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ORIGINAL RESEARCH ARTICLE

Application of Mixed Hydrotropic Solubilization Technique for Simultaneous Spectrophotometric Estimation of Metronidazole and Miconazole Nitrate from Different Pharmaceutical Dosage Forms

S. R. Kadam[2], Minakshi V. Janjale*[1], S. B. Akole[2], S. S. Bhosale[2]

[1]S.S.J.I.P.E.R, Jamner, Jalgaon-424206, Maharastra, India
[2]NDMVP, Nasik, Maharastra, India

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ABSTRACT

Quantitative estimation of poorly water-soluble drugs involves use of organic solvents. Major drawbacks of organic solvents include high cost, volatility and toxicity. In the present investigation, hydrotropic solubilization is employed to enhance the aqueous solubilities of poorly water-soluble drugs metronidazole and miconazole nitrate in two-component pharmaceutical formulations for simultaneous spectrophotometric determination. Three novel, simple, accurate, sensitive and economical procedures employed are simultaneous equation method, absorbance ratio method, and dual wavelength method. All methods utilize solution containing 40% urea and 10% sodium benzoateas, hydrotropic solubilizing agent. The solubility of drugs increases more than 14 times in mixed hydrotropic solution as compared to solubility in distilled water.In the solution containing 40% urea and 10% sodium benzoate, metronidazole and miconazole nitrate show maximum absorbance at a wavelength of about 325&285 nm respectively and isobestic point is observed at 296 nm. The results of analysis have been validated statistically and by recovery studies. Parameters such as linearity, precision, accuracy, specificity and robustness were studied as reported in the International Conference on Harmonization guidelines. So this method can be successfully employed in the routine analysis of metronidazole and miconazole nitrate in bulk drug and dosage forms like ovules and gel.

Keywords: Metronidazole and Miconazole nitrate, mixed hydrotropic solution (urea & sodium benzoate), spectrophotometry, validation.

INTRODUCTION

Imidazoles are five membered ring structures containing two nitrogen atoms with a complex side chain attached to one of the nitrogen atoms. Imidazoles in current clinical use are clotrimazole, miconazole, econazole and ketoconazole[1,2]. Miconazole nitrate (MIZ), 1-[2,4-dichloro-(b-(2,4-dichlorobenzyloxy) phenethyl] imidazole, possesses a wide antifungal spectrum. It is administered by the troche dosage form or by the intravenous infusion in the treatment of severe systemic fungal infections. It is also applied as a 2.0% cream or powder in infections of nails and skin [3,4]. Metronidazole (MNZ), 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 [5]. Literature survey reveals that both MIZ and MNZ are official in U.S.P. [6] and B.P. [7]. Several methods are available for the determination of the latter compounds by high-performance liquid chromatography (HPLC) in different pharmaceutical preparations, either alone [8-13], in combinations of MIZ and MNZ [14,15] or with other active ingredients [16-23]. Various Spectrophotometric methods have been reported for the determination of MIZ [4, 20, 24-27] and MNZ [3, 28-35] from its individual and combined formulations with other active ingredients. Derivative spectrophotometric methods have been reported for the simultaneous determination of MIZ and MNZ in combined dosage forms [14, 36]. HPTLC [37-39], quantitative NMR [40], chemometric [41] and titrimetric [42] methods have been described for the determination of MIZ and MNZ from its individual and combined formulations with other active ingredients. MNZ

***Corresponding Author:** Minakshi V.Janjale**, Email:** minakshijanjale@gmail.com, **Phone No:** +91- 9665123490

has been determined by chemiluminescence [43], biamperometry [5] and electro analysis (voltammetry and polarography) [44-49] in different pharmaceutical dosage forms. MIZ was determined in its oral gel formulation by GC on a column (11 m × 0.22 mm) of CP Sil 5CB (0.12 mm) at 270°C with N_2 as carrier gas (1 mL min-1) and N-P detection [50].However instrumental facilities of HPTLC, NMR,chemometry, chemiluminescence, biamperometry, polarography, GC and HPLC and voltammetry being rare as compared to UV spectroscopy and there is no method for the simultaneous determination of MIZ and MNZ by UV using mixed hydrotrophy technique, So a new method for the simultaneous determination of MIZ and MNZ from pharmaceutical preparations containing these combinations by UV using mixed hydrotrophy technique is developed.

The term hydrotropy has been used to designate the increase in solubility of various substances in water, due to the presence of large amounts of additives. A large number of poorly water-soluble drugs have been solubilized using various hydrotropic solutions sodium benzoate, niacinamide, sodium salicylate, sodium acetate, sodium citrate, and urea have been employed to enhance the aqueous solubility of many poorly water-soluble drug [51-55]. HPLC and spectrophotometric methods for simultaneous and separate estimation of metronidazole and miconazole nitrate in binary formulations whichrequire use of costlier and toxic organic solvents are reported in literature, but no method has been reported using hydrotropic solubilization. The aim of the present work was to develop a novel, simple, rapid, accurate, precise, reproducible and economical method for the simultaneous estimation of the binary drug formulation using simultaneous equation method, absorbance ratio method and dual wavelength method.

MATERIALS AND METHODS

Pure miconazole nitrate and metronidazole were obtained from Cipla Pharmaceutical Limited, Goa and SEIMENS Laboratories Gurgaon, India, respectively as a gift samples. A Chemito UV-visible recording spectrophotometer (model- UV-2600) - with 1 cm matched quartz cells were used for spectrophotometric analysis. All the chemicals were used of analytical grade.

Preliminary solubility studies of drugs

Solubilities of metronidazole and miconazole nitrate were determined at 28±1°. An excess amount of drug was added to screw capped 30 ml glass vials containing various hydrotropic solutions and distilled water, separately. The vials were shaken mechanically for 12 h at 28±1°, in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hr, and then centrifuged for 5 min at 2000 rpm. The supernatant of each vial was filtered through Whatmann filter paper No. 41. The filtrates were diluted suitably, and analyzed spectrophotometrically against corresponding solvent blank.

Preparation of standard metronidazole and miconazole nitrate solutions

Pure 25 mg of metronidazole and 10 mg miconazole nitrate were dissolved in 40 ml of solution containing 40% urea and 10% sodium benzoate separately and stirred for 15 min to solubilize the drug and the final volume of both solutions was made up to 100ml with distilled water to get standard solution with concentrations of 250 & 100 µg/ml of metronidazole and miconazole nitrate respectively. Each solution was divided into two parts A and B. Part A was kept at room Temperature for 48 hrs to check its chemical stability and precipitation, if any. Part B of both solutions was further diluted with distilled water separately to get various dilutions having concentration range of 2-40 µg/ml of both the drugs. Absorbances of resulting metronidazole and miconazole nitrate dilutions were noted at 325 & 285 nm respectively against corresponding reagent blanks. Calibration curve was plotted as concentration versus absorbance. Both the drugs obeyed Beer's law within the concentration range of 5-30 µg/ml for metronidazole as well as formiconazole nitrate. The absorptivity values (A 1%, 1 cm) of both drug at selected wavelengths was determined. Part A solution was analyzed in the same way as part B solution.

Development of UV method

Three simple, accurate spectrophotometric methods Simultaneous equations, Absorbance ratio or Q-analysis method, Dual wavelength have been developed for the simultaneous determination of metronidazole and miconazole nitrate in gel and ovule dosage forms.

Method 1: Simultaneous equations method

Two wavelengths selected for the method are 285 nm and 325 nm that are absorption maximas of miconazole nitrate and metronidazole respectively in solution containing 40% urea and 10% sodium benzoate. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1

cm) for both the drugs at both wavelengths were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations-

$$Cx = \frac{A1ay2 - A2ay1}{ax1ay2 - ax2ay1} \quad \text{--------Eq 1}$$

$$Cy = \frac{A1ax2 - A2ax1}{ay1ax2 - ay2ax1} \quad \text{---------Eq 2}$$

Where, A1 and A2 are absorbances of mixture at 285 nm and 325 nm respectively, ax1 and ax2 are absorptivities of miconazole nitrate at λ1 and λ2 respectively and ay1 and ay2 are absorptivities of metronidazole at λ1 and λ2 respectively. Cx and Cy are concentrations of miconazole nitrate and metronidazole respectively.

Method II (Absorbance ratio or Q-analysis method)

From the overlain spectrum of miconazole nitrate and metronidazole (fig. 1), two wavelengths were selected one at 296 nm which is the isoabsorptive point for both the drugs and the other at 285 nm which is λ max of miconazole nitrate. The absorbances were measured and the absorptivity i.e. A(1%, 1cm) values for both drugs at the selected wavelengths were also calculated. The method employs Q values and the concentrations of drugs in sample solution were determined by using the following formula,

For miconazole nitrate

$$Cx = \frac{Qm - Qx}{Qy - Qx} \times \frac{A1}{ax1}$$

For metronidazole

$$Cy = \frac{Qm - Qy}{Qx - Qy} \times \frac{A1}{ay1}$$

Where,

$$Qm = \frac{\text{Absorbance of sample at 282 nm}}{\text{Absorbance of sample at 296 nm}}$$

$$Qx = \frac{\text{Absorptivity of miconazole nitrate at 282 nm}}{\text{Absorptivity of miconazole nitrate at 296nm}}$$

$$Qy = \frac{\text{Absorptivity of metronidazole at 282 nm}}{\text{Absorptivity of metronidazole at 296nm}}$$

A1 = Absorbance of sample at isoabsorptive point,

ax1 and ay1 = Absorptivities of miconazole nitrate and metronidazole respectively at isoabsorptive point.

Method 3: **Dual Wavelength Method**

For estimation of one component, two wave lengths were selected, where the absorbances of other component were same. Therefore the difference in the absorbances in the mixed spectra at the corresponding wavelengths will be directly proportional to the concentration of that component. For miconazole nitrate, 300 nm (λ1) and 333 nm (λ2), for metronidazole 283 nm (λ1) and 303 nm(λ2) were selected. The difference in the absorbances at the selected wavelengths, were plotted against the respective concentration to obtain the calibration curves. The concentration in sample solutions of each component was obtained from the calibration curves of the respective drugs.

Application to gel

Accurately weighed amount of gel equivalent to 25 mg of metronidazole (12.5 mg miconazole nitrate) was dissolved in 40 ml of solution containing 45% urea and 10% sodium benzoate and sonicated in bath sonicator for 15 min to solubilize the drug and the final volume of both solutions was made up to 100ml with distilled water. It was filtered through Whatman filter paper # 41. Filtered extract was appropriately diluted with distilled water to obtain mixed standards in the linearity range for each drug. The sample solutions were scanned in the selected wavelength region for respective methods,(method I, II and III) and the results were obtained are reported in the (**Table 1**).

Application to gel

Twenty ovules containing miconazole nitrate and metronidazolewere accurately weighed and cut. Accurately weighed amount of cutting ovules equivalent to 25 mg of metronidazole (12.5 mg miconazole nitrate) was dissolved in 40 ml of solution containing 40% urea and 10% sodium benzoate and the mixture was mixed on a hot plate stirrer at 60^{o}Cfor 15min. After this period, the solution was cooled andsonicated in bath sonicator for 15 min to solubilize the drugand the final volume of both solutions was made up to 100ml with distilled water. It was filtered through Whatman filter paper # 41. Filtered extract was appropriately diluted with distilled water to obtain mixed standards in the linearity range for each drug. The sample solutions were scanned in the selected wavelength region for respective methods,(method I, II and III) and the results were obtained are reported in the (**Table 2**).

Recovery studies

To evaluate the validity and reproducibility of the proposed method, recovery experiments were carried out. Recovery study was carried out as per ICH Q Guidelines at three different concentration levels- 80%, 100%, 120% by replicate analysis (n=3). Here to a preanalysed sample solution,

standard drug solutions were added as spiked concentrations and drug contents were determined by the above proposed analytical methods. The results of analysis of recovery studies are presented in (**Table 3**).

Validation of developed method

The methods were validated statistically as per ICH guidelines for parameter like accuracy, precision, specificity, LOD, LOQ, ruggedness, linearity and range.

Accuracy

Accuracy of the developed method was confirmed by doing recovery study as per ICH norms at three different concentration levels- 80%, 100%, 120% by replicate analysis (n=3). Here to a preanalysed sample solution, standard drug solutions were added and then percentage of drug content was calculated. The result of accuracy study was reported in Table 3. From the recovery study it was clear that the method is very accurate for quantitative estimation of metronidazole and miconazole nitrate in tablet dosage form.

Precision

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated. Repeatability was performed for six times with tablets formulation. The results of statistical evaluation are given in Table3. Intermediate Precision was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the solutions of miconazole nitrate and metronidazole i.e. dilutions for miconazole nitrate (4 µg/ml, 8 µg/ml, 12 µg/ml) and for metronidazole (10 µg/ml, 20 µg/ml, and 30 µg/ml) for three times in the same day. Inter-day precision was determined by analyzing the same concentration range of solutions daily for three days, results were recorded.The %RSD of interday and intraday precision was determined and reported in table 4. From the data obtained, the developed spectroscopic method was found to be precise and accurate.

Specificity

The specificity of the method was checked for the interference of impurities in the analysis of a drug solution. As there was no interference of impurities, the method was found to be specific.

Linearity and Range

Appropriate dilutions of standard stock solutions were analysed as per the developed methods.

Both the drugs obeyed Beer's law within the concentration range of 5-30 µg/ml for metronidazole and 2-10 µg/ml for miconazole nitrate. The linearity data are presented in (**Table 4**).

LOD and LOQ

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 3∂/S and 10 ∂/S, respectively as per ICH guidelines, where∂ is the standard deviation of the response and S is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified. The result was reported in Table 4.

Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments and by different operators. It was observed that there were no marked changes in the results, which demonstrated that the spectroscopic method developed, are rugged and robust.

RESULTS AND DISCUSSION

Based on the solubility and stability and spectral characteristics of the drug, mixed hydrotropic solution, containing 40% urea and 10% sodium benzoate was selected as hydrotropic agent. Part A solution of drugs i.e.miconazole nitrate and metronidazole in hydrotropic solution was kept at room temperature for 48 hrs. There was no precipitation of drug in Part A solution within 48 hrs.In addition, drug contents of Part A solutions (after 48 hrs) were same as those of Part B of both solutions (fresh solutions). This study reveals that the estimations can be done within 48 hrs at least, without having any detrimental effect on drug stability.

It is evident from Table 1 & 2 that the value of mean percent drugs (miconazole nitrate & metronidazole) estimated by proposed spectrophotometric methods I, II & III for both formulations gel and ovule are very close to 100.0, indicating the accuracy of the proposed method of analysis. Low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method. The percent recoveries estimated ranged from 98 to 101%. The values are close to 100 indicating the accuracy of the proposed method Table 3.

Table 1: Analysis data of Commercial ovule of Miconazole nitrate and Metronidazole

Method Name	Lable claim (Mg/tab)		% lable claim estimated (Mean±SD)*		% COV		SE	
	MNZ	MIZ	MNZ	MIZ	MNZ	MIZ	MNZ	MIZ
SEM	500	150	99.43±0.229	98.34±0.559	0.232	0.363	0.402	0.250
ARM	500	150	98.82±0.210	99.89±o.527	0.410	0.633	0.0939	0.235
DWM	500	150	99.78±0.535	99.77±0.553	0.536	0.454	0.239	0.447

SEM: *Simultaneous Equation Method,* ***ARM:****Absorbance Ratio Method,* ***DWM: Dual*** *Wavelength Method* ***MIZ:*** *Miconazole nitrate,* ***MNZ:*** *Metronidazole, SD: Standard deviation,* ***COV****: Coefficient of variation, SE: Standard error*
**Average of three determination*

Table 2: Analysis data of Commercial GEL of Miconazole nitrate and Metronidazole

Method Name	Lable claim %w/w		% lable claim estimated (Mean±SD)*		% COV		SE	
	MNZ	MIZ	MNZ	MIZ	MNZ	MIZ	MNZ	MIZ
SEM	1	2	99.64±0.269	98.57±0.559	0.594	0.875	0.974	0.559
ARM	1	2	99.72±0.236	99.36±o.250	0.350	0.269	0.358	0.259
DWM	1	2	99.74±0.845	99.32±0.304	0.763	0.369	0.852	0.358

SEM: *Simultaneous Equation Method,* ***ARM:****Absorbance Ratio Method,* ***DWM:*** *Dual Wavelength Method, MIZ: Miconazole nitrate* ***MNZ:*** *Metronidazole ,* ***SD****: Standard deviation,* ***COV****: Coefficient of variation,* ***SE****: Standard error*
**Average of three determination*

Table 3: Recovery Study for Spiked Concentration of Miconazole nitrate and Metronidazole added to Prenalyzed sample solution

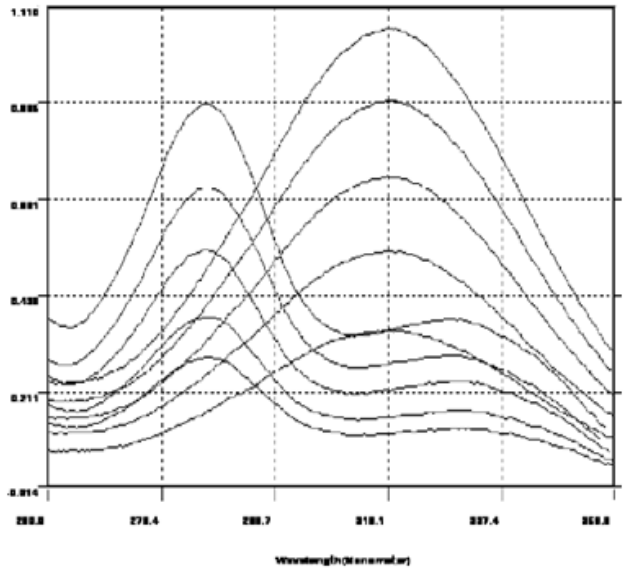
Method Name	Level of % Recovery	% Recovery Found ± (SD)*		% COV		Standard error	
		MNZ	MIZ	MNZ	MIZ	MNZ	MIZ
SEM	80	98.85±0.467	99.82±0.548	0.425	0.549	0.269	0.245
	100	99.04±0.282	99.83±0.431	0.291	0.432	0.166	0.194
	120	99.83±0.321	99.54±0.289	0.325	0.289	0.184	0.129
ARM	80	99.75±0.382	99.26±0.231	0.382	0.214	0.220	0.094
	100	100.2±0.421	99.59±0.436	0.423	0.433	0.243	0.176
	120	99.60±0.403	99.23±0.765	0.416	0.774	0.238	0.313
DWM	80	99.70±0.703	98.98±0.729	0.688	0.765	0.404	0.212
	100	99.60±0.720	99.75±0.564	0.722	0.452	0.415	0.180
	120	99.53±0.543	98.04±0.691	0.551	0.926	0.317	0.240

** Average of three determination*

Table 4: Summary of optical characteristics and validation paramters

Parameters	SEM		ARM		DWM	
	MNZ	MIZ	MNZ	MIZ	MNZ	MIZ
Λ max	282nm	325nm	-	-	-	-
Beer's law limit(range)	2-10µg/ml	5-30 µg/ml	-	-	-	-
Linearity (r^2)	0.9989	0.9991	0.9992	0.9988	0.9986	0.9992
Specificity*	Specific	Specific	Specific	Specific	Specific	Specific
Accuracy (Recovery)	98.89%	99.82%	99.75%	99.83%	99.70%	99.54%
Precision Intraday(%RSD)	0.577	0.728	0.690	0.243	0.394	0.314
Interday(%RSD)	0.291	0.571	0.415	0.114	0.149	0.421
LOD	0.675	0.978	0.657	0.978	0.108	0.104
LOQ	2.04	2.964	1.981	2.964	0.330	0.317
Intercept	0.592	0.148	0.029	0.0111	0.146	0.0462
Slope	0.0018	0.0019	0.0003	0.0283	0.0041	0.0480

Fig 1: Overlain spectra of metronidazole and miconazole nitrate



CONCLUSION

Most of the organic solvents like ethanol, methanol, acetonitrile, hexane, cyclohexane, diethyl ether, chloroform and toluene find wide use in spectrophotometic analysis of poorly water-soluble drugs. Most of these organic solvents are toxic in nature, costlier and responsible for pollution. Inaccuracy in spectrophotometric estimation due to volatility is another drawback of organic solvents. Since urea and sodium citrate are cheaper than most of the organic solvents, therefore other poorly water-soluble drugs can also be estimated by similar mixed hydrotropy avoiding the use of organic solvents. It is, thus, concluded that the proposed method is new, simple, cost-effective, safe, accurate, precise and environmentally friendly. This method can be successfully employed in the routine analysis of

metronidazole and miconazole nitrate in tablet dosage form. Like this method other hydotrophes can also be tried by combining them to exert synergistic effect on solubility of poorly water soluble drugs to be applied in different fields of analysis. Mixed hydrotropy may find wide use in development of aqueous formulations of poorly water soluble drugs in future.

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