Spectrophotometric method for determination of metronidazole in pure and tablet dosage forms via Schiff's base formation by coupling with crotonaldehyde

Aminu Musa[1], Auwal A. Bala[2], Ibrahim Nasir[3], Vivian Mamman[1], and Manasse Sallau[1].
[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. [2]Department of Pharmacology, College of Medical and Health Sciences, Federal University Dutse, Nigeria.[3]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Corresponding author: Aminu Musa
Email: pharmamin@gmail.com, Phone: +2348032872718

ABSTRACT

Background: UV-visible spectrophotometric methods are popular because of their simplicity, accuracy, precision and low cost.

Objectives: In this study a new simple, accurate, economical and reproducible spectrophotometric method for the quantitative determination of metronidazole was developed and validated.

Methods: The method is based on the reduction of the nitro group in metronidazole with zinc powder under acidic condition (5N HCl) at 27°C in methanol for 15 minutes followed by coupling the resulting amine with crotonaldehyde to form a yellow chromogen with a λmax of 408nm. The proposed method was validated according to international conference on harmonization (ICH) guidelines and used to assay a sample of standard metronidazole powder and four different brands (A, B, C and D) of metronidazole tablets.

Results: The assay results were validated by comparing with British pharmacopeia B.P method for assay of metronidazole. Beer's law was obeyed within a concentration range of 2.0 to 12.0µg/mL with correlation coefficient of 0.9934.The precision (0.4 and 0.9 % RSD), accuracy (% Er =0.16), recovery (100 %), limit of detection (0.29 µg/mL) and limit of quantitation (0.89 µg/mL) of the method were all found to be satisfactory. No significant difference ($p > 0.05$) was observed between the content of metronidazole assayed using the developed method and the BP method in all the samples.

Conclusion: The proposed method can be used for quantitative determination of metronidazole in pure and tablet dosage forms.

Keywords: Metronidazole, spectrophotometry, method development, crotonaldehyde

Méthode de spectrophotométrie pour la détermination du métronidazole dans les formes pures et de dosage de comprimés par la formation de base de Schiff par couplage avec le crotonaldéhyde

Aminu Musa[1], Auwal A. Bala[2], Ibrahim Nasir[3], Vivian Mamman[1], and Manasse Sallau[1].
[1]Département de chimie pharmaceutique et médicinale, Faculté des sciences pharmaceutiques, Université Ahmadu Bello, Zaria, Nigeria. [2]Département de pharmacologie, Collège des sciences médicales et de la santé, Université fédérale de Dutse, Nigéria. [3] Département de chimie pharmaceutique et médicinale, Faculté des sciences pharmaceutiques, Université Usmanu Danfodiyo, Sokoto, Nigéria.

Auteur correspondant: Aminu Musa
Email: pharmamin@gmail.com, Téléphone: +2348032872718

RESUME

Contexte: Les méthodes de spectrophotométries UV-visible sont populaires en raison de leur simplicité, de leur exactitude, de leur précision et de leur faible coût.

Objectifs : Dans cette étude, une nouvelle méthode de spectrophotométrie simple, précise, économique et reproductible pour la détermination quantitative du métronidazole a été développée et validée.

Méthodes: La méthode est basée sur la réduction du groupe nitro dans du métronidazole avec de la poudre de zinc dans des conditions acides (HCl 5N) à 27° C dans du méthanol pendant 15 minutes, suivie du couplage de l'amine obtenue avec du crotonaldéhyde pour former un chromogène jaune avec aλmax de 408nm. La méthode proposée a été validée conformément aux directives de la conférence internationale sur l'harmonisation (ICH) et a été utilisée pour doser un échantillon de poudre de métronidazole standard et quatre marques différentes (A, B, C et D) de comprimés de métronidazole.

Résultats: Les résultats du test ont été validés par comparaison avec la méthode de pharmacopée britannique B.P pour le dosage du métronidazole. La loi de Beer a été respectée dans une plage de concentrations de 2,0 à 12,0 µg/mL avec un coefficient de corrélation de 0,9934. La précision (0,4 et 0,9% RSD), la précision (% Er = 0,16), la récupération (100%), la limite de détection (0,29 µg/mL) et la limite de quantification (0,89 µg/mL) de la méthode se sont tous révélés satisfaisants. Aucune différence significative (p> 0,05) n'a été observée entre la teneur en métronidazole testée en utilisant la méthode développée et la méthode BP dans tous les échantillons.

Conclusion: La méthode proposée peut être utilisée pour la détermination quantitative du métronidazole sous forme posologique pure et en comprimé.

Mots-clés: métronidazole, spectrophotométrie, développement de méthodes, crotonaldéhyde

INTRODUCTION

Metronidazole (Figure 1), chemically known as 2 - (2-Methyl-5-nitro-1H-imidazol-1-yl) ethanol is a 5 Nitroimidazoles used extensively in the treatment of amoebic, protozoal and bacterial infections. Standard Metronidazole is a white to pale yellow, odourless crystals or crystalline powder.It darkens on exposureto light. Sparingly soluble in water and in alcohol.[1] Metronidazole is on the WHO's list of essential medicines. It is used the treatment of infections caused by microorganisms, including Bacteroides, Clostridium sp, Endolimaxnana, Entame bahistolytica, Fuso bacterium vincentii, Gardnerellavaginalis, Giardia lamblia, Peptostreptococcus andTrichomonassp. Metronidazole is used to eradicate Helicobacter pylori in peptic ulcer disease (with other antimicrobials, and either bismuth compounds or proton pump inhibitors) and in the management of malodorous tumours and ulcers where there is anaerobic infection amongst many other indications.[1]

Metronidazole is officially assayed by titrimetric, potentiometric and high performance liquid chromatographic methods HPLC.[2,3] The reported HPLC methods are expensive, requiring sophisticated and complex instrumentation. These limitations make UV-Visible spectrophotometric methods useful and popular in resource challenged environments because of their simplicity, accuracy, precision and low cost.[4,5] Spectrophotometric methods have been reported to utilized the reduction of the nitro group in metronidazole and condensation reaction with aromatic aldehyde such as vanillin, p-dimethyl amino benzaldehyde (PDAB) and 1,10-Phenanthrolin to yield yellow colored Schiff's bases that can be quantified spectrophotometrically.[6] Similarly Other methods have been reported, that 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 can absorb light.[7] However, some of the reported spectrophotometric methods for metronidazole determination presents some limitations like extraction and use of buffer system,[8] multistep procedures,[9] and use of complex/expensive reagent.[10,11]

In this study a simple, accurate, economical and reproducible spectrophotometric method for the quantitative determination of metronidazole in pure and tablet dosage forms using crotonaldehyde as coupling reagent was developed and validated.

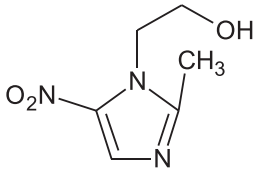

Figure 1: Metronidazole

METHODS

Equipment

AE 240 digital analytical balance (Mettlertoledo, USA).A double scanning UV/VIS spectrophotometer, Helios Zeta, Model 164617 (Thermo scientific, USA).

Materials and Reagents

Standard Metronidazole Powder (Sigma Aldreich, Germany), 5N HCI, Methanol, Deionised water, Crotonaldehyde, 0.1M perchloric acid, acetone and Zinc dust. Four brands of Metronidazole tablets (200mg) labeled as samples A, B, C and D respectively.

Preparation of stock solution of reduced metronidazole

Metronidazole standard powder (100mg) was dissolved in hot methanol (30mL) in a beaker followed by addition of 5N Hydrochloric acid (10mL) and zinc dust (0.5g) while shaking. The mixture was placed on water bath at 70°C for 20 minutes. Thereafter, it was then filtered and washed with three10mL portions of methanol into a volumetric flask (100mL) and made up to mark with methanol to obtain a 1mg/mL solution.

Preparation of crotonaldehyde in methanol

Crotonaldehyde 8%w/v was prepared by accurately weighing and dissolving 8g in 100mL methanol.

Formation of Schiff's base and determination of analytical wavelength

Portions (2mL) of the diluted reduced metronidazole (100µg/mL) were transferred to five different test tubes followed by 2mL of 8%w/v crotonaldehyde solution as coupling reagent. The mixture was heated on water bath at different temperature and time ranges; heating at 27°C for 15 minutes was optimum for formation of the Schiff's base. The yellow coloured chromogen formed (stable for more than 3 hours) was scanned against the blank on the spectrophotometer in the range 200 to 600nm to determine the wavelength of maximum absorption (λmax).

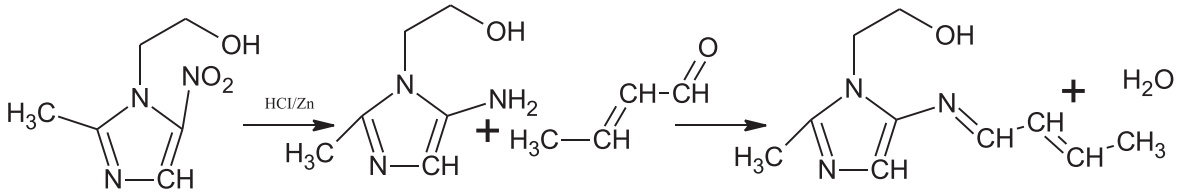

Figure 2: Proposed equation for the reactions

Method validation

From a solution (100µg/mL) of the reduced metronidazole, serial dilutions were done to obtain solutions of concentrations 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0µg/mL.A portion (2mL) of each of these solutions was treated with 2mL of 8%w/v crotonaldehyde solution and the mixture was heated on water bath at 27°C for 15 minutes. The absorbance of each mixture was determined at the λmax. A plot of absorbance against the corresponding concentration gave the calibration curve. The method was validated with respect to precision, accuracy, percentage recovery, limit of detection and quantitation limit according to ICH guideline for validation of analytical procedures [12].

The utility of the proposed method was checked by assaying the content of metronidazole in the four brands (A, B C and D)of metronidazole tablets (200mg). This was done by weighing 20 randomly selected tablets from each brand separately and grounding them into fine powder as specified by British pharmacopeia. An amount of each powder equivalent to 100mg metronidazole was weighed and treated with 2mL of 8%w/v crotonaldehyde solution and the mixture was heated on water bath at 27°C for 15 minutes and their respective absorbance determined at the λmax. Triplicate assays were conducted on each sample. The validity of this assay was checked by assaying the same samples using the official BP method and comparing the two results using the student's t-test on Graph Pad Prism 5 software.

RESULTS

The wave length of maximum absorption of the deep yellow coloured chromogen of metronidazole with crotonaldehyde and its calibration parameters are shown in Table 1. The calibration curve obeys Beer's law in the concentration range from 2.0 - 12.0µg/mL (Figure 3). The accuracy, precision and other validation parameters are presented in Table 2. The comparative assay of the content of metronidazole in the four tablet brands (A, B, C and D) using the developed method and the BP method are shown in Table 3.

Table 1: Calibration parameters and λmax of developed method

Parameter	Value
λmax (nm)	408
Linearity range (µg/mL)	2.0 – 12.0
Correlation coefficient (r)	0.9934
Regressionequation	$A = Cy + x$
Slope (y)	0.1042
Intercept (x)	0.281

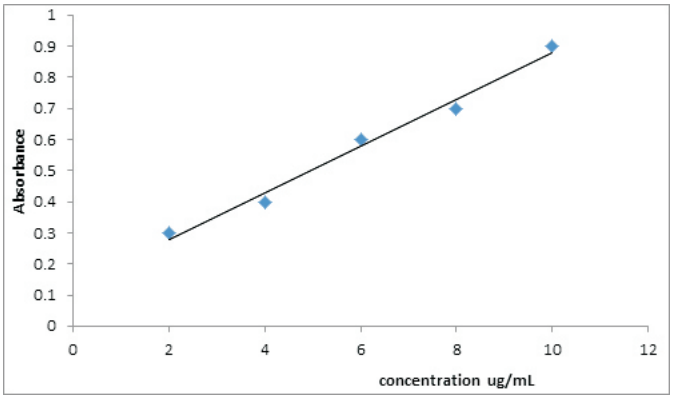

Figure 3: Standard curve (Calibration curve)

Table 2: Validation parameters of the developed method

Parameter	Value
Precision within day (%RSD)	0.41
Precision between day (%RSD)	0.90
Accuracy (%Er)	0.16
Percentage recovery (%)	100.06
Limit of Detection (µg/mL)	0.29
Limit of Quantitation (µg/mL)	0.89

A = absorbance, C = Concentration, % RSD -The percentage coefficient of variation (Relative Standard Deviation)

Table 3: Comparison of assay results of metronidazole using this method and BP method

Tablet Samples	% Content of Metronidazole ± SEM	
	Proposed method	BP method
A	99.3 ± 0.55	101 ± 0.23
B	*89.8 ± 0.45	*86.5 ± 0.34
C	98.6 ± 0.44	96 ± 0.14
D	103.0 ± 0.05	101.7 ± 0.50

*Outside the BP specification for metronidazole content.

DISCUSSION

The regression analysis of the calibration curve gave a good coefficient of correlation, thus making it suitable for the estimation of the drug. Linear regression equation is of the type shown in Table 1 defining the relationship between absorbance (A) and concentration (C in µg/mL). The accuracy of the proposed method expressed as the measure of percentage relative error (%Er) was found to be within the acceptable range (1 – 5 %) for moderately accurate procedure (Table 2).[13] The accuracy was better than the1.72, 3.97 and 2.30 reported in other spectrophotometric methods for metronidazole determination.[10] The average percentage recovery for the method was also found to be 100.16% and is better than the 99.15 and 99.54% reported in spectrophotometric methods for metronidazole determination.[14] Both within and between day precisions of the method were less than 1% (Table 2).This shows that the precision of the proposed method is satisfactory as the acceptable range is <15 %.

The content of metronidazole assayed using the developed method and the BP method was not significant difference (p>0.05) in all samples analyzed (Table 3). Thus, the developed method can be interchanged with the official method. However, the content of metronidazole in sample B fell outside the BP specification (95.0 – 105.0 %) using both the developed method and the BP method (Table 2). This is indicating that sample B might be a substandard product.

Our method involved the use of a spectrophotometer which may not be as sensitive as other methods that involved high performance apparatus such as, high performance liquid chromatography (HPLC).

CONCLUSION

The spectrophotometric method developed for the determination of metronidazole is simple, precise, accurate, reproducible and cost effective and can be used routinely for quantitative estimation of metronidazole in pure and tablet dosage forms. The stability of the yellow coloured chromogen formed will provide sufficient time to perform the analysis, thereby making the methodology feasible in resource challenged laboratories.

ACKNOWLEDGEMENT

The authors will like to acknowledge Malam Iliyasu Salisu, the Chief Technologist Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria for running all the samples.

REFERENCES

1. Sweetman SC. (Eds), Martindale (2002). The complete drug reference 35th edition: The Royal pharmaceutical society of Great Britain, London. Electronic version;
2. Joyani D. and Manabendra D (2014). UV Spectrophotometric Assay Method Development and Validation of Metronidazole in Bulk and Tablet Formulation. J*ournal of Pharma. Sci. Tech.3: (2) 2321-4376*
3. British Pharmacopoeia Volll (2002). Printed for Her Majesty's Stationary office at University Press, Cambridge, Appendix VII.
4. United States Pharmacopoeia. Asian Edition 30(2006). United States Pharmacopoeial Convention. Inc: electronic version.
5. Musa A, Nasir, I, and Garba M (2014). A Novel Spectrophotometric Method for Determination of Lamivudine in Pure and Tablet Dosage Form by Formation of a Coloured Hydrazone Using 2,4-dinitrophenyl Hydrazine. *African Journal of Pharmaceutical Research and Development6: (1) 1-5.*
6. Siddappa K, Mallikarjun M, Reddy PT, and Tambe M (2008).Spectrophotometric Determination of Metronidazole through Schiff's Base system using vanillin and PDAB reagents in pharmaceutical preparations. Ecl. Quím., São Paulo. 33(4): 41-46,
7. Zeeshan M, Muhammad T, Ansari SA, Muhammad AS, Muhammad F, and Mueenv A (2016). Development and application of spectrophotometric method for quantitative determination of Metronidazole in pure and tablet formulations. *Parkistan journal of pharmaceutical research2 (1):28-32*
8. Khaled MD, Ismail S, Samia M, and Mohamed E (2012).Extractional Spectrophotometric Analysis of Metronidazole, Tinidazole, Ornidazole and Secnidazole Bases through Acid-dye Complexation

using bromothymol blue dye. *Pakistani Journal of Pharmaceutical Sciences 25 (1): 207 – 208.*

9. Khalaf FA (2012). Spectrophotometric Determination of Metronidazole via Diazotization Reaction with p Hydroxy Benzaldehyde as a Coupling Reagent. *Research Journal of Pharmacology 6 (2):25–29*
10. Saffaj, T, Charrouf M, Abourriche A, Abboud Y, Bennamara A, and Barrada M(2004). Spectrophotometric Determination of Metronidazole and Secnidazole in Pharmaceutical Preparations. Societa ChemicaItaliana 59 (10):843 –846.
11. Adegoke, OA, Umoh OE, and Soyinka J O (2010). Spectrophotometric Determination of Metronidazole and Tinidazole via Charge Transfer Complexation using Chlorannilic acid. Journal of Iran Chemical Society 7 (2): 259 – 360.
12. International Conference on Harmonization "Q29R1 (1995). Text on Validation of Analytical Procedures", Federal register CPMP/ICH/381/95.
13. Havey, D (2000). Modern analytical Chemistry. The McGraw-Hill company, Inc. University of California, United States. p 39.
14. Thalasamma, P, and Vankateswarlu P (2009). Spectrophotometric Method for Determination of Metronidazole in Pharmaceutical Pure and Dosage