Application Note : 1901

Urine LDTD Shotgun Screening:

Drugs of abuse in Urine at 9 seconds per sample using Dry and Dissolve easy preparation

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Introduction

Screening various drug classes in urine samples traditionally requires several different immunoassay reagents. In order to reduce the number of screening assays, some laboratories are transitioning to mass spectrometry to allow accurate and broad detection of drugs using a single screening method. The drugs of interest have different polarities and require a long LC chromatographic analysis to separate and elute these different chemical families. To increase the sample analysis throughput, LDTD® -MS/MS using fast ß-glucuronide digest and simple extraction method is developed as screening tool.

Goal: Use Dry and Dilute sample preparation method for LDTD-MS/MS screening of all compounds in a single operation.

LDTD®-MS/MS offers specificity combined with an ultra-fast analysis for an unrivaled screening method. To develop this application, we focused on performing a fast and simple preparation method. 124 drugs of abuse from different classes (opioids, benzodiazepines, amphetamines, barbiturates, cocaine, etc.) are analyzed simultaneously with quantitative screening results obtained in less than 9 seconds per sample. Attained cut off values are specific to the individual drugs (not a family) and generally at a much lower value than with EIA.

Luxon Ionization Source

The Luxon Ion Source® (**Figure 1**) is the second-generation sample introduction and ionization source based on the LDTD® technology for mass spectrometry. Luxon Ion Source® uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity giving more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high intensity molecular ion signal in less than 1 second sample to sample and allows working with very small volumes.

Figure 1 - Luxon Ion Source®

Figure 2 - Schematic of the Luxon ionization source

Sample Preparation Method

Sample hydrolysis process in a 96 Deep Well plate:
10 µL urine sample
25 µL internal standard mixture with

- 5 % internal standard solution in methanol
- 20 % ß-glucuronidase
- 75 % Hydrolysis buffer

Vortex and incubate at 55°C for 30 minutes.

- Add 400 µL of MeCN containing 0.5% Formic Acid
- Vortex
- Evaporate to dryness
- Dissolve with 250 µL of MTBE containing 0.05% HCl 1N
- Vortex
- Add 250 µL MTBE containing 0.1% NaOH 1N
- Vortex

Option 1

- Spot 5 µL on a LazWell™96 plate
- Dry 1 minute at room temperature

Option 2 (acidic compounds)

- Transfer 150 µL to a new 96-well plate
- Add 50 µL of KH_2PO_4 solution at 1.25 mM in MeOH-H_2O (80:20%)
- Vortex
- Spot 5 µL on a LazWell™96 plate
- Dry 8 minutes with convection at 40 °Celsius

LDTD-MS/MS Parameters

LDTD

Model: Phytronix, Luxon S-960
Carrier gas: 3 L/min (air)
Laser pattern: 6 second ramp to 55% power and hold 2 seconds

MS/MS

MS model: Sciex 5500 QTrap®
Scan Time: 5 msec
Total run time: 9 seconds per sample
Ionization: APCI
Analysis Method:

- Selected MRM transition with 5 msec dwell time for up to 60 compounds per desorption
- Possibility of switching the polarity of the MRM method

"A la carte" Drug list

Toxicology laboratories can determine their own drug list to screen choosing the associated MRM in the list of compounds from **Table 1**. They can also add transitions for molecules that are not listed or newly designed drugs. We recommend a maximum of 60 transitions of 5 msec dwell time per desorption to achieve the best sensitivity and throughput.

Examples of screening choices:

- Short list of 40 compounds with polarity switching, 1 desorption.
- Best cut-off sensitivity of all compounds separated in 3 desorptions.
- All-in-one analysis of 124 compounds at slightly higher cut off values with a 3 msec dwell time, 1 desorption.

Results and Discussion

Precision

Spiked samples around the decision point and blank solutions are used to validate the precision of the method. Each concentration must not exceed 20% CV and the mean concentration ± 2 times the standard deviation must not overlap with other concentrations at the decision point. The peak area against the IS ratio was used to normalize the signal. Replicate extractions are deposited on a LazWell™ plate and dried before analysis. No overlapping at the decision point is observed for all curves and the CV% was below 15% for within-run experiments. Results using the ± 2 STD overlay are plotted at **Figure 3.** The results of within-run test for Fentanyl allow a cut off value of 2 ng/mL in urine. All cut-offs of different drugs are determined this way.

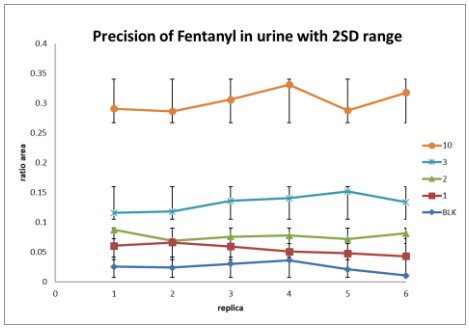

Figure 3 – Within-run Precision curves for Fentanyl

Table 1 - Compounds tested with associated cut off value

Compounds	Luxon Screen (ng/mL)	Compounds	Luxon Screen (ng/mL)
25I-NBOMe	5	Ritalinic Acid	100
6-MAM	10	Secobarbital	100
Alprazolam	10	Tapentadol	8
Aminoflunitrazepam	50	Temazepam	20
Amitriptyline	20	THC-COOH	25
Amobarbital	100	Tramadol	25
Amphetamine	50	Zaleplon	10
Benzoylecgonine	10	Zolpidem	4
Buprenorphine	10	Duloxetine	100
Bupropion	10	Norquetiapine	25
Butalbital	100	Paroxetine	10
Carisoprodol	10	Quetiapine	5
N-Desmethyl Citalopram	10	Sertraline	10
Citalopram	10	Trazodone	10
Clonidine 1	100	5F-PB-22	10
Codeine	50	AB-FUBINACA	10
Cotinine	10	AB-PINACA 5-Pentanoic aci	10
Cyclobenzaprine	5	AM-2201 4-HYDROXYPENT	10
Desipramine	10	JWH-018	5
Desmethyltapentadol	100	JWH-073	2
Desmethyltramadol	10	MAM-2201	(hydroxypentyl) 10
Dextromethorphan	5	PB-22	10
Dextrorphan	10	UR-144 5 Pentanoic Acid	50
Diazepam	20	XLR-11	(hydroxypentyl) 10
Doxepin	100	4-Ethylmethcathinone	10
EDDP	150	ALPHA-PVP	20
Fentanyl	2	Butylone	10
Fluoxetine	20	Ethylone	10
Haloperidol	10	MDPV	10
Hydrocodone	50	Mephedrone	10
Hydromorphone	50	Methylone	10
Hydroxyalprazolam	100	Naphyrone	10
Hydroxybupropion	40	Pentylone	10
Imipramine	10	Mitragynine	10
Ketamine	4	O-Desmethyl Tramadol	100
Lorazepam	20	Venlaflaxine	20
MDA	40	Risperidone	10
MDEA	5	7-Hydroxy-Mitraginine	10
MDMA	10	9-Hydroxy-Risperidone	10
Meperidine	20	Dehydroxy-Aripiprazole	40
Meprobamate	100	4-ANPP	5
Methadone	50	N-Desmethyl-U 47700	5
Methamphetamine	50	U 47700	10
Methylphenidate	10	Clonazepam	20
Morphine	50	Phentermine	25
N-desmethyl mirtazapine	10	mCPP	40
Naloxone	10	Acetyl Norfentanyl	5
Naltrexone	20	Butyryl Norfentanyl	20
6-B-Naltrexole	20	Cys-3- methyl Norfentanyl	20
Norbuprenorphine	10	Nor-Carfentalyl	10
Nordiazepam	5	Acetyl Fentanyl	2
Norfentanyl	8	Acryl Fentanyl	2
Norketamine	8	Norfluoxetine	40
Normeperidine	20	Butyryl Fentanyl	10
Norpropoxyphene	50	Cys-3-Methyl Fentanyl	10
Nortriptyline	10	Furafentanyl	2
Oxazepam	100	Carfentanyl	5
Oxycodone	20		
Oxymorphone	20	Creatinine (dilution only)	50 (µg/mL)
Phencyclidine	10	EtG (dilution only)	500
Phenobarbital	100	Gabapentin (dilution only)	500
Propoxyphene	20	Pregablin (dilution only)	500

Conclusion

Luxon Ion Source® combined to Q-Trap 5500 mass spectrometer system allows ultra-fast (**9 seconds per sample**) screening of drugs in urine sample using a simple generic sample preparation method.

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