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Evaluation of Potential Drug Interference during LDTD-MS/MS analysis

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OVERVIEW

Purpose
- Evaluation of the potential for signal interference during drug quantification

Method
- Different active molecules are spiked into human plasma batches
- Six different potential interference drugs are added in a QC sample
- Different extraction techniques are used

Results
- Excellent linearity over the calibration range ($R^2 > 0.99$)
- Excellent accuracy ranging from 85.5 and 110.4% using area ratio values
- Excellent precision ranging from 0.5 and 18.5 % using area ratio values
- No interference from potential interfering drugs (Caffeine, Ibuprofen, Nicotine, Ethanol, Warfarin, Acetaminophen)
- **All those samples are analyzed with a run time of 9.6 seconds sample-to-sample using LDTD-MS/MS technique**

INTRODUCTION

The Laser Diode Thermal Desorption is a rapid analysis approach in which samples are thermally desorbed by a laser diode. Molecules are channeled, using a carrier gas, to a corona discharge region for ionization prior to detection via a mass spectrometer. Compounds of interest as well as other potential drug interfering compounds are desorbed at the same time during analysis. To verify the cross-talk effect, different potentially interfering molecules (Caffeine, Ibuprofen, Nicotine, Warfarin and Acetaminophen) were spiked at the maximum expected concentration in QC plasma sample of a drug of interest (Dextrorphan). All QCs are compared to the reference to evaluate the potential effect.

METHOD

Procedure 1 (Dextrorphan: Protein Precip)
- 25 µL Plasma (Spiked with Dextrorphan)
- 50 µL Internal standard (25 ng/ml Dext-d3 in Acetonitrile)
  - Vortex 30 seconds
- 25 µL NaCl (sat in water)
  - Vortex 30 seconds
- Centrifuge 14000 rpm / 2 minutes
- Spot 2 µL of upper phase in LazWell plate.

Procedure 2 (Dextrorphan: Liq-Liq)
- 25 µL Plasma (Spiked with Dextrorphan)
- 10 µL Internal standard (25 ng/ml Dext-d3 in Acetonitrile)
- 25 µL NaOH (0.1N) in water
  - Vortex 30 seconds
- 100 µL Ethyl acetate
  - Vortex 30 seconds
- Centrifuge 14000 rpm / 2 minutes
- Spot 5 µL of upper phase in LazWell plate.

Procedure 3 (Dextrorphan: SPE)
Cartridge: Waters, Oasis HLB (1cc)
- Activation: 0.5 ml MeOH, 0.5 ml NaOH (0.01N in water)
- Load: Mixture
  - 50 µL Plasma (Spiked with Dextrorphan)
  - 20 µL Internal standard (25 ng/ml Dext-d3 in Acetonitrile)
  - 100 µL NaOH (0.1N) in water
- Wash: 1 ml (MeOH/Water/NaOH(0.1N) : 50/50/10)
- Elution: 1 ml MeOH (Evaporate to dryness)
  - Reconstitute: 100 µL MeOH/Water (75/25)
- Spot 2 µL in LazWell plate.

Procedure 4 (PCP: Liq-Liq)
- 100 µL Plasma (Spiked with PCP)
- 20 µL Internal standard (250 ng/ml PCP-d5 in Methanol)
- 100 µL NaOH (0.1N) in water
  - Vortex 30 seconds
- 600 µL 1-Chlorobutane
  - Vortex 30 seconds
- Centrifuge 3500 rpm / 5 minutes
- Transfer 400 µL of upper phase
  - Add 20 µL HCl (0.1N)
  - Evaporate to dryness
- REC with 40 µL Methanol/Water (75/25)+ HCl (0.1N)
- Spot 5 µL in LazWell plate.

Procedure 5 (Dextromethorphan: Liq-Liq)
- 100 µL Plasma (Spiked with Dextromethorphan)
- 20 µL Internal standard (100 ng/ml Dext-d3 in Methanol)
- 100 µL NaOH (0.1N) in water
  - Vortex 30 seconds
- 600 µL 1-Chlorobutane
  - Vortex 30 seconds and Centrifuge 14000 rpm / 4 minutes
- Transfer 400µL organic phase and add 20µL HCl(0.1N)
- Spot 5 µL in LazWell plate.

Instrumentation
- LDTD model T-960, Phytronix Technologies
- TSQ® Vantage, Thermo Fisher Scientific

	LDTD Parameters		Mass Spectra Parameters			
	Laser pattern*	Carrier gas flow	Mode	S-Lens	CE	Transition (SRM)
Procedure 1	3-35-2	3 L/min (Air)	APCI (+)	95	25	Dextrorphan: 258 -> 199 Dextrorphan-d3: 258 ->199
Procedure 2	3-45-2	3 L/min (Air)	APCI (+)	95	25	Dextrorphan: 258 -> 199 Dextrorphan-d3: 258 ->199
Procedure 3	3-45-0	3 L/min (Air)	APCI (+)	95	25	Dextrorphan: 258 -> 199 Dextrorphan-d3: 258 ->199
Procedure 4	3-45-2	3 L/min (Air)	APCI (+)	50	15	PCP: 244 -> 159 PCP-d5: 249 -> 164
Procedure 5	3-45-2	3 L/min (Air)	APCI (+)	105	35	Dextromethorphan: 272 -> 147 Dextromethorphan-d3: 275 -> 147

Table 1 LDTD and Mass spectra Parameter

*Laser pattern
-First number: Slope time to reach the maximum power
-Second number: Maximum Laser Power value
-Third number: Time of Maximum Laser Power plateau

RESULTS:

Potentiel Drug identification		Plasma conc.
Caffeine	Caf-L	6 µg/ml
	Caf-H	60 µg/ml
Ibuprofen	Ibu-L	35 µg/ml
	Ibu-H	350 µg/ml
Nicotine	Nic-L	30 ng/ml
	Nic-H	300 ng/ml
Ethanol	EtOH-L	5 µg/ml
	EtOH-H	50 µg/ml
Warfarin	War-L	340 ng/ml
	War-H	3400 ng/ml
Acetaminophen	Acet-L	150 µg/ml
	Acet-H	1500µg/ml

Table 2 Final concentration value of Potential Drug interference in QC sample

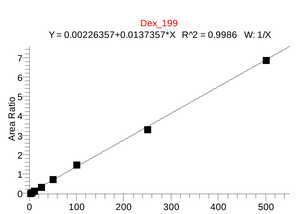

Figure 1 Typical standard curve for drug quantification

	Caf-L	Caf-H	Ibu-L	Ibu-H	Nic-L	Nic-H	EtOH-L	EtOH-H	War-L	War-H	Acet-L	Acet-H
Nominal conc. (ng/ml)	95	95	95	95	95	95	95	95	95	95	95	95
N	6	6	6	6	6	6	6	6	6	6	6	6
Mean (ng/ml)	103.6	100.5	99.7	104.9	100.5	96.8	96.3	92.9	104.3	98.5	98.2	91.7
RSD (%)	1.5	4.5	1.4	2.7	2.0	1.3	6.1	1.6	9.9	1.7	1.6	2.9
%Nom. conc.	109.0	105.8	104.9	110.4	105.8	101.9	101.3	97.8	109.7	103.7	103.3	96.5

Table 3 Procedure 1 (Dextrorphan: Protein Precipitation)

	Caf-L	Caf-H	Ibu-L	Ibu-H	Nic-L	Nic-H	EtOH-L	EtOH-H	War-L	War-H	Acet-L	Acet-H
Nominal conc. (ng/ml)	95	95	95	95	95	95	95	95	95	95	95	95
N	6	6	6	6	6	6	6	6	6	6	6	6
Mean (ng/ml)	90.0	96.5	92.5	92.6	90.3	93.1	95.7	89.9	90.9	95.4	103.2	98.5
RSD (%)	2.5	3.9	6.3	6.4	2.6	5.9	6.1	5.1	4.6	12.4	4.8	6.0
%Nom. conc.	94.7	101.6	97.4	97.5	95.1	98.0	100.7	94.6	95.7	100.5	108.6	103.7

Table 4 Procedure 2 (Dextrorphan: Liq-Liq)

	Caf-L	Caf-H	Ibu-L	Ibu-H	Nic-L	Nic-H	EtOH-L	EtOH-H	War-L	War-H	Acet-L	Acet-H
Nominal conc. (ng/ml)	95	95	95	95	95	95	95	95	95	95	95	95
N	6	6	6	6	6	6	6	6	6	6	6	6
Mean (ng/ml)	88.2	87.3	90.4	95.3	89.3	82.7	89.3	101.4	98.3	96.8	81.3	86.8
RSD (%)	2.2	2.9	5.4	7.3	4.6	7.8	9.8	2.4	3.2	2.3	3.8	4.0
%Nom. conc.	92.9	91.9	95.2	100.3	94.0	87.0	94.0	106.8	103.5	101.9	85.6	91.4

Table 5 Procedure 3 (Dextrorphan: SPE)

	Caf-L	Caf-H	Ibu-L	Ibu-H	Nic-L	Nic-H	EtOH-L	EtOH-H	War-L	War-H	Acet-L	Acet-H
Nominal conc. (ng/ml)	98	98	98	98	98	98	98	98	98	98	98	98
N	3	3	3	3	3	3	3	3	3	3	3	3
Mean (ng/ml)	101.7	90.6	99.9	104.7	102.4	102.8	108.2	94.8	100.6	106.5	95.8	99.3
RSD (%)	0.5	18.1	8.1	9.2	5.3	7.1	4.4	4.0	7.1	9.2	11.3	4.5
%Nom. conc.	103.8	92.5	101.9	106.8	104.5	104.9	110.4	96.8	102.6	108.6	97.7	101.3

Table 6 Procedure 4 (PCP: Liq-Liq)

	Caf-L	Caf-H	Ibu-L	Ibu-H	Nic-L	Nic-H	EtOH-L	EtOH-H	War-L	War-H	Acet-L	Acet-H
Nominal conc. (ng/ml)	98	98	98	98	98	98	98	98	98	98	98	98
N	3	3	3	3	3	3	3	3	3	3	3	3
Mean (ng/ml)	95.1	94.4	96.8	93.1	95.5	92.3	96.5	93.6	93.4	91.6	94.7	95.0
RSD (%)	2.7	11.6	6.7	6.8	1.6	5.4	5.4	1.6	8.8	3.2	5.4	2.9
%Nom. conc.	97.1	96.3	98.8	95.0	97.5	94.2	98.5	95.5	95.3	93.5	96.6	96.9

Table 7 Procedure 5 (Dextromethorphan: Liq-Liq)

CONCLUSIONS

- No interference is observed with common drugs at C_{max} value or even 10 times C_{max} value during the molecule quantitation
- No interference is observed with different extraction techniques for multiple molecules.
- Similar results are obtained with Methadone and EDDP in plasma. Results not shown here.
- LDTD provides specific High-Throughput analysis of sample extract in **9.6 seconds sample-to-sample**