Impurity Structural Identification/Confirmation and Profiling of Carbamazepine

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Overview

Purpose: Develop a simple assay for accurate and robust impurity analysis of carbamazepine using the Accela UHPLC system and a high performance Hypersil GOLD CN (cyano) column. And combining the high resolution, high accurate mass spectrometry and Mass Frontier software is a powerful solution for definitive identification and structural confirmation of drug impurities.

Methods: 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). For LC-MS analysis the LC set up was coupled to an Orbitrap Velos Pro.

Results: A simple, accurate and robust quantitative HPLC/UV assay for carbamazepine purity assessment was developed. A high-resolution, accurate mass LC-MS method was used for identification and confirmation of impurities in carbamazepine drug substance.

Introduction

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. However, UV-based methods alone neither provide unequivocal identification of known impurities nor enable identification of unexpected contaminants. Liquid chromatography in combination with multi-stage mass spectrometry is the method of choice for the structural elucidation of impurities.

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 500 ng/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 1.5 mg/mL of carbamazepine with 3 µg/mL of each impurity was used.

Liquid Chromatography
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
18.00	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

Mass Spectrometry

Full MS Scan		MS/MS	
Mass Range	m/z 150 to 270	Ion Trap AGC	1e4
Resolution	30,000	FT AGC	5e4
Injection Time	50 ms	FT Injection	50 ms
AGC Target	7e5	Microscans	1

Results

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), 10,11-dihydro-dibenz[b,f]azepine-5-carbonyl chloride (impurity B), *N*-carbamoylcarbamazepine (impurity C), iminostilbene (impurity D), 9-methylacridine (impurity E), and iminodibenzyl (impurity F). See figure 1 for structures. 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. 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 2). 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 3)

FIGURE 1. Structures and molecular weights (in Da) of Carbamazepine and Impurities A, D and F.

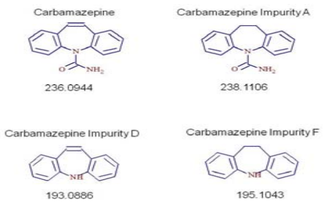


FIGURE 2. UHPLC separation of 12 carbonyl-DNPH derivatives at 20 µg/mL concentration

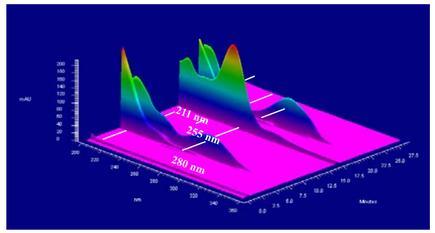
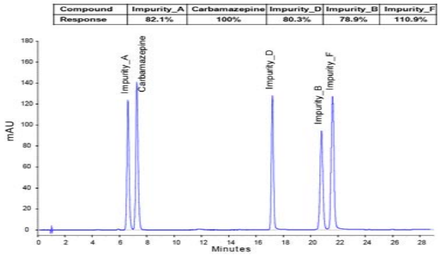


FIGURE 3. HPLC separation of a mixture of carbamazepine and its related impurities (100µg/mL) using a 3 µm Hypersil GOLD CN column, 2.1mm x 150 mm. Mobile phase: A- Water; B – Acetonitrile. UV detection: 211 nm.



A standard solution of 1 mg/mL carbamazepine was analyzed and 600 ng/mL of impurity A (0.06%) was detected at 211nm wavelength (Figure 4. The resolution between the carbamazepine and impurity A peaks was 2.3 which exceeds the minimum resolution specified by USP. Figure 5 shows the chromatogram of the standard 1 mg/mL carbamazepine solution spiked with 0.05% of impurities A, B, D and F at 211 nm wavelength. 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 4. 1.0 mg/mL of carbamazepine standard was analyzed and 0.06% impurity A (600 ng/mL) was detected.

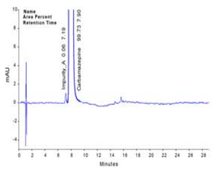
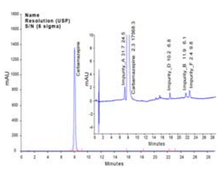


FIGURE 5. Impurities are baseline-separated and easily detected at the ICH reporting threshold (0.05%).



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).
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

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

3. Structural Identification and Confirmation by LC/MS/MS

Using an integrated Accela-Orbitrap Velos Pro LC-MS platform, a Hypersil GOLD™ CN (cyano) column (3 µm, 2.1 × 100 mm) and a simple, MS-compatible acetonitrile/water gradient, a mixture containing 1.0 mg/mL carbamazepine and 0.15 mg/mL each of impurities A, B, and D was baseline-separated and detected within 16 minutes (Figure 6a). High mass accuracy measurements enabled identification of all analytes. To minimize potential memory effects, a divert valve in the mass spectrometer was configured to re-direct the sample flow away from the ion source during elution of carbamazepine (Figure 6b).
The [M+H]+ ion of impurity A was selected for fragmentation by collision induced dissociation (CID) and higher-energy collisional dissociation (HCD) for structure confirmation by LC/MS/MS. See figure 7 (a) & (b).

FIGURE 6. (a) Extracted ion chromatogram with MH+ ions of high-resolution, accurate mass LC/MS analysis of carbamazepine and related impurities. (b) Potential memory effects are minimized using a divert valve in the Orbitrap Velos Pro mass spectrometer to reduce unnecessary contamination of the ion source by carbamazepine.

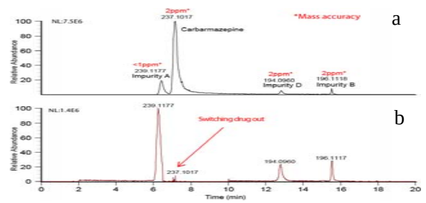
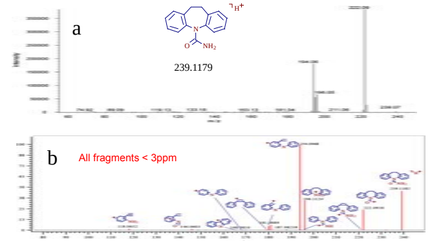


FIGURE 7. MS/MS spectra of carbamazepine impurity A with MH+ ions. (a) CID MS/MS acquired in the ion trap. (b) HCD MS/MS acquired in the mass spectrometer with chemically intelligent automatic fragment annotation from Mass Frontier 7.0 software.



Conclusion

- A much more optimized and simplified Carbamazepine and impurities analysis method was developed compared to the reported USP method.
- With the specific selectivity of Hypersil Gold CN column, a desired method development strategy was successfully achieved.
- 0.05% impurities relative to main drug were easily detected and fulfilled the USP requirements.
- 0.2% impurities relative to main drug were accurately quantified and fulfilled all the USP requirements.
- The relative standard deviation for RT and peak area were all under 2% which is within the limitation of regulatory requirements for a quality control environment.
- Accurate mass measurements of molecular ion species enabled identification of analytes.
- CID and HCD MS/MS generated complementary fragmentation information for unambiguous structural characterization. Accurate mass assignment of each of the fragment ions in the HCD MS/MS spectrum and automatic fragmentation annotation using Mass Frontier 7.0 provided definitive structural confirmation.

References

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