Application Note: 419

Quantitative Analysis of Pseudoephedrine Tablets by UHPLC/MS

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Key Words

- Accela™
- MSQ Plus™
- Illicit Drug
- MDMA
- Methamphetamine

Introduction

Pseudoephedrine is frequently used as a decongestant in common cold products including Sudafed®, Advil® Cold, Drixoral®, Benadryl®, Aleve®, and Claritin®. Pseudoephedrine hydrochloride and sulfate salts are found in these products either as single-ingredient preparations or, more commonly, in combination with antihistamines, naproxen, paracetamol (acetaminophen), and/or ibuprofen.

Pseudoephedrine and ephedrine, both List I chemicals, are highly coveted by drug traffickers who use them to manufacture methamphetamine, a Schedule II controlled substance, for the illicit market. The diversion of over-the-counter pseudoephedrine-containing products is one of the major contributing factors to the methamphetamine situation in the United States.[1] The separation and identification of pseudoephedrine from illicit drug mixtures, especially the methamphetamine group compounds including amphetamine, methamphetamine, and 3,4-methylenedioxy-*N*-methamphetamine (3,4-MDMA), will help to identify the sources and the manufacturing pathway of the methamphetamine seized in the illicit market.

The Thermo Scientific Accela ultra high performance liquid chromatograph (UHPLC) performs separations 5 to 10 times faster than conventional HPLC by employing sub-2 µm diameter stationary phases and ultra high pressure instrumentation. The 1 to 2 second peak widths and relatively high mobile phase flow rates that are typical of UHPLC methods demand a robust, fast scanning MS detector. The fast scanning Thermo Scientific MSQ Plus single quadrupole mass spectrometer meets this UHPLC requirement and has been used for similar applications.[2] This application note describes an effective UHPLC/MS method for high throughput separation, identification, and quantitation of pseudoephedrine.

Experimental Conditions

Standard Preparation

Ephedrine, pseudoephedrine, amphetamine, methamphetamine, and 3,4-MDMA standards (1 mg/mL in methanol) were purchased from Alltech-Applied Science (State College, PA, USA). Figure 1 displays the chemical structures of these five compounds. Calibration standards were prepared by mixing all five compounds and then diluting the stock solution with methanol to a series of concentrations before analysis.

Sample Preparation

Three common over-the-counter cold products (brand names A, B, and C) containing pseudoephedrine as one of the active ingredients were analyzed.

Assay solution A: The cold tablet A was disintegrated and then dissolved in 20 mL methanol. The mixture was sonicated for 15 minutes. A portion of the supernatant was centrifuged at 12,000 RPM for 3 minutes and the clear supernatant was diluted to approximately 120 ng/mL with methanol.

Assay solutions B and C: The appropriate cold tablet (B or C) was disintegrated and then dissolved in 20 mL methylene chloride. The mixture was sonicated for 15 minutes. A 1 mL sample of the slurry was diluted to 20 mL with methanol. An aliquot of this solution was centrifuged at 12,000 RPM for 3 minutes and a portion of the clear supernatant was diluted to approximately 120 ng/mL with methanol.

Chromatographic Conditions

Instrument: Thermo Scientific Accela UHPLC system
Column: Hypersil GOLD™ PFP (perfluorinated phenyl) 1.9 µm, 100 × 2.1 mm (Thermo Fisher Scientific, Bellefonte, PA)
Flow Rate: 1 mL/min
Mobile phase: A: Water with 0.06% acetic acid
B: Acetonitrile (ACN) with 0.06% acetic acid

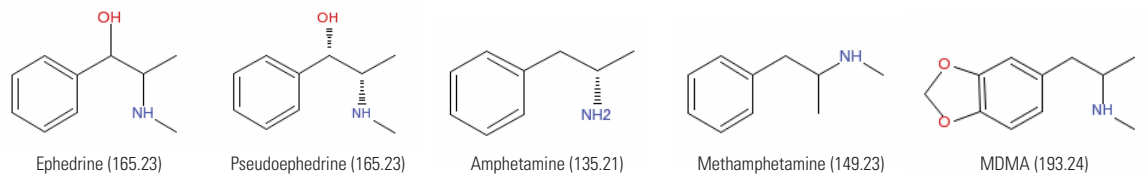

Figure 1: Chemical structures of the five drug compounds

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Gradient:

Gradient I: Separation of five drugs							
t (min)	0	1.5	3.5	3.51	3.99	4.0	5.0
A (%)	99	99	60	5	5	99	99
B (%)	1	1	40	95	95	1	1

Gradient II: Sensitivity evaluation and quantitation						
t (min)	0	3.0	3.1	3.9	4.0	5.0
A (%)	90	75	5	5	90	90
B (%)	10	25	95	95	10	10

Injection volume: 1 µL partial loop injection,
25 µL loop size
Column Temperature: 45 °C
Maximum column backpressure observed: 750 bar

Mass Spectrometer Conditions

Instrument: Thermo Scientific MSQ Plus
Ionization: Electrospray (ESI)
Polarity: Positive
Probe Temperature: 450 °C
Cone Voltage: 55.0 V
Scan Mode: Full scan with mass range of 100 to 200 *m/z*
or selected ion monitoring (SIM) at
m/z 150.18, 166.18, 177.15, and 194.15.
ESI Voltage: 4.5 kV
Scan Time: 0.2 s

Results and Discussion

Separation, MS Detection, and Pseudoephedrine Confirmation

The separation of the five drug compounds is shown in Figure 2A. Ephedrine elutes first at 2.41 minutes, followed by pseudoephedrine at 2.60 minutes, amphetamine at 2.93 minutes, methamphetamine at 3.38 minutes, and 3,4-MDMA at 3.64 minutes. The analytes are baseline separated with excellent peak efficiency and resolution. The MS spectra of the drug standards show both $[M+ACN+H]^+$ and $[M+H]^+$ ion signals. The most abundant ions are the $[M+H]^+$ ions at *m/z* 166.18, 166.18, 150.18, and 194.15 for ephedrine, pseudoephedrine, methamphetamine, and 3.4-MDMA, respectively. The $[M+ACN+H]^+$ ion has the most intense signal at *m/z* 177.15 for amphetamine. Full scans (100 to 200 *m/z*) were employed for the confirmation of the five compounds, and SIM modes were used for sensitivity and quantitation studies.

Pseudoephedrine was identified as the major active ingredient for all three brand name drugs by the UHPLC/MS method. The chromatograms are illustrated in Figure 2B-D. The retention times of 2.62 minutes for all three samples matched very well with the retention time of the pseudoephedrine standard at 2.60 minutes. The confirmation of pseudoephedrine at 2.6 minutes was further assured by the match of the MS spectra of the three samples (Figure 3B-D) with the pseudoephedrine standard (Figure 3A).

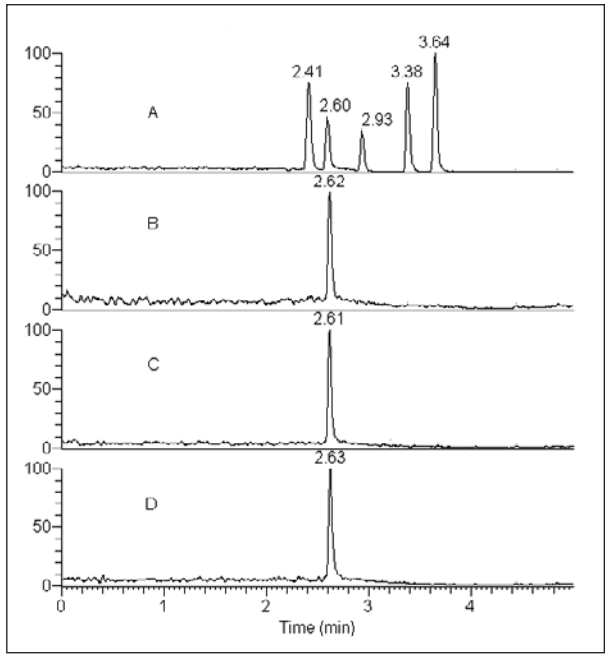

Figure 2: Chromatograms of drug standards (A), with the elution order of ephedrine, pseudoephedrine, amphetamine, methamphetamine and 3,4-MDMA, and assay samples (B-D), extracted from over-the-counter cold products with brand names A, B, and C.

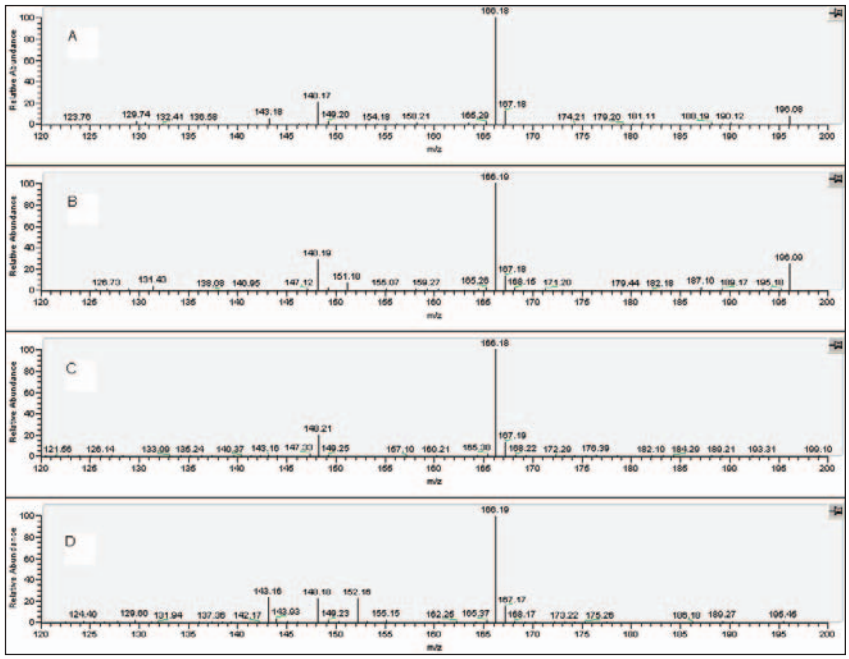

Figure 3: MS spectra of pseudoephedrine standard (A) and assay samples (B-D) from over-the-counter (OTC) drugs with brand names A, B, and C. Molecular ion of pseudoephedrine at *m/z* 166.18 was the major MS peak. Fragmentation ion of pseudoephedrine at *m/z* 148.17, created by the in-source CID of the MSQ Plus with a cone voltage of 55 volts, provided the additional MS confirmation of the assay samples.

Calibration Curve and Sensitivity

Calibration curves of the five drug standards were constructed over the concentration range of 1.25 to 1667 ng/mL (equivalent to 1.25 to 1667 pg on column) with 10 calibration levels (Figure 4). Each calibration level was injected three times and the mean area responses were plotted against the concentrations. Correlation coefficients with R^2 = 0.996 or better were achieved for the five drug standards.

The limit of quantitation (LOQ) and the limit of detection (LOD) of the five drug compounds were determined based on the calibration curve of signal-to-noise ratio versus concentration and the definitions of LOQ and LOD using s/n = 10 and 3. LOQs for all five drugs ranged from 0.96 to 1.7 ng/mL, while LODs ranged from 0.29 to 0.53 ng/mL (Table 1). The outstanding sensitivity of this method was highlighted by the achievement of picogram level quantitation for all five analytes.

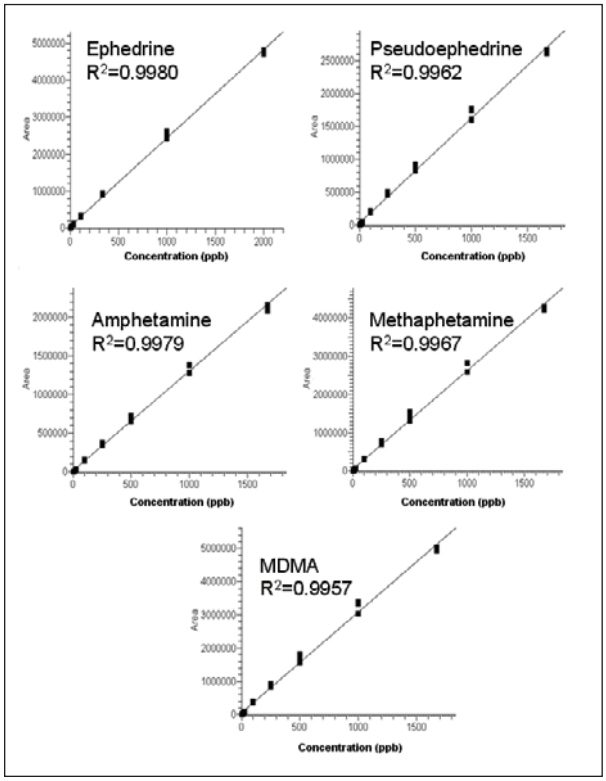

Figure 4: Calibration curves constructed for five drug standards at 1.25, 2.5, 5.0, 10, 25, 100, 250, 500, 1000 and 1667 ng/mL (ppb).

	LOQ (ng/mL)	LOD (ng/mL)
ephedrine	1.21	0.36
pseudoephedrine	1.25	0.38
amphetamine	1.78	0.53
methamphetamine	0.96	0.29
3,4-MDMA	1.09	0.33

Table 1: LOQ and LOD of the five drug compounds with 1 µL sample injection

Quantitation Using Internal Standard

An internal standard method was used for the quantitative determination of pseudoephedrine in its tablet form. Amphetamine was used as an internal standard (100 ng/mL). It was added to the freshly prepared assay sample solutions and pseudoephedrine standard solutions at 50 ng/mL, 100 ng/mL, 150 ng/mL, and 200 ng/mL. Six repeated injections for each standard and assay solution were conducted. Pseudoephedrine and amphetamine were well separated in four standard solutions at 1.73 minutes and 2.13 minutes (Figure 5A-D). The chromatograms of three assay samples are shown in Figure 5E-G. The concentrations of the assay samples were determined based on the calibration curve of peak area ratio against concentration (Figure 6). Excellent linearity with a correlation coefficient of R^2 = 0.997 was obtained. The experimental concentrations of the assay solutions were in good agreement with the reported values (Table 2).

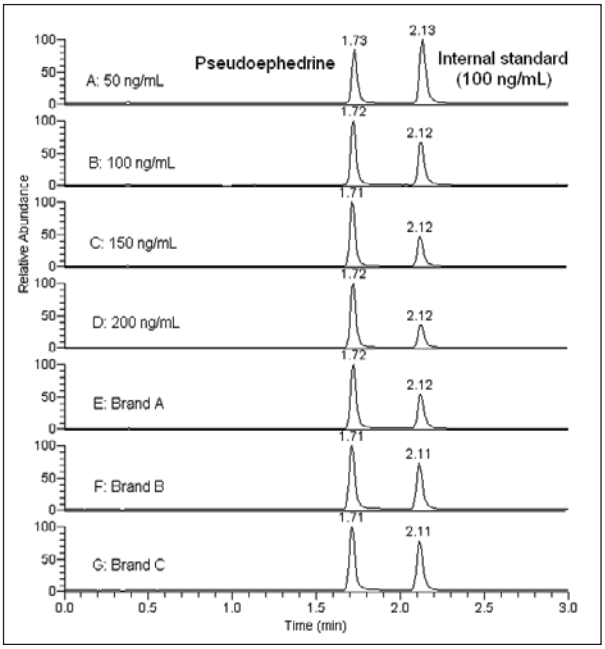

Figure 5: Chromatograms of four pseudoephedrine standard bracket solutions at 50 ng/mL (A), 100 ng/mL (B), 150 ng/mL (C) and 200 ng/mL (D), and three assay sample solutions from cold products with brand name A (E), brand name B (F), and brand name C (G). Pseudoephedrine was eluted at 1.73 min. Amphetamine (100 ng/mL), the internal standard, was added and eluted at 2.13 min.

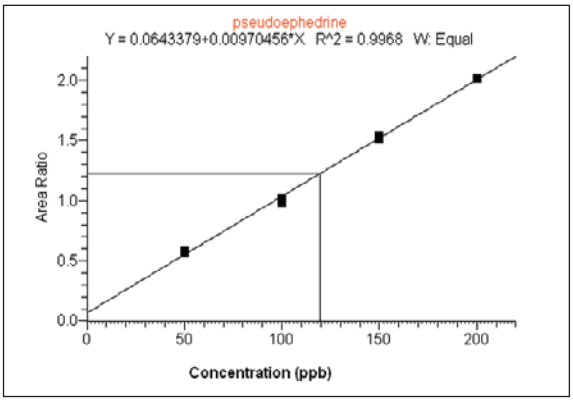

Figure 6: Calibration curve for pseudoephedrine quantitation using internal standard method.

	Experimental value (mg/tablet)	Reported value (mg/tablet)	% Recovery	%RSD (n = 6)
Brand name A	120.09	120	100.1	1.9
Brand name B	112.33	120	93.6	5.6
Brand name C	104.49	120	87.1	2.3

Table 2: Pseudoephedrine quantitation using amphetamine as internal standard

Conclusions

A simple, fast, and reliable separation and identification method for five drugs (pseudoephedrine, ephedrine, amphetamine, methamphetamine, and 3,4-MDMA) using UHPLC/MS was developed. The ppb (ng/mL) level sensitivity and accuracy of this method provides great opportunity to identify and quantify pseudoephedrine and/or other components in illicit drug samples. It also offers an efficient tool to determine the source and manufacturing pathway of drugs seized in the illicit market.

References

[1] Pseudoephedrine Notice, Office of Division Control, US Department of Justice, Drug Enforcement Administration.

[2] G Jiang, R. Chen, and C. Loran, Quantitative Measurement of Simvastatin Using High Speed LC/MS, Application Note 402, Thermo Fisher Scientific, San Jose, CA, USA.

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