

Comparison of two multi-residue methods for simultaneous quantitative screening and qualitative confirmation of 90 pesticides using the 4000 Q-TRAP LC/MS/MS system

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Introduction

The use of a new hybrid quadrupole/linear ion trap known as the QTRAP™ combines the capabilities of a triple quadrupole mass spectrometer and ion trap technology on a single platform. Product ion scans are conducted in a hybrid fashion with the fragmentation step accomplished via acceleration into the collision cell followed by trapping and mass analysis in the Q3 linear ion trap [1]. Furthermore, the instrument allows to confirm the detected compound by using Information Dependent Acquisition (IDA) of Enhanced Product Ion scans (EPI) triggered by Multiple Reaction Monitoring.

The Multiple Reaction Monitoring (MRM) mode is preferred scan type for multi-residue method. However, at least two MRM transition are required for positive confirmation of the pesticide residues. Typically, one MRM transition is used for quantification and the second transition is used to confirm analytical results. Therewithal, the ratio of transitions has to pass the criteria defined by the document N° SANCO/2007/3131 [2].

Our multi-residue method originally with two MRM transitions for each compound has been modified by replacing of the second (confirmation) MRM transition with an enhanced product ion scan (EPI). The Information Dependent Acquisition (IDA) parameters decide whether an MRM transitions trigger and EPI of the precursor ion. The EPI spectra were recorded with collision energy spread (CES) instead of three spectra with different collision energies (CE) [3]. The MS/MS library, contains CES spectra of 90 pesticides in positive mode, was established for identification unknown compounds.

This poster presents the implementation and application possibilities of the Information Dependent Acquisition on a hybrid quadrupole-linear ion trap mass spectrometer (4000 QTRAP™) to maximize data collection in pesticide analysis.

Overview of the Method Development

- **Aim:** Development of an LC-MS/MS screening method for 90 pesticides in samples of fruit and vegetables with library searching using a QTRAP™ 4000.
- Optimization of MRM and IDA settings (IDA threshold, selection of 90 MRM transition)
- Development of and MS/MS library from spectra obtained using of "collision energy spread" (CES) with positive ESI.
- Application to fruit and vegetable samples and comparison with our previously developed method with two MRM transition for each compound.

Conclusion

- The QTRAP™ mass spectrometer demonstrated powerful capabilities in generating MS/MS spectra for pesticides when operated in the Information Dependent Acquisition (IDA) mode.
- The method operated in the IDA mode was evaluated as a potential tool for extending the list of pesticides included in the method. Using one transition for each analyte in combination with scan speeds of 4000 amu/sec allow to reduce the scan cycle approximately about 25% in comparison to the method with two MRM transitions for each analyte. Thus, the amount of compound could be increased and the amount of data points across a peak stay within criteria defined by the document N° SANCO/2007/3131.
- The reliability of the IDA method to produce comparable and reproducible EPI spectra was tested on the group of matrix-matched standards. This experiment showed that the amount of correctly matched pesticides depends on the concentration level and is almost unaffected by matrix. The compounds that were not matched were sorted out the list of requested EPI scans. The confirmation of these compounds in a samples was based on two MRM transitions. The final method has been developed as a combination of IDA method and method using two MRM transitions for minor group of previously selected compound.

References

- [1] Hager, J. W.; Leblanc, Y., *Journal of Chromatography A*. 1020 (2003) 3-9
- [2] Method validation and quality control procedures for pesticide residues analysis in food and feed. Document No. SANCO/2007/3131
- [3] Application Note 3200 QTRAP™ LC/MS/MS System. <http://www.appliedbiosystems.com>

Methods and Experiments

Liquid Chromatography/Mass Spectrometry:

• **Instrument:** Applied Biosystems 4000 Q TRAP® LC-MS/MS System with TURBO V™ source, Agilent 1200 LC Binary SL Pump, Agilent 1200 G1367C High Performance Autosampler SL, Agilent 1200 Thermostatted Column Compartment SL.

• **HPLC Conditions:** Column: Discovery® C18 column (15 cm × 2.1 mm, 5 µm); Column oven temperature: 35 °C; Injection volume: 10 µL; Flow rate: 0.2 mL/min; Solvent A: 0.1% HCOOH + 2 mmol NH₄COOH; Solvent B: methanol; LC gradient: 0 min: 80% of solvent A + 20% of solvent B, 3 min: 37% A + 63% B, 18 min: 5% A + 95% B, 18 - 31 min: 5% A + 95% B, 31 min: 80% A + 20% B.

• **MS Parameters:**

• **Parameters common for both method:**

ESI positive, Curtain gas: 25 psi, IonSpray Voltage: 5500 V, Temperature: 550 °C, Ion Source Gas1 (GS1): 60 psi, Ion Source Gas2 (GS2): 60 psi, Dwell time: 5, 10 or 20 ms

• **Original method with two MRM transition for each compound:**

Collision gas pressure: Medium; Pause between mass ranges: 1 ms

• **Method with Enhanced Product Ion Scan mode (EPI):**

Survey scan: MRM, one transitions per analyte; Collision gas pressure: High

Dependent scan: EPI with CES 35±15 eV; dynamic fill time (DFT); mass scan range of 65-520 amu using a scan rate of 4000 amu/sec

IDA-criteria: EPI triggered for MRM transitions with intensity exceeding 5000 cps

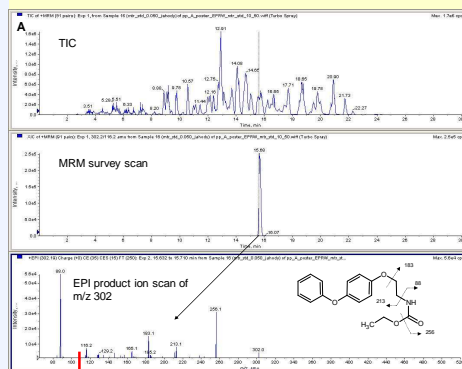


Figure 1A. IDA chromatogram of matrix-matched standard of strawberry with concentration 50 ng/mL.

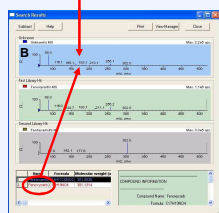


Figure 1B. Detail match results for fenoxycarb presented in matrix-matched standard.

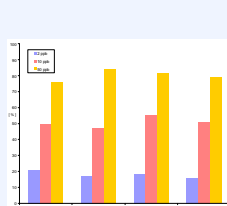


Figure 2. The relationship between the concentration level of four matrix-matched standards of pesticides and amount of pesticides correctly matched against the library expressed in %.

Table 1. Pesticides sorted by the ability to produce EPI spectra

Pesticides without the library entry	Aldicarb-sulfonide, Aldicarb, Bifenox, Demeton-S-methyl, Diethofencarb, Disulfoton, Methidathion, Oxamyl, Terbufos, Trifluralin
Pesticides without library match at any tested levels	3-hydroxycarbofuran, Aldicarb-sulfone, Etofenprox, Lufenuron, Methiochlorproprate, Metolachlor, Pendimethalin, Picoxystrobin, Pymetrozine, Terbufos-sulfonide, Thiodicarb
Pesticides matched at all tested levels	Acetamiprid, Carbendazim, Chloroxuron, Dimethomorph, Fenprophate, Fensulfoton, Fluoxastrobin, Imazalil, Mepronil, Methiochlorproprate, Panoxazole, Pymethanil, Spirocycloproprate, Tebuconazole, Tebufenpyrad, Tetraconazole, Thiabendazole, Triamizole
Pesticides matched at the level 10 and 50 ng/mL	Buprofezin, Cadusafos, Carbofuran, Cyproconazole, Cyprodinil, Diniconazole, Disulfoton-sulfonide, Fenhexamid, Fensulfoton-PO-sulfone, Flusilazole, Hexaconazole, Mepanipyrim, Metamitrol, Linuron, Pitracarb, Piflufenfos, Propiconazole, Pyraclostrobin, Pyriproxyfen, Pyrimorfen, Trifloxystrobin, Vamidothion
Pesticides matched only at the level 50 ng/mL	Azoxystrobin, Carbaryl, Chlorpyrifos, Chlorpyrifos-methyl, Clifentazine, Dicrotophos, Dimoxystrobin, Epoxiconazole, Fenoxycarb, Flufenoxuron, Heptylthiazox, Imidacloprid, Kresoxim-methyl, Methiochlorproprate-sulfone, Phorate-sulfonide, Prochloraz, Pyridaben, Terbufos-sulfone, Thiocloprid, Vamidothion-sulfonide

• **Sample preparation:** 1 kg or 10 pieces of sample were minced and homogenized with dry ice; 10±0.1 g of homogenized sample was blended with 50 mL acetonitrile for 2 min (in case of recovery sample, 1 mL of standard solution was added prior addition of acetonitrile); filtered and filtration paper rinsed with 20 mL of fresh portion of acetonitrile; the supernatant was gently dried under low pressure (140 mbar, 40 °C); residue in distillation flask was redissolved in 100 mL of CH₂Cl₂/acetone (4/1 v/v); intensive shaking (3 min) with 50 mL of buffer (pH = 8); lower layer was collected and upper water layer reextracted with 80 mL of fresh portion of CH₂Cl₂/acetone (4/1 v/v); collected lower layers were combined and gently dried under low pressure (350 mbar, 40 °C); residue after solvent removal was redissolved in 10 mL of methanol/water (1/1 v/v) and filtered through a syringe filter (PTFE, 0.45 µm).

Q-TRAP Information Dependent Acquisition (IDA)

Samples of food and vegetables were analyzed using a Q-TRAP mass analyzer with MRM triggers information-dependent acquisition (IDA). When the signal from an MRM transition reaches a specified threshold (5000 cps), it triggers a dependent acquisition of an enhanced precursor ion scan (EPI). The EPI spectrum obtained with collision energy spread (35 ± 15 eV) provides maximum information in a single scan, by collecting product ion generated by 20, 35 and 50 eV. The acquisition time is as long as the time for one spectrum with a single collision energy.

The Library

For setting up the library, the pesticide mixture with concentration of 100 ng/mL was injected on column and MRM triggered EPI spectra of the precursor ions were collected with collision energy spread (35 ± 15 eV). The spectra corresponding with the precursor ion and collected at previously known retention time was assigned to individual pesticide and used for setting up the library.

Results and Discussion

To detect the presence of pesticides and their metabolites an Information Dependent Analysis (IDA) method was used. Figure 1A shows target analysis of pesticides using an MRM survey scan that were followed by an Enhanced Product Ion (EPI) experiment with Dynamic Fill Time (DFT) for structural elucidation and confirmation of any pesticides. Dynamic Fill Time (DFS) is a software selectable option to determine the optimal fill time for linear ion trap to ensure high data quality over a wide dynamic range. The EPI scan was performed at 4000 amu/s. The MRM survey scan was based on the quantification transition with the same dwell times adopted from original method with two MRM transition for each compound. The cycle time for these 90 MRM transition was 1 second and in combination with the EPI scan with fast scan rates all of the required information could be collected within an 1.4 seconds long cycle time. This value is approximately about one-quarter shorter than the scan cycle necessary for the simultaneous measurement of 180 MRM transitions in the original method. This two seconds long scan cycle could be considered to be the limiting because for approximately 30 seconds wide peaks ensure the amount of data points across the peak within the criteria defined by the document N° SANCO/2007/3131 [2].

The library was set up by collecting previously identified EPI spectra obtained with collision energy spread (35 ± 15 eV) into a library that is used to confirm pesticide presence. Query spectra are compared to references in the library to find the ones that are most similar (see Figure 1B). The library was created on the base of injection of standard mixture of pesticides with concentration 100 ng/mL. Only seven pesticides did not provide utilizable EPI spectra for setting up the library.

To evaluate the capability of the IDA method with EPI scan, this method was tested on matrix-matched standard prepared on three concentration levels 2, 10 and 50 ng/mL which were prepared from lettuce, mandarin, flour and baby food. The evaluation was based on the amount of pesticides that produced the EPI spectra utilizable for comparison with the library. The results are shown in Figure 2 and Table 1. Compounds that were not matched either are producing the useless EPI spectra (due to low fragmentation and/or low concentration) for comparison with the library or the parameters of comparison were outside criteria limit (Fit < 70). On the base of this experiment, the method has been modified and for these compound two MRM transition was used instead of the collecting of EPI spectra. Therefore, the final method is based on the collecting EPI spectra in combination with two MRM transition for selected compounds. This method is used for routine analysis.