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Towards a Universal Bioanalytical Workflow for Volumetric Absorptive Microsampling: The Application of Impact-Assisted Extraction for the Elimination of Recovery Bias due to Blood HCT Exemplified in the Analysis of 16 Anti-Epileptic Drugs of Disparate logP

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OVERVIEW

PURPOSE

To demonstrate the applicability of Volumetric Absorptive Microsampling (VAMS) coupled with Impact-Assisted Extraction (IAE) for the elimination of HCT recovery bias in the determination of 16 anti-epileptic drugs (AEDs) using a single assay incorporating LC-sMRM with polarity cycling.

METHOD

Human blood was sampled onto a Mitra® microsampling device (10 µL), dried for 24 hours at RmT in the presence of desiccant, and extracted by IAE using 80% methanol. Data were acquired using a SCIEX Triple Quad 5500 operated in scheduled MRM (sMRM) mode whilst polarity cycling the ESI source.

RESULTS

The IAE method demonstrated excellent linearity for all 16 AEDs with intra- and inter-assay precision and accuracy meeting all acceptance criteria. Recoveries of AEDs of disparate logP (0.11 - 3.42) from dried blood on Mitra® substrate ranged from 84% to 99%, obtained without hematocrit bias.

INTRODUCTION

LC-MS assays for anti-epileptic drug (AED) panels are often required in clinical trials for new AEDs to ensure adherence to protocol inclusion/exclusion criteria, or for therapeutic drug monitoring to optimize and individualize combined AED therapies. In pediatric populations, current sampling is invasive with large volumes of blood typically drawn, making this procedure less attractive in clinical practice. Consequently, an alternative microsampling strategy is required for these applications.

Volumetric Absorptive Microsampling (VAMS) using the Mitra® device from Neoteryx represents a dried blood technique which eliminates sampling volume bias as a function of hematocrit level (HCT). However, several research laboratories have reported recovery bias from the Mitra® substrate due to blood HCT. Recently, we reported a novel approach to the elimination of this recovery bias using Impact-Assisted Extraction (IAE). In the current research, the applicability of VAMS coupled with IAE is investigated for the development of a single assay supporting the determination of 16 AEDs of at therapeutically relevant levels requiring only 10 µL of blood.

METHODS

SAMPLE PREPARATION

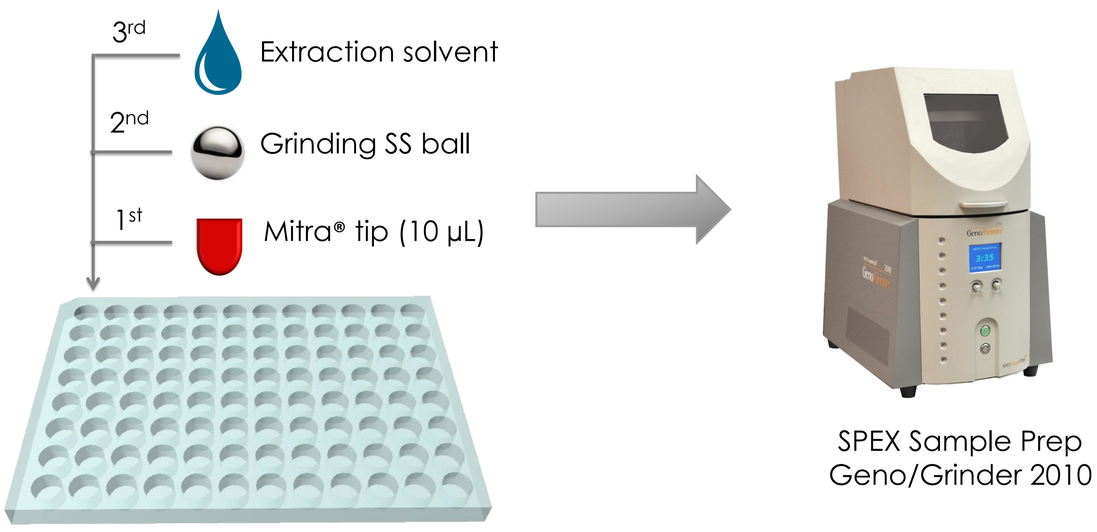

Figure 1. Order of addition and apparatus required for the Impact-Assisted Extraction (IAE) of AEDs from Mitra® substrate. Optimal extraction solvent was MeOH:H2O (4:1) used under IAE conditions of 1750 rpm for 10 minutes.

LC-MS/MS DETECTION

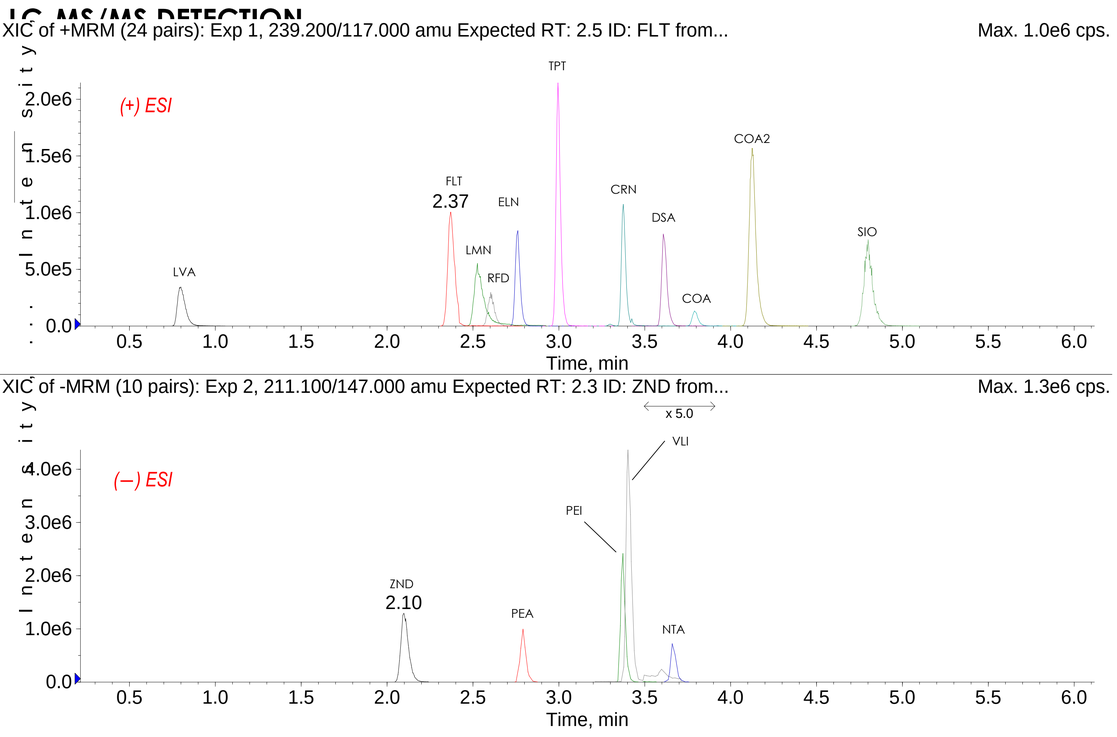

Figure 2. Overlaid XICs of 16 AEDs. Separation was achieved using a Synergi Polar RP column with ammonium acetate/MeCN mobile phase. AEDs were detected using a SCIEX TripleQuad 5500 operated in sMRM mode (30 sec window, 150 msec scan time) with dynamic polarity cycling from (-) 4.5 kV to (+) 5.5 kV.

AED	Abbrev.	LogP	MRM Transition	t_r (min)	Range (µg/mL)	r^2
Levatiracetam	LVA	0.59	171.1 > 126.1	0.80	0.200 – 20.0	0.9966
Zonisamide	*ZND*	*0.11*	*211.1 > 147.0*	*2.10*	*0.080 – 8.00*	*0.9989*
Felbamate	FLT	0.68	239.2 > 117.0	2.37	2.00 – 200.0	0.9927
Lamotrigine	LMN	1.43	256.0 > 211.0	2.53	0.160 – 16.0	0.9979
Rufinamide	RFD	1.93	239.3 > 221.8	2.60	0.075 – 7.50	0.9979
S-Licarbazepine	ELN	1.73	255.1 >194.4	2.76	0.200 – 20.0	0.9935
Phenobarbital	*PEA*	*2.14*	*231.1 > 42.0*	*2.79*	*0.080 – 8.00*	*0.9988*
Topiramate	TPT	0.13	357.3 > 264.1	3.00	0.200 – 20.0	0.9957
Carbamazepine	CRN	2.77	237.1 > 194.3	3.37	0.080 – 8.00	0.9973
Phenytoin	*PEI*	*3.40*	*251.1 > 101.9*	*3.37*	*0.080 – 8.00*	*0.9989*
Valproic Acid	*VLI*	*2.80*	*143.2 > 143.2*	*3.40*	*1.25 – 125.0*	*0.9942*
Desmethylclobazam	DSA	3.42	287.0 > 245.1	3.61	0.010 – 1.00	0.9990
Nitrazepam	*NTA*	*2.55*	*280.0 > 252.2*	*3.66*	*0.001 – 0.100*	*0.9912*
Clonazepam	COA	3.15	316.1 > 270.2	3.79	0.0025 - 0.250	0.9944
Clobazam	COA2	2.55	301.2 > 259.0	4.13	0.010 – 1.00	0.9978
Stiripentol	SIO	3.12	217.0 > 187.0	4.80	0.200 – 20.0	0.9972

RESULTS

RECOVERY

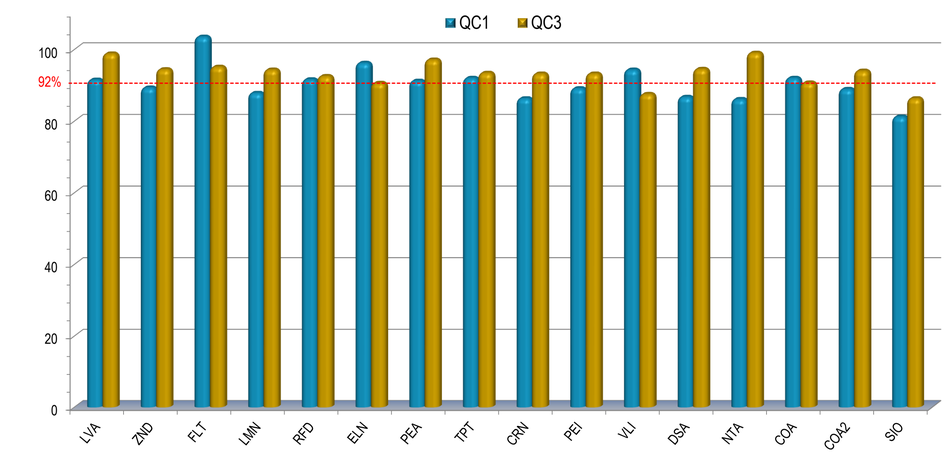

Figure 3. AED recovery determined from QC-1 and QC-3 concentrations with average recovery indicated in red. Despite the disparity in logP (0.11 – 3.4), the majority of AEDs were recovered in > 90% yield when using IAE with MeOH:H_2O (4:1).

HEMATOCRIT EFFECT

(3 replicates/ QC level/ HCT level)*

AED	QC-1				QC-3			
	HCT 30%		HCT 66%		HCT 30%		HCT 66%	
	CV [%]	Nominal [%]	CV [%]	Nominal [%]	CV [%]	Nominal [%]	CV [%]	Nominal [%]
Levatiracetam	3.6	103.3	5.1	106.7	4.1	109.7	5.4	105.2
Zonisamide	*1.9*	*103.2*	*3.9*	*100.8*	*4.0*	*109.7*	*8.4*	*105.7*
Felbamate	2.1	110.1	4.1	96.5	6.3	118.0	8.5	94.7
Lamotrigine	5.6	103.2	8.4	101.6	2.4	107.1	6.8	101.7
Rufinamide	1.5	100.6	5.8	107.9	6.7	100.3	9.7	105.1
S-Licarbazepine	9.1	105.4	8.7	106.9	6.9	109.0	4.7	100.2
Phenobarbital	*3.2*	*102.0*	*5.1*	*107.5*	*6.0*	*104.3*	*9.3*	*107.2*
Topiramate	2.8	104.6	4.2	109.3	6.2	106.2	12.0	111.0
Carbamazepine	0.4	106.5	6.3	107.8	7.3	98.8	6.4	98.4
Phenytoin	*2.2*	*104.5*	*2.2*	*108.4*	*7.4*	*106.6*	*8.8*	*106.1*
Valproic Acid	*6.9*	*98.9*	*2.6*	*103.2*	*5.9*	*101.0*	*2.1*	*95.4*
Desmethylclobazam	2.0	108.6	5.7	104.4	4.4	102.6	8.7	103.8
Nitrazepam	*0.9*	*101.0*	*8.2*	*101.3*	*3.2*	*96.9*	*10.7*	*97.8*
Clonazepam	7.0	109.0	3.4	111.3	4.1	106.1	6.9	104.0
Clobazam	1.3	105.1	4.0	111.7	4.8	105.5	6.3	103.3
Stiripentol	2.3	105.6	6.5	106.6	10.4	104.9	3.9	102.2

* back-calculated P&A data derived from calibrant curve prepared with 40% blood HCT

BETWEEN-RUN PRECISION AND ACCURACY

(*n* = 3 batches, 6 replicates/QC level; Blood HCT 40%)

AED	QC -LOQ		QC-1		QC-2		QC-3	
	CV [%]	Nominal [%]	CV [%]	Nominal [%]	CV [%]	Nominal [%]	CV [%]	Nominal [%]
Levatiracetam	5.1	104.1	6.0	102.5	3.9	103.7	4.7	104.5
Zonisamide	*7.3*	*102.8*	*5.7*	*102.6*	*3.0*	*101.3*	*4.8*	*102.9*
Felbamate	8.5	101.2	3.8	107.8	7.6	99.8	6.2	99.5
Lamotrigine	8.0	99.4	6.3	103.8	5.1	103.8	5.8	103.6
Rufinamide	9.0	102.9	5.2	103.5	4.3	102.4	6.9	103.1
S-Licarbazepine	9.7	97.5	7.9	105.6	5.2	101.6	7.5	100.5
Phenobarbital	*7.2*	*104.8*	*5.1*	*104.5*	*3.4*	*103.1*	*5.0*	*104.4*
Topiramate	6.1	102.8	7.7	100.7	4.1	100.5	5.4	103.0
Carbamazepine	8.7	99.5	6.5	106.5	5.0	101.3	6.8	99.5
Phenytoin	*8.1*	*102.2*	*6.0*	*102.7*	*4.1*	*102.5*	*4.8*	*104.3*
Valproic Acid	*11.4*	*99.3*	*5.6*	*100.4*	*4.3*	*101.6*	*5.1*	*96.5*
Desmethylclobazam	6.8	94.6	6.0	104.0	3.7	110.9	4.5	102.3
Nitrazepam	*7.7*	*99.0*	*6.1*	*102.3*	*4.7*	*101.8*	*5.5*	*97.0*
Clonazepam	11.0	98.3	9.2	103.3	3.0	104.8	4.9	103.2
Clobazam	6.1	100.9	5.7	103.3	3.3	102.7	4.0	103.4
Stiripentol	7.0	97.2	7.7	103.9	3.8	101.7	4.4	100.1

The combination of sMRM with rapid polarity switching allowed optimized ionization conditions for each AED, whilst furnishing a minimum of 18 scans across each chromatographic peak. This sampling frequency ensured precision and accuracy data met all acceptance criteria for a quantitation method supporting AED concentration ranges typical of circulating therapeutic levels. Further, high recovery by IAE coupled with TripleQuad 5500 sensitivity supported an extract dilution factor (80-fold) which facilitated a high-throughput "dilute-and-shoot" workflow. Six control donors evaluated for matrix effect and specificity met all acceptance criteria, as did precision and accuracy data for low and high QCs fortified at blood HCT levels of 30% and 66% quantified against a calibration curve prepared at 40% HCT. Notably, the successful quantitation of QCs at 30% and 66% HCT indicates an elimination of both sampling and recovery bias when coupling VAMS with IAE. Additionally, the ability to recover all 16 AEDs in high yield despite logP's ranging from 0.11 to 3.42 suggests IAE represents a universal sample preparation approach.

CONCLUSION

The research herein represents the first report exemplifying not only the application of VAMS to an extensive AED panel, but the successful extraction of such a large number of analytes with disparate logP from the Mitra® substrate. The ability to implement a single-assay as opposed to historical dual-assay approaches (one +ESI, the other –ESI) was feasible by continuous polarity switching at a rate supporting the sampling frequency required for reliable quantitation.

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