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Short communication

Study of nitrophenols preconcentration using quinolin-8-ol immobilized on controlled-pore glass in the presence of iron(III) Chromatographic determination of dinoseb in lemon juice

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Abstract

The efficiency of an adsorbent consisting of quinolin-8-ol immobilized on controlled-pore glass which was packed in a minicolumn to preconcentrate nitrophenols in the presence of iron(III) has been established. Retention was carried out at acidic pH in the presence of 20 m*M* iron(III). Methanol–30 m*M* formic acid–sodium formate aqueous solution (pH 4.2) (65:35) was used in a one-step elution. Determinations were carried out by using liquid chromatography with detection at 350 nm. Determination of trace amounts of dinoseb in lemon juice was carried out with a minimum sample preparation. Recoveries were between 89 and 100% at 200–33 $\mu g\ l^{-1}$. The maximum admissible concentration in fruit juice dictated by the Spanish and European Community legislation can be measured.

Keywords: Adsorbents; Nitrophenols; Dinoseb; Quinolin-8-ol

1. Introduction

Nitrophenols are used as pesticides and synthetic intermediates; moreover, nitrophenols are also degradation products of organophosphorus pesticides. These organic micropollutants typically have to be determined at the low sub $\mu g\ l^{-1}$ level in water, and at the low mg kg^{-1} level in fruit or vegetables. Analysis at these levels requires a concentration step. Solid-phase isolation has proved to be a good alternative to liquid–liquid extraction because of its simplicity, robustness and potential automation. In addition, most modern pesticides are fairly soluble in water and so are less amenable to extraction with organic solvents.

On-line and off-line solid-phase extraction (SPE) has been applied to the determination of phenols using small pre-columns or disks containing different adsorbents, such as octadecyl-bonded silica [1–3], styrene–divinylbenzene copolymers [2–9], graphitized carbon black [8,10–11], chemically modified polymeric sorbent with a benzoyl group [9], and quinolin-8-ol (oxine) immobilized on controlled pore glass [12]. These methods have been applied to the extraction of phenols mainly from water samples. Liquid chromatography (LC) in combination with

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these preconcentration techniques using small cartridges [2,3,6–11,13] or membrane extraction disks [2,4] is preferred, although gas chromatographic techniques [14] or supercritical fluid chromatography [15] could be used. Octadecyl-bonded silica (C_{18}) is one of the most popular reversed-phase sorbents, but the most hydrophilic phenols have a low tendency to be retained on the C_{18} surface [2,10]. Styrene–divinylbenzene copolymers were found to be the most suitable sorbents, but problems such as low recoveries for the most polar analytes still occur [7,8]. In order to decrease detection limits and increase the reproducibility and potential automation, several authors have used on-line instead of off-line SPE to determine phenolic compounds using C_{18}, PLRP-S [2] or more specific sorbents such as ENVI-Chrom P [16].

Quinolin-8-ol (oxine) has been used in immobilized form for the preconcentration of pentachlorophenol [12], as well as for LC separation of the anions [17], metal-assisted separation of phenols [18], preconcentration of ethynyl steroids [19] and preconcentration of buturon [20]. Controlled-pore glass (CPG) has been the support of choice in some of these applications because the immobilization reactions on this support are relatively simple and also because it exhibits the good mechanical strength and swelling stability required for the flow systems.

The purpose of this paper is to study the retention of nitrophenols such as 2-nitrophenol, 4,6-dinitro-2-methylphenol and 2,4-dinitro-6-secbutylphenol (dinoseb) for preconcentration on oxine immobilized covalently on CPG and assisted by Fe(III) for chromatographic separation. The method has been applied to the analysis of dinoseb in lemon juice.

2. Experimental

2.1. Equipment

The chromatographic experiments were performed with a Perkin-Elmer isocratic LC pump coupled with a Perkin-Elmer Model LC 290 UV–Vis detector. Chromatographic separation of the nitrophenols was performed with a reversed-phase Spherisorb 5 ODS (2) column (150×4.6 mm). The analyte quantitation was carried out with the Nelson software package. For preconcentration purposes, a Wiz programmable peristaltic pump connected to a minicolumn (20×2.5 mm) filled with oxine immobilized on CPG was used.

2.2. Reagents

All chemicals were of analytical reagent grade and purified water was obtained using a Milli-Q apparatus (Millipore). Standards of 2-nitrophenol (>99% pure, Fluka), 4,6-dinitro-2-methylphenol (98% pure, Chem Service) and 2,4-dinitro-6-secbutylphenol (99% pure, Riedel-de Haën) were used for preparing 500 mg l^{-1} stock standard solutions in $1.0 \cdot 10^{-3}$ *M* sodium hydroxide solution. Diluted working solutions were prepared daily by diluting with Milli-Q water. All solutions were stored at 4°C in a refrigerator. (Aminophenyl)trimethoxysilane (95% pure, ABCR) was supplied as mixed isomers and stored under refrigeration. Controlled pore glass (CPG-240-200, Sigma) was boiled in 5% nitric acid for 30 min, filtered on a glass filter, washed with deionized water and dried in an oven at 95°C. HPLC-grade methanol (Carlo Erba), formic acid (Carlo Erba) and Milli-Q water were used to prepare the mobile phase. Hydrochloric acid (Probus) and iron(III) nitrate were used to condition the preconcentration column.

2.3. Immobilization procedure and storage

Immobilization on CPG was achieved following the procedure described by Marshall and Mottola [21]. The product was packed in a glass microcolumn (20×2.5 mm I.D.) so that the length of the immobilized oxine zone was 15 mm. The column filled with the immobilized oxine was stored in a refrigerator at 4°C and pH 1. The dried material was stored in a desiccator. The efficiency of the immobilization procedure was evaluated indirectly [12].

2.4. Chromatographic conditions

A reversed-phase Spherisorb 5 ODS (2) analytical column (150×4.6 mm I.D.) was used. Isocratic elution was carried out with a mobile phase of 30 m*M* formic acid–sodium formiate (pH 4.2)–methanol (35:65). The wavelength for the determination of

the analytes was 350 nm. Quantitation was performed by using external standard calibration methods.

2.5. Off-line liquid–solid extraction

The glass minicolumn (20×2.5 mm I.D.) was packed from a slurry of oxine–CPG in water with the help of a peristaltic pump at a flow-rate of 1.2 ml min^{-1}. Conditioning of the preconcentration column was carried out with 10.0 ml of 0.1 *M* hydrochloric acid, then with 10 ml of 0.4 *M* hydrochloric acid and finally with 10 ml of 20 m*M* iron(III) nitrate at 1.0 ml min^{-1} for retention of 2-nitrophenol and 4,6-dinitro-2-methylphenol. Conditioning of the column for the retention of dinoseb was carried out with 10 ml of 20 m*M* iron(III) nitrate at 1.0 ml min^{-1}. Analytes were directly eluted by the mobile phase used for the chromatographic separation. Nitrophenols were eluted with 2.0 ml of mobile phase at 1.0 ml min^{-1}, and 20 μl was injected into the chromatographic system. Before the next preconcentration, the column was washed with 10 ml of acetonitrile at 1.0 ml min^{-1}.

3. Results and discussion

3.1. Optimization of chromatographic conditions

Because the pK_a values for dinoseb, 4,6-dinitro-2-methylphenol and 2-nitrophenol are 4.4, 4.31 and 7.23, respectively, separation studies were carried out at pH values between 3.5 and 4.4, buffered with acetic acid–sodium acetate or with formic acid–sodium formiate. Several water–methanol mixtures were studied as mobile phases. At the same water–methanol ratio, retention times at pH 3.5 were lower when aqueous solutions were buffered with formic acid–sodium formiate than those obtained when solutions were buffered with acetic acid–sodium acetate. From these results, a 30 m*M* formic acid–sodium formiate (pH 4.2) aqueous solution–methanol (35:65) mobile phase was chosen for further studies. Fig. 1 shows the chromatogram in the optimum conditions.

3.2. Analytical characteristics for standards

A good linear response in the range 0.1–30 mg l^{-1} (linear regression coefficient between 0.9996 and 0.9998) was achieved for the three nitrophenols. The limits of detection (3/1 signal-to-noise ratio) were 60 μg l^{-1} for dinoseb, 10 μg l^{-1} for 4,6-dinitro-2-methylphenol and 20 μg l^{-1} for 2-nitrophenol. The reproducibility of the chromatographic method for 1.5 mg l^{-1} (n=5) of each compound was between 1.2 and 1.3%.

3.3. Off-line nitrophenol enrichment

On a previous study [12], nitrophenols showed some retention on oxine depending on the number of substituents and their relative positions in the aromatic rings. Furthermore, the presence of an alkyl group resulted in a greater retention on the oxine. When the preconcentration column was conditioned with 0.1 *M* hydrochloric acid and then with iron(III) solution retention on immobilized oxine increases.

As indicated above, an iron(III)-loaded 8-quinolinol silica gel adsorbent was used as stationary phase for the HPLC separation of the phenols [18]; iron(III) was chosen as the metal ion because it has a large formation constant with oxine and also because it has a high affinity for oxygen and should interact readily with the hydroxyl moiety of the phenols. When the minicolumn was conditioned first with 0.1 *M* hydrochloric acid and then with 10 ml of 10 m*M* iron(III), preconcentration of 1.5 mg l^{-1} of each nitrophenol yielded recoveries of 4, 50 and 29% for 4,6-dinitro-2-methylphenol, 2-nitrophenol and dinoseb, respectively. The influence of the concentration of iron(III) solutions was studied in the 10–30 m*M* range. Recoveries did not experiment changes at concentrations higher than 20 m*M*; with this concentration, recoveries of 10, 50 and 85% were obtained for 4,6-dinitro-2-methylphenol, 2-nitrophenol and dinoseb respectively; therefore, 20 m*M* was used for further studies. Further conditioning studies show that it is not necessary to use hydrochloric acid for retention of dinoseb (recoveries around 93%), but if no hydrochloric acid is used, the recoveries for 2-nitrophenol and 4,6-dinitro-2-methylphenol were 29 and 6%, respectively. Hydrochloric acid solutions 0.1, 0.2 and 0.4 *M* were

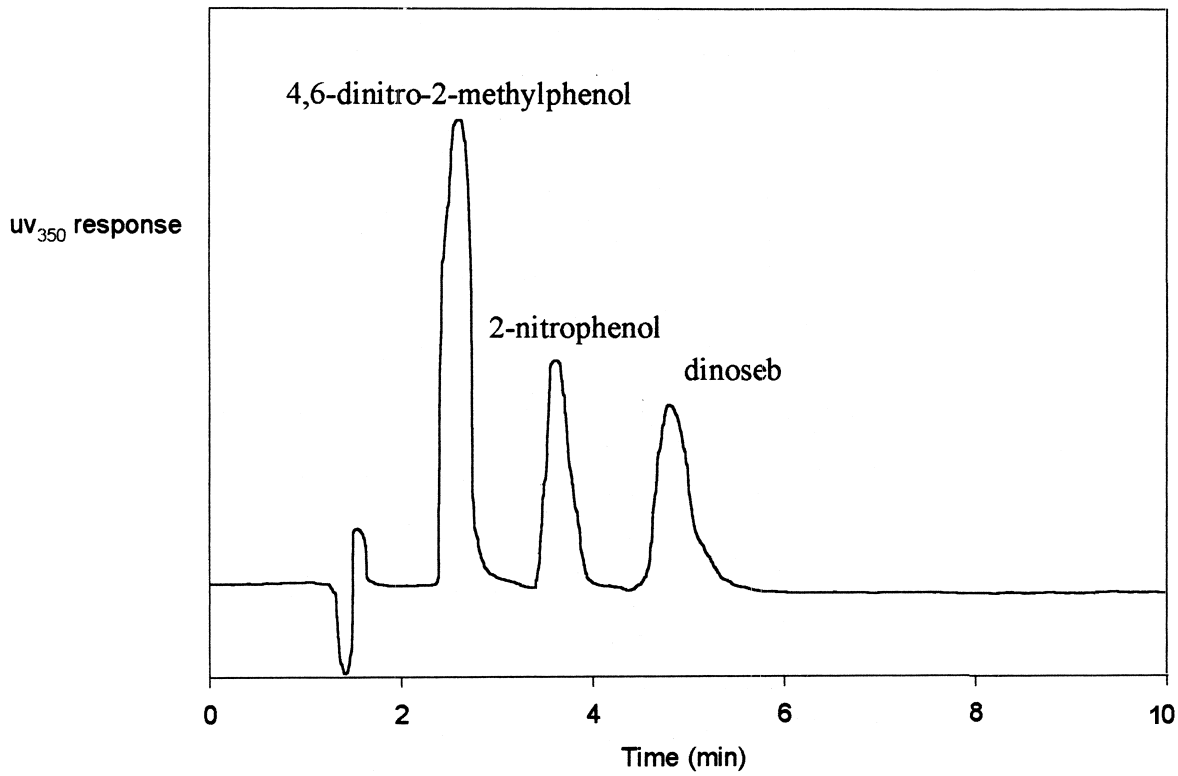

Fig. 1. HPLC–UV (350 nm) chromatogram of nitrophenols; 2-nitrophenol and 4,6-dinitro-2-methylphenol, 1.0 mg l^{-1}; dinoseb, 2 mg l^{-1}. HPLC conditions, see Section 2.4.

studied as preconditioning solutions. The best results for 2-nitrophenol and 4,6-dinitro-2-methylphenol, recoveries of 64 and 35% respectively, were obtained when two aliquots of this acid (0.1 and 0.4 *M*) were used. It seems that the use of these two solutions produce the protonation of the free silanol groups of the controlled pore glass, thus minimising the electrostatic repulsion between controlled pore glass and nitrophenols. Elution was carried out with the mobile phase used in the chromatographic separation.

Before the next preconcentration, the oxine minicolumn was washed with 10 ml of acetonitrile at 1.0 ml min^{-1}; a lower amount of acetonitrile cannot be used because it would produce lower recoveries in the subsequent preconcentration Once the conditioning step was established for each nitrophenol, the reproducibility for the recovery of 1.6 mg l^{-1} of each nitrophenol was 10% for 4,6-dinitro-2-methylphenol, 6% for dinoseb and 3% for 2-nitrophenol; in all cases, variability of the preconcentration process became unacceptable when the preconcentration column was used more than four times. Under the retention conditions tested, 2-nitrophenol and 4,6-dinitro-2-methylphenol are not ionized and several types of interactions may be involved in the retention: complex formation with the oxine–iron(III), hydrogen bonding and $\pi-\pi$ and dispersion interactions. In the case of dinoseb, the presence of hydrochloric acid, and hence the pH, seems not to affect the retention of the nitrophenol, but the presence of iron(III) seems to be essential for retention.

The influence of the flow-rate on the retention of nitrophenols was evaluated between 0.6 and 1.2 ml min^{-1}. At flow-rates higher than 1.0 ml min^{-1}, retention decreased, being only about 42% for dinoseb at 1.2 ml min^{-1}. For flow-rates between 0.6 and 1.0 ml min^{-1}, retention was higher than or around 90%; therefore, 1.0 ml min^{-1} was chosen for

Table 1
Determination of dinoseb in lemon juice

Lemon juice volume (ml)	Dinoseb added (μg)	Dinoseb found (μg)	Recovery (%) (SD[a])
10	2.0	1.8	89 (9)
10	0.86	0.84	98 (8)
10	1.40	1.34	96 (5)
25	1.23	1.23	100 (4)
25	0.86	0.83	95 (7)

[a] $n=3$.

further studies. The elution flow-rate was studied between 0.6 and 1.0 ml min^{-1}; no influence was observed in this range, so 1.0 ml min^{-1} was selected for further studies.

The capacity of retention of the column was studied between 0.8 and 6.0 mg l^{-1} of dinoseb. The average recovery was 94% for concentrations in the 0.8–4.0 mg l^{-1} range with standard deviation (SD) of 6%. The maximum amount of nitrophenols retained by the column per gram of oxine–CPG was established as 0.4 mg when dinoseb was used.

The breakthrough volume was determined by studying the recovery of 10 μg of dinoseb in volumes between 10 and 100 ml. For volumes of 10, 25 and 50 ml, recoveries were 93, 88 and 82%, respectively, with SDs equal to or lower than 9%. Volumes higher than 50 ml produce lower recoveries, but, taking into account that a volume of 2

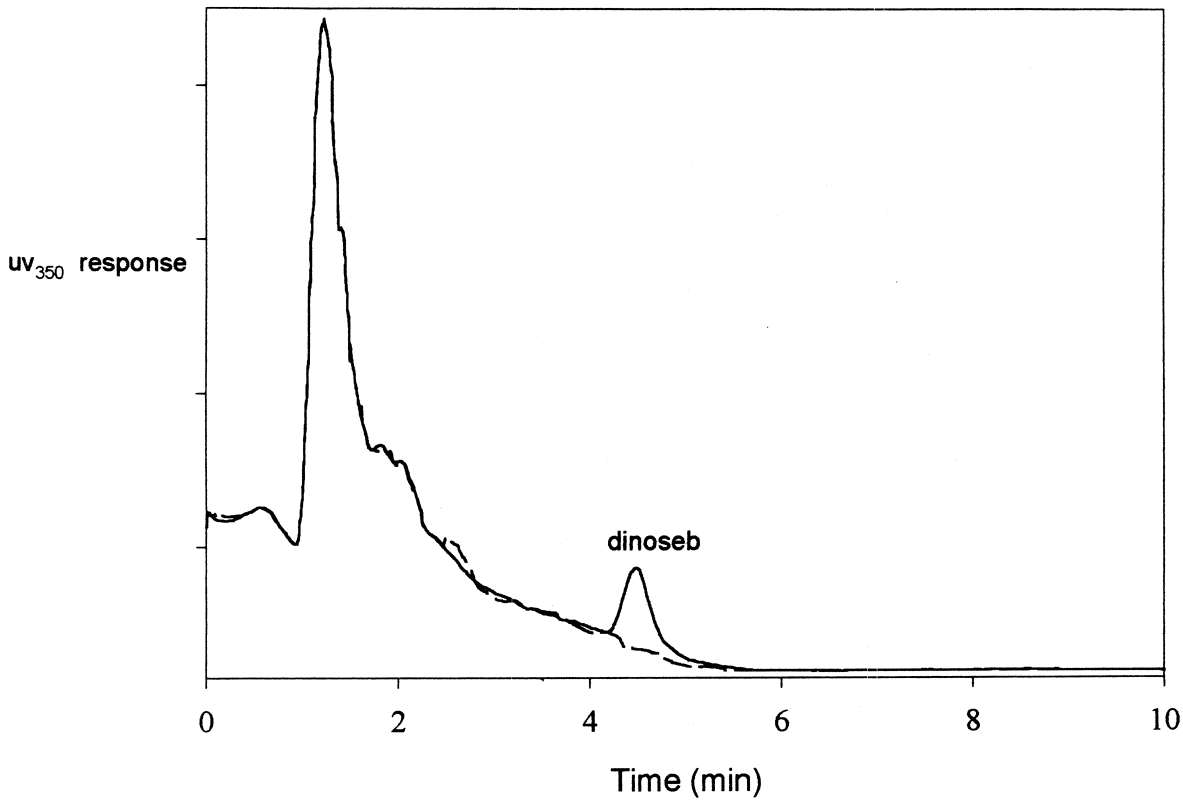

Fig. 2. HPLC–UV (350 nm) chromatograms of 25 ml of lemon juice. Solid line: sample spiked with 1.23 μg of dinoseb after preconcentration; dotted line: non-spiked sample after preconcentration. For conditions, see Section 2.4.

ml of mobile phase is enough to completely elute the nitrophenols, a preconcentration factor of 25 can be achieved.

In the optimum conditions established, limits of detection in distilled water for 2-nitrophenol, 4,6-dinitro-2-methylphenol and dinoseb were 3.3, 2.3 and 5.0 μg l^{-1}, respectively.

3.4. Determination of dinoseb in lemon juice

To evaluate the applicability of the proposed method, commercial lemon juice samples were spiked with different amounts of dinoseb. The amounts added are around the maximum level established by the Spanish legislation, which is 50 μg l^{-1} [22]. Just after filtration, the preconcentration process was carried out and dinoseb was determined by the chromatographic separation described above. Results are shown in Table 1. Final recoveries were in the 89–100% range, with SDs between 9 and 4%. The chromatograms (Fig. 2) of the analytical solution resulting from the oxine preconcentration step show a dinoseb peak clearly separated from the endogenous compounds of the lemon juice. Detection limit (3/1 signal-to-noise ratio) was 17 μg l^{-1} for dinoseb in lemon juice.

4. Conclusions

The retention of nitrophenols on oxine immobilized on controlled pore glass can be controlled through the conditioning step. The retention of these nitrophenols in the presence of iron(III) is higher when the 2-position in the aromatic ring is occupied with a nitro-group. Retention of 2-nitrophenol and 4,6-dinitro-2-methylphenol was not possible without previously conditioning the preconcentration column at acidic pH. The acid–base characteristics seem not to be so important, since pK_a of dinoseb and 4,6-dinitro-2-methylphenol are very similar, while pK_a of 2-nitrophenol is higher; but the presence of Fe(III) is essential for preconcentration purposes. The application to lemon juice requires a minimum sample preparation.

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References

[1] S. Burtz, H.J. Stan, Anal. Chem. 67 (1995) 620–630.
[2] D. Puig, D. Barceló, Chromatographia 40 (1995) 435–444.
[3] M. Castillo, R. Domingues, M.F. Alpendurada, D. Barceló, Anal. Chim. Acta 353 (1997) 133–142.
[4] O. Jáuregui, M.T. Galceran, Anal. Chim. Acta 340 (1997) 191–199.
[5] C. Elvira-Cozar, P. Cano-Faura, L.V. Pérez-Arribas, M.E. León-González, L.M. Polo-Díez, Chromatographia 40 (1995) 91–95.
[6] J. Slobodnik, M.G.M. Groenewegen, E.R. Brouer, H. Lingeman, U.A.Th. Brinkman, J. Chromatogr. 642 (1993) 359–370.
[7] E. Pocurull, R.M. Marcé, F. Borull, J. Chromatogr. A 738 (1996) 1–9.
[8] D. Puig, D. Barceló, J. Chromatogr. A 733 (1996) 371–381.
[9] N. Masqué, M. Galiá, R.M. Marcé, F. Borull, Analyst 122 (1997) 425–428.
[10] A. Di Corcia, S. Marchese, R. Samperi, G. Cecchini, L. Cirilli, J. AOAC Int. 77 (1994) 446–453.
[11] A. Di Corcia, A. Bellioni, M. Diab Madbouly, S. Marchese, J. Chromatogr. A 733 (1996) 383–393.
[12] C. Moreno-Román, M.R. Montero-Escolar, M.E. León-González, L.V. Pérez-Arribas, L.M. Polo-Díez, Anal. Chim. Acta 288 (1994) 259–264.
[13] M. Castillo, D. Puig, D. Barceló, J. Chromatogr. A 778 (1997) 301–311.
[14] Z. Voznakova, J. Podehradska, M. Kohlickova, Chemosphere 33 (1996) 285–291.
[15] E. Pocorull, R.M. Marce, F. Borull, J.L. Bernal, L. Toribio, M.L. Serna, J. Chromatogr. A 755 (1996) 67–74.
[16] E.R. Brouwer, U.A.Th. Brinkman, J. Chromatogr. A 678 (1994) 223–231.
[17] M.-S. Kuo, H.A. Mottola, Anal. Chim. Acta 120 (1989) 255–266.
[18] G.J. Shahwan, J.R. Jezorek, J. Chromatogr. 256 (1983) 39–48.
[19] M.W.F. Nielen, R.E.J. van Soest, H.E. van Ingen, A. Farjam, R.W. Frei, U.A.Th. Brinkman, J. Chromatogr. 417 (1987) 159–167.
[20] M.W.F. Nielen, H.E. van Ingen, A.J. Valk, R.W. Frei, U.A.Th. Brinkman, J. Liq. Chromatogr. 10 (1987) 617–633.
[21] M.A. Marshall, H.A. Mottola, Anal. Chem. 55 (1983) 2089–2093.
[22] Ministerio de la Presidencia, R.D. 280 (1994), Productos Vegetales, Límites Máximos y Control de Residuos de Plaguicidas B.O.E. No. 58 2004, 1994, (Transposition of the Directives of the European Council Nos. 90/642/CEE, 93/57/CE, 93/58/CEE, 97/700/CEE)