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Evaluation of the Matrix Effects in Herbal-Based Potions in Pesticide Residues Analysis by Dispersive Liquid-Liquid Microextraction Followed by Gas Chromatography-Mass Spectrometry

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The quantitative results in the pesticide residue analysis by gas chromatography-mass spectrometry are adversely affected by the phenomenon known as the matrix effects. A matrix effect may be noticed as an increase or decrease in the response of the detector signal compared with the response produced by solvent solutions of the analytes. The purpose of this research is to evaluate and compare the matrix effects in two nutraceutical samples (Alpa and Alpa Lesana), containing alcohol and herbal extracts. Samples were extracted by dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry analysis. Thirty-eight pesticides from different chemical classes were used to evaluate the matrix effects. Matrix effects were studied by calculating the matrix factor for each pesticide for the two herbal-based potions. The method was validated in terms of trueness (recoveries 70-120%), precision (below 20%), linearity (correlation coefficients for Alpa higher than 0.98; for Alpa Lesana in the range of 0.83 to 0.99), and the limits of detection (0.001-0.780 µg l^{-1} (Alpa); 0.014-0.812 µg l^{-1} for Alpa Lesana). The present study revealed the strong dependence of matrix effects on the sample type and the complexity of the matrix. Most of the pesticides showed strong signal enhancement in the case of Alpa Lesana analysis. On the other hand, most of the pesticides were influenced only minimally in the case of Alpa analysis. To compensate the matrix effects, the utilization matrix match standard solutions for calibration is recommended for both samples.

Keywords: Matrix effect, Matrix factor, Herbal potions, Pesticides GC-MS

INTRODUCTION

It is well known that a variety of herbal dietary supplements currently available on market may benefit consumers in the management of their health and provide a safer alternative to conventional medical treatment [1]. Nowadays, the relationship between food and drugs is getting closer. Nutraceuticals are components of dietary origin, with claimed beneficial therapeutic activities [2]. Nutraceuticals are commodities derived from food, mostly herbal-based, but used in the medicinal form of pills, capsules, potions, and liquids [3]. The assertion is that nutraceuticals enhance health and well-being, provide disease prevention, treatment, and survival benefits to the users, and as a result commercialization of these products is on a massive scale [4]. Consumer interest in complementary and alternative therapies, including the use of botanical dietary supplements, continues to increase all around the world [5].

Nutraceuticals are herbal-based products, therefore many of these products are farmed using conventional agricultural practices, including pesticide application to control insects and other pests during their cultivation and/or storage. Bearing in mind that most of these products are prepared by evaporation of the solvents used during the

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extraction from the raw material, pesticides or other contaminant compounds may also be concentrated and stabilized in the final product, and therefore, these should be monitored in nutraceutical products to ensure their safety [6].

The contaminants in nutraceuticals exist at trace levels, and these products have a complex matrix. For this reason, sample preparation and isolation of the analytes are very important for nutraceutical analysis. An important aspect of routine analytical methods is their environmental impact. Therefore, major advances have been achieved on sample preparation techniques within a green chemistry context, including minimal solvent and reagent consumption; elimination or reduction of the use of toxic substances; application of factors as pressure, microwave, and ultrasound radiation with the aim to reach high process effectiveness in a short time interval; and lower residues generation [7]. In the past few decades, several miniaturized methods, including, dispersive liquid-liquid microextraction (DLLME), have gained spreading popularity. DLLME is based on a ternary component solvent system, which involves the rapid injection of an appropriate mixture of disperser (water-soluble solvent) and a few microliters of extracting solvent into an aqueous solution. It results in the dispersion of water-immiscible extracting solvent throughout the aqueous phase as fine droplets and the analytes are enriched into extractant droplets [8,9]. Samples containing alcohol such as nutraceutical potions are promising samples for the DLLME technique, because ethanol natively contained in the sample may with an advantage serve as a dispersive solvent in the extraction step, and the use of additional solvent is eliminated [9,0].

Gas chromatographic (GC) techniques coupled with mass spectrometry (MS) are the most powerful analytical tools currently available for monitoring pesticide residues. In the multi-residue pesticide analysis using GC instruments, the matrix effect (ME) is a major problem that reduces the accuracy and precision of analytical results. In general, the term “matrix effect” is defined as “the direct or indirect alteration or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample” [11,12]. Analytes could be adsorbed or thermally degraded on the active sites of the system or matrix compounds may block the active sites and fewer analyte molecules will be adsorbed, consequently enhancing their signal [13]. Analytes may decompose at active sites in the liners, column, and detector, giving losses, which may result in suppression of the chromatographic signal.

Numerous methods have been proposed to reduce ME. The most obvious one is additional sample workup or extensive sample clean-up, leading to the selective removal of potential co-eluting interferences [14-18]. Other approaches used for the prevention of ME include the use of analyte protectants [19,20], coated inlet liners, compensation factors, different injection techniques, dilution, GC priming, internal standards, and isotopically labelled internal standards [21,22]. The matrix-matched calibrations are the most applied method to correct ME [9, 13,23-28].

The work aims to develop and validate a DLLME-GC-MS method for the determination of pesticides in nutraceutical potions. In this paper, the extent and variability of matrix effects in two alcohol containing herbal potions differing in the amount of herbal extract content (Alpa and Alpa Lesana) are determined and compared. The influence of the matrix on the recovery, precision, linearity, limits of detection, and quantification are also discussed.

EXPERIMENTAL

Chemicals and Reagents

Pesticide standards with purity higher than 95% from various chemical groups were purchased from different sources (Dr. Ehrenstorfer, Augsburg, Germany; Bayer, Leverkusen, Germany; Cheminova, Harboore, Denmark; Agrovita, Ivanka pri Dunaji, Slovak Republic).

Thirty-eight pesticides with different physical and chemical properties, belonging to various chemical groups, such as organochlorine, organophosphorus, chloracetamides, amines, phenols, dinitroanilines, carbamates, triazoles, azoles, pyrimidines, and pyrethroids were investigated in this study. At first, the working solution of standards at concentration 1 ng μl^{-1} was analyzed in full scan (FS) mode, and then, pesticides were classified into SIM groups. Table 1 summarizes the studied pesticides, their properties (chemical group, pesticide action, polarity expressed by K_{ow}), retention times, and monitored ions.

Table 1. List of Pesticides, their Chemical Classes, Properties, and GC-MS Parameters (Retention Time, Monitored Ions)

No.	Pesticide	Chemical group	Function	$logK_{ow}$	Retention time (min)	Monitored ions (m/z) [b]
1	Propham	Carbamate	Herbicide	2.60	3.596	**179**, 93, 120
2	*o*-Phenylphenol	Phenol	Fungicide	3.18	3.865	**170**, 169, 141
3	Diphenylamine	Amine	Herbicide	3.82	4.294	**169**, 168, 167
4	Trifluralin	Dinitroaniline	Herbicide	5.27	4.380	**306**, 264, 307
5	Chlorpropham	Carbamate	Herbicide	3.4	4.385	**127**, 171, 213
6	Hexachlorobenzene	OCP	Fungicide	3.93	4.651	**284**, 286, 249
7	Terbuthylazine	Triazol	Fungicide	3.70	4.889	**214**, 229, 173
8	Diazinon	OPP	Insecticide	3.69	4.901	**304**, 179, 107
9	Lindane	OCP	Insecticide	3.50	4,861	**181**, 219, 183
10	Pirimicarb	Carbamate	Insecticide	1.70	5.118	**166**, 72, 238
11	Acetochlor	Chloracetamid	Herbicide	4.14	5.290	**149**, 146, 223
12	Vinclozolin	Dicarboximid	Fungicide	3.02	5.341	**212**, 285, 178
13	Dimethachlor	Chloracetamid	Herbicide	2.17	5.261	**197**, 134, 149
14	Alachlor	Chloracetamid	Herbicide	3.09	5.370	**160**, 188, 146
15	Tolclofos-methyl	OPP	Fungicide		5.394	**265**, 267
16	Parathion-methyl	OPP	Insecticide	3.00	5.228	**263**, 125, 109
17	Pirimiphos-methyl	OPP	Insecticide	3.90	5.519	**290**, 305, 276
18	Malathion	OPP	Insecticide	2.75	5.616	**173**, 127, 93
19	Chlorpyrifos	OPP	Insecticide	4.70	5.696	**314**, 197, 199
20	Triadimefon	Triazole	Fungicide	2.77	5.776	**208**, 181, 210
21	Cyprodinyl	Pyrimidine	Fungicide	4.00	5.976	**224**, 225, 86
22	Tolylfluanid	Phenyl sulfamid	Fungicide	3.90	6.039	**137**, 238, 101
23	Penconazole	Triazole	Fungicide	4.64	6.028	**248**, 159, 161
24	Triflumizole	Imidazol	Fungicide	3.67	6.137	**278**, 206, 179
25	Bromophos-ethyl	OPP	Insecticide	6.15	6.188	**359**, 303, 242
26	Fludioxonil	Benzodioxol	Fungicide	4.12	6.062	**248**, 249, 86
27	Procymidone	Dicarboximid	Fungicide	3.30	6.125	**283**, 285, 96
28	Myclobutanil	Triazole	Fungicide	2.89	6.554	**179**, 245, 288
29	Trifloxystrobin	Strobilurin	Fungicide	4.50	7.029	**172**, 116, 187
30	*p,p*-DDT	OCP	Insecticide	7.00	7.138	**235**, 165, 237
31	Bifenthrin	Pyrethroid	Insecticide	6.60	7.476	**181**, 165, 166
32	Bromopropylate	Benzilate	Acaricide	5.40	7.521	**341**, 339, 343
33	Fenazaquin	Quinazoline	Acaricide	5.51	7.659	**145**, 160, 146
34	Fenarimol	Pyrimidine	Fungicide	3.69	8.025	**139**, 251, 207
35	Pyridaben	Pyrethroid	insecticide	6.37	8.322	**147**, 309, 207
36	Difenoconazole	Dioxolane	Fungicide	4.40	8.752	**32**, 207, 281
37	Azoxystrobin	Metoxyacrylate	Fungicide	2.50	9.576	**344**, 207, 224
38	Famoxadone	Oxazolidinedione	Fungicide	4.59	9.813	**330**, 207, 224

A stock solution of pesticide standards at a concentration of 1 mg ml^{-1} was prepared by weighing the individual pesticides and dissolving them with ethanol (Suprasolv grade, Merck KGaA, Darmstadt, Germany). A working standard composite solution (0.020 mg ml^{-1}) of all pesticides was prepared in ethanol and stored at 4 °C. Tetrachloroethane, methanol, and purified water of pesticide residue grade purity (from Sigma Aldrich, Steinheim, Germany) were used. The working pesticide solutions were prepared by an appropriate dilution of a mixture of stock solutions in ethanol. Samples of nutraceutical potions, Alpa and Alpa Lesana, with alcohol content at 60% and 70% respectively (prepared by producer according to patented license) were obtained from local stores (Bratislava, Slovakia) and stored at 5 °C until the moment of analysis.

Instrument and Apparatus

Chromatographic analyses were carried out on an Agilent 6890N GC system (Agilent Technologies, Little Falls, DE, USA) connected with an Agilent 5975 mass-selective detector. An Agilent 7683B autosampler and programmable temperature vaporization (PTV) injector were used for the injection of 2 µl of solutions in solvent vent injection mode. The PTV injector was operated under the temperature program: the initial temperature was set at 80 °C (hold 0.20 min), then the temperature was increased at the gradient of 400 °C min^{-1} to the temperature 300 °C, which was held for 2.00 min, and then, the temperature was increased at the gradient of 400 °C min^{-1} to reach the temperature of 350 °C, which was hold for 5.00 min. The GC separation was performed by a narrow-bore capillary column CP-Sil 8 CB (Agilent Technologies) with 5% diphenyl 95% dimethylsiloxane stationary phase, 15 m × 0.15 mm I.D. × 0.15 µm film thickness. A deactivated retention gap (1 m × 0.32 mm) from Supelco (Bellefonte, Pennsylvania, USA) was used as a pre-column. At the beginning of the injection, the column temperature was set at 100 °C held for 1.75 min, increased with the gradient at 60 °C min^{-1} to 150 °C, then increased at 23.8 °C min^{-1} to 300°C, final isothermal part was held for 2.90 min. To reach the initial injector temperature as fast as possible, for the next injection a cryogenic CO_2 cooling was set on. The overall run time was 11.25 min. Helium was used as the carrier gas in the mode of constant flow rate at 1.2 ml min^{-1}. MS detection was carried out by an Agilent 5975 mass-selective detector (Agilent Technologies) operating in electron ionization mode (EI, 70 eV). The temperatures of the ionization source and the quadrupole were set at 280, and 150 °C, respectively. For the full scan, the mass range of 40-550 *m/z* was selected. In SIM, a solvent delay of 3 min was applied.

Pesticides were weighed by Sartorius Analytic MC1 scales (Sartorius, Göttingen, Germany). Rotofix 32 centrifuge (Hettich, Tuttlingen, Germany) and Vortex Heidolph Multi Reax Shaker (Heidolph, Berlin, Germany) were used for the sample preparation.

DLLME Extraction

The samples were prepared according to the previously developed DLLME method for the extraction of pesticides from samples containing 40% of alcohol [9]. Before the extraction, the alcohol content of the samples was adjusted by water addition to 40%. The DLLME procedure for both samples was the following, using 950 µl of the sample with alcoholic content adjusted to 40%, spiked with 50 µl of standard reference pesticide solution. The spiked sample with 40% ethanol content was placed in a 15 ml centrifuge tube. 1.75 ml ultra-pure water and 0.01 g of NaCl were added to the spiked sample. Subsequently, the mixture of 80 µl of tetrachloroethane (extraction solvent) and 187.5 µl of methanol (additional dispersive solvent) was quickly injected. A cloudy solution resulting from the dispersion of the fine tetrachloroethane droplets in the aqueous solution was formed in the centrifuge tube. The closed centrifuge probe was immediately vortexed at 1800 rpm for 3 min, followed by centrifugation at 4000 rpm for 2 min. The final extract sedimented at the bottom of the centrifuge probe was transferred to the insert in a 2 ml vial and taken for GC-MS analysis.

Method Validation

Trueness and precision in terms of average recovery and relative standard deviation (RSD) were evaluated for the proposed method by conducting recovery experiments at three concentration levels for both samples (Alpa and Alpa Lesana). The recovery experiments were realized with spiked samples at concentration levels: 10, 50, and 100 µg l^{-1}. Samples with a volume of 950 µl were spiked to

the corresponding concentration level of pesticides by adding 50 µl of solution at the corresponding concentration level and left to stand 20 min before the extraction. Precision was assessed by five replicate experiments at each concentration level, in addition, for each of them, three replicate GC-MS measurements were performed.

The results from the recovery study were assessed for compliance with SANTE/12682/2019 European Union guidelines [29], according to which recovery should fall within the range of 70-120%, with an associated RSD less than or equal to 20%.

The linearity was evaluated using matrix-matched calibration curves for both samples (Alpa and Alpa Lesana) at concentrations ranging from 0.01 µg l^{-1} to 250 µg l^{-1}. The lowest calibration level (LCL) was selected individually for all pesticides depending on the pesticide response. Linear minimal square regression analysis was applied using peak area as an analytical signal. LOD and LOQ were calculated using signal-to-noise ratio (S/N) criteria.

Evaluation of Matrix Effects

The ME was investigated by calculating the matrix factor (MF) for each pesticide for both samples of herbal-based potions. The MFs were calculated for each studied pesticide by comparing analytes response in matrix-matched solution vs. the pesticide response obtained in pure solvent solution at concentration level 50 µg l^{-1}. For the calculation of MFs, peak areas for quantification ion in SIM mode of GC-MS analysis of each pesticide (shown in bold in Table 1) were utilized. The order of the injection in the sequences for the study of matrix effects was as follows: (1) pure solvent solution of the analytes (2) matrix-matched solution (Alpa) with the following three repetitions. In the same way, the sequences of Alpa Lesana followed. Exceeding the MF higher than 20% or smaller than -20% indicates the peak signal suppressing or enhancing due to the matrix effects in the calibration procedure.

RESULTS AND DISCUSSION

The studied samples of the nutraceutical products, Alpa and Alpa Lesana are alcohol-containing herbal solutions. The basis of all Alpa recipes is an alcoholic solution of essential oils, herbs, and natural menthol. Alpa is a solution of natural plant essential oils, menthol, linalool, neroli oil, and other aromatic substances. Contrary to Alpa, Alpa Lesana contains a higher amount of natural menthol, and additionally, it contains an extract of fir-needles from Coniferales trees. The primary utilization of these nutraceuticals is inhalation, mouth washing, low-amount consumption or it can be applied to the skin during the massage to soften the pain due to rheumatism or the physical workload. The higher concentration of menthol in Alpa Lesana in comparison with Alpa increases its cooling effects and provides more effective relaxation for painful muscles. Both samples are used mostly for external use, for rubbing into the skin, massages, as an additive to baths of the entire body as well as footbaths, it is also used for bandages and gargling or as slight disinfection means. The active substances contained in both products have antiseptic and disinfection effects, they mitigate pains at muscle and joint rheumatism, removing tiredness after physical load.

Method Validation

Sample preparation of both samples (Alpa and Alpa Lesana) was carried out following an optimized DLLME procedure [9] since it provided adequate extraction and clean-up. Shortly, the following parameters were searched: type of extraction solvent (Tetrachloroethane, Chloroform, Dichloromethane, Tetrachloromethane, n-Hexane with Tetrachlororethane selected option); Volume of extraction solvent from 60 to 160 µl with 80 µl as an optimum; Type of additional dispersant (Acetone, Acetonitrile, Methanol, Ethanol, while Methanol was selected); Volume of dispersive solvent 60-375 µl (while 187.5 µl was optimum); Volume of water 1.25 to 2.5 ml (1.75 ml was used); Salt addition was tested between 0-30% (10% was optimal); Extraction time from 5 to 60 min, while 5 min was selected; various mixing methods (vortex at 1800 rpm was optimal). pH adjustment between 2-10 was tested and no pH adjustment was chosen.

Validation of the method was carried out according to SANTE/12682/2019 guidelines [29]. Recovery assays were performed on five replicates at three spike levels. The blank samples were spiked by the corresponding amount of working standard solution at the concentrations of 10, 50, and 100 µg l^{-1} individual pesticides in the sample. The extracts were analyzed by GC-MS three times and the peak

areas were averaged. The extraction recoveries (ER) (Eq. (1)) were expressed as the ratio of the analyte amount in sediment phase (n_{sed}) and the total analyte amount (n_0) in the original analyzed sample aliquot, expressed in %:

$$ER = \frac{n_{sed}}{n_0} \times 100\% = \frac{c_{sed} \times V_{sed}}{c_0 \times V_{aq}} \times 100\% \quad (1)$$

where V_{sed} and V_{aq} are the volume of sediment phase and sample solution, respectively. c_{sed} is the analyte concentration in the sedimented phase and c_0 is the initial analyte concentration in the sample. All compounds presented satisfactory recoveries from tested matrices within the range between 70% and 120% except some pesticides at the lowest concentration level (10 µg kg^{-1}), malathion in case of Alpa with the recovery of 121%, alachlor and tolclofos-methyl in case of Alpa Lesana with recoveries 56% and 54%, respectively. The precision of the method was expressed as RSD, and the values were generally below 20% for all pesticides in both types of samples, fulfilling the established requirements for pesticide residue analysis. At concentration level 10 µg kg^{-1} the recoveries of pesticides ranged from 73-121% for Alpa and in the range 54-120% for Alpa Lesana. The low recoveries of the pesticides isolated from Alpa Lesana should be explained by the high complexity of the sample. The recoveries of pesticides at concentration level 50 µg kg^{-1} were between 74% and 119% for Alpa and between 71% and 120% for Alpa Lesana and at 250 µg kg^{-1} between 73 and 107% for Alpa and 72% and 113% for Alpa Lesana. The recoveries are summarized in Table 2.

The linearity was studied in the range of 10 concentration levels from 0.01 to 250 µg l^{-1}. In the case of Alpa, good linearity results with satisfactory correlation coefficients were obtained for all pesticides. The correlation coefficients were higher than 0.99, except for trifluralin and parathion-methyl, while the correlation coefficients for these two pesticides were above 0.98. Alpa Lesana represents a more complex sample, because of the higher amount of herbal content in comparison to Alpa. This complexity influenced the linearity results, the correlation coefficients were lower than in the case of Alpa. The correlation coefficients fall in the range from 0.96-0.99 for most of the pesticides except for chlorpropham, trifluralin, acetochlor, and tolylfluanid, which showed lower linearity with correlation coefficients from 0.83 (chlorpropham) to 0.92 (tolylfluanid).

LODs were evaluated using a signal-to-noise (S/N) ratio of 3:1 and LOQs using S/N of 10:1. LOD and LOQ values for both kinds of samples are summarized in Table 3. The LODs for Alpa ranged between 0.001 µg l^{-1} (hexachlorobenzene) and 0.780 µg l^{-1} (propham), on the other hand, for Alpa Lesana the LODs were higher and ranged between 0.014 µg l^{-1} (hexachlorobenzene) and 0.812 µg l^{-1} (propham). The LODs and LOQs for most of the pesticides were lower for Alpa, except for seven pesticides, namely dimethachlor, acetochlor, chlorpyrifos, tolylfluanid, triflumizole, difenoconazole, and famoxadone. The values of LOD and LOQ also confirmed the complexity of Alpa Lesana. The obtained LOQs were more than 10 times higher for some pesticides (o-phenylphenol, tolclofos-methyl, cyprodinil, tolylfluanid, penconazole, bromophos-ethyl, trifloxystrobin, *p,p*-DDT, fenazaquin), and all these pesticides were influenced by strong matrix effects in the case of Alpa Lesana.

Matrix Effects

ME may be noticed as an increase or decrease in the response of the detector signal given by the presence of analytes in the sample (containing matrix components) compared with the response produced by solvent solutions of the analytes. The MFs were calculated for each studied pesticide by comparing analytes peak areas in matrix-matched standard solutions and the pesticide peak area obtained in a pure solvent at a concentration level of 50 µg/L for both of the samples (Alpa and Alpa Lesana), according to the following equation (Eq. (2)):

$$MF = \left(\frac{peak\ area\ in\ matrix - matched\ solution}{peak\ area\ in\ solvent\ solution} \right) \times 100\% \quad (2)$$

Exceeding the MFs higher than 20% or smaller than -20% indicates a peak signal enhancing or suppressing respectively due to the ME. MEs were classified into three types: minimal signal suppression or enhancement effects (MF interval -20% to 20%), moderate effects (MF interval -50% to -20% or 20% to 50%), and strong matrix effects (less than -50% or greater than 50%).

Table 2. The Extraction Recoveries (ER) at Three Concentration Levels with Precision Expressed as Relative Standard Deviation (RSD) for Alpa and Alpa Lesana (Concentration Level is Declared as a Subscript)

Pesticide	Alpa						Alpa Lesana					
	ER_{10} (%)	RSD_{10} (%)	ER_{50} (%)	RSD_{50} (%)	ER_{100} (%)	RSD_{100} (%)	ER_{10} (%)	RSD_{10} (%)	ER_{50} (%)	RSD_{50} (%)	ER_{100} (%)	RSD_{100} (%)
Propham	86	7	79	15	79	4	117	7	73	7	81	12
o-Phenylphenol	73	1	74	9	73	6	86	10	71	3	73	3
Diphenylamine	89	4	87	3	75	10	101	6	82	1	79	6
Trifluralin	92	10	85	2	75	7	108	12	79	0	78	11
Chlorpropham	103	7	96	1	85	5	74	7	73	6	83	19
Hexachlorobenzene	79	2	80	2	82	1	73	3	75	2	78	7
Terbuthylazine	103	20	110	18	76	10	109	16	89	1	95	18
Diazinon	101	16	115	6	96	17	93	9	120	3	113	2
Lindane	117	11	103	18	92	8	110	3	73	4	76	6
Pirimicarb	88	19	90	6	80	20	83	14	76	7	81	6
Acetochlor	97	11	116	3	94	16	86	11	74	5	76	11
Vinclozolin	73	4	80	1	72	1	114	17	79	14	112	4
Dimethachlor	99	4	106	3	85	5	96	19	72	2	86	1
Alachlor	79	10	85	8	92	6	73	15	79	10	82	5
Tolclofos-methyl	111	1	107	1	85	6	56	7	100	19	92	2
Parathion-methyl	99	10	107	5	92	7	54	12	73	3	72	4
Pirimiphos-methyl	117	5	119	1	90	11	70	17	86	2	91	4
Malathion	121	7	118	4	87	3	113	6	83	1	101	9
Chlorpyrifos	103	18	114	6	107	19	75	16	83	10	106	5
Triadimefon	110	2	103	19	88	18	117	36	76	3	82	1
Cyprodinyl	103	2	99	3	85	10	96	3	81	0	84	7
Tolylfluanid	117	2	116	14	91	12	108	5	84	5	84	8
Penconazole	109	1	105	5	85	9	119	19	116	5	113	9
Triflumizole	103	8	100	1	84	10	91	3	81	1	82	4
Bromophos-ethyl	108	6	117	2	101	12	114	5	96	4	100	5
Fludioxonil	105	4	106	6	87	6	101	2	84	3	84	5
Procymidone	100	1	95	0	82	10	98	1	85	3	81	4
Myclobutanil	87	18	76	6	95	11	104	1	87	5	82	9
Trifloxystrobin	90	9	84	0	76	8	120	2	89	8	80	3
p,p-DDT	120	1	115	2	95	12	93	9	95	1	90	5
Bifenthrin	105	2	95	0	84	11	120	2	89	4	90	3
Bromopropylate	119	6	103	2	87	13	98	3	84	5	81	6
Fenazaquin	115	7	105	3	87	11	109	1	113	2	101	0
Fenarimol	106	2	115	1	95	9	113	8	93	0	87	7
Pyridaben	94	15	100	3	86	9	115	3	85	5	85	4
Difenoconazole	108	15	110	18	106	3	112	4	87	4	86	9
Azoxystrobin	107	11	98	4	90	10	97	3	86	3	83	4
Famoxadone	119	19	115	1	93	13	114	19	103	1	95	7

Table 3. Validation Results of the Developed Method, Limit of Detection (LOD), the Limit of Quantification (LOQ), and low Calibration Levels (LCL) for Pesticides in Alpa and Alpa Lesana

	Alpa			Alpa Lesana		
	LCL ($\mu g\ l^{-1}$)	LOD ($\mu g\ l^{-1}$)	LOQ ($\mu g\ l^{-1}$)	LCL ($\mu g\ l^{-1}$)	LOD ($\mu g\ l^{-1}$)	LOQ ($\mu g\ l^{-1}$)
Propham	5	0.781	2.574	5	0.812	2.680
o-Phenylphenol	0.1	0.010	0.033	0.5	0.091	0.300
Diphenylamine	0.05	0.015	0.050	0.5	0.060	0.198
Trifluralin	0.5	0.039	0.129	0.5	0.068	0.224
Chlorpropham	0.05	0.005	0.017	5	0.315	1.040
Hexachlorobenzene	0.01	0.001	0.003	0.5	0.014	0.046
Terbuthylazine	0.1	0.015	0.050	0.5	0.115	0.380
Diazinon	0.5	0.011	0.036	0.5	0.036	0.119
Lindane	0.5	0.018	0.059	0.5	0.090	0.297
Pirimicarb	0.05	0.007	0.023	0.5	0.070	0.231
Acetochlor	1	0.263	0.868	1	0.164	0.541
Vinclozolin	5	0.595	1.964	1	0.203	0.670
Dimethachlor	0.1	0.017	0.056	0.5	0.128	0.422
Alachlor	0.5	0.093	0.307	5	0.306	1.010
Tolclofos-methyl	0.1	0.022	0.073	1	0.132	0.436
Parathion-methyl	0.01	0.003	0.010	0.5	0.071	0.234
Pirimiphos-methyl	0.5	0.033	0.109	0.5	0.055	0.182
Malathion	0.5	0.027	0.089	0.5	0.033	0.109
Chlorpyrifos	0.5	0.027	0.089	0.5	0.026	0.086
Triadimefon	0.1	0.016	0.053	0.5	0.066	0.218
Cyprodinyl	0.1	0.004	0.013	0.5	0.038	0.125
Tolylfluanid	0.1	0.013	0.043	1	0.136	0.449
Penconazole	0.5	0.129	0.426	0.5	0.014	0.046
Triflumizole	0.5	0.013	0.043	0.5	0.076	0.251
Bromophos-ethyl	1	0.217	0.716	0.5	0.095	0.314
Fludioxonil	0.01	0.002	0.007	0.5	0.032	0.106
Procymidone	0.5	0.058	0.191	0.5	0.141	0.465
Myclobutanil	0.1	0.018	0.059	1	0.221	0.729
Trifloxystrobin	0.1	0.011	0.036	1	0.195	0.644
p,p-DDT	0.01	0.001	0.003	0.5	0.036	0.119
Bifenthrin	0.5	0.04	0.132	1	0.159	0.525
Bromopropylate	0.05	0.009	0.030	0.5	0.042	0.139
Fenazaquin	0.05	0.005	0.017	1	0.085	0.281
Fenarimol	0.1	0.007	0.023	0.1	0.024	0.079
Pyridaben	0.5	0.034	0.112	0.5	0.073	0.241
Difenoconazole	5	0.264	0.871	0.5	0.100	0.330
Azoxystrobin	1	0.029	0.096	0.5	0.062	0.205
Famoxadone	1	0.268	0.884	0.5	0.102	0.337

As it can be seen in Fig. 1 for Alpa Lesana, stronger ME in comparison with Alpa was observed. In the case of all pesticides except fenarimol, higher MFs in Alpa Lesana were recorded. It can be seen in the case of Alpa Lesana that most of the MFs are out of the suitable range, indicating significant differences between the matrix-matched standards and the solvent-matched standards. The MFs for this type of sample were in the range from -84% to 607%. Only one pesticide, hexachlorobenzene shows minimal ME with MF 2%.

Signal suppression is less common in GC analysis, in this study only 1 pesticide fludioxonil in Alpa Lesana showed strong signal suppression (-84%). However, in the case of Alpa, 19 pesticides showed signal suppression, but the fact is, that 12 pesticides indicate only minimal signal suppression with MF in the range from -1% to-17%, and no more than four pesticides, terbuthylazine, vinclozolin, parathion-methyl, and difenoconazole showed strong matrix suppression with MF between -55% and -96%.

Evaluating the number of pesticides showing minimal, moderate, and strong ME in Alpa *vs*. Alpa Lesana is represented in Fig. 2. Only one pesticide (hexachlorobenzene) showed minimal ME with MF = 2% in the case of Alpa Lesana. On the other hand, for Alpa minimal ME was observed for most of the pesticides, twenty pesticides indicated minimal ME ranging between -17% and 19%.

The number of pesticides with moderate and strong ME was higher for Alpa Lesana, 11 pesticides showed MF between 22% and 48% and 28 pesticides expressed MF ranging from 53-607% thus showing strong ME. The highest ME was obtained for tolylfluanid. It is expected, that amino groups contained in tolylfluanid are responsible for this ME due to the interactions of amino groups with the active sites in the GC system. In turn, the number of pesticides influenced with moderate and strong matrix effects was lower for Alpa, with moderate ME for 7 pesticides with MF between -27% and -20% and between 20% and 34% and strong ME with MF going down up to -96% and going above 50% up to 166% (13 pesticides). It can be concluded, that Alpa Lesana contains a greater amount of interfering substances, causing higher matrix effects.

Comparing the influence of Alpa and Alpa Lesana

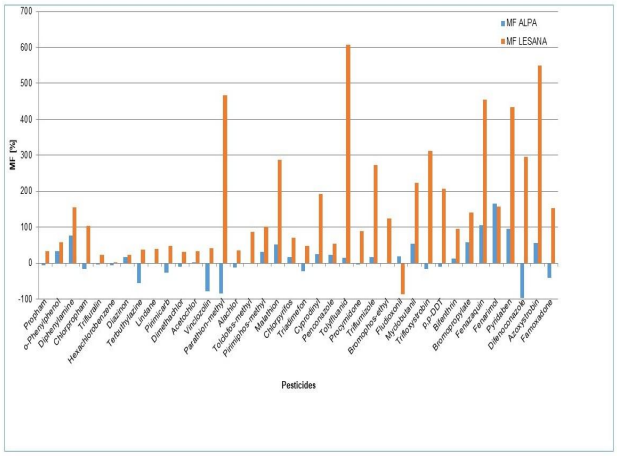

Fig. 1. Comparison of matrix factors (MF) observed for pesticides in Alpa and Alpa Lesana.

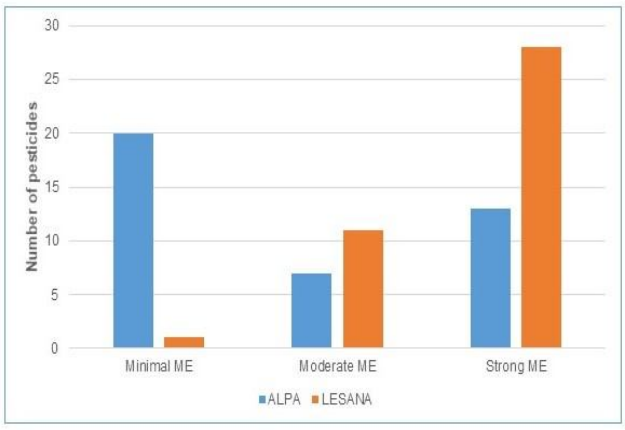

Fig. 2. The number of pesticides showed minimal, moderate, or strong matrix effect in Alpa and Alpa Lesana.

matrix components to the peak area of individual pesticides, the following observations were noticed. For vinclozolin and terbuthylazine strong signal suppression in Alpa, with MF -77% and -55%, respectively were observed, on the other hand, moderate ME with MF 36% and 42%, in Alpa Lesana were obtained. Except for these pesticides, all the studied analytes were influenced by the matrix stronger in the case of Alpa Lesana. The highest difference between the MF can be seen for tolylfluanid, which showed MF 14%, which is minimal ME for Alpa, and 607%, which was the

strongest MF, for Alpa Lesana. Only ten pesticides were simultaneously influenced strongly by the matrix in both samples, from the chemical structure of these samples we could conclude that six from these pesticides contain heterocyclic amines (fenazaquin, fenarimol, pyridaben, difenoconazole, azoxystrobin, and myclobutanil), parathion-methyl and malathion contain phosphate functional group, bromopropylate contains hydroxyl and diphenylamine amino functional group. Pesticides containing hydroxy, amino, and phosphate functional groups, are likely retained on the glass and metal surfaces in a heated gaseous state. In the presence of plant-derived matrix components in the extract, the interaction of pesticides with active sites is considerably reduced as such matrix components, which are present in excess, occupy most of the active sites, allowing the pesticide molecules to pass through the GC system with strongly reduced surface interactions. The presence of a matrix thus leads to reduced pesticide decomposition and sharper peaks [21,29].

It can be concluded, that Alpa Lesana, which contains more herbal compositions represents a more complex sample than Alpa, therefore, stronger ME in this type of sample was provided. ANOVA was used to confirm the results, considering the data normal distribution and employing 0.05 of the significance level. The obtained results show that evaluating ME significant differences were observed for the two sample types and also the compounds had significant differences between their area values in solvent and matrix-matched solutions in both samples. Therefore, there are significant ME for Alpa and Alpa Lesana, respectively. For the elimination of ME in both samples, matrix-matched standard solutions are proposed to be used.

Comparison of the Matrix Effect in Different Types of Samples

The developed DLLME method is a simple microextraction method without a clean-up step. Therefore, higher matrix effects are observed in the case of matrices with complex structures (such as Alpa Lesana). DLLME method without cleanup based on solidification of a floating organic drop (DLLME-SFO) was applied by Marube and coworkers [30] for isolation of pesticides from drinking water samples. Drinking water is not a very complex matrix with a well-established chemical composition, therefore, extensive MEs were not expected. Low MEs were obtained for pesticides difenoconazole and azoxystrobin, these pesticides showed strong MF in both nutraceutical potions (Alpa and Alpa Lesana) in our study. DLLME for complex samples such as baby food, herbs, vegetables, and fruits was applied mostly after QuEChERS extraction as an enrichment step. The determination of bifenthrin was reported to be minimally influenced by the matrix from palm dates, with an MF of 11% [31], the same situation was observed in the case of Alpa with an MF of 13% for bifenthrin in our study. On the other hand, the pesticides showed moderate or strong matrix effects despite QuEChERS-DLLME extraction [32-35].

In some cases, an extreme cleanup during the QuEChERS extraction could help to reduce the ME. The use of the mixture of sorbents (PSA, Envi Carb, C18) minimalized the ME in some kinds of herbs [13,36], vine leaves [37], and tea samples [38]. On the other hand, the mixture of PSA, C18, and GBC was not enough for the elimination of ME in brown rice and peanut samples [21]. As it can be seen in Table-S1, the MFs for this type of sample were higher than 90%. Two pesticides, triflumizole, and diphenylamine showed minimal ME in all kinds of studied samples. In comparison with our study, minimal ME was observed for triflumizole in Alpa, but strong in the case of Alpa Lesana. Diphenylamine shows strong ME in Alpa and Alpa Lesana. For the rest of the pesticides, the ME varied from minimal to strong, depending on the sample type and the sample preparation procedure.

It can be concluded that the matrix effects do not depend only on the chemical structure of the compounds, but mostly on the type of the sample and the sample preparation method used for the extraction of the analytes.

CONCLUSIONS

This work was devoted to the study of the matrix effect in two herbal potions, namely Alpa and Alpa Lesana. Herbal potions represent very complex matrices for pesticide residue analysis differing in the amount of herbal matrix content. The DLLME for sample preparation followed by fast GC-MS was established as an analytical methodology for pesticide residue analysis in this type of

liquid samples. The complexity of Alpa Lesana was proven by the matrix factor, which shows strong matrix effects for most of the pesticides. Alpa Lesana contains a higher variety of herbs extract and higher alcohol content than Alpa, which indicated higher matrix effects in comparison to results in Alpa. In the case of Alpa, 27 pesticides out of the studied total of 38 pesticides, indicated minimal or moderate matrix effects. The DLLME-GC-MS method was validated for both samples, providing satisfactory linearity, recovery, and precision. Recoveries for both sample types were in the range of 70-120% with precision below 20%. Linearity in the concentration range from 0.01 to 250 μg l^{-1} was evaluated by correlation coefficients for Alpa higher than 0.98; for Alpa Lesana in the range from 0.83 to 0.99. The limits of detection were in the range 0.001-0.780 μg l^{-1} for Alpa; and 0.014-0.812 μg l^{-1} for Alpa Lesana. It can be concluded that the use of matrix match standards is strongly recommended in the case of complex matrices, such as nutraceutical potions.

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Conflict of Interests

The authors declare no conflict of interest.

Ethical Approval and Informed Consent

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent: Not applicable.

REFERENCES

[1] T. Tuzimski, J. AOAC Int. 98 (2015) 1141.

[2] H. Heba-Alla, A.G. Medhat, E.Z. Hala, A.I. Mohamed, Food Chem. 156 (2014) 94.

[3] A. Páleníková, S. Hrouzková, Sep. Purif. Rev. 45 (2016) 288.

[4] S. Mahabir, Pharma. Nutrition 2 (2014) 120.

[5] S. Barnes, J. Prasain, Curr. Opin. Plant. Biol. 8 (2018) 324.

[6] R.D. Alves, R. Romero-González, R. López-Ruiz, M. L. Jiménez-Medina, A. Garrido Frenich, Anal. Bioanal. Chem. 408 (2016) 8089.

[7] M.H. Petrarca, A. Ccanccapa-Cartagena, A. Masiá, H. T. Godoy, Y. Picó, J. Chromatogr. A 1497 (2017) 28.

[8] M. Rezaee, Y. Yamini, M. Faraji, J. Chromatogr. A 1217 (2010) 2342.

[9] A. Szarka, D. Turková, S. Hrouzková, J. Chromatogr. A 1570 (2018) 126.

[10] S. Hrouzková, M. Brišová, A. Szarka, J. Chromatogr. A 1506 (2017) 18.

[11] K. Akutsu, M. Yoshimitsu, Y. Kitagawa, S. Takatori, N. Fukui, M. Osakada, S. Yamaguchi, K. Kajimura, H. Obana, T. Watanabe, J. Sep. Sci. 40 (2017) 1293.

[12] D.R. Erney, C.F. Poole, J. High Resolut. Chromatogr. 16 (1993) 501.

[13] E. Rutkowska, B. Łozowicka, P. Kaczyński, Food Anal. Methods 11 (2018) 709.

[14] F.J.L. Ortega, J. Robles-Molina, S. Brandt, A. Schütz, B. Gilbert-López, A. Molina-Díaz, J.F. García-Reyes, J. Franzke, Anal. Chim. Acta 1020 (2018) 76.

[15] A. Páleníková, G. Martínez-Domínguez, F.J. Arrebola, R. Romero-González, S. Hrouzková, A.G. Frenich, Food Anal. Methods 8 (2015) 2194.

[16] A. Páleníková, G. Martínez-Domínguez, F.J. Arrebola, R. Romero-González, S. Hrouzková, A.G. Frenich, Food Chem. 173 (2015) 796.

[17] G. Giacinti, Ch. Raynaud, S. Capblancq, V. Simon, J. Chromatogr. A 1483 (2017) 8.

[18] P. Kaczyński, Food Chem. 230 (2017) 524.

[19] M. Kirchner, R. Húšková, E. Matisová, J. Mocák, J. Chromatogr. A 1186 (2008) 271.

[20] E. Rutkowska, B. Łozowicka, P. Kaczyński, Food Chem. 279 (2019) 20.

[21] T. Tsuchiyama, M. Katsuhara, M. Nakajima, J. Chromatogr. A 1524 (2017) 233.

[22] M.Y. Azis, L. Asia, A. Piram, B. Buchari, P. Doumenq, H. Setiyanto, J. Physics 1013 (2018) 1.

[23] S. Chawla, H.K. Patel, H.N. Gor, K.M. Vaghela, P.P. Solanki, P.G. Shah, J. AOAC Int. 100 (2017) 616.

[24] Y.A. Kim, A.M.A. El-Aty, M. Rahman, J.H. Jeong, S.

Ho-Chul, J. Wang, S. Shin, J. H. Shim, J. Chromatogr. A 1076 (2018) 130.

[25] B. Łozowicka, E. Rutkowska, M. Jankowska, Environ. Sci. Pollut. Res. 24 (2017) 7124.

[26] N.S. Pano-Farias, S.G. Ceballos-Magana, R. Muniz-Valencia, J. Gonzalez, J. Food Drug Anal. 25 (2017) 501.

[27] M. Andraščíková, S. Hrouzková, Food Anal. Methods 9 (2016) 2182.

[28] M. Andraščíková, S. Hrouzková, S.C. Cunha, Food Addit. Contam. 30 (2013) 286.

[29] H. Kwon, M. Anastassiades, D. Dörk, S.M. Hong, B. Moon, Anal. Bioanal. Chem. 410 (2018) 5481.

[30] L.C. Marube, S.S. Caldas, K.L. Soares, E.G. Primel, Microchim. Acta 182 (2015) 1765.

[31] S.B.A. Ghani, S.S. Alhewairini, S. Hrouzková, Food Anal. Methods 11 (2018) 354.

[32] J. Li, M. Sun, Q. Chang, X. Hu, J. Kang, C. Fan, Chromatographia 80 (2017) 1447.

[33] M.H. Petrarca, J.O. Fernandes, H.T. Godoy, S.C. Cunha, Food Chem. 212 (2016) 528.

[34] Y. Qin, L. Chen, X. Yang, Y. Tang, S. Li, C. Liu, Chromatographia 79 (2016) 875.

[35] Y. Wang, X. Miao, H. Wei, D. Liu, G. Xia, X. Yang, Food Anal. Methods 9 (2016) 2133.

[36] X. Duan, L. Tong, D. Li, Z. Yu, Y. Zhao, Chromatographia 81 (2018) 545.

[37] B. Maestroni, A.A. Alnaser, I. Ghanem, M.D. Islam, V. Cesio, H. Heinzen, S. Kelly, A. Cannavan, J. Agric. Food Chem. 66 (2018) 6421.

[38] A. Beneta, M.D. Pavlović, I. Periša, M. Petrović, Int. J. Environ. Anal. Chem. 98 (2018) 987.

[39] Y. Shin, J. Lee, J.H. Kim, J. Appl. Biol. Chem. 61 (2018) 543.

[40] S.M. Taha, S.A. Gadalla, Talanta 174 (2017) 767.

[41] E. Lehmann, C. Oltramare, L.F. de Alencastro, Anal. Chim. Acta 999 (2018) 87.

[42] A. Lozano, S. Uclés, A. Uclés, C. Ferrer, A.R. Fernández-Alba, J. AOAC Int. 101 (2018) 374.

[43] T.R.O. Stremel, C.E. Domingues, R. Zittel, C.P. Silva, P.L. Weinert, F.C. Monteiro, S.X. Campos, J. Environ. Sci. Health B 53 (2017) 246.