Seamless automated sample preparation and analysis of DUID samples using high-resolution DIA QTOF library screening

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Overview

- A fully automated sample preparation and quantitative analysis for screening Driving Under the Influence of Drugs, Alcohol and Medicines samples (commonly used acronyms include DRUID/DUID/DUI) was initially developed for blood and tested for oral fluid analysis using a high resolution QTOF LC-MS/MS instrument coupled with a CLAM-2030 sample preparation station.

1. Introduction

Oral fluid has several key advantages in testing for Driving Under the Influence of Drugs, Alcohol and Medicines samples (in this work referred to as DUID). It is non-invasive, rapid, observed sampling at the time of the traffic stop or crash. In this work, a method was developed to evaluate an automated sample preparation station integrated with a high resolution QTOF LC-MS/MS to evaluate a method developed for emergency screening.

2. Materials and Methods

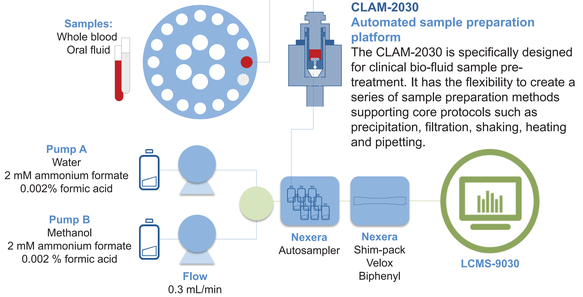

Figure 1 Schematic representation of the automated screening platform for DUID sample analysis.

Sample preparation

- Samples for DUID analysis were submitted for a cocaine, amphetamine and opioid panel screen (CAO Panel). Oral fluid was sampled using a dry swab (FLOQSwab) and afterwards placed in 2 mL phosphate buffer. Prior to analysis, swabs were sonicated (5 min) then heads centrifuged (5 min 3000 rpm).

Automated sample preparation platform; CLAM-2030 (Shimadzu, Japan)

- 20 µL methanol added to activate the sample filter, 50 µL of sample, 20 µL internal standard mixture (stable isotope labelled), 90 µL acetonitrile. The sample was mixed at 1900 rpm (2 min), filtered (2 min, 0.45 µm filter pore size) then directly injected (5 µL) on to the LCMS/MS system.

High Resolution QTOF Mass spectrometry; LCMS-9030 (Shimadzu, Japan)

- MS and DIA-MS/MS analysis; MS scan m/z 70-1000; 100 msecs
  - DIA-MS/MS mass scans m/z 40-1000; 25 msecs for each precursor isolation window; isolation width 20 Da; collision energy spread 5-55V; 36 mass scan events. Scan cycle time 0.975 second (36 mass scan in total).

3. Results

3.1 Automated screening applied to oral fluids in DUID

A batch of oral fluid samples was submitted for DUID screening using a targeted triple quadrupole LC-MS/MS method (previously validated for blood, plasma and serum samples) and tested against a high resolution QTOF LC-MS/MS analysis. The clinical request focused on a targeted CAO panel (cocaine, amphetamines and opioids).

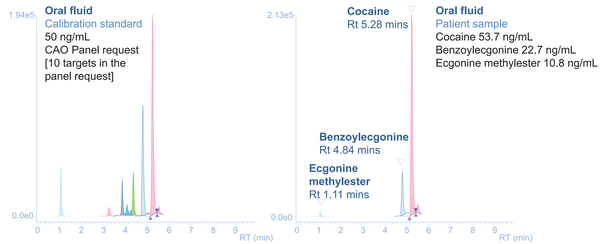

Figure 2 The automated sample preparation method was tested against a CAO panel request (targets included 6-MAM, amphetamine, benzoylecgonine, cocaine, ecgonine methylester, MDA, MDEA, MDMA, methamphetamine and morphine). The oral fluid blank matrix was spiked with a CAO panel over a concentration of 0.5-100 ng/mL (the 50 ng/mL calibration standard is shown on the right highlighting the detection of benzoylecgonine, cocaine, ecgonine methylester.

Target CAO panel	Rt (mins)	Formula	QTOF LCMS-9030	Triple quadrupole LCMS-8050
			Quantitation m/z	MRM transitions m/z
6-MAM	3.901	C19H21NO4	**328.1543**	328.20>165.20
Amphetamine	3.647	C9H13N	**136.1121**	136.20>91.10
Benzoylecgonine	4.843	C16H19NO4	**290.1387**	290.20>168.25
Cocaine	5.283	C17H21NO4	**304.1543**	304.00>182.15
Ecgonine methylester	1.108	C10H17NO3	**200.1281**	200.20>182.20
MDA	3.883	C10H13NO2	**180.1019**	180.20>105.15
MDEA	4.409	C12H17NO2	**208.1332**	208.20>163.20
MDMA	4.124	C11H15NO2	**194.1176**	194.20>163.10
Methamphetamine	3.914	C10H15N	**150.1277**	150.20>91.10
Morphine	3.310	C17H19NO3	**286.1438**	286.20>152.10
			Automated processing	QuEChERS sample preparation

Method assessment

- The automated sample preparation method was tested against a validated triple quadrupole LC-MS/MS method using an established, validated, routinely used QuEChERS protocol.
- Both methods used the corresponding stable isotope labelled internal standards (D3 or D5 depending on the target) and the same LC conditions with a Shim-pack Velox Biphenyl column chemistry.
- As one example of repeatability, in a 48h batch analysis peak area variance for cocaine-D3 was 4.6% RSD and mass accuracy error was less than 5 ppm (n=90 patient samples).

3.2 Comparing an automated analysis approach to a validated QuEChERS protocol

A QuEChERS protocol has previously been successfully validated for blood, serum, plasma and oral fluids and the oral fluid same samples tested by an automated sample preparation method using a high resolution QTOF detection.

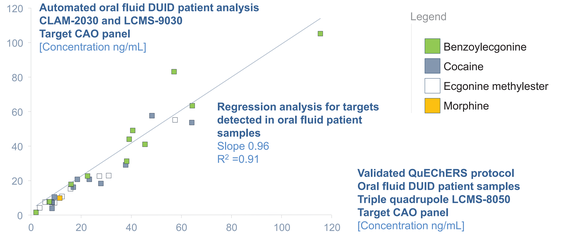

Figure 3 CAO panel patient sample requests were used in the evaluation of an automated sample preparation approach. Both methods were in close agreement (slope 0.96). In this patient group of samples cocaine and benzoylecgonine were commonly detected in oral fluids.

The advantages of high-resolution LC-MS/MS analysis

- High reporting confidence (accurate mass error, isotope fidelity, retention time variance and library similarity score).
- Library verification using in-house MS/MS product ion spectral libraries or other data repositories such as Wiley (Registry of Tandem Mass Spectral Data: MS for ID, Maurer/Meyer/Helfer/Weber LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites), HighResNPS.

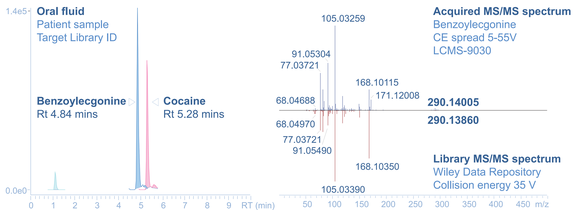

Figure 4 Library verification for benzoylecgonine detected in oral fluid patient sample analysis. In this example, the Wiley data repository was also used for a compound identification (despite different instruments and different CE values there is high value in each library providing further evidence for compound identification).

To help review data quickly with a high reporting confidence the LabSolutions Insight software can be configured to show color-based flagging to highlight correspondence with mass accuracy, isotope matching score, retention time and library similarity score.

3.3 Retrospective analysis

The clinical need may change if the patient is unconscious, or the scope of the drug search may require a check for other target panels. In this case, an untargeted workflow is considered on the same data file without reacquiring further sample data.

Untargeted data analysis

- For the query patient sample, the data processing initially uses a component detection algorithm to find all ion signals that behave in a covariant manner (as a chromatographic peak). A suspect search is submitted and if a match is found within set tolerances (including mass error, isotope fidelity, retention time) the candidate is tested against a high-resolution MS/MS library.
- If a library match results in a high similarity the candidate can be reported with a higher confidence.

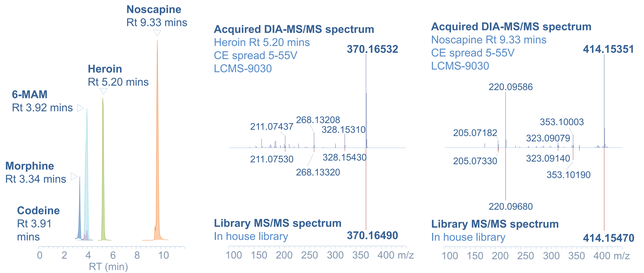

Figure 5 A patient sample was reprocessed to search for other suspected drugs. In this CAO panel request, morphine and 6-MAM was initially detected and reported. Extending the search space in retrospective analysis also identified drug compounds consistent with illicit heroin use (including noscapine and codeine). The suspect screening compounds were confirmed by library identification using an in-house library;

- MS/MS data acquired using authentic standards with a fragmentation energy of 5-55V.
- All product ion spectra corrected to theoretical accurate mass values using Insight Assign application. MS/MS spectra acquired using targeted MS/MS and a precursor ion isolation width of unit mass. Spectra were acquired positive and negative ion modes
- Retention time mapped to a Shim-pack Velox Biphenyl LC column.

4. Conclusions

- A validated triple quadrupole LC-MS/MS using a QuEChERS sample preparation protocol was tested against an automated sample preparation method using the CLAM-2030 integrated with the LCMS-9030 high resolution LC-MS/MS system for the emergency analysis of oral fluids in DUID cases. For the patient sample group tested there was close agreement between the two methods (regression analysis between the two methods resulted in a slope equal to 0.96).
- The key advantages in using a high-resolution LC-MS/MS in emergency screening relate to high reporting confidence (accurate mass error, isotope distribution score, retention time and product ion MS/MS spectra from in house library repositories or third-party data bases) and retrospective data analysis to search for suspect screening.