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Application Note

The Utility of MS^E for Toxicological Screening With waters_connect™ and the Xevo™ G3 QTof Mass Spectrometer

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Este é um Resumo de aplicações e, por isso, não inclui uma seção de experimento detalhada.

For forensic toxicology use only.

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 High-Resolution Mass Spectrometry (HRMS) 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. Therefore, additional information *e.g.*, an associated retention time (RT) and confirmatory fragment ions should be used, where possible, 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.

This application brief includes examples of MS^E data for toxicological compounds and summarizes some of the key benefits of this acquisition mode in comparison to conventional data-dependent techniques. We describe the flexibility around data processing and summarize the contents of the Waters™ Forensic Toxicology HRMS Screening Solution and the Library.

Experimental

LC-MS System Configuration

ACQUITY™ UPLC™-I-Class (FTN) System in combination with the Xevo™ G3 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

waters_connect™ informatics package was used in combination with the Waters Forensic Toxicology HRMS Scientific Library.

Results and Discussion

Certified reference material (CRM) for toxicologically -relevant compounds were obtained from Merck (Dorset, UK) and were analysed using UPLC-QToF 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, retention time (RT) can also be included in the identification criteria.

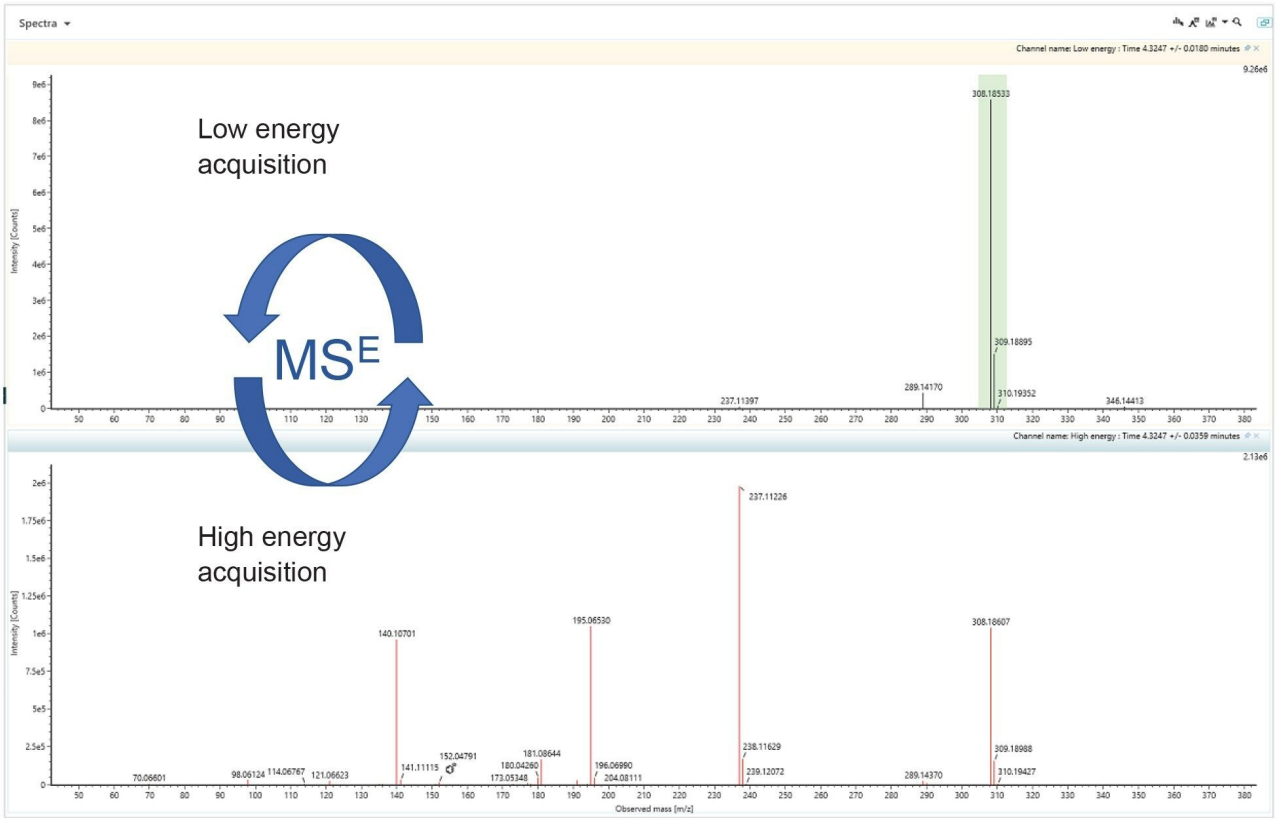

Figure 1. With MS^E the dataset is complete; full accurate mass data for both precursors (shown in the low energy spectrum) and fragment ions (high energy spectrum) is always acquired, even in the case of closely or coeluting analytes.

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 minimum 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, therefore, 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 re-acquire 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.

Targeted analysis:

Comparison to the toxicology library (retention time and diagnostic fragment ions) for >2000 characterized drugs and metabolites

Semi-targeted analysis

A Molfile is a file which describes the structure of a molecule including the atoms and the bonds. Within waters_connect, it can be used to screen for substances by:

1) Providing the expected *m/z* for a substance, so that the low energy MS data can be automatically mined for evidence of the compound
2) Preforming *in-silico* fragmentation of the molecule structure to yield theoretical fragment ions, which are then automatically sought in the high energy MS data. The presence of any corresponding ions in the MS data would support identity

Non-targeted analysis:

A submission of an 'unknown peak' to the 'Discovery' toolset automates the proposal of an elemental formulae, searches external online libraries for likely substances and subsequently launches the *in-silico* fragmentation process to compare the theoretical fragments for the proposed candidate with fragment ions in the high energy data

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 waters_connect Forensic Toxicology HRMS 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 HRMS Library.

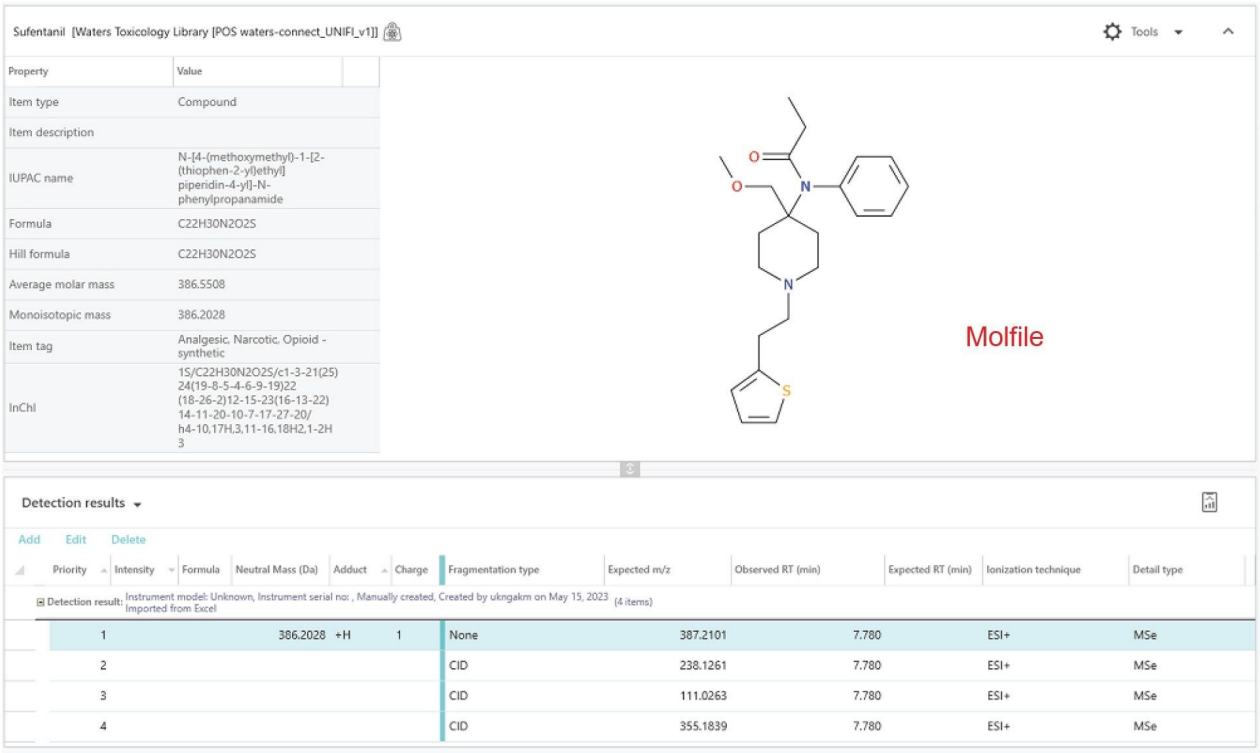

Figure 3. Example library item for a representative compound in the waters_connect Forensic Toxicology Library.

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.

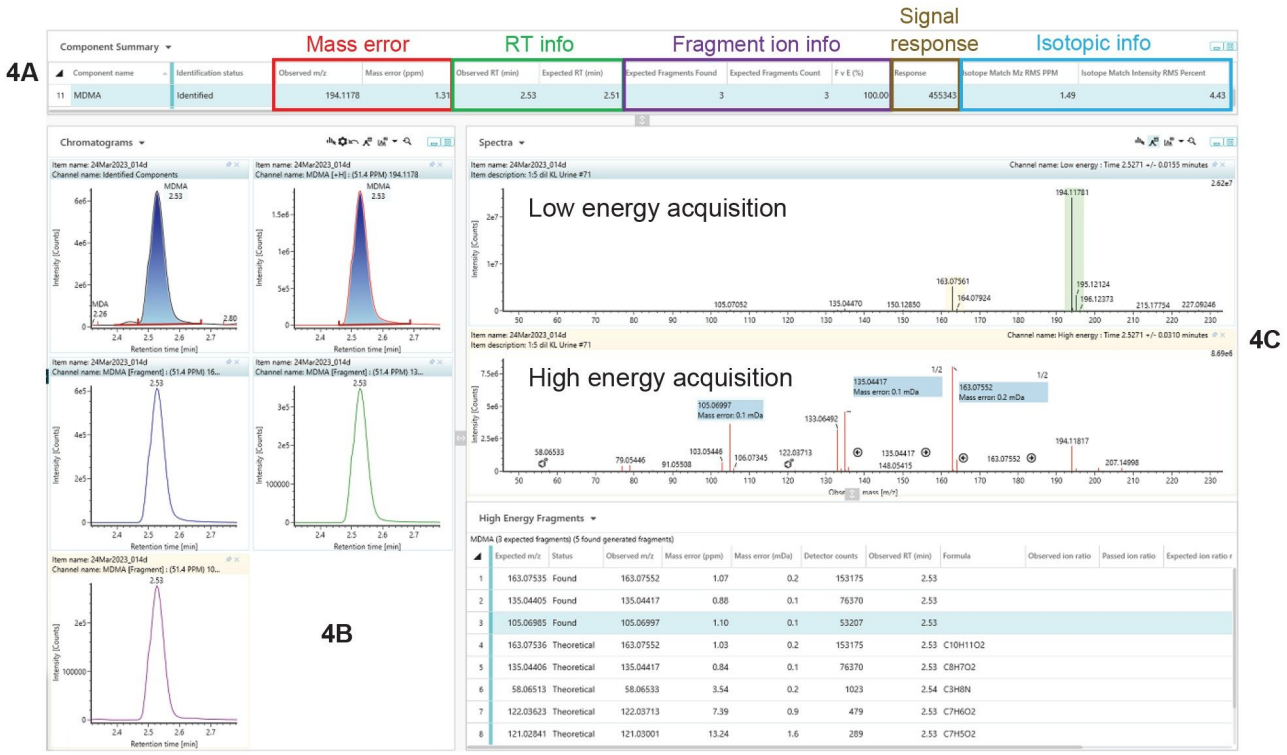

Figure 4. Detection of MDMA in a sample following targeted analysis. A wealth of information is available and may be viewed if desired. In this figure the upper table (4A) details the results of the comparison of acquired data against the reference information contained in the Toxicology Library. Panel 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 waters_connect 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.

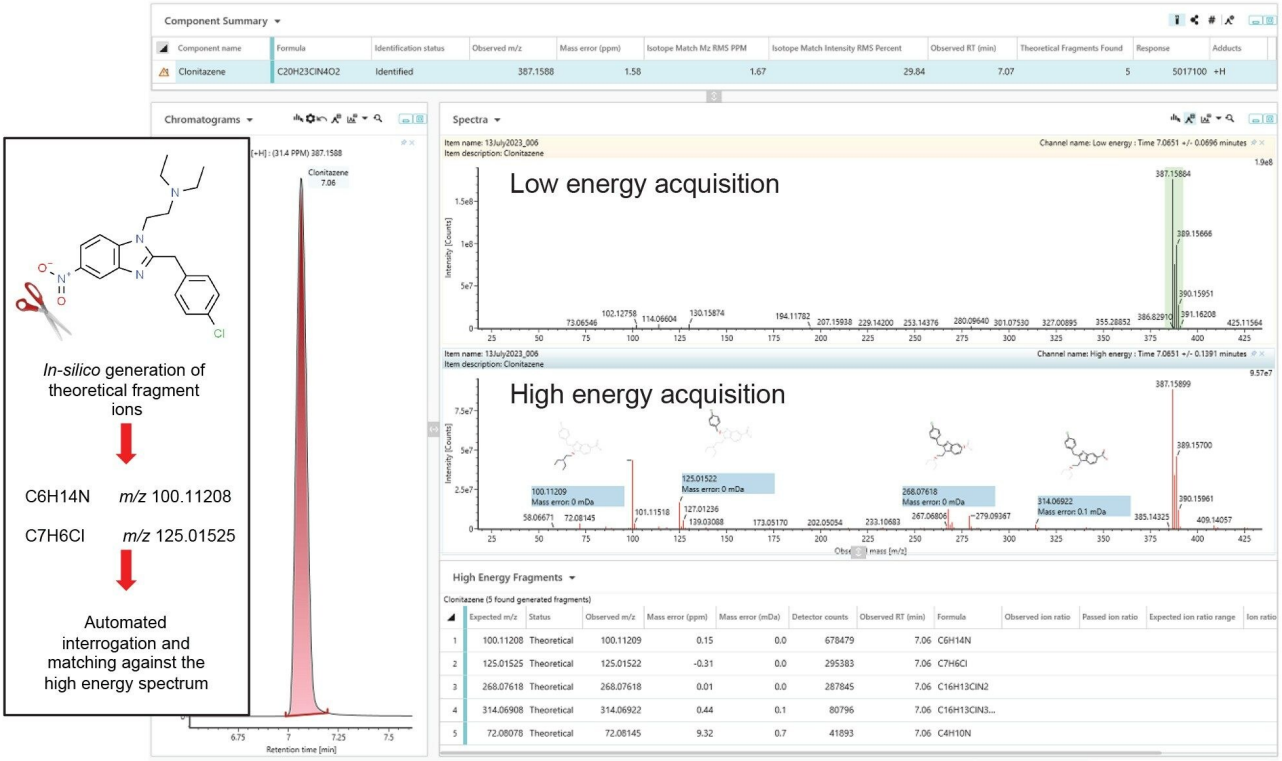

Figure 5. Tentative detection of clonitazene in a mixed benzimidazole (nitazene) opiate standard sample by screening using a Molfile. This class of synthetic opioids can exhibit potency up to several hundred times that of morphine. In this sample, the processing highlighted an unknown peak with a mass of m/z 387.1588. 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. The tentative detection of clonitazene was subsequently confirmed following analysis of CRM.

Non-targeted (discovery) Analysis

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, waters_connect 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; waters_connect 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. waters_connect 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, waters_connect 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 isotonitazene (N,N-diethyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1H-benzimidazol-1-yl]ethanamine), using the discovery workflow. A more detailed description of the discovery workflow, with illustrated examples, is available.[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 an external Scientific libraries e.g., FDA UNII-NLM, to provide proposal of likely substances which correspond to the measured accurate mass and matching of theoretical fragments with the observed fragment ions. In this example the proposal of isotonitazene (N,N-diethyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1H-benzimidazol-1-yl]ethanamine), based on measured mass m/z 411.2400 together with the isotopic information was supported by 26 matched fragment ions.

Conclusion

MS^E is a powerful acquisition mode that provides a complete catalogue of accurate mass data. Identification by this technique is based on a combination of retention time and an accurate mass fingerprint of the analyte. While the use of Tof instruments provide the capability to assign masses to four decimal places and offers improved specificity over nominal mass data, the additional information of the characteristic fragments provided by use of MS^E represents further specific identification parameters and minimizes false positive detections. Ion ratios of the fragment ions can also be incorporated into the user's identification criteria.[5] Furthermore, fragment ions can be useful to differentiate isomers. Together, these capabilities translate into a faster and easier data review for the user and an overall, higher confidence in identification.

As a complete, non-restricted catalogue of accurate mass information, MS^E also enables discovery workflows which can be applied to facilitate identification of substances that may be new emerging psychoactive substances and analogues.

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