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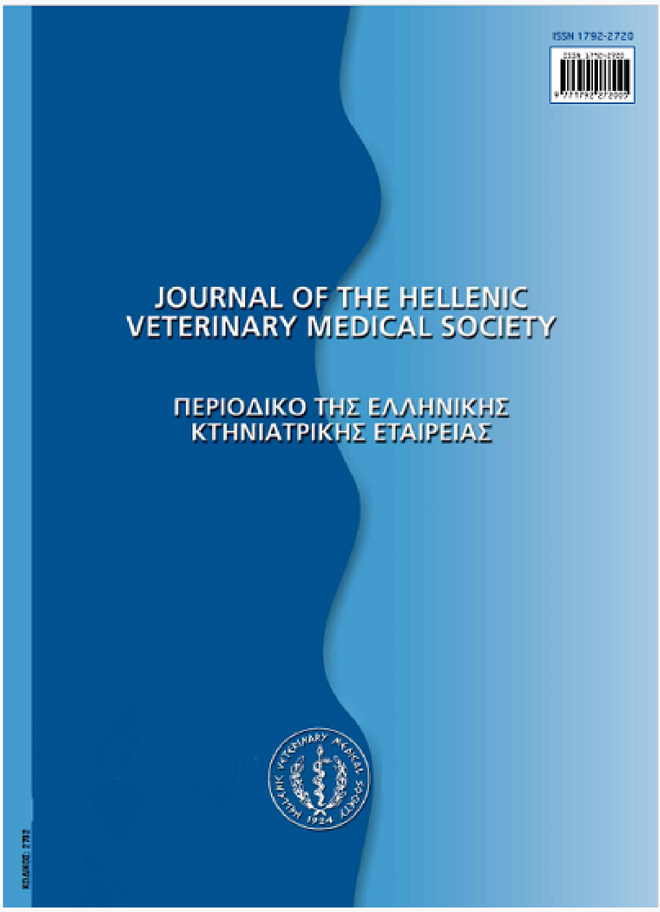

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Problems encountered in LC-MS/MS analysis for the determination of pesticide residues in food

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Προβλήματα στον προσδιορισμό υπολειμμάτων φυτοφαρμάκων με τη χρήση υγρής χρωματογραφίας-φασματομετρίας μάζας τριπλού τετραπόλου

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ABSTRACT. The most common problems encountered in LC-MS/MS analysis of food are cases of compounds with low recovery, low sensitivity in one transition, common transition with matrix or other analytes, as well as solvent and matrix effects. The significance of each case was experimentally investigated in this study by the use of representative compounds. The QuEChERS approach was used for the extraction of pesticides. The study involved analysis of 128 pesticides representing various chemical classes, polarities and molecular masses, in 8 products representing different commodities of food matrices. Blank matrices fortified at 33.3 and 167 μg/kg, were analysed using different LC-MS/MS conditions in order to evaluate the significance of each parameter. Additionally long term LC-MS/MS system suitability data of 18 months were used to evaluate the variability of retention time and ion ratio at the limit of detection. This was assessed by the use of 6 representative compounds and the respective control charts.

Keywords: pesticide residues, food, LC-MS/MS

ΠΕΡΙΛΗΨΗ. Μελετήθηκαν τα πιο κοινά προβλήματα που παρουσιάζονται στις αναλύσεις τροφίμων με τη χρήση της υγρής χρωματογραφίας – φασματομετρίας μάζας τριπλού τετραπόλου (LC-MS/MS). Αφορούσαν σε ενώσεις με χαμηλές ανακτήσεις, χαμηλή ευαισθησία στη μια από τις δυο μεταπτώσεις, μία κοινή μετάπτωση με το υπόστρωμα ή με άλλους

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αναλύτες, καθώς και με το διαλύτη. Επιπλέον, μελετήθηκε το φαινόμενο της επίδρασης του υποστρώματος στη μέτρηση (matrix effect). Η σημαντικότητα του κάθε προβλήματος μελετήθηκε πειραματικά με τη χρήση αντιπροσωπευτικών ενώσεων. Για την εκχύλιση των φυτοφαρμάκων χρησιμοποιήθηκε η προσέγγιση QuEChERS. Η μελέτη περιλαμβάνει 128 αναλύτες που αντιπροσωπεύουν διαφορετικές κατηγορίες χημικών ομάδων, με διαφορετικές πολικότητες και μοριακές μάζες σε 8 προϊόντα τροφίμων που αντιπροσωπεύουν διάφορες κατηγορίες υποστρωμάτων. Λευκά υποστρώματα εμβολιάστηκαν στα 33.3 μg/kg και 167 μg/kg, τα οποία και αναλύθηκαν χρησιμοποιώντας διαφορετικές συνθήκες LC-MS/MS προκειμένου να αξιολογηθεί η σημαντικότητα της κάθε παραμέτρου. Επιπλέον, χρησιμοποιήθηκαν μακροπρόθεσμα δεδομένα 18 μηνών ελέγχου καταλληλότητας του συστήματος LC-MS/MS προκειμένου να αξιολογηθεί η μεταβλητότητα του χρόνου έκλουσης των αναλυτών και η αναλογία ιόντων στο όριο ανίχνευσης. Η αξιολόγηση αυτή έγινε με χρήση 6 αντιπροσωπευτικών ενώσεων και με τα αντίστοιχα διαγράμματα ελέγχου.

Λέξεις ευρετηρίασης: υπολείμματα φυτοφαρμάκων, τρόφιμα, LC-MS/MS

INTRODUCTION

QuEChERS (Quick Easy Cheap Effective, Rugged and Safe) (EN 15662) is one of the most commonly applied approaches for pesticide residue analysis that provides recoveries between 70 and 120% for the majority of pesticide/matrix combinations. QuEChERS includes sodium chloride to limit polar interferences and several buffering reagents to preserve base sensitive analytes. For high water content matrices, the European version of the method is extraction with acetonitrile in the presence of citrate buffering salts, to obtain pH-values between 5 and 5.5. However for high oil content food extraction is performed without the citrate buffer (Anagnostopoulos and Miliadis, 2013). Freezing is a critical part of the extraction procedure of high fat content food, as it helps to partly remove some additional co-extractives with limited solubility in acetonitrile, while the major part of fat and waxes solidify and precipitate. Also the removal of chlorophyll from commodities with a high chlorophyll content, which is performed by the addition of graphitized carbon black, results at a sacrifice of approximately 25% reduced recovery of some planar pesticides.

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) allows the simultaneous rapid and efficient determination of many compounds. Selectivity and sensitivity are notably improved compared to traditional chromatographic techniques, the sample pre-treatment steps can be minimized, and reliable quantitation and confirmation can be easily achieved at the low concentration levels required for residues analysis. However limitations exist in certain compounds, as low sensitivity for one or both transitions, common transitions with compounds of the matrix and solvent effects.

The purpose of this study is through validation to detect analytes among 128 representative pesticides that present matrix effects and require matrix matched calibration, or with low recovery, even when matrix matched. Also to investigate: limitations of sensitivity in LC-MS/MS determination, solvent effects, as well as ways to overcome these problems. Additionally, long term LC-MS/MS system suitability data were collected for 18 months, in order to evaluate the variability of retention time and ion ratio at 0.01mg/kg, which is the limit of detection (LOD). This was attained by the use of 6 representative compounds and the respective control charts.

MATERIALS AND METHODS

Pesticide reference standards (purity>98%) of analytes were purchased from Ehrenstorfer GmbH, Augsburg, Germany, Sigma-Aldrich, Saint Louis, USA and ChemService, West Chester, USA. All solvents, namely acetonitrile, methanol and water were of HPLC grade. Primary secondary amine sorbent (PSA, 40 μm, Bondesil) was purchased from Varian Inc., USA. Magnesium sulfate dried was purchased from Acros Organics and Graphitized Carbon Black (GCB) from Sigma-Aldrich Chemie GmbH. Stock solutions of 1000 μg/mL were prepared, usually in acetone. The different stock solutions were combined into a mixed standard solution containing 128 pesticides, each at 1 μg/mL in acetonitrile. The solutions were stored at -20°C.

Samples were extracted using the European version of the QuEChERS method, EN 15668. For food of high water content 10 g of sample were used, while for cereals, olives and olive oil 5 g. Citrate buffer was used for the extraction with acetonitrile and PSA for dispersive solid phase extraction. No citrate buffer was used for olives and olive oil.

An Agilent 1200 Liquid Chromatograph combined with a Waters Quattro Premier triple quadrupole Mass Spectrometer at the ESI+ mode, with an Eclipse XDB C-18, 2.1×150 mm column were used. Gradient elution with solvent A: 0.1% HCOOH, 5 mM HCOONH4, 20% methanol in water and solvent B: 0.1% HCOOH, 5 mM HCOONH4 in methanol was used as in the Table 1. Column temperature was set at 40oC. Flow rate was set at 0.25 mL/min and 5 μL of sample were diluted in the autosampler with 20 μL of water before injection so that the injected solvent to simulate the initial composition of the mobile phase. MS/MS was operated at the MRM mode.

For the within-laboratory validation, representative matrices were used as to cover a wide range of commodities. Based on the SANCO document 12571/2013 a selection of representative products was used, as to cover a wide category of products. Eight products were used from different commodities for this study; i.e. flour from the cereals, olive oil from the high oil content food, olives from the high oil and intermediate water content food, 4 different commodities of fruit and vegetable matrices from the high water content food (apples, cabbage, pepper and potatoes) and oranges from the high acid and high water content food, (DG-SANCO 2013). Blank matrices were selected from previously analysed samples with not detected residues of the analytes.

Table 1: Elution program of LC analysis.

Retention time (min)	% solvent A	% solvent B
0	100	0
2	100	0
12	50	50
30	0	100
40	0	100
40.5	100	0

RESULTS AND DISCUSSION

Accuracy, precision, LOD, solvent and matrix effects were assessed in order to detect analytes presenting problems in their detection or quantitation. The study included data covering 128 pesticides and 8 matrices. As blank matrices, products known not to have been treated with pesticides were used. Blank matrices were analysed to confirm the absence of pesticides and then were fortified at two different concentrations, 0.0333 mg/kg and 0.167 mg/kg. The selected pesticides represent a significant variety of chemical classes and physicochemical properties as shown in Table 2.

The recoveries obtained for all matrices except olive oil were in the acceptable range (70-120%) for 114 out of 128 analytes; the exceptions represent 11% of the studied compounds and included 9 sulfonylureas and 5 other analytes, as described below. However for olive oil, 34 out of the 128 studied analytes failed to produce acceptable recoveries, showing that the applied extraction was not efficient for 27% of the studied compounds. As for the 7 other matrices, the pH-sensitive sulfonylureas had low to very low recovery values (10-60%), even when the extract was not acidified following PSA or even when quantitation was performed with matrix matching. Additionally benfuracarb from the carbamates presented low recovery when in matrix and dichlofluanid from sulfamides. They both had very low recoveries in most matrices, even when quantitated with matrix matching, except dichlofluanid in apples and oranges, were recoveries were acceptable. Diafenthiuron also presented very low recovery in all 7 matrices, except for oranges, and ethoxyquin also (~20%) in potatoes, apples, flour and cabbage but not in the other products, in which it could be quantified satisfactorily by matrix matching. Finally tolyfluanid presented very low recovery in potatoes and cabbage, while in the rest products it could be quantified accurately with matrix matching. Among the studied analytes, azinphos methyl and phosmet presented the same retention time, a common quantitative transition, and a similar but different qualitative transition. This could result in false negative results, since the ion ratio of

Table 2: Number of compounds studied for each chemical class and the range of physicochemical properties within each class. (University of Hertfordshire, 2007)

Chemical group	No of studied compounds	Ranges of values		
		Solubility in H_2O, mg/L	$\log k_{ow}$	Vapour pressure, mPa
Amides	5	0.9 – 26×10^{3}	0.67 - 2.17	2×10^{-4} – 3.3×10^{-3}
Esters	2	0.05-7.9	4-4.5	5.5×10^{-2}
Benzoylureas	3	0.004 - 111	2.28 - 4	6.52×10^{-12} – 1.2×10^{-4}
Bendimidazoles	3	8 - 30	1.5 - 2.4	8.8×10^{-6} – 1.5×10^{-4}
Carbamates	14	7.74 – 28×10^{4}	-0.44 – 4.6	7.7×10^{-8} – 1.3×10^{-2}
Neonicotinoids	4	185 - 4250	-0.13 - 1.26	4×10^{-10} – 1×10^{-6}
Organophosphorous	20	1 – 10^{6}	-0.9 - 3.85	1.03×10^{-5} – 0.123
Pyretrhins (natural)	6	0.35 - 1038	2.85 - 5.62	4.6×10^{-7} – 2.02×10^{-5}
Sulfonylureas	8	3.7 - 3293	-0.78 – 0.646	4.2×10^{-11} – 2.8×10^{-6}
Strobylurines	3	1.9 - 6	2.5 – 3.99	1.1×10^{-10} – 2.3×10^{-6}
Triazines	5	6.2 – 13×10^{3}	-0.1 – 3.21	4.48×10^{-7} – 1.5×10^{-4}
Triazoles	8	0.2 - 156	3.08 – 4.1	2.2×10^{-10} – 0.056
Phenylureas	8	0.06 - 735	1.6 – 5.76	5×10^{-6} – 4.3×10^{-3}
Various	39	0.075 – 2×10^{5}	-0.5 – 5.6	7.9×10^{-7} – 0.267

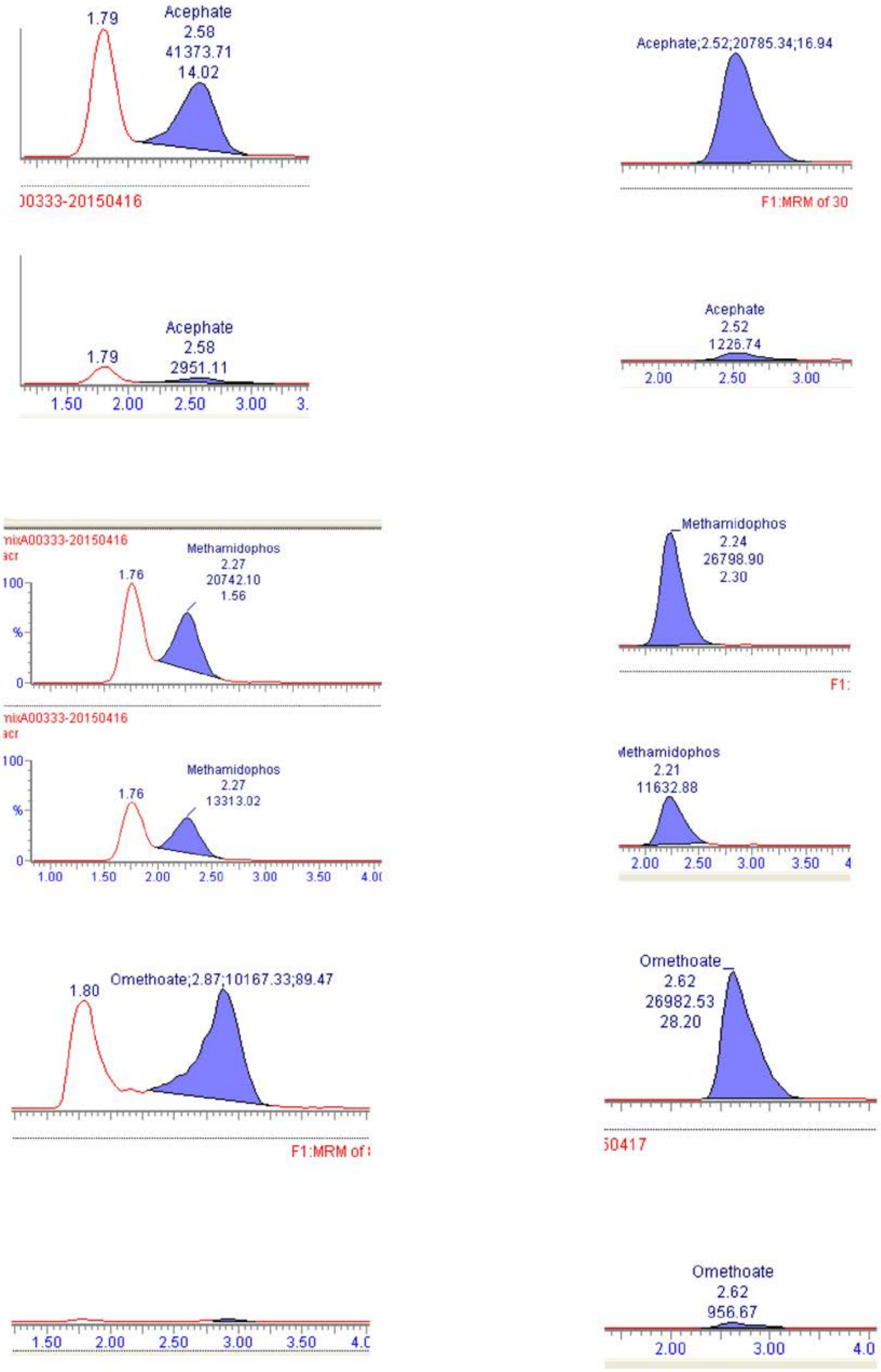
the 2 transitions is dependent on the simultaneous or not presence of the 2 compounds in the sample or the standard mixture, e.g the ion ratio for azinphos methyl transitions is 0.8 when it is alone, and 0.08 when it is in the same solution with phosmet.

Since acceptable accuracy and precision were attained for most analytes at 0.03 mg/kg, this level was considered as the limit of quantitation (LOQ). Therefore in most cases 0.01 mg/kg was considered as the limit of detection, since LOD and LOQ are related with the equation LOQ=3.3×LOD. Low sensitivity at both transitions presented aclonifen, fenitrothion, parathion and parathion methyl; while low sensitivity in one transition presented florasulam, pendimethalin, pyrifenox. Compounds with significant signal enhancement when in matrix, e.g. diclofluanid, could not be detected at the 0.01 mg/kg level, when standard solutions in solvent were used. Thiophanate methyl in dilute solutions was found unstable and needed to be prepared fresh.

The very polar compounds as acephate, aldicarb sulfoxide, formetanate, methamidophos, omethoate, propamocarb, that are early eluting, they all presented two peaks for each transition, one common for all compounds at approximately 1.8 min and a second peak at various retention times depending on the compound as shown in Figure 1. This happened when the injected solutions were in acetonitrile, a convenient solvent since it is the QuEChERS extraction solvent. This phenomenon often resulted in bad peak resolution, thus affecting quantitation. The first common peak disappeared as shown in Figure 1, when the injected solutions were in a solvent same in composition as the initial mobile phase (20% methanol in water). Another effect possibly originating from the reagents, was the second transition of malathion (m/z 331>99) that was observed in all studied matrices.

The stability of the retention time and the ion ratio at the limit of detection over 18 months period were studied under the same conditions. System suitability data of LC-MS/MS were used for this purpose. LC-MS/MS system was assessed for its suitability for everyday use, by injecting a mixture of 6 representative analytes at 0.01 μg/mL, i.e. the limit of detection. The data collected for the retention time (RT) and the ion ratio of these analytes were monitored in control charts. Long term data collected for 18 months were then statistically treated, in order to evaluate the variability of retention time and ion ratio. As the same column was used throughout the whole period of the 18 months, the S/N

Fig 1: LC-MS/MS chromatograms of very polar pesticides in acetonitrile (left), and in 20% MeOH 1 in H2O (right).



ratio and the shape of the peaks were also monitored, in order to detect any column deterioration an to take the proper corrective actions, as cleaning of the frit, flush or back flush of the column with proper solvents. It was found that the S/N at 0.01 mg/L, the shape and the width of the peaks did not present significant differences through the 18-months period. The RSD values of the RT, as seen from Table 3, were found within the 18 months period between 1.01 and 2.59%, except for the very polar and early eluting oxamyl with 7.26%, for which chromatography is poor. It is also seen that with increasing retention time RSD values decrease.

As for the second critical confirmation criterion in LC-MS/MS, which is the ion ratio (IR), as seen from Table 4, the RSD values of 18 months data, were found between 19.8% and 45.7%, not showing dependence on the relative intensity of the product ion, a criterion set in Council Directive 96/23/EC and Document N° SANCO/12495/2011.

CONCLUSIONS

Accuracy, precision, LOD, solvent and matrix effects were assessed in a study that included 128 representative pesticides in 8 matrices. The recoveries obtained were in the acceptable range for 89% of the studied pesticides in all matrices except olive oil, 34 in which the applied extraction was not efficient for 27% of the studied compounds. Sulfonylureas was a class of pesticides, all presenting low recoveries. Cases of pesticides presenting one transition with low sensitivity, a common transition with the matrix or other analytes were detected. The very polar analytes were found to suffer from solvent effect, splitting into two peaks when injected in acetonitrile. From 18 months data, under constant LC conditions, the RSD values of the retention time ranged between 1.0 and 2.6% with one exception, while the respective ion ratio RSD values between 19.8% and 45.7%, not showing any dependence on the relative intensity of the product ion.

Table 3:%RSD values of the retention time (RT) for selected pesticides.

	oxamyl	acetamiprid	pirimicarb	metalaxyl	diazinon	etoxazol
RT_{aver}, min	4.1	10.9	14.7	17.9	24.2	29.0
s	0.3	0.3	0.4	0.2	0.3	0.2
%RSD	7.3	2.4	2.6	1.0	1.1	0.6

Table 4: %RSD values of the ion ratio (IR) for selected pesticides.

	oxamyl	acetamiprid	pirimicarb	metalaxyl	diazinon	etoxazol
IR (aver)	0.951	0.242	0.460	0.245	0.258	0.189
s	0.3	0.05	0.2	0.05	0.1	0.04
RSD	27.4	19.8	38.5	21.6	45.7	22.2

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