Extraction of Barbiturates from Oral Fluid using Supported Liquid Extraction (SLE) after collection with the Quantisal, Intercept & Oral-Eze Collection Devices prior to GC/MS Analysis

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Introduction

Oral fluid testing is gaining approval in the forensic toxicology community as a suitable tool to supplement urine and blood testing where misuse of drugs is suspected. A quick, dignified specimen can be obtained from a person relatively easily in workplace applications, drug driving incidents and other applications.

Barbiturates are a group of compounds which were originally developed to alleviate anxiety and nervousness. Today they have more relevance prescribed as anticonvulsant and migraine treatments in the form of phenobarbital and butalbital, respectively.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Ammonium hydroxide (28-32%), 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 'B' columns; p/n 820-0055-B and 1 mL capacity 'C' columns; p/n 820-0140-C.

Matrix Pre-treatment:

Intercept®: Add 10 µL of 0.5% NH_4OH (v/v, aq) to each device.

Oral-eze®: Add 10 µL of 4% NH_4OH (v/v, aq) to each device.

Quantisal™: Add 15 µL of conc. NH_4OH (aq) to each device.

Sample Application:

Intercept®: 300 µL (equivalent to 100 µL of OF) was applied to the ISOLUTE SLE+ B column, or the complete contents were applied to the ISOLUTE SLE+ C column.

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

Quantisal™: 400 µL (equivalent to 100 µL of OF) was applied to the ISOLUTE SLE+ B column, or 1 mL (equivalent to 250 µL of OF) was applied to the ISOLUTE SLE+ C column.

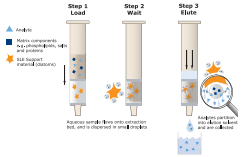

***Figure 1**. Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.*

Analyte Extraction, Quantisal and Intercept:

ISOLUTE SLE+ B columns: 2 x 1 mL aliquots of MTBE.
ISOLUTE SLE+ C columns: 2 x 2.5 mL aliquots of MTBE.

Analyte Extraction, Oral-eze:

ISOLUTE SLE+ B column: 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.

Post Extraction:

The extracts were evaporated to dryness at 40 °C. Extracts were reconstituted in 80 µL EtOAc and 20 µL TMAH 0.2M (trimethylanilinium hydroxide).

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: 150 °C;
Injection *volume*: 1 µL
Oven conditions: 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.
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: Ionization was performed using EI. Signals were acquired using selected ion monitoring (SIM) in 5 groups, as shown in ***Table 1.***

***Table 1.** MS acquisition parameters*

SIM Group	Analyte	Target (Quant) Ion	1st Qual Ion	2nd Qual Ion
1	Butalbarbital	196	195	181
1	Butabarbital	169	184	211
2	Amobarbital	169	184	225
2	Pentobarbital	169	184	225
3	Secobarbital	196	195	181
4	Hexobarbital	235	81	169
4	Phenobarbital	232	146	175

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. Initial experiments involved loading these buffers into ISOLUTE SLE+ after drug spiking, but without any modification or dilution, prior to elution with a water immiscible solvent.

Figures 2 & 3. demonstrate the outcome. Taken in isolation, the recovery data shows some possible elution strategies, e.g. 100% DCM following a Quantisal sample load, however with no modification, it was observed that the methodology resulted in substantial residue and non-optimal cleanliness after evaporation, especially with MTBE and ethyl acetate. To overcome this, the pH environment of the matrix was evaluated.

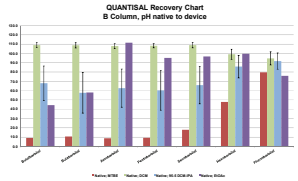

***Figure 2.** Recoveries using Quantisal devices and 4 elution solvents at the native pH.*

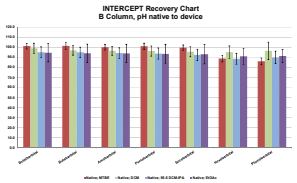

***Figure 3.** Recoveries using Intercept devices and 4 elution solvents at the native pH.*

Control of loading pH was investigated using minimal volumes of concentrated NH_4OH, to enable maximum matrix loading of the pre-buffered oral fluid. It was found that a pH range of 8-8.5 was effective at delivering significantly cleaner extracts and much improved recoveries for Quantisal and reduced RSDs for Intercept. This is shown in ***Figures 4&5.***

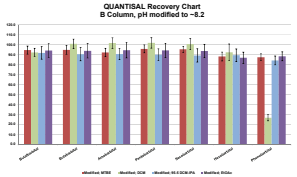

***Figure 4.** Recoveries using Quantisal devices and 4 elution solvents at higher pH.*

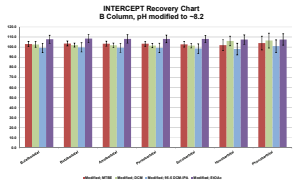

***Figure 5.** Recoveries using Intercept devices and 4 elution solvents at higher pH.*

The third device to be evaluated was Oral-eze. ***Figure 6.*** shows the recoveries with and without pH control, with a 95/5 DCM/IPA elution solvent. Due to time constraints, testing with this particular device was not extensive. In addition to improved barbiturates results and extract cleanliness, the optimized pH approach also accommodates the extraction of common basic drugs from the same collection device, as described in drugs of abuse poster P39.

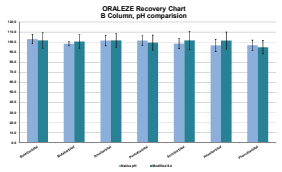

***Figure 6.** Recoveries using Oral-eze devices at its native pH and increased pH.*

Increased loading volume was evaluated on the Quantisal and Intercept devices using the ISOLUTE SLE+ C column. The optimum elution solvents used were MTBE and DCM/IPA (95:5, v/v). For the Quantisal device, recoveries were 100-106% and 89-105% for the two solvents respectively, with all RSDs under 10%. For the Intercept device the recoveries were 92-95% for MTBE and 92-94% for DCM/IPA with RSDs similarly under 10%.

The lower limits of quantitation are displayed in ***Table 2.***

***Table 2.** LLOQ values for barbiturates for Quantisal and Intercept devices when loading on B or C ISOLUTE SLE+ columns*

Analyte	ISOLUTE SLE+ B Format LLOQ (ng/mL)		ISOLUTE SLE+ C Format LLOQ (ng/mL)	
	Quantisal	*Intercept*	*Quantisal*	*Intercept*
Butalbarbital	25	25	10	10
Butabarbital	10	10	4	4
Amobarbital	10	5	4	2
Pentobarbital	10	10	4	4
Secobarbital	25	10	10	4
Hexobarbital	10	10	4	4
Phenobarbital	10	25	4	10

Calibration curves were constructed from a concentration range of 5-500 ng/mL on B columns and 2-200 ng/mL on C columns. The Quantisal devices demonstrated r^2 values of 0.9998 to 0.9935 and the Intercept devices demonstrated values of 0.9980 to 0.9960. Representative curves can be seen in ***Figures 7 & 8.***

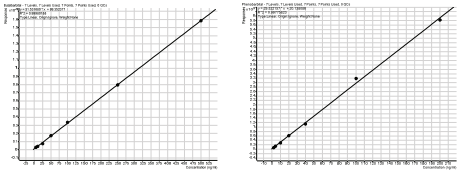

***Figure 7 & 8.** Calibration curves for barbiturate analytes extracted from Quantisal by ISOLUTE SLE+ 400 µL columns and Intercept by ISOLUTE SLE+ 1 mL columns respectively.*

Conclusion

- This poster demonstrates the suitability of ISOLUTE SLE+ for the rapid and reliable extraction of barbiturates from three oral fluid collection devices, prior to GC/MS.
- The control of the pH environment is important to avoid the presence of residue, post-evaporation. Each device has a unique pre-loading step that is tailored to achieve this.

Part Number: P111