Improved Limits of Detection for the Analysis of Microcystins Using Prototype Quadrupole Geometry and Tuning

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ABSTRACT

Purpose: A prototype triple-stage quadrupole mass spectrometer based on the Thermo Scientific™ TSQ Endura™ MS has been upgraded with segmented quadrupole mass filters. The performance of the instrument has been characterized on component and system levels. New tune and calibration routines were developed to better match the new optical design.

Methods: The prototype instrument was used to run a mixture of microcystins and nodularin in complex environmental samples according to EPA Method 544.

Results: Significant gains in performance were measured and are reported. In addition, improvements in robustness and reproducibility are expected from the new TSQ instrument.

INTRODUCTION

Optics of modern triple-stage quadrupole mass spectrometers are very efficient in delivering ions from the source to the detector. Any further improvements must address not only fractional transmission gains, such as quad isolation efficiency, but also guarantee fast and reproducible tune settings to achieve and maintain optimal instrument performance. These benefits are particularly appreciated by routine applications such as the quantitation of microcystins in environmental samples according to the EPA guidelines.

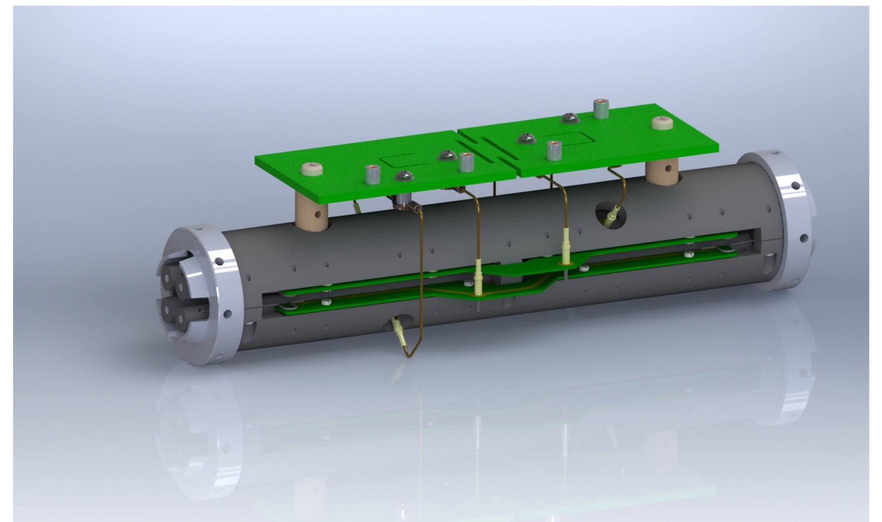
The performance of a prototype triple-stage quadrupole mass spectrometer was characterized using both standards and complex environmental samples. The enhancement to the quadrupole geometry, tuning, and calibration provided considerable improvement in limits of detection and reproducibility.

MATERIALS AND METHODS

Hardware Setup

A prototype triple-stage quadrupole mass analyzer based on the TSQ Endura instrument was constructed using improved RF-only quadrupole sections (Figure 1). Additional PCBs were designed to decouple DC voltages from the main mass analyzing section and transfer RF voltages to the pre- and post-filter.

Figure 1. New quadrupole design for a prototype system with a capacitor and DC distribution boards.



The quadrupole has 22 mm long segments carrying approximately two-thirds of the RF voltage on them. The DC offsets on the front and back segments are maintained at the same level. The quadrupole keeps the same parabolic ground surface on round rods and the same r_0 of 4 mm as on the TSQ Endura MS. Ion optics element geometry and tolerances of the mechanical design have been updated to better match the new system geometry. This included adjustments to all three sets of lenses that control ion transfer in and out of Q1 and Q3 as well as the collision cell.

New RF coil boxes and DC rod drivers with improved robustness and speed have been developed. The details on speed aspects are presented in a separate Poster (TP 387) on Tuesday. A balanced RF design has been used for this iteration, which allows for easy quadrupole voltage polarity changes for optimal performance.

In addition to changes to the analyzer and electronics sections, the prototype was equipped with the next generation long-life ion detector (see Poster ThP 360 on Thursday) as well as a number of other improvements that were targeting robustness for routine applications.

Quadrupole Transmission Measurements

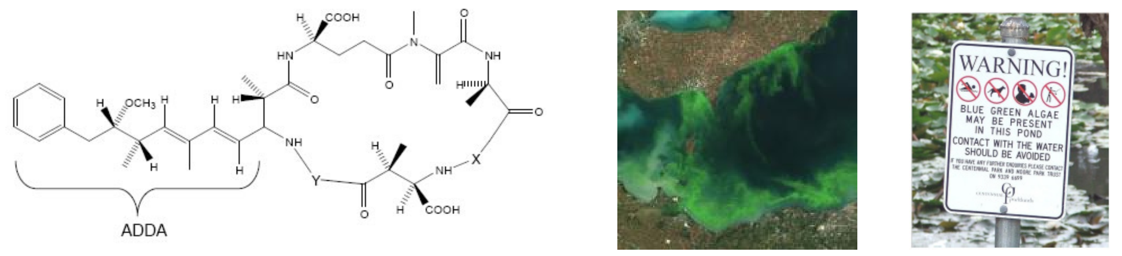
The quadrupole transmission efficiency measurements were performed by using two different techniques. In the first one, full instrument tune and calibration was done initially, and the calibrant peak intensity was compared in the isolation mode (at 0.7 Da) vs. full transmission mode (RF only on the specific quadrupole). For Q1 performance analysis, a precursor ion scan was used, and for Q3, the product ion scan was employed.

In an even more convenient regime, the peak width could be varied continuously using a diagnostic routine by making small adjustments of RF/DC values. The efficiency of the isolation at narrow peak width (such as 0.4 Da) was compared to the performance at normal peak width.

EPA Method: Application Setup

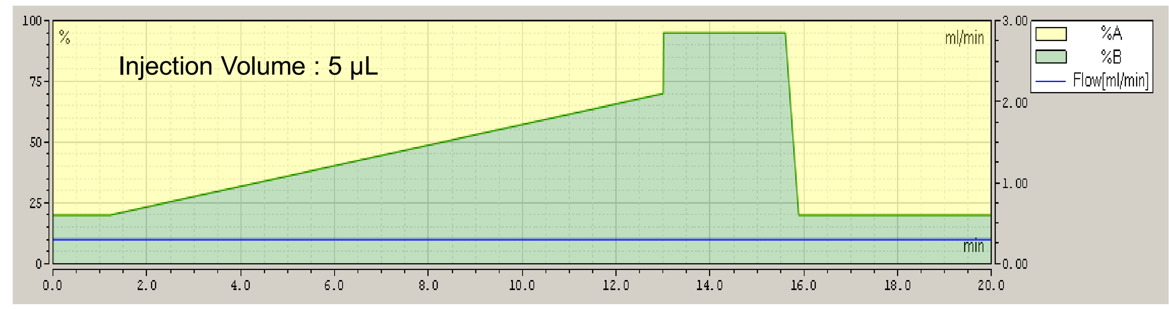
The prototype instrument was used to run a mixture of microcystins and nodularin in complex environmental samples following the EPA Method 544. This protocol addresses one of the most critical needs to control the quality of water in natural reservoirs against the presence of toxic chemicals of biological origin. As an example of a recent outbreak, a half-million people were without drinking water in 2014 in the state of Ohio due to high toxin levels caused by algae blooms in Western Lake Erie (Figure 2).

Figure 2. Typical microcystin peptide structure alongside with the related algae bloom images from Ohio Lake Erie area (2014).



A total run time of 8 minutes was used to separate the mixture of microcystins with good sensitivity (ng/L) and reproducibility.

Figure 3. LC method gradient and the injection amount.



Phase A: 100 mM Acetic Acid in Water
Phase B: Methanol

Column: Accucore C18 100 x 2.1 mm, 2.6 µm
Column Temperature: 30 ºC

The method was run on a standard TSQ Endura system as well as the prototype system with the same front end so that a direct comparison would be possible. Table 3 provides a list of typical compounds that were monitored. A series of standards were analyzed along with the water samples from local sources.

RESULTS

System Tune and Calibration

Routine quantitative analysis requires reproducible and stable performance from a mass spectrometer-based system with minimal user intervention or maintenance. One of the key factors in the day-to-day reproducibility is the stability of system tune and calibration.

The new segmented quadrupole model along with the improved optics alignment can simplify the need for tuning. Some curves are more flat and the variation ranges are reduced (see Figure 4 for examples). In many cases, the tuning is such that it is possible to assign a fix voltage to certain optical elements.

As shown in Table 1, it is possible to set all but one of the DC lens voltages in the system to fixed or mass-independent values. This is in contrast with the case of the TSQ Endura system where several more voltages must be adjusted based on *m/z*. Notably, the elements with fixed voltages are in the vicinity of the new segmented quadrupoles. This is to be expected based on higher acceptance phase space of this type of mass filters.

Figure 4. Typical tuning curves for two key DC lens elements showing flat tuning characteristics for several mass calibrant ions in a range of 69 to 1522. Red arrows indicate voltage settings that work well independent of mass.

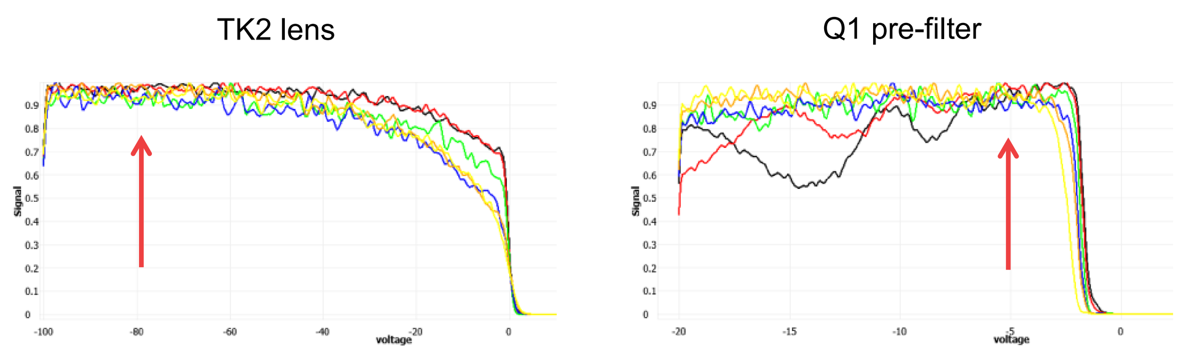


Table 1. List of select optical element DC voltages on the new prototype vs. the standard TSQ Endura system. Many tuned and mass-dependent parameters have been eliminated due to better ion beam control.

Element	New system	Old systems
MP00	fixed	mass-dependent
L0	mass-independent	mass-dependent
MP0	fixed	mass-dependent
TK1	mass-dependent	mass-dependent
TK2	fixed	mass-dependent
EL21	fixed	mass-dependent
Pre-filter DC	mass-dependent	-

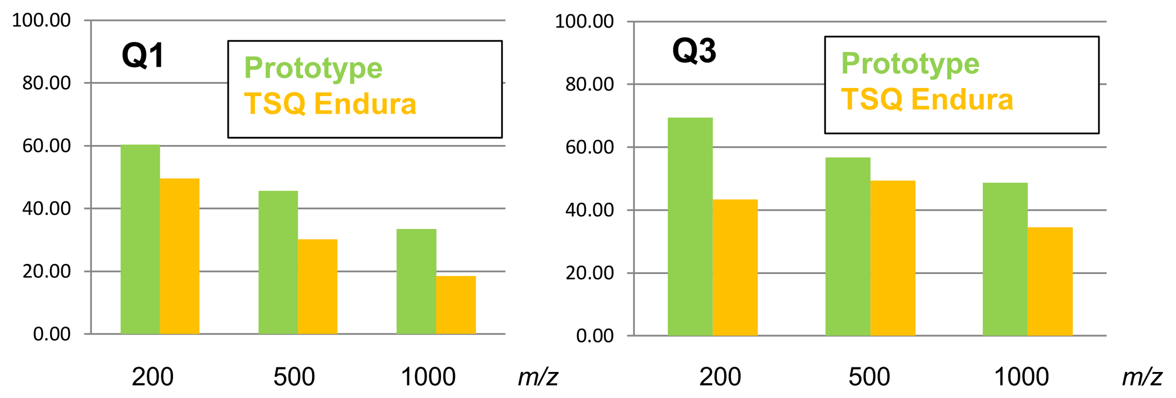
Quadrupole Transmission Efficiency in Normal (0.7 Da) Resolution Mode

Figure 5 illustrates the observed improvement of quadrupole transmission efficiency compared to the TSQ Endura system. Data from two TSQ Endura systems have been averaged to get a better representation of the typical performance.

One can see gains across the mass range with average improvement of **1.5x** for both Q1 and Q3. As a result, one could expect a cumulative gain of **2x** or higher in SRM mode for most compounds.

At even higher ion *m/z,* bigger gains may be expected as the presence of RF-only quadrupole sections typically brings additional benefits to high mass performance. See Figure 6 that suggests that this is in fact true as shown by the comparative cyclosporine injection data.

Figure 5. Transmission ratios (0.7 Da vs. RF-only) for Q1 and Q3.



Quadrupole Transmission Efficiency in Narrow (0.4 Da) Resolution Mode

Better isolation efficiency pays off for running quadrupoles in high resolution mode as well (0.4 Da FWHM). Table 2 shows typical improvement in 0.4/0.7 peak intensity ratio for the new prototype.

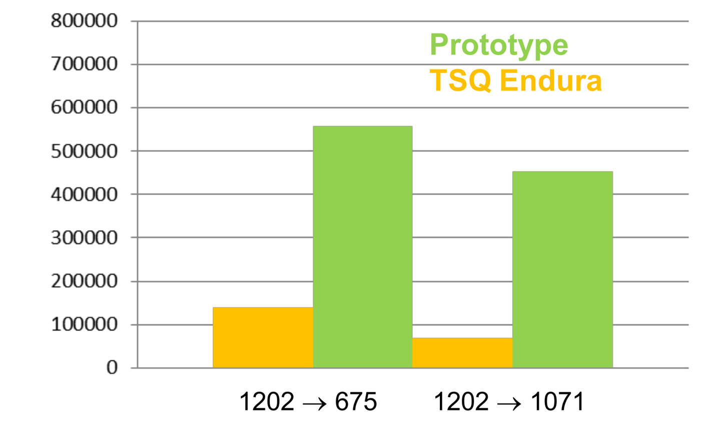
Table 2. Comparison of 0.4/0.7 transmission ratios for TSQ Endura system and the prototype system. Considerable improvements can be seen for the wide mass range while bigger benefits are observed for higher *m/z* ions.

0.4/0.7 Ratios	Endura	Prototype
Mass 182	70%	88%
Mass 508	54%	80%
Mass 997	42%	73%
Mass 180	72%	80%
Mass 506	57%	76%
Mass 995	37%	68%

Quadrupole Transmission Efficiency for High *m/z* Ions

From the analytical standpoint, one should also expect bigger gains for the analysis of compounds featuring large *m/z* precursor and product ions, as in peptide quant applications. A test case was run using injections of cyclosporine that with two high *m/z* transitions: 1202 → 675 and 1202 → 1071. In both cases, peak area gains of a factor of four or more were obtained for the prototype instrument as shown in Figure 6 below.

Figure 6. Peak area comparison for cyclosporine injections.



Microcystin Peptide Analysis

The EPA method was run on a set of standards as well as samples from California locations. Even though the injection amount was five times lower than the one specified by the EPA method, the results of the tests came back positive for all samples.

Relative comparison between the performance of the standard TSQ Endura system and the new prototype demonstrated average peak area gains of about 4x (see factors for individual compounds in the last column of Table 3). No obvious correlation between the precursor/product *m/z* and the gain in signal was observed. This suggests relatively uniform improvement of ion transmission in a broad mass range. Additionally, the ion signal increase may be due to overall better optical alignment of the new prototype.

Table 3. Data on microcystin peptides tested. Also shown are signal gains for the prototype.

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	Prototype signal gains
MC-RR-D	519.9	135.029	27.4	**5.8**
MC-YR-D	523.362	135.1	10.23	**9.3**
NOD	825.412	135.1	54.89	**3.9**
NOD	825.412	389.3	38	
MC-LA	910.412	389.014	36.73	**4.1**
MC-LA	910.412	402.137	23.16	
MC-LA	910.412	776.304	17.73	
MC-LF	986.425	478.208	24.37	**4.0**
MC-LF	986.425	776.4	28.61	
MC-LF	986.425	852.292	19.17	
MC-LR	995.512	135.075	54.47	**9.5**
MC-LY	1002.425	135.1	46.96	**3.7**
MC-LY	1002.425	868.304	19.4	
SURR	1028.512	135.118	49.24	**2.7**
MC-RR-S	1040	135.1	55	
MC-YR-S	1045.475	135.1	55	**4.3**

CONCLUSIONS

- The new implementation of the mass filter design for a TSQ mass spectrometer has been evaluated. The hardware changes have been accompanied by improvements in tuning and calibration procedures.
- Measurements of ion isolation efficiency in Q1 and Q3 quadrupoles showed noticeable enhancement in a broad mass range. Gains of up to a factor of two or more have been demonstrated.
- Application of a new autotune provides a more stable, simple, and reproducible operation with fixed voltage values for the majority of optical elements. In fact, essentially only the DC offset on quadrupole optics is being tuned and is set as mass-dependent parameter, and even then the range of adjustments is small.
- Improved sensitivity of the prototype instrument was validated for the analysis of microcystin cyclopeptides. The standard EPA 544 Method was run on a prototype system. The performance required by the method was verified while using injection amounts which were as much as five times lower that the ones specified by the EPA method.
- In comparison to the TSQ Endura system performance, average signal gains of 4x were measured, which was in line with expectations based on individual quadrupole testing results.

ACKNOWLEDGEMENTS

We would like to thank Ali Haghani from Eurofins Eaton Analytical Inc. for supplying the samples for the microcystin cyclopeptide analysis.

TRADEMARKS/LICENSING