

0043 - Ephedra for Ephedrine Alkaloids by HPLC

Botanical Name: *Ephedra sinica Stapf*; *Ephedra equisetina*; *Ephedra intermedia*; *Ephedra distachya*

Common Names: Ma Huang

NOTE: *Ephedra nevadensis* (Mormon tea), *Ephedra antisyphilitica*, and *Ephedra trifurca* are also ephedra species; however, they do not contain the ephedrine alkaloids for which ephedra is associated and are not covered here.

Parts of Plant Used: Aerial parts

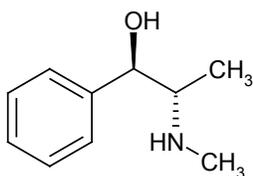
Uses: Treatment of asthma and bronchial spasms; as a stimulant and diaphoretic.

Modes of Action:

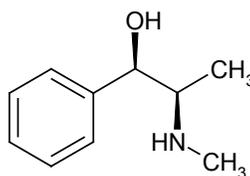
Ephedrine and pseudoephedrine, two of the biogenic amines found in ephedra are potent stimulants. Ephedrine is a sympathomimetic compound that stimulates both α - and β -adrenergic receptors. Administration of ephedrine or pseudoephedrine causes cardiac stimulation, elevated systolic and diastolic blood pressure, and CNS stimulation. These biogenic amines are also believed to cause glycogenolysis and increased oxygen consumption.

Chemical Markers:

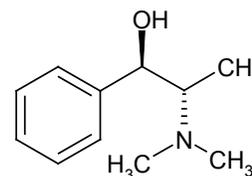
Ephedra is known to contain up to six bioactive alkaloids: (-)-ephedrine, (+)-pseudoephedrine, (-)-methylephedrine, (+)-methylpseudoephedrine, (-)-norephedrine, and (+)-norpseudoephedrine. These alkaloids constitute about 1 wt% to 2.5 wt% of the plant on a dry weight basis, with (-)-ephedrine accounting for between 30% and 90% of this total. Typically, ephedrine and pseudoephedrine combined account for more than 90% of the total alkaloids in ephedra. The species *E. nevadensis*, *E. antisyphilitica*, and *E. trifurca*, all found in North America, have thus far been found to be free of these alkaloids.



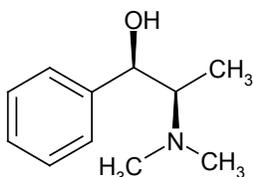
Ephedrine



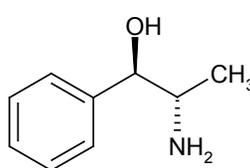
Pseudoephedrine



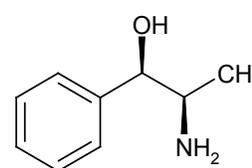
Methylephedrine



Methylpseudoephedrine



Norephedrine



Norpseudoephedrine



Methods of Analysis

Analysis of the ephedrine alkaloids in ephedra presents a number of challenges. All six ephedrine alkaloids are structurally similar. They are hydrophilic amine compounds that have poor retention on a traditional reversed-phase HPLC system. Their basic nature often leads to excessively broad peaks and peak tailing on chromatographic systems. Lastly, they have poor UV absorption above about 210 nm. Usually, sample cleanup utilizing solid-phase extraction is required to reduce interferences. Nevertheless, several methods have been published for the analysis of ephedrine alkaloids in dietary supplements. HPLC–UV, HPLC–MS, GC, and cation-exchange (CE) methods have all been utilized for the analysis of ephedra products. AOAC International recently adopted an HPLC–UV method for the analysis of ephedrine alkaloids in dietary supplements.

Method 1:

The AOAC International-approved HPLC–UV method¹ for the analysis of ephedrine alkaloids in botanicals and dietary supplements utilizes strong cation-exchange (SCX) solid-phase extraction (SPE) as a clean-up step and short-wavelength UV detection. This method is able to separate all six ephedrine alkaloid diastereomers and to quantify ephedrine and pseudoephedrine, but it cannot separate the individual enantiomers. This method underwent a collaborative study involving 10 international laboratories. A disadvantage to this method is that caffeine is a very late eluting peak; therefore, samples that contain caffeine require a column flush with high organic concentration after every fourth injection.

Sample Preparation:

Prepare samples in a diluent consisting of 97% 10 mM KH_2PO_4 and 3% methanol. Treat all samples to a SPE step utilizing a SCX column to remove interferences. Then load 5 mL of the sample solution onto a conditioned SPE cartridge and wash with 1 mL of 50 mM H_3PO_4 , then with 2 mL of methanol. Elute the ephedrine alkaloids from the SPE column into a 10-mL volumetric flask with a solution of 5% NH_4OH in methanol, and dilute to volume with 500 mM H_3PO_4 . An internal standard is not used.

Chromatography:

Column: Phenomenex Synergi Polar RP, 150 × 4.6 mm.
Mobile phase: 3% Methanol–97% KH_2PO_4 buffer (100 mM).
Flow rate: 1.5 mL/minute
Injection volume: 20 μL
Detection wavelength: 210 nm
Run time: 20 minutes

Validation Data:

Linearity: 3 to 200 mcg/mL, $r^2 > 0.9979$, recovery = 98.0% to 101%.
Accuracy: 84.6% to 98.2% recovery (two spike levels of negative control).
Repeatability: 0.98% to 2.6% RSD for ephedrine, 2.0% to 4.1% RSD for pseudoephedrine, 0.85% to 3.0% RSD for ephedrine + pseudoephedrine.
Reproducibility: 2.1% to 6.6% RSD for ephedrine, 9.0% to 11.2% RSD for pseudoephedrine, 2.0% to 7.5% RSD for ephedrine + pseudoephedrine.
Selectivity: Niacinamide was demonstrated to be a potential interferent with norpseudoephedrine.
Robustness: Not specified
LOD/LOQ: LOD = ~0.5 mcg/g of each alkaloid.

Method 2:

Hurlbut et al.² developed an HPLC–UV method that also utilized SPE with a SCX cartridge for sample cleanup. This method has a detection wavelength of 255 nm, which improves selectivity but greatly reduces the sensitivity of the method. Detection limits are estimated to be approximately 0.5 mg/g.

Sample Preparation:

Extract about 0.5 g of sample in 25 mL of aqueous diluent. Filter the solution, and treat 1 mL of the filtrate to SPE on a cation-exchange column. Elute the ephedrine alkaloids with 4 mL of buffer containing 16.4 g of sodium acetate in 970 mL of water with 8 mL of acetic acid, 3 mL of triethylamine, and 20 mL of acetonitrile (pH 4.8). Dilute this solution to 10 mL with water.

Chromatography:

Column: YMC Phenyl, 5 µm, 3.0 × 250 mm.

Mobile phase: 16.4 g of sodium acetate in 1.94 L of water with 16 mL of acetic acid, 6.0 mL of triethylamine, and 40 mL of acetonitrile.

Flow rate: 0.8 mL/minute

Injection volume: 20 µL

Detection wavelength: 255 nm

Validation Data:

Linearity: 4 to 300 mcg/mL for each ephedrine alkaloid with correlation coefficients greater than 0.9999.

Accuracy: 71.5% to 102%, depending on the ephedrine alkaloid.

Precision: 1.5% to 15% RSD (ephedrine + pseudoephedrine).

Selectivity: Three herbal matrix “blanks” showed no interferences with ephedrine alkaloids.

Ruggedness: A second laboratory analyzed the same samples with good agreement with the first laboratory.

Robustness: Changes in column temperatures, flow rates, acetonitrile concentration, HPLC column lots, and SPE column lots did not significantly affect results.

LOD/LOQ: LOD = 0.5 mg of ephedrine alkaloid per g.

Method 3:

Krol³ developed a method that was considered by AOAC for adoption. The Krol method can be considered complementary to Method 1. Krol utilizes a basic mobile phase (pH 9.45) requiring a column that can withstand basic conditions. The advantage to this approach is that it allows for a higher organic concentration in the mobile phase, resulting in less carryover. Caffeine elutes before the ephedrine alkaloids in this system, so a column wash is not needed for samples that contain caffeine. The disadvantage of this method is that the mobile phase is near the pKa of the ephedrine alkaloids: small changes in pH can lead to large changes in retention time. Solid-phase extraction using a mixed-mode SPE cartridge in cation-exchange mode is performed on the samples, and 1-methyl-3-phenylpropylamine (MPPA) is used as an internal standard.

Sample Preparation:

Add about 1 g of sample to a 100-mL volumetric flask. Then add 20 mL of water, 50 mL of methanol, and 1 mL of internal standard solution (1000 mcg/mL MPPS) and sonicate the sample for 1 hour. Cool, and then dilute the sample to volume with methanol. Acidify 2.0 mL of this sample solution with 5 mL of 1% formic acid and apply to a Waters Oasis MCX cartridge (3 mL). Wash the column, in sequence, with 1 mL of 0.1 N HCl, 1 mL of methanol,

1 mL of water, and 1 mL of 25% methanol–75% NH₄OH (5%). Elute the ephedrine alkaloids with 1.5 mL of 95% methanol–5% NH₄OH into a 5-mL volumetric flask and dilute to volume with 10 mM NH₄CO₃.

Chromatography:

Column: Waters XTerra Phenyl, 3.5µm, 150 × 2.1 mm.
Mobile phase: 10% Acetonitrile–90% 10 mM NH₄HCO₃, pH 9.45.
Flow rate: 0.23 mL/minute
Injection volume: 5 µL
Detection wavelength: 210 nm

Validation Data:

Linearity: 2.5 to 250 mcg/mL for ephedrine, 2.0 to 200 mcg/mL for pseudoephedrine, 0.5 to 50 mcg/mL for the minor ephedrine alkaloids. Correlation coefficients 0.9983 to 0.9996, depending on the ephedrine alkaloid.
Accuracy: 86.9% to 121.4% average recovery, depending on the ephedrine alkaloid; from spiked matrix (Red Rose black tea).
Precision: 1.9% to 6.6% RSD for ephedrine, 4.4% to 9.8% RSD for pseudoephedrine.
Selectivity: Black tea “blank” matrix showed no interferences. In addition, selectivity was shown using UV diode-array and MS analysis.
Ruggedness: Not determined
Robustness: Not specified
LOD/LOQ: LOQ = < 50 mcg/g, LOD = < 10 mcg/g

Method 4:

Gurley et al.⁴ used ion-pair HPLC with UV detection to quantify ephedrine alkaloids in nine different products. Ion-pairing increases the retention of the ephedrine alkaloids on reversed-phase columns, increasing selectivity from other polar, nonalkaloid analytes. No solid-phase extraction was performed, but an internal standard (dextroamphetamine sulfate) was incorporated. Disadvantages of the method are a long sample preparation procedure and the use of the dextroamphetamine internal standard, a DEA Schedule II controlled substance.

Sample Preparation:

Transfer the contents of a single tablet (crushed) or a hardshell capsule or a whole softgel to a round-bottom flask. Add 30 mL of mobile phase and reflux the contents at 80°C for 30 minutes. Centrifuge the mixture for 10 minutes at 36×g. Decant the supernate into a 50-mL volumetric flask, and wash the residue in the centrifuge tubes with two additional 10-mL volumes of mobile phase. Add the washings to the volumetric flask, and dilute to volume with mobile phase. Transfer 500 µL of this solution to a 1.5-mL microcentrifuge tube and spike with 25 µL of internal standard solution.

Chromatography:

Column: Alltech Altima C18, 5 µm, 250 × 4.6 mm.
Mobile phase: Acetonitrile–tetrahydrofuran–water (38:5:57) with 5 mM sodium lauryl sulfate.
Flow rate: 0.7 mL/minute
Injection volume: 25 µL
Detection wavelength: 208 nm

Validation Data:

Linearity: 6.25 to 400 mcg/mL, correlation coefficients 0.9990 to 0.9992.

Accuracy: 92% to 99.8% recovery.

Precision: 1.6% to 6.5% intraday, depending on the alkaloid; from spiked recovery samples.

0.27% to 2.8% interday, depending on the alkaloid; from spiked recovery samples.

Selectivity: Not specified

Robustness: Not specified

LOD/LOQ: LOQ = 6.25 mcg/mL

References:

1. Roman MC. Determination of ephedra alkaloids in urine and plasma by HPLC-UV: collaborative study. *J AOAC Int.* 2004 Jan-Feb;87(1):15-24.
2. Hurlbut JA, Carr JR, Singleton ER, et al. Solid-phase extraction cleanup and liquid chromatography with ultraviolet detection of ephedrine alkaloids in herbal products. *J AOAC Int.* 1998;81(6):1121-7.
3. Krol Sr J. The analysis of ephedra alkaloids at alkaline pH in botanicals and dietary supplements using LC/UV/MS. Presented at: AOAC International annual meeting; September 2002; Anaheim, CA.
4. Gurley BJ, Wang P, Gardner SF. Ephedrine-type alkaloid content of nutritional supplements containing *Ephedra sinica* (Ma-huang) as determined by high performance liquid chromatography. *J Pharm Sci.* 1998;87:1547-53.