

Calibration of Shahid's Analytical Method for Adulterated Zn-EDTA Fertilizers by Ion Chromatography and Atomic Absorption Spectroscopy

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Summary: Chelated zinc fertilizers are usually recommended in calcareous alkaline soils to provide Zn nutrition in order to prevent possible Zn precipitation. In Punjab (Pakistan), Zn EDTA products are being manufactured, and marketed to meet the zinc requirement of various crops grown in Zn deficient soils. Under fertilizer control order, 1973 (amended), their quality has to be monitored by the Agriculture Department, Government of the Punjab. None of the traditional method was found suitable which can separate the mineral fraction from that of chelated adulterated fertilizer except for those methods based on ion chromatography. Calibration of ion chromatography method was carried out by determining the mineral Zn fraction leading to estimate remaining Zn.EDTA fraction in fertilizer samples of adulterated nature *i.e.* mixture of chelated and mineral fraction. In order to achieve the objective atomic absorption spectroscopy was coupled with ion chromatography. The method offers a specific, reliable technique for determination of chelated zinc in fertilizers. In the first step chelation was broken down with concentrated sulphuric acid treatment and total zinc contents were determined by atomic absorption spectroscopy. In second step, non-chelated (mineral) portion of zinc was determined by ion chromatography using cation column and conductivity detector. Chelated zinc was calculated by subtracting non-chelated (mineral) fraction from total zinc contents.

Introduction

Zinc solubility is highly pH dependent and decreases by a factor of 100 for each unit increase in pH [1]. The calcareous soils particularly exhibit zinc nutritional deficiency problem. Zinc adsorption by carbonates or precipitation of zinc as Zn(OH)₂ or ZnCO₃ is responsible for the unavailability of zinc in calcareous soils [2]. The deficiencies of micro-nutrients (Fe, Zn, Mn, B, Cu, and Mo) can be removed by the use of respective mineral fertilizer. However, chelates offer the highest nutrient use efficiency that bring the trace elements into a plant available form at relatively slow rate to the roots and shoots [3]. Different sources of nutrient Zn are mentioned in Table-1.

Most commonly used chelating agents are ethylene diamine tetraacetic acid (EDTA), 2-hydroxyethyl-ethylene diamine triacetic acid (HEEDTA), diethylene triamine pentaacetic acid

(DTPA), ethylenediamine-di-(2-hydroxyphenyl) acetic acid (EDDHA), ethylenediamine (*o*-hydroxy-*p*-methylphenylacetic) acid (EDDHMA) and ethylene diamine-di-(2-carboxy-5-hydroxyphenyl acetic) acid (EDDCHA). These all belong to the class of amino-carboxylic acids. The feature of controlling the concentration of free form of metal ions is fundamental while selecting the chelating agent. Best known is EDTA which is widely used [3]. Punjab Government fertilizer control order, 1973 (amended) regulates the manufacturing, and marketing of chelated zinc fertilizers through agriculture department.

Results and Discussion

In the analysis of cations through ion chromatography (IC) the retention time is considered a reasonably unique identifying characteristic and it

Table-1: Different sources of nutrient zinc.

Micronutrient	Commonly used fertilizers	Chemical formula	Nutrient contents (%)
Zinc	Zinc Sulphate	ZnSO ₄ .H ₂ O	35.0
		ZnSO ₄ .7H ₂ O	22.7
	Zinc chelates	Na ₂ ZnEDTA.4H ₂ O	13.8
		C ₁₀ H ₁₂ N ₂ Na ₂ O ₂ Zn.4H ₂ O	9.0

Source: Murphy, and Walsh [6].

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depends upon pump speed (*i.e.* mobile phase flow rate), concentration of element in analyte, nature of mobile phase and column packing material. Specifications of cation column used for analysis are presented in Table-2. Retention time of mineral zinc ranged from 9.79–10.32 minutes with a mean value of 10.14 and standard deviation of 0.17 minutes (Table-3).

Table-2: Specifications of Shodex cation column.

Specifications	Nomenclature
	Shodex IC YK-421
Column Size (I.D × Length)	4.6 mm × 125 mm
Theoretical plates	2500 min.
Packing material	Silica gel bounded with carboxylic group.
Max. Temp	60 C°
Max. Pressure	150 Kgcm ⁻²
Max. Flow rate	1.5 mLmin ⁻¹
Mobile phase limit (pH)	2 – 7
Mobile phase	1.5 mM Tartaric acid, and 1 mM Oxalic acid

Source : www.shodex.com/english/db0306.html

Compatibility of mobile phase was checked by running both the mineral zinc ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ M.W.= 287.54 g / mol, 22.7 % Zn) and chelated zinc standards ($\text{Na}_2\text{ZnEDTA} \cdot 4\text{H}_2\text{O}$, Zinc Titriplex GR, 471.63 g / mol, $\text{C}_{10}\text{H}_{12}\text{N}_2\text{Na}_2\text{O}_8\text{Zn} \cdot 4\text{H}_2\text{O}$, MERCK, 13.8% Zn). When mineral zinc standard was run through ion chromatography (IC) with different concentrations, peaks of zinc developed at specific mean retention time of 10.14 ± 0.17 minutes (Fig. 1). Peak area increased with direct proportion to the concentration of mineral zinc. Doubling the quantity of mineral zinc peak area also doubled indicating that IC is directly responding to the concentration of mineral zinc (Table-4 and Fig. 5) *e.g.* peak area of mineral zinc was 1480.3715 mVs which doubled to 2974.3410 mVs when Zn were doubled from 114 mgL^{-1} to 228 mgL^{-1} . To cross check the zinc concentration in mineral zinc standard, it was also analysed by atomic absorption spectroscopy (AAS) for total zinc contents and value was found 22.76 % Zn with standard deviation of 0.16 in the same way regarding IC results for mineral zinc standard, mean value was 22.93 % Zn with standard deviation of 0.30 (Table-6). Analysis results of zinc sulphate hepta or mono hydrate, either using AAS or IC, were more or less the same.

During chelated zinc ($\text{Na}_2\text{ZnEDTA} \cdot 4\text{H}_2\text{O}$) analysis by IC, no peak of mineral zinc was developed although peak of sodium was developed at

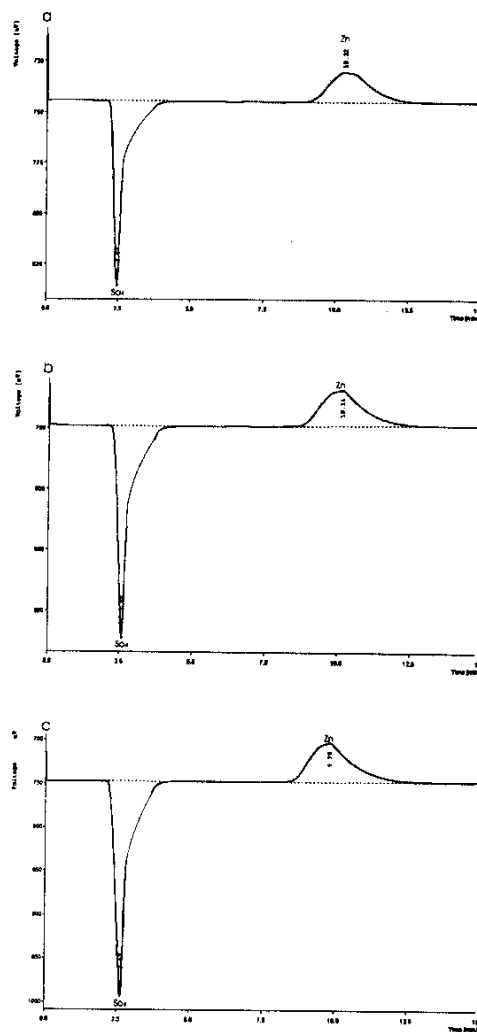


Fig. 1: Response of different concentrations of mineral Zinc ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ standard 22.7%) (a) Mineral Zinc 114, SO_4 168 mg/L (b) Mineral Zinc 228, SO_4 334 mg/L (c) Mineral Zinc 324, SO_4 504 mg/L.

Table-3: Range, mean, and standard deviation of retention time of cations by IC.

Element	Retention Time (minutes)							Range	Mean	S.D.	
Mineral Zn^{2+}	10.32	10.14	9.79	10.20	10.18	10.22	10.00	10.30	9.79-10.32	10.14	0.17
Na^+	7.26	7.28	7.33	7.21	7.15	7.11	7.32	7.23	7.11-7.33	7.24	0.08

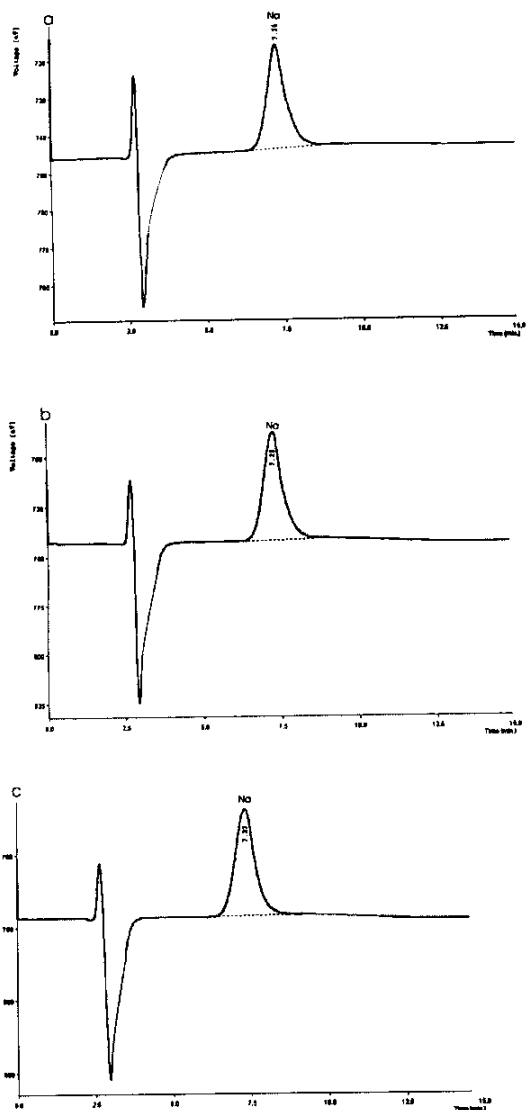


Fig. 2: Response of different concentrations of Na₂Zn EDTA 4H₂O (Chelated Zn Standard) (a) Na 50 mg/L (b) Na 90 mg/L (c) Na 148 mg/L.

mean specific retention time of 7.24 ± 0.08 minutes (Fig. 2, Table-3). For total zinc contents of chelated standard firstly digestion was performed using concentrated H₂SO₄ in digestion block (DK-20, Velp sceintifica). After suitable dilution it was aspirated through AAS (Varian SpectrAA 250 plus). Mean value calculated was 13.72 with standard deviation of 0.12 (Table-7).

Mixtures of chelated and mineral zinc standards in different weight based ratios were also prepared and were run through AAS and IC to determine the recovery percentage of both. Analytical results were compared with standard results

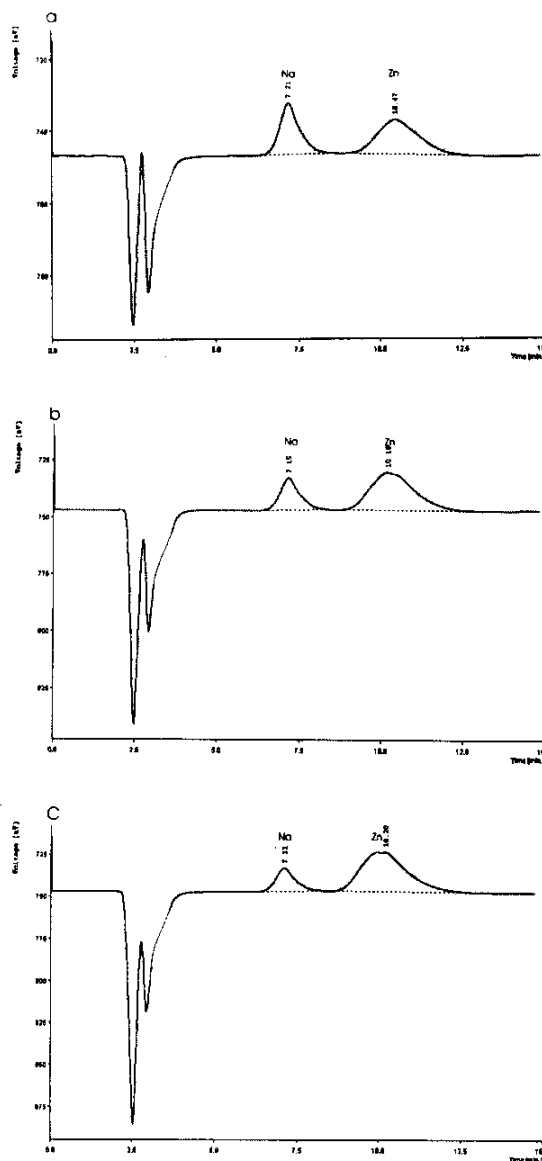


Fig. 3: Separation of Na and mineral Zn in mixtures of chelated and mineral Zn standards.(a) Mixture 1. Na 25, Zn 57 mg/L (b) Mixture 2. Na 25, Zn 114 mg/L (c) Mixture 3. Na 25, Zn 171 mg/L.

Table-4: Retention time, and concentration proportional peak area of cations.

Description	Cations	Calculated Concentration (mg L ⁻¹)	Retention Time (min)	Peak Area (mVs)
ZnSO ₄ .7H ₂ O (Standard 22.7%) 25mg/50 mL	Zn ²⁺	114	10.32	1480.3715
ZnSO ₄ .7H ₂ O (Standard 2.7 %) 50mg/50 mL	Zn ²⁺	228	10.14	2974.3410
ZnSO ₄ .7H ₂ O (Standard 22.7 %) 75mg/50 mL	Zn ²⁺	342	9.79	4539.1128
Na ₂ ZnEDTA.4H ₂ O (Standard 13.8%) 25mg/50 mL	Zn ²⁺ Na ⁺	70 50	-- 7.26	-- 1189.0006
Na ₂ ZnEDTA.4H ₂ O (Standard 13.8%) 50mg/50 mL	Zn ²⁺ Na ⁺	13.8 98	-- 7.28	-- 2363.5674
Na ₂ ZnEDTA.4H ₂ O (Standard 13.8%) 75mg/50 mL	Zn ²⁺ Na ⁺	210 148	-- 7.33	-- 3313.2167
Mixture-1	Zn ²⁺ Na ⁺	57 25	10.20 7.21	850.7984 604.8114
Mixture-2	Zn ²⁺ Na ⁺	114 25	10.18 7.15	1576.9814 586.7434
Mixture-3	Zn ²⁺ Na ⁺	171 25	10.22 7.11	2367.2860 580.9073
Mixture-4	Zn ²⁺ Na ⁺	57 50	10.00 7.32	882.8511 1177.5112
Mixture-5	Zn ²⁺ Na ⁺	57 75	10.30 7.23	915.4708 1775.5349

Table-5: Analytical results of mineral, chelated zinc standards and their mixtures.

Description	Analytical Results (%)			Standard Analysis (claimed %)	
	Total Zinc (AAS)	Mineral Zinc (EC.Detector Cation column)	Chelated Zinc (Total zinc minus Mineral zinc)	Mineral Zinc**	Chelated Zinc**
Mineral Zinc Standard	22.7	22.7	0	22.7	0
Zn.EDTA.4H ₂ O Standard	13.7	0	13.7	0	13.8
Mixture-1, 1:1* Chelated Zn:Mineral Zn	18.5	11.7	6.8	11.3***	6.9***
Mixture-2, 1:2* Chelated Zn:Mineral Zn	20.8	16.7	4.1	15.1	4.6
Mixture-3, 1:3* Chelated Zn:Mineral Zn	21.4	17.6	3.8	17.0	3.4
Mixture-4, 2:1* Chelated Zn:Mineral Zn	17.1	8.7	8.4	7.6	9.2
Mixture-5, 3:1* Chelated Zn:Mineral Zn	16.3	5.6	10.7	5.7	10.3
Unknown Sample -1	9.4	5.3	4.1	--	--
Unknown Sample -2	9.6	5.8	3.8	--	--
Unknown Sample -3	1.9	0	1.9	--	--

*Ratios on weight basis. ** Calculation on actual weight basis.

***Mixture-1, Mineral Zinc = 1/2 × 22.7 = 11.3 %, Chelated Zinc = 1/2 × 13.8 = 6.9 %

Table-6: Recovery percentage of total Zn by AAS and mineral Zn by IC.

Detail	Replicate	Atomic Absorption Spectroscopy (AAS)		Ion Chromatography (IC)	
		Analytical Results (Total Zinc %)	Zn Recovery (%)	Analytical Results (Mineral Zn %)	Zn Recovery (%)
ZnSO ₄ .7H ₂ O Standard 22.7 %	1	22.7	100.0	22.9	100.8
	2	23.0	101.3	23.3	102.6
	3	22.9	100.9	22.4	98.7
	4	22.6	99.5	23.0	101.3
	5	22.8	100.4	23.1	101.7
	6	22.6	99.5	22.9	100.9
Mean	--	22.76	--	22.93	--
Standard Deviation	--	0.16	--	0.30	--
ZnSO ₄ .7H ₂ O Standard 35.0 %	1	35.2	100.6	35.8	102.3
	2	34.9	99.7	35.2	100.6
	3	35.1	100.3	34.6	98.8
	4	35.2	100.6	35.1	100.3
	5	34.8	99.4	35.0	100.0
	6	34.9	99.7	35.3	100.8
Mean	--	35.02	--	35.16	--
Standard Deviation	--	0.17	--	0.39	--

(calculated on actual weight basis) and were found almost same (Table-5, Figs. 3 and 4).

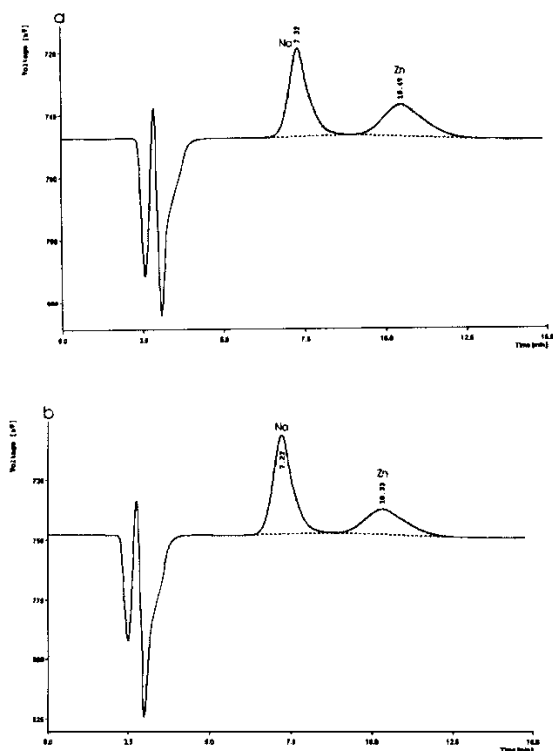


Fig. 4: Separation of Na and mineral Zn in mixtures of chelated and mineral Zn standards. (a) Mixture 4. Na 50, mineral Zn 57 mg/L (b) Mixture 5. Na 75, mineral Zn 57 mg/L.

Experimental

Principle

Mineral zinc fraction of an adulterated Zn.EDTA sample was separated by ion chromatography (IC) using suitable mobile phase, cation column and conductivity detector. Chelated zinc content was estimated by subtraction of mineral fraction from total Zn contents, determined by AAS.

Reagents and Solutions

High performance liquid chromatography (HPLC) grade solvents purchased from Merck were used for analytical purpose. These chemicals were degassed by using Perkin Elmer vacuum degasser series 200.

The choice of suitable mobile phase is vital in ion chromatography. Successful chromatographic separation depends upon differences in the interaction of solute with mobile phase and stationary phase. For relatively simple separations, it is often possible to use a single solvent for the whole analysis *i.e.* isocratic operation. Zinc was analyzed isocratically. Mobile phase (eluent) was prepared by dissolving 0.225 g of highly purified L-tartaric acid (1.5 mM, C₄H₆O₆) and 0.126 g of oxalic acid 2-hydrate (1 mM, (COOH)₂.2H₂O) into a 1 liter volumetric flask. Volume was raised upto the mark by de-ionized water. After dissolving the salts using ultrasonic vibrator (ultrasonic LC 30 H, Elma, Germany) filtration of eluent was done through vacuum filtration assembly with 0.45 μm pore size filter.

Table-7: Recovery percentage of total Zn by AAS and mineral Zn by IC.

Detail	Replicate	Atomic Absorption Spectroscopy (AAS)		Ion Chromatography (IC)	
		Analytical Results (Total Zinc %)	Zn Recovery (%)	Analytical Results (Mineral Zn %)	Zn Recovery (%)
ZnSO ₄ Solution 10.0 %	1	10.2	102.0	9.8	98.0
	2	10.1	101.0	10.7	107.0
	3	9.9	99.0	9.5	95.0
	4	10.3	103.0	9.6	96.0
	5	10.2	102.0	10.4	104.0
	6	9.7	97.0	10.2	102.0
Mean	--	10.06	--	10.03	--
Standard Deviation	--	0.22	--	0.48	--
Na ₂ ZnEDTA. 4H ₂ O Chelated Zinc Standard 13.8 %	1	13.7	99.3	0	0
	2	13.8	100.0	0	0
	3	13.6	98.5	0	0
	4	13.9	100.7	0	0
	5	13.7	99.3	0	0
	6	13.6	98.5	0	0
Mean	--	13.72	--	--	--
Standard Deviation	--	0.12	--	--	--

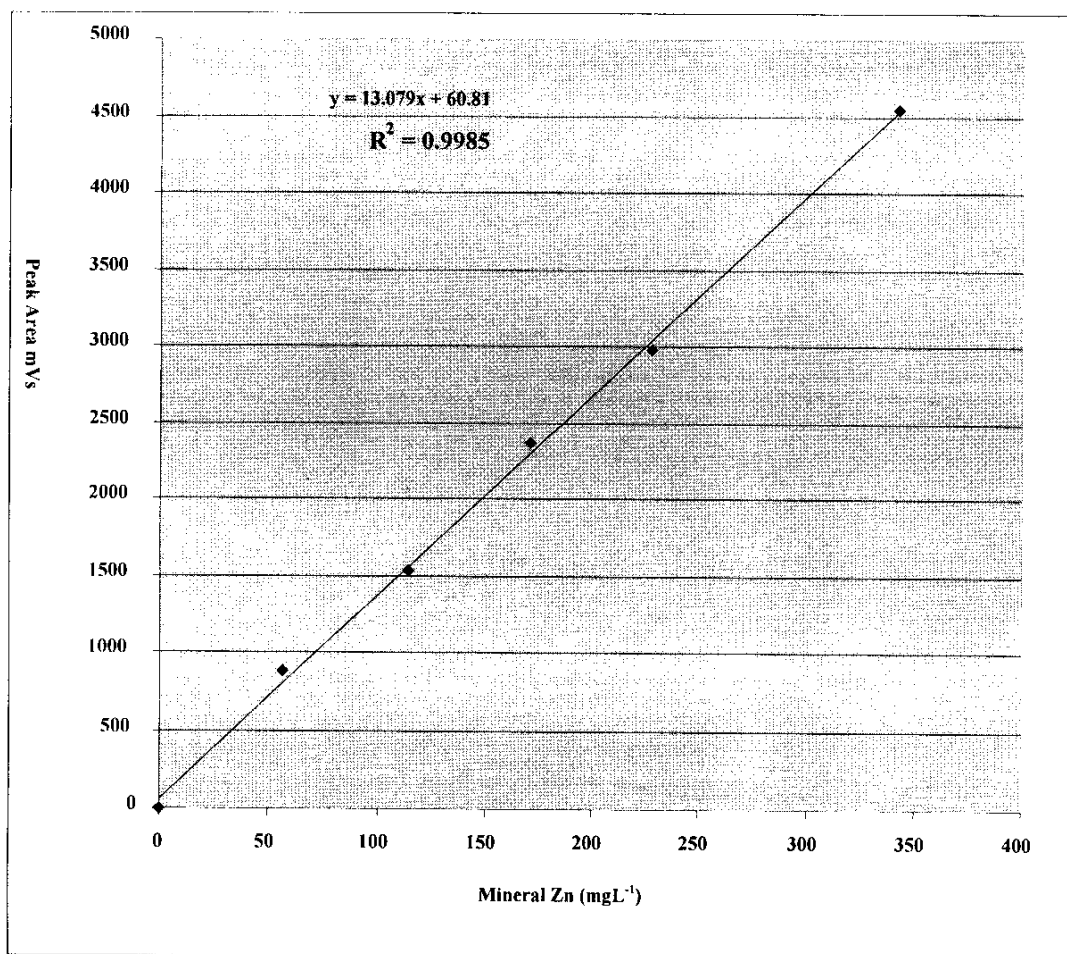


Fig. 5: Trend line showing relationship between mineral Zn concentration and peak area.

The pH level of mobile phase affects the degree of dissociation of acidic/basic compounds and can be used as a mean of controlling the degree of interaction between a polar/ionic analyte and the stationary phase. Thus pH can be used to influence the retention time and peak shape. Therefore at the beginning of method development it is recommended to use an acidic eluent [4]. The pH of mobile phase was kept as 2.4 measured by Hanna pH meter model 212 and it was within the range of requirement of column YK-421 (pH 2 to 7). The electrical conductivity of mobile phase was $740 \mu\text{Scm}^{-1}$.

Sample Preparation

Appropriate quantity of sample was dissolved in mobile phase. After one ultrasonication

sample was filtered through $0.45 \mu\text{m}$ pore size filter (Sartorius Minisart® single use filter unit).

Determination of Total Zn Contents by Atomic Absorption Spectroscopy (AAS)

Total zinc contents were determined by AAS after digestion of chelated zinc standard ($\text{Na}_2\text{ZnEDTA}\cdot 4\text{H}_2\text{O}$, $\text{C}_{10}\text{H}_{12}\text{N}_2\text{Na}_2\text{O}_8\text{Zn}\cdot 4\text{H}_2\text{O}$, MERCK). Percentage of total zinc in chelated zinc standard was 13.72. Standard curve was developed by aspirating standards of 5, 10 and 15 mgL^{-1} of Zn prepared from certified reference material. Air-acetylene flame was used for the determination of zinc as it attains temperature of about 2400 K [5].

Determination of Mineral Zn Contents by Ion Chromatography (IC)

Ion chromatography is simply a high-performance version of ion exchange chromatographies. It can be performed on standard HPLC equipment rather than using a dedicated ion chromatograph system. Ion chromatograph is a form of column chromatographic machine used frequently in analytical chemistry to separate, identify and quantify compounds. It has a column, a pump, and detector that show retention time of the molecules and analyte. Retention time varies depending on the interactions between the stationary phase (column), analyte being analyzed and the mobile phase used.

High performance liquid chromatography (HPLC) used for ion chromatography in the soil and water testing laboratory for research, Lahore, is from Perkin Elmer series 200 with conductivity detector and cation column IC YK-421 that is specially designed for the simultaneous separation of monovalent and divalent cations. Shodex (IC YK-G) guard column (4.6 mm × 10 mm) was installed immediately upstream of the main column to protect it from contamination by sample. The guard column is intended to maintain the column performance as designed for a long period of time and not to improve its resolving power. Twenty microliter (20 µL) sample was injected manually and flow rate was adjusted to 0.5 mLmin⁻¹ after examining the chromatograms developed at different flow rates. Best results were found at flow rate of 0.5 mLmin⁻¹ at which separation of cations occur efficiently.

Peak area was used for calculation of mineral zinc.

Mineral Zinc% = (sample peak area/standard peak area) X (weight of standard/weight of sample) X standard purity

Chelated Zn fraction was calculated as followings:

Chelated zinc% = Total zinc (AAS) – Mineral zinc (IC)

Calculation of uncertainty of chelated zinc measurement (Table-7)

Standard Deviation, $\sigma = \{\sum (x_i - \bar{x})^2 / (n - 1)\}^{1/2}$

Confidence level is 95 % *i.e.* k = 2

Uncertainty of total zinc (U_t) by AAS for Na₂ZnEDTA.4H₂O = 0.12

Uncertainty of mineral zinc (U_m) by IC for Na₂ZnEDTA.4H₂O = 0.00

Combined Uncertainty U_c = $\{(U_t)^2 + (U_m)^2\}^{1/2} = 0.12$

Expanded Uncertainty U_E = U_c × k = ± 0.24

Conclusion

This method can be follow to determine the chelated zinc portion in zinc fertilizers containing upto 14% chelated zinc with uncertainty value of ± 0.24.

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