MODERN TECHNIQUES OF ANALYSIS FOR THE PESTICIDE RESIDUES ASSESSMENT IN PLANT MATERIALS

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Abstract
Organochlorine pesticides were tested in order to establish optimal conditions for rapid detection in dried leafy matrices, using DRS software. In tests we have used dried Trifolium pratense herba fortified with OC pesticide mix, processed with modified QuEChERS method. Instrumental analysis was carried out complying Agilent "key" condition, column of 15 m, in which case the retention time for chlorpyrifos-methyl was 8,296 min. DRS allows linear calibration in the concentrations range 0,05-1 mg/kg for most of pesticides; for concentrations ≤ of 0.05 mg / kg is needed most advanced purification method or acquisition in SIM mode.

Keywords: DRS, organochlorine, pesticides residues, Quechers.

INTRODUCTION

The pesticide residues presence in plant products is a current problem and implementation of quick and accurate determination methods is of real importance.

Although organochlorine pesticide (OC) treatments was forbidden for the most part, however, such residues are found quite frequently in products. Monitoring OC has been justified by their persistence and the possibility of bioconcentration in organisms or extracts, through certain manufacturing stages. Nowadays, the pesticide monitoring is expanding beyond food, for example, to botanical dietary supplements (Meng et al., 2007). Food extracts for pesticide residue analysis contain a lot of chemical compounds. Consequently, one risks removing pesticides along with endogenous compounds if elaborate cleanup steps are used. So, the challenge is to detect traces of pesticides in samples that contain a lot of interferences (Wylie, 2008). Thus, monitoring of OCs residues in food becomes a routine analysis of pesticides monitoring laboratories. Official pesticides datasets showed that persistent organochlorine esidues were surprisingly common in certain foods despite being off the market for over 30 years. Residues of dieldrin, in particular, posed substantial risks in certain root crops. About one quarter of samples of organically labelled fresh produce contained pesticides residues, compared with about three quarters of conventional samples (Panseri, 2013). Although QuEChERS started out as a method for the extraction of multiclass, multiresidue pesticides from fruits and vegetables, it now is being used for a wide variety of analytes (for example, vet drugs, PAHs and antibiotics) in a wide variety of matrices (for example, plasma, meat and soil (Lehotay et al., 2010). Could be mentioned diverse applications for determination of pesticide residues, i.e. flax samples, nuts and pastry (Urairat et al., 2010), green tea, red tea and chamomile (Lozano et al., 2012) fresh spices (Sadowska-Rociek et al., 2012) honey and beeswax (Mullin et al., 2010), gingko-biloba leaf etc (Zhou, 2009). **D**econvolution **R**eporting **S**oftware (DRS) allows the identification of target compounds eliminating interferences and involves four consecutive steps: (I) noise analysis, (II) separation of pure components, (III) deconvolution of spectra, (IV) identification of

pure compounds. Determination is based on comparison with a library of spectra acquired in standard conditions. DRS is a mathematical technique that separates overlapping mass spectra into deconvoluted spectra of the individual components (Meng et al., 2007). The technique began to be used in laboratories, and the underlying problem is the limit of detection (LOD) of the subjected residues, considering that the technique is recommended for "dirty" samples analyzis. However, setting the limit at which residues can be detected is the first step to the method develop. Kirchner et al. estimated the lowest concentration of pesticide residues (mix formed by 18 residues) in non-fatty food matrix at which the residues can be successfully identified by automatic spectral deconvolution software and they found that the identification was successful at concentration levels ranged between 4 and 0.4 mg kg^{-1} in real matrix samples (apples). Also, with decreasing concentration, the number of identified pesticides and the quality of deconvoluted spectra decreased. The calculated limits of full-scan detection ranged from 0.20 ng for chlorpyrifos to 1.10 ng for captan (Kirchner et al., 2007).

MATERIALS AND METHODS

Testing technique, in order to establish the lower limit of determination was performed using red clover (*Trifolium pratense*) matrix and OC pesticide standards. Extraction was performed with modified QuEChERS protocol. Extraction step (1 g sample) was performed with 10 mL acetonitrile working variant "without citrates". Clean-up step was performed using 1 ml extract with a mixture of sorbents (50 mg Primary Secondary Amine (PSA) 12 mg active C and 150 mg MgSO4 anh) by SPD (solid phase dispersion) technique. The analysis was performed using an Agilent GC-MS equipment (7890A-5975C) - SCAN mode. The acquisition parameters were enforced by the software algorithm (HP-5MS column, 15 m

x 0.25 mm, 0.25 μm, MMI Inlet - 60° C (0.35 min) -900 ^{O}C/ min to 280 ^{O}C (15 min) to 900 ^{O}C to 300 ^{O}C (0 min); 2 μL injection volumes; Oven profile: 70 ^{O}C (1 min) -50 ^{O}C/ min to 150 ^{O}C, 6 ^{O}C/ min to 200 ^{O}C, 16 ^{O}C/ min to 280 ^{O}C (5 min), run time = 22 min; He, constant pressure.

Identification and quantification through DRS using the specific file associated to separation by 15m column (2Xdata1X.cal); retention time of chlorpyrifos -methyl was 8,296 min.

Steps to lock and adjust the retention time, for DRS applications are:

1) running a sample representing the analyzed mixture, which contains chlorpyrifos methyl
2) required chromatograms acquisition for the **R**etention **T**ime **L**ocking (RTL), using Chemstation software specific option
3) RT checking for chlorpyrifos methyl and its adjustment, if necessary at RT = 8.29 min
4) lock method towards chlorpyrifos methyl
5) running the standard mixture for verification

RESULTS

- Analytes can be separated and properly identified by DRS (standard chromatogram and DRS report for 1μg/ml mixture concentration is represented in figure 1 and 2 respectively).
- DRS allows linear calibration for the pesticide residues concentrations in the range 0.05-1 mg/kg for the majority of the studied analytes .
- analyzed pesticides for which the DRS allowed quantitation at the concentration values less than maximum residue limit (MRL) **()** were: α-HCH, β-HCH, γ-HCH, δ-HCH, dieldrin, p,p-DDE, p,p-DDD, p,p-DDT, β-endosulfan, endosulfan sulphate, methoxychlor.
- QuEChERS extraction protocol and DRS quantitation enable the determination at certain concentrations for: *α-HCH, β-HCH, γ-HCH, δ-*HCH, heptachlor, adrin, heptachlor epoxide, chlordane-*trans*, chlordane-*cis*, dieldrin, p,p-DDE, p,p-DDD, p,p-DDT, endrin, β-endosulfan, endosulfan sulphate, endrin-ketone, methoxychlor.
- a DRS quantitation report for 1 ppm concentration in sample is represented in figure 3.
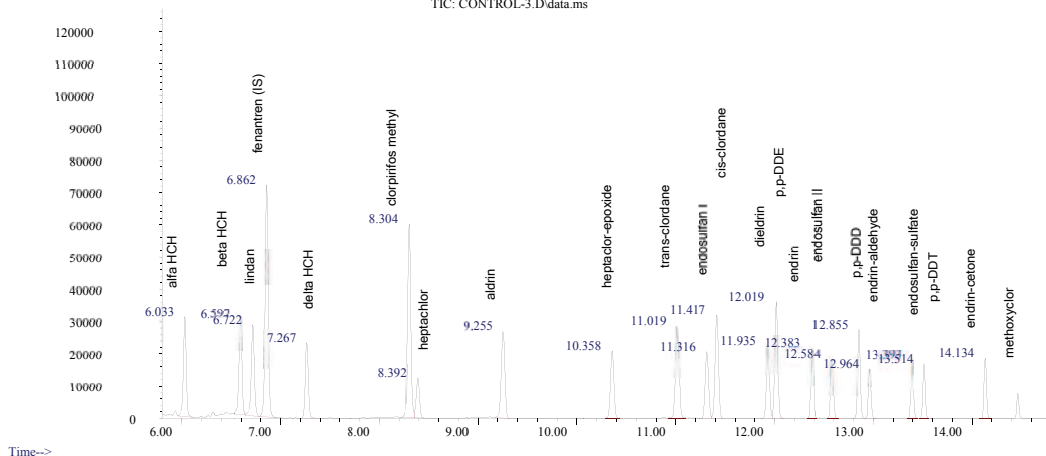
Figure 1. Standard chromatogram for 1μg/ml mixture concentration

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MSD Deconvolution Report
Sample Name: MIX-OC
Data File: C:\msdchem\1\DATA\2014\22.01.2014\MIX-OC.D
Date/Time: 2:45:27 PM Monday, January 27, 2014

Adjacent Peak Subtraction = 2
Resolution = Medium
Sensitivity = High
Shape Requirements = Medium

The NIST library was searched for the components that were found in the AMDIS target library.

R.T.	Cas #	Compound Name	Amount (μg/ml) Chem station	AMDIS	AMDIS Match	AMDIS R.T. Diff sec.	NIST Reverse Match	NIST Hit Num.
4.9870	84662	Diethyl phthalate			99	1.0	94	1
6.0298	319846	BHC alpha isomer	1	0.95	99	-1.5	88	1
6.5898	319857	BHC beta isomer	1	0.87	97	-1.2	89	1
6.7155	58899	Lindane	1	0.98	84	-1.8	84	3
6.8571	1517222	Phenanthrene-d10			100	-0.7	84	2
7.2621	319868	BHC delta somer	1	0.89	98	-1.2	90	1
7.9729	84695	Diisobutyl phthalate			86	3.1	80	30
8.3002	5598130	Chlorpyrifos Methyl	1.9	1.74	98	0.4	90	1
8.3882	76448	Heptachlor	1	0.83	91	-1.2	33	1
9.254	309002	aldrin	1	0.59	95	-0.9	85	1
10.3569	1024573	Heptachlor exo-epoxide isomer B	1	0.57	95	-0.2	36	1
11.0128	5103742	trans-Chlordane	1	0.88	97	-1.0	38	1
11.3122	959988	Endosulfan (alpha isomer)	1	0.97	88	-0.8	31	2
11.4137	5103719	cis-Chlordane	1	0.92	96	-0.3	36	2
11.933	60571	dieldrin	1	0.81	86	0.0	90	1
12.0165	72559	p,p'-DDE	1	0.91	99	0.7	89	1
12.1646	53190	o,p'-DDD			82	-1.4	73	6
12.3764	72208	Endrin	1	0.74	90	0.5	81	1
12.5812	33213659	Endosulfan (beta isomer)	1	0.82	88	0.3	81	2
12.8531	72548	p,p'-DDD	1	0.91	99	1.2	87	1
12.9602	7421934	Endrin aldehyde	1	0.8	86	0.6	83	1
13.391	1031078	endosulfar sulfat	1	0.55	92	1.4	79	1
13.5099	50293	p,p'-DDT	1	0.89	96	2.3	85	2
14.1286	53494705	Endrin ketone	1	0.85	85	1.8	78	1
14.4592	72435	Methoxychlor	1	0.81	90	3.4	78	1
6.859		fenantren	1					

Figure 2. DRS report for 1μg/ml mixture concentration

MSD Deconvolution Report
Sample Name: proba TRF-OC-1 mg/kg
Data File: C:\msdchem\1\DATA\2014\22.01.2014\TRF-1.D
Date/Time: 10:04:53 AM Tuesday, January 28, 2014

Adjacent Peak Subtraction = 1
Resolution = Medium
Sensitivity = Medium
Shape Requirements = Medium

The NIST library was searched for the components that were found in the AMDIS target library.

R.T.	Cas #	Compound Name	Amount (mg/kg) Chem station	AMDIS	AMDIS Match	R.T. Diff sec.	NIST Reverse Match	Hit Num.
2.6818	87627	2,6-Dimethylaniline			57	9.9	62	25
2.7069	91203	Naphthalene			64	4.1	79	2
3.1240	89838	Thymol			96	7.4	88	3
3.4080	97530	Eugenol			95	1.2	88	1
3.9490	131113	Dimethylphthalate			61	-0.4		
3.9490	33933798	4-Octanol, 2,4-dimethyl-					78	1
4.2953	33704619	Cashmeran			68	-1.8	66	49
4.9964	84662	Diethyl phthalate			100	2.1	96	1
5.3409	119619	Benzophenone			97	0.9	91	1
6.0317	319846	BHC alpha isomer	1	0.96	93	-1.2	88	1
6.5908	319857	BHC beta isomer	1	0.77	85	-1.1	77	3
6.7184	58899	Lindane	1	0.56	89	-1.5	82	3
6.9046	85018	Phenanthrene			55	-0.8		
6.9046	0000	Benzo[f]isoindol-1,3(1H,3H)-dione, 3a,4,9,9a-tetrahydro-4,9-O-benzeno-2-hexyl-					57	1
7.2668	319868	BHC delta isomer	1	0.95	89	-0.6	83	2
7.6639	106025	Exaltolide [15-Pentadecanolide]			45	13.1		
7.6639	502692	2-Pentadecanone, 6,10,14-trimethyl-					94	1
7.9721	84695	Diisobutyl phthalate			100	2.9	88	1
8.2937	5598130	Chlorpyrifos Methyl	1.88	1.79	83	-0.3	77	1
8.3831	76448	Heptachlor	1	0.74	72	-1.8	66	1
9.2085	84742	Di-n-butylphthalate			63	0.1	72	53
9.2518	309002	Aldrin	1	0.84	76	-1.5	65	1
10.355	1024573	heptaclor epoxid	1	0.41	76	-0.4	64	1
11.0124	5103742	trans-Chlordane	1	0.77	84	-1.1	72	2
11.3062	959988	Endosulfan (alpha isomer)	1	0.31	59	-1.5	53	2
11.4105	5103719	cis-Chlordane	0.99	0.88	84	-0.7	72	3
11.9323	60571	Dieldrin	1	0.91	56	-0.3	66	2
12.0157	72559	p,p'-DDE	1	0.94	96	0.6	89	1
12.1939	53190	o,p'-DDD			60	2.2	42	24
12.3816	72208	Endrin	1	0.95	63	1.1	57	1
12.5799	33213659	Endosulfan (beta isomer)	1	0.64	64	0.1	61	1
12.8499	72548	p,p'-DDD	1	0.9	92	0.8	85	1
13.3876	1031078	Endosulfan sulfate	1	0.67	73	0.9	64	1
13.5095	50293	p,p'-DDT	1	0.83	86	2.2	75	4
14.1273	53494705	Endrin ketone	1	0.88	46	1.6	54	2
14.4597	72435	Methoxychlor	1	0.88	82	3.4	70	2
14.8605	117817	Bis(2-ethylhexyl)phthalate			93	4.4	86	3

Figure 3. DRS quantitation report for 1 ppm concentration in sample

CONCLUSIONS

- DRS is a time saving method which allows the determination of the OC residues in complex matrices.
- In case of exceeding the MRL, DRS can be used to confirm the results for most pesticides residues.

- Using the DRS at lower detection limits is conditioned by the optimization of the clean-up method and/ or the use of SIM (Selected Ion Monitoring) mode acquisition.

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