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SIMULTANEOUS DETERMINATION OF IRON(II) AND -(III) BY ION CHROMATOGRAPHY WITH POST-COLUMN REACTION

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SUMMARY

A method for the simultaneous determination of Fe(II) and Fe(III) which combines ion chromatography and a post-column reaction is described. Using 0.05 *M* tartaric acid (pH 4.5) or 0.15 *M* lactic acid (pH 4.5) as eluent, Fe(II) and Fe(III) were first separated on a column (50 × 4.6 mm I.D.) packed with cation-exchange resin, and then transferred to a post-column reactor. The colour formation of Fe(II) with bathophenanthrolinedisulphonic acid disodium salt was then observed at 530 nm. Fe(III) was reduced to Fe(II) by ascorbic acid, and then the colour formation was again observed.

INTRODUCTION

Iron is widely distributed in nature and is one of the extremely important elements in the biological system. The behaviour of its various oxidation states in oxidation–reduction reactions is complicated. However, it is important to be able to establish the chemical state accurately and quickly. The oxidation states of iron are mainly 2+ and 3+.

A spectrophotometric method has been widely used for the determination of Fe^{2+} and Fe^{3+}. However, this procedure is complicated and slow. Although the separation of transition metals using reversed-phase chromatography has been reported[1–5], there are few methods for the simultaneous determination of Fe^{2+} and Fe^{3+}, and the accuracy of determination is poor.

Recently, some experiments have been reported in which various metals were separated by ion chromatography (IC) using a conductivity detector[6]. However, the sensitivity of the detection is not sufficient. Riviello and Pohl[7] and Wang *et al.*[8] reported the analysis of transition metals by a post-column reaction method, using a visible spectrophotometric detector and 4-(2-pyridylazo)resorcinol (PAR) as reagent. This method has the advantage of being able to detect various metals simultaneously because PAR reacts with many metal ions unselectively. However, in the case where metal ions other than iron coexist in high concentration, these ions interfere in the determination of Fe^{2+} and Fe^{3+}; therefore, the accuracy of this determination is low.

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The aim of this study was the simultaneous determination of Fe^{2+} and Fe^{3+} a combination of IC and post-column reaction. The Fe^{2+} is treated with bathophenanthrolinedisulphonic acid disodium salt (BDAD) and examined by visible spectrophotometric Fe^{3+} is first reduced to Fe^{2+} with ascorbic acid and then treated in the same way. The effect of interfering ions, and also some applications are described.

EXPERIMENTAL

Apparatus

A Dionex Model 2010i ion chromatograph and a Yokokawa Hokushin Electric Co. IC-Model 100 were used, with an Hewlett-Packard spectrophotometric detector 1040A for high-performance liquid chromatography and a Dionex UV–Vis detector.

The separating columns are 50 × 4.6 mm I.D., packed with 10-μm 0.03 mequiv./g cation-exchange resin TSK-gel IC Cation (Toyo Soda) and 250 × 4.6 mm I.D., packed with 10-μm 0.03 mequiv./g cation-exchange resin SCX-1 (Yokokawa Hokushin Electric Co.).

Standard solution

For Fe^{2+} and Fe^{3+}, the standard solutions were prepared by dissolving highly purified $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ (Merck) in 50 m*M* hydrochloric acid just prior to use. The experiments confirmed that Fe^{3+} was not present in the standard solution of Fe^{2+}, by comparing the chromatogram of the standard solution of Fe^{2+} containing added ascorbic acid as a reducing reagent and that of the standard solution without ascorbic acid, no change in the peak of Fe^{2+} was observed. For other transition metals, Cu^{2+}, Ni^{2+}, Zn^{2+} and Co^{2+}, the standard solutions were prepared just prior to use from standard 1000 ppm solution for atomic absorption spectrophotometric analysis (Waco Junyaku Co.).

Eluent

The eluents were prepared by dissolving oxalic acid, citric acid, tartaric acid or lactic acid in water, adjusting the pH with lithium hydroxide and deaerating by ultrasonication under reduced pressure.

Colour reagent

The colour reagent was prepared by adding ascorbic acid as a reducing agent is bathophenanthrolinedisulphonic acid disodium salt (Nakarai Chemical Co.).

RESULTS AND DISCUSSION

Colour reagent

Fig. 1 shows a flow diagram of the apparatus employed for the determinations described. The standard measurement conditions are summarized in Table I.

The requirements of the colour reagent for the simultaneous determination of Fe^{2+} and Fe^{3+} by IC are as follows: (i) water-soluble; (ii) complex formation is very rapid; (iii) Fe^{2+} and Fe^{3+} can be detected at the same wavelength with high sensitivity; (iv) the pH range for complex formation is wide; (v) the complex formed is

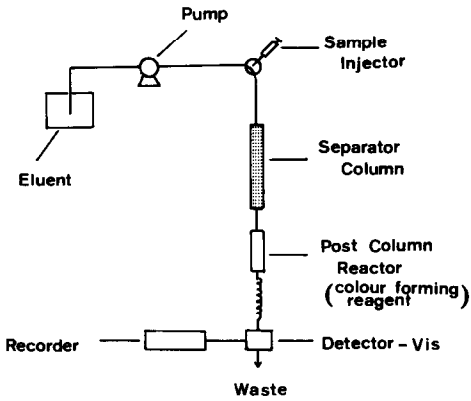

Fig. 1. Schematic diagram of the flow system for the determination of Fe^{2+} and Fe^{3+}.

TABLE I

ANALYTICAL CONDITIONS FOR DETERMINATION OF Fe(II) AND Fe(III)

Separating column	:	50 × 4.6 mm I.D., cation-exchange resin
Eluent	:	0.05 *M* tartaric acid (pH 4.5) or 0.15 *M* lactic acid (pH 4.5)
Sample volume	:	50 μl
Flow-rate	:	1.0 ml/min (eluent), 1.0 ml/min (colour reagent)
Colour reagent	:	$1 \cdot 10^{-3}$ *M* bathophenanthrolinedisulphonic acid disodium salt + 0.2 *M* ascorbic acid
Mixing temperature	:	40°C
Detector	:	Visible 530 nm

very stable. PAR is widely used in high-performance liquid chromatography (HPLC) as a colour reagent for metal ions. However, it has a tendency to react non-selectively with many metal ions. Especially, Cu^{2+} is eluted close to Fe^{3+}, and the sensitivity for Cu^{2+} is approximately ten times that for Fe^{3+}. Therefore, it is difficult to determine Fe^{3+} in a sample containing Cu^{2+}.

It is known that BDAD (Fig. 2) reacts with Fe^{2+} to yield a maximum absorption at 530 nm. Our experiment showed similar results (Fig. 3). Fig. 3 shows the visible absorption spectra of Fe^{2+} + BDAD (B), Fe^{3+} + ascorbic acid + BDAD (A_2) and Fe^{3+} + BDAD (A_1). The spectra B and A_2 had maximum absorption at 530 nm, but A_1 did not.

The conditions for colour formation was investigated, namely the temperature and the mixing ratio between the eluent and the colour reagent. Fig. 4 shows the measured absorbances for the colour reaction of 1×10^{-3} *M* BDAD and 0.2 *M*

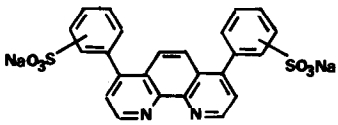

Fig. 2. Structural formula of bathophenanthrolinedisulphonic acid disodium salt.

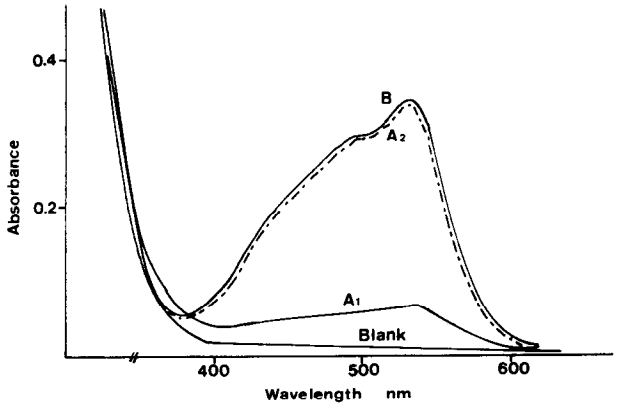

Fig. 3. Absorption spectra of Fe^{2+} (5 ppm) + 1 m*M* BDAD (B), Fe^{3+} (5 ppm) + 0.2 *M* ascorbic acid + 1 m*M* BDAD (A_2) and Fe^{3+} (5 ppm) + BDAD (A_1). The pH of the sample was 1.5.

ascorbic acid (eluent–colour reagent, 1:0.7) and 0–10 ppm of Fe^{2+} and Fe^{3+} at 20, 30 and 40°C. The dependence of the absorbance of Fe^{2+} on concentration was linear at all temperatures studied. However, that for Fe^{3+} deviated from linearity below 30°C. This may indicate that the reduction of Fe^{3+} by ascorbic acid is incomplete at 20 and 30°C.

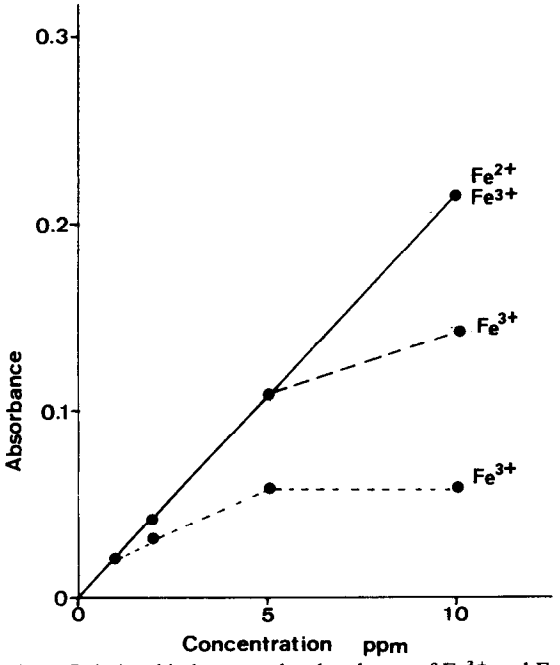

Fig. 4. Relationship between the absorbance of Fe^{3+} and Fe^{2+} (0–10 ppm) and the temperature at which colour formation occurs. Eluent: 50 m*M* tartaric acid (pH 4.5). Colour reagent: 1.0 m*M* BDAD + 0.2 *M* ascorbic acid, flow-rate 0.7 ml min^{-1}. Temperatures: ———, 40, 50°C; – – –, 30°C; ---, 20°C. The pH of the sample was 1.5.

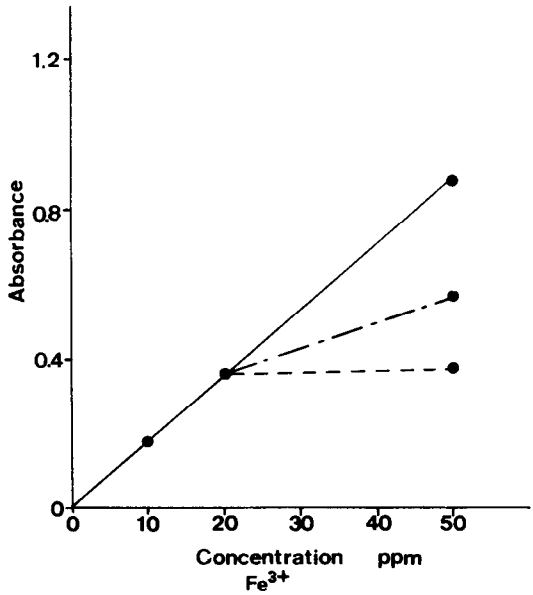

Fig. 5. Relationship between the absorbance of Fe^{3+} and the mixing ratios of the eluent and colour reagent: ---, 1:0.4; ---, 1:0.7; ———, 1:1.0. Temperature: 40°C. Eluent: 50 m*M* tartaric acid (pH 4.5). The pH of the sample was 1.5.

Fig. 5 compares the linearity of the absorbance of Fe^{3+} at mixing ratios of the eluent and colour reagent of 1:0.4, 1:0.7 and 1:1.0, at 40°C. A straight line was obtained within the range of 0–50 ppm when the mixing ratio was 1:1. Also, even at increased mixing ratio, there was no variation in the baseline.

Effect of column length

Generally, in IC, when the inner diameter of the column is constant, the separation ability tends to increase with increasing column length. However, when separating complexes, etc., the interactions among the sample, the column packing and the eluent are complicated. Fig. 6 compares chromatograms for the standard solution of Fe^{2+}, using the columns of 4.6 mm I.D. and 250 and 50 mm in length. With the longer column (A), the baseline level was elevated close to the position of elution of Fe^{3+} (t_R = 3 min); this phenomenon was not noted with the shorter column (B).

It may be considered[9] that the Fe^{3+} results from the oxidation of Fe^{2+} in contact with the column packing, and can be prevent shortening the length of the column and hence the contact time between the sample and the column packing.

Eluent conditions

The investigation was conducted using oxalic acid, citric acid, tartaric acid or lactic acid as the eluent.

Effect of pH. Various pH values < 5 were investigated. It was found that pH 4.5 was appropriate to maintain an interval of *ca.* 2 min between the elution times of Fe^{2+} and Fe^{3+}. Below pH 4.5, because the elution time increases, the phenomenon of baseline elevation occurred. Therefore, all subsequent measurements were conducted at pH 4.5.

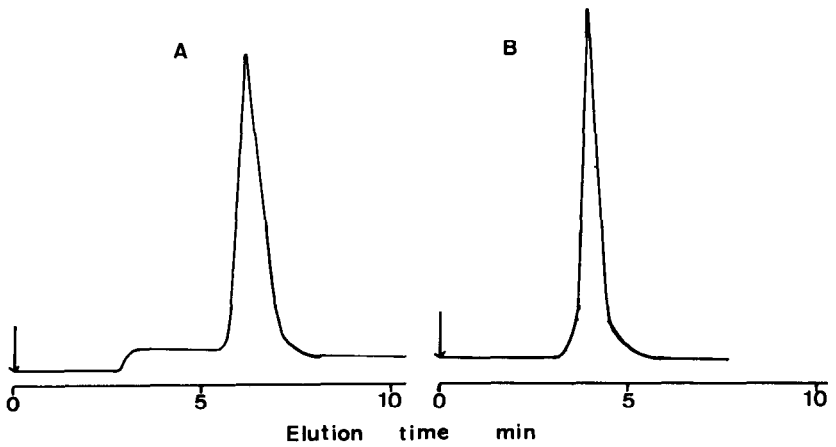

Fig. 6. Chromatograms of Fe^{2+} using 250 × 4.6 mm I.D. (A) and 50 × 4.6 mm I.D. (B) columns. Eluent, 50 m*M* tartaric acid (pH 4.5); flow-rate, 1.0 ml min^{-1}; detection, 530 nm.

Mixed eluents. Fig. 7 shows the chromatogram of the standard solution of Fe^{2+} and Fe^{3+}, using 10 m*M* oxalic acid and 7.5 m*M* citric acid as eluent. An unknown peak at t_R = 3 min was detected between those of, Fe^{3+} (t_R = 2.5 min) and of Fe^{2+} (t_R = 4 min). Since this unknown peak interfered with the simultaneous determination of Fe^{2+} and Fe^{3+}, each peak was identified.

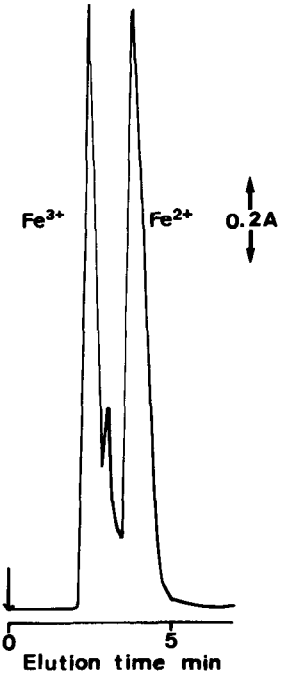

Fig. 7. Chromatogram of the standard solution of Fe^{2+} and Fe^{3+} (5 ppm). Eluent: 10 m*M* oxalic acid + 7.5 m*M* citric acid, pH 4.5. Column, 50 × 4.6 mm I.D.; flow-rate, 1.0 ml min^{-1}; detection, 530 nm.

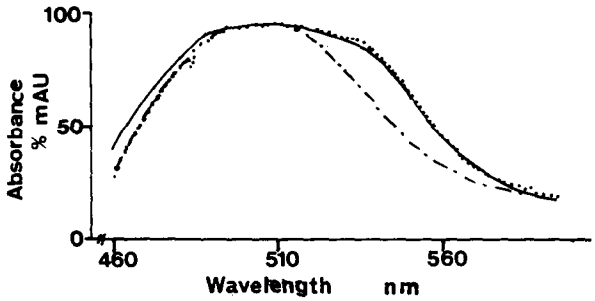

Fig. 8. Absorption spectra at 460–600 nm of the three peaks: —, Fe^{3+}; –.–, Fe^{2+}; ----, unknown.

First, the spectrophotometric detector 1040A was employed instead of the UV–Vis detector and the absorbance was measured after colour formation (with PAR). Then, using an automatic program, absorption spectra at 460–600 nm were plotted for each peak as shown in Fig. 8. From the analysis of the spectra, the unknown peak seemed to be that of the Fe^{3+}–PAR complex.

Next, the spectrophotometric detector 1040A was connected after the column and the absorbance measured before colour formation. Fig. 9 shows spectra at 200–400 nm for each peak. From the results it was inferred that the unknown peak is due to one of the complexes of Fe^{3+} with oxalic acid, or citric acid, which has a maximum absorption at 215 nm.

Also, iron was detected by plasma emission spectrochemical analysis of this unknown peak.

Comparison of single organic acid as eluents. When two kinds of organic acids were employed together as the eluent the number of components being detected increased. Therefore, the chromatograms obtained by using each organic acid by itself were compared as shown in Fig. 10.

When oxalic acid (A) and citric acid (B) were used as eluent, shoulders appeared before and after the peak of Fe^{3+}. Such a phenomenon was not found in the cases of lactic acid (C) and tartaric acid (D). It may be inferred that; at pH 4.5, two kinds of complex ions are produced with Fe^{3+} in the cases of oxalic acid and citric acid which are eluted at somewhat different elution times, whereas in the cases of lactic acid and tartaric acid, one kind of complex ion is produced because of the dissociation constants of these organic acids.

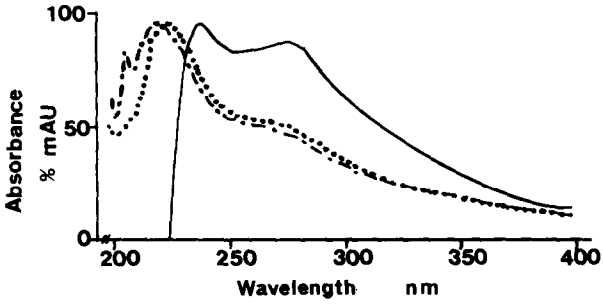

Fig. 9. Absorption spectra at 200–400 nm of the three peaks. Details as in Fig. 8.

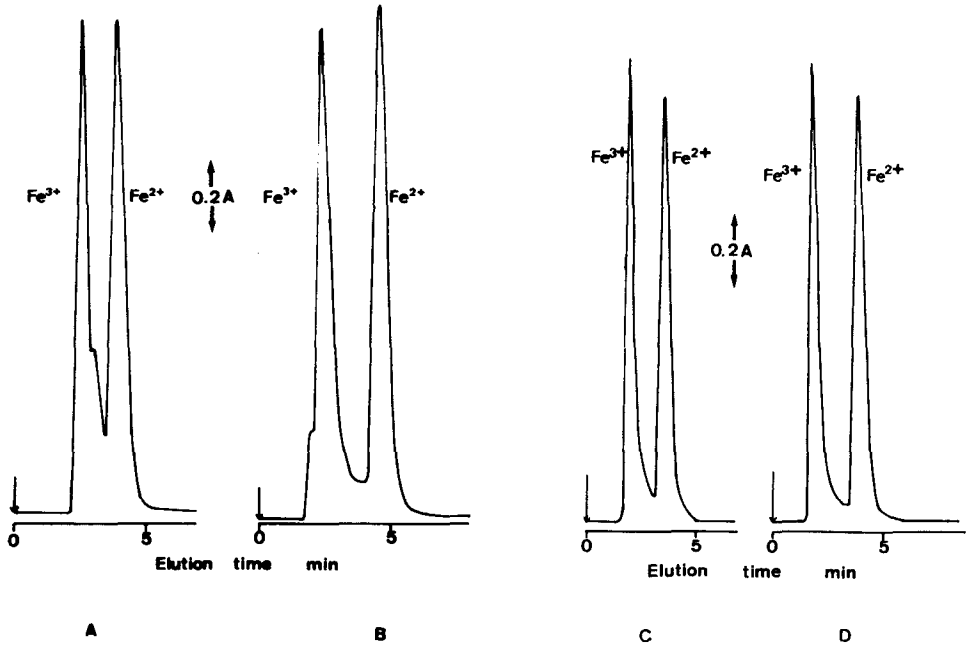

Fig. 10. Chromatograms of the standard solution of Fe^{2+} and Fe^{3+} (5 ppm) using different eluents (pH 4.5): A, 15 m*M* oxalic acid; B, 25 m*M* citric acid; C, 150 m*M* lactic acid; D, 50 m*M* tartaric acid.

Reproducibility and detection limits

The reproducibility of the determination of Fe^{2+} by itself and of Fe^{3+} by itself (5 ppm) as sufficient, with standard deviations (n = 7) of 3.4 and 4.2% respectively. The detection limits for Fe^{2+} and Fe^{3+} were 10 ppb at a signal-to-noise ratio of 3.

Interference from other metal ions

Using 50 m*M* tartaric acid (pH 4.5) as eluent, the peaks of Fe^{2+} and Fe^{3+} were compared when standard solutions (each 100 ppm) of Cu^{2+}, Ni^{2+}, Zn^{2+} and Co^{2+} were added to a standard solution of Fe^{2+} and Fe^{3+} (5 ppm) with the peaks obtained in the absence of the interfering species. No effect of these metal ions on the peaks of Fe^{2+} and Fe^{3+} was detected.

Simultaneous determination of Fe^{2+} and Fe^{3+} in hot spring-water

Fig. 11 shows the chromatograms for iron ions in Toya hot spring-water: a–c are chromatograms of the spring-water from the source; d is a chromatogram for water from the reservoir into which the spring-water was pumped up from source. It is clear that Fe^{2+} in the water from the source is oxidized to Fe^{3+} in the reservoir.

Table II showed the results of a comparison between a conventional spectrophotometric detection method (*o*-phenanthroline method) and the IC method. Samples were immediately adjusted to pH 1.0 with hydrochloric acid after sampling. In the *o*-phenanthroline method, Fe^{2+} and total iron were measured, and the values for Fe^{3+} were calculated. The values from the two methods were in satisfactory agreement.

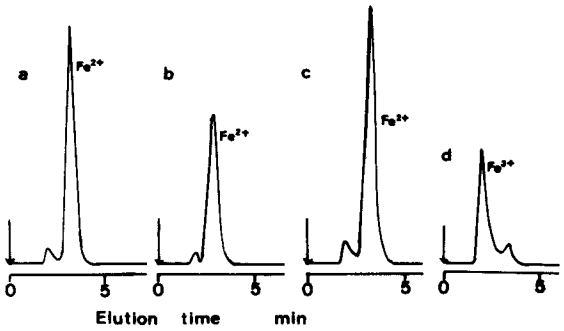

Fig. 11. Chromatograms of Fe^{2+} and Fe^{3+} in Toya hot spring-water, from the source (a–c) and reservoir (d). Column: TSK-gel IC, cation-exchange resin. Eluent: 50 m*M* tartaric acid, pH 4.5; flow-rate 1.0 ml min^{-1}. Detection: visible, 530 nm.

TABLE II

ANALYTICAL RESULTS (mg/kg, $n = 3$) FOR Fe^{2+} AND Fe^{3+} IN HOT SPRING-WATER

Hot spring-water	*IC method*			*o-Phenanthroline method*		
	Fe^{2+}	*Fe^{3+}*	*Total Fe**	*Fe^{2+}*	*Fe^{3+}***	*Total Fe*
Toya (reservoir)	0.26 ± 0.02	0.79 ± 0.05	1.05 ± 0.04	ND***	0.6 ± 0.42	1.0 ± 0.15
Toya (No. 1)	0.72 ± 0.01	0.15 ± 0.00	0.87 ± 0.01	0.8 ± 0.05	ND***	0.9 ± 0.00
(No. 2)	1.23 ± 0.02	0.26 ± 0.03	1.49 ± 0.05	1.2 ± 0.05	0.3 ± 0.11	1.5 ± 0.05
(No. 3)	1.36 ± 0.11	0.42 ± 0.07	1.78 ± 0.16	1.3 ± 0.05	0.5 ± 0.22	1.9 ± 0.25
Kuttsyaro	0.25 ± 0.02	2.42 ± 0.06	2.67 ± 0.08	0.2 ± 0.15	2.5 ± 0.20	2.7 ± 0.05
Tokati	0.13 ± 0.00	1.39 ± 0.02	1.52 ± 0.02	0.1 ± 0.05	1.5 ± 0.10	1.6 ± 0.05
Sukawa	30.4 ± 0.11	0.63 ± 0.05	31.0 ± 0.15	28.4 ± 0.45	0.6 ± 0.45	29.0 ± 0.00

* $Fe^{2+} + Fe^{3+}$.
** Total Fe minus Fe^{2+}.
*** ND = not detected (<0.1).

CONCLUSIONS

A method for the simultaneous determination of Fe^{2+} and Fe^{3+} by ion chromatography and post-column reaction has been established. Fe^{2+} and Fe^{3+} are separated by a column (50 × 4.6 mm I.D.) packed with cation-exchange resin, using 0.05 *M* tartaric acid (pH 4.5) or 0.15 *M* lactic acid (pH 4.5) as eluent, and then transferred into a post-column factor containing 1×10^{-3} *M* bathophenanthroline-disulphonic acid disodium salt plus 0.2 *M* ascorbic acid. The temperature is raised to 40°C, and the colour is recorded at 530 nm using a visible spectropohotometric detector.

The analysis time is about 5 min and the accuracy is up to 10 ppb for each kind of iron ions. This method was applied for the simultaneous determination of Fe^{2+} and Fe^{3+} in hot spring-water, and the results compared with those obtained by a conventional spectrophotometric detection method; good agreement was found.

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