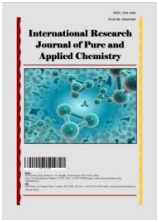

International Research Journal of Pure & Applied Chemistry

22(3): 34-42, 2021; Article no.IRJPAC.68162
ISSN: 2231-3443, NLM ID: 101647669

Novel Rapid Validated TLC- Densitometry for the Simultaneous Determination of Three Co-formulated Drugs used for Deep Tooth Inflammation Treatment

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Authors' contributions

This work was carried out in collaboration among all authors. Author GK performed the experiments and wrote the first draft of the manuscript. Author AAS put the protocols for pharmaceutics and samples preparation, contributed to the interpretation of data and revised the manuscript. Author MM managed the project in all stages and revised the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2021/v22i330394
Editor(s):
(1) Dr. Wolfgang Linert, Vienna University of Technology, Austria.
Reviewers:
(1) S. Parthasarathy, Mahatma Gandhi Medical College and Research Institute, India.
(2) C. Kalaimagal, Bharathidasan University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68162

Original Research Article

Received 01 March 2021
Accepted 05 May 2021
Published 13 May 2021

ABSTRACT

A new use of a tertiary antibiotic combination of ciprofloxacin, clindamycin and metronidazole is currently being studied as an intracranial (intracranial) drug in an effort to disinfect the root canal system to revitalize the tooth with dead pulp. It is necessary to developing analytical method for the new drug combination.

Herein we developed a rapid validated thin-layer chromatography (TLC)–densitometric method has been developed for the simultaneous determination of 3 co-formulated drugs used for deep tooth inflammation Treatment. The studied drugs are Ciprofloxacin.HCL (CIP), Clindamycin phosphate (CLI) and Metronidazole(MET). The separation was achieved by using silica gel 60 F254 plates (20*20 cm) as stationary Phase and the developing system of Dichloromethane:n-Hexane:methanol:Ethylamine:Triethylamine (40:20:32:3:5)v/v.

Densitometry scanning was performed at 220 nm. The R_f values of CIP, CLI and MET were found

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to be 0.588, 0.776 and 0.898 respectively.
The method was validated as per the International Conference on Harmonization (ICH) guidelines and was successfully applied for the analysis of ternary lab made mixture, containing the cited ternary mixture without interference from excipients. There is no previously published TLC–densitometry method for the determination of the previously mentioned ternary mixture. The suggested method is novel, rapid, accurate, reproducible and of low cost, so; thus, it can be used for quality control analysis of these formulations.

Keywords: Ciprofloxacin HCL; clindamycin phosphate; metronidazole; thin-layer chromatography–densitometry; deep tooth inflammation treatment.

1. INTRODUCTION

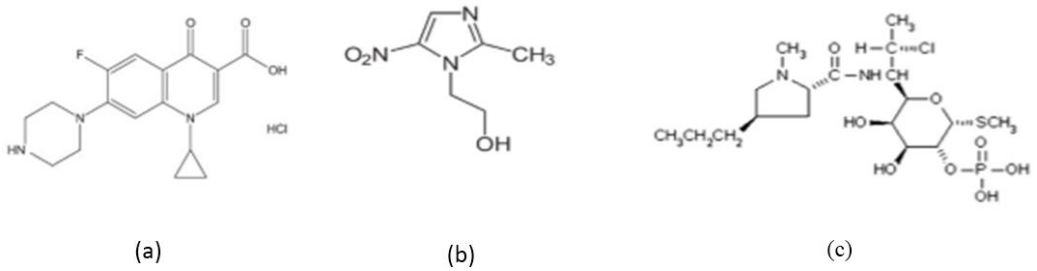
Ciprofloxacin Hydrochloride (CIP), is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid]; Fig(1,a) [1,2] . Ciprofloxacin hydrochloride exerts its bactericidal effect by interfering with the bacterial DNA gyrase, thereby inhibiting the DNA synthesis and preventing bacterial cell growth [3,4]. Ciprofloxacin has been linked to rare but convincing instances of liver injury that can be severe and even fatal.

Metronidazole (MET), is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol Fig (1,b). It is used as antibacterial and antiamaebiasis [1,2]. It is a commonly used antibiotic, belonging to the nitroimidazole class of antibiotics. It is frequently used to treat gastrointestinal infections as well as trichomoniasis and giardiasis, and amebiasis which are parasitic infections [5]. Metronidazole has been used as an antibiotic for several decades, with added antiparasitic properties that set it apart from many other antibacterial drugs, allowing it to treat a wide variety of infections. It is available in capsule form, tablet form, and topical form, and suppository preparations for the treatment of various infections [6].

Clindamycin phosphate (CLI) is the 2-phosphate ester of clindamycin. Clindamycin (methyl-7-chloro-6,7,8-trideoxy-6-{[(4R)-1-methyl-4-propyl-L-prolyl]amino}-1-thio-L-threo-α-D-galacto-octopyranoside) Fig (1,c) [1,2] . It is a semi-synthetic derivative of lincomycin. It is an antibiotic effective against Gram-positive aerobes as well as Gram-negative and Gram-positive anaerobic pathogens. Topically, it is used for the treatment of acne vulgaris and bacterial vaginosis, which typically leads to the suppression of cutaneous propionibacterium acnes [7-9]. Due to the inhibition of propionicbacterium acnes, the free fatty acid level on the skin surface decreases. Clindamycin phosphate applied topically penetrates to a very great extent into open comedogenes and thus, produces a high percentage of comedogenes [10].

The literature survey reveals that many methods concerning the determination of separate formulations containing either CIP, CLI and MET. Various analytical methods have been reported for the estimation of CIP, CLI and MET as alone as well as in combination with other drugs. The three drugs were determined separately using various techniques and in combination with other drugs. however, no method has been reported for the simultaneous estimation of ciprofloxacin, clindamycin phosphate and metronidazole in pharmaceutical mixture.

Several methods were reported for the determination of each component of this formulation. For CIP there are spectrophotometry [11–21], TLC [22,23], HPTLC [24,25], HPLC [26,27], CE [28], Electrochemistry [29-33]. The methods for MET determination include Ion Selective Electrodes [34-38], HPLC [39-43], HP-TLC [44,45], Spectrophotometric methods [46-50], Fluorescence [51,52], Photo-Fenton Oxidation Technology [53], Charge-Transfer Complexes Formation [54], Glassy Carbon Electrode Modified with Gold-Copper Nanoparticles as Novel Electrochemical Sensor for Determination of Metronidazole [55], and Cerium doped magnetite nanoparticles for highly sensitive detection of metronidazole via chemiluminescence [56]. Various methods for CLI determination have been reported in the literature, such as high-performance liquid chromatography (HPLC) [57,58], spectrophotometry [59], capillary electrophoresis [60], chemi-luminescence [61], electrochemi-luminescence [62], electrochemistry [63] and electrochemical sensors [64,65].



(a) (b) (c)

Fig. 1. The chemical structure of (a) Ciprofloxacin hydrochloride, (b) Metronidazole, and (c) Clindamycin phosphate

To date, to the best of our knowledge, there is no reported thin-layer chromatography (TLC)–densitometry method for the simultaneous determination mixture of CIP, CLI and MET. The TLC method has the advantages over the HPLC method of being less cost and time-saving and not requiring pH adjustment of the mobile phase or tedious cleanup procedures. Thus, the aim of our present work was to conduct TLC-densitometry for the simultaneous determination of the 3 co-formulated drugs in bulk powder and lab made pharmaceutical preparation.

The novel of this article, a triple antibiotic mixture of ciprofloxacin, clindamycin, and metronidazole was used as an intracranial (intracanal) medicament in an attempt to disinfect the root canal system for revascularization of a tooth with a necrotic pulp. However, discoloration developed after applying the triple antibiotic mixture [66-73].

2. MATERIALS AND METHODS

2.1 Apparatus

Shimadzu “dual wavelength flying spot scanning” densitometer CS-9301 PC (Tokyo, Japan, 2000) (program version 2.00) was used for TLC plates scanning. Digital Water Bath of Heidolph Laborota 4001-Rotary Evaporator, Germany) was used to incubate solutions. UV-254 nm chamber was used for UV experiments. Pre-coated TLC plates, silica gel 60 GF-254 (20 × 20 cm) (Merck, Germany). Hamilton 5-µL micro-syringe (Switzerland) was used to apply samples on TLC plates. Glass TLC developing chamber (20 × 20 × 10 cm).

2.2 Materials and Reagents

Pharmaceutical grade Ciprofloxacin HCl, Metronidazole and Clindamycin phosphate (99%) (supplied by Lyphar, Shaanxi, China). Methanol, isocratic HPLC grade (Scharlab S.L., Spain). Dichloromethane, n-Hexane, Ethylamine, Triethylamine, (Merck, Germany). All the reagents used were of AR grade.

2.3 Procedure

2.3.1 Preparation of standard stock solutions

Stock solutions prepared by dissolving 250 mg of CIP, MET, and CLI separately in 25mL volumetric flask, in least amount of mixture methanol and complete to 25 mL. to obtain solutions contain (10 mg/mL) of CIP, CLI and MET.

2.3.2 Preparation of standard mixture solutions

Suitable amounts of the last mentioned standard stock solutions, were taken in series of 10mL volumetric flasks, made up to the mark with methanol, to prepare a standard mixture solutions of CIP, CLI and MET, in the concentration range (0.5-8.0) mg/mL for CIP, CLI and MET.

2.3.3 Optimization of chromatographic conditions

Few trials were carried to determine CIP, CLI and MET, in dosage form. The optimum condition of separation was determined.

The pre-coated TLC plates silica gel 60 F254 (20 cm × 20 cm, 250 µm thickness) were used. 1 µL on spots from each standard mixture solutions and sample solution, were applied on TLC plates. The Chromatograms were run to the solvent front of 85 mm by ascending development in the chamber previously saturated for 30 min with Dichloromethane: n-Hexane:methanol:Ethylamine:Triethylamine (40:20:32:3:5) v/v. as the mobile phase (run time 30 min). After development, the plates were removed immediately and dried in an oven at 60°C for 1 hr.

Densitometry scanning at λ = 220 nm was performed with a Shimadzu TLC Scanner in the absorbance mode. The silt dimension was kept at 4.0 mm × 0.45 mm and a scanning rate of 20 mm s–1 was employed. The chromatograms were integrated using the densitometer .

3. RESULTS AND DISCUSSION

The aim of this work was to develop a TLC–densitometric method for the simultaneous determination of 3 co-formulated drugs, namely, CIP, CLI and MET, in bulk powder and in lab made mixture. To the best of our knowledge, there was no reported TLC–densitometric method for the simultaneous determination of CIP, CLI and MET. TLC–densitometry has the advantages of being simple, cost-effective (for the instrument and the solvents used), and rapid, when compared to HPLC.

Densitometry scanning at λ = 220 nm was performed for standard mixtures of CIP, CLI, MET (0.5, 1.0, 2.0, 4.0, 6.0, 8.0) g/spot for each compound Fig. (2,3).

System suitability test parameters must be checked to ensure that the system was working correctly during the analysis. Method performance data including retardation factor (R_f), resolution (R_s), Selectivity (α) and Theoretical plates Number (N) are listed in Table 1.

3.1 Method Validation

3.1.1 Linearity and range

Five concentrations were chosen in the ranges of corresponding of to the analytical concentration of CIP, CLI and MET. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equations were as shown in Table 2.

3.1.2 Accuracy

Accuracy was assessed using 9 determinations over 3 concentration levels covering the specified range (75,100 and 125%). Accuracy was reported in Table 3 as percent recovery by the assay of known added amount of analyte in the sample.

Fixed dose of lab made combination of CIP, CLI and MET was prepared. The ratio is maintained at 250, 250, and 250 mg in tablet respectively. The resultant sample solution was used for chromatographic development and scanning followed by analysis. The analysis was repeated in triplicate, Table (4).

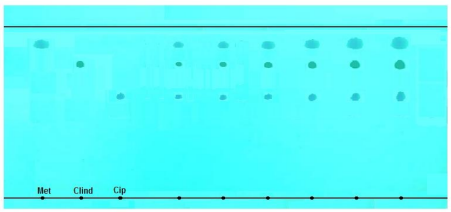


Fig. 2. UV Photo of TLC silica gel 60 F_{254} used for separation of a standard mixtures of CIP, CLI, MET (0.5, 1.0, 2.0, 4.0, 6.0, 8.0) g/spot for each compound

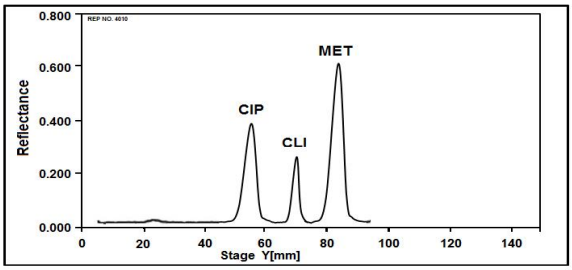


Fig. 3. Densitometry TLC chromatogram of standard mixture of CIP, CLI, MET with concentration (6 g/spot for each compound, using Dichloromethane:n-Hexane:methanol:Ethylamine:Triethylamine (40:20:32:3:5)v/v as the developing system measured at 220 nm

Table 1. System suitability parameters of the developed TLC–densitometry method

Parameter	CIP	CLI	MET
Retardation factor, R_f	0.588±0.004	0.776±0.005	0.898±0.005
Resolution (R_S)	-	3.05	1.54
Selectivity (α)	-	2.434	2.428
Theoretical plates Number (N)	1444	2788	1322

Table 2. Linear regression data for analysis of CIP, MET, and CLI by the developed TLC method (n = 3)

Item	CIP	CLI	MET
Linear range, µg/spot	0.5 – 8.0	0.5–8.0	0.5–8.0
Detection limit , µg/spot	0.09	0.11	0.08
Quantitation limit , µg/spot	0.27	0.33	0.24
Regression Data:			
Slope (a)	158.04	153.70	266.57
Intercept (b)	332.36	175.52	283.32
Correlation Coefficient , r^2	0.9947	0.9924	0.9944

y = a C+b where a is the slope, b is the intercept point, C is the concentration of the compound (µg/spot) and Y is the drug peak area

Table 3. Accuracy (Recovery%) of drugs in sample

Concentration	AV ± SD%		
	CIP	CLI	MET
75%	101.26±0.17	98.58±0.21	98.86±0.18
100%	99.48±0.25	99.83±0.26	99.68±0.23
125%	100.52±0.28	101.13±0.42	100.02±0.40

Table 4. Assay of CIP, CLI and Met in Lab made combination (n=3)

Drug	Label claim (mg/tab)	Amount found (mg/tab)	Recovery%	SD%
CIP	250	254.05	101.62	1.37
CLI	250	252.30	100.92	1.52
MET	250	249.30	99.72	1.28

4. CONCLUSION

The present work described the successful simultaneous quantitative analysis of CIP, CLI and MET in their laboratory-prepared mixtures used for dental treatment using TLC–densitometry as per ICH [74] guidelines for the simultaneous estimation of CIP, MET and CLI. The results showed that the developed TLC–densitometry had the advantages of being simpler than the HPLC, as it used simple mobile phase with no pH adjustment, sensitive, and economic, as it saves cost (inexpensive apparatus and solvents) and time (up to 20 samples could be applied onto a single plate per one development). The developed method can be successfully used in routine quality control testing, allowing qualitative and quantitative determination with high accuracy and precision.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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