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Assessment of the Quality of Antimalarial Medicines Sold in Bukavu, DRC

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Abstract

According to 2021 estimates, 247 million people suffered from malaria and 619,000 died from it worldwide. The World Health Organization (WHO) receives reports of substandard or falsified products, of which antimalarials and antibiotics are the most cited. The quality of antimalarial drugs is an important factor contributing to the emergence of resistance in Plasmodium falciparum. This is the reason for this study on the quality control of antimalarial drugs marketed in the Democratic Republic of Congo (DRC) market, in South Kivu/Bukavu. A cross-sectional study was conducted with an analytical aim, which consisted of analyzing the quality of the most consumed antimalarials in Bukavu, including artemether-lumefantrine, sulfadoxine-pyrimethamine, quinine sulfate, and dihydroartemisinin-piperaquine, in tablet form, and quinine bihydrochloride in injectable form. Organoleptic tests, pharmacotechnical tests, and Raman spectroscopy identification were performed on all samples, and quantitative analysis by UV-Visible spectrophotometry and near-infrared spectroscopy in transmission mode was performed. Non-conformity was found during visual examination and pharmacotechnical tests (mass uniformity, friability, and hardness). Lumefantrine, quinine, sulfadoxine, pyrimethamine, dihydroartemisinin, and piperaquine were identified in the samples except artemether, because lumefantrine dominates the Raman spectrum due to the low concentration of artemether in the tablet, according to the method used for identification. For quantification, there was no non-conformity related to the dosage of quinine sulfate and bihydrochloride; however, with regard to artemether-lumefantrine, the lumefantrine concentration was

outside the specification for the first five batches, but the sixth batch was conforming according to the specification. It is recommended to enhance packaging and labeling controls to reduce tablet breakage and improve appearance, ensuring compliance prior to market launch. Additionally, refine pharmacotechnical processes.

Keywords

Quality Control, Antimalarials, Artemether-Lumefantrine, Quinine Sulfate and Bichlorhydrate, Sulfadoxine-Pyrimethamine, Dihydroartemisinin-Piperaquine

1. Introduction

Malaria is a parasitic infection caused by a protozoan of the genus Plasmodium, transmitted to humans by the bite of a mosquito (Anopheles) [1]. *Plasmodium falciparum* is the most widespread and pathogenic. Malaria attacks caused by *Plasmodium vivax, P. ovale, and P. malariae* are generally less severe. *Plasmodium knowlesi* can cause severe attacks but is sensitive to chloroquine [2].

According to the latest World Health Organization (WHO) report on malaria worldwide, progress in the fight against malaria is still stagnating, particularly in African countries where the burden is high. In 2021, an estimated 247 million people suffered from malaria and 619,000 died from it worldwide [3] [4]. Malaria remains one of the most devastating infectious diseases, with approximately 212 million infections and 429,000 deaths each year in sub-Saharan Africa, and 25 million cases in the DRC, resulting in 46,800 deaths in 2018.

In the Democratic Republic of Congo (DRC), an estimated 97% of the population lives in areas of stable malaria transmission for 8 to 12 months a year; the remaining 3% are exposed to epidemic malaria in the high mountains of the eastern part of the country. Approximately 80% of deaths occur at home, representing millions of deaths annually [5] [6]. According to the PNLP (Programme National de Lutte contre le Paludisme), out of approximately 6 million inhabitants of the province of South Kivu, in the year 2022, the number of malaria patients was 1,522,657, including 997 deaths [7].

The proliferation of poor-quality medicines has been described as a global pandemic that threatens the lives of millions of people. Poor-quality medicines can have a devastating impact on patients' lives, leading to prolonged illness, treatment failure, side effects, loss of income, or death [8].

According to new research from the WHO, an estimated 1 in 10 medicines in circulation in low- and middle-income countries is either substandard or falsified. This means patients are taking medicines that can neither completely treat nor prevent disease [9].

It is understood that the quality of antimalarial drugs is an important factor contributing to the development of resistance of Plasmodium falciparum to artemis-

inin-based combination therapies (ACTs). However, until now, it has been difficult to accurately estimate the extent of the problem and assess changes over time [8].

An analysis by the London School of Hygiene and Tropical Medicine for the WHO estimates that substandard and fake antimalarial drugs could cause an additional 116,000 deaths from the disease each year in sub-Saharan Africa, at an average cost of $38.5 million per year to patients and health systems. In 2015, a study published in the American Society of Tropical Medicine and Hygiene estimated that more than 122,000 children under five died each year from substandard antimalarial drugs in sub-Saharan Africa [10].

2. Methods

2.1. Study Design

Our study was cross-sectional with analytical aims. It consisted of analyzing the quality of the most consumed antimalarials in the Democratic Republic of Congo, including artemether-lumefantrine, quinine sulfate and bihydrochloride, sulfadoxine-pyrimethamine, and dihydroartemisinin-piperaquine, all in tablet form with the exception of quinine bihydrochloride, which was in injectable form, particularly in the province of South Kivu, city of Bukavu. The samples were collected in the city of Bukavu, capital of the province of South Kivu; they were subsequently analyzed at the laboratories of the Department of Pharmaceutical Sciences of the University of Liège in Belgium and at the analytical chemistry laboratory of the Official University of Bukavu.

It assessed the pharmaceutical technology requirements regarding labeling and physical examination (checklist), verification of those registered and authorized on the DRC market, and pharmacotechnical tests (disintegration time, friability, mass uniformity, hardness, and dissolution).

UV spectrophotometry and near-infrared spectroscopy in transmission mode allowed us to measure the active ingredients (quinine sulfate, quinine bihydrochloride, and artemether-lumefantrine) and infrared spectroscopy (NIR) and Raman for the identification of the molecules, using the guidelines of the European Pharmacopoeia. The methods used for the analysis of quinine sulfate and bihydrochloride have been validated in the laboratory of Liège University, and the one used for the quantification of artemether-lumefantrine has been verified beforehand to ensure the accuracy of the results.

As pharmacotechnical tests on solid forms, we performed mass uniformity, friability, hardness, and disintegration time. For injectable forms, we performed the extractable volume.

For the qualitative analysis, the visual inspection test was carried out using a checklist focused on labeling, packaging, drug color, etc. and 85.7 mg of Lumefantrine standard - 13.3 mg of artemether standard were weighed, but also identification by infrared spectroscopy. RAMAN spectroscopy was used to compare the different spectra with the spectra in the database. A spectral range of 250 - 1800 cm^{-1}, spectra preprocessing (AsLS baseline correction, SG smoothing with win-

dow size 7), and a maximum normalization of 1 were used [11]-[13]. The UV-Vis spectrophotometry method validated in the laboratory of the Pharmaceutical Sciences Department of the University of Liège was used to quantify quinine sulfate and bichlorhydrate. Dilutions of quinine sulfate were made up to 8 µg/ml (standards and samples). Three solutions/samples with methanol as well as two solutions/standards with milliQ water were prepared separately, and six readings of standard 1 to assess the system suitability test by evaluating instrument repeatability and sensitivity before sample analysis, two readings of standard 2 to confirm system suitability throughout the analytical session, and two readings per sample to ensure result reproducibility. Three solutions were prepared and tested for each batch [14]. This method was compared to the near-infrared spectroscopy method validated by Ciza *et al.* in the same laboratory, where the sample (10 mg/100 ml) was prepared in 1 M HCl, decanted, and filtered with the 4.5 µm filter. The assay was done by NIR spectrometry in transmission mode using a 2 mm thick cuvette, 32 scans, 16 PGA, and 3 tests with 3 readings per test [12]. Artemether-lumefantrine was measured by the UV-Vis spectrophotometry method verified in the analytical chemistry laboratory of the Official University of Bukavu, where 100 mg of artemether and lumefantrine powder, taking into account the average weight of each batch, and 85.7 mg of Lumefantrine standard, 13.3 mg of artemether standard were weighed. The powder was dissolved in 100 ml of ethanol, dilutions were made to obtain solutions with a concentration of 25 µg/ml, and then the reading of the standards (artemether and lumefantrine) and samples was carried out at wavelengths of 212 and 262 nm, respectively (Karajgi *et al.*, 2016).

2.2. Sample Collection

A selection of different products from different manufacturers of the most commonly consumed antimalarials in the province of South Kivu was made.

A simple random strategy was employed to select 18 batches, including 6 batches for artemether-lumefantrine, 4 batches for quinine sulfate, 3 batches for quinine bihydrochloride, 3 batches for sulfadoxine-pyrimethamine, and 2 batches for dihydroartemisinin-piperaquine. Pharmaceutical stores, pharmacies, and hospital pharmacies have been selected as sampling facilities.

2.3. Equipment, Reagents and Solvents

2.3.1. Equipment

Num	Equipment	Specifications
1	Analytical balance	Mettler Toledo XS 203 S-GM0121 et Sartorius CA 102
2	Friabilator	F2GM0074, Sotax; Denekamp, Netherlands
3	Hardness tester	GM0207
4	Disintegration tester	DT3 GM0074, Sotax; Denekamp, Netherlands
5	Near Infrared	MPA (Multiple Propose Analyse)

Continued

6	Near Infrared	NIR-S-G1, Reflective Spectrometer, InnoSpectra, Li Hsing Rd., Science Park, Hsinchu, Taiwan Region
7	Near Infrared	NIR-M-T1
8	Spectro RAMAN	Thermo Scientific Truscan
9	Ultrasonic bath	Branson 2510 C.A.060 and 2210 C.A.061
10	Spectrophotometer UV-Vis	Lambda 40, C.A.010
11	Spectrophotmeter UV-Vis	Drawell, DU-8200, SN 201907DF003

2.3.2. Reagents and Solvents

Num	Réactifs	Spécifications
1	Methanol	J.T.Baker, UN 1230, CAS: 67-56-1, lot: 2314205854, Israel
2	Ethanol	EMSURE, UN1170, CAS No.: 64-15-5, Merck KGaA, 64271 Darmstadt, USA
3	Quinine sulphate dihydrate	Cat: 418780050, Lot/A0437825, CAS: 6119-70-6 Thermo Scientific, Germany
4	Quinine dihydrochloride	Q5-5G, Lot 0000145028, CAS N˚6119-47-7, Germany
5	Lumefantrine	L0256, Lot LOECT-RO, CAS RN: 82186-77-4, >98.0% , Switzerland
6	Milli-Q water	A2190, Lot. 65WVM-LK, CAS RN: 71963-77-4, >98.0% , Switzerland

2.4. Statistical Analysis

PCA was used to visualize the spectral data of different samples, and Excel software was used to determine the concentration of active ingredients in each sample. The Excel sheet with a validated formula was used to determine the concentration of the active ingredient after the assessment of the recovery, the coefficient of variation, and the System Suitability Test. PLS_Toolbox v9.3 was used for Principal Component Analysis (PCA) and spectral preprocessing.

3. Results and Discussion

3.1. Pharmacotechnical Tests, Extractable Volume, and Checklist

For these pharmacotechnical tests, non-conformities were observed at 5.8% for mass uniformity, 46.66% for friability, and 46.66% for hardness. Compared to the extractable volume result and referring to the standard from the European Pharmacopoeia that the deviation should be less than 5%, our samples were within the standard. According to the elaborated checklist, samples categorized as "quarantined" were obtained because they included tablets that were not whole in the blister, peeled-off film, and non-intact inner packaging, which represented 10% non-compliance with the elaborated checklist (**Figure 1**). These results corroborate those of Baba Moussa *et al.*, who found respective non-compliances of 97.5% and 5% for the mass uniformity and disintegration tests [15]. This is similar to Nnanga *et al.*, who also detected this type of non-conformity in hardness in 06 batches (19.67%), friability in 03 batches (10%), visual control in 03 batches (9.99%), and labeling in 03 batches with the men-

tion "Cannot be sold outside Nigeria," but at different percentages [16].

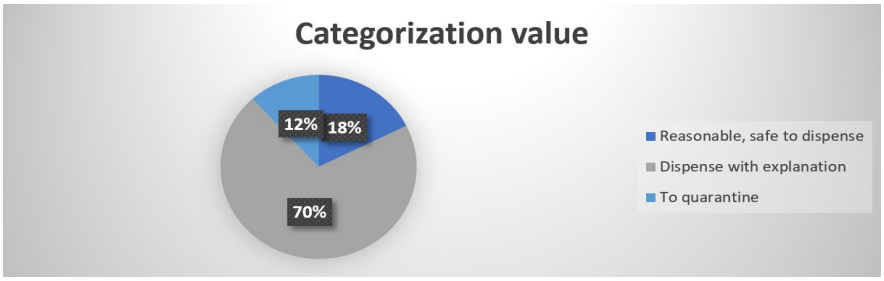
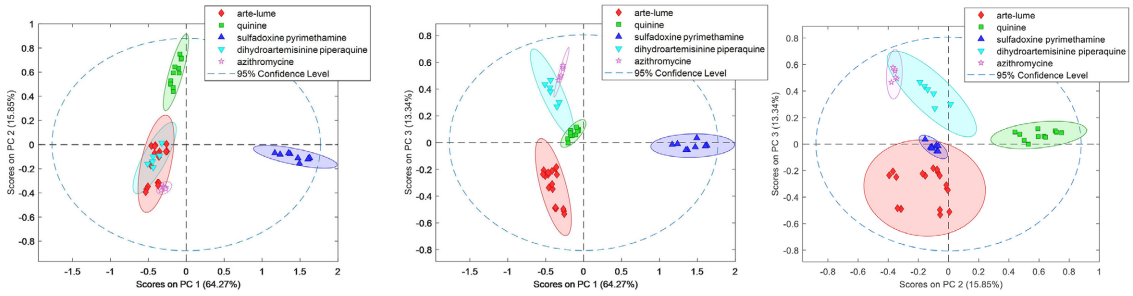


Figure 1. Categorization of samples based on the checklist.

3.2. Identification Results

3.2.1. Analysis Results of Samples Inside and Outside Blister Packs by MPA

➢ **Analysis of PCA in blister packs**

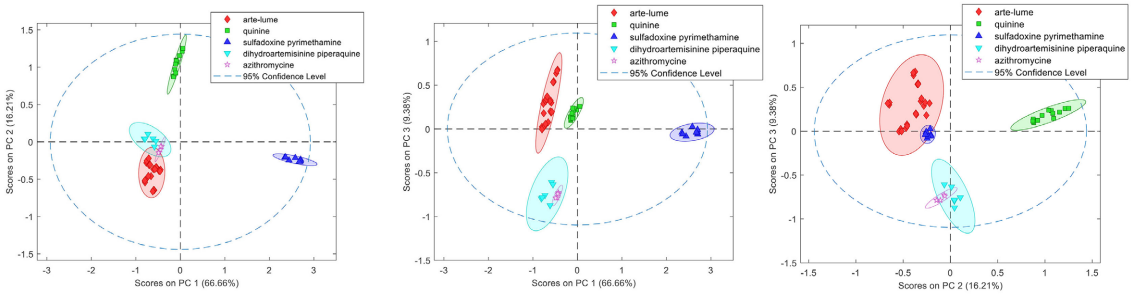


(a) (b) (c)

PCA: Principal Component Analysis.

Figure 2. PCA analysis in the blister with MPA. (a) Scores in PC1-PC2, (b) scores in PC1-PC3, (c) scores in PC2-PC3.

➢ **Analysis of PCAs outside the blister pack**



(a) (b) (c)

Figure 3. Analysis of non-blister PCA with MPA. (a) Scores in PC1-PC2, (b) scores in PC1-PC3, (c) scores in PC2-PC3.

3.2.2. Results of the Analysis of Samples in and Out of the Blister by NIR-S-G1

➢ **Analysis of PCA in blister packs**

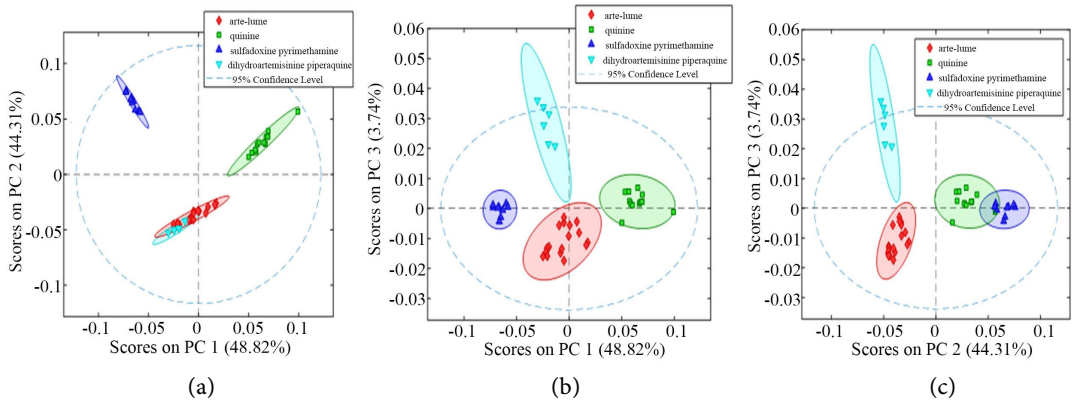


Figure 4. PCA analysis in the blister with NIR-S-G1. (a) Scores in PC1-PC2, (b) scores in PC1-PC3, (c) scores in PC2-PC3.

➢ Analysis of PCAs outside the blister pack

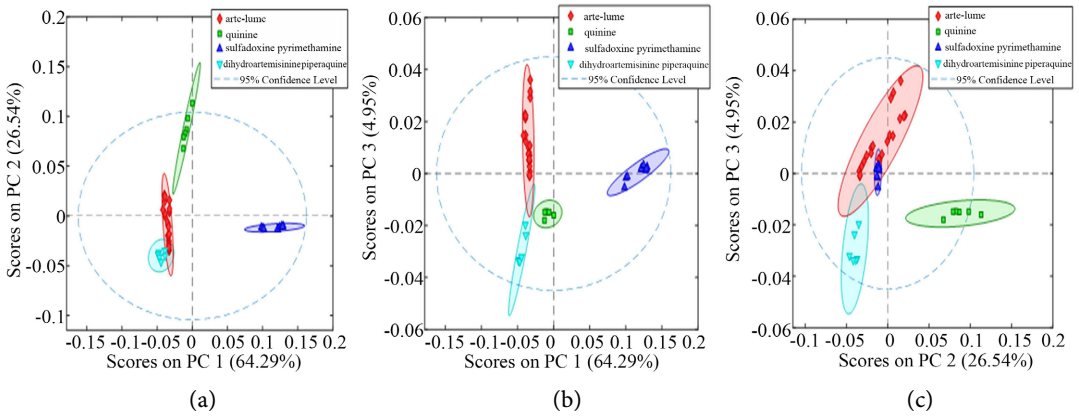


Figure 5. Analysis of non-blister PCAs with NIR-S-G1. (a) scores in PC1-PC2, (b) scores in PC1-PC3, (c) scores in PC2-PC3.

3.2.3. TruScan in- and Out-of-Blister Sample Analysis Results

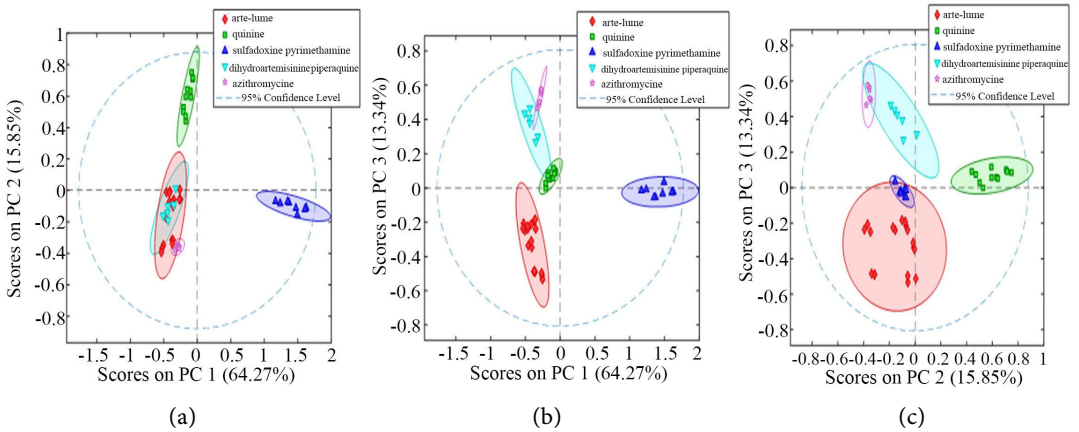
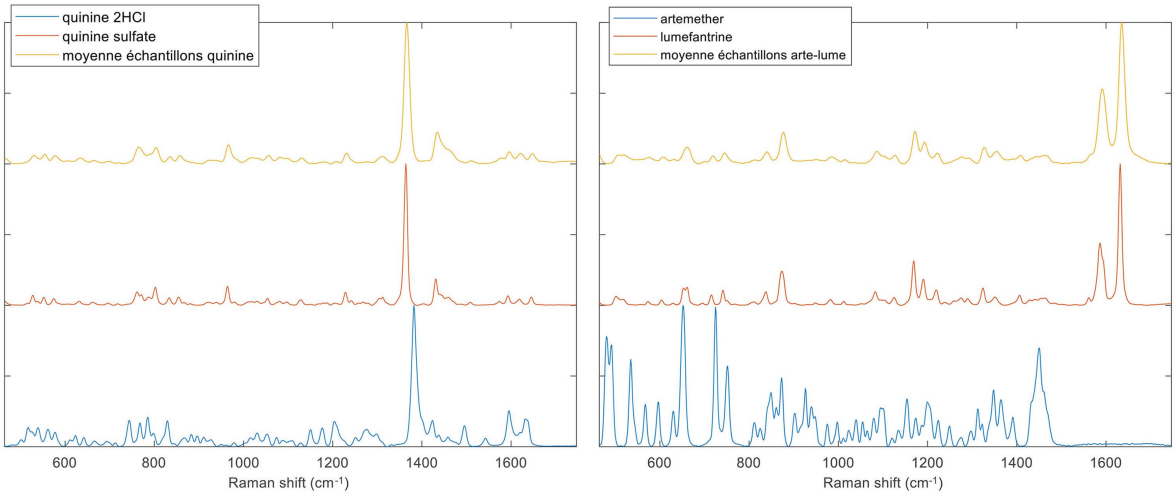


Figure 6. Analysis of PCAs in and out of the blister with Truscan. (a) scores in PC1-PC2, (b) scores in PC1-PC3, (c) scores in PC2-PC3.

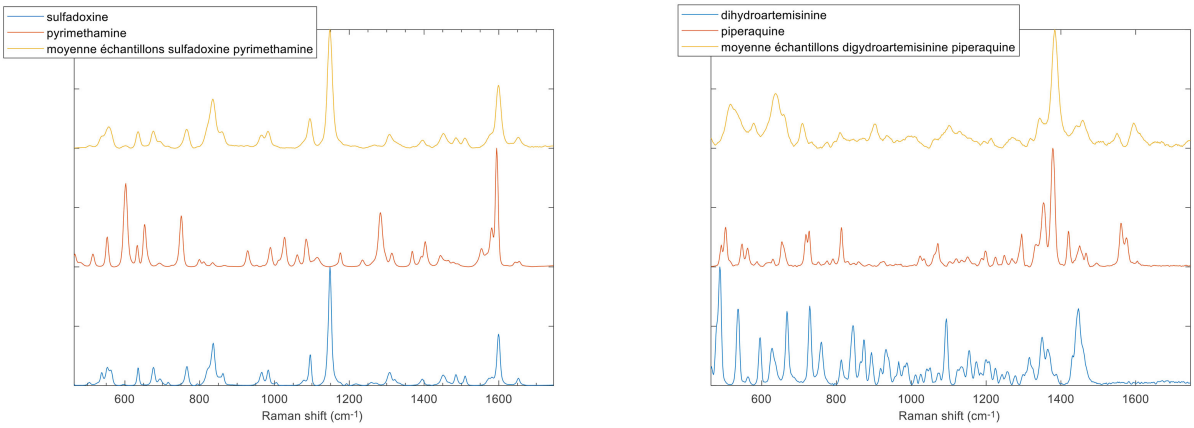
Near infrared spectroscopy (NIR-S-G1 and MPA) and Raman spectroscopy (Truscan) were used for the PCA of tablets in the blister and out of the blister after preprocessing the spectral data to improve the noise/signal ratio, in order to check the trends. The following results were obtained: with MPA, after analysis of the first three principal components giving more information on the data, there is an influence related to PVC (**Figures 1-3**); with NIR-S-G1, there was less influence of the blister as the main absorbance bands occur out of the PVC signal (**Figure 4** and **Figure 5**); and with Truscan, there was no influence of the blister (the spectra are superimposable) (**Figure 6**). This could be explained by the fact that the tablet samples were in the transparent blister. In addition, PVC blister packaging absorbs IR with MPA in transmission mode, which masks the spectrum of the tablet over the entire length of the path, but surface analysis bypasses the PVC with NIR-S-G1 and Raman in reflection mode.

The identification was not carried out with near infrared because there was not a model for this purpose, but rather with Truscan, which allowed us to identify the molecules with a high concentration in the different samples in associations. This corroborates the results of Patient *et al.* during their study on the comparison of the qualitative performances of portable near infrared and Raman spectrophotometers on the detection of falsifications of pharmaceutical products, where they noticed that with Raman, it is only possible to find the signal with highly dosed compounds because they identified lumefantrine alone in the Artemether-lumefantrine association [13]. Yabré *et al.* also used NIR-S-G1 for PCR and developed the DD-SIMCA model for the identification of their samples. Thus, they found some falsifications of Artemether-lumefantrine purchased on the illicit market, as well as samples of sulfadoxine-pyrimethamine and dihydroartemisinin-piperaquine after confirmation by thin-layer chromatography [17] [18].

3.2.4. Identification by RAMAN Spectroscopy by Comparison of Average Spectra with Spectra in the Database



(a) (b)

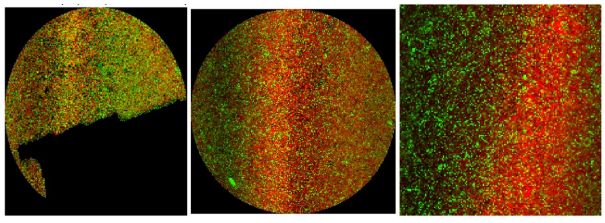


(c) (d)

Figure 7. Identification of samples by Raman spectroscopy.

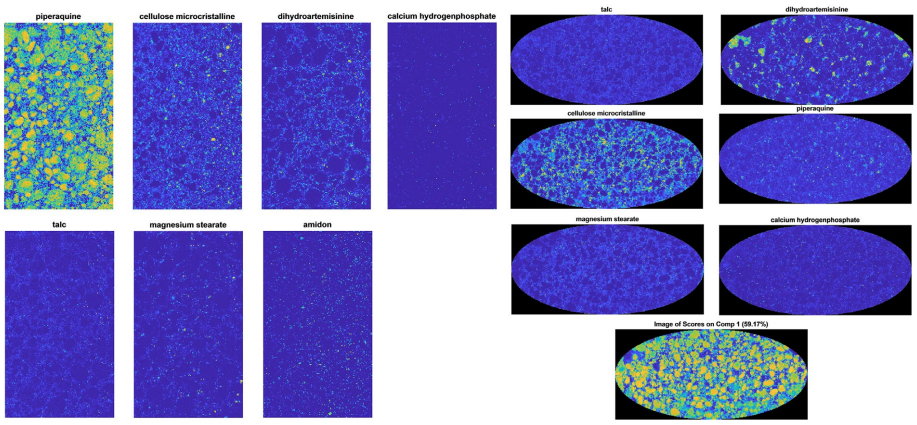
By comparing our spectra with the spectra in the database, lumefantrine has been identified in all artemether-lumefantrine samples, quinine in all quinine samples, sulfadoxine and pyrimethamine in all sulfadoxine-pyrimethamine combinations, and piperaquine in all dihydroartemisinin-piperaquine samples (**Figure 7**).

3.2.5. Raman Imaging Identification of Sulfadoxine-Pyrimethamine and Dihydroartemisinin-Piperaquine



(a) (b) (c)

Figure 8. Identification of sulfadoxine-pyrimethamine by Raman imaging. (a) Falcidox, (b) maladox 2510111, (c) maladox 2520001.



(a) (b)

Figure 9. Identification of dihydroartemisinin-piperaquine. (a) Artecure, (b) Pinax.

Tablets have been identified by Raman spectroscopy, and upon identification, lumefantrine was present in all artemether-lumefantrine samples, quinine in all quinine samples, pyrimethamine in all sulfadoxine-pyrimethamine combinations, and piperaquine in all dihydroartemisinin-piperaquine samples. Raman imaging allowed us to identify both components of the sulfadoxine-pyrimethamine and dihydroartemisinin-piperaquine combinations (Figure 8 and Figure 9).

3.3. Quantitative Analysis Results

➢ **Quinine sulfate by UV-Visible and NIR spectrophotometry**

Table 1. Concentration of quinine sulfate samples by UV-Vis.

Code	Statement	Average % in QS/ET by UV-Vis	Average % per NIR	Specification (90% - 110%) Decision
A008	500 mg	95.5 ± 2.53	97.59 ± 4.02	Compliant
A009	500 mg	99.7 ± 2.25	101.32 ± 0.76	Compliant
A010	500 mg	96.3 ± 2.10	95.25 ± 1.17	Compliant
A011	300 mg	104.8 ± 3.66	94.1 ± 0.20	Compliant

QS: quinine sulfate ET: standard deviation.

➢ **Quinine bihydrochloride by UV-Vis spectrophotometry**

Table 2. Concentration of quinine sulfate samples by UV-Vis.

Code	Statement	Content in %/Test	Average % in QB	Specification (90% - 110%) Decision
		99.8		
	300 mg/ml	100.5	100.1 ± 0.36	Compliant
		100		
		98.7		
	500 mg/2ml	98.1	98.4 ± 0.31	Compliant
		98.5		
		98.3		
	500 mg/2ml	98.1	98.4 ± 0.2	Compliant
		97.9		

All batches of quinine sulfate and bihydrochloride dosed were within the specification standard (90% - 110%) (Table 1 and Table 2). This coincides with the results of "Mbinze *et al.*" on the evaluation of the quality of quinine sulfate and bihydrochloride, metronidazole, and amoxicillin after validation of the method by using the total error strategy, the method we also used to analyze our samples. "Farid *et al.*" also found similar results in the quality control of antimalarials sold on the illicit market of Porto-Novo. The results also coincide with those of "Ciza *et al.*," who worked on the "Application of NIR handheld transmission spectros-

copy and chemometrics to assess the quality of locally produced antimalarial medicines in the Democratic Republic of Congo" [12] [14] [15].

➢ **Artemether-lumefantrine by UV-Vis spectrophotometry**

Based on these results, all six batches had an artemether concentration within the specification range, but only batch A006 had a lumefantrine concentration within the specification range. The first five batches had an underdose of lumefantrine (Table 3).

Table 3. Concentration of artemether-lumefantrine samples.

Code Statement	Artemether		Lumefantrine		Specification (90% - 110%) Decision
	Content in %/Test	Average % in Art/ET	Content in %/Test	Average % in Lum/ET	
	99.1		81.4		
A001 20 - 120 mg	98.8	99.03 ± 0.21	81.8	81.6 ± 0.2	Non-compliant
	99.2		81.6		
	100.1		81.5		
A002 80 - 480 mg	99.9	99.9 ± 0.57	81.9	81.7 ± 0.2	Non-compliant
	99.7		81.7		
	98.8		88		
A003 80 - 480 mg	98.4	98.6 ± 0.23	86.4	87 ± 1.1	Non-compliant
	98.8		85.8		
	94.8		87.8		
A004 20 - 120 mg	94.8	94.73 ± 0.12	85.8	85 ± 1.5	Non-compliant
	94.6		84.8		
	99.2		84.8		
A005 20 - 120 mg	98.6	99 ± 0.35	85.2	85.5 ± 0.9	Non-compliant
	99.2		86.6		
	99.7		93.5		
A006 20 - 120 mg	98	98.46 ± 1.08	90.5	91.5 ± 1.5	Compliant
	97.7		91.5		

4. Conclusions

At the end of this end-of-training work in the quality control of antimalarials sold on the Bukavu market in the Democratic Republic of Congo, non-conformities related to the checklist (labeling and visual examination) were noted, where two batches of quinine (breakage of the tablets in two in the blister and poor presentation of the internal label) and one batch of dihydroartemisinin-piperaquine (detachment of part of the coating in the blister) were qualified as "quarantined"; to pharmacotechnical tests including mass uniformity, friability, and hardness. Non-compliance was observed for one batch of quinine with regard to mass uniformity, two batches of quinine, three batches of artemether-lumefantrine, and two

batches of sulfadoxine-pyrimethamine for friability, and one batch of sulfadoxine-pyrimethamine, three batches of quinine, and one batch of dihydroartemisinin-piperaquine for hardness.

Vibrational spectroscopy was used to analyze samples by making a comparison in and out of the blister with near-infrared spectroscopy (MPA and NIR-S-G1) and Raman. During the analysis of the principal components PC1, PC2, and PC3 with MPA, there was an influence of the PVC constituent of the blister, but with NIR-S-G1 and Raman, there was no influence of the PVC because the main absorbance bands were outside the PVC signal. The identification of the active ingredients was carried out by Raman spectroscopy by comparing the spectra of the samples with the spectra of the database. Lumefantrine was identified in all artemether-lumefantrine samples, quinine in all quinine samples, sulfadoxine and pyrimethamine in all sulfadoxine-pyrimethamine combinations, and piperaquine in all dihydroartemisinin-piperaquine samples. Regarding the extractable volume of quinine bihydrochloride, all batches had a deviation of less than 5% and therefore were within the standard. Raman imaging allowed us to confirm the presence of all the constituents found in the sulfadoxine-pyrimethamine and dihydroartemisinin-piperaquine combinations.

Regarding the quantification of samples, the UV-Vis spectroscopic method and infrared were used for the analysis of quinine sulfate, quinine bihydrochloride, and artemether-lumefantrine, and according to the results obtained, there was non-conformity related to the dosage of quinine sulfate and bihydrochloride; all samples had a concentration within the specification interval (90% - 110%). However, regarding artemether-lumefantrine, all six batches had an artemether concentration within the specification interval, but the lumefantrine concentration was out of specification for the first five batches. It is advised to strengthen packaging and labeling procedures to prevent tablet damage and enhance product appearance, ensuring regulatory compliance before market launch. Furthermore, improve pharmacotechnical parameters like uniformity of weight, friability, and hardness through more stringent in-process quality checks and excipient optimization. To address lumefantrine under-dosing issues, conduct thorough investigations into formulation problems and implement corrective manufacturing actions to guarantee effective medication delivery.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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