

Accelerating drug screening in forensic hair analysis by applying high-speed polarity switching in HR LC-MS/MS

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1. Introduction and Aim

In 2023, the Society of Hair Testing (SoHT) published updated consensus guidelines for the forensic analysis of drugs of abuse in hair, defining recommended targets across eight major drug classes: opiates, cocaine, amphetamines, cannabinoids, opioids, methadone, buprenorphine, and ketamine. Legal drivers, accepted use cases, and degree of prescription differ between US and Europe but in both jurisdictions, mass spectrometry is widely applied to support legally defensible conclusions. The challenge for any drug testing in hair samples, whether it is in forensic and safeguarding contexts, workplace testing or a judicial setting is the need to develop methods capable of detecting expanded search panels with the increased prevalence of new psychoactive substances (NPS) and non-medical prescription drug use. The approach applied in this work considered a LC/QTOF analysis using DDA-MS/MS with rapid polarity switching and a novel data processing application, Insight Profiler. The software was designed to support a single click approach to automate data processing of complex data sets in non-targeted analysis from feature detection to compound identification.

2. Methods

2.1 Data acquisition

Sample Preparation

- Hair samples (each donor providing a single 6 cm sample) were segmented into six 1 cm samples and were washed in dichloromethane then dried overnight before milling. Internal standard was added to 10 mg powdered hair (± 0.5 mg) followed by extraction using 1% HCl in methanol. Extracts were dried under oxygen free nitrogen (OFN) at 40°C then reconstituted in 200 μ L 80:20 H₂O:MeOH (2 μ L injection volume).

Reversed phase LC separation: Nexera X2

- Column: Shim-pack Velox™ Biphenyl (100 x 2.1 mm, 2.7 μ m); column temp. 40°C, flow rate: 0.3 mL/min, 17 min total analysis time
- Binary gradient; H₂O:MeOH gradient with 2 mM ammonium formate and 0.002% formic acid

LC/QTOF Mass Spectrometry Detection: LCMS-9050

- MS scan m/z 100-1000, 100 msec scan time. Polarity switching time 800 msec.
- MS/MS DDA: 4 scans in positive ion mode (m/z 40-1000, 33 msec), 2 scans in negative ion mode (m/z 40-1050, 33 msec) CE 5-55V (1.998 sec total cycle time).

2.2 Automated data processing in Insight Profiler

For non-targeted screening, the processing parameters in a single method were set up for feature detection, alignment and compound identification using multiple libraries including NIST, Wiley, and the Shimadzu Forensic Toxicology database.

Feature Detection and Alignment
Detects and aligns all ion signals that behave as a peak.

Statistics and filters
Applied to find ion signals of significance and to remove ions of high variance.

Compound identification
Using large scale screening lists and multiple libraries to identify ion signals of interest.

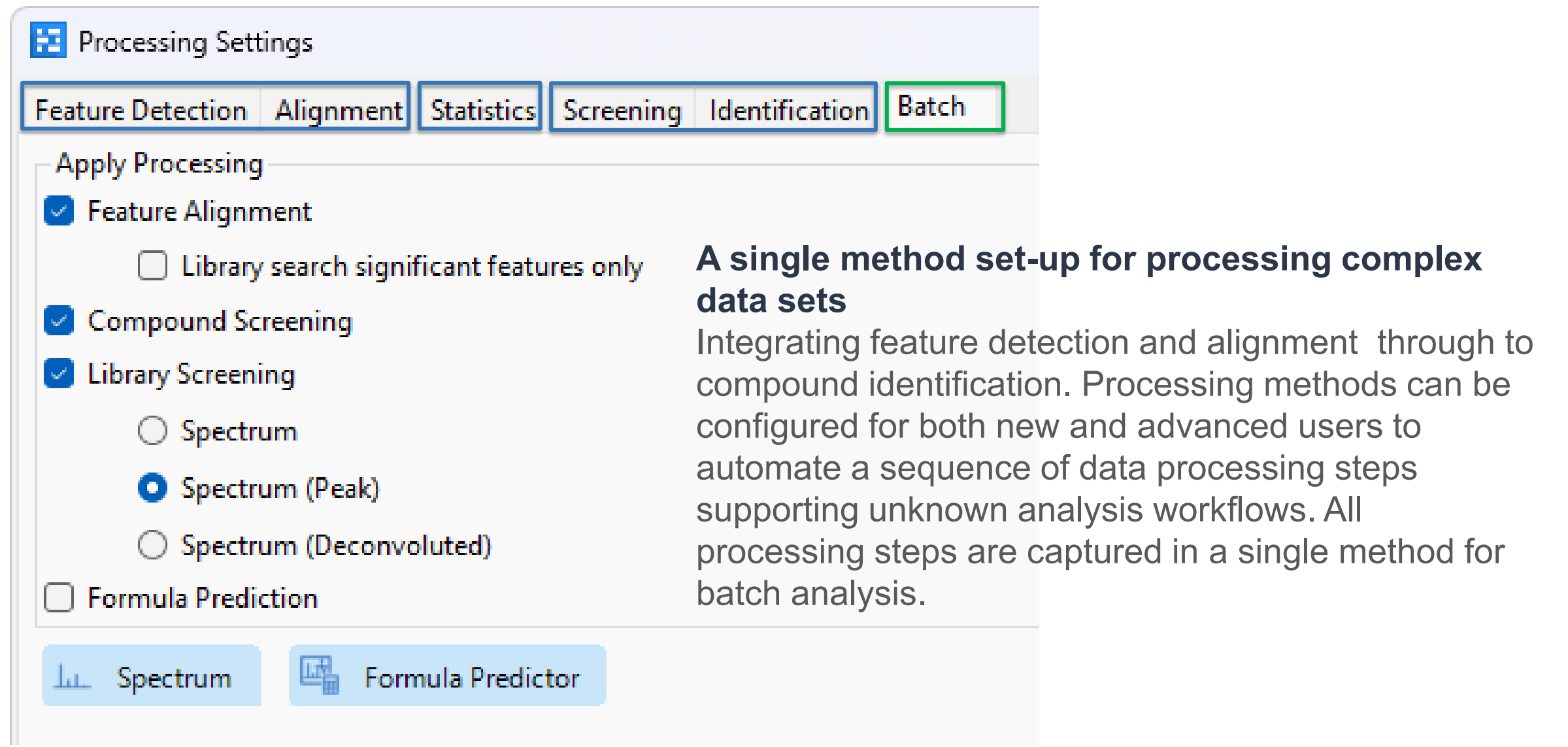


Figure 1. The Insight Profiler processing application method editor, designed to create a single workflow for feature detection of unknowns to compound identification.

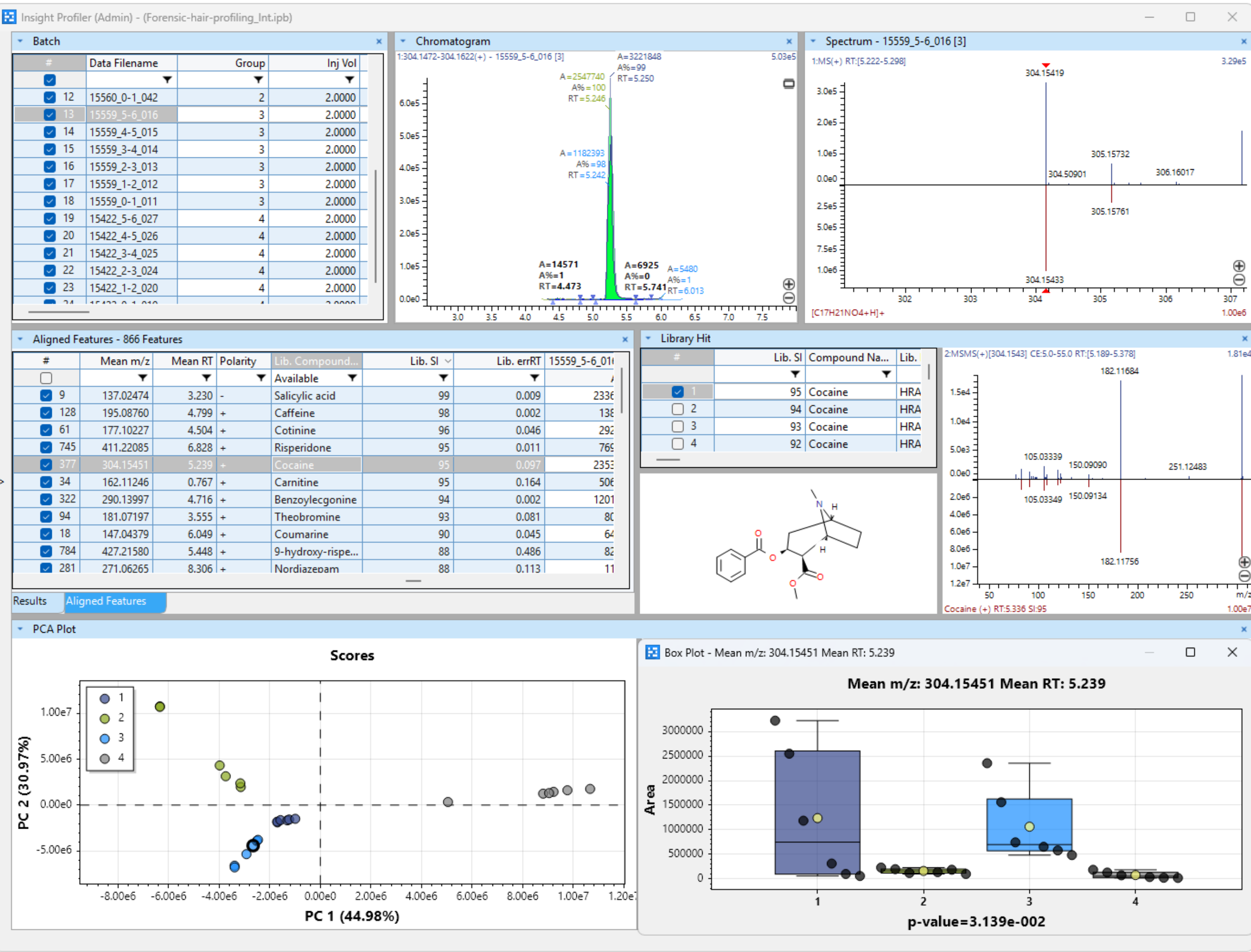


Figure 2. Insight Profiler data review highlighting the detection of cocaine in hair samples. The box plot highlighted compliance to court rulings as cocaine concentration was reduced over a 6-month time frame in two donors, group labels 1 and 3. Two other donors, group labels 2 and 4 confirmed compliance. In each box plot, the sample order from left to right indicate distance from the head: 5-6 cm, 4-5 cm, 3-4 cm, 2-3 cm, 1-2 cm, 0-1 cm.

3. Results

3.1 Monitoring abstinence during supervision and compliance to court ruling

Each scalp hair follows a growth cycle: anagen (active growth), catagen (regression) and telogen (resting) phase, lasting several years, a few weeks and several months, respectively. Scalp hair grows at a rate of 0.6–1.4 cm per month, averaging 1 cm per month. In one donor, hair samples taken over 6 months provided evidence for abstinence during supervision and compliance to court ruling following cocaine use (Figure 3).

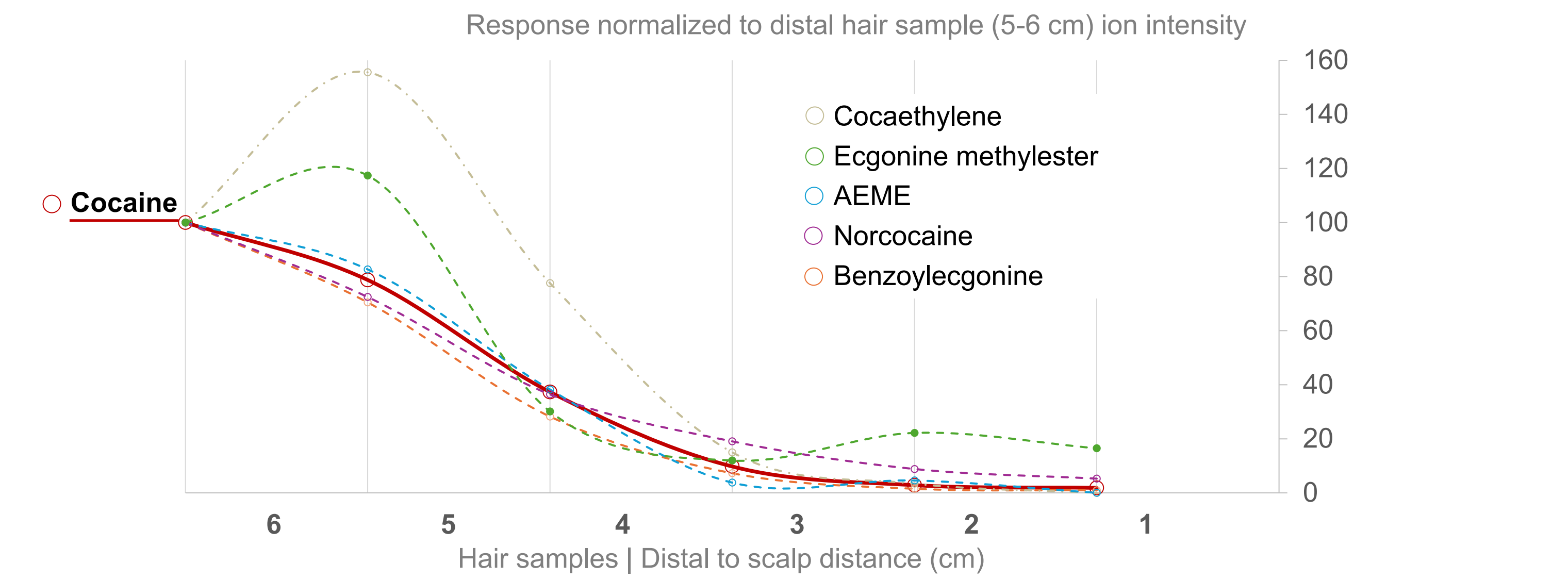


Figure 3. In this donor, the distribution of cocaine and associated metabolites was consistent with abstinence; with targeted LC/QTOF analysis the concentration of cocaine was 1.24 ng/mg in the distal hair segment (5–6 cm) compared with <0.01 ng/mg in the proximal segment (0–1 cm). This pronounced proximal–distal gradient indicates declining historical exposure, with concentrations in newly grown hair well below the 0.5 ng/mg reporting cutoff.

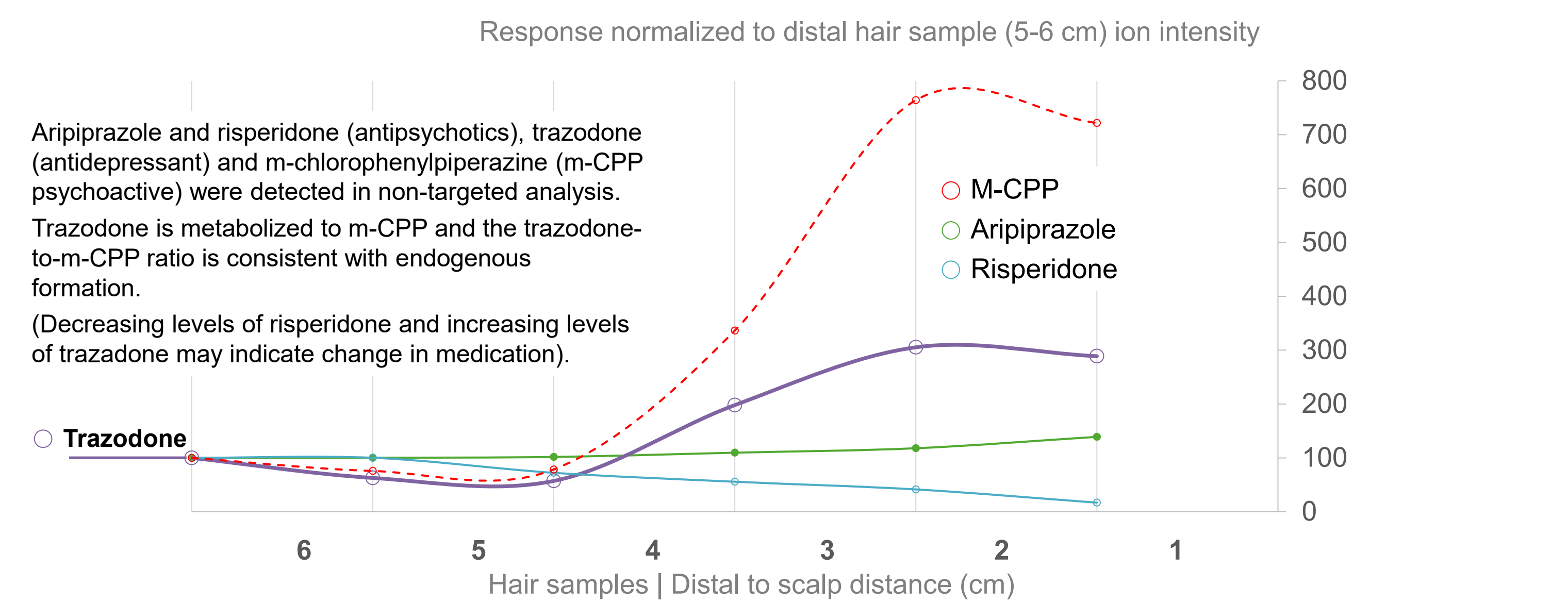


Figure 4. Outside the scope of providing evidence in monitoring abstinence during supervision and compliance to court rulings, Insight Profiler non-targeted analysis also detected other compounds in donor hair samples related to medication.

3.2 Hair analysis revealing exposure to chemicals

In the donor hair samples in this work, Insight Profiler non-targeted analysis screening detected compounds outside the scope of drug misuse and compliance with court-ordered abstinence including naturally occurring compounds, prescribed or over-the-counter medications, personal-care product ingredients, or environmental contaminants (including certain pesticides).

Table 1. Distribution of compounds detected in hair donor samples. (Salicylic acid was detected in negative ion mode and was likely due to use of anti-dandruff shampoo).

Natural Products	Pharmaceuticals	Restricted Compounds	Pesticides
Adenosine Caffeine Carnitine Coumarine Cytosine Harman MHPC Nicotinic acid Quinine Riboflavin Salicylic acid Theobromine Theophylline	Aripiprazole Cetirizine Citalopram Codeine Diazepam Diphenhydramine Hydrocodone Paracetamol Paroxetine Promethazine Quetiapine Risperidone Trazodone	Cocaine Ketamine Lidocaine Procaine Zopiclone Metabolites AEME Benzoylcegonine Cocaethylene Ecgonine methylester Norcocaine	1-(4-chlorophenyl)urea Azoxystrobin Carbendazim DEET

4. Conclusions

- The targeted LC/QTOF polarity switching approach successfully identified a significant number of unknown compounds in the samples not routinely targeted by triple quadrupole analysis.
- The method using DDA-MS/MS was easily configured to support an expanded DoA target panel to meet the needs of forensic toxicology screening.
- Non-targeted analysis using Insight Profiler accelerated data analysis by aligning identified compounds from the same hair donor showing direction of change for pharmaceutical and illicit compounds detected.