Development of an Assay for Besylate in Amlodipine Besylate by IC and a Second Assay to Simultaneously Determine Amlodipine and Besylate by HPLC

Brian De Borba and Jeffrey Robrer,
Thermo Fisher Scientific, Sunnyvale, CA, USA
Overview

**Purpose:** Amlodipine besylate is a calcium channel blocker that is used for the treatment of hypertension and angina. Similar to other calcium channel blockers, amlodipine works by relaxing the arterial muscles, which decreases peripheral resistance and therefore reduces blood pressure. Active pharmaceutical ingredients (APIs) such as amlodipine are commonly manufactured into acid addition salts to improve bioavailability. Amlodipine besylate is the salt form of amlodipine, which is produced from the reaction of amlodipine (a weak base) and benzenesulfonic acid. It is critical to quantify the concentration of the API and counterion in the drug formulation to establish stoichiometry, completeness of salt formation, and mass balance.

**Method:** We developed an isocratic ion chromatography (IC) method with suppressed conductivity detection for determining besylate in amlodipine besylate. A second method was developed for the simultaneous determination of amlodipine and besylate using a trimode (reversed-phase, anion-exchange, cation-exchange) high-performance liquid chromatography (HPLC) column with UV detection. The HPLC method used a mobile phase containing 70% 100 mM ammonium acetate/30% acetonitrile. The sample was prepared in deionized (DI) water for the IC method and mobile phase for the HPLC method.

**Results:** The linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, and accuracy are presented for both methods. Correlation coefficients for the standard curves ranged from 0.9984 to 0.9995 with peak area RSDs <1. The recovery of besylate spiked into the API ranged from 99% to 104% using the IC method. In addition, the amount of besylate in amlodipine besylate ranged from 28.1–28.4% with the IC method and 27.6–28.2% with the HPLC method, which is comparable to the theoretical amount of 27.9% based on the molecular weight of the drug formulation.

**Conclusions:** These results demonstrate the ability to accurately determine the counterion in a drug formulation by IC with suppressed conductivity detection. In addition, comparable results can be obtained for the counterion when using HPLC with UV detection. The HPLC method also provides additional advantages by simultaneously determining the API and counterion, which is a cost-effective approach for many laboratories. The two methods are shown to have good linearity, sensitivity, precision, and accuracy.

Introduction

Reversed-phase liquid chromatography (RPLC) is the most common approach used to analyze the API, but it often fails to retain the more hydrophilic analytes that are commonly used as counterions during the drug manufacturing process. For the determination of counterions, IC has proven to be a reliable, sensitive, and selective technique.¹

The current U.S. Pharmacopeia (USP) method for determining amlodipine besylate (Figure 1) describes RPLC for separating the API using 50 mM triethylamine (pH 3)/MeOH/MeCN at a ratio of 50:35:15 with UV detection at 237 nm.² However, there is currently no USP method for determining the besylate counterion. Mixed-mode chromatography combines RPLC and ion-exchange properties to simultaneously separate the drug molecule and counterion. 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., KOH or MSA), which eliminates the labor and potential errors of manual eluent preparation.

In this study, amlodipine and besylate (i.e., benzenesulfonic acid) were separated on a Thermo Scientific™ Acclaim™ Trinity™ P1 column. 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. After separation, amlodipine was detected by UV at 237 nm and besylate was detected at 262 nm. In addition, a separate IC method was developed and compared to the HPLC method for determining besylate in the drug substance.

**FIGURE 1. Structure of amlodipine besylate.**

```
H3C  N  H3CO
H2CH2          CH3
O               O
Cl               S

Besylate
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1. Thermo Scientific™ Dionex™ IonPac™ AG18/AS18, 2 mm
2. SRD-3600 Integrated Solvent and Degasser Rack
3. HPG-3400RS Binary Rapid Separation Pump with Solvent Selector Valves
4. DAD-3000RS Rapid Separation Diode Array Detector
5. Table 1: Linearity, LOD, and LOQ for besylate.

<table>
<thead>
<tr>
<th>Analyte Range</th>
<th>LODµg/mL</th>
<th>LOQµg/mL</th>
<th>(r2)</th>
<th>n</th>
<th>a</th>
<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Besylate</td>
<td>0.05</td>
<td>0.15</td>
<td>0.9995</td>
<td>5</td>
<td>0.02</td>
<td>0.75</td>
<td>10</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>0.05</td>
<td>0.15</td>
<td>0.9995</td>
<td>5</td>
<td>0.02</td>
<td>0.75</td>
<td>10</td>
</tr>
</tbody>
</table>

These results demonstrate the ability to accurately determine the counterion in a drug formulation by IC with suppressed conductivity detection. In addition, comparable results can be obtained for the counterion when using HPLC with UV detection. The HPLC method also provides additional advantages by simultaneously determining the API and counterion, which is a cost-effective approach for many laboratories. The two methods are shown to have good linearity, sensitivity, precision, and accuracy.

**Conclusion**

These results demonstrate the ability to accurately determine the counterion in a drug formulation by IC with suppressed conductivity detection. In addition, comparable results can be obtained for the counterion when using HPLC with UV detection. The HPLC method also provides additional advantages by simultaneously determining the API and counterion, which is a cost-effective approach for many laboratories. The two methods are shown to have good linearity, sensitivity, precision, and accuracy.
Methods

Sample Preparation
Amlodipine besylate (1 mg/mL): Weigh approximately 10.0 mg of amlodipine besylate on an analytical balance and dissolve the solid in 10 mL of DI water for the IC method or 70% NH₄OAc/30% MeCN for the HPLC method. Perform serial dilutions to achieve the desired target concentration.

Method A: IC
A Thermo Scientific Dionex ICS-5000+ HPIC™ Ion Chromatography system was used for determination of besylate. The system included an SP Single Pump, EG Eluent Generator, DC Detector/Chromatography Compartment, and an AS-AP Autosampler.

Method B: HPLC
Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) System including:
- SRD-3600 Integrated Solvent and Degasser Rack
- HPG-3400RS Binary Rapid Separation Pump with Solvent Selector Valves
- WPS-3000TRS Rapid Separation Wellplate Sampler Thermostatted
- TCC-3000RS Rapid Separation Thermostatted Column Compartment
- DAD-3000RS Rapid Separation Diode Array Detector

TABLE 1. Chromatographic conditions.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method A IC</th>
<th>Method B HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columns</td>
<td>Thermo Scientific™ Dionex™ IonPac™ AG18/AS18 set, 2 mm</td>
<td>Acclaim, Trinity P1, 3 µm</td>
</tr>
<tr>
<td>Mobile Phases</td>
<td>KOH delivered using a Thermo Scientific Dionex EGC 500 KOH Eluent Generator Cartridge with a Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column</td>
<td>A: 100 mM NH₄OAc, pH 5 B: 100% MeCN</td>
</tr>
<tr>
<td>Conditions</td>
<td>60 mM KOH</td>
<td>0–8 min 30% B</td>
</tr>
<tr>
<td>Total Run Time</td>
<td>15 min</td>
<td>8 min</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.25 mL/min</td>
<td>0.50 mL/min</td>
</tr>
<tr>
<td>Inj. Volume</td>
<td>5 µL</td>
<td>5 µL</td>
</tr>
<tr>
<td>Column Temp</td>
<td>30 ºC</td>
<td>30 ºC</td>
</tr>
<tr>
<td>Detection</td>
<td>Suppressed conductivity, Thermo Scientific™ Dionex™ ASRS™ 300 Anion Self-Regenerating Suppressor™, 2 mm, recycle mode, 38 mA</td>
<td>UV at 237 nm (amlodipine) and 262 nm (besylate)</td>
</tr>
</tbody>
</table>

Results
This study demonstrates two methods: an IC method to determine the besylate counterion; and a separate HPLC method to simultaneously determine amlodipine and besylate.

Method A: Determination of Besylate by IC
The Figure 2 chromatogram demonstrates the determination of besylate in amlodipine besylate using a Dionex IonPac AS18 column with suppressed conductivity detection. As shown, besylate is separated in approximately 12 min using 60 mM electrolytically generated KOH eluent. At this eluent concentration, trace anions (if present) elute near the void and therefore do not interfere with the determination of the counterion. To validate the method, the linearity, precision, and accuracy were evaluated.
Table 2: Linearity, LOD, and LOQ for besylate.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Range (µg/mL)</th>
<th>Coefficient of Determination ($r^2$)</th>
<th>LODb (µg/mL)</th>
<th>LOQc (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Besylate</td>
<td>0.5–20</td>
<td>0.9995</td>
<td>0.02</td>
<td>0.07</td>
</tr>
</tbody>
</table>

a Quadratic Fit  
b LOD = 3x S/N  
c LOQ = 10x S/N

FIGURE 2: Determination of besylate in amlodipine besylate using Method A.

<table>
<thead>
<tr>
<th>Source</th>
<th>Flow Rate</th>
<th>Eluent</th>
<th>Inj. Volume</th>
<th>Detection</th>
<th>Eluent</th>
<th>Eluent Source</th>
<th>Columns</th>
<th>Sample</th>
<th>Peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dionex EGC 500 KOH with Dionex CR-ATC</td>
<td>0.25 mL/min</td>
<td>60 mM KOH</td>
<td>5 µL</td>
<td>Suppressed conductivity, Dionex ASRS 300, 2 mm, recycle mode</td>
<td>4 mM KOH</td>
<td>Dionex IonPac AG18/AS18, 2 mm</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Columns: Dionex IonPac AG18/AS18, 2 mm  
Eluent: 60 mM KOH  
Inj. Volume: 5 µL  
Detection: Suppressed conductivity, Dionex ASRS 300, 2 mm, recycle mode  
Sample: USP Amlodipine Besylate RS  
Peaks: 1. Besylate 2.8 µg/mL

Precision

The method precision was determined by performing six replicate injections of a 10 µg/mL amlodipine besylate USP standard that was prepared on three separate days.

TABLE 2: Precision and recovery of the besylate counterion in amlodipine besylate (Method A).

<table>
<thead>
<tr>
<th>Day</th>
<th>Analyte</th>
<th>Measured Besylate (µg/mL)</th>
<th>% Besylate in Amlodipine Besylate</th>
<th>Peak Area RSDa</th>
<th>Recovery (%b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Besylate</td>
<td>2.84</td>
<td>28.4</td>
<td>0.64</td>
<td>102</td>
</tr>
<tr>
<td>2</td>
<td>Besylate</td>
<td>2.82</td>
<td>28.2</td>
<td>0.81</td>
<td>101</td>
</tr>
<tr>
<td>3</td>
<td>Besylate</td>
<td>2.81</td>
<td>28.1</td>
<td>0.45</td>
<td>101</td>
</tr>
</tbody>
</table>

a n = 6  
b calculated relative to the theoretical 27.9% besylate

Accuracy

Amlodipine besylate was spiked at 50%, 100%, and 150% of the target besylate concentration and the recovery was calculated. The recovery ranged from 98.7–104.4%.

Rapid Separation of Besylate by High Pressure IC

To increase sample throughput, a 4 µm Dionex IonPac AS18 column (2 × 150 mm) was briefly evaluated for the determination of besylate in amlodipine besylate. As shown in Figure 3, besylate is separated in approximately 5 min on the 4 µm column compared to approximately 12 min using the column and conditions described in Method A.
the HPLC method. In addition, the amount of besylate in amlodipine besylate ranged from 28.1–28.4% with besylates spiked into the API ranged from 99% to 104% using the IC method. In comparison to the HPLC method for determining besylate in the drug substance.

Was detected at 262 nm. In addition, a separate IC method was developed and compared to the HPLC method for determining besylate in the drug substance. This study demonstrates two methods: an IC method to determine the besylate counterion and the ability to use electrolytically generated eluents (i.e., KOH or MSA), which provides spatial separation of the anion-exchange, cation-exchange, and the ability to use electrolytically generated eluents (i.e., KOH or MSA), which reduces time and labor associated with running a second method. Although both compounds can be quantified simultaneously, the primary focus of this method was to determine the besylate counterion. To validate the method, the linearity, precision, and accuracy were evaluated.

**TABLE 3: Linearity, LODs and LOQs for amlodipine and besylate.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Range (µg/mL)</th>
<th>Coefficient of Determination ((r^2))</th>
<th>LOD(^c) (µg/mL)</th>
<th>LOQ(^d) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipinea</td>
<td>10–150</td>
<td>0.9998</td>
<td>0.14</td>
<td>0.47</td>
</tr>
<tr>
<td>Besylate(b)</td>
<td>10–100</td>
<td>0.9984</td>
<td>2.3</td>
<td>7.7</td>
</tr>
</tbody>
</table>

\(a\) Prepared as amlodipine besylate according to USP protocol; detected at 237 nm
\(b\) Detected at 262 nm
\(c\) LOD = 3x S/N
\(d\) LOQ = 10x S/N

**FIGURE 4: Simultaneous determination of amlodipine and besylate.**

Column: Acclaim Trinity P1, 3 µm, 3.0 × 50 mm
Eluents: A. 100 mM NH\(_4\)OAc, pH 5.0
B. 100% MeCN
Isocratic: 30% B from 0–8 min
Flow Rate: 0.5 mL/min
Inj. Vol.: 5 µL
Detection: A. UV, 237 nm; B. UV, 262 nm
Sample: A. 50 µg/mL USP Amlodipine Besylate RS
B. 200 µg/mL USP Amlodipine Besylate RS
Peaks:
1. Amlodipine 50 µg/mL
2. Besylate 60 µg/mL
Accuracy
Amlodipine besylate was spiked at 50% and 100% of the target besylate concentration and the recoveries were calculated. The recovery at the 50% spiked concentration was 103% and 98.1% for the 100% spiked concentration.

Precision
The method precision was determined by performing six replicate injections of a 200 µg/mL amlodipine besylate USP standard that was prepared on three separate days.

TABLE 4: Precision and recovery of the besylate counterion in amlodipine besylate using Method B.

<table>
<thead>
<tr>
<th>Day</th>
<th>Analyte</th>
<th>Measured Besylate (µg/mL)</th>
<th>% Besylate in Amlodipine Besylate</th>
<th>Peak Area RSDa</th>
<th>Recovery (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Besylate</td>
<td>55.6</td>
<td>27.8</td>
<td>0.90</td>
<td>99.6</td>
</tr>
<tr>
<td>2</td>
<td>Besylate</td>
<td>55.2</td>
<td>27.6</td>
<td>0.75</td>
<td>98.9</td>
</tr>
<tr>
<td>3</td>
<td>Besylate</td>
<td>56.5</td>
<td>28.2</td>
<td>0.51</td>
<td>101</td>
</tr>
</tbody>
</table>

a n = 6  

b calculated relative to the theoretical 27.9% besylate

Conclusion
- Besylate (i.e., benzenesulfonate) was reliably determined in amlodipine besylate by anion-exchange chromatography using a simple sample prep and electrolytically generated KOH eluent.
- Amlodipine and besylate were simultaneously determined on a trimode HPLC column, which reduces time and labor associated with running a second method.
- The results for determining the besylate counterion were comparable between the IC and HPLC methods.

References