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Short communication

Matrix solid-phase dispersion based on magnetic ionic liquids: An alternative sample preparation approach for the extraction of pesticides from vegetables

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ABSTRACT

In this study, we propose, for the first time, the direct use of a magnetic ionic liquid (MIL) in a matrix solid-phase extraction procedure. Because of the magnetic properties, the MIL can be harvested directly after the extraction step, using a magnet, while its hydrophobic nature makes feasible the extraction of analytes. Raw vegetables of high water content can be analyzed without any pretreatment. The viscous nature of the selected MIL assists in blending with the matrix, while its hydrophobicity facilitates easier separation and retrieval. Additionally, no solid dispersing materials or co-sorbents are needed. A simple, low-cost analytical method for the determination of multi-class pesticides residues in raw vegetables was developed, with satisfactory recoveries.

1. Introduction

Sample preparation is one of the most challenging steps in an analytical procedure [1]. In order to overcome time-consuming and laborious steps, research groups continue to put a lot of effort into developing new procedures, based on different extraction principles. For liquid samples, many techniques can be employed [2]. However, solid matrices often present a number of complexities. As it is difficult to directly extract the target analytes from a solid matrix, often a preliminary step precedes the main extraction, in which the analytes are transferred from the solid phase to a liquid one [3]. This is often considered to be a limiting step in many sample preparation procedures. To overcome these difficulties, matrix solid-phase dispersion (MSPD) has been extensively developed [3,4].

MSPD is particularly useful as it can be used for solid, semi-solid or highly viscous samples and transfers the analytes to a liquid phase. [5]. Many different sorbent materials have been proposed, for MSPD procedures, including carbon-based, molecularly-imprinted polymers, nanoparticles etc. [3]. Recently, our research group has proposed an alternative to the classical MSPD, using magnetic octadecyl-based nanoparticles for the multiresidue determination of pesticides in carrots [6].

Ionic liquids (ILs) have been used as extraction solvents or sorbents in many microextraction procedures [7]. Recently, Wang et al. proposed two new MSPD procedures that employ ILs [8,9]. In their original work, they added 1-hexyl-3-methylimidazolium tetrafluoroborate ([C_6MIM][BF_4]) along with diatomite in a mortar containing condiment samples. After blending and extraction, they added the ion-pairing agent NH_4PF_6 to form the [C_6MIM][PF_6] IL, which was easily separated from the eluent [8]. In subsequent work, they immobilized another IL on the surface of silica gel and used it as the extractant phase [9]. Aside from ILs, magnetic ionic liquids (MILs) have been developed and used for sample preparation [10,11]. Wang et al. used MILs in a dispersive liquid-liquid microextraction step of the eluent, after MSPD of oilseeds, for the detection of triazines [12]. Although this was the first attempt to combine MILs with MSPD, the MIL was not actually used in the MSPD step.

In this study, we propose, for the first time, the integration of MILs into a MSPD procedure. The magnetic properties of the MIL make it possible to magnetically harvest with an ordinary magnet, negating the need for the blended mixture to be transferred and packed into a cartridge. The viscous nature of the selected MIL assists in blending with the matrix, while its hydrophobicity facilitates easier separation and retrieval. As a proof-of-concept, an analytical method for the determination of multi-class pesticide

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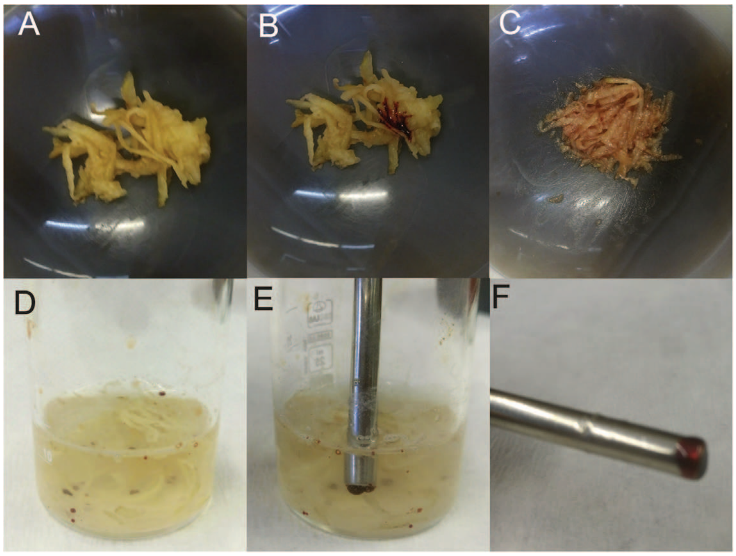

Fig. 1. Representative pictures of the various steps of the developed procedure: (A) chopped potato in a mortar, (B) chopped potato with a drop of MIL before mixing, (C) chopped potato and MIL after mixing, (D) Mixture of potato and MIL in saturated sodium chloride solution after ultrasonication, (E) harvesting the MIL droplets with a magnetic rod and (F) the collected MIL droplets on the magnetic rod.

residues in vegetables is developed, which constitutes a high level of simplicity, low cost and satisfactory recoveries.

2. Materials and methods

2.1. Chemicals and reagents

Trihexyl(tetradecyl) phosphonium chloride (97.7%) was purchased from Strem Chemicals (Newburyport, MA, USA). Cobalt(ll) chloride hexahydrate (98.0%), (99.9%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,1,1,5,5,5-hexafluoroacetylacetone (99%) was purchased from Acros Organics (Morris Plains, NJ, USA). All other chemicals and pesticide standards (purities >99%) were purchased from Aldrich (Sigma–Aldrich–Hellas, Greece).

2.2. Instrumentation

A Shimadzu HPLC system coupled to a diode array detector (DAD) was used for separation and detection of pesticides. The system consisted of a LC20AD pump, a CTO 10AS column oven, a SPD-M20 A DAD and a Hypersil ODS column (250 × 4.6 mm, 5 μm) kept at 30 °C. Injection volume was 20 μL. The mobile phase consisted of water (A) and ACN (B), containing 0.1% (v/v) formic acid. Pesticides were separated using the following gradient elution program: from 35% to 70% B (the MIL is completely soluble in this composition range of mobile phase) in 30 min. The flow rate of the mobile phase was set at 0.8 mL min^{-1}. The detector was set at a wavelength range of 220–360 nm. Peak identification was based on the comparison of retention times and UV spectra with those of the authentic compounds.

2.3. MIL synthesis

All MILs investigated in this study were synthesized and characterized using previously reported procedures [13] and they were the following: $[P_{66614}{}^{+}][Gd(III)(hfacac)_4{}^{-}]$, $[P_{66614}{}^{+}][Dy(III)(hfacac)_4{}^{-}]$, $[P_{66614}{}^{+}][Co(II)(hfacac)_3{}^{-}]$, $[P_{66614}{}^{+}][Mn(II)(hfacac)_3{}^{-}]$ and $[P_{66614}{}^{+}][Ni(II)(hfacac)_3{}^{-}]$, where $P_{66614}{}^{+}$ and hfacac denote the (trihexyl(tetradecyl)phosphonium and hexafluoroacetylacetonate cation and anion, respectively.

2.4. Sample preparation

Raw potatoes and other vegetables and fruits were purchased from a local market at Ioannina, Greece. Samples were prepared according to European Council Directive 2002/63/EC [14]. Potatoes were washed with distilled water and wiped dry using a paper towel. A representative portion (500 g) was processed using an electric food chopper mixer, keeping the peel intact. The chopped sample was analyzed directly. Spiked potato samples were prepared by adding an appropriate volume of pesticide stock solution to the homogenized sample and were analyzed directly.

2.5. Microextraction procedure

For the microextraction procedure, 1.0 g of chopped vegetable sample was weighed out and transferred into a mortar. One drop of $[P_{66614}{}^{+}][Co(II)(hfacac)_3{}^{-}]$ MIL (15 ± 1 mg) was added to the sample and dispersed using a pestle for 1 min, to form tiny droplets. Then, 10 mL of a saturated sodium chloride solution was added to the mixture, homogenized for 30 s and transferred into a glass beaker. This step was repeated once more to quantitatively receive all of the sample. The beaker containing the mixture was ultrasonicated for 30 s in an ultrasonic bath and the MIL droplets were harvested using a neodymium rod magnet. The magnet was soaked first in distilled water (as a washing step) and then in an Eppendorf tube containing 1 mL of acetonitrile in order to dissolve the harvested MIL drops. The solution was evaporated up to 100 μL using a gentle nitrogen stream and injected into the HPLC-DAD system.

3. Results and discussion

3.1. Selection of the MIL

As with any sample preparation procedure, the selection of the proper extraction phase is crucial for the success of the procedure. In our case, we examined the potential of some rare earth-based MILs and some transition metal-based MILs to be used directly as extractants in a MSPD procedure. The selected MILs differ in the metal and the number of anionic ligands. Their potential for extraction was tested using spiked potato samples with a mixture of ten organophosphate, organochloride and triazine pesticides (the

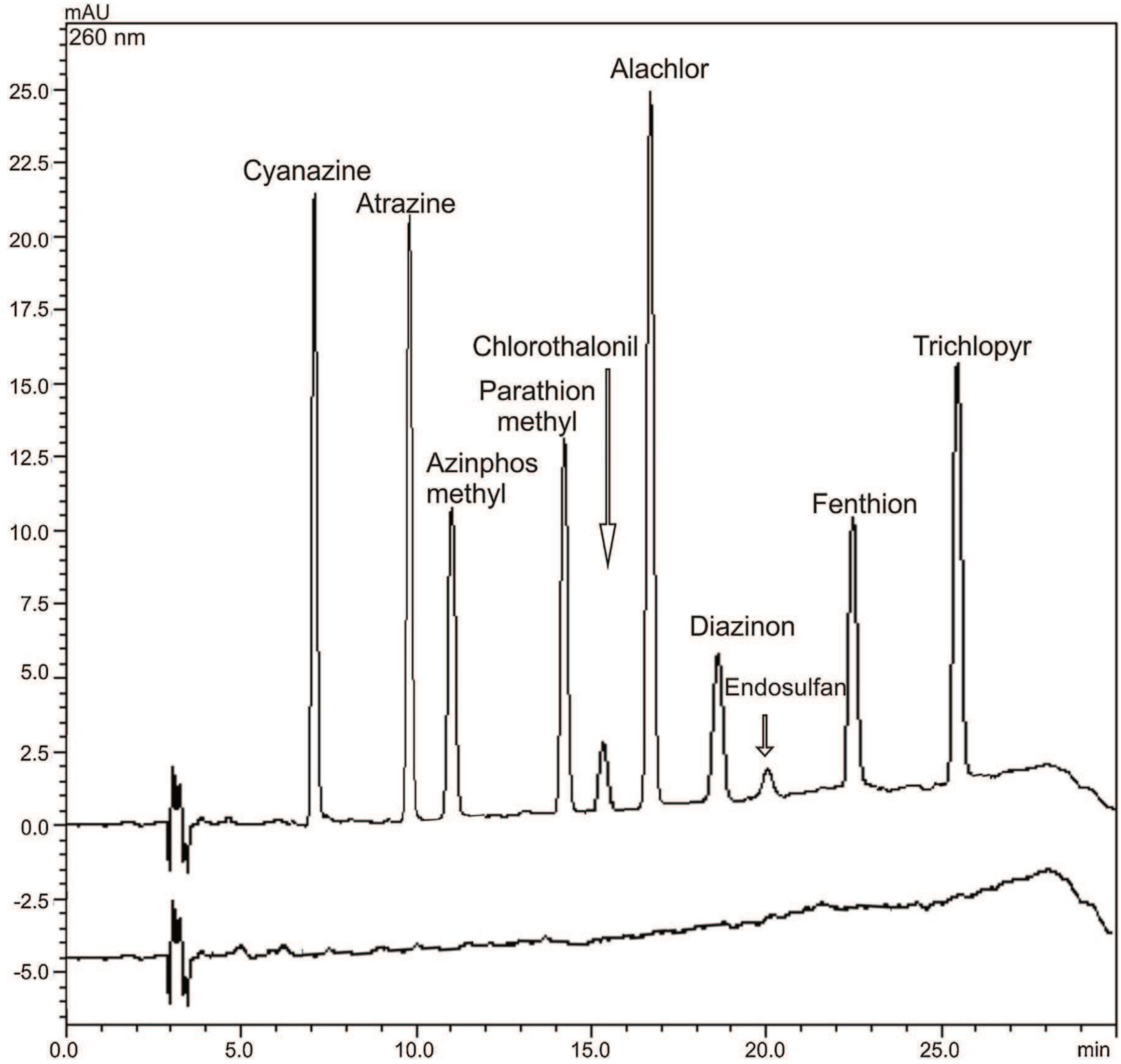

Fig. 2. Chromatogram of a spiked with 0.2 mg kg^{-1} (upper) and a blank (lower) potato sample with the developed procedure.

concentration of each was 0.05 mg kg^{-1}). The pesticides examined herein were selected to be representative from each pesticide class. Results showed that all five tested MILs had similar extraction behavior toward the examined pesticides. It can be deduced that neither the metal nor the anion (hexafluoroacetylacetonate) ligand plays a major role in the extraction of pesticides. Thus, the extraction is dependent, mainly, on the cation. Due to the hydrophobic nature of both the cation, as a major component of MILs and the examined pesticides ($\log K_{ow}$ of examined pesticides ranges between 2.22 and 4.62), hydrophobic interactions reasonably favor the extraction.

As the magnetic retrieval of the MIL droplets is based, mainly, on their visual discrimination from the rest of the system, their collection from a mixture containing the matrix depends on the potential color of the processed sample. At first, Gd- and Dy-based MILs were examined. However, their retrieval from the examined matrices was rather difficult as they are light yellow to colorless. This was not the case with Co- and Mn-based MILs, which were more easily retrieved using a magnetic rod, in contrast to Ni-based analogue, which was poorly harvested. This is ratified by the low effective magnetic moment of the latter as compared to the two former MILs [13]. Eventually, between the dark red $[P_{66614}{}^{+}][Co(II)(hfacac)_3{}^{-}]$ and pale yellow $[P_{66614}{}^{+}][Mn(II)(hfacac)_3{}^{-}]$ the first was selected for further studies, as its color made visual discrimination and harvesting from the solution easier. However, both MILs can be used interchangeably, without loss in the efficiency of the MSPD procedure.

3.2. Effect of vegetable matrix

Before establishing the analytical method, we examined vegetables and fruits that can serve as matrices for the proposed MIL-based MSPD procedure. In this context, we conducted experiments using cabbage, potatoes, zucchini and cucumber. All of them were tested as raw matrices, either as such or after overnight drying to remove most of their water content. At low water content, the recoveries of pesticides were low (i.e. almost up to three times lower than those of the high-water content matrices) for all studied matrices. This was due to the fact that the MIL, instead of forming tiny droplets, was embedded deeply in the matrix, so that its detachment was then impossible. A highly homogeneous mixture was formed and the ensuing ultrasonication was proved to be helpless for MIL separation. To strengthen the findings of low recoveries under such conditions, the procedure was applied to raisins and apples, which are matrices of inherently low to moderate water content (~5% and 60%, respectively). The reduced recoveries

Table 1
Analytical figures of merit of the developed microextraction procedure.

Analyte	QW[*] (nm)	linear equation[**]	Coefficient of determination (R^2)	LOQ[***] (mg kg^{-1})	Matrix effect (%)	RSD (%)[****]	
						within-day (n = 5)	between-day (n = 3 × 5)
Cyanazine	260	y = 893633x+3190	0.9996	0.003	−11	5.8	5.9
Trichlopyr	286	y = 407976x+578	0.9988	0.006	8	5.5	7.0
Atrazine	260	y = 320152x+2205	0.9987	0.006	7	6.0	6.5
Azinphos methyl	276	y = 62925x+3293	0.9989	0.006	−2	6.9	7.9
Parathion methyl	274	y = 644394x+2677	0.9980	0.006	−7	7.1	8.0
Chlorothalonil	232	y = 263294x+2586	0.9988	0.009	−13	5.3	5.5
Alachlor	266	y = 391373x+753	0.9980	0.007	4	6.2	6.3
Endosulfan	220	y = 1724423x+17908	0.9980	0.002	14	5.2	6.4
Diazinon	245	y = 442646x+2995	0.9995	0.007	−9	6.4	8.0
Fenthion	254	y = 162787x+4783	0.9988	0.003	−12	5.4	6.8

[*] QW: quantification wavelength.
[**] Matrix matched calibration curves of spiked potatoes were prepared in the range of LOQ-1.25 mg Kg^{-1} for all pesticides.
[***] LOQ: limit of quantification. Calculated by decreasing the analyte concentration up to a signal-to-noise ratio = 10.
[****] RSD: relative standard deviation. Calculated using samples spiked with 0.01 mg kg^{-1} of each analyte.

Table 2
Relative recoveries of the examined pesticides from spiked potato, zucchini and cucumber.

Analyte	Relative Recovery (%) from potato		Relative Recovery (%) from zucchini		Relative Recovery (%) from cucumber	
	0.01 mg kg^{-1}	0.10 mg kg^{-1}	0.01 mg kg^{-1}	0.10 mg kg^{-1}	0.01 mg kg^{-1}	0.10 mg kg^{-1}
Cyanazine	96	98	92	96	93	97
Trichlopyr	97	101	95	98	93	99
Atrazine	95	101	101	105	94	96
Azinphos methyl	95	99	98	102	96	105
Parathion methyl	94	98	97	98	102	104
Chlorothalonil	95	97	96	99	99	105
Alachlor	98	101	98	99	94	99
Endosulfan	99	101	101	104	94	96
Diazinon	94	97	101	105	99	105
Fenthion	96	98	101	103	93	100

were found to be even more pronounced at the low-water content matrices substantiating our previous observations. Therefore, the proposed procedure is applicable to raw matrices of high water content (almost >60%). This can, eventually, be advantageous to the analysis, as time-consuming steps for removing water are not needed.

With regard to cabbage, its recoveries were less satisfactory (lower by almost 20%) than those of the rest tested vegetables. Its tissue was not disrupted completely during chopping and in the course of mixing with MIL it was not effectively blended, thus hindering the extraction. On the contrary, the “soft” texture of the other vegetables allowed complete disruption of the plant tissue giving rise to a good dispersion of MIL in the homogeneous sample. Taking into consideration the above discussion, water content above 60% in the matrix ensures a smooth and unimpeded application of the proposed procedure. Matrices such as potatoes, zucchini and cucumber possess water content ranging between 65% and 85% and are suitable for the proposed procedure [15,16].

3.3. Effect of dispersion material

Although the MIL was easily dispersed in the selected matrices and the addition of solid dispersing materials was not necessary, we examined the potential of adding a solid dispersing material or a soluble inorganic salt in an effort to improve the recoveries or simplify the procedure. Along these lines, we examined silica (35–70 mesh), quartz silica (50–70 mesh particle size), sodium chloride and sodium sulfate as dispersion materials in the developed method. All tested materials were added to potato samples and, after blending them for 1 min, the MIL was added. It was found that the addition of both salts resulted in poor dispersion of MIL compared to the procedure in the absence of salt. This is due to the tendency of both salts to absorb water, which was found to be important for the efficient dispersibility of the MIL. However, it was noticed that MIL droplets were more readily separated after ultrasonication, when a salt was added. Taking advantage of this effect, a saturated solution of sodium chloride was used to transfer the blend from the mortar to the glass beaker, prior to ultrasonication, instead of adding water. In this way, the formed droplets coalesced, forming fairly larger drops, which were easily separated from the bulk phase and harvested. Most importantly, the reproducibility of the procedure was improved (without the salt solution, average relative standard deviation (RSD) of five measurements was 9.5% and with the salt solution the average RSD was 6.0%), while pesticide extraction recoveries were not influenced.

When silica was used, the formation of MIL droplets during the blending step was hindered, resulting in reduced number of droplets. Moreover, it was noticed that silica particles attached to the viscous MIL droplets interfered with the sorption process. When quartz silica was employed, although the MIL was dispersed after blending, again, a large number of droplets were attached to the quartz silica particles, which were not separated in a reasonable amount of time, after ultrasonication. Therefore, they could not be retrieved quantitatively. Based on the above, no solid dispersing material was used during the extraction/blending step for further experiments. Representative pictures of the different steps of the developed procedure are given in Fig. 1.

3.4. Effect of MIL-to-matrix ratio

To maximize the adsorption of a sample preparation procedure, the optimum ratio of MIL-to-matrix ratio should be found. Due to its high viscosity, the weighing of MIL is rather challenging. Consequently, the MIL was added directly to matrices already placed in the mortar, using a pipette tip to produce a drop of 15 ± 1 mg. Our experiments showed that when more than one drop was instilled, with the amount of matrix held constant, significantly larger droplets were formed and the extraction yield was almost unaltered. As there was not any significant improvement using more than one drop, we examined the quantity of sample keeping the amount of MIL fixed at one drop per tested sample. Quantities of 0.5, 1.0 and 1.5 g of chopped potato were then used, and spiked with the same quantity of analytes. The results revealed that for 0.5 and 1.0 g of sample, the mean extraction was nearly the same (~95%), while a pronounced decrease in the extraction (~15% decrease) was observed for 1.5 g of sample. Next, the same sample quantities (i.e., 0.5, 1.0 and 1.5 g) were used previously spiked with the same concentration of analytes. The analytes from samples of 0.5 and 1.0 g were almost quantitatively extracted. When 1.5 g of sample was used, extraction percentages of the ten pesticides ranged between 55% and 70%. Therefore, a sample amount of 1.0 g was selected for further experiments.

3.5. Analytical characteristics of the method

Based on the aforementioned optimum parameters, an analytical method was developed for the determination of pesticides. Fig. 2, shows a chromatogram of a spiked and a blank potato sample. It can be seen that no interfering peaks overlap those of the examined compounds at the retention times, highlighting that the proposed procedure can serve both as an extraction and a clean-up step. Analytical characteristics of the method are listed in detail in Table 1. Quantification of the analytes was performed at different wavelengths (shown in Table 1). As it can be seen, coefficients of determination were higher than 0.9980 in all cases, suggesting good linearity. The limits of quantification were between 0.002 and 0.009 mg kg^{-1}. These values are lower than the general default maximum residue limits (MRLs) established for pesticides (0.01 mg kg^{-1}), according to EU 62/2018 European Commission legislation [17]. Intraday RSD and interday RSD values (calculated using samples spiked with 0.01 mg kg^{-1} of each analyte) were between 5.2–7.1% and 5.5–8.0%, respectively. The relative recoveries of the examined compounds (no residues were detected in the original matrix) calculated from potato samples spiked at the MRL (i.e. 0.01 mg kg^{-1}) and ten times higher were in the range of 94–101% and 97–101%, for the high and low tested concentration, respectively (Table 2). Finally, matrix effects were evaluated, using samples spiked with 2 × LOQ concentration of each analyte, according to our previous study, [18]. From Table 1, it can be seen that these values range between −13% and 14% for all pesticides studied. As the matrix effect values were found to be lower than 20%, the respective effect is considered insignificant [19]. Therefore, the calibration curves developed herein can be used for the quantification of the selected pesticides in other vegetables. To further elucidate the applicability of the method, relative recoveries were also calculated from spiked zucchini and cucumber samples, which were in the range of 92–101% and 93–105%, respectively. The above results advocate for a procedure which adheres to SANTE/11814/2017 guidelines [20].

4. Conclusions

In this study, the direct use of MIL under the principles of MSPD is presented for the first time and a sample preparation procedure was developed for the extraction of ten pesticides from vegetables. Raw vegetables of high water content were analyzed without any other pretreatment prior to or after extraction, which is completed in a single step, without the need for solid dispersing materials or co-sorbent for clean-up. The proposed MSPD procedure benefits from the simplicity, low cost and satisfactory recoveries while it is amenable to improvement for more challenging applications.

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