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USE OF A PRE-COLUMN PACKED WITH MERCURY(II)–8-HYDROXYQUINOLINE FOR THE SELECTIVE ON-LINE TRACE ENRICHMENT OF 2-MERCAPTOBENZIMIDAZOLE IN LIQUID CHROMATOGRAPHY

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SUMMARY

A small pre-column packed with a mercury(II)–8-hydroxyquinoline phase is used for the selective trace enrichment and clean-up of 2-mercaptobenzimidazole, a thiol model compound. Strong covalent bond formation between the analyte and the metal-loaded sorbent allows the introduction of a wash step with methanol–water (1:1). This results in a superior selectivity to that observed with the frequently used reversed-phase packing materials.

Desorption of the pre-concentrated thiol from the pre-column is effected with an eluent containing cysteine as a displacer. The elution profiles are inefficient, however, so that peak compression of the analyte on the top of the C_{18} separation column is necessary. With UV absorbance detection at 254 nm, the detection limit is 1 ppb. The excellent selectivity of the on-line pre-column sample handling is demonstrated in the analysis of river water and industrial waste water samples.

INTRODUCTION

Sample pre-treatment based on liquid–solid sorption techniques has been shown[1] to be very useful for the trace enrichment of environmental samples in column liquid chromatography (LC). In our group, C_{18}-modified silica[2], styrene–divinylbenzene copolymers[3] and carbon-based[4] sorbents have been used for on-line trace enrichment of many non-polar and moderately polar solutes from aqueous samples, utilizing pre-columns with geometrical volumes of 30–80 μl. For the efficient trace enrichment of highly polar compounds such as polar anilines and phenol, ion-exchange sorbents have been used[5,6]. When using pre-columns packed with reversed-phase materials, however, together with the analyte many other interfering compounds will usually also be concentrated. The selectivity then has to be provided during the detection step, utilizing, *e.g.*, reaction detectors.

As an alternative, we have used[5,6] ion-exchange resins as pre-column packing materials in order to increase the selectivity during the sample handling step. In

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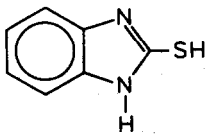

Fig. 1. Structure of 2-mercaptobenzimidazole.

addition, metal-loaded sorbents have been shown to have good potential for the selective sorption of complexing species[7]. Unfortunately, until now the application of these metal-loaded sorbents has been successful only in the selective removal (sorption) of interfering compounds; efficient sorption plus desorption for on-line analysis has been problematic.

From the literature it is known[8–10] that thiol compounds can form very strong covalent mercaptide bonds with mercury(II). Once a thiol has been sorbed on a mercury-loaded phase, several organic–water solvent mixtures can be utilized as flushing solvents to increase the selectivity and clean-up, without seriously affecting the mercaptide bond strength, *i.e.*, the retention of the analyte.

In this study, we immobilized mercury(II) on a commercially available 8-hydroxyquinoline (oxine)-modified hydroxyalkylmethacrylate gel, Spheron oxine, which has a strong affinity for heavy metal ions (but not for the ubiquitous alkali and alkaline earth metal ions), with distribution constants of the order of 10^5 (ref. 11). Retention of mercury(II) on an oxine phase will occur[12] at pH > 4.0. With Spheron oxine, the capacity for several metal ions at pH 5.0 is reported[11] to be 0.1–0.4 mmol g^{-1}, loading with metals being complete within 5–10 min. Alkaline media should be avoided in order to prevent the formation of (hydr)oxides of mercury(II).

2-Mercaptobenzimidazole (Fig. 1) was chosen as a model analyte because of its thiol functionality and its chromophore, which allows simple, although non-selective, UV absorbance detection. This imidazole can occur in the environment as a consequence of its application as a corrosion inhibitor[13] for several metals and as an antioxidant in tyres (and other rubber products). However, as a result of toxicological studies[14] it is considered to be a potential carcinogen, and a method for its selective trace determination in industrial waste water and surface water is therefore of interest.

EXPERIMENTAL

Apparatus

A Kontron (Zürich, Switzerland) LC system consisting of a Model 410 pump and a Uvikon 720 LC UV absorbance detector operated at 254 nm was used in combination with a Gilson (Villiers le Bel, France) Model 302 pump, two home-made six-port switching valves and a Rheodyne (Berkeley, CA, U.S.A.) Model 5011 six-way solvent selection valve. Chromatograms were analogue recorded on a Kipp & Zonen (Delft, The Netherlands) BD 40 recorder and processed manually.

Stationary phases and columns

Trace enrichment was carried out on a slightly modified 4 × 2.0 mm I.D.

Chrompack (Middelburg, The Netherlands) pre-concentration column. The pre-column was hand-packed[15] by using a syringe filled with a slurry of 25–40 μm Spheron oxine 1000 (Lachema, Brno, Czechoslovakia) in water–methanol (1:1). For reasons of comparison, several experiments were performed using a home-made 2 × 4.6 mm I.D. pre-column[15] packed with the spherical 10 μm styrene–divinylbenzene copolymer PRP_1 (Hamilton, Reno, NV, U.S.A.). The analytical column was a 250 × 4.6 mm I.D. stainless-steel column pre-packed with 5-μm LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.).

Chemicals

HPLC-grade methanol and analytical reagent grade sodium acetate, acetic acid, mercury(II) acetate and mercury(II) chloride were obtained from J. T. Baker (Deventer, The Netherlands) and cysteine from Merck. 2-Mercaptobenzimidazole was a gift from Dr. F. Iverson (Health Protection Branch, Ottawa, Canada). Demineralized water was purified in a Milli-Q (Millipore, Bedford, MD, U.S.A.) filtration system to obtain LC-grade water for use in eluents and standard solutions. Eluents were degassed in an ultrasonic bath under vacuum prior to use.

Preparation of the mercury(II)-loaded sorbent

Spheron oxine (1 g) was suspended in 20 ml of a 0.1 *M* acetate buffer (pH 5.0), 320 mg (*ca.* 1 mmol) of mercury(II) acetate were added and the mixture was mechanically shaken for 2 h. The mercury-loaded phase was collected on a glass filter and washed with acetate buffer (pH 5.0), LC-grade water and methanol. Finally it was dried under vacuum and stored at room temperature.

Procedures

Stock solutions of 2-mercaptobenzimidazole were prepared by weighing and dissolving in methanol, and stored at −20°C. The solutions were diluted with LC-grade water to obtain standard solutions at the (sub)-ppb level. Thiols are easily oxidized to disulphides[16]. Solutions of the model compound and of cysteine therefore had to be degassed in an ultrasonic bath, purged with nitrogen and stored in the dark; in addition, fresh dilutions were prepared daily.

Breakthrough curves of 2-mercaptobenzimidazole on the mercury(II)-loaded phase were recorded according to the procedure reported in ref. 2 using 250 ppb standard solutions in water–methanol (4:1) and a flow-rate of 1 ml min^{-1}.

The river and waste water samples were filtered over a 0.8 μm membrane filter prior to their loading onto the mercury(II)–oxine phase.

RESULTS AND DISCUSSION

Characteristics of the mercury(II)–oxine precolumn

Retention. The retention of a thiol such as 2-mercaptobenzimidazole on the mercury(II)–oxine phase tested will be governed by a least two different mechanisms. Firstly, strong complexation will occur between the mercury ion and the SH group of the test solute. Second, the aromatic rings of the Spheron oxine may be expected to display a distinct hydrophobic interaction towards the phenyl ring of the benzimidazole.

In this retention study, we always prepared the sample solution with 30% methanol. That is, trace enrichment was carried out under conditions in which retention will be predominantly due to covalent bond formation between the thiol group and the divalent mercury. In this situation, the breakthrough volume of 2-mercaptobenzimidazole on a 4 × 2 mm I.D. pre-column was found to be more than 100 ml, which was the maximum sample volume tested.

Desorption. Elution of thiols from a mercury(II)-loaded phase is believed to occur only under one of the following conditions[8]:

(1) the use of a displacer such as another thiol which has a stronger affinity for Hg(II) than the thiol under investigation and/or is present in excess;

(2) the use of a strong mineral acid such as 0.1 *M* hydrochloric acid to break the mercaptide and the mercury(II)–oxine bond;

(3) the use of an Hg(II)-containing solution which will compete with the immobilized mercury for the thiol compound.

We preferred to use a thiol displacer rather than the aggressive hydrochloric acid or the toxic mercury(II) salt solution. In the literature, cysteine and mercaptoethanol have been suggested as effective displacers in off-line applications of an organomercurial–agarose phase[9]. For practical reasons (odour!), we preferred to work with cysteine and used it in all desorption studies.

Desorption from the mercury(II)-loaded pre-column with subsequent on-line transfer to a C_{18} analytical column with water–methanol (3:1) containing 0.05 *M* cysteine was found to result in a huge peak at the start of the chromatogram, which was caused by the Hg(II)–cysteine complex. The imidazole could be desorbed by a 0.5 ml plug of 0.1 *M* cysteine and eluted with a mobile phase containing water–methanol (3:7) without interferences from the early eluting peak, but the peak profile was poor. Although the use of back-flush instead of forward-flush elution slightly improved the situation, we preferred to reconcentrate the broad 2-mercaptobenzimidazole peak on a second, 4 × 4.6 mm I.D., pre-column, packed with C_{18}-modified silica in order to remove the interfering Hg(II)–cysteine complex and to obtain peak compression prior to the actual separation on the C_{18} analytical column.

The suggested approach was only partly successful. 2-Mercaptobenzimidazole indeed eluted as a very narrow peak with a retention time of only 5 min, and without any serious interference from the Hg(II)–cysteine complex. Unfortunately, however, the recovery was only 30–40%, which was due to a partial breakthrough on the second pre-column. Replacing the C_{18} phase in this pre-column by PRP_1 was not sufficient to increase the recovery towards 100%. We therefore decided to apply the peak compression principle on the analytical column itself in all further experiments. This approach turned out to be fully satisfactory (for details, see *Final procedure*).

Regeneration. After a single desorption with cysteine, it was impossible to preconcentrate a second sample on the same mercury(II)–oxine pre-column. Obviously, the divalent mercury had been removed or deactivated by the displacer.

We attempted to perform an on-line regeneration by pumping 10 ml of 0.05 *M* mercury(II) acetate solution through the pre-column. Unfortunately, however, we observed a decreasing recovery of the test solute, probably due to bonding with mercury(II) oxide deposited in the pump, switching valve and/or capillaries of the LC system. Regeneration with 3 ml of 0.05 *M* mercury(II) acetate (or chloride) solution, introduced via a plastic syringe, eliminated the problems and the subsequent

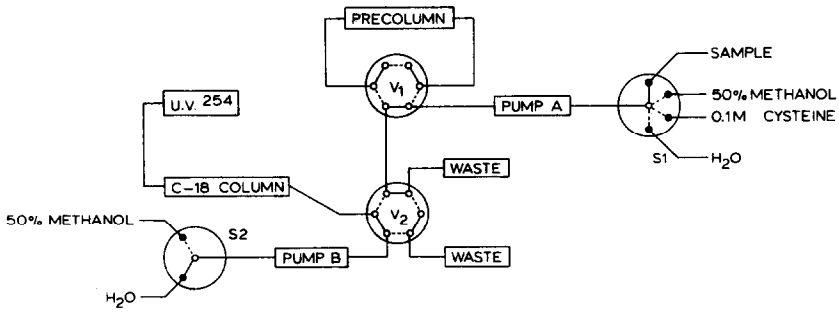

Fig. 2. Experimental set-up for the selective on-line trace enrichment of 2-mercaptobenzimidazole from water samples. S, Low-pressure solvent selection valve; V, high-pressure switching valve. Pre-column, 4 × 2.0 mm I.D. mercury(II)–oxine. Analytical column, 250 × 4.6 mm I.D. packed with 5 μm LiChrosorb RP-18. Eluents as indicated. Flow-rate of pump B, 1 ml min^{-1}. Flow-rate of pump A, as in final procedure.

recoveries now became constant and were comparable to those obtained with fresh mercury(II)–oxine pre-columns. Owing to the obvious difficulty of automating such a regeneration system[7], we recommend the use of an automated cartridge exchange device, such as the Advanced Automated Sample Processor (AASP) (Varian, Sunnyvale, CA, U.S.A.) for metal-loaded sorbents.

Final procedure

The selective on-line trace enrichment of 2-mercaptobenzimidazole was carried out with the experimental set-up shown in Fig. 2. The following procedure was adopted:

(1) 10 ml of aqueous sample solution are loaded onto the mercury(II)-loaded pre-column using pump A at 2 ml min^{-1};

(2) after the sample introduction, the pre-column is flushed with 3 ml of water–methanol (1:1) in order to selectively eliminate interferences retained by a hydrophobic mechanism;

(3) the methanol is removed with 5 ml of LC-grade water;

(4) the analyte is eluted from the pre-column towards the C_{18} analytical col-

TABLE I

ANALYTICAL DATA FOR THE SELECTIVE ON-LINE TRACE ENRICHMENT OF 2-MERCAPTOBENZIMIDAZOLE

Conditions: 10-ml sample solutions analysed according to the final procedure (*cf.*, Fig. 2); data based on peak-area measurements.

Criterion	*Level*	*Result*
Repeatability	11 ppb (n = 10)	±2.6%
Recovery	16 ppb (n = 10)	97%
Linearity (r)	0.5–500 ppb (n = 7)	0.9994
Detection limit	Signal-to-noise ratio = 3:1	10 ng or 1 ppb

umn in the forward-flush mode with 5 ml of 0.1 *M* cysteine in LC-grade water via pump A at 1 ml min^{-1};

(5) the C_{18} analytical column is flushed with 10 ml of LC-grade water via pump B at 1 ml min^{-1} in order to remove the excess of Hg(II)–cysteine;

(6) separation is performed with a step gradient from water to water–methanol (1:1), and 2-mercaptobenzimidazole is detected by UV absorbance at 254 nm.

In order to achieve on-column peak compression in step 4, the C_{18} analytical column has to be conditioned with 9 ml of LC-grade water via pump B at 1 ml min^{-1} prior to the introduction of the pre-column eluate. Fortunately, this conditioning step can be performed during the sample handling on the pre-column (steps 1–3), so the total analysis time is only 28 min. In this study, the experiments were carried out manually, and owing to a lack of an automated cartridge exchange device, a new pre-column cartridge was inserted by hand after each analysis. The entire procedure can, however, easily be automated using a simple microprocessor and a time-based column- and solvent-switching programme, as demonstrated previously[5].

General performance

Table I summarizes the analytical data obtained by means of the final procedure using the set-up in Fig. 2. The recovery was calculated by peak-area comparison with a 100 µl loop injection directly on to the C_{18} analytical column. It should be noted that because of the large breakthrough volume of over 100 ml, the concentration sensitivity can easily be increased by one order of magnitude by introducing larger sample volumes.

Application to real samples

Fig. 3 shows chromatograms of (a) an LC-grade water standard solution, (b) a surface water sample from the river Amstel (Amsterdam, The Netherlands) and (c)

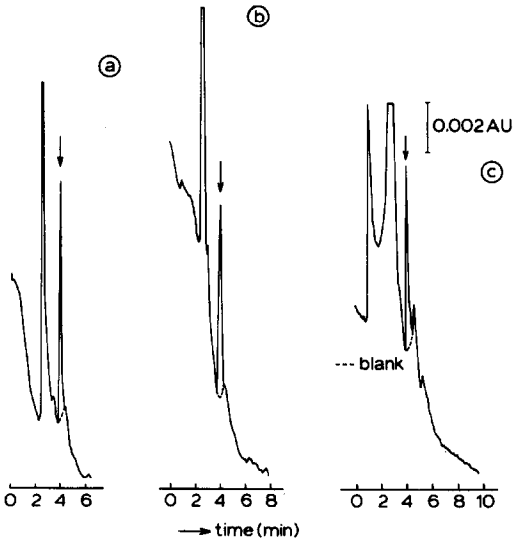

Fig. 3. Chromatograms of 10-ml water samples without and spiked with 11 ppb of 2-mercaptobenzimidazole, analysed using the set-up in Fig. 2 and the final procedure described in the text. (a) LC-grade water; (b) river Amstel water; (c) industrial waste water. Detection at 254 nm, 0.02 a.u.f.s. Other conditions as in Fig. 2.

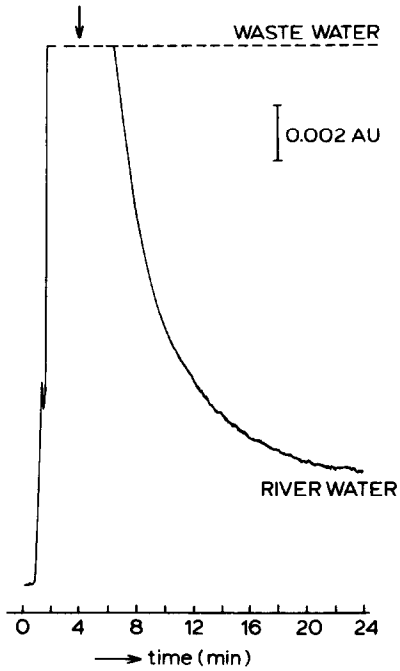

Fig. 4. Chromatograms of 10 ml of the same spiked river and waste water samples (11 ppb of 2-mercaptobenzimidazole) as used in Fig. 3b and c, obtained after on-line trace enrichment on a 2 × 4.6 mm I.D. pre-column packed with PRP_1, flushing with 10 ml of LC-grade water and direct elution to the analytical column with water–methanol (1:1). Other conditions as in Fig. 3.

an industrial waste water sample. Results for the blanks and samples spiked with 11 ppb of 2-mercaptobenzimidazole are given. The early eluting peaks (t_r = 0–3 min) are mainly due to Hg(II)–cysteine, which is not completely eluted before the step gradient (see above). 2-Mercaptobenzimidazole elutes 4 min after the introduction of this step gradient. The selectivity of the method is evident when we consider the close similarity of the chromatograms obtained for the surface and waste water samples and for the LC-grade water sample. The recovery of the test solute in spiked river and waste water compared with that in LC-grade water was 100%.

The good selectivity of the proposed method is also well demonstrated by comparing Figs. 3 and 4. The latter represents the on-line trace enrichment of the same spiked river and waste water samples on a 2 × 4.6 mm I.D. pre-column, packed with the non-selective, highly hydrophobic PRP_1, with an additional flush step with 10 ml of LC-grade water. In this instance, retention was only due to hydrophobic interaction and the pre-column could be eluted with water–methanol (1:1) onto the C_{18} analytical column. However, there is now a definite lack of selectivity, which illustrates the urgent need for the development of selective sorbents for on-line trace enrichment in reversed-phase LC.

CONCLUSIONS

Selective on-line trace enrichment of thiol compounds can be carried out on

small pre-columns packed with mercury(II)-loaded sorbents. Elution has to be carried out using a thiol displacer such as cysteine, with subsequent re-concentration using the on-column peak compression principle because of the poor elution profile.

The excellent selectivity shows up when real samples are analysed and the results are compared with those obtained by a conventional non-selective trace enrichment on a reversed-phase pre-column. The present method is linear over three orders of magnitude and allows the detection of 2-mercaptobenzimidazole in environmental samples at the low parts per billion level with a recovery of almost 100%. Future experiments should prove the general usefulness of the mercury(II)–oxine phase for the selective isolation of other sulphur-containing compounds such as pesticides, drugs and metabolites.

An evaluation of several packing materials loaded with different metal ions is currently being carried out with special emphasis on silver(I)-loaded pre-columns to be used for the selective on-line trace enrichment of compounds containing an acetylenic bond.

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