Determination of Benzenesulfonic Acid Counterion in Amlodipine Besylate by Ion Chromatography

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Keywords
Drug Substance, Active Pharmaceutical Ingredient (API), Dionex IonPac AS18 2 mm Column, Suppressed Conductivity Detection

Introduction

Nearly half of all pharmaceuticals are produced in their salt form. Pharmaceutical salt formation is a critical step in the development process to help promote solubility, improve stability, decrease toxicity, and reduce hygroscopicity. Although the percentage of sulfonate salts used as active pharmaceutical ingredient (API) counterions is relatively low compared to other salt forms, their usage has increased significantly over the past 20 years. This increase is attributed to the decrease in aqueous solubility of new drug candidates.[1]

Amlodipine besylate (Figure 1) is the salt form of amlodipine that is produced from the reaction of amlodipine (a weak base) and besylate (the common name for the benzenesulfonic acid anion). This potent API is a calcium channel blocker used for the treatment of hypertension and angina (i.e., chest pain). Similar to other calcium channel blockers, amlodipine works by blocking the transport of calcium into the smooth muscles of the coronary and other arteries. Therefore, the smooth muscle relaxes, decreasing its peripheral resistance and thereby decreasing blood pressure.[2]

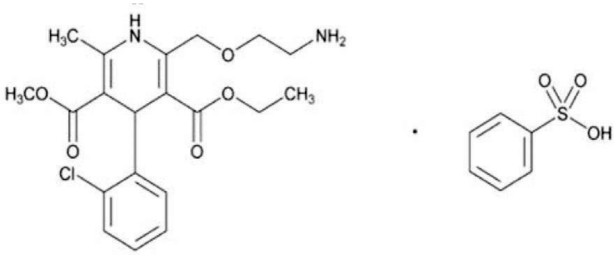

Figure 1. Structure of amlodipine besylate.

The current U.S. Pharmacopeia (USP) monograph for determining amlodipine besylate describes a reversed-phase liquid chromatography method for separating the API using 50 mM triethylamine (pH 3)/CH_3OH/CH_3CN at a ratio of 50:35:15 with UV detection at 237 nm.[3] However, there is currently no USP method for determining the benzenesulfonic acid counterion. It is important to determine the concentration of the counterion in the drug substance to establish the stoichiometry, the correct molecular mass of the drug, and the completeness of salt formation. Counterion determination is also important in drug authenticity studies.

Ion chromatography (IC) has proven to be a reliable, sensitive, and selective technique for the determination of a wide range of inorganic and organic ions in a variety of matrices, including pharmaceuticals. Previous studies have successfully demonstrated the ability of IC to determine counterions, impurities, and degradation compounds in a variety of drug substances and products.[4–9]

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Goal

To develop a simple, rapid, and accurate IC method for the determination of the benzenesulfonic acid counterion in amlodipine besylate

Equipment

- Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ system,* capable of supporting high-pressure IC, including:
  - SP Single Pump
  - EG Eluent Generator
  - DC Detector/Chromatography Compartment
- Thermo Scientific Dionex AS-AP Autosampler with a Sample Syringe, 250 µL (P/N 074306) and 1200 µL Buffer Line Assembly (P/N 074989)
- Thermo Scientific Dionex EGC 500 Potassium Hydroxide (KOH) Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column (P/N 075550)
- Vial Kit, 10 mL, Polystyrene with Caps and Blue Septa (P/N 074228)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.2

* This application can also be performed on any Dionex ICS system capable of eluent generation (excluding the method parameters shown in Figure 5).

Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistance or better
- Benzenesulfonic Acid, Sodium Salt, 98% (Fisher Scientific P/N AC401851000)

Sample

Amlodipine Besylate (USP P/N 1029501)

Conditions	
Columns:	Thermo Scientific™ Dionex™ IonPac™ AG18 2 mm Guard, 2 × 50 mm (P/N 060555) Dionex IonPac AS18 2 mm Analytical, 2 × 250 mm (P/N 060553)
Eluent:	60 mM KOH
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column
Flow Rate:	0.25 mL/min
Injection Volume:	5 µL
Detection:	Suppressed Conductivity, Thermo Scientific™ Dionex™ ASRS™ 300 Anion Self-Regenerating Suppressor™ (2 mm), recycle mode, 38 mA current
System Backpressure:	~2500 psi
Background Conductance:	~0.6 µS
Noise:	~1–2 nS/min, peak-to-peak
Run Time:	15 min

Preparation of Solutions and Reagents

Benzenesulfonate Stock Solution, 1000 µg/mL

Accurately weigh 0.1146 g of benzenesulfonic acid, then transfer the solid to a 100 mL volumetric flask and dilute to the mark with DI water.

Benzenesulfonate Secondary Stock Solution, 10 µg/mL

Transfer 1 mL of the 1000 µg/mL benzenesulfonate stock solution to a 100 mL volumetric flask and dilute to volume with DI water.

Working Standard Solutions

Prepare working standard solutions at concentrations of 0.5, 1.0, 5.0, 10, 15, and 20 µg/mL. To prepare concentrations between 1 and 20 µg/mL, add the appropriate volume of benzenesulfonate stock solution to a 100 mL volumetric flask and dilute to volume with DI water.
To prepare the lowest standard (i.e., 0.5 µg/mL), add an appropriate volume of the 20 µg/mL benzenesulfonate standard to a 100 mL volumetric flask and dilute to the mark with DI water. Store these standards at 4 °C when not in use.

Sample Preparation

To prepare 1 mg/mL of amlodipine besylate, weigh 10 mg of USP amlodipine besylate on an analytical balance in a 20 mL glass bottle with a screw cap and add 10 mL of DI water. Vortex the solution for at least 1 min followed by sonication for 5 min to fully dissolve the solid. To prepare 10 µg/mL of amlodipine besylate, transfer 1 mL of the 1 mg/mL amlodipine besylate solution to a 100 mL volumetric flask and dilute to the mark with DI water.

System Preparation and Configuration

Install and configure the Dionex EG by first installing the backpressure tubing in place of the columns to produce a total backpressure of 2000–2500 psi at a flow rate of 1 mL/min. Install a Dionex EGC 500 KOH cartridge and condition the cartridge by setting the KOH concentration to 50 mM at 1 mL/min for 45 min. After the conditioning process is complete, disconnect the backpressure tubing temporarily installed in place of the column set. Install a Dionex CR-ATC 500 trap column between the Dionex EGC 500 KOH cartridge and the Dionex EGC degasser. Hydrate the Dionex CR-ATC 500 trap column prior to use by following the instructions outlined in the product manual (Document No. 079684-01).

Install and configure the Dionex AS-AP Autosampler in Push Full mode. Follow the instructions in the Dionex AS-AP Autosampler Operator's Manual (Document No. 065361-07) to calibrate the sample transfer line to ensure accurate and precise sample injections.

Install the Dionex IonPac AG18 2 mm Guard (2 × 50 mm) and the Dionex IonPac AS18 2 mm Analytical (2 × 250 mm) columns in the lower compartment of the DC compartment. Ensure that the system pressure displayed by the pump is between 2000 and 2500 psi when 60 mM KOH is delivered at 0.25 mL/min to allow the Degas Assembly to effectively remove hydrolysis gases from the eluent. If necessary, install additional backpressure tubing to adjust the pressure. For the Dionex IonPac AS18-4 µm Analytical column, no additional restriction tubing is needed after the Degas Assembly because the pressure produced by the column is sufficient to effectively remove hydrolysis gases from the eluent.

Prepare the Dionex ASRS 300 suppressor for use by hydrating the suppressor. Use a disposable plastic syringe and push ~0.75 mL of degassed DI water through the ELUENT OUT port and 2 mL of degassed DI water through the REGEN IN port. Allow the suppressor to sit for ~20 min to fully hydrate the suppressor screens and membranes. Install the Dionex ASRS 300 suppressor for use in Recycle mode according to the product manual for the Dionex ASRS 300 suppressor (Document No. 031956-07). Alternatively, the Thermo Scientific Dionex AERS 500 (2 mm) Anion Electrolytically Regenerated Suppressor can be used as a direct replacement for the Dionex ASRS 300 suppressor to produce equivalent or improved results.

Equilibrate the Dionex IonPac AS18 2 mm column with 60 mM KOH at 0.25 mL/min for ~60 min. Analyze a matrix blank by injecting DI water. An equilibrated system will have a background conductance of ~0.6 µS.

Results and Discussion

Benzenesulfonic acid is a strong acid with a pKa of *ca.* –2.5. When dissolved in water, the compound completely dissociates to form benzenesulfonate, which can be separated by anion-exchange chromatography followed by suppressed conductivity detection. However, the presence of the benzene group increases the hydrophobicity and thus retention time relative to most other common anionic counterions. To separate benzenesulfonate in amlodipine besylate, a hydroxide-selective Dionex IonPac AS18 2 mm Analytical column was used with an electrolytically generated 60 mM KOH eluent, followed by suppressed conductivity detection. Figure 2 shows the separation of a 10 µg/mL benzenesulfonate standard separated under these conditions with a retention time of ~12 min. The method was validated for specificity, linearity, limit of detection and quantification, precision, and accuracy.

Column:	Dionex IonPac AG18/AS18, 2 mm
Eluent:	60 mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge with Dionex CR-ATC 500 trap column
Flow Rate:	0.25 mL/min
Inj. Volume:	5 µL
Detection:	Suppressed conductivity, Dionex ASRS 300 suppressor, 2 mm, recycle mode
Peak:	1. Benzenesulfonate 10 µg/mL

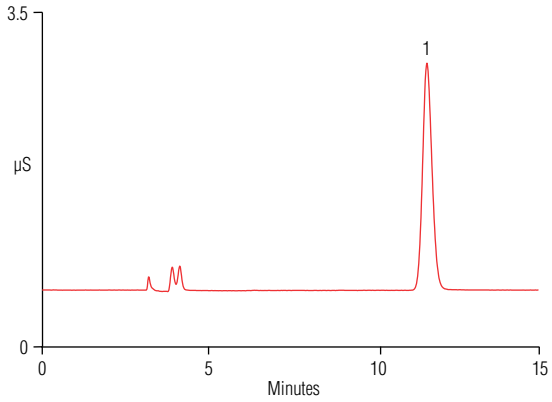

Figure 2. Separation of a 10 µg/mL benezenesulfonate standard.

Specificity

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) defines specificity as the ability to unequivocally assess the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.[10] The specificity of the method was initially evaluated by injecting a DI water blank to check for potential interferences at the retention time of benzenesulfonate. Furthermore, Figure 3 demonstrates that the retention time of benzenesulfonate in the standard is nearly identical to the USP amlodipine besylate sample, indicating the method is specific for its intended use.

Linearity, Limit of Detection, Limit of Quantification

To determine the linearity of the method, calibration standards were injected in duplicate at six concentration levels in the range of 0.5–20 µg/mL of benzenesulfonate. A plot of peak area versus concentration produced a coefficient of determination (r^2) of 0.9995 using a quadratic fit (Figure 4).

The USP compendial method for validation General Chapter <1225> specifies a signal-to-noise ratio (S/N) of 10 for the determination of the limit of quantification (LOQ).[11] The baseline noise was determined by measuring the peak-to-peak noise in a representative 1-min segment of the baseline where no peaks elute. Typical baseline noise for this method using the Dionex ASRS 300 suppressor in Recycle mode is ~1–2 nS/min. The LOQ for benzenesulfonate was 0.07 µg/mL (S/N = 10). The limit of detection (LOD) based on a S/N of 3 for benzenesulfonate was estimated to be 0.02 µg/mL. The linearity, LOD, and LOQ are summarized in Table 1.

Table 1. Calibration, LOD, and LOQ for benzenesulfonate.

Analyte	Range (µg/mL)	Coefficient of Determination (r^2)[a]	LOD[b] (µg/mL)	LOQ[c] (µg/mL)
Benzenesulfonate	0.5–20	0.9995	0.02	0.07

[a] Quadratic Fit
[b] LOD = 3 × S/N
[c] LOQ = 10 × S/N

Column: Dionex IonPac AG18/AS18, 2 mm
Eluent: 60 mM KOH
Eluent Source: Dionex EGC 500 KOH cartridge with Dionex CR-ATC 500 trap column
Flow Rate: 0.25 mL/min
Inj. Volume: 5 µL
Detection: Suppressed conductivity, Dionex ASRS 300 suppressor, 2 mm, recycle mode
Samples: A. 1 µg/mL benzenesulfonate standard
B. 10 µg/mL amlodipine besylate

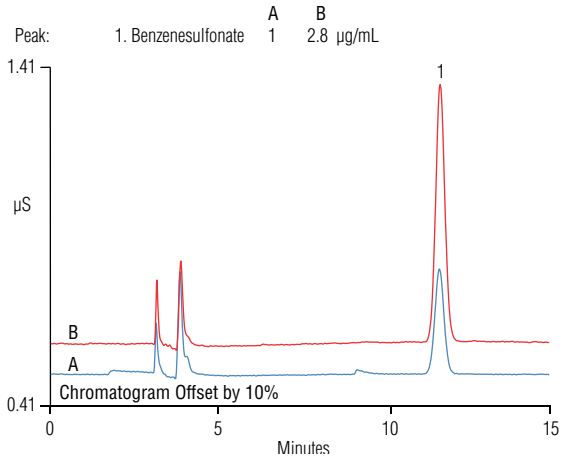

Figure 3. Comparison of (A) benzenesulfonate standard to (B) benzenesulfonate in amlodipine besylate.

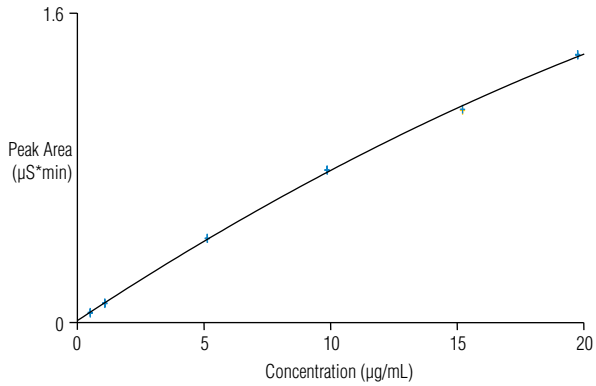

Figure 4. Calibration curve for benzenesulfonate.

Accuracy

A preparation of 10 µg/mL amlodipine besylate was made on three different days (i.e., one preparation per day). The concentration of benzenesulfonate determined in the sample ranged from 2.81 to 2.84 µg/mL, which is equivalent to 28.1 to 28.4% besylate in the drug substance. Therefore, the experimental values were within 2% of the theoretical amount of besylate in the sample, which is 27.9% according to the molecular weight. The accuracy of the method was studied by spiking amlodipine besylate at 50, 100, and 150% of the target concentration. The calculated recoveries for the sample spiked at these concentrations were 98.7, 104.4, and 103.6%, respectively.

Precision

The precision of the method was determined by performing six replicate injections from three independent preparations of 10 µg/mL amlodipine besylate prepared over three days. The intraday retention time and peak area precisions for six replicate injections were <0.05 and <0.9%, respectively. The between-day retention time and peak area precisions for the replicate injections (n = 18) were 0.03 and 0.7%, respectively. The peak area precision and recovery of besylate relative to the theoretical amount are summarized in Table 2.

Rapid Separation of Benzenesulfonate by High-Pressure IC

To increase sample throughput, a Dionex IonPac AS18-4 µm Analytical column (2 × 150 mm) was briefly evaluated for the determination of benzenesulfonate in amlodipine besylate. This column uses the same ion-exchange chemistry as the Dionex IonPac AS18 column described in this study but with smaller substrate particles. The smaller particle size provides higher peak efficiencies and improved resolution. This and the Dionex ICS-5000+ HPIC system allow higher flow rates to be used that produce faster run times and increased productivity. Figure 5 demonstrates the separation of benzenesulfonate in ~5 min on the 4 µm column operated at 0.38 mL/min compared to ~12 min using the standard Dionex IonPac AS18 column at 0.25 mL/min. The higher flow rate and shorter 4 µm column length decreased the retention time of the target analyte by nearly 60%. The decreased run times enable analysis of nearly twice the number of samples per hour, thereby increasing sample throughput.

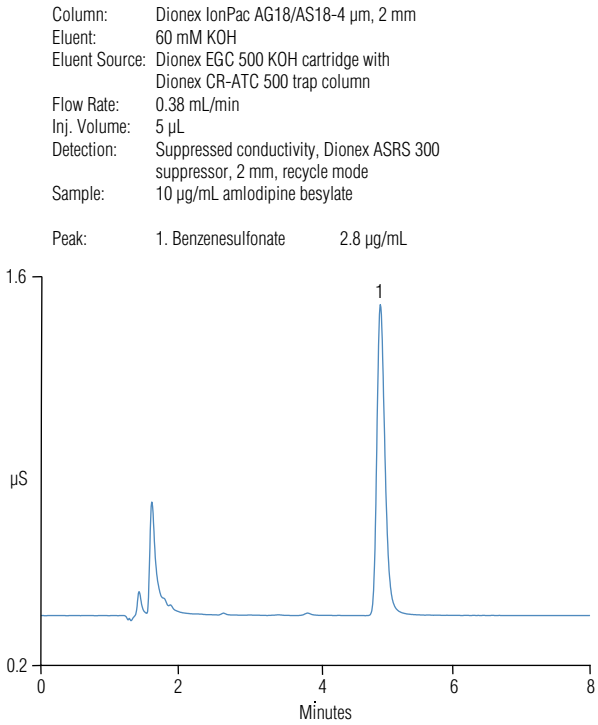

Figure 5. Rapid separation of benzenesulfonate in amlodipine besylate on the Dionex IonPac AS18-4 µm column.

Table 2. Precision and recovery of the benzenesulfonate counterion in amlodipine besylate.

Day	Analyte	Measured Besylate (µg/mL)	Benzenesulfonate in Amlodipine Besylate (%)	Peak Area RSD[a]	Recovery (%)[b]
1	Benzenesulfonate	2.84	28.4	0.64	102
2	Benzenesulfonate	2.82	28.2	0.81	101
3	Benzenesulfonate	2.81	28.1	0.45	101

[a] n = 6

[b] Calculated relative to the theoretical 27.9% amount of besylate

Conclusion

This study demonstrates the determination of benzenesulfonate in amlodipine besylate using an electrolytically generated potassium hydroxide eluent with a hydroxide-selective Dionex IonPac AS18 anion-exchange column and suppressed conductivity detection. This method provides a simple and reliable approach to determine the counterion in the drug substance. The electrolytically generated hydroxide eluent enhances the level of automation and ease-of-use of the IC system, improving inter- and intralaboratory data reproducibility. The method proved its accuracy by generating data that is within 2% of the theoretical amount of benzenesulfonate. In addition, this method also demonstrated good precision and spiked recoveries for determining the counterion in amlodipine besylate. Furthermore, the Dionex IonPac AS18-4 µm column can be used in place of the standard (9 µm) column to increase sample throughout by reducing the run time by nearly 60%.

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