

Screening and Quantitation of Drugs from Blood and Urine by Paper Spray MS

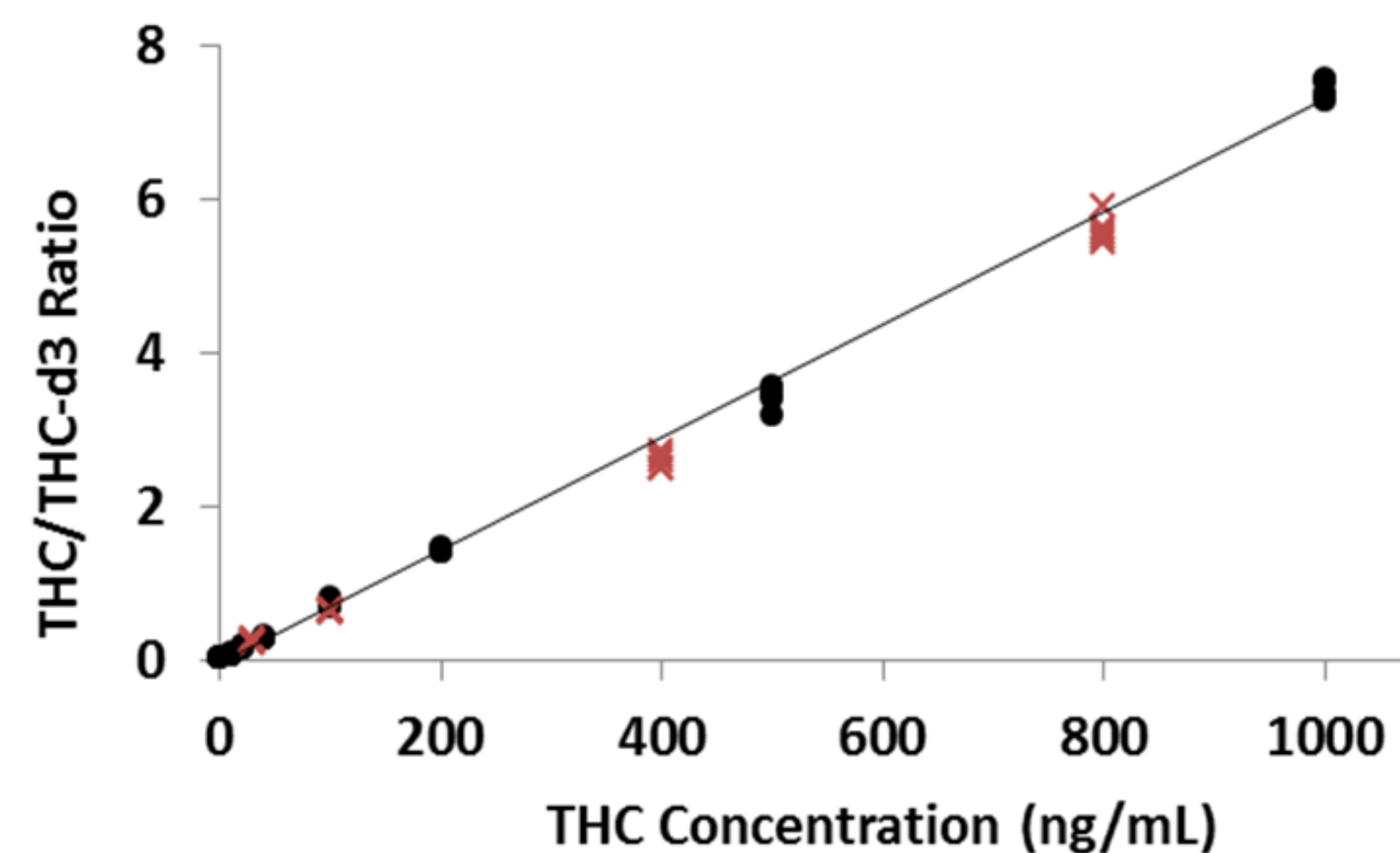
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Overview

- Paper spray is a method for performing rapid drug analysis by mass spectrometry without sample preparation
- Analyte extraction and ionization is performed by applying a spray solvent by initiating an electrospray plume from a porous substrate (i.e. paper) after application of solvent and high voltage
- The sample (blood, urine, saliva, etc) is dried and stored on the paper
- Here, results for three experiments related to drug analysis by paper spray are shown:
 - Screening of drugs and drug metabolites in urine
 - Quantitative analysis of drugs in dried blood spots
 - Separation of three opiate isomers using FAIMS ion mobility filtering coupled to a triple quad

Experiment 2: Quantitative Analysis Directly from Dried Blood Spots



Drug	Paper Spray LOD directly from DBS (ng/mL)
Amphetamine	1
Methamphetamine	0.3
MDA	2
MDMA	0.04
MDEA	0.3
Morphine	12
Cocaine	0.05
D9-THC	4

Figure 6. The eight compounds shown to the left were quantitated from dried blood spots by paper spray MS/MS. Isotope labeled internal standards were mixed into the blood sample prior to spotting it onto the paper. The first 7 were quantitated simultaneously. THC was analyzed separately because it required a different solvent

Instrument: Thermo TSQ Access Max
Solvent: 95-5 methanol-water with 0.2% hydroxylamine

Ryan D. Espy, Bas Teunissen, Nicholas E. Manicke, Yue Ren, Zheng Ouyang, Arian van Asten, R. Graham Cooks. *Analytical Chemistry*. 2014

Experiment 3: Opiate Separation by FAIMS Filtering Prior to MS/MS

- Paper spray increases throughput and decreases sample preparation
- However, removal of chromatography decreases selectivity
- FAIMS (high field asymmetric waveform ion mobility spectrometry) is an ambient pressure ion mobility technique that can be coupled inline with mass spectrometry to improve selectivity without increasing analysis time
- In some cases, it can resolve structural isomers that cannot be distinguished by MS/MS
- Paper spray was tested on a Thermo TSQ to separate 3 opiate isomers: morphine, norcodeine, and hydromorphone

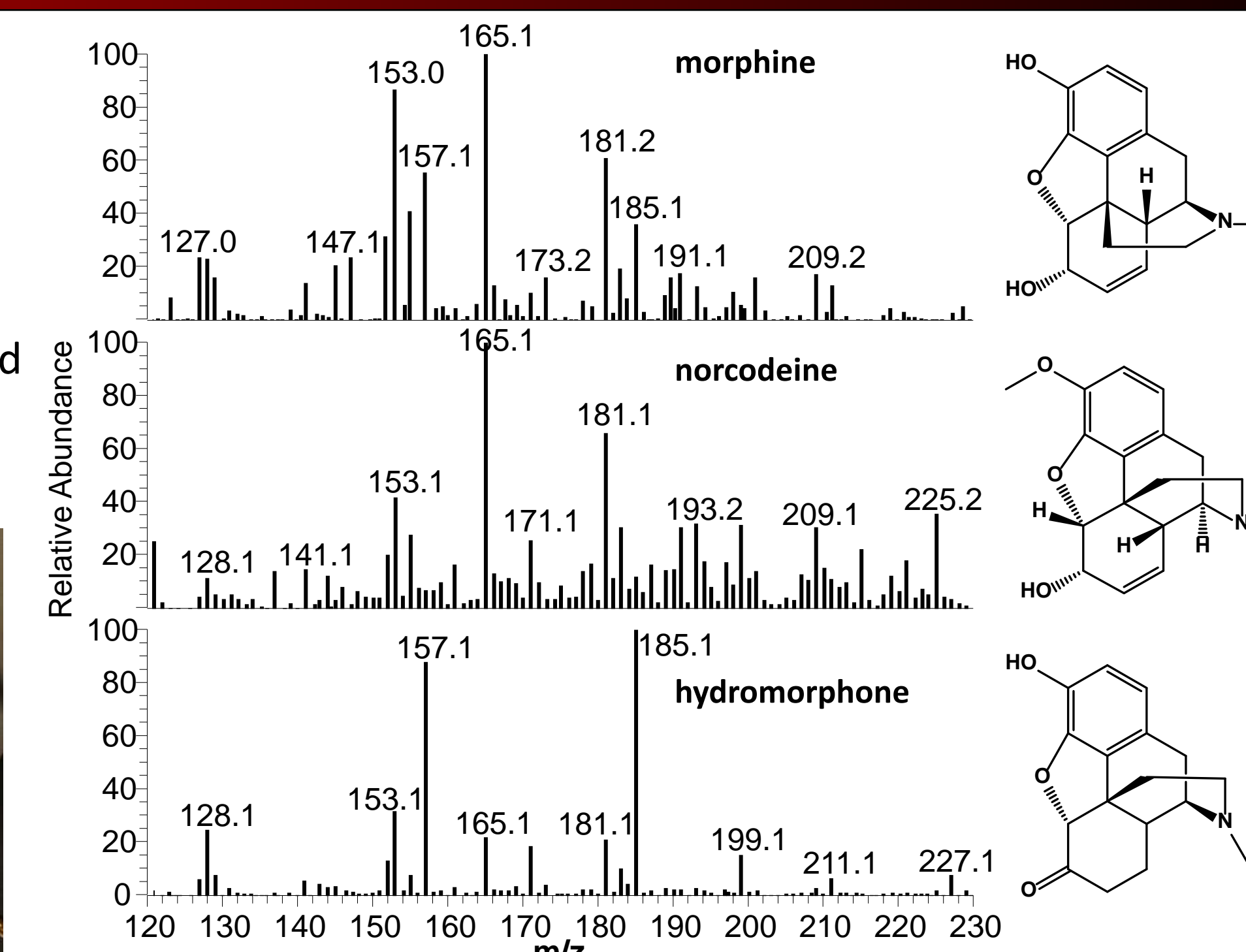
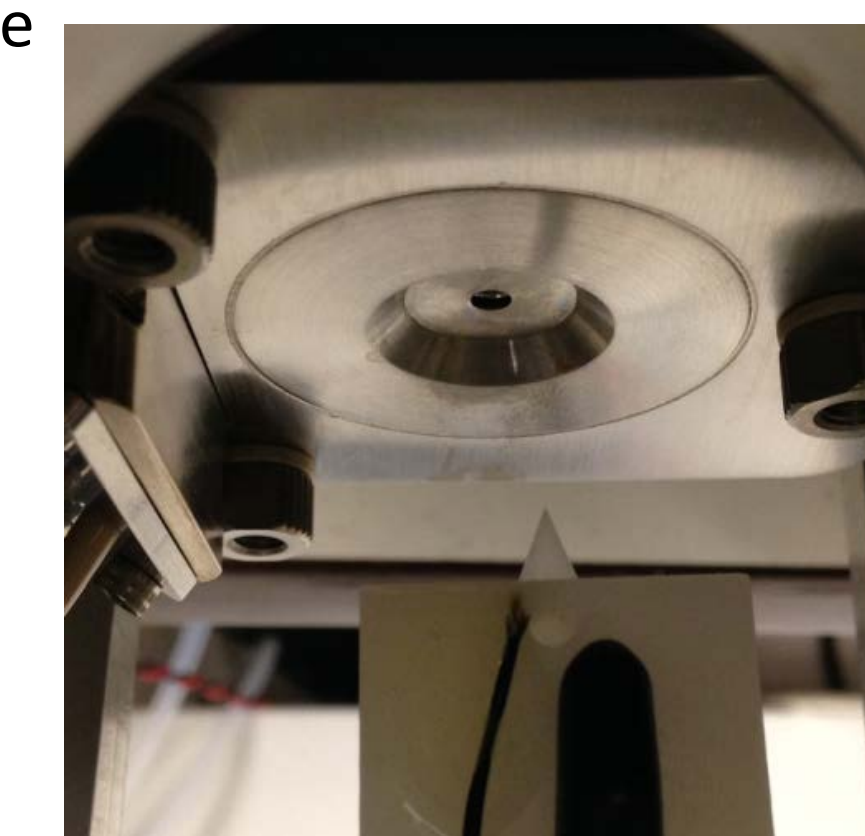


Figure 8. paper spray MS/MS spectra of three opiate isomers. They cannot be distinguished adequately by MS/MS

Figure 7. Picture of a prototype paper spray cartridge held in front of the FAIMS inlet for analysis. The FAIMS is attached to a triple quad mass spectrometer



Introduction

- A cone-jet of charged solvent droplets can be generated from wet paper by applying high voltage (figure 1)
- The spray solvent that is applied to the paper also acts as the extraction solvent to extract drugs and other compounds from the dried sample (figure 2)
- Applied Voltage = 3500 to 5000V
- Typical solvents:
 - Methanol or acetonitrile with a small amount of water (<10% usually)
 - Chloroform or dichloromethane can be added to the spray solvent to extract more hydrophobic analytes
 - Addition of acetic or formic acid (~0.01%) improves ionization
 - Sodium or ammonium acetate can be substituted for the acid if adduct formation is desired rather than protonation

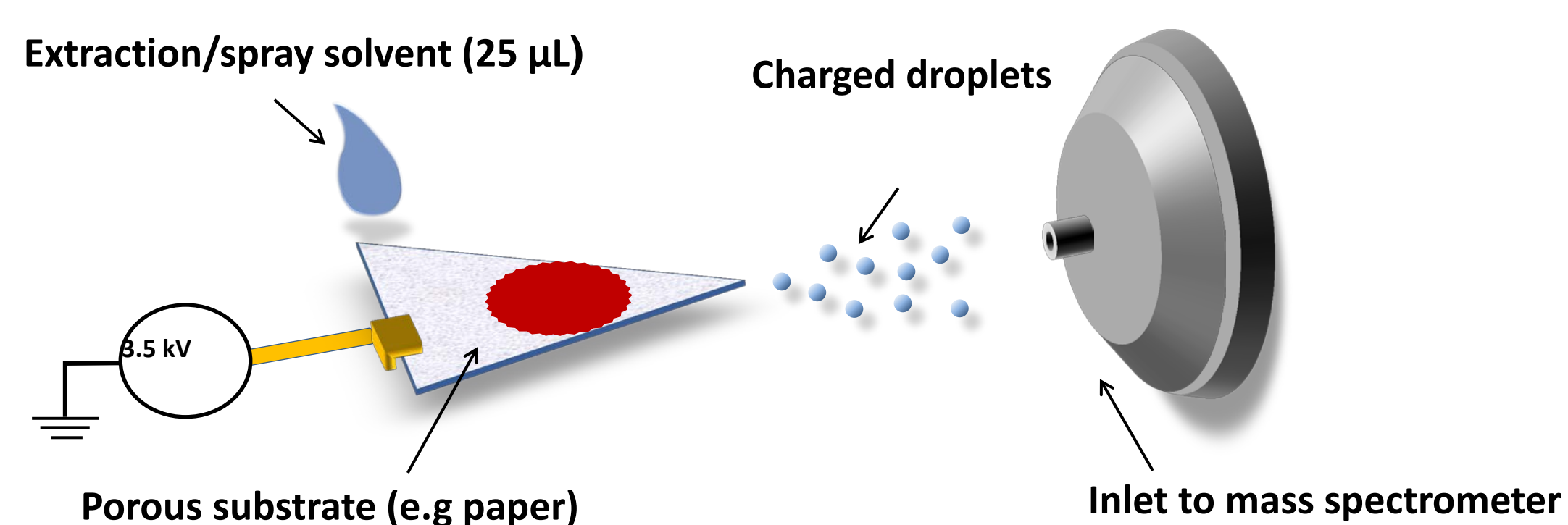
Figure 1. Picture of cone-jet generated from paper



Figure 3. Picture of an automated paper spray MS attachment and the disposable paper spray cartridge

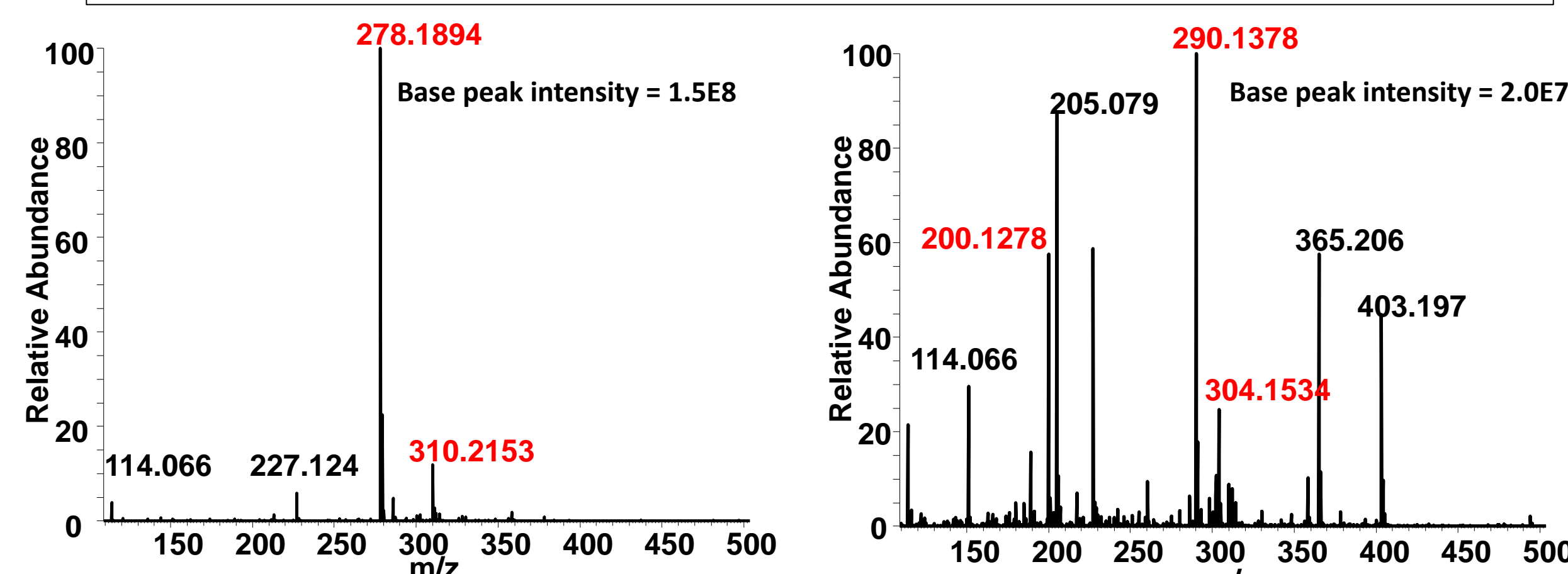


Figure 2. Schematic of paper spray analysis of a dried blood spot



Experiment 1: Drug Screening in Urine using Paper Spray on an Exactive

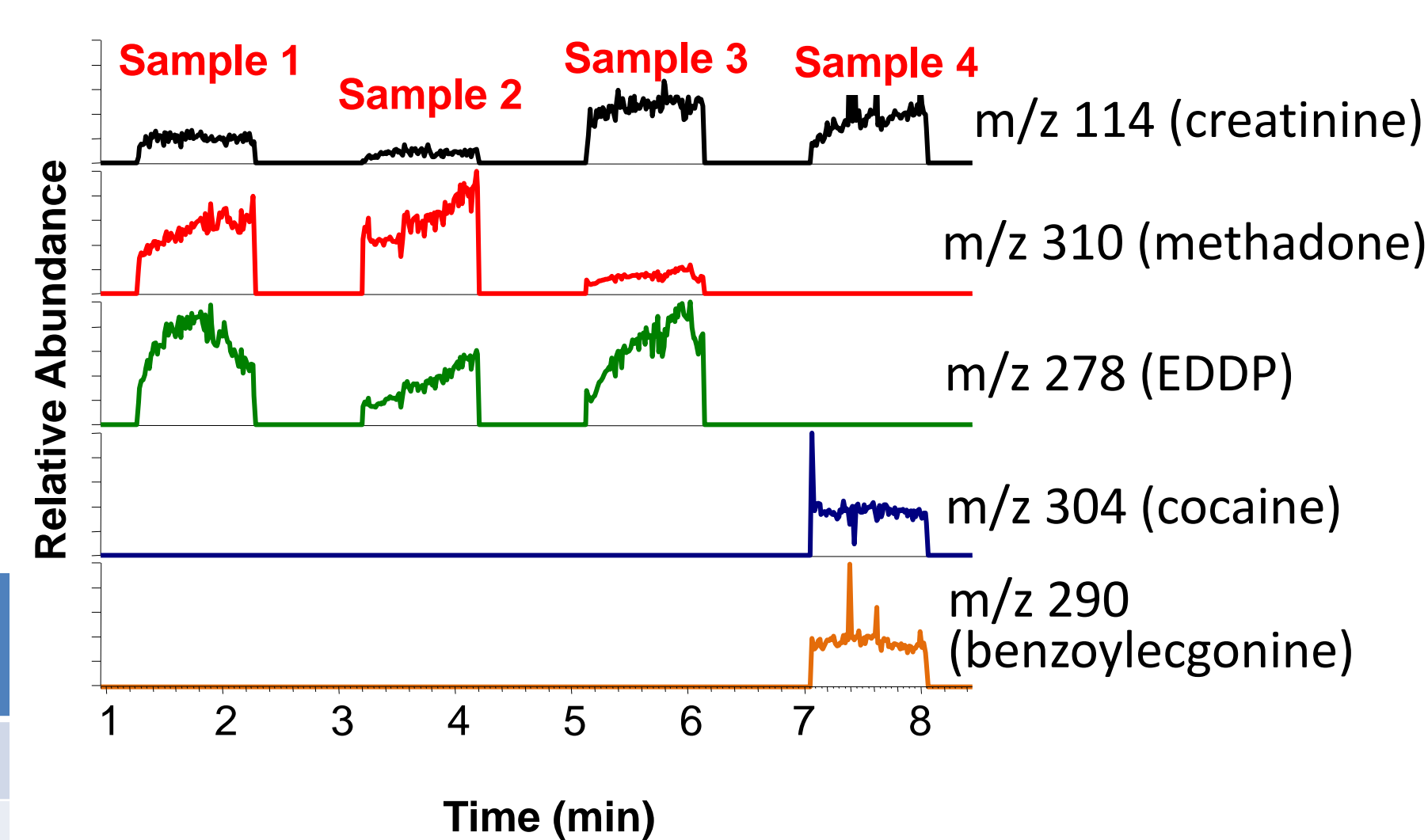
Figure 4. Two spectra obtained from paper spray MS of urine samples obtained from a methadone clinic. Analysis was performed on a Thermo Exactive MS



Compound	Theoretical m/z (M+H) ⁺
EDDP	278.1903
Methadone	310.2165

Compound	Theoretical m/z (M+H) ⁺
Ecgonine methyl ester	200.1281
Benzoyllecgonine	290.1387
Cocaine	304.1543

Figure 5. Extracted ion chromatograms (5ppm window) for several compounds detected from the urine. Four samples are shown on each chromatogram



FAIMS Separation with Lower Field Electrodes

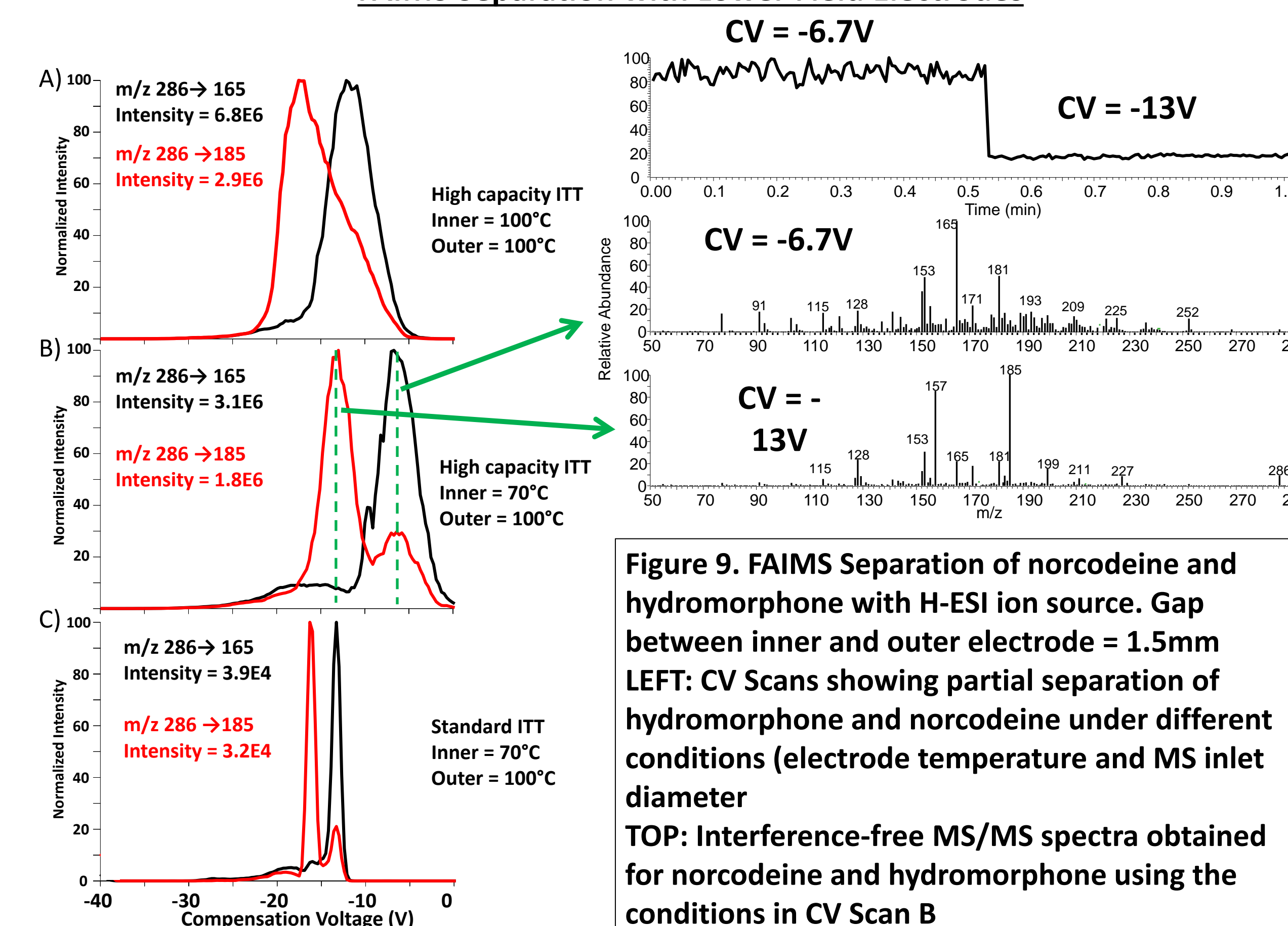


Figure 9. FAIMS Separation of norcodeine and hydromorphone with H-ESI ion source. Gap between inner and outer electrode = 1.5mm
LEFT: CV Scans showing partial separation of hydromorphone and norcodeine under different conditions (electrode temperature and MS inlet diameter)
TOP: Interference-free MS/MS spectra obtained for norcodeine and hydromorphone using the conditions in CV Scan B

FAIMS Separation with Higher Field Electrodes

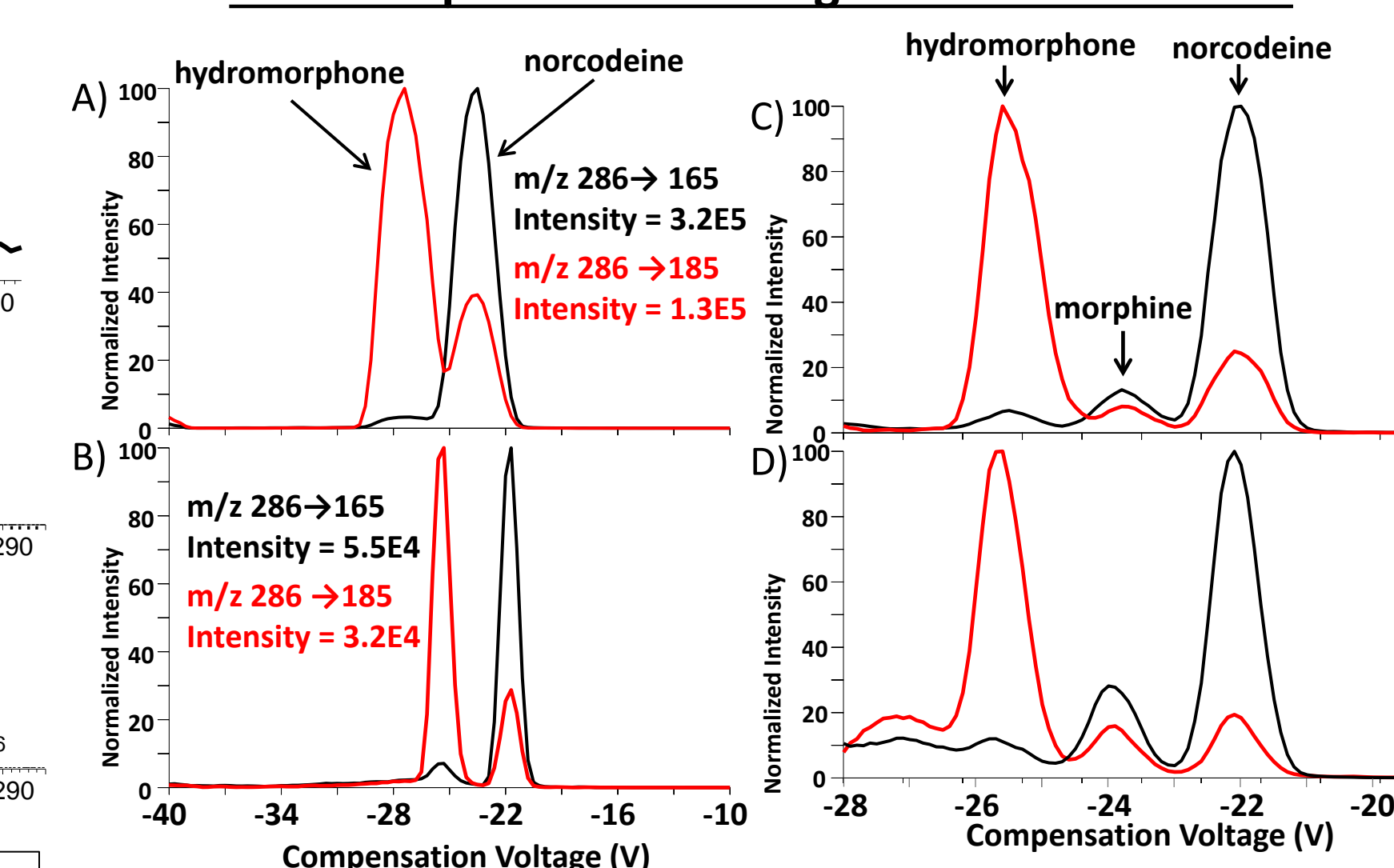


Figure 10. Separation of hydromorphone and norcodeine using the higher field electrodes (electrode gap = 1.25 mm) using the H-ESI ion source. A: both electrodes at 100°C. B: inner electrode decreased to 70°C
C: Three opiate mixture analyzed by H-ESI using the same conditions as B
D: Paper spray FAIMS separation of the three opiate mixture
Red line: m/z 286 → 185; black line: m/z 286 → 165