

Evaluation of Drugs of Abuse Extraction from Oral Fluid using Supported Liquid Extraction (SLE+) prior to GC/MS and LC/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 the extraction of a range of drugs of abuse prior to GC/MS or LC/MS analysis. The target analyte list includes barbiturates, THC and metabolites, benzodiazepines, z drugs, amphetamines, cathinones, opiates, cocaine, buprenorphine, PCP, fentanyl and ketamine.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Ammonium acetate, ammonium hydroxide, HCl, formic acid and GC derivatizing agents were purchased from Sigma-Aldrich (Dorset, UK). Oral fluid collection devices were purchased from their respective companies. 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+ 400 µL capacity; 820-0055-B.

Matrix Pre-treatment:

Intercept®: Add 10 µL of 0.5% NH₄OH (aq) to each device.

Oral-eze®: Add 10 µL of 4% NH₄OH (aq) to each device.

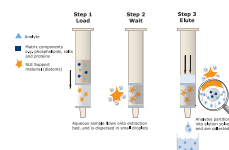
Quantisal™: Add 15 µL of conc. (28-32%) NH₄OH (aq) to each device.

Sample Application:

Intercept® and Oral-eze®: 300 µL (equivalent to 100 µL of OF) was applied to the ISOLUTE® SLE+ column.

Quantisal™: 300-400 µL (equivalent to 75-100 µL of OF) was applied to the ISOLUTE® SLE+ column.

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



Analyte Extraction: 2 x 1 mL aliquots of DCM/IPA (95:5, v/v). Each aliquot was allowed to flow under gravity for 5 minutes before

applying a pulse of vacuum for 10-20 seconds to completely remove the final aliquot.

GC/MS Post Extraction, Barbiturates & THC: The extracts were evaporated to dryness at 40 °C.

Barbiturates: Extracts were reconstituted in 80 µL EtOAc and 20 µL TMAH 0.2M (trimethylanilinium hydroxide).

THC: Extracts were reconstituted in 50 µL EtOAc and 25 µL MTBSTFA:TBDMCS 99:1.

LC/MS Post Extraction: 100 µL of 50 mM HCl in methanol was added to the collection plate. The extracts were evaporated to dryness at 40 °C and reconstituted in 200 µL of 80:20 mobile phase prior to analysis.

GC/MS Conditions

GC: 7890A GC with QuickSwap (Agilent Technologies Inc.). Column: Phenomenex Zebron ZB-Semivolatiles, 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.

Temperature: Barbiturates 150 °C; THC 250 °C.

Injection volume: Barbiturates 1 µL, THC 2 µL.

Oven conditions:

Barbiturates: Initial 120 °C, hold for 1 min, ramp 12 °C/min to 192 °C then ramp 120 °C/min to 330 °C, hold for 0.85 min.

THC: Initial 100 °C, ramp 100 °C/min to 280 °C, hold for 10.5 min, then ramp 100 °C/min to 330 °C, hold for 0.5 min.

Backflush: 3 void volumes (2.76 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 SIM.

UPLC-MS/MS Conditions

UPLC: Waters Acquity UPLC (Waters Assoc., Milford, USA).

Column: Acquity UPLC BEH C18 column (1.7 µ, 100 x 2.1 mm id) (Waters Assoc., Milford, MA, USA).

Mobile Phase: 5 mM NH₄OAc (aq) and 5 mM

NH₄OAc/MeOH at a flow rate of 0.3 mL/min.

Gradient: 90/10 increasing to 10/90 over 10 minutes.

Initial starting conditions resumed at 11.4 minutes.

Injection Volume: 10 µL (partial loop with overflow).

Column Temperature: 40 °C.

MS: Quattro Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive and negative ions were acquired in the MRM mode.

Desolvation Temperature: 450 °C.

Ion Source Temperature: 150 °C.

Collision Gas Pressure: 3.5 x 10⁻³ mbar.

Results

Each oral fluid collection device is made up of different combinations of additives. Control of pH was investigated using minimal volumes of NH₄OH to enable maximum matrix loading of the pre-buffered oral fluid. Loading pH was optimized to be between 8-8.5 to allow extraction of basic drugs while eliminating the conversion of 6-MAM to morphine. Additionally the extraction of native oral fluid from these devices demonstrated substantial residue and non-optimal cleanliness, so pH control was a necessity.

The analysis of barbiturates and THC was performed using GC/MS analysis. Both analyte suites extract under a variety of pH conditions (data not shown). However, the purpose of the poster was to provide a generic set of conditions to enable the simultaneous extraction of THC and barbiturates along with a variety of basic drugs of abuse. **Figures 2 and 3.** demonstrate barbiturate and THC extraction recoveries obtained at pH conditions between 8-8.5 and extraction using 95/5 DCM/IPA or MTBE. All 3 devices provide good extraction recoveries and low RSDs at all concentration levels.

Figure 2. Barbiturate recoveries from various oral fluid collection devices.

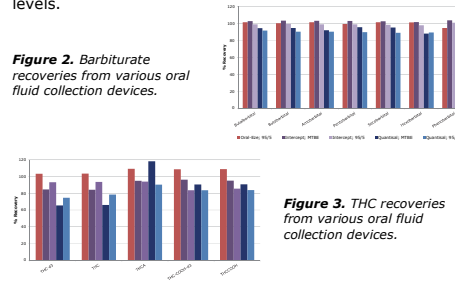


Figure 3. THC recoveries from various oral fluid collection devices.

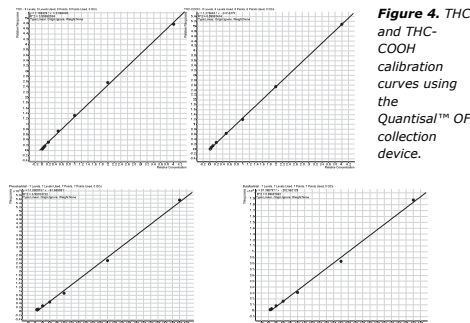


Figure 4. Phenobarbital and Butalbital calibration curves using the Intercept® OF collection device.

Analysis of a multiclass suite of drugs of abuse was performed using LC-MS/MS. **Figures 4, 5 and 6.** demonstrate extraction recoveries obtained with various extraction solvents from the Intercept®, Oral-eze® and Quantisal™ collection devices, respectively. The cocaine metabolite, BZE required DCM combinations as the extraction solvent, preferentially 95/5 DCM/IPA. MTBE as a non-chlorinated solvent showed good recoveries of the majority of analytes investigated.

Figure 4. DOA recoveries from Intercept® oral fluid collection device.

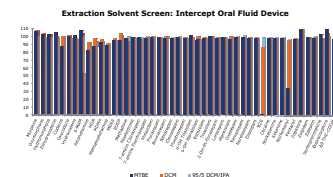


Figure 5. DOA recoveries from Oral-eze® oral fluid collection device.

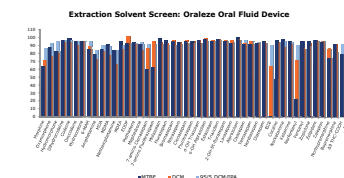
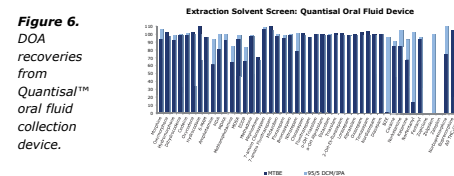


Figure 6. DOA recoveries from Quantisal™ oral fluid collection device.



Conclusion

- This poster demonstrates the suitability of ISOLUTE® SLE+ for the rapid and reliable extraction of multiple drugs of abuse and metabolites from oral fluid.
- BZE extraction requires the use of DCM/IPA as the optimal extraction solvent. If BZE is not in the suite the use of other non-chlorinated solvents, such as MTBE can be substituted.

Further Work

- Quantisal®: Investigation of loading volumes to achieve optimized extract cleanliness.
- Calibration lines for DOA multisuite LC-MS/MS method with all oral fluid collection devices.