Alternative Forensic Matrices: Evaluation of Simplified Workflow for Drugs of Abuse Extraction from Nail Samples Prior to LC-MS/MS Analysis

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Introduction

The testing of alternative matrices in forensic and/or clinical toxicology is gaining popularity, partly due to less invasive means of collection. Blood and urine testing are still far more prevalent. However, traditional testing in combination with other matrices such as hair or nail can provide a more rounded picture of abstinence or abuse and associated timeframes. Solid matrix analysis by LC/MS or GC/MS is generally more involved due to the necessity of multiple manual steps to convert the sample into an extractable form. This poster aims to demonstrate workflow advantages for fingernail analysis; multi-sample homogenization, extraction and analysis for a range of drugs of abuse.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Ammonium hydroxide (28-32%), ammonium formate, hydrochloric acid (37%) and formic acid were purchased from Sigma-Aldrich Company ltd. (Gillingham, UK). HPLC and LC/MS grade solvents were from Honeywell Research Chemicals (Bucharest, Romania). Water (18.2 MΩ.cm) was drawn fresh daily from a Direct-Q 5 water purifier (Merck Millipore, Watford, UK). Nail samples were kindly donated by healthy human volunteers.

Sample Preparation

ISOLUTE® SLE+ Procedure (*Figure 1.*)

ISOLUTE® SLE+ 400 µL capacity 96-well plates or columns.

Matrix Preparation:

Weigh 10 mg of nail into 2 mL Biotage® Lysera tubes containing 5 x 2.4 mm stainless steel beads.

Internal Standard ISTD: 1 ng/mg of nail.

Direct solvent extraction post pulverization: Add 1 mL methanolic 0.1% (v/v) NH_4OH to each nail sample.

Pre-concentration post pulverization: Add 1 mL of methanol to each nail sample.

Micropulverisation (MPE) Procedure:

Biotage® Lysera: 8 x 45 s cycles at 6.95 m/s with 45 s dwell.

Centrifuge extracts for 10 minutes at 13,300 rpm. Remove aliquot for pre-concentration or proceed for direct extraction.

Sample Application:

200/400 µL of supernatant was applied directly to ISOLUTE® SLE+ sorbent in the case of direct extraction methodology. For pre-concentration methodology, up to 400 µL of supernatant was transferred and evaporated using either: Biotage® SPE Dry (plate processing) or TurboVap® LV (column processing) at 20 °C to reduce evaporative losses of amphetamines. Samples were reconstituted in up to 400 µL 50:50 methanol:water and loaded.

Analyte Extraction:

DCM/IPA (95/5, v/v, 600 µL) was applied and allowed to flow under gravity for 5 minutes. MTBE (600 µL) was applied and allowed to flow under gravity for 5 minutes. A pulse of positive pressure at 10 psi (10-20 seconds) allowed complete removal of the final aliquot.

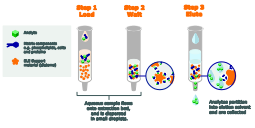

Figure 1. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.*

Post extraction: Extracts were evaporated at 40 °C in the presence of 100 µL of 50 mM HCl in MeOH (in order to avoid evaporative losses of amphetamines) and reconstituted with 200 µL of 70:30 mobile phase.

Manual Sample Preparation

All extraction protocols were developed using a semi-automated 48/96 position positive pressure manifold prior to method conversion to the Biotage Extrahera™ platform.

Biotage Extrahera™ Automated Sample Preparation Platform

The optimized extraction protocols were transferred to an automated sample preparation platform, equipped with an 8 channel pipetting head and positive pressure processing functionality. The system is interconvertible between 4 and 8 channel pipetting into 24 (6 x 4 arrangement) columns or 96-well plates, respectively. The Extrahera™ platform is shown in ***Figure 2***.

Figure 2. *Biotage Extrahera™ automated sample preparation platform.*

LC/MS Conditions

Instrument: Shimadzu 8060 Triple Quadrupole mass spectrometer equipped with and ES interface for mass analysis (Shimadzu Europa GmbH, Duisburg, Germany). Positive ions were acquired in the multiple reaction monitoring (MRM) mode.

Column: Restek Raptor™ Biphenyl 2.7 µm (100 x 2.1 mm) with EXP guard cartridge (Thames Restek UK Ltd., Saunderton, UK.)

Mobile Phase: A: 2 mM Ammonium Formate (aq) 0.1% formic acid

Mobile Phase B: 2 mM Ammonium Formate (MeOH), 0.1% formic acid

Flow Rate: 0.4 mL/min

Gradient and MRM transitions: Details on Biotage.com

Heat Block Temp: 400° C; **Interface Temp:** 300 °C;

DL Temp: 250 °C

Nebulizing Gas: 3 L/min; **Drying Gas:** 3 L/min;

Heating Gas: 17 L/min; **CID Gas:** 270 kPa

Results

Figure 3. illustrates the work-flow options investigated for nail analysis in our study. MeOH was added to nail samples allowing drug release post pulverization. Aliquots were then either directly extracted or pre-concentrated prior to extraction.

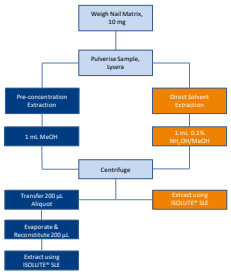

Figure 3. *Workflow investigation for nail analysis.*

Figures 4 and 5. demonstrate 96-well plate recovery profiles using direct solvent extraction or analyte pre-concentration. For both options a range of extraction solvents provided high reproducible recoveries for most analytes. However, the best extraction was provided by 600 µL DCM or DCM/IPA (95/5, v/v) followed by 600 µL MTBE.

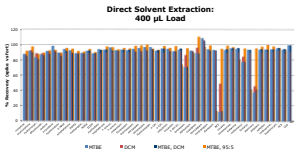

Figure 4. *Recovery profiles from direct extraction of pulverized nail.*

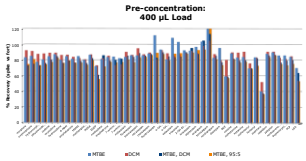

Figure 5. *Recovery profiles from pre-concentration of pulverized nail.*

Manual methods were then converted to the Extrahera™ automated sample preparation platform. ***Figures 6 and 7.*** demonstrate recovery profiles of the two best elution solvent combinations loading either 200 or 400 µL of pulverized nail extract.

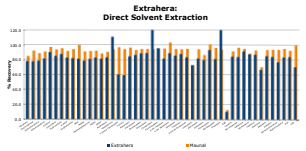

Figure 6. *Recovery profiles comparing manual vs automated sample preparation for direct loading methodology.*

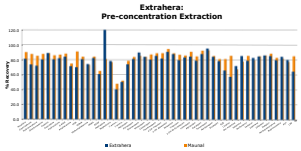

Figure 7. *Recovery profiles comparing manual vs automated sample preparation for pre-concentration methodology.*

Method performance was replicated using the ISOLUTE® SLE+ 400 µL capacity columns (recovery data not shown). Calibration curves constructed from 1-1000 pg/mg of nail demonstrated good linearity with all analytes returning coefficients of determination (r^2) greater than 0.99. ***Figures 8 and 9.*** demonstrate typical calibration curves for direct and pre-concentrated nail extracts using 95/5 DCM/IPA (v/v) followed by MTBE elution.

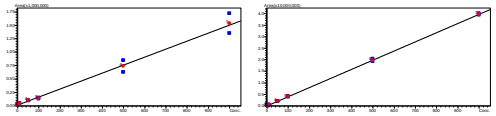

Figure 8. *Calibration curve for methamphetamine and BZE using direct solvent extraction.*

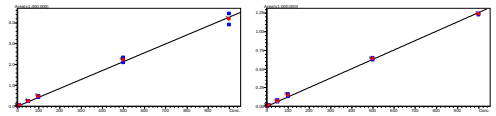

Figure 9. *Calibration curve for 6-MAM and buprenorphine using pre-concentration methodology.*

Lower limits of quantitation (LLOQ) for direct solvent extraction using 95/5 followed by MTBE elution are summarized in ***Table 1.***

Table 1. *Drug LLOQ values.*

Drug Analyte	LLOQ pg/mg	Drug Analyte	LLOQ pg/mg
Morphine	1	α-OH Triazolam	< 5
Oxymorphone	< 1	α-OH Alprazolam	5
Hydromorphone	< 1	Estazolam	1
Dihydrocodeine	< 1	Triazolam	< 1
Codeine	< 1	2-OH-Et-flurazepam	1
Oxycodone	< 1	Lorazepam	10
Hydrocodone	< 1	Alprazolam	5
6-MAM	< 1	Oxazepam	5
Amphetamine	1	Temazepam	1
MDA	5	Nordiazepam	1
MDMA	< 1	Diazepam	1
Methamphetamine	< 1	BZE	< 1
MDEA	< 1	Cocaine	< 1
EDDP	< 1	Nor-ketamine	< 1
Methadone	5	Ketamine	< 1
Mephedrone	< 1	Norfentanyl	< 1
7-amino Clonazepam	< 1	Fentanyl	< 1
7-amino Flunitrazepam	1	Zopiclone	1
Midazolam	< 1	Zolpidem	< 1
Flurazepam	< 1	Zaleplon	< 1
Bromazepam	< 5	Norbuprenorphine	5
Nitrazepam	1	Buprenorphine	< 1
Clonazepam	1	PCP	< 5
Flunitrazepam	1	LSD	5

Conclusion

- This poster describes an improved workflow for the analysis of drugs of abuse from nail samples.
- Implementation of bead homogenization along with automated sample preparation allowed for simplified methodology.
- The direct solvent extraction approach avoids the need for pre-concentration while maintaining desired LOQs with either 200 or 400 µL of nail extract.

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