Quantification of the therapeutic peptide exenatide in human plasma

MRM³ quantitation for highest selectivity in complex mixtures on the SCIEX QTRAP® 5500 System

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With increasing focus on biotherapeutics, there is greater interest in using LC-MS for the quantitative analysis of proteins and peptides in pharmaceutical research. Exenatide is a large therapeutic peptide that has been approved for the treatment of Diabetes mellitus type 1 and 2. This peptide enhances glucose-dependent insulin secretion by the pancreatic beta-cell, acting as a regulator of glucose metabolism and insulin secretion.

In recent years, the plasma concentrations of exenatide were measured by ligand-binding assays, such as immune-enzymatic assays used for pharmacokinetic studies. However, there are specificity and selectivity risks with these types of analysis since certain compounds may have similar physiochemical properties. For this reason, an MRM³ LC-MS strategy[1] was explored here as a way to obtain higher selectivity in peptide using the SCIEX QTRAP® 5500 System.

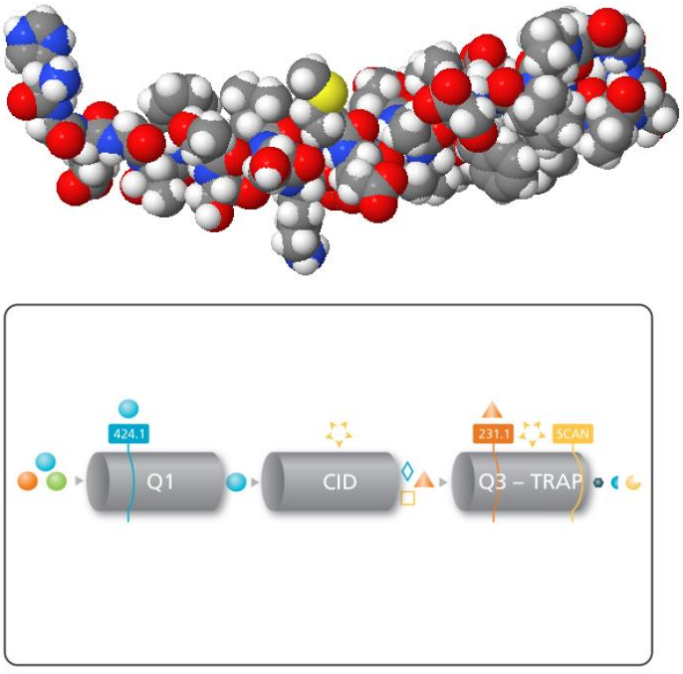

Figure 1. Structure of exenatide. (Top) Exenatide is a large 39 amino acid peptide (MW = 4186.6 Da) that acts as a regulator of glucose metabolism and insulin secretion. (Bottom) MRM³ scan mode for quantitative analysis by LC-MS. Parent ion is first selected in the Q1 quadrupole, then fragmented in Q2 collision cell. Product ions are trapped then isolated in the linear ion trap, followed by excitation to perform the second fragmentation step. 2nd generation product ions are scanned out to the detector.

Key features of MRM³ for quantifying large therapeutic peptides

- Because of the multiple fragmentation steps used in MRM³, higher selectivity is typically achieved.
- Improvements to the QTRAP® 5500 Systems have enabled faster and more sensitivity MRM³ analysis
- Detection limits in very complex matrices can often be improved using MRM³ analysis by removing interferences at the low end of the concentration curve, improving signal/noise.
- Unlike MS³ on traditional ion traps, the unique hybrid triple quadrupole – linear ion trap design allows Q1 to be used for precursor ion selection (unit resolution), and Q2 for the first fragmentation step in a transmission mode. This allows higher speed and more flexibility in the choice of the first product ion since there is no low mass cut-off associated with the first fragmentation step in Q2, and higher collision energies can be used.

Methods

Sample preparation: Exenatide was extracted from human plasma, dried in a TurboVap under nitrogen and reconstituted. In all steps, pH values and organic phase were carefully controlled.

Liquid chromatography:

- LC: Shimadzu UFLC LC-20ACXR
- Column: Reverse phase C-18 2.0 x 30 mm, 5 µm
- Flow rate: 0.6 mL / min
- Injection volume: 5 µL
- Mobile Phase A
  - 0.1% Formic acid in water
- Mobile Phase B
  - 0.1% Formic acid in Methanol
- Gradient: 2 – 95% B in 5 minutes

Mass spectrometry: LC-MS analysis was done on the QTRAP® 5500 System (SCIEX) using the MRM^3 acquisition strategy[1]. The principle of MRM^3 analysis as performed on the QTRAP® 5500 System is demonstrated in Figure 1. Using the MS^3 scan type, the trap was filled using Dynamic Fill Time (DFT) and the instrument was scanned at 10,000 Da/sec. The trap excitation time was 25 ms, giving a total cycle time of 0.17 sec. The transition ions used for the MRM^3 analysis were 838 → 396 → 202.

Assay development results

In Enhanced MS (EMS) mode, the multiple charged parent ion $[M+5H]^{5+}$ at m/z 838.3 was selected as the first precursor (Figure 2, top). When this charge state is fragmented, the predominant product ion m/z 396.4 was chosen as the second precursor (Enhanced Product ion (EPI) scan, Figure 3, middle). The m/z 396.4 was fragmented in LIT to generate MS/MS/MS spectrum (Figure 2, bottom). The major fragment ion m/z 202.2 was selected as the second generation product ion for the MRM^3 quantification.

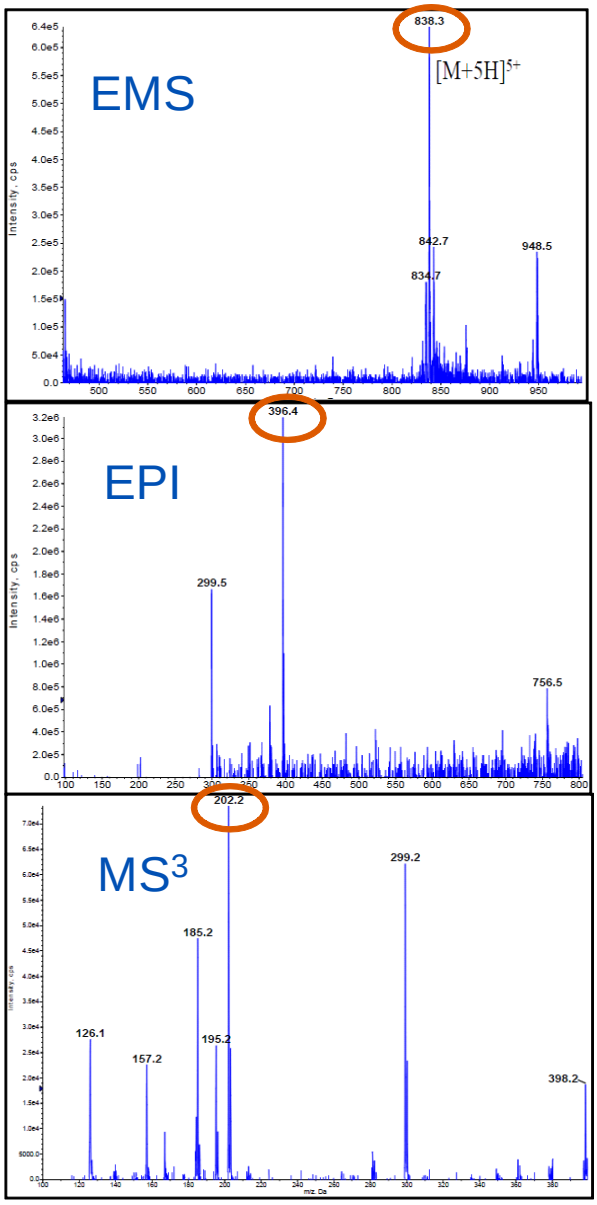

Figure 2. MRM^3 assay design. EMS scan (top) is used to select the dominant parent ion, the most intense fragment ions are identified using the EPI mode (middle), and MS/MS/MS fragmentation is used to select the best secondary fragments to extract for MRM^3 quantitation.

Assay performance for exenatide

Use of MRM^3 analysis resulted in significantly improved selectivity of detection for exenatide in human plasma extracts. Figure 3 shows a comparison of MRM^3 vs. traditional MRM quantitation. Baseline was lower and chromatographic interference from the plasma matrix was significantly reduced in MRM^3. The fast scanning speed of the QTRAP® 5500 System (10 000 Da/sec) provided a sufficient number of data points across the analyte chromatographic peak for good reproducibility of quantitation.

The improved detection performance resulted in excellent assay performance at the LLOQ and four QC levels as shown in Table 1. Accuracy and %CV for six replicates demonstrate that the MRM^3 approach is capable of quantitative performance suitable for development-grade bioanalytical assays.

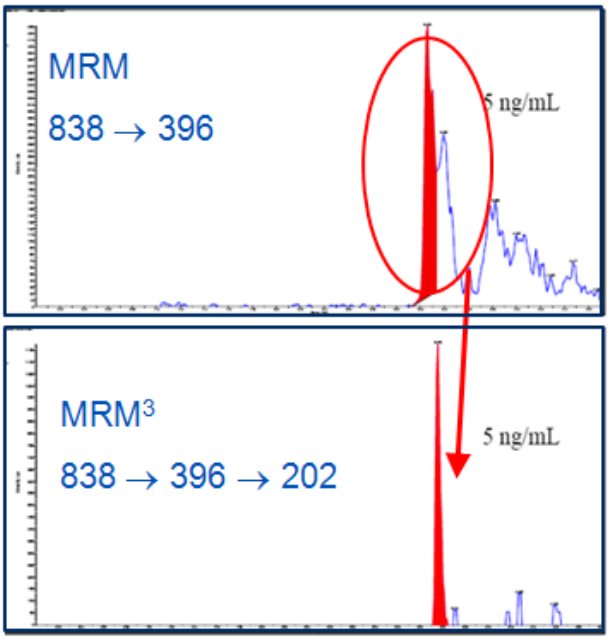

Figure 3. MRM^3 significantly improved selectivity of detection in plasma. Elimination of chromatographic interferences and background noise improves the LOQ for exenatide in plasma.

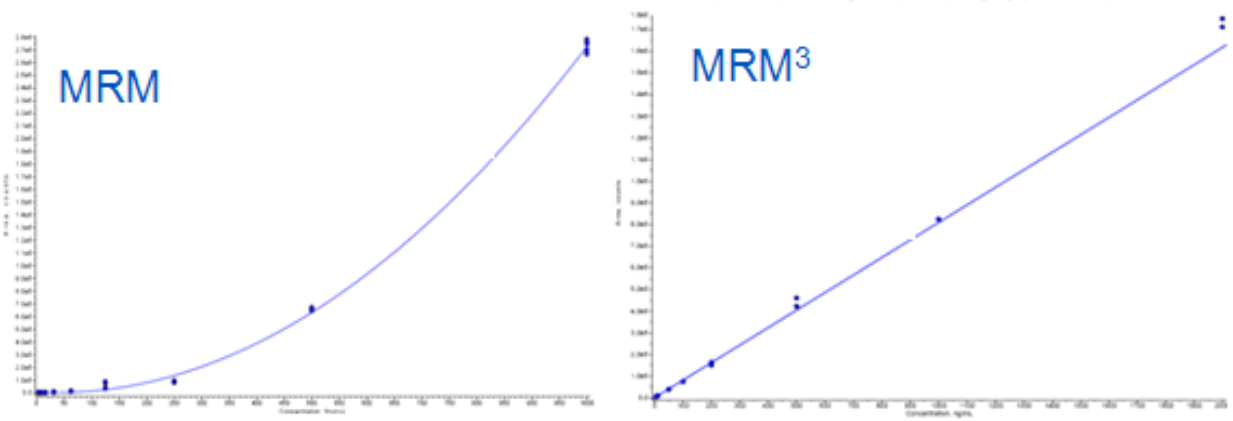

Figure 4. Calibration curves for exenatide in human plasma using MRM and MRM^3 analysis. (Left) MRM from 250-1000 ng/mL and (Right) MRM^3 from 5 – 1000 ng/mL showing significantly better linearity (R2 = 0.996).

Conclusions

- A bioanalytical assay for Exenatide in human plasma was successfully developed using MRM^3 analysis.
- The increased selectivity of MRM^3 allowed for the significant reduction of baseline noise and chromatographic interference, resulting in improved analytical performance compared to the MRM approach in this example.
- MRM^3 demonstrated the potential for excellent linearity achieving a calibration range of 5-2000 ng/mL, compared with a range of less than 250-1000 ng/mL for traditional MRM with this analyte due to interferences.
- Accuracy and reproducibility of the MRM^3 assay was compatible with the requirements for a development stage bioanalytical assay.

Table 1. Accuracy and reproducibility of the MRM^3 assay. The reproducibility achieved on the quality control samples demonstrates that MRM^3 analysis is compatible with validated bioanalysis.

	LLOQ	LQC	MQC	MHQC	HQC
Conc (ng/mL)	5.00	15.0	50.0	800	1800
	5.14	15.9	46.4	779	1650
	4.32	16.9	47.2	767	1549
	5.65	12.0	41.7	821	1521
	4.54	13.5	43.7	729	1641
	3.69	17.1	50.0	658	1745
	4.22	17.4	45.3	751	1672
Mean	4.59	15.5	45.7	751	1630
SD	0.701	2.22	2.85	54.8	82.4
CV	15.3%	14.3%	6.2%	7.3%	5.1%
RE	-8.2%	3.2%	-8.6%	-6.1%	-9.5%

References

1. MRM^3 Quantitation for Highest Selectivity in Complex Matrices. SCIEX Technical Note RUO-MKT-02-2739-A.

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