Extraction of Δ⁹-THC, THCA and 11-nor-9-carboxy-Δ⁹-THC from Oral Fluid using Supported Liquid Extraction (SLE) after collection with the Quantisal, Intercept & Oral-Eze Collection Devices prior to GC/MS Analysis.

Rhys Jones[1], Lee Williams[1], Helen Lodder[1], Geoff Davies[1], Adam Senior[1], Alan Edgington[1], Steve Jordan[1], Claire Desbrow[1], Victor Vandell[2].

[1]Biotage GB Limited, Distribution Way, Dyffryn Business Park, Ystrad Mynach, Cardiff, CF82 7TS, UK.
[2]Biotage, 10430 Harris Oaks Blvd., Suite C, Charlotte North Carolina 28269, USA.

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. Cannabis misuse continues widely all over the world, and this has led to the necessity for rapid and reliable methods for the analysis and quantitation of THC and its main metabolite 11-nor-9-carboxy-THC (THC-COOH). In addition, THCA is a marker which can show a distinction between *Cannabis Sativa* use and synthetic medicinal Dronabinol.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Ammonium hydroxide (28-30%), 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; 820-0055-B
ISOLUTE® SLE+ 1 mL capacity 'C' columns; 820-0140-C

Matrix Pre-treatment:

Intercept°: Add 10 µL of 0.5% of commercial NH_4OH (v/v aq) to each device.
Oral-eze°: Add 10 µL of 4% of commercial NH_4OH (v/v aq) to each device.
Quantisal™: Add 15 µL of conc. commercial (28-30%) NH_4OH (v/v 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. 1 mL (equivalent to 250 µL of OF) was applied to the ISOLUTE SLE+ C column.

Analyte Extraction:

B columns: 2 x 1 mL aliquots of DCM/IPA (95:5, v/v).
C columns: 2 x 2.5 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 positive pressure (10 psi) for 10-20 seconds to completely remove the final aliquot.

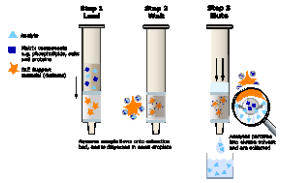


Figure 1. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction procedure.*

Post Extraction:

The extracts were evaporated to dryness at 40 °C. Extracts were reconstituted in 50 µL EtOAc and 25 µL MTBSTFA:TBDMCS 99:1.

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: 250 °C
Injection volume: 2 µL
Oven conditions: Initial 100 °C, ramp 100 °C/min to 280 °C, hold for 10.5 minutes, ramp 100 °C/min to 330 °C, hold for 0.5 min
Backflush: 3 void volumes (2.4 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 3 groups, as shown in ***Table 1.***

Table 1. *MS acquisition parameters*

SIM Group	Analyte	Target (Quant) Ion	1st Qual Ion	2nd Qual Ion
1	Δ⁹THC-D3	374	431	348
1	Δ⁹THC	371	428	345
2	THCA	530	631	455
3	THC-COOH-D3	416	518	575
3	THC-COOH	413	515	572

Results

Early experiments were performed with elutions of MTBE, DCM, DCM/IPA (95/5) and ethyl acetate. All solvents produced analyte recoveries exceeding 79% with the exception of 100% DCM, as demonstrated in ***Figure 2.*** DCM provided insufficient recovery of the carboxy-THC metabolite but addition of 5% polar modifier, isopropanol (IPA) provided high recoveries.

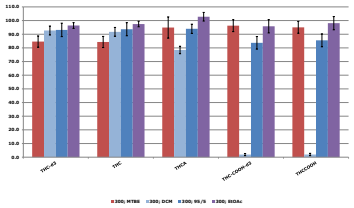


Figure 2. *Recoveries using 4 elution solvents*

This initial elution solvent testing involved pH adjustment using minimal volumes of concentrated NH_4OH in the buffered oral fluid, to enable maximum load of the matrix.

This pH control was performed to increase the environment to a value of 8-8.5. This accommodates any basic drug extractions from the same OF sample, such as morphine or amphetamine, if the laboratory so requires as described in previous drugs of abuse posters. THC and its metabolites are lipophilic so potentially they are soluble enough to extract into the water immiscible solvents even when fully ionized.

In addition to providing a pH environment amenable to other test suites, the pH control also had the effect of improving cleanliness post-evaporation. An extraction without pH modification was performed, i.e. loading under native pH conditions, and there was substantial residue within the collection tubes, especially with MTBE and ethyl acetate. The recovery and RSD data from Quantisal testing is also shown from this test in ***Figure 3.*** and from Oral-eze in ***Figure 4.*** Both data sets are with DCM/IPA 95/5 as the elution solvent. Due to time constraints, testing with the Oral-eze device was not extensive.

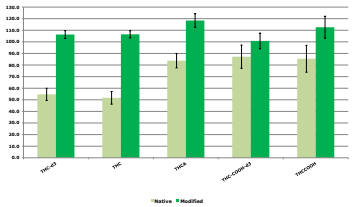


Figure 3. *Recoveries using DCM/IPA 95/5 from a Quantisal load at native and ~ pH 8.2.*

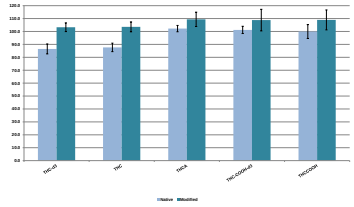


Figure 4. *Recoveries using DCM/IPA 95/5 from an Oral-eze load at native and ~ pH 8.2*

Increased loading volume was evaluated on the Quantisal and Intercept devices using the ISOLUTE SLE+ 1 mL C column. ***Figure 5.*** demonstrates analyte recoveries greater than 77% when scaling to larger load volumes. Corresponding RSDs are below 10%.

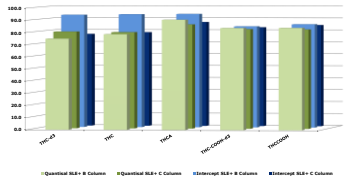
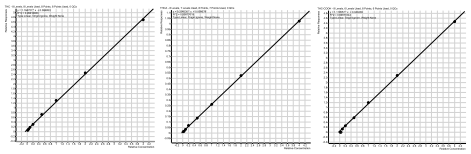


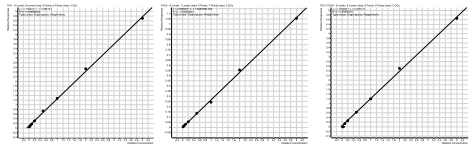
Figure 5. *Recovery comparison scaling up to ISOLUTE® SLE+ 1 mL columns, loading from Quantisal and Intercept devices.*

Calibration curves were constructed from a concentration range of 4-800 ng/mL using 1 mL C columns. The Quantisal devices demonstrated r^2 values > 0.999 for each analyte as can be seen in ***Figures 6-8.***



Figures 6-8. *Calibration curves for extracted levels of spiked oral fluid after collection with Quantisal devices using 1 mL ISOLUTE® SLE+ format from 4 ng/mL to 800 ng/mL. Analyte r^2 values are > 0.9991, 0.9997 and 0.9997 for THC, THCA and THC-COOH respectively.*

Calibration curves performance using the Intercept device demonstrated values > 0.998 for all analytes as can be seen in ***Figures 9-11.***



Figures 9-11. *Calibration curves for extracted levels of spiked oral fluid after collection with Intercept devices using 1 mL ISOLUTE® SLE+ format from 4 ng/mL to 800 ng/mL. Analyte r^2 values are > 0.998 for THC, THCA and THC-COOH.*

The lower limits of quantitation for the assay using the Quantisal and Intercept devices extracted with the SLE+ 1 mL capacity 'C' columns are displayed in ***Table 2.***

Table 2. *LLOQ values for Δ⁹THC, THCA and THCA for Quantisal and Intercept devices when loading on 1 mL ISOLUTE®SLE+ columns.*

Analyte	ISOLUTE® SLE+ 1mL C Column LLOQ (ng/mL)	
	Quantisal	*Intercept*
Δ⁹THC	4	4
THCA	10	10
THC-COOH	20	20

Conclusion

- This poster demonstrates the suitability of ISOLUTE® SLE+ for the rapid and reliable extraction of THC, THCA and THC-COOH from three oral fluid collection devices, prior to GC/MS analysis.
- The control of the pH environment is important to avoid the presence of matrix residue, post-evaporation. Each device has a unique pre-loading step that is tailored to achieve this.
- If chlorinated solvents are prohibited, MTBE is a suitable substitute to DCM/IPA (95/5).
- Lower limits of quantitation will be possible using updated GC/MS equipment.

This poster was first presented at MSACL EU, Florence, 2015
Part Number: P134