Simultaneous Determination of Amlodipine and Its Counterion Besylate by HPLC

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Acclaim Trinity P1 Column, Pharmaceutical, API, UV Detection

Introduction

Amlodipine besylate (Figure 1) is a potent calcium channel blocker used for the treatment of hypertension and angina (i.e., chest pain). These types of medications function by blocking calcium transport into the smooth muscle of the coronary and other arteries in the body. The smooth muscle then relaxes, decreasing its peripheral resistance, and thereby decreasing blood pressure.[1] Active pharmaceutical ingredients (APIs) such as amlodipine are commonly manufactured as their acid addition salts to promote solubility and improve both stability and bioavailability.

High-performance liquid chromatography (HPLC) is the most commonly used approach to analyze the API, but often fails to retain the more hydrophilic analytes typically used as counterions during the drug manufacturing process. For the determination of counterions, ion chromatography (IC) has proven to be a reliable, sensitive, and selective technique.[2] Previously, Thermo Scientific Application Note 1078 demonstrated use of the Thermo Scientific™ Dionex™ IonPac™ AS18 column with an electrolytically generated hydroxide eluent and suppressed conductivity detection for the determination of benzenesulfonic acid anion (besylate) in amlodipine besylate drug substance.[3]

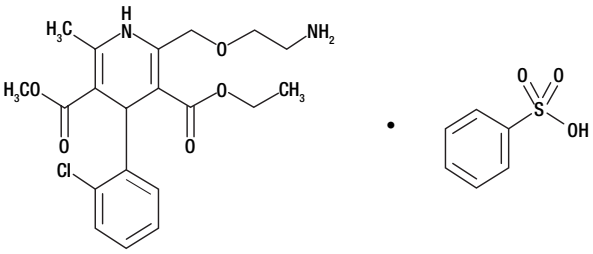

Figure 1. Structure of amlodipine besylate.

The current U.S. Pharmacopeia (USP) method for determining amlodipine besylate describes an HPLC method for separating the API using a 50 mM triethylamine (pH 3)/CH_3OH/CH_3CN mobile phase at a ratio of 50:35:15, with UV detection at 237 nm.[4] However, there is currently no method in the USP monograph for determining the besylate counterion. Mixed-mode chromatography combines reversed-phase and ion-exchange properties to simultaneously separate the drug molecule and counterion. Due to the complexity and versatility of pharmaceutical analytes, it is advantageous to use a stationary phase that provides cation-exchange, anion-exchange, and reversed-phase properties. The primary benefit of using a trimode column is that the API and counterion can be determined in a single analysis, which increases throughput and decreases mobile phase consumption and waste generation. However, the determination of pharmaceutical counterions by IC provides other benefits, such as improved sensitivity and the ability to use electrolytically generated eluents (i.e., potassium hydroxide or methanesulfonic acid), which eliminates potential errors from manual eluent preparation and increases method automation.

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This study focuses on an HPLC method to simultaneously separate the API and counterion of the drug substance amlodipine besylate. Amlodipine and besylate are separated using a Thermo Scientific™ Acclaim™ Trinity™ P1 column with an ammonium acetate buffer (100 mM NH_4OAc, pH 5) and acetonitrile (70:30, v/v) mobile phase at 0.50 mL/min, with simultaneous UV detection at 237 nm and 262 nm. This trimode column contains unique construction that provides spatial separation of the anion-exchange, cation-exchange, and reversed-phase modes, which allows each to be independently controlled.

Goal

To develop a simple and accurate HPLC method for the simultaneous determination of amlodipine and besylate in an amlodipine besylate drug substance

Equipment

- Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system, including:
  - SRD-3600 Integrated Solvent and Degasser Rack, 6 Channels (P/N 5035.9230)
  - HGP-3400RS Binary Pump with Solvent Selector Valves (P/N 5040.0046)
  - WPS-3000TRS Wellplate Sampler, Thermostatted (P/N 5840.0020)
  - Sample Loop, 25 µL (P/N 6820.2415)
  - TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
  - DAD-3000RS Photodiode Array Detector (P/N 5082.9920)
  - Semi-Micro Flow Cell for DAD-3000 and MWD-3000 Series, SST, 2.5 µL Volume, 7 mm Path Length (P/N 6080.0300)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, release 7.2

Consumables

- Acclaim Trinity P1, 3 µm Analytical, 3.0 × 50 mm (P/N 071388).
- Thermo Scientific™ Dionex™ Viper™ UHPLC Capillary Fingertight (for 10-32) Fitting, i.d. 0.13 mm/0.005", Length 250 mm, SST (P/N 6040.2325)
- Viper UHPLC Capillary Fingertight (for 10-32) Fitting, i.d. 0.18 mm/0.007", Length 450 mm, SST (P/N 6040.2365)
- Mixer for 400 µL Mixing Volume for SD/RS Pumps for Pressures up to 103.4 MPa/15,000 psi (P/N 6040.5310)
- Vial Kit, 1.5 mL, Glass with Caps and Septa (P/N 055427)

Reagents and Standards

- Deionized (DI) water, Type I reagent grade, 18 M -cm resistance or better
- Benzenesulfonic Acid, Sodium Salt, 98% (Fisher Scientific P/N AC401851000)
- Amlodipine Besylate (USP P/N 1029501)
- Ammonium Acetate (NH_4OAc), Certified ACS, ≥97% (Fisher Scientific P/N A637-500)
- Acetonitrile (CH_3CN), Optima™ LC/MS (Fisher Scientific P/N A955-4)
- Acetic Acid, Glacial, Optima (Fisher Scientific P/N A465-1)

Conditions (Applicable to Figure 2)	
Column:	Acclaim Trinity P1, 3 µm Analytical, 3.0 × 50 mm (P/N 071388)
Mobile Phase:	A. 100 mM NH_4OAc (pH 5) B. 100% CH_3CN
Isocratic Conditions:	30% B from 0–8 min
Flow Rate:	0.50 mL/min
Injection Volume:	5 µL
Temperature:	30 °C
Detection:	UV, absorbance at 237 nm and 262 nm
System Backpressure:	~1300 psi
Run Time:	8 min

Preparation of Solutions and Reagents

NH_4OAc (100 mM), pH 5

Dissolve 7.70 g of NH_4OAc in ~800 mL of DI water, then add 2 g of glacial acetic acid. Dilute to the mark with DI water. Sonicate the resulting mobile phase for 10 min to remove dissolved gases.

Mobile Phase Diluent (70% 100 mM NH_4OAc, pH 5/30% CH_3CN)

Mix 175 mL of 100 mM NH_4OAc with 75 mL of CH_3CN in a 250 mL HDPE bottle. Use this solution for sample and standard dilutions.

Besylate Stock Solution (1000 µg/mL)

Accurately weigh 0.1146 g of benzenesulfonic acid (sodium salt) and transfer to a 100 mL volumetric flask. Dilute to the mark with mobile phase diluent.

Amlodipine Stock Solution (1000 µg/mL)

Prepare amlodipine as amlodipine besylate according to the USP monograph by dissolving 10 mg of USP amlodipine besylate in 10 mL of mobile phase diluent.

Besylate Working Standard Solutions

Prepare working standard solutions for besylate at concentrations of 10, 20, 50, 75, and 100 µg/mL. To prepare concentrations between 10 and 100 µg/mL, add the appropriate volume of besylate stock solution to a 100 mL volumetric flask and dilute to volume with mobile phase. Store these standards at 4 °C when not in use.

Amlodipine Working Standard Solutions

Prepare working standard solutions for amlodipine at concentrations of 10, 25, 50, 100, and 150 µg/mL amlodipine besylate. To prepare concentrations between 10 and 150 µg/mL, add the appropriate volume of stock solution to a 100 mL volumetric flask and dilute to volume with mobile phase. 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 mobile phase diluent. Vortex the solution for at least 1 min, followed by sonication for 5 min to fully dissolve the solid. To prepare 50 µg/mL of amlodipine besylate, transfer 75 µL of the 1 mg/mL amlodipine besylate solution to a 1.5 mL glass autosampler vial and add 1.42 mL of mobile phase diluent. To prepare 200 µg/mL of amlodipine besylate, transfer 300 µL of the 1 mg/mL amlodipine besylate solution to a 1.5 mL glass autosampler vial and add 1.2 mL of mobile phase diluent.

Results and Discussion

Separation and Detection

Amlodipine belongs to the dihydropyridine class of molecules, which are well known in pharmacology as calcium channel blockers for the treatment of hypertension. This drug substance is typically manufactured in the besylate salt form (i.e., benzenesulfonate), which is considered significantly safer than the maleate counterion due to the absence of toxic impurities in the final product.[5] Several methods in the literature demonstrate the determination of amlodipine alone or in combination with other drugs using a traditional C18 reversed-phase HPLC column with UV detection.[1] However, the hydrophilic nature of besylate makes it challenging to obtain separation, adequate retention, and good peak shape using traditional reversed-phase columns. Therefore, a stationary phase containing multiple separation modes is needed to simultaneously determine the hydrophobic API and the charged hydrophilic counterion in the same analysis.

The Acclaim Trinity P1 column is a trimode column specifically developed for the simultaneous separation of APIs and counterions. The inner-pore area of the stationary phase is modified with an organic layer that provides reversed-phase and anion-exchange properties, whereas the outer-pore area is modified with cation-exchange functionality. These properties enable the retention of amlodipine by the reversed-phase mode and besylate by the weak anion-exchange mode.

To determine the maximum UV absorbance for amlodipine and besylate, the photodiode array detector was set in 3D mode with a scan range from 230 to 350 nm. A USP standard containing 1 mg/mL amlodipine besylate (equivalent to 721 µg/mL amlodipine and 279 µg/mL besylate) was used to simultaneously assess the respective maximum UV wavelengths. Amlodipine and besylate showed absorbance maxima at approximately 237 nm and 262 nm, respectively. Figure 2 shows the separation of amlodipine and besylate on the Acclaim Trinity P1 column, followed by detection at their respective UV wavelengths. The compounds were separated within 8 min using an isocratic NH_4OAc and CH_3CN mobile phase. The volatile mobile phase also allows the method to be compatible with other detection systems, such as charged aerosol and mass spectrometry.

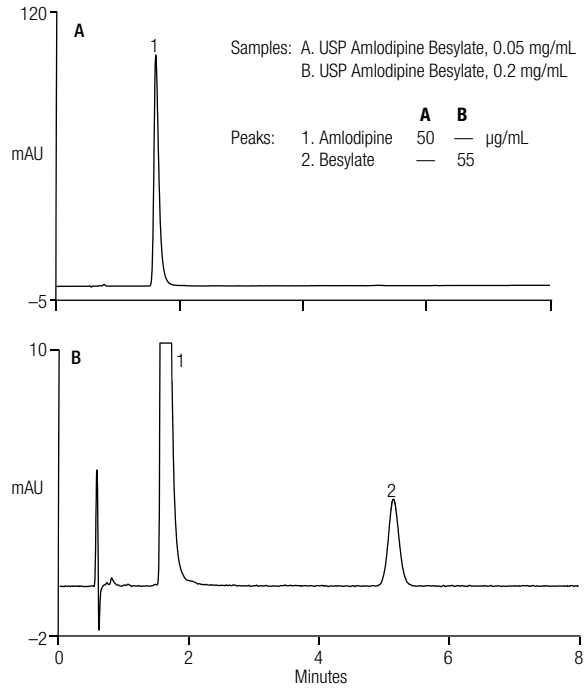

Figure 2. Simultaneous determination of amlodipine and besylate.

Method Calibration

The method was calibrated separately for amlodipine and besylate. For amlodipine, five concentration levels ranging from 10 to 150 µg/mL amlodipine besylate were injected in duplicate. A least squares regression curve of peak area vs concentration was plotted for amlodipine, which produced a coefficient of determination (r^2) value of 0.9998. For besylate, five concentration levels were also used, but the calibration ranged from 10 to 100 µg/mL, with each concentration level injected in duplicate. The coefficient of determination based on the calibration curve was 0.9999.

Although not required for the assay, the limit of detection (LOD) and limit of quantification (LOQ) were also determined for this method. The LOD and LOQ were determined based on 3× signal-to-noise ratio (S/N) and 10× S/N, respectively. For amlodipine, the LOD and LOQ were calculated at 0.14 and 0.47 µg/mL (as amlodipine besylate), respectively. The calculated LOD and LOQ for besylate were 2.3 and 7.7 µg/mL, respectively. Table 1 summarizes the method calibration, LOD, and LOQ data.

Table 1. Method calibration, LODs, and LOQs for amlodipine and besylate.

Analyte	Range (µg/mL)	Coefficient of Determination (r^2)	LOD[c] (µg/mL)	LOQ[d] (µg/mL)
Amlodipine[a]	10–150	0.9998	0.14	0.47
Besylate[b]	10–100	0.9984	2.3	7.7

[a] Prepared as amlodipine besylate according to USP protocol; detected at 237 nm
[b] Detected at 262 nm
[c] LOD = 3× S/N
[d] LOQ = 10× S/N

Table 2. Precision and recovery of the besylate counterion in 200 µg/mL amlodipine besylate.

Day	Measured Besylate (µg/mL)	Besylate in Amlodipine Besylate (%)	Peak Area RSD[a]	Recovery (%)[b]
1	55.6	27.8	0.90	99.6
2	55.2	27.6	0.75	98.9
3	56.5	28.2	0.51	101

[a] n = 6
[b] Calculated relative to the theoretical 27.9% besylate

Table 3. Precision of three independent preparations of 50 µg/mL amlodipine besylate prepared over three days.

Day	Analyte	n	Amlodipine Concn (µg/mL)	Retention Time RSD	Peak Area RSD
1	Amlodipine	6	50	<0.01	0.35
2	Amlodipine	6	50	0.08	0.64
3	Amlodipine	6	50	<0.01	1.16

Method Precision and Accuracy

The precision of the method was determined by performing six replicate injections from three independent preparations of 50 and 200 µg/mL amlodipine besylate over three days. The higher amlodipine besylate concentration was used to determine the percentage of besylate in the drug substance and to assess the intra- and interday precision. The besylate concentration in 200 µg/mL amlodipine besylate ranged from 55.2 to 56.5 µg/mL (Table 2). This is equivalent to 27.6–28.2% besylate in the drug substance, which is 98.9–101.1% of the theoretical 27.9% besylate, based on the molecular weight of amlodipine besylate.

The intraday retention time and peak area precisions for besylate (n = 6) were ≤0.1 and <1%, respectively. The between-day retention time and peak area precisions for besylate (n = 18) were 0.24 and 1.25%, respectively. For amlodipine besylate prepared at 50 µg/mL, the intraday retention time and peak area precisions for amlodipine (n = 6) were <0.1 and <1.2%, respectively (Table 3). The between-day retention time and peak area precision for amlodipine (n = 18) were 0.19 and 1.03%, respectively.

Method accuracy was also evaluated by spiking 50 and 100% of the target besylate concentration in 200 µg/mL amlodipine besylate. The calculated recoveries at the two concentration levels were 102.7 and 98.1%. The retention time and peak area precisions (n = 6) for the spiked sample injections were <0.1 and <0.7%, respectively.

Conclusion

This study demonstrates a rapid, accurate, and reliable method for the simultaneous determination of amlodipine and its counterion besylate. The method requires only 8 min per analysis using an Acclaim Trinity P1 column and UV detection. In addition, combining the analysis of the API and counterion into a single method saves times, reduces mobile phase consumption, and minimizes waste.

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