Characterization of new psychoactive substances using high resolution mass spectrometry structural assignment software

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Overview

- Seized drug samples were analysed by high resolution QTOF LC-MS/MS to identify new psychoactive substances (NPS).
- A second-generation component detection algorithm was applied to the untargeted analysis of seized drug samples. The algorithm has been enhanced to reduce the impact of noise artifacts and increase the probability of detecting all ion signals which behave in covariant manner.
- The untargeted discovery workflow identified putylone and bk-4MA-NBOMe by LC-MS/MS QTOF and confirmed by complimentary techniques including NMR.

1. Introduction

The growing impact of new psychoactive substances (NPS) in a constantly evolving drug market represents an increasing challenge in forensic toxicology. At the end of 2023, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) was monitoring around 930 new psychoactive substances (NPS), 41 of which were first reported in Europe in 2022. In this work a high-resolution LC-MS/MS QTOF was applied to the analysis of seized drug samples using a second-generation component detection algorithm with peak identification by MS/MS and NMR.

2. Materials and Methods

Seized drug samples were measured using an established high resolution forensic toxicological screening workflow using a second-generation component detection algorithm and peak identification by MS/MS. Results were screened against the Shimadzu Toxicology Database, a spectral library containing over 1200 retention time defined compounds and curated MS/MS spectra. Third party databases were also considered including the HighResNPS spectral library.

- **Reverse phase LC Separation**
  - 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 data dependant acquisition, up-to 20 dependant scans m/z 40-1000 CE 5-55 V, 40 msec (0.9 sec total cycle time).
- **Untargeted Component detection**
  - A second-generation component detection algorithm was applied to the TOF MS scan event for peak detection. The algorithm has been enhanced and optimized to locate ions that behave as a recognized chromatographic feature (ion intensities rise and fall in abundance in a covariant manner) to give a simplified ‘single component’ output when multiple ion species are present.
  - To further minimize the impact of idiosyncratic ion behaviors and to maximize reporting of high-quality chromatographic peaks quality, components are automatically assessed for jaggedness (may be encountered in saturation), symmetry and noise specifically at trace levels to report true components.

3. Results

The untargeted discovery workflow considers several key steps;

- **Component Detection**. Create a series of overlapping spectral data bins for the TOF MS scan event. For each spectral data bin, combine all scan data into a single average spectrum. Check neighboring spectral data windows for the same ions to find the peak apex and locate a component. Remove candidate components which do not meet the reporting criteria for jaggedness, symmetry and intensity thresholding.
- **MS and MS/MS Identification**. For each ion signal considered to be a chromatographic peak suspect screening lists, high resolution library searching, and automated formula prediction can be applied to help identify candidates with high reporting confidence. For NPS which are new to the market complimentary analytical techniques are required for positive identification including NMR, FTIR and GC-EI-MS.

3.1 Identifying Putylone in Drug Seizure Samples

In this sample, two components were reported following automated component detection and screening against the Shimadzu Toxicology Database.

Component 1 | Rt 4.990 mins. Precursor mass accuracy, isotope pattern, Rt and MS/MS library matching score reported caffeine with high confidence.

Component 2 | Rt 5.051 mins. Insight Discovery highlighted a review status. Precursor mass accuracy, isotope pattern and MS/MS library matching score reported N-ethylpentylone (a substituted cathinone and stimulant drug, in the US it is a Schedule I controlled substance since June 2018). However, the Rt was flagged as a review status given the disparity between the library Rt and experimental Rt (Figure 1).

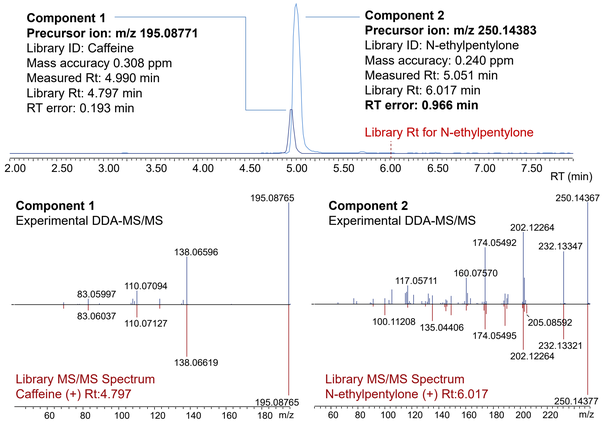

Figure 1. Seized drug analysis detected 2 components in this sample; component 1 was positively identified as caffeine whilst component 2 was inconsistent with a library Rt corresponding to N-ethylpentylone requiring further evidence for a positive identification.

Although the DDA-MS/MS spectrum for Component 2 resulted in a high dot product similarity score consistent with N-ethylpentylone the library Rt was inconsistent. The presence of m/z 191.07084 in the experimental DDA-MS/MS was consistent with putylone (a stimulant and empathogen compound first detected in 2023). The identification was verified by comparing a synthesized standard of putylone with LC-MS/MS, NMR, FTIR and GC-EI-MS.

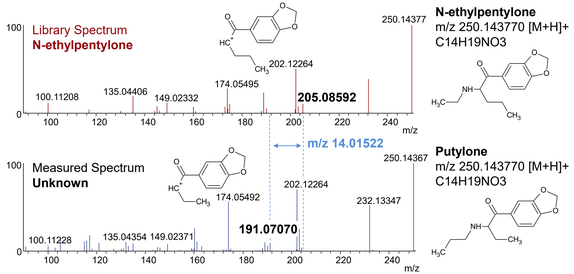

Figure 2. The library DDA-MS/MS spectrum N-ethylpentylone differed from the experimental DDA-MS/MS of component 2 with the presence of m/z 191.07070 and absence of m/z 205.08592. Insight Assign structural assignment application reported a structure consistent with the loss of CH2 from the ion at m/z 205.08592.

3.2 Identifying bk-4MA-NBOMe in Drug Seizure Samples

In a second sample, a single component was detected. The DDA-MS/MS fragment ions are consistent with a relatively new class of psychedelic compounds referred to as NBOMe (or 25X-NBOMe) which have appeared on the illegal drug market. The most frequently reported drugs from this group are 25I-NBOMe, 25B-NBOMe, and 25C-NBOMe[1]. NBOMe compounds are ultrapotent and highly efficacious agonists of serotonin 5-HT2A and 5-HT2C receptors.

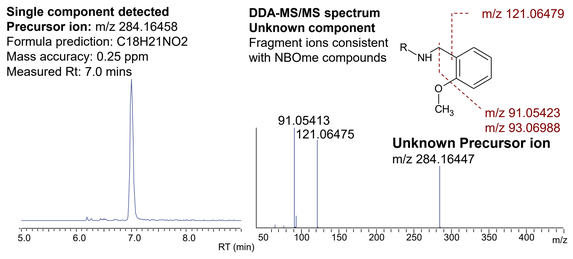

Figure 3. In this seized drug sample, a single NPS component was detected. The fragment ions at m/z 91.05423 and 121.06479 agreed with the NBOMe class of psychedelic compounds but could not be confirmed by existing library MS/MS repositories.

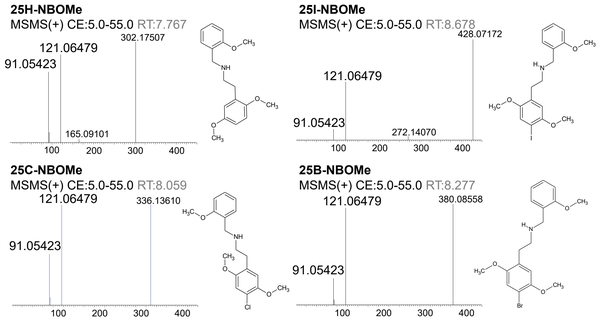

Figure 4. MS/MS spectra for 4 NBOMe compounds highlighting the characteristic fragment ions at m/z 91.05423 and 121.06479 for this class of psychedelic compounds from the Shimadzu Toxicology Database. 25I-NBOMe, 25B-NBOMe, and 25C-NBOMe, accounted for 0.03% of the total quantity of hallucinogens (other than ketamine) seized globally between 2011 and 2017[1].

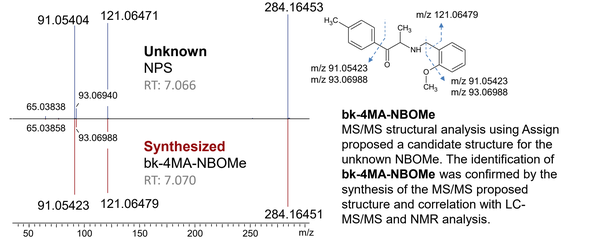

Figure 5. Positive identification of the unknown NBOMe NPS compound; bk-4MA-NBOMe (protonated molecular ion m/z 284.16451).

4. Conclusions

- Drug seizure samples were analysed by high resolution LC-MS/MS. In one case, the unknown compound was positively identified as putylone by reviewing MS/MS and Rt data with a forensic toxicology library.
- In another case, MS/MS data helped to propose a candidate structure which was synthesised for a positive identification as bk-4MA-NBOMe.
- In both cases, complimentary techniques such as NMR and GC-EI-MS provided evidence for a positive identification.

[1]Reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7054380/.