Extraction Efficiency Table.

A Generic Extraction Method for Basic Drugs of Abuse from Urine Samples

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Abstract

The rationale behind using the Plexa DAS SPE cartridge is to allow a generic extraction method to be used across a range of drug classes (Opiates (inc. 6-MAM) /Amphetamines /Cocaine(Benzoylecgonine) /Methadone /Benzodiaziapine /Ketamine /Zopiclone from urine.

Allowing single extraction methods to be utilized across a range of drugs thus minimizing the number of different solvents and buffers required. This minimises the possibility of human errors being made when working up the samples.

This work was undertaken using an Agilent 6410 LC-MS/MS to analyse the samples. The triple quad does not necessarily require complete resolution of the target compounds by the LC as the MS detector works by filtering the targeted ions through the MS. The initial filter only allows the target (precursor ions) through into the collision cell. Once fragmented only the quantification and qualification ions are passed through to the detector

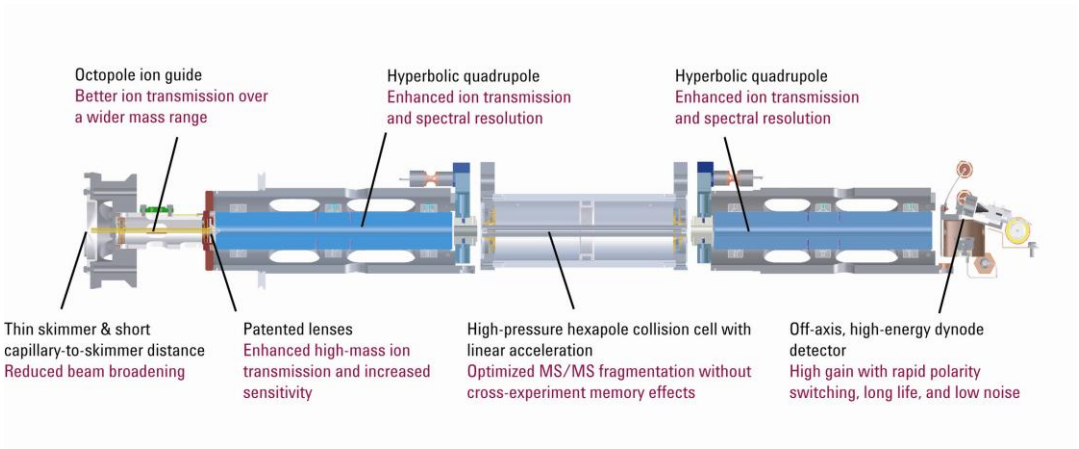


Figure 1: Ion optics are optimised for ion transmission across a broad mass range

Extraction method and Analysis Parameters

Sample Pretreatment : Each class will have a different initial work up, if an acid or enzymatic hydrolysis is required. So long as the sample is buffered to pH6 and below the sample can be placed straight on the DAS cartridge.

The SPE cartridges were:
Conditioned with 1mL of Methanol followed by 1mL Water prior to sample being loaded.

SPE Cartridge Wash: 1mL 0.1mol/L Hydrochloric acid, followed by 1mL 60:40 Methanol/0.1mol/L Hydrochloric acid solution.

The cartridges were then dried for 2 minutes.

The samples were extracted with 150µL of Methanol/Acetonitrile (50/50), followed by two aliquots 150µL of Methanol/Acetonitrile/Ammonium Hydroxide (50/50/2).

Once extracted the samples were dried down and then re-constituted into 150µL of Methanol/water solvent (ratio dependent on the class).

HPLC Method / Instrumentation

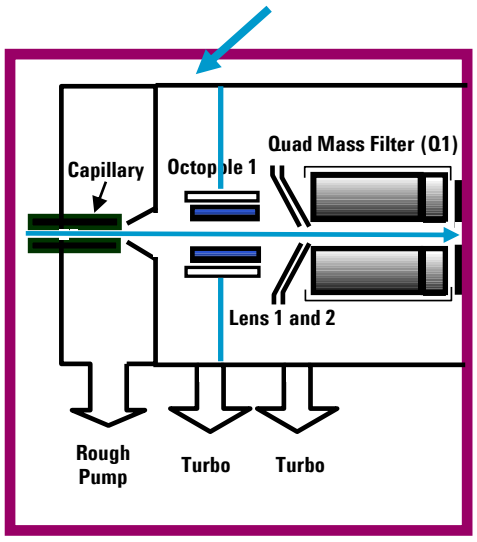
The chromatography was performed on an Agilent Zorbax C18 XDB 1.8u, 50 x 4.6mm reverse phase column at 50ºC.

Mobile phase composition:
Solvent A: 5mM Ammonium Acetate with 0.001% Formic acid
Solvent B: Acetonitrile with 0.001% Formic acid
Isocratic flow with ...(different ratios of solvent A and B respectively dependant on analyte)
Flow rates: 0.5 to 0.8mL/min

Extraction Efficiency Table

Compound	Water extracted		Urine extracted	
	% Recovery	% RSD	% Recovery	% RSD
6-MAM	109.23	10.7	105.6	2.02
Dyhydrocodeine	105.03	3.09	102.61	1.22
Codeine	108.52	3.30	108.22	1.99
Morphine	107.63	2.61	104.48	1.65
Oxazepam	50.30	4.54	49.74	6.52
Nor-diazepam	67.13	6.15	66.33	3.92
Flunitrazepam	89.72	3.64	92.23	3.73
Diazepam	82.44	2.79	81.68	3.08
7 Aminonitrazepam	38.22	2.34	34.98	18.51
7 Aminoflunitrazepam	61.91	5.06	53.37	5.78
Temazepam	70.66	2.33	72.24	3.28
Amphetamine	64.13	10.20	63.19	6.77
Methamphetamine	87.66	8.51	87.76	2.42
MDA	50.56	14.67	48.45	10.86
MDMA	78.90	8.21	81.10	2.95
MDEA	81.11	8.38	82.56a	2.18
Benzoylecgonine	92.95	6.54	93.71	2.70
Methadone	85.94	7.06	85.08	6.77
EDDP	8.16	26.47	9.19	23.16
Ketamine	100.77	0.54	98.47	0.65
Nor-Ketamine	106.51	5.68	86.24	6.10
Zopiclone	71.85	7.50	48.25	12.60
Zopiclone-Oxide	143.92	3.84	70.30	12.86

Innovations in Front-End Ion Optics Deliver Better Sensitivity Across a Broad Mass Range



10X sensitivity advantage

Key components contributing to sensitivity

- Dielectric capillary
- Small diameter octopole ion guide
- High frequency RF octopole
- Lens 2 RF (transmission of higher masses)
- Hyperbolic post-filter and quadrupole

Summary.

This work demonstrates that it is possible to use a single generic extraction method for a range of basic drugs from urine, using the cut off limits from the UK Workplace and SAMSHA guidelines. While some of the drugs may have poor recoveries; this is off set by the reproducibility and sensitivity of the Agilent 6410 LC-MS/MS.