Two approaches for MRM^3 data acquisition using the SCIEX 7500 system

Shaokun Pang
SCIEX, USA

ABSTRACT

SCIEX QTRAP systems have long boasted the ability to “trap” product ions in the linear ion trap, induce secondary fragmentation with excitation in the trap, and scan out second-generation product ions for selection in an MRM^3 workflow. The latest SCIEX 7500 system, which can be upgraded to a QTRAP system, features updated front-end technology and software control that allows for the manipulation of conditions to produce fragmentation upstream of the first quadrupole (Q1) in the ion path, called Q0 dissociation, which enables a different MRM^3 workflow. This study aimed to compare the 2 strategies using clenbuterol as a test compound. The Q0 dissociation-enhanced MRM^3 workflow is shown to be a viable option for targeted quantification when additional specificity is required.

INTRODUCTION

Analysis of small molecules in complex samples using mass spectrometry can often lead to quantitative and qualitative analytical challenges due to low analyte concentrations, presence of high background and interfering components of similar structure and mass. Generating a second-generation product ion for a target analyte reduces the potential for isobaric interference and elevated baseline signal by adding another layer of specificity to the assay for the target species. SCIEX QTRAP systems have long featured the ability to “trap” product ions in the linear ion trap, induce secondary fragmentation with excitation in the trap, then scan out second-generation product ions for quantification in an MRM^3 workflow. The SCIEX 7500 system[1] features updated front-end technology and software control relative to older models that allow for the generation of second-generation product ions in a different way, using the Q0 dissociation feature (Figure 1). This provides an effective alternative workflow to the trap-based MRM^3 workflow to maximize the quantitative selectivity of an assay.

This study aimed to compare 2 strategies for producing higher specificity MRM^3 assays using an example pharmaceutical compound, clenbuterol. Data were either generated using this alternative workflow, in which fragmentation was produced first before Q0 using the Q0 dissociation-enhanced feature and later in the collision cell, or using a conventional MRM^3 workflow, in which fragmentation was produced first in the collision cell and later in the linear ion trap. The data generated using these 2 workflows were compared based on quantitative analysis parameters including signal, sensitivity and linear response in a calibration curve.

MATERIALS AND METHODS

Sample preparation: Clenbuterol was spiked in artificial urine matrix to prepare a standard calibration curve.

Chromatography: An ExionLC AD system was used with the analytical Kinetex C18 (50 x 2.1 mm, 2.6 mm) column. The injection volume used for all experiments was 2 µL and the column temperature was held at 30° C. The mobile phase A for LC separation was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile.

Mass spectrometry: Using the SCIEX 7500 system upgraded to a QTRAP system, both Q0 dissociation-enhanced (Q0DE-MRM^3) and linear ion trap-based MRM^3 (LIT-MRM^3) experiments were conducted and the quantitative performance metrics of the 2 methods were compared. A typical MRM workflow was also assessed as a baseline of comparison by which to assess the MRM^3 data. The acquisition methods used for the Q0DE-MRM^3 and LIT-MRM^3 workflows are shown in Figures 2 and 3, respectively. The effect of Q0 trapping on method performance was tested. The LIT-MRM^3 method was run with Q0 toggled on and off for this comparison.

Data processing: All standard curve data were processed using the Analytics module of SCIEX OS software and the AutoPeak algorithm. The saturation correction was set at 8e7 and calibration curve regressions were calculated to fit a quadratic model with 1/x^2 weighting. All concentration units reported within this dataset are pg/mL. For data processing, 2 different second-generation product ions were monitored and for the assessment of the method performance, the data traces of the individual ions and the sum of the 2 ions were used.

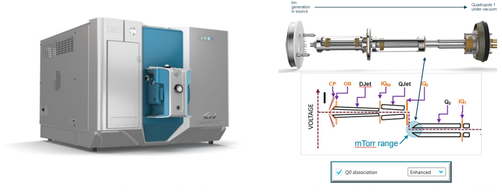

Figure 1. Q0 dissociation (Q0D) on the SCIEX 7500 system. Q0D is a single parameter in the SCIEX OS software that acts on all ions entering the vacuum region. In “Enhanced” mode (Q0DE), this parameter represents a voltage differential between the IQ0 and the Q0 lenses. It is a rampable or optimizable setting that can be tuned for a particular method or analyte.

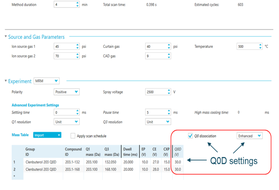

Figure 2. QTRAP 7500 system acquisition method for Q0DE-MRM^3. Only 1 Q0DE parameter needs to be optimized. Multiple compounds can be multiplexed into same MRM experiment table as other regular MRM transitions.

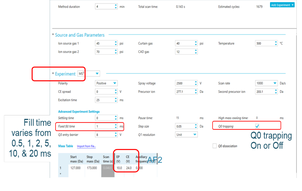

Figure 3. QTRAP 7500 system acquisition method for QTRAP MRM^3. Multiple parameters, such as AF2, FT and Q0 trapping, need to be optimized. Every compound requires a separate MS/MS/MS experiment in the method.

RESULTS

The data generated by the novel Q0DE-MRM^3 method were first compared to the data generated by a typical and widely accepted MRM method. The LLOQ, LDR and %CV of the Q0DE-MRM^3 and MRM data were comparable (Table 1) in the absence of isobaric interference and elevated baseline complexity, demonstrating minimal negative impact of this workflow.

Next, the data generated by the Q0DE-MRM^3 and LIT-MRM^3 approaches were compared. This comparison required additional experiments to consider the impact of Q0 trapping. As seen in the metrics summarized in Table 1, the LIT-MRM^3 results varied in LLOQ and LDR and were of lower quality compared to the MRM and Q0DE-MRM^3 data quality.

The time for filling the ion trap with product ions for excitation must be considered when designing and optimizing MRM^3 methods. The fixed fill time (FFT) is predominantly selected for methods used for quantification, to allow for comparability between standards and samples. Adjustments to the user-defined FFT, however, can affect the quantitative performance metrics of the overall method. To assess this effect, a series of FFTs was applied to the LIT-MRM^3 method. The resulting LLOQs and LDRs were compared between the LIT-MRM^3 quantification results (Table 2, Figure 4).

Table 1. Comparison of MRM, Q0DE-MRM^3 and LIT-MRM^3 methods on the SCIEX 7500 system. Little difference was observed in sensitivity and reproducibility between the 2 MRM^3 workflows. Both approaches achieved similar limits of quantification with similar %CVs when Q0 trapping is used, however, Q0 trapping limitited the LDR (Figure 4).

Experiment Type	Transition	LLOQ (pg/mL)	ULOQ (pg/mL)	%CV
MRM	*277-203*	*3*	*100,000*	*0.52-2.76*
	277-168	*3*	*100,000*	*0.59-11.29*
	Sum	*3*	*20,000*	*0.57-6.32*
Q0DE-MRM^3	*277-203-168*	*3*	*100,000*	*0.20-15.53*
	277-203-132	*3*	*100,000*	*0.07-20.67*
	sum	*3*	*20,000*	*0.39-9.61*
LIT-MRM^3, Q0 trapping off	*277-203-168*	*20*	*10,000*	*3.29-21.02*
	277-203-132	*20*	*3,000*	*2.14-9.05*
	sum	*3*	*3,000*	*2.83-10.66*
LIT- MRM^3, Q0 trapping on	*277-203-168*	*3*	*3,000*	*1.49-25.29*
	277-203-132	*3*	*1,000*	*1.62-12.47*
	sum	*3*	*3,000*	*1.68-19.54*

Table 2. Effects of fixed fill time (FFT) and Q0 trapping on quantification performance for the LIT-MRM^3 workflow.

FFT (ms)	Without Q0 trapping								
	277-203-132			*277-203-168*			Sum		
	LLOQ (pg/mL)	ULOQ (pg/mL)	%CV	LLOQ (pg/mL)	ULOQ (pg/mL)	%CV	LLOQ (pg/mL)	ULOQ (pg/mL)	%CV
0.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2	N/A	N/A	N/A	N/A	N/A	N/A	3,000	100,000	2.77-8.67
5	20	20,000	0.7-10.13	200	20,000	2.38-8.50	20	20,000	1.26-6.66
10	20	20,000	2.22-14.14	30	20,000	0.38-12.08	20	20,000	1.16-6.44
20	20	20,000	1.34-23.64	30	20,000	2.02-15.83	30	20,000	0.87-14.63
FFT (ms)	With Q0 trapping								
	277-203-132			*277-203-168*			Sum		
	LLOQ (pg/mL)	ULOQ (pg/mL)	%CV	LLOQ (pg/mL)	ULOQ (pg/mL)	%CV	LLOQ (pg/mL)	ULOQ (pg/mL)	%CV
0.5	200	100,000	2.62-23.52	200	100,000	1.28-20.79	30	20,000	1.37-19.56
1	30	20,000	1.08-20.13	200	100,000	1.86-12.44	30	20,000	1.53-7.15
2	20	10,000	1.14-13.80	200	20,000	0.92-6.36	20	20,000	0.38-8.51
5	20	3,000	0.82-3.54	20	3,000	2.08-13.23	20	3,000	0.82-5.19
10	20	3,000	1.33-7.68	20	3,000	0.90-11.71	20	3,000	1.24-8.79
20	20	3,000	0.47-7.59	20	3,000	0.99-15.33	20	3,000	0.29-3.82

The addition of Q0 trapping also facilitated the ability to detect lower levels of analyte at lower FFTs. However, the signal tended to become saturated with Q0 trapping as FFT was increased (Figure 4) and LT-MRM^3 needs more parameter optimization (Table 3). The speed of the analysis varied between approaches. For the traditional MRM and the Q0DE-MRM^3 approaches, all method information is stored in a single MRM table (Figure 2), making it efficient to monitor multiple compounds with multiple transitions. Fast dwell times can therefore be achieved, as the instrument operates with all elements continuously transmitting.

With the LIT-MRM^3 approach, however, a separate MS/MS/MS experiment must be set up for each primary fragment ion to be monitored (Figure 3). Additionally, the time required for analysis includes both the excitation time and the time to scan the secondary product ions out of the ion trap. Thus, the acquisition time per compound is longer and fewer compounds can be multiplexed into a single assay with the LIT-MRM^3 approach.

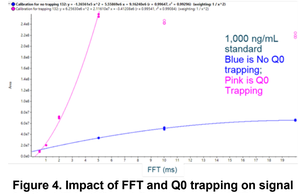

Figure 4. Impact of FFT and Q0 trapping on signal for the LIT-MRM^3 workflow. Increasing FFT increased the signal intensity for the MRM^3 data trace. Turning on Q0 trapping quickly saturated the detector and therefore limited the LDR. The same pattern was observed for both secondary product ions assessed.

Table 3. Tunable parameters for the different targeted workflows. While Q0DE-MRM^3 requires little extra optimization work, LT-MRM^3 needs more parameter optimization.

Parameter	MRM	Q0DE-MRM^3	LIT-MRM^3
Collision energy (CE)	*Ramp*	*Ramp*	*Ramp*
Q0 dissociation (Q0D)	*-*	*Ramp*	*-*
AF2	*-*	*-*	*Ramp*
Fill time	*-*		*Set*
Q0 trapping	*-*		*On / off*

Note: Source conditions were optimized and held constant across these experiments.

CONCLUSIONS

Here, the clenbuterol example was used to show proof-of-concept evidence that the Q0DE-MRM^3 workflow, available on the SCIEX 7500 system, has potential to be a sensitive, reproducible and easy-to-set-up option for addressing analytical challenges of selectivity.

- Compared to the historically utilized LIT-MRM^3 workflow that leverages the QTRAP system, the Q0DE-MRM^3 workflow requires much less development and optimization and can be higher multiplexed
- The Q0DE-MRM^3 method produced data with quality comparable to that of the MRM-based acquisition method in terms of LLOQ, ULOQ and %CV and outperformed the LIT-MRM^3 workflow
- Multiple targeted workflows are available on the SCIEX 7500 system, upgradable to a QTRAP system, that allow method developers to select the right tool for quantification studies when additional selectivity is needed.

References

1. MRM^3 Quantitation for Highest Selectivity in Complex Matrices. SCIEX technical note, RUO-MKT-02-2739-B.
2. Enabling new levels of quantification. SCIEX technical note, RUO-MKT-02-11886-B.

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