Linear Dynamic Range Improvement in TOF-MS/MS Mode on X500 QTOF system

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ABSTRACT

Quantitation on the X500 QTOF system can be performed in both TOF-MS and TOF-MS/MS modes. Because detectors used in QTOF reach saturation at lower ion currents than those used in triple quadrupole instruments, the upper limit of quantitation (ULOQ) in QTOF instrument can be lower. This results in comparatively limited Linear Dynamic Range (LDR) in QTOF instrument for quantitation analysis. In order to improve ULOQ, a technique named Enhance Dynamic Range (EDR), previously implemented for TOF-MS only in SCIEX 5600 and 6600 instruments, has now extended to TOF-MS/MS on the SCIEX X500 platform controlled by SCIEX OS software 1.3. This poster demonstrates the improvement of LDR in TOF-MS/MS on SCIEX X500 instruments.

INTRODUCTION

The QTOF mass spectrometer is gaining popularity in qualitative and quantitative analysis because of its higher selectivity and lower background. Triple quadrupole instruments are generally able to achieve much higher signal levels and therefore greater ULOQ than QTOF instruments owing to differences in detector technologies. One effective method to extend the ULOQ is through dynamic pulsing of a lens element in the front end in the instrument to quantitatively gate ions coming into the quadrupole optics. This has been employed in the TOF-MS mode of operation on the SCIEX Triple TOF 5600 and 6600 instruments. This method has been called Ion Transmission Control, or ITC.

With ITC, the ion current attenuation is adjusted dynamically by the software scan by scan to ensure the detector saturation point is not reached. The attenuation is controlled on both of total ion current and the individual peak intensity. For any detection cycle, when a total ion current target and/or an individual peak intensity target is reached, the ion current will be attenuated accordingly in the next cycle. Then a corresponding correction factor, ion transmission coefficient, will be applied to the data output to account for the beam attenuation (Figure 1). Because most often the quantitation is performed in TOF-MS mode, and total ion current in TOF-MS/MS mode is generally low due to Q1 isolation, the ITC has traditionally only been applied in TOF-MS mode.

With the increasing demands in doing quantitative analysis in TOF-MS/MS mode, the ITC technique is extended for both TOF-MS and TOF-MS/MS modes in SCIEX X500 QTOF instrument controlled by SCIEX OS software 1.3 using the Enhanced Dynamic Range (EDR) feature. In this poster we demonstrate that the application of EDR to TOF-MS/MS mode increased the LDR by extending the ULOQ without any impact on lower limit of quantitation (LLOQ) and other aspects of performance.

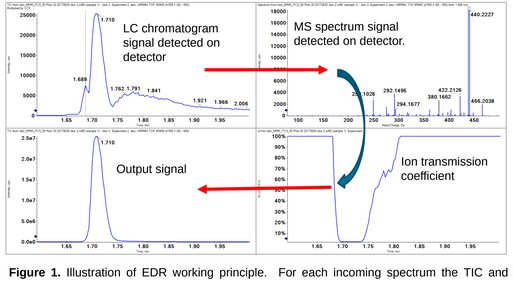

Figure 1. Illustration of EDR working principle. For each incoming spectrum the TIC and base peak intensities are monitored and used to determine the beam attenuation (if necessary) for the next MS cycle.

MATERIALS AND METHODS

Sample Preparation:
Seventeen calibration standards were prepared by making 3-fold serial dilutions of 100 µg/mL Alprazolam/Atorvastatin in 50/50 acetonitrile/water containing 10ng/mL Alprazolam-D5/Atorvastatin-D5, respectively. The analyte concentration covered the range of 0.00077400 to 33300 ng/mL, and internal standard concentration were calculated accordingly and spanned the range of 6.67 ng/mL to 10 ng/mL.

LC-MS/MS Conditions:

An Agilent 1290 LC system with Phenomenex Kinetex®, C18 2.6 µm 100A° (50 x 2 mm) column was used at 40 ºC. Mobile phase were A: water/acetonitrile (98:2) + 2mM ammonium acetate + 0.1% formic acid, and B: water/acetonitrile (2:98) + 2mM ammonium acetate + 0.1% formic acid. A gradient as shown in Table 1 was used at flow rate of 500µL/min. The injection volume was set to 3µL.

Three SCIEX X500 QTOF systems with Turbo V™ source and TwinSpray probe were used. MRMHR workflow was used for quantitation of both Alprazolam and Atorvastatin. MS and MSMS conditions are shown in Table 2. Atorvastatin was also analyzed with MRMHR in negative ion mode, and Scheduled MRMHR and SWATH® workflows. For Atorvastatin in negative ion mode, the same LC and MS conditions were used, except the deprotonated ions at 557.3 and 562.3 were used as MSMS precursors. Scheduled MRMHR conditions are the same as in MRMHR, with a scheduled window of 30s around the elution time of Atorvastatin. For SWATH® acquisition, two Q1 selection windows of 500-550 and 549 -600 Da were used and the other conditions were the same as in MRMHR. For all methods, both MS and MSMS spectra were acquired and used for quantitation.

For each workflow, the experiment is designed to run in back to back comparison by toggling between two MS methods: one with EDR on for TOF-MS/MS by check the “Enhance dynamic range” box in the method editor; another one with EDR off by NOT checking the “Enhance dynamic range” box. For all experiment method, EDR for TOF-MS was always on by default.

Table 1. LC gradient.

Total Time(min)	Flow Rate (µl/min)	A (%)	B (%)
0.1	500	90	10
2	500	2	98
2.5	500	2	98
2.55	500	90	10
3.55	500		stop

Table 2. TOF-MS and TOF-MS/MS conditions for MRM HR

MS Experiment	Precursor ion	Start Mass	Stop Mass	Accumulation Time (ms)	DP	CE	CES	EDR
Aprazolam								
TOF MS	N/A	150	650	50	80	10	0	N/A
TOF MS2	309.1	80	650	50	80	41	10	On/off
TOF MS2	314.1	80	650	50	80	41	10	On/off
Atorvastatin								
TOF MS	N/A	150	650	25/35/50	150	10	0	N/A
TOF MS2	559.3	80	650	25/35/50	150	35	0	On/off
TOF MS2	564.3	80	650	25/35/50	150	35	0	On/off

Data were processed with Analytics in SCIEX OS software 1.3. The TOF-MS and TOF-MS/MS masses and the XIC window width for each compound are described in Table 3. The XIC window width can be adjusted according to resolution of the instrument and background interference as long as the accuracies and %CVs meet bioanalysis requirements[1], %CV within +/-15% except +/-20% for LLOQ and accuracy between 85-115% except 80-120% for LLOQ. The LDR with EDR on and off are used to evaluate the improvement of EDR function.

Table 3. quantitation process conditions

	Alprazolam	XIC window	Atorvastatin positive	XIC window	Atorvastatin negative	XIC window
MS ion analyte	309.08	0.06	559.27	0.06	557.2	0.06
MS ion IS	314.09	0.06	564.29	0.06	562.2	0.06
MSMS ion analyte	281.07	0.06	440.22	0.06	397.12	0.06
MSMS ion IS	286.09	0.06	445.25	0.06	402.15	0.06

RESULTS

The experiment is designed to run in back to back comparison by toggling between two MS methods: one with EDR on for TOF-MS/MS and another one with EDR off for each calibration standard to minimize any variation from instruments and samples.

When EDR was off, the ion transmission for TOF-MS/MS was not attenuated therefore the detector saturation could occur if the analyte concentration is high. Figure 2 A and B shows such an example. At concentration of 11100 ng/mL for Atorvastatin, XIC of the most sensitive transition 559.3-440.2 (A) showed a deteriorated peak shape with peak top collapsed. The TOF-MS/MS spectrum (B) also showed a strange pattern in terms of relative intensities of different fragment peaks, compared to a normal TOF-MS/MS spectrum (D). All these phenomena suggested that the detection subsystem was well saturated. As a result of the saturation, the LDR obtained was only 3.4 order, with LLOQ at 0.0627 ng/mL and ULOQ at 137 ng/mL (shown in Figure 3 A).

When EDR was on, the ion transmission for TOF-MS/MS was attenuated dynamically to prevent saturation. As shown in Figures 2 C and D, a normal XIC peak and MSMS spectrum were obtained for the same sample at concentration of 11100 ng/mL, and in the TOF-MS/MS spectrum, peak at 440.2 is displayed as base peak because it presents the most intense fragment ion. Please also note that the peak height of the XIC is at 9e6 cps, 30 times more than the peak height obtained with EDR off (Figure 2 A). This is the output signal after correction with ion transmission coefficient of detected signal. As a consequence, the obtained LDR was 5.2 order, with LLOQ at 0.0627 ng/mL and ULOQ at 11100 ng/mL (shown in Figure 3 B), a 1.8 order increase.

The LDR obtained with EDR on and off are summarized in Table 4. For all workflows the LLOQ was not affected by EDR, and the improvement of LDR is due to extension of ULOQ to higher concentration samples as expected. Accumulation times down to 25 ms were tested and did not show a significant impact on LDR.

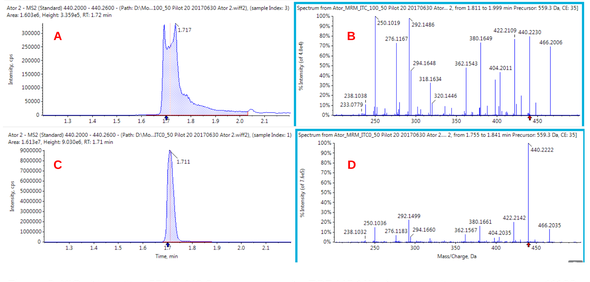

Figure 2. XIC of transition 559.3-440.2 and spectrum for TOF-MS/MS of atorvastatin at concentration 11100 ng/mL in MRMHR. A and B with EDR off, and C and D with EDR on.

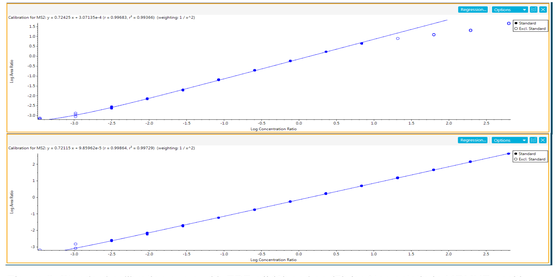

Figure 3. Standard calibration curve with EDR off (A) and on (B) for Atorvastatin in MRMHR positive. Transition 559.3-440.2, with a XIC window of 0.06 Da, was used for quantitation.

Table 4. summary of LDR obtained with EDR on and off on 3 instruments for different workflows

	Alprazolam positive MRMHR		Atorvastatin positive MRMHR		Atorvastatin negative MRMHR		Atorvastatin positive sMRMHR		Atorvastatin positive SWATH®		Atorvastatin positive MRMHR, 25 ms	
	EDR off	EDR on	EDR off	EDR on	EDR off	EDR on	EDR off	EDR on	EDR off	EDR on	EDR off	EDR on
instrument 1	2.4	4.3	3.3	5.2	3.3	4.3	2.9	5.2	2.9	4.8		
instrument 2	2.4	4.3	2.9	5.2	2.9	4.3	2.9	5.2	2.9	4.3		
instrument 3	3.3	4.8	3.3	4.8	3.3	4.3	2.4	4.8	2.3	4.3	3.3	4.8
average LDR improvement	1.8		1.9		1.1		2.3		1.8		1.5	

CONCLUSIONS

- The EDR feature for MSMS improves the LDR performance (> 1 order) for tested compounds and workflows by extending the ULOQ to higher concentration without effects on LLOQ.
- Shorter accumulation, 25 ms and 35 ms in MSMS mode has not caused any loss of LDR performance comparing with 50 ms accumulation time.

REFERENCE

[1]. Guidance for Industry Bioanalytical Method Validation, FDA. May 2018.

TRADEMARKS/LICENSING

AB Sciex is doing business as SCIEX.

Document number: [RUO-MKT-10-7838]