Evaluation of Sample Preparation Approaches for the Extraction of Amphetamine, Methamphetamine, MDMA and Metabolites from Urine prior to GC/MS Analysis

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Introduction

Amphetamine, methamphetamine and Ecstasy continue to be widely abused in many parts of the world. EMCDDA reported in March 2019 that the latest drug taking habits of over 70 European cities show an increase in the detection of these stimulants in 2018 compared to 2017. Urine analysis continues to be among the most popular approach to determining drug intake. This poster will examine various sample preparation approaches in the analysis of amphetamines prior to gas chromatography-mass spectrometry. Comparisons were performed between silica-based and polymer-based SPE as well as supported liquid extraction (SLE).

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Ammonium hydroxide, formic acid, hydrochloric acid 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

Extractions were developed using supported liquid extraction, silica-based and polymer-based SPE in column format.
ISOLUTE® SLE+ was used in the 1 mL capacity column format (P/N 820-0140-CG) following a load-wait-elute procedure (***Figure 1***).

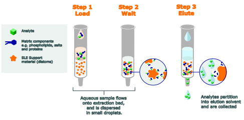


Figure 1. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.*

EVOLUTE® EXPRESS CX and ISOLUTE® HCX were used in the 60 mg/3 mL (P/N 611-0006-BX) and 130 mg/3 mL (902-0013-B) column formats, respectively. Traditional SPE procedures incorporating optimized wash steps (***Figure 2***) were compared along with streamlined load-wash-elute using the polymer based SPE phase.

Figure 2. *Schematic of a Typical SPE Procedure.*

Full method optimization was performed for each sample preparation technique with final extraction protocols for each shown in ***Table 1***.

Post extraction: Extracts were evaporated at 40 °C in the presence of 100 µL 200 mM HCl in MeOH. Samples were then derivatized in-vial with ethyl acetate (50 µL) and pentafluoropropionic anhydride (PFPA) (50 µL) at 50 °C for 15 minutes. Further evaporation was performed at ambient temperature followed by reconstitution in ethyl acetate prior to GC/MS analysis.

Table 1. *Optimized Extraction Protocols.*

Step	ISOLUTE® SLE+	EXPRESS® CX	ISOLUTE® HCX
Condition	-	-	MeOH 3 mL
Equilibration	-	-	Water 3 mL
Equilibration	-	-	0.1M $NaHPO_4$ pH6 (aq) 1mL
Sample load	1:1 0.1% NH_4OH (aq) 1mL	1:1 0.1M NH_4OAc pH6 (aq) 2 mL	1:3 0.1M $NaHPO_4$ pH6 (aq) 4 mL
Wash 1	-	0.1M ammonium acetate pH6 (aq) 2 mL	H_2O 3 mL
Wash 2	-	1% Formic acid (aq) 1mL	0.1M acetic acid (aq) 1 mL
Wash 3	-	MeOH 2 mL	MeOH 3 mL
Elution	Ethyl Acetate 4 mL	78/20/2 DCM/IPA/NH_4OH (1 mL)	78/20/2 DCM/IPA/NH_4OH (3 mL)

GC Conditions

GC: 7890A GC with QuickSwap (Agilent Technologies Inc.)
Column: Restek Rxi-5ms, 30 m x 0.25 mm ID x 0.25 µm
Carrier Gas: Helium 1.2 mL/min (constant flow)
Inlet: 250 °C, Splitless, purge flow: 50 mL/min at 1.0 min
Injection volume: 1 µL
Oven conditions: Initial temperature 60 °C, hold for 1 minute. Ramp 25 °C/min to 215 °C, hold for 1 minute.
Backflush: 2 void volumes (1.6 mins)
Transfer Line: 280 °C

MS Conditions

MS: 5975C MSD (Agilent Technologies Inc.)
Source Temperature: 230 °C
Quadrupole Temperature: 150 °C
Monitored Ions: EI signals were acquired using selected ion monitoring (SIM) mode. ***Table 2.*** shows the monitored ions for each analyte.

Table 2. *Selected ion Monitoring MS Parameters.*

SIM Group	Analyte	Target (Quant) Ion	Qual Ion
1	Amphetamine-D5	194	123
1	Amphetamine	118	190
2	Methamphetamine	204	118
3	MDA	162	135
4	MDMA	162	204
4	MDEA	218	162

Results

ISOLUTE® SLE+ Optimization

Initial investigations focused on the evaporative losses of Amphetamines. Various volumes and strengths of HCl were investigated to prevent analyte losses during evaporation. ***Figure 3.*** demonstrates the recovery performance of increasing the amount of HCl during the evaporation.

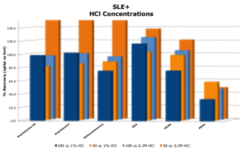


Figure 3. *Evaporative losses comparing varying HCl concentration.*

Sample pre-treatment using 0.1% NH_4OH was previously demonstrated to yield good extraction efficiencies (data not shown). Elution solvent investigation demonstrated the ability of various solvents to provide effective results. Overall the use of ethyl acetate provided optimum performance as detailed in ***Figure 4.***

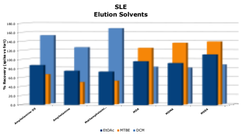


Figure 4. *Elution solvent recovery profiles.*

Following solvent
selection, minimum elution volumes were investigated. ***Figure 5.*** demonstrates the recovery performance using multiple elution volumes.

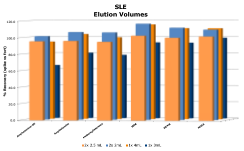


Figure 5. *Elution solvent volume recovery profiles.*

SPE Optimization

Traditional SPE methods for the extraction of amphetamines from urine have been based on the use of silica-based mixed-mode SPE. Initial experiments involved optimization of load and wash parameters using ISOLUTE® HCX for potential streamlining of the methodology. ***Figures 6 and 7.*** demonstrate recovery performance using various wash combinations along with determination of the necessity of an acidic interference wash using ISOLUTE® HCX.

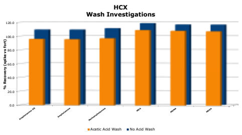


Figure 6. *Acidic interference wash recovery profiles using ISOLUTE® HCX.*

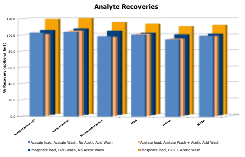


Figure 7. *Wash combination recovery profiles using ISOLUTE® HCX.*

Figure 8. demonstrates the comparison of acidic additive during washing on amphetamine recoveries using polymer-based mixed-mode SPE, EVOLUTE EXPRESS® CX.

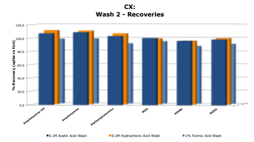


Figure 8. *Acidic additive wash combination recovery profiles using EVOLUTE® EXPRESS CX.*

Polymer-based mixed-mode SPE allowed reduced sorbent bed volumes compared to silica-based equivalents. This allowed method streamlining in terms of wash volumes, waste and elution volumes. Minimum elution solvent volume investigations demonstrated 1 mL 78/20/2 DCM/IPA/NH_4OH gave optimum amphetamines recovery (data not shown) using a 60 mg sorbent bed. Polymer-based SPE also allowed further method streamlining due to fully water wettable components. Full optimized SPE methodology was compared with a streamlined Load-Wash-Elute (LWE) procedure. ***Figure 9.*** demonstrates equivalent performance using EVOLUTE EXPRESS® CX allowing the elimination of column conditioning and equilibration steps.

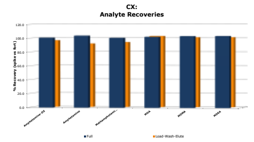


Figure 9. *LWE and full SPE recovery profiles using EVOLUTE EXPRESS® CX.*

Figure 10. compares the final protocols for HCX, CX and SLE.

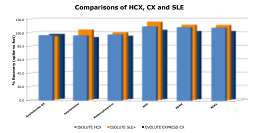
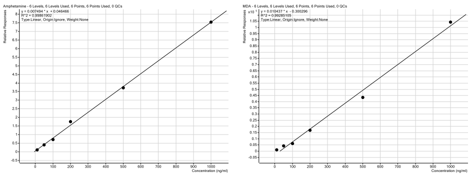
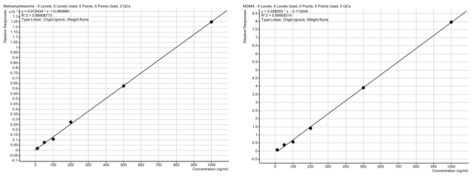


Figure 10. *shows recoveries comparing HCX, CX and SLE*

Calibration curves constructed from 10-500 ng/mL of human urine demonstrated good linearity with all analytes returning coefficients of determination (r^2) greater than 0.99. ***Figures 11 and 12.*** demonstrate calibration curves for amphetamine and MDA extracted using ISOLUTE® SLE while ***Figures 13 and 14.*** show equivalent curves for methamphetamine and MDMA extracted using EVOLUTE® EXPRESS CX elution.



Figures 11 and 12. *Calibration curves for amphetamine and MDA extracted using ISOLUTE® SLE+.*



Figures 13 and 14. *Calibration lines for methamphetamine and MDMA extracted using EVOLUTE® EXPRESS CX.*

Final calibration performance is summarized in ***Table 3.***

Table 3. *Drug LLOQ (estimated) values for SPE and ISOLUTE SLE.*

Analyte	HCX r^2	CX r^2	SLE+ r^2	SPE LLOQ ng/mL	SLE+ LLOQ ng/mL
Amphetamine	0.997	0.999	0.999	10	~25
Methamphetamine	0.998	0.999	0.999	~4	~20
MDA	0.999	0.999	0.997	~6	~22
MDMA	0.996	0.998	0.999	~6	~33
MDEA	0.997	0.998	0.998	~3	~45

Conclusion

- This poster demonstrates a range of approaches for the extraction and cleanup of amphetamine, methamphetamine, MDMA and metabolites from urine.
- Each technique demonstrates the required method performance.
- Streamlined approaches have been presented in order to save time, cost, solvent/reagent use and associated waste disposal all of which add to the overall assay costs.

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