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The ECHO technique – the more effective way of data evaluation in liquid chromatography–tandem mass spectrometry analysis

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Abstract

The aim of this study was to evaluate the applicability of ECHO technique in pesticide residue analysis using LC/MS/MS instruments with atmospheric pressure chemical (APCI) and electrospray (ESI) ionization. The technique is based on simultaneous injections of reference standards and samples in one run. First and second injections are made ahead and behind a precolumn, respectively, thus resulting in a short difference of retention times between standard and sample peak. The obtained couple of peaks were applied to the easy detection of pesticides and simultaneous estimation of the residue content in real samples in a single run. If residues were not observed, the second sample peak did not occur and the ECHO peaks were used to demonstrate instrument performance in each run and for each analyte. Another advantage of ECHO technique is its potential to compensate matrix effects. The occurrence and compensation of matrix effects using APCI was tested with four matrix types (water containing, acidic, dry and sugar containing) and 22 pesticides. The same matrix types but 58 pesticides were used tests with electrospray ionization. Most often matrix effects had been observed with lemon. The percentage of pesticides showing significant matrix effects did not differ between APCI and ESI. But these effects caused signal enhancement in APCI measurements and signal suppression, when ESI was used. The ECHO technique was able to compensate many matrix effects in measurements with both types of ion sources.

Keywords: Pesticides; Atmospheric pressure ionization; Matrix effects; Calibration; ECHO peak technique; Internal standards

1. Introduction

Fruit and vegetables are traded worldwide and it is generally not known which pesticides might have been applied in their agricultural production. Consumers, however, demand produce to be "free of harmful pesticide residues". Therefore, food chemists are expected to check whether maximum legal residue levels (MRLs) have been exceeded for all pesticides. Multiresidue methods applying gas chromatography–mass spectrometry coupling (GC–MS) are established for monitoring more than 400 thermally stable pesticides [1–3]. These pesticide residues are identified in screening analyses by means of the dedicated mass spectral libraries containing reference mass spectra and retention times of more than 400 active ingredients and also their metabolites. Software programs are available for automated screening [4].

A great number of pesticides, however, are not thermally stable and therefore not amenable to GC–MS. These active ingredients include a great proportion of the modern pesticides, the control of which is most relevant. In the last few years, the development of liquid chromatography (LC) interfacing with mass spectrometry (LC–MS) and tandem mass spectrometry (LC–MS/MS) has resulted in instruments of spectacular performance in comparison with instruments of previous generations. The historical development up to the breakthrough in the mid-nineties is described by Niessen in a monograph on LC–MS [5] and a review [6], both being very good sources for understanding the basics of the ionization and interface techniques used in modern LC–MS and LC–MS/MS equipment. Recent and future developments of LC in pesticide trace analysis have been reviewed by Hogedoorn and van Zoonen [7] and two pesticide multiresidue methods have been presented by Jansson et al. [8] and Klein and Alder [9] who demonstrated the simultaneous screening of about 100 pesticide residues in crops applying LC–MS/MS with ESI in

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the positive mode and additional 10 pesticide residues in the negative mode at the 0.01 mg/kg concentration level.

The pesticide residue concentration level expected in foodstuffs is between 0.01 mg/kg and a few mg/kg in the presence of a great quantity of natural compounds from the food matrix. The new LC–MS/MS techniques, however, provide the necessary selectivity and detection sensitivity to allow the unequivocal identification of these trace level concentrations in the extracts of food samples.

While the detection of pesticide residues at trace level concentration in food matrices applying GC–MS or LC–MS/MS usually poses no longer any problems, the production of reliable quantitative results is one of the great challenges to the pesticide residue analyst.

The enhancement or suppression of signal intensity of the analytes in the presence of matrix compounds poses a major problem, which has not yet finally been solved.

Calibration of GC–MS as well as LC–MS systems can be carried out in different ways. Very popular, because easily to realize, is the use of external calibration with reference standards in solvent. The quantitative results obtained using external standard calibration, however, frequently show poor accuracy of results. The reason for this effect is the presence of matrix compounds in the final sample extracts, which may cause suppression or enhancement of the analyte signal [10]. Therefore, matrix-matched standard calibration has been established in GC–MS and LC–MS and LC–MS/MS where the extract of a non-contaminated foodstuff of the same kind of the analyzed food sample is spiked with the pesticides under investigation. The other possibility is the standard addition method where the same sample extract is spiked with the analytes detected and run again under the same conditions. Both procedures are obviously time consuming.

The best method to compensate for recovery variations as well as matrix effects in GC–MS and LC–MS/MS is the addition of stable isotope labeled compounds at the beginning of sample cleanup. The analyte and its isotope labeled analogue possess the same chemical structure and hence the same behavior during cleanup and chromatography and they appear at the same time with exactly the same co-elutes in the ion source of the mass spectrometer where they suffer exactly the same amount of signal alteration. The quantitation is easily done by calculating the analyte's concentration from the known concentration of the internal standard. This method is obviously not applicable to pesticide multiresidue analysis because a restricted number of labeled pesticides is available only. And even if all needed pesticides would be available as labeled compounds, such general use of labeled standards in multiresidue methods would be very expensive.

ECHO peak technique, representing a new interesting alternative to the internal standard concept, was first presented by Powley et al. in 2000 at the European Pesticide Workshop in York [11]. With this new technique, two injections were carried out in each analysis, namely within a short time period the unknown sample and a standard solution. As a result, the peak of the analyte from the standard elutes in close proximity to the peak from the analyte from the sample, thus forming the so-called ECHO peak. It is expected that both peaks elute so closely that they are affected in the same manner by the co-eluted matrix components. The ECHO technique has recently been described in detail by Zrostliková et al. [12] and a review of matrix effects in pesticide residue analysis was presented by Hajslová and Zrostliková [10]. In both the papers, a description and extensive discussion of the various interpretations connecting the origin of the matrix effects and their possible compensation are given.

In this paper, we discuss the extent of matrix effects obtained in LC–MS/MS measurements with atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) and its compensation by the ECHO technique. This technique was applied to the analysis of 70 pesticides in five different foodstuffs, representative for a wide range of food matrices. Additionally, ECHO technique offers some advantages in the evaluation of chromatograms of routine samples. Some examples will be discussed.

2. Material and methods

2.1. Chemicals

All organic solvents and other chemicals were either HPLC grade or analytical reagent grade. Most of the pesticides used are obtained from Ehrenstorfer, Augsburg, Germany and Riedel de Haen, Seelze, Germany. Others are gifts from those companies producing the active substance of the pesticide products (BASF, Bayer CropScience, Syngenta and Tomen Agro). ChemElut disposable extraction columns with 5 mL sample capacity (Part no. 1219-8006) were obtained from Varian GmbH, Analytical Instruments (Darmstadt, Germany).

2.2. Extraction

Preparation of matrix-matched standards were performed with the five foodstuff matrices: tomato, cucumber, lemon, raisins and wheat flour. The samples were obtained from local supermarkets, paying special attention to obtain "non-contaminated" foodstuffs. The test samples were homogenized in the presence of dry ice. After evaporation of carbon dioxide in a freezer to an aliquot of 10 g of tomato, cucumber or lemon, 0.5 mL, 0.5 mL and 1 mL water were added, respectively, to obtain an amount of 10 mL water as sum of natural and added water. In the case of raisins and wheat flour, the mass of the test portion was 5 g and the amount of added water was 8 mL and 9 mL, respectively. Such water enriched test portions were extracted with 20 mL methanol.

Preparation and extraction of spiked cucumber samples and pears with incurred residues was performed in the same way after addition 0.5 mL water.

2.3. Cleanup

Six milliliters of the methanol/water extract were well mixed with 2 mL of an aqueous solution of NaCl (20 g in 100 mL). An aliquot of 5 mL was transferred to a Chem-Elut column with 5 mL sample capacity. After a 5 min period of equilibration, the column was eluted with 16 mL dichloromethane and the collected eluate was evaporated at 40 °C to dryness. The residue was redissolved in 250 μL methanol with the help of an ultrasonic bath and mixed with 1 mL water containing 5 mmoles/L ammonium formate. The resulting final extracts (1.25 mL) contained the residues of 1 g water-rich (tomato, cucumber, lemon) or 0.5 g dry sample (wheat flour, raisins) per milliliter. Finally, they were filtered through a 0.45 μm filter into glass vials.

2.4. HPLC

Liquid chromatography was carried out using an Agilent 1100 system equipped with a binary pump (G1312A), a column oven with six-port switching valve (G1316A) and an autosampler (G1313A, Agilent Technologies Deutschland GmbH, Waldbronn, Germany). The chosen column was an Aqua 5 μ C18 125 Å, 50 mm × 2 mm and an Aqua 5 μ C18 125 Å, 10 mm × 2 mm precolumn (Phenomenex, Aschaffenburg, Germany).

The degassed (degasser G1322 A) mobile phase A consisted of 80% water and 20% methanol, mobile phase B of 90% methanol and 10% water. Both phases A and B contained 5 mmoles/L ammonium formate. For the first injection, a pure mobile phase A was used.

In APCI experiments, the flow rate was 0.4 mL/min and the mobile phase composition was changed during the run after the second injection as follows: starting with 0%, the percentage of B was increased linearly to 100% over 25 min and then kept constant for another 6 min. Equilibration time prior to the next injection was 10 min.

During ESI measurements, some minor changes were necessary. The flow rate was reduced to 0.2 mL/min and the linear increase of phase B from 0% to 100% was finished after 29 min. Hundred percent B was kept constant for 10 min.

In both types of measurements, the injection volume was 20 μL.

2.5. Mass spectrometry

2.5.1. General

The effluent from the HPLC system was introduced to an Applied Biosystems API 2000 triple quadrupole mass spectrometer (Applera Deutschland GmbH, Weiterstadt, Germany) equipped with a Heated Nebulizer (APCI) or TurboIonSpray (ESI) interface, respectively. The source parameters were optimized in preliminary experiments and kept constant for all analytes of this study. They are summarized in Table 1.

Table 1
Source parameters

Parameter	Optimum APCI conditions	Optimum ESI conditions
Curtain gas pressure (CUR; psi)	50	50
Heater gas temperature (TEM; °C)	400	350
Collision gas pressure (CAD; psi)	4	4
Needle current (NC; μA)	2	–
Ion spray voltage (IS; V)	–	5500
Auxillary (APCI) or nebulizer (ESI) gas pressure (GAS 1; psi)	70	60
Nebulizer (APCI) or heater (ESI) gas pressure (GAS 2; psi)	35	60

2.5.2. MRM

Each analyte was tuned individually. Source optimization and tuning were performed by introducing the analytes into the mass spectrometer through direct infusion via a syringe pump at a flow rate of 0.2 mL/min and a solvent composition of water/methanol (1 + 1) with 5 mmoles/L ammonium formate. Suitable mass transitions selected, together with the most important analyte-dependent parameters, declustering potential (DP) and collision energy (CE) thus found are summarized in Table 2.

2.6. Calibration standards

Separate calibration standards were prepared in solvent (mobile phase A) and in matrix extracts. The extracts were free from residues. Identical stock solutions containing all pesticides in methanol at a level of 1 μg/mL were used for both types of standards. The stock solutions for APCI experiments contained 24 pesticides and the standard mix used for ESI experiments included 58 analytes. As a consequence of using two different amounts of test portion, namely 10 g or 5 g, a particular calibration level e.g. of 0.1 mg/kg corresponds to different standard concentrations, i.e. 0.1 μg/mL and 0.05 μg/mL. If possible, standards were used immediately after preparation, otherwise the vials were kept at −20 °C until use (normally within 2–3 days).

2.7. Principle of ECHO technique in pesticide multiresidue analysis

The ECHO technique applies the injection of a reference standard solution of a pesticide mixture (the ECHO standard) followed by an injection of the unknown sample into the LC column of the LC–MS/MS system within a short time period applying an instrumental setup as shown in Fig. 1. The intention is to elute the reference standard and the analyte from the sample closely in time, thus forming the so-called ECHO peak. If the retention times of the two peaks are close enough then they should both be affected by the co-eluting matrix compounds from the sample in the same manner and thus matrix effects are compensated provided that the matrix peak is sufficiently wide to affect both two peaks. Due to the low elution strength of the first mobile

Table 2
Analyte specific parameters and source of pesticides used

Pesticide	RT (min)	Transition	DP (V)	CE (V)
Ionization: APCI positive				
Bendiocarb	11.4	224 → 167	11	13
Carbaryl	12.7	202 → 145	11	15
Carbofuran	11.2	222 → 165	11	17
Clethodim	14.0/17.7	360 → 164	6	27
Cycloxydim	11.2/17.7	326 → 280	11	19
Diflubenzuron	20.7	311 → 158	11	19
Flutriafol	14.9	302 → 123	11	39
Haloxyfop	17.1	362 → 316	11	23
Imidacloprid	5.1	256 → 175	11	27
Mesotrione	1.4	340 → 228	36	23
Methiocarb	17.3	226 → 169	11	13
Methoxyfenozide	18.6	369 → 149	1	23
Metobromuron	13.8	259 → 170	11	25
Monolinuron	12.7	215 → 126	11	25
Boscalid (Nicobifen)	17.4	343 → 307	16	25
Promecarb	17.6	208 → 109	11	21
Propoxur	10.7	210 → 111	6	19
Pymetrozine	3.7	218 → 106	21	29
Tebuconazole	21.2	308 → 69	16	39
Teflubenzuron	24.8	381 → 158	11	23
Thiamethoxam	2.9	292 → 211	11	17
Trichlorfon	4.7	257 → 109	21	23
Triflumuron	22.4	359 → 156	11	23
Triforine	16.8/17.5	435 → 390	6	19
Ionization: ESI positive				
3-Hydroxy-carbofuran	11.1	238 → 163	16	19
5-Hydroxy-clethodim sulfone	9.3	408 → 204	16	27
Aldicarb[a]	14.5	208 → 89	1	21
Atrazine	21.2	216 → 174	21	25
Azoxystrobin	25.1	404 → 372	36	19
Butocarboxim[a]	14.3	208 → 75	1	15
Carbaryl	20.0	202 → 145	11	15
Carbofuran	18.2	222 → 165	16	17
Clethodim	21.6/25.5	360 → 164	41	25
Clethodim-imin sulfone	11.9	302 → 98	71	41
Clethodim-imin sulfoxide	11.5/12.4	286 → 208	26	21
Clethodim sulfone	13.7	392 → 164	1	33
Clethodim sulfoxide	12.9/14.0	376 → 206	1	19
Cyprodinil	28.9	226 → 93	61	45
Demeton-s-methyl[a]	18.7	248 → 89	6	17
Demeton-s-methyl sulfone	4.8	263 → 169	66	21
Dimethoate	10.6	230 → 199	16	13
Ethiofencarb sulfone[a]	7.9	275 → 107	11	25
Ethiofencarb sulfoxide	8.4	242 → 107	41	23
Fenhexamid	27.0	302 → 97	91	33
Fenoxycarb	28.5	302 → 88	66	29
Fenpropimorph	35.5	304 → 147	46	39
Fluazifop-P-butyl	31.9	384 → 282	61	25
Furathiocarb	32.0	383 → 195	51	23
Imazalil	29.3	297 → 159	26	31
Imidacloprid	9.6	256 → 209	51	21
Imidacloprid hydroxide	7.5	272 → 191	46	23
Imidacloprid olefine	7.2	254 → 171	56	23
Indoxacarb	31.1	528 → 203	76	51
Iprovalicarb	26.4/26.6	321 → 119	46	23
Isoproturon	22.2	207 → 165	46	19
Isoxaflutole[a]	22.7	377 → 251	26	25
Linuron	24.8	249 → 160	66	23
Metalaxyl	22.3	280 → 220	46	19
Methiocarb[a]	25.1	243 → 169	11	17
Methoxyfenozide	26.2	369 → 149	36	23

Table 2 (*Continued*)

Pesticide	RT (min)	Transition	DP (V)	CE (V)
Metolachlor	27.2	284 → 252	16	19
Monocrotophos	6.3	224 → 127	46	21
Omethoat	2.2	214 → 125	51	29
Oxamyl[a]	3.2	237 → 72	1	21
Oxydemeton-methyl	3.9	247 → 169	21	19
Phorate sulfoxide	21.2	277 → 199	51	15
Picoxystrobin	28.3	368 → 145	36	27
Pirimicarb	20.8	239 → 72	16	31
Promecarb	25.4	208 → 109	11	21
Propamocarb	6.5	189 → 102	16	23
Propoxur	17.7	210 → 111	11	19
Pymetrozin	6.8	218 → 105	56	27
Pyraclostrobin	30.0	388 → 194	6	19
Pyridate metabolite (6-chloro-3-phenyl-pyridazine-4-ol)	6.8	207 → 104	66	31
Pyrimethanil	24.5	200 → 107	61	33
Quinmerac	4.4	222 → 204	21	23
Spiroxamine	29.6	298 → 144	41	27
Tebuconazole	29.1	308 → 70	21	39
Tebufenozid	28.2	353 → 133	41	23
Thiabendazole	18.1	202 → 175	56	35
Thiacloprid	14.7	253 → 126	81	29
Vamidothion	11.2	288 → 146	16	17

[a] Precursor ion is $[M + NH_4]^+$. In all other cases a protonated molecular ion $[M + H]^+$ was chosen as precursor ion.

phase A, analytes are retained in the front of the separation column as a narrow band. After a short time period of about 1 min, the column switch valve is changed to direct the mobile phase through a short precolumn – filled with exactly the same separation phase as the main column – connected now directly with the main column. Now a second injection is carried out and the eluent gradient is started. As a result of this setup, the two analytes, one from the ECHO standard mixture and one from the sample elute closely. There are four possibilities of orders of injections, of which the following was found to give best results in multiresidue pesticide analysis, namely to inject the ECHO standard first into the chromatographic (main) separation column followed after 0.3 min by the injection of the sample into the precolumn.

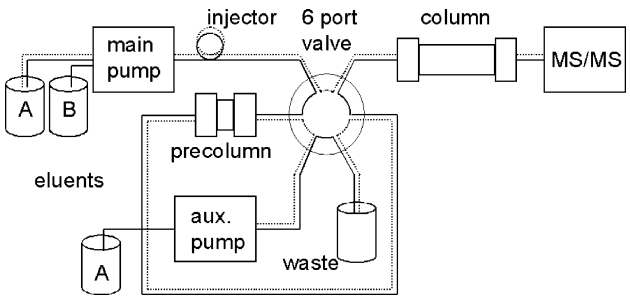

Fig. 1. Instrumental set-up for the ECHO peak technique in a multiresidue analysis. Dotted line: column switch valve position to allow the mobile phase A flow directly into the separation column – ECHO standard is injected, duration of run is 18 s. Solid line: column switch valve position directs the flow of mobile phase A through the precolumn – sample is injected 50 s after first injection and the eluent gradient of main run is started.

3. Results and discussion

3.1. Selection of suitable MS/MS conditions

In a preceding paper on pesticide multiresidue analysis [9], it was shown that most of the around 100 pesticides under investigation exhibited a better response with electrospray ionization than with APCI. A recent, more extensive, comparison of both ionization techniques resulted in a more differentiated picture. APCI response increases with eluent flow rate while, with our instrument, ESI response begins to decrease if the flow rate exceeds 0.1 mL/min. Therefore, a flow rate of 0.4 mL/min was used for APCI, but a flow rate of 0.2 mL/min was used for ESI measurements.

Suitable transitions from precursor to product ions (MRM transitions) were identified with the help of the automatic tune function of instrument software. Transitions from most abundant precursor to most abundant product ions were usually selected. The most abundant precursor for most pesticides was the $[M + H]^+$ ion. Small fragments with m/z ratios <80 were omitted if alternative product ions were available. In order (i) to achieve a stable and high abundance of precursor ions, (ii) to select two suitable mass transitions and (iii) to optimize the yield of product ions, each analyte was tuned individually. Since preliminary experiments had shown an influence of the flow rate on the declustering potential, the syringe pump was operated at a flow rate of 0.2 mL/min. In order to detect interference with such solvent clusters, which may occur during an LC run, water/methanol (1 + 1) with 5 mmoles/L ammonium formate was chosen as a solvent for tuning.

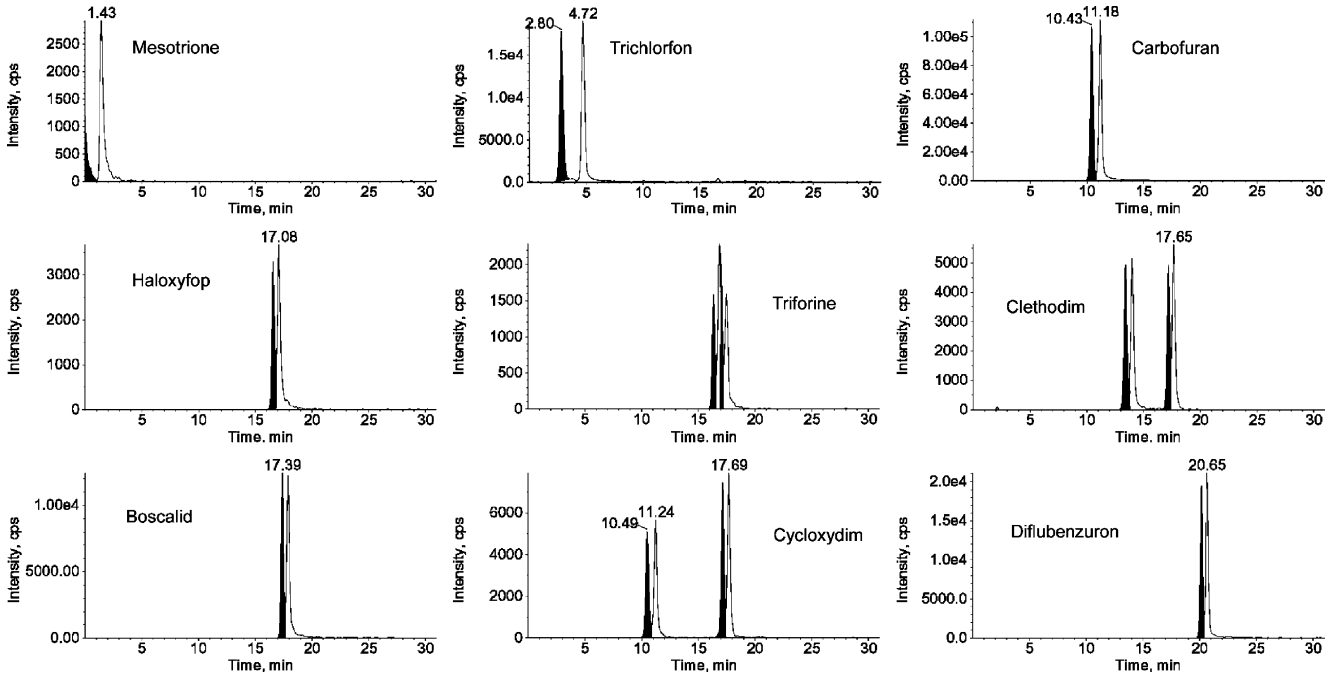

Fig. 2. MRM traces of nine pesticides obtained with HPLC/APCI–MS/MS using ECHO technique. The same standard in solvent containing the analytes at 0.1 μg/mL is used in first (echo) and second (sample) injection. The injected amount is 2 ng/analyte, which is corresponding to a residue concentration of 0.1 mg/kg.

The most important analyte-dependent parameters DP and CE thus found are summarized in Table 2. Dwell time for the MRMs was set to 75 ms and 25 ms in APCI and ESI measurements, respectively. Both values allowed the generation of enough signal points for a good description of the peaks of the individual pesticides in the chromatogram. A sufficient APCI response was obtained for 24 pesticides. Using alternative electrospray ionization 58 analytes gave adequate signal intensity. From these compounds, 12 pesticides were investigated with both ionization techniques.

3.2. ECHO technique applied to identical standard injections

A chromatogram of nine pesticides obtained by applying the ECHO peak technique is presented in Fig. 2. The same standard mixture vial was used as ECHO standard and for sample injection. Except for the very early eluting compounds the ECHO peaks appear in close proximity throughout the whole chromatogram, which is an important prerequisite for achieving satisfactory results. Fig. 2 allows at a glance the match of any peak to be seen along with the corresponding ECHO peak or their difference in size. Further, this kind of presentation is not effected by the variation in the ionization response between the various pesticides.

In Fig. 2, a specific problem of very early eluting pesticides is demonstrated in the first trace. When including very polar pesticides such as mesotrione, the peak of first injection is lost. With our LC/MS software, only one autosampler can be controlled and the mass spectrometric detector is not able to continue acquisition of ECHO standard data while the instrument is cycling to the second injection. As a result, most of first injected mesotrione has already passed through the chromatographic column before the mass spectral data acquisition is started following the second (sample) injection. One simple solution for improving the retardation of mesotrione at the front of the chromatographic column is to increase the water content in mobile phase A to over 80%. But this approach was found not to be feasible because the sample extract after cleanup had to be taken up in some methanol to dissolve all pesticide residues in the sample, resulting in a methanol/water ratio of 20:80 in the final extract. Additionally, preliminary chromatographic experiments with a higher water proportion in mobile phase A, applying reference standards, resulted in a clear distortion of the peak shape of many analytes because the less polar compounds are not sufficiently soluble in water.

Another problem occurs if pesticides consist of two (or more) isomers with similar retention. In Fig. 2, the trace of triforine may serve as an example. In the chromatogram of its MRM transition, the peak resulting from the second triforine isomer of the first injection elutes simultaneously with the peak of the first isomer of the second injection. Consequently, only three instead of four peaks are obtained and ECHO technique is hardly usable for quantitation of that compound. In the case of clethodim and cycloxydim each of which consists of two isomers with different retention, such problems did not occur. In the MRM traces of these two compounds, both peak pairs are observed to be well separated and each isomer peak shows its own ECHO peak.

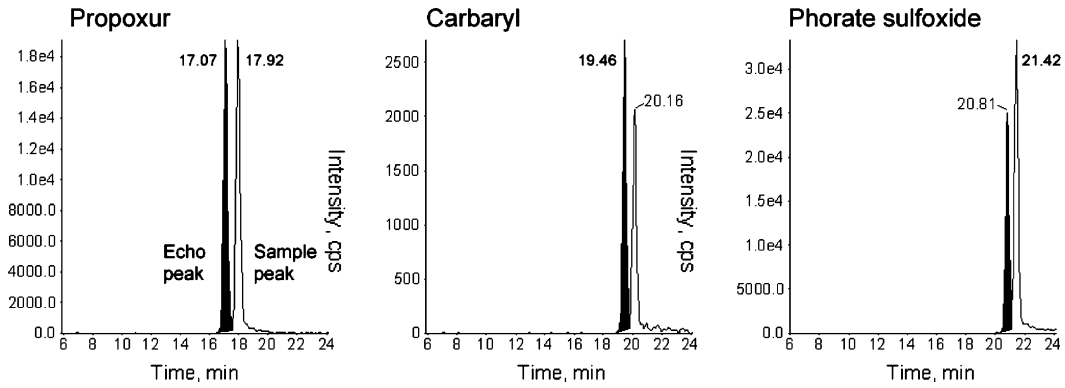

Fig. 3. Three selected MRM windows (ESI) from the extract of lemon fortified with 58 pesticides at 0.1 mg/kg. Assuming a recovery of 100% the obtained analyte concentration in the sample extract is 0.1 μg/mL. First (echo) injection: standard in solvent containing the analytes at 0.1 μg/mL (black peaks). Second (sample) injection: extract of spiked lemon (transparent peaks).

For the reasons explained above, mesotrione and triforine cannot be analyzed with the ECHO technique and are excluded from further discussion.

3.3. Application of ECHO technique to foodstuffs

In Fig. 3, an extract of a chromatogram from a recovery experiment is shown with 3 of the 58 pesticides added to lemon at a residue concentration level of 0.1 mg/kg. The three pesticides carbaryl, phorate sulfoxide and propoxur were selected for presentation. It can be seen that the ECHO standards and the pesticides added to the food sample elute closely and exhibit similar peak areas. The first eluting ECHO standard is marked by shading the peak area. In this experiment, the concentration of the ECHO standard is chosen to be the same as the spike concentration. Furthermore, the three MRM windows, which are representative for all the other pesticides under investigation show no interfering peaks in the whole chromatogram between 2 min and 30 min. This means, that the general use of a confirmatory second transition may not be necessary with this matrix. In the case of a violation of a MRL, however, the confirmatory second transition can be easily performed.

In Fig. 4, results from an extract of a lemon sample from the market, which was found to be free of any pesticide residue are shown. In this case, the ECHO standard mixture of 58 pesticides is applied at a concentration level of 0.1 mg/kg again. The three ECHO signals of carbaryl, phorate sulfoxide and propoxur can be easily recognized but second peak is neither shown in any of the three MRM windows nor in the whole chromatogram. As may be drawn from Figs. 2 and 3, however, a positive pesticide residue detection must appear very closely behind the corresponding reference standard. The same procedure is applied for checking the method at the lowest calibration levels, which is meaningfully carried out in pesticide multiresidue analysis in foodstuffs at 0.01 mg/kg. At a glance, it is possible to see if all the calibrated pesticides in the method are found with sufficient sensitivity and recovery at the lowest concentration level of the MRLs.

This brings us to another real sample from the market. Pears were analyzed with the ECHO technique by adding the ECHO standard at the 0.01 mg/kg concentration level. One of the 22 pesticides under investigation was easily recognized as can be drawn from Fig. 5. While 21 MRM windows showed only one peak, two peaks appeared in close formation in the window of triflumuron with the second sample peak

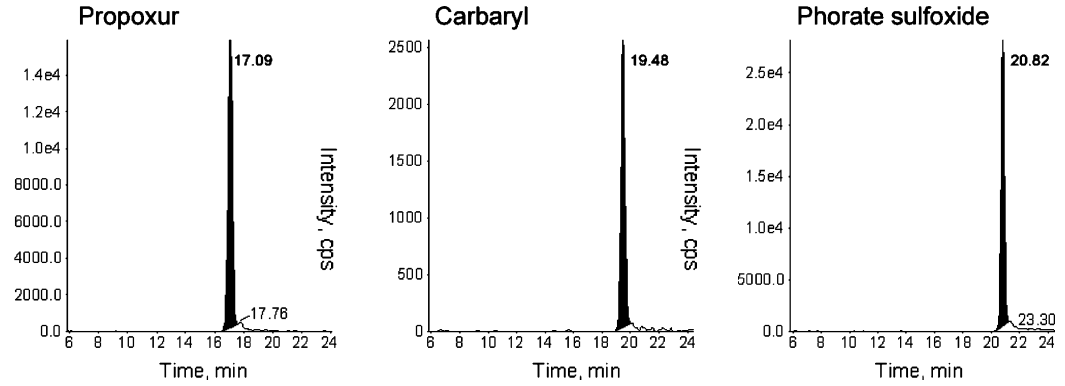

Fig. 4. Identical three MRM windows (ESI) from the extract of non-fortified lemon using the ECHO technique. Sample peaks are not observed. First (echo) injection: standard in solvent containing the analytes at 0.1 μg/mL (black peaks). Second (sample) injection: extract of blank lemon.

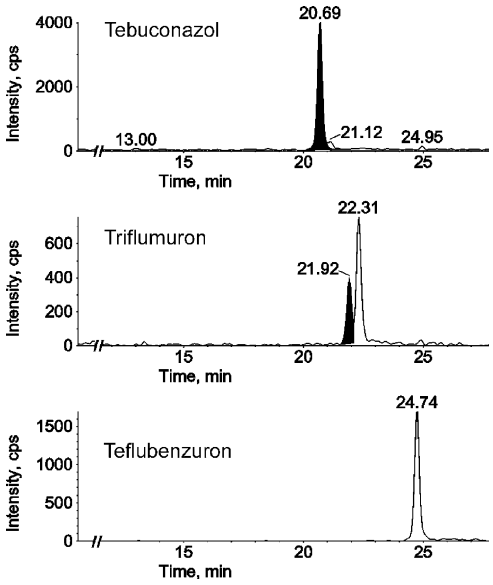

Fig. 5. Pears from the market. Check for 24 pesticides with APCI at the limit of 0.01 mg/kg by means of the ECHO technique. The MRM windows of tebuconazol (no residue), triflumuron (residue of more than 0.01 mg/kg) and teflubenzuron (apparently no residue; see text and Fig. 6) are extracted. First (echo) injection: standard in solvent containing the analytes at 0.01 μg/mL (black peaks). Second (sample) injection: extract of pears (transparent peaks).

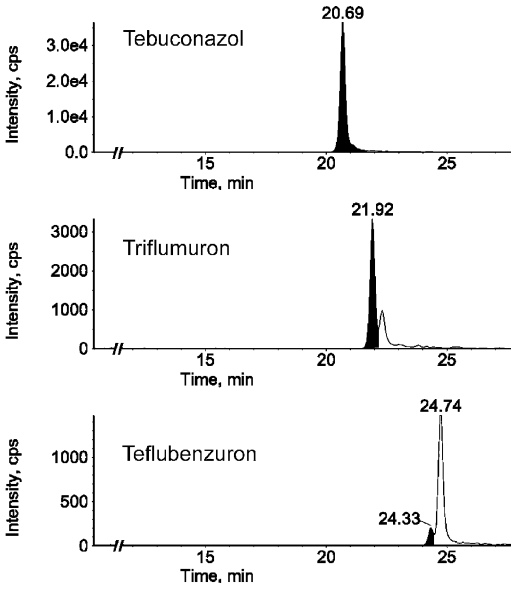

Fig. 6. Same extract of pears as in Fig. 5 checked with ten times higher concentration of ECHO standard. Again the MRM windows of tebuconazol (no residue), triflumuron (residue of less than 0.1 mg/kg) and teflubenzuron (residue ≫ 0.1 mg/kg) are displayed. First (echo) injection: standard in solvent containing the analytes at 0.1 μg/mL (black peaks). Second (sample) injection: extract of pears (transparent peaks).

exhibiting a larger peak area than the leading ECHO standard. Obviously triflumuron was found as pesticide residue in the pear sample at a concentration level clearly higher than 0.01 mg/kg.

At first glance, the absence of a marked ECHO peak in the teflubenzuron trace of Fig. 5 is surprising. The data system normally marks an ECHO peak by shading its peak area. A closer inspection of the teflubenzuron peak lead to a comparison of recorded retention time with the expected value and shows that the recorded peak indeed does not appear at the regular retention time of the teflubenzuron ECHO standard. The large peak in the teflubenzuron MRM window in Fig. 5 eluted 0.4 min later. Due to the high reproducibility of the retention times of ECHO standards and following sample peaks, it became evident that the large peak must have overlapped the ECHO standard at 0.01 mg/kg. The analysis was repeated with an ECHO standard mixture at the concentration level 0.1 mg/kg, MRM windows of this second run are shown in Fig. 6. Again, the ECHO standard of teflubenzuron is quite difficult to spot. It is the tiny peak forming just a front shoulder on the following large peak, which was the pesticide residue teflubenzuron, finally determined at 0.75 mg/kg. This example shows that large differences in concentration levels of ECHO standard and pesticide residue in the sample may lead to some pesticide residues being overlooked. This kind of misinterpretation is favoured by the normalization of the peak height in each MRM window, which gives no indication of the amount of the analyte present in the sample. Therefore, inspection of retention times and comparison of peak area values of standards are indispensable even in this more convenient type of inspection of chromatographic results. However, the evaluation of the MRM chromatograms by the analyst is supported by marking the ECHO standards when appearing at the correct retention times as shaded peaks.

3.4. Occurrence and compensation of matrix effects

The most accurate quantitation method in LC/MS is the application of matrix-matched standards, the simplest method is the application of standards in solvent. Unfortunately, serious matrix effects may occur in atmospheric pressure ionization. Since these effects are caused by co-extracted matrix components, they do not occur in calibration runs of standards in solvent. For this reason, systematic errors may result from the most simple type of calibration. Therefore, a special interest exists in studying the ability of the ECHO technique to compensate matrix effects [12]. Such compensation is expected if ECHO peaks (from standard in solvent) elute with minor time differences to sample peaks, thus being influenced by the same matrix co-eluents.

According to the analytical procedure described, a matrix-matched standard corresponding to the level of 0.1 mg/kg was prepared for each of the food matrices under investigation. The frequency of matrix effects as well as the compensation of these effects by the ECHO technique was tested with the sample setup as outlined in Fig. 7.

In the first experiment, the first and following second injection are made from the same vial containing the standard in solvent, which is mobile phase A. As a result, the chromatograms presented in Fig. 2 were obtained. For the subsequent experiment, firstly the standard in solvent was injected,

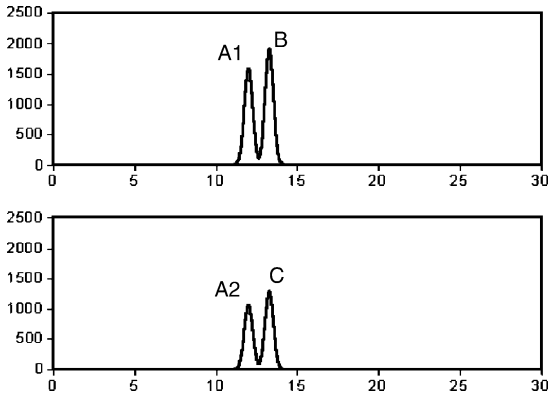

Fig. 7. Sample setup to test the compensation of matrix effects. Top: the same standard in solvent is used in first (echo) and second (sample) injection resulting in peaks A1 and B. Bottom: first (echo) injection of standard in solvent resulting in peak A2 and second (sample) injection of standard in matrix giving peak C. Matrix effect has reduced peak height in A2 and C.

this was followed by an injection of the corresponding standard in matrix 50 s later. One series of measurements was related to one matrix and consisted of three repetitions of the first experiment (both injections with standard in solvent) and six runs of the second experiment (first injection: standard in solvent; second injection: standard in matrix).

If the matrix affects the signal intensity, a difference between the peak areas of peak B (standard in solvent) and peak C (matrix-matched standard) will be observed. We classify such matrix effects as significant if the peak area obtained in the presence of matrix differs more than 20% from the area acquired without matrix. In such cases the area ratio C/B is outside the range 0.8–1.2. Using the APCI source significant matrix effects had been observed in 32% of all measurements with 22 pesticides in 4 matrices. As shown in Table 3, in all significant cases (printed in bold) an enhancement of signal intensity was observed in the presence of matrix. Most frequent matrix effects occurred with extracts of lemon. With this matrix, 80% of signals were found to exhibit a significant enhancement. In the extracts of the other matrices, namely raisins, tomato and wheat flour 27%, 14% and 5% of 22 pesticides exhibited significant matrix effects when applying APCI for MS/MS detection.

With ESI, 58 pesticides were investigated. The results are presented in Table 4. Very similar patterns of matrix effects were observed with ESI and APCI. Again significant matrix effects, that means area ratios C/B < 0.8 or C/B > 1.2, were observed in 32% of all measurements. Even the ranking of matrices with respect to their impact on the signal intensity of the various pesticides is similar when sorting the matrices according to the frequency of matrix effects observed. Most frequent significant matrix effects were observed with lemon. About 70% of all pesticides investigated in lemon was found to be significantly influenced in their signal intensity by matrix. From 58 pesticides in the extracts of raisins, cucumber and wheat flour 38%, 12% and 10%, respectively, showed significant matrix effects with ESI. There is, however, an important difference with respect to the effects on signal intensity between APCI and ESI. Whilst in the application of APCI the signal intensity of pesticides is increased by the co-eluting matrix compounds, in contrast, with ESI the signal intensities of pesticides are usually found to be reduced in the presence of matrix. [Since we did not investigate the mechanism of matrix effects and the published literature does not provide a clear answer, we are not able to explain this observation.]

If the ECHO technique compensates matrix effects, then peak area of ECHO standard A2 and the peak area of matrix-matched standard C should differ to a lesser extent than peaks B (standard in solvent) and C. The best match for any quantitation is found if the ratio C/A2 is close to 1.0. The first question, however, was whether the ratio of two reference standards determined with the ECHO technique is close to 1.0 as can be calculated from B/A1. Similar peak area in this experiment are a prerequisite for precise quantitation of pesticide residues by the ECHO technique. Obviously this requirement is not perfectly fulfilled. From the chromatograms shown in Fig. 2, it can be drawn that often the second peak is larger than the first, although both injections were made from the same vial of reference standard. The peak area ratios B/A1 are also presented for APCI in Table 3 and for ESI measurements in Table 4. With both ionization techniques, the peak areas of the second peaks B are larger than those of the first injection A1. There is no easy explanation of this unexpected observation. Since analytes of the second injection (which results in larger peaks) are passing through precolumn and not those of the first injection, losses within the precolumn can be excluded. However, we noticed that the deviation is often larger in cases of lower resolution between an ECHO peak, which elutes first and the sample peak, which runs behind the ECHO standard. At least in some extent the larger peak area of the second peak in this situation is caused by tailing of the first one not being adequately resolved which thus adds to the area of the second. Some efforts have been made to optimize the LC eluent gradient with respect to sufficient resolution. However, the divergent goals with this technique cannot be ignored: the sample peaks and the ECHO peaks must be close to be affected by the same matrix co-eluents but they should also be adequately resolved to avoid the described tailing phenomenon. This reproducible deviation of the peak area ratio B/A1 from the expected value 1.0 is a systematic error of the ECHO technique. This deviation of B/A1 from 1.0 also shifts the peak area ratios C/A2 to higher values. In a precise study of the compensation of matrix effects this systematic shift must be corrected. Such correction is obtained by dividing the area ratio C/A2 by the systematic error B/A1. This ratio C/A2/B/A1 is presented for each pesticide in each matrix in Tables 3 and 4. These normalized values reflect the compensation of matrix effects without influence of the systematic error.

Significant matrix effects are most often observed in extracts of lemon. Applying APCI, 18 out of 22 pesticides exhibit major effects (bold numbers in Table 3). ECHO technique is able to compensate these effects of lemon matrix in

Table 3
Matrix effects using APCI ionization and its compensation by ECHO technique

Pesticide	Systematic error[b] (ratio B/A1)	Matrix[a]											
		Tomato			Lemon			Wheat flour			Raisins		
		Matrix effect (ratio C/B)	Matrix effect corrected by ECHO peak (ratio C/A2)	Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)	Matrix effect (ratio C/B)	Matrix effect corrected by ECHO peak (ratio C/A2)	Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)	Matrix effect (ratio C/B)	Matrix effect corrected by ECHO peak (ratio C/A2)	Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)	Matrix effect (ratio C/B)	Matrix effect corrected by ECHO peak (ratio C/A2)	Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)
Bendiocarb	1.10	1.02	1.02	0.92	**1.25**	1.12	1.02	0.97	1.11	1.01	1.12	1.08	0.98
Carbaryl	1.14	1.09	1.10	0.97	**1.24**	1.17	1.03	0.94	0.98	0.86	1.08	1.12	0.98
Carbofuran	1.09	1.04	1.03	0.94	**1.28**	1.09	1.00	0.95	1.01	0.93	1.13	1.07	0.98
Clethodim[c]	1.06	0.98	1.06	1.00	**1.54**	0.88	0.83	[d]			[d]		
Cycloxydim[c]	1.06	0.96	1.00	0.95	1.10	0.63	**0.60**	[d]			[d]		
Diflubenzuron	1.15	1.00	1.19	1.03	**1.35**	1.22	1.05	1.05	0.93	0.81	**1.22**	1.11	0.96
Flutriafol	1.24	1.03	1.12	0.90	**1.20**	1.43	1.15	0.92	1.07	0.86	1.10	1.25	1.00
Haloxyfop	1.32	1.12	1.10	0.83	**2.21**	1.31	0.99	1.09	1.06	0.80	**1.36**	1.18	0.89
Imidacloprid	1.21	**1.44**	1.02	0.85	**1.62**	1.11	0.92	1.02	0.92	**0.76**	**1.22**	1.08	0.89
Methiocarb	1.06	1.09	1.07	1.01	**1.45**	1.14	1.07	1.11	1.07	1.01	1.10	1.00	0.94
Methoxyfenozide	1.23	1.05	1.25	1.02	**1.43**	1.26	1.02	1.04	1.06	0.86	**1.21**	1.27	1.03
Metobromuron	1.05	0.97	1.02	0.97	0.93	0.91	0.87	1.02	1.02	0.98	1.11	1.04	0.99
Monolinuron	1.07	0.96	1.01	0.95	1.17	1.11	1.03	0.91	0.94	0.88	1.11	1.06	0.99
Nicobifen	1.14	0.97	1.17	1.03	**1.30**	1.16	1.02	1.04	1.00	0.87	1.15	1.11	0.97
Promecarb	1.06	1.03	1.11	1.04	**1.32**	1.04	0.98	0.99	0.96	0.91	1.05	1.03	0.96
Propoxur	1.07	1.05	1.03	0.96	**1.24**	1.06	0.99	0.91	0.95	0.89	1.04	1.01	0.95
Pymetrozine	1.15	**1.41**	0.92	0.80	**1.36**	1.09	0.95	1.08	1.10	0.96	**1.23**	0.94	0.82
Tebuconazole	1.25	1.03	1.13	0.90	**1.28**	1.32	1.06	1.04	1.10	0.89	0.94	1.05	0.84
Teflubenzuron	1.33	1.02	1.28	0.96	**1.44**	1.21	0.91	1.15	1.20	0.90	**1.49**	1.29	0.97
Thiamethoxam	1.00	1.12	1.06	1.06	**1.88**	1.05	1.05	**1.21**	1.08	1.08	1.16	0.92	0.91
Trichlorphon	1.02	**1.22**	0.95	0.94	**1.38**	1.03	1.01	0.98	1.01	0.99	1.16	0.99	0.97
Triflumuron	1.26	0.99	1.26	1.00	**1.38**	1.34	1.06	1.07	1.05	0.83	1.08	1.13	0.90
No. of ratios outside the range 0.8–1.2		3		0	18		1	1		1	6		0

[a] Mean of six determinations. Ratios outside the range 0.8–1.2 printed in bold.
[b] Mean of 15 determinations.
[c] Sum of peak area of both isomers.
[d] Standard in matrix not sufficiently stable.

Table 4
Matrix effects using ESI ionization and its compensation by ECHO technique

Pesticide	Systematic error[b] (ratio B/A1)	Matrix[a]											
		Cucumber			Lemon			Wheat flour			Raisins		
		Matrix effect (ratio C/B)	Matrix effect corrected by ECHO peak (ratio C/A2)	Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)	Matrix effect (ratio C/B)	Matrix effect corrected by ECHO peak (ratio C/A2)	Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)	Matrix effect (ratio C/B)	Matrix effect corrected by ECHO peak (ratio C/A2)	Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)	Matrix effect (ratio C/B)	Matrix effect corrected by ECHO peak (ratio C/A2)	Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)
3-Hydroxycarbofuran	1.05	0.94	0.96	0.92	**0.76**	0.89	0.84	0.93	1.07	1.02	1.00	1.05	0.99
5-Hydroxy-clethodim-sulfone	1.22	1.11	1.29	1.06	**1.39**	1.11	0.92	1.00	1.24	1.02	1.13	1.17	0.96
Aldicarb	1.07	0.89	0.97	0.90	**0.67**	1.20	1.12	0.99	1.13	1.06	0.80	0.94	0.88
Atrazine	1.30	**0.77**	1.17	0.90	**0.41**	1.68	**1.29**	0.92	1.30	1.00	**0.67**	1.14	0.87
Azoxystrobin	1.09	0.86	1.08	0.99	**0.78**	1.26	1.15	0.90	1.06	0.97	0.88	1.11	1.02
Butocarboxim	1.06	0.94	1.01	0.95	**0.69**	1.13	1.07	1.03	1.13	1.06	0.82	0.96	0.90
Carbaryl	1.05	0.95	1.02	0.97	**0.53**	0.84	**0.80**	0.88	1.07	1.02	0.99	1.25	1.19
Carbofuran	1.11	0.91	1.11	1.00	**0.56**	1.16	1.05	0.96	1.14	1.03	**0.79**	0.99	0.90
Clethodim[c]	1.15	1.11	1.45	**1.26**	**0.52**	0.88	**0.76**	0.91	1.05	0.91	**0.76**	0.99	0.86
Clethodim-imin-sulfone	1.08	1.00	1.06	0.98	1.11	1.15	1.07	0.96	1.10	1.02	1.06	1.06	0.99
Clethodim-imin-sulfoxide[c]	1.03	1.00	1.03	1.00	0.88	1.06	1.03	0.92	1.00	0.97	0.99	1.00	0.97
Clethodim-sulfone	1.27	1.15	1.33	1.04	**0.66**	0.76	**0.60**	0.96	1.31	1.03	0.97	1.30	1.02
Clethodim-sulfoxide[c]	1.16	1.18	1.38	1.19	**0.56**	0.77	**0.66**	1.01	1.23	1.06	1.01	1.26	1.09
Cyprodinil	1.23	0.92	1.24	1.01	0.80	1.15	0.93	**0.72**	1.13	0.92	**0.79**	1.27	1.03
Demeton-s-methyl	1.19	0.86	1.07	0.90	**0.62**	1.09	0.92	1.05	1.32	1.11	**0.58**	0.80	**0.68**
Demeton-s-methyl-sulfone	1.02	0.97	1.09	1.07	0.86	1.26	**1.23**	0.97	1.07	1.06	0.96	1.17	1.15
Dimethoate	1.14	0.91	1.06	0.93	**0.60**	0.95	0.83	1.00	1.14	0.99	**0.78**	1.07	0.93
Ethiofencarbsulfone	1.07	0.90	0.97	0.91	**0.75**	0.98	0.92	0.94	1.04	0.98	0.93	1.08	1.01
Ethiofencarbsulfoxide	1.07	0.96	1.06	0.99	**0.74**	1.02	0.95	0.95	1.07	1.00	1.11	1.30	**1.21**
Fenhexamid	1.12	**0.69**	0.88	**0.79**	**0.73**	1.08	0.97	0.85	1.03	0.92	0.80	1.07	0.96
Fenoxycarb	1.17	0.84	1.13	0.97	0.90	1.24	1.06	0.80	1.14	0.98	0.85	1.31	1.12
Fenpropimorph	1.19	**0.74**	1.13	0.95	0.90	1.19	1.00	**0.43**	1.18	0.99	**0.64**	1.19	1.00
Fluazifop-P-butyl	1.50	0.87	1.38	0.92	0.88	1.42	0.94	**0.71**	1.33	0.88	**0.66**	1.24	0.82
Furathiocarb	1.22	0.97	1.26	1.03	0.86	1.15	0.94	**0.69**	0.98	0.81	**0.67**	0.98	0.81
Imazalil	1.39	**0.79**	1.18	0.85	0.81	1.49	1.07	0.89	1.81	**1.30**	**0.54**	0.92	**0.66**
Imidacloprid	1.07	1.03	1.05	0.98	1.06	1.30	**1.21**	1.01	1.12	1.04	0.99	1.08	1.01
Imidacloprid hydroxide	1.11	0.90	1.16	1.04	0.97	0.85	**0.76**	0.89	1.05	0.94	**1.22**	1.37	**1.24**
Imidacloprid olefin	1.08	1.20	1.25	1.16	1.15	1.13	1.04	1.12	1.30	1.20	0.95	1.05	0.97
Indoxacarb	0.72	0.91	0.80	1.11	0.95	0.70	0.98	0.93	0.95	**1.33**	1.05	0.81	1.14
Iprovalicarb[c]	1.08	0.89	1.11	1.03	**0.77**	1.13	1.05	0.94	1.13	1.04	0.88	1.16	1.07
Isoproturon	1.10	0.85	1.07	0.97	**0.60**	0.97	0.88	0.96	1.08	0.98	0.85	1.27	1.15
Isoxaflutole	1.23	0.97	1.13	0.92	**0.55**	0.92	**0.75**	0.96	1.17	0.95	**0.36**	0.44	**0.36**
Linuron	1.03	0.80	1.04	1.01	**0.67**	1.46	**1.42**	0.84	1.05	1.03	0.83	1.07	1.05
Metalaxyl	1.10	0.84	1.04	0.95	**0.71**	1.44	**1.31**	0.91	1.09	0.99	0.89	1.14	1.04
Methiocarb	1.16	0.84	1.16	0.99	**0.73**	1.37	1.18	0.90	1.11	0.96	0.85	1.26	1.09
Methoxyfenozide	1.04	0.87	1.08	1.04	**0.70**	1.02	0.99	0.93	1.02	0.98	0.83	1.05	1.01
Metolachlor	1.17	0.81	1.19	1.02	**0.72**	1.13	0.96	0.87	1.13	0.96	**0.78**	1.11	0.95
Monocrotophos	1.07	0.92	1.00	0.93	**0.79**	1.05	0.98	0.98	1.10	1.02	0.94	1.10	1.02
Omethoat	1.00	0.89	0.87	0.88	**0.70**	0.73	**0.73**	0.90	0.95	0.95	**0.71**	0.70	**0.70**
Oxamyl	1.10	0.95	1.45	**1.32**	0.85	2.23	**2.03**	0.93	1.82	**1.66**	0.88	2.11	**1.92**
Oxydemeton-methyl	1.02	1.03	1.13	1.11	**0.76**	1.27	**1.24**	0.91	1.14	1.12	0.95	1.65	**1.62**
Phorate sulfoxide	1.18	0.89	1.09	0.92	**0.53**	1.31	1.11	0.93	1.22	1.04	**0.79**	1.10	0.93
Picoxystrobin	1.13	0.94	1.13	1.00	0.86	1.18	1.04	0.89	1.20	1.07	0.83	1.11	0.99
Pirimicarb	1.16	0.87	1.11	0.96	**0.53**	1.84	**1.59**	0.93	1.12	0.96	**0.79**	1.06	0.91
Promecarb	1.31	**0.73**	1.09	0.83	**0.66**	1.20	0.92	0.87	1.21	0.92	**0.65**	1.09	0.84
Propamocarb	1.12	0.95	1.06	0.95	**0.79**	1.14	1.01	0.95	1.10	0.98	0.98	1.13	1.01
Propoxur	1.09	0.88	1.00	0.92	**0.56**	0.99	0.92	0.96	1.11	1.03	0.88	1.14	1.05
Pymetrozin	1.08	1.00	1.06	0.98	**0.77**	0.91	0.84	0.96	1.08	0.99	0.94	1.11	1.02
Pyraclostrobin	1.33	**0.75**	1.10	0.83	0.81	1.47	1.11	**0.69**	1.09	0.82	**0.74**	1.27	0.96
Pyridat-metabolite	0.96	0.89	1.01	1.06	**0.58**	0.92	0.96	**0.70**	0.73	**0.77**	**0.75**	1.02	1.07
Pyrimethanil	1.08	**0.78**	1.07	0.99	**0.64**	1.25	1.15	0.97	1.13	1.05	**0.76**	1.01	0.93
Quinmerac	1.19	0.89	1.48	**1.25**	**0.64**	1.17	0.99	0.95	1.51	**1.28**	**0.79**	2.07	**1.75**
Spiroxamine	1.35	0.85	1.22	0.90	0.93	1.46	1.08	0.85	1.24	0.92	0.84	1.29	0.96

Table 4 (*Continued*)

Pesticide	Systematic error[b] (ratio B/A1)	Matrix[a] Cucumber: Matrix effect (ratio C/B)	Cucumber: Matrix effect corrected by ECHO peak (ratio C/A2)	Cucumber: Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)	Lemon: Matrix effect (ratio C/B)	Lemon: Matrix effect corrected by ECHO peak (ratio C/A2)	Lemon: Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)	Wheat flour: Matrix effect (ratio C/B)	Wheat flour: Matrix effect corrected by ECHO peak (ratio C/A2)	Wheat flour: Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)	Raisins: Matrix effect (ratio C/B)	Raisins: Matrix effect corrected by ECHO peak (ratio C/A2)	Raisins: Matrix effect corrected by ECHO peak and by systematic error (ratio C/A2/B/A1)
Tebuconazole	1.15	0.89	1.12	0.97	**0.78**	1.13	0.98	0.80	1.43	**1.24**	**0.54**	0.84	**0.73**
Tebufenozid	1.06	0.91	1.00	0.94	0.88	1.11	1.05	0.85	1.08	1.02	0.80	1.05	0.99
Thiabendazole	1.50	0.88	1.55	1.03	**0.52**	1.29	0.86	0.97	1.53	1.02	0.82	1.66	1.11
Thiacloprid	1.13	0.85	1.01	0.90	**0.49**	0.90	0.80	0.96	1.13	1.01	0.86	1.12	1.00
Vamidothion	1.08	0.92	1.03	0.95	**0.71**	0.92	0.85	0.95	1.09	1.00	0.92	1.04	0.96
No. of ratios outside the range 0.8–1.2		7		4	40		14	6		6	22		10

[a] Mean of six determinations. Ratios outside the range 0.8–1.2 printed in bold.
[b] Mean of 15 determinations.
[c] Sum of peak area of both isomers.

nearly all cases if the systematic error is taken into consideration with the exception of the ever problematic pesticide cycloxydim. In extracts of tomato and raisins, no significant matrix effects remained if ECHO technique was applied and the correction of the systematic error was performed. In extracts of wheat flour, the impact of matrix on the signal intensity of pesticides is not very distinctive when applying APCI and with the ECHO technique no improvement was obtained with respect to quantitative determination.

When applying ESI, matrix effects are compensated by ECHO technique to a somewhat lesser extent. A significant reduction of signal intensity in the presence of lemon extracts was observed for 40 pesticides (bold ratios C/B in Table 4). With 26 analytes, these matrix effects disappeared when applying the ECHO technique instead of separate runs of standard in matrix and standards in solvent. In extracts of cucumber, the use of the ECHO technique in our ESI measurements reduced the small number of significant matrix effects from seven to only four. The somewhat more significant matrix effects seen with extracts of raisins were also clearly reduced by the ECHO technique from 22 to 10. Extracts of wheat flour showed less frequent matrix effects and therefore little changes were found with respect to the quantitative results when applying ESI.

Our results demonstrate that there is no obvious difference between the frequency of matrix effects observed in APCI and ESI measurements. But the way the quantitative outcome is affected by the matrix is opposite. The presence of matrix enhances analyte peak intensity when APCI is applied and causes reduction of signal intensity in ESI measurements. In total, more than 90% of all major matrix effects observed with APCI and more than 50% of significant effects detected in ESI measurements were found to be compensated by application of the ECHO technique.

4. Conclusion

In this paper, the new ECHO technique has been applied in a multiresidue pesticide analysis with about 70 pesticides. Studies were performed with these pesticides in four different foodstuff matrices. It could be demonstrated that calibration by ECHO technique produces in >70% of the cases of significant matrix effects better results compared to standards in solvent, which are injected in a separate run. Therefore, application of ECHO technique is the better alternative in first screening measurements to identify samples with residues above maximum residue limits. Such screening does not need perfect calibration and can be performed without matrix-matched standards.

There are, however, additional important advantages of the ECHO technique:

1. a retention time shift is easily recognized for each of the pesticides included in the method;

2. a permanent check of the lowest calibration level is achieved by injecting the reference standard at the lowest calibration level;
3. it is much cheaper to use normal reference standards than stable isotope labeled reference standards and easily to perform because the reference standards must be at hand anyway for the development and the daily application of the LC–MS/MS method;
4. a direct comparison with critical concentration levels is easily performed by applying the ECHO standards at the relevant concentration level, e.g. the MRLs of the individual pesticides in the specified foodstuffs or the lowest MRL for all pesticides (e.g. at the 0.01 mg/kg concentration level);
5. a screening at the relevant concentration levels allows fast recognition of samples free of pesticide residues (no double peak appears in any of the MRM windows) and those with pesticide residues present (a double peak appears in one or several of the MRM windows, allowing a crude estimation of the pesticide residue concentration level by comparison with reference standard concentration level used in the ECHO technique);
6. separate injections of reference standard solutions may be omitted, which makes sequences faster.

The only limitation of ECHO technique is the inability to estimate the amount of an incurred residue, if the difference between the pesticide concentration in the sample and the ECHO standard does not allow the integration of the smaller peak.

Summarizing the merits of the ECHO technique, we want to emphasize that, after a simple cleanup, the differentiation between uncontaminated food samples and those with pesticide residues can be achieved in one single analytical screening run. Additionally, by comparison of the peak areas of sample peaks and ECHO peaks an estimation of the concentration levels of the recognized pesticides can easily be carried out. In our opinion, these points make a marked contribution to the enhancement of productivity in the daily routine work of pesticide residue analysis applying LC–MS/MS.

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