Application of a HRMS polarity switching method for targeted quantitation and unknown screening for drugs of abuse in urine

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Overview

- High-Resolution Q-TOF LC-MS/MS based fast polarity switching was applied to the analysis of urine samples in routine clinical toxicology.
- The method was developed using a calibration test kit of 108 drugs of abuse including amphetamines, barbiturates, benzodiazepines, cocaine, opiates/opioids and used to screen clinical urine samples.
- Targeted analysis with internal standards allowed the identification of drugs in the clinical samples above the clinical reporting concentration. Non-targeted screening provided evidence of poly-substance use beyond the scope of the clinical targeted panel using a single polarity switching data acquisition method.

1. Introduction

The identification of drugs of abuse in urine by LC-MS/MS is commonly used to monitor substance misuse, detect drug intoxication or improve clinical management. To increase sample throughput for routine clinical and forensic toxicology applications, a high-resolution LC-MS/MS method has been developed using fast polarity switching to reduce sample throughput by two-fold. The method was applied to the analysis of urine samples in a routine clinical toxicology laboratory.

2. Materials and Methods

Urine samples including standards at six concentrations and clinical samples were analyzed by LC-MS/MS on a QTOF (LCMS-9050, Shimadzu Corporation). The target panel included 108 drugs of abuse compounds (6PLUS1® Multilevel Urine Calibrator Set Mass Tox® Drug of Abuse Testing in Urine, Chromsystems) and 29 internal standards. High resolution QTOF LC-MS/MS (LCMS-9050, Shimadzu Corporation, Japan) was applied to urine samples to screen for drugs of abuse, medications and dietary drugs using a non-targeted screening (NTS) workflow with fast polarity switching.

Reversed phase LC Separation.

- Shim-pack Velox™ Biphenyl (2.1x100mm 2.7µm); 40ºC, flow rate 0.3 mL/min.
- Binary gradient; water + 2mM ammonium formate + 0.002% formic acid, and methanol + 2mM ammonium formate + 0.002% formic acid.
- Cycle time 17 minutes.

LC-MS/MS Mass Spectrometry Detection.

- Positive ion mode TOF MS survey scan (m/z 100-1000; 100 msecs) followed by four DDA MS/MS scans (m/z 40-1000; 33 msec).
- Negative ion mode TOF MS survey scan (m/z 100-1000; 100 msecs) followed by two DDA MS/MS scans (m/z 40-1000; 33 msec).
- Collision energy spread 5-55V; External mass calibration.
- Total cycle time <2 seconds.

Data Processing.

- LabSolutions Insight software was used in quantitative analysis of targets with internal standards.
- LabSolutions Insight Discovery software was applied for NTS to identify unknown compounds in the samples from the Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology.

3. Results

3.1 Assessing the impact of polarity switching

Mass accuracy error

- To assess the stability of the QTOF system with fast polarity switching a panel of 61 drugs of abuse (DoA) compounds was spiked into plasma and extracted using QuEChERS and acquired with polarity switching and single polarity.

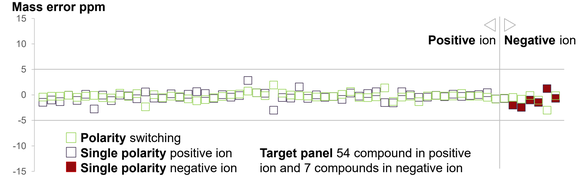

Figure 1. Precursor mass error for a target panel of DoA acquired with three different modes; polarity switching, single polarity in positive ion and single polarity in negative ion. The three data acquisition methods generated precursor ion data less than 5 ppm mass error (external mass calibration) for a 10 ng/mL plasma extract.

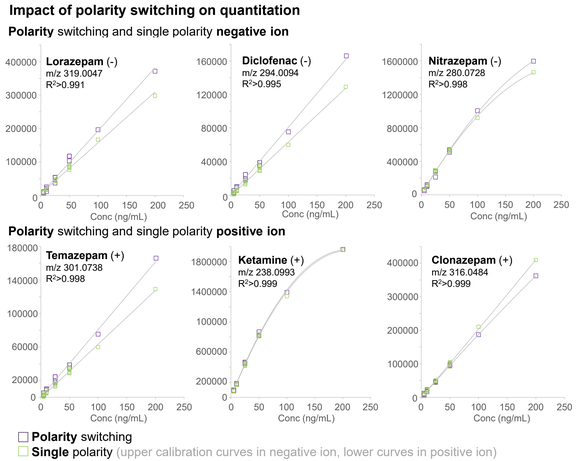

Figure 2. Calibration curves for several DoA compounds acquired with fast polarity switching compared to single polarity mode in negative (upper calibration curves) and positive ion (lower calibration curves). Polarity switching resulted in comparable signal response and linearity (all calibration curves delivered R^2>0.99).

3.2 Polarity switching method applied to drugs of abuse analysis in urine samples

Targeted quantitative analysis

- **Compounds routinely detected**; included 6-MAM, alprazolam, amphetamine, benzoylecgonine, buprenorphine, codeine, EDDP, gabapentin, lorazepam, methadone, morphine, nitrazepam, nordiazepam, oxazepam, pregabalin, temazepam, and zopiclone. Compounds detected within the calibration range meet the reporting criteria, compounds detected above are considered for review.

Reporting criteria

- **Precursor ion**; with a mass error less than 5 ppm, isotope distribution score greater than 50, retention time tolerance within 0.2 mins of the library retention time.
- **DDA-MS/MS**; library verification, with a dot product similarity index (SI) score greater than 75 (reverse fit).
- **Concentration**; above the clinical reporting threshold concentration.

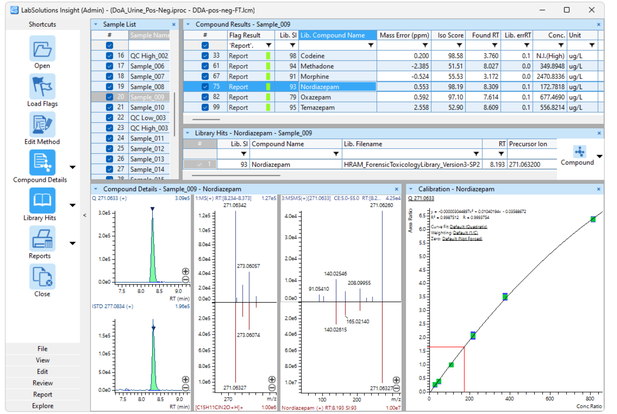

Figure 2. LabSolutions Insight quantitative data browser.

3.3 Polarity switching method applied to NTS

Non-targeted screening using Insight Discovery software

- **Insight Discovery**; requires no prior knowledge of a compound list. Discovery software has two key steps involving a novel component detection algorithm with advanced feature filtering which can be applied to positive and negative ion and a flexible search engine for MS/MS library matching.
- **Finding compounds outside the target panel**; Insight Discovery was applied to search for compounds from a list of over 1,200 compounds (part of the Shimadzu HRAM Library for Forensic Toxicology).

Non-targeted screening using Insight Discovery software

- **Screening list**; over 1,200 DoA compounds included in the screening list which includes the compounds name (or simply formula), m/z and Rt (mins).
- **Compound reporting**; following component detection and removing false positive features, the detected feature list was matched against the screening list. Compounds were reported with high confidence with a library spectral match.

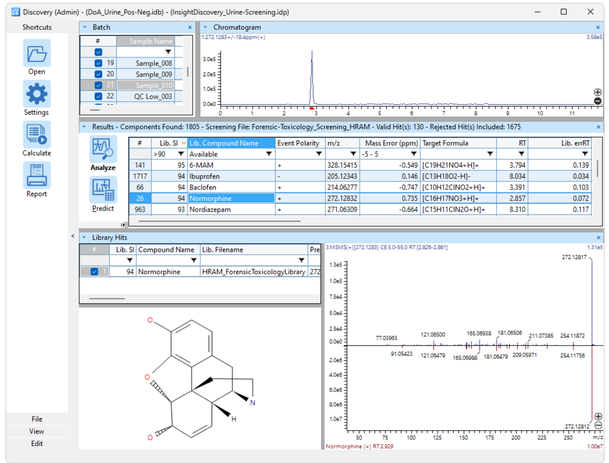

Figure 3. Applying Insight Discovery software to search for compounds in an unknown sample using polarity switching data acquisition and a search list of DoA compounds. Following this approach, additional opiate metabolites (normorphine and morphine-6-glucuronide) and cocaine related compounds (ecgonine methylester and anhydroecgonine methyl ester) were positively identified in the unknown samples, supporting the targeted results.

4. Conclusions

- To accelerate sample throughput for routine clinical and forensic toxicology laboratories, a high resolution QTOF LC-MS/MS method was developed with fast polarity switching to reduce batch analysis times by 2-fold without compromising precursor quantitation or DDA-MS/MS identification.
- The method was applied to the analysis of urine samples for targeted therapeutic drug monitoring and non-targeted screening. DDA-MS/MS spectra resulted in high reporting confidence with library searching against the Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology.