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MONITORING IN OILS PESTICIDES RESIDUES AND POLYCYCLIC AROMATIC HYDROCARBONS FOR SAFETY OF VEGETABLE OILS

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Abstract: Multiresidue method for determination of pesticides in vegetable oils was modified, based on complex sample preparation using QuEChERS and GC- MS (DRS-AMDIS) and LC-MS/MS, for determination of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, chrysene was used method of donor-acceptor complex chromatography (HPLC) with fluorescence detector. Methods can control of residues of the 60 pesticides (chloro-organic pesticides, phosphor organic pesticides, triazoles, pyrethroids) and 4 PAHs in vegetable oils, hydrogenated fats and margarines. Over the last year found 65 samples of sunflower oil containing pirimiphos-methyl at 0.005 - 0.02 mg/kg. About 150 samples of sunflower oil, the content of four PAHs in excess of 2-4 times the norm Commission Regulation (EC) Regulation SANCO/10616/2009 MRL for PAHs in foodstuffs.
Keywords: pesticide residue, polycyclic aromatic hydrocarbons, vegetable oil, QuEChERS, GC-MS, LC-MS/MS, HPLC/FLD.

INTRODUCTION

Vegetable oil - important ingredients of the daily diet and health food person. At the same time, the lipid component of vegetable oils is an accumulator of xenobiotics, especially dioxins, POPs, nonpolar and weakly polar pesticides, polycyclic aromatic hydrocarbons (PAHs), most of which have substantial toxicological, particularly mutagenic and carcinogenic properties. This necessarily requires the regulation of pesticides and PAHs in vegetable oils, cooked food and implementation mandatory analytical input and output controls, agricultural products, food processing and diet. MRL for pesticides and PAHs are developed and regularly reviewed (SSRaN/2001; SANCO/10616/2009). Existing methods for determining multiresidues of pesticides in vegetable oils is not possible to simultaneously analyze the list of pesticides due to regulatory (72 a.i), a standard method to determine PAH in vegetable oils (ISO 4689:2006) has been developed to determine a concentration - benzo (a) pyrene , while the rest PAHs which normalized of the EU - not analyzed. Standard method laborious, lengthy, costly, since bases on the alkaline hydrolysis of the test sample, extraction of BaP and unsaponified of lipids with hexane, clean extract re-extraction BaP from hexane mixture water - N, N-dimethylformamide with sequential extraction with hexane, concentration, and purification on florizile determining the mass of BaP by HPLC/FLD. It should be noted that due to the constant revision of regulations, increased requirements for pesticide residue control methods and PAH, in the first instance to determine the accuracy and reliability of identification, improving metrological characteristics.

Thus, the aim of this study was to improve the methods for determining trace PAHs and PESTICIDE RESIDUE in vegetable oils in accordance with SANCO 12495:2011, by participating in the inter-laboratory comparison to prove their compliance with international

standards and perform routine measurements of mass fraction of pesticides and PAHs in samples of vegetable oils

MATERIAL AND METHODS

The objects of research are vegetable oils (sunflower, olive, soybean, corn, canola) and hydrogenated vegetable fat. Sample preparation of vegetable oils for pesticide residue analysis performed by the technique developed based on the method ***EN 15662:2008*** (anhydrous variant of the QuEChERS). Chromatographic analysis was carried out using extracts oil ***GC-MS*** (DRS-AMDIS) and ***LC-MS\MS*** on the devices:

1. ***Agilent Technologies 7890 MSD 5975S*** (Column HP-5 MS 15m x 0.25 mm ID x 0.25um; GC-MS method DRS Scan / Sim; injector temperature 250 º C, column temperature 70 º C (2min) 20 º C \ min to 270 º C (0 min); interface temperature 280 º C, the temperature of the filament 230 º C, quadrupole temperature 150 º C, flow 1:50, 50 ml\min, the pressure of the carrier gas (helium) 60.7 kPa (constant pressure); Full Scan 45-500 amu).

2. ***Dionex-SUMMIT (MS-3200 Q-Trap)*** (column temperature – 20 º C; autosampler temperature – 20 º C, flow rate - 0.5 ml/min; gradient - acetonitrile/water [H^+]. For determination of some pyrethroids applied additional purification of the extract oil on alumina using a mixture of diethyl ether-light petroleum as eluent.

Analysis of PAHs through the introduction and use in ULQSAP method of donor-acceptor complex chromatography HPLC/FLD, is carried out by direct entry into the chromatograph 80 *µl* sample of oil. The device: ***Dionex-UltiMate 3000.2 Dual (UV)*** (column temperature - 30 º C; autosampler temperature - 40 º C, speed and chemical composition of the eluent in a gradient mode: on the right pump - isopropanol, acetonitrile, on the left - acetonitrile/water, acetonitrile).

RESULT AND DISCUSSION

The complexity of the matrix of the vegetable oils (sunflower, rape, olive, soybean, corn, etc.) make difficulties at identification and determination pesticide residues. All known methods for the determination of pesticide residues in oils have certain advantages, disadvantages and limits of application, including even the most popular EN 15662:2008.

For solve the problem of determining residues of 60 pesticides in vegetable oils, hydrogenated fats and margarines in ULQS AP a comprehensive approach which combine GC-MS (OCP, FOS, triazoles, pyrethroids) with LC-MS\MS (triazoles, carbamates, neonicotinoids) was used. For GC-MS analysis was developed the anhydrous version of method QuEChERS. Extraction of pesticides from sunflower oil was carried out using a mixture of solvents, followed by concentration of extracts on a rotary evaporator and purified with solid-phase dispersion (mix of PSA, C18, and $MgSO_4$). To improve the determination of some pyrethroids the additional purification on alumina column with a mixture of diethyl ether-light petroleum as eluent was made.

Analysis of sunflower oil content neonicotinoids, carbamates and triazoles was performed using traditional QuEChERS followed by tandem mass spectrometry, HPLC-MS\MS in positive ionization mode.

Processing GC-MS spectra was performed using method of automatic deconvolution - DRS (Deconvolution Reporting Software) and identification system AMDIS (Automated mass spectral deconvolution and identification system). System was locked by a retention time of chlorpyrifos-methyl, RTL-8.299min. A typical deconvolution report of the extract presented in

table 1., it demonstrates that the Agilent MSD ChemStation software does not detect pirimiphos-methyl, while DRS-AMDIS system identifies pirimiphos-methyl in oil extract with high match (82%) at the level of 0.14 *ng/ul.*

Table.1 The DRS report of sunflover oil extract

			Amount (ng)		AMDIS		NIST	
R.T.	Cas #	Compound Name	Chem station	AMDIS	Match	R.T. Diff sec.	Reverse Match	Hit Nu
7.974	84695	Diisobutyl phthalate	1.75	1.25	79	3.2	83	17
9.163	29232937	Pirimiphos-methyl		0.14	82	1.1	70	1
9.228	84742	Di-n-butylphthalate	8.82	7.53	92	2.5	90	3
10.459	206440	Fluoranthene		0.24	92	4.8	88	1
11.144	129000	Pyrene		0.39	59	5.2		
11.144	0000	trans-13-Octadecenoic acid, methyl ester					87	1

For developing method pesticides of different chemical groups were added to oil matrix at three spiking levels (0.02, 0.05, 0.1 *mg/kg*) to evaluate recovery and quantitation.

The results of the recoveries obtained for listed pesticides at spiking level 0.05 *mg/kg* in oil samples presents in Table 2.

Table.2 The results of the recoveries

Pesticide	***RT min, m\z***	***Rec, %***	***MRL EU, mg\kg***
aldrin	9.25 m\z 66, 263	27	0.02
tetramethrin	14.44 m\z 164	100	-
fludioxanil	12.16 m\z 248	116	-
ethion	13.02 m\z 97, 231	92	-
oxadixyl	12.98 m\z 163	93	-
metalaxyl	8.66 m\z 206, 160	102	-
fipronil	10.99 m\z 213	98	-

malathion	9.42 m\z 173	89	-
chlorpyrifos-ethyl	9.62 m\z 97,197	75	0.05
chlorpyrifos-methyl	8.29 m\z 282, 284	67	0.05
lambda-cyhalothrin	15.23 m\z 181, 197	110	0.05
cypermethrin(Σisomers)	16.41,16.52 m\z 163,165	75	-
4,4´-DDE	12.02 m\z 246,248	17	0.05
4,4´-DDD	12.86 m\z 235, 237	48	0.05
4,4´-DDT	13.51 m\z 235, 237	42	0.05
deltamethrin	18.08 m\z 181,253	70	0.2
diazinon	m\z 179,137	97	0.02
alpha-endosulfan	11.32 m\z 241,239	129	-
beta-endosulfan	12.59 m\z 195,241	65	-
endosulfan sulfate	13.39 m\z 387,272	105	-
endrin	12.38 m\z 263, 281	68	0.01
tefluthrin	7.53 m\z 197	80	-
hexachlorobenzene	6.17 m\z 284, 286	12	0.02
alfa-hexachlorocyclohexane	6.02 m\z 181, 183	67	0.02
beta-hexachlorocyclohexane	6.59 m\z 181, 183	70	0.02
gamma-hexachlorocyclohexane	6.61 m\z 181, 183	76	0.01
heptachlor	8.38 m\z 181, 183	75	0.01
cis-heptachlorepoxide	14.01 m\z 353,355	45	0.01
4,4´-methoxychlor	14.46 m\z 227, 228	110	-
permethrin(Σisomers)	15.73, 15.84 m\z 183	70	0.05

The spiking samples of the other fatty matrix (margarine and hydrogenated vegetable oils) also were analyzed.

Monitoring samples of sunflower oil using HPLC/MS/MS method based on EN 15662:2008 sample preparation. Limits of quantification - 0,010-0,005 *mg/kg*. To verify the method of continuously analyzes the oil samples spiking standard solutions of pesticides at 0.01-0.1 *mg/kg* were used. Recovery most of the components meet the requirements of SANCO 12495-2011 (70-120 %). Organochlorine pesticides, due to its high lipophilicity, give the smallest, but always stable recovery of 40-60 %. To calculate the amount of those pesticides residues in samples it needs introduce corrective factor for recovery.

Table.3 Carbamates, triazoles, neonicotinoids (LC-MS\MS). spiking level 0,01 *mg/kg*

№	Pest	1-st trans	2-st trans	Rec, %
1	Azoxystrobin	404.1/371.9	404.1/343.9	76
2	Carbaryl	202.1/144.9	202.1/127.0	119
3	Carbendazim	192.1/160.0	192.1/132.0	73
4	Chlorpyrifos	349.9/96.9	349.9/198.0	35
5	Chlorpyrifos-methyl	321.9/125.1	321.9/289.8	47
6	Carbofuran	222.1/165.1	222.1/123.0	119
7	Carboxin	236.1/142.9	236.1/86.9	88
8	Cyproconazole	292.1/70.0	292.1/125.1	83
9	Diazinon	305.1/169.1	305.1/96.6	66
10	Dichlorvos	220.9/127.1	220.9/108.9	97
11	Difenconazol	406.1/250.9	406.1/337.0	98
12	Epoxiconazole	330.1/121.0	330.1/101.2	94
13	Malathion	331.0/127.0	331.0/99.0	87
14	Malaoxon	315.0/127.1	315.0/99.2	112
15	Methamidophos	142.0/124.9	142.0/93.9	80
16	Oxadixyl	279.1/219.2	279.1/133.3	81
17	Pirimiphos-meth	306.1/164.1	306.1/108.1	67
18	Tebuconazol	308.1/70.0	308.1/124.9	95
19	Thiametoxam	292.0/211.0	292.0/181.0	60
20	Thiophanate-meth	343.0/151.0	343.0/192.0	107

Proposed approach was applied to monitor the residue of 60 pesticides in 107 samples vegetable oil, margarine and hydrogenated fat. At 65 samples of sunflower oil pirimiphos-methyl at levels 0.005-0.02 *mg/kg* was found. A few samples contained a small amount of chlorpyrifos at 0.005-0.01 *mg/kg* and fludioxonil. The customer MRL requirements for pirimiphos-methyl in oil were 0.11 *mg/kg*, for chlorpyrifos 0.11 *mg/kg*, respectively. Results of of some samples analysis was confirmed in the arbitrage laboratory SOFIA (Hamburg-Barmbek, Germany), the results were good agreement. Extended measurement uncertainty is ± 50 %. The proposed rapid method for the screening and quantification of pesticide residues in vegetable oil has been successfully used in Proficiency Test Fapas 0585(2012). Was confirmed target compounds: endosulfan sulfate, hexachlorobenzene, permethrine, PCB 101, PCB 180 at concentrations 45-116 *mg/kg*.

Applying of the donor-acceptor complex chromatography with fluorescence detector allowed analyzed the PAHs in various vegetable oils without prior sample preparation of samples. The developed method for the determination of PAHs in vegetable oils (sunflower, corn, olive, flaxseed, pumpkin seed oil and walnuts) was validated in 2011, according with ISO 8466 obtained performance characteristics statistical evaluation of the linear calibration function. PAHs extracted from lipid matrix by used specific donor-acceptor binding sorbent with different functional groups. Column packed with sorbent entering into a specific donor-acceptor interaction (DAI) with PAHs molecules is placed directly into the chromatograph, which automates the process of extraction of PAHs. For 20 minutes oil sample with the mobile phase (isopropanol) is moved to DAI column during that time achieved a complete separation of lipid molecules and PAHs. PAHs adsorbed on a column giving, for the next 40 minutes of back flow in gradient mode (acetonitrile-water) and fed to elute connected in two chromatographic columns. Process chromatography with injection of 80 *µl* of oil is held in a gradient, and detection - programmable change wavelengths for 50 minutes. To check the efficiency of the chromatographic system, used in the extraction and separation of mixtures of PAHs from different lipid matrices. During the year, chromatographic analysis of the test solution. Chromatography of the resulting calculated and compared to the parameters by which carried out monitoring indicators chromatographic system. For example, at the peak of benzo(a)pyrene is given in Fig. 1 control: complete extraction of the analyte from the oil, in violation of the chromatographic column packing and efficiency.

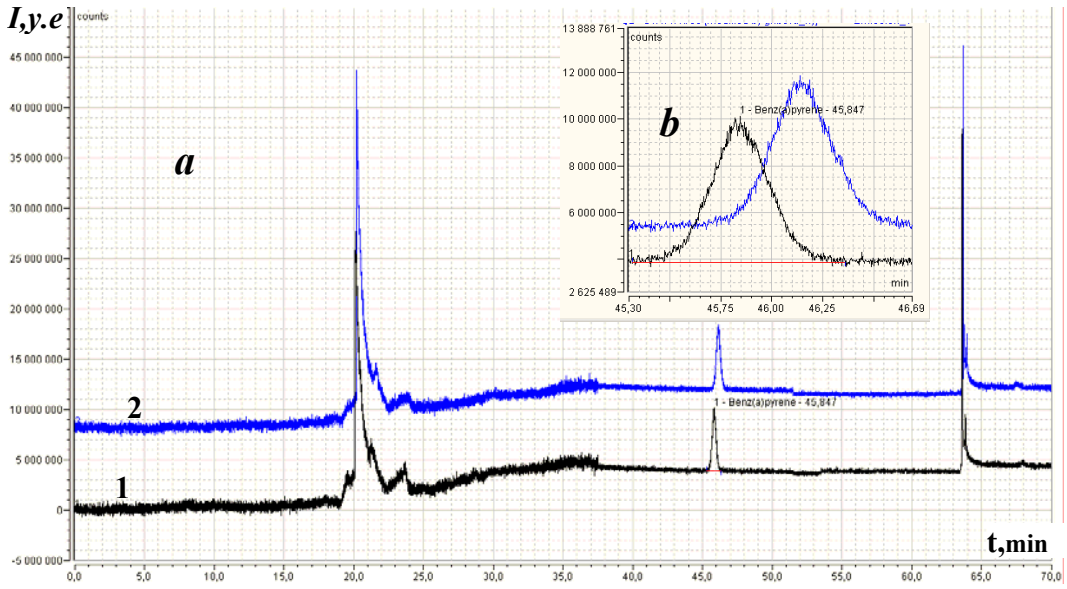

Fig. 1.-***a)*** The chromatogram of the matrix of sunflower oil containing 2.0 µg/kg BaP. The measurements were performed: 1 - 10.06.2011 2 - 14.06.2012. ***b)*** The peak of benzo(a)pyrene (1:5 magnification).

Practical tasks to establish the number of regulated in Ukraine BaP and regulated in the EU BaA, BbF, chrysene, were solved with the use of reference materials, obtaining calibrated function have been carried out on a oils solution with 4 PAH content, regression parameters are given in table 4.

Table 4.The parameters of the regression equation based on the analytical signal of four PAHs in the matrix of sunflower oil

Substance	*Regression a + bx*	*Standard deviation (Sxo=Sy/b)*	*Variation coefficient Vxo = Sxo/Xcp,(%)*	*Detection limit*
BaP	1.8 E4+1.6 E11	2.7 E-8	1.46	9.4 E-8
BaA	10.8 E4+4.6 E12	3.1 E-8	2.36	3.3 E-8
Chrysene	9.6 E4+4.4 E12	3.2 E-8	1.60	2.4 E-8
BbF	1.8 E4+1.1 E11	3.1 E-8	1.45	1.7 E-7

At injection 80 *µl* calibration oil solutions were determine linear dependence of the analytical signal of PAH concentrations in the range 0.5 *µg/kg* – 10.0 *µg/kg*. The lower limit of calibration standards is due to MAC on BaP content of fat-containing foods for infants and young children, top - the actual demand analysis of contaminated oils. The measurement results are processed in the software package "Spline" (**Makarchuk, 2006**) Detection of PAHs in vegetable oils (Fig. 3, Fig. 4) physico-chemical properties which meet the requirements to appropriate state standards and specifications stated, saying that oil is not dependent on the technology, and the fatty acid composition susceptible to contamination of PAHs (Fig. 2).

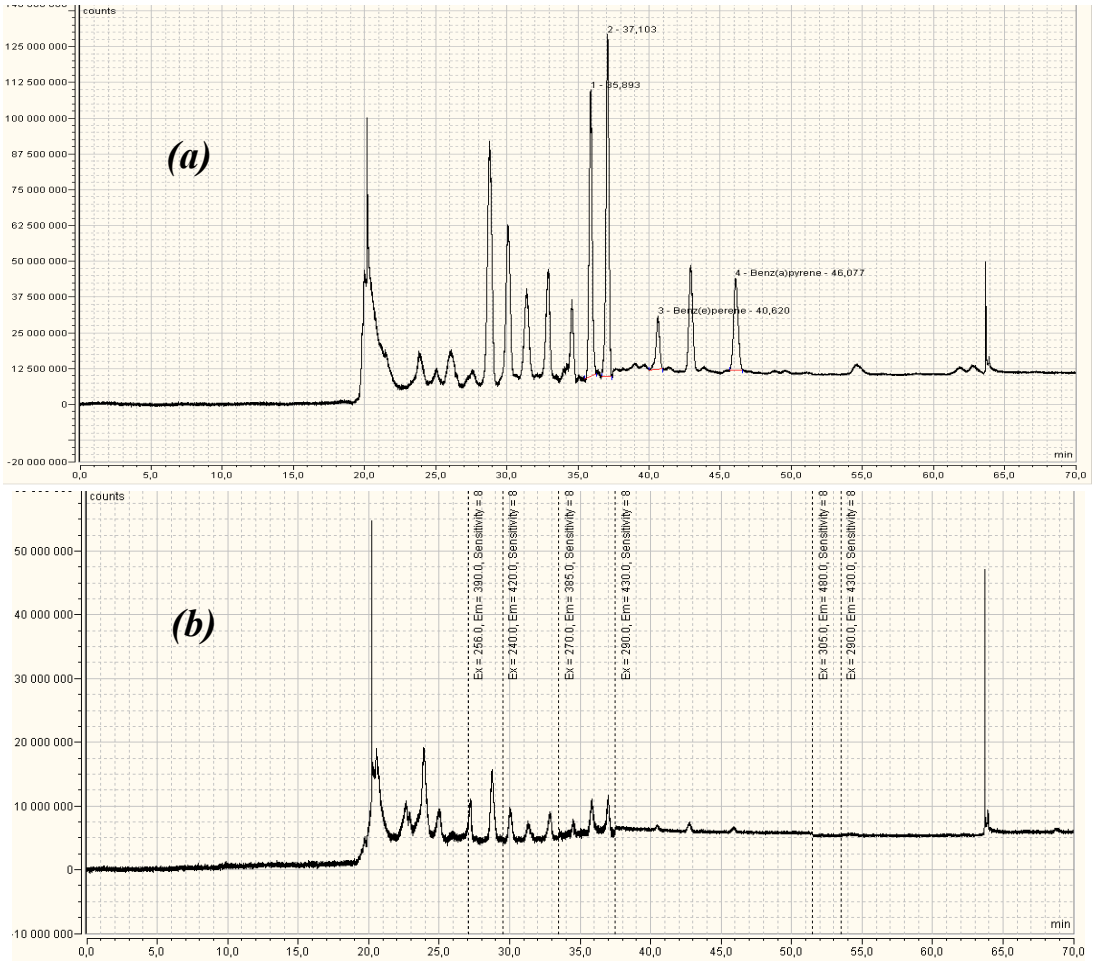

Fig. 2. - Chromatogram of a sample of sunflower (***a***), pumpkin (***b***) oil.

According to chromatographic data can see that in the oils contain mixtures of different qualitative and quantitative composition of 12 to 16 polyaromatic compounds.
During the last year were found of 150 samples sunflower oil which contained PAHs in concentration in excess of 2-4 times norm of SANCO / 10616/2009 MRL.

To determine the cause of the high content of PAHs in oils must identify the critical points of production vegetable oil, as well as studied the mechanism of accumulation of PAHs oilseed crops during the growing season. Thus, except in the complex and lengthy extraction step, was worked out method of analyzing the content of PAHs in vegetable oils by HPLC/FLD. Technique allowed, compared to the method of DSTU 4689:2006, reduce in 8 times the analysis time, avoided loss of analyte and achieve high reproducibility.

Determination of PAHs in vegetable oils at the level of 0.5 to 10.0 mg/kg is carried out which total relative error of ± 20 %. The accuracy of the method and the stability of the chromatographic system was confirmed receipt satisfactory metrological characteristics. Method meets requirements of DSTU 17025 and EU SANCO 12495-2011, which is confirmed by the appropriate validation characteristics. The developed method has been successfully applied in the inter-laboratory comparisons, performing routine analyzes for 2011-2012.

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