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Spectrofluorimetric Determination of Diiodohydroxyquinoline in Presence of Metronidazole in Pharmaceutical Formulation and Spiked Human Plasma

Nouruddin W. ALI[1], Mohammed GAMAL[1,♠], Mohammed ABDELKAWY [2]
Hassan Y. ABOUL-ENEIN[3]

[1]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, AlshaheedShehata Ahmed Hegazy St., 62574 Beni-Suef, Egypt*
[2]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St., 11562, Cairo, Egypt*
[3]*Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, Cairo 12311, Egypt*

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ABSTRACT

Accurate and sensitive spectrofluorimetric method was developed for determination of diiodohydroxyquinoline in presence of metronidazole. In this method the native fluorescence of diiodohydroxyquinoline in water solvent at λ = 495 nm when excitation was at 250 nm is used for its determination. Linear correlation was obtained in the concentration range of 400 to 900 ng mL^{-1}. The proposed method was successfully applied for determination of diiodohydroxyquinoline in bulk powder with mean accuracy of 100.21±1.13 or in spiked human plasma with mean accuracy of 100.53±1.42 without interference of metronidazole.

Keywords: spectrofluorimetry, Diiodohydroxyquinoline and Metronidazole.

1. INTRODUCTION

Metronidazole (MTN) is 2-methyl-5-nitroimidazole-1-ethanol [1]. It is a 5-nitroimidazole derivative with activity against anaerobic bacteria and protozoa. MTN acts as an amoebicide at all sites of infection of *Entamoebahistolytica* infection. Because of its rapid absorption, it is probablyless effective against parasites in the bowel lumen and is therefore used with a luminal amoebicide, in the treatment of invasive amoebiasis[2].

Diiodohydroxyquinoline (DIHQ) or iodoquinol[3] is 5, 7-diiodoquinolin-8-ol [1], is a halogenated hydroxyquinolinewhich can be used in the treatment of amoebiasis. DIHQ is poorly absorbed from the gastrointestinal tract and is amebicidal at the site of infection [2].This combination of two amoebicidal agents (DIHQ and MTN) is used for treatment of extra and intra-intestinal amoebiasis.The structural formulas of DIHQ and MTN are shown in Figure 1.

♠Corresponding author, e-mail: mgamalm3000@yahoo.com

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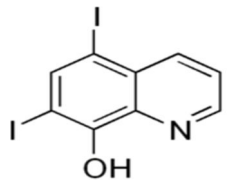

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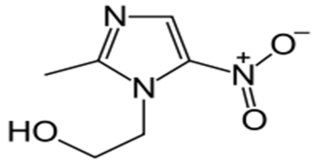

Figure 1. Chemical structureof DIHQ (A) and MTN (B)

There are many reports for the determination of DIHQ and MTN either separately or in combination with other drugs including spectrophotometry [4-12], electrochemistry [13, 14], high performance liquid chromatograpgy (HPLC) [15–22], thin layer chromatography (TLC) [23] , gas chromatography(GC) [24], atomic absorption spectrometry [25], iodometric titration [26] and non aqueous titration [27, 28].

Few methods have been reported for determination of DIHQ and MTN in binary mixture. The first method determined the two drugs spectrophotometrically using modified Vierodt equation [29]. The second method used the bivariate calibration method for resolution of the binary mixture and compared results to those of derivative spectrophotometry [30]. The third method determined DIHQ and MTN in pharmaceutical preparation by thin layer chromatography (TLC) [31], in which a very complicated process was applied where the spots were detected by spraying 20 % w/v methanloic solution of KOH and heating to 80º C till development of reddish pink color then the spots scrapped and dissolved in 0.1 MHCl and finally the absorbencies of supernatant were measured spectrophotometrically at 280 nm.

The objective of this work is to develop highly sensitive, selective and reproducible method for determination of DIHQ in presence of MTN. Spectrofluorimetric method are well known for providing high selectivity and sensitivity when used for determination of pharmaceutical drugs. In this paper, the spectrofluorimetric method has been proposed for determination of DIHQ in presence of MTN that can be used for routine quality control analysis of DIHQ in bulk powder, pharmaceutical formulation and spiked human plasma.

2. EXPERIMENTAL

2.1. Instruments

1-Jasco FP-6200 Spectrofluorimeter equipped with a xenon lamp and 1 cm quartz cuvette (Japan).
• The following requirements are taken into consideration:
-Band width (Ex): 5 nm. -Band width (Em): 20 nm.
-Response: 0.02 sec. -Sensitivity: medium.
-Scanning speed: 500 nm.min^{-1}
2- Sonix TV ss-series ultrasonicator (USA).

2.2. Materials

2.2.1. Pure samples

1- DIHQ and MTN were kindly supplied by Chemical Industries Development Co (CID), Giza, Egypt. Their purities were found to be 100.01±0.84 and 99.21±1.60, respectively, according to the spectrophotometric method of (CID) company.

2.2.2. Market samples

Paramibe compound® tablet (Batch No 114336W) labeled to contain 250 mg of DIHQ and 250 mg of MTN, is obtained from CID Co., Giza, Egypt.

2.2.3. Reagents

All the chemicals and the solvents used were of analytical grade.
1- Ethanol, hydrochloric acid, NaOH, sulfuric acid all are from (El - Nasr Pharmaceutical Chemicals Co, Abu-Zabaal, Cairo, Egypt).
2- Deionized Water (SEDICO Pharmaceutical Co., 6th October City, Egypt).
3- Methanol and Acetonitrile (E.Merck, Germany).
4-Human plasma obtained from VACSERA (Cairo, Egypt).

2.3. Preparation of standard solutions

1. Stock standard solution of DIHQ and MTN (0.5 mg.mL^{-1}) : weigh accurately 0.05 gram of DIHQ and MTN into two separate 100-mL volumetric flasks, add 50 mL methanol to each flask, stir magnetically for 30 minutes to dissolve then complete the volume to the mark with methanol.
2. Working standard solution(1) (100 µg.mL^{-1}): transfer accurately 20 mL of DIHQ and MTN stock standard solutions respectively into two 100-mL volumetric flasks, then complete the volume to the mark with methanol.
3. Working standard solution (2) (1 µg.mL-1): Transfer accurately 1 mL of working standard solution (1) to a100-mL volumetric flask, then the volume was completed to the mark with methanol.

3. PROCEDURE

3.1. Method development and Optimization

(i) Effect of solvent

Record the fluorescence spectra of 500 ng.mL^{-1} of DIHQ in deionized water , 0.1 M H_2SO_4, 0.1 M HCl, 0.1 MNaOH, methanol, acetonitrile and ethanol at λ_{ex}= 250 nm for all solvents.

(ii) Effect of excitation wavelength

Record the fluorescence spectra of 500 ng.mL^{-1} of DIHQ in water at different excitation wavelengths (230, 240, 246, 248, 250, 252, 254, 256, 258, 260 , 270, 280 and 290 nm).

(iii) Stability of the fluorescence intensity by time

Measure DIHQ 500 ng.mL^{-1} fluorescence intensity at different time intervals for 1 hours, using λ_{ex}= 250 nm and at λ_{em} = 495 nm in water, to study the stability of DIHQ fluorescence intensity by time**.**

3.2. Linearity and construction of calibration curve

Transfer aliquots equivalent to 400 – 900 ng of DIHQ from its working solution (2) into asset of 10-mL volumetric flasks .Complete to the mark using water. The fluorescence intensity was recorded at λ_{em} = 495 nm using λ_{ex}= 250 nm. Construct the calibration curve by plotting the fluorescence intensity /100 at λ_{em} = 495 nm versus the corresponding concentrations and compute the regression equation**.**

3.3. Analysis of laboratory prepared mixtures of DIHQ and MTN by the proposed spectrofluorimetric method.

Into a series of 10-mL volumetric flasks, transfer accurately aliquots equivalent to 400-900 ng of DIHQ from its final working standard solutions (1 µg.mL^{-1}), add from MTN working solutions equivalent to 100 - 900 ng, complete to volume with water . Measure the fluorescence intensity of the prepared solutions was at λ_{em} = 495 nm using λ_{ex}= 250 nm. Calculate the concentrations of DIHQ from its corresponding regression equation.

3.4. Application of the proposed spectrofluorimetric method to pharmaceutical formulation.

Repeat the same procedure under 3.3. Appropriate dilution was made to bring up concentrations of 500 and 800 ng.mL^{-1} of DIHQ. The proposed Spectrofluorimetric method was applied for the determination of DIHQ concentrations.

3.5. Application of the proposed spectrofluorimetric method to spiked human plasma.

Into a series of 10-mL volumetric flasks, spike 1-mL blank (drug free) plasma sample with different concentrations of DIHQ; complete the volume to mark with methanol to provide final concentrations from 400-900 ng of DIHQ. shake vigorously then centrifuge the samples at 3000 rpm for 15 min. transfer 1-mL of the protein free supernatant to a series of 10-mL volumetric flasks then complete to the volume with water and the suggested method was applied as described under linearity .

4. RESULT AND DISCUSSION

DIHQ shows native fluorescence in water. Solutions of DIHQ exhibit their strongest fluorescence at 495 nm when excited at 250 nm.

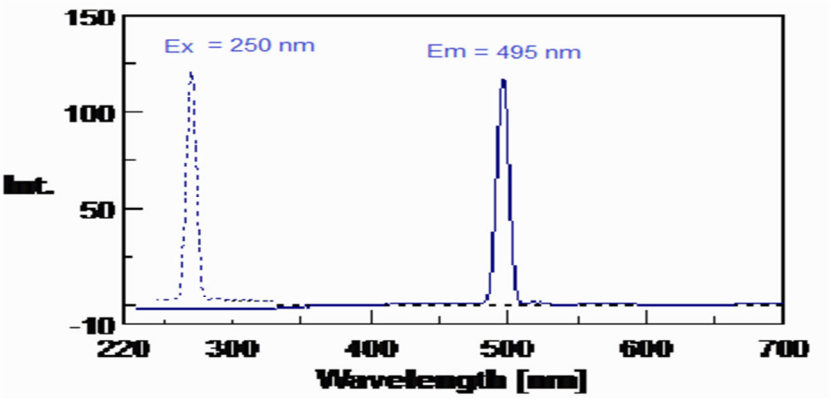

Figure 2. Emission spectrum (λ_{ex}= 495 nm) and excitation spectrum (λ_{em}= 250 nm) of DIHQ in water .

Substitution on quinoline structure by two iodine atoms generally decreases the fluorescence intensity [32]. This is explained by high linearity range of DIHQ from 400 to 900 ng.mL^{-1}.

4.1. Method Development and Optimization

The aim of this work is to develop a sensitive method that can be applied successfully for quantification of DIHQ in pharmaceutical formulation and in spiked human plasma.

The reaction conditions were optimized in order to maximize fluorescence intensity and drug sensitivity. Different factors affecting fluorescence intensity were examined including effect of solvent, use of different excitation wavelengths and effect of time.

Different solvents were used and their effect on the fluorescence intensity of DIHQ was studied. Maximum fluorescence intensity was obtained in water.

The fluorescence intensity was strongly decreased in acetonitrile, 0.1 M H_2SO_4, 0.1 MHCl, methanol and ethanol Figures (3).

Different excitation wavelengths were tested in order to enhance the sensitivity. It was found that excitation at 250 nm gave the maximum intensity. Figure (4)

The effect of time on the stability of fluorescence intensity was studied and DIHQ fluorescence intensity at 495 nm was found to be stable over 1 hour in water solvent, as shown in Figure (5).

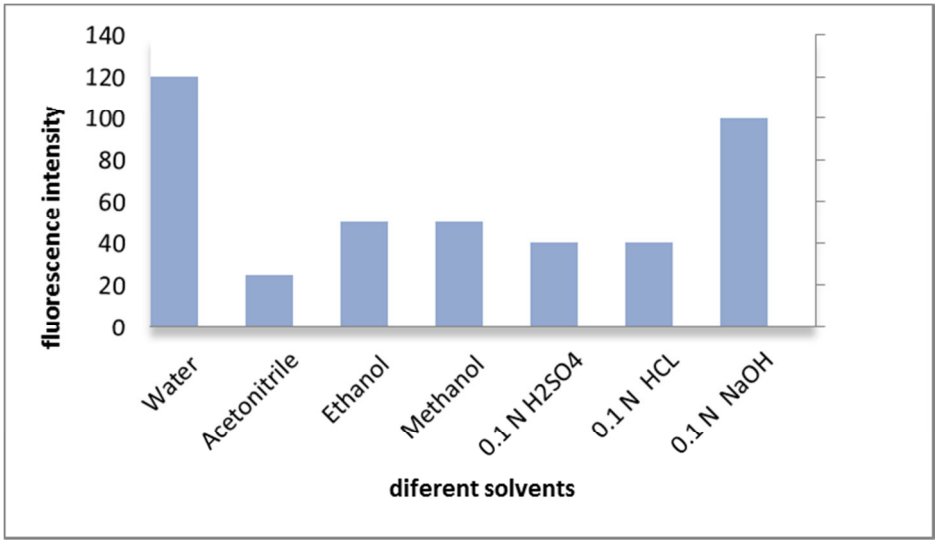

Figure 3. Effect of different solvents on fluorescence intensity of DIHQ (800 ng.mL^{-1}) at λ_{em}=495 nm and λ_{ex}=250 nm.

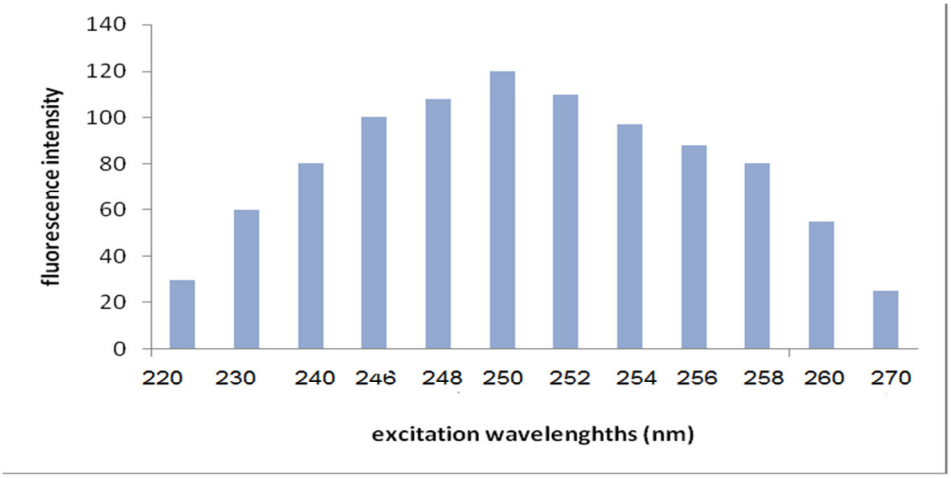

Figure 4. Effect ofdifferent excitation wave length from 220 to 270 nm on fluorescence intensity ofDIHQ (800 ng.mL^{-1}) in water.

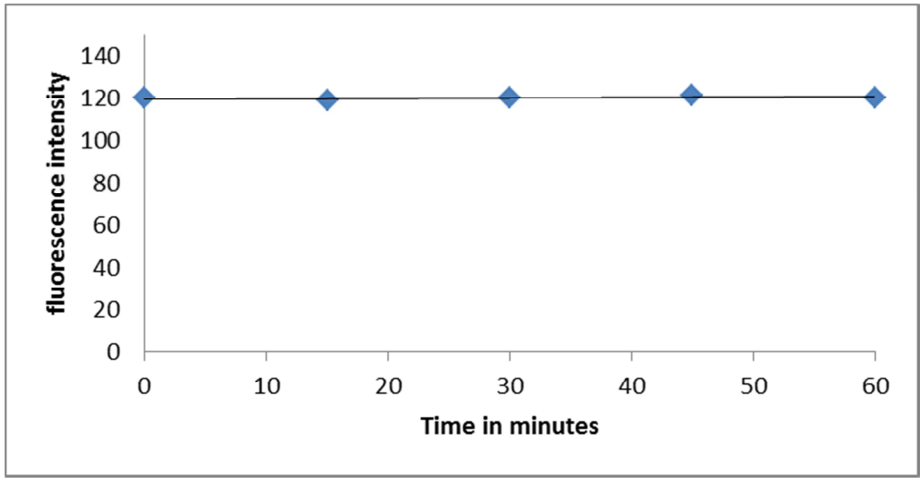

Figure 5.Effect of time on stability of fluorescence intensity of DIHQ (800 ng.mL^{-1}) (when λ_{em} = 495 nm and λ_{ex}= 250 nm) in water.

4.2. Method Validation

Method validation was performed according to the International Conference on Harmonization (ICH) guidelines [33].

Linearity of the proposed method was evaluated and found to be in the concentration range of 400 – 900 ng.mL^{-1} for DIHQ in case of this spectrofluorimetric method by measuring the florescence intensity of DIHQ /100 at λ_{em}= 495 nm with λ_{ex}at 250 nm in without interference of MTN, Figure (5). The high value of the correlation coefficient and the low intercept value indicate the good linearity the good linearity of the proposed method.

The regression equation was computed and found to be:

F= 0.002 C - 0.700 , r = 0.9992

Where F is the fluorescence intensity at λ_{em}= 495 nm while C is the concentration of DIHQ in ng.mL^{-1}, r the correlation coefficient.

Accuracy of the proposed method was checked by its application for determination of different blind samples of DIHQ. The concentrations were calculated from the corresponding regression equations. The results obtained as shown in Table (1).

Table 1. Results of accuracy for determination of **DIHQ** by the proposed spectrofluorimetric method.

Taken (ng.mL^{-1})	Found* (ng.mL^{-1})	Recovery %
400.00	406.00	101.50
500.00	506.00	101.20
600.00	594.00	99.00
700.00	704.00	100.57
800.00	790.00	98.50
900.00	900.20	100.22
Mean ±SD		100.21± 1.13

*Average of three determinations

Specificity of the proposed method was checked by its application for determination of different blind samples of DIHQ in presence of MTN. The concentrations were calculated from the corresponding regression equations. The results obtained as shown in Table (2).

Table 2. Determination of DIHQ in laboratory prepared mixtures by the proposed spectrofluorimetric method.

Mixture ratio DIHQ : MTN**	Concentration ($ng.mL^{-1}$)	Recovery %*
1:1	500 : 500	100.50
2: 1	800 : 400	99.60
1: 2	400 : 800	102.21
3:1	600 : 200	99.33
1: 3	400 : 1200	98.88
4 :1	800 : 200	101.95
Mean ±SD		100.41±1.399

* Average of three determinations
** The ratio of pharmaceutical formulation.

Accuracy of the method was assured by applying the standard addition technique on Paramibe compound® tablets where good recoveries were obtained as shown in Table (3) revealing no interference from both MTN and excipients and good accuracy of the proposed method.

Table 3.Application of standard addition technique to the analysis of DIHQ by spectrofluorimetric method.

Preparation	Claimed taken $ng.mL^{-1}$	Claimed found* $ng.mL^{-1}$	Recovery of DIHQ	Pure added $ng.mL^{-1}$	Purefound* $ng.mL^{-1}$	Recovery of added
Paramibe compound ® Batch No. 114336W	400.00	405.00	101.25 ±1.023	50.00 100.00 150.00	49.00 97.00 148.50	98.00 97.00 99.00
Mean±S.D.						98.00± 1.000
Paramibe compound ® Batch No. 114336W	500.00	494.00	98.80 ±1.244	50.00 100.00 150.00	49.00 99.00 147.50	98.00 99.00 98.30
Mean±SD.						98.43± 0.51

*Average of 3 determinations

Precision of the proposed spectrofluorimetric method was evident as shown in Table (4).

The low value of %RSD shows that the method are robust and that deliberate small changes in the studied factors did not lead to a significant change in fluorescence intensity or wavelength shift as shown in Table (4).

The high sensitivity attained by the proposed spectrofluorimetric method allows the determination of DIHQ in spiked human plasma. The concentrations of DIHQ were calculated from the following regression equation.

$F= 0.243C – 1.650,\ r = 0.999$

The proposed spectrofluorimetric method is valid for the determination of DIHQ in spiked human plasma with mean percentage recovery 100.53±1.417 as shown in Table (5).

Table 4.Results of assay validation parameters of the proposed Spectrofluorimetric method for the determination of DIHQ.

Parameters	Spectrofluorimetric method
Range ($ng.mL^{-1}$)	400 – 900 $ng.mL^{-1}$
Slope	0.002
Intercept	0.700
Correlation coefficient (r)	0.999
Accuracy (mean ± SD)	100.21± 1.129
Repeatability*	0.521
Intermediate precision*	0.876

*The intra-day and inter-day relative standard deviations of the average of concentration 500, 600 and 750 $ng.mL^{-1}$ ofDIHQ

Table 5.Determination of pure DIHQ by the proposed Spectrofluorimetric method in spiked human plasma.

Taken ($ng.mL^{-1}$)	Found* ($ng.mL^{-1}$)	Recovery %**
400.00	408.00	102.00
500.00	492.00	98.40
600.00	610.00	101.67
700.00	708.00	101.14
800.00	794.00	99.25
900.00	906.50	100.72
Mean ±SD		100.53± 1.42

* using equation: F= 0.243C – 1.650

** Average of 3 determinations

5. CONCLUSION

The suggested method provides selective, accurate and sensitive analytical procedure for the determination of DIHQ. The method described proved appropriate for the routine analysis and quality control of DIHQ in presence of MTN either in its pharmaceutical formulation or in spiked human plasma.

Statistical analysis was performed by comparing the results of the proposed method with those of manufacturer method. Results indicate that there is no significant difference observed regarding accuracy and precision, as shown in Table (6).

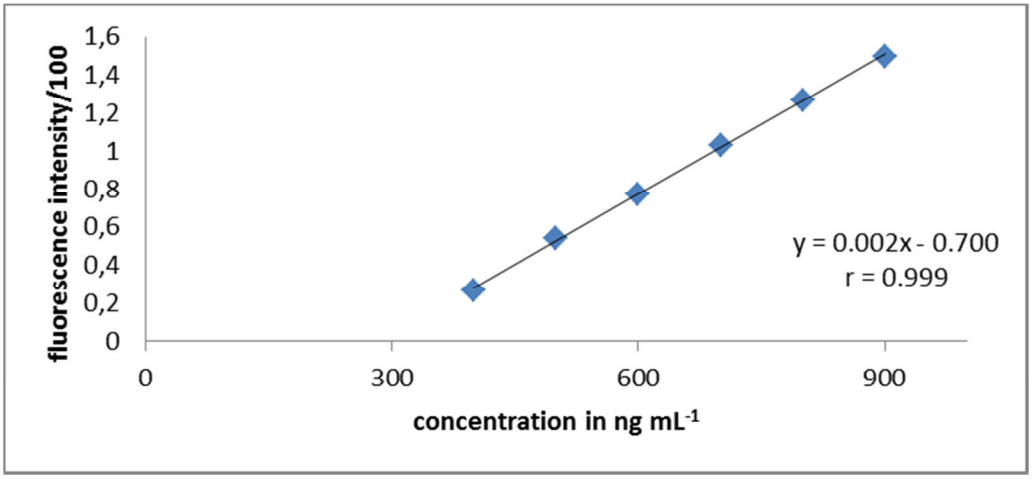

Figure 6. Linearity of fluorescence intensity ofDIHQ to the corresponding concentrations (when λ_{em} = 495 nm and λ_{ex}= 250 nm) in water solvent.

Table 6.Statistical comparison of results obtained by the suggested method and the reference method.

Data	Spectrofluorimetric method	Reference method[a]
Mean %	100.21	100.01
SD	1.13	0.84
N	6	6
Student 's t-test (2.23)	0.349	
F(*5.05*)	2.420	

a-manufactured method personal communications
b- Figures in parenthesis represent the corresponding tabulated values for *t* and *F* at *P*=0.05.

REFERENCES

1. ***Merck Index***: An Encyclopedia of Chemicals, Drugs and Biological 14th Ed., S. Budavari (Ed.), Merck & Co., Inc., Whitehouse Station, NJ(2006).

2. ***Martindale***: The Extra Pharmacopoeia, the Complete Drug Reference 35th Ed., K. Profitt (Ed.), Royal Pharmaceutical Society, London, UK(2006).

3. United States Pharmacopeia "***The National Formulary USP***", United States Pharmacopeia convention Inc (2009).

4. Nagaraja, P, Sunitha, K R, Vasantha RA, Yathirajan H.S., Spectrophotometric determination of metronidazole and tinidazole in pharmaceutical preparations. ***Journal of Pharmaceutical and Biomedical Analysis*** 28(3-4), 527–535 (2002).

5. Erk N, Altun M L N., Spectrophotometric resolution of metronidazole and miconazole nitrate in ovules using ratio spectra derivative spectrophotometry and RP-LC.***Journal of Pharmaceutical and Biomedical Analysis***, 25(1)115-122 (2001).

6. Khashaba PY, Refaat I H, Emara K M and Gaber H M. , Utility of Diazotized 4 - Amino-6-Chloro-1,3-Benzene Disulphonamide for the Spectrophotometric Determination of 8-Hydroxquinolines and Dibenzazepines.***Analytical Letters***, 32(15) 3029-3047 (1999).

7. Shingbal D M, Natekar G B., Determination of diiodohydroxyquinoline in pharmaceutical dosage forms. ***Indian Journal of Pharmaceutical Sciences,*** 42(6)181-183 (1980).

8. Rizk M, Belal F, Ibrahim F, Ahmed S, Sheribah Z A., Derivative UV spectrophotometric analysis of some pharmaceutically important halogenated 8-Hdroxyquinoline derivatives via their Pd (II)-Complexes. ***ScientiaPharmaceutica,*** 68 (3) 297-307 (2000).

9. Bhowal SK, Das TK., Spectrophotometric determination of clioquinol, iodoquinol and broxyquinoline in pharmaceutical preparations after complexation with some bivalent metal ions. ***Indian Journal of Pharmaceutical Sciences***, 52(4) 200-204 (1990).

10. Saha U, Sen A K, Das T K., Spectrophotometric determination of clioquinol and diodoquin in pharmaceutical preparations using uranyl acetate as a

chromogenic agent. ***Analyst,*** 113 (11) , 1653-1655.(1988).

11. Belal F., Spectrophotometric determination of halogenated 8-hydroxyquinoline derivatives. ***Talanta***, 31 (8) 648-650 (1984).

12. Windheuser JJ, Chu DY., Colorimetric determination of iodochlorhydroxyquin and diiodohydroxyquin. ***Journal of Pharmaceutical Sciences***, 56 (4)519-521 (1976).

13. Lu SF, Wu KB, Dang XP, Hu SS., Electrochemical Reduction and Voltammetric Determination of Metronidazole at a Nanomaterial Thin Film Coated Glassy Carbon Electrode. ***Talanta***, 63: 653-657 (2004).

14. Jin WR, Dong Q, Yu DQ, Ye XY, Li W. (2000), Capillary electrophoresis /electrochemical detection system with on-line deoxygenation. ***Electrophoresis,*** 21(8) 1540–1544 (2000).

15. Yu HY, Xia XP, Xu LY, He J, Chen FY., Studies on determination of metronidazole and tinidazole in breast-feeding women. ***YaowuFenxiZazhi***, 22(2) 274–277 (2002).

16. Frasey AM, Boyer-Grand A, Pouget MP, Vennat B, Lartigue C, Galmier MS., Validation of a Reversed-Phase Liquid Chromatographic Method for the Determination of Metronidazole in Transparent Oil-Water-Gel Formulations. ***MicrochimicaActa***, 144(1) 171–176 (2004).

17. Liu G, Zhang QM, Wu W, Zhang J, Jiang R, Tan ST., HPLC Determination of Metronidazole, Chloramphenicol and Hydrocortisone in Nasal Cavities Lotion. ***YaowuFenxiZazhi***, 23(4) 317–319 (2003).

18. Shen JZ, YhangY, Zhang SX, Ding SY, Xiang XH., Determination of Nitroimidazoles and Their Metabolites in Swine Tissues by Liquid Chromatography. ***J. AOAC Int***, 86 (3) 505–509 (2003).

19. Storms M L and Stewart J T., Development of a reversed-phase liquid chromatographic method for the analysis of amoxicillin, metronidazole, and pantoprazole in human plasma using solid-phase extraction. ***Journal of Liquid Chromatography and Related Technologies,*** 25 (16) 2433–2443 (2002).

20. Daeseleire E, De-Ruyck H, Van-Renterghem R., Rapid confirmatory assay for the simultaneous detection of ronidazole, metronidazole and dimetridazole in eggs using liquid chromatography-tandem mass spectrometry. ***Analyst,*** 125(9)1533–1535 (2000).

21. Rizk M, Belal F, Ibrahim F, Ahmed S, Sheribah Z A., LC of pharmaceutically important halogenated 8-hydroxyquinolines after precolumnderivatization with Pd(II).***Journal of Pharmaceutical and Biomedical Analysis***., 27(5) 813-820 (2002).

22.Wojtowicz EJ., Reverse-phase high-performance liquid chromatographic determination of halogenated 8-hydroxyquinoline compounds in pharmaceuticals and bulk drugs. ***Journal of Pharmaceutical Sciences***, 73 (10) 1430-1433 (1984).

23. Argekar AP, Raj SV, Kapadia SU., Simultaneous Determination of Metronidazole and Nalidixic Acid in Pharmaceutical Dosage Forms by HPTLC. ***Indian Drugs***, 33: 167-170.(1996).

24. Wang J H., Determination of three nitroimidazole residues in poultry meat by gas chromatography with nitrogen–phosphorus detection. ***J. Chromatogr. A,*** 918(2) 435–438 (2001).

25. Nejem R M, Issa M M, El-Abadla N S, Al-Kholy M, Saleh A A. (2008), Determination of diiodohydroxyquinoline using UV-visible and atomic absorption spectrometry. ***Asian Journal of Chemistry***, 20 (5) 3849-3856 (2008).

26. Soliman SA., A simple method for the determination of 5,7-diiodoquinolin-8-ol and 5-chloro-7-iodoquinolin-8-ol (clioquinol). ***Analyst***, 100 (1195) 696-702.(1975) .

27. Kavarana H H., Assay of diiodohydroxyquin by non-aqueous titration.***American Journal of Pharmacy and the Sciences Supporting Public Health***, 131 (5) 184-187 (1959).

28. Paranjothy K L K, Banerjee S K., Estimation of diiodohydroxyquin in nonaqueous media. ***Journal of Pharmaceutical Sciences***, 62 (10) 1697-1698 (1973).

29. Chatterjee P K, jain C L, sethi P D., Simultaneous spectrophotometric estimation of di-iodohydroxyquinoline and metronidazole or their analogues derivatives in combined dosage forms***.Indian Journal of Pharmaceutical Sciences,*** 48 (6)195-197 (1986).

30. Lopez-De-Alba P L, Wrobel K, Lopez-Martinez L, Wrobel K, Yepez- Murrieta M L, Amador-Hernandez J., Application of the bivariate spectrophotometric method for the determination of metronidazole, furazolidone and di-iodohydroxyquinoline in pharmaceutical formulations. ***Journal of Pharmaceutical and Biomedical Analysis,*** 16 (2) 349-355 (1997).

31. Parimoo P, Umapathi P, Ravikumar N, Rajasehar S., Determination of metronidazole and di-iodohydroxyquinoline in pharmaceutical preparations by quantitative thin layer chromatography. ***Indian Drugs***, 29(5) 228-230 (1992).

32. John .A,***Analytical Chemistry Handbook*** ,Wiley, New York,NYUSA (2005).

33. ***ICH Harmonized Tripartite Guideline***: Validation of Analytical Procedures: Text and Methodology, Q2 (R1) Geneva (2005).