Analysis of Melamine and Metformin Hydrochloride by HPLC-UV

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Abstract

The United States Pharmacopeia (USP) monograph for the analysis of metformin stipulates that an L9 column must be used. Thermo Scientific BioBasic SCX fulfills the “strongly acidic cation exchanger” requirement for the L9 packing and, when used for the metformin method, achieves the system suitability criteria specified in the monograph.

Introduction

Melamine has many uses in both the industrial and agricultural fields. Mixed with resins melamine exhibits fire retardant properties and is frequently used to produce cooking utensils and fabrics. Melamine contains 66% nitrogen by mass and can be used in crop fertilization, however due to elevated costs in comparison to other fertilizers it is economically impractical. Due to the high nitrogen content, melamine has in the past been used in China as an adulterant for feedstock and milk. The addition of nitrogen-rich melamine increases the apparent protein content of poor quality food. Exposure to melamine can result in kidney damage, renal failure and subsequently death.

Metformin is primarily used in the treatment of type 2 diabetes and insulin resistant conditions such as polycystic ovary syndrome (PCOS). Similarly to melamine, metformin has a polar, nitrogen-rich structure, hence the two compounds are regularly detected within the same chromatographic method.

Based on sulfonic acid chemistry, BioBasic™ SCX is a strong cation exchange phase which lends itself to the separation of melamine and metformin according to the USP monograph for metformin hydrochloride. We have demonstrated that this column successfully separates melamine and metformin, satisfying the USP criterion that the resolution is >10.

Experimental Details

Key Words
- BioBasic SCX
- Melamine
- Metformin
- Clinical
- USP

Chemicals and Reagents

<table>
<thead>
<tr>
<th>Chemicals and Reagents</th>
<th>Part Number</th>
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<tbody>
<tr>
<td>Fisher Scientific buffer: Ammonium dihydrogen phosphate</td>
<td>A886-500</td>
</tr>
<tr>
<td>Fisher Scientific HPLC grade water</td>
<td>W/0106/17</td>
</tr>
<tr>
<td>Sigma Aldrich metformin</td>
<td>D5035</td>
</tr>
<tr>
<td>Sigma Aldrich melamine</td>
<td>M2659</td>
</tr>
</tbody>
</table>

Preparation of mobile phase: 17 g of ammonium dihydrogen phosphate was dissolved into 1 L of HPLC grade water. The pH was adjusted to 3.0 using phosphoric acid.

Mobile phase: 1.7% ammonium dihydrogen phosphate pH 3.0
Flow rate: 1.0 mL/min
Run time: 16 minutes
Column temperature: 40°C
Injection wash solvent: Water
UV detector wavelength: 218 nm

Solutions

Samples were prepared according to the USP specification:
The working standard contained 5 µg/mL of metformin and 2 µg/mL of melamine in mobile phase.

Data processing

Software: Thermo Scientific ChromQuest 5.0 software
Results
When separating melamine and metformin with BioBasic SCX using the method specified in the USP monograph, better peak tailing factors were achieved when the column was temperature controlled (Table 1). Note that there is no specification with regards to tailing factor in the USP monograph.

The resolution value achieved with BioBasic SCX was 26, which comfortably exceeded the value of 10, specified in the monograph.

Replicate injections of the melamine/metformin system suitability mix showed that BioBasic SCX produced reproducible retention and peak shape (Table 2 and Figure 1).

Conclusions
- BioBasic SCX can be used to successfully resolve melamine and metformin in accordance with USP system suitability criteria.
- Better peaks shapes and good retention time stability was achieved when the column was thermostatically controlled (Figure 1).

Table 1: The effect of temperature on peak shape

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Peak 1: Melamine (Tf)</th>
<th>Peak 2: Metformin (Tf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22°C (ambient)</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>40 °C</td>
<td>1.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table 2: Statistical assessment based upon data derived from six replicate injections at 40°C

<table>
<thead>
<tr>
<th>Results</th>
<th>Peak 1: Melamine</th>
<th>Peak 2: Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time, tR/min</td>
<td>4.38</td>
<td>10.08</td>
</tr>
<tr>
<td>%RSD tR</td>
<td>0.22</td>
<td>0.45</td>
</tr>
<tr>
<td>%RSD Area</td>
<td>0.33</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Figure 1: Separation of melamine and metformin using BioBasic SCX with a column temperature of 40°C