Testing and Quantitation of Opioids in Urine and Serum by LC-TOF

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1 Introduction

The study presented here details the use of UHPLC-TOF mass spectrometry to detect and quantify a class of natural compounds, known as opioids. The opioids are studied as structural models for drug design and their reliable measurement remains an essential need in research and forensic laboratories today. Screening of these compounds is commonly done using immunoassays, which suffer from non-specificity and poor sensitivity hence, requiring additional confirmation with GC-MS assays. The GC-MS assays require derivatization of the analytes which can be time consuming. However, LC/MS based techniques do not require derivatization. Among the LC techniques, LC/MS/MS is often used to quantitate compounds in biological fluids however, these assays are only suitable for targeted analysis. TOF mass spectrometers unlike MS/MS instruments collect full spectrum information which allows for both targeted and non-targeted analysis making them ideal to use in laboratories.

2 Experimental conditions

A PerkinElmer Flexar™ FX-15 LC pump with a PerkinElmer AxION® 2 TOF was used for UHPLC separation and detection of the compounds. The separation was achieved on a PerkinElmer Brownlee SPP C-18, 2X100 mm, 2.7 µm column using a mobile phase gradient of water and acetonitrile containing 0.1% formic acid. The TOF was operated in positive mode.

Opiate standards were purchased from Cerilliant (Round Rock, TX, US). Calibration curves were set up for urine by spiking urine with varying concentrations of analyte compounds and diluted with water (1:1) and injected on column (7 µL). For serum samples, after spiking with varying concentrations of compound mixture, protein was precipitated with acetonitrile containing 1% acetic acid, centrifuged, supernatant dried to ~100 µL and final volume brought to 1mL and injected on column.

3 Results

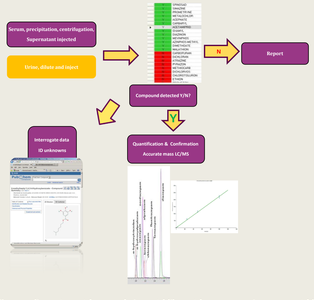


Fig. 1. Workflow for testing, identification and quantification of opioids in urine and serum

Testing

The full spectral information provided by the TOF allows for examining of the data for hundreds of compounds that may be present in the sample without pre-defining them prior to analysis. Powerful software tools, such as AxION Solo™ software, was utilized to rapidly identify the presence or absence of compounds in large batches of samples (Figure 2). The software identifies the presence of a compound based on accurate mass and isotope profile ratio as shown in Figure 3. In addition to searching against spectral information, the software can also search for target analytes based on user defined retention time windows which further improves the specificity of detection. Even after acquisition of data, the samples can be re-examined for presence of other compounds that may be in the sample by simply adding these to the target list in the software and extracting their masses from the individual chromatograms. The analysis of opioids was complete in 6 minutes with all the compounds eluting in < 5 minutes (Figure 4).

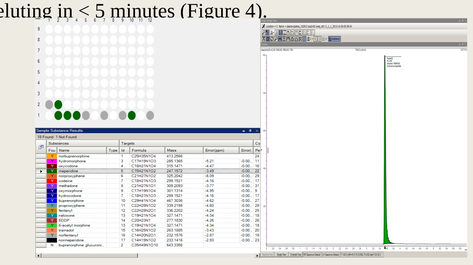


Fig. 2. Axion Solo Software: The top left hand corner shows the presence (green) and the absence (grey) of meperidine in different serum samples (vials). The remaining opioids detected in the selected vial are displayed in the table (bottom left).

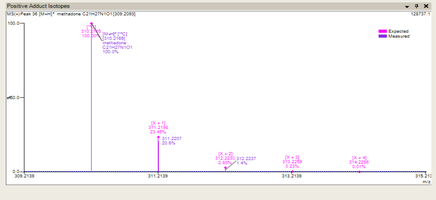


Fig 3. The accurate mass of methadone for A, A+1, are < 3ppm. The isotope ratios for A+1, A+2 are within 3% of expected ratio

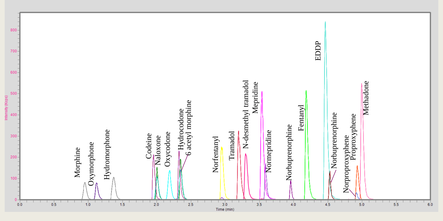


Fig. 4. Analysis of opioids by UHPLC-TOF MS spiked in serum < 5 mins.

Confirmation/quantification

The overall assay sensitivity was determined to be in the 1-10 ng/mL range for all of the compounds spiked into serum, (Table 1). The limit of quantifications (LOQs) measured by the TOF instrument were 200- 2000 times more sensitive than what is required by the non-specific EMIT immunoassays for majority of the opioids (with the exception of 6-acetyl morphine, which has a cut off of 10 ng/mL). When analyzing such low levels of compound carryover must be assessed to ensure that the assay is suitable for use. In spite of the low LOQs provided by the TOF MS, 0% carryover was observed after injection of 1 µg/ml (1000 ng/ml) standard for most of the opioids.

The linearity of a representative compound, normeperidine is shown in Figure 5. The assay showed linearity over four orders with an r^2 value of 0.997. The majority of the opioids analyzed showed linearity between 3-4 orders of dynamic range with r^2 values of 0.99 demonstrating that the assay was valid over the clinically relevant range required (Table 2). Multiple injections (n=5) of each calibration level showed excellent reproducibility (RSDs < 15%) for each of the compounds. The presence of a given compound sample can be confirmed by accurate mass and the isotope profile provided by TOF MS. The accurate masses of each of the opioids are < 5 ppm.

Analyte	serum LOQs (ng/mL)	Urine LOQs (ng/mL)
normepridine	1	1
tramadol	1	2
norproxyphene	2	10
proxyphene	2	1
EDDP	1	1
methadone	2	1
Meperidine	1	1
norfentanyl	2	2
fentanyl	1	1
naloxone	5	10
oxymorphone	10	10
hydromorphone	5	5
hydrocodone	10	10
norbuprenorphine	5	2
buprenorphine	2	2
6-acetyl morphine (6-AM)	5	5
codeine	5	10
oxycodone	5	
N-desmethyl tramadol	1	
morphine	10	10

Table 1. Shows the LOQs of the opioids in urine and serum

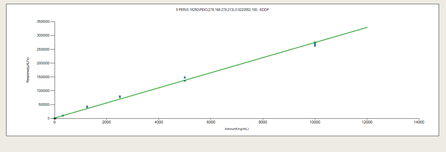


Fig. 5. Shows linearity for EDDP spiked in serum over 1- 10,000 ng/mL concentration range(r^2 = 0.997, n=5 for each calibration level).

Analyte	Concentration range (ng/mL)	r^2
normepridine	1-10,000	0.997
tramadol	1-10,000	0.995
norproxyphene	2-10,000	0.999
proxyphene	2-10,000	0.998
EDDP	1-10,000	0.997
methadone	2-10,000	0.995
Meperidine	1-10,000	0.997
norfentanyl	2-10,000	0.997
fentanyl	1-10,000	0.998
naloxone	5-10,000	0.993
oxymorphone	10-20,000	0.996
hydromorphone	5-20,000	0.991
hydrocodone	10-20,000	0.994
norbuprenorphine	5-10,000	0.991
buprenorphine	2-10,000	0.997
6-acetyl morphine (6-AM)	5-10,000	0.996
codeine	5-10,000	0.998
oxycodone	5-20,000	0.998
N-desmethyl tramadol	1-10,000	0.997
morphine	10-20,000	0.997

Table 2. Shows the linear dynamic range and regression for each of the opioids spiked in serum as matrix

4 Conclusions

1. Even in a challenging matrix such as urine or serum, the method required little to no sample preparation or method development, saving hours of time and the use of costly reagents and consumables.
2. The AxION 2 TOF was easily able to identify 1-10 ng/mL concentration of opioids spiked in urine or serum.
3. The detection limits of these compounds were 200-2000 times lower than that required by immunoassays.
4. The AxION 2 TOF with the ADC detector technology provides wide dynamic range capabilities similar to that of a triple quadrupole mass spectrometer and also offers the screening of untargeted compounds.
5. For rapid large scale screening of batches of samples PerkinElmer AxION Solo software provides a quick and easy platform to detect the presence or absence of opioids.

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