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EVALUATION OF THE POTENTIAL OF A QUINIDINE-BASED MONOLITHIC COLUMN ON THE ENANTIOMERIC SEPARATION OF HERBICIDES BY NANO-LIQUID CHROMATOGRAPHY

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Abstract

The use of agrochemicals as pure enantiomers avoids the presence of non-active pesticide compounds in the environment. The quality control of these agrochemicals requires the development of new chiral methodologies. In this work, the performance of the monolithic column named poly(O-9-[2-(methacryloyloxy)-ethylcarbamoyl]-10,11-dihydroquinidine-co-2-hydroxyethyl methacrylate-co-ethylene dimethacrylate) (poly(MQD-*co*-HEMA-*co*-EDMA)) was assessed as stationary phase for the enantiomeric separation of seven phenoxy acid herbicides (dichlorprop, 2-(4-chlorophenoxy)propionic acid, 2-(3-chlorophenoxy)propionic acid, mecoprop, fenoprop, 2-phenoxypropionic acid, and fenoxaprop) in a nano-liquid chromatography (nano-LC) system. Under optimized conditions (60/40 (v/v) ACN/0.1 M ammonium acetate (pH 6.0) at a flow rate of 20 µL/min), it was possible to obtain baseline separation for most of the studied compounds. The analysis of an enantiomerically pure mecoprop commercial herbicide sample which also contained three achiral phenoxy acid herbicides (MCPA, 2,4-D and dicamba) commonly present in these kind of formulations showed that the use of a 80/20 (v/v) ACN/0.1 M ammonium acetate (pH 5.3) enabled the simultaneous separation of these compounds and mecoprop enantiomers in less than 8 min. The analytical characteristics of the methodology were evaluated in terms of selectivity, linearity, precision, accuracy, LOD, and LOQ. Results demonstrated that determined amounts of R-mecoprop in commercial formulations were in agreement with the labeled content and the enantiomeric impurity was below the LOQ. The poly(MQD-*co*-HEMA-*co*-EDMA) capillary monolithic column exhibits great potential as a useful and environmental-friendly tool for the quality control of agrochemicals by nano-LC.

Keywords: chiral separation / phenoxy acid herbicide / mecoprop / nano-liquid chromatography / quinidine monolithic column

1. Introduction

Chirality receives high interest in several fields such as pharmaceutical, food, and environmental analysis. The fact that about 25 % of the active compounds in agrochemicals are chiral demonstrates the impact of chirality in the environment [1]. Due to the inherent stereospecificity of natural occurring biological processes, enantiomeric composition of agrochemicals must be controlled. In fact, when only one of the enantiomers is active, the employ of racemic mixtures indicates that around 50 to 75 % of unnecessary product is released to the environment [2]. For these reasons, regulatory agencies are aware of the impact of chirality and demand not only full information regarding enantiomers' activity but also the commercialization of pure active enantiomer when differences in their activity are found [3,4].

Among environmental chemical pollutants, herbicides are compounds widely used for killing unwanted plants in agriculture, gardening, homes, and soil treatment. For example phenoxy acids are one of the most employed herbicides due to their strong activity and also for their high selectivity against undesired weeds [5,6]. Phenoxy acids have good solubility in water, which allows them to move in agriculture ecosystems, cause surface and ground water pollution, and then lead into flora and fauna degradation [7]. Several phenoxy acids compounds are optically active, however, R-enantiomer is usually the only active form [8]. This means that the use of enantiomerically pure formulations can considerably decrease the amount of agrochemicals in the environment.

Separation techniques are the most useful tools to achieve enantiomeric separations. Among them, the use of HPLC [9], GC [10], supercritical fluid

chromatography (SFC) [11], CE [12], capillary electrochromatography (CEC) [13] or nano-liquid chromatography (nano-LC) [14] can be highlighted. The use of capillary separation techniques such as CE, CEC and nano-LC presents the advantages of using low sample and reagent consumption being environmental-friendly techniques [15]. The addition of chiral selectors to the separation buffer in CE enables the development of efficient methodologies for enantiomeric separations although the chiral selector employed can be not compatible with the use of mass spectrometry detection [16]. This is why the use of chiral stationary phases in CEC and nano-LC is attractive. An important drawback that CEC and nano-LC present is the lack of commercially available chiral columns to be used. This confers a high interest to the investigation of the potential of new stationary chiral phases [15].

Among chiral stationary phases for capillary columns, monolithic columns are presented as an alternative to packed columns due to their high permeability, low back-pressure, low resistance to mass transfer, and easy preparation [14,17]. Lämmerhofer *et al.* developed a carbamoylated quinidine-based chiral monolithic column, namely poly(O-9-[2-(methacryloyloxy)-ethylcarbamoyl]-10,11-dihydroquinidine-co-2-hydroxyethyl methacrylate-co-ethylene dimethacrylate) ((poly(MQD-*co*-HEMA-*co*-EDMA)) for CEC [18]. They presented the potential of this monolithic column in the enantioseparation of a group of N-derivatized amino acids [18] and for three phenoxy acid herbicides by CEC [19] although the application to the real samples was not reported. More recently, this column was re-optimized in order to provide better permeability, efficiency, and selectivity for nano-LC applications and it successfully enantioresolved a wide range of N-derivatized amino acids [20]. However, to the best of our knowledge, the enantiomeric separation of phenoxy acids herbicides by nano-LC with quinidine-based capillary monolith columns has never been reported.

In this work, the potential of poly(MQD-*co*-HEMA-*co*-EDMA) monolithic column was evaluated for the enantioseparation of a group of seven phenoxy acid herbicides (dichlorprop, 4-CPPA, 3-CPPA, mecoprop, fenoprop, 2-PPA, and fenoxaprop) by nano-LC. The monolithic column was then applied for the analysis of enantiomerically pure mecoprop commercial herbicide formulations which also contained three achiral herbicides (MCPA, 2,4-D, and dicamba). It is worth highlighting that nano-LC or quinidine-base stationary phases have never been used to determine mecoprop enantiomers in real samples.

2. Materials and methods

2.1. Reagents and samples

All reagents employed for the preparation of mobile phase and samples were of analytical grade. Acetonitrile (ACN) and acetic acid were obtained from Scharlau Chemie (Barcelona, Spain) and ammonium acetate was obtained from Merck (Darmstadt, Germany). 3-(Trimethoxysilyl)-propyl methacrylate (γ-MAPS), 2,2'-azobisisobutyronitrile (AIBN), 2-hydroxyethyl methacrylate (HEMA), ethylene dimethacrylate (EDMA), methanol (MeOH), 1-dodecanol, and cyclohexanol were all purchased from Aladdin Chemicals (Shanghai, China). MilliQ water from Millipore (Bedford, MA, USA) was employed to prepare all solutions. 2-(3-Chlorophenoxy)propionic acid (3-CPPA) and 2-(4-chlorophenoxy)propionic acid (4-CPPA) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). 2-Phenoxypropionic acid (2-PPA) and (R,S)-2-(2,4-dichlorophenoxy)propanoic acid (R,S-dichlorprop) were acquired in Chem Service (West Chester, PA, USA) and 2-(2,4,5-trichlorophenoxy)propionic acid (fenoprop), 2-[4-(6-chloro-1,3-benzoxazol-2-yloxy)phenoxy]propionic acid (fenoxaprop), R,S-mecoprop, (3,6-dichloro-2-

methoxybenzoic acid (dicamba), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) were obtained from Fluka (Buchs, Switzerland). Commercial herbicide sample containing mecoprop labeled as pure enantiomer (R-mecoprop) and containing three achiral herbicides (dicamba, 2,4-D, and MCPA) was purchased in a market from Madrid (Spain).

2.2. Instrumentation

All nano-LC experiments were performed on a laboratory self-assembled nano-LC system that consisted of a LC-10AS HPLC pump (Shimadzu, Kyoto, Japan), a four port injection valve with 20 nL internal loop (Cheminert, Valco Instruments Houston, Texas, USA) and a UV-Vis detector model 200 (Linear instruments, Fremont, California, USA). In order to split the mobile phase, a stainless steel tee (Cheminert, Valco Instruments Houston, Texas, USA) with flow split capillary (250 mm × 20 µm I.D) was employed before the injection valve. The data acquisition and data handling were performed using the software Chromatostation N200 from Zhejiang University (China). The analytes were all detected at a wavelength of 210 ± 2 nm.

A pH meter model 744 Metrohn (Herisau, Switzerland) was employed to adjust the pH of the mobile phases. For preparing and degassing mobile phases an ultrasonic bath model B200 Branson Ultrasonic Corporation (Danbury, USA) was used.

2.3. Preparation of poly(MQD-co-HEMA-co-EDMA) monolithic columns

To provide anchoring sites for the polymeric bulk, the inner wall of the fused silica capillary was pretreated as described in our previous work [20]. In brief, a γ-MAPS/MeOH (50/50, v/v) solution was introduced into the capillary, and the capillary was then submerged in a water bath at 60 ^{o}C for 12 h with both ends sealed with

silicone rubbers. After that, the capillary was rinsed with MeOH to flush out the residuals and was then dried by a nitrogen stream again for further use.

The monomers (MQD, HEMA, and EDMA), the polymerization initiator AIBN (1 % (w/v) with respect to the monomers) and the binary porogenic mixture (1-dodecanol and cyclohexanol) were mixed ultrasonically into a homogenous solution in a 2-mL vial. The composition of the polymerization mixture was chosen according to our previous work [20]. The obtained polymerization mixture was ultrasonicated in a bath for 5 min to degas, and then introduced into the γ-MAPS pretreated capillary. By sealing the both ends of the capillary with rubbers, the capillary was incubated at 60 ^{o}C for 20 h. Finally, the prepared monolithic capillary column was washed with MeOH to remove unreacted monomers and porogens. An on-column detection window was then created at a distance of 4 cm from the end of the column using a thermal wire stripper. The physical and chromatographic properties of the column have been investigated in our previous work [20].

2.4. Nano-LC conditions

Ammonium acetate buffer solutions were prepared by dissolving an appropriate amount of the salt in deionized water and adjusting to the desired pH value with acetic acid. The mobile phase was freshly prepared by mixing the buffers with ACN in a selected proportion. Stock standard solutions of racemic and enantiomerically pure herbicides were prepared by dissolving appropriate amount of the compound in ACN. In order to study the enantiomer migration order for mecoprop and dichlorprop, these solutions were enriched in a molar ratio of R- to S-enantiomer of 3:1. In the analysis of commercial herbicide sample, the corresponding aliquot was taken and diluted to desired concentration with ACN. This solution was injected into the system after 200

times dilution with ACN. Prior to the nano-LC experiments, both mobile phase and samples solutions were filtered through a 0.22-μm membrane.

At the beginning of each working day, the pump was purged with the mobile phase at a flow rate of 5 mL/min for 5 min to avoid the presence of any bubbles that might entry into the capillary column. Then, the poly(MQD-*co*-HEMA-*co*-EDMA) monolithic capillary column was conditioned by flushing it with the mobile phase at a total flow rate of 50 μL/min. Consequently, the mobile phase flow was reduced to the working flow rate (20 or 10 μL/min) until obtaining a stable pressure and baseline. At the end of each working day, the poly(MQD-*co*-HEMA-*co*-EDMA) monolithic capillary column was flushed with the mobile phase at a flow of 20 μL/min for 5 min.

The optimal experimental conditions for the enantioseparation of phenoxy acid herbicide compounds as following: a mobile phase was a mixture of ACN/0.1 M ammonium acetate buffer (pH 6.0)(60/40, v/v); the total flow rate was set at 20 μL/min and the injection volume was 20 nL; the column dimension is 200 mm x 100 μm I.D; analytes were detected at 210 ± 2 nm wavelength.

2.5. Data treatment

Enantioresolution (Rs) of the chromatographic peaks was determined according to eq. 1:

$$R_s = 1.18\frac{t_2 - t_1}{w_1 + w_2} \text{ (eq. 1)}$$

where t_1, t_2, w_1 and w_2 are the elution time and the peak width at half height of the first and second eluting enantiomers, respectively.

The content (%) of mecoprop determined with respect to the labeled amount in the analyzed samples was calculated using eq. 2:

$$\text{Content}\ (\%) = \frac{[C]_{exp}}{[C]_{labeled}} \cdot 100\ (\text{eq. } 2)$$

where $[C]_{exp}$ is the amount of R-mecoprop determined experimentally and $[C]_{labeled}$ indicated the labeled amount of R-mecoprop in the commercial product. Data analysis and composition of graphics were performed in Excel Microsoft Office 2007, Statgraphics Plus 5 and Origin Pro 7.

2.6. Study of the analytical characteristics of the developed methodology for the determination of mecoprop enantiomers

Linearity was determined by the external standard calibration method by means of the triplicate injection of standard solutions at eight different concentration levels (linear range: 5-1250 mg/L for R-enantiomer and 0.5-125 mg/L for minor S-enantiomer). The effect of matrix interferences was investigated by comparing the calibration slopes obtained by the external standard and the standard addition calibration methods [21]. The standard additions calibration curve was obtained by spiking the commercial sample with known concentrations of R-enantiomer (0, 45, 90, 210, 360 and 600 mg/L) and S-enantiomer (0, 35, 50, 60, 80 and 100 mg/L) of mecoprop, each solution injected three times. Precision was studied as instrumental repeatability and intermediate precision. Instrumental repeatability was determined from six repeated injections (n = 6) of a standard solution at concentration values of 1000 mg/L for R enantiomer and 100 mg/L for S enantiomer. Intermediate precision was assessed by three standard solutions containing 1000 and 100 mg/L of R- and S-mecoprop, respectively, injected by triplicate on three different days (n = 9). LODs for mecoprop enantiomers were calculated as 3.29 times the standard deviation of the intercept (S_a) divided by the slope of external standard calibration method [21]. LOQs were calculated

as 10 times the S_a divided by the slope of external standard calibration method [21]. Moreover, in the case of S-mecoprop the LOD and LOQ values were also experimentally evaluated. Relative LOD (RLOD) was calculated considering the lowest amount of enantiomeric impurity (S-mecoprop) that could be detected in presence of the maximum concentration of R-mecoprop (LOD experimentally evaluated was employed in this calculation). Accuracy was evaluated for mecoprop enantiomers as the recovery obtained for four solutions of a commercial herbicide sample (n = 4) containing 210 mg/L of R-mecoprop (as labeled amount) spiked with 600 and 60 mg/L of R- and S-mecoprop, respectively.

3. Results and discussion

3.1. Evaluation of the potential of poly(MQD-co-HEMA-co-EDMA) capillary monolithic column to enantioseparate phenoxy acid herbicides by nano-LC.

In this work, the potential of the poly(MQD-*co*-HEMA-*co*-EDMA) capillary monolithic column (see **Fig. 1**) to enantioseparate chiral phenoxy acid herbicides by nano-LC was evaluated using selected analytes, including 3-CPPA, 4-CPPA, mecoprop, dichlorprop, fenoprop, fenoxaprop, and 2-PPA. **Fig. 2** displays their chemical structures marking the asymmetric carbons for each herbicide.

In order to study the effect of ACN content, the proportions of ACN and ammonium acetate buffer in the mobile phase was varied between 60/40 and 40/60 (v/v). It was observed that the retention time of all analytes increased as the ACN content decreased from 60% to 40%, while Rs for all studied compounds, except for 3-CPPA remained almost constant. A higher Rs in less time was obtained with a mobile

phase containing 60% of ACN and it was chosen for continuing with the method development.

The pH of mobile phase has a significant impact on retention and enantioselectivity by affecting the ionization state of solutes and stationary phase. The influence of the apparent pH on the enantioresolution of phenoxy acid herbicides was also investigated by adjusting the pH values of buffer solution to 6.0, 5.3, and 4.0 before mixing with ACN. It was observed that both retention factor and Rs of the studied analytes decreased with decreasing apparent pH. The enantioselectivity values remained almost constant between pH 6.0 and 5.3, and then decreased significantly at pH 4.0. This effect is related to the changes in the ionization state of these analytes what affects the enantioselectivity. This is in concordance to what was previously reported where the enantioselectivity of quinidine-based stationary phases is mainly due to the electrostatic interactions between the quinidine and acid analytes [18, 22]. The pKa value of quinidine is 8.7, thus the stationary phase should remain positively charged while the phenoxy acid compounds were negatively charged within the studied pH range. When decreasing apparent pH, the electrostatic interactions between the carboxylate function of phenoxy acids and the positively charged tertiary nitrogen of quinidine decreased. Thereby, an apparent pH of 6.0 was selected because it offered the best Rs for these compounds.

Under the optimal experimental conditions (ACN/0.1 M ammonium acetate (pH 6.0) (60/40, v/v) at a flow rate of 20 µL/min), five tested phenoxy acids were baseline separated while 2-PPA and fenoxaprop were partially enantioresolved (**Fig. 3**). This observation is in accordance with the proposed mechanism by M. Lämmerhofer et al. [18, 22]. The enantioselectivity for the chiral acid molecules could be attributed to a global effect, including ion-pair formation between chiral analytes and selector, π-π and

steric interactions, etc. The lack of electron-withdrawing groups in the aromatic ring of 2-PPA (see **Fig. 2**) impede the π-π interactions, and then decrease the interaction with the selector. The larger size of fenoxaprop compared to the other herbicides (see **Fig. 2**) may disturb the interaction between the carboxylate moiety and the basic nitrogen of the selector, and second, the benzoxazole group of this herbicide might stabilize the electron cloud on the aromatic ring of quinidine and thus, decrease the selector-selectand π-π interactions [22].

Moreover, the enantiomer migration order of phenoxy acids mecoprop and dichlorprop were further evaluated (see *section 2.4*). The obtained chromatograms confirmed that R-enantiomer was eluted before S-enantiomer in both cases (see **Fig. 3**).

3.2. Study of the analytical characteristics of the chiral method for the determination of mecoprop enantiomers

To evaluate the potential of the quinidine column in the analysis of real samples mecoprop was chosen as a test analyte due to its elevated use as herbicide (the Environmental Protection Agency estimates that approximately 5 million pounds are used worldwide each year [23]). A commercial herbicide sample containing mecoprop labeled as pure enantiomer (R-mecoprop) and containing three achiral herbicides (dicamba, 2,4-D, and MCPA) was analyzed. The chemical structures of these achiral herbicides are also shown in **Fig. 2**.

In order to study the analytical characteristics of the developed method, selectivity, linearity, precision, accuracy, LODs and LOQs were evaluated as described in *section 2.6*. Under the above optimal conditions (ACN/0.1 M ammonium acetate (pH 6.0) (60/40, v/v) at a flow rate of 20 µL/min), the compounds present in the commercial herbicide interfered with mecoprop enantiomers (**Fig. 4A**). Thereby, slight

modifications of the separation conditions had to be carried out. First, the effect of mobile phase flow rate was studied keeping the rest of conditions constant, finding that 20 µL/min was the optimum value as lower values (10 µL/min) caused an increase in analysis time without improving the Rs between peaks (**Fig. 4B**). By decreasing the pH from 6.0 to 5.3 a better Rs between peaks was found, however just three peaks were observed (**Fig. 4C**). Finally, a change in the proportion of ACN/0.1 M ammonium acetate buffer (pH 5.3) of the mobile phase from 60/40 to 80/20 (v/v) was proved to reveal the full separation of the compounds of the herbicide sample in the shortest analysis time. Under these new conditions (**Fig. 4D**), the Rs of mecoprop enantiomers decreased (Rs from 2.36 to 1.90) but the simultaneous separation of all compounds present in the herbicide sample was achieved. Moreover, a small peak corresponding to the mecoprop impurity (S-mecoprop) could be detected (zoom of **Fig. 4D**). Identification of each peak could be possible by means of spiking the real sample with standard solution of the corresponding achiral phenoxy acid herbicides (**Fig. 4D**).

Under the optimized conditions to analyze the commercial herbicide sample (ACN/0.1 M ammonium acetate (pH 5.3) (80/20, v/v) at a flow rate of 20 µL/min), method linearity for mecoprop enantiomers was evaluated. As shown in **Table 1**, linearity was adequate in all cases as R^2 values were ≥ 99.0 %, confidence interval for the slope did not include the zero value and confidence interval for the intercept included the zero value (for a 95 % confidence level). When comparing the slopes of external standard and standard addition calibration methods the P-values of t-test were > 0.05 (for a 95 % confidence level) as shown in **Table 1**. These results demonstrated the absence of matrix interferences, therefore, the external standard calibration method was used to quantify mecoprop content in the real sample.

Precision of the method was evaluated as instrumental repeatability and intermediate precision. Regarding the instrumental repeatability, the RSD values (%) were lower than 1 % for peak areas and lower than 2 % for elution times. In the case of the intermediate precision, the RSD values (%) achieved for the enantiomers of mecoprop were between 1.9 and 2.2 for elution times and between 2.3 and 3.0 for peak areas as it can be observed in **Table 1.**

LOD and LOQ for R-mecoprop (calculated as explained in section 2.6) were 6.6 and 20.0 mg/L, respectively, as seen in **Table 1**. Regarding the S-enantiomer, calculated values were 1.7 and 5.1 mg/L for LOD and LOQ, respectively. However, when these last values were tested experimentally, they were higher: 20.0 and 35.0 mg/L, respectively, being these the values shown in **Table 1**. Moreover, RLOD value was found to be 2.0 % as seen in **Fig. 5.**

Accuracy was determined as the recoveries obtained for both mecoprop enantiomers. The obtained recovery was 99 ± 3 % for R-enantiomer and 108 ± 9 % for S-enantiomer as seen in **Table 1.**

3.3. Application of the nano-LC method to determine mecoprop enantiomers in a commercial herbicide product

Finally, once the analytical characteristics of the nano-LC method were evaluated, it was then applied to the determination of R-mecoprop in a commercial herbicide product under the optimized conditions for this purpose employing standard calibration method to quantify the amount of mecoprop. The determined amount of R-mecoprop was 42.06 ± 0.08 g/L which is translated into a 100.14 % of the labeled content. In the case of mecoprop enantiomeric impurity content (S-mecoprop) it could be detected, however this value was below the LOQ as can be seen in **Fig. 4D**. The

obtained results indicated that the poly(MQD-*co*-HEMA-*co*-EDMA) capillary monolithic column can be considered as a useful tool in the determination of the optical purity of agrochemicals by nano-LC.

4. Concluding remarks

The poly(MQD-*co*-HEMA-*co*-EDMA) capillary monolithic column employed in this work was proven for the first time to offer enantiodiscrimination in a nano-LC system towards different phenoxy acid compounds. Five out of seven chiral compounds could be baseline separated (resolutions from 1.9 to 3.5) under optimized conditions (60/40 (v/v) ACN/0.1 M ammonium acetate (pH 6.0) at a flow rate of 20 µL/min) in less than 20 min. The analytical characteristics of the method developed for the determination of mecoprop in commercially available herbicide formulations were evaluated. Since the analyzed commercial herbicide sample contained three more achiral herbicides which interfered with peaks of mecoprop enantiomers the method had to be re-optimized. Under these conditions (80/20 (v/v) ACN/0.1 M ammonium acetate (pH 5.3) at a flow rate of 20 µL/min) the simultaneous separation of mecoprop enantiomers from the other three achiral herbicides could be carried out in less than 8 min. Results demonstrated that determined R-mecoprop amounts in commercial formulations were in agreement with the labeled content and that the enantiomeric impurity was below the LOQ showing the potential of the poly(MQD-co-HEMA-co-EDMA) capillary monolithic column as a useful and environmental-friendly tool for the quality control of agrochemicals by nano-LC.

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Figure captions

Figure 1. Simplified chemical structure of the quinidine-based monolithic chiral stationary phase.

Figure 2. Chemical structures of the studied phenoxy acid compounds. The asymmetric carbon is denoted by *.

Figure 3. Chromatograms of the separation of the seven chiral phenoxy acid compounds under optimized conditions for standard solutions and Rs values. Analytes concentration: 2000 mg/L for mecoprop and dichlorprop, 1000 mg/L for fenoprop, fenoxaprop, 2-PPA, and 4-CPPA, and 500 mg/L for 3-CPPA. Experimental conditions: ACN/0.1 M ammonium acetate (pH 6.0) (60/40, v/v) at a flow rate of 20 µL/min, detection at 210 ± 2 nm. Experiments were performed at room temperature.

Figure 4. Chromatograms of the analyzed commercial herbicide sample diluted 200 times having a concentration of R-mecoprop of 420 mg/L according to the labeled content. (Experimental conditions: ACN/0.1 M ammonium acetate (pH 6.0) (60/40, v/v) at a flow rate of 20 µL/min (A); ACN/0.1 M ammonium acetate (pH 6.0) (60/40, v/v) at a flow rate of 10 µL/min (B); ACN/0.1 M ammonium acetate (pH 5.3) (60/40, v/v) at a flow rate of 20 µL/min (C); ACN/0.1 M ammonium acetate (pH 5.3) (80/20, v/v) at a flow rate of 20 µL/min (D). Other conditions as in **Fig. 3**. Peak identification: mecoprop enantiomers are denoted by R and S, MCPA (1), 2,4-D (2), and dicamba (3). Achiral phenoxy acid herbicides (1-3) were only identified in (D).

Figure 5. Chromatograms corresponding to the RLOD (%) for S-mecoprop in a solution containing 1000 mg/L of R-mecoprop and 20 mg/L of S-mecoprop. Experimental conditions as in **Fig. 4D**.

Figure 1.

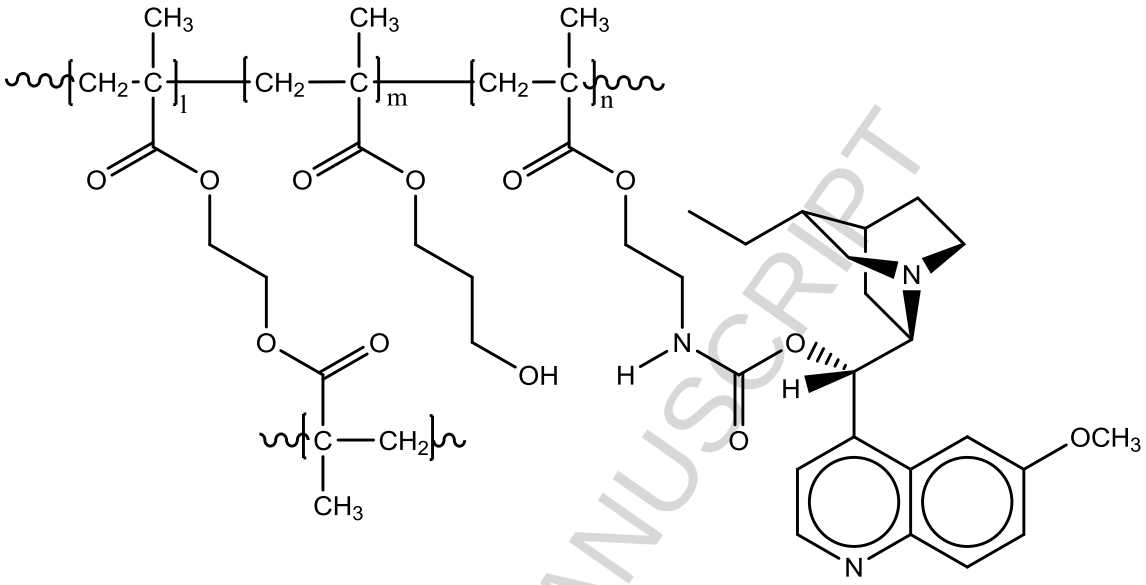

Figure 2.

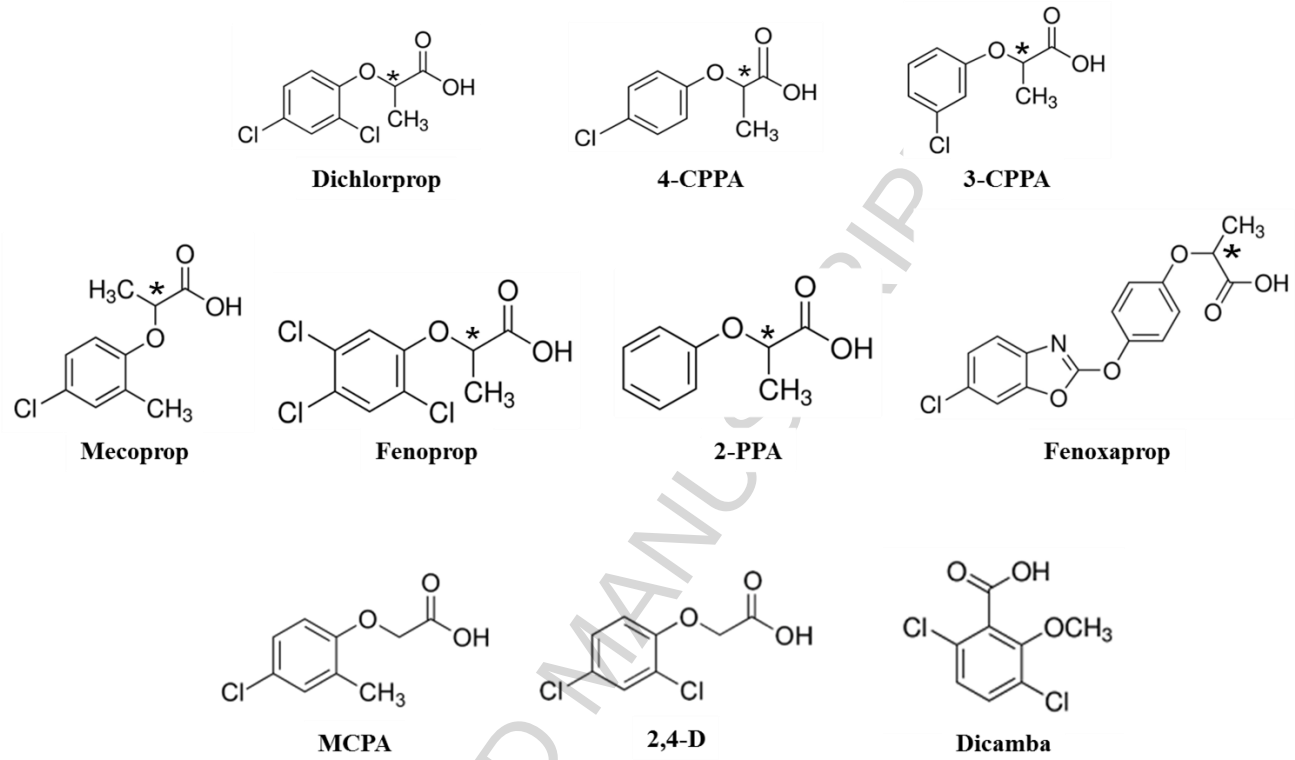

Figure 3.

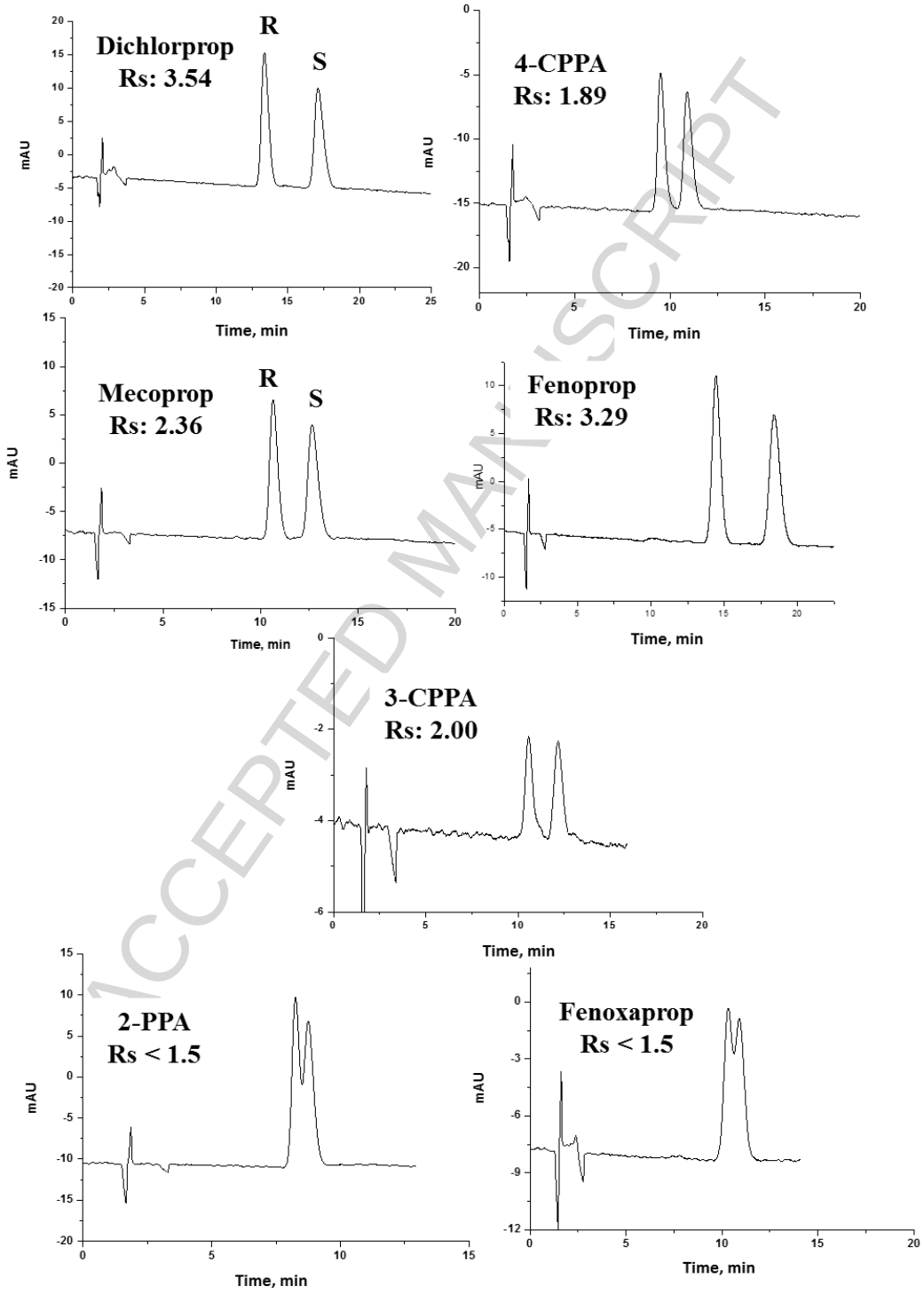

Figure 4.

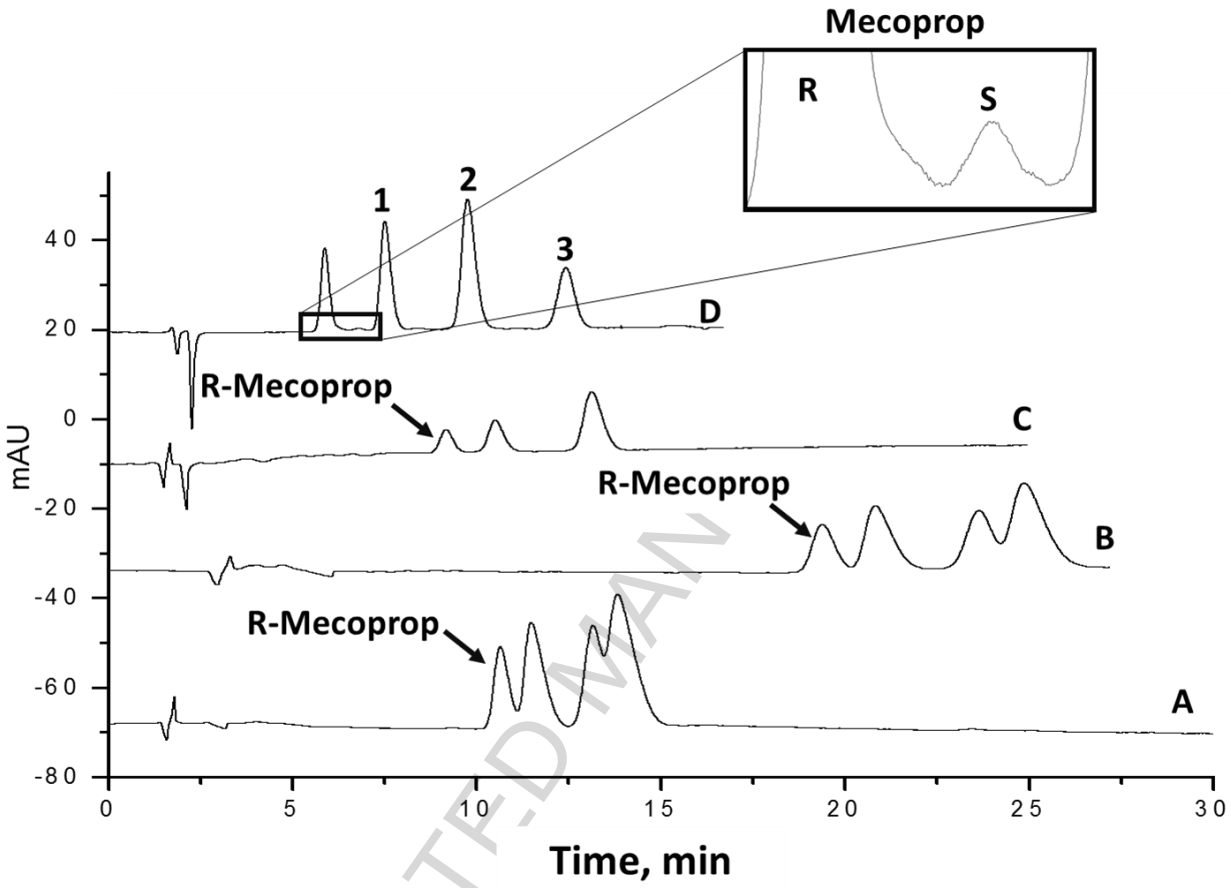

Figure 5.

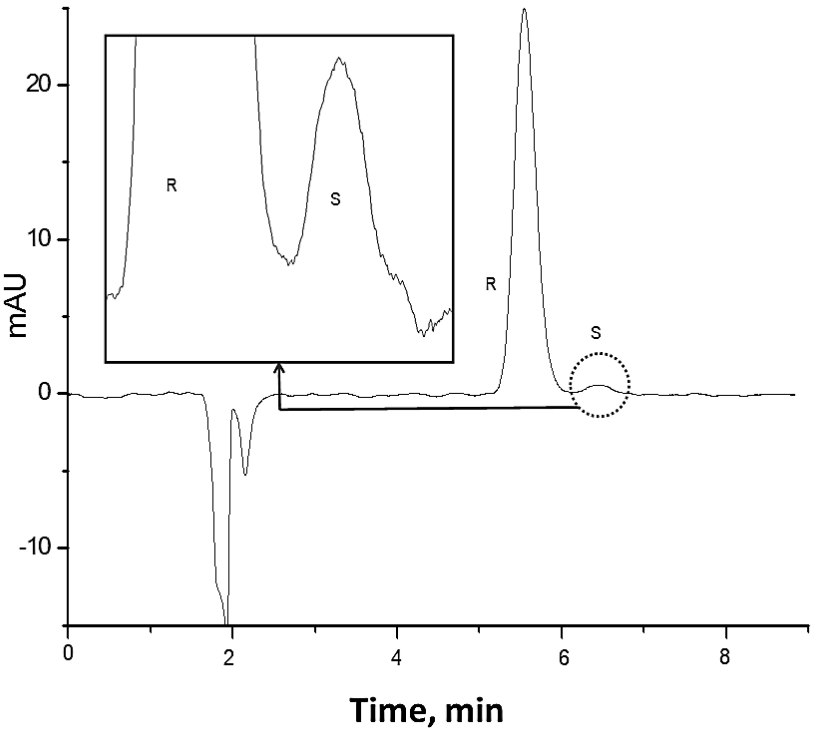

Table 1. Analytical characteristics of the developed method. Experimental conditions as in Fig. 4D.

	R-mecoprop	**S-mecoprop**
External standard calibration method		
Linear range (mg/L)	10-1000	1-100
Linear equation	4241 + 243.826 C	46.4 + 227.3 C
Standard errors	S_a=488 S_b= 0.998	S_a=116 S_b=2.4
R^2	99.9 %	99.7 %
Standard additions calibration method		
Study of matrix interferences (p-value)	0.6200	0.3204
Accuracy		
Recovery	99 ± 3 %	108 ± 9 %
Precision		
Instrumental repeatability		
Time, RSD (%)	1.7	1.4
Area, RSD (%)	0.9	0.9
Intermediate precision		
Time, RSD (%)	5.7	5.6
Area, RSD (%)	7.0	9.6
LOD (mg/L)	6.6	20.0
LOQ (mg/L)	20.0	35.0

S_a and s_b: standard deviation of the intercept and slope, respectively.

Highlights

- Poly(MQD-co-HEMA-co-EDMA) monolithic column was prepared for its use in nano-LC
- Enantiomers of seven phenoxy acid herbicides were separated
- Mecoprop enantiomers were separated from other three achiral herbicides
- R-Mecoprop was quantified in commercial herbicide formulations
- Enantiomeric impurity of R-mecoprop commercial formulation was below the LOQ