The QuEChERS Approach to Determine Pharmaceuticals and Toxins in Whole Blood

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Introduction

- Blood is the most complex of the biological fluids

- Determination of drugs in whole blood is often necessary in forensic analysis because of the difficulty in obtaining serum or plasma

- Conventional procedures to analyze drugs in complex matrices like whole blood involve tedious, time consuming, expensive, and complex steps, and possible sample loss and contamination problems are not unusual

- QuEChERS is a sample preparation technique that was developed for the extraction of multi-class pesticide residue from fruits and vegetables, in 2003

- Although biological fluids may seem vastly different than a piece of fruit they follow parallel paths

Comparison

Biological Fluids

- Complex matrices
- Disrupt cell architecture
  - Lyses, vortexing. organic solvent, hydrolysis
- Analytes with a wide range of polarities
- Remove analyte or matrix
- Analysis by LC/MS/MS, GC/MS or GC/MS/MS

Fruits and Vegetables

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Basic SPE Procedure for Blood Sample

- Pretreatment of whole blood involves dilution with an acidic solution, phosphate buffer or water/FA
- Sonicate 15 minutes; disrupts cell membranes
- Centrifuge
  - SPE procedure
    - Condition cartridge: methanol, water
    - Load cartridge: sample
    - Wash cartridge: aqueous methanol or buffer % methanol
    - Elut cartridge: eluent for analysis

Basic QuEChERS Procedure

15 g homogenized sample, (spike)

Add 15 mL ACN (1% AA), vortex

Add AOAC Extraction Salts, shake

Centrifuge

Transfer 1 or 8 mL to AOAC d-SPE tube, vortex, centrifuge

Transfer to Analysis vial

Advantages of QuEChERS Procedure

- Liquid-liquid type extraction
- Use of acid to interrupt protein binding
- Excess salts to lyses cellular components
- Use of acetonitrile to facilitate protein precipitation
- Use of dispersive SPE to adsorb matrix interferences

Plossl, et.at., J Chrom A, 1135 (2006), 19-26

Disadvantages of QuEChERS Procedure

- Sample and solvent amount required in the method, are not “biological friendly”

Opposing Sample Preparation Ideas

Compatible Sample Preparation Techniques

Purpose Behind the mini-Q Approach

- Offer a more biological friendly approach
- Small sample and volume requirements
- Range of drug compounds, various polarities and pKa
- Evaluation of background interferences
- Extraction and recovery data in acceptable range

mini-QuEChERS: mini-Q

Procedure:

- Add 2 ceramic homogenizers
- 1 or 2 mL whole blood added to a centrifuge tube with screw cap
- Add std soln or IS soln, vortex
- Add 2 mL acidified ACN, vortex 30 sec
- Add 500 mg of extraction salts, vigorously shake 1 minute, centrifuge 5000 rpm, 1 min
- Transfer 1 mL of extract to d-SPE material in centrifuge tube, vortex, centrifuge
- Add 200 uL to 800 uL water in LC vial, vortex analyze

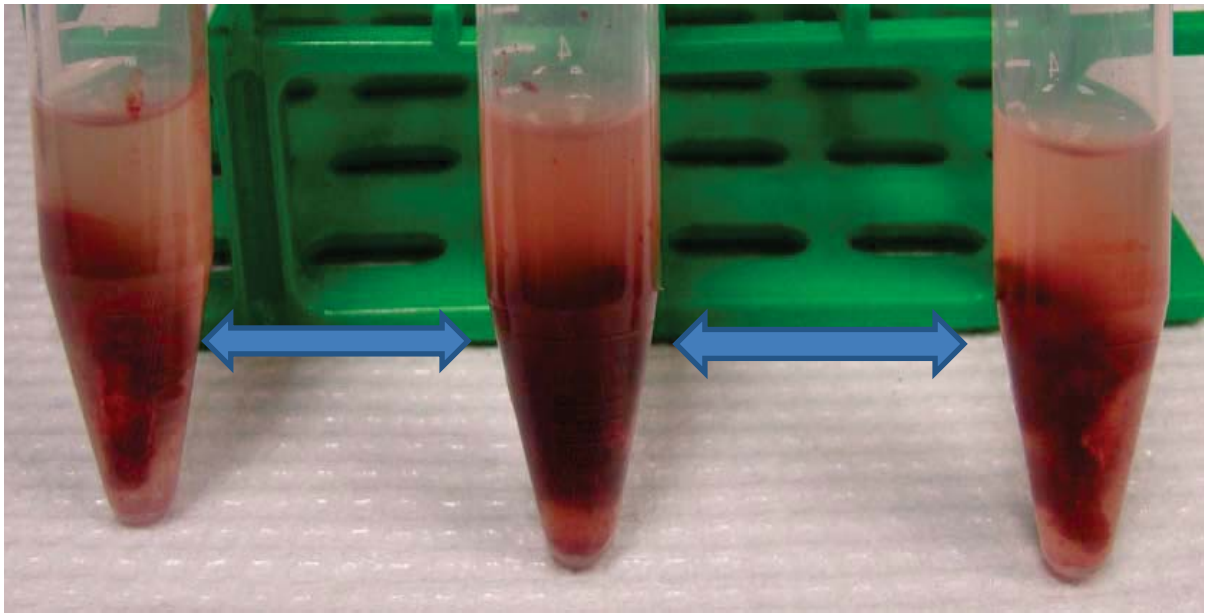
Refinement of mini-Q Procedure

Parameters evaluated in the mini-Q technique

- Whole blood sample
  - Most complicated biological matrix
- Addition of acid, type and amount
  - Effect on partitioning and extraction
- Addition of QuEChERS salts, non-buffered, NaAcetate, or Citrate buffered
  - Effect on extract, visual inspection
- Dispersive SPE
  - Matrix cleanup and compound recoveries

Addition of Acid to Acetonitrile extraction solvent

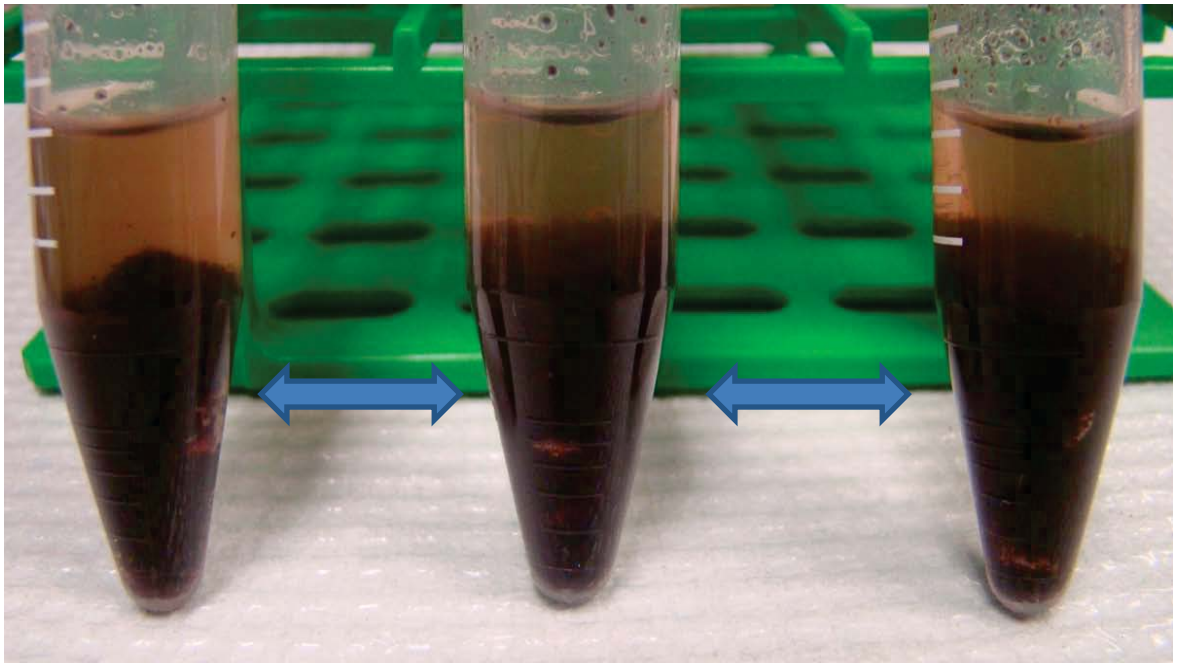
- Use of acetic acid versus formic acid
  - Evaluation of a series of acidic acetonitrile solutions in which the percentage of acid was increased



ACN 1% AA/ACN ACN

Clumping and sticky results

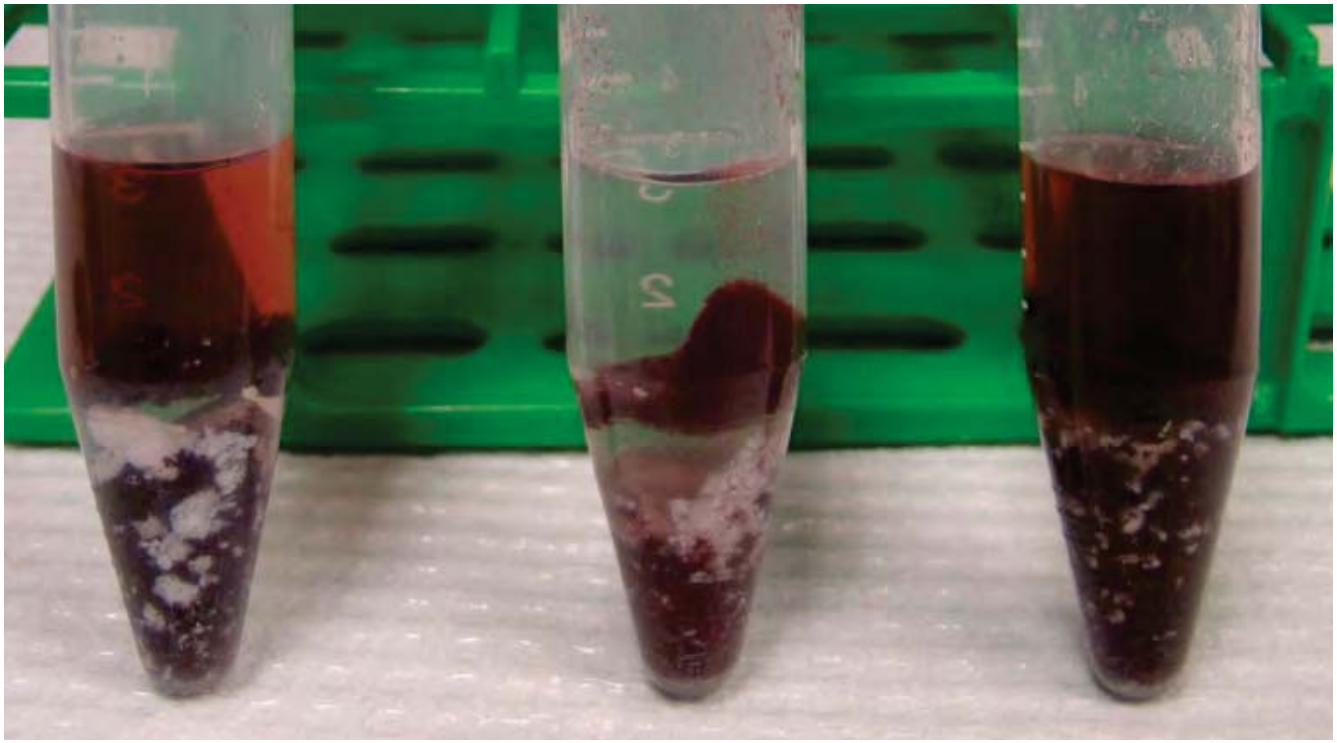
- Addition of 0.4% formic acid/acetonitrile



EN: citrate buffers AOAC: NaAcetate Non-buffered: NaCl

Lyses of cellular structure, increased particulate nature

- Evaluation of QuEChERs salts: Partitioning/Extraction
  - EN: NaCl, Sodium citrate buffers, Mg SO4
  - AOAC: NaAcetate and MgSO4
  - Nonbuffered: NaCl and MgSO4



EN: Citrate salts AOAC: NaAcetate Non-buffered: NaCl

- Dispersive SPE
  - Remove matrix interferences: PSA*, C18, GCB, MgSO4
  - D-SPE: PSA and MgSO4

d-SPE	EN: Citrate	AOAC: NaAcetate	Non-buffered: NaCl
	EN d-SPE	AOAC: d-SPE	AOAC: d-SPE	AOAC: d-SPE

*PSA: Primary and secondary amine

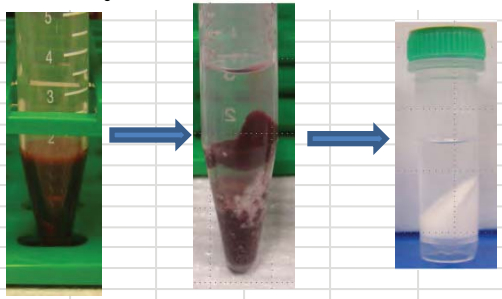
Pharmaceuticals Used in Study

Compound	CAS number	Log P (o/w)	pKa	Therapeutic Use
Lidocaine	137-58-6	2.4	8.01	Local anesthetic, antiarrhythmic
Tramadol	27203-92-5	2.5	9.41	Analgesic
Amitriptyline	50-48-6	4.92	9.4	Antidepressant
Biperidene	514-65-8	4.0	10.8	Anticholinergic
Oxazepam	604-75-1	2.23	1.7, 11.3	Antianxiety
Lorazepam	846-49-1	2.47	1.3, 11.5	Antidepressant
Chlorpromazine	50-53-3	5.18	9.3	Antipsychotic
Diltiazem	42399-41-7	3.63	7.7	Calcium channel blocker
Naloxone	465-65-6	1.45	7.9	Opioid Receptor Antagonist
Nortriptyline (IS)	72-69-5	5.65	9.7	

5 ug/mL stock solution (9 compounds) and IS in methanol

LC/MS/MS Acquisition Data

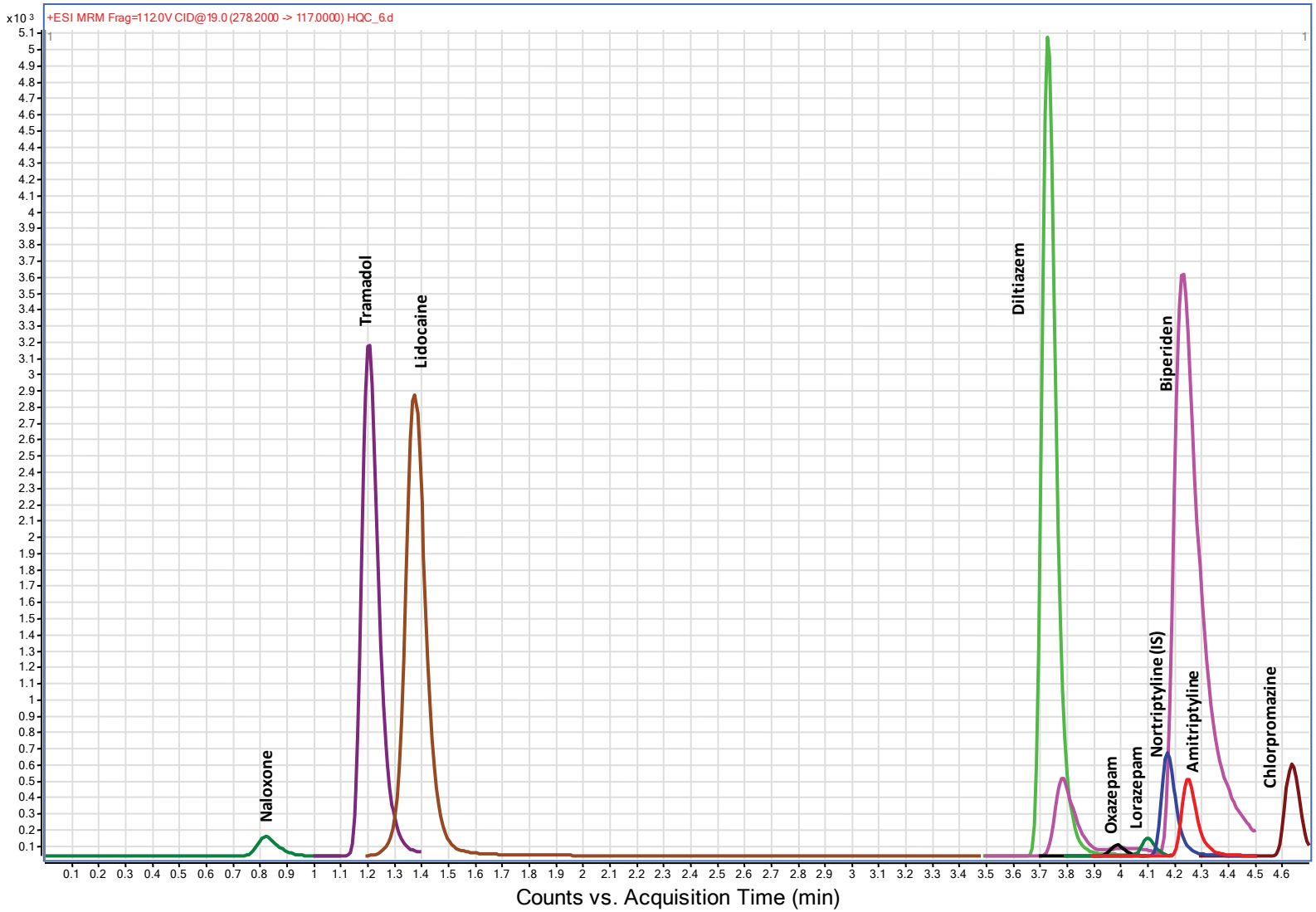
Compound	MRM channels (m/z)	Fragmentor (V)	CE (V)	RT (min)	Delta RT
Lidocaine	1) 235.18 > 86.1 2) 235.18 > 58.1	97	11 35	1.37	0.4
Tramadol	1) 264.2 > 58.1 2) 264.2 > 246.1	97	15 3	1.20	0.4
Amitriptyline	1) 278.2 > 117 2) 278.2 > 105	112	19 19	4.25	0.4
Biperidene	1)312.23 > 98.1 2) 312.23 > 55.1	123	19 60	4.23	0.7
Oxazepam	1) 287.06 > 240.9 2) 287.06 > 268.9	112	19 7	3.99	0.4
Lorazepam	1) 321.02 > 274.9 2) 321.02 > 302.9	113	15 7	4.09	0.4
Chlorpromazine	1) 319.11 > 86.1 2) 319.11 > 58.1	112	15 43	4.63	0.4
Diltiazem	1) 415.17 > 177.9 2) 415.17 > 149.9	128	19 43	3.73	0.4
Naloxone	1) 328.16 > 310 2) 328.16 > 212	123	15 39	0.82	0.4
Nortriptyline (IS)	1) 264.18 > 233 2) 264.18 > 91	97	7 19	4.17	0.4



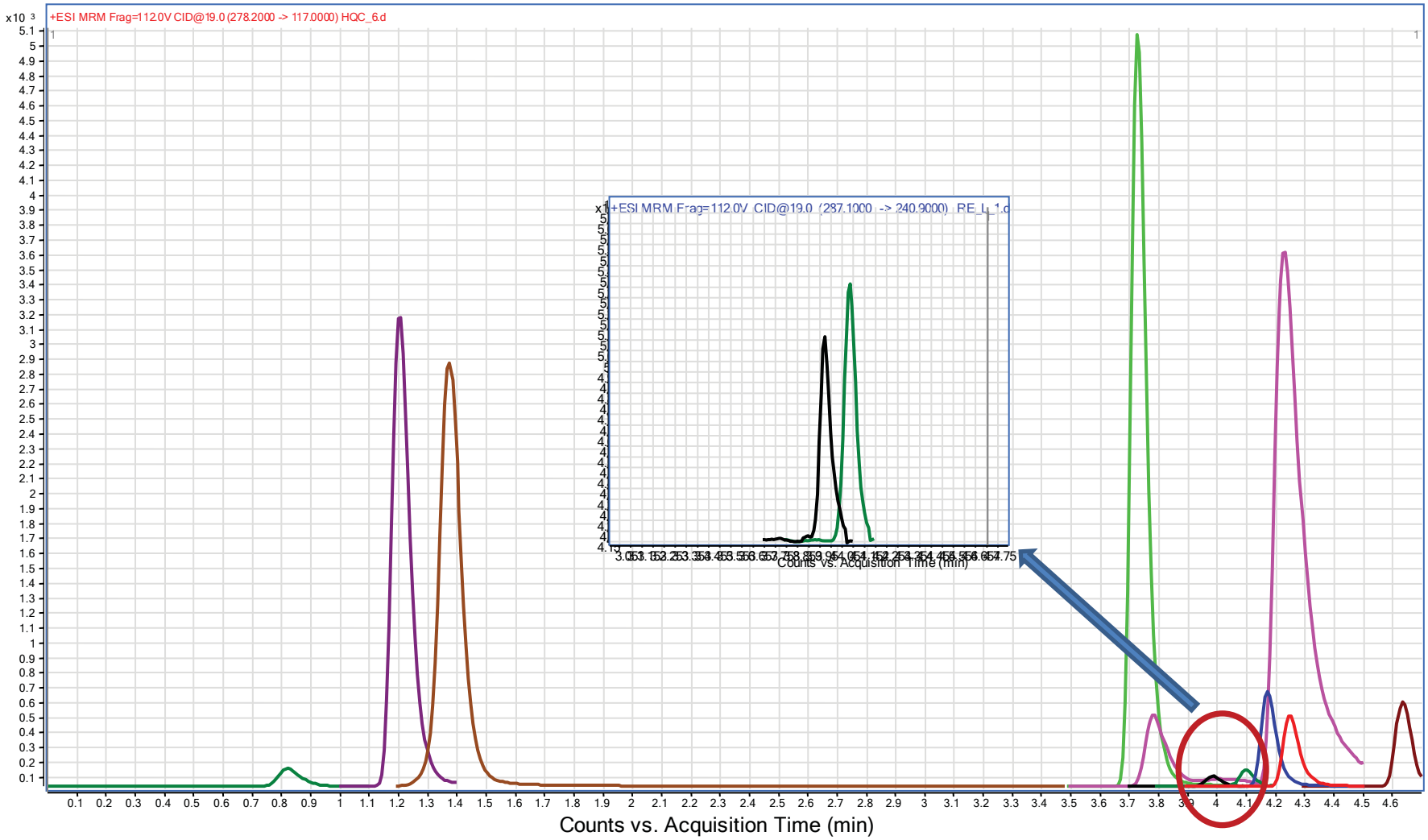
Poroshell 120 EC-C18, 2.7 µ, 2.1 x 100 mm, PN 695775-902
Mobile Phase A: 5 mM Ammonium acetate, pH 5; 20:80 MeOH:Water
B: 5 mM Ammonium Acetate in ACN
GT (ºC): 300
GF (l/min): 7
Nebulizer (psi): 40
SGT (ºC): 400
SFG (l/min): 12
Capillary (V): 3500
NV (V): 500

Counts vs. Acquisition Time (min)

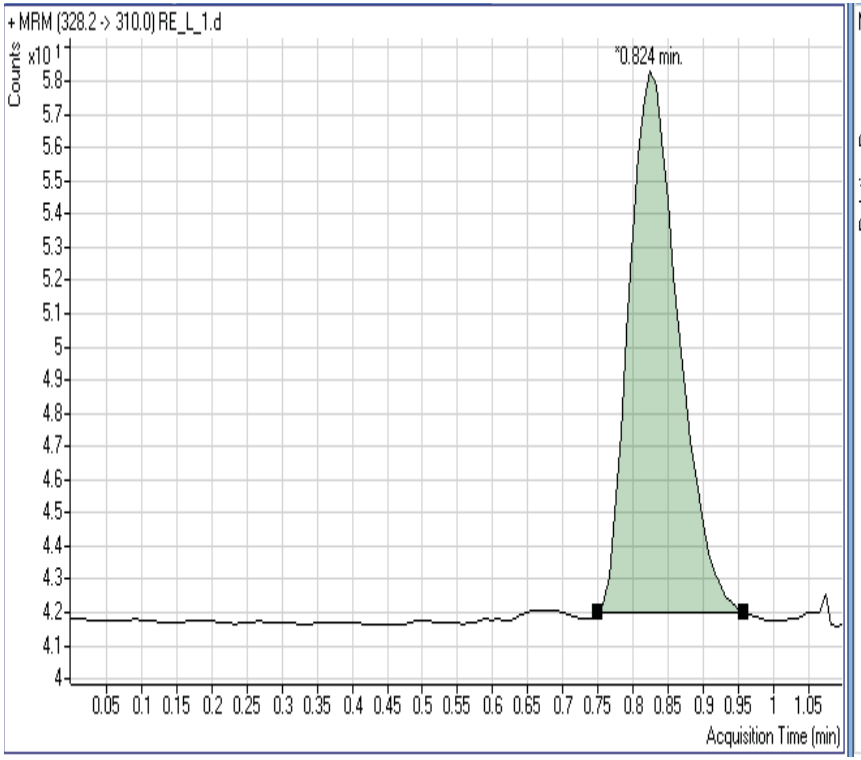
QuEChERS Extraction of Whole Blood MS Scan mass range 100-800, scan time 20 sec, Fragmentor 80 V, positive polarity. Injection volume 10 µL, 20% B to 75% B over 5.5 minutes. Hold 75% B for 2 minutes.

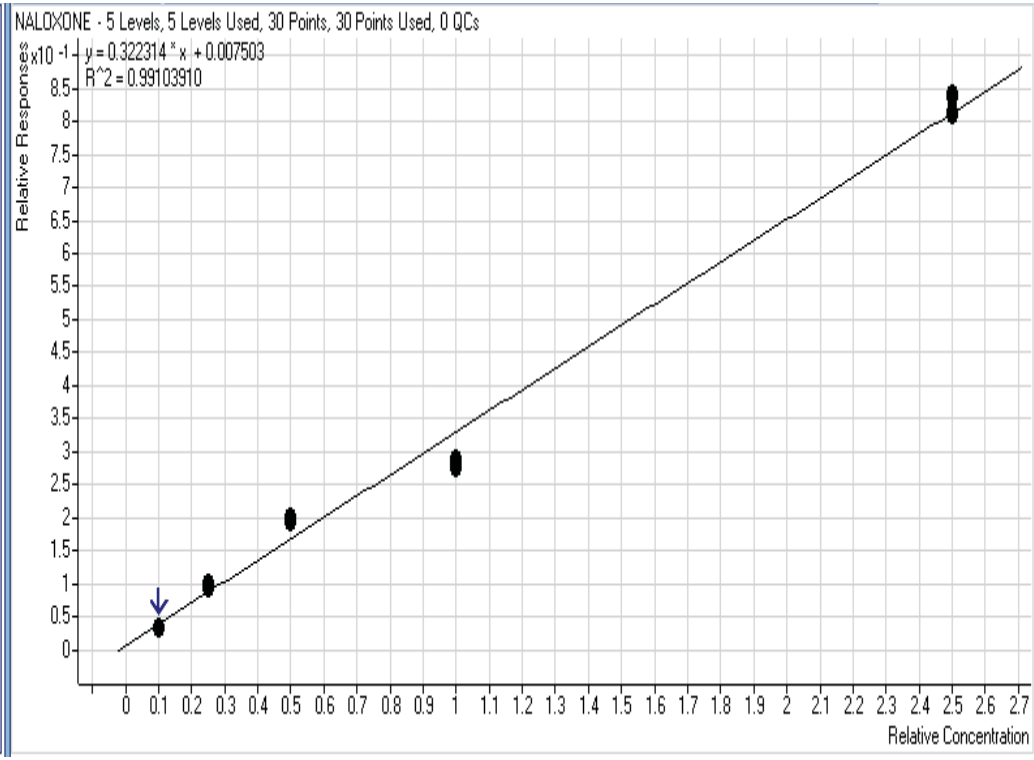


LC/MS/MS Chromatograms of 100 ng/mL spiked whole blood sample after mini-QuEChERS extraction; AOAC (NaAc) and d-SPE (PSA)



Signal-to-noise for Oxazepam and Lorazepam, at 10 ng/mL (10 ppb), SN = 3.0





Example of mini-QuEChERS extracted standard linear curve for Naloxone from 10-250 ng/mL (ppb), $R^2 = 0.991$.

Recovery and Reproducibility

Compound	25 ng/mL Spiked		50 ng/mL Spiked		100 ng/mL Spiked	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Lidocaine	81.6	35.3	98.7	15.7	100	11.8
Tramadol	97.2	18.6	105	3.0	104	8.2
Amitriptyline	85	13.6	104	2.1	104	8.2
Biperidene	75.5	14.8	97	4.5	99	8.2
Oxazepam	60.4	17.3	77.0	9.2	78	8.6
Lorazepam	68.4	17.0	81.9	6.8	81.8	8.6
Chlorpromazine	75	14.1	110	10.3	105	6.3
Diltiazem	63.7	15.8	88.1	2.7	91.7	8.3
Naloxone	68	12.1	80.6	9.0	75.5	7.7

Conclusion and Future Work

- mini-QuEChERS approach for the extraction of pharmaceutical compounds from whole blood offers an alternative to other sample preparation techniques
- mini-QuEChERS sample preparation is a simple, easy and cost effective approach, requiring minimal sample preparation expertise and equipment
- Extend the number and classifications evaluated
- Other biological matrices, postmortem blood
- Future work will involve convenient form factors for both the mini-QuEChERS extraction salts and d-SPE