Customer application note | 000572

Liquid chromatography

Topiramate impurity analysis: Method migration from a legacy HPLC system to modern instrumentation

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Keywords

Topiramate, ESA Corona CAD, Vanquish Charged Aerosol Detector, pharmaceutical quality assurance, HPLC impurity analysis, pharmaceutical manufacturing

Application benefits

Demonstration of the suitability of the Thermo Scientific™ Vanquish™ Flex UHPLC system with a Thermo™ Scientific™ Vanquish™ Charged Aerosol Detector for topiramate impurity analysis

Goal

This application note examines the suitability of an LC system with a Vanquish Charged Aerosol Detector (CAD) for impurity analysis of topiramate as a replacement for the legacy LC system with an ESA Corona CAD used in the European Pharmacopoeia (Ph. Eur.) monograph 2616.

Introduction

Topiramate is an anticonvulsant drug used primarily to treat epilepsy. It is also used to prevent migraines and in the treatment of trigeminus neuralgia and obesity. Typical doses range from 50 mg to 400 mg (max), which determines the reporting threshold of the impurity analysis.[1] This is defined by the ICH Q3A (R2) guideline and set to 0.05% for substances with a daily intake less than 2 g. According to this guideline, for topiramate the identification threshold is set to 0.10% and the quantification threshold is set to be 0.15%.[2]

As topiramate and its impurities lack a suitable chromophore, measurement by HPLC-UV is not feasible and an alternate approach is required for their detection. Charged aerosol detection is a universal technique capable of measuring analytes that do not possess a suitable chromophore. In this application note, we examine the impurity analysis of topiramate (according to the Ph. Eur.)[3] by means of two different generations of CAD and their suitability for this analysis.

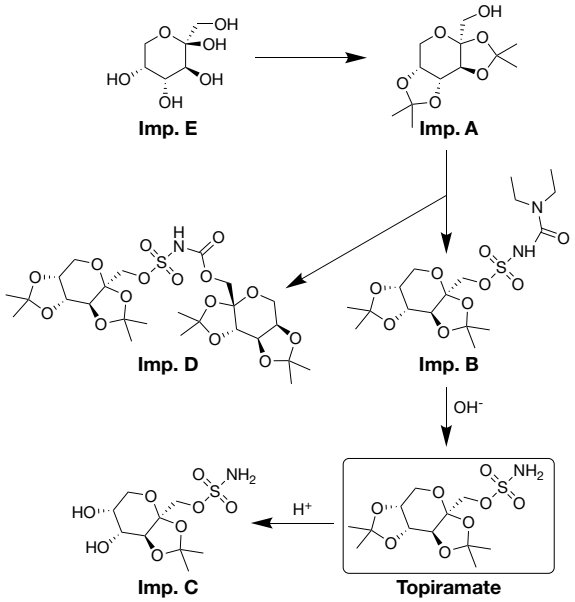

Figure 1. Impurity profile of topiramate with respect to the Ph. Eur. 10.5 and to Ilko *et al.*[3,4]

Experimental

Chemicals

Chemical name	Part number
Deionized water, 18.2 MΩ·cm resistivity or higher	N/A
Acetonitrile, Optima™ LC/MS grade (ACN), Fisher Scientific™	A955-212
Ammonium acetate, Optima™ LC/MS grade, Fisher Scientific™	A114-50
Acetic acid, Optima™ LC/MS grade, Fisher Scientific™	A113-50
Formic acid, 99,0+%, Optima™ LC/MS grade, Fisher Scientific™	A117-50
Topiramate Certified Reference Standard (CRS)	EDQM Y0002134
Methanol, Optima™ LC/MS grade (MeOH), Fisher Scientific™	A456-212
Impurity A CRS	EDQM Y0002133
Impurity E CRS (Fructose)	EDQM Y0002132
Impurity B	EDQM
Impurity C	EDQM
Impurity D	EDQM

Sample handling

Item name	Part number
Fisher Scientific™ Fisherbrand™ Mini Vortex Mixer	14-955-152
Vials (amber, 2 mL), Fisher Scientific™	03-391-6
Cap with Septum (Silicone/PTFE), Fisher Scientific™	13-622-292

Sample preparation

All test and reference solutions were prepared in accordance with the Ph. Eur. monograph for topiramate.[3] The sample solution was prepared by accurately weighing and dissolving 50.0 mg of the substance to be examined in 10.0 mL of solvent equal to the chromatographic starting conditions. Reference solution (a) was obtained by diluting the test solution (1:100 followed by a 1:10 dilution step). Reference solution (b) contains topiramate impurity E CRS (fructose) and is prepared by dissolving and diluting 15.0 mg of fructose to 50.0 mL with a solvent equal to the chromatographic starting conditions. A 5.0 mL volume of this solutions was again diluted to 200.0 mL to obtain the reference solution (b). The preparation process of reference solution (c) includes the *in situ* preparation of impurity C. Therefore, a 5 mg amount of topiramate was dissolved in 1 mL of a mixture of acetonitrile, water, and formic acid (2:4:4, v:v:v) and incubated at 60 °C for 30 min. A 20 µL volume of that solution was diluted to 1 mL with a solution of impurity E (fructose) (25 mg/L) to obtain reference solution (c).

Additionally, a solution of topiramate (5 mg/mL) and its impurities A–E (0.1% with reference to the test solution) was prepared by spiking the test solution with an impurity stock solution (1 mg/mL of each impurity).

Instrumentation

Module	Part number
Vanquish Flex UHPLC system consisting of:	
Vanquish System Base	VH-S01-A
Vanquish Dual Pump F	VF-P32-A
Vanquish Split Sampler FT	VF-A10-A
Vanquish Column Compartment H	VH-C10-A-02
Vanquish Charged Aerosol Detector H	VH-D20-A
Corona Nitrogen 1010 Nitrogen Generator	6295.0200

Topiramate method (Ph. Eur.)

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

Chromatography Data System

Thermo Scientific™ Chromeleon™ Chromatography Data system (CDS), version 7.3 was used for data acquisition and analysis.

Legacy instrumentation

Originally, the method was developed on the ESA Corona Charged Aerosol Detector (ESA, Chelmsford, MA, USA), using a Phenomenex™ Kinetex™ PFP 100 × 4.6 mm, 2.6 µm column on an Agilent™ 1100 chromatographic system consisting of an online vacuum degasser, a binary pump G1312A, an autosampler G1313A, and a thermostatted column compartment G1316A (Agilent Technologies Deutschland GmbH, Waldbronn, Germany) coupled to the charged aerosol detector by a 0.25 mm internal diameter PEEK capillary and a 0.22 µm stainless-steel inlet-frit. An ESA nitrogen generator (Thermo Fisher Scientific, ESA, Chelmsford, MA, USA) was used to produce highly pure nitrogen (99.9%). The gas inlet pressure was set to 35.0 psi. Filter was set to “none” and range set to 100 pA. Agilent ChemStation™ Rev. B03.02 software was used for data processing.

Results and discussion

With regards to the impurity profile of topiramate (Figure 1), impurity A and impurity E can potentially occur at levels above 0.15% and therefore must be regarded as specified according to the Ph. Eur. monograph and the ICH Q3A (R2) guideline.[2,3] To guarantee a pure compound, impurity A is limited to no more than 0.2%, while for impurity E a maximum content of 0.15% is allowed. In this monograph, the content of impurity A is determined by TLC and not via HPLC-CAD due to its semi-volatile characteristics.[3]

The method was migrated by reproducing the legacy method developed years back[4] on the Vanquish Flex UHPLC system with a Vanquish CAD by adapting the instrument parameters with minimal necessary changes. Comparable results were achieved with the Vanquish instrumentation with respect to the impurity analysis of topiramate (Figure 2). With regards to the elution order, there were no differences in the chromatograms. The system suitability criteria (resolution between impurity E and impurity C of at least 4.5) was met by both instruments. The Vanquish Flex UHPLC system achieved a resolution of 6.25, which was slightly better than the 4.96 achieved with the legacy system. This is due to differences in the column performance from the two manufacturers. In addition, sharper peaks could be obtained with the Vanquish UHPLC-CAD system. Consequently, potential additional impurities, if present, are more readily detected.

In general, due to a partial overlapping of the sodium peak and topiramate impurity E, the amount of the latter can be determined by evaluating the respective peak heights. Contamination with sodium may lead to an overestimate of impurity E level. Therefore, the peak pair of sodium and impurity E will need to be carefully evaluated. Higher sodium concentrations can occur if water of insufficient quality is used (e.g., the usage of purified water instead of ultra-pure water). Furthermore, for the best CAD performance, the highest grade chemicals commercially available—for example, LC-MS grade—should be used.

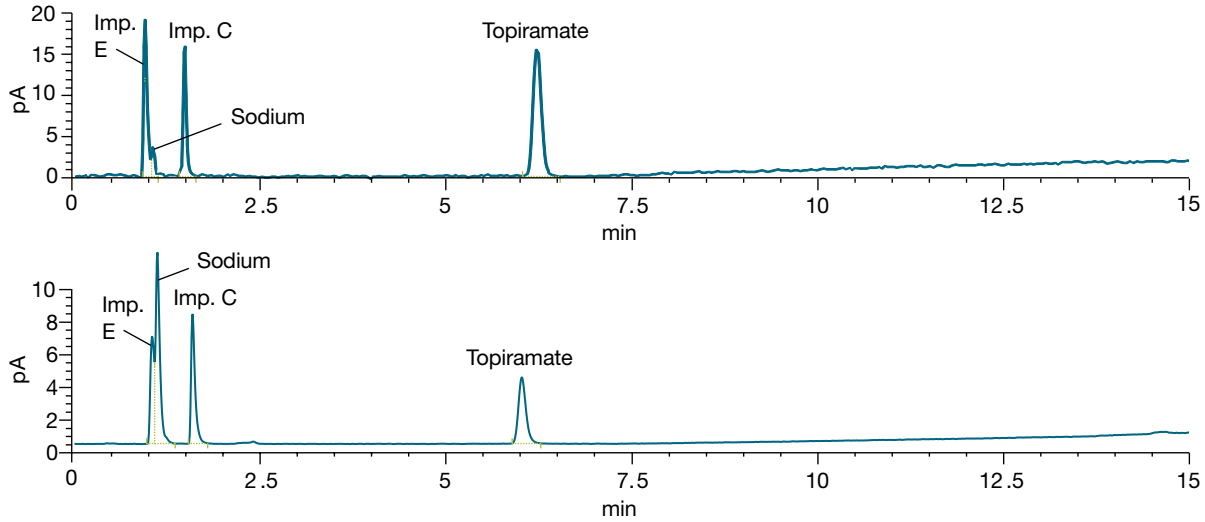

Figure 2. Comparison of the chromatograms obtained with two different topiramate reference solutions measured with different laboratory instrumentation at different times (c). Top: Vanquish Flex UHPLC system; bottom: legacy HPLC system.

Visual comparison shows a higher noise level for the Vanquish CAD compared to the older generation Corona CAD. The derived apparent signal-to-noise ratio (S/N) of a reference solution (a) (a solution of 0.1% topiramate referred to the test solution), which is 30.5 for the Corona CAD and 17.2 for the Vanquish CAD, indicates a better sensitivity for the older generation model. Care must be taken when interpreting these values, as the calculation is only accurate for a linear response behavior. In literature it is well described that earlier CAD models show a diminished response at the low mass concentration range.[4] As a consequence, the noise levels are underestimated, leading to higher apparent S/N values. It has been demonstrated that the Vanquish CAD shows a superior sensitivity throughout the whole mass range,[5] and this effect is most pronounced for low mass concentrations. In practical terms, it is erroneous to rely on the S/N or higher-level standards to derive limits of quantitation and detection, but rather calibration standards, which are close to the LOD/LOQ, should be used. Additionally, the sensitivity can be improved for the Vanquish CAD by optimization of the evaporation temperature, which is adjustable with the Vanquish Charged Aerosol Detector, but not with the earlier model. (Figure 3).

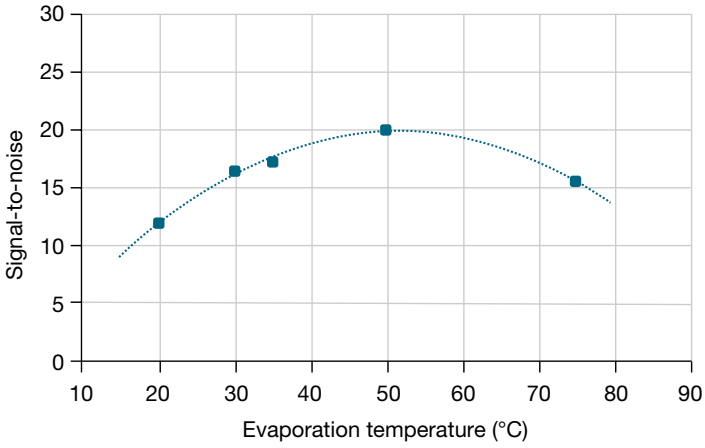

Figure 3. Influence of the evaporation temperature on the signal-to-noise ratio of a 0.1% topiramate solution with an optimum value at 50 °C (see References 5 and 6 for more details)

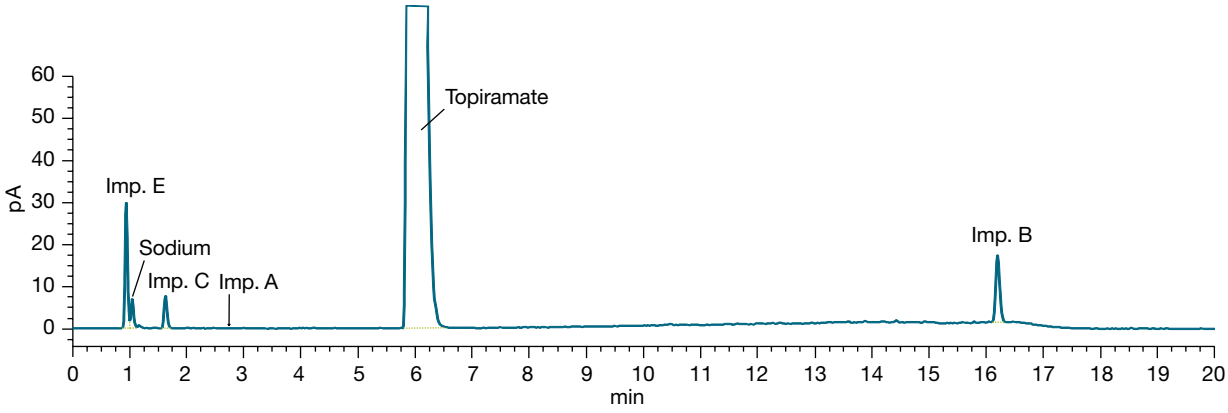

Figure 4. Impurity spiked solution of topiramate measured by means of the current related substances test for topiramate of the Ph. Eur. on the Vanquish Flex UHPLC system

A solution of topiramate spiked with its impurities A–E was measured following the Ph. Eur. method (Figure 4). The impurities B, C, and E can easily be detected. As mentioned above, impurity A is a semi-volatile compound and cannot be quantified by the CAD.

Interestingly, the sensitivity of the CAD can be increased by using post-column addition of ACN achieved with a T-piece and flow from the second pump.[4] As can be seen in Figure 5, the detection sensitivity of impurity A can be significantly increased. However, although the sensitivity was improved, it was still not sufficient (at least 100-fold lower compared to the other impurities) to quantify impurity A, so TLC quantification described in the Ph. Eur. monograph is still recommended.

Impurity D could not be determined within the runtime given by the gradient in the Ph. Eur. method using either the Kinetex column or the Accucore column (see exemplary chromatogram in Figure 4). Although it remains unclear as to why impurity D does not elute within the original run time, this might be due to subtle differences between commercial batches of columns, i.e., a slightly different column chemistry compared to the column batch used for the method development from several years ago.[4] This chromatographic problem is readily solved by using an additional hold for 5 minutes with 50% mobile phase B at the end of the gradient before the recalibration step (Figure 6).

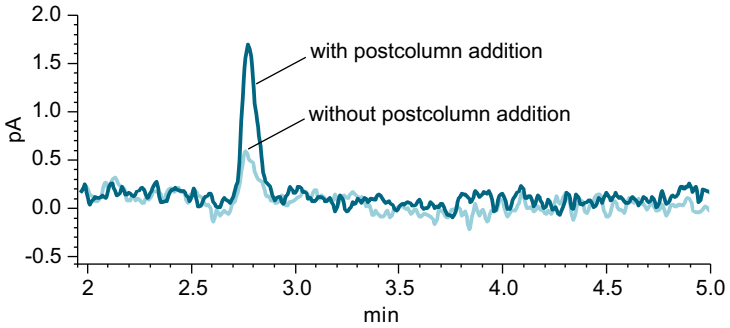

Figure 5. Influence of post-column addition with isocratic flow of ACN (0 vs 1 mL/min) on the response of impurity A

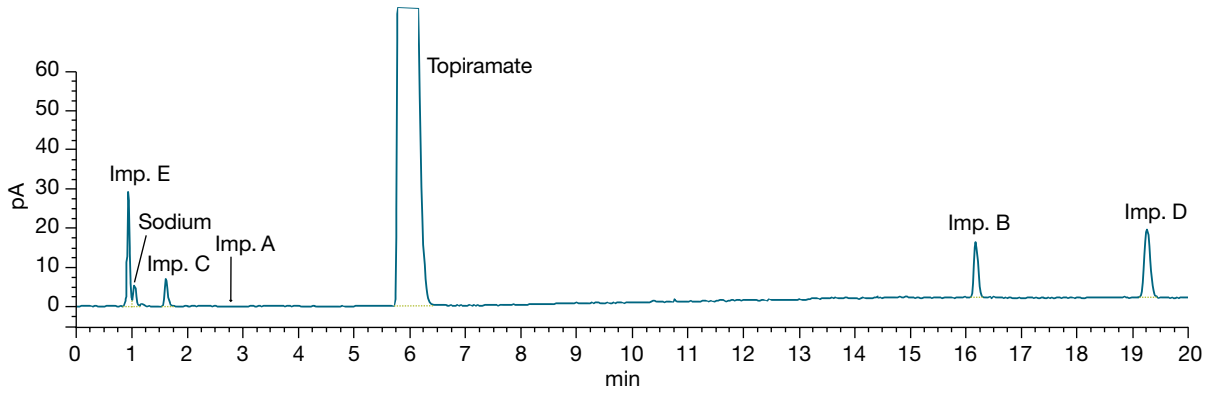

Figure 6. Chromatogram of an impurity spiked test solution of topiramate performed on the Vanquish Flex UHPLC system; gradient conditions: 0–5 min 20% B; 5–15 min 20→50% B; 15–20 min 50% B; 20–20.1 min 50→20% B; 20.1–25 min 20% B

Conclusion

- Successful method migration from the ESA CAD system to the Vanquish Flex UHPLC system configured with Vanquish CAD was demonstrated. Both instrumental approaches met the requirements of the Ph. Eur. “related substances” test for the impurity analysis of topiramate.
- It is recommended that impurity E be quantified using peak height.
- Post-column addition of ACN can improve signal-to-noise ratios.
- The Vanquish Flex UHPLC system with the Vanquish Charged Aerosol Detector is more versatile. For example, the ability to adjust evaporation temperature enables improved performance.
- The gradient needs to be prolonged to elute impurity D by adding a 5 min hold at the high organic step.
- Usage of LC-MS chemicals is recommended.

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