

API and Counterions in Adderall® Using Multi-mode Liquid Chromatography with Charged Aerosol Detection

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Key Words

Acclaim Trinity P2, Corona Veo charged aerosol detector; pharmaceutical, Adderall, amphetamine, counterions

Abstract

This work demonstrates the determination of the API (amphetamine) and counterions (aspartate, saccharin and sulfate) in a medicine Adderall® within a single analysis using a Thermo Scientific™ Acclaim™ Trinity™ P2 column and Thermo Scientific™ Dionex™ Corona™ Veo™ Charged Aerosol Detector (CAD).

Introduction

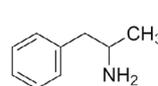
Adderall is used to treat Attention Deficit and Hyperactivity Disorder (ADHD) and narcolepsy. It is a formulation of dextro-amphetamine sulfate, dextro-amphetamine saccharate, racemic amphetamine sulfate and racemic amphetamine aspartate monohydrate. The structures of amphetamine, aspartate, and saccharin are shown in Figure 1. The combination of dextro-amphetamine sulfate, dextro-amphetamine saccharate, racemic amphetamine sulfate and racemic amphetamine aspartate monohydrate optimizes the bioavailability profile over time. For quality purposes, it is necessary to measure the mass balance of the APIs versus the counterions. This set of analytes imposes analytical challenges which cannot be met by any reversed-phase columns, and mixed-mode HPLC permits the measurement of all the components in a single run.

The Acclaim Trinity P2 column is based on Nanopolymer Silica Hybrid (NSH™) technology, which consists of high-purity porous spherical silica particles coated with charged nanopolymer particles. The inner-pore area of the silica particles is modified with a covalently bonded hydrophilic layer that provides cation exchange retention, while the outer surface is modified with anion-exchange nano-polymer beads. This chemistry ensures spatial separation of the anion exchange and cation exchange regions.

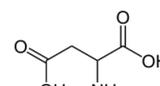
The Corona Veo Charged Aerosol Detector (CAD) is the third generation of this technology. It converts the column effluent into a dry aerosol and applies an electrical charge to the particles; the amount of charge measured by the electrometer is proportional to the mass of analyte.



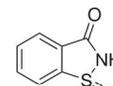
The Corona Veo and the diode-array UV detector are used for complementary detection. For the detection of UV-transparent analytes, CAD offers detection limits superior to evaporative light scattering detection, and unlike refractive index detection, gradient compatibility.



Amphetamine



Aspartate



Saccharin

Figure 1: Structures of API and counterions in Adderall®

Experimental Details

Consumables	Part Number
Polypropylene vials, 1.5 mL	079812
Acetonitrile, Fisher Optima™ LC/MS grade	A955
Formic acid, >98%	
Ammonium formate, 99.995%	
(+/-)-Amphetamine, 1.00 mg/mL in methanol, Cerilliant	NC9909702
Deionized water	

Sample Preparation

A standard was prepared to mimic 200 µg/mL Adderall® XR in water. Stock solutions of amphetamine, aspartic acid, sodium saccharin, and ammonium sulfate (each 1000 µg/mL) were diluted in water to make the expected concentrations of 122 µg/mL amphetamine, 24 µg/mL aspartate, 24 µg/mL saccharate, and 26 µg/mL sulfate.)

Separation Conditions	Part Number
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 System
Column:	Acclaim Trinity P2, 3 µm, 50 × 3 mm 085433
Mobile phase A:	Acetonitrile
Mobile phase B:	Water
Mobile phase C:	100 mM ammonium formate, pH 3.65 (6.35 g/L NH ₄ HCO ₂ + 4.5 g/L HCO ₂ H)

Time (min)	A	B	C
-8.0	35	59	6
0.0	35	59	6
0.5	35	59	6
5.0	35	0	65
10.0	20	0	80
12.0	20	0	80

Table 1: LC gradient conditions

Flow rate:	0.60 mL/min
Column temperature:	30 °C
Injection volume:	5 µL
Detector 1:	Thermo Scientific™ Dionex™ DAD-3000RS Diode Array 200–400 nm; UV 254 nm shown (data rate 5 Hz, filter 0.5 sec)
Detector 2:	Corona Veo Charged Aerosol Detector (evaporator temperature 55 °C, gas pressure 60 psi, data rate 5 Hz, filter 2 s, power function 1.50)

Data processing

Software:	Thermo Scientific™ Dionex™ Chromeleon™ 6.8 SR13. Blank subtraction applied.
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Results

Since Acclaim Trinity P2 columns provide both cation-exchange and anion-exchange retention mechanisms at the same time, they can adequately retain both amphetamine (cationic) and three counterions (anionic) under the same chromatographic conditions. The unique chemistry of the Acclaim Trinity P2 column, in which cation-exchange and anion-exchange regions are spatially separated, allows for great flexibility in method optimization by adjusting mobile phase buffer concentration, pH, and/or organic solvent content. For this particular application, aspartate, saccharin, sulfate and sodium were easily resolved, but amphetamine required certain amount of solvent to elute. Various buffer concentrations, solvent levels and gradient slopes were examined using three mobile phase bottles containing acetonitrile, 100 mM ammonium formate buffer and de-ionized water. The best result was achieved by the condition described in “Separation Conditions”, according to the criteria of retention ($k > 2$), resolution ($R_s > 2$) and analysis time (<15 min), as shown in Figure 2.

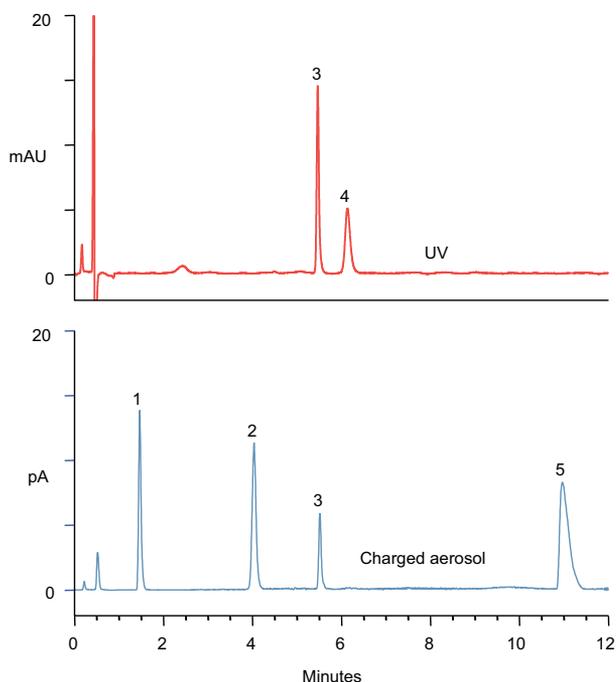


Figure 2: API and counterions of Adderall®.

Peaks: 1. aspartate (24 µg/mL), 2. sodium, 3. saccharin (24 µg/mL), 4. amphetamine (122 µg/mL), 5. sulfate (26 µg/mL)

Conclusion

- The Acclaim Trinity P2 column provides solutions for simultaneous determination of API (amphetamine) and counter-ions (aspartate, saccharin and sulfate).
- The separation is carried out using a simple mobile phase system of acetonitrile and ammonium formate buffer.
- The Corona Veo Charged Aerosol Detector and the UltiMate 3000 Diode Array Detector provide complementary detection for the API and its counterions.

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