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

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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 benzoate as 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 ketoconazole¹,². 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
Preliminary solubility studies of drugs were used of analytical grade for spectrophotometric analysis. All the chemicals and SEIMENS Laboratories Gurgaon, India, visible recording spectrophotometer (model - UV-2600) - with 1 cm matched quartz cells were used and SEIMENS Laboratories Gurgaon, India, visible recording spectrophotometer (model - UV-2600) - with 1 cm matched quartz cells were used and SEIMENS Laboratories Gurgaon, India, visible recording spectrophotometer (model - UV-2600) - with 1 cm matched quartz cells were used and SEIMENS Laboratories Gurgaon, India, visible recording spectrophotometer (model - UV-2600) - with 1 cm matched quartz cells were used and SEIMENS Laboratories Gurgaon, India, 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.

**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 100 ml 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 for miconazole nitrate. The absorbity 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 maxima 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) - 384
Absorptivity of miconazole nitrate at 282 nm

Method 3

Dual Wavelength Method

For estimation of one component, two wavelengths 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 metronidazolere were 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°Cfor 15min. After this period, the solution was cooled and sonicated 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,
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 3δ/S and 10 δ/S, respectively as per ICH guidelines, whereδ 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.
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Table 1: Analysis data of Commercial ovule of Miconazole nitrate and Metronidazole

<table>
<thead>
<tr>
<th>Method Name</th>
<th>Lab claim (Mg/tab)</th>
<th>% Lab claim estimated (Mean±SD)*</th>
<th>% COV</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEM</td>
<td>500 150</td>
<td>99.43±0.229</td>
<td>0.232</td>
<td>0.365</td>
</tr>
<tr>
<td>ARM</td>
<td>500 150</td>
<td>98.82±0.210</td>
<td>0.410</td>
<td>0.633</td>
</tr>
<tr>
<td>DWM</td>
<td>500 150</td>
<td>99.78±0.535</td>
<td>0.536</td>
<td>0.454</td>
</tr>
</tbody>
</table>

*Average of three determination

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

<table>
<thead>
<tr>
<th>Method Name</th>
<th>Lab claim %w/w</th>
<th>% Lab claim estimated (Mean±SD)*</th>
<th>% COV</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEM</td>
<td>1 2</td>
<td>99.64±0.269</td>
<td>0.594</td>
<td>0.875</td>
</tr>
<tr>
<td>ARM</td>
<td>1 2</td>
<td>99.72±0.236</td>
<td>0.350</td>
<td>0.269</td>
</tr>
<tr>
<td>DWM</td>
<td>1 2</td>
<td>99.74±0.845</td>
<td>0.763</td>
<td>0.369</td>
</tr>
</tbody>
</table>

*Average of three determination

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

<table>
<thead>
<tr>
<th>Method Name</th>
<th>Level of % Recovery</th>
<th>% Recovery Found ±(SD)*</th>
<th>% COV</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEM</td>
<td>80</td>
<td>98.85±0.467</td>
<td>0.425</td>
<td>0.549</td>
</tr>
<tr>
<td>ARM</td>
<td>100</td>
<td>99.04±0.282</td>
<td>0.291</td>
<td>0.432</td>
</tr>
<tr>
<td>DWM</td>
<td>120</td>
<td>99.83±0.321</td>
<td>0.325</td>
<td>0.289</td>
</tr>
</tbody>
</table>

*Average of three determination

Table 4: Summary of optical characteristics and validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MINZ</th>
<th>SEM</th>
<th>Arm</th>
<th>DWM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A max</td>
<td>282nm</td>
<td>325nm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Beer’s law limit(μg/ml)</td>
<td>2-10</td>
<td>5-30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linearity (r²)</td>
<td>0.9989</td>
<td>0.9991</td>
<td>0.9992</td>
<td>0.9988</td>
</tr>
<tr>
<td>Specificity*</td>
<td>Specific</td>
<td>Specific</td>
<td>Specific</td>
<td>Specific</td>
</tr>
<tr>
<td>Accuracy (Recovery)</td>
<td>98.89%</td>
<td>99.82%</td>
<td>99.75%</td>
<td>99.83%</td>
</tr>
<tr>
<td>Precision Intraday(%RSD)</td>
<td>0.577</td>
<td>0.728</td>
<td>0.690</td>
<td>0.243</td>
</tr>
<tr>
<td>Interday(%RSD)</td>
<td>0.291</td>
<td>0.571</td>
<td>0.415</td>
<td>0.114</td>
</tr>
<tr>
<td>LOD</td>
<td>0.675</td>
<td>0.978</td>
<td>0.657</td>
<td>0.978</td>
</tr>
<tr>
<td>LOQ</td>
<td>2.04</td>
<td>2.964</td>
<td>1.981</td>
<td>2.964</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.592</td>
<td>0.148</td>
<td>0.029</td>
<td>0.0111</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0018</td>
<td>0.0019</td>
<td>0.0003</td>
<td>0.0283</td>
</tr>
</tbody>
</table>

CONCLUSION

Most of the organic solvents like ethanol, methanol, acetone, hexane, cyclohexane, diethyl ether, chloroform and toluene find wide use in spectrophotometric 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 hydrotropes 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.

REFERENCES


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