Journal of Chromatography, 367 (1986) 69–76
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 825

2,4-DIHYDROXYBENZOIC ACID AS A NOVEL ELUENT IN SINGLE COLUMN ANION CHROMATOGRAPHY

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(First received February 13th, 1986; revised manuscript received May 20th, 1986)

SUMMARY

2,4-Dihydroxybenzoic acid has been studied as an eluent for single column ion chromatography with indirect UV detection. The advantages of this eluent are discussed. Only low concentrations of 2,4-dihydroxybenzoic acid in the mobile phase are needed. Eighteen anions could be detected. The early eluting anions such as silicate and fluoride show well separated peaks and can be determined simultaneously with other common anions. The detection limit is, *e.g.*, 150 ppb for silicate (as Si) and 50 ppb for fluoride. It is shown that this method can be applied to the determination of anions in tap- and mineral-water samples. A baseline separation is obtained in about 20 min. Many other applications are possible because of the high resolution of this system, thus also silicate, phosphate and arsenate can be separated. The loss of ion-exchange capacity poses no problem since a decrease in the concentration of 2,4-dihydroxybenzoic acid results in almost the same chromatogram as before.

INTRODUCTION

In recent years, ion chromatography has steadily been developed and is now widely applied to the determinations of ions. Haddad and Heckenberg[1] reviewed anion chromatography by high-performance liquid chromatography (HPLC). Frequently a modular system with a single column technique is used, thus there is no need for a suppressor and all components can later be used in a different configuration. The system is also more flexible in the use of different organic and inorganic salts as eluents[2,3]. In most cases the detection limit is much lower when using a suppressor[4], but a single column system is generally sufficient.

The present paper describes a new single column system, applied to the determination of silicate, fluoride, chloride and sulphate in drinking and mineral waters. Other anions can also be detected and separated.

The problem with earlier systems lay in the separation of peaks at the beginning of the chromatogram; it was possible only to separate the major anions, for example in mineral-water samples[5]. The determination of fluoride can be achieved only in standard solutions. Attempts to separate fluoride from the injection peak failed.

0021-9673/86/$03.50

Special methods were developed for determining fluoride and other difficult anions by ion chromatography. The disadvantage of these systems is that they are only capable of detecting small groups or single anions, *e.g.*, anions like fluoride, borate and silicate eluted early with 3.2 m*M* potassium hydroxide at pH 11.5[6]. Other anions could not be determined by this system.

Dionex developed a system appropriate to the determination of silicate together with other anions (by conductivity detection) with a post-column reaction detector[4]. A single-column technique and only one detector is required. This system can be realized only by using a column which is stable at pH > 8, otherwise some anionic species are insufficiently dissociated.

Okada and Kuwamoto[7,8] described such a chromatographic system where potassium hydroxide is the eluent and detection is achieved with a conductivity detector. The retention time is about 30 min for sulphate, consequently the determination of late eluting anions is time-consuming. The separation is not very selective in all cases, *e.g.*, silicate and fluoride, therefore greater differences in concentrations present difficulties. Its adaption to different separation problems is also difficult because a change in the eluent concentration always implies a change in pH. Greater flexibility would result if both parameters were to be varied independently.

Lee[6] described a new anion-exchange resin which has favourable characteristics for anion determinations. The present paper reports a detailed investigation of 2,4-dihydroxybenzoic acid as an eluent for this phase. Fundamental studies on improving the efficiency of the separation of anions have been made. The aim was to devise a simple and efficient system for the determination of early eluting anions simultaneously with other anions in a reasonable time. This system is applicable to water samples.

EXPERIMENTAL

Apparatus

The HPLC equipment consisted of a dual-head reciprocating pump (Bischoff 2200), a syringe-loading sample injector (Bischoff 7125) and a variable-wavelength UV detector (Bischoff 8201) linked to a pen recorder (Linseis L 6510). The variable-loop injector with a 100-μl loop was integrated in a thermobox (Bischoff 4000). A water-bath (Lauda mgw k 4) was used to keep the temperature constant at 30°C. The flow-rate of the HPLC pump was set to 3.0 ml/min. The chart speed of the recorder was 1 cm/min. The column (Bischoff Hyperchrome NC, 250 mm × 4.6 mm I.D.) was filled with Hamilton PRP-X 100 strongly basic anion-exchange resin. The particle size of this resin was 10 μm and the exchange capacity was 0.17 mequiv./g.

Reagents

The degree of purity of 2,4-dihydroxybenzoic acid was "purum" (Fluka, >98%). The eluent was prepared every day. Potassium hydroxide (analytical grade, Fluka) was added to obtain the required pH. Water for preparing the eluent and the standard solutions was first de-ionized and then distilled. Water samples were pretreated with a cation exchanger (Serva, Dowex 50W-X8, analytical grade, 100–200 mesh, H^+), 1–2 g per 10 ml.

RESULTS AND DISCUSSION

2,4-Dihydroxybenzoic acid is used for the determination of early eluting anions simultaneously with late eluting ones in an acceptable time, *i.e.*, a total retention time of about 20 min for sulphate. It was chosen as the eluent for the following reasons. *p*-Hydroxybenzoic acid and Hamilton PRP-X 100 anion-exchange resin provide a reliable system for the determination of many anions[6]. The ion-exchange resin consists of polystyrene–divinylbenzene with chemically bonded trimethylammonium groups. Fluoride is only detectable in standard solutions by this system. Experiments showed that salicylic acid, *o*-hydroxybenzoic acid, is a more powerful eluent, but the separation of anions was not very satisfactory. An increase in eluting power resulting from the presence of two hydroxyl groups and a carboxyl group was expected, and the advantasgeous characteristics of *p*-hydroxybenzoic acid should be maintained. The results obtained in the present study bear this out.

The dissociation constants (Table I) of 2,4-dihydroxybenzoic acid suggest that all its ionizable groups influence the elution strength at pH *ca.* 10. The similarity of the dissociation constants of 2,4-dihydroxybenzoic acid to those of *p*-hydroxybenzoic acid and salicylic acid is remarkable, thus a fusion of the characteristics becomes evident. The eluting power of 2,4-dihydroxybenzoic acid exceeds those of the primary substances. This is very important, because it is now possible to decrease the concentration of the organic salt in order to detect the early eluting anions, simultaneously with the late eluting ones, as sharp and well separated peaks.

TABLE I

DISSOCIATION CONSTANTS[12] OF THE ACIDS USED AS ELUENTS

pK	*2,4-Dihydroxy-benzoic acid*	*p-Hydroxy-benzoic acid*	*Salicylic acid*
pK_1	3.29	4.58	3.00
pK_2	8.98	9.23	13.4
pK_3	13	–	–

Since only minimum concentrations of organic salts are necessary and 2,4-dihydroxybenzoic acid shows high UV absorbance, an indirect UV detection is possible. This detection mode for "transparent" ions was described in detail by Small and Miller[9] and Naish[10].

The pH has to be adjusted to around 10, pH 10.1 being preferred. If the pH is higher than 10.2, difficulties arise with the determination of sulphate, because a new negative system-peak appears directly in front of the sulphate peak. If the eluent pH is lower than 9.8, the peak height for silicate will decrease and its separation from the injection peak will deteriorate. This fact can be explained by a higher polymerization rate of silicate[11] and the weaker ion dissociation. At pH 10.0–10.2, monosilicic acid will be the predominant form of silicate.

The wavelength for the indirect UV detection does not coincide with the ab-

sorption maximum of the eluent. A wavelength of 312 nm is used at which the signal-to-noise ratio is most favourable.

The chromatogram, in Fig. 1 shows the detection of seven important ions with high resolution; all peaks are baseline separated. The total retention time for most water analyses is represented by that of the sulphate peak. Fig. 2 presents a chromatogram of an "old" column, *i.e.*, on which 600 injections had been made. The eluent concentration had to be decreased in order to achieve a separation comparable to that shown in Fig. 1. However, the retention times in the two cases are equal. Only the sensitivity of sulphate detection deteriorates. The detection limit for sulphate is 500 ppb* on a "new" column and 2 ppm on the "old" column; the eluent concentration has little influence on the detection limit of, *e.g.*, fluoride and silicate.

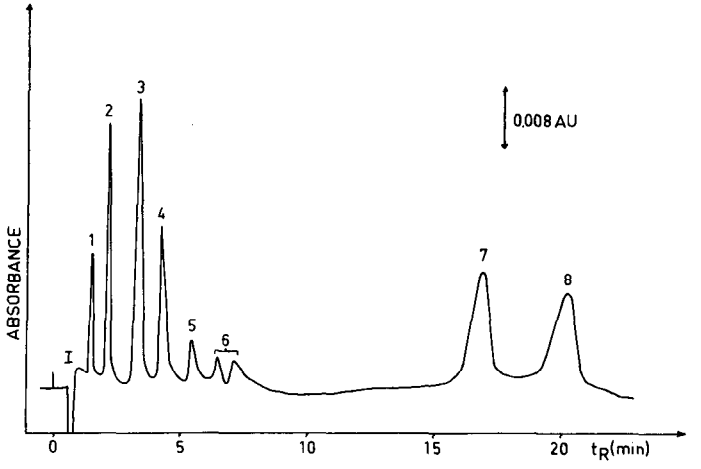

Fig. 1. HPLC separation of seven important anions. Eluent: 70 mg/l 2,4-dihydroxybenzoic acid adjusted to pH 10.1 with potassium hydroxide. Column: Bischoff Hyperchrome NC (250 mm × 4.6 mm I.D.) filled with Hamilton PRP-X 100 anion-exchange resin. Flow-rate: 3 ml/min. Temperature: 30°C. Sample size: 100 µl. UV Detector: wavelength 312 nm. Peaks: I = injection peak; 1 = silicate (10 ppm); 2 = fluoride (3 ppm); 3 = chloride (10 ppm); 4 = nitrite (10 ppm); 5 = bromide (10 ppm); 6 = system peak; 7 = phosphate (20 ppm); 8 = sulphate (20 ppm).

The system can be adjusted to every stage of diminishing ion-exchange capacity of the column. We cannot yet explain the diminishing ion-exchange capacity and hitherto all attemps to regenerate the column have failed. It is expected that a column used in routine analysis under unchanged conditions can be utilized more frequently until the eluent concentration has to be decreased to 25 mg/l 2,4-dihydroxybenzoic acid, as in Fig. 2.

Tap-water samples can be analyzed (Fig. 3), although in most cases they contain only small quantities of silicate and fluoride. The total retention time is 20 min for sulphate, with simultaneous baseline separation. The detection limit is 150 ppb for silicate (as Si) and 50 ppb for fluoride (based on three times the baseline noise).

* Throughout this article the American billion (10^9) is meant.

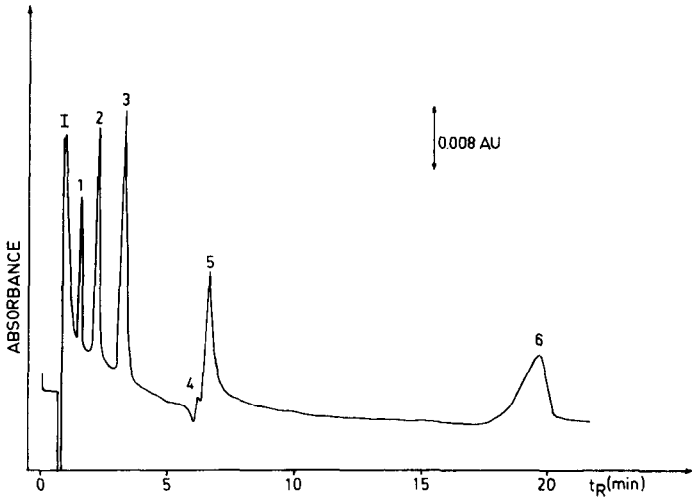

Fig. 2. HPLC separation of anions and an "old" column (600 injections). Eluent: 25 mg/l 2,4-dihydroxybenzoic acid adjusted to pH 10.1 with potassium hydroxide. Other conditions as in Fig. 1. Peaks: I = injection peak; 1 = silicate (10 ppm); 2 = fluoride (3 ppm); 3 = chloride (10 ppm); 4 = system peak; 5 = nitrate (10 ppm); 6 = sulphate (20 ppm).

These limits are low enough for analyzing water samples. As shown in Fig. 4, samples of mineral water can also be determined. A lower detection limit can be obtained if a high-performance conductivity detector is used.

The determination of silicate and fluoride in water samples is only possible if the sample is pretreated with a cation-exchange resin (see Experimental), because

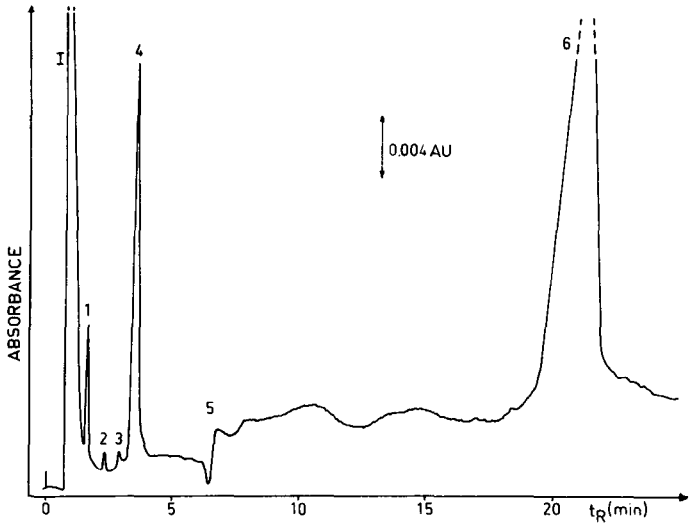

Fig. 3. Anion analysis in tap-water. For conditions see Fig. 1. Peaks: I = injection peak; 1 = silicate (3.9 ppm); 2 = fluoride (110 ppb); 3 = unknown; 4 = chloride (8 ppm); 5 = system peak; 6 = sulphate (40.5 ppm).

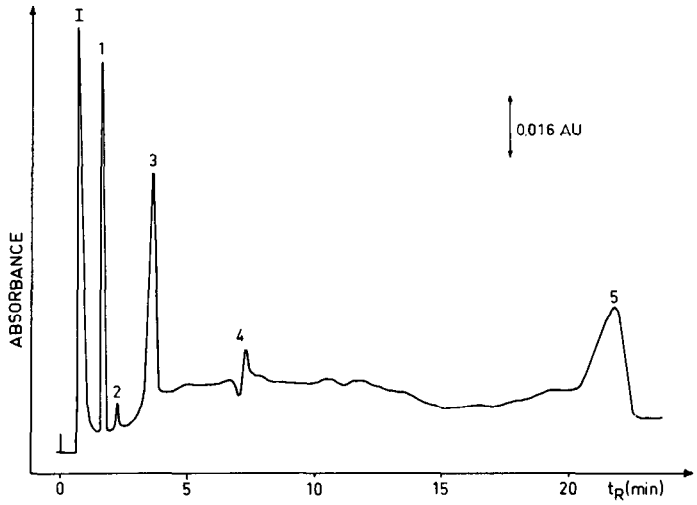

Fig. 4. Anion analysis in mineral water "Teinacher Sauerbrunnen". For conditions see Fig. 1. Peaks: I = injection peak; 1 = silicate (55.4 ppm); 2 = fluoride (580 ppb); 3 = chloride (22.9 ppm); 4 = system peak; 5 = sulphate (41 ppm).

cations exhibit a large negative signal directly after the injection peak. This peak interferes with the determination of the first anion peaks. The pretreatment results in different pH values for the samples, depending on the amounts of ions contained. The difference in pH does not affect the separation and sensitivity. Samples of mineral water containing an high quantity of sulphate (> 1 g/l) can be analyzed in the same way. For the pretreatment of tap-water, 1 g cation-exchange resin per 10 ml is sufficient, whereas 2 g of cation-exchange resin are needed for the mineral-water samples due to the large cation concentrations. The sample and the cation-exchange resin were mixed, degassed and filtered through a 0.45-μm membrane filter before injection.

TABLE II

RETENTION TIMES OF ANIONS

Chromatographic conditions as in Fig. 1. The standard deviations of the retention times are about 5%.

Anion	*Retention time (min)*	*Anion*	*Retention time (min)*
Silicate	1.55	System peak	6.5
Borate	2.05	Hydrogen carbonate	6.5
Fluoride	2.15	Nitrate	6.75
Iodate	2.35	Chlorate	10.8
Chloride	3.25	Phosphate	15.9
Cyanide	3.8	Selenite	16.9
Nitrite	4.1	Arsenate	17.8
Bromate	4.5	Sulphate	18.8
Bromide	5.2	Selenate	20.8
Azide	5.7	Molybdate	25.5

TABLE III

INFLUENCE OF ELUENT CONCENTRATION ON RETENTION

Concentration of 2,4-dihydroxybenzoic acid (mg/l)	*Retention time (min)*	
	Fluoride	*Sulphate*
25	3.4	No peak after 50 min
40	2.8	32
70	2.1	18.8

All anions tested are listed in Table II together with their retention times in minutes. Sulphate has a retention time of 18.8 min and not of about 20 min as shown in the chromatograms below. This can be explained by a loss of ion-exchange capacity (120 injections on this column), the eluent concentration remaining the same. Tests with a standard solution containing fluoride, silicate, chloride and sulphate showed that the retention time does not vary upon five successive injections. With the exception of iodide, all anions tested were detectable.

Different eluent concentrations were tested on a new column to determine the dependence of the retention on the reagent concentration (Table III).

Since a number of anions can be determined, a wide application is expected. Many mixtures of anions can be separated. As an example, the separation and simultaneous determination of silicate, phosphate and arsenate is shown in Fig. 5. This separation is of interest for the simultaneous determination of very low concentrations of these anions after preconcentration as heteropoly acids with molybdate. This method is an adequate alternative to the more complicated photometric determination. A detailed description of the possibilities for the simultaneous determination of phosphate, arsenate and silicate by single column ion chromatography will be published elsewhere.

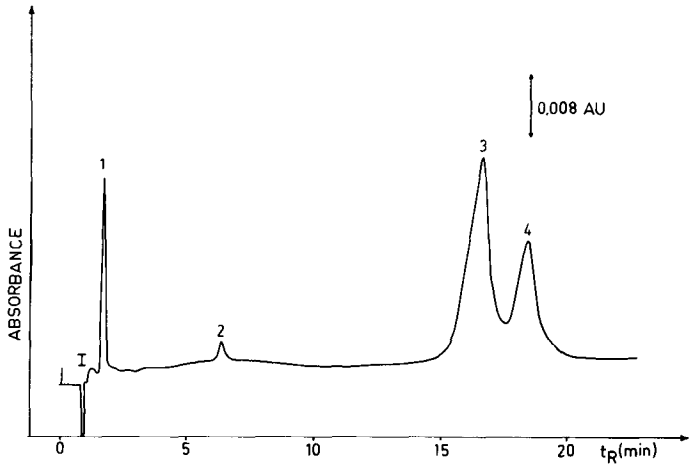

Fig. 5. HPLC separation of some special anions. For conditions see Fig. 1. Peaks: I = injection peak; 1 = silicate (20 ppm); 2 = system peak; 3 = phosphate (50 ppm); 4 = arsenate (50 ppm).

Due to baseline separation minor difficulties arise respecting large differences in concentration of neighbouring anions. In future studies we will investigate systematically the correlation of eluting characteristics and functional groups of substances used as eluents for single column anion chromatography on anion-exchange resins, *e.g.*, other dihydroxybenzoic acids, phthalates, benzenetricarboxylate, etc.

ACKNOWLEDGEMENTS

Our thanks are due to the Deutsche Forschungsgemeinschaft (DFG), Bonn-Bad Godesberg and to Bischoff Analysentechnik, Leonberg (F.R.G.) for their support of this work.

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