Evaluation of Simplified Workflow for Hair Matrix Extraction Prior to UPLC-MS/MS Analysis

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Introduction

Hair analysis is growing in popularity due to the non-invasive nature of the sample collection. Although not used as routinely as other matrices such as blood or urine it does have advantages in that the matrix can indicate prolonged drug exposure. This can provide valuable information with respect to therapeutic drug regimens or in abused drug abstinence cases. As a result hair analysis can be viewed as a complementary technique to traditional blood and urine analysis. Sample preparation for hair analysis is often lengthy involving multiple manual labor steps from cutting, washing, homogenization/pulverization, digestion, sample extraction and analysis. This poster aims to demonstrate a streamlined sample preparation workflow for hair analysis.

Experimental

Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Ammonium hydroxide (28-32%), ammonium formate, hydrochloric acid (37%) formic and acetic acids 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). Hair samples were kindly donated by healthy human volunteers.

Sample Preparation

ISOLUTE® SLE+ Procedure (*Figure 1.*)

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

Matrix Preparation:

Weigh 20 mg of hair into 2 mL Biotage® Lysera tubes containing 4 x 2.4 mm stainless steel beads.

Internal Standard ISTD: 1 pg/mg of hair.

Direct solvent extraction post pulverization: Add 1 mL MeOH (cannabinoids) or methanolic 0.1% (v/v) NH_4OH (DoA) to hair samples.

Pre-concentration post pulverization: Add 1 mL of MeOH to samples.

Micropulverisation (MPE) Procedure:

Biotage® Lysera: 3 x 5.3 m/sec for 3 minutes with a 20s dwell. Centrifuge tubes 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 the pre-concentration methodology, extracts were transferred and evaporated at 20 °C followed by reconstitution in up to 400 µL 70/30 (cannabinoids) or 50:50 Methanol:Water (drugs of abuse panel).

Analyte Extraction:

MTBE (cannabinoids) or DCM/IPA (95/5, v/v, 600 µL) was applied and allowed to flow under gravity for 5 minutes. For both panels a second aliquot of 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.

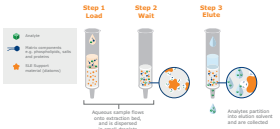

Figure 1. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.*

Post extraction: Extracts were evaporated at 40 °C in the presence of 100 µL of 50 mM HCl in MeOH (drugs of abuse panel only) in order to avoid evaporative losses of amphetamines.

LC/MS Conditions

Instrument: Shimadzu Nexera x2 UHPLC and an 8060 Triple Quadrupole mass spectrometer equipped with an ES interface for mass analysis (Shimadzu Europa GmbH, Duisburg, Germany). Positive/negative ions were acquired in the multiple reaction monitoring (MRM) mode. Full gradient and MRM available on Biotage.com.

Table 1. *LC/MS instrument parameters.*

Conditions	Drugs of Abuse Panel	THC panel
Column	Restek Raptor™ Biphenyl 2.7 µm (100 x 2.1 mm) with	ACE EXCEL C18 50 x 2.1 mm, 1.7 µm (ACT, UK)
Guard Column	EXP guard cartridge (Thames Restek UK Ltd., Saunderton, UK.)	
Mobile Phase A	2 mM Ammonium Formate (aq) 0.1% formic acid	0.01% Acetic Acid (aq)
Mobile Phase B	2 mM Ammonium Formate (MeOH) 0.1% formic acid	0.01% Acetic Acid (MeOH)
Flow Rate	0.4 ml/min	0.3 mL/min
Heat Block Temp.	400 °C	500 °C
Interface Temp	300 °C	400 °C
DL Temp	250 °C	300 °C
Nebulising gas	3 L/min	3 L/min
Drying Gas	3 L/min	5 L/min
Heating Gas	17 L/min	15 L/min
CID Gas	270 kPa	270 kPa

Results

Figure 2. illustrates work-flow options investigated for hair analysis in this study. MeOH was used to swell hair samples allowing drug release during micropulverization. Aliquots were either directly extracted or pre-concentrated prior to extraction as identified previously.

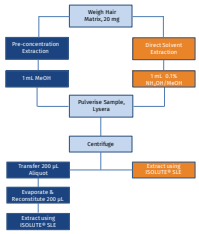

Figure 2. *Workflow investigation for hair analysis.*

Figures 3 and 4. demonstrate recovery profiles using direct solvent extraction or analyte pre-concentration for a drugs of abuse panel. A range of extraction solvents provided high reproducible recoveries for the majority of analytes using both loading regimens. For our panel 600 µL DCM or DCM/IPA (95/5, v/v) followed by 600 µL MTBE provided the best extraction performance.

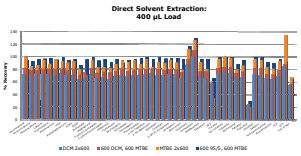

Figure 3. *Recovery profiles from direct extraction of pulverized hair.*

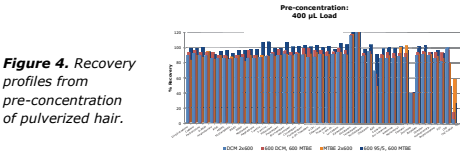

Figure 4. *Recovery profiles from pre-concentration of pulverized hair.*

Figure 5. demonstrates the final method performance using DCM/IPA (95/5, v/v) followed by MTBE with both pre-concentration and direct loading.

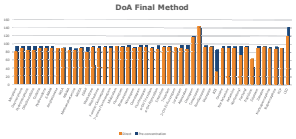

Figure 5. *Recovery profiles of the final methods.*

Initial investigation of the THC panel involved evaluation of non-specific binding during evaporation. As demonstrated in ***Figure 6.*** Non-specific binding to collection plates were reduced by increasing methanol to above 50% during reconstitution.

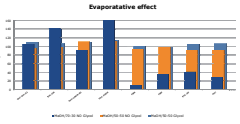

Figure 6. *Non-specific binding profiles during evaporation from 96-well collection plates.*

Figure 7. demonstrates elution solvent performance for analyte pre-concentration loading option. MTBE gave good reproducibility and recoveries as well as provided the best matrix factors and signal.

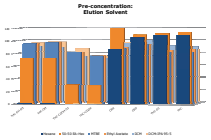

Figure 7. *Recovery profiles comparing elution solvents for analyte pre-concentration loading.*

Similar trends were observed using a direct loading strategy (data not shown). ***Figure 8.*** demonstrates final recovery comparison between direct and pre-concentrated hair matrix loading. Final calibration was only performed using pre-concentration loading regimen due to sensitivity.

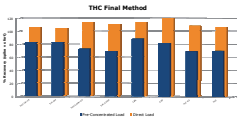

Figure 8. *Recovery profiles for final optimized loading methods.*

Figures 9 and 10. demonstrate typical calibration curves for direct and pre-concentrated hair extracts (DoA, 1-1000 pg/mg) while ***Figure 11.*** Details pre-concentration only (cannabinoids, 0.1-200 pg/mg). Excellent linearity was observed with all analytes returning coefficients of determination (r^2) greater than 0.99.

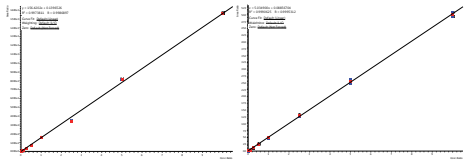

Figure 9. *Calibration curve for methamphetamine and BZE using direct hair solvent extracts.*

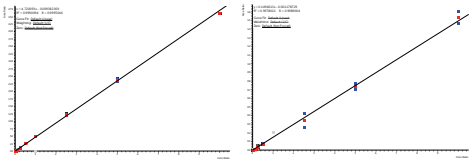

Figure 10. *Calibration curve for 6-MAM and buprenorphine using pre-concentrated hair extracts.*

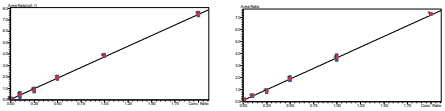

Figure 11. *Calibration curve for THC-COOH and THC using pre-concentrated hair extracts.*

Limits of quantitation for direct (DoA) and pre-concentration loading regimens (cannabinoids) are summarized in ***Table 2.***

Table 2. *Drug LLOQ values (**cannabinoids separate analyses**).*

Drug Analyte	LOQ pg/mg	Drug Analyte	LOQ pg/mg
Morphine	1	α-OH Triazolam	5
Oxymorphone	1	α-OH Alprazolam	10
Hydromorphone	1	Estazolam	<1
Dihydrocodeine	<1	Triazolam	1
Codeine	<1	2-OH-Et-flurazepam	<10
Oxycodone	1	Lorazepam	10
Hydrocodone	1	Alprazolam	<1
6-MAM	1	Oxazepam	1
Amphetamine	1	Temazepam	1
MDA	25	Nordiazepam	5
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	5
Midazolam	1	Zolpidem	<1
Flurazepam	<1	Zaleplon	1
Bromazepam	5	Norbuprenorphine	50
Nitrazepam	5	Buprenorphine	1
Clonazepam	5	PCP	1
Flunitrazepam	10	LSD	1
THC	***10***	***CBN***	***10***
THC-OH	***10***	***CBD***	***0.5***
THC-COOH	***0.2***	***THCAA***	***<10***

Conclusion

- This poster describes an improved workflow for the analysis of various drugs of abuse panels from hair.
- Implementation of bead homogenization allowed for simplified matrix processing.
- Loading is dependent on analyte panel and LOQ requirements.

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