[TECHNOLOGY BRIEF]

The Utility of MS^E for Toxicological Screening.

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GOAL

To investigate the utility of the innovative data acquisition mode MS^E for the screening of toxicants in human urine.

BACKGROUND

Laboratories are frequently required to perform broad screening techniques on complex biological samples to identify drugs of abuse and other toxicants. In recent years there has been an increased interest in the use of Time-of-Flight (Tof) instruments for this purpose owing to the high level of specificity offered by exact mass data.

Whilst exact mass libraries can be automatically generated without reference material *i.e.,* from molecular formulae, the lack of additional information can lead to false positive results in the analysis of authentic samples. Thus, where possible, additional information *e.g.,* an associated retention time (RT) and confirmatory fragment ions should be used to increase confidence in drug identification and to improve the ease and speed of data review and reporting.

MS^E is a novel, patented mode of data acquisition that permits the seamless collection of a comprehensive catalog of information for both precursor and fragment ions in a single analysis. This is achieved by rapidly alternating between two functions *i.e.,* the first, acquired at low energy provides exact mass precursor ion spectra; the second, at elevated energy provides high energy exact mass of the fragment ions. In addition to providing increased confidence in identification, fragmentation can help to differentiate between isobaric compounds.

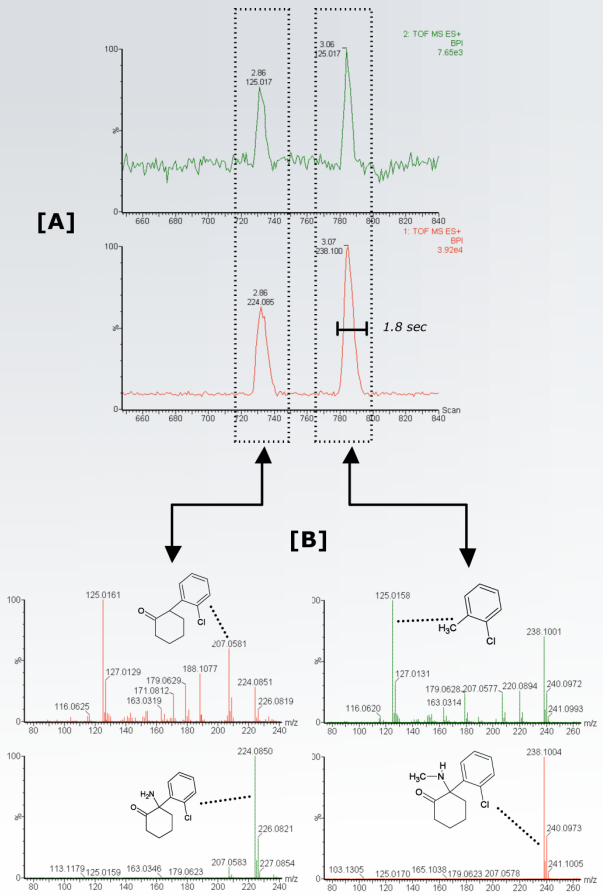

Figure 1. MS^E analysis of an authentic urine sample.

Panel A shows the chromatograms for the low (lower-trace) and high (higher-trace) energy data. The displayed data focuses on two unknown analytes eluting at 2.9 and 3.1 min, respectively. A minimum of 12 data points are collected for each analyte and for each energy condition.

Panel B shows the underlying exact mass spectra for the low (lower-traces) and high energy (upper-traces) condition. If desired, any ions within these spectra can be submitted for Elemental Composition Analysis which uses a combination of exact mass and isotope patterns to propose likely elemental formulae. MassFragment™ can be used to verify and assign logical molecular structures for a given measured mass.

In contrast to the more conventional data dependent acquisition (DDA), MS^E has no duty cycle restrictions and therefore better addresses the problems associated with co-elution in complex biological mixtures and the challenges of the sharper, narrower peaks associated with UPLC separation.

THE SOLUTION

LC/MS System Configuration

ACQUITY UPLC® System in combination with the Xevo™ G2 QTof Mass Spectrometer

LC/MS Conditions

Column: ACQUITY UPLC® HSS C_{18} Column

Run time: 15 min gradient elution

Ionization Mode: ESI Positive

Acquisition range: 50-1200

Resolution: 20,000 FWHM

MS^E conditions: Collision energy ramp 10-40 eV

Software and Database

ChromaLynx™ XS application manager was used in targeted mode for automated comparison with an in-house database comprising more than 700 toxicologically-relevant compounds/metabolites. The database includes precursor ion mass and RT and is supplemented with fragment ion information (Figure 3).

Doxepine	C19H21NO	6.93	f:107.0497	f:141.0704	f:235.1123
Ecgonine methyl ester	C10H17NO3	0.82	f:182.1128	f:82.0657	f:150.0919
EDDP	C20H23N	7.34	f:249.1517	f:234.1283	f:186.1283

Figure 3. Excerpt from the in-house toxicology database. Data includes the following information (from left to right): elemental formula; RT and exact mass information of specific fragment(s).

RESULTS

Diluted urine samples were analysed using UPLC/TOF analysis in MS^E mode. The increased speed and resolution associated with UPLC separation results in a significant reduction in peak width. Figure 1 shows an example of a typical peak, where widths (half-height) can be less than 2 sec, but data quantity and quality remains uncompromised. With MS^E, data is collected continually at both low, and elevated, energy thus a full data set for both precursors and fragment ions is always acquired. Even in the case of closely or co-eluting analytes, full exact mass spectra are available.

The data was processed automatically using ChromaLynx XS and compared to an in-house database. The compounds eluting at 2.9 and 3.1 min were identified as norketamine and ketamine respectively. The sample was also positive for theophylline, another ketamine metabolite *i.e.*, dehydronorketamine, the recreational drug MDMA (Ecstasy) and two of its metabolites (HMMA and MDA) (Figure 4.). The combination of MDMA and ketamine are commonly-abused in a drug practice known as 'kitty-flipping'.

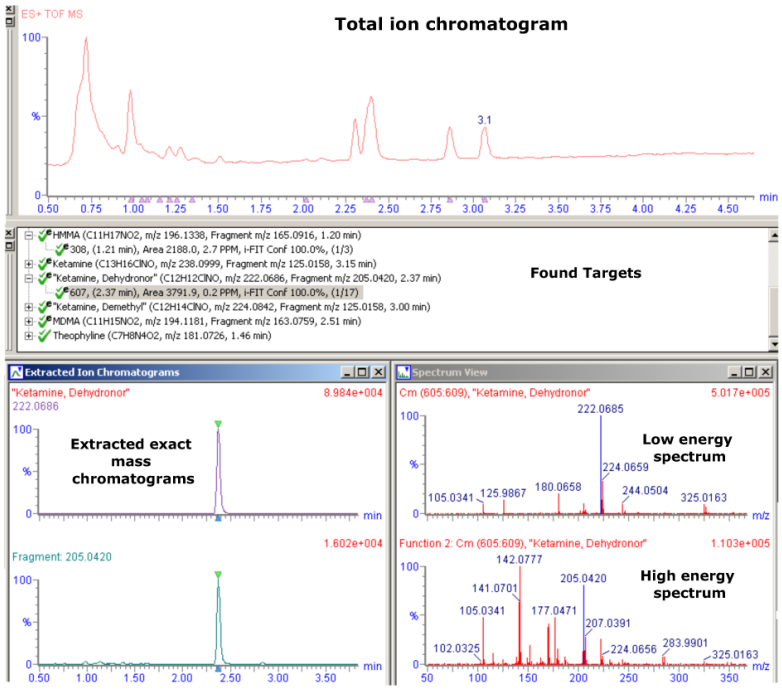

Figure 4. ChromaLynx browser showing details for identified drugs. With the exception of theophylline all analytes were identified and confirmed with additional fragment ions (denoted by the bold 'e'). Where extra confirmatory ions are not specified in the target list (as in the case of theophylline), identification is based on exact mass, isotope ratios and RT.

SUMMARY

MS^E was successfully used to analyse authentic urine samples. Fragment ion confirmation provides superior confidence in analyte identification and minimises the opportunity for false positives thus improving the ease and speed of review and reporting.

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