Forensic

Fast and Selective Quantification of Opiates in Urine using LDTD-TripleTOF® 5600 System

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Overview

- Quantification methods for Opiate analysis in urine using a high-resolution accurate mass spectrometer.
- Sample preparation consists of a solid phase extraction procedure in a 96-well format.
- **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 LC-MS/MS or GC-MS methods for the quantification of opiate drugs. The methods used typically have long run times, often longer than 10 minutes, in order to achieve an efficient separation of the isobaric compounds. The cost of wash solvents, mobile phase and columns also amount to be very expensive as well as having to consider the hazardous nature of the solvents used. The high throughput LDTD™ ion source coupled with a high resolution accurate mass AB SCIEX TripleTOF® 5600 System constitutes a Fast and Specific system for the quantification of opiates.

Sample Preparation

The following Opiate drugs (Codeine, Morphine, Hydrocodone, Hydromorphone, Oxycodone and Oxymorphone) were spiked in urine to obtain a standard curve from 50 ng/mL to 5,000 ng/mL.

An internal standard mixture containing the following solutions was prepared:

- 1.5 mL Deuterated solution (Morphine-d6, Codein-d6, Hydrocodone-d6, Hydromorphone-d6, Oxycodone-d6 and Oxymorphone-d3) at 4 µg/mL in Methanol
- 6 mL β-Glucuronidase solution
- 1.5 mL Sodium Acetate buffer (1M, pH5)

The glucuronide hydrolysis of 250 µL Urine sample:

- 75 µL Internal Standard mixture
  - Incubated at 60 °C for 2 hours.

MOX derivatization was performed as follows:

- 0.7 ml of MOX solution was added to the hydrolysed sample
  - 7% Methoxyamine(25-30%) in HCl solution (0.7%)
- The sample was mixed and Incubated at room temperature for 20 minutes.
- Then centrifuged for 3 minutes at 3500 rpm

The solid phase extraction (Clean screen, CSDAU, 100mg, 96 well) was performed as follows:

- Activation:
  - 1 ml Methanol
  - 1 ml Water
- Loading:
  - 1 ml hydrolyzed sample
- Wash:
  - 1 ml Water
  - 1 ml Sodium acetate (100mM, pH 4.5)
  - 1 ml Methanol
- Elution:
  - 1.5 ml Ethyl Acetate/Isopropanol/NH_4OH (80/15/5)
  - Evaporate to dryness.

The final derivatization and reconstitution was performed as follows:

- 100 µL PFB reagent was added
  - 0.1%(v/v)2,3,4,5,6-Pentafluorobenzyl bromide and 0.5%(v/v) N,N-diisopropylethylamine in Acetonitrile.
- The sample was capped, mixed and incubated at 40 °C for 30 minutes.
- Evaporated to dryness
- Reconstituted with 250µL of Methanol/Water (75/25)
- Spotted 2 µL in LazWell plate.

LDTD parameters

The analysis was performed using a volume of 2 µL. The sample extract was spotted onto the 96 LazWell plate and allowed to dry at room temperature. The carrier gas flow was set at 3 L/min and the laser pattern was characterized by: 2 seconds at 0 % of laser power, 3 seconds ramp to 55 % of power, 1 second plateau at 55 % and shut down to 0 % in 0.01 seconds. All the drugs were desorbed simultaneously, and their results extracted from the same file.

TOF-MS/MS parameters

The analysis was performed using the Product Ion mode on a TripleTOF® 5600 System. The inlet parameters for the source are: Ion Source Gases (GS1/GS2) at 0, Curtain Gas (CUR) at 10, Temperature (TEM) at 0 and Nebulizer Current (NC) at 3.0.

All product ions use 100 as Declustering Potential (DP), an accumulation time of 20 ms and a scan window from 100 to 350 amu in positive mode. **Table 1** summarizes the specific transitions for the compounds.

Drug ID	Precursor Ion	Product Ion	CE
Morphine	268	161.06 ± 0.01	40
		211.08 ± 0.01*	
		178.08 ± 0.01*	
		179.09 ± 0.01*	
Morphine-d6	274	163.07 ± 0.01	40
Codeine	282	175.07 ± 0.01	40
Codeine-d6	288	253.14 ± 0.01	40
Hydrocodone	329	170.09 ± 0.01	50
Hydrocodone-d6	335	173.11 ± 0.01	50
Hydromorphone	315	272.13 ± 0.01	30
Hydromorphone-d6	321	274.14 ± 0.01	30
Oxycodone	345	254.12 ± 0.01	40
Oxycodone-d6	351	260.15 ± 0.01	40
Oxymorphone	331	300.15 ± 0.01	30
Oxymorphone-d3	334	303.16 ± 0.01	30

*Transition used for confirmation only

Table 1 Analysis of Opiates on TripleTOF® 5600 System

Using the parameters in the previous table, standard curves (**Figure 2**) are generated for the quantification of unknown samples (r^2>0.99). 1007 real samples were tested and gave a good correlation between LC-MS/MS results and LDTD-MS/MS results. An average correlation of 0.9529 was found for all concentrations of molecules.

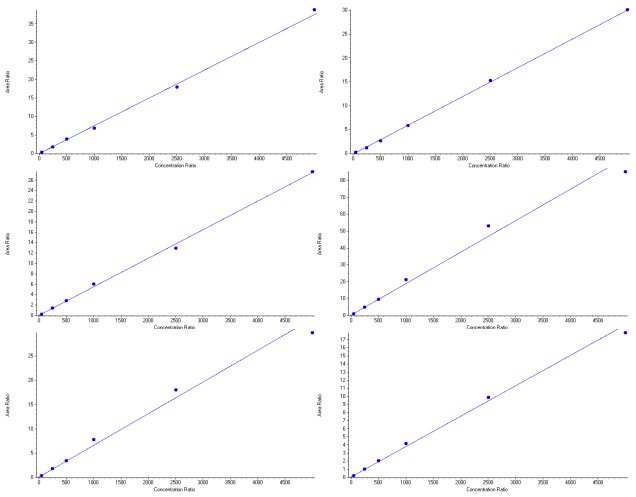

Figure 2 (From top to bottom, left to right) Hydromorphone, Codeine, Hydrocodone, Oxymorphone, Oxycodone and Morphine calibration curves.

Results and Discussion

The LDTD-TripleTOF® 5600 System was operated in the product ion mode. In this mode, a specific mass is selected in the Q1 quadrupole. The selected mass is then fragmented in the LINAC® collision cell and a High Resolution product ion scan is generated using the Accelerator TOF™ Analyzer. The Accelerator TOF™ Analyzer was set to scan with a mass range between 100 to 350 amu. To reach optimal signal specificity, the product ion signal is extracted with a mass window of 10 ppm.

The use of chemical properties during the sample preparation helped us gain better specificity for the mass of each opiate. The derivatization of each 4 of the 6 molecules eliminated the interference between the isobars.

Conclusion

With the High-Resolution versatility of the TripleTOF® 5600 System and the high-throughput of the LDTD™ ion source, we achieved an ultra fast quantification method running 1 sample **every 7 seconds** for confirmation of opiate drugs in urine. Sample preparation consists of an automated Solid Phase Extraction followed by reconstitution with the extract spotted directly onto the LazWell plates.

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