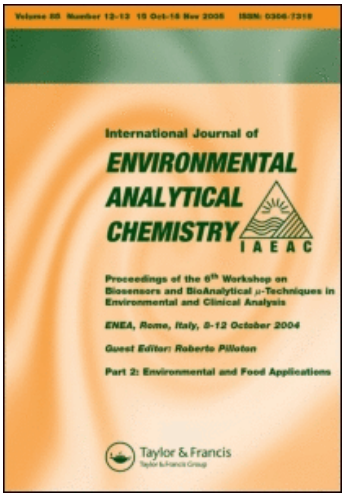

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Fast GC-MS of endocrine disrupting chemicals

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An analytical method has been developed and evaluated for the determination of endocrine disrupting chemicals (EDCs) – pesticides in non-fatty food. Twenty-nine analytes of different chemical classes according to EDCs draft list of European Commision were selected. The method is based on fast GC-MS with bench top quadrupole MS detector and electron impact (EI) ionisation (70 eV). For the effective separation programme temperature vaporiser (PTV) injector and narrow-bore column were used. Slightly modified QuEChERS sample preparation technique was applied for the preparation of apple extracts for matrix-matched standards, synthetic sample and real sample extracts. Two internal standards (I.S.) – heptachlor (HPT) and triphenylphosphate (TPP) – were used. The results needed for the method validation were calculated from absolute peak areas and normalised areas to HPT and TPP. The important part of the study is the utilisation of analyte protectants (APs). Three calibrations approaches: matrix-matched standards without/with APs and acetonitrile (MeCN) standards with APs were used. The best results were obtained with matrix-matched standards without APs. The developed and validated analytical method was used for the identification and quantification of EDCs pesticides in real samples (orange, lettuce, strawberry and plum).

Keywords: fast GC-MS; endocrine disrupting pesticides; calibration approaches; analyte protectants; non-fatty food; ultratrace analysis

1. Introduction

Endocrine disrupting chemicals (EDCs) are substances that can cause adverse effects by interfering in some way with body hormones or chemical messengers. These substances are therefore called hormone disruptors or endocrine disruptors, as it is the endocrine glands that secrete the hormones. Hormones play a crucial role in guiding normal cell differentiation in early life forms so that exposure to the endocrine disrupting substances in the egg or in the womb can alter the normal process of development. Mature animals can also be affected but mainly the developing organism is especially vulnerable. Exposure at this sensitive time may cause effects that are not evident until later in life, such as effects on learning ability, behaviour, reproduction and increased susceptibility to cancer and other diseases [1]. EDCs compounds include a wide variety of pollutants such as pesticides, polycyclic aromatic hydrocarbons, phthalate plasticisers, alkylphenols and natural and synthetic hormones [2]. Pesticides are used

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widely to kill unwanted organisms in crops, public areas, homes and gardens and medicinally to kill parasites. Many are proven or suspected to be EDCs [3].

According to the status of all active pesticide substances on the European Union (EU) market [4] more than 1100 pesticides are currently registered. The increasing public concern in recent years about possible health risks due to the pesticide residues in the diet has deeply modified the strategy for the crop protection, with emphasis on food quality and safety. This led to the strict regulation of maximum residue limits (MRLs) in food commodities. To date, more than 17.000 MRLs have been set for various commodities and pesticides [5]. MRLs vary ordinarily within the interval 0.0008–50 $mg\,kg^{-1}$ [4], typically between 0.01 and 10 $mg\,kg^{-1}$ for adult population. MRLs in baby food are set to the level of 10 $\mu g\,kg^{-1}$ which corresponds to the required limit of quantification $\leq 5\,\mu g\,kg^{-1}$ [6]. There is a wide range of suspected or bona fide EDCs, including pesticides. Their negative ecotoxicological influences require to monitor EDCs at much lower concentration than the concentration, at which their toxicity was proved (e.g. in water already at concentrations of $ng\,L^{-1}$ [7]).

The European Commission has published a draft list of chemicals, including pesticides, which are believed to damage health by interfering with the way hormones work [8]. The EDCs draft list is a good starting point for action but is not comprehensive and fails to include several chemicals which have otherwise been identified as threats to health [8]. The draft list of high and medium priority pesticides is published. High priority pesticides (for example amitrole, atrazine, lindane, linuron, vinclozolin, zineb, etc.) are endocrine disruptors, and medium priority pesticides (for example diazinon, dicofol, dimethoate, diuron, endosulfan, iprodione, etc.) are potential endocrine disruptors [8,9]. Therefore, research activity in the area of EDCs pesticide residues analysis leads towards development of more efficient multiresidue analytical methods.

The most widely used method for the analysis of EDCs compounds is based on gas chromatography (GC). GC in the combination with mass spectrometry (MS)-based detection [10–17] techniques has become powerful instrument for EDCs compounds analysis. For detection and separation of EDCs compounds with GC-MS techniques, EI ionisation (70 eV) was preferred and the column mainly with dimensions 30 m × 0.25 mm (I.D.) × 0.25 μm film of stationary phase was used. This represents conventional capillary GC-EI-MS methods for the analysis of EDCs compounds. Different sample preparation techniques have been applied to the extraction and analysis of EDCs compounds: EDCs pesticides [10–17], EDCs phtalates [10,14,15] and EDCs phenols [13], polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) [17] in environmental samples, mainly water samples [10,11,14–17], tissues (frog and fish) [12] and human cord blood [13]. The most commonly used method for liquid samples is solid-phase extraction (SPE) – off-line [10,13,15,16], on-line [14] or automated SPE [11] with different types of sorbent. Other sample preparation techniques which were applied for extraction of EDCs compounds are LPE (Liquid Phase Extraction) [12] and SBSE (Stir-Bar Sorptive Extraction) [17]. SPE can be used to determine a broad range of pesticides in one analysis. SPE methods are rapid, efficient (good recoveries and low detection limit), use less solvent than other methods and consequently have lower laboratory expenses [11].

Fast GC techniques satisfy the present-day demands on faster and cost-effective analysis [18–20]. There are obvious advantages of faster GC compared to conventional capillary GC, mainly shorter analysis time (in minutes range), increased laboratory throughput, reduced costs per sample and improved precision and sensitivity.

Nowadays fast GC can be performed on commercial gas chromatographs with standard equipment for high-speed injection, electronic gas pressure control, rapid oven heating/cooling and fast detection [21].

Despite the great efforts in the research of GC amenable pesticide residues analysis the problematic issues are matrix effects and mainly matrix-induced chromatographic response enhancement [22]. Anastassiades *et al*. [23] revised the previous attempt [24] to find masking agents that would mask active sites in the GC system and thus would provide strong response enhancement of pesticides. Three compounds (ethylglycerol, gulonolactone and sorbitol) have been chosen. These compounds have been termed as 'analyte protectants' [23].

In this study, a fast GC-MS method with narrow-bore column was developed to analyse ultra-trace concentration levels of 29 EDCs pesticides with 2 I.S. – heptachlor and triphenylphosphate in non-fatty food (apple matrix). Fast GC-MS equipped with a PTV injector and a bench-top quadrupole MS detector in combination with slightly modified sample preparation QuEChERS method (quick, easy, cheap, effective, rugged, and safe) [25] was used for the analysis of selected EDCs pesticides. The goal was to evaluate different calibration approaches based on matrix-matched standardisation and application of analyte protectants. The developed method was used for analysis of real samples.

2. Experimental

2.1 *Reagents and materials*

Standards of pesticides and internal standards (I.S.) were obtained from various sources (Bayer, Leverkusen, Germany; Dr. Ehrensdorfer, Augsburg, Germany; Cheminova, Harboore, Denmark; Ciba-Geigy, Basel, Switzerland; Shering, Kenilworth, NJ, USA; Dow AgroScience, Indianapolis, IN, USA; Agrovita, Ivanka pri Dunaji, Slovak Republic) and were of purity >95%. A list of pesticides and I.S. is given in Table 1. Solutions of individual pesticides were prepared in toluene (Merck KGaA, Darmstadt, Germany) at an approximate concentration of $10\,mg\,mL^{-1}$. Stock solution of pesticide mixture at a concentration of $0.02\,mg\,mL^{-1}$ was prepared in toluene. An internal standard solution of TPP ($1\,mg\,mL^{-1}$) and HEPT ($1\,mg\,mL^{-1}$) was also prepared in toluene. Working standard pesticide mixtures and I.S. solutions with lower concentration were prepared in toluene by dilution.

APs used for experiment were 3-ethoxy-1,2-propanediol (98%), D-sorbitol (99%), L-gulonic acid γ-lactone (97%) (Aldrich-Chemie GmbH, Steinheim, Germany). Mixture solution of APs was prepared: $200\,mg\,mL^{-1}$ of 3-ethoxy-1,2-propanediol, $20\,mg\,mL^{-1}$ of D-sorbitol, $20\,mg\,mL^{-1}$ L-gulonic acid γ-lactone in acetonitrile (MeCN) (Merck KGaA, Darmstadt, Germany) : water (7 : 3), which represents the optimal ratio of APs (10 : 1 : 1) as determined in the previous research [22,26,27].

All stock solutions were stored at −18°C and diluted solutions at +4°C. Standards and analyte protectants were weighed on Sartorius Analytic MC1 scales (Sartorius, Göttingen, Germany).

Magnesium sulfate ($MgSO_4$) – clean, anhydrous and sodium chloride (NaCl) – per analysis were from Lachema (Lachema a.s., Brno, Czech Republic). $MgSO_4$ was annealed at 500°C (5 hours) and NaCl at 600°C (6 hours). Primary and secondary amine (PSA) sorbent – Bondesil 40 μm was obtained from Varian (Varian Inc., Harbor City, USA).

Table 1. List of used EDCs pesticides and internal standards (*triphenylphosphate* and *heptachlor*), their chemical class, retention times, monitored ions, SIM group start times and data acquisition rate.

Compound	Chemical class	Retention time (min)	Monitored ions[1]	SIM group start time (min)	Data acquisition rate (scans/s)
1. Diuron	Urea	3.85	**187**;189;124	3.00	11.68
2. Trifluralin	Dinitroaniline	5.13	**306**;264;307		
3. Hexachlorbenzen	Organochlorine	5.41	**284**;286;282	5.30	6.64
4. Dimethoate	Organophosphorous	5.51	**87**;93;125		
5. Atrazine	Triazine	5.63	**200**;215;202		
6. Lindan	Organochlorine	5.67	**181**;183;109		
7. Acetochlor	Chloroacetamide	6.06	**146**;162;223	5.90	4.89
8. Chlorpyrifos-methyl	Organophosphorous	6.09	**286**;288;125		
9. Vinclozolin	Dicarboximide	6.13	**212**;187;124		
10. Alachlor	Chloroacetamide	6.14	**160**;188;146		
11. Metribuzin	Dicarboximide	6.16	**198**;199;103		
12. *Heptachlor* (*I.S.*[2])	–	6.20	**272**;100;274		
13. Dicofol	Organochlorine	6.63	**139**;251;253	6.28	8.47
14. Malathion	Organophosphorous	6.37	**173**;127;93		
15. Linuron	Urea	6.44	**61**;248;160		
16. Diazinon	Organophosphorous	6.91	**287**;302;288	6.78	5.44
17. Procymidone	Dicarboximide	6.93	**96**;283;285		
18. Folpet	Phthalimide	6.98	**260**;104;262		
19. Chlordane	Organochlorine	7.12	**373**;375;377		
20. Endosulfan-alfa	Organochlorine	7.12	**241**;239;195		
21. Myclobutanil	Triazole	7.36	**179**;245;288	7.30	6.68
22. Nitrofen	Organochlorine	7.52	**283**;285;202		
23. Endosulfan-beta	Organochlorine	7.63	**241**;239;195		
24. Chlordecone	Organochlorine	7.81	**272**;274;270		
25. *Triphenylphosphate* (*I.S.*[2])	–	8.06	**326**;325;215	8.00	8.49
26. Bifenthrin	Pyrethroid	8.24	**181**;165;166		
27. Iprodione	Dicarboximide	8.30	**314**;316;187		
28. Mirex	Organochlorine	8.76	**272**;274;270	8.60	11.85
29. Prochloraz	Imidazole	9.18	**308**;310;266		
30. Cypermethrin	Pyrethroid	9.44	**163**;165;181	9.30	13.51
31. Deltamethrin	Pyrethroid	10.18	**181**;253;251		

[1]Target ions in bold.
[2]I.S. – internal standard.

2.2 *QuEChERS – sample preparation method*

The apples with peel (samples – non-treated with pesticides) and real samples of fruits and vegetable (lettuce, orange, strawberry and plum) were mixed with blender Braun MX 2050 (Braun GmbH, Kronberg, Germany). In the original QuEChERS procedure [25] certain changes were made according to our needs and resources [28]: comminution with a chopping device with dry ice was replaced by mixing in a blender, and therefore, the homogenisation with Ultra Turrax was used at the extraction step instead of shaking to ensure good extraction efficiencies. Ten grams of a sample weighed into a 50 mL centrifuge tube (polypropylene; Bio-Chrom s.r.o., Bratislava, Slovak Republic)

was extracted with 10 mL of MeCN using Ultra-Turrax T 25 basic (IKA-Werke GmbH, Staufen, Germany) homogeniser at 19,000 rpm for 3 min. This was followed by liquid–liquid partitioning (LLP): 1 g NaCl and 4 g $MgSO_4$ were added and the mixture was shaken by hand for 1 min. Subsequently, the mixture was centrifuged (ROTOFIX 32; Hettich centrifugen, Tuttlingen, Germany) at 4000 rpm for 5 min. Portion of the upper layer was transferred into a 15 mL centrifuge tube (polypropylene; Bio-Chrom s.r.o., Bratislava, Slovak Republic) containing 25 mg PSA sorbent and 150 mg $MgSO_4$ per 1 mL of the cleaned extract. The mixture was shaken by hand for 1 min, and then centrifuged for 5 min at 4000 rpm to separate solids from solution. The cleaned extract of apples not-treated with pesticides (blank apple sample extract) was used for the preparation of matrix-matched standard solutions and for the preparation of synthetic sample solutions; the cleaned extracts of real samples with/without APs were analysed.

2.3 *GC-MS*

GC-MS measurements were performed on an Agilent 6890N GC system (Agilent, Little Falls, DE, USA) coupled to an Agilent 5975 mass-selective detector equipped with a PTV and an Agilent 7683B autoinjector. The PTV was operated in the solvent vent mode; its temperature programme was: 40°C (hold 0.20 min), 400°C min^{-1} to 300°C (hold 2.00 min), 400°C min^{-1} to 350°C (hold 5.00 min). The flow through split-valve was 50 mL min^{-1} and it was closed after 0.2 min and opened after 1.75 min. Chromatographic separation was performed using the following column temperature programme: 60°C (hold 1.75 min), 60°C min^{-1} to 150°C, 23.8°C min^{-1} to 300°C (hold 1.90 min). Total time of analysis was 11.45 min. The injection volume was 2 μL and after each injection, the syringe was washed with MeCN. Helium with purity 5.0 (Linde Technoplyn, Bratislava, Slovak Republic) was used as a carrier gas in constant flow mode (1.2 mL min^{-1}). Microbore chromatographic column CP–Sil 8 CB (Varian, Middelburg, The Netherlands) with 5% diphenyl 95% dimethylsiloxane stationary phase 15 m × 0.15 mm I.D. × 0.15 μm was utilised. It was connected to a nonpolar deactivated precolumn (1 m × 0.32 mm I.D.) for focusation purposes and better ruggedness of the chromatographic system, via a 'swaging nut' connector (Agilent Technologies, USA). MS with electron impact ionisation mode (70 eV) was operated in selective ion monitoring (SIM) mode. For each pesticide 3 specific ions were selected and sorted into groups; the used dwell time was 10 ms. The retention times, target ions, qualifier ions, start times of SIM groups and frequency of data acquisition for pesticides are given in Table 1.

2.4 *Solutions preparation for calibration, synthetic and real sample measurements*

Seven concentration levels of pesticides were used for calibration: 1; 5; 10; 50; 100; 250 and 500 ng mL^{-1} (pesticide concentrations in ng mL^{-1} correspond to 1; 5; 10; 50; 100; 250 and 500 μg kg^{-1} in a sample). Three types of calibration solutions were prepared: (1) matrix-matched standards without APs – Matrix; (2) matrix-matched standards with APs – Matrix + APs and (3) MeCN standards with APs – MeCN + APs. All solutions contained 150 ng mL^{-1} TPP and 500 ng mL^{-1} HEPT. The volume 25 μL of APs mixture

solution was added to matrix-matched standard solutions and MeCN standards of pesticides ((2) and (3)).

(1) Preparation of matrix-matched standard solutions without APs: 925 μL blank apple sample extract + 25 μL TPP (6000 ng mL^{-1}) + 25 μL HEPT (20,000 ng mL^{-1}) + 25 μL pesticides in toluene (various concentrations of dilute solutions corresponding to the above mentioned seven concentration levels).
(2) Preparation of MeCN standard and matrix-matched standard solutions with APs: 900 μL MeCN/blank apple sample extract + 25 μL TPP (6000 ng mL^{-1}) + 25 μL HEPT (20,000 ng mL^{-1}) + 25 μL pesticides in toluene (various concentrations of dilute solutions corresponding to the above mentioned concentration levels) + 25 μL APs mixture solution.

The preparation, concentrations and the order of the injections in the sequences for synthetic and real sample measurements are given in Table 2.

3. Results and discussion

A series of experiments was carried out to characterise the analytical methodology proposed for the present work. In the first phase, 29 EDCs pesticides covering a wide range of chemical classes including organochlorine (hexachlorbenzen, lindan, dicofol, chlordane, endosulfan alfa and beta, nitrofen, chlordecone, mirex), organophosporous (dimethoate, chlorpyrifos-methyl, malathion, diazinon), dicarboximide (vinclozolin, metribuzin, procymidone, iprodione), pyrethroid (bifenthrin, cypermethrin, deltamethrin), urea (diuron, linuron), chloracetamide (acetochlor, alachlor), dinitroaniline (trifluralin), triazine (atrazine), phthalimide (folpet), triazole (myclobutanil), imidazole (prochloraz) were selected as EDCs pesticides model compounds. In the second phase, 2 I.S. – heptachlor and triphenylphosphate, were used. GC-MS results were evaluated utilising absolute peak areas of EDCs pesticides and peak areas normalised to two different I.S. The selection of I.S. was performed according to their application in the literature [22,26,27]. Quantitative analysis in daily routine pesticide residues analysis is usually performed by external calibration (with matrix-matched standards) without I.S., as matrix effects can influence signal of internal standard and signals of analytes in different way. In the third phase, analyte protectants were applied for the reduction/elimination of the matrix effects [23,26,27]. Matrix-induced response enhancement effects are well known to seriously affect measurement accuracy in GC applications, mostly leading to over-estimated results, when using the convenient matrix free calibration standards [26,31]. The addition of the APs to the solvent standard solutions used for calibration as well as to the matrix extracts (prepared with QuEChERS sample preparation method) significantly improves the peak shapes and resolution and also helps to significantly reduce quantification errors of 'difficult' GC compounds [32]. Trace analysis is performed to analyse traces in the samples with high concentration of co-extractants constituting the matrix of the sample. In spite of careful sample preparation procedure, some parts of the matrix are unavoidably present in the final extract [20]. The contamination can result in the degradation of ruggedness and stability of GC system. Therefore, liner and pre-column were replaced during experiments (liner: before individual calibration approaches, before synthetic sample measurements and before individual standards approaches for real samples; pre-column: before calibration and synthetic sample measurements).

Table 2. Preparation of synthetic and real samples, standards concentration, order of injections in sequences and replicates of GC-MS measurements.

	Preparation/sequences	GC-MS
Synthetic sample		
- Apple matrix – QuEChERS extraction:		
without APs	Analogically as matrix-matched standards without APs (subchapter 2.4 – (1))	
with APs	Analogically as matrix-matched standards with APs (subchapter 2.4 – (2))	
- Pesticides concentration in standards and synthetic sample	50 ng mL^{-1} (50 μg kg^{-1})	
- Injections order in sequence	1. Standard – Matrix[a] 2. Synthetic sample without APs	6 replicates
	1. Standard–Matrix+APs[b] 2. Synthetic sample with APs	6 replicates
	1. Standard – MeCN+APs[c] 2. Synthetic sample with APs	6 replicates
Real sample		
- Fruit and vegetable matrices (orange, strawberry, plum, lettuce) – QuEChERS extraction – two test portions (P1, P2):		
without APs	950 μL real sample extract + 25 μL TPP (6000 ng mL^{-1}) + 25 μL HEPT (20, 000 ng mL^{-1})	
with APs	925 μL real sample extract + 25 μL TPP (6000 ng mL^{-1}) + 25 μL HEPT (20, 000 ng mL^{-1}) + 25 μL APs mixture solution	
- Standards concentration	50 ng mL^{-1} (50 μg kg^{-1}) – for lettuce, orange and strawberry 250 ng mL^{-1} (250 μg kg^{-1}) – for plum	
- Injection order in sequence	1. Standard – Matrix[a] 2. P1, P2 (lettuce) without APs 3. Standard –Matrix[a] 4. P1, P2 (orange) without APs 5. Standard – Matrix[a] 6. P1, P2 (strawberry) without APs 7. Standard – Matrix[a] 8. Standard – Matrix[a] 9. P1, P2 (plum) without APs (16) 10. Standard – Matrix[a]	- 3 replicates (1. – 10.) - In the same way the sequences of standards (Matrix + APs[b], MeCN + APs[c]) and real sample extracts followed[d]

[a]Matrix – matrix-matched standard without APs.
[b]Matrix+APs – matrix-matched standard with APs.
[c]MeCN+APs – acetonitrile standard with APs.
[d]Utilising bracketing [28–30].

3.1 *Calibration*

Instrumental quality parameters (linearity, limit of detections – LODs and limit of quantifications – LOQs, repeatability of measurements) for the analysed EDCs pesticides were determined. Calibration standards were prepared at the concentration levels from 1 to 500 ng mL^{-1} (1, 5, 10, 50, 100, 250 and 500 ng mL^{-1} corresponding to the range of 1–500 μg kg^{-1} in a real sample) and were measured in six replicates starting with the lowest concentration.

For the evaluation of linearity of calibration curves, calibration experiments were performed utilising the following sequence of measurements: (1) matrix-matched calibration standards without addition of APs; (2) matrix-matched calibration standards with addition of APs and (3) calibration standards in acetonitrile with addition of APs. The study of linearity of calibration curves provided best results for matrix-matched calibration standards without the addition of APs. Generally, the values of coefficient of determination (R^2) calculated from absolute peak areas were in the range of 0.9820–0.9999, 17 EDCs pesticides measured in this way have the values of $R^2 > 0.999$. The median value was 0.9991. Matrix-matched calibration standards with the addition of APs provided very similar results (0.9757–0.9999, median value 0.9986). The difference is in the number of $R^2 > 0.999$ (12 EDCs pesticides) but this difference is not as significant as in the case of results obtained with calibration standards in MeCN with addition of APs (0.9649–0.9998, median value 0.9949). Prevailing of matrix effects over analyte protectants in solvent calibration standards probably caused lower values of R^2 as compared to other calibration approaches. Normalisation to HPT and TPP provided similar values of R^2 in each calibration approach, generally, normalisation of peak areas to HPT and TPP makes the results close to $R^2 = 1.0000$. Median value of R^2 calculated from normalised areas to HPT for matrix-matched standards without APs was 0.9993, to TPP – 0.9991; for matrix-matched standards with APs 0.9988 to HPT, 0.9987 to TPP; for acetonitrile standards with APs 0.9955 to HPT, to TPP – 0.9952. The normalisation to both I.S. does not provide a significantly improvement in linearity, but the utilisation of HPT provides slightly better results in comparison to the results calculated from absolute peak areas. Chromatograms of target ions of EDCs pesticides in different standard solutions analysed by fast GC-MS in SIM mode at the concentration level of 50 ng mL^{-1} (50 μg kg^{-1}) are presented in Figure 1. Numbering of peaks is identical with the number of compounds given in Table 1. In spite of identical concentration level in A, B and C (Figure 1), we may observe a great difference in analyte response between matrix-matched calibration standards with/without APs and MeCN calibration standards with APs. The y-axis scales in Figure 1 are not identical.

Instrumental LODs and LOQs calculated from calibration measurements at the lowest calibration (concentration) levels (LCLs) are presented in Table 3. LODs were calculated from signal to noise (S/N) ratios (1 : 3), LOQs (1 : 10). In general, the LCL for each calibration approach was at the concentration level of 1 ng mL^{-1} (1 μg kg^{-1}) for majority of EDCs pesticides. In case of problematic compounds (for example – pesticides with weak response or most volatile compounds such as diuron, dicofol, linuron, folpet, chlordecone, prochloraz, cypermethrin) the LCLs were 5–10 times higher, in case of MeCN calibration standards with addition of APs they were even 50–100 times higher. Presented LODs and LOQs reflect instrumental LODs and LOQs, as they were calculated from data obtained from analysis of standards in spiked matrix extracts and standards in a solvent. For the majority of compounds in this study, the lowest LODs and LOQs were obtained from

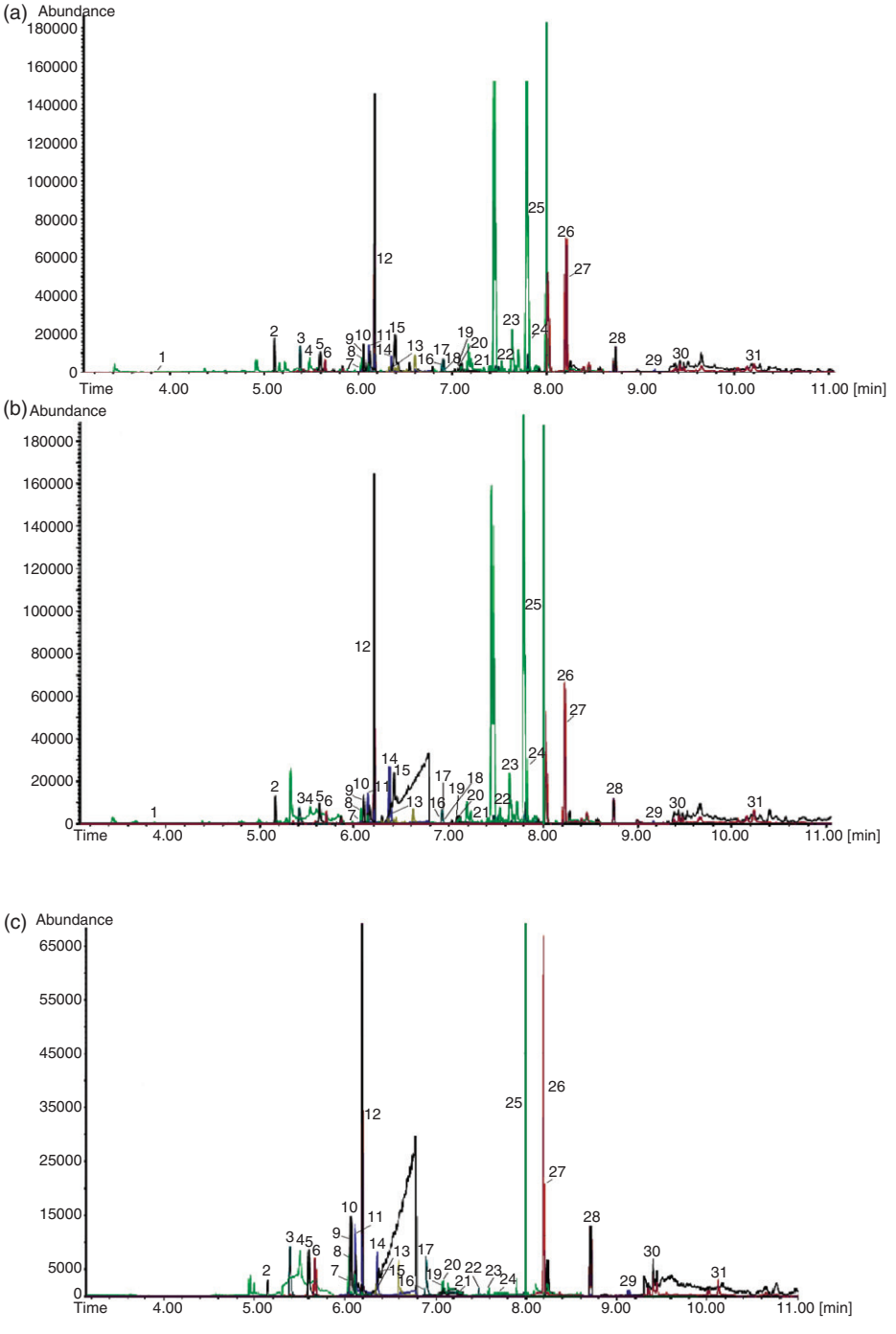

Figure 1. Chromatograms of target ions of EDCs pesticide in various standard solutions (50 ng mL^{-1}) analysed by fast GC-MS in SIM mode: (a) – matrix-matched standard solution without APs; (b) – matrix-matched standard solution with APs; (c) – MeCN standard solution with APs. Numbering of peaks is identical with the number of compounds given in Table 1.

standards prepared in the matrix extracts with/without addition of APs. LOQs are lower than $5\,\mu g\,kg^{-1}$, which is the required LOQs for the analysis of pesticide residues in baby food with MRLs of $10\,\mu g\,kg^{-1}$ with the exception of pesticides such as diuron, dicofol, linuron, prochloraz and cypermethrin.

Very good results of repeatability of peak area measurements expressed as the relative standard deviations (RSDs) were obtained for the majority of analytes in the matrix standards without/with APs and in the MeCN calibration standards with APs (RSDs $< 10\%$, observed at the LCLs). Median values of RSDs were in the range of 5.9–7.7% for all types of calibration standards, where the RSDs of pesticides were calculated from absolute peak areas and normalised areas to TPP and HPT except for a few compounds in MeCN standards with APs. All three types of calibration standards at all LCLs met the EU criterion of RSD $\leq 20\%$ [33].

Table 3. Instrumental LODs[a], LOQs[b] and LCLs for all types of calibration standards.

	Matrix			Matrix+APs			MeCN+Aps		
Compound	LCL $ng\,mL^{-1}$	LOD $ng\,mL^{-1}$	LOQ $ng\,mL^{-1}$	LCL $ng\,mL^{-1}$	LOD $ng\,mL^{-1}$	LOQ $ng\,mL^{-1}$	LCL $ng\,mL^{-1}$	LOD $ng\,mL^{-1}$	LOQ $ng\,mL^{-1}$
Diuron	10	2.63	8.77	10	3.59	11.97	100	14.37	57.86
Trifluralin	1	0.07	0.24	1	0.10	0.32	1	0.12	0.39
Hexachlorbenzen	1	0.02	0.08	1	0.03	0.09	1	0.03	0.11
Dimethoate	1	0.16	0.53	1	0.22	0.73	5	2.27	7.49
Atrazine	1	0.85	2.83	1	1.16	3.87	1	1.41	4.70
Lindan	1	0.52	1.73	1	0.71	2.37	1	0.80	2.67
Acetochlor	1	0.77	2.57	1	1.05	3.51	1	1.19	3.96
Chlorpyrifos-methyl	1	0.08	0.27	1	0.06	0.20	1	0.12	0.41
Vinclozolin	1	0.29	0.97	1	0.22	0.73	5	3.45	11.38
Alachlor	1	0.09	0.30	1	0.07	0.23	1	0.13	0.44
Metribuzin	1	0.10	0.33	1	0.08	0.25	1	0.15	0.49
Dicofol	10	3.56	11.87	10	4.80	16.01	10	5.21	17.37
Malathion	1	0.34	1.13	1	0.27	0.89	1	0.60	2.00
Linuron	10	6.32	21.07	10	9.20	30.68	50	18.15	66.07
Diazinon	1	0.09	0.28	1	0.13	0.44	1	0.16	0.53
Procymidone	1	0.27	0.90	1	0.39	1.31	1	0.48	1.59
Folpet	10	1.05	3.50	10	1.53	5.10	100	21.54	75.17
Chlordane	1	0.10	0.32	1	0.15	0.49	1	0.25	0.87
Endosulfan-alfa	1	0.46	1.53	1	0.32	1.07	5	2.72	9.41
Myclobutanil	1	0.07	0.22	1	0.05	0.16	1	0.11	0.37
Nitrofen	5	1.09	3.62	5	0.76	2.54	10	1.92	6.42
Endosulfan-beta	1	0.01	0.04	1	0.02	0.05	5	1.02	3.69
Chlordecone	10	0.91	3.03	10	0.61	2.02	50	3.61	12.82
Bifenthrin	1	0.02	0.08	1	0.03	0.11	1	0.04	0.12
Iprodione	1	0.10	0.33	1	0.17	0.55	1	0.16	0.53
Mirex	1	0.11	0.32	1	0.18	0.61	1	0.26	0.87
Prochloraz	10	5.37	17.90	10	3.21	10.70	50	7.83	26.12
Cypermethrin	5	2.05	6.83	5	2.87	9.56	5	3.24	10.79
Deltamethrin	1	0.07	0.24	1	0.06	0.20	5	1.11	3.88

[a]Calculated as 3 : 1 S/N ratio.
[b]Calculated as 10 : 1 S/N ratio.

3.2 *Analysis of synthetic samples*

A synthetic sample is a spiked blank sample extract (of apples that were not treated with any pesticides). Their absence was confirmed in prior experiment [22]. Synthetic sample represents a simulated real sample but with the known concentration in order to evaluate various calibration approaches. For each of three types of calibration standards the QuEChERS extract of apples was used. The concentration of pesticides selected for this experiment was 50 ng mL^{-1} (50 µg kg^{-1}). This is five times higher than the MRLs for baby food. In order to simulate routine conditions these experiments were intentionally carried out utilising a moderately dirty chromatographic system with a total of 195 performed injections (140 injections of matrix). Figure 2 represents the graph of calculated concentrations of pesticides in synthetic sample using matrix-matched standards without/with APs and MeCN standards with APs *vs.* the expected 50 µg kg^{-1} concentration. Synthetic sample and relevant calibration standards were measured six times. In the case of MeCN calibration standards with APs the concentration of EDCs pesticides is overestimated for a number of compounds. Diuron and folped were not detected, their LCLs in MeCN+APs standards were at the concentration of 100 ng mL^{-1} (100 µg kg^{-1}) – Table 3. For this approach, the maximal value of error of determination of concentration of pesticides obtained from the absolute peak areas was for endosulfan-beta and was found to be 22%. In some cases also underestimation of quantity was observed. As TPP and also HPT undergo matrix effects, the overestimation and/or underestimation is moderately lower. According to our studies, the enhancement of calculated concentration depends on the type of pesticide, number of injections and the type of matrix [22,34]. The median value of concentration (given in terms of % *vs.* 50 µg kg^{-1}) calculated from absolute peak areas is 108%, normalised to TPP 98% and 100% for HPT.

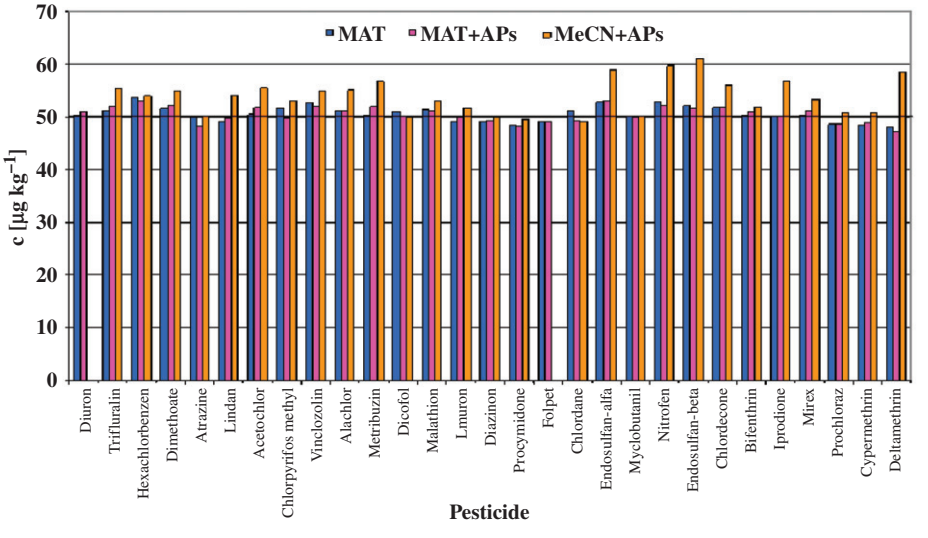

Figure 2. Graph of calculated concentrations of pesticides in synthetic sample using matrix-matched standards without/with APs and MeCN+APs *vs.* the expected 50 µg kg^{-1} concentration. For each of three types of calibration standards the QuEChERS extract of apples was used. Six GC-MS measurements was performed for synthetic sample and relevant calibration standards.

Matrix-matched standards without/with APs are appropriate for quantification of EDCs pesticides, as the results from these calibration approaches best mach the actual content in the sample. The median value of concentration (given in terms of% *vs.* 50 μg kg^{-1}) calculated from absolute peak areas is 101%, normalised to TPP 98% and 103% to HPT, respectively.

3.3 *Analysis of real samples*

To demonstrate the applicability of the method four types of non-fatty food were analysed: orange, lettuce, strawberry and plum. The results are listed in Table 4. Average pesticide concentrations (calculated from triplicate analysis of two parallel samples) obtained from absolute peak areas and areas normalised to TPP and HPT and relative standard deviations for parallel samples (RSD_{PA}) and GC-MS analysis (RSD_{GC}) are presented (RSDs < 9.5%). Positive findings of pesticides used for the treatment of real samples were the following: malathion (orange) and iprodione (lettuce, strawberry, plum). Matrix-matched standards (apple matrix) without/with APs and MeCN standards with APs at the concentration of 50 ng mL^{-1} (50 μg kg^{-1} in original sample) for orange, lettuce and strawberry and 250 ng mL^{-1} (250 μg kg^{-1} in original sample) for plum were used for quantification. Concentration of both EDCs pesticides was in the range of 41–246 μg kg^{-1}. The presence of EDCs pesticides in real samples was confirmed at fairly similar concentration levels for all calibration approaches but for MeCN standards with APs the results were slightly overestimated. Chromatograms of target ions of EDCs pesticides analysed by fast GC-MS in real sample extracts are given in Figure 3.

4. Conclusions

The developed and validated method of fast GC-MS with the utilisation of a PTV injector, a narrow-bore column and a bench top quadrupole MS detector (with EI ionisation in SIM mode) combined with QuEChERS sample preparation technique was shown to be fast (separation in 11.45 min.) and sensitive for the analysis of selected EDCs pesticides at the trace concentration levels in non-fatty food (1–500 ng mL^{-1} corresponds to 1–500 μg kg^{-1} in real samples). Calibration with matrix-matched standards provides the best results compared to other calibration approaches in terms of linearity of measurements expressed as R^2 (median 0.9991), instrumental LODs (0.01–21.54 ng mL^{-1}), LOQs (0.04–75.17 ng mL^{-1}) and the repeatability of absolute peak area measurements at LCL expressed as RSDs (≤10%). Utilisation of pesticide standards in a neat solvent (MeCN) with addition of APs is the simplest approach for routine use. However, it provides higher values of LODs and LOQs, particularly for the most volatile and problematic analytes (diuron, dimethoate, dicofol, linuron, folpet, nitrofen, chlordecone, prochloraz, cypermethrin). Analysis of synthetic sample at pesticide residues concentration of 50 μg kg^{-1} yielded overestimation of a number of EDCs pesticides under study (trufluralin, acetochlor, metribuzin, endosulfan-alfa, nitrofen, endosulfan-beta, chlordecone, iprodione, deltamethrin) with maximal errors up to 22%. The degree of overestimation depends on a compound and its concentration [35] and seems to depend on the number of injections [20,22] and the

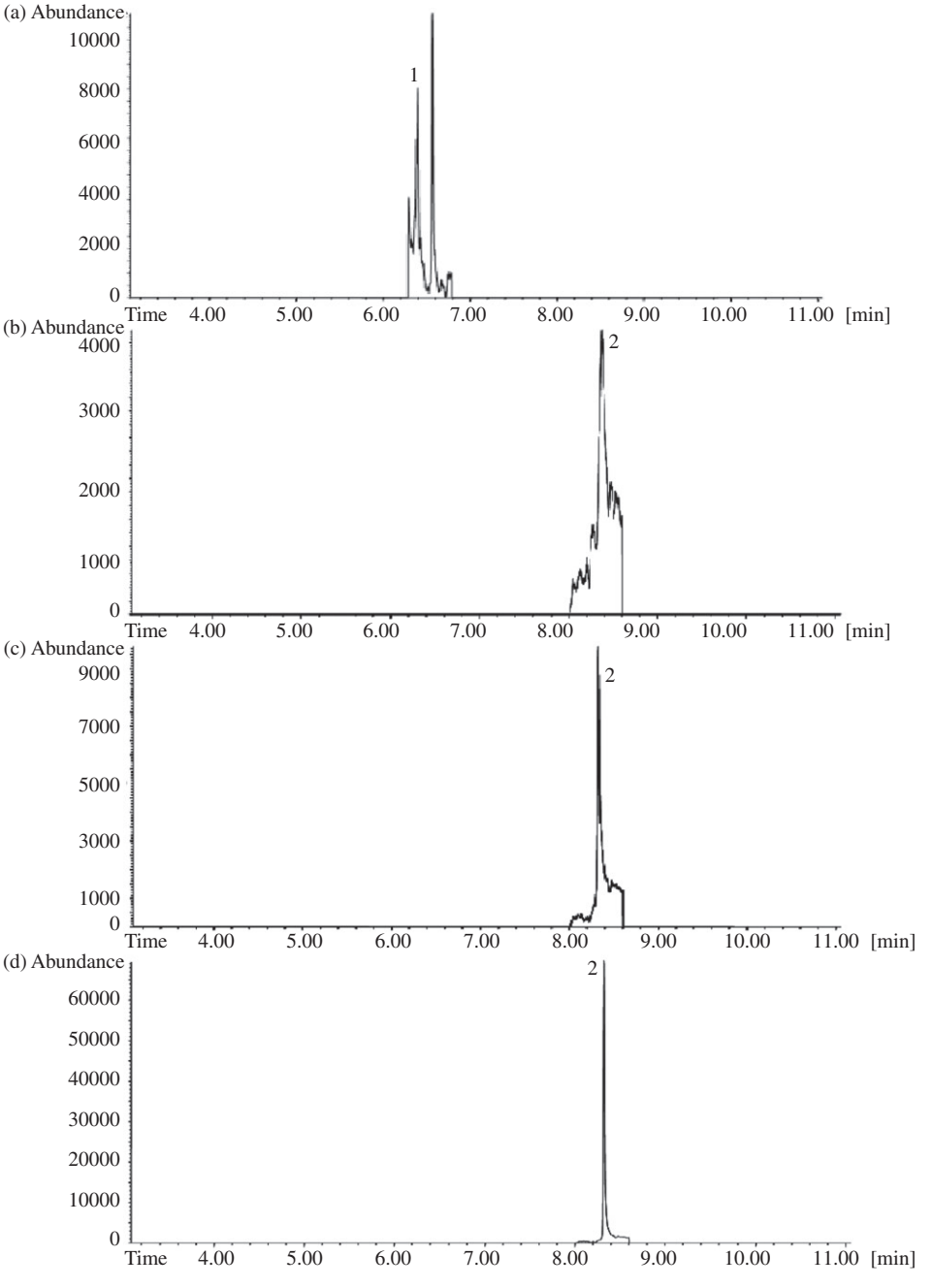

Figure 3. Chromatograms of target ions of EDCs pesticides analysed by fast GC–EI–MS in SIM mode in real sample extracts: (a) – orange (1 - malathion, $m/z = 173$); (b) – lettuce, (c) – strawberry, (d) – plum (2 – iprodione, $m/z = 314$).

Table 4. Concentration (c_i, $\mu g\,kg^{-1}$) of pesticide residues determined in various real samples with different calibration standards and calculated using absolute peak areas (A_i) and areas normalised to TPP (A_i/A_{TPP}) and HPT (A_i/A_{HPT}) and relative standard deviations (RSDs).

Matrix Compound	A_i			A_i/A_{TPP}			A_i/A_{HPT}		
	c_i	RSD_{PA}[a]	RSD_{GC}[b]	c_i	RSD_{PA}[a]	RSD_{GC}[b]	c_i	RSD_{PA}[a]	RSD_{GC}[b]
					Matrix				
Orange									
Malathion	53	4	9	53	4	9	53	3	9
Lettuce									
Iprodion	42	3	6	41	4	6	42	4	5
Strawberry									
Iprodion	41	3	4	42	5	5	41	4	7
Plum									
Iprodion	241	3	4	244	4	6	242	4	5
					Matrix + APs				
Orange									
Malathion	51	7	9	51	7	8	51	5	9
Lettuce									
Iprodion	42	3	7	44	4	7	43	3	6
Strawberry									
Iprodion	38	4	7	36	4	7	38	5	6
Plum									
Iprodion	232	5	6	239	6	8	238	6	5
					MeCN + APs				
Orange									
Malathion	53	4	10	53	4	8	53	3	8
Lettuce									
Iprodion	45	7	9	46	8	9	45	6	8
Strawberry									
Iprodion	43	4	4	42	4	6	43	3	5
Plum									
Iprodion	246	3	5	245	3	5	246	3	7

[a]$n = 2$, RSD_{PA} is the RSD for parallel extraction and analysis of two real samples (parallel analysis).
[b]$n = 3$, RSD_{GC} is the RSD for triplicate GC-MS analysis of one extract of a real sample.

GC system maintenance (how often the liner and precolumn is changed). The column capacity in fast GC with narrow-bore columns is strongly influenced by matrix co-extractants. Normalisation of peak areas to I.S. (HPT, TPP) does not bring a significant improvement in quantification of pesticides in model and real sample measurements.

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