A validated normal phase HPLC method for simultaneous determination of drotaverine hydrochloride and omeprazole in pharmaceutical formulation

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A simple, precise, specific and accurate normal phase HPLC method has been developed for the simultaneous determination of drotaverine hydrochloride and omeprazole in tablet dosage form. The chromatographic separation was achieved on HiQsil column using UV detector. The mobile phase consisting of n-heptane: dichloromethane: methanolic ammonia (5%): methanol at a flow rate of 1.0 ml/min was used. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

Keywords: Drotaverine hydrochloride, Omeprazole, Normal phase HPLC.

INTRODUCTION

Drotaverine hydrochloride is chemically known as 1-[(3, 4-[diethoxyphenyl) methylene]-6, 7 diethoxy-1, 2, 3, 4 - tetrahydroisoquinoline hydrochloride [1]. Omeprazole is chemically known as 6-methoxy-2-[(4-methoxy-3, 5- dimethylpyridin-2-yl) methylsulfinyl]-1H-benzimidazole. Drotaverine hydrochloride is highly potent spasmyloytic agent [2]. Omeprazole is used as an antiulcer drug and against other acid-related diseases [3]. Literature survey reveals that both USP 2007 [4] and IP 2007 [5] report HPLC method for assay of omeprazole. Several analytical methods that have been reported for estimation of drotaverine hydrochloride are spectrophotometry [6, 7, 8], HPLC [9, 10, 11], thin layer chromatography [12, 13] and voltametry [14]. Analytical methods reported for the estimation of omeprazole are HPLC [15-21], LC-MS [22] and HPTLC [23]. An RP-HPLC method has also been reported for this combination [24]. To the best of our knowledge, there is no published NP-HPLC method for this combination. The present paper describes a simple, accurate and precise method for simultaneous estimation of drotaverine hydrochloride and omeprazole in combined tablet dosage form. The present NP-HPLC method was validated following the ICH guidelines [25].

MATERIALS AND METHODS

Reagents and chemicals

Dichloromethane, n-heptane, methanol and water of HPLC grade were procured from Ashnoj specialties Pvt. Ltd., Navi Mumbai. Working standard of drotaverine hydrochloride and omeprazole was procured from Zydus Cadila Healthcare Ltd. India.

Equipment

Chromatographic separation was performed on a Jasco HPLC system equipped with a Jasco PU-2080 plus intelligent pump, Jasco UV-2075 plus UV detector and Rheodyne injector with 50 μl loop volume.

Chromatographic conditions

The mobile phase consisting of n-heptane:dichloromethane:methanolic ammonia (5%):methanol (50:25:1:4) at a flow rate of 1.0 ml/min was used. Mobile phase was prepared by first mixing the methanolic ammonia with methanol. Then dichloromethane was added to it followed by n-heptane. Precaution must be taken while mixing the solvents. The mobile phase was then filtered through membrane.
filter and sonicated for 15 min in ultrasonic bath. The column HiQSil (4.6mm x 250mm) was used at ambient temperature.

**Preparation of standard stock solution**

Standard stock solutions of drotavamine hydrochloride and omeprazole of strength 1mg/ml were prepared using dichloromethane. Appropriate amounts of these stock solutions were then further diluted to get the required concentrations of standard stock solutions.

**System suitability studies**

The resolution, number of theoretical plates, retention time and peak asymmetry were calculated for the working standard solutions and is as shown in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Drotavamine hydrochloride</th>
<th>Omeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates</td>
<td>34075.88</td>
<td>15192.87</td>
</tr>
<tr>
<td>Asymmetry Factor</td>
<td>1.01</td>
<td>1.18</td>
</tr>
<tr>
<td>HETP (cm)</td>
<td>0.00073</td>
<td>0.00164</td>
</tr>
<tr>
<td>Resolution*</td>
<td>4.53</td>
<td></td>
</tr>
</tbody>
</table>

*With respect to previous peak

The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination. The typical chromatogram of standard solution is as shown in Figure 1.

**ASSAY**

**Preparation of sample solutions**

Twenty tablets were weighed and powdered. Powder equivalent to 10 mg of omeprazole was weighed and transferred to 10 ml volumetric flask. Dichloromethane about 8 ml was added and sonicated for 10 min, volume was made up with the same solvent. This solution was then filtered through membrane filter paper. Further dilutions were made in dichloromethane to get concentrations in Beers law range. The retention times of drotavamine hydrochloride and omeprazole were found to be 6.0 ± 0.02 and 8.01 ± 0.03 respectively. The assay was calculated from the equation of regression line for each drug. The percentage assay of individual drug was calculated and presented in Table 2.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount present (mg/tab)</th>
<th>Amount found (mg/tab)</th>
<th>% label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drotavine Hydrochloride</td>
<td>40</td>
<td>39.70</td>
<td>99.25</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>10</td>
<td>10.01</td>
<td>100.13</td>
</tr>
</tbody>
</table>

**METHOD VALIDATION**

As per ICH guidelines, the method validation parameters checked were specificity, linearity, precision, accuracy, limit of detection, limit of quantitation and robustness.

**Specificity**

A blank solution (mobile phase) was injected and the chromatogram showed no inferring peaks at retention time of the two drugs. The chromatogram of drotavamine hydrochloride and omeprazole extracted from the tablet were compared with those acquired from drotavamine hydrochloride and omeprazole standards, correlation was good (in terms of t_R and area) indicates specificity of method. Common tablet excipients like starch, lactose, magnesium stearate were dispersed in dichloromethane, filtered and injected. There was no interference found.
Linearity and range

Aliquots of standard stock solutions of drotaverine hydrochloride and omeprazole were taken in 10 ml volumetric flasks and diluted with dichloromethane to get final concentrations in range of 2.5-12.5 μg/ml for omeprazole and 10-50 μg/ml for drotaverine hydrochloride. Triplicate injections were made five times for each concentration for each drug separately and chromatographed under the conditions as described above. The plots of peak area versus respective concentrations of drotaverine hydrochloride and omeprazole were found to be linear in the concentration range of 10-50 μg/ml and 2.5-12.5 μg/ml respectively. The linear regression equations of the lines are:

For drotaverine hydrochloride -

\[ y = 48166x + 491336, \quad (r = 0.9964) \]

For omeprazole -

\[ y = 82804x + 163070, \quad (r = 0.9953) \]

Precision

Precision study was performed to find out intra-day and inter-day variations. The percent relative standard deviation for intra-day precision was 0.288% for drotaverine hydrochloride and 0.232% for omeprazole and inter-day precision was 1.107% for drotaverine hydrochloride and 1.312% for omeprazole. Both the values were well within the limit of 2% as per ICH guidelines.

Accuracy

The accuracy was determined by recovery studies. The recovery studies were performed by standard addition method, at 80%, 100%, 120% level. Percent recovered was calculated using regression equation. For both the drugs, recovery was performed in same way and in triplicate. The percentage recovery were calculated and presented in Table 3.

Limit of detection and limit of quantitation

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.

\[
\text{LOD} = \frac{3.3 \times \text{Standard deviation of } y \text{ intercept}}{\text{Slope of calibration curve}}
\]

Drotaverine Hydrochloride - 1.13 μg/ml

Omeprazole - 0.27 μg/ml

The LOQ is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy.

\[
\text{LOQ} = \frac{10 \times \text{Standard deviation of } y \text{ intercept}}{\text{Slope of calibration curve}}
\]

Drotaverine Hydrochloride - 3.42 μg/ml

Omeprazole - 0.81 μg/ml

Robustness

Robustness of the method was determined by making slight deliberate changes in chromatographic conditions like 1% change in ratio of mobile phase constituents, ± 1nm change in detection wavelength and 0.05% change in flow rate. It was observed that there were no marked changes in the chromatogram. It suggests that the developed method is robust.

Table 3. It shows recovery studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level of recovery</th>
<th>Amount present (mg)</th>
<th>Amount added (mg)</th>
<th>Amount recovered</th>
<th>% recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drotaverine Hydrochloride</td>
<td>80</td>
<td>20</td>
<td>16</td>
<td>35.95</td>
<td>99.88 + 0.82</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>20</td>
<td>20</td>
<td>40.47</td>
<td>101.17 + 0.28</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>20</td>
<td>24</td>
<td>44.41</td>
<td>100.93 + 1.04</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>5</td>
<td>4</td>
<td>8.83</td>
<td>98.15 + 0.11</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>100</td>
<td>5</td>
<td>5</td>
<td>10.10</td>
<td>101.08 + 0.70</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>5</td>
<td>6</td>
<td>10.88</td>
<td>98.98 + 1.00</td>
</tr>
</tbody>
</table>
REFERENCES


5. Indian Pharmacopoeia, Volume II, Published by the controller of Publication, Delhi, 2007, 1473-1475.


