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UV-SPECTROPHOTOMETRIC DETERMINATION OF METRONIDAZOLE IN BULK AND PHARMACEUTICAL DOSAGE FORM USING HYDROTROPIC SOLUBILIZATION TECHNIQUE

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ABSTRACT

Hydrotropic solvents may proper choice to preclude the use of organic solvents so that, a simple, accurate, novel, safe and precise method could developed for estimation of poorly water soluble drug, metronidazole. Solubility of metronidazole is increased by using 8M urea as a hydrotropic agent. Metronidazole showed the maximum absorbance at 318 nm in method A , 314-322 nm in method B and 314 nm in method C. At these wavelengths, hydrotropic agent and other tablet excipients did not show any significant interference in the spectrophotometric assay. The developed methods were found to be linear in the range of 2-16 µg/ml with correlation coefficients (R) of 0.999, 0.999 and 0.998 respectively. The mean percent label claim of metronidazole in formulation estimated by the proposed methods were found to be 98.00%(A), 96.50% (B), 97.60% (C). The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical parameters were found to be good accordance with the prescribe values. As hydrotropic agent was used in the proposed methods, these methods were eco-friendly and it can be used in routine quantitative analysis of drug in bulk and dosage form in industries.
Key words: Metronidazole; urera; AUC, Hydrotropic solubilization technique; derivative spectroscopy.

INTRODUCTION:

The term hydrotropic agent was first introduced by Neuberg (1916), to designate anionic organic salts which, at high concentrations, considerably increase the aqueous solubility of poorly soluble solutes. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds. Hydrotropic agents consist generally of two essential parts, an anionic group and hydrophobic aromatic ring or ring system. The anionic group is obviously involved in bringing about high aqueous solubility, which is prerequisite for a hydrotropic substance.
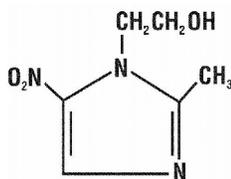

Fig. 1: Structure of Metronidazole

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On the other hand, planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization. Hydrotropes commonly used includes sodium benzoate, sodium acetate, sodium salicylate, nicotinamide, urea, trisodium citrate, sodium ascorbate, piperazine, caffeine, potassium citrate etc. hydrotropic agents have been observed to enhance the solubility of various substances in water. Metronidazole [2-(2-methyl-5-nitro-1H-imidazol-1yl) is an amaebicide, antiprotozoal and antibiotic effective against anaerobic bacteria and certain parasites. It is the drug of choice for first episodes of mild-to-moderate clostridium difficile infection. Metronidazole exerts rapid bactericidal effects against anaerobic bacteria, with a killing rate proportional to the drug concentration. Concentration-dependent killing has also been observed with Entamoeba histolytica and trichomonas vaginalis. Metronidazole kills bacteroides fragilis and clostridium perfringens more rapidly than clindamycin.[1,2] Metronidazole is officially determined by titrimetry, potentiometry, GC [4], spectrophotometric [1,2] and HPLC [3], UPLC [5] methods. IP [7] descrbes the non- aqueous titration method using perchloric acid as titrant for the assay of metronidazole. BP [8] describes potentiometric titrations. USP [9] describes HPLC and non aqueous titration method for metronidazole. The objective of present investigation is to develop simple, precise, accurate and ecofriendly UV-spectrophotometric, AUC and first order derivative methods for determination of metronidazole in bulk and in tablet dosage form using

piperazine as a hydrotropic solubilizing agent. The developed methods were validated as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Metronidazole (99.4%) working standard was obtained as gift sample from Padmaza laboratories, Vijayawada, India. Pharmaceutical tablet formulation of METROGYL tab 400 mg purchased from local pharmacy. Urea (A.R Grade;Qualigens) and distilled water used for the study.

Instrumentation

Shimadzu UV -1800 double beam spectrophotometer with 1cm path length supported by shimadzu UV-probe software, version 2.21 was used for spectral measurements with 10mm matched quartz cells. Shimadzu balance (BL-220H) was used for weighing.

Selection of solvent

8M urea solution was used as a solvent for developing spectral characterstics of a drug. The selection was made after assessing the solubility in different hydrotropic solvents like sodium acetate, sodoium benzoate, urea, sodium chloride, citric acid and piperazine. The drug showed complete solubility in 1M piperazine, 8M urea among these solvents 8M urea showed maximum stability than 1M piperazine.

Preparation of diluent

8M urea solution was prepared by 4.86 gm of urea pure chemical was weighed and dissolved in 10 ml distilled water and the volume was made upto the mark with distilled water in 10 ml volumetric flask.

Preparation of standard stock solution

Working standard metronidazole 10 mg was weighed accurately and transferred to a 10 ml volumetric flask and dissolved in 1 ml of 8 M urea solution. The flask was shaken and volume was made up to the mark with distilled water to give a solution of 1000µg/ml. It was further diluted with distilled water to get the concentration of 100µg/ml. From this solution a series of aliquots were prepared for further method development.

Method A:

Absorption maxima method:

For the selection of analytical wavelength 10µg/ml solution of metronidazole was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. From the spectrum λ_{max} of metronidazole 318 nm was selected for the analysis. The calibration curve was prepared in concentration range of 2-16 µg/ml at 318 nm. The calibration curve for metronidazole was plotted in the concentration v/s absorbance and regression equation was calculated.(fig. 2A&2B)

Method B:

Area under curve method:

For the selection of analytical wavelength 10µg/ml solution of metronidazole was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. Area under curve (AUC) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 313-323 nm. Area calculation processing item calculates area bound by curve and horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. From this regression eqation was calculated for the determination of amount of metronidazole in tablet formulation.(fig.3A&3B)

Method C:

First order derivative spectroscopy:

It involves the conversation of normal spectrum to its zero, first, second or higher derivative spectrum. In derivative spectrophotometry, spectra are obtained by plotting the first or a higher order derivative of absorbance with respect to wavelength as a function of wavelength. Often, these plots reveal spectral detail that is the lost in an ordinary spectrum. In addition, concentration measurements of an analyte in the presence of interference or of two or more analytes in a mixture can sometimes be made more easily accurately using derivative methods. In this method, 10µg/ml solution of metronidazole was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200-400 nm. The absorption spectra thus obtained were derivatised from zero to second order. First order derivative spectra of drug showed a sharp peak at 314 nm, which was selected for its quantification. The calibration curve for metronidazole was plotted in the concentration range of 2-16 µg/ml at 314 nm. The concentration of drug present in the tablets was determined against the calibration curve in quantization mode. (fig. 4A&4B)

Estimation of metronidazole in tablet formulation:

For the estimation of metronidazole in the commercial formulation, 20 tablets, each containing 400 mg of metronidazole were weighed and average weight was calculated. Triturate the tablets, for the analysis of drug, Quantity of powder equivalent to 100 mg of metronidazole was transferred to100 ml volumetric flask and dissolved in 8 M urea solution shake for 5min and volume made up to the mark with distilled water . Then it was filtered through whatman filter paper no.41. Further dilutions of the stock solution were made in distilled water to get required concentration. In method A,the concentration of metronidazole was determined by measuring absorbances of sample solution at 318 nm .in method B, the concentration of Metronidazole was determined by measuring absorbances of sampel solution in wavelength range of 313-323 nm .in method C, first order derivative spectroscopy the concentration of metronidazole was determined by measuring amplitude difference at λ_{max}314 nm. Result of tablet analysis are shown in table no.1 the assay procedure was repeated 6 times using each method (n=6) (table.1)

Method validation

The method was validated according to ICH guidelines to study accuracy, linearity and precision.

Linearity:

In order to find out linearity range of proposed UV-spectrophotometric methods, studies were carried out by plotting absorbances of analyte against concentrations of the analyte.a good linear relationship (r^2=0.999, 0.999 &0.998 for method A,B&C respectively) was observed between concentrations of metronidazole and the corresponding absorbance. the regression of metronidazole concentration over its absorbance was found to be y=0.055x+0.004, 0.626x+0.068 &0.065x+0.029 for method A,B&C (where y is the absorbance and x is the concentration of metronidazole).the slope ,intercept and the correlation coefficient of the drug were shown in table.2

Accuracy

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts to determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (2μg, 4μg, 6μg) of bulk sample to the pre-analyzed formulation .the solutions were suitably diluted in the range and then each of the dilution was observed 6 times. the % recoveries of the drug was found to be 99.87, 95.15 &98.60% in method A, B &C respectively. The results were shown in the table.3

Precision

Precision is the level of repeatability of results as reported between samples analyzed on the same day (intra-day) and samples run on 3 different days (inter-day).to check the intra-day and inter-day variation of the method, solution containing 10 μg/ml metronidazole were subjected to the proposed spectrophptometric method of analysis and the recoveries obtained were noted. the precision of proposed method i.e. the intra and inter-day variations in the absorbance of the drug solutions was calculated in terms of % RSD and the results were presented in the table.3 stastical revolution

revealed that relative standard deviation of drugs at different concentration levels for 6 times was less than 2.0 (intra day-0.177inter day-0.147).

LOD

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conclusions. the detection limit is usually expressed as the concentration of analyte. The standard deviation and response of the slope-

LOD=3.3 * standard deviation (σ)/ s

LOQ

The quantitation limit of an analytical procedure is the lowest amount of an analyte of a sample which can be quantitatively determined with suitable precision and accuracy. The standard deviation and response of the slope-

LOQ=10* standard deviation (σ)/ s

RESULTS AND DISCUSSION

For quantitative estimation of metronidazole in bulk and tablet dosage form three validated methods was proposed for method A, the absorbance maxima was found to be 318 nm, for method C λ_{max} at 314 nm was selected and for method B area under curve in the range of 314-322 nm were selected for the analysis. The % assay by the three methods was found to be 98.00% in method A, 96.50% in method B and 97.60% in method C. No interference was observed from the pharmaceutical excipients. The % recovery obtained for absorption maxima, first order derivative spectroscopy and area under the curve was found to be in the range of 91.22%, 95.15%, 98.60%. Hence, the proposed were validated in terms of linarity, precision, and accuracy. The present work provides an accurate and sensitive method for the analysis of metronidazole in bulk and tablet dosageforme.

Table-1: Results of marketed formulation analysis

Proposed methods	Label claim(mg)	Test conc(μg/ml)	Amount found (μg/ml)	%Assay	%RSD
A	400mg	10	9.80	98.00	0.55
B	400mg	10	9.65	96.50	0.46
C	400mg	10	9.76	97.60	0.62

Table-2: Optical characteristics of the proposed methods

S.no	Parameter	Method A	Method B	Method C
1	Linearity(μg/ml)	2-16	2-16	2-16
2	Linearity eqution	y=0.055x+0.004	Y=0.626x+0.068	Y=0.065x+0.029
3	Slope±SD	0.055±0.0026	0.626±0.0045	0.065±0.0028
4	Intercept ±SD	0.004±0.0046	0.068±0.0069	0.029±0.0096
5	Correlation coefficient	0.999	0.999	0.998

Table-3: Recovery studies of proposed methods

Method	Level of recovery	Pre anlyzed conc(µg/ml)	Amount added(µg/ml)	Amount found(µg/ml)	%Recovery
Method A	50	4	2	5.98	99.66
	100	4	4	7.90	98.75
	150	4	6	10.12	101.2
Method B	50	4	2	5.52	92
	100	4	4	7.46	93.25
	150	4	6	10.02	100.2
Method C	50	4	2	5.82	97
	100	4	4	7.65	95.62
	150	4	6	10.32	103.2

Table-4 Precision studies of proposed methods:

	Intra day			Inter day		
Method	Concentration(µg/ml)	Mean ±SD	%RSD	Concentration(µg/ml)	Mean ±SD	%RSD
A	10	9.86±0.0076	0.127	10	9.16±0.0025	0.165
B	10	9.89±0.0092	0.155	10	8.90±0.0045	0.144
C	10	9.96±0.015	0.251	10	9.21±0.00	0.134
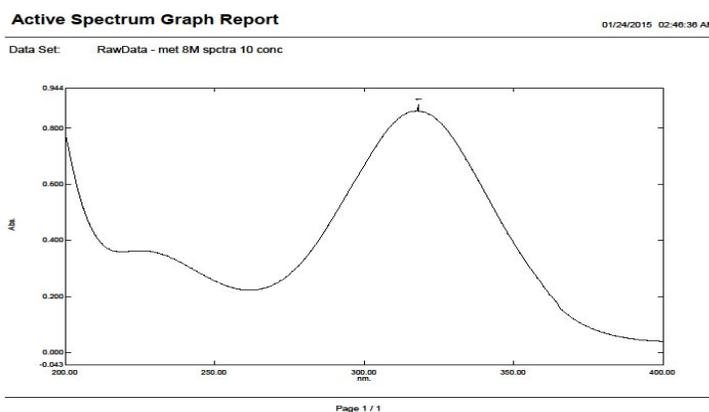
Fig. 2A Absorption maxima spectrum of metronidazole
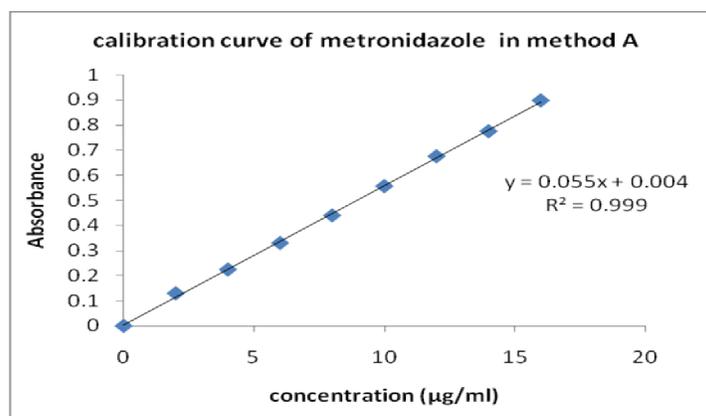
Fig. 2B calibration curve of Metronidazole in method A

Fig.3A AUC spectrum of metronidazole

Active Spectrum Graph Report

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Data Set: RawData - met 8M spctra 10 conc
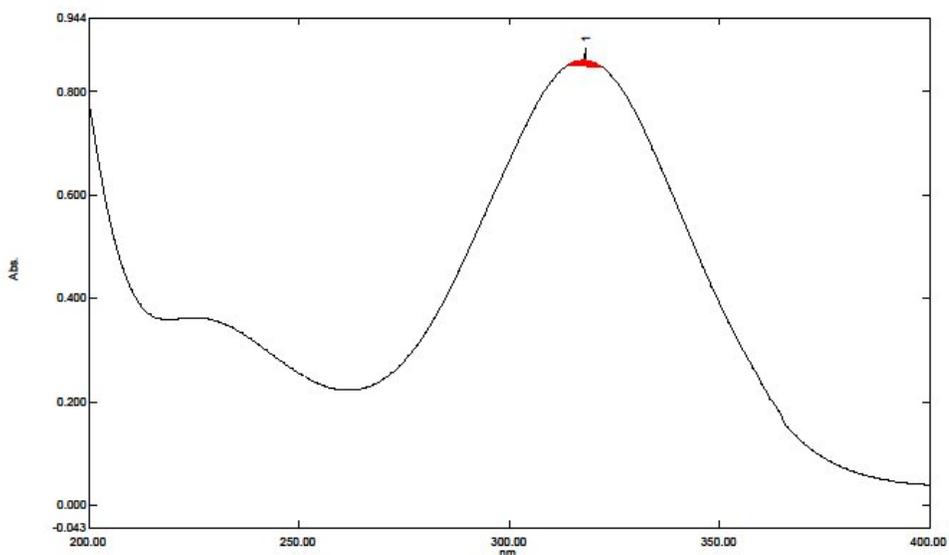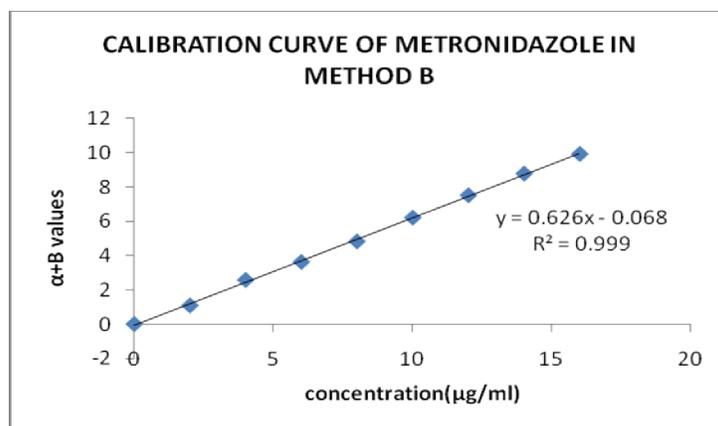
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Fig. 3B calibration curve of Metronidazole in method B

Data Set: Dataset 025003 - met 8M spctra 10 conc
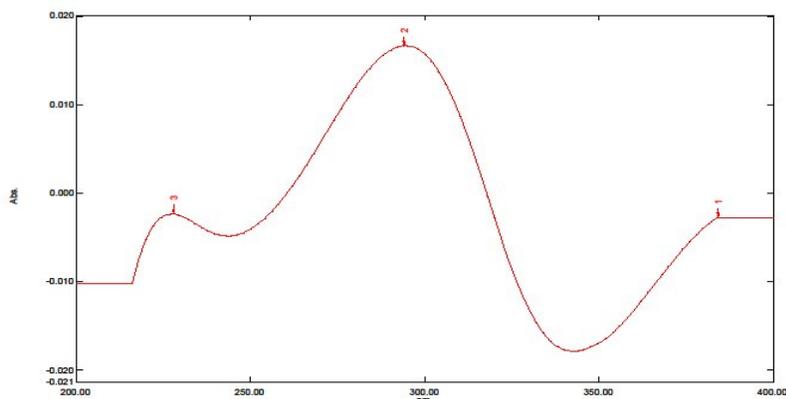
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Fig.4A first order derivative spectrum of metronidazole

CALIBRATION CURVE OF METRONIDAZOLE IN METHOD C

$y = 0.065x + 0.030$
$R^2 = 0.998$

dA/dλ values
concentration(μg/ml)
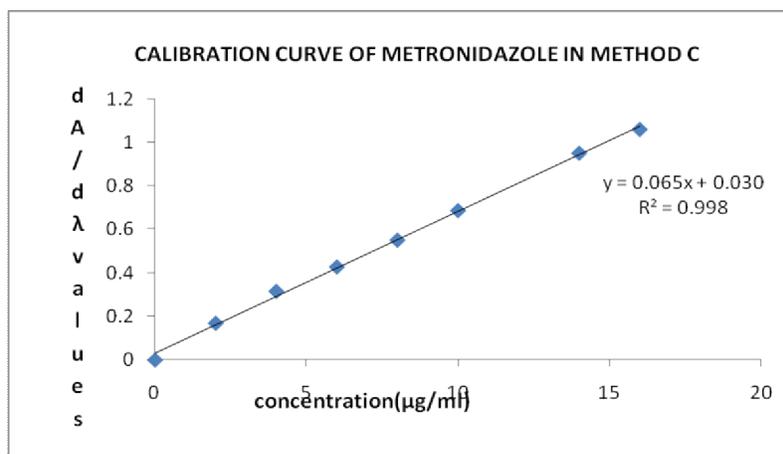
Fig. 4B Calibration curve of Metronidazole in method C

CONCLUSION

The three spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and %RSD calculated for the methods are within the limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence it can be conducted that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of metronidazole in its bulk and formulation. The proposed methods were found to be simple, economical, eco-friendly, rapid, precise and accurate for the determination of metronidazole in tablet dosage form. There is good scope for other poorly water soluble drugs which may be tried to get solubilised in 8 M urea solution (as hydrotropic agent) to carry out their spectrophotometer analysis excluding the use of costlier and unsafe organic solvents. Thus, it can be easily and conveniently adopted for routine quality control analysis.

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