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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 kinds 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.

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.

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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 °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 °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.