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A highly selective and simple spectrophotometric method for the determination of zinc at nano-trace levels in some environmental, biological, food, and pharmaceutical samples using 2-hydroxynaphthaldehydebenzoylhydrazone

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RESEARCH ARTICLE

KEYWORDS

ABSTRACT

A simple, ultra-sensitive, and highly selective spectrophotometric method has been established for the trace quantification of zinc (Zn), using 2-hydroxynaphthaldehyde benzoylhydrazone (HNABH). Zn forms a pale yellowish-green complex (maximum absorption at 426 nm) with HNABH (1:1, *v:v*) in a marginally acidic solution (0.00005-0.00023 M H_2SO_4). The average molar absorption coefficient and Sandell's sensitivity were found: 2.87×10^5 L/mol.cm and 12 ng/cm^2 of Zn, respectively. The observed linearity range for Zn was 0.01-50 mg/L with a detection limit of 1 μg/L. The analysis of biological, food, and vegetable samples using the suggested method were found to be in tremendous accord with those acquired by Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The method has high precision and accuracy (s = ±0.01 for 0.5 mg/L). The limit of quantification of the proposed method was 10 μg/L.

1. Introduction

Zinc is an essential micronutrient that plays a vital role in human growth [1]. It is vital for the metabolic action of a significant number of the body's protein. It is also important for our cell division, immune system, DNA, and RNA synthesis [2]. Thus, a balanced diet with the recommended level of zinc should be ensured to overcome any deficiency syndrome related to zinc. Sometimes malignant conditions and diabetes can result in zinc deficiency in the human body [3]. Zinc deficiency can lead to several clinical illnesses. Dysfunction in the immune system, neurological disorder, pneumonia, and growth retardation are closely related to zinc deficiency. Zinc is found in the human body at a different level depending on the age and gender of the people [4]. Zinc is not abundantly present in all sorts of foodstuffs. Animal protein contains much bioavailable zinc than different vegetable sources [5]. When consumed at a high level it leads to zinc toxicity. Thus, zinc quantification in a trace and ultra-trace level in the biological, environmental, and food samples is of optimal importance.

Atomic absorption spectroscopy (AAS), spectrofluorimetry, and inductively coupled plasma spectroscopy (ICP) are presently being used for quantification of Zn [6-9]. These are very sophisticated machines which include continuous operating costs. Especially, inductively coupled plasma-optical emission spectroscopy (ICP-OES) and inductively coupled plasma-mass spectroscopy (ICP-MS) involve much complex operating condition. One the other hand, spectrophotometric analysis is for a routine check of a metal that is very simple and quick [10,11]. It is also very accurate and assesses metal concent-ration in a versatile amount of sample in a quick manner [12]. Thus, among all the analytical techniques spectrophotometry is one of the most efficient and rapidly used techniques for trace analysis. Spectrophotometric analysis is quick and simple for metal quantification in several environmental samples [13-16].

In this study, we developed a very simple method for the quantification of zinc using a spectrophotometer. We used 2-hydroxynaphthaldehydebenzoylhydrazone (HNABH), a ligand for trace determination of zinc using a spectrophotometer with a high degree of sensitivity and selectivity. The ligand HNABH reacts with zinc in a mild acidic condition (0.00005-0.00023 M H_2SO_4) forming a pale-yellowish green complex that can be directly measured spectrophotometrically with a high degree of selectivity for Zn. Optimization of the method was done in such a way that the method is suitable for using at room temperature. The standard procedure is relatively simpler and less laborious.
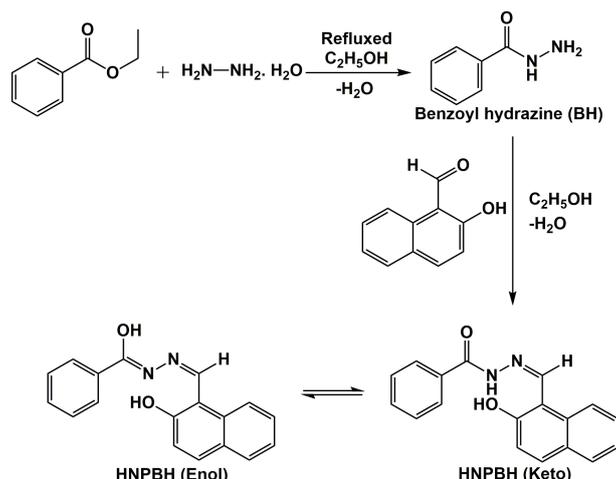
Scheme 1. Synthesis of 2-hydroxynaphthaldehydebenzoylhydrazone (HNABH).

The method was tested with standard reference materials, synthetic mixtures, and with several real samples: water, blood, urine, soil, milk, and pharmaceuticals. These samples were from different locations of the Chittagong, Bangladesh and were collected to check the applicability of the proposed method to determine the amount of Zn accurately. The aim of the study is not to assess the current environmental situation but to show the feasibility of the proposed method to monitor Zn in a certain sample with complex matrix. The results were compared with the atomic absorption spectroscopy, inductively coupled plasma-optical emission spectroscopy and with the claimed values in some products.

2. Experimental

2.1. Apparatus

A Shimadzu (Kyoto, Japan) (Model-1800) double beam UV/VIS spectrophotometer was used for the measurements of absorbance. A Shimadzu (Model: AA7000) atomic absorption spectrophotometer equipped with a microcomputer-controlled air-acetylene flame and a Shimadzu (Japan) (Model: 9800) Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), (λ = 418 nm, plasma gas flow rate (L/min) = 15, LOD: below 1 µg/L of Mo, RF Power (W) = 1400, Nebulizer gas flow rate (L/min) = 1-10) were used for comparison of the results was used for the comparison of the results. An FT-IR spectrophotometer, Shimadzu (Model-IR Prestige 21) was used to record the infrared spectrum.

2.2. Synthesis of the reagent

The reagent 2-hydroxynaphthaldehydebenzoylhydrazone (HNPBH) was synthesized in two steps according to the method of Sacconi and Salam [17,18]. At first, ethyl benzoate (700 mmol) reacts with hydrazine hydrate (700 mmol) at 140 °C to give benzoyl hydrazine (BH). The reaction takes roughly 20 h to complete. The melting point of the product was 115 °C. A mixture of 1:1 molar ratio of 2-hydroxynaphthaldehyde (HNP) and the synthesized benzoyl hydrazine (BH) was used for the preparation of the reagent 2-hydroxynaphthaldehydebenzoyl hydrazone with a reaction yield of 80%. The reaction is represented in Scheme 1. The melting point of the reagent was 204 °C [17]. The findings of the elemental analysis (C = 70.10%, O = 10.05 %, N = 9.70 %, H = 5.06 %) of the reagent was in excellent agreement with the expected values (C = 74.45 %, O = 11.03 %, N = 9.65 %, H = 4.83 %). From the FT-IR spectrum of

HNPBH, the peak at 1622.20 cm^{-1} was due to the characteristic C=N double bond peak ($v_{C=N}$, 1590-1660 cm^{-1}) [19].

2.3. Reagents and solutions

Analytical grade reagents of all the chemicals were used without further purification. Ethanol (HPLC grade), doubly distilled deionized water was used throughout the analysis. Certified Reference Materials (CRMs) of alloys, steels, and brass for zinc were obtained from the National Research Council Canada (https://nrc.canada.ca/en/certifications-evaluations-standards/certified-reference-materials/contact-us-crm) and CRMs of the bovine liver, human serum, and hair were obtained from the National Research Council Canada.

2.4. HNABH (3.9×10^{-3} M) and Zn standard solution (1.53×10^{-2} M)

A requisite amount of HNABH was dissolved in a fixed volume of distilled absolute ethanol to prepare the reagent solution. Before any analysis, a newly prepared reagent solution (1×10^{-4} M) was used. 399.88 mg of zinc from zinc nitrate tetrahydrate (ZnNO$_3$.4H$_2$O) was dissolved in doubly distilled deionized water to prepare a stock solution of volume 100 mL (1 mg/mL). The standardization of the solution was done with EDTA using Eriochrome Black T as an indicator. Before any analysis, the required solution was prepared from this standard stock solution.

2.5. General procedure

0.1-500 µg of zinc in a 10 mL volumetric flask was mixed with a 1:10 to 1:20 fold molar excess (preferably 1 mL of 3.45×10^{-3} M) of 2-hydroxynaphthaldehydebenzoylhydrazone (HNABH). About 1.5 mL of 0.0005 M sulfuric acid was added to the solution and mixed well. After 1 min, 3 mL of ethanol was added to the former solution. The mixture was made 10 mL with the addition of deionized water. The absorbance was measured at 426 nm against a corresponding reagent blank. A simultaneously prepared calibration curve was used to determine Zn from any unknown solution.

2.6. Sample collection and preservation

Polythene bottles were used to collect water samples from several locations of the Chittagong area, Bangladesh, and HNO$_3$ (1 mL/L) were employed as a preservative.

Table 1. Summary of selected analytical parameters obtained with optimization experiments.

Parameters	Studied value	Selected value
Wavelength, λ_{max} (nm)	200-800	426
Solvent (mL)	0 - 6	2.5 - 4 (preferably 3)
H_2SO_4 (M)	0.000005-0.005	0.00005-0.00023 (preferably 0.00005)
pH	3.5 - 6	5 (preferably)
Temperature (ºC)	10 - 90	25±5
Reagent (fold molar excess, M:R)	1:1 - 1:50	1:10 - 1:20 (preferably 1:10)
Linear range (mg/L)	0.001-100	0.01-50
Molar absorptivity (L/mol.cm)	$1.06×10^6$ - $1.23×10^5$	$2.87×10^5$
Detection limit (µg/L)	0 - 100	1.0
Sandell's sensitivity (ng/cm²)	0 - 100	12
Reproducibility (% RSD)	0 - 5	0 - 2
Regression coefficient, R^2	0.9987-0.9997	0.9998
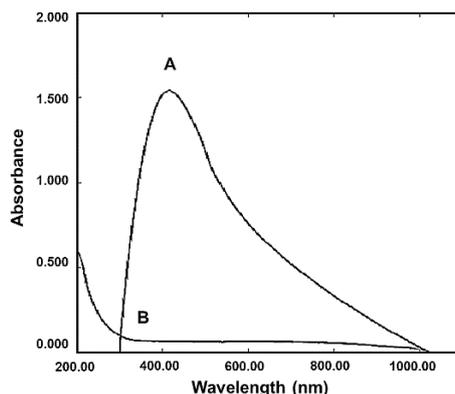
Figure 1. A and B absorbance spectra of the Zn-HNABH and the reagent blank (λ_{max} = 426 nm) in aqueous solutions.

Polypropylene bottles were used to collect blood and urine samples from the patient of Treatment Centre, Center for Specialized Care and Research (CSCR) Hospital, and Chittagong Medical College Hospital, Bangladesh with proper concern to the authorities. The samples were stored at -20 °C. Soil (surface) samples were collected from different locations in the Chittagong region, Bangladesh which includes the coastal area near the Bay of Bengal, Chittagong, University of Chittagong campus, and several industries in Chittagong region. Air-dried soil samples were homogenized with a ceramic mortar. Leotsinidis's method was employed for the sampling of breast milk [20]. Manually expressed 20 mL of milk was poured into a plastic container and frozen at -20°C. Infant formula samples of different major brands were from the local market in Chittagong with preparation described by Hua *et al.* [20].

3. Results and discussion

3.1. Optimization of some parameters on the absorbance

3.1.1. Effect of solvent

The absorption spectra of a Zn-HNABH have a maximum absorbance at 426 nm at 25±5 °C and average molar absorptivity of $2.87×10^5$ L/mol.cm (Figure 1). The absorbance remains unchanged for over 24 h. An earlier report illustrates the reaction mechanism of the current method [21]. A decline in the absorbance was observed outside that temperature range. HNABH is poorly water-soluble. A (30±2%) (*v*:*v*) ethanol/water medium generated the maximum absorbance. When the solvent effect was checked, 20-60% (2-6 mL) ethanol delivered a steady-maximum absorbance of the Zn-HNABH system. For all successive measurements, 30% ethanol was added.

3.1.2. Effect of acidity and reagent concentration

Several acids (H_2SO_4, H_3PO_4, HCl and HNO_3) with a variant concentration were tested for the maximum absorbance of the

system and H_2SO_4 was best among them. Those acids were tested for gaining the highest absorbance and the consistency of the absorbance value. From the test, it is evident that beginning with 0.5 mL of 0.0005 M H_2SO_4 the absorbance value increased and was almost plateaued until 2.25 mL of the acid. The decrease in the absorbance at lower acidity may be due to a lower possibility of complex formation of the ligand with the present amount Zn in the solution. Thus, a range of acid volume was good enough to produce higher and persistent absorbance of the complex. As a result, for all the following analyses 1.5 mL of 0.0005 M sulfuric acid was added. Several amounts of HNABH were checked against a definite metal concentration and a reagent molar ratio of 1:10 to 1:20 delivered a constant and maximum absorbance of Zn complex. 1 mL of $3.9×10^{-3}$ M HNABH reagent was added for all later analysis.

3.1.3. Calibration graph and composition of the complex

0.01-100 mg/L (broken into four sets: 0.01-0.1, 0.1-1.0, 1.0-10, and 10.0-100.0 mg/L) of the metal was analyzed using the optimum condition. The linear range was obtained for 0.01-50 mg/L of Zn. The final calibration curve with the limit of the linearity is given in Figure 2. The molar absorption coefficient and the Sandell's sensitivity were observed to be $2.87×10^5$ L/mol.cm and 12 ng/cm² of Zn, respectively. The parameters selected for the experiments are summarized in Table 1. The detection limit of the method was found to be 1.0 µg/L. The limit of quantification of the proposed method was 10 µg/L. Job's method [21] of continuous variation indicted a Zn - HNABH (1:1) complex with the reagent (Figure 3). The molar ratio method [22] was also applied to ascertain the stoichiometric composition of the complex. Both methods yielded similar results. The probable structure is given in Scheme 2.

3.1.4. Effect of foreign ions

50 ions and complexing agents were checked for any influence on the determination of 1 mg/L of Zn.

Table 2. Effect of interfering radicals.

Species x	Tolerance ratio, x/Zn (w/w)	Species x	Tolerance ratio, x/Zn (w/w)
Aluminum b	100	Lead(II)	50
Arsenic(III)	50	Magnesium	100
Antimony	100	Mercury(II)	100
Azide	100	Molybdenum(VI)	100
Bismuth(III)	100	Manganese(II), (VII)	100
Bromide	100	Nickel(II)	80
Barium	100	Nitrate	100
Cadmium	100	Oxalate	100
Cobalt(II)	100	Phosphate	100
Cobalt(III)	100	Potassium	100
Calcium	80	Selenium(IV)	50
Chloride	100	Selenium(VI)	50
Citrate	100	Strontium	50
Chromium(VI)	100	Sulphate	100
Chromium(III)	80	Sodium	100
Cesium	100	Tartrate	1000
Copper(II) a	100	Tin(II)	100
Cerium(III)	20	Tin(IV)	100
EDTA	1000	Titanium(IV)	100
Fluoride	100	Tellurium(IV)	50
Iron(II) c	100	Thiocyanate	100
Iron(III)	100	Tungsten(VI)	50
Iodide	100	Vanadium(V)	100
Lithium	80	Uranium	100

[a] With 10 mg/L EDTA.
[b] With 10 mg/L tartrate.
[c] With 10 mg/L 1,10-phenonthroline.
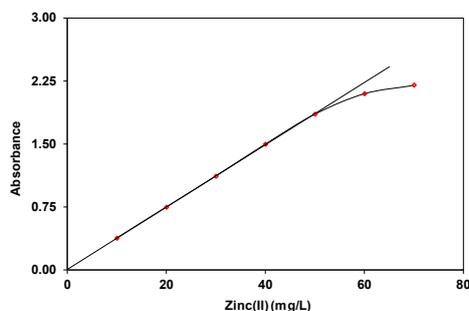
Figure 2. Calibration graph of 10-50 mg/L of Zn.
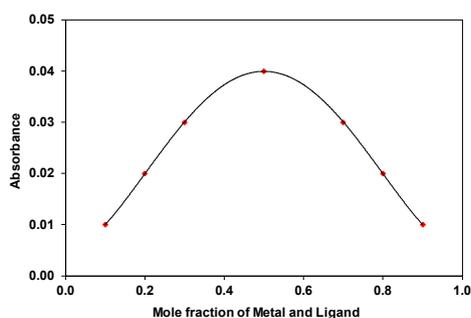
Figure 3. Job's method for determining the composition of the Zn: HNABH (1:1) complex.
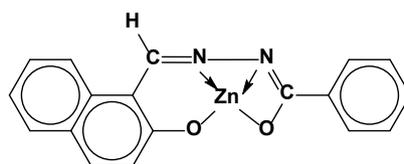
Scheme 2. Probable structure of [Zn(HNABH)] complex.

The ion present in the solution is taken as interfering when the absorbance value deviated by more than 5% of the expected value for only Zn [23]. The results are summarized in Table 2. Most of the ions did not show any substantial interference to the quantification of Zn. Cu(II), Al, and Fe(II) ions interfered with the Zn determination to some extent, probably due to some complex formation with the reagent. Masking agents have been used to eliminate any interference to those ions: EDTA for Cu(II), Al by tartrate, and finally, 1,10-phenanthroline was used to mask Fe(II).

Table 3. Determination of Zn in some synthetic mixtures.

Mixture	Composition of mixtures (mg/L)	Zn (mg/L)		
		Added	Found [a] (n=5)	Recovery±SD [b] (%)
A	Zn	0.50	0.50	100±0.00
		1.00	0.99	99±0.6
B	As in A + Na + Cd + Ce³⁺ + Li	0.50	0.49	98±0.8
		1.00	1.00	100±0.00
C	As in B + K + Mn²⁺ + Cr³⁺ + As³⁺ + EDTA (50)	0.50	0.51	102±0.9
		1.00	0.99	99±1.0
D	As in C + Hg²⁺ + Ca +Ni + Sn²⁺ + EDTA (50)	0.50	0.52	102±1.0
		1.00	1.03	106±1.2
E	As in D + Mg + Mn(VII) + Ag + Sr + EDTA (50)	0.50	0.53	106±1.5
		1.00	1.05	105±1.6
F	As in E + Sb³⁺ + Se³⁺ + Te⁴⁺	0.50	0.54	108±1.8
		1.00	1.08	108±2.0

[a] Average of five analysis of each sample.
[b] The measure of precision is the standard deviation (SD).

Table 4. Determination of Zn in some certified reference materials.

Certified reference materials, (Composition, %)	Zn (%)		
	Certified value	Found (n=5)	RSD [a] (%)
BAS-CRM-10g : High tensile brass (Cu=60.8, Fe=1.56, Pb=0.23; Ni=0.16, Sn=0.21, Al=3.34, Zn=32.0, Mn=0.12)	32.00	31.92	1.35
BAS-CRM-5g : Brass (Cu=67.4, Sn=1.09, Pb=2.23, Zn=28.6 and Ni=0.33)	28.60	28.35	1.85
CRM-Brass-5f : Brass (Cu=70.8,Zn=24.2, Sn=1.84, Fe=0.31, Ni=0.17 and Mn=0.12)	24.20	24.10	2.00
CRM-NIST-1640 : Trace metals in water (Al=105, As=89, Cd=105, Cr=95, Zn=101, Cu=102, Fe=105, Mn=101, Pb=104)	101.00	100.50	2.20
CRM-GHENT-SERUM : Human serum [b]	4.20	4.12	1.80
NIST-SRM-1577b : Bovin liver [c]	6.50	6.47	2.10
CRM-BCR-397 : Human hair [c]	2.50	2.48	2.00

[a] The measure of precision is the relative standard deviation (RSD).
[b] Values in mg/L.
[c] Values in mg/kg.

Table 5. Determination of Zn in some surface soil.

Sample source [c]	Zinc (mg/kg) (n=5) [a]	RSD [b] (%)
Marine soil (*Bay of Bengal, Chittagong, Bangladesh*)	2.05±0.5	1.8
Agricultural Soil (*University of Chittagong campus*)	3.60±1.0	1.5
Industrial Soil (*Bangladesh Welding Electrodes Ltd., Sreerampur, Sutipara, Dhaka*)	155.0±1.0	2.0
Industrial soil (*PHP Steels Ltd. Bara Kumira, Chittagong, Bangladesh*)	146.0±1.2	2.1
Industrial soil (*Elite Paint and Chemical Industries Ltd, Bayezid Bostami Road, Chittagong*)	120.5±1.5	2.5
Industrial soil (*T.S.P. Complex Ltd., Patenga , Chittagong*)	95.6±1.8	2.6
Industrial soil (*Eastern Refinery, North Patenga, Chittagong*)	79.5±1.0	2.3
Industrial soil (*Transcom Beverages Ltd., Kalurghat Heavy Industrial Area, Chittagong*)	85.0±1.5	2.5
Industrial soil (*GlaxoSmithKline Bangladesh Limited, North Kattali, Chittagong*)	110.5±1.2	2.8
Estuarine Soil (*Halda River, Chittagong, Bangladesh*)	17.5±1.5	2.1
Roadside soil (*Chittagong-Dhaka Highway*)	7.9±0.5	1.8
Roadside soil (*New market, Chittagong*)	10.8±1.0	2.0

[a] Average of five replicate analysis of each sample.
[b] Measure of precision is the standard deviation±s.
[c] Composition of the soil samples: C, N, P, K, Na, Ca, Mg, Fe, Pb, Cu, Zn, Mn, Mo, Co, NO_3, NO_2, SO_4, etc.

3.2. Application of the proposed method

3.2.1. Synthetic mixtures and certified reference materials

A few mixtures of varying compositions comprising zinc and individual ions of known concentrations were analyzed by the present method utilizing EDTA or tartrate as a masking agent and precise results were achieved. The results are represented in Table 3. The "% recovery" using the method was excellent and it was between 98±0.8 to 108±2.0 for every synthetic mixture.

To validate the proposed method, brass, alloys, and some CRMs were analyzed. The sample solution preparation was done by a method recommended by Parker [23]. 1 mL of the solution was taken in a 10 mL volumetric flask and analyzed by the proposed method using EDTA or tartrate as a masking agent. The results obtained were excellent and summarized in Table 4. A total of 7 CRM samples were analyzed for the Zn content to validate the current method. The relative standard deviation (RSD) was very small in every analysis and it was between 1.35% to a maximum of 2.2%.

3.2.2. Soil and water

Marine, industrial, roadside, and riverine soil samples were taken from several areas of Chittagong and Dhaka, Bangladesh.

Air-dried soil samples (100 g) were digested using a method recommended by Hesse [24]. The digested solution was taken in a volumetric flask and measured by the proposed method using tartrate or EDTA as a masking agent. The results are shown in Table 5. The maximum Zn content (155.0±1.0 mg/kg) was found in industrial soil from Bangladesh Welding Electrodes Ltd., Sreerampur, Sutipara, Dhaka. The lowest amount Zn was obtained from marine soil from the Bay of Bengal, Chittagong, Bangladesh. It indicates that the Zn contamination has been done near the former industry and eventually lead to abnormally higher Zn content in the soil.

Water samples were prepared by a method proposed by Greenberg *et al.* [25]. 1 mL of the pre-concentrated water sample was transferred in a 10 mL volumetric flask and the zinc content was determined using tartrate or EDTA as a masking agent. The results of the Zn content in the water sample are summarized in Table 6. Several tap, well, pond, rainwater, river water, seawater, and drain water from different industries were analyzed. The standard addition method was used to check the % recovery of the samples. The recovery was excellent, and it was in between 100.00±0.0 to 102.75±0.7%. As expected, the highest Zn content (800.00 µg/L) was found in drain water from Bangladesh Welding Electrodes Ltd., Sreerampur, Sutipara, Dhaka.

Table 6. Determination of Zn in some environmental water samples.

Samples		Zinc (μg/L)		Recovery±s (%)	Sr [b] (%)
		Added	Found [a]		
Tap water		0	260.00		
		100	365.00	101.39±0.6	0.35
		500	770.00	101.32±0.5	0.32
Rainwater		0	45.00		
		100	145.00	100.00±0.0	0.00
		500	560.00	102.75±0.7	0.36
Pond water		0	150.00		
		100	260.00	104.00±1.0	0.29
		500	650.00	100.00±0.0	0.00
Well water		0	180.00		
		100	280.00	100.00±0.0	0.00
		500	690.00	101.47±0.3	0.25
River water	Karnaphuli (upper)	0	75.00		
		100	180.00	102.86±0.8	0.24
		500	585.00	101.74±0.9	0.28
	Karnaphuli (lower)	0	80.00		
		100	180.00	100.00±0.0	0.00
		500	590.00	101.72±0.5	0.19
	Halda (upper)	0	60.00		
		100	165.00	103.13±0.7	0.08
		500	570.00	101.79±0.8	0.15
	Halda (lower)	0	50.00		
		100	150.00	100.00±0.0	0.00
		500	560.00	101.82±1.0	0.21
Sea water	Bay of Bengal (upper)	0	25.00		
		100	130.00	104.00±0.8	0.45
		500	525.00	100.00±0.0	0.00
	Bay of Bengal (lower)	0	20.00		
		100	120.00	100.00±0.0	0.00
		500	530.00	101.92±0.6	0.35
Drain water	PHP Steels Ltd. [c]	0	550.00		
		100	650.00	100.00±0.0	0.00
		500	1060.00	100.95±0.6	0.30
	Eastern Refinary [d]	0	275.00		
		100	380.00	101.33±0.9	0.25
		500	775.00	100.00±0.0	0.00
	K.P.M. [e]	0	600.00		
		100	710.00	101.43±0.5	0.35
		500	1120.00	101.82±1.0	0.49
	Welding Industry [f]	0	800.00		
		100	910.00	101.11±1.5	0.29
		500	1330.00	102.31±1.8	0.39
	GSK Bangladesh Ltd. [g]	0	750.00		
		100	850.00	100.00±0.0	0.00
		500	1265.00	101.20±1.0	0.49

[a] Average of five replicate determinations of each sample.
[b] The measure precision is the relative standard deviation (S_r).
[c] PHP Steel Mill, Bara Kumira, Chittagong.
[d] Eastern Refinery, North Patenga, Chittagong.
[e] Karnaphuli Paper Mill, Chandraghona, Chittagong.
[f] Bangladesh Welding Electrodes Ltd., Sreerampur, Sutipara, Dhaka.
[g] GlaxoSmithKline Bangladesh Limited, North Kattali, Chittagong.

3.2.3. Biological and pharmaceutical samples

Digestion of the biological samples was done according to Ahmed *et al.* [26] 1 mL of the digested solution was poured into a 10 mL volumetric flask and the zinc content was determined using the proposed method. The blood and urine samples are taken from the hospital with prior permission from the respective patients and the hospital authority with full discloser of the analysis to be done. The blood and urine samples are taken from different patients and cannot be compared for the total Zn amount to the respective patient. The total amount of Zn in the patients' blood and urine was different due to the individual patient's condition. The variation in the result may also be due to abnormality due to the respective disease and other health conditions of the patient. The results are summarized in Table 7. Blood and urine samples from several patients and normal adults were taken and analyzed for Zn concentration. Blood samples contain higher Zn than the respective urine samples. Blood from liver cirrhosis patient contained the highest Zn with a concentration of 24.22 mg/L while a male normal adult contains only 4.18 mg/L in his blood.

The Zn content in the biological samples was very comparable to that found by atomic absorption spectroscopy.

Pharmaceutical samples were digested following a method recommended by Ahmed *et al.* [26]. 1 mL of the digested sample was taken into a 10 mL volumetric flask and Zn content was measured using the proposed method using tartrate as a masking agent. Tablet, syrup, ointment, and powder from several pharmaceutical brands were analyzed using the proposed method (Table 8). The Zn content found from the suggested method was comparable to the reported value.

3.2.4. Vegetable, food, and milk

The vegetable and food samples were digested using a method recommended by Stahr [27]. The digested sample was measured using a volumetric flask and the zinc content was determined as described using tartrate as a masking agent [28]. The Zn contents in the vegetable and food samples were compared with the conventional atomic absorption spectroscopy and the results were in excellent agreement (Table 9). The highest Zn content (10.9 mg/kg) was found in pumpkin seed and the lowest (2.8 mg/kg) in cabbage.

Table 7. Determination of Zn in some biological samples.

Sample source	Sample	Zinc (mg/L)					
		AAS (n=5)		Spectrophotometry (Proposed method) (n=5)		ICP-OES (n=5)	
		Found	RSD (%)	Found	RSD (%)	Found	RSD (%)
Normal adult (Male) (Non-smoker)	Blood	4.18	1.2	4.20	1.3	4.22	1.5
	Urine	1.32	1.0	1.51	1.0	1.52	1.0
Kidney disease patient (Male)	Blood	12.50	1.5	12.80	1.5	13.10	1.8
	Urine	3.20	1.2	3.30	1.2	3.50	1.1
Diarrhea patient (Male)	Blood	1.18	0.6	1.22	0.5	1.25	1.5
	Urine	0.25	0.2	0.51	0.8	0.78	0.9
Skin disease patient (Male)	Blood	1.74	1.2	1.80	1.5	1.82	1.6
	Urine	0.45	0.9	0.47	1.0	0.51	0.8
Liver cirrhosis patient (Male)	Blood	24.22	1.5	24.52	1.7	24.82	1.8
	Urine	6.21	1.0	6.32	1.0	6.35	1.2
Diabetic patient (Male)	Blood	13.39	1.6	13.45	1.8	13.52	1.8
	Urine	3.41	1.3	3.48	1.5	3.51	1.6
Pregnant woman	Blood	3.71	1.5	3.75	1.7	3.79	1.5
	Urine	0.92	1.0	0.95	1.2	0.97	1.0
Normal female adult (Non-smoker)	Human hair (Female)	1.35 [d]	1.0	1.56	1.1	1.49	1.2
Normal male adult (Non-smoker)	Human nail (Male)	2.50 [d]	1.5	2.80	1.8	2.90	2.0

[a] Samples were collected from Chittagong medical college hospital and Treatment hospital, Chittagong.
[b] Average of the five replicate determinations.
[c] Values in mg/kg.

Table 8. Determination of Zn in some pharmaceutical samples.

Brand name	Sample type	Composition of sample	Trade name	Zn (mg/kg or mg/L or µg/g)			RSD * (%)
				Reported value	Found (n = 5)	Recovery (%)	
Square	Tablet [a]	$ZnSO_4$: 10 mg/kg	ZDT-20	10.0	9.98	99.8±0.5	1.5
Beximco	Tablet [a]	$ZnSO_4$: 10 mg/kg	ZEDEX-20	10.0	9.97	99.7±0.8	1.6
Square	Syrup [b]	$ZnSO_4$: 10 mg/ 5 mL	ZESUP	2.0	1.99	99.5±1.0	1.8
Incepta	Syrup [b]	$ZnSO4$: 10 mg/ 5 mL	ZIFLU	2.0	2.00	100.0±0.00	1.0
Square	Ointment [a]	ZnO : 400 mg/kg	Nebanol	400.0	398.5	99.6±1.0	1.6
Incepta	Ointment [a]	ZnO : 400 mg/kg	Napguard	400.0	397.8	99.5±1.2	1.8
Medicon Lab.	Powder [c]	ZnO (20 µg)	Mebalon	20.0	19.95	99.8±1.5	2.0
Opsonin Pharma	Powder [c]	ZnO (20 µg)	Neocin	20.0	19.98	2.9± 1.0	2.5

* The measure of precision is the relative standard deviation.
[a] Values in mg/kg.
[b] Values in mg/L.
[c] Values in µg/g.

Table 9. Determination of Zn in some vegetable and food samples.

Sample	Zinc (mg/kg) Found [a]± s (n=5)			
	ICP-OES (n=5)		Proposed method (n=5)	
	Found	RSD [b]	Found	RSD [b]
Pumpkin Seed (*Cucurbita pepo*)	10.7	1.2	10.9	2.0
Carrot (*Daucus carota*)	12.5	1.5	12.8	1.8
Mushroom (*Agaricus bisporus*)	4.8	1.8	4.95	1.5
Peanut (*Arachis hypogaea*)	3.1	1.0	3.20	1.4
Lentils (*Lens culinaris*)	2.8	1.3	3.00	1.0
Potato (*Solanum tuberosum*)	7.5	1.5	7.8	1.8
Cabbage (*Brassica oleracea*)	2.6	1.7	2.8	1.6
Garlic (*Allium sativum*)	3.12	1.8	3.15	1.5
Brown rice (*Oryza sativa*)	5.15	2.1	5.25	2.1
Spinach (*Spinacia oleracea*)	6.5	2.5	6.62	2.3

[a] Average of five replicate analyses of each sample.
[b] The measure of precision is the relative standard deviation (RSD).

Table 10. Determination of Zn in some milk samples.

Milk samples * (with brand)	Zinc (mg/kg - mg/L)			
	Claimed value	Found	Recovery (%)	RSD (%)
Marks [a]	0.81±0.04	0.85±0.05	104±0.5	1.2
Arong [a]	0.84±0.03	0.88±0.02	105±0.8	1.5
Dano [a]	1.25±0.5	1.30±0.6	104±1.0	2.2
Nido [a]	0.95±0.01	0.98±0.03	103±0.6	2.0
Diploma [a]	1.00±0.05	1.05±0.08	105±0.9	2.1
Lactogen [a]	1.28±0.2	1.33±0.3	104±0.8	2.3
Cow milk [b]	1.75±0.3	1.81±0.5	103±1.0	2.5
Goat milk [b]	2.12±0.5	2.15±0.6	101±1.1	2.0
Human milk [b]	2.98±0.35	2.95±0.25	99±0.5	1.5
Horlics [a]	0.98±0.2	1.00±0.3	102±1.0	2.0

* Samples were collected from local market and hospital of Chittagong.
[a] Values in mg/kg.
[b] Values in mg/L.

The milk samples were prepared using a method proposed by Hua *et al.* [20]. 1 mL of the final milk solution was taken into a 10 mL volumetric flask and the zinc content was determined using tartrate as a masking agent by the proposed method. The results are shown in Table 10. Human breast milk contained the maximum amount (2.95±0.25 mg/L) of Zn content among all the other sample analyzed.

4. Conclusions

A novel, simple, highly selective, and a cost-effective method using Zn-HNABH complex was proposed for the detection of Zn metal ions. The method can ascertain Zn content in a versatile matrix with a high degree of accuracy and precision. This method has great potential for routine analysis of Zn where sophisticated and high-cost instruments are hard to obtain. Since the method can work at room temperature with a degree of accuracy it can be adopted very easily. This novel spectrophotometric method has advantages with almost no operating cost, easy to conduct immediately. Finally, the method will of great potential for the determination of Zn in versatile complex matrices with greater sensitivity and high selectivity.

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Disclosure statement ⓈⓈ

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