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Development and validation of a hydrotropic UV–Visible spectrophotometric method for the simultaneous assay of ornidazole and ofloxacin tablets

Shabani, M. L.[1], Ngoyi, M. N.[5], Tweni, B. E.[2], Mbinze, K. J.[3], & Marini, D. R.[4]

[1]*Drug Analysis Laboratory, Department of Galenics and Drug Analysis, Higher School of Pharmaceutical Technician, Higher Institute of Medical Techniques (ISTM-Bukavu), Democratic Republic of the Congo* [2]*Department of Pharmacy, Faculty of Medicine and Pharmacy, University of Kisangani, Democratic Republic of the Congo* [3]*Drug Analysis Laboratory, Department of Galenic and Drug Analysis, Faculty of Pharmaceutical Sciences, University of Kinshasa, Democratic Republic of the Congo* [4]*Pharmaceutical Analytical Chemistry Laboratory, Department of Pharmacy, CIRM, Faculty of Medicine, University of Liège, Belgium* [5]*National Laboratory for Quality Control of Drugs and Health Products (LNCQM), Kinshasa, Democratic Republic of the Congo.*

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Correspondence to:
Luc Shabani
shamilamitongo@gmail.com

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ABSTRACT

Introduction
In the Democratic Republic of Congo (DRC), weaknesses in pharmaceutical distribution systems contribute to the circulation of substandard and falsified medicines. The fixed-dose combination of ofloxacin and ornidazole is commonly prescribed for the treatment of mixed bacterial and protozoal infections; however, it lacks harmonized monographs in major international pharmacopoeias (USP, BP, and EP).

Purpose
This study aimed to develop and validate an alternative, environmentally friendly analytical method for the simultaneous determination of ofloxacin and ornidazole using UV–Visible spectrophotometry.

Methods
Solubilization was achieved using an effective ternary mixture (10% urea/10% nicotinamide, w/v), alkalinized to pH 9.5 with NaOH and measured using a calibrated digital pH meter. This alkaline condition exploits the amphoteric nature of ofloxacin by inducing a bathochromic shift to 330 nm. Quantification was based on Vierordt's simultaneous equation spectrophotometric method. Validation was conducted in accordance with ICH Q2(R2) guidelines using a Total Error approach (accuracy profile) over a range of 80% to 120% of target concentrations.

Results
The method demonstrated specificity in the presence of common excipients. Metrological performance showed excellent linearity (R^2 = 0.9923 for ornidazole and R^2 = 0.9913 for ofloxacin), supported by 45 experimental observations and mean recovery rates of 99.61% and 99.95%, respectively. The lower limit of quantification (LLOQ) was graphically established from the accuracy profile at 8.6000 µg/mL and 3.4000 µg/mL, ensuring 95% decisional reliability. Expanded uncertainty (k = 2, 95% CI) remained within acceptable limits (3.68% and 3.50%). Assay of a commercial batch confirmed compliance with pharmacopoeial specifications.

Conclusion
The validated method provides a cost-effective alternative for routine quality control laboratories. By using an aqueous hydrotropic system instead of hazardous organic solvents, this protocol offers a robust and sustainable screening tool for market surveillance in resource-limited settings while significantly reducing the laboratory's environmental footprint.

INTRODUCTION

Ensuring medicine quality requires reliable analytical methods for the quantification of active pharmaceutical ingredients (APIs) (Kelani et al., 2021). The circulation of substandard and falsified (SF) medical products remains a significant global health challenge, particularly affecting anti-infectives in developing regions (World Health Organization [WHO], 2024). In these settings, weaknesses in pharmaceutical distribution systems contribute to the prevalence of SF medicines, with some estimates suggesting a market share exceeding 30% in several low- and middle-income countries (WHO, 2024).

In the Democratic Republic of Congo (DRC), fragmented supply chains and porous boundaries between formal and informal sectors foster a vulnerable pharmaceutical environment. A major public health incident in 2015, which resulted in the hospitalization of more than 1,000 individuals due to falsified diazepam, underscored the urgent need for robust market surveillance and accessible quality control tools (Lunsevila et al., 2023). Among commonly used anti-infectives, the fixed-dose combination of ofloxacin and ornidazole is frequently prescribed for the treatment of mixed bacterial and protozoal infections (Indian Pharmacopoeia Commission, 2022). Despite its clinical importance, this formulation lacks harmonized monographs in major international pharmacopoeias such as the USP, BP, or EP, leaving local regulatory authorities without standardized protocols for routine verification.

Current analytical approaches for this combination frequently rely on high-performance liquid chromatography (HPLC) or UV spectrophotometry using hazardous organic solvents such as methanol and acetonitrile. These solvents pose toxicological risks to laboratory personnel and create environmental challenges related to waste management (Patil et al., 2021). Although recent studies have explored advanced mathematical approaches such as wavelet transforms or chemometric models to resolve overlapping spectra (Mahmoud et al., 2023; Prajapati & Bari, 2021), a methodological gap remains in the availability of simplified, fully aqueous protocols. Specifically, there is a need for methods that optimize spectral resolution through selective ionization using hydrotropic solubilization, without requiring expensive chromatographic equipment or hazardous reagents.

This study addresses this gap by evaluating whether a synergistic nicotinamide–urea hydrotropic mixture can serve as an effective substitute for green spectrophotometric determination of ofloxacin and ornidazole in tablet dosage forms. The objective is to provide a cost-effective and environmentally friendly analytical tool suitable for routine market surveillance by national regulatory authorities, ensuring the quality of essential anti-infectives while minimizing the laboratory's environmental footprint.

METHODS

Materials and Sampling

Reference standards of ornidazole (Batch 22/EDP/ORN/142) and ofloxacin (Batch 11489003-OF) were used. Concentrations were adjusted based on certified values (99.41% for ornidazole and 99.72% for ofloxacin) to ensure metrological traceability. A commercial batch of ORNID-OX® tablets (500/200 mg) was obtained from an authorized pharmacy. To ensure a representative sample, 20 tablets were randomly selected from this batch, weighed, and finely pulverized to obtain a homogeneous composite powder for analysis.

Instrumentation

Measurements were performed using an Agilent Cary 60 (WinUV Pharma v5.0) double-beam spectrophotometer (wavelength accuracy ± 0.06 nm; spectral bandwidth 1.5 nm). The pH was measured using a calibrated digital pH meter.

Preparation of Solutions

The hydrotropic solution (10% urea/10% nicotinamide, w/v) was heated to 68–70°C to ensure complete solute integration and to prevent urea crystallization upon cooling, as the concentration exceeds the solubility limit at room temperature. Stock solutions were prepared using 40% hydrotropic agent, followed by a 1:250 dilution in water to reduce the concentration below the minimum hydrotropy concentration (MHC). All concentrations are reported in µg/mL.

Analytical Protocol and Vierordt's Simultaneous Equations

The spectral range from 200 to 400 nm was scanned to identify the absorption characteristics of the analytes in

the hydrotropic medium. Absorbances were measured at 320 nm (λ_1) and 330 nm (λ_2). These wavelengths were selected to maximize absorptivity ratios for each component, thereby ensuring numerical stability and selectivity of the system.

Based on the principle of additivity of absorbances, Vierordt's simultaneous equation spectrophotometric method was applied. The following system of equations was established:

$A1=0.09887C_1 + 0.01019C_2$(1)

$A2 =0.00208C_1 + 0.24312C_2$ (2)

where A_1 and A_2 represent the absorbances of the mixture at 320 nm and 330 nm, respectively, and C_1 (ornidazole) and C_2 (ofloxacin) represent the concentrations in µg/mL.

Method Validation and Sensitivity Limits

Method validation was performed in accordance with ICH Q2(R2) guidelines and the SFSTP (2007) approach, based on 45 experimental observations (n = 15 per day over three consecutive days). The practical lower limit of quantification (LLOQ) was established graphically from the accuracy profile, defined as the intersection of the 95% β-expectation tolerance limits (integrating both bias and dispersion) with the ±10% acceptance criteria. The limit of detection (LOD) was derived from the LLOQ using:

$$LOD = \frac{LLOQ}{3.3} \quad (3)$$

Measurement Uncertainty Analysis

Measurement uncertainty was estimated at each concentration level. The combined standard uncertainty (ucu_cuc) was calculated as the square root of the sum of the variances of trueness (bias) and intermediate precision:

$$U_c = \sqrt{s^2IP + u^2bias} \quad (4)$$

sIPs_{IP}sIP is the standard deviation of intermediate precision and ubiasu_{bias}ubias is the uncertainty associated with method bias. Expanded uncertainty (U) was obtained using a coverage factor of k=2k = 2k=2 (95% confidence interval):

$k = 2$ (95% CI): $U = k \times u_c = 2 \times u_c$ (5)

All concentrations and associated uncertainty limits are expressed in µg/mL.

RESULTS

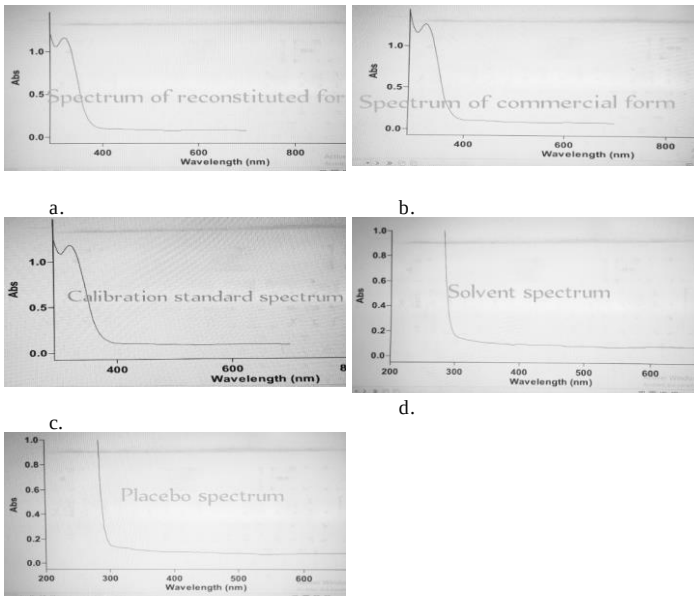
Hydrotropic Solvent System Performance

Visual inspection confirmed the immediate and complete dissolution of both ornidazole and ofloxacin powders. The resulting solution remained clear and homogeneous, with no microprecipitation observed over a 24 h period. The baseline at 800 nm remained at 0.000 absorbance units, confirming an optically transparent medium. This condition is essential (*sine qua non*) for rigorous application of the Beer–Lambert law and supports intermediate precision.

Table 1:
Solubilization Tests and Metrological Validation

Evaluated parameter	Observation/result	Conclusion/justification
Ornidazole solubility	Immediate dissolution	Effective hydrotropic effect.
Ofloxacin solubility	Immediate dissolution	Confirms formation of the carboxylate form at pH 9.5.
Combined appearance	Clear and homogeneous	No incompatibility; colloidal stability maintained.
Baseline at 800 nm	Absorbance = 0.000	No Rayleigh scattering; supports intermediate precision.

Figure 1:
Comparative UV–Visible absorption spectra (200–400 nm): (a) reconstituted laboratory form; (b) commercial tablet sample (ORNID-OX®); (c) working standard solution (ornidazole 10 µg/mL and ofloxacin 4 µg/mL); (d) hydrotropic solvent blank; (e) placebo matrix solution. The identical spectral profiles of the standards and commercial samples (a–c) confirm the chemical identity of the APIs, while the baseline response of the placebo and solvent (d–e) at 320 nm and 330 nm demonstrates method specificity and the absence of matrix interference following dilution below the MHC.



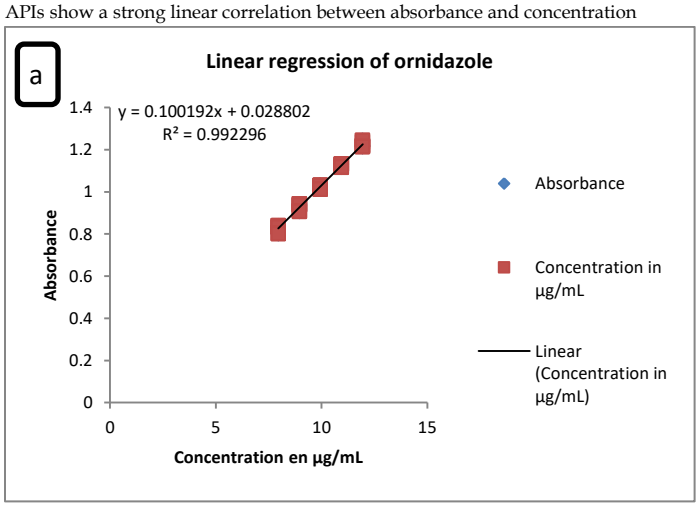
Linearity, Regression Statistics, and Residual Analysis

Regression statistics and residual analysis supported the homoscedasticity of the data. Coefficients of determination ($R^2 > 0.991$), obtained over 45 observations, indicated a robust linear relationship between absorbance and concentration. Regression parameters are summarized in Table 2.

Table 2:
Linearity, Detection Limits, and Sensitivity (n = 45)

Parameter	Ornidazole	Ofloxacin
Linearity range (µg/mL)	7.9600–11.9400	3.1940–4.7900
Coefficient of determination (R^2)	0.9923	0.9913
LLOQ (accuracy profile) (µg/mL)	8.6000	3.4000
LOD (LLOQ/3.3) (µg/mL)	2.6061	1.0303
Sandell's sensitivity (µg/mL)	0.0100	0.0041

Figure 2:
Linear regression curves: (a) ornidazole at 320 nm and (b) ofloxacin at 330 nm. Both APIs show a strong linear correlation between absorbance and concentration



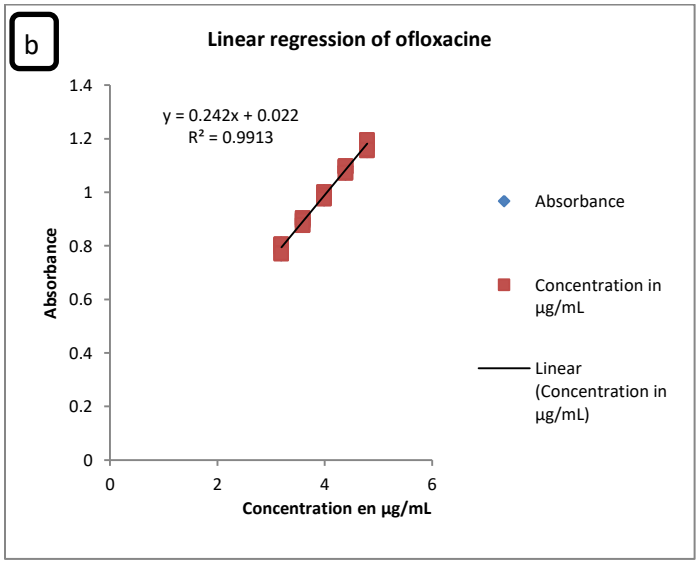
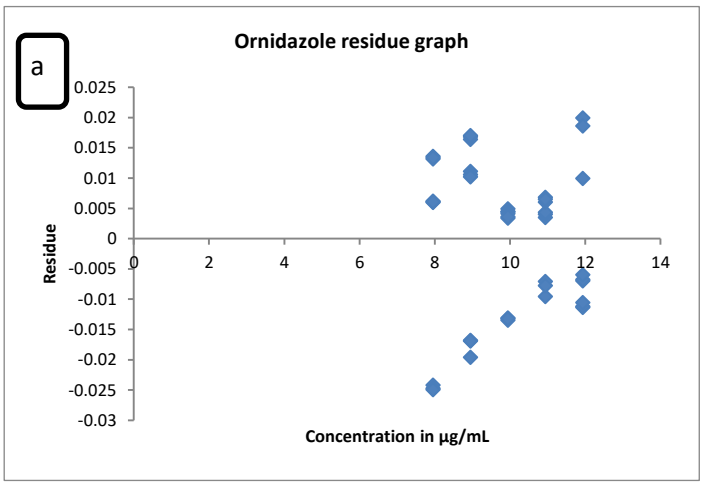
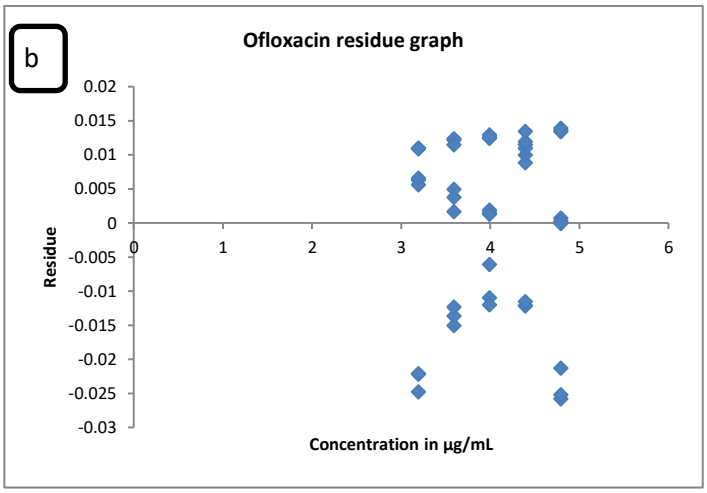


Figure 3:
Residual plots: (a) ornidazole and (b) ofloxacin. The random distribution of residuals around the zero line confirms homoscedasticity and the absence of systematic bias





Accuracy and Precision Profile

Statistical noncompliance at Level 1 (80%), where tolerance limits exceeded ±10%, indicates reduced robustness at low concentrations. This finding justified setting the practical LLOQ at 8.6000 µg/mL for ornidazole and 3.4000 µg/mL for ofloxacin to ensure 95% decisional reliability.

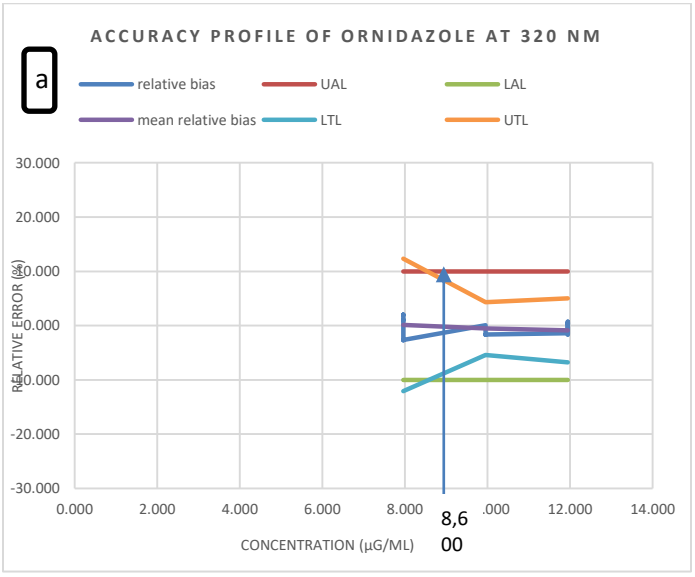
Table 3:
Validation Results for Ofloxacin (Accuracy and Precision)

Evaluated parameter	Level 1 (80%)	Level 2 (100%)	Level 3 (120%)
Theoretical concentration (µg/mL)	3.1940	3.9920	4.7900
Recovery rate (%)	99.9895	99.8385	99.0419
Intermediate precision (RSD, %)	2.318	1.136	1.642
Lower tolerance limit (%)	−11.5250	−5.8010	−9.1130
Upper tolerance limit (%)	11.5040	5.4790	7.1970
Compliance (±10%)	Noncompliant	Compliant	Compliant

Table 4:
Validation Results for Ornidazole (Accuracy and Precision)

Evaluated parameter	Level 1 (80%)	Level 2 (100%)	Level 3 (120%)
Theoretical concentration (µg/mL)	7.9600	9.9500	11.9400
Recovery rate (%)	100.1312	99.4680	99.1336
Intermediate precision (RSD, %)	2.453	0.979	1.191
Lower tolerance limit (%)	−12.0540	−5.4030	−6.7760
Upper tolerance limit (%)	12.3160	4.3290	5.0430
Compliance (±10%)	Noncompliant	Compliant	Compliant

Figure 4:
Accuracy profiles for simultaneous determination of the fixed-dose combination: (a) ornidazole; (b) ofloxacin. Solid lines represent relative bias, and dashed lines represent upper and lower 95% β-expectation tolerance limits. Horizontal dotted lines represent ±10% acceptance limits. The intersection of tolerance limits with acceptance limits at Level 1 confirms the practical LLOQ (8.6000 µg/mL for ornidazole and 3.4000 µg/mL for ofloxacin), ensuring a 95% probability that future measurements will fall within predefined specifications.



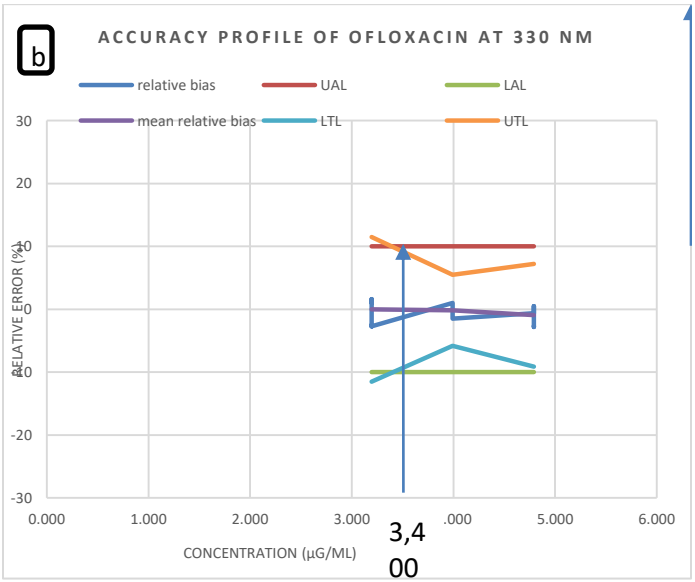
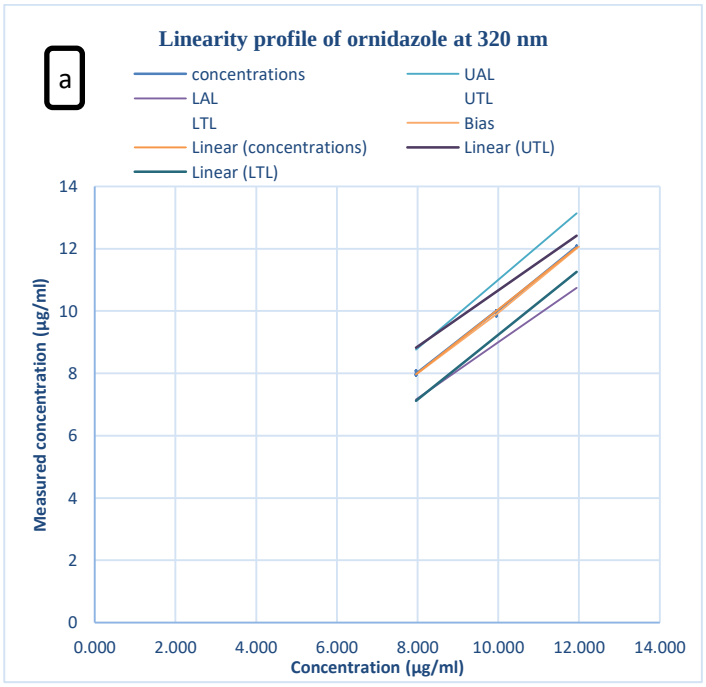
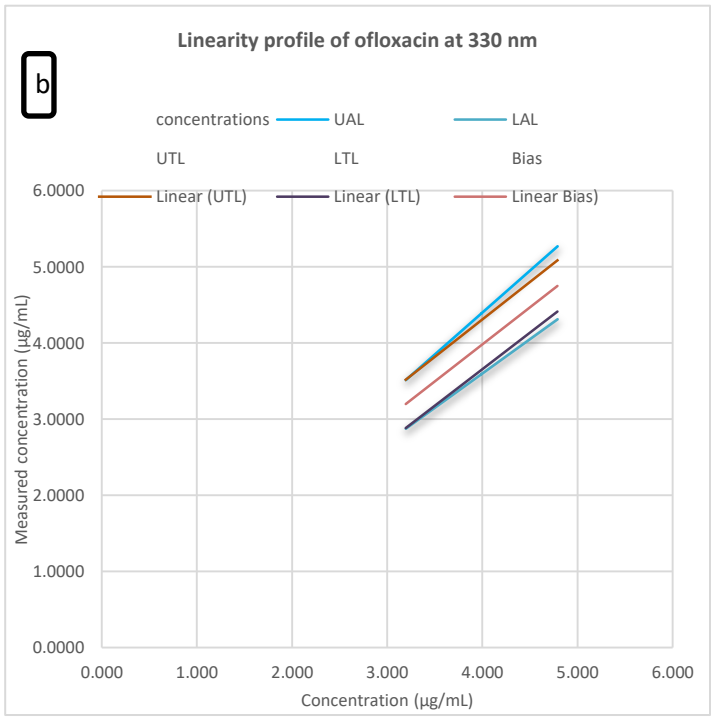


Figure 5:
Method linearity profiles for simultaneous determination: (a) ornidazole; (b) ofloxacin. Dashed lines represent the identity line (y = x), and solid lines represent back-calculated concentrations. Alignment across the 80%–120% range confirms the absence of proportional bias and validates method accuracy throughout the analytical window ($R^2 > 0.991$).





Commercial Assay and Stability

Expanded uncertainty (±3.50% to ±3.68%) remained well below the ±10% acceptance limits, providing a safety

margin greater than 6%. Assay results for ORNID-OX® were 100.42% for ornidazole and 95.74% for ofloxacin, confirming compliance with pharmacopoeial specifications.

Table 5:
Commercial Batch Assay and Solution Stability (24 h)

Parameter	Ornidazole	Ofloxacin	Decision
Amount found	502.085 mg	191.48 mg	Compliant
Stability (refrigerated, 24 h)	100.31%	105.20%	Stable
Stability (room temperature, 24 h)	99.85%	101.02%	Stable

DISCUSSION

Physicochemical Rationale of Solubilization and Selectivity

The effectiveness of the ternary hydrotropic system (10% urea/10% nicotinamide, pH 9.5) is driven by the chaotropic effect of urea and the selective ionization of ofloxacin **(Table 1)**. At pH 9.5, deprotonation of the carboxyl group (pKa ≈ 6.1) increases ofloxacin aqueous solubility and induces a bathochromic shift to 330 nm (Figure 1). This spectral shift improves physicochemical selectivity, enabling accurate mathematical resolution using Vierordt's equations and minimizing signal overlap between the two APIs. These findings are consistent with hydrotropic principles described by Patil et al. (2021) regarding the replacement of hazardous solvents in pharmaceutical analysis.

Statistical Reliability and Residual Analysis

The robustness of the proposed method is supported by excellent linearity (R^2 = 0.9923 for ornidazole and R^2 = 0.9913 for ofloxacin) across 45 observations **(Table 2)**. Residual analysis (Figure 3) and regression curves **(Figure 2)** further validate the linear model. The random distribution of residuals around the zero line confirms homoscedasticity, demonstrating constant error variance throughout the analytical range (80%–120%). This absence of systematic bias supports reliable concentration estimation, as also confirmed by the method linearity profiles (Figure 5).

Analytical Role of the MHC and Decisional Reliability

The sequential 1:250 dilution is a critical analytical step that reduces the hydrotropic agent concentration below the MHC. This disrupts the molecular solubilization complex and minimizes matrix effects, which may explain the high recovery rates (99.04%–100.13%) obtained for tablet formulations (**Tables 3** and **4**). Furthermore, graphical determination of the LLOQ from the accuracy profile **(Figure 4)** ensures 95% decisional reliability, providing an analytical safety margin greater than 6% during routine quality control of commercial batches **(Table 5)**.

Comparison With Literature and Limitations

Compared with recent green analytical strategies, such as the smartphone-based TLC method proposed by Kelani et al. (2021) or advanced spectrophotometric approaches using wavelet transforms and chemometric methods (Mahmoud et al., 2023; Prajapati & Bari, 2021), the present protocol provides a simpler mathematical approach suitable for routine laboratories. However, the method has limitations. Its restricted linearity range (80%–120%) makes it unsuitable for impurity profiling, and its sensitivity is lower than chromatographic techniques (e.g., HPLC–UV), limiting its application to pharmaceutical dosage forms rather than trace-level analysis.

CONCLUSION

This study developed and validated an environmentally friendly spectrophotometric method for the simultaneous determination of ofloxacin and ornidazole. By using an alkaline hydrotropic system (pH 9.5), the method provides a practical approach for quantifying anti-infectives without hazardous organic solvents such as methanol or acetonitrile. The method fulfilled ICH Q2(R2) requirements, demonstrating excellent linearity (R^2 > 0.991) and high recovery rates. This low-cost screening tool may support regulatory authorities in the DRC in conducting active market surveillance against substandard and falsified medicines, thereby improving public health protection while reducing the laboratory's environmental footprint.

Data Availability: All data generated or analyzed during this study are included in this published article. Raw spectrophotometric data are available from the corresponding author upon reasonable request.

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(Gemini 1.5 Pro) was used for writing assistance and translation from French to English during preparation of this manuscript. The authors supervised the process, made all necessary revisions, and assume full responsibility for the final text.

Ethical Approval: This study was conducted in accordance with applicable ethical standards for research. The research protocol was reviewed and approved by the Research Ethics Committee of the University of Kisangani under authorization number UNIKIS/CER/042/2023. All procedures were performed in accordance with the ethical standards of the institutional and national research committee.

Conflicts of Interest: None declared.

ORCID iDs:

Shabani, M. L.[1]: Nil idntified.
Ngoyi, M. N.[5]: Nil idntified.
Tweni, B. E.[2]: Nil idntified.
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