Impurity Profiling of Carbamazepine by HPLC/UV

Terry Zhang, Guifeng Jiang, Sergio a Guazzotti and Diab Elmashati Thermo Fisher Scientific, San Jose, USA

Abstract
Carbamazepine is a medication indicated for use in epilepsy, trigeminal neuralgia, and bipolar disorder.1 This widely prescribed tricyclic antidepressant is administered orally in usual doses of 400-1200 mg/day and is available in several commercial tablet, capsule and suspension forms.2 Synthesis-related organic impurities are usually present in bulk forms as well as in pharmaceutical formulations of carbamazepine. Pharmacopeial and batch standards for the purity of carbamazepine to ensure drug efficacy and safety. The United States Pharmacopeia (USP) establishes 0.2% as the maximum limit for any individual impurity and 0.5% as the total of all impurities relative to the active pharmaceutical ingredient.3 The USP describes a quantitative HPLC/UV procedure for impurity profiling of carbamazepine that utilizes a 4.6 mm x 250 mm column with L10 packing (5-10 µm silica particles with cyano bonded phase) and a mobile phase consisting of water, methanol, tetrahydrofuran, formic acid and triethylamine. However, the utility of this method for routine quality control analysis is limited by the complexity of the mobile phase, which could lead to poor reproducibility, as well as by the difficulty in achieving the required resolution (R2 ≥ 1.7) between carbamazepine and the impurity 10,11-dihydrocarbamazepine (impurity A), which has comparable polarities and differ widely in their concentrations. Routine drug purity analysis requires simple and robust analytical techniques that deliver exceptional resolution, sensitivity, and accuracy. In this presentation, we describe a simple HPLC/UV assay, accurate and robust impurity analysis of carbamazepine using the Acela UPLC system and a high performance Hypersil GOLD CN (cyano) column, and demonstrate that the method meets USP requirements.

Materials and Methods

Sample preparation
Carbamazepine and related impurities A, B, D and F were purchased from Sigma Aldrich. Stock solutions of 2 mg/mL were prepared in methanol. Solutions of 100 µg/mL concentration were prepared by diluting the stock solution in methanol and were used for method development. Calibration standards were prepared by serial dilution of the stock solutions in methanol, at concentrations of 250 ng/mL – 400 µg/mL for each impurity and 5 µg/mL – 100 µg/mL for carbamazepine. A mixture containing 1 mg/mL of carbamazepine and 500 ng/mL of each impurity was prepared to examine method suitability at a 0.05% reporting threshold. For assessments of system suitability and precision, a mixture of 1.5 mg/mL of carbamazepine was used.

LC/UVAAnalysis

Institutional HPLC separations were performed on an Acela 1250 LC system and an Acela autosampler (Thermo Fisher Scientific, San Jose, CA, USA). UV absorbance was monitored at 211 nm at 20 Hz using an Acela PDA detector (Thermo Fisher Scientific, San Jose, CA, USA).

LC Parameters

Column: Hypersil GOLD CN column (2.1 x 150 mm, 3 µm particle size) Mobile Phase: [A] Water [B] Acetonitrile

Column temperature: 30°C Sample injection volume: 1 µL Needle wash: 80:20 (v/v) acetonitrile/water Gradient: 0.00 88.9 12.5 400.0 10.00 88.9 12.5 400.0 14.00 75.0 25.0 400.0 28.00 75.0 25.0 400.0 19.00 62.5 37.5 400.0 30.00 62.5 37.5 400.0 31.00 88.9 12.5 400.0

Concentration

85.0

Results and Discussion

1. HPLC separation of carbamazepine and related impurities

The major organic impurities that arise from the synthesis of carbamazepine are 10,11-dihydrocarbamazepine (impurity A, also referred to as USP carbamazepine related compound A), 9-methylcarbazine (impurity B), N-carbamoylcarbamazepine (impurity C), iminostilbene (impurity D), iminodibenzyl (impurity E), and 5-chlorocarbonyliminostilbene (impurity F). An HPLC/UV assay that can accurately and reproducibly detect, identify and quantify these compounds at two levels (as low as 0.05%-0.2% relative to the parent drug carbamazepine) is required to comply with USP requirements and ICH guidelines.

The USP chromatographic method for determining carbamazepine purity specifies the use of a 4.6 mm x 250 mm L10 column (cyano column with 5-10 µm silica particles), a mobile phase composed of 1000 mL water/methanol/tetrahydrofuran (85:12:3) with 0.22 mL formic acid and 0.5 mL triethylamine, a flow rate of approximately 1.5 mL/min, and detection at 230 nm. Dynamic selection of the wavelength for quantitation was based on the best S/N for all analytes. Figure 1. Selection of the wavelength for quantitation was based on the best S/N for all analytes.

2. Method Validation

System suitability was investigated by analyzing six replicate injections of a solution containing 1500 µg/mL of carbamazepine and 3 µg/mL of each impurity, corresponding to 0.2% of the parent drug. Retention time RSDs ranged from 0.01-0.02%, while peak area RSDs ranged from 1 - 2% (Table 1), indicating excellent method reproducibility. According to Equation 1, signal-to-noise ratio of 25 is required to comply with the 52% RSD criterion specified by the USP method: % RSD = 50(S/N) (Equation 1)

Mean signal-to-noise ratios of the impurities at 0.2% levels relative to the parent drug ranged from 28.5 to 73.4 (Table 1), ensuring method reliability for quantification and compliance with USP requirements. Mean resolutions ranged from 2.2 to 11.1, and the mean resolution of 2.2 between carbamazepine and impurity A exceeded the USP resolution requirement of 1.7 (Table 1).

Quantitative accuracy for all impurities was excellent. Ranging from 102 to 111% (Table 1), Figure 2 shows that excellent linearity in detector response was observed over the range of 0.25-400 µg/mL (ppm) for all impurities and 5-1500 µg/mL (ppm) for carbamazepine, with correlation coefficients ≥ 0.997 for all analytes.

Conclusions
A simple, accurate and robust quantitative HPLC/UV assay for carbamazepine purity assessment was developed. Separation and detection of carbamazepine and four of its related synthetic impurities was achieved within 22 minutes using the Acela 1350 UPLC system, a Hypersil GOLD CN column and a simple acetonitrile/water gradient. The efficient and selective Hypersil GOLD CN column enhanced resolution between carbamazepine and impurity A, provided sensitivity gains through improved peak shapes, and eliminated the need for ion-pairing agents. Trace levels of impurities (0.05% of parent drug) were easily detected. Impurities at levels of 0.2% of carbamazepine were quantifiable with 2% RSD. This simple HPLC/UV assay meets USP criteria and ICH guidelines and provides a powerful alternative to the HPLC-based procedures currently recommended by regulatory agencies for QACQ of carbamazepine purity.

References


Table 1. Method validation parameters were assessed by analyzing six replicate injections of each compound. CA: corrected area accuracy

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration µg/mL</th>
<th>R2</th>
<th>Area RSD</th>
<th>Mean Rs</th>
<th>Mean S/N CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurity A</td>
<td>0.25 - 400</td>
<td>0.9999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>5.00 - 1500</td>
<td>0.9973</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impurity B</td>
<td>0.25 - 400</td>
<td>0.9999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impurity F</td>
<td>0.25 - 400</td>
<td>0.9998</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 1. Selection of the wavelength for quantitation was based on the best S/N for all analytes.

FIGURE 2. HPLC separation of a mixture of carbamazepine and four of its impurities (100µg/mL) using a 3 µm Hypersil GOLD CN column, 2.1 mm x 150 mm. Mobile phase: A: Water, B: Acetonitrile. UV detection: 211 nm.

Figure 4. Impurities are baseline-separated and easily detected at the ICH reporting threshold (0.05%). Mixture contains 1.5 mg/mL of carbamazepine and 400 ng/mL of each impurities A, B, D, and F.

Table 2. Excellent linearity of detector response was achieved, with correlation coefficients 0.997 for all compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration µg/mL</th>
<th>Correlation Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurity A</td>
<td>0.25 - 400</td>
<td>0.9999</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>5.00 - 1500</td>
<td>0.9973</td>
</tr>
<tr>
<td>Impurity B</td>
<td>0.25 - 400</td>
<td>0.9999</td>
</tr>
<tr>
<td>Impurity F</td>
<td>0.25 - 400</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.