A rapid liquid chromatographic method was developed for the determination of oxatomide in its finished active pharmaceutical ingredient form and in the presence of its process impurities. The method was developed on a sub 2 µm Hypersil Zorbax XDB C18 column (30 × 4.6 mm, i.d., 1.8 µm). The rapid method employed a gradient mobile phase consisting of solvent A: 0.01 M tetrabutylammonium hydrogen sulfate and 0.5% (v/v) ammonium acetate in water and solvent B: acetonitrile. A flow rate of 2 mL/min was employed with the diode-array detector set at 230 nm. The original method supplied by Janssen Pharmaceuticals Ltd was run on a Thermo Scientific octadecylsilica gel C18 column (100 × 4.6 mm, i.d., 5 µm) with an analysis time of 20 min. The main aim was to substantially reduce the analysis time while maintaining good efficiency. Run-time was reduced to 6.5 min with a total loss in analysis time of 68%. Solvent consumption was also reduced by 68%. Validation according to the International Conference of Harmonization guidelines was undertaken. The parameters examined were accuracy, precision, linearity, selectivity, robustness, limit of detection and limit of quantification; all criteria were met. Sample stability testing was also carried out. Oxatomide proved stable under ambient and 4°C temperatures and in the presence of light for up to 24 h.

Introduction

Oxatomide 1-[3-[4-(diphenylmethyl)-piperazin-1-yl] propyl]-1,3-dihydro-2H-benzimidazol-2-one is a derivative of the organic compound piperazine (1). It was first discovered by Janssen in 1975. Oxatomide is a histamine H1 (sedating) receptor antagonist which also inhibits mast cell degranulation.

It has been tested in gel and cream form and has proven to be efficient as an anti-itching agent in vulvar itching in females (2). Oxatomide has also been tested in oral suspension in pediatric bronchial asthma. It proved quite significant in the reduction of persistent coughing, wheezing and shortness of breath (3). However, other authors disagree with these findings and claim that even though some lung function was improved the results within these studies are not consistent (4). It is mainly used in the treatment of long-lasting hives, chronic itching and scaling of the skin and dermographism urticaria (skin writing) (5).

While oxatomide has been around for a long period, it is still extensively used as an anti-allergy agent (6) and an anti-inflammatory agent (7). It has also proven to be beneficial as an anti-asthmatic agent (8). Furthermore, it has been adopted to incorporate more delivery mechanisms and its usage in combination with a number of other drug products (9) and with natural products has been extensively investigated (10). A number of methods are detailed within the literature; Fujii et al. (11) developed a reproducible high-performance liquid chromatography (HPLC) method to determine oxatomide in blood plasma in humans with an analysis time of < 30 min. Kikuchi et al. (12) determined oxatomide and its metabolite M-11 in rat plasma and also both human plasma and serum employing HPLC along with a fluorometric detector. Petrollini et al. (13) employed HPLC to determine the oxatomide levels in serum of young children who had been overdosed with the drug to determine its effect on their heart rate. A conventional spectrofluorimetric method was employed by Nepote et al. (14) to determine oxatomide in the presence of phenylephrine and paracetamol. More recently, spectrofluorimetry was utilized to determine oxatomide by Balraj et al. (15). The molecular complexes of both ketoconazole and oxatomide were successfully characterized by employing spectrofluorimetry along with UV–vis, FT-IR and 1H NMR.

HPLC is currently one of the most widely used analytical techniques within the pharmaceutical industry. Due to the demand for faster analysis times and reduction of waste, ultra high-performance systems and smaller particle size column technology have been introduced. Increased flow rates and shorter analysis times have been observed with little or no effect to resolution and efficiency (16–18).

The analysis time and solvent consumption within this study were reduced substantially by almost 68%. The new rapid method was validated according to the International Conference on Harmonization (ICH) guidelines. The parameters examined were accuracy, precision, linearity, selectivity, robustness, limit of detection and limit of quantification (LOQ). Sample stability testing was also carried out.

Experimental

Chemicals and reagents

Oxatomide standard (active pharmaceutical ingredient, API) and its four process impurities (Figure 1) were received from Janssen Pharmaceutical Ltd., Cork, Ireland. HPLC-grade acetonitrile was purchased from Sigma-Aldrich Ireland Ltd. Ammonium acetate and tetrabutylammonium hydrogen sulfate were purchased from Van Waters and Rogers International. Ultra-pure water was obtained using a TKA water purification system.


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Chemical names

(i) Oxatomide standard: 1-[3-[4-(diphenylmethyl)-1-piperazinyl] propyl]-1,3-dihydro-2H-benzimidazol-2-one; (ii) impurity A:
1,3-dihydro-1-[3-(1-piperazinyl)propyl]-2H-benzimidazol-2-one; (iii) impurity B: 1-(3-chloropropyl)-1,3-dihydro-2H-benzimidazol-2-one; (iv) impurity C: alpha-phenylbenzenemethanol; and (v) impurity D: 1-[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl) propyl]-3-[3-[4-(diphenylmethyl)-1-piperazinyl]propyl]-1,3-dihydro-2H-benzimidazol-2-one monohydrate.

Instrumentation
An Agilent 1200 Rapid Resolution Liquid Chromatograph was used for this analysis. It contains a 1200 series binary pump SL, a vacuum degasser, a 1200 series high-performance auto sampler, a 1200 series thermostated column compartment SL, a 1200 series diode-array detector SL for up to 80 Hz operation which were controlled by ChemStation B.02.01-SR1 data acquisition and evaluation software.

Chromatographic conditions
Table I illustrates the gradient elution program for the elution of oxatomide and its related impurities utilizing the original Janssen method. A Thermo Scientific octadecylsilyl silica gel (ODS) C$_{18}$ column (30 × 4.6 mm, i.d., 5 μm) and injection volume of 10 μL were employed for this method. Table IB contains the gradient elution for the developed rapid method for the determination of oxatomide and its process impurities. This rapid method employed a Hypersil Zorbax XDB C$_{18}$ column (30 × 4.6 mm, i.d., 1.8 μm). A mobile phase consisting of solvent A: 0.01 M tetrabutylammonium hydrogen sulfate and 0.5% (w/v) ammonium acetate in water and solvent B: acetonitrile was used. A flow rate of 2 mL/min and UV detection of 230 nm were employed. The column was at ambient temperatures, and an injection volume of 2 μL was used. Solvent A was filtered through 0.2 μm nylon filters.

Preparation of sample solutions
A stock solution of 1,000 μg/mL was prepared by weighing 10 mg of the oxatomide standard and each impurity into the same 10 mL volumetric flask. Five concentrations ranging from 200 to 1,000 μg/mL were made from the stock solution in interval of 200 μg/mL. These concentrations were used to determine linearity, precision and accuracy.

Method validation
Linearity
Linearity test solutions ranging from 200 to 1,000 μg/mL in increments of 200 μg/mL were prepared (as per method in previous paragraph). Triplicate injections were carried out at each concentration. The mean average of the areas observed were determined and plotted against concentration. The equation of the line, along with correlation coefficient was then determined by means of least-squares linear regression.

Accuracy
Accuracy was derived from the linearity data employing the same concentrations 200 to 1,000 μg/mL. Each concentration was injected in triplicate. Accuracy is measured in % recovery and was calculated according to the following equation:

$$\text{% recovery} = \left( \frac{\text{actual yield}}{\text{theoretical yield}} \right) \times 100.$$

The acceptance criterion according to Janssen Pharmaceuticals Ltd is between 80 and 120%.

Limit of detection and limit of quantification
LOD is the amount of substance that can be detected but not quantified. LOQ is the amount of substance that can
be quantified. According to the ICH one method employed to determine LOD and LOQ is the ratio of signal visible to baseline noise. A ratio of 3:1 and 10:1, respectively, is specified and was determined by injecting in triplicate dilutions of known concentration. These dilutions were determined manually from the 1,000 µg/mL concentration and injected in triplicate.

**Precision**
Precision is a measure of the reproducibility of the method. It is expressed as percent Relative Standard Deviation (% RSD). It is presented as intra- and interday precision. Intraday precision was determined by analysis repeatability employing the 200 and 600 µg/mL concentrations and injection repeatability employing the 400 and 800 µg/mL concentrations. Both repeatability analysis results were determined by six replicate injections all carried out on the same day under identical conditions. Interday or intermediate precision was determined employing 1,000 and 300 µg/mL concentrations. It was carried out on a different day by a different analyst and was determined by injecting six replicate injections.

**Selectivity**
Selectivity is the ability to measure accurately the analyte of interest in the presence of other components such as impurities and degradation products. A selectivity solution made up of a 100:1 ratio API and impurities, respectively, was prepared by weighing 100 mg of the oxatomide standard and 1 mg of each impurity into a 10 mL volumetric flask and making it up to the mark with methanol. This selectivity solution was injected in triplicate.

**Robustness**
Robustness, according to ICH guidelines is the ability to marginally alter a parameter of the method without it affecting the results. If the results of a method are affected then those parameters should be controlled and a statement added to the method documentation. Flow rate, column temperature and percent organic on the initial and final mobile phase composition were altered and resolution between the two critical pairs was observed. Each individual parameter altered was investigated using the 200, 400 and 600 ppm standard samples, each containing the API and each impurity. All injections were carried out in triplicate.

**Sample stability**
The stability of the standard solutions was determined by storing four identical 800 µg/mL standard samples in clear and brown vials in the 4°C fridge and at ambient temperatures for 4 weeks after preparation. All four vials were injected after 24 h, 48 h, 1, 2 and 4 weeks, and the results were compared with fresh sample.

**Results**

**Method development and optimization**
Figure 2 shows the resulting chromatogram for the analysis of oxatomide standard and its process-related impurities.

**Method validation**

**Linearity**
Table II shows linearity data for oxatomide standard and its process-related impurities.

**Accuracy**
Table III shows recovery data for oxatomide standard.

**LOD and LOQ**
Table IV shows LOD and LOQ values for oxatomide standard and its related impurities.

**Precision**
Table V shows % RSD for precision studies of API and impurities.

**Selectivity**
Figure 3 shows the resulting chromatogram for selectivity under optimum conditions using the sub 2 µm XDB column.

**Robustness**
This method was deemed robust once flow rate was altered by no more than 20%, column temperature was altered by no >13% and the initial % organic was altered by no >4% from the validated method conditions. Once these limits were exceeded, resolution was below an acceptable level.

**Sample stability**
Table VI lists % difference for laboratory stability studies after 48 h.

**Discussion**

**Method development and optimization**
Initially, as detailed by Janssen specification, oxatomide and its impurities were determined utilizing a Thermo Scientific ODS C18 Column (100 × 4.6 mm, i.d., 5 µm) and an injection volume of 10 µL. A flow rate of 2 mL/min and a run-time of 20 min were employed with the API eluting at 8.575 min (Figure 2A).

Following an extensive survey of available columns and some initial experimental work, a sub 2 µm, XDB column was chosen. The method was optimized by altering the flow rate, injection volume, gradient steepness and column temperature, separately and in increments. Achieving the shortest run time with acceptable resolution and efficiency was the main aim. After an extensive testing, a gradient, as per Table I, was found to give the shortest possible time of 6.5 min with the best possible resolution. The 6.5 min run-time included a wash step of 1 min and also a 1 min re-equilibration step. UV detection of 230 nm and injection volume of 2 µL were employed. The column was at ambient temperature. Resolution between the two critical pairs was above the specified limit of 1.5 set out by Janssen (Figure 2B).

**Method validation**
The new rapid method was validated according to the ICH guidelines. The parameters examined were linearity, accuracy, precision, LOD, LOQ, specificity and robustness. Some stability studies were also undertaken.
Calibration plots for the API: oxatomide standard and each of the impurities gave a linear response over the range of 200–1,000 mg/mL. The mean values of the slopes, intercepts and correlation coefficients are given in Table II.

**Accuracy**

The percent recovery was calculated from the linearity solutions in the range of 200–1,000 µg/mL. Percent recoveries ranging from 98.6 to 101.7% were observed for the API and can be seen in Table III.

**Linearity**

Figure 2. The resulting chromatogram for the analysis of oxatomide standard and its process-related impurities utilizing (A) Janssen’s current specification (B) the optimized rapid method on the sub 2 µm XDB column (chromatographic conditions as in Table IB).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Slope</th>
<th>Y-intercept</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imp A</td>
<td>1.5011</td>
<td>-14.1790</td>
<td>0.9996</td>
</tr>
<tr>
<td>Imp B</td>
<td>1.7548</td>
<td>-9.8342</td>
<td>0.9998</td>
</tr>
<tr>
<td>Imp C</td>
<td>1.1429</td>
<td>13.5670</td>
<td>0.9997</td>
</tr>
<tr>
<td>Imp D</td>
<td>1.8233</td>
<td>-11.0820</td>
<td>0.9997</td>
</tr>
<tr>
<td>Oxa std</td>
<td>2.1082</td>
<td>-8.5248</td>
<td>0.9997</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>99.2</td>
</tr>
<tr>
<td>400</td>
<td>101.7</td>
</tr>
<tr>
<td>600</td>
<td>98.6</td>
</tr>
<tr>
<td>800</td>
<td>100.2</td>
</tr>
<tr>
<td>1,000</td>
<td>100.1</td>
</tr>
</tbody>
</table>
Limit of detection and limit of quantification
The LOD and LOQ for oxatomide standard and its related impurities were determined according to the ICH guidelines. The signal-to-noise ratio was measured experimentally based on the 3:1 and 10:1 ratio for LOD and LOQ, respectively. The concentrations found are presented in Table IV.

Precision
Injection repeatability results presented RSD values ranging from 0.10 to 0.60% and analysis repeatability results with RSD values ranging from 0.11 to 0.31% were observed. The intermediate precision results ranged from 0.48 to 1.45%. All results demonstrate RSD values of < 2.0 recommended by the ICH guidelines.

Selectivity
The oxatomide standard and each of the standard impurities were injected separately in triplicates. All samples displayed different retention times. A selectivity solution containing the API and all impurities in a ratio of 100:1, respectively, was also injected in triplicate. All peaks were well resolved, and no interference from impurities or degradation products was observed (Figure 3).

Robustness
The robustness of this method was determined by altering flow rate, column temperature and initial % organic. A 20% flow rate increase saw a slight resolution reduction, which was within the specification. However, any further increase in flow rate beyond this lowered the resolution between the oxatomide and impurity C peaks to unacceptable levels.

The maximum acceptable column temperature increase was an increase of 13% from ambient temperature (average of 29.3 °C) to 33 °C, while a reduction of resolution was observed, it was within specification. Further temperature increases led to coelution of the critical pair.

Marginally, reduced resolution occurred when the initial % organic content was lowered by 4%. Any further % organic content reductions showed unacceptable resolution levels.

This method was therefore deemed robust, once the above parameter guidelines are adhered to.

Table IV
LOD and LOQ Values for Oxatomide Standard and Its Related Impurities

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD (mg/mL)</th>
<th>LOQ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imp A</td>
<td>0.16</td>
<td>0.60</td>
</tr>
<tr>
<td>Imp B</td>
<td>0.20</td>
<td>0.62</td>
</tr>
<tr>
<td>Imp C</td>
<td>0.30</td>
<td>0.98</td>
</tr>
<tr>
<td>Imp D</td>
<td>0.13</td>
<td>0.28</td>
</tr>
<tr>
<td>Oxa std</td>
<td>0.24</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Table V
% RSD for Precision Studies of API and Impurities

<table>
<thead>
<tr>
<th>Compound</th>
<th>Injection repeatability</th>
<th>Analysis repeatability</th>
<th>Intermediate repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% RSD 200 μg/mL 600 μg/mL</td>
<td>% RSD 400 μg/mL 800 μg/mL</td>
<td>% RSD 1,000 μg/mL 300 μg/mL</td>
</tr>
<tr>
<td>Imp A</td>
<td>0.33 0.17</td>
<td>0.31 0.17</td>
<td>0.48 1.30</td>
</tr>
<tr>
<td>Imp B</td>
<td>0.35 0.13</td>
<td>0.11 0.13</td>
<td>0.55 1.07</td>
</tr>
<tr>
<td>Imp C</td>
<td>0.60 0.16</td>
<td>0.20 0.18</td>
<td>0.86 1.45</td>
</tr>
<tr>
<td>Imp D</td>
<td>0.46 0.12</td>
<td>0.12 0.13</td>
<td>0.65 1.36</td>
</tr>
<tr>
<td>Oxa std</td>
<td>0.33 0.10</td>
<td>0.12 0.11</td>
<td>0.73 1.25</td>
</tr>
</tbody>
</table>

Table VI
Percent Difference for Laboratory Stability Studies after 48 h

<table>
<thead>
<tr>
<th>Compound</th>
<th>Clear vial 4°C</th>
<th>Brown vial 4°C</th>
<th>Clear vial ambient temperature</th>
<th>Brown vial ambient temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imp A</td>
<td>0.71</td>
<td>0.79</td>
<td>1.08</td>
<td>1.10</td>
</tr>
<tr>
<td>Imp B</td>
<td>0.98</td>
<td>0.96</td>
<td>1.30</td>
<td>1.25</td>
</tr>
<tr>
<td>Imp C</td>
<td>0.99</td>
<td>1.01</td>
<td>1.27</td>
<td>1.30</td>
</tr>
<tr>
<td>Imp D</td>
<td>1.36</td>
<td>1.30</td>
<td>1.60</td>
<td>1.57</td>
</tr>
<tr>
<td>Oxa std</td>
<td>1.01</td>
<td>0.90</td>
<td>1.21</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Figure 3. The resulting chromatogram for selectivity under optimum conditions using the sub 2 μm XDB column (100:1 API:impurity, chromatographic conditions as in Table IB).
Sample stability

As can be seen from Table VI, the oxatomide standard and each impurity remained stable for up to 48 h, indicating that oxatomide can be used in long sequences of up to 48 h. Janssen recommend a cut-off point of 2% difference between fresh solution and stability solutions. There is no significant difference between the amber or clear vials at 4°C or at ambient temperature, demonstrating that oxatomide standard and its process impurities are not light sensitive. There is a significant difference between the 4°C temperature and ambient temperature demonstrating that oxatomide and its process impurities are temperature dependent.

Conclusion

A new rapid LC method for the analysis of oxatomide and its main process impurities were successfully developed and validated. This rapid method demonstrated a 68% reduction in analysis time in comparison to the original method. As a result, solvent consumption was also reduced by the same value proving the rapid method to be both economically and environmentally friendly. The reduction in analysis time was achieved by employing a sub 2 μm XDB column with resolution and efficiency being sufficiently maintained. The method was validated according to ICH guidelines, and all parameters were within specification proving the method to be accurate, precise and specific. The new rapid method proved reliable for the determination of oxatomide and its process impurities.

Acknowledgment

The authors thank the management of Shannon Applied Biotechnology Centre and Janssen Pharmaceutical Ltd., Cork for supporting this work.

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