

# Application of the 1,2-Dihydroxybenzene-3,5-Disulfonic Acid for Separation and Preconcentration of Trace Iron and Determination by Eight Order Derivative Spectrophotometry

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**ABSTRACT:** Iron is quantitatively retained on an adsorbent made by coprecipitation of 1,2-dihydroxybenzene-3,5-disulfonic acid (Tiron) and tetradecyldimethylbenzylammonium chloride (TDBA) on microcrystalline naphthalene as a slurry or packed in a column, in the pH range 6.0-9.0 from a large volumes of aqueous solutions (up to 200 ml on microcrystalline naphthalene and up to 500 ml of aqueous solutions in the case of column method) of various samples. After filtration, the solid mass consisting of iron complex and naphthalene is dissolved in 5 ml of dimethylformamide and the metal determined by eight derivative spectrophotometry. Alternatively iron can be quantitatively adsorbed on Tiron-TDBA-naphthalene adsorbent packed in a column at a flow rate of 0.5 to 5.0 ml/min and determined similarly. About 0.05 µg of iron can be concentrated in a column from 500 ml of aqueous sample, where its concentration is as low as 0.1 ng/ml. Various parameters such as the effect of pH, volume of aqueous phase and interference of a number of metal ions on the determination of iron have been studied in detail to optimize the conditions for its determination in standard alloys and biological samples.

**KEY WORDS:** Trace iron determination, Eight derivative spectrophotometry, Tiron-TDBA-naphthalene

## INTRODUCTION

Numerous quaternary ammonium salts such as cetyltrimethylammonium chloride, trioctylmethylammonium chloride, and tetradecyldimethylbenzylammonium chloride (TDBA) have been widely used as the counter ions of anionic metal complexes or metal chelates in the solvent extraction spectrophotometric determination of

metals [1,2]. The use of high molecular weight amines provides high sensitivity and selectivity.

1,2-Dihydroxybenzene-3,5-disulfonic acid (Tiron) is a well-known chelating agent for the colorimetric determination of titanium [3], molybdenum [4], niobium [5], vanadium [6], etc. This reagent has two sulfonic

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acid groups and reacts with various metal ions to form anionic chelates. The metal chelate anions form water-insoluble complexes with quaternary ammonium salts like TDBA which can be extracted into nonaqueous solvents. Matsuo *et al.* [7] have reported the spectrometric determination of iron with SCN in the presence of TDBA. The sensitivity and selectivity of the method are greatly enhanced over the conventional thiocyanate method while interference due to foreign ions is also diminished. However, some of the organic solvents are partly miscible with water thereby, limiting the degree of the concentration of the metal ion into the organic solvent. The proposed reagent is fairly sensitive and more selective than most of the reported reagents in the literature for the estimation of iron [8-12].

Derivative spectrophotometry offers the advantages of increased selectivity and sensitivity compared with normal spectrophotometry [13-20]. Spectrophotometers equipped with suitable derivative units enable not only the derivative spectra of the first and second orders to be obtained but also higher orders.

The increased selectivity in derivative spectrophotometry results from the fact that bands which overlap in the normal absorption spectra appear as separated bands in the derivative spectra. Derivative spectrophotometry can increase the sensitivity owing to the amplification of derivative signals and lowering of noise, easier improvements in selectivity and sensitivity in cases where the bands in the normal absorption spectra are fairly sharp [21-22].

A survey of the literature reveals that metal ions may be preconcentrated on various adsorbents such as thiol cotton [23] silanized glass beads [24], silica gel [25], Amberlite XAD-4 resin [26], cellulose [27], green tea leaves [28], polythioether foams [29], C<sub>18</sub> column [30] and organic solvent-soluble membrane filters [31]. Some of these adsorbents may be fairly effective for preconcentration of metal ions, but their methods of preparation are lengthy and involve severe control of the operational conditions. The desorption of the metal is carried out by the slow process of elution (the metal complex is probably held by the interior surfaces of the adsorbent

and thus the adsorbed complex is not easily eluted), hence the procedure is time consuming. Solvent extraction is a simple and convenient technique for separating and concentrating metal ions, but it can not be applied directly to metal ions which form stable complexes with the chelating agents only at elevated temperatures. Solid-liquid separation after adsorption of metal chelates on microcrystalline naphthalene is more rapid and convenient and can be applied to different types of metal complexes [32,33]. The preconcentration of iron ion is also possible by passing its aqueous solution over naphthalene-Tiron-TDBA adsorbent packed in a column. The microcrystalline naphthalene method is rapid but the column method gives a better preconcentration factor [18,19].

## EXPERIMENTAL

### Apparatus

A Shimadzu UV 160 spectrophotometer with 1.0 cm quartz cell were used. A Beckman pH meter was employed for pH measurements. A funnel-tipped glass tube (60 mm × 6 mm) was used as a column for preconcentration. It was plugged with polypropylene fibers and then filled with the adsorbent to a height of 1.0 - 1.2 cm after pressing lightly with a flat glass rod. All atomic absorption measurements were made with an atomic absorption spectrometer (Shimadzu AA 670). All glassware were washed with a mixture of concentrated sulphuric and nitric acids (1+1) before use.

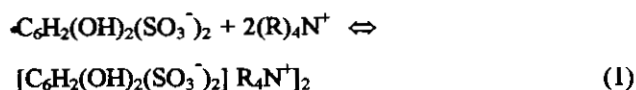
### Reagents

All the reagents were of analytical reagent grade. Standard iron solution (1000 ppm) was prepared by dissolving 0.723 g of Fe(NO<sub>3</sub>)<sub>3</sub> · 9H<sub>2</sub>O in 100 ml of 0.3 M nitric acid. A working solution (3 ppm) was prepared by appropriate dilution. This solution was standardized directly using an atomic absorption spectrometric standard iron solution. A 1% solution of tetradecyldimethylbenzylammonium chloride (TDBA) and 0.2% 1,2-dihydroxybenzene-3,5-disulfonic acid (Tiron) were prepared in distilled water. Buffer solutions of pH 3 - 6, 6 - 8 and 8 - 11 were prepared by mixing appropriate ratios

of a 0.5 M acetic acid and 0.5 M sodium acetate, 0.1 M; sodium dihydrogen phosphate and 0.1 M dipotassium hydrogen phosphate; and 0.5 M ammonia and 0.5 M ammonium acetate solution, respectively. A 20% solution of naphthalene was prepared in acetone. Solutions of alkali metal salts (1%) and various metal salts (0.1%) were used for studying the interference of anions and cations respectively.

#### Retention characteristics of Tiron-TDBA

The TDBA chloride is soluble in water and is used as a counter-cation for the metal complex or metal chelate anions in the solvent extraction of metals [34]. Tiron has two sulfonic acid group capable of dissociation and thus it can form ion pairs (Tiron : TDBA = 1: 2) with quaternary ammonium cations as follows:



The ion pair formed between the Tiron anion and TDBA<sup>+</sup> cation is easily coprecipitated with microcrystalline naphthalene. The ion-pair reaction between Tiron and several well known quaternary ammonium salts were therefore tried in order to develop new adsorbents. Preliminary results indicated that only TDBA<sup>+</sup> and cetyltrimethylammonium (CTMA<sup>+</sup>) can form ion pairs with Tiron. In this study, TDBA<sup>+</sup> was selected as the counter ion as it is cheaper than CTMA<sup>+</sup> and has excellent retention characteristics for metal ions.

#### Reaction conditions

These were established with the use of 4 µg iron. The adsorption of iron on this adsorbent was found to be a maximum in the pH range 6.0 - 9.0. In subsequent studies, the pH was maintained at approximately 7.5. Addition of 0.2-3 ml of the buffer did not affect the retention of iron and the use of 1 ml was chosen. Various amounts of 0.2% solution of Tiron were tried. Iron was quantitatively adsorbed on the adsorbent in the range 0.4 - 4.0 ml of the reagent. Therefore, 2.0 ml of the reagent was used in the present study.

Various amounts of naphthalene (20% solution of naphthalene in acetone) were added to the sample solu-

tions keeping other variables constant. It was observed that the signal height remained constant with the addition of 1.0 - 4.0 ml of 20% naphthalene solution. Therefore, 2.0 ml of 20% naphthalene solution was used in subsequent studies. The effect of shaking time on the adsorption indicated that the signal height remained constant over a range of 0.5-5.0 min. Therefore, 1.0 min of shaking time was maintained in the present work.

In the case of column method, the flow rate was varied from 0.5 to 8 ml/min. It was found that a flow rate of 0.5 - 5.0 ml/min did not affect adsorption. A flow rate of 1 ml/min was chosen in all experiments.

The volume of the aqueous phase was varied in the range of 10 - 700 ml under the optimum conditions, keeping other variables constant. It was observed that the signal height was almost constant up to 200 ml (preconcentration factor of 40). However, for convenience, all the experiments were carried out with 40 ml of the aqueous phase. Whereas, in the case of column method, signal height was almost constant up to an aqueous phase volume of 500 ml. Therefore, a preconcentration factor of 100 can be achieved by the column.

#### Choice of solvent

A number of solvents were examined to dissolve the metal complex along with naphthalene. Since the solid mass is dissolved in a small volume (3 - 5 ml) of solvent, it is essential to select a solvent in which the chelate is highly soluble and also sensitive for UV-vis spectrophotometric measurements. The solid material is insoluble in ordinary organic solvents such as toluene, 1, 2-dichloroethane, n-hexane, nitrobenzene, isoamyl alcohol, n-amyl alcohol, ethyl acetate, methyl isobutyl ketone, chloroform and dioxane, but soluble in dimethyl sulfoxide, dimethylformamide (DMF), and propylene carbonate. DMF was preferred because of the high solubility and stability of metal complex on naphthalene. It was found that 2 - 3 ml of this solvent was sufficient to dissolve the entire mixture, thus further enhancing the sensitivity of the method. Since only a small volume (3 - 5 ml) of the solvent is required to dissolve the solid mass, it was essential to study the effect of the excess water attached to the adsorbent. It was found that the

excess water caused 10-12% decrease in absorbance leading to an error in the determination. Thus, it was necessary to eliminate the water attached to naphthalene in the column completely by aspirating the column for 2-3 min.

#### *Retention capacity of the adsorbent*

The retention capacity of adsorbent was determined by a batch method. The experiment was performed by adding 500  $\mu\text{g}$  of iron, 1 ml of the buffer (pH 7.5) and 40 ml of water in a beaker. This solution was transferred a separatory funnel and a suitable amount of the adsorbent naphthalene-Tiron-TDBA was added. The separatory funnel was vigorously shaken on a mechanical shaker for 5 min. The solid mass was separated by filtration and iron was quantitated from the filtrate by FAAS. The solid mass on the filter paper was dried in an oven, kept in a desiccator, and weighed to determine the mass of the adsorbent. The maximum amount of iron retained was 5.5 mg/g of the adsorbent. It was also found that the retention capacity depends on the amounts of TDBA and Tiron supported on the naphthalene. From the stoichiometry in the above reaction, the molar ratio of Tiron to the TDBA is 1:2. From the observations on the preparation of the adsorbent, it was found that the use of Tiron (1.5-2.0 g) and TDBA (2.5-4.0 g) for 20 g of naphthalene was adequate for the complete retention of the metal ions. In the present work, 1.5 g of Tiron and 3.6 g of TDBA were used for convenience.

#### *Preparation of Tiron-TDBA-naphthalene adsorbent*

A solution of naphthalene was prepared by dissolving 20 g of naphthalene in 40 ml of acetone on a hot plate-stirrer at approximately 35 °C. This solution was transferred into 1400 ml of distilled water containing 3.5 g (0.0098 moles) of TDBA under constant stirring at room temperature, and to it was added a 500 ml solution of Tiron (1.5 g, 0.0048 moles). The naphthalene mixture coprecipitate (adsorbent) was stirred for a few hours and allowed to stand overnight. It was drained off by decantation and the mixture washed twice with distilled water

in the same way. The slurry of adsorbent in water was stored in a bottle.

#### *General procedure for microcrystalline naphthalene*

Sample of iron solution (up to 200 ml of containing 0.20 - 75  $\mu\text{g}$ ) was placed in an Erlenmeyer flask with tightly fitted stopper and to this were added 1.0 ml of 0.5% potassium sodium tartrate solution, followed by 2 ml of 0.2% of the reagent (Tiron). The pH was adjusted to 7.5 with 1.0 ml of the phosphate buffer solution, and 2 ml of 1% TDBA solution was added. The solution was mixed well and allowed to stand for a few seconds. Then, 2 ml of a 20% solution of naphthalene in acetone was added while continuous shaking. The solid mass thus formed consisting of naphthalene and the metal complex was separated by filtration on a Whatman filter paper (No.1041). The residue was dried in the folds of a filter paper and transferred to the Erlenmeyer flask. The solid mass consisting of the metal complex along with naphthalene was dissolved in 5 ml of dimethylformamide (DMF). Finally, the eight derivative absorption spectra was recorded in the range 450 - 650 nm against a blank solution prepared in the same way.

#### *General procedure for the column method*

Sample solution (up to 500 ml) containing 0.20 - 75  $\mu\text{g}$  of iron was taken in a beaker and to this were added 1.0 ml of 0.5% potassium sodium tartrate solution. The pH was adjusted to 7.5 with 1.0 ml of the phosphate buffer solution. The column loaded with the adsorbent Tiron-TDBA-naphthalene was conditioned to pH 7.5 by passing 2-5 ml of the buffer solution at a flow rate of 1 ml  $\text{min}^{-1}$ . The iron sample solution prepared above was then passed at a flow rate of 1 ml  $\text{min}^{-1}$ . The packing in the column was washed with a few milliliters of distilled water and then aspirated strongly for 2-3 min, pushing down the solid mass in the column with a glass rod in order to eliminate the excess water attached to the adsorbent. The metal complex was removed from the column along with naphthalene with 5 ml of DMF. The eight order derivative absorption spectra in the range

450-650 nm were recorded against a blank solution prepared in the same way. The signal was measured between  $\lambda_3 = 484$  nm and  $\lambda_2 = 520$  nm. A calibration curve was prepared by taking various known amounts of iron under the conditions given above.

## RESULTS AND DISCUSSION

### Spectrophotometric measurements

The zero order (normal spectrophotometric) and eight order derivative spectra of the complex are shown in Figs. 1 and 2. As can be seen, the higher wavelength peaks of the derivative spectra are more significant. The sensitivity of the eighth order derivative is much higher than that of the zero order (normal). The eighth derivatization leads to sharper zero order bands (normal spectrophotometric bands) and gives higher signals in the resulting spectra. The characteristics of derivative spectra, such as peak height and noise level, depend on the choice of parameters such as order of derivative, scan speed and integration time during recording of the spectra. The optimum parameters were chosen from preliminary experiments. The best results were obtained from the eighth order derivative spectra due to high signals at  $\lambda_1$ ,  $\lambda_2$  and  $\lambda_3$  with a length interval of 9 nm. The amount of iron can be determined by measuring the signal between the baseline and the corresponding peaks. It could also be determined from the signal between  $\lambda_1$

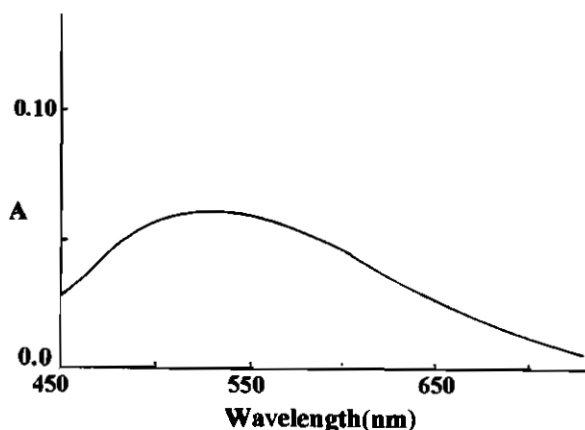


Fig. 1: Zero order spectrum of the naphthalene-Fe(III)-Tiron-TDBA complex. Fe(III), 4  $\mu$ g; buffer, 1 ml; pH, 7.5; Tiron, 2 ml (0.2%); solvent, 5 ml DMF; reference, reagent blank.

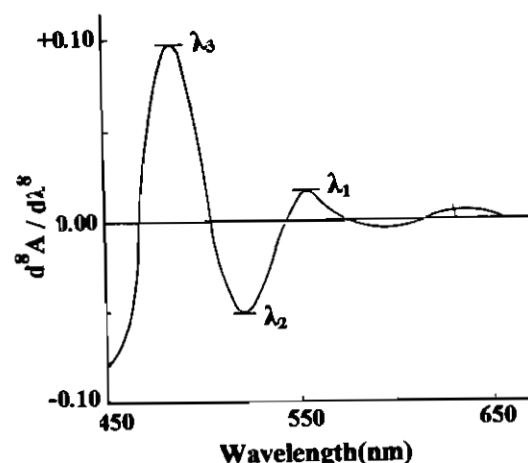


Fig. 2: Eighth derivative spectrum of the naphthalene-Fe(III)-Tiron-TDBA complex. For conditions, see Fig. 1.

and  $\lambda_2$  or  $\lambda_3$  and  $\lambda_2$ . In the present work, a peak-to-peak method between  $\lambda_3=484$  nm and  $\lambda_2=520$  nm was applied.

### Calibration, sensitivity, precision and stability

Considering that it is possible to retain 0.05  $\mu$ g of iron from 500 ml of solution passed through the column, the dissolution with 5.0 ml DMF gives a detection limit of 0.1 ng/ml for iron at the minimum instrumental settings. The linearity was maintained in the concentration range of 0.4 ng/ml to 7.5  $\mu$ g/ml iron in aqueous solution or 0.04 to 15  $\mu$ g/ml iron in final DMF solution with a correlation factor of 0.9996. Eight replicate determinations of 4  $\mu$ g of iron in 5 ml DMF solution gave a mean intensity in the eighth derivative spectrum, measured from the peak-to-peak signal between  $\lambda_3$  and  $\lambda_2$ , of 0.147 with a relative standard deviation of  $\pm 1.2\%$ . The sensitivity was 0.174 ( $d^8A/dnm^8$ )ml/ $\mu$ g from the slope of the calibration curve. The solution was stable for more than 8 days.

### Interference of foreign ions

Various salts and metal ions were added individually to a solution containing 4  $\mu$ g of iron and the general procedure was applied. The tolerance limit (error < 3%) is given in Table 1. Among the salts examined, most did not interfere in the range mg-g level. Among the metal ions studied, many did not interfere even at the milli-

Table 1: Effect of foreign ions

Salt or Ion	Tolerance Limit
CH <sub>3</sub> COONa.3H <sub>2</sub> O, KNO <sub>3</sub> , L-ascorbic acid	1 g
KSCN, Na <sub>2</sub> SO <sub>4</sub>	400 mg
KClO <sub>4</sub> , NaCl, Thiourea	350 mg
Sodium tartrate	200 mg
Sodium citrate	50 mg
Sodium oxalate	8 mg
Zn(II), Hg(II), Pb(II)	2 mg
Cd(II), Ca(II), Al(III), Mn(II)	3 mg
Cr(III), V(V), Mg(II), Co(II)	1 mg
Ni(II), Sn(II), Zr(IV), Pd(II), Ru(III), Cu(II)	500 µg
W(VI), Mo(VI), Rh(III), Ti(IV)	200 µg
	100 µg

*Fe(III): 4 µg, pH: 7.5, Tiron: 2 ml of 0.2%.*

gram levels. Thus the method is highly selective without the use of any masking agents. The proposed procedure has therefore been applied to the determination of iron in alloys and biological samples without any prior separations.

#### Analysis of iron in standard alloys

The proposed method has been applied to the determination of iron in Nippon Keikinzoku Kogyo (NNK) 916; NNK 3A30 Aluminum Alloys; NNK 1021 Al, Si, Cu, Zn Alloy; and National Institute of Standards Technology and Standard Reference Materials NIST, SRM 929 Zinc Alloy; NIST SRM 94C Zn-Base Die Casting Alloy; NIST SRM 627 Zn-Base Alloy CE and NIST SRM 627 Zn-Base Alloy E. A 0.1 g sample of each standard alloy was completely dissolved in 4 - 5 ml of hydrochloric acid (1+1) by heating on a water-bath and then 1 ml of 30% hydrogen peroxide was added to the solution. The excess of peroxide was decomposed by heating the solution on a water-bath. The solution was cooled and filtered, if needed, and the filtrate diluted to 100 ml with doubly distilled water in a calibrated flask. An aliquot (1 - 2 ml) of this solution was taken and iron was determined by the general procedure (Table 2).

#### Analysis of iron in standard biological samples

The proposed method has been applied to the determination of iron in National Institute for Environmental studies (NIES) No. 1 Pepperbush; NIES, No. 3 Chlorella; NIES, No. 5 Human Hair; NIES, No. 6 Mussels and NIES, No. 2 Pond Sediment. Pepperbush or chlorella or pond sediment (0.1 g) or mussel (0.5 g) was taken individually in a beaker and dissolved in concentration nitric acid (5 ml) with heating. The solution was cooled, diluted and filtered. The filtrate was made to 100 ml with distilled water in a calibrated flask. An aliquot (1-2 ml) of the sample solution was taken individually and iron was determined by the general procedure. The results are given in Table 3 and as can be seen they are in good agreement with their certified specifications.

#### CONCLUSIONS

A simple, economical and highly selective adsorbent was obtained simply by mixing the aqueous solutions of Tiron and TDBA<sup>+</sup> Cl<sup>-</sup> with an acetone solution of naphthalene and applied in the preconcentration of iron from a large volume of the aqueous solutions of various samples like alloys, steel and those of biological origin. As Tiron reacts with several metal ions, similar procedure may also be developed for them. The reagent is cheap and highly, selective for iron and the use of eighth derivative spectrophotometry further increases the sensitivity and selectivity of the method. Iron may also be determined by direct aspiration of the DMF solution of the metal complex (after the preconcentration steps) into the flame of an AAS instrument or by pulse polarography. Although adsorption onto the microcrystalline naphthalene is more rapid, the preconcentration factor is comparatively lower than the column method.

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Table 2: Analysis of iron in standard alloys

Sample	Composition %	Concentration of Iron (%)	
		Certified Value	found* <sup>+</sup>
NKK 916 Aluminum Alloy	Si, 0.41; Cu, 0.27; Mn, 0.11; Mg, 0.10; Cr, 0.05; Ni, 0.06; Zn, 0.30; Ti, 0.10; Sn, 0.05; Pb, 0.04; V, 0.02; Zr, 0.05; Bi, 0.03; Co, 0.03; Sb, 0.01; B, 0.0006	0.54	0.53±0.02 <sup>b</sup>
NKK 3A30 Aluminum Alloy	Si, 9.96; Cu, 0.059; Mn, 0.042; Mg, 0.01; Zn, 0.043; Ni, 0.042; Ti, 0.038; Sn, 0.038	0.633	0.635±0.014 <sup>b</sup>
NIST SRM 629 Zinc Alloy	Cu, 1.50; Al, 5.15; Mg, 0.094; Pb, 0.0135; Cd, 0.0155; Sn, 0.012; Cr, 0.0008; Mn, 0.0017; Ni, 0.0075; Si, 0.078	0.017	0.018±0.002 <sup>a</sup>
NKK No. 1021 Al, Si, Cu, Zn Alloy	Pb, 0.18; Ni, 0.14; V, 0.007; Zn, 1.76; Sn, 0.10; Cu, 2.72; Mg, 0.29; Cr, 0.03; Mn, 0.20; Ti, 0.04; Si, 5.56; Sb, 0.01; Zr, 0.01; Bi, 0.01; Ca, 0.004	0.99	0.96±0.04 <sup>b</sup>
NIST SRM 94C Zn-Base Die Casting Alloy	Ni, 0.006; Mn, 0.014; Mg, 0.042; Sn, 0.006; Al, 4.13; Cu, 1.10; C, 0.002; Pb, 0.006;	0.018	0.019±0.001 <sup>a</sup>
NIST SRM 627 Zn-Base Alloy CE	Mg, 0.030; Sn, 0.0042; Cr, 0.0038; Mn, 0.014; Ni, 0.0029; Si, 0.021; Al, 3.88; Si, 0.021; Cd, 0.005; Cu, 0.132	0.0023	0.0021±0.0002 <sup>a</sup>
NIST SRM 627 Zn-Base Alloy E	Mg, 0.094; Sn, 0.012; Cr, 0.0008; Mn, 0.017; Ni, 0.0075; Si, 0.078; Al, 5.15; Cu, 0.50; Pb, 0.0135; Cd, 0.015	0.17	0.16±0.01 <sup>b</sup>

\* Average of five determinations, ± standard deviation

+ Standard addition method was applied.

a) Column method was applied.

b) Microcrystalline naphthalene method was applied.

Table 3: Analysis of iron in biological samples

Sample	Composition	Concentration ( $\mu\text{g/g}$ )	
		Certified Value	Found <sup>*†</sup>
NIES, No. 1 Pepperbush	K, $1.51\pm 0.06$ ; Mn, $0.203\pm 0.107$ ; Mg, $0.408\pm 0.020$ ; Ca, $1.38\pm 0.07\%$ ; Cd, $6.7\pm 0.5$ ; Ni, $8.7\pm 0.6$ ; Cu, $12\pm 1$ ; Co, $23\pm 3$ ; Pb, $5.5\pm 0.8$ ; Zn, $340\pm 20$ ; Rb, $75\pm 4$ ; Ba, $165\pm 10$ ; Na, $106\pm 13$ ; Sr, $36\pm 4$ ; As, $2.3\pm 0.3$ ; P, (1100); Cr, (1.3); Cs, (1.2); Tl, (0.13); Hg, (0.056) $\mu\text{g/g}$	$205\pm 17$	$201\pm 6^b$
NIES, No. 3 Chlorella	K, $1.24\pm 0.06$ ; Ca, $0.49\pm 0.03$ ; Mg, $0.33\pm 0.02$ ; P, (1.7)% Zn, $20.5\pm 1.0$ ; Sr, $40\pm 3$ ; Co, $0.87\pm 0.05$ ; Mn, $69\pm 5$ ; Cu, $3.5\pm 0.3$ ; Cd, (0.026); Pb, (0.60); Sc, (0.013) $\mu\text{g/g}$	$0.185\pm 0.010^a$	$0.182\pm 0.008^{a,c}$
NIES, No. 5 Human Hair	Pb, 6.0; Cd, 0.20; Sb, 0.07; Zn, 169; Al, 240; Cu, 16.3; Mg, 208; Hg, 4.4; K, 34; Rb, 0.19; Sc, 0.05; Se, 1.4; Na, 26; Sr, 2.3; Ti, 3.2; Ca, 728; Cr, 1.4; Ba, 2.2; Ni, 1.8 $\mu\text{g/g}$	225	$227\pm 5^b$
NIES, No. 6 Mussels	Na, $1.00\pm 0.03$ ; K, $0.54\pm 0.02$ ; Ca, $0.13\pm 0.01$ ; Mg, $0.21\pm 0.01$ ; P, (0.77) % Zn, $106\pm 6$ ; As, $9.2\pm 0.5$ ; Ni, $0.93\pm 0.06$ ; Pb, $0.91\pm 0.04$ ; Cd, $0.82\pm 0.03$ ; Cr, $0.63\pm 0.07$ ; Ag, $0.027\pm 0.003$ ; Cu, $4.9\pm 0.3$ ; Mn, $16.3\pm 1.2$ ; Al, (220); Sr, (17); Se, (1.5); Co, (0.37); Hg, (0.05) $\mu\text{g/g}$	$158\pm 8$	$155\pm 4^b$
NIES, No. 2 Pond Sediment	Al, $10.6\pm 0.5$ ; Ca, 0.81; K, 0.68; Na, 0.57 % Zn, 343; Pb, 105; Cr, 75; Ni, 40; Cu, 210; Co, 27; As, 12; Cd, 0.82 $\mu\text{g/g}$	$6.53\pm 0.35^a$	$6.48\pm 0.08^{a,c}$

\* Average of five determinations,  $\pm$  standard deviation, a) %

+ Standard addition method was applied.

NIES: National Institute of Environmental Studies reference materials.

NIES, No. 1, No. 3 & No. 6 (Values in parentheses were approximate and not certified).

b) Column method was applied.

c) Microcrystalline naphthalene method was applied.



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