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Rapid and sensitive determination of phenylurea herbicides in water in the presence of their anilines by extraction with a Carbopack cartridge followed by liquid chromatography

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ABSTRACT

A rapid and sensitive method for determining phenylurea herbicides in environmental aqueous samples in the presence of their anilines is described. The water sample is preconcentrated by passage at a flow-rate of *ca.* 150 ml/min through a 250-mg graphitized carbon black (Carbopack B) cartridge. After washing with 0.6 ml of methanol, the Carbopack B trap is connected with a cartridge containing a strong cation exchanger. Organics trapped by the Carbopack cartridge are eluted by passage of 6 ml of methylene chloride–methanol (95:5, v/v). Anilines and other basic compounds are quantitatively substracted from the solvent system while flowing through the cation-exchange cartridge. After evaporation and redissolution, the sample is subjected to reversed-phase gradient elution high-performance liquid chromatography with UV detection at 250 nm. Recoveries of phenylureas added to water at levels between 30 and 3000 ng/l were higher than 92%. The limit of detection was about 1 ng/l, for a 2-l sample. With respect to an octadecyl (C_{18})-bonded silica cartridge, the Carbopack B cartridge had a far better extraction efficiency for polar phenylureas.

INTRODUCTION

Substituted phenylureas are selective herbicides used extensively in agriculture and are fairly persistent in the aquatic environment. This has led to the development of many analytical procedures for determining phenylurea residues in aqueous samples. Mostly gas chromatography [1–3] and liquid chromatography [4–8] have been used.

A particular problem which may be encountered in determining phenylureas is the simultaneous presence in water of their degradation products, *i.e.*, anilines, which can interfere with the determination of the parent pesticides. Goewie *et al.* [5] succeeded in eliminating anilines from the aqueous samples by adopting a special platinum phase packed in a short precolumn.

Carbopack B is a graphitized carbon black (GCB) which has proved to be a valuable material for the liquid–solid extraction (LSE) of organochlorine insecticides [9,10] and triazine [11,12] and phenoxy acid [13] herbicides from water. Carbopack B cartridges proved to be more efficient than commonly used octadecyl (C_{18})-bonded silica cartridges for the LSE of phenols [14] and chloroanilines [15].

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This paper describes a sensitive and rapid procedure for determining residues of phenylureas in drinking and surface waters in the presence of their anilines. The method first involves LSE of phenylureas by a Carbopack cartridge. The eluate is then passed through a strong cation exchanger to remove anilines and other bases prior to evaporation, redissolution and measurement by high-performance liquid chromatography (HPLC). Concentration factors of more than 6000 allowed the detection of phenylureas at concentrations of about 1 ng/l.

EXPERIMENTAL

Reagents and chemicals

Authentic phenylureas were purchased from both Riedel-de Haën (Seelze, Germany) and Eurobase (Milan, Italy): fenuron (N′-phenyl-N,N-dimethylurea); metoxuron (N′-3-chloro-4-methoxyphenyl-N,N-dimethylurea); monuron (N′-4-chlorophenyl-N,N-dimethylurea); monolinuron (N′-4-chlorophenyl-N-methoxy-N-methylurea); fluometuron (N′-3-fluoromethylphenyl-N,N-dimethylurea); chlortoluron (N′-3-chloro-4-methylphenyl-N,N-dimethylurea); metobromuron (N′-4-bromophenyl-N-methoxy-N-methylurea); difenoxuron (N′-4-methoxy-4-phenoxyphenyl-N,N-dimethylurea); isoproturon (N′-4-isopropylphenyl-N,N-dimethylurea); diuron (N′-3,4-dichlorophenyl-N,N-dimethylurea); linuron (N′-3,4-dichlorophenyl-N-methoxy-N-methylurea); chlorbromuron (N′-3-chloro-4-bromophenyl-N-methoxy-N-methylurea); chlorouxoron (N′-4-chloro-4-phenoxyphenyl-N,N-dimethylurea); and neburon (N-3,4-dichlorophenyl-N-butyl-N-methylurea). Individual standard solutions were prepared by dissolving 100 mg of each herbicide in 100 ml of methanol. A composite working standard solution was prepared by mixing 0.5 ml of each standard solution and diluting to 100 ml with methanol.

Except for 4-chloroaniline and 3,4-dichloroaniline, anilines of interest were prepared from the corresponding phenylureas by catalytic hydrolysis on silica [3].

For HPLC, distilled water was further purified by passing it through a Norganic cartridge (Millipore, Bedford, MA, U.S.A.). Acetonitrile and methanol of HPLC grade were obtained from Carlo Erba (Milan, Italy). All other solvents were of analytical-reagent grade (Carlo Erba) and were used as supplied.

Apparatus

A 250-mg amount of Carbopack B (120–400 mesh) (Supelco, Bellefonte, PA, U.S.A.) was packed in a polypropylene tube (6 cm × 1.4 cm I.D.) (Supelco). A 200-mg amount of Amberlite CG-120-I (100–200 mesh) (Fluka, Buchs, Switzerland) was packed in a plastic tube (6 cm × 0.5 cm I.D.) (Supelco). Polyethylene frits (20-μm pore size) (Supelco) were located above and below each sorbent bed. To avoid crushing of the Carbopack B particles, which results in a decrease in the permeability of the cartridge, the upper frit was gently rested above the sorbent bed. The connection between the two cartridges was made with a plastic adapter (Supelco). Before use, the Carbopack cartridge was washed sequentially with 4 ml of methylene chloride–methanol (95:5, v/v), 2 ml of methanol and 6 ml of water. The cation-exchange material was converted from the Na^+ to the H^+ form by washing it with 10 ml of 1 mol/l hydrochloric acid, 3 ml of water and 3 ml of acetonitrile. This cartridge was reused several times by restoring it with 2.5 ml of 1 mol/l methanolic potassium

hydroxide solution to elute trapped basic compounds, followed by 2 ml of water. From this point, the same sequence as reported above was followed to activate the cation exchanger cartridge. The Carbopack B cartridge was fitted into a side-arm filtering flask and liquids were forced to pass through the cartridge by vacuum (water pump).

Procedure

Aquous samples were fortified with known volumes of the composite working standard solution of phenylureas. When analysing hypochlorite-containing tap water samples, hypochlorite was reduced in advance by adding 0.4 g/l of sodium sulphite to avoid oxidation of the analytes. Water samples were then shaken for 1 min and, after 10 min, poured into a glass reservoir which was connected to the Carbopack cartridge. When necessary, after supplying surface water samples with the analytes, they were filtered through Whatman GF/C glass-fibre pads (pore size 10 μm) to remove algae and debris. Water was forced to pass through the cartridge at flow-rates of 130–150 ml/min. Just after the sample had passed through the column, the cartridge was filled with 6 ml of distilled water, which was allowed to pass through the cartridge at a flow-rate of 10–20 ml/min. This operation serves to eliminate drops of sample water adhering to the plastic walls of the cartridge, whose presence can decrease the efficiency of the organic solvent system used for desorbing the analytes.

Following the passage of large water volumes, some shrinkage of the sorbent bed may occur. In such an event, before washing with distilled water, the upper frit was pushed against the top of the sorbent bed. This expedient facilitates the subsequent removal of water from the sorbent bed and improves the effectiveness of the eluent system as it can permeate the sorbent bed more homogeneously.

After the distilled water had passed through the trap, most of it was removed by reducing to the minimum the pressure in the flask for 30 s. The water pump was then disconnected, 0.6 ml of methanol was poured into the cartridge, the pump was linked to the flask again and methanol was passed slowly through the sorbent bed to eliminate residual water. Thereafter, the Carbopack cartridge was connected to the cation exchanger, a conical glass vial was located below the two cartridges and the pesticides were eluted with 6 ml of methylene chloride–methanol (95:5, v/v) at a flow-rate of about 2 ml/min). The last drops of the eluent system were collected by using the vacuum. The extract was dried in a water-bath at 40°C under a stream of nitrogen and the residue was reconstituted with 0.3 ml of water–methanol (60:40, v/v). A 50-μl volume of this solution was injected into the HPLC apparatus.

HPLC apparatus

A Model 5000 liquid chromatograph (Varian, Walnut Creek, CA, U.S.A.) equipped with a Rheodyne Model 7125 injector having a 50-μl loop with a Model 2550 UV detector (Varian) was used, with a 25 cm × 4.6 mm I.D. column filled with 5-μm LC-18 reversed-phase packing (Supelco). The organic modifier was methanol–acetonitrile (85:15, v/v). Gradient elution of phenylureas was performed by increasing linearly the percentage of the organic modifier from 47% to 70% in 20 min. Phenylureas were detected with the UV detector set at 250 nm.

The concentrations of the herbicides in water samples were calculated by comparing the heights of the peaks obtained with the sample and with a standard solution. The latter was prepared by drying a known volume of the composite working standard solution and dissolving the residue in 0.3 ml of water–methanol (60:40, v/v).

RESULTS AND DISCUSSION

Chromatographic conditions

Reversed-phase HPLC fractionation of phenylureas was performed using a concave gradient with methanol as organic modifier [16]. Under the same chromatographic conditions as above, but using an HPLC column from a different manufacturer, we observed that chlortoluron and metobromuron were eluted as a single peak and isoproturon and diuron were poorly separated from each other.

In order to fractionate the fourteen phenylureas considered, the effect of various methanol–acetonitrile mixtures as organic modifiers on the relative retention of the analytes was studied. The results are reported in Fig. 1. The phenylureas having a methoxy substituent on the urea nitrogen, namely monolinuron, metobromuron, linuron and chlorobromuron, exhibited the lowest mobilities as the eluotropic strength of the mobile phase was increased by increasing the acetonitrile content in the organic modifier. It was found that 15% acetonitrile in methanol gave the best results in terms of fractionation of the phenylureas considered.

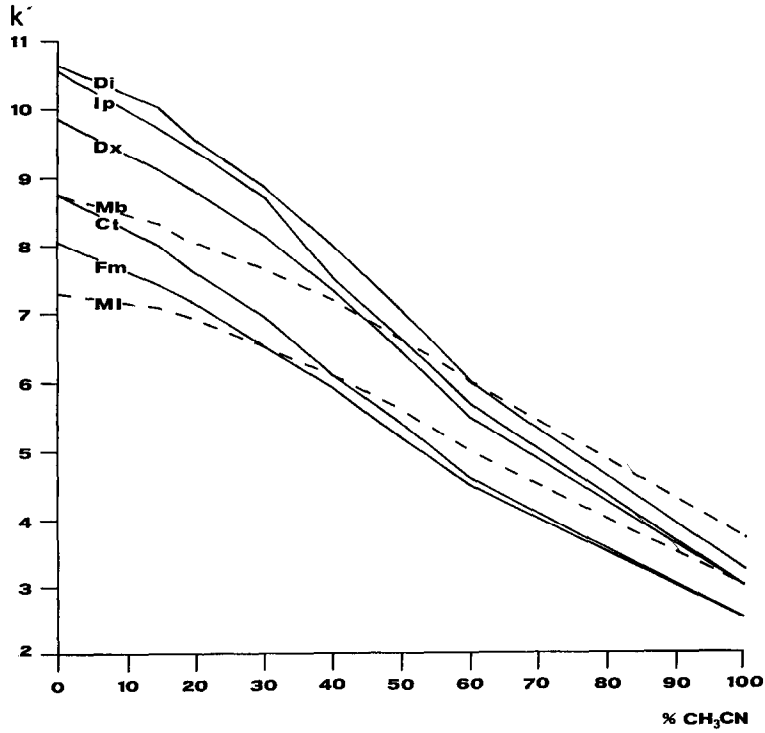

Fig. 1. Plots of retention (capacity factor, k') on a C_{18} silica column of some selected phenylureas *vs.* composition of the acetonitrile–methanol mixture used as organic modifier. Compounds: Ml = monolinuron; Fm = fluometuron; Ct = chlortoluron; Mb = metobromuron; Dx = difenoxuron; Ip = isoproturon; Di = diuron. The solvent programme was from 47 to 70% organic modifier in 20 min.

Recovery studies

In order to achieve sufficiently high enrichment factors, which enable pesticides to be monitored in drinking water samples in the ng/l range, extraction of large volumes of water is a prerequisite. In this respect, the ability of the Carbopack cartridge to retain quantitatively phenylureas on passing through it increasing volumes (1, 2 and 4 l) of a groundwater sample spiked with the herbicides at 150 ng/l was evaluated. The recovery data obtained from three determinations for each water volume considered showed that only when 4 l of groundwater were extracted was about 10% of fenuron, which is the most water-soluble phenylurea, lost in the water effluent.

The extraction efficiency of the Carbopack cartridge was compared with that of a 0.5-g C_{18} disposable extraction column (Supelco). The size of the plastic tube containing the siliceous material was the same as that containing Carbopack. Phenylureas were extracted in triplicate from 2 l of the same water sample used for experiments with the Carbopack cartridge and eluted with two 2-ml aliquots of methanol. Except for the most hydrophobic compounds, large losses of the other phenylureas were observed, the percentage recoveries ranging from 6.3% for fenuron to 69.6% for fluometuron. Hence, it appears that Carbopack is more suitable than the C_{18} material for extracting polar pesticides from aqueous samples. The extraction of large volumes of water by the C_{18} cartridge was also time consuming, as about 100 min were needed to pass 2 l of water through it, whereas the same operation with the Carbopack cartridge required only about 15 min.

Accuracy and precision

The recovery and the within-run precision of the proposed method with various concentrations of the fourteen herbicides considered were assessed. A sample of tap water made 0.4 g/l in sodium sulphite was divided into two portions, which were spiked with the analytes at levels of 30 and 3000 ng/l. Each portion was divided into six 2-l aliquots, each of which was analysed six times by the procedure. The quantitative results showed that the recovery of all phenylureas was independent of the herbicide concentration, demonstrating the absence of any adverse effect of irreversible adsorption by the materials composing the extraction apparatus. A slight loss of fenuron (recovery 83.2%) occurred at the highest phenylurea concentration considered. This can be accounted for by displacement chromatography of fenuron by the other more strongly retained phenylureas. The relative standard deviations (R.S.D.) at a concentration of 30 ng/l ranged from 3.76% for difenoxuron to 8.93% for fluometuron, and at 3000 ng/l the R.S.D. ranged from 0.69% for metoxuron to 2.64% for chlorouxoron. Quantitative results obtained by analysing water samples spiked with 30 ng/l of phenylureas showed that this method is well suited for monitoring compliance with the European Community standard for drinking water.

Matrix effect

Although LSE procedures with small cartridges have become popular during the last decade, this technique suffers mainly from the disadvantage that, when sampling large volumes of highly contaminated environmental aqueous samples, saturation effects due to organics present in the water may lead to abrupt and unpredictable decreases in the breakthrough volumes of the analytes of interest, as measured by

extracting them from pure water. The matrix effects on the recovery of phenylureas was evaluated by extracting in triplicate with the Carbopack cartridge 1- and 2-l aliquots of both river and sea water samples spiked with phenylureas at individual concentrations of 30 ng/l. As measured with a Beckman 915 A TOC Analyzer, the total organic carbon contents in the river and sea water samples were 6.5 and 1.7 mg/l, respectively. From these experiments, it was evident that only fenuron, among the phenylureas considered, was severely lost (recovery 53.5%) on extraction from 2 l of the river water sample.

Interferences

The analysis of phenylureas may be complicated by the presence in the water sample of their main degradation products, anilines. In addition, anilines may be

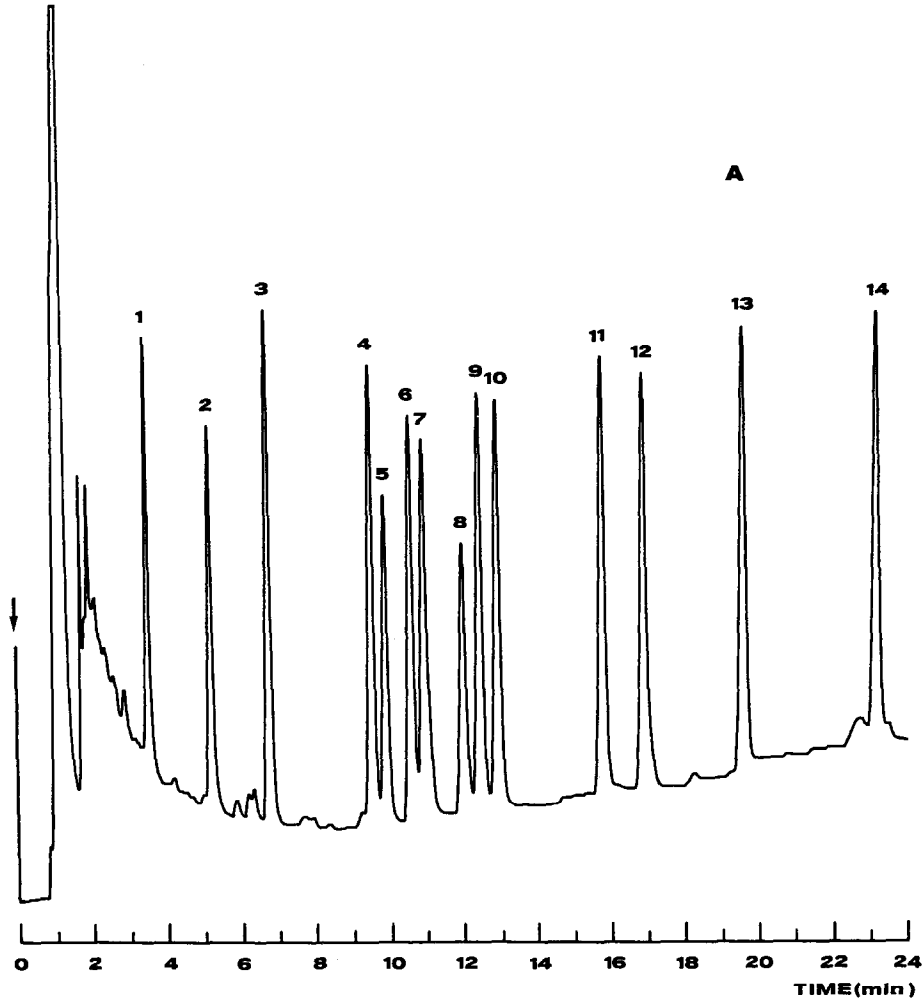

Fig. 2.

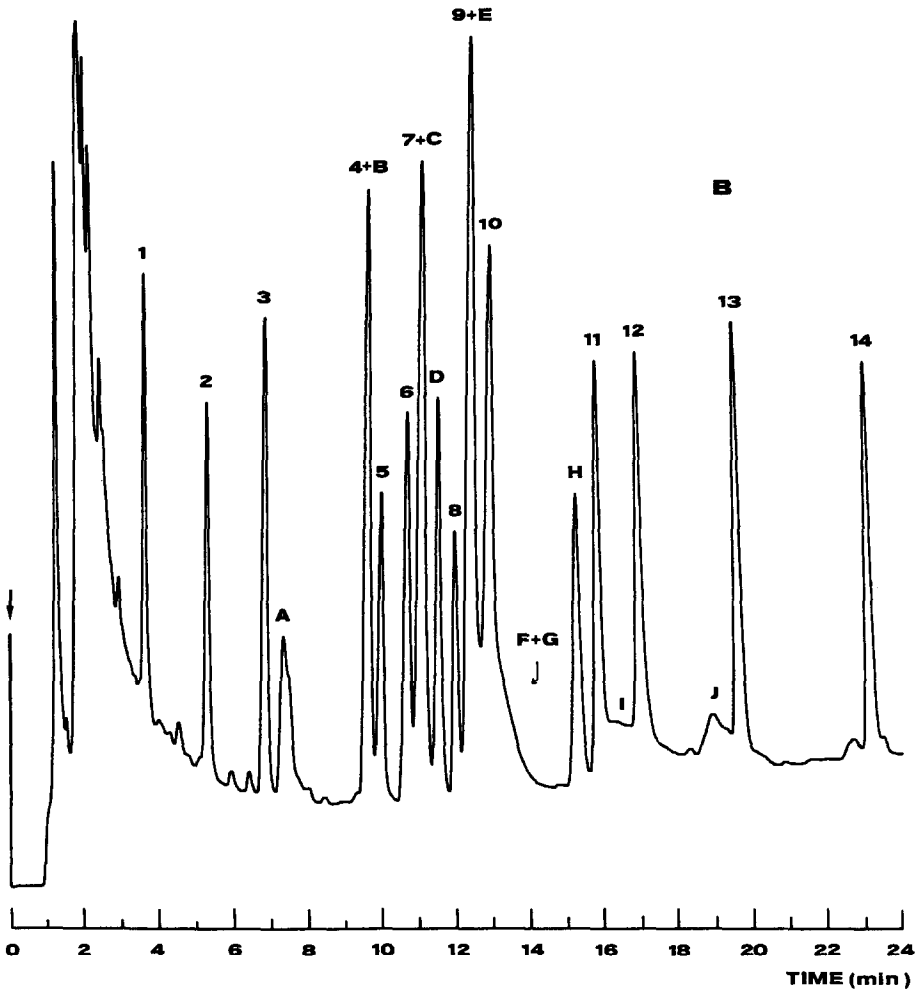

Fig. 2. Chromatograms obtained on analysing 1-l aliquots of river water (Tevere, February 1990) spiked with 300 ng/l of each phenylurea, 1000 ng/l of each corresponding aniline and 2000 ng/l of simazine, atrazine and propazine (A) by the proposed procedure and (B) with the Carbopack trap alone. Compounds: 1 = fenuron; 2 = methoxuron; 3 = monuron; 4 = monolinuron; 5 = fluometuron; 6 = chlortoluron; 7 = metobromuron; 8 = difenoxuron; 9 = isoproturon; 10 = diuron; 11 = linuron; 12 = chlorbromuron; 13 = chlorouxoron; 14 = neburon; A = simazine; B = 3-trifluoromethylaniline; C = atrazine; D = 3,4-dichloroaniline; E = 3-chloro-4-bromoaniline; F = 4-chloroaniline; G = 4-bromoaniline; H = propazine; I = 3-chloro-4-methoxyaniline; J = 3-chloro-4-methylaniline. The other anilines were eluted as very skewed peaks.

present in environmental aqueous samples as a result of industrial discharges from factories using anilines as synthetic intermediates. This problem was eliminated by incorporating a second cartridge containing a strong cation exchanger, having an exchange capacity of about 4 mequiv./g, which was connected in series with the Carbopack cartridge before desorbing the analytes. In such way, while phenylureas passed completely unretained through the second cartridge, any basic compound was

re-adsorbed on the exchanger from the methylene chloride–methanol mixture flowing through it. This double trap system has the advantage over that making use of a platinum phase [5] of being of more general use because, in addition to primary anilines, it also eliminates any other basic compound. As an example, Fig. 2 shows typical chromatograms obtained on analysing 1 l of a Tevere river water sample fortified with phenylureas, their corresponding anilines and simazine, atrazine and propazine, which are weakly basic herbicides, by the proposed procedure and with a single Carbopack cartridge.

Limit of detection

Under the chromatographic conditions selected and by extracting 2 l of drinking water samples, the limit of detection (signal-to-noise ratio = 3) for phenylureas was

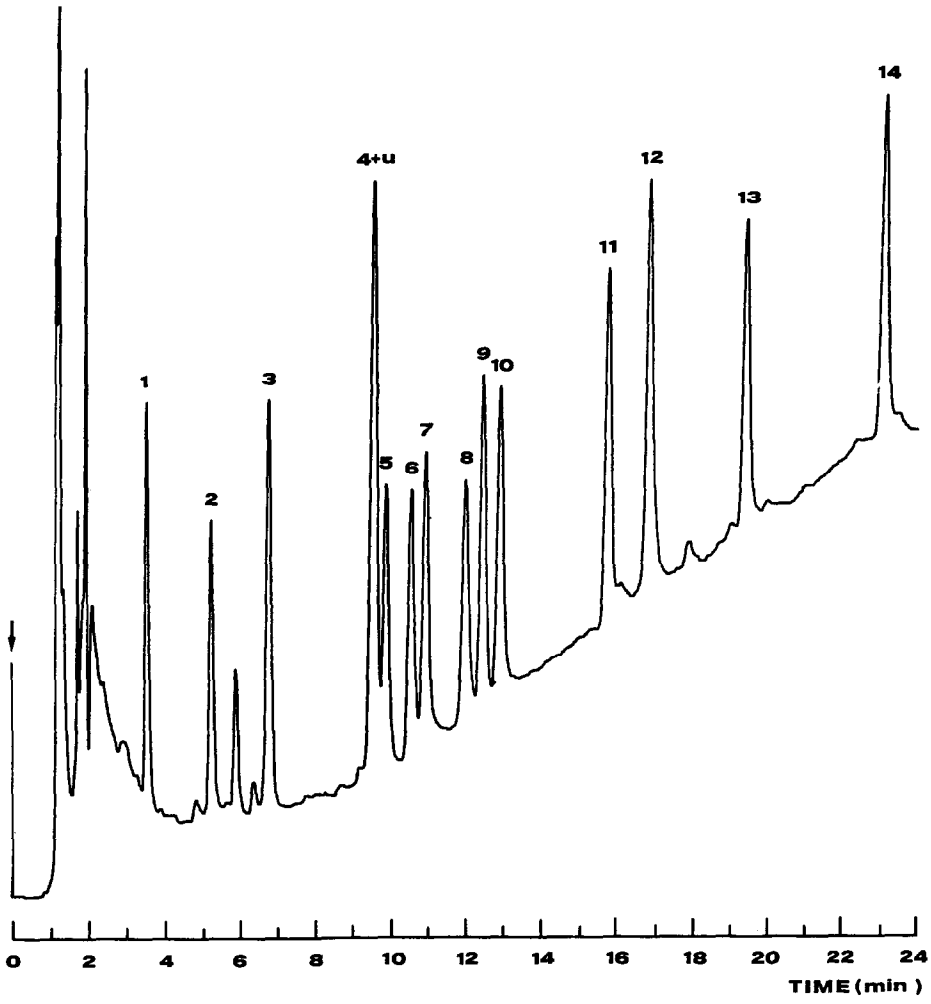

Fig. 3. Chromatogram obtained on analysing 2 l of a tap water specimen spiked with 30 ng/l of each phenylurea. Peaks as in Fig. 2; U, unknown compound eluted together with monolinuron.

estimated to be about 1.0 ng/l. Fig. 3 shows a typical chromatogram obtained on analysing 2 l of a drinking (tap) water sample spiked with 30 ng/l of phenylureas.

Sample storage

One of the advantages of liquid–solid extraction over liquid–liquid partitioning is that sampling and extraction of a water sample can be done simultaneously by passing the water through a sorbent trap as it is pumped at the sampling site. The small-volume cartridge could then be transported to the laboratory for desorption and chromatographic analysis. On the other hand, the original composition of extracts of heavily contaminated water samples might be altered after prolonged contact with the sorbent surface owing to some reactions catalysed by the adsorbing material itself. For phenylureas, the effect of storage was evaluated by extracting 1-l aliquots of a Tevere river sample spiked with 300 ng/l of each herbicide. After the water had been passed through them, the cartridges were stored at ambient temperature and analysed in duplicate after 5, 10 and 15 days of storage. In every case the recoveries of phenylureas were not significantly different from those obtained with unstored cartridges.

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