Impurity Profiling of Carbamazepine by HPLC/UV

Terry Zhang, Guifeng Jiang, Sergio a Guazzotti and Diab Elmashni, 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 anticonvulsant is administered orally in usual doses of 400-1200 mg/day and is available in several commercial tablet, capsule and suspension forms.[1]

Synthesis-related organic impurities are usually present in bulk forms as well as in pharmaceutical formulations of carbamazepine. Pharmacopoeias set strict 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.[2] 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 phases) 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 (Rs ≥ 1.7) between carbamazepine and the impurity 10,11-dihydroxycarbamazepine (carbamazepine related compound A), which have 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 for accurate and robust impurity analysis of carbamazepine using the Accela UHPLC system and a high performance Hypersil GOLD CN (cyano) column, and demonstrate that the method meets USP requirements.

Experimental

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 solutions 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 – 1.5 mg/mL for carbamazepine. A mixture containing 1mg/mL of carbamazepine and 500ng/mL of each impurity was prepared to examine method suitability at a 0.05% reporting threshold. For assessments of system suitability and quantification, a mixture of 1.5mg/mL of carbamazepine with 3µg/mL of each impurity was used.

LC/UV Analysis

Instrumentation

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

LC parameters:

Columns: Hypersil GOLD CN column (2.1 x 150 mm, 3.0 µm particle size)
Mobile Phase: [A] Water
[B] Acetonitrile
Column temperature: 30°C
Sample injection volume: 1 µl
Needle wash: 80:20 (v/v) acetontrile:water

Gradient:

Time	A %	B %	µL/min
0.00	88.0	12.0	400.0
10.00	88.0	12.0	400.0
14.00	75.0	25.0	400.0
28.00	70.0	30.0	400.0
19.00	15.0	85.0	400.0
30.00	15.0	85.0	400.0
31.00	88.0	12.0	400.0

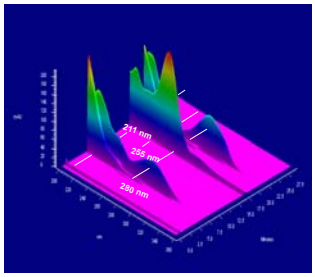
Results and Discussion

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-methylacridine (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 quantitate these compounds at trace levels (as low as 0.05%-0.20% 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 0.22mL formic acid and 0.5mL triethylamine, a flow rate of approximately 1.5 mL/min, and detection at 230 nm. Drawbacks of this assay for QA/QC applications include the use of a complex mobile phase with an ion-pairing agent, which could lead to operational difficulties and poor reproducibility, and the difficulty in meeting the resolution requirement (Rs ≥ 1.7) for carbamazepine and impurity A. Resolution and method performance may be improved by using high efficiency HPLC columns with smaller (≤ 3 µm) particles and by modifying the mobile phase.

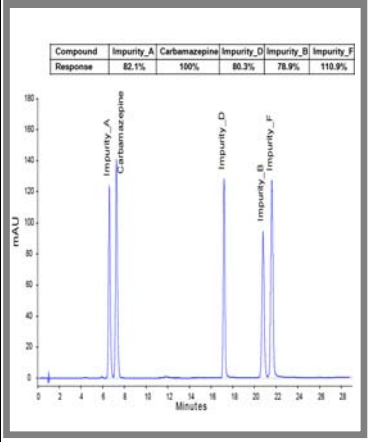
Figure 1. Selection of the wavelength for quantitation was based on the best S/N for all analytes.



The Hypersil GOLD CN (cyano) column offers alternative selectivity in reversed phase chromatography with lower hydrophobicity compared to C18 alkyl chain phases. The optimal wavelength of 211 nm was determined based on the best signal-to-noise ratio (S/N) for all analytes (Figure 1). Baseline separation of carbamazepine and the impurities at 100 µg/mL concentrations was achieved in 22 minutes using a Hypersil CN column (3 µm, 2.1 x 150 mm) and a simple acetonitrile/water gradient (Figure 2). Carbamazepine, impurity A and impurity D were less retained on the cyano phase and the elution order of carbamazepine and impurity A was reversed with this column. Exceptionally sharp and narrow peaks were obtained, resulting in excellent UV responses of similar magnitude for all analytes (Figure 2). The impurity responses were calculated relative to carbamazepine and were applied later for impurity analysis and quantification of a standard carbamazepine solution. Maximizing peak heights leads to sensitivity gains and is especially beneficial in trace analysis at ICH reporting and identification thresholds (0.05% and 0.10%, respectively).

A standard solution of 1 mg/mL carbamazepine was analyzed and 600 ng/mL of impurity A (0.06%) was detected (Figre 3). The resolution between the carbamazepine and impurity A peaks was 2.3, which exceeds the minimum resolution specified by USP (Figure 3). Figure 4 shows the chromatogram of the standard 1 mg/mL carbamazepine solution spiked with 0.05% of impurities A, B, D and F. All impurities were easily detected at these trace amounts, and the stronger UV response exhibited by impurity A was attributed to the 0.06% that was already present in the carbamazepine standard. As the majority of aqueous and matrix contaminants usually elute early at void volume, elution of the first analyte of interest after the sixth minute ensures a robust quantitation method.

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



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 ratios of ≥ 25 are required to comply with the ≤2% 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). Table 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

FIGURE 3. 1.0 mg/mL of carbamazepine standard was analyzed and 0.06% impurity A (600 ng/mL) was detected. Excellent resolution is attained between carbamazepine and impurity A on the Hypersil GOLD CN column. The resolution between this critical pair is 2.3, which exceeds the USP criterion of > 1.7.

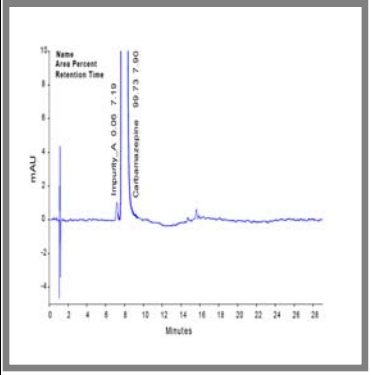


Figure 4. Impurities are baseline-separated and easily detected at the ICH reporting threshold (0.05%). Mixture contains 1.0 mg/mL of carbamazepine and 500 ng/mL each of impurities A, B, D, and F.

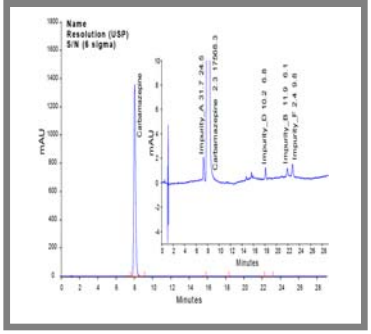


Table 1. Method validation parameters were assessed by analyzing six replicate injections of each compound.

CAA*: corrected area % accuracy

Compound	Conc. µg/ml	n	RT RSD %	Area RSD%	Mean Rs	Mean S/N	CAA*
Impurity_A	3.00	6	0.01	2	NA	73.4	111
Carbamazepine	1500	6	0.01	1	2.2	19720.8	NA
Impurity_D	3.00	6	0.01	2	9.1	44.1	108
Impurity_B	3.00	6	0.02	2	11.0	28.5	113
Impurity_F	3.00	6	0.01	1	2.2	34.6	102

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

Compound	Concentration µg/mL	Correlation Coefficients
Impurity A	0.25 - 400	1.0000
Carbamazepine	5.00 - 1500	0.9973
Impurity D	0.25 - 400	0.9995
Impurity B	0.25 - 400	0.9999
Impurity F	0.25 - 400	0.9998

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 Accela 1250 UHPLC system, a Hypersil GOLD CN column and a simple water/acetonitrile gradient. The highly 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 QA/QC of carbamazepine purity

References

1.http://www.merckmanuals.com/professional/lexicomp/carbamazepine.html
2. U.S. Pharmacopeia Monograph for Carbamazepine, USP32-NF27, page 1784.