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Pharma

Analysis of impurities in topiramate by HPLC with charged aerosol detection and a single quadrupole mass spectrometer

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Keywords

Topiramate, impurity profiling, Vanquish Core HPLC, charged aerosol detection, ISQ EM mass spectrometer, single quadrupole mass spectrometer, Mass Frontier 8.1 software, in-source CID

Application benefits

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) supports Thermo Scientific™ ISQ™ EM single quadrupole mass spectrometer and Thermo Scientific™ Vanquish™ Charged Aerosol Detector (CAD) control and data processing simultaneously.
- Sensitive detection of semi-volatile impurity A which exhibits low CAD response
- Peak purity confirmation to rule out co-elution
- Straightforward peak assignment based on *m/z*
- Identity confirmation of known impurities and prediction of molecular weight and putative structure proposal for unknown impurities

Goal

To demonstrate the additional benefits of coupling a single quadrupole mass spectrometer to an HPLC/CAD system by providing peak purity identification, molecular weight information, and peak confirmation for topiramate and its impurities.

Introduction

Impurities are inevitably present in small molecule drugs and can arise during the different stages of the products, from raw materials and side reactions in the manufacturing process to drug product storage. Different side effects caused by impurities were reported over the past few decades, including decreasing the therapeutic effect, showing toxicity, and altering properties of the active pharmaceutical ingredient (API), etc.[1-2] In alignment with the ICH Q3 guideline, it is necessary to identify the structure and assess the safety of the impurity when the content of one impurity is above the threshold (0.10% for ≤2 g/day dose for drug substances).[3] Additionally, to ensure the safety of the drug, impurities should be monitored during the entire product life cycle, from early research and development to commercial manufacturing.

HPLC-UV is one of the most common analytical techniques used in different labs to determine impurity concentration. Charged aerosol detection is a universal detection technique that can be utilized to detect non-volatile and some semi-volatile compounds with or without a UV chromophore. Compared with other universal detectors, such as evaporative light scattering detectors (ELSD) and refractive index (RI) detectors, CAD has higher sensitivity, broader dynamic range, and consistent analyte response, and can be used for gradient elution.[4] Specifically for the topiramate application, CAD shows three to nine times higher sensitivity than ELSD.[5] Now CAD has been included in the U.S. Pharmacopeia (USP) and European Pharmacopoeia (EP) for several drugs and their impurities analysis without a UV chromophore.[6] Mass spectrometry is an ideal tool for molecular weight and structural analysis. This information is commonly used for impurity peak confirmation and identification. The ISQ EM single quadrupole mass spectrometer is an easy-to-use and reliable instrument, designed for routine applications, which can be seamlessly integrated in GMP environments and operate under the 21 CFR Part 11 compliance-ready Chromeleon CDS, and provide a better understanding of analyzed samples in the lab.

Topiramate is an anticonvulsant drug mainly used for the treatment of different types of seizures and prophylactic treatment of migraines.[7] As topiramate and its impurities lack a suitable chromophore, the official method described in the EP for the determination of the drug substance and its impurities content employs HPLC-CAD (not including impurity A). In our previous application note, we showed the suitability of using the Thermo Scientific™ Vanquish™ Flex UHPLC system configured with Vanquish CAD for topiramate and its impurity analysis.[8] Here, to demonstrate the additional benefits of adding a single quadrupole mass spectrometer to the lab, an ISQ EM mass spectrometer was coupled with the HPLC-CAD.

Although the CAD is the detector of choice to meet the EP requirements, it does not allow for the measurement of impurity A, which is done by thin-layer chromatography (TLC). Adding an ISQ EM mass spectrometer allows for the analysis of all impurities within the same chromatographic run and provides some additional information. The molecular weight information provided by the system can be used for peak assignment and peak purity identification to rule out the co-elution of impurities with the API. The in-source collision induced dissociation (CID) function of the ISQ EM mass spectrometer generates molecular fragments, which can be used to further confirm known impurities or deduce putative structures for unknown impurities.

Experimental

Instrumentation

- Thermo Scientific™ Vanquish™ Core HPLC system consisting of:
  - Vanquish System Base Core (P/N VC-S01-A)
  - Vanquish Quaternary Pump CN (P/N VC-P21-A)
  - Vanquish Split Sampler CT (P/N VC-A12-A)
  - Vanquish Column Compartment C (P/N VC-C10-A)
  - Vanquish Charged Aerosol Detector F (P/N VF-D20-A)
- ISQ EM single quadrupole mass spectrometer (P/N ISQEM-ESI-APCI)

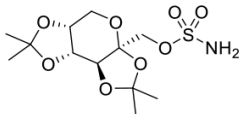
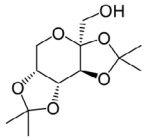
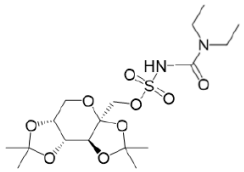
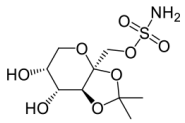
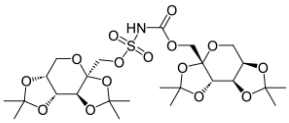
Software

- Chromeleon CDS, version 7.3
- Thermo Scientific™ Mass Frontier™ software, version 8.1

Reagents and consumables

- Water purified by a Thermo Scientific™ Barnstead™ GenPure™ Pro water purification system with a resistivity of 18.2 MΩ·cm or higher
- Thermo Scientific™ SureSTART™ 2 mL glass vials (amber) (P/N 6ASV9-2P)
- Thermo Scientific™ SureSTART™ 9 mm vial caps with septum (P/N 6ASC9ST1)
- Acetonitrile, HPLC grade, Fisher Scientific™ (P/N A998-4)
- Acetic acid, Optima™ LC/MS grade, Fisher Scientific™ (P/N A113-50)
- Ammonium acetate, Optima™ LC/MS grade, Fisher Scientific™ (P/N A114-50)
- Topiramate API and its impurities were obtained from various suppliers, as listed in Table 1.

Table 1. Summary of topiramate and its impurities listed in the Ph. Eur. 10.0

Name	Chemical name	Purity	Supplier	Chemical structure	Average molecular weight (Da)
Topiramate	2,3:4,5-Bis-O-(1-methyl ethylidene)-β-D-fuctopyranose 1-sulfamate	99.6%	Anpel		339.36
Topiramate impurity A	2,3:4,5-Di-O-isopropylidene-β-D-fructopyranose	99.0%	Aladdin		260.28
Topiramate impurity B	1-O-[(Diethylcarbamoyl)sulfamoyl] -2,3:4,5-Bis-O-(1-methyl ethylidene)-β-D-fructopyranose	98.0%	TRC		438.49
Topiramate impurity C	2,3-O-(1-Methylethylidene)-1-O-sulfamoyl-β-D-fructopyranose	97.0%	TRC		299.30
Topiramate impurity D	2,3:4,5-Bis-O-(1-methyl ethylidene)-1-O-[[[2,3:4,5-bis-O-(1-methyl ethylidene)-β-D-fructopyranose	98.0%	TRC		625.64

Sample and eluent preparation

Topiramate stock solution with a concentration of 10.0 mg/mL was prepared in an 8 mL amber glass vial by dissolving the appropriate standard sample with the equivalent initial gradient mixture of the liquid chromatography separation. Impurities stock solutions were prepared in 8 mL amber glass vials with a final concentration of 1.0 mg/mL with the same solvent.

The topiramate solution with a concentration of 5.0 mg/mL was prepared by diluting the topiramate stock solution with the solvent equal to the chromatographic starting conditions. A mixture solution of topiramate (5.0 mg/mL) and its impurities A–D (spiked at 0.1% weight relative to the test solution) was prepared by adding 1.0 mL topiramate stock solution, 10 µL impurities A-D stock solution, and 960 µL solvent into an 8 mL amber glass vial.

Eluent A was prepared by weighing 1.927 g ammonium acetate in a 1.0 L glass bottle, adding 1.0 L water into the bottle, then adjusting the pH to 3.5 using acetic acid. To avoid contamination of the eluents, the pH of eluent A was adjusted with a pH meter by decanting a small amount of the solution into a beaker and inserting the probe in the beaker to test the pH. Eluents were re-prepared every five days to minimize background noise and baseline drift.

Chromatographic conditions (EP 10.0)

Parameter	Value
Column	Thermo Scientific™ Accucore™ PFP, (100 × 4.6 mm, 2.6 µm), P/N 17426-104630
Mobile phase	A: 25 mM ammonium acetate in water, pH 3.5 (adjusted with acetic acid) B: Acetonitrile
Gradient	Time (min) — Solvent B (%) 0–5 — 20 5–15 — 20–50 15–15.1 — 50–20 15.1–20 — 20
Flow rate	1.0 mL/min
Column temperature	40 °C, still air
Autosampler temperature	8 °C
Autosampler wash solvent	Mixture of methanol and water (50:50, v:v)
Injection volume	20 µL
Detector settings (CAD)	Evaporation temperature: 35 °C Power function value: 1.0 Filter constant: 3.6 s Data collection rate: 10 Hz

ISQ EM mass spectrometer settings

Parameter	Value
Ionization mode	HESI, Positive
Source setting	Easy mode. Setting for sensitivity was 1; setting for mobile phase volatility was 1; setting for thermally labile sample was 1
Full scan	
Time	0–20 min
Mass range	*m/z* 100–800
Source CID voltage	0, 20, 30, 40, 50, 60 V
Compound scans	
Compound Time Mass Source CID voltage	Topiramate impurity C 0–2.5 min $[M+Na]^+$: 322.0 *m/z* 0 V
Compound Time Mass Source CID voltage	Topiramate impurity A 2.5–4.0 min $[M+NH_4]^+$: 278.2 *m/z* 0 V
Compound Time Mass Source CID voltage	Topiramate impurity B 8.0–10.0 min $[M+H]^+$: 439.0 *m/z* 10 V
Compound Time Mass Source CID voltage	Topiramate impurity D 10.1–15.0 min $[M+NH_4]^+$: 643.4 *m/z* 20 V

Results and discussion

The HPLC-CAD method listed in Ph. Eur. 10.0 was used to detect the API topiramate and its impurities. To acquire compound mass information, an ISQ EM mass spectrometer was added to this system. For this, a T-piece was used to split the flow from the analytical column between the mass spectrometer and the CAD with a split ratio of 1:3 as we described before.[9] The topiramate solution with a concentration of 5.0 mg/mL was initially analyzed; the CAD response and total ion chromatogram (TIC) with a scan range of *m/z* 100–800 are shown in Figure 1. The API topiramate was eluted at 7.75 min, and no impurities at a level higher than 0.10% relative to the API were detected from the CAD chromatogram (the areas of the unknown peak eluted at 10.45 min on the CAD chromatogram and 12.9 min on the TIC are about 0.075% and 0.089% of the area of topiramate, respectively). To confirm the absence of additional components co-eluting with the API, peak purity was assessed by comparing the mass spectra of the peak front, peak apex, and peak tail. Figure 2 shows the results of peak purity analysis, where $[M+NH_4]^+$ (*m/z* 357.1) of the topiramate was the dominant peak in all cases. No other peaks were detected, which indicates a high purity of the topiramate peak.

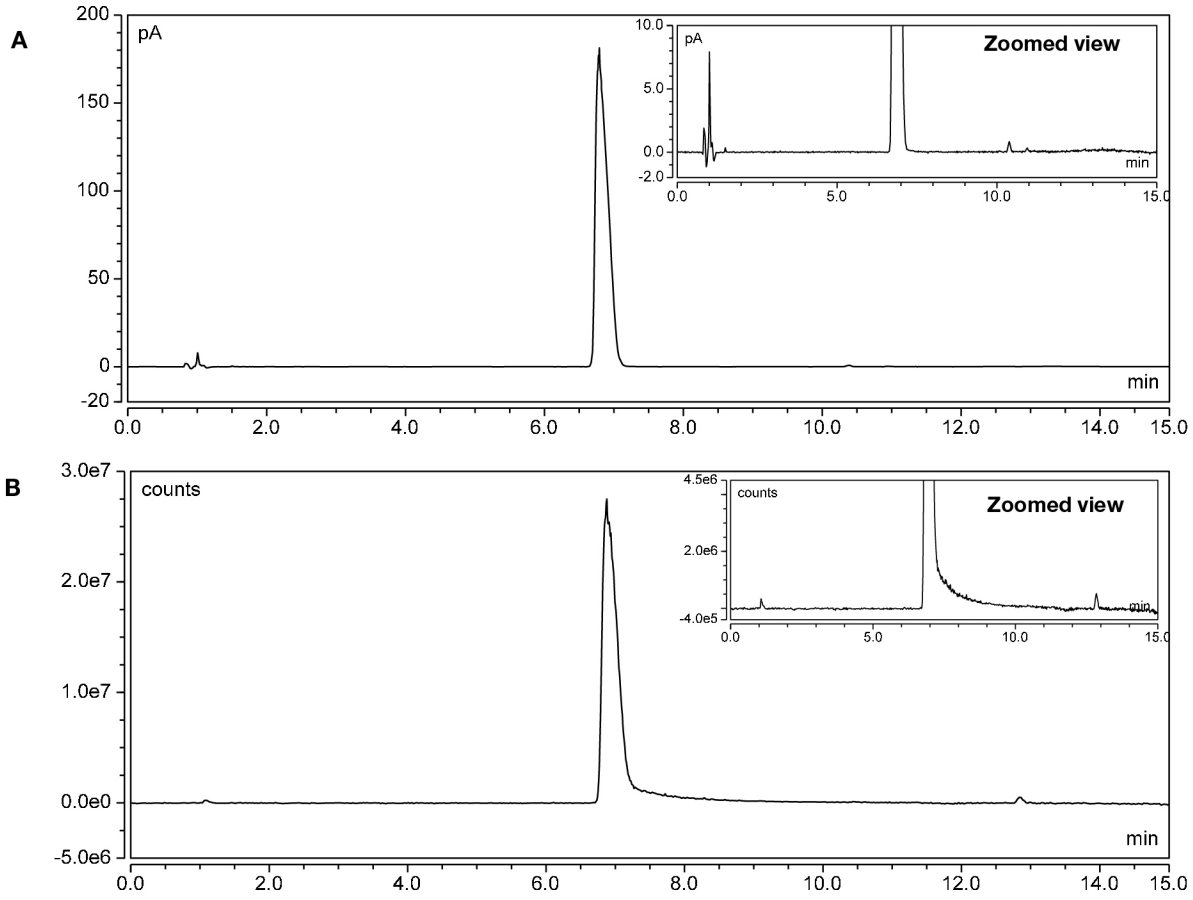


Figure 1. CAD chromatogram (A) and TIC (B) of topiramate with zoom into the baseline

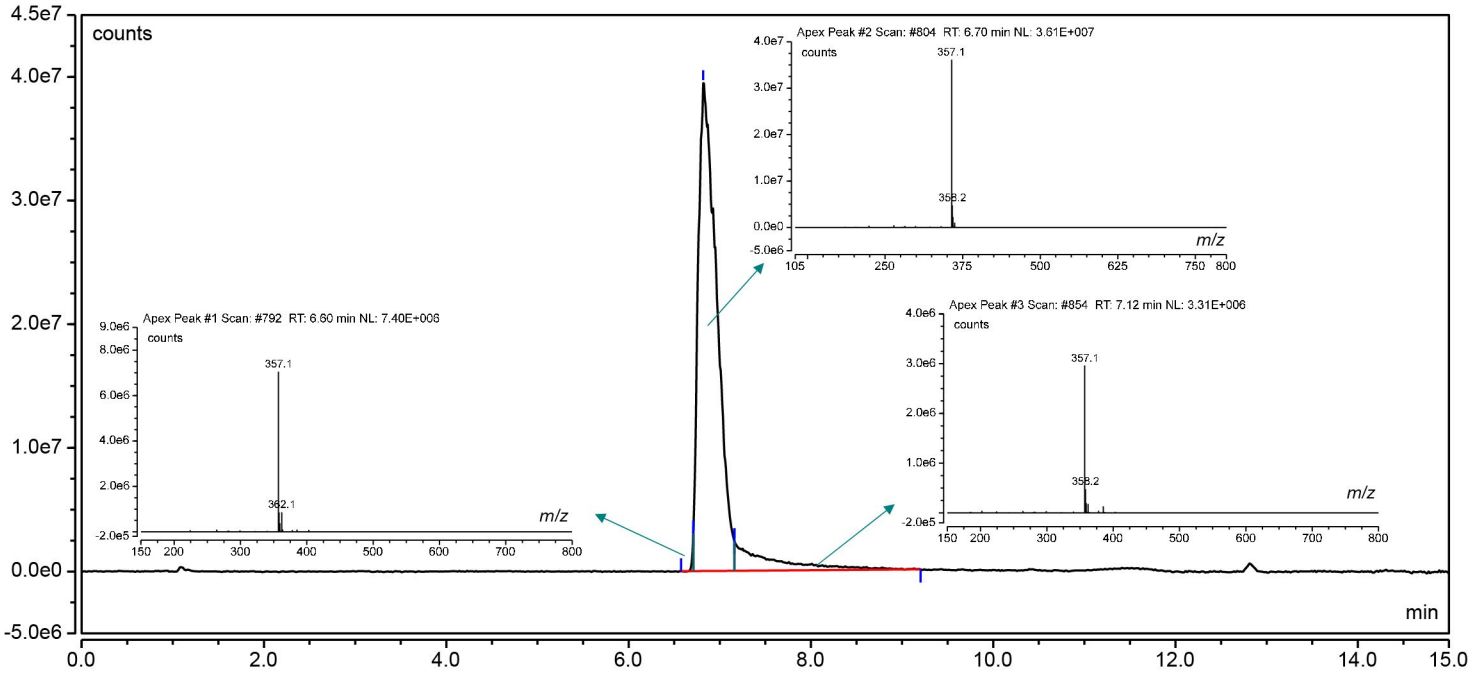


Figure 2. Peak purity analysis of topiramate. Mass spectra of the peak front, peak apex, and peak tail of topiramate are shown. Mass spectra for the peak front and the peak tail represent 10% of the peak height. The concentration for topiramate is 5.0 mg/mL and for impurities A to D is 0.005 mg/mL.

To simulate an analysis of API with impurities, impurities A to D listed in Ph. Eur. 10.0 were spiked into topiramate solution at a reference concentration of 0.10%. Figure 3 shows the CAD response and TIC of this solution, with the API and three impurities detected using CAD. Four impurities and the API were detected by using the mass spectrometer. These peaks could be quickly assigned to impurities listed in Ph. Eur. 10.0 based on the *m/z* information obtained from the mass spectrometer, as shown in Figure 3C. As mentioned in our previous application note, charged aerosol detection is a suitable technique for topiramate and its impurities B, C, and D analysis. Impurity A is a semi-volatile compound and cannot be quantified by the CAD at the 0.1% level due to its low CAD response.[8] Instead, the method listed in Ph. Eur. 10.0 for impurity A detection is TLC. Here, the TIC results indicate that the mass spectrometer shows a good response for these four impurities and can be used for impurity A analysis even at a very low level (0.1% of API). This provides a solution, allowing the analysis of the complete impurity profile in one injection.

The sensitivity of the CAD and ISQ EM mass spectrometer was further investigated. To get a better response, compound mode (selected ion monitoring) was used on the ISQ EM mass spectrometer with the conditions listed in the Experimental section. Figure 4 shows the response of impurities A to D from CAD and the ISQ EM mass spectrometer with different concentrations. For CAD, the limit of the detection (LOD) of impurities C and D was 0.0625 µg/mL with signal-to-noise ratios (S/N) of 8.7 and 5.1, respectively, and for impurity B was 0.25 µg/mL with an S/N of 10.7. Compared with CAD, at the 0.0625 µg/mL level, the S/N values for the impurities A, B, C, and D from the ISQ EM mass spectrometer were 6.2, 65.9, 12.2, and 98.5, respectively. At the 0.0125 µg/mL level, the S/N of impurities C, B, and D from the mass spectrometer were 3.6, 13.8, and 17.3, representing a significant improvement of the method's sensitivity by incorporating the ISQ EM mass spectrometer into the analysis. The high sensitivity of the ISQ EM mass spectrometer enables the detection of hidden components with low concentration and/or insufficient CAD response in drug products.

In addition to providing molecular weight information, the ISQ EM mass spectrometer can be used to obtain structural information using in-source CID, creating pseudo-MS^2 fragmentation spectra. This can be used to further confirm known impurities or deduce putative structures for unknown impurities. Using in-source CID, an additional voltage offset is added to the ion transfer tube and the skimmer cone optics, causing a faster acceleration of the ions into the Q00 octopole, enhancing the collision with residual nitrogen molecules in a similar way to the collision cell of a triple quadrupole mass spectrometer.[10]

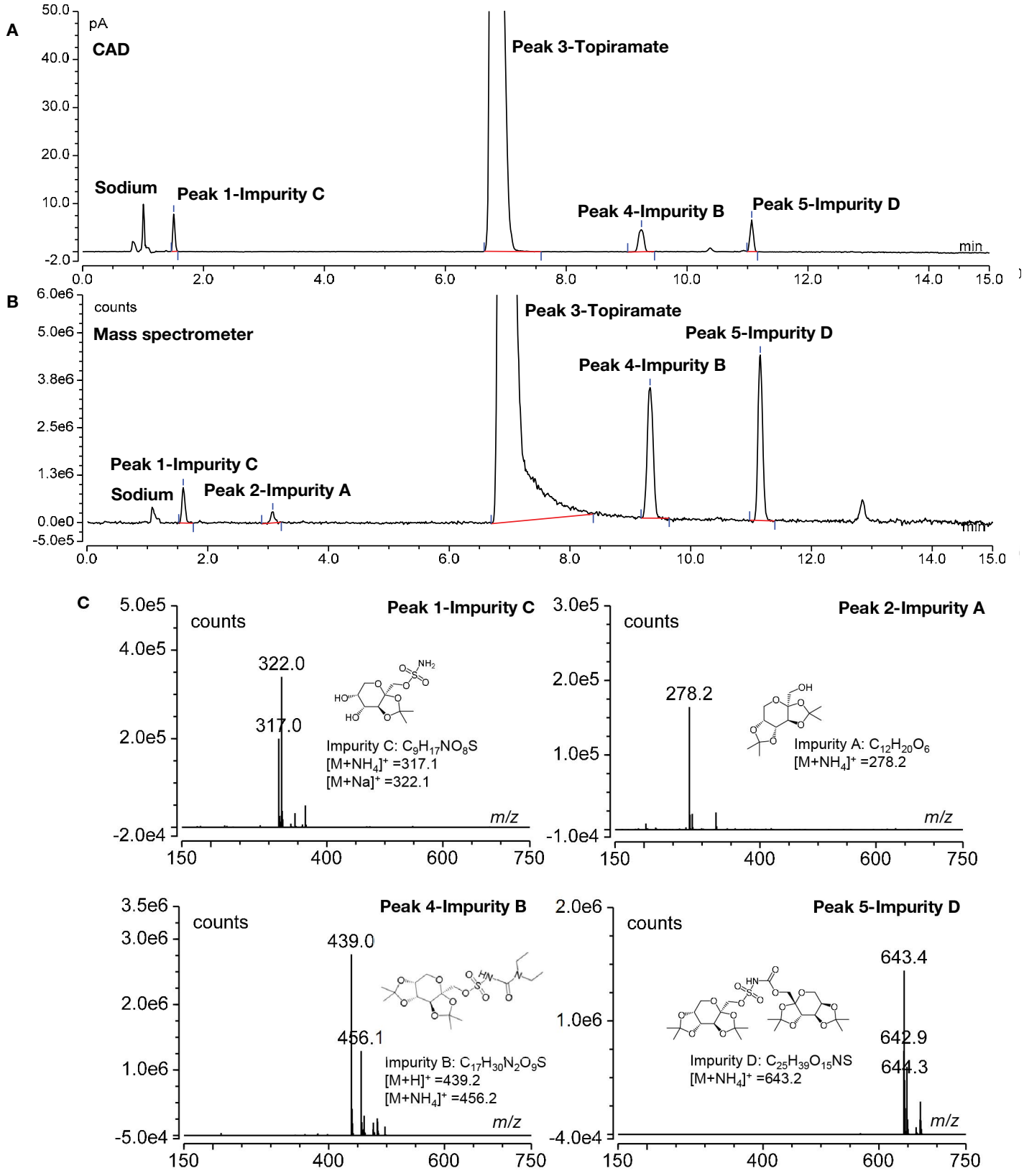


Figure 3. CAD chromatogram and TIC of topiramate and its impurities. (A) CAD response of topiramate and its impurities A to D, (B) TIC of topiramate and its impurities A to D, (C) mass spectra of impurities A to D. The concentration for topiramate is 5.0 mg/mL and for impurities A to D is 0.005 mg/mL.

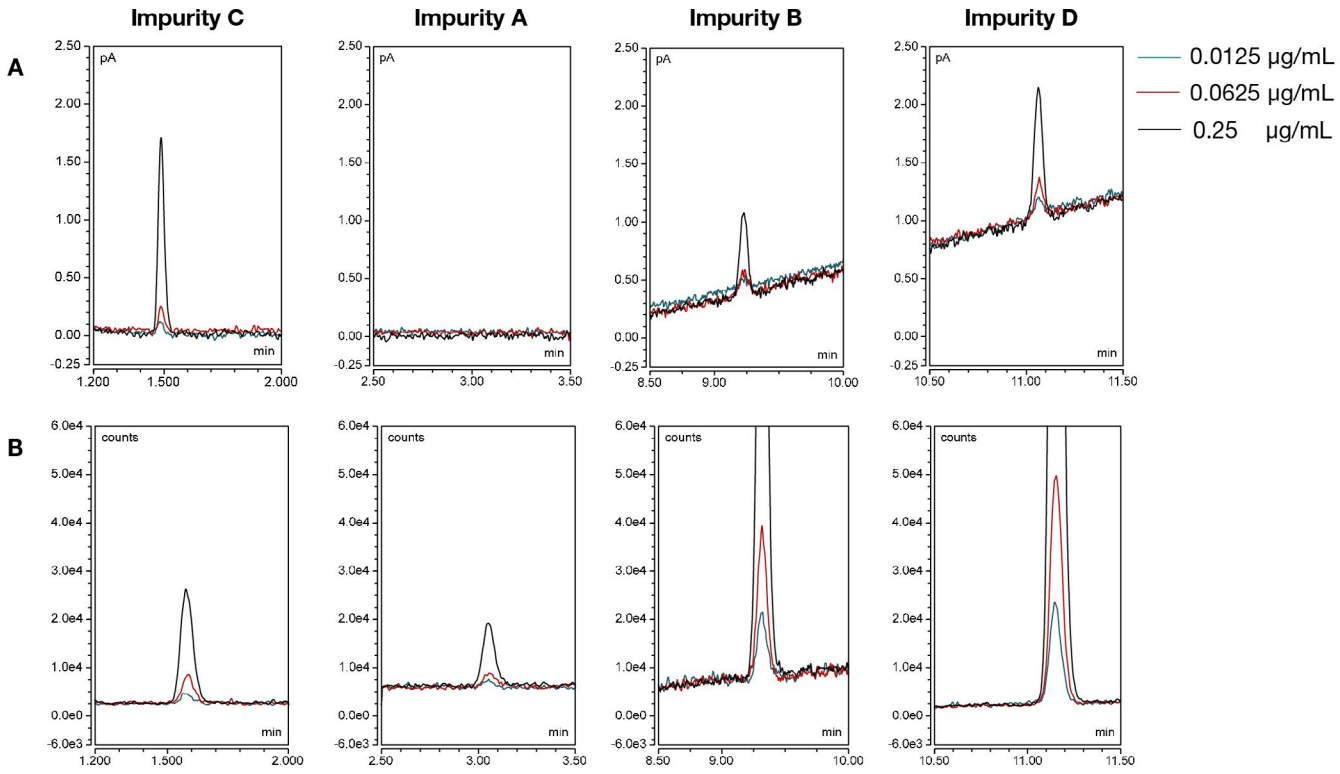


Figure 4. Comparison of the sensitivity of the CAD (A) and ISQ EM mass spectrometer (B) for impurities A–D based on the CAD trace and SIM trace, respectively

As the energy required to break molecular bonds is different depending on the compounds, the in-source CID voltage needs to be optimized. At low CID voltage, bonds within the molecule cannot break. If the in-source CID voltage is too high, multiple bonds break, which makes it difficult to deduce the molecular structure. The voltage from 10 to 60 V was tested in this experiment. The results show that the optimum voltage for the impurities A and B was 30 V and for the impurities C and D was 40 V. Under these conditions, some of the labile bonds were broken and generated fragments in addition to the intact compounds. The mass spectra obtained with in-source CID for impurities A to D are shown in Figures 5 to 8. Mass Frontier software was used to assist in the elucidation of the fragments. Based on their known chemical structures, *in silico* fragments could be predicted in the Mass Frontier software based on general fragmentation rules and literature fragmentation pathways. These can be used to annotate the observed fragments and explain the mechanism. The structures of fragments were annotated in Figures 5–8, which show that almost all fragments generated by in-source CID can be predicted and explained in Mass Frontier software. These results could be used to further confirm the structure of the impurities.

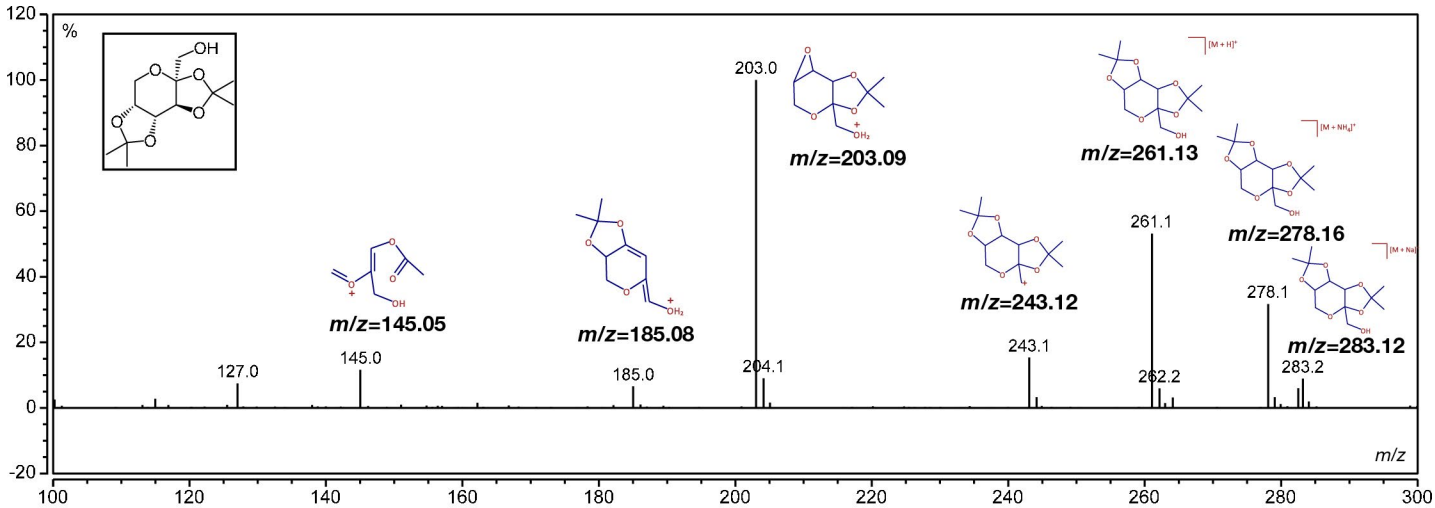


Figure 5. Mass spectrum of impurity A at an in-source CID of 30 V with fragments annotated

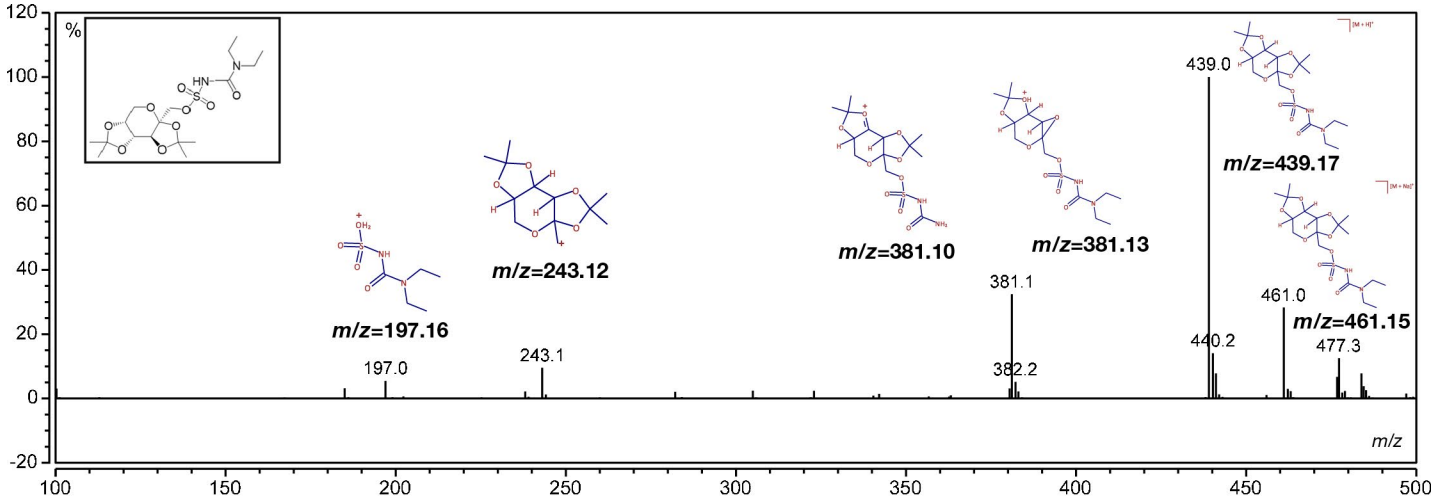


Figure 6. Mass spectrum of impurity B at an in-source CID of 30 V with fragments annotated

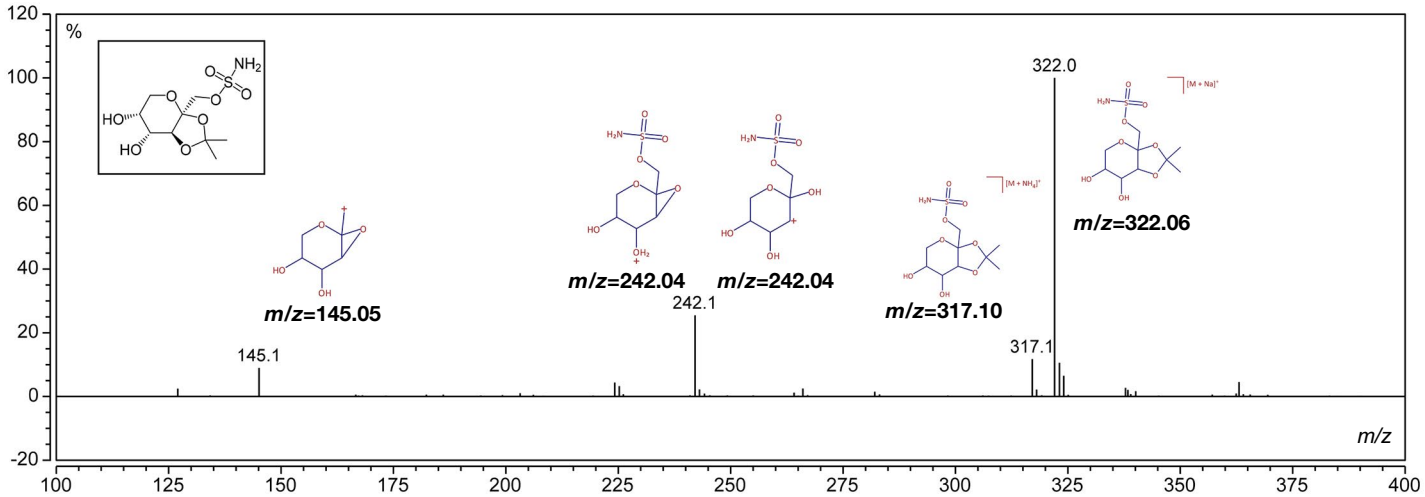


Figure 7. Mass spectrum of impurity C at an in-source CID of 40 V with fragments annotated

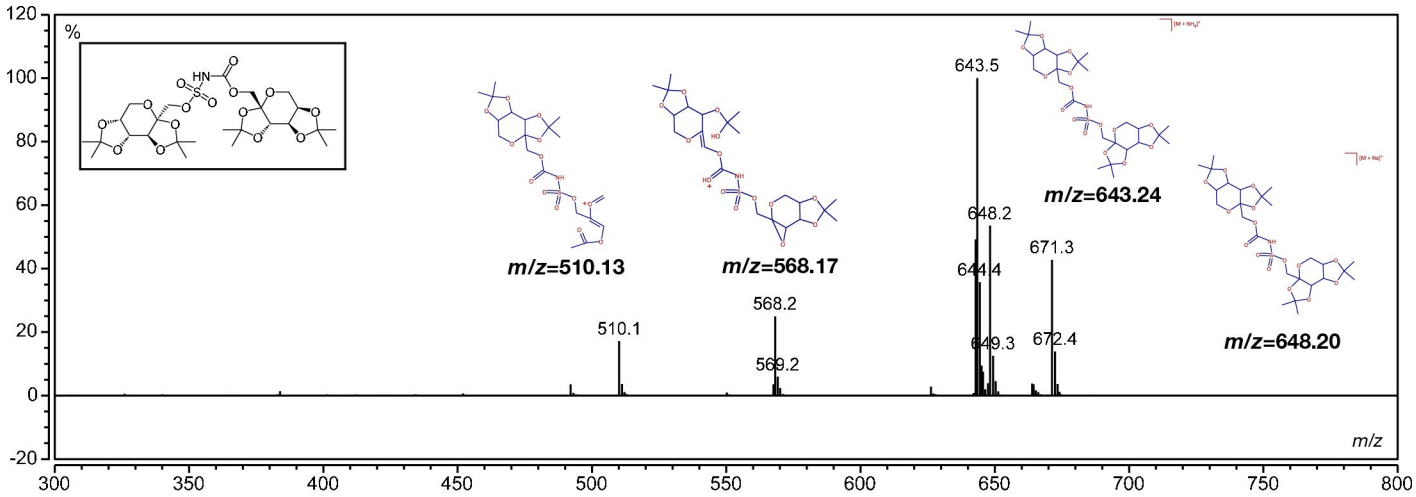


Figure 8. Mass spectrum of impurity D at an in-source CID of 40 V with fragments annotated

Conclusion

CAD is the official detector described in the EP for the sensitive and accurate quantitative analysis of the drug topiramate and its impurities B-E. In this application note, an ISQ EM mass spectrometer was added to the HPLC-CAD for topiramate and its impurity analysis. The single quadrupole mass spectrometer brings the following additional benefits:

- Impurity A is a semi-volatile compound and generates a limited response to CAD; TLC was recommended for impurity A analysis in Ph. Eur. 10.0. The ISQ EM mass spectrometer has a good response for topiramate and impurities A to D, providing an alternative for impurity A analysis.
- The peak purity of API topiramate was identified using the full scan results from the ISQ EM mass spectrometer.
- The high sensitivity of the ISQ EM mass spectrometer enables the detection of hidden components with low concentration and/or insufficient CAD response in drug products.
- The ISQ EM mass spectrometer provided molecular weight and molecular structure information, which can be used to confirm the known impurities and putatively deduce unknown impurities.

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