Pakistan Journal of Pharmaceutical Research
Faculty of Pharmacy, Bahauddin Zakariya University Multan, Pakistan
ISSN: 2410-6275 January, 2016 Vol: 02 Issue: 01: PP 28-32

Research Article

Development and application of spectrophotometric method for quantitative determination of Metronidazole in pure and tablet formulations

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Received: Oct 7, 2015
Revised: Dec 30, 2015
Accepted: Jan 11, 2016
Online: Jan 27, 2016

ABSTRACT

A rapid, simple and sensitive spectrophotometric method has been developed for the determination of metronidazole in pharmaceutical pure and dosage forms. The method depends on alkaline hydrolysis of metronidazole releases the nitro group as nitrite ion and yielded nitrite ions can be used to give a colored complex that absorbs maximally at 505 nm. Beer's law is obeyed in the concentration ranges 9-100 g/ml with molar absorptivity of 1.14 ×10^3 L mole^{-1} cm^{-1}. The proposed method is precise, accurate and specific for the quantitative determination of drug in bulk and dosage forms. The results of analysis of commercial formulations and the recovery study of metronidazole suggested that there is no interference from any excipients, which are present in pharmaceutical formulations of metronidazole. Statistical comparison of the results was performed with regard to accuracy and precision using student's t-test and F-ratio at 95% confidence level. There is no significant difference between the reported and proposed methods with regard to accuracy and precision.

Keywords: Metronidazole; Spctrophptometric; Pharmaceutical analysis; Alkaline hydrolysis;

INTRODUCTION:

Metronidazole 2-(2-methyl-nitroimidazol-1-yl) ethanol, is a substance that has a wide range of uses due to its activity against protozoa and anaerobic bacteria, largely used (Simões *et al*., 2006) as the active ingredient of anthelmintic (Edwards, 1993). Thus, there is an important demand for rapid and simple method for determination of metronidazole. Several methods have been reported for determination of metronidazole which includes titrimetry, polarographic (Sankar and Reddy, 1990) high performance chromatography.

(Sun *et al*., 2007, Al Shaalan, 2007, Tavakoli *et al*., 2007, Tao *et al*., 2006), voltammetric (Rege and Sathe, 2011), derivative spectrophotometry, and flow injection analysis(Simões *et al*., 2006). Most of the spectrophotometric methods reported suffer from the disadvantages, like narrow range of determination requires heating or extraction, long time for the reaction to complete, use of non-aqueous systems, stability of the colored product formed etc.

This paper reports a simple and fast method for the determination of metronidazole in pure bulk and pharmaceutical dosage form by ultraviolet visible spectrophotometry providing accurate and precise results. This method is based upon simple release of nitrite ions in sodium hydroxide solution, used to determine metronidazole.

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EXPERIMENTAL

Apparatus

All spectrophotometric measurements were carried out using a spectrophotometer (U 1100

Hitachi, Japan) with silica glass cell of 1 cm thickness. Officially calibrated Pyrex glassware was used throughout this study.

Reagent and standard solution

All chemicals used were of analytical grade. Metronidazole was supplied by Lahore Chemical and Pharmaceutical Works, Lahore, Pakistan. Metronidazole tablets were purchased from a local market. Sodium hydroxide was purchased from BDH, England. 0.1 N sodium hydroxide solution was prepared and standardized. Metronidazole standard stock solution was prepared by dissolving 100 mg in 0.1 N sodium hydroxide.

Procedure for pure drug

Aliquots of 9-100 g/ ml of the Metronidazole stock solution were pipetted into a series of 10 ml standard volumetric flasks. Then, the reaction mixtures were heated at 30±5 C in water bath in for 1 minute. The volume was made up to the mark with 0.1 N sodium hydroxide solution. The absorbance was measured within the stability period of 1 hour after dilution at 505 nm against a reagent blank

Procedure for dosage forms

An accurately weighed portion of powdered tablet equivalent to 100 mg of metronidazole was stirred well with 20 ml 0.1 N sodium hydroxide and left standing for five minutes. The residue was filtered on Whatman filter No. 42 paper and washed with 0.1 N sodium hydroxide. The filtrate and washings were diluted to the volume in 50 ml measuring flask with 0.1 N sodium hydroxide. The procedure reported under "Construction of calibration curve" was followed for the determination of drug content.

RESULTS AND DISCUSSION

Determination of absorption maximum

Drug when treated with 0.1 N sodium hydroxide, develops color, which absorbs maximally at 505 nm. The absorption spectrum of product against blank is shown in Figure 1.

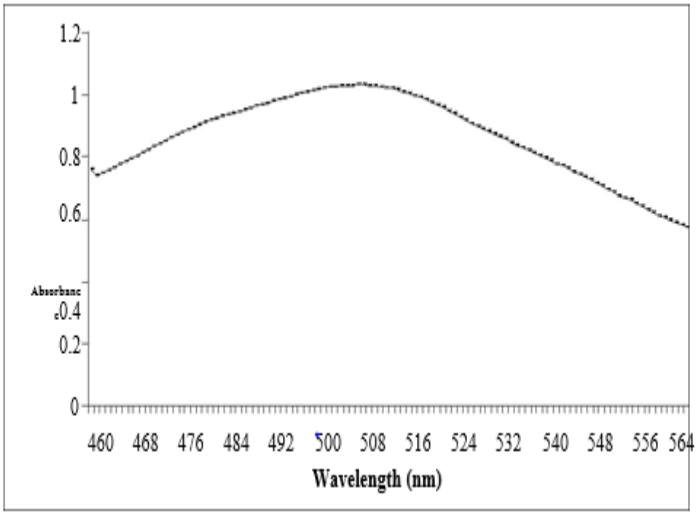

Figure 1: Absorption spectrum of metronidazole

Reaction sequence

The alkaline hydrolysis of metronidazole releases the nitro group as nitrite ion and the reaction can be carried out in a quantitative way by controlling the temperature. Thus, a simple measurement of the yielded nitrite ions can be used to determine metronidazole(Sanyal, 1987, Lau *et al.*, 1969).

Interference studies

To study the potential interference from the commonly used excipients and other additives such as microcrystalline cellulose, magnesium stearate, hypermellose, titanium dioxide, starch, recovery studies were carried out. Under the experimental conditions employed, to a known amount of drug (Metronidazole 20 g/ml), excipients in different concentrations were added and analyzed. Results of recovery analysis are present in Table 1. Excipients at concentration shown in Table 1 do not interfere with assay.

Table 1: Determination of metronidazole in the presence of excipients

No.	Excipients	Amount taken (g/ml)	Rcovery (%) ± RSD (n =5)
1	Talc	50	99.64±0.82
2	Microcrystalline cellulose	300	101.32±0.74
3	Sodium starch glycolate	100	100.7±0.25
4	Lactose	300	100.2±0.52
5	Magnesium stearate	200	99.58±0.56
6	Hypermellose	100	101.5±0.75
7	Titanium dioxide	80	99.1±0.41

20 g /ml of Metronidazole was taken for interference studiesIn additions recoveries in most cases were around 100% and lower relative standard deviation (RSD) values indicates the good precision of the reference method.

Optical Characteristics and validation of the method

Optical Characteristics for metronidazole, such as Beer's law limits, molar absorptivity and Sandell's sensitivity are given in Table 2. The results compared with reference method are shown in Table 3(Edwards, 1993). Statistical comparison of the result was performed with regard to accuracy and precision using t-test and F-ratio at 95% confidence level is shown in Table 3. There is no significant difference between the reported and proposed method with regard to accuracy and precision. The calibration curve is linear over range of 9-100 g/ml as shown in Figure 2. A comparison of performance of the proposed method with already reported spectrophotometric method is given in Table 4.

Table 2: Optical characteristics and statistical data for the regression equation of the proposed method.

Parameter	Value
λ_{max} (nm)	505
Stability of color (minutes)	60
Beer's law limit (μg/ml)	9-100
Sandell's sensitivity (μg/ml per 0.001 A)	1.55×10^{-1}
Molar absorptivity (L mol^{-1} cm^{-1})	1.15×10^{3}
Regression equation (Y^{*})	
Slope(b)	0.0017
Intercept (a)	0.0909
Correlation coefficient (r)	0.9978
RSD^{**}	0.868

.Y^{*} = a+bC

Table 3: Determination of metronidazole formulations by the proposed and reference methjod.

Brand Name	Label claim	% found* ± SD Proposed method	Reference method	t-test	F-test
Metrogyl[a]	200 mg	100.1±3.2	99.98±2.1	1.85	3.11
	400 mg	101.5±2.5	100.9±1.8	1.61	2.17
Metason[b]	200 mg	99.6±1.8	99.6±2.1	1.11	4.10
	400 mg	100.8±2.3	100.2±3.2	1.72	1.13
Meronide[c]	200 mg	100.4±2.3	101. ±1.9	2.40	3.25
	400 mg	101.2±1.7	100.8±2.1	0.50	1.48

* Average of five determinations.

[a] Unexolabs (Pvt) Ltd,Lahore, [b] Life line pharmaceuticals, Lahore [c] Irza pharma (Pvt) Ltd.,Lahore.

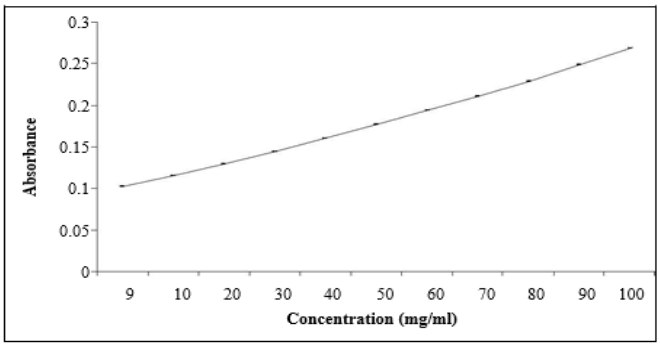

Figure 2: Calibration curve of metronidazole

Table 4: Comparison of performance of the proposed method with reported spectrophotometric methods.

Sr. #	Reagent Used	λ_{man} (nm)	Limitations	References
1	β-Naphthol	510	Time consuming process involving reduction and derivitization	(Saffaj et al., 2006)
2	Bromocresol Green	654	Involves extraction with chloroform and buffer pH 9.5	(Amin, 1997)
3	Bromocresol Purpule	618	Involves extraction with chloroform and buffer pH 10.0	(Amin, 1997)
4	8-Quinolinol	500	Costly reagent	(Saffaj et al., 2004)
5	Metol and Potassium dichromate	502	Involves reduction, use of buffer pH 2.9 and less stable due to pH dependent	(Sastry et al., 1988)
6	p-dimethyl amino cinnamaldehyde	510	Involves reduction with Zn-HCl and low sensitivity	(Moussa, 1982)
7	MBTH	500	Involves reduction with Zn-HCl and costly reagent	(Nagaraja et al., 2002)
8	NEDA	520	Involves reduction with Zn-HCl and low sensitivity	(Nagaraja et al., 2002)
9	NQS	510	Costly reagent	(Dinesh et al., 2004)
10	NaOH	505	Highly sensitive, time and cost effective	Proposed Method

CONCLUSION

It may be concluded that developed spectrophotometric method for the determination of metronidazole is reliable, simple, sensitive, accurate, rapid and economical. The results are good agreement with reference method. The proposed method can be applied in quality control laboratories for routine analysis of metronidazole.

REFERENCES

Al shaalan n (2007). Determination of diloxanide furoate and metronidazole in binary mixture using first derivative of the ratio-spectra and high-performance liquid chromatography-uv methods. *J appl sci.*, 4**:** 66-72.

Amin as (1997). Quantitative determination of some pharmaceutical veterinary formulations using bromocresol purple and bromocresol green. *Analyt. Lett.*, 30**:** 2503-2513.

Dinesh nd, nagaraja p and rangappa ks. (2004). A sensitive spectrophotometric assay for tinidazole and metronidazole using a pd-c and formic acid reduction system. *Turkish j. Chem.*, 28**:** 335-343.

Edwards di (1993). Nitroimidazole drugs-action and resistance mechanisms i. Mechanism of action. *J. Antimicrob. Chemother.*, 31**:** 9-20.

Lau ep, yao c, lewis m, and senkowski b (1969). Colorimetric determination of some n-1-substituted nitroimidazoles. *J. Pharm. Sci.*, 58**:** 55-57.

Moussa ba (1982). Colorimetric determination of parasiticides hycanthone and metronidazole. *Int. J. Pharm.*, 10**:** 199-207.

Nagaraja p, sunitha k, vasantha r and yathirajan h (2002). Spectrophotometric determination of metronidazole and tinidazole in pharmaceutical preparations. *J. Pharm. Biomed. Anal.*, 28**:** 527-535.

Rege p and sathe p (2011). Simultaneous quantification of ofloxacin and ornidazole in combined drug formulation by a simple electro analytical technique. *Int. J. Pharm. Sci. Res.*, 2: 2226-31.

saffaj t, charrouf m, abourriche a, abboud y, bennamara a and berrada m (2004). Spectrophotometric determination of metronidazole and secnidazole in pharmaceutical preparations. *Il. Farmaco.*, 59: 843-846.

saffaj t, charrouf m, abourriche a, aboud y, bennamara a and berrada m (2006). Spectrophotometric determination of metronidazole and secnidazole in pharmaceutical preparations based on the formation of dyes. *Dyes pig.*, 70**:** 259-262.

Sankar ps and reddy sj (1990). Polarographic determination of metronidazole. *Asian j. Chem.*, 2**:** 245.

Sanyal a (1987). Rapid and selective method for quantitation of metronidazole in pharmaceuticals. *J. Assoc. Off. Anal. Chem.*, 71**:** 849-851.

sastry c, aruna m and rao arm (1988). Spectrophotometric determination of some antiamoebic

and anthelmintic drugs with metol and chromium (vi). *Talanta.*, 35**:** 23-26.

Simões ss, medeiros ep, gaião en, lyra ws, moreira pn, araújo mc, silva ec and nascimento vb (2006). Flow injection determination of metronidazole through spectrophotometric measurement of the nitrite ion produced upon alkaline hydrolysis. *J. Brazilian chem. Soc.* 17**:** 609-613.

Sun hw, wang fc and ai lf (2007). Simultaneous determination of seven nitroimidazole residues in meat by using hplc-uv detection with solid-phase extraction. *J. Chromatogr. B.*, 857**:** 296-300.

Tao d, jinzhong x, chongyu s, jiang y, huilan c, bin w, zengyun z, gonghai l., zhang j and fei l (2006). Determination of three nitroimidazole residues in royal jelly by high performance liquid chromatography-tandem mass spectrometry. *Chinese j. Chromatogr.*, 24: 331-334.

Tavakoli n, varshosaz j, dorkoosh f and zargarzadeh mr (2007). Development and validation of a simple hplc method for simultaneous in vitro determination of amoxicillin and metronidazole at single wavelength. *J. Pharm. Biomed. Anal.,* 43**:** 325-329.