Quantitation and Identification of the Pesticide Malathion in Fruit Samples using MRM^3 Quantitation

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Introduction

LC/MS/MS instruments operating in Multiple Reaction Monitoring (MRM) are widely used for targeted quantitation on triple quadrupole and hybrid triple quadrupole linear ion trap (QTRAP®) systems because of their well known selectivity and sensitivity. In MRM mode the first quadrupole (Q1) filters a specific precursor ion; the collision cell (Q2) generates fragments (product ions) which are filtered in the third quadrupole (Q3). Although this double mass filtering greatly reduces noise there is always a chance that elevated background levels or matrix signals interfere with the targeted analyte.

One possibility of improving quantitative results is using a more selective detection mode, such as MRM^3. When a QTRAP® System is operated in MRM^3 mode first Q1 filters the first precursor ion; than Q2 generates product ions which are trapped in Q3 operating as a linear ion trap (LIT). Afterwards the LIT isolates the second precursor ion and generates the second generation of product ions which are scanned out towards the detector. In comparison to MRM mode MRM^3 provides higher selectivity due to one additional fragmentation step. Both modes of operation are illustrated in Figure 1.

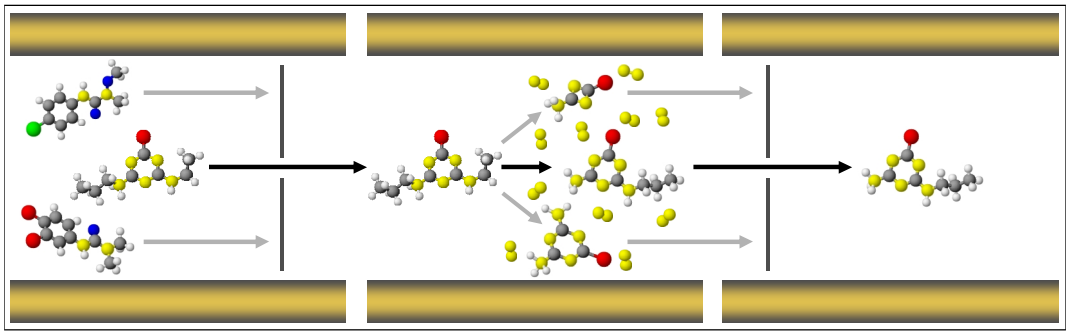
The novel QTRAP® 5500 system uses the new and patented Linear Accelerator™ Trap designed to reduce the fragmentation time and to increase the MS/MS/MS excitation efficiency delivering a whole new level of MRM^3 performance. This together with the high sensitivity of the mass spectrometer and faster scanning of up to 20000 Da/s allows performing MRM^3 with lower detection limits and with shorter cycle times than previous generations of QTRAP® Systems.

Method Details

- Ultra High Pressure Liquid Chromatography using a Shimadzu UFLC$_{XR}$ system with a Phenomenex Synergi Fusion-RP (2.5 µm) column and a fast gradient of water and methanol with ammonium formate buffer
- AB SCIEX QTRAP® 5500 System with Turbo V™ Source and ESI probe
- Experiment 1: detection of two MRM transitions with 100 ms dwell time
- Experiment 2: detection of two MRM^3 scans at 20000 Da/s with 20 ms fill time and 25 ms excitation time, total cycle time of only 0.33 s

The detected masses in MRM and MRM^3 mode with compound dependent parameters are shown in Figure 2.

Multiple Reaction Monitoring



MRM^3

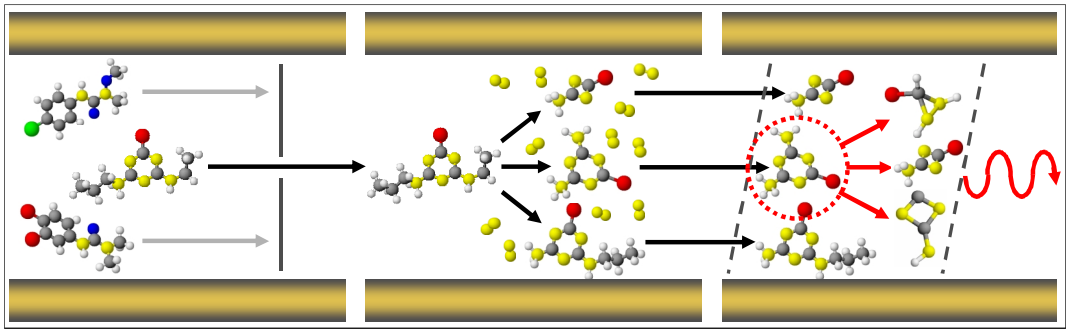


Figure 1. Principles of MRM and MRM^3 performed on a 5500 QTRAP® LC/MS/MS System

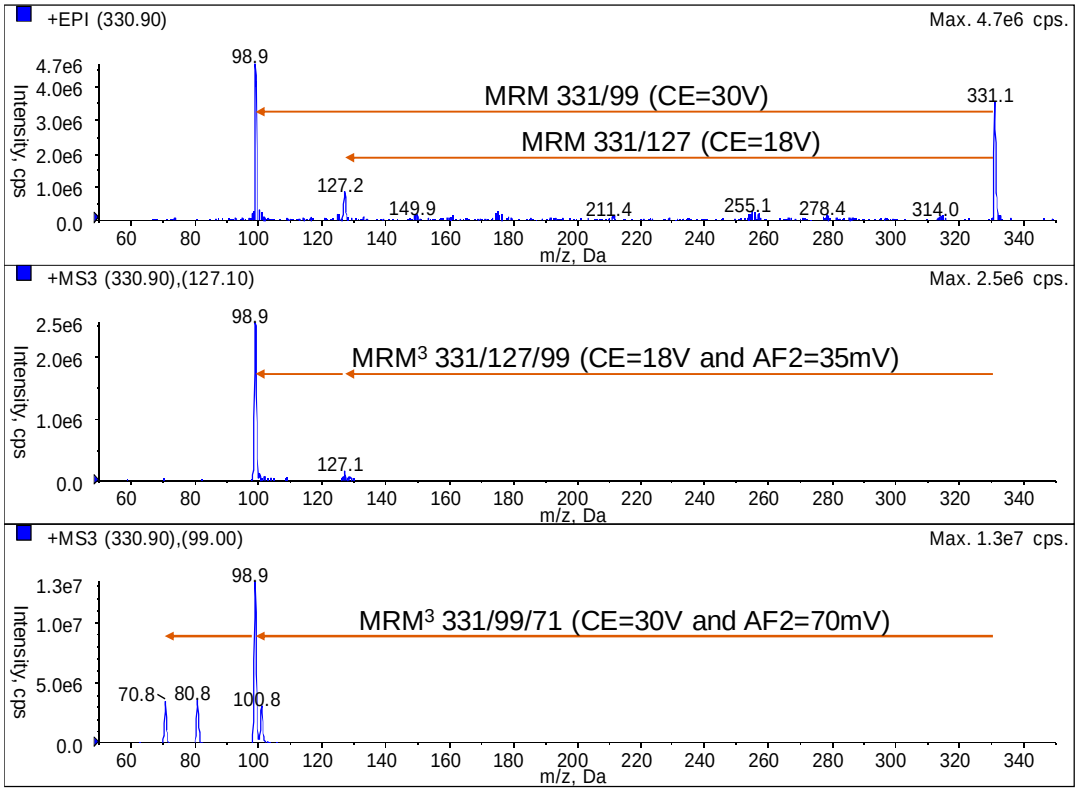


Figure 2. Detection of Malathion using the following MS experiments: MRM of 331/127 and 331/99 and MRM^3 of 331/127 quantifying the 2nd product ion 99 and 331/99 quantifying the 2nd product ion 71

Results

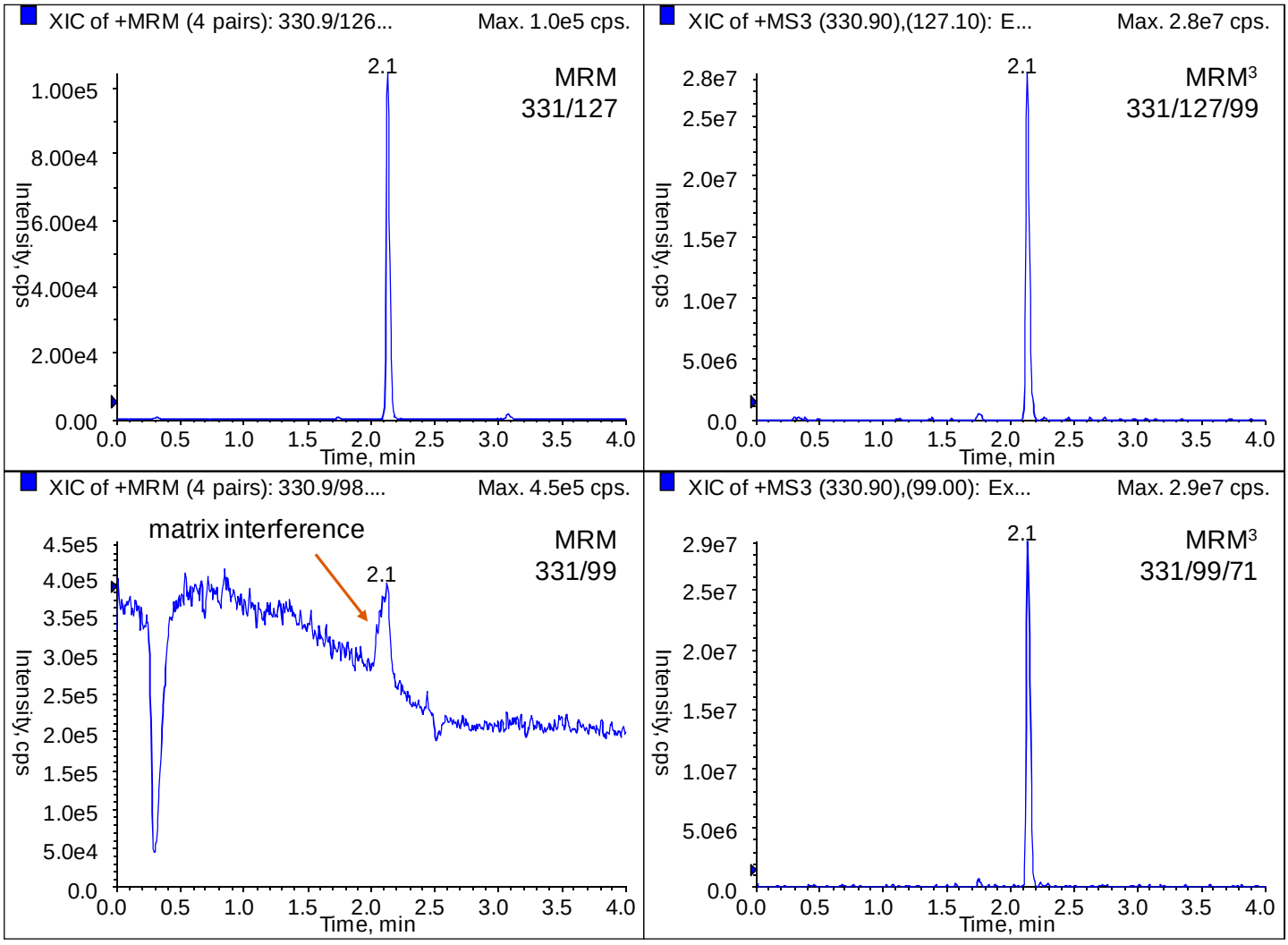


Figure 3: Comparison of selectivity detecting Malathion in apple extract (10 ppb) – while the MRM transition 331/127 showed expected selectivity the second transition 331/99 had an elevated background level and also matrix interference; in contrast both MRM^3 experiments showed superior selectivity

Selectivity

A homogenized apple was spiked with 10ppb Malathion, extracted using a QuEChERS procedure, diluted 50 times to minimize matrix effects, and analyzed by LC/MS/MS. The resulting chromatograms using two MRM transitions and two MRM^3 experiments are shown in Figure 3. The MRM transition 331/127 showed expected selectivity, while the second transition 331/99 had an elevated background level and also matrix interference. In contrast both MRM^3 experiments showed superior selectivity for reliable quantitation.

Sensitivity

Table 1 shows signal-to-noise ratios (S/N) of the chromatograms described above.

Table 1: Signal-to-noise ratios (S/N) of MRM and MRM^3 of 10 ppb Malathion in a 50 times diluted QuEChERS extract of apple

MRM	S/N	MRM^3	S/N
331/127	922	*331/127/99*	497
331/99	8 high background	*331/99/71*	147

The quantifier MRM and the quantifier MRM^3 had very similar sensitivity. Both experiments allowed quantifying Malathion at sub ppb levels. However, pesticide testing requires identification. Thus, a second MRM or MRM^3 signal has to be recorded to allow ratio calculation (qualifier/quantifier). In our example, the second MRM showed a dramatic loss in S/N over the qualifier MRM^3 signal because of the elevated background. In this case the quantitation and identification of Malathion in fruit matrix using MRM^3 was much more sensitive than in MRM mode.

Reproducibility

MS^3 on traditional ion traps and MRM^3 on older QTRAP® Systems suffer from its long duty cycles. Typically, this long cycle time results in lower accuracy and reproducibility. The QTRAP® 5500 System with the new Linear Accelerator™ Trap can perform MRM^3 experiments much faster than any other ion trap. For example, the method used in this study performed two MRM^3 scans with a cycle time of only 0.33 s, which allowed ~15 data points across the UHPLC peaks that had a base-to-base peak width of only 5.

Several fruit samples were fortified with 10 ppb Malathion and analyzed in replicates. While MRM detection suffered from matrix interference, MRM^3 resulted in more accurate and reproducible data. The %CV values in apricot, apple, pear, and orange were <5% with accuracies ranging from 90% to 110%. In addition, the MRM^3 ratio calculation clearly identified the presence of Malathion in the analyzed fruits.

Summary

LC/MS/MS methods were developed to quantify and identify the organophosphorus pesticide Malathion in fruit samples using the new QTRAP® 5500 LC/MS/MS System. These methods used traditional MRM and MRM^3 mode. Both methods were compared regarding selectivity, sensitivity, accuracy and reproducibility. The results show that the higher selectivity of MRM^3 eliminates background and matrix interference, resulting in better data quality. MRM^3 gave comparable data versus MRM for the quantifier signal but much better sensitivity, accuracy, and reproducibility for the qualifier signal in fruit matrix.

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