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Comparison of tandem-in-space and tandem-in-time mass spectrometry in gas chromatography determination of pesticides: application to simple and complex food samples

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Abstract

Gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) is one of the most powerful techniques in pesticide residue analysis. MS/MS can be conceived in two ways: tandem in space (e.g. triple quadrupole, QqQ) or in time (e.g. ion trap, IT). QqQ and IT are commonly interfaced to GC; however, there has not been any direct comparison between them in pesticide residue analysis so far. In the present work, the performance of GC coupled to these two analyzers (GC-QqQ-MS/MS and GC-IT-MS/MS) was studied and compared for pesticide residue analysis, as well as its application in food analysis. The large volume injection (LVI) technique together with programmed-temperature vaporization (PTV) was applied. For this purpose, 19 pesticides, including organochlorine and organophophorus pesticides and pyrethroids, were analyzed in both systems. Mass spectrometric data, performance characteristics (linearity, intra-day and inter-day precision) and the influence of the matrix nature on the analysis of low concentrations were compared. The target compounds were analysed in solvent and in two representative food matrices such as cucumber (high water content) and egg (high fat content). MS data and intra-day precision was similar in QqQ and IT, whereas inter-day precision was significantly worse in QqQ. Linearity (expressed as determination coefficient, R^2) in the range 10-150 µg L^{-1} was adequate in both systems; however, better R^2 values were obtained with the QqQ analyzer in high and low concentration ranges (1-50 and 1-750 µg L^{-1}, respectively). The influence of the matrix nature on the analysis of low concentrations of each analyzer was also evaluated. The QqQ and IT performance was similar in cucumber and solvent. However, the QqQ provided better sensitivity in egg working in selected reaction monitoring (SRM).

Keywords: Triple quadrupole; Ion trap; Tandem in space; Tandem in time; Mass spectrometry; Pesticide; Food analysis

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1. Introduction

A few years ago, the determination of pesticides in complex samples was usually carried out by gas chromatography (GC) coupled to classical detectors, such as electron capture detection (ECD) [1-3] or nitrogen-phosphorus detection (NPD) [4-6]. In spite of their selectivity, these detectors are often subject to matrix components, and do not provide unequivocal confirmation of the identity of the compound. Nowadays, the use of more selective detectors in GC, such as mass spectrometry (MS) detectors is widely extended due to their advantages in comparison with the classical detectors. The use of MS detectors in the selected ion monitoring (SIM) mode [7] allows reducing background noise, although this mode does not eliminate matrix interferences in all cases. Certain MS detectors can perform tandem mass spectrometry (MS/MS), which provides higher selectivity and sensitivity. This increase in selectivity permits the analysis of the target compounds without the need of obtaining complete chromatographic resolution between these ones and matrix components, even at trace levels in complex samples. As a consequence, sample pre-treatment procedures can be minimized or even removed, depending on the matrix nature.

The applicability of GC-MS/MS for the determination of pesticide residues at trace levels in complex food samples, such as vegetables or fatty foods, has been widely demonstrated in a number of manuscripts [8-13]. In addition, international guidelines for pesticide residues analyses in food [14] indicate that confirmatory methods giving structural information about target analytes are requested. In this sense, GC-MS/MS performs the simultaneous identification, confirmation and quantification of the compounds and, therefore, it is a powerful technique in trace analysis.

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MS/MS basically consist in two stages of mass analysis: the first one involves the selection of a precursor ion, after the ionization of the molecules either with a dissociation process or a chemical reaction. In the second stage, the fragments (product ions) obtained by collision induced dissociation (CID) of the precursor ion with an inert gas (helium or argon) are analysed. These steps can be carried out by either tandem in time (by performing a sequence of events in an ion storage device) or in space (by coupling two different instruments). In tandem in-time instruments, the different MS/MS stages are carried out successively inside the same physical space but separately in time. On the contrary, tandem in-space instruments involve two mass analyzers in series, thus the different stages of the process occur sequentially in separate physical regions.

Two of the most common MS/MS analyzers used in pesticide residue analysis are the ion trap (IT) and the triple quadrupole (QqQ) analyzer. IT and QqQ analyzers are representative of MS/MS in time and MS/MS in space, respectively. Both instruments present advantages and disadvantages. Briefly, among the main advantages of the IT are the possibility of performing multiple MS experiments to obtain higher order MS^n spectra and its better sensitivity in scan mode. However, some limitations of the IT (depending on the manufacturer) are its inability to trap ions below *m/z*

50, the possibility of interfering side reactions owing to all reactions which occur in the same space [15], or the fact that the product ion scan is the only available MS/MS mode. On the contrary, the QqQ can operate in the four MS/MS modes: product ion scan, precursor ion scan, neutral loss scan and selected reaction monitoring (SRM). This last mode is faster than the product ion scan (used in IT) and it permits the simultaneous measurement of high number of MS/MS transitions or reactions (up to a number of 70-80 transitions per time segment, mainly depending on the dwell time and chromatographic separation) providing chromatographic peaks with adequate number of scans [16-18]. In practice, this scan speed implies that the number of target compounds which can be simultaneously determined by the QqQ in SRM is about 25-30 [17, 19], whereas this number is about 3 times lower in the case of IT (7-8 compounds) [20, 21]. Nowadays, this is a very important issue in routine applications when choosing and developing an analytical method since the instrumental method speed allows increasing the sample throughput in laboratories.

In spite of both GC-QqQ-MS/MS and GC-IT-MS/MS successfully determine pesticide residues in food samples, there has not been any direct comparison between them in this field. In fact, there are only a few studies which compare them in the analysis of alkylphosphonates [22] and muramic acid [23]. Therefore, the aim of this study is to compare the performance of these two systems for the analysis of 19 pesticide residues with very different properties, including organochlorine (OCPs) and organophophorus (OPPs) pesticides and pyrethroids, as well as its application in food samples, such as cucumber and egg. This comparison also includes the use of the large volume injection (LVI) technique together with the application of programmed-temperature vaporization (PTV), combination commonly used in trace analysis as injection technique. We present the results of this comparison with respect to the mass spectral characteristics, precision (intra-day precision and inter-day precision), linear range and influence of the matrix nature on the analysis of low concentrations for the compounds under study. Finally, the most appropriate routine application of each instrument is also described, according to the obtained results.

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2. Experimental

2.1. Reagents and chemicals

Acrinathrin, bifenthrin, buprofezin, chlorfenvinphos, chlorpyriphos ethyl, cyfluthrin, cypermethrin, deltamethrin, endosulfan α, endosulfan β, endosulfan sulphate, ethion, fenthion, isocarbophos, isofenphos, isofenphos methyl, malathion, parathion ethyl and parathion methyl standards as well as the internal standard (IS), caffeine, were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Riedel-de-Haën (Seelze-Hannover, Germany); purities were always > 96.0 %, except for cypermethrin (91.0 %). Acetonitrile (AcN) and *n*-hexane were supplied by J.T. Baker (Deventer, The Netherlands); ethyl acetate (EtAc), cyclohexane, and

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dichloromethane were supplied by Riedel-de Haën; acetone was provided by Fluka (Steinheim, Germany), and methanol was supplied by Sigma–Aldrich (Steinheim, Germany), always in residue analysis grade. Stock standard solutions of individual compounds (with concentrations between 250 and 500 µg L^{-1}) were prepared by exact weighing of the powder or liquid and dissolution in 50 mL of acetone; these solutions were stored under refrigeration (T ≤ 5ºC). A multi-compound working standard solution (2 µg L^{-1} concentration of each compound) was prepared by appropriate dilutions of the stock solutions with acetone and stored in a fridge (T ≤ 5ºC). A caffeine working solution at a concentration of 20 µg L^{-1} was prepared by dilution of the corresponding volume of the stock solution with acetone. An additional caffeine solution at a concentration 500 µg L^{-1} was prepared by exact weighing of the powder and dissolution in 50 mL of cyclohexane. Reagent-grade anhydrous sodium acetate (NaAc, 99.5 %), magnesium sulphate ($MgSO_4$, 97.0 %) and glacial acetic acid (HAc, 99.5 %) were purchased from Panreac (Barcelona, Spain). Preparative-grade (100 g, bulk) C18-bonded silica material with 40-µm particle size, 18 % carbon load and end capped, 12-mL reservoirs for solid phase extraction (SPE) and polyethylene frits were provided by Varian (Harbour City, CA, USA). Florisil sorbent with 150–250 µm particle size, 60–100 mesh (250 g, bulk) and pesticide residue grade was obtained from Fluka. Primary secondary amine (PSA) bonded silica (100 g, bulk) was supplied by Supelco (Bellefonte, PA, USA).

2.2. Sample procedure

Vegetable samples: The method based on the QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction reported by J.L. Fernández-Moreno *et al.* [19] was applied. Briefly, an aliquot of homogenized sample (10 g) was exactly weighed into a 50-mL Teflon tube. Then, AcN with 1% HAc (10 mL) was added; the tube was sealed and vigorously shaken for 1 min. Next, anhydrous NaAc (1 g) and anhydrous $MgSO_4$ (4 g) were added and the tube was shaken again. The sample was centrifuged at 4000 rpm (2750 g) for 6 min in a high-volume centrifuge. An aliquot of the upper layer (1 mL) was placed in a 2.0-mL Eppendorf safe-lock micro test tube containing PSA (25 mg) and anhydrous $MgSO_4$ (150 mg). The tube was sealed and carefully shaken before being centrifuged at 6000 rpm (3500 g). Finally, the clean extract (800 µL) was transferred into a 2-mL GC vial to be taken to dryness under a gentle nitrogen stream. The residue was re-dissolve with a cyclohexane solution (400 µL) which contained the IS (500 µg L^{-1}).

Egg samples: The method developed by Plaza-Bolaños *et al.* [16] was used in the preparation of egg extracts. Briefly here, 0.5 g of homogenized egg sample was weighed in a glass mortar. Next, C18 sorbent (2.0 g) and anhydrous $MgSO_4$ (1.0 g) were added. The sample was blended using a

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glass pestle with moderated pressure and transferred into a 12-mL SPE reservoir containing activated Florisil (2.0 g). The mixture was fitted with a glass bar and a frit was placed on top. The elution of the cartridges was performed with 1.5 mL of AcN saturated in n-hexane and 8.5 mL of EtAc in a SPE vacuum manifold. The extracts were collected in 10-mL test tubes and evaporated to near dryness with a nitrogen stream. The residue was re-dissolved with 975 µL of cyclohexane and 25 µL of the IS working solution (20 µg L^{-1}).

2.3. Instrumentation

A Varian 3800 gas chromatograph (Varian Instruments, Sunnyvale, CA, USA) equipped with electronic flow control (EFC) and cryogenic cooling with carbon dioxide (CO_2, 99.9 %) was coupled to a Varian 1200L QqQ mass analyzer. Samples were injected with a Combi Pal (CTC Analytics AG, Zwingen, Switzerland) into a 1079 split/splitless septum-equipped programmable injector (SPI) which operated in the LVI technique, using a 100 µL syringe. The QqQ mass spectrometer was operated in electron ionization (EI, 70 eV). The QqQ analyzer was calibrated with perfluorotributylamine every three days. After the ionization process, ions were passed through a hexapole ion guide to the mass analyzers (mass range from *m/z* 10 to 1500). The curved collision cell presented a 180º degree path. Helium (99.9999 %) at a flow rate of 1 mL min^{-1} was used as carrier gas. Argon (99.999 %) was used as collision gas.

Another Varian 3800 gas chromatograph also equipped with EFC and the aforementioned injector was coupled to a Saturn 2000 IT mass analyzer. Samples were injected with a Varian 8200 auto-sampler with a syringe of 100 µL, operating in the LVI. The IT mass analyzer also operated in EI (70 eV) and it was weekly calibrated with perfluorotributylamine. Helium (99.9999 %) at a flow rate of 1 mL min^{-1} was used as carrier and collision gas.

In both instruments, GC-QqQ-MS and GC-IT-MS, the glass liner was equipped with a plug of Carbofrit (Resteck Corp., Bellefonte, PA, USA) and a fused silica untreated capillary column (2 m × 0.25 mm i.d.) from Supelco. This column was used as guard column connected to a Factor Four VF-5ms analytical column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) from Varian Instruments. The computers which controlled both systems held an EI-MS-MS library specifically created for the target analytes under the experimental conditions. Other EI-MS libraries were also available. Varian Workstation software was used for instrument control and data processing.

Samples were chopped and homogenised using a kitchen blender from Braun MX32 (Barcelona, Spain). An analytical balance AB204-S from Mettler Toledo (Greifensee, Switzerland) was used to weigh samples and powder reagents. A multi-tube shaker (12 tubes) Multi Reax from Heidolph

(Nürnberg, Germany) and a minishaker from IKA-Works (Wilmington, NC, USA), were used to shake the 50-mL Teflon and 2-mL Eppendorf tubes. Centrifugations were performed in two different apparatus: a high-volume centrifuge equipped with a bucket rotor (4 x 250 mL) from Orto Alresa, mod. Consul (Madrid, Spain) and a microcentrifuge equipped with an angular rotor (24 x 2.0 mL) from Biofuge Pico (Heraeus Hanau, Germany). A six-port solvent evaporator from Supelco was used to take extracts to dryness. SPE extractions were performed with an SPE manifold system supplied by Waters (Milford, MA, USA).

2.4. Triple quadrupole mass analyzer conditions

Aliquots of 10 µL were injected into the gas chromatographic system operating at a syringe injection flow rate of 10 µL s^{-1}. The injector temperature program was as follows: 70ºC (hold for 0.5 min) → 310ºC (100ºC min^{-1}, hold for 10 min). The injector split ratio was initially set at 20:1. Splitless mode was switched on at 0.5 min until 3.5 min. At 3.5 min, the split ratio was 100:1 and at 10 min, 20:1. The column oven program was: 70ºC (hold for 3.5 min) → 180ºC (50 ºC min^{-1}) → 300ºC (25ºC min^{-1}, hold 10 min). A cryogenic cooling with CO_2 was applied when the injector temperature was 170ºC in order to reach the initial injector temperature as fast as possible before continuing with the next injection.

The QqQ mass spectrometer operated in the SRM mode. The temperatures of the transfer line, manifold and ionization source were 300, 40, and 280ºC, respectively. A filament-multiplier delay of 4.5 min was set in order to prevent instrument damage. The emission current for the ionisation filament was set at 50 µA. The electron multiplier voltage was set at 1400 V (+200V offset above the value obtained in the auto-tuning process). The isolation window set in the first quadrupole (Q1) was 1.5 atomic mass unit (amu). The specific MS/MS parameters used are shown in Table 1.

2.5. Ion trap mass analyzer conditions

Aliquots of 10 µL of sample were injected into the gas chromatograph operating at a syringe injection flow rate of 10 µL s^{-1}. The injector temperature program and split ratio set were the same as in the GC-QqQ-MS system. The column oven program applied was: 70ºC (hold for 3.5 min) → 150ºC (50ºC min^{-1}) → 168ºC (5ºC min^{-1}) → 270ºC (6ºC min^{-1}) → 300ºC (90ºC min^{-1}, hold for 5 min).

The transfer line, manifold and trap temperatures were 280, 50 and 200ºC, respectively. A filament-multiplier delay of 6.40 min was established in order to prevent instrument damage. The automatic gain control (AGC) was set with an AGC-target of 5000 counts. The emission current for

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the ionisation filament was set at 80 µA, generating electrons with energy of 70 eV. The axial modulation amplitude was 4.0 V. The MS/MS process was carried out by CID with a non-resonant excitation for all the studied compounds since the response obtained was adequate and slightly more reproducible than using resonant mode. The electron multiplier voltage was 1700 V (+200 V offset above the value obtained in auto-tuning process). The specific MS/MS parameters used are shown in Table 2.

Results and discussion

3.1. Comparison of MS data obtained by ion trap and triple quadrupole analyzers

A comparison of the MS spectra obtained in full scan mode with both analyzers was carried out. The oven temperature program was slightly different in both systems since QqQ analyzers have a scan speed higher than IT scan speed, which influences in the development of the chromatographic analysis. The confirmation from the MS spectra was carried out applying the identification point criteria (IPs) established in the European Commission Decision 2002/657/EC [24]. Briefly, this Decision introduces the use of a number of IPs, depending on the class of compound analysed and the spectrometric technique used. For a QqQ analyzer, a minimum of 3 IPs are requested for pesticide residue analysis. The monitoring of either two MS/MS transitions or 3 SIM transitions achieves this minimum. The confirmation stage is more exhaustive when using an IT because a product ion scan is always registered and, thus high number of characteristic ions can be monitored. The confirmation is carried out by direct comparison of the sample spectrum and the reference spectrum.

In relation to the studied compounds, in general, higher fragmentation was observed in the IT full scan spectra, as previously reported [22], and the precursor ions obtained from both systems were normally the same. Nevertheless, a number of exceptions were observed: buprofezin, isocarbophos and isofenphos methyl. In the case of buprofezin, fragmentation was significantly different for both analyzers (Figure 1): the precursor ion selected for the QqQ analyzer was the ion *m/z* 172, whose intensity in the IT was much lower. On the contrary, the precursor ion for IT, *m/z* 249, showed a very poor signal in the QqQ. Whether both product ion spectra were compared, the fragmentation in the IT was higher than the QqQ fragmentation, showing several ions which were not present in the QqQ spectra, such as *m/z* 106, 201 and 277. Another exception was isocarbophos. In this compound, the full scan spectra obtained by both analyzers were very similar for the most important ions; the ion *m/z* 230 was selected as precursor ion in both systems (Figure 1). However, this precursor ion did not provide any suitable product ion by the QqQ analyzer since the sensitivity reached was insufficient to ensure the quantification-confirmation of this compound at low

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concentrations. In consequence, SIM was applied instead of SRM for the detection and confirmation of isocarbophos. The monitoring of three ions provided the requested sensitivity and it complied with the identification-confirmation criteria previously fixed. The last exception corresponded to isofenphos methyl, whose fragmentation obtained by both IT and QqQ showed considerable variations (Figure 1). The selected precursor ion was *m/z* 231 in the IT; however, this ion showed lower intensity in the QqQ analyzer. An alternative strategy was applied in the optimization of the QqQ-MS/MS conditions for isofenphos methyl: two precursor ions were used. According to the full scan spectrum obtained by this analyzer, two possible precursor ions could be used: *m/z* 199 and 241. Theoretically, *m/z* 199 was the best option in terms of sensitivity since it showed the highest intensity. The second one (*m/z* 241) was the most adequate in terms of selectivity due to its high *m/z* ratio. The QqQ analyzer permits the use of different precursor ions for the same compound. The CID conditions for both precursor ions were tested and the most appropriate MS/MS transitions (Figure 2) were chosen in order to reach a compromise solution between sensitivity and selectivity.

The aforementioned differences are mainly a consequence of the different way in which the MS/MS process is performed in both QqQ and IT. In the case of the QqQ analyzer, the fragmentation and selection of the ions occurs sequentially in separate regions or quadrupoles. The first (Q1) and third quadrupoles (Q3) are mass analyzers, acting as filters for the ions generated in both the ionization source and the collision cell (second quadrupole). This quadrupole, which is usually indicated by lower-case q in literature, operates in RF-only mode and acts like a lens for all the ions and not like a mass filter. As result, a continuous beam of ions is transmitted through the instrument while mass selection and fragmentation of the precursor ions by CID is continuously produced in separate parts of the instrument. This transmission of ions can lead to scattering losses and thus, a decrease of sensitivity for certain ions. On the contrary, these transmission losses are not a problem in the case of tandem in time since all the MS/MS processes occur in the same region.

On the other hand, inside the IT, the generation and selection of the precursor ion and the following CID process and product ions mass analysis are implemented by a timing sequence or scan function. For this reason, the IT shows higher sensitivity operating in the product ion scan mode and spectra with more fragments are obtained [25]. The unique feature of this analyzer is the possibility of obtaining higher order MS^n spectra. However, there is normally a lack of sensitivity whether MS^3 is performed due to the reduction in the amount of ions available for the following fragmentation. In fact, this mode is seldom applied in pesticide residue analysis [15, 22], although considerable increase of selectivity can be obtained for other applications whenever the generation of a sensitive sequence of product ions is possible.

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3.2. Comparison of validation data

The comparison was established in terms of precision, linearity and influence of the matrix nature on the analysis of low concentrations. Precision was expressed as relative standard deviation (RSD), and it was evaluated as intra-day and inter-day precision. For this study, in the first place, five samples in solvent at a concentration of 50 µg L^{-1} were injected in both systems during the same day to evaluate the intra-day precision. Secondly, other five samples in solvent at the same concentration were injected in both instruments in five different days to evaluate the inter-day precision. The results are shown in Figure 3. RSD values were always < 18 % (except for bifenthrin in the QqQ analyzer). In general, both analyzers showed similar intra-day precision, with adequate RSD values, in the range 2-16 % and 1-14 % for the QqQ and IT analyzer, respectively. However, 70 % of the compounds under study had RSD values higher in QqQ. In the case of pyrethroids, RSD values obtained by the IT analyzer were always lower than the corresponding values in QqQ (except for acrinathrin). The RSD values obtained for the inter-day precision were in the range 4-20 % for QqQ and 3-14 % for IT. Although the range of RSD was very similar, higher values were obtained by QqQ in 60 % of the compounds studied. The difference is significantly higher for a number of pyrethroids (acrinathrin, bifenthrin, cypermethrin and cyfluthrin); the RSD value obtained by QqQ was more than twice as high as the RSD value obtained by IT. As possible reasons, it must be noticed that dwell times were maybe too low; however, probably the main cause was due to the design of each analyzer. The MS/MS process in the QqQ itself may have some influence in the precision since ions must be transmitted through the quadrupoles, whereas in the IT, all processes happens inside the same space. Moreover, to our experience, the QqQ is not as robust as the IT, which can also contribute to these differences in the precision values. In any case, RSD values are adequate, according to the typical maximum value of 20 % in pesticide residue analysis.

Linearity was evaluated in solvent in the entire range from 1 to 750 µg L^{-1}. Other ranges were also studied, such as 10-150 µg L^{-1} (a normal range in routine analysis), and finally, a range including low concentrations: 1-50 µg L^{-1}. The corresponding concentration levels were 1, 5, 10, 25, 50, 150, 300, 500 and 750 µg L^{-1}. The same samples were injected in both instruments during the same day. Linear calibration graphs were plotted by least-squares regression of concentration versus relative peak area (analyte/IS) of the calibration standards and the corresponding determination coefficients (R^2) were calculated. The results are summarized in Table 3.

In the case of the QqQ analyzer, the linearity in the widest range (1-750 µg L^{-1}) was adequate for the majority of compounds, except for isocarbophos. On the contrary, five compounds (buprofezin, chlorfenvinphos, chlorpyriphos ethyl, ethion and isofenphos methyl) showed a lower linear range, 1-500 µg L^{-1} in the IT. This result was expected since the IT itself is a reduced space and it has a

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maximum storage capacity, beyond which mass resolution and spectral quality deteriorate and sensitivity decreases [25]. This effect can appear in spite of the application of an electronic tool, the AGC, which allows one to control the ionization time in order to maintain the number of ions in the trap at the optimum level. As a result, depending of the compound, the IT can be saturated when working at very high concentrations. In the QqQ, the amount of ions present in both the ionization source and the collision cell (q) do not influence in the same way as in the IT since the QqQ analyzer performs the MS/MS fragmentation in separated places.

In general, differences in R^2 values for the range 10-150 µg L^{-1} were not significant: both the QqQ and IT suitably work in this range. Nevertheless, the aforementioned differences become higher in a low concentration range such as 1-50 µg L^{-1}. The QqQ analyzer provided better values: 63.2 % of the compounds showed $R^2 \geq 0.99$ and 26.3 %, $R^2 \geq 0.98$. The R^2 values obtained by IT were ≥ 0.99 for 36.8 % of the compounds studied and ≥ 0.98 for 47.4 %. Therefore, R^2 values were better with the QqQ analyzer at low concentration ranges.

Finally, the influence of the matrix nature on the analysis of low concentrations was evaluated by injecting low concentration standards prepared both in solvent and blank extracts of two different matrices: cucumber and egg. The first one is a matrix with high water content usually applied as reference matrix in pesticide residue analysis of vegetables. Secondly, egg is a matrix with high fat content, thus it is a more complex matrix which can be useful to evaluate the MS/MS performance when analysing low concentrations of both instruments in the case of "dirty" samples. Cucumber and egg matrix-matched standards and solvent standards were prepared at low concentrations and injected in both systems during the same day. The results summarized in Figure 4 are the concentrations corresponding to chromatographic peaks with a S/N=3. The capability of analysing low concentrations in solvent was very similar in both instruments for the target compounds. Concentrations lower than 1 µg L^{-1} were properly analysed (S/N = 3) for 84 % and 79 % of the analytes by the IT and QqQ analyzer, respectively. In the case of cucumber (vegetable matrix), although these percentages were lower (64 % in IT and 63 % in QqQ), the performance was also similar in both cases. On the contrary, the capability of analysing these low concentrations decreased clearly in egg. Only 22 % of the studied compounds were properly analysed at a concentration lower than 1 µg L^{-1} by IT, whereas this value was 32 % by QqQ. In this matrix, the percentage compounds detected at concentrations > 10 µg L^{-1} in IT (50 %) was significantly higher than the corresponding value in QqQ (26 %). Summarizing these results, the differences between the IT and QqQ analyzers when analysing low concentrations is proportional to the complexity of the matrix (Figure 4). These two analyzers provide adequate results in simple matrices similar to solvent and vegetable matrices such as cucumber. However, the performance of the QqQ analyzer is more adequate for the analysis of fatty matrices, such as egg, where an easy sample pre-treatment

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was applied. The applied extraction method for egg has a minimum clean-up step which may have influence on the worse performance provided by IT in these samples. The use of additional clean-up stages is requested to obtain detect lower concentrations. Moreover, the better QqQ performance can be explained due to the selectivity of this analyzer working in SRM, which permit to eliminate high number of interferences (Figure 5). It must be noticed that sensitivity in SRM depends on the efficiency of the isolation of the precursor ions, the CID fragmentation yield and the specificity of the selected transitions.

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Conclusions

The performance of GC-QqQ-MS/MS and GC-IT-MS/MS in pesticide residue analysis has been compared. The MS data obtained by each analyzer were very similar within the group of studied compounds, although higher spectral information (complete product ion spectrum) was provided by the IT analyzer. This is an important advantage in comparison to QqQ, especially in the confirmation of complex positive samples, since the IT analyzer provides more exhaustive confirmation.

The study of validation parameters showed that the intra-day precision was slightly better in IT and the inter-day precision was clearly worse in QqQ, especially in the case of some pyrethroids. This last result can mean that GC-IT-MS/MS shows higher robustness than GC-QqQ-MS/MS. Linearity was similar in the range 10-150 L^{-1}; however, this figure of merit was better for high (1-750 µg L^{-1}) and low concentration ranges (1-50 µg L^{-1}) in the QqQ analyzer. In relation to the influence of the matrix nature on the capability of analysinf low concentrations, there was not significant differences between the IT and QqQ in the analysis of pesticide residues in vegetable matrices, such as cucumber, as well as in clean matrices similar to solvent. However, the QqQ showed better performance in complex and "dirty" matrices such as egg due to its higher selectivity working in SRM. Consequently, it is possible to reduce or even eliminate the clean-up stage and, therefore, increase the sample throughput. On the contrary, the low selectivity and sensitivity reached by IT in matrices with high fat content means that additional clean-up stages should be included to obtain similar results to those of the QqQ analyzer. This would increase the total analysis time: extraction sample plus instrumental determination. In this sense, the QqQ shows high scan speed which allows the development of faster instrumental methods with higher number of compounds. In contrast, one of the main drawbacks of the IT analyzer is the low scan speed which affects to the instrumental analysis time, depending on the number of analytes. In economic terms, the GC-IT-MS/MS instrument shows an interesting price for pesticide residue laboratories: it is much cheaper than the GC-QqQ-MS/MS equipment. Although the QqQ is more versatile than the

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IT since it can operate in the four MS/MS modes in GC, its price can result prohibitive for some laboratories.

In conclusion, GC-IT-MS/MS is the best option, provided that the number of analytes and the instrumental analysis time is not a key factor in the performance of the laboratory. In other words, the limit of this instrument could be set at a maximum of 80-100 compounds in 30-40 min, approximately. This system is the most suitable option for the routine analysis of pesticide residues in vegetables, having in mind the aforementioned limitations. Its applicability in fatty matrices depends on the sample pre-treatment applied and target compounds. GC-QqQ-MS/MS could be the most adequate option whenever the number of compounds is very high and fast analysis time is required. This analyzer permits the application of easy sample pre-treatments and the reduction of the number of clean-up steps. Its lower robustness in comparison with the IT should be also taken into account.

Acknowledgements

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438 **FIGURE CAPTIONS**

439

440 **Fig. 1.** Full scan spectra of buprofezin, isocarbophos and isofenphos methyl obtained by GC-QqQ-
441 MS/MS and GC-IT-MS/MS

442 **Fig. 2.** Development of the CID conditions for isofenphos methyl in the QqQ analyzer. The selected
443 collision energy is pointed out by a square in each case

444 **Fig. 3.** Inter-day (a) and intra-day (b) precision values (expressed as relative standard deviation,
445 RSD) obtained with the QqQ and the IT analyzer **for solvent standards at 50 µg kg^{-1}.**

446 **Fig. 4.** Influence of the matrix on the capability of analysing low concentrations in (a) QqQ and (b)
447 IT. Percentage of compounds under study yielding a S/N=3 at a concentration ≤1, 1-5, 5-10 and >
448 10 µg kg^{-1} is shown.

449 **Fig. 5.** Total ion chromatogram (TIC) obtained by (a) GC-QqQ-MS/MS and (b) GC-IT-MS/MS of
450 a standard prepared in (1) solvent, (2) cucumber and (3) egg (10 µg kg^{-1}), as well as (4) a cucumber
451 blank and (5) an egg blank sample

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Figure

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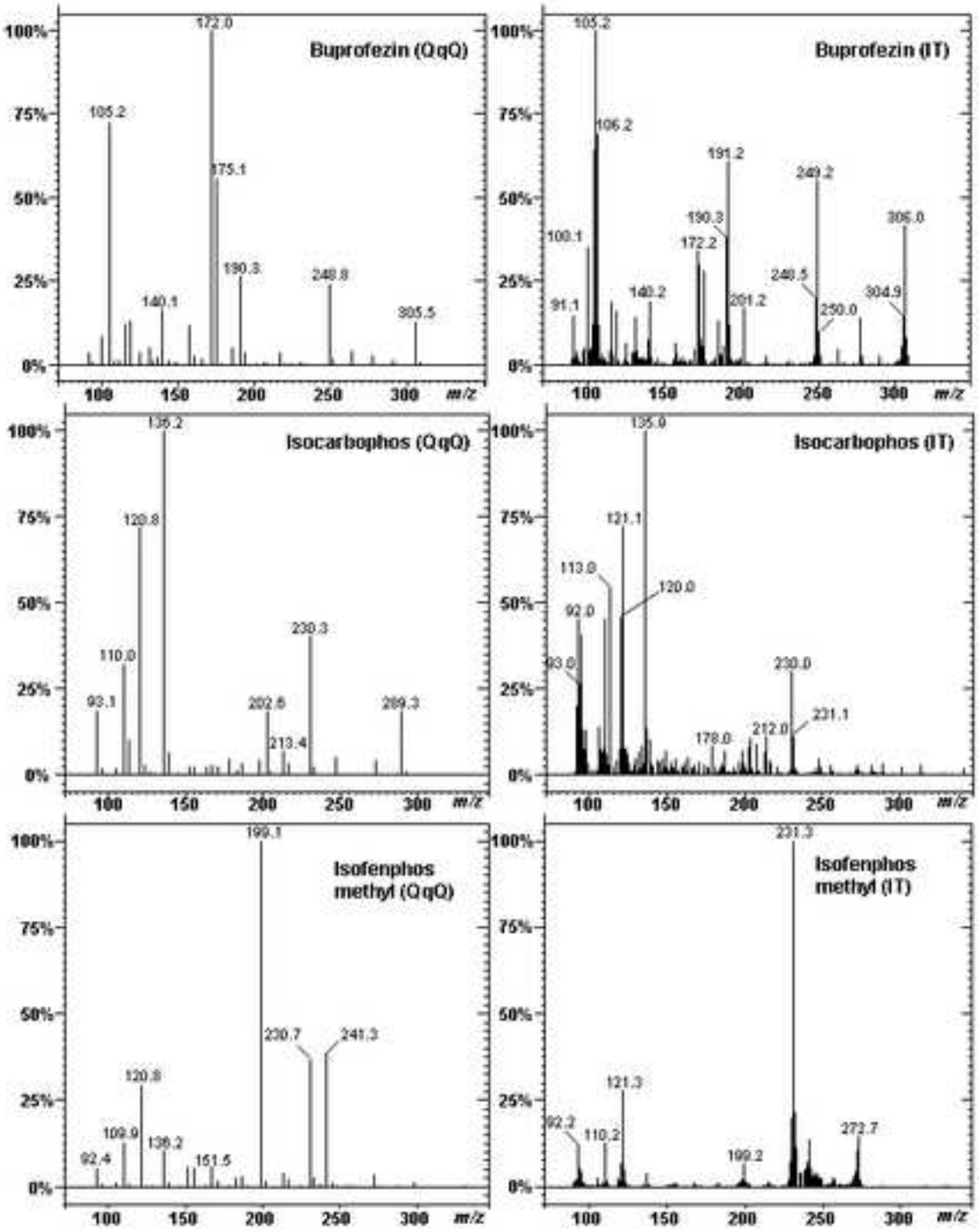

Figure

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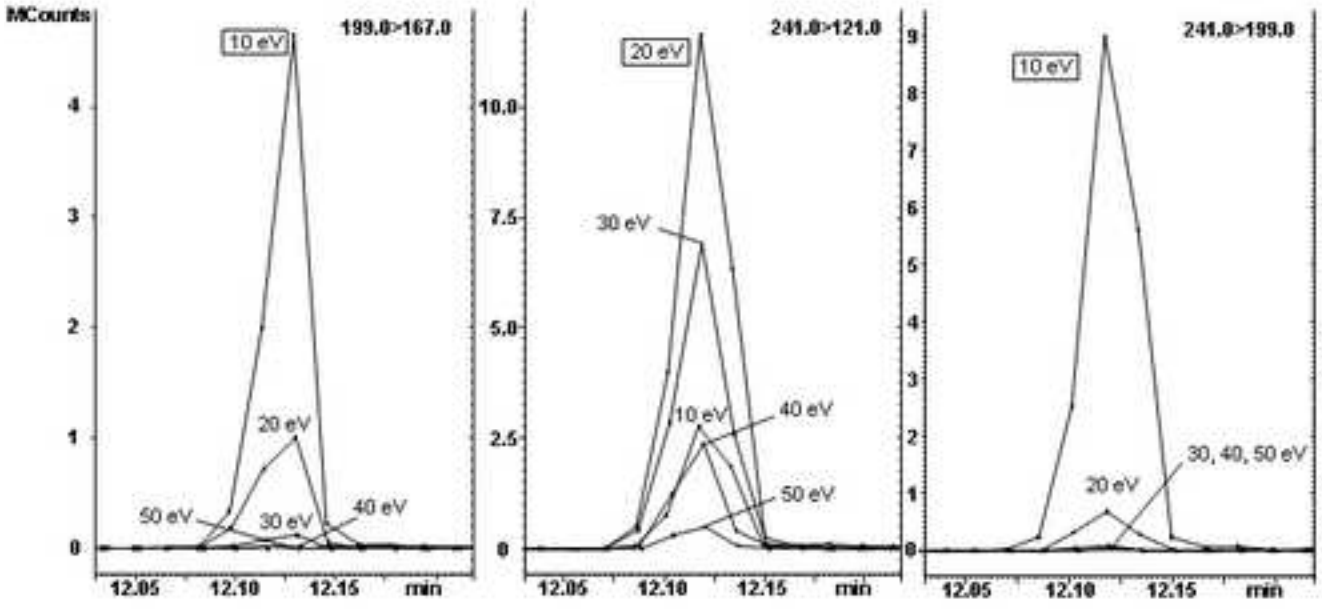

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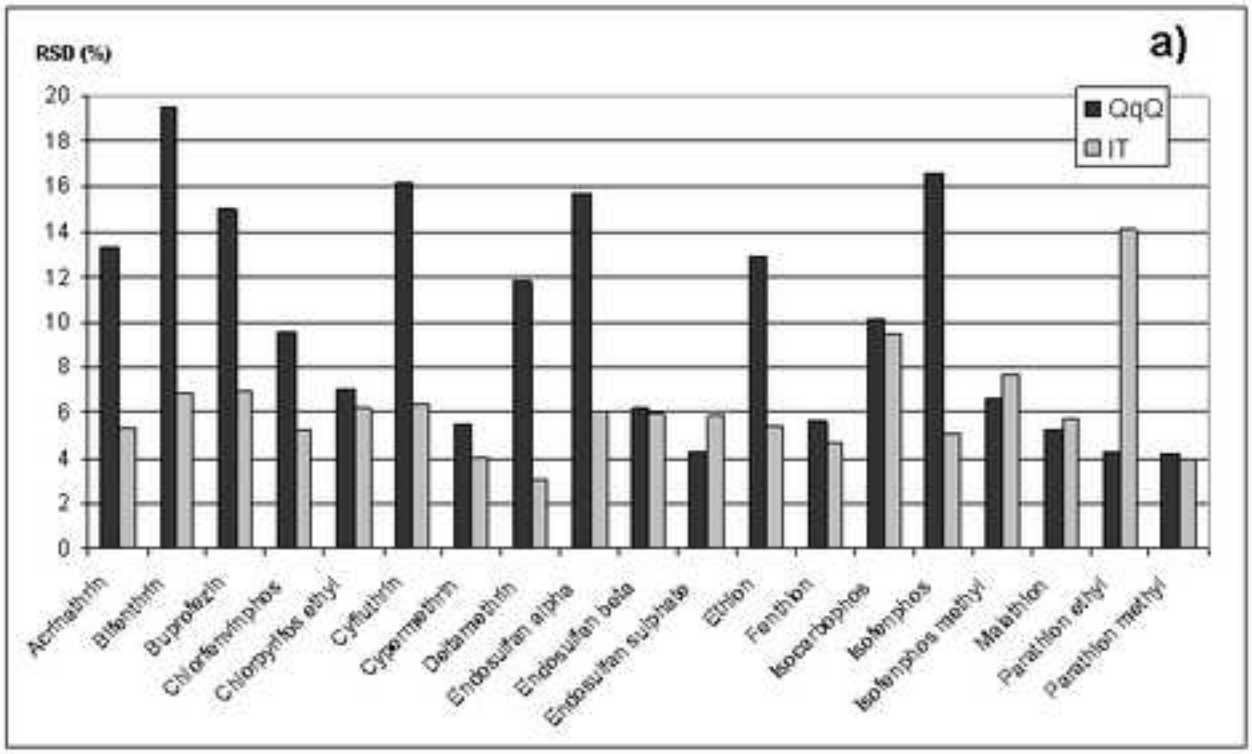

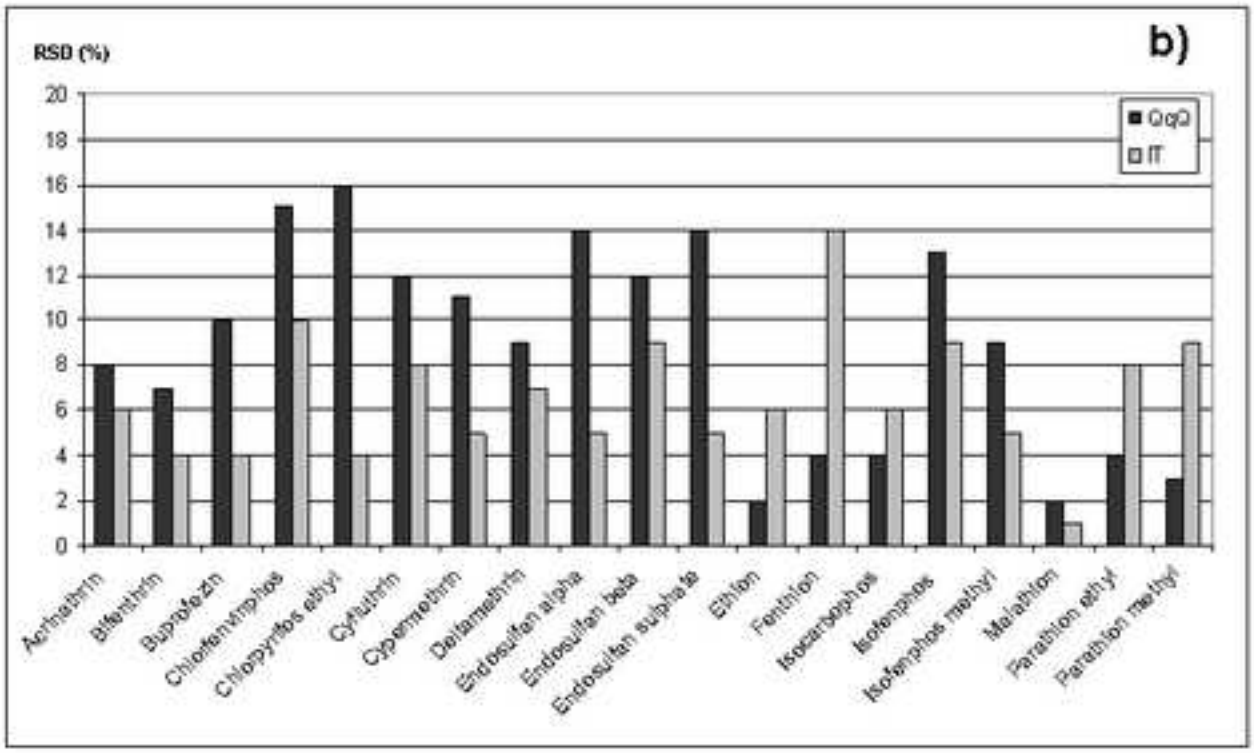

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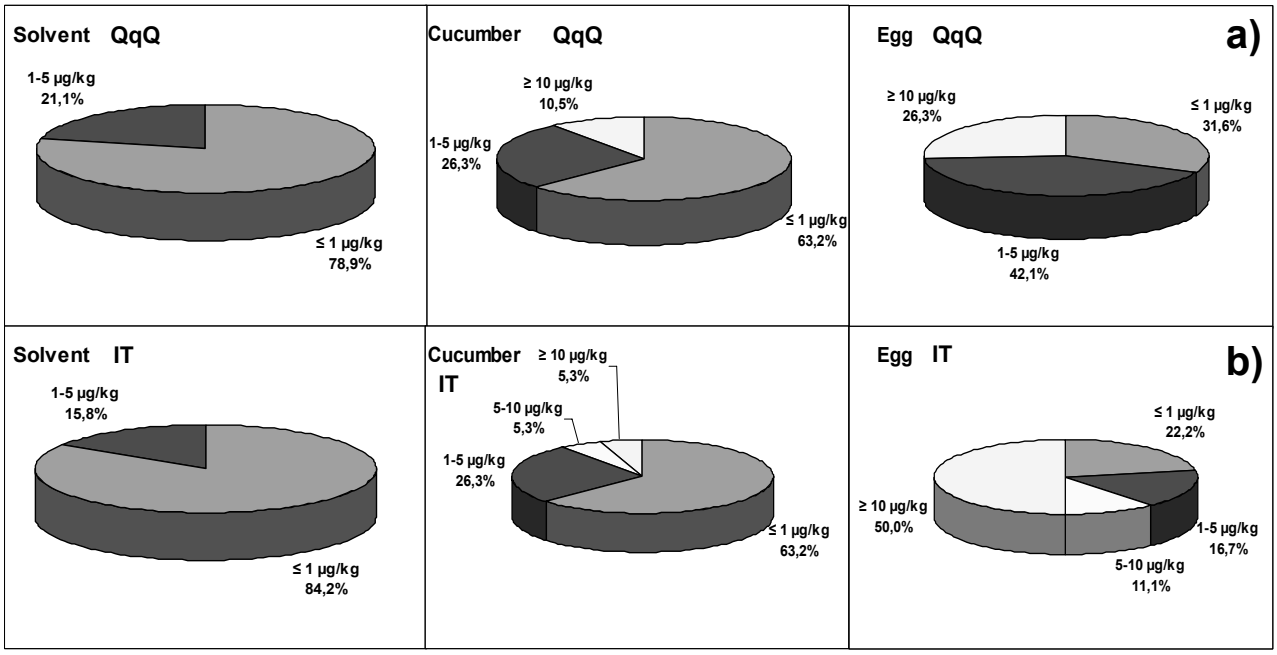
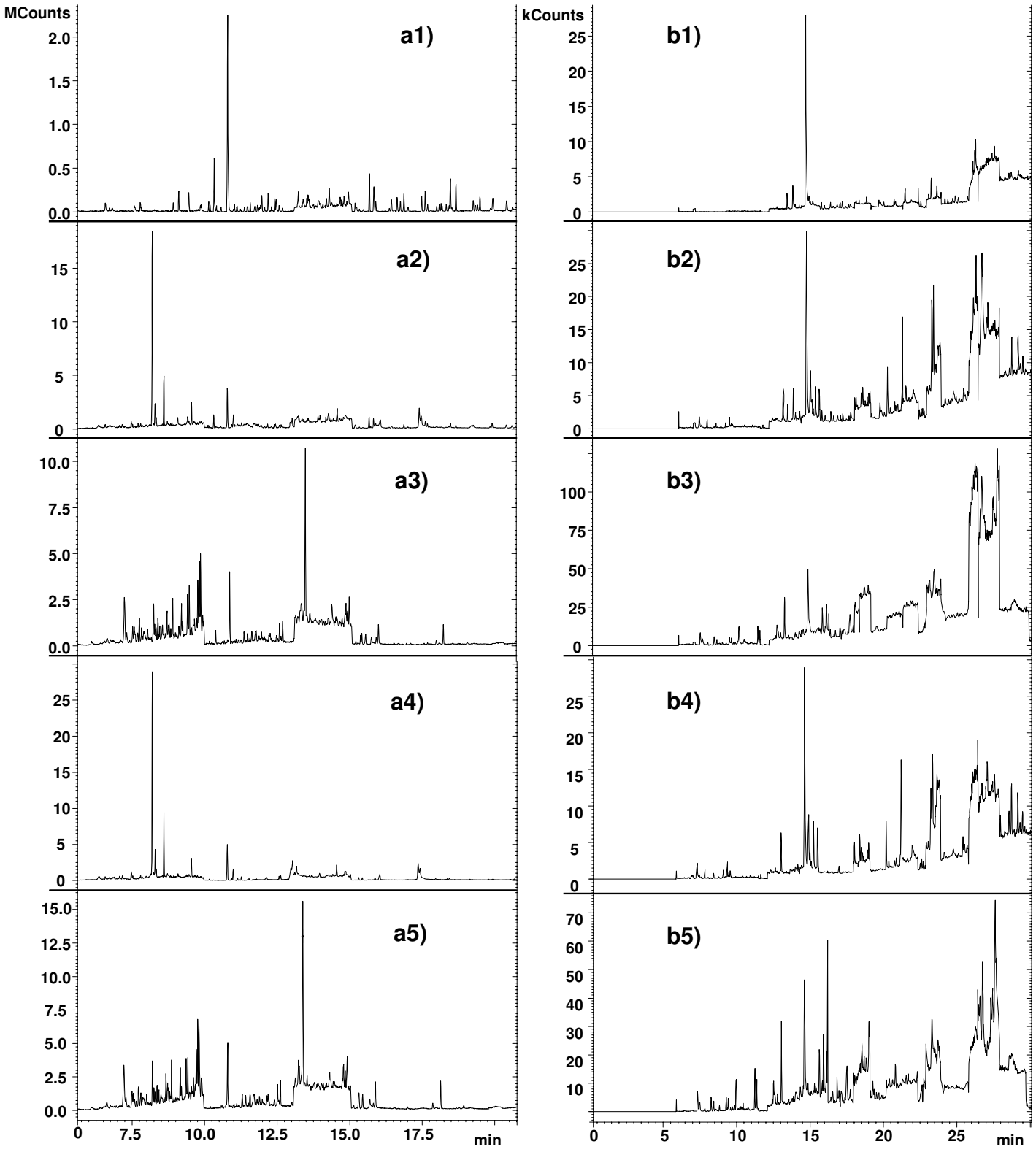

Solvent QqQ
1-5 µg/kg
21,1%
≤ 1 µg/kg
78,9%
Cucumber QqQ
≥ 10 µg/kg
10,5%
1-5 µg/kg
26,3%
≤ 1 µg/kg
63,2%
Egg QqQ
a)
≥ 10 µg/kg
26,3%
≤ 1 µg/kg
31,6%
1-5 µg/kg
42,1%
Solvent IT
1-5 µg/kg
15,8%
≤ 1 µg/kg
84,2%
Cucumber IT
≥ 10 µg/kg
5,3%
5-10 µg/kg
5,3%
1-5 µg/kg
26,3%
≤ 1 µg/kg
63,2%
Egg IT
b)
≤ 1 µg/kg
22,2%
≥ 10 µg/kg
50,0%
1-5 µg/kg
16,7%
5-10 µg/kg
11,1%

Figure



Tables

Table 1. GC-QqQ-MS/MS conditions

Compound	Precursor ion (*m/z*)	Produt ion (*m/z*)	Collision energy (eV)	Dwell time (ms)
Acrinathrin	181	126	50	10
		152	40	10
Bifenthrin	181	115	50	10
		166	20	10
Buprofezin	172	115	20	5
		131	10	5
Chlorfenvinphos	267	123	40	6
		159	30	6
Chlorpyriphos ethyl	314	258	20	5
		286	10	5
Cyfluthrin	206	150	40	17
		177	20	17
Cypermethrin	163	91	20	17
		127	10	17
Deltamethrin	253	93	10	17
		174	10	17
Endosulfan α	241	136	40	5
		170	30	5
		206	20	5
Endosulfan β	241	136	40	5
		170	30	5
		206	20	5
Endosulfan sulphate	272	141	40	5
		237	10	5
Ethion	231	185	20	5
		203	10	5
Fenthion	278	109	10	5
		153	50	5
Isocarbophos*	121			5
	136			5
	289			5
Isofenphos	255	121	20	5
		213	10	5
Isofenphos methyl	199	167	10	5
	241	121	20	5
	241	199	10	5
Malathion	173	99	10	6
		127	10	6
Parathion ethyl	291	109	10	5
		137	10	5
Parathion methyl	263	109	10	6
		153	10	6
Caffeine (IS)	194	109	20	5

* SIM transitions are shown

Tables

Table 2. GC-IT-MS/MS conditions

Compound	Parent ion (*m/z*)	CID Amplitude (V)	CID Storage level (*m/z*)	Quantification ion (*m/z*)	*m/z* Range	Scan time (s)
Acrinathrin	181	90	80	152	80-335	0.28
Bifenthrin	181	40	50	165	80-350	0.28
Buprofezin	249	50	80	191:195	90-375	0.28
Chlorfenvinphos	267	82	100	159	80-425	0.29
Chlorpyriphos ethyl	314	100	170	258+286	80-425	0.27
Cyfluthrin	206	96	86	151	90-315	0.27
Cypermethrin	163	53	70	127	90-315	0.27
Deltamethrin	253	57	90	172:174	90-360	0.28
Endosulfan α	241	84	80	170:172+204:206+136	80-260	0.38
Endosulfan β	241	63	100	170:172+204:206+136	90-275	0.27
Endosulfan sulphate	272	64	80	235:237	80-360	0.28
Ethion	231	63	100	175+203	90-275	0.27
Fenthion	278	92	112	135	90-350	0.28
Isocarbophos	231	95	100	155	90-350	0.28
Isofenphos	213	52	93	185	80-425	0.28
Isofenphos methyl	199	57	95	121+167	80-425	0.28
Malathion	173	51	75	99	90-321	0.27
Parathion ethyl	291	61	128	263	90-350	0.28
Parathion methyl	263	48	80	136+246	80-300	0.27
Caffeine (IS)	194	56	60	120+108	80-300	0.27

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Tables

Table 3
Determination coefficients (R^2) obtained by GC-QqQ-MS/MS and GC-IT-MS/MS

Compound	TRIPLE QUADRUPOLE				ION TRAP			
	1-50 µg kg^{-1} (R^2)	10-150 µg kg^{-1} (R^2)	Linear range		1-50 µg kg^{-1} (R^2)	10-150 µg kg^{-1} (R^2)	Linear range	
			(µg kg^{-1})	R^2			(µg kg^{-1})	R^2
Acrinathrin	0,9960	0,9995	1-750	0,9975	0,9958	0,9989	1-750	0,9996
Bifenthrin	0,9948	0,9992	1-750	0,9986	0,9880	0,9984	1-750	0,9981
Buprofezin	0,9774*	0,9842	5-750	0,9958	0,9913	0,9800	1-500	0,9811
Chlorfenvinphos	0,9959	0,9866	1-750	0,9954	0,9840	0,9997	1-500	0,9996
Chlorpyriphos ethyl	0,9888	0,9932	1-750	0,9880	0,9894	0,9995	1-500	0,9969
Cyfluthrin	0,9981	0,9986	5-750	0,9940	0,9851*	0,9895	10-750	0,9996
Cypermethrin	0,9958	0,9991	10-750	0,9928	0,9672	0,9984	10-750	0,9990
Deltamethrin	0,9898*	0,9883	5-750	0,9913	0,9727	0,9886	10-750	0,9976
Endosulfan α	0,9980	0,9994	5-750	0,9996	0,9857	0,9996	1-750	0,9998
Endosulfan β	0,9903	0,9994	5-750	0,9940	0,9921	0,9993	1-750	0,9989
Endosulfan sulphate	0,9496*	0,9920	5-750	0,9899	0,9922	0,9969	5-750	0,9976
Ethion	0,9940	0,9850	1-750	0,9970	0,9854	0,9996	1-500	0,9977
Fenthion	0,9973	0,9996	1-750	0,9851	0,9886	0,9976	1-750	0,9987
Isocarbophos	0,9879	0,9951	10-500	0,9910	0,9912	0,9900	1-750	0,9962
Isofenphos	0,9961	0,9962	1-750	0,9954	0,9991	0,9999	1-750	0,9961
Isofenphos methyl	0,9963	0,9974	1-750	0,9891	0,9740	0,9998	1-500	0,9906
Malathion	0,9951	0,9998	1-750	0,9968	0,9872	0,9962	1-750	0,9943
Parathion ethyl	0,9860	0,9986	5-750	0,9919	0,9809*	0,9967	10-750	0,9861
Parathion methyl	0,9888	0,9944	1-750	0,9984	0,9905	0,9975	5-750	0,9953

*R^2 value for range 5-50 µg kg^{-1}