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Metronidazole Analysis: Method Development and Validation via Hydrotropic Solubilization

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ABSTRACT

A quick, easy, cost-effective, efficient and safe UV-visible spectrophotometric method is described for the assay of metronidazole in reference powders and tablet formulations via hydrotropic solubilization.1M nicotinamide solution was employed to solubilize the metronidazole and the absorbance at 320 nm was measured. Linearity, precision, accuracy, and specificity were determined in accordance with the ICH guidelines. Metronidazole followed Beer's law in the concentration range 1–15µg/ml with an r^2 of 0.9994. LOD and LOQ were 0.4277 µg/ml and 1.2961 µg/ml respectively. The percentage content of reference metronidazole powder was 99.36± 0.053 with % RSD of 0.05 whilst that in tablet dosage was 96.71± 0.106 with % RSD of 0.11. The precision study was found to be 96.98± 0.136 with % RSD of 0.140 and accuracy by recovery method was 98.80 ± 0.122 with % RSD of 0.12. There was no statistical difference between the results obtained by the proposed method and that of the official British Pharmacopoeial method for the assay of metronidazole. It may be conveniently deduced that the developed validated method can be applied in a routine analysis of metronidazole in reference powders and tablet dosage forms.

1. INTRODUCTION:

Metronidazole, 2-(2-Methyl-5-nitro-1*H*-imidazole-1-yl) ethan-1-ol is poorly soluble, synthetic antiprotozoal and the bactericidal drug which belongs to the nitroimidazole class of compounds. Metronidazole is active against organisms such as *Trichomonas vaginitis, Giardia lamblia, Balantadium coli, Helicobacter pylori* and*Gardnerellavaginilis.* Metronidazole is quantitatively analyzed officially by non-aqueous titrimetry, potentiometry and HPLC methods [1-3]. Several other tedious, cumbersome and expensive methods have been reported in the literature for the determination of metronidazole including UV-visible spectrophotometry and differential Pulse Polarography (DPP) Technique [4-6]. Non-aqueous titration involves the use of reagents such as perchloric acid which is corrosive, dangerous, pungent and expensive for chemical analysis. HPLC is very expensive and requires special skills to operate. It also requires the use of expensive reference standards and HPLC grade reagents which are not readily available in most third world countries.

Specific solvents/reagents and standards availability and cost are constant challenges in industry and research institutions. In such situations, quality control monitoring of drug both in-process and post-market becomes difficult for both industry and regulatory bodies to secure the ultimate interest of the consumer. There is, therefore, the need to investigate alternative methods to enhance the aqueous solubility of poorly water-soluble drugs for effective quantitative analysis. The solubilizing properties of hydrotropes can be exploited for this purpose.

Hydrotropic solubilization is a molecular phenomenon which introduces a second solute called the hydrotrope that causes a several-fold increase in aqueous solubility of an aqueous insoluble solute under normal conditions [7-9]. Examples include sodium salicylate [10], ibuprofen sodium [11], nicotinamide [12] and sodium benzoate [5].Nicotinamide has widely been used in the field of hydrotropic solubilization to solubilize several sparingly soluble drugs including progesterone, testosterone, diazepam, griseofulvin, saquinavir, riboflavin, nifedipine, Ketoprofen and others [13]. It is a water-soluble, amide derivative of nicotinic acid (niacin; vitamin B_3). Nicotinic acid is an old medication and was mainly used to treat hyperlipidemia. It has been used clinically (1) to treat schizophrenia and psoriasis; (2) to prevent type I diabetes mellitus; and (3) as a potent radiosensitizer.

This hydrotropic solubilization method eliminates the setbacks associated with the official analytical procedures for metronidazole [14], in that it only requires mixing the drug with the hydrotrope in water. It does not require chemical modification of hydrophobic drugs and use of expensive organic solvents and finally, the hydrotropic character is selective for only the analyte in question. The method of hydrotropic solubilization is therefore quick, easy, cost-effective, efficient and safe.

The present work describes the utilization of nicotinamide as hydrotrope to solubilize metronidazole in aqueous media and the solubilized metronidazole analyzed by UV-visible spectrophotometry.

2. MATERIALS AND METHODS:

2.1 Standards and Reagents: Referencemetronidazole sample was obtained as a gift from-Ernest Chemist Limited-Tema, Ghana. All other chemicals and reagents used were of analytical grade. Commercial tablets of metronidazole were procured from local pharmacies in Kumasi, Ghana.

2.2 Method Development

***2.2.1 Selection of Hydrotropes for Metronidazole*:** 2M solutions of reference ibuprofen sodium, sodium benzoate, nicotinamide, trisodium citrate and urea were prepared. 20ml of each solution was measured into separate 100 ml beakers and 200 mg quantity of reference metronidazole added. The mixtures were sonicated for 30 minutes and those that yielded clear solutions as a sign of solubilization were selected as the hydrotrope(s).

***2.2.2 Stability of Color Complex Formed by Metronidazole and NaOH*:** 200mg of metronidazole was accurately weighed and solubilized in the 20ml solution of 1M Nicotinamide in a 250 ml beaker. 10 ml 0.5M NaOH was immediately added, transferred into a 100ml volumetric flask and diluted to volume. Further dilutions were made to obtain 10µg/ml with distilled water. The absorbance was measured at 320nm after every 3 minutes up to 20 minutes in order to determine the time required for stable complex formation between metronidazole and NaOH. A graph of absorbance vs. time was plotted to ascertain the maximum time required for complete coloration.

2.2.3 Determination of Maximum Time of Solubilization: 200 mg of reference metronidazole was added to 20ml 1M nicotinamide sonicated for 5, 10, 15 and 20 minutes. The resultant solutions were filtered and the residue rinsed with 2x10ml of the hydrotrope and the filtrates analyzed for percentage drug content to determine the maximum time of solubilization.

***2.2.4 Determination of Minimum Hydrotropic Concentrations (MHC)*:** 20ml each of the following concentrations of nicotinamide; 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 1.75 and 2.00M were measured into 100ml beakers and 200mg quantity of reference metronidazole was added and sonicated for 30 minutes. The mixtures were filtered through Whatman's Filter Paper No. 90 and 10ml of 0.5M NaOH was added to each of the filtrates and allowed to stand for up to 20 minutes. The solutions were transferred into a 100ml volumetric flasks and diluted to volume with distilled water. A 1ml portion of each of these solutions was pipetted into a 200ml volumetric flask and diluted to volume. The absorbance values were measured at 320nm using 0.05M NaOH in 0.2M nicotinamide as blank with a T90+ UV-visible spectrophotometer (PG Instruments Limited, UK). The quantity of metronidazole solubilized was then estimated from the absorbance to ascertain the least concentration of nicotinamide with which the drug has maximum solubility.

2.3 Preparation of Standard Solutions: 50mg of reference metronidazole powder was solubilized in 20ml of 1M nicotinamide. 10ml of 0.5M NaOH was added and allowed to stand for 10 minutes to generate a deep wine color. The solution was then diluted to 100ml with distilled water to make a stock solution of 500µg/ml.

2.4 Analytical Method Validation: The novel method was validated in compliance with International Conference on Harmonization [15] by determining the linearity, limits of detection and quantification, precision (Intra-day and Inter-day), accuracy and specificity.

2.4.1 Linearity. 1ml of the stock solution (500µg/ml) was serially diluted to obtain concentrations of 15µg/ml, 12.5µg/ml, 10µg/ml, 7.5µg/ml, 5µg/ml, 2.5µg/ml and 1µg/ml with distilled water. The absorbance values of the final solutions were measured with a UV-visible spectrophotometer at 320nm against a blank of 0.05M NaOH in 0.2M nicotinamide solution. The Linearity of the novel method was determined from the correlation coefficient of the regression line. The procedure was carried out in triplicate.

***2.4.2 Limits of Detection and Quantification*:** The limit of detection (LOD) and the limit of quantification (LOQ) of the novel method were determined by using the standard deviation of the response and slope of the linear regression curve as defined in equations (1) and (2) respectively.

$$LOD = \frac{3.3\delta}{S} \quad \text{.....(1)}$$

$$LOQ = \frac{10\delta}{S} \quad \text{..... (2)}$$

Where δ is the standard deviation of the response and S is the slope of the calibration curve.

***2.4.3 Precision Study of Method*:** This involves Intra-day and Inter-day Precision. In both situations, the %contents were calculated as; (Average weight of drug found (mg) / Label Claim (mg))*100%. The Intra-day precision (reproducibility) was determined on a formulated tablet on the same day and in the same laboratory at three (3) different times. The Inter-day (intermediate) precision of the method was determined on the formulated tablet by three different analysts and on three (3) different days and in the same laboratory.

***2.4.4 Accuracy by Recovery Method*:** The accuracy of the method was determined by the spiked recovery method where 80, 100 and 120% levels of the reference drug powder were accurately weighed and added to the labeled claimed formulated tablet. The mixture was solubilized and analyzed via the developed method. Three (3) samples were prepared for each recovery level and the percentage of recovery was determined.

***2.4.5 Specificity*:** The developed method was examined for specificity by scanning the 1M nicotinamide as blank followed by a solution of metronidazole in the 1Mnicotinamide hydrotrope. The spectra were evaluated.

2.5 Application of Developed Method to Metronidazole Assay: Twenty tablets of metronidazole were weighed and finely powdered. The quantity of the tablet powder equivalent to 200mg of metronidazole was accurately weighed and solubilized in a 20ml solution of 1M Nicotinamide by sonicating for 10 minutes. The resultant solution was filtered with Whatman's filter Paper No. 90 and the residue rinsed with 2 x 10ml of the hydrotrope. A 10ml solution of 0.5M NaOH was added to the combined filtrate and allowed to stand for 10 minutes to generate the wine color. The wine solution was diluted to 100ml with distilled

water and 1ml pipetted and diluted to 200ml with distilled water to make a concentration of 10µg/ml. The absorbance was measured at 320nm against a reagent blank and the actual concentration obtained from the regression equation of the calibration graph. The procedure was carried out in triplicate.

2.6 Official Assay of Metronidazole Powder and Tablets (BP, 2013): The quantity of pure Metronidazole powder equivalent to 200mg was accurately weighed and dissolved in 50ml of glacial acetic acid in a 250ml beaker. The resultant solution was potentiometrically titrated against 0.1M Perchloric acid from the burette. 1ml of 0.1M Perchloric acid is equivalent to 17.12 mg of metronidazole. For the formulation's assay, 20 tablets were accurately weighed and finely powdered using mortar and pestle. The quantity of powdered tablets containing 200mg of metronidazole was transferred to a sintered-glass crucible and extracted with 6 x 10ml quantities of hot acetone. The extracts were cooled and 50ml of acetic anhydride was added. This solution was potentiometrically titrated against 0.1M Perchloric acid. 1ml of 0.1M Perchloric acid is equivalent to 17.12mg of metronidazole.

2.7 Statistical analysis

The results from the developed method were statistically evaluated using the British Pharmacopoeia's non-aqueous acidimetric method of analysis of reference and formulated metronidazole tablets (BP, 2013) by the paired *students t-test* at 95% confidence level.

3.0 RESULTS AND DISCUSSION:

3.1 Method Development: Hydrotropic solubilization phenomenon was applied in the UV-visible spectrophotometric analysis to study poorly water-soluble metronidazole in aqueous media by solubilizing in a 1M solution of nicotinamide. This UV-visible spectrophotometric method was performed divulging that there was no chemical interaction between the drug and the hydrotrope and that there was no interference from the hydrotrope as confirmed by Figure 1 (A & B). Starting with the selection of a suitable hydrotrope as in Table 1, metronidazole solubilized in both nicotinamide and sodium benzoate solutions but that of nicotinamide was selected for analysis because the broad absorption bands observed for sodium benzoate from 250-320nm interfered greatly with the metronidazole absorbance at 315-320nm after a series of dilution. Because of these findings, sodium benzoate was concluded not suitable as hydrotrope for metronidazole in quantitative analysis.

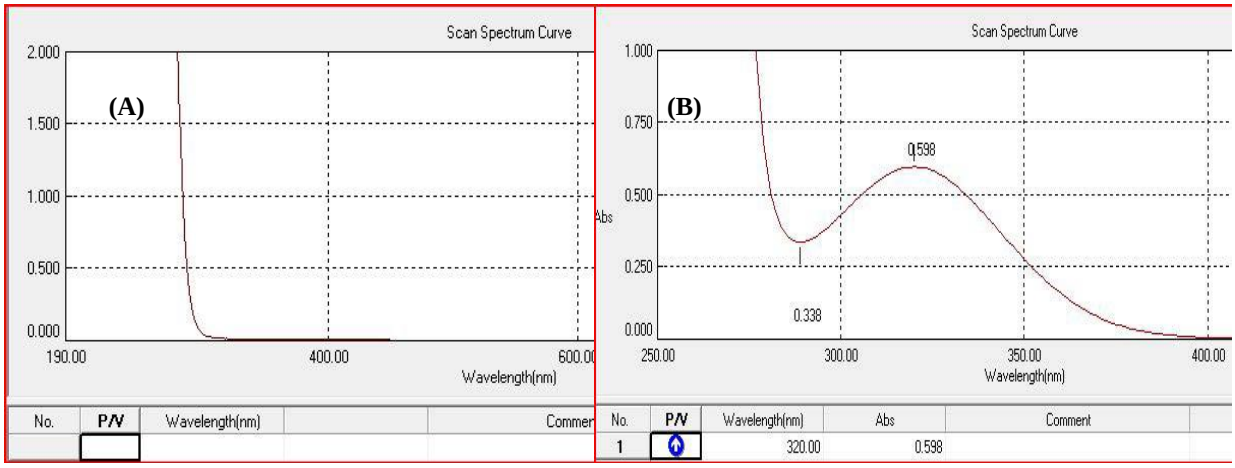

Figure 1: Absorption spectra of 1M Nicotinamide (A) and Metronidazole in hydrotrope (B)

Table 1. Selection of Hydrotropes for Metronidazole

Hydrotrope (2M)	Observation	Inference
Ibuprofen Sodium	Particles suspended in solution	Insoluble
Sodium Benzoate	*Clear Solution*	*SOLUBLE*
Nicotinamide	*Clear Solution*	*SOLUBLE*
Trisodium Citrate	Particles suspended in solution	Insoluble
Urea	Particles suspended in solution	Insoluble

The 0.1M NaOH was observed gradually forming a wine color with the metronidazole in the solution. The colored complex was observed to be a result of metronidazole and sodium hydroxide alone and has no effect from the nicotinamide. The alkaline hydrolysis of metronidazole releases nitro group as nitrite ion and yielded nitrite ions that result in a colored complex [16]. The method has been used to determine metronidazole through spectrophotometric measurement of the nitrite on produced upon alkaline hydrolysis by flow injection [17]. Figure 2 shows the maximum time required for the complex formation is 10 minutes.

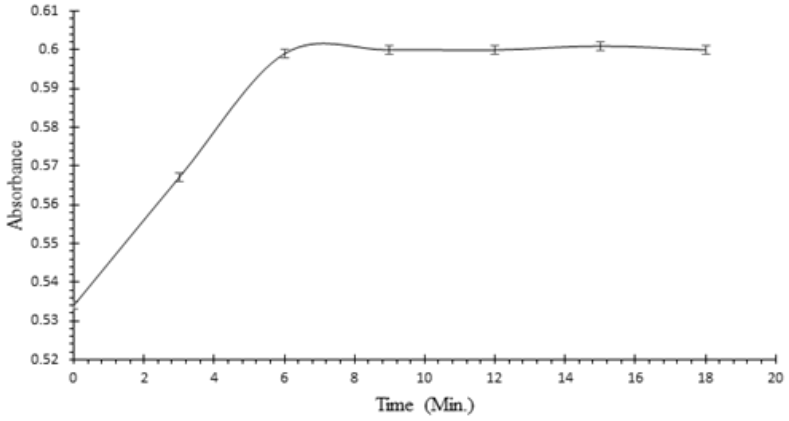

Figure 2: Graph of Absorbance vs. time (min) of Metronidazole/NaOH colored complex

From Table 2, 10 minutes was observed to be the maximum time (min) required for solubilization of metronidazole. The minimum hydrotropic concentration (MCH) of the nicotinamide solution was from 1.00M where the percentage solubilized metronidazole contents found was above 99%. 1M nicotinamide was therefore selected for quantitative analysis of metronidazole.

Table 2: Determination of MHC of Hydrotropes and Maximum Time of Solubilization

MHC of Hydrotropes			Maximum Time (min) of solubilization		
Concentration (M)	Amt. found (mg)	(%) Content	Time (min)	Amt. found (mg)	% Content
0.25	47.57	23.79	5	182.73	91.36
0.50	109.53	54.76	10	198.64	99.32
1.00	198.98	99.49	15	198.49	99.24
1.50	199.00	99.50	20	198.71	99.35
2.00	198.97	99.49			

(Average of three(3) determinations)

Based on these, the developed method required solubilizing a specified amount of metronidazole in 1M nicotinamide for 10 minutes, add 0.1M NaOH, allow to stand for 10 minutes, dilute to an appropriate concentration and measure absorbance at 320nm. Although a maximum absorbance at 505nm for metronidazole in 0.1N NaOH has been reported [16], the current work observed an absorption maximum at 320nm due to the dilution of the solubilized metronidazole in 1M Nicotinamide. Naveed et al., [18] however reported analyzing metronidazole in 1N sodium hydroxide at a wavelength maximum of 278nm.

3.2 Analytical Method Validation: The novel method was validated according to ICH guidelines. The method followed Beer's law in the concentration range of 1 –15µg/ml with a regression equation of y = 0.059x + 0.0115 and r^2 of 0.9994 (Figure 3).

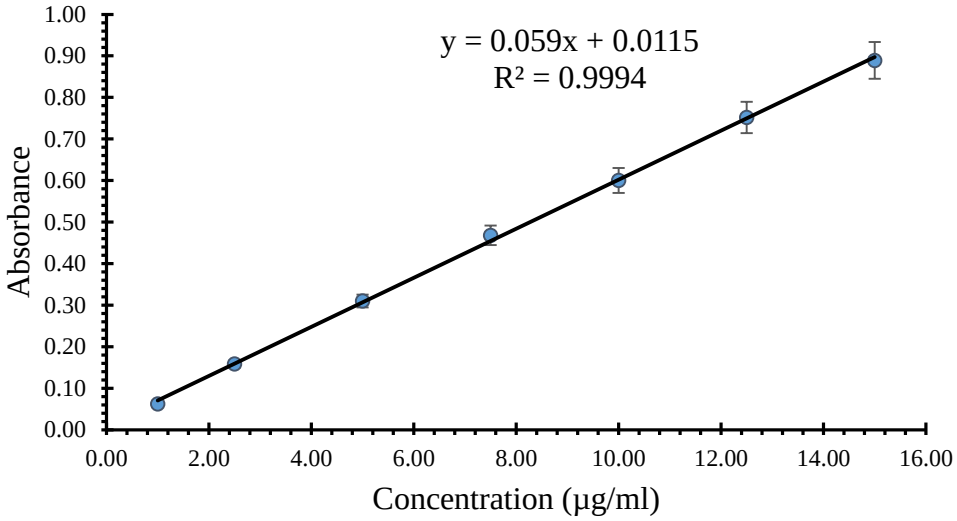

Figure 3: Calibration Curve of Metronidazole in 1M Nicotinamide.

LOD was 0.4277µg/ml and LOQ was 1.2961µg/ml as shown in Table 3 that elaborate sufficient sensitivity of the method. The %RSD values of intra-day and inter-day precision were 0.15, and 0.16 respectively as in Table 4. The new method was found to be precise in the specified range with all the %RSD values below the acceptable limit of 2%. The method was found to be accurate for the quantitative determination of metronidazole with mean percentage recovery of 98.80 ± 0.122 and RSD of 0.12 from the recovery studies (Table 5). There was a satisfactory agreement between the estimated amount and the label claim of the manufacturer. The developed method was found to be specific for the determination of metronidazole (Figure 1).

Table 3: Deductions from Calibration Curve

No.	Parameter	Result
1.	Working λ	315 – 320 nm
2.	Linearity Range (µg/ml)	1.00 – 15.00
3.	Regression equation	y = 0.059x + 0.0115
4.	Correlation Coefficient, r^2	0.9994
5.	Average Standard Error, δ	0.0075671
6.	Limit of Detection (LOD)	0.4277 µg/ml
7.	Limit of Quantification (LOQ)	1.2961 µg/ml

Table 4: Intra-day and Inter-day precision showing %RSD

Batch	Label Claim (Mg)	Intra-day Average of 3 time	Inter-day Average of 3 days
1	200	96.94	96.94
2	200	96.99	97.03
3	200	96.72	96.72
4	200	96.72	96.72
Mean		96.84	96.85
0.287		0.287	0.287
SD		0.144	0.159
% RSD		0.15	0.16

Table 5: Accuracy study of the proposed method

Level (%)	Label Claim (mg)	Amt. of pure drug added (mg)	Total Metronidazole analyzed (mg)	Mean Amount Recovered (mg)	Mean %Recovery ± s	% RSD
80	200	160	360	355.2674	98.69 ± 0.116	0.12
100	200	200	400	395.9760	98.99 ± 0.102	0.10
120	200	240	440	434.3768	98.72± 0.149	0.15
Overall Average					*98.80 ± 0.122*	*0.12*

(Average of four (4) determinations)

3.3Application of Developed Method to Metronidazole Assay: The developed method yielded percentage content of reference metronidazole powder as 99.36± 0.053 with RSD of 0.05 whilst that of British Pharmacopoeia method was 99.37± 0.064 with RSD of 0.06. Corresponding analysis of metronidazole in tablet dosage form by the developed method yielded 96.71± 0.106 with RSD of 0.11 and the British Pharmacopoeia method gave 96.71± 0.071 with RSD of 0.07 as shown in Table 6.

Table 6: Assay of metronidazole

Item.	**Reference Metronidazole Powder (%)**		**Metronidazole Tablet (%)**	
	Developed Method	**B.P. Method**	**Proposed Method**	**B.P. Method**
1	99.36	99.30	96.70	96.66
2	99.43	99.40	96.84	96.63
3	99.31	99.45	96.72	96.78
4	99.33	99.35	96.58	96.76
Mean ± s	*99.36± 0.053*	*99.37±0.064*	*96.71± 0.106*	*96.70± 0.071*
% RSD	*0.05*	*0.06*	*0.11*	*0.07*

t-test at 95% confidence level	*t-calculated = 0.2695* *t-statistical = 1.943*	*t-calculated = 0.0970* *t-statistical = 1.943*

(Average of four (4) determinations)

Statistical evaluation of the analysis of metronidazole by the proposed and the standard B.P. methods on an average of four (4) determinations was conducted (Table 6). *Student's t-test* calculated were 0.2695 and 0.0970 in the reference metronidazole powder and formulation respectively with *statistical t-value* of 1.943 at 95% confidence level. By conventional criteria, this difference is considered to be not statistically significant. The %contents obtained for both reference and formulated tablets of metronidazole were within the stipulated limits in the British Pharmacopoeia, (BP, 2013) and this attests to the fact that the method is accurate and reliable. This developed UV-visible spectrophotometric method compares favorably with the traditional non-aqueous titrimetric method which requires the use of toxic, detrimental and pungent organic/inorganic solvents. In addition, the developed method employs water as the solvent and does not incorporate errors by excipients since hydrotropic solutions show solubility for only the compound of interest. Niacinamide in the presence of multi-cosolvents propylene glycol and PEG 400 have been used to solubilize metronidazole in an aqueous environment [19]. However, the use of a single hydrotrope in this work to improve aqueous solubility of metronidazole presents an eco-friendly as well as the cost-effective analytical procedure for the assay of metronidazole. From the data observed, it is suggested that the novel method may be applied in a routine analysis of metronidazole in reference and tablet formulations.

CONCLUSION:

The 1M solution of nicotinamide was used to solubilize metronidazole and was successfully analyzed by UV-visible spectrophotometry. The developed method is statistically comparable to existing assay methods for routine quality control analysis. All the standard deviations and relative standard deviations obtained were satisfactorily low. Results of precision at different levels were found to be within acceptable limits of less than 2 (limits < 2). The present work was undertaken with a view to making the quantification of metronidazole quick, easy, cost-

effective, efficient and safe, precluding the use of sophisticated analytical instruments, toxic and harmful organic/inorganic solvents. Thus, exploring the application of hydrotropic solubilization phenomenon devoid of interference by the hydrotropic agent during analysis.

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