

# Monitoring Of Copper Content In Some Food Samples Using Micro Extraction Combined With Spectrophotometric Technique

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## ABSTRACT

An organic reagent namely 2-(2-bromophenyl)imino)methyl)-4- (5,6-dimethylpyridin-2-yl)diazanyl)phenol (R) was synthesized" ,characterized" and used for the determination" of copper" after preconcentration using "Dispersive liquid- liquid microextraction(DLLME) .In this preconcentration method ,ethanol and acetone were used as extraction and disperser solvents respectively and" the ligand" (2-(2-bromophenyl)imino)methyl)-4- (5,6-dimethylpyridin-2-yl)diazanyl)phenol" was used as a chelating agent for the extraction of" Cu(II),Uv-Vis spectrophotometry was applied for the quantitation of the analyte after preconcentration. The effect of various parameters on the extraction was investigated,such as disperser and extraction solvent type and volume,,pH and concentration of chelating agent.At optimum condition,the enrichment factors of (105) was obtained ,the calibration graph was linear in the range (10-100) µg L-1 Cu +2" with detection limit of" (2.79) µg L-1 and "relative standard deviation" (RSD) for seven "replicate measurements" of 20 µg L-1 of Cu +2 was (1.75)%."The method was applied to the determination of" Cu +2 in some Bee honey samples.

**Keywords:** azo dyes, pyridine, shiff base, chelate complexes

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## INTRODUCTION

Copper is widely distributed in nature and is nutritionally essential metal, it plays an important role in carbohydrate and lipid metabolism but it comes toxic to human if a large amount is accumulated in the tissue, the main sources of this metal intake are drinking water and food (1-5) . "the quantification of copper in food is very important considering the toxicity of this metal therefore the monitoring of copper in food samples even at ultra trace level is very essential , but such analyses are difficult because such samples contain low concentration of copper . several preconcentration procedures to determine copper have been devised involving separation techniques such as" cloud point extraction (6) , solid phase extraction (7) , coprecipitation (8) , and dispersive Liquid - Liquid microextraction (9) "Dispersive liquid-liquid microextraction (DLLME) is an extraction technique developed within the last decade, which involves the dispersion of fine droplets of extraction solvent in an aqueous sample. Partitioning of analytes into the extraction phase is instantaneous due to the very high collective surface area of the droplets. This leads to very high enrichment factors and very low solvent consumption", relative to other solid or liquid phase extraction methods<sup>(10)</sup>.

Different conventional spectrophotometric" techniques have been already combined with DLLME for trace-metals analysis"<sup>(11-16)</sup>, Among them, UV-Vis " has been, by far, the most widely used due to its" simplicity, availability versatility, speed, precision ,accuracy, and cost-effectiveness. "This technique is normally used in analytical chemistry for quantitative determination of different analytes such as transition metal ions, biological macromolecules and highly conjugated organic compounds", "In the present" work, a new azo -schiff base reagent, namely 2-(2-bromophenyl)imino)methyl)-4- (5,6-dimethylpyridin-2-yl)diazanyl)phenol was

synthesized ,characterized, and" exploited as a laboratory-made complexing agent to investigate the Dispersive liquid-liquid microextraction methodology for preconcentration of ultra trace amounts of copper ion using ethanol (extractant solvent) and acetone (disperser solvent) "and their determination by UV- Vis spectrophotometry. . The developed method was applied for the determination of " ultra trace amounts of copper(II) in bee honey samples"

## experimental Apparatus

"(FTIR)Spectra(4000-400cm-1)in KBr disk were recorded on SHIMADZU FTIR-8400S fourier. transform. melting point were measured using Stuart, UK. Elemental Analysis 3764,carlo erba Europ. 1HNMR were recorded on fourier transformation bruker spectrometer, operating at (400MHz) with (DMSO-ds) measurements were made at Department of chemistry, kashan university, Iran" . For pH determinations, a Philip PW model 9421pHmeter with a combined glass electrode was used"

## Reagents and Solutions

"All the chemicals used were of analytical reagent grade, and used without further purification. Distilled and deionized water was used for diluting the samples and reagents. A 2-amino-5,6dimethylpyridine, salicylaldehyde , 2-bromoaniline and ethanol were purchased from (GCC, England). Stock solutions of Cu(II) ion (1000 mg L<sup>-1</sup>) were prepared by dissolving (2.686g)CuCl<sub>2</sub>·2H<sub>2</sub>O (Merck) in deionized water, respectively. Working standard solutions of metal ion were freshly prepared by appropriate dilution of the stock standard solution.. A acetate buffer solution (0.1 mol L<sup>-1</sup>) was prepared from acetic acid and sodium acetate at different pH.."

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### Synthesis procedure of 2-((2-bromophenyl)imino)methyl)-4-((5,6-dimethylpyridin-2-yl)diazenyl)phenol (R)

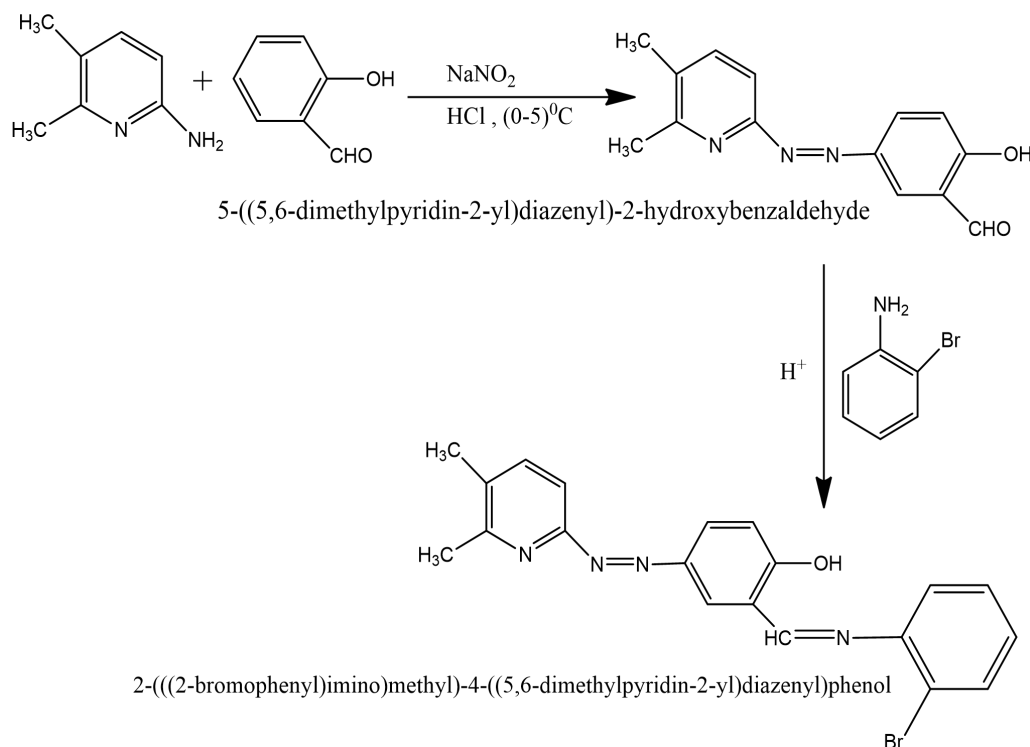
#### Synthesis of 5-((5,6-dimethylpyridin-2-yl)diazenyl)-2-hydroxybenzaldehyde" (1)<sup>17-19</sup>

" dissolved 2-Amino-4,6-dimethylpyridine (0.01mol) in 50 ml distilled water and 4 ml hydrochloric acid . The solution was diazotized with (0.75 gm, 0.01 mol in 25 ml distilled water) sodium nitrate (NaNO<sub>2</sub>)was cooled and added drop - wise to solution of 2-Amino-4,6-dimethylpyridine. ""The resulting reaction mixture was stirred of"" 20 "minutes ,formed a clear yellow solution .In the resulting diazonium chloride solution,drop-wise, with cooled condition and stirring continuously at(0-5)<sup>o</sup>C,was added to solution of salicylaldehyde (0.01 mol) dissolved in 100 ml ethanol .

"The reaction mixture was stirred for another 2 hours at (0-5)<sup>o</sup>C " in ice-bath. After completion of "reaction ,the reaction mixture was added to the ice cold water" ( 150 ml) " with stirring .The crude product was" "separated by filtration , washed with distilled water and dried .The solids obtained recrystallized with ethanol to" get brown crystals colored .The purity of the azo dye ligand (1) was determined by thin layer chromatography "(TLC)". " The yield of the reaction was 88%"".

#### "Synthesis of 2-(((2-bromophenyl)imino)methyl)-4-((5,6-dimethylpyridin-2-yl)diazenyl)phenol (R)"<sup>20-21</sup>

"A mixture of ( 0.01mol) of 2-bromobenzaldehyde and (0.01mol) (1) was refluxed for 3h in 20 mL of ethanol and Add drops of acetic acid .The reaction mixture was cooled and kept for 24 hs.The crystals found was filtered, dried and recrystallized from ethanol to give compound (R)" .



**Fig. "1: Synthetic path of reagent (2-((2-bromophenyl)imino)methyl)-4- ((5,6-dimethylpyridin-2-yl)diazenyl)phenol (R)"**

#### Dispersive liquid-liquid microextraction procedure:

"The pH of a 10 ml of sample solution containing Cu<sup>+2</sup> in the rang (50-100) ng/ml was adjusted to (pH=6) with 1.0 mol/ L acetate buffer and 1ml of reagent R (1.0×10<sup>-4</sup>mol L<sup>-1</sup>)was placed in 10 ml glass test tube with conical bottom . then (400) µL of acetone(as disperser solvent) containing (100) µL ethanol (as extraction solvent) was injected rapidly in to the sample solution by using a (1mL) syringe.. A cloudy solution (water,acetone and ethanol) was formed in the test tube " , in this step Cu<sup>+2</sup> complex was extracted into very fine droplets of ethanol in few seconds,""The mixture was then centrifuged for 5 min at "5000" rpm,after this process the dispersed fine droplets of" ethanol "were sedimented at the bottom of the test tube","then the sediment was diluted by 0.5 ml of methanol and "the concentration of Cu (II) ion was determined spectrophotometry at λ<sub>max</sub> "(590) nm.

#### Preparation of Bee honey samples : (<sup>22</sup>)

"The honey samples were heated in a water bath at 40oC for 2 h. After cooling, aliquots containing 1 g of each sample were weighed directly into PTFE flasks, to which 0.5 mL of HNO<sub>3</sub> and 0.5 mL of H<sub>2</sub>O<sub>2</sub> were added and the mixture allowed to stand for 12 h. Subsequently, the flasks were closed with screw caps and heated to 100oC for 3 h. After cooling to room temperature, the flasks were opened, the resulting solution transferred to graduated polypropylene vials and the volume brought to 25 mL by adding 0.5 M HCl. The aliquots of the final solution were extracted and analyzed for copper content according to the prescribed general procedure for" DLLME

#### Infrared spectra

"The synthesized ligand and its complexes were characterized by FT-IR , compound (1) show absorption at (1724) cm<sup>-1</sup> for (C=O), (2707) cm<sup>-1</sup> for (C-H)aldehyde,( 1450) cm<sup>-1</sup> (-N=N-),(3309) cm<sup>-1</sup> (OH) for phenol,and show band at (3008) for (C-H)aromatic and

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band for (C-H) aliphatic at  $(2823)\text{cm}^{-1}$ ,  $(1535)\text{cm}^{-1}$  for (C=N)pyridine,  $1627\text{cm}^{-1}$  for (C=C) aromatic".

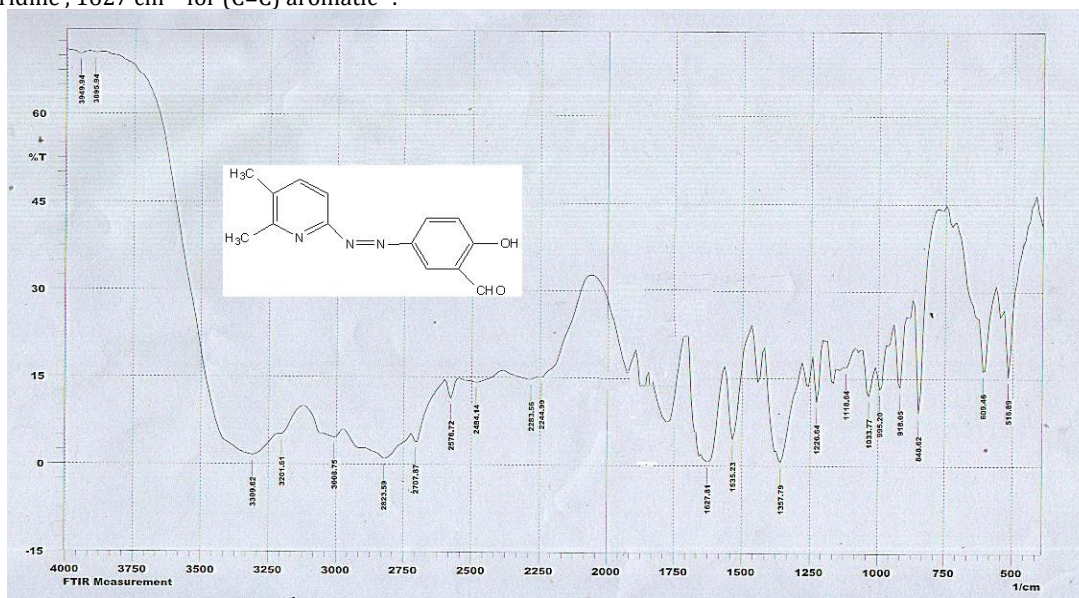


Fig (2) FT-IR spectra of compound (1)

The synthesized ligand (2) were characterized by FT-IR show absorption at  $(1650)\text{cm}^{-1}$  for new (C=N),  $(1488)\text{cm}^{-1}$  (-N=N-),  $(3394)\text{cm}^{-1}$  (OH) for phenol, and show band

at  $(3062)\text{cm}^{-1}$  for (C-H)aromatic and band for (C-H) aliphatic at  $(2923)\text{cm}^{-1}$ .

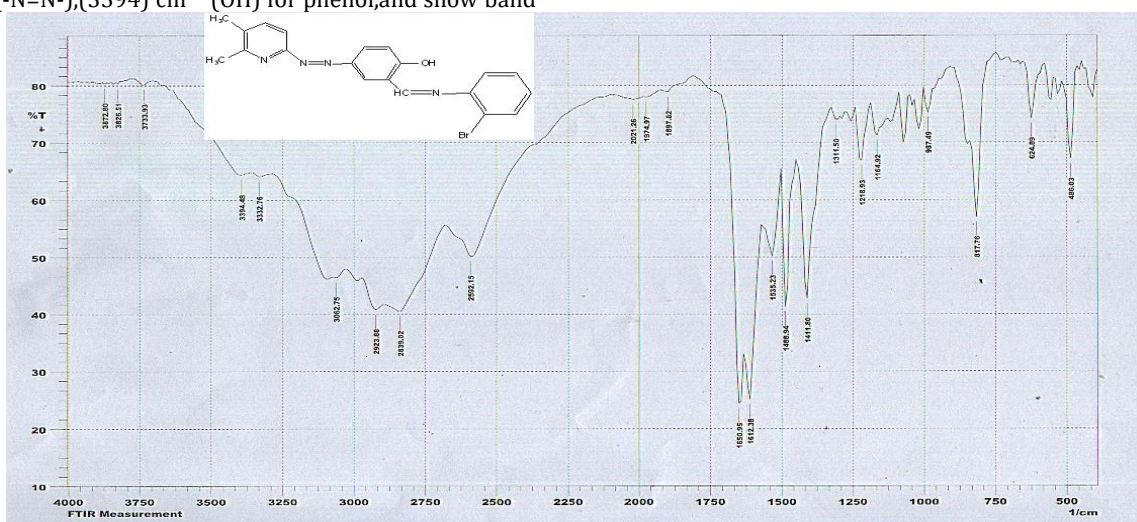
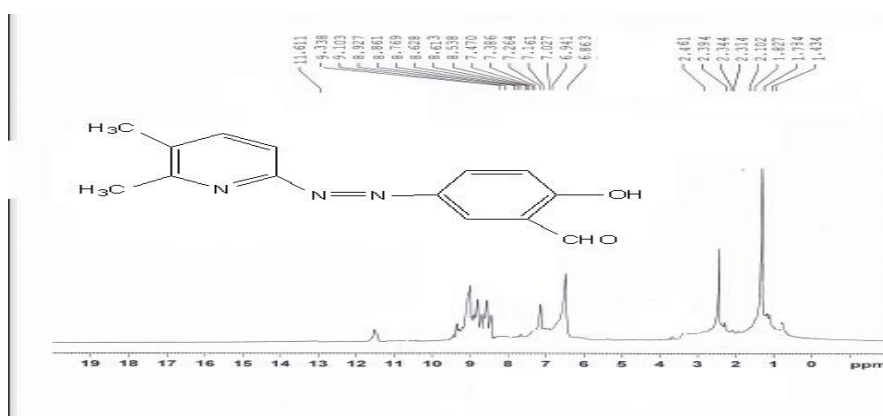


Fig (3) "FT-IR spectra of ligand , compound (R) "

### <sup>1</sup>H NMR Spectra "

"The <sup>1</sup>H-NMR(DMSO) spectrum data of compound (1) show  $\delta$ : 6.8-8.9 (m, 5H, Ar-H), 1.7 (s, 6H, -CH<sub>3</sub>), 9.3 (s, 1H, OH), 11.6 (s, 1H, C-H) Ald".

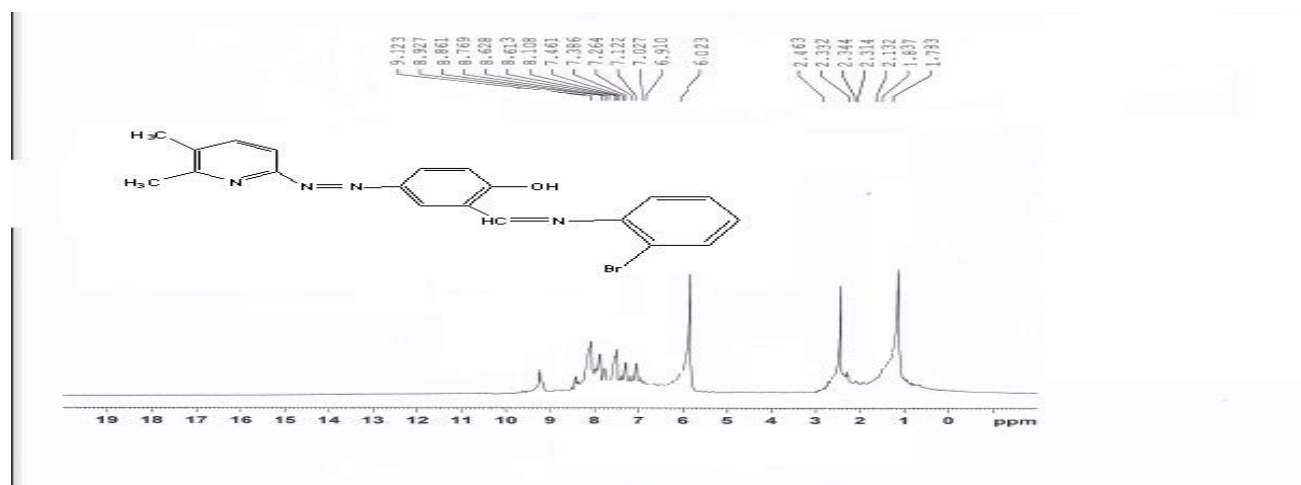




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**Fig ( 4 )<sup>1</sup>H NMR Spectra For compound (1)''''**

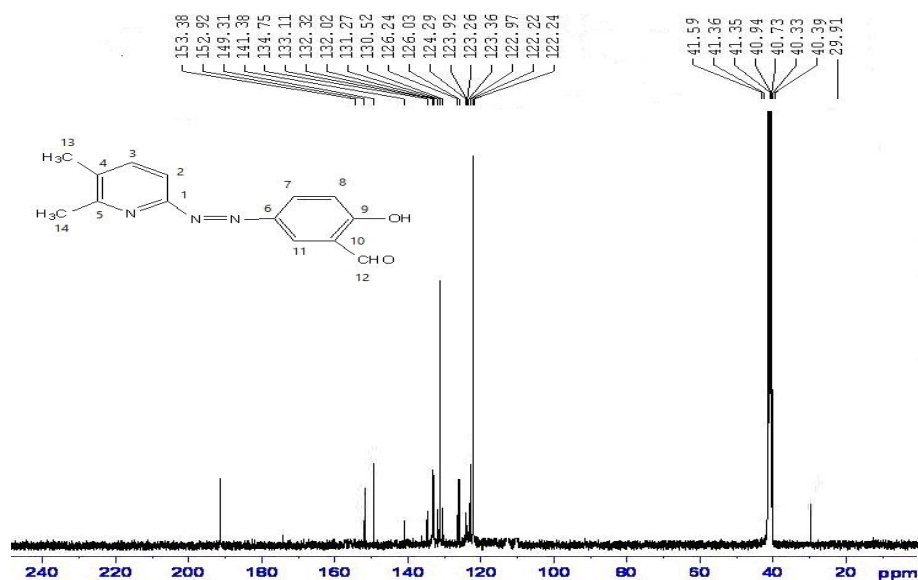
"The<sup>1</sup>H-NMR(DMSO) spectrum data of compound (2) show  $\delta$ :6.9-8.9 ( m , 9H , Ar-H ),1.8 ( s , 6H , -CH<sub>3</sub> ) ,6.02 ( s , 1H,CH-N )".



**Fig ( 5 )<sup>1</sup>H NMR Spectra For compound (R)''''**

### <sup>13</sup>C-''''NMR Spectra'' ''

"The<sup>13</sup>C-NMR(DMSO) spectrum data of compound (1) show  $\delta$ :191(C12) , 153 (C9) ,152(C1) , 149(C5) , 141(C6) 134(C4) ,29(C13,C14) , 133-122(C aromatic)"



**Fig ( 6 )<sup>13</sup>C'''' NMR Spectra For compound (1) ''**

"The<sup>13</sup>C-NMR(DMSO) spectrum data of compound (2) show  $\delta$ :135(C13) , 151 (C9) ,150(C1) , 149(C5) , 147(C6) , 135(C4) ,25(C13,C14) , 112-132(C aromatic)" .

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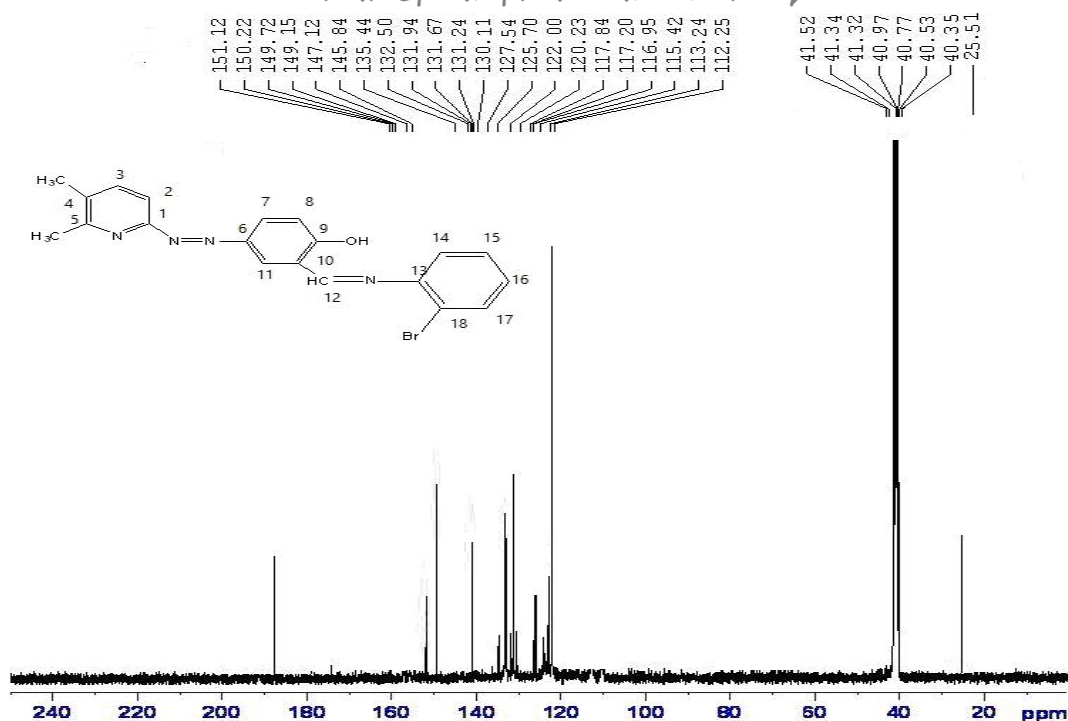


Fig ( 7) <sup>13</sup>C NMR Spectra compound (R)

### Absorption spectra

"The absorption spectra of [(R)<sub>2</sub>Cu] complex was recorded against a reagent blank prepared under the identical conditions. The spectra of Cu(II) complex show

the absorption maxima of 590 nm with molar absorptivities of  $0.11 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ . Whilst, the ligand (R) gave the absorption maxima of 484 nm as depicted in Figure 8".

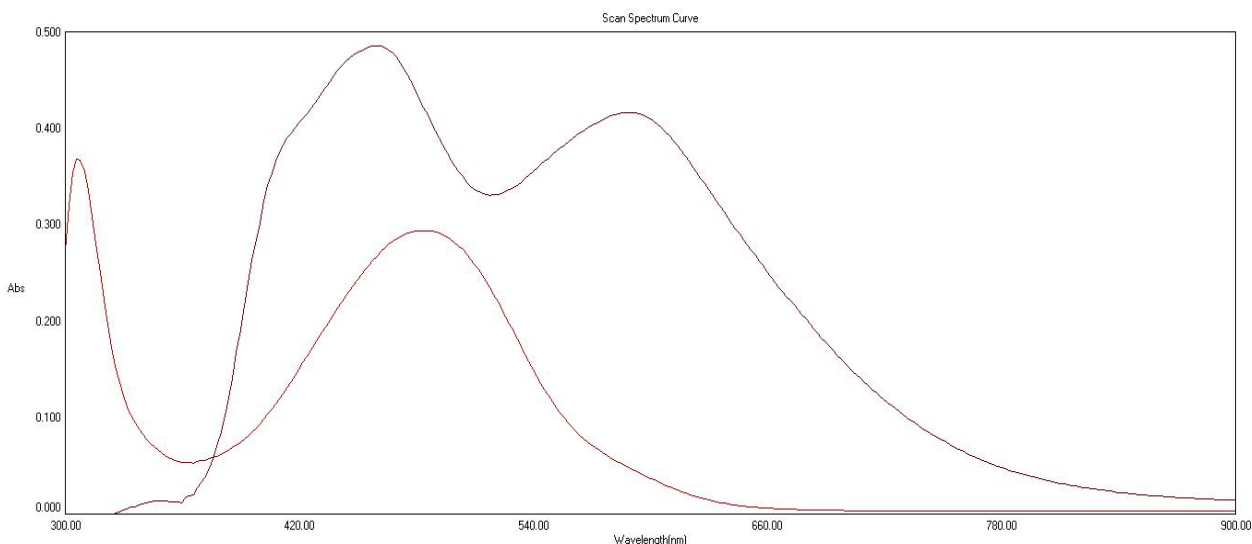


Fig. 8:" Absorption spectra (a) Reagent =  $1 \times 10^{-3} \text{ M}$  (b) [(R)<sub>2</sub>Cu complex, Cu(II) =  $60 \text{ ng mL}^{-1}$ , [R] =  $0.3 \text{ mL}$ " of  $1 \times 10^{-3} \text{ M}$ , Buffer pH = 6"

"The Reagent (R) reacts with Cu(II) ion at pH 6 forming a deep green complex, and the absorbance reached its maximum within 5 min and remained stable, for at least 24 h. The stoichiometry of [(R)<sub>2</sub>Cu] complex was studied, under the established experimental conditions, by Job's and mole ratio methods. The

obtained results indicated that the composition of complexes was (1: 2) with stability constant of  $1.9 \times 10^{10} \text{ L}^2 \text{ M}^{-1}$ . In addition this complexes was characterized on the basis of spectroscopic techniques and the suggested related chemical structure is shown in Figure 9"

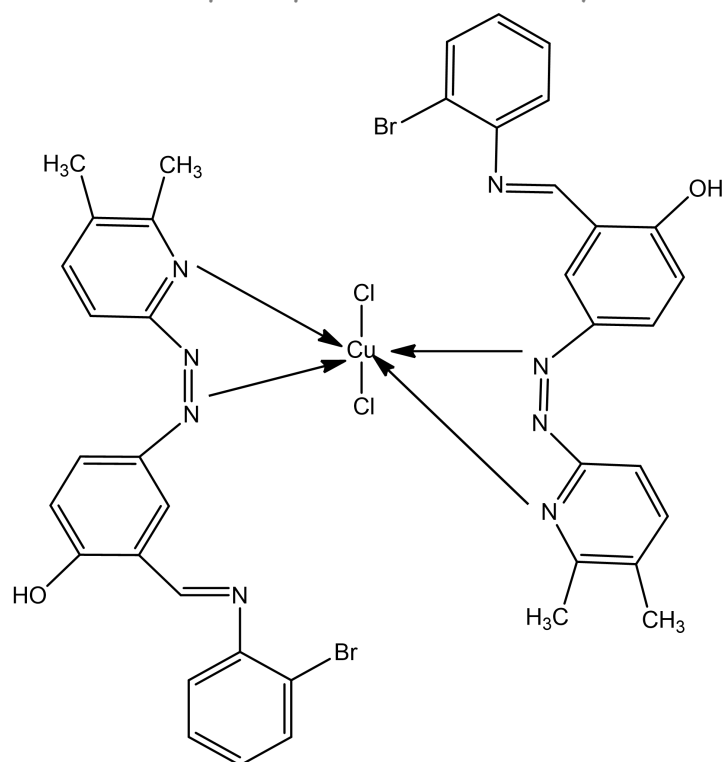


Fig. 9:" The probable chemical structure of the complex "

#### Optimization of DLLME Procedure

"The effect of different experimental variables which impact the DLLME procedure for  $\text{Cu}^{+2}$  complex such as type of the extraction and disperser solvents, pH and the concentration of reagent, amount, were investigated using one variable-at-a-time (OVAT) strategy in searching of the optimum conditions, to maximize recovery percentage and other analytical figures of merit such as sensitivity and detection limit of  $\text{Cu}^{+2}$  complex in the selected matrices. Each experiment of the following variable was conducted followed the general DLLME procedure"

#### Effect of type of the extraction and disperser solvents:

When we selected the extraction solvent some properties must be considered. The extraction solvent should have a higher density than water, low solubility in water and an extraction capability of the interested compound. To study this effect four different solvents such as methanol, ethanol, chloroform and carbon tetrachloride were tested and according to the absorbance signals at (590) nm, the most suitable solvent was ethanol ( Fig10 ) .. In the DLLME, disperser solvent should be miscible with both extraction solvent and water. Therefore ethanol, acetone, acetonitrile and methanol were tested as disperser solvent. Under the same conditions, and according to the absorbance signals at (590) nm, the most suitable solvent was acetone. ( Fig11)

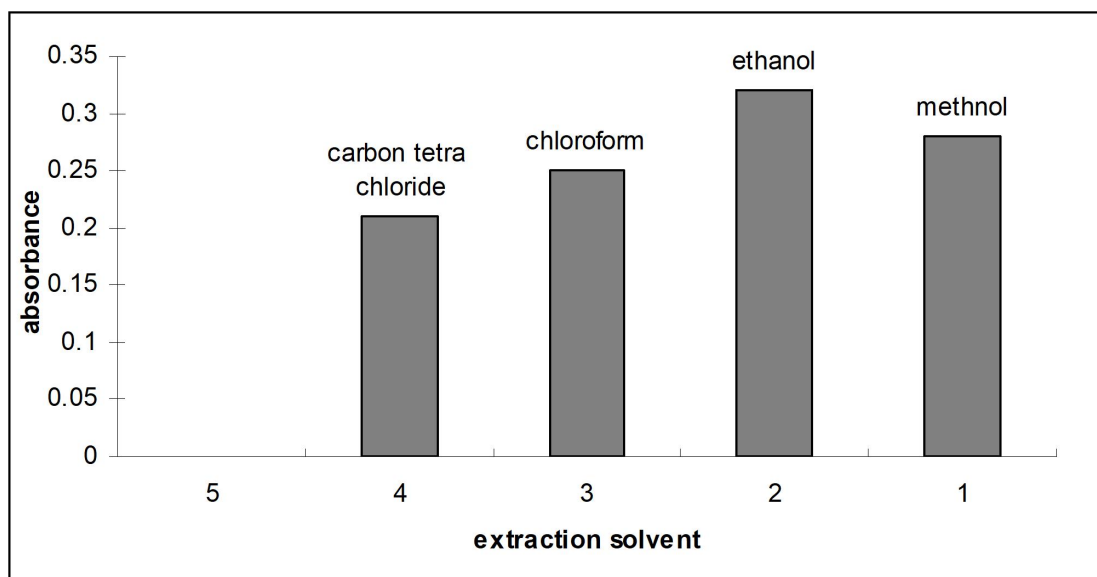


Fig. 10 :Effect of type of the extraction solvent on the formation of Cu (II) complex by DLLME [Conditions: 40 ng mL<sup>-1</sup> Cu(II) , 0.3 mL of 1x 10<sup>-3</sup>M (R)

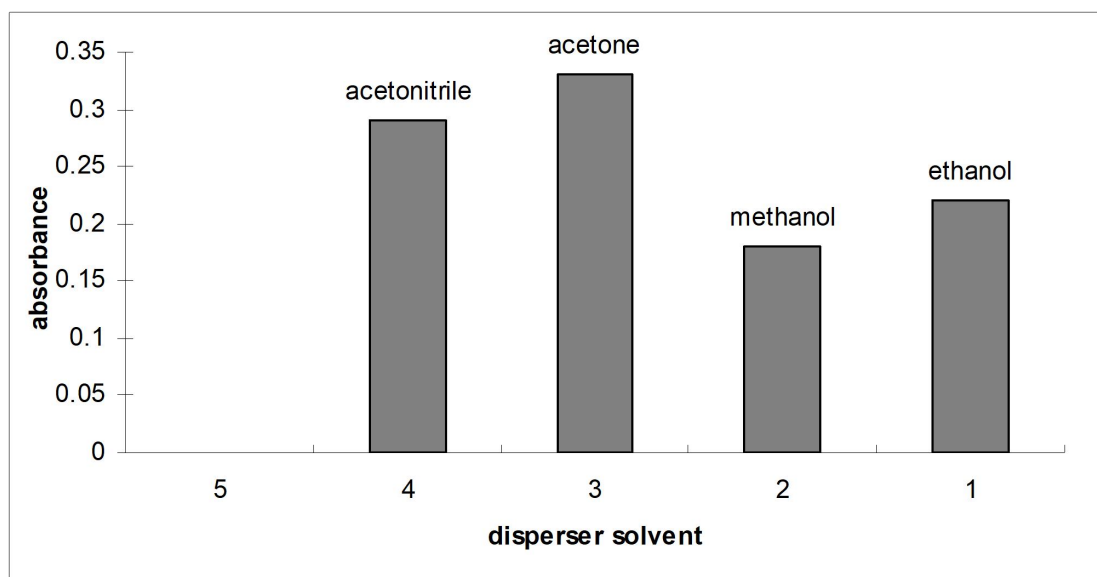


Fig. 11 :Effect of type of the disperser solvent on the formation of Cu (II) complex by DLLME [Conditions: 40 ng mL<sup>-1</sup> Cu(II) , 0.3 mL of 1x 10<sup>-3</sup>M (R)

#### Effect of pH

"The solution pH plays an important role in the formation of metal complex with the chelating agent and their subsequent extraction by DLLME methodology. Thus, the effect of pH was studied in the range of 2 to 8 using different pH acetate buffer solutions. The results are depicted in Figure 4. As can be seen from Fig.4 that the

absorbance first increased with increasing pH and reached a maximum at pH 6.0. Thereafter, the absorbance gradually decreased because of partial dissociation of the complexes at higher pH, which may result in incomplete extraction of complexes. Therefore, pH 6.0 was selected as the optimum pH' for complete formation of for Cu(II) complex".

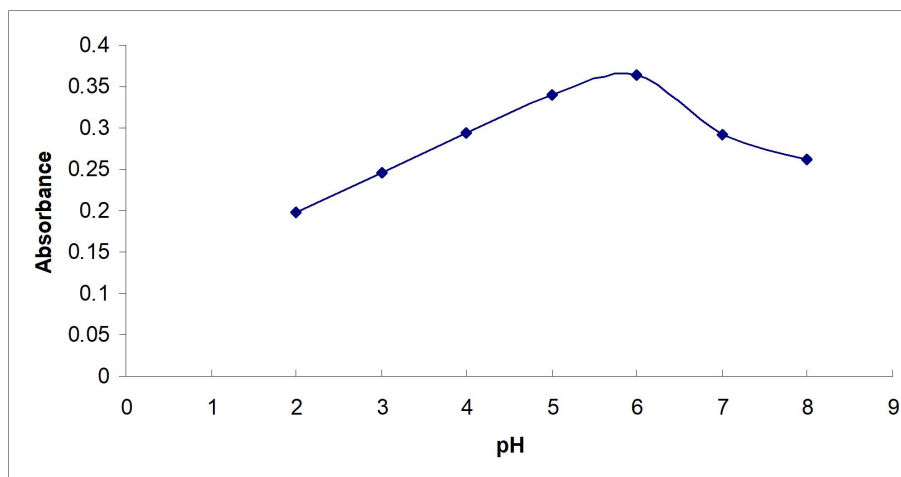


Fig. 12: Effect of pH on the formation of Cu (II) complex by DLLME [Conditions: 50 ng mL<sup>-1</sup> Cu(II) , 0.3 mL of 1x 10<sup>-3</sup>M (R)]

#### Effect of " (2-(2-bromophenyl)imino)methyl)-4- (5,6-dimethylpyridin-2-yl)diazenyl)phenol ) (R) " concentration

"The effect of the (2-(2-bromophenyl)imino)methyl)-4-(5,6-dimethylpyridin-2-yl)diazenyl)phenol concentration was investigated by measuring the absorbance signal according to the general DLLME procedure of solution containing 50 ng mL<sup>-1</sup> Cu(II) and varying volume from 0.1 to 0.5 mL of 1 x 10<sup>-3</sup> mol L<sup>-1</sup> from this reagent. the

analytical responses increase rapidly as the volume of reagent increases and reach maximum up to 0.3 mL and decrease thereafter with further increase in the chelating agent indicating that any excessive amount of chelating reagent was not necessary (Fig.13.). Consequently, 0.3 mL of 1 x 10<sup>-3</sup> mol L<sup>-1</sup> of 2-(2-bromophenyl)imino)methyl)-4-(5,6-dimethylpyridin-2-yl)diazenyl)phenol ) was chosen as optimum for Cu(II) ion".

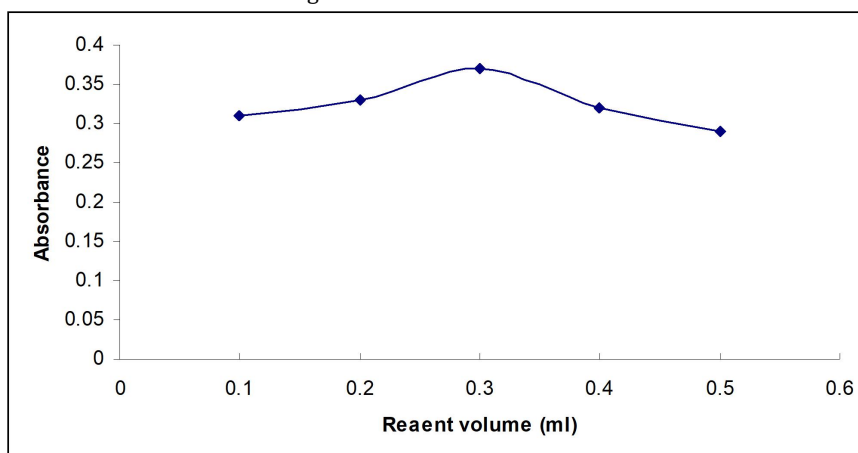


Fig. 13" Effect of concentration of " reagent (R) on the DLLME of Cu(II) [Conditions: 50 ng mL<sup>-1</sup> Cu (II), X mL of 1x 10<sup>-3</sup>M reagent , pH =6 ]

#### Calibration graphs

"Under the optimized conditions established by DLLME procedure, a series of standard Cu(II) solutions ranging from 10-100 ng mL<sup>-1</sup> respectively, was taken and subjected to the general DLLME in order to test the

linearity of the method.. The statistical evaluation for the calibration graphs has shown that a strong correlation between signal and Cu(II) concentration may exist (r = 0.9999). The statistical analytical results for the calibration data for Cu(II) is summarized in Table 1".

"Table 1 Method validation of the determination Cu complex"

Cu(II)	"Parameter"
y = 0.0459+ 0.0065 x	"Regression equation"
0.9999	"Correlation coefficient(r)"
0.00605	"Std. dev. of regression line (s <sub>y/x</sub> )"
10-100	"Concentration range ( ng mL <sup>-1</sup> )"
2.79	"Limit of Detection ( ng mL <sup>-1</sup> )"
9.30	"Limit of Quantitation ( ng mL <sup>-1</sup> )"
153x10 <sup>-3</sup>	"Sandell's sensitivity (µg cm <sup>-2</sup> )"
0.11× 10 <sup>4</sup>	"Molar absorptivity (L.mol <sup>-1</sup> .cm <sup>-1</sup> )"
1 : 2	"Composition of complex (M: L)*"



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1.75 %	"RSD% (n=7)"
98.01	Recovery%
105	"Enrichment factor(EF)***"

\*\*\* EF is calculated as the ratio of slope of calibration curves obtained with and without DLLME"

"The proposed method has achieved enriching factor of 105 fold and this is what was allowed to get on the detection limit of 2.79 ng mL<sup>-1</sup> for Cu(II) in aqueous solution. It can be concluded that the prepared ligand in this work beside DLLME -Spectrophotometry gave

satisfactory analytical figures of merit for Cu(II) which were much better than with those obtained by some previous studies (Table 2). But, they were in harmony with most studies that used analytical methods in combination with DLLME"

"Table 2: Comparison of the proposed of DLLME Method with reported methods in chemical literatures for the determination of Cu (II) ion".

preconcentration technique	LOD µg / L	Linear range µg / L	sample	Ref
SI-DLLMG-FAAS	0.04	-	water	23
DLLME-Fo-LADS	0.34	(2-70)	water+ human urine	24
DLLMG-FAAS	3	(50-2000)	water	25
DLLME-HPLC	3	(10-4000)	water	26
DLLME-UV-Vis	5	(20-90)	water	27
DLLME-SQTFAAS	0.7	-	environmental	28
DLLME-FAAS	0.1	0.5-500	Rice and millet	29
DLLME-UV-Vis	0.5	-	water and food	30
DLLME-IL-µE	0.132	-	drinking water + serum	31
DLLME-UV-Vis	2.79	10-100	Bee honey	This work

### Recovery Test

Since the certificate reference materials (CRM's) for the determination of the copper in samples are not available, "accuracy in term of recovery percent was studied by spiking of 20, 30, 40 ng.mL<sup>-1</sup> Cu(II) to appropriate

amount of honey sample solution and the same steps were followed by general DLLME procedure, The results were tabulated in Table 3 "

"Table 3 Accuracy of the proposed method."

E <sub>rel</sub> (%)	Recovery (%)	amount metal ion found(ng.mL <sup>-1</sup> )	amount metal ion taken (ng.mL <sup>-1</sup> )
-2.0	98	19.6	20
-2.7	97.3	29.2	30
-1.25	98.75	39.5	40

### Interference Study

"The effect of most diverse ions expected in the honey matrices on the determination of 50 ng /mL-Cu (II) solutions were studied following the general DLLME procedure. It is agreed that an extraneous ion deemed to interfere seriously when it gives a relative error percent of more than ± 5%. The results indicated that some of "

metal ions like, Ca (II), Na (I), K (I), Mg(II),Co(II) , Ni( II)and Zn(II) have no appreciable effect on the copper ion responses, while the other metal ions such as Mn (II), and Cd(II) have exceeded the allowable limits of interferences for Cu(II) as shown in Table 4"

Table 4. Effect of divers ions on the absorption signal of Cu(II) (50 ng mL<sup>-1</sup>, Abs= (0.364) by DLLME-spectrophotometry"

E <sub>rel</sub> (%)	ΔA	A	Interferent / Cu(II)	Interfering ion
-0.55	-0.002	0.362	1000	Na <sup>+</sup>
0.27	0.001	0.365	1000	K <sup>+</sup>
0.81	0.003	0.367	1000	Ca <sup>+2</sup>
1.35	0.005	0.369	1000	Mg <sup>+2</sup>

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0.54	0.002	0.366	1000	Cd (II)
4.2	0.016	0.38	500	Ni (II)
5.2	0.02	0.384	500	Mn(II)
6.9	0.027	0.391	500	Cd (II)
1.08	0.004	0.368	1000	Zn (II)

A mixture of 0.01M oxalic acid, tartaric acid and sodium fluoride used to control the interferences of Cd(II) and Mn(II) without any appreciable masking of Cu Ion

### Applications Study

According to the considerable analytical features that have been achieved in this method, such as, high recoveries, low detection limit and interference-free, the

method was employed for the detection of copper ions in bee honey samples after the digestion procedures that described in experimental work and measured in triplicate. At the same time, the sample solutions were also determined by flame atomic absorption spectrometric method (FAAS). The results are presented in Tables 5.

**Table5:** Results of the estimation of Cu (II) ion in different Bee honey samples.

### CONCLUSION

In this piece of work, a new DLLME coupled with traditional spectrophotometric method using synthesized ligand was established for the determination of Cu(II) in Bee honey samples. The separation of copper ion was easily conducted in single extraction by DLLME using homemade organic reagents for the first time. The

determination of copper in food and water samples using amino acid as the complexing agent J. Food Compos Anal. 23, 95.

- S. J. Wang, H. Zheng, B. X. Ye, 2008. Simultaneous Determination of Cd (II), Cu (II), Pb (II) and Zn (II) in Human Plasma by Potentiometric Stripping Analysis J. chin. chem. Soc, 55, 1080.

Bee honey samples	Concentration of Cu ( $\mu\text{g}\cdot\text{g}^{-1}$ ) ""	
	Proposed method	FAAS
" Iraqi 1"	0.096 $\pm$ 0.0031	0.091 $\pm$ 0.0025
" Iraqi 2"	0.075 $\pm$ 0.0012	0.088 $\pm$ 0.0017
" Iraqi 3"	0.099 $\pm$ 0.0045	0.092 $\pm$ 0.0057
Turkish 1	0.078 $\pm$ 0.0016	0.073 $\pm$ 0.0033
Turkish 2	0.073 $\pm$ 0.0023	0.076 $\pm$ 0.0064
Turkish 3	0.089 $\pm$ 0.0015	0.082 $\pm$ 0.0051
Iranian 1	0.094 $\pm$ 0.0064	0.097 $\pm$ 0.0092
Iranian 2	0.071 $\pm$ 0.0038	0.079 $\pm$ 0.0013
Iranian 3	0.080 $\pm$ 0.0091	0.073 $\pm$ 0.0027

established method gave the distinct features which were represented by acceptable analytical figures of merit and high reliability compared with other sophisticated techniques (Table 2).

### REFERENCES

- M. J. Ahmed, I. Jahan and S. Banoo, 2002. A simple spectrophotometric method for the determination of copper in industrial, environmental, biological and soil samples using 2,5-dimercapto-1,3,4-thiadiazole. Anal. Sci., 18, 805
- M. I. Toral, P. Richter, C. Rodriguez, 1997. Simultaneous determination of copper and iron by second derivative spectrophotometry using mixtures of ligands Talanta 45, 147.
- P. Liang, J. Yang, 2010. Cloud point extraction preconcentration and spectrophotometric determination of copper in food and water samples using amino acid as the complexing agent J. Food Compos Anal. 23, 95.
- S. J. Wang, H. Zheng, B. X. Ye, 2008. Simultaneous Determination of Cd (II), Cu (II), Pb (II) and Zn (II) in Human Plasma by Potentiometric Stripping Analysis J. chin. chem. Soc, 55, 1080.
- D. Citak, M. Tuzen, 2010. A novel preconcentration procedure using cloud point extraction for determination of lead, cobalt and copper in water and food samples using flame atomic absorption spectrometry Food Chem. 119, 1399.
- N. Baghban, A. Mohammad Itaji, S. Dalfarnig and A. Ali Jafari, 2012. "Cloud Point Extraction of Trace Amounts of Copper and Its Determination by Flow Injection Flame Atomic Absorption Spectrometry". Croat Chem Acta 85, 1. (ca1803-3).
- Y. Guoi, H. Zhao, Y. Han, X. Liu, S. Guan, Q. Zhay, X. 2017. Simultaneous spectrophotometric determination of trace copper, nickel, and cobalt ions in water samples using solid phase extraction coupled with partial least squares approaches. J. Anal. Spectrosc. 173, 173.

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8. D.Citak ,M.Tuzen, M. Soylak ,2009. Simultaneous coprecipitation of lead, cobalt, copper, cadmium, iron and nickel in food samples with zirconium(IV) hydroxide prior to their flame atomic absorption spectrometric determination. Food and chemical toxicology 47, 9, 2302-2307".
9. Asadollahi .T, Ali M.H. Shabani, S. Dadfarnia, J. Ghasemi, , Curr., 2012. Application of Chemometrics Methods for the Simultaneous Determination of Zinc and Copper after Preconcentration and Separation by Liquid Liquid Microextraction Based on Solidification of Floating Organic Drop Coupled to Flow Injection Spectrophotometry Anal. Chem. 8. 373-381".
10. Ahad.B, Ahmad.R, 2012,Development of a Dispersive Liquid-Liquid Microextraction Technique for the Extraction and Spectrofluorimetric Determination of Fluoxetine in Pharmaceutical Formulations and Human Urine, *Advanced Pharmaceutical Bulletin*, " 2(2), 157-164
11. Farajzadeh, M.A., Djozan, D., Bakhtiyari, R.F., 2010 b. Use of a capillary tube for collecting an extraction solvent lighter than water after dispersive liquid-liquid microextraction and its application in" chromatography - flame ionization detection. Talanta 81 (5), 1360-1367.
12. Gharehbaghi, M., Shemirani, F., 2011. Ionic liquid-based dispersive liquid-liquid microextraction and enhanced spectrophotometric determination of molybdenum (VI) in water and plant leaves samples by FO-LADS. Food and Chemical Toxicology 49 (2), 423-428"
13. Jia, X., Han, Y., Liu, X., Duan, T., Chen, H., 2010. Dispersive liquid-liquid microextraction combined with flow injection inductively coupled plasma mass spectrometry for simultaneous determination of cadmium, lead and bismuth in water samples. Microchimica Acta 171 (1-2), 49-56".
14. Jia, X., Han, Y., Liu, X., Duan, T., Chen, H., 2011. Speciation of mercury in water samples by dispersive liquid-liquid microextraction combined with high performance liquid chromatographyinductively coupled plasma mass spectrometry. Spectrochimica Acta Part B: Atomic Spectroscopy 66 (1), 88-92".
15. Liang, P., Peng, L., 2010. Determination of silver(I) ion in water samples by graphite furnace atomic absorption spectrometry after preconcentration with dispersive liquid-liquid microextraction. Microchimica Acta 168 (1-2), 45-50".
16. RusnaI kovaI , L., Andruch, V., Balogh, I.S., S̃ krlıI kova I , J., 2011. A dispersive liquid-liquid microextraction procedure for determination of boron in water after ultrasound-assisted conversion to tetrafluoroborate. Talanta 85 (1), 541-545".
17. Khalid.J.Al-adilee and Shaimaa Adnan , 2017. Synthesis and Spectral Properties Studies of Novel Hetrocyclic Mono Azo dye Derived from Thiazole and Pyridine with Some Transition Complexes orientjchem, 33 (4):1-13".
18. RomanGrzeskowiak. Author links open the author workspace.MichaelGoldstein,1979. Inorganica Chimica Acta 33, : 153-156"
19. 19- "Guessan Kouakou Nobel, Kafoumba Bamba, Ouattara Wawohinlin Patrice, Nahossé Ziao , Computational Chemistry, 2017, 5, 51-64"
20. Shaimaa Adnan, Ahmed Jasim Mohammed and Hassan Thamer,2015. Synthesis and identification of some derivatives of 1,3,4-thiadiazole J. Chem. Pharm. Res, 7(10):1000- 1011"
21. Shaimaa Adnan Behget , European Journal of Scientific Research ,2016. Synthesis and Characterization of Some Heterocyclic Compounds from 8-Hydroxy quinoline and Studying of their Biological activity , 141 (2): 154-170"
22. ZUHAIR A, AZHAR A, KASIM" H ,2012 Combined cloud-point extraction and spectrophotometric detection of lead and cadmium in honey samples using a new ligand, *Int. J. Chem. Sci.*: 10(3), 1185-1204"
23. Anthemidis, A.N., Kallirroy-Ioannou, I.G., 2009. On-line sequential injection dispersive liquid-liquid microextraction system for flame atomic absorption spectrometric determination of copper and lead in water samples. Talanta 79 (1), 86-91".
24. Ezoddin, M., Shemirani, F., Jamali, M.R., 2010. Fiber optic-linear array detection spectrophotometry in combination with dispersive liquid-liquid microextraction for preconcentration and determination of copper. Journal of Analytical Chemistry 65, 153-158"
25. Farajzadeh, M.A., Bahram, M., Mehr, B.G., Joı nsson, J.A., 2008 Optimization of dispersive liquid-liquid microextraction of copper (II) by atomic absorption spectrometry as its oxinate chelate: application to determination of copper in different water samples. Talanta 75 (3), 832-840".
26. Farajzadeh, M.A., Bahram, M., Vardast, M.R., 2010a. Central composite design applied to optimization of dispersive liquid- liquid microextraction of Cu(II) and Zn(II) in water followed by high performance liquid chromatography determination. CLEAN- Soil, Air, Water 38 (5-6), 466-477".
27. S̃ krlıI kovaI , J., Andruch, V., Balogh, I.S., KocuI rova I , L., Nagy, L., Bazal, Y., 2011. A novel, environmentally friendly dispersive liquid-liquid microextraction procedure for the determination of copper. Microchemical Journal 99, 40-45".
28. Gözde. Ö, Sezin. E, Dotse .S, Merve .F, ... Sezgin Bakırdere ",2017 Sensitive determination of copper in water samples using dispersive liquid-liquid microextraction-slotted quartz tube-flame atomic absorption spectrometry, microchemical journal, 132 , 406-410".
29. Wu, W.X., Wu, Q.H., Wang, C., Wang, Z., 2011. A novel method for the determination of trace copper in cereals by dispersive liquid- liquid microextraction based on solidification of floating organic drop coupled with flame atomic absorption spectrometry. Chinese Chemical Letters 22 (4), 473-476".
30. Wen.X, Yang.Q Yan, Z., Deng, Q., 2011. Determination of cadmium and copper in water and food samples by dispersive liquid-liquid microextraction combined with UV-vis spectrophotometry Microchemical Journal 97 (2), 249-254"
31. Salma .A, Tasneem .G , Hassan .I, Mariam .S, ... Faheem .S , "2016 A new dispersive liquid-liquid microextraction using ionic liquid based microemulsion coupled with cloud point extraction for determination of copper in serum and water

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