Application Note: 423

A Comparison of Binary and Ternary Gradients Using Stage 1 Illicit Drugs

Guifeng Jiang; Thermo Fisher Scientific, San Jose, CA

Key Words

- Accela™ High Performance LC System
- MSQ Plus™ MS Detector
- Drugs of Abuse
- Ternary Solvent Gradient
- UHPLC/MS

Introduction

When attempting to separate a complex drug mixture by HPLC, a simple binary solvent gradient might fail to provide good resolution of all analytes. To improve the resolution, the separation factor can be manipulated by using a third solvent. However, this approach is not convenient with typical high pressure binary solvent mixing equipment.

The heart of the Accela Ultra-High Performance Liquid Chromatograph (UHPLC) is its low pressure gradient quaternary mixing pump. With this pump, up to four solvents can be used for convenient method development, system flushing, and eluent preparation. Because changing solvents, buffers, and pH values is quick and easy, the method development process is greatly simplified.

In this application note, we show that using three solvents in the separation and detection of a mixture of 14 drugs/metabolites by UHPLC/MS provides better performance than is possible with a simple binary gradient. The drugs are separated within 8 minutes on a Hypersil GOLD™ PFP 1.9 µm, 100 × 2.1 mm column and detected by a fast scanning (12,000 amu/s) single quadrupole mass spectrometer.

Goal

Improve the separation of 14 drugs of abuse in complex drug mixtures by employing ternary solvent gradient.

Experimental Conditions

Drug Standard Preparation

Pseudoephedrine, ephedrine, amphetamine, methamphetamine, 3,4-methylenedioxy-N-methamphetamine (3,4-MDMA), oxycodone, hydrocodone, clonazepam, noscapine, cocaine, caffeine, tetrahydrocannabinol (THC), cannabinol, and cannabidiol standards (1 mg/mL in methanol) were purchased from Alltech-Applied Science (State College, PA). The 14 compounds were mixed with the optimized molar ratio in the range of 1 to 100 and diluted to 0.1 ppm with methanol to make the drug mixture standards.

Chromatographic Conditions

Chromatographic analysis was performed using the Accela UHPLC system (Thermo Scientific, San Jose, CA). The chromatographic conditions were as follows:

Column: Hypersil GOLD PFP (perfluorinated phenyl), 1.9 µm, 100×2.1 mm

Flow rate: 1 mL/min

Mobile phase: A: 0.06% (v/v) acetic acid in water
B: 0.06% (v/v) acetic acid in acetonitrile
C: 0.06% (v/v) acetic acid in methanol

Injection volume: 1 µL partial loop injection, 25 µL loop size

Column temperature: 45 °C

Method 1: Binary gradient method

t (min)	0	5.0	5.1	7.0	8.6	9.9	10.0	12.0
A (%)	98	80	50	40	5	5	98	98
B (%)	2	20	50	60	95	95	2	2

Method 2: Ternary gradient method

t (min)	0	4.0	4.1	10.0	11.0	11.1	12.0
A (%)	95	75	50	5	5	95	95
B (%)	1	5	10	19	19	1	1
C (%)	4	20	40	76	76	4	4

Mass Spectrometer Conditions

MS analysis was carried out on an MSQ Plus single quadrupole LC/MS detector (Thermo Scientific, San Jose, CA). The MS conditions were as follows:

Ionization: Electrospray (ESI)
Polarity: Positive
Probe temperature: 450 °C
Cone voltage: 60 V
Scan mode: Full scan (100-500 *m/z*)
ESI voltage: 4.5 kV
Scan time: 0.2 s

Results and Discussion

The separation and detection of 14 illicit drugs by UHPLC/MS was conducted by two methods. In Method 1, a conventional binary solvent gradient was employed. The mobile phases were water with 0.06% (v/v) acetic acid and acetonitrile with 0.06% (v/v) acetic acid. In Method 2, a ternary solvent gradient was used. The mobile phases were water with 0.06% (v/v) acetic acid, acetonitrile with 0.06% (v/v) acetic acid, and methanol with 0.06% (v/v) acetic acid.

The separation of the 14 illicit drugs by Method 1 using binary solvents is illustrated in Figure 1. Most of the drug compounds were separated with adequate resolution. A few pairs of compounds, such as oxycodone and methamphetamine (peaks 5 and 6), hydrocodone and 3,4-MDMA (peaks 7 and 8), and cocaine and noscapine (peaks 10 and 11), were not baseline resolved. The separation of the drug mixtures was dramatically improved by using ternary solvents in Method 2 (Figure 2). Baseline resolution of all 14 drugs was achieved. Methanol, a

weaker eluent compared to acetonitrile, provided better resolution for most of the drug compounds. However, the separation speed had to be reduced to accommodate the high column backpressure caused by the high viscosity of methanol. To maintain the same separation speed as in Method 1, acetonitrile was used to manipulate the separation factor and to reduce the column backpressure.

Conclusions

An efficient separation method that resulted in better resolution was developed with a quaternary mixing pump for the separation of 14 illicit drugs. The method development process was simplified by using a quaternary mixing pump in an UHPLC system.

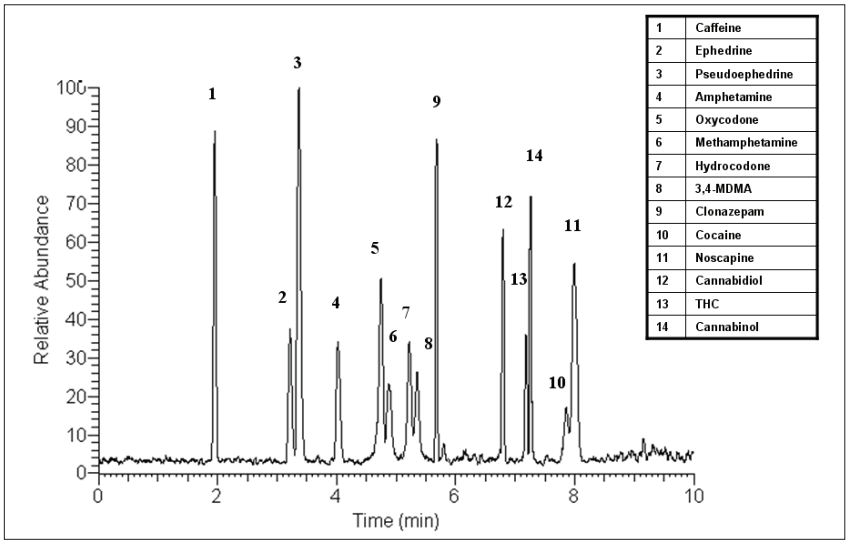

Figure 1: Separation of 14 illicit drugs with binary solvent gradient.

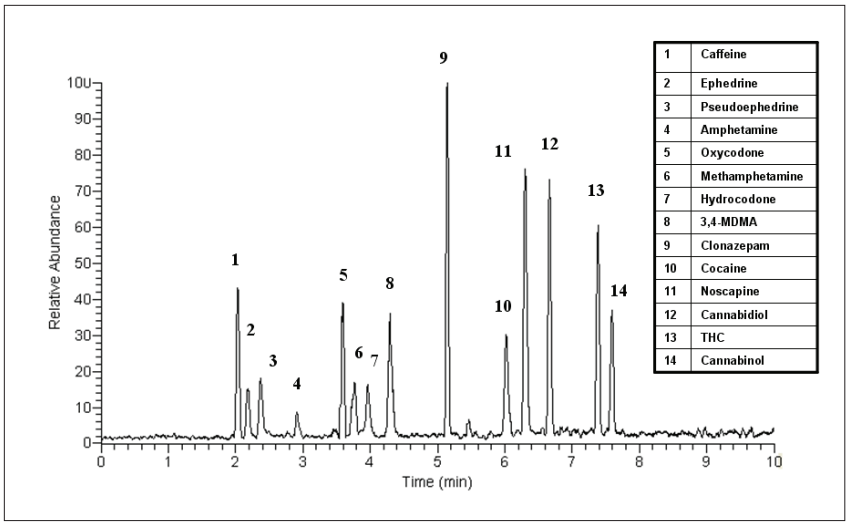

Figure 2: Separation of 14 illicit drugs with ternary solvent gradient.

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