Mass spectrometry

Expedite confident SRM transition creation

Integration of the Thermo Scientific mzCloud database with the Thermo Scientific TSQ and TSQ Plus mass spectrometers

My targeted compound methods are continually evolving to include more compounds deemed experimentally important and I must create effective SRM transitions. Is there an easier way to develop targeted methods to maintain productivity?

Yes, with the continued development of spectral libraries and compound databases that are created from exhaustive analysis of purified standards, new software routines can leverage the empirical information to build SRM transitions and bypass lengthy and costly optimization routines.

With the recent introduction of the Thermo Scientific™ TSQ™ Plus mass spectrometers, SRM transition information can be directly imported from the Thermo Scientific™ mzCloud advanced mass spectral databases. The mzCloud database is one of the world's largest databases built on extensively curated, high-quality mass spectral fragmentation acquired on Orbitrap mass spectrometers. Each entry includes exhaustive high-resolution MS, MS/MS, and for most compounds, multi-stage MS^n spectra which have been acquired at various collision energies. Collectively, each entry enables determination for the set of experimental information critical for highly confident analysis and detection for compounds associated with a wide range of market applications.

How does mzCloud determine the optimal parameter for SRM transitions?

The mzCloud mass spectral library is created using commercially available standards that are individually analyzed using Thermo Scientific™ Orbitrap™ Hybrid and Thermo Scientific™ Orbitrap Tribrid™ mass spectrometers, ensuring high resolution and high mass accuracy for the greatest confidence in the resulting MS, MS/MS, and multi-stage MS^n data. Structural characterization is performed using beam-type, higher energy collisional dissociation (HCD) to acquire product ion spectra. Additionally, MS/MS and multi-stage MS^n using on-resonance, low-energy collision induced dissociation (CID) is also performed using the Orbitrap Tribrid mass spectrometer (For more information, go to www.mzCloud.org). Figure 1 shows an example entry for Monensin. Each entry contains curated data fully characterizing the compound. In addition, multiple instances may be included, covering polarity and methods of dissociation.

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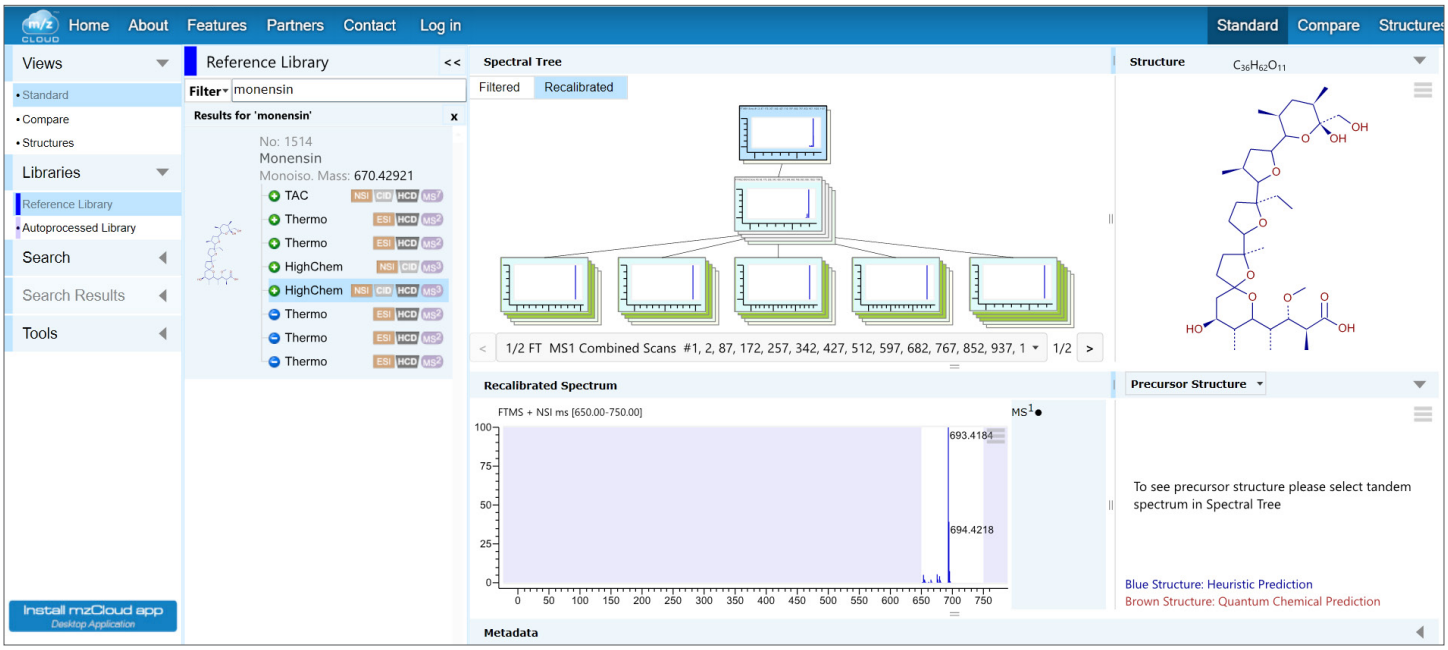


Figure 1. Screen capture from mzCloud showing the Monensin entry. The interactive entry enables multiple spectral views based on the instance for ionization polarity and MS^n stage performed and form of dissociation used to generate the resulting ion tree.

Each MS^n stage contains all spectra for manual evaluation, for example, the MS/MS spectra acquired at each HCD/CID normalized collision energy (NCE) setting is contained as well as an overall breakdown curve. An example for Abendazole is shown in Figure 2 for the set of five product ions measured from HCD dissociation. The product ion abundance rank determines the quantifying and qualifying ions used to generate the resulting SRM transitions and the apex NCE values can be converted to voltage values used on the Thermo Scientific™ TSQ™ and TSQ Plus mass spectrometers.

By enabling intelligent data acquisition, MS^n data and breakdown curves can be evaluated for both positive and negative ESI polarity as well as exploration for optimal precursor ion formation, including protonation or adduct formation. All of which can be accumulated under a primary compound structure and used to create novel opportunities for SRM transition determination for each targeted compound.

The mzCloud database contains over 19,000 entries covering 16 different compound classes. Compounds are continually being added to the database to address emerging research requests.

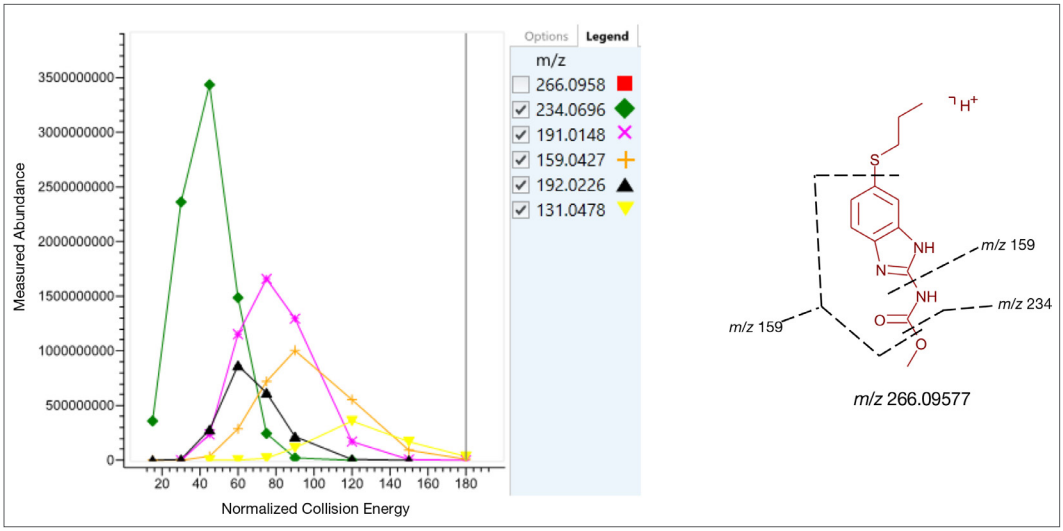


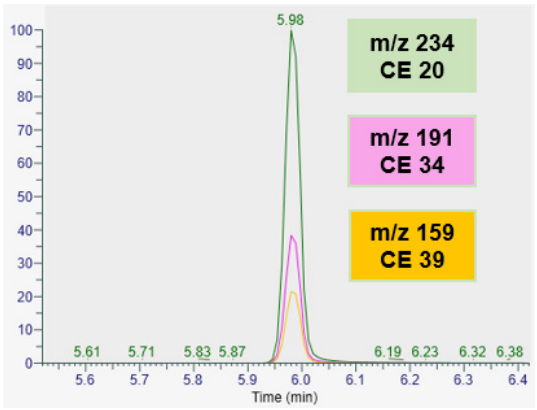
Figure 2. Breakdown curve from measured structural fragments for the veterinary drug albendazole. The breakdown curve is generated by acquiring full scan HRAM product ion spectra at 10 V steps and the ion intensity measured for each product ion per NCE value. The protonated albendazole structure and predicted fragments shown on the right were generated in Mass Frontier 8.0 software.

How well does the product ion information contained in the mzCloud database correlate with triple quadrupole dissociation?

While Orbitrap-based mass spectrometers and the TSQ and TSQ Plus mass spectrometers use different neutral collision gases, nitrogen (N_2) and argon (Ar) respectively, the relative energetics for unimolecular fragmentation pathways will overlap generating similar product ions. To test this hypothesis, a set of veterinary drugs were infused and optimized on the TSQ Plus mass spectrometer from standards to determine up to three product ions, collision energy settings per product ion, and the resulting product ion ratios. In addition, similar SRM transition information was imported directly from the mzCloud database using the NCE-to-CE equation to establish the resulting TSQ CE value per product ion transition. No further method optimization was performed on the experimental values derived from mzCloud.

Figure 3 shows the LC-MS/MS analysis of Albendazole using each method. Each figure shows the CE value per SRM transition determined by each method and the extracted SRM chromatograms relative to the base peak. Note the similar CE values per product ion optimized from direct infusion on the Thermo Scientific™ TSQ Quantis Plus™ mass spectrometer and CE values read in from the mzCloud entry as they differ only by 2 V or less.

A



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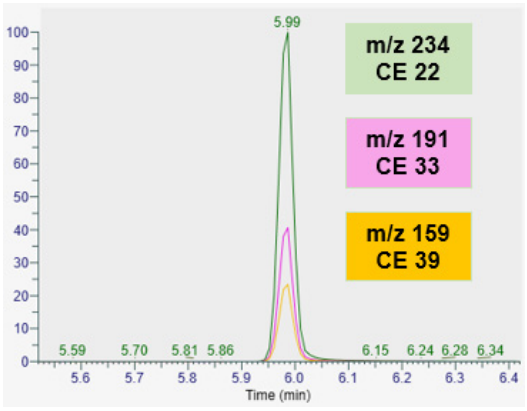
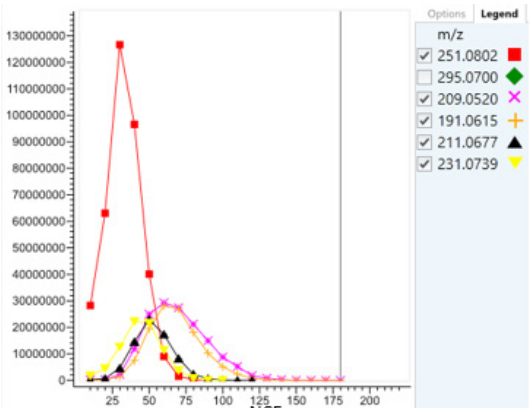


Figure 3. Measured albendazole response based on SRM transitions empirically determined by 2A) direct infusion optimization and 2B) imported directly from the mzCloud database. The product ions are color coded with the breakdown curve shown in Figure 2.

As noted, the compound characterization steps used to create the mzCloud compound entry is also performed in -ESI mode based on the proposed chemical structure. Since small molecule market applications generally target groups of compounds covering widely different structures and functional groups, the final method often requires polarity switching to maximize the number of target compounds analyzed in a single run. Figure 4 shows that the resulting SRM performance for flunixin that imported parameters for -ESI. Again, the CE values agree nicely between the manually optimized SRM parameters and those imported from the mzCloud database.

A



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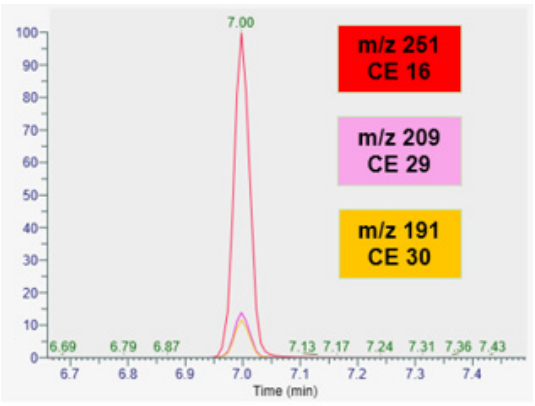

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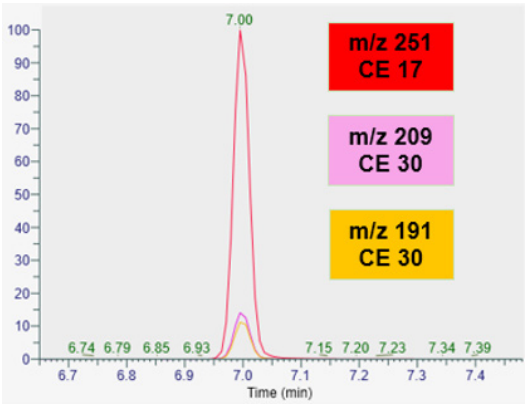


Figure 4. Analysis of flunixin in negative ESI mode. Figure 4A shows the mzCloud database entry with the product ions and breakdown curves. The comparative SRM response for flunixin using empirically determined SRM transitions from 4B direct infusion analysis and 4C) from the mzCloud database entry.

Figure 5 shows the resulting area-under-curve (AUC) values for each product ion based on the SRM transitions for each compound. Since the CE values are similar with differences generally only 1 or 2 V, the expected performance and ion ratios should be similar. In fact, Figure 5 confirms the measured product ion ratios for each compound are almost identical providing additional levels of confidence in compound detection.

Evaluation of 35 veterinary drugs shows that for 66 measured SRM transitions, the median difference in CE settings between predicted and those empirically determined by direct infusion on the TSQ Plus mass spectrometer was 1.63 V indicating the equation used to translate NCE to CE is highly accurate. Similar research has been published determining collision energy conversion equations between triple quadrupole mass spectrometers and Q-TOFs (Ye, 2016) as well as Orbitrap ID-X Tribrid mass spectrometers. (Schwaiger-Haber, 2021)

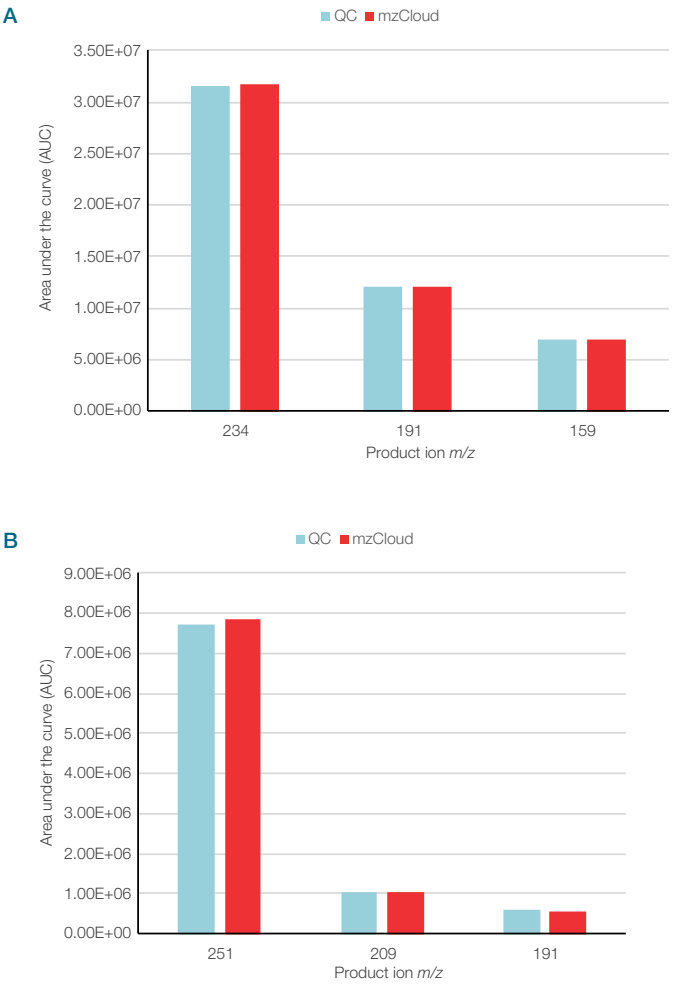


Figure 5. Comparative ion ratios for A) albendazole measured in +ESI mode and B) flunixin measured in -ESI mode where the QC response represents the SRM transition parameters determined from direct infusion analysis and values imported directly from the mzCloud database entry.

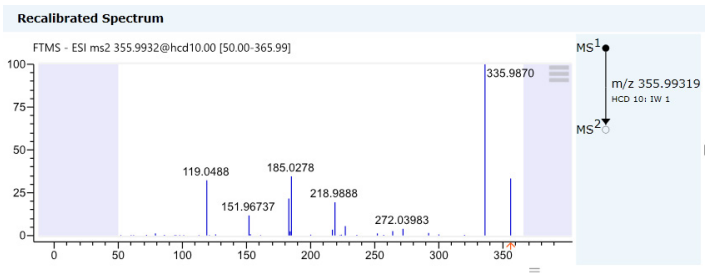
What other advantages does this approach provide?

The goal of developing targeted SRM transitions is to identify the best sets of precursors and product ions that ensure selectivity, specificity, and most of all, quantitative sensitivity. For small molecules, optimal SRM transitions could result from positive or negative mode, as shown above. In addition, some compounds preferentially form adducts that can modify unimolecular dynamics creating new product ions that are more selective and sensitive, while maintain specificity relative to the matrix. Compound entries into the mzCloud database can account for each of these cases and the importing software routine introduced on the TSQ Plus mass spectrometers reads in multiple instances enabling the user to perform direct analysis on endogenous compounds using the predicted instrument settings to quickly determine which SRM transition sets are optimal.

Figure 6 shows the benefit of reading in SRM transition information from both polarities for the same compound, florfenicol. Performing the manual optimization may start with one polarity to determine the set of potential product ions, and then perform CE optimization whereas importing mzCloud database information provides information for both polarities if it exists, and/or for additional precursor/product ion pairs. Determination of the optimal SRM transitions can be performed in one experiment utilizing the fast polarity switching speeds of the TSQ Plus mass spectrometers. Figure 6D plots the measured AUC values from both polarities to enable comparative responses. Further, the simultaneous evaluation in both polarities provides an opportunity to evaluate putative matrix interference to make final determinations with minimal time or effort.

In addition to polarity, adduct formation can modify the SRM transition response and effectiveness. Purified compounds are used to generate comprehensive mzCloud database entries. Compound analysis leverages untargeted HRAM MS that directs sampling precursors and adducts in both polarities in both polarities that generates extensive product ion spectra. This routine provides further transition information to a user that typically has not been investigated when performing compound characterization on a triple quadrupole mass spectrometer. This advantage becomes extremely important to determine optimal SRM transitions as certain transitions could provide better quantitative accuracy and/or more effective qualitative specificity in a given sample matrix.

A



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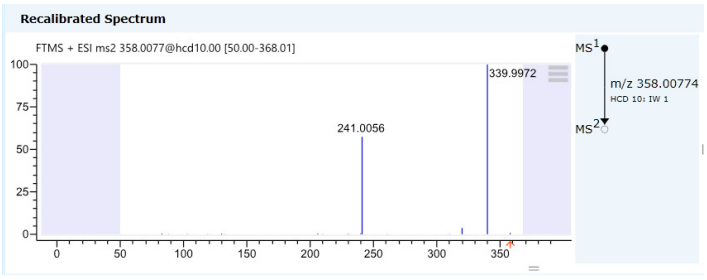

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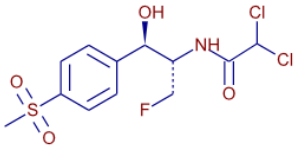


Product ion *m/z*	336	185	119
Opt CE	11.0	20.0	32.0
mzCloud	9.5	19.8	30.0

D

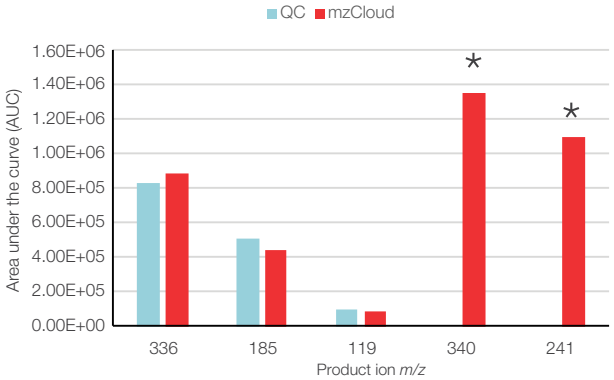
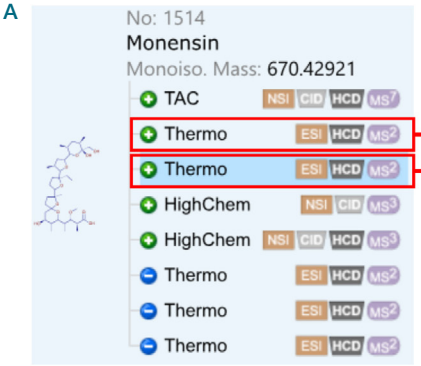


Figure 6. The mzCloud database entry for florfenicol showing the curated product ion spectrum from A) -ESI and B) +ESI analysis as well as the comparative set of CE values (shown in C), and the measured response using optimized CE values and those derived from mzCloud (D). The product ions at *m/z* 336, 185, and 119 were measured in -ESI mode while the measured response plotted from the product ions at *m/z* 340 and 241 were measured in +ESI mode acquired in the same method.

A



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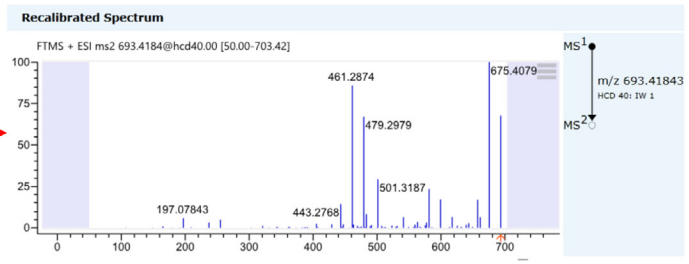

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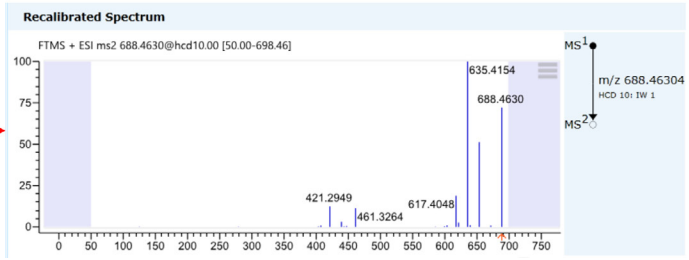

D

Product ion *m/z*	675	461	479
Opt CE	36.0	52.4	53.8
mzCloud	43.8	53.6	53.6

E

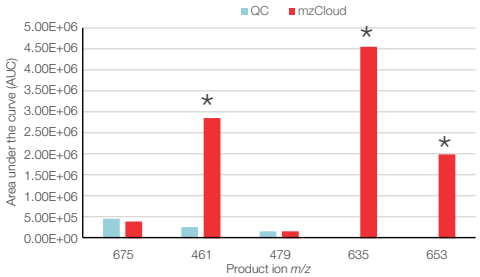

Figure 7. The mzCloud database entry for monensin and the different instances in +ESI and -ESI (A). The representative HCD product ion spectra are from precursors formed by two instances resulting from B) a sodium and C) an ammonium adduct were evaluated using the import function on the TSQ Plus mass spectrometer in the same method. The resulting comparison of CE values used for the sodium adduct are shown in D) and E) the comparative AUC responses measured for each SRM transition. The transitions with asterisks were acquired using the monensin precursor formed by an ammonium adduct.

Figure 7 shows the comparative analysis for monensin. The mzCloud database provides multiple instances derived from different precursor adduct formation. The two different sets of SRM transitions were included in the final method for comparative analysis. The results demonstrated a significant improvement in AUC values using the ammonium adduct as compared to the SRM transitions derived from sodium adduct. Specifically, the *m/z* 461 product ion can be formed for both precursor adducts. The AUC responses for SRM transitions from various precursor adducts are often available from the mzCloud database, but it should be noted that the resulting AUC response may be different from what is predicted depending on the mobile phase or solution composition used during the data acquisition.

How can I learn more about the new workflow option?

Thermofisher.com/TSQPlus provides detailed description of new capabilities introduced on the TSQ Plus mass spectrometers increasing the analytical productivity as well as simplifying instrument operation. The website contains new applications demonstrating workflow performance with detailed settings.

Thermofisher.com/mzCloud and mzCloud.org provide more information about the mzCloud mass spectral database and applications. The websites contain more information about relevant features, statistics, and functionalities to support a multitude of small molecule workflows.

Learn more at **thermofisher.com/TSQPlus** and **thermofisher.com/mzCloud**