Separation and determination of scandium by reversed-phase high-performance liquid chromatography with diacetyl-*N*,*N*-bis(4-hydroxybenzoylhydrazone) as a pre-column chelating agent

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An HPLC method for the determination of scandium(III) was developed, with diacetyl-*N*,*N*-bis(4-hydroxybenzoylhydrazone) (DBHB) as a pre-column chelating agent. Tetradentate DBHB formed a 1 : 1 chelate with Sc^{III} ion. The Sc^{III}–DBHB chelate was separated on a C_{18}–silica gel column with a mobile phase of acetonitrile–water (30 + 70 m/m) containing 5×10^{-3} mol kg^{-1} tetramethylammonium bromide and 2.5×10^{-3} mol kg^{-1} hexamethylenetetramine buffer. Using a 100 mm^3 injection at 420 nm, the detection limit for Sc^{III} was 8.4×10^{-10} mol dm^{-3} (37 ng l^{-1}). Rare earth elements gave no peaks on the chromatogram. The method can detected Sc^{III} in river water without preconcentration.

Introduction

One of great concerns in geology and metallurgy is the determination of scandium in ores, rocks, alloys and pure metals. In addition, as toxicity of scandium ion against giant algae has been reported,[1] the demand for measuring scandium in environmental samples has also been recognized.

Spectrometry, fluorimetry, inductively coupled plasma atomic emission spectrometry (ICP-AES), electrothermal atomic absorption spectrometry (ETAAS) and neutron activation analysis (NAA) have been used to determine scandium. However, most of these techniques have poor sensitivity and suffer from matrix interferences. For example, the determination of scandium by ICP-AES suffers seriously[2,3] from spectral interference from the rare earth elements. Also, the spectrophtometric determination of scandium with chromogenic agents such as 5,7-dichloroquinolin-8-ol,[4] Xylenol Orange,[5] Chromazurol S,[6] Arsenazo III[7] and Nile Blue[8] shows a lack of selectivity and sensitivity, so that it was necessary to combine preseparation and preconcentration techniques for the determination of scandium. ETAAS must overcome the memory effect due to the formation of non-volatile scandium oxide[9] to achieve satisfactory sensitivity. Although NAA[10–13] possesses high sensitivity and selectivity, there is the practical limitation that the apparatus requires a neutron source. Only a few studies[14,15] on the determination of scandium by ICP-MS seem to have been performed.

On the other hand, the determination of metal ions by reversed-phase high-performance liquid chromatography (HPLC) following derivatization to metal chelates with an organic chelating agent possesses attractive advantages such as high selectivity, simplicity, low cost and simultaneous detectability. This technique was called pre-column chelation HPLC. Most metal ions have been determined by this technique and some reviews have been published.[16–19] However, scandium has not previously been determined by this technique. Only ion-exchange chromatography using HPLC techniques has been used to separate scandium together with rare earth elements.[20,21]

We have previously reported[22,23] that the coordinative function of the silanol group on silica-based stationary phases seriously influences the elution behavior of some metal chelates. We also found that the bis(hydrazone) group could be used to detect scandium with pre-column chelation HPLC, in screening metal chelates whose elution behavior was influenced by the coordinative function of the silanol group. In this work, we separated and determined scandium by HPLC with the bis(hydrazone) diacetyl-*N*,*N*-bis(4-hydroxybenzoylhydrazone) as a chelating agent. Although a few bis(hydrazones) have already been used as chelating agents for HPLC,[24–26] their potential has not been fully explored.

Experimental

Synthesis of DBHB

Diacetyl-*N*,*N*-bis(4-hydroxybenzoylhydrazone) (DBHB) was synthesized using the procedure reported by Silva *et al.*[27] and Lever.[28] Diacetyl (0.05 mol, 4.3 g) and twice the amount of salicylhydrazide (0.1 mol, 15.2 g) were dissolved in 200 cm^3 of ethanol and the solution was refluxed for 2 h. The precipitate of a white DBHB compound was separated by filtration and washed with hot ethanol. Scheme 1 shows the synthesis of DBHB.

The elemental analysis was as follows: calculated for DBHB ($C_{18}H_{18}N_4O_4$), C 61.01, H 15.12, N 15.81; found, C 60.67, H 15.40, N 15.35%. The melting-point of DBHB could not determined because the white crystal changed to a yellow color when it was heated above 270 °C which was below the melting-point. The identity of DBHB was confirmed by IR and NMR spectroscopy.

Reagents and apparatus

All reagents were of analytical-reagent grade unless stated otherwise. A DBHB solution (5×10^{-3} mol dm^{-3}) was prepared by dissolving the reagent in dimethylformamide. A standard solution (1000 ppm) of Sc^{III} was obtained from Kanto Chemicals (Tokyo, Japan). Analytical-reagent grade acetonitrile was purified by distillation. Water was purified with a

Milli-Q system (Millipore, Bedford, MA, USA) after distillation and de-ionization.

The HPLC system consisted of a Hitachi (Tokyo, Japan) L-4000 pump, a Hitachi L-6000 spectrophotometric detector, a Hitachi D-2500 integrator, a YMC (Tokyo, Japan) packed ODS-AM column (150 × 4.6 mm id) and a Rheodyne (Cotati, CA, USA) Model 7125 loop injector (100 mm^3 loop). A Hitachi U-2000A spectrophotometer was used for spectrophotometric studies.

The mobile phase was acetonitrile–water (30 + 70 m/m) containing 5×10^{-3} mol kg^{-1} tetramethylammonium bromide and 2.5×10^{-3} mol kg^{-1} hexamethylenetetramine buffer. The pH of the mobile phase was adjusted to 6.0 prior to the addition of methanol. The flow rate of the mobile phase was 0.8 cm^3 min^{-1}. The eluate was monitored at 420 nm.

Procedure

Volumes of 3 cm^3 of sample solution, 2 cm^3 of 5×10^{-3} mol dm^{-3} DBHB solution and 1 cm^3 of 1 mol dm^{-3} hexamethylenetetramine buffer (pH 6.0) were placed in a 10 cm^3 calibrated flask and diluted to volume. Volumes of 100 mm^3 of the solution were injected with a loop injector.

Results and discussion

Chelate formation studies

In our preliminary research, other chromogenic agents for Sc^{III} such as Chlorophosphoazo-III, PAN, TAM, other related azo dyes and quinoin-8-ol derivatives were unsuccessful as pre-column HPLC chelating agents for Sc^{III}. They seemed to be decomposed by a ligand exchange reaction with silanol group on the silica gel in the ODS column, probably because Sc^{III} ion is a 'Hard' metal ion, with strong oxophilicity and silanophilicity. The adsorption isotherm of Sc^{III} on silica gel shown in Fig. 1 was measured to estimate the silanophilicity of the Sc^{III} ion. The formation constant (log K_{M-O-Si}) of the Sc^{III}–silanol complex was deduced form the formation reaction of the Sc^{III}–silanol chelate [eqn. (1)] and calculated by eqn. (2).

$$M^{3+} + HO\text{–}Si \rightleftharpoons M(\text{–}O\text{–}Si) + H^+ \quad (1)$$

$$K_{M\text{-}O\text{-}Si} = \frac{[M\text{-}O\text{-}Si][H^+]}{[M^{3+}][HO\text{-}Si]} \quad (2)$$

The value of $K_{Sc–O–Si}$ [= 1.64 (ref. 23)] was the largest of the metal ions adsorbed on silica gel (Al^{III}; −0.78, Ga^{III}; 0.62, Fe^{III}; 0.31, In^{III}; −0.72 and Zn^{II}; −3.34). The strong silanophilicity of Sc^{III} also made the Sc^{III} chelate undergo ligand exchange reactions with the silanol group on silica gel in the batch system (*i.e.*, in a beaker, not in the column). The pre-column chelating agent for Sc^{III} must have the stability and inertness to resist ligand exchange reactions with silanol group.

DBHB was found in our previous systematic search for a suitable ligand to undergo an exchange reaction between the silanol groups on silica gel and metal chelates. Fig. 2 shows that the Sc^{III}–DBHB chelate gave a sharp peak on the chromatogram. DBHB, which is a tetradentate ligand, formed a 1 : 1 (metal-to-ligand) chelate with Sc^{III} ion. The structure of the chelate is shown in Scheme 2. The Sc^{III}–DBHB chelate was stable and formed over a wide pH range, as shown in Fig. 3. Fig.

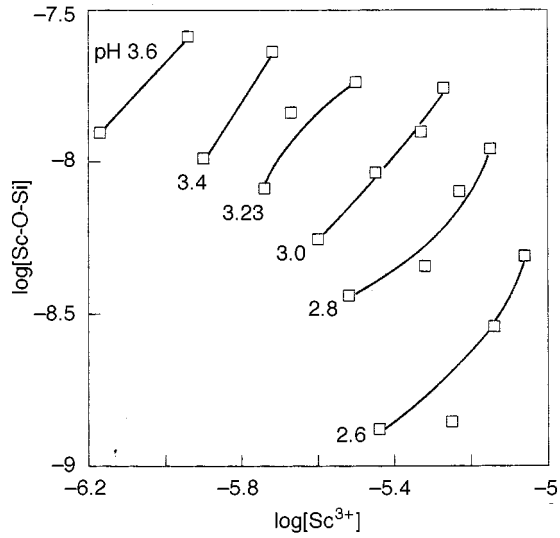


Fig. 1 Adsorption isotherm of Sc^{III} ion on silica gel at different pH values at an ionic strength of 0.1 ($NaNO_3$) and 25 °C.

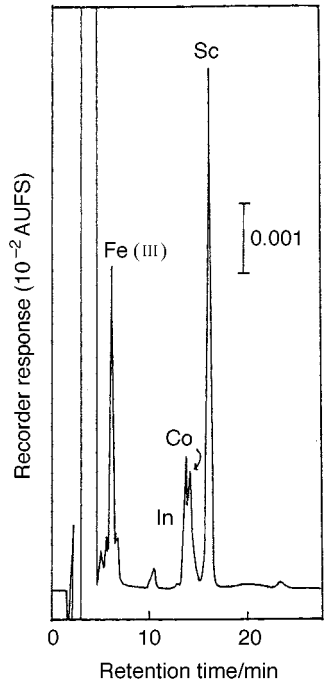
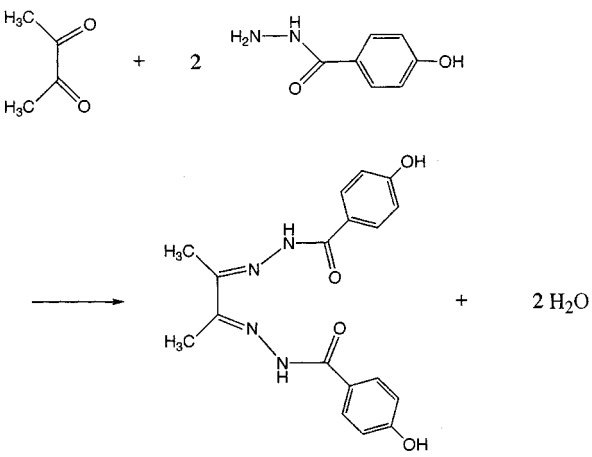


Fig. 2 Typical chromatogram of DBHB chelate. Column, YMC pack ODS AM (150 × 4.6 mm id); eluent, acetonitrile–water (30 + 70 m/m) containing 5×10^{-3} mol kg^{-1} tetramethylammonium bromide and 2.5×10^{-3} mol kg^{-1} hexamethylenetetramine buffer (pH 6.0); detection wavelength, 420 nm; flow rate, 0.8 cm^3 min^{-1}; sample solution, 2.5×10^{-5} mol dm^{-3} metal ions (Bi^{III}, Cd^{II}, Co^{II}, Cu^{II}, Fe^{III}, In^{III}, Ni^{II}, Mn^{II}, Pb^{II}, Pd^{II}, Sc^{III}, Ti^{VI}, Y^{III}, Zn^{II}, and Zr^{VI}), 1.0×10^{-3} mol dm^{-3} DBHB, 0.1 mol dm^{-3} hexamethylenetetramine buffer (pH 6.0); dimethylformamide–water (50 + 50 v/v).

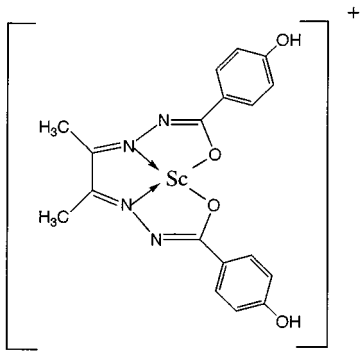


Scheme 1 Synthesis of DBHB.

3 also shows the plots of pH *versus* absorbance for the Sc^{III} chelate of salicylaldehyde benzoylhydrazone (SAB), which is an *O,N,O*-tridentate hydrazone, for comparison. The tetradentate hydrazones formed more stable chelates than the tridentate hydrazones. The stability of the Sc^{III}–DBHB chelate influenced the detectability by HPLC.

The kinetic properties of metal chelates also influence the detectability of the HPLC separation of metal chelates. Although a labile chelate dissociates when migrating in the column, a kinetically inert chelate could survive even if it was thermodynamically unstable. Fig. 4 shows the ligand exchange reaction between the Sc^{III}–DBHB chelate and EDTA to determine the kinetic properties of the chelate. The Sc^{III}–DBHB chelate dissociated gradually in the presence of EDTA. On the other hand, Sc^{III} chelates of tridentate ligand *e.g.*, SAB and bidentate ligand *e.g.*, quinolin-8-ol, derivatives dissociated within 1 s when EDTA was added to the batch. The inertness of the Sc^{III}–DBHB chelate enabled a rate constant of 0.768×10^{-3} s^{-1} to be calculated, assuming a pseudo-first-order reaction.

The stability and inertness of the Sc^{III}–DBHB chelate seemed to be due to the three chelation rings and the appropriate cavity size of DBHB to fit the Sc^{III} ion, as shown in Scheme 2. However, DBHB and its analogues have not been considered as spectrophotometric reagents because of their poor spectrophotometric properties such as low selectivity, low sensitivity and poor color change. The absorption spectra of metal–DBHB chelates are shown in Fig. 5. They show that DBHB reacts with many transition metal ions, and the spectra are similar to each other. Utilizing HPLC was an effective way to overcome the problems. Furthermore, the overlap of the spectra between the chelates and the ligand would not be a problem when the mobile phase contained no bis(hydrazone) ligand.[29]



Scheme 2 Structure of Sc–DBHB chelate.

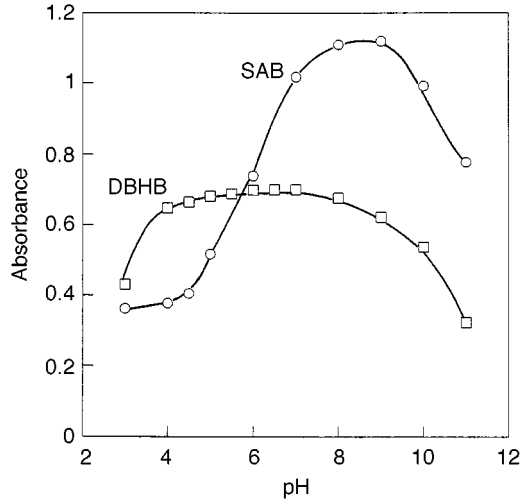


Fig. 3 Plots of absorbance of Sc chelates *versus* pH. □ DBHB; ○ SAB. Sample solution, 2.5×10^{-5} mol dm^{-3} Sc^{III} ion; 1.0×10^{-3} mol dm^{-3} DBHB, 0.1 mol dm^{-3} hexamethylenetetramine buffer (pH 6.0); dimethylformamide–water (50 + 50 v/v); wavelength, 420 nm.

HPLC studies

A typical chromatogram obtained for a solution containing 15 metal–DBHB chelates (Bi^{III}, Cd^{II}, Co^{II}, Cu^{II}, Fe^{III}, In^{III}, Ni^{II}, Mn^{II}, Pb^{II}, Pd^{II}, Sc^{III}, Ti^{VI}, Y^{III}, Zn^{II} and Zr^{VI})–is shown in Fig. 2. Only Co^{II}, Fe^{III}, In^{III} and Sc^{III} chelates were detected spectrophtometrically. The other DBHB chelates decomposed in the HPLC column owing to the ligand exchange reaction with the EDTA added to the mobile phase and silanol groups on the stationary phase. The Sc^{III} chelate was separated from the other chelates detected. It is emphasized that Y^{III} and Zr^{VI} chelates had similar properties to the Sc^{III} chelate but gave no peaks on the chromatogram in this HPLC system.

As the Sc^{III}–DBHB chelate had a positive charge, $[M^{III}L]^+$, the cation exchange function of the silanol groups also affected the elution of the chelate. This resulted in a broad peak of the Sc^{III}–DBHB chelate. Tetramethylammonium bromide (TMA) was added to suppress the silanol effect. Fig. 6 shows the plots of the retention time of the chelates *versus* the concentration of TMA. As the concentration of TMA increased, the retention time of each metal chelate decreased and the peak shape became sharp. TMA seemed to act not only as a silanol-blocking agent

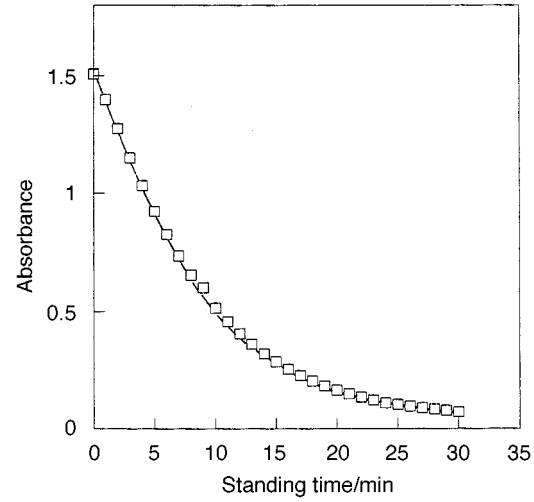


Fig. 4 Plots of absorbance of Sc^{III} chelate *versus* standing time after mixing with EDTA. 5×10^{-5} mol dm^{-3} Sc^{III} ion; 5×10^{-4} mol dm^{-3} DBHB, 1×10^{-3} mol dm^{-3} EDTA, 0.1 mol dm^{-3} hexamethylenetetramine buffer (pH 6.0); dimethylformamide–water (50 + 50 v/v), wavelength, 420 nm.

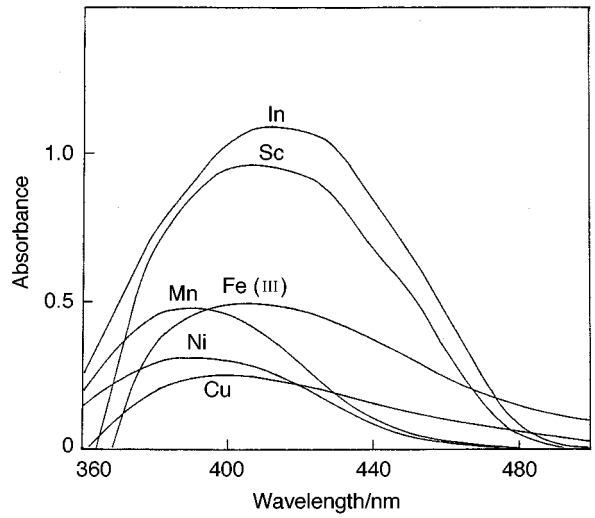


Fig. 5 Absorbance spectra of DBHB chelates. Sample solution, 2.5×10^{-5} mol dm^{-3} metal ion; 1.0×10^{-3} mol dm^{-3} DBHB, 0.1 mol dm^{-3} hexamethylenetetramine buffer (pH 6.0); dimethylformamide–water (50 + 50 v/v).

but also as an ion-exclusion agent.[30] TMA gave the best chromatogram of DBHB chelates among the tetra(C_1–C_5-alkyl)ammmonium bromides examined.

The acetonitrile content in the mobile phase also influenced the retention of each metal chelate, because the DBHB chelates were retained in the reversed-phase mode. As the acetonitrile content increased, the retention time of each metal chelate decreased rapidly.

Influence of foreign ions, calibration curves and application

The influence of foreign ions on the HPLC determination of trace amount of scandium is shown in Table 1. Metal ions such as Fe, Co and In gave peaks on the chromatogram that seriously interfered with the determination of scandium. Addition of EDTA to the mobile as a masking agent suppressed the interference of foreign ions. Although the effect of Co^{II} was not clear, a redox reaction of Co^{II} seemed to influence the interference. The peak of Sc^{III} disappeared when potassium iodate was added to the sample solution as Co^{II} was oxidized. It should be noted that yttrium, lanthanides and zirconium, which interfered with the spectrophotometric determination of scandium, could be tolerated at levels up to 100-fold. As these metal ions have larger ionic radii than the cavity of DBHB, they formed less stable chelates with DBHB and decomposed in the column. The titanium chelate eluted gradually, so that it gave a very broad response that was not usually recognized as a peak. However, injection of high concentrations of titanium chelate caused the baseline to be unstable. Phosphate ion formed a stable complex with scandium and coexisting phosphate ion gave a negative interference. Judging from the recovery test with river water, these foreign ions did not interfere with the determination of scandium in river water.

The calibration curve obtained using peak height with 100 mm^3 injections was linear from 1×10^{-8} to over 5×10^{-7} mol dm^{-3}. No blank peak was observed for scandium. The detection limit defined as three times the baseline noise (S/N = 3) was 8.4×10^{-10} mol dm^{-3} (37 ng l^{-1}). The detection limit might be improved if the baseline noise were suppressed electronically. The detection limit of this method was lower than that of ICP-AES and was almost equal to that of ICP-MS[14] (5.7×10^{-8} g l^{-1}, *i.e.*, 1.3×10^{-9} mol dm^{-3}). The reproducibility (relative standard deviation) of the method was 7.8% for four replicate analyses of 1.0×10^{-8} mol dm^{-3} (0.45 μg l^{-1}) scandium solution.

The chromatogram of a river water sample is shown in Fig. 7. The peak of scandium could be identified, but it was not possible to determine the concentration in the river water sample as it was below the determination limit (S/N = 10, 2.8×10^{-9} mol dm^{-3}). The recovery was checked by adding a standard solution of scandium to a river water sample. The results are summarized in Table 2. The difference between the

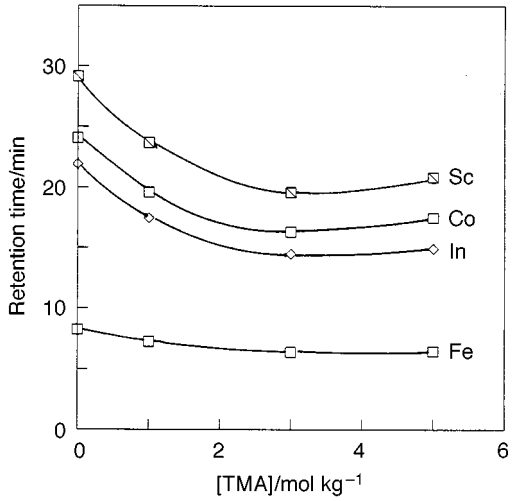


Fig. 6 Plots of retention time of DBHB chelates *versus* tetramethylammmonium bromide in the mobile phase. Other conditions in the carrier solution as in Fig. 2.

Table 1 Tolerance limits of foreign ions in the determination of scandium. ([Sc^{III}] = 1.0×10^{-7} mol dm^{-3})

Tolerance limit [ion]/[Sc][a]	Ion
< 1000	Na^+, K^+, Br^- Cl^-, I^-, NO_3^-
500	Al^{III}, Ca^{2+}, Ba^{2+}, Mg^{2+}
200	Cu^{II}, SO_4^{2-}
100	Bi^{III}, Pb^{II}, Pd^{II}, Zn^{II}, Zr^{IV}
50	Cd^{II}, Ni^{II}
20	Mn^{II}
10	In^{III}, Ti^{IV}, PO_4^{3-}
1	Co^{II}, Fe^{III}
EDTA masking[b]—	
50	Cd^{II}, Ni^{II}, Fe^{III}, Mn^{II}
20	In^{III}, Ti^{IV}
10	PO_4^{3-}
5	Co^{II}

[a] Tolerance limit is defined as the molar ratio which gives not more than a ±5% error in the determination. [b] $[EDTA]_{mobile\ phase}$ = 1.0×10^{-4} mol dm^{-3}.

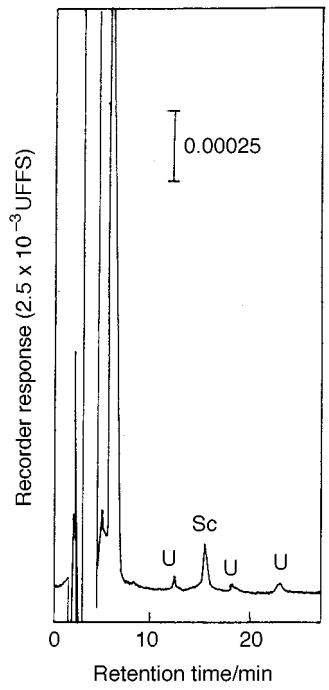


Fig. 7 Chromatogram of river water sample. Sample solution, river water sampled at Watarase River (Kusaki dam Gunma, Japan) on 17th December 1996. Conditions as in Fig. 2. U represents unknown species.

Table 2 Recovery test of scandium in river water. Sample: river water sampled at Watarase River (Kusaki dam Gunma, Japan) on 17th December 1996.

Sc added/ 10^{-8} mol dm^{-3}	Sc found/ 10^{-8} mol dm^{-3}
1.00	1.00
1.00	1.05
1.00	1.14
1.00	1.19
	Mean: 1.10 ± 0.09

added and the recovered concentrations corresponds to the Sc^{III} ion contained originally in the river water sample. A suitable preconcentration method should be utilized to determine Sc^{III} in river water.

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