Nota applicativa

Accurate Mass Screening and Discovery of Benzimidazole Opioids With the Xevo™ G3 QTof

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Questa relazione è un Application Brief e non contiene una sezione dettagliata sull'esperimento.

For forensic toxicology use only.

Abstract

The application of the Forensic Toxicology High Resolution Mass Spectrometry (HRMS) Screening Solution to demonstrate semi-targeted and discovery workflows with the analysis of benzimidazole opioids, using waters_connect™ informatics platform and the Xevo G3 QTof (Figure 1).

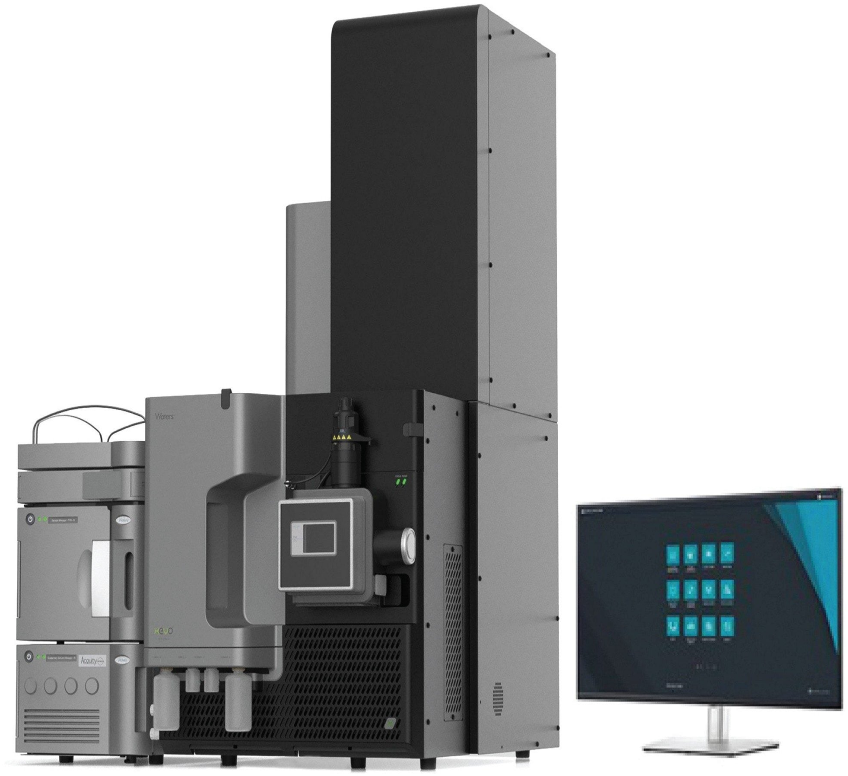


Figure 1. ACQUITY UPLC I-Class with the Xevo G3 QTof.

Benefits

Utilizing the suite of discovery parameters within Elucidation Toolset of the waters_connect informatics platform to demonstrate the simplicity of using the software when creating and updating custom libraries with the addition of new analytes.

Introduction

Time-of-flight Mass Spectrometry (Tof-MS) has become the instrument of choice for screening, as accurate mass presents a significant advantage over its nominal mass counterpart, a key benefit is the ability to implement screening methods without the need of reference material. In addition to high specificity, for substance identification together with isotopic data, accurate mass spectrometry can provide the user the opportunity to propose likely elemental compositions. The proposal of elemental formulae is often the starting point for a complex multi-stage elucidation of chemical structures. In a toxicological setting, this can prove invaluable and assist the analyst in targeting emerging designer drugs, novel psychoactive substances, and metabolites where certified reference material may not yet be available.

In this workflow, we analyze a series of benzimidazole opioids (nitazenes), a class of synthetic opioids that can exhibit potency up to several hundred times that of morphine. These nitrogen-containing heterocyclic compounds are derived from the fusion of the aromatic compounds benzene and imidazole. The analysis of these substances presented an opportunity to evaluate the semi-targeted and discovery workflows within the waters_connect informatics package.

Experimental

Materials

Five benzimidazole opioids certified reference material were obtained from Merck (Dorset, UK) at a concentration of 1 mg/mL stock solutions, supplied in methanol; Butonitazene, Clonitazene, Etonitazene, Isotonitazene, and N-Pyrrolidino Etonitazene.

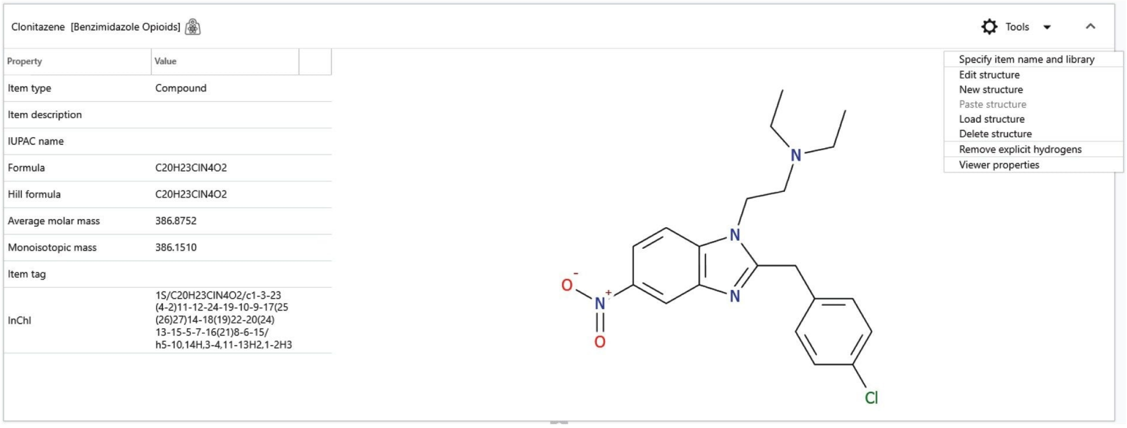
Sample Preparation

Individual stock solutions of the benzimidazole opioids were initially prepared, by dilution with methanol, to a concentration of 10 µg/mL. Prior to analysis, the stock solutions were further diluted with 5 mM ammonium formate pH 3.0 to yield a sample for injection at a concentration of 500 ng/mL. The sample was vortex mixed prior to analysis.

Results and Discussion

Prior to analysis, a custom library was created for the benzimidazole opioids, by simply entering the names of the five analytes. A MOL file describing the structure of each substance was added to each entry in the library (Figure 2). Each benzimidazole opioid was injected individually and data were acquired using the ACQUITY™

UPLC™ I-Class (FTN) System in combination with the Xevo G3 QTof based on the established Forensic Toxicology HRMS Screening Solution, with chromatographic separation achieved within 15 minutes using a gradient elution setup.[1,2] The Xevo G3 QTof Mass Spectrometer was operated in MS^E acquisition in positive ionization mode.[2] This mode of acquisition facilitates collection of full MS spectra and involves the rapid alternations between two collision-cell voltages: the first, acquired at a low voltage, provides accurate mass of the precursor ion; the second, as a ramped voltage (10–40 eV), provides accurate masses of the fragment ions. Following the analysis of the benzimidazole opioid standards, the acquired data was subsequently screened against the custom benzimidazole opioid library. The acceptance criteria for an identification of each analyte were as follows: three dimensional (3D) low energy ion count intensity greater than 250; retention time to be within 0.35 min of reference; the observed precursor mass to be within 5 ppm of expected.



Clonitazene [Benzimidazole Opioids]

Property	Value
Item type	Compound
Item description	
IUPAC name	
Formula	C20H23ClN4O2
Hill formula	C20H23ClN4O2
Average molar mass	386.8752
Monoisotopic mass	386.1510
Item tag	
InChI	1S/C20H23ClN4O2/c1-3-23(4-2)11-12-24-19-10-9-17(25(26)27)14-18(19)22-20(24)13-15-5-7-16(21)8-6-15/h5-10,14H,3-4,11-13H2,1-2H3

Figure 2. Entry for Clonitazene contained in the custom benzimidazole opioids library. Existing MOL file structures can be appended (Load structure) or created by standard chemical drawing packages and subsequently appended (New structure).

Semi-targeted Workflow: Identification Through the Application of in-silico Fragmentation

Each of the benzimidazole opioids were confirmed through the mass accuracy of the protonated precursor ion in combination with the theoretical fragment ions that were generated automatically from the MOL file structure, during processing and matched to the observed fragment ions in the high-energy spectrum. Figure 3 illustrates an example of *in-silico* fragmentation for Clonitazene on the waters_connect component summary page. The low-energy ions assigned to this analyte are highlighted in green within the spectrum and correspond to the protonated isotope cluster. The high-energy spectrum is annotated with sub-structures of Clonitazene, as generated automatically by waters_connect and associated to the high-energy spectral peaks as fragment ions.

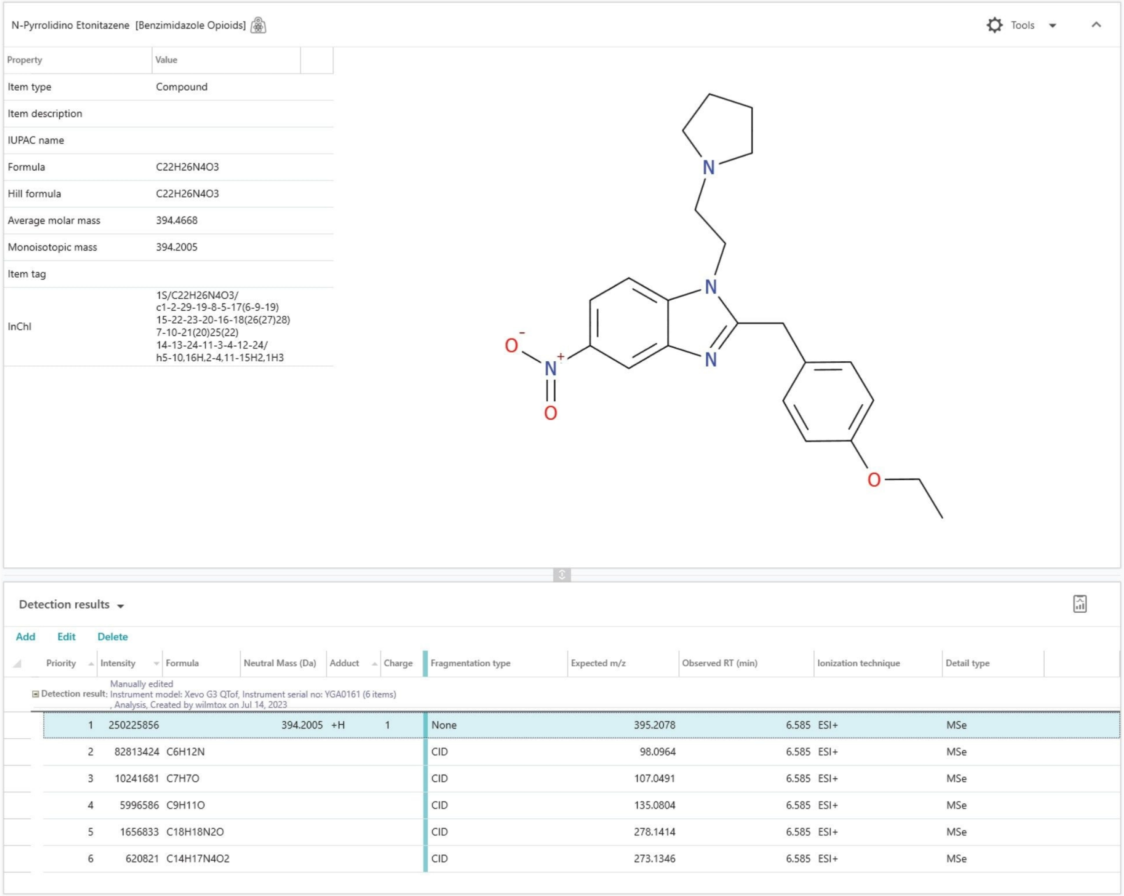
Component Summary

Component name	Formula	Identification status	Observed m/z	Mass error (ppm)	Isotope Match Mz RMS PPM	Isotope Match Intensity RMS Percent	Observed RT (min)	Theoretical Fragments Found	Response	Adducts
Clonitazene	C20H23ClN4O2	Identified	387.1588	1.58	1.67	29.84	7.07	5	5017100	+H

Figure 3. Identification of Clonitazene using waters_connect informatics platform.

Updating Library Entries

All five benzimidazole opioids were detected and identified based on the information entered into the custom library, this included the addition of the theoretical fragment ions which were generated during the processing step and a retention time that was also observed for each analyte. Library entries can be easily updated directly from this analysis to contain the expected retention time and the expected *m/z* value for the assigned adduct and associated fragment ions. Following the library update, a typical entry would contain information similar to that shown in Figure 4 for N-Pyrrolidino Etonitazene. This additional information can be used to target the substance in subsequent analyses.



N-Pyrrolidino Etonitazene [Benzimidazole Opioids]

Property	Value
Item type	Compound
Item description	
IUPAC name	
Formula	C22H26N4O3
Hill formula	C22H26N4O3
Average molar mass	394.4668
Monoisotopic mass	394.2005
Item tag	
InChI	1S/C22H26N4O3/ c1-2-29-19-8-5-17(6-9-19) 15-22-23-20-16-18(26(27)28) 7-10-21(20)25(22) 14-13-24-11-3-4-12-24/ h5-10,16H,2-4,11-15H2,1H3

Detection result: Manually edited
Instrument model: Xevo G3 QTof, Instrument serial no: YGA0161 (6 items)
, Analysis, Created by wilmtox on Jul 14, 2023

Priority	Intensity	Formula	Neutral Mass (Da)	Adduct	Charge	Fragmentation type	Expected m/z	Observed RT (min)	Ionization technique	Detail type
1	250225856		394.2005	+H	1	None	395.2078	6.585	ESI+	MSe
2	82813424	C6H12N				CID	98.0964	6.585	ESI+	MSe
3	10241681	C7H7O				CID	107.0491	6.585	ESI+	MSe
4	5996586	C9H11O				CID	135.0804	6.585	ESI+	MSe
5	1656833	C18H18N2O				CID	278.1414	6.585	ESI+	MSe
6	620821	C14H17N4O2				CID	273.1346	6.585	ESI+	MSe

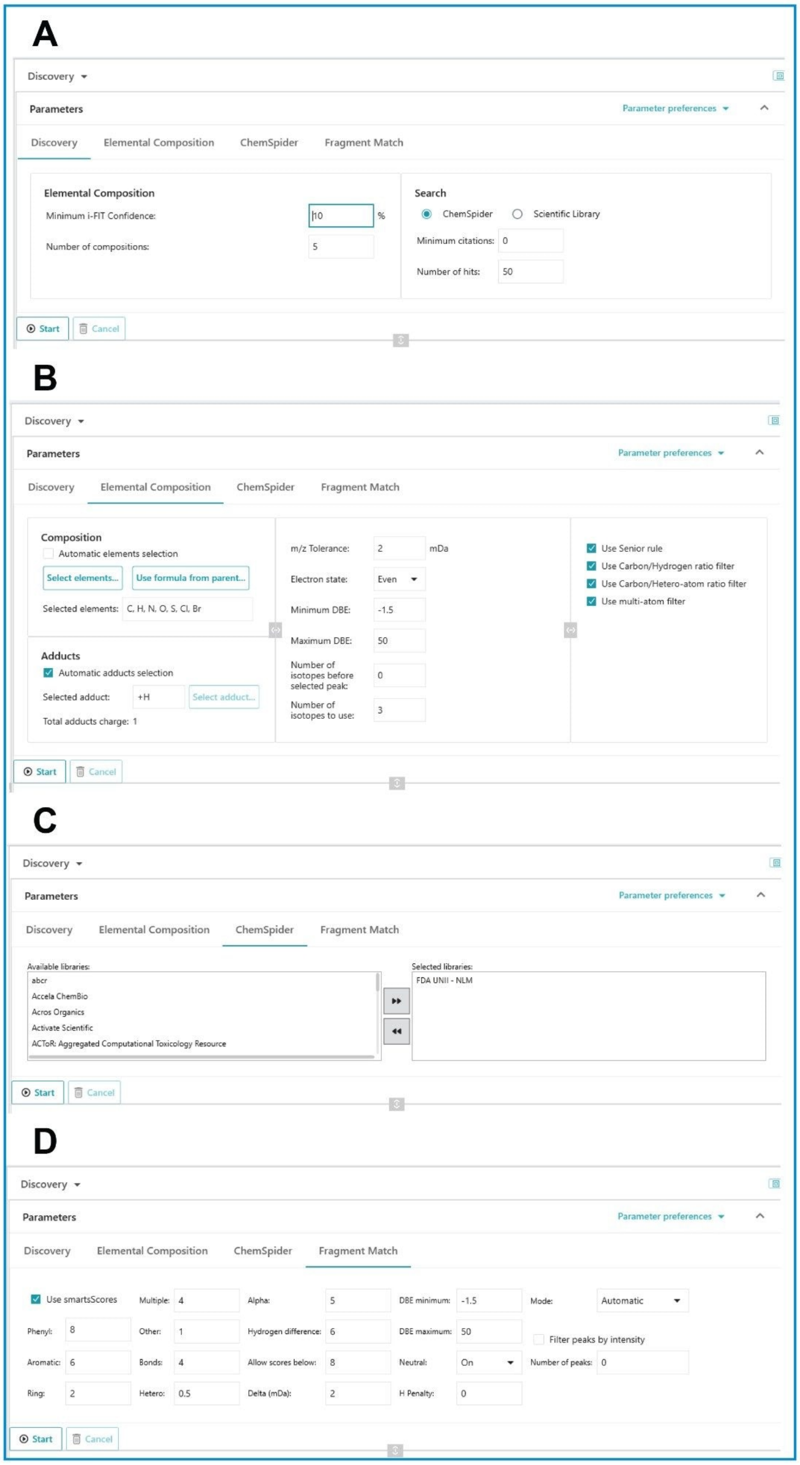
Figure 4. Library entry for N-Pyrrolidino Etonitazene. The lower section of the composite is now populated with the retention time information and expected m/z values of the precursor and fragment ions.

Discovery Workflow

A feature of waters_connect is the Elucidation Toolset, which makes use of discovery parameters; elemental composition, library searching and fragment match functionality into a single step process, making it simple to obtain the identity of unknown substances within a sample. The discovery parameters used are shown in Figure 5A–D.

Figure 5A shows the maximum number of elemental compositions to be returned for each component and the number of library hits returned for each elemental composition. For each component selected, the measured *m/z* value is submitted to the elemental composition application, Figure 5B illustrates the parameters used. Each scientific formula returned by the elemental composition application is then automatically submitted to a list of selected libraries. These libraries can either belong to the waters_connect repository or ChemSpider, if connected to the internet. The ChemSpider library selection is displayed in Figure 5C.

Each scientific formula that is returned from the library search is automatically submitted to the fragment match application, provided the library hit has an associated structure in the form of a MOL file. The fragment match application performs a systematic bond dissociation for each structure, applying the parameters selected in Figure 5D and matches the *m/z* values of the theoretical sub-structures to measured high-energy fragment ions. The number of fragment ions matched and the percentage of the intensity of the high-energy spectrum accounted for by those matches are both determined.



A

Elemental Composition

Minimum i-FIT Confidence: 10 %

Number of compositions: 5

Search

● ChemSpider ○ Scientific Library

Minimum citations: 0

Number of hits: 50

B

Composition

☐ Automatic elements selection

Selected elements: C, H, N, O, S, Cl, Br

Adducts

☑ Automatic adducts selection

Selected adduct: +H

Total adducts charge: 1

m/z Tolerance: 2 mDa

Electron state: Even

Minimum DBE: -1.5

Maximum DBE: 50

Number of isotopes before selected peak: 0

Number of isotopes to use: 3

☑ Use Senior rule

☑ Use Carbon/Hydrogen ratio filter

☑ Use Carbon/Hetero-atom ratio filter

☑ Use multi-atom filter

C

Available libraries:

- abcr
- Accela ChemBio
- Acros Organics
- Activate Scientific
- ACToR: Aggregated Computational Toxicology Resource

Selected libraries:

- FDA UNII - NLM

D

☑ Use smartsScores

Phenyl: 8

Aromatic: 6

Ring: 2

Multiple: 4

Other: 1

Bonds: 4

Hetero: 0.5

Alpha: 5

Hydrogen difference: 6

Allow scores below: 8

Delta (mDa): 2

DBE minimum: -1.5

DBE maximum: 50

Neutral: On

H Penalty: 0

Mode: Automatic

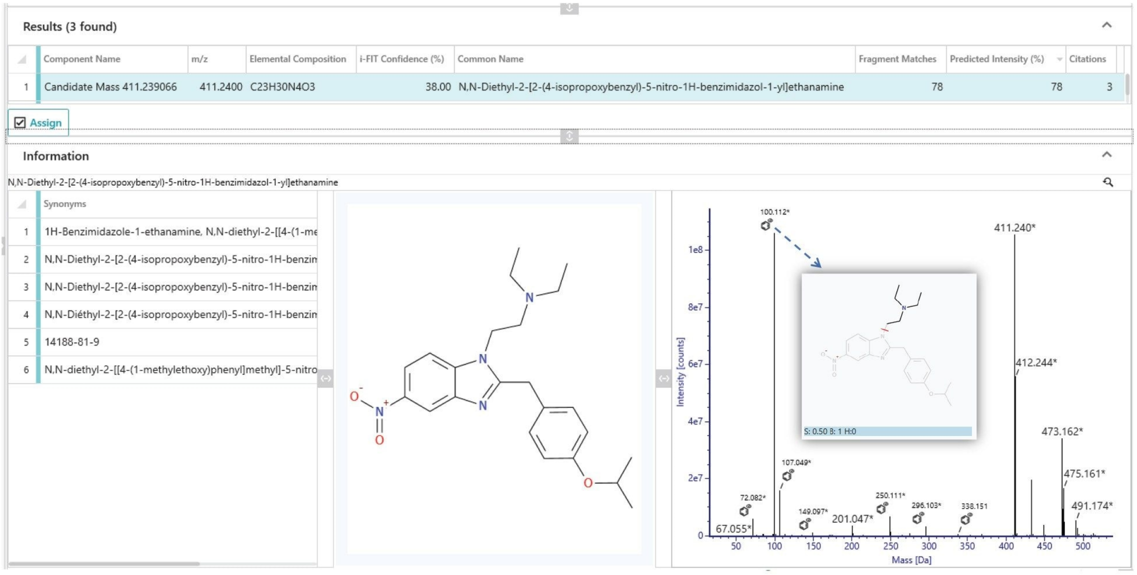
☐ Filter peaks by intensity

Number of peaks: 0

Figure 5. Elucidation Toolset in waters_connect. A) General discovery parameters. B) Elemental

composition parameters. C) ChemSpider parameters. D) Fragment match parameters.

For the purposes of illustration, the candidate component identified as Isotonitazene in the targeted analysis was submitted for structural elucidation. The results, upon running the application with respect to the parameters which are shown in Figure 5A–D, are presented in Figure 6.



Results (3 found)

	Component Name	m/z	Elemental Composition	i-FIT Confidence (%)	Common Name	Fragment Matches	Predicted Intensity (%)	Citations
1	Candidate Mass 411.239066	411.2400	C23H30N4O3	38.00	N,N-Diethyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1H-benzimidazol-1-yl]ethanamine	78	78	3

☑ Assign

Information

N,N-Diethyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1H-benzimidazol-1-yl]ethanamine

	Synonyms
1	1H-Benzimidazole-1-ethanamine, N,N-diethyl-2-[[4-(1-me
2	N,N-Diethyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1H-benzin
3	N,N-Diethyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1H-benzin
4	N,N-Diéthyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1H-benzin
5	14188-81-9
6	N,N-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro

Figure 6. A typical result generated using the Elucidation Toolset within waters_connect informatics package.

The component submitted for the structural elucidation was Candidate Mass *m/z* 411.239066. The results show that one elemental composition $C_{23}H_{30}N_4O_3$, with an i-FIT™ confidence above the threshold set was found. The elemental composition was automatically submitted to the FDA UNII-NLM library, within ChemSpider and a hit for Isotonitazene (N,N-Diethyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1H-benzimidazol-1-yl]ethanamine) was returned with a list of synonyms, a structure, and the number of citations. The structure was used automatically in conjunction with fragment match and appropriate sub-structures were assigned to the high-energy spectrum associated with Candidate Mass *m/z* 411.239066, as displayed in Figure 6. The number of high-energy fragment ions matched by sub-structures and the percentage of the intensity of the high-energy spectrum, accounted for by those fragment matches, are shown for the library hit.

Access to this information for a range of components, elemental compositions, and library hits enables the analyst to make an informed decision with respect to the identity of the unknown substances in their samples.

Conclusion

In this study we have utilized the Forensic Toxicology HRMS Screening Solution with waters_connect to acquire data for a selection of benzimidazole opioids. The acquired data was used to demonstrate the ease by which a custom scientific library can be created and updated. The waters_connect informatics platform was used to process MS^E data using a semi-targeted workflow. The fragment match functionality was able to assign sub-structures to high-energy ions. Furthermore, the Elucidation Toolset has been shown to enhance the discovery workflow.

References

1. M. Wood. The Utility of MS^E for Toxicological Screening; Waters Application Brief. 720005198. March 2022.
2. HRMS Forensic Toxicology Screening solution media available at Forensic Toxicology Application Solution Media by Waters | Marketplace <https://marketplace.waters.com/apps/159226/forensic-toxicology-application-solution-media#!overview> .

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo G3 QTof Mass Spectrometer </nextgen/it/it/products/mass-spectrometry/mass-spectrometry-systems/xevo-g3-qtof.html>

Screening Platform Solution with UNIFI <https://www.waters.com/waters/nav.htm?cid=134682903>

waters_connect <https://www.waters.com/waters/nav.htm?cid=135040165>

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