DETERMINATION OF MOSAPRIDE AND PANTOPRAZOLE IN A FIXED-DOSE COMBINATION BY UV SPECTROPHOTOMETRIC METHODS AND RP-HPLC

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ABSTRACT

An accurate and reproducible UV-spectrophotometric methods and liquid chromatographic assay method were developed and validated for the determination of mosapride and pantaprazole in capsule formulation. Two wavelengths were selected for each UV method, first simultaneous equation 274 nm, 288.2 nm and second Q value analysis method 274 nm, 302 nm was the isobestic point for both the drugs. The 30 mM ammonium sulphate buffer : acetonitrile (50:50, v/v) was used for reverse-phase liquid chromatography to determine the contents of mosapride and pantaprazole in combination-capsule dosage form. The UV and HPLC methods were validated by determining parameters such as specificity, linearity, LOD and LOQ, precision, accuracy, ruggedness and robustness. The methods were found to be specific against placebo interference. Linearity was evaluated over the concentration range of 5-50.0 µg/mL by UV and 0.5 to 5.0 µg/mL by HPLC method respectively, for mosapride and pantaprazole (the value of R² 0.999 found were by both the methods for mosapride and pantaprazole). Both the intraday and interday precision values of the systems and methods were determined. The accuracy of the methods ranged from 99.99 to 102.24 % for mosapride and from 100.45 to 101.22 % for pantaprazole. The proposed methods were found to be robust when slight but deliberate changes were made in analytical conditions. The developed methods were found suitable for the simultaneous estimation of mosapride and pantaprazole in capsule formulation as well in raw materials for quality control.

INTRODUCTION

The combined dosage form of any pharmaceuticals is for the synergistic effect or to give longer time effect. In present study Mosapride citrate and Pantoprazole combination is used as antacid to decrease acidity (excess secretion of acid in stomach). Mosapride citrate (MOSP), known as (±) 4-amino-5-chloro-2-ethoxy-N-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl] benzamide 2-hydroxy-1,2,3-propanetricarboxylate citrate, dihydrate is a novel gastroprokinetic agent plays an important role in conjunction with life-style modifications in short and long term management of gastroesophageal reflux disease and dyspepsia in many of the asian countries. Pantoprazole (PANT), 5-(difluoromethoxy)- 2- [(3, 4- dimethoxy -2-pyridyl)methylsulfinyl][1H-benzimidazole, is a selective and long-acting proton pump inhibitor (1). The literature review reveals that only a few methods have been developed for the quantification of individual drug MOSP and PANT. Determination of related-substances of MOSP in bulk drugs and pharmaceuticals by HPLC (2-4), identification of a polar impurity in MOSP by LC- MS/MS and by LC- MS in equine tissues (5,6), structural identification and characterization of potential impurities of PANT (7), determination of PANT by HPLC in human plasma (8), HPLC separation of domperidone and pantoprazole (9), enantiomeric determination of PANT in human plasma by HPLC (10) are the current analytical methods for analysis of MOSP and PANT. In proposed method carried out for the simultaneous determination of MOSP and PANT in capsule formulation by two UV-spectrophotometric methods and RP-HPLC method. The validation of methods were carried out as per ICH guidelines (11-13). The chemical structures of the drugs are as shown in Figure 1.

Experimental:
Instrumentation
HPLC
The chromatographic separation was performed on the Waters liquid chromatographic system equipped with (Waters-1515) isocratic solvent delivery system pump with (Waters-2487) dual wavelength absorbance UV-detector and Rheodyne 7725i injector with 50 µL loop volume. Breeze 3.3 data station was applied for data collecting and processing. A phenomenox C18 column (250mm x 4.6mm i. d., 5µm particle size) was used for the separation.
A Shimadzu UV-Visible Spectrophotometer-160A was used. The mobile phase was prepared daily, filtered, sonicated before use and delivered at the flow rate of 1.0 mL/min.

**UV-Spectrophotometer**

The mobile phase consisted of a mixture of acetonitrile-30 mM ammonium sulphate buffer (pH 5.5) in the ratio of (50:50, v/v) [without adjustment of pH by any other buffer, it avoids complications of pH adjustment is the advantage of this buffer] The mobile phase was prepared daily, filtered, sonicated before use and delivered at the flow rate of 1.0 mL/min. The optimal conditions for recording the spectra to achieve good reproducibility included scan speed at 60nm/s, slit width at 2 nm. The detection wavelength fixed at 275 nm as isobestic point for both drugs as shown in Figure 2.

**Chemicals and reagents**

The pharmaceutical grade gift reference standard of Mosapride citrate (99.84 %) obtained from HAB Pharmaceutical & Research Ltd. Worli, Mumbai, India. Pantoprazole (99.18 %) and Hydrochlorothiazide (99.42 %) (Internal standard) were procured from M/S. Apex pharmaceuticals (Andrapradesh), India., MOSA PLUS Capsule formulations from market. Acetonitrile and methanol used were of HPLC grade; all analytical grade chemicals and solvents were obtained from E Merck (India) Ltd, Mumbai. Ammonium sulphate AR grade was procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system.

**Figure 1. Chemical Structures of Mosapride citrate, Hydrochlorothiazide (IS), Pantoprazole**

**Figure 2 Overlay UV-spectrum for Mosapride citrate and Pantoprazole**
Standard solutions and calibration curves

For HPLC

Standard stock solutions were prepared at concentration of 1 mg/mL of MOSP and PANT separately using a mixture of water and acetonitrile (1:1, v/v) for HPLC method. The working standard solutions were prepared of different concentrations ranging from 0.5 to 5.0 µg/mL for MOSP and PANT respectively, by maintaining the concentration of hydrochlorothiazide (IS) at a constant level of 10 µg/mL. From the above each mixture 50 µL was injected in triplicate for the estimation of each standard drugs, under the optimized chromatographic conditions, a steady baseline was recorded; the typical chromatogram was recorded for internal standard, pantoprazole and mosapride standards as shown in Figure 5. The retention times of internal standard, pantoprazole and mosapride were found to be 3.58, 5.68 and 8.85 min, respectively. The calibration curve was obtained by simple linear regression of concentration of drug to the response factor.

For UV-Spectrophotometry

Standard stock solutions were prepared at concentration of 1 mg/mL of MOSP and PANT separately using a mixture of water and methanol (1:1, v/v) for spectrophotometric measurements. From stock solutions, the working standard solutions were prepared of different concentrations ranging from 5.0 to 50.0 µg/mL for MOSP and PANT respectively and scanned in the wavelength range of 200-400 nm. Two wavelengths selected for simultaneous equation were 274, 288.2 nm respectively as shown in Figure 2. $\varepsilon (A_{\text{1%}}, 1\text{cm})$ is calculated for each standard drug by measuring absorbance of 1% solution at 1cm path length. Similarly, mixed
standard solutions were used for UV-
spectrometric analysis by simultaneous equation
and Q-analysis method.

**Analysis of formulation by HPLC**

Twenty capsules were weighed and a quantity of powder equivalent to one capsule was weighed and transferred to 50 ml of mixture of acetonitrile and water (1:1, v/v) dissolved and filtered. The combined extracts were made up to 100 mL by adding required amount of IS with same mixture. The required amount of solution was centrifuged and further dilutions were made with mobile phase to get a concentration of MOSP, PANT and hydrochlorothiazide (IS) 2 µg/mL, 4 µg/mL and 10 µg/mL respectively. From the above mixture 50µL was injected in triplicate for the estimation of drugs in sample, under the optimized chromatographic conditions, a steady baseline was recorded; the typical chromatogram for sample was recorded as shown in Figure 6. The retention times of sample hydrochlorothiazide (IS), pantaprazole and mosapride were found to be 3.56, 5.67 and 8.84 min respectively. The detection wavelength was fixed at 275 nm.

![Figure 5. Typical chromatogram of standard solution with IS:](image1)

1) Peak of Hydrochlorothiazide (IS) at 3.58 min  
2) Peak of Pantoprazole at 5.68 min  
3) Peak of Mosapride citrate at 8.85 min.

![Figure 6. Typical chromatogram of sample solution with IS:](image2)

1) Peak of Hydrochlorothiazide at 3.56 min 
2) Peak of Pantoprazole at 5.67 min  
3) Peak of Mosapride citrate at 8.84 min.
Analysis of formulation by UV

Twenty capsules were weighed and a quantity of powder equivalent to one capsule was weighed and transferred to 50 ml of mixture of methanol and water (1:1, v/v) dissolved and filtered. The combined extracts were made up to 100 ml with same mixture. Further dilutions were made to get sample solution containing 15 µg/mL, 20 µg/mL MOSP, PANT respectively and scanned in the wavelength range of 200-400 nm. Two wavelengths selected for simultaneous equation method were 274 nm, 288.2 nm and for Q value analysis 274 nm and 302 nm was the isobestic point for both the standard drugs.

Method 1: Simultaneous Equation method

For simultaneous equation method the absorbance of standard individual solutions were measured at two wavelengths 274 nm, 288.2 nm and from that calculated absorptivity of MOSP and PANT respectively. The sample solution was measured at two wavelengths 274 nm, 288.2 nm as A₁ and A₂ respectively and concentration of the individual two drugs in sample was calculated using simultaneous equation. The method employs solving of simultaneous equations using Cramer’s rule and matrices.

The simultaneous equations were

\[ A_1 = C_{1m} \times C_1 + C_{1p} \times C_2 \] (1)
\[ A_2 = C_{2m} \times C_1 + C_{2p} \times C_2 \] (2)

C₁ₙ and C₂ₙ absorptivity values of MOSP 274 nm wavelength
C₁ₚ and C₂ₚ absorptivity values of PANT 288.2 nm wavelength
C₁ and C₂ are concentrations of MOSP and PANT respectively in sample solution.

Method 2: Q value analysis method

The overlay spectrum of standard solutions observed for MOSP and PANT were as shown in Figure 2. The two wavelengths were selected for measure absorbance, first at 302 nm as an isoabsorptive point for both the drugs and second at 274 nm wavelength of MOSP. The dilutions of the standard and sample solutions were carried out as reported in simultaneous equation method. The absorptivity values for both drugs at the selected wavelength were calculated and employed for Q analysis, the concentration of drugs in sample solution were determined by using the following formula.

For MOSP

\[ Q_0 = \frac{A}{Q_1 - \frac{Q_0}{Q_2 - Q_1} \times \frac{C_1}{a_1}} \]
Absorptivity of sample at 274nm

For PANT

\[ Q_0 = \frac{A}{Q_1 - \frac{Q_0}{Q_2 - Q_1} \times \frac{C_1}{a_1}} \]
Absorptivity of sample at 302nm

In the above equation A was absorbance of sample at isoabsorptive point and a₁ and a₂ were absorptivity values of MOSP and PANT respectively at isoabsorptivity point.

Results and discussion

The aim of the present work was to develop simple and reproducible UV-spectrophotometric and RP-HPLC with ultraviolet detection for the simultaneous determination of MOSP and PANT in solid pharmaceutical dosage forms. As the solubility of MOSP and PANT was sparingly soluble in water therefore mixture of acetonitrile and water used (1:1, v/v) for HPLC, which improved resolution and peak shape. The mixture of methanol and water (1:1, v/v) for spectrophotometry was used as solvent for preparation of all standard and sample solutions as easily available and cost effective by using methanol and water.

HPLC Method development

Several attempts were performed in order to get satisfactory resolution of MOSP and PANT in different mobile phases with various ratios mixture of organic phases and buffers by using C₁₈ column. Initially the mobile phase used was
mixture of water and methanol followed by water and acetonitrile in different ratios. The mobile phase used was acetonitrile-ammonium acetate buffer (pH 5.5) in the ratio (60:40, v/v) by isocratic elution could not give satisfactory resolution. Further Acetonitrile and 30 mM ammonium sulphate buffer (pH 5.5) in ratio of (50:50, v/v) mobile phase was used by isocratic elution shown satisfactory and good resolution with internal standard hydrochlorothiazide. The effect of solvent composition by changing the ratio of acetonitrile-ammonium sulphate buffer in the ratio of (60:40, v/v) and (80:20, v/v) could not gave satisfactory resolution. Therefore this method was sensitive to mobile phase ratio. The effect of change in pH of mobile phase by ± 0.2 does not shown significant change in retention time of each analyte. The retention time of MOSP and PANT with hydrochlorothiazide (IS) on C18 column was found satisfactory with above mobile phase at a flow rate of 1.0 mL/min. The resolution was found reproducible and satisfactory.

**Selection of UV wavelength and internal standard**

The detector wavelength of the present study was selected on the basis of higher sensitivity. The internal standard was selected due to its suitable retention time, recovery and lack of interference with endogenous peaks and also not much affected by the mobile phase pH. These phenomena helped their good separation with other peaks.

**UV Method development**

Ultraviolet absorption spectrum of each standard drug was scanned at 200-400 nm. The observed maximum absorbance of MOSP at 274 nm and PANT at 288.2 nm. The standard of each drug (1%)solutions were prepared and measured absorbance at both the wavelengths for calculating absorptivity of each drug with respect to its wavelengths C_1m, C_2m and C_1p, C_2p. The absorbance of sample solutions was also noted at the respective wavelengths for both drugs.

Similarly for Q-analysis method two wavelengths selected for the formulation one was wavelength of MOSP at 274 nm and the other was an isoabsorptive point at 302 nm. Absorbances were noted for standard and sample solutions. By calculating Q-values for each drug by formulae and content of each drug was determined in the formulation. Statistical parameters were reported in Table 2. Recovery study carried out for both the methods were performed by spiking the known standard drug in powdered formulations. The results of the recovery analysis were represented in Table 1.

**Method validation**

**Linearity and range**

**HPLC**

The linearity and range for HPLC method was determined at six concentration levels for MOSP and PANT. The linearity and range of MOSP and PANT were found as 0.5-5.0 µg/mL and 0.5-5.0 µg/mL respectively. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept values of calibration curve for MOSP y = 0.0512X-0.0057 (R^2 = 0.9994) and for PANT y = 0.0502 X-0.0053 (R^2 = 0.9986) where Y represents the ratio of peak area ratio of analyte to IS and X represents analyte concentration.

**UV Method**

The linearity and range for UV method was determined at six concentration levels for MOSP and PANT. The linearity and range of MOSP and PANT were found as 5-50.0 µg/mL and 5 - 50.0 µg/mL respectively. The calibration curve was constructed by plotting absorbance against concentration of drugs. The slope and intercept values of calibration curve for MOSP y = 0.0233X+ 0.0217 (R^2 = 0.999) and for PANT y = 0.039 X +0.0369 (R^2 = 0.999).

**Accuracy and precision**

The accuracy of the methods was determined by the method of standard addition at three different levels. The recovery studies were carried out for capsules by spiking standard of each drugs equivalent to 80%, 100%, and 120% to the original amounts present in each drug formulations. The average recoveries were as reported in Table 1.
The precision of the method was assessed by replicate analysis of pharmaceutical preparations. The precision and accuracy of HPLC and UV methods were obtained by analyze on the same day (intra-day) and analyze on the different days by triplicate analysis (inter-day) precision and expressed as relative standard deviation percentage (R.S.D. %). The correlation coefficient and the data on precision and accuracy are reported in Table 2.

**LOD and LOQ**

The sensitivity of MOSP and PANT was estimated as limit of detection (LOD) and limit of quantification (LOQ), they were calculated by the use of the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of the noise, and B is the slope of the corresponding calibration plot by HPLC method and where N is the standard deviation of the absorbance of the drugs (n=3) and B is the slope of the corresponding calibration plot by UV-spectrophotometric method. The LOD and LOQ Values were reported in Table 2.

**Recovery and stability**

The recovery studies were carried out for capsules by spiking standard of each drugs equivalent to 80%, 100%, and 120% to the original amounts present in each drug formulations.

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The retention time and peak area of MOSP and PANT remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 h.

### Table 1: Results of analysis of formulation and recovery studies

<table>
<thead>
<tr>
<th>Methods</th>
<th>Label claim mg/Capsule</th>
<th>Amount found mg/Capsule</th>
<th>% Recovery ± SD (n=6)</th>
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<tr>
<td></td>
<td>MOSP</td>
<td>PANT</td>
<td>MOSP</td>
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<tr>
<td>Simultaneous Equation Method</td>
<td>15 20</td>
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<td>19.52</td>
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<td>Q-Value Analysis Method</td>
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<td>19.75</td>
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<td>RP-HPLC Method</td>
<td>15 20</td>
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### Table 2: Statistical analysis of determination of MOSP and PANT in raw material by UV and HPLC methods

<table>
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<tr>
<th>Statistical parameters</th>
<th>Simultaneous Equation</th>
<th>Q-analysis</th>
<th>RF-HPLC</th>
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<tr>
<td></td>
<td>MOSP</td>
<td>PANT</td>
<td>MOSP</td>
</tr>
<tr>
<td>Linearity and range (µg/mL)</td>
<td>5.50</td>
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<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
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<tr>
<td>Standard deviation</td>
<td>0.024</td>
<td>0.042</td>
<td>0.022</td>
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<tr>
<td>LOD (µg/mL)</td>
<td>0.15</td>
<td>0.12</td>
<td>0.15</td>
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<tr>
<td>LOQ (µg/mL)</td>
<td>0.45</td>
<td>0.35</td>
<td>0.45</td>
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<tr>
<td>Accuracy (%)</td>
<td>99.81</td>
<td>100.2</td>
<td>99.81</td>
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<tr>
<td>Precision %RSD</td>
<td>Interday</td>
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<td>0.53</td>
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<tr>
<td></td>
<td>Intraday</td>
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<td>0.93</td>
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CONCLUSIONS
In conclusion, a novel HPLC and two simple reproducible UV-spectrophotometric methods were developed and validated for the simultaneous determination of MOSP and PANT in solid dosage form. It assured the satisfactory precision and accuracy and has high analytical potential. The proposed methods were found to be simple, accurate, economical and reproducible and can be applied for routine analysis in laboratories. They are suitable for the quality control of the raw materials, formulations, dissolution studies and HPLC method can be employed for bioequivalence studies for the same formulation.

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