ISSN 1061-9348, Journal of Analytical Chemistry, 2020, Vol. 75, No. 7, pp. 859–868. © Pleiades Publishing, Ltd., 2020.

ARTICLES

Application of Zirconium Based Sorbent for the Xenobiotics Determination in Food of Animal Origin

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Received November 13, 2017; revised January 25, 2018; accepted January 2, 2020

Abstract—This paper reports the application of a new type of material for matrices with high lipid content – zirconium based sorbent (**Z-Sep+**) which improves fat removal from the extracts for the simultaneous determination of polycyclic aromatic hydrocarbons (**PAHs**) and organochlorine pesticide (**OCPs**) residues in food of animal origin with gas chromatography–mass spectrometry (selected-ion monitoring) detection. The sample preparation was based on the modified QuEChERS method and was evaluated in terms of analyte recoveries, linearity, selectivity, repeatability, limits of detection and quanfication. The obtained results showed that the recovery ratios for both groups of investigated compounds were fit to EU specified ranges with the relative standard deviation lower than 10% for most compounds. In both cases acetonitrile turned out to be better solvent than ethyl acetate taking into account the recovery ratio as well as the purity of the samples. The results show that Z-Sep+ can be successfully applied for the simultaneous determination of OCPs residue and PAHs in food of animal origin.

Keywords: polycyclic aromatic hydrocarbons, organochlorine pesticides, QuEChERS method, Z-Sep+, food of animal origin, GC-SIM–MS

DOI: 10.1134/S1061934820070175

Pollution by persistent chemicals is potentially harmful to the organisms at higher trophic levels in the food chain. The chronic effects of pesticides from food intake on human health are not well defined, but there is an increasing evidence of carcinogenicity and genotoxicity, as well as disruption of hormonal functions [1, 2]. While pesticide compounds tend to accumulate in the fat and muscle tissues of animals, they can also reach other organs such as the liver, lungs and brain. They usually are transferred from plants to animals via the food chain [3]. Polycyclic aromatic hydrocarbons (**PAHs**) are very well known ecotoxicants harmful to human health. Inhalation of polluted air or cigarette smoke together with dietary intake represent the major pathways for human organism exposure [4, 5]. In food, for example in meat products, they are formed during thermal processes like smoking, roasting, barbecuing, and grilling as the effect of insufficient combustion or thermal decomposition of the organic material [6–9].

The literature review demonstrates that a number of sample preparation techniques and methods of analyses have been implemented for the quantification of PAHs and pesticide residues in a wide range of foodstuffs and other agricultural products [10–12]. However, none of these reports highlighted the problems, pitfalls, and achievements in food of animal origin. Several authors reported the successful **QuEChERS** (quick, easy, cheap, effective, rugged, and safe) method application for PAHs [13–19] and pesticide residues [19–27] extraction from various food matrices of animal origin. This methodology shows advantages over traditional sample preparation techniques as it requires only a small amount of reagents, in addition, sample isolation and clean-up are achieved in a single step instead of series of time-consuming solvent extractions. The QuEChERS method has achieved worldwide acceptance due to its simplicity and high throughput enabling laboratories to process significantly more samples in a given time compared with earlier methods [28]. This two-step extraction technique based on salting out (extraction) followed by dispersive solid phase extraction (**d-SPE**) (clean-up) was originally developed as a multiclass residue method for the pesticide residues determination in food of plant origin [29, 30] but their effectiveness and popularity contributed to their application for other matrices like food of animal origin which is the most complex matrix with high fat content. It is challenging to extract pesticides without the co-extraction of lipids which are difficult to remove from the extract and may affect the detection system [31–33]. The high amount of fat residues in the final extract would deteriorate the column and the mainte-

nance of the instrument in working conditions would be complicated. Since lipids deposit on the source, the analyte sensitivity is highly reduced due to ion suppression. Due to the fact that many of target pesticides are fat-soluble, non-polar compounds and they tend to remain in fat, the main focus is put on removing interfering lipids without losing certain analytes. The amount of fat in the final extract depends on the extraction solvent as well as on the clean-up procedure applied. Lipids are readily soluble in ethyl acetate (**EtAc**), *n*-hexane or diethyl ether [34]. Due to the limited fat transfer into the extract, acetonitrile (**MeCN**) is a better solvent. Nevertheless, certain amounts of lipids may still find their way into the extract. Therefore, an effective clean-up technique like dispersive solid phase extraction should be applied. Much depends on the capability of sorbents used. Primary secondary amine (**PSA**), octadecylsilane (**C_{18}**) or graphitized carbon black (**GCB**) are the most popular sorbents used in the clean-up step [13, 14, 17, 20–22, 24]. We also applied them in previous experiments for evaluating the QuEChERS method for organochlorine pesticides (**OCPs**) [27] and PAHs [18] determination in food of animal origin. Although all analytical parameters evaluated were excellent, the main drawback of these methods was the significant matrix effect for most compounds. The new promising sorbent which has been applied in the determination of pesticide residues for the purification of extracts with high oil content was zirconium dioxide coated silica sorbent (**Z-Sep+**) [26, 35]. Due to presence of Lewis acid sites, Bronsted acid-base sites, and octadecylsilane groups on the surface on these new materials, they could be a good adsorbent of fatty acids and proteins. Therefore, the purpose of this work was to evaluate the utility of Z-Sep+ sorbent for a clean-up step in the simultaneous determination of organochlorine pesticide residues and polycyclic aromatic hydrocarbons in food of animal origin (pork ham) with gas chromatography-selected ion monitoring–mass spectrometry (**GC-SIM–MS**) detection using the QuEChERS method. Different types of sorbents (silica gel, florisil, bondesil-ENV, Z-Sep+, Z-Sep+/C_{18}) and solvents (acetonitrile, ethyl acetate) were applied. Due to our best knowledge, such research has not been performed yet according to the available literature.

EXPERIMENTAL

Reagents and materials. Acetonitrile of HPLC grade was purchased from Merck KGaA, Germany. Magnesium sulphate anhydrous (p.a.) and sodium chloride (p.a.) were purchased from POCh SA, Poland. Florisil200 UM (FL), silica gel60 (0.063–0.100 mm) (**SG**), C_{18} (octadecylsilane), and ENV 125 UM (styrene-divinylbenzene) SPE Bulk Sorbent were derived from Agilent Technologies, USA. Supel™ QuE Z-Sep+ was obtained from Sigma-Aldrich Chemie, Germany. Organochlorine pesticide mix: α-hexachlorocyclohexane (**α-HCH**), β-hexachlorocyclohexane (**β-HCH**), lindane, δ-hexachlorocyclohexane (**δ-HCH**), heptachlor, aldrin, heptachlor epoxide, γ-chlordane, α-chlordane, endosulfan, 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethene (**o,p'-DDE**), dieldrin, endrin, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (**4,4'-DDD**), endrin aldehyde, endosulfan sulfate, methoxychlor; EPA 525 PAH Mix-B: acenapthylene, fluorene, phenanthrene, anthracene, pyrene, benzo[a]-anthracene, chrysene, benzo[b]fluoranthene, benzo[k]-fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[c,d]pyrene; internal standards (**IS**): mirex and anthracene d_{10}; and syringe standard (**SS**) chrysene d_{12}; were obtained from Supelco, USA. Stock, intermediate, and working standard solutions of PAHs (1 μg/mL), OCPs (2 μg/mL) and mirex, chrysene d_{12}, and anthracene d_{10} solutions at concentrations of 1 μg/mL were prepared in acetonitrile.

Equipment. GC analyses were carried out on a Varian 4000 GC/MS (Varian, Inc., USA) system consisting of a 3800 gas chromatograph and a 4000 Ion Trap MS detector. The column was a Phenomenex Zebron Multiresidue-1 (30 m L × 0.25 mm i.d. × 0.25 μm df; Phenomenex, USA). The GC oven was operated with the following temperature program: 50–300°C (5°/min). The total analysis time was 32 min. Helium (Linde Gas, Poland) was used as the GC carrier gas at a flow rate of 1.0 mL/min. The autosampling injector was a CP-1177 Split/Splitless Capillary Injector with the temperature of 270°C, 1.0 μL volume, and the splitless time of 1.0 min for all standards and samples. Each injection was repeated three times.

The ion trap mass spectrometer operated in the internal ionization mode, scan range from m/z 45 to 500. Analysis was conducted in the selected ion monitoring mode based on the quantitative ions. The trap and transfer line temperatures were set at 180 and 220°C, respectively. The analyses were carried out with the solvent delay of 10 min. The emission current of the ionization filament was set at 15 μA. Data acquisition and processing were performed using a Varian Start Workstation software and a NIST 2.0 library.

For solvent evaporation and extracts concentration an Accu™ Thermoblock (Labnet, USA) with nitrogen (Linde Gas, Poland) was used. An MPW 350 R Centrifuge (MPW Med. Instruments, Poland) was used for sample preparation.

Sample preparation method for polycyclic aromatic hydrocarbons and organochlorine pesticides simultaneous determination. The main aim of this work was to evaluate the utility of the QuEChERS method for PAHs and OCPs simultaneous determination in food of animal origin. Afterwards, the optimized procedure was applied for selected real samples of ham, the same that were tested in previous experiments [18, 27].

QuEChERS method development for fat samples of animal origin. A series of experiments were performed for the sample preparation techniques optimization.

Table 1. Scheme of the experiment conducted in the study

Tested factor	Chemical	Quantity	Variant of the method									
			1	2	3	4	5	6	7	8	9	10
Extraction solvent, mL	MeCN	10	+	+	+	+	+	–	–	–	–	–
	EtAc	10	–	–	–	–	–	+	+	+	+	+
Sorbent, mg	SG	300	+	–	–	–	–	+	–	–	–	–
	FL	300	–	+	–	–	–	–	+	–	–	–
	ENV	300	–	–	+	–	–	–	–	+	–	–
	Z-Sep+	500	–	–	–	+	+	–	–	–	+	+
	C_{18}	300	–	–	–	–	+	–	–	–	–	+

SG, silica gel; Z-Sep+, zirconium dioxide; C_{18}, octadecylsilane; FL, florisil; ENV, styrene-divinylbenzene SPE bulk sorbent; MeCN, acetonitrile; EtAc, ethyl acetate.

There are two factors affecting the results of QuEChERS in terms of recovery: (1) the solvent chosen for extraction and (2) the type, quantity and purity of salts and sorbents for d-SPE. In this experiment, the type of solvent and sorbent were studied. The amount of added salts was investigated elsewhere and resulted in the lack of the influence on the analyte recovery. Acetonitrile and ethyl acetate were used for the extraction step. This choice of solvents was due to the fact that in our previous tests MeCN and EtAc were turned out to be the most efficient for extraction OCPs and PAHs, respectively [18, 27]. As a clean-up sorbent we decided to apply not earlier used materials, namely, silica gel, florisil, ENV, and new promising Z-Sep+ and Z-Sep+ with C_{18} addition. The combination of the methods is presented in Table 1.

The usefulness of the method was verified using the analyte recovery in spiked samples. Homogenized pork ham samples with no PAHs and OCPs detected previously were used for recovery studies. Recovery study involved pork ham sample being spiked with the standard solutions to the fortification level of 0.008 mg/kg for OCPs and 0.002 mg/kg for PAHs. Both levels were adapted to the maximum residue level (**MRL**) limits set in EU: 0.01 [36] and 2 μg/kg [37], respectively.

The extraction process was conducted as follows: 8 g of a representative portion of ham was weighted into a 50 mL centrifuge tube and spiked with a mixture of PAHs, OCPs, and both internal standards, mixed, and left to stand for 15 min at room temperature prior to extraction. Then, 2 mL of water and 10 mL of an appropriate solvent were added to each tube and vigorously shaken for 1 min. After that, 1 g of NaCl and 4 g of $MgSO_4$ were added, and the tube was shaken immediately after addition of the salt. Then, each sample was shaken vigorously for 1 min and centrifuged for 15 min at 8700 RCF. Next, 6 mL of the supernatant was transferred into a 15 mL polypropylene (**PP**) tube containing appropriate amount of the SPE bulk sorbent (according to Table 1) for d-SPE to clean-up the extracts and 0.9 g of $MgSO_4$. After shaking for 30 s and 5 min centrifugation at 5000 RCF, 4 mL volume from each extract was transferred into a 4 mL tube and evaporated to dryness in the concentrator at 40°C. The residues were dissolved in 1 mL of hexane, and the syringe standard was added. Each sample was prepared in triplicate. Afterwards, the optimized procedure was applied for selected real samples of ham. For better illustration, the final sample preparation method is shown in Fig. 1. Blank samples were prepared in acetonitrile and ethyl acetate, respectively. Matrix-matched calibration standards at concentrations ranging from 2 to 400 ng/mL were prepared by diluting the standard mixture solution to the corresponding blank sample extracts.

Real samples analysis. To verify the effectiveness of the method, it was decided to examine 5 smoked and roasted pork ham samples investigated previously (S2, S5, S6, S14, and S15) [17, 18]. Selection was made on the basis of some PAHs presence in the samples. A few OCPs were also present in the selected samples. All products were purchased from local shops and were manufactured by leading meat companies in Poland. The final sample preparation method 4 was applied for all tested samples.

RESULTS AND DISCUSSION

Gas chromatography-selected ion monitoring–mass spectrometry determination. Target compounds were identified according to their qualitative ions and retention times. Calibration curves were constructed by plotting the ratio of the integrated peak area divided by the peak area of the internal standard and syringe standard against analyte concentration. For matrix effect elimination, peak areas were reduced by the area of the peaks of compounds derived from blank. Therefore, calibration curves were calculated without y-intercept, which excluded systematic errors. The retention times and characteristic ions for all analyzed compounds are shown in Table 2.

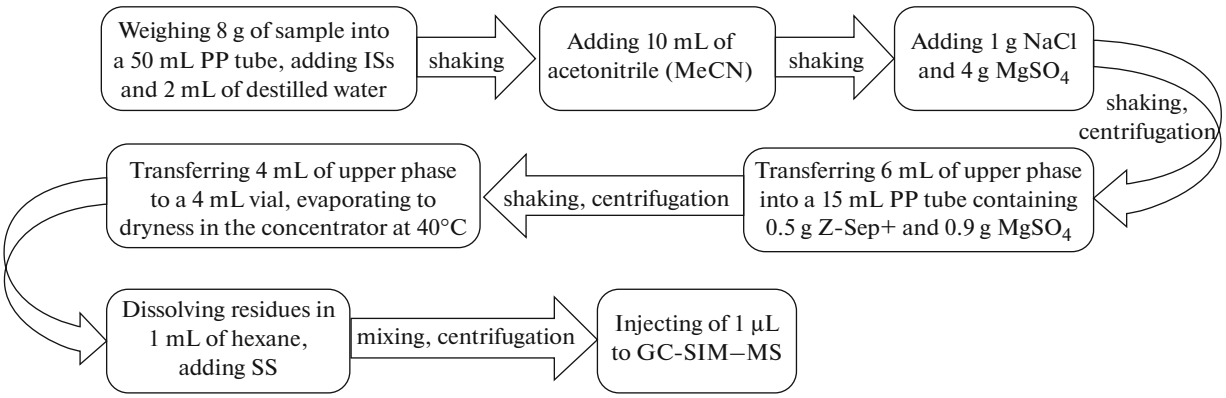

Fig. 1. Scheme of the experiment conducted in the study.

Recovery study. Recovery studies conducted after fortification to the level of 0.008 mg/kg for OCPs and 0.002 mg/kg for PAHs resulted in various recovery values presented in Table 3.

The obtained results showed that the best recovery ratios were in the specified ranges from 70 to 120% [38] and from 50 to 120% [39] for OCPs and PAHs, respectively, with the relative standard deviation (**RSD**) lower than 15% for all tested analytes for the method 4. The method is based on extraction with acetonitrile and extract clean-up with zirconium based sorbent (Z-Sep+). The recovery ratios in the chosen method ranged from 70 to 120% for organochlorine pesticide residues and from 52 to 111% for polycyclic aromatic hydrocarbons with RSD lower than 10% for most compounds. In both cases, taking into account the recovery ratio and also purity of the samples, MeCN turned out to be a better solvent than EtAc. After evaporation, in the residues of the samples extracted with ethyl acetate there were much more impurities than in the residues of the samples after extraction with acetonitrile. It was caused by the fact that the use of EtAc resulted in the extraction of contaminants from the sample. Additionally, MeCN has suitable miscibility with water which allows good penetration into the aqueous fraction of samples, and it can be easily separated from water by adding salt. Moreover, the extraction of lipophilic materials, waxes, fats and pigments is greatly reduced when using MeCN [21]. Furthermore, acetonitrile is the preferred solvent for QuEChERS instead of toxic organochlorine compounds, which makes it friendly to environment and human. In most cases, these procedures are laborious and require high volumes of toxic solvents. This also results in high costs of analyses.

In the other variants of the QuEChERS method, the recovery values did not fit within the prescribed range. The obtained values were more diversified, and the RSDs exceeded 20% more than once. Overall, it was observed that the methods in which MeCN was used as an extraction solvent were characterized by higher recovery rates for individual analytes than those using ethyl acetate. In view of the above, 15 analytes (5 PAHs and 10 OCPs) were recovered in the specified ranges using method 1 with silica gel, for method 2 using florisil—19 compounds (6 PAHs and 13 OCPs), for method 3 applying ENV—11 PAHs, and for method 5 using Z-Sep$^+$ and C_{18}—only 3 analytes (2 PAHs and 1 OCPs). The lowest recovery values for the tested compounds were obtained in method 5 where zirconium based sorbent with addition of octadecylsilane (300 mg) was applied. Such low recovery values were most likely due to the excessive amount of the sorbent used which also bound the analytes apart from extracts contaminants [24]

In PAHs determination, for all sorbent combinations and both extraction solvents the recovery ratios for fluorene and phenantrene fitted in the specified ranges except two cases. On contrary, dibenzo[a,h]anthracene and indeno[c,d]pyrene had very low recoveries ranging from 0 to 29% and from 5 to 26%, respectively. This can mean that low values of the recovery ratios in case of heavy PAHs were probably influenced by the use of sorbents that might remove from the samples some compounds with planar structure.

In pesticides residue determination, two of them, namely endosulfan and dieldrin, had recovery values exceeding 100% almost in all sorbent combinations, while DDD had low recoveries (6–42%) for most tested sorbents. The exception was the recovery values received for methods (5 and 9) using zirconium based sorbents for extract clean-up and they were 77 and 89%, respectively. For almost all methods (1–4) with the use of MeCN as an extraction solvent the recovery ratios for all HCH isomers either stayed in the set range of 70–120% or were even higher.

Comparison with other studies. The methodology for pesticide residues determination in environmental and plant origin samples is very well documented and many examples are available in the literature. Nevertheless, samples with more complex matrices, such as products of animal origin, usually demand a two-step

Table 2. Retention times and data of the quantification of target compounds

Rt	Compound	Quantification ion, *m/z*	Confirmation ion, *m/z*	R^2	LOD, mg/kg	LOQ, mg/kg
10.23	Acenaphthylene	152.1	151.1, 151.3, 153.1	0.9989	0.0002	0.0006
13.24	Fluorene	166.1	164.1, 165.1, 165.3	0.9991	0.0002	0.0007
15.78	Phenanthrene	178.1	166.1, 178.2, 179.1	0.9988	0.0002	0.008
15.83	Anthracene d_{10} (IS_1)	188.0	188.1, 177.9, 189.2	–	–	–
15.86	Anthracene	178.1	165.1, 178.2, 179.1	0.9993	0.0004	0.0013
16.35	α-HCH	181.0	181.1, 183.0, 219.0	0.9991	0.0007	0.0022
16.84	β-HCH	183.0	181.1, 183.0, 219.0	0.9994	0.0007	0.0021
17.07	Lindane	183.0	181.1, 183.0, 219.0	0.9989	0.0013	0.004
17.64	δ-HCH	183.0	181.1, 183.0, 219.0	0.9987	0.0012	0.004
19.06	Pyrene	202.1	200.1, 202.3, 203.1	0.9995	0.0001	0.0003
20.02	Heptachlor	236.9	272.1, 274.0, 237.0	0.9989	0.009	0.0029
20.78	Aldrin	263.0	263.1, 66.1, 265.0	0.9986	0.0015	0.005
21.52	γ-Chlordane	374.7	375.1, 373.2, 377.0	0.9985	0.0006	0.0020
21.90	α-Chlordane	374.7	373.2, 375.0, 241.1	0.9987	0.0004	0.0012
22.01	Benzo[a]anthracene	228.1	226.1, 228.3, 229.1	0.9984	0.0003	0.009
22.01	Chrysene d_{12} (SS)	240.1	240.2, 239.2, 241.2	–	–	–
22.05	Chrysene	228.1	226.1, 228.3, 229.1	0.9986	0.0002	0.0006
22.78	Benzo[b]fluoranthene	252.1	250.1, 253.1, 253.3	0.9982	0.0002	0.0007
22.83	Benzo[k]fluoranthene	252.1	250.1, 250.4, 253.1	0.9979	0.0002	0.0005
23.41	Benzo[a]pirene	252.1	250.1, 250.3, 253.2	0.9994	0.0003	0.009
23.85	Heptachlor epoxide	352.9	353.1, 81.0, 355.0	0.9978	0.0014	0.004
24.27	DDE	246.1	246.2, 248.1, 318.2	0.9997	0.0003	0.0011
24.55	Dieldrin	263.0	79.1, 81.0, 263.1	0.9989	0.009	0.0029
24.77	Endrin	243.0	245.2, 263.1, 243.2	0.9993	0.0011	0.004
24.95	Endosulfan	193.1	195.0, 193.1, 241.1	0.9996	0.0012	0.004
26.07	DDD	165.2	235.2, 165.1, 237.1	0.9998	0.0004	0.0015
26.13	Endrin aldehyde	243.0	245.1, 243.1, 67.1	0.9985	0.0006	0.0020
26.46	Endosulfan sulphate	236.9	272.0, 239.0, 229.1	0.9986	0.0003	0.0009
27.03	Metoxychlor	227.0	227.2, 228.2, 165.1	0.9993	0.0002	0.0007
27.98	Mirex (IS_2)	237.0	91.1, 75.1, 93.0	–	–	–
29.19	Indeno[c,d]pyrene	276.1	274.1, 277.1, 277.5	0.9987	0.0006	0.0019
29.29	Dibenzo[a,h]anthracene	278.2	276.0, 276.5, 279.1	0.9990	0.0005	0.0016

R^2, coefficient of determination; LOD, limit of detection; LOQ, limit of quantification.

clean-up. Fatty food analysis is usually laborious and not fully effective in cleaning-up the sample if the compounds should be evaluated below l g/kg of lipid weight. The separation of lipophilic compounds, such as organochlorines, from bulk of fatty material is a necessary step prior to their quantification.

Several approaches for sample preparation for organochlorine pesticide residues determination have been applied. Direct solid–liquid extraction (**SLE**) was the most widely used extraction technique from foods of animal origin, especially from meat [40–43], followed by Polytron extraction [44–46], matrix solid phase dispersion (**MSPD**) [45, 47], accelerated solvent extraction (**ASE**) [44] and finally classical Soxhlet extraction [44]. To eliminate co-extracted interferents from extracts, various clean-up techniques have been used. It appears from the literature review that the most popular technique seems to be gel permeation chromatography (**GPC**) [41, 42, 44–47] followed by solid phase extraction. Florisil [40, 45, 47] and C_{18} [40, 43] were the most widely used adsorbents in applied cartridges. Acidified silica gel column [48] and semipermeable membranes [49] were also used.

Table 3. Polycyclic aromatic hydrocarbons and organochlorine pesticides recoveries (%) as relative standard deviations (%, in parentheses) for all tested methods

Compound	Variant of the method									
	1	2	3	4	5	6	7	8	9	10
Acenaphthylene	81.1 (5.0)	82.3 (7.4)	67.1 (5.2)	68.5 (7.5)	19.5 (3.4)	46.5 (4.3)	38.9 (9.5)	63.3 (5.6)	43.8 (3.8)	65.3 (6.1)
Fluorene	93.7 (7.4)	97.0 (4.7)	96.3 (4.3)	111.0 (7.1)	74.8 (6.3)	67.6 (6.2)	107.9 (11.0)	80.7 (5.6)	114.6 (10.4)	145.9 (12.8)
Phenanthrene	47.7 (6.3)	98.3 (5.3)	76.4 (5.6)	65.0 (4.8)	80.0 (7.5)	102.4 (11.4)	108.8 (11.1)	131.2 (12.6)	60.7 (5.9)	90.3 (7.5)
Anthracene	55.0 (9,6)	74.4 (6.2)	0.0 (0.0)	55.5 (4.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	38.8 (3.4)	125.8 (9.8)
α-HCH	124.3 (18.7)	89.6 (9.6)	146.6 (2.9)	111.4 (2.5)	146.6 (5.8)	121.7 (5.8)	76.5 (13.1)	86.9 (6.7)	48.9 (7.6)	63.3 (3.0)
β-HCH	78.4 (8.3)	82.3 (6.0)	80.8 (10.0)	93.8 (12.1)	12.5 (24.4)	91.2 (11.3)	70.2 (12.9)	50.7 (8.4)	42.3 (10.4)	62.7 (9.9)
Lindane	77.5 (9.3)	72.3 (0.3)	79.4 (10.9)	92.9 (10.1)	11.2 (10.0)	86.4 (4.3)	71.3 (11.8)	52.3 (3.4)	45.8 (0.3)	58.7 (6.8)
δ-HCH	105.0 (12.2)	105.5 (2.9)	116.1 (8.0)	106.0 (5.1)	27.2 (13.2)	122.1 (8.9)	105.8 (6.1)	69.0 (10.5)	65.8 (13.9)	90.5 (3.5)
Pyrene	60.8 (4)	62.7 (7.1)	23.1 (1.7)	65.3 (3.1)	11.0 (2,3)	76.7 (5.6)	35.5 (6.1)	79.4 (5.8)	8.9 (8.5)	8.1 (7.1)
Heptachlor	83.9 (14.3)	70.2 (9.6)	77.5 (3.2)	101.4 (7.0)	62.7 (21.1)	86.3 (6.9)	110.1 (14.2)	65.1 (25.5)	58.5 (13.5)	75.0 (4.5)
Aldrin	65.2 (21.5)	73.5 (1.0)	60.3 (9.7)	74.7 (3.5)	18.2 (8.9)	80.0 (13.5)	67.1 (4.7)	47.1 (21.0)	41.8 (11.3)	59.8 (7.1)
γ-Chlordane	73.6 (3.0)	83.9 (6.4)	68.6 (5.6)	86.1 (8.4)	5.9 (26.8)	92.6 (1.6)	42.7 (8.1)	4.0 (20.3)	3.8 (0.9)	7.5 (2.2)
α-Chlordane	72.9 (9.5)	72.1 (8.4)	60.1 (9.3)	80.4 (7.2)	10.6 (18.5)	74.9 (13.7)	36.7 (8.4)	4.1 (9.5)	4.5 (11.2)	6.1 (8.2)
Benzo[a]anthracene	47.5 (4.6)	39.4 (3.8)	35.5 (3.1)	56.5 (3.9)	0.0 (0.0)	49.0 (4.2)	19.5 (11.3)	44.5 (3.6)	21.8 (5.3)	79.8 (9.4)
Chrysene	69.1 (7.2)	34.5 (3.2)	0.0 (0.0)	54.8 (3.5)	0.0 (0.0)	26.9 (3.1)	47.6 (23.2)	59.5 (15.4)	0.0 (0.0)	58.5 (10.8)
Benzo[b]fluoranthene	0.0 (0.0)	57.9 (4.5)	7.9 (6.5)	52.1 (6.8)	7.6 (4.8)	29.9 (2.7)	59.0 (13.4)	70.5 (6.4)	45.7 (8.9)	43.0 (9.8)
Benzo[k]fluoranthene	33.0 (3.5)	34.5 (3.7)	14.6 (2.3)	63.6 (8.5)	13.4 (2.1)	18.3 (2.3)	70.4 (6.2)	0.0 (0.0)	26.4 (9.1)	44.5 (4.2)
Benzo[a]pirene	41.5 (3.9)	44.2 (13.2)	19.6 (2.7)	71.0 (5.9)	21.2 (3.6)	27.6 (1.9)	14.9 (2.1)	22.8 (3.5)	71.0 (4.3)	81.1 (6.7)
Heptachlor epoxide	82.7 (9.8)	87.2 (6.8)	70.2 (2.8)	84.4 (3.5)	6.4 (16.4)	91.8 (13.6)	78.4 (9.1)	33.9 (19.8)	0.0 (0.0)	3.1 (8.6)
DDE	67.6 (0.4)	68.7 (6.4)	41.5 (7.7)	73.0 (9.8)	3.2 (28.4)	85.3 (13.7)	67.8 (15.1)	51.6 (27.5)	46.1 (6.6)	56.2 (8.6)
Dieldrin	126.4 (4.2)	103.1 (6.2)	139.8 (3.3)	114.7 (11.1)	31.2 (8.3)	137.7 (13.8)	107.4 (17.4)	142.1 (3.5)	122.3 (0.8)	72.5 (6.2)
Endrin	134.2 (19.5)	85.7 (12.7)	95.6 (2.4)	69.9 (9.5)	19.5 (14.5)	127.2 (42.8)	79.9 (8.3)	105.5 (15.1)	76.8 (5.5)	48.4 (11.3)
Endosulfan	142.3 (3.8)	139.0 (33.7)	140.1 (11.1)	120.1 (6.1)	115.0 (14.8)	147.9 (45.0)	117.3 (9.7)	142.2 (12.4)	108.3 (8.3)	75.2 (11.4)

Table 3. (Contd.)

Compound	Variant of the method									
	1	2	3	4	5	6	7	8	9	10
DDD	41.8 (14.2)	40.7 (6.5)	26.6 (6.0)	77.1 (9.4)	5.7 (29.5)	35.7 (5.1)	30.4 (17.5)	30.6 (8.3)	89.0 (7.8)	25.6 (8.8)
Endrin aldehyde	87.1 (2.5)	89.3 (3.0)	80.0 (19.2)	88.7 (7.7)	31.2 (19.0)	59.1 (7.2)	57.8 (9.2)	63.8 (9.2)	72.4 (9.3)	62.5 (19.7)
Endosulfan sulphate	75.3 (24.6)	68.9 (24.5)	59.2 (51.2)	99.6 (1.7)	19.5 (4.8)	72.2 (10.4)	69.1 (0.7)	67.5 (15.8)	12.2 (0.0)	16.4 (23.0)
Metoxychlor	103.0 (3.8)	99.0 (5.0)	84.9 (3.8)	92.3 (4.8)	5.7 (3.7)	99.3 (2.2)	84.1 (16.4)	76.4 (12.7)	58.6 (11.6)	60.2 (16.4)
Indeno[c,d]pyrene	25.6 (3.1)	13.0 (10.1)	5.1 (11.1)	55.8 (7.1)	18.9 (10.9)	16.2 (11.8)	21.9 (10.9)	14.0 (11.2)	25.7 (2.1)	21.9 (2.3)
Dibenzo[a,h]anthracene	0.0 (0.0)	21.2 (1.9)	4.3 (5.5)	56.2 (4.5)	0.0 (0.0)	17.6 (2.1)	23.2 (6.4)	29.4 (8.2)	0.0 (0.0)	23.1 (13.1)

Recovery values in all presented above works reported for investigated extraction and clean-up methods are in good agreement with EU specified range (70–120%) for all tested analytes, except one report with MSPD and Florisil clean-up [45]. In this case, recovery values were in the range of 58–99%. However, these conventional extraction techniques are laborious and time-consuming, moreover, expensive materials and large solvent volumes are usually needed. Furthermore, evaporation of toxic solvents is a source of analyte loss and atmospheric and environmental pollutions [3]. As a result, the QuEChERS method has found a lot of interest in recent years. Various combinations of sorbents, mostly based on PSA, for extracts clean-up and MeCN as an extraction solvent for OCPs determination were used [20–24, 27]. In two reports [19, 26], Z-Sep cartridges were also applied. However, Parrilla-Vázquez Enhanced Matrix Removal–Lipid (**EMR–Lipid™**) was found as the most appropriate sorbent but the recoveries did not fit to the prescribed level (70–120%) and were from 34 to 88% [25]. Comparing received in our study recovery values of analytes with our previous study we noted that only 4 investigated pesticides (δ-HCH, heptachlor epoxide, DDE and endosulfan sulphate) had significantly lower values. Two of them (α- and β-HCH) significantly exceeded values obtained previously. But in both cases all values were in good agreement with EU specified range (70–120%). Other OCPs recovery values did not differ significantly from the ones investigated previously [27]. Moreover, the received extracts were cleaner than those cleaned up with PSA, which was also confirmed by Rajski et al. [26]. Sapoznikova and Lehotay reported the lowest chromatographic background after zirconium based sorbent application as well [19]. Comparing the obtained results with other works is a little difficult because sometimes they differ in the spectrum of target compounds. Despite this, Omar et al. observed very similar recoveries for HCH isomers [22]. Slightly higher values were noted for chlordane and DDT and its metabolites. Our results are also in agreement with Savi et al. tests, where recoveries ranged between 75 and 105% [20]. Molina-Ruiz et al. in the research on chicken livers additionally used SAX sorbent additive, which resulted in recoveries in the range of 72–117% [23]. While researching fish, SAX, NH_2 and C_{18} addition was applied, which resulted in a decrease in recoveries to 63–91 and 61–122% for carp and sturgeon, respectively [24].

It is well known that one of the main difficulties in the analysis of fatty matrices is due to their high fat content (e.g., lipids, triglycerides, and fatty acids). Therefore, the extraction of PAHs from these complex matrices is usually laborious and time-consuming. The conventional sample treatment and extraction methods including solid-liquid extraction [16, 50, 51], ultrasonic extraction [50, 52], microwave assisted extraction (**MAE**) [52], accelerated solvent extraction [53], pressurized liquid extraction (**PLE**) [54], saponification [55], enzyme immunoassay [56], followed by SPE with silica gel cartridges [16, 50, 52–55], and GPC [53, 54] were applied to clean-up samples. New approaches for the determination of PAHs including dispersive solid phase extraction which is the basis of the QuEChERS method have been investigated and developed and the following sorbents were used: silica gel [15], PSA especially for remove fatty acids and sugars [17], C_{18} to remove non-polar materials [13, 14, 19], and Z-Sep to minimize interferences from fatty materials and pigments [19].

Considering our results, in PAHs group only fluorene had recovery value not significantly differing from the previous research. Recovery values for other PAHs were significantly lower than investigated before but still perfectly fitted to EU specified range (50–

Table 4. Polycyclic aromatic hydrocarbons and organochlorine pesticides content (mg/kg) in analyzed samples

Compound	S2[a]	S5	S6	S14	S15
Acenaphthylene	n.d.[b]	n.d.	0.005	n.d.	n.d.
Fluorene	n.d.	n.d.	0.004	0.003	0.0029
Phenanthrene	0.0014	0.0028	0.0018	n.d.	0.0027
Anthracene	0.003	0.006	n.d.	0.006	0.0065
α-HCH	n.d.	0.0010 (0.3156)	0.004 (0.2529)	<LOQ	n.d.
β-HCH	n.d.	n.d.	n.d.	n.d.	n.d.
Lindane	0.003 (0.0943)	n.d.	n.d.	n.d.	0.0020 (0.1000)
δ-HCH	n.d.	n.d.	n.d.	n.d.	n.d.
Pyrene	n.d.	0.0006	n.d.	n.d.	n.d.
Heptachlor	<LOQ	<LOQ	<LOQ	n.d.	<LOQ
Aldrin	n.d.	n.d.	n.d.	n.d.	n.d.
γ-Chlordane	n.d.	n.d.	n.d.	n.d.	n.d.
α-Chlordane	n.d.	0.005 (0.1469)	n.d.	n.d.	n.d.
Benzo[a]anthracene	n.d.	n.d.	n.d.	n.d.	n.d.
Chrysene	0.0023	n.d.	n.d.	n.d.	n.d.
Benzo[b]fluoranthene	n.d.	n.d.	<LOQ	n.d.	n.d.
Benzo[k]fluoranthene	<LOQ	n.d.	<LOQ	0.0009	n.d.
Benzo[a]pirene	n.d.	<LOQ	0.0021	n.d.	n.d.
Heptachlor epoxide	n.d.	n.d.	n.d.	n.d.	n.d.
DDE	0.0017 (0.0486)	0.0017 (0.0531)	0.0012 (0.0706)	0.0013 (0.0361)	0.0016 (0.0800)
Dieldrin	n.d.	n.d.	n.d.	n.d.	n.d.
Endrin	n.d.	n.d.	n.d.	n.d.	n.d.
Endosulfan	n.d.	n.d.	n.d.	n.d.	n.d.
DDD	<LOQ	n.d.	0.006 (0.3529)	<LOQ	<LOQ
Endrin aldehyde	n.d.	n.d.	n.d.	n.d.	n.d.
Endosulfan sulphate	n.d.	n.d.	n.d.	n.d.	n.d.
Metoxychlor	n.d.	n.d.	n.d.	n.d.	n.d.
Indeno[c,d]pyrene	n.d.	n.d.	n.d.	n.d.	n.d.
Dibenzo[a,h]anthracene	0.0025	0.007	n.d.	0.004	0.003

[a]S, sample; [b]n.d., not detected.

120%) [18]. The most similar recovery values were also obtained by Johnson [14] who quantified the investigated analytes in shrimp (72–113%) and oysters (78–116%). Slightly higher values, from 88 to 113%, were obtained by Sapoznikova and Lehotay [19] and from 92 to 99% by Urban and Lesueur [13], which most likely results from the use of different sorbents.

In summary, the achieved recovery results indicate that MeCN is a more suitable solvent for the investigated analytes (PAHs and OCPs) extraction although EtAc has greater ability to extract PAHs in general.

Analytical performance of the method. The analytical performance of the chosen variant of the QuEChERS method outlined above (method 4) was examined by looking at its linearity, selectivity, recovery, repeatability, limit of detection (**LOD**), and limit of quantification (**LOQ**). The least squares method was used to obtain equations of calibration curves ($y = ax + b$). Linearity (coefficient of determination, R^2, higher than 0.99) was observed for target compounds in the concentration range from 2 to 400 ng/mL (Table 2). Recovery studies were conducted after pork

ham sample fortification with selected OCPs at the level of 0.008 mg/kg and PAHs at the level of 0.002 mg/kg. The received recovery values from 70 to 120% for OCPs and from 50 to 120% for PAHs were in good agreement with appropriate documents, SANTE/11945/2015 and Commission Recommendation (EU) No. 836/2011 for OCPs and PAHs, respectively [38, 39].

The repeatability, expressed as the relative standard deviation of the analyzed samples, was lower than 15% for all target analytes according to documents mentioned above. The limit of detection was calculated based on the standard deviation of the response (s) of the curve and the slope of the calibration curve (a) (according to the formula LOD = 3.3 s/a), and the limit of quantification was equal to three times the LOD. The level of noise was measured from the chromatograms obtained for the standard solutions with the lowest concentration, i.e., 2 ng/mL. LOD and LOQ for the used method calculated as a signal-to-noise ratio showed values from 0.0001 and 0.0003 mg/kg for pyrene to 0.0006 and 0.019 mg/kg for indeno[c,d]pyrene in PAHs group. In OCPs family, LOD and LOQ were from 0.0003 and 0.0009 mg/kg for endosulfan sulphate to 0.0015 and 0.0047 mg/kg for aldrin (Table 2). LOQ values for all detected compounds were in good agreement with the value specified in the Commission Recommendation (EU) No. 836/2011 [17] for PAHs and in SANTE/11945/2015 for OCPs [22, 23] and were lower than the MRLs established by EU regulation (0.01 mg/kg). Compared with previous experiments [18, 27], received values were in most cases lower, 4 values (for chrysene, benzo[b]fluoranthene, DDE and endosulfan sulphate) were not significantly higher and 2 values (for pyrene and heptachlor epoxide) were at the same level. The sensitivity calculated as the calibration slope coefficient was the highest for chrysene and fluorantene derivatives (benzo[b]- and benzo[k]-) and the lowest for indeno[c,d]pyrene in PAHs family, and the highest for metoxychlor and DDT metabolites (DDD and DDE) and the lowest for endrin, endosulfan, endosulfan sulphate, and heptachlor in OCPs group.

Real samples analysis. The results of real sample analysis are presented in Table 4. The contents for both studied groups of compounds were presented in milligrams per kilogram of tested ham. Moreover, OCPs residue concentration in extractable fat was calculated based on fat content published elsewhere [27] and shown in brackets.

There is no reason to discuss the content of analytes in particular real samples because it has already been done elsewhere. The only thing that can be mentioned is that there were no exceedances of MRLs for the determined PAHs and OCPs in any of the analyzed samples [36, 37]. However, it should be noted that the received contents of PAHs and OCPs in investigated samples did not differ significantly from those obtained in the previous works [18, 27]. This proves that the method used can be successfully applied for the simultaneous determination of selected PAH and OCP residues in food of animal origin. The experiment revealed that the use of zirconium based sorbent (Z-Sep+) in extract clean-up step is the optimal variant of QuEChERS method for the simultaneous PAHs and OCPs determination in food of animal origin. Moreover, in this case no low temperature precipitation (freezing-out) was applied as before, which resulted in a shortening of sample preparation time. Furthermore, analyzed extracts showed higher purity and fat was removed at the purification step.

FUNDING

This research was performed with the financial support from Ministry of Science and Higher Education of Republic of Poland within the statutory R & D activities (DS-3707/15/KTGiK).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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