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The Utility of MS^E for Toxicological Screening

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For forensic toxicology use only.

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief investigates the utility of the innovative data acquisition mode MS^E for the screening of toxicants in human specimens.

Introduction

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 accurate mass data.

While theoretical or 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 catalogue of information for both precursor and fragment ions in a single analysis.[1–3] This is achieved by rapidly alternating between two functions ***i.e.***, the first, acquired at low collision energy provides an accurate mass measurement of the precursor ion. The second, at elevated energy provides accurate masses of the fragment ions. In addition to providing increased confidence in identification, fragmentation can help to differentiate between isobaric compounds.

Experimental

LC-MS System Configuration

ACQUITY™ UPLC™ I-Class PLUS (FTN) System in combination with the Xevo™ G2 XS QTof Mass Spectrometer.

LC-MS Conditions

Column:	ACQUITY UPLC HSS C_{18}, 1.8 µm, 2.1 x 150 mm (p/n: 186003534)
Run time:	15 min
Ionization mode:	ESI+
Acquisition range:	*m/z* 50–1000
MS^E conditions	Collision energy function 1:6 eV Collision energy function

2:ramp 10–40 eV

Software and Library

UNIFI Software was used in combination with the Waters Forensic Toxicology Scientific Library.

Results and Discussion

Certified reference material (CRM) for toxicologically-relevant compounds were obtained from Merck (Dorset, UK) and were analysed using UPLC-Tof in MS^E mode. Figure 1 shows the MS^E data obtained following analysis of a representative substance, buflomedil. The figure illustrates how a confident identification can be obtained from the ability to measure the mass of the precursor ion to four decimal places (precursor mass is shown in the low energy spectrum). When MS^E is utilized, even greater confidence in identification can be achieved by additional incorporation of the masses of the specific fragment ions which are generated when the collision energy is ramped (high energy spectrum). Furthermore, RT can also be included in the identification criteria.

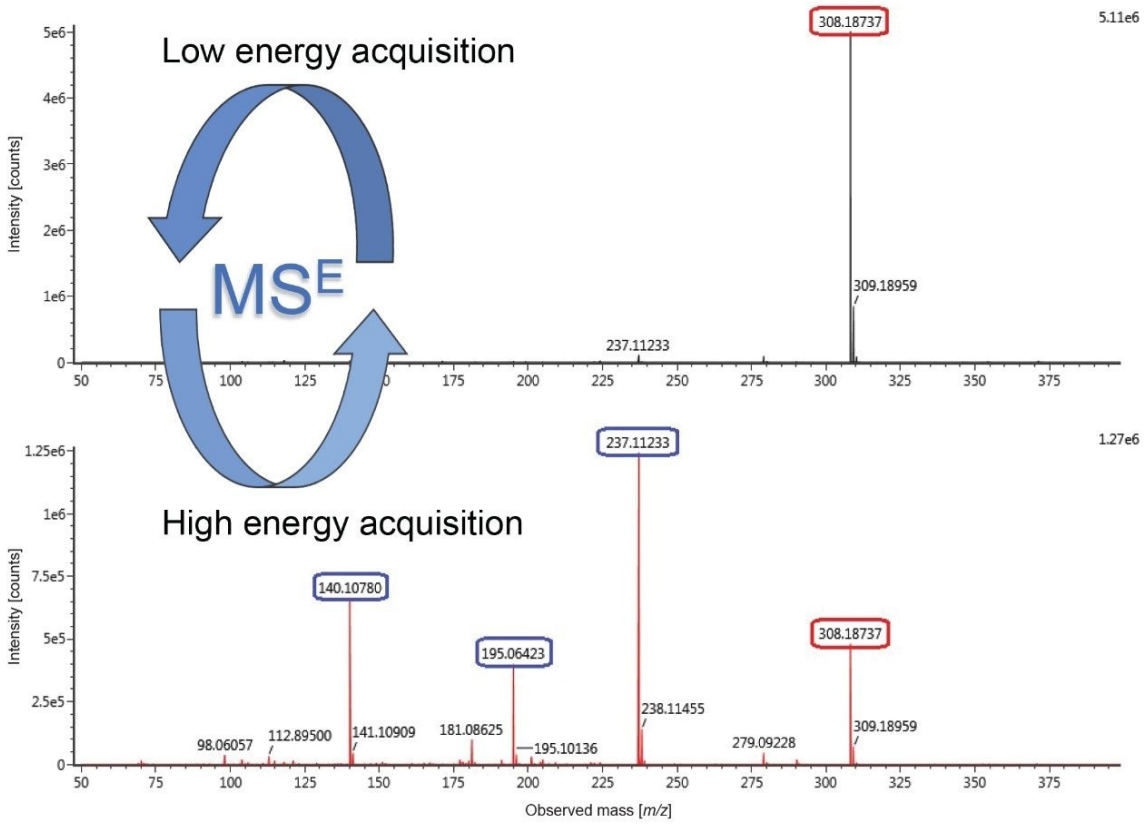

Figure 1. With MS^E the dataset is complete; full accurate mass data for both precursors (shown in red boxes) and fragment ions (blue boxes) is always acquired, even in the case of closely or co-eluting analytes.

MS^E is a data-independent technique meaning that full accurate mass data is being acquired continually under both low and high energy conditions throughout the analysis. This is in contrast to data-dependent (or information-dependent) techniques, where typically the instrument commences collecting full accurate mass data at low energy to provide mass information of the intact molecules until a 'trigger' is received which then instructs the instrument to switch to collecting data in tandem mass spectrometry (MS/MS) mode. The trigger could be any precursor ion exceeding a response threshold, or alternatively the instrument may be set to trigger on detection of specifically targeted precursor ion(s). The disadvantage of data-dependent approaches can be that while the instrument is collecting MS/MS - it is not collecting full scan MS data, thus the data is incomplete. In forensic toxicology a complete and unrestricted dataset is particularly advantageous as it provides the ability to retrospectively examine the data without fear that potentially relevant data has been omitted by use of

targeted acquisition techniques such as data-dependent analysis such as conventional MS/MS. In other words, the user can reprocess existing data without the need to reanalyze or reacquire additional data for the sample.

However, the key benefit of having the complete data means that it opens up the ability to process data using three complementary workflows as summarized in Figure 2.

Figure 2. These complementary workflows are possible owing to the complete nature of the MS^E data.

Targeted Analysis

Targeted analysis is the most straightforward approach, where the acquired data is simply matched against a reference library. The UNIFI Forensic Toxicology Screening Solution includes a comprehensive library, in which each library entry comprises a reference RT together with the exact mass of the precursor ion and verified

diagnostic exact mass fragment ions. Figure 3 shows an image of a representative entry from the Waters Forensic Toxicology Library.

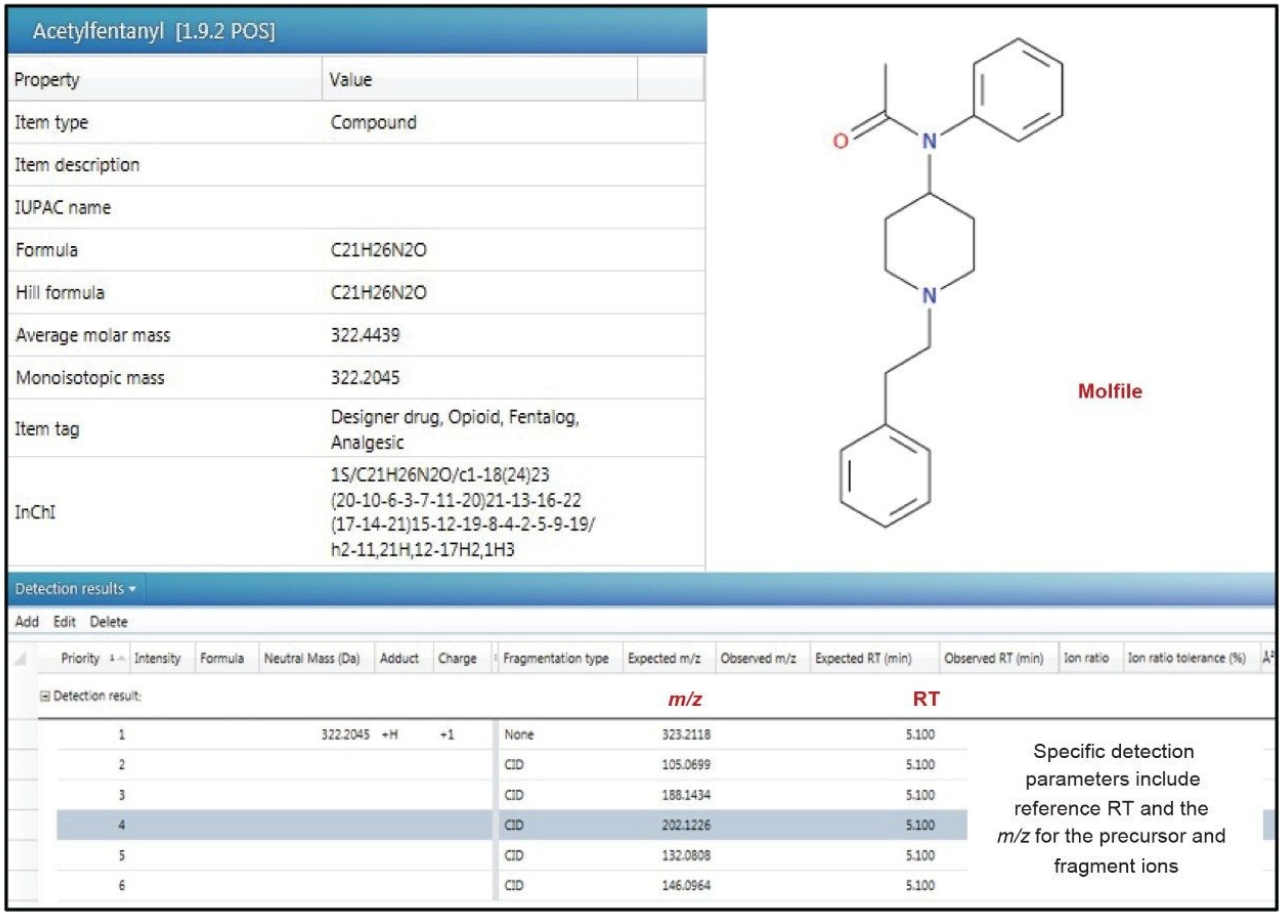

Figure 3. Example library item for a representative compound in the UNIFI Forensic Toxicology Library.

Figure 4 shows the results browser for a typical sample processed in this way and demonstrates the wealth of information that is available for use in the library matching process, and which provides the user with a fast, clear, and confident identification.

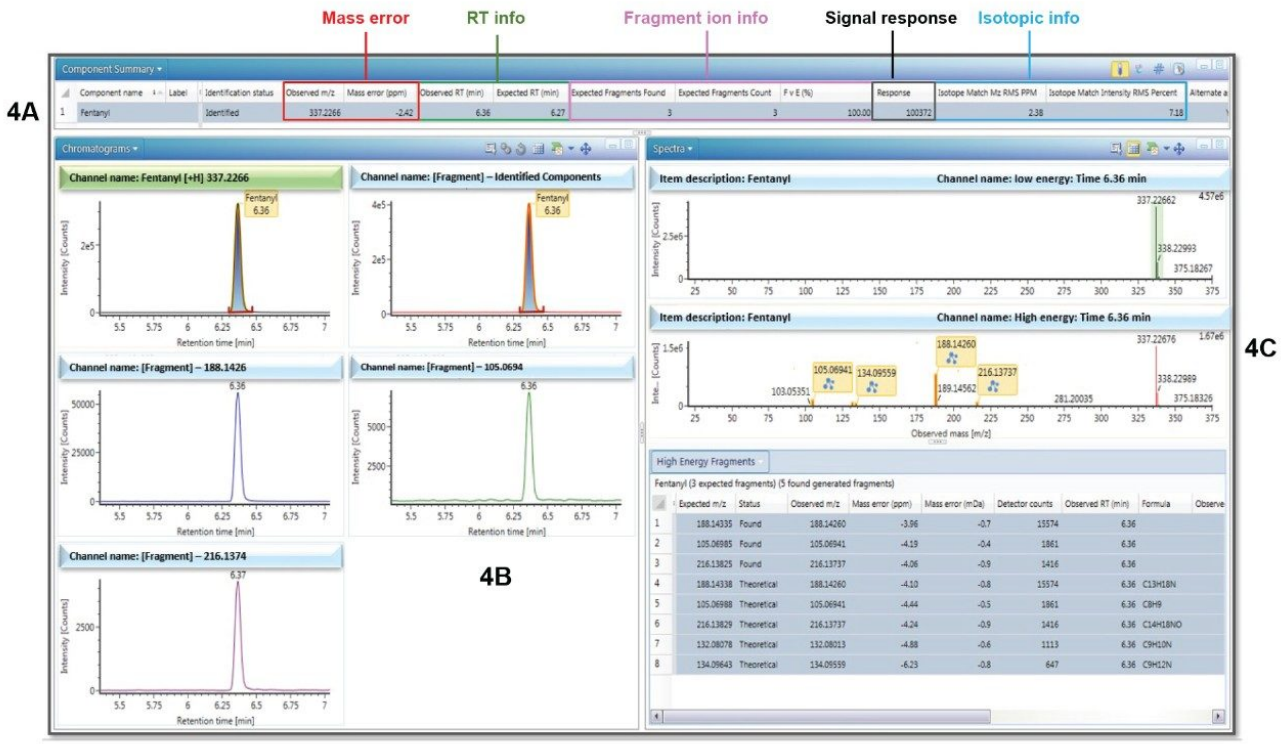

Figure 4. Detection of fentanyl in a sample following targeted analysis. A wealth of information is available and may be viewed if desired. In this figure the upper table (4A) details the results of the comparison of acquired data against the reference information contained in the Toxicology Library. Panel 4B displays the extracted ion chromatograms for the targeted precursor and the 3 diagnostic fragment ions and demonstrates that all ions are time-aligned at 6.36 min. The low and high energy spectra are shown in Panel 4C (upper). Details of the fragment ions are listed in the lower table.

Semi-targeted Analysis

One of the key benefits of high resolution/accurate mass instruments is that even in the absence of a fully characterized library entry, the user still has the ability to screen for drug substances on the basis of their exact mass. This is especially beneficial for forensic toxicology laboratories as it enables the user to screen for novel or emerging drug substances without the requirement of CRM; this is invaluable as access to commercial reference material for new analogues is often delayed. In semi-targeted processing, a Molfile is utilized; this file describes the elemental formula and overall arrangement of the bonds of the compound of interest. During the automated processing, evidence of the *m/z* for the precursor ion (as determined from the Molfile) is sought in the low

energy trace (Figure 5). While this information alone, is useful for a tentative identification, in addition UNIFI performs *in-silico* fragmentation of the Molfile to generate theoretical fragment ions which are then sought in the high energy data. A sample containing evidence of both precursor ***and*** theoretical fragment ions for a particular drug substance, demonstrates a higher confidence in likely identity.

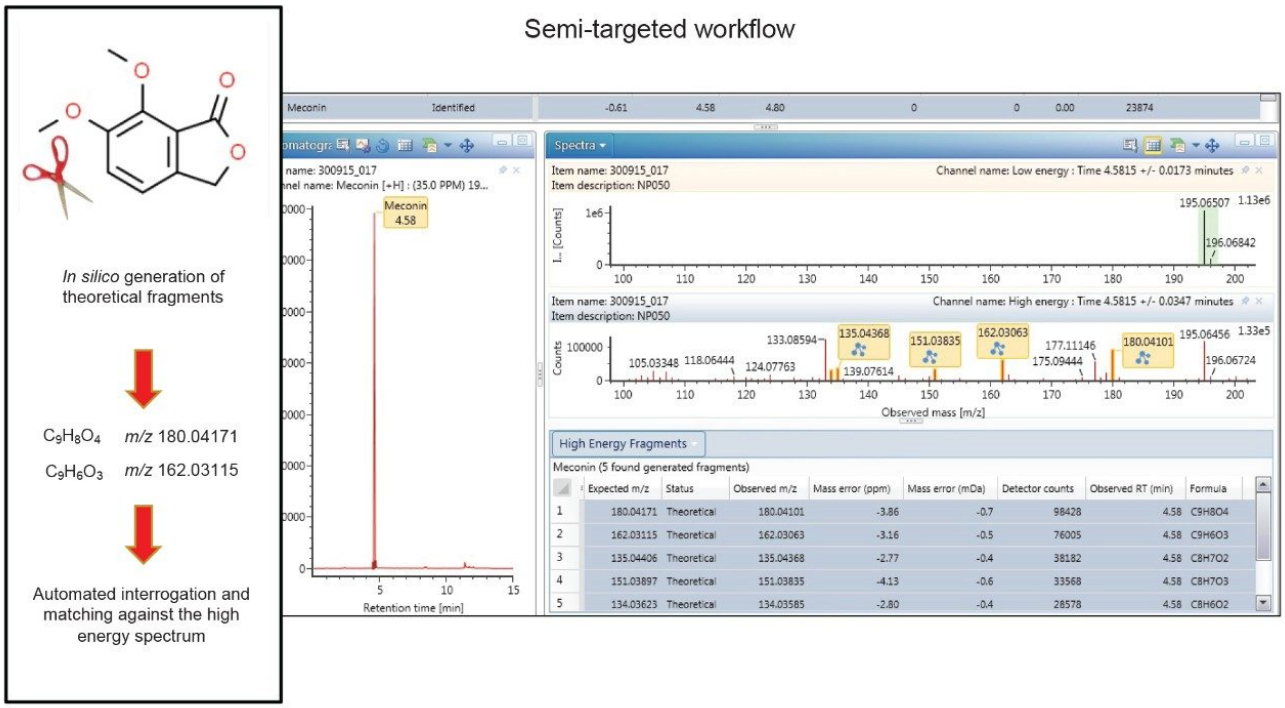

Figure 5. Tentative detection of meconin in a urine sample by screening by use of a Molfile. In this sample processing highlighted an unknown peak with a mass of m/z 195.0651, which was consistent with that of the noscapine metabolite meconin. Meconin has been proposed as a marker for illicit opiate use. In-silico fragmentation is automatically performed for all Molfiles added to the library and yields theoretical fragments which are then sought in the high energy MS^E data. This sample also screened positive for several opiates including noscapine, papaverine and heroin. The tentative detection of meconin was subsequently confirmed following analysis of CRM.

Non-targeted (discovery)

Discovery workflows can be applied where there is an unknown peak in the data that is not identified by either targeted, or semi-targeted, workflows. Under these circumstances, UNIFI offers a full suite of discovery tools that

can be used to elucidate the structure of the unknown. The first step in the discovery process is to determine the likely elemental formula(e) of the substance; UNIFI achieves this based on the accurate mass and isotopic information of the precursor mass in the low energy trace of the MS^E data. The second step is to assign any likely substances that correspond to that measured formula. UNIFI achieves this by searching online chemical databases such as those contained within Chemspider and simultaneously accessing the Molfile associated with that substance. In the third step and final step, UNIFI performs the *in-silico* fragmentation process for any proposed substances and compares the theoretical ions with the fragment ions observed in the high energy trace of the unknown substance. The greater the similarity of the acquired to the theoretical, the higher the confidence in the proposed identity. Further confirmation of this type of preliminary tentative identification would require verification of retention time and fragment ions through analysis of CRM. Figure 6 shows an example of a tentative identification of cyclopropylfentanyl using the discovery workflow. A more detailed description of the discovery workflow, with illustrated examples, is available in another Application Note.[4]

Figure 6. On selection of the component peak of the unknown for the elucidation process, an automated discovery sequence is triggered which includes searching external Scientific libraries e.g., Drugbank,ChEMBL, to provide proposal of likely substances which correspond to the measured accurate mass and matching of theoretical fragments with observed fragment ions. In this example the proposal of cyclopropylfentanyl , based on measured mass m/z 349.2272 together with the isotopic information was supported by 26 matched fragment ions.

Conclusion

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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