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Selective high performance liquid chromatography imprinted-stationary phases for the screening of phenylurea herbicides in vegetable samples

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Abstract

Molecularly imprinted polymers (MIPs) for the determination of phenylurea herbicides have been synthesized by polymerisation of the appropriated reagents mixture within the pores of preformed spherical silica particles leading to a silica-MIP composite material. Subsequently, the silica matrix was etched away resulting in MIP beads which can be considered the "mirror image" of the original silica mold. The MIP particles were packed in stainless steal HPLC columns (125 mm × 4.6 mm I.D.) and the materials were evaluated as imprinted-stationary phases for phenylurea herbicides. The imprinting effect of the originated specific binding sites for the selective recognition of phenylurea herbicides was clearly demonstrated. An efficient separation of a mixture of phenylurea herbicides in two groups, with or without a methoxy group in the chemical structure, was achieved and well shaped and defined peaks were obtained. Finally, the optimum imprinted column (prepared using linuron as template, 2-(trifluoromethyl)-acrylic acid as monomer, 72 h of polymerisation time and the subsequent dissolution of silica matrix) was used for the LC-UV screening of phenylurea herbicides directly from vegetable sample extracts without any previous clean-up step at low concentration level in less than 10 min.

Keywords: Phenylureas; Molecular imprinting; Stationary phases; HPLC; Vegetable samples; Screening

1. Introduction

The synthesis of artificial materials with specific recognition properties toward certain selected molecules possesses an enormous interest in many fields of modern chemistry. Molecular imprinting technique has emerged during the last years as one of the most interesting strategies to design and synthesize tailor-made materials able to mimic the specific recognition properties of enzymes and antibodies. Since the original works reported by Wulff [1] and Mosbach [2] (responsible of the covalent and non-covalent approach, respectively), an increasing number of papers on the preparation and use of molecularly imprinted polymers (MIPs) in different fields (i.e. catalysis [3], solid-phase extraction [4] and chromatography [5], among others) have been reported [6]. This increasing interest has been due to the relatively simple synthesis, low cost and physical and chemical stability of MIPs in comparison to that achieved for biological specific receptor molecules (i.e. antibodies).

Whereas MIPs perform reasonably well in solid-phase extraction procedures, the use of MIP stationary phases in liquid chromatography still have to sort out some important problems. Originally, one of the main disadvantages of using MIPs as stationary phases was the irregular size and shape of the imprinted particles obtained. Bulk polymerisation was the first strategy developed to synthesize imprinted polymers and, after the mandatory grinding and sieving steps, the obtained particles possessed invariably a heterogeneous particle size and binding site distribution with poor site accessibility for the target analyte. In addition, the resulting material produced high backpressure and low mass transfer kinetics when packed on HPLC columns, resulting in the obtainment of broad and tailed peaks. In order to overcome the mentioned drawbacks, different strategies to prepare MIP beads with improved chromatographic properties have been proposed such as suspension polymerisation [7], multi-step

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swelling and polymerisation [8], imprinting in core-shell particles [9] and precipitation polymerisation [10]. However, even using these techniques, it is still difficult to tune the system to obtain both the desired particle characteristics (size, porosity, pore volume, surface area) and the high affinity of binding sites required. Alternatively, the use of preformed beads of well known and optimum morphology as supports for the preparation of imprinted polymers have been proposed following mainly two different strategies: (i) grafting of thin MIP films on the surface of porous modified silica [11–13] and (ii) polymerisation within the pores of preformed silica beads [14,15]. Polymerisation inside the pores of preformed silica is a simple method as it just consists of filling the pores with the polymerisation mixture and subsequently heating of particles to allow polymerisation. This strategy uses the pores as micro-vessels in which conventional micro-bulk polymerisation takes place. This leads to a composite material with the same size and shape as the former silica beads. Additionally, the silica matrix can be etched away resulting in MIP beads which can be considered the "mirror image" of the original silica mold.

The different polymerisation strategies described above have been mainly employed in the synthesis of MIPs to be used as chiral stationary phases, which represents a rather small area de application. Besides this, systematic studies comparing imprinted-stationary phases prepared by different polymerisation strategies are scarce [16,17], making difficult to decide a priori which strategy will perform better for a certain application. Thus, keeping this comments in mind, the aim of the present paper is the synthesis, evaluation and comparison of different MIPs based on the use of preformed silica beads to be used as selective HPLC stationary phases able to recognize and separate several phenylurea herbicides. At the present, phenylurea herbicides are widely used for the protection of different crops which have made authorities to establish maximum residue limits (MRLs) of this compounds in vegetables ranging, within Europe, from 0.02 to 0.1 mg kg^{-1}, depending upon the herbicide and the crop [18]. In consequence, it is not surprising that routine laboratories have seen an important increase of the amount of parameters and samples to be analysed, which makes desirably the development and implementation of rapid-response (screening) analytical tools. Such screening systems should be capable of processing a large number of samples in short time, allowing the analyst to select only those samples with the target characteristics (i.e. the presence of analytes above or below a given concentration threshold) for further confirmation [19]. Thus, in this sense, a second goal of the present paper is the use imprinted columns for the LC-UV screening of phenylurea herbicides at low concentration levels directly from vegetable sample extracts without any previous clean-up step.

2. Experimental

2.1. Reagents

Fenuron (FEN), metoxuron (MXN), chlortoluron (CTN), isoproturon (IPN), metobromuron (MBN) and linuron (LIN) were purchased from Dr. Ehrenstofer (Augsburg, Germany) and the corresponding chemical structures are shown in Fig. 1. Stock standard solutions (1 g l^{-1}) were prepared in acetonitrile and stored at −22 °C. Methacrylic acid (MAA), 2-(trifluoromethyl)-acrylic acid (TFMAA), ethylene glycol dimethacrylate (EDMA) and 2,2′-azobis methylbutyronitrile (AIMN) were purchased from Sigma–Aldrich (Madrid, Spain). Anhydrous toluene was purchased from Sigma–Aldrich (Madrid, Spain), HPLC grade water, acetonitrile and methanol were purchased from Lab-Scan (Dublin, Ireland). All other chemicals were of analytical reagent grade. EDMA and MAA were freed from stabilizers by distillation under reduced pressure and AIMN was recrystallized from methanol prior to use. All other chemicals were used as received.

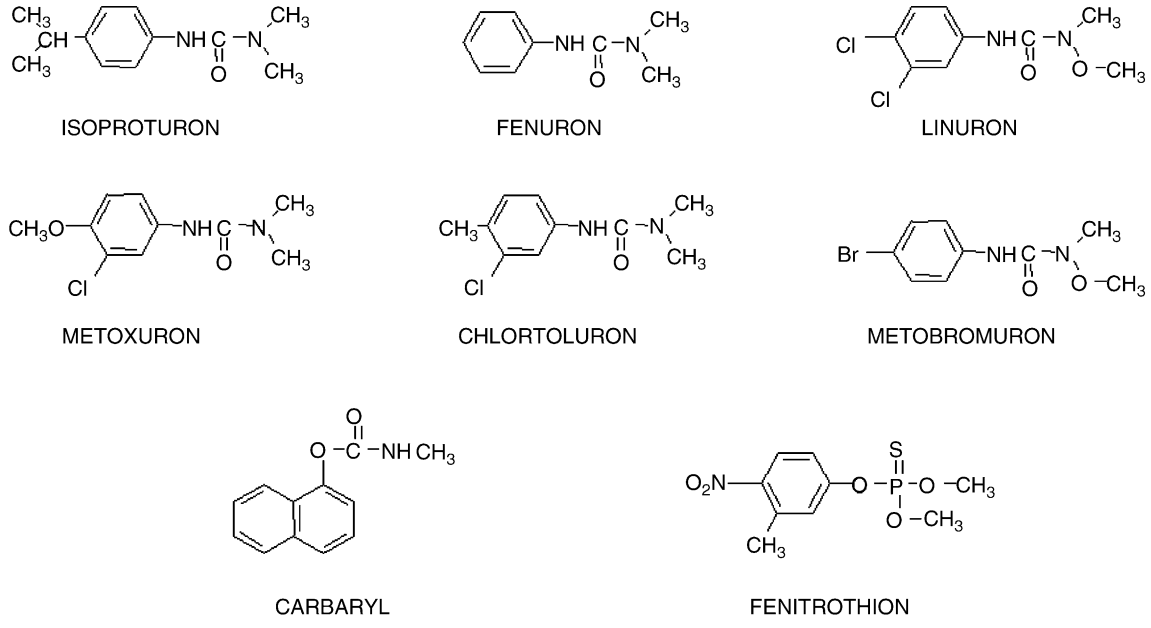

Fig. 1. Chemical structures of selected pesticides.

The porous silica gel (specific surface area $S = 380\,m^2\,g^{-1}$, particle size $p_s = 10\,\mu m$, pore volume $V_p = 1.083\,ml\,g^{-1}$ and pore diameter $d_p = 10.5\,nm$) was synthesized according to Fresne et al. [20] and kindly supplied by Dr. Cedric du Fresne (University of Mainz).

2.2. Polymers preparation

2.2.1. Pore-filled silica-molecularly imprinted composite material

A polymerisation mixture was prepared containing template molecule (IPN or LIN, 1 mmol), functional monomer (TFMAA or MAA, 4 mmol), EDMA (20 mmol), AIMN (0.5 mmol) and 2 ml of dry toluene. Then, porous silica beads were left in contact with the polymerisation mixture, keeping the proportion of 1 g of silica per 1 ml of polymerisation mixture, and shaken vigorously in order to allow the polymerisation mixture to penetrate the pores of the silica. After purging with nitrogen, polymerisation inside the pores was thermally initiated at 65 °C and allowed to continue for 24, 48 or 72 h at this temperature. Finally, the template was removed by extraction with methanol in a Soxhlet apparatus for 16 h. Non-imprinted polymers were prepared as described above but without addition of template.

2.2.2. MIP beads

Pore filled silica-MIP composite materials (c-MIPs) were prepared as described above and then silica (1 g) was etched away by treatment with a 3 M aqueous solution of NH_4HF_2 (10 ml) for 12 h under agitation. After dissolution, the spherical imprinted beads (dis-MIPs) were washed with water to remove unreacted salts.

Table 1 summarize the different experimental conditions combinations (template/monomer/polymerisation time) used and the corresponding c-MIPs and dis-MIPs evaluated in the present study.

2.3. Characterization techniques

2.3.1. Elemental analysis and infrared spectroscopy

Elemental microanalyses were performed using a LECO CHNS-932 analyser. Transmission infrared spectra were obtained using a Spectrum BX FTIR Spectrometer (Perkin-Elmer, Boston, MA).

Table 1
Molecularly imprinted polymers evaluated in the present study

MIPs obtained[a]	Experimental conditions		
	Monomer	Template	Polymerisation time
c-MIP1/dis-MIP1	MAA	IPN	24
c-MIP2/dis-MIP2	MAA	IPN	48
c-MIP3/dis-MIP3	MAA	IPN	72
c-MIP4/dis-MIP4	TFMAA	IPN	48
c-MIP5/dis-MIP5	TFMAA	LIN	48
c-MIP6/dis-MIP6	TFMAA	LIN	72

[a] c-MIP: Composite material; dis-MIP: MIP beads obtained after silica dissolution of the corresponding c-MIP.

2.3.2. Nitrogen sorption and scanning electron microscopy measurements

Nitrogen sorption measurements were performed on an ASAP 2020 Accelerated Surface Area and Porosimetry analyser (Micromeritics Instrument Corporation, Norcross, GA). Before measurements, 100–150 mg of the samples were heated at 100 °C under high vacuum (10^{-5} Pa) for at least 3 h. The specific surface areas (S) were evaluated using the BET method, and specific pore volumes (V_p) and the average pore diameter (d_p) using the BJH theory.

The scanning electron micrographs were carried out at Centro de Microscopía Electrónica "Luis Bru" (Universidad Complutense de Madrid) using a JEOL JM-6400 (Peabody, MA).

2.4. Chromatographic evaluation of polymers

Polymer particles were dry-packed in HPLC stainless steel columns (125 mm × 4.6 mm). The columns were then connected to a Hewlett Packard 1100 Series HPLC system equipped with a quaternary high-pressure pump and a photo diode-array detection (DAD) system. A volume of 20 μl of 0.5–10 $\mu g\,ml^{-1}$ solutions of phenylurea herbicides in acetonitrile were injected by a Rheodyne 7725i injection valve and the retention times and areas were recorded at 244 nm with an isocratic flow rate of $1\,ml\,min^{-1}$. The retention factors (k) were calculated as $k = (t_R - t_0)/t_0$, where t_0 is the retention time of acetone (used as a void marker) and t_R is the retention time of the analyte. The imprinting factors (IF) for each analyte were calculated from the retention factors of each analyte obtained on the MIP and NIP columns ($IF = k_{MIP}/k_{NIP}$). Theoretical plate numbers (N) were calculated for each analyte using classical chromatography theory assuming ideal peaks according to Eq. 1:

$$N = 5.54\left(\frac{t_R}{W_{0.5}}\right)^2 \quad (1)$$

2.5. Screening of phenylurea herbicides in vegetable samples

A volume of 40 ml of acetonitrile was added to 10 g of dry sample and, after manual shacking during 10 min, the mixture was centrifuged for 30 min. The supernatant was filtered through a 0.45 μm filter, and evaporated to dryness. The dried extract was redissolved in 1 ml of acetonitrile containing a mixture of phenylureas used in this study at a concentration level of $1\,mg\,l^{-1}$ and directly injected onto the optimum imprinted-chromatographic column (dis-MIP6).

3. Results and discussion

The work presented herein focuses on the preparation of phenylurea-imprinted polymers, based on the use of fully characterized preformed porous silica particles, to be used as stationary phases in HPLC for the screening of several phenylurea herbicides from real vegetable samples. In this way, theoretically, the resulting imprinted polymer particles should possess a controlled and homogeneous morphology with optimum char-

Table 2
Characteristics of pore filled silica polymer composite materials (c-MIP) and those obtained after dissolution of silica mold (dis-MIP)[a]

	C (%)	H (%)	S ($m^2\,g^{-1}$)	V_p ($cm^3\,g^{-1}$)	d_p (Å)
Silica	–	–	380	1.08	105
c-MIP1	24.37	3.28	251.2	0.26	65.4
c-MIP2	23.07	3.24	238.6	0.30	76.5
c-MIP3	23.06	3.17	159.1	0.20	76.7
dis-MIP1	52.44	6.53	315.2	0.27	35.0
dis-MIP2	55.65	6.71	394.8	0.31	40.4
dis-MIP3	54.63	6.66	366.0	0.45	58.2

[a] BET surface area (S), specific pore volume (V_p) and average pore diameter (d_p) were calculated from nitrogen adsorption measurements.

acteristics allowing the separation of target analytes from matrix components.

3.1. Characterization of molecularly imprinted polymers

Initially, different composite materials (c-MIPs) were prepared at different polymerisation times (24, 48 and 72 h) with the same porous silica using IPN as template and MAA as monomer. Subsequently, the silica mold was etched away leading to MIP beads (dis-MIPs). Table 2 shows the physical characteristics (specific pore volume (V_p), surface area (S) and average pore diameter (d_p)) of MIP materials compared to those of initial silica estimated from the adsorption isotherms and the corresponding elemental analysis data. As can be seen, a decrease in S and V_p, with respect to the original silica, was observed in the composite materials, whereas there was again an increase of these parameters (especially of S values) for dis-MIPs. This fact can only be attributed to the dissolution of the silica. The success of the dissolution of silica is clearly observed according to the increase of carbon and hydrogen content, together with a mass loss of around 50% in the composite material after treatment with NH_4H_2F (aq). In addition, the removal of silica was confirmed by the corresponding IR spectra (not shown) where the band corresponding to the silica (Si–O–Si, $\sim$1040 cm^{-1}) disappeared, whereas the intensities of the bands corresponding to the carbonyl ($\sim$1726–1730 cm^{-1}) and vinyl ($\sim$2974 cm^{-1}) stretching vibration increased after silica dissolution. Similar data were observed for the corresponding non-imprinted polymers. All these data confirm the success of the polymerisation methodology used in this work and thus, as a result, the structure and morphology of the materials dis-MIP can be considered the "mirror image" of the original silica mold.

3.2. Chromatographic evaluation of molecularly imprinted polymers

As an example, Fig. 2 shows the scanning electron micrograph of dis-MIP3 material prepared in this work. It can be observed that spherical particles with an approximate mean diameter of 10 μm were obtained. In addition, according to the physical characteristics reported in Table 2, all the materials present permanent porosity making them suitable for chromatographic purposes. Thus, materials were packed into HPLC

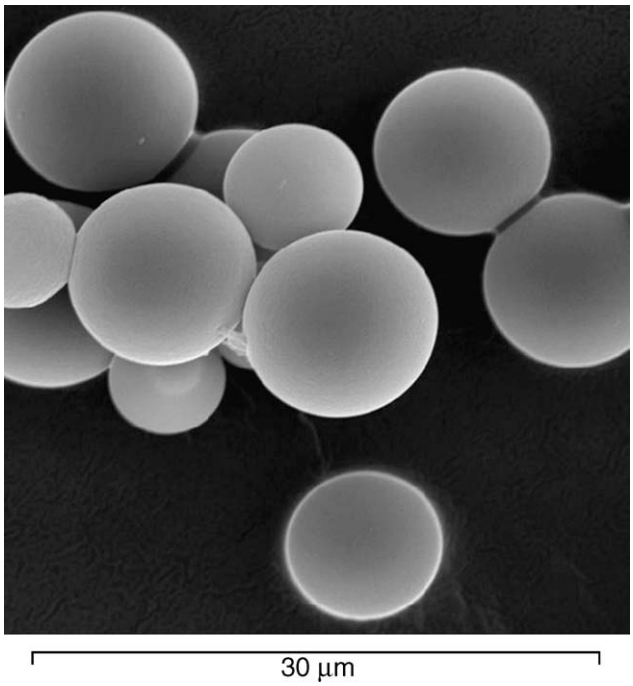

Fig. 2. Scanning electron micrograph of dis-MIP3.

columns for a preliminary evaluation as chromatographic stationary phases. After equilibrating the columns with acetonitrile, the elution of several phenylurea herbicides was performed in isocratic mode and retention factors on the imprinted (k_{MIP}) and non-imprinted (k_{NIP}) stationary phases, as well as the corresponding imprinting factor (IF = k_{MIP}/k_{NIP}), were calculated. This study showed that both c-MIP and dis-MIP materials exhibited imprinting effects, however, the IF values obtained for c-MIP materials were rather low (<1.2). These results might be attributed to the decrease of surface area and pore volume values observed in c-MIPs (see Table 2) making binding sites not easily available. However, an increase in such values, as that observed after silica dissolution, leads to more accessible binding sites. Consequently, c-MIP materials were discarded for further experiments.

3.2.1. Influence of polymerisation time

Table 3 shows the imprinting factors (IF) and the theoretical plate numbers (N) assuming ideal peaks obtained for the polymers dis-MIP1, dis-MIP2 and dis-MIP3 which were prepared

Table 3
Chromatographic characteristics (IF and N) of dis-MIP1, dis-MIP2 and dis-MIP3 stationary phases obtained in the analysis of phenylurea herbicides

	dis-MIP1		dis-MIP2		dis-MIP3	
	IF	N (m^{-1})	IF	N (m^{-1})	IF	N (m^{-1})
FEN	1.21	1300	1.80	2300	2.70	3000
CTN	1.29	1200	1.86	2200	2.41	2900
IPN	1.43	6300	2.35	2000	3.35	2800
MXN	1.46	5800	1.96	2500	2.88	3000
LIN	2.28	3200	3.28	3800	2.71	6600
MBN	2.22	10700	2.89	3500	2.44	5900

with different polymerisation times (24, 48 and 72 h, respectively).

From these data, it is clear that the three materials exhibit molecular recognition properties when compared to the corresponding non-imprinted materials not only for the template molecule (IPN) but also for other phenylurea herbicides (IF values higher than 1 in all cases). On the other hand, it can be observed that the longer polymerisation time the higher IF and *N* values are obtained. This result would be in concordance with the percentage of polymer formed (72% for dis-MIP1, 80% for dis-MIP2 and 86% for dis-MIP3) taking into account the mass of polymerisation mixture used (the same in all cases) and the mass of polymer obtained after etched out the silica matrix, demonstrating that imprinting effect increased with the amount of polymer formed. However, it is important to stress that not only the amount of polymer but also its physical properties have undoubtedly an influence on the chromatographic performance of MIPs. In this sense, according to the data reported in Table 2, dis-MIP3 exhibits the larger pore volume which, as suggested above, makes easier analytes to access to binding sites and thus this material shows the higher IF and *N* values of the tested polymers.

3.2.2. *Influence of functional monomer and template molecule*

In previous studies carried out by our group toward the synthesis of imprinted polymers able to selectively retain phenylurea herbicides [21–23], it was observed that both the template and the functional monomer did influence the performance of the materials obtained, both in terms of affinity and selectivity for the target analytes. Thus, a new set of polymers using TFMAA as functional monomer, instead of MAA, was prepared. The polymerisation time was fixed in 48 h, and IPN or LIN were used as template molecules (see Table 1). After silica matrix dissolution, the obtained materials (dis-MIP4 and dis-MIP5, respectively) were evaluated in order to study the influence of the employed monomers over the chromatographic performance.

Fig. 3A and B show the IF and *N* values obtained for all the analytes tested in the different dis-MIP materials. As can be observed, dis-MIP5 (LIN/TFMAA system) exhibits higher IF and *N* values for FEN, IPN, CTN and MXN compared to materials dis-MIP4 (IPN/TFMAA system) and dis-MIP2 (IPN/MAA system). However, the studied TFMAA-based materials shown lower IF for MBN and LIN than those obtained in dis-MIP2. Looking at the chemical structures of these compounds shown in Fig. 1, this result can only be attributed to the fact that both compounds contain a methoxy group near to the urea group, which is likely the responsible of the monomer:template interaction through hydrogen bonding during the prearrangement step. Thus, it seems clear that the presence of $–OCH_3$ groups affects the interaction of these compounds with imprinted sites in certain manner either by steric repulsion or by unspecific interactions inside the imprinted cavities (i.e. methoxy group may also interact by hydrogen bonding with the acidic moieties inside the cavities) as suggested previously [23]. Apart from these theoretical considerations, the obtained results open the possibility of using these materials for the separation of phenylurea herbicides

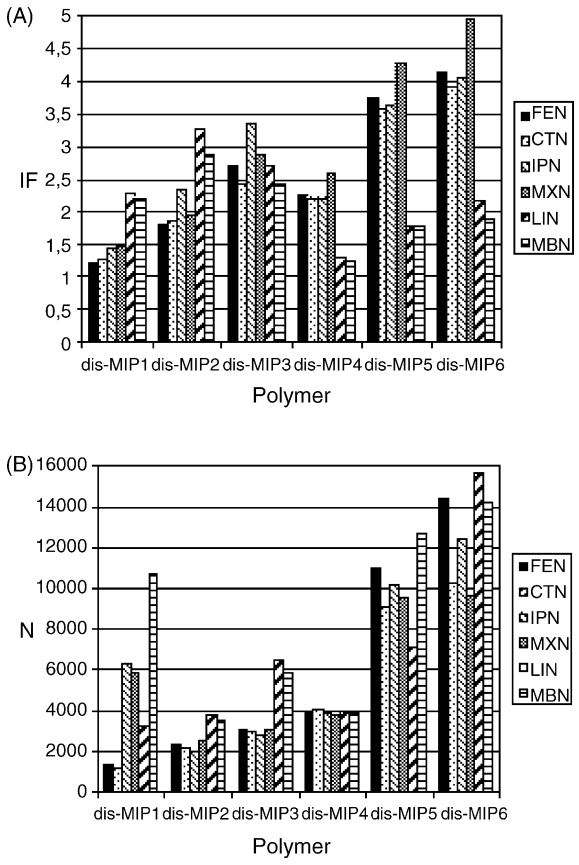

Fig. 3. Imprinting factor values (A) and theoretical plate numbers (B) calculated for dis-MIP materials of each phenylurea herbicide.

in two different groups depending upon the presence or absence of a methoxy group in its chemical structure.

According to the results reported above regarding the template/monomer system and polymerisation time, it was decided to prepare a new material (dis-MIP6) using LIN as template, TFMAA as functional monomer and 72 h of polymerisation time, and its chromatographic performance was evaluated as described in Section 2. The obtained IF and *N* values are shown and in Table 4, and also in Fig. 3 for comparison purposes with those values obtained for the other dis-MIP materials.

Table 4
Chromatographic characteristics of dis-MIP6 stationary phase obtained in the analysis of phenylurea herbicides[a]

	t_R MIP (min)	t_R NIP (min)	k_{MIP}	k_{NIP}	IF	N (m^{-1})
Acetone	2.14	1.83	–	–	–	–
FEN	4.16	2.26	0.95	0.23	4.13	14400
CTN	4.06	2.26	0.90	0.23	3.91	10300
IPN	4.21	2.27	0.97	0.24	4.05	12400
MXN	4.26	2.20	0.99	0.20	4.96	9700
LIN	2.74	2.08	0.28	0.14	2.17	15700
MBN	2.78	2.12	0.30	0.16	1.87	14300

[a] t_R (MIP): retention time on imprinted polymer; t_R (NIP): retention time on non-imprinted polymer; k_{MIP} and k_{NIP}: retention factors on imprinted and non-imprinted polymers using acetone as void marker; IF: imprinting factor calculated as $IF = k_{MIP}/k_{NIP}$; *N*: theoretical plate number. The parameters were calculated after injection of 20 μl of 10 mg l^{-1} solution of phenylurea herbicides in acetonitrile. For other chromatographic conditions, see Section 2.

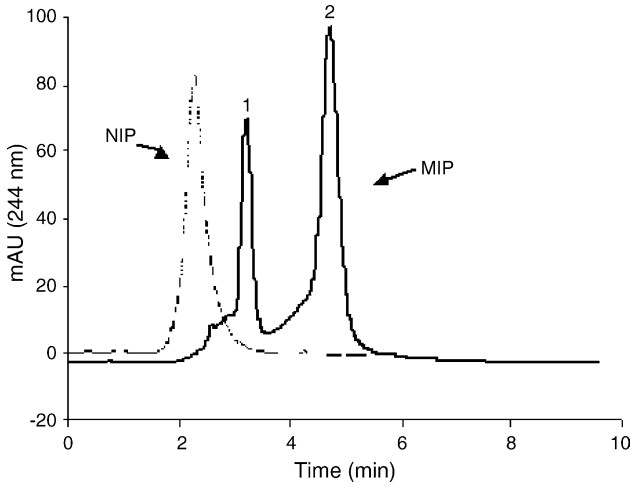

Fig. 4. Chromatograms obtained after the injection of a mixture of herbicides at 5 μg ml^{-1} concentration level onto dis-NIP6 and dis-MIP6 columns. Injection volume was 20 μl, mobile phase 100% acetonitrile at a flow rate of 1 ml min^{-1}. Peak numbers: (1) LIN and MBN; (2) FEN, CTN, IPN and MXN.

As expected, a general improvement of both IF and *N* values was obtained using dis-MIP6 material as chromatographic stationary phases and thus this polymer was selected for further studies.

3.2.3. Separation of phenylurea herbicides

Once the superior potential of dis-MIP6 for the selective recognition of phenylurea herbicides was demonstrated, the ability of such material for separating a mixture of analytes in two groups as suggested above was evaluated. In order to perform this study, a standard solution containing 5 μg ml^{-1} of each phenylurea in acetonitrile was injected both onto HPLC imprinted and non-imprinted columns and the chromatograms obtained are shown in Fig. 4. It can be observed that dis-MIP6 column is able to separate phenylureas herbicides in two different groups depending upon the presence (LIN and MBN, peak 1) or absence (FEN, CTN, IPN and MXN, peak 2) of a methoxy group in its chemical structure. It is clear that the presence of this chemical group disrupt in some extent the interaction of LIN and MBN with the imprinted binding sites allowing their separation from the rest of analytes under study.

In a parallel experiment, in order to further evaluate the selective recognition of the imprinted column, a mixture of fenitrothion and carbaryl (Fig. 1), pesticides of organophosphorous and carbamates families, respectively, at the same concentration was injected. These compounds were eluted with a retention time close to t_0, which confirmed the high selectivity provided by dis-MIP6 column.

Finally, calibration graphs were constructed using the dis-MIP6 by injecting 20 μl of solution of mixtures of LIN and IPN (representing both phenylurea groups) in acetonitrile within the range 0.5–10 mg l^{-1} of each pesticide. The correlation coefficients were satisfactory ($R^2 > 0.998$), which would allow the screening of phenylurea herbicides in real samples at concentration levels according to current legislation.

Table 5
Recoveries (%) and RSDs (%) obtained after the direct injection of vegetable sample extracts spiked with LIN and IPN ($n = 5$) at 100 ng g^{-1} concentration level in dis-MIP6 column

Sample	LIN	IPN
Potato	97 ± 9	95 ± 5
Pea	86 ± 9	94 ± 7
Corn	102 ± 9	110 ± 13

3.3. Screening of phenylurea herbicides in plant sample extracts

As stated in Section 1, the main goal of the present work was the use imprinted columns for the LC-UV screening of phenylurea herbicides at low concentration levels directly from vegetable sample extracts without any previous clean-up step. Thus, vegetable samples (potato, corn and pea) were treated as described in Section 2 and sample extracts, both unspiked and spiked with LIN and IPN at a concentration level of 1 μg ml^{-1} (equivalent to 100 ng g^{-1}), were directly injected without any previous clean-up step in dis-MIP6 column. The recoveries obtained (shown in Table 5) were quantitative in all cases with relative standard deviations (RSDs) lower than 15% demonstrating the suitability of the proposed method for the screening of phenylurea herbicides in vegetable samples.

Fig. 5 shows the chromatograms obtained of blank and spiked (100 ng g^{-1} concentration level of LIN and IPN) potato sample extracts. As can be observed, the high selectivity of dis-MIP6 allows the detection of both analytes, being them retained in the column whereas interferences are rapidly eluted. It is clear that the use of this MIP permits the screening of these analytes at concentration level required by nowadays legislation in less than 10 min using typical instrumentation (LC-UV) available in any routine laboratory. It is important to point out that, in parallel experiments, sample extracts were directly injected in commercial C_{18} columns using the same chromatographic conditions

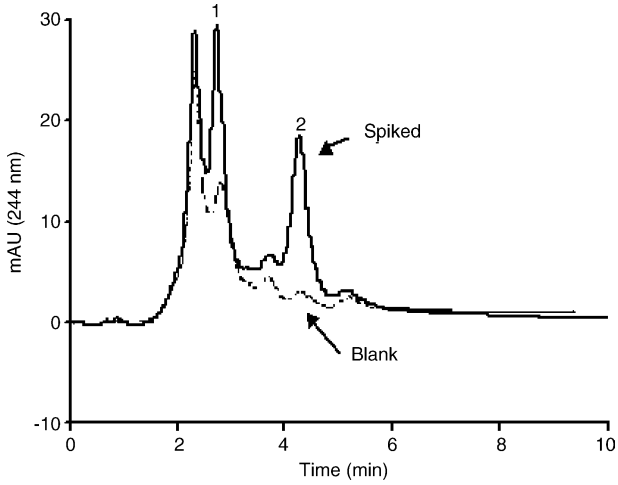

Fig. 5. Chromatograms obtained after the injection of a blank and spiked potato sample extract directly onto the imprinted column dis-MIP6. Injection volume was 20 μl, mobile phase 100% acetonitrile at a flow rate of 1 ml min^{-1}. Peak numbers: (1) LIN; (2) IPN.

(isocratic elution with acetonitrile) or performing a suitable elution gradient. In both cases, the detection of analytes was not possible due to the coelution of matrix components with target analytes.

Finally, it is important to stress that no looses on columns performance were observed along this work, demonstrating the physical and chemical stability of the proposed imprinted-stationary phases.

4. Conclusions

In this work, it has been demonstrated the suitability of several imprinted-stationary phases for the separation and the selective recognition of phenylurea herbicides. Their preparation based on the use of preformed silica beads has proved to be a facile method to obtain spherically particles with appropriated chromatographic characteristics to be used as stationary phases in HPLC.

Besides this, an imprinted-stationary phase was successfully employed for the screening of phenylurea herbicides in plant sample extracts by LC-UV without any further clean-up. From our knowledge, this is the first work dealing with the use of MIPs as stationay phases in HPLC allowing the direct injection of complex samples with the analytes not only becoming separated from each other (in two groups in this case) but also from the matrix-interfering compounds. In this sense, from our point of view, the incorporation of MIPs to simple screening systems will ease the obtainment of the growing amount of information required in a variety of fields (i.e. health, food, environment) and a further development in this area is expected in the near future.

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