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 LCG Standards (Teddington, UK). Ammonium hydroxide, formic acid, hydrochloric acid and GC derivatizing agents were purchased from Sigma-Aldrich (Dorset, UK). NeoSal™ OF devices and buffering were kindly donated by Agylphor (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.
Do A: pH control using 18 µL of conc. NH4OH.
Synthetic Cannabinoids: No pH modification.
Sample Application: Apply 1 mL of OF matrix to each column (equivalent to 250 µL of OF).
Analyte Extraction:
Do A: 2 x 25 mL aliquots of DCM/IPA.
Synthetic cannabinoids: 2 x 2.5 mL 95/5 methanol/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.

Analyte LLOQ Values.

Table 2. Analyte LLOQ values.

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 DOA was used to reconstitute for transfinal to high recovery GC vials for further evaporation and derivatization. Extracts were derivatized as shown in Table 1.

Figure 3. Recovery profile chart comparing elution solvent selection.

Figure 4. Recovery profile chart for synthetic cannabinoid extraction procedure.

Figure 5. Calibration lines: methamphetamine, 2C-B, methanol, phenol/phenol, ramaquapine, ADX, morphine, THC and THAB.

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. 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.

Figure 2. 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 6-14: Calibration lines: methamphetamine, 2C-B, methanol, phenol/phenol, ramaquapine, ADX, morphine, THC and THAB.

Conclusion

Supported Liquid Extraction provides simple, rugged and reliable sample preparation analysis 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.