

An Accurate and Robust LC/MS Method for the Identification of Illicit Drug Salt Forms

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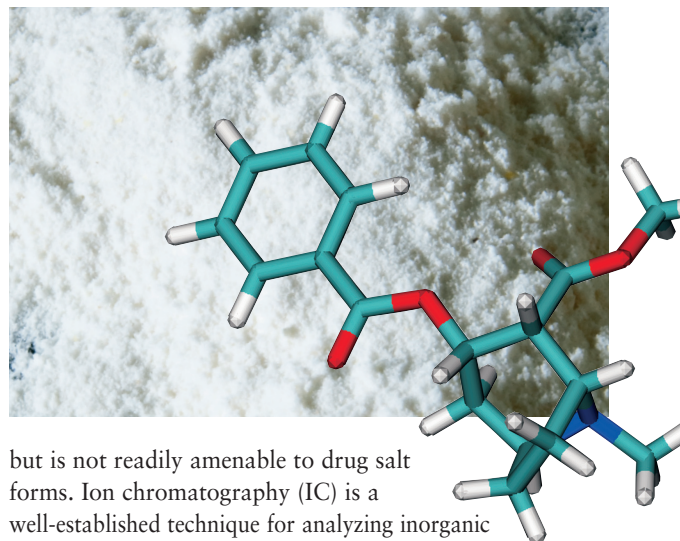
Goal

Develop a robust LC/MS method for the analysis of illicit drug salt forms.

Introduction

Between 172 and 250 million people worldwide used illicit drugs at least once in 2007, with 18–38 million estimated to be heavy drug users.¹ Among the most widely abused controlled substances are the stimulants. Cocaine, the most potent stimulant derived from natural sources, was consumed by an estimated 15.6–20.8 million people worldwide in 2007, representing 0.4–0.5% of the world's population aged 15–64.¹ While cocaine use is declining in North America, it is still the world's largest cocaine market with an estimated 6.87 million users.¹ Production and use of synthetic amphetamine-group stimulants (predominantly methamphetamine and amphetamine) are on the rise, most notably in developing countries where law enforcement is often more lax. Clandestine manufacture of amphetamine-type substances, particularly of methamphetamine, has been reported in over sixty countries, and seizures have increased globally. The availability of inexpensive precursor chemicals is a major contributing factor to the proliferation of clandestine methamphetamine operations in the United States.² In 2007, between 15.8 and 50.6 million people worldwide aged 15–64 were estimated to have consumed amphetamines-group substances (0.4%–1.2% of the population).¹ The number of ecstasy-group (predominantly 3,4-methylenedioxymethamphetamine, or 3,4-MDMA) drug users was reported to be between 11.6 and 23.5 million worldwide (0.3%–0.5% of the population).¹ Inappropriate and extended use of any of these drugs can lead to devastating health outcomes. Moreover, the illicit drug trade fuels crime and violence and can destabilize whole communities. Techniques to accurately identify drugs, drug precursors and new drug derivatives are crucial to law enforcement efforts to curb the black market in controlled substances.

Illicit cocaine, amphetamine, methamphetamine and MDMA are often encountered in the form of salts, and identification of the specific counterion present may provide valuable information about the source of production and the manufacturing pathway. Screening and identification of these stimulants in seized evidence requires an analytical method that is capable of detecting the salt forms. Gas chromatography-mass spectrometry (GC/MS) is a commonly used technique for the identification of controlled substances,



but is not readily amenable to drug salt forms. Ion chromatography (IC) is a well-established technique for analyzing inorganic and organic ions, but separate columns and methods are required for cations and anions. Moreover, IC instrumentation and consumables are costly and not readily available in most forensic laboratories.

High performance liquid chromatography with mass spectrometry (LC/MS) is a powerful alternative to GC/MS for drug analysis, enabling exceptionally sensitive detection and accurate identification of a wider range of compounds without the need for derivitization. Thermo Scientific Hypercarb columns, packed with porous graphite carbon particles, are ideally suited for highly polar and ionized analytes such as drug salt forms. The flat, crystalline and polarizable Hypercarb™ surface retains very polar species and separates structurally-related compounds via hydrophobic and electrostatic retention mechanisms. The Thermo Scientific Accela LC system offers the flexibility of performing both conventional and high-speed LC separations on a single platform, and seamlessly integrates with the Thermo Scientific MSQ Plus Mass Detector, a sensitive and fast scanning single quadrupole mass spectrometer (MS). The rapid polarity switching capability of the MSQ™ Plus Mass Detector enables simultaneous detection of cations and anions in a single analysis. This application note describes an accurate and robust LC/MS method developed for simultaneous separation and detection of cations and anions in illicit drug salt forms. Importantly, the method enables drug salt forms in seized materials to be identified and confirmed by two independent parameters (retention time and mass spectral signature).

Key Words

- Accela
- Hypercarb
- MSQ Plus Mass Detector
- Drug Analysis
- Forensics
- Salt Forms

Materials and Methods

Sample Preparation

Amphetamine, methamphetamine, 3,4-MDMA and cocaine standards (1 mg/mL in 1 mL of methanol) were purchased from Alltech-Applied Science (State College, PA, USA). Potassium chloride, potassium bromide, potassium iodide, potassium nitrate, potassium phosphate and ammonium sulfate were obtained from Sigma. A stock solution of the drug mixture was prepared by mixing the four standards at 5 µg/mL concentration in methanol. The stock mixture of the salt standards was prepared using 6 µg/mL iodide, 160 µg/mL chloride, 60 µg/mL bromide, 20 µg/mL sulfate, 10 µg/mL phosphate, and 3 µg/mL nitrate. The standard sample used in LC injections was prepared by mixing the drug stock solution and the salt stock solution (1:1, v/v) and diluting five-fold in water with 1% formic acid.

LC/MS Analysis

Instrumentation

LC/MS analysis was performed on an Accela™ UHPLC system coupled to the MSQ Plus Mass Detector, a single-stage quadrupole mass spectrometer.

LC Parameters

Column:	Thermo Fisher Scientific Hypercarb column (50 × 2.1 mm, 3 µm particle size)			
Mobile Phase:	A: Water with 1% formic acid B: Methanol (MeOH) with 1% formic acid			
Flow Rate:	300 µL/min			
Column Temperature:	60 °C			
Sample Injection Volume:	2 µL			
Gradient:	Time (min)	A% Water/ 1% FA	B% MeOH/ 1% FA	Flow Rate µL/min
	0.0	92	8	300
	2.0	92	8	300
	2.1	70	30	300
	5.5	30	70	300
	5.6	92	8	300
	15.0	92	8	300

MS Parameters

Polarity Switching Mode Detection	
Heated Electrospray Ionization Source Conditions:	
Spray Voltage:	3500 V
Probe Temperature:	450 °C
Full Scan:	130–200 amu, positive mode, cone 55 V
Full Scan:	30–130 amu, negative mode, cone 130 V

Results and Discussion

Separation and MS Detection of Drug and Inorganic Anion Standards

Cocaine and the synthetic stimulants amphetamine, methamphetamine and 3,4-MDMA are frequently found as salt forms in suspected materials. Common salt forms of cocaine are chloride and bromide. Amphetamine is usually found in the chloride and sulfate forms. Bromide and chloride forms of methamphetamine are common, but iodide, phosphate and nitrate forms may also be encountered. For 3,4-MDMA, bromide and chloride are the most common salt forms, although a phosphate form may occasionally be detected.

Separation of a mixture of cocaine, amphetamine, methamphetamine and 3,4-MDMA standards as well as a mixture of six inorganic anions was achieved using a Hypercarb column and a single LC/MS method (Figure 1). Gradient elution was used to control selectivity and retention of the inorganic anions and the organic cations. The inorganic anions were separated within 3 minutes with an elution order of phosphate, chloride, bromide, nitrate, iodide and sulfate. The drug standards were baseline-resolved in 7 minutes and eluted in order of increasing hydrophobicity: amphetamine, methamphetamine, 3,4-MDMA and cocaine. Retention times are summarized in Table 1.

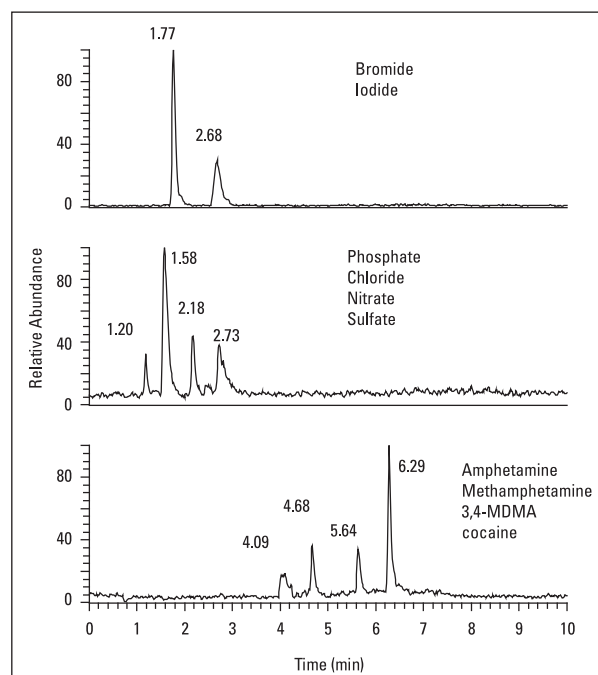


Figure 1: HPLC/MS separation and detection of the four illicit drug standard and their possible counter ions with ESI ionizations. a) Extracted ion chromatogram at m/z of 80.95 and 126.98; b) Extracted ion chromatogram at m/z of 34.95, 61.97, 79.00 and 79.99; c) Extracted ion chromatogram at m/z of 136.12, 150.10, 194.15 and 303.95

The mass spectra of the stimulants and their common counterions are shown in Figure 2. The most abundant ions for the drug standards are the $[M+H]^+$ ions, at m/z 136.12, 150.10, 194.15 and 303.95 for amphetamine, methamphetamine, 3,4-MDMA, and cocaine, respectively. The acetonitrile adduct of amphetamine, at m/z of 177.26, was observed as the second intense peak in its spectrum. The fragment ions at m/z 62.97 and 79.00 in the phosphate mass spectrum represent PO_2^- and PO_3^- , respectively. The dominant fragments at m/z 45.97 and 61.97 observed in the nitrate spectrum represent the NO_2^- and NO_3^- ions, respectively. In the sulfate mass spectrum, the product ion at m/z 79.99 represents SO_3^- while the ion at m/z 97.00 represents HSO_4^- . The iodide mass spectrum is characterized by the single I^- fragment ion at m/z 126.98. The mass spectra of the chloride and bromide ions exhibit their characteristic isotopic signatures.

Compound	Retention Time (min)	RSD% (RT,n = 3)	Linearity Range ng/mL (ppb)	Correlation Coefficients	LOQ ng/mL (ppb)	LOD ng/mL (ppb)
Phosphate	1.20	0.46	1.14–22800	0.9991	3.3	1.1
Chloride	1.58	0.36	50–5000	0.9999	100	30
Bromide	1.79	0.65	5–10000	0.9971	30	10
Nitrate	2.18	0.94	4–1000	0.9961	25	8
Iodide	2.68	0.97	5–1000	0.9962	35	10
Sulfate	2.73	0.35	2.94–29400	0.9992	6	2
Amphetamine	4.09	0.37	0.5–2000	0.9979	2	0.5
Methamphetamine	4.68	0.37	0.5–2000	0.9967	1	0.3
3,4-MDMA	5.64	0.37	0.5–2000	0.9957	1	0.3
Cocaine	6.29	0.46	0.125–1000	0.9969	1	0.3

Table 1: LC/MS data for the drug and inorganic anion standards

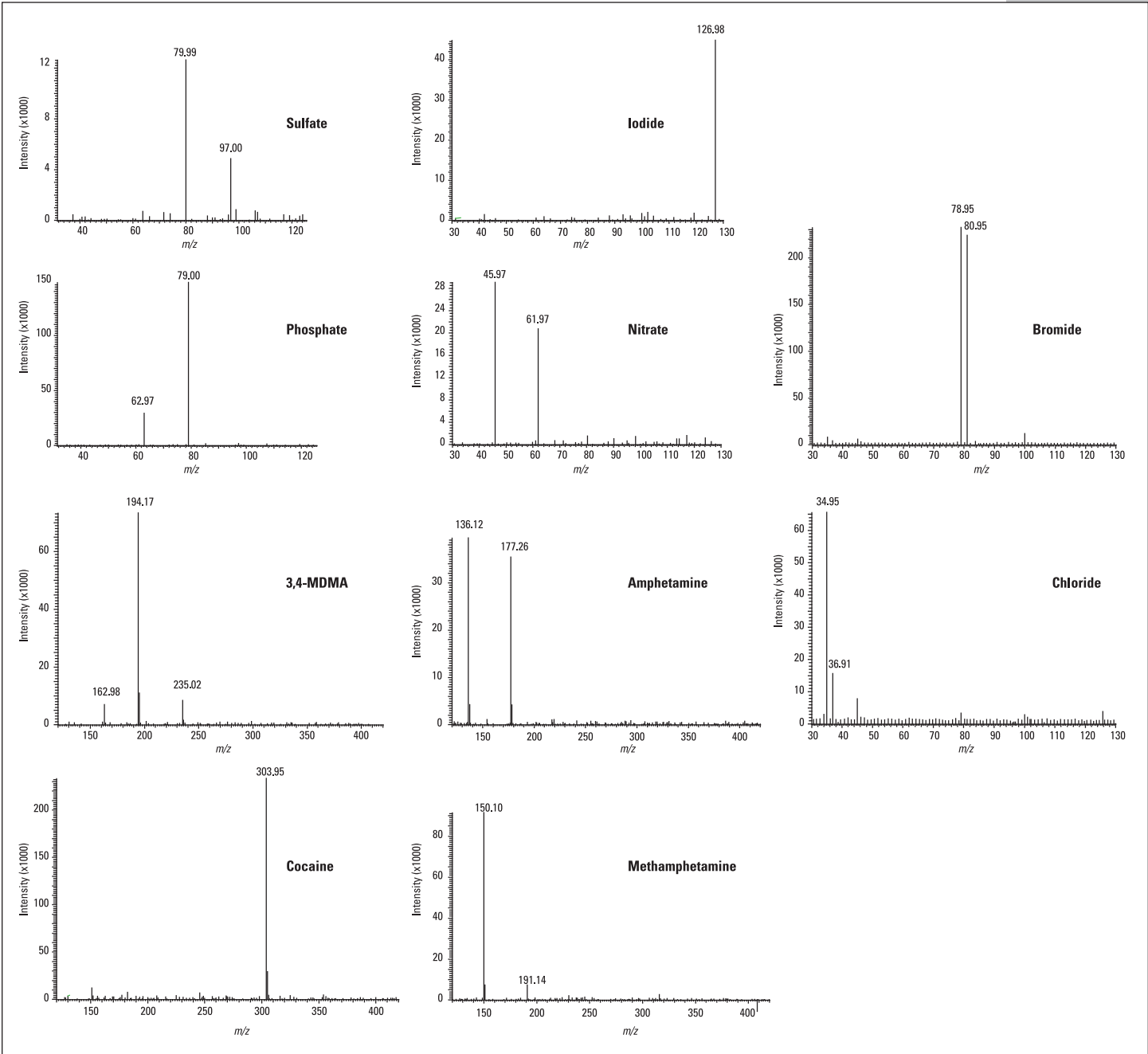


Figure 2: Mass spectra of the drug and inorganic anion standards

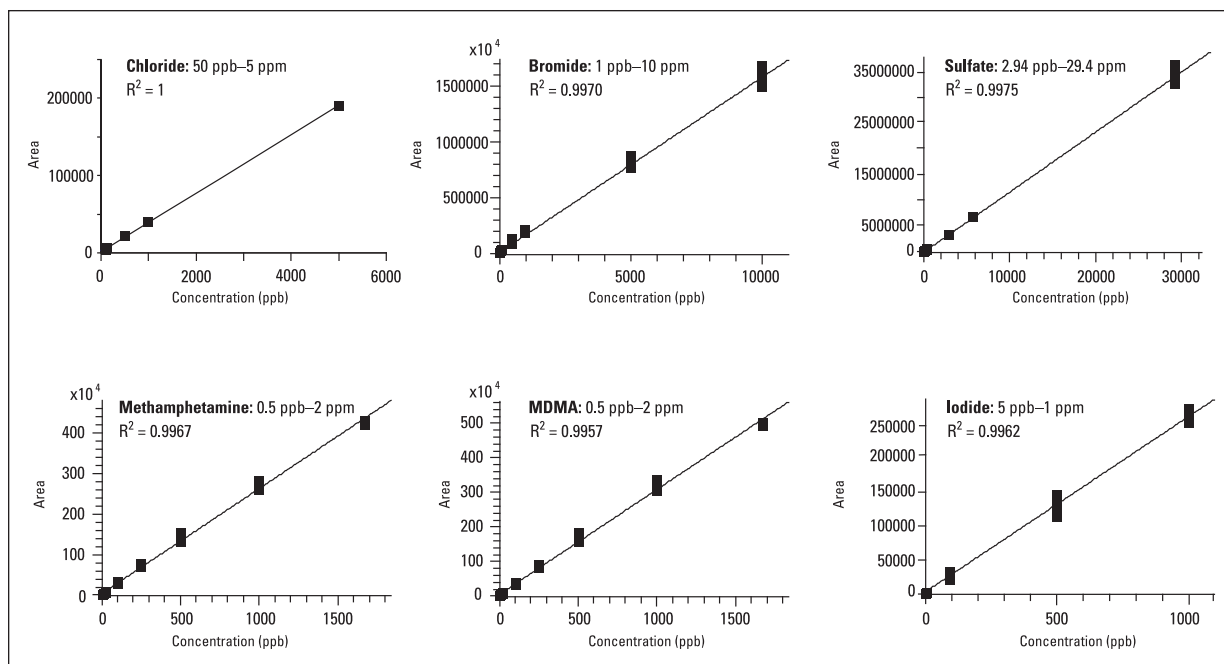


Figure 3: Representative calibration curves of drug and inorganic anion standards

Linearity and Sensitivity

Excellent linearity in detector response was observed over the range of 0.125 – 2000 ng/mL (ppb) for the drug standards and over the range of 1.14 – 29400 ng/mL (ppb) for the inorganic ions, with correlation coefficients greater than 0.996 for all analytes (Table 1). Representative calibration curves are shown in Figure 3.

Limits of detection (LODs) and limits of quantitation (LOQs), defined as S/N ratio of 3 and 10, respectively, are shown in Table 1. For drug standards, LODs ranged from 0.3 – 0.5 ng/mL (ppb), and LOQs ranged from 1 – 2 ng/mL (ppb). For the inorganic ions, LODs were between 1.1 – 30 ng/mL (ppb), and LOQs ranged from 3.3 – 100 ng/mL (ppb).

Reproducibility

Reproducibility was investigated by analyzing three replicate injections of each analyte. Retention time RSDs ranged from 0.35 – 0.92%, indicating excellent method reproducibility, particularly of the Accela UHPLC pump.

Conclusion

An accurate and robust LC/MS method for the identification of illicit drug salt forms was developed. Simultaneous separation and detection of cations and anions was achieved using a Hypercarb LC column and the polarity switching mode of the MSQ Plus Mass Detector. Furthermore, this method utilizes two uncorrelated parameters – retention time and mass spectral signature – to establish the identity of drug salt forms.

References

1. World Drug Report 2009, United Nations Office on Drugs and Crime.
2. Methamphetamine National Drug Threat Assessment 2009, National Drug Intelligence Center, US Dept of Justice. <http://www.justice.gov/ndic/pubs31/31379/meth.htm>

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