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MOLECULARLY IMPRINTED SOLID PHASE EXTRACTION FOR TRACE ANALYSIS OF DIAZINON IN DRINKING WATER

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ABSTRACT

Amongst organophosphate pesticides, the one most widely used and common environmental contaminant is diazinon; thus methods for its trace analysis in environmental samples must be developed. Use of diazinon imprinted polymers such as sorbents in solid phase extraction, is a prominent and novel application area of molecular imprinted polymers. For diazinon extraction, high performance liquid chromatography analysis was demonstrated in this study. During optimization of the molecular imprinted solid phase extraction procedure for efficient solid phase extraction of diazinon, Plackett-Burman design was conducted. Eight experimental factors with critical influence on molecular imprinted solid phase extraction performance were selected, and 12 different experimental runs based on Plackett-Burman design were carried out. The applicability of diazinon imprinted polymers as the sorbent in solid phase extraction, presented obtained good recoveries of diazinon from LC-grade water. An increase in pH caused an increase in the recovery on molecular imprinted solid phase extraction. From these results, the optimal molecular imprinted solid phase extraction procedure was as follows: solid phase extraction packing with 100 mg diazinon imprinted polymers; conditioning with 5 mL of methanol and 6 mL of LC-grade water; sample loading containing diazinon (pH=10); washing with 1 mL of LC-grade water, 1 mL LC-grade water containing 30% acetonitrile and 0.5 mL of acetonitrile, respectively; eluting with 1 mL of methanol containing 2% acetic acid. The percentage recoveries obtained by the optimized molecular imprinted solid phase extraction were more than 90% with drinking water spiked at different trace levels of diazinon. Generally speaking, the molecular imprinted solid phase extraction procedure and subsequent high performance liquid chromatography analysis can be a relatively fast and proper approach for qualitative and quantitative analysis of diazinon in drinking water.

Key words: Non-covalent imprinting approach, Molecularly imprinted solid phase extraction, Experimental design, Plackett-Burman design, Diazinon

INTRODUCTION

Presently, pesticides are an important group of environmental pollutants and many of them are easily found in: drinking water, surface water, air, and food hence resulting in human exposure in various ways. Increased global worry and sensitivity about adverse effects of exposure to the environmental pollutants has led to create a tendency for developing new analytical techniques for qualitative and quantitative analysis of the compounds in environmental samples. In spite of the fact that several developed analytical techniques have emerged, sample preparation methods are still an essential step in most analysis, and are now an ongoing concern. There is a general concept that sample preparation is the most important step in analytical methods. After the sampling and sample preservation steps, sample preparation is the third step in an analytical technique (Mitra, 2003).

A common approach for sample preparation, which has become a candidate of choice in many analytical fields for handling and analysis

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of complex samples (Stevenson, 1999), is solid phase extraction (SPE). SPE has been designed for concentrating and clean-up of samples, as well as for the removal of toxic or valuable substances from a variety of predominantly aqueous solutions (Huck and Bonn, 2000). SPE compared to liquid-liquid extraction (with relatively clean extractions) has many benefits such as being is: cheap, quite fast, less hazardous and expensive solvents requirments, good recovery potential, and being automatic can be automated (Blomgren *et al*., 2002). Our previous works reported successful use of SPE for sample preparation of some compounds (Shahtaheri *et al*., 2007; 2008).

Most SPEs are based on adsorption analytes on a solid phase. The solid phase is then washed from interference compounds and the analytes of interest are desorbed by elution using a liquid. Sorbents for SPE are divided into three groups including: inorganic oxides, low-specificity sorbents and compound-specific and class-specific sorbents (Poole, 2003). In the last decades, due to insufficient selectivity of inorganic oxides and low specific sorbents, a growing trend has been focused on the last sorbents. Immunosorbents and molecularly imprinted polymers (MIP) are the commonly used compound-specific and class-specific sorbents. Although immunosorbents show high selectivity to target molecules, they are less stable, difficult to prepare, and expensive, so their usages are reduced (He *et al*., 2007). Recently, because of stability, low cost and ease of preparation, MIPs have become an interesting research field to the preparation of specific sorbents for SPE of compounds in environmental and occupational samples (Haup, 2003). It should be mentioned that usage of MIPs in SPE is a hopeful and novel method development in analytical chemistry.

MIPs are artificial polymers with recognition binding sites able to bind a molecule or its structural analogues from a complex sample (Mayes and Whitcombe, 2005;Tamayo *et al*., 2007). The polymers are formed by polymerization of functional monomers and a cross-linker in a complementary shape around a template molecule. Template extraction will allow recognition binding sites to remain in the polymer matrix. The application of MIPs as sorbents in solid-phase extraction, namely molecularly imprinted solid-phase extraction (MISPE), have been successfully reported for many compounds. Some of them are; atrazine (Koohpaei *et al*., 2008), nitrophenol (Masque *et al*., 2000), sulfonylureas (Zhu et al., 2002), benzo(a)pyrene (Lai *et al*., 2004) and carbamazepine (Beltran *et al*., 2007), etc.

It has become increasingly apparent that the performance of MISPE and its recovery are dependent on many experimental variables. For this reason, optimization of the MISPE procedure is an essential step in the analysis of an analyte. However, it can be tedious, time consuming and cost consuming if a proper method is not selected. Statistical experimental design is an important tool for improving the performance of a process (Montgomery, 2000); therefore it has been used to develop an optimized process for MISPE (Koohpaei *et al*., 2008).

Statistical experimental design is a beneficial tool for choosing experiments efficiently and systematically to give reliable and utilized information (Gotti *et al*., 2000). It gives an optimization method over the classical one-at-a-time experiment (Yan and Ramström, 2005). In those experiments, optimization processes are tedious, time consuming and can produce erroneous optimums in techniques where there are interactions between factors (Wu and Hamada, 2000).

The aim of this work was to synthesize diazinon imprinted polymers for use as a sorbent in the SPE cartridge. Then, the MISPE procedure for extraction of the trace amount of diazinon in drinking water was developed and itssubsequent its HPLC analysis was optimized. During the MISPE optimization, a simple statistical experimental design, called Plackett-Burman design, was applied to evaluate the influence of experimental factors on the MISPE performance.

MATERIALS AND METHODS

Reagents

Analytical grade diazinon was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Acetonitrile, methanol, chloroform, dichloromethane and glacial acetic acid were purchased from Merck and were analytical or HPLC grade. Methacrylic acid (MAA) as the functional monomer and ethylene glycol dimethacrylate (EDMA) as the cross-linker, were purchased from Merck (Hohenbrunn, Germany). MAA was distilled under vacuum to remove the inhibitor prior to polymerization. To clean the inhibitor from EDMA, it was washed with 10% aqueous sodium hydroxide three times, followed by washing with pure water, and then dried over anhydrous sodium sulfate. 2, 2′-Azobis (2-methylpropionitrile) (AIBN) was provided from ACROS (New Jersey, USA). Ultra pure water used for HPLC analysis and MISPE process were obtained from a Direct–Q3 Water Purification System (Millipore Corporation, USA). Diazinon standard solutions were prepared by dilution of the stock solution (100 µg/mL in acetonitrile) with LC- grade water.

Analysis of diazinon

For analyzing diazinon, high performance liquid chromatography (JASCO LC- 2000 series HPLC) was used. The instrument was equipped with CO-2060 column oven, PU–2080 pump, and UV/VIS 2075 detector. The detector was set at 245 nm. The chromatographic column was C-18 (250×4.6 mm i.d.; Supelco, USA). The mobile phase was a mixture of acetonitrile, methanol, and water (60:20:20) containing 5 µL H_3PO_4 at a flow rate of 1.2 mL/ min. The column temperature was fixed at 40 ºC. The injection volume was 10 µL.

Preparation of molecularly imprinted and non-imprinted polymers

Non-covalent imprinting approach, which is widely used in molecularly imprinted technology, was conducted for diazinon imprinted polymer preparation. Briefly, MAA (3.07 mmol) and diazinon (1 mmol) as the template were placed in a glass tube. The tube was agitated on a shaker (100 rpm) at room temperature. After 2 h, EDMA (17.85 mmol), AIBN (16.21 mg), and solvent (chloroform: 7.38 mL) were added to the tube. Following 2 mins of ultrasonication, the pre-polymerization mixture was purged with pure nitrogen for 5 min. The tube was sealed under a N_2 stream. For polymerization, the tube was transferred to a water bath set at 55 ºC for 24 h. The bulk polymers were ground and particles, sizing from 53-106 µm, were excessively washed with methanol, using Soxhlet extractor until no diazinon was detected in the effluent. Then, the particles were dried at 50 ºC over night. After washing with 10 mL methanol and 10 mL acetonitrile, particles were dried at 70 ºC. Non-imprinted polymers (NIP) were also synthesized using the same procedure without the addition of diazinon.

MISPE procedure optimization

Plackett-Burman experimental design (Plackett et al., 1944) was applied during the optimization process for the evaluation of the main experimental factors influencing the MISPE procedure. The selected experimental factors were; sorbent mass, sample volume, analyte concentration, sample pH, washing solvent volume (water), acetonitrile in the washing solvent, elution solvent volume (methanol), acetic acid in the elution solvent. For each factor a high (+) and low (-) level were examined. Total experimental runs to be carried based on the Plackett-Burman experimental design are 12 (Vaidya et al., 2003). Table 1 shows the selected factors and their levels used in the Plackett-Burman design. Table 2 depicts the experimental runs required for an eight-factor Plackett-Burman design. Percentage recovery of diazinon on MISPE in each run was taken as the model response. Minitab statistical software package (release version 14) was used to design and analyze the Plackett-Burman experimental design.

Table 1: The selected factors and their levels used in Plackett-Burman experimental design

Factor name		Low level (-)	High level (+)	Factor name		Low level (-)	High level (+)
A	sorbent mass (mg)	50	100	E	washing solvent volume (mL of water)	1	4
B	sample volume (mL)	3	8	F	Acetonitrile percentage in the washing solvent	5%	30%
C	analyte concentration (µg/mL)	1	5	G	elution solvent volume (mL of methanol)	1	3
D	sample pH	2	10	H	acetic acid percentage in elution solvent	2%	10%

For each run in Table 2, the MISPE procedure was carried out. In run number 1, for example, 50 mg of dry diazinon imprinted polymers were packed into an empty SPE cartridge between two filters. The cartridge was connected to a vacuum extraction unit equipped with a regulator to set flow rate of about 1 mL/min in all SPE steps. In all cases, before their usage, MISPE cartridges were regenerated with 3 mL of methanol; acetic acid (90:10), 10 mL of dichloromethane, and 5 mL of acetonitrile followed by conditioning with 5 mL of methanol and 6 mL of LC-grade water. Then, 3 mL of a 1 µg/mL of diazinon in LC- grade water (pH adjusted to 10 with 20% aqueous NaOH) was passed through the MISPE. After, the cartridge was washed with 1 mL of LC- grade water, 4 mL LC- grade water containing 30% acetonitrile, and 0.5 mL of acetonitrile, respectively. Diazinon was eluted from the MISPE cartridge using 1 mL of methanol containing 10% acetic acid. After the elution, the amount of diazinon recovered from the MISPE was determined with HPLC system using the method described above. For each run in Table 2, as a control, a non-imprinted SPE (NISPE) cartridge was also prepared and a SPE procedure was conducted in the same manner. The optimized MISPE procedure was tested for different concentrations (10, 50, 100, and 200 ng/mL) of diazinon in LC-grade water (60 mL).

Table 2: Experimental runs required for an eight-factor Plackett-Burman design

Run*	A	B	C	D	E	F	G	H	Run	A	B	C	D	E	F	G	H
1	-1	-1	-1	1	1	1	-1	1	7	1	-1	1	1	-1	1	-1	-1
2	-1	1	1	-1	1	-1	-1	-1	8	1	-1	-1	-1	1	1	1	-1
3	-1	-1	-1	-1	-1	-1	-1	-1	9	-1	1	-1	-1	-1	1	1	1
4	1	1	-1	1	-1	-1	-1	1	10	1	1	-1	1	1	-1	1	-1
5	-1	-1	1	1	1	-1	1	1	11	-1	1	1	1	-1	1	1	-1
6	1	1	1	-1	1	1	-1	1	12	1	-1	1	-1	-1	-1	1	1

* Run order was randomized.

MISPE procedure of spiked drinking water

The performance of the optimized MISPE procedure was evaluated for trace analysis of diazinon in drinking water. Drinking water was collected from a tap in the laboratory and its pH was adjusted to 10 with 20% aqueous NaOH (optimized pH obtained in this study). The drinking water was filtered to remove particulate and spiked with diazinon at: 1, 50, 100, and 200 ng/mL. Then, MISPE of 500 mL of 1 ng/mL (sample loading flow rate: 1.5 mL/min) and as well as 20 mL of 50, 100, and 200 ng/mL (sample loading flow rate: 1 mL/min) were carried out.

Method validation

The validity of the optimized MISPE procedure was proven via intra- and inter-day method validation. For this purpose, drinking water was spiked with diazinon to receive a final concentration of: 50, 100, 150, 200, and 250 ng/mL. Intra-day method validation was obtained by MISPE of each spiked sample, six times during a day. Inter-day method validation was also obtained by MISPE of the spiked samples, six times on five different days over a period of one week.

RESULTS

In this study molecularly imprinted polymers were prepared via non-covalent imprinting technology, and were used as the specific sorbent in SPE for trace analysis of diazinon. During optimization of MISPE procedure for efficient SPE of diazinon, Plackett-Burman design was conducted. Eight experimental factors, with critical influence on MISPE performance were selected and 12 different experimental runs based on the Plackett-Burman design carried out. Table 3 illustrates the percentage recovery of the MISPE and NISPE in the 12 experimental runs of thePlackett-Burman design.

The obtained results were analyzed with analyze factorial design menu in Minitab. The estimated effects and coefficients for response are shown in Table 4. The default amount of p-value (0.05) was selected for the model. The R^2 value for the model was 98.74%. From the results, the effect of experimental factors D and H were statistically significant while, the effects of the other experimental factors were insignificant.

Table 3: The percentage recovery of the MISPE and NISPE in the 12 experimental runs of Plackett-Burman design [a]

Run	Recovery on MISPE	Recovery on NISPE	Run	Recovery on MISPE	Recovery NISPE
1	71.53±7.92	25.41±1.58	7	99.82±1.04	18.42±1.34
2	56.24±3.30	12.68±0.85	8	0.00	0.00
3	14.97±0.54	7.16±1.85	9	0.00	0.00
4	72.89±1.47	14.58±2.41	10	100.24±2.50	31.64±4.84
5	99.61±2.58	25.84±2.21	11	100.84±1.80	35.12±1.11
6	0.27±0.01	0.00	12	0.00	0.00

[a] All experiments were carried out in triplicate and the results are expressed as mean ± standard deviation.

Table 4: Estimated effects and coefficients for response[a]

Term	Effect	Coefficient	S. D.[b]	T	P-value	Term	Effect	Coefficient	S. D.	T	P-value
Constant	-	10.7	15.39	0.70	0.53	E	4.4	2.2	1.86	1.17	0.32
A	-0.5	-0.2	0.1118	-2.09	0.12	F	-95.3	-47.7	22.36	-2.13	0.12
B	3.0	1.5	1.1185	1.33	0.27	G	-2.5	-1.3	2.79	-0.45	0.68
C	8.1	4.0	1.3981	2.90	0.06	H	-532.5	-266.3	69.90	-3.81	0.03
D	19.7	9.9	0.6991	14.11	0.00						

[a] The analysis was done using coded units. S=9.68, R^2= 98.74%.

[b] S. D.: Standard deviation

As explained elsewhere in this study, it was decided to take the condition of run 7 as the optimized MISPE procedure. The obtained results from conducting the optimized MISPE procedure on LC- grade water spiked with different amounts of diazinon, are summarized in Table 5.

In order to evaluate the applicability of the optimized MISPE procedure for trace analysis of diazinon existing in real samples, the procedure was applied for analysis of drinking water spiked to the final concentration of: 1 ng/mL (sample volume:500 mL), 50, 100, and 200 ng/mL (sample volume:20 mL). The obtained results from MISPE of drinking water spiked with different amounts of diazinon are summarized in Table 6.

In order to validate the optimized MISPE procedure, intra- and inter- day method validation was carried out. The results are portrayed in Table 7.

Table 5: The percentage recovery of diazinon on MISPE and NISPE of LC-grade water (60 mL) spiked with different amounts of diazinon [a]

Analyte concentration (ng/mL)	10	50	100	200
Recovery on MISPE	92.78±2.86	90.96±1.53	92.85±0.50	91.10±1.38
Recovery on NISPE	N.D.	N.D.	14.06±0.30	15.17±0.36

[a] All experiments were carried out in triplicate and the results are expressed as mean ± standard deviation. N.D.: diazinon was not detected in elution solvent.

Table 6: The percentage recovery of diazinon on MISPE and NISPE of drinking water spiked with diazinon

Analyte concentration (ng/mL)	1	50	100	200
Recovery on MISPE	91.77±2.15	90.16±4.2	90.15±0.49	94.21±2.04
Recovery on NISPE	N.D.	N.D.	20.39±0.96	19.76±0.63

N.D: diazinon was not detected in elution solvent

Table 7: Iinter- and intra-day method validation of diazinon spiked in drinking watersample volume:15 mL (N=6)

	Concentration (ng/mL)									
	50		100		150		200		250	
	Inter	Intra	Inter	Intra	Inter	Intra	Inter	Intra	Inter	Intra
Mean	46.34	46.02	92.85	93.67	141.66	143.03	183.44	184.48	238.62	240.24
S.D.	0.39	0.52	0.51	0.44	0.40	0.57	0.38	0.46	0.32	0.90

Fig. 1 shows the chromatograms obtained before and after MISPE of LC-grade and drinking water (pH= 10) spiked with diazinon. It has been shown that HPLC can not detect diazinon without the MISPE procedure.

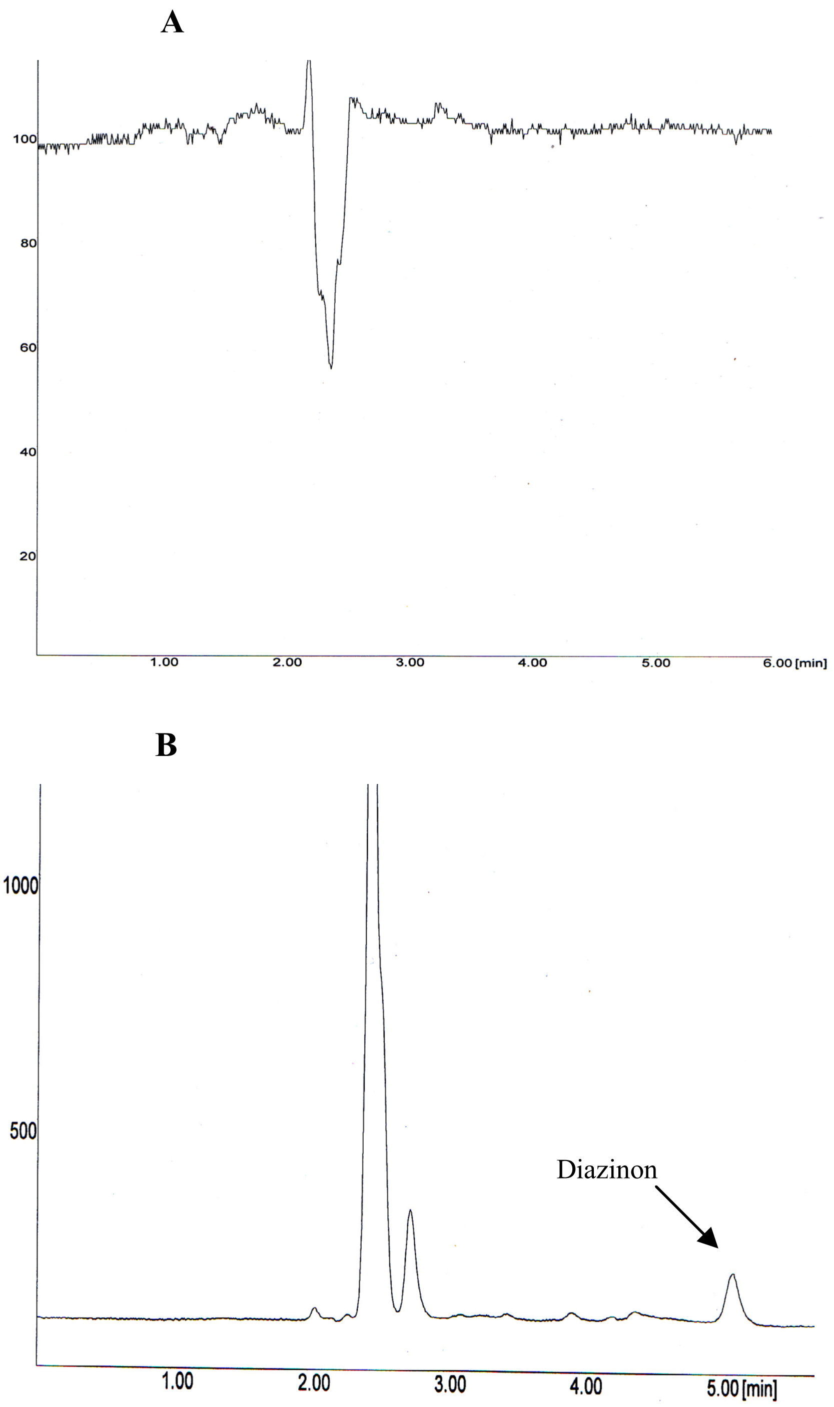
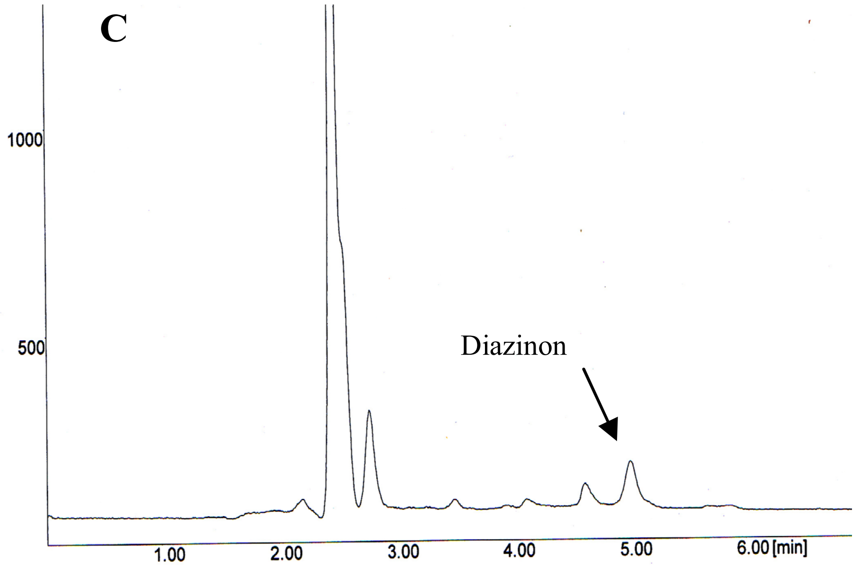


Fig. 1 Chromatograms obtained before and after MISPE. A: before MISPE of LC-grade water spiked with 50 ng/mL of diazinon. B: after MISPE of 10 mL of LC-grade water spiked with 50 ng/mL of diazinon. C: after MISPE of drinking water (500 mL) spiked with 1 ng/mL of diazinon.



DISCUSSION

Diazinon imprinted polymers were synthesized with MAA as the functional monomer, and EDMA as the cross-linker via non-covalent imprinting technology. Due to its preparation being less complex and the broad selection of functional monomers, the non-covalent imprinting technology has proven to be more versatile than the alternative covalent technology (Zhang et al., 2006). In this study, MAA as the carboxylic acid-based functional monomers was used for polymerization.

Until now, carboxylic acid-based monomers, principally methacrylic acid functional monomer has been the most successful and common monomer in non-covalent imprinting. It relies upon the ability to interact in various ways with the template as; H-bond donors, H-bond acceptors and through formal ion-pair formation, as well as weaker dipole–dipole interactions. The methyl group in MAA restricts its rotation and conformational flexibility and also provides additional van der Waals interactions (Mayes and Whitcombe, 2005). Following the polymer preparation, its application as the sorbent in solid phase extraction of trace amounts of diazinon was tested in this work. While MISPE performance depends on different experimental factors such as; sorbent mass, sample pH, the type and amounts of washing solvent, the type and amounts of eluting solvent etc, the experimental factors must be carefully optimized to extract the analyte by MISPE with high selectivity.

Twelve different experimental runs based on Plackett-Burman design were carried out. As shown in Table 3, compared to NISPE, application of diazinon imprinted polymers as sorbent in SPE has generally led to reach a good recovery of diazinon from LC-grade water. This reveals that the MIPs had binding sites for successful template (diazinon) trapping from sample matrix. In addition, this difference in performance demonstrates that MIPs exhibited specific binding for the template, but it could also demonstrate that the template binding in MIPs is due to a combination of both specific and non-specific adsorption. The results demonstrate different MISPE procedures can extensively change the MIP performance in SPE. However, the percentage recovery of MISPE procedure obtained in runs: 1, 4, 5, 7, and 11, were better than the percentage recovery of the others MISPE procedures.

The results in Table 4 explain that the effects of experimental factors D (19.7) and H (-532.5) were statistically significant. The effect of the sample pH, the first most important factor, is very noticeable. An increase in pH causes an increase in the recovery on MISPE. The low recovery of diazinon at pH 2 may be related to the protonation of the MAA functional groups in the binding sites of the polymer matrix. This phenomenon leads to less diazinon binding with the polymer binding sites. Furthermore, the MIP binding sites contain the MAA functional group (an organic acid), and in small amounts of pH, ionization of these sites is low. Hence, the electrostatic force between carboxyl groups and diazinon is weak (Liang et al., 1999). The recovery of the MISPE is also influenced by the addition of acetic acid in the elution solvent. The effects of experimental factors A (-0.5), B (3), C (8.1), E (4.4), F (-95.3), and G (-2.5) were insignificant. R^2 value for the model (98.74%) was high enough to predict the response. It means 98.74% of the variation in response is explained by the model and predictions would be accurate. In other words, eight selected experimental factors could explain 98.74% of the variation in the recovery. It should be mentioned that the R^2 value for a valid model is 60% or greater. Therefore, this model would be reasonably accurate.

Although, the primary aim of Plackett-Burman design is to evaluate and screen the influence of the experimental factors on a specific response (Srinivas *et al*., 1994), a striking phenomenon was found in this study. In addition to determine the influence of the experimental factors on the recovery, the design effectively assisted us to reach the optimized MISPE procedure. Consider runs 5, 7, 10, and 11 in Table 3. These runs have resulted acceptable and remarkable recovery. Although all the mentioned runs are well for obtaining acceptable recovery of diazinon, run 7 has resulted the lowest non-specific binding on NISPE. Therefore, we decided to take run 7 as the optimized MISPE procedure. From these results, the optimal MISPE procedure was as follows: SPE packing with 100 mg diazinon imprinted polymers; MISPE regeneration with 3 mL of methanol; acetic acid (90:10), 10 mL of dichloromethane, and 5 mL of acetonitrile respectively. Conditioning with 5 mL of methanol and 6 mL of LC-grade water; sample loading containing diazinon (pH adjusted to 10 with 20% aqueous NaOH), washing with 1 mL of LC-grade water, 1 mL LC- grade water containing 30% acetonitrile, and 0.5 mL of acetonitrile, respectively; eluting with 1 mL of methanol containing 2% acetic acid.

The results in Table 5 and 6, show the optimized MISPE could successfully extract diazinon from LC-grade water as well as from drinking water. It should be noted these recoveries are indicative of good performance of the MISPE. The percentage recoveries were more than 90% when LC-grade and drinking water was spiked at different levels of diazinon. As the results indicate, an enhancement of the molecular pre-concentration by SPE can be achieved using diazinon imprinted polymers. In this study, diazinon was determined in drinking water spiked with diazinon at 1 ng/mL by passing 500 mL of the sample. The MISPE procedure developed here possessed the requirements necessary for health applications aimed at the water quality assessment. Hence, this MISPE procedure can be used to determine the compliance or non-compliance of water drinking properties with the environmental protection agency (EPA) standard for diazinon in drinking water.

As explained in the method validation test, the MISPE procedure was performed with different concentrations of diazinon prepared in drinking water for determining intra- and inter-day accuracy and precision data. Relative standard deviation for intra- and inter-day method validation was less than 1.12. Relative standard deviations were; 0.84, 0.54, 0.28, 0.20, and 0.13 for 50, 100, 150, 200, and 250 ng/mL, respectively for inter-day, and 1.12, 0.46, 0.39, 0.24, and 0.37 at the same concentration, respectively for intra-day. The results prove that diazinon can be determined with high accuracy and precision. The chromatograms verify that MISPE enables diazinon to be detected at trace levels via isolation, purification and pre-concentration of diazinon in drinking water.

Experimental design - Plackett-Burman, showed excellent ability to evaluate the effect of different experimental factors on recovery, as well as to reach the optimized MISPE procedure by carrying out few experimental runs. The recoveries obtained in this study by the optimized MISPE procedure of spiked drinking water were satisfactory and acceptable. Generally, the MISPE procedure and subsequent HPLC analysis can be a relative fast and proper approach for qualitative and quantitative analysis of diazinon in drinking water.

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