Evaluation of Drugs of Abuse Extraction from Oral Fluid Using Supported Liquid Extraction (SLE) Prior to UPLC-MS/MS Analysis

Biotage®

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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 UPLC-MS/MS analysis. The target analyte list includes benzodiazepines, z drugs, amphetamines, cathinones, opiates, cocaine, buprenorphine, PCP, THC-COOH, fentanyl and ketamine.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Ammonium acetate, ammonium hydroxide (28-32%), HCl, formic acid 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+ Optimized Procedure (Figure 1.)
Columns: ISOLUTE®SLE+ 400 µL capacity B columns; 820-0055-B
ISOLUTE® SLE+ 1 mL capacity C columns; 820-0140-C

Matrix Pre-treatment:
Intercept®: Add 10 µL of 0.5% NH_4OH (v/v, aq) per device and dilute 1:1 with HPLC H_2O
Quantisal™: Add 15 µL of conc. NH_4OH (aq) per device

Sample Application:
Quantisal™: 200 or 500* µL was applied to the ISOLUTE® SLE+ B or C columns, respectively. * *maximum load for DCM.*
Intercept®: 480 µL was applied to the ISOLUTE® SLE+ C column.

Analyte Extraction: Quantisal™ (B and C) and Intercept®(C only)
ISOLUTE® SLE+ B columns: 2 x 1 mL aliquots of DCM
ISOLUTE® SLE+ C columns: 2 x 3 mL aliquots of DCM or EtOAc

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.

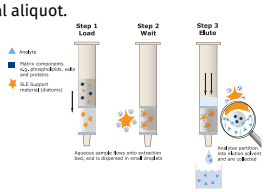

Figure 1. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction procedure.*

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

UPLC Conditons

Instrument: Waters Acquity UPLC (Waters Assoc., Milford, MA, USA)
Column: ACE EXCEL 1.7 µm C18, 100 mm x 2.1 mm id, (Advanced Chromatography Technologies, Aberdeen, UK)
Mobile Phase: 5 mM NH_4OAc (aq) and 5 mM NH_4OAc/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 overfill).*
Column Temperature: 40 °C

Mass Spectrometry

Instrument: 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 multiple reaction monitoring mode (MRM).
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 to desorb the drugs from the collection pad and preserve the oral fluid matrix. Control of pH was investigated using minimal volumes of NH_4OH 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. ***Figures 2. and 3***. demonstrate extraction recoveries obtained with various extraction solvents from the Quantisal™ and Intercept® 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.

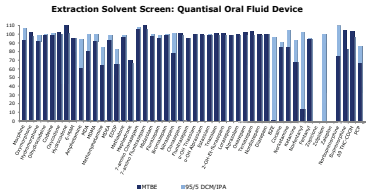

Figure 2. *DOA recoveries from Quantisal™ oral fluid collection device.*

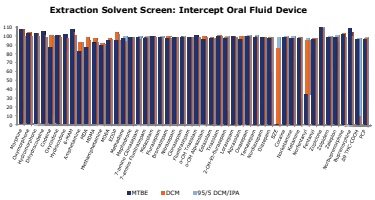

Figure 3. *DOA recoveries from Intercept® oral fluid collection device.*

The oral fluid collection device additives provide an interesting challenge when using LC-MS analysis. When performing full scans on the extracts it was noticed that substantial interference was observed when using DCM and 95/5 DCM/IPA as the extraction solvents. MTBE and EtOAc demonstrated less interference as shown in ***Figures 4 and 5.*** for Quantisal™ and Intercept® collection devices, respectively. For both collection devices the use of 95/5 DCM/IPA was discontinued. Optimization of loading volume provided the optimal balance of extract cleanliness and recoveries when using DCM.

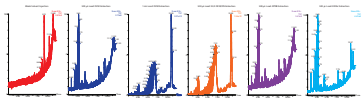

Figure 4. *Full scan TIC when using the Quantisal™ oral fluid collection device.*

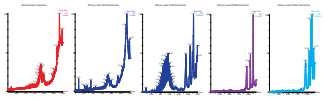

Figure 5. *Full scan TIC when using the Intercept® oral fluid collection device.*

Figure 6. demonstrates analyte recoveries when using the Quantisal™ device with optimized loading volumes (200 µL and 500 µL for the ISOLUTE 400 µL and 1 mL columns, respectively) and eluting with DCM.

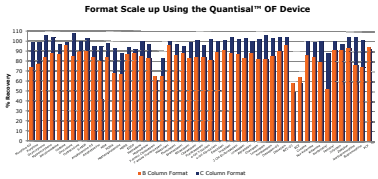

Figure 6. *DOA recovery format scale up using the Quantisal™ oral fluid collection device.*

Figure 7. demonstrates the optimal extraction conditions for the Intercept® OF device; loading 500 µL onto the ISOLUTE® SLE+ 1 mL capacity column and eluting with 2 x 3 mL of EtOAc.

This method demonstrates optimal extract cleanliness and good recoveries for the majority of analytes. BZE recovery is below 10% although reproducibility is good down to sub ng/mL levels.

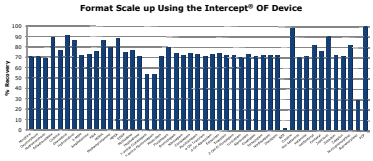

Figure 7. *DOA recovery format scale up using the Intercept® oral fluid collection device.*

Figure 8. demonstrates typical calibration curves from 1-500 ng/mL obtained using the Quantisal™ device. Quadratic function was observed at high concentrations for a number of analytes. However, dilution and internal standards helped and ultimately demonstrated excellent linearity and coefficients of determination (r^2 > 0.99). RSDs were below 10% at all concentration levels.

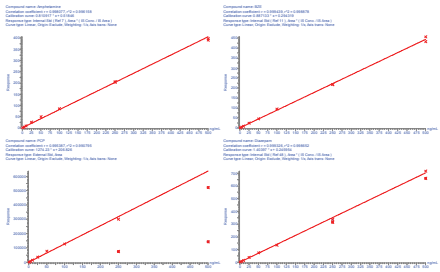

Figure 8. *Calibration curves extracting 200 µL of oral fluid using the Quantisal™ collection device using the ISOLUTE® SLE+ 400 µL capacity columns.*

Figure 9. demonstrates typical calibration curves from 1-500 ng/mL obtained using the Intercept® device. Again some quadratic function was observed at high concentrations for a number of analytes. Overall excellent linearity and coefficients of determination (r^2 > 0.99) were returned for all analytes. RSDs were below 10% at all concentration levels.

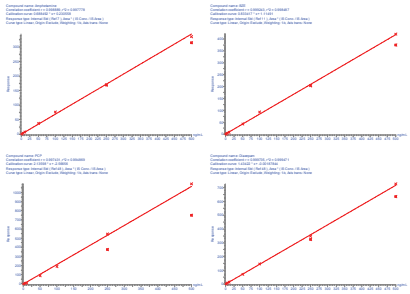

Figure 9. *Calibration curves extracting 480 µL of buffered oral fluid using the Intercept® collection device using the ISOLUTE® SLE+ 1 mL capacity columns.*

All LLOQs for both oral fluid collection device methods were sub ng/mL levels.

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. If BZE is not in the suite the use of other non-chlorinated solvents, such as MTBE or EtOAc can be substituted.
- The use of 95/5 DCM/IPA although providing good extraction of most drug suites, suffers from non-optimal extract cleanliness associated with co-extraction of OF buffer additives.
- When using the Intercept® OF device the use of DCM required a substantial under-load of the column. EtOAc provided sufficient LLOQs for BZE even though < 10% recovery was returned.

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