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SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF METRONIDAZOLE IN PHARMACEUTICAL PURE AND DOSAGE FORMS

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ABSTRACT

Two simple, precies, rapid, sensitive and accurate Spectrophotometric methods have been developed for the estimation of metronidazole either in pure form or in tablet dosage forms. The proposed methods are based on the reduction of metronidazole was carried out with Zinc powder and 5N HCl at room temperature in methonal.The resulting amine was used to two methods. Method A is based on oxidation Coupling with 1,10- Phenanthrolin to form Orange red colored chromogen exhibiting absorption maxima at 510 nm with apparent molar absorptivity of $2.32x10^3(Lm^{-1}cm^{-1})$ and obeyed beer's law in the concentration range 5-55 µg/ml.Method B is based on diazotization and coupling reaction with $NaNo_2$ and 4-chloro3-nitro Aniline to form Yellow colored chromogene exhibiting absorbance maximum at 480 nm with apparent molar absorptivity of $2.71x10^3(Lm^{-1}cm^{-1})$ and obeyed beer's law in the concentration range of 5-60 µg/ml.The assay of results was found to be in good agreement with label claim. The proposed methods were Simple, Sensitive, Precise, Accurate, quick and useful for routine quality control.

Key Words: Spectrophotometry, Metronidazole, 1, 10- Phenanthrolin, Ferric Chloride, Sodium Nitrite, 4-Chloro3-Nitro Aniline, Zinc powder

INTRODUCTION

5-Nitroimadazoles Such as Metronidazole are extensively used as antiamoebic, antiprotozoal and antibacterial drugs. The discovery of the antibacterial and antitrichomonal properties of the antibiotic azomycin led to the investigation of nitroimidazoles as antiparasitic agents[1, 2] .Nitroimidazole derivatives Presents biological activity against anaerobic micro-organisms, being largely used as active ingredient of antihelminthic medicine[3]. The discovery of the antitrichromal properties of metronidazole revolutionized the treatment of disease. The properties of metronidazole were studied; it was not clinically tested until some years later. In laboratory tests, Metronidazole is effective against intestinal amoebiasis in rats and hepatic amoebiasis in hamsters and also active against Entamoeba histolytica in vitro[4].The initial clinical tests of metronidazole indicated that it was capable of Curing invasive amoebic dysentery and amoebic liver abscess[5]. Subsequent clinical tests have established metronidazole as the drug of choice in the treatment of all forms of amoebiasis in humans[6, 7].

Metronidazole is officially determined by titrimetry, potentiometry and HPLC methods. Indian Pharmacopoeia[8] describes the non-aqueous titration method using Perchloric acid as titrant and malachite green as indicator for the assay of metronidazole. British Pharmacopoeia[9] describes potentiometric and non-aqueous methods using perchloric acid as titrant.United states Pharmacopoeia [10] describes HPLC and non aqueous titration methods for the assay of metronidazole. Several methods have been reported for the determination of metronidazole including Spectrophotometry[11, 12, 13], Polarograpy[14]. Most of Spectrophotometric methods found in the literature for the determination of metronidazole in the visible region involve initial reduction by treatment with Zn Powder and HCl[15,22] followed by the diazotization and coupling of the resulting amine.

All these methods are less sensitive[21] involve tedious Procedures such as heating and extraction[19, 22, 23] utilize costly reagents.

In present Study, two Spectrophotometric methods for the quantitative estimation of metronidazole have been developed after converting it to its reduced form by using Zn powder and HCl as well as the reaction of its reduced Product with 1, 10- Phenanthrolin and 4-Choloro3-Nitro Aniline followed by diazotisation was studied to establish the optimum reaction Conditions, optical characterictics, Precision and accuracy of the Proposed methods.

EXPERMENTAL

Instrumentation:

Shimadzu UV – Visible double beam Spectrophotometer (model 2450) with 1cm matched quartz cells were used for all the spectral measurements. All the chemicals used were of A.R.grade.

Chemicals and Reagents:

Methanol, 1, 10- Phenanthrolin, Ferric Chloride, Phosphoric acid, HCl, $NaNo_2$ is obtained from Sigma Aldrich and SD fine chemicals, India. Sample of drug and Internal Standard were obtained from J.B Chemicals&Pharamaceutical Ltd Mumbai, India respectively. Double distilled Water was used to prepare all Solutions for the method.

Reduction of Nitro group and Preparation of the Standard Solution:

About 100 mg of metronidazole Pure or equivalent tablet powder was accurately Weighed and dissolved in 20 ml of Methanol. The methonolic Solution metronidazole was treated with 10 ml of 5N HCl and 0.5 gr of Zn powder was added in portions While Shaking. After Standing for 1 hour at room temperature, the Solution Was filtered using a Whattman filter paper 41 to remove the insoluble matter. The residue was washed with 10 ml portions of Methanol three times and the total Volume of the filtrate was made upto 100 ml with methanol. The final Working Standard Solution of reduced mentronidazole containing 1mg/ml was prepared by using further dilutions.

General Procedure:

Method A

Aliquots of solution 0.5 to 5.5 ml (5-55µg/ml) were transferred in to a Series of 10 ml graduated tubes 1.8 ml of 0.2% Ferric Chloride and 1 ml of 0.2% 1,10- Phenanthrolin are added in each tube and made up to volume with distilled water. The absorbance of the orange red colored chromogen was measured at 510 nm against reagent blank. The amount of metronidazole Present in the sample Solution was computed from its calibration curve.

Method B

Aliquots of metronidazole ranging from 0.5 to 6 ml (5-60µg/ml) were transferred in to a series of 10 ml graduated tubes, to each 1ml of HCl (0.1N) and 0.5 ml $NaNo_2$ (1%) were added at room temperature .After 5 min 1.5 ml of 4-chloro3-nitro Aniline was added. The volumes were made up to the mark with distilled Water. The absorbance of yellow colored chromogen was measured at 480 nm against a reagent blank .The amount of the drug in a sample was calculated from the calibration graph shown in Fig.1.

RESULTS AND DISCUSSION

The optical characteristics such as beer's law limits, molar absorptivity, sand ell's sensitivity, the regression analysis was made for the slope, intercept correlation & the results are in Table.1.

The Proposed methods for the determination of Metronidazole were simple, accurate, linear, precise and reproducible. Hence the methods can be used for routine analysis of metronidazole in bulk and solid dosage form.

Application:

Application of the proposed method to the determination of Metronidazole drug in its dosage form was successfully made, the results are presented in Table. 2. The excellent recoveries obtained indicated the absence of any interference from the excipients

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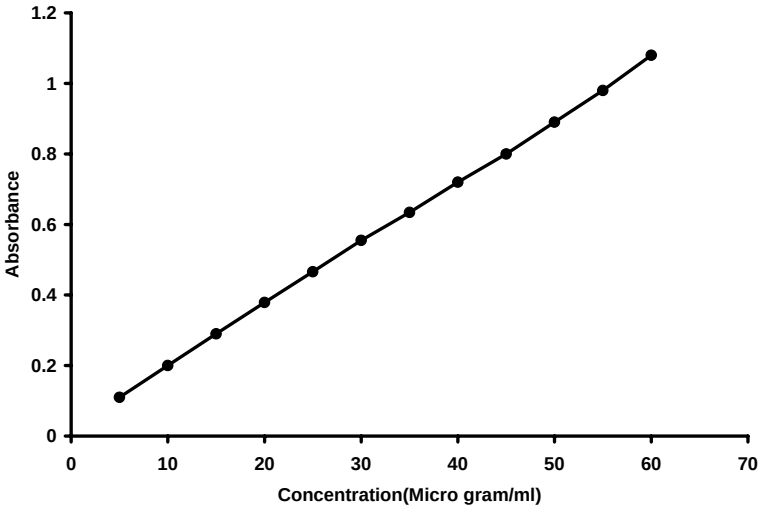

Fig-1: Calibration curve of metronidazole (Method B)

Table-1:.Optical Characteristics and Validation data Spectrophotometric methods

Parameters	Method A	Method B
λmax nm	510	480
Beer's Law limit (μg ml^{-1})	5-55	5-60
Molar absorptivity (L $m^{-1}cm^{-1}$)	2.32X 10^3	2.71X10^3
Specific absorptivity	0.0135	0.0158
Sand ell's sensitivity (μg ml^{-1})	0.0740	0.0632
Regression equation **(Y=bX+C)		
Slope (b)	0.0228	0.0174
Intercept (C)	0.0219	0.0275
Correlation coefficient (r)	0.9996	0.9997
% Relative Standard Deviation (R.S.D)*	1.93	2.50
Colour	Orange Red	Yellow

*Average of Five determination
**Y=bX+C, Where Y is the absorbance and X is the concentration of the drug μg ml^{-1}

Table- 2: Determination of Metronidazole in dosage form.

Pharmaceutical formulations	Labeled amount (mg)	Amount found in mg* by		Percent recovery	
		Method A (mg) mean ± SD	Method B (mg) mean ± SD	Method A	Method B
Metrozyl	200	198 ± 0.63	199 ± 0.34	99.15	99.54

*Mean of five determinations.

REFERENCES

1. M.E.Wol, John Wiley and Sons, "Text book of Burgers Medicinal Chemistry", fourth ed., New York, 979.

2. C.Coser, L.Julon, *Ann.inst.Pasteur*.**96**, 238(1958)
3. D.I.Edwards., *J.antimicrob.chemother*, **31**, 9(1993)
4. C.Cosar, C.Cusan, R.Horclosis, R.M.Jacob, J.Robert, J.STchelitche, R.Vapure, *Arzneim –Forsch*, **16**, 23. (1966)
5. S.J.Powell, I. McLeod, A.J. Wilmot, R. Elsdon- Dew, *Lancet*, **2**, 1329. (1969)
6. S.J.Powell, Bull. N.Y. *Acad.Med* ,**47**, 469(1971)
7. R.B.Khambatta, *Ann.Trop. Med. Parasitol*, **62**,139(1968)
8. Indian Pharmacopoeia, Controller of Publications, New Delhi, **2**, 764. (1996)
9. British Pharmacopoeia, Her Majesty's Stationery Office, London, **2 ,1257** (2003)
10. The United States Pharmacopoeial Convention, Rockville, MD, The United States Pharmacopoeia 24th edn. The National Formulary ,**19** , 1104 (2000)
11. P. D. Panzade, K. R. Mahadlik, *East Pharm*, **43** ,115 (2000)
12. P. Nagaraja, K. R. Sunitha, R. A. Vasantha, H. S. Yathirajan, *J. Pharm. Biomed. Anal*, **28** ,527 (2002)
13. T. Saffaj, A. Charrouf, Y. Abourriche, A. abboud, M. B. Bennamara, *Formaco*, **59,** 843 (2004)
14. D. M. Joshi, A. P. Joshi, *J. Indian. Chem. Soc*,**74**, 585 (1997)
15. B. A. Moussa, *Int. J. Pharm*, **10** ,199 (1982)
16. T.P. Gandhi, P. R. Patel, V. C. Patel, S. K. Patel, *J. Inst. Chem*,**56** ,127 (1984)
17. C.S.P. Sastry, M. Aruna, A. R. M. Rao, *Talanta*, **35**, 23 (1988)
18. C.S.P. Sastry, M. Aruna, A. R. M. Rao, A. S. R. P. Tipirneni, *Chem. Anal*, **36**, 153 (1991)
19. A. S.Amin, *Anal. Lett*, **30**, 2503(1997)
20. P. Nagaraja, K. R. Sunitha, R. A. Vasantha, H. S. Yathirajan, *J. Pharm. Biomed. Anal*, **28** ,527 (2002)
21. N. D. Dinesh, P. Nagaraj, K. S. Rangappa, *Turk. J. Chem*, **28**, 335 (2004)
22. A. Rehaman, A. S. Ijaz, A. Raza, *J. Iran. Chem. Soc*, **2**, 197 (2005)
23. F. Bluemenhal, *Biochem.Z*, **28**, 1321 (1956)
24. W. Frieber, *Centr. Baktenol. Parasitenk*, **87** ,254 (1922)
25. *Chem. Rev*, **26**, 324 (1940)
26. R. Wasicky, *Z. Anal. Chem*, **54** (1915)
27. Raymond-Hamlet, *Bull. Sci. Pharmacol*, **33**, 447 (1926)
28. A. E. A. Werner, *Sci. Proc, Roy. Dulbin Soc,* **23**, 214 (1944)
29. K. Kapse, G. Prabhakar, S. Appala raju, *Indian. J. Pharm. Sci*, **3**, 403 (2006)
30. B. H. Ahmed, O.O. Johnson, *J. Pharmacy and Bioresources*, **1**,1(2004)
31. B. H. M. Mruthyunjayaswamy, S. M. Malipatil, S. Appala Raju, *J. Indian. Chem. Soc* ,**18**, 60 (2001)

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