Strategies for Extraction of Drugs from Whole Blood

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Introduction

Whole blood is often the specimen of choice for detection of drugs in the forensic laboratory. Samples are typically extracted to remove interferences and isolate the analytes of interest prior to GC-MS or LC-MS/MS analysis. Sample clean-up reduces interferences that can suppress MS response and decrease column lifetime or clog chromatography instrumentation. Here two "pass through" solid phase extraction techniques (protein removal/phospholipid depletion and dual mode extraction) and supported liquid extraction were evaluated for a group of about 100 compounds in whole blood. These DOA compounds included drugs from multiple drug classes: anticonvulsants, barbiturates, benzodiazepines, opioids, antidepressants, stimulants, hallucinogens, cannabinoids and antipsychotics.

Methods

All standards were purchased from Cerilliant (Round Rock, TX). HPLC grade water, methanol (MeOH), dichloromethane (DCM), formic acid (FA), ammonium hydroxide (NH_4OH) methyl tert-butyl ether (MTBE), isopropanol (IPA), ethyl acetate (EtOAc) and acetonitrile (ACN) were purchased from Sigma Aldrich (St. Louis, MO). Biotage® ISOLUTE® HYDRO DME+ dual mode extraction plates (970-0400-PZ01), ISOLUTE® PLD+ protein removal/phospholipid depletion extraction plates (918-0050-P01) and ISOLUTE® SLE+ 400 µL supported liquid extraction plates (820-0400-P01) were used for the extractions. Samples were processed using a Biotage® PRESSURE+ 96 position positive pressure manifold (PPM-96), and a Biotage® SPE Dry 96 (SD-9300-DHS-NA). LC columns were generously provided by Restek.

Whole blood was provided by UTAK (Valencia, CA). Fortified samples were prepared at 100 ng/mL for all compounds. An unextracted no matrix standard prepared in reconstitution solvent was also prepared at the same concentration and analyzed to calculate process efficiency (1). After extraction, samples were analyzed by LC-MS/MS using a Nexera UPLC (Shimadzu, Kyoto Prefecture, Japan) and a SCIEX 5500 triple quadrupole mass spectrometer with Turbo Ionspray® Ion Interface (Sciex, Framingham, MA) in scheduled MRM mode.

Table 1. LC Parameters

Column	Restek Raptor Biphenyl 2.7 µm, 50 x 3.0 mm
MPA	0.1% Formic Acid (aq)
MPB	0.1% Formic Acid in MeOH
Flow Rate	0.45 mL/min
Column Temp	40 °C
Sample Temp	20 °C
Injection Volume	2.5 µL

The LC gradient started with 95% MPA and gradually decreased to 5% MPA over a 9.0 minute total run time.

Optimized source parameters are shown in Table 2 (sMRM transition parameters not shown, but available upon request). Retention window for sMRM was set at 45 seconds with a target scan time at 2.85 seconds.

Table 2. MS Parameters

Ionization Spray Voltage	+1500(V)	**CAD**	Medium
Source Temp	600 °C	**GS1**	50
Curtain	30 (V)	**GS2**	70

Extraction Protocols

Samples were extracted following the manufacturer's protocol. ISOLUTE® SLE+ samples were pretreated with 1% formic acid, water, or 0.5M NH_4OH prior to extraction. Samples were extracted and analyzed in triplicate and average values are reported. A negative control and an extraction blank were also extracted and analyzed.

DME+ Protocol

Prepared whole blood at 100 ng/mL
↓
Added 100 µL whole blood sample to DME+ plate
↓
Added 10 µL of formic acid to samples with FA
↓
Added 600 µL ACN
↓
Applied pressure 6-8 psi
↓
Dried eluent under N_2 at 40°C upper/50°C lower
↓
Reconstituted in 100 µL 90:10 MPA:MPB

PLD+ Protocol

Prepared whole blood at 100 ng/mL
↓
Added 400 µL ACN to PLD+ plate for samples without formic acid
↓
Added 400 µL ACN with 1% formic acid to PLD+ plate for samples with FA
↓
Added 100 µL of whole blood sample to PLD+ plate and mixed
↓
Applied positive pressure 6-8 psi
↓
Dried eluent under N_2 at 40°C upper/50°C lower
↓
Reconstituted in 100 µL 90:10 MPA:MPB

SLE+ Protocol

Prepared whole blood at 100 ng/mL
↓
Pretreated 150 µL of whole blood with 150 µL of pretreatment solution
↓
Loaded 300 µL total volume onto 400 µL SLE+ plate, waited 5 minutes
↓
Eluted with 2 x 800 µL elution solvent, waited 5 minutes after addition of each aliquot
↓
Dried eluent under N_2 at 40°C upper/50°C lower
↓
Reconstituted in 150 µL of 90:10 MPA:MPB

Results

Results for selected drug classes are in Figures 1 and 2.

Figure 1. Process Efficiency for PLD+ and DME+

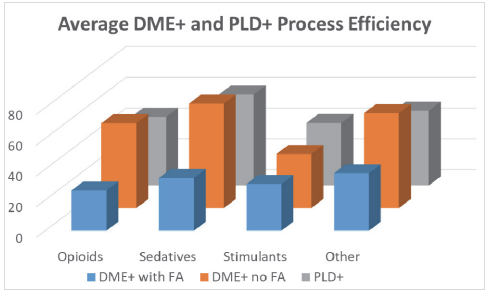
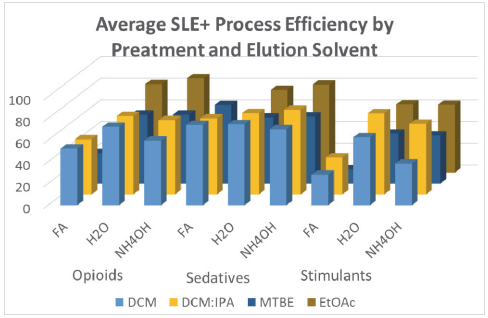


Figure 2. Process Efficiency SLE+



Opioids included 6-AM, buprenorphine, codeine, EDDP, fentanyl, hydrocodone, hydromorphone, meperidine, methadone, morphine, naloxone, norbuprenorphine, norfentanyl, oxycodone, oxymorphone, tapentadol, tramadol. Sedatives consisted of 7-aminoclozepam, alprazolam, clonazepam, diazepam, lorazepam, midazolam, nordiazepam, oxazepam, phenobarbital, secobarbital, temazepam, and triazolam. Stimulants were comprised of amphetamine, benzoylecgonine, cocaethylene, cocaine, mCPP, MDA, MDEA, MDMA, methamphetamine, methcathinone, and ritalinic acid. "Other" was a selection from the remaining drug classes: amitriptyline, aripiprazole, atomoxetine, bupropion, buspirone, carbamazepine, carisoprodol, chlorpheniramine, chlorpromazine, clomipramine, clonidine, clozapine, cyclobenzaprine, dextromethorphan, duloxetine, fluoxetine, haloperidol, imipramine, ketamine, lamotrigine, levetiracetam, lidocaine, meprobamate, methaqualone, mirtazapine, nortriptyline, olanzapine, oxcarbazepine, paroxetine, phenytoin, quetiapine, risperidone, sertraline, topiramate, trazodone, trimipramine and venlafaxine.

The whole blood used in this study contained high amounts of gabapentin, so process efficiency could not be determined.

Results (continued)

Table 3. Process Efficiency Data for DME+ and PLD+

Value	Opioids			Sedatives			Stimulants			Other		
	DME+	DME+ FA	PLD+	DME+	DME+ FA	PLD+	DME+	DME+ FA	PLD+	DME+	DME+ FA	PLD+
min	31.8	2.1	16.7	13.8	0.7	30.3	0.1	1.8	20.9	20.9	0.1	14.2
max	99.6	89.1	87.1	129.7	129.7	129.7	81.3	62.8	74.3	87.7	69.5	74.9
average	55.2	26.2	44.6	67.9	34.4	51.1	35.2	30.4	40.6	61.7	37.4	48.6
median	53.9	24.6	41.2	62.3	34.9	52.6	32.0	27.9	38.1	62.4	37.6	50.3

Table 4. Process Efficiency Data for SLE+

Pre-treatment	Value	Opioids				Sedatives			
		DCM	DCM:IPA	MTBE	EtOAc	DCM	DCM:IPA	MTBE	EtOAc
FA	min	3.2	2.5	1.7	-	27.9	26.9	39.5	-
	max	94.5	101.1	92.5	-	92.1	94.1	91.2	-
	average	52.1	50.7	27.9	-	74.0	70.0	72.3	-
	median	65.9	53.3	13.7	-	76.3	75.2	73.9	-
H2O	min	23.0	17.4	17.4	59.0	62.4	61.1	50.3	64.3
	max	100.7	121.3	82.6	99.6	95.3	92.2	73.2	90.2
	average	72.2	72.1	63.3	81.5	74.6	74.8	60.8	76.1
	median	77.9	76.3	63.5	83.5	74.6	74.3	61.8	78.6
NH_4OH	min	20.2	33.2	17.4	44.8	7.7	59.6	50.7	66.6
	max	91.3	127.5	82.6	120.9	88.4	92.0	71.1	91.9
	average	59.6	68.4	63.3	87.0	70.0	77.8	61.6	81.1
	median	65.4	68.7	63.5	86.7	72.5	80.4	63.8	84.6
Pre-treatment	Value	Stimulants				Other			
		DCM	DCM:IPA	MTBE	EtOAc	DCM	DCM:IPA	MTBE	EtOAc
FA	min	0.0	2.8	0.0	-	41.2	26.9	3.4	-
	max	94.6	93.3	60.4	-	100.5	115.3	103.7	-
	average	28.2	34.3	12.6	-	75.0	81.5	56.1	-
	median	14.2	19.3	4.4	-	80.0	82.0	63.3	-
H2O	min	0.0	4.8	0.0	0.4	20.4	31.5	2.4	29.2
	max	87.2	107.2	73.2	89.3	114.9	120.4	89.7	106.4
	average	62.6	74.6	45.9	62.7	78.7	76.6	65.0	79.4
	median	72.6	86.4	56.1	76.2	82.2	78.7	67.4	80.6
NH_4OH	min	0.0	3.6	0.0	0.3	12.1	25.0	2.7	24.6
	max	70.1	105.5	66.6	89.0	98.2	127.6	93.0	102.6
	average	38.4	65.0	44.2	62.4	69.9	75.2	66.6	81.0
	median	51.3	73.2	54.8	78.5	71.8	78.5	69.9	84.0

Samples pretreated with formic acid and extracted with PLD+ were dirty and not injected. Samples pretreated with formic acid and extracted using SLE+ and ethyl acetate were dirty and not injected.

Conclusions

- Acetonitrile with formic acid can't be used as an extraction solvent for whole blood on ISOLUTE® PLD+, but whole blood can be treated with formic acid prior to extraction using ISOLUTE® HYDRO DME+
- Ritalinic acid and pregabalin showed low recovery under all conditions, but were detected
- Best results for basic analytes were without formic acid on ISOLUTE® HYDRO DME+ and using NH_4OH pretreatment using ISOLUTE® SLE+
- Acidic drugs had the highest process efficiencies using formic acid pretreatment on ISOLUTE® HYDRO DME+ and ISOLUTE® SLE+
- Overall the best process efficiencies for most compounds used neutral or basic pretreatment and ISOLUTE® SLE+
- Minimal cleanup of whole blood with pass through SPE or SLE+ resulted in very clean samples and detection of most compounds

References

1. Matuszewski BK et al, *Anal. Chem.* 75, 3019-3030 (2003)

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