

RESEARCH ARTICLE

NON-EXTRACTIVE SPECTROPHOTOMETRIC DETERMINATION OF COPPER(II) IN LEAFY VEGETABLE SAMPLES USING SALICYLALDEHYDE ACETOYLHYDRAZONE(SAAH)

Renuka, M* and Hussain Reddy, K

Chemistry lecturer, Loyola Degree collage, pulivendula, Y. S. R (Dist)

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ABSTRACT

A very simple, highly selective and non-extractive spectrophotometric method for the trace amounts of copper(II) has been developed. salicylaldehyde acetoethylhydrazone(SAAH) has been proposed as a new analytical reagent for the direct non-extractive spectrophotometric determination of copper(II). The reagent reacts with copper(II) in acidic medium(pH 5.0) to form a pale yellow coloured 1: 1 (M : L) complex. The reaction is instantaneous and the complex shows maximum absorbance at 372 nm. The molar absorptivity and Sandell's sensitivity are found to be $1.0 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.635 \mu\text{g cm}^{-2}$ respectively. The system obeys Beer's law in the range, 1.0- 9.0 $\mu\text{g/ml}$ of copper(II). The method is highly selective for copper and successfully used for determination of copper in leafy vegetable samples.

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INTRODUCTION

Hydrazones are potential and interesting analytical reagents [1-3]. These reagents are frequently used for the spectrophotometric determination of metal ions. However, salicylaldehyde acetoethylhydrazone (SAAH) is not so far used for the spectrophotometric determination of copper. Therefore, it is of interest to develop spectrophotometric method for the determination of Copper(II). Copper is an essential nutrient to all plants and animals. It is an industrially important metal used in coin making, wire making, medicine, alloys, fashioning metal products, transportation industry and thermal conductance. On the other hand increasing accumulation of copper(II) in the environment through numerous industrial sources, poses danger to public health. The amount of copper that contaminates various biological and environmental substances is of accumulation of copper in the human liver is a characteristics of Wilsons disease, Jaundice which produces neurologic and psychiatric defects. The levels of copper in biological samples may indicate malefaction. This paper describes the non - extractive spectrophotometric determination of copper (II) as SAAH complex in aqueous medium. In continuation of our ongoing work, we report here the spectrophotometric determination of copper in various leafy vegetable samples. A close literature survey reveals that SAAH are so far not been employed for the spectrophotometric determination of copper (II). This

method is far more selective, simple and rapid than all of the existing spectrophotometric methods.

In the light of the above, and in continuation of our previous work [4-6], herein we report synthesis, characterization and spectrophotometric properties of SAAH. The developed SAAH method is applied for the determination of copper(II) in leafy vegetables.

MATERIALS AND METHODS

Materials

salicylaldehyde and acetoethylhydrazide were procured from Merck, India. Ethanol of AR grade, Merck and used as received. Solvent like N,N-dimethylformamide used after distillation.

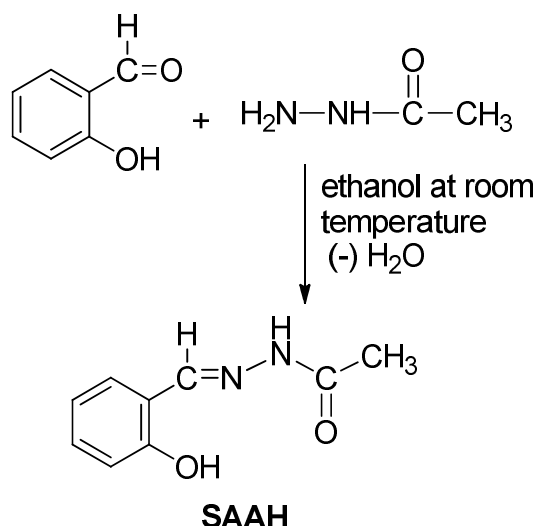
Synthesis of reagent salicylaldehyde acetoethylhydrazone(SAAH)

Synthesis of salicylaldehyde acetoethylhydrazone (SAAH)

Salicylaldehyde (1.05 ml, 0.01m mol) and acetoethylhydrazide (0.74g, 0.01 mol) were mixed in a clean round bottom flask dissolved in 10 ml of ethanol and the contents were heated under reflux for 2 hours. Light pale yellow coloured product was separated out on cooling. It was collected by filtration, washed several times with hot water. This compound was recrystallized from methanol and dried in vacuo. yield 2.5 gm, melting point 202 – 204°C.

*✉ Corresponding author: Renuka, M

Chemistry lecturer, Loyola Degree collage, pulivendula, Y. S. R (Dist)



Preparation of reagent solution

The reagent solution (0.01M) was prepared by dissolving 0.045gm of the compound in 10 ml of dimethylformamide (DMF) in 25-ml volumetric flask. The reagent solution was found to be stable for 10hrs.

Preparation of copper(II) solution

A 1×10^{-2} M stock solution of divalent copper was prepared by dissolving requisite quantity (0.20 g) of $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ in doubly distilled water containing few drops of glacial CH_3COOH and made up to mark in 100- ml volumetric flask. The stock solution was standardised gravimetrically [7]. Dilute solutions were prepared from this stock solution. Solutions of large number of inorganic ions, complexing agents were prepared from their analaR grade (or) equivalent grade water soluble salts.

Procedure

An aliquot of the solution containing copper in optimum concentration range, 10ml of buffer solution (pH 5.0) and 1ml of 0.01 M reagent solution were combined in 25ml volumetric standard flask and resulting solution was diluted to the mark with distilled water. The absorbance of the solution was measured at 372 nm against reagent (SAAH) blank. The measured absorbance was used to compute the amount of copper from predetermined calibration plot.

Preparation of leafy vegetable sample

The established optimized conditions of extractive spectrophotometric method were applied to leafy vegetables, for the determination of copper(II) content. The leafy vegetables analyzed were brought from the local market during the month of January. The samples were cleaned and dried in open air, protecting them from mineral contamination. The dried sample was pulverized in a mortar for the purpose of analysis, to a convenient size. Ten grams of each powdered sample was taken into a silica crucible, heated to oxidize the organic matter, and ashed at 550 °C in a muffle furnace for 4-5 h. The ash was dissolved by heating with 10 mL 2N hydrochloric acid and filtered through an acid-washed filter paper (Whatman no. 41), and then the residue was washed with

hot water. The filtrate and washings were collected in a 25 mL volumetric flask and finally made up to the mark with double-distilled water.

Procedure

An appropriate aliquot was analyzed for copper (II) by the recommended procedure using SAAH from the leafy vegetable was extracted and absorbance values are measured against reagent blank prepared under the similar conditions at 372nm. The process was repeated four times for each sample, and the results obtained were confirmed by direct atomic absorption spectrometer. The results obtained are presented in (Table 4).

Apparatus

A Perkin – Elmer (Lambda 25), UV – Visible spectrophotometer equipped with 1.0- cm (path length) quartz cell and ELICO model LI- 610 pH meter were used in the present study.

RESULTS AND DISCUSSION

Characterization of reagent 2,4-Dihydroxyacetophenone acetoylhydrazone (DAAH)

The newly synthesized reagent salicylaldehyde acetoylhydrazone (SAAH) is characterized using IR, NMR and Mass spectral data.

IR spectra

The reagent (SAAH) are easily obtained by condensation reaction. It has been characterized using IR, NMR and Mass spectral data. The infrared spectrum of SAAH showed bands (cm^{-1}) 3074(m), 2946(m), 1681(s), 1616(s), 1574(m), 14879 (m),1339(S), 1266(S), 1218(S), 759(m), and 698(s) are respectively assigned to $\nu(\text{CH})$ aromatic stretch, $\nu(\text{CH})$ aliphatic stretch, $\nu(\text{C} = \text{N})$ Hydrazine stretch, $\nu(\text{C} = \text{O})$ azomethine stretch, $\nu(\text{CH})$ aromatic ring asymmetric bending, $\nu(\text{CH})$ symmetric bending, $\nu(\text{OH})$ plane bending, $\nu(\text{C} - \text{N})$ stretch, $\nu(\text{CH})$ in plane bending, $\nu(\text{CH})$ aromatic oop bend and $\nu(\text{N} - \text{N})$ stretch vibrations respectively. $^1\text{H} - \text{NMR}$ spectra ($\text{DMSO} - d_6$) showed signals (δ ppm) at 1.95, 6.82 - 6.90, 7.46 - 7.49, 8.31 and 11.60 due to $-\text{CH}_3$, phenyl ring, $-\text{OH}$, $-\text{CH}$ and $-\text{NH}$ (imino) protons of SAAH respectively. Mass spectra shows molecular ion signal at m/z 178. Other peaks are observed at m/z values of 177, 163 and 135 respectively. Due to the loss of methyl, acetyl and CH_3CONH_2 radicals. Based on above spectral data the structure of the reagent is given in Fig 1.

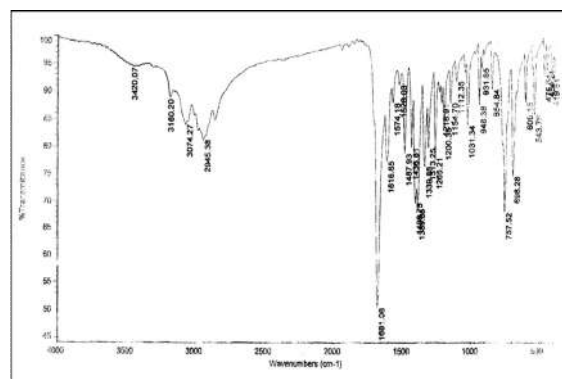


Fig. 1. Infrared Spectrum of SAAH in KBr disc

¹H – NMR spectra

The ¹H – NMR spectrum (Fig . 2)of SAAH (in DMSO –d₆) showed signals at (δ ppm) 1.954, 6.82 – 6.90, 7.47, 8.31 and 11.60 due to -CH₃ protons, ring protons, -OH protons, aldehydic CH protons and -NH protons respectively..

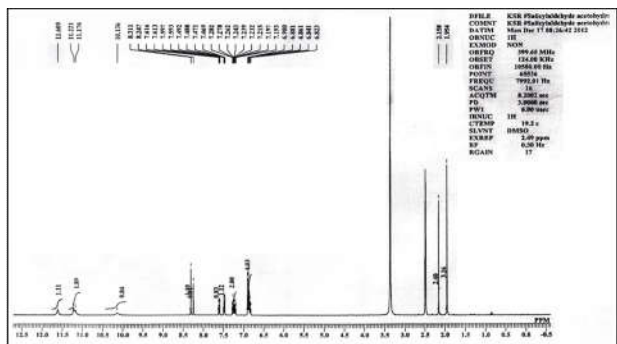


Fig. 2 ¹H-NMR Spectrum of SAAH in DMSO – d₆ medium

Mass spectra

Mass spectrum (Fig. 2) of SAAH shows signal at m/z 178 due to the formation of molecular ion. The peaks observed at m/z values of 177 ,163 and 135 are due to the loss of H, –CH₃ and –COCH₃ radicals respectively.

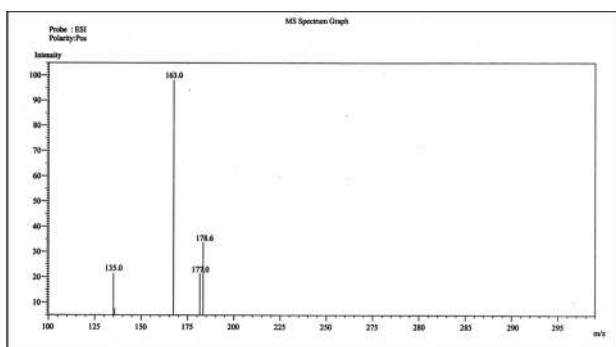


Fig. 3 Mass spectrum of SAAH

UV-Visible spectra

Absorption spectra (Fig. 4) of 2 × 10⁻⁵M DAAH solution at different pH values were recorded and pKa values were determined spectrophotometrically using Phillip and Merritt method [8]. The bathochromic shift from 305 – 345 nm indicates that in solution on increasing pH the >C=O group of the reagent (SAAH) is enolised and dissociated.

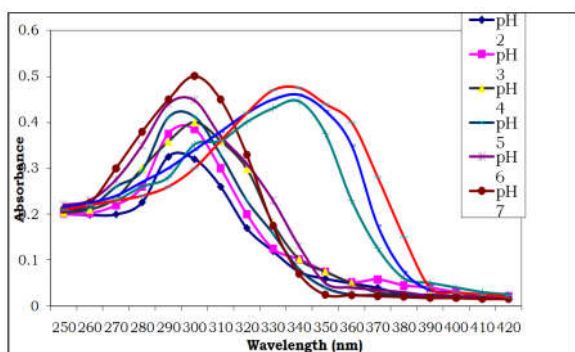


Figure .4 Absorption spectra of 2 × 10⁻⁵ M of SAAH at different pH values

The values of SAAH are 2.5 (pK₁) and 8.0 (pK₂) respectively. The pK₁ and pK₂ values are presumably due to keto – enol tautomerism and deprotonation of - NH group respectively.

Effect of reagent concentration

The data (Table .1) indicate that a 10- fold molar excess of reagent is sufficient for full colour development.

Table 1 Effect of reagent (SAAH) concentration on the absorbance of the complex at 372 nm

Cu(II) : SAAH	Absorbance
1 : 05	0.360
1 : 10	0.393
1 : 20	0.405
1 : 40	0.410
1 : 60	0.464

Effect of time

The absorbance of Cu(II) – SAAH complex was measured at different time intervals to ascertain the time stability of the complex .The absorbance of the Cu(II) complex was measured at 372 nm. The colour development is instantaneous and the absorbance of the complex remains constant for 3 hrs and there after showed gradual decrease in intensity with increasing time.

Effect of pH

The effect of pH on the colour intensity of Cu(II) – SAAH complex is studied . A plot between absorbance of the complex at 372 nm and pH is shown in Fig 5. The graph indicates that the complex shows maximum and constant absorbance in the pH range 4.5 – 5.5. Hence, buffer solution of pH 5.0 is chosen for subsequent studies.

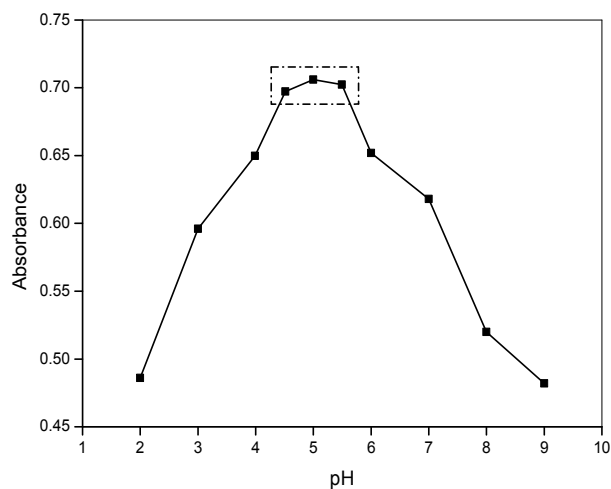


Figure 5 Effect of pH on the absorbance of Cu(II)-SAAH complex

Adherence to to Beers law, Molar absorptivity, and Sandell's sensitivity

The system obeys Beer's law in the range of 1.0 - 9.0 µg/ml of copper (II). The calibration plot is shown in Fig 6. Molar absorptivity and Sandell's sensitivity of the method are found to be 1.0 × 10⁴ L mol⁻¹ cm⁻¹ and 0.635 µg . cm² respectively.

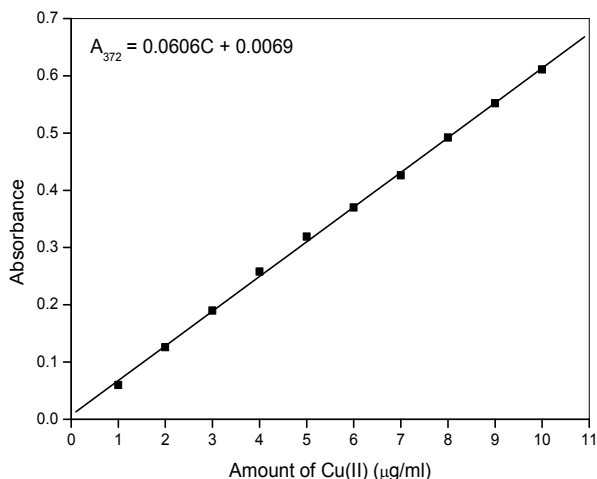


Fig. 6. Calibration plot for Cu(II) determination

Precision

The precision of the method was checked by ten replicate analysis of sample each containing 1.27 ppm of copper. The standard deviation and relative standard deviation are found to be ± 0.0059 and $\pm 1.75\%$ respectively.

Effect of foreign ions

The validity of the method was assessed by investigating the effect of various cations and anions on the determination of copper(II). The amount of foreign ion which brings about a change in absorbance by $\pm 2\%$ was taken as its tolerance limit and results are given in Table 2.

Table 2 Tolerance limit of foreign Ions in the determination of 1.27 µg/ml of copper

Ion added	Tolerance limit µg/ml	Ion added	Tolerance limit µg/ml
Tartrate	254	Ag(I)	13
Hypo	254	Ni(II)	5.0
Nitrate	254	Fe(II)	4.0
Sulphate	254	Mn(II)	2.5
Thiourea	254	Co(II)	2.5
Urea	203	Fe(III)	2.5 ^a
Fluoride	178	Zn(II)	2.5
Phosphate	127	Pb(II)	2.8
Iodide	127	Fe(II)	2.8
Chloride	50.8	Mo(II)	2.5
Bromide	25.4	Hg(II)	2.0 ^b
Oxalate	2.3	Sn(IV)	2.0
EDTA	0.5	Pd (II)	1.5
Citrate	0.5		

^a Masked with 200 µg/ ml of iodide.
^b Masked with 2S50 µg/ ml of cyanide.

Larger amounts of Hg(II) and Fe(III) do not interfere in the presence of masking agents. Interference of mercury(II) and iron(III) are masked with iodide and cyanide respectively.

Determination of composition of the complex

The composition of the complex (M : L = 1 : 1) was determined by Job’s continuous variation method and molar ratio method . The plots are shown in Fig.7 and Fig. 8 respectively, The dissociation constant (α) and concentration (c) of the reagent at intersecting point were used in the calculation of stability constant of the complex. Stability constant of the complex 1:1 (M : L) is given by $1-\alpha / \alpha^2 c$ and

value is 3.76×10^4 . The predicted structure of Cu(II) – SAAH is given in Fig .9.

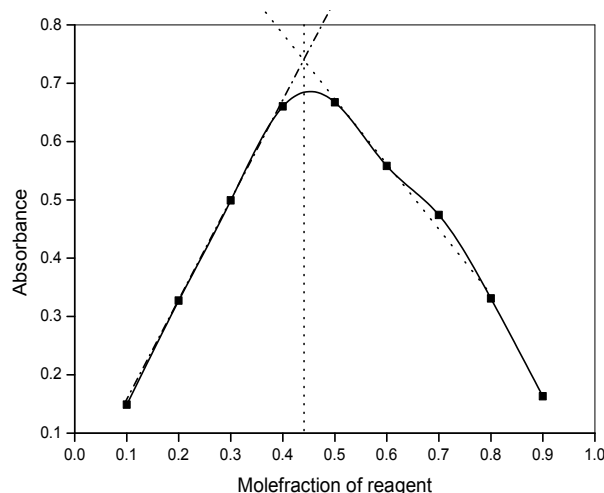


Figure 7 Job’s curve

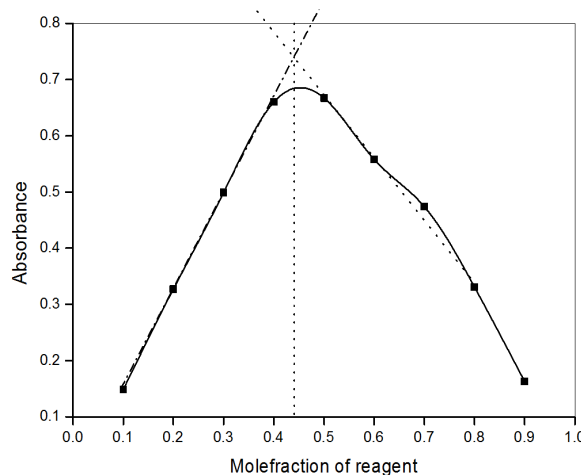


Figure .8 Molar ratio plot.

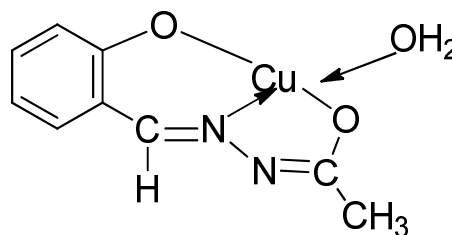


Fig .9 Structure of Cu – SAAH complex

Various physico – chemical and analytical characteristics of copper complex are summarized in Table 3.

Applications

The amount of copper present in certain leafy vegetable samples was determined by the developed method. The amount of the metal ion in leafy vegetable samples given in Table 4.

Table 3 Physico – Chemical and Analytical Characteristics of Cu(II) Complex with SAAH

S. No.	Characteristics	Results
1	λ_{\max} (nm)	372
2	pH range (optimum)	4.5 – 5.5
3	Mole of reagent required per mole of metal ion for full colour development	10 fold
4	Time stability of the complex (in hrs)	3
5	Beer's law validity range ($\mu\text{g/ml}$)	1.0 – 9.0
6	Molar absorptivity ($\text{lit mol}^{-1}\text{cm}^{-1}$)	1.0×10^4
7	Specific absorptivity ($\text{ml g}^{-1}\text{cm}^{-1}$)	0.157
8	Sandell's sensitivity μg of Cu(II) cm^{-2}	0.635
9	Composition of the complex as obtained in Job's and molar ratio methods	1 : 1
10	Stability constant of the complex	3.76×10^4
11	Mean absorbance	0.234 ± 0.0002
12	Standard deviation in the determination of $1.27 \mu\text{g/ml}$ of Cu(II) for ten determinations	0.0061
13	Relative Standard deviation (RSD) %	2.60
14	Y–intercept	0.0069
15	Angular coefficient	0.0606
16	Detection limit ($\mu\text{g/ml}$)	0.0782
17	Determination limit ($\mu\text{g/ml}$)	0.235

Table 4 Determination of Copper(II) in leafy vegetable samples using SAAH

Name of the sample	Amount of copper(II) added ($\mu\text{g/g}$)	Amount of cooper(ii) found ^a ($\mu\text{g/g}$)		SD	RSD(%)
		AAS method	Present method		
Cucumber (<i>Cucumis sitivas</i>)	10.00	10.52	10.51	0.03	0.28
Green peas (<i>Pisum sativam</i>)	10.00	11.15	11.14	0.05	0.43
Fresh bean (<i>Dolichos lablab</i>)	10.00	10.88	10.87	0.04	0.37
White raddish (<i>Raphanus sativus</i>)	10.00	14.21	14.20	0.06	0.42

a= average of four replicants. n = 4

Table 5 Comparison of Spectrophotometric Methods For The Determination of copper(II)

S.No	Name of the reagent	λ_{\max} (nm)	pH range	Determination of Cu(II) ($\mu\text{g/ml}$)	$\epsilon \times 10^4$ ($\text{L mol}^{-1}\text{cm}^{-1}$)	Ref
1	Eosin-Perindopril	535	-	-	6.55×10^3	9
	Ramipril	535	-	-	4.0×10^3	
2	3-{2-[2-(2-hydroxy imino-1-methyl-propylidene amino) -ethyl-imino-butan-2-one oxime	570	alkaline	-	0.16×10^4	10
3	1-phenyl-1-hydrazoneyl-2 - oximino propane-1,2-dione	345	9.4	-	0.35×10^3	11
4	Acetophenone-p-chloro – phenyl thiosemicarbazone	4.0-9.0	0.25-6.35	-	5.5×10^3	12
5	Cefixime	336	1.015-1.822	-	8.29×10^3	13
6	N-(o-hydroxy benzilidene – 4- methyl aniline) arabanoze	-	5.8 – 6.8	1.0 – 20.0	0.89×10^3	14
7	2,4-Dihydroxyacetophenone acetoylehydrazone	370	4.0	0.2-2.0	1.0×10^4	PM

PM – Present method

CONCLUSION

A comparison of spectrophotometric methods [9 - 14] for the determination copper is given in

Table 5. The data suggest that the present method seems to rank among the sensitive methods.

The present method is simple, rapid and more sensitive than other reported methods for determination of copper. It is successfully applied for the determination of copper in leafy vegetable samples.

Acknowledgement

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