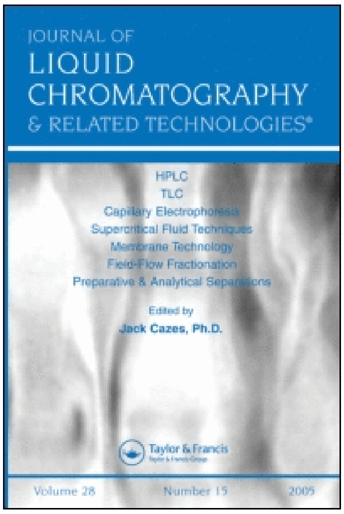

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DIRECT DETERMINATION OF ALUMINUM IN DRINKING AND NATURAL WATERS AS 8-HYDROXYQUINOLINE CHELATE BY RP-HPLC

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ABSTRACT

Determination of trace aluminum in the form of 8-hydoxylquinoline chelate by reversed phase high performance liquid chromatography and spectrophotometric detection is described in this paper. The optimization of the experimental conditions including precolumn complexation, chromatographic separation, and detection wavelength is comprehensively investigated. The response is linear from 0.002 to 0.3 μg/mL with a detection limit of 0.002 μg/mL. The relative standard deviation is 3.6% at an Al concentration of 0.2 μg/mL. The reliability of the entirely optimized procedure is confirmed by the direct analyses performed on ten real drinking and natural water samples.

Key Words: Aluminum; 8-Hydroxyquinoline; Chelation; Drinking water; Natural water; HPLC

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INTRODUCTION

The ecological and biological significance of aluminum has been an important subject during the last two decades or so. Dissolved Al in natural waters at elevated concentration resulting from acid deposition, is found to be toxic to aquatic and terrestrial biota. The existence of this element in drinking waters is also, at present, of major concern for the potential threat to the health of a number of species including humans (1–3). Unfortunately, the Al salts such as alum, are often utilized in the water supply system as flocculating agents. Al pots are used daily to boil drinking water and to cook foods especially in many developing countries. Al is taken into human bodies every day by way of drinking waters, beverages, foods, etc.

It has been generally recognized that there is significant relationship between the levels of Al in waters and some human diseases. Therefore, the sensitive and direct methods for determination of Al in real water samples are of obvious importance to investigate the ecological and biological effects including the toxicity of this element. Many different analytical techniques have been developed to this end. A number of electrochemical methods have received much interest in recent years as well (4–10). The most commonly used methods for the same purpose are colorimetry using a variety of spectrophotometric reagents, AAS employing graphite furnace atomization, and ICP-AES (11–22).

The colorimetry is time-consuming and labor intensive for the extraction procedure, and ICP-AES requires complicated and expensive equipment. RP-HPLC of metal chelates has been successfully applied to trace metal analysis using various derivatizing reagents, because the method gives good and wide calibration curves compared to above-mentioned procedures.

In addition, sensitivity and reproducibility are also good, and the method requires no special technique, except for usual HPLC apparatus. On the other hand, the metal such as Al that is relatively difficult to atomize, is expected to be determined more sensitively by HPLC than AAS (23). 8-Hydroxyquinoline (8-HQ) is an excellent chelating agent for a large number of metal ions (24,25). A few papers have been published about the separation and determination of Al-8-HQ chelate by RP-HPLC, most of which only regarded the simultaneous determination of several metal ions and paid little attention to analysis of trace Al. The thorough optimization of the complexation reaction, the chromatographic condition, and the detection wavelength has not been studied so far (26–35).

The purpose of this work is to optimize, systemically, the experimental conditions for direct determination of Al in the form of 8-HQ chelate by RP-HPLC with UV-detection. A number of valuable definite results are obtained in this paper. Also, the application of the proposed method to analyze Al in drinking and natural waters, without sample preconcentration, is reported.

EXPERIMENTAL

Apparatus

A Varian 5060 high performance liquid chromatograph (Varian, Walnut Creek, CA, USA) coupled to a Rheodyne 7725i injector valve with a 50-μL loop (Rheodyne, Cotati, USA), and a Waters 486 tunable UV–Vis absorbance detector (Waters, Mildford, MA, USA) is used.

The column used is a stainless steel tube (150 mm × 4.6 mm I.D.) packed with Phase Separation Spherisorb ODS 2, 5 μm (Dalian Institute of Chemical Physics, Academia Sinica, Dalian, PRC), and a Waters Guard-Pak precolumn module with a Nova-Pak C_{18} insert (Waters) is used to protect the analytical column. Chromatograms were recorded on a Yokogawa Hokushin Electric Type 3066 pen recorder (Sino–Japanese Sichuan Fourth Meter Factory, Chongqing, PRC), together with a Model JS-3030 chromatographic working station (Dalian Johnsson Separation Science & Technology Corporation, Dalian, PRC).

An inductively coupled plasma quantometer, Model 1100 (Jarrell–Ash Company, Boston, MA, USA), is employed for the determination of the total metals in all water samples. A Waters 510 pump equipped with an IC-Pak Anion precolumn insert in Guard-Pak precolumn module, an IC-Pak Anion HC analytical column, and a Waters 432 Conductivity detector (Waters) is employed to chromatographically analyze the concentrations of anions in partial water samples. The pH values of the solutions are measured by a Mettler Toledo 320 pH meter (Mettler–Toledo Instruments Shanghai Co. Ltd, Shanghai, PRC).

Reagents

Methanol is HPLC grade (Institute for Fine Chemical Engineering of Huaiyin Plastic Product Factory, Huaiyin, PRC, WHO Collaborating Center for Research in Human Reproduction, Tianjin, PRC). All other chemicals are analytical-reagent grade unless stated otherwise, and used without further purification.

Twice distilled water from a quartz device is used for all solutions, dilution and mobile phase. The mobile phase is filtered through a Millipore Milli-Q system with 0.45-μm membranes (Millipore, Bedford, MA, USA) and degassed in an ultrasonical bath before use.

The standards of Al and other metal ions are prepared from ICP-AES grade stock solutions (1000 μg/mL) provided by ICP-AES Laboratory, Center of Materials Analysis, Nanjing University.

Chromatographic Conditions

The mobile phase consists of 4.0 mmol/L ammonium acetate–acetic acid buffer (pH 6.0) plus 3.2 mmol/L 8-HQ in a mixture of 57% (v/v) methanol and 43% (v/v) water; with a flow rate of 1.0 mL/min. The sample injection volume is 50 μL and the column temperature is 30°C. The detection wavelength is 380 nm.

Precolumn Chelation

To a suitable amount of the metal ions in a 10-mL volumetric flask, 0.5 mL of 1.0 mmol/L acetate buffer (pH 6.0) and 1.0 mL 20 mmol/L 8-HQ solution in methanol are added, sonicated for 15 min, and diluted to the mark with methanol. 8-HQ is sparingly soluble in water and form chelates of metals that are insoluble. The content of methanol is more than 40% (v/v) in order to maintain the solubility of hydrophobic chelates in the resulting aqueous mixture.

A part of the mixture is filtered through a membrane with 0.45 μm micropore (Milipore), and 50 μL of the filtered solution is directly injected onto the column. The blank test is necessary to surmount the possible interference from metals, particularly Al, at trace level in the chelating reagent and reaction medium, including the acetate buffer solution.

Sample Treatment

An adequate amount of nitric acid (guaranteed reagent, Shanghai First Chemical Reagent Factory, Shanghai, PRC) is added to 5.0 mL of water samples, the pH of the samples being adjusted to 1.5–2.0. The other procedures for the acidified samples are the same as described in the "precolumn chelation" section.

All glassware is carefully treated with around 2 mol/L nitric acid and rinsed several times with twice distilled water from quartz.

RESULTS AND DISCUSSION

Chromatogram of Al-8-HQ Complex

Figure 1a shows a typical chromatogram relative to a synthetic solution of Al, Zn, Cu, Fe, and Mn. As can be seen, Al–8-HQ chelate is distinctly separated and recognized from unreacted 8-HQ, Fe, and Mn chelates of this ligand. The coelution of Zn and Cu chelates with unreacted 8-HQ makes peaks of Zn and Cu disappear in the chromatogram. Ca, Mg, Na, K, Si, and Sr which are the abundant or

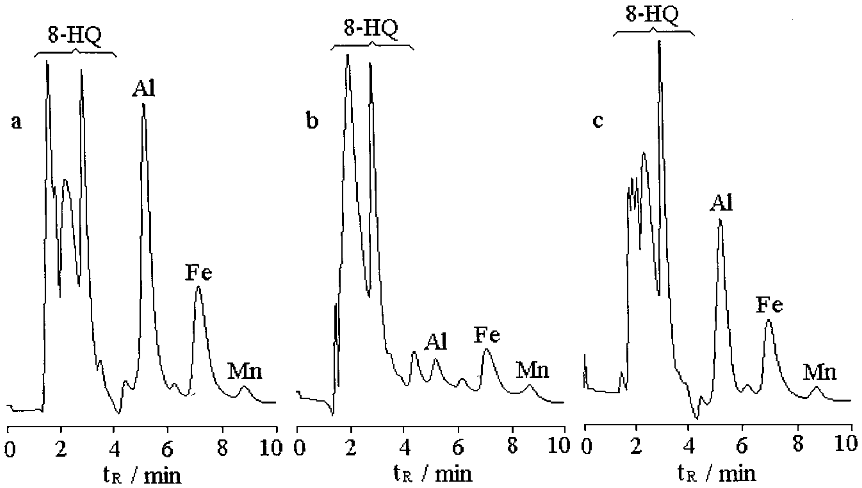

Figure 1. Chromatogram of metal–8-HQ chelates: a) 0.2 μg/mL of each metal ion; b) Robust natural mineral water; c) Zhangjiajie mountain cave water. Experimental conditions are presented in the text.

sub-abundant elements in drinking and natural waters, do not interfere in the assay because no complexing reaction happens to these metals under the conditions of the experiment. Therefore, the precolumn complexation is satisfactory for quantitation based on the peak height measurement.

In fact, because the concentration of major interfering elements present in drinking and natural fresh waters are very low, they will not cause any interference for determining Al (36,37). It is found in the present experiment that accurate and precise determination is somewhat difficult for Fe and Mn, presumably because of (1) the ions released by the metallic components of the system (27), and (2) the conversion of the oxidation states of these two metal ions (32,33).

Optimization of Precolumn Complex Formation

Effect of the Quantity of 8-HQ

For the determination of 0.2 μg/mL (about 0.008 mmol/L) of Al, as long as the molar ratio of 8-HQ to Al exceeds 20:1, the peak height of the chelate is stable under the optimum chromatographic conditions. This result is slightly different from previous work in which the optimum ratio was 10:1. The highest limit of molar ratio of 8-HQ to Al is not checked, but the peak shape is still constant when the ratio is up to 2000:1.

In consideration of unreacted 8-HQ which has very large molar absorptity at the wavelength of 380 nm, the most suitable wavelength for Al–8-HQ in this experiment, the excess ligand in the sample solution should be minimized. Thus, the molar ratio of 50–500 is recommended for the chelate formation prior to HPLC analysis.

Effect of pH and Buffer Concentration in Reaction Medium

The influence of pH and concentration of acetate buffer, are examined by using different pH or different concentration ammonium acetate–acetic acid in the complexing medium. Al peak height increases with increasing pH values in the range of 4.0–5.5, then becomes stable at the pH more than 5.5; and that of 8-HQ is just the opposite. This result indicates that the pH below 5.0 is unfavorable to the complete chelation for RP-HPLC, though suitable for colorimetry. At pH 6.0, the concentration of ammonium acetate in the range of 0–100 mmol/L has little effect on the peak height of Al, while the height of 8-HQ decreases with decreasing acetate buffer concentration in the range studied.

A negative peak appears at the position of unreacted 8-HQ when the acetate concentration is below 5.0 mmol/L, and fortunately does not hinder Al peak. 8-HQ is an amphoteric substance, so it is found, experimentally, that (1) a group of peaks, not a single peak, appear in chromatograms corresponding to unreacted 8-HQ, and (2) the pH and concentration of the buffer result in the change of the existing form of 8-HQ on which the chromatographic behavior and the UV absorptive spectrum depend. Thus, an ammonium acetate concentration of 50 mmol/L at pH 6.0 is selected in order to harmonize the media of all samples before chelation, although it is not influential in the Al chelate of 8-HQ whether or not the buffer (pH 6.0) is added.

Effect of Sonication and Heating on Chelating Reaction

Heating results in not only the volatilization loss of methanol and the insolubilization of 8-HQ and its chelates in sample solution, but also the conversion of the unreacted 8-HQ. In addition, heating and cooling prior to analysis is time-consuming. Sonication is a simple, convenient, and mild technique. As long as sonication time exceeded 15 min, the concentration of the chelate does not vary at least in 30 days. In suitable medium, the fast kinetics of Al with 8-HQ allows complete chelation and good stability at room temperature, which is the important requirement of quantitative analysis including "on-line" sample preconcentration and "on-line" chelation of Al by the cited procedure (31,33,35).

Optimization of Chromatographic Conditions

Effect of Methanol Content in Mobile Phase

Acetonitrile is most widely utilized as organic modifier in mobile phase for the determination of metal ions as 8-HQ chelates by RP-HPLC. However, this reagent is very expensive in many developing countries. Methanol is recommended as the organic modifier in the mobile phase instead of acetonitrile in present work for its great separation capacity and low price, although only one or two previous authors used it as the sole organic modifier in the HPLC technique mentioned here. As usual, an increase in methanol content in the mobile phase results in a decrease in the retention of the chelate. The effect of the methanol content on retention time and peak height of the chelate is investigated.

When the methanol concentration is raised above 58%, the separation of Al–8-HQ chelate and other components including 8-HQ and its Fe, Mn chelates is unsatisfactory, although the peak heights are rather high. If the methanol concentration is lower than 54%, the peak widths of the chelates are seriously increased and the peak shapes changed randomly. The result seems to indicate that 8-HQ chelates of metals decompose in a low methanol concentration. The best separation is obtained when the methanol concentration is 57% with the resolution between Al and Fe chelates of more than 1.38. This mixture, as compared to the reaction medium, is enriched in methanol content for the benefit of resistance to on-column decomposition of the chelate.

Effect of Concentration of 8-HQ in Mobile Phase

The degradation of Al–8-HQ during the elution on a reversed-phase column is a knotty problem owing to its high lability when there is strong hydrophobic interaction between the chelate and the stationary phase in aqueous mobile phase. An appropriate amount of the ligand is added in the mobile phase to prevent dissociation of the chelate. Figure 2 shows the influence of concentration of 8-HQ on peak height of Al chelate under different methanol content in mobile phase. The lowest 8-HQ concentration (critical point) that makes the peak height stable increases with decreasing content of methanol.

When the methanol contents are 58%, 57%, 56%, and 54%, the critical points are 2.68, 2.80, 2.96, and 3.14 mmol/L, respectively. It is likely that these two factors are mutually complementary in maintaining the stability of the chelate. The optimum 8-HQ concentration is selected at 3.2 mmol/L in the mobile phase in which 57% (v/v) of methanol exists.

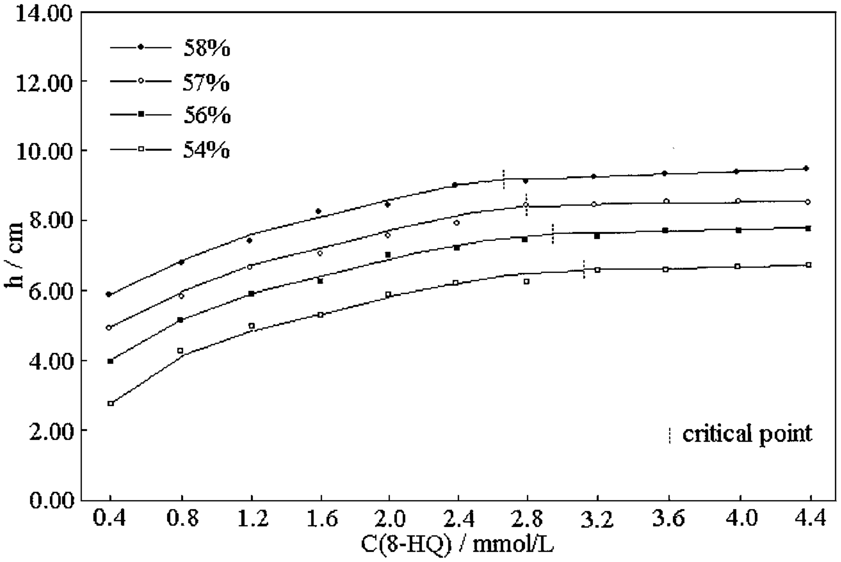

Figure 2. Dependence of peak height on 8-HQ concentration in mobile phase. Other conditions as in Figure 1.

Effect of pH and Buffer Concentration in the Mobile Phase

By respectively adjusting different pH and different concentration acetate buffer in the methanol–water solution (57:43 v/v) containing 3.2 mmol/L 8-HQ, the effects of pH and concentration of buffer are investigated experimentally. No effects of pH and concentration of ammonium acetate–acetic acid on the retention time of Al–8-HQ chelate are found because of its hydrophobicity and neutrality. However, the effect of the buffer on peak height is significant. The peak height of Al increases with increasing pH values in the range studied, but remains stable at the pH between about 5.5 and 6.5, while that of unreacted 8-HQ decreases rapidly with increasing pH values. Additionally, the baseline is unstable when the pH is below 5.0.

This result is not in accordance with a previous conclusion that pH ranging from 4.5–7.0 does not gives any significant difference in the chromatographic results (29). The peak height of Al sharply increases with the ammonium acetate concentration up to about 1.0 mmol/L and then becomes constant over the acetate concentration range 1.0–8.0 mmol/L before decreasing slowly at the buffer concentration higher than 8.0 mmol/L. When ammonium acetate concentration is less than 4.0 mmol/L, an inverse peak occurs. Furthermore, the less the concentration is, the broader, the higher, and the closer to Al the inverse peak is, interfering

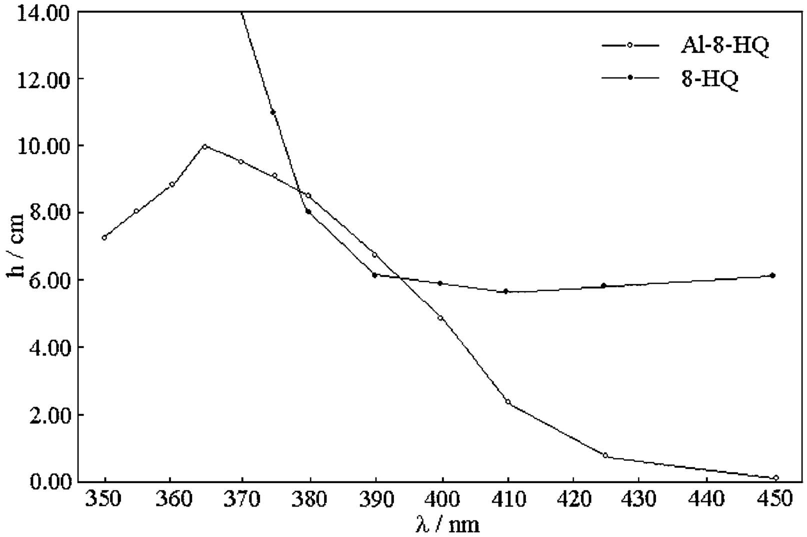

Figure 3. Dependence of peak height on wavelength. Other conditions as in Figure 1.

with Al peak. This phenomenon verifies, further, the conclusion that the buffer can convert the existing form of 8-HQ as described above. Therefore, an ammonium acetate concentration of 4.0 mmol/L and pH 6.0 are chosen as the optima.

Selection of Wavelength for Monitoring the Chelate

The peak height of Al chelate of 8-HQ at the wavelength range of 350–450 nm is shown in Figure 3; the maximum appearing around 365 nm, however, the excess of ligand having a very strong absorption at the same wavelength range. The shorter the wavelength is, the noisier the baseline is. It is well known that the wavelength of maximum absorption of the chelate is around 390 nm by spectrophotometry (14,38). Accordingly, most of analysts employed 390–400 nm as the monitoring wavelength for RP-HPLC determination of Al-8-HQ, ignoring the difference of the spectra obtained with different blanks.

Experimentally, the mobile phase used in the present experiment may shift the maximum about 30 nm towards the short ultraviolet zone. The detection of Al is achieved by monitoring the absorption of Al-8-HQ complex in the eluent at 380 nm for getting higher sensitivity, instead of 365 nm. The noise, which is too high, is caused by the absorption of the mobile phase containing 3.2 mmol/L of 8-HQ at 365 nm. In addition, the chelate has the highest peak at 380 nm on the basis of the same noise level as that at other wavelengths.

The Calibration Graph and Detection Limit

Standards at concentrations of 0.002–0.4 μg/mL of Al are prepared and injected into the chromatographic column under the optimum experimental conditions. The linear relationship of peak height versus concentration of Al is obtained from 0.002 to 0.3 μg/mL. The regression equation is h (cm) $= -0.005952 + 41.9714$ C with correlation coefficient of 0.9991. The relative standard deviation for five replicate determinations of 0.2 μg/mL Al is 3.6%. The detection limit, calculated as the concentration that gives a signal triple background noise, is 0.002 μg/mL (0.1 ng in the injected solution volume) under the optimum chromatographic conditions.

Practical Analysis of Drinking and Natural Water Samples

The Al concentrations in drinking and natural water samples are examined according to the general procedure described above. The real samples are prepared

Table 1. Analysis of Al in Drinking and Natural Water Samples

No	Sample	Determined Concentration (μg/mL)	RSD (%($n=3$))	Added (μg/mL)	Found Concentration (μg/mL)	RSD (%($n=3$))	Recovery (%)	ICP-AES (μg/mL)
Drinking waters								
1	Robust purified water	0.016	7.8	0.080	0.095	1.0	93.8	0.017
2	Robust natural mineral water	0.023	6.1	0.080	0.102	1.6	95.6	0.020
3	Nong Fu Shan Quan natural water	0.070	1.8	0.080	0.153	2.8	104.3	0.064
4	Tap water	0.202	5.0	0.040	0.224	0.9	91.1	0.183
Natural waters								
5	Zhangjiajie stream water	0.034	7.3	0.080	0.111	3.1	91.2	0.030
6	Nanjing rain water	0.107	0.9	0.080	0.193	1.0	105.6	0.110
7	Nanjing snow water	0.107	5.4	0.080	0.188	1.7	100.9	0.094
8	Zhangjiajie mountain cave water	0.121	3.5	0.040	0.165	2.2	103.3	0.113
9	Zhangjiajie river water	0.145	3.7	0.040	0.172	2.5	91.0	0.140
10	Yellow Mountain pool water	0.156	1.8	0.040	0.184	1.5	92.3	0.175

Table 2. The Water Quality Data of the Water Samples (μg/mL)

No[a]	1	2	3	4	5	6	7	8	9	10
Ba	<0.001	0.024	0.010	0.028	0.006	0.006	0.004	0.006	0.007	0.006
Ca	<0.01	3.47	5.48	40.46	6.20	3.64	1.20	83.50	75.40	5.37
Cd	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Co	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Cr	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Cu	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Fe	<0.015	<0.015	<0.015	0.032	<0.015	0.026	0.043	<0.015	0.110	0.020
K	<0.3	2.7	0.6	1.7	<0.3	2.4	1.0	<0.3	<0.3	1.0
Li	<0.006	0.008	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Mg	<0.015	1.064	0.786	9.364	1.170	0.396	0.137	4.120	11.60	0.909
Mn	<0.001	0.006	<0.001	0.006	<0.001	0.004	0.002	<0.001	<0.001	0.014
Mo	<0.005	0.007	0.007	0.013	<0.005	0.007	0.007	<0.005	<0.005	<0.005
Na	0.16	7.46	1.57	10.88	0.88	0.71	0.22	0.99	0.99	2.49
Ni	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
P	<0.06	0.10	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	0.13
Pb	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
Si	<0.01	25.26	1.66	4.09	2.69	0.27	0.24	3.14	3.04	3.37
Sr	<0.001	0.023	0.018	0.198	0.027	0.024	0.008	0.190	0.380	0.021
Ti	<0.002	<0.002	<0.002	0.003	<0.002	0.005	0.004	<0.002	<0.002	<0.002
V	<0.002	0.002	0.003	<0.002	<0.002	0.005	0.003	<0.002	<0.002	0.002
Zn	<0.002	0.009	0.163	0.041	<0.002	0.028	0.016	<0.004	<0.002	0.019
F^-		–		0.2						
Cl^-		0.7		9.0						
NO_3^-		–		11.6						
SO_4^{2-}		–		34.5						

[a] For sample number see Table 1.

as follows: Drinking waters are obtained from the supermarkets in Nanjing, tap water drawn from the local supply system. Natural waters are collected from rain and snow in the vicinity of Nanjing, and river, cave, and stream in Zhangjiajie (a national natural protective zone), and pool in Yellow Mountain (a famous natural scenic spot in China).

Typical chromatograms obtained for Robust natural mineral water and Zhangjiajie mountain cave water are given in Figures 1b and c, respectively. Table 1 gives the results obtained for various drinking and natural waters, accompanied by those for these samples spiked with Al standard to ascertain the reliability of the proposed method.

There is good, satisfactory accord between the results obtained by the present method and those obtained by ICP-AES. The recoveries are between 91.0–105.6%, and the relative standard deviation 0.9–7.8%. The water quality data of the samples listed in Table 2 indicates that no obvious interference is found from foreign ions.

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