Evaluation of Drugs of Abuse Extraction from Whole Blood Using Supported Liquid Extraction (SLE) Prior to GC/MS Analysis

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Introduction

Whole blood continues to be a valuable tool in forensic toxicology for the immediate and near-term detection of illicit drugs, and in cases where no other sample is available. Screening drugs of abuse can be complicated due to the wide variation of functional groups associated with different analyte classes. Most extraction techniques cannot extract all analytes using a single procedure without using non-optimal extraction protocols resulting in compromised extract cleanliness. Supported liquid extraction allows for the simultaneous analysis of cross functional analytes in a single extraction protocol without forfeiting extract cleanliness.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Ammonium hydroxide, formic acid, hydrochloric acid and GC derivatizing agents were purchased from Sigma-Aldrich (Dorset, UK). Blank whole blood was purchased from Sera Labs International (Sussex, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q (Merck Millipore, Germany) water used throughout.

Sample Preparation

ISOLUTE® SLE+ Procedure (*Figure 1.*)
Columns: ISOLUTE® SLE+ 1 mL capacity 'C' columns; 820-0140-C.

Matrix Pre-treatment:
400 µL of whole blood was pre-treated with 400 µL of 1% ammonium hydroxide in water (NH_4OH)

Sample Application:
750 µL of pre-treated blood was applied to the columns.

Analyte Extraction:
2 x 2.5 mL aliquots of DCM.
Each aliquot was allowed to flow under gravity for 5 minutes before applying a pulse of positive pressure for 10-20 seconds to completely remove the final aliquot.

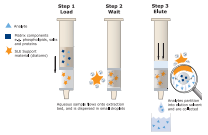


Figure 1. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.*

Post Extraction:
The extracts, with the exception of amphetamine compounds, were evaporated to dryness at 40 °C. Amphetamines were evaporated at ambient temperature. Extracts were derivatized as shown in ***Table 1.***

Table 1. Solvents used post-evaporation to derivatize analytes.

Analyte Group	Pre-reconstitution derivatization	Heating step	Reconstitution	Heating step
Amphetamines	50 µL EtOAc 50 µL PFPA	30 minutes at 70 °C then evaporation	50 µL EtOAc	No
Barbiturates	N/A	N/A	80 µL EtOAc 20 µL TMAH	No
Benzodiazepines	N/A	N/A	50 µL EtOAc 50 µL MTBSTFA	30 minutes at 70 °C
Cocaine Opiates	N/A	N/A	50 µL EtOAc 50 µL MTBSTFA	30 minutes at 70 °C

GC/MS Conditions

GC: 7890A GC with QuickSwap (Agilent Technologies Inc.)
Column: Agilent J&W DB-5, **30 m x 0.25 mm ID x 0.25 µm**
Carrier Gas: Helium 1.2 mL/min (constant flow)
Inlet: Splitless, purge flow at 50 mL/min at 1 min. Temp: 250 °C;
Injection volume: **1 µL**
Oven conditions: See Biotage.com application notes section for oven temperature profiles.
Backflush: 2 void volumes (1.6 mins)
Transfer Line: 280 °C
MS: 5975C MSD (Agilent Technologies Inc.).
Source Temperature: 230 °C
Quadrupole Temperature: 150 °C
Monitored Ions: EI signals were acquired using selected ion monitoring (SIM) mode. See Biotage.com application notes section for monitored ions for each analyte.

Results

Whole blood was spiked with standards and ISTDs and allowed to bind for 1 hour before pre-treatment and subsequent processing. Early whole blood method optimization focused on pre-treatment 1:1 with buffer, loading 300 µL onto the 400 µL capacity SLE+ column. pH control at and above neutrality was investigated with 50 mM ammonium acetate at around pH8, or NH_4OH (aq) at 0.1% or 1%. Increased loading volumes onto the 1 mL capacity format was performed with the same 1:1 dilution and using a total of 750 µL. Elution solvents used in development were MTBE, DCM, 95:5 DCM:IPA (v:v) and ethyl acetate.

Ammonium acetate and 0.1% NH_4OH (aq) pre-treatment options demonstrated sub-optimal extract cleanliness which affected baseline noise during MS acquisition. Likewise, 95:5 DCM:IPA and ethyl acetate demonstrated similar extract cleanliness issue and were not considered moving forward.
Prior to investigating drug recovery, a combination of 1% NH_4OH (aq) pre-treatment with MTBE or DCM solvents were chosen based on extract cleanliness; the higher pH providing less matrix components from portioning into the organic water-immiscible solvent.

Figures 2-6. demonstrate the various drug panels using 1% NH_4OH pre-treatment and elution with 2x 2.5 mL MTBE or DCM. ***Figure 5.*** demonstrates BZE recovery reliance on DCM as the elution solvent.

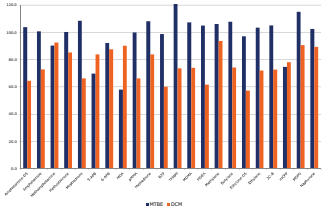


Figure 2. *Amphetamine recoveries comparing elutions with MTBE or DCM.*

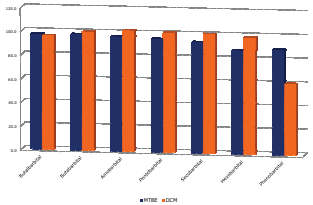


Figure 3. *Barbiturate recoveries comparing elutions with MTBE or DCM.*

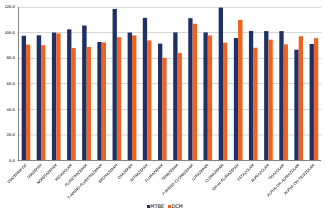


Figure 4. *Benzodiazepine recoveries comparing elutions with MTBE or DCM.*

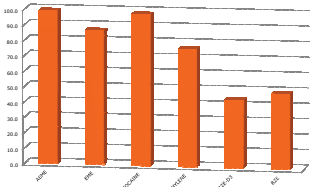


Figure 5. *Cocaine and metabolite recoveries when using DCM as an elution solvent.*

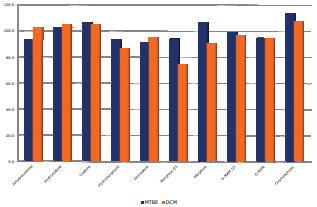
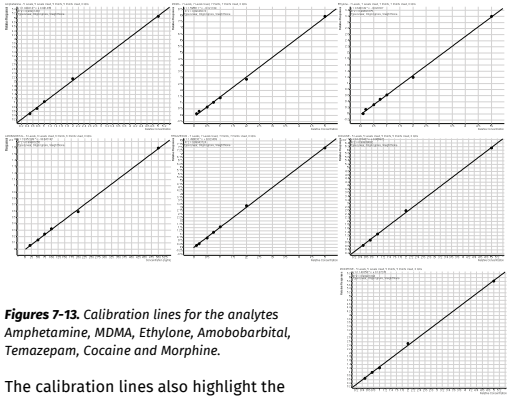


Figure 6. *Opiates recoveries comparing MTBE and DCM elution*

The data from the charts show the majority of analytes can be extracted from whole blood using MTBE as an extraction solvent. However, no BZE is quantifiable until DCM or a DCM-containing option is used. DCM also has the advantage of being slightly cleaner than MTBE. With the development of an optimized extraction method, calibration lines were constructed from 10- 500 ng/mL of whole blood. The internal standard, where appropriate, was spiked at 100 ng/mL. ***Figures 7-13.*** demonstrate the linearity of some representative analytes. The coefficient of determination (r^2) for each analyte was greater than 0.99.



Figures 7-13. *Calibration lines for the analytes Amphetamine, MDMA, Ethylone, Amobobarbital, Temazepam, Cocaine and Morphine.*

The calibration lines also highlight the lower limit of quantitation (LLOQ). These are summarized for the optimized method in ***Table 2.***

Table 2. *Drug LLOQ values.*

Drug Analyte	LLOQ ng/mL	Drug Analyte	LLOQ ng/mL
Amphetamine	50	Flunitrazepam	75
Methamphetamine	50	7-amino-flunitrazepam	100
Methcathinone	50	Bromazepam	50
Mephedrone	50	Oxazepam	10
5-APB	10	Nitrazepam	50
6-APB	10	Flurazepam	20
MDA	20	Temazepam	10
pMMA	20	7-amino-clonazepam	50
Methedrone	20	Lorazepam	20
BZP	100	Clonazepam	75
TFMPP	20	2-OH-Et-Flurazepam	20
MDMA	10	Estazolam	100
MDEA	20	Alprazolam	75
Methylone	10	Triazolam	100
Butylone	<10	Alpha-hydroxy-alprazolam	20
Ethylone	10	Alpha-hydroxy-triazolam	50
2C-B	20	AEME	50
mCPP	50	EME	20
MDPV	20	Cocaine	50
Naphyrone	20	Cocaethylene	50
Butalbarbital	50	BZE	50
Butabarbital	20	Dihydrocodeine	50
Amobarbital	20	Hydrocodone	100
Pentobarbital	20	Codeine	50
Secobarbital	50	Hydromorphone	75
Hexobarbital	20	Oxycodone	200
Phenobarbital	100	Morphine	50
Diazepam	20	6-MAM	50
Nordiazepam	<10	Oxymorphone	100
Midazolam	20		

Conclusion

» This poster demonstrates a fast, reliable protocol to extract multiple drug of abuse panels from whole blood using a common methodology. This benefits laboratory workflow where multiple assays are run each day, thus saving both worker hours and consumable costs.

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