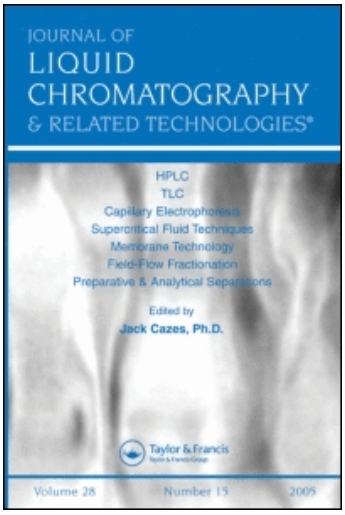

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Ichinoki, Susumu , Miyanaga, Sachie , Hattori, Maya and Fujii, Youichi(2005) 'Selective Determination of Iron in River Water and Standard Bovine Liver by Solvent Extraction with N-Benzoyl-N-Phenylhydroxylamine Followed by Reversed-Phase HPLC', Journal of Liquid Chromatography & Related Technologies, 28: 9, 1417 — 1429
To link to this Article: DOI: 10.1081/JLC-200054901
URL: http://dx.doi.org/10.1081/JLC-200054901

Journal of Liquid Chromatography & Related Technologies®, 28: 1417–1429, 2005

ISSN 1082-6076 print/1520-572X online
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Selective Determination of Iron in River Water and Standard Bovine Liver by Solvent Extraction with N-Benzoyl-N-Phenylhydroxylamine Followed by Reversed-Phase HPLC

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Abstract: A selective method for determination of iron ions in river water and iron in standard bovine liver has been developed by reversed-phase HPLC. An Iron (Fe) ion was quantitatively extracted into 4-methyl-2-pentanone at pH 3.0 ± 0.5 as *N*-benzoyl-*N*-phenylhydroxylamine (BPHA) chelate. The extracted Fe-BPHA chelate was then separated on a ODS column with an eluent of methanol/water/0.2 mol/L BPHA (78 : 21 : 1), and detected at 443 nm. The correlation coefficients of the calibration curves obtained with 5 mL Fe standards were more than 0.999 over the range of 10 ng/mL (ppb) to 10 μg/mL (ppm). The detection limit of Fe ion in 5 mL water was 2 ppb, which corresponded to 3 times the standard deviation of the blank peak area. Relative standard deviations of peak areas ($N = 5$) for 5, 0.5, and 0.05 ppm Fe standards were 0.9, 0.8, and 0.9%, respectively. Analytical results of Fe ion in river water obtained by the presented method, showed good agreement with those by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Fe concentration in standard bovine liver was determined to be 193 μg/g, which was coincident with the reference value (194 ± 20 μg/g). Effects of foreign ions on the method were investigated with 55 metal ions. Almost none of the ions interfered, except for V(V), Ti(IV), and Sn(II).

Keywords: N-Benzoyl-N-phenylhydroxylamine, Iron ion, Solvent extraction, High-performance liquid chromatography, Bovine liver, River water, Photometric detection

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INTRODUCTION

The application of high performance liquid chromatography (HPLC) in the separation and determination of metal ions has increased in recent years.[1–5] HPLC is not such an expensive apparatus and the running cost is minimal. In addition, operation of the HPLC is easy, and a more sensitive quantitative analysis is possible by combining pre-column derivatization HPLC with a simple solvent extraction. Many metal ions have been determined as metal chelates with various chelating reagents. The authors also determined various metal ions by HPLC as metal chelates.[6–8]

The reagent *N*-benzoyl-*N*-phenylhydroxylamine (BPHA) was used as a chelating agent for Fe ion, as well as zinc, cobalt, vanadium (V), and molybdenum (Mo) ions. The metal-BPHA chelates were extracted into some organic solvents, and then determined by gravimetric analysis,[9] paper chromatography,[10] polarography,[11] spectrophotometry,[12–14] normal-phase high performance liquid chromatography (NP-HPLC),[15–17] and reversed-phase (RP) HPLC.[18] Although Mo, and V were determined as BPHA chelates by HPLC,[15–18] determination of Fe was not found except in polarography.[11]

In this paper, analytical conditions, such as extraction pH, shaking time, and eluent composition were studied for selective and sensitive determination of Fe ion. The analytical results of Fe in river water and standard bovine liver samples, showed good agreement with the results by ICP-AES and the certified value, respectively.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Jasco PU-2080i inert pump (Japan Spectroscopic Co., Ltd, Tokyo, Japan), a Rheodyne 7125 injector (Cotati, CA) equipped with a 200 μL sample loop of polyether etherketone (PEEK), a Jasco UVIDEC-100-VI photometric detector, a Cosmosil 5 C_{18}-MS PEEK column (250 × 4.6 mm ID, Nacalai Tesque, Kyoto, Japan), a Shimadzu Chromatopac C-R6A integrator (Shimadzu Co., Kyoto, Japan), and a thermostat water bath (Taitec Co., Koshigaya, Japan). A Yamato SA-31 auto-shaker (Yamato Scientific Co., Ltd., Tokyo, Japan) was used for solvent extraction. A Plasma-Spec I ICP-AES (Leeman Labs Inc., MA) was also used for Fe analysis in river water. Micropipettes were used for 1 mL or less volume of solutions.

Reagents

All reagents used were of analytical-reagent grade unless otherwise stated. Milli-Q water was used for aqueous solution preparation and wet ashing

procedure. The chelating reagent BPHA (CAS No. 304-88-1, $C_{13}H_{11}NO_2 = 213.24$) was obtained from Tokyo Kasei Kogyou Co. Ltd. (Tokyo, Japan) for use as a 4-metyl-2-pentanone (methyl isobutyl ketone, MIBK) solution. A Fe standard solution of 1000 μg/mL (ppm) for Atomic Absorption Spectrometry was obtained from Wako Pure Chemical Industries (Osaka, Japan). The other Fe solutions were prepared by dilution of the above solution (1000 ppm) with 0.1 mol/L (M) HNO_3. The metal solutions used are summarized in Table 1. Methanol was distilled and filtered through a membrane filter (pore size, 0.45 μm). An acetate buffer solution of pH 4.0

Table 1. Metal standard solutions used

Metal	Salt	Medium	Metal	Salt	Medium
Ag(I)	$AgNO_3$	0.1 M HNO_3	Mn(II)	$Mn(NO_3)_2$	0.1 M HNO_3
Al(III)	$Al(NO_3)_3$	0.5 M HNO_3	Mo(VI)	$(NH_4)_6Mo_7O_{24}$	H_2O
As(III)	As_2O_3	NaOH in water pH 5 with HCl	Na(I)	NaCl	H_2O
Au(III)	$HAuCl_4$	1 M HCl	Nd(III)	$Nd(NO_3)_3$	1 M HNO_3
Ba(II)	$BaCl_2$	1 M HCl	Ni(II)	$Ni(NO_3)_2$	0.1 M HNO_3
Be(II)[a]	$BaSO_4$	0.03 M HNO_3	Pb(II)	$Pb(NO_3)_2$	0.1 M HNO_3
Bi(III)	$Bi(NO_3)_3$	0.5 M HNO_3	Pd(II)	$PdCl_2$	1 M HCl
Ca(II)	$CaCO_3$	0.1 M HNO_3	Pr(III)	$Pr(NO_3)_3$	1 M HNO_3
Ce(III)	$Ce(NO_3)_3$	1 M HNO_3	Pt(IV)	H_2PtCl_6	1 M HCl
Cd(II)	$Cd(NO_3)_2$	0.1 M HNO_3	Rh(III)	$Rh(NO_3)_3$	2 M HNO_3
Co(II)	$Co(NO_3)_2$	0.1 M HNO_3	Sb(III)	$SbCl_3$	3 M HCl
Cr(VI)	$K_2Cr_2O_7$	0.1 M HNO_3	Sc(III)[a]	Sc (metal)	1 M HNO_3
Cs(I)	$CsNO_3$	0.5 M HNO_3	Se(IV)	SeO_2	0.1 M HNO_3
Cu(II)	$Cu(NO_3)_2$	0.1 M HNO_3	Si(IV)	Na_2SiO_3	0.2 M Na_2CO_3
Dy(III)	$Dy(NO_3)_3$	1 M HNO_3	Sm(III)	$Sm(NO_3)_3$	1 M HNO_3
Er(III)	$Er(NO_3)_3$	1 M HNO_3	Sn(II)	$SnCl_2$	6 M HCl
Eu(III)	$Eu(NO_3)_3$	1 M HNO_3	Sr(II)	$Sr(NO_3)_2$	1 M HNO_3
Fe(III)	$Fe(NO_3)_3$	0.1 M HNO_3	Tb(III)	$Tb(NO_3)_3$	1 M HNO_3
Ga(III)	$Ga(NO_3)_3$	1 M HNO_3	Te(IV)	$TeCl_4$	6 M HCl
Gd(III)	$Gd(NO_3)_3$	1 M HNO_3	Ti(IV)	$Ti(SO_4)_2$	1 M H_2SO_4
Ge(IV)	GeO_2	H_2O	Tl(I)	$TlNO_3$	1 M HNO_3
Hg(II)	$HgCl_2$	0.02 M HCl	Tm(III)	$Tm(NO_3)_3$	1 M HNO_3
Ho(III)	$Ho(NO_3)_3$	1 M HNO_3	V(V)	NH_4VO_3	0.45 M H_2SO_4
In(III)	In (metal)	0.5 M HNO_3	W(VI)	Na_2WO_4	H_2O
K(I)	KCl	H_2O	Y(III)	$Y(NO_3)_3$	1 M HNO_3
La(III)	$La(NO_3)_3$	1 M HNO_3	Yb(III)	$Yb(NO_3)_3$	1 M HNO_3
Lu(III)	$Lu(NO_3)_3$	1 M HNO_3	Zn(II)	$Zn(NO_3)_2$	0.1 M HNO_3
Mg(II)	$Mg(NO_3)_2$	0.1 M HNO_3	Zr(IV)	$ZrO(NO_3)_2$	1 M HNO_3

[a]Concentrations of the metal ions were 1000 ppm, except for Be and Sc (100 ppm).

was prepared with 2 M acetic acid and 2 M sodium acetate solutions. River water was collected from the Kakehashi River (Komatsu, Japan). Concentrated hydrochloric acid was added to the river water immediately to adjust the pH to 1. The solution was then filtered through a membrane filter (0.45 μm pore size) and used as the river water sample for HPLC and ICP-AES analysis. A standard reference material (Bovine Liver) was obtained from National Institute of Standards and Technology (NIST, SRM 1577a). HCl, HNO_3, and $HClO_4$ used were super special grade for metal analysis purchased from Wako.

Recommended Extraction Procedure and HPLC Conditions

Transfer a 5.0-mL sample solution into a 10-mL centrifuge tube with a stopper. Adjust the pH of the solution to 1, if necessary. For calibration curves, transfer a Fe standard solution and 0.1 M HCl (total volume 5.0 mL) into a centrifuge tube. Add 2 mL of acetate buffer (pH 4.0) and 500 μL of 0.02 M BPHA solutions. Shake the contents for 10 min. After left standing for 5 min, collect the organic layer. Determine the Fe-BPHA chelate in the extract under the following HPLC conditions:

Column: Cosmosil 5 C_{18}-MS PEEK column (250 × 4.6 mm I.D., particle size 5 μm), Column temp.: 40°C, Eluent: methanol/water/0.2 M BPHA (78 : 21 : 1, v/v), Flow rate: 1.2 mL/min, Injection volume of organic layer: 5 μL, Detection wavelength: 443 nm.

Recommended Wet Ashing Procedure for Bovine Liver

Place 200 mg of Bovine Liver sample in a 50 mL glass conical beaker, and add 5 mL of mixed acid ($HNO_3/HClO_4 = 1:1$,v/v) to the beaker. Place a watch glass on the beaker, and let it stand over night. After standing, heat the contents in the beaker on a hot plate as follows: 50°C 20 min → 75°C 20 min → 100°C 20 min → 125°C 20 min. After cooling, add 5 mL of $HClO_4$ into the beaker, and place a watch glass on the beaker. Heat the solution again on a hot plate as follows: 150°C 20 min → 200°C 60 min. After cooling, wash the watch glass with water, and add the wash solution to the beaker. Evaporate the solution to dryness on a hot plate (125°C) without the watch glass. After drying and cooling, add 2.5 mL of 2 M HCl, and heat the contents (75°C 20 min → 100°C 10 min). Transfer the solution to a 50 mL volumetric flask, and add the washing solution from the beaker into the flask. Add water up to 50 mL. The pH of the resulting solution should be about 1.

A blank test must be carried out to correct the Fe contamination from the beaker and acids used.

Recovery Tests with a River Water Sample

A river water sample (pH 1) of 4.0 mL and 1.0 mL of 0.1 M HCl were taken into a centrifuge tube. To another centrifuge tube, 4.0 mL of a river water sample and 1.0 mL of 2.5-ppm Fe standard were added. Fe concentrations in these solutions were determined according to the recommended procedure. The recovery percentage was calculated from the two results. A similar experiment was carried out by ICP-AES (Ar plasma, 40 MHz) with 238.20 nm for detection of Fe.

RESULTS AND DISCUSSION

HPLC Conditions

Because Fe-BPHA chelate was not stable in a methanol/water eluent, addition of the chelating reagent (BPHA) into the eluent was required. Thus, the effect of BPHA concentration in the eluent on the peak height of Fe-BPHA chelate was investigated at concentrations of 0, 0.1, 0.2, 0.5, 1.0, 2.0, and 4.0 mM. The Fe height was plotted against the BPHA concentration in the eluent (Figure 1). The optimum BPHA concentration was 2 mM because a maximum peak height was obtained at that level. Therefore, the eluent of methanol/water/0.2 M BPHA (78 : 21 : 1, v/v) was used for the remainder of the work.

Column temperature was set at 40°C considering the flow rate (1.2 mL/min) and backpressure of the PEEK column.

The peak height of the Fe-BPHA chelate was measured at 360–540 nm, and the result is shown in Figure 2. Detection wavelength was set at 443 nm, which gave the maximum peak height of Fe-BPHA chelate.

Injection volume of the organic layer (MIBK) was also investigated in the range of 2-20 μL. Peak area was proportional to injection volume in the

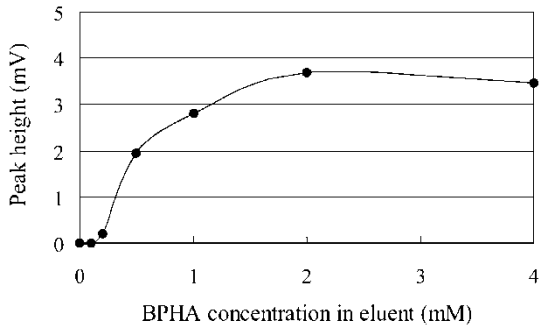

Figure 1. Effect of BPHA concentration in eluent on peak height of Fe-BPHA chelate.

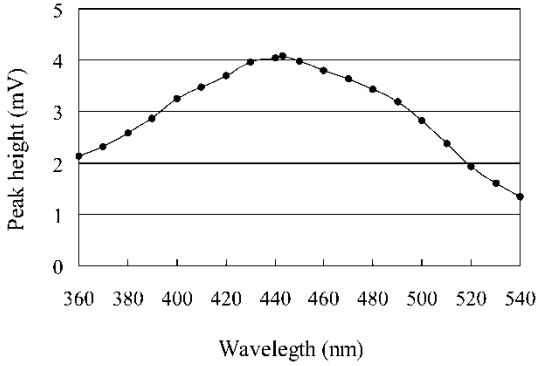

Figure 2. Effect of detection wavelength on peak height of Fe-BPHA chelate.

range of 2–10 μL. Because a 10 μL injection gave base line drift in front of the Fe chelate peak, 5 μL was selected as the optimum injection volume. The base line drift was probably caused by the large amount of free BPHA.

Extraction Conditions

In order to extract Fe ion quantitatively into MIBK as BPHA chelate, extraction conditions must be optimized. Thus, the extraction pH and shaking time were investigated according to the recommended procedure. The effect of pH on extraction of Fe-BPHA chelate is shown in Figure 3. Since constant and maximum peak areas were obtained over the pH range of 2.3 to 3.9, pH 3.0 ± 0.5 was employed as the optimum pH. This pH is easily controlled by addition of 2 mL of 2 M acetic acid/2 M sodium acetate buffer solution (pH 4.0) to a 5 mL sample solution of pH 1.

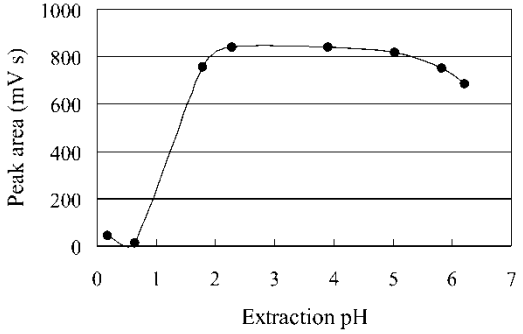

Figure 3. Effect of extraction pH on extraction of Fe(III) ion. The pH was measured after shaking and phase separation.

Because Fe(III) ion was quantitatively extracted into MIBK by shaking for 5–30 min, 10 min was selected.

The extracted Fe-BPHA chelate in MIBK solution was stable for at least 7 hours, because the peak area of Fe chelate did not change. Thus, immediate injection of the organic layer was not required.

Calibration Curves, Detection Limit, and Reproducibility

Calibration curves for Fe ion were prepared with Fe standards of varying concentrations by the recommended procedure. Correlation coefficients of the calibration curves were more than 0.999 over the concentration range of 10 ppb to 10 ppm, as shown in Table 2. A typical calibration curve is shown in Figure 4b.

The detection limit of Fe ion in a 5 mL solution was 2 ppb, which corresponded to 3 times the standard deviation of the blank peak area. Relative standard deviations (RSDs) of the peak area ($N = 5$) for 5, 0.5, and 0.05 ppm Fe standards were 0.9, 0.8, and 0.9%, respectively. It is noted that the excellent values of RSDs were obtained by a manual injection procedure.

Effect of Foreign Ions

The effects of 55 foreign ions on the determination of 0.5-ppm Fe(III) ion (5 mL) were investigated. Table 3 shows that 41 metal ions did not interfere at 400 times or more the concentration of Fe ion. Sn(II) ion of 5 ppm, 2-ppm Ti(IV), and 1-ppm V(V) interfered with the determination of 0.5 ppm Fe ion. The V ion was partly extracted into MIBK as BPHA chelate and the peak overlapped with the Fe chelate peak. More details are shown in Table 3.

Determination of Fe Ion in River Water and Recovery Tests by HPLC and ICP-AES

The concentration of Fe ion in river water was determined according to the recommended procedure. The Fe concentration was 0.334 ppm. To the river water, 0.5 ppm Fe was added, and the total Fe concentration was measured at 0.835 ppm. Thus, the recovery of the Fe ion was 100.1%. Similar experiments were carried out with ICP-AES. Fe concentration in the same river water was 0.329 ppm, and recovery of 0.5 ppm Fe was 102.7%. The Fe concentration and recovery of 0.5 ppm Fe, obtained by HPLC from another day, were 0.330 ppm and 102.0%, respectively. The agreement of the results obtained by HPLC and ICP-AES indicated that the BPHA reacted

Table 2. Calibration curves for Fe(III) ion and reproducibilities of peak areas.

Calibration curves for Fe ion			
Concentration range	Equation of line	Correlation coefficient	Measuring point (ppm)
1–10 ppm	$y = 18.373x - 1.392$[a]	0.9998	0, 1, 2, 4, 6, 8, 10
0.1–1 ppm	$y = 140.27x - 0.3001$[b]	0.9998	0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0
0.01–0.1 ppm	$y = 298.8x + 1.0741$[c]	0.9992	0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.10

Reproducibilities of peak areas (mV sec)					
5 ppm Fe Range: 0.16 AUFS		0.5 ppm Fe Range: 0.02 AUFS		0.05 ppm Fe Range: 0.01 AUFS	
No. of run	Peak area	No. of run	Peak area	No. of run	Peak area
1	91.025	1	69.631	1	14.962
2	89.579	2	68.908	2	14.675
3	91.175	3	69.811	3	14.574
4	89.943	4	69.810	4	14.864
5	91.485	5	70.506	5	14.848
Average	90.641	Average	69.733	Average	14.785
SD[d]	0.83	SD[d]	0.57	SD[d]	0.16
RSD[e] (%)	0.92	RSD[e]	0.82	RSD[e]	0.90

y: peak area (mV sec), x: concentration of Fe ion (ppm).
Detector range (AUFS): [a]0.16, [b] 0.02, [c] 0.01.
[d]Standard deviation.
[e]Relative standard deviation.

with all Fe ion species in the river water sample. More details are shown in Table 4.

We think that the preparation of a calibration curve every day is not required, because the difference between the two equations of the calibration curves in Table 4 was negligible.

Determination of Fe in Bovine Liver

According to the certified value of NIST, standard reference material bovine liver contains Fe at $194 \pm 20\,\mu g/g$. When Fe in 200 mg of the sample was

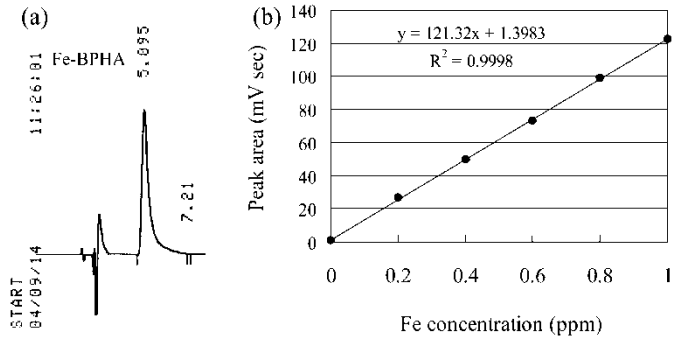

Figure 4. Typical chromatogram of Fe-BPHA chelate (a) and calibration curve (b). Concentration of Fe ion shown in a 5 mL standard solution.

resolved in 50 mL solution, the concentration of Fe ion was calculated to 0.776 ppm. Thus, the calibration curve of 0.1 – 1.0 ppm was prepared for Fe determination in bovine liver (Figure 4b). Though a smaller amount of the sample could be used, 200 mg was used considering the homogeneity of the sample.

Table 3. Effects of foreign metal ions on determination of 0.5-ppm Fe(III) ion

Tolerance limit	Metal ion
200 ppm	As(III), Al(III), Bi(III), Ca(II), Cd(II), Ce(III), Co(II), Cr(VI), Cs(I), Cu(II), Dy(III), Eu(III), Er(III), Ga(III), Gd(III), Ge(IV), Hg(II), Ho(III), In(III), K(I), La(III), Lu(III), Mg(II), Mn(II), Na(I), Ni(II), Nd(III), Pb(II), Pd(II), Pr(III), Pt(IV), Rh(III), Si(IV), Se(IV), Sm(III), Sr(II), Tb(III), Tl(I), Tm(III), Yb(III), Zn(II)
100 ppm	Ba(II), Y(III)
50 ppm	Ag(I), Sb(III), W(VI)
20 ppm	Au(III), Be(II), Sc(III), Zr(IV)
10 ppm	Mo(VI), Te(IV)
5 ppm	Sn(II)
2 ppm	Ti(IV)
1 ppm	V(V)

Note: The tolerance limit value of the foreign ion concentration was taken as the value that caused an error of less than 10% in the recovery of Fe(III) ion (0.5 ppm).

Table 4. Results of Fe(III) ion determination in river water and recovery tests.

No. of run	Sample[a] (ppm)	Added (ppm)	Found (ppm)	Recovery (%)[b]	Equation of calibration curve	Correlation coefficient
Result 1						
1	0.334	0.500	0.842	101.6	y = 140.27x − 0.3001	0.9998
2	0.338	0.500	0.833	99.8		
3	0.330	0.500	0.837	100.6	y: peak area (mV sec)	
4	0.331	0.500	0.829	99.0	x: Fe conc. (ppm)	
5		0.500	0.834	100.0		
Average	0.334	0.500	0.835	100.2		
SD[a]	0.004		0.005	1.0		
RSD[b]	1.15		0.60	1.0		
Result 2						
1	0.328	0.500	0.859	105.1	y = 142.6x − 0.2635	0.9997
2	0.332	0.500	0.849	103.0		
3	0.325	0.500	0.846	102.3	y: peak area (mV sec)	
4	0.334	0.500	0.822	97.6	x: Fe conc. (ppm)	
Average	0.330	0.500	0.844	102.0		
SD[a]	0.004		0.016	3.2		
RSD[b]	1.21		1.88	3.1		

Flow rate of eluent: 1.0 mL/min.
[a]Sample: Fe concentration in river water.
[b]Recovery (%): (Found-Sample) × 100/0.5, average value of sample was used.

The wet ashing procedure with nitric and perchloric acids was investigated, and we agreed with the recommended procedure described above. It was found that a heating procedure was required to resolve the Fe completely. When 0.1 or 2 M HCl was added to the bovine liver ash without heating, very low results were obtained compared with the certified value.

The analytical chromatogram of Fe in bovine liver is shown in Figure 4a. The analytical results obtained by the recommended procedure agreed with the certified value of the NIST (Table 5). Two results obtained on another day (Result 1 and 2) showed good agreement with the certified value.

Table 5. Analytical results of Fe in Bovine Liver

No. of run	Found (μg/g)	Certified value (μg/g)	Accuracy (%)[a]	Equation of calibration curve	Correlation coefficient
Result 1					
1	194.6	194 ± 20	100.3	y = 121.32x + 1.3983	0.9999
2	194.7	194 ± 20	100.4		
3	190.0	194 ± 20	98.0	y: peak area (mV sec)	
4	187.8	194 ± 20	96.8	x: Fe conc. (ppm)	
5	198.8	194 ± 20	102.5		
Average	193.1	194 ± 20	99.6		
SD[b]	4.3		2.2		
RSD[c]	2.2		2.2		
Result 2					
1	191.6	194 ± 20	98.8	y = 121.32x + 1.3983	0.9999
2	190.4	194 ± 20	98.2		
3	191.0	194 ± 20	98.5	y: peak area (mV sec)	
4	193.7	194 ± 20	99.8	x: Fe conc. (ppm)	
5	192.9	194 ± 20	99.4		
6	194.8	194 ± 20	100.4		
Average	192.4	194 ± 20	99.2		
SD[b]	1.7		0.9		
RSD[c]	0.9		0.9		

[a]Accuracy (%) = (Found/Certified value) × 100.
[b]Standard deviation.
[c]Relative standard deviation (%).

CONCLUSION

A conventional RP-HPLC apparatus equipped with a popular photometric detector can carry out this method. The proposed extraction and HPLC procedure is simple and easy. Shaking time for extraction is 10 min, and analysis time by HPLC is 8 min. In addition, the presented method for Fe analysis does not use chlorinated solvents for extraction or HPLC separation. When a 5 mL solution was used for analysis, calibration curves were linear between 10 ppb to 10 ppm with correlation coefficient of more than 0.999,

and the detection limit was 2 ppb. The HPLC method was applied to the determination of Fe in river water and standard bovine liver samples with precise results.

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Received November 22, 2004
Accepted December 29, 2004
Manuscript 6533