Reducing Matrix Effects in Biological Samples Using Thermal Gradient Focusing Extractive Electrospray Ionization

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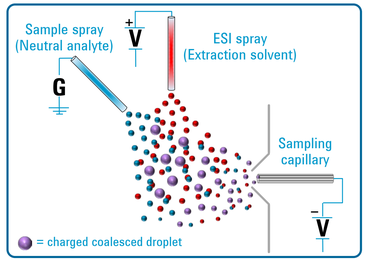
Introduction

The analysis of drugs and related compounds in biological fluids using LC/MS/MS has become an essential technique in the pharmaceutical industry. However, the suppression or enhancement of analyte signals due to endogenous matrix interferences can adversely affect quantitative results. This is especially true with protein precipitation protocols (PPT) that fail to remove phospholipids, a major source of matrix effects in plasma. Although sample dilution (by 10-fold or more) is generally effective at minimizing matrix effects, this approach is constrained by analyte detection limits.

Extractive Electrospray Ionization (EESI) is an indirect electrospray process where analytes are first aerosolized, then ionized by collision/fusion with charged droplets generated by an auxiliary ESI spray of pure solvent. EESI is highly tolerant of sample matrices, but applications to date have been limited to capillary flow conditions[1]. In this study, we compare the performance of a thermal gradient focusing extractive electrospray source (TGF-EESI) with a 10-fold sample dilution protocol for the analysis of forensic drugs in biological matrices at LC flows up to 0.5 mL/min.



Experimental

Sample Preparation

The Agilent LC/MS Toxicology Test Mixture (5190-0470) is a mixture of 26 compounds from a range of forensic drug classes, including amphetamines, opiates, benzodiazepines and others at 1 µg/mL analyte concentration in methanol. This standard was used to prepare sample concentrations at 10, 50, 100, 500, 1000, 5000, and 10000 fg/µL in both pure solvent (30% methanol) and precipitated human plasma.

Experimental

Sample Preparation, continued

The following PPT protocol was used to prepare standards for the matrix effect study: 100 µL of human plasma was precipitated with 300 µL acetonitrile, then centrifuged at 16,000 x g for 10 minutes. 200 µL of the supernatant was diluted with 600 µL water, spiked with the forensic drug mixture, and diluted to 1000 µL with 30% methanol to the desired sample concentration.

1290 UHPLC Conditions

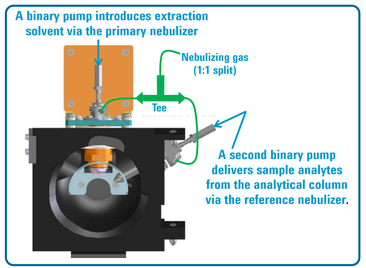
Mobile phases (Analytical Pump):
A = 0.01% formic acid + 5mM NH_4COOH in Water
B = 0.01% formic acid + 5mM NH_4COOH in Methanol
Gradient: 10%B at 0 min, 15%B at 0.5 min, 50%B at 3 min, 98%B at 4 to 6 min, 10%B at 6.1 min; Stop time: 8 min
Flow rate: 0.5 mL/min

Column: Agilent ZORBAX RRHD Eclipse Plus C18
2.1 x 100 mm, 1.8 µm (P.N. 959758-902)
Column temperature: 60 °C
Injection volume: 1 µL

Mobile phases (Extraction Solvent Pump):
A = 0.1% acetic acid in Water
B = 0.1% acetic acid in Methanol
Isocratic: 10%A: 90%B at 0 min; Stop time: 8 min
Flow rate: 0.5 mL/min

TGF-EESI Source Configuration

A dual-spray Agilent Jet Stream (AJS-ES) source was configured for TGF-EESI use by introducing analytes through the reference nebulizer and using the primary nebulizer to introduce the extraction solvent. The reference nebulizer and tee were removed for standard AJS-ES use.



Experimental

6490 QQQ MS Conditions

(AJS-ES Conditions)
Nebulizer pressure: 20 psi
Sheath gas: 12 L/min @ 400 °C
Drying Gas: 16 L/min @ 220 °C
Capillary voltage: 2800 V (+); 2500 V (–)
Nozzle voltage: 0 V (+); 0 V (–)

(TGF-EESI Conditions)
Primary nebulizer pressure: 25 psi
Reference nebulizer pressure: 25 psi
Sheath gas: 12 L/min @ 400 °C
Drying gas: 16 L/min @ 220 °C
Capillary voltage: 2800 V (+); 2500 V (–)
Nozzle voltage: 1500 V (+); 2000 V (–)

Dynamic MRM: 1 Quantifier + 1 Qualifier per analyte
Polarity Switching Delay: 50 msec
Fragmentor: 380 V
Delta EMV: 200 V
Resolution: MS1 (Low); MS2 (Low)
Collision cell acc: 4V

Experimental Setup

After establishing the LC/MS gradient method conditions using Agilent publication 5990-4265EN a starting point[2], precursor ion scans (m/z 184) and neutral loss scans (m/z 141) were performed using blank PPT human plasma matrix to identify retention time of various phospholipid species.

Quantitative ME [%] calculations using AJS-ES conditions were determined by comparing area responses of the toxicology test mixture spiked in pure solvent, in undiluted post-PPT human plasma, and with 10-fold dilution of the spiked plasma PPT samples with n = 5 replicates for each level.

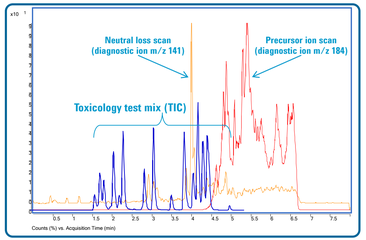
Quantitative ME [%] calculations using TGF-EESI conditions were determined by comparing area responses of the toxicology test mixture spiked in pure solvent and undiluted human plasma PPT with n = 5 replicates for each level.

ME [%] = [(avg. area response in post-PPT spiked plasma/ avg. area response in solvents) – 1] x 100

ME [%] > 0: Ionization Enhancement
ME [%] < 0: Ionization Suppression
ME [%] ± 15% is considered acceptable

Results and Discussion

The precursor ion scan indicates that the bulk of the phospholipids elute after the toxicology test mixture compounds. However, the neutral loss scan indicates potential matrix interference peaks between 2.9 and 3.4 minutes that coincide with the elution times of meperidine and PCP. Additional matrix peaks from 3.8 minutes onward coelute with a number of compound classes, including benzodiazepines, opiates, proadifen and d9-THC.

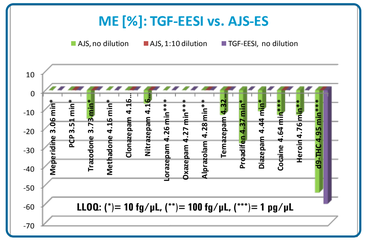


In all, 15 compounds in the toxicology test mixture were found to elute with phospholipids in PPT human plasma and were selected to study the utility of TGF-EESI for reducing matrix effects at narrow-bore chromatographic flow rates. TGF-EESI performance was compared with results obtained using a 10-fold sample dilution protocol as well as undiluted samples using AJS-ES. In addition to matrix effects, the TGF-EESI approach was also evaluated for response, reproducibility, and signal-to-noise.

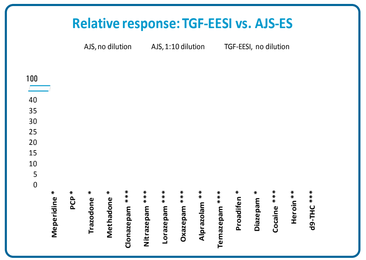
Compound	RT (min)	Precursor (m/z)	Quantifier (m/z)	Qualifier (m/z)
Meperidine	3.06	248.2	220.1	174.1
PCP	3.51	244.2	91.0	86.1
Trazodone	3.73	372.2	176.0	148.0
Methadone	4.16	310.2	265.1	105.0
Clonazepam	4.16	316.0	270.0	214.0
Nitrazepam	4.16	282.1	236.1	180.0
Lorazepam	4.26	321.0	275.0	194.0
Oxazepam	4.27	287.0	269.0	241.0
Alprazolam	4.28	309.1	281.0	205.0
Temazepam	4.32	301.1	255.1	177.0
Proadifen	4.37	354.2	167.0	91.1
Diazepam	4.44	285.1	193.0	154.0
Cocaine	4.64	304.2	182.1	77.0
Heroin	4.76	370.2	165.0	58.1
d9-THC	4.95	315.2	193.2	123.3

Results and Discussion

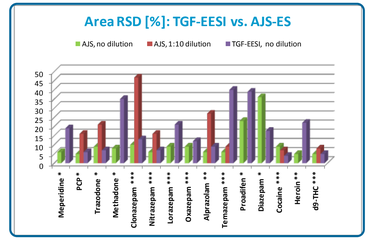
Of the 15 compounds selected for evaluation 8 were found to exhibit some matrix effect with undiluted samples using AJS-ES. However, only Proadifen and delta-9-THC exceed the 15% acceptability limit. Matrix effects were eliminated using a 10-fold sample dilution, but only 8 of the compounds were detectable at the LLOQ used for the study. All compounds were detected using TGF-EESI, and matrix effects were eliminated for all samples except delta-9-THC.



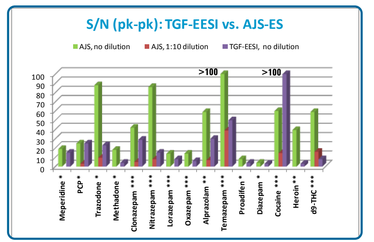
Relative (peak area) response using TGF-EESI was compared with AJS-ES using undiluted and 10-fold diluted samples in PPT human plasma. Normalized peak area responses for the 10-fold sample dilutions were approximately 10% of the AJS-ES peak area responses for undiluted samples that did not exhibit matrix suppression, and correspondingly higher when matrix suppression was eliminated by sample dilution. By contrast, it is clear that TGF-EESI response characteristics are different than AJS-ES with sample dilution. Normalized peak area responses for the TGF-EESI data range from 1% for delta-9-THC to 30% for PCP and Proadifen, with an average relative response of 15% for all analytes.



With the exception of temazepam, TGF-EESI yielded better signal reproducibility than using a 10-fold sample dilution with AJS-ES.



A comparison of analyte signal-to-noise (S/N) values yielded similar results. TGF-EESI yielded better S/N performance for all analytes except delta-9-THC when compared with a 10-fold sample dilution using AJS-ES.



Conclusions

A dual-spray Agilent Jet Stream (AJS-ES) source was successfully configured for TGF-EESI use for the analysis of forensic drugs in biological matrices at 0.5 mL/min. The TGF-EESI configuration eliminated matrix effects for all analytes except delta-9-THC. The TGF-EESI configuration also exhibited better analyte response, better reproducibility and an average of 3.5x better signal-to-noise than diluting samples 10-fold prior to analysis with AJS-ES.

[1] H. W. Chen, A. Venter and R. G. Cooks, Chem. Commun., 2006, 2042–2044.

[2] "Agilent G1734AA MassHunter Forensics and Toxicology Dynamic MRM Database Kit Quick Start Guide." Agilent Technologies publication 5990-4265EN