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# Determination of chlorotriazines in aqueous environmental samples at the ng/l level using preconcentration with a cation exchanger and on-line high-performance liquid chromatography

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ABSTRACT

On-line precolumn sample handling was applied to preconcentrate chlorotriazines in aqueous samples prior to their high-performance liquid chromatographic separation. Chlorotriazines are ionizable compounds (p$K_a$ between 1.6 and 2) and a strong cation exchanger is used for their concentration. As the direct percolation of large aqueous sample volumes through a cation-exchange precolumn is impossible owing to the high concentrations of inorganic cations in natural waters, a two-step preconcentration has to be carried out. First, triazines are trapped in their neutral form by direct percolation of the aqueous sample through a first precolumn packed with the copolymer-based PRP-1 sorbent. In the second step, the PRP-1 precolumn is coupled to a second precolumn packed with a cation exchanger. A small volume of deionized water–acetonitrile mixture at pH 1 allows the triazines to be desorbed from the PRP-1 precolumn and concentrated on the cation exchange precolumn in their protonated form. The content of the cation exchange precolumn is analysed on-line using an acetonitrile–water gradient. As the PRP-1 precolumn also acts as a powerful filter to many neutral interferents, detection limits are below the 10 ng/l level in drinking water.

## INTRODUCTION

The extensive use of chlorotriazines as selective herbicides in agriculture and their relatively high persistence [1] imply that these compounds are now present in the environment. Contamination of surface and groundwater and of sediments by the most commonly applied chlorotriazine herbicides, simazine and atrizine, has been reported [2–6]. In European countries, the drinking water ordinance demands a limited concentration of 0.5 $\mu$g/l (ppb) for the sum of all pesticides and 0.1 $\mu$g/l with respect to each compound [7]. Sensitive analytical methods are required to detect such low concentrations, including a preconcentration step before the analysis. Reported detection limits of chlorotriazines obtained by UV detection at 220 nm are between 0.5 and 5 ng. Therefore, determinations at levels below 0.1 $\mu$g/l require the preconcentration of 100–500-ml water samples.

Sample enrichment based on liquid–solid sorption techniques has been shown to be a good alternative to liquid–liquid extractions. Off-line procedures with prepacked cartridges and subsequent gaschromatographic (GC), GC–mass spectrometric or high-performance liquid chromatographic (HPLC) analyses have been described [8–15]. On-line enrichment analysis has some advantages, especially when applied to trace analysis. For example, selectivity can be increased by coupling several precolumns packed with different sorbents in series [16]. On-column technology requires the precolumn dimensions and packing to be adapted to those of the analytical column and the precolumns to be packed with a small-granule sorbent in order to avoid band broadening of analytes during their

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transfer from the precolumn to the analytical column [17]. Grob and Li [18] have described an on-line atrazine preconcentration coupled with GC analysis [18].

Octadecylsilica can be used for the concentration of chlorotriazines but breakthrough occurs rapidly for the more polar simazine. Chlorotriazines are more retained by PRP-1 copolymer [19,20]. This sorbent could be used alone for chlorotriazine preconcentration, but the desired detection limit could not be reached, owing to many interfering peaks present in the matrix of natural aqueous samples and also concentrated by this sorbent, giving rise to a noise baseline.

A solution to reducing the number of matrix components concentrated together with chlorotriazines is to use a selective sorbent such as a cation exchanger, as chlorotriazines are ionizable compounds ($pK_a$ between 1.6 and 2). Their direct concentration on a cation exchanger from water samples has not been reported. One reason is that a pH lower than 0.5 is necessary for their complete ionization in water and it is known that some triazines are not stable at very low pH. Another reason is that it is not possible to percolate large volumes of water samples through a cation exchanger owing to the high concentration of inorganic cations in natural waters. In previous work dealing with determination of polar aniline derivatives in water, we showed that direct percolation could be avoided by carrying out a two-step extraction, which consists of first trapping the analytes in their neutral form by PRP-1 sorbent and subsequent transfer to a cation exchanger in their protonated form [21]. The application of this procedure to chlorotriazines requires very acidic conditions for the transfer to the cation exchanger, so that the analyte stability has to be assessed. In the literature, there is one example of the use of a cartridge prepacked with a silica-based cation exchanger, which was not used as a cation exchanger but under strictly anhydrous conditions that involved complete removal of water and consequently off-line preconcentration [3].

## EXPERIMENTAL

### *Apparatus*

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

### *Stationary phases and columns*

The analytical column was a 15 cm × 4.6 mm I.D. stainless-steel column prepacked with spherical 5-μm Nucleosil $C_{18}$ octadecylsilica (Macherey, Nagel & Co., Düren, Germany). Samples were preconcentrated on a 15 mm × 3.2 mm I.D. precolumn prepacked with 7-μm PRP polystyrene–divinylbenzene polymer (Brownlee Columns; Applied Biosystems, San Jose, CA, USA). The cation-exchange precolumn was a 10 mm × 2 mm I.D. stainless-steel precolumn available from Chrompack (Middelburg, Netherland) which was packed manually with BC-X8 sulphonic acid-type resin-based cation exchanger (15–20 μm) (Benson, Reno, NV, USA) using a thick slurry and a microspatula.

### *Chemicals*

HPLC-grade acetonitrile was purchased 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 obtained 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% methanol.

### *Procedure*

The experimental set-up is described in ref. 18. The following procedure was adopted.

(1) Percolation of a natural water sample adjusted to a pH between 6 and 8 (if necessary) through the PRP-1 precolumn via the percolation pump at a flow-rate of 10 ml/min.

(2) Flushing the PRP-1 precolumn with 2 ml of LC-grade water via the same pump at a flow-rate of 1 ml/min. The 2-ml volume was measured with a 5-ml burette.

(3) Transfer of the cationic compounds to the exchanger precolumn. The PRP-1 precolumn was coupled with the 10-mm long cation-exchange precolumn and 3 ml of a mixture containing 25% of acetonitrile and water acidified to pH 1 with perchloric acid were percolated through the two precolumns in series with the same pump and flow-rate as in (2).

(4) Flushing of the two precolumns with 2 ml of LC-grade water as in (2).

(5) Backflush desorption from the cation exchanger to the $C_{18}$ analytical column by an acetonitrile gradient with lithium perchlorate–perchloric acid (0.05 *M*) at pH 4 via the gradient HPLC pump with the experimental conditions described in Fig. 14.

(6) Regeneration of the PRP-1 precolumn with 10 ml of pure acetonitrile and then with 20 ml of LC-grade water at a flow-rate of 10 ml/min.

(7) Regeneration of the exchanger precolumn with 25 ml of $10^{-3}$ *M* perchloric acid at a flow-rate of 10 ml/min;

Drinking water samples were analysed without any filtration. River water samples were filtered over a glass-fibre filter (Whatman GF/F).

## RESULTS AND DISCUSSION

Table I reports the characteristics of the four chlorotriazines tested. In the first step of the concentration procedure, analytes are adsorbed under neutral conditions and the maximum water sample volume that can be percolated through the PRP-1 precolumn depends on their retention in water. In the second step, they are desorbed from the PRP-1 sorbent at pH 1, which is the lowest recommended pH for this sorbent. As simazine and atrazine are only partially ionized at pH 1, their desorption from the PRP-1 sorbent, their fixation on the cation exchanger and their stability have to be carrefully assessed.

### *Preconcentration on the PRP-1 precolumn*

Chlorotriazines are well retained by the PRP-1 sorbent in their neutral form. Breakthrough volumes higher than 500 ml were measured on a 15 mm × 3.2 mm I.D. precolumn packed with the PRP-1 copolymer for the four chlorotriazines according to the procedure described in ref. 19. A consequence is that a conventional on-line analysable precolumn can be used and the sample volume can be increased to 500 ml for the first step of the preconcentration procedure.

### *Desorption from PRP-1 and transfer to the cation exchanger*

Desorption and transfer of protonated solutes are carried out at the same time by flushing the two precolumns in series with a solution named in this work "transfer solution" and consisting of a few millilitres of deionized water adjusted to pH 1 and a certain amount of acetonitrile. This transfer solution must allow the complete desorption of solutes from the PRP-1 precolumn and their fixation by the cation-exchange precolumn. The first point can be verified by on-line analysis of the PRP-1 precol-

TABLE I

CHARACTERISTICS OF 2-CHLORO-1,3,5-TRIAZINES

| Compound | Abbreviation | Substituent in position | | p$K_a$ |
|---|---|---|---|---|
| | | 2- | 4- | |
| Simazine | S | $-NHC_2H_5$ | $-NHC_2H_5$ | 1.65 |
| Atrazine | A | $-NHC_2H_5$ | $-NHCH(CH_3)_2$ | 1.68 |
| Propazine | P | $-NHCH(CH_3)_2$ | $-NHCH(CH_3)_2$ | 1.85 |
| Terbutylazine | T | $-NHC_2H_5$ | $-NHC(CH_3)_3$ | 1.95 |

umn, and the second by knowing the breakthrough volumes of solutes dissolved in the transfer solution on the cation-exchange precolumn.

The breakthrough volumes on the cation-exchange precolumn depends mainly on the ionization state of the solute. They were not measured directly but were estimated in the following way: 5-ml transfer solutions made of pure water at pH 1 and 1.5 were spiked with 0.5 μg of each chlorotriazine and percolated directly though the cation-exchange precolumn, which was subsequently analysed on-line. By comparing the results with those of a 0.5-μg loop injection, breakthrough volumes were found to be lower than 5 ml at pH 1.5 (60% of atrazine protonated) and larger than 5 ml at pH 1 (82% of atrazine protonated).

These results imply that it is possible to retain the compounds on the small cation-exchange precolumn. However, as seen above, the transfer solution must also contain a certain amount of organic solvent for the desorption from PRP-1. The effect of the transfer solution composition was studied by using the whole procedure: a 50-ml LC-grade water sample spiked with 10 μg/l of chlorotriazines at pH 6 was preconcentrated through the PRP-1 precolumn; the two precolumns were flushed with transfer solutions having different compositions (reported in Table II) and each precolumn was analysed separately on-line. Recoveries were calculated by comparison with a 0.5-μg direct injection onto the analytical column, as described above, for both precolumns. Experiments were first made at pH 1.5. With a 5-ml transfer solution made of water at pH 1.5 and 20% of acetonitrile, simazine and atrazine were completely desorbed from PRP-1 but not propazine and terbutylazine. On adding more acetonitrile (25%), the desorption was still not complete for the two late-eluted triazines, but there was only a 20–25% loss. The volume was then decreased to 3 ml (line 3 in Table II) and the recoveries increased on the cation exchanger, being above 75% for all compounds except terbutylazine. A lower pH was then necessary. With 5 ml of water at pH 1 and 25% of acetonitrile, no compound was left on the PRP-1 precolumn but there was still a beginning of elution from the cation-exchange precolumn. On decreasing the volume to 3 ml, all recoveries were above 85% owing to a small breakthrough from the cation-exchange precolumn for simazine and atrazine and an incomplete desorption for the two late-eluted compounds. It is not necessary to know these recoveries accurately because all the quantitative analyses are made by spiking water samples and preconcentrating them via the whole method, but in trace analysis it is important to have the highest possible amount on the precolumn. This composition of the transfer solution (3 ml, pH 1 and 25% of acetonitrile) was selected for the final procedure.

Chlorotriazines are stable during the acidic transfer, as the recoveries reported above were calculated

TABLE II

EFFECT OF TRANSFER SOLUTION ON THE DESORPTION FROM THE PRP-1 PRECOLUMN AND ON THE ADSORPTION ON THE CATION-EXCHANGE PRECOLUMN

Recoveries (%) calculated on the PRP-1 precolumn ($R_a$) and on the cation-exchange precolumn ($R_b$) by comparison between a 0.5-μg direct injection and the preconcentration of a 50-ml of LC-grade water sample spiked with 10 μg/l of each compound via the whole procedure; mean values from triplicate measurements.

| Transfer solution | | | Compound | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Acetonitrile concentration (%) | pH | Volume (ml) | S | | A | | P | | T | |
| | | | $R_a$ | $R_b$ | $R_a$ | $R_b$ | $R_a$ | $R_b$ | $R_a$ | $R_b$ |
| 20 | 1.5 | 5 | 0 | 65 | 0 | 70 | 40 | 5 | 54 | 5 |
| 25 | 1.5 | 5 | 0 | 60 | 0 | 70 | 20 | 50 | 25 | 75 |
| 25 | 1.5 | 3 | 0 | 83 | 0 | 87 | 25 | 75 | 50 | 50 |
| 25 | 1 | 5 | 0 | 70 | 0 | 80 | 0 | 90 | 0 | 100 |
| 25 | 1 | 3 | 0 | 88 | 0 | 91 | 5 | 90 | 10 | 85 |

by comparing peak areas with those obtained by a direct 20-$\mu$l injection of standard solution in methanol. The 10–15% loss of simazine and atrazine could be due to a certain instability, but is also easily explained by incomplete retention by the cation exchanger owing to their partial ionization. This is confirmed by an increase in the transfer solution volume (line 4), which increases the breakthrough of these two analytes. Terbutylazine has the highest $pK_a$ value and is more ionized at pH 1, so that the observed recovery is 100% when it is completely desorbed from the PRP-1 precolumn (line 4). These experiments just indicate that chlorotriazines are stable during the 3 min necessary for the transfer. Their stability was also observed at pH 1 for a period of up to 30 min in the experiments described above (direct percolation through the cation exchanger). Stability over a longer period was not studied.

*Desorption from the cation exchanger*

In order to have detection limits as low as those obtained by direct injections, band broadening is undesirable when analysing the solutes preconcentrated on the cation exchange precolumn by the analytical mobile phase. Fig. 1a shows the chromatogram obtained on injecting 0.5 $\mu$g directly onto the analytical column with a 20-$\mu$l loop with UV detection at 230 nm (close to the maximum absorbance of chlorotriazines) and Fig. 1b shows the chromatograms obtained on preconcentrating and analysing on-line a 500-ml drinking water sample spiked with 0.1 $\mu$g/l of each compound. Considering the chlorotriazine peaks, no band broadening is observed: the peak heights differ only because of the different amounts injected (0.5 and 0.05 $\mu$g, respectively) and the 85–90% recoveries. Therefore, the precolumn–column coupling is correct and chlorotriazine desorption is rapid under the chromatographic conditions selected. Some aniline derivatives were included in the standard solution to ensure the good working of an electrochemical detector, which was in series with the UV detector. Chlorotriazine are not oxidizable and cannot be detected electrochemically. Nevertheless, as we can expect some aniline derivatives to be also concentrated by

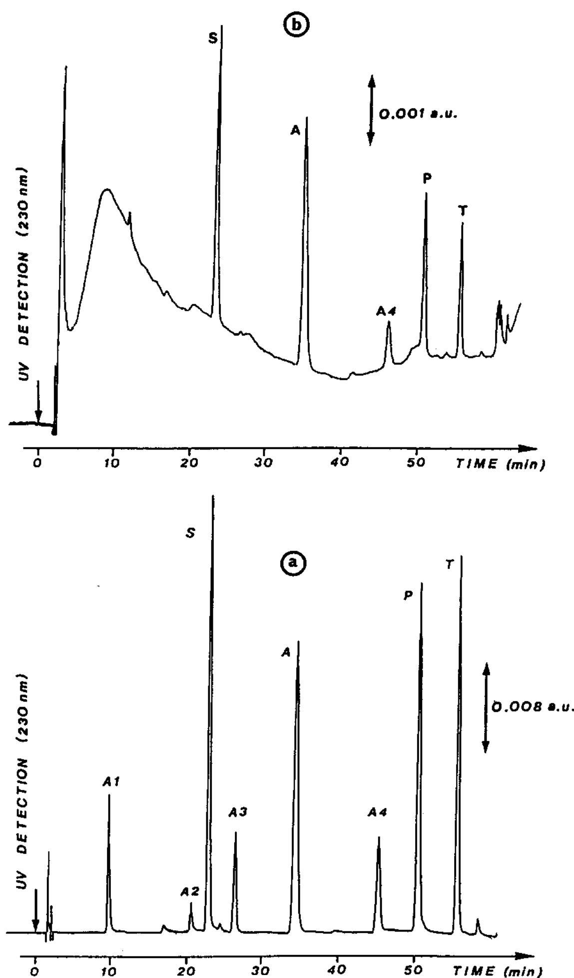


Fig. 1. (a) Direct 20-$\mu$l loop injection of each chlorotriazine reported in Table I and of four aniline derivatives (A1 = 3-chloro-4-methoxyaniline, A2 = 3-chloro-4-methylaniline, A3 = 4-isopropylaniline, A4 = 3,4-dichloroaniline). (b) Chromatogram corresponding to the on-line elution of the cation-exchange precolumn after preconcentration of a 500-ml drinking water sample spiked with 0.1 $\mu$g/l of each compound. Analytical column, 15 cm × 0.46 cm I.D. packed with 5-$\mu$m Nucleosil $C_{18}$; mobile phase, acetonitrile gradient with a 0.05 $M$ solution of perchloric acid–lithium perchlorate at pH 4 at a flow-rate of 1.5 ml/min; gradient 15% to 23.5% acetonitrile from 0 to 20 min, 25.3% from 20 to 35 min and up to 46% at 60 min; UV detection at 230 nm, preconcentration at pH 6 through a 15 mm × 3.2 mm I.D. precolumn packed with 7-$\mu$m PRP-1, transfer to 10 mm × 2 mm I.D. precolumn packed with 15–20-$\mu$m BC-X8 cation exchanger with 3 ml of perchloric acid (pH 1) containing 25% of acetonitrile.

the procedure and to interfere in the analysis, the use of the two detectors helps to identify chlorotriazine with a peak at 230 nm and the absence of a peak with the electrochemical detector.

*Detection limits and application to drinking water samples*

The chromatogram in Fig. 1b indicates that the determination at the 0.1 μg/l level required by the EEC ordinance is easy and that the detection limits are much lower. The baseline contains very little background, owing to an efficient removal of neutral interferents left on the PRP-1 precolumn and the absence of cationic interfering material detected at 230 nm. The detection limits and the linearity of solute peak height or surface response were assessed by adding increasing amounts to 500 ml of LC-grade and drinking water samples and by using the whole preconcentration and on-line analysis procedure. Owing to this baseline quality, it is possible to use a more sensitive range of the UV detector and increase the peak heights. The calibration graphs for the chlorotriazines with UV detection at 230 nm were linear ($r$ = 0.998 with six data points) over the whole range tested (0.02–1 ng/l). The repeatability ranged between 3 and 8% (relative standard deviation) ($n$ = 4) at the 0.1 μg/l level. Detection limits were calculated for a signal-to-noise ratio of 3 and were 3 ng/l for simazine and atrazine and 5 ng/l for terbutylazine and propazine when 500-ml samples were analysed. Therefore, the analysis of a 100-ml sample is sufficient for monitoring drinking waters with detection limits of 15 ng/l for atrazine and simazine, which allows accurate determinations at the 0.1 μg/l level. Nevertheless, it could be of interest to handle 500-ml samples to detect lower concentrations, *e.g.*, for metabolite studies. Fig. 2a illustrates the low detection limits obtained with real samples. This sample was not spiked and simazine and atrazine were detected at 10 and 15 ng/l concentrations, respectively. In Fig. 2b, a 500-ml sample of the same drinking water was spiked with 2 ng/l of each compound.

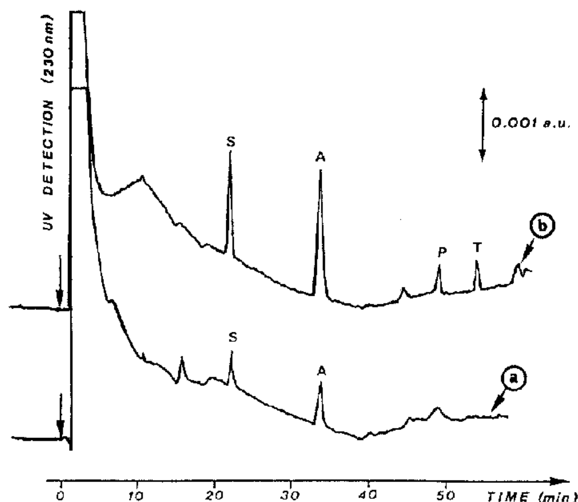


Fig. 2. Preconcentration and on-line analysis of a 500-ml drinking water sample from Paris, April 1990: (a) non-spiked; (b) spiked with 20 ng/l of each compound. Experimental conditions as in Fig. 1b.

*Interferent filter effect of the PRP-1 precolumn: application to river water samples*

Compared with other methods, the low detection limits obtained here with drinking water sample are due to the efficient removal of many neutral interferences by the PRP-1 precolumn. This is more noticeable when analysing river waters with more complex matrices than those of drinking waters. Fig. 3 shows the analysis of a non-spiked 500-ml sample of water from the river Seine, taken in Paris at the end of April. The transfer solution contained 25% of organic solvent, both ionic and some neutral compounds are desorbed from the PRP-1 precolumn, and, as the cation-exchange precolumn is a styrene–divynilbenzene polymer-based sorbent, we can expect the retention of some neutral aromatic organic compounds together with the ionic compounds. However, the baseline obtained in Fig. 3 on eluting the cation-exchange precolumn is free from interferents and is similar to that obtained on analysing drinking water samples, showing that the neutral organics desorbed by the 25% acetonitrile content are not recovered on the cation-exchange precolumn. Therefore, the trapping of ionic chlorotriazines is very selective under the selected experimental conditions. This also indicates that the matrix of the river Seine does not contain many ionizable compounds showing absorption at 230 nm and having polarities similar to those of chlorotriazines. The electrochemical response, not represented here, does not show the presence of oxidizable com-

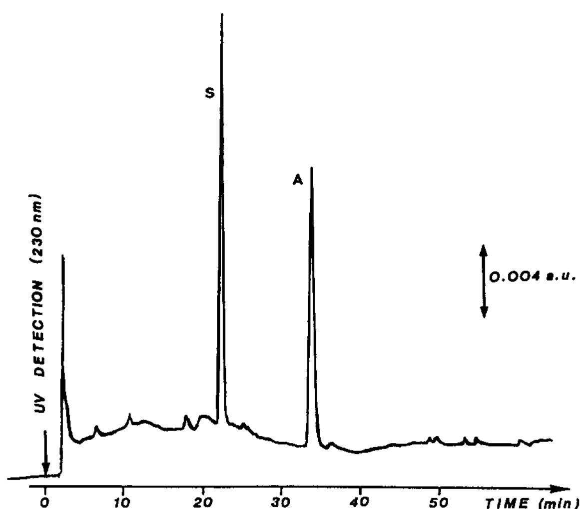


Fig. 3. Preconcentration and on-line analysis of a non-spiked 500-ml sample taken from the river Seine, Paris, April 1990. On-line analysis of the cation-exchange precolumn. Experimental conditions as in Fig. 1b.

pounds. This sample contained 0.7 and 0.8 ng/l of simazine and atrazine, respectively.

## CONCLUSIONS

The on-line preconcentration and analysis of aqueous environmental samples carried out with two precolumns allows chlorotriazine determinations at very low levels without pretreatment of the samples. This method is sensitive, selective and can be easily automated. The identity of compounds is reinforced by the fact that the solutes are ionizable, by their retention time, their UV response at 230 nm and the absence of electrochemical response. Compared with other methods, the detection limits are lower and the handling of a 100-ml sample produces accurate determinations at the 0.1 ng/l level. However, the main conclusion of this work is that when looking for trace compounds, a low level can be obtained only with efficient removal of interferences and selective trapping of the relevant compounds, rendering the precolumn selection and coupling the most important step of the methodology.

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