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Mass spectrometry

Productivity taken to the next level with the TSQ Plus triple quadrupole mass spectrometer portfolio

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From addressing the complexities of molecules and matrices, to ensuring ease-of-use while satisfying regulatory requirements, today's challenges in targeted quantitation involve more than just measuring concentrations of target analytes. Gains in productivity are achieved through the introduction of new hardware and software features with the Thermo Scientific™ TSQ™ Plus triple quadrupole mass spectrometer (MS) portfolio, enabling ultimate confidence in data quality with robust, sensitive, reproducible, and reliable quantitative methodologies.

Translation of MS and MS/MS data from the Thermo Scientific™ mzCloud™ mass spectral library, collected with Thermo Scientific™ Orbitrap™-based mass spectrometer technology, into a selected reaction monitoring (SRM) list to be utilized with the Thermo Scientific™ TSQ™ and/or TSQ Plus mass spectrometer portfolio was made possible, facilitating the setup of TSQ MS-based methods without purified standards. Additionally, the data quality of analytes at lower abundance can be improved with the instrument control software feature that enables the prioritization of the instrument dwell time for those analytes.

Improvement in the efficiency of Q2 ion transmission for low mass range is due to a new collision cell design with innovative electrode profiles and overall mechanical design, which enhances several aspects of performance including sensitivity and robustness. Faster Pos/Neg switching times are achieved with the introduction of a highly accurate and stable power supply, whereas sample throughput is improved by allowing the measure of a large panel of analytes in both positive and negative ion modes during fast LC-MS/MS runs

without sacrificing sensitivity. In addition, the Thermo Scientific™ TSQ Altis™ Plus mass spectrometer also features new QR5 Plus segmented hyperbolic-surface quadrupoles with an improved geometry which contributes to both spectral quality and long-term temperature stability, increasing robustness for a range of mass isolation widths down to 0.2 Da FWHM.

Integrated with the TSQ Plus triple quadrupole mass spectrometers, these technology features result in enhanced instrument performance which enables users to achieve maximum robustness, sensitivity, reproducibility, and reliability of their quantitative methods for the most demanding applications.

Simplified development of SRM-based methodology with mzCloud mass spectral library and dwell time weighting

Two new software features are being introduced with the Thermo Scientific TSQ Plus triple quadrupole MS portfolio: translation of MS/MS data from the mzCloud mass spectral library into SRM information customized for the Thermo Scientific™ triple quadrupole MS systems, and enhanced data quality of low abundant analytes with the dwell time factor weighting option.

With the increasing demand for high 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 transition from instrument installation to producing sample results. It can also lead to faster implementation of new assays in the laboratory. Utilizing the mzCloud database can reduce the time required to build confident SRM tables by importing the precursor ion *m/z* values, the most abundant product ions, and their corresponding collision energy (CE) value. The CEs are derived from the optimal normalized collision energy (NCE) generated in the mzCloud database higher-energy collisional dissociation (HCD) breakdown curves. Both positive and negative ions are imported, if they are in the mzCloud database.

Figure 1 compares the response between an SRM method derived from experimental optimization via a classic infusion experiment and SRMs imported from the mzCloud database. The correlation is very close between the two methods, in terms of product ions selected, CEs, and the intensity of SRMs. Importing from the mzCloud database can dramatically decrease method development times, and methods can even be developed when analytical standards are not available.

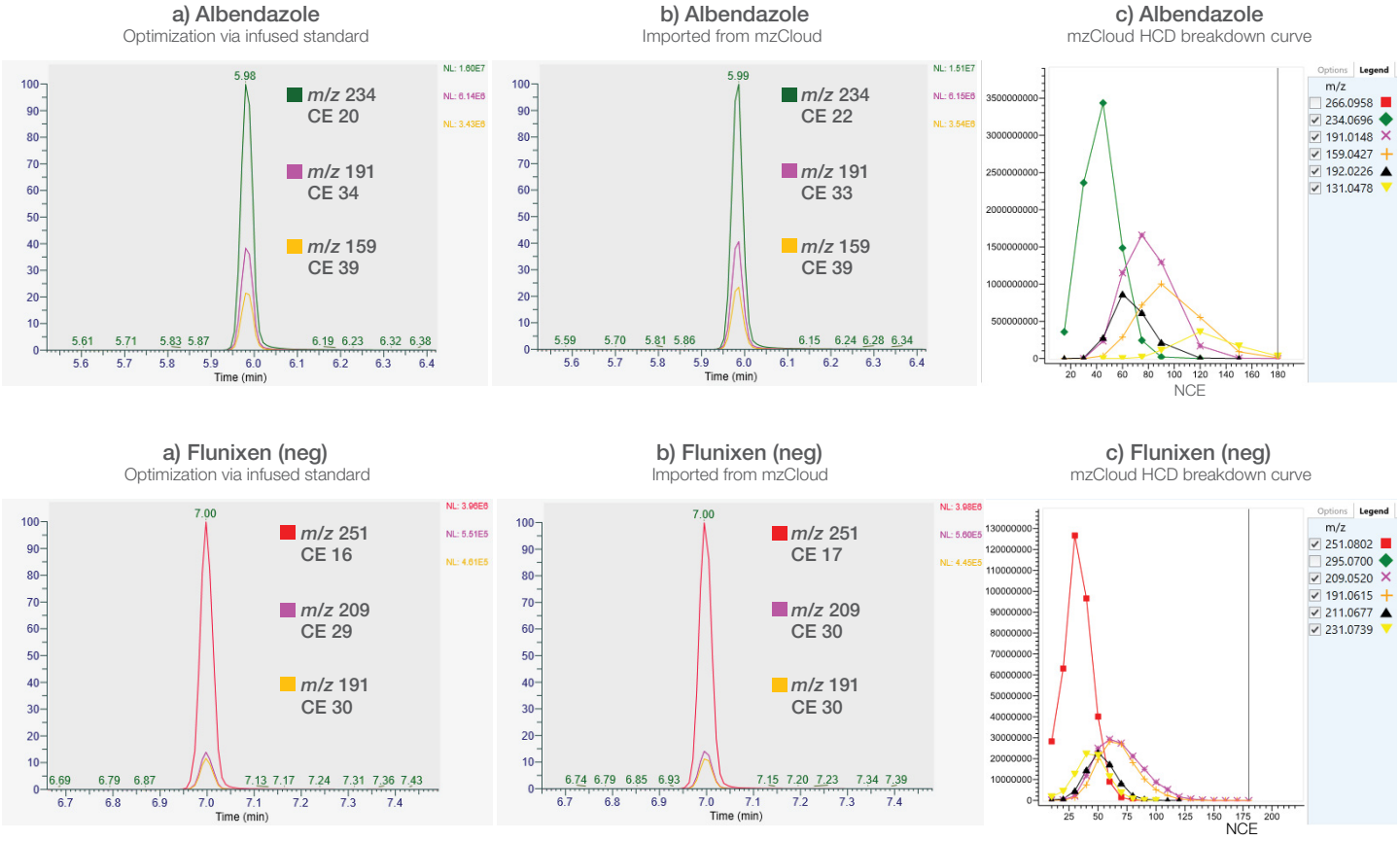

Figure 1. An overlay of extracted ion chromatograms (XIC) of a) SRMs generated by infusion of an analytical standard and experimentally determined product ions and CEs. b) SRMs generated by importing from the mzCloud database. c) The HCD breakdown curve from the mzCloud database, with precursor ion de-selected.

In the case of compounds that may not ionize well, or compounds that are less abundant in a given sample, better data quality is achieved with longer dwell times. Increasing the dwell time of these compounds will lead to better ion statistics and, therefore, better reproducibility for these analytes. The new software feature allows for several levels of prioritization, dependent on the user preferences and the assay. Once the prioritization settings are adjusted for the target compounds, the dwell times for all SRM transitions in the method (for unscheduled acquisition) or within the scheduled window are recalculated based on the cycle time. The automated global dwell time settings expedite the method optimization.

A 284 multi-residue pesticide LC-MS/MS method using the dwell time weighting feature showed improved %RSDs for compounds that were set to a priority of "high," while keeping the priority of the rest of the compounds at Priority 4 (below "normal"). For instance, the %RSD was improved from 35% to less than 15% in the response of the compound 2,4-D with the scan time priority set to 1 (high priority) enabling its quantitation in leek below the MRL level (Figure 2). Conversely, lowering the priority of Fluquinconazole, an analyte with high abundance that elutes in a chromatographic region with the highest number of co-eluting analytes, from Priority 3 to Priority 4 kept the %RSD below 15%, even with dwell time below 2 ms (Figure 3). This demonstrates that the dwell time factor weighting option can improve analytical precision for problematic compounds, without adversely affecting the remaining panel of analytes, even below MRL.

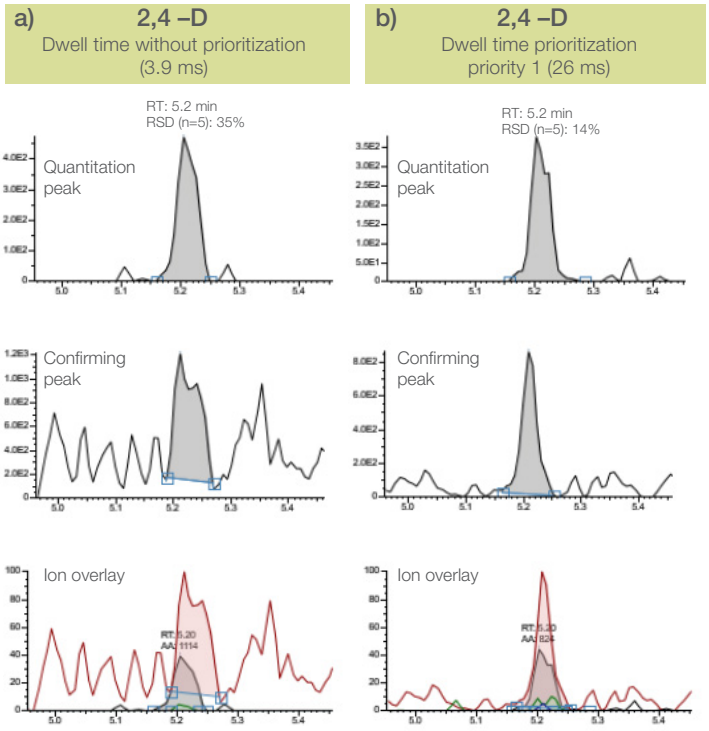

Figure 2. Extracted Ion Chromatograms of 2,4-D quantitation and confirming ions in leek below the MRL: a) scan time priority set to 3 (normal, 3.9 ms) and b) scan time priority set to 1 (high, 26 ms).

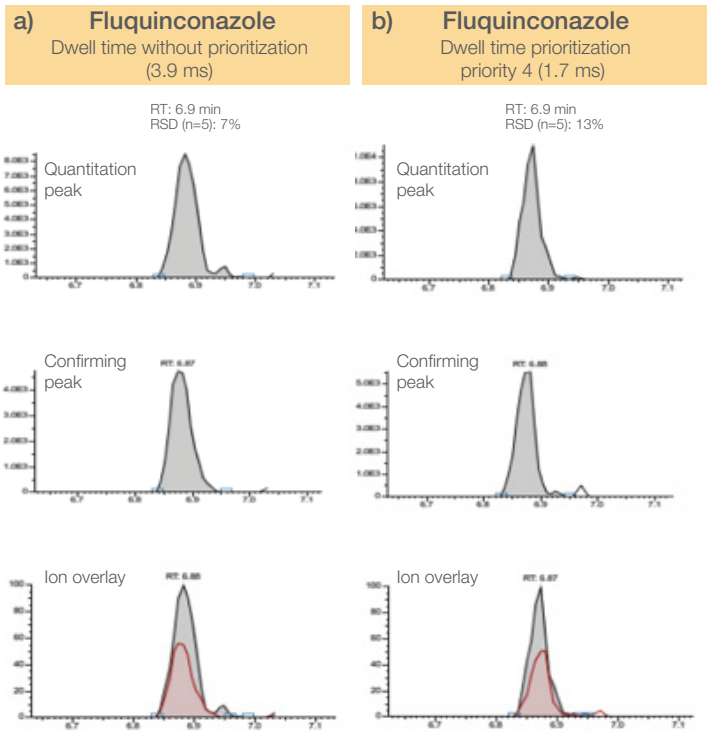

Figure 3. Extracted Ion Chromatograms of Fluquinconazole quantitation and confirming ions in leek at a concentration below MRL: a) scan time priority set to 3 (normal, 3.9 ms) and b) scan time priority set to 4 (1.7 ms).

Improved sensitivity with increased Q2 transmission for low mass range

The performance of a collision cell has a significant impact on the production of product ions by collision-induced dissociation (CID) for MS^2 analysis, where both parent and product ions need to maintain stable trajectories inside the cell to ensure maximum transmission. Improved sensitivity and robustness in SRM mode are achieved with the Active Reaction Collision Cell II (ARC II) with innovative electrode profiles that were optimized to achieve a wide *m/z* transmission range. The robustness of the ARC II design also provides more uniform performance with less need for optics tuning. Here, two studies were performed using low mass range analytes to test Q2 transmission: quantitation of haloacetic acids (HAAs) in drinking water and quantitation of nitrosamine impurities in metformin drug products.

HAAs, which are by-products formed when drinking water goes through an extensive disinfection process to ensure high quality, can present health risks. A two-fold improvement of Low Limit of quantitation (LLOQ) was achieved for most of the analytes with low fragment masses with the Thermo Scientific™ TSQ Fortis™ Plus mass spectrometer. (Figure 4) These results highlight that instrument sensitivity is improved with the increased Q2 transmission for low-mass fragment ions.

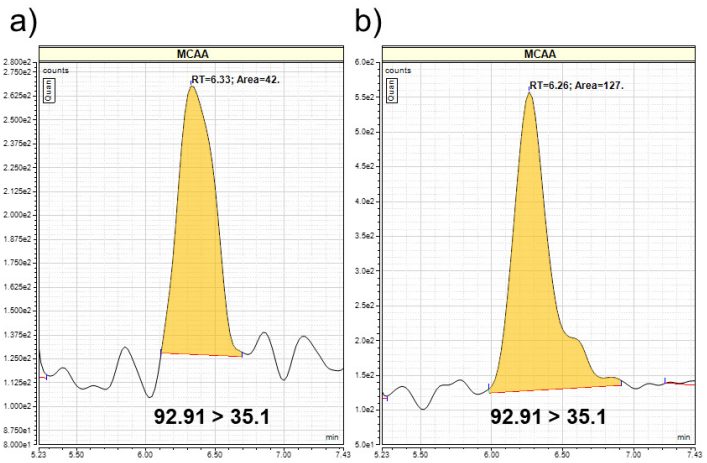

Figure 4. Example XIC of MCAA quantifier ion spiked at 0.25 ppb in water: a) data acquired with a TSQ Fortis MS and b) data acquired with a TSQ Fortis Plus MS.

After the discovery of unacceptable levels of nitrosamine contaminations in certain drug products over the last two years, regulatory agencies around the world have mandated stringent guidelines and analytical testing of all pharmaceuticals, especially for chemically synthesized ones, to control and limit the level of these genotoxic impurities in drugs. In the example below, nitrosamines, which are analytes of low mass range were quantified in metformin drug products using a Thermo Scientific™ TSQ Quantis™ Plus triple quadrupole mass spectrometer. An improvement in the signal response for most of the monitored nitrosamines was observed, demonstrating the enhancement in sensitivity for low-mass range analytes with the ARC II cell (Figure 5).

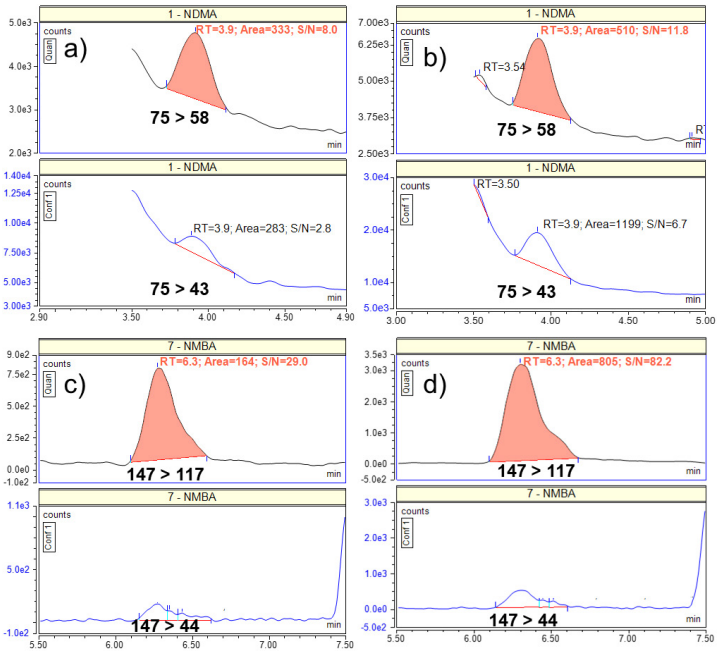

Figure 5. NDMA and NMBA spiked at 20 ppb in 100 mg/mL metformin: a) NDMA data acquired with TSQ Quantis MS and b) NDMA data acquired with TSQ Quantis Plus MS. c) NMBA data acquired with TSQ Quantis MS and d) NMBA data acquired with TSQ Quantis Plus MS.

Increased throughput with faster Pos/Neg switching time

Robust and reliable analytical methods for the simultaneous analysis of several analytes in both positive and negative ionization modes combined with fast LC-MS runs continue to pose challenges for every analytical laboratory seeking high-throughput sample analysis. The TSQ Plus MS portfolio features a new, highly accurate and stable power supply that allows Pos/Neg switching times in less than 5 ms. Pos/Neg switching means that all transitions with the same polarity (e.g., positive ESI) are monitored sequentially and then the system performs polarity switching (negative ESI) for the remaining set of SRM transitions prior to switching polarity back at the start of the next cycle. Thus, Pos/Neg switching time is only applied twice in one cycle as shown in Figure 6. A fast LC-MS/MS experiment that included 10 SRM transitions (5 SRM transitions per ionization mode) with dwell times of 10 ms was designed to investigate the signal stability with Pos/Neg switching. The first SRM transitions in positive and negative modes immediately acquired after the Pos/Neg switching event in this experiment corresponded to atrazine and tolbutamide, and their signal responses provided insights of the new power supply stability and accuracy.

The same signal response for atrazine and tolbutamide was obtained with Pos/Neg switching time of 5 ms when compared to the responses obtained on the TSQ Altis MS where the Pos/Neg switching time is 25 ms (Figure 7). Additionally, a higher number of data points across the chromatographic peak was obtained for the data collected with a Pos/Neg switching time of 5 ms, as expected (Figure 8). These results highlight that the instrument's improved Pos/Neg switching times do not adversely affect measured signal response, providing increased throughput for faster LC-MS/MS methods.

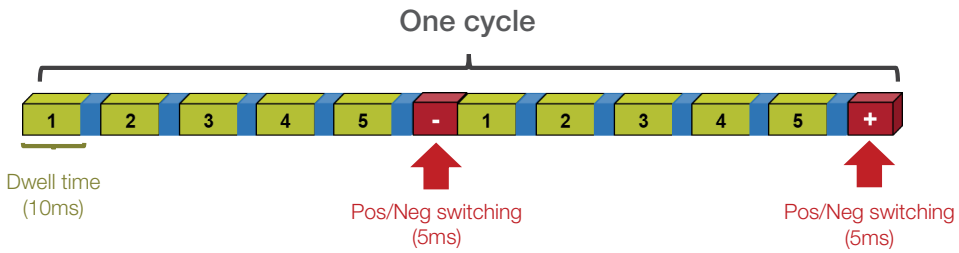

Figure 6. The Pos/Neg switching time was tested monitoring 10 SRM transitions, 5 in positive and 5 in negative mode, with 10 ms dwell times.

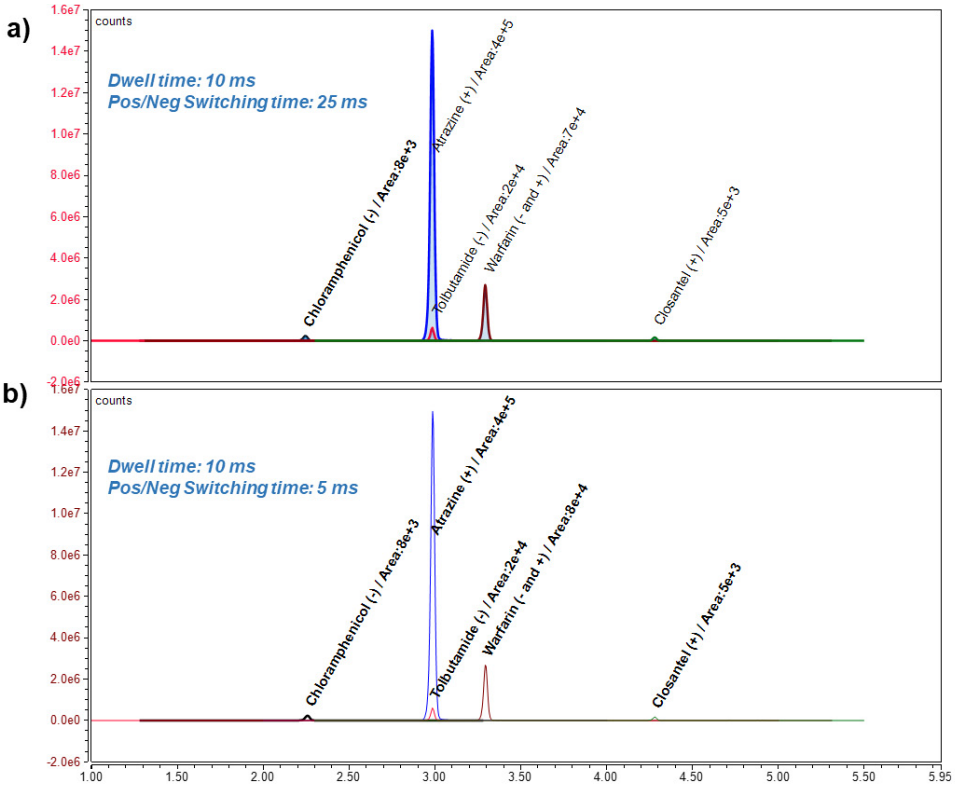

Figure 7. Overlaid extracted ion chromatograms of selected compounds used for the Pos/Neg switching test: a) experiment conducted with TSQ Altis mass spectrometer where the Pos/Neg switching time is 25 ms; b) experiment conducted with TSQ Altis Plus mass spectrometer where Pos/Neg switching time is 5 ms.

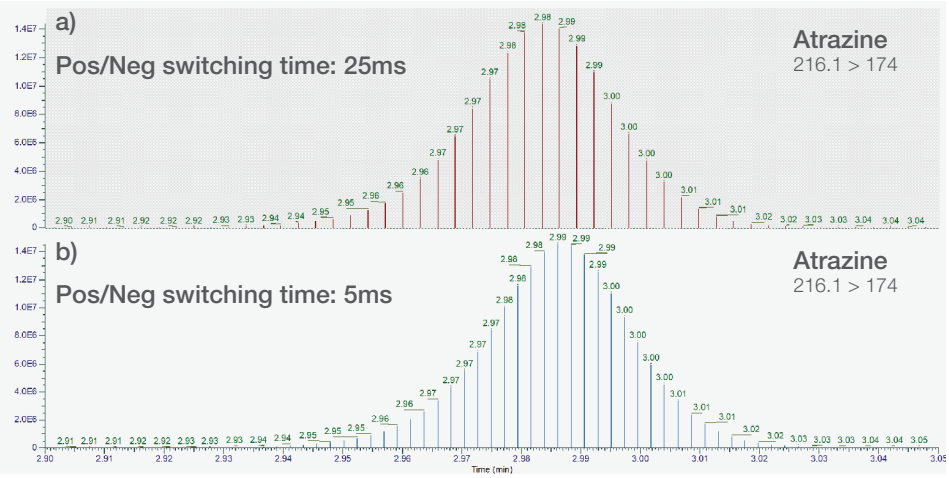

Figure 8. SRM acquisition points for Atrazine with Pos/Neg switching time of: a) 25 ms and b) 5 ms.

Excellent mass stability with the new QR5 Plus segmented hyperbolic-surface quadrupoles of the TSQ Altis Plus MS

Mass accuracy and stability of the quadrupole mass analyzers are critical for robust operation of triple quadrupole instruments in SRM operation. This is particularly important for applications that involve a series of continuous utilization of the system with minimized downtime between batches of samples. The improved geometry of the QR5 Plus segmented hyperbolic-surface quadrupoles contributes to both spectral quality and long-term mass stability. Here, the analysis of immunosuppressant drugs, which are analytes of high mass range, in whole blood for clinical research during five consecutive days was used to investigate the instrument long-term stability. The daily sequence of samples comprised an initial set of eight calibrators followed by repeated sets of five controls and twenty blank blood samples, and both Q1 and Q3 isolation windows were set to 0.7 Da FWHM. Figure 9 shows reproducible calibration curves for cyclosporin-A and stable response of internal standard cyclosporin-D for more than 1000 injections demonstrating that the TSQ Altis Plus mass spectrometer with the QR5 Plus quadrupoles is highly precise and robust for challenging SRM assays.

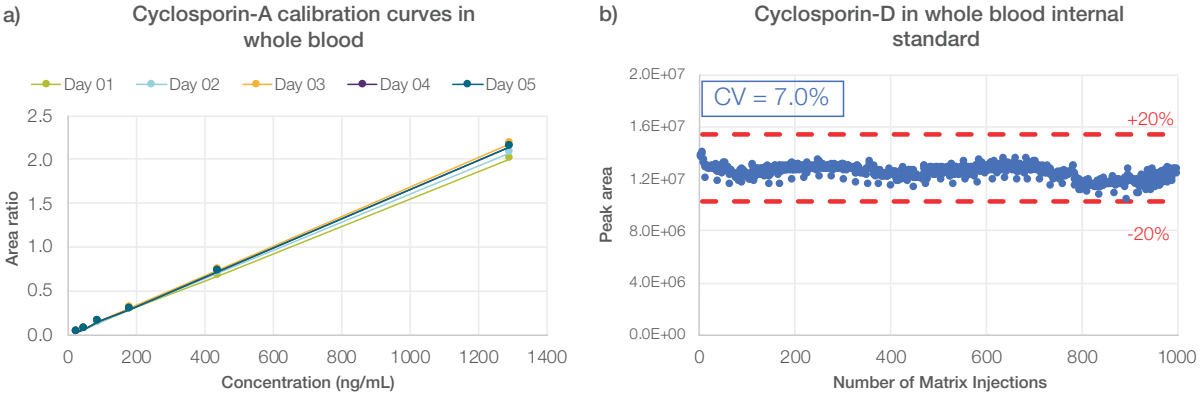

Figure 9. a) Cyclosporin-A calibration curves in whole blood obtained during 5 consecutive days. b) Peak area reproducibility across 5 consecutive days from different batches of whole blood for cyclosporin-D internal standard: 0.7 Da isolation window in both Q1 and Q3.

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