Applicability of a Novel Automation Platform for Column-based Drug of Abuse Extraction prior to LC/MS or GC/MS Analysis

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Introduction

Historically, silica-based solid phase extraction (SPE) columns have been used for drugs of abuse testing, the exact choice being dependent on drug functionality. GC/MS sensitivity requirements traditionally dictated larger volume sampling resulting in the use of columns capable of accepting volumes up to 10 mL. Reduced sample size has been achieved due to increased specificity using GC-MS/MS or the movement to LC-MS/MS analysis. This poster will present a dedicated sample preparation platform capable of processing volumes associated with drugs of abuse testing whether using GC/MS or LC/MS end points.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Sodium hydroxide, buffers, acetic, formic, hydrochloric acids, and GC derivatizing agents were purchased from Sigma-Aldrich (Dorset, UK). Negative urine was provided by healthy volunteers. All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q (Merck Millipore, Germany) water used throughout.

Sample Preparation

Non-hydrolyzed Urine: Urine was spiked with standards/ISTDs and pre-treated 1:1 or 1:3 with 100 mM pH 6 buffer.
Hydrolyzed Urine LC/MS Panel: Urine was buffered 1:1 with 100 mM ammonium acetate pH 5 and β-glucuronidase enzyme (95/5) and hydrolyzed at 60 °C for 2 hrs.
Hydrolyzed Urine THC: Hydrolysis was performed by adding 100µL NaOH to a 2 mL urine sample and heated at 60°C for 25 minutes. After cooling pH control was achieved using 1mL of acetic acid.

Extractions were performed using silica-based mixed-mode SPE in 3 mL column format. ISOLUTE® HCX 130 mg/3 mL (cation exchange) was used for the extraction of amphetamines and full drug panel while ISOLUTE® HAX 200 mg/3 mL (anion exchange) was used for the extraction of THC-COOH. Extraction protocols are shown in ***Table 1***.

Table 1. *Solid Phase Extraction Protocols.*

Step	ISOLUTE® HCX (LC/MS DoA)	ISOLUTE® HCX (Amphetamines)	ISOLUTE® HAX (THC-COOH)
Condition	MeOH 2 mL	MeOH 3 mL	MeOH 3 mL
Equilibration	0.1M $NaHPO_4$ pH6 2 mL	i) Water 3 mL ii) 0.1M $NaHPO_4$ pH6 1 mL	i) Water 3 mL ii) 0.1M NaOAc pH3 1 mL
Sample load	2 mL	4 mL	3.1 mL
Wash 1	1M Acetic acid 2 mL	H_2O 2 mL	H_2O 2 mL
Wash 2	MeOH 1 mL	0.1M Acetic acid 2 mL	0.1M HCl (aq):ACN 70:30 2 mL
Wash 3	-	MeOH 3 mL	100 µL Hexane
Elution	78/20/2 DCM/MeOH/NH_4OH 3 mL	78/20/2 DCM/IPA/NH_4OH 3 mL	2% Formic acid Hexane:EtOAc 50:50 3 mL

Post extraction:
LC/MS extracts were evaporated at 40 °C in the presence of 100 µL of 50 mM HCl in MeOH to avoid volatile amine losses followed by reconstitution in 0.1% formic acid aq/MeOH (90/10). Amphetamines were evaporated at 25 °C, reconstituted with 50 µL ethyl acetate (EtOAc), 50 µL pentafluoropropionic anhydride (PFPA) and derivatized at 50 °C for 25 minutes. A second evaporation step was employed followed by reconstitution with 200 µL EtOAc for GC/MS analysis. THC-COOH was evaporated at 40 °C, reconstituted with 20 µL EtOAc, 20 µL BSTFA:TMCS 99:1 and derivatized at 70 °C for 25 minutes. After cooling samples were ready for direct injection.

Biotage® Extrahera™ Automated Sample Preparation Platform

Extraction protocols were transferred to the Extrahera™ High-volume HV-5000 sample preparation platform (shown in ***Figure 1***.) equipped with a 4 channel pipetting head for 5000 µL tips and positive pressure processing functionality. Columns were processed in a 24 or 48 position arrangement.

Figure 1. *Biotage® Extrahera™ HV-5000 automated sample preparation platform.*

GC/MS Conditions

GC: 7890A GC (Agilent Technologies Inc.).
Column: Agilent J&W DB-5ms, 30 m x 0.25 mm ID x 0.25 µm.
Carrier Gas: Helium 1.5 mL/min (constant flow).
Inlet: Splitless.
Temperature: Amphetamines 250 °C; THC 260 °C.
Injection volume: Amphetamines 1 µL, THC 1.5 µL.
Oven conditions:
Amphetamines: Initial 60 °C, hold for 1 min, ramp 25 °C/min to 215 °C hold for 4 min.
THC: Initial 60 °C, ramp 25 °C/min to 325 °C, hold for 2 min.
Backflush: 3 void volumes.
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.

UHPLC-MS/MS Conditions

UHPLC: Nexera X2 UHPLC (Shimadzu Europa GmbH, Duisburg, Germany).
Column: Restek Raptor Biphenyl column (2.7 µ, 100 x 2.1 mm id).
Mobile Phase: 2 mM ammonium formate containing 0.1% formic acid in aq (A) and MeOH (B) at a flow rate of 0.4 mL/min.
Column Temperature: 30 ˚C. **Injection Volume:** 5 µ
Gradient: 80/20 hold 2 min; increasing to 40/60 over 5.5 minutes, hold 3.75 min; increase to 100% B over 1.5 min; hold 0.75 min. Initial starting conditions resumed at 13.5 minutes.

MS: Nexera 8060 Triple Quad MS (Shimadzu Europa GmbH).
Heat Block Temp: 300 ˚C. **Interface Temp:** 150 ˚C. **DL Temp:** 250 ˚C.
Nebulizing/Drying Gas Flows: 3 L/min. **Heating Gas Flow:** 17 L/min.
CID Gas Pressure: 270 kPa.

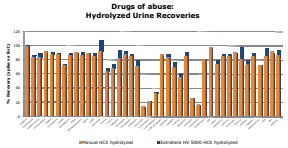
Results

Previously developed silica-based SPE protocols for each analyte panel were compared for performance using offline positive pressure processing or the Biotage® Extrahera™ High-volume HV-5000 sample preparation platform. Flow control and processing parameters were optimised for both techniques.

LC/MS Drug Panel

Excellent correlation in terms of analyte recoveries, matrix factors and RSDs were returned as demonstrated in ***Figures 2-4***.

Figure 2. DoA *recoveries comparing manual vs automated processing using ISOLUTE® HCX columns.*



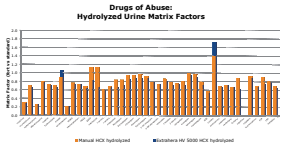
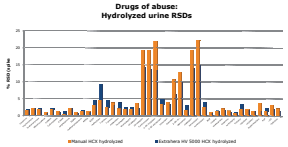


Figure 3. DoA *Matrix Factors comparing manual vs automated processing using ISOLUTE® HCX columns.*

Figure 4. DoA *RSDs comparing manual vs automated processing using ISOLUTE® HCX columns.*



Calibration curves demonstrated excellent linearity and coefficients of determination (r^2) > 0.99. Due to instrument sensitivity some analytes demonstrated quadratic function at high concentration levels. Calibration curve examples from EH processing is demonstrated in ***Figures 5-6.***

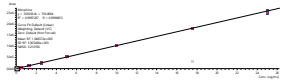


Figure 5. *Morphine calibration curve from automated processing.*

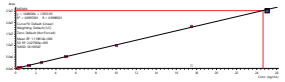
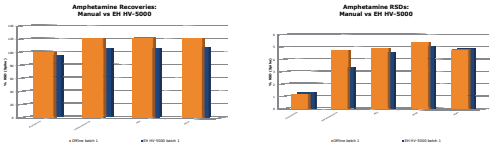


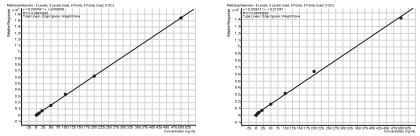
Figure 6. *MDMA calibration curve from automated processing.*

GC/MS Urine analysis

Amphetamines were extracted from non-hydrolyzed urine. Better precision was obtained using the HV-5000 compared to manual positive pressure processing. ***Figures 7-8.*** demonstrate recovery and RSD performance while ***Figures 9-10.*** compare calibration curve (5-500 ng/mL) performance for methamphetamine. Excellent linearity was observed for all analytes, r^2 > 0.999.



Figures 7-8. *Amphetamine recovery and RSD profiles comparing manual vs automated processing using ISOLUTE® HCX columns.*



Figures 9-10. *Methamphetamine calibration curve from automated (LHS) and manual processing (RHS).*

THC-COOH was extracted from base hydrolyzed urine. Recoveries and RSDs are demonstrated in ***Figure 11.***, while calibration curves (1-100 ng/mL) are shown in ***Figures 12-13.***

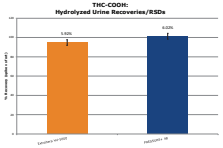
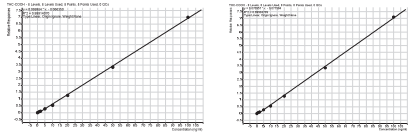


Figure 11. THC-COOH *recovery and RSD profiles comparing manual vs automated processing using ISOLUTE® HAX columns.*



Figures 12-13. *THC-COOH calibration curve from automated (LHS) and manual processing (RHS).*

Sample throughput was dependent on several factors: processing volumes, extraction sorbent geometry (column size) and automation bed layout. 6 mL (C) column sizes were processed in 24 positions while 3 mL (B) was compared in 24 or 48 deck positions. Where sample/elution volumes allow, moving from 24 to 48 positions increase method time by approximately 50% depending on assay complexity.

Conclusion

- This poster demonstrates the effectiveness of the Extrahera™ HV-5000 for the automated extraction of drugs of abuse for LC/MS or GC/MS analysis.
- Automation throughput was drastically improved using 5000 µL tips for assays using column formats and larger sample/solvent processing volumes.

SOFT 2022, Cleveland, OH
Part Number: P241. V.1