Fast and Selective screening of 34 drugs of abuse in urine using LDTD-TripleTOF™ 5600 System

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Overview

- Screening and quantification methods for drug analysis in urine using high resolution mass spectrometer
- Sample preparation consists of a quick liquid-liquid extraction procedure
- **Run time of 7 seconds sample to sample**

Instrumentation (Figure 1)

- Phytronix Technologies LDTD ion source (model S-960);
- AB SCIEX, TripleTOF™ 5600 System.

Figure 1 LDTD interfaced to AB SCIEX TripleTOF™ 5600 System

Introduction

Toxicology Laboratories generally use screening methods based on immunoreactivity. The selectivity of these automated instruments unfortunately allows for cross-interference caused by similar drugs. The cost of the reactants (enzymes or antibodies) could also amount to be very expensive. The high throughput LDTD™ ion source coupled with a high resolution mass spectrometer TripleTOF™ 5600 System constitutes an ultra-fast screening system for small molecules in urine. In this application we demonstrate how 34 drugs of abuse are quantified simultaneously in 7 seconds per sample.

Samples Preparation

The following drugs were spiked in urine at two times the cut off value used in typical screening procedure: Fentanyl, Norfentanyl, Propoxyfene, Norpropoxyfene, Methadone, EDDP, Codeine, Morphine, Oxycodone, Oxymorphone, Diazepam, Estazolam, Hydroxyalprazolam, Hydroxyethylflurazepam, Hydroxymidazolam, Hydroxytriazolam, Lorazepam, Nordiazepam, Oxazepam, Temazepam, 7-aminoclonazepam, 7-aminoflunitrazepam, Chlordiazepoxide, Meparidine, Normeperidine, THC-COOH, amobarbital, butalbital, Phenobarbital, Secobarbital, JWH-018 5-OH, JWH-073 4-OH, JWH-018 5-COOH, JWH-073 4-COOH. The urine samples are diluted two-fold in order to obtain the cut off concentration and a second dilution is done to obtain 50% of the cut off.

Liq-Liq extraction (Basic drug)

- 50 µL urine sample
- 50 µL Internal standard solution dissolved in NaOH (0.2N)
  - Vortex
- 200 µL Ethyl acetate
  - Vortex and centrifuge (14000rpm/2min)
  - Spot: 4 µL on 96 LazWell*

Liq-Liq extraction (Acid drug)

- 50 µL urine sample
- 50 µL Internal standard solution dissolved in HCl (0.2N)
  - Vortex
- 200 µL 1-chlorobutane
  - Vortex and centrifuge (14000rpm/2min)
  - Spot: 4 µL on 96 LazWell*

***LazWells should be pre-coated with EDTA:**

- Spot onto LazWell plate 8µL of an EDTA solution (216 µg/ml in MeOH/Water/NH_4OH (75/24/1))
- Evaporate to dryness

LDTD parameters

The analysis is performed using a volume of 4 µL. The sample extract is spotted into the 96 LazWell plate and allowed to dry at room temperature. The carrier gas flow is set at 3 L/min and the laser pattern is characterized by: 2 seconds at 0 % of laser power, 3 seconds ramp to 45 % of power, 1 seconds plateau at 45 % and shut down to 0 % in 0.01 seconds. ***Figure 2*** shows the extracted mass from TIC chromatogram for Nordiazepam. The four (4) concentration samples are analyzed sequentially following the Laser pattern demonstrated underneath the figure. All the other drugs are desorbed simultaneously, and their results are extracted from the same file. Acquisition may be conducted in one sample per file or all samples in one file at the user's preference. Multiquant™ software allows quantification in both modes.

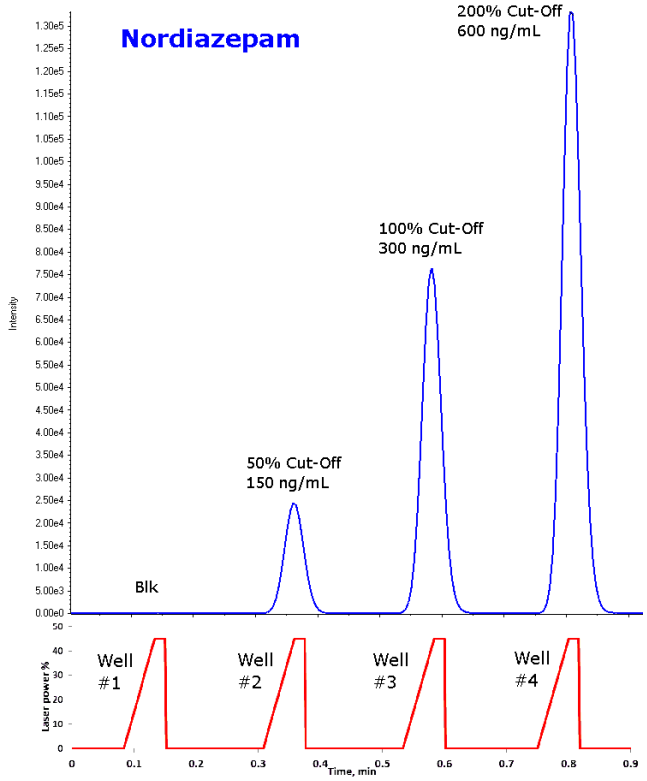

Figure 2: Nordiazepam curve from urine extract (XIC of 271.073 ± 5ppm). Each desorption is done in 7 seconds and desorbs all 34 compounds simultaneously.

Table 1 : Results of 34 compounds extracted in urine sample and calculated attainable cut off values at 50% blank interference level.

	Range (ng/ml)	Typical Cut Off (ng/ml)	Estimated Cut Off Acidic conditions (ng/mL)	Estimated Cut Off Basic Conditions (ng/mL)
7-aminoclonazepam	150 - 600	300	34	5.6
7-aminoflunitrazepam	150 - 600	300	-	17
Amobarbital	150 - 600	300	19	-
Butalbital	150 - 600	300	31	-
Chlordiazepoxide	150 - 600	300	-	8.6
Codeine	150 - 600	300	-	18
Diazepam	150 - 600	300	6.3	3.6
EDDP	150 - 600	300	243	70
Estazolam	150 - 600	300	2.9	1.4
Fentanyl	100 - 400	200	-	10
JWH 018 5-COOH	5 - 20	10	3.5	1.4
JWH 018 5-OH	5 - 20	10	4.4	4.0
JWH 073 4-COOH	5 - 20	10	4.7	-
JWH 073 4-OH	5 - 20	10	5.4	6.7
Lorazepam	150 - 600	300	0.7	0.6
Meparidine	50 - 200	100	-	5.0
Methadone	150 - 600	300	8.8	1.8
Morphine	150 - 600	300	-	122
Nordiazepam	150 - 600	300	13	2.5
Norfentanyl	100 - 400	200	-	14
Normeperidine	50 - 200	100	-	13
Norpropoxyfene	150 - 600	300	-	10
OH-alprazolam	150 - 600	300	13	1.4
OH-ethylflurazepam	150 - 600	300	0.8	0.5
OH-midazolam	150 - 600	300	-	4.7
OHtriazolam	150 - 600	300	0.9	0.9
Oxazepam	150 - 600	300	2.1	1.3
Oxycodone	150 - 600	300	-	10
Oxymorphone	150 - 600	300	-	59
Phenobarbital	150 - 600	300	18	-
Propoxyfene	150 - 600	300	-	114
Secobarbital	150 - 600	300	59	-
Temazepam	150 - 600	300	1.5	1.5
THC-COOH	25 - 100	50	10	-

Results and Discussion

LDTD-TripleTOF™ 5600 System operated in scanning mode (High Resolution) allows measurement of all ionizable compounds in the extract. The system scans with a mass range between 100 to 900 amu. Extracting the signal from a mass window of 10 ppm gives sufficient specificity to quantify molecules of the same nominal mass. The APCI ionization performed in positive or negative mode covers the properties of the set of molecules targeted. Analysis of the 34 drugs in a urine sample needs 2 desorptions (positive and negative) of 7 seconds. With this technique, all the drugs are screened in the spiked urine sample in a range well below their cut off values (**Table 1**).

Conclusion

With the high resolution versatility of the new TripleTOF™ 5600 System and the high-throughput of the LDTD™ ion source, we achieved an ultra fast analysis method running 1 sample every 7 seconds to screen 34 common toxicology drugs in urine. Sample preparation consists of a quick liquid-liquid extraction spotted directly into Lazwell plates. This method detects the 34 examined drugs and quantifies them at concentrations well below their cut off values.