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Trace-level determination of polar phenolic compounds in aqueous samples by high-performance liquid chromatography and on-line preconcentration on porous graphitic carbon

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ABSTRACT

The use of porous graphitic carbon (PGC) was investigated for the trace enrichment and the on-line liquid chromatographic separation of polar phenolic compounds (phenol, di- and trihydroxybenzenes, aminophenols, etc.) from aqueous samples. Comparison between retentions obtained with PGC and with the copolymer-based sorbent PRP-1 showed similar variations of the capacity factors with the mobile phase composition, but an inverse retention order. The capacity factor of a very polar analyte, such as 1,3,5-trihydroxybenzene (phloroglucinol), is 1000 in pure water, whereas this analyte is not retained by C_{18}-silica and is poorly retained by PRP-1 (k' = 3 in water). A precolumn packed with PGC can be coupled to a PGC analytical column for simple separation in the reversed-phase mode. This methodology has been applied to the direct determination of pyrocatechol, resorcinol and phloroglucinol below the 0.1 μg/l level in a 50-ml sample.

INTRODUCTION

There is an increasing need for trace-level determinations of high-polarity compounds in aqueous environmental samples. On-line preconcentration on short precolumns prior to liquid chromatography has been shown to be useful in the trace determination of organic compounds in aqueous environmental or biological samples. As a first approach, liquid–solid extraction can be described as a simple chromatographic process with aqueous media as the mobile phase and the sorbent as the stationary phase; during the enrichment step, the analytes should be well retained by the sorbent and not eluted by water. Convenient sorbents are therefore reversed-phase materials, such as the widely used octadecyl-bonded silicas (C_{18}), some divinylbenzene–styrene copolymers and carbon-based sorbents. Trace enrichment of apolar compounds can be effected efficiently with C_{18}-silicas. Carbon-based materials and copolymer-based sorbents (*e.g.*, PRP-1 or PLRP-S) were found to have an enhanced affinity for medium-polarity compounds such as mono- and dichlorophenols [1–4].

Graphitized carbon black (GCB) has proved to be as an excellent gas chromatographic adsorbent in a wide variety of applications [5], but practical application in high-performance liquid chromatography (HPLC) is prevented by its poor mechanical properties [6]. GCB has also been employed in prepacked cartridges for off-line preconcentrations of medium-polarity organic compounds [7–9]. Colin *et al.* [10] strengthened GCB by deposition of pyrolytic carbon. The first attempts to pack precolumns with carbon materials for on-line preconcentration were made by Werkhoven-Goewie and co-workers [1–3] with these pyrocarbon sorbents. Although this material was never commercially available, their studies have shown that pyrocarbon sorbents are much better suited than C_{18}-silicas for preconcen-

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trating analytes containing polarizable substituents, such as nitro, phenyl or halogen. Nevertheless, similar results have been obtained by using PRP-1 sorbent for the preconcentration of these compounds [2].

Significant progress in the preparation of carbonaceous stationary phases for HPLC has been made in the last decade [11], and columns with high-efficiency porous graphitic carbon have become commercially available, but no development of their use for on-line preconcentration has been reported. It has been shown that PGC acts as a strong reversed-phase sorbent; it behaves like C_{18}-silica but requires eluents containing a lower proportion of water for equivalent retention. PGC also has unique separation qualities, such as selectivity for diastereoisomers and for closely similar geometric isomers [11–19]. Nevertheless, Colin and co-workers [12,13] reported that the retention order can be different from that obtained with C_{18}-silica for hydroxybenzenes and nitrobenzenes, indicating differences in the adsorption of polar solutes. In this work, PGC was investigated as a sorbent for preconcentration of these compounds from aqueous media and also as a stationary phase for HPLC. The retentions of phenol and di- and trihydroxybenzenes were studied with mobile phases having a high water content and compared with values obtained with C_{18}-silicas and PRP-1 copolymer.

EXPERIMENTAL

Apparatus

On-line percolation of water was performed with a Milton Roy pump (LDC, Riviera Beach, FL, USA). Precolumn elutions and analyses were carried out with a Varian (Palo Alto, CA, USA) Model 5060 liquid chromatograph equipped with a variable-wavelength UV 200 spectrophotometer and a Coulochem Model 5100 electrochemical detector (ESA, Bedford, MA, USA). Precolumns and analytical column switching were connected with two Rheodyne (Berkeley, CA, USA) valves. Quantitative measurements of peak areas were provided by a CR3A integrator–computer from Shimadzu (Kyoto, Japan).

Stationary phases and columns

A commercial column packed with Hypercarb porous graphitic carbon (100 × 4.6 mm I.D., 7 μm particle size) (Shandon, Runcorn, UK), a column laboratory-packed with PRP-1 copolymer (100 × 4.6 mm I.D., 10-μm particle size) (Hamilton, Reno, NV, USA) and a column laboratory-packed with LiChrosorb RP-18 (150 × 4.6 mm I.D., 5-μm particle size) (Merck, Darmstadt, Germany) were used. Stainless-steel precolumns (10 × 2 mm I.D.) from Chrompack (Middelburg, Netherland) and laboratory-made stainless-steel precolumns (22 × 4.6 mm I.D. or 27 × 4.6 mm I.D. were used for high-retention measurements in water-rich mobile phases and for on-line preconcentration. Precolumns were laboratory-packed using a thick slurry and a microspatula.

Chemicals

HPLC-grade acetonitrile was obtained from Rathburn (Walkerburn, UK) and methanol from Prolabo (Paris, France). LC-grade water was prepared by purifying demineralized water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). Other chemicals were purchased from Prolabo, Merck or Fluka (Buchs, Switzerland). Stock solutions of selected solutes were prepared by weighing and dissolving them in methanol. LC-grade water samples were spiked with these solutions at the μg/l or ng/l level. The final standard solutions did not contain more than 0.5% of methanol.

Procedure

Retention volumes were measured for each mobile phase composition and reported values are the means of three measurements, made using either long columns for low retention volumes or shorter columns for longer retention volumes in water-rich eluents. Mobile phases were obtained by mixing methanol and a 0.05 *M* solution of perchloric acid–lithium perchlorate (pH 4) in different proportions. The flow-rate was 1 ml/min and the temperature was 25°C. The void volume was determined by injection of a 2 *M* solution of sodium nitrate for each mobile phase composition and capacity factors were calculated with this value for each mobile phase. Columns were equilibrated by percolating at least 100 ml of mobile phase before injection. Solutes were dissolved in the mobile phase at 25 ppm concentration and 20 μl were injected.

The on-line experimental set-up was as described

in ref. 4, for instance, the stainless-steel precolumn being placed in the sample-loop position of the six-port liquid switching valve. The water sample was adjusted to pH 6 with perchloric acid and percolated through the PGC precolumn. The precolumn was flushed with 2 ml of LC-grade water adjusted to pH 6 with perchloric acid and was then backflush-eluted with an analytical methanol–water or acetonitrile–water gradient.

Drinking water samples were analysed without any filtration.

RESULTS AND DISCUSSION

Comparison of retentions on PGC, PRP-1 and C_{18}-silica

These three stationary phases are non-polar materials used in reversed-phase chromatography. However, differences exist between these packings. With C_{18}-silicas, solute–stationary phase interactions are weak and non-selective whereas they play an important role with the two other sorbents. Fig. 1 shows the plots of the logarithm of the capacity factor (log k') against the methanol volume fraction (φ) in the water–methanol mobile phase. The addition of methanol to the mobile phase results in the same dependences for the three packings, showing a reversed-phase behaviour. The differences are in the retention values and retention order. It is clear that both PRP-1 and PGC are much more hydrophobic than C_{18}-silica, as shown by considering the retention of phenol. When comparing the plots obtained with C_{18}-silica (Fig. 1a) with those obtained with PRP-1 (Fig. 1b), we observe the same decreasing retention order with the solute polarity from phenol to phloroglucinol. Plots for phloroglucinol with C_{18}-silica have not been reported; this polar analyte is not retained by C_{18}-silica with methanol-rich mobile phases and has been proposed as an experimental probe for the determination of the void vol-

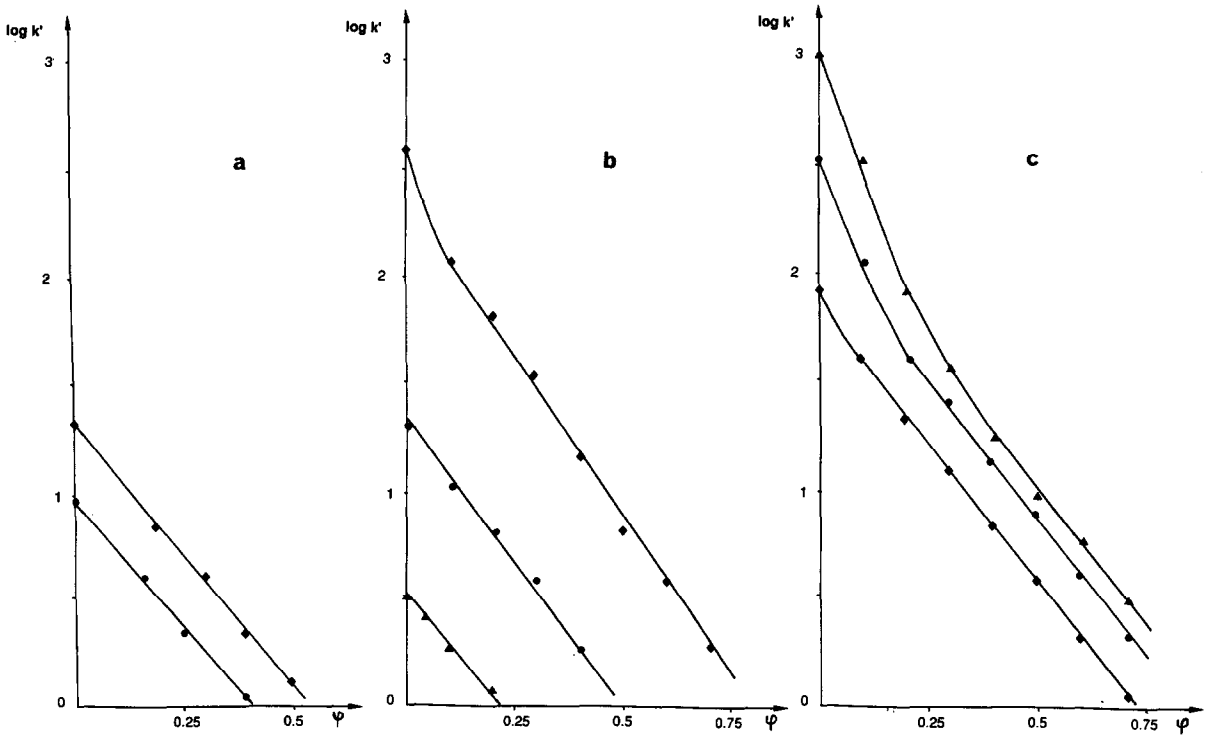

Fig. 1. Variations of capacity factors (log k') with the mobile phase composition obtained on (a) LiChrosorb RP-18, (b) PRP-1 and (c) PGC. Solutes: ◆ = phenol; ● = resorcinol; ▲ = phloroglucinol; φ is the volume fraction of methanol in the mobile phase [mixture of methanol and a 0.05 M solution of perchloric acid–lithium perchlorate (pH 4)]. Flow-rate, 1 ml/min; unretained compound, 2 M sodium nitrate.

ume of the column [20]. In pure water, it is very slightly retained (log k'_w = 0.5).

The capacity factors with PRP-1 are much higher than those with C_{18}-silica for the three solutes represented, k'_w of phenol being 15 times higher with PRP-1. The observed stronger retentions are explained by the π–π interactions between these aromatic compounds and the styrene–divinylbenzene matrix of the PRP-1 sorbent, which do not exist with the *n*-alkyl chains of C_{18}-silicas. When comparing plots obtained with PGC (Fig. 1c) with those obtained with C_{18}-silica and PRP-1, it can be observed that the retention order is reversed and increases from the less polar phenol to the more polar phloroglucinol. Phenol is four times more retained by PRP-1 than by carbon, indicating that interactions between phenol and PRP-1 are stronger than those between phenol and PGC. This result is in agreement with the results of Werkhoven-Goewie *et al.* [2], showing the superiority of PRP-1 over pyrocarbon-modified carbon black for preconcentrating monochlorophenol and dichlorophenols.

More surprising is the great difference in retention for phloroglucinol with k'_w values of 1050 with PGC, only 3 with PRP-1 and 0.3 with C_{18}-silica. This can only be explained by different retention mechanisms of polar solutes and by a strong interaction of hydroxy groups with polar species at the carbon surface. Further work is in progress to explain these interactions.

One can see easily the potential use of this sorbent for preconcentrating these non-volatile and very polar phenolic compounds directly from water samples. Chlorophenols are an important class of environmental pollutants and some of their degradation products by photolytic dehalogenation are less chlorinated and more hydroxylated phenols. One can expect in environmental waters that some of the degradation products of many pollutants will be hydroxylated.

Other hydroxylated phenolic compounds are now being tested and the first results are presented in Table I. Capacity factors measured in pure water are reported for PRP-1 and for PGC stationary phases. Retention volumes were calculated for a 2.2 cm × 0.46 cm I.D. precolumn in order to investigate the sample volume that can be handled for preconcentration because, to a first approximation, breakthrough volumes can be calculated from retention volumes in water [2–4]. Except for phenol, the solutes tested are much more retained by the PGC sorbent than by the PRP-1 sorbent. Their retention in water is sufficient for an on-line preconcentration and HPLC analysis. As indicated above, the capacity factor increases with increasing number of hydroxy substituents on the aromatic ring but, when examining capacity factors of di- and trihydroxybenzenes, 1,3- and 1,4-dihydroxybenzene give similar results (331 and 228) whereas 1,2-dihydroxybenzene has a capacity factor of 120, closer to that of phenol (81); the capacity factor of 1,2,3-trihydroxybenzene is between those of phenol and resorcinol or hydroquinone. When two hydroxy groups are substituted on vicinal carbons, only one seems to be taken into account for retention, indicating that the steric effects influence the adsorption of these phenolic hydroxy compounds.

Comparing now the variations of log k' obtained with the three sorbents with the composition of the water–methanol mixtures in Fig. 1, we can observe linear relationships between log k' and the methanol volume fraction for each sorbent, except for mobile phases containing more than 80% of water, where similar deviations to those described for C_{18}-

TABLE I

EXPERIMENTAL CAPACITY FACTORS OBTAINED IN WATER (k'_w) FOR PRP-1 AND PGC STATIONARY PHASES AND CORRESPONDING CALCULATED RETENTION VOLUMES (V_r) WITH A 2.2 × 0.46 cm I.D. PRECOLUMN

See Experimental for conditions.

Compound	PRP-1		PGC	
	k'_w	V_r (ml)	k'_w	V_r (ml)
Phenol	400	92	81	21
1,2-Dihydroxybenzene (pyrocatechol)	46	11	120	31
1,3-Dihydroxybenzene (resorcinol)	21	5	331	86
1,4-Dihydroxybenzene (hydroquinone)	7	1.8	288	75
1,2,3-Trihydroxybenzene (pyrogallol)	5.6	1.5	172	45
1,3,5-Trihydroxybenzene (phloroglucinol)	3	0.9	1050	273
4-Aminophenol	12	3.1	112	30
3-Aminobenzoic acid	37	9	151	40

silicas are obtained. For the same compound, the slopes of the linear plots of log k' *vs.* methanol volume fraction obtained with the three sorbents are very similar or identical, indicating that the changes in log k' observed on just decreasing the methanol content of the mobile phase are similar for the three stationary phases.

On-line preconcentration with PGC

The measurements of retention volumes in water indicated that it was possible to preconcentrate di- and trihydroxybenzenes by percolating water samples directly through the 2.2 cm long precolumn. As these polar analytes are strongly adsorbed on PGC, it was necessary to investigate their desorption for on-line transfer to an analytical column packed with the same sorbent. Fig. 2a shows the analytical separation when injecting 20-μl of a mixture of hydroxybenzenes directly into the Hypercarb column eluted by a methanol–water gradient. The first peak is broad and the resolution between the first two peaks is not very good, but we did not made further attempts to optimize the separation of all hydroxy derivatives, as our aim was to illustrate the potential of carbon for the on-line enrichment of these compounds. Nevertheless, it must be pointed out that the observed plate number decreases when using mobile phases having a high water content for the separation of more polar analytes, compared with the plate number obtained with less polar analytes eluted with mobile phases having a low water content. This is not peculiar to PGC and this variation can also be observed for some other reversed-phase materials.

Fig. 2b shows the chromatogram obtained after preconcentration of a 50-ml sample of LC-grade water spiked with 10 μg/l of each compound through the 2.2-cm long precolumn and on-line elution with the same analytical gradient as that used in Fig. 2a. Peak heights cannot be compared between the chromatograms in Fig. 2a and b because the amounts injected were not exactly known with direct injection, the point of interest being the comparison of peaks widths. The chromatograms are similar and we can just observe a small band broadening for the first two peaks. Generally band broadening comes from desorption of the solute from the precolumn to the analytical column and can be suppressed by compressing the relatively broad profile in the precolumn at the top of the analytical column by the choice of a proper mobile phase and by a backflush desorption. From a geometrical point of view, in order to avoid band broadening, it is necessary to use precolumn of small dimensions compared with those of the analytical column [21]. First using a 1 cm × 0.2 cm I.D. precolumn we studied the on-line preconcentration and elution of 2-chlo-

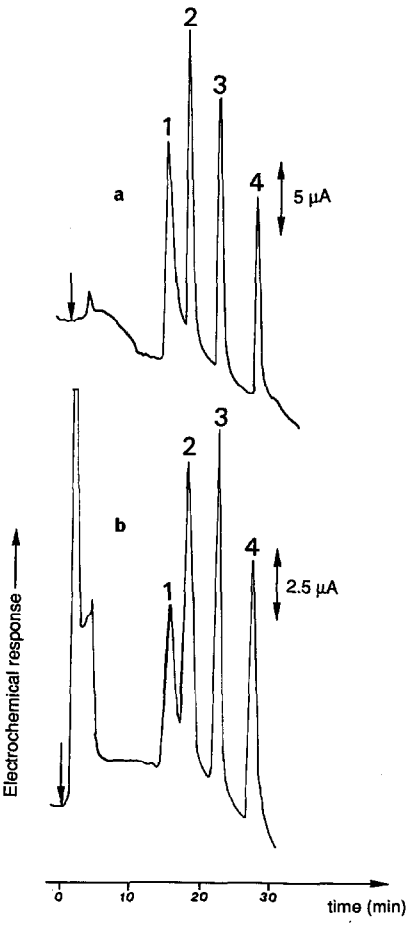

Fig. 2. (a) Direct 20-μl loop injection of hydroxybenzene mixture. (b) Chromatogram corresponding to the on-line elution of the PGC precolumn after preconcentration of a 50-ml LC-grade water sample spiked with 10 μg/l of each compound. Peaks: 1 = pyrocatechol; 2 = resorcinol; 3 = phloroglucinol; 4 = 2-chlorophenol. Analytical column, Hypercarb (10 cm × 0.46 cm I.D.) packed with 7-μm PGC; mobile phase; methanol gradient with a 0.05 M solution of perchloric acid–lithium perchlorate (pH 4) at a flow-rate of 1 ml/min; gradient 25 to 100% methanol from 0 to 40 min; electrochemical detection at 1.1 V (*vs.* Ag/AgCl); preconcentration at pH 6 through a 2.2 cm × 0.46 cm I.D. precolumn packed with 7-μm PGC at a flow-rate of 3 ml/min.

rophenol; no band broadening occurred under the experimental conditions selected. Nevertheless, possibly owing to the 7 μm particle size of PGC, the pressure in the precolumn increased rapidly, preventing its re-use. In previous on-line studies, 10-μm packings were selected in order to percolate water samples at a flow-rate of at least 5 ml/min. It was then decided to take a larger diameter of the precolumn, and a 2.2 cm × 0.46 I.D. precolumn was selected. This precolumn is too long for good coupling to the analytical column and this is certainly the reason for the observed band broadening in Fig. 2b. On another hand, this precolumn had the advantage of handling a larger sample volume, owing to stronger retentions of analytes, with an acceptable band broadening for trace determination as illustrated below. The same precolumn was reused more than 30 times with reproducible results.

Breakthrough volumes were not measured directly but calculated by comparing the chromatograms obtained after preconcentration of increasing sample volumes [22]. The peak areas were first measured on the chromatogram when preconcentrating a 10-ml volume spiked with 50 μg/l. A comparison with a direct injection of the same amount of analytes indicated that breakthrough did not occur with any of the analytes tested. The sample volume was then increased and the concentration decreased in order always to have the same amount preconcentrated. Recoveries were calculated as the ratio of the corresponding peak area to that obtained with the 10-ml samples and are reported in Table II. Breakthrough volumes (V_b) can be approximately calculated when the recoveries begin to decrease. V_b is less than 20 ml for pyrocatechol, between 20 and 50 ml for resorcinol and between 50 and 100 ml for phloroglucinol and 2-chlorophenol. These values are lower than the experimental retention volumes calculated in Table I (31, 86 and 273 ml for pyrocatechol, resorcinol and phloroglucinol, respectively). This can be easily explained by the fact that breakthrough curves can be spread over a large volume range. When determining experimental breakthrough curves on PRP-1 sorbent, we measured a breakthrough volume of 130 ml for simazine, whereas the retention volume was 207 ml and the end of the breakthrough curve 280 ml [22]. Another reason is that when several compounds are present together in the sample, they do not behave as if they were alone in LC-grade water, and we can expect lower V_b values also for this reason. This is why it is not necessary to measure V_b for each solute and it is better to calculate them by preconcentrating real samples spiked with the analytes of interest, following the method described above. It is important also to note that owing to the spreading of the breakthrough curves, the amount of analytes preconcentrated increases when the sample volume is increased above V_b, even if the corresponding recovery decreases. As an example, the peak height of phloroglucinol is higher for a 100-ml than for a 50-ml sample, even if breakthrough occurred between 50 and 100 ml.

Application to drinking water samples

A 50-ml sample of drinking water was spiked with 0.2 μg/l each of pyrocatechol, resorcinol and phloroglucinol and the preconcentration procedure was applied. The corresponding chromatogram is shown in Fig. 3. There is a large interfering peak during the first 30 min of the gradient, which appears also with the non-spiked water sample (blank run). We did not investigate any further clean-up but just modified the gradient shape in order to delay the elution of analytes after this interfering peak. The most important point here is that combination of preconcentration using PGC, on-line elution with water–acetonitrile and electrochemical detection allows a very simple determination of these very polar analytes with low detection limits, calculated to be at the 0.05 μg/l level under the selected experimental conditions. The large interfering peak shows also that PGC is a non-specific sorbent, as

TABLE II

PERCENTAGE RECOVERY OF COMPOUNDS DEPENDING ON THE SAMPLE VOLUME PERCOLATED THROUGH A 2.2 × 0.46 cm I.D. PRECOLUMN

See text for calculation; mean relative standard deviation < 3% ($n=3$). Each sample contained 0.5 μg of each compound and concentrations were 25, 10, 5 and 2 μg/l in the 20-, 50-, 100- and 250-ml samples, respectively.

Compound	Sample volume (ml)			
	20	50	100	250
Pyrocatechol	87	52	19	6.5
Resorcinol	98	66	34	13
Phloroglucinol	100	94	62	33
2-Chlorophenol	99	93	68	36

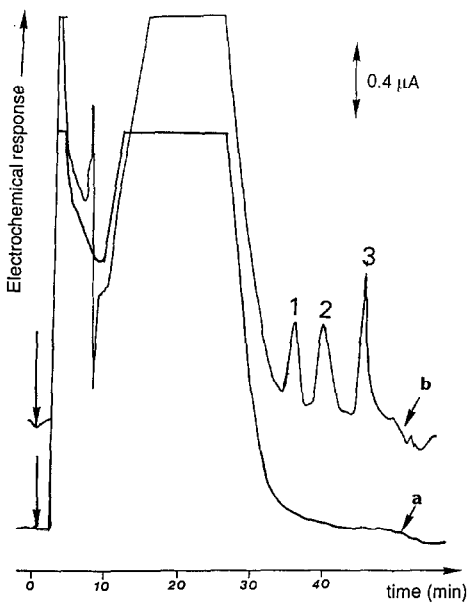

Fig. 3. Preconcentration and on-line analysis of 50 ml of drinking water (a) non-spiked and (b) spiked with 0.2 μg/l of each compound. Peaks: 1 = pyrocatechol; 2 = resorcinol; 3 = phloroglucinol. Analytical column Hypercarb (10 cm × 0.46 cm I.D.) packed with 7-μm PGC; mobile phase acetonitrile gradient with a 0.05 *M* solution of perchloric acid–lithium perchlorate (pH 4) at a flow-rate of 1 ml/min; gradient, 5% of acetonitrile from 0 to 18 min and to 25% of acetonitrile at 58 min; electrochemical detection at 0.85 V (*vs.* Ag/AgCl); preconcentration at pH 6 through a 2.7 cm × 0.46 cm I.D. precolumn packed with 7-μm PGC at a flow-rate of 3 ml/min.

are other reversed-phase materials. Nevertheless, as it retains analytes that are not retained at all by C_{18}-silica, it is possible to couple two precolumns on-line during the preconcentration step [23]. The first, packed with C_{18}-silica, should retain many apolar to moderately polar interfering compounds and not phenolic hydroxy compounds, which would then be recovered on the PGC precolumn.

CONCLUSIONS

The potential of PGC for both trace enrichment and on-line elution of very polar phenolic compounds has been demonstrated. This is an unexplored field of potential applications to some water-soluble, non-volatile and very polar solutes that are impossible to concentrate by the usual techniques. Applications to the handling of aqueous environmental samples are promising.

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