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Simultaneous Determination of Metronidazole and Diiodohydroxyquine in Bulk Powder and Paramibe Compound Tablets by TLC-Densitometry and HPLC

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Abstract

Two sensitive and precise chromatographic methods were developed and validated for simultaneous determination of metronidazole (MTR) and diiodohydroxyquin (DIQ) in pharmaceutical preparation. The techniques adopted for quantification are coupled TLC-densitometry and HPLC. A mixture of chloroform, toluene, ethanol and acetic acid (9:9:1:1, v/v/v/v) was used as the developing solvent for TLC-densitometry. A mixture of methanol and acetonitrile, (96:4, v/v) was used as a mobile phase for HPLC at 0.6 mL min^{-1} flow rate and UV detection at 254 nm. Linearity was obtained in concentration range of 0.5-10 µg $spot^{-1}$ for DIQ and 1-20 µg $spot^{-1}$ for MTR applying TLC-densitometry and 0.005-0.5 mg mL^{-1} for DIQ and 0.01-0.5 mg mL^{-1} for MTR applying HPLC. The selectivity of the proposed methods was checked using laboratory prepared mixtures. The proposed methods were successfully applied to the analysis of MTR and DIQ in their mixture and in pharmaceutical dosage forms without interference from other additives.

Keywords: Metronidazole; Diiodohydroxyquinoline; TLC-Densitometry; HPLC; Tablets dosage form

Introduction

Metronidazole (MTR), is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol (Figure 1a). It is used as antibacterial and antiamaebiasis [1]. Metronidazole is metabolized by oxidation to 2–hydroxymethyl metronidazole and 2–methyl–5–nitroimidazol–1–acetic acid, and by conjugation with glucuronic acid. About 70 to 80% of a dose is excreted in the urine in 48 hr with less than 10% of the dose as unchanged drug, up to 10% as conjugated MTR, about 27% as 2–hydroxymethylmetronidazole, 10% as the conjugated 2–hydroxymethyl metabolite, and 20% as the acid metabolite [2]. Diiodohydroxyquinoline (DIQ), 5,7-diiodoquinolin-8-ol (Figure 1b). It is widely known by the trade name Diodoquin, is a quinoline derivative which can be used in the treatment of amoebiasis. Iodoquinol is poorly absorbed from the gastrointestinal tract and is amebicidal at the site of infection. It acts by chelating ferrous ions essential for metabolism [3].

The literature survey reveals several analytical methods for quantitative estimation of MTR in body fluids and in pharmaceutical formulations these methods include ultaviolet spectrophotometry [4-6], high-performance liquid formulations (HPLC) [7,8] and voltammetry [9]. Quantitation of metronidazole and spiramycin in human plasma, saliva and gingival crevicular fluid by LC–MS/MS [10]. Simultaneous multi residue determination of metronidazole and spiramycin in fish muscle using high performance liquid chromatography with UV detection [11]. Microsized Graphite Sensors for potentiometric determination of metronidazole and spiramycin [12]. DIQ was determined in pharmaceutical formulations using HPLC [13].

The present work aimed to develop simple instrumental methods for simultaneous determination of MTR and DIQ in combination. These methods include as chromatographic methods; namely, TLC-densitometry method and HPLC [14].

Experimental

Instruments

Camag TLC scanner 3S/N 130319 with winCATS software. Camag

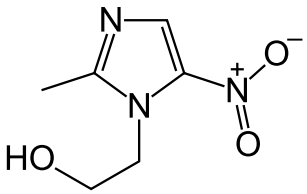
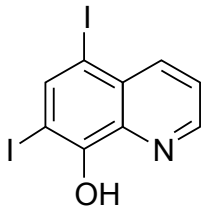


Figure 1a: Chemical structure of Metronidazole (MTR).



M.W. 396.951gm

Figure 1b: Chemical structure of Diiodohydroxyquine (DIQ).

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linomat autosampler (Switzerland), Camag microsyringe (100 µL). A liquid chromatography consisted of an quaternary pump (Agilent Model G1316A/G1316B), a diode array multiple wavelength detector (Model G1316 C/D and G1365C/D, Agilent 1200 Series), standard and preparation autosamplers (Agilent 1200 series) equipped vacuum degasser, Agilent. Stationary phase (250 mm×4.6 mm, 10 µm) C_{18} Lichrosorb™ 10 µm analytical column, Alltech (USA). Mobile phase; methanol and acetonitrile (96:4, v/v) isocratically at 0.6 mL min^{-1}. The mobile phase was filtered through a 0.45 µm millipore membrane filter and was degassed for ~15 min in an ultrasonic bath prior to use. UV-detection was done at 254 nm. The samples were filtered also through a 0.45 µm membrane filter.

Solvents, authentics and pharmaceutical preparation

Reference metronidazole hydrochloride powder (MTR) and reference diiodohydroxyquine (DIQ) were kindly donated by Al Qahir Pharmaceuticals Co. The potency was found to be 1002 µg mg^{-1}. Pharmaceutical dosage form (Paramibe compound, 500 mg tablets were kindly supplied by Chemical Industries Development (CID) and were claimed to contain 250 mg of MTR and 250 mg of DIQ per each tablet. Methanol and acetonitrile HiPerSolv®, HPLC-grade E. Merck (Darmstadt, Germany), BDH-laboratory supplier (Poole, England). De-ionized water: Bidistilled from "Aquatron" Automatic Water Still A4000, Bibbysterillin Ltd. (Staffordshire, UK), methanol analytical grade.

Standard solutions

MTR standard solution and DIQ standard solution (0.5 mg mL^{-1} each) in mobile phase for the HPLC method and MTR standard solution (1 mg mL^{-1}) and DIQ (0.5 mg mL^{-1}) in methanol for TLC-densitometric method. The standard solutions were freshly prepared on the day of analysis and stored in a refrigerator to be used within 24 hr.

Procedures

TLC-densitometric method:

- **Linearity:** Aliquots 1-20 µL of MTR standard solution (1 mg mL^{-1}) and DIQ (0.5 mg mL^{-1}) were applied in the form of bands on a TLC plate. The band length was 4 mm apart from each other and 10 mm from the bottom edge of the plate. Linear ascending development was performed in a chromatographic tank previously saturated with chloroform, toluene, ethanol and acetic acid (9:9:1:1, v/v/v/v) for 1 hr at room temperature. The developed plates were air-dried and scanned at 311 nm using deuterium lamp, absorbance mode at 3 mm×0.45 mm slit dimension and scanning speed of 20 mms^{-1}. Calibration curves relating the optical density of each spot to the corresponding concentration of MTR and DIQ were constructed. The regression equations were then computed for the studied drugs and used for determination of unknown samples containing them (Table 2).

Liquid chromatographic method:

- **Linearity:** Portions 0.1–2 mL from MTR standard solution (0.5 mg mL^{-1} in the mobile phase) and DIQ (0.5 mg mL^{-1}) were transferred separately into a series of 10-mL volumetric flasks and completed with mobile phase. The contents of each flask were completed to volume with the mobile phase to get the concentrations of 5–1000 mg mL^{-1} of MTR and 5-500 mg mL^{-1} of DIQ. The samples were then chromatographed using the following chromatographic condition. Stationary phase (250 mm×4.6 mm 10 µm) C_{18} Lichrosorb™ 10 µm analytical column, Alltech (USA), mobile phase; methanol and acetonitrile, (96:4, v/v). The mobile phase was filtered through a 0.45 µm millipore membrane filter and was degassed for about 15 min in an ultrasonic bath prior to use, flow rate; 0.6 mL min^{-1} [isocratically at ambient temperature (~25°C)], with UV-detection at 254 nm. The samples were filtered also through a 0.45 µm membrane filter. To reach good equilibrium, the analysis was usually performed after passing ~50–60 mL of the mobile phase, just for conditioning and pre-washing of the stationary phase. The relative peak area ratios (by using 0.04 mg mL^{-1} MTR and 0.0375 mg mL^{-1} DIQ as divisor) were then plotted versus the corresponding concentrations of MTR and DIQ to get the calibration graphs and to compute the corresponding regression equations. Concentrations of unknown samples of MTR and DIQ were determined using the obtained regression equations.

Analysis of laboratory prepared mixtures containing different ratios of MTR and DIQ: Aliquots were mixed to prepare different mixtures containing different ratios (1:1, 1:4, 1:3, 2:1) of MTR and

Parameter	TLC-densitometric method		HPLC method	
	MTR	DIQ	MTR	DIQ
Range	1-20 µg $spot^{-1}$	0.5-10 µg $spot^{-1}$	0.01-1 mg mL^{-1}	0.005-0.5 mg mL^{-1}
Slope	0.079	0.438	39.69	38.16
Intercept	0.092	0.738	- 0.486	0.011
Mean	99.87	100.15	99.92	100.15
± S.D.	0.365	0.593	0.433	0.483
Variance	0.133	0.352	0.187	0.233
Coefficient of variation	0.365	0.592	0.433	0.482
Correlation coefficient (r)	0.9998	0.9999	0.9999	0.9999
R.S.D. (%)	0.365	0.592	`0.433	0.482

Table 1: Assay parameters and validation sheet for determination of metronidazole (MTR) and diiodohydroxyquine (DIQ).

Preparation	TLC-densitometric method	HPLC method
Paramibe compound tablets (0.5 g)		
MTR mean ± S.D.	99.67 ± 0.165	99.995 ± 0.313
DIQ mean ± S.D.	99.61 ± 0.098	100.008 ± 0.121

Table 2: Determination of MTR and DIQ in paramibe compound tablet by proposed methods.

DIQ, respectively. The concentrations were calculated from the corresponding regression equations (Table 3).

Assay of pharmaceutical formulations (Paramibe compound tablets): Twenty tablets were weighed and grinded to determine the average weight per tablet. Aliquot of the powder tablet equivalent to 250 mg of MTR and DIQ each was extracted by shaking with methanol for 15 minutes then the volume was completed to the mark with methanol to get a concentration of 1 mg mL^{-1} of MTR and DIQ and proceed as described under each method.

Results and Discussion

TLC-fractionation

TLC-monitoring of MTR and DIQ was done on thin layer plates of silica gel F254 using chloroform, toluene, ethanol and acetic acid (9:9:1:1, v/v/v/v) as the developing solvent. The developed plates were visualized under short UV-lamp. MTR (R_f value=0.13); could be separated from DIQ (R_f value=0.741).

Chromatographic methods

TLC-densitometry: A TLC-densitometric method is described for the determination of MTR in the presence of DIQ without prior separation. Different solvent systems were tried for the separation of MTR and DIQ. Satisfactory results were obtained by using a mobile phase composed of chloroform, toluene, ethanol and acetic acid (9:9:1:1, v/v/v/v), where R_f=0.13 and 0.740 for MTR and DIQ, respectively. The separation allows the determination of MTR with no interference from DIQ (Figure 4). The linearity was confirmed by plotting the measured peak area versus the corresponding concentrations at 311 nm over a range of 1–20 µg $spot^{-1}$ and 0.5-10 µg $spot^{-1}$ for MTR and DIQ, respectively where a linear response was obtained (Figures 2 and 3). The regression equation was found to be: A=0.079C+0.092, r=0.9998; where A is the area under the peak and C is the concentration of MTR in µg $spot^{-1}$ and r is the correlation coefficient and for DIQ, the regression equation was found to be: A_2=0.438C_2+0.738, r_2=0.9999; where A_2 is the area under the peak and C_2 is the concentration of DIQ in µg $spot^{-1}$ and r_2 is the correlation coefficient (Table 4).

Method	TLC-densitometric method	HPLC method
MTR (Mean ± SD)	99.97 ± 0.320	100.10 ± 0.469
DIQ (Mean ± SD)	100.40 ± 0.253	100.28 ± 0.085

Table 3: Determination of metronidazole and diiodohydroxyquine in laboratory prepared mixtures by the proposed methods.

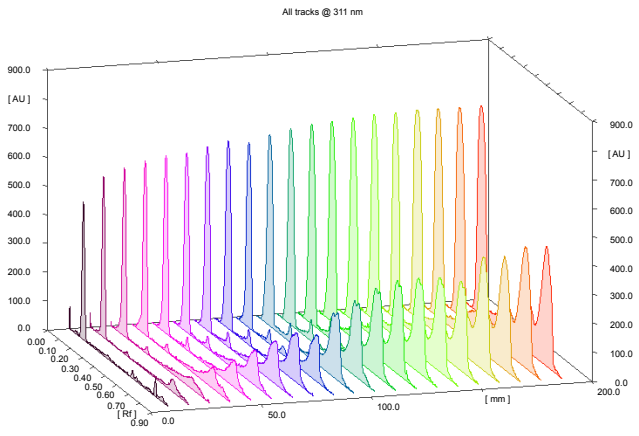

Figure 2: Linearity of MTR (1-20) µg $spot^{-1}$ (R_f value=0.13) DIQ (0.5-10) µg $spot^{-1}$ (R_f value=0.74) measured at 311 nm.

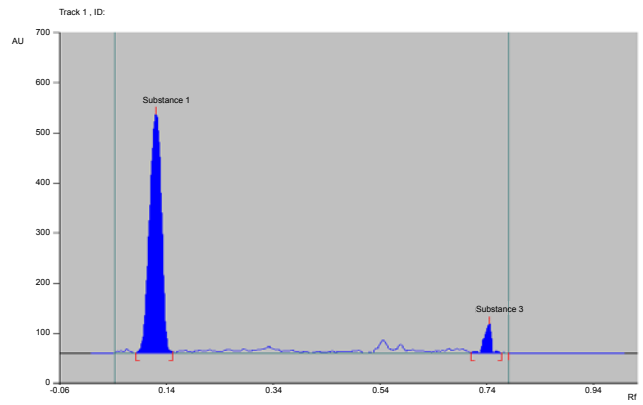

Figure 3: Separation of MTR (R_f value=0.13) from DIQ (R_f value=0.74) upon using chloroform+toluene+ethanol+acetic acid (9:9:1:1, v/v/v/v).

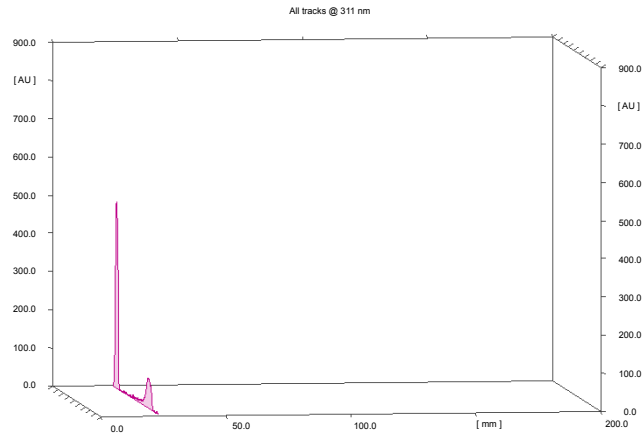

Figure 4: Separation of MTR (R_f value=0.13) from DIQ (R_f value=0.74).

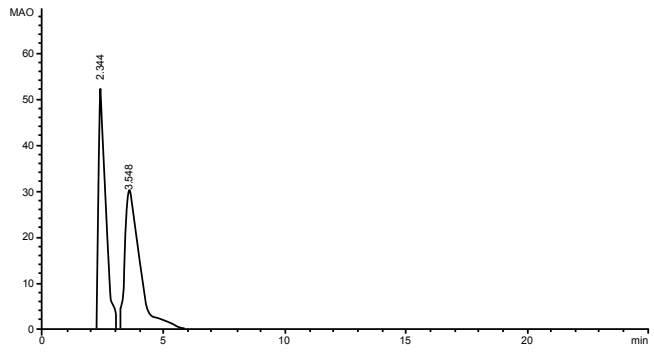

Figure 5: Liquid chromatographic separation of metronidazole (2.344 min) and diiodohydroxyquine (3.548 min) upon using methanol+acetonitrile, (96:4, v/v) as a mobile phase.

The precision of the proposed method was checked by the analysis of different concentrations of authentic samples in triplicates. The

Parameters	TLC-densitometry		HPLC		official method[1]	Reference method
	MTR	DIQ	MTR	DIQ	MTR	DIQ
Mean	99.87	100.15	99.92	100.15	100.07	100.18
± S.D	0.365	0.593	0.433	0.483	0.507	0.641
Variance	0.133	0.352	0.187	0.233	0.257	0.411
F-test	1.93 (2.71)[a]	1.17 (2.71)[a]	1.37 (4.88)[a]	1.76 (4.95)[a]		
Student's *t*-test	0.899 (2.06)[a]	0.102 (2.06)[a]	0.583 (2.18)[a]	0.094 (2.20)[a]		
N	20	20	8	7	6	6

[a]The values in the parenthesis are corresponding the oretical t- and F-values at P=0.05 [14]

Table 4: Statistical comparison for the results obtained by the proposed methods and the official method for analysis of MTR and official method for analysis of DIQ.

mean percentage recovery was found to be 99.87 ± 0.365 for MTR and 100.15 ± 0.539 for DIQ (Table 1).

High-performance liquid chromatography: A simple isocratic high-performance liquid chromatographic method was developed for the determination of MTR and DIQ in pure form and in pharmaceutical preparation using (250 mm×4.6 mm, 10 µm) C_{18}lichrosorb™ analytical column. The mobile phase was consisting of methanol and acetonitrile, (96:4, v/v). The mobile phase was chosen after several trials to reach the optimum stationary/mobile-phase matching. The average retention times under the conditions described are 2.344 min for MTR, 3.548 min for DIQ (Figure 5). One sample can be chromatographed in less than 6 min. Peak purity was confirmed for the HPLC peaks of both MTR and DIQ by a pilot run using a photodiode array detector. Calibration graph was obtained by plotting the relative peak area ratios (by using 0.04 mg mL^{-1} MTR and 0.0375 mg mL^{-1} DIQ as divisor) against concentration of MTR and DIQ (mg mL^{-1}). Linearity range was found to be 0.01–1 mg mL^{-1} for MTR and 0.005-0.5 mg mL^{-1} of DIQ. The regression equation for MTR: A=39.69C-0.486 (r=0.9999) where A is the relative peak area ratio, C is the concentration of MTR (mg mL^{-1}) and r is the correlation coefficient and for DIQ A_2=38.16C_2+0.011 (r=0.9999). The mean percentage recovery of pure sample was found to be 99.92 ± 0.433 for MTR and 100.15 ± 0.483 for DIQ (Table 1).

Conclusion

The suggested methods are found to be sensitive and precise. Application of the proposed methods to the analysis of MTR and DIQ in their pharmaceutical formulation shows that excipient do not interfere with the determination. The proposed methods can be used for routine analysis of metronidazole and diiodohydroxyquine in quality control laboratories.

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