

Determination of Sulfate and Sulfamate in Topiramate Using a Reagent-Free Ion Chromatography System

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Key Words

Antiepileptic Drugs, Drug Product, Epilepsy, IonPac AS11, RFIC, Seizures, Suppressed Conductivity

Introduction

Epilepsy is a common neurological disorder that can cause anywhere from a brief cessation of responsiveness to severe muscle spasms with a loss of consciousness.¹ This condition is usually controlled with antiepileptic drugs (AEDs). However, approximately 20% of patients with epilepsy are resistant to therapy with first-generation AEDs.² Topiramate (Topamax®) is a second-generation AED approved by the FDA for the treatment of partial seizures (with or without secondarily generalized seizures), seizures associated with Lennox Gastaut Syndrome, and primary generalized tonic-clonic seizures in patients that are inadequately controlled by conventional first-generation AEDs.³ Topiramate has also been shown to be effective in the prophylactic treatment of migraine headaches.

Topiramate (2,3:4,5-bis-O-methylethylidene-β-D-fructopyranose sulfamate) is structurally distinct from other AEDs in that it is a sulfamate-substituted monosaccharide with a molecular formula of $C_{12}H_{21}NO_8S$ and a structural formula shown in Figure 1. Under ambient storage conditions, topiramate is very stable in the solid state. However, at elevated temperatures and humidity, the active pharmaceutical ingredient (API) degrades to produce

organic degradation products, insoluble polymeric products, and the inorganic anions sulfamate and sulfate.⁴ When the degraded product is assayed, a decrease in the topiramate concentration is observed.⁵ Determination of the inorganic degradation products, sulfamate and sulfate, can be used to monitor and confirm topiramate degradation.

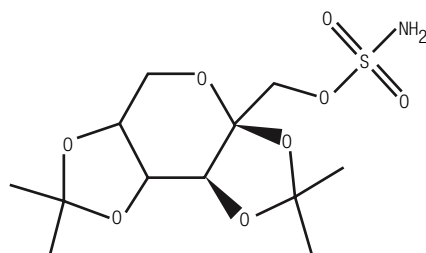
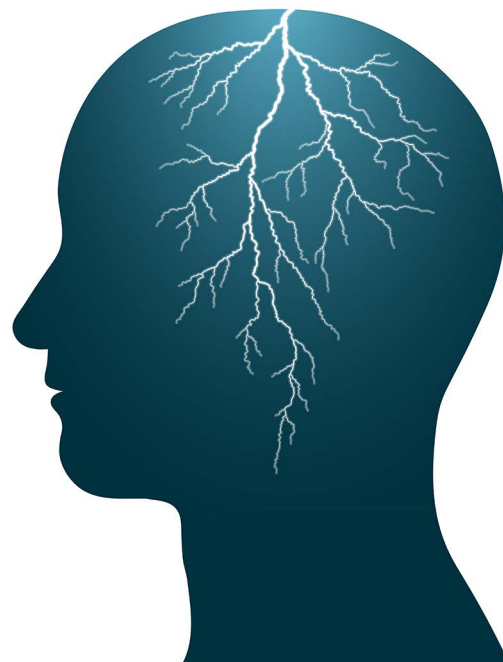


Figure 1. Chemical structure of topiramate.



The current U.S. Pharmacopeia (USP) compendial method for determining the limit of sulfamate and sulfate in topiramate describes an anion-exchange chromatography method using a gradient of manually prepared sodium hydroxide, followed by suppressed conductivity detection.⁶ In addition, a proposed USP method for determining the limit of sulfamate and sulfate in topiramate tablets uses a PRP-X100 anion-exchange column (Hamilton®, Reno, NV) with a *p*-hydroxybenzoic acid/methanol eluent (97.5:2.5, adjusted pH 9.4 ± 0.5) with conductivity detection.⁷ The use of manually prepared NaOH eluent in the current USP method imposes some potential limitations that can affect the integrity of the analytical results, such as undesirable baseline shifts, high detection limits, and irreproducible retention times. Also, the proposed USP method has a peak area precision specification of less than 15% for sulfamate and sulfate, suggesting that the method is imprecise.

In this application note (AN), we demonstrate the use of a Thermo Scientific™ Dionex™ Reagent-Free™ Ion Chromatography (IC) system with the Thermo Scientific™ Dionex™ IonPac™ AS11 column and suppressed conductivity detection for the determination of sulfamate and sulfate in topiramate. The Dionex IonPac AS11 (USP L61) column has been successfully used in the pharmaceutical industry and is currently specified in several USP methods, including an assay for citric acid/citrate and phosphate in different pharmaceutical formulations, phosphite in etidronate disodium, and free sulfate in enoxaparin sodium injection.^{8–10}

The Dionex IonPac AS11 column consists of a 13 µm microporous resin bead with a highly crosslinked core, and a Thermo Scientific™ Dionex™ MicroBead™ anion-exchange layer that is functionalized with hydrophilic quaternary ammonium groups. The selectivity of the Dionex IonPac AS11 column is optimized for the separation of a large number of inorganic anions and organic acid anions using a hydroxide eluent gradient. Because the selectivity of sulfamate is similar to low molecular weight organic acids, the Dionex IonPac AS11 column is ideal for this application. The column enables the resolution of the target analyte from other excipients in a topiramate sample while also allowing the separation of sulfate within a reasonable time.

An automated potassium hydroxide eluent generator eliminates the problems typically encountered when manually preparing hydroxide eluents, as described in the current USP method. In addition, no organic solvents are required, which further simplifies this analysis. In this AN, the linearity, detection limits, precision, and recovery of the sulfamate and sulfate degradation products in a topiramate sample are demonstrated.

Equipment

- Thermo Scientific™ Dionex™ ICS-3000 RFIC™ system* consisting of:
 - DP Dual Pump module (an SP Single Pump module can also be used)
 - EG Eluent Generator module
 - DC Detector/Chromatography module (single or dual temperature zone configuration)
 - AS Autosampler
- Thermo Scientific™ Dionex™ EluGen™ EGC II KOH cartridge (P/N 058900)
- Thermo Scientific™ Dionex™ CR-CTC Continuously Regenerated Cation Trap Column (P/N 060477)
- Thermo Scientific™ Dionex™ Chromeleon™ 6.8 Chromatography Data System
- Glass injection vials with caps, 1.5 mL (vial kit, Dionex P/N 055427)
- Glass scintillation vials (20 mL, VWR® P/N 66021-624)

* Any Thermo Scientific™ Dionex™ RFIC-EG™ system can be used for this application.

Reagents and Standards

- Deionized water, Type I reagent grade, 18 MΩ-cm resistivity or better
- Sulfamic acid, NH₂SO₃H (Sigma-Aldrich® 242772)
- Sulfate standard 1000 mg/L, 100 mL (Thermo Scientific P/N 037160 or Ultra Scientific®, VWR P/N ULICC-006)

Samples

Topiramate (C₁₂H₂₁NO₈S, USP Catalog # 1672206)

Conditions

Column:	Dionex IonPac AG11 guard, 2 × 50 mm (P/N 044079), Dionex IonPac AS11 analytical, 2 × 250 mm (P/N 044077)
Eluent:	0.5 mM potassium hydroxide from 0–2 min, 0.5–5 mM from 2–5 min, 5–38 mM from 5–15 min, 38 mM from 15–20 min**
Eluent Source:	Dionex EGC II KOH with CR-ATC
Flow Rate:	0.25 mL/min
Temperature:	30 °C (lower compartment) 30 °C (upper compartment)
Inj. Volume:	5 µL
Detection:	Suppressed conductivity, Thermo Scientific™ Dionex™ ASRS™ 300 Anion Self-Regenerating Suppressor™ (2 mm), recycle mode, 24 mA current
Noise:	~1–2 nS/min peak-to-peak
Run Time:	20 min
System Backpressure:	~2400 psi
Background Conductance:	~0.5–0.7 µS

** The column equilibrates at 0.5 mM KOH for 5 min prior to the next injection.

Preparation of Solutions and Reagents

Stock Standard Solutions

Purchase a certified 1000 mg/L sulfate standard from Thermo Fisher Scientific or another reputable source. Alternatively, prepare a 1000 mg/L sulfate stock solution by dissolving 0.1479 g of sodium sulfate in deionized water and dilute to 100 mL. To prepare 1000 mg/L sulfamate stock solution, dissolve 0.1000 g of sulfamic acid in 100 mL of deionized water. Store the stock solutions in high-density polyethylene or polypropylene bottles at 4 °C.

Primary Dilution Standards

Prepare 5 mg/L of sulfamate in a 20 mL scintillation vial by combining 100 μ L of the 1000 mg/L sulfamate stock solution with 19.9 mL of deionized water. Prepare 50 mg/L of sulfate in a separate 20 mL scintillation vial by combining 1 mL of the 1000 mg/L sulfate stock solution with 19 mL of deionized water.

Calibration Standards

Prepare calibration standards of sulfamate and sulfate from their respective 5 mg/L and 50 mg/L primary dilution standards using appropriate dilutions from each standard. Five levels of calibration standards were used in this study. Store the calibration standards at 4 °C when not in use.

Sample Preparation

Topiramate is soluble in alkaline solutions containing sodium hydroxide or sodium phosphate with a pH between 9 and 10. Topiramate is also soluble in organic solvents, such as acetone, chloroform, dimethylsulfoxide, and ethanol. The solubility in water is 9.8 mg/mL.¹¹ Although samples can be analyzed in organic solvent matrices by IC, samples dissolved in deionized water are easier to prepare and are without potential matrix interferences. Therefore, topiramate was prepared in deionized water at a concentration of 6 mg/mL, which is well below its solubility limit. To prepare the sample, dissolve 12 mg of topiramate in 2 mL of deionized water and then vortex the solution to completely dissolve the solid.

Results and Discussion

Previously, it was shown that sulfamate esters, such as topiramate, undergo hydrolysis at pH 3 and elevated temperature (50 °C) in aqueous media to produce an organic degradation product and sulfamic acid.⁸ However, hydrolysis of these esters can also occur at higher pH values. In a separate study, the authors showed two possible degradation pathways for topiramate, both producing sulfamate and sulfate as byproducts.⁴ An increase in these inorganic degradation products is indicative of a decrease in the potency of the API. Therefore, it is critical to determine the amount of sulfamate and sulfate in topiramate to assess its purity and stability under different storage conditions over time. The current USP monograph specifies the limit of sulfamate and sulfate to $\leq 0.1\%$ in topiramate⁶ and $\leq 0.25\%$ in the proposed USP method for topiramate tablets.⁷

This AN describes the development of an IC method for the determination of sulfamate and sulfate in topiramate

using a 2 mm Dionex IonPac AS11 column with an electrolytically generated potassium hydroxide eluent and suppressed conductivity detection. The 2 mm column format was chosen to reduce the consumption of the pharmaceutical product by lowering the required sample volume to 5 μ L, relative to the 20 or 70 μ L sample volumes specified in the USP methods. In addition, this column format enables the system to operate at lower flow rates, which reduces eluent consumption and waste. Because sulfamate is weakly retained on the Dionex IonPac AS11 column, a weak KOH eluent concentration gradient from 0.5 to 5 mM was initially used to separate the target analyte from excipients in the sample. This was followed by an increase in the hydroxide concentration to 38 mM to elute the more strongly retained sulfate. Figure 2 shows a standard separation of 0.5 μ g/mL sulfamate and 2.5 μ g/mL sulfate on the Dionex IonPac AS11 column. The retention times for sulfamate and sulfate using the gradient conditions shown were approximately 6.5 min and 12 min, respectively.

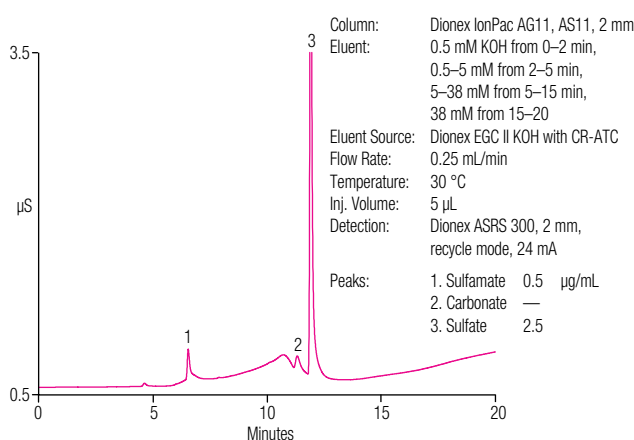


Figure 2. Separation of sulfamate and sulfate on the Dionex IonPac AS11 column.

Linearity, Limit of Quantitation, Limit of Detection

To determine the linearity of the method, calibration standards were injected in duplicate at five concentration levels in the range of 0.1–1.0 μ g/mL of sulfamate and 0.5–10 μ g/mL of sulfate. A plot of peak area vs. concentration produced correlation coefficient (r^2) values of 0.9999 and 0.9995 for sulfamate and sulfate respectively, using least squares regression fits (Table 1). The USP compendial method for validation <1225> specifies a signal-to-noise (S/N) ratio of 10 for the determination of the limit of quantitation (LOQ).⁹ The baseline noise was determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute. Typical baseline noise for this method using the Dionex ASRS 300 suppressor in the recycle mode is ~ 1 –2 nS/min. The LOQs for sulfamate and sulfate were determined to be 0.06 μ g/mL and 0.016 μ g/mL (S/N = 10) respectively. These data demonstrate that the method described in this AN can quantify 0.001% (w/w) sulfamate and 0.0003% (w/w) sulfate in a 6 mg/mL topiramate sample, which is well below the USP specification of 0.1%. The limits of detection (LOD) based on a S/N of 3 for sulfamate and sulfate were estimated to be 0.02 μ g/mL and 0.005 μ g/mL, respectively.

Table 1. Linearity, LOQs, and LODs for sulfamate and sulfate.

Analyte	Range (µg/mL)	Linearity (r ²)	Limit of Detection ^a (µg/mL)	Limit of Quantitation ^b (µg/mL)
Sulfamate	0.10–1.0	0.9999	0.02	0.060
Sulfate	0.50–10	0.9995	0.005	0.016

^aLODs estimated from $3 \times S/N$ ^bLOQs estimated from $10 \times S/N$

Accuracy and Precision

Method performance was evaluated with replicate injections of standard and sample solutions, and the recovery of known concentrations of sulfamate and sulfate added to the topiramate sample. The relative standard deviations (RSDs) of the retention times and measured peak areas were calculated from six replicate injections of a standard solution containing 0.5 µg/mL sulfamate and 2.5 µg/mL sulfate. The calculated peak area precisions for replicate injections of the standard were 1.2% and 0.22%, respectively. These RSD values are less than the required 2% specification in the current USP method and significantly less than the 15% requirement in the proposed USP method. The retention time precisions for the replicate injections were 0.21% and 0.02% for sulfamate and sulfate, respectively.

The method was used to assay a sample solution prepared at 6 mg/mL topiramate from a single lot of a USP Reference Standard (RS). The average sulfamate and sulfate concentrations detected in topiramate RS were 0.004% (0.255 ± 0.006 µg/mL) and 0.019% (1.18 ± 0.01 µg/mL), respectively. These concentrations were determined after a five-fold dilution of the topiramate sample. This reduced the response of an unknown to avoid interference with the quantification of sulfamate and, therefore, effectively reduced the topiramate concentration from 6 mg/mL to 1.2 mg/mL. The sulfamate and sulfate USP concentrations determined in this AN meet the $\leq 0.1\%$ specification for topiramate according to the USP 32-NF 27 monograph.⁶ The retention time precisions ($n = 6$) were 0.19% and 0.03% for sulfamate and sulfate, respectively. The peak area precisions of the replicate injections for sulfamate and sulfate were 2.46% and 0.64%, respectively.

The accuracy of the method was evaluated by spiking known concentrations of sulfamate and sulfate in the sample and calculating the recoveries based on the difference in response between the topiramate sample and spiked topiramate sample. For the sample spiked with 0.140 µg/mL sulfamate and 0.486 µg/mL sulfate, the average recoveries for six replicate injections were $104.5 \pm 1.5\%$ and $102.8 \pm 1.6\%$, respectively, suggesting that the method is accurate. Figure 3 compares the separation of a topiramate sample to the same sample spiked with known concentrations of sulfamate and sulfate.

Column: Dionex IonPac AG11, AS11, 2 mm
 Eluent: 0.5 mM KOH from 0–2 min, 0.5–5 mM from 2–5 min, 5–38 mM from 5–15 min, 38 mM from 15–20
 Eluent Source: Dionex EGC II KOH with CR-ATC
 Flow Rate: 0.25 mL/min
 Temperature: 30 °C
 Inj. Volume: 5 µL
 Detection: Dionex ASRS 300, 2 mm, recycle mode, 24 mA
 Sample: A) 6 mg/mL Topiramate
 B) 6 mg/mL Topiramate spiked
 Sample Prep: 1:5 dilution in DI water

Peaks:	A	B
1. Sulfamate	0.255	0.985 µg/mL*
2. Carbonate	—	—
3. Sulfate	1.8	3.67

*Calculated concentrations are shown

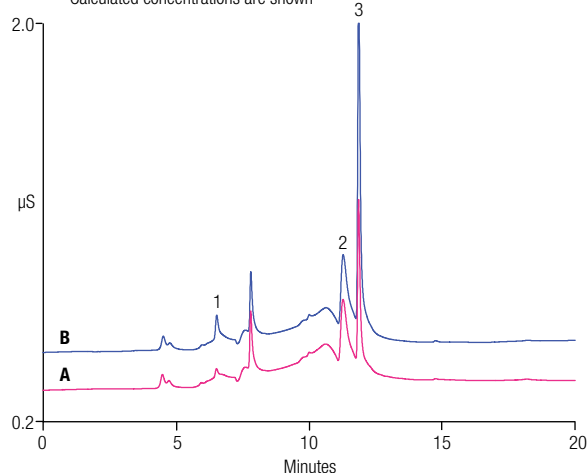


Figure 3. Comparison of the separation of sulfamate and sulfate in A) topiramate sample and B) spiked topiramate sample.

Sample Stability

Topiramate is reported to be very stable under normal storage conditions, but at elevated temperatures and humidity, degradation produces sulfamate, sulfate, and organic products. An assay of degraded topiramate produces an expected decrease in the topiramate concentration. This study examined the stability of topiramate based on the increase in sulfamate and sulfate concentrations when stored at room temperature (25 °C), at 4 °C, and at 60 °C for up to five consecutive days. Three independently prepared solutions containing 6 mg/mL topiramate each were subjected to the different temperature environments. When topiramate was stored at room temperature or 4 °C, no differences were observed between the initial sulfamate and sulfate values, and the values after five days. This indicates that the API is very stable under these conditions, as previously reported. However, when topiramate was stored at 60 °C, the sulfamate concentration tripled within 48 h, while sulfate remained unchanged from the initial value. After 120 h of storage at 60 °C, the sulfamate concentration significantly increased to 8.5% and sulfate increased to 0.18%. This demonstrates the degradation of the API at elevated temperatures, as reported in the literature.

Conclusion

This application note shows the determination of sulfamate and sulfate in topiramate using an electrolytically generated potassium hydroxide eluent with the Dionex IonPac AS11 column and suppressed conductivity detection. This method provides a simpler approach compared to the current drug substance and drug product methods proposed by the USP because it avoids (i) the manual preparation of eluents, which can produce inconsistencies in retention times and peak areas within and between laboratories, and (ii) the use of organic solvents. The RFIC system enhances the level of automation and ease-of-use of the method, improving inter- and intra-laboratory data reproducibility. The exceptionally low background and baseline noise achieved using a hydroxide eluent combined with suppressed conductivity enabled the quantification of 0.001% sulfamate and 0.0003% sulfate in topiramate. In addition, this method also demonstrated good linearity, precision, and accuracy for determining the inorganic degradation byproducts of topiramate.

List of Suppliers

- Sigma-Aldrich, P.O. Box 14508, St. Louis, MO 63178 USA. Tel: 1-800-325-3010.
www.sigma-aldrich.com
- U.S. Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852, USA. Tel: 1-800-227-8772.
www.usp.org
- VWR International, 1310 Goshen Parkway, West Chester, PA, 19380, USA. 1-800-932-5000.
www.vwr.com

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