Automated tool for optimal quantitative analysis conditions using mzCloud Spectral Library

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Abstract

Purpose: Introduction of a new tool that allows for automatic generation of best the quantitative conditions for multiple compounds at time.

Methods: Internally developed software tool utilizing information from Thermo Scientific™ mzCloud™ mass spectral library with features of quality control, identification of characteristic transition ions and collision energy recalculations.

Results: Automatic generation of quantitative mass spectrometry methods validated on established toxicology, veterinary drugs and pesticide MRM libraries.

Introduction

Here we present a swift and handy tool that automates the determination of optimal spectral conditions for quantitative analysis of compounds. This tool **identifies the most intensive fragment ions** of MS2 HRAM spectra and the **best collision energies** for each compound, streamlining the analysis process. Utilizing a database of 32,000 compounds and 200,482 MS2 ion tree experiments, it ensures only highest-quality spectral tree are considered. It significantly reduces time and cost of compound optimizations in laboratory settings.

Materials and methods

Data Preparation

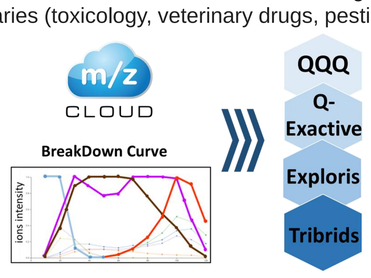
The tool employs an algorithm to search the mzCloud mass spectral library, performing rigorous quality checks on available MS2 HRAM spectra. Key parameters, such as the number of collision energy steps and the breakdown curve shape of each fragment ion, are evaluated. By scrutinizing the selected parameters, the algorithm ensures that all off the potential fragment ions are provided to the user.

Transition ion selection and collision energy recalculation

From high-quality data the best quantitative conditions were selected. This process encompasses the identification of the most intense fragment ions and extracting the normalized collision energies for each ion at the point of highest absolute intensity. At last, the normalized collision energy (NCE) values are recalculated to collision energy (CE).

Data validation

The extracted m/z values of the transition ions and the calculated values of CE were validated using established MRM libraries (toxicology, veterinary drugs, pesticides).

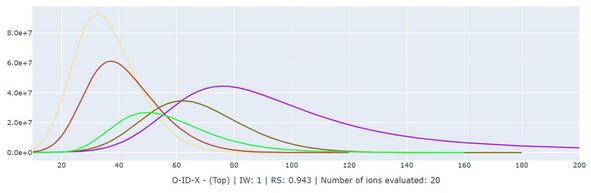


Results

Quality control system for spectral libraries

- The mzCloud database comprises 32,000 compounds (reference and auto-processed libraries).
- From an initial 3,462,578 of MS2 spectra belonging to 200,482 ion tree experiments, 168,486 met highly stringent quality criteria and were selected for further analysis.
- Parameters evaluated:
  - quality of the breakdown curve (BDC) with well-defined ion kinetics
  - comprehensive set of collision energy levels
  - well defined m/z range during data acquisition
- By implementing a rigorous quality control system, it is ensured that the solution is providing the most comprehensive information about quantitative mass spectrometry conditions for every compound of interest (figure 1).

Figure 1. Example of high-quality experiment with well defined BDCs for the five most intense transition ions. Each curve represent a transition ion. The value of the absolute intensities are on the y-axis and the values of NCE are on the x-axis.



Extraction of m/z values of transition ions and collision energies

The mzCloud mass spectral library comprise of diverse information for the compounds recorded. A molecule can be present in the libraries multiple times with different polarity, ion activation or precursor type. This diversity can be represented with the molecules of Genistein with total 33 records in the libraries (figure 2).

Figure 2. Representation of the diversity of the recorded information in mzCloud libraries. The compound Genistein have been recoded 33 times with 2 polarities, 2 ion activations, 6 different precursor types on multiple instruments.

To be able to **capture and provide a comprehensive information** about he queried compounds the solution:

• Provides m/z values of transition ions and corresponding collision energies.

• Collision energy selected based on maximum absolute intensity of the ion trough the range of measured energies (figure 3).

• Covers unique combinations of polarity, ion activation, and ion type.

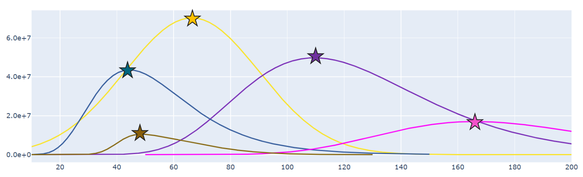
• The number of transition ions depends on the compound and experimental settings.

• Ensures meaningful m/z values by meeting key conditions:
  - Good signal-to-noise ratio.
  - Presence of ion in multiple consecutive scans.

• Can provide detailed information on:
  - Relative intensity of selected ion to the base peak or most intense ion.
  - Ratio between two arbitrarily selected ions in the spectra.

• Outputs list of transition ions with recommended collision energies.

Figure 3. Examples of identified characteristic ions with corresponding NCE at the maximum intensity of the BDC



List of m/z values of characteristic ion extracted from BDCs with their corresponding NCE at maximum value:

- ★ m/z – 118.07, NCE - 70
- ★ m/z – 91.05, NCE - 110
- ★ m/z – 132.08, NCE - 40
- ★ m/z – 65.04, NCE - 170
- ★ m/z – 69.07, NCE - 50

Recalculation of collision energies between different instruments

The current version of the tool recalculates normalized collision energy (NCE) to collision energy (CE) for a variety of instruments, including Thermo Scientific™ Tribrid™ mass spectrometers and Thermo Scientific™ hybrid mass spectrometers, extending compatibility to triple quadrupole mass spectrometers. This broad applicability enhances the tool's utility across diverse MS platforms.

Validation of m/z values of transition ions and collision energies

The generated m/z values and their corresponding collision energies were validated against established toxicology, veterinary drugs and pesticides MRM libraries.

- In total 876 compounds were validated, from those 844 were positive and 32 were negative polarity.
- The total number validated transition ions was higher because several compounds are recoded multiple times the mzCloud libraries and in average 2 transition ions were compared for each experiment.
- The total number of validated datapoints for positive polarity was 3520 and the median difference of collision energies between the reference data and mzCloud was 3.12 eV (figure 4).
- For the negative polarity, the number validated datapoints were 118 with a median difference of the collision energies between the reference data and mzCloud was 1.77 eV (figure 5).
- The distribution of the validation data shows exceptional correlation of the information in the mzCloud mass spectral libraries with the established MRM libraries.

Figure 4. Distribution of the differences of the CE (eV) between reference MRM libraries and mzCloud libraries for positive polarity

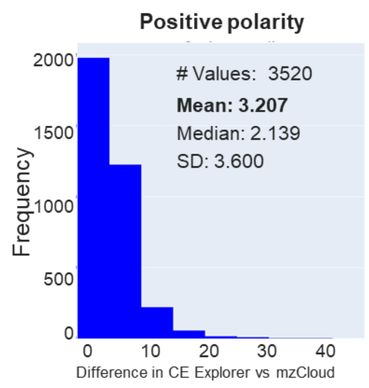
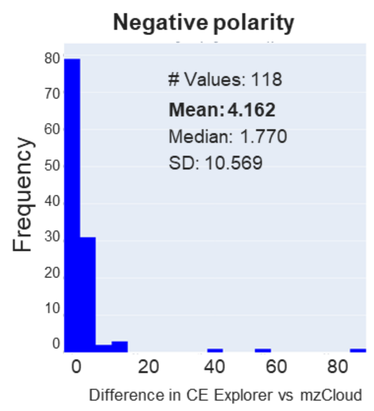


Figure 5. Distribution of the differences of the CE (eV) between reference MRM libraries and mzCloud mass spectral libraries for negative polarity

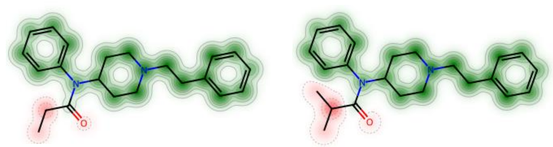


Utilization of mzCloud mass spectral libraries to provide extra information not established in the reference MRM libraries

In traditional MRM libraries, information on transition ions is often limited to the two most intense ions, lacking comprehensive m/z values for all transition ions. Exploring new m/z values can be time-consuming and resource-intensive. By using mzCloud mass spectral libraries, we can ease the burden of exploratory analysis, allowing users to focus on quantitative experiments.

The demand for fast and reliable quantitation of fentanyl analogs is high. However, when different compounds produce the same transition ions (cross-talk), it complicates MS experiment setup and speed. Typically, an HPLC system is coupled with the MS instrument to address this, requiring significant financial and time investments. Leveraging mzCloud mass spectral libraries reduces these costs and provides additional information for compounds of interest.

Figure 6. Similarity map between fentanyl (left) and isobutyryl fentanyl (right)



For example, in traditional MRM libraries, both fentanyl and isobutyryl fentanyl share transition ions of 188.196 and 105.191, limiting concurrent analysis. By utilizing mzCloud mass spectral library, we can identify a specific transition ion for fentanyl with an m/z value of 216.138 and formula $C_{14}H_{18}NO$ (figure 8) as well for isobutyryl fentanyl with an m/z value of 230.153 and formula $C_{15}H_{20}NO$ (figure 9) thus, enhancing analysis efficiency.

Figure 7. Comparison of collision energies of specific compounds between the reference MRM libraries and mzCloud mass spectral libraries.

	Tox/Pest Explorer Lib		mzCloud data export		
	Ions	CE	Ions	NCE	CE
Sufentanil 387.210	355.21	20.0	355.21	30	21.05
	238.13	20.0	238.13	30	21.05
Terbutryn 242.143	186.054	18.18	186.054	30	15.00
	91.071	26.65	91.071	60	20.31
Fenantyl 337.227	188.196	28.0	188.196	40	26.29
	105.191	42.0	105.191	70	40.91
		Fentanyl analogs share the same product ions	**216.138**	40	**Cross-talk**
Isobutyryl fentanyl 351.243	188.196		188.196	40	**solved**
	105.191		105.191	70	26.29
			230.153	40	40.91

Figure 8. Fentanyl – conventionally monitored ions (top), additionally extracted ions (bottom) with a specific ion of $C_{14}H_{20}NO$ - 216.138 m/z

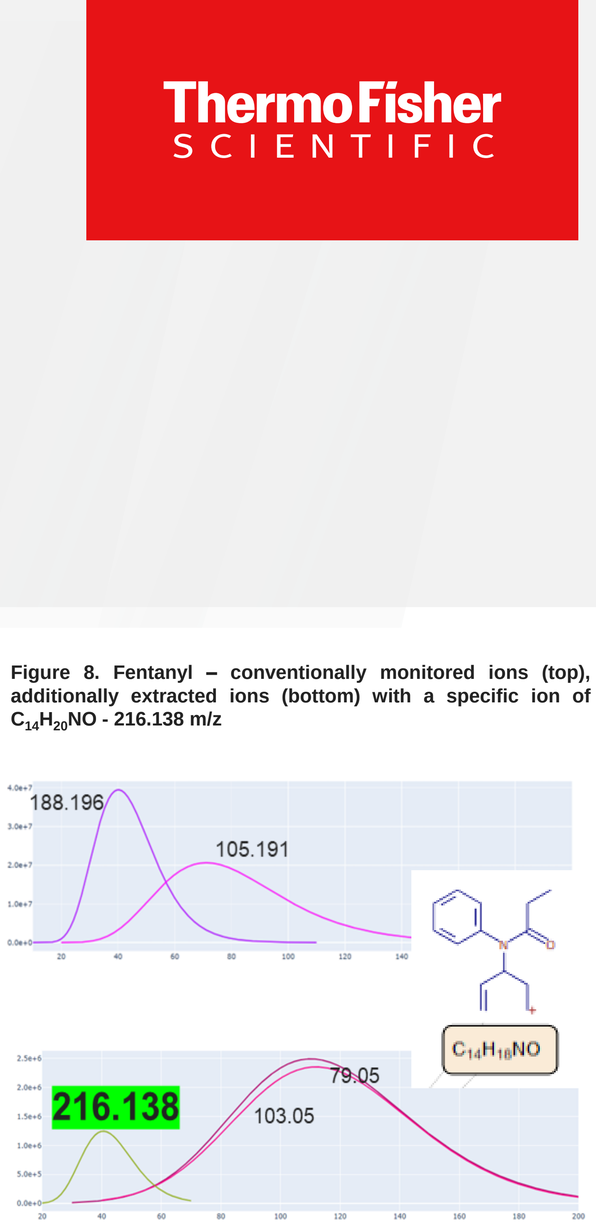
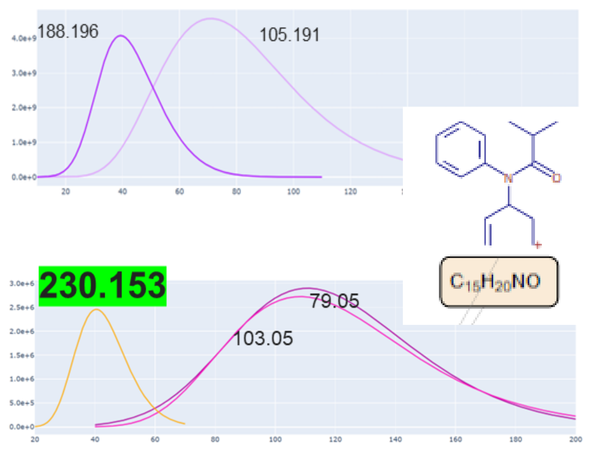


Figure 9. Isobutyryl fentanyl - conventionally monitored ions (top), additionally extracted ions (bottom) with a specific ion of $C_{15}H_{20}NO$ - 230.153 m/z



Conclusions

- The implementation of a novel quality control system for spectral libraries ensures the highest standards of data integrity and reliability, enabling the extraction of precise m/z values of transition ions. This system not only recalculates collision energies (CE and NCE) for compatibility across various instruments but has also undergone successful validation against established MRM libraries, confirming its accuracy and robustness.
- By leveraging the extensive mzCloud mass spectral libraries, our solution provides significant added value. It offers comprehensive information on transition ions and their collision energies, reducing the time and resources required for exploratory analysis. This enhancement facilitates the fast and reliable quantitation of complex compounds, such as fentanyl analogs, and accommodates the specific needs of quantitative mass spectrometry experiments.
- Ultimately, our approach delivers a more efficient and cost-effective solution, advancing the capabilities of mass spectrometry analysis and providing users with the critical data necessary for their research and applications.

Acknowledgements

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