Efficacy of Sample Preparation Techniques on Whole Blood Prior to High Resolution Mass Spectrometry (HRMS) Drug Screening

Jessica Lynn Ayala*, M.S., D-ABFT-FT and Sarah Kerrigan, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

SH™ MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

SH™ MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

INTRODUCTION

The shift from immunoassay to mass spectrometry-based toxicological screening presents a number of challenges for operational laboratories. Sample preparation is just one of those challenges, because electrospray ionization (ESI) techniques are susceptible to matrix effects that can negatively impact assay performance. During toxicological screening, fast, efficient extraction protocols are vital. Supported liquid extraction (SLE) is an alternative to conventional solid phase or liquid/liquid extraction techniques. In this study, liquid chromatography-quadrupole/time-of-flight mass spectrometry (LC-Q/TOF MS) was used for toxicological screening of whole blood specimens. SLE in combination with protein precipitation and lipid removal were evaluated for Tier I and select Tier II drugs encountered in impaired driving investigations (1). Tier I drugs include the most commonly encountered compounds in impaired drivers, while Tier II drugs encompass more regional prevalence, and may require more advanced instrumentation for analysis.

Simultaneous mass-spectrometry based screening technologies for multiple drug classes (including cannabinoids) is still a challenge for most laboratories. In this preliminary evaluation of sample preparation techniques, compounds of forensic interest with diverse physicochemical properties were evaluated (**Table 1**). Incorporation of protein precipitation and the use of lipid removal devices prior to SLE was investigated.

MATERIALS AND METHODS

Tier I drugs at the recommended cutoffs, and other selected compounds from representative categories within Tier II were fortified into blood (**Table 1**). Acetaminophen and salicylate were included due to their importance in medicolegal death investigation. Prior to SLE, blood (0.5 mL) was subjected to i) no pre-sample clean-up (control), ii) protein precipitation (PPT), or iii) lipid removal (Agilent Captiva EMR-Lipid) using replicate analysis (n=3). Three solvents were selected for PPT (acetone, acetonitrile and methanol) using a 2:1 ratio of organic solvent:blood (with vortex mixing). Lipid removal devices were utilized with and without the use of 1% formic acid (as recommended by the manufacturer). Following completion of the sample pre-treatment step, 500 µL of 0.1% formic acid in water was added and 750 µL of sample was extracted using a 1 mL SLE column (Biotage Isolute SLE+).

After sample adsorption to the SLE sorbent, drugs were eluted with varying amounts of 90:10 dichloromethane/isopropanol and methyl-tert-butyl ether (MTBE). The extracts were then fortified with acidic methanol and evaporated to dryness under nitrogen at 50°C. Extracts were reconstituted in 20 µL of a 60:40 mixture of mobile phase A:B (5mM ammonium formate with 0.01% formic acid in water/0.01% formic acid in methanol). After centrifugation, the supernatant was transferred to a clean autosampler vial and analyzed in positive and negative ESI under All Ions acquisition using an Agilent 1290 Infinity II/ 6530 LC-Q/TOF-MS equipped with an Agilent Poroshell 120 EC-C18 (2.1 X 100mm, 2.7µm) column.

RESULTS

Table 1: Selected Drugs

Drug	Concentration (ng/mL)	Tier
7-Aminoclonazepam	10	I
α-Hydroxyalprazolam	10	I
Alprazolam	10	I
Amphetamine	20	I
Benzoylecgonine	50	I
Buprenorphine	1	I
Carisoprodol	500	I
Clonazepam	10	I
Diazepam	50	I
Fentanyl	1	I
Lorazepam	10	I
Methadone	50	I
Methamphetamine	20	I
Morphine	10	I
Nordiazepam	50	I
Oxazepam	50	I
Oxycodone	10	I
Temazepam	50	I
THC-COOH	10	I
Tramadol	100	I
Zolpidem	10	I
AB-CHMINACA	5	II
α-PVP	20	II
Amitriptyline	50	II
Diphenhydramine	20	II
Gabapentin	1000	II
Ketamine	20	II
LSD	5	II
PCP	20	II
Phenobarbital	100	II
Phenytoin	1000	II
Quetiapine	200	II
Sertraline	200	II
Valproic acid	50000	II
Acetaminophen	10000	
Salicylic acid	50000	

Drugs in **bold** and *italicized* were analyzed in Negative ESI.

Table 2: Normalized Analyte Response (N=3)

Drug	SLE only	Acetone	Acetonitrile	Methanol	EMR	EMR (1% FA)
7-Aminoclonazepam	100%	71%	140%	109%	97%	1%
AB-CHMINACA	100%	101%	95%	133%	125%	230%
Acetaminophen	100%	64%	65%	63%	65%	59%
Alprazolam	100%	59%	123%	117%	118%	128%
Amitriptyline	100%	72%	72%	48%	33%	12%
Amphetamine	100%	68%	34%	83%	62%	44%
Benzoylecgonine	100%	147%	183%	173%	142%	163%
Buprenorphine	100%	143%	101%	72%	74%	50%
Carisoprodol	100%	81%	27%	135%	87%	171%
Clonazepam	100%	42%	61%	63%	66%	37%
Diazepam	100%	84%	175%	103%	200%	160%
Diphenhydramine	100%	630%	647%	837%	427%	345%
Fentanyl	100%	54%	49%	50%	53%	22%
Gabapentin	100%	72%	130%	125%	131%	110%
Ketamine	100%	72%	97%	61%	109%	87%
Lorazepam	100%	56%	37%	38%	60%	27%
LSD	100%	26%	91%	38%	90%	73%
Methadone	100%	32%	291%	186%	153%	58%
Methamphetamine	100%	68%	64%	66%	68%	99%
Morphine	100%	94%	7%	67%	26%	35%
Nordiazepam	100%	59%	43%	57%	63%	63%
Oxazepam	100%	39%	82%	24%	87%	62%
Oxycodone	100%	68%	74%	93%	64%	47%
PCP	100%	116%	107%	73%	115%	128%
Phenobarbital	100%	78%	28%	49%	37%	23%
Phenytoin	100%	83%	86%	82%	88%	78%
Phenytoin	100%	137%	138%	166%	96%	146%
Quetiapine	100%	28%	30%	22%	26%	15%
Salicylic acid	100%	132%	134%	106%	139%	5%
Sertraline	100%	21%	26%	20%	53%	30%
Temazepam	100%	55%	157%	150%	268%	273%
THC-COOH	100%	0%	41%	103%	58%	8%
Tramadol	100%	276%	331%	119%	175%	189%
Valproic acid	100%	0%	50%	1%	4%	10%
Zolpidem	100%	69%	37%	50%	70%	54%
α-Hydroxyalprazolam	100%	46%	134%	70%	125%	99%
α-PVP	100%	155%	146%	113%	134%	120%

Figure 1: Effect of Solvent Protein Precipitation Coupled with SLE

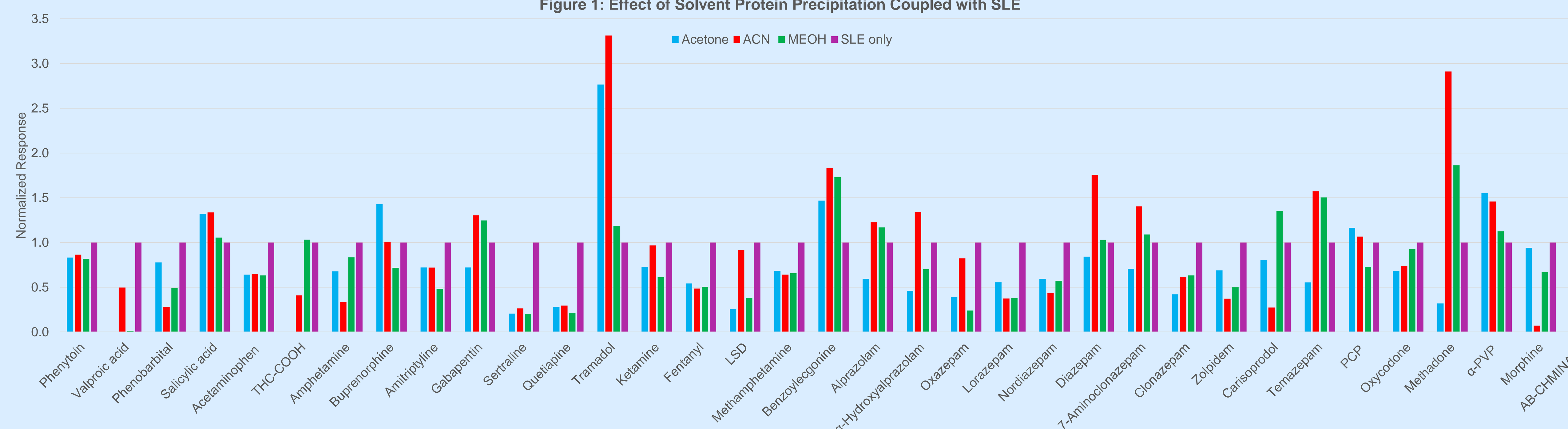
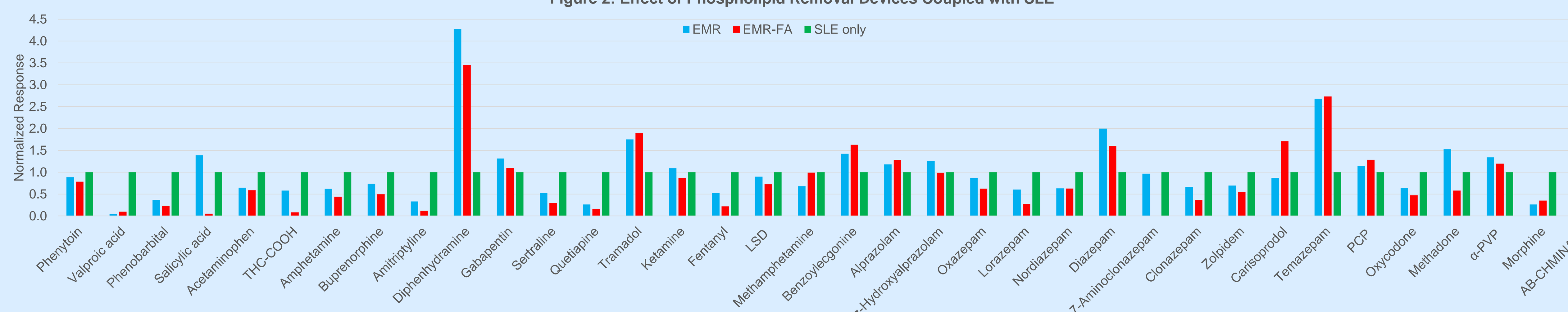


Figure 2: Effect of Phospholipid Removal Devices Coupled with SLE



DISCUSSION

Protein precipitation exhibited the greatest variability in analyte response for the acidic drugs (analyzed in negative ESI) when normalized to the control group. Analyte loss following SLE pre-treatment was particularly evident for 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) and valproic acid (VPA). VPA showed little to no recovery following sample preparation by protein precipitation or the lipid removal device. Simultaneous identification of low-concentration analytes with very different physicochemical properties is very challenging. Although additional sample preparation can reduce matrix effects and potentially improve signal, noticeable losses were observed for several drugs (**Figures 1 and 2**).

Decreases in signal intensity were also observed for some of the most challenging basic drugs, due to their low recommended cutoffs (e.g. fentanyl and buprenorphine at 1 ng/mL) (**Table 2**). Overall abundance was improved for several drugs following sample pre-treatment prior to SLE. However, this was accompanied by increased imprecision for some of the most challenging drugs (e.g. buprenorphine).

Use of formic acid during cartridge-based lipid removal requires careful consideration since it has the potential to negatively influence drug recovery for some compounds.

Although the SLE protocol was used to identify compounds at the recommended cutoff concentrations, optimum conditions for elution and overall robustness (including lot-to-lot variability) were not investigated.

CONCLUSIONS

Although additional sample pre-treatment exhibited improved performance for some basic and neutral drugs, SLE alone provided satisfactory performance for the majority of drugs using the preliminary conditions tested. Optimum SLE conditions and overall robustness require further investigation.

REFERENCES

1. Logan, BK, D'Orazio, AL, Mohr, ALA, Limoges, JF, Miles, AK, Scarneo, CE, Kerrigan, S, Liddicoat, LJ, Scott, KS and Huestis, MA (2017) Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities-2017 Update. Journal of Analytical Toxicology, 42(2), 63-68.

ACKNOWLEDGEMENTS

This project was supported by Award No. 2018-75-CX-0040 from the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.