SHIMADZU

# Forensic hair analysis of illicit drugs using a sequential TOF MS, DDA-MS/MS and DIA-MS/MS method combining quantitative and qualitative workflows

[1]Benjamin Barrett, [2]Alan Barnes, [2]Emily G. Armitage, [1]Ethan Webster, [2]Neil J. Loftus
[1]AttoLife, Scottow Enterprise Park, Norwich, UK; [2]Shimadzu Corporation, Manchester, UK

## Overview

- A HRMS LC-MS/MS method was developed to optimize targeted and non-targeted analysis and applied to forensic drug screening in hair samples using a mixed-data acquisition mode.
- The mixed-data acquisition mode combined a TOF MS mass scan with both data dependent (DDA-MS/MS) and data independent (DIA-MS/MS) acquisition in a single method.
- This approach resulted in high reporting confidence for targeted analysis with the capability of retrospective DIA-MS/MS analysis for not-targeted screening (NTS).

## 1. Introduction

Methods developed to analyze hair samples in a forensic toxicology context continue to expand in capability and scope given the evolving landscape of novel synthetic opioids and new psychoactive substances in addition to meeting a need for robust routine detection of commonly encountered drugs of abuse and misuse of existing pharmaceuticals. To meet this need, a high-resolution LC-MS/MS method was developed with a sequential series of mass scan events; TOF MS mass scan (for precursor quantitation and screening analysis), DDA-MS/MS (targeted for high reporting confidence in library searching) and DIA-MS/MS mass scans (for retrospective screening and expanded search panels). In this work the method was applied to the analysis of hair samples as proof of abstinence for family court matters.

## 2. Materials and Methods

Hair samples from 5 donors (each donor providing a 6 cm segmented sample representing approximately 6 months) were submitted for high resolution LC-MS/MS analysis (QTOF LCMS-9050, Shimadzu Corporation). Data were acquired using mixed-data acquisition involving a series of sequential mass scan events; TOF MS mass scan followed by DDA-MS/MS and MS, DIA-MS/MS in positive ion mode. LabSolutions Insight™ and Insight Discovery were used for data processing targeted and non-targeted analysis. Compounds were reported with high reporting confidence using library searching (Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology).

### Sample preparation

- Hair samples were washed in dichloromethane and dried overnight before milling. Internal standards were added to 10 mg powdered hair (±0.5 mg) and extracted in methanol + 1% HCl by ultrasonication. Extracts were dried under OFN at 40 ºC.
- Samples were reconstituted and vortexed in 200 µL 80:20 water:methanol.

**Reversed phase LC Separation:** Nexera X2 (Shimadzu Corporation, Japan).

- Column: Shim-pack Velox™ Biphenyl (100 x 2.1 mm, 2.7 µm); column temp. 40 ºC, flow rate: 0.3 mL/min, 17 min total analysis time.
- A: water + 2 mM ammonium formate + 0.002% formic acid.
- B: methanol + 2 mM ammonium formate + 0.002% formic acid.

**High resolution QTOF analysis:** LCMS-9050 (Shimadzu Corporation, Japan).

- MS scan m/z 100-1000, 100 msec scan time.
- MS/MS DDA, up-to 4 dependent scans m/z 40-1000 CE spread 5-55 V, 33 msec.
- MS/MS DIA 35 consecutive scans (m/z 100-500 20 Da precursor isolation width, m/z 500-920 35 Da precursor isolation width), 25 msec scan time (1.2 sec total cycle time).

## 3. Results

## 3.1 Targeted quantitative analysis

Mixed-data acquisition was applied to forensic hair samples using a TOF MS scan followed by DDA-MS/MS for a specific target panel of drugs of abuse (DoA) and included a series of DIA-MS/MS mass scans to support retrospective data analysis.

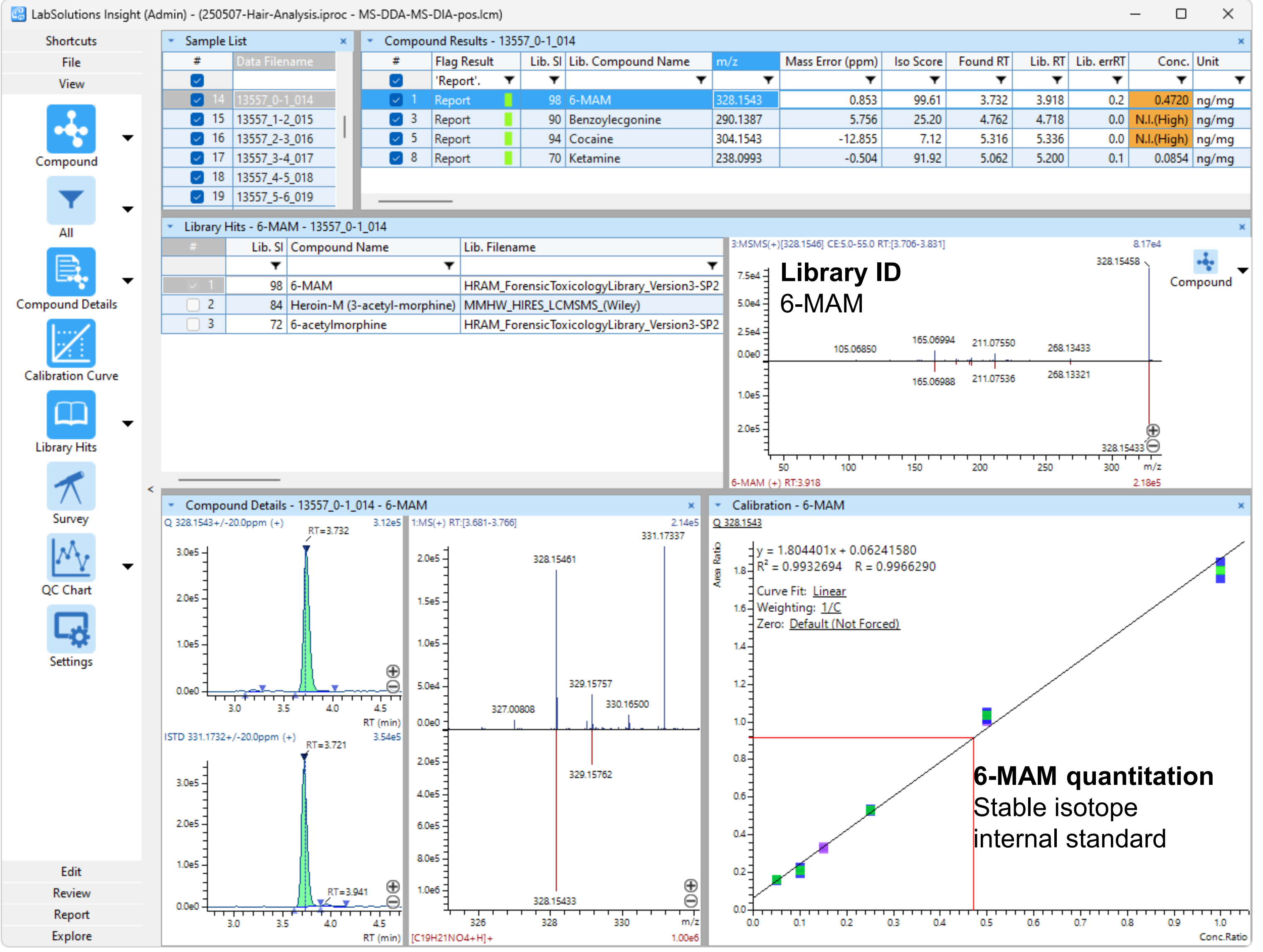
### Target panel

- 41 drugs of abuse targets, in addition to 21 stable isotope labelled internal standards.
- The panel was based largely on the Society of Hair Testing (SoHT) consensus for drugs of abuse in hair.

### LabSolutions Insight Quantitative Data Review | Unknown Hair Sample Analysis

Reporting criteria included;

- **Precursor ion**; with a mass error less than 5 ppm, isotope distribution score greater than 40, retention time tolerance within 0.25 mins of the library retention time.
- **DDA-MS/MS**; library verification, with a dot product similarity index (SI) score greater than 70 (reverse fit).



LabSolutions Insight (Admin) - (250507-Hair-Analysis.iproc - MS-DDA-MS-DIA-pos.lcm)

Sample List

| # | Data Filename |
|---|---|
| ☑ 14 | 13557_0-1_014 |
| ☑ 15 | 13557_1-2_015 |
| ☑ 16 | 13557_2-3_016 |
| ☑ 17 | 13557_3-4_017 |
| ☑ 18 | 13557_4-5_018 |
| ☑ 19 | 13557_5-6_019 |

Compound Results - 13557_0-1_014

| # | Flag Result | Lib. SI | Lib. Compound Name | m/z | Mass Error (ppm) | Iso Score | Found RT | Lib. RT | Lib. errRT | Conc. | Unit |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ☑ | 'Report'. | | | | | | | | | | |
| ☑ 1 | Report | 98 | 6-MAM | 328.1543 | 0.853 | 99.61 | 3.732 | 3.918 | 0.2 | 0.4720 | ng/mg |
| ☑ 3 | Report | 90 | Benzoylecgonine | 290.1387 | 5.756 | 25.20 | 4.762 | 4.718 | 0.0 | N.I.(High) | ng/mg |
| ☑ 5 | Report | 94 | Cocaine | 304.1543 | -12.855 | 7.12 | 5.316 | 5.336 | 0.0 | N.I.(High) | ng/mg |
| ☑ 8 | Report | 70 | Ketamine | 238.0993 | -0.504 | 91.92 | 5.062 | 5.200 | 0.1 | 0.0854 | ng/mg |

Library Hits - 6-MAM - 13557_0-1_014

| # | Lib. SI | Compound Name | Lib. Filename |
|---|---|---|---|
| 1 | 98 | 6-MAM | HRAM_ForensicToxicologyLibrary_Version3-SP2 |
| ☐ 2 | 84 | Heroin-M (3-acetyl-morphine) | MMHW_HIRES_LCMSMS_(Wiley) |
| ☐ 3 | 72 | 6-acetylmorphine | HRAM_ForensicToxicologyLibrary_Version3-SP2 |

Calibration - 6-MAM

Q 328.1543
y = 1.804401x + 0.06241580
R² = 0.9932694 R = 0.9966290
Curve Fit: Linear
Weighting: 1/C
Zero: Default (Not Forced)

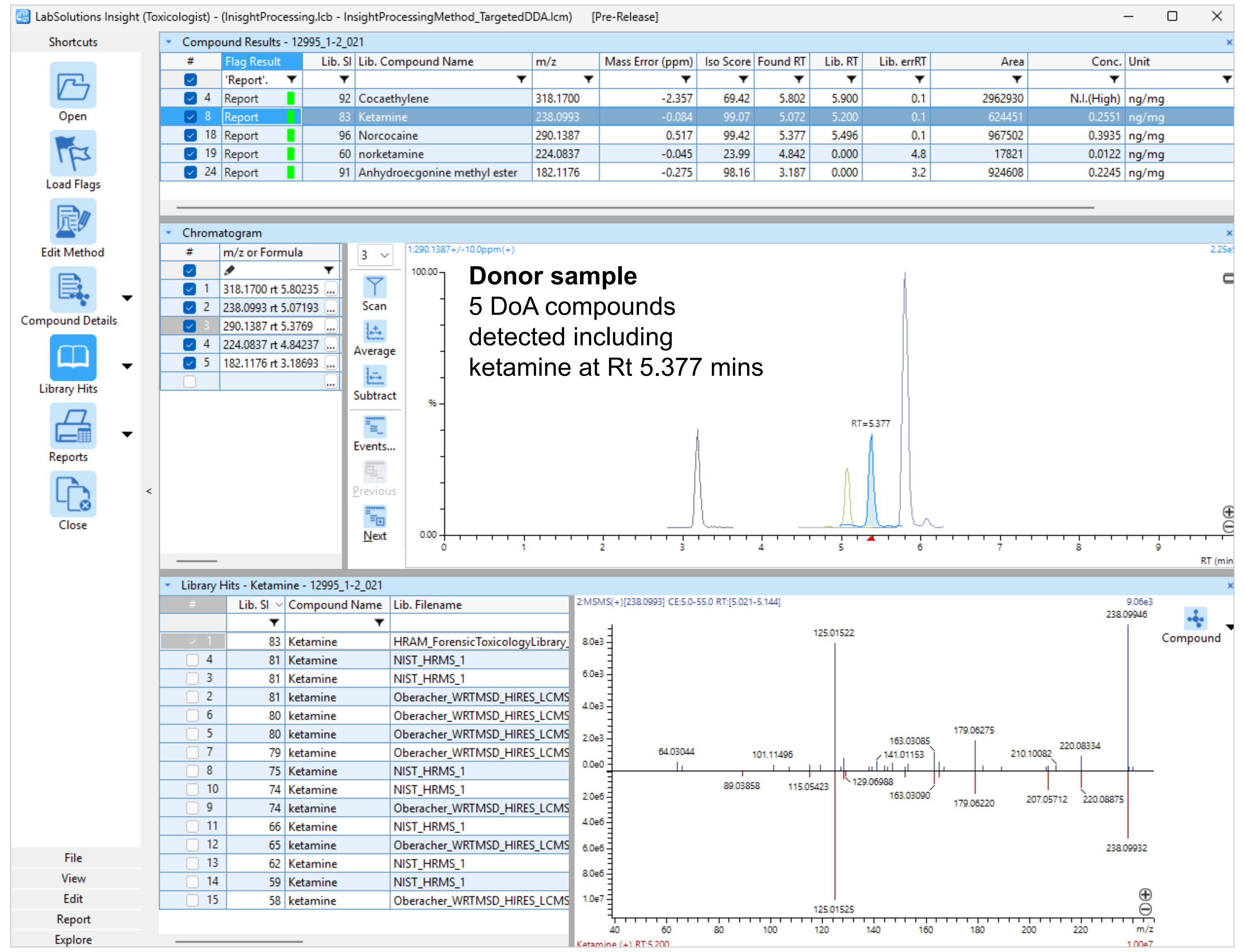
**Figure 1.** LabSolutions Insight quantitative data review software highlighting the detection of 6-MAM, ketamine, benzoylecgonine and cocaine with concentrations ranging from 0.047 ng/mg to over 2 ng/mg in one donor sample (concentrations above reporting cut off flagged yellow). Each compound identification was verified using the HRMS MS/MS data repositories (in this example, the Shimadzu High Resolution Accurate Mass Library for Forensic Toxicology and Wiley MS/MS data repositories) and each compound quantified using stable isotope labelled internal standards.

## 3.2 Targeted analysis of donor hair samples

- **DoA compounds detected in hair samples from 5 donors;** included 6-MAM, anhydroecgonine methyl ester, benzoylecgonine, cocaethylene, cocaine, codeine, ketamine, norcocaine, norketamine, MDMA, m-hydroxycocaine, m-hydroxybenzoylecgonine and tramadol.
- **Exposure and compliance monitoring:** Hair sample analysis is widely applied in forensics to retrospectively document exposure to substances over a prolonged period of up to several months. In this work, donor hair samples were sectioned into six segments of 1 cm and analyzed as individual samples.

**Table 1.** DoA concentrations for 6-MAM, morphine, ketamine, codeine and cocaethylene are consistent with reduced drug use over a period of approximately 6 months. Cocaine, benzoylecgonine, ADME and norcocaine were also detected at high concentrations.

| Sample length (cm) | **Concentration (ng/mg)**<br>6-MAM | Morphine | Ketamine | Codeine | Cocaethylene | |
|---|---|---|---|---|---|---|
| 5-6 | 1.2696 | 0.7535 | 0.1612 | 0.0655 | 0.0772 | **5-6 cm sample**<br>DoA concentrations above cut off values |
| 4-5 | 1.1975 | 0.7651 | 0.1757 | 0.0472 | 0.0869 | |
| 3-4 | 1.0524 | 0.6979 | 0.1744 | 0.0404 | 0.0764 | |
| 2-3 | 1.0093 | 0.5398 | 0.1514 | 0.0359 | 0.0873 | |
| 1-2 | 0.7802 | 0.3382 | 0.1283 | 0.0051 | 0.074 | |
| 0-1 | 0.4719 | 0.185 | 0.0854 | 0.0000 | 0.0515 | **0-1 cm sample**<br>DoA concentrations reduced, falling to a cut off value |
| Reporting cut off | 0.2 | 0.2 | 0.2 | 0.2 | 0.05 | |



LabSolutions Insight (Toxicologist) - (InisghtProcessing.lcb - InsightProcessingMethod_TargetedDDA.lcm) [Pre-Release]

Compound Results - 12995_1-2_021

| # | Flag Result | Lib. SI | Lib. Compound Name | m/z | Mass Error (ppm) | Iso Score | Found RT | Lib. RT | Lib. errRT | Area | Conc. | Unit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ☑ | 'Report'. | | | | | | | | | | | |
| ☑ 4 | Report | 92 | Cocaethylene | 318.1700 | -2.357 | 69.42 | 5.802 | 5.900 | 0.1 | 2962930 | N.I.(High) | ng/mg |
| ☑ 8 | Report | 83 | Ketamine | 238.0993 | -0.084 | 99.07 | 5.072 | 5.200 | 0.1 | 624451 | 0.2551 | ng/mg |
| ☑ 18 | Report | 96 | Norcocaine | 290.1387 | 0.517 | 99.42 | 5.377 | 5.496 | 0.1 | 967502 | 0.3935 | ng/mg |
| ☑ 19 | Report | 60 | norketamine | 224.0837 | -0.045 | 23.99 | 4.842 | 0.000 | 4.8 | 17821 | 0.0122 | ng/mg |
| ☑ 24 | Report | 91 | Anhydroecgonine methyl ester | 182.1176 | -0.275 | 98.16 | 3.187 | 0.000 | 3.2 | 924608 | 0.2245 | ng/mg |

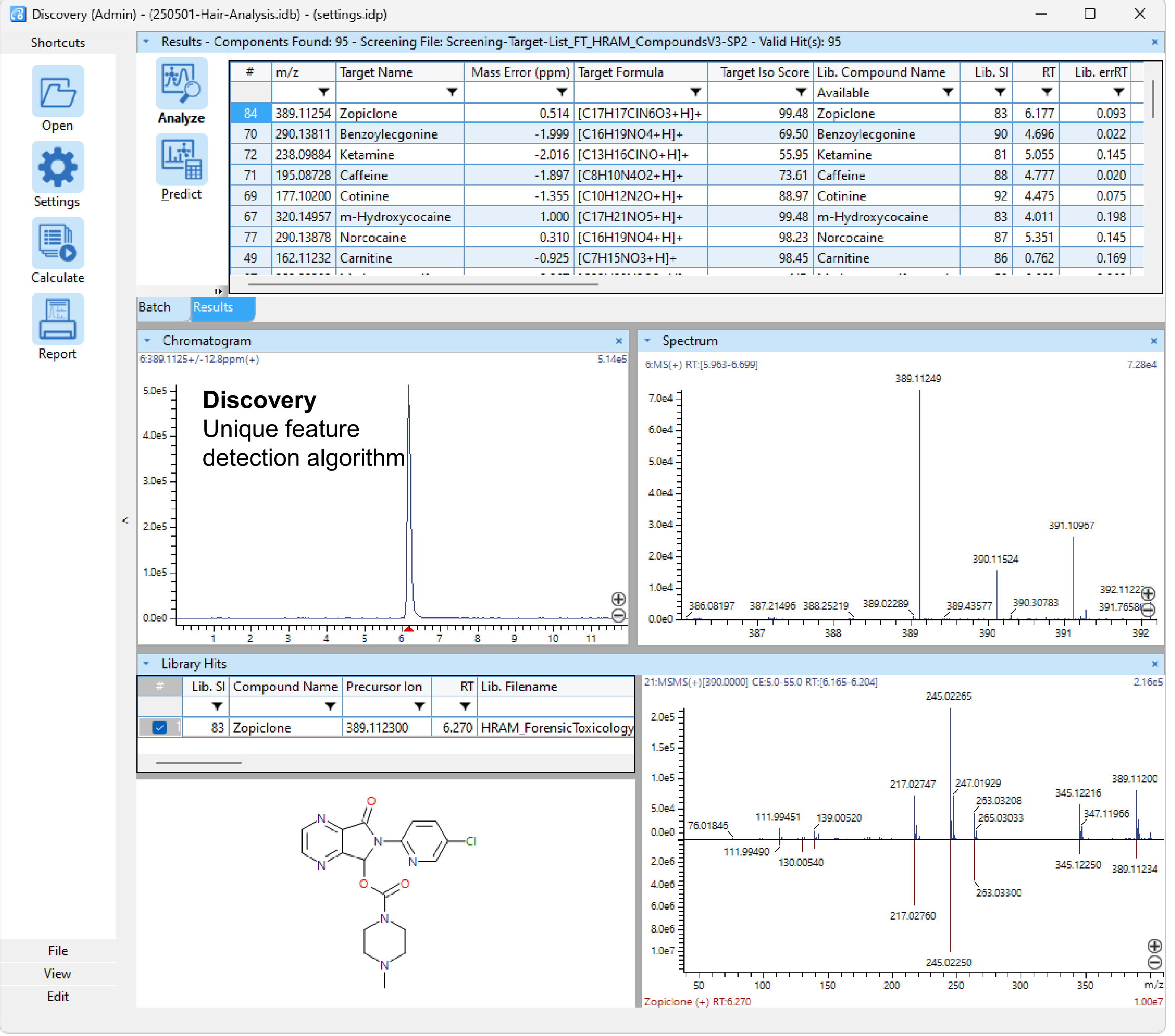
Library Hits - Ketamine - 12995_1-2_021

| # | Lib. SI | Compound Name | Lib. Filename |
|---|---|---|---|
| 1 | 83 | Ketamine | HRAM_ForensicToxicologyLibrary_ |
| ☐ 4 | 81 | Ketamine | NIST_HRMS_1 |
| ☐ 3 | 81 | Ketamine | NIST_HRMS_1 |
| ☐ 2 | 81 | ketamine | Oberacher_WRTMSD_HIRES_LCMS |
| ☐ 6 | 80 | ketamine | Oberacher_WRTMSD_HIRES_LCMS |
| ☐ 5 | 80 | ketamine | Oberacher_WRTMSD_HIRES_LCMS |
| ☐ 7 | 79 | ketamine | Oberacher_WRTMSD_HIRES_LCMS |
| ☐ 8 | 75 | Ketamine | NIST_HRMS_1 |
| ☐ 10 | 74 | Ketamine | NIST_HRMS_1 |
| ☐ 9 | 74 | ketamine | Oberacher_WRTMSD_HIRES_LCMS |
| ☐ 11 | 66 | Ketamine | NIST_HRMS_1 |
| ☐ 12 | 65 | ketamine | Oberacher_WRTMSD_HIRES_LCMS |
| ☐ 13 | 62 | Ketamine | NIST_HRMS_1 |
| ☐ 14 | 59 | Ketamine | NIST_HRMS_1 |
| ☐ 15 | 58 | ketamine | Oberacher_WRTMSD_HIRES_LCMS |

**Figure 2.** DoA compounds detected in a donor hair sample identified with high reporting confidence by using multiple libraries including NIST23 HRMS LC-MS/MS library.

## 3.3 Non-targeted screening

- **Non-targeted screening (NTS) data processing was applied to the same donor hair samples;** Insight Discovery software is a workspace focused on unknown analysis (requires no prior knowledge of any compound list).



Discovery (Admin) - (250501-Hair-Analysis.idb) - (settings.idp)

Results - Components Found: 95 - Screening File: Screening-Target-List_FT_HRAM_CompoundsV3-SP2 - Valid Hit(s): 95

| # | m/z | Target Name | Mass Error (ppm) | Target Formula | Target Iso Score | Lib. Compound Name | Lib. SI | RT | Lib. errRT |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Available | | | |
| 84 | 389.11254 | Zopiclone | 0.514 | [C17H17ClN6O3+H]+ | 99.48 | Zopiclone | 83 | 6.177 | 0.093 |
| 70 | 290.13811 | Benzoylecgonine | -1.999 | [C16H19NO4+H]+ | 69.50 | Benzoylecgonine | 90 | 4.696 | 0.022 |
| 72 | 238.09884 | Ketamine | -2.016 | [C13H16ClNO+H]+ | 55.95 | Ketamine | 81 | 5.055 | 0.145 |
| 71 | 195.08728 | Caffeine | -1.897 | [C8H10N4O2+H]+ | 73.61 | Caffeine | 88 | 4.777 | 0.020 |
| 69 | 177.10200 | Cotinine | -1.355 | [C10H12N2O+H]+ | 88.97 | Cotinine | 92 | 4.475 | 0.075 |
| 67 | 320.14957 | m-Hydroxycocaine | 1.000 | [C17H21NO5+H]+ | 99.48 | m-Hydroxycocaine | 83 | 4.011 | 0.198 |
| 77 | 290.13878 | Norcocaine | 0.310 | [C16H19NO4+H]+ | 98.23 | Norcocaine | 87 | 5.351 | 0.145 |
| 49 | 162.11232 | Carnitine | -0.925 | [C7H15NO3+H]+ | 98.45 | Carnitine | 86 | 0.762 | 0.169 |

Library Hits

| # | Lib. SI | Compound Name | Precursor Ion | RT | Lib. Filename |
|---|---|---|---|---|---|
| ☑ 1 | 83 | Zopiclone | 389.112300 | 6.270 | HRAM_ForensicToxicology |

**Figure 3.** Compounds detected by Insight Discovery non-targeted analysis software included: benzocaine and phenacetin commonly used as adulterants in cocaine samples; noscapine detection indicates possible use of illicit heroin. Other compounds detected outside the DoA target list included caffeine, nicotine, paracetamol, phenacetin but also zopiclone and noscapine (both compounds were subsequently added to the DoA target list).

## 4. Conclusions

- Mixed-data acquisition was applied to targeted and non-targeted analysis to detect drugs of abuse (DoA) in donor hair samples as part of a legal need to show abstinence for family court matters.
- The targeted method using DDA-MS/MS can easily be configured to support expanded DoA target panels and meet a growing need in forensic screening.
- Non-targeted analysis with DIA-MS/MS resulted in the detection of compounds outside the scope of the DoA panel .