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Toxicology Screening of Human Blood using Quadrupole-Time of Flight (QTOF) Mass Spectrometry

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Overview

This method focuses on the use of high-resolution mass spectrometer for screening blood samples for commonly abused drugs using data independent analysis and library matching.

Introduction

Initial toxicology screening is performed on all toxicology samples to screen for the presence of certain drug classes and compounds. Traditionally, toxicology screening has been done using immunoassay which is limited to specific drug classes and can result in false positives. With the addition of many new novel psychoactive substances and unknown compounds, the demand to identify these unknown compounds has increased. To meet the increasing demand for a more rapid and effective toxicology screening method, a high-resolution accurate mass quadrupole-time of flight (Q-TOF) mass spectrometer with a comprehensive library was used to develop a screening workflow. A method was developed on a Q-TOF to screen toxicologically significant compounds in blood extracts.

Methods

Solid phase extracted blood samples were spiked with a panel of commonly abused drugs (benzoylecgonine, codeine, fentanyl, hydrocodone, hydromorphone, methadone, methamphetamine, morphine, and temazepam) at concentrations ranging from 5-5000 ng/mL. All components were separated using a Shim-pack Velox column (2.1 x 100 mm; 2.7µm) with a mobile phase of water and methanol with 2 mM ammonium formate and 0.002% formic acid. Data was acquired using a MS TOF scan event and DIA-MS/MS in positive ion mode. MS scan range was *m/z* 40-900 and each DIA-MS/MS mass scan had a variable precursor isolation width and a collision energy spread of 5-55 V. The acquired data allows for simultaneous highly specific targeted quantitation and non-targeted screening with library verification.

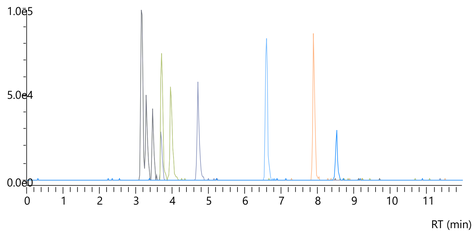


Figure 1. Chromatogram of the abused drug panel (benzoylecgonine, codeine, fentanyl, hydrocodone, hydromorphone, methadone, methamphetamine, morphine, and temazepam)

Results and Discussion

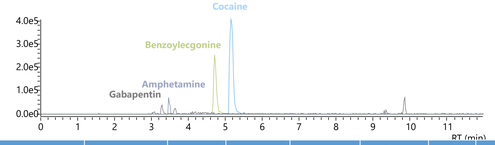
A panel of commonly abused drugs (benzoylecgonine, codeine, fentanyl, hydrocodone, hydromorphone, methadone, methamphetamine, morphine, and temazepam) was used to develop a routine toxicology screening workflow by high resolution LC-MS/MS. For targeted workflows, the method included a toxicologically relevant compound database with a predefined retention time and MS/MS fragmentation energy for over 900 compounds. Each accurate mass product ion spectrum in the compound database was acquired using targeted MS/MS and a precursor ion isolation width of 1 Da.

Figure 2. LabSolutions Insight data review application shows the results of the sample analysis, 9 drugs of abuse compounds have been positively identified (within the criteria for reporting a compound with high confidence).

Library verification in forensic toxicology

Using the test panel, all compounds were successfully detected and positively identified in each calibration standard 5, 50, 500, and 5000 ng/mL. Spiked toxicology compounds amphetamine (*m/z* 136.1110; RT=3.47 min), benzoylecgonine (*m/z* 290.1384; RT=4.71 min), cocaine (*m/z* 304.1547; RT=5.15 min), gabapentin (*m/z* 172.1327; RT=3.28 min), alprazolam (*m/z* 309.0903; RT=8.71 min), fluoxetine (*m/z* 310.1420; RT=8.712 min), methamphetamine (*m/z* 150.1283; RT=3.70 min), and morphine (*m/z* 286.1450; RT=3.17 min) were detected in the blood samples. Highly confident compound identification was reported for all targets with similarity scores higher than 80 using library search paraments weighted for both mass accuracy and ion signal intensity with limited retention time windows.

Blood Sample A



Compound	Formula	Conc. (ng/mL)	Pos/Neg	m/z	Mass Error (ppm)	Lib. SI	Found Rt	RT diff
Amphetamine	$C_9H_{13}N$	5	Positive	136.1121	-7.714	93	3.47	-0.16
Benzoylecgonine	$C_{16}H_{19}NO_4$	100	Positive	290.1387	-0.931	91	4.71	-0.01
Cocaine	$C_{17}H_{21}NO_4$	25	Positive	304.1543	1.381	90	5.15	-0.18
Gabapentin	$C_9H_{17}NO_2$	1000	Positive	172.1332	-3.079	85	3.28	-0.11

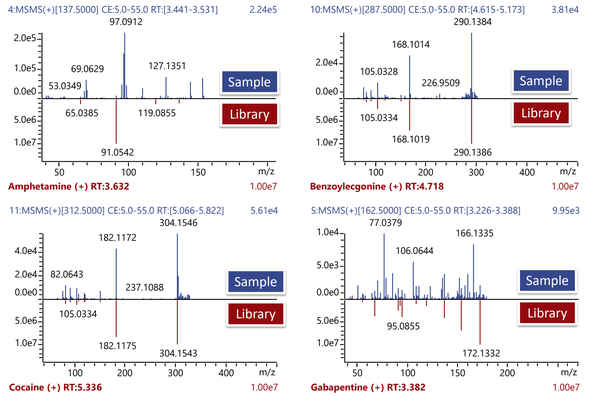
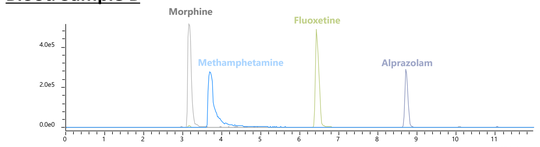


Figure 4. Chromatogram and the MS/MS spectra of the spiked toxicology compounds in blood sample A acquired on a LCMS-9030 using a DIA method. Compound information and results are shown in the table above.

Blood Sample B



Compound	Formula	Conc. (ng/mL)	Pos/Neg	m/z	Mass Error (ppm)	Lib. SI	Found Rt	RT diff
Alprazolam	$C_{17}H_{13}ClN_4$	10	Positive	309.0902	0.226	90	8.71	0.09
Fluoxetine	$C_{17}H_{18}F_3NO$	50	Positive	310.1413	2.225	99	6.41	-0.16
Methamphetamine	$C_{10}H_{15}N$	5000	Positive	150.1277	4.130	87	3.70	-0.17
Morphine	$C_{17}H_{19}NO_3$	100	Positive	286.1438	4.299	83	3.17	-0.11

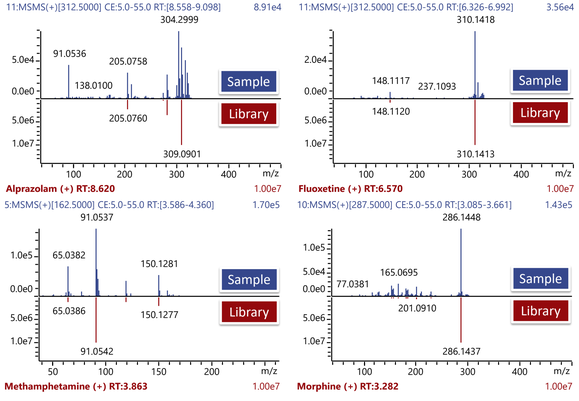


Figure 5. Chromatogram and the MS/MS spectra of the spiked toxicology compounds in blood sample B acquired on a LCMS-9030 using a DIA method. Compound information and results are shown in the table above.

Conclusions

The toxicology workflow was optimized for both targeted LC-MS/MS analysis and untargeted toxicology screening to detect and identify a range of targets including illicit drugs, adulterants, unregulated supplements, and prescription medications. To increase reporting confidence in compound identification for targeted analysis the accurate mass, isotopic distribution, accurate mass MS/MS library verification on the product ion spectrum, and retention time (RT) were used to target large panels of compounds of interest. In this method, the chromatographic separation was optimized for a diverse chemical space. Method parameters for peak integration and spectrum processing considered both trace level and component saturation to consider likely toxicology workflows. This workflow demonstrated highly confident reporting in routing toxicology screening for over 900 compounds using a QTOF mass spectrometer.

Acknowledgement

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