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The Behavior of Some Pesticide Residues in Stored Medicinal Plants Samples

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Abstract Quantification of pesticide residues in dried plants or processed products is of utmost importance for their quality control. In this respect, pesticide residues should be accurately quantified, from the time of sampling/harvesting until the time of analysis or effective consumption. For teas and spices this approach is important, since on the one hand, they are kept dry (10-12% moisture, at 15-25 degrees Celsius) and are consumed as such, and these raw material can be processed by extraction after longer periods of time, even after years. Knowing the behavior of residues in dried plants is a benefit for the direct consumer, for the producer, and for the analyst who must know the stability of samples during analytical methods validation and testing. The assessment of the recovery level of deliberately added pesticides has been carried out on samples that have been kept for 4 years under normal temperature and humidity conditions. The remaining pesticide concentration was analyzed by GC-MS (SIM) at four time intervals; each time the stored sample was compared to a freshly fortified sample of the same concentration. The data was statistically processed (t-test). The rate of pesticide movement has been evaluated for three calculated percent of remaining thresholds: below 20%: for some organophosphorous and organochlorine pesticides, between 20-80% interval: for some organophosphorous, organochlorine and acetamide pesticides and more than 80 %: for some organochlorine and pyrethroid pesticides.

Keywords pesticide residues, QuEChERS, GC-MS, SIM mode, remaining concentration

1. Introduction

Nowadays, the presence of pesticides in animal and vegetal commodities is a topic of public concern regarding the potential health hazards derived from them [1, 2, 3]. Most analyses of pesticide residues in foods are being performed in raw agricultural commodities for a variety of purposes, which include regulatory monitoring, import/export certification, risk assessment, field-application trials, organic food verification, and marketing to consumers [4].

However, basic processes acting on pesticide residues in the field can continue to operate after crops are harvested. These include: volatilization, hydrolysis, penetration, metabolization, enzymatic transformation, and oxidation. The use of various physical processes on yielded products such as washing, trimming, peeling or juicing, can split the residues between various processed food fractions. This is often leading to direct reductions in the levels of residues in remaining edible portions. Processes involving heat or use of chemicals can increase volatilization, hydrolysis or other chemical degradation processes, hence reducing residue levels. Some processes can lead to a higher level of contaminants, a good example being the loss of moisture, which can lead to a higher concentration of some residues

in the dry raw material; another case is that of lipophilic pesticides that tend to concentrate in lipid structures where residue levels can increase in certain processing fractions such as vegetable oils [4].

These considerations suggest that effects of post-harvest practices and food processing should be taken into account on the rate of pesticide movement and dissipation, during dietary exposure assessments, so as to ensure realistic consumer safety regarding the pesticide residues intake.

Herbs have an important role in our health and our food, and have a variety of culinary and medicinal uses [5]. Similar to other crops, herbal plants may be contaminated by toxic substances such as mycotoxins, mildews, heavy metals, and pesticides. It is well known that there are many contaminants and residues that may cause harm to the consumers of herbal medicines. It is therefore essential to establish a convenient quality control method to certain the safety of herbal products [1].

Controls on pesticide residues in crops are generally based on Maximum Residue Limits (MRL's). The analytical determination of pesticides in herbs involves the identification and quantification of single or combinations of compounds in the presence of complex matrices, hence being a difficult task. The complexity of the herb matrix resides in the presence of phenolic compounds, carotenoids, chlorophyll, fats, waxes and essential oils. Herbal sample preparation is a crucial step in pesticide residue analysis. In recent times, research has been focusing on those methods which allow for reduction of the organic solvent used for extraction, and the elimination of the additional sample clean-up and pre-concentration steps before chromatographic analysis [6]. The sensitivity requirement for this analysis is determined by the regulatory background. The analysis of pesticide residues in tea and herbal products follows the regulations of the European Commission Directorate [7]. The maximum permissible level of pesticide residues in herbs, teas and similar products is regulated by EU Reference Laboratories [8] and Codex Alimentarius [9]. Furthermore, this kind of products should comply with quality standards in relevant European Pharmacopoeia or USP monographs, or those in pharmacopoeia of a Member State, according to the Directive 2004/24/EC [10].

Most studies in the literature related to the monitoring of pesticides in products of plant origin focuse on highlighting their degradation / removal after or during processing or storage of the raw materials, and refers to plant material with high water content [11], in which case the degradation is favored. The usefulness of studying the fate of pesticide residues in dried plant materials (e.g. medicinal plants/spices, 10-12% moisture) in testing laboratories can be extended. However, it is an important reference in the validation of analytical methods as well as for establishing the recommended preservation time of samples/ reference samples. Also, this kind of tests are very useful for setting the stability term of the CRM in order to obtain minimal differences between spiking values versus consensus assigned values [12, 13].

In this respect, the aim of our work was to evaluate the stability of multi-residues of pesticides in dried plants - e.g. herbs. The pesticides chosen for testing are included in the main groups analyzed by laboratories, in compliance with the basic condition that the limit of quantification (LOQ) is less/ equal MRL[8], for which have been made comprehensive studies for method validation. Assessing the stability of residues in the sample, as part of the validation was achieved by repeated analysis at timed intervals, by comparing a freshly fortified sample with a fortified sample stored in proper conditions, according to WHO guidelines on good agricultural and collection practices for medicinal plants [14].

2. Materials and Methods

Reagents

A multistandard mixture containing solutions of individual pesticide standards, prepared in HPLC-grade acetonitrile (from Sigma-Aldrich) at certain concentrations was used. This solution was used for the fortified samples preparation; the certified pesticide analytical standards were purchased from Dr. Ehrenstorfer. Extraction and cleanup have been made by using the QuEChERS method (for samples with high pigments content) with primary-secondary amine (PSA from Agilent) (45µm), Supelclean ENVI Carb (Supelco) and anhydrous magnesium sulphate (Sigma). Blanc matrix (sage - Salvia officinalis) was purchased from a certified organic.

Samples Preparation

The initial sample for stability testing of the pesticide residues (Salvia officinalis leaves) was prepared by fortification of 50 g blank matrix with a solution of the exact concentration, to obtain a concentration in the plant close to MRL, homogenized in a rotary evaporator flask of 1 L, at 40° C and high vacuum conditions. The fortified samples were stored in paper bags under controlled temperature and humidity conditions (15-25 degrees C and 20-65% humidity respectively). The behavior of residues for this sample was assessed by repeated analysis at different time points (one week, one month, 2 years and 4 years), and comparison was made with a freshly spiked sample of the same concentration. From each type of sample, at specific time intervals 10 g were weighed and grounded, from which six repetitions were analyzed each time.

The plant material was ground to a fine powder (500 µm screen) using a laboratory mill, Foss-Cyclotech 1093 model.

After numerous tests, the QuEChERS method ("Quick, Easy, Cheap, Effective, Rugged, and Safe") for sample preparation has proven to be a robust method with numerous and varied areas of application [15-19], justifying our choice in the proposed experimental protocol. Briefly, 1g of spiked sample was extracted with 10 mL acetonitrile in the presence of 1 g of magnesium sulfate (anhydrous) and 0.5 g of sodium chloride. Magnesium sulphate provides phase separation by absorption of water, whilst sodium chloride helps to remove co-extractives from the matrix so as to make more selective extraction for pesticide. For further cleanup, an aliquot was used in the dispersive solid phase extraction step, containing both magnesium sulfate (fine) for further water absorption, PSA to remove acids (including fatty acids), sugars, and activated charcoal for pigments (e.g. chlorophyll).

GC-MS Analysis

The Agilent 7890A GC equipment coupled with 5975C inert MS was used for the samples analysis. Acquisition parameters were: DB-5 MS column (60 m x 0.25 mm x 0.25µm), oven program (70 ºC isotherm for 3 min, then varying the temperature ramp up to 300 °C, and isothermal run for 22 min), splitless injector, 280 ºC, injection volume 1 µL, flow rate adjusted by the RTLOCK system blocked at retention time =20.6 min for phenanthrene (internal standard), single quadrupol, automated injection with 7693 autosampler. The quantitative method for determination of pesticide residues was the GC-MS-SIM mode, for each pesticide being chosen ions of maximum intensity which fulfill the criteria of selectivity (in the blank sample at the pesticides' retention times chromatographic peaks should not be higher than 30% of responses for the LOQ spiked samples) [8].

Data analysis

The data were analyzed using the ChemStation G1701EA Rev. E.02.00 SP2 software, the integration method being set for each compound.

Method validation showed that at LOQ level for the investigated pesticide residues, the recovery was 70-120%, in agreement with the enforced EU regulations.[8] The SIM method allowed to increase sensitivity and selectivity of the method, gathering data for masses of interest without interfering with chromatographic signals resulted from the complex analyzed matrix. Although the chromatographic integration possibilities are reduced, considering the situations of peaks overlapping, they can be individually integrated using software options, including QEdit Quant Results.

Tested compounds, their retention time (RT) and concentrations are listed in Table 1.

Table 1: Tested compounds, retention time and concentration

N^{o}. crt.	**Pesticide / quantitation ion for SIM detection**	**RT (min)**	**Concentration (mg/kg)**
1	dichlorvos / 185	12.45	0.32
2	α-HCH / 217	18.83	0.08
3	HCB / 284	19.04	0.08
4	β-HCH / 217	19.57	0.08
5	quintozen / 237	19.64	0.34
6	diazinon / 199	19.79	0.22

7	γ-HCH (lindan) / 217	19.97	0.08
8	fonofos / 246	20.09	0.09
9	δ-HCH / 183	20.89	0.08
10	chlorpyrifos-methyl / 286	21.81	0.08
11	alachlor / 188	22.00	0.04
12	parathion methyl / 263	22.09	0.17
13	pirimiphos-methyl / 290	22.62	0.15
14	heptachlor / 337	22.74	0.08
15	fenitrothion / 277	22.96	0.24
16	malathion / 285	23.07	1.72
17	chlorpyrifos / 197	23.53	0.12
18	parathion / 139	23.87	0.76
19	aldrin / 265	24.26	0.08
20	chlorfenvinphos / 267	25.11	0.46
21	heptachlor- epoxide / 353	26.00	0.08
22	methidathion / 302 (145)	26.46	0.33
23	chlordane-cis / 375	26.81	0.08
24	chlordane-trans / 373	27.36	0.08
25	α-endosulfan / 170	27.46	0.08
26	4,4'-DDE / 246	8.01	0.08
27	dieldrin / 263	28.67	0.08
28	ethion / 384	29.63	0.4
29	endrin / 317	29.73	0.08
30	4,4'-DDD /235	30.01	0.08
31	β-endosulfan / 339	30.09	0.08
32	4,4'-DDT / 235	31.77	0.08
33	endosulfan sulfate / 272	31.88	0.08
34	piperonyl butoxide / 338	32.29	4.03
35	bromopropylate / 341	33.86	0.86
36	phosalone / 367	35.39	0.15
37	azinphos methyl / 160	35.75	1.43
38	permethrin / 183	38.09	1.61
39	cypermethrin / 181	41.01	1.68
40	fenvalerate / 125	44.69	2.35
41	deltamethrin / 181	47.48	0.8

The study was based on the method validation results of the multi-residue pesticide method and was initially aimed at assessing the stability of the samples up to two years. Finding for some classes of pesticides, unexpectedly high values of remaining residues, the study later extended over a period of 4 years.

The determination of concentrations was done by one point calibration, consisting in the average of injections of the freshly prepared samples. Stability assessment of pesticides is based on calculating the difference in concentration between stored samples and freshly prepared samples (expressed in percentage). Was calculated average concentrations, standard deviation (SD) and relative standard deviation (RSD) for each compound in freshly prepared (FP) versus stored sample (SS), for one week, one moon, two and four years respectively.

Statistical analysis of results was based on the comparison of the two averages obtained in each analysis time point, by checking the statistical significance of differences. This was done by the t-test: Two-Sample Assuming Unequal Variances (Excel) and comparing the calculated probability with the 0,05 threshold.

Evaluations were reported as % remaining after storage (% R) for the stored samples, according to JMPR recomended procedure.[20] This was calculated at each measurement interval with the F1 formula (see below), and the resulting values were reported using combined uncertainty (% sM) associated to each % R value, calculated according to F2 (see below), and were graphically represented for each analysed pesticide.

F1: % remaining after storage = $100+\frac{(\overline{x1}-\overline{x2})}{\overline{x1}}*100$, were:

$\overline{x1}$, $\overline{x2}$ - mean of the freshly prepared / stored samples responses

F2: relative combined uncertainty % (% sM) = $\frac{\sqrt{\frac{2*S1^2}{\sqrt{N1}}+\frac{2*S2^2}{\sqrt{N2}}}}{\overline{x1}}*100$, were:

S_1, S_2 - standard deviation for the data sets

N_1, N_2 – number of determinations on each data set

$\overline{x1}$ - mean of the freshly prepared samples responses

3. Results and Discussion

In Figure 1 is shown the total chromatogram of the compounds separated / identified at levels close to MRL in the elution order, with each chromatographic peak integration; the chromatographic run was divided into 2 time intervals for proper view of the signals (labels and retention times).

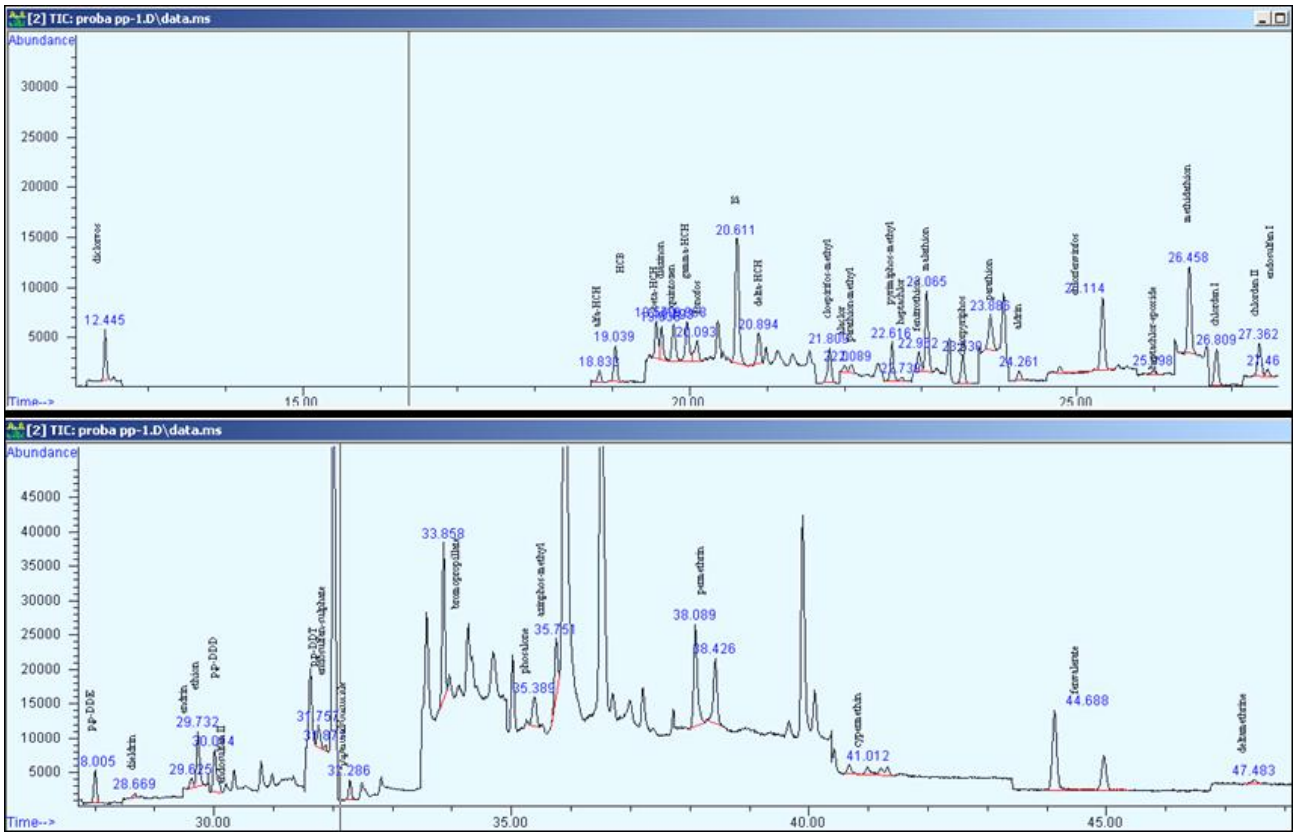


Figure 1: Total chromatogram of the separated compounds with retention times and integrations aspects

The data in Table 2 show the detected amounts of pesticide residues-R(%) at each time interval, with associated uncertainty-U(%). The data in Table 3 present the returned probability calculated for differences at each stage of testing.

Table 2: Percentage of remaining after storage for each pesticide residue at specified time interval, and the associated uncertainty

No. crt.	Compound Name	One week		One month		Two years		Four years	
		R (%)	U(%)	R (%)	U (%)	R (%)	U (%)	R (%)	U (%)
1	dichlorvos	86,53	6,74	14,08	16,40	0	3,01	0	7,95
2	alpha HCH	103,70	5,14	100,00	6,67	45,57	3,09	49,37	6,87
3	HCB	100,00	3,72	80,52	5,16	35,29	4,09	44,74	8,08
4	beta HCH	96,15	5,82	102,56	3,99	90,91	5,96	82,67	6,80
5	diazinone	100,00	3,10	66,51	3,29	10,00	3,00	0	5,94
6	quintozene	101,45	1,85	84,91	3,81	57,01	5,38	17,52	8,07
7	gamma HCH	102,53	3,12	101,39	6,01	74,68	7,48	65,75	9,46
8	fonofos	100,00	2,90	81,52	4,44	21,05	3,33	12,50	7,19
9	delta HCH	105,13	6,53	101,25	3,56	88,89	4,38	70,93	6,31
10	chlorpyrifos methyl	101,27	2,09	78,38	4,24	14,08	2,78	6,67	7,90
11	alachlor	102,70	8,43	87,50	6,47	45,95	7,96	27,03	8,17
12	parathion methyl	100,54	6,06	82,16	4,46	0	6,20	0	8,77
13	pirimiphos methyl	102,03	2,21	87,33	1,99	40,54	3,34	27,40	8,05
14	heptachlor	103,85	3,32	102,74	7,19	64,38	7,16	51,85	5,55
15	fenitrothion	101,18	5,97	85,32	2,60	8,58	4,21	8,18	7,82
16	malathion	103,04	5,81	82,66	3,34	21,95	4,02	4,46	7,01
17	chlorpyrifos	102,52	2,49	88,99	4,60	81,97	4,06	74,58	8,94
18	parathion	101,65	5,43	94,72	1,88	87,31	2,69	83,01	9,98
19	aldrin	98,80	7,68	101,45	8,76	55,29	5,58	45,00	6,96
20	chlorfenvinphos	98,28	9,84	92,58	5,67	90,49	10,8	63,05	8,00
21	heptachlor epoxide	103,85	7,24	101,28	6,03	12,50	3,24	0	6,42
22	methidathion	105,75	6,53	90,60	7,80	0	5,15	0	6,35
23	chlordan cis	105,13	4,39	102,53	2,83	91,25	3,84	88,46	7,85
24	clordan trans	101,27	6,21	101,27	1,94	92,11	4,49	90,91	8,78
25	endosulfan I	97,53	6,78	98,73	6,77	69,44	7,41	61,84	6,81
26	p,p-DDE	103,80	7,59	102,56	2,96	96,15	3,90	89,87	8,36
27	dieldrin	101,27	6,50	96,30	8,43	88,75	4,74	81,01	8,73
28	endrin	100,00	6,45	98,80	8,23	77,63	5,81	93,51	4,62
29	ethion	105,33	6,59	94,87	2,61	74,40	6,87	3,42	6,72
30	p,p-DDD	102,38	7,10	102,53	2,29	103,80	5,63	93,75	5,13
31	endosulfan II	98,81	7,42	94,67	7,51	51,43	8,27	66,67	9,29
32	p,p-DDT	98,72	10,35	93,75	5,59	110,77	12,2	56,63	8,45
33	endosulfan sulfate	101,18	7,38	97,30	10,56	54,02	6,87	0	8,56
34	piperonyl butoxide	105,91	5,31	97,04	4,37	99,30	4,76	70,04	6,62
35	bromopropylate	103,20	4,16	93,07	2,77	96,08	5,05	91,77	7,20
36	phosalone	105,48	5,07	96,34	6,36	92,45	5,03	77,30	8,45
37	azynphos methyl	103,67	8,27	85,86	7,50	16,85	4,60	0	5,99
38	permethrin	104,87	6,68	93,29	7,48	95,63	5,98	91,84	6,83
39	cypermethrin	107,99	8,33	87,11	5,84	85,10	10,4	91,65	3,86
40	fenvalerate I+II	104,54	8,62	91,78	8,24	90,04	7,88	87,88	6,27
41	deltamethrin	103,99	9,56	99,41	2,01	99,23	9,52	87,23	7,82

Table 3: The calculated probability (t-test); Values highlighted in green show that differences are statistically significant, with values of calculated probability $P<0.05$.

No. crt.	Compound Name	Tested after:			
		One week	One month	Two years	Four years
1	diclorvos	0,00811	0,00000	0,00000	0,00000
2	alpha HCH	0,11686	0,85663	0,00000	0,00000
3	HCB	0,80184	0,00004	0,00000	0,00001
4	beta HCH	0,25747	0,17967	0,02310	0,00055
5	diazinone	0,94290	0,00000	0,00000	0,00000
6	quintozene	0,12610	0,00002	0,00000	0,00000
7	gamma HCH	0,11522	0,65832	0,00027	0,00018
8	fonofos	0,91927	0,00001	0,00000	0,00000
9	delta HCH	0,10869	0,28481	0,00103	0,00000
10	chlorpyrifos methyl	0,79533	0,00006	0,00000	0,00000
11	alachlor	0,67025	0,00318	0,00000	0,00006
12	parathion methyl	0,86690	0,00002	0,00000	0,00002
13	pirimiphos methyl	0,06564	0,00000	0,00000	0,00000
14	heptachlor	0,10125	0,41723	0,00001	0,00000
15	fenitrothion	0,63389	0,00000	0,00000	0,00000
16	malathion	0,32853	0,00000	0,00000	0,00000
17	chlorpyrifos	0,07180	0,00439	0,00001	0,00024
18	parathion	0,55832	0,00021	0,00013	0,00694
19	aldrin	0,88071	0,82847	0,00000	0,00000
20	chlorfenvinphos	0,72838	0,02499	0,11690	0,00000
21	heptachlor epoxide	0,43843	0,56825	0,00000	0,00000
22	methidathion	0,12533	0,03345	0,00000	0,00000
23	chlordan cis	0,10552	0,06828	0,00173	0,02124
24	clordan trans	0,54270	0,07249	0,00764	0,06125
25	endosulfan I	0,70080	0,75878	0,00001	0,00004
26	p,p-DDE	0,37042	0,13486	0,14715	0,04967
27	dieldrin	0,60837	0,36237	0,00548	0,00196
28	endrin	0,88411	0,71673	0,00002	0,03408
29	ethion	0,14462	0,00289	0,00002	0,00000
30	p,p-DDD	0,47202	0,05898	0,27601	0,04753
31	endosulfan II	0,82186	0,15496	0,00001	0,00010
32	p,p-DDT	0,75618	0,07559	0,13535	0,00002
33	endosulfan sulfate	0,66455	0,69730	0,00000	0,00002
34	piperonyl butoxide	0,05592	0,21600	0,77859	0,00001
35	bromopropylate	0,17005	0,00057	0,15549	0,04889
36	phosalone	0,09268	0,29142	0,01055	0,00056
37	azinphos methyl	0,40504	0,00680	0,00000	0,00000
38	permethrin	0,18934	0,12069	0,17854	0,04229
39	cypermethrin	0,10172	0,00213	0,02182	0,00163
40	fenvalerate I+II	0,31654	0,06691	0,02984	0,00356
41	deltamethrin	0,42575	0,57423	0,88393	0,00855

The rate of pesticide movement and dissipation profile was evaluated for three levels of remaining thresholds (%) and graphically presentated in Figure 2, as well as in the chromatography diagram (Figure 3).

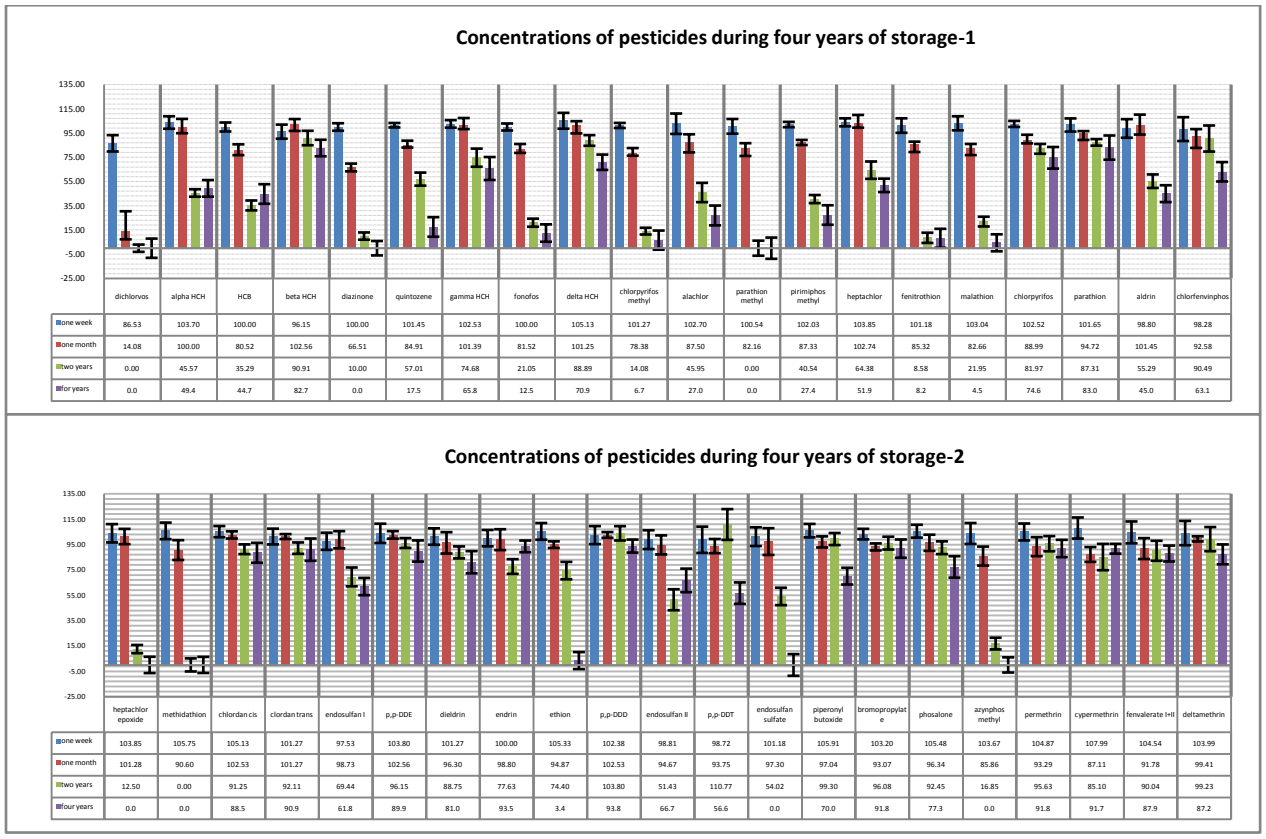
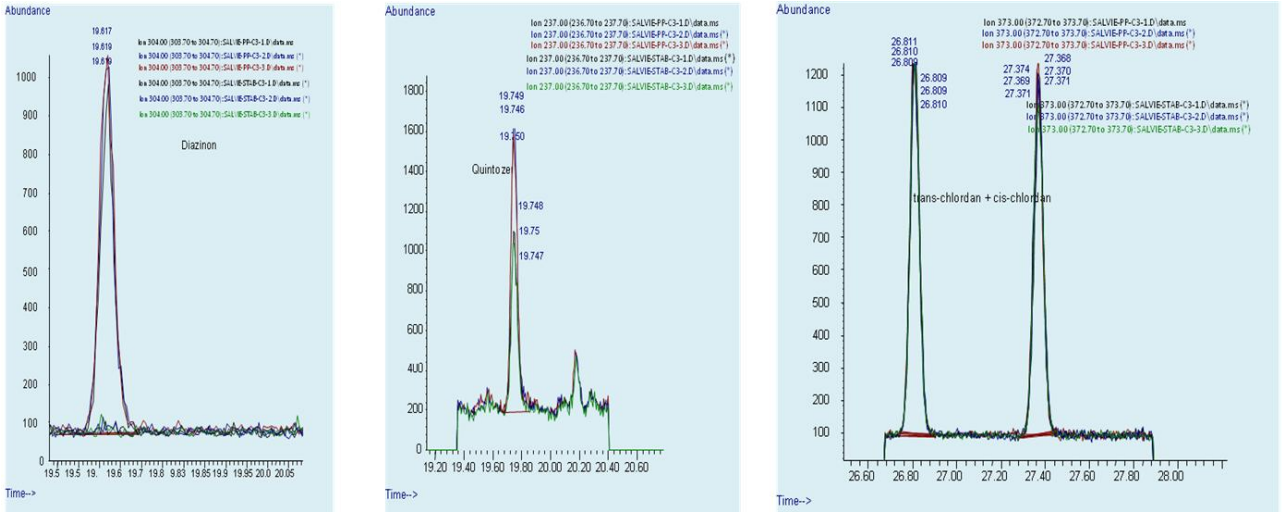


Figure 2: Concentration of pesticide residues during four years of study



a)- *lower than 20% threshold* *b) 20-80% interval* *c) more than 80 % threshold*

Figure 3: Comparative chromatograms representing the compounds in freshly prepared samples/two years stored samples, showing the concentration profile for each category of degradation threshold (a-diazinon, b-quintozen, c-chlordane isomers)

Pesticide residues (% of remaining) for the «lower than 20% threshold»

This profile was assessed for the following compounds: dichlorvos, diazinon, fonofos, fenitrothion, azinphos-methyl, chlorpyrifos-methyl, parathion-methyl, methidathion, malathion, ethion, belonging to the organophosphorous pesticide class (OP), the organochlorine (OCl) heptachlor-epoxide and endosulfan-sulphate.

Pesticide residues (% of remaining) for the 20-80% interval.

At the established 20-80 % interval of remaining residues in stored samples we found the organochlorine : HCH isomers, hexachlorbenzene, heptachlor, aldrin, endosulfan alpha and beta isomers, the organophosporous: pirimiphos-methyl, chlorfenvinfos, chlorpirifos, (OP), and alachlor from acetamide class.

Pesticide residues (% of remaining) for the more than 80 % threshold

Persistent compounds identified and quantified for this threshold were mainly organochlorines: chlordane-isomers cis and trans, DDT isomers, dieldrin, and pyrethroids : permethrin, cypermethrin, fenvalerate, deltamethrin, and from bromobenzilate class, bromopropylate.

Organochlorine pesticides (OCl) are broad-spectrum, high efficient, inexpensive pesticides widely used worldwide. The strong stability makes organochlorine pesticides to accumulate largely through the food chain, which eventually influences human beings health. Because of the structural similarities, showing chlorine-substituted aliphatic or aromatic cyclic rings, these pesticides share certain physicochemical characteristics such as persistence, toxicity, bioaccumulation and long-range transport potential. In this respect, residual OCl pesticides are often identified, sometimes exceeding MRLs [21]. In our study, about 40% of the OCls have similar and constant concentration profile in freshly prepared samples versus stored samples, and more than 70% have the remaining threshold over 50%.

Organophosphoric pesticides (OPs) are the most commonly used pesticides that still form the largest group of the world wide sales [22], although other newer and more specific insecticides were developed. The extensive use of OPs may be due to their mode of action, physical properties and metabolism. This group of pesticides tend to degrade rapidly on exposure to sunlight, air and soil, and some of them have high volatility which limits their persistence after foliar application. More than 60% of the studied OPs showed a pronounced descending concentration profile in samples kept under proper storage conditions.

In contrast to OP insecticides, pyrethroids exhibit low toxicity to mammals and birds, while also demonstrating strong selectivity for insects and invertebrates. They have both a low potential to bioaccumulate and proficient detoxification in mammalian receptors. The low vapor pressures and high octanol-water coefficients of pyrethroids indicate a low propensity to volatilize and a high affinity for organic matter [23]. Thereby, in our study, 100% of the pyrethroids proved to be highly persistent compounds in stored samples comparing with the freshly prepared ones.

4. Conclusions

The fate of pesticide residues during storage varies to a great extent, according to numerous factors. The chemical structure is determinant for the detected levels of pesticides. Chlorinated molecules with high stability and persistence were recovered in high concentration in the preserved samples.

The low moisture content in dry plants favored the conservation of some pesticide concentrations.

The determined levels of the remaining pesticide residues after four years can be used as reference for assessing the degree of stability of the analyzed pesticides in this type of samples (useful information for method validation) and in setting / determining the time range for samples storage.

Taking into account that some pesticides do not degrade on their own, if they are present in dry vegetable samples, the methods of processing the plant material at industrial level can be chosen to allow for efficient removal of pesticide residues.

This study could continue with the analysis of metabolites / pesticide degradation products found during sample storage to identify the toxic compound that could occur in a sample declared free of pesticides. For this kind of analysis, advanced steps of purification and concentration of the sample will be followed by a complex scanning analysis.

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References

1. Quality control methods for medicinal plant materials-World Health Organization Geneva, 1998
2. Patel, P.; et al. (2011).*Guidelines on Quality Control of Herbal Medicine*. IJRAP, 2(4), 1148-1154;
3. Pérez-Parada A.; Colazzo M.; Besil N.; Dellacassa E.; Cesio V.; Heinzen H.; & Fernández-Alba, A.R. (2011*). Pesticide Residues in Natural Products with Pharmaceutical Use: Occurrence, Analytical Advances and Perspectives. In Pesticides in the Modern World-Trends in Pesticide Analysis*, Ed. L.M. Stoytcheva, InTech Publisher, Rijeka, 357-390
4. Elpiniki G. A. (2011). *Fate of Pesticide Residues on Raw Agricultural Crops after Postharvest Storage and Food Processing to Edible Portions*. Pesticides. IntechOpen. Ed. Margarita Stoytcheva chapter 28.
5. Sasidharan, S.; Chen, Y.; Saravanan, D.; Sundram K. M.; and Yoga Latha L. (2011). Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. *Afr J Tradit Complement Altern Med*. 2011, 8(1): 1–10.
6. Łozowicka, B.; Jankowska, M.; Rutkowska. E.; Hrynko, I.; Kaczyński, P.; and Miciński, J. (2014). The evaluation of a fast and simple pesticide multiresidue method in various herbs by gas chromatography. *J.Nat Med*., 68(1): 95–111.
7. European Commission Directorate-General for Health and Food Safety (http://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727&LabID=100&Lang=EN
8. SANTE 11945/2015- Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed
9. *CODEX ALIMENTARIUS (*www.fao.org/fao-who-codexalimentarius/en/*)*
10. Directive 2004/24/EC of the European Parliament and of the Council, 2004 amending, as regards traditional herbal medicinal products, Directive 2001/83/EC on the Community code relating to medicinal products for human use.
11. El-Behissy, E.Y.; King, R.D.; Ahmed, M.M.; Youssef, A.M.(2001). Fate of Postharvest-Applied Dichlorvos in Stored and Processed Dates, *J Agric Food Chem*., 49 (3), 1239-45
12. Sykes, M.; Thompson, M.; and Reynolds, S. (2013).Pesticide Residues in Food-Based Proficiency Test Materials, Spiking Values versus Consensus Assigned Values. *J. Agric. Food Chem.*, 61 (18), pp 4205
13. Saldanha, H. M. (2009). Investigations of the feasibility of producing a new "natural" matrix Reference Material for the analysis of pesticide residues in products of plant origin. Ph.D. Thesis.
14. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants (2003). World Health Organization Geneva
15. Steiniger D.; Butler J.; Phillips, E.(2009) Multi-residue Pesticide Analysis in Onion by a Modified QuEChERS Extraction and Ion Trap GC/MSn Analysis LC-GC Application Notebook
16. Sahoo, K. S.; Battu, R.; Singh, B. Development and Validation of QuEChERS Method for Estimation of Propamocarb Residues in Tomato (*Lycopersicon esculentum* Mill) and Soil (2011). *American Journal of Analytical Chemistry*, 2, 26-31.
17. Sadowska-Rociek A.; Surma, M.; Cieslik, E. (2013).Application of QuEChERS Method for Simultaneous Determination of Pesticide Residues and PAHs in Fresh Herbs. *Bull Environ Contam Toxicol*., 90:508–513
18. Hao Z. C. (2012). Analysis of pesticide residues in green tea, using Agilent Bond Elut QuEChERS EN kit,by LC-MS/MS detection. Application Note (http://www.chem.agilent.com/Library/applications/5990-6400EN.pdf)

19. Urairat K.; Lehotay, S. J.; Mastovska, K.; Dorweiler, K. J. (2010). Extension of the QuEChERS Method for Pesticide Residues in Cereals to Flaxseeds, Peanuts, and Doughs. *J. Agric. Food Chem.*, 58 (10), pp 5950–5958
20. FAO Manual on the Submission and Evaluation of Pesticide Residues Data, 2009; Chapter 5 – JMPR practices in estimating maximum residue levels and proposing maximum residue limits, Section 5.5: Stability of pesticide residues in stored analytical samples.
21. Kang, J.Ho and Chang Y.S. (2011). Organochlorine Pesticides in Human Serum, Pesticides -Strategies for Pesticides Analysis. *IntechOpen*
22. Chen, J.; Duan, C.; Guan, Y. (2010). Sorptive extraction technique in sample preparation for organophosphorus pesticide in complex matrices. *J. Chromatogr. B*, 878, 1216–1225
23. Palmquist, K.; Salatas, J.; and Fairbrother A. (2012). Pyrethroid Insecticides: Use,Environmental Fate, and Ecotoxicology, Insecticides - Advances in Integrated Pest Management. *IntechOpen*