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Difference Spectrophotometric Method for the Determination of Metronidazole in Tablets

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In this work, a simple, sensitive and rapid spectrophotometric method is proposed for determination of metronidazole. The method is based on the induced spectral changes upon changing the pH of the medium and measured the difference in absorbance at 326 nm. The difference absorbance (ΔA) is linear in the concentration over the range 2.0-40.0 µg/mL (r^2 = 0.999), with a detection limit 0.76 µg/mL. The method was successfully applied to the commercial pharmaceutical drug without interference from common ingredients accompanying the drug. The obtained results are in good agreement with those obtained by official methods.

Key Words: Spectrophotometry, Metronidazole, Tablets, Excipients.

INTRODUCTION

Metronidazole (Fig. 1), 2-methyl-5-nitroimidazole-1-ethanol[1,2] is a broad spectrum antibiotic targeting gram positive bacterial, gram negative bacteria and anaerobic protozoa.

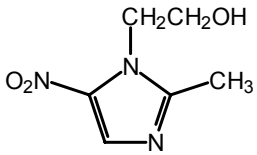

Fig. 1. Chemical structure of metronidazole

Clinically, it is used frequently for treatment of pseudomembranous enterocolit caused by Clostridium difficile. It is also used in combination with other antibiotics for eradication of *Helicobacter pylori* infections. Metronidazole also possesses useful amebicidal activity and is in fact,

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effective against both intestinal and hepatic amebiasis. It is widely used in the treatment of vaginal infections causes by amoeba such as trichomoniasis, giardiasis and gardnerella vaginalis. Metronidazole is also becoming increasingly useful in other antibiotic-resistant infections[3,4].

A survey of literature reveals that there are various methods available for quantitative determination of metronidazole in pharmaceutical dosage forms, human plasma, saliva and gastric juice. These methods use high performance liquid chromatography[5]. Although HPLC methods are sensitive, but they are laborious, expensive and need trained staff.

Colorimetric methods have traditionally been the most widely used for the determination of metronidazole alone or in their combination with other drugs in dosage forms[6-8].

Most of the reported colorimetric methods suffer from the several disadvantages like narrow range of determination and required heating or extraction, long time for the reaction to complete, use of non-aqueous systems, stability of the coloured product formed. First derivative spectrophotometry is used for metronidazole determination in mixture with ciprofloxacin[9]. Simoes *et al.*[10] have used a flow injection analysis for determination of metronidazole based on the spectrometric monitoring of nitrite ions released from an alkaline hydrolysis of the sample.

A voltammetric method has used for the determination of metronidazole in dosage forms. The method is based on the electrochemical reduction of the drug at a glassy carbon electrode[11]. Non-aqueous volumetric titration is recommended by the pharmacopoeia for the direct determination of metronidazole[1]. Although this method is simple, being daily practiced in many routine analysis laboratories, it is time consuming and tedious as it is based on the use of acetic acid.

The selectivity and accuracy of spectrophotometric analysis of samples containing absorbing interferents may be markedly improved by the technique of difference spectrophotometry. The essential feature of a difference specrophotometic assay is that the measured value is the difference absorbance (ΔA) between two equimolar solutions of the analyte in different chemical forms, which exhibit difference spectral characteristics[12]. The simplest and most commonly employed technique for altering the spectral properties of an analyte is the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers. The ultraviolet-visible absorption spectra of many substances containing ionisable functional groups, *e.g.*, phenols, aromatic carboxylic acids and amine are dependent on the state of ionization of the functional groups and consequently on the pH of the solution[12]. Difference spectrophotometric method has used to determine metronidazole in presence of nalidixic acid[13]. In this work, the pH induced difference spectrophotometic method used for determination of the metronidazole drug in tablets.

EXPERIMENTAL

A Jena Model UV-VIS spectrophotometer (Jena, Germany) with 1.0 cm matched quartz cells was used.

Pharmaceutical grade metronidazole was kindly provided by Prof. F. Belal (Faculty of Pharmacy, Mansoura University, Mansoura, Egypt). The drug was used without further purification. Sodium hydroxide and hydrochloric acid (were from Sigma, St. Louis, MO, USA) (0.1 M solution), glacial acetic acid (Panreac Quimica SA, Barcelona, Spain), acetic anhydride (Codex Carlo Erba, Milan. Italy), potassium dihydrogen orthophosphate (Fluka-Garantie, Sigma-Aldrich, Milan, Italy), di-sodium hydrogen phosphate (East Anglia Chemicals), perchloric acid (Riedel-De Haen AG, Sigma-Aldrich Laborchemikalein, Germany), billarent green (Gurr Certistain, England). Double distilled water was always used.

Phosphate buffer pH 7 (0.1 M): The buffer was prepared by dissolving 1.361 g of potassium dihydrogen orthophosphate in sufficient water to produce 100 mL and adjust the pH using a 3.5 % (w/v) solution of disodium hydrogen phosphate[1].

Perchloric acid: (0.1 M solution in glacial acetic acid).

Brilliant green: (1 % w/v) solution in glacial acetic acid).

Commercial dosage forms, Flazol fort (500 mg metronidazole, Asia Pharmaceutical Industries, Syria), Supplin (500 mg metronidazole film coated tablets, Biochemie GmbH, Kundi, Austria), Amrizole (250mg metronidazole tablet, Amriya Pharmaceutical Industries, Alexandria, Egypt), Nidazole (500 mg metronidazole tablet, Alhkma, Jordan), were purchased from local drug stores.

Procedure: Stock solution of metronidazole was prepared by dissolving 12.5 mg of pure metronidazole in 50 mL of water. Working standard solutions with concentration ranging from 2-40 µg/mL in water were prepared by transfer appropriate volumes of stock solution to 25 mL volumetric flasks in duplicate. The volume was then adjusted with 0.1 M HCl and 0.1 M NaOH to give a series of equimolar solutions of metronidazole in different pH medium.

Procedure for the assay of metronidazole in tablet: The average mass of ten tablets was determined and the tablets were grounded in a mortar. An amount of powder (accurately weighed) equivalent to 12.5 mg of metronidazole was transferred into 50 mL volumetric flask and made up to the mark with water. The content of the flask was stirred magnetically for 10 min and then the solution was filtered through Whatmann No. 1 filter paper. The first filtrate was removed then 1 mL of the filtrate was transferred to 25 mL volumetric flask in duplicate. The volume was then adjusted with 0.1 M HCl and 0.1 M NaOH. The absorbance difference

(ΔA) between the acidic solution and equimolar 0.1 M NaOH solution of pure drug and samples were measured at 326 nm using UV-Vis spectrophotometer with two 1.0 cm matched cells, by placing the acidic solution as reference and NaOH solution as sample.

RESULTS AND DISCUSSION

This work describes a simple pH-induced difference spectrophotometric method for the determination of metronidazole in tablets (in presence of tablet excipients).

The absorption spectra of equimolar solutions of metronidazole in 0.1 M hydrochloric acid (pH 1), 0.1 M NaOH (pH 13) and phosphate buffer (pH 7) are shown in Fig. 2.

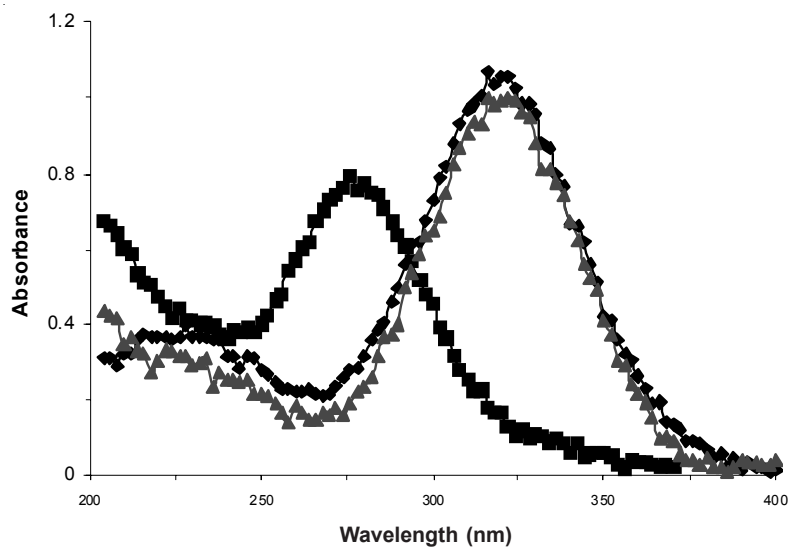

Fig. 2. The absorption spectra of equimolar solution of metronidazole (20 µg/mL) in (◆) 0.1M NaOH, (■) 0.1M HCl and phosphate buffer (pH7) (▲).

Metronidazole is a weak base (having a pK_a of 2.5)[4,14] so its absorption spectra in NaOH solution (pH 13) and buffer solution (pH 7) are identical. The absorption spectrum of metronidazole in acid medium, (Fig. 2), shows hypsochromic shift and hypochromic effect (decrease in the intensity of the absorption)[15,16]. The hypsochromic shift (blue shift) is due to the protonation of the imidazole ring in metronidazole molecule. Hence, the pair of the electrons is no longer available for the conjugated structure which is available in alkaline medium.

Fig. 3 shows the difference absorption spectrum of metronidazole solution. It is generated by measure the absorbance of equimolar metronidazole solution at pH 13 (NaOH solution) in sample cell against the metronidazole at pH 1 (HCl) in the reference (blank) cell.

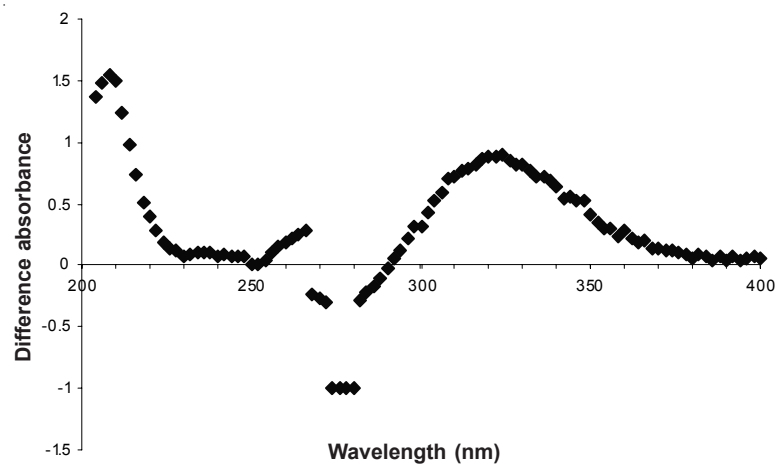

Fig. 3. The difference absorption spectrum of equimolar solution of metronidazole (20 µg/mL) in 0.1 M HCl *vs.* 0.1 M NaOH

At 294 and 380 nm both acidic and alkaline solutions of metronidazole have identical absorbance and consequently exhibit zero difference absorbance. Such wavelengths of equal absorptivity of the two species are called isobestic or iso-absorptivity points[15,16]. Above 294 nm the alkaline solution absorbs more intensely than the acidic solution and the ΔA is therefore positive. Below 294 nm the acidic solution absorbs more intensely than alkaline solution, so the ΔA is negative. The suitable wavelength for quantitative difference spectrophotometric measurements of metronidazole is at 326 nm (λ_{max})[13].

Calibration plot: A plot of difference absorbance (at 326 nm) *vs.* metronidazole concentration was seen to be linear over the concentration range 2.0-40.0 µg/mL ($r^2 = 0.9988$) with a slop of 4.38×10^{-2} and intercept of 1.62×10^{-2} (Fig. 4).

The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be detected. The LOD was found to be 0.76 µg/mL, according to the 3 s/m definition[17], where s is the standard deviation (n = 5) of the signal from 5 µg/mL metronidazole aliquots and m is the slop of the calibration graph.

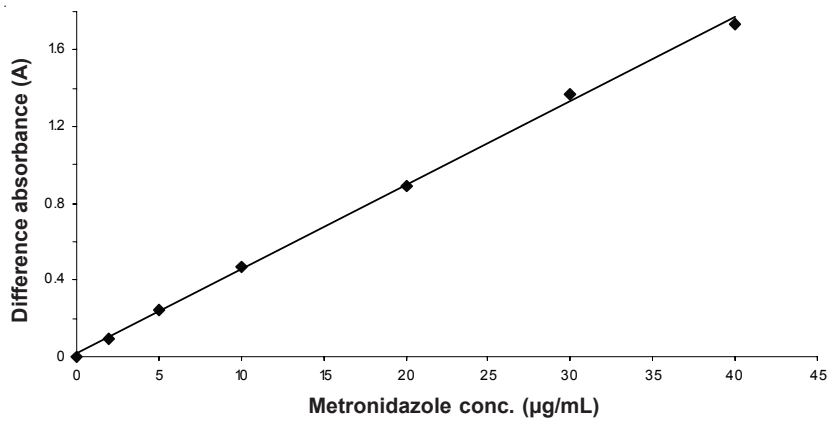

Fig. 4. The difference absorption calibration curve of metronidazole in 0.1 M NaOH and 0.1 M HCl. The linear regression equation is: Y= 0.0438X + 0.0162 (r^2 = 0.9988)

Interference studies: The effect of foreign substances, inactive excipient materials, that commonly accompanying the drug in pharmaceutical formulation, such as tablets, (starch, mannitol, cellulose, polyvinylpyrrolidone, calcium phosphate, magnesium stearate, hydroxypropyl-methyl cellulose, polyethylene glycol, titanium dioxide) was studied by comparison the absorption spectra of metronidazole in standard solution and in solution of some tablets extract (for example: supplin and nidazole tablets). The obtained absorption spectra are identical. Fig. 5, confirmed that tablet excipients have no interference effect on the measurements of the ΔA values at λ_{max}.

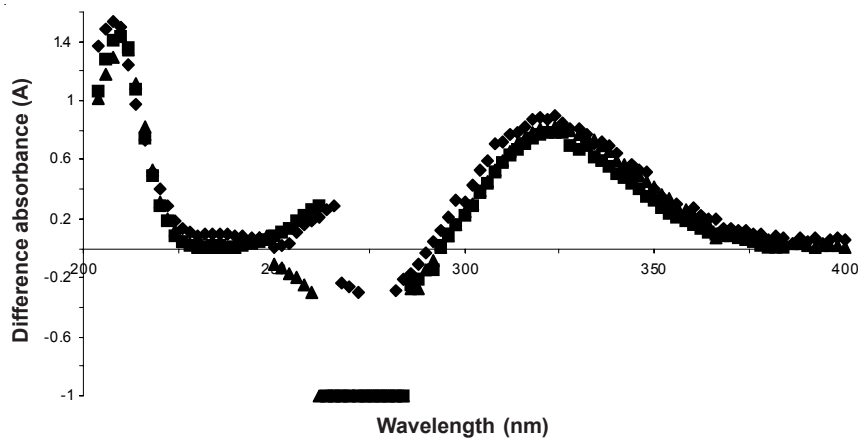

Fig. 5. The difference absorption spectra of equimolar solution of metronidazole (20 µg/mL) in 0.1 M HCl *vs.* 0.1 M NaOH, using standard solution (◆), nidazole (■) and Supplin solution (▲)

Analysis of commercial tablets: Difference spectrophotometric method was applied to four brands of metronidazole tablets well known in the market. The results of analysis are reported in Table-1. The reproducibility of the method was checked by five replicate determinations and then the relative standard deviation (RSD) was calculated. The results obtained were compared with those obtained from a reference method (non-aqueous titration)[1]. The precision and accuracy of the method was further compared statistically using Student's t-test and variance ratio test at the 95 % confidence level, the calculated t-values and F-values do not exceed the tabulated values (2.78 and 9.12, respectively). So there is no significant difference between the two methods regarding accuracy and precision.

TABLE-1
DETERMINATION OF METRONIDAZOLE IN COMMERCIAL TABLETS USING THE PROPOSED PROCEDURE AND NON-AQUEOUS TITRATION

	Difference spectrophotometric method			
	Supplin (500 mg)	Frazol Fort (500 mg)	Amrizole (250 mg)	Nidazole (500 mg)
Mean[a] ± SD	504.16 ± 9.87	502.40± 9.13	245.26 ± 4.63	503.60 ± 8.82
RSD[b]	1.960	1.820	1.890	1.750
Recovery (%)[c]	101.800	100.400	99.000	101.600
t-test[e]	2.035	0.433	-1.153	2.060
F-test[e]	0.756	0.491	0.361	0.767
	Non-aqueous Titration method[d]			
	Supplin (500 mg)	Frazol Fort (500 mg)	Amrizole (250 mg)	Nidazole (500 mg)
Mean[a] ± SD	495.18 ± 11.5	500.63 ± 5.85	247.65 ± 7.62	495.48 ± 7.19
RSD[b]	2.31	1.17	3.08	1.45

[a]Mean of five values.
[b]R.S.D., relative standard deviation.
[c]Recovery (%)= [Amount found by difference spectrophotometric method/ Amount found by official method] × 100.
[d]Tablets Analyzed by non-aqueous titration [B.P., 1998].
[e]t-test and F-test statistical analyses were performed using SPSS10 programme.

Conclusion

The method is found to be simple, economical, selective and sensitive. The statistical parameters clearly indicate the reproducibility and accuracy of the method. Analysis of the metronidazole in its dosage forms showed no interference from the common excipients and additives. Difference spectrophotomerty by inducing pH of the medium may be recommended for routine and quality control analysis of the investigated drug in tablets.

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