

An on-line turbulent flow extraction LC-MS/MS screen for the quantitative analysis of multiple classes of illicit drugs in both plasma and urine

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Overview

Purpose: To investigate the use of turbulent flow extraction chromatography for the detection of multiple drugs of abuse directly from plasma and urine.

Methods: A Turboflow™ Transcend TLX1 extraction system was used with a Quantum Ultra™ triple quadrupole mass spectrometer to analyse raw spiked rat plasma and human urine solutions.

Results: The turboflow extraction system allowed direct injection of spiked plasma and urine solutions onto the LC-MS/MS system. The assay was fast (data acquisition time < 10 minutes) and covered a wide concentration range (0.5 – 500 ng mL⁻¹).

Introduction

Recently, forensic and toxicology laboratories have moved to LC-MS analyses for drugs of abuse to eliminate the derivatisation step associated with GCMS, but a rigorous solid-phase extraction step is still required. In addition, many LC-MS/MS methods for the analysis of drugs of abuse only identify one class of drug compound. The Transcend™ TLX system allows the direct injection of biological matrices onto the LC-MS system, minimizing sample preparation (Figures 1, 2 and 3). Selected reaction monitoring (SRM) on the TSQ Quantum Ultra™ triple quadrupole mass spectrometer (Figure 4) is used here for quantification of over twenty compounds from many classes of drugs of abuse from both plasma and urine.

Methods

Step 1: 28 drug of abuse compounds were spiked into rat plasma or human urine to generate solutions across the concentration range of the assay. Samples were centrifuged prior to analysis.

Step 2: Inject 10 µL of raw plasma/urine onto Cyclone-MAX™ 0.5 x 50 mm extraction column. Back-flush onto Hypersil™ Gold aQ 2.1 x 50 mm, 5 µm analytical column.

Step 3: Heated electrospray (HESI™) ionisation was utilised with a triple quadrupole mass spectrometer in positive ion SRM mode.

Turboflow extraction chromatography

High turbulence liquid chromatography (HTLC) - a system for online sample preparation and sample purification

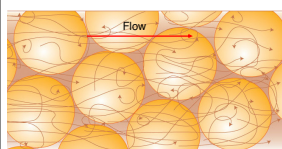


FIGURE 1.

Large particle columns (30 µm or larger) allow a high flow rate with low back pressure

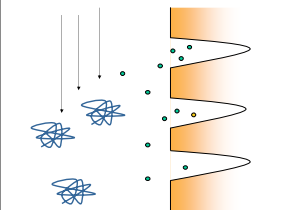


FIGURE 2.

Small molecules diffuse into pores of particles, large molecules (from matrix) do not diffuse and are washed to waste

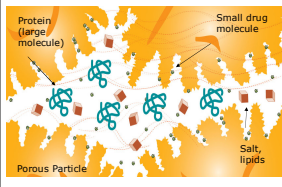


FIGURE 3.

The separation is bimodal, since additional retention is achieved with different column chemistries

FIGURE 4. Turboflow online extraction with a Thermo Scientific Quantum triple quadrupole MS.



Results

Drug type	Analyte	LOQ ng mL ⁻¹	%RSD
Depressant	7-aminonitrazepam	1	19.8
	Norketamine	1	14.4
	Ketamine	0.5	19.1
	Oxazepam	5	16.0
	Nordiazepam	5	19.7
	Nitrazepam	1	21.6
	Alprazolam	5	18.9
	Temazepam	50	12.85
	Clonazepam	10	22.4
	Mirtazapine	0.5	9.3
Hallucinogen	MDEA	0.5	9.1
	LSD	0.5	12.4
	PCP	0.5	13.5
	MBDB	0.5	17.1
	Morphine	5	11.6
Opioid	Codeine	5	21.4
	Methadone	0.5	3.4
	Buprenorphine	5	10.9
	Buprenorphine glucuronide	100	27.9
	Norbuprenorphine	5	18.7
	Norbuprenorphine glucuronide	0.5	17.6
	EDDP	0.5	9.2
Stimulant	Amphetamine	5	13.06
	Metamphetamine	0.5	18.0
	MDA	10	7.8
	Benzoylcegonine	0.5	15.7
	Cocaine	0.5	4.5
MDMA	1	5.1	

Table 1. Calculated limits of quantitation (LOQ) for each drug of abuse analyte spiked into rat plasma. Intra assay variability at the LOQ is presented as %RSD from five replicate injections.

Drug type	Analyte	LOQ ng mL ⁻¹	%RSD
Depressant	7-aminonitrazepam	1	12.9
	Norketamine	1	23.9
	Ketamine	1	19.2
	Oxazepam	5	16.3
	Nordiazepam	0.5	4.9
	Nitrazepam	1	14.5
	Alprazolam	1	14.6
	Temazepam	1	6.8
	Clonazepam	5	24.3
	Mirtazapine	0.5	17.9
Hallucinogen	MDEA	0.5	13.6
	LSD	0.5	5.1
	PCP	0.5	6.4
	MBDB	1	19.9
	Morphine	5	14.4
Opioid	Codeine	5	8.1
	Methadone	0.5	2.8
	Buprenorphine	10	26.8
	Buprenorphine glucuronide	100	27.9
	Norbuprenorphine	50	12.5
	Norbuprenorphine glucuronide	10	20.6
	EDDP	0.5	4.3
Stimulant	Amphetamine	5	11.6
	Metamphetamine	0.5	11.0
	MDA	1	18.2
	Benzoylcegonine	1	19.4
	Cocaine	0.5	9.6
MDMA	0.5	15.6	

Table 2. Calculated limits of quantitation (LOQ) for each drug of abuse analyte spiked into human urine. Intra assay variability at the LOQ is presented as %RSD from five replicate injections.

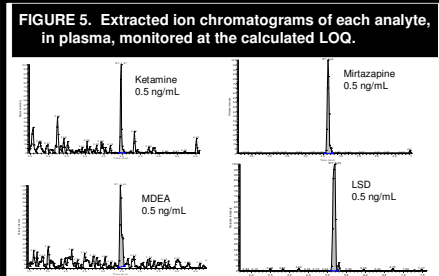
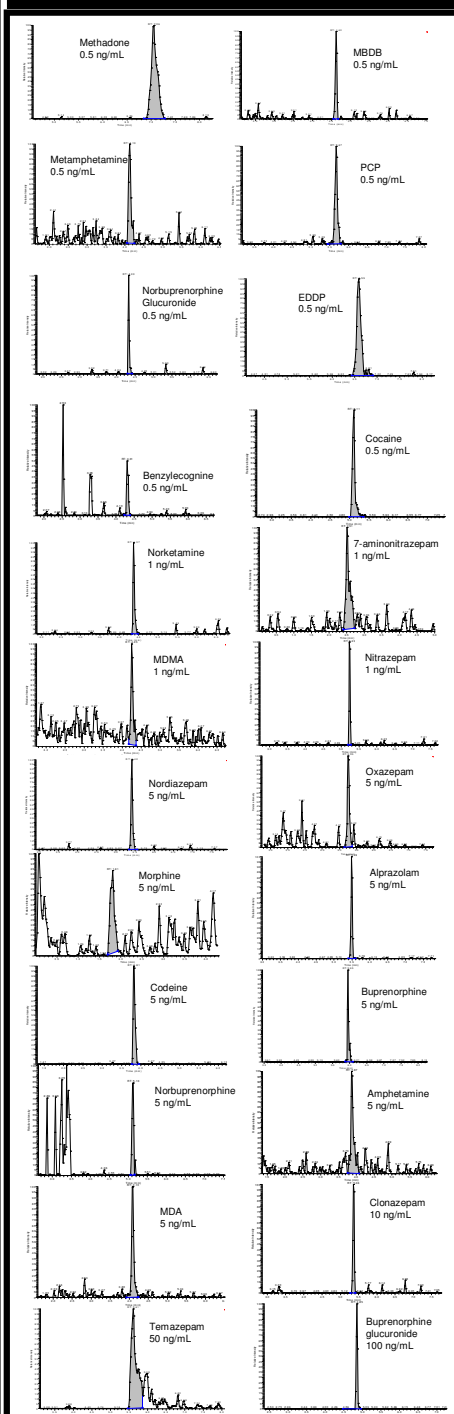


FIGURE 5 continued:



Conclusions

A fast (data acquisition time < 10 min) and quantitative method for the detection of multiple drug of abuse compounds in rat plasma and human urine is described (Figure 5). Minimal sample preparation is required and the assay covers a wide concentration range (0.5 – 500 ng mL⁻¹). Intra assay variability (< 20% RSD for 5 replicate injections) was assessed at the lower limit of quantitation (Tables 1 and 2).