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CHARACTERIZATION OF MINERAL WATERS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

G. P. CARTONI* and F. COCCIOLI
Dipartimento di Chimica, Università di Roma, Piazzale Aldo Moro, Rome (Italy)

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SUMMARY

Mineral waters can be characterized by high-performance liquid chromatographic analysis after an on-line trace enrichment of the organic fraction. The major components of these non-volatile organic substances are the humic compounds found at a wide range of concentration. Phenylindole has been identified and determined in mineral waters stored in plastic containers.

INTRODUCTION

Mineral water is a natural spring-water which should be uncontaminated and bottled as such without any physical or chemical treatment. Only pure carbon dioxide can be added and declared on the bottle label. There is a large consumption of mineral waters in Italy, especially in hot regions and during the summer, and consequently many types of mineral waters are marketed.

For all these water types, the label should report a description of their therapeutical properties and bacteriological purity, the physico-chemical characteristics and the amounts of the more common ions. Very little information is provided about the dissolved organic matter; in some cases it is only written: "Organic substances absent". In a few cases the amount of potassium permanganate needed to oxidize all the organic compounds is reported.

In this paper are reported the results obtained for some Italian mineral waters by high-performance liquid chromatography (HPLC) with an on-line trace enrichment of the non-volatile organic fraction. HPLC studies have been carried out on the analysis of surface waters, especially for pollutant determinations. Priority pollutant phenols have been determined at trace levels[1]. In drinking water, the non-volatile organic compounds were analyzed by HPLC and some compounds identified by mass spectrometry[2]. The on-line trace enrichment by HPLC has been applied to identify some pollutants in lake water[3] and in mineral and river waters[4]. Reversed-phase HPLC has been applied to the determination of dissolved organic matter in estuarine water[5]. High-performance size exclusion chromatography was employed to study aquatic humic substances, and their molecular weight range was estimated[6].

The addition of chlorine to drinking water produces chlorinated compounds

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from the humic substances, and those have been identified by gas chromatography[7].

Finally, the contamination of water stored in plastic bottles has been investigated by HPLC[8].

EXPERIMENTAL

Equipment

Analyses were performed with a Perkin-Elmer Series 2 liquid chromatograph equipped with two independent pumps, a gradient controller and a variable-wavelength UV detector Model LC-55 with a potentiometric recorder. The column (250 mm × 4.6 mm I.D.) was slurry packed in our laboratory with LiChrosorb RP-18, 10 μm (Merck, Darmstadt, F.R.G.).

Reagents

Acetonitrile for HPLC was obtained from Carlo Erba (Milan, Italy). Pure water was prepared in our laboratory by redistillation of distilled water from potassium permanganate in all-glass apparatus.

Humic substances used as references were prepared in our laboratory by extraction of a peat sample, using a sodium pyrophosphate solution according to a procedure similar to that previously reported[7]. A standard solution was prepared by dissolving the dried compounds in twice distilled water.

Procedure

About 100 ml of the water sample were filtered through a 0.45-μm Millipore filter, degassed under vacuum for 15 min and placed in the reservoir of one pump of the liquid chromatograph. All the instrument lines had previously been cleaned and filled with twice distilled water. The flow-rate was 1.5 ml/min and the water under analysis was pumped for 30 min. During this period the traces of dissolved organic substances are accumulated in the first part of the column. The sample bottle was then replaced by a bottle of twice distilled water and pumping continued for 8 min.

A gradient from 0 to 100% acetonitrile in 20 min was then employed at a flow-rate of 1.5 ml/min. In this period the organic compounds are eluted, and at the end of the gradient pure acetonitrile was passed through the column to clean it and all the other parts of the instrument. The chromatograph was then reset to 0% acetonitrile and twice distilled water was pumped for another 5 min prior to a new analysis. Recoveries better than 90% were obtained for water samples spiked with less than 1 ppm of aromatic compounds such as benzene, toluene and naphthalene.

RESULTS AND DISCUSSION

In Fig. 1 a chromatogram is shown obtained with a mineral water, following the described procedure, compared with the blank. It is very important periodically to carry out some blanks with freshly twice distilled water, using exactly the same analysis conditions as for the mineral-water samples. It is seen that for the blank there are only small peaks at the end of the chromatogram which are not due to water because their heights do not change with the sampling time of the water, but are probably due to small impurities in the acetonitrile. The sampling time of 30

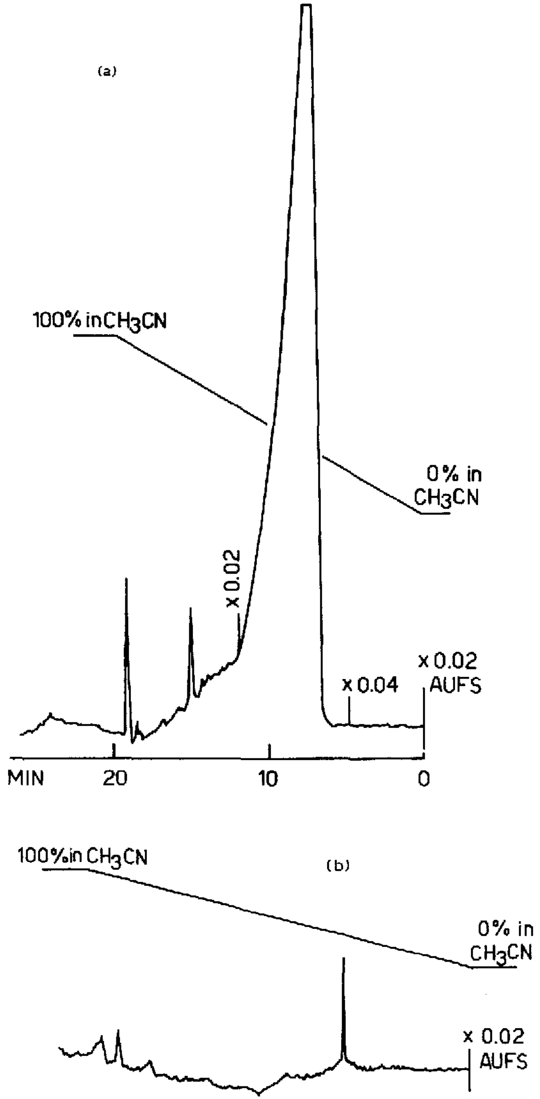

Fig. 1. (a) Chromatogram of Sangemini mineral water after on-column trace enrichment. UV detection at 254 nm. Gradient from 0 to 100% acetonitrile in 20 min. Column: 25 cm × 4.6 mm I.D., LiChrosorb RP-18, 10 μm. Flow-rate: 1.5 ml/min. (b) Blank chromatogram of twice distilled water recorded under the same conditions.

min corresponding to a water volume of 45 ml has been selected as a compromise between the need to process enough sample to achieve a high sensitivity and the fact that with too large amounts of water some of the more polar compounds can be eluted during the sampling time; if this happens an increase in the instrument baseline is observed.

TABLE I

RETENTION TIMES AND PEAK AREAS (mm^2) FOR SOME MINERAL WATERS

a and b represent different bottles.

Water sample	*Retention time (min)*					*Container*
	6.5	*14*	*15*	*18*	*18.5*	
Sacra	1500	51	34	29		Glass
Levissima	700	60		14	134	Plastic
Levissima	650					Glass
Sangemini (a)	5800	20		33		Glass
(b)	6200	32		47		Glass
Fiuggi (a)	900		18	26		Glass
(b)	900	10	58	25		Glass
Laurentina (a)	1400	15		45		Glass
(b)	1600	28		45		Glass
Panna	1100	36		16	45	Plastic

The retention times and the corresponding peak areas for the various samples of mineral waters are reported in Table I. For all the samples there is a large peak with a retention time of 6.5 min which has been shown to contain a mixture of humic substances. The identification was achieved by comparison of retention times and the UV spectra. A standard solution of 1 mg/l of humic acids in twice distilled water was analysed according to the reported procedure; a peak with the same retention time as that of the samples was observed. Also the UV spectra recorded for the chromatographic peak with the stoped-flow technique were identical for the samples and the standard.

These humic substances comprise a mixture of complex, polymeric substances with a broad range of molecular weights and are not separated under our chromatographic conditions. Their presence in drinking water is well known[6]. From the peak areas of the sample and the standard the concentrations of these humic compounds in some mineral waters have been calculated and the values are reported in

TABLE II

CONCENTRATIONS OF HUMIC SUBSTANCES IN SOME MINERAL WATERS

a and b represent different bottles.

Water	*Concentration (mg/l)*
Sacra	0.75
Levissima	0.35
Sangemini (a)	2.9
(b)	3.1
Fiuggi (a)	0.45
(b)	0.45
Laurentina (a)	0.7
(b)	0.8
Panna	0.55

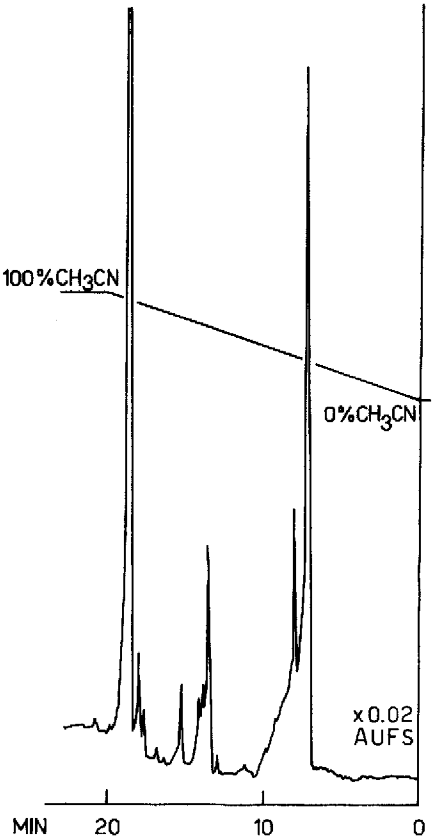

Fig. 2. Chromatogram of Levissima mineral water stored in plastic containers. Working conditions as in Fig. 1.

Table II. A standard deviation of ± 5% was obtained from repeated analysis. There are large differences in the concentrations for the examined mineral waters. "Sangemini" water shows the largest concentration, which can be explained by the nature of the soil where the spring is located.

TABLE III

CONCENTRATIONS OF PHENYLINDOLE IN SOME MINERAL WATERS STORED IN PLASTIC CONTAINERS

Water	*Concentration ($\mu g/l$)*	*Date of bottling*	*Date of analysis*
Levissima	5.8	March 29, 1982	March 7, 1983
Panna	0.15	September 13, 1982	March 20, 1983
Panna	1.8	February 12, 1981	March 31, 1983
Fabia	1.2	December 9, 1982	March 31, 1983
Boario	0.52	March 5, 1982	April 1, 1983
Evian	0.19	October 28, 1981	March 7, 1983

Owing to their high complexing properties, the humic substances can influence the therapeutical characteristics of the mineral water.

Many other peaks have also been observed for these waters but it is not clear whether they are of natural origin or arise from contamination or micropollution phenomena. Their concentrations are well below the ppb (μg/l) level and identification is not easy.

A large number of peaks was observed for mineral water samples stored in plastic bottles, which can be attributed to constituents of the container. Among these peaks we have identified one of the more abundant found only in samples stored in plastic bottles. This peak with a retention time of 18.5 min is due to phenylindole, identified by comparing the retention time and the UV spectra, recorded for the eluted peak, for the water samples and for a standard solution analysed under the same conditions (Fig. 2). In Table III are reported the concentrations of phenylindole in mineral waters stored in plastic bottles together with the date when the water was bottled and when it was analyzed. Phenylindole is used as an additive to plastic materials and consequently it is slowly released into water; its concentration depends on the plastic quality and on time and storage conditions.

This procedure of trace enrichment on the top of the analytical column is very advantageous for clear diluted samples such as mineral waters. With the described method the risk of contamination from solvents and laboratory manipulation is strongly reduced. No instrument modification is required and any commercial apparatus can be used. The chromatograms obtained are very characteristic for each type of mineral water examined, and as it is required to determine the chemical composition of inorganic constituents, an HPLC analysis of the dissolved organic materials should be also carried out. With the danger of contamination of a spring from industrial or agricultural products and the increasing use of plastic containers, it is advisable periodically to monitor the content of organic material in bottled mineral waters.

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