Detection and identification of new psychoactive substances in pooled urine using High Resolution All-Ions MS/MS

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1. Introduction

New Psychoactive Substances (NPS) are substances that mimic effects of illicit drugs like cocaine, cannabis and amphetamines– and are synthetized to evade law enforcement by introducing slight modifications to chemical structures of controlled substances. NPS are easily acquired legally through online vendors and smart shops where they are sold under false labels with misleading information about their effects and safety. They are considered a growing problem in many communities and are responsible for numerous fatal intoxications. Detection of NPS is a challenge due to their rapid transience on the drug scene, creating a continually moving analytical target.

Liquid chromatography (LC) coupled to high-resolution mass spectrometry (HRMS) based on a quadrupole time-of-flight (QTOF) provides sensitive, full-spectrum MS data with high mass resolution and accuracy. All-ions MS/MS mode (based on QTOF-MS) also refered to as MSE enables fragmentation at two collision energies (low and high) without single isolation of specific precursors in a single analytical run. This allows the possibility of detecting a large number of compounds. Furthermore, linked to sophisticated post-acquisition data processing strategies previously applied[1,2], it allows the tentative identification of compounds detected without the need of acquiring reference standards (which are sometimes not available).

The approach presented here demonstrates the application of all-ions MS/MS mode as a useful tool for combined post-acquisition targeted analysis and suspect screening strategies for identification of NPS in pooled urine samples.

2. Materials and methods

Pooled urine samples collected from various locations in London were prepared by hydrolysis and protein precipitation with acetonitrile (1:2). The LC system consisted of an Agilent 1290 Infinity LC, coupled to an Agilent 6530 Accurate-Mass Q-TOF MS. The LC separation was performed using a Phenomenx Biphenyl (100 x 2.1 mm, 2.6 µm) at a flow rate of 0.4 mL/min. The mobile phase consisted of (A) H_2O with 0.04% HCOOH and (B) 80/20 AcN/H_2O with 0.04% HCOOH. The applied gradient, in function of B, was: 0 min: 2%; 2 min: 2%,18 min: 40%; 25 min: 90%; 29 min: 90%; 29.5 min: 2%; 33 min: 2%. Acquisition was performed in positive and negative MS mode with 3 scan segments at 0, 15, and 35 eV with fragmentor at 100 V at a rate of 2.5 spectra/s. Source parameters were as follows: gas temperature 350 °C, gas flow 10 L/min, nebulizer 40 psi, capillary voltage 4000 V. Acquired data were processed using Find by Formula (FbF) alogarithm in MassHunter Qualitatitive Analysis B.06.00 linked to Personal Compound Database Library (PCDL) containing an in-house developed database with >1500 compounds, e.g., phenethylamines, piperazines, synthetic cannabinoids, synthetic cathinones, tryptamines, and some of their metabolites.

3. Results and discussion

Data processing (*Fig 1)* shows how the FbF alogarithm is applied to examine mass peaks in the low energy (LE) channel against the PCDL for compounds with the same accurate mass values. A match

score is calculated based on a compound's accurate mass, isotopic abundance and spacing. If the compound passes the match score filter, it is labelled as 'identified'. The detected compound's isotope pattern is also extracted displaying abundance and spacing. For fragment identification, the resultant ions in the MS/MS spectrum are extracted as chromatograms and a co-elution score is generated indicating confidence of the correlation between parent-ion and fragment ions based on abundance, peak shape, retention time and labelled as 'qualified'. For targeted confirmation, MS/MS spectra in the PCDL are compared to ions detected in high energy (HE) channel to confirm presence of fragments. If no MS/MS spectrum is available, a tentative confirmation is made by elucidating 'qualified' fragments based on parent compound as shown in *Fig 1* for the two fragments.

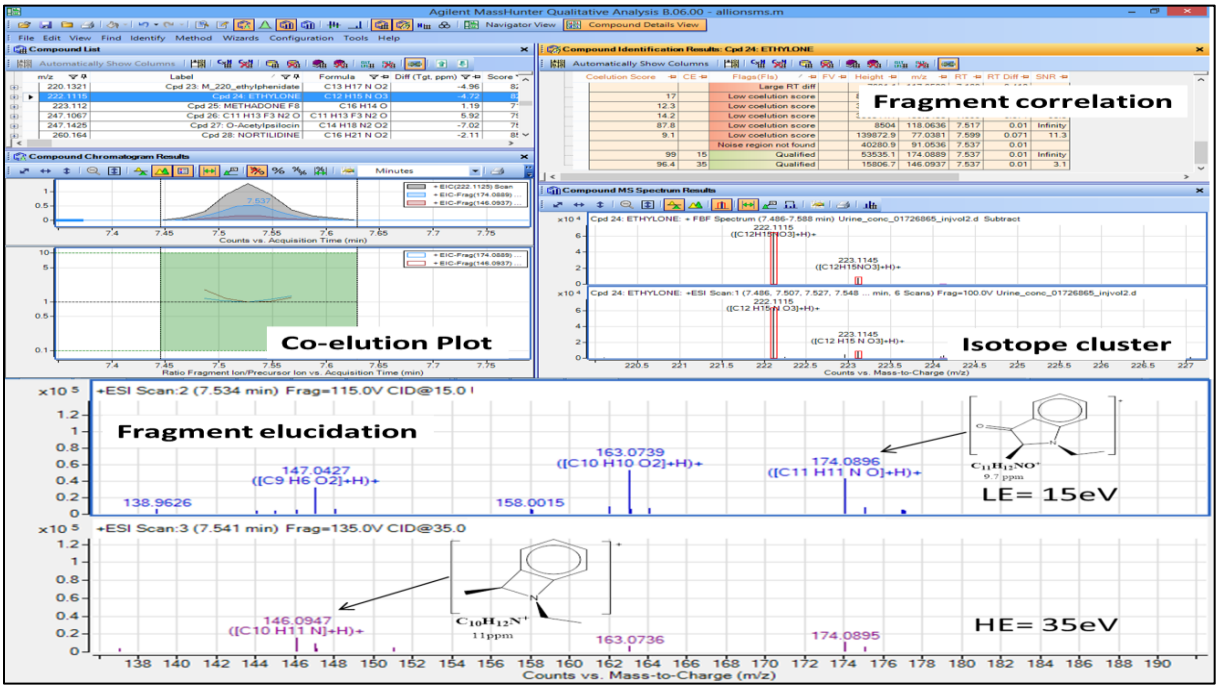

Figure 1: Detected compounds detailed view showing tentative confirmation of ethylone in a pooled urine sample

A total of 13 samples were tested using the workflow design described and on average we had 60 compounds detected per sample of which 70% were false positive, 28% tentatively confirmed and 2% confirmed with MS/MS spectra in the PCDL from available reference standards (targeted).

4. Conclusions

The all-ions MS/MS workflow proves to be a powerful tool in determining which NPS are commonly found in a certain area which is useful when deciding which reference standards to prioritze for purchase. In addition, data can be reanalyzed in the future to look for additional compounds without re-injections.

5. References

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