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Review Article

Green RP-HPLC for Fluoroquinolone–Nitroimidazole Combinations: Methods for Ciprofloxacin, Levofloxacin and Ornidazole and a Framework for a Stability-Indicating Ternary Method

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Abstract: Ciprofloxacin (CIP) and levofloxacin (LEV) are fluoroquinolone drugs with ornidazole (ORN) being a 5-nitroimidazole; fluoroquinolone and nitroimidazole drugs are often used together for treating infections caused by bacteria and protozoans. Quality of products using such combinations is determined by using reversed-phase high performance liquid chromatography (RP-HPLC); however, available procedures makes references to both drugs together, use acetonitrile or methanol in the composition of the mobile phase and do not report their greenness in majority of cases. We have not been able to find literature about a method of RP-HPLC for determination of the three compounds together, including method validation and greenness analysis. The review aims to outline (i) the difficulty of separating the three drugs at the same time, (ii) the comparative analysis of the established methods for determining similar combinations of fluoroquinolones and nitroimidazoles, (iii) analysis of mobile phases containing ethanol together with eco-friendliness analysis via Application Eco-scale, GAPI and AGREE methods, and (iv) creating analytical quality-by-design (AQbD) concept based on the validation and forced degradation test plan for a ternary analysis using alcohol and acid.

Keywords: Ciprofloxacin, Levofloxacin, Ornidazole, RP-HPLC, Green Analytical Chemistry, Ethanol, AGREE, GAPI, Analytical Quality by Design, Stability-Indicating Method.

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INTRODUCTION

Analytical techniques are key to pharmaceutical quality control, as they allow accurate measurement of each active ingredient in a product as well as separating it from any excipients and possible impurities and degradation products. This situation, however, is complicated for fixed-dose combinations of antimicrobial agents, as the components in these products differ in terms of polarity, ionization and UV properties and degradation pathways.

Ciprofloxacin and levofloxacin block the activity of bacterial DNA gyrase and topoisomerase IV, two enzymes responsible for the process of DNA supercoiling in bacteria [1]. Levofloxacin is known as the S-enantiomer of ofloxacin. Ornidazole is a member of the 5-nitroimidazole class of drugs; its nitro group is reduced to reactive species in anaerobic bacteria and protozoa, which results in DNA damage [2]. This is why the combination of fluoroquinolone and nitroimidazole classes of agents is common for treatment of mixed infections and simultaneous assay of their concentration is a

requisite in quality-control labs. The compound of ciprofloxacin has attracted widespread attention among scientists and practitioners alike [3].

The use of RP-HPLC with UV or diode-array detection (DAD) has increased because of its functionalist usefulness as an important method. It has been noted that many protocols published use varying proportions of these solvents as well as phosphate buffering systems resulting in methods being run isocratically for ten and more minutes. Green analytical chemistry (GAC) requires that the use of dangerous solvents is minimised, energy consumption is reduced and waste is minimised [4-6]. There is also discussion of the replacement of acetonitrile in RP-HPLC with ethanol that is biodegradable and renewable [7, 8].

Additionally, assay methods used during stability testing should be introduced with the concept of stability indicating methods: they should allow for the measurement of the target drug while taking into account the effect of degradation products formed in the conditions of testing following ICH Q1A(R2) and Q1B [9,10]. Validation is performed in accordance with ICH Q2(R2) and development of the method can be done according to ICH Q14 [11,12].

In this article, the goal is to unify the discussed aspects specifically in the case of one green RP-HPLC method that can determine the stability of CIP, LEV, and ORN. Specifically, the paper encompasses: (i) analyzing the physicochemical reasons that result in difficulty in the analyzed mixture; (ii) comparing chromatographic methods that are published in literature for similar mixtures; (iii) reviewing the use of ethanol-based mobile phases and greenness metrics; and (iv) providing an AQbD-based framework in addition to the validation plan.

2. Profiles of Drugs and Physicochemical Characteristics

The three drugs have structural differences that determine retention and peak shapes in RP-HPLC (Table 1). Ciprofloxacin has cyclopropyl at N-1 and piperazinyl at C-7 position of the fluoroquinolone core. Levofloxacin contains a fused chiral benzoxazine ring and has methylpiperazinyl substitution. Both contain a carboxylic acid and basic nitrogen piperazine that ensure they are amphoteric and exist as cation, zwitterion or anion depending on the pH value. Ornidazole contains a 2methyl-5-nitroimidazole and chlorohydroxypropyl side chain and lacks any acid-base pair that could be relevant in the mean HPLC pH range; this drug is only weakly basic.

Table 1: Structural and chromatographic characteristics of the three analytes

Property	Ciprofloxacin (CIP)	Levofloxacin (LEV)	Ornidazole (ORN)
Class	Fluoroquinolone	Fluoroquinolone (S-ofloxacin)	5-Nitroimidazole
Key groups	Carboxylic acid; cyclopropyl; piperazine	Carboxylic acid; benzoxazine ring; methylpiperazine	Nitroimidazole; hydroxyalkyl chain; chlorine
Ionisation behaviour	Amphoteric (acid pKa ≈ 6, amine pKa ≈ 8–9)	Amphoteric (acid pKa ≈ 6, amine pKa ≈ 8)	Weakly basic (pKa ≈ 2.5)
Expected behaviour on C18 at pH 3	Cationic; moderate retention, tailing risk	Cationic; close to CIP	Largely neutral; retained longer
Typical UV maxima (nm)	≈ 275–280, 320–330	≈ 290–295	≈ 310–320
Mechanism	Inhibits DNA gyrase / topoisomerase IV	Inhibits DNA gyrase / topoisomerase IV	Nitro reduction; DNA damage

pKa and UV maxima are approximate values from the general literature and vary with medium; they are given to show trends.

There are three practical implications. The first point is that the ionization state of the CIP and LEV changes dramatically over the pH range of 5-9, meaning that small changes in pH around these values can cause big differences in retention, which is why most techniques are used at a pH of 2.5-4, where the two acids are almost entirely protonated and the ionized silanols are suppressed. The second point is that the CIP and LEV compounds are very similar in terms of their size and polarity, and therefore one is bound to get resolution for them. The third point is that ORN is less polar compared to the others and works in a longer wavelength range, so one will have to make a compromise and use a single wavelength for the detection.

3. Chromatographic Obstacles of the Ternary Mixture

3.1 Silanol Interactions and Peak Shape

The low pH aqueous phase or the addition of amine modifiers like triethylamine or the use of finished columns or hybrid particle columns are some techniques that could be applied to cure the tailing caused by the presence of residual silanol interactions between silica-based C18 phases and basic amines such as those obtained from the

piperazine moiety of the CIP and LEV. Because of green principles, it is preferable to use volatile acids (acetic or formic acid) or low-concentration ammonium salts instead of triethylamine and phosphate since this reduces waste and hazards and still allows further analysis via LC-MS.

3.2 Elution Window and Run Time

ORN stays longer than CIP and LEV while in an acidic environment; therefore, an isocratic mobile phase that works well enough to elute ORN first would dissipate CIP and LEV. On the other hand, a mobile phase that can separate these compounds may elongate ORN elution. One of the examples is a method for ofloxacin and ornidazole determination: on C18 column with acetonitrile–ammonium acetate as a mobile phase, having proportions of 25:75 and a pH of 5.8, ofloxacin eluted at 4.28 minutes whereas ornidazole at 6.75 minutes [15]. A ternary method should set parameters of the mobile-phase composition, temperature and flow rate allowing achieving a good elution window for these 3 components. If isocratic conditions do not work, gradient elution can be employed, but it will increase equilibration time.

3.3 Detection

The fluoroquinolones show a strong absorption between 275 and 295 nm, and ORN has a strong absorption between about 310 and 320 nm. The method makes use of a compromise wavelength which is usually established in the range of 293 to 311 nm. Thus, the above-mentioned method of ofloxacin-ornidazole used the wavelengths of 293 and 311 nm [15]. Ethanol has the absorption higher than about 210 nm, which means that the absence of absorption shows no effect on the detection.

3.4 Requirement of Stability-Indicating Method

A stability-indicating method needs to separate the active compounds from the degradation products. Ciprofloxacin is sensitive to light and oxidation, while its major photoproduct has been quantified with the help of capillary electrophoresis and UPLC [16]. Ornidazole is sensitive to both alkali and light, while levofloxacin is resistant to these factors. Thus, the method must be optimized for various degradation modes, while the degradation product of one substance should not be found together with the active substance of other compound. The above requirements will be discussed in Section 8.

4. Reported RP-HPLC Parameters for Some Combinations

There do not exist any prior studies pertaining to the ternary mixture in question. However, we do have at hand studies pertaining to the nearest analogue (Table 2). For instance, the study of Chamseddin and Jira on the separation of fluoroquinolones demonstrated that CIP, LEV, and moxifloxacin can be separated without going through any difficulties when a sub-3 µm C18 column is utilized along with methanol–phosphate mobile phase at pH 3. D'Souza *et al*., separated fluoroquinolone and ornidazole using an acetonitrile–ammonium acetate mix with linearity being available in the concentration range of 2–16 µg/mL for both drugs. The work by Fares *et al*., and the stability-indicating technique by Kelani *et al*., were not dedicated to the mentioned drugs, but they still demonstrate green and AQbD strategies that we would like to use for the purpose of communicating our approaches.

Table 2: Selected published RP-HPLC methods relevant to the CIP–LEV–ORN problem

Reference	Analytes	Column	Mobile phase / flow	Detection	Notes
Chamseddin & Jira, 2011 [13]	CIP, LEV, moxifloxacin	BDS Hypersil C18, 100 × 4.6 mm, 2.4 µm	Methanol: 25 mM phosphate pH 3 (28: 72); 1.0 mL/min	UV	Effect of methanol, pH, buffer strength studied; compared with calixarene and monolithic phases
D'Souza *et al.,* 2021 [15]	Ofloxacin, ORN	Hyperclone BDS C18, 250 × 4.6 mm, 5 µm; 25 °C	Acetonitrile: ammonium acetate pH 5.8 (25: 75); 1.0 mL/min	293 and 311 nm	Rt 4.28 and 6.75 min; 2–16 µg/mL; stability-indicating; tablets and dissolution
Kelani *et al.,* 2023 [18]	Ibuprofen, phenylephrine (not our analytes)	YMC-C8, 100 × 4.6 mm, 3 µm; 35 °C	Gradient 0.1% hexanesulfonic acid: acetonitrile 80:20 to 60:40; 1.0 mL/min	DAD 220 nm	Eco-Scale 88 vs 84 for a reported method; 7 min run
Fares *et al.,* 2022 [17]	Deferasirox, deferiprone (not our analytes)	XBridge RP-C18; 25 °C	Ethanol: acidic water pH 3.0 (70: 30); 1.0 mL/min	UV 225 nm	AQbD; design space about 65.8–68.8% ethanol, pH 3.0–6.0; eight greenness tools

Compiled from the cited papers. Conditions for the ternary mixture have not been published and are not implied by this table.

There are two points to note. Firstly, none of the methods involving fluoroquinolone and nitroimidazole works with ethanol; they employ either acetonitrile or methanol. Secondly, the system suggested by Fares *et al*., based on AQbD is implemented at 25°C with isocratic flow at 1 mL/min, the mobile phase consisting of two-thirds ethanol mixed with acidic water in a typical 4.6 mm column [17]. This is the system we expect to test during the analysis of CIP, LEV and ORN.

5. Environmentally Friendly Analytical Chemistry and Mobile Phase Using Ethanol

5.1 Ideas behind the Principles and Choosing Solvent

The twelve principles of green analytical chemistry embody the principles of green chemistry for analytical field: direct analysis wherever possible, minimum sample and reagents, minimum waste, maximum safety of reagents and energy consumption and safety of the operator. In case of HPLC, choice and consumption of solvents provides the most important indicator to estimate overall environmental impact. According to solvents selection, ethanol is identified one of the best solvents and acetonitrile and methanol appear less favourable in terms of health, safety and environment [4-6].

5.2 Ethanol's Properties Critical to RP-HPLC

Ethanol acts as a more potent solvent than methanol for a number of constituents and offers dissimilar selectivity as compared to acetonitrile, implying the need for experimental determination of isoeluotropic composition. Ethanol's main inconveniences are its substantial viscosity leading to increased back-pressure as well as its low value of UV cutoff at about 210 nm (Table 3). This viscosity issue can be tackled through moderate flow rates (approximately 0.6 to 1.0 mL/min), higher temperature of the column (25 to 40 °C), and utilization of columns with greater size or advanced dimensions, backing the capacity of the machine [7-14].

Table 3: Approximate properties of common RP-HPLC organic modifiers (textbook values)

Property	Acetonitrile	Methanol	Ethanol
UV cut-off (nm)	≈ 190	≈ 205	≈ 210
Viscosity of pure solvent at 20 °C (mPa·s)	≈ 0.37	≈ 0.59	≈ 1.2
Maximum viscosity of aqueous mixture (mPa·s, 20 °C)	≈ 1.0 (at about 15–20% organic)	≈ 1.8 (at about 40%)	≈ 2.7 (at about 40–50%)
Hazard profile	Flammable, toxic; made as a by-product of acrylonitrile	Flammable, toxic	Flammable; low toxicity; can be bio-derived
Green solvent rating	Usable, less favourable	Usable, less favourable	Preferred

Values are rounded, widely tabulated figures [8-14] and should be rechecked against the solvent supplier's data before reporting.

5.3 Greenness Metrics

There are three most common tools for chromatographic methods (see Table 4). The Analytical Eco-Scale gives the score by deducting penalty points due to reagents and energy consumption, occupational health and safety and waste from 100; as a result the scores above 75 are considered excellent, and the ones above 50 – acceptable [19]. The GAPI is a five-part pictogram considered to be helpful in terms of sampling, sample preparation, reagents, instruments and waste [20]. AGREE gives the score for the method from 0 to 1 according to the twelve GAC principles and produces a diagram resembling a clock face [21]. The use of all three tools in the analysis eliminates the pitfall of using any one of them only.

Table 4: Greenness assessment tools proposed for the ternary method

Tool	Output	Interpretation	Main limitation
Analytical Eco-Scale [19]	Score = 100 − penalty points	> 75 excellent; > 50 acceptable	Semi-quantitative; penalties depend on reagent amounts
GAPI [20]	Pictogram with 15 fields in 5 pentagons	More green fields = greener	Qualitative; no single number
AGREE [21]	0–1 score with 12-segment diagram	Closer to 1 = greener	Weighting is adjustable and somewhat subjective

According to the findings of Kelani *et al*., the Analytical Eco-Scale score for their stability-indicating HPLC technique to measure ibuprofen and phenylephrine is 88 in comparison to a score of 84 for an earlier method, with scores greater than 75 being excellent [18]. Greenness scores alone do not indicate analytical quality: any method that fails on accuracy, precision or robust criteria will not be valuable even

though it qualifies as green. Hence, greenness scores must be presented in conjunction with ICH validation scores.

6. The Principle of Analytical Quality by Design

AQbD implements the same reasoning behind ICH Q8(R2) in the case of validation of a method: it establishes the desired performance then finds the reasons influencing it while defining a region of operation where such performance is obtained [12-23]. The procedure follows several actions shown schematically in Figure 1:

1. Establish the analytical target profile: establish the values of CIP, LEV and ORN and the specificities regarding degradation products.
2. Identify the critical method parameters: solving the issue of resolving two close peaks – CIP and LEV, evaluating the retention times of ORN and the analysis, calculating tailing factors and peak purity.
3. Assess risk using the Ishikawa diagram and screen the factors with the Plackett–Burman design or fractional design.
4. Optimize the methods by using a response surface design.
5. Define the MODR and determine the system suitability criteria.
6. Validate the method within the MODR.

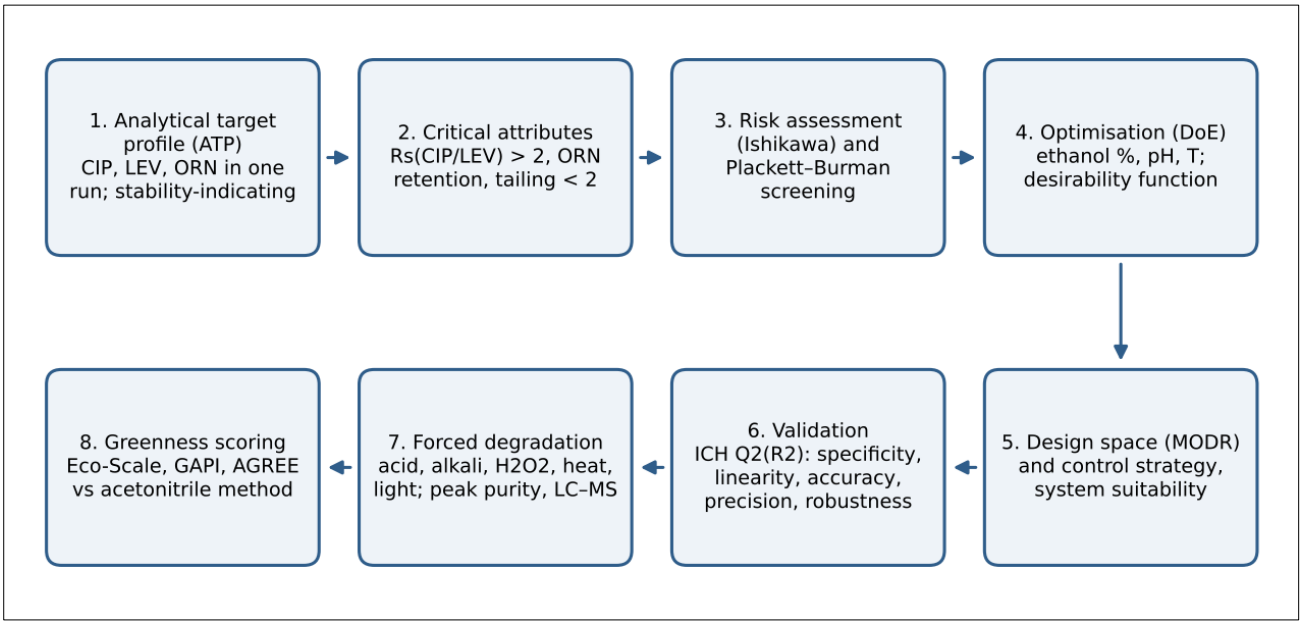


Figure 1: Proposed AQbD workflow for a green, stability-indicating ternary RP-HPLC method (schematic; no experimental data)

Fares *et al*., have proposed an innovative idea. They examined the flow rate, temperature, organic percentage, pH, and wavelength using a Plackett-Burman design. They determined that the % ethanol, pH, and temperature were key factors and used them in an optimized design where 16 trials were carried out and two levels were used for three factors (i.e., 60-70% ethanol, 3.0-7.0 pH, and 25-35°C), resulting in responses of resolution, tailing, and run time. This studying of ternary mixtures would mean that the CIP/LEV resolution and ORN retention time should also be included as response variables and pH range lowered to around 2.5 due to fluoroquinolone chemistry.

7. Proposed Framework for a Green Ternary Method

7.1 Starting Conditions

Table 5 presents the starting conditions along with the factor variations we intend to test. These conditions are based on the references in Table 2, but have not yet been tested for this mix. The expected elution order when using C18 at acidic pH is CIP first followed by LEV and then ORN. However, the actual order, retention times and resolution must be verified through experiment.

Table 5: Proposed starting conditions and factor ranges (hypotheses for experimental testing)

Parameter	Starting point	Range to explore	Rationale
Column	C18, 250 × 4.6 mm, 5 µm, end-capped	Also base-deactivated C18 or C8	Base-deactivation reduces fluoroquinolone tailing [14]; Kelani *et al*., used a C8 for an unrelated pair [18]
Organic modifier	Ethanol	Ethanol 15–45% in aqueous phase	Green solvent [7]; isoeluotropic composition to be found
Aqueous phase	Water with acetic or formic acid	pH 2.5–4.0	Suppress silanols; volatile; LC–MS compatible
Elution	Isocratic	Gradient if ORN elutes too late	Minimise solvent and complexity
Flow rate	0.8 mL/min	0.6–1.0 mL/min	Limit back-pressure from ethanol–water
Temperature	30 °C	25–40 °C	Lowers viscosity; check analyte stability
Detection	DAD, extract at about 295 nm	275–320 nm	Compromise wavelength; DAD for peak purity
Injection volume	10–20 µL	—	Typical for 4.6 mm column

7.2 Sample Preparation

The bulk reference standard is dissolved in mobile phase or ethanol–water. The tablets are weighed and powdered and the amount corresponding to the desired concentration is extracted by sonication in suitable diluent. The prepared solution is further diluted and filtered through a 0.45 or 0.22 mm membrane filter. The use of the mobile phase as a diluent avoids the problem of solvent-mismatch peak distortion and does not add to the greenness of the process. The stock solution thus prepared is in the concentration of 1 mg/mL prepared in a suitable diluent and stored away from the light at 2 to 8 °C before preparing the working standard at the required concentration from the linearity range.

8. Validation and Forced Degradation Plan

8.1 Validation According to ICH Q2(R2)

This guidance outlines the required validation characteristics for both assay and impurity testing methods, while also permitting the use of different multivariate and platform strategies [11]. The proposed validation plan and acceptance parameters are summarised in Table 6though these parameters represent targets, by whether they are met or not.

Table 6: Validation plan and proposed acceptance criteria

Characteristic	Design	Proposed criterion
Specificity	Blank, placebo, individual drugs, mixtures, stressed samples; DAD peak purity	No interference; Rs > 1.5 for every pair; purity angle < threshold
Linearity	At least five levels, each drug, triplicate	$r^2 \geq 0.999$; residuals randomly distributed
Range	Typically 80–120% of test concentration for assay	Linearity, accuracy and precision met across range
Accuracy	Spiked placebo at 80, 100 and 120%, three replicates each	Mean recovery 98–102%
Precision	Repeatability (n = 6) and intermediate precision (different days or analysts)	RSD ≤ 2%
LOD / LOQ	Signal-to-noise or calibration slope and SD of response	S/N ≈ 3 and ≈ 10; precision at LOQ checked
Robustness	Deliberate changes in % ethanol, pH, temperature, flow within MODR	System-suitability limits maintained
System suitability	Replicate injections of the mixed standard	Rs (CIP/LEV) > 2; tailing < 2; plates > 2000; RSD of area ≤ 2%
Solution stability	Standards and samples at room temperature and 2–8 °C	Change ≤ 2% over study period

8.2 Forced Degradation

Stress tests are conducted according to ICH Q1A(R2) for hydrolysis, oxidation and heat degradation, and according to Q1B for light degradation [9, 10]. The purpose is to achieve 10–30 % degradation of the drug, which allows for the generation of degradants without forming secondary products. Since the drugs differ in terms of their

sensitivity, appropriate conditions should be applied for each drug and a mixture and single drug tests done too. The plan is shown in Table 7 where the behaviour is reported that justifies each decision.

Table 7: Forced-degradation plan for the ternary method and the behaviour that motivates each stress

Stress	Starting conditions to tune	Expected behaviour (literature trend)
Acid	0.1–1 N HCl, 60–80 °C, hours	LEV and ORN degrade appreciably; CIP moderately
Alkali	0.01–0.1 N NaOH, room temperature to 60 °C, minutes to hours	ORN is highly labile (use mild conditions); LEV stable; CIP moderate
Oxidation	3% H2O2, room temperature to 70 °C	CIP most sensitive; LEV forms N-oxide; ORN comparatively stable
Thermal	Solid and solution at 60–80 °C	Generally small for all three
Photolysis	ICH Q1B exposure of solid and solution	CIP and ORN highly photolabile; protect samples when not testing light
Controls	Unstressed, blank and placebo, protected from light	Mass balance 90–110%; DAD peak purity; LC–MS of major degradants

Trends are from the published stability literature on these drugs; verify each against its primary study before reuse.

ORN may be completely degraded in a few minutes by strong alkali, therefore too strong of an alkaline condition will get rid of ORN peak, leaving no information regarding specificity. Light must be applied for solutions and solids independently; also stressed solutions not being tested for light sensitivity should be isolated from light exposure.

9. Greenness Evaluation Plan

The proposed ternary method will be assessed in terms of Analytical Eco-Scale, GAPI and AGREE, and compared with conventional acetonitrile-phosphate method for the same analytes under the same conditions. Input data should be recorded: solvent volume per analysis (flow rate and time of the test), type of the solvent and its danger, required energy used by the instrument and for sonication, wastes obtained, number of investigations per hour, and operator hazard. Numeric data must be reported in the supplementary table, so that results can be reproduced. Solvent use per injection is the most simple comparative item: 1mL/min for 10 minutes of the test means that about 10mL of mobile phase is used, and all organic parts of phase would be replaced with ethanol-based and acetonitrile.

10. Limitations and Anticipated Challenges

- There are no experimental data available on the ternary mixture in this manuscript, and all conditions, ranges, and criteria mentioned in this article have yet to be tested.
- Ethanol's viscosity may impose limitations on flow rate or column length in some instruments, and cut-off value of 210 nm limits low-wavelength detection.
- CIP and LEV have close selectivity, if ethanol–water at an acidic pH cannot offer Rs > 2, a deactivated with base column, different pH, or ternary organic phase might need to be used, which lowers greenness value.
- Drugs that are photolabile make it difficult to work with the samples, and all solutions must be light-protected during the validation.
- Results on greenness values are semi-quantitive, thereby various approaches yield different ranks for one method.
- Stability results published in the literature originate from single drug studies under various conditions, so that the coexistence of the degradation products may differ from that of the individual drugs.

11. Future Perspectives

There are three advancements that will improve the field. The first is the use of UHPLC utilizing sub-2 µm particles. This technique can enhance the run times even further, and minimize the usage of solvents, although the pressure limitations would become more crucial due to the viscosity of ethanol. Another possibility is the use of LC-MS with the aim of performing analysis of the degradants so that one can establish what products will be formed and if there are any products that co-elute with any other drug. The last advancement would be the formulation of new assessment schemes and the incorporation of requirements of ICH Q14, which directs analytical processes towards lifecycle management.

12. CONCLUSIONS

So far, there have been no reported methods for the determination of ciprofloxacin, levofloxacin and ornidazole using a method that is not only green but also indicates stability. The chemistry appears to support this view; acidic medium, which controls ionization and the tailing of the fluoroquinolones, has so far been used in methods with other analytes

including during AQbD; and according to the ICH Q1A(R2), Q1B and Q2(R2) guidelines, one can successfully demonstrate the stability-indicating capability of the method being suggested. This paper provides a framework comprising factors, ranges, forced degradation design, validation criteria and greenness that need to be checked to prove the feasibility of the approach. The next step consists in conducting the laboratory work to obtain data that will pave the way for a full-fledged research article.

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REFERENCES

1. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev. 1997;61(3):377–392.
2. Edwards DI. Nitroimidazole drugs—action and resistance mechanisms. I. Mechanisms of action. J Antimicrob Chemother. 1993;31(Suppl A):9–20.
3. Conley ZC, Bodine TJ, Chou A, Zechiedrich L. Wicked: the untold story of ciprofloxacin. PLoS Pathog. 2018;14(3):e1006805.
4. Anastas PT, Warner JC. Green Chemistry: Theory and Practice. Oxford: Oxford University Press; 1998.
5. Gałuszka A, Migaszewski Z, Namieśnik J. The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. TrAC Trends Anal Chem. 2013;50:78–84.
6. Tobiszewski M, Mechlińska A, Namieśnik J. Green analytical chemistry—theory and practice. Chem Soc Rev. 2010;39(8):2869–2878.
7. Welch CJ, Wu N, Biba M, Hartman R, Brkovic T, Gong X, et al. Greening analytical chromatography. TrAC Trends Anal Chem. 2010;29(7):667–680.
8. Capello C, Fischer U, Hungerbühler K. What is a green solvent? A comprehensive framework for the environmental assessment of solvents. Green Chem. 2007;9(9):927–934.
9. ICH Harmonised Tripartite Guideline. Q1A(R2): Stability Testing of New Drug Substances and Products. International Council for Harmonisation; 2003.
10. ICH Harmonised Tripartite Guideline. Q1B: Photostability Testing of New Drug Substances and Products. International Council for Harmonisation; 1996.
11. ICH Harmonised Guideline. Q2(R2): Validation of Analytical Procedures. International Council for Harmonisation; adopted 2023.
12. ICH Harmonised Guideline. Q14: Analytical Procedure Development. International Council for Harmonisation; adopted 2023.
13. Chamseddin C, Jira T. Comparison of the chromatographic behavior of levofloxacin, ciprofloxacin and moxifloxacin on various HPLC phases. Pharmazie. 2011;66(4):244–248. doi:10.1691/ph.2011.0314
14. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Hoboken: Wiley; 2010.
15. D'Souza K, Syeda A, Khatal P, Sathyanarayana MB, Vasantharaju SG. Stability indicating assay method development and validation for simultaneous estimation of ofloxacin and ornidazole by RP-HPLC in bulk: an application to tablet formulation and dissolution studies. Indian J Pharm Educ Res. 2021;55(2):607–613. doi:10.5530/ijper.55.2.100
16. Alabbas AB, Abdel-Gawad SA. Stability-indicating quantification of ciprofloxacin in the presence of its main photo-degradation product by CZE and UPLC: a comparative study. Separations. 2023;10(7):391.
17. Fares MY, Hegazy MA, El-Sayed GM, Abdelrahman MM, Abdelwahab NS. Quality by design approach for green HPLC method development for simultaneous analysis of two thalassemia drugs in biological fluid with pharmacokinetic study. RSC Adv. 2022. doi:10.1039/D2RA00966H
18. Kelani KM, Fayez YM, Abdel-Raoof AM, Fekry RA, Hassan SA, et al. Development of an eco-friendly HPLC method for the stability indicating assay of binary mixture of ibuprofen and phenylephrine. BMC Chem. 2023;17:141. doi:10.1186/s13065-023-01056-4
19. Gałuszka A, Migaszewski ZM, Konieczka P, Namieśnik J. Analytical Eco-Scale for assessing the greenness of analytical procedures. TrAC Trends Anal Chem. 2012;37:61–72.
20. Płotka-Wasylka J. A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. Talanta. 2018;181:204–209.
21. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE—Analytical GREEnness metric approach and software. Anal Chem. 2020;92(14):10076–10082.
22. Peraman R, Bhadraya K, Padmanabha Reddy Y. Analytical quality by design: a tool for regulatory flexibility and robust analytics. Int J Anal Chem. 2015;2015:868727.
23. ICH Harmonised Tripartite Guideline. Q8(R2): Pharmaceutical Development. International Council for Harmonisation; 2009.