Identifizierung von minderwertigen und gefälschten Arzneimitteln in Ländern mit niedrigem und mittlerem Einkommen durch Screening-Technologien

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vorgelegt von

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Erklärung

Ich erkläre hiermit, dass ich die zur Promotion eingereichte Arbeit mit dem Titel:

„Identifizierung von minderwertigen und gefälschten Arzneimitteln in Ländern mit niedrigem und mittlerem Einkommen durch Screening-Technologien“

selbständig verfasst, nur die angegebenen Quellen und Hilfsmittel benutzt und Zitate als solche gekennzeichnet habe. Ich erkläre, dass die Richtlinien zur Sicherung guter wissenschaftlicher Praxis der Universität Tübingen (Beschluss des Senats vom 25.5.2000) beachtet wurden. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Mir ist bekannt, dass die falsche Abgabe einer Versicherung an Eides statt mit Freiheitsstrafe bis zu drei Jahren oder mit Geldstrafe bestraft wird.

Tübingen, den 27.02.2023

Gesa Gnegel

Abkürzungsverzeichnis

BP	British Pharmacopoeia, Britisches Arzneibuch
COVID-19	Coronavirus disease 2019, Coronavirus-Krankheit 2019
CQ	Chloroquin
DC	Dünnschichtchromatographie
DR Kongo	Demokratische Republik Kongo
Difäm	Deutsches Institut für Ärztliche Mission
EPN	Ecumenical Pharmaceutical Network, Ökumenisches Pharmazeutisches Netzwerk
GC-MS	Gaschromatographie mit Massenspektrometrie Kopplung
GMP	Good manufacturing practice, Gute Herstellungspraxis
GPHF	Global Pharma Health Fund, Globaler Pharma-Gesundheitsfonds
HPLC	High performance liquid chromatography, Hochleistungsflüssigkeitschromatographie
HPMC	Hydroxypropylmethylcellulose
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, Internationaler Rat für die Harmonisierung der technischen Anforderungen an Humanarzneimittel
LC-HR-MS	Liquid Chromatography High Resolution Mass Spectrometry, Flüssigchromatographie mit hochauflösender Massenspektrometrie Kopplung
LMIC	Low- and Middle-Income countries, Länder mit niedrigem und mittlerem Einkommen
NIR	Nahes Infrarot
PC	Principal component, Hauptkomponente
PCA	Principal component analysis, Hauptkomponentenanalyse
pH	Potential hydrogenii, Potential des Wasserstoffs
Ph. Int.	International Pharmacopoeia, Internationales Arzneibuch
RFDA	Rwanda Food and Drugs Authority, Rwandische Aufsichtsbehörde für Lebensmittel und Medikamente
SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2, Schweres akutes Atemwegssyndrom Coronavirus Typ 2
US$	United States Dollar, Dollar der Vereinigten Staaten von Amerika
USP	United States Pharmacopeia, Arzneibuch der Vereinigten Staaten von Amerika
WHO	World Health Organization, Weltgesundheitsorganisation

Zusammenfassung

Minderwertige und gefälschte Arzneimittel bedrohen die Gesundheit von Patient:innen weltweit aber besonders in Ländern mit niedrigem und mittlerem Einkommen (LMICs). Schätzungen der WHO zufolge ist in diesen Regionen jedes zehnte Arzneimittel minderwertig oder gefälscht. Für die rasche Identifizierung solcher Produkte ist in den letzten Jahren eine Vielzahl von Screening-Technologien entwickelt worden. Daten über Kosten, Nutzen und Limitationen in der praktischen Anwendung fehlen jedoch für viele dieser Technologien. Diese Doktorarbeit wertet daher den routinemäßigen Einsatz der Screening-Technologie GPHF-Minilab® durch 16 kirchliche Gesundheitsorganisationen in 13 LMICs im Zeitraum 2019-2020 aus. Insgesamt wurden 34 vermutlich gefälschte Produkte identifiziert und an die WHO gemeldet. Dies führte zu mehreren (inter-)nationalen Warnmeldungen. Im Jahr des Ausbruches der COVID-19 Pandemie (2020) wurden mehr Fälschungen gefunden als im Vorjahr (2019). Der Anstieg ging insbesondere auf gefälschte Chloroquin-Präparate zurück. Chloroquin wurde zu Pandemiebeginn als Behandlungsoption bei COVID-19 diskutiert. Mit Verbrauchsmaterialkosten von 7,45€ pro Probe erwies sich das Minilab® als preisgünstige und wirksame Screening-Technologie.

Mit tragbaren NIR-Spektrometern können schnelle und nicht-destruktive Probenmessungen durchgeführt werden. Die für ihren Einsatz als Screening-Technologien benötigten Spektraldatenbanken und multivariaten Auswertungsverfahren sind derzeit Gegenstand der Forschung. In dieser Doktorarbeit wurde für das tragbare Spektrometer NIR-S-G1 eine auf dem Verfahren der Hauptkomponentenanalyse basierende Methode zur Verifizierung unentbehrlicher Wirkstoffe in Tabletten erstellt, validiert und auf ihre Eignung zur Identifizierung von Arzneimittelfälschungen untersucht. Mit der entwickelten Methode konnten Tabletten anhand der enthaltenen Wirkstoffe unterschieden und alle untersuchten Fälschungen, die den deklarierten Wirkstoff nicht oder in deutlich zu geringen Mengen enthielten, als solche identifiziert werden.

In einem Nebenprojekt dieser Doktorarbeit, wurde der Gehalt des Konservierungsmittels Benzylalkohol in Oxytocin-Injektionen bestimmt, die im Rahmen einer Feldstudie in Rwanda gesammelt wurden. Es zeigte sich, dass bei einem Hersteller der Benzylalkoholgehalt zwischen 0,9% und 0,0018% schwankte, was grobe Verstöße gegen die Gute Herstellungspraxis nahelegt.

Summary

Substandard and falsified medicines threaten the health of patients worldwide, but especially in low- and middle-income countries (LMICs). According to WHO estimates, one in ten medicines in these regions is substandard or falsified. A variety of screening technologies have been developed in recent years to rapidly identify such products. However, data on costs, benefits and limitations in practical application are lacking for many of these technologies. This doctoral thesis therefore evaluates the routine use of the GPHF-Minilab® screening technology by 16 faith-based health organizations in 13 LMICs in the years 2019 and 2020. A total of 34 suspected falsified products were identified and reported to WHO. This resulted in several (inter-)national alerts. More falsified products were found in the year of the COVID-19 pandemic outbreak (2020) than in the previous year (2019). The increase was particularly due to falsified chloroquine preparations. Chloroquine had been discussed as a treatment option at the onset of the pandemic. With consumable costs of 7.45€ per sample, the Minilab® proved to be an inexpensive and effective screening technology.

Portable NIR spectrometers can be used to perform rapid and non-destructive sample measurements. The spectral databases and multivariate evaluation procedures required for their use as screening technologies are currently a subject of research. In this doctoral thesis, a method for the NIR-S-G1 portable spectrometer with the purpose of verifying essential active ingredients in tablets based on principal component analysis was created, validated, and evaluated for its suitability in identifying falsified medicines. With the method developed, tablets could be distinguished based on the active ingredients they contained, and all falsified medicines examined that did not contain the declared active ingredient or contained it in quantities that were significantly too low could be identified as such.

In a side project of this doctoral thesis, the content of the preservative benzyl alcohol was determined in oxytocin injections collected during a field study in Rwanda. It was found that for one manufacturer, the benzyl alcohol content varied between 0.9% and 0.0018%, suggesting gross violations of good manufacturing practice.

Publikationen und Präsentationen

Akzeptierte Publikationen

„Identification of Falsified Chloroquine Tablets in Africa at the Time of the COVID-19 Pandemic"

Gnegel, G.; Hauk, C.; Neci, R.; Mutombo, G.; Nyaah, F.; Wistuba, D.; Häfele-Abah, C.; Heide, L.

The American Journal of Tropical Medicine and Hygiene, 2020; 103(1):73-76

„Quality of oxytocin and misoprostol in health facilities of Rwanda"

Bizimana, T.; Hagen, N.; **Gnegel, G.**; Kayumba, P.C.; Heide, L.

PloSOne, 2021; 16(1):e0245054

„Surveillance for substandard and falsified medicines by local faith-based organizations in 13 low- and middle-income countries using the GPHF Minilab"

Gnegel, G.; Häfele-Abah, C.; Neci, R.; Difäm-EPN Minilab Network; Heide, L.

Scientific Reports, 2022; 12(1):13095

Noch nicht eingereichte Manuskripte

„Verification of the Active Pharmaceutical Ingredient in Tablets Using a Low-Cost Near-Infrared Spectrometer and Principal Component Analysis"

Gnegel, G.; Gabel, J.; Kessler, W.; Heide, L.

Mündliche Präsentationen

„Spektroskopische Methoden zur Arzneimittelqualitätsprüfung: NIR und Raman“

Vortrag im Rahmen des Netzwerktreffens der in der pharmazeutischen Entwicklungszusammenarbeit tätigen Organisationen, Tönisvorst, Februar 2020

„Implementing detection technologies in low-resource settings”

Vortrag im Rahmen der Konferenz “Technologies to Tackle Substandard & Falsified Medical Products in Global Health”, Wellcome Centre for Ethics and Humanities (Ethox Centre), University of Oxford, UK (online), November 2021

„Alles fake? Arzneimittelfälschungen in Niedriglohnländern“

Workshop im Rahmen des Neuaufgenommenen-Wochenendes des Cusanuswerkes, online, Januar 2022

„Workshop: Visuelle Arzneimittelinspektion“

Workshop im Rahmen des Kurses „Pharmacy in Global Health“ der Universität Tübingen, online, März 2022

„Universitäre Pharmazie und Globale Gesundheit“

Vortrag im Rahmen der Jahrestagung des Vereins demokratischer Pharmazeutinnen und Pharmazeuten, Bonn, Juni 2022

„Exercise: Quality control of tablets by near-infrared spectroscopy”

Praktische Demonstration und Vortrag im Rahmen des Workshops „Drug lifecycle control in Sub-Saharan Africa“, Arusha (Tansania), September 2022

Erklärung der Eigenanteile

„Identification of Falsified Chloroquine Tablets in Africa at the Time of the COVID-19 Pandemic"

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The American Journal of Tropical Medicine and Hygiene, 2020; 103(1):73-76

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  - Durchführung der Untersuchungen mit dem GPHF-Minilab®
- Dorothee Wistuba
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PloSOne, 2021; 16(1):e0245054

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„Surveillance for substandard and falsified medicines by local faith-based organizations in 13 low- and middle-income countries using the GPHF Minilab"

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„Verification of the Active Pharmaceutical Ingredient in Tablets Using a Low-Cost Near-Infrared Spectrometer and Principal Component Analysis“

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  - Projektplanung und -betreuung
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Einleitung

Gefälschte und minderwertige Arzneimittel – Prävalenz, Ursachen und Konsequenzen

Das Recht auf Gesundheit gehört zu den 1948 von den Vereinten Nationen verabschiedeten Menschenrechten.[1] Um dieses zu erreichen ist auch eine „allgemeine Gesundheitsversorgung, einschließlich […] Zugang zu sicheren, wirksamen, hochwertigen und bezahlbaren unentbehrlichen Arzneimitteln und Impfstoffen“ (Vereinte Nationen, 2015, S.17) notwendig, wie das Unterziel 3.8 der Ziele für eine nachhaltige Entwicklung der Vereinten Nationen erneut betont.[2] Noch sind diese Ziele aber nicht erreicht: 2017 hatten rund zwei Milliarden Menschen keinen Zugang zu grundlegenden Arzneimitteln.[3] Faktoren wie ein eingeschränkter Zugang zu erschwinglichen, qualitätsgesicherten Arzneimitteln, ungenügende staatliche Regulierung des Arzneimittelmarktes durch Gesetze und Kontrollsysteme sowie limitierte Kapazitäten in den Bereichen Personal, Fachwissen und Ausrüstung, begünstigen ihrerseits das Auftreten von minderwertigen und gefälschten Arzneimitteln.[4,5] So sind insbesondere Menschen in Ländern mit niedrigem und mittlerem Einkommen der Gefahr von minderwertigen und gefälschten Arzneimitteln ausgesetzt.[6,7] Die WHO schätzt, dass 10,5% der Arzneimittel in diesen Ländern minderwertig oder gefälscht sind.[6] Die Konsequenzen einer Behandlung mit Medikamenten von ungenügender Qualität sind vielschichtig und betreffen sowohl individuelle Patient:innen als auch ganze Gesundheitssysteme. So kann es zu längeren und schwereren Krankheitsverläufen oder sogar zum Tod von Patient:innen kommen.[6,8] Grund hierfür können nebst fehlenden oder unterdosierten Wirkstoffen auch Kontaminationen sein: Im Oktober und November 2022 veröffentlichte die WHO zwei internationale Warnmeldungen zu pädiatrischen Erkältungssäften, die unzulässige Mengen an Diethylenglykol und Ethylenglykol enthalten und die damit behandelten Kinder in Lebensgefahr bringen.[9,10] Bezogen auf die Gesamtbevölkerung, führen minderwertige und gefälschte Arzneimittel also zu einer gesteigerten Morbidität und Mortalität sowie Prävalenz von Erkrankungen. Weitere Schäden entstehen Patient:innen und Gesundheitssystemen auf finanzieller Ebene, indem Ressourcen für den Kauf solcher Arzneimittel verschwendet werden. Schätzungen der WHO zufolge, werden jährlich umgerechnet 30,5 Milliarden US$ für Arzneimittelfälschungen und

minderwertige Arzneimittel ausgegeben. Werden solche Arzneimittel in öffentlichen Gesundheitseinrichtungen eingesetzt, kann dies das Vertrauen in diese Gesundheitssysteme nachhaltig schädigen.[6] Weiterhin können insbesondere minderwertige und gefälschte Antibiotika und Malariamedikamente zur Entstehung von Arzneimittelresistenzen beitragen.[6,11] Damit wirksame Maßnahmen zur Bekämpfung von minderwertigen und gefälschten Arzneimitteln implementiert werden können, sind verlässliche Daten zur Prävalenz von gefälschten und minderwertigen Arzneimitteln nötig.[4] Eine Anzahl von Studien hat sich mit dieser Fragestellung beschäftigt und eine überraschend heterogene Datenlage geschaffen: In einer Übersichtsarbeit aus dem Jahr 2018 geben Ozawa et al. die Prävalenz von minderwertigen und gefälschten Arzneimitteln in Ländern mit niedrigem und mittlerem Einkommen mit 14% an.[7] In einer unabhängig davon veröffentlichten Übersichtsarbeit von McManus und Naughton aus dem Jahr 2020, finden diese für Länder mit niedrigem und mittlerem Einkommen hingegen eine mehr als doppelt so hohe Prävalenz von 30%.[12] In Einzelstudien können bisweilen noch größere Schwankungen beobachtet werden: So wird die Prävalenz minderwertiger Malariamedikamente in Malawi je nach Studie mit 88% oder auch nur mit 11% angegeben.[13,14] Eine Ursache für diese Heterogenität liegt in methodischen Unterschieden bei der Planung und Durchführung der Forschungsarbeiten. Hierzu zählen das Verwenden unterschiedlicher Studiendesigns, Definitionen minderwertiger und gefälschter Arzneimittel sowie Grenzwerte für analytische Untersuchungen wie Gehalts- und Freisetzungsuntersuchungen.[15,16] In den letzten Jahren wurden daher Richtlinien zur Durchführung von Arzneimittelqualitätsstudien eingeführt[15,17] und harmonisierte Grenzwerte vorgeschlagen.[16] Weiterhin arbeitete die WHO 2017 eindeutige Definitionen für minderwertige und gefälschte Arzneimittel aus: Mit Arzneimittelfälschungen sind solche Produkte zu bezeichnen, die falsche Angaben zu ihrer Identität, Zusammensetzung oder Herkunft aufweisen. Diese Falschangaben müssen hierbei vorsätzlich und in betrügerische Absicht gemacht worden sein. Minderwertige Produkte sind hingegen solche, die den geforderten Qualitätsansprüchen nicht gerecht werden, wobei die Ursache der Qualitätsmängel in versehentlichen Fehlern beispielsweise bei Herstellung, Verpackung, Transport oder Lagerung der Arzneimittel liegt.[18] Da in der praktischen Anwendung nicht immer eindeutig ist, ob ein Qualitätsmangel auf vorsätzliche oder unabsichtliche Ursachen zurückzuführen ist, haben Hauk et al.[16] Kriterien zur Klassifizierung von Produkten vorgeschlagen, die beispielsweise im Rahmen von Feldstudien identifiziert wurden.

Als Fälschungen sollen hier solche Produkte behandelt werden, die entweder von einer zuständigen Behörde oder dem deklarierten Hersteller als solche bestätigt wurden. Weiterhin sind auch solche Produkte als Fälschungen zu betrachten, die keinen oder einen nicht-deklarierten Wirkstoff anstelle des angegebenen Wirkstoffes enthalten oder bei denen die Verpackung eindeutige Falschangaben aufweist. Letzteres kann beispielsweise dann gegeben sein, wenn der deklarierte Hersteller nicht existiert. Auch bei Produkten, die weniger als 50% des deklarierten Wirkstoffgehaltes enthalten, handelt es sich wahrscheinlich um Fälschungen, sofern keine Hinweise auf einen bereits stattgefundenen Zersetzungsprozess, beispielsweise in Form von Abbauprodukten, vorliegen.[16]

Screening-Technologien zur raschen Identifizierung von gefälschten und minderwertigen Arzneimitteln

Die von der WHO vorgeschlagene Strategie zur Bekämpfung von gefälschten und minderwertigen Arzneimitteln umfasst drei Säulen: Vorbeugen, Aufdecken und Reagieren.[4] Insbesondere für die zweite Säule spielen analytische Untersuchungen von Arzneimitteln eine große Rolle. Der Goldstandard hierfür sind die Methoden der international anerkannten Arzneibücher, wie das Britische Arzneibuch (BP), das Arzneibuch der Vereinigten Staaten von Amerika (USP) oder das Internationale Arzneibuch (Ph. Int.), die in der Regel teures Laborequipment wie HPLC-Anlagen voraussetzen. Solch umfassend ausgestattete Labore, entsprechend ausgebildetes Personal und die notwendigen finanziellen Ressourcen, sind in vielen Ländern mit niedrigem und mittlerem Einkommen rar.[19] In der Konsequenz können verdächtige Arzneimittel nicht oder nur mit deutlicher zeitlicher Verzögerung untersucht werden. Ein Baustein zur Schließung dieser analytischen Lücke sind Screening-Technologien.[4,20] Der Begriff Screening-Technologien umfasst eine Reihe von vereinfachten Untersuchungsverfahren und -geräten, mit deren Hilfe ausgewählte Aspekte der Qualität eines Arzneimittels analysiert werden können. Häufige Anwendungen von Screening-Technologien sind die Verifizierung der äußeren Erscheinung des Produktes, die Identifizierung sowie die Quantifizierung von Wirkstoffen, Hauptinhaltsstoffen oder Verunreinigungen.[21] Idealerweise sind Screening-Technologien preiswert in der Anschaffung und Anwendung, einfach in der Handhabung, transportierbar oder tragbar und haben eine kurze Analysendauer. In den letzten Jahren ist eine Vielzahl von Screening-Technologien entwickelt worden,

die auf so verschiedenen Analyseprinzipien wie bspw. der Kolorimetrie, der Chromatographie, der Spektroskopie oder der visuellen Inspektion beruhen.[21,22] Der Vielzahl an Technologien steht eine dünne Datenlage hinsichtlich Aspekten der praktischen Anwendbarkeit gegenüber: viele Screening-Technologien wurden bis dato nur an einer kleinen Anzahl von Wirkstoffen getestet, nicht in Feldstudien und nicht im Vergleich miteinander untersucht. Weiterhin fehlen oft Daten zu Sensitivität und Spezifität der Screening-Ergebnisse sowie zum Kosten-Nutzen-Verhältnis.[22,23] Ebensolche Informationen sind aber bspw. für Arzneimittelaufsichtsbehörden von großer Relevanz, um eine fundierte Auswahl einer für ihre jeweils beabsichtigte Anwendung passenden Technologie zu treffen.

Vergleichsweise gut untersucht ist die vom Global Pharma Health Fund (GPHF) entwickelte Screening-Technologie GPHF-Minilab®: Mit fast 900 Exemplaren in 99 Ländern, ist das Minilab® die weltweit am häufigsten eingesetzte Screening-Technologie zur Überprüfung der Arzneimittelqualität in Ländern mit niedrigem und mittlerem Einkommen.[22,24,25] Diese „Kofferlabore“ werden beispielsweise bei Arzneimittelaufsichtsbehörden oder an Universitäten in Forschung und Lehre eingesetzt.[14,26,27] Darüber hinaus kommen sie auch bei den Mitgliedern eines vom Deutschen Institut für Ärztliche Mission (Difäm) und vom Ecumenical Pharmaceutical Network (EPN) gegründeten Netzwerkes zum Einsatz. Bei dem Difäm-EPN Minilab Netzwerk handelt es sich um einen Zusammenschluss kirchlicher Zentral- und Krankenhausapotheken, die mittels Minilab® routinemäßig Arzneimittel aus ihrem Lagerbestand oder Wareneingang, sowie auffällige Arzneimittelproben, beispielsweise von Gesundheitseinrichtungen oder vom Schwarzmarkt, untersuchen.[28,29] Gemäß GPHF-Minilab® Handbuch[30] umfasst das Screening vier Schritte: eine visuelle Prüfung von Etikett, Verpackung und Darreichungsform, eine Überprüfung des Gesamt- bzw. Füllgewichtes der Darreichungsform, einen vereinfachten Zerfallstest sowie eine dünnschichtchromatographische Analyse zur qualitativen und semiquantitativen Untersuchung des Wirkstoffs. Bei Letzterer werden auf eine DC-Platte nebst zwei Spots der im vorgeschriebenen Lösungsmittel dispergierten Arzneimittelprobe auch zwei Referenzspots aufgetragen. Diese enthalten den deklarierten Wirkstoff in Konzentrationen, die 100% und 80% der auf dem Etikett angegebenen Menge entsprechen. Nach Entwicklung der Platte in der vorgeschriebenen mobilen Phase und unter entsprechender Detektion (z.B. UV-Licht, Iodfärbung) kann anhand von Retentionsfaktor und Farbe der Spots die Anwesenheit des deklarierten Wirkstoffes

überprüft werden, wohingegen die Größe und Stärke der Spots Hinweise auf die enthaltene Dosierung geben.[30] Auf diese Weise können derzeit 107 Wirkstoffe in Tabletten, Kapseln, Injektionen und in einigen flüssigen oralen Darreichungsformen untersucht werden.[30] Während sich das Minilab® bei der Identifizierung von Arzneimitteln, die den angegebenen Wirkstoff nicht enthalten, in Feld- und Laborstudien als sehr empfindlich und spezifisch erwiesen hat, ist es weniger empfindlich beim Nachweis von Produkten, die eine unzureichende Menge des Wirkstoffs enthalten oder eine unzureichende Wirkstofffreisetzung aufweisen.[31,32] Im Rahmen dieser Dissertation wurden die Ergebnisse einer zweijährigen Routineanwendung des Minilabs® in 13 Ländern ausgewertet.

Als nachteilhaft bei der Anwendung des Minilabs® können die Destruktivität der Methode (d.h. die Zerstörung der Arzneimittelprobe im Rahmen der Analyse), der Bedarf an Chemikalien und Referenzstandards und die für eine Screening-Technologie verhältnismäßig lange Analysendauer empfunden werden. Eine andere Screening-Methodik die ein rasches, nicht-destruktives Screening ermöglicht, weder zusätzlichen Reagenzien noch umfassende Schulung des anwendenden Personals erfordert, stellt die Nahinfrarotspektroskopie mit tragbaren Spektrometern dar.[22,33-35] Da bei der Nahinfrarotspektroskopie Oberton- und Kombinationsschwingungen von Molekülen angeregt werden, resultieren Absorptionsspektren mit breiten, überlappenden Banden. Zudem sind Nahinfrarotspektren nicht nur von chemischen Informationen wie der Wirk- und Hilfsstoffzusammensetzung eines Arzneimittels, sondern auch von physikalischen Eigenschaften wie der Partikelgröße, Feuchtigkeit oder Kristallform abhängig.[36,37] In der Regel können die Spektren daher nicht ohne Zuhilfenahme mathematisch-statistischer Verfahren, der sogenannten Chemometrie, und auf die jeweilige Fragestellung angepasster Spektraldatenbanken ausgewertet werden.[34,36,37] Die Erstellung und kontinuierliche Aktualisierung der erforderlichen Datenbanken mit Referenzspektren kann erheblichen Aufwand verursachen[36,38] und setzt den Zugang zu qualitätsgesicherten, authentischen Präparaten aller relevanten Arzneimittel voraus. Während die benötigten tragbaren Spektrometer kommerziell erhältlich sind, ist die Auswahl entsprechender chemometrischer Verfahren und Etablierung von Datenbanken derzeit noch Gegenstand der Forschung und auch Teil dieser Dissertationsschrift.

Arzneimittelversorgung und -qualität während der COVID-19 Pandemie

Im Dezember 2019 wurde in Wuhan, China ein Ausbruch von schweren Pneumonien unbekannter Genese beobachtet. Im Januar 2020 wurde als Erreger der Infektionen ein mit SARS-CoV-2 bezeichnetes Virus ausgemacht.[39] Dieses neuartige Coronavirus breitete sich in den darauf folgenden Wochen schnell über den Globus aus und am 11. März 2020 rief die WHO den Zustand einer Pandemie aus.[40] Bis Oktober 2022 kostete die sogenannte COVID-19 Pandemie mehr als 6,5 Millionen Menschen das Leben.[41] Unmittelbar nach der Identifizierung des Virus, wurden verschiedene bereits bei anderen Indikationen etablierte Wirkstoffe hinsichtlich ihrer möglichen Wirksamkeit gegen Sars-CoV-2 diskutiert, darunter auch die Wirkstoffe Chloroquin und Hydroxychloroquin.[42,43] Hierbei handelt es sich um zwei Wirkstoffe, die indikationsgemäß bei Malaria in Gebieten mit günstiger Resistenzlage sowie bei Autoimmunerkrankungen eingesetzt werden. Verstärkt durch Effekte wie gesteigerte mediale Berichterstattung, Falschinformationen („fake news“) und Panikkäufe, stieg die Nachfrage nach Arzneimitteln wie Chloroquin und Hydroxychloroquin in den ersten Monaten der Pandemie stark an.[44,45] Parallel hierzu kam es weltweit zu Beeinträchtigungen und Unterbrechungen von Lieferketten[46-48] woraus Lieferengpässe bei mehreren unentbehrlichen Arzneimitteln resultierten.[44,49] In diesem Szenario von gesteigerter Nachfrage und eingeschränkter Verfügbarkeit, wurde ein Anstieg von gefälschten und minderwertigen Arzneimitteln vorhergesagt[43] und zahlreiche Berichte über Qualitätsmängel bei Arzneimitteln und Medizinprodukten, die im Zusammenhang mit COVID-19 stehen, wurden ab Januar 2020 veröffentlich.[50] Die COVID-19 Pandemie führte also zu einer globalen Verschlechterung der Versorgung von Patienten mit qualitativ hochwertigen Medikamenten. Die anfänglichen Hoffnungen bezüglich Chloroquin und Hydroxychloroquin erwiesen sich als unberechtigt: im Juni 2020 wurde der mit diesen Wirkstoffen bestückte Arm der von der WHO geleiteten klinischen Studie „Solidarity“ vorzeitig aufgrund fehlender Wirksamkeit eingestellt.[51] Welche Auswirkungen die Pandemie auf die Arzneimittelqualität in afrikanischen Ländern hatte, wird im Rahmen dieser Dissertation an konkreten Fallbeispielen illustriert.

Qualität von Oxytocin- und Misoprostol-Präparaten in Rwanda

Im Jahr 2017 starben weltweit 295 000 Frauen bei der Geburt, die meisten von ihnen in Subsahara Afrika.[52] Die häufigste Geburtskomplikation mit tödlichem Ausgang sind Nachgeburtsblutungen, bei denen die Mütter einen starken Blutverlust erleiden.[53] Mittel der Wahl zur Prävention und Behandlung von Nachgeburtsblutungen in Ländern mit niedrigem und mittlerem Einkommen sind Oxytocin und Misoprostol.[53,54]

Bei Oxytocin handelt es sich um ein zyklisches Nonapeptid mit einer Disulfidbrücke.[55] Oxytocin ist gegenüber hohen Temperaturen sehr empfindlich und kann sich während der Lagerung bei hohen Temperaturen rasch zersetzen.[56,57] Die WHO empfiehlt daher die Aufbewahrung im Kühlschrank bei 2-8°C.[56] Einige Hersteller geben jedoch abweichende Lagerungsbedingungen wie bspw. Raumtemperatur auf ihren Produkten an.[58] Weiterhin hat Oxytocin ein Stabilitätsmaximum bei pH 3-5, weshalb Injektionslösungen auf diesen pH-Bereich eingestellt werden sollten.[59]

Misoprostol ist ein Analogon des Prostaglandins E1. Eine Zersetzung dieses Wirkstoffes findet insbesondere unter dem Einfluss von Feuchtigkeit leicht statt.[60] Die Verpackung von Misoprostol-Tabletten in Alu-Alu-Blistern bietet einen guten Schutz vor Feuchtigkeit und verhindert einen vorzeitigen Wirkstoffabbau.[61] Weiterhin bewirkt die Formulierung von Misoprostol als 1%ige Dispersion in Hydroxypropylmethylcellulose (HPMC) eine Verbesserung der Wirkstoffstabilität.[62]

Die Qualität von im Handel befindlichen Oxytocin- und Misoprostol-Präparaten wurde in einem systematischen Review aus dem Jahr 2020 von Torloni et al.[63] ausgewertet. In den Review wurden 14 Studien zur Qualität von Oxytocin-Injektionen und drei Studien zu Misoprostol-Tabletten einbezogen. 39,7% der Oxytocin-Proben und 38,7% der Misoprostol-Proben entsprachen nicht den Qualitätsstandards.[63] Am Beispiel von Oxytocin und Misoprostol wird besonders deutlich, welchen Schaden minderwertige und gefälschte Arzneimittel anrichten können: Eine mit solchen Präparaten behandelte Nachgeburtsblutung kann unmittelbar den Tod der Patientin zur Folge haben. Daher wurden im Rahmen dieser Dissertation Untersuchungen zur Überprüfung der Qualität von Oxytocin-Proben aus Rwanda durchgeführt.

Zielsetzung

Da Screening-Technologien eine wichtige Rolle bei der raschen Identifizierung von Arzneimittelfälschungen spielen und somit Patient:innen vor potenziell schädlichen Produkten schützen können, widmet sich diese Doktorarbeit der praktischen Anwendung und Weiterentwicklung solcher Technologien. Wie in aktuellen Übersichtsarbeiten hervorgehoben, fehlen zu vielen Screening-Technologien verlässliche Daten zu Kosten, Nutzen und Limitationen.[22,23] Die Screening-Technologie GPHF-Minilab® ist zwar verhältnismäßig gut untersucht, jedoch lagen vor dieser Doktorarbeit wenige Daten über deren langfristige, routinemäßige Anwendung vor. Diese Lücke sollte in Zusammenarbeit mit 16 lokalen Gesundheitsorganisationen geschlossen und Voraussetzungen sowie Kosten des erfolgreichen Betriebes dieser Screening-Technologie aufgezeigt werden. Weiterhin sollte ausgewertet werden, welche Art von problematischen Arzneimitteln so identifiziert werden können, und wie häufig diese auftreten. Die COVID-19 Pandemie war zu Beginn dieser Doktorarbeit nicht absehbar, dennoch konnte das Monitoring der Minilab®-Anwendung Daten zur Häufigkeit und Art von Arzneimittelfälschungen im Pandemiekontext generieren. Die Identifizierung und gründliche Untersuchung von fünf Chloroquin-Fälschungen, die kurz nach Pandemieausbruch in Kamerun und der DR Kongo gefunden wurden, zeigt auf, dass die Pandemie das Risiko einer Verschlechterung der globalen Arzneimittelsicherheit mit sich brachte.

Mit NIR-S-G1 ist ein preiswertes, tragbares Spektrometer kommerziell erhältlich. Damit dieses als Screening-Technologie eingesetzt werden kann, werden auf die jeweilige Fragestellung angepasste Spektraldatenbanken und chemometrische Verfahren benötigt. Ziel dieser Doktorarbeit war der Aufbau einer Spektraldatenbank und die Erarbeitung eines chemometrischen Verfahrens für die Verifizierung von unentbehrlichen Wirkstoffen in Tabletten. Um für den Einsatz in Kontexten mit limitierten Ressourcen geeignet zu sein, sollte letzteres Verfahren idealerweise unabhängig von teurer Software anwendbar und die Ergebnisdarstellung auch für Spektroskopie-Laien verständlich sein.

Die Gehaltsbestimmung des Konservierungsmittels Benzylalkohol in Oxytocin-Injektionen, die in einer Feldstudie in Rwanda gesammelt wurden, sollte die Qualitätsprüfung dieser Proben vervollständigen, da Qualitätsmängel nicht auf Identität und Quantität des Wirkstoffes begrenzt sind.

Ergebnisse

Routinemäßiger Einsatz der Screening-Technologie GPHF-Minilab® vor und während der COVID-19 Pandemie

Im Rahmen dieser Doktorarbeit wurde die routinemäßige Anwendung des GPHF-Minilabs® durch das Difäm-EPN Minilab Netzwerkes hinsichtlich der damit identifizierten minderwertigen und gefälschten Arzneimittel sowie der erforderlichen finanziellen und strukturellen Voraussetzungen für den erfolgreichen Einsatz dieser Screening-Technologie ausgewertet. Im Zeitraum 2019-2020 sammelten und analysierten die 16 Mitgliedsorganisationen des Netzwerkes insgesamt 2055 Arzneimittelproben, von denen 1919 die Einschlusskriterien dieser Studie erfüllten (Abbildung 1).

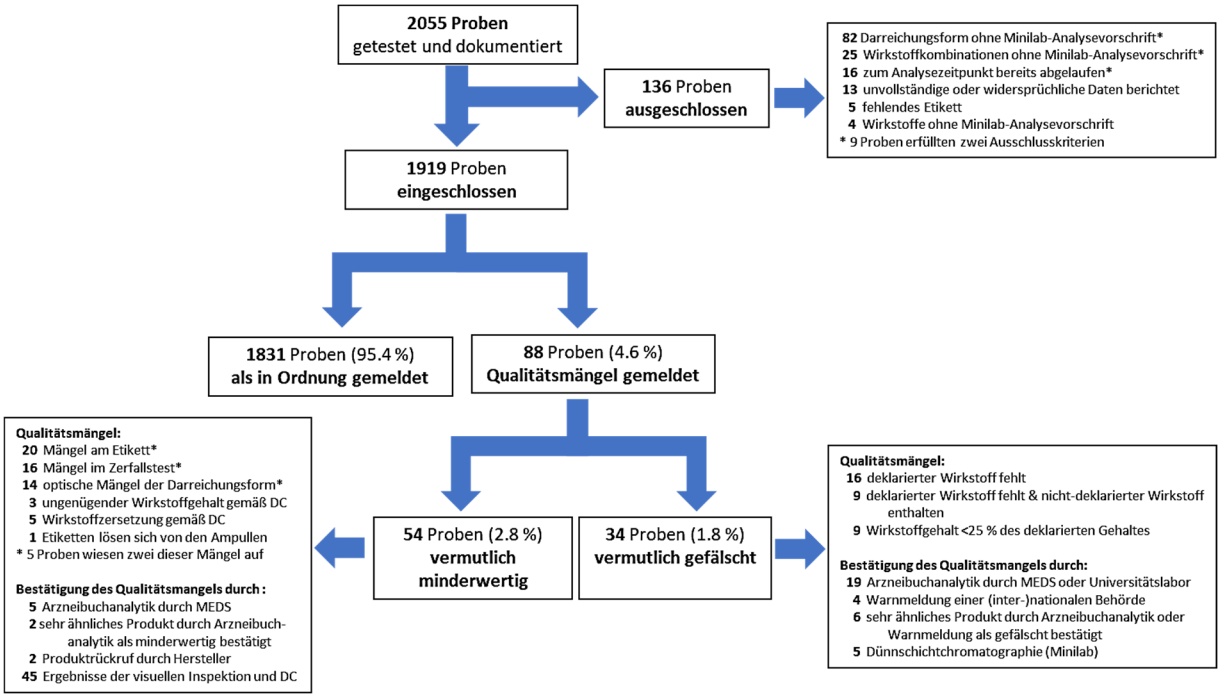
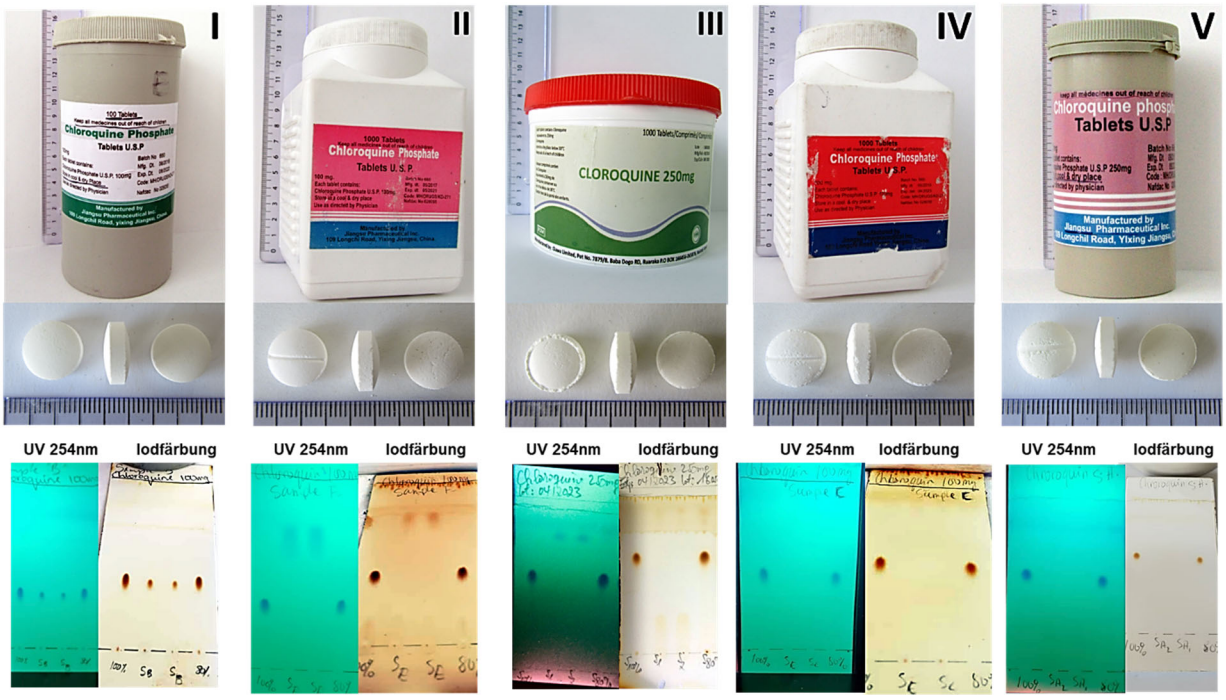


Abbildung 1: Flussdiagramm der mit dem Minilab® untersuchten Präparate, inklusive Ausschlusskriterien und identifizierter Qualitätsmängel. *(Modifiziert nach Gnegel et al.[29])*

Die Mitgliedsorganisationen hatten ihren jeweiligen Sitz in zwölf afrikanischen Ländern sowie in Indien und alle Proben wurden in diesen 13 Ländern gesammelt. Da die Organisationen das Minilab® für ihre Wareneingangskontrolle verwenden, stammte ein Großteil der untersuchten Proben (1591 Produkte) aus den Lagerbeständen der Partner oder von privaten (Groß-)Händlern, bei denen die Mitgliedsorganisationen

einkaufen. Weitere 111 Proben wurden auf dem Schwarzmarkt erstanden. Die übrigen 217 Proben stammten aus Gesundheitseinrichtungen oder wurden den Netzwerkmitgliedern als Spenden überlassen. Gemäß den Angaben auf den Etiketten, stammten 50,3% der Proben aus Indien, 22,2% wurden in verschiedenen afrikanischen Ländern produziert und 16,3% waren chinesische Produkte. Mit 42,1%, stellen Antibiotika den größten Teil der untersuchten Proben dar, gefolgt von Malaria-Medikamenten (17,7%) und Schmerzmitteln (11,7%). Mit 1831 Proben (95,4%) war der Großteil der untersuchten Produkte in der Minilab®-Untersuchung unauffällig. Bei 88 Proben (4,6%) wurden hingegen Qualitätsmängel festgestellt. Hiervon wurden 34 Produkte (1,8% aller eingeschlossenen Proben) als vermutlich gefälscht eingestuft, da sie den deklarierten Wirkstoff nicht enthielten oder weniger als 25% der deklarierten Menge. Neun Produkte enthielten zusätzlich nicht-deklarierte Wirkstoffe (Abbildungen 1 und 2). Die übrigen 54 Produkte (2,8% aller eingeschlossenen Proben) wurden als vermutlich minderwertig eingestuft. Diese Produkte wiesen visuelle Mängel an Etikett oder Darreichungsform auf, enthielten gemäß dünnschichtchromatographischer Untersuchung eine ungenügende Wirkstoffmenge oder bestanden den vereinfachten Zerfallstest nicht. Die Termini „vermutlich gefälscht“ und „vermutlich minderwertig“ kommen hier zum Einsatz, da in vielen aber nicht in allen Fällen die Ergebnisse der Screening-Technologie GPHF-Minilab® durch eine Arzneibuchanalytik abgesichert werden konnten. Alle 34 Fälschungen wurden in den fünf Ländern Kamerun, Tschad, DR Kongo, Zentralafrikanische Republik und Nigeria gefunden. Von den 111 auf dem Schwarzmarkt erworbenen Arzneimitteln wurden 12,6% (14 Proben) als vermutlich gefälscht eingestuft. Dieser Anteil ist signifikant höher ($p < 0,0001$) als bei den 1808 Arzneimittel aus legalen Quellen (1,1%, 20 Proben). Von den 970 Arzneimitteln aus den Lagerbeständen und Wareneingängen der Netzwerkmitglieder, wurden nur drei (0,3%) als vermutlich gefälscht eingestuft, was auf eine weitgehend erfolgreiche Produkt- und Lieferantenqualifizierung durch die teilnehmenden Zentral- und Krankenhausapotheken hindeutet. Weiterhin waren 22 (64,7%) der 34 vermutlich gefälschten Präparate bereits in der visuellen Inspektion auffällig, während visuelle Mängel nur bei 34 (1,8%) der 1885 nicht-gefälschten Präparate beobachtet wurden. Der statistisch signifikante Unterschied ($p<0,0001$) zwischen diesen beiden Gruppen zeigt die Bedeutung einer gründlichen visuellen Inspektion von Arzneimittelproben auf. Bei 32 der 34 vermutlich gefälschten Präparate handelt es sich um Mittel zur Behandlung von Infektionskrankheiten.

Eine besonders interessante Serie von Fälschungsfunden stellten hierbei fünf gefälschte Präparate dar, die gemäß den Etiketten den Wirkstoff Chloroquin enthalten sollten. Dieser Wirkstoff wird indikationsgemäß für die Behandlung von Malaria sowie bei rheumatischen Erkrankungen eingesetzt, war jedoch in den ersten Monaten der COVID-19 Pandemie auf seine Wirksamkeit bei Infektionen mit SARS-CoV-2 untersucht worden und hatte hierbei starke mediale Aufmerksamkeit erfahren. Alle fünf Präparate waren im Zeitraum 31. März bis 4. April 2020 von zwei Mitgliedern des Difäm-EPN Minilab Netzwerkes in Kamerun und der DR Kongo entdeckt worden, wobei drei der Präparate in privaten Apotheken, die beiden übrigen auf dem Schwarzmarkt verkauft wurden. Alle fünf Produkte wurden einem Screening mittels GPHF-Minilab® unterzogen. Bereits bei der visuellen Inspektion ergaben sich erste Zweifel an Qualität und Authentizität der Produkte. Drei Produkte wiesen Rechtschreibfehler auf dem Etikett auf, in einem dieser Fälle war sogar der Wirkstoffname falsch geschrieben. Vier Produkte waren mit ungültigen Registrierungsnummern der nigerianischen Arzneimittelaufsichtsbehörde bedruckt und vier der fünf Produkte trugen die gleichen Chargennummer, obwohl sie sich hinsichtlich Verfallsdaten, Dosierungen und Verpackungen deutlich voneinander unterschieden. Die anschließend durchgeführte dünnschichtchromatographische Untersuchung bestätigte die ersten Zweifel: Nur in einem Fall deutete das Screening auf eine geringe Menge des deklarierten Wirkstoffes hin, in zwei Fällen ließ die Untersuchung die Anwesenheit von nicht-deklarierten Wirkstoffen vermuten und in den letzten beiden Fällen konnte auf diese Weise die Anwesenheit keines Wirkstoffes, inklusive des deklarierten, festgestellt werden. An der Universität Tübingen wurden im Rahmen dieser Doktorarbeit weitere Untersuchungen vorgenommen: Nicht deklarierte Wirkstoffe wurden mittels LC-HR-MS identifiziert. Gehaltsbestimmungen erfolgten gemäß den Vorschriften der USP 42 mittels HPLC. Wie bereits vermutet, enthielt nur eine der fünf Proben den deklarierten Wirkstoff Chloroquin, jedoch nur 21,7% der auf dem Etikett angegebenen Menge. Zwei weitere Proben enthielten verschiedene Mengen (126,5mg und 14,1mg) Metronidazol. 35,7mg Paracetamol wurden in der vierten und 1,6mg Paracetamol sowie 14,6mg Metronidazol in der fünften Probe identifiziert. Die WHO wurde über diese Funde informiert und veröffentlichte eine entsprechende weltweite Warnmeldung.[64]



Deklarierter Chloroquin (CQ) Gehalt:

100 mg CQ Phosphat	100 mg CQ Phosphat	250 mg CQ	100 mg CQ Phosphat	250 mg CQ Phosphat

Identifizierte Wirkstoffe:

21,7 mg CQ Phosphat	kein CQ 35,7 mg Paracetamol	kein CQ 126,5 mg Metronidazol	kein CQ 14,1 mg Metronidazol	kein CQ 1,6 mg Paracetamol 14,6 mg Metronidazol

Abbildung 2: Verpackungen, Tabletten und Ergebnisse des dünnschicht-chromatografischen Screenings sowie der weiterführenden Laboranalytik von fünf Chloroquin (CQ)-Fälschungen, die in Kamerun und der DR Kongo gefunden wurden. Auf jeder Dünnschichtchromatographie-Platte sind links und rechts Referenzstandards entsprechend 100% und 80% des deklarierten Wirkstoffgehaltes aufgetragen. In der Mitte sind zwei Spots der Probe aufgetragen. *(Modifiziert nach Gnegel et al.[65])*

Im weiteren Verlauf der Studie wurden noch fünf weitere Chloroquin-Fälschungen identifiziert und mit insgesamt zehn gefälschten Präparaten stellten Chloroquin-Tabletten die häufigste gefundene Fälschung dar. Bemerkenswerterweise wurden alle zehn Chloroquin-Fälschungen im Jahr 2020, also nach dem Ausbruch der COVID-19 Pandemie, identifiziert. Dadurch stieg der Anteil von vermutlich gefälschten Arzneimitteln von 1,3% im Jahr 2019 auf 2,2% im Jahr 2020 an (Abbildung 3).

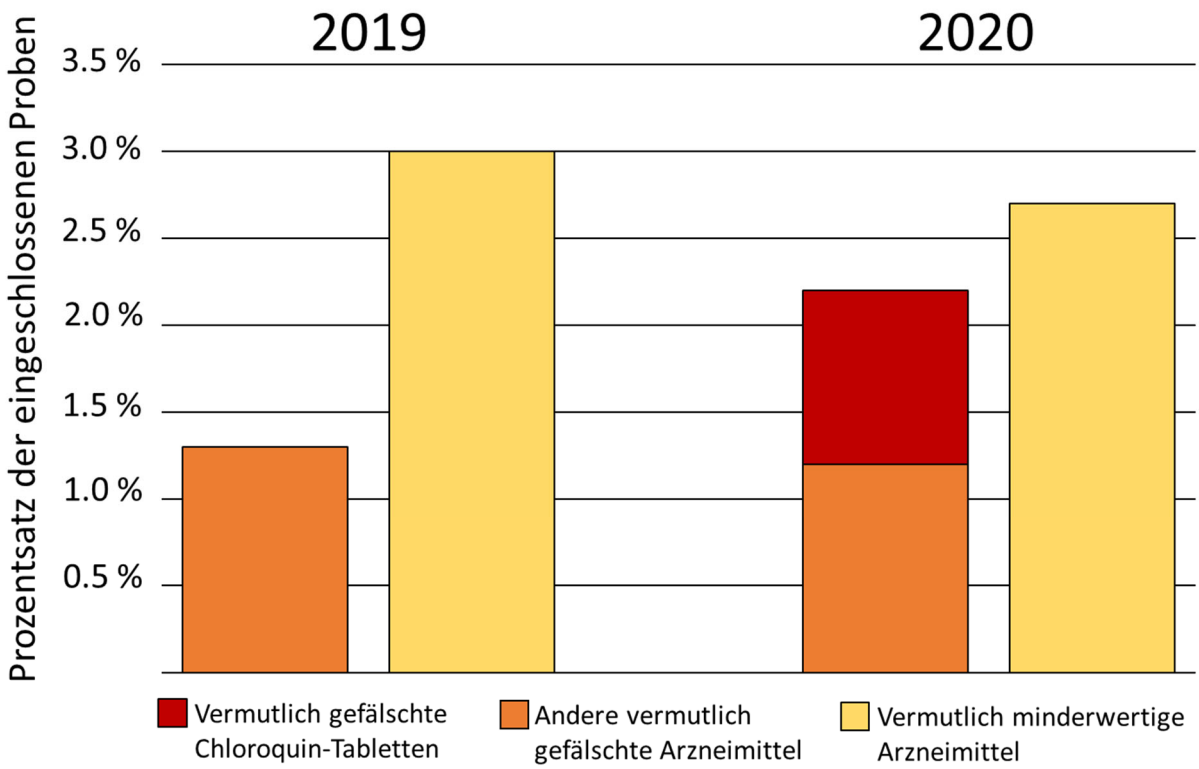


Abbildung 3: Veränderungen im Auftreten von vermutlich gefälschten und vermutlich minderwertigen Arzneimitteln im Zusammenhang mit der COVID-19 Pandemie. *(modifiziert nach Gnegel et al.[29])*

Alle identifizierten Fälschungen wurden der WHO gemeldet und mit insgesamt vier internationalen[64,66-68] und mehreren nationalen Warnmeldungen[69-73] über diese Arzneimittelfälschungen, hat die Arbeit des Minilabnetzwerkes und dieses Forschungsprojekt zur Verbesserung der globalen Arzneimittelsicherheit beigetragen. Die hierfür benötigten externen Kosten beliefen sich auf 49.600€, die zur Beschaffung von Chemikalien, Referenzstandards und Labormaterialien, zur Finanzierung von Bestätigungsanalysen gemäß Arzneibuchmonographien in einem externen Labor, für eine Schulung zur Handhabung des Minilabs® für ein neues Netzwerkmitglied sowie für die Koordination des Netzwerkes eingesetzt wurden. Dies entspricht verhältnismäßig günstigen Gesamtkosten von 25,85€ oder Materialkosten von 7,45€ pro untersuchte Probe, wobei lokal entstandene Kosten wie Gehälter des Laborpersonals sowie die Bereitstellung einer Laborräumlichkeit nicht berücksichtigt sind.

Entwicklung einer Methode zur Wirkstoffverifizierung in Tabletten mit dem tragbaren Spektrometer NIR-S-G1

In einem weiteren Forschungsprojekt dieser Doktorarbeit, wurde eine auf dem chemometrischen Prinzip der Hauptkomponentenanalyse (principal component analysis, PCA) beruhende Methode der Wirkstoffverifizierung in Tabletten für das preisgünstige, tragbare Spektrometer NIR-S-G1 (InnoSpectra – Hsinchu, Taiwan) entwickelt. Hierzu wurden neun PCA-Modelle zur vom Hersteller und der Marke eines Präparates unabhängigen Verifizierung von zehn unentbehrlichen Wirkstoffen in Tabletten erstellt, validiert und anschließend auf ihre Eignung und Limitationen bei der raschen Identifizierung von Arzneimittelfälschungen untersucht. Die hierfür erstellte Spektraldatenbank umfasste 170 Arzneimittelproben, die größtenteils während Feldstudien in Kamerun, DR Kongo, Tschad und Nigeria gesammelt wurden. Spektren von 59 qualitätsgesicherten Produkten bildeten den für die Berechnung der neun PCA-Modelle verwendeten Trainingssatz. Für jeden der zehn untersuchten Wirkstoffe sowie für Placebo wurden Tabletten von drei bis sechs verschiedenen Herstellern in diesen Trainingssatz eingeschlossen. Das erste PCA-Modell wurde auf der Grundlage aller Trainingssatzspektren berechnet und die Ergebnisse als Hauptkomponente (principal component, PC-)1 vs. PC-2 Scores-Diagramm dargestellt. Wie in Abbildung 4A dargestellt, bilden sämtliche Penicillin-V Datenpunkte ein Cluster, das räumlich klar von allen übrigen Datenpunkten abgetrennt werden kann. Eine Verifizierung des Wirkstoffes Penicillin V ist daher anhand dieses PCA-Models (PCA I) möglich. Für die Erstellung des zweiten PCA-Modells (PCA II) wurden alle Spektren von Penicillin-V-Tabletten aus dem Trainingssatz entfernt, und PCA II unter Verwendung der Spektren der verbleibenden 53 Produkte berechnet. Das Scores-Diagramm von PCA II (Abbildung 4B) zeigt, dass dieses Model die Verifizierung der Wirkstoffkombination Sulfamethoxazol/Trimethoprim ermöglicht, da die zugehörigen Datenpunkte hier ein räumlich gut abtrennbares Cluster bilden. Auf diese Weise wurde eine Sequenz von insgesamt neun PCA-Modellen erstellt, die auch die Verifizierung der weiteren Wirkstoffe Ciprofloxacin, Furosemid, Metronidazol, Metformin und Hydrochlorothiazid ermöglichen. Hingegen bildeten Präparate die entweder Amoxicillin oder die Kombination Amoxicillin/Clavulansäure enthielten ein überlappendes Cluster. Sie konnten zwar von allen übrigen Präparaten, nicht aber voneinander getrennt werden. Weiterhin war es nicht möglich Doxycyclin-Präparate sauber von Placebo-Präparaten zu trennen.

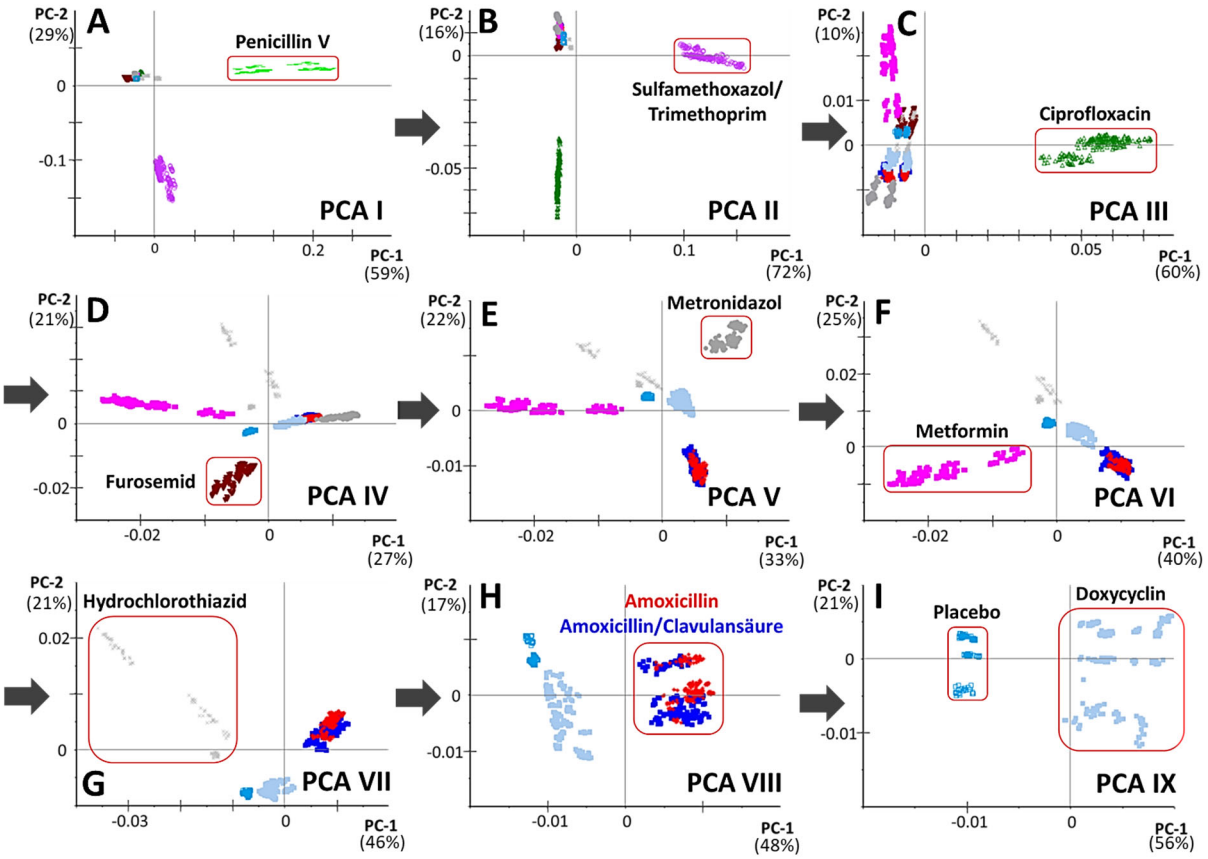


Abbildung 4: Sequenz von neun PCA-Modellen zur Verifizierung verschiedener Wirkstoffe in Tabletten. *(Modifiziert nach Gnegel et al., "Verification of the active pharmaceutical ingredient in tablets using a low-cost near-infrared spectrometer and principal component analysis", 2022, Noch nicht eingereichtes Manuskript)*

Der gemäß den Empfehlungen der ICH Guideline Q2(R2) zur Validierung analytischer Prozesse[74] erstellte externe Validiersatz wurde auf die neun PCA-Modelle projiziert. Die insgesamt 20 Validierproben enthielten die jeweils gleiche Menge der gleichen Wirkstoffe wie die Produkte des Trainingssatzes, waren jedoch von anderen Herstellern produziert worden. In 18 Fällen wurden die Datenpunkte der Validierproben in die erwarteten Cluster projiziert, in zwei Fällen jedoch auf Positionen in der Nähe aber nicht in das entsprechende Cluster. Als Ursache hierfür konnten Unterschiede im Massenanteil des Wirkstoffes zwischen Trainingssatz und Validiersatz ausgemacht werden, die durch unterschiedliche Tablettengewichte verursacht wurden. In beiden Fällen hatten die Validierproben einen niedrigeren Massenanteil als die Präparate im Trainingssatz, was deren Lage nahe dem entsprechenden Cluster erklärt. In keinem Fall wurde eine Validierprobe dem Cluster eines anderen Wirkstoffes zugeordnet.

Nach der Erstellung und Validierung der PCA-Modelle, wurden diese mithilfe verschiedener Testsätze genauer untersucht. Der erste Testsatz enthielt Spektren von 18 Produkten, die sich von denen im Trainingssatz lediglich in ihrer Charge unterschieden. 17 dieser Produkte wurden in die erwarteten Cluster und in die unmittelbare Nähe der jeweils herstellergleichen Produkte aus dem Trainingssatz projiziert. Ein Metronidazol-Produkt wurde hingegen in die Nähe aber außerhalb des Metronidazol-Clusters projiziert. Untersuchungen von Tablettengewicht und Rohspektren der im Trainings- und im Testsatz enthaltenen Chargen wiesen deutliche Unterschiede auf. Dies lässt vermuten, dass der Hersteller zwischen der Produktion der beiden Chargen seine Herstellungsverfahren geändert hat.

Der zweite Testsatz enthielt 21 Produkte, die sich in der Wirkstärke und in 17 Fällen auch im Hersteller, von denen im Trainingssatz unterschieden. Die Mehrzahl dieser Produkte wurde in die Cluster der jeweiligen Wirkstoffe projiziert. Dieses Ergebnis war bei der Projektkonzeption nicht erwartet worden, ließ sich jedoch durch die enthaltenen Wirkstoffmassenanteile gut erklären: bei Präparaten mit hohem Wirkstoffgehalt war oft auch eine größere Menge an Hilfsstoffen verwendet worden, sodass der Massenanteil konstant blieb. Produkte mit einem Massenanteil, der dem vom Trainingssatz abgedeckten Bereich entsprach, wurden in die entsprechenden Cluster projiziert. Produkte, die außerhalb des Clusters projiziert wurden, wichen in ihrem Massenanteil von denen im Trainingssatz ab.

Der dritte Testsatz enthielt 32 Produkte mit insgesamt 30 nicht im Trainingssatz enthaltenen Wirkstoffen. Die meisten dieser Spektren wurden in den PCA-Modellen auf deutlich von allen Wirkstoff-Clustern des Trainingssatzes getrennte Positionen projiziert und konnten so rasch von diesen unterschieden werden. Eine Fehlzuordnung trat im Fall von Moxifloxacin Hydrochlorid auf, welches dem Cluster des chemisch verwandten Ciprofloxacin Hydrochlorid zugeordnet wurde. Spektren von Tabletten die freies Moxifloxacin enthielten, wurden hingegen auf eine vom Ciprofloxacin Hydrochlorid-Cluster deutlich verschiedene Position projiziert. Zwei weitere Fehlzuordnungen traten bei den Wirkstoffen Acetazolamid und Ranitidin auf. Da beide Präparate nur wenige charakteristische Peaks in ihren Rohspektren aufwiesen, wurden sie fälschlicherweise dem Doxycyclin-Cluster zugeordnet. Für Wirkstoffe mit wenigen spektralen Charakteristika ist die hier vorgeschlagene Methode daher nicht geeignet.

Der vierte Testsatz enthielt 20 gefälschte Produkte die entweder keinen Wirkstoff, einen nicht-deklarierten Wirkstoff oder zu geringe Mengen des deklarierten Wirkstoffes enthielten oder aber bei korrekter Wirkstoffidentität und -menge aufgrund von Manipulationen am Etikett als gefälscht eingestuft wurden. Während alle 13 Produkte mit fehlendem oder falschem Wirkstoff sowie ungenügender Wirkstoffmenge durch die Projektion auf die PCA-Modelle rasch als Fälschungen erkannt werden konnten, ließen sich die sieben Fälschungen mit dem manipulierten Etikett mit dieser Methode nicht identifizieren.

Qualität von Oxytocin- und Misoprostol-Präparaten in Rwanda

Im Rahmen einer Studie zur Qualität von Oxytocin-Injektionen und Misoprostol-Tabletten in Rwanda, wurden in 46 Gesundheitseinrichtungen, Apotheken sowie bei Großhändlern insgesamt 57 Oxytocin-Proben (7 Chargen, 4 Hersteller) und 25 Misoprostol-Proben (10 Chargen, 6 Hersteller) gesammelt. Alle Oxytocin-Proben wurden einer Bestimmung von Identität, Gehalt und pH-Wert nach USP 40 zugeführt. Misoprostol-Proben wurden hinsichtlich Identität, Gehalt sowie Wirkstofffreisetzung nach Ph. Int. 2017 untersucht. Zudem wurde ein Temperaturmonitoring der Oxytocin-Lagerplätze in den Gesundheitseinrichtungen über einen Zeitraum von 6 Monaten durchgeführt.

Alle 57 Oxytocin-Präparate bestanden die Identitäts- und pH-Prüfung nach USP. Die Gehaltsprüfung bestanden 48 Proben. Bei den neun Proben mit vom Sollbereich abweichendem Gehalt, handelte es sich um alle gesammelten Proben derselben Charge des Herstellers Jiangxi Xierkangtai Pharmaceutical Co. Ltd, China. Mit einem Mediangehalt von 118% waren diese Proben deutlich überdosiert. Bei der zweiten untersuchten Charge desselben Herstellers war der Oxytocin-Gehalt unauffällig, jedoch wurden stark schwankende Mengen des Konservierungsmittels Benzylalkohol nachgewiesen (s.u.).

Alle 25 untersuchten Misoprostol-Proben enthielten den deklarierten Wirkstoff und bestanden somit die Identitätsprüfung. Jedoch waren zehn Proben mit Gehalten von <50% stark unterdosiert und Misoprostol-Abbauprodukte konnten identifiziert werden. Bei diesen zehn Proben handelte es sich um alle im Rahmen dieser Studie untersuchten Proben der Hersteller Corona Remedies Pvt., Ltd, Indien und Maxtar Bio-Genics, Indien. Bei beiden Herstellern gaben die Verpackungen keinen Aufschluss darüber, ob der für die Stabilität von Misoprostol wichtige Hilfsstoff HPMC enthalten

war. Die 15 Proben, die den Gehaltstest bestanden hatten, waren auch im Freisetzungstest konform, während die zehn Proben, die den Gehaltstest nicht bestanden hatten, auch eine ungenügende Wirkstofffreisetzung aufwiesen. Letztere Produkte wurden von der Rwandischen Arzneimittelaufsichtsbehörde (RFDA) zurückgerufen, nachdem diese über die Analysenergebnisse in Kenntnis gesetzt wurde.
Von wenigen kleineren Abweichungen abgesehen, wurden die auf den Oxytocin-Präparaten jeweils angegebenen Lagertemperaturen von 2-8°C bzw. Raumtemperatur in den Gesundheitseinrichtungen gut eingehalten.

Im Rahmen dieser Dissertation, wurde die Untersuchung der Benzylalkoholkonzentrationen in den Oxytocin-Injektionen des Herstellers Jiangxi Xierkangtai Pharmaceutical Co. Ltd, China vorgenommen. Hierzu wurde eine von Rego und Nelson[75] entwickelte HPLC-Methode adaptiert und Linearität sowie Präzision gemäß der ICH Q2(R1) Richtlinie[76] bestimmt. Die relative Standardabweichung der Messungen (Wiederholbarkeit) betrug 0,2%. Die zuvor mittels GC-MS erfolgte Identifizierung des nicht-deklarierten Konservierungsmittels wurde in der HPLC-Analyse anhand der identischen Retentionszeit von Probe und Referenzstandard bestätigt und der Gehalt betrug in nahezu allen Proben 0,9%. Alle untersuchten Ampullen der Probe QOR04 enthielten abweichend davon nur 0,004% Benzylalkohol (Abbildung 5). Diese Probe war bereits durch ihren niedrigen Wirkstoffgehalt (90,3%) aufgefallen, der an der unteren Grenze des vom USP als zulässig definierten Gehaltsbereiches liegt. Abgesehen von Probe QOR04 fand sich unter allen untersuchten Ampullen nur noch eine weitere mit einer abweichenden Benzylalkoholkonzentration: Während zwei von drei untersuchten Ampullen der Probe QOR75 0,9% Benzylalkohol sowie 99,7 bzw. 100,2% Oxytocin enthielten, enthielt die dritte Ampulle nur 0,0018% des Konservierungsmittels und 88,3% des deklarierten Oxytocin-Gehaltes.

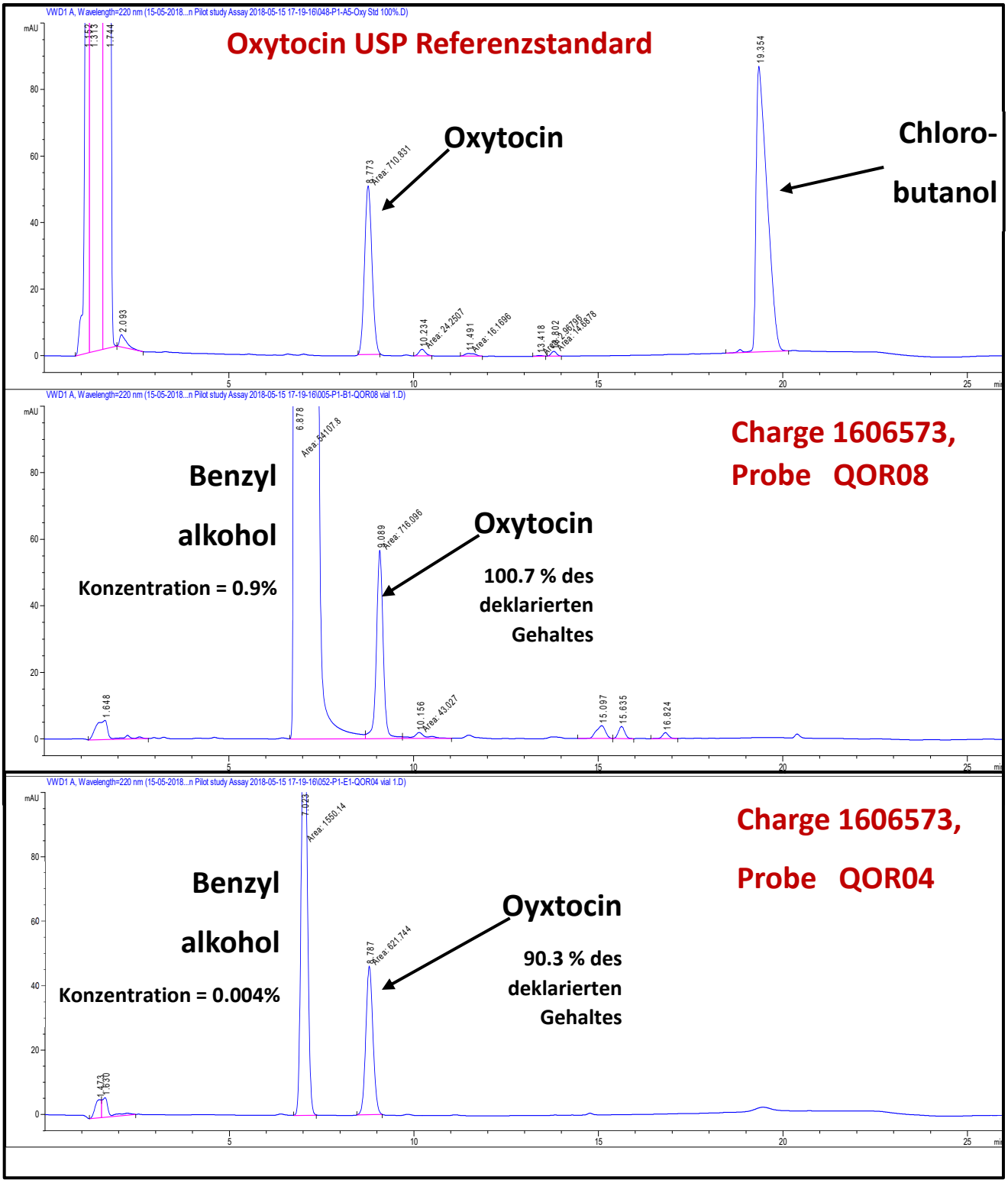


Abbildung 5: HPLC-Untersuchung des nicht deklarierten Konservierungsmittels Benzylalkohol, das in unterschiedlichen Konzentrationen in Oxytocin-Proben derselben Charge vorhanden ist, die gemäß Etikett von Jiangxi Xierkangtai Pharmaceuticals Co. Ltd (China) hergestellt wurde. *(Modifiziert nach Bizimana et al.[77])*

Diskussion

Routinemäßiger Einsatz der Screening-Technologie GPHF-Minilab® vor und während der COVID-19 Pandemie

Im Rahmen dieser Doktorarbeit wurde erstmalig die routinemäßige Anwendung einer Screening-Technologie über einen langen Zeitraum (zwei Jahre) und einen großen geographischen Raum (zwei Kontinente) ausgewertet und so zum Kenntnisstand über die praktische Anwendbarkeit von Screening-Technologien im Feld beigetragen. Weiterhin wurden 34 vermutlich gefälschte Arzneimittel als solche identifiziert und an die WHO gemeldet. Durch daraufhin veröffentlichte nationale und internationale Warnmeldungen, wurde ein Beitrag zur globalen Arzneimittelsicherheit geleistet. Auf lokaler Ebene wurden diese Produkte von den Mitgliedern des Minilabnetzwerkes unter Quarantäne gestellt und so die Abgabe an Patient:innen ausgeschlossen.

Die identifizierten Fälschungen stellen in mehrfacher Hinsicht eine Gefahr für Patient:innen dar, die anhand der fünf unmittelbar nach Ausbruch der COVID-19 Pandemie identifizierten Chloroquin-Fälschungen erläutert wird: Aufgrund des fehlenden oder stark unterdosierten Wirkstoffes, kann der gewünschte therapeutischer Effekt von diesen Produkten nicht erwartet werden. Vier der fünf Produkte enthielten subtherapeutische Mengen von antiinfektiven Wirkstoffen, was zur Entwicklung von Resistenzen gegen diese Wirkstoffe beitragen kann. Möglicherweise wurde eine zu geringe Menge Chloroquin in einem der Präparate verwendet, um Produktionskosten zu sparen. Metronidazol als nicht deklarierter Wirkstoff könnte zum Einsatz gekommen sein, um den bitteren Geschmack von Chloroquin zu imitieren. Die sehr geringen detektierten Mengen Paracetamol, lassen eine Verunreinigung der Ausgangsmaterialien oder der Produktionsgeräte durch zuvor produzierte Chargen vermuten. Durch den Fund dieser Proben im März und April 2020 ist ein unmittelbarer zeitlicher Zusammenhang mit der COVID-19 Pandemie gegeben. Scheinbar haben Kriminelle schnell auf die mediale Aufmerksamkeit rund um den Wirkstoff Chloroquin reagiert und entsprechende Fälschungen produziert. Ein weiterer Fund eines Chloroquin-Präparates mit einem Verkaufspreis von umgerechnet 414 US$ für 100 Tabletten (Gnegel et al.,[65] Supplementary Figure S1) verdeutlicht die Gewinnaussichten, die Kriminelle sich durch solcherlei Fälschungen versprachen.

Weder stellt die vorliegende Forschungsarbeit eine Studie zur Prävalenz von Arzneimittelfälschungen dar, noch wurden alle eingeschlossenen Arzneimittelproben einer Vollanalytik gemäß Arzneibuch unterzogen. Dennoch reiht sich der gefundene Prozentsatz von 1,8% vermutlich gefälschten Produkten nahtlos in die Ergebnisse kürzlich veröffentlichter Arzneimittelqualitätsstudien ein: so fanden Hauk et al. eine Fälschungsprävalenz von 1,7% in Kamerun und DR Kongo[16] und Rahman et al. konnten eine Prävalenz von 1,2% in Bangladesch feststellen.[78] Dies sowie die bereits in vorhergegangenen Studien belegte gute Sensitivität des Minilabs® hinsichtlich der Identifikation von Arzneimittelproben mit groben Abweichungen von den vorgegebenen Qualitätsstandards[22,31] legen nahe, dass derartige Mängel im Rahmen des vorliegenden Minilab®-Screenings zuverlässig und vollständig aufgedeckt wurden. Insgesamt wurden 4,6% der untersuchten Arzneimittelproben als wahrscheinlich minderwertig oder gefälscht eingeschätzt. Diese Rate liegt deutlich unter der Prävalenzschätzung der WHO (10,5%)[6] oder den 18,7% die in einem Review von Ozawa et al. als Prävalenz minderwertiger und gefälschter Arzneimittel in Ländern mit niedrigem und mittlerem Einkommen gefunden wurden.[7] Es ist daher wahrscheinlich, dass ein Teil der minderwertigen Arzneimittel in diesem Screening-Ansatz nicht identifiziert wurden, zumal eine geringere Sensitivität des Minilabs® hinsichtlich der Detektion von Arzneimitteln mit ungenügendem Wirkstoffgehalt oder Problemen bei der Wirkstofffreisetzung bekannt ist.[31,32] Ebenfalls führen die Mitglieder des Difäm-EPN Minilab Netzwerkes weitere Maßnahmen zur Auswahl ihrer Lieferanten und Sicherung der Qualität ihrer Arzneimittel durch, was zu der niedrigen Rate an minderwertigen Produkten beigetragen haben könnte.

Das Minilab® und die darum geschaffene Netzwerkstruktur ermöglichten den lokalen Partnerorganisationen sich über das Minilab®-Screening hinausführend gegen minderwertige und gefälschte Arzneimittel einzusetzen: Nachdem sich Tabletten im Rahmen des vereinfachten Zerfallstests nach zwei Tagen nicht aufgelöst hatten, präsentierte der nigerianische Netzwerkpartner dem lokalen Hersteller das Ergebnis und veranlasste diesen das Produkt zurückzurufen (Gnegel et al.,[29] Supplementary Figure S1). Weiterhin konnten zwei Fälschungen aufgedeckt werden, deren deklarierte Wirkstoffe, Vitamin A und Carbamazepin, nicht mittels Minilab® untersucht werden können. Die Vitamin A-Kapseln waren dem tschadische Netzwerkpartner aufgrund von Unregelmäßigkeiten auf dem Etikett aufgefallen und eine daraufhin veranlasste Vollanalyse zeigte, dass sich der Wirkstoff bereits stark zersetzt hatte. Der

anschließend informierte Hersteller bestätigte, dass bei dem Produkt das Verfallsdatum von Kriminellen manipuliert und um mehrere Jahre verlängert worden war. Die WHO veröffentlichte zu diesem Fall eine internationale Warnmeldung.[79] Die Carbamazepin-Tabletten hingegen waren einem kamerunischen Minilabpartner aufgrund von ausbleibender Wirksamkeit bei Patienten aufgefallen und eine nasschemische Farbreaktion[80] deutete auf die Abwesenheit des Wirkstoffes hin. Dieser Verdacht konnte in einer anschließend durchgeführten Vollanalyse bestätigt werden und eine nationale Warnmeldung wurde von der Gesundheitsbehörde Kameruns veröffentlicht (Gnegel et al.,[29] Supplementary Figure S4).

In diesem Forschungsprojekt konnte gezeigt werden, dass das Arzneimittelqualitäts-Screening mittels Minilab® einen wichtigen Beitrag zur Arzneimittelqualitäts-überwachung leisten kann. Das Minilab® ist hierbei ein vergleichsweise erschwingliches Instrument, dass zusätzlich lokale Partner aktiv in den Kampf gegen gefälschte und minderwertige Arzneimittel einbindet und hier Bewusstsein und Handlungsspielräume schafft. Als Screening-Technologie ist das Minilab® nicht dafür ausgelegt Vollanalysen gemäß Arzneibuchmonografien zu ersetzen, jedoch kann es wichtige Hinweise auf Qualitätsmängel liefern und in Kontexten von begrenzten Ressourcen für die Vorauswahl von Produkten verwendet werden, die solch einer kostspieligen Vollanalyse zugeführt werden. Als Voraussetzungen für den langfristig erfolgreichen Einsatz des Minilabs® konnten der konstante Nachschub an benötigten Chemikalien, Referenzstandards und Labormaterialien, der Zugang zu einem gut ausgestatteten Labor mit der Option Vollanalysen durchzuführen, ein hohes Engagement der beteiligten Partner sowie sichere Berichtserstattungsstrukturen identifiziert werden, die das Melden von Arzneimittelfälschungen bei Wahrung der Anonymität des Berichterstatters ermöglichen.

Entwicklung einer Methode zur Wirkstoffverifizierung in Tabletten mit dem tragbaren Spektrometer NIR-S-G1

Für Kontexte, in denen die oben aufgeführten Voraussetzungen nicht gegeben sind, oder in Ergänzung zum Minilab®, sind alternative Screening-Technologien sinnvoll. In dieser Doktorarbeit wurde daher das tragbare Spektrometer NIR-S-G1 untersucht und eine PCA-basierte Methode zur Wirkstoffverifizierung in Tabletten für dieses Gerät entwickelt. Grundlage hierfür ist, dass sich die NIR-Spektren wirkstoffgleicher Tabletten unter den verwendeten Bedingungen im PCA-Scores-Diagramm zu Clustern

zusammenlagern, obwohl sie von verschiedenen Herstellern produziert wurden und sich daher in Faktoren wie der Hilfsstoffzusammensetzung, oder der Oberflächenstruktur unterscheiden. Mithilfe einer Sequenz von neun PCA-Modellen ließen sich diese Cluster schrittweise voneinander trennen, wobei die Verwendung der jeweils ersten beiden Hauptkomponenten ausreichend war. Die Anwesenheit eines Wirkstoffes in zu screenenden Tabletten lässt sich nun durch Aufnahme von NIR-Spektren und nach Projektion derselben auf die PCA-Modelle anhand der Lage der zugehörigen Datenpunkte in den Scores-Diagrammen einfach prüfen.

Voraussetzung für die Trennbarkeit der Tabletten anhand ihrer Wirkstoffe ist das Vorhandensein wirkstoffspezifischer, spektraler Merkmale im NIR-Absorptionsspektrum, wie es bei dem Großteil der untersuchten Wirkstoffe der Fall war. Doxycyclin und Clavulansäure hingegen wiesen unter den hier verwendeten Bedingungen nicht ausreichend charakteristische Merkmale in den Absorptionsspektren auf und die Versuche Doxycyclin- von Placebo-, und Amoxicillin- von Amoxicillin/Clavulansäure-Tabletten zu trennen scheiterten. Einschränkungen für die Anwendbarkeit von NIR-S-G1 für Wirkstoffe mit wenigen spektralen Charakteristika wurden auch von Zambrzycki et al. für das Antibiotikum Ofloxacin beschrieben.[81] Probleme bei der Trennung von Amoxicillin und Amoxicillin/Clavulansäure traten auch bei Tie et al. auf, obwohl hier ein Benchtop-NIR-Spektrometer verwendet wurde.[82] Die fehlende Anwendbarkeit der hier vorgestellten Methode bei Wirkstoffen wie Doxycyclin und Clavulansäure wurde daher wahrscheinlich nicht durch den verwendeten chemometrischen Ansatz oder das untersuchte Spektrometer verursacht.

Ein interessanter Fund ist, dass die Massenanteile der Wirkstoffe in den Produkten für das spektrale Ergebnis und damit die Position der Datenpunkte im Scores-Diagramm relevanter sind als die angegebene Wirkstoffmenge in Milligramm. Bei der Anwendung dieser Methode sollte daher für alle Proben der (nominale) Massenanteil des Wirkstoffs anhand der auf dem Etikett angegebenen Wirkstärke und des Tablettengewichts berechnet werden. Liegt dieser Massenanteil außerhalb des vom Trainingssatz abgedeckten Bereich, ist die Methode nur sehr eingeschränkt anwendbar.

In diesem Projekt wurde keine Probe mit weniger als 12,5mg oder 12,4% Massenanteil des Wirkstoffes analysiert. Es ist wahrscheinlich, dass die vorgestellte Methode für Produkte mit einem sehr geringen Wirkstoffgehalt nicht geeignet ist. Allerdings werden

71,8% aller Wirkstoffe (Vitamine und Mineralstoffe ausgenommen) die als feste orale Darreichungsformen in der Kernliste der „22. World Health Organization Model List of Essential Medicines“[83] aufgeführt sind in Dosierungen von 12,5mg oder höher eingesetzt. Dies deutet darauf hin, dass diese Methode grundsätzlich für die meisten Produkte geeignet sein könnte.

Um eine zukünftige Anwendbarkeit in ressourcenarmen Umgebungen zu ermöglichen, wurden die verwendeten Geräte und Methoden so einfach wie möglich gehalten. Das tragbare Spektrometer NIR-S-G1 wurde ausgewählt, weil es trotz seines geringen Preises von 1600 US$ pro Gerät gute Ergebnisse in Bezug auf die photometrische Linearität, die Wellenlängengenauigkeit und das spektroskopische Rauschen lieferte.[84,85] Bei der verwendeten chemometrischen Methode PCA handelt es sich nicht um eine Klassifizierungsmethode im eigentlichen Sinne, sie wird üblicherweise für eine erste Datenexploration eingesetzt. Hier wurde die PCA ausgewählt, da sie auch in der weitverbreiteten Software MS Excel ausgeführt werden kann.[86] Weiterhin kann die graphische Ergebnisdarstellung durch die Lage der Datenpunkt im Scores-Diagramm zusätzliche Hinweise auf Identität und Gehalt des Wirkstoffes einer unbekannten Probe liefern.

Diese neue Methode wurde exemplarisch an 20 Arzneimittelfälschungen untersucht. Alle 13 Fälschungen, die den deklarierten Wirkstoff nicht oder in stark abweichenden Mengen enthielten, konnten identifiziert werden. Wie hingegen von einer Methode zur Wirkstoffverifizierung zu erwarten, wurden solche Fälschungen mit manipuliertem Etikett aber korrekter Wirkstoffidentität und –menge nicht erkannt. Um auch solche Fälschungen sicher identifizieren zu können, muss nicht nur der Wirkstoff verifiziert, sondern das gesamte Produkt authentifiziert werden. Solche Ansätze wurden in der Literatur für NIR im Allgemeinen[87-91] und für NIR-S-G1 im Speziellen[92,93] beschrieben. Allerdings erfordern derlei Modelle umfangreiche Referenzdatenbanken, die idealerweise Spektren aller in einem Land oder einer Region für die betreffenden Wirkstoffe registrierten Marken beinhalten. Eine zugängliche und stets aktuelle, produktspezifische Datenbank scheint für die meisten Länder mit niedrigem und mittlerem Einkommen in absehbarer Zeit nicht realisierbar zu sein und müsste von oder in Zusammenarbeit mit den Arzneimittelzulassungs-behörden und den Herstellern erstellt und gepflegt werden. Ein wirkstoffspezifischer Ansatz, wie er hier vorgestellt wurde, ist einfacher umsetzbar und kann dennoch zur Identifizierung vieler

Fälschungen beitragen. Während ein solcher Ansatz, ebenso wenig wie andere Screening-Technologien, Vollanalysen gemäß Arzneibuchmethoden ersetzen kann oder soll, könnte er für Mitarbeiter:innen von kirchlichen, privaten oder zivilgesellschaftlichen Organisationen, die routinemäßig die Qualität von Arzneimitteln untersuchen, oder für risikobasierte Überwachungsmaßnahmen nach der Markteinführung, die einen hohen Probendurchsatz erfordern, von großem Nutzen sein. In beiden Kontexten könnten die Vorteile einer schnellen, preiswerten und einfach durchführbaren Methode deren Limitationen überwiegen. Letztere sollten jedoch bei der Anwendung dieser Methode im Auge behalten werden, um ein übermäßiges Vertrauen in die Ergebnisse zu vermeiden. Empfehlenswert ist eine Kombination dieser Methode mit anderen grundlegenden Qualitätsprüfungen, wie z.B. der visuellen Inspektion oder dem GPHF-Minilab®-Screening sowie eine Analyse nach Arzneibuchmethoden derjenigen Produkte, die im Screening auffällig waren.

Qualität von Oxytocin- und Misoprostol-Präparaten in Rwanda

Die durchgeführte Qualitätsuntersuchung von Oxytocin- und Misoprostol-Proben in Rwanda zeigte eine im Vergleich zu Berichten aus anderen Ländern[94-96] gute Lagerpraxis der temperaturempfindlichen Oxytocin-Präparate in den Gesundheitseinrichtungen. Dies hat dazu beigetragen, dass sich unter den 57 gesammelten Proben kein degradiertes Produkt befand. Auf der anderen Seite konnte im Rahmen dieser Studie eine Charge mit deutlich zu hohem Wirkstoffgehalt identifiziert werden. Alle neun untersuchten Produkte dieser Charge enthielten mehr als 110% des Wirkstoffsollgehaltes. Für Patientinnen bedeutet das Auftreten solcher Präparate das Risiko eine Überdosierung. Beunruhigenderweise wurden innerhalb einer weiteren Charge des gleichen Herstellers, Proben mit einer Konzentration des nicht-deklarierten Konservierungsmittels Benzylalkohol von 0,9% aber auch mit der zweihundertfach niedrigeren Konzentration 0,004% identifiziert. Dies legt eine schwere Verletzung der Guten Herstellungspraxis nahe und wirft generelle Fragen über andere Qualitätsaspekte der Produkte dieses Herstellers auf.

Weiterhin wurde bei allen hier untersuchten Misoprostol-Proben zweier Hersteller eine massive Unterdosierung bei gleichzeitiger Anwesenheit von Zersetzungsprodukten festgestellt. Es muss daher vermutet werden, dass ein Abbauprozess stattgefunden hat, der für Patientinnen das Risiko einer wirkverminderten Therapie bedeutet. Die Etiketten aller unterdosierten Produkte gaben keinen Aufschluss über die Anwesenheit

des für die Wirkstoffstabilität entscheidenden Hilfsstoffe HPMC, was ein möglicher Grund für den Abbau sein kann.

Die hier beschriebenen Qualitätsmängel wurden nicht mit Screening-Technologien, sondern mittels der Goldstandard-Technologie HPLC untersucht und identifiziert. Bis dato ist noch keine Screening-Technologie für die Untersuchung von Misoprostol- oder Oxytocin-Präparaten entwickelt worden. Zudem fokussieren viele Screening-Technologien die Identifizierung von Präparaten, die den deklarierten Wirkstoff nicht oder in einer ungenügenden Konzentration enthalten, sodass überdosierte Produkte und solche mit schwankender Hilfsstoffkonzentration in diesen Fällen unentdeckt bleiben.

Diese Publikation zeigt, dass Arzneimittelqualität von vielen Faktoren abhängt und an vielen Stellen gesichert werden muss: Die Bedeutung von Formulierung, Herstellung und Lagerung wurde an dieser Stelle anschaulich illustriert.

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Beteiligung

Abbildung 5 wurde von Bizimana et al. erstellt und modifiziert in diese Arbeit eingebracht.

Appendix

Akzeptierte Publikationen und noch nicht eingereichte Manuskripte jeweils mit ergänzendem Material (Supplementary Material). Da es sich bei der ergänzenden Tabelle Supplementary Table 2 der noch nicht eingereichten Publikation „Verification of the Active Pharmaceutical Ingredient in Tablets Using a Low-Cost Near-Infrared Spectrometer and Principal Component Analysis“ um eine sehr große und im Din A4 Format unleserliche Tabelle handelt, ist diese nicht beigefügt.

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Identification of Falsified Chloroquine Tablets in Africa at the Time of the COVID-19 Pandemic

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Abstract. Reports that chloroquine and hydroxychloroquine may be effective against COVID-19 have received worldwide attention, increasing the risk of the introduction of falsified versions of these medicines. Five different types of falsified chloroquine tablets were discovered between March 31, 2020 and April 4, 2020, in Cameroon and the Democratic Republic of Congo by locally conducted thin layer chromatographic analysis. Subsequent investigation by liquid chromatography and mass spectrometry in Germany proved the absence of detectable amounts of chloroquine and the presence of undeclared active pharmaceutical ingredients, that is, paracetamol and metronidazole, in four of the samples. The fifth sample contained chloroquine, but only 22% of the declared amount. Such products represent a serious risk to patients. Their occurrence exemplifies that once medicines or vaccines against COVID-19 may be developed, falsified products will enter the market immediately, especially in low- and middle-income countries (LMICs). Timely preparations for the detection of such products are required, including the establishment of appropriate screening technologies in LMICs.

In February 2020 and March 2020, reports that chloroquine (CQ) and hydroxychloroquine (HCQ) may be effective against COVID-19[1–4] received massive political and media attention worldwide, despite limited evidence.[5,6] Concerns have been raised that the premature off-label use of CQ and HCQ in COVID-19 may result in shortages of these medicines in their established, approved indications (i.e., against autoimmune diseases and, in case of CQ, *Plasmodium vivax* malaria).[7,8] The demand for CQ and HCQ quickly outstripped the supply, exacerbating the risk of falsified medicines entering the market.[8] We here report the recent occurrence of falsified CQ, detected in Cameroon and the Democratic Republic (DR) of Congo.

The Ecumenical Pharmaceutical Network (EPN), among other tasks, monitors medicine quality using the Global Pharma Health Fund (GPHF) Minilab,[9] a screening methodology based on thin layer chromatography (TLC) which is easy to conduct in resource-limited environments.[10] In March 2020, local member organizations of the EPN reported that both in private pharmacies and in informal markets, several types of falsified CQ tablets were appearing which, in local GPHF Minilab analysis,[11] were found not to contain CQ. Through the German Institute for Medical Mission (Difaem), the member organization of EPN which coordinates the Minilab network, the WHO Rapid Alert System, was informed, and the WHO published an international Medical Product Alert about falsified CQ tablets.[12]

In the following days, further falsified CQ samples were identified in Cameroon. Five samples were forwarded by commercial courier from Cameroon and the DR Congo to Tuebingen University, Germany. They are depicted in Figure 1, together with photos of their TLC analysis, according to the GPHF Minilab procedure.[11] Details of the samples are listed in Table 1.

Thin layer chromatography readily showed the presence of CQ in the reference solutions, visible both under UV light and in subsequent detection with iodine vapor. By contrast, CQ was not detectable in four of the investigated samples. The fifth sample showed a spot of CQ, but the compound was apparently present only in a low amount (Figure 1, sample I). For samples II and III (Figure 1), TLC analysis with UV detection showed the presence of further, undeclared compounds with a higher retention factor than CQ. The undeclared compound in sample II was also detectable by iodine staining, but the compound in sample III was not (Figure 1), indicating that these two compounds were chemically different.

These observations were confirmed at Tübingen University by high-performance liquid chromatography (HPLC) according to the U.S. Pharmacopeia.[13] As shown in Figure 2, no CQ was detected in four of the samples. By contrast, in sample I, CQ was present in an amount corresponding to 21.7 mg CQ phosphate, that is, only 21.7% of the amount stated on the label. Samples II and V showed an unknown compound with a retention time of 4.7 minutes in HPLC, and samples III, IV, and V showed a further unknown compound with a retention time of 4.5 minutes.

Liquid chromatography (LC) coupled with high-resolution mass spectrometry (HR-MS) showed that the two unknown compounds had exact molecular masses of 152.0709 and 172.0719, consistent with the masses of paracetamol and of metronidazole, respectively. Their identity was confirmed in comparison with authentic reference compounds of paracetamol and of metronidazole, showing identical retention times, molecular masses, and mass spectrometric fragmentation as the references (Supplemental Table S4, Supplemental Figures S2 and S3, Supplemental Information). The quantities of these compounds were determined as 35.7 mg paracetamol per tablet for sample II and as 126.5 mg metronidazole per tablet for sample III. Samples IV and V were found to contain smaller amounts of metronidazole, that is, 14.1 mg and 14.6 mg per tablet, respectively. Sample V additionally contained traces of paracetamol (1.6 mg per tablet).

The labeling of the five samples showed mistakes and spelling errors (Table 1), suggesting that they were produced not by established manufacturers but by criminals. The stated manufacturer of sample III, Dawa Limited, Kenya, was contacted by the local partners in the DR Congo and confirmed

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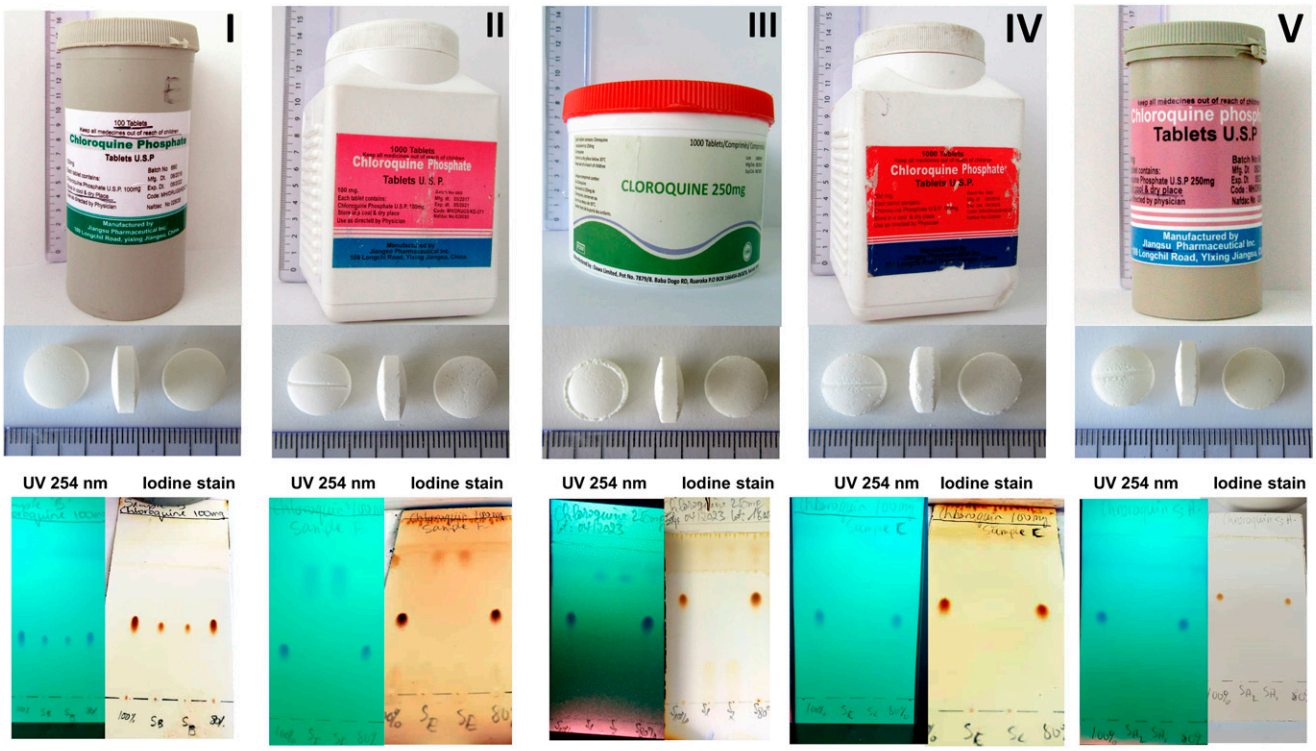

Chloroquine (CQ) amount declared:

100 mg CQ phosphate	100 mg CQ phosphate	250 mg CQ	100 mg CQ phosphate	250 mg CQ phosphate

Active principles detected:

21.7 mg CQ phosphate	no CQ 35.7 mg paracetamol	no CQ 126.5 mg metronidazole	no CQ 14.1 mg metronidazole	no CQ 1.6 mg paracetamol 14.6 mg metronidazole

FIGURE 1. Falsified samples of chloroquine (CQ) tablets identified in Cameroon and the Democratic Republic Congo, and their thin layer chromatographic (TLC) analysis[11]; see Supplemental Information for details of the analytical procedure. Each TLC plate shows two spots of the respective sample in the middle and two spots of authentic CQ (corresponding to 100% and 80% of the declared amount of the sample) on the left and the right, respectively. Thin layer chromatography plates were photographed in Cameroon and the Democratic Republic Congo with locally available equipment; therefore, the angle of photography is not uniform. The active principles listed at the bottom were identified by high-performance liquid chromatography according to the U.S. Pharmacopeiea and by liquid chromatography–high-resolution mass spectrometry analysis (see text). The CQ amount in sample I was calculated as CQ phosphate; the identity of the counterion (phosphate or sulfate) was not determined. (Photos: packaging, © G. G., C. H., and L. H.; TLC analysis, © F. N. and G. M.)

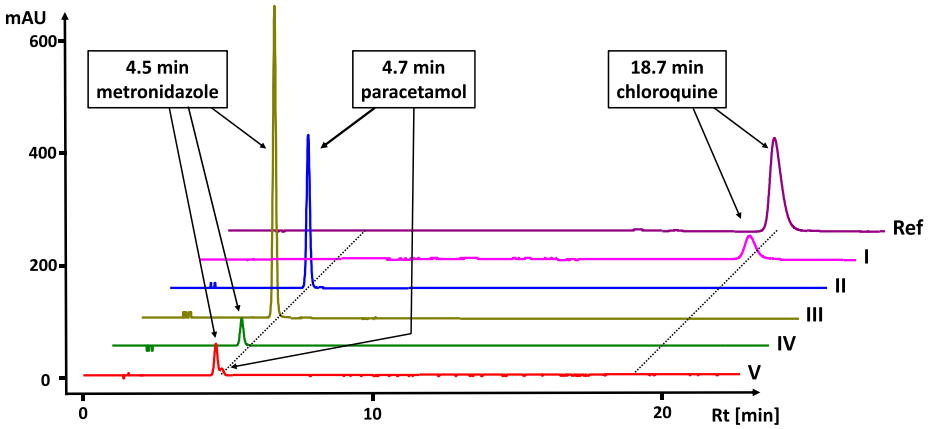

FIGURE 2. High-performance liquid chromatography analysis of falsified samples of chloroquine (CQ) tablets. Analysis was carried out according to the U.S. Pharmacopeia[13]; see Supplementary Information for details of the analytical procedure. Ref = CQ authentic reference substance; I, II, III, IV, and V = falsified samples of CQ tablets (see Figure 1, Table 1).

TABLE 1
Falsified samples of chloroquine tablets identified in Cameroon and the DR Congo

Sample code (Figures 1 and 2)	I	II	III	IV	V
Stated product name	Chloroquine phosphate tablets U.S.P	Chloroquine phosphate tablets U.S.P.	Cloroquine [*sic*] 250 mg	Chloroquine phosphate tablets U.S.P.	Chloroquine phosphate tablets U.S.P
Stated strength	100 mg	100 mg	250 mg	100 mg	250 mg
Stated manufacturer	Jiangsu Pharmaceutical Inc., China	Jiangsu Pharmaceutical Inc., China	Dawa Limited, Kenya	Jiangsu Pharmaceutical Inc., China	Jlangsu [*sic*] Pharmaceutical Inc., China
Batch number, mfg date, exp date	660, August 2018, August 2022	660, May 2017, May 2021	1605059, May 2019, April 2023	660, May 2019, April 2023	660, September 2018, September 2022
Found in	Limbe, Cameroon	Douala, Cameroon	Bukavu, DR Congo	Douala, Cameroon	Douala, Cameroon
Type of facility found in	Private pharmacy	Private pharmacy	Informal vendor	Private pharmacy	Informal vendor
Date of discovery	April 3, 2020	March 31, 2020	April 4, 2020	April 4, 2020	March 31, 2020
Labeling inconsistencies					
Spelling errors	+	–	+	–	+
Invalid NAFDAC registration number	+	+	–	+	+
Same batch number for different products	+	+	–	+	+

DR = Democratic Republic; NAFDAC = National Agency for Food and Drug Administration and Control, Nigeria. NAFDAC registration numbers were checked using the NAFDAC Registered Products Database available at www.nafdac.gov.ng/our-services/registered-products/.

that this sample had not been produced by them. Samples I, II, IV, and V were stated to be produced by "Jiangsu Pharmaceutical Inc., China," but no company with that name, or with the address stated on the labels, could be identified on the internet.

Notably, while this report was in preparation, Cameroon customs authorities reported the seizure of 210 cartons of falsified CQ tablets.[14]

The low amount of CQ in sample I is likely to reflect the attempt by the criminal producers to save costs in the purchase of the active pharmaceutical ingredient. The inclusion of paracetamol, as in sample II, has been reported previously in a falsified medicine from Cameroon, also identified by members of EPN.[10,15] Both in sample II and in that previous case, the amount of paracetamol was too low to achieve a relevant therapeutic effect. Metronidazole is very bitter and was included in samples III, IV, and V probably to mimic the bitter taste of CQ. The antibacterial and antiprotozoal compound metronidazole is usually formulated in tablets of 200–500 mg each. Therefore, samples III, IV, and V contain a subtherapeutic dose, which may contribute to the emergence of antimicrobial resistance. The additional presence of traces of paracetamol in sample V may represent a contamination from a prior production batch, reflecting poor manufacturing standards.

The absence of CQ in four of the five investigated samples, the subtherapeutic amount of CQ in the fifth sample, and the presence of undeclared active pharmaceutical ingredients in four of these samples represent serious health risks for the patients in Cameroon and the DR Congo. The authorities in Cameroon and the DR Congo, and the WHO Rapid Alert System were informed about these findings.

Such products may furthermore cause financial hardships to the patients: sample III was sold in the DR Congo for US$200 for a package of 1,000 tablets, that is, 15 times more expensive than the international procurement price.[16] In Cameroon, the EPN partner organization even reported the occurrence of a further package of 100 CQ tablets with a stated price of 250,000 CFA, that is, US$413 (Supplemental Figure S1, Supplemental Information).

The occurrence of such falsified CQ samples at this time of the COVID-19 pandemic also has wider implications. For any medicine or vaccine which may be reported to be effective against this disease, a frantic demand is to be expected, resulting in a serious danger of the appearance of falsified medicines. Low- and middle-income countries (LMICs) will be especially vulnerable: with their constrained access to essential medicines, their often weak technical capacity for medicine quality assurance and control, and their challenges in the maintenance of appropriate standards of governance in healthcare facilities and national medicines regulatory authorities, they show exactly those conditions which the WHO has identified as favoring the occurrence of substandard and falsified medicines.[17] Because of the recent disruption of the production and supply chains in India and China, which are the most important producer countries of generic medicines for LMICs, this problem will not remain restricted to medicines for the treatment and prevention of COVID-19 but encompass many types of medicines.

The rapid installation of simple, inexpensive screening technologies which can detect substandard and falsified medicines, such as TLC or near infrared or Raman spectroscopy,[8,9,18] may represent an important part of the response to the COVID-19 pandemic in LMICs. The data displayed in Figure 1 are a good example for the possibilities and limitations of the GPHF Minilab[11] in the identification of falsified medicines in future screening programs.

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Supplementary Information for

Covid-19 and the identification of falsified chloroquine tablets in Africa

Figure S1: *Package of 100 chloroquine phosphate tablets 100 mg found in Yaoundé, Cameroon, on April 9, 2020, with a stated price of 250,000 CFA, i.e. 414 US $ (Photo: © F. Nyaah). Due to the exorbitant price, this sample was not purchased and not analyzed.*

Methods of thin-layer chromatography and quantitative HPLC analysis

Method	Chloroquine phosphate and sulfate (GPHF Minilab Manual 2020)[1]
Stationary phase	Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, 5x10 cm
Mobile phase	Ethyl acetate/methanol/25% aqueous ammonia solution 1:4:0.1 (v/v)
Applied volume of sample and standard	2 µl
Detection	1) UV light, 254 nm; 2) Exposure to iodine vapor and visual evaluation in daylight
Standards	chloroquine phosphate in water, 2.5 and 2.0 mg/ml
Sample preparation	Tablets with a declared content of 100 mg chloroquine phosphate: one tablet was finely ground with a pestle and suspended in 20 ml of water. (Tablets with a declared content 250 mg chloroquine phosphate: one tablet was finely ground with a pestle and suspended in 50 ml of water.) After three minutes of shaking, the solution was allowed to sit for additional five minutes. 2 ml of the supernatant were removed and diluted with 2 ml of water.

Table S1: *Method for thin-layer chromatography*

Method	Chloroquine Phosphate Tablets (USP 42 monograph, 2019)[2]
Instrument	HPLC (Agilent 1100 Series)
Column/stationary phase	Reprospher 100 C18, 250 x 4 mm, 5µm (Dr. Maisch GmbH, Ammerbuch, Germany)
Mobile phase	Methanol/aqueous buffer 22:78 (v/v) (aqueous buffer contained 6.8 g monobasic potassium phosphate and 1 ml perchloric acid per liter water; pH 2.5)
Flow rate	1.2 ml/min
Oven temperature	30 °C
Injection volume	10 µl
Detector	UV, 224 nm
Standard	0.15 mg/ml chloroquine phosphate Pharmaceutical Secondary Standard (Sigma-Aldrich LOT #LRAB3715) in water.
Sample preparation	One tablet was finely ground in a mortar. An aliquot of approx. 100 mg was weighed into a 100 ml volumetric flask. 50 ml of water were added. The flask was sonicated for 15 minutes and then filled up with water to 100 ml. For each sample, two independent experiments were carried out.

Table S2: *Method for quantitative HPLC analysis of chloroquine and paracetamol*

Method	Metronidazole Tablets (USP 42 monograph 2019)[3]
Instrument	HPLC (Agilent 1100 Series)
Column/stationary phase	Reprospher 100 C8, 150 x 4.6 mm, 5µm (Dr. Maisch GmbH, Ammerbuch, Germany)
Mobile phase	Methanol/water 20:80 (v/v)
Flow rate	1.0 ml/min
Oven temperature	30 °C
Injection volume	5 µl
Detector	UV, 254 nm
Standard	0.56 mg/ml Metronidazole Analytical Standard (Sigma-Aldrich LOT #MKBZ3056V) in methanol/water 20:80 (v/v).
Sample preparation	Three tablets were finely ground in a mortar. An aliquot of approx. 100 mg was weighed into a 100 ml volumetric flask. 50 ml of methanol were added. The flask was sonicated for 10 minutes and then filled up to 100 ml with mobile phase. For each sample, two aliquots were weighted and analyzed.

Table S3: *Method for quantitative HPLC analysis of metronidazole*

High resolution liquid chromatography-mass spectrometry

HR-HPLC/MS(/MS) was carried out using a Thermofisher UltiMate 3000 HPLC with a Phenomenex Luna 3µm Polar C18 100 Å column 150 x 2 mm, column temperature 30°C. Eluent A: 0.1% formic acid in water; eluent B: 0.1% formic acid in methanol. Gradient 5-100% B over 20 min followed by 100% B isocratic for 10 min; flow rate 0.3 ml/min. UV detection with a diode array detector. HR mass spectrometry: ESI-TOF Bruker MaXis 4G. The sample solutions were investigated in comparison to authentic paracetamol and metronidazole reference in H_2O/methanol 2:1. In samples III, IV and V, peaks at 5.0 min (metronidazole) were detected. In samples II and V, peaks at 5.4 min (paracetamol) were detected. UV spectra of the samples and the respective references were identical. The molecular ion of the respective samples showed the same exact mass as the molecular ion from paracetamol and/or metronidazole (Table S4). These were consistent with the molecular formula $C_8H_9NO_2$ of paracetamol and $C_6H_9N_3O_2$ of metronidazole.

Sample	Retention time	$[M+H]^+_{theoretical}$	$[M+H]^+_{measured}$	relative mass accuracy
Metronidazole reference	5.0 min	172.0717	172.0719	1.3 ppm
Paracetamol reference	5.4 min	152.0706	152.0709	2.0 ppm
Sample II	5.4 min	152.0706	152.0709	1.8 ppm
Sample III	5.0 min	172.0717	172.0721	2.3 ppm
Sample IV	5.0 min	172.0717	172.0720	1.9 ppm
Sample V	5.0 min	172.0717	172.0718	1.0 ppm
	5.4 min	152.0706	152.0708	1.2 ppm

Table S4: *Retention times, and theoretical and calculated exact masses, for the investigated samples and for metronidazole and paracetamol reference substances. HPLC conditions for HPLC-MS are different from those for quantitative analysis according to USP, therefore retention times are different from those shown in Figure 2.*

MS/MS analysis showed the presence of the characteristic fragments of metronidazole (Fig. S2) in samples III, IV and V, and the presence of the characteristic fragments of paracetamol (Fig. S3) in samples II and V. The observed fragmentation of the samples and the respective references were identical.

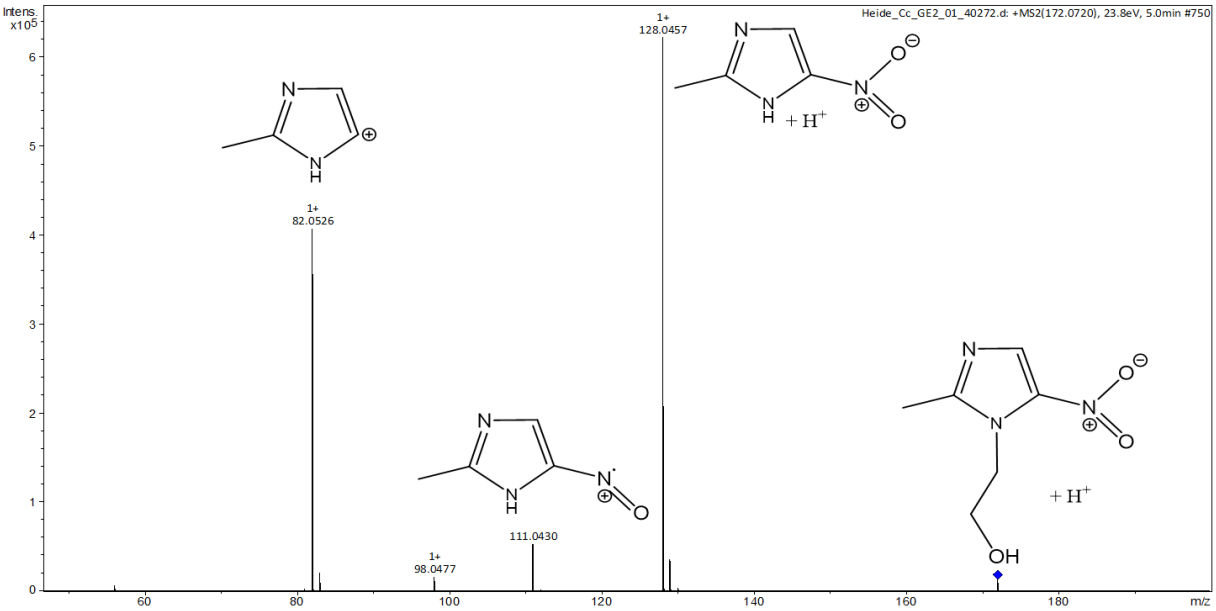

Figure S2: *MS/MS fragmentation of metronidazole in sample IV.*

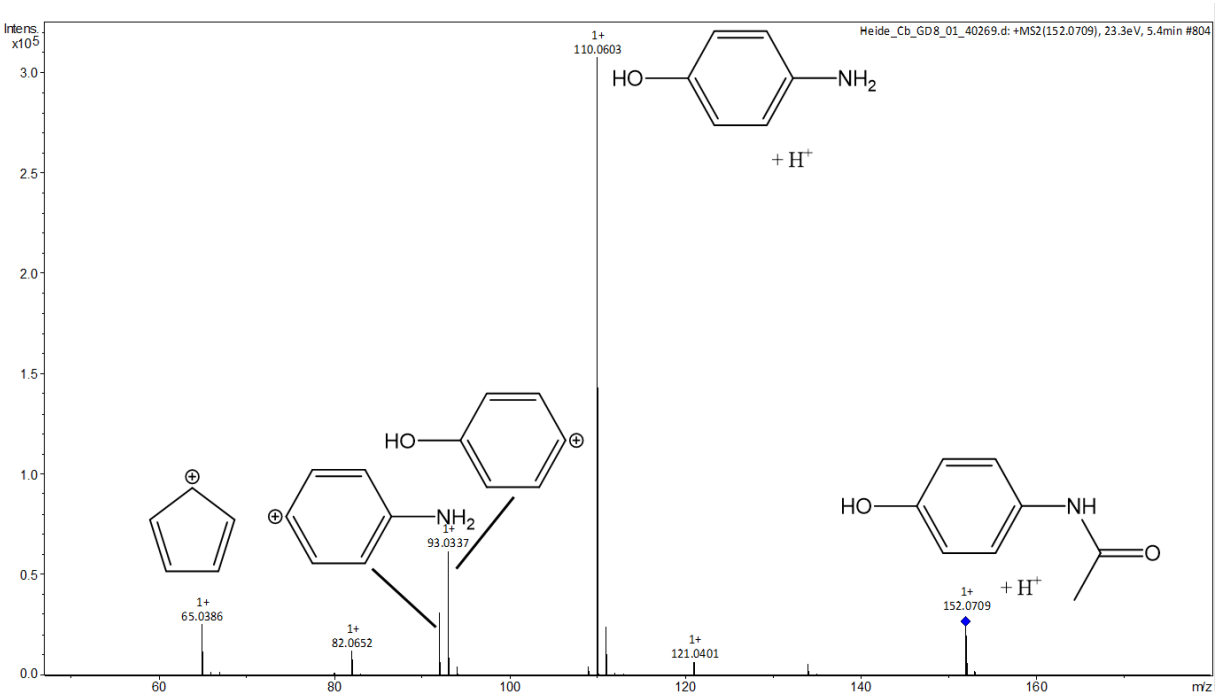

Figure S3: *MS/MS fragmentation of paracetamol in sample II.*

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PLOS ONE

RESEARCH ARTICLE

Quality of oxytocin and misoprostol in health facilities of Rwanda

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Abstract

Sustainable Development Goal 3.1 calls for a reduction of the maternal mortality ratio to less than 70 per 100,000 live births by 2030. The most important cause of maternal mortality is post-partum haemorrhage (PPH). Oxytocin injections and misoprostol tablets are medicines of first choice for the management of PPH in low- and middle-income countries (LMICs). Unfortunately, both substances are chemically unstable, and previous studies have revealed serious quality problems of these medicines in LMICs. The present study is the first report on their quality in Rwanda. From 40 randomly selected health facilities (hospitals, health centers, retail pharmacies and private clinics) in different parts of Rwanda, as well as from six wholesalers and government stores, oxytocin injections and misoprostol tablets were collected. Oxytocin storage temperatures in the health facilities were monitored for six months using temperature data loggers, and found to correctly follow the storage requirements stated by the manufacturers (2–8˚C, or room temperature) with few minor deviations. Oxytocin injections (57 samples, representing seven batches of four brands) were tested for their oxytocin content and pH value according to the United States Pharmacopeia. Twenty-four samples from three European manufacturers passed all tests. However, all nine samples of one batch of a Chinese manufacturer showed an excessive content of oxytocin (range 117.2–121.5% of the declared amount). Another batch of the same manufacturer showed extreme variations of the concentration of the preservative benzyl alcohol. Misoprostol tablets (25 samples, representing ten batches of six brands) were tested for content and dissolution according to the International Pharmacopoeia. Fifteen samples passed, but all 10 samples of two brands from India failed with extreme deviations, containing only 42.5–48.7% of the stated amount of misoprostol. In conclusion, oxytocin quality in Rwanda was better than reported from other African countries. However, two extremely substandard brands of misoprostol tablets were found. The Rwandan authorities reacted quickly and efficiently, and recalled these substandard medicines from the market. For oxytocin and misoprostol, with their well-known problems of quality and stability, procurement should possibly

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be restricted to medicines which are WHO-prequalified or which have been manufactured in countries with stringent regulatory authorities.

Introduction

In the year 2017, an estimated number of 295,000 women around the world died due to complications of pregnancy and childbirth [1]. The highest maternal mortality ratio is observed in the sub-Saharan region of Africa [1, 2]. Post-partum hemorrhage (PPH) is the most important cause of maternal mortality, and 50% of all cases of PPH worldwide occur in Africa [3]. Rwanda, a low-income country in sub-Saharan Africa [4], has successfully lowered its maternal mortality ratio from 1160 down to 248 per 100,000 live births in the years from 2000 to 2017 [1]. Efforts are made by the government of Rwanda to further reduce the maternal mortality ratio to less than 70 per 100,000 live births by 2030, in accordance with the Sustainable Development Goal (SDG) 3.1 [5, 6]. To achieve this target, oxytocic medicines which are used to treat and prevent PPH are of principal importance. Oxytocin injections and misoprostol tablets are among the medicines of first choice for the prevention and treatment of PPH [3, 7]. They are included as oxytocics (uterotonics) in the WHO model list of essential medicines [8] and in the Rwanda National List of Essential Medicines for adults [9]. Also, they have been included in the list of 13 life-saving items prepared by the United Nations Commission on Life-Saving Commodities for Women and Children (UNCoLSC) [10], as the only medicines for the management of PPH.

The use of substandard and falsified medicines has been shown to result in serious public health problems [11]. Especially in LMICs, the quality of medicines often fails to meet the pharmacopeial specifications, and this has far-reaching adverse consequences for patients, families, national health systems and the economy [11, 12]. The use of substandard oxytocin or misoprostol preparations in the management of PPH may lead to therapeutic failure in the treatment of excessive bleeding, and even to the death of the patient [13, 14]. Avoiding such preventable deaths is one of the key measures required to reach SDG 3.1.

Unfortunately, oxytocics are sensitive to environmental conditions. Oxytocin itself is a peptide hormone containing nine amino acids with an intramolecular disulfide bridge (Fig 1) [15]. It is highly sensitive to elevated temperatures and may degrade quickly when inappropriately stored, especially in tropical climates [16, 17]. The World Health Organization (WHO) recommends to store all preparations of oxytocin in the refrigerator, i.e. between 2˚C and 8˚C [16]. However, some commercial oxytocin preparations carry recommendations for non-refrigerated storage [18, 19]. Oxytocin stability furthermore depends on the pH value, with an optimum stability at pH 4.5 [20]. Both the United States Pharmacopeia (USP) and the International Pharmacopeia (Ph. Int.) demand that oxytocin injections must have a pH value between 3.0 and 5.0 [19].

A systematic review published by Torloni et al. in 2016 [13] listed eight studies on the quality of oxytocin conducted in LMICs. In a subsequent systematic review published by the same authors in 2020 [14], the number of included oxytocin quality studies had increased to 14. Overall, 39.7% of the oxytocin samples investigated in all these studies had been reported to fail quality testing. An insufficient content of the active pharmaceutical ingredient (API) represented by far the most frequently found deficiency. The overall percentage of failing samples had been 31.4% for studies conducted in the time period of 2000–2011 (n = 363 samples), but had increased to 44.4% for studies conducted from the year 2012 onwards (n = 611 samples) [14]. This indicates that in the last two decades, the quality problems of oxytocin have increased rather than decreased.

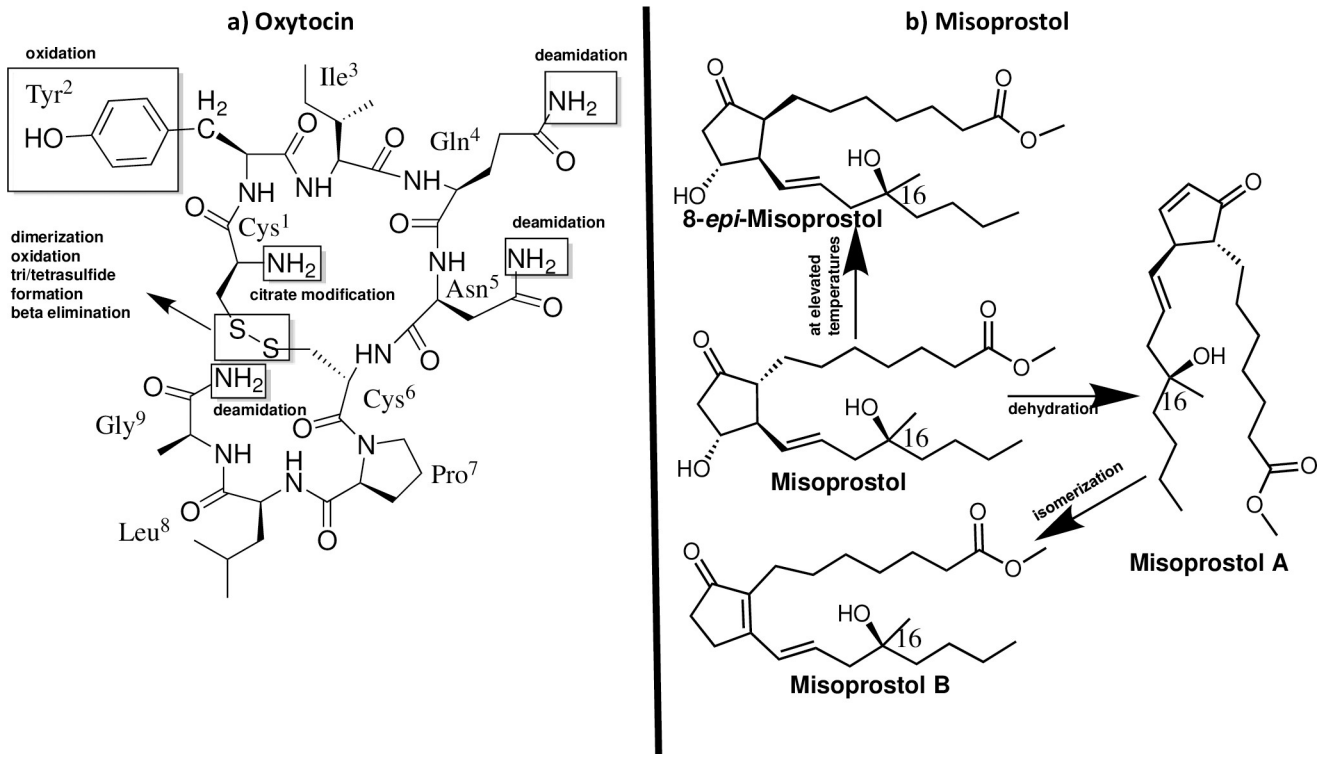

Fig 1. Structures of oxytocin (modified from [21]) and misoprostol, and their typical degradation mechanisms. Commercial misoprostol is a mixture, containing the depicted structure, its epimer at C16, and the enantiomers of both compounds. Likewise, the degradation products of misoprostol contain the corresponding stereoisomers.

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The percentage of oxytocin samples reported to fail quality testing varies notably between different studies. Anyakora et al. [22] reported that 74.2% of the 159 oxytocin samples collected in Nigeria failed quality testing. Similarly, Stanton et al. [23] reported a failure rate of 73.9% upon investigation of 46 oxytocin samples from Ghana. On the other hand, Hagen et al. [24] showed that only 11% out of 65 oxytocin samples collected in Malawi failed testing, and only with moderate deviations from the pharmacopeial specifications. Another study conducted in Ethiopia reported a failure rate of only 4% within 45 oxytocin samples [25]. In all these studies, the failure rate resulted nearly exclusively from an incorrect API amount determined in the samples [14]. Therefore, the different failure rates reported are not due to different parameters being tested in the different studies.

Misoprostol (Fig 1) is an analog of prostaglandin E1. Commercial preparations contain a mixture of both its epimers at C-16, and their enantiomers [26]. Misoprostol is a viscous oil at room temperature and is extremely unstable in the presence of water [27]. Both the raw material and the finished products have to be carefully protected from humidity [27, 28]. In finished pharmaceutical products, misoprostol must be stabilized in form of a 1% dispersion in hydroxypropyl methylcellulose (HPMC) [29], since in the absence of HPMC misoprostol quickly undergoes dehydration, isomerization and epimerization reactions (Fig 1), resulting in a loss of activity. A study by Hall [28] on 215 misoprostol samples, collected in 15 LMICs, reported an incorrect API content in 45% of the samples, and a decomposition of misoprostol in those samples which were packaged in plastic-aluminium blisters. Therefore, it has been strongly recommended that misoprostol tablets should be packaged in double-sided aluminium blisters to protect them from moisture [28]. Storage of misoprostol tablets outside the blisters exposes

them to moisture and has been shown to quickly decrease the amount of the active ingredient, and also to reduce hardness and increase friability of the tablets [27]. In spite of these well-documented problems, misoprostol quality in LMICs has received much less attention than oxytocin quality. The above-mentioned review published by Torloni et al. [14] lists, besides the study by Hall, only two further studies which investigated the quality of misoprostol tablets: Anyakora et al. [22] reported that 56 (33.7%) out of 166 misoprostol samples collected in Nigeria failed quality testing due to incorrect API content, but the study did not state the exact amount of API detected in the samples. Hagen et al. [24] reported that 5 (17%) out of 30 misoprostol samples from Malawi failed pharmacopeial specifications, notably all five with extreme deviations since they contained only 12.7–30.2% of the declared amount.

So far, no data on the quality of oxytocin injections and misoprostol tablets in Rwanda have been published, although the above-mentioned findings from other LMICs indicate that the presence of substandard preparations is likely. Therefore, in the present study samples of oxytocin injections and misoprostol tablets were collected from randomly selected government, faith-based and private health facilities and drug outlets, as well as from government medical stores and private wholesalers in Rwanda, and were investigated for their quality according to the United States Pharmacopeia (USP) and the International Pharmacopeia (Ph. Int.), respectively. In parallel to this study, an evaluation of the availability and prices of essential medicines in health facilities of Rwanda, also beyond oxytocin and misoprostol, has been carried out, and the results have been published elsewhere [30].

Methods

Study design and ethical approval

The study protocol and the methods for collection and investigation of the samples were designed following the MEDQUARG guidelines [31] and the WHO Guidelines on the Conduct of Surveys of the Quality of Medicines [32]. Ethical approval to conduct this study was obtained from the College of Medicine and Health Sciences Institutional Review Board (CMHS-IRB) of the University of Rwanda with approval notice No. 026 /CMHS IRB/2018. An authorization to access health facilities and to conduct this study was kindly granted by the Ministry of Health, Rwanda (reference No. 20/1361/DGPHFIS/2018). In fulfilment of the requirements for sample transfer from Rwanda to Germany, a Material Transfer Agreement (MTA) was signed between investigators and the Rwandan Ministry of Health. Consent to import medicine samples for analysis was also obtained from German authorities (Regierungspräsidium Tübingen, Leitstelle Arzneimittelüberwachung).

Selection of sampling sites

Samples of oxytocin and misoprostol were collected in Kigali city and in five districts representing the provinces of Rwanda, i.e. Bugesera district (Eastern Province), Karongi district (Western Province), Musanze district (Northern Province), and Muhanga and Kamonyi districts (Southern Province). Government, faith-based and private facilities were included, i.e. government district hospitals and health centers, faith-based district hospitals and health centers, private retail pharmacies and private clinics/hospitals. A list of these health facilities in Kigali city and in the five selected districts was obtained from the Ministry of Health, comprising altogether 13 district hospitals (government or faith-based), 77 government health centers, 44 faith-based health centers, 36 private clinics, and 234 private retail pharmacies. For each of the five districts and for Kigali city, two hospitals and two health facilities of each of the other categories (government health centers; faith-based health centers; private retail pharmacies; private clinics) were randomly selected using the RAND function of Microsoft Excel.

However, in each of the two districts Muhanga and Kamonyi only a single district hospital existed; these were included into the study. In Musanze district, no district hospital existed, but a government referral hospital, and this was included. Therefore, a total of 57 health facilities and private retail pharmacies were selected. In the course of the study visits, it turned out that one of the selected district hospitals was a specialized orthopedic hospital and did not stock oxytocin or misoprostol. Also, 11 of the 12 selected private clinics stated that they did not store these medicines, and 5 of the 12 retail pharmacies had none of these two medicines available. Therefore, oxytocin and/or misoprostol could be collected from 40 health facilities and retail pharmacies, and these are listed in S1 Table.

In addition to health facilities and retail pharmacies, oxytocics were also collected from the government central medical store (Medical Procurement and Production Division [MPPD]), from two government district pharmacies and from three large private wholesalers. Therefore, samples were collected from a total of 46 different facilities. Fig 2 shows the location of the facilities on a map of Rwanda.

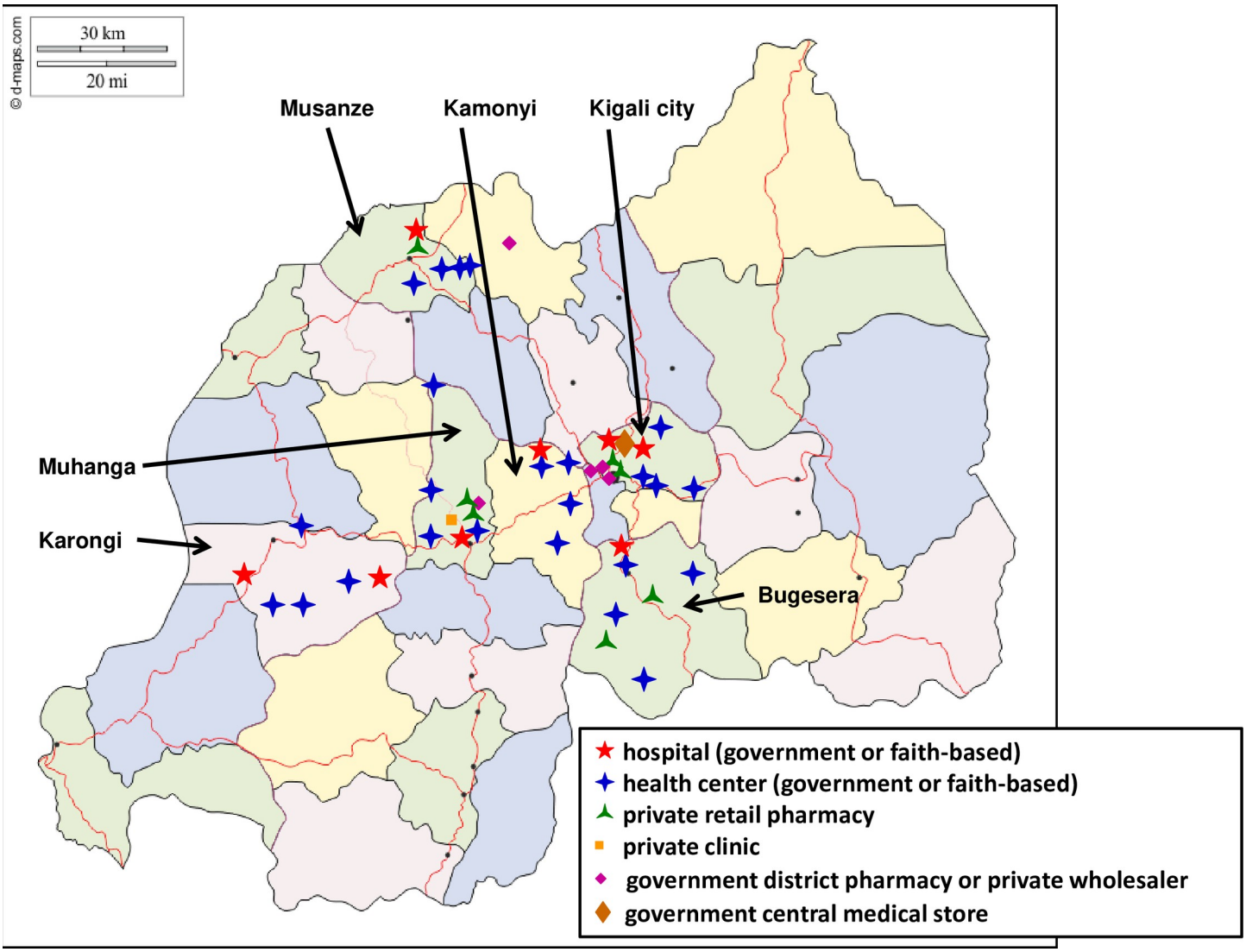

Fig 2. Map of the location of the 46 facilities from which oxytocics were collected. Reprinted (with modifications) from https://d-maps.com/conditions.php?lang=en under a CC BY license, with permission from d-maps.com, original copyright 2007–2020.

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In health facilities which stored oxytocics both in their pharmacy stores and in their maternity wards, samples were collected from both sites if these medicines were available there. Thereby in total samples were collected from 44 storage rooms and 20 maternity wards, as listed in S1 Table.

Sample collection

Sampling was done by the investigator T.B. in March 2018 for the pilot study in Muhanga district, and in September-October 2018 for the main study in the other districts and Kigali city. At each sampling site, from every available brand and batch, 10 vials of oxytocin and 50 tablets of misoprostol were collected if available. A minimum amount of 5 vials of oxytocin and 10 tablets of misoprostol was collected.

When sampling from government and faith-based health facilities, the collected vials and tablets were replaced with medicines which had been purchased by the investigator from the government central medical store or two government district pharmacies, in order to avoid causing stock-outs in the health facilities by the sampling. Samples collected from private facilities (retail pharmacies, private clinics and wholesalers) were paid for in cash. In health facilities and retail pharmacies, samples were collected by an overt approach, i.e. the investigator informed the staff about the purpose of the visit, and the "Sample Site & Drug Purchase Records" shown in S1 Fig were filled and signed by the investigator and by the responsible person at the health facility. Samples from private wholesalers were obtained through a local retail pharmacy using a mystery shopper approach, and no "Sample Site & Drug Purchase Records" were prepared for these purchases.

Upon sample collection, each sample was immediately labeled with a unique sample code. All samples of oxytocin were transported to a central collection site in Rwanda in a 12 V plug-in refrigerator, and subsequently stored between 2˚C and 8˚C until shipment to Germany. Misoprostol tablets were transported and stored at a temperature not exceeding 25˚C. Samples were hand-carried to Tübingen University, Germany, by the investigator T. B. on commercial passenger flights in May 2018 (for the pilot study) and in November 2018 (for the main study). They were placed into appropriate storage conditions at Tübingen University within 24 hours after departure from Rwanda. A temperature data logger was kept with the samples at all times to record the temperature during transport and intermediate storage.

Assessment of oxytocin storage temperatures

Oxytocin storage temperatures were recorded using temperature data loggers (Tempmate M1 by imec Messtechnik GmbH, Heilbronn, Germany). These were placed by the investigator at the storage sites of oxytocin at the time of sample collection, and they automatically recorded the temperature every 10 minutes. Private wholesalers were contacted by a mystery shopper approach, and therefore no temperature data logger could be placed. Temperature data loggers were recollected by the investigator after six months, and the mean kinetic temperature (MKT) was calculated by the imec Messtechnik software.

Registration status of collected medicines

To enquire the registration status of the medicines collected in Rwanda, the Rwanda Food and Drug Authority (RFDA) was contacted. However, the full registration process of medicines by RFDA had not yet been in effect at the time of sample collection. It could not be established which of the collected preparations had been pre-registered according to the previous procedures.

Packaging examination

The information stated on the packaging and in the package inserts of the samples were examined visually for the presence of irregularities or inconsistencies, such as spelling mistakes, unusual batch numbers, unexpected or modified manufacturing or expiry dates, or signs of repacking. The individual dosage units (oxytocin vials and misoprostol tablets) were inspected for visible deficiencies, like color changes, suspended particles within vials, etc. In addition, for misoprostol tablets the material of the primary packaging was recorded, as this is important for misoprostol stability [28].

HPLC analysis

Oxytocin injections were analyzed for identity, assay and pH value following the respective monograph of the United States Pharmacopoeia 40 (USP 40). The assay was performed using High Performance Liquid Chromatography (HPLC; Agilent Infinity 1260 II with binary pump, refrigerated autosampler, integrated column compartment and variable wavelength detector; Agilent Technologies, Santa Clara, CA, USA). A Reprospher column 100 C18, 5 μm; 12.5 cm x 4.6 mm (Dr. Maisch GmbH, Ammerbuch, Germany) was used with a column temperature of 21˚C. A linear gradient of mobile phase A (0.1 M aqueous NaH_2PO_4 buffer) and mobile phase B (acetonitrile/water 1:1) was used: 0 min, 30% B; 10 min: 40% B; 17.5 min: 65% B; 20.5 min, 65% B; 23.5 min, 30% B; 26 min, 30% B. Flow rate: 1.5 ml/min. Detection: 220 nm. Injection volume: 70 μl. The diluent used to prepare standard solutions was prepared by dissolving 500 mg of chlorobutanol in 0.5 ml glacial acetic acid, adding 500 mg of ethyl alcohol, 110 mg of sodium acetate and filling up to 100 ml with bi-distilled water. Analysis was performed for three different vials per sample, each injected twice, yielding six measurements per sample. Oxytocin USP Reference Standard purchased from Merck KGaA, Darmstadt, Germany, was used for comparison. The pH value was measured twice for each vial, testing three vials from each sample of oxytocin, and the average value was calculated.

The concentration of the preservative benzyl alcohol, which was only contained in the oxytocin samples manufactured by Jiangxi Xierkangtai Pharmaceutical Co. Ltd, China, was determined using a method modified from Rego and Nelson [33]. The analysis was carried out using HPLC (Agilent 1200 Series with a diode array detector; Agilent Technologies, Santa Clara, CA, USA), with the same column as described above. The mobile phase was composed of 20% acetonitrile and 80% water. Flow rate: 1.5 mL/min. Detection: 254 nm. Injection volume: 50 μl. Samples were diluted 1:30 with bi-distilled water prior to injection, except for sample QOR04 which was injected without dilution. Analysis was performed for at least two different vials per sample, each injected twice. Benzyl alcohol pharmaceutical secondary standard (SIGMA-ALDRICH, St. Louis, MO, USA; Lot LRAC1678; certified purity of 99.98%) was used as reference material.

Misoprostol tablets were analyzed following the respective monograph of the International Pharmacopoeia 2017 (Ph. Int. 2017) for identity, assay and dissolution testing. The Agilent Infinity 1260 II HPLC instrument described above was used with a stainless-steel column packed with ReproSil-XR 120 C18, 5 μm, 150 mm x 4.6 mm (Dr. Maisch GmbH, Ammerbuch, Germany) and a guard column containing the same material. The column oven was kept at 35.0˚C. A premixed mobile phase composed of acetonitrile and bi-distilled water in a ratio of 45:55 was used, at a flow rate of 1.5 ml/min in isocratic mode. The injection volume was 100 μl for assay and 250 μl for dissolution testing. HPLC vials were kept in a cooled autosampler at 4˚C until sample injection to avoid degradation. Detection was carried out by UV at 200 nm. Sample and standard solutions were freshly prepared using the mobile phase as diluent. For misoprostol assay, two separate determinations were carried out for each sample. For each determination, five tablets were placed into 50 ml of the mobile phase. In the case of three of

the collected samples, the number of available tablets was insufficient for this procedure, and in these cases for each of the two determinations one tablet was placed into 10 ml of the mobile phase. Misoprostol was dissolved from the tablets using an ultrasonic bath, and ice was added to the bath to avoid degradation by heat. The solution was filtered through Rotilabo PTFE 0.20 μm filters (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) and injected into the HPLC, with three injections of each of the two solutions, yielding six measurements per sample. European Pharmacopoeia Reference Standard (batch N˚ 3.0) from the European Directorate for the Quality of Medicines (EDQM) was used for comparison.

Misoprostol tablets were analyzed for related substances according to Ph. Int., using the same HPLC instrumentation and the same column as described above for the assay. The mobile phases consisted of acetonitrile, water and methanol in the ratio 28:69:3 (mobile phase A) and 47:50:3 (mobile phase B), and were used in the gradient mode described in Ph. Int., at a flow rate of 1.5 ml/min and a column temperature of 35˚C. The injection volume was 200 μl. Detection was carried out by UV at 200 nm. Sample solutions were prepared as described in Ph. Int. For comparison, a misoprostol standard solution 20 μg/ml in mobile phase A, and the same standard solution heated for 1 hour at 80˚C, were used. Each of the sample and standard solutions was injected twice. The different degradation products of misoprostol (Fig 1) were identified as described by Ph.Int., based on their relative retention times compared to misoprostol.

Dissolution testing was conducted according to Ph. Int. using a dissolution tester PT-WS 610 (Pharma Test Apparatebau AG, Hainburg, Germany). Into each of the six dissolution vessels filled with 500 ml of de-ionized water, one tablet was placed. The test was conducted at 37 ± 0.5˚C with a paddle rotational speed of 50 revolutions per minute. Samples were withdrawn after 30 min through an in-line filter, and 250 μl of these solutions were injected into the Infinity 1260 II HPLC system described above.

The system suitability for the method for oxytocin assay was verified according to USP 40, and for the methods for misoprostol assay and dissolution according to Ph. Int.

For the determination of the benzyl alcohol concentration, linearity and precision of the applied method were validated according to the International Council for Harmonization (ICH) guideline Q2(R1) [34]. Relative standard deviation of the measurements (repeatability) was 0.2%.

Sample analysis was conducted unblinded to packaging. All oxytocin and misoprostol samples were analyzed before reaching their expiry dates.

Mass spectrometric analysis

Gas chromatography-mass spectrometry (GC-MS) was carried out using a Hewlett Packard/Agilent HP6890 GC system coupled with a HP5973 mass selective detector. The injector temperature was 280˚C. An Agilent HP-5ms Ultra Inert (5%-phenyl)-methylpolysiloxane column 30 m x 0.25 mm with a film thickness 0.25 μm was used. The temperature gradient was 40 to 320˚C with 10˚C/min, followed by 10 min 320˚C isothermal. Helium was used as carrier gas with a flow rate of 1.2 ml/min. Electron impact ionization (EI) was carried out with 70 eV, and a single quadrupole analyzer was used.

HPLC-MS/MS analysis was conducted on a Thermo Scientific UltiMate 3000 HPLC-System coupled with an ESI-TOF Bruker maXis 4G (Bruker Daltonics, Bremen, Germany) in the positive mode.

Definitions for substandard and falsified medicines

Samples were classified as within specification or out of specification (= substandard) based on the criteria of USP 40 for assay and pH value in case of oxytocin injections, and based on the criteria of the Ph. Int. 2017 for assay and dissolution in case of misoprostol tablets. According

to these pharmacopoeias, both oxytocin injections and misoprostol tablets must contain not less than 90.0% and not more than 110.0% of the declared amount of the active pharmaceutical ingredient (API). For oxytocin vials, the pH value must be between 3.0 and 5.0. For dissolution testing of misoprostol tablets, the amount in solution must not be less than 80% (Q) of the amount declared on the label.

Following the terminology introduced by earlier studies of WHO, assay results deviating more than 20% from the declared API content, and dissolution results falling more than 25% below the pharmacopeial Q value, were considered as extreme deviations [35, 36]. Lesser deviations from pharmacopeial specifications were considered as moderate deviations. As per definition of WHO [11], products that deliberately/fraudulently misrepresent their identity, composition or source were considered falsified.

Data analysis

Excel (Microsoft Office Professional Plus 2019) was used to calculate means, medians and percentiles, and relative standard deviations (RSD). Figures of the distribution of the assay test results for oxytocin injections and misoprostol tablets, and the dissolution test results for misoprostol tablets, were generated using the statistical software JMP 14.2 (SAS GmbH, Heidelberg, Germany).

Information of national authorities and stakeholders

Following the request stated in the permission No 20/1361/DGPHFIS/2018 from the Ministry of Health of Rwanda to conduct the present study, the authors have submitted on December 2, 2018, an alert letter to the Rwandan authorities about two extremely substandard brands of misoprostol tablets found to circulate in Rwanda (see Results section). Furthermore, this manuscript was shared with the Rwandan Food and Drug Authority (RFDA) and with the WHO Rapid Alert System.

Results

Overview of sampling sites

As shown in Table 1, oxytocics could be collected from 40 of the 57 randomly selected health facilities and retail pharmacies, as well as from three government medical stores and three private wholesalers (see Methods section). In health facilities, samples were collected both from medicine storage rooms and from maternity wards if available.

Notably, oxytocin injections were available in every visited government and faith-based health facility, i.e. both in hospitals and health centers, consistent with the recommendations of the Rwanda National List of Essential Medicines (REML) [9]. According to the REML, misoprostol tablets are expected to be available as oxytocics in hospitals but not in health centers. Indeed, all eight hospitals but only three of the 24 visited health centers, had misoprostol tablets available in a sufficient amount for sampling.

A total of 12 retail pharmacies had been randomly selected and visited, but only seven had misoprostol tablets available, and none stored oxytocin injections. Of the 12 randomly selected private clinics, most stated that they do not offer maternity services, and oxytocin could be collected only from a single private clinic.

A detailed list of all sampling sites, with the numbers of oxytocin vials and misoprostol tablets collected at each site, is shown in S1 Table.

Overview of collected oxytocin samples

A total of 57 oxytocin samples were collected. All of these were packaged in vials of 1 ml, with a concentration of 10 IU/ml. As shown in Table 2, the 57 samples represented only four

Table 1. Overview of sampling sites for oxytocin injections and misoprostol tablets.

Category of health facility	Number of facilities	Sites in facility	Number of sites	Number of oxytocin samples collected	Number of misoprostol samples collected
Government hospitals	3	storage rooms	3	3	4*
		maternity wards	2	2	1
Faith-based hospitals	5	storage rooms	5	5	5
		maternity wards	5	5	2
Government health centers	12	storage rooms	11	11	2
		maternity wards	6	6	0
Faith-based health centers	12	storage rooms	11	11	1
		maternity wards	7	7	0
Private clinics	1	storage rooms	1	1	0
Retail pharmacies	7	storage rooms	7	0	7
Government central medical store	1	storage rooms	1	2*	0
Government district pharmacies	2	storage rooms	2	1	2
Private wholesalers	3	storage rooms	3	3	1
Total	**46**		**64**	**57**	**25**

* Two brands collected in one sampling site.

https://doi.org/10.1371/journal.pone.0245054.t001

different brands and a total of seven different batches. In addition to the facilities listed in Table 1, three further private pharmaceutical wholesalers in Kigali were contacted, as well as the procurement organization for faith-based health facilities, i.e. the Bureau de Formations

Table 2. List of oxytocin brands and batches collected in this study.

Brand name and stated manufacturer	WHO Pre-Qualified/ SRA	Stated storage temperature requirement	Batch N°	Manufacture/ Expiry Date	Number of samples
Oxytocin injection	-	room temperature [a]	1606573	Jun 16 / Jun 19	24
Jiangxi Xierkangtai Pharmaceutical Co. Ltd			1604521	Apr 16 / Apr 19	9
China					
Steroxine 10 IU/1 ml [b]	SRA	2–8˚C	160042	Feb 16 / Jan 19	1
Laboratoires Sterop					
Belgium			160269	Sep 16 / Aug 19	1
Oxytocin 10 IU/ml	WHO-PQ	2–8˚C	37611016	Oct 16 / Oct 20	4
AS Grindeks					
Latvia [c]			37711116	Nov 16 / Nov 20	1
Oxytocin 10	SRA	2–8˚C	70779A	Sep 17 / Sep 20	17
Rotexmedica GmbH Arzneimittelwerk					
Germany					

WHO-PQ = WHO-prequalified medicine; SRA = manufactured in a country with stringent regulatory authority.

[a] Storage requirement stated on packaging: "Store in a cool dry place, away from light"; storage requirement stated on the package insert: "Store in a dark place at room temperature, protect from light."

[b] Batch 160269 was labeled with the unbranded generic name "Oxytocin 10 IU/1 ml", all other information was identical as in batch 160042.

[c] Marketing authorization holder: Peckforton Pharmaceuticals Ltd., United Kingdom.

https://doi.org/10.1371/journal.pone.0245054.t002

Médicales Agréés du Rwanda (BUFMAR), but none of these had any other oxytocin brands or batches in stock than those shown in Table 2. Therefore, the preparations listed in Table 2 appear to represent most (or all) oxytocin batches which were in circulation in Rwanda at the time of sample collection.

The most frequently encountered preparation, representing 33 samples, was stated to be manufactured by Jiangxi Xierkangtai Pharmaceutical Co. Ltd, China. On its secondary packaging, the storage requirement was stated as "Store in a cool dry place, away from light", while in the package insert, the storage requirement showed a slightly different wording: "Store in a dark place at room temperature, protect from light."

The three other brands (Table 2) were stated to be manufactured in European countries with stringent regulatory authorities (SRAs) [37], and all of them were labeled for refrigerated storage (i.e. storage at 2–8˚C). The product manufactured by AS Grindeks, Latvia (marketing authorization holder: Peckforton Pharmaceuticals Ltd., UK) was a WHO-prequalified medicine [38, 39].

The stated shelf life of three out of the four brands was three years, and four years in case of the preparation by Grindeks. The package inserts of the preparations by Sterop (Belgium), Grindeks (Latvia) and Rotexmedica GmbH Arzneimittelwerk (Germany) stated the excipients, i.e. sodium chloride, different buffering agents, water for injection, and in case of the Sterop preparation also chlorobutanol, a preservative and stabilizing agent [40]. In contrast, no excipients were stated for the product by Jiangxi Xierkangtai.

The preparations by Jiangxi Xierkangtai (China), by Rotexmedica (Germany) and by Grindeks (Latvia) were all found in both government and faith-based facilities. In contrast, the preparation by Sterop (Belgium) was only found in two private pharmaceutical wholesalers at the time of sample collection. No expired samples of oxytocin were found to be in circulation.

Oxytocin storage conditions

Out of 57 samples of oxytocin, 33 were labeled for room temperature storage, and 24 for refrigerated storage. The actual storage place in the facilities could be inspected at 52 of the 56 sampling sites; it could not be inspected at the pharmaceutical wholesalers which were contacted by a mystery shopper approach. Notably, in all inspected sites the actual storage place (i.e. inside or outside the refrigerator) correctly corresponded to the storage recommendation of the manufacturer.

Temperature data loggers were placed at the storage places of oxytocin and recollected after six months. In five cases, the loggers failed to record data or got lost in the facility, but from 47 oxytocin storage sites temperature recordings were obtained. These comprised 18 refrigerated and 29 non-refrigerated storage places. The results recorded at the individual sites are listed in S1 Table.

In 13 out of the 18 refrigerated oxytocin storage sites, the recorded mean kinetic temperature (MKT) ranged from 4.3˚C to 7.3˚C, compliant with the manufacturers' storage requirement of 2–8˚C. In one site, the recorded storage temperature was too low (MKT = -1.3˚C). At this site, except for short temperature spikes (possibly due to opening of the refrigerator), the recorded temperature was constantly around -2˚C. Though this indicates an incorrect temperature setting of the refrigerator, it is unlikely to have caused freezing of the preparation, due to the presence of excipients in the oxytocin vials. In three sites, the recorded MKTs in the refrigerators were slightly too high (8.5˚C, 9.1˚C and 10.5˚C, respectively). And in one further site (a maternity ward of a faith-based hospital), oxytocin was not stored in a refrigerator but in a cool box, reportedly only for immediate use and for not more than 24 hours. Indeed, the temperature data logger at that site recorded alternating periods of cold temperature and room

temperature. The MKT in this cool box over the entire recording period resulted as 15.8˚C, but the storage temperature at the times of oxytocin storage may well have been correct.

In most of the other storage sites, the temperatures were largely constant over the entire recording period. Only in one case larger fluctuations (between +15˚C and -2˚C) of longer duration were observed. Five sites showed few very brief periods of temperatures above 8˚C which may have been due to occasional power failures, as also mentioned by the staff of these facilities.

At the 29 non-refrigerated oxytocin storage sites, the median value of the recorded MKTs was 23.5˚C (range 19.8–26.3˚C), reflecting the temperate climate of Rwanda, most of which is situated at an altitude of approximately 1500 m.

Packaging examination and visual inspection of oxytocin samples

Packaging examination revealed no irregularities except for a few minor mistakes in the use of upper and lower case letters on the packaging and in the package inserts of the product stated to be manufactured by Jiangxi Xierkangtai (China). However, one sampling site (private clinic) stored the vials of oxytocin not in their original secondary packaging but in a box labeled as dexamethasone. Visual inspection showed no deficiencies like color changes or suspended particles.

Chemical analysis of oxytocin samples

The presence of oxytocin could be confirmed for all samples, therefore neither packaging examination nor chemical analysis indicated the presence of any falsified oxytocin samples. Also the pH value which is important for oxytocin stability was within USP specifications (3.0–5.0) for all samples (S2 Table). Fig 3 shows the distribution of the assay results for the investigated brands and batches. Notably, none of the 57 samples showed an insufficient content of oxytocin.

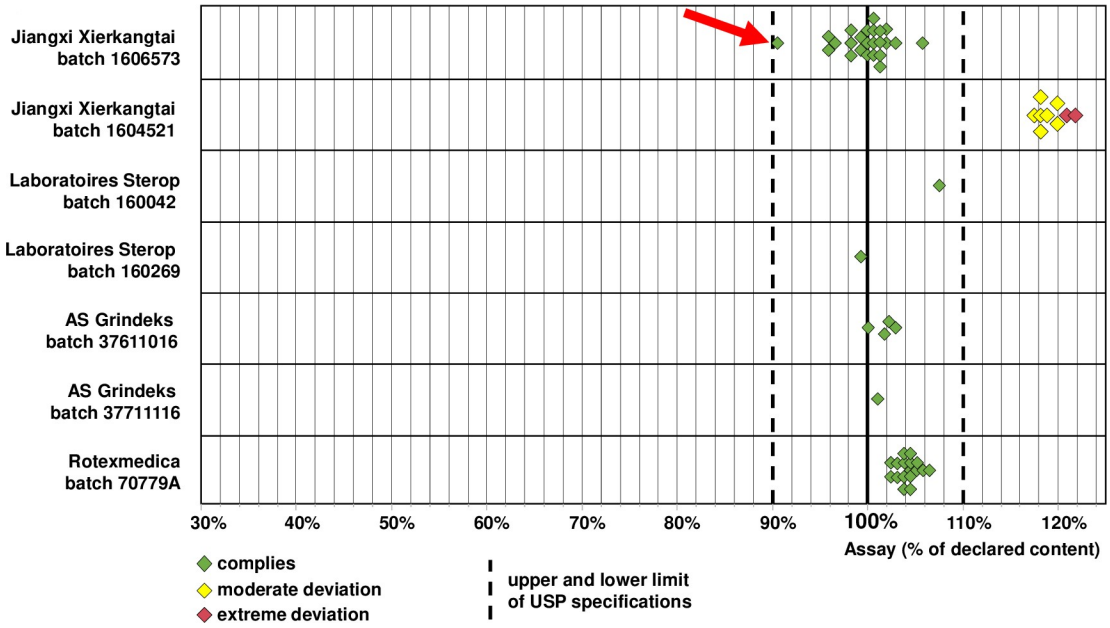

Fig 3. Content of oxytocin determined in each of the investigated samples. The arrow marks a sample with deviating content of the active pharmaceutical ingredient oxytocin and of the preservative benzyl alcohol (see main text and Fig 4).

https://doi.org/10.1371/journal.pone.0245054.g003

The 17 samples of Rotexmedica (Germany) all belonged to one single batch and showed a median content of 103.8% of the declared amount. These samples had been collected at different points of the supply chain (government central medical store; hospitals; government and faith-based health centers), but nevertheless showed a very uniform API content (range 102.0–105.9% of the declared amount). Likewise, the samples of AS Grindeks (Latvia) showed a very uniform content (range 99.9–102.8% of the declared amount). The two samples of Sterop (Belgium), belonging to different batches, contained 99.6 and 107.8% of the declared amount.

In sharp contrast, all nine samples of batch no. 1604521, stated to be manufactured by Jiangxi Xierkangtai (China) were found to deviate from USP specifications (90–110% of the declared amount) by containing too high amounts of oxytocin (median 118.0%). For two of these samples, the obtained assay result even exceeded the declared content by more than 20%, which represents an extreme deviation following the definition used in previous studies of WHO [35, 36].

In the other batch by Jiangxi Xierkangtai (batch no. 1606573), 23 of the 24 samples ranged in their content from 95.6 to 105.5% of the declared amount, well inside the content range specified by USP. However, for one sample of this batch, collected from a private wholesaler (sample no. QOR04; collected from facility no. 44 in S2 Table), the oxytocin content determined for three investigated vials was 89.7%, 90.4% and 91.0% of the declared amount, respectively, therefore on the borderline of USP specifications.

Fig 3 shows that the oxytocin content varied between different brands and batches. The results of the chemical analysis of each oxytocin sample, and the age of the samples at the time of analysis, are shown in S2 Table. In case of Jiangxi Xierkangtai batch no. 1606573, samples which were collected during the pilot study in March 2018 were 24 months old at the time of analysis. These did not show a higher content than those samples which were collected in the main study in September and October 2018 and were 30 months old at time of analysis. This, and the other data in in S2 Table, provide no evidence that differences in the age of the samples were important for the observed differences in oxytocin content.

Unexpectedly, the HPLC assay of the samples stating Jiangxi Xierkangtai as manufacturer showed a very large peak of an unknown substance eluting approximately two minutes earlier than oxytocin (Fig 4). Neither the packaging nor the package insert gave any information on the identity of this substance. An enquiry was sent to the three e-mail addresses given on the website of the stated manufacturer, but remained unanswered. Therefore, the identity of the unknown substance was investigated by mass spectrometry. GC-MS analysis showed the following mass and fragmentation: m/z 108 (M^+; 92%), 107 (67%), 91 (17%), 79 (100%), 77 (63%), 65 (7%), 51 (12%), 39 (8%). Comparison to the database of the National Institute of Standards and Technologies, Gaithersburg, MD, USA (NIST; http://webbook.nist.gov) showed that these data were identical to those of benzyl alcohol, a commonly used preservative in parenteral pharmaceutical preparations [41]. Subsequently, the identity of the unknown substance was confirmed by both GC-MS and HPLC-MS investigation in comparison to authentic benzyl alcohol, showing identical retention times and mass spectrometric fragmentations. The concentration of benzyl alcohol was determined as 0.9% in nearly all samples.

However, the oxytocin sample no. QOR04 (which had already been noticed to contain the lowest oxytocin amount of all investigated samples, see above) was found to contain only 0.004% benzyl alcohol, showing identical concentrations in all vials. This sample had been collected from a private wholesaler in Kigali. It is highlighted in Fig 3 by an arrow, and in S2 Table by bold print. Its batch number as well as primary and secondary packaging and package insert were identical to those from the 23 other samples which stated Jiangxi Xierkangtai as manufacturer (S2 Fig).

Within all other investigated samples by Jiangxi Xierkangtai only a single vial was found with a deviating concentration of benzyl alcohol. It belonged to a sample (no. QOR75) which

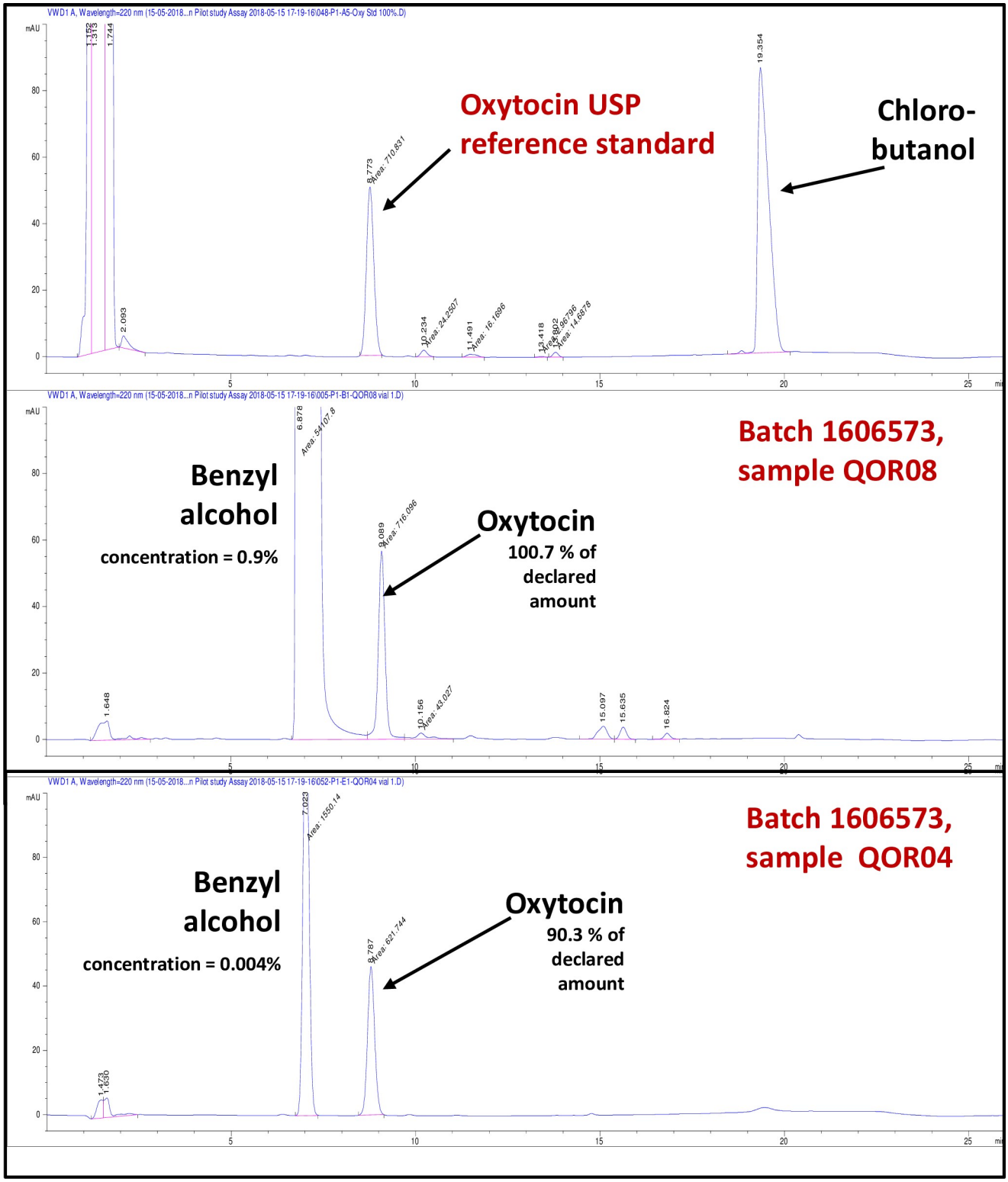

Fig 4. HPLC detection of the undeclared preservative benzyl alcohol, present in different concentrations in oxytocin samples of the same batch, stated to be manufactured by Jiangxi Xierkangtai Pharmaceuticals Co. Ltd (China).

https://doi.org/10.1371/journal.pone.0245054.g004

had been collected in a government district hospital (listed as facility no. 2 in S1 Table). That vial showed a concentration of 0.018% benzyl alcohol and an oxytocin content of 88.3% of the declared amount. The other two investigated vials of the same sample QOR075 carried the same batch number and had the same appearance, but showed a concentration of 0.9% benzyl alcohol and oxytocin contents of 99.7 and 100.2% of the declared amount, respectively.

Overview of collected misoprostol samples

Twenty-five samples of misoprostol tablets were collected. All of them had a stated content of 200 μg per tablet. These samples represented six brands and a total of ten batches (Table 3). As mentioned above for oxytocin, also three further wholesalers as well as the faith-based procurement agency BUFMAR were contacted, but had no additional brands or batches of misoprostol tablets in stock. The preparations listed in Table 3 therefore appear to represent most (or all) of the batches of misoprostol tablets which were in circulation in Rwanda at the time of sample collection.

The most frequently encountered preparation, representing ten of the 25 collected samples, was the originator product Cytotec®. For two of the three collected batches, the marketing authorization holder was Pfizer Holding (France), and for the third batch it was Continental

Table 3. List of misoprostol brands and batches collected in this study.

Brand name and stated manufacturer	WHO Pre-qualified/ SRA	Stated storage requirements	Batch N°	Manufacture/ Expiry Date	Number of samples
	SRA	No special storage requirements [c]	B15445 [a]	Dec 16 [d] / Nov 19	2
Cytotec® 200 μg			B17173 [a]	Jul 17 [d] / Jun 20	6
Piramal Healthcare UK Limited		Store at room temperature (15–25˚C)	B18097 [b]	Nov 17 [d] / Oct 20	2
United Kingdom					
Ace Miso®	WHO-PQ	Do not store above 30˚C, protect from light	ACE160963	Sep 16 / Aug 18	1
Acme Formulation Pvt. Ltd.					
India					
MIZO®	-	Store at a temperature not exceeding 30˚C at a dry place	E6SGFT010	Jun 16 / May 18	1
SYNOKEM Pharmaceuticals LTD India			E6SGLT004	Dec 16 / Nov 18	1
Misoprostol 200 mcg Tablets	WHO-PQ	Store at a temperature not exceeding 30˚C	45180301	Feb 18 / Feb 20	2
China Resources,					
ZIZHU Pharmaceuticals Co Ltd					
China					
C-stol®	-	Store below 30˚C. Protect from light and moisture	ERW-005	Mar 18 / Feb 21	3
CORONA Remedies Pvt Ltd					
India					
Cynomax®	-	Store at 20 to 25˚C in a dry area	M8TAB1801	May 18/ Apr 20	4
MAXTAR BIO-GENICS					
India			MTYX-1604	Aug 16 / Jul 18	3

WHO-PQ = WHO-prequalified medicine; SRA = manufactured in a country with stringent regulatory authority.

[a] Marketing authorization holder: Pfizer Holding, France.

[b] Marketing authorization holder: Continental Pharma Inc., Belgium.

[c] Package insert: "Tenir hors de la vue et de la portée des enfants. Pas de précaution particulière de conservation". I.e.: "Keep out of sight and reach of children. No special storage requirements."

[d] Manufacturing date not stated on packaging. Shelf-life listed according to information from the websites www.hpra.ie and www.medicines.org.uk/emc.

https://doi.org/10.1371/journal.pone.0245054.t003

Pharma Inc., Belgium (see Table 3). For all three batches, the stated manufacturer of the collected samples was Piramal Healthcare, based in the United Kingdom and therefore in a country with a stringent regulatory authority (SRA). Three further samples were WHO-prequalified medicines [38, 39] and had been produced by Acme Formulation Pvt Ltd, India, or by China Resources Zizhu Pharmaceutical Co, Ltd, China, respectively. The remaining 12 samples represented three generic preparations which were manufactured in India (Table 3), a country without an SRA, and were not WHO-prequalified.

Storage requirements were stated on the packaging and/or in the packaging insert of most misoprostol samples, but the indicated temperatures varied (Table 3): two samples were labeled for storage at 15–25˚C, seven samples at 20–25˚C, and eight samples at ≤ 30˚C. For the remaining eight samples, representing two of the three batches of the originator product Cytotec®, the package insert surprisingly stated: "No special storage requirements."

Misoprostol is very unstable and must be formulated as a 1% dispersion in hydroxypropyl methylcellulose (HPMC; hypromellose) to protect it from degradation [29]. For both the originator product and the WHO-prequalified product by Acme (India), exactly this formulation was stated in the package insert. Zizhu Pharmaceuticals, Co (China), listed hypromellose as an excipient for its product. The Indian company Synokem Pharmaceuticals Ltd (Mizo®) mentioned that misoprostol was contained as a "1% dispersion", but failed to mention what it was dispersed in. And notably, for the products by the Indian companies Corona Remedies Pvt., Ltd and Maxtar Bio-Genics, no information on excipients was given, and it remained unclear whether or not HPMC was contained therein.

The shelf life of four of the brands was given as two years, while for C-stol® (Corona Remedies, India) the stated shelf life was three years. For the originator medicine Cytotec® (Piramal Health Care, UK), only an expiry date but no manufacturing date was given on the packaging, but internet databases stated a shelf life of three years for this brand (see Table 3).

Misoprostol blister materials and storage conditions

While oxytocin degradation is primarily caused by elevated storage temperatures, misoprostol degradation is especially caused by exposure to moisture [27, 28]. Therefore, misoprostol tablets must be packaged in double-sided aluminium blisters, not in conventional plastic-aluminium blisters. Indeed, all collected samples were correctly packaged in double-sided aluminium blisters.

In the present study, storage temperatures were systematically recorded for oxytocin but not for misoprostol. However, the storage temperatures recorded at the 29 non-refrigerated oxytocin storage sites, as well as in four of the investigated retail pharmacies (S1 Table) may present a good approximation for the misoprostol storage temperatures in health facilities and drug outlets of Rwanda. As mentioned above, the recorded mean kinetic temperatures (MKTs) were moderate, and only in five out of 33 sites they slightly exceeded 25˚C (with recorded MKTs between 25.1 and 26.3˚C). From one of these sites (MKT 25.4˚C), a misoprostol sample with a stated storage requirement of 20–25˚C was collected (S1 Table), i.e. the MKT exceeded the demanded storage temperature by 0.4˚C. Therefore, in the present study no serious violations of the recommendations for packaging and for storage temperatures of misoprostol tablets were observed.

Chemical analysis of misoprostol samples

The presence of misoprostol was confirmed in all samples, but assay and dissolution testing revealed dramatic shortcomings in two of the six investigated brands. As shown in Fig 5, the assay results were within Ph. Int. specification (90–110% of the declared amount) for all samples of the originator product, of the two WHO-prequalified brands and of the product by

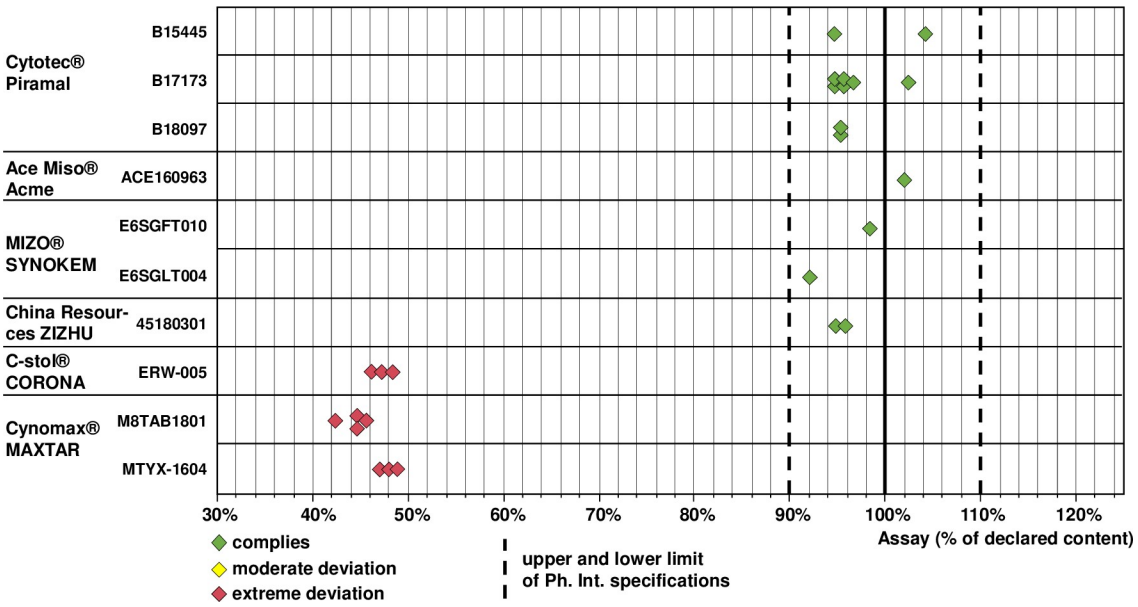

Fig 5. Content of misoprostol determined in each of the investigated samples.

https://doi.org/10.1371/journal.pone.0245054.g005

Synokem Pharmaceuticals, India. In sharp contrast, all three samples of C-stol® and all seven samples of Cynomax® showed less than 50% of the declared content, i.e. failed assay testing with extreme deviations. Therefore, an additional HPLC analysis was carried out for these samples, using the method of Ph. Int. for detection of related substances. This clearly showed large amounts of the typical degradation products of misoprostol (S3 Fig), suggesting that the low content of misoprostol in these two preparations was due to degradation of the API. The two investigated batches of Cynomax® had different ages at the time of analysis (7 and 22 months since the date of manufacture; S3 Table) and showed different API contents (mean 44.4% and 47.8% of the declared content). However, contrary to expectations the higher content was recorded for the older sample, indicating that the difference in content may not have been due to different age but due to differences in manufacture; different transport and storage conditions represent another possible reason.

All misoprostol samples were also tested for dissolution of the API. The International Pharmacopoeia demands that from misoprostol tablets at least 80% of the declared API amount must be released in 30 min under the defined conditions. As shown in Fig 6, all samples which had passed assay testing also passed dissolution testing. However, the samples of C-stol® and Cynomax®, which had been shown already in assay testing to contain less than 50% of the declared API amount, obviously failed dissolution testing with extreme deviations from USP specifications. From the C-stol® samples, approximately one quarter of the contained amount of the API did not dissolve, proving shortcomings in dissolution in addition to the extreme non-compliance in the assay. The results of the chemical analysis of each misoprostol sample, and the age of the samples at the time of analysis, are shown in S3 Table.

The two extremely non-compliant brands had been collected both from government and from faith-based health facilities, and from one retail pharmacy.

Product recall in Rwanda

The Rwanda Food and Drug Authority (RFDA) was alerted by the authors of this study about the two extremely substandard brands of misoprostol tablets by e-mail on December 2, 2018.

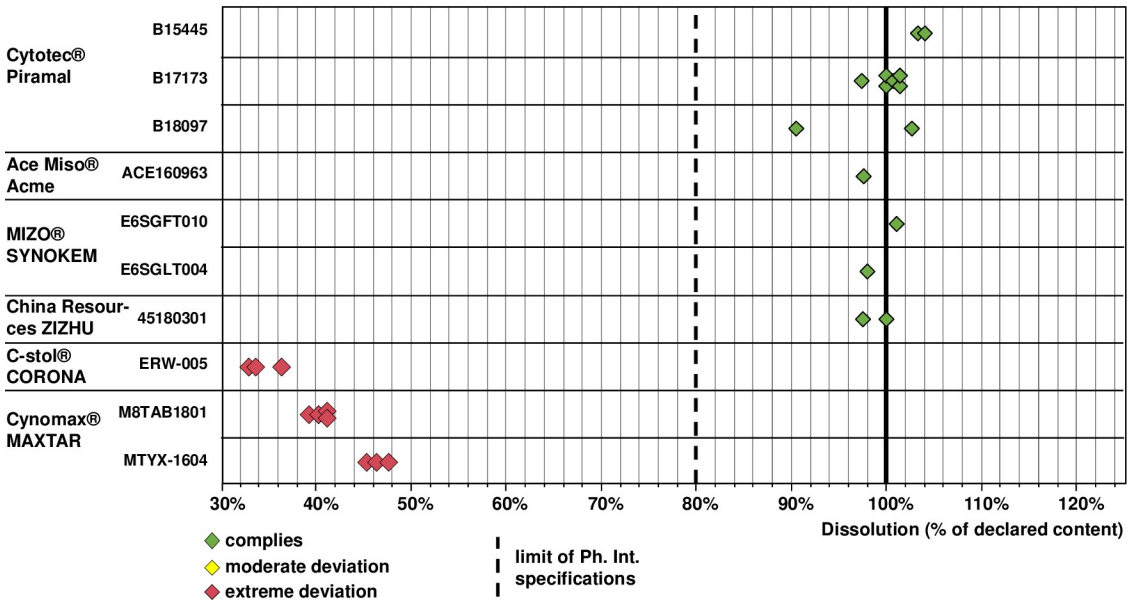

Fig 6. Dissolution of misoprostol determined in each of the investigated samples.

https://doi.org/10.1371/journal.pone.0245054.g006

RFDA invited the authors to a meeting at RFDA which took place on December 4, 2018. On December 7, 2018 RFDA issued an alert (Ref 079/Rwanda FDA/2018) requesting all suppliers and retailers to stop the distribution of these products and to put them in quarantine; all public and private hospitals, health centers, clinics and retail pharmacies were instructed to stop dispensing these products until investigations on the quality issues by Rwanda FDA were completed. Subsequently, RFDA issued a formal recall (Ref 0108/Rwanda FDA/2019 of 13 February 2019), stating that RFDA's investigations had revealed the indicted batches of misoprostol tablets to be substandard, and instructing all wholesalers, retailers, district pharmacies, public and private health facilities that the indicted batches should be returned to the supplier for suitable disposal.

Discussion

The present study showed excellent (100%) availability of oxytocin injections in the investigated government and faith-based health facilities. Also misoprostol tablets were available as oxytocics in all investigated hospitals, as foreseen by the Rwanda National List of Essential Medicines [9]. This proves remarkable success of the health authorities of Rwanda in assuring the availability of these life-saving commodities in hospitals and health centers.

Oxytocin storage inside or outside the refrigerator was found to exactly follow the manufacturers' instructions. For samples labeled for storage at 2–8˚C, the correct storage temperature was maintained well in most of the sites, with only few and probably inconsequential deviations. Again, this proves success of the health authorities of Rwanda, and stands in positive contrast to reports on oxytocin storage conditions in many other LMICs [19, 24, 25, 42–45]. A direct consequence of this success may be the fact that not a single sample of oxytocin was found which had an insufficient content of the API, again in contrast to reports from many other countries [22, 24, 25]. Of course, it cannot be excluded that knowledge of being monitored in this study may have influenced the behavior of health facility staff.

The number of different brands of oxytocin and misoprostol circulating in Rwanda at the time of sample collection was remarkably small. This may be related to the small market size of

Rwanda: many manufacturers and international distributors may not see sufficient economic incentive to engage in medicine sales in this country. Possibly, this can limit the ability of public and private stakeholders to select good-quality, affordable medicines in their procurement.

Out of ten brands collected in total (4 of oxytocin injections, 6 of misoprostol tablets), three were prequalified by WHO [38, 39], and three more were manufactured in countries with stringent regulatory authorities (SRAs) [37]. Together, these six brands represented 45% of the samples collected in this study, and notably all these samples passed the quality tests in this study with good results, indicating that prequalification by WHO, as well as production in a country with an SRA, is a reliable predictor of good quality of medicines.

In stark contrast, three out of the four brands which were neither WHO-prequalified nor produced in a country with an SRA showed serious quality deficiencies. Most alarmingly, every investigated sample of the misoprostol products Cynomax® (stated manufacturer Maxtar Bio-Genics, India) and C-stol® (stated manufacturer Corona Remedies, India) failed assay and dissolution testing with extreme deviations from the pharmacopeial specifications, likely to result in clinical inefficacy. Large amounts of misoprostol degradation products were detected in these two brands, indicating that the API had degraded to a large extent. Notably, the package inserts of both preparations did not mention whether or not HPMC (hypromellose) had been used in the formulation of these tablets, which is essential for misoprostol stability [29]. Also in Malawi, extremely substandard misoprostol brands, however from different stated manufacturers, have been found [24].

The quality deficiencies observed for the oxytocin injection stating Jiangxi Xierkangtai (China) as manufacturer were not as extreme as those observed for the above-mentioned misoprostol brands, but still alarming. All six samples of one of the two investigated batches of that oxytocin brand exceeded the content limit specified by USP, two of the samples even contained more that 120% of the stated amount. In pharmaceuticals with unstable APIs, a content slightly higher than 100% of the stated amount is often intentionally included to ensure that the content is still above the lower pharmacopeial limit towards the end of the shelf life. However, the pharmacopeias define an upper limit to avoid overdosing of the therapy, and this limit was clearly exceeded in the batch in question.

The oxytocin injections stating Jiangxi Xierkangtai as manufacturer were found to contain benzyl alcohol in a concentration of 0.9%. This compound in this concentration is perfectly acceptable as a preservative in parenteral preparations [39, 46]. However, in most countries regulations demand that the presence of such excipients must be declared in the package insert [47], but for the product in question no excipients were declared at all.

The most worrying observation regarding this product, however, was the detection of vials with a two-hundred-fold lower benzyl alcohol concentration, carrying the same batch number as the vials with 0.9% of that preservative. While this may not cause direct harm to a patient treated with that product, it indicates gross violations of good manufacturing practice, raising strong doubts also about other quality aspects of this product. This kind of problem has not been reported in previous studies of oxytocin quality. However, this problem may easily escape detection in a medicine quality study as it has been visible only in a small number of the investigated vials.

The oxytocin injections by Jiangxi Xierkangtai were labeled for non-refrigerated storage, as also is the case for oxytocin from many other manufacturers [19, 24, 42–44]. Obviously, the use of oxytocin products which do not have to be stored in a refrigerator appears to be an attractive option, especially in LMICs. However, recent studies have shown that oxytocin products labeled for non-refrigerated storage may not have any better stability than products labeled for refrigerated storage, and on the contrary may even be less stable [40, 44]. Therefore,

international stakeholders including WHO have issued the recommendation "Buy quality oxytocin, keep it cool" and recommended that all oxytocin products should be stored at 2–8˚C [16, 48], irrespective of the manufacturer's storage recommendation. Health authorities in Rwanda and elsewhere may consider this recommendation. A heat-stable formulation of the oxytocin analogue carbetocin has recently been added to the WHO Essential Medicines List, and in future it may become a further treatment option for post-partum hemorrhage for use in facilities where storage at 2–8˚C is problematic.

Several previous studies have reported problems of oxytocin quality in LMICs, but the present study shows for Rwanda a decidedly different situation than reported from other countries. The present data suggest that the Rwandan authorities have successfully assured the availability and (in most cases) the appropriate storage of oxytocin according to manufacturers' instructions. The detected problems of oxytocin and especially misoprostol quality must be addressed not so much by improved storage and transportation conditions of the medicines, but by improvements of the supplier qualification in medicine procurement. It may be considered whether for oxytocin and misoprostol, with their well-known problems of quality and stability, procurement should be restricted to WHO-prequalified medicines and to medicines manufactured in countries with stringent regulatory authorities. And as a simple "rule of thumb", the results of this study suggest that medicines for which the excipients are not stated in the package insert should be regarded as doubtful in quality.

So far, misoprostol quality has received much less attention in scientific studies than oxytocin quality [14]. However, the results of this study, of the study of Hall [28] and of a recent study from Malawi [24], show quality problems of different brands of misoprostol which are even much more serious than those reported for oxytocin, with API contents below (or far below) 50% of the stated amount, and this problem needs attention in future studies.

Rwanda has only recently established its national drug regulatory agency, i.e. the Rwanda Food and Drug Authority (RFDA), and this will certainly contribute to increased patient safety. The extremely quick and efficient action which RFDA took on the reported substandard misoprostol brands holds good promise for the future development of medicine quality in this country.

Supporting information

S1 Fig. Sample site & drug purchase record.
(PDF)

S2 Fig. Photos of two samples of oxytocin injections, carrying the same batch number but containing different concentrations of benzyl alcohol.
(PDF)

S3 Fig. HPLC analysis for related substances in two misoprostol samples.
(PDF)

S1 Table. List of included health facilities, number oxytocin vial and misoprostol tablets collected, and recorded oxytocin storage conditions.
(PDF)

S2 Table. Results of chemical analysis of all oxytocin samples.
(PDF)

S3 Table. Results of chemical analysis of all misoprostol samples.
(PDF)

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SAMPLE SITE & DRUG PURCHASE RECORD

Name of survey site: _____Date and time of visit: _____
Description of location: _____
Temperature data logger Location: _____ Serial number: _____
Photo taken (5): YES ☐ NO ☐

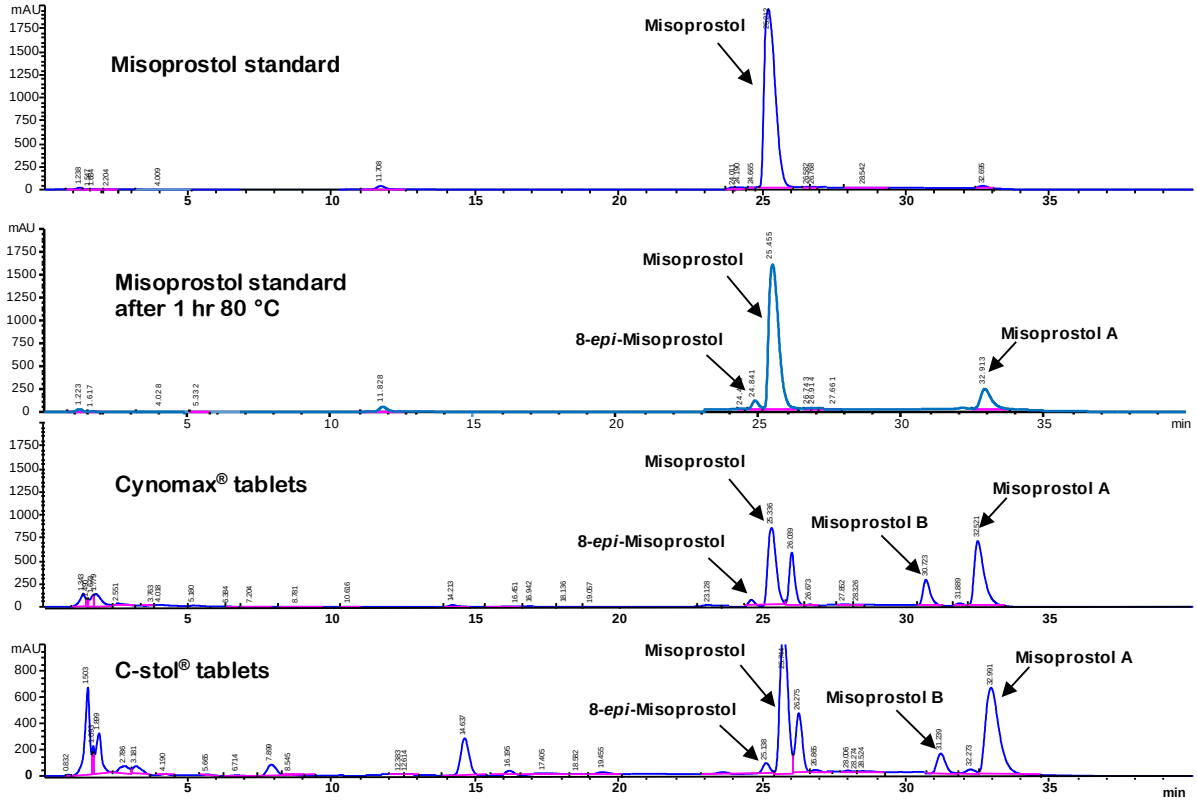
Drug samples collected	Desired quantity	Obtained quantity	QOR reference number	Reason for not obtaining the desired quantity	Brand name	Batch no	Expiry date	Manufacturer, country	Price in Rwf per tabs/vial (if applicable)	Storage conditions according to the manufacturer		Refrigerated (r) / not refrigerated (nr) in original package (op)/out of original package (nop) protected from light (pr) /not protected from light (npr)	1) Thermometer kept with oxytocics? 2) Temperature recorded daily? If yes, please take picture
										Temperature in °C	Relative humidity in %		
Misoprostol 0,2mg tab.	50											(r) ☐ (nr) ☐ (op) ☐ (nop) ☐ (pr) ☐ (npr) ☐	1) yes ☐ no ☐ 2) yes ☐ no ☐
Oxytocin 10 IU	10											(r) ☐ (nr) ☐ (op) ☐ (nop) ☐ (pr) ☐ (npr) ☐	1) yes ☐ no ☐ 2) yes ☐ no ☐

- **If samples are taken without original package, please take picture of original package (showing the name, batch number, expiry date, manufacturing date, name / address of manufacturer)!**
- Collected samples were replaced: YES ☐ NO ☐ Collected samples were paid for: YES ☐ (Attach receipt!) NO ☐
- Name of sampling person: _____Signature: _____
- Name of person responsible for health facility: _____Signature: _____

S1 Fig. Sample site & drug purchase record.

S2 Fig: Photos of two samples of oxytocin injections, carrying the same batch number but containing different concentrations of benzyl alcohol.

Top: Sample No. QOR04, containing 0.004% benzyl alcohol.
Bottom: Sample No. QOR05, containing 0.9% benzyl alcohol.



S3 Fig: HPLC analysis for related substances in two misoprostol samples.

Cynomax® tablets (batch M8TAB1801) and C-stol® tablets (batch ERW-005) were investigated. See Methods section for experimental details, and Results section for details of the investigated brands and batches.

S1 Table. List of included health facilities, number oxytocin vial and misoprostol tablets collected, and recorded oxytocin storage conditions

Facility No	Type of facility	District	Sampling site (maternity ward or storage room)	Number of collected misoprostol tablets	Misoprostol storage conditions stated on label	Number of collected oxytocin vials	Oxytocin storage conditions stated on label	Oxytocin storage site	Oxytocin storage shelf: mean kinetic temperature measured over 6 months (°C)	Refrigerator: mean kinetic temperature measured over 6 months (°C)
1	Government district hospital	Kigali city	maternity ward	50	20-25°C	10	2-8°C	refrigerator	-	**9.1**
			storage room	50; 50[e]	<30°C	10	room temperature [f]	shelf	25.3	-
2			storage room	50	<30°C	10	room temperature	shelf	24.2	-
3	Government referral hospital	Musanze	maternity ward	0	-	10	2-8°C	refrigerator	-	**8.5**
			storage room	60	<30°C	10	2-8°C	refrigerator	-	6.5
4	Faith-based district hospital	Bugesera	maternity ward	0	-	6	room temperature	shelf	26.3	-
			storage room	20	20-25°C	10	room temperature	shelf	23.4	-
5		Kamonyi	maternity ward	0	-	10	2-8°C	refrigerator	-	5.4
			storage room	51	<30°C	10	2-8°C	refrigerator	-	6.1
6		Karongi	maternity ward	0	-	10	2-8°C	cool box [a]	-	**15.8[a]**
			storage room	50	15°-25°C	10	2-8°C	refrigerator	-	not det.
7			maternity ward	10	15°-25°C	10	2-8°C	refrigerator	-	6.7
			storage room	50	<30°C	10	2-8°C	refrigerator	-	7.1
8		Muhanga	maternity ward	51	<30°C	10	room temperature	shelf	22.2	-
			storage room	50	20-25°C	10	room temperature	shelf	21.5	-
9	Government health center	Bugesera	maternity ward	0	-	5	room temperature	shelf	23.7	-
			storage room	13	20-25°C	10	room temperature	shelf	24.8	-
10			maternity ward	0	-	10	room temperature	shelf	26.0	-
			storage room	51	<30°C	10	room temperature	shelf	25.4	-
11		Kamonyi	maternity ward	0	-	8	room temperature	shelf	23.5	-
			storage room	0	-	10	room temperature	shelf	24.4	-
12			maternity ward	0	-	10	room temperature	shelf	not det.	-
			storage room	0	-	10	room temperature	shelf	25.0	-
13		Karongi	storage room	0	-	10	2-8°C	refrigerator	-	5.8
14			storage room	0	-	10	2-8°C	refrigerator	-	4.5
15		Kigali city	storage room	0	-	10	room temperature	shelf	23.7	-
16			maternity ward	0	-	10	room temperature	shelf	24.2	-
17		Muhanga	maternity ward	0	-	9	room temperature	shelf	22.3	-
			storage room	0	-	10	room temperature	shelf	21.4	-
18			storage room	0	-	10	room temperature	shelf	21.4	-
19		Musanze	storage room	0	-	10	2-8°C	refrigerator	-	7.3
20			storage room	0	-	10	2-8°C	refrigerator	-	5.7
21	Faith-based health center	Bugesera	maternity ward	0	-	10	room temperature	shelf	23.8	-
			storage room	0	-	10	room temperature	shelf	not det.	-
22			maternity ward	0	-	10	room temperature	shelf	25.1	-
			storage room	30	20-25°C	10	room temperature	shelf	**25.4 [g]**	-
23		Kamonyi	storage room	0	-	10	room temperature	shelf	22.7	-
24			maternity ward	0	-	10	room temperature	shelf	22.2	-
			storage room	0	-	10	room temperature	shelf	22.1	-
25		Karongi	storage room	0	-	10	2-8°C	refrigerator	-	4.3
26			maternity ward	0	-	10	2-8°C	refrigerator	-	**-1.3**
27		Kigali city	storage room	0	-	6	2-8°C	refrigerator	-	6.5
28			storage room	0	-	10	2-8°C	refrigerator	-	**10.5**
29		Muhanga	maternity ward	0	-	9	room temperature	shelf	21.0	-
			storage room	0	-	10	room temperature	shelf	21.9	-
30			maternity ward	0	-	10	room temperature	shelf	22.7	-
			storage room	0	-	10	room temperature	shelf	22.4	-
31		Musanze	maternity ward	0	-	10	2-8°C	refrigerator	-	not det.
			storage room	0	-	10	2-8°C	refrigerator	-	5.1
32			storage room	0	-	10	2-8°C	refrigerator	-	4.7
33	Private clinic	Muhanga	storage room	0	-	10	room temperature	shelf	not det.	-
34	Retail pharmacy	Bugesera	storage room	25	no requirements	0	-	n.a.	24.1 [c]	-
35			storage room	29	no requirements	0	-	n.a.	24.0 [c]	-
36		Kigali city	storage room	50	no requirements	0	-	n.a.	24.2 [c]	-
37			storage room	28	no requirements	0	-	n.a.	23.9 [c]	-
38		Muhanga	storage room	50	no requirements	0	-	n.a.	-	-
39			storage room	48	20-25°C	0	-	n.a.	-	-
40		Musanze	storage room	50	no requirements	0	-	n.a.	-	-
41	Government central medical store	Kigali city	storage room	0	-	100;200[d,e]	2-8°C	refrigerator	-	not det.
42	Government district pharmacy	Burera	storage room	250[d]	<30°C	0	-	n.a.	-	-
43		Muhanga	storage room	200[d]	20-25°C	160[d]	room temperature	shelf	19.8	-
44	Private wholesaler	Kigali city	storage room	0	-	100[d]	room temperature	not det. [b]	-	-
45			storage room	0	-	100[d]	2-8°C	not det. [b]	-	-
46			storage room	252[d]	no requirements	100[d]	2-8°C	not det. [b]	-	-

Storage conditions which deviate from manufacturers' requirements are given in **bold, underlined** letters.
n.a. = not applicable
not det. = not determined (temperature logger not placed, lost after placement, or no data recorded).

[a] Oxytocin was stored in this maternity ward in a cool box, reportedly only ordered for immediate use.

[b] From private wholesalers, samples were purchased through a retail pharmacy using a mystery shopper approach. Therefore, storage conditions were not determined, and temperatures data loggers were not placed.

[c] In a few pharmacies, temperature loggers were placed although they did not stock oxytocin.

[d] Higher number of tablets/vials purchased as replacement samples and for additional stability testing.

[e] Two brands collected in one sampling site.

[f] Storage requirement stated on packaging: "Store in a cool dry place, away from light". Storage requirement stated on the package insert: "Store in a dark place at room temperature, protect from light."

[g] Exceeds storage temperature of misoprostol recommended by the manufacturer of the respective sample.

S2 Table. Results of chemical analysis of all oxytocin samples

Brand name and stated manu-facturer	Stated storage tempera-ture require-ment	Batch N°	Manu-facture/ Expiry Date	Faci-lity No.	Facility type	Site in facility	Mean assay (% of declared content)	RSD assay	Mean pH value	RSD pH value	Age of samples (months) [a]
Oxytocin injection; Jiangxi Xier-kangtai Pharma-ceutical Co. Ltd; China	Room tempe-rature	1606573	Jun 16/ Jun 19	2	gov. hospital	stor.	96.1% [b]	6.62% [c]	4.14	0.98%	30
				4	faith-b. hospital	mat.	102.7%	1.56%	4.03	0.48%	30
						stor.	105.5%	2.22%	4.00	0.68%	30
				8	faith-b. hospital	mat.	99.2%	0.68%	4.48	2.17	24
						stor.	99.9%	1.31%	4.40	0.96	24
				9	gov. HC	mat.	101.3%	0.55%	4.05	0.89%	30
						stor.	101.1%	1.66%	4.05	0.76%	30
				11	gov. HC	mat.	100.5%	1.73%	4.05	0.34%	30
						stor.	100.7%	1.73%	4.05	0.33%	30
				12	gov. HC	mat.	100.4%	2.29%	4.08	0.62%	30
						stor.	99.8%	1.29%	4.07	0.73%	30
				17	gov. HC	mat.	95.8%	1.71%	4.56	3.27	24
						stor.	97.9%	0.52%	4.50	1.33	24
				18	gov. HC	stor.	99.0%	0.88%	4.44	0.97	24
				23	faith-b. HC	stor.	99.1%	1.05%	4.12	0.18%	30
				24	faith-b. HC	mat.	100.4%	1.99%	4.06	0.48%	30
						stor.	95.6%	4.94%	4.19	4.38%	30
				29	faith-b. HC	mat.	98.2%	0.75%	4.46	0.73	24
						stor.	98.0%	0.79%	4.48	0.84	24
				30	faith-b. HC	mat.	99.5%	1.32%	4.38	1.18	24
						stor.	99.7%	1.18%	4.39	0.77	24
				33	private clinic	stor.	100.7%	0.96%	4.41	0.94	24
				43	distr. pharm.	stor.	101.5%	0.45%	4.06	0.32%	23
				44	**wholesaler**	**stor.**	**90.3% [b]**	**0.72%**	**4.08**	**0.84%**	**23**
		1604521	Apr 16/ Apr 19	1	gov. hospital	stor.	119.3%	2.09%	3.85	0.13%	32
				10	gov. HC	mat.	118.0%	1.98%	3.83	0.48%	32
						stor.	117.2%	0.52%	3.82	0.51%	32
				15	gov. HC	stor.	119.9%	3.25%	3.84	0.30%	32
				16	gov. HC	mat.	120.6%	2.24%	3.82	0.11%	32
				21	faith-b. HC	stor.	117.6%	1.02%	3.84	0.44%	32
						stor.	121.5%	1.61%	3.82	0.48%	32
				22	faith-b. HC	mat.	117.3%	1.38%	3.80	0.26%	32
						stor.	117.8%	2.31%	3.78	0.47%	32
Steroxine 10 IU/1 ml [d]; Labora-toires Sterop; Belgium	2-8°C	160042	Feb 16/ Jan 19	46	wholesaler	stor.	107.8%	0.62%	3.79	0.22%	27
		160269	Sep 16/ Aug 19	45	wholesaler	stor.	99.6%	1.11%	3.98	0.57%	20
Oxytocin 10 IU/ml; AS GRINDEKS, Latvia [e]	2-8°C	37611016	Oct 16/ Oct 20	1	gov. hospital	mat.	102.8%	1.63%	4.11	0.13%	26
				5	faith-b. hospital	mat.	102.1%	1.99%	4.09	0.15%	26
						stor.	101.9%	3.29%	4.10	0.22%	26
				20	gov. HC	stor.	99.9%	0.74%	4.11	0.10%	26
		37711116	Nov 16/ Nov 20	41	CMS	stor.	100.9%	0.82%	4.09	0.64%	18
Oxytocin 10; Rotex-medica GmbH Arznei-mittelwerk; Germany	2-8°C	70779A	Sep 17/ Sep 20	3	gov. hospital	mat.	104.1%	2.96%	4.06	0.39%	15
						stor.	102.0%	0.26%	4.02	0.20%	15
				6	faith-b. hospital	mat.	103.7%	2.09%	4.09	0.40%	15
						stor.	103.1%	1.53%	4.06	0.35%	15
				7	faith-b. hospital	mat.	103.9%	1.92%	4.02	0.10%	15
						stor.	103.8%	1.84%	4.03	0.10%	15
				13	gov. HC	stor.	103.8%	1.67%	4.02	0.19%	15
				14	gov. HC	stor.	104.2%	1.93%	4.05	0.30%	15
				19	gov. HC	stor.	102.6%	0.44%	4.00	0.10%	15
				25	faith-b. HC	stor.	103.6%	1.76%	4.02	0.24%	15
				26	faith-b. HC	mat.	103.9%	1.67%	4.01	0.19%	15
				27	faith-b. HC	stor.	105.9%	3.96%	3.97	0.19%	15
				28	faith-b. HC	stor.	105.2%	2.37%	3.99	0.21%	15
				31	faith-b. HC	mat.	103.4%	0.86%	4.00	0.14%	15
						stor.	105.4%	2.44%	3.99	0.19%	15
				32	faith-b. HC	stor.	104.6%	2.51%	4.01	0.20%	15
				41	CMS	stor.	102.4%	0.89%	3.99	0.47%	8

RSD = relative standard deviation; gov. = government; faith-b. = faith-based; HC = health center; CMS = government central medical store; stor. = storage room; mat. = maternity ward. The sample found to contain only 0.004 % benzyl alcohol is shown in **bold print**.

[a] Age of sample at time of analysis.
[b] Deviating concentration of the preservative benzyl alcohol observed, see main text.
[c] See text for explanation of the high standard deviation observed for this specific sample.
[d] Batch 160269 was labeled with the unbranded generic name “Oxytocin 10 IU/1 ml”, all other information was identical as in batch 160042.
[e] Marketing authorization holder: Peckforton Pharmaceuticals Ltd., United Kingdom

S3 Table. Results of chemical analysis of all misoprostol samples

Brand name and stated manufacturer	Stated storage requirements	Batch N°	Manu-facture/ Expiry Date	Faci-lity no.	Facility type	Site in faci-lity	Mean assay (% of declared content)	RSD assay	Mean dissolution (% of declared content)	RSD disso-lution	Age of samples (months) at time of analysis
Cytotec® 200 µg, Piramal Healthcare UK Limited; United Kingdom [a]	No special storage requirements [b]	B15445 [a]	Dec 16[c] / Nov 19	38	retail pharm.	stor.	104.2%	0.13%	104.2%	0.61%	18
				40	retail pharm.	stor.	94.7%	0.27%	103.5%	2.79%	24
		B17173 [a]	Jul 17[c] / Jun 20	1	gov. hospital	stor.	95.9%	0.19%	100.1%	1.45%	17
				34	retail pharm.	stor.	94.8%	0.29%	101.2%	2.19%	17
				35	retail pharm.	stor.	94.6%	0.35%	100.1%	2.81%	17
				36	retail pharm.	stor.	94.8%	0.58%	100.8%	1.59%	17
				37	retail pharm.	stor.	95.6%	0.48%	101.5%	3.05%	17
				46	wholesaler	stor.	102.4%	1.13%	97.5%	2.82%	10
	Store at room temperature (15-25 °C)	B18097 [b]	Nov 17[c] / Oct 20	6	faith-b. hospital	stor.	95.1%	0.56%	90.6%	4.49%	13
				7	faith-b. hospital	mat.	95.5%	3.80%	102.8%	2.69%	13
Ace Miso®, Acme Formulation Pvt. Ltd.; India	Do not store above 30°C, protect from light	ACE160963	Sep 16/ Aug 18	42	gov. district pharm.	stor.	102.0%	0.44%	97.7%	2.59%	20
MIZO®, SYNOKEM Pharmaceuticals LTD; India	Store at a temperature not exceeding 30°C at a dry place	E6SGFT010	Jun 16/ May 18	8	faith-b. hospital	mat.	98.4%	0.02%	101.2%	3.51%	24
		E6SGLT004	Dec 16/ Nov 18	10	gov. HC	stor.	92.1%	1.62%	98.1%	3.18%	24
China resources, ZIZHU Pharmaceuticals Co Ltd; China	Store at a tempera-ture not exceeding 30 °C	45180301	Feb 18/ Feb 20	1	gov. hospital	stor.	95.8%	0.20%	97.7%	2.31%	10
				3	gov. hospital	stor.	94.8%	0.16%	100.1%	2.64%	10
C-stol®, CORONA Remedies Pvt Ltd; India	Store below 30°C. Protect from light and moisture	ERW-005	Mar 18/ Feb 21	2	gov. hospital	stor.	46.8%	1.33%	32.9%	15.61%	9
				5	faith-b. hospital	stor.	46.2%	0.14%	33.6%	6.82%	9
				7	faith-b. hospital	stor.	48.5%	2.96%	36.4%	4.53%	9
Cynomax®, MAXTAR BIO-GENICS; India	Store at 20° to 25°C in a dry area	M8TAB1801	May 18/ Apr 20	1	gov. hospital	mat.	42.5%	0.51%	39.8%	1.35%	7
				4	faith-b. hospital	stor.	44.6%	0.95%	39.3%	4.15%	7
				9	gov. HC	stor.	45.6%	1.07%	41.1%	3.53%	7
				22	faith-b. HC	stor.	44.9%	0.27%	41.2%	2.37%	7
		MTYX-1604	Aug 16/ Jul 18	8	faith-b. hospital	stor.	48.6%	0.06%	46.4%	3.36%	22
				39	retail pharm.	stor.	47.1%	0.04%	45.3%	1.57%	22
				43	gov. district pharm.	stor.	47.6%	0.24%	47.6%	4.49%	21

RSD = relative standard deviation
gov. = government
faith-b. = faith-based
HC = health center
pharm. = pharmacy
stor. = storage room
mat. = maternity ward.

[a] Marketing authorization holder: Pfizer Holding, France.

[b] Marketing authorization holder: Continental Pharma Inc., Belgium.

[c] Package insert: "Tenir hors de la vue et de la portée des enfants. Pas de précaution particulière de conservation". I.e.: "Keep out of sight and reach of children. No special storage requirements."

[d] Manufacturing date not stated on packaging. Shelf-life listed according to information from the websites www.hpra.ie and www.medicines.org.uk/emc.

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Surveillance for substandard and falsified medicines by local faith-based organizations in 13 low- and middle-income countries using the GPHF Minilab

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This study evaluates the use of the Global Pharma Health Fund (GPHF) Minilab for medicine quality screening by 16 faith-based drug supply organizations located in 13 low- and middle-income countries. The study period included the year before the COVID-19 pandemic (2019) and the first year of the pandemic (2020). In total 1,919 medicine samples were screened using the GPHF Minilab, and samples showing serious quality deficiencies were subjected to compendial analysis in fully equipped laboratories. Thirty-four (1.8%) of the samples were found not to contain the declared active pharmaceutical ingredient (API), or less than 50% of the declared API, or undeclared APIs, and probably represented falsified products. Fifty-four (2.8%) of the samples were reported as substandard, although the true number of substandard medicines may have been higher due to the limited sensitivity of the GPHF Minilab. The number of probably falsified products increased during the COVID-19 pandemic, especially due to falsified preparations of chloroquine; chloroquine had been incorrectly advocated as treatment for COVID-19. The reports from this project resulted in four international WHO Medical Product Alerts and several national alerts. Within this project, the costs for GPHF Minilab analysis resulted as 25.85 € per sample. Medicine quality screening with the GPHF Minilab is a cost-effective way to contribute to the global surveillance for substandard and falsified medical products.

Substandard and falsified (SF) medicines pose a severe risk for patients worldwide, especially in low- and middle-income countries (LMICs) where 10.5% of all medicines have been estimated by the World Health Organization (WHO) to be substandard or falsified[1,2]. According to the current definitions of the WHO, falsified medicines are products which "deliberately/fraudulently misrepresent their identity, composition, or source"[1]. Substandard medicines are products which, without deliberate/fraudulent intent, fail to meet their quality standards[1], e.g. due to poor manufacturing practice, poor packaging, or inappropriate transportation and storage conditions. SF medicines frequently fail to cure the patients and may thereby cause prolonged illness or even death. They may also lead to severe adverse effects[1–5]. In addition, under-dosed anti-infectives contribute to the global emergence of antimicrobial resistance[1,2,6].

The SARS-CoV-2 pandemic has affected the supply of medicines especially in LMICs, both by an increased demand for medical products for the treatment and prevention of COVID-19 and by the disruption of supply chains worldwide[7–11]. This has created prospects for criminals to introduce illegal products into the supply chains. An imminent increase in the occurrence of SF medicines was predicted early in the course of the pandemic[12]. Indeed, reports on the seizure of huge amounts of SF medical products, including products related to COVID-19, have been published shortly thereafter[13].

Pharmaceutical analysis for the identification of SF medicines is usually carried out according to the methods of pharmacopoeias. These analytical methods, also called "compendial analysis", require well-equipped

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laboratories and highly educated personnel, and are costly and time-consuming[14]. In many low-resource settings, the timely performance of compendial analyses is challenging or even impossible. Therefore, the introduction of simple, low-cost screening technologies which allow the rapid detection of SF medicines, and their subsequent removal from supply chains, represent one suitable intervention in this context[15–20]. However, a recent publication comparing available screening technologies concluded that "the evaluation of medicine quality screening devices in laboratory and in real-life-settings is [still] in its infancy"[19], and stated that more research is required to explore the respective benefits, prerequisites and limitations of such instruments[20].

With almost 900 devices distributed to 98 countries, the Global Pharma Health Fund (GPHF) Minilab is the most frequently used screening technology for medicine quality in LMICs[18,21,22]. According to the GPHF Minilab manual[23], the analysis comprises three steps: a visual inspection of label, packaging and product; a simplified disintegration test; and a thin-layer chromatographic analysis for qualitative and semi-quantitative examination of the active pharmaceutical ingredients (APIs)[23]. While the GPHF Minilab was found to be highly sensitive and specific in the identification of products which do not contain the stated API, it is less sensitive in the detection of products which contain an insufficient amount of the API, or show insufficient dissolution of the API[24,25].

In 2015 a medicine quality study[26] was carried out by ten faith-based drug supply organizations located in seven African and Asian countries which were using the GPHF Minilab. Each organization collected medicine samples from private local medicine outlets, including the informal sector, within a six month period. A total of 869 samples were collected and tested. Samples failing the GPHF Minilab analysis were subsequently investigated by compendial analysis in international laboratories. This study resulted in the identification of 12 samples which did not contain the stated API[26]. The successful completion of that study encouraged the German Institute for Medical Mission (Difäm; Tübingen, Germany), and the Ecumenical Pharmaceutical Network (EPN; Nairobi, Kenya) to further expand the use of the GPHF Minilab for basic medicine quality screening by faith-based drug supply organizations in Africa and Asia. The resulting Difäm-EPN Minilab Network comprised 16 member organizations in the period between January 2019 and December 2020. All of them hold valid licenses in their countries, allowing them to procure and distribute medicines to faith-based healthcare facilities. They routinely use the GPHF Minilab for basic quality testing of selected medicines procured for distribution in their organization, and of a certain number of samples from external sources, such as informal vendors, for comparison.

The present report analyses the operation of this Network from January 2019 to December 2020. We purposefully included the year before the COVID-19 pandemic, and the first year of the pandemic, to allow some insight into the effect of the pandemic on the occurrence of SF medicines. In this period, the Network analyzed approximately 2000 medicine samples. We here report on the numbers and types of identified SF medicines, and provide information on costs and organizational requirements for this approach of a low-cost, basic medicine quality screening by local organizations in LMICs. The present study was not designed as a study of the prevalence of SF medicines with a prospective, systematic sampling design, but it reports the results of a routine use of the GPHF Minilab in medicine quality assurance by the involved organizations, and the requirements therefor.

Methods

Study design. Starting from 2010 and with support from the faith-based aid organization Bread for the World (Berlin, Germany), Difäm has provided drug supply organizations (DSOs) in Africa and India with GPHF Minilabs and trained them in their use. In 2019 and 2020, the resulting Difäm-EPN Minilab Network comprised 16 member organizations who routinely used the GPHF Minilab for medicine quality screening. These DSOs report all test results to Difäm in form of standardized Excel tables which are filled and submitted every three months. However, the DSOs are encouraged to report samples failing Minilab analysis to Difäm immediately. Difäm organizes confirmatory compendial testing (see below) for samples for which Minilab analysis had indicated serious quality deficiencies. Furthermore, Difäm provides the DSOs with standards, reagents and equipment required for the operation of the Minilabs, and organizes trainings and network meetings. Therefore, the results of the Minilab testing by the network partners, and of the confirmatory compendial analyses, as well as data on the requirements of funds and materials for the network operation are available at Difäm. For the present study, a research pharmacist (G.G.) retrospectively analyzed these data for the period January 2019 and December 2020, including the results of all samples which had been tested by the network members in this period.

Location of the involved organizations, and qualification of the personnel responsible for GPHF Minilab screening. The 16 DSOs are located in 13 LMICs in Africa and Asia (see "Results" section). All DSOs employed at least one pharmacist or pharmacy technician, and at least two staff members of each DSO had been trained in the use of the GPHF Minilab[26]. Fourteen out of the 16 DSOs were active during the entire investigated period, one organization left the Network in 2019, another one joined in 2020.

Sample collection. In the Difäm-EPN Minilab Network, each DSO signs a Memorandum of Understanding pledging to collect and analyze at least 75 medicine samples yearly; a smaller number than 75 is considered inefficient in view of the requirements for the regular provision of consumables, equipment and training. However, for one relatively small organization, a number of 50 samples was agreed.

The DSOs are requested to collect medicines for which Minilab protocols exist. The GPHF Minilab manual[23] contains protocols for the analysis of 100 APIs mainly in the forms of tablets, capsules and injectables, as well as for frequently used fixed combinations of these APIs. Minilab protocols for seven further APIs have been developed in 2021 and 2022, i.e. after the period under investigation[27].

Difäm and EPN provide guidance to the DSOs for medicine procurement in accordance to WHO recommendations[28], including supplier selection and prequalification. Respective trainings are offered to the DSOs on a regular basis. Depending on the resources of the respective DSO, and on the possibilities and

limitations for medicine procurement in the respective country, the degree of adherence to the WHO recommendation varies between DSOs.

The process of sample collection is left in the responsibility of the individual DSOs. The DSOs use the Minilab primarily to screen the quality of those medicines which they routinely procure and distribute to their member health facilities and patients. The sources of these medicines include faith-based, governmental, and private vendors, i.e. licensed medicine sellers. A very small number of samples were received by the DSOs as donations from foreign charitable organizations.

Some additional samples were collected from health facilities, obtained either during routine supervisory visits by DSO staff or sent by health facilities to the DSOs for testing purposes, e.g. in cases of doubt about the quality of the respective product. In addition, Difäm encouraged the DSOs to collect and analyze some samples from external sources such as informal vendors. Previous studies have shown that informal vendors are an especially important route of entry of SF medicines into the market[24,29], and analyzing medicine samples from informal vendors is a useful way to sensitize the DSOs towards the occurrence of such SF medicines. A refund of the purchase costs of such samples was offered by Difäm to the DSOs, but the number of samples collected from external sources was left to the DSOs to decide. The DSOs were free to use either an open or a mystery shopper approach when purchasing from informal vendors; the mystery shopper approach was most frequently chosen.

Prior to the Minilab testing, collected medicine samples were stored in the medicine storage facilities of the respective DSOs under appropriate conditions.

Minilab analysis. According to the GPHF Minilab manual[23], medicine samples were investigated by visual inspection, disintegration testing (if applicable) and thin-layer chromatography (TLC). In most organizations, all three steps were performed by the same person, hence no blinding was applied.

Visual inspection was carried out as specified in the chapter "Visual Inspection" of the GPHF Minilab manual[23]. Especially, product labelling was checked for completeness and plausibility of information, and correct spelling. The primary packaging was examined for adequate protection of the medicine, and the dosage units were screened for visual deficiencies.

Disintegration testing was carried out as specified in the respective chapter of the GPHF Minilab manual[23]. It should be noted that this is not a compendial test but a simplified procedure, and that no thermostated device is used. Six tablets and capsules were immersed in a flask containing 100 mL water of 37 °C, and the liquid was stirred or shaken from time to time. Immediate release tablets and capsules were considered as compliant if all six units fully disintegrated within 30 min, while slow-release and enteric-coated products have to withstand this test and must not disintegrate before 30 min. This test is not applicable for injectables, dry syrups and chewable tablets.

TLC analysis: Sample and reference tablets were crushed (capsules were opened, injectables and dry syrups were used as such) and extracted with a defined volume of the solvent described in the respective monograph of the GPHF Minilab manual. After a dilution step, 2 µL of the solution were applied to a TLC plate (Merck silica gel 60 F254, 0.2 mm thickness, 5 × 10 cm) using a microcapillary. On each plate, two reference solutions were applied, one corresponding to 100% of the stated amount of the respective API, the other corresponding to 80%. In addition, two spots of sample solution were applied on the plate. After development of the plate in the mobile phase described in the respective monograph of the manual, the spots were detected using UV light (254 or 366 nm) or a chemical staining method (iodine vapor; ninhydrin or sulfuric acid solutions) as described in the GPHF Minilab manual. The sample was rated compliant if the sample spots showed the same travel distance (relative retention factor) as well as the same color, size, and shape as the reference spots, were not weaker than the 80% reference spot and did not show additional spots indicating the presence of undeclared compounds or contaminants.

Additional/confirmatory testing. When Minilab testing revealed severe quality deficiencies (such as absence of the declared API; presence of a non-declared API; grossly insufficient amount of the declared API; or disintegration times of > 2 h), the DSOs reported these to the investigators in Tübingen using a standardized Microsoft Word form, together with photographs of the product and the developed TLC plate. The reports were checked by G.G. and C.H. to exclude possible false alerts, e.g., due to misinterpretation of TLC results, and further investigations were initiated. In eight cases G.G. and C.H. considered that the Minilab analysis by the first partner organization did not allow an unequivocal classification as compliant or non-compliant. In these cases, the suspect samples were sent to a second partner organization for re-testing. If test or re-test clearly revealed severe quality deficiencies, samples were forwarded to a fully equipped laboratory for compendial analysis using commercial courier services. In the study period, compendial analysis was conducted in 25 cases, either at the WHO prequalified laboratory of Mission for Essential Drugs and Supplies (MEDS; Nairobi, Kenya) or in the Pharmaceutical Institute of Tübingen University. No blinding was employed, i.e., persons performing the re-tests or compendial analyses were aware of previous testing results. Compendial testing was carried out following the current monographs of the United States Pharmacopeia (USP) or British Pharmacopoeia (BP), or according to in-house procedures established by MEDS if no respective USP or BP monograph was available. Identity and assay tests were performed in all 25 compendial analyses. Dissolution testing was carried out for solid oral dosage forms; however, it was omitted for samples not containing the declared active ingredient, and for samples containing less than 80% of the stated amount of API. Further tests, e.g., for related products, were performed as necessary. In cases where the identification of non-declared compounds was required, high resolution mass spectroscopy was performed[30].

Due to budget constraints, samples which showed minor quality deficiencies in the Minilab testing (such as spelling mistakes on the label or a disintegration time between 30 min and 2 h) were not submitted to compendial

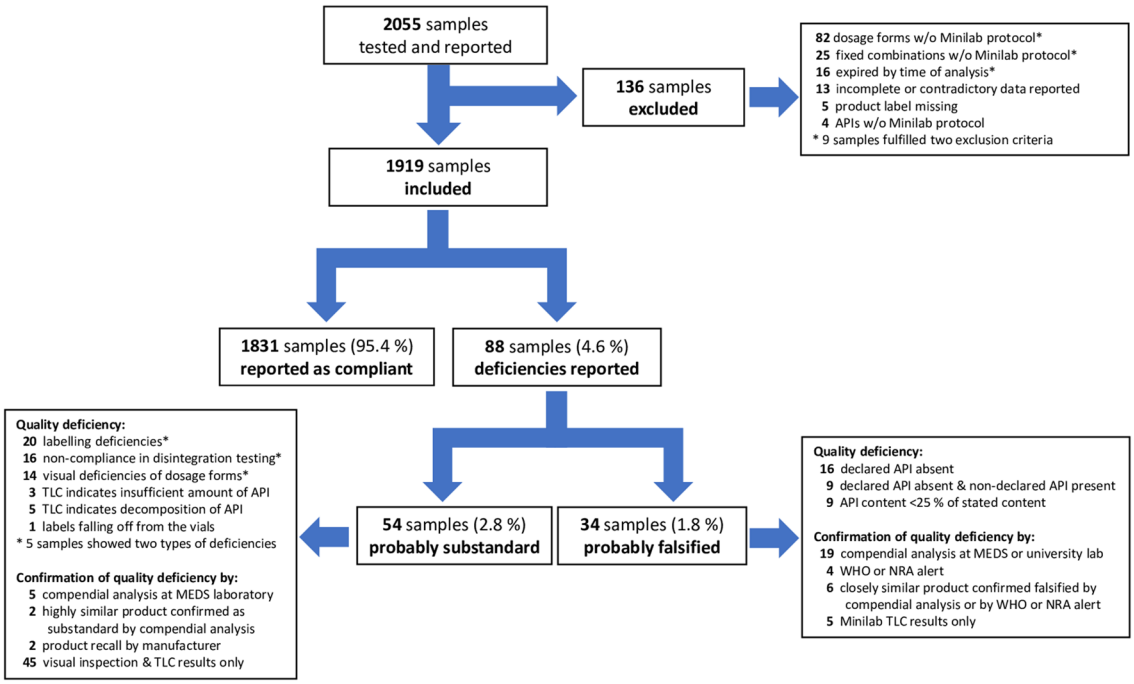

Figure 1. Flow chart showing the evaluation of the reported medicine quality data.

analysis. In some cases, the re-test and/or compendial analysis could not be performed due to an insufficient amount of remaining dosage units.

Routine documentation of samples, of test results and of costs. The results of all performed analyses were sent by the DSOs to the research pharmacists at Tübingen quarterly. In a standardized drop-down Microsoft Excel sheet, the source of the collected sample, product name, API, strength, manufacturing date, expiration date, batch number, name of stated manufacturer, country of manufacturing, and the month of testing were reported. Receipts and invoices of all expenditures related to the activities of the Difäm-EPN Minilab Network were collected, and costs were analyzed by the research pharmacist (G.G.).

Definitions. "Substandard" and "falsified" medical products were defined as suggested by WHO[1]. Since many but not all detected deficient products could be forwarded to an unequivocal compendial analysis, and in many cases the (stated) manufacturers could not be reached to confirm suspected falsifications, we use the cautious terms "probably falsified" and "probably substandard" for most of the results of the present evaluation. As suggested by Hauk et al.[31], samples containing no API, an incorrect API, or less than 50% of the stated API (without presence of degradation products) were rated as "probably falsified". The twelve African countries investigated in this research were assigned to five geographic regions of Africa as defined by the Organisation of African Unity (the predecessor of the African Union) in 1976 (CM/Res.464QCXVI)[32]. For the purpose of this research, "sample" is defined as a medical product of a specific brand and batch, collected at the same time and same place and subsequently subjected to one or several analyses.

Statistical methods. Data collection and basic evaluation were performed using Excel by Microsoft Office Professional Plus 2016. Statistical analyses were conducted using MedCalc (MedCalc Software, Ostend, Belgium)[33]. Comparisons of proportions were evaluated by the two-sided N-1 Chi-squared test as recommended by Campbell and Richardson for two-by-two tables with small sample sizes[34,35]. All p-values < 0.05 were considered significant.

Results

Overview of collected samples. As shown in Fig. 1, 2055 samples were tested and reported in the course of this study. Each of the eight samples sent to a second partner organization for re-testing (see "Methods") was counted as a single sample. A total of 136 samples were excluded from the present data analysis, most frequently because they represented oral liquid dosage forms. With the exception of protocols for dry syrups containing artemether/lumefantrine, amoxicillin or amoxicillin/clavulanic acid, no Minilab protocols for oral liquid dosage forms exist, as the excipients present in syrups and suspension may interfere with the TLC analysis and may preclude the reliable interpretation of the result. As shown in Fig. 1, analytical results correctly based on GPHF Minilab protocols were reported for 1,919 samples, and these were included into the data analysis.

Samples were collected and analyzed in 13 countries by 16 faith-based DSOs, as summarized in Table 1. Fifteen of these organizations are located in sub-Saharan Africa, one in India. Of the 1919 samples included

Region and country of collection	Sources of samples				Total no. of samples included	Results of analysis			
	Private vendors or own stock	Health facilities	Donations or unknown	Informal vendors		No. of probably falsified samples	%	No. of probably substandard samples	%
East Africa	**152**	**5**	**1**	**3**	**161**	**0**	**0.0%**	**3**	**1.9%**
Kenya	43	0	0	0	43	0	0.0%	3	7.0%
Rwanda	9	0	0	0	9	0	0.0%	0	0.0%
Tanzania	14	5	1	0	20	0	0.0%	0	0.0%
Uganda	86	0	0	3	89	0	0.0%	0	0.0%
Central Africa	**1126**	**68**	**0**	**106**	**1300**	**31**	**2.4%**	**48**	**3.7%**
Burundi	241	1	0	0	242	0	0.0%	7	2.9%
Cameroon	686	19	0	40	745	11	1.5%	20	2.7
Central African Rep	22	11	0	17	50	3	6.0%	8	16.0%
DR Congo	142	11	0	10	163	6	3.7%	3	1.8%
Chad	35	26	0	39	100	11	11.0%	10	10.0%
West Africa	**171**	**103**	**0**	**2**	**276**	**3**	**1.1%**	**3**	**1.1%**
Ghana	0	27	0	0	27	0	0.0%	0	0.0%
Nigeria	171	76	0	2	249	3	1.2%	3	1.2%
Southern Africa	**78**	**29**	**11**	**0**	**118**	**0**	**0.0%**	**0**	**0.0%**
Malawi*	78	29	11	0	118	0	0.0%	0	0.0%
Southeast Asia	**64**	**0**	**0**	**0**	**64**	**0**	**0.0%**	**0**	**0.0%**
India	64	0	0	0	64	0	0.0%	0	0.0%
Total	**1,591**	**205**	**12**	**111**	**1919**	**34**	**1.77%**	**54**	**2.81%**

Table 1. Overview of collected medicine samples, and results of analysis. Three faith-based drug supply organizations in Cameroon contributed to this study, and two in the Democratic Republic of the Congo (DRC). *For two samples from Malawi, the source is unknown.

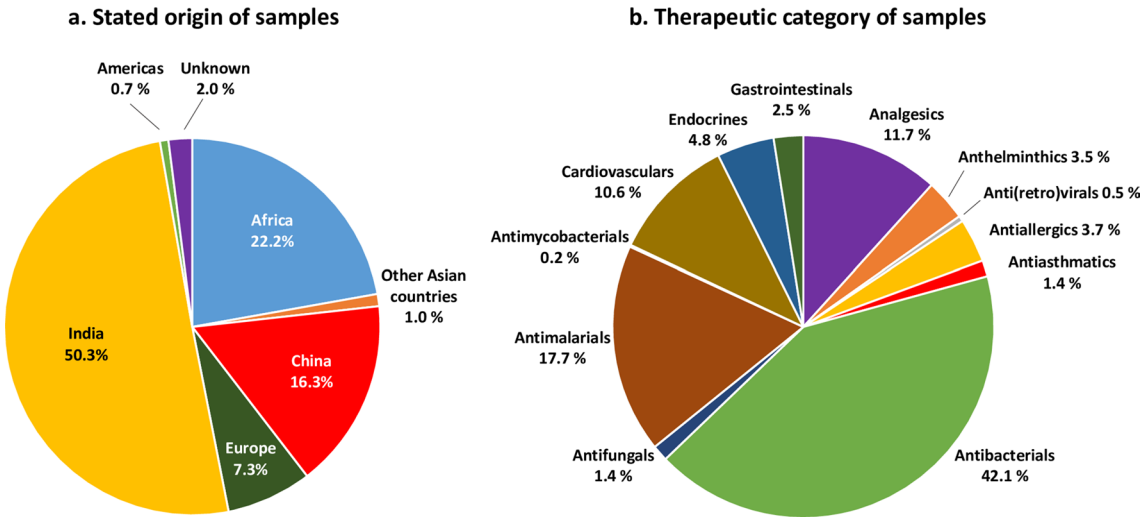

Figure 2. Stated origin (**a**) and therapeutic categories (**b**) of the 1919 included samples.

in the data analysis, 1591 (82.9%) were collected from the own stock of these DSOs, or from private vendors. Private vendors are commercial sources from which these organizations purchase medicines for distribution to health facilities, therefore the two categories "own stock" and "private vendors" are combined in Table 1. A total of 205 samples (10.7%) were collected from health facilities. Only 10 samples (0.5%) were products donated to the DSOs. Further 111 samples (5.8%) were collected from informal vendors, with 106 of these collected in four of the five included countries of Central Africa.

The different organizations involved in this study had different staffing capacities for their contribution to the Minilab surveillance project. The most active organization, located in Cameroon, contributed 512 samples. The partner organization in Rwanda joined the surveillance only in the second project year, and therefore contributed only nine samples.

The stated countries of origin of the included medicine samples are depicted in Fig. 2a. Half of the samples (966 samples; 50.3%) were stated to be produced in India, and 313 (16.3%) in China. Further 426 samples (22.2%)

were stated to be produced in Africa, with Nigeria (159 samples; 8.3%), Kenya (98 samples; 5.1%) and Uganda (72 samples; 3.8%) as the most important African producer countries.

As shown in Fig. 2b, 1252 samples (65.2%) were medicines for the treatment of infectious diseases, with antibacterials (808 samples; 42.1%) and antimalarials (339 samples; 17.7%) as most frequent categories. Among the medicines for non-communicable diseases, analgesics were included most frequently (225 samples; 11.7%). The most frequently tested dosage forms were tablets (1467 samples; 76.4%), followed by injections (250 samples; 13.0%), capsules (168 samples; 8.8%), and dry syrups (34 samples; 1.8%).

In total, 71 different APIs were tested according to the protocols of the GPHF Minilab manual. A detailed overview of the different APIs and dosage forms of the medicines included into the data analysis is given in Supplementary Table S1.

Results of sample analysis. A research pharmacist (G.G.) cross-checked the data reported by the partner organizations from Africa and Asia, and the categorization of the results as compliant or non-compliant by the partners. Corrections by the research pharmacist were required only in 15 cases (0.8%). Most frequently (six cases), a failure in disintegration testing had been incorrectly reported for modified release tablets; as stated in the GPHF Minilab manual[23], these are in fact not expected to disintegrate under the test conditions specified in the Minilab protocol.

Samples which showed major quality deficiencies in the TLC analysis (absence of stated API, presence of undeclared substances, underdosage of declared API) and/or in the disintegration test (i.e., disintegration time > 2 h) were sent for compendial analysis to MEDS or Tübingen University. In eight ambiguous cases (0.4%) a re-test by a second Network member was sought. Three of these cases were found to be compliant in the Minilab re-test, in the other five cases further investigation was considered necessary. Subsequently conducted compendial analysis at the MEDS laboratory revealed that two suspected products were compliant with the specifications while three were not.

After these corrections, eventually 1831 samples (95.4%) were reported as compliant, while for 88 samples (4.6%) quality deficiencies were reported and these were considered as SF products (Fig. 1). Of the 88 samples reported to show deficiencies, 34 (1.8% of evaluated samples) were rated as probably falsified by the research pharmacist: for 16 of these 34 samples, TLC analysis showed that the stated API was absent. In another 9 samples the stated API was absent, but they contained a different, undeclared API. In the remaining 9 cases, visual analysis of the TLC results suggested that the API was present in a much smaller amount than stated on the label, and indeed compendial analysis confirmed for these samples that the API content was < 25% of the stated amount (see Table 2), and at the same time no decomposition products were detected. Examples of TLC results for different types of quality deficiencies are depicted in Fig. 3.

For 19 of the 34 samples rated as probably falsified, the quality deficiencies detected in the GPHF Minilab analysis were confirmed by compendial analysis in the WHO-prequalified laboratory of MEDS or in the laboratory of Tübingen University. For another four samples, compendial analysis was considered unnecessary since reports published by WHO or by a national medicines regulatory authority confirmed that these samples were falsified. For six samples, closely related products had previously been identified as falsified by compendial analysis or an (inter-)national alert. For the remaining five samples, the low number of tablets remaining after the Minilab testing did not allow a confirmation by compendial analysis, but the evidence from TLC analysis and packaging analysis was considered unequivocal.

In total 54 samples (2.8% of evaluated samples) were rated probably substandard (Fig. 2) because of one or several of the following reasons. The most common reason (20 samples) was deficient labelling, such as missing batch numbers or orthographic mistakes, however without conclusive evidence for falsification as described by Hauk et al.[31]. Fourteen samples showed visual deficiencies of the dosage forms, such as discolorations or cracks in case of tablets, or agglomeration of capsules. Sixteen samples showed non-compliance in disintegration testing. In two of these cases, the tablets had not disintegrated even after two days. The faith-based drug supply organization decided to contact the local manufacturer, presented the test result, and the manufacturer thereupon issued a product recall, as depicted in Supplementary Fig. S1.

In three out of the 54 samples rated as probably substandard, TLC analysis indicated an insufficient amount of the API, estimated to be in the range of 50–80% of the declared amount by visual inspection of the TLC plate. Unfortunately, in these three cases the sample size was insufficient to allow for compendial analysis. In five further samples, representing different batches of captopril tablets from two manufacturers, TLC analysis indicated decomposition of the API (Fig. 3a). Compendial analysis conducted for one sample from each manufacturer respectively proved API contents of only 66.1% and 50.7% of the declared amount, as well as elevated, non-compliant quantities of the decomposition product captopril disulfide.

The limited funds available for the present project did not permit to subject all samples rated as probably substandard to compendial analysis.

Samples identified as probably falsified in this study. Table 2 lists the 34 samples rated as probably falsified, with their declared APIs, their countries of discovery, their stated country of manufacture, and the result of their chemical analysis. Supplementary Table S2 provides further details on these samples, including the brand names of the products, batch numbers, expiry dates and names of the stated manufacturers.

Ten of these 34 samples were labeled to contain chloroquine as API, eight to contain quinine, and another eight to contain sulfamethoxazole/trimethoprim. Out of the 34 probably falsified medicines 32 were anti-infectives. Probably falsified samples were found only in five of the 13 countries where this study was conducted, i.e., in Cameroon, Chad, DRC, and CAR (Central Africa) and in Nigeria (West Africa). Out of the 111 medicines collected from informal vendors, 14 (12.6%) were rated as probably falsified, contrasting to 20 (i.e., only 1.1%)

No.	Country of discovery	Declared active APIs, and dosage form	Stated country of origin	API content (% of stated amount); + undeclared APIs
1	Cameroon	Ampicillin/cloxacillin sodium capsules	India	0%/0%
2	Chad	Artemether/lumefantrine tablets	India	0%/0%
3	DR Congo	Ceftriaxone sodium inj. 1 g	Spain	23.5%
4	DR Congo	Ceftriaxone sodium inj. 1 g	Spain	23.8%
5	DR Congo	Ceftriaxone sodium inj. 1 g	Spain	< 23% (weight of vial content = 230 mg)
6	DR Congo	Chloroquine tablets	Kenya	0%; + 126.5 mg metronidazole
7	Cameroon	Chloroquine phosphate tablets	India	0%
8	Nigeria	Chloroquine phosphate tablets	Nigeria	0%
9	Cameroon	Chloroquine phosphate tablets	Nigeria	0%
10	Cameroon	Chloroquine phosphate tablets	China	21.7%
11	Cameroon	Chloroquine phosphate tablets	China	0%; + 14.1 mg/tablet metronidazole
12 & 13	Cameroon	Chloroquine phosphate tablets	China	0%; + 35.7 mg/tablet paracetamol
14	Cameroon	Chloroquine phosphate tablets	China	0%; + 14.6 mg/tablet metronidazole + 1.6 mg/tablet paracetamol
15	Cameroon	Chloroquine phosphate tablets	Nigeria	12.2%
16	Cameroon	Hydrochlorothiazide tablets	Belgium	0%; + 5 mg/tablet glibenclamide
17	Cameroon	Paracetamol/diclofenac sodium tablets	India	95.2% paracetamol/0% diclofenac
18	Nigeria	Proguanil tablets	Malta	< 25% (estimate from TLC)
19	DR Congo	Quinine tablets	India	0%
20	Chad	Quinine tablets	Nigeria	0%
21	Central Afr. Rep	Quinine sulphate tablets	Nigeria	0%
22	Central Afr. Rep	Quinine sulphate tablets	Nigeria	0%
23	Central Afr. Rep	Quinine sulphate tablets	Bulgaria	0%
24	Chad	Quinine sulphate tablets	Cyprus	0%; + 12 mg/tablet chloroquine
25	DR Congo	Quinine sulphate tablets	Uganda	0%
26	Chad	Quinine sulphate tablets	Norway	0%
27	Chad	Sulfamethoxazole/trimethoprim tablets	Nigeria	0%/0%
28	Nigeria	Sulfamethoxazole/trimethoprim tablets	Nigeria	0%/0%
29	Chad	Sulfamethoxazole/trimethoprim tablets	Not stated	0%/0% + paracetamol (TLC analysis)
30	Chad	Sulfamethoxazole/trimethoprim tablets	Nigeria	0%/0%
31	Chad	Sulfamethoxazole/trimethoprim tablets	Nigeria	0%/0%
32	Chad	Sulfamethoxazole/trimethoprim tablets	Nigeria	47.7%/21.2%
33	Chad	Sulfamethoxazole/trimethoprim tablets	Nigeria	17.6%/16.3%
34	Chad	Sulfamethoxazole/trimethoprim tablets	Nigeria	< 25%/< 25% (estimate from TLC)

Table 2. Medicine samples identified in this study as probably falsified. In the cases 12 and 13, two samples of this medicine were identified independently in the course of this study. Supplementary Table S2 provides further details on these samples, including the brand names of the products, batch numbers, expiry dates and names of the stated manufacturers.

out of the 1,808 medicines from legal sources ($p < 0.0001$). Out of the 970 medicines collected from the own stock of the participating faith-based DSOs, only three (0.3%) were rated as probably falsified, indicating a largely successful product and supplier selection by the DSOs.

Fifteen of the 34 probably falsified samples were stated to be produced in Africa (13 of these in Nigeria), and the others in Europe (8), India (5), and China (5). For one sample no country of manufacture was indicated. However, the manufacturer and the country of origin stated on the label of a falsified medicine may obviously be incorrect. Some manufacturers named in Supplementary Table S2, such as Strides Arcolab, India, have an excellent international reputation which falsifiers may have criminally misused in the labelling of their falsified medicines. For the artemether/lumefantrine preparation listed in Supplementary Table S2, Strides Arcolab confirmed to the authors and to WHO that this product is a falsification. Some other manufacturers listed in Supplementary Table S2, such as "Enitop Pharmaceuticals Nig. Ltd" and "Pharmachim Bulgaria" are non-existing companies[37].

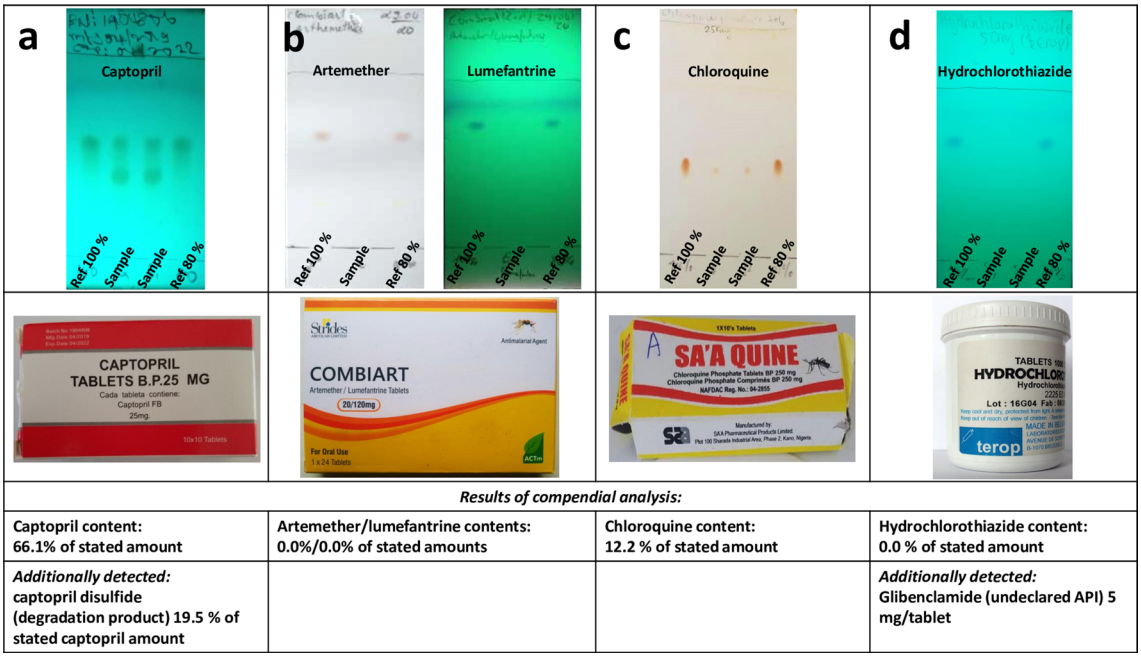

Figure 3. Examples of TLC analysis of samples of the present study, showing (**a**) decomposition of the API; (**b**) absence of the declared APIs; (**c**) API content 12.2% of the stated amount; (**d**) absence of the declared API, and presence of a non-declared API (the non-declared API glibenclamide is not visible in the depicted TLC plate, but was discovered by the local partner in an additional, specific TLC analysis for glibenclamide, prompted by the observed hypoglycemic effect of the falsified medication[36]). (Photos: Gesa Gnegel, Lutz Heide and Difäm-EPN Minilab Network).

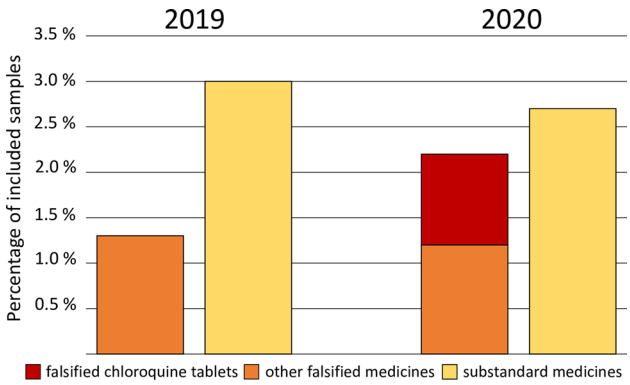

Figure 4. Changes of the occurrence of probably falsified and probably substandard medicines in the course of the COVID-19 pandemic.

Notably, out of the 34 probably falsified medicines, 22 samples (64.7%) were reported to show deficiencies already in visual inspection, such as missing data or mistakes in the labelling, or visible deficiencies of the dosage forms. In contrast, out of the 1885 medicines considered non-falsified, 34 (i.e., only 1.8%) were reported to show visual deficiencies. This difference is statistically significant ($p < 0.0001$) and emphasizes that careful visual inspection is an important and powerful tool in the screening for falsified medicines[26,38,39].

Changes of the occurrence of substandard and falsified medicines in the course of the COVID-19 pandemic. Figure 4 compares the number of probably falsified and probably substandard samples reported in the two investigated years. In 2019 (before the pandemic), 11 samples (1.3%) out of 871 were probably falsified. In 2020 (during the pandemic) this increased to 23 (2.2%) out of 1048 samples. Though this difference does not reach statistical significance ($p = 0.14$), it indicates a trend towards an increase in the occurrence of falsified medicines, as has been predicted at the outset of the pandemic[12]. Notably, the observed increase was nearly entirely due to the occurrence of ten falsified chloroquine samples (nine samples in the first half of 2020, one in the second half).

External funding:	
14,300 €	Consumables for GPHF Minilab analysis: 41.7% for solvents (procured locally); 32.9% for reference standards and 19.7% for TLC plates, glassware and GPHF Minilab manuals (procured and shipped through TTM Technologie Transfer Marburg e.V.; Coelbe, Germany); 5.6% for packaging and shipment
10,500 €	17 confirmatory compendial analyses at MEDS laboratory, Nairobi (offered at reduced rates)
2,000 €	GPHF Minilab training course in Rwanda
22,800 €	Personnel costs for research pharmacist/network coordinator at Difäm, Tuebingen, Germany (0.2 full-time equivalents)
=49,600 €	**Total external funding** (47,600 € from Bread for the World, Berlin, Germany; 2,000 € from Difäm, Tübingen, Germany)
Contributions by network participants	
Staff time for GPHF Minilab analyses and documentation by 16 participating drug supply organizations	
Acquisition costs of medicine samples by 16 participating drug supply organizations	
Purchase of one GPHF Minilab (5,500 €) by one participating drug supply organization, using its own funds	
8 confirmatory compendial analyses at Tuebingen University laboratory, Germany, provided at no charge	

Table 3. Funding requirements for the surveillance for substandard and falsified medicines in the reporting period (Jan. 2019–Dec. 2020).

Sharing data with stakeholders. All probably falsified samples listed in Table 2 and Supplementary Table 2, as well as four products rated as severely substandard (i.e. amount of the API estimated as 50–80% of the declared amount by visual inspection of the TLC plate; or TLC indicating major decomposition of the API, as shown in Fig. 3a) were reported to the WHO Global Surveillance and Monitoring System for SF Medical Products (Rapid Alert System). These reports were made by G.G., without mentioning the names of the partner organizations to protect their anonymity. WHO decided about further actions, such as contacting the stated manufacturers, informing responsible national regulatory authorities, and in very serious cases publishing international WHO Medical Product Alerts. The medicine quality analyses conducted within this study resulted in the release of four WHO Medical Product Alerts[36,37,40,41], warning about the cases listed as No. 6, 7, 10–14, 16, 20, 21, 23 and 24 in Table 2/Supplementary Table S2. Several national drug regulatory authorities also published alerts about these cases, e.g. the National Authority for Food and Drug Administration of Nigeria (NAFDAC)[42–44], the Ministère de la Santé et de la Population of CAR (Supplementary Fig. S2), the Laboratoire National de Contrôle de Qualité des Médicaments et d'Expertise (LANACOME) of Cameroun (Supplementary Fig. S3) and the Drug Regulatory Authority of Pakistan[45]. NAFDAC also published an alert about the case listed as No. 33 in Table 2/Supplementary Table S2[46].

Funding requirements for the surveillance for substandard and falsified medicines. As reported by Petersen et al.[26], the initial provision of GPHF Minilabs for most partners of the Difäm-EPN Minilab Network in the years 2010–2015 had required approximately 5600 US$ per Minilab, and the initial training of the personnel required approximately 2300 US$ per partner organization. As summarized in Table 3, in the present reporting period (2019–2020) external funding was required for consumables, confirmatory compendial analyses, training, and for the research pharmacist (G.G.) at Difäm who also acted as network coordinator (total 0.2 full-time equivalents). All personnel costs required for the local GPHF Minilab analyses were borne by the faith-based DSOs in Africa and Asia themselves. Most of the tested medicine samples were obtained from the own stocks of the participating DSOs, or from the private vendors they used as source of their supplies (Table 1); no external funds were provided for the acquisition of these samples. A refund of the purchase costs for samples from external sources had been offered out of the project budget, but none of the DSOs claimed such refunds in the reporting period, probably due to the comparatively small sums involved. In the original project budget, one yearly network meeting had been foreseen and budgeted at 6,400 € each. Due to the outbreak of the COVID-19 pandemic, however, these meetings were held online, and this budget line remained untouched.

Based on the 1919 samples included into the data analysis (Fig. 1), the costs for consumables for GPHF Minilab analysis (Table 3) resulted as 7.45 € per sample on average. However, this varied between the partners: DSOs testing a small number of samples but including a high number of different APIs required higher costs per sample, e.g. since many reference standards needed to be replaced upon expiry.

Based on the total external funding of 49,600 € (Table 3), the total external costs amounted to 25.85 € per sample. Notably, a compendial analysis of all 1919 samples, even at the reduced rates offered by MEDS for this project (618 € per sample on average), would have costed approximately 1.2 million €, i.e., 23 times more than the actual external funding of the project.

Based on the 34 probably falsified medicines listed in Table 3, the external costs of the identification of one such product resulted as 1459 €.

Discussion

This study describes procedures, results, costs, and limitations of a routine medicine quality screening using the GPHF Minilab by faith-based DSOs in Africa and Asia. As described recently, the evaluation of medicine quality screening devices, especially in real-life-settings, is still in its infancy[19,20], and to our knowledge the present

study is the first systematic investigation of the routine use of a medicine quality screening device covering a large geographic area (two continents, 13 countries) and a longer period of time (two years).

Since the screening project and its present evaluation were carried out with a minimal budget, many but not all detected deficient products could be forwarded to an unequivocal compendial analysis. Furthermore, it was not possible in many cases to contact the (stated) manufacturers to confirm the observed falsifications. Therefore, we use the cautious terms "probably falsified" and "probably substandard" in the results of the present evaluation. A total of 2,055 medicine samples were screened with the GPHF Minilab by the 16 participating organizations, of which 1,919 samples met the inclusion criteria and were included in the present evaluation (Fig. 1). Of these, 34 samples (1.8%) were classified as probably falsified, since they did not contain the declared API, contained undeclared APIs, or contained less than 50% of the declared API without presence of decomposition products. The quality deficiencies of these samples are summarized in Table 2 and clearly illustrate the huge public health threat posed by such preparations. Similar percentages of falsified medicines as in the present study have been reported in previous studies in LMICs, carrying out compendial analysis for all samples. e.g., Rahman et al.[47] found 1.1% falsified samples in Bangladesh, and Hauk et al.[31] found 1.7% in Cameroon and the DR Congo. No falsified medicines were found by Seitzer et al.[48] among 88 samples from Burkina Faso, Cote d'Ivoire, Ghana and Tanzania. The GPHF Minilab has been proven to be highly sensitive in the detection of the gross deficiencies described above[18,24], and it appears likely that such deficiencies have been detected reliably and completely in the present GPHF Minilab screening.

In the present study, 54 samples (2.8%) were reported to be probably substandard. Together with the 34 samples rated as probably falsified, this results in 88 SF samples (4.6%). However, we cannot exclude that a considerable number of substandard samples have been missed out, due to the known, limited sensitivity of the GPHF Minilab in the detection of products which contain an insufficient amount of the API, or show insufficient dissolution of the API[24,25]. Notably, an average prevalence of 10.5% SF medicines in LMICs was reported in a review by WHO[1], and a rate of 18.7% in a review by Ozawa et al.[49].

In total 38 cases of SF medicines from the present study have been reported to the WHO. Four international Medical Product Alerts were subsequently issued by WHO[36,37,40,41], as well as alerts by national authorities (Supplementary Figs. S2, S3 and S4). The routine use of the GPHF Minilab in faith-based DSOs has therefore successfully contributed to the WHO Global Surveillance and Monitoring System for SF Medical Products[2].

Any medicines identified as probably falsified within the stock of the faith-based DSOs were quarantined and not distributed to patients, and deficient medicines offered by private suppliers to the DSOs were excluded from future drug procurement. Thereby, the project helped to prevent the spread of SF medicines in the health facilities supplied by the DSOs, and it increased the awareness of the problem of SF medicines. This is also evidenced by the low rate (0.3%) of probably falsified medicines found within the own stock of the involved DSOs.

The effects of the project extended even beyond the 100 APIs included in the GPHF Minilab manual: in 2020, the participating DSO from Chad discovered vitamin A capsules which failed visual inspection due to suspicious labelling mistakes. Compendial analysis at MEDS was initiated, and the products were found to be strongly degraded. The stated manufacturer confirmed that manufacturing and expiration date had been altered, apparently by criminals, to extend the stated product shelf life. WHO was informed and issued an international Medical Product Alert[50]. Furthermore, suspicious batches of carbamazepine tablets were reported by project partners in Cameroon. No Minilab monograph is available for this API, however the product failed a color reaction carried out according to the WHO handbook on basic test for pharmaceutical substances[51]. Subsequent compendial analysis conducted at MEDS showed absence of the declared API. The Cameroonian Ministry of Public Health was informed an issued an alert (Supplementary Fig. S4).

The medicine quality screening approach described here represents a very cost-effective way to contribute to the global surveillance for SF medical products. The total external funding of 49,600 € (Table 3) is extremely low compared to other international programs in health and/or in development cooperation. At the same time, this approach empowers local stakeholders to assume an active role in the surveillance for SF medicines. Of course, its limited sensitivity in the detection of substandard medicines must be kept in mind, and the GPHF Minilab can neither replace compendial analysis nor should it be used as a sole quality assurance (QA) measure in DSOs but rather as one part of a comprehensive QA system.

The Difäm-EPN Minilab Network operates since 2010 and has been growing constantly in this time. The success of this program depended especially on four factors: first, the existence of a well-established network of organizations with similar values and high commitment to the project. Second, the assurance of the continuous supply of the required consumables for GPHF Minilab analysis, funded by an external donor organization. Third, the possibility for confirmatory compendial analysis of suspected poor-quality samples, provided primarily by the WHO-prequalified medicine quality laboratory of MEDS, Nairobi, at moderate costs. The Certificates of Analysis provided by such a laboratory allow the involved DSOs to report their finding to national and international authorities, and to suppliers and manufacturers, as described in the present report. And fourth, the availability of an academically trained network coordinator (0.2 full-time equivalents) located at Difäm who supported organizational aspects, communications and training activities.

The role and the limitations of the GPHF Minilab in medicine quality assurance in low-resource settings need to be considered responsibly. The compliance with pharmacopeial standards can only be proven conclusively by (expensive) pharmacopeial methods. As mentioned, the GPHF Minilab is one of several available medicine quality screening techniques, and reviews of their respective strengths and limitations have been published[16–20,22].

GPHF Minilab analysis is fast in comparison to full compendial analysis, but it still requires considerable staff time from the involved DSOs. It will certainly be worthwhile to further investigate the possibilities of a complementation of the use of the GPHF Minilab with the use of low-cost near-infrared and/or Raman spectroscopic devices[52]. Such devices hold promise for simple, inexpensive medicine quality screening and do not require consumables. However, spectral reference libraries still need to be created and maintained, and the possibilities

and limitations of the application of spectroscopic devices in the quality screening of many different medicines from many different sources need to be investigated in field studies.

The involvement of private or civil society organizations, as described here, clearly offers the prospect to increase the outreach and speed of the detection and removal of SF medicines, especially in low-resource settings where government institutions may not be sufficiently equipped and staffed for the comprehensive completion of these tasks. However, such activities need to be carefully and diplomatically established, as government institutions may perceive them as an intrusion into their own responsibilities from the side of the civil society organizations. In fact, during the reporting period of the present study, one of the involved DSOs was informed by respective national Ministry of Health that the DSO is not authorized to control the quality of medicines. The appearance of a WHO Medical Product Alert about a falsified medicine in the respective country, based on a report from that DSO and a compendial analysis by MEDS, may have caused irritation in the government authorities. This DSO has since then stopped to share the results of their GPHF Minilab analyses with other stakeholders, which is a regrettable development for the Network. Possibly, institutions like the WHO can help to mediate a constructive dialogue between national authorities and civil society organizations to foster the development of a mutually beneficial and acceptable mode of cooperation in the surveillance for SF medicines.

For this study, data from 2019 and 2020 were evaluated, hence from the year before the outbreak of the COVID-19 pandemic, and from the first year of the pandemic. Notably, in 2020 ten falsified chloroquine products were found. This is most likely due to the "hype" of chloroquine and hydroxychloroquine as possible treatments for COVID-19, which strongly increased the demand and prices of these products[30,53–55]. Apparently, criminal falsifiers responded swiftly to this opportunity.

Data availability

The dataset generated during and analyzed during the current study are not publicly available. An anonymized version of the dataset is available from the corresponding author on reasonable request.

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Author contributions
Data were produced and documented by the members of the Difäm-EPN-Minilab Network and collected by G.G. and C.H., G.G. evaluated the data and wrote the first draft of the manuscript, L.H., C.H. and R.N. revised the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare no competing interests.

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Surveillance for substandard and falsified medicines by local faith-based organizations in 13 low- and middle-income countries using the GPHF Minilab

Supplementary Information

Gesa Gnegel[1,2], Christine Häfele-Abah[2,3], Richard Neci[3], Difäm-EPN Minilab Network[2,3], and Lutz Heide[1,3]*

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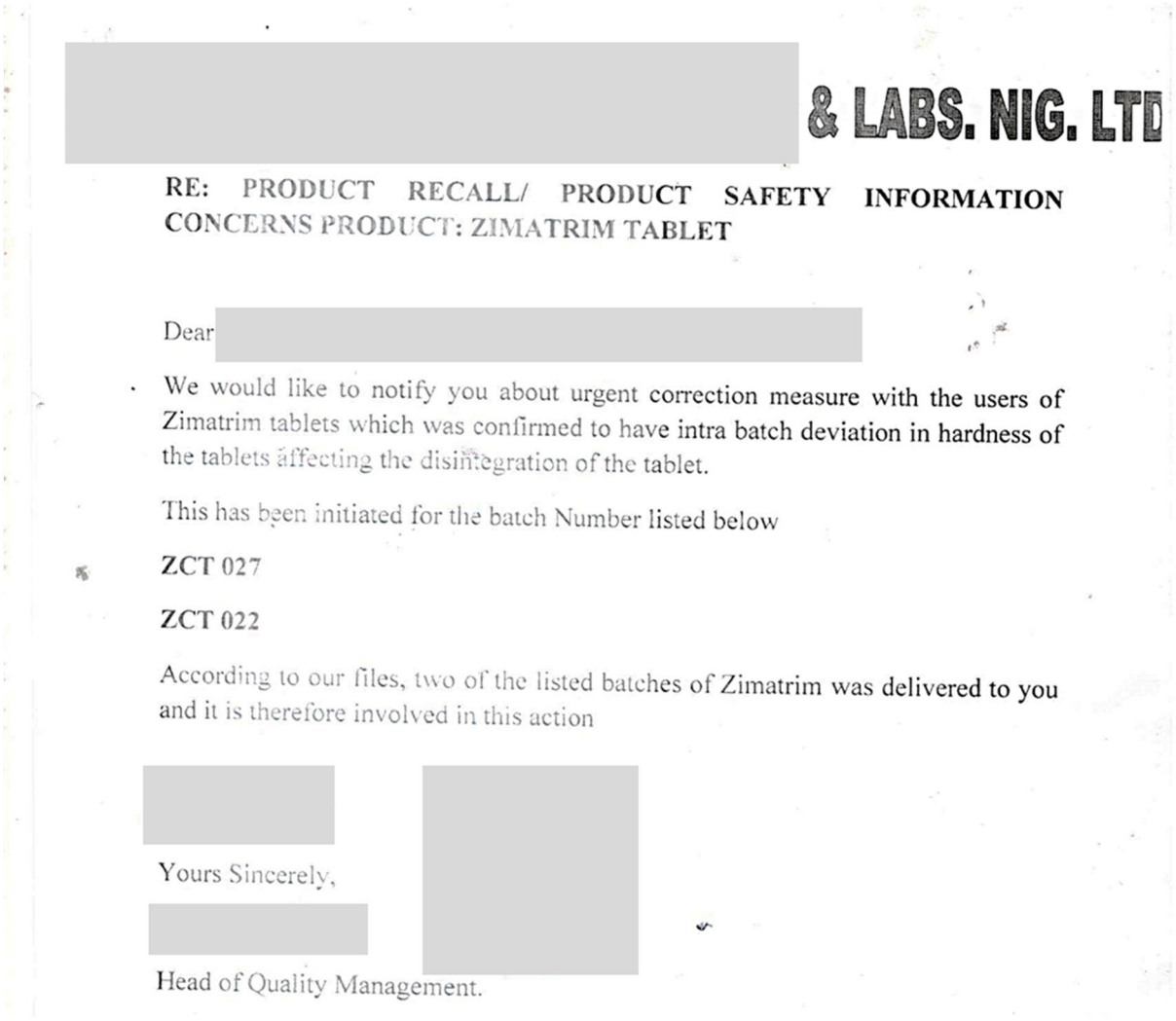


& LABS. NIG. LTD

RE: PRODUCT RECALL/ PRODUCT SAFETY INFORMATION CONCERNS PRODUCT: ZIMATRIM TABLET

Dear

We would like to notify you about urgent correction measure with the users of Zimatrim tablets which was confirmed to have intra batch deviation in hardness of the tablets affecting the disintegration of the tablet.

This has been initiated for the batch Number listed below

ZCT 027

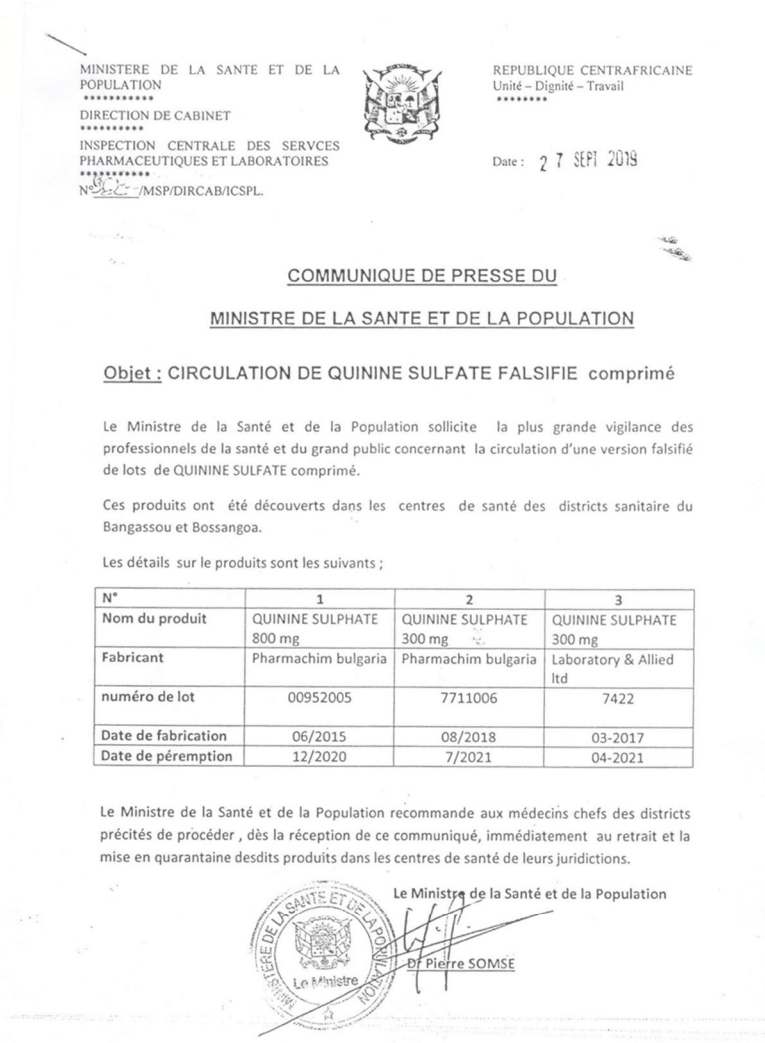
ZCT 022

According to our files, two of the listed batches of Zimatrim was delivered to you and it is therefore involved in this action

Yours Sincerely,

Head of Quality Management.

Supplementary Figure S1: Product recall issued by a Nigerian manufacturer



MINISTERE DE LA SANTE ET DE LA POPULATION
****
DIRECTION DE CABINET
****
INSPECTION CENTRALE DES SERVCES PHARMACEUTIQUES ET LABORATOIRES
****
N°____/MSP/DIRCAB/ICSPL.

REPUBLIQUE CENTRAFRICAINE
Unité – Dignité – Travail
****

Date : 27 SEPT 2019

COMMUNIQUE DE PRESSE DU

MINISTRE DE LA SANTE ET DE LA POPULATION

Objet : CIRCULATION DE QUININE SULFATE FALSIFIE comprimé

Le Ministre de la Santé et de la Population sollicite la plus grande vigilance des professionnels de la santé et du grand public concernant la circulation d'une version falsifié de lots de QUININE SULFATE comprimé.

Ces produits ont été découverts dans les centres de santé des districts sanitaire du Bangassou et Bossangoa.

Les détails sur le produits sont les suivants ;

N°	1	2	3
Nom du produit	QUININE SULPHATE 800 mg	QUININE SULPHATE 300 mg	QUININE SULPHATE 300 mg
Fabricant	Pharmachim bulgaria	Pharmachim bulgaria	Laboratory & Allied ltd
numéro de lot	00952005	7711006	7422
Date de fabrication	06/2015	08/2018	03-2017
Date de péremption	12/2020	7/2021	04-2021

Le Ministre de la Santé et de la Population recommande aux médecins chefs des districts précités de procéder , dès la réception de ce communiqué, immédiatement au retrait et la mise en quarantaine desdits produits dans les centres de santé de leurs juridictions.

Le Ministre de la Santé et de la Population

Le Ministre

Dr Pierre SOMSE

MINISTERE DE LA SANTE ET DE LA POPULATION
****
DIRECTION DE CABINET
****
INSPECTION CENTRALE DES SERVCES PHARMACEUTIQUES ET LABORATOIRES
****
N°____/MSP/DIRCAB/ICSPL.

REPUBLIQUE CENTRAFRICAINE
Unité – Dignité – Travail
****

Date : 27 SEPT 2019

INSTRUCTION MINISTERIELLE

Suite à la notification de l'alerte internationale de l'OMS réf : **N° 10/2019 relative à des lots de QUININE SULPHATE FALSIFIES** circulant sur le territoire centrafricain, le Ministre de la Santé et de la Population instruit tous les médecins chefs de district de procéder à une recherche active de ces différents lots dans toutes les formations sanitaires de leurs juridictions afin de procéder à leur retrait et mise en quarantaine et d'en informer la direction de la pharmacie et du médicament.

-Copie DPLMT pour suivi

Le Ministre de la Sante et de la Population

Le Ministre

Dr Pierre SOMSE

Supplementary Figure S2: National medical product alert published by the Ministry of Health and Population of the Central African Republic

REPUBLIQUE DU CAMEROUN
Paix-Travail-Patrie

Laboratoire National de Contrôle de Qualité des Médicaments et d'Expertise
---
DIRECTION GENERALE

REPUBLIC OF CAMEROON
Peace-Work-Fatherland

National Drug Quality Control and Valuation Laboratory

GENERAL MANAGEMENT

Réf : 1340 **/20/L/LANACOME/DG/.-** *Yaoundé, le* 30 MARS 2020

LE DIRECTEUR GENERAL

1) Aux Directeurs des:
- ***Hôpitaux Généraux,***
- ***Hôpitaux Centraux,***
- ***Hôpitaux de District***
- ***Cliniques privées***

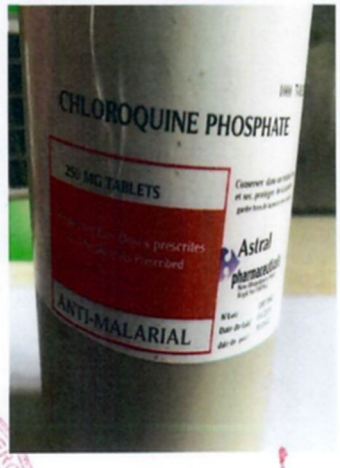
2) Aux Pharmaciens d'Officines et Pharmaciens Chefs des Hôpitaux et Cliniques privées
3) Aux Grossistes agréés

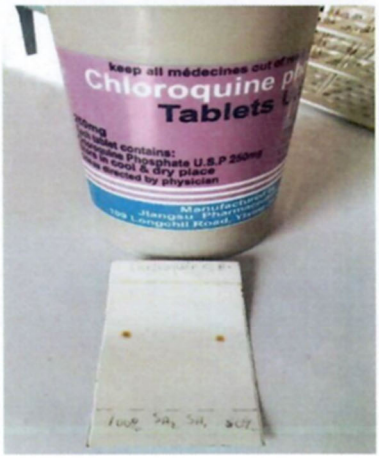
Objet : Circulation de la Chloroquine sans principe actif.

Mesdames et Messieurs les Directeurs des établissements hospitaliers,

Le Laboratoire National de Contrôle de Qualité des Médicaments et d'Expertise informe les populations et les professionnels de la santé que deux présentations de chloroquine issue des circuits de contrebande sont actuellement en circulation au Cameroun et se retrouveraient déjà dans certaines formations sanitaires.

Les résultats des tests de ces deux présentations de chloroquine ci-dessous révèlent l'absence de toute substance active pharmaceutique.





Supplementary Figure S3: National medical product alert published by the National Drug Quality Control and Valuation Laboratory of Cameroun

REPUBLIQUE DU CAMEROUN
Paix – Travail – Patrie

MINISTERE DE LA SANTE PUBLIQUE

CABINET DU MINISTRE

INSPECTION GENERALE DES SERVICES PHARMACEUTIQUES ET DES LABORATOIRES

D13-79

REPUBLIC OF CAMEROON
Peace – Work – Fatherland

MINISTRY OF PUBLIC HEALTH

MINISTER'S CABINET

GENERAL INSPECTORATE FOR PHARMACEUTICAL SERVICES AND LABORATORIES

Yaoundé, le 18 AVR 2019

COMMUNIQUE PRESSE

Le Ministre de la Santé Publique informe le public que deux **faux médicaments** sont en circulation au Cameroun avec les mentions suivantes :

	1er Lot	2ème Lot
Nom du produit	CARBAMAZEPINE TABLETS 200 mg	CARBAMAZEPINE TABLETS 200 mg
Présentation	Boite de 1000 comprimés	Boite de 1000 comprimés
Fabricant inscrit sur le conditionnement	Swiss Pharma GIOC NV.Baiva Dist.Ahmedabad 380220 Gujarat, india	Swiss Pharma GIOC NV.Baiva Dist.Ahmedabad 380220 Gujarat, india
Numéro de lot	09C011	09C010
Date de péremption	10/2022	08/2019
Date de fabrication	10/2017	08/2014

Des analyses faites au laboratoire ont confirmé que ces deux lots de médicaments ne contiennent pas de **Carbamazépine**, principe actif attendu et sont des **FAUX MEDICAMENTS**.

La contrefaçon d'un médicament ne garantissant ni sa qualité, ni son innocuité, ni son efficacité, le Ministre de la Santé Publique invite chacun à plus de vigilance et le cas échéant, à arrêter l'utilisation de ce faux médicament, puis à communiquer rapidement l'information à l'Inspection Générale des Services Pharmaceutiques et des Laboratoires : Mail **igpharmacie@yahoo.com** et Mobile **655 97 85 00**.

Le Ministre de la Santé Publique

Dr MANAOUDA Malachie

Site Web: www.minsante.cm/ www.minsante.gov.cm
Ministère de la Santé Publique/Inspection Générale des Services Pharmaceutiques et des Laboratoires (IGSPL)

Supplementary Figure S4: National medical product alert published by the Cameroonian Ministry of Health

API	Total number of samples	Number of stated manufacturers	Stated strength	Dosage Form	Number of samples	Probably substandard samples	Probably falsified samples
Acetylsalicylic acid	22	13	75mg	tablet	4		
			81mg	tablet	5		
			100mg	tablet	2		
			300mg	tablet	3		
			500mg	tablet	6	3	
			500mg	injection	1		
			1000mg	injection	1		
Acetylsalicylic acid + paracetamol	6	2	400mg	tablet	3		
			325+400mg	tablet	1		
			200+400mg	tablet	1		
			300+600mg	tablet	1		
Aciclovir	9	4	400mg	tablet	6		
			800mg	tablet	3		
Albendazole	36	26	200mg	tablet	1		
			400mg	tablet	35	1	
Aminophylline	19	11	100mg	tablet	15		
			250mg/10mL	injection	4		
Amlodipine	50	30	5mg	capsule	1		
			5mg	tablet	23		
			10mg	tablet	26		
Amodiaquine	1	1	200mg	tablet	1		
Amoxicilline	101	49	125mg	tablet	1		
			250mg	tablet	17	3	
			500mg	tablet	15	1	
			250mg	capsule	24	1	
			500mg	capsule	36	1	
			1000mg	injection	1		
			125mg/5ml	dry syrup	2		
			250mg/5ml	dry syrup	5		
Amoxicilline + clavulanic acid	47	33	250+125mg	capsule	1		
			500+125mg	capsule	2		
			1000+200mg	injection	4		
			250+125mg	tablet	2		
			500+62.5mg	tablet	1		
			500+125mg	tablet	25		
			875+125mg	tablet	5	1	
			200+28.5mg/5ml	dry syrup	2		
			250+31.25mg/5ml	dry syrup	1		
			250+62.5mg/5ml	dry syrup	4		
Ampicilline	15	8	500mg	injection	1		
			1000mg	injection	14		
Ampicilline + cloxacilline	6	5	250+250mg	capsule	5		1
			250+250mg	tablet	1		
Artemether	26	20	20mg	injection	4		
			40mg	injection	2		
			80mg	injection	20		
Artemether + lumefantrine	130	62	20+120mg	tablet	66		1
			40+240mg	tablet	1		
			80+480mg	tablet	43		
			15+90mg/5ml	dry syrup	10		
			20+120mg/5ml	dry syrup	10		
Artesunate	28	15	60mg	injection	27		
			120mg	injection	1		

API	Total number of samples	Number of stated manufacturers	Stated strength	Dosage Form	Number of samples	Probably substandard samples	Probably falsified samples
Artesunate + amodiaquine	2	2	100+270mg	tablet	2		
Atenolol	25	16	25mg	tablet	3		
			50mg	tablet	16		
			100mg	tablet	6		
Azithromycin	51	31	250mg	capsule	2		
			250mg	tablet	14		
			500mg	tablet	35		
Benzylpenicillin benzathine	16	6	1000mg	injection	3		
			2.4 mega	injection	13		
Benzylpenicillin procain	1	1	3 mio IU	injection	1		
Benzylpenicillin sodium	2	2	1 mega	injection	2		
Bisoprolol	1	1	10mg	tablet	1		
Captopril	14	9	25mg	tablet	14	4	
Cefalexin	9	7	250mg	capsule	4		
			500mg	capsule	5	1	
Cefixime	42	28	100mg	tablet	2		
			200mg	tablet	23		
			400mg	tablet	16		
			500mg	tablet	1		
Cefotaxime	4	1	1000mg	injection	4		
Cefpodoxime	1	1	200mg	tablet	1		
Ceftriaxone	66	32	250mg	injection	1		
			500mg	injection	1		
			1000mg	injection	64		3
Cefuroxime	3	2	250mg	tablet	1		
			500mg	tablet	2		
Cetirizine	5	4	10mg	tablet	5		
Chloramphenicol	10	7	250mg	capsule	10		
Chloroquine	45	15	100mg	tablet	11	1	5
			250mg	tablet	34		5
Chlorphenamine	16	12	4mg	tablet	16		
Ciprofloxacin	85	52	200mg/100ml	injection	3		
			250mg	tablet	3		
			500mg	tablet	77	2	
			750mg	tablet	1		
			500mg	capsule	1		
Clarithromycin	14	10	500mg	tablet	14		
Clindamycin	3	3	300mg	capsule	2		
			300mg	tablet	1		
Clomifene	7	4	50mg	tablet	7		
Cloxacillin	45	18	1000mg	injection	1	1	
			500mg	injection	7		
			250mg	capsule	13		
			500mg	capsule	21	1	
			500mg	tablet	3		
Dapsone	3	1	100mg	tablet	3		
Diclofenac	51	40	25mg	injection	1		
			75mg	injection	9		
			50mg	tablet	30	4	
			100mg	tablet	11	1	
Diclofenac + paracetamol	5	5	50+325mg	tablet	1		
			50+500mg	tablet	4		1
Diclofenac + paracetamol + chlorphenamine	1	1	50+500+4mg	tablet	1		

API	Total number of samples	Number of stated manufacturers	Stated strength	Dosage Form	Number of samples	Probably substandard samples	Probably falsified samples
Dihydroartemisinin + piperaquine	7	4	40+320mg	tablet	7		
Doxycycline	37	13	100mg	capsule	11	1	
			100mg	tablet	25		
			200mg	tablet	1		
Erythromycin	34	21	250mg	tablet	17	3	
			500mg	tablet	17		
Fluconazole	10	9	200mg	capsule	6		
			150mg	tablet	1		
			200mg	tablet	2		
			200mg/100ml	injection	1		
Furosemide	34	23	20mg/ml	injection	1		
			20mg/2ml	injection	4		
			40mg	tablet	29		
Gentamicin	12	11	80mg/2ml	injection	12		
Glibenclamide	27	16	5mg	tablet	27		
Griseofulvin	16	9	250mg	tablet	6		
			500mg	tablet	10		
Hydrochlorothiazide	26	13	25mg	tablet	18		
			50mg	tablet	8	1	1
Levofloxacin	32	25	250mg	tablet	1		
			500mg	tablet	30		
			750mg	tablet	1		
Lisinopril	12	5	5mg	tablet	1		
			10mg	tablet	1		
			20mg	tablet	10		
Mebendazole	27	16	100mg	tablet	23	1	
			400mg	tablet	1		
			500mg	tablet	2		
			500mg/100ml	injection	1		
Mefenamic acid	2	1	250mg	tablet	1		
			500mg	tablet	1		
Metformin	59	35	500mg	tablet	45	2	
			750mg	tablet	1		
			850mg	tablet	9		
			1000mg	tablet	4		
Metoclopramide	8	8	10mg	tablet	7		
			10mg	injection	1		
Metronidazole	66	41	500mg/100ml	injection	4		
			200mg	tablet	22		
			250mg	tablet	28	1	
			400mg	tablet	5		
			500mg	tablet	7	1	
Naproxen	2	2	500mg	tablet	2		
Nifedipine	26	20	10mg	tablet	4		
			20mg	tablet	20		
			25mg	tablet	1		
			30mg	tablet	1		
Ofloxacin	13	10	200mg	injection	1		
			200mg	tablet	8		
			400mg	tablet	4		
Omeprazole	34	25	20mg	capsule	22		
			20mg	tablet	1		
			40mg	injection	11		

API	Total number of samples	Number of stated manufacturers	Stated strength	Dosage Form	Number of samples	Probably substandard samples	Probably falsified samples
Paracetamol	147	64	400mg	capsule	1		
			10mg/ml	injection	2		
			300mg/2ml	injection	2		
			1000mg/100ml	injection	8		
			100mg	tablet	8		
			300mg	tablet	1		
			500mg	tablet	125	6	
Phenoxymethylpenicillin	23	6	250mg	tablet	21	1	
			500mg	tablet	2		
Piperaquine	1	1	375mg	tablet	1	1	
Praziquantel	4	3	600mg	tablet	4		
Prednisolone	50	26	5mg	tablet	50	2	
Proguanil	5	4	100mg	tablet	5		1
Quinine	74	39	300mg/ml	injection	1		
			500mg	injection	3		
			600mg/2ml	injection	7		
			100mg	tablet	9		
			300mg	tablet	41	2	7
			400mg	tablet	1		
			500mg	tablet	11	1	
			800mg	tablet	1		1
Ranitidine	6	4	50mg	injection	1		
			150mg	tablet	1		
			300mg	tablet	4		
Salbutamol	7	6	2mg	tablet	2		
			4mg	tablet	5		
Simvastatin	5	3	40mg	tablet	5		
Sulfadoxine + pyrimethamine	20	13	500+25mg	tablet	20	1	
Sulfamethoxazole + trimethoprim	68	36	100+20mg	tablet	6		
			400+80mg	tablet	55	4	8
			800+160mg	tablet	7		
Tetracycline	2	2	250mg	tablet	1		
			250mg	capsule	1		
Total	**1919**	**495**			**1919**	**54**	**34**

Supplementary Table S1: Overview of all samples included in this study

No.	Country of discovery	Declared active pharmaceutical ingredient (API)	Name of product	Batch no	Expiry date	Stated manufacturer	Stated country of origin	Quality deficiency
1	Cameroon	Ampicillin trihydrate, Cloxacillin sodium	Amcloxin 250/250mg cps	A015050	04.20	MAXHEAL Pharmaceuticals(India)Ltd.	India	declared API absent
2	Chad	Artemether, Lumefantrine	COMBIART 20/120mg tbl	7225119 (carton) 7225500 (blister)	08/2021	Strides ARCOLAB LIMITED	India	declared API absent
3	DR Congo	Ceftriaxone sodium	Ceftriaxone 1g inj	J-104	06/2021	LDP – Laboratoirios TORLAN S.A	Spain	API content 23.5 % of stated content
4	DR Congo	Ceftriaxone sodium	Ceftriaxone 1g inj	J-104	06/2020	LDP - Laboratoirios TORLAN S.A	Spain	API content 23.8 % of stated content
5	DR Congo	Ceftriaxone sodium	Ceftriaxone 1g inj	M-63	01/2022	LDP - Laboratoirios TORLAN S.A	Spain	total vial content less than 230 mg
6	DR Congo	Chloroquine	CLOROQUINE 250mg tbl	1605059	04/ 2023	Dawa Limited	Kenya	declared API absent; 126.5 mg metronidazole
7	Cameroon	Chloroquine phosphate	CHLOROQUINE PHOSPHATE 250mg tbl	EBT 2512	10/2022	Astral pharmaceuticals	India	declared API absent
8	Nigeria	Chloroquine phosphate	ENI-QUIN 250mg tbl	CQLL	July 2023	ENITOP PHARMACEUTICAL Nig. L	Nigeria	declared API absent
9	Cameroon	Chloroquine phosphate	CHLOROQUINE PHOSPHATE 100mg tbl	h-659	07/04/21	Enitop Pharmaceutical Nig. Ltd.	Nigeria	declared API absent
10	Cameroon	Chloroquine phosphate	Chloroquine Phosphate 100mg tbl	660	08/2022	Jiangsu Pharmaceuticals Inc.	China	API content 21.7 % of stated content
11	Cameroon	Chloroquine phosphate	Chloroquin Phosphate 100mg tbl	660	04/2023	Jiangsu Pharmaceuticals Inc.	China	declared API absent; 14.1 mg metronidazole
12 &13	Cameroon	Chloroquine phosphate	Chloroquine Phosphate 100mg tbl	660	05/2021	Jiangsu Pharmaceuticals Inc.	China	declared API absent; 35.7 mg paracetamol
14	Cameroon	Chloroquine phosphate	Chloroquine phosphate 250mg tbl	660	09/2022	Jlangsu Pharmaceuticals Inc.	China	declared API absent; 14.6mg metronidazole 1.6 mg paracetamol
15	Cameroon	Chloroquine phosphate	SA'A QUINE 250mg tbl	SQ 19024	10/2022	SA'A Pharmaceutical Products Limited.	Nigeria	API content 12.2 % of stated content

No.	Country of discovery	Declared active pharmaceutical ingredient (API)	Name of product	Batch no	Expiry date	Stated manufacturer	Stated country of origin	Quality deficiency
16	Cameroon	Hydrochlorothiazide	HYDROCHLOROTHIAZIDE 50mg tbl	16G04	30/05/2021	LABORATOIRES STEROP	Belgium	declared API absent; 5mg glibenclamide
17	Cameroon	Paracetamol, Diclofenac sodium	Gabamol 500/50mg tbl	MP9820	09/2023	McCoy Pharma Pvt. Ltd.	India	declared API diclofenac absent (95.2% paracetamol)
18	Nigeria	Proguanil	PROGUANIL 100mg tbl	P.626	05 / 2021	PHARMAMED	Malta	only traces of API contained
19	DR Congo	Quinine	QUININE 300mg tbl	T459Q	10/2022	-	India	declared API absent
20	Chad	Quinine	QUININE SULPHATE 300mg tbl	7711006	7/2021	Enitop Pharmaceutical Nig. Ltd.	Nigeria	declared API absent
21	Central Afr. Rep.	Quinine sulphate	QUININE SULPHATE 300mg tbl	7711006	7/2021	Enitop Pharmaceutical Nig. Ltd.	Nigeria	declared API absent
22	Central Afr. Rep.	Quinine sulphate	QUININE SULPHATE 300mg tbl	7711006	05/2022	Enitop Pharmaceutical Nig. Ltd.	Nigeria	declared API absent
23	Central Afr. Rep.	Quinine sulphate	QUININE SULPHATE 800mg tbl	00952005	12/2020	Pharmachim	Bulgaria	declared API absent
24	Chad	Quinine sulphate	QUININE SULPHATE 300mg tbl	44680	04/ 2021	Remedica Ltd	Cyprus	declared API absent; 12 mg chloroquine
25	DR Congo	Quinine sulphate	Quinine Sulphate 300mg tbl	022157	10/2021	RENE INDUSTRIES LTD.	Uganda	declared API absent
26	Chad	Quinine sulphate	QUININE SULPHATE 300mg tbl	8858	-	WEIDER FARMASOYTSIKE A/S	Norway	declared API absent
27	Chad	Sulfamethoxazole + Trimethoprim	Arveltrim 80/400mg tbl	A.M 420	06/22	Arvel Marris Pharmaceutical Nig Ltd.	Nigeria	declared API absent
28	Nigeria	Sulfamethoxazole + Trimethoprim	GILTRIM 80/80mg tbl	SCT:002	Jan. 2023	GIL INDUSTRIES LTD.	Nigeria	declared API absent
29	Chad	Sulfamethoxazole + Trimethoprim	KOLLYTRIM 80/400mg tbl	-	-	KOLLINTON PHARMACEUTICAL IND. LTD.	-	declared APIs absent; small amount paracetamol
30	Chad	Sulfamethoxazole + Trimethoprim	MEDITRIM 80/400mg tbl	C 10M	03/23	MEDIVILLE PHARMACEUTICAL NIG. LTD.	Nigeria	declared API absent
31	Chad	Sulfamethoxazole + Trimethoprim	Optrim 80/400mg tbl	OLL20	12/23	OPTIMAL LAB LTD.	Nigeria	declared API absent

No.	Country of discovery	Declared active pharmaceutical ingredient (API)	Name of product	Batch no	Expiry date	Stated manufacturer	Stated country of origin	Quality deficiency
32	Chad	Sulfamethoxazole + Trimethoprim	POLETRIM 80/400mg tbl	409.38	07/23	MAOBISON INTER – Link & ASSOCIATES LTD.	Nigeria	sulfamethoxazole 47.7 % trimethoprim 21.2 % of stated content
33	Chad	Sulfamethoxazole + Trimethoprim	SA'A TRIM 80/400mg tbl	ST191097	10/2022	SA'A Pharmaceutical Products Ltd	Nigeria	sulfamethoxazole 17.6 % trimethoprim 16.3 % of stated content
34	Chad	Sulfamethoxazole + Trimethoprim	SA'A TRIM 80/400mg tbl	-	-	SA'A Pharmaceutical Products Ltd	Nigeria	API content less than 25% (see sample above)

Supplementary Table S2: Medicine samples identified in this study as probably falsified.
In the cases no. 12 & 13, two samples of this medicine were identified independently in the course of this study.

Verification of the Active Pharmaceutical Ingredient in Tablets Using a Low-Cost Near-Infrared Spectrometer and Principal Component Analysis

Short title: **Verification of the Active Ingredient in Tablets Using NIR Spectroscopy**

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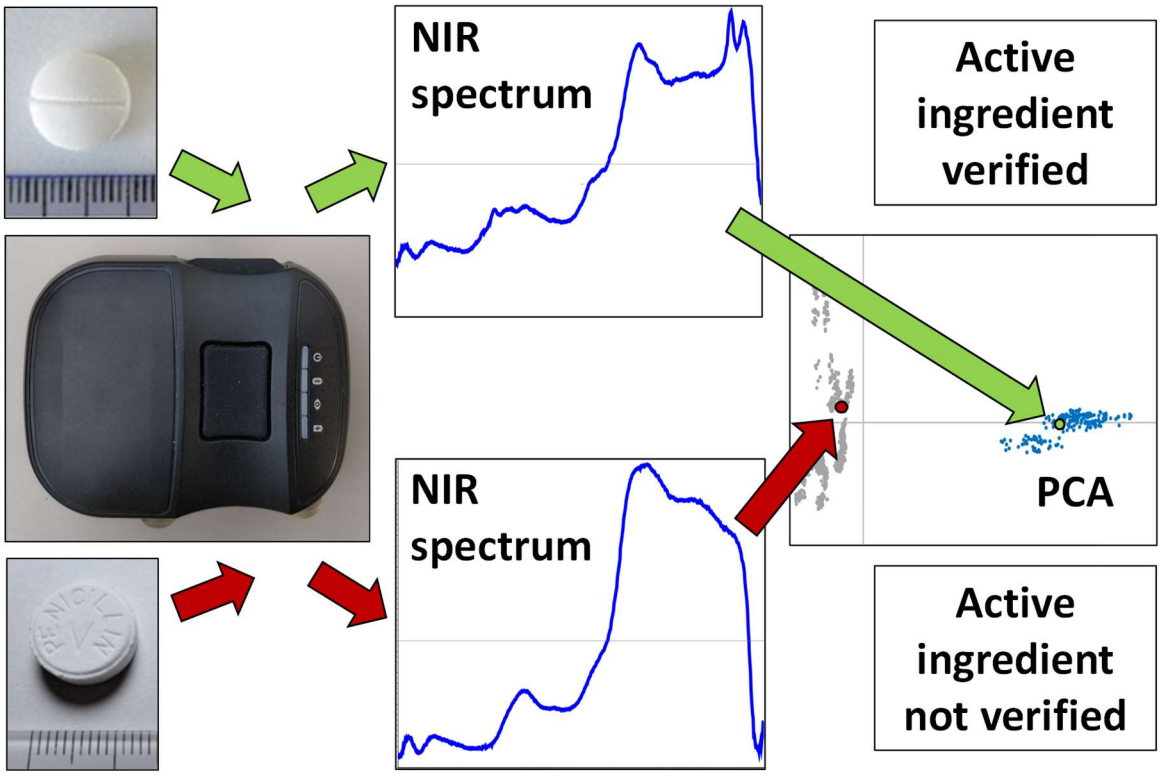
Abstract

Screening technologies for the identification of falsified medicines are important especially in low- and middle-income countries. Near-infrared (NIR) spectroscopy offers striking advantages for this purpose, including speed of analysis and independence from reagents or solvents. A key limitation, however, is the need for large and regularly updated libraries of NIR reference spectra required for the authentication of different brands of pharmaceuticals. In the present study, a method was developed for the verification of the presence of the declared active pharmaceutical ingredients (APIs) in tablet preparations, irrespective of brands. A low-cost NIR spectrometer was used, and multivariate data analysis comprised a series of nine principal component analyses (PCAs), arranged in a binary decision tree. The method was developed and tested using 170 pharmaceutical products, most of them collected in medicine quality studies in African countries and including 20 falsified medicines. Tablets containing penicillin V, sulfamethoxazole/trimethoprim, ciprofloxacin, furosemide, metronidazole, metformin, and hydrochlorothiazide could be reliably distinguished. Falsified medicines containing no API, incorrect APIs, or grossly incorrect amounts of the declared

APIs could be readily identified by projection of their spectra onto the PCA models. The scores plots of principal component 1 (PC-1) and PC-2 were found to be sufficient for this purpose. The separation of NIR spectra in PCA was influenced by the mass percentage of the APIs in the total weight of the respective tablets. Therefore, tablet weight should be measured and considered in addition to the recording of NIR spectra. Future research can further expand the range of products covered, and the present method may become a useful approach in screening programs for falsified medicines in low resource settings.

Graphical abstract



Introduction

Substandard and falsified (SF) medicines expose patients worldwide to the risk of prolonged illness, economic loss or even death, due to ineffective therapy or toxic effects. With an estimated

prevalence of 10.5% SF medicines, low- and middle-income countries (LMICs) are especially affected.[1,2]

In LMICs, the national medicine regulatory authorities and other stakeholders in medicine quality assurance are often underfunded, and they lack both personnel and access to fully equipped medicine quality control laboratories. This allows medical products to enter the market without sufficient quality control, or even through illicit channels.[3,4] Rapid, low-cost medicine quality screening technologies have therefore been recommended in the fight against SF medicines in LMICs,[5] and several countries have started implementing such programs.[6-8] Easy-to-use screening technologies may be used also by personnel specialized in other disciplines such as such as supply chain staff and health care workers. We recently reported on the use of the Global Pharma Health Fund (GPHF)-Minilab® by faith-based medicine supply organizations in Africa.[9] The GPHF-Minilab® employs thin-layer chromatography for the identification of active pharmaceutical ingredients (APIs), and is currently the most widely used medicine quality screening tool worldwide.[10,11]

In recent years, portable near-infrared (NIR) spectrometers have received attention as medicine quality screening technologies.[10,12-16] They require neither consumables nor sample preparation/destruction, the time required for analysis is very short, and only minimal training is necessary for their operation.[10,17,18] However, raw spectra are difficult to interpret as such, and usually a multivariate evaluation of the spectral data must be carried out, e.g. by principal component analysis (PCA).[19-22]

NIR spectra of pharmaceutical products depend both on the chemical composition, i.e. on the APIs as well as on the excipients, and on physicochemical characteristics such as crystallinity, particle size, moisture etc.[19] Extensive research has been carried out on the use of NIR spectroscopy for the discrimination of authentic, usually branded products of a given manufacturer from falsified products, even in cases when both have a similar composition.[23-30] Obviously, this approach requires a complete and up-to-date library of the NIR spectra of all authentic products of interest, and the creation of such libraries requires considerable effort.[19,31] For many stakeholders involved in

medicine procurement in LMICs, such as the faith-based medicine supply organizations mentioned above,[9,32] complete and up-to-date libraries of the NIR spectra of all relevant authentic products are impossible to obtain, since these organizations procure generic medicines at low prices from a large and ever-changing number of manufacturers located in India, China and many other countries.[9,33] Unfortunately, the occurrence of falsified medicines which contain no APIs, grossly insufficient amounts of APIs or even incorrect APIs remains a constant problem in such settings.[1,34]

In the present study, we investigated whether a low-cost NIR spectrometer combined with basic chemometric tools may offer a useful technology for the verification of the APIs in pharmaceutical tablets, irrespective of specific brands, in low-resource settings. While some previous studies have addressed API verification by NIR spectroscopy, they used more expensive equipment or included only small numbers of products.[35-37] We now investigated 170 pharmaceutical products. 110 of these had been collected in African countries, and all of these had been investigated in our laboratory for the identity, quantity and dissolution of the APIs using compendial high-performance liquid chromatography (HPLC) methods.[9,33,38] The present study also included 20 products which had been identified as falsified. We employed the low-cost handheld NIR-S-G1 spectrometer (InnoSpectra, Taiwan) which has been evaluated in previous studies for its use in low-resource settings.[28-30,39-41] For chemometric analysis, we took advantage of the simplicity of PCA and demonstrated that a sequence of PCA models arranged in a binary decision tree is suitable for the verification of the contained APIs, and can be used for the identification of falsified medicines.

Theory

1. Principal component analysis

Principal component analysis[21,22] is an unsupervised chemometric method that reduces dimensionality of a data matrix *X* (IxJ; with I being the rows or samples and J being the columns or data points measured) by decomposing it into a scores matrix *T* (IxA; with A being the number of

principal components calculated), a loadings matrix *P* (JxA) and a residuals matrix *E* (IxJ) following the equation:

$$X = TP^t + E \quad (1)$$

The principal components are orthogonal, i.e. uncorrelated to each other, and their number can be chosen depending on the desired application. Vectors out of the matrices *T* and *P* can be visualized as scores and loadings plots. In particular, the scores plots allow a simple and intuitive understanding of the relationships between the data: closely related data form clusters in the scores or loadings plots, respectively. Many algorithms for chemometric discrimination of groups have been developed.[20,42] PCA itself is not a discrimination algorithm, but often used for initial data exploration. However, in the present study we have successfully used the advantages of PCA, such as the graphical evaluability of the results which is intuitively understandable even to non-experts in spectroscopy, for our objective of API verification in tablets.

2. Decision trees

Decision trees are tree-like representations of a hierarchical sequence of decisions.[43] Starting with an initial decision at the root node and continuing with subsequent decisions at each internal node, the decision tree branches out until each branch ends in a so-called leaf, which at the same time represents the result. In the case of binary decision trees, at each node two outcomes are possible. We arranged a sequence of PCAs in a binary decision tree for API verification in tablets, each PCA representing one node. In each PCA, the presence of a particular API is queried. If the API is successfully verified, the decision tree ends. Otherwise, the next decision node is passed through, and the process continues with the next PCA and the next API. Figure 1 illustrates the binary decision tree developed in this study.

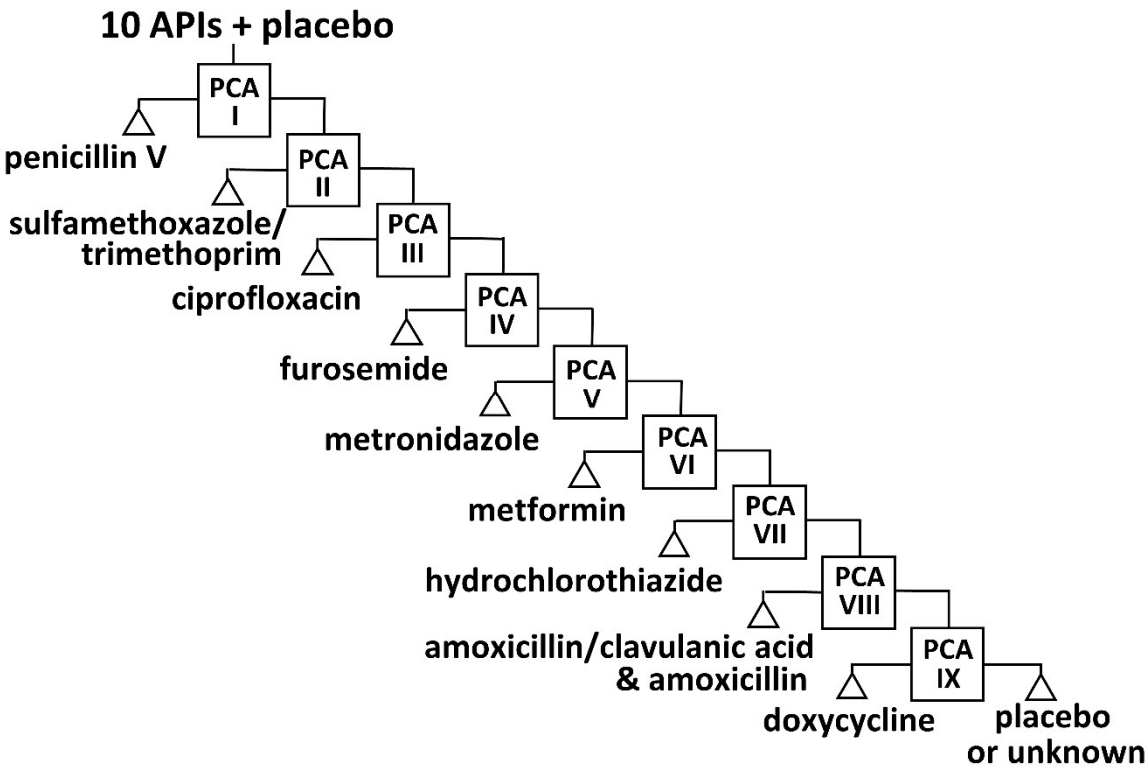


Figure 1: Principal component analysis (PCA) models arranged in a binary decision tree for active pharmaceutical ingredient verification.

Methods

1. Collection of pharmaceutical products

Table 1 shows the types of pharmaceutical products included in this study, and Supplementary Table S1 gives information on each of the investigated products. Of these, 105 products had been collected in Cameroon, the Democratic Republic (DR) of Congo and Chad during recently published medicine quality studies of our group; the procedures for their collection are described in the respective publications.[9,33,38] Additionally, five falsified medicines had been purchased in an ongoing study in August 2021 in Nigeria, from different commercial suppliers in Anambra and Enugu states. These medicines had been shipped to Germany by commercial courier services, and were stored in Tübingen University at 21°C until analysis. Most of the samples collected in African countries had

been sold without a package leaflet, and no information was available on the excipients they contained.

Furthermore, 60 products were purchased from the pharmacy of Tübingen University Hospital and from a licensed retail pharmacy in Tübingen, and represented medicines licensed and marketed in Germany. If more than one brand of a certain type of medicine was purchased in Germany (Table 1), brands were selected which differed from each other in their excipient composition.

Table 1: *Overview of the pharmaceutical products investigated in this study. All products were formulated as tablets.*

Stated API(s)	Stated strength (mg)	Number of products:					Collected in:		Total number	Mass % API(s) [$]
		Training set	Validation set	Different batch	Different strength	Falsified	Africa*	Germany		
Medicine types included in training and validation sets:										
Amoxicillin	500	6	2	3	2	0	11	2	13	52-82
Amoxicillin/ Clavulanic acid	500/125	5	2	0	1	2	8	2	10	45-55/ 8-13
Ciprofloxacin	500	6	2	1	2	0	7	4	11	59-73
Doxycycline	100	6	2	3	0	0	10	1	11	32-68
Furosemide	40	6	2	2	0	4	12	2	14	21-38
Hydrochlorothiazide	50	3	1	1	5	1	7	4	11	12-50
Metformin	500	6	2	2	4	0	12	2	14	77-94
Metronidazole	250	6	2	3	3	2	13	3	16	34-85
Penicillin V	250	6	2	2	3	1	12	2	14	71-89
Sulfamethoxazole/ Trimethoprim	400/80	6	2	1	1	8	16	2	18	58-77/ 12-15
Placebo	-	3	1	0	0	0	0	4	4	
Total		**59**	**20**	**18**	**21**	**18**	**108**	**28**	**136**	
Further medicine types:										
Falsified "chloroquine" [§]						2	2	0	2	
30 additional APIs [#]						0	0	32	32	32-92
Grand total						**20**	**110**	**60**	**170**	

** Cameroon, Democratic Republic of Congo, Chad, and Nigeria*[9,33,38]

[$] Proportion of active pharmaceutical ingredient(s) in total tablet weight, expressed as percent. Calculated including the tablets of training and validation sets and the tablets of different batches and strength but excluding the falsified products.

[§] Two products labeled as chloroquine tablets, containing no chloroquine but 124 mg or 14 mg of metronidazole, respectively[38]

[#] 32 products containing 30 additional active pharmaceutical ingredients from the WHO Essential Medicines List 2021,[44] *purchased in Germany and listed in Supplementary Table S1.*

Supplementary Table S1 gives detailed information on each of the 170 products.

2. HPLC Analysis

Compendial analysis was carried out according to the monographs of the United States Pharmacopeia 2018 (USP 41) for the respective medicines. This comprised identification and quantification of the API as well as dissolution testing. For the 105 products collected in Cameroon, the DR Congo and Chad, the procedures and results of this testing have been published.[9,33,38]

Additionally, five falsified products labelled as sulfamethoxazole/trimethoprim tablets 400/80 mg had been collected in Nigeria (see above). They were investigated by HPLC-UV analysis for identity and quantity of the APIs, using an Agilent 1260 Infinity II HPLC system (Agilent Technologies, Santa Clara, California, USA), with columns and solvents as specified in the USP 41. Certified pharmaceutical secondary reference standards from Sigma-Aldrich (St. Louis, Missouri, USA) were used. The results of this analysis are listed in Supplementary Table S1.

3. Creation of training, validation, and challenging sets

As shown in Table 1, the products were split into a training set for PCA model creation, a validation set for external model validation, and various challenging sets. For inclusion in the training and validation sets, in most cases eight products of the same API(s) and strength could be obtained. Using the RAND function of MS Excel®, two of these products were selected randomly for the validation set. The assignment of different products to challenging sets C1 – C7 is shown in Table 2.

Table 2: *Challenging sets for testing the PCA models developed in this study*

Set	**Products**
C1	Different batches of brands comprised in the training set
C2	Products of different strength than those comprised in the training set
C3	Tablet preparations of 30 additional APIs not contained in the training set
C4	Falsified medicines containing no API at all
C5	Falsified medicines containing a different API instead of the declared one
C6	Falsified medicines containing the correct amount of the declared API but carrying a label misrepresenting the source or the expiry date of the product
C7	Falsified medicines containing the declared API in an incorrect amount

4. NIR spectrometers

Five NIR-S-G1 spectrometers (devices A-E), produced by InnoSpectra (Hsinchu, Taiwan), were purchased; however, device A had to be excluded due to a technical defect. As described by Crocombe,[45] the NIR-S-G1 uses the digital light processing (DLP) technology of Texas Instruments (Dallas, Texas, USA), i.e. it contains a digital micromirror device and a single 1mm uncooled InGaAs detector.[46] It performs measurements in diffuse reflection mode, in the wavelength range of 900-1700 nm. A performance evaluation of NIR-S-G1 devices, including wavelength and photometric accuracy and repeatability, as well as spectroscopic noise, has been published recently.[47]

5. Software

ISC WinForms SDK GUI (v3.7.2, InnoSpectra, Hsinchu, Taiwan) was used to control the NIR-S-G1 devices. The Aspen Unscrambler® (V12.1, Aspen Technology Inc, Bedford, Massachusetts, USA) was utilized for data preprocessing and PCA. Both the Aspen Unscrambler® and Microsoft Excel® (V16.0, Microsoft Corporation, Redmond, Washington, USA) were employed for data visualization.

6. Spectra acquisition

For spectra acquisition, the NIR-S-G1 device was fixed in an upright position, either by using a 3D printed device holder (Supplementary Fig. S1) or by attaching self-adhesive plastic feet to the rounded bottom of the device. The device was connected to a computer using a USB cable. Tablets were removed from blisters and placed directly onto the sapphire scan window for spectra acquisition. Any vibrations or movements were avoided during the measurement.

The spectrometer was switched on one hour before the first measurement. Ten initial blind scans were then performed to ensure a system temperature between 30 and 40 °C for all measurements. The employed instrument settings are given in Supplementary Table S2. Spectra were saved as comma separated values (CSV) files.

Four spectra were acquired of each tablet, two each from the bottom and the top side, with a vertical flip in between the two measurements. Of each product, two tablets were investigated. Training set products were first measured by investigator BM using the devices C and D. After two weeks, measurement was repeated by investigator GG using devices C and B. Thereby, 32 spectra were acquired from each training set product. Another two weeks later, validation set products were measured by investigator JG using devices C and E. Thereby, 16 spectra were acquired from each validation set product. In this way, procedures were validated by measurements carried out on different days, by different persons and using different pieces of equipment, following the recommendations of the International Council for Harmonisation (ICH)[48] and the USP.[49]

Products of the challenging sets were measured by investigator BM using device C. Eight spectra were acquired from each product. However, for one challenging set product (a falsified medicine), only one tablet was left after chemical analysis, and therefore only four spectra were recorded.

Between measurements, tablets were stored at 21 °C in a dark place, in low-density polyethylene zip bags which were placed in a high-density polyethylene box together with desiccant silica gel.

7. Spectral data pretreatment

The raw NIR spectra were pretreated using a standard normal variate (SNV) transformation[50] followed by application of a Savitzky-Golay algorithm[51] for smoothing and derivative calculation (second derivative, second order polynomial). According to the NIR-S-G1 manufacturer's information,[46] the photosensitivity of the device is low at wavelengths <950 nm, and it changes with temperature at wavelengths >1650 nm, resulting in noise in these ranges. Furthermore, the common excipient talcum exhibits a prominent absorption peak at 1391 nm (Supplementary Fig. S2) which interfered with the desired classification of spectra according to the APIs (rather than according to excipients). Therefore, only the spectral ranges 950-1375 nm and 1405-1650 nm were used in PCA.

8. Principal component analysis and data projection

PCA models were computed using pretreated, mean-centered spectral data. The singular value decomposition (SVD) algorithm was applied with cross validation. As explained in the Results, a sequence of nine PCA models was established. Spectra of validation set products or challenging set products were projected onto each of the PCA models successively according to Eq. 1. The results were visualized in the respective PC-1 vs. PC-2 scores plots.

9. Definitions

In this study, the current definitions of substandard and falsified medicines by the World Health Organization (WHO) were used.[1,2] Where necessary, additionally the criteria suggested by Hauk et al.[52] were applied.

Results

1. Investigated pharmaceutical products

Table 1 shows the types of medicines investigated in the present study. All of these are listed in the 2021 WHO Model List of Essential Medicines,[44] and all were formulated as tablets. Most of these products had been collected in Cameroon, the Democratic Republic of Congo (DRC), Chad or Nigeria, and had been analyzed at Tuebingen University.[9,33,38] Only products which complied with the USP specifications for identity, assay (= quantity) and dissolution of the API(s) were included into the training and validation sets. Additional products were purchased in Germany. For inclusion into training and validation sets, eight different brands of identical strength could be obtained for most types of medicines (Table 1). Additionally, four brands of placebo products were included.

Challenging sets were created from tablets belonging to different batches of the brands comprised in the training set, or from tablets of different strength than those comprised in the training set as shown in Table 2. These products had been confirmed to comply with pharmacopeial specifications. Furthermore, challenging sets were created from tablets which had been identified as falsified in the course of the

mentioned medicine quality studies[9,33,38] or in the present study (see Methods). Finally, a challenging set was created from tablet preparations of 30 additional APIs which were contained in the 2021 WHO Model List of Essential Medicines[44] and were commercially available in Germany.

As shown in Supplementary Table S1, 143 of the products were white tablets, while the remaining 27 products were green (1), yellow (12), blue (2), brown (2), beige (3), grey (1), pink (4) or orange (2). In twelve products, the coating had a different color than the tablet core.

A total of 45 products showed smooth top and bottom sides, 83 had a score line or an embossing on one side, and 40 on both sides. For two falsified products, tablets from the same container showed different kinds of embossing and score lines.

In total, samples of 170 different pharmaceutical products were included into this study. Supplementary Table S1 gives detailed information about each of these medicines, including brand names and stated manufacturers, as well as quality deficiencies found for the falsified samples.

2. Acquisition of NIR spectra

Acquisition of the NIR spectra for training and validation sets followed the recommendations of the ICH[48] and the USP.[49] Therefore, independent validation samples were analyzed by a different person on a different spectrometric device, and at a different time point compared to the training set (see Methods). In total, 2928 spectra were recorded and included into the data analysis. The complete 2928 raw spectra are given in Supplementary Table S2.

3. Visual comparison of acquired NIR spectra

Figure 2 shows the NIR spectra of different brands of penicillin V, amoxicillin, ciprofloxacin, and placebo tablets. NIR spectra of tablets depend both on the chemical composition, i.e. on the APIs as well as on the excipients, and on physicochemical characteristics such as crystallinity, particle size, moisture etc.[19] Nevertheless, Fig. 2 clearly suggests that for tablets containing e.g. penicillin V or ciprofloxacin, the spectral features in the investigated wavelength range predominantly depend on the respective API. Amoxicillin tablets show fewer distinctive absorption peaks in this wavelength

range, and the investigated placebo tablets showed even less. Supplementary Fig. S2 depicts the spectra of all products included into the training set of this study. Most of them show API-related differences to each other and to the spectra of the placebo tablets. This suggests that spectra recorded on the low-cost NIR-S-G1 device may allow the verification of the presence and type of APIs contained in tablet products, provided the respective API shows absorption bands in the range of 900-1700 nm. Interestingly, the spectra of penicillin V and amoxicillin tablets are very different (Fig. 2), despite the considerable chemical similarity between these two beta-lactam antibiotics.

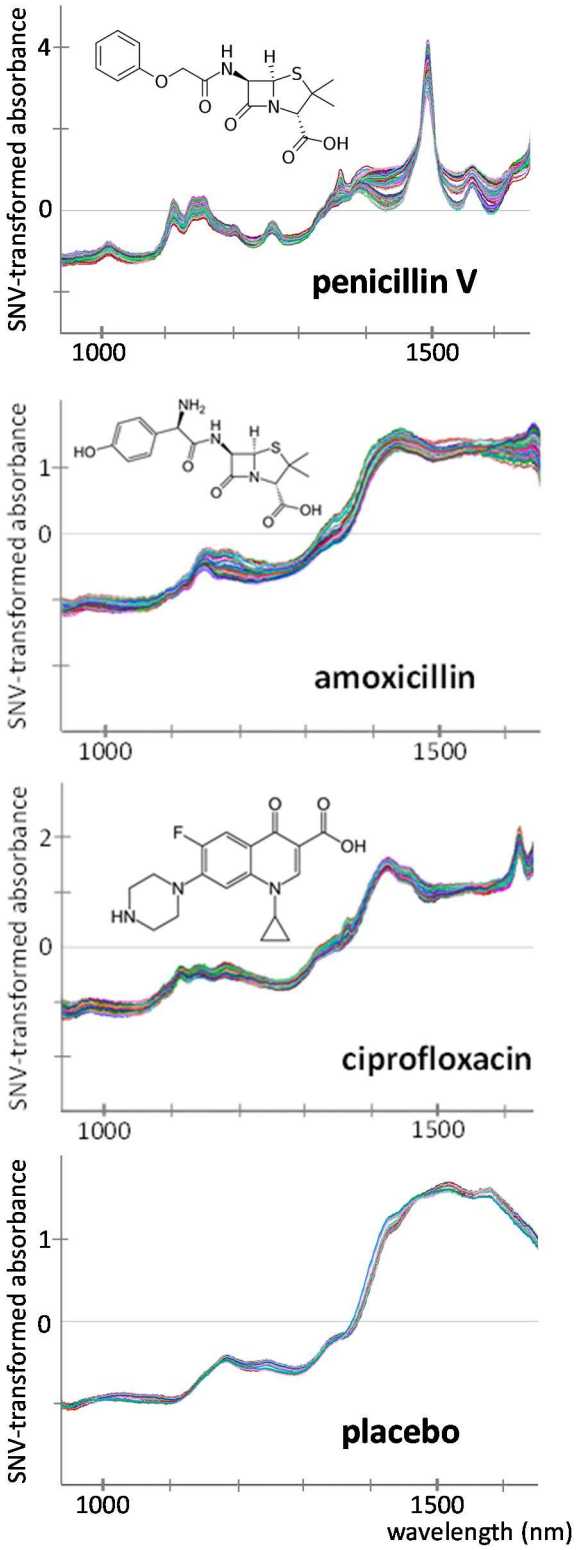


Figure 2: Near-infrared spectra (950 - 1650 nm) of penicillin V tablets (11 products), amoxicillin tablets (10 products), ciprofloxacin tablets (10 products) and placebo tablets (4 products).

4. Building a hierarchical binary decision tree with PCA models for verification of the presence of different APIs

The NIR spectra were pretreated as described in the Methods. Subsequently, a sequence of nine PCA models (from PCA I to PCA IX) was established, and arranged in a binary decision tree (see Theory section). For the creation of the first PCA model (PCA I), all 1888 training set spectra were used, and the results were displayed as principal component 1 (PC-1) vs. PC-2 scores plot (Fig. 3A). The data points of penicillin V tablets were clearly separable from all other data points. For the computation of the second PCA model (PCA II), all spectra of penicillin V tablets were removed from the calculation. In the plot of PCA II (Fig. 3B), the data points of sulfamethoxazole/trimethoprim tablets formed a cluster, allowing their removal from the calculation before computing PCA III. In the scores plot of PCA III (Fig. 3C), the data points of ciprofloxacin tablets were clearly separable from all other data points, while they had overlapped inseparably with the data points of other APIs at the stage of PCA I (Fig. 3A).

In the same manner, subsequent PCA models (PCA IV – PCA VII) were established which allowed to group the spectra of tablets containing furosemide, metronidazole, metformin, or hydrochlorothiazide (Fig. 3D-G), respectively, with clear spatial separation of their data points in the PC-1/PC-2 scores plots from the spectra of tablets containing other APIs.

After removal of the spectra of the tablets containing the above-mentioned APIs, the subsequent PCA VIII showed an overlap of the data points of amoxicillin and amoxicillin/clavulanic acid tablets (Fig. 3H). Even when the PCA was recalculated without the spectra of doxycycline and placebo tablets (Supplementary Fig. S3), no separation could be achieved, neither using PC-1 and PC-2, nor using PC-3 and PC-4. Therefore, the present method is not suitable to reliably distinguish amoxicillin tablets from amoxicillin/clavulanic acid tablets.

Before calculating the final PCA IX, the spectra of amoxicillin and amoxicillin/clavulanic acid tablets were removed from the dataset. The resulting scores plot (Fig. 3I) showed a separation of the data points of the investigated doxycycline and placebo tablets. However, the observed differences in the

PC scores between these two types of tablets were small. In subsequent investigations (see below), data points of some tablets containing no API were projected to a position within the data points of doxycycline tablets. Therefore, the present method is not able to reliably distinguish doxycycline tablets from tablets containing no API.

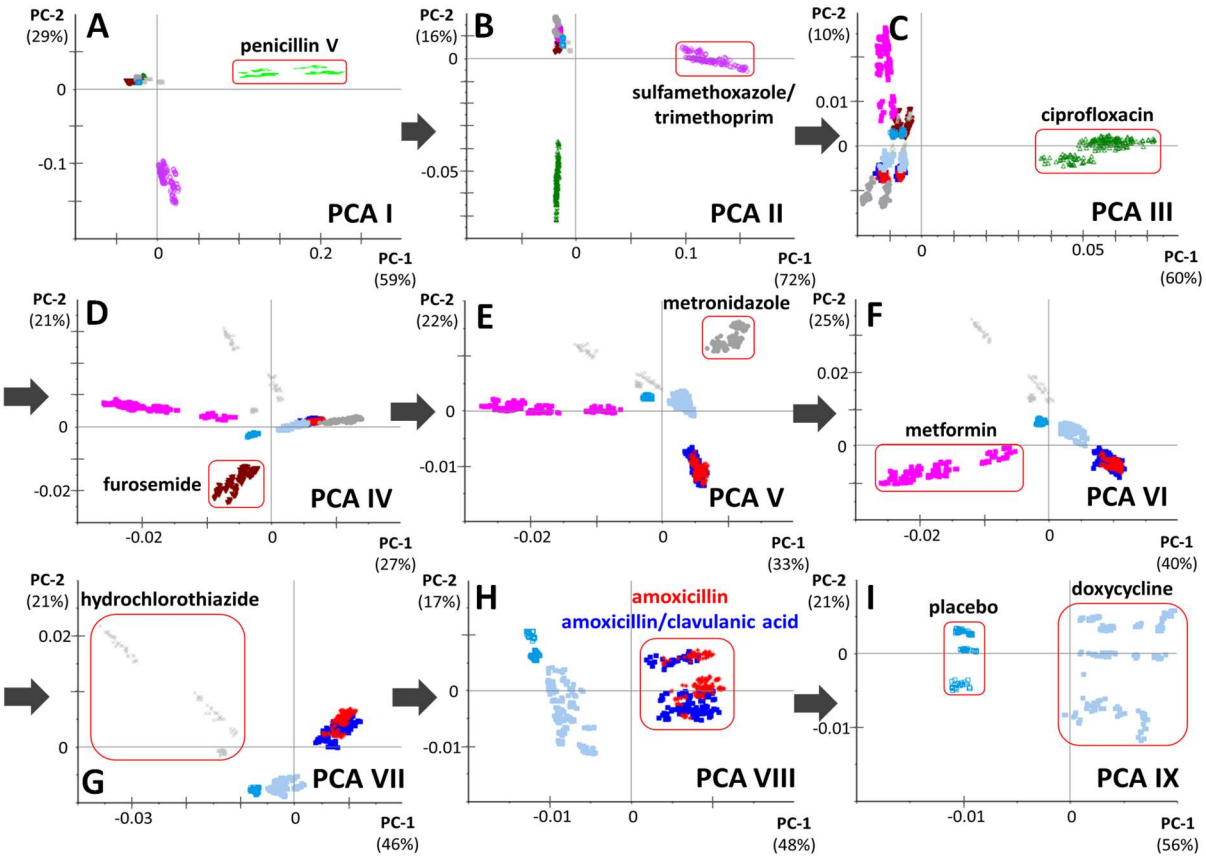


Figure 3: Series of nine principal component analyses (PCAs) for the verification of the presence of different active pharmaceutical ingredients in tablets. As depicted, only principal components 1 and 2 (PC-1 and PC-2) were used in this analysis.

5. Factors influencing the principal component scores of tablets with identical pharmaceutical ingredients

The NIR spectra of tablets containing the same API in the same amount still showed differences from each other in their PC scores. As an example, Fig. 4 shows an enlargement of the data points

obtained for the metformin tablets of the training set, and illustrates factors contributing to this variance.

Data points from tablets of different brands formed identifiable though not completely separable subclusters (Fig. 4A). All six brands comprised in the training set contained 500 mg metformin per tablet, but the average tablet weight of the brands varied from 533.4 to 649.1 mg (Supplementary Table S1), due to different amounts of excipients used by the manufacturers. Fig. 4B shows the mass percentage of metformin in the total tablet weight for each brand. As clearly visible, the PC scores are highly correlated to the mass percentage of the API in the tablets. Similar observations were made for other APIs. Of all products included in this study, hydrochlorothiazide tablets showed the largest differences in their API mass percentages (Table 1). Correspondingly, they also showed the largest differences in their PC scores (Fig. 3G).

Figure 4C shows that spectra acquired with three different NIR-S-G1 spectrometric devices showed small but consistent differences. However, these differences did not prevent the correct classification of tablets according to the different APIs. If the tablet spectra lacked distinctive absorption peaks in the investigated wavelength range, the separation of the spectra according to the different devices became more prominent (Fig. 3I): the data points of doxycycline and placebo tablets each form three horizontal clusters, corresponding to the three different devices used to record these spectra. In contrast, no influence was detected regarding the investigator operating the device, neither in the example depicted in Fig. 4C nor in other parts of this study.

No difference was observed between spectra recorded from the top or the bottom sides of the tablets, even though two of the metformin brands had a score line on their top side (Fig. 4D). The same observation was made for tablets with an embossing on one side. Notably, also colored tablets (e.g. of furosemide, hydrochlorothiazide, and metronidazole; Supplementary Table S1) did not show noticeable differences from white tablets in their PC scores.

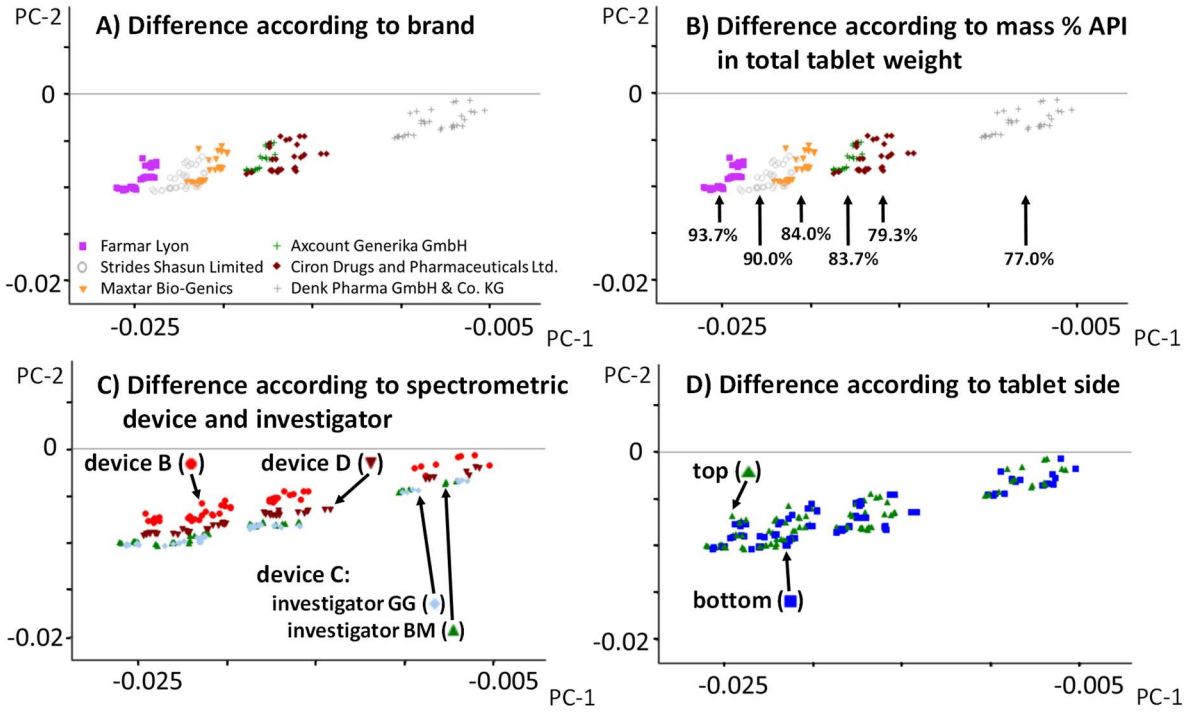


Figure 4: Variance of principal component (PC) scores of near-infrared spectra of metformin tablets according to A) brands; B) mass percentages of active pharmaceutical ingredient (API) in the total tablet weight; C) spectrometric device and investigator; and D) tablet side. Tablets of Maxtar Bio-Genics and of Ciron Drugs and Pharmaceutical Ltd. had a score line on the top side, the other four brands had smooth surfaces on both sides.

6. External validation of the PCA models

Spectra of the products comprised in the validation set (Table 1) were projected on the respective PCA models. In nearly all cases, this projection allowed a correct assignment of the validation set products to the respective APIs. In two cases, however, all eight spectra recorded from one of the two validation set products were projected to a position outside of (although still close to) the respective clusters of the training set spectra (Fig. 5A and B). Notably, in both cases the mass percentages of the API in the total tablet weight were found to be out of the range of the mass percentages covered in the training set: furosemide tablets (stated manufacturer: Holden Medical Laboratories Pvt. Ltd., India) had an API mass percentage of 20.5%, compared to 24.1 - 35.4% in the

six samples of the training set. Sulfamethoxazole/trimethoprim tablets (stated manufacturer: Strides Arcolab Limited) had a sulfamethoxazole mass percentage of 57.9%, compared to 65.9 – 71.9% in the six samples of the training set; the trimethoprim mass percentage was 11.6%, compared to 13.2-14.4% in the training set. Consistent with their lower API mass percentage, the spectra of these two validation set products were projected to a position between the respective cluster of the training set and the placebo tablets (Fig. 5A and B). Therefore, the present method should not be used for tablets which contain an API mass percentage outside the range covered by the respective reference products.

The projections of all validation set products are depicted in Supplementary Fig. S4. In no case, any of the samples of the validation set was misplaced to the cluster of a different API.

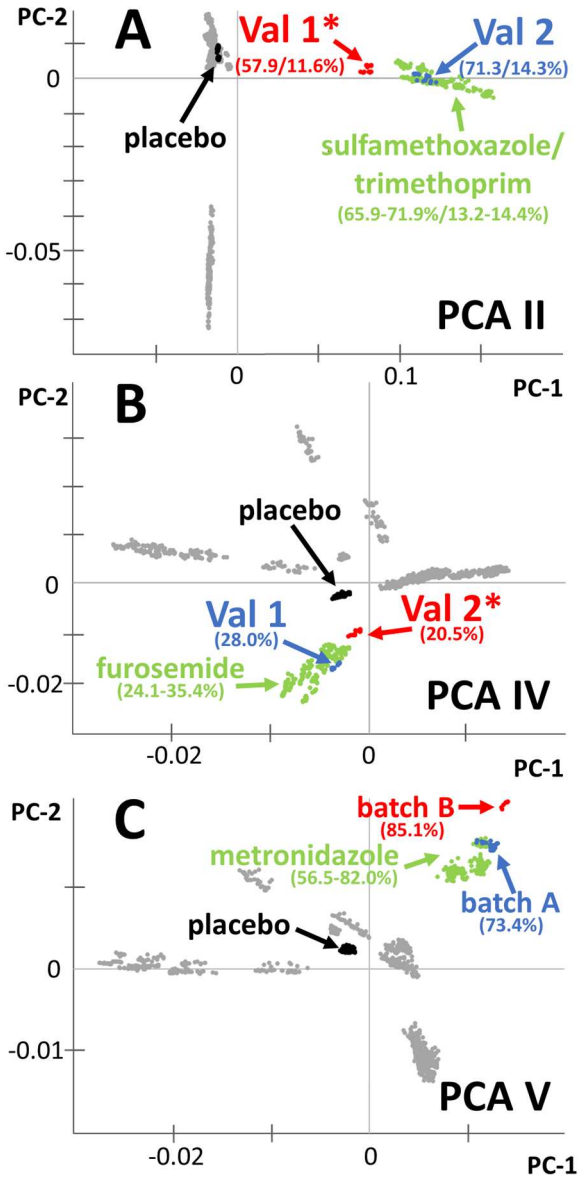


Figure 5: Influence of mass percentage of active pharmaceutical ingredient (API) in total tablet weight on the principal component (PC) scores of near-infrared spectra. **A)** and **B)** Experiment for the external validation of PCA models II and IV. For both furosemide and sulfamethoxazole/trimethoprim tablets, two validation set products (Val 1 and Val 2) were investigated, respectively. The validation set products labeled with an asterisk (*) had a lower mass percentage of API than all six samples of the training set. **C)** Testing of PCA model V with a different batch (batch B) of a metronidazole brand than contained in the training set (batch A). Unexpectedly, batch B was found to have a higher mass percentage of API than batch A, and than all other samples of the training set.

7. Testing of the PCA models with different batches of brands contained it the training set

Eighteen samples were available which represented different batches of brands contained in the training set (Table 1). In 17 out of these 18 cases, their spectra were found to be projected into the correct clusters, closely coinciding with the spectra of the corresponding brands of the training sets. This confirmed the precision and robustness of the developed method. In one case, however, all eight spectra of the investigated batch were projected to a position outside of the corresponding training set cluster, different from the position of the corresponding batch in the training set (Fig. 5C). This case concerned metronidazole 250 mg tablets, stated manufacturer CSPC Ouyi Pharmaceutical Co. Ltd., China, batch no. 825160701 (training set; hereafter called batch A) and batch no. 825170302 (challenging set; hereafter called batch B). Both samples had been collected in the DR Congo, and both had passed analysis for correct identity, content, and dissolution of the API in our laboratory.[33] In view of this unexpected result, the HPLC analysis was repeated. This confirmed the correct identity and quantity of the active ingredient. However, it was noticed that the average tablet weight of batch B was 293.7 mg, clearly lower than the average weight of 340.6 mg measured for batch A. The most likely explanation for these observations is that the manufacturer had used different manufacturing protocols for these two batches of the same brand.

The low tablet weight of batch B resulted in a metronidazole mass percentage of 85.1% of the total tablet weight, higher than the 56.5 – 82.0% range covered by the six samples of the training set (Supplementary Table S1). Correspondingly, the spectra of batch B were projected to a position more distant from the placebo tablets than the spectra of the training set (Fig. 5C).

8. Testing of the PCA models with tablets of different strength than those comprised it the training set

A total of 21 products were available with the same API but different strength than those in the training set (Table 1) and 17 of these were from different manufacturers than those comprised in the training set (Supplementary Table S1). Notably, projection of their spectra onto the respective PCA

models allowed their correct assignment to the respective APIs in most cases, especially if their API mass percentages in the total tablet weight were within the range covered by the training set (Supplementary Fig. S5). In contrast, two metronidazole products had markedly lower mass percentages of the API than those contained in the training set (i.e. 33.5 and 44.4%, compared to 56.5 – 82.0% covered in the training set). Their spectra were projected to a position between the clusters of the metronidazole training set and the placebo tablets (Supplementary Fig. S5, panel D). Similar observations were made for hydrochlorothiazide tablets with lower API mass percentages than those contained in the training set (Supplementary Fig. S5, panel F).

9. Testing of the PCA models with tablets containing different APIs than those comprised in the training set

Tablet preparations had been purchased containing 30 different APIs which were not comprised in the training set (Table 1 and Supplementary Table S1). Their spectra were projected successively onto the nine PCA models. Most of these spectra were clearly separated from the data point clusters of the training set. Therefore, they were readily identified as different from all products contained in the training set. Supplementary Fig. S6 shows as examples projections onto the scores plots of PCA III and PCA V. However, the spectra of moxifloxacin hydrochloride tablets were not separated from the data point cluster of ciprofloxacin hydrochloride tablets in PCA III, probably related to the fact that the chemical structures of these two compounds are very similar. Supplementary Fig. S6 further shows that tablets containing moxifloxacin as free base were clearly separated from those containing moxifloxacin hydrochloride. Likewise, the training set tablets containing doxycycline hyclate (i.e. doxycycline hydrochloride hemiethanolate hemihydrate) were clearly separated from tablets containing doxycycline monohydrate.

In contrast, the spectra of acetazolamide and ranitidine tablets, which showed few distinctive NIR absorption bands in the investigated wavelength range, could not be separated from those of the training set and were projected into the doxycycline cluster in PCA IX. Furthermore, the spectra of

azithromycin and praziquantel were only narrowly separated from those of other APIs. These observations illustrate the limitations in the discrimination power of the present method.

10. Testing of the PCA models with falsified medicines

The key purpose of the present study was to examine whether the developed method may be useful for the identification of falsified medicines in low-resource settings. Twenty samples of falsified medicines were available (Table 1 and Supplementary Table 1), collected in African countries and analyzed in our laboratory (see Methods). Four of these products had been found to contain no API at all, while their labels claimed a content of sulfamethoxazole/trimethoprim 400/80 mg (three products) or amoxicillin/clavulanic acid 500/125 mg (one product). Spectra of these tablets were projected successively onto the nine PCA models. All their data points were found to be located clearly outside of the clusters of sulfamethoxazole/trimethoprim and amoxicillin/clavulanic acid tablets, and outside of any other API clusters in the scores plots of PCA models I – VIII. They were therefore readily identified as falsified products. However, as mentioned above, the spectra of these tablets (containing no API) were projected to positions near to or within the data points of doxycycline tablets in PCA IX (data not shown).

Another falsified product contained 93 mg metronidazole benzoate instead of the declared 200 mg free metronidazole.[33] Metronidazole benzoate is an ester of metronidazole. As shown in Fig. 6A and B, this product was readily recognized as different from the ones included into the training set, due to a very different position from all other data points.

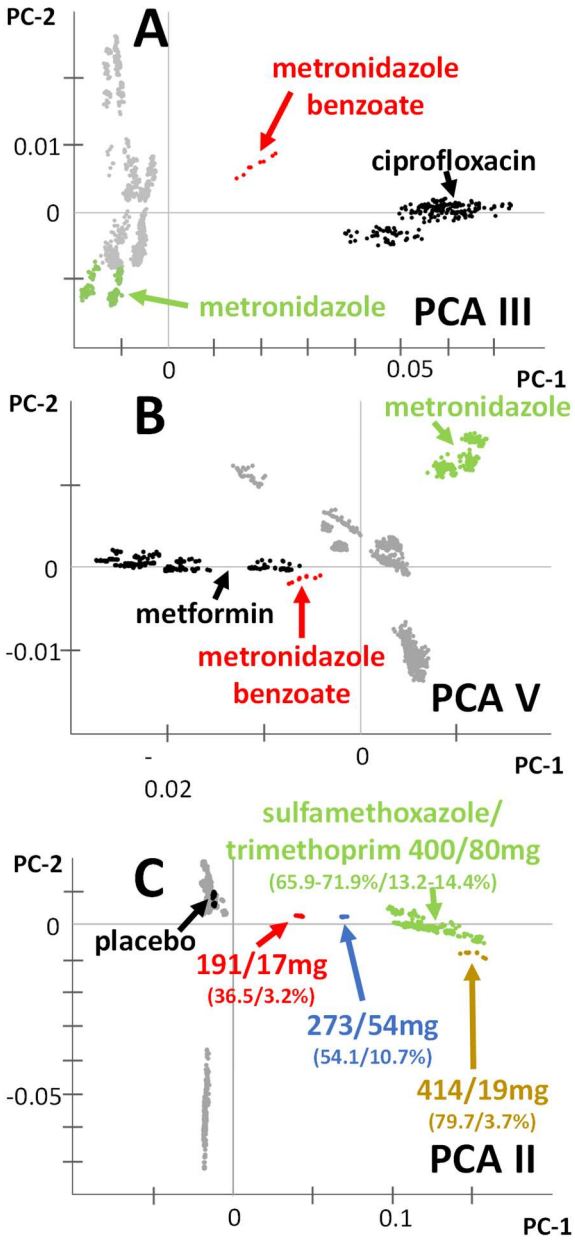


Figure 6: Detection of falsified tablets. **A)** and **B)** Projection of spectra of falsified tablets containing 93mg metronidazole benzoate instead of the declared 200mg free metronidazole onto PCA models III and V. **C)** Projection of the spectra of three falsified sulfamethoxazole/trimethoprim products containing incorrect amounts of the declared active pharmaceutical ingredients (APIs) onto PCA model II. The true tablet strength and the resulting API mass percentages in the total tablet weight are indicated.

A further falsified product contained 5 mg glibenclamide instead of the declared 50 mg hydrochlorothiazide.[9,53] Its spectra were projected to a position clearly different from those of hydrochlorothiazide tablets in PCA VII, readily identifying this product as falsified. Its spectra were projected to a position close to those of the placebo tablets in PCA IX (data not shown).

Two further falsified products contained low amounts of paracetamol (50 mg or 27 mg, respectively) instead of the declared 500 mg penicillin V[33] or 400/80 mg sulfamethoxazole/trimethoprim (see Methods). All their data points fell clearly outside the clusters of penicillin V and sulfamethoxazole/trimethoprim in the respective PCA models, and therefore they were readily identified as falsified. In PCA IX, their data points were located within the datapoints of doxycycline tablets (data not shown).

Another two falsified products were labelled as chloroquine tablets but contained 126 mg and 14 mg of metronidazole, respectively.[38] Chloroquine tablets were not contained in the training set of the present study, but metronidazole tablets were. The falsified product containing 126 mg metronidazole was projected to a position between the clusters of the metronidazole 250 mg tablets and the placebo tablets in PCA V, whereas the sample containing only 14 mg metronidazole was projected in PCA IX to a position within the datapoints of doxycycline tablets (data not shown).

Two falsified products were labelled to contain sulfamethoxazole/trimethoprim 400/80 mg; however, chemical analysis in this study (see Methods) proved that they contained only 191/17 mg and 273/54 mg, respectively. Correspondingly, their data points were projected to a position between the training set clusters of sulfamethoxazole/trimethoprim tablets and placebo tablets (Fig. 6C). These products, containing APIs in different amounts than declared, could therefore be identified by the method developed in this study. A third falsified product labelled as sulfamethoxazole/trimethoprim 400/80 mg, was found to contain a slightly excessive amount of sulfamethoxazole (414 mg), but only 19 mg trimethoprim. Due to its low tablet weight, it had a sulfamethoxazole mass percentage of 79.7%, higher than the range covered in the training set (65.9-

71.9%). It was projected to a position nearby but outside of the training set cluster of sulfamethoxazole/trimethoprim tablets (Fig. 6C).

Further seven falsified medicines had been found to contain the correct type and quantity of the declared APIs, but in five cases their expiry dates had been manipulated (illegally extending the stated shelf life), and in two cases the entire product (tablets and packaging) was falsified.[52] These products represented tablets of furosemide, metronidazole, sulfamethoxazole/trimethoprim and amoxicillin/clavulanic acid (Supplementary Table S1). Their spectra were found to be projected into the expected clusters of the training set, consistent with their correct API content.

Discussion

In the present study, it was investigated whether a low-cost NIR spectrometer combined with basic chemometric methods can be used for the verification of APIs in medicines in low-resource settings. Three principal conclusions can be drawn from the results:

First, the NIR spectra of nearly all tablet products investigated could be well separated according to the contained APIs, even though the products were from different brands, produced by different manufacturers and contained different excipients. The separation was achieved by a sequence of PCAs arranged in a hierarchical binary decision tree (Figs. 1 and 3) and only required the use of the PC-1 vs. PC-2 scores plots. Falsified medicines with qualitative or major quantitative deviations from the declared API content could be readily identified by projecting their NIR spectra onto these PCAs.

Second, the NIR spectra of a few of the investigated products could not be separated according to the contained API. Most notably, doxycycline tablets could not be separated reliably from tablets containing no API. A similar observation has been reported by Zambrzycki et al.[13] for ofloxacin tablets in NIR spectroscopy. In the present study, also the spectra of amoxicillin/clavulanic acid tablets could not be separated from those of amoxicillin tablets. Notably, Tie et al.[36] had likewise reported problems in the separation of NIR spectra of amoxicillin/clavulanic acid tablets and amoxicillin tablets, even using benchtop NIR spectrometers in addition to handheld devices, and using

supervised data analysis with different algorithms in addition to PCA. Therefore, the limitations observed in the present study are probably not the due to shortcomings of the instrumentation or of the chemometric approach, but result from the lack of sufficient differences in the NIR absorption of the examined samples in the investigated wavelength range.

Third, the position of the spectra in the PCA scores plots was found to be strongly influenced by the mass percentage of the APIs in the total tablet weight. In a screening for falsified medicines using the present method, the (nominal) API mass percentage should be determined for all samples, calculated from the strength of the tablet stated on the label and from the tablet weight. The latter can be measured readily also in resource-limited settings using an inexpensive digital scale.

In screening programs for falsified medicines, the products to be investigated carry labels stating the declared type and quantity of the API. The principal aim of the screening is to confirm or to refute the label claim. In the present study, the label claims of eleven falsified medicines with qualitative or with major quantitative deviations from the stated content were all correctly refuted, resulting in 100% sensitivity[54] for this (small) dataset. As expected, seven falsified medicines which contained the correct type and quantity of the APIs were not detected. Of the 32 medicines containing other APIs than those comprised in the training set, moxifloxacin hydrochloride was misassigned to ciprofloxacin hydrochloride in PCA III, and acetazolamide and ranitidine were misassigned to doxycycline in PCA IX. Azithromycin and praziquantel were only narrowly separated from the other APIs of the training set. Therefore, in that experiment the sensitivity for a reliable recognition of an incorrect API was 27/32 = 84%.

Out of the 19 medicines of the validation set, 17 were correctly assigned to the respective API, resulting in a specificity[54] of 89%. The two samples which could not be assigned had API mass percentages out of the range covered in the training set.

Zambrzycki et al.[13] also investigated the sensitivity and specificity of the NIR-S-G1 device for medicine quality screening, using seven antimalarials and antibiotics. These authors reported sensitivities of

91.5% or 30.6% in the detection of medicines containing either no or incorrect APIs, or containing insufficient quantities of APIs, respectively. However, their data are not directly comparable to the present study. Zambrzycki et al.[13] used a smartphone app termed PillScanNIR, which at that time was under development by a non-profit organization (Zambrzycki et al.,[13] S4 Appendix). This app aims at the authentication of brands, not at the verification of APIs (Zambrzycki et al.,[13] S5 Appendix). Details on the chemometric approach of the PillScanNIR app, or on the application of this app, have not been published yet.

The method developed in this study offers the typical advantages of NIR spectroscopy, e.g. simplicity and speed of data acquisition, independence from solvents and reagents, and performance without sample destruction. The employed NIR-S-G1 spectrometer was purchased for 1.600 USD per unit for the present study, and even lower prices have been reported in the literature.[13]

Due to the focus on API verification, the present method requires only a moderate number of brands of each API for the reference spectra library. This is a principal advantage in comparison to methods for brand authentication, which require a complete and regularly updated library of all authentic medicines to be investigated in a given market. In many low- and middle-income countries, which have to import most of their medicines from a multitude of sources and have relatively weak medicine regulatory agencies, such complete libraries will be very difficult to establish and to maintain.[31] Even if established, those complete libraries may not become available at affordable costs to non-governmental stakeholders involved in medicine procurement. In contrast, the governments of China and Russia, i.e. countries with well-controlled domestic markets and strong governance, reportedly have established large surveillance systems for falsified and substandard medicines based on NIR-spectroscopic verification of brand authenticity.[6,55]

Data analysis by PCA alone, as presented in this study, may offer the possibility of future implementation without expensive proprietary software, e.g. using available chemometric toolboxes for MS Excel®.[56] Furthermore, the graphic representation of PCA scores plots is intuitively

understandable and the position of a screened sample in these plots reveals considerably more information than the simple pass/fail result provided by many discriminant algorithms.

The complete spectral data generated during this study are provided in Supplementary Table S2 to allow further data analysis. As an example the present study has shown differences between the spectra recorded on different NIR-S-G1 devices; calibration transfer methods may be used to minimize the influence of such differences on data analysis.[57-60] Furthermore, the separation of the spectra of different groups of samples may be investigated using algorithms such as data-driven soft independent modelling of class analogy (DD-SIMCA) or others.[36,61-63]As depicted in Fig. 3 A-G, the method developed in this study can currently be used for the verification of the presence of seven APIs in tablet preparations. Future research may be aimed at increasing the number of APIs covered by this approach. Supplementary Fig. S6 suggest that many more APIs can be separated reliably.

However, also the limits of the present method must be recognized. The method is not suitable for APIs lacking characteristic absorption in the investigated wavelength range (such as doxycycline). Further, it is expected to be unsuitable for medicines with very low API amounts, such as oral contraceptives. In the present study, only samples with $\geq$12.5 mg API per dosage unit were included. In the core list of the 22nd WHO Model List of Essential Medicines 2021,[44] 71.8% of the medicines used as solid oral dosage forms (excluding vitamins and minerals) have an API content of $\geq$12.5 mg per unit. Another 10.0% of the medicines are listed with strengths both higher and lower than 12.5 mg API per unit, and for 18.2%, all listed solid oral dosage forms contain less than 12.5 mg. Therefore, the present method can be suitable for many but certainly not for all medicines of interest. Further research is required to establish its applicability to different dosage forms, and also to investigate to what extent the presence or absence of individual components in fixed combinations of two or more APIs can be verified.

Like most screening technologies, the present method cannot provide definitive evidence of compliance or non-compliance of a medicine with pharmacopeial specifications. In practice it may best be employed together with other screening methods, such as visual inspection and the GPHF-

Minilab, as well as together with confirmatory analysis by compendial methods for samples which fail in the screening. The speed of analysis by NIR spectroscopy may allow the screening of a much larger number of medicine samples than investigated using previous technologies.

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We are grateful to Benedikt Mannstadt and Katharina Beck for assistance in the recording of the spectra. We would also like to thank LuxFlux GmbH, Reutlingen, Germany, and Roland Winzen, nir-support, Bad Neuenahr-Ahrweiler, Germany, for advice and assistance in the initial steps of this study. The contribution by G.G. was kindly supported by a PhD scholarship from Cusanuswerk e.V., Bonn, Germany. We acknowledge support by the Open Access Publishing Fund of the University of Tübingen.

Data availability

All original data are available as Supplementary Material.

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Supplementary Figures - Verification of the Active Pharmaceutical Ingredient in Tablets Using a Low-Cost Near-Infrared Spectrometer and Principal Component Analysis

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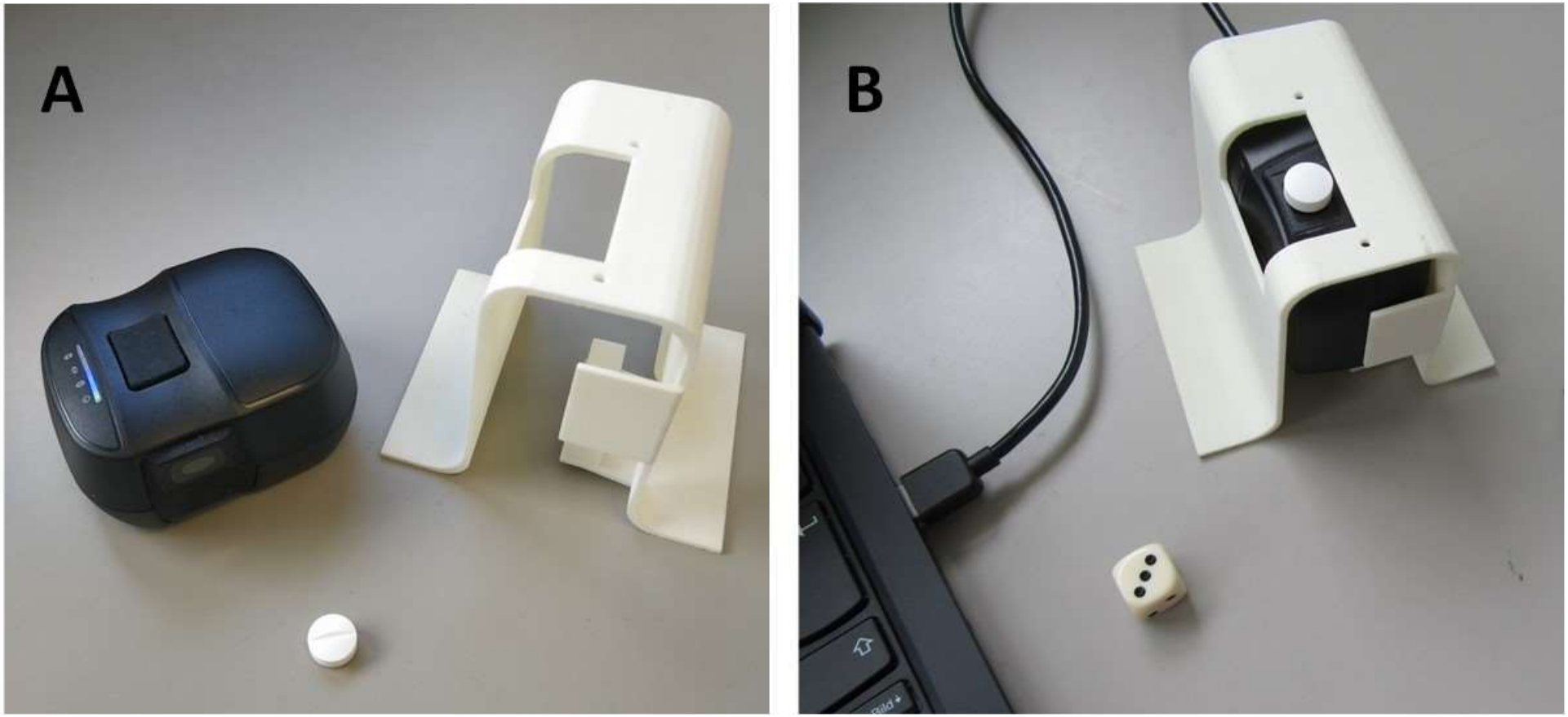
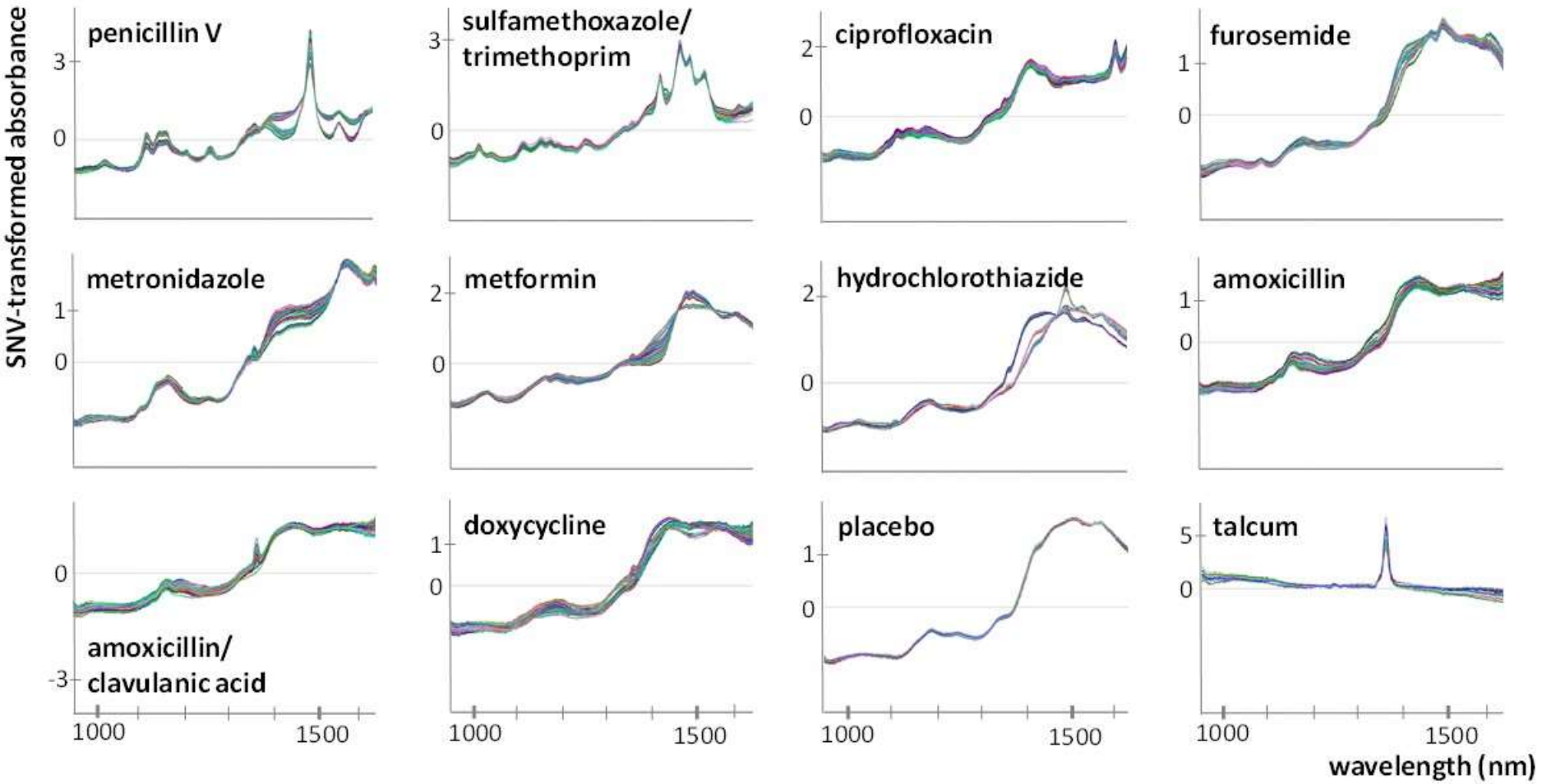
Table of contents

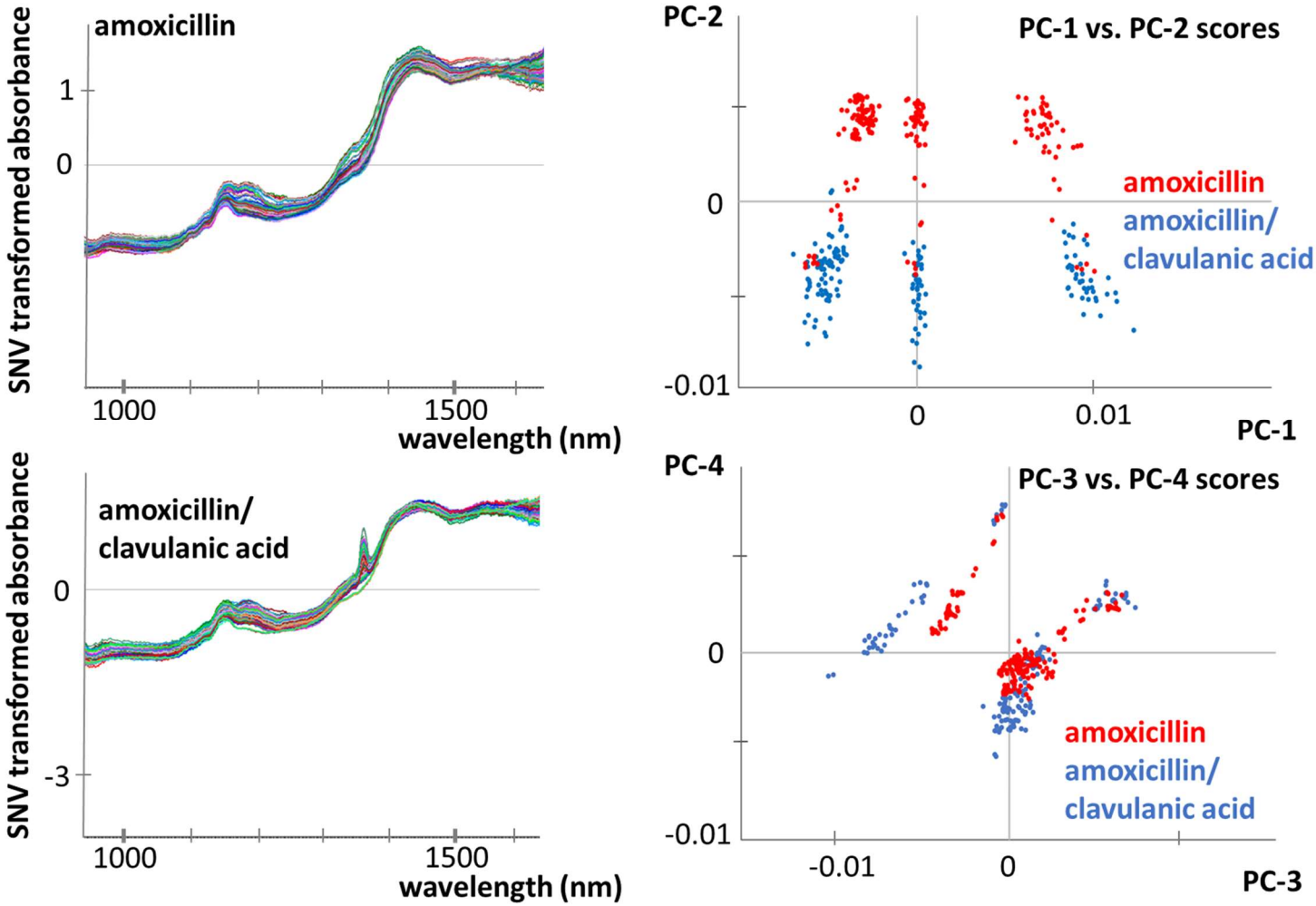
Please note that the documents "**Supplementary Table S1 – Sample information**" and "**Supplementary Table S2 – Spectra**" are provided as separate Excel-files in the online version of the journal.



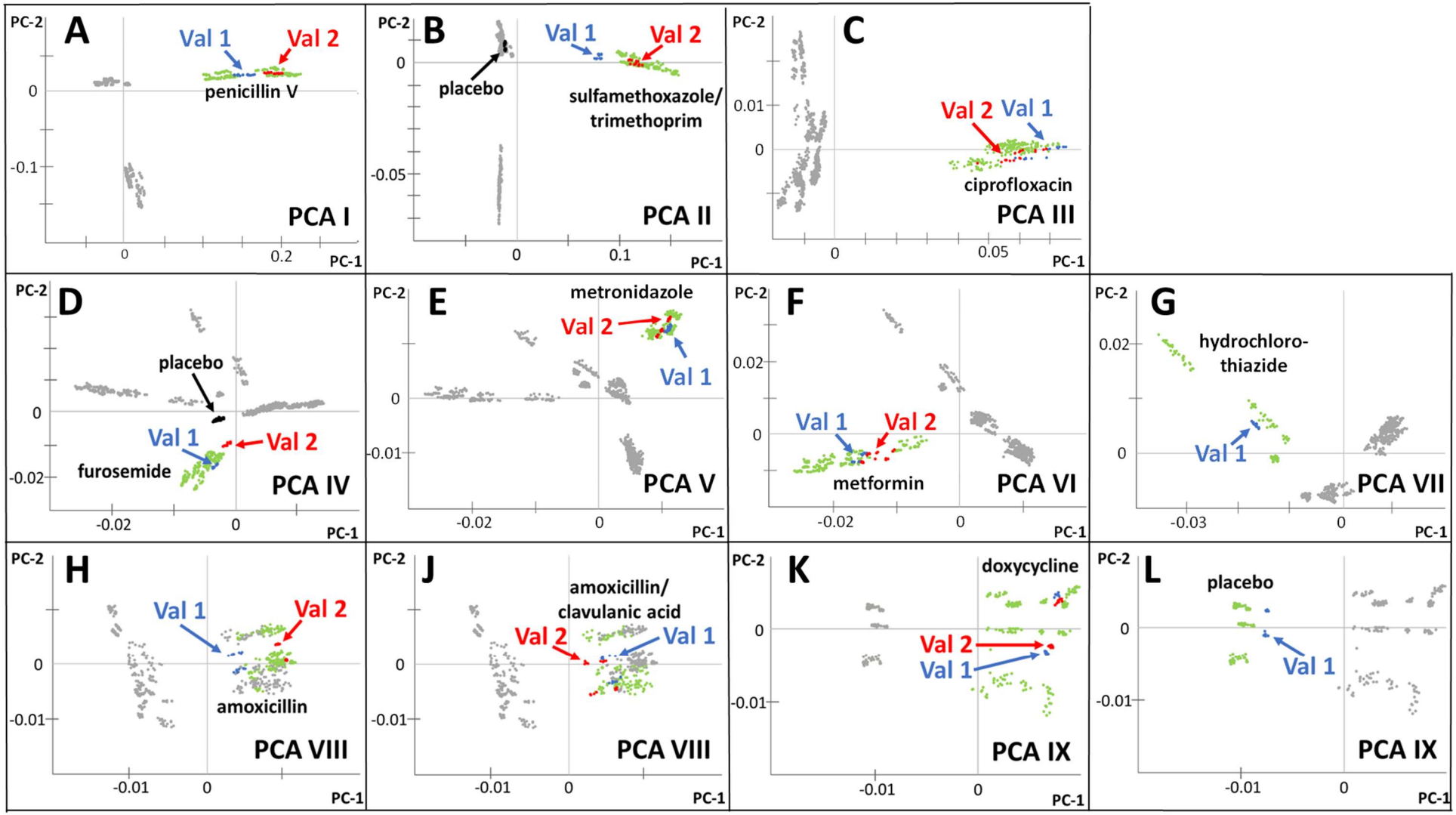
Supplementary Figure S1: (A) The NIR-S-G1 spectrometer, the 3D-printed device holder (designed by LuxFlux GmbH, Reutlingen, Germany), and a pharmaceutical tablet. (B) Assembly for spectra acquisition. The dice is included to illustrate the size of the device. (Photo: Gesa Gnegel, Tübingen University)



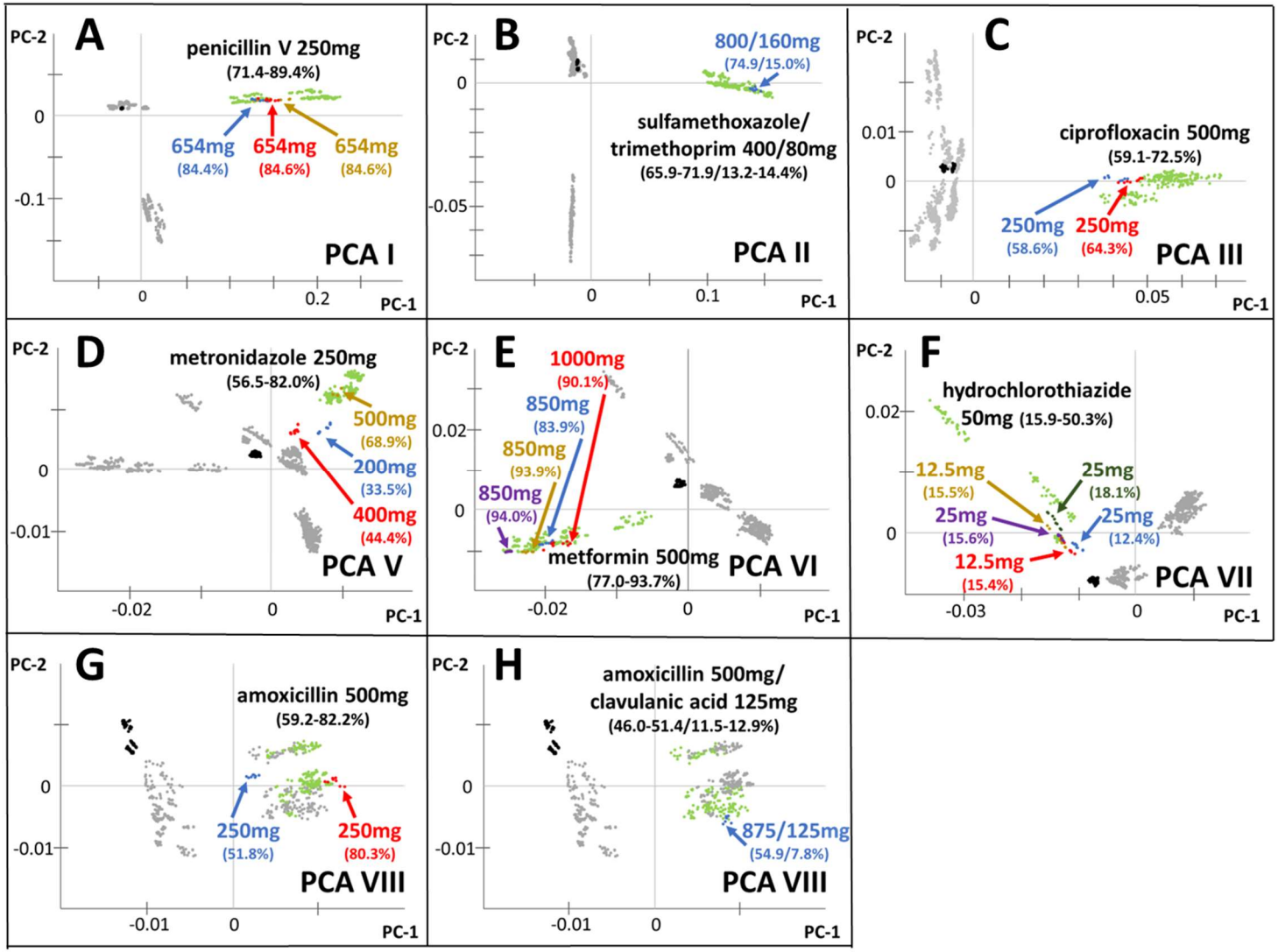
Supplementary Figure S2: Near-infrared spectra (950-1650 nm) of all tablet products included into the training set, and of talcum powder measured in a quartz cuvette.



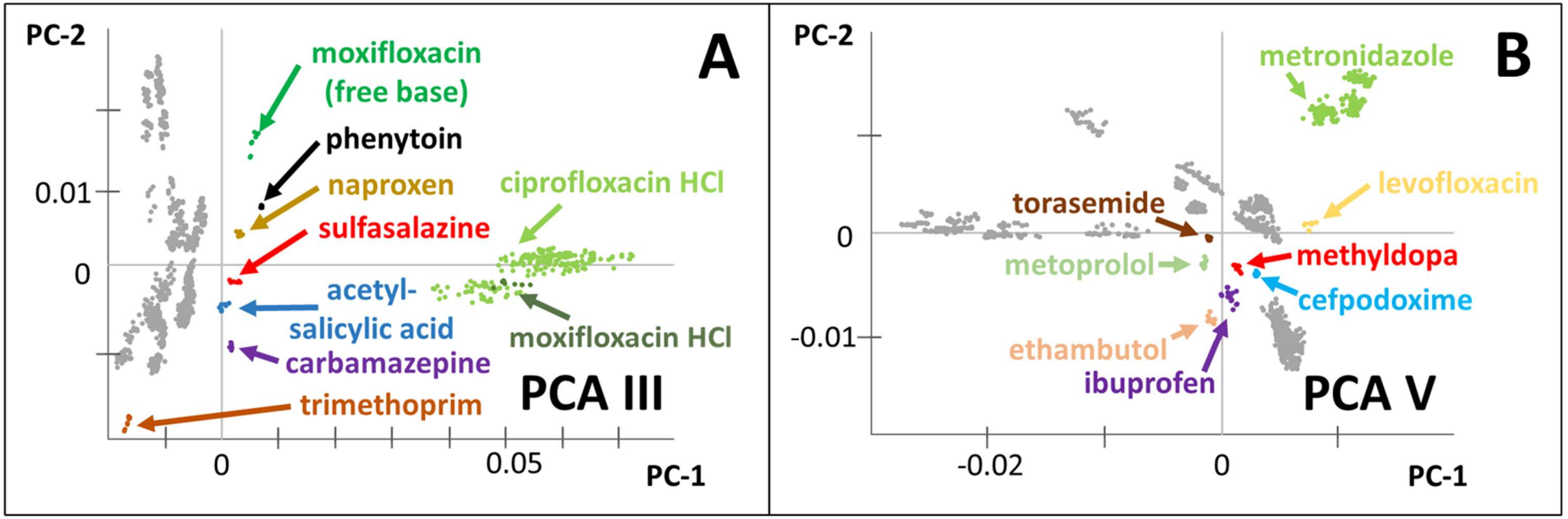
Supplementary Figure S3: Near-infrared spectra of the amoxicillin and amoxicillin/clavulanic acid products of the training set, and PC1/PC-2 and PC-3/PC-4 score plots of a PCA computed from only the spectra of these products. A complete separation could not be achieved. Several of the amoxicillin/clavulanic acid products show a peak at 1391 nm resulting from the excipient talcum (see Supplementary Figure S2). The range 1375-1405 nm was excluded in the PCA computation (see Methods).



Supplementary Figure S4: External validation of PCA models. The data points of the training set samples of the respective active pharmaceutical ingredient (API) are marked in green color. For most APIs, two validation set products (Val 1 and Val 2), containing the same API at the same strength as in the training set, were investigated. However, only one product was available for the validation sets for hydrochlorothiazide and placebo tablets. As also shown in Supplementary Figure S3, spectra of amoxicillin and of amoxicillin/clavulanic acid tablets could not be separated in PCA VIII, but are depicted here in two panels for reasons of clarity. Also, PCA IX is depicted in two panels, for validation of doxycycline and of placebo, respectively.



Supplementary Figure S5: Testing of the PCA models with tablets of different strength than those comprised in the training set. The tablet strength and the active pharmaceutical ingredient (API) mass percentages in the total tablet weight are indicated. The data points of the training set of the respective API are marked in green color, and the data points of the placebo tablets in black. As also shown in Supplementary Figs. S3 and S4, tablet spectra of amoxicillin and of amoxicillin/clavulanic acid could not be separated in PCA VIII, but are depicted here in two panels for reasons of clarity. For furosemide and doxycycline, no tablets of different strength were available. 654 mg penicillin V correspond to 1 million I.U.



Supplementary Figure S6: Projection of the spectra of tablet products containing different active pharmaceutical ingredients than those comprised in the training set onto PCA III (A) and PCA V (B). The depicted products are identified as different form all training set products, with the exception of moxifloxacin hydrochloride tablets the spectra of which were projected into the data point cluster of ciprofloxacin hydrochloride tablets in PCA III.

Supplementary Table S1 - Sample Information

An Excel version of this table has been uploaded as "Research Data" and will be available as supplementary information on the journal website.

Abbreviation	Set	Stated API	Stated strength [mg]	Determined strength [mg], if different	Tablet weight [mg]	m/m% API 1	m/m% API2	Strength API 1	Strength API 2	Product name	Stated manufacturer	Stated distributor (if different from manufacturer)	Batch	Country of collection	Stated country of manufacturing	Stated country of distributor	Dosage form	Color tablet coating	Color tablet core (if different from coating)	Top side	Bottom side	Shape
AC_Heu	Training Set	Amoxicillin-Trihydrate/Potassiun	500/125	-	1058.0	47.3%	11.8%	500	125	-	Heumann Pharma	-	8HEN03B	Germany	Germany	-	tablet	white	-	break notch	break notch	oblong
AC_Hex	Training Set	Amoxicillin-Trihydrate/Potassiun	500/125	-	988.8	50.6%	12.6%	500	125	Amoclav 500 Plus	Sandoz GmbH	Hexal AG	HT3893	Germany	Austria	Germany	tablet	white	-	break notch	break notch	oblong
AC_Mem	Training Set	Amoxicillin-Trihydrate/Potassiun	500/125	-	1080.2	46.3%	11.6%	500	125	Co-Amoxiclav	Medopharm Pvt. Ltd.	-	1680002	Cameroun	India	-	tablet	white	-	smooth	smooth	oval
AC_PIL	Training Set	Amoxicillin-Trihydrate/Potassiun	500/125	-	1087.6	46.0%	11.5%	500	125	Co-Amoxiclav	PIL Pharmaceuticals Pvt. Ltd.	Imres	AAGB6027	Cameroun	India	The Netherlands	tablet	white	-	break notch	smooth	oblong
AC_San	Training Set	Amoxicillin-Trihydrate/Potassiun	500/125	-	972.0	51.4%	12.9%	500	125	Curam 625mg	Sandoz GmbH	-	FL5158	DR Congo	Austria	-	tablet	white	-	break notch	break notch	oblong
Amo_1A	Training Set	Amoxicillin-Trihydrate	500	-	670.6	74.6%	0.0%	500	0	-	Sandoz GmbH	1A Pharma GmbH	JX8820	Germany	Austria	Germany	tablet	white	-	break notch	break notch	oblong
Amo_CSZ	Training Set	Amoxicillin-Trihydrate	500	-	608.4	82.2%	0.0%	500	0	-	CSPC Zhongnuo Pharmaceuticals Co. Ltd.	IDA Foundation	678150103	DR Congo	China	The Netherlands	tablet	white	-	break notch	smooth	round
Amo_Heu	Training Set	Amoxicillin-Trihydrate	500	-	681.4	73.4%	0.0%	500	0	-	Heumann Pharma	-	5068001M	Germany	Germany	-	tablet	white	-	break notch	break notch	oval
Amo_Nin	Training Set	Amoxicillin-Trihydrate	500	-	747.4	66.9%	0.0%	500	0	AMOXZEM TAB	Ningbo Shuangwei Pharmaceutical Co. Ltd	ZMC Hamburg GmbH	151121	Cameroun	China	Germany	tablet	white	-	embossing "AMOX ZMC" & break notch	smooth	oblong
Amo_San	Training Set	Amoxicillin	500	-	676.1	74.0%	0.0%	500	0	Amoxycillin Sandoz	Sandoz GmbH	-	HD4437	Cameroun	Austria	-	tablet	white	-	break notch	break notch	oblong
Amo_Str	Training Set	Amoxicillin-Trihydrate	500	-	844.2	59.2%	0.0%	500	0	-	Strides Arcolab Limited	-	AG-064	Cameroun	India	-	tablet	white	-	break notch	smooth	oblong
Cip_Cip	Training Set	Ciprofloxacin HCl	500	-	690.1	72.5%	0.0%	500	0	CIPLOX-500	Cipla Ltd.	-	ID55812	Cameroun	India	-	tablet	white	-	embossing "CP 500" & break notch	smooth	oblong
Cip_CSO	Training Set	Ciprofloxacin HCl	500	-	815.6	61.3%	0.0%	500	0	-	CSPC Ouyi Pharmaceutical Co. Ltd.	Imres	784160201	Cameroun	China	The Netherlands	tablet	white	-	break notch	smooth	oblong
Cip_Gre	Training Set	Ciprofloxacin HCl	500	-	795.1	62.9%	0.0%	500	0	CIPROMAX FORT 500	Greenfield Pharmaceuticals (Jiang Su) Co. Ltd.	Climax Pharmaceutical Chemists Ltd.	173121091	Cameroun	China	not known	tablet	white	-	embossing "CIPRO 500"	smooth	oval
Cip_Hex	Training Set	Ciprofloxacin HCl H2O	500	-	846.1	59.1%	0.0%	500	0	CiproHEXAL	S.C. SANDOZ S.R.L.	Hexal AG	HX9434	Germany	Rumenia	Germany	tablet	white	-	embossing "Cip 500" & break notch	break notch	oblong
Cip_Max	Training Set	Ciprofloxacin HCl	500	-	755.0	66.2%	0.0%	500	0	WINCIP-500	Maxheal Laboratories Pvt. Ltd.	Aphantee Pharmaceuticals Nig. Ltd	WC6001	Cameroun	India	Nigeria	tablet	white	-	embossing "MAXHEAL"	embossing "CIPRO 500"	oval
Cip_Sta	Training Set	Ciprofloxacin HCl H2O	500	-	767.7	65.1%	0.0%	500	0	-	STADA Arzneimittel AG	STADApharm GmbH	11GJAD	Germany	Germany	Germany	tablet	white	-	embossing "C500"	break notch	oblong
Dox_AL	Training Set	Doxycycline-Hyclat	100	-	261.9	38.2%	0.0%	100	0	-	STADA Arzneimittel AG	ALIUD PHARMA GmbH	75218	Germany	Germany	Germany	tablet	greenish yellow	-	smooth	smooth	round
Dox_CSO	Training Set	Doxycycline-Hyclat	100	-	186.5	53.6%	0.0%	100	0	-	CSPC Ouyi Pharmaceutical Co. Ltd.	Missionpharma	06140911	DR Congo	China	Denmark	tablet	greenish yellow	-	smooth	smooth	round
Dox_Fou	Training Set	Doxycycline-Hyclat	100	-	311.9	32.1%	0.0%	100	0	-	Fourrts (India) Laboratories Pvt.Limited	-	E1193	Cameroun	India	-	tablet	yellow	-	smooth	smooth	round
Dox_Mem	Training Set	Doxycycline-Hyclat	100	-	149.8	66.8%	0.0%	100	0	-	Medopharm Pvt. Ltd.	Amstelpharma	6MF123	DR Congo	India	The Netherlands	tablet	light blue	greenish yellow	smooth	smooth	round
Dox_Men	Training Set	Doxycycline-Hyclat	100	-	308.3	32.4%	0.0%	100	0	-	Medicamen Biotech Ltd.	-	NT7540	Cameroun	India	-	tablet	orange	greenish yellow	smooth	smooth	round
Dox_Nan	Training Set	Doxycycline-Hyclat	100	-	177.0	56.5%	0.0%	100	0	-	Nanjing Baijingyu Pharmaceutical Co. Ltd.	Missionpharma	DHA17001	DR Congo	China	Denmark	tablet	greenish yellow	-	smooth	smooth	round
Fur_1A	Training Set	Furosemide	40	-	164.5	24.3%	0.0%	40	0	-	Salutas Pharma GmbH	1A Pharma GmbH	JK7772	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
Fur_Asc	Training Set	Furosemide	40	-	115.4	34.7%	0.0%	40	0	Furosemide Tabrad	Asence Pharma Pvt. Ltd.	TABRAD SPRL	T-799002	Cameroun	India	Belgium	tablet	beige	-	break notch	smooth	round
Fur_Ast	Training Set	Furosemide	40	-	113.1	35.4%	0.0%	40	0	Asix	Astra Lifecare Pvt. Ltd.	-	031	DR Congo	India	-	tablet	white	-	break notch	smooth	round
Fur_Rat	Training Set	Furosemide	40	-	146.0	27.4%	0.0%	40	0	-	Teva Operations Poland Sp. z o.o.	ratiopharm GmbH	16411018	Germany	Poland	Germany	tablet	white	-	break notch	smooth	round
Fur_San	Training Set	Furosemide	40	-	159.0	25.2%	0.0%	40	0	-	Sandoz US	-	FT4986	Cameroun	USA	-	tablet	white	-	embossing "GG 201" & break notch	smooth	round
Fur_Zen	Training Set	Furosemide	40	-	166.0	24.1%	0.0%	40	0	ZENAMIDE	Zenufa Laboratoire	-	15T-75	DR Congo	DR Congo	-	tablet	white	-	embossing *Zenufa-Logo*	break notch	round
Hyd_Dex	Training Set	Hydrochlorothiazide	50	-	314.5	15.9%	0.0%	50	0	-	Dexcel Pharma GmbH	-	2001157	Germany	Germany	-	tablet	white	-	break notch	smooth	round
Hyd_Hon	Training Set	Hydrochlorothiazide	50	-	143.0	35.0%	0.0%	50	0	-	Hongkong Prost Medicines and Health Products Co. Ltd	-	160815	Cameroun	Hong Kong	-	tablet	yellow	white	break notch	smooth	round
Hyd_Pha	Training Set	Hydrochlorothiazide	50	-	99.5	50.3%	0.0%	50	0	-	Pharmaquick	-	992801	Cameroun	Benin	-	tablet	white	-	smooth	smooth	round
Metf_Axc	Training Set	Metformin HCl	500	-	597.3	83.7%	0.0%	500	0	-	Axcount Generika GmbH	-	MAU1C96	Germany	Germany	-	tablet	white	-	smooth	smooth	round
Metf_Den	Training Set	Metformin HCl	500	-	649.1	77.0%	0.0%	500	0	Metformin Denk	Denk Pharma GmbH & Co. KG	-	9C7	Cameroun	Germany	-	tablet	white	-	smooth	smooth	round
Metf_Fam	Training Set	Metformin HCl	500	-	533.4	93.7%	0.0%	500	0	Glucophage 500mg	Famar Lyon	Merck Serono /Merck Sante s.a.s	F0554	Cameroun	France	France	tablet	white	-	smooth	smooth	round
Metf_Mat	Training Set	Metformin HCl	500	-	595.0	84.0%	0.0%	500	0	Maxformin-500	Maxtar Bio-Genics	-	MXTEJ1701	Cameroun	India	-	tablet	white	-	break notch	smooth	round
Metf_Cir	Training Set	Metformin HCl	500	-	630.3	79.3%	0.0%	500	0	Shalformin	Ciron Drugs and Pharmaceuticals Ltd.	Shalina Healthcare DMCC/Laboratoire	5E01015	DR Congo	India	Dubai	tablet	white	-	break notch	smooth	round
Metf_Str	Training Set	Metformin HCl	500	-	553.2	90.4%	0.0%	500	0	-	Strides Shasun Limited	-	7352132	Cameroun	India	-	tablet	white	-	smooth	smooth	round
Metr_Aur	Training Set	Metronidazole	250	-	356.0	70.2%	0.0%	250	0	MEGYL	Aura Pharmaceuticals Pvt. Ltd	New Cesamex S.P.R.L.	006	DR Congo	India	DRC	tablet	yellow	-	smooth	smooth	round
Metr_CSO	Training Set	Metronidazole	250	-	340.6	73.4%	0.0%	250	0	-	CSPC Ouyi Pharmaceutical Co. Ltd.	-	825170302	DR Congo	China	-	tablet	white	-	smooth	smooth	round
Metr_DrW	Training Set	Metronidazole	250	-	352.0	71.0%	0.0%	250	0	Arilin	Dr. August Wolff GmbH & Co. KG Arzneimittel	-	806172	Germany	Germany	-	tablet	white	-	smooth	smooth	round
Metr_Ent	Training Set	Metronidazole	250	-	389.5	64.2%	0.0%	250	0	-	Entrance Pharmaceuticals & Research Centre	-	NT17I22	Cameroun	Ghana	-	tablet	white	-	smooth	smooth	round
Metr_Jiz	Training Set	Metronidazole	250	-	305.0	82.0%	0.0%	250	0	-	Jinzhou Jiuyang Pharmaceutical Co. Ltd	CMEC Hongkong Co. Ltd.	T20160801	DR Congo	China	Hong Kong	tablet	yellow	-	break notch	smooth	round
Metr_Str	Training Set	Metronidazole	250	-	442.5	56.5%	0.0%	250	0	-	Strides Arcolab Limited	-	7751013	Cameroun	India	-	tablet	white	-	smooth	smooth	round
Pen_CSZ	Training Set	Phenoxymethylpenicillin-Potassi	250	-	306.0	81.7%	0.0%	250	0	-	CSPC Zhongnuo Pharmaceuticals Co. Ltd.	-	688151109	DR Congo	China	-	tablet	white	-	smooth	smooth	round
Pen_Far	Training Set	Phenoxymethylpenicillin-Potassi	250	-	306.6	81.5%	0.0%	250	0	Peni-V	Farmasino Pharmaceutical (Jiangsu) Co. Ltd.	New Cesamex S.P.R.L.	W160939	DR Congo	China	DRC	tablet	white	-	smooth	smooth	round
Pen_Reg	Training Set	Phenoxymethylpenicillin-Potassi	250	-	279.5	89.4%	0.0%	250	0	UNIPEN 250	Regal Pharmaceuticals Ltd.	-	170093	DR Congo	Kenya	-	tablet	white	-	break notch	smooth	round
Pen_Shg	Training Set	Phenoxymethylpenicillin-Potassi	250	-	311.7	80.2%	0.0%	250	0	-	Shandong Yikang Pharmaceutical Co. Ltd.	not known	170322	DR Congo	China	not known	tablet	white	-	break notch	smooth	round
Pen_Sin	Training Set	Phenoxymethylpenicillin-Potassi	250	-	298.8	83.7%	0.0%	250	0	Peni-V	Sinochem Jiangsu Co. Ltd	New Cesamex S.P.R.L.	170504	DR Congo	China	DRC	tablet	white	-	break notch	smooth	round
Pen_Spa	Training Set	Phenoxymethylpenicillin-Potassi	250	-	350.3	71.4%	0.0%	250	0	SPENIV TABLETS 250	Sparsh Bio-Tech Pvt. Ltd.	-	PT460	DR Congo	India	-	tablet	white	-	break notch	smooth	round
ST_Ago	Training Set	Sulfamethoxazole/Trimethoprim	400/80	-	607.2	65.9%	13.2%	400	80	TRIMAGO	Agog Pharma Ltd.	-	T71155	DR Congo	India	-	tablet	white	-	embossing "COTRIM 480" & break notch	embossing "AGOG"	round
ST_AL	Training Set	Sulfamethoxazole/Trimethoprim	400/80	-	556.3	71.9%	14.4%	400	80	-	STADA Arzneimittel AG	ALIUD PHARMA GmbH	80120	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
ST_Lin	Training Set	Sulfamethoxazole/Trimethoprim	400/80	-	579.7	69.0%	13.8%	400	80	Co-trimoxazole	Lincoln Pharmaceuticals Ltd.	Ubithera Pharma Pvt. Ltd.	GM6006	Cameroun	India	India	tablet	white	-	break notch	smooth	round
ST_Kam	Training Set	Sulfamethoxazole/Trimethoprim	400/80	-	559.2	71.5%	14.3%	400	80	KAM COTRI	Kampala Pharmaceutical Industries	-	1816	DR Congo	Uganda	-	tablet	white	-	embossing "KPI" & break notch	embossing "C"	round
ST_Rat	Training Set	Sulfamethoxazole/Trimethoprim	400/80	-	600.4	66.6%	13.3%	400	80	Cotrim-ratiopharm	Merckle GmbH	ratiopharm GmbH	U30851D	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
ST_Sha	Training Set	Sulfamethoxazole/Trimethoprim	400/80	-	556.1	71.9%	14.4%	400	80	Sulfatrim	Shalina Laboratories Pvt. Ltd.	-	BNJ7007	DR Congo	India	-	tablet	white	-	embossing "SULFATRIM 480"	break notch	round
AC_Asc	C2_different strength	Amoxicillin-Trihydrate/Potassiun	875/125	-	1592.4	54.9%	7.8%	875	125	TAMCLAV 1G	Asence Pharma Pvt. Ltd.	TABRAD SPRL	PT7088	Cameroun	India	Belgium	tablet	white	-	break notch	break notch	oblong
Amo_CSZCa	C2_different strength	Amoxicillin-Trihydrate	250	-	483.0	51.8%	0.0%	250	0	-	CSPC Zhongnuo Pharmaceuticals Co. Ltd.	Missionpharma	797160908	DR Congo	China	Denmark	tablet	white	-	break notch	smooth	oval
Amo_CSZCb	C2_different strength	Amoxicillin	250	-	311.3	80.3%	0.0%	250	0	-	CSPC Zhongnuo Pharmaceuticals Co. Ltd.	IDA Foundation	677150802	DR Congo	China	The Netherlands	tablet	white	-	break notch	smooth	round
Cip_1A	C2_different strength	Ciprofloxacin HCl H2O	250	-	426.9	58.6%	0.0%	250	0	Cipro - 1A	S.C. SANDOZ S.R.L.	1A Pharma GmbH	JF8242	Germany	Rumenia	Germany	tablet	white	-	embossing "CIP 250" & break notch	smooth	round
Cip_StaC	C2_different strength	Ciprofloxacin HCl H2O	250	-	388.9	64.3%	0.0%	250	0	-	STADA Arzneimittel AG	STADApharm GmbH	45333	Germany	Germany	Germany	tablet	white	-	embossing "C 250" & break notch	break notch	round with notch
Hyd_1A	C2_different strength	Hydrochlorothiazide	12.5	-	81.0	15.4%	0.0%	13	0	-	Salutas Pharma GmbH	1A Pharma GmbH	JC2540	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
Hyd_AAA	C2_different strength	Hydrochlorothiazide	12.5	-	80.5	15.5%	0.0%	13	0	-	Artesan Pharma GmbH & Co. KG	AAA-Pharma GmbH	16L008	Germany	Germany	Germany	tablet	white	-	smooth	smooth	round
Hyd_Nov	C2_different strength	Hydrochlorothiazide	25	-	138.5	18.1%	0.0%	25	0	Esidrex	Novartis Farmacéutica S.A.	Novartis Pharma AG	BL801	Cameroun	Spain	Switzerland	tablet	white	-	embossing "CE" & break notch	embossing "CG"	round
Hyd_Rat	C2_different strength	Hydrochlorothiazide	25	-	201.0	12.4%	0.0%	25	0	-	Merckle GmbH	ratiopharm GmbH	R24234	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
Hyd_Sal	C2_different strength	Hydrochlorothiazide	25	-	162.0	15.4%	0.0%	25	0	-	Salutas Pharma GmbH	Sandoz GmbH	GN5244	Cameroun	Germany	Austria	tablet	white	-	break notch cross-shaped	smooth	round
Metf_Axo	C2_different strength	Metformin HCl	850	-	1013.6	83.9%	0.0%	850	0	Asur-850	Axon Drugs Pvt. Ltd.	Indus Lifescience Pvt. Ltd.	16ASU01	DR Congo	India	India	tablet	white	-	break notch	smooth	oblong
Metf_Blu	C2_different strength	Metformin HCl	850	-	905.6	93.9%	0.0%	850	0	Metformina Bluefish	Bluefish Pharmaceuticals AD	-	5150657	DR Congo	Sweden	-	tablet	white	-	embossing "61"	embossing "A"	round
Metf_DenC	C2_different strength	Metformin HCl	1000	-	1109.7	90.1%	0.0%	1000	0	Metformin Denk	Denk Pharma GmbH & Co. KG	-	20384	DR Congo	Germany	-	tablet	white	-	break notch	break notch	oblong
Metf_Mek	C2_different strength	Metformin HCl	850	-	904.7	94.0%	0.0%	850	0	Glucophage	Merck	-	18670	DR Congo	Belgium	-	tablet	white	-	smooth	smooth	round
Metr_DrWC	C2_different strength	Metronidazole	500	-	726.0	68.9%	0.0%	500	0	Arilin	Dr. August Wolff GmbH & Co. KG Arzneimittel	-	601351	Germany	Germany	-	tablet	white	-	break notch	smooth	round
Metr_Sta	C2_different strength	Metronidazole	400	-	901.5	44.4%	0.0%	400	0	-	STADA Arzneimittel AG	STADAPHARM GmbH	72201	Germany	Germany	Germany	tablet	white	-	break notch	break notch	oblong
Metr_StrC	C2_different strength	Metronidazole	200	-	597.0	33.5%	0.0%	200	0	Metrosim-200	Strides Shasun Limited	-	7352053	DR Congo	India	-	tablet	yellow	-	embossing "Metro Metro" & break notch	smooth	round
Pen_AL	C2_different strength	Phenoxymethylpenicillin-Potassi	654	-	731.5	89.4%	0.0%	654	0	Penicillin V AL	STADA Arzneimittel AG	ALIUD PHARMA GmbH	92218	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
Pen_Rat	C2_different strength	Phenoxymethylpenicillin-Potassi	654	-	774.8	84.4%	0.0%	654	0	Penicillin V-ratiopharm 1 M	Merckle GmbH	ratiopharm GmbH	S28551	Germany	Germany	Germany	tablet	white	-	break notch	break notch	oblong
Pen_San	C2_different strength	Phenoxymethylpenicillin-Potassi	654	-	773.1	84.6%	0.0%	654	0	Ospen	Sandoz GmbH	-	HC8534	Cameroun	Austria	-	tablet	white	-	break notch	break notch	oblong
ST_Bai	C2_different strength	Sulfamethoxazole/Trimethoprim	800/160	-	1067.5	74.9%	15.0%	800	160	Cotrim Fort	Laboratoire Bailly-Creat	-	CR479	Cameroun	France	-	tablet	white	-	embossing "CREAT"	break notch	oblong
Amo_NinC	C1_different batch	Amoxicillin-Trihydrate	500	-	743.2	67.3%	0.0%	500	0	AMOXZEM TAB	Ningbo Shuangwei Pharmaceutical Co. Ltd	ZMC Hamburg GmbH	170617	Cameroun	China	Germany	tablet	white	-	embossing "AMOX ZMC" & break notch	smooth	oblong
Amo_SanC	C1_different batch	Amoxicillin	500	-	672.8	74.3%	0.0%	500	0	Amoxycillin Sandoz	Sandoz GmbH	-	HD4445	Cameroun	Austria	-	tablet	white	-	break notch	break notch	oblong
Amo_StrC	C1_different batch	Amoxicillin-Trihydrate	500	-	841.8	59.4%	0.0%	500	0	-	Strides Arcolab Limited	-	AG-044	Cameroun	India	-	tablet	white	-	break notch	smooth	oblong
Cip_CSOC	C1_different batch	Ciprofloxacin HCl	500	-	825.6	60.6%	0.0%	500	0	-	CSPC Ouyi Pharmaceutical Co. Ltd.	-	784150904	DR Congo	China	-	tablet	white	-	break notch	smooth	oblong
Dox_CSOC	C1_different batch	Doxycycline-Hyclat	100	-	173.0	57.8%	0.0%	100	0	-	CSPC Ouyi Pharmaceutical Co. Ltd.	-	503150911	DR Congo	China	-	tablet	greenish yellow	-	smooth	smooth	round
Dox_MemC	C1_different batch	Doxycycline-Hyclat	100	-	147.6	67.8%	0.0%	100	0	-	Medopharm Pvt. Ltd.	IDA Foundation	5MJ146	DR Congo	India	The Netherlands	tablet	light blue	greenish yellow	smooth	smooth	round
Dox_NanC	C1_different batch	Doxycycline-Hyclat	100	-	182.1	54.9%	0.0%	100	0	-	Nanjing Baijingyu Pharmaceutical Co. Ltd.	Missionpharma	DHA15007	DR Congo	China	Denmark	tablet	greenish yellow	-	smooth	smooth	round
Fur_AstC	C1_different batch	Furosemide	40	-	106.0	37.7%	0.0%	40	0	Asix	Astra Lifecare Pvt. Ltd.	-	028	DR Congo	India	-	tablet	white	-	break notch	smooth	round
Fur_ZenC	C1_different batch	Furosemide	40	-	165.7	24.1%	0.0%	40	0	ZENAMIDE	Zenufa Laboratoire	-	14T-35	DR Congo	DR Congo	-	tablet	white	-	embossing *Zenufa-Logo*	break notch	round
Hyd_PhaC	C1_different batch	Hydrochlorothiazide	50	-	99.8	50.1%	0.0%	50	0	-	Pharmaquick	-	992701	Cameroun	Benin	-	tablet	white	-	smooth	smooth	round
Metf_MatC	C1_different batch	Metformin HCl	500	-	597.8	83.6%	0.0%	500	0	Maxformin-500	Maxtar Bio-Genics	-	MT3M-1607	Cameroun	India	-	tablet	white	-	break notch	smooth	round
Metf_StrC	C1_different batch	Metformin HCl	500	-	547.4	91.3%	0.0%	500	0	-	Strides Arcolab Limited	-	7351219	Cameroun	India	-	tablet	white	-	smooth	smooth	round
Metr_AurC	C1_different batch	Metronidazole	250	-	342.2	73.1%	0.0%	250	0	MEGYL	Aura Pharmaceuticals Pvt. Ltd	New Cesamex S.P.R.L.	009	DR Congo	India	DRC	tablet	yellow	-	smooth	smooth	round
Metr_CSOC	C1_different batch	Metronidazole	250	-	293.7	85.1%	0.0%	250	0	-	CSPC Ouyi Pharmaceutical Co. Ltd.	Imres	825160701	DR Congo	China	The Netherlands	tablet	white	-	smooth	smooth	round
Metr_EntC	C1_different batch	Metronidazole	250	-	389.6	64.2%	0.0%	250	0	-	Entrance Pharmaceuticals & Research Centre	-	NT17145	Cameroun	Ghana	-	tablet	white	-	smooth	smooth	round
Pen_RegC	C1_different batch	Phenoxymethylpenicillin-Potassi	250	-	278.1	89.9%	0.0%	250	0	UNIPEN 250	Regal Pharmaceuticals Ltd.	-	170888	DR Congo	Kenya	-	tablet	white	-	break notch	smooth	round
Pen_SpaC	C1_different batch	Phenoxymethylpenicillin-Potassi	250	-	350.0	71.4%	0.0%	250	0	SPENIV TABLETS 250	Sparsh Bio-Tech Pvt. Ltd.	-	PT448	DR Congo	India	-	tablet	white	-	break notch	smooth	round
ST_AgoC	C1_different batch	Sulfamethoxazole/Trimethoprim	400/80	-	603.7	66.3%	13.3%	400	80	TRIMAGO	Agog Pharma Ltd.	-	T64108	DR Congo	India	-	tablet	white	-	embossing "COTRIM 480" & break notch	embossing "AGOG"	round

Pla_Fag	Training Set	Placebo	0	-	506.9	0.0%	0.0%	0	0	-	Fagron GmbH & Co. KG	-	212C2/1	Germany	Germany	-	tablet	white	-	break notch	smooth	round
Pla_Cae	Training Set	Placebo	0	-	750.7	0.0%	0.0%	0	0	-	Caelo & Loretz GmbH	-	21003767001	Germany	Germany	-	tablet	white	-	smooth	smooth	oblong
Pla_Win	Training Set	Placebo	0	-	127.1	0.0%	0.0%	0	0	P-Tabletten	Artesan Pharma GmbH & Co. KG	Winthrop Arzneimittel GmbH	123233	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
Pla_Utu	Validation Set	Placebo	0	-	220.9	0.0%	0.0%	0	0	-	University Tübingen (Department of Pharmaceutical Technology	-	-	Germany	Germany	-	tablet	white	-	smooth	smooth	round
DoxM_Hex	C3_different API	Doxycycline Monohydrat	100	-	195.8	51.1%	0.0%	100	0	DoxyHEXAL tabs 100mg	S.C. SANDOZ S.R.L.	Hexal AG	HT6705	Germany	Rumenia	Germany	tablet	grey	-	break notch	smooth	round
Lev_Den	C3_different API	Levofloxacin-Hemihydrat	500	-	727.8	68.7%	0.0%	500	0	-	Rottendorf Pharma GmbH	DENK PHARMA GmbH & Co. KG	20588	Germany	Germany	Germany	tablet	light pink	white	break notch	break notch	oblong
Lev_Hex	C3_different API	Levofloxacin-Hemihydrat	500	-	667.8	74.9%	0.0%	500	0	-	Lek Pharmaceuticals d.d.	Hexal AG	JR1927	Germany	Slovenia	Germany	tablet	pink	white	break notch	smooth	oblong
Mox_Pur	C3_different API	Moxifloxacin HCl	400	-	702.9	56.9%	0.0%	400	0	-	APL Swift Services (Malta) Limited	PUREN Pharma GmbH & Co. KG	MLSA17006-A	Germany	Malta	Germany	tablet	pink	light yellow	embossing "E18"	smooth	oblong
Mox_Rat	C3_different API	Moxifloxacin	400	-	715.7	55.9%	0.0%	400	0	-	Merckle GmbH	ratiopharm GmbH	1736013	Germany	Germany	Germany	tablet	light pink	light yellow	smooth	smooth	oblong
Tor_1A	C3_different API	Torasemide	50	-	102.4	48.8%	0.0%	50	0	-	Lek S.A.	1A Pharma GmbH	LE5583	Germany	Poland	Germany	tablet	white	-	smooth	smooth	round
Tri_InP	C3_different API	Trimethoprim	100	-	202.8	49.3%	0.0%	100	0	INFECTOTRIMET	INFECTOPHARM Arzneimittel und Consilium GmbH	-	R072001.2	Germany	Germany	-	tablet	white	-	break notch	smooth	round
Xip_Rat	C3_different API	Xipamide	40	-	102.1	39.2%	0.0%	40	0	-	Merckle GmbH	ratiopharm GmbH	XO4624C	Germany	Germany	Germany	tablet	white	-	embossing "40"	break notch	round
Ace_Mep	C3_different API	Acetazolamide	250	-	463.9	53.9%	0.0%	250	0	Acemit	medphano	-	1601018	Germany	Germany	-	tablet	white	-	break notch	smooth	round
Acv_Bas	C3_different API	Aciclovir	200	-	303.4	65.9%	0.0%	200	0	Aciclo Basics	Sun Pharmaceutical Industries Europe B.V. or Terapia S.A.*	Basics GmbH	2994670	Germany	The Netherlands or Ru	Germany	tablet	white	-	embossing "ACV"	embossing "200"	oblong
All_Hex	C3_different API	Allopurinol	100	-	175.6	57.0%	0.0%	100	0	-	Lek Pharmaceuticals d.d.	Hexal AG	JV3906	Germany	Slovenia	Germany	tablet	white	-	break notch	smooth	round
Ass_1A	C3_different API	Acetylsalicylic acid	500	-	623.5	80.2%	0.0%	500	0	ASS 500	Salutas Pharma GmbH	1A Pharma GmbH	JL4799	Germany	Germany	Germany	tablet	white	-	break notch cross-shaped	smooth	round
Azi_Rat	C3_different API	Azithromycin 2 H2O	250	-	400.7	62.4%	0.0%	250	0	-	Merckle GmbH or PLIVA HRVATSKA d.o.o.*	ratiopharm GmbH	189037	Germany	Germany or Croatia*	Germany	tablet	white	-	embossing "AI 250"	smooth	oval
Car_Des	C3_different API	Carbamazepin	200	-	269.4	74.2%	0.0%	200	0	Timonil 200	Desitin Arzneimittel GmbH	-	17000399	Germany	Germany	-	tablet	white	-	embossing "T"	break notch	round
Cef_Sta	C3_different API	Cefpodoxim Proxetil	200	-	584.1	34.2%	0.0%	200	0	-	STADA Arzneimittel AG	STADApharm GmbH	92354	Germany	Germany	Germany	tablet	white	-	smooth	smooth	oblong
Cla_Hex	C3_different API	Clarithromycin	250	-	409.8	61.0%	0.0%	250	0	-	S.C. SANDOZ S.R.L.	Hexal AG	JG1596	Germany	Rumenia	Germany	tablet	white	-	break notch	break notch	oblong
Cmy_MIP	C3_different API	Clindamycin HCl	150	-	304.5	49.3%	0.0%	150	0	Clinda-saar	MIP Pharma GmbH	-	275400	Germany	Germany	-	tablet	white	-	smooth	smooth	round
Eth_Rie	C3_different API	Ethambutol 2HCl	400	-	579.1	69.1%	0.0%	400	0	EMB - Fatol 400 mg	SW Pharma GmbH	RIEMSER Pharma GmbH	13068	Germany	Germany	Germany	tablet	white	-	embossing "400"	break notch	round
Ibu_Rat4	C3_different API	Ibuprofen	400	-	571.2	70.0%	0.0%	400	0	Ibu-Ratiopharm 400mg	Merckle GmbH	ratiopharm GmbH	V16666A	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
Ibu_Rat6	C3_different API	Ibuprofen	600	-	843.9	71.1%	0.0%	600	0	Ibu-Ratiopharm 600mg	Merckle GmbH	ratiopharm GmbH	V08455A	Germany	Germany	Germany	tablet	apricot	white	break notch	break notch	oblong
Iso_Rie	C3_different API	Isoniazid	100	-	109.1	91.7%	0.0%	100	0	ISOZID 100 mg	SW Pharma GmbH	RIEMSER Pharma GmbH	003118	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
Mdo_Sta	C3_different API	Methyldopa 1.5 H2O	250	-	404.6	61.8%	0.0%	250	0	-	STADA Arzneimittel AG	STADAPHARM GmbH	83942	Germany	Germany	Germany	tablet	orange	white	embossing "STADA"	embossing "B8"	round
Mpr_1A	C3_different API	Metoprolol Tartrate	100	-	251.8	39.7%	0.0%	100	0	-	Salutas Pharma GmbH	1A Pharma GmbH	JL9316	Germany	Germany	Germany	tablet	white	-	break notch cross-shaped	smooth	octagonal
Nap_1A	C3_different API	Naproxen	250	-	380.5	65.7%	0.0%	250	0	-	Salutas Pharma GmbH	1A Pharma GmbH	JW4697	Germany	Germany	Germany	tablet	beige	-	break notch	smooth	round
Par_Rat	C3_different API	Paracetamol	500	-	644.8	77.5%	0.0%	500	0	-	Merckle GmbH	ratiopharm GmbH	V03478A	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
Phe_Neu	C3_different API	Phenobarbital	100	-	213.3	46.9%	0.0%	100	0	-	neuraxpharm Arzeinmittel GmbH	-	171089	Germany	Germany	-	tablet	white	-	break notch cross-shaped	break notch cross-shaped	round
Phy_Tev	C3_different API	Phenytoin	100	-	149.1	67.1%	0.0%	100	0	Phenytoin AWD	Merckle GmbH	Teva GmbH	19B038A	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
Prq_Bay	C3_different API	Praziquantel	600	-	930.3	64.5%	0.0%	600	0	Biltricide	Bayer AG	Bayer Vital GmbH	314419	Germany	Germany	Germany	tablet	white	-	embossing "BAYER" break notch	embossing "LG" & break notch	oblong
Pyr_Rie	C3_different API	Pyrazinamide	500	-	609.9	82.0%	0.0%	500	0	PYRAFAT 500mg	SW Pharma GmbH	RIEMSER Pharma GmbH	014107	Germany	Germany	Germany	tablet	white	-	break notch	smooth	round
Ran_Sta	C3_different API	Ranitidine HCl	150	-	316.5	47.4%	0.0%	150	0	-	STADA Arzneimittel AG	STADApharm GmbH	82337	Germany	Germany	Germany	tablet	white	-	embossing "150"	break notch	round
Sul_Saf	C3_different API	Sulfasalazine	500	-	621.3	80.5%	0.0%	500	0	Colo-Pleon	Sanofi-Aventis Deutschland GmbH	-	1036721	Germany	Germany	-	tablet	white	-	smooth	smooth	oval
Vor_Hex	C3_different API	Voriconazole	200	-	621.1	32.2%	0.0%	200	0	-	Lek Pharmaceuticals d.d.	Hexal AG	JK2908	Germany	Slovenia	Germany	tablet	white	-	embossing "200"	smooth	oval
AcF_Gla	C4_no API	Amoxicillin-Trihydrate/Potassiun	500/125	0	1311.9	0.0%	0.0%	0	0	Augmentin	SmithKline Beecham Pharmaceuticals	GSK	562626	Cameroun	United Kingdom	not known	tablet	white	-	embossing "AC" & break notch	smooth	oval
AcF_Med	C6_manipulated label	Amoxicillin-Trihydrate/Potassiun	500/125	-	1078.8	46.3%	11.6%	500	125	-	Medopharm Pvt. Ltd.	IDA Foundation	F456733	Cameroun	India	The Netherlands	tablet	white	-	smooth	smooth	oval
ChlF_Daw	C5_wrong API	Chloroquine	250	126mg metron	468.2	26.9%	0.0%	126	0	-	Dawa Limited	-	1605059	DR Congo	Kenya	-	tablet	white	-	circular edge	smooth	round
ChlF_Jia	C5_wrong API	Chloroquine phosphate	100	14mg metron	499.3	2.8%	0.0%	14	0	-	Jiangsu Pharmaceutical Inc.	-	660	Cameroun	China	-	tablet	white	-	break notch	smooth	round
FurF1_Mic	C6_manipulated label	Furosemide	40	-	143.0	28.0%	0.0%	40	0	-	Micro Labs Ltd.	IDA Foundation	FRIH0077	Cameroun	India	The Netherlands	tablet	white	-	break notch	smooth	round
FurF2_Mic	C6_manipulated label	Furosemide	40	-	142.6	28.0%	0.0%	40	0	-	Micro Labs Ltd.	IDA Foundation	FRIH0077	Cameroun	India	The Netherlands	tablet	white	-	break notch	smooth	round
FurF3_Mic	C6_manipulated label	Furosemide	40	-	142.7	28.0%	0.0%	40	0	-	Micro Labs Ltd.	IDA Foundation	FRIH0077	Cameroun	India	The Netherlands	tablet	white	-	break notch	smooth	round
FurF4_Mic	C6_manipulated label	Furosemide	40	-	142.6	28.0%	0.0%	40	0	-	Micro Labs Ltd.	IDA Foundation	FRIH0077	Cameroun	India	The Netherlands	tablet	white	-	break notch	smooth	round
HydF_Ste	C5_wrong API	Hydrochlorothiazide	50	5mg glibencla	68.8	7.3%	0.0%	5	0	-	Laboratoires Sterop	-	16G04	Cameroun	Belgium	-	tablet	white	-	embossing "S"	smooth	round
Metr_Mac	C5_wrong API	Metronidazole	200	93mg metron	431.2	21.6%	0.0%	93	0	Metronyl	MAC'S Pharmaceuticals Ltd.	-	L3028	DR Congo	Kenya	-	tablet	white	-	smooth	smooth	round
MetrF_Mem	C6_manipulated label	Metronidazole	250	-	354.2	70.6%	0.0%	250	0	-	Medopharm Pvt. Ltd.	-	4MJ164	DR Congo	India	-	tablet	white	-	break notch	smooth	round
PenF_Oxf	C5_wrong API	Phenoxymethylpenicillin-Potassi	500	50mg paracet	628.5	8.0%	0.0%	50	0	-	Oxford Pharma	-	190	Cameroun	Belgium	-	tablet	white	-	embossing "500000 IU" & break notch	embossing "PENICILLIN V"	round
STF_Cit	C4_no API	Sulfamethoxazole/Trimethoprim	400/80	0	551.2	0.0%	0.0%	0	0	C Cotrim - 480	Citicare Laboratories Ltd	-	018	Nigeria	Nigeria	-	tablet	white	-	embossing "480 CT"	smooth	round
STF_Gau	C7_insufficient API	Sulfamethoxazole/Trimethoprim	400/80	414/19	519.7	79.7%	3.7%	414	19	Zimatrim	Gauze Pharm. & Lab. LTD.	-	ZGT037	Nigeria	Nigeria	-	tablet	white	-	embossing"ZIMATRIM"	embossing "GAUZE"	round
STF_Mao	C7_insufficient API	Sulfamethoxazole/Trimethoprim	400/80	191/17	523.7	36.5%	3.2%	191	17	Poletrim	Maobison Inter-Link & Associates Ltd.	-	409.38	Chad	Nigeria	-	tablet	white	-	embossing "CT+480" & break notch	embossing *Logo MIA*	round
STF_Opt	C4_no API	Sulfamethoxazole/Trimethoprim	400/80	0	579.0	0.0%	0.0%	0	0	Optrim	Optimal Lab. Ltd	-	OLL20	Chad	Nigeria	-	tablet	white	-	Tab1: embossing "COTRIM 480" & break notch"; Tab2	smooth	round
STF_Rot	C4_no API	Sulfamethoxazole/Trimethoprim	400/80	0	563.1	0.0%	0.0%	0	0	Rotrim	Rotac Medical Lab.	-	RML478	Nigeria	Nigeria	-	tablet	white	-	Tab1: smooth, tab2: embossing	smooth	round
STF_Spr	C6_manipulated label	Sulfamethoxazole/Trimethoprim	400/80	-	573.3	69.8%	14.0%	400	80	Co-Trimoxazole	Sprukfield	-	I3617	Cameroun	Togo	-	tablet	white	-	embossing "COTRI 480" & break notch	smooth	round
STF_Wel	C5_wrong API	Sulfamethoxazole/Trimethoprim	400/80	27mg paracet	638.9	4.2%	0.0%	27	0	Weltrim	Weltec Healthcare Ltd	-	NHL-1001	Nigeria	Nigeria	-	tablet	white	-	embossing "COTRIM 480"	smooth	round
STF_Zun	C7_insufficient API	Sulfamethoxazole/Trimethoprim	400/80	273/54	504.2	54.1%	10.7%	273	54	Zuntrim	Zunamediks Pharm. LTD.	-	RT0003C	Nigeria	Nigeria	-	tablet	white	-	embossing "Zuntrim 480"	embossing "ZMPL"	round
AC_Cin	Validation Set	Amoxicillin-Trihydrate/Potassiun	500/125	-	1112.1	45.0%	11.2%	500	125	Cinclamox	Cinpharm	-	DW3312	Cameroun	not stated	-	tablet	white	-	smooth	smooth	oval
AC_Int	Validation Set	Amoxicillin-Trihydrate/Potassiun	500/125	-	1104.4	45.3%	11.3%	500	125	-	Intermed	Unimed International Ltd.	QTN02	DR Congo	India	UK	tablet	white	-	break notch	smooth	oblong
Amo_Mem	Validation Set	Amoxicillin-Trihydrate	500	-	647.6	77.2%	0.0%	500	0	-	Medopharm Pvt. Ltd.	-	16144002	Cameroun	India	-	tablet	white	-	break notch	smooth	round
Amo_Nan	Validation Set	Amoxicillin-Trihydrate	500	-	893.1	56.0%	0.0%	500	0	-	Nanjing Sino Pharmaceutical Ltd.	-	160103	Cameroun	China	-	tablet	white	-	break notch	smooth	oblong
Cip_mem	Validation Set	Ciprofloxacin HCl	500	-	783.8	63.8%	0.0%	500	0	-	Medopharm Pvt. Ltd.	-	217090001	Cameroun	India	-	tablet	white	-	break notch	smooth	oblong
Cip_Mey	Validation Set	Ciprofloxacin HCl	500	-	713.1	70.1%	0.0%	500	0	Ecoflox-500	Medley Pharmaceuticals Ltd.	-	D60263	DR Congo	India	-	tablet	white	-	smooth	smooth	oval
Dox_Bai	Validation Set	Doxycycline-Hyclat	100	-	228.5	43.8%	0.0%	100	0	Doxycreat	Laboratoire Bailly-Creat	-	45	Cameroun	France	-	tablet	brown	greenish yellow	smooth	smooth	round
Dox_Jia	Validation Set	Doxycycline-Hyclat	100	-	177.6	56.3%	0.0%	100	0	-	Jiangsu Ruinian Qianjin Pharm. Co.Ltd	Sprukfield	141110	Cameroun	China	not known	tablet	brown	greenish yellow	break notch	smooth	round
Fur_Ely	Validation Set	Furosemide	40	-	142.9	28.0%	0.0%	40	0	Frusemide	Elys Chemical Industries Ltd.	-	4G68	DR Congo	Kenya	-	tablet	green	-	break notch	smooth	round
Fur_Hol	Validation Set	Furosemide	40	-	194.8	20.5%	0.0%	40	0	-	Holden Medical Laboratories Pvt. Ltd.	Imres	HE16B52	DR Congo	India	The Netherlands	tablet	white	-	break notch	smooth	round
Hyd_Glo	Validation Set	Hydrochlorothiazide	50	-	185.6	26.9%	0.0%	50	0	-	Global Pharma Healthcare Pvt. Ltd.	-	TE399	Cameroun	India	-	tablet	white	-	break notch	smooth	round
Metf_Ber	Validation Set	Metformin HCl	500	-	550.7	90.8%	0.0%	500	0	Siofor	BERLIN-CHEMIE AG	-	81002B	Germany	Germany	-	tablet	white	-	smooth	smooth	round
Metf_Mem	Validation Set	Metformin HCl	500	-	606.6	82.4%	0.0%	500	0	-	Medopharm Pvt. Ltd.	-	7MA42	Cameroun	India	-	tablet	white	-	smooth	smooth	round
Metr_Daw	Validation Set	Metronidazole	250	-	346.2	72.2%	0.0%	250	0	Eflaron 250	DAWA Limited	-	1607130	DR Congo	Kenya	-	tablet	yellow	-	smooth	smooth	round
Metr_Mem	Validation Set	Metronidazole	250	-	353.2	70.8%	0.0%	250	0	-	Medopharm Pvt. Ltd.	IDA Foundation	5MA91	DR Congo	India	The Netherlands	tablet	white	-	break notch	smooth	round
Pen_Ast	Validation Set	Phenoxymethylpenicillin-Potassi	250	-	316.6	79.0%	0.0%	250	0	As-V	Astra Lifecare Pvt. Ltd.	-	185	DR Congo	India	-	tablet	white	-	break notch	smooth	round
Pen_NCP	Validation Set	Phenoxymethylpenicillin-Potassi	250	-	301.2	83.0%	0.0%	250	0	-	NCPC North Best	IDA Foundation	150923	DR Congo	China	The Netherlands	tablet	white	-	smooth	smooth	round
ST_Mem	Validation Set	Sulfamethoxazole/Trimethoprim	400/80	-	560.6	71.3%	14.3%	400	80	Co-trimoxazole BP	Medopharm Pvt. Ltd.	IDA Foundation	4MB107	DR Congo	India	The Netherlands	tablet	white	-	embossing "COTRI 480" & break notch	smooth	round
ST_Str	Validation Set	Sulfamethoxazole/Trimethoprim	400/80	-	690.6	57.9%	11.6%	400	80	Co-trimoxazole Tablets BP	Strides Arcolab Limited	-	7750175	Cameroun	India	-	tablet	white	-	embossing "COTRIIM 480" & break notch	smooth	round

* Stated manufacturer as indicated on the secondary packaging or on the package leaflet. In two cases (lines 110 and 113), two different manufacturers were indicated, stating that the product was produced by either of them. In these cases, both manufacturers (and countries of manufacture) were included in this table.