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Fluorometric determination of serum and urinary aluminium with 8-quinolinol by kinetic-differentiation-mode micellar chromatography

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Abstract

A new fluorometric method has been developed for the determination of aluminium with 8-quinolinol by kinetic-differentiation-mode micellar chromatography. The proposed method enabled the determination of aluminium down to 1 μg/l in human serum and urine without preliminary deproteinization. The most remarkable point of this method is that only aluminium ion selectively responds among metal ions. The complex formation of aluminium in serum with 8-quinolinol was completed within a few minutes at room temperature. The serum matrix and aluminium chelate were separated on a reversed-phase column with an eluent containing acetonitrile, sodium dodecylsulfate, and Triton X-100, and aluminium chelate was detected at Ex 370 nm, Em 504 nm. The values obtained by this method were in good agreement with those of Zeeman graphite-furnace atomic absorption spectrometry. The proposed method will provide a simple and rapid technique for the determination aluminium in medical fields. © 1997 Elsevier Science B.V.

Keywords: Kinetic differentiation liquid chromatography; Micellar liquid chromatography; Serum; Urine; Aluminium; Quinolinol; Metal chelates

1. Introduction

The toxicity of aluminium in animal cell biology is well recognized. Several recent studies have identified aluminium as a potential, although highly controversial, contributory factor in the pathology of Alzheimer disease, amyotrophic lateral sclerosis, and dialysis dementia [1–4]. Therefore, the determination of aluminium in human serum of these patients has attracted considerable attention in the fields of clinical chemistry and physiological chemistry. Although the most commonly used method for aluminium in the medical field is graphite-furnace atomic absorption spectrometry (GF-AAS) [5,6], the use of this technique is often limited due to matrix interference and insufficient precision. Aluminium concentrations in the samples are usually close to the detection limit of line-source GF-AAS. The most powerful methods for metal ions, inductively-coupled plasma (ICP) atomic emission spectrometry (AES) and ICP-MS, cannot be used for such samples because of its relatively lower sensitivity for aluminium and inherent interference by phosphate. Furthermore, the maintenance of these instruments is rather costly. We previously reported a sensitive method based on ion-pair reversed-phase HPLC with

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spectrophotometric detection using 2,2′-dihydroxyazobenzene (DHAB) after serum protein precipitation [7]. The use of HPLC for the determination of aluminium has been gaining popularity in recent years [7–15].

We briefly communicated the unexpected finding that 8-quinolinol acts as a specific reagent for aluminium in kinetic differentiation (KD)-mode HPLC with fluorometric detection [14]. This finding prompted us to construct a method for aluminium based on the novel combination of KD-mode HPLC and micellar HPLC, which is expected to have desirable properties, such as being specific for aluminium, highly sensitive because of the amplification of fluorescence intensity with micellar media, and the separation of aluminium chelate from matrices such as serum proteins by micellar partition. In this paper, we report a detailed investigation of the new method and its application to human sera of 113 healthy subjects and urine samples. In KD-mode HPLC [16–18], the chelating reagent is not added in the eluent, therefore kinetically labile chelates are decomposed on the column as a result of separation of the chelates and their reagents. The KD mode is useful for detecting small signals without a background caused by the reagent stream. Contrary to the conventional on- and post-column detection HPLC with reagent stream, the sensitivity is determined by the inherent signal-to-noise ratio of the detection system. As described in a number of investigations on the micellar HPLC of metallo–organic chelates [19,20], micelles provide a great variety of interactions with the solute in the mobile phase, and have been adopted to obtain a desired resolution [21]. The separation is judiciously controlled by variation of the molecular structure, charge (negative, positive, or neutral), hydrophobicity, and the concentrations of micelle-forming surfactant used. Consequently, this method enabled us to determine 1 μg/l of aluminium in human serum and urine without any pretreatment.

2. Experimental

2.1. Chemicals

Analytical-reagent grade of 8-quinolinol was used as obtained from Kanto Chemical Co. Sodium dodecyl sulfate (SDS) was obtained from Wako Pure Chemical Industries, and Triton X-100 was obtained from Nacalai Tesque. The standard solution of aluminium, copper, iron, zinc, and calcium were obtained from Kanto Chemical Co. The multielement standard solution (Al, Cu, Fe, Mn, Pb, and Zn) was obtained from Wako Pure Chemical Industries. Each standard solution was diluted with 0.01 mol/l nitric acid (ultrapure grade, Tama Chemicals) to the required concentration in use. All other reagents used were of analytical-reagent grade. The ultrapure grade water used was obtained from Tama.

2.2. Instrumentation

The HPLC system consisted of a Nanospace SI-1/2001 pump, an SI-1/2013 fluorescence detector with a 16-μl cell, an SI-1/2002 UV–Vis detector, an SI-1/2014 column oven, an SI-1/2003 autosampler and a Nanospace system controller with data processor (Shiseido, Tokyo, Japan). Column temperature was controlled at 16°C. A Capcell Pak MF ph-1 (150×1.5 mm I.D. from Shiseido, Tokyo, Japan) column was used. The packing materials of this column was silicone polymer-coated mixed-functional silica developed for the direct determination of drugs in serum and plasma [22]. The column life in this system was approximately 100 injections of human sera without deproteinization. Flow-rate was 100 μl/min; the mobile phase compositions are given in the figure captions. For GF-AAS, a model Hitachi Zeeman 8000, equipped with an autosampler was used. Light source was an Hitachi hollow cathode lamp of Al (wavelength 309.3 nm), and sample volume was 20 μl. The other parameters have been described elsewhere [23]. Use of glassware, the most potential source of external aluminium contamination, was avoided. Instead, PTFE and polyethylene wares cleaned by filling with 1 mol/l nitric acid and leaving overnight were used throughout the study.

2.3. Procedure

The recommended procedure is as follows: 50 μl of 0.01 mol/l 8-quinolinol (17%, w/w acetonitrile, 1 mol/l hydrochloric acid) solution and 250 μl of 3 mol/l N,N-bis(2-hydroxyethyl)-2-aminoethanesul-

fonic acid (BES) buffer solution (pH 7.5) containing 0.01 mol/l SDS were added to 150-μl aliquots of serum or urine samples. Then, the mixture was filtered with a 0.22-μm membrane filter (Millipore, Tokyo, Japan). A 20-μl aliquot of the filtrate was injected onto the HPLC column with the autosampler. Serum matrix and aluminium chelate were separated on the reversed-phase column with an eluent containing 16 wt.% acetonitrile, 0.01 mol/l SDS, 1 wt.% Triton X-100, and 0.1 mol/l BES buffer (pH 7.0). Fluorescence of aluminium chelate was detected at Ex=370 nm and Em=504 nm.

3. Results and discussion

The reagent, 8-quinolinol, has been extensively used for the determination of metal ions including aluminium in conventional extraction spectrophotometry. Methods for simultaneous determinations of metal ions with 8-quinolinol by RP-HPLC were also proposed [8–11]. On the course of our systematic studies on KD-mode HPLC, it was found that 8-quinolinol (HL) gave peaks only for aluminium and cobalt by spectrophotometric detection among the common metal ions: Fe(III), Cu(II), Zn(II), Mg(II), Ca(II), F(I), Mn(II), Pb(II), and Co(II) [14]. This selectivity is attributed to the kinetic nature of solvolysis reaction:

$$ML + mH_2O \rightarrow M(H_2O)_m^{n+} + nL^-$$

In the case of the 8-quinolinol system, only aluminium and cobalt survived and were detected. The peaks of cobalt and aluminium were very close to each other (Fig. 1a), however, only aluminium was selectively detected by fluorometry. Consequently, the fluorometric detection KD-HPLC system with 8-quinolinol gives a specific signal for aluminium (Fig. 1b). The proposed method is based on the unique combination of the KD- and micellar-modes in addition to conventional chromatographic sepa-

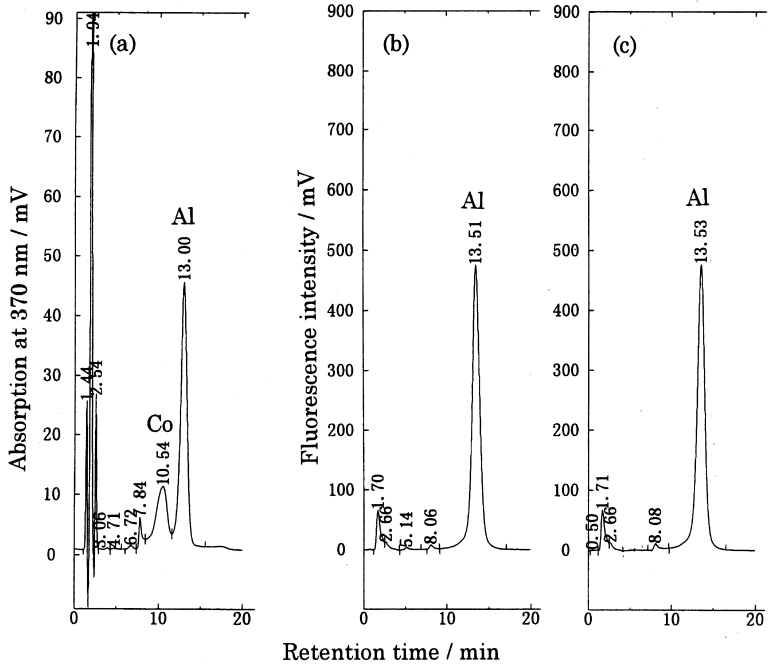

Fig. 1. Chromatograms of 8-quinolinolato–metal complexes. Column, Capcell Pak MF ph-1 (150×1.5 mm I.D.); mobile phase, 16% (w/w) acetonitrile, 0.01 mol/l SDS, 1% (w/w) Triton X-100, and 0.1 mol/l BES (pH 7.0); detection, (a) spectrophotometric detection Abs. at 370 nm; (b) and (c) fluorescence detection Ex=370 nm, Em=504 nm; flow-rate, 100 μl/min; column temperature, 16°C; sample volume, 10 μl; sample, (a) 10 mg/l Co and 10 mg/l Al; (b) 10 mg/l Co and 1 mg/l Al; (c) Al^{3+}, Fe^{3+}, Zn^{2+} 1 mg/l; Cu^{2+}, Mn^{2+}, Pb^{2+} 0.1 mg/l.

ration, as described above. The surfactant, SDS, used at the concentration of 0.01 mol/l in the mobile phase brought about effective separation of aluminium from the serum and urine matrixes. Under these conditions, the matrix peaks were eluted within 10 min, and complete separation was achieved, as shown in Fig. 3.

3.1. The effect of surfactants on the fluorescence intensity and retention behavior of the solute

The addition of several kinds of surfactants (SDS, CA-2330, Triton X-100, Emulgen 20T, Emulgen-911, Emulgen-507, Briji-35, and Tween-20) to 8-quinolinolato–aluminium chelate solution brought about increase in fluorescence intensity, as well as inducing slight shifts in wavelength of emission and excitation maximum of the complex. The fluorescence intensity increased with increasing concentration of surfactants, and reached to a constant and maximum intensity by the addition of more than approximately 5 wt.% of each surfactant in the mobile phase. For example, typical results for SDS are shown in Fig. 2, where the fluorescence intensity and capacity factor (k') of the solutes were measured as a function of SDS concentration at 22°C on a Capcell Pak MF ph-1 column. Increasing the SDS concentration resulted in shortened retention time and an increase in fluorescence intensity, and reached constant and maximum values at 0.03 mol/l SDS in the mobile phase.

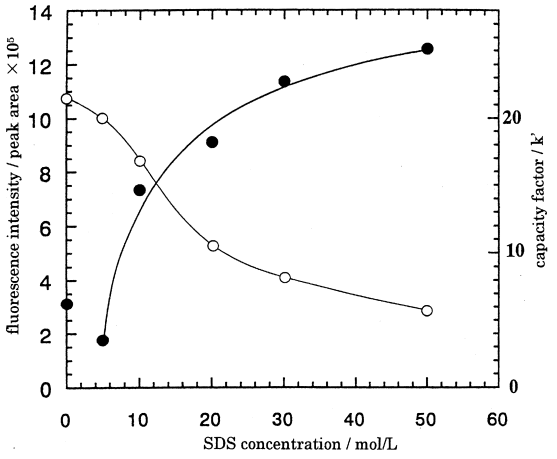

Fig. 2. Effect of SDS concentration on the fluorescence intensity and capacity factor (k') of the aluminium chelate; fluorescence intensity (●), capacity factor (○).

3.2. Chromatography

The SDS concentration in the mobile phase was adjusted in order to achieve separation of the chelate peak from that of the matrix. A typical chromatograms for serum and urine samples are shown in Fig. 3; the blank serum produced a background response that was eluted within 10 min, and the separation of the chelate from the matrix was successfully achieved.

3.3. Sensitivity and precision

The calibration curve showed good linearity over the range tested, up to 1000 μg/l. The coefficients of variation were 1.2% at 10 μg/l aluminium solution ($n=20$), 2.4% at 10 μg/l in the serum sample ($n=20$), and 1.0% at 40 μg/l in the serum sample ($n=20$). The relative standard deviation for the background was 1.9% ($n=20$) at 0.01 mol/l nitric acid. The detection limit in pure solution, defined as three-fold of the standard deviation of the blank signal, was 1 μg/l. The determination of aluminium in human serum samples by GF-AAS was also carried out. As shown in Fig. 4, there was a good agreement between the values obtained using the HPLC method (y) and those using GF-AAS (x).

$$y = 0.889x + 1.105$$

Here, the correlation coefficient was 0.927 ($n=41$). A lower concentration range for serum aluminium in the normal sera group was more easily observed using the HPLC method than using GF-AAS. Such a high performance of the HPLC method is related to the careful removal of contamination, minimized by the use of high-purity chemicals, aluminium-minimized doubly distilled water, and undeviating attention to the use of carefully rinsed tubes and vessels. The recovery of aluminium added to the human sera was between 100 and 104%, that to the rat sera was 100–105%, and that to the human urine was 90–102% (Tables 1–3).

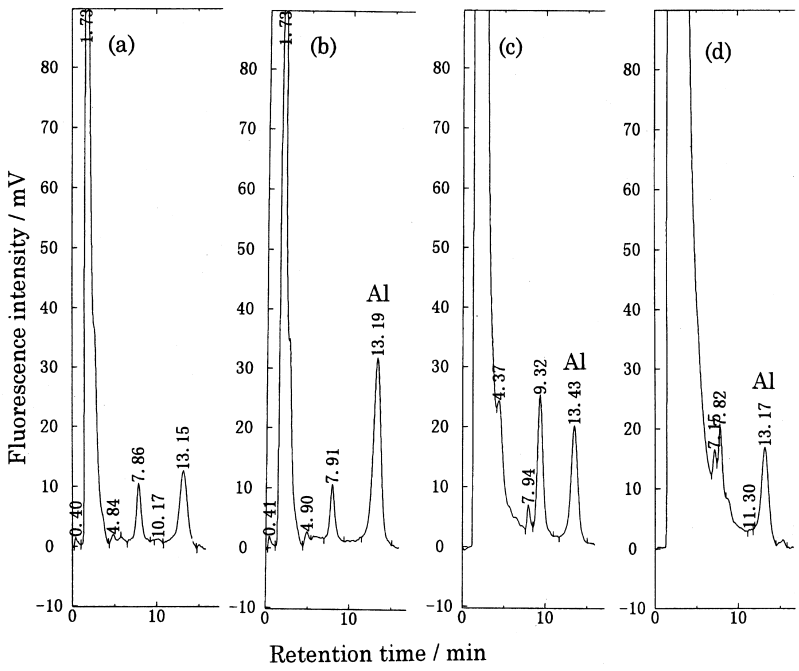

Fig. 3. Chromatograms of serum and urine samples. Column, Capcell Pak MF ph-1 (150×1.5 mm I.D.); mobile phase, 16% (w/w) acetonitrile, 0.01 mol/l SDS, 1% (w/w) Triton X-100, and 0.1 mol/l BES (pH 7.0); fluorescence detector, Ex=370 nm, Em=504 nm; flow-rate, 100 μl/min; column temperature, 16°C; sample volume, 10 μl; sample, (a) reagent blank (0.01 mol/l HNO_3); (b) aqueous calibrator 10 μg/l Al; (c) human serum 2 μg/l Al; (d) human urine 1 μg/l Al.

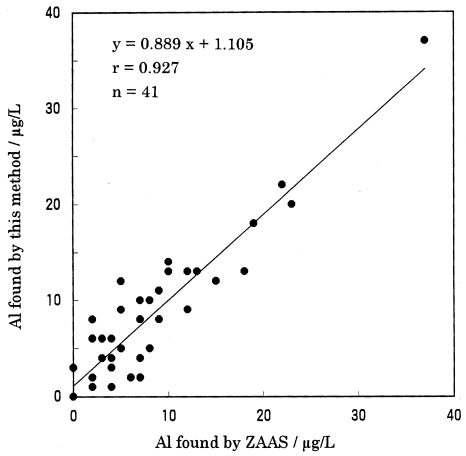

Fig. 4. Comparison of aluminium concentrations found using the HPLC method and those using polarized Zeeman atomic absorption spectrometry.

3.4. Effect of foreign substances

This method is essentially free from the interference of other common metal ions. The effect of foreign substances of biological importance on the aluminium signal response was tested (Table 4). No interferences were observed for iron(III), copper(II), and zinc(II) up to 10 mg/l, and for magnesium(II) and calcium(II) up to 100 mg/l. These concentrations are about 5 times larger, or more, than those normally found in human serum.

3.5. Reagent blank

The sensitivity is limited by the dilution of the samples and the blank contribution from the reagents used. The practical blank value normally corresponds to less than 10 μg/l. The largest source of aluminium contamination was found to be from im-

Table 1
Recovery of aluminium spiked in human sera

Sample no.	Addition to sample (μg/l)	Al found (μg/l)	Recovery (%)
1	0	2	
2	10	12	100
3	25	28	104
4	50	52	100

Table 2
Recovery of aluminium spiked in rat sera

Sample no.	Addition to sample (μg/l)	Al found (μg/l)	Recovery (%)
1	0	9	
2	10	20	105
3	25	34	100
4	50	60	102

Table 3
Recovery of aluminium spiked in human urine

Sample no.	Addition to sample (μg/l)	Al found (μg/l)	Recovery (%)
1	0	0	
2	10	9	90
3	25	25	100
4	50	51	102

purities in the BES buffer solution, and the secondary contribution was from the 8-quinolinol solution. However, the fluctuation of background was so small (standard deviation was ±0.3 μg/l) that the signals superimposed on it were measured with sufficient precision.

3.6. Determination of aluminium in the standard reference materials and healthy subjects

Standard reference materials (SRM 1598 Bovine serum) offered by NIST (National Institute of Standards & Technology) was measured by the HPLC method. The value obtained for SRM 1598 was 4.5±0.5 μg/l ($n=5$), which was sufficiently close to the certified value of 3.7±0.9 μg/l. The serum aluminium in the healthy group measured by the HPLC method was 2±3 μg/l (mean±SD, $n=113$), which was in good agreement with those reported by other investigators, 0.5–4 μg/l [15] and 4–11 [23] μg/l. The value reported by Frederik [6], 7.3±2.0 μg/l, measured by GF-AAS is higher than these values.

Table 4
Tolerance limits of foreign substances

Metals (mg/l)	Metals (mg/l)	Other substances (mg/l)
Fe (10)	F (2)	Ascorbic acid (200)
Cu (10)	Mn (0.1)	Bilirubin (400)
Zn (10)	Pb (0.1)	Hemoglobin (5000)
Mg (100)	Co (10)	Uric acid (400)
Ca (500)		Glucose (5000)

4. Conclusions

The KD-mode HPLC is useful for selective detection of metal complexes, and the sensitivity for trace metal ions has been significantly improved. Micellar chromatography using SDS in the mobile phase allowed the direct injection of serum, without deproteinization, onto the column for rapid moni-

toring of aluminium. This method is recommended for the routine determination of aluminium in human serum and urine because of its operational simplicity and its general convenience for monitoring the health of hemodialysis patients at the bedside.

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