Drugs of Abuse Extraction from Oral Fluid Using Supported Liquid Extraction (SLE) Following Collection with NeoSal™ Prior to GC/MS Analysis

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Introduction

Drug screening using oral fluid has gained popularity over recent years due to its simple, non-invasive collection means. 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. This poster demonstrates protocols for the determination of a range of drugs of abuse following collection with the NeoSal™ oral fluid device and GC/MS analysis. The drug suites included amphetamines and synthetic cathinones, barbiturates, benzodiazepines, cocaine, opiates, cannabinoids and synthetic cannabinoids.

Experimental

Reagents

Drug standards and associated internal 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). NeoSal™ OF devices and buffer were kindly donated by Agriyork (York, 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: ISTDs were spiked into the OF collection device at respective concentrations for each drug panel.

DoA: pH control using 18 µL of conc. NH_4OH.
Synthetic Cannabinoids: No pH modification.

Sample Application: Apply 1 mL of OF matrix to each column (equivalent to 250 µL of OF).

Analyte Extraction:
DoA: 2 x 2.5 mL aliquots of DCM/IPA.
Synthetic Cannabinoids: 2 x 2.5 mL 95/5 hexane/EtOAc.

Each aliquot was allowed to flow under gravity for 5 minutes into an appropriate collection tube. A pulse of positive pressure for 10-20 seconds was applied to completely remove the final aliquot.

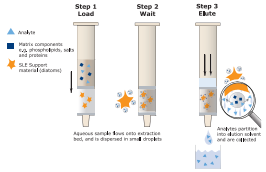


Figure 1. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.*

Post Extraction:
All extracts were evaporated to dryness at 40 °C. Amphetamine panel was evaporated in the presence of methanolic HCl (100 µL, 0.2M) to avoid analyte losses due to volatility. 250 µL of EtOAc was used to reconstitute for transferral to high recovery GC vials for further evaporation and derivatization. Extracts were derivatized as shown in ***Table 1.***

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

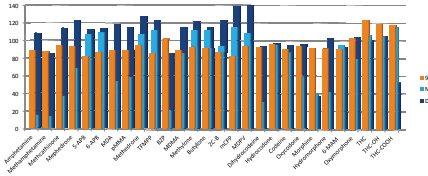
Analyte Group	Pre-reconstitution derivatization	Heating step	Reconstitution	Heating step
Amps	25 µL EtOAc 25 µL PFPA	20 minutes at 70 °C then evaporation	50 µL EtOAc	No
Barbs	N/A	N/A	80 µL EtOAc 20 µL TMAH	
Benzos			50 µL EtOAc 50 µL MTBSTFA (99/1 t-BDMCS),	30 minutes at 70 °C
Cocaine				
Opiates			25 µL EtOAc 25 µL BSTFA (99/1 TMCS),	
THC			40 µL EtOAc 20 µL BSTFA (99/1 TMCS),	25 minutes at 70 °C
Synthetic Cannabinoids				

GC/MS Conditions

GC: 7890A GC with QuickSwap (Agilent Technologies Inc.)
Column: Agilent J&W DB-5MS, **30 m x 0.25 mm ID x 0.25 µm**
Carrier Gas: Helium 1.2 mL/min (constant flow)
Inlet: Splitless, Temp: 250-275 °C *
Injection volume: 1-2 **µL** *
Oven: Various gradients *
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 for full GC/MS conditions for each panel.

Results

Initial method development focused on OF pre-treatment and elution solvent conditions to allow simultaneous extraction of multiple drugs of abuse classes. Investigation of the NeoSal™ device demonstrated the necessity of 18 µL of NH4OH to allow adequate pH control to 8-8.5.This pH does not induce conversion of 6-MAM to morphine while being high enough to allow suitable extraction of multiple classes of acidic, neutral and basic drug panels. ***Figure 2.*** demonstrates extraction recovery comparing various elution solvent combinations: MTBE, DCM and 95/5 DCM/IPA. The latter solvent performed best for a wide analyte panel and was selected going forward. It is also worth noting that the cocaine metabolite, BZE requires DCM solvent combinations for effective extraction.



Figures 2. *Recovery profile chart comparing elution solvent selection.*

Figure 3. demonstrates final analyte recoveries following ISOLUTE® SLE+ 1 mL column extraction using the optimized procedure. Each analyte panel was run separately as detailed in ***Table 1.***

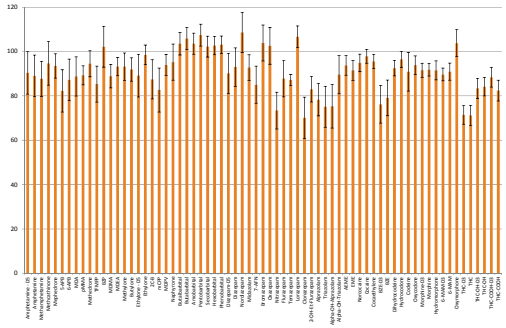


Figure 3. *Recovery profile chart for analytes using a common extraction procedure.*

Synthetic cannabinoids could not be combined with the DoA extraction protocol due largely to differing polarities and solubilities. ***Figure 4.*** demonstrates analyte extraction recoveries using the optimized synthetic cannabinoid method at un-modified pH pre-treatment and 95/5 hexane/EtOAc elution solvent combinations.

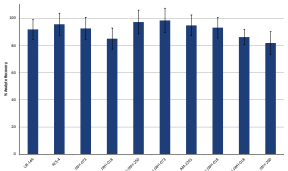
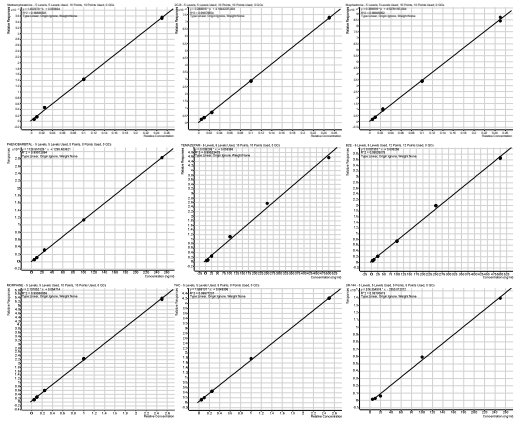


Figure 4. *Recovery profile chart for synthetic cannabinoids extraction procedure.*

Calibration lines were constructed from 5 to 250 ng/mL with the optimized methods. ***Figures 5-13.*** demonstrate the linearity of some representative analytes. The coefficient of determination (r^2) for each analyte was greater than 0.99 for concentration range to the limit of quantitation of each analyte. The LLOQs are summarized for the optimized methods in ***Table 2.***



Figures 6-14. *Calibration lines: methamphetamine, 2C-B, mephedrone, phenobarbital, temazepam, BZE, morphine, THC and UR-144.*

Table 2. *Analyte LLOQ values.*

Drug Analyte	LLOQ (ng/mL)	Drug Analyte	LLOQ (ng/mL)
Amphetamine, Cathinones, synthetic amphetamines	5	***Barbiturates***	5
Benzodiazepines			
Diazepam	5	***THCs***	< 5
Nordiazepam	5	***Synthetic Cannabinoids***	
Midazolam	5	UR-144	5
7-AFN	25	RCS-4	~65
Bromazepam	100	JWH-073	25
Oxazepam	100	JWH-018	25
Nitrazepam	100	5-OH-pentyl-JWH-250	5
Flurazepam	5	3-OH-butyl-JWH-073	10
Temazepam	5	AM-2201	25
Lorazepam	5	4-OH-pentyl-JWH-018	25
Clonazepam	100	5-OH-pentyl-JWH-018	25
2-OH-Et-Flurazepam	5	JWH-200	10
Alprazolam	100	***Opiates***	
Triazolam	100	Dihydrocodeine	5
α -OH-Alprazolam	25	Hydrocodone	100
α -OH-Triazolam	25	Codeine	10
Cocaines		Oxycodone	100
AEME	5	Morphine	5
EME	100	Hydromorphone	25
Norcocaine	10	6-MAM	5
Cocaine	5	Oxymorphone	100
Cocaethylene	5		
BZE	5		

Conclusion

- Supported Liquid Extraction provides simple, rugged and reliable sample preparation approach for the extraction of drugs of abuse from oral fluid collected using the NeoSal™ OF device.
- This poster demonstrates a common procedure for extraction of: amphetamine, cathinone, designer amphetamine, barbiturate, benzodiazepine, cocaine, opiate and cannabinoid drug panels.
- Synthetic cannabinoids required separate optimization.

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