Determination of Aluminium in Human Serum by Kinetic Differentiation Mode Reversed Phase Micellar HPLC with Fluorometric Detection

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A highly sensitive and selective fluorometric method for aluminium in humn serum with 8-quinolinol has been developed by the use of kinetic differentiation mode micellar high performance liquid chromatography. The most remarkable point of this method is that only aluminium ion responses to this detection system, as well as no deproteinization is required prior to analysis. The detection limit, 3SD, was 1 ppb for aluminium.

Highly controversial discussions had been made on the role of aluminium in the pathology of Alzheimer disease and dialysis dementia, however, recent studies have identified aluminium as one of the key substances in these disease.[1] Determination of aluminium in human serum of these patients has attracted considerable attention in the fields of clinical and physiological chemistry. The most commonly used analytical technique for aluminium in this field is graphite-furnace atomic absorption spectrophotometry (GF-AAS),[2] which is often limited due to serum matrix interference,[3] and the detection limit is 5 ppb for aluminium in human serum. We previously reported a sensitive method based on ion-pair reversed-phase partition (RP) HPLC with spectrophotometric detection by the use of 2,2'-dihydroxyazobenzene after serum protein precipitation.[4] In this study, a simple, sensitive, and selective method which requires no deproteinization process prior to analysis has been proposed.

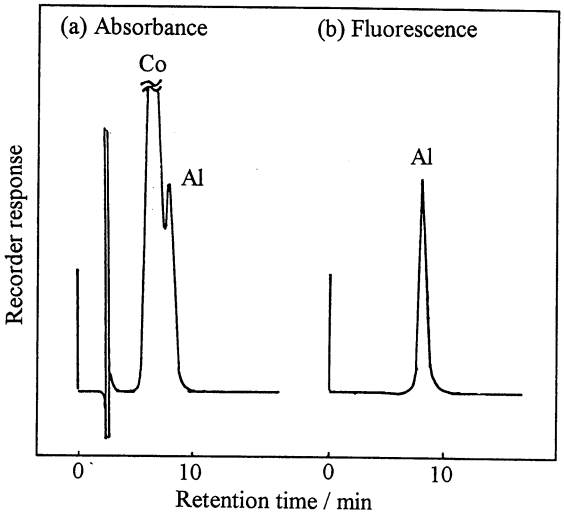

Figure 1. Chromatogram of 8-qinolinolato - metal complexes. Column: Yanapak ODS-T; Mobile phase : 65 wt% aq-methanol, 1×10^{-4} mol Kg^{-1} EDTA, 5×10^{-3} mol Kg^{-1} NaOAc ; Detector: (a) Absorbance at 370 nm, (b) Fluorescence at Ex. = 370 nm, Em. = 504 nm ; Flow rate : 1cm^3/min ; Sample volume : 0.1 cm^3; Sample : Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, and Pb^{2+} 4×10^{-5} mol dm^{-3}, Al^{3+} 4×10^{-6} mol dm^{-3}.

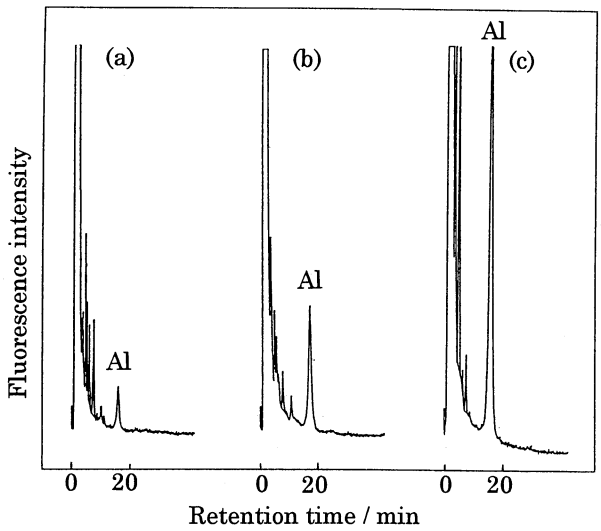

Figure 2. Typical chromatogram for aluminium determination. Column : CAPCELL PAK MF ph-1 (4.6 mm i.d.×150 mm length); Mobile phase: 20 wt% actonitrile, 0.01 mol dm^{-3} SDS, 1×10^{-4} mol dm^{-3} EDTA, 0.01 mol dm^{-3} BES (pH 7.0) ; Fluorescence detector: Ex. = 370 nm, Em. = 504 nm ; Flow rate: 1 cm^3/min ; Column temp : 22 ℃ ; Sample volume: 0.1 cm^3 ; Sample : (a) human serum 9 ppb Al, (b) human serum 132 ppb Al, (c) huma serum 593 ppb Al.

A reagent, 8-quinolinol, has been used for determination of metal ions including aluminium in conventional wet chemical analysis. Recently, simultaneous determinations of them with 8-quinolinol by RP-HPLC were proposed.[5] On the course of our systematic studies on kinetic differentiation (KD) mode HPLC,[6] in which chelating reagent is not added in the eluent, we have found that 8-quinolinol gives peaks only for aluminium and cobalt among the common metal ions including those indicated in Figure 1, even though their peaks were very close each other in KD-HPLC with spectrophotometric detection (Figure 1a). In order to detect aluminium selectively, fluorometric detection was applied to the system. Consequently, it was found that the fluorometric detection KD-HPLC system with 8-quinolinol is specific for aluminium (Figure 1b).

The proposed method is based on the combination of two separation mechanisms in addition to that of usual RP-HPLC system: one is the KD mode [6] and the other is the micellar mode.[7] Surfactants and its micelles provide a variety of interactions with the solute in the mobile phase, based on the molecular structure

of sur- factants,[8,9] and they are effective to obtain a desired separation of aluminium species in the samples.

In order to separate the aluminium chelate peak from the matrix peaks of human serum samples, sodium dodecylsulfate (SDS) was used at the concentration of 0.01 mol dm^{-3} in the mobile phase. At this conditions, the matrix peaks were eluted within 10 min, and the separation was achieved as shown in Figure 2. The addition of SDS, as well as several other detergents to the aluminium chelate solution, increases fluorescence intensity (Figure 3), therefore, SDS micelle acts as a useful media for highly sensitive fluorometric determination of aluminium.

The recommended procedure [10] is as follows : To 300 μ l of human serum, 50 μ l of 1 mol dm^{-3} hydrochloric acid was added to liberate aluminium from carrier protein in serum, and 250 μ l of 0.005 mol dm^{-3} 8-quinolinol (20 wt% acetonitrile solution) was added. Then, 300 μ l of 2.5 mol dm^{-3} N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) buffer solution (pH 7.0) was added to form its aluminium chelate. The reaction mixture was injected onto the HPLC column with the 100 μ l loop injector. The serum matrix and aluminium chelate are separated on the reversed-phase column with an eluent containing 20 wt% acetonitrile, 0.01 mol dm^{-3} sodium dodecyl sulfate, 1×10^{-4} and mol dm^{-3} EDTA, and 0.01 mol dm^{-3} BES buffer (pH 7.0). Then the aluminium complex is detected with fluorescence detector (Ex. 370 nm, Em. 504 nm).

By the use of this method, the serum samples from 49 patients on hemodialysis were analyzed. There was a good agreement between the values obtained by this HPLC method (y) and those by GF-AAS (x) :

$$y = 1.171\,x - 26 \quad (r = 0.987)$$

and the detection limit, 3SD, was 1 ppb for aluminium (n = 5).

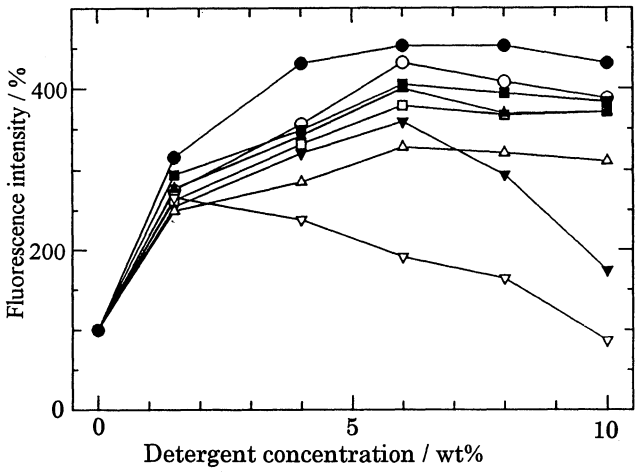

Figure 3. Effect of detergents on fluorescence intensity (blank = 100%). Detergents added : SDS (●), Emulgen- 20T (○), Briji 35 (□), Emulgen911 (■), Emulgen 507 (◆), TritonX-100 (▼), Tween 20 (▽), CA 2330 (△).

The proposed method will provide a new approach for many investigators to carry out aluminium analysis in medical field. Particularly, this method is recommended for monitoring the health conditions of hemodialysis patients at the bed side. Furthermore, due to its operational simplicity and general convenience, the method must be adopted for the routine use for monitoring aluminium in human serum.

References and Notes

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3 ICP-AES and ICP-MS can not be used for such samples, due to relatively lower sensitivity for Al and interference by phosphate, recpectively.

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10 8-Quinolinol, SDS, and all other reagents used were of analytical reagent grade. The water used was doubly distilled by an all PYREX glass apparatus. The HPLC system was consisted from JASCO Corporation components and a CAPCELL PAK MF ph-1 (4.6 mm i.d. × 150 mm from Shiseido Company, Ltd.,) column. Teflon and polyethylene wares cleaned by filling with 1 mol dm^{-3} nitric acid and leaving overnight were used throughout the study.