

Chromatography

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UHPLC-MS: An Efficient Method for the Determination of Illicit Drugs

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Introduction

The illicit drugs trade generates one of the largest global revenues at around \$322 billion, according to the UN World Drug Report, 2009¹. Posing a major threat to the pharmaceutical industry and patients worldwide, illicit drugs are falsified medicinal products that contain either sub-standard or falsified ingredients, or ingredients in the wrong dosage. The main illicit drugs are the opiates (mostly heroin), cocaine, cannabis, and amphetamine-type stimulants (ATS) such as amphetamines, methamphetamine and ecstasy. Illicit drugs are listed under five categories namely narcotics, stimulants, depressants, hallucinogens and cannabis. These categories include many drugs legally produced and prescribed by doctors as well as those illegally produced and sold outside of medical channels.²

Illicit drugs have serious health implications as well as political and economic consequences and as a result, their production, trade and use are strictly regulated. Considering the different policies that are adopted globally, The World Drug Report 2009, produced by the United Nations Office on Drugs and Crime (UNODC), presents comprehensive information on the illicit drug market. Providing detailed estimates and trends on the production, trafficking and consumption of drugs on the illicit drug market, one of the main recommendations made in the World Drugs Report was greater efficiency in law enforcement by focusing more on the small number of high profile, high volume and violent drug traffickers instead of the large numbers of petty offenders.

As in previous years, the 2009 Report contains wide-ranging data collated and analyzed by the UNODC and suggests some encouraging reductions in the production of cocaine and heroin. However, it also reports that there are concerns that the production and use of synthetic drugs may be increasing in the developing world.

To counter the growing numbers of medicinal products that are altered in relation to their identity or source, stringent legislation is being enforced. European Parliament wants to establish an effective legislative basis to reduce falsified medicinal products on the illicit drug market by introducing better safety features and track and trace systems for medicinal packaging³.

Under the Council Framework Decision 2004/757/JHA⁴, the need for legislative action to tackle illicit drug trafficking has been recognized. The regulation outlines minimum provisions on the constituent elements of criminal acts and provides a framework for penalties in relation to the quantities and the type of drugs trafficked. As penalties are dependant on the amount of the controlled substance found in a drug mixture, a method is required that is capable of separating, detecting and quantifying illicit drug mixtures in an accurate manner.

Analytical Techniques

Liquid chromatography-mass spectrometry (LC-MS) methods often simplify and eliminate the need to derivatize sample preparation, saving time and resources. Nevertheless, long run times and low separation efficiency limit the utility of conventional high performance liquid chromatography

(HPLC). Ultra high performance liquid chromatography (UHPLC) provides an accurate and sensitive method of identifying and quantifying components in illicit drug samples. It also offers an efficient tool to determine the source and manufacturing pathway of drugs seized on the illicit drug market. The relatively high separation efficiency of UHPLC with capillary GC makes ultra high performance liquid chromatography-mass spectrometry (UHPLC-MS) a viable alternative method for illicit drug analysis, performing separations five to 10 times faster than conventional HPLC by employing sub-2 μm diameter particles.

This article illustrates the separation and detection of a mixture of 14 illicit drugs/metabolites by UHPLC-MS.

Experimental

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, USA). These 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 produce the drug mixture standards.

Chromatographic analyses were performed using the Thermo Scientific Accela UHPLC system (Thermo Fisher Scientific, San Jose, CA). MS analysis was carried out on the Thermo Scientific MSQ Plus single quadrupole LC-MS detector (Thermo Fisher Scientific, San Jose, CA).

Results and Discussion

MS Detection

Both positive and negative electrospray analysis were performed using the polarity switch function of Windows® based Thermo Scientific Xcalibur software platform (Thermo Fisher Scientific, San Jose, CA). All of the analytes exhibited higher ionization efficiency in the positive ion mode compared with the negative mode. The MS spectra of the drug standards demonstrated both molecular ion signals of $[M+H]^+$ and acetonitrile adducts of the form $[M+ACN+H]^+$. For 13 of the analytes, the signal from the molecular ion was more intense than the signal from the acetonitrile adduct. For amphetamine, the most intense signal was from the acetonitrile adduct $[M+ACN+H]^+$ at m/z of 177.2.

Separations with Standard Stationary Phases

The Thermo Scientific Hypersil GOLD, Hypersil GOLD aQ and Hypersil GOLD PFP (Thermo Fisher Scientific, San Jose, CA) were evaluated to separate the illicit drug mixtures (Figure 1). The UHPLC method with each column type was optimized individually. Hypersil GOLD aQ, a polar endcapped C18 phase which offers more retention of polar compounds, did not resolve the early eluting compounds including methamphetamine, oxycodone, caffeine, MDMA and hydrocodone. The separation on the column may have been impaired by interactions between the polar endcapped stationary phase and the polar analytes.

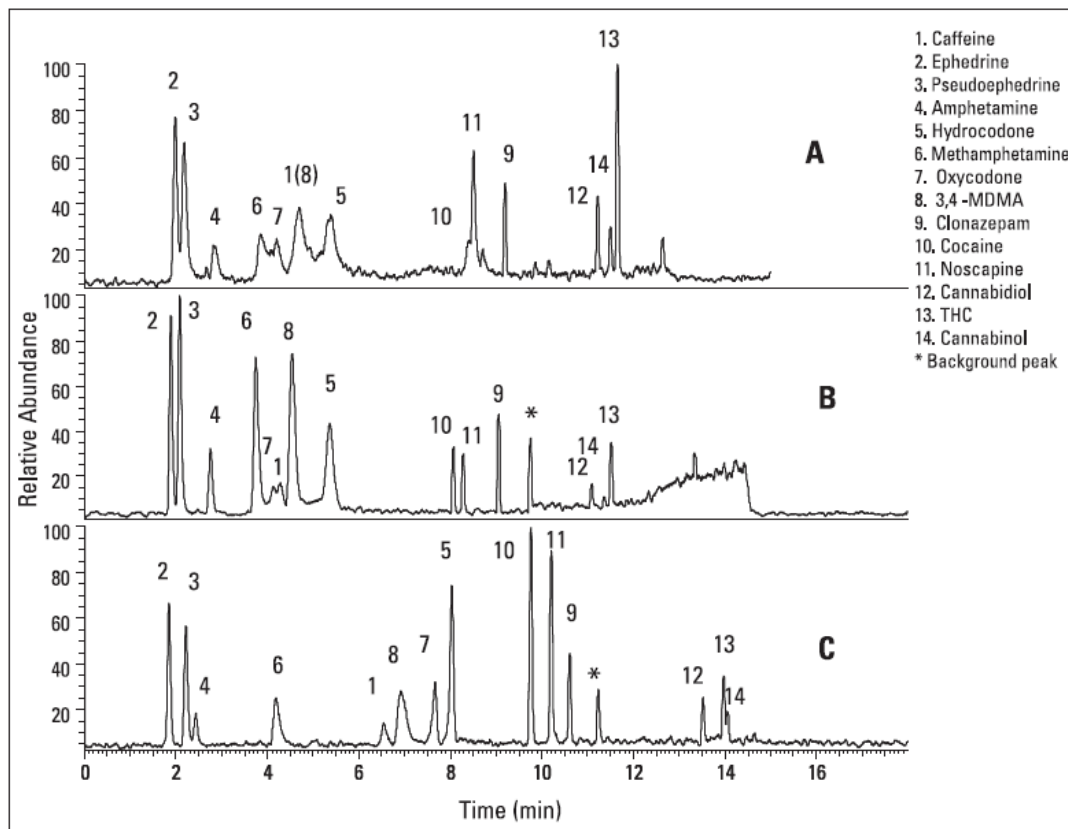


Figure 1: Comparison of 1.9 µm Hypersil GOLD stationary phases for the UHPLC separation of 14 illicit drugs. A) Hypersil GOLD aQ, B) Hypersil GOLD, C) Hypersil GOLD PFP.

Hypersil GOLD, with LI or C18 selectivity, showed improved selectivity for all analytes except caffeine (peak 1) and oxycodone (peak 7). The column uses highly pure silica and endcapping procedure to minimize unwanted interactions between analytes and the acidic silanols of the silica support. Hypersil GOLD PFP enabled the optimal separation of all 14 analytes by improving the resolution of the earlier eluting compounds. The column introduces a fluorine group into the stationary phase to improve selectivity towards halogenated compounds, as well as polar compounds containing hydroxyl, carboxyl, nitro or other polar groups.⁵

Separations using Acetic Acid and Trifluoroacetic Acid (TFA) as Eluent Modifier

Trifluoroacetic acid, formic acid and acetic acid can be added into the mobile phase to generate differences in selectivity. Separation of 14 illicit drugs on a Hypersil GOLD PFP column was evaluated by using either trifluoroacetic acid, formic acid or acetic acid as eluent modifier. The separation method with 0.02% TFA (Figure 2A) provided fast separation performance with good resolution and sharp peaks. However, the use of TFA is generally not recommended with MS detection due to its effect on signal suppression.

All of the analytes were well resolved with 0.1% formic acid as modifier (Figure 1C), but only when 100% water was used at the beginning of the gradient method. However, prolonged use of 100% water may degrade the stationary phase and shorten the column lifetime, so this gradient method is not suited for routine use. Most of the analytes were well separated with adequate resolution using 0.06% acetic acid as eluent modifier (Figure 2B). However, under such conditions, a few pairs of compounds, such as oxycodone and methamphetamine (peaks 7 and 6), hydrocodone

and 3, 4-MDMA (peaks 5 and 8), cocaine and noscapine (peaks 10 & 11) were not baseline resolved.

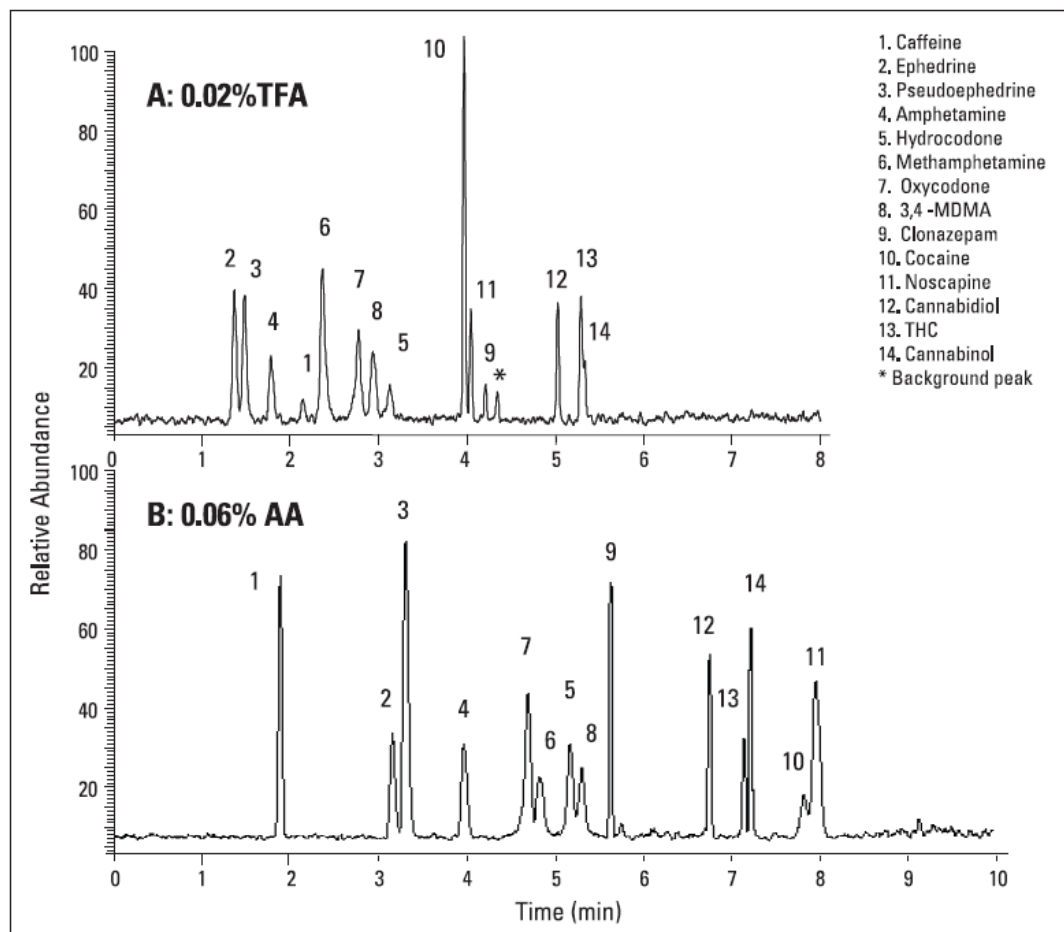


Figure 2: UHPLC/MS chromatograms of the 14 illicit drugs with acidic solvent modifiers. A) 0.02%, B) 0.06% acetic acid.

Separations with Hybrid Column Phases

Three hybrid stationary phases were evaluated after connecting different stationary phase columns in series (Figure 3). Separations of 14 illicit drugs with these three hybrid stationary phases demonstrated great variation in selectivity. In general, the hybrid column phases improved selectivity between THC and cannabinol, cocaine and noscapine, but reduced selectivity between earlier eluting compounds, such as oxycodone, MA, hydrocodone and MDMA, compared with the Hypersil GOLD PFP phase.

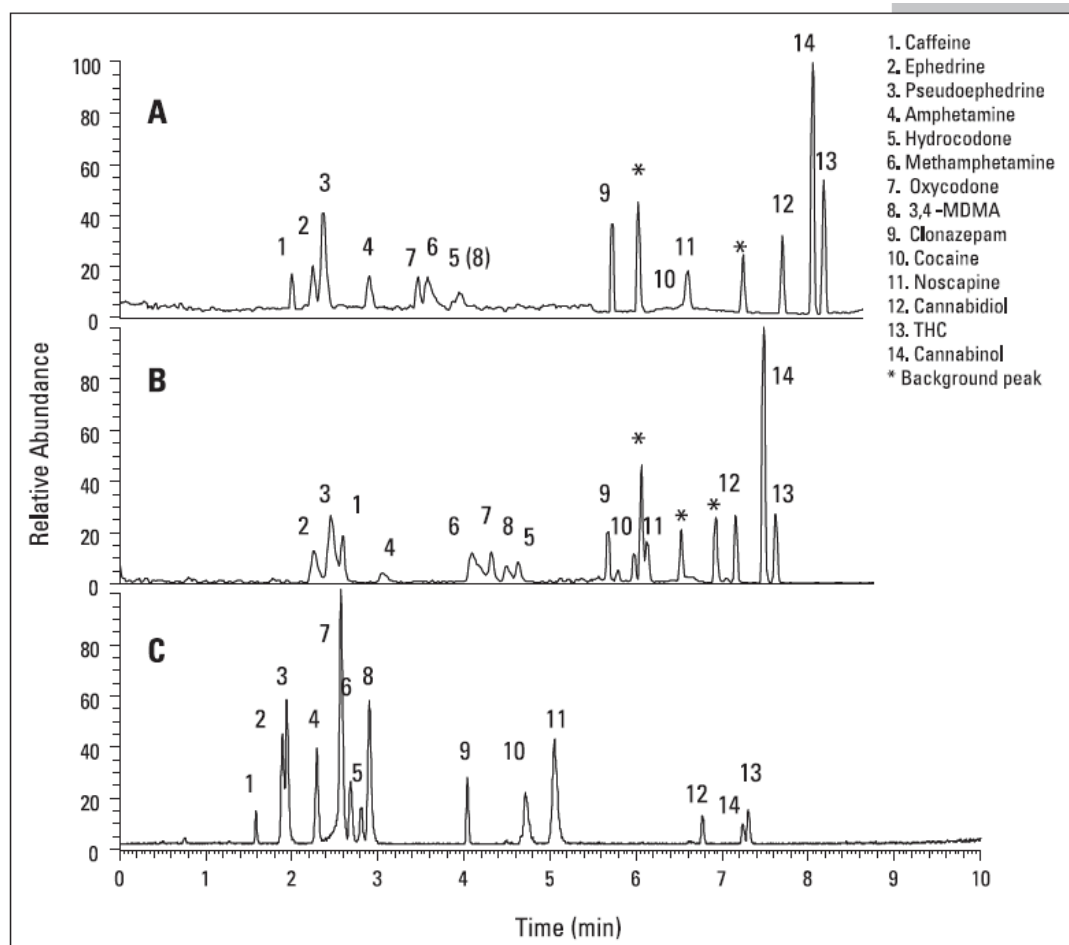


Figure 3: Comparison of hybrid stationary phase chemistry for the separation of 14 illicit drugs. A) 50 x 2.1 mm Hypersil GOLD + 50 x 2.1 mm Hypersil GOLD PFP, B) 50 x 2.1 mm Hypersil GOLD PFP + 50 x 2.1 mm Hypersil GOLD, C) 100 x 2.1 mm Hypersil GOLD PFP + 20 x 2.1 mm Hypersil GOLD.

Separation with Ternary Gradient

The separation of the drug mixtures was dramatically improved by using three solvents: water, acetonitrile and methanol (Figure 4). Baseline resolution of all 14 drugs was achieved. Methanol, a weaker eluent compared with acetonitrile, provided better resolution for most of the analytes. However, the flow rate had to be reduced to accommodate high column backpressure caused by the high viscosity of methanol. Adding acetonitrile reduced the column backpressure so as to maintain the same separation speed.

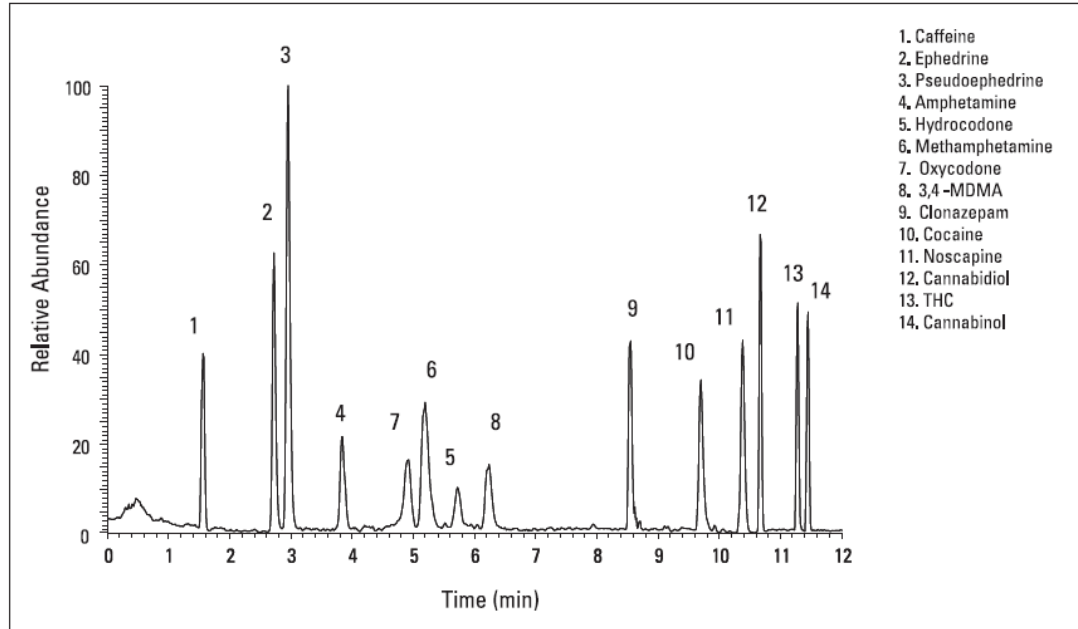


Figure 4: Optimized UHPLC/MS separation of 14 illicit drugs with ternary gradient.

Calibration Curve and Sensitivity

Calibration curves for the drug standards were constructed over the pre-specified concentration range with 10 calibration levels (Figure 5). Each calibration level was injected three times and the mean area responses were plotted against the concentrations. Correlation coefficients with $R^2 = 0.995$ or better were achieved for all illicit drug compounds.

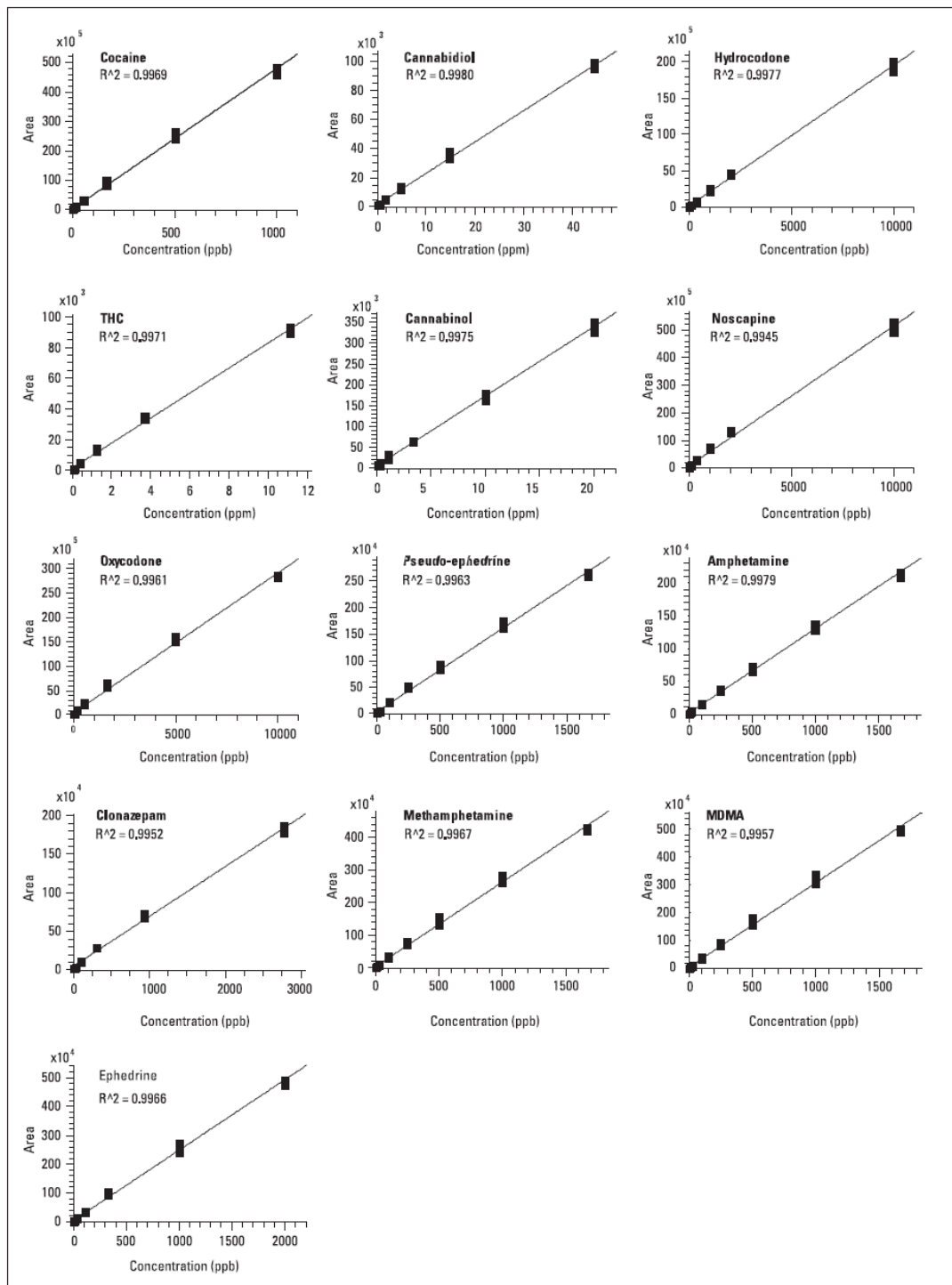


Figure 5: Calibration curves for illicit drugs.

The limit of quantitation (LOQ) and the limit of detection (LOD) of the 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 , respectively. LOQs for all drugs were in the

range of 0.96-300 ng/mL, while LODs were from 0.29 to 90.0 ng/mL. The outstanding sensitivity of this method was highlighted by the achievement of picogram level quantitation for 10 illicit drugs with 1 µL sample injection.

Conclusion

In order to protect human health and comply with stringent legislation, fast, efficient and reliable separation and identification of illicit drugs and metabolites is required in line with EU regulations. Using UHPLC-MS with a ternary solvent gradient provides improved separation resolution, reducing the overall analytical time and increasing laboratory productivity. Various selectivities are achieved by employing different column surface chemistries, acidic solvent modifiers and eluent systems. Identification of components from illicit drug mixtures by single quadrupole MS at the ppb (ng/mL) level helps to identify the source and manufacturing pathway of drugs seized on the illicit drug market.

For more information about the Thermo Scientific UHPLC/MS solutions, please call +1 866-463-6522, e-mail analyze@thermofisher.com or visit www.thermoscientific.com/accela.

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