Development of a Simplified User Interface for SRM Method Creation

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ABSTRACT

Purpose: The demand for analytical instrumentation that is easy to use continues to increase over time. Introduction of dwell time factors and mzCloud interfacing leads to simplified development of SRM transition lists. Additionally, a method visualization feature allows simple viewing of the final method to see the number of transitions per cycle the distribution of dwell times across the analytical run.

Methods: A conversion tool has been developed enabling the translation of MS and MS/MS data from mzCloud, and collected on Orbitrap based technology, into an SRM list (including precursor and product masses, as well as collision energies) on triple quad-based technology.

For compounds that are less abundant in samples, additional scan time can lead to better data quality. Increasing the dwell time for these compounds can lead to better reproducibility for these analytes. The software allows for several levels of prioritization, allowing users to quickly group different analytes in various prioritization levels.

Results: Predicted collision energy values from the mzCloud database were compared to collision energy values obtained by traditional syringe-infusion compound optimization. The values were found to be within 1V-2V of each other and integrated peaks that were obtained by both methods were also found to be extremely comparable.

Trial runs for the dwell time prioritization showed improved %CVs for compounds that were set to a priority of "high," while keeping the priority of the rest of the compounds at "normal."

INTRODUCTION

With the increasing demand for sample throughput on triple quadrupole technology, it is important that users are not hindered by complex instrument method user-interfaces. Simplifying the user interface will lead to a faster transfer from instrument installation to producing sample results. It can also lead to faster implementation of new assays in the laboratory.

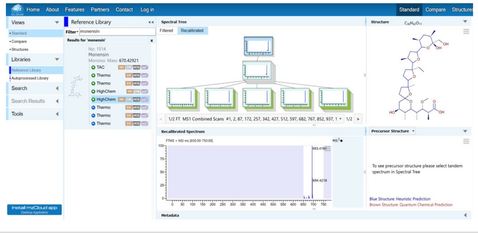
Utilizing mzCloud can reduce the time required to build SRM tables by selecting from a list of pre-defined compounds and readily available MS and MS/MS information. Additionally, instrument scan time can be prioritized for compounds that may be of lower abundance to improve the data quality of those analytes. The final method can be easily viewed in two easy-to-read charts showing the number of transitions per cycle and the and the distribution of dwell times across the analytical run.

MATERIALS AND METHODS

mzCloud integration

With the recent introduction of the Thermo Scientific™ TSQ™ Plus mass spectrometers, SRM transition information can be directly utilized from entries within 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. The mzCloud database contains over 19,000 entries covering 16 different compound classes. Each class has a range of curated entries such as 3,724 endogenous metabolites. Compounds are continually being added to the database to address emerging research requests. Each entry includes exhaustive high-resolution MS, MS/MS, and for most compounds, multi-stage MSn spectra which have been acquired at various collision energies. Each entry contains curated data fully characterizing the compound. Figure 1 shows an example entry for Monensin.

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 MSn stage performed and form of dissociation used to generate the resulting ion tree.



MATERIALS AND METHODS continued

mzCloud integration continued

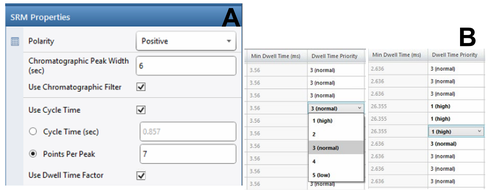
The product ion abundance rank determines the quantifying and qualifying ions used to generate the resulting SRM transitions and the apex normalized collision energy (NCE) values can be converted to collision energy (CE) voltage values used on the TSQ and TSQ Plus mass spectrometers.

While Orbitrap-based mass spectrometers and the TSQ and TSQ Plus mass spectrometers use different neutral collision gases - nitrogen (N2) and argon (Ar) respectively - the relative energetics for unimolecular fragmentation pathways will overlap generating similar product ions. To test the hypothesis, a set of veterinary drugs were infused and optimized on the TSQ Plus mass spectrometer from purified 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 CE value per product ion transition. No further method optimization was performed on the experimental values derived from mzCloud.

Dwell Time Prioritization

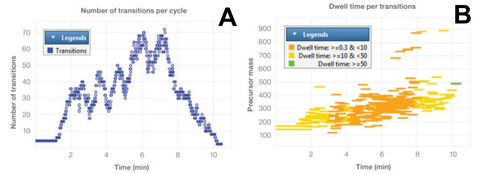
Depending on the nature of specific compounds in assays, it can sometimes be necessary to improve the reproducibility of low abundant compounds and enhance the data quality. Increasing scan time for specific transitions can accomplish this. However, with overlapping scan cycles it can be somewhat challenging to calculate changing dwell times manually and keep a desired number of scans per chromatographic peak. Improvements in SRM table parameter definitions can remove the burden of these calculations from the customer. Figure 2 shows the new enhanced features of the TSQ Plus method editor that makes these calculations easier for customers.

Figure 2. New method editor features that allow customers to explicitly state the number of points per peak desired (A). Dwell times will be automatically calculated based on chromatographic peak width and number of transitions per cycle. Dwell time priority assignments (B) give further control over dwell times for specific transitions, if desired.



As the customer is building the SRM method, a method visualization feature can be toggled to display the number of transitions per cycle and the number of transitions that fall within certain dwell time ranges (0.3msec – 10msec; 10msec – 50msec; >50msec), as shown in Figure 3.

Figure 3. Method visualization feature showing (a) the number of transitions per scan cycle and (b) the dwell time grouping of each transition throughout the run. The dwell time is also displayed relative to the precursor mass.



RESULTS

mzCloud integration

Figure 4 shows the LC-MS/MS analysis of Albendazole using a method derived from method on-system compound optimization and a method derived by using the transition data from mzCloud. 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 TSQ Altis Plus mass spectrometer and CE values read in from the mzCloud entry as they differ only by 1 and 2 V.

Figure 4. Measured albendazole response based on SRM transitions empirically determined by (A) direct infusion optimization and (B) imported directly from the mzCloud database.

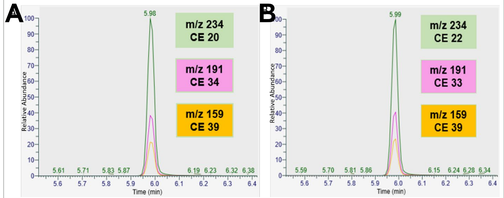
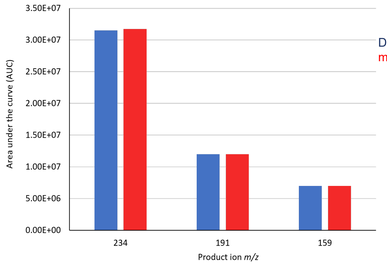


Figure 5 shows the resulting area-under-curve (AUC) values for each product ion based on the SRM transitions for each compound. Since the CE values are similar with differences generally only 1 or 2 V, the expected performance and ion ratios should be similar. In addition, the measured product ion ratios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

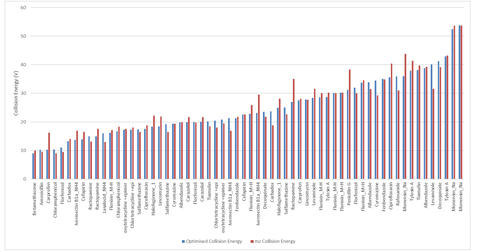
Figure 5. Comparative ion ratios for albendazole measured in +ESI mode where the QC response represents the SRM transition parameters determined from (blue) direct infusion analysis and (red) values imported directly from the mzCloud database entry.



Evaluation of a set of veterinary drugs shows that for measured SRM transitions, the average difference in CE settings between predicted and those empirically determined by direct infusion on the TSQ Plus mass spectrometer is 2.23 V while the median value was 1.63 V indicating the equation used to translate NCE to CE is accurate. Figure 6 shows a graphical representation of the NCE to CE comparisons.

RESULTS continued

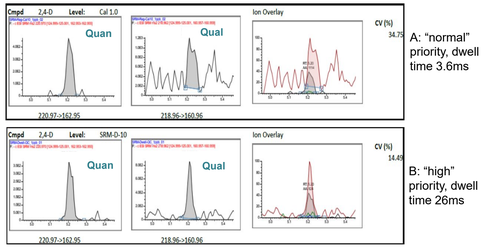
Figure 6. NCE vs CE values for a panel of veterinary drugs. The average difference in CE settings between predicted and those empirically determined by direct infusion is 2.23V while the median value was 1.63V



Dwell time prioritization

A pesticide method was developed using information provided by the mzCloud tool. Trial runs for the dwell time weighting showed increased peak areas and improved %CV's for compounds that were set to a priority of "high," (Priority 1) while keeping the priority of the rest of the compounds at "normal" (Priority 3). For this experiment, a method that included 284 pesticides was used. For 2,4-D, changing the priority from 3 (normal) to 1 (high) increases the dwell time from 3.9ms to 26ms. Subsequently, the %CV dropped from 34.75% to <15%, enabling its quantitation at the 1 µg/kg level. Example chromatograms of both priorities are shown in Figure 7.

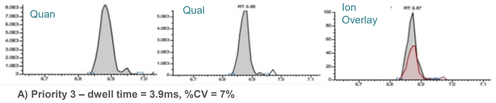
Figure 7. Data collected from a multi-residue pesticide method with 284 compounds, changing the dwell time priority for 2,4-D from (A) "normal" to (B) "high." %CVs and overall signal abundance showed improvement with the higher dwell times.



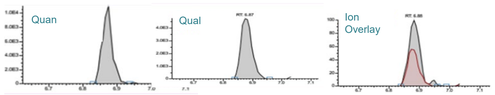
Conversely, lowering the priority of high abundance compounds can provide additional dwell time to other compounds in the method. The TSQ Plus systems still offer superior instrument performance even at very low dwell times, so data quality is minimally impacted with lower dwell times for these higher abundant compounds. As shown in Figure 8, lowering the priority of fluquinconazole from (A) 3 to (B) 4 kept the data quality intact, even with the decreased dwell time. At Priority 3, the dwell time for the two transitions was 3.9ms. With 5 replicates, the %CV for this compound was 7%. When the priority was lowered to Priority 4, the dwell time decreased to 1.7ms, and while the %CV increased to 13%, this was still below the 15% level that is standard in most applications/regulatory guidelines. There was also minimal impact on overall peak intensity and ion ratios.

RESULTS continued

Figure 8. Lowering the dwell time for a highly abundant compound like fluquinconazole from (A) "3" to (B) "4" kept the data quality intact, even with decreased dwell time.



A) Priority 3 – dwell time = 3.9ms, %CV = 7%

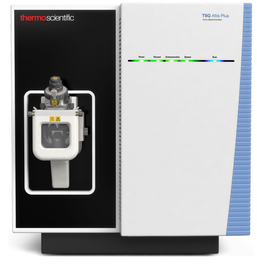


B) Priority 4 – dwell time = 1.7ms, %CV = 13%

CONCLUSIONS

- Implementation of mzCloud into the TSQ Plus method editor reduces method development time, allowing the customer to transition faster from instrument installation to producing results.
- Collision energies and peak intensities/ratios for methods created via compound optimization and importing from mzCloud are essentially identical.
- Dwell time prioritization functionality removes the burden of complex cycle time calculations from the customer.
- Increasing dwell times for challenging compounds can lead to improved data quality.
- Decreasing dwell times for more abundant compounds does not greatly impact data quality.

TRADEMARKS/LICENSING



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