Development and Validation of HS-GC Method For Determination of Residual Solvents in Montelukast Sodium Bulk

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Residual solvents in pharmaceutical samples are monitored using gas chromatography with head space. Based on good manufacturing practices, measuring residual solvents is mandatory for the release testing of all active pharmaceutical ingredients (API). The analysis of residual organic solvents (Methanol, Acetonitrile, Acetone, DMSO and Toluene) in Montelukast sodium, an active pharmaceutical ingredient was investigated. The Head space gas chromatography (HSGC) method described in this investigation utilized a DB-624 Capillary (30.0 m x 0.53 mm ID, 3.0 µm F.T.) column with total run time 15 min. using DMSO as sample diluents. The injector temperature was set at 150°C to prevent degradation. Nitrogen/air at a constant flow rate of 4.5mL/min was used as a carrier gas. The method was validated to be specific, linear, precise, sensitive and showed excellent recovery.

Keywords: Headspace-gas chromatography, Method validation, Residual solvents, Montelukast sodium.

INTRODUCTION

The determination of residual solvents in drug substances, excipients or drug products is known to be one of the most difficult and demanding analytical tasks in the pharmaceutical industry. Furthermore, the determination of polar residual solvents in pharmaceutical preparations continues to present an analytical challenge mainly because these compounds are quite difficult to remove from water or polar solvents.[1-3] Many pharmaceutical products must be analyzed for residual solvents at different stages of their development (raw materials, intermediate products, and final product). Organic solvents such as methanol, aceton, dichloromethane, isopropyl alcohol and toluene are frequently used in the pharmaceutical industry. The manufacturing of new active pharmaceutical ingredients (APIs) under GMP conditions commands to control adequately the quality of the different ingredients happening in the synthesis. Organic residual solvents have therefore to be controlled and their purity has to be determined before any GMP synthesis. Headspace gas chromatography (HS-GC) method has been used for the determination of residual solvents in pharmaceutical compounds.[4-13] Direct injection of analytes evaporated through equilibration between liquid (or solid) phase and gas phase to GC system minimized the contamination of GC system and the deterioration of GC column.[12] Volatile residual solvents are accumulated prior to analysis.[13, 14]

Montelukast is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. It is usually administered orally. Montelukast is a CysLT₁ antagonist; that it blocks the action of leukotriene D₄ (and secondary ligands LTC₄ and LTE₄) on the cysteiny l leukotriene receptor CysLT₁ in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation. Montelukast Sodium (1-[(1R)-1-[3-(1E)-2-(2-chloro-2-quinolinyl) ethenyl] phenyl]-3-[1-hydroxy-1-methylethyl] phenyl] -propyl] thio] methyl] cyclopropanecarboxylic acid, monosodium salt is a white colored powder and it is freely soluble in ethanol, methanol, and water and practically insoluble in acetonitrile. Molecular weight of Montelukast Sodium is 608.2 g/mol and formula is C₇₇H₆₅ClNO₂SNa[15, 16].

The review of literature revealed that several methods are available for the determination of montelukast sodium. Reported method for estimation Montelukast sodium dosage form are spectrophotometry[17-19], spectrophotometry[18], Voltametric method[21], RP-HPLC[22-26] and HPTLC.[27,29]

But, there is no any residual solvent method has been reported yet for montelukast sodium. There for the present research work aims to develop a HSGC method for analysis of residual solvents in Montelukast sodium. The residual solvents were compared to standard solvents and the ICH standard residual solvents limit.

MATERIALS AND METHODS

Chemicals and reagents

Used Chemicals were obtained from the following suppliers: methanol (sigma-aldrich, Mumbai, India), aceton, toluene and dimethyl sulfoxide (DMSO) were obtained from Merck-Mumbai. Active pharmaceutical ingredient of Montelukast

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Sodium was obtained as a gift sample from Calida pharmaceutical Pvt. Ltd.

Instrumentation and chromatographic conditions
The analysis was performed on Shimadzu GC-2014 + head space HT15468 autosampler (software: GC solution) with flame-ionization detector. The injector temperature was 150 °C and detector temperature was 290°C. Column was DB-624 (30.0 m × 0.53 mm ID, 3.0 µm F.T.). Split ratio of injection 1:5. Total run time was 15 min. Nitrogen/air at a constant flow rate of 4.5mL/min was used as a carrier gas.

<table>
<thead>
<tr>
<th>Name</th>
<th>Retention time (min)</th>
<th>Theoretical plates</th>
<th>Resolution</th>
<th>Tailing factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>2.58</td>
<td>8664.563</td>
<td>0</td>
<td>1.103</td>
</tr>
<tr>
<td>Acetone</td>
<td>4.186</td>
<td>11906.983</td>
<td>11.737</td>
<td>1.101</td>
</tr>
<tr>
<td>ACN</td>
<td>4.778</td>
<td>18512.102</td>
<td>4.879</td>
<td>1.054</td>
</tr>
<tr>
<td>Toluene</td>
<td>11.189</td>
<td>122754.509</td>
<td>47.816</td>
<td>1.009</td>
</tr>
<tr>
<td>DMSO(blank)</td>
<td>14.978</td>
<td>171637.709</td>
<td>27.739</td>
<td>0.75</td>
</tr>
</tbody>
</table>
volume 5 mL was transferred into head space (HSS) vial and sealed it with aluminum closure.

**Preparation of Test solution**
Weighed accurately 0.1g of the test sample into HSS vials, and add 5 ml of DMSO solvent and seal the vials with aluminum closure.

**Blank Preparation**
A fixed volume 5.0 mL of DMSO was transferred into HSS vial and it was sealed.

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**Table 2: Summary of Validation parameters**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Methanol</th>
<th>Acetone</th>
<th>Toluene</th>
<th>Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity Range (µg/ml)</td>
<td>1500-4500 µg/ml</td>
<td>2500-7500 µg/ml</td>
<td>445-1335 µg/ml</td>
<td>205-615 µg/ml</td>
</tr>
<tr>
<td>2</td>
<td>Straight line equation</td>
<td>Y = 24.3486x + 2599.8800</td>
<td>Y = 107.6993x + 8775.5200</td>
<td>y = 752.9373x + 14038.6400</td>
<td>y = 23.2211x + 129.5600</td>
</tr>
<tr>
<td>3</td>
<td>Correlation coefficient</td>
<td>R² = 0.9990</td>
<td>R² = 0.9991</td>
<td>R² = 0.9961</td>
<td>R² = 0.9989</td>
</tr>
<tr>
<td>4</td>
<td>Intraday precision (n=6)</td>
<td>1.65</td>
<td>1.84</td>
<td>1.66</td>
<td>2.38</td>
</tr>
<tr>
<td></td>
<td>Interday precision (n=6)</td>
<td>1.72</td>
<td>1.86</td>
<td>1.77</td>
<td>2.29</td>
</tr>
<tr>
<td>5</td>
<td>Accuracy (%recovery)</td>
<td>99.54-100.36</td>
<td>99.60-100.71</td>
<td>100.54-101.45</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Limit of Detection (µg/ml)</td>
<td>56.83</td>
<td>124.72</td>
<td>9.89</td>
<td>0.29</td>
</tr>
<tr>
<td>7</td>
<td>Limit of Quantification (µg/ml)</td>
<td>172.22</td>
<td>377.93</td>
<td>29.97</td>
<td>0.89</td>
</tr>
<tr>
<td>8</td>
<td>Robustness (% RSD)</td>
<td>6.21</td>
<td>7.92</td>
<td>9.06</td>
<td>4.27</td>
</tr>
</tbody>
</table>

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**Procedure**
The system was set up as mentioned under chromatographic conditions. Then, 1.0 mL of gaseous phase from blank followed by standard solution and test solution were injected. Figure 2 and Table 1 shows typical chromatogram of Montelukast sodium by HSGC method and System suitability parameters.
VALIDATION OF ANALYTICAL METHOD

1) Linearity
Solutions of analyte solvent, having different concentration, were made separately 50% concentration to 150% concentration of limit. Three replicates were performed at each level. The calibration curves were obtained with the average of peak area ratios of three replicates. The correlation coefficient (R²) values for all residual solvents were found to be higher than 0.997 and the calibration curves were linear within the range. Figure 3,4,5,6 shows the linearity curves of different residual solvent.

2) Precision
For the system precision, a single injection of blank and six replicate injections of standard solution were observed. Intermediate precision study was carried out by a different analyst, on a different instrument and on another day. The percentage %RSD was calculated. The % RSD for each solvent was found to be less than 10 and system suitability was passed.

3) Accuracy
Accuracy of the method was ascertained by standard addition method at 3 levels. Standard solution quantity equivalent to 75%, 100% and 125% were added in Sample.
- Methanol standard concentration - 3000 ppm (ICH guidelines)
- Acetone standard concentration - 5000 ppm (ICH guidelines)
- Toluene Standard Concentration- 890 ppm (ICH guidelines)

Stock standard Solution-I
A volume 0.380 mL of methanol (equivalent to 300 mg), 0.641 mL of acetone (equivalent to 500 mg) and 0.102 mL of toluene (equivalent to 89 mg) were transferred into a 100 mL volumetric flask containing about 70 mL of DMSO to the flask and dilute up to the mark with same solvent. From the stock standard solution I, 1.5, 2.00 and 2.5 mL were transferred into 100 mL volumetric flask to prepare 75 %, 100 % and 125 % limit solution. From that solutions 5 mL solution was transferred into H5 vial and the vial was sealed.

Spike Sample
Accurately weighed 0.1 g of sample was taken in head space vial. To it 5 mL of each level of standard solution was transferred into a HSS vial and sealed the vial.

4) LOD and LOQ
The LOD and LOQ were calculated by instrumental and statistical methods. For the instrumental method, LOD is determined as the lowest amount to detect, and LOQ is the lowest amount to quantify, by the detector. The LODs of residual solvents in Omeprazole API were determined based on a signal-to-noise ratio of 3:1. The LOQs of residual solvents were determined based on a signal-to-noise ratio of 10:1. The values for the LOD and LOQ for methanol, toluene, acetone and acetone are shown in Table 2.

5) Robustness
To demonstrate the robustness of the method, the system suitability criteria with slight variations in method parameters, was verified. The following parameters were changed: column oven temperature ± 5 °C from the ideal conditions (initial column oven temperature at 35 °C and 45 °C), the flow rate ± 10% from the ideal conditions (flow rate 0.9 mL/min and 1.1 mL/min), the split ratio ± 10% from the ideal conditions (the split ratio of 1:4 and 1:6), two columns of different serial numbers. The results are shown in Table 2. For the robustness study, individual % RSD should not exceed 10.0 and cumulative (overall) % RSD should not exceed 15.0, for each component, and system suitability should pass.

RESULTS AND DISCUSSION
The system suitability parameters and system precision are evaluated and found within the limits. A plot is drawn between concentration of the component and the instrument response; It is found to be linear in the concentration range with good correlation coefficient greater than (R²=0.999), Precision and accuracy of the developed method are expressed in %RSD and % of recovery of the active pharmaceutical ingredient respectively. Low %RSD value and high percent of recovery indicate that the method is highly precise and accurate. All system suitability parameters were found within the standard limit.

CONCLUSION
A single, rapid and highly selective HSGC method was developed and validated for the quantification of residual solvents present in Montelukast sodium through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns. The residual solvents methanol, acetone, acetonitrile and toluene were determined. The developed method is specific, accurate, precise and robust as per ICH guidelines.

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REFERENCES
1. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1), 2005
9. Legrand S, Dugay. Use of solid-phase microextraction coupled with gas Chromatography for the determination of residual solvents in...

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