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Journal of Chromatography A, 1076 (2005) 202–206
JOURNAL OF CHROMATOGRAPHY A
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Short communication

Capillary liquid chromatography of chlorophenoxy acid herbicides and their esters in apple juice samples after preconcentration on a cation exchanger based on polydivinylbenzene-*N*-vinylpyrrolidone

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Received 27 December 2004; received in revised form 7 March 2005; accepted 12 April 2005

Abstract

A capillary liquid chromatography (cLC) method with gradient elution has been used to determine chlorophenoxy acid herbicides: 2,4-dichlorophenoxyacetic acid, 4-chloro-2-methylphenoxyacetic acid, 2-(2,4-dichlorophenoxy)propanoic acid, 2-(4-chloro-2-methylphenoxy)propanoic acid, 4-(2,4-dichlorophenoxy)butanoic acid, 4-(4-chloro-2-methylphenoxy)butanoic acid, 2-(2,4,5-trichlorophenoxy)propanoic acid, 2,4-dichlorophenoxyacetic-1-methyl ester and 2,4-dichlorophenoxyacetic-1-butyl ester in spiked apple juice samples with amounts between 0.025 and 0.150 mg kg^{-1} of each herbicide. Clean-up and preconcentration of acid and esters were carried out in an Oasis MCX polymer. Detection limits obtained by cLC, between 0.005 and 0.018 mg kg^{-1}, allowed the determination of chlorophenoxy acids and their esters in apple juice samples around the levels permitted by the European Regulations, with recoveries in the range 84–99% and RSDs between 1 and 4%.

Keywords: Chlorophenoxy acid herbicides; Capillary high performance liquid chromatography; Apple juice; Solid phase extraction; Cation exchanger polymer

1. Introduction

Chlorophenoxy acid herbicide formulations, alkyl esters and free carboxylic acids, are widely applied to get rid of unwanted plants. Due to their toxicity, which depends on their chemical form, the European Community has established legal directives to restrict the use and to control their maximum residue levels in several matrices [1].

A number of papers have reported multi-residue methods for the determination of phenoxy acid herbicides in various matrices. High-performance liquid chromatography (HPLC) methods are generally preferred over gas chromatography (GC) ones [2,3], because HPLC can be used without derivatization. Among all the detection techniques, ultraviolet detection (UV) continues to be the most common one [4–8]. Moreover, HPLC allows the simultaneous determination of herbicides in acid and ester forms, which is an important advantage because herbicides in ester form are more hazardous than their acid forms [9].

The scale down of analytical liquid chromatography methods from conventional size LC columns (3.0–4.6 mm I.D.) to capillary columns of 0.15–0.30 mm I.D. promises several advantages, such as significant reduction in the consumption and disposal of solvents. The lack of sensitivity due to the small injection volumes required can be overcome by using larger injection volumes of samples in low elution strength solutions [9–15].

Regarding to clean-up and sample preconcentration, the recently introduced macroporous copolymer poly(divinylbenzene–co-*N*-vinylpyrrolidone) can be suitable to preconcentrate these analytes which included quite strong acids and low polarity esters. It has been used for the solid phase extraction of phenoxy acids and other herbicides from water samples [16]. This polymeric sorbent exhibits both

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doi:10.1016/j.chroma.2005.04.026

hydrophilic and lipophilic retention characteristics, remains wetted by water.

The aims of the present study are to explore the determination of phenoxy acid herbicides in acid and ester form by cLC and to assess the capability of commercial cartridges packed with poly(divinylbenzene–co-*N*-vinylpyrrolidone) macroporous copolymer and functionalized with sulfonic acid groups for their SPE from apple juice samples.

2. Experimental

2.1. Reagents and standards

All reagents and solvents were of analytical reagent grade. HPLC grade methanol and acetonitrile were supplied by Scharlab (Barcelona, Spain), and purified water was obtained from a Milli-Q system from Millipore (Bedford, MA, USA). Pesticides were of a purity between 95 and 99%. 2,4-Dichlorophenoxyacetic acid (2,4-D), 2-(2,4,5-trichlorophenoxy)propanoic acid (2,4,5-TP), 4-chloro-2-methylphenoxyacetic acid (MCPA) were supplied by Aldrich; 2-(2,4-dichlorophenoxy)propanoic acid (2,4-DP), 2,4-D-1-methyl ester, 4-(2,4-dichlorophenoxy)butanoic acid (2,4-DB) were from Sigma and finally 2-(4-chloro-2-methylphenoxy)propanoic acid (MCPP), 4-(4-chloro-2-methylphenoxy)butanoic acid (MCPB) and 2,4-D-1-butyl ester were supplied by Riedel-de-Häen.

Stock solutions were prepared by dissolving 20 mg of each herbicide in 100 mL of methanol. These solutions were stored at 4 °C in the dark for 3 months maximum. Working standard solutions were prepared in methanol by diluting the stock solutions as required. In order to prevent the influence of the possible degradation of pesticides on the results, the working solutions were prepared daily.

2.2. SPE and filtration materials

Solid phase extraction was carried out with Oasis MCX cartridges ([poly(divinylbenzene–co-*N*-vinylpyrrolidone)] functionalized with sulfonic acid groups) (60 mg–3 mL, 30–60 μm particle size, 0.80–1.20 mequiv./g, 7.3–8.9 nm pore size), supplied by Waters (Milford, MA, USA) Material for sample filtering was Osmonics polyester membranes (2.0 μm pore size) from Scharlab (Barcelona, Spain).

2.3. Instrumentation

The SPE procedure was carried out by means of a vacuum manifold supplied by Varian (Harbor City, CA, USA) and connected to a Selecta membrane vacuum pump (Selecta, Barcelona). Simultaneous extraction of twenty samples can be performed.

The cLC system used was an Agilent Model 1100 Series (Agilent Technologies, Spain), which was equipped with a solvent delivery system composed of a binary capillary pump G1376 A and a degasser G1379 A, a Beckman UV detector with programmable variable wavelength 166 Detector System Gold, and a microcell (35 nL, 8.0 mm pathlength) (Beckman, Fullerton, CA, USA). An analytical Column packed with 3 μm Hypersil C_{18} BDS 150 mm × 0.3 mm I.D. (LC Packings, Amsterdam, The Netherlands) was employed for the analyses. All components were interfaced to a Dell computer equipped with a MMX Pentium Processor and Gold Nouveau Chromatography Workstation Software (version 1.6) for Windows (Beckman).

2.4. Procedure

2.4.1. SPE preconcentration

The Oasis MCX cartridge was conditioned with 1.5 mL of methanol to solvate the functional groups of the sorbent, and further with 1.5 mL of purified water. Once the conditioning step had ended, the evaluation of the SPE sorbent to retain the phenoxy acid herbicides was made by passing through the cartridge 25 mL of an aqueous solution containing a mixture of 1.5 μg of 2,4-D-1-butyl ester and 0.3 μg of all the other analytes, at a flow rate of 2 mL min^{-1}. For the retention step, pH was adjusted below 2 by addition of few drops of concentrated H_2SO_4. After loading, elution was accomplished with 1.5 mL of 0.8% phosphoric acid in methanol. The eluate was diluted to 10 mL with purified water to decrease its solvent strength for focusing purposes.

2.4.2. Chromatographic determination of chlorophenoxy acids

A total volume of 20 μL of both standard and samples solutions containing only 15% of methanol for focusing purposes was injected into the LC system and determined under selected chromatographic conditions. Separation of the herbicides was performed using the C_{18} analytical column at a flow rate of 8 μL min^{-1}. For gradient elution, the aqueous component of the mobile phase was 0.8% H_3PO_4 and the organic one was methanol. The following multistep gradient was employed: 40:60 (v/v) methanol:0.8% H_3PO_4 aqueous solution for 25 min, then a linear increase to 70% methanol for another 15 min, and a final isocratic mode at 70% methanol till the end of the chromatogram. This gradient allows the separation in 55 min. UV detection was at 232 nm for all the herbicides with the exception of 2,4-D-1-butyl ester, which was detected at a wavelength of 283 nm.

2.4.3. Preparation of apple juice samples

Six grams of commercial apple juice samples were spiked with the target analytes in a concentration range between 0.025 and 0.150 mg kg^{-1}; then they were left to stabilize for 20–30 min and were diluted to 20 mL with purified water. The resulting solution was adjusted to pH 1 with concentrated H_2SO_4 and filtered through a polyester membrane. The filtered residue was carefully washed three times with 5 mL of pH 1 aqueous solution each, and the resulting final

solutions (around 50 mL) were then percolated through the preconditioned SPE cartridge at 2 mL min^{-1}. A washing step was carried out as follows: 2 mL of the pH 1 aqueous solution, then 2 mL of the 50% methanol aqueous solution, and finally 2 mL of an aqueous solution containing 15% methanol and 0.8% phosphoric acid. The pesticides were eluted with 1.5 mL of methanol containing 0.8% phosphoric acid and diluted to 10 mL for focusing purposes with purified water before the chromatographic analysis by cLC.

3. Results and discussion

3.1. Solid phase extraction of standards

Chlorophenoxy acid herbicides are ionizable compounds (pK_a values 2.7–4.8) that exist in the ionic state at most environmental pH values. Retention on the copolymer sorbent was studied at pH values between 0–2. In this range, the analytes showed recoveries between 80 and 100%. At pH 0, 2,4-D yielded recoveries around 140%, whereas its butyl and methyl esters yielded around 35 and 55%, respectively. This fact was attributed to the hydrolysis of the herbicides in the ester form at strongly acidic media. As a compromise between high recoveries and slow hydrolysis reaction of the esters, pH 1 was the optimum for the retention procedure. At this pH, all phenoxy acid herbicides are neutral molecules and presumably the predominant retention mode is exclusively based on a reversed-phase mechanism [17].

The elution of the retained herbicides at pH 1 was accomplished with 1.5 mL of methanol–60 mM HC1, acetonitrile–60 mM HC1 and methanol–0.8% phosphoric acid. As can be observed in Fig. 1, when the elution was made with 60 mM HC1 in acetonitrile, low recoveries were obtained for some of them, especially for 2,4-D-1-butyl ester. By using 60 mM HC1 in methanol, better recoveries were observed, but only 0.8% phosphoric acid in methanol allowed to achieve recoveries higher than 90% for 2,4-D and their esters. When the elution was made with 2 mL of this solvent, significant differences in the recoveries were found for 2,4-DP only.

With the aim to establish the maximum aqueous volume that can be percolated through the Oasis MCX sorbent, volumes between 25 and 1000 mL containing 1.5 μg of 2,4-D-1-butyl ester and 0.3 μg of all the other herbicides were passed through the cartridges. Breakthrough volumes found were around 700 mL for MCPA, MCPB and 2,4-D-1-methyl ester, and 600 mL for 2,4-DB. For the other herbicides, breakthrough volumes were higher than 1000 mL. This data shows the very strong retention of all the studied compounds.

3.2. Determination of chlorophenoxy add herbicide residues in apple juice samples

The ability of the Oasis MCX cartridge for extraction and preconcentration of phenoxy acid herbicides and their esters

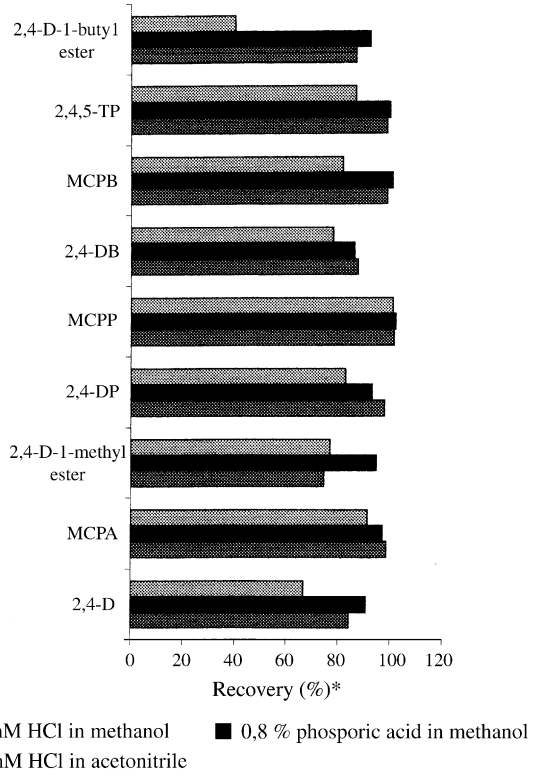

Fig. 1. Influence of solvent on the elution process. (*) Mean of three determinations, volume of solvent 1.5 mL.

was firstly evaluated by passing 6 g of un-spiked and spiked apple juice samples at the levels established by the Spanish and European regulations (0.10 mg kg^{-1} for 2,4-D and MCPA, and 0.05 mg kg^{-1} for all the other herbicides) [1]. In order to avoid clogging of the cartridges, a previous filtration step of the spiked sample was necessary. When nylon, cellulose ester and MicronSep cellulosic membranes were employed, considerable losses of some of herbicides were observed and the recoveries achieved were between 9 and 70%. On the contrary, polyester membranes, showed negligible retention, and a matrix clean-up effect, and thus, this were used for sample filtering. Several amounts of samples were tested in the range between 6 and 10 g in order to achieve the lower limits required and finally, 6 g of apple juice were chosen as suitable sample size. To minimize the effect of co-extracted organic matter, several washing sequences with 2 mL of aqueous solutions containing different ratios of methanol, ammonium hydroxide solution and phosphoric acid were tested. The best results were achieved by the procedure described in Section 2.4.3, allowing to obtain acceptable recoveries for all the studied herbicides.

Analytical characteristics such as detection limits (LODs) and linearity range expressed as mg kg^{-1} were determined in the extracts of 6 g of apple juice. Calibration plots were obtained by preconcentration of free herbicide apple juice samples. The extracts were spiked with increasing amounts of the pesticides assuming that solution volume remained constant after each herbicide addition. The analytical characteristics

Table 1
Analytical characteristics and recoveries of chlorophenoxy acid herbicides added to apple juice samples

Herbicide	LOD (mg kg^{-1})	Linearity range[a] (mg kg^{-1})	Added[b] (mg kg^{-1})	Recovery (%) ± RSD (%)[c]
2,4-D	0.007	0.03–0.17	0.050–0.150	94 ± 3
MCPA	0.010		0.050–0.150	93 ± 2
2,4-D-1-methyl ester	0.010		0.050–0.150	98 ± 2
2,4-DP	0.010	0.02–0.12	0.025–0.100	84 ± 4
MCPP	0.010		0.025–0.100	86 ± 2
2,4-DB	0.005		0.025–0.100	84 ± 3
MCPB	0.005		0.025–0.100	99 ± 1
2,4,5-TP	0.005		0.025–0.100	85 ± 4
2,4-D-l-butyl ester	0.018	0.07–0.17	0.075–0.150	84 ± 2

6 g of apple juice. Injected volume 20 μL.
[a] Five determinations.
[b] Three levels spiked at five determinations for each level.
[c] $n = 15$.

achieved can be observed in Table 1. All analytes showed good linearity in the concentration range investigated, and the correlation coefficients for all the peak area measurements were in the range 0.992–0.999. Regarding LODs, cLC with large injection volumes and focusing solutions provided suitable sensitivity for all the herbicides, even taking into account the dilution needed after SPE to obtain this focusing solution, which contains 85% of water.

To evaluate the applicability of the proposed method, apple juice samples were spiked at various levels, one at the maximum level allowed, another one above it and a third one below it. Since most of the analytical methods involve a previous hydrolysis step, the European and Spanish regulations established limits for these herbicides expressed as their acid forms in vegetable samples; therefore, we have taken the same limits for the ester forms. As can be seen in Table 1, acceptable recoveries between 84 and 99% with RSD in the range 1–4%, were obtained. Similar recoveries for the three studied spiking levels were found for MCPA, 2,4-D-1-methyl ester, MCPP, MCPB, 2,4,5-TP and 2,4-D-1-butyl ester. In the case of 2,4-DP and 2,4-DB, a considerable decrease in the recovery was observed at the lower spiking level. Fig. 2 shows the cLC chromatogram obtained from a standard solution of chlorophenoxy acid herbicides and for an apple juice sample with and without spiked herbicides at the maximum level allowed.

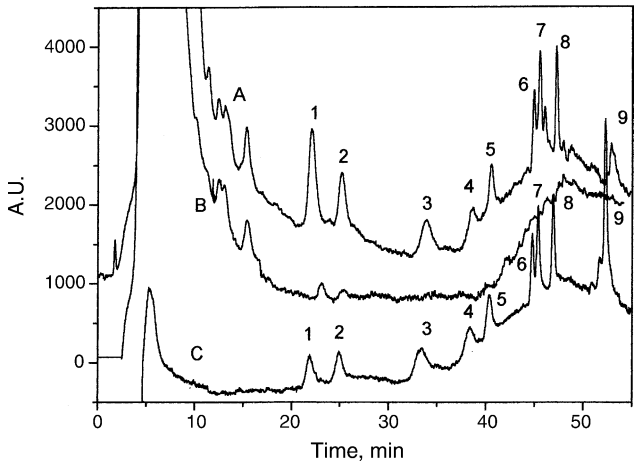

Fig. 2. Chromatograms obtained by cLC under the optimum conditions described in Section 2. (A) Apple juice spiked at the level permitted by regulations (0.10 mg kg^{-1} for 2,4-D and MCPA, and 0.05 mg kg^{-1} for all the other herbicides; (B) apple juice without spiked chlorophenoxy acids, (C) standard solution corresponding to concentrations of 300 μg L^{-1} for 2,4-D-1-butyl ester and 30 μg L^{-1} for all the other herbicides. (1) 2,4-D; (2) MCPA; (3) 2,4,-D-1-methyl ester; (4) 2,4-DP; (5) MCPP; (6) 2,4-DB; (7) MCPB; (8) 2,4,5-TP; (9) 2,4-D-1-butyl ester.

4. Conclusions

Solid phase extraction on poly(divinylbenzene–co-*N*-vinylpyrrolidone) copolymer combined with cLC allows the determination of residues of chlorophenoxy acid and ester herbicides in commercial apple juice samples spiked at levels around the maximum ones permitted by the European regulations, with recoveries between 84 and 99% and reproducibilities between 1 and 4%. The cLC method has provided good LODs in a complex matrix such as apple juice, in addition it involves a lower consumption of hazardous organic solvents and produces fewer residues.

Acknowledgements

Authors wish to thank the Spanish Ministerio de Ciencia y Tecnología (Dirección General de Investigación) for financial support (project BQU-2003-00667). N.R.-C. wishes to thank the Complutense University for support through a predoctoral fellowship.

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