COMPARISON OF MRM AND MRM^3 FOR THE QUANTITATIVE DETERMINATION OF MISOPROSTOL ACID IN HUMAN PLASMA

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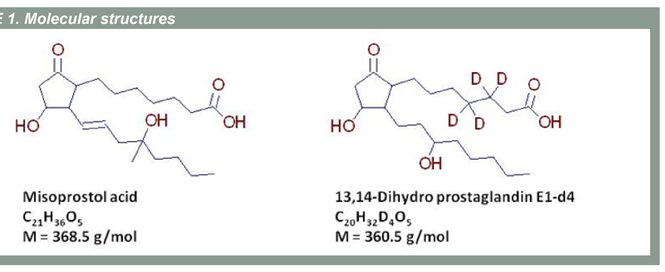
INTRODUCTION

Multiple-Reaction-Monitoring (MRM) is traditionally the dedicated sensitive and selective mode for quantitation using triple quadrupole MS/MS instruments. However, in complex matrices MRM selectivity may suffer from high background interferences making reproducible peak integration difficult. A triple quadrupole combined with linear ion trap (LIT) technology allows additional fragmentation in the Linear Accelerator Trap generating secondary fragments (MRM^3). Therefore MRM^3 provides the opportunity for increased selectivity and due to accumulation of secondary fragments sensitivity may be comparable to MRM mode.

Misoprostol acid (figure 1) is the biologically active metabolite of Misoprostol, a synthetic analogue of Prostaglandin E1 (PGE1) commonly used for the prevention and treatment of gastric ulcers. A highly sensitive Ultraperformance Liquid Chromatography - Tandem Mass spectrometry (UPLC-MS/MS) method was established in our laboratory for the determination of Misoprostol acid in human plasma down to 5 pg/mL. Misoprostol acid was extracted from human plasma by means of automated liquid-liquid extraction and quantified using d4-labeled 13,14-Dihydro prostaglandin E1 (figure 1) as internal standard. Analysis was performed on an AB Sciex Q-Trap® 5500 using traditional MRM mode for quantitation. All aspects of the bioanalytical method including sample preparation, chromatography and ionisation were explored during method development in order to optimize analyte sensitivity and selectivity.

FIGURE 1. Molecular structures



Whilst method performance using MRM was generally good, method robustness was found to be occasionally compromised by selectivity issues. In order to potentially overcome these issues an additional level of selectivity was added to the method using secondary fragmentation by MRM^3.

Both approaches to quantitation (MRM and MRM^3) for this analyte are compared with respect to sensitivity, selectivity, precision and linearity. The potential benefits and limitations of each approach are outlined and discussed.

METHOD SUMMARY

Human plasma (K2 EDTA) was fortified with 2.5% by volume of analyte solution containing Misoprostol acid in appropriate concentrations. Both the fortification and subsequent sample preparation (table 1) were performed on ice in order to minimize instability issues observed at room temperature. As no deuterated Misoprostol acid was available and Misoprostol acid is a closely related analogue of Prostaglandin E1, 13,14-Dihydro prostaglandin E1-d4 was considered appropriate to be used as an internal standard.

TABLE 1. Sample preparation

Extraction method	Liquid-liquid extraction (LLE) automated on Tomtec Quadra 96
Matrix	Human plasma (K2 EDTA)
Sample volume required	200 µL
Extraction solvent	1-Chlorobutane/Diethylether (80/10 v/v)
Analytical range	5.00 – 500 pg/mL
Quantitation	Peak area ratio
Regression model	linear, 1/concentration^2 weighting

Chromatographic separation (table 2) was achieved using a gradient elution. After an initial 1.4 min at 10% mobile phase B, it is increased to 90% over 3.5 min, kept at 90% for 0.5 min and then returned to initial conditions for 1 min in order to re-equilibrate the column. A neutral mobile phase system provided the best compromise between chromatographic resolution and sensitivity.

TABLE 2. Chromatographic conditions

UPLC-System	Acquity UPLC-System (Waters)
Analytical column	Acquity BEH C18, 100x2.1 mm, 1.7 µm (Waters)
Column temperature	50°C
Mobile phase A	2 mM Ammonium acetate in HPLC grade water (Baker)
Mobile phase B	2 mM Ammonium acetate in Methanol
Flow rate	0.430 mL/min
Injection volume	100 µL
Run time	6.5 min

An AB Sciex Q-Trap® 5500 mass spectrometer equipped with a Turbo Ion Spray source was used for analysis. Negative ions of analyte and internal standard were detected in both, MRM and MRM^3 detection mode. All quadrupole, LIT and ion source parameters were optimized to achieve best compromise between maximum sensitivity and appropriate selectivity (table 3).

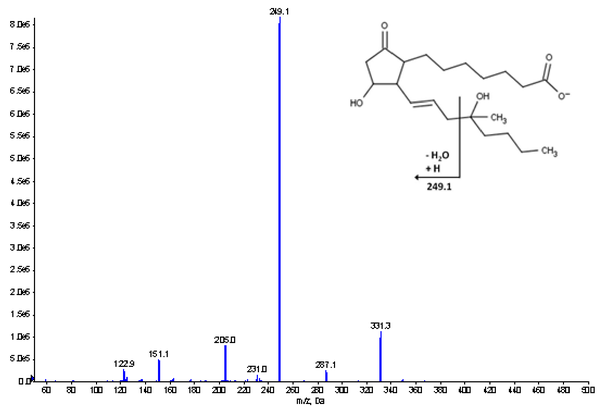
TABLE 3. AB Sciex Q-Trap® 5500 parameters

	AB Sciex Q-Trap 5500
Ion source / polarity	ESI / negative
CAD	5
CUR	25
TEM	600°C
Gas 1	50
Gas 2	50
Ion spray voltage	– 4200 V
	MRM
Transitions	367.1 / 249.1 (Misoprostol acid) 359.2 / 341.1 (IS)
Dwell time	200 msec
Resolution Q1 / Q3	unit / unit
	MRM^3
Transitions	367.1 / 249.1 / 101.17 – 201.17 (Misoprostol acid) 359.2 / 341.7 / 259.23 – 235.23 (IS)
LIT fill time	250 msec (Misoprostol acid) 100 msec (IS)
Resolution Q1 / Q3	unit / LIT
Scan rate	1000 Da/s
Q0 trapping	active

MASS SPECTROMETRY OPTIMIZATION

Mass spectrometry was optimized for both, MRM and MRM^3, using pure analyte solutions for identification of compound characteristic mass transitions. Figure 2 shows a typical product ion scan for negative ion precursor 367.1. The most abundant fragment was evident at 249.1.

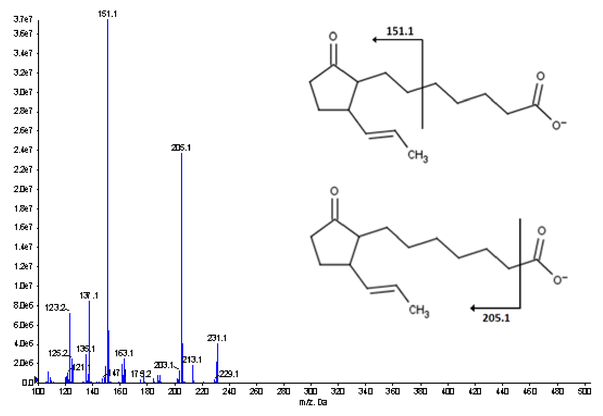
FIGURE 2. Product ion scan of Misoprostol acid



Primary product ion scan of Misoprostol acid (367.1) at a collision energy of –30 eV. Suggested fragmentation to give product ion at 249.1.

Secondary fragmentation of 367.1 / 249.1 was achieved within the LIT. As figure 3 illustrates two potential secondary fragments were identified. LIT parameters (such as scan rate, fill time, dynamic fill, Q0-trapping, mass range width) were optimized according to table 3 using replicate injections onto full chromatographic system. This is necessary as LIT parameters influence such factors as total signal and peak sampling time. Overall the best compromise between sensitivity and selectivity could be achieved at maximum fixed fill times with Q0 trapping activated and a center mass range width of +/- 50 Dalton. The use of dynamic fill time reduced the absolute response by a factor of 2 with no significant benefits to selectivity and precision.

FIGURE 3. MS/MS/MS scan of Misoprostol acid



Secondary product ion scan of Misoprostol acid (367.1/249.1) with proposed fragmentation pattern.

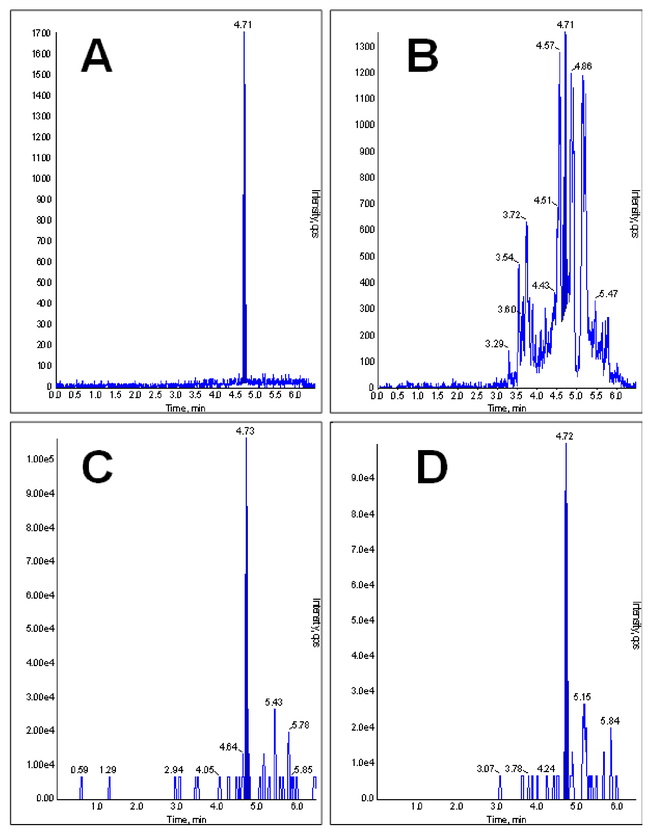
Further increases in sensitivity in MRM^3 mode could be achieved by lowering the Q1 resolution from unit to low or open. However the increase in absolute response (up to a factor of two) that could be achieved in pure solution was offset by a comparable decrease in selectivity in extracted matrix samples.

Likewise the alternate secondary fragment of 205.1 was found to be nonselective in matrix extracts. Whilst its absolute response was comparable to the fragment 151.1, a consistent interference peak was observed at the retention of analyte even in matrix blank injections.

EXPERIMENTAL DATA

In order to compare the performance of both quantitation modes a set of human plasma extracts was prepared containing replicate numbers of standards, quality control samples and blanks allowing the assessment of sensitivity, linearity, precision, accuracy and selectivity. Detection was performed in MRM and MRM^3 modes using respective optimized conditions as described in table 3. In order to ensure absolute comparability the same set of samples was injected in subsequent runs using identical UPLC conditions. Representative example chromatograms are shown in figure 4. Method performance results are presented in table 4.

FIGURE 4. Example chromatograms in MRM and MRM^3 modes



A: LLOQ, 5.00 pg/mL Misoprostol acid in pure solution using MRM mode, B: LLOQ, 5.00 pg/mL Misoprostol acid in extracted Human plasma using MRM mode, C: LLOQ, 5.00 pg/mL Misoprostol acid in pure solution using MRM3 mode, D: 15.0 pg/mL of Misoprostol acid in extracted Human plasma using MRM3 mode

TABLE 4. Mean accuracies and precision of spiked quality control samples

Parameter	Type of QC and spiked level (pg/mL)				
	PS LLOQ*	LLOQ	Low	Medium	High
	5.00	5.00	15.0	75.0	400
			MRM		
Mean accuracy (%)	–	97.0	89.5	107.9	86.5
Precision (CV%)	4.7	19.9	5.9	1.7	2.8
n	6	6	6	6	6
			MRM^3		
Mean accuracy (%)	–	133.6	84.5	98.2	83.6
Precision (CV%)	11.6	52.9	10.8	15.3	7.8
n	6	6	6	6	6

* LLOQ in pure solution

DISCUSSION

Figures 4A and 4B illustrate the selectivity issues observed for the assay when using MRM mode for quantitation. Analysis of pure solution (containing analyte and internal standard at plasma extract equivalent concentrations) provided excellent sensitivity in both, absolute and relative response (S/N at LLOQ = 40) with good precision (CV = 4.7%). However, plasma extracts performed significantly worse as accurate automatic integration of the target peak is complicated by additional background peaks surrounding the peak of interest. Whilst the absolute response remains comparable, an accurate assessment of the signal to noise is hardly possible. Furthermore inconsistent peak integration leads to significant loss in precision at low concentration levels (CV = 19.9% at LLOQ).

As figures 4C and 4D demonstrate MRM selectivity issues can be significantly reduced by applying optimized MRM^3 conditions. However several constraints on method performance must be noted. At first, the overall baseline noise was considerably reduced showing almost no interfering peaks within the surrounding area of the peak of interest. This certainly contributes to accurate peak identification and consistent peak integration. However this improvement in selectivity was offset by a significant impact on overall sensitivity with associated loss of precision at low concentrations. (Note that the determination of signal to noise becomes difficult in MRM^3 mode since no true background noise is detected. Precision therefore becomes the only parameter available to determine appropriate LLOQ sensitivity). In fact in this example the precision at all concentrations is reasonably affected when compared to corresponding MRM mode results. As a result the actual LLOQ had to be raised up to 15.0 pg/mL when using MRM^3 mode for quantitation.

It is interesting to note that the observed loss in sensitivity and precision only occurred in matrix samples whilst pure solutions at corresponding concentration levels gave acceptable results in both, signal and CV. The reason for this observation has not been explored to date, although potential impacts of disturbing matrix components cannot be excluded.

CONCLUSION

MRM^3 quantitation provides a promising alternative to traditional MRM quantitation whenever selectivity issues cause complications to accurate peak integration. Nevertheless potential constraints must be taken into account, especially as outlined within this poster a loss in sensitivity and overall method precision.

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