Confirmation of drugs of abuse in pseudo drug mixes using UHPLC-ESI TOF MS

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1 Introduction

Forensic crime labs are responsible for the qualitative confirmation of drugs of abuse for use in court cases. Gas chromatography mass spectrometry (GCMS) has been the gold standard for confirmation of cocaine, procaine, and morphine in confiscated illicit drugs. GCMS analysis requires extraction, derivatization, and long run times. In this study, pseudo drug mixes were made to mimic typical composition of street drug mixtures. We demonstrate the use of ultra high performance liquid chromatography coupled to electrospray time-of-flight mass spectrometry (UHPLC-ESI TOF MS) for confirmation of cocaine, procaine, and morphine in pseudo drug mixes in 1 min. Using high resolution and exact mass capabilities of a TOF mass spectrometer coupled to uHPLC we can rapidly confirm the identity of drugs of abuse in complex mixtures.

2 Method

A PerkinElmer Flexar™ FX-15 LC pump with PerkinElmer AxION™ TOF MS was used for separation and detection of cocaine, procaine, and morphine.

Sample preparation: Morphine and cocaine standards were obtained from Restek (Bellefonte, PA) with concentration of 1mg/ml in 100% methanol. Samples were diluted 1:1000 with 50/50 methanol/water with 0.1 % formic acid to make a final concentration of 0.001 mg/ml. Procaine standard was obtained from Sigma Aldrich (St. Louis, MO) and was prepared to a concentration of 1 mg/ml in 50/50 methanol/water with 0.1 % formic acid and diluted 1:1000 to make a final concentration 0.001mg/ml. All other reagents were received from Sigma Aldrich. Pseudo drug mix 1 contained a mixture of 25 % w/w cocaine, 20 % procaine, 50 % bicarbonate, and 5% caffeine with the final concentration of 25 µg/mL cocaine. 1µL of sample was injected. Pseudo drug mix 2 contained a mixture of 15 % w/w morphine, 80 % lactose, and 5%caffeine with the final concentration of 15 µg/mL morphine.

Liquid chromatography conditions:

Pump type: Flexar FX-15
Column: PerkinElmer Brownlee Hres column C-18 (1.9µm, 2.1 x 50mm)
Mobile phase:
A: Water with 5 mM Ammonium Acetate and 0.1% Formic Acid
B: Acetonitrile with 0.1% Formic acid
Column temp: 55°C
Flow rate: 0.8 mL/min
Injection volume: 1 µL
Gradient conditions:

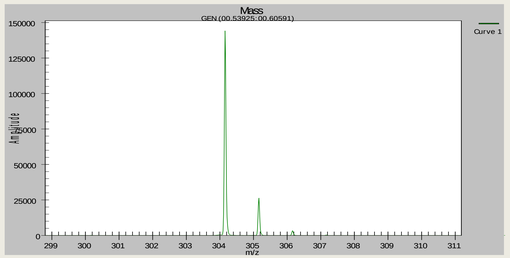
Time (min)	%A	%B
0	85	15
1	5	95

Mass spectrometer conditions

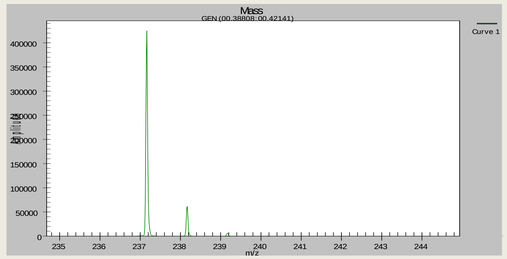
The ESI TOF MS was operated in positive ion mode (*m/z* 100 - 1000) at 3 spectra/sec for detection with capillary exit voltage set to +90V.

Compound	Formula	Monoisotopic Mass	$[M+H]^+$
Cocaine	$C_{17}H_{21}NO_4$	303.14705	304.15487
Procaine	$C_{13}H_{20}N_2O_2$	236.15247	237.16029
Morphine	$C_{17}H_{19}NO_3$	285.13648	286.14431

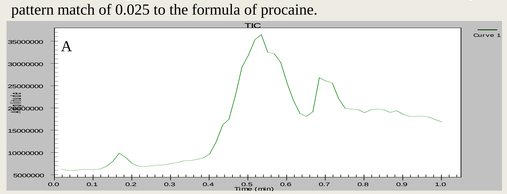
3 Results

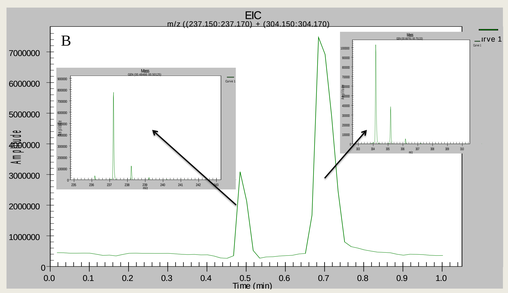


Cocaine standard injected at 1 µg/mL. Perkin Elmer ESI-TOF-MS measured 304.1547 with a <2ppm mass error to the $[M+H]^+$ of 304.1543 and an isotopic pattern match of 0.023 to the formula of cocaine.



Procaine standard injected at 1 µg/mL. Perkin Elmer ESI-TOF-MS measured 237.1604with a <1ppm mass error to the $[M+H]^+$ of 237.1602 and an isotopic pattern match of 0.025 to the formula of procaine.

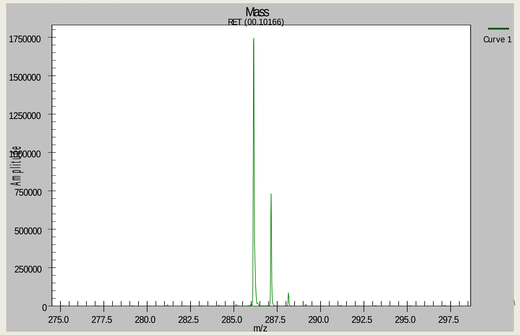




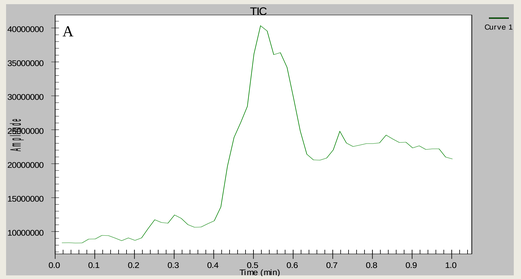
A) Total ion chromatogram (TIC) of pseudo drug mix 1

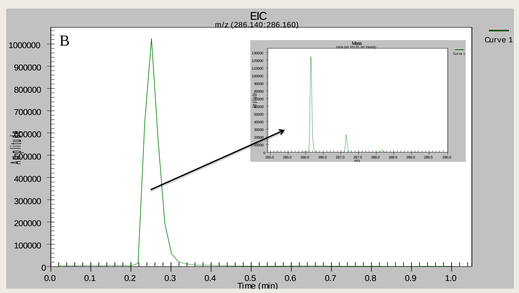
B) Extracted ion chromatogram (EIC) of procaine (peak 1) and cocaine (peak 2) from pseudo drug mix 1 and averaged mass spectra under the EICs.

The high mass accuracy capabilities of the PerkinElmer AxION™ TOF MS provides exact masses of cocaine (RT 0.7 min) and procaine (0.5 min)standards with <2 ppm mass accuracy. By using the PerkinElmer Flexar™ FX-15 LC pump with PerkinElmer AxION™ TOF MS and EICs to extract out cocaine and procaine we are able to provide 2 modes of identity confirmation required for positive identification for crime labs in less than 1 min per sample with little to no sample prep.



Morphine standard injected at 1 µg/mL. Perkin Elmer ESI-TOF-MS measured 286.1443 with a <1ppm error to the $[M+H]^+$ of 286.1443 and an isotopic pattern match of 0.012 to the formula of morhpine.



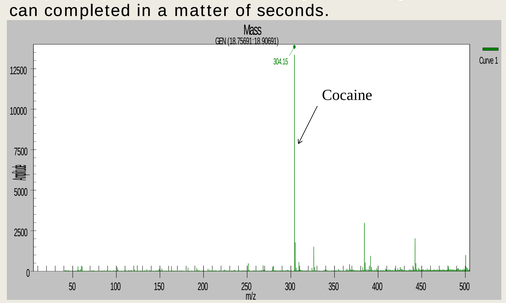


A) TIC of pseudo drug mix 2

B) Extracted ion chromatogram of morphine from pseudo drug mix 2 and averaged mass spectra under the EICs.

4 New Developments in Forensics Analysis

When running a large volume of samples, sometimes run time per sample can be the rate limiting step. By using a new direct sample analysis (DSA) source, the need for sample prep is eliminated and analysis for drugs of abuse can completed in a matter of seconds.



Average spectrum on a 20$ bill using the Perkin Elmer DSA-TOF. Cocaine is found to be the most abundant peak on the bill and is confirmed by exact mass.

6 Summary

Cocaine, procaine, and morphine standards were analyzed by PerkinElmer Flexar™ FX-15 LC pump with PerkinElmer AxION™ TOF MS . The chromatographic retention time combined with the accurate mass of <2ppm for all reference standards along with the isotopic pattern confirming the chemical formula gave a reference for running unknown samples. The identity of the unknown drugs of abuse can be confirmed in pseudo drug mixes by matching the chromatographic retention time, mass accuracy and isotopic pattern of the drugs of abuse against the reference standards. By using the high resolution and mass accuracy of the TOF, we are able to eliminate the need for sample extraction and derivatization by analyzing extracted ion chromatograms with a narrow mass window of 0.005Da. The accurate mass was found to be <2ppm for the drugs of abuse in pseudo drug mixes. This aided the ability to confirm the chemical formula of the drugs in a complex matrix that could not be done with traditional methods of GCMS or LCMS. The combination of chromatographic separation by UHPLC and the accurate mass of the TOF should satisfy the SWGDRUG recommendations for drugs of abuse analysis recommending 2 separate confirmations such as UHPLC retention time and exact mass of the TOF.
The DSA –TOF source adds an additional benefit of a no sample prep analysis workflow for a quick confirmation of presence of compounds such as cocaine on dollar bills.

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