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

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 Neosafe™ oral fluid device and GC/MS analysis. The drug suites included amphetamines and synthetic cathinones, barbiturates, benzodiazepines, cocaine, opiates, cannabinoids and synthetic cannabinoids.

Experiment

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). NeoSaltTM 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.

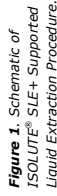
ISOLUTE® SLE+ Procedure (Figure 1.)

Matrix Pre-treatment: ISTDs were spiked into the OF collection device at respective concentrations for each drug panel.

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

DoA: 2 x 2.5 mL aliquots of DCM/IPA.

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.



and was selected going forward. It is also worth noting that the cocaine metabolite, BZE requires DCM solvent combinations for effective extraction.

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 78 μ l 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.

Analyte Group	Pre-reconstitution derivatization	Heating step	Reconstitution	Heating step
Amps	25 μ l EtOAc 25 μ l PPA	20 minutes 30 minutes Evaporation	50 μ l EtOAc 80 μ l EtOAc 20 μ l TMAH	No
Barbit			50 μ l EtOAc 20 μ l TMAH 99 (1-LB-TMCS)	30 minutes at 70 °C
Benzois			25 μ l EtOAc 25 μ l BSFA 99 (1-TMCS)	25 minutes at 70 °C
Cocaine	N/A	N/A		
Opiates				
THC				
Synthetic cannabinoids				

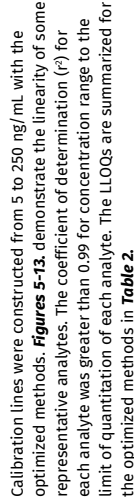
GC: 7890A GC with QuickSwap (Agilent Technologies Inc.)
Column: Agilent J&W DB-5MS, 30 m x 0.25 mm ID x 0.25 µm
Carrier Gas: Helium 1.2 mL/min (constant flow)

* See Biotage.com application notes for full GC/MS conditions for each panel.

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 4. Recovery profile chart for synthetic cannabinoids extraction procedure.



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

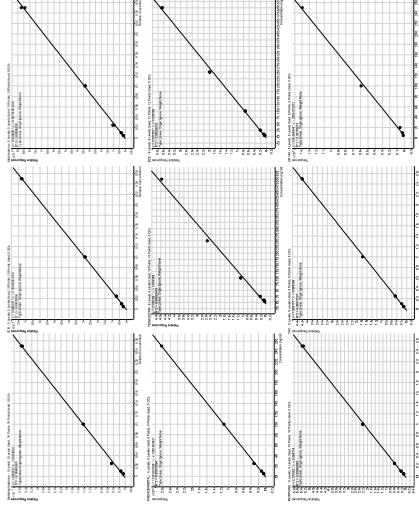


Table 2. Analyte LLOQ values.

Drug Analyte	Drug Analyte	Drug Analyte	Drug Analyte	Drug Analyte
Amphetamine, Cathinones, synthetic amphetamines	Barbiturates	THCs	Synthetic Cannabinoids	LL00 (ng/mL)
Benzodiazepines				
Diazepam	5	THCs		<5
Nordiazepam	5			
Rhizoxolam	5	Ultrax		5
7-ACN	25	RES-4		48
Bromazepam	100	JWH-073		23
Oxazepam	100	JWH-078		23
Nitrazepam	100	5-OH-pentyl- JWH-250		5
Flurazepam	5	3-OH-butyl- JWH-073		10
Temazepam	5	AKC-201		25
Lorazepam	5	4-OH-pentyl- JWH-078		25
Clonazepam	100	5-OH-pentyl- JWH-078		25
2-OH-Et-Flurazepam	5	JWH-200	Opines	10
Alprazolam	100			
Triazolam	100	Dihydrocodone		5
α -OH-Alprazolam	25	Hydrocodone		100
α -OH-Triazolam	25	Codone		10
Cocaines		Oxycodone		100
AEME	5	Morphine		5
EME	100	Hydromorphone		25
Norcocaine	10	6-MAM		100
Cocaine	5	Oxymorphone		100
Cocathylene	5			
BZE	5			