Quantitation and identification of the pesticide malathion in fruit samples using MRM^3 workflow

Using the QTRAP® 5500 System

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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. 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 to use a more selective detection mode, such as MRM^3 workflow[1]. In comparison to MRM mode, MRM^3 often provides higher selectivity due to one additional fragmentation step, effectively reducing interferences and improving quantitation.

Improved selectivity for quantitation

Here, a homogenized apple was spiked with the organophosphorus pesticide Malathion (10 ppb), then 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 1. 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. Table 1 shows signal-to-noise ratios (S/N) of the chromatograms described above.

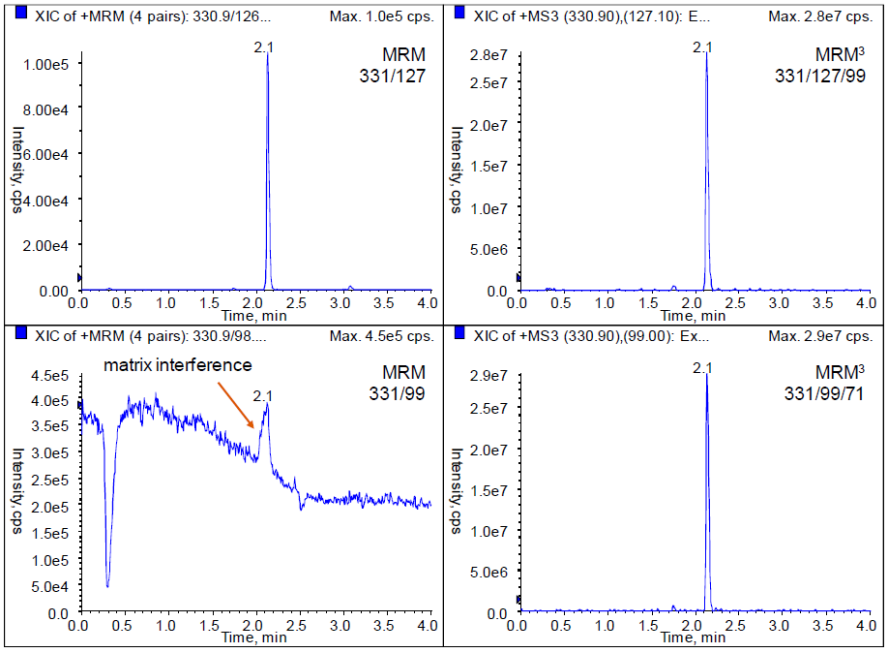

Figure 1: Comparison of MRM vs MRM^3 for detecting malathion (10ppb) in apple extract. (Left) While the MRM transition 331/127 showed expected selectivity, the second transition 331/99 had an elevated background level and also matrix interference. This results in incorrect ion ratios and prevents confident identification. (Right) In contrast, both MRM^3 experiments showed minimal interferences and higher selectivity.

Improved confirmation

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 this example, the second MRM showed a dramatic loss in S/N 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.

Methods

Chromatography: Separation was performed using a Shimadzu UFLCXR system with a Phenomenex Synergi Fusion-RP (2.5 µm) column and a fast gradient of water and methanol with ammonium formate buffer.

Mass spectrometry: A looped MS experiment was performed using the SCIEX QTRAP 5500 System with Turbo V™ Source and ESI probe. The first experiment consisted of two MRM transitions of 100 msec dwell time each. The second experiment was two MRM^3 scans using a scan speed of 20000 Da/s with 20 msec fill time and 25 msec excitation time. Total cycle time of only 0.33 sec which allowed ~15 data points across the UHPLC peaks that had a base-to-base peak width of only 5 sec. The detected masses in MRM and MRM$_3$ mode with compound dependent parameters are shown in Figure 2.

Data processing: Data was processed with MultiQuant™ Software.

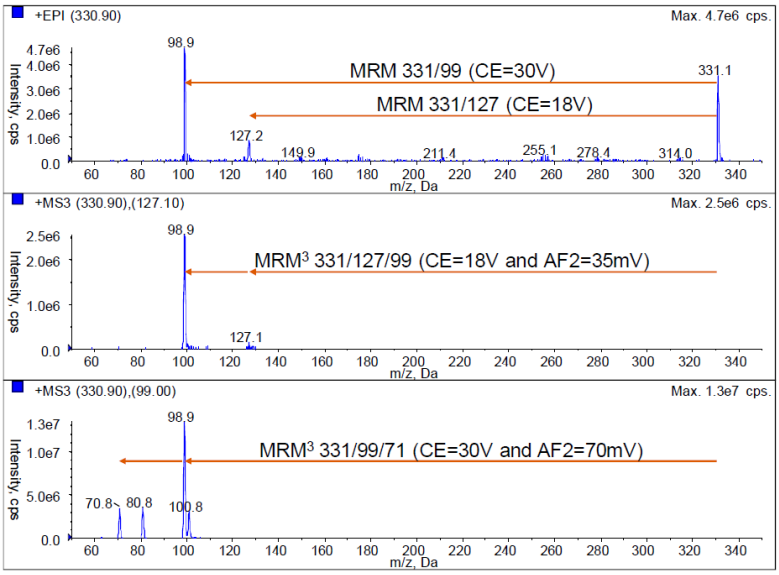

Figure 2. Developing the MRM and MRM^3 workflow methods. (Top) Full scan MS/MS of Malathion shows two fragments at m/z 127 and 99 for use in the MRM experiment. (Middle) MS/MS/MS of 331/127 shows a fragment at m/z 99 for use in the first MRM^3 experiment. (Bottom) MS/MS/MS of 331/99 shows a fragment at m/z 71 for use in the second MRM^3 experiment.

Improved reproducibility

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.

Table 1. Improvements in detection Using MRM^3 Workflow. Signal-to-noise ratios (S/N) of MRM and MRM3 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**	*331 / 127 / 71*	*147*

****** High background – see Figure 2***

Summary

LC-MS/MS methods were developed to quantify and identify the organophosphorus pesticide Malathion in fruit samples using the QTRAP® 5500 System. The traditional MRM and MRM^3 modes 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.

References

1. MRM^3 quantitation for highest selectivity in complex matrices. SCIEX Technical Note RUO-MKT-02-2739-A.

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