Pergamon

Talanta, Vol. 41, No. 2, pp. 279–282, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0039-9140/94 $6.00 + 0.00

SEPARATION AND DETERMINATION OF TRACE AMOUNTS OF BERYLLIUM(II), ALUMINIUM(III) AND CHROMIUM(III) WITH CHROMOTROPE 2C CHELATES BY RP-HPLC

LIU SHOUPU, ZHAO MINGQIAO and DENG CHUANYUE
Department of Chemistry, Southwest China Teachers University, Chongching, People's Republic of China

(*Received* 7 *January* 1993. *Revised* 28 *June* 1993. *Accepted* 2 *July* 1993)

Summary—A reversed-phase high-performance liquid chromatographic separation and determination of beryllium(II), aluminium(III) and chromium(III) with chromotrope 2C chelates on a C18-bonded stationary phase is reported. Methanol–water (45:55 v/v) containing $6 \times 10^{-3}M$ tetra-*n*-butylammonium bromide (TBAB) and $2 \times 10^{-2}M$ acetate buffer solution (pH 6.0) as mobile phase and with spectrophotometric detection at 530 nm was applied. The method has high sensitivity, the detection limits being 0.2 ppb for beryllium(I), 1 ppb for aluminium(III) and 2 ppb for chromium(III). Under the optimum conditions, most other metal ions did not interfere, *e.g.* up to 2 mg of Hg(II), Sn(II, IV), Pb(II), Bi(III), Ag(I), Zn(II), Cd(II), Cu(II), 1.5 mg of Fe(II), Co(II), Ni(II), 1.2 mg of Ca(II), Mg(II), Sr(II), Ba(II), 1 mg of Ga(III), In(III), 0.5 mg of Fe(III), 1 mg of Ga(III), In(III), 0.5 mg of Fe(III), 0.4 mg of Th(IV), Zr(IV). The method can be applied to the simultaneous determination of trace amounts of beryllium(II), aluminium(III) and chromium(III), in water, rice, flour and human hair samples.

The chromotrope 2C [or named as carboxyazo I, disodium salt 2-(2-carboxyphenylazo)-1,8-dihydroxy naphthalene-3,6-disulfonic acid] is a monoazo compound of the chromotropic acid.

It was first synthesized by Majumder and Savariar[1] as a metallochromic indicator for the complex titration of thorium, zirconium and iron with EDTA, and as a colour reagent for spectrophotometric determination of thorium and aluminium.[1,2] The colour reaction of it with other metal ions has not been investigated, and the use as a chromatographic reagent for separation and determination of metal ions has not been reported. Our experiments found that it also formed red chelates with Be(II), Cr(III) and V(IV), *etc.* However, these red chelates sensitivities are not high and show poor selectivities in the determination of these ions by spectrophotometry, because the overlapping of their absorption bands lead to mutual interference. In this work we found that the RP-HPLC determination of Be(III), Al(III) and Cr(III) with chromotrope 2C chelates had high sensitivity and very good selectivity, the usual metal ions and anions do not interfere in the determination. The method can be applied in the determination of beryllium(II), aluminium(III) and chromium(III) in water, rice, flour and human hair samples.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Model LC-6A pump, a Model C-R3A reporting integrator (Shimadzu, Kyoto, Japan), a Rheodyne Model 7125 injector (Cotati, CA, USA) with a 20-μl sample loop, a column peaked with 9–10 μm YWG-C18 (250 × 4.0 mm I.D.) (Dalian Institute of Chemical Physics, Academia Sinica, China), a Model SPD-6AV UV–VIS spectrophotometric detector (Hitachi, Kyoto, Japan), a Model SA-720 pH meter (Orion, Cambridge, MA, USA), a Model CQ-50 ultrasonicator for degassing the mobile phase (Shanghai, Ultrasonic Instrument Factory, Shanghai, China) and a Model 8800 centrifuge (Shanghai, China) were used.

A Model WF-1A atomic absorption spectrometer (Beijing Second Optical Instrument Factory, Beijing, China) was used for the determination of chromium and beryllium using a Model WF-4A graphite furnace.

A Model U-3400 spectrophotometer

Hitachi, Kyoto, Japan) was used for the determination of aluminium.

Reagents

The reagent chromotrope 2C 0.1% (v/v) was synthesized according to Refs. 1, 2.

A stock standard solution of various metal ions at a concentration of 1 mg/ml was prepared by the usual method from highly pure metal oxides or salts. Working standard solutions at a concentration of 1 μg/ml were prepared by dilution with water.

The mobile phase was methanol–water (45:55 v/v) containing $5 \times 10^{-3}M$ TBAB and $2 \times 10^{-2}M$ acetate buffer (pH 6.0). The mobile phase was filtered through a 0.45 μm membrane filter and degassed using a model CQ-50 ultrasonic bath before use.

General procedure

Place 1 μg of Be(II) and 2 μg of Al(III) and Cr(III) in a 25-ml standard flask. Add 2.0 ml of acetic acid–sodium acetate buffer solution (pH 6.0), 2.0 ml of 0.1% chromotrope 2C solution, mix and dilute to about 20 ml with water. After heating in a water-bath at boiling temperature for 15 min, the solution was cooled to room temperature, diluted to the mark with water, and centrifuged for 3 min at 3000 rpm. Then, an aliquot of 20 μl of the solution was injected into the chromatograph with the 20-μl loop injector. The flow-rate of the mobile phase was 0.9 ml/min and the eluate was monitored at 530 nm with an SPD-6AV UV–VIS spectrophotometric detector. The sensitivity was set at 0.01 a.u.f.s. The amounts of metal ions eluted were determined from the peak areas.

Determination of Be(II), Al(III) and Cr(III) in water was as follows.

Clean, filtered 15-ml water samples were put in a 25-ml standard flask, then Be(II), Al(III) and Cr(III), as determined and described above in the general procedure, were added.

Determination of Be(III), Al(III) and Cr(III) in rice, flour and human hair samples was accomplished in the following manner.

A 1.000 g amount of oven-dried rice flour or human hair samples was weighed in a crucible, covered and ashed in an electric oven at 750°C for 3 hr. The ash was dissolved in nitric acid and the solution was evaporated nearly to dryness. The residue was dissolved with 5 ml water, filtered into a 25-ml standard flask and diluted to the mark with water, then Be(II), Al(III) and Cr(III) were determined as depicted above in the general procedure.

RESULTS AND DISCUSSION

Colour conditions and wavelength of the chelates

Only Be(II), Al(III), Th(IV) and V(IV) developed colour with chromotrope 2C in pH 5 acetic acid–sodium acetate buffer solution at ambient temperature; Cr(III) also formed a red chelate after heating, and the colour reaction can be completed after heating for 15 min in a water-bath at boiling temperature. However, only the peaks of the chelates of Al(III), Be(II) and Cr(III) were shown in the chromatographic system. The characteristics of the chelates of chromotrope 2C with Be(II), Al(III) and Cr(III) and their colour conditions are shown in Table 1.

The wavelength of maximum absorption of the chelates are between 530 and 535 nm with the mobile phase as blank. Therefore, Be(II), Al(III) and Cr(III) can be satisfactorily separated and determined in the presence of other interfering ions by RP-HPLC with spectrophotometric detection at 530 nm.

Effect of the composition and pH of the mobile phase

A number of binary or tertiary combinations of organic solvents and water such as methanol–water, methanol–acetonitrile, methanol–acetonitrile–water and methanol–tetrahydrofuran–water were investigated as potential mobile phases. It found that the differences in retention time of the reagent and the chelates were small. For this reason, the separation of these chelates was unsatisfactory.

When an organic cation such as TBAB as a

Table 1. The colour conditions and the characteristics of the chromotrope 2C chelates with Be(II), Al(III) and Cr(III)

Metal ions	pH	Temperature	$\varepsilon \times 10^{-4}$ (*l/mol·cm*)	λ_{max} (*nm*)	Composition ratio
Be(II)	5.5–7.0	R.T.	1.35	530	1:1
Al(III)	4.5–6.5	R.T.	2.15	535	1:1
Cr(III)	4.0–6.5	100°C (15 min)	1.35	535	1:1

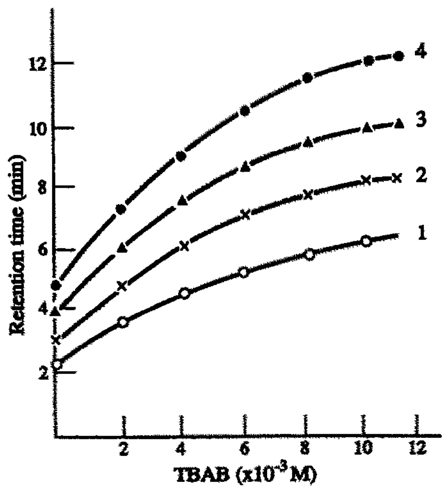

Fig. 1. Effect of the concentration of TBAB on retention time. (1) Be(II)–chromotrope 2C. (2) Al(III)–chromotrope 2C. (3) Cr(III)–chromotrope 2C. (4) Chromotrope 2C.

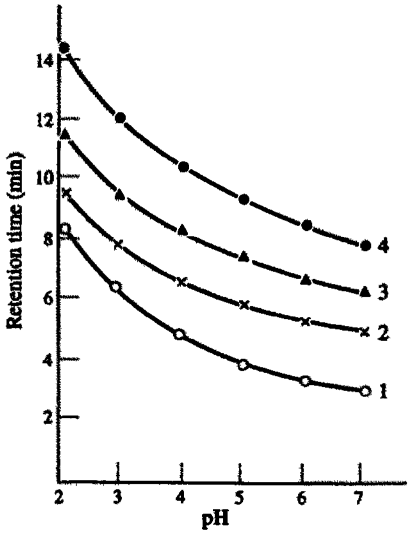

Fig. 3. Effect of pH on retention time. (1) Be(II)–chromotrope 2C. (2) Al(III)–chromotrope 2C. (3) Cr(III)–chromotrope 2C. (4) Chromotrope 2C.

counter ion was added to the mobile phase, because the cation has formed the ion pairs with the anionic chelates, each chelate retention time and their differences were all increased. The optimum concentration of TABA is 6m*M* and the best separation was obtained using methanol–water as a mobile phase (see Figs 1 and 2).

The effect of the ethanol concentration on the retention time of the chelates was investigated in the mobile phase. It is shown in Fig. 2. When the methanol concentration is between 42 and 48%, the best separation of the chelates was obtained. The effect of pH on the retention time was studied by using different pH acetic acid–sodium acetate buffer solutions in methanol–water solution (45:55 v/v). The retention time of the chelates were decreased with the increase of pH in the mobile phase in the presence of TBAB (see Fig. 3). The optimum pH ranges from 5.0 to 7.0. It is also a suitable pH for forming the chelates and so a higher sensi-

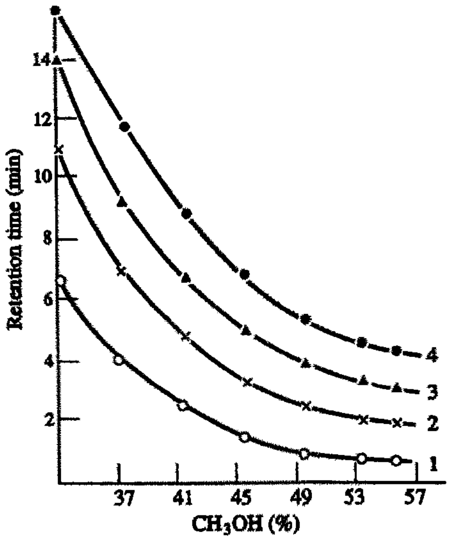

Fig. 2. Effect of the concentration of methanol on retention time. (1) Be(II)–chromotrope 2C. (2) Al(III)–chromotrope 2C. (3) Cr(III)–chromotrope 2C. (4) Chromotrope 2C.

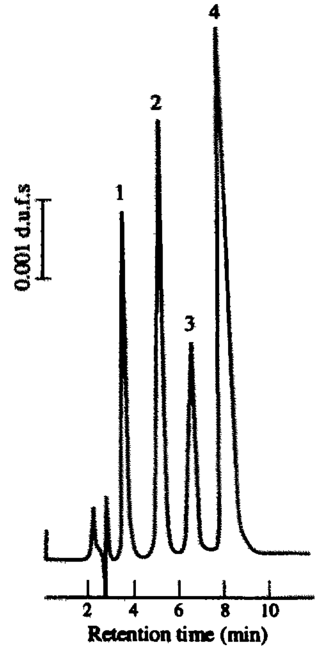

Fig. 4. Standard chromatogram. Be(II), 1.0 μg/25 ml; Al(III) and Cr(III), 2.5 μg/25 ml peaks: 1 = Be(II). 2 = Al(III). 3 = Cr(III). 4 = Chromotrope 2C.

Table 2. Determination of Be(II), Al(III) and Cr(III) in water, rice, flour and human hair

Sample	Element	Content determined ($\mu g/l$)‡	Average content ($\mu g/l$)‡	Relative standard deviation (%)	AAS* method ($\mu g/l$)‡
Spring water	Be	0.22, 0.21, 0.22, 0.22	0.22	2.3	0.24
	Cr	9.8, 10.2, 10.4, 10.2	10.21	2.5	10.06
	Al	94.8, 95.3, 97.2, 98.0	96.3	1.6	94.4†
Pool water	Be	0.21, 0.20, 0.21, 0.22	0.21	3.9	0.21
	Cr	31.4, 30.7, 31.2, 30.9	31.05	1.0	30.24
	Al	72.8, 73.5, 74.1, 74.3	73.67	0.9	72.56†
Tap water	Be				
	Al	49.5, 48.7, 49.6, 48.8	49.15	0.9	52.49†
	Cr	23.3, 22.7, 23.5, 23.4	23.2	1.6	20.72
Rice	Be				
	Cr	84.3, 84.8, 85.2, 84.6	84.8	0.4	84.30
	Al	48.7, 48.9, 47.6, 47.8	48.5	1.3	50.3†
Human hair	Be	2.2, 2.1, 2.2, 2.2	2.2	2.3	2.2
	Cr	1.51, 1.57, 1.56, 1.53	1.55	1.8	1.50
	Al	9.4, 9.2, 9.3, 9.4	9.35	1.2	9.25†
Flour	Be				
	Cr	16.5, 16.7, 16.2, 16.7	16.65	2.5	16.40
	Al	8.72, 8.35, 8.32, 8.42	8.40	0.8	8.20†

*Atomic absorption spectrometry.[3]

†Spectrophotometry with chromazol KS and cetylpyridinium bromide.[4]

‡The content determined were μg/g for rice, human hair and flour samples.

tivity was obtained. In subsequent experiments acetate buffer solutions (pH 6.0) were used.

Effect of foreign ions

The effect of foreign ions on the determination of 1 μg of beryllium(II), 2 μg of aluminium(III) and chromium(III) was studied under the optimum conditions. The following ions do not interfere with a relative error of less than 5%, up to 2 mg of Hg(II), Sn(II, IV), Pb(II), Sb(III, V), Bi(III), Ag(I), Zn(II), Cd(II), Cu(II), 1.5 mg of Fe(II), Co(II), Ni(II), 1.2 mg of Ca(II), Mg(II), Ba(II), 1 mg of Ga(III), In(III), 0.5 mg of Fe(III), 0.4 mg of Th(IV), Zr(IV). Hence the method has high selectivity.

Calibration graph and detection limits

Calibration graph of peak area *vs*. metal ion concentration were linear in the concentration ranges 0–200 ppb for Be(II), 0–400 ppb for Al(III) and Cr(III).

The detection limits, calculated as the concentration that gave a signal triple the background noise (signal-to-noise ratio 3), were 0.2 ppb for Be(II), 1 ppb for Al(III) and 2 ppb for Cr(III). A standard chromatogram is shown in Fig. 4.

Real samples were analysed

The results for the determination of Be(II), Al(III) and Cr(III) in water, rice, flour and human hair are shown in Table 2. The relative standard deviations are from 0.4 to 3.9%.

REFERENCES

1. A. K. Majumder and C. P. Savariar, *Z. Anal. Chem.*, 1960, **174**, 267.
2. Z.-F. Liu, S.-P. Liu and X.-C. Wang, *Metallurgical Analysis*, 1990, **10**, 9.
3. H.-T. Zhang, *The Complete Works of Water Analysis*, The Documental Press of Chinese Science and Technology, Chongqing, 1989, 204.
4. S.-P. Liu, *Analyst*, 1982, **107**, 484.