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Factors Affecting the Accurate Quantification of Pesticide Residues in Non-Fatty Matrices

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1. Introduction

The presence of pesticide residues is regarded as a potential chemical hazard in several foodstuffs, such as fruits and vegetables. Based on the increasing consumers' concern about the residues persistence in their food, a large number of multiresidue extraction methods (MRMs) has been evaluated to ensure accurate residues determination (Greve, 1988; van Zoonen, 1996; Schenck & Wong, 2008). The widely diffused MRMs in the analysis of non-fatty matrices apply extraction with appropriate solvents (e.g., ethyl acetate – EtOAc, acetone, acetonitrile – ACN, methanol) in the first step and gas chromatography (GC) with sensitive and selective detectors (e.g., nitrogen phosphorus – NPD, electron capture – ECD, flame photometric – FPD, mass spectrometry – MS) in the final part of determination (Motohashi et al., 1996; Seiber, 1999; Beyer & Biziuk, 2008; Sannino, 2008; Schenck & Wong, 2008). A large number of modifications in the possible additional clean-up of the organic solvent extract are also included to result in more accurate result of analysis (Tekel & Hatrik, 1996; Schenck & Lehotay, 2000; Lee & Richman, 2002; Schenck et al., 2002). Nowadays, most of the approaches applied are effective in detecting and quantifying several analytes in a large scale of matrices within a relatively short period by minimizing reagents consumption (Lee et al., 1991; Hajšlová et al., 1998; Egea González et al., 2002; Majors, 2007).

However, the MRMs application has sometimes increased the result inaccuracy caused by several parameters, such as matrix analysed, concentration level of pesticide identified, extraction solvent and/or determination technique applied and phenomena like "matrix-induced enhancement effect" (Erney et al., 1993; Cai et al., 1995; Hajšlová et al., 1998; Schenck & Lehotay, 2000; Anastassiades et al., 2003a; Maštovská & Lehotay, 2004; Menkissoglu-Spiroudi & Fotopoulou, 2004; Georgakopoulos et al., 2007). These factors, individually or combined, are able to lead in several adverse effects by under- or over-estimation analysis result, detection of unknown peaks, masking of analysed residue peak by co-extract components etc. (Hajšlová et al., 1998; Hajšlová & Zrostlíková, 2003; Poole, 2007). Concerning the above, a lot of studies involving the factors affecting the quantification of the residue(s) have been applied aiming to: (a) determine the parameter(s) introducing the inaccuracy of the result, (b) evaluate and correct the effect of factor(s) influencing the results of the determination, (c) suggest more optimal analytical conditions in cost-effective MRMs and (d) evaluate some critical parameters for possible method validation.

The objective of this chapter is to review some important findings from the evaluation of the critical factors affecting the accurate residues quantification in non-fatty matrices,

incorporating recent results of our laboratory dealing with the validation of an MRM. More specifically, there were efforts to investigate whether the collection of validation data from a single product of a botanical category and then validate the method for lots of commodities of the same botanical group is or not an erroneous practice. Finally, some future perspectives are also referred with purpose both to generalize the findings and validate the same MRM in the same laboratory for lots of commodities with limited error occurrence.

2. Critical factors introducing uncertainty of the residue analysis result

As already mentioned, there are a lot of parameters influencing the accuracy/precision of an analytical measurement. Provided the fact that analytical GC instrumental parameters, such as capillary analytical column, injector and detector are suitable for the separation and the identification/quantification of residues, these agents are able to significantly affect the final result. The factors with their possible effects, not always be predicted or corrected, are analysed in the following paragraphs.

2.1 Extraction solvent suitability for pesticide residues determination

The extraction's step objective is to separate most of the non-ionic residue(s) quantity from the plant matrix components by the application of organic solvent(s). Several solvents have been used for the extraction techniques with acetone, dichloromethane, methanol, EtOAc, petroleum ether and ACN to be the most popular (Luke et al., 1975; Ambrus et al., 1981; Greve, 1988; Hernández et al., 1990; Andersson & Pålsheden, 1991; Cai et al., 1995; van Zoonen, 1996; Anastassiades et al., 2003b; Maštovská & Lehotay, 2004; Schenck & Wong, 2008). EtOAc plus aliquots of salt (e.g., anhydrous sodium sulfate) to bind the water content of the plant product from the organic phase (Greve, 1988; Cai et al., 1995; Dorea et al., 1996; Pugliese et al., 2004; Berrada et al., 2006; Georgakopoulos et al., 2007), acetone with the addition of non-polar solvents, such as mixtures of dichloromethane-petroleum ether (van Zoonen, 1996; Bempelou & Liapis, 2006; Cengiz et al., 2006; Georgakopoulos et al., 2009), hexane-methylene chloride (Andersson & Pålsheden, 1991), dichloromethane-hexane (Lacassie et al., 1997) etc. and ACN combined with salts addition (anhydrous magnesium sulfate and sodium chloride) and dispersive solid phase extraction (dSPE) techniques (Anastassiades et al., 2003a; Schenck & Hobbs, 2004; Leandro et al., 2005; Lehotay et al., 2005; Hernández-Borges et al., 2009) represent the most commonly extraction procedures.

The efficiency of those mentioned MRMs to determine residues of different physicochemical properties has been compared in a lot of researches. Although EtOAc and acetone partition of several fruit extracts generally gave acceptable organophosphorus pesticides (OPs) recoveries (%R) of 70 to 110%, the EtOAc procedure resulted in better values for polar molecules (e.g., methamidophos, omethoate, acephate); a higher co-extracts number was also observed in EtOAc extracts (Andersson & Pålsheden, 1991). For instance non-acceptable low mean recovery of 58% was observed in the extremely polar methamidophos with acetone, plus hexane-methylene chloride, method compared to the respective 96% with the EtOAc method. From different solvents evaluated (methanol, acetone with and without partition in dichloromethane-petroleum ether and EtOAc), EtOAc was the most preferable for the extraction of polar OPs (acephate, methamidophos, oxydemeton-methyl etc.) from grape and cabbage matrices (Mol et al., 2003). It is notable that acetone partition resulted in recoveries of 12 to 76% for such OPs. Although the majority of 90 pesticide recoveries for various fruits and vegetables were higher than 80% in concentration ranges from 0.01 to 0.5

mg/kg with the rapid extraction of acetone using vortex mixing and solid phase extraction (SPE), the most polar OPs could not be determined (Štajnbaher & Zupančič-Kralj, 2003). EtOAc extraction provided better average %R, with satisfactory validation parameters, than dichloromethane extraction for 16 organochlorine pesticides (OCs) (Yenisoy-Karakaş, 2006). EtOAc non-fatty, fruit-based baby food extracts provided (a) higher recoveries for polar dimethoate, (b) lower recoveries for semi- and non-polar chlorpyrifos, methidathion, diazinon and phosalone and (c) higher amount of lipophilic compounds affecting the measurement than the relevant acetone partition extracts (Georgakopoulos et al., 2009). Among different extraction solvents, known to result in acceptable %R for a wide range of pesticides, ACN was chosen to the modern method named QuEChERS, as an acronym of quick, easy, cheap, effective, rugged and safe (Anastassiades et al., 2003a). This was due to the lower degree of matrix co-extracts in fatty matrices and higher %R of certain pH-dependent pesticides compared to the "dirtier" EtOAc extracts. The larger amount of remained co-extracts seems to be the major disadvantage of the EtOAc method (Ambrus & Thier, 1986; Greve, 1988); the amount of lipophilic co-extracts decreases in the order EtOAc>acetone>>ACN compared with the respective amount of sugar interferences (decreasing order of acetone>ACN>EtOAc) (Maštovská & Lehotay, 2004).

The slightly water-miscible EtOAc with the addition of anhydrous sodium sulfate aliquots to remove co-extracted water and force the polar pesticides into the organic phase (Schenck & Wong, 2008) is proved to be the favourable MRM for the analysis of polar and semi-polar analytes from non-fatty matrices, containing zero or minimum amounts of non-volatile compounds (Georgakopoulos et al., 2007). The acceptable validation parameters combined with the properties of easy and quick to handle and cost-effective (Andersson & Pålsheden, 1991; Fernandez-Alba et al., 1994) have made this MRM as one of the most favourable in the residue analysis. The more complex the matrix (containing more lipophilic co-extracts), the more the need for an extra clean-up step, such as gel permeation chromatography (Hajšlová et al., 1998) or Florisil column (Dorea et al., 1996). The polar, miscible with water acetone, requiring a series of liquid-liquid partition steps with non-polar solvents, seems to give accurate analysis results for a more wide range of pesticides, except for extremely polar OPs (Majors, 2007), in the analysis of non-fatty matrices containing or not non-volatile components. Thus, there is elimination of undesirable interference effects in the final residue result even if no further clean-up is applied (van Zoonen, 1996; Georgakopoulos et al., 2009). The new approach of QuEChERS, employing shaking of the matrix with ACN, followed by the addition of salts and dSPE with appropriate sorbent amounts (Anastassiades et al., 2003a; Majors, 2007; Schenck & Wong, 2008) represents the most suitable MRM for the analysis of polar, semi- and non-polar analytes in non-fatty and low-fatty matrices (e.g., containing 2 to 20% of fat) with large amounts of non-volatile compounds.

2.2 Pesticide residue physicochemical properties

The use of pesticides has rapidly increased over the last 60 years; nowadays over 1100 substances are registered as pesticides (Anonymous, 2006) and around 2.5 million tones of their formulations per year are applied (Tadeo et al., 2008). These compounds belong to different chemical groups (e.g., OCs, OPs, carbamates, pyrethroids, benzoylureas) (van der Hoff & van Zoonen, 1999; Sannino, 2008), presenting much different physicochemical properties, such as water solubility (w.s.), polarity, vapor pressure (v.p.), melting point etc. Since great differences among molecules even belonging to the same group are observed (e.g., extremely polars methamidophos and acephate contrary to non-polars chlorpyrifos

and parathion of Ops; Noble, 1993), the selection of an MRM to accurate determine a large variety of pesticides seems very difficult. The most significant properties, apart from volatility indicating the effective detection by GC, are polarity and resistance to different pH ranges determining parameters such as the extraction solvent selection and the type of possible clean-up step (Anastassiades et al., 2003a; Schenck & Wong, 2008).

The type of the analysed pesticides is proved to influence both its %R and occurrence of matrix effect ($p<0.05$), as the recoveries for the same matrix GC-extracts vary with the different pesticides (Erney et al., 1993; Hajšlová et al., 1998; Georgakopoulos et al., 2007). Acetone partition and ACN, both plus clean-up using SPE cartridges, fruit extracts gave different OPs recoveries (Schenck & Lehotay, 2000). Specifically, pesticides containing amides and/or multiple polar P=O bonds, such as omethoate, monocrotophos and dicrotophos, presented excessively high recoveries ranged from >110% to >200%. Furthermore, compounds containing single P=O bonds, such as acephate and methamidophos rather than non-polar P=S bonds, such as chlorpyrifos and malathion, were identified as tending to give particularly high %R. Regarding the physicochemical properties, especially values of w.s. and logarithm of *n*-octanol partition coefficient ($\log k_{ow}$) which are a degree of polarity (Hajšlová et al., 1998), methamidophos and dimethoate are more polar than methidathion and chlorpyrifos and for this reason their recoveries were higher in EtOAc extracts (Georgakopoulos et al., 2007). In this study, methamidophos recoveries were of poorer precision in the independent replicates; similar behavior with higher recoveries in combination with high relative standard deviation (RSD) values has been reported for captan and other polar analytes (Cai et al., 1995; Hajšlová et al., 1998). Moreover, many commonly applied pesticides are sensitive to specific pH values ($p<0.05$) (Lehotay et al., 2005; Payá et al., 2007). For instance, base-sensitive compounds (e.g., tolylfluanid, captan and folpet) degrade rapidly at high pH-extracts (Lehotay & Maštovská, 2009); an adjustment of acidic pH should be performed to avoid partial loss of those residues (Anastassiades et al., 2003a; Lehotay et al., 2005). Similarly, basic compounds, such as thiabendazole and imazalil, are generally poorly recovered from matrix extracts of low pH (Anastassiades, 2003a; Anastassiades et al., 2006). ACN apple juice extracts of pH values ranging from 2.5 to 7.0 gave negligible loss (recoveries of 90 to 100%) of such analytes in acidic solutions compared with the significant losses (recoveries of 50 to 70%) in the respective EtOAc extracts (Anastassiades et al., 2003a).

Therefore, the largest chemical group of OPs, covering a wide range of polarity from e.g. the extreme polar methamidophos of negative $\log k_{ow}$ (-0.8) to non-polar ethion of high $\log k_{ow}$ (5.1) may be successfully analysed by simple, cost-effective MRMs, such as EtOAc and acetone partition. It should be reminded that polars are better extracted by EtOAc in comparison with medium- and non-polars better extracted by the acetone partition method (van Zoonen, 1996; Mol et al., 2003; Maštovská & Lehotay, 2004; Georgakopoulos et al., 2009). The selection of the more appropriate MRM should be based on the physicochemical properties of target compounds. To cover the analysis of more compounds, including troublesome analytes in terms of polarity and/or acidity, QuEChERS application with ACN as the extraction solvent (Anastassiades et al., 2003a; Lehotay et al., 2005; Majors, 2007) with or without slight modifications seems to be one of the best modern MRM approaches.

2.3 Concentration level of the analysed residue(s)

The ratio of analyte and matrix concentration in the GC-extract seems to be a crucial point in the accuracy of the final residue result, since differences in the recovery portions among the

fortification levels of the same pesticide are commonly observed ($p<0.05$). Hajšlová et al. (1998), Jiménez et al. (2001) and Anastassiades et al. (2003b) noticed unacceptable %R and matrix-induced enhancement effects at lower concentration levels of target pesticides and/or at higher matrix components. Higher apparent recoveries of >200% were obtained for certain susceptible to matrix enhancement effect analytes (e.g. captan, iprodione) with solvent standard quantification at the low concentrations of ≤0.02 mg/kg in vegetable matrices (Menkissoglu-Spiroudi & Fotopoulou, 2004). From recent results presented (Georgakopoulos et al., 2007), it was concluded that the lower the fortification level, the higher the %R. The phenomenon was more evident in the Maximum Residue Levels (MRLs) of 0.01 and 0.02 mg/kg especially for the polars methamidophos and dimethoate in almost all the examined fruits and vegetables with solvent standards quantification. It should also be addressed that significant matrix effects were obtained for five pesticides tested (dimethoate, parathion methyl, chlorothalonil, diazinon and fenitrothion) in all fruit extracts of the low concentration equal to 0.05 mg/kg (Freitas & Lanças, 2009). This repeatable behavior can be attributed to the lower competitive effect of the pesticide standards, when they are found in trace fortification levels, for covering the active sites of the injection liner (Hajšlová & Zrostlíková, 2003); a phenomenon connected with the presence of matrix effects extensively analysed in a following paragraph.

2.4 Chemical composition and co-extracts of analysed matrix

MRMs should be able to effectively quantify lots of residues in several matrices presenting a large variety of components and remained co-extracts. The water, protein, fat and sugar content of commonly commodities analysed is much different, as shown in Dorea et al. (1996), Hajšlová et al. (1998), Egea González et al. (2002), Lesueur et al. (2008) etc. The choice of the appropriate MRM is strongly associated with the composition of the matrix, and especially the fat content (Motohashi et al., 1996). According to Greve (1986) non-fatty samples contain less than 5% total fat contrary to fatty samples. Food and Drug Administration (FDA) extraction methods are designed for fatty, containing ≥2% fat, and non-fatty, containing <2% fat, matrices (Sannino, 2008). Lehotay et al. (2005) presented a more suitable matrix taxonomy for MRMs; non-fatty samples contain <2%, low fatty contain 2 to 20% and fatty ≥20% total fat. Non-fatty products have been divided according to the water percentage as moist (containing >80% water), medium water content (containing and samples presenting sugars of 5 to 30%) and dry (Greve, 1988; Tekel & Hatrik, 1996). Furthermore, with purpose to choose the more suitable MRM for several matrices, plant products have been categorized according to their chemical composition (Ambrus et al., 1981) or botanical characteristics (Bates & Gorbach, 1982). Thus, it has been proposed that collecting validation parameters deriving from only one representative commodity (e.g., orange from citrus fruits) should provide validation ability for lots of products belonging to the same botanical category (e.g., lemon, mandarin, kiwi fruit).

The recovery portion of pesticides depends greatly on the chemical composition of the examined matrix ($p<0.05$). Lemon and onion, recognized as high acid and high sulfur content respectively, gave much lower %R for ≈150 pesticides by QuEChERS plus GC-MS compared with tomato and grape extracts (Lesueur et al., 2008). Unacceptably high %R were observed to non-fatty extracts containing more non-volatile compounds, such as chlorophylls in leafy vegetables, carotenoids in fruiting vegetables (e.g., lycopene in tomatoes), essential oils in citrus peels, waxes in grapes (Georgakopoulos et al., 2007). Freitas & Lanças (2009) indicated that the enhancement or decrease of the response and %R

significantly differed from matrix to matrix among 6 fruits tested having variable chemical composition. Furthermore, the type of co-extracts that remains in the final sample leads to a markedly different detector response and causes false positive results (Erney et al., 1993; Hajšlová et al., 1998; Poole, 2007). Scientific evidence suggests that more distinct matrix effects have been reported for matrix extracts rich in pigments and lipids (Hajšlová et al., 1998; Godula et al., 1999; Anastassiades et al., 2003b). Organosulfur compounds, not removed by the SPE columns evaluated, of cabbage interfered with the detection of early eluting OPs in the GC-FPD analysis (Schenck et al., 2002). Among 21 kinds of vegetables tested, only garlic, onion and leek extracts gave "unknown" peaks in GC-FPD due to large amounts of sulfur constituents (Cai et al., 1995). A peak in orange extracts appeared in every fruit (by GC-NPD), either of organic produce or of conventional crops, and detected in the peel orange extract analysed itself compared with the analysis of orange juice (Georgakopoulos et al., 2007) may be the reason why citrus peels require an extract clean-up, described by Dorea et al. (1996). EtOAc fruit purée and cocktail extracts, presenting more complicated components and higher co-extract amounts, influenced negatively both the NPD response and the accurate determination in contrast to the respective fruit juice extract (Georgakopoulos et al., 2009). To overcome these co-extracts effects, additional clean-up steps, compatible with pesticides analysed and solvent(s) applied, have been proposed (Tekel & Hatrik, 1996; Schenck & Lehotay, 2000; Schenck & Wong, 2008). Their application represents a compromise between the time and cost required on the one hand and the "cleaner" (containing less constituents affecting the %R and detection limit) extract on the other hand (Seiber, 1999; Lee & Richman, 2002).

2.5 Matrix-induced enhancement effects

The quantification of certain analytes by GC is strongly affected by a phenomenon known as matrix-induced chromatographic response enhancement, which was first described by Erney et al. (1993) and causes excessively high recovery results. The phenomenon takes place during the analysis of samples containing a wide range of components (e.g., pigments, lipids, waxes) that may remain after the preparation of the extract and its possible clean-up (Godula et al., 1999; Hajšlová & Zrostlíková, 2003). Such non-volatile constituents accumulate in the GC inlet and/or in the front part of a capillary column, resulting in the reduction of the loss and protection of the analyte(s) from adsorption and thermal degradation (Erney et al., 1993; Poole, 2007). Particularly, during analysis of a pesticide(s) standard solution, more active sites, especially in the injection liner, are available for the analyte(s) molecules compared with those available during analysis of an extract also containing matrix components (Schenck & Lehotay, 2000). This is because the latter components block the active sites both presenting in the (a) liner and (b) connection of the injector with the capillary column (Erney et al., 1993), increasing the transfer of analyte(s) to the separation column and detector (Poole, 2007). Therefore, when free-matrix standard solutions are injected, poor peak shapes combined with peak tailing and low response results for some affected compounds, such as those presented in Poole (2007), are observed contrary to the respectives of matrix extract solutions (Anastassiades et al., 2003b).

Nowadays, the matrix effect is considered as one of the most persistent sources of uncertainty in pesticide residue analysis (Egea González et al., 2002) by increasing the level of random errors and/or introducing a systematic effect on the result (Cuadros-Rodríguez et al., 2002). Available studies involving the analysis of various residues in different matrices (Erney et al., 1993; Erney et al., 1997; Johnson et al., 1997; Jimenez et al., 2001; Menkissoglu-

Spiroudi & Fotopoulou, 2004) prove that its presence and extent depends on several parameters, most of which were previously reported. More specifically, many thermolabile compounds, containing polar structure/functional groups, quantified in low concentration (e.g., <0.1 mg/kg), are referred as "troublesome analytes" (e.g., methamidophos, acephate, captan, chlorothalonil, monocrotophos, folpet) since they are susceptible to matrix enhancement (Lee et al., 1991; Bernal et al., 1997; Hajšlová et al., 1998; Godula et al., 1999; Hajšlová & Zrostlíková, 2003; Poole, 2007). Moreover, many non-fatty matrices are identified as tending to give matrix effects, such as apple, tomato, banana, orange peel, stone fruits, carrot, leafy vegetables, wheat, wine etc. (Miyahara et al., 1994; Egea González et al., 2002; Navarro et al., 2002; Patel et al., 2004; Georgakopoulos et al., 2007; Freitas & Lanças, 2009), due to the high co-extracts amount persisting in the GC analytical sample, necessitating the application of clean-up step(s) (Dorea et al., 1996; Hajšlová et al., 1998; Schenck & Lehotay, 2000; Li et al., 2008). It should also be addressed that matrix effects are difficult to study because of the different analysis conditions for the samples, since the effects of simple maintenance application (e.g. changing the injection liner, cutting the front part of capillary column) are unpredictable (Godula et al., 1999; Schenck & Lehotay, 2000). Thus as Hajšlová et al. (1998) indicated the history of the GC system, especially changes in the injection port, plays an important role in the occurrence of such phenomena. As a consequence, recoveries of several pesticides are not reproducible and the effects of co-extracts cannot be considered as stable and foreseeable (Georgakopoulos et al., 2007).

Several injection techniques have been proposed to compensate for matrix effects and eliminate the uncertainty of the final result; these are not always available for analytical laboratories due to the increasing cost required (Schenck & Wong, 2008). For instance, the use of cold on-column injection is considered as one of the most practical approaches by which pesticides thermolysis and decomposition or adsorption inside the inlet could be avoided (Wylie & Uchiyama, 1996; Godula et al., 1999). Furthermore, polar pesticides and matrices containing non-volatile constituents could be analysed by on-column injection with the parallel use of a packed column or a deactivated pre-column (to keep most of matrix components) connected to the injector site. However, the main disadvantage of on-column injection is related with the much increased maintenance necessity of the column, being impractical for complex or relatively un-cleaned matrices compared with the conventional hot splitless injection (Anastassiades et al., 2003b). Programmable temperature vaporization (PTV) may result both in decreased analyte discrimination during injection and limited adverse effects of non-volatiles by introducing large volumes of sample (Grolimund et al., 1998; Godula et al., 2001; Poole, 2007). Pulsed splitless injection, involving an increasing of column head pressure for 1 to 2 min during the injection, reduces the residence time of analyte(s) in the inlet and minimizes solvent expansion volumes (Wylie & Uchiyama, 1996; Godula et al., 1999). The main drawbacks of these techniques are related with the (a) increasing amount of non-volatile components into the column more than the desirable and (b) reducing but not eliminating the occurrence of matrix effects (Godula et al., 1999; Anastassiades et al., 2003b).

An alternative approach dealing with the preparation of the analytical sample is the application of an extensive clean-up step after the extraction. Its use may result in several benefits, such as elimination of matrix interferences causing such phenomena, high recoveries, detection and quantification limits (LODs and LOQs, respectively), reduction of maintenance needs for the GC instrument due to the relatively clean extract (e.g., lower changes of liners and capillary columns, smaller detector contamination by the impurities)

and restriction of enhancement effects (Hajšlová et al., 1998; Schenck & Lehotay, 2000; García-Reyes et al., 2007). The major disadvantage is the demanding for extra labor time and cost (Greve, 1988; Egea González et al., 2002; Beyer & Biziuk, 2008; Schenck & Wong, 2008) because of the increasing needs for additional solvent amounts, columns (Florisil, SPE) and sorbents (primary secondary amine-PSA, octadecyl-C_{18}, graphitized carbon black-GCB) (Dorea et al., 1996; Schenk et al., 2002; Anastassiades et al., 2003a; Li et al., 2008). It should also be noticed that the more the steps of an MRM, the higher the possibility for analytes partial loss and the increase of the combined uncertainty during the procedure (Hajšlová et al., 1998; Menkissoglu-Spiroudi & Fotopoulou, 2004; Poole, 2007).

Among the mentioned and other suggested approaches, such as the use of correction functions (Egea González et al., 2002; Cuadros-Rodríguez et al., 2002) or analyte protectants (Anastassiades et al., 2003b; Poole, 2007), the most practical solution to eliminate matrix effects seems to be the application of matrix-matched standard solutions (Erney et al., 1993; Erney et al., 1997; Poole, 2007). These standards are prepared by adding appropriate aliquots from solvent standard solutions in blank matrix extracts (Erney et al., 1997; Štajnbaher & Zupančič-Kralj, 2003; Lesueur et al., 2008; Freitas & Lanças, 2009; Georgakopoulos et al., 2009). Their application has nowadays been included in the calibration step for pesticides quantification (Erney et al., 1993; Bernal et al., 1997; Egea González et al., 2002; Martínez Vidal et al., 2004). For calibration by comparing the quantity of a fortified extract with the respective of a matrix-matched standard, the concentration of the standard should be equal to the final concentration of the extract; otherwise the result is incorrect (Erney et al., 1997; Georgakopoulos et al., 2009). When applied as reference materials, matrix standards have provided acceptable %R and overcome enhancement in detector response, since the interferences effects were approximately similar to the fortified extracts analysed (Erney et al., 1997). Available studies prove their effectiveness in hundreds of residues analysis for various product extracts. Indicatively, excessively high OP recoveries of >120 to 240% were reduced to 81 to 97% with matrix standard calibration for potato extracts (Lehotay & Eller, 1995), extremely high recoveries of >200 to 1000% for lots of analytes in honey extracts were also corrected to the acceptable range with controlled spiked blank extracts (Jiménez et al., 1998), recoveries approaching the 300% were reduced to 70 to 110% in white wine (Holland et al., 1994), recoveries much higher than 110% for some pesticides (e.g., parathion methyl) as a result of non-matrix calibration plots were significantly reduced by matrix-matched vegetable calibration curves (Johnson et al., 1997), standard solutions of blank fruiting vegetables were found to correct the high recoveries of >200% of most pesticides to the acceptable 70 to 110% (Menkissoglu-Spiroudi & Fotopoulou, 2004) etc. The disadvantages of this technique have mainly to do with the increasing demands for more blank extracts (larger quantities of matrix and extraction solvent), more labor time for preparation and larger needing for GC maintenance.

2.6 Confirmatory results of the factors affecting residues quantification

The presence and the extent of matrix effects in pesticide residue analysis were assessed by the application of an official MRM (acetone partition with dichloromethane-petroleum ether) and GC-NPD. Recoveries of 5 OPs presenting different polarity were evaluated in non-fatty matrix and fortification level (MRL and one multiple of it) combinations by standards prepared both in solvent and matrix-matched solutions (Tables 1 to 5). Unacceptably high %R combined with pronounced matrix effects were observed to the more polar dimethoate (Table 1) and to the lower fortification levels of <0.1 mg/kg (Tables 1 to 3).

The matrix standards single point determination resulted in recoveries of the acceptable 70 to 110% contrary to the relevant of solvent standards determination in lots of the examined combinations ($p<0.05$). This is more evident in the analytes dimethoate (Table 1) and phosalone (Table 5), in specific matrix extracts (especially those of lettuce) and in various low fortification level – analyte – matrix combinations (Tables 1 to 5). It is notable that all calculated by matrix-matched standards recoveries of chlorpyrifos (Table 2) and fenitrothion (Table 3) and almost all of diazinon (Table 4) and phosalone (Table 5) were found in the acceptable range. The results also proved that when the purpose is the identification and the monitoring of MRLs, conventional, cost-effective quantification by solvent standards could be successfully utilized if the triptych non-polar analyte (e.g., chlorpyrifos, fenitrothion, diazinon) – concentration (higher MRL values of >0.1 mg/kg) – plant product analysed tends to give no matrix effects and results overestimation (Tables 1 to 5). In those conditions described the effects of factors are limited; thus the analysis may be accurate both without spending more laborious time and cost for matrix standards or clean-up step(s) and without demanding the application of mass spectrometry techniques not available by many laboratories.

Matrix	Recoveries ± RSDs (%) (*n*=3) of dimethoate					
	C_1 (mg/kg)	Solvent standards	Matrix standards	C_2 (mg/kg)	Solvent standards	Matrix standards
Pear	0.02	nd*	nd*	0.2	**93.5 ± 1.4a**	**76.7 ± 3.0b**
Orange	0.02	158.3 ± 3.3a	**103.8 ± 5.1b**	0.2	**72.0 ± 0.8a**	63.2 ± 3.6b
Tomato	0.02	320.4 ± 0.9a	118.7 ± 3.0b	0.2	**75.9 ± 2.1a**	**82.7 ± 3.2b**
Lettuce	0.5	**100.4 ± 2.7a**	**71.5 ± 1.5b**	0.05	**77.8 ± 6.3a**	**109.6 ± 6.6b**
Peach	0.02	164.5 ± 3.5a	**102.1 ± 4.2b**	0.2	115.7 ± 6.2a	57.9 ± 10.0b

nd*: non detectable

a, b: within a specific matrix and fortification level, those values lacking a common letter are different ($p<0.05$)

Table 1. Recoveries ± RSDs (%) of dimethoate in matrix – fortification level (C_1 equal to MRL established by European legislation, C_2 a multiple of it) combinations using both solvent and matrix-matched standard solutions (*n*=3)

Matrix	Recoveries ± RSDs (%) (*n*=3) of chlorpyrifos					
	C_1 (mg/kg)	Solvent standards	Matrix standards	C_2 (mg/kg)	Solvent standards	Matrix standards
Pear	0.5	**88.9 ± 1.9a**	**86.8 ± 0.8a**	0.05	**85.9 ± 4.6a**	**95.7 ± 0.3b**
Orange	0.3	**100.1 ± 3.2a**	**100.4 ± 1.2a**	0.03	141.2 ± 2.3a	**79.0 ± 1.1b**
Tomato	0.5	**100.1 ± 2.9a**	**105.7 ± 0.6a**	0.05	117.0 ± 2.0a	**76.4 ± 5.6b**
Lettuce	0.05	205.5 ± 5.0a	**76.3 ± 0.6b**	0.5	**85.4 ± 1.6a**	**96.4 ± 5.7b**
Peach	0.2	**72.2 ± 1.4a**	**82.3 ± 2.7b**	0.02	235.1 ± 2.0a	**103.3 ± 4.3b**

a, b: within a specific matrix and fortification level, those values lacking a common letter are different ($p<0.05$)

Table 2. Recoveries ± RSDs (%) of chlorpyrifos in matrix – fortification level (C_1 equal to MRL established by European legislation, C_2 a multiple of it) combinations using both solvent and matrix-matched standard solutions (*n*=3)

Matrix	Recoveries ± RSDs (%) (*n*=3) of fenitrothion					
	C_1 (mg/kg)	Solvent standards	Matrix standards	C_2 (mg/kg)	Solvent standards	Matrix standards
Pear	0.5	**87.6 ± 2.8a**	**79.1 ± 2.5b**	0.05	**99.7 ± 6.7a**	**90.2 ± 3.6a**
Orange	2.0	65.2 ± 1.1a	**71.9 ± 4.0b**	0.02	**104.4 ± 1.5a**	**111.0 ± 1.1b**
Tomato	0.5	**99.4 ± 1.1a**	**104.6 ± 1.6a**	0.05	149.3 ± 2.2a	**105.4 ± 4.7b**
Lettuce	0.5	**105.7 ± 0.5a**	**91.7 ± 0.5b**	0.05	173.7 ± 1.4a	**110.5 ± 7.1b**
Peach	0.5	**84.1 ± 3.5a**	**75.8 ± 2.6b**	0.05	180.5 ± 3.8a	**97.9 ± 6.0b**

a, b: within a specific matrix and fortification level, those values lacking a common letter are different ($p<0.05$)

Table 3. Recoveries ± RSDs (%) of fenitrothion in matrix – fortification level (C_1 equal to MRL established by European legislation, C_2 a multiple of it) combinations using both solvent and matrix-matched standard solutions (*n*=3)

Matrix	Recoveries ± RSDs (%) (*n*=3) of diazinon					
	C_1 (mg/kg)	Solvent standards	Matrix standards	C_2 (mg/kg)	Solvent standards	Matrix standards
Pear	0.3	**88.5 ± 0.8a**	**107.2 ± 5.9b**	0.03	180.4 ± 3.6a	**102.4 ± 1.6b**
Orange	1.0	**85.2 ± 4.0a**	**80.3 ± 3.2b**	0.01	**102.5 ± 1.5a**	**102.9 ± 1.9b**
Tomato	0.5	**74.0 ± 3.2a**	**92.1 ± 3.7b**	0.05	**111.5 ± 3.1a**	**97.0 ± 5.2b**
Lettuce	0.02	56.7 ± 4.9a	**87.5 ± 1.9b**	0.2	**99.0 ± 1.0a**	**105.0 ± 5.3b**
Peach	0.02	**97.0 ± 2.0a**	122.9 ± 4.3b	0.2	**85.7 ± 1.7a**	119.7 ± 3.4b

a, b: within a specific matrix and fortification level, those values lacking a common letter are different ($p<0.05$)

Table 4. Recoveries ± RSDs (%) of diazinon in matrix – fortification level (C_1 equal to MRL established by European legislation, C_2 a multiple of it) combinations using both solvent and matrix-matched standard solutions (*n*=3)

Matrix	Recoveries ± RSDs (%) (*n*=3) of phosalone					
	C_1 (mg/kg)	Solvent standards	Matrix standards	C_2 (mg/kg)	Solvent standards	Matrix standards
Pear	2.0	43.0 ± 1.4a	**107.5 ± 4.0b**	0.2	53.1 ± 3.2a	119.7 ± 7.2b
Orange	1.0	44.8 ± 2.0a	**90.0 ± 1.1b**	0.1	64.8 ± 2.7a	**107.0 ± 1.8b**
Tomato	1.0	46.5 ± 3.1a	**109.9 ± 1.1b**	0.1	57.8 ± 1.5a	**108.7 ± 3.8b**
Lettuce	1.0	41.9 ± 4.4a	65.8 ± 2.4b	0.1	**87.2 ± 1.0a**	**75.5 ± 1.9b**
Peach	2.0	37.9 ± 4.3a	**70.9 ± 9.4b**	0.2	62.4 ± 2.2a	**110.8 ± 2.4b**

a, b: within a specific matrix and fortification level, those values lacking a common letter are different ($p<0.05$)

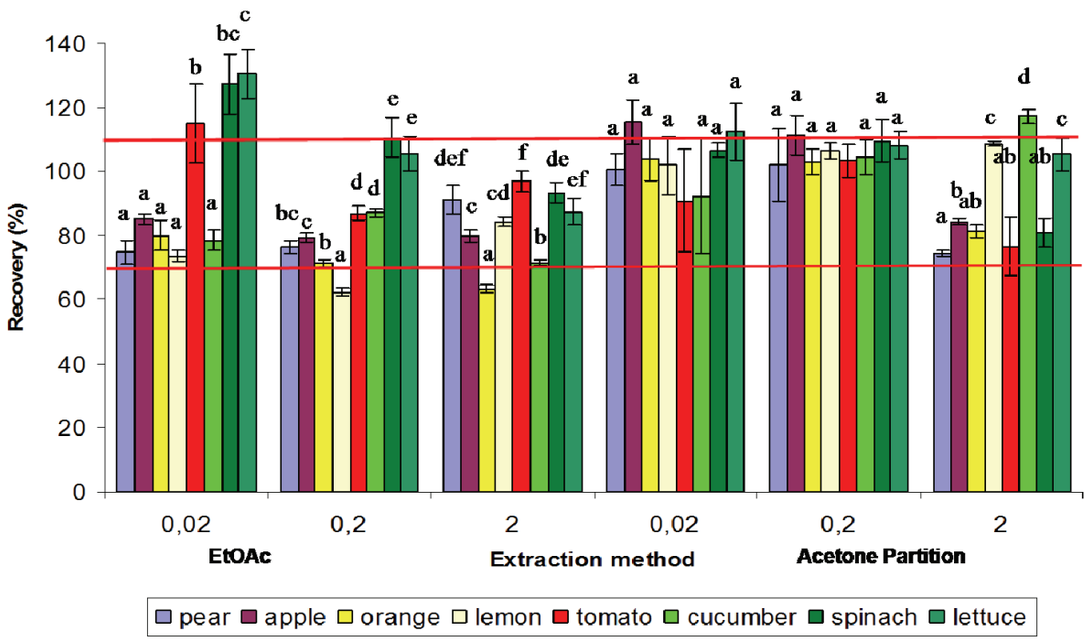
Table 5. Recoveries ± RSDs (%) of phosalone in matrix – fortification level (C_1 equal to MRL established by European legislation, C_2 a multiple of it) combinations using both solvent and matrix-matched standard solutions (*n*=3)

3. Validation of an MRM in plant products belonging to the same botanical group: Is a correct or an erroneous practice?

The validation of an MRM is a continuous procedure including the performance verification of critical parameters, such as accuracy, precision, sensitivity, repeatability and reproducibility during its application (Jensen, 1988; Hill & Reynolds, 1999; Ambrus, 2004). Basic concepts of the validation criteria have been developed by EURACHEM, AOAC International and national organizations (Ambrus, 2008). The general guidelines ensure the method reliability under the prescribed conditions and requirements of the validation protocol being available in the records of every validated laboratory (Huber, 1998; Fong, 1999). It should also be noticed that validation is a complicated procedure, demanding the accurate evaluation of some analytical parameters, such as %R, LOD, LOQ, %RSD, linearity, repeatability, reproducibility, matrix effects, combined uncertainty, random and systematic errors, referred in European standards (ISO 17025;2005), being recorded for the analytes – matrix combinations in fixed periods of time (Huber, 1998; Fong, 1999; Wood, 2006).

The appropriate MRM choice depends on all mentioned parameters, the main of which seem to be the effects of matrix. This is due to the significantly different %R and LODs-LOQs of specific analytes among the different examined commodities because of the co-extracts remained even after the application of clean-up step(s). However, the application of different approaches in the MRMs within the same laboratory among the matrices analysed is practically impossible. Therefore, there are increasing demands to extend a specific procedure to variable matrices with parallel acceptable analytical parameters. For this reason, several commodities have been categorized according to different criteria of taxonomy. Ambrus et al. (1981) suggested six major groups of plant products according to their chemical composition (e.g., group I including tuberous and root vegetables such as carrot, potato, garlic, onion, group II containing products with absence or low chlorophyll and fat content such as stone fruits, fruiting vegetables, banana, radish etc.) (Tekel & Hatrik, 1996). Bates & Gorbach (1982) applied the differences of botanical characteristics in the plant products, as adopted by Codex Alimentarius (e.g., lettuce, spinach, radish etc belong to leafy vegetables, orange, mandarin, lemon etc are in the group of citrus fruits), to classify plant products for the appropriate MRM selection. Some extensions or limitations of MRM validation to more matrices are reported to Hill & Reynolds (1999); for example analytical data for grains are not enough to prove its effectiveness for beer or data for one brassica vegetable may be applicable to similar products of this group. In our previous study, there were significant differences in the recoveries of pesticides in extracts derived from matrices belonging to the same botanical group (especially in the categories of pome fruits and citrus) under the examined conditions (Georgakopoulos et al., 2007). Therefore, obtaining analytical data, by EtOAc method without additional clean-up and GC-NPD solvent standard single point determination, from only one representative matrix with the purpose to validate the procedure in its botanical category was proved an erroneous practice.

Based on the information described and taking into consideration that the influence of each matrix on the chromatographic response should not always be correlated with its botanical characteristics, there were evaluations of when this practice is or not efficient while still using cost-effective MRMs and GC-NPD. Analytical parameters, such as recovery data, LOD, LOQ, repeatability and combined uncertainty were generally within the acceptable



In all Figs: (a) error bars designate %RSDs for the %R in the three independent replicates (n=3) and (b) a, b, c, d, e, f: within a specific MRM and fortification level, those values lacking a common letter are different ($p<0.05$)

Fig. 1. Recovery ± RSD (%) of dimethoate among the different matrix – fortification level – extraction method combinations.

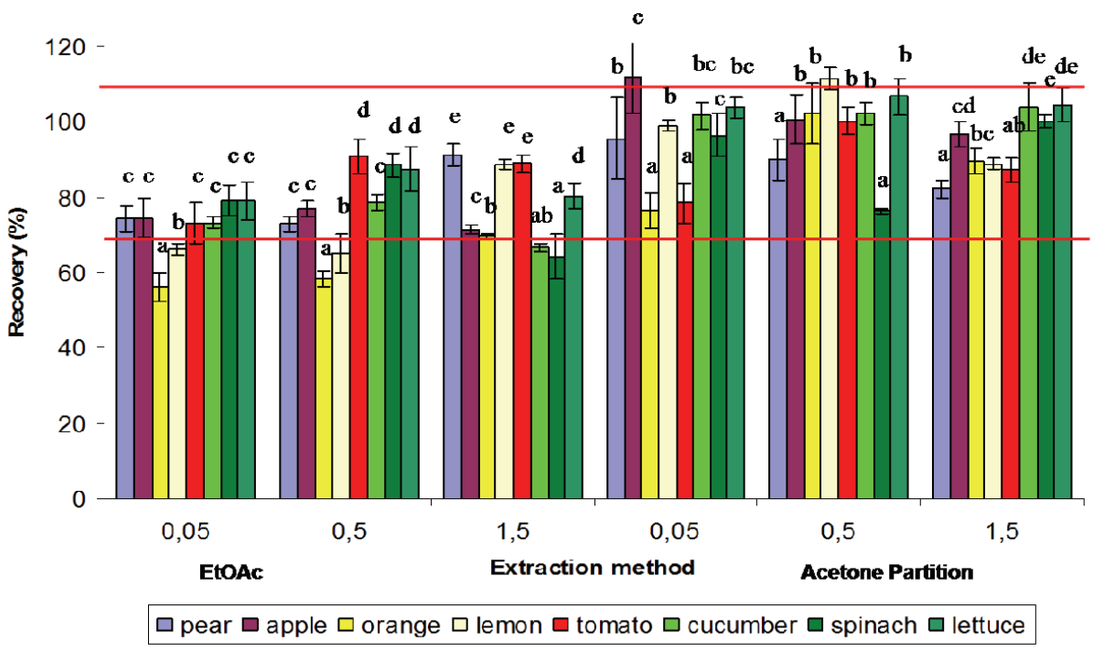


Fig. 2. Recovery ± RSD (%) of chlorpyrifos among the different matrix – fortification level – extraction method combinations.

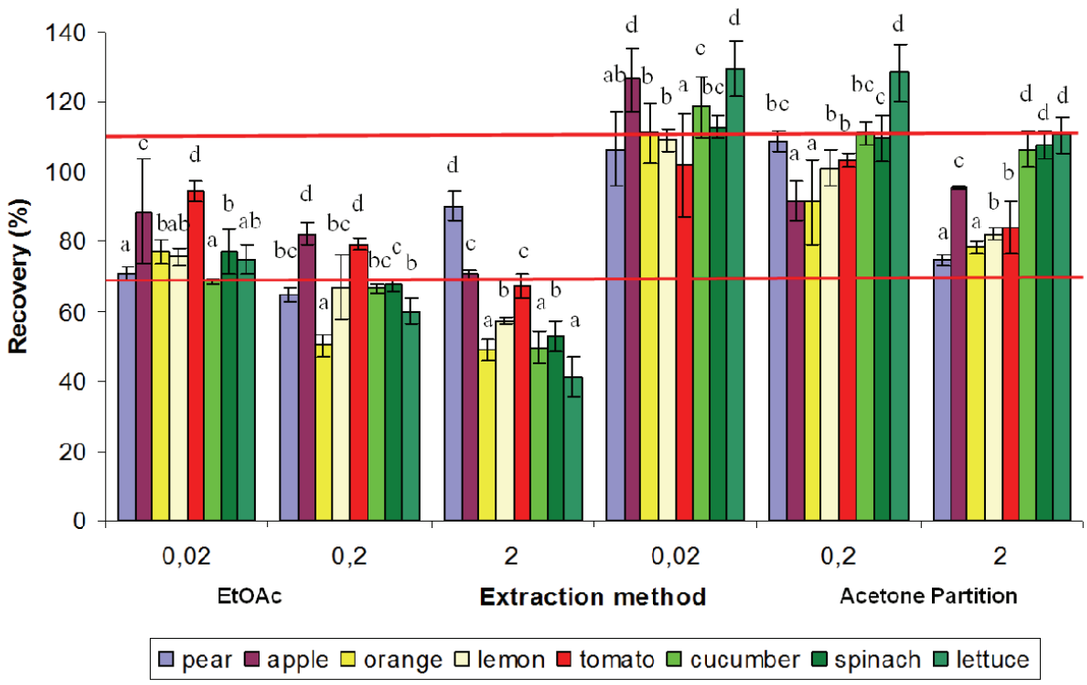


Fig. 3. Recovery ± RSD (%) of methidathion among the different matrix – fortification level – extraction method combinations.

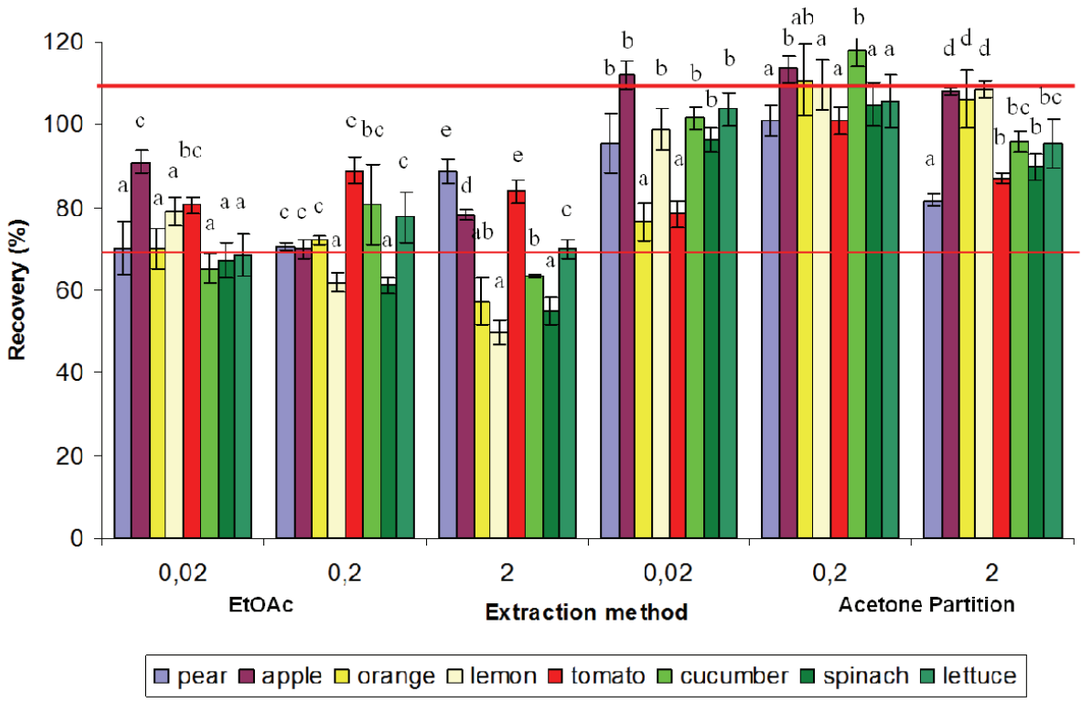
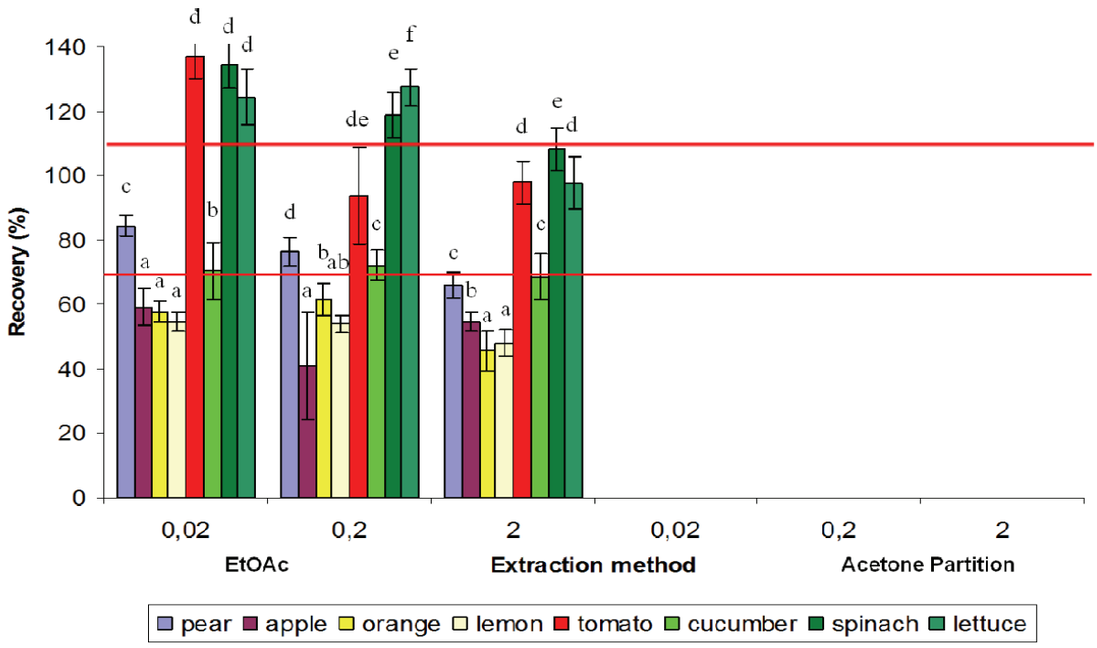


Fig. 4. Recovery ± RSD (%) of diazinon among the different matrix – fortification level – extraction method combinations.



Bars are not presented in acetone partition for methamidophos since it was not even detected in the relevant extracts.

Fig. 5. Recovery ± RSD (%) of methamidophos among the different matrix – fortification level – extraction method combinations.

ranges by the application of matrix-matched standard solution determination for EtOAc and acetone partition method (Figs 1 to 5 and Table 6). Thus, the effects of factors suspected for further uncertainty of the final result seem to be limited without much increasing demands for additional costs and techniques. However, the method of acetone resulted in higher recoveries than the EtOAc ($p<0.05$); this was more intense in semi- and non-polar analytes (acetone partition extracts generally gave %R of 90 to 110%) contrary to dimethoate and especially methamidophos (not detected in any acetone partition extract) (Figs 1 to 5). Furthermore, chromatograms of acetone partition extracts were free of unknown peaks in contrast to the respective of EtOAc leafy vegetable and citrus extracts (Fig. 6).

Recoveries derived from the matrices of the same botanical group did not appear differences ($p\geq0.05$) in acetone partition contrary to the respective of EtOAc ($p<0.05$) (Figs 1 to 4). For instance, concentrations of 0.02 and 0.2 mg/kg did not present any %R difference in among the eight products examined (Fig. 1). Similar behavior was observed to the majority of the examined combinations, such as those of diazinon and acetone partition extracts. Therefore, this MRM may be successfully applied to collect data from a single product and extend the validation for lots of commodities of the same botanical group.

4. Conclusions and future perspectives

The accurate determination of analytes is significantly affected by several factors, which may induce false results about the quantity of residues. The most important factors leading in various adverse effects are summarized as follows: (a) solvent and other materials (e.g., type of sorbents for clean-up) applied for the residues extraction from the matrix analysed,

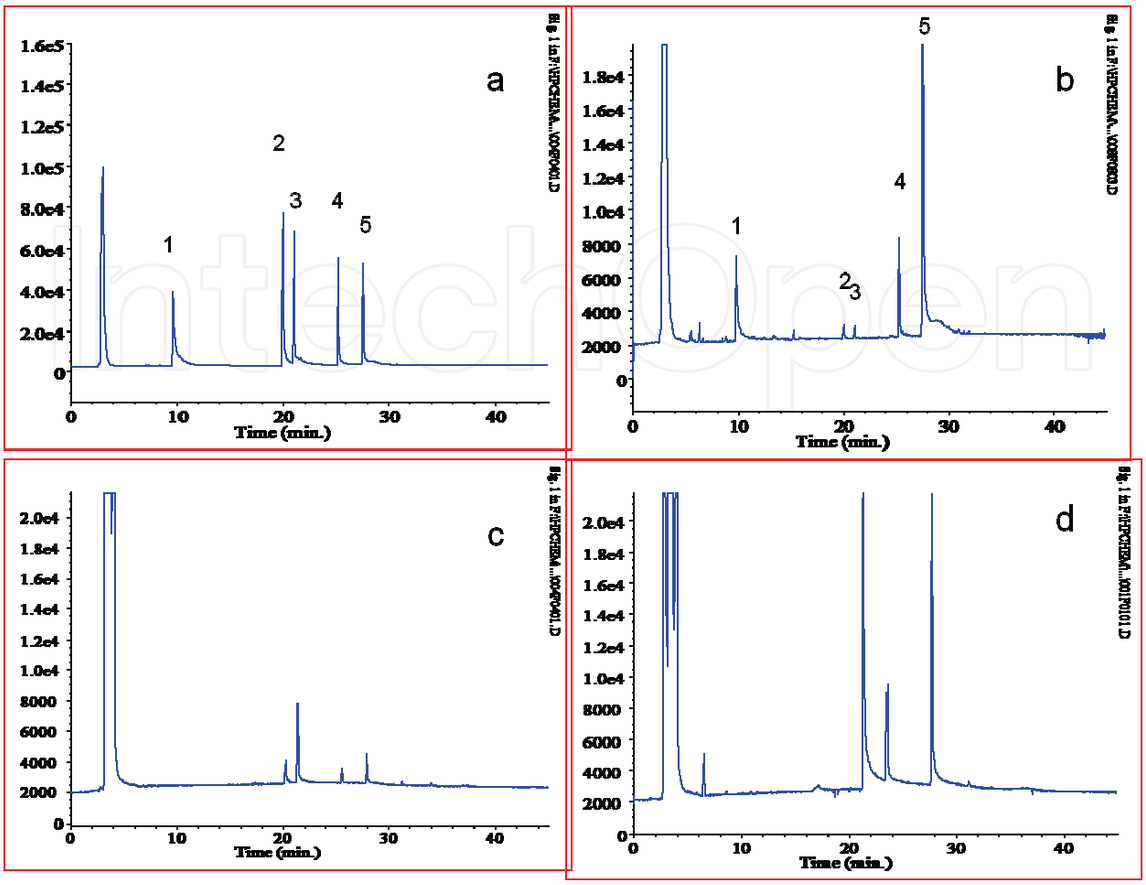


Fig. 6. GC-NPD chromatograms of fortified with the pesticides mixture (a) tomato – EtOAc, (b) orange – EtOAc, (c) tomato – acetone partition and (d) orange – acetone partition extracts. Peaks identification: 1. methamidophos, 2. dimethoate, 3. diazinon, 4. chlorpyrifos and 5. methidathion.

(b) detected molecule polarity and its determined concentration level, (c) matrix chemical composition and remained co-extracts in the final GC-extracts leading in enhancement effects, (d) GC system history related with the appropriate maintenance application. Even after the application of alternative approaches, such as additional clean-up step, on-column injection, GC-MS/MS, the phenomena of matrix-induced effect may not always be predicted or minimized; matrix-matched standard solutions are likely proved to reduce the extents of such effects. Some efforts should also be applied to the cost-effective MRMs, since they are able to provide adequate validation data without the extra needing for modern expensive techniques not always being available by many analytical laboratories. Moreover, with the purpose to validate an MRM to several commodities of the same botanical characteristics, the application of acetone partition plus GC-NPD with matrix standard single point determination was proved as an encouraging practice contrary to the failure techniques of previous studies. However, in order to generalize the findings, a higher number of pesticides, including much more analytes from the different polarity categories, should be utilized in the fortification procedures of the plant products belonging in different groups of botanical categories.

Pesticide	Matrix-MRM	LOD (mg/kg)*	LOQ (mg/kg)*	Repeatability (%RSD)**	Combined uncertainty (%RSD)**
Chlorpyrifos	Pear-Ac. Part.	0.002	0.023	0.8 (0.5)	6.5
	Apple-Ac. Part.	0.003	0.032	6.7 (0.5)	9.4
	Orange-Ac. Part.	0.004	0.027	3.8 (0.3)	10.8
	Lemon-Ac. Part.	0.004	0.031	2.7 (0.2)	5.3
	Tomato-Ac. Part.	0.003	0.030	4.1 (0.5)	6.2
	Cucumber-Ac. Part.	0.003	0.028	3.1 (0.05)	4.2
	Spinach-Ac. Part.	0.004	0.033	8.5 (0.05)	11.4
	Lettuce-Ac. Part.	0.004	0.032	9.1 (0.05)	12.7
	Pear-EtOAc	0.002	0.020	1.8 (0.5)	3.3
	Apple-EtOAc	0.003	0.027	2.2 (0.5)	3.7
	Orange-EtOAc	0.001	0.015	1.8 (0.3)	3.5
	Lemon-EtOAc	0.001	0.015	3.7 (0.2)	6.5
	Tomato-EtOAc	0.001	0.021	4.3 (0.5)	6.6
	Cucumber-EtOAc	0.001	0.020	2.5 (0.05)	3.8
	Spinach-EtOAc	0.003	0.022	6.6 (0.05)	8.9
	Lettuce-EtOAc	0.003	0.025	7.9 (0.05)	9.3
Diazinon	Pear-Ac. Part.	0.001	0.024	1.5 (0.3)	4.6
	Apple-Ac. Part.	0.001	0.019	3.4 (0.3)	8.1
	Orange-Ac. Part.	0.002	0.018	2.6 (1.0)	4.4
	Lemon-Ac. Part.	0.002	0.027	3.1 (LOQ)	5.1
	Tomato-Ac. Part.	0.001	0.024	5.2 (0.5)	5.5
	Cucumber-Ac. Part.	0.001	0.024	9.7 (LOQ)	10.5
	Spinach-Ac. Part.	0.002	0.025	10.4 (LOQ)	13.4
	Lettuce-Ac. Part.	0.002	0.028	9.9 (LOQ)	12.8
	Pear-EtOAc	0.003	0.023	1.2 (0.3)	2.6
	Apple-EtOAc	0.001	0.022	2.3 (0.3)	3.9
	Orange-EtOAc	0.001	0.009	0.9 (1.0)	2.5
	Lemon-EtOAc	0.001	0.015	4.9 (0.02)	5.8
	Tomato-EtOAc	0.001	0.024	3.4 (0.5)	6.1
	Cucumber-EtOAc	0.005	0.032	8.0 (LOQ)	12.8
	Spinach-EtOAc	0.003	0.026	8.3 (LOQ)	13.1
	Lettuce-EtOAc	0.003	0.025	7.7 (LOQ)	12.6
Methidathion	Pear-Ac. Part.	0.007	0.044	3.3 (0.3)	4.7
	Apple-Ac. Part.	0.003	0.033	4.2 (0.3)	7.4
	Orange-Ac. Part.	0.007	0.041	6.2 (2.0)	10.7
	Lemon-Ac. Part.	0.003	0.031	2.7 (2.0)	4.4
	Tomato-Ac. Part.	0.009	0.033	9.3 (LOQ)	17.4
	Cucumber-Ac. Part.	0.004	0.030	9.4 (LOQ)	11.9
	Spinach-Ac. Part.	0.009	0.037	11.0 (LOQ)	15.6
	Lettuce-Ac. Part.	0.008	0.040	13.4 (LOQ)	16.8
	Pear-EtOAc	0.001	0.021	2.2 (0.3)	3.6
	Apple-EtOAc	0.002	0.025	3.9 (0.3)	5.4
	Orange-EtOAc	0.001	0.013	2.7 (2.0)	4.5
	Lemon-EtOAc	0.004	0.026	2.2 (2.0)	3.2
	Tomato-EtOAc	0.004	0.024	16.8 (LOQ)	21.9
	Cucumber-EtOAc	0.001	0.017	3.6 (0.02)	4.7
	Spinach-EtOAc	0.003	0.026	10.0 (LOQ)	14.4
	Lettuce-EtOAc	0.004	0.028	9.4 (LOQ)	14.1
Dimethoate	Pear-Ac. Part.	0.003	0.030	5.8 (LOQ)	7.8

Pesticide	Matrix-MRM	LOD (mg/kg)*	LOQ (mg/kg)*	Repeatability (%RSD)**	Combined uncertainty (%RSD)**
	Apple-Ac. Part.	0.002	0.024	8.7 (LOQ)	9.6
	Orange-Ac. Part.	0.004	0.035	7.3 (LOQ)	10.1
	Lemon-Ac. Part.	0.006	0.039	7.4 (LOQ)	11.8
	Tomato-Ac. Part.	0.009	0.047	13.3 (LOQ)	20.7
	Cucumber-Ac. Part.	0.010	0.052	14.2 (LOQ)	23.0
	Spinach-Ac. Part.	0.010	0.050	12.6 (LOQ)	18.2
	Lettuce-Ac. Part.	0.009	0.048	11.7 (LOQ)	17.4
	Pear-EtOAc	0.002	0.021	5.9 (LOQ)	7.3
	Apple-EtOAc	0.001	0.019	3.3 (LOQ)	5.3
	Orange-EtOAc	0.002	0.023	5.0 (LOQ)	7.2
	Lemon-EtOAc	0.001	0.017	7.4 (LOQ)	7.9
	Tomato-EtOAc	0.008	0.051	15.1 (LOQ)	19.6
	Cucumber-EtOAc	0.003	0.030	10.4 (LOQ)	11.0
	Spinach-EtOAc	0.008	0.047	13.1 (LOQ)	15.8
	Lettuce-EtOAc	0.008	0.050	11.6 (LOQ)	15.4
Methamidophos***	Pear-EtOAc	0.006	0.031	13.0 (LOQ)	20.0
	Apple-EtOAc	0.004	0.022	14.7 (0.05)	20.2
	Orange-EtOAc	0.002	0.016	7.0 (0.02)	8.7
	Lemon-EtOAc	0.002	0.015	4.2 (0.2)	5.3
	Tomato-EtOAc	0.003	0.030	10.9 (0.5)	18.6
	Cucumber-EtOAc	0.002	0.015	2.8 (1.0)	7.0
	Spinach-EtOAc	0.006	0.034	13.4 (LOQ)	19.6
	Lettuce-EtOAc	0.007	0.039	8.9 (0.2)	12.4

*: LOD was estimated as the analyte concentration resulted in signal (S) to noise (N) ratio of 3 (S/N=3) (Huber, 1998) and verified by the analysis of the pesticide mixture fortified at 0.01 mg/kg (six independent replicates) as three times the standard deviation (LOQ=3*SD) (Yenisoy-Karakaş, 2006; Barriada-Pereira et al., 2007; Georgakopoulos et al., 2009); LOQ was defined as the analyte concentration resulting in S/N of 10 (Huber, 1998) and verified by the afore-mentioned procedure applied for LOD. LOQ equals "mean+10*SD", where mean is the average of concentration levels determined in the six independent replicates by the analysis procedures (Yenisoy-Karakaş, 2006; Barriada-Pereira et al., 2007; Georgakopoulos et al., 2009).

**: Repeatability determination was based on the %RSDs derived from six independent replicates prepared by the same analyst, analytical method and analysis day (Ambrus, 2004) of matrix extracts fortified with the MRL level (in mg/kg) as established by the European legislation shown in parenthesis of every combination; LOQ level was used in the cases that the MRL was lower than the LOQ; the same levels were used for the determination of combined uncertainty the calculation of which included the major sources of uncertainty, such as (Huber, 1998; Cuadros-Rodríguez et al., 2002; Ambrus, 2004).

***: Methamidophos validation data are not presented for acetone partition, since this analyte was not even detected in any extract of this MRM.

Table 6. Critical analytical parameters for MRM validation among different pesticide – matrix – extraction method combinations

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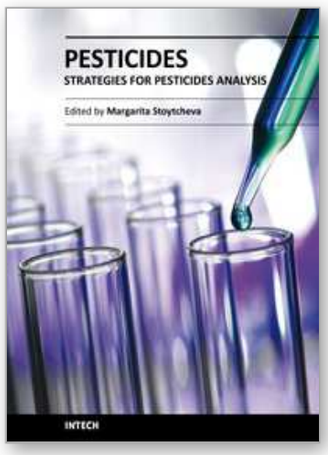

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This book provides recent information on various analytical procedures and techniques, representing strategies for reliability, specificity, selectivity and sensitivity improvements in pesticides analysis. The volume covers three main topics: current trends in sample preparation, selective and sensitive chromatographic detection and determination of pesticide residues in food and environmental samples, and the application of biological (immunoassays-and biosensors-based) methods in pesticides analysis as an alternative to the chromatographic methods for "in situ" and "on line" pesticides quantification. Intended as electronic edition, providing immediate "open access" to its content, the book is easy to follow and will be of interest to professionals involved in pesticides analysis.

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