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Am. J. Trop. Med. Hyg., 00(00), 2023, pp. 1–9
doi:10.4269/ajtmh.23-0045
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Detection of Substandard and Falsified Antibiotics Sold in the Democratic Republic of the Congo Using Validated HPLC and UV-Visible Spectrophotometric Methods

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Abstract. The access to afford safe, effective, and genuine medications is a major challenge for people in low- to middle-income countries. This study aimed at developing and validating simple, accurate, and inexpensive analytical liquid chromatography and ultraviolet-visible spectrophotometric methods to ensure quality control of antibiotics sold in formal and informal pharmaceutical markets. It focused on four antibiotics (azithromycin [AZT], cefadroxil [CFD], cefixime [CFX], and erythromycin [ERH]) used to treat infectious diseases in the region of Haut-Katanga in the Democratic Republic of the Congo (DRC). The total error strategy (accuracy profile) matching with the validation requirements of International Council on Harmonization was used for the validation. The validation results showed that three analytical methods of AZT, CFD, and ERH were validated according to the accuracy profile obtained, whereas the proposed method of CFX was not validated. Therefore, the United State Pharmacopoeia method permitted to quantify CFX samples. The dosage intervals ranged from 25 to 75 µg/mL for CFD, from 750 to 1,500 µg/mL for AZT, and from 500 to 750 µg/mL for ERH. The application of the validated method to samples collected ($N = 95$) allowed the detection of 25% substandard antibiotics with a rate of poor quality much higher in the informal circuit compared with the formal one (54% versus 11%; $P < 0.05$). The routine application of these methods will strengthen the quality control of drugs marketed in DRC. This study gives evidence for the availability of poor-quality antibiotics in the country, requiring the immediate attention of the national medicine regulatory authority.

INTRODUCTION

Substandard and falsified (SF) medications represent a permanent threat that compromises the improvement of health in the world.[1] They are responsible for several cases of treatment failures, mortality, toxicity, and the sudden uprising of resistance to antibiotics used to eradicate pathogenic bacteria.[2,3] Although SF medications affect all parts of the world, the African continent has the highest prevalence of SF medications worldwide.[4] Nearly one out of five medicines sold across the continent is either falsified or of low quality.[4] Several factors may explain this situation, such as the inadequacy of the quality assurance system (good manufacturing practices, good distribution practices and good pharmacy practices), the medications sale in inappropriate places, the lack of analytical strategies to detect substandard medicines, improper manufacturing, and poor monitoring and surveillance systems to verify the quality of pharmaceutical products.[5–7]

In the context of the COVID-19 pandemic, several cases of falsification of vaccines and drugs (e.g., chloroquine) intended to treat COVID-19 have been reported in some African countries, namely Cameroon, Chad, the Democratic Republic of the Congo (DRC), Nigeria, and South Africa.[4,8–10] Democratic Republic of the Congo, being the second largest country in Africa, is seriously affected by the problems of substandard medications.[11] In fact, it is surrounded by nine neighboring countries. This situation makes it particularly vulnerable to the entry of medicines by smuggling and partly explains the circulation of medicines of dubious quality in this country. Previous studies carried out in DRC have already addressed the issues associated with SF medicines and their magnitude within the country.[12–14] Most of them focused on antimalarials, antibiotics, and medicines for non-communicable diseases in the region of Kinshasa (the capital city of DRC). However, data on SF medicines in the Region of Haut-Katanga (one of the four largest provinces of the country) remain barely available.

To effectively combat the scourge of SF medicines, simple and accurate analytical methods, able to detect and quantify suspected SF antibiotics, are necessary. These analytical methods are described in pharmacopoeias and involve techniques such as high-performance liquid chromatography (HPLC) or gas chromatography coupled to detection systems such as ultraviolet spectroscopy (UV), mass spectrometry, fluorescence, or chemiluminescence.[15–17] However, in low- and middle-income countries (LMICs) such as DRC, these methods are hardly achievable because of the high cost of analysis because most of them require high-grade solvents and expertise.[17,18] This makes their use difficult in routine analysis carried out in quality control laboratories. In this context, LMICs urgently need inexpensive, easy-to-use, and rapid methods to detect low-quality medicines and diagnostics throughout the supply chain.[18] High-performance liquid chromatography and UV-visible (UV-vis) spectrophotometry are among the most widely used techniques for quality control of essential medicines in LMICs.[19,20] Therefore, this work focused on these two analytical techniques. Hence, the overall objective of the present study was to develop and validate simple, rapid, and accurate HPLC and UV spectrophotometric methods suitable for routine quality control testing of four widely used antibiotics (azithromycin [AZT], cefadroxil [CFD], cefixime [CFX], and erythromycin [ERH]) in the Haut-Katanga region. Methods were

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validated according to guidelines of the International Council on Harmonization (ICH) Q_2 (R1). The strategy of total error represented by the accuracy profile has been used as a decision tool for the validation. The following validation criteria were studied: selectivity (specificity), accuracy, fidelity (repeatability and intermediate fidelity), accuracy, linearity, limit of detection (LOD) and limit of quantification (LOQ), and dosage interval.[21,22]

MATERIALS AND METHODS

Sampling. Sample collection took place in March 2020 in both formal and informal sectors. Lubumbashi was selected as the study area simply because it is the second largest urban zone in DRC after Kinshasa and is the main hub for importation and trade. Lubumbashi is the capital city of the Haut-Katanga province and covers an area of 747 km^2, located in the southeastern part of DRC. The expansion of mining and commercial activities made Lubumbashi an attractive city and the main hub for importation and trade.[23]

Medicines were purchased from private retail drug stores, including retail shops and wholesale shops selling retail medicines. Pharmaceutical outlets were classified into formal and informal sectors. The formal sector represented all the pharmaceutical outlets having the authorization from National Medication Regulatory Authorities (NMRA) to sell medicines; the informal circuit included only retail private shops where medicines are sold without authorization from the NMRA. In DRC, the NMRA is called "Autorité Congolaise de Réglementation Pharmaceutique" (ACOREP). Therefore, all 35 licensed wholesalers in Lubumbashi were eligible. They play a central role by supplying the public and private sectors across the region of Haut-Katanga. Concerning retail private shops, the ACOREP listed only 12 that were licensed in Lubumbashi. Twenty-five started processes to obtain the license. In the present study, all 12 licensed retail drug shops and the 25 that have started processes to obtain the license were considered.

Elsewhere, the informal circuit included only retail private shops where medicines were sold without the license from the ACOREP; the exact number of unlicensed pharmaceutical outlets was not available. A survey conducted in 2017 on the informal sector in Haut-Katanga revealed that 333 pharmaceutical outlets are available in this region.[24] In this report, we focused only on informal drug vendors where drugs can be found spread out on shelves or the ground. The informal drug vendors operated in small shops located away from the main shopping roads and mainly in markets.

The targeted antibiotics were azithromycin, CFX, CFD, and ERH. Therefore, to distinguish samples, we considered active pharmaceutical ingredient (API), dosage form, and batch number as mentioned by the manufacturer. In the case where samples had the same API, dosage form, and batch number, a careful visual inspection was performed to make sure that other elements (manufacturer's address, hologram, manufacturer's logo, notice, physical appearance of dosage form, packaging color, barcode, etc.) were also identical. In this context, medicines collected from the same outlet and labeled with the same international nonproprietary name, brand name, strength, size, batch/lot number, and manufacturing and expiry dates were considered as one sample. We also checked whether the samples collected were registered and authorized to be marketed in DRC by consulting the official list of registered drugs.

The sample size was estimated using the following equation[25]:

$$N = p(1-p)z^2/E^2$$

where p is prevalence, z is the confidence interval, and E is the margin of error. The exact prevalence of antibiotics is not available. We assumed a prevalence of 30% ($P = 0.3$) for substandard medicines in some regions of sub-Saharan Africa. With the margin of error E (10%) and 95% of confidence interval ($z = 1.96$), the random sample size estimated is 81 samples.

The collected samples were then immediately encoded in a sample library using physical inspection of the sample (appearance, integrity, attribution of a code) and storage of the samples according to the manufacturer's specifications. In this case, samples were stored at room temperature (15°C–25°C), and the temperature was monitored during the storage of samples throughout this study.

Reagent and standard. Ammonium acetate (98%), methanol (HPLC gradient grade), ammonium hydroxide 32%, hydrochloric acid 37%, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Starch, crospovidone, lactose monohydrate, magnesium stearate, and microcrystalline cellulose were purchased from Fagron N.V. (Waregem, Belgium). AZT (purity 98%), CFD (purity 98%), CFX (purity 98%), and ERH (purity 98%) were purchased from TCI Belgium (Waregem, Belgium). Ultrapure water was produced with a Milli-Q Plus 185 water purification system (Millipore, Billerica, MA).

The HPLC system consisted in a Waters 2695 separation module connected to a photodidode array (model no. 2996; Waters Corporation, Eschborn, Germany).

Preparation of stock standard solution.

Stock standard solution for HPLC method. AZT and CFD were determined by the HPLC method. A generic and nonvalidated liquid chromatography method developed by Mbinze et al.[26] was used at the start. Two stock solutions were prepared for the development phase. The first stock solution was prepared by dissolving 50 mg of AZT in a 10-mL volumetric flask with methanol. This solution was diluted five times to reach a final concentration of 1,000 μg/mL using a mixture of water and methanol (92%/8%, v/v) prior to injection at the HPLC system. AZT was used at a higher concentration (1,000 μg/mL) level due to its weak absorptivity in the UV range. The second stock solution was prepared in methanol by dissolving 20 mg of CFD in a 20-mL volumetric flask. This solution was diluted 20 times to reach a final concentration of 50 μg/mL by using the same solvent mixture as for AZT.

In the development phase, gradient elution mode was used for CFD and AZT. For CFD, the proportion of methanol in the mobile phase varied from 8% to 95%, and gradient time was set to 40 minutes.[26] Then, the isocratic elution mode was carried out with 95% methanol and 5% aqueous solution of ammonium acetate buffer for 10 minutes. However, considering that the characteristics of our column (xBridge Shield C18 100 mm by 4.6 mm, 3.5 μm) were different from the generic method (xBridge Shield C18 250 mm by

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4.6 mm diameter, particle size 5 μm), we had to proceed to adjustment of the flow according to the following equation:

$$F_1F_2 = \frac{l_2d_2^2}{l_1d_1^2}$$

where F_1 is the flow indicated in the monograph (minutes), F_2 is the flow adjusted (minutes), L_1 is the length of the column indicated in the monograph (mm), L_2 is the length of the column used (mm), D_1 is the internal column diameter indicated in monograph (mm), and d_2 is the internal column diameter used (mm).

Regarding AZT, the same mobile phase was used as for the method proposed for AZT. The gradient time was fixed at 10 minutes (the proportion of methanol in the mobile phase changing from 5% to 80%). The isocratic elution mode was performed with 80% methanol and 20% aqueous solution of the ammonium acetate buffer for 10 minutes. Variations were made on three factors: pH of the aqueous phase, column temperature, and gradient time. The temperature was varied at three different levels (25°C, 30°C, and 35°C), as was pH (3, 6, and 10). These factors were modified one after the other until optimal experimental conditions were achieved.

Stock standard solution for the UV-vis spectrometric method. Cefixime and ERH were concerned for UV-vis method. For CFX, a serial of three different stock solutions was prepared by dissolving 20 mg of CFX in a 20-mL volumetric flask using distilled water, 0.1 N hydrochloric acid, and 0.1 N sodium hydroxide, respectively. These three solutions were subsequently diluted 50, 33.3, and 25 times to reach final concentrations of 20, 30, and 40 μg/mL of CFX. The absorbance of these solutions was measured using the UV spectrophotometer at 220, 254, and 257 nm for CFX and at 215 and 305 nm for ERH. For ERH, two final concentrations of 1,000 and 500 μg/mL were obtained by diluting a stock solution of 2,000 μg/mL two and four times.

The robustness of the developed method was assessed by varying slightly selected critical parameters (e.g., pH and temperature) to verify whether they have an impact on the results obtained. These variations were set at ±0.2 for pH and temperature (i.e., 5.8 and 6.2 and 24.8 and 25.2°C for the CFD method; 9.8 and 10.2 and 34.8 and 35.2 for AZT method).

Working standard solutions for HPLC method validation. A stock solution of calibration standard (CS) of AZT and CFD was prepared as indicated above. From this, calibration standard solutions were prepared at three concentrations levels: Level 1 (50%), 25 μg/mL (CFD) and 500 μg/mL (AZT); Level 2 (100%), 50 μg/mL (CFD) and 1,000 μg/mL (AZT); and Level 3 (150%), 75 μg/mL (CFD) and 1,500 μg/mL (AZT).

A stock solution of validation standard (VS) was prepared by incorporating the matrix into the standard to simulate as much as possible the real drug product composition to evaluate the matrix effect. This matrix was composed of commonly used excipients in solid dosage forms. First, the average weight of tablets and capsules of the selected samples was determined to find out the proportion of the API and the excipients in the dosage form. For the AZT tablets, the proportions of API and excipients were 58.5% and 41.5%, respectively. These proportions were 90.3% and 9.7%, respectively, for CFD capsules.

Concerning AZT, a matrix stock solution was prepared by dissolving in methanol 26.64, 10.65, 12.43, 152.71, and 152.71 mg, respectively, of starch, magnesium stearate, crospovidon, monohydrate lactose, and microcrystalline cellulose in a 25-mL volumetric flask, and volume was increased with the same solvent. Then 1 mL of this stock solution was transferred to a 10-mL volumetric flask containing the standard stock solution, and volume was increased with methanol.

For CFD, 13.94, 6.43, and 194.03 mg of starch, magnesium stearate and lactose monohydrate, respectively, were weighed and then dissolved in a 100-mL volumetric flask, and volume was increased with methanol; 1 mL of this solution was transferred to a volumetric flask containing the standard stock solution. All the validation solutions were filtered through 0.45-μm polytetrafluoroethylene prior to further dilution to obtain aliquots to be analyzed.

The stock solution of VS was obtained in the same way as indicated in the CS. From that five different levels of concentration were achieved: Level 1 (50%), 25 μg/mL (CFD) and 500 μg/mL (AZT); Level 2 (75%), 37.5 μg/mL (CFD) and 750 μg/mL (AZT); Level 3 (100%), 50 μg/mL (CFD) and 1,000 μg/mL (AZT); Level 4 (125%), 62.5 μg/mL (CFD) and 1,250 μg/mL (AZT); and Level 5 (150%), 75 μg/mL (CFD) and 1,500 μg/mL (AZT).

Independently, three solutions for each concentration level were prepared. The preparation of these solutions was repeated for three consecutive days corresponding to three different series. Accordingly, a total of 27 tests for the CS and 45 tests for the VS were carried out. All these solutions were freshly prepared, ultra-sonicated for complete dissolution, and kept away from light to avoid the degradation of light-sensitive antibiotics.

Chromatographic conditions. An analytical column XBridge Shield (Waters Corporation) C18 (100 mm × 4.6 mm inner diameter, 3.5 μm particle dimension) was used to perform the separation. Analytes were monitored photometrically at a wavelength of 220 nm. The flow rate was 1 mL/minute. The collected chromatographic data were the UV spectra of the analytes, retention times, peak areas, and peak heights.

The buffer solution of the mobile phase (buffer/methanol) consisted of 10 mM ammonium acetate. The pH was adjusted with hydrochloric acid 5 N or hydroxide sodium 1 N depending on the targeted pH value. The pH of mobile phase and buffer was measured with a Seven Easy S20 pH meter (Mettler Toledo, Columbus, OH).

For the development of the method of AZT, variations were done on pH of the aqueous phase, temperature of the column, and gradient time. These factors were modified one after the other until an optimal experimental condition was obtained.

Software. Empower 2.0 software (Waters Corporation, Milford, MA) for Windows was used to control the LC system, record the signals from the detector, and interpret the generated chromatograms. Data were treated using Excel version 2013. The accuracy profiles, validation results, and uncertainty estimation were obtained using e-novalV3.0 software (Arlenda, Belgium).

Working standard solutions for spectrophotometric method validation. The preparation procedure was the same as for the HPLC method. However, the proportions of API

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and matrix were 59% and 41% for CFX and 80% and 20% for ERH, respectively. For CFX, dissolution in a 25-mL volumetric flask of starch (25.6 mg), magnesium stearate (10.2 mg), polyvinylpyrrolidone (11.9 mg), lactose monohydrate (11.9 mg), and microcrystalline cellulose (146.7 mg) was carried out with water. Then 1 mL of this solution was transferred to a 20-mL volumetric flask containing 20 mg of CFX standard, and water was added to achieve the target volume.

For ERH, dissolution in a 50-mL volumetric flask of starch (18.9 mg), magnesium stearate (75 mg), lactose monohydrate (111.9 mg), and microcrystalline cellulose (111.9 mg) was done with methanol. Then 1 mL of this solution was transferred to a 10-mL volumetric flask containing 20 mg of the ERH standard.

From the above solution, five different levels of concentration were prepared: Level 1 (50%), and 20 μg/mL (CFX) and 250 μg/mL (ERH); Level 2 (75%), 30 μg/mL (CFX), 375 μg/mL (ERH); Level 3 (100%), 40 μg/mL (CFX) and 500 μg/mL (ERH); Level 4 (125%), 50 μg/mL (CFX) and 62.5 μg/mL (ERH); and Level 5 (150%), 60 μg/mL (CFX) and 750 μg/mL (ERH).

Sample solutions for routine analyses. The samples collected were treated the same as the reference substances. They were prepared in the same conditions as the standard at the concentration matching the third level of the VS (100%), which corresponds to 50 μg/mL for CFD, 1,000 μg/mL for AZT, 40 μg/mL for CFX, and 500 μg/mL for ERH. Vortexing (5 minutes) and sonication (5 minutes) in the ultrasonic bath were necessary to ensure a complete dissolution of the samples. The obtained samples were centrifuged (2,500 rpm, 5 minutes) and filtered through a 0.45-μm PTFE syringe prior to injection into the LC system. The specifications taken into account are those of the USP: 90–110% for AZT (tablet et capsule) and CFX (tablet) and 90–120% for CFD (capsule and oral suspension), CFX (oral suspensions), and ERH (tablet and oral suspension). Samples out of specification were analyzed a second time by another analyst to reach a final decision. The matrix used simulated the composition of capsule and tablet dosage forms. Supplemental Table 1 gives details of the LC methods described in the United States Pharmacopoeia for the analysis of the oral suspension antibiotics that were used.

Regarding the identification of API, both retention time and overlapping of UV-vis spectra of the analyte of interest with regard to reference substance were used for HPLC methods. REgarding weight uniformity, the compliance criteria required that at most two units may deviate from the average mass of a specified percentage and that the mass of no unit may deviate from double of this percentage.[27] Regarding content uniformity, the acceptance value (AV) taken into account was calculated according to the following equation[28]:

$$AV = |M - x| + KS$$

where x is the mean of an individual content expressed as a percentage of label claim, M is the reference value, K is acceptability constant, and S is the sample standard deviation. The AV must be $\leq$ 15.0%.

RESULTS AND DISCUSSION

Overview of collected samples. A total of 95 samples were collected. From 137 pharmaceutical outlets visited, 16 (11.6%) outlets were excluded because they were either closed at the time of sampling, refused to sell, or lacked the antibiotics studied. Samples from the formal sector accounted for 73% of the total samples collected; 27% of the samples were collected from the informal sector. Samples from wholesale shops represented 66% of the total samples collected, and 34% were from pharmaceutical outlets selling retail medicine; 58% of the collected samples were manufactured in India, 17% domestically, 11% in China, and 9% in France (Supplemental Figure 1). This finding also corroborates with previous studies reporting that India is among the biggest suppliers of drugs circulating in Africa.[29]

Regarding the drug products, CFX represented 38% of the collected samples, followed by CFD (23%), AZT (21%), and ERH (18%). Indeed, 11 different manufacturers of CFX were identified in the region, versus eight for AZT, seven for CFD, and six for ERH. Tablets accounted for 41% of samples, followed by oral suspension (30%) and hard capsules (29%). Overall, 37% of samples were neither registered nor authorized to be marketed in the region. The proportion of samples not authorized and collected in the informal sector was higher (64.3% versus 35.1%; $P < 0.05$) than in the formal sector.

Quantification method development.

LC methods. The estimated flow rate of 0.4 mL/minute was obtained after applying the equation indicated above. The retention time of CFD was 6.7 minute. However, because our column could resist flow to a rate greater than 0.4 mL/minute, the flow rate of 1 mL/minute was finally used to reduce the analysis time, and the retention time of CFD was found to be 2.68 minutes (Figure 1). The isocratic elution mode was therefore retained because it gives a shorter retention time. Table 1 gives the conditions selected for the analysis of CFD.

Table 2 gives the selected experimental conditions for AZT. The retention time of AZT was 4.95 minutes at these conditions (Figure 2).

Spectrophotometric method. A series of experiments was carried out to determine the target concentration, the wavelength, and the diluent to be used for sample preparation (Supplemental Table 2). These analytical conditions were selected by considering the linearity range, with a coefficient of determination (R^2) nearing 1 and the absorbance between 0.2 and 0.8 (middle of the Beer-Lambert range).

To implement CFX, the following conditions were selected: water as solvent, detection and quantification at 220 nm, and 40 μg/mL as the target concentration. For ERH, the conditions were methanol as solvent, detection and quantification at 215 nm, and 500 μg/mL as the target concentration.

Validation of the proposed methods. It was necessary to demonstrate that these methods provide accurate and reliable results. Validation was carried out according to the guidelines of ICH Q2 (R1). The strategy of total error represented by the accuracy profile has been used as decision tool for the validation.[30]

Selectivity/specificity. The selectivity of AZT and CFD was evaluated by injecting separately the blank, the matrix, the standard, and the standard with the matrix (Supplemental Figure 2). No interference was observed between the peak of AZT and CFD, showing the selectivity of two methods. The retention time and UV spectra of these two antibiotics were not affected by the matrix used. Concerning the

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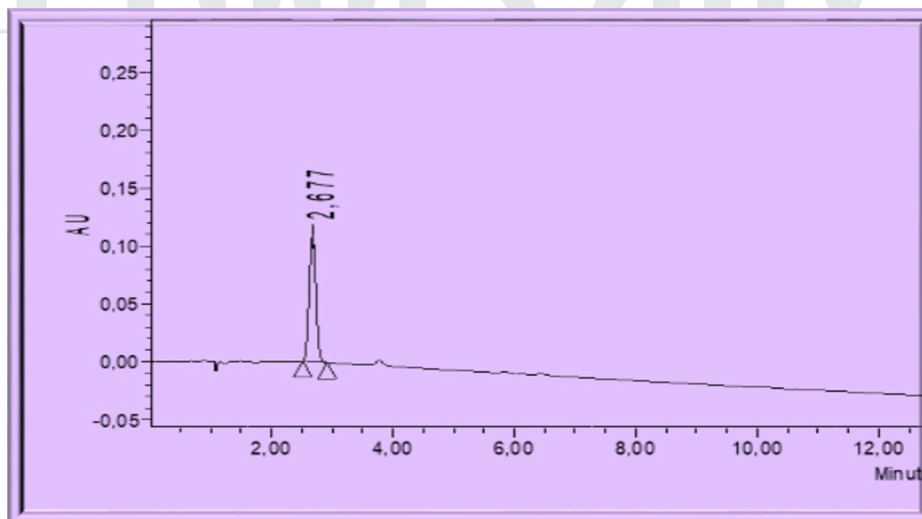

FIGURE 1. Chromatogram obtained for cefadroxil (retention time: 2.68 minutes).

specificity of UV-vis as the spectrophotometric method of ERH, no interference was observed between the matrix used and the ERH standard at the chosen wavelength. Moreover, there was no significant difference (t-calculated < t-table) between the average of the absorbance values obtained with the standard as compared with standard with the matrix solutions. However, the matrix has a significant effect with CFX. Indeed, the absorbance increased considerably for CFX standard solution alone in comparison to CFX solution with the matrix. The value of t-calculated was much higher than the t-table (Supplemental Table 3). Therefore, spectrophotometric method of CFX was not considered to be specific.

Linearity. Linearity was determined by evaluating the relationship between the calculated concentration (VS) and the introduced concentration (CS). Both methods are linear, as shown in Figure 3. The tolerance limits for each concentration level were found within the acceptance limits. The slope (0.95 for AZT and 1.01 for CFD) and the correlation coefficient ($R^2 =$ 0.9956 for AZT and 0.9970 for CFD) are close to 1.

Trueness. The trueness was evaluated by considering the mean of bias observed between the series of measurements and the reference concentrations. Using the calibration curve of each analyte, the concentrations of the VS were back-calculated and expressed in terms of absolute bias (μg/mL) and relative bias (%). The results obtained showed that the trueness was acceptable for the developed methods for AZT, CFD, and ERH because the relative bias of all concentration levels was not greater than 3% (Supplemental Table 4). In contrast, the trueness of the CFX method was not acceptable. Indeed, except for the third, fourth, and fifth concentration level, which gave a relative bias of less than 15%, the relative bias for two other concentration levels (first and second) was greater than 15%.

Precision. Precision was expressed in terms of relative standard deviation (%) for repeatability (intraday variations) and intermediate precision (inter-day variations) at each concentration level. The precision was found acceptable for all methods, as shown in Supplemental Table 5.

Accuracy. The accuracy was determined by the 95% β-expectation tolerance interval to observe the closeness of agreement of every concentration measured by the method and the assumed true value of this concentration. It combines the uncertainties of trueness and precision and is expressed both as an actual value and as a percentage of the targeted concentration.

For CFD, all the concentration levels considered fall within the 10% acceptance limits fixed for all target concentration levels, guaranteeing that 95% of the future quantification results will be accurate in the evaluated dosage interval (Figure 4).

Regarding the accuracy profile of AZT, the lower and upper limits of the first concentration level (500 μg/mL) are outside of the acceptance limits. Therefore, this method is valid only for the second, third, fourth, and fifth concentration levels.

TABLE 1
Chromatographic conditions selected for the analysis method of CFD: isocratic elution mode

Characteristics	xBridge C18 (100 mm by 4.6 mm; particle dimension, 3.5 μm)
Mobile phase	buffer (ammonium acetate): methanol (92:8)
Flow	1 mL/minute
Injection volume	10 μL
Column temperature	25°C
System detection	photodiode array, 220 nm
pH of buffer	6

CFD = cefadroxil.

TABLE 2
Chromatographic conditions retained for the analysis method of AZT: isocratic elution mode

Characteristics	xBridge C18 (100 mm by 4.6 mm inner diameter; particle dimension, 3.5 μm)
Mobile phase	methanol:buffer (ammonium acetate), 80:20
Flow	1 mL/minute
Injection volume	10 μL
Column temperature	35°C
System detection	photodiode array, 220 nm
pH of buffer	10

AZT = azithromycin.

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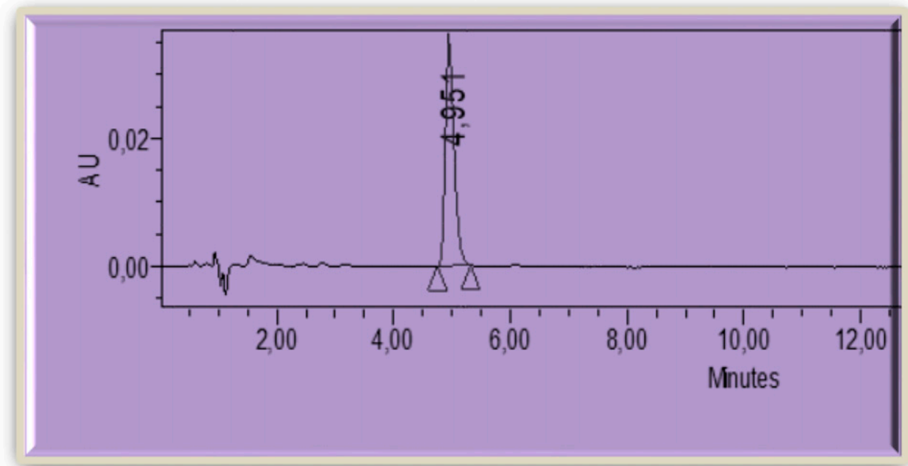

FIGURE 2. Chromatograms obtained for azithromycin (retention time: 4.95 minutes).

CFD

Back calculated concentration (µg/mL)

Introduced concentration (µg/mL)

AZT

Back calculated concentration (µg/mL)

Introduced concentration (µg/mL)

ERH

Back calculated concentration (µg/mL)

Introduced concentration (µg/mL)

FIGURE 3. Linearity profile. Dashed blue line indicates the 95% β-expectation intolerance interval, dashed black line indicates acceptance limit, and blue dots indicate back-calculated concentrations.

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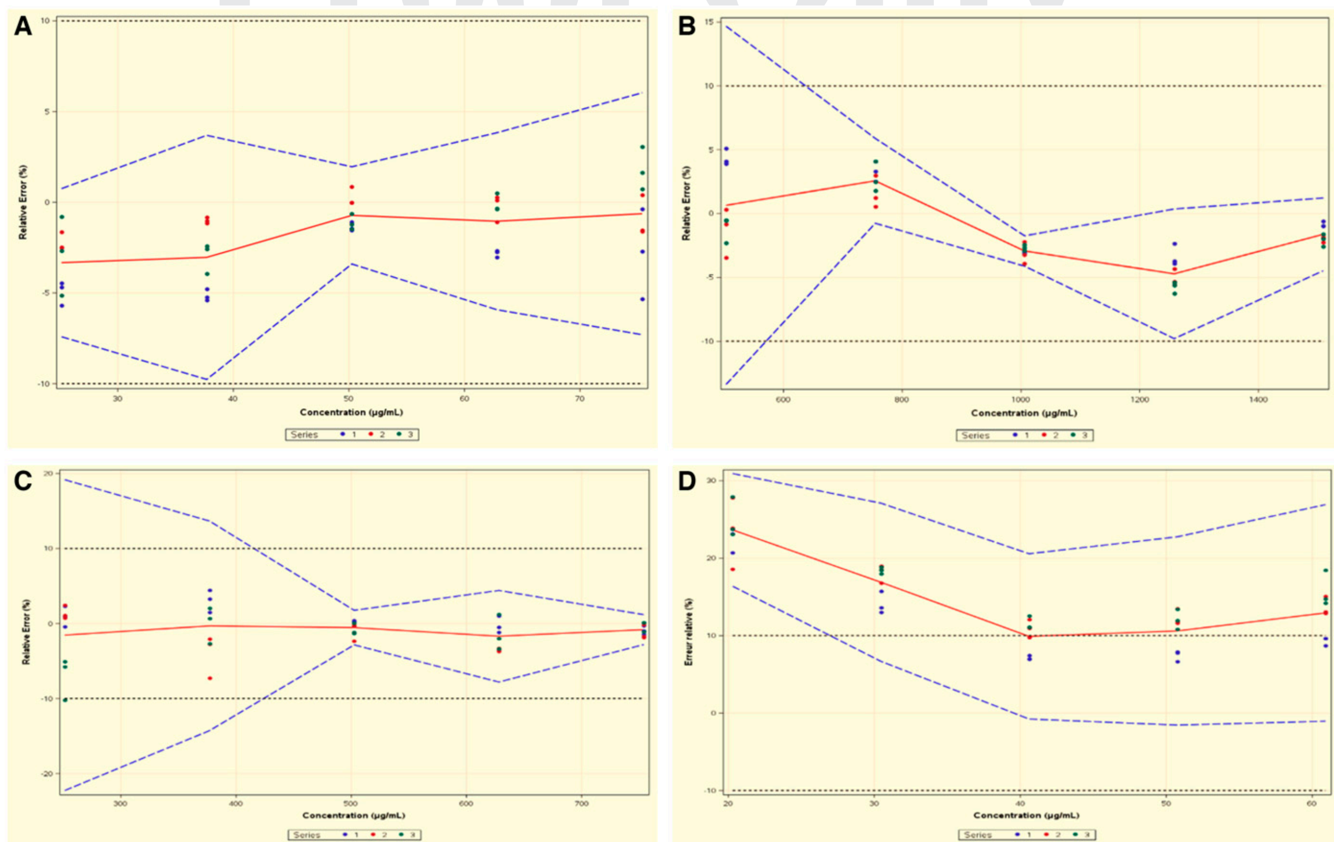

FIGURE 4. Accuracy profile (**A**) cefadroxil, (**B**) azithromycin, (**C**) erythromycin, and (**D**) cefixime. Solid red line: relative bias (%); dashed blue line: 95% β-expectation tolerance interval; dots: relative back-calculated concentrations; dashed black line: acceptance limits (±10%).

Regarding the accuracy profile of ERH, only the third, fourth, and fifth concentration levels are valid, whereas the first and second levels are not included in the acceptance limits. Conversely, the method is not valid for CFX because this range of concentration (20–60 µg/mL) did not fall between the acceptance limits.

LOD and LOQ. The LOQs were evaluated by calculating the lowest and the highest concentrations beyond which the accuracy limits or β-expectation tolerance limits go outside the acceptance criteria. The lower and upper LOQ values obtained (µg/mL) were 25.1 and 75.4 for CFD, 640 and 1,509 for AZT, and 423.9 and 753.80 for ERH. The LOD was evaluated experimentally by considering the signal-to-noise ratios of 3. The values of LOD obtained (µg/mL) were CFD (4.87), AZT (57.4), and ERH (11.99). Methods developed were robust because no significant variations were observed when variations of the experimental conditions were performed.

Application. The validated methods of AZT, CFD, and ERH were applied to samples collected to assess and consolidate their use in routine analysis.

The results of quantitative estimations for the 95 samples show that 25% of the samples contained an amount of API that was out of specifications according to the USP (Supplemental Table 6). All of-out-specifications samples were underdosed (ranging from 62.5% to 88.7%). More than half of-out-specifications samples (54%) found were related to tablets, followed by capsules (29%) and oral suspensions (17%). Elsewhere, samples unregistered in the country represented 54% of nonconformities. Furthermore, the proportion of substandard samples sold in the informal sector was higher than those collected in the formal sector (54% versus 11%; $P < 0.05$).

Twelve out of 36 samples of CFX drug products had unqualified drug contents ranging from 62.5% to 88.6%. For CFD, of 22 samples analyzed, six were found to be substandard, and 2 out 17 samples of ERH drug products were noncompliant. The results of weight uniformity showed that all samples analyzed (70 samples) were compliant according to the European Pharmacopoeia and USP specifications.[27,28] The same finding was obtained for content uniformity, where the AV of all samples was less than 15.0%.

Limitations of this study. The selection of antibiotics did not take into account those administered parenterally. Only four antibiotics were considered, whereas several antibiotics are used in the management of infectious diseases. Therefore, the results are not representative of injectable antibiotics and other types of orally administered antibiotics that were not included in this study.

Sample collection did not include all drug outlets in detail; therefore, it is likely to be confirmed that several samples were not collected in this study. However, the results presented in this work give a real picture about the quality of antibiotics marketed locally.

In addition to the preceding portraits, interlaboratory studies were not carried out in this study to deepen the data on random errors. This implies that these methods will only be

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used within a laboratory. Furthermore, these methods did not take into account the impurities likely to accompany the molecules of antibiotics studied, which means that there are no guarantees on the detection of impurities that may be present in drug samples.

This study confirms that low-quality medications affect mostly developing countries and are responsible for many cases of morbi-mortality. The detection of SF medicines can contribute to strengthen the surveillance and decrease the rate of falsification. In this framework, fighting against SF medicines, three new analytical methods (two HPLC and one UV-vis spectrophotometric) were validated for the quantitative analysis of three antibiotics (AZT, CFD, and ERH) present in pharmaceutical products. This method has been validated in terms of selectivity, trueness, precision (repeatability and intermediate precision), accuracy, linearity, limit of detection/limit of quantitation, and dosage interval. The strategy of the total error has been used to validate these methods that present advantages of being also simple, rapid, and affordable. The methods have been successfully applied to samples ($N = 95$) containing the above-mentioned antibiotics marketed in DRC. Twenty-five percent of samples contained insufficient amounts of API. Finally, the described methods demonstrate their suitability for quality control of both high- and low-quality antibiotics. They are fast and not labor intensive. We believe that the routine application of these methods will strengthen the quality control of drugs marketed in DRC. Equally, this study documents the availability of low-quality antibiotics, demonstrating the need for immediate attention by the ACOREP.

Received January 20, 2023. Accepted for publication May 11, 2023.

Published online June 20, 2023.

Note: Supplemental materials appear at www.ajtmh.org

Acknowledgments: We thank the ARES (Academy of Research and Higher Education-Commission for Development Cooperation) for support. The American Society of Tropical Medicine and Hygiene (ASTMH) assisted with publication expenses.

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