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Journal of Chromatography A, 1000 (2003) 181–197
JOURNAL OF CHROMATOGRAPHY A
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Review

Matrix effects in (ultra)trace analysis of pesticide residues in food and biotic matrices

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Abstract

If one has to determine multiple residues of modern pesticides and/or other groups of (semi)polar and/or thermally unstable contaminants with a wide range of physico-chemical properties within a single analytical run, it can be complicated to obtain low limits of quantitation (LOQ), good precision as well as relevant trueness of results for all the target analytes. Matrix components, which are unavoidably present in analyzed samples (even after the thorough clean-up step), may be responsible for miscellaneous adverse affects impairing different stages of the GC and/or LC determinative step. In this review, the nature of various types of matrix effects are discussed together with suggestions for prevention, reduction and/or compensation of their occurrence when determining troublesome analytes in foods and other complex biotic matrices.

Keywords: Reviews; Matrix effects; Accuracy; Calibration; Trace analysis; Injection techniques; Pesticides; Contaminants

Contents

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0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00539-9

1. Introduction

Identification/quantitation of organic contaminants in biotic matrices is the subject of analytical work in many laboratories worldwide. Food commodities probably constitute the major part of examined samples. Several groups of contaminants (such as pesticide residues, residues of veterinary drugs, industrial pollutants, mycotoxins) which may have a negative impact on food quality can occur in food as a result of the various stages of its production, packaging, transport, holding and/or environmental contamination. As the presence of such compounds not intentionally added to food is a matter of concern for public health, great attention is paid to an official residue control by the competent national authorities. Surveillance/compliance programs represent one of the important measures taken by all developed countries to minimise contaminants in foodstuffs. The present paper attempts to address some of the issues closely related to the accuracy of the generated data. Their quality is obviously a prerequisite of effective risk analysis and therefore any potential source of errors, such as matrix effects discussed below, has to be closely investigated and characterised.

It should be noted that analytical procedures commonly used in food analysis can also be employed without any fundamental modifications for examination of various matrices of plant/animal origin and/or human tissues. On that account the same strategies to prevent/minimise potential matrix effects should be applied when analysing organic contaminants in various environmental or other biotic samples.

2. General strategies in residue analysis

Trace analysis of organic contaminants in food and/or biotic samples typically consist of the following consecutive steps: (i) isolation of analytes from sample matrix, (ii) removing of bulk co-extracts from crude extract and (iii) identification and quantification of target analytes. As far as not sufficiently specific detector is used i.e., when the combination of retention time with detection principle does not avoid false positive results, additional (iv) confirmation step should be realised. Separation techniques represented nowadays mainly by gas, GC, and liquid chromatography, LC, are mainly applied to accomplish final determinative steps [1]. Although the instrumental configuration and setting of operational conditions greatly predetermine performance characteristics of respective method, the key role in many cases plays a choice of appropriate sample preparation strategy corresponding to steps (i) and (ii) above.

In practice, cost-effective as well as labour saving multiresidue methods enabling determination of multiple analytes within a single run are widely used [2]. However, in general terms, the wider the range of physico-chemical properties of target analytes; the more complicated is an efficient removing of co-isolated matrix components from a particular crude extract. Depending on their nature (molecular size, polarity, thermal stability, volatility etc.), these substances may interfere in various stages of chromatographic process. Since the character of phenomena responsible for adverse effects on the quality of analytical data in GC and LC based methods is different, relevant issues are discussed in two separate sections.

Residues of modern pesticides were selected as an example of target analytes, the determination of which may suffer from poor accuracy because of various effects that are briefly reviewed in the next sections. One should emphasize that the same problems exist in the determination of many other target analytes when occurring in particular samples at (ultra)trace concentration levels.

3. Matrix effects in gas chromatography

In performing analyses of complex samples, miscellaneous problems can be encountered, which are caused by the matrix unavoidably present in the sample injected into the GC system. Such problems may occur both at the detector and the injector site. Good understanding of their nature and in-depth knowledge of the general principles of preventive/corrective measures that can be applied under real-life conditions is undoubtedly of great importance for obtaining high quality results. It should be noted that some phenomena related to an impaired accuracy in

the determination of these (semi)polar/thermally unstable analytes are not encountered when ''classic'' environmental contaminants, typically very hydrophobic compounds, are the subject of GC analysis.

3.1. Interferences of matrix with detection process

As far as conventional-element, structure or functional group selective detectors are used (electron capture, ECD, nitrogen phosphorus, NPD, and flame photometric, FPD, detectors are the most common in residue analysis), elution of volatile impurities with an identical retention time as the analyte may result in manifold unfavourable consequences, the most pronounced being: (i) the peak of the analyte is obscured (masked) → false negative result; (ii) the impurity is falsely identified as analyte, which is in reality absent → false positive result; (iii) detector signal is increased → overestimation of the result; (iv) quenching of the detector signal (this problem is serious for the ''classic'' flame photometric detector, FPD; significant improvement is provided by pulsed FPD) → underestimation of result. While, as discussed later, the last two problems can be compensated for by adopting an appropriate strategy for calibration; false positive and false negative results can both be hardly avoided if confirmation of the results by an independent alternative analytical procedure is not carried out. Mass spectrometric detectors, MSDs (particularly those with specific ion monitoring) obviously provide higher specificity based on a more detailed reflection of the molecular structure of a particular compound. However, adding a further dimension to the chromatogram does not necessarily guarantee positive handling of co-elution problem. Common low resolution MSDs employing quadrupole and/or ion trap analyzers can under some circumstances fail to provide unbiased confirmation of analyte identity. This might be a case especially for compounds yielding non-specific ions of low m/z values by electron impact ionisation process. Under these circumstances achieving of unbiased identification and accurate quantitation alike may become unfeasible because of interfering matrix ions. Both the use of a chromatographic system with increased separation power and employing a mass spectrometric detector possessing high detection potential particularly in terms of mass resolution and/or data acquisition rate theoretically offer conceivable solutions. Regarding the first option, unfortunately, in spite of growing number of high resolution GC columns coated with phases dedicated for specific groups of contaminants and generally improved performance of these capillaries (''extra low'' bleed, good stability even at very high temperatures etc.), the complete separation of all the sample components is practically not attainable for very complex samples in a single, one dimensional run.

The breaking innovation in many analytical areas including trace analysis of organic contaminants occurring in food and other biotic samples represents application of so-called comprehensive (or orthogonal) gas chromatography. Although this form of two-dimensional technique (GC×GC) was introduced by Liu and Phillips [1] already in 1991 it has become commercially available only recently. Comprehensive GC×GC shows remarkably enhanced separation potential [3–7] since it enables the chromatogram to spread into two independent dimensions in which the compounds in the mixture are separated by independent mechanisms. Thanks to separation of analytes from ''chemical noise'' and also due to obtaining significantly narrower peaks in a second dimension hence increased signal to noise, the gain of detection sensitivity [8,9] provides an important input in residue analysis. Until recently the detection in GC×GC was limited to the use of fast analogue detectors such as flame ionisation detector, FID or ECD. However, recent developments in mass spectrometry, particularly commercialisation of time-of-flight, TOF, mass spectrometers providing very fast acquisition rate considerably enlarged the application field of GC×GC techniques [10–12]. A cogent example of an application of GC×GC separation for overcoming problems caused by co-eluting bulky matrix component yielding the same fragmentation ions as those originated from target analyte is shown in Fig. 1. Although still in the state of development (and also still rather expensive), GC×GC/TOF-MS will become undoubtedly a technique of choice in the near future whenever unbiased determination of multiple trace analytes in very complex matrices (including unpurified crude extracts of foodstuffs) is needed. The main prerequisite for routine application of this technique is obviously a further improvement

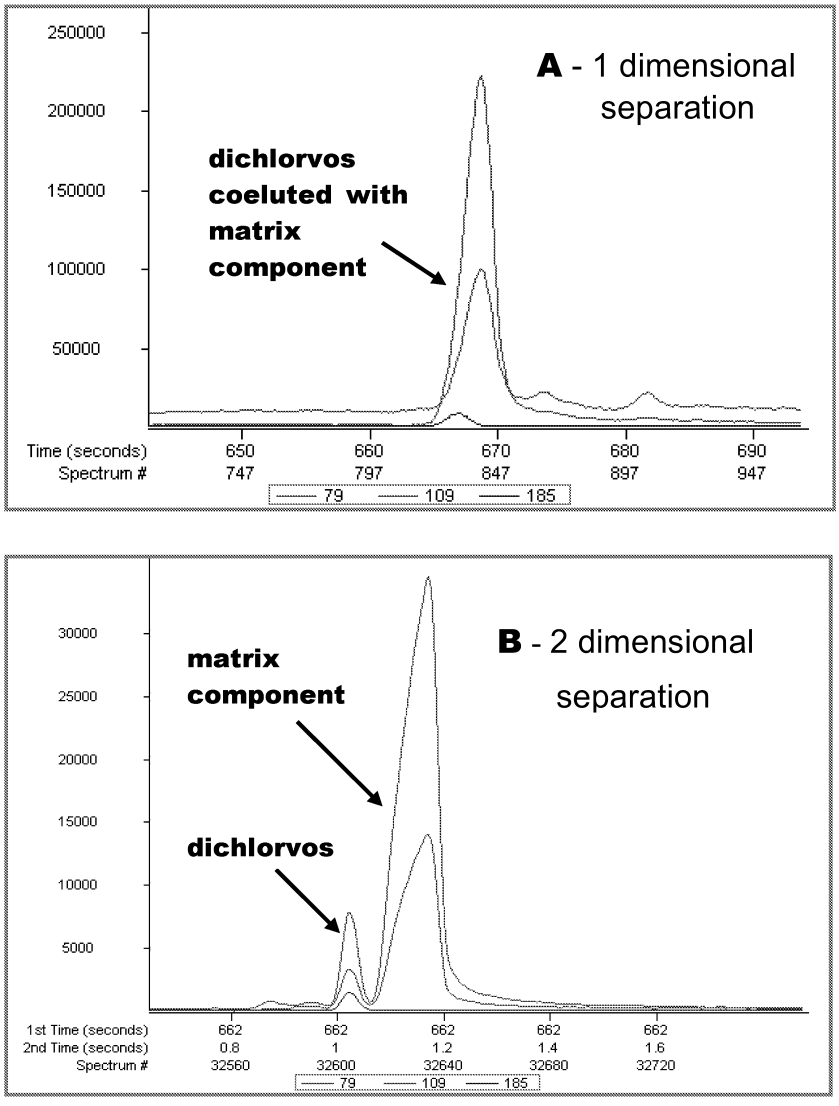

Fig. 1. Illustration of solving co-elution problem in determination of dichlorvos (0.01 mg/kg) in apples. A 1-μl volume of purified extract injected (pulsed splitless). Pegasus 4D (Leco) system consisting of Agilent 6890N GC and Pegasus III MS-TOF used; columns set: 1st DB-XLB (30 m×0.25 mm×0.25 μm), 2nd DB-XLB (1 m×0.1 mm×0.1 μm); TOF setting: acquisition rate 5 Hz, mass range 45–400, detector voltage 1800 V, modulation in 2D separation: time 2 s, modulation temperature offset 30 °C [unpublished results]. A—*m*/*z* 185 is the only available selective ion for quantitation, confirmation of analyte impossible, *m*/*z* 109 and 79 present also in co-eluted compound. B—Analyte resolved from interference, unbiased identification possible (reverse factor match 920).

of respective software to allow substantial reduction of the time needed for data processing.

3.2. Matrix-induced chromatographic response enhancement

Besides the above mentioned detection difficulties occurring in a particular single run due to analyte–analyte and/or analyte–volatile matrix component co-elution, other severe problems might be encountered because of co-injection of non-volatile matrix constituents. Considering common sample preparation procedures, lipids (waxes, triacyl glyceroles, phospholipids etc), various pigments (chlorophylls,

carotenoids, melanoidines etc.) and other higher molecular mass components (e.g. plant resins) soluble in solvent used for extraction represent typical bulk co-extracts, part of which can be contained even in purified samples. Depending on an employed injection technique, their deposits in a GC inlet (and often also in a front part of separation capillary) may lead to successive, in most cases adverse changes in performance of chromatographic system. The loss of analytes, tailing of their peaks hence occurrence of integration problems and impaired detectability (i.e. increased limits of detection, LODs) are distinctive implications of built-up dirt. Fig. 2. clearly demonstrates the dependence of robustness of the GC

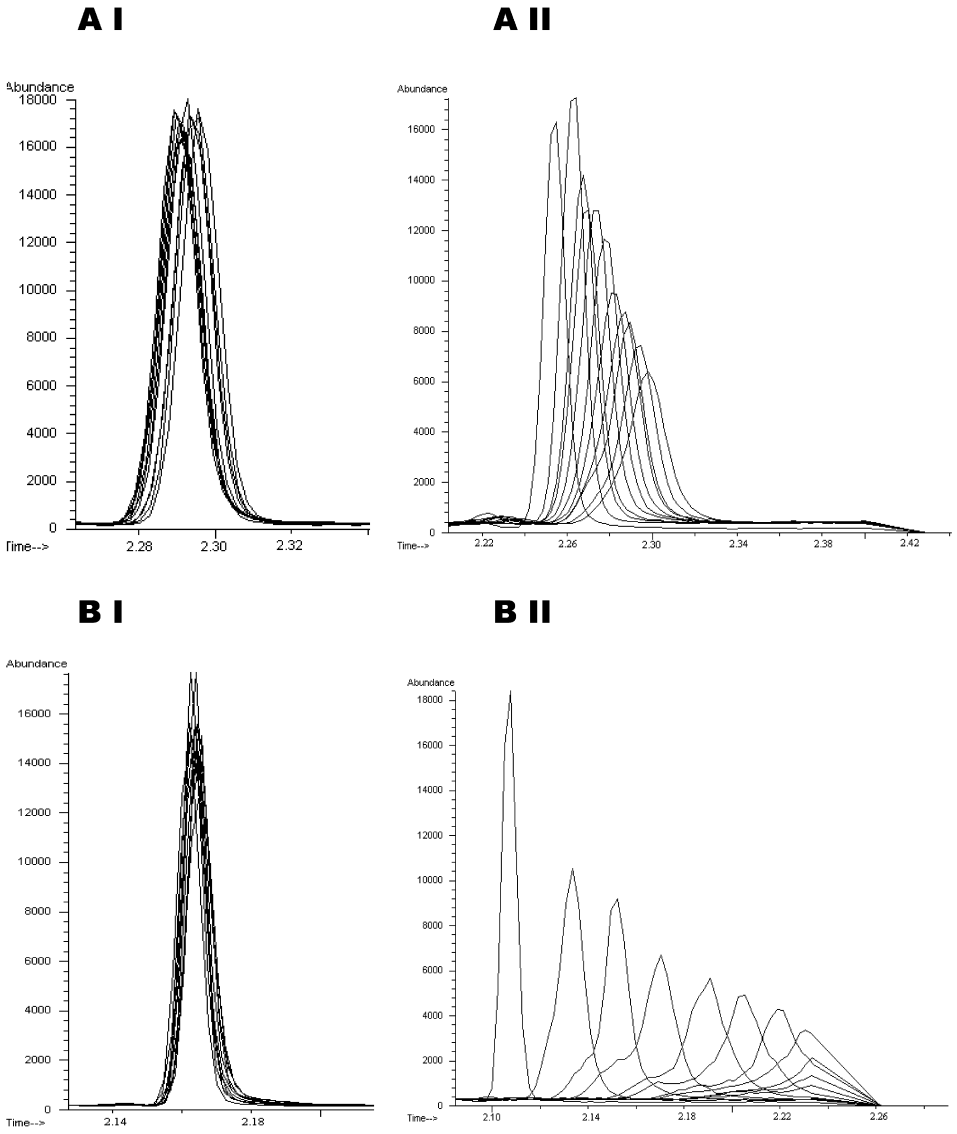

Fig. 2. Illustration of the influence of column size (A has higher capacity compared to B) and efficiency of sample clean-up (in procedure I it was more thorough compared to II) on stability chromatographic systems, repeated ($n = 11$) GC–MS (Agilent 6890 GC coupled with Agilent 5973 MSD) analyses of lindane (m/z 181) in carrot extract [unpublished results]. Columns: A—DB-5 MS, 10 m×0.53 mm, 1 μm film. B—DB-5 MS 10×0.25 mm, 0.25 μm film. Sample preparation procedures: I—Ethyl acetate extraction→HPGPC clean-up (final carrot content = 1 g/ml toluene); II—acetone extraction→dichloromethane–hexane (1:1,v/v) partition (final carrot content = 1 g/ml toluene).

system on experimental set-up (intensity of sample clean-up and capacity of GC column shown here as an example). Both the procedure used for preparation of analytical sample (better efficiency of high performance gel permeation chromatography, HPGPC, in removing of interfering matrix) and parameters of GC column (better tolerance of injected matrix by wide-bore column) are important factors. On the other hand, as mentioned later (masking phenomena), up to certain limits, co-injection of sample components may exhibit certain kinds of beneficial effects in terms of peak shapes and sizes. Actually, priming of GC by injection of ''real'' i.e., matrix containing sample before starting a daily program used to be a basic recommendation provided to analysts as early as in the era of packed columns.

The sources of these phenomena, which are called ''matrix-induced chromatographic response enhancement'', were discussed in-depth for the first time by Erney et al. [14]. In this and following studies [15,16], authors explained the rationale for a poor accuracy of some data generated by routine GC methods employing ''traditional'' calibration strategies for quantitation of more polar residues (in particular case quantitation of organophosphorus pesticides was the subject of study). Rather troublesome quantitation [17] can be experienced for such analytes whenever hot injection, mainly splitless technique representing undoubtedly one of the most popular sample introduction strategies is employed. The problem consists of the presence of various active sites in the injection port (and also in a separation column) that can be responsible for irreversible adsorption and/or catalytic (thermo)decomposition of susceptible analytes. Besides free silanol groups and metals potentially present in a surface of even high quality glass injection liner declared by producers as ''deactivated'', additional active sites can (in a largely unpredictable manner) originate from non-volatile co-extracts in a front part of GC system during repeated analyses of real-life samples. As schematically illustrated in Fig. 3, the amount of molecules of respective analyte introduced into GC column is lower when injected in a net solvent compared to injection realised in the presence of matrix. Molecules of impurities (in trace analysis they are typically the most abundant components of sample) effectively compete for active

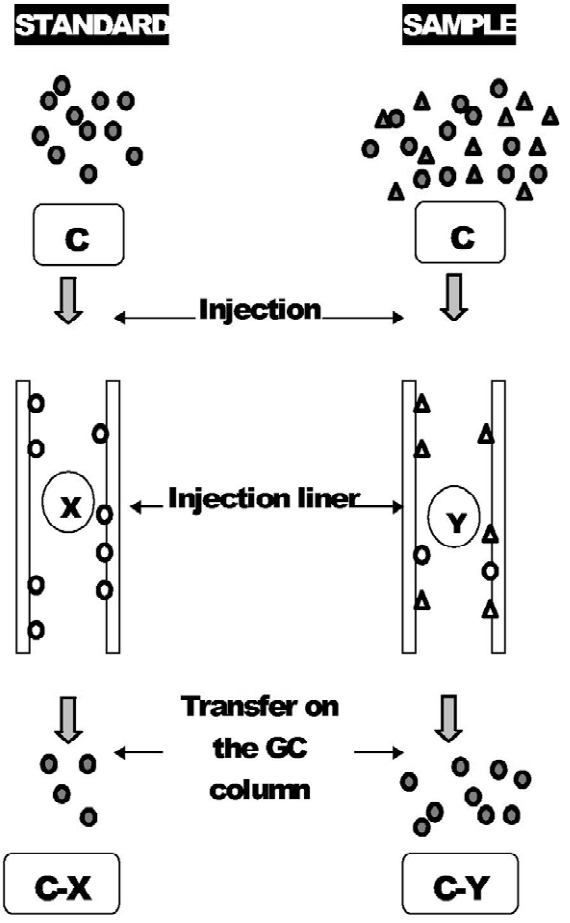

Fig. 3. Simplified illustration of the cause of matrix induced chromatographic enhancement effect; C—number of injected analyte molecules; X, Y—number of free active sites for their adsorption in injector; ● molecules of analyte in injected sample; ○ portion of analyte molecules adsorbed in GC injector; ▲ molecules of matrix components in injected sample; △ portion of matrix components adsorbed in GC liner; (C–X)<(C–Y).

sites with analyte hence reduce its interactions and consequent losses in injector. Similarly the existence of some masking effects of active sites can be presumed for volatile impurities overlapping analyte peak on separation column. As far as—in accordance with common calibration practices—standard in net solvent is used for calibration, overestimation of results may occur. As mentioned earlier, compounds prone to matrix-induced chromatographic enhancement effects are either thermolabile or rather polar and they are typically capable of hydrogen bonding.

Using modern pesticides as a problem demonstration group, compounds containing the following characteristic structures/functional groups in their molecule are typically troublesome in this context [17–19]: (i) organophosphates (–P=O); (ii) carbamates (–O–CO–NH–); (iv) hydroxy compounds (–OH); (v) amino compounds (R–NH–); (vi) imidazoles, benzimidazoles –N=); urea derivatives (–NH–CO–NH–). Examples of severe distortion of results documented as recoveries substantially exceeding 100% are shown in Table 1. It is worth to notice that hydrophopbic, non-polar substances such persistent organochlorine contaminants (with some exceptions such as DDT that may thermally degrade in a dirty injector) are not prone to injection related problems. It is also important to emphasize at this point, that not only the chemical structure of the analyte but also its concentration are factors playing an important role under particular experimental conditions. Generally, with decreasing analyte concentration the overestimation of results becomes more pronounced. The rationale for this effect is obvious from Fig. 3. The ratio (C–Y)/C–X) may dramatically increase when analyte approaches (ultra)trace levels, in marginal case the quantification of analytes is not feasible since the peak of analyte in solvent falls below LOD. Fig. 4 shows an example of such a close to critical situation. Another important factor is of course the composition of examined matrix or better expressed the character and amount of co-extracts left in the sample taken for chromatographic analysis.

3.3. Approaches to avoid overestimation of results

While the use of labelled standards that are affected by analytical procedure in the same extent as native analytes is a common practice in analysis of various groups of persistent environmental contaminants such as PCBs, PCDDs/PCDFs, PAHs etc.,

Table 1
Overestimation of results (calibration based on external matrix-free standards) due to matrix-induced chromatographic enhancement reported for some modern pesticides in various studies

Pesticide	Reported recovery	Ref.	Pesticide	Reported recovery	Ref.
Acephate	114–118	[20]	Heptenophos	233–255	[21]
	136–183	[15]	Chlorothalonil	119–155	[24]
	117–125	[22]	Chlorpyrifos	132–138	[15]
	206–270	[24]		111–121	[24]
Azinphos-Me	194–364	[21]	Iprodione	118–204	[21]
Bromopropylate	114–130	[24]	Malaoxon	111–132	[22]
Captan*	293–1011	[15]	Malathion	112–132	[24]
	145–178	[24]	Methamidophos	128–151	[15]
Carbaryl	174–286	[24]		191–237	[24]
Coumaphos	140–246	[21]	Methidathion	126–159	[24]
Cypermethrin	151–319	[15]	Monocrotophos	192	[15]
Diazion	106–127	[15]		114–145	[22]
Dichlofluanid	129–454	[15]	Omethoate	123–178	[15]
	111–114	[24]		115–145	[22]
	127–296	[21]		157–289	[24]
Dimethoate	111–144	[15]	Phosalone	138–161	[24]
	192–296	[24]	Phosmet	110–129	[22]
	116–130	[22]	Procymidone	152–243	[15]
	112–189	[24]	Propham	115–262	[24]
Etrimfos	125–140	[22]	Tetradifon	129–199	[15]
Fenoxon	133–225	[23]	Tolylfluanid	114–123	[24]
Fenthion-sulfoxide	136–304	[23]	Triadimefon	212–431	[15]
Fenthion-sulphone	114–173	[23]	Triadimenol	230–420	[15]
Folpet*	250–931	[15]	Trichlorphon	150	[15]

Compounds marked by * are thermally unstable.

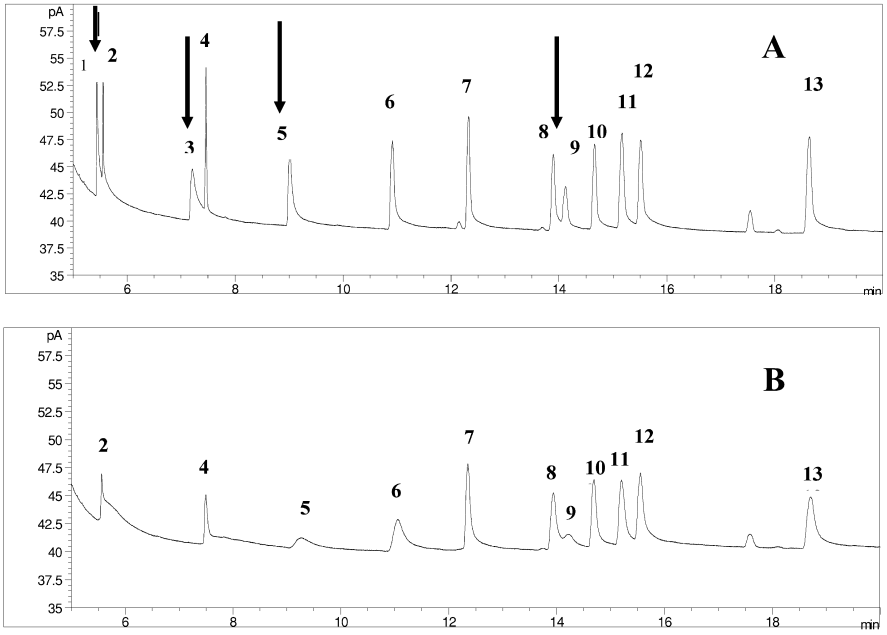

Fig. 4. Long-term stability of the GC/NPD system (Agilent 6890) employed for determination of organophosphorus pesticides in wheat samples. A 1-μl volume of purified extract in toluene containing equivalent to 1 mg of original sample and 10–100 pg of analytes were injected using pulsed splitless injection technique [13]; A—10 injections of wheat samples, B—87 injections of wheat samples. Analytes: 1. Methamidophos, 2. Dichlorvos, 3. Acephate, 4. Propham, 5. Omethoate, 6. Dimethoate, 7. Etrimfos, 8. Tolclofos-Me, 9. Carbaryl, 10. Pirimiphos-Me, 11. Malathion, 12. Chlorpyrifos, 13. Methidathion. Peaks of troublesome analytes are marked by arrows. Note: when using Agilent NPD, some tailing is observed for nitrogen-containing analytes.

this approach is, unfortunately, not practicable in routine multiresidue analysis in which the number of analytes typically approaches one hundred. At present only a very limited number of certified pesticide standards is commercially available and, in addition, because of their inherently lower stability compared to persistent organochlorine pesticides, the expiry period of such standards is shorter. Under real-life conditions, the frequent purchase of expensive standards (even if available) could hardly be managed in a cost-effective way. Because of these problems, other scientifically sound solutions have to be searched.

Two alternative approaches to avoid overestimation of results due to this kind of matrix effects can be considered: (i) elimination of their primary causes and/or (ii) their effective compensation. In practice, these theoretical prerequisites are either poorly achievable or their solution may create a heavy burden for expenses and/or labour. Therefore, reasonable compromises have to be adapted in routine work while still keeping the accuracy of measurements within the range required by regulatory guidelines.

3.3.1. Minimisation of the primary causes: no active sites in GC system, no matrix in sample

The concept of the GC system free of active sites is in principle not viable not only because of the unavailability of commercial virtually inert materials stable even under long term exposure to high temperatures (those set in ‘‘hot’’ injection port typically exceed 200 °C) but also due uncontrollable formation of new active sites from deposited non-volatile matrix.

A more conceivable strategy might be based on avoiding sample matrix to be injected into the GC system. Unfortunately, the presence of residual sample components in sample can be hardly eliminated by common clean-up procedures such as liquid–liquid partition or (often in combination with) frac-

tionation employing gel permeation, adsorption and/or ion-exchange chromatography [17]. As far as sufficiently high recoveries for target analytes are required, at least part of co-extracts possessing similar properties like analytes penetrates into their fraction. In one of the recent studies addressing accuracy issue in pesticide residue analysis Schenk and Lehotay [22] have shown that even extensive clean-up of crude extracts obtained from various vegetables, fruit and cereals carried out by combination of several types of SPE cartridges (three types of sorbents – graphitized carbon, primary/secondary amine and strong anion-exchanger were used in series) cannot assure that no recoveries exceeding 100% will be found for any organophosphorus pesticides/matrix combination, although in many cases substantially reduced matrix enhancement effects were obtained in purified samples.

In any case, the importance of efficient clean-up should not be underestimated since not only matrix effects but the overall performance of a GC system is unfavourably affected by non-target matrix components (also shown in Fig. 2).

3.3.2. Calibration employing masking of active sites

Another apparently straightforward approach to deal with the problem created by active sites in injector is their intentional ‘‘masking’’ during the injection of calibration standard. The use of extract prepared from residue-free matrix for dissolving the standard provides the solution [14,16,18–20]. This strategy is referred to in Commission Document 2002/657/EC, paragraph 2.4.1: ‘‘In case of external calibration, it is mandatory that calibration standards are prepared in a solution that matches as closely as possible the composition of sample solution’’. To fulfil this requirement for a wide range of pesticide/matrix combinations which are typically examined in food control laboratories is fairly difficult because of (i) limited stability of pesticides during long-term storage in the presence of matrix components [25], (ii) problems to obtain blank samples of some commodities (e.g. oranges), (iii) increased demand for instrumentation maintenance (added contamination burden to GC system). In addition to these practical limitations, additional workload when realising this type of calibration and, consequently, increased cost of analyses might be arguments for objections.

The first attempt to simplify the protection of analytes against adverse effects in injection port was undertaken by Erney and Poole [15], who investigated the possibility of mimicking the presence of the matrix by addition of suitable additives to the calibration standard solutions. Unfortunately, no long-term stable positive effect was experienced. In their very recent extensive study, Anastassiades et al. [26] re-introduced the concept of analyte protectants (reagent masking agents). In their experiments, standard solutions of 30 common modern pesticides (including some of those recognized as prone to matrix effects, see Table 1) containing low concentrations of additives capable of hydrogen bonding were examined for response enhancement effects. Among 93 potential analyte protectants, compounds containing multiple polar/ionisable groups such as various polyols and their derivatives, carboxylic acids, amino acids, derivatives of basic nitrogen containing heterocycles, etc. were included in the study. The potential to overcome errors in quantitation caused by matrix-induced enhancement effects was shown namely for compounds containing multiple-hydroxy groups such as sugars and some of their derivatives, also gluconolactone appeared to be effective. When added to extracts and matrix-free standards alike, dramatic enhancement (up to one order of magnitude) of analyte peaks lowers substantially their LODs and facilitates their quantitation. Considering the current regulatory policy of US federal agencies precluding the use of matrix matched standards calibration, the implementation of these findings into routine practice is an even more challenging option. In any case, the evaluation of the long term influence of analyte protectants on the GC system has to be completed. It should be noted that using the unique method multiresidue (QuEChERS) developed by authors [27] for simple sample preparation purified extract in acetonitrile is obtained in the final step. Since in most of the other existing multiresidue methods purified sample is transferred to a less polar solvent (ethyl acetate, toluene and/or isooctane) prior to GC analysis, straightforward application of the above strategy, is not directly applicable. Probably another type of protectants should be used in this case.

3.3.3. Choice of an optimal injection technique

An elegant way to overcome the problems caused by the analysis of dirty samples has been recently enabled by introduction of a novel large volume injection (LVI) technique called Dirty (Difficult) Matrix Introduction (DMI), sometimes referred to as Direct Sample Introduction (DSI) [28,29]. Its realisation involves the following steps: (i) transfer of a sample (crude extract can be used) aliquot into a microvial located in a special holder (adapted glass injector liner), (ii) gentle venting of the solvent from the sample, preferably at a relatively low injector temperature, (iii) brief heating of the injector to the desired temperature needed for achieving intra-injector thermal extraction and volatilisation of the target analytes (other matrix components can be volatilized too); (iv) focusing of semi-volatile sample components in the front part of the separation column followed by conventional GC separation. While the sample microvial is disposed after use, re-using the particular liner is possible. The main advantages of DMI are: (i) reduced demands for GC system maintenance (contrary to other injection techniques, contamination by non-volatile matrices does not occur), (ii) reduced laboriousness of sample preparation (elimination of clean-up step is conceivable); and (iii) possibility to shorten the GC analysis time (i.e., increase of the sample throughput) thanks to regulated transfer of less volatile late eluting matrix components in the latter case a lower upper GC oven temperature might also be appreciated for some GC phases) [30]. At the same time, it is important to notice that also volatile matrix components introduced from the sample into the injector may still influence the quantitative aspects of the injection process. Generally, optimisation of DMI, especially in case of multiresidue methods, is a rather demanding task. Nevertheless, the applicability of DMI in routine analysis of multiple pesticides in plant matrices was recently demonstrated [31,32]. With using modern autosamplers, reproducible DMI injections can be realised in a fully automated way.

While only few laboratories nowadays have DMI available, one can also compensate for matrix-induced chromatographic enhancement by optimising the injection conditions. The injection temperature and the residence time of the analytes in the heated region are the main parameters governing undesirable interactions with active sites. The instrumental parameters that are to be tuned are column head pressure, flow rate of the carrier gas, volume of the injected sample and also the character of the sample solvent. The choice of chromatographic column is also important.

At present, the use of pulsed splitless injection for the analysis of organic contaminants has been often reported [33–36]. This set-up (an increase in column head pressure for a short time (usually 1 or 2 min) during sample injection leads to a several times higher carrier gas flow-rate through the injector and thus faster transport of sample vapour onto the GC column. Under these conditions, the residence time of the analytes in the injection chamber is much shorter than for classic splitless injection. As a result, in some cases a significant suppression of analyte adsorption and/or degradation (but also discrimination) occurs in the inlet port [37]. The responses of troublesome compounds (mentioned earlier in Section 3.2) obtained with pulsed splitless injection are thus significantly higher than those obtained with common splitless injection [38]. In addition, due to the increased pressure, higher volumes of sample can be injected (up to 5 μl) without the risk of back flash. Consequently, lower detection limits can be achieved.

Reduced temperature stress put on the analytes during the injection period can also be achieved by using a Programmable Temperature Vaporiser (PTV). Injection of a normal or large volume of sample released into a cold liner (temperature set below or near the solvent boiling point) is followed by an increase in the temperature, evaporation of solvent and transfer of analytes onto the GC column. PTV can be operated in various modes, the splitless and solvent split are the most common techniques in residue analysis. The advantages of PTV injection over other sample introduction techniques consist of a decreased analyte discrimination during injection step, better recoveries of thermo-degradable compounds and generally less pronounced adverse effects of non-volatile substances present in the sample during the injection process [39,40]. In trace analysis of sensitive contaminants the use of glass wool or any other filling of the liner should be strictly omitted to avoid an increase in the amount of active sites [13]. Another advantage of using a PTV in the

large volume injection (LVI), mode is saving the time otherwise needed for sample concentration.

Fig. 5 compares the potential of three injection techniques to diminish matrix effects for susceptible analytes, PTV being superior in this respect (relatively polar organophosphates such as acephate, methamidophos or omethoate are obviously troublesome compounds). On the other hand, very intensive matrix-induced chromatographic enhancement was obtained for on-column injection. This technique is obviously unsuitable for particular purpose because of rapid formation of matrix deposits in the front part of the chromatographic column that makes quantitation impossible after several injections of real sam-

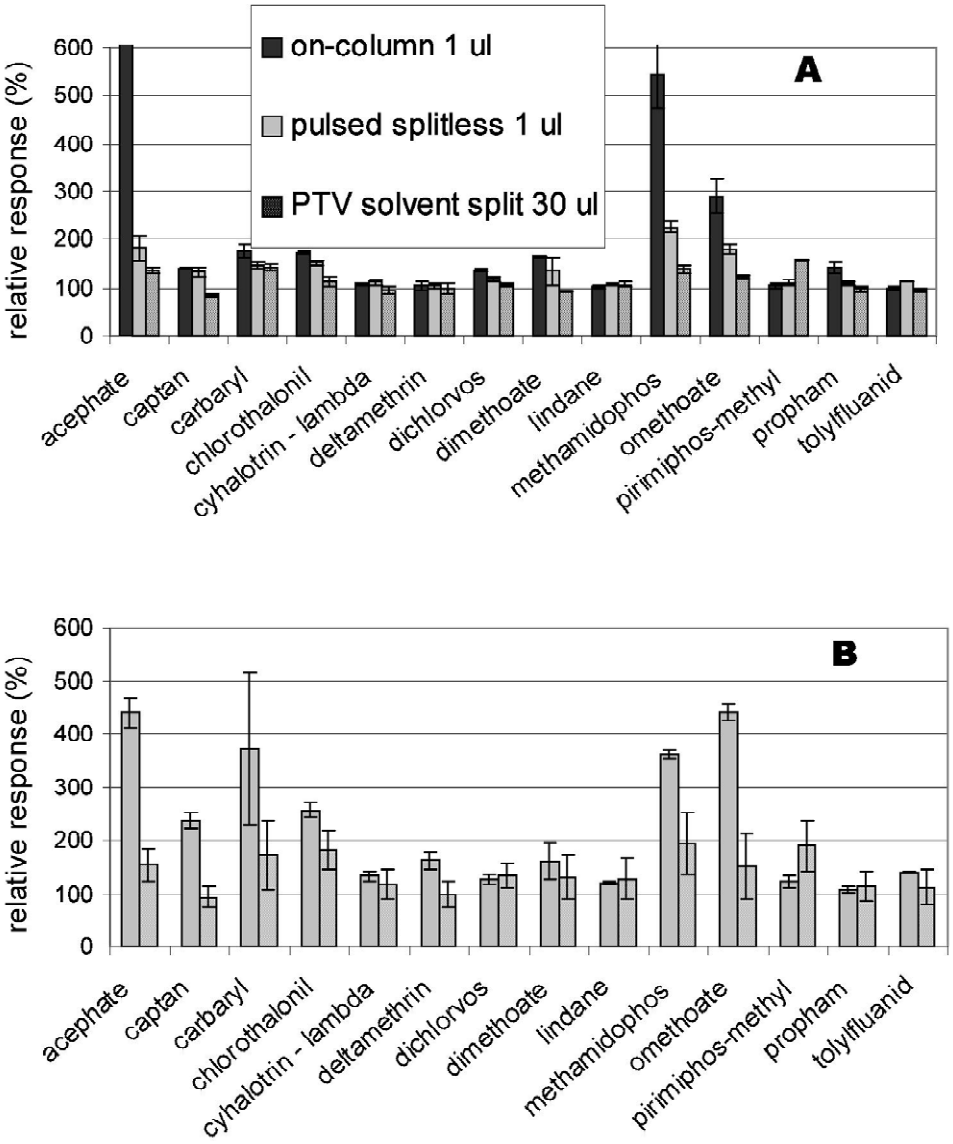

Fig. 5. Comparison of alternative injection techniques; GC–MS determination of pesticide residues in wheat samples; 1 μl of extract in toluene containing the equivalent to 1 mg of original sample and 10–100 pg of analytes were injected; A—''clean'' system,10 injections of real samples; B —''dirty system'', 87 injections of real samples; 100%=response of standard in net solvent [13].

ples. PTV is clearly shown as the most powerful injection technique in dealing with matrix effects in case of particular target compounds.

3.3.4. Correction function on biased results

Recently, two comprehensive papers [41,42] proposing a new strategy for correction of analytical results generated by calibration based on standards in net solvent (hence potentially affected by matrix-induced chromatographic enhancement) have been published. The calculated correction function was validated by statistical treatment of a large data set obtained by GC/ECD analyses of multiple residues in various vegetable commodities in a 4-month period. Calibration curves obtained by using external standards (i) in pure solvents and (ii) matrix-matched standards were used for comparison. Application of correction function for prediction of results would reduce both the cost of analyses and the time needed to their accomplishment thanks to avoiding the preparation of matrix-matched standards. Additional benefit could be seen in increased instrument maintenance interval due to a lower amount of matrix burden onto GC. However, the use of correction factor may become unreliable in case of a large variation of levels of pesticides in respective commodities. Therefore, the application of this correction approach will probably stay limited only to laboratories focusing on analyses of large series of samples with analytes varying only in a narrow concentration range.

4. Matrix effects in liquid chromatography

In routine control of food contaminants, high-performance liquid chromatography, HPLC, obviously represents a method choice whenever analysis of thermally unstable, polar or non-volatile contaminants is required. However, the demand for extensive clean-up when using this technique as a determinative step is urgent provided the most common detectors such as refractometric or UV/DAD are used. These detectors are neither sensitive and/or selective enough to enable unbiased determination of low levels of multiple residues of very different chemical structures in complex matrices. The lack of a relevant detection technique has been overcome by combining liquid chromatography with mass spectrometric detection (LC–MS). Although the first functional coupling of LC to MS was reported more than 25 years ago, technical difficulties in interfacing the high flow volumes of mobile phase with the high vacuum in mass spectrometric analyser prevented for many years a routine use of LC–MS methods in food control laboratories. The situation was basically improved by introduction and commercialisation of robust and reliable interfaces.

Nowadays, LC–MS instruments employing atmospheric pressure ionisation (API) are probably the most commonly used in trace analysis (among API ionisation techniques, electrospray ionisation, ESI, and atmospheric pressure chemical ionisation, APCI, are the most often applied ionisation techniques).

Analogously to GC, co-eluting matrix components may interfere with the detection process. To achieve high sensitivity and selectivity of target analytes detection, tandem mass spectrometry (MS–MS) employing either tandem-in-time MS–MS (ion trap analyzers) or tandem-in-space MS–MS (e.g. triple stage quadrupoles) is a preferred option by most experts working in the field of trace analysis. In any case, as a part of the validation procedure, the assessment of an influence of sample matrix on the quality of generated data is a crucial issue.

4.1. Matrix effects in LC–MS and their compensation

It is a well documented fact that the performance of API LC–MS interface is considerably influenced by the composition of liquid entering the detector, i.e., not only the type and amount of organic mobile phase modifiers and volatile buffers, but also the type and amount of sample matrix components play an influential role. Co-extracted substances present in the injected sample can cause serious quantitation problems when co-eluted with the analyte of interest; either suppression or enhancement of the analyte signal are typical symptoms. An example of diverse trends in matrix effect (i.e., overestimation or underestimation of results) for pesticides representing several classes of chemical structures is shown in Fig. 6. These phenomena have been observed by many authors in analyses of complex samples [43–52] and are referred to as, alike in GC, matrix

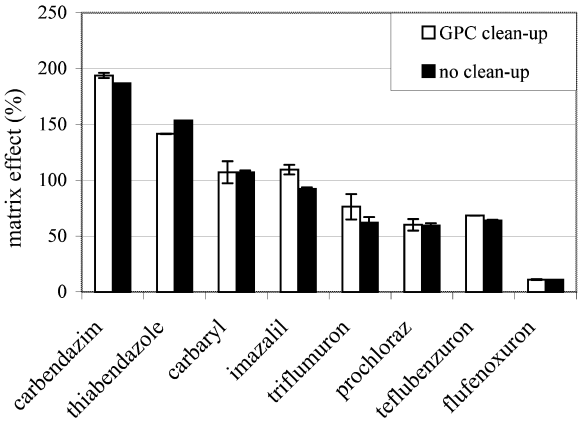

Fig. 6. Matrix effects measured in LC–MS analysis of pesticide residues in apples (LCQ Deca ion trap instrument, Finnigan, USA, ESI ionisation) in: A—GPC purified crude extracts, B—crude extracts; concentration level of target pesticides—0.005 μg/ml; sample aliquot—1 g apple/ml of extract [59]. Note: compounds responsible for matrix effects were obviously not removed by purification procedure.

effects. Although the exact mechanism of matrix suppression/enhancement phenomena is not known, it is assumed, that matrix components influence the effectivity of the ionisation processes in API interface (causing a mutual positive or negative effect in the amount of ions formed from the target analyte) [53]. Kebarle et al. [54,55] suggested that organic compounds present in the sample in concentrations exceeding approx. 10^{-5} *M* may compete with the analyte for an access to the droplet surface for gas phase ion emission. In some instances, the decrease of ion intensities of MH^+ ions of an analyte can be attributed to the gas-phase proton transfer between the electrosprayed gas-phase molecules and evaporated molecules of the stronger gas-phase base. Another hypothesis given in literature refers to the radius of droplets from which gas-phase ions are emitted. If samples contain non-volatile matrix components, droplets are prevented from reaching their critical radius and surface field, hence the ionisation efficiency is decreased and reduction of ion signal for an analyte occurs [43]. Matrix components may also influence the effectivity of the ion formation in the ionisation process by altering the surface tension of electrospray droplets and by building adduct ions or ion pairs with the analytes [50].

As a result of matrix suppression/enhancement phenomena the response of an analyte in pure solvent standard differs significantly from that in matrix sample. Therefore, for quantitation purposes, calibration by solvent-based external standards can provide biased results, especially in the analysis of complex samples such as food. To address matrix effects in LC–MS, several approaches have been discussed in the literature. The possible strategies leading to elimination or reduction of matrix effects are briefly summarized below.

4.1.1. Improved sample preparation, optimised HPLC conditions

Various factors influence the ionisation of analytes in interface; hence the selection of sample preparation as well as separation strategy should be harmonized with the requirements for optimal performance of ionisation phenomena that are closely related to identification/quantitation process. Following suggestions for optimisation of this phase might be considered. (i) Reduction of the amount of matrix components in injected sample. This task can be accomplished by employing a more selective extraction procedure [49,56] or a more extensive sample clean-up [45]. This approach is, however, time consuming and, in addition, a risk of the loss of analytes during several consecutive clean-up steps is generally increased. (ii) Decreasing the amount of injected sample aliquot is another possibility. Although this approach may lead to the reduction of matrix effects [47], it is not—for obvious reasons—a method of choice in trace level analysis. (iii) Improving HPLC separation efficiency is also a conceivable approach. As far as the change of stationary phase does not provide the required resolution of analyte from sample components, column switching technology (two-dimensional chromatography) may provide the reduction of number of overlapping peaks [47,51,52]. (iv) Modification of the mobile phase composition is also an alternative.

Choi et al. [48] observed a good correlation between responses of some analytes in the solvent standard and those measured in matrix containing standard when low concentrations of mobile phase additives, such as formic acid, ammonium formate or ammonium hydroxide were used. However, the reduction of matrix effects appeared at concentrations of buffer additive, when the signal response

from the standard was already significantly suppressed by the buffer. In other words matrix effects were compensated on the account of increased LODs.

4.1.2. Selection of optimal calibration strategy

If matrix suppression/enhancement phenomena cannot be eliminated by one of the above described ways, appropriate calibration technique compensating as much as possible for matrix effects should be used. The following options can be offered: (i) calibration using external matrix-matched standards—supposing, the standards with the same or similar matrix composition as that of analysed sample, are used, practically full compensation of matrix effects occurs. This approach (as already discussed in the GC Section) is relatively laborious and, moreover, the appropriate blank (i.e., material free of residues of target analyte) may not be always available; (ii) the use of internal standard could be considered too. General prerequisite to achieve efficient compensation of matrix effects is identity or almost agreement of retention time of internal standard and that of respective analyte. To get accurate data, co-eluted matrix should affect both compounds to (as much as possible) the same extent. Isotopically labelled internal standards are very well suited for this purpose; unfortunately, their use is rather expensive, especially in a multicomponent analysis, where a separate internal standard for each analyte is theoretically required. Not only because of economic reasons but mainly due to the lack of commercial standards (see also discussion in Section 3.3.1), the use of isotopically labelled standards is mostly restricted to single-residue analyses [57].

Under the above circumstances, post-column addition of structure analogue of a target analyte, i.e., internal standard of similar properties as possesses the target analyte(s) can be added in a constant flow-rate to the effluent from LC separation column which enters the MS detector. The response of an analyte in a particular sample is proportional to the response of internal standard at the retention time of analyte. For the determination of a small group of structurally related analytes this technique was proven to be a simple and reliable approach [46]. However, the application of such a strategy to multiclass multicomponent analysis has not been applied in practice yet. A technical obstacle of this method might be seen in the need to have an additional pump available.

Echo-peak technique represents a novel interesting alternative of internal standard concept. With this technique each analysis comprises two injections into the LC–MS system. Unknown sample and a standard solution are injected consecutively within a short time period, under specific experimental conditions. As a result, the peak of analyte from the standard elutes in the close proximity to the peak of analyte from the sample, thus forming the ‘‘echo peak’’. Provided that retention times of these two peaks are close enough to be affected by the co-eluted sample components in the same manner, matrix effects are compensated [58,59]. As an internal standard method the echo-peak technique provided the possibility of monitoring a signal reduction during the analytical sequence and to compensate this decrease by relating sample peak area to this internal standard. To illustrate the potential of alternative calibration approaches the results of analysis obtained from several types of pesticide residues in apple extract by means of several above mentioned techniques are shown in Fig. 7. Although the echo approach is undoubtedly a viable alternative, one should always bear in mind relatively low peak capacity of LC as compared to GC hence doubled number of peaks in the chromatogram may lead to problems under some circumstances.

5. Conclusions

In general terms, the accuracy of results in trace analysis of (semi)polar and/or thermally unstable contaminants potentially occurring in foods or other biotic matrices may be very poor provided standards of analytes in net solvent are used for external calibration. No matter whether gas or liquid chromatography are used for quantitation, the in-depth understanding of the nature of adverse matrix effects is a basic prerequisite of taking effective measures to prevent/compensate occurrence of encountered problems. Considering the virtual impossibility to use isotopically labelled analogues as internal standards for each of the target analyte, external calibration employing matrix-matched standards for GC and/or

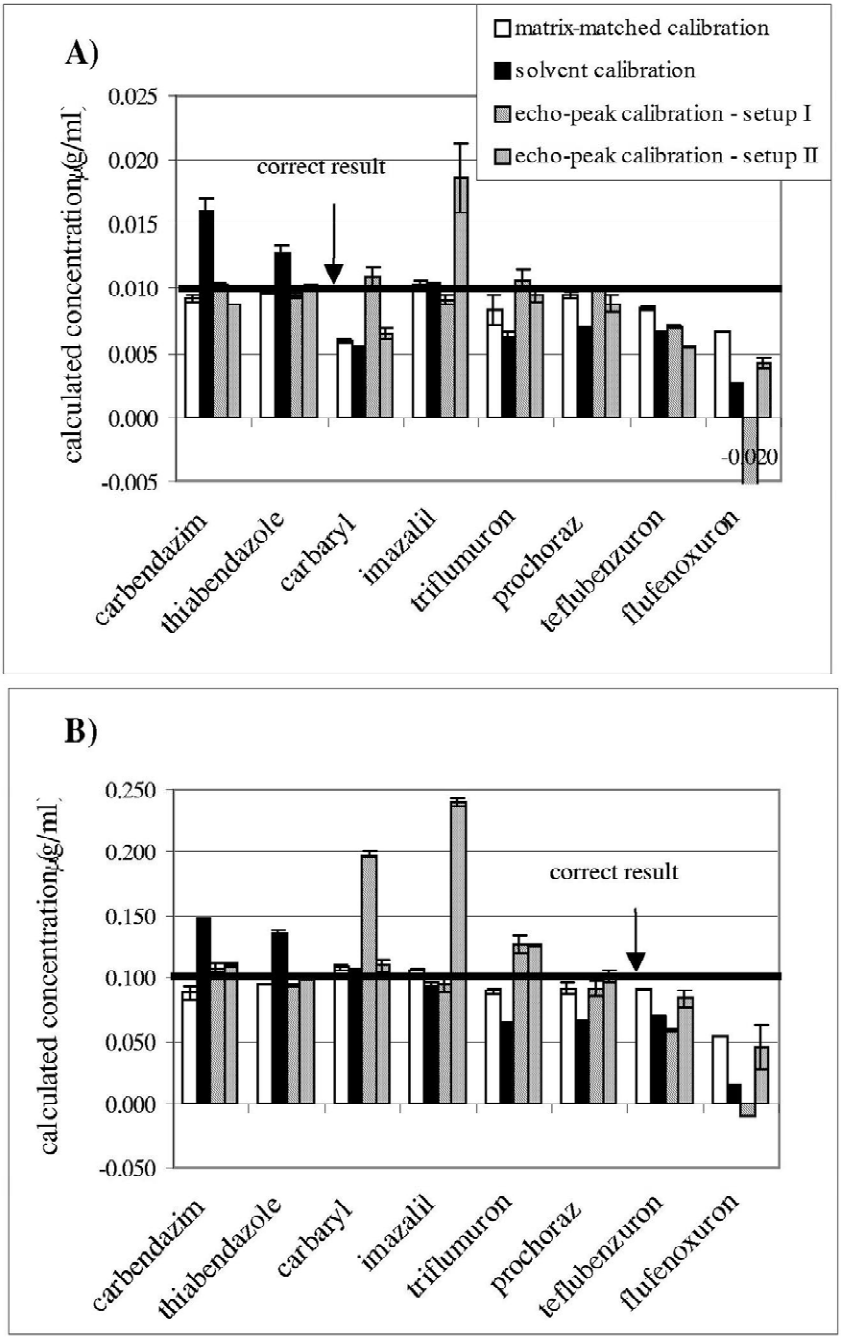

Fig. 7. Accuracy of data obtained by LC–MS (LCQ Deca ion trap instrument, Finnigan, USA, ESI ionisation) analysis of modern pesticides in crude apple extract (5 g/ml); four alternative calibration techniques used; spiking levels: A—0.01 μg/ml, B—0.1 μg/ml; injection order in echo-peak calibration: setup I—reference standard before sample, setup II—reference standard after sample [59]. Note: in case of flufenoxuron, set-up I, ionisation of reference standard was probably not affected by co-eluting interference that resulted in ''overcompensation'' of calculated value.

LC quantitation is a scientifically sound but rather impractical approach. Therefore alternative strategies might be used. In GC, an efficient separation of matrix components avoiding both the identification and quantitation problems can be achieved by comprehensive GC×GC. Coupling this technique with fast TOF-MS is a challenging option for further developments in residue analysis. As regards injector-related problems the use of the PTV injection technique may fairly reduce matrix induced chromatographic enhancement. A smart solution of problems caused by non-volatile matrix can be achieved by the novel, Dirty Matrix Introduction Technique, DMI.

In LC–MS that represents nowadays the prominent analytical technique in determination of polar and/or thermolabile residues, great attention has to be paid to the compensation of enhancement/suppression of analyte signal by co-eluting matrix. The use of echo-peak calibration may represent a good compromise in solution of these adverse phenomena.

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