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COMBINED CLOUD-POINT EXTRACTION AND SPECTROPHOTOMETRIC DETECTION OF LEAD AND CADMIUM IN HONEY SAMPLES USING A NEW LIGAND

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ABSTRACT

A new, simple and reliable cloud-point extraction (CPE) combined with spectrophotometric method has been developed for the separation, preconcentration and determination of lead and cadmium ions. The method involved reaction of metal ions with newly synthesized 7-(6-methoxy 2-benzothiazolyl azo)-8-hydroxyquinoline [7-(6MBTA8HQ)] as chelating agent to form hydrophobic complexes. The formed 7-(6MBTA8HQ)$_2$ Pb and 7-(6MBTAHQ)$_2$Cd complexes were extracted at specific pH by cloud-point procedure using nonionic surfactant Triton X-114 as extraction medium. The complexes were subsequently entrapped in surfactant micelles. The surfactant-rich phases were diluted with suitable solvent and the lead and cadmium contents were measured spectrophotometerically at their respective wavelengths maxima. The effect of several experimental parameters which impact the CPE efficiency was optimized by one-factor-at-a-time. Under the optimized conditions, enrichment factors of 114 and 84 were achieved for Pb (II) and Cd (II) ions respectively. The linear range of 5.00-100 ng mL^{-1}, 0.32-7.50 ng mL^{-1} along with limit of detection 3.9 ng mL^{-1}, 0.19 ng mL^{-1}, precision expressed as relative standard deviation for seven replication measurements (10 ng Pb mL^{-1} and 0.94 ng Cd mL^{-1}) of (1.12%) and (1.79%) and the recovery range of (96.27 ± 3.53) and (97.57 ± 3.48) were obtained for Pb (II) and Cd (II) ions, respectively. The developed method was applied for the determination of Pb and Cd in honey samples.

Key words: Cloud-point extraction, Molecular spectrophotometry, Lead and cadmium, Honey samples.

INTRODUCTION

Trace amounts of toxic heavy metals in the bee honey come most probably from contamination during the bees' nutrition in polluted area where they fly or from the equipment which employed for processing and storage containers. However, some researchers have concurred that honey may be useful for the collection of information which

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pertain the environment where the bees live and consider as biological indicator for environmental pollution[1-3]. In fact, there is no information concerning the maximum tolerable levels of these metals in bee honey by national / international standards because it cannot be considered a complete food by human but food supplement. However, several national standards recommend that the bee honey should be free from heavy metals in amounts which may represent a hazard to human health. Whatever the case is the determination of toxic heavy metals like a lead and cadmium in honey is by far fruitful and gives information about the metals content of a variety of honey, which will be useful for the purposes of quality control, health and safety aspects.

Lead and cadmium are not essential and undesirable elements in human and animal organisms as they do not perform any physiological functions but show strong toxic properties even when present at very low levels, causing several health effects. Moreover, the continuous exposure to low levels of these metals can result in bioaccumulation and negative health effects[4]. However, FAO/WHO recommend that a tolerable intake for lead of (25 μg/Kg) and for cadmium of (7 μg/Kg) body weight/week[5].

Bee honey is widely known as a natural product contains very complex ingredients, depending on plant species for bees' nutrition and environmental conditions, ranging from the great amount of carbohydrate mixtures, water with minor amount of protein, and trace amount of vitamins and minerals[6]. Trace metals determination in sugar-rich like honey has been a challenging analytical task, mostly due to the low concentrations of these metals in most samples and the complexity of the matrices, which requires sensitive instrumental techniques and often a pre-concentration step[7].

Recently, the metals determination in various grades of honey by atomic spectrometric techniques (FAAS, ETAAS and ICP-OES) is well reviewed[8]. Inductively coupled plasma-mass spectrometry[2,9], total reflection X-ray fluorescence spectrometry[10,11], and ion chromatography and voltammetry[12,13] have also been applied for the determination metals in honey. Although some of these techniques are sophisticated and have a high detection power, but they are relatively expensive and not available in all laboratories. In contrast, ultraviolet-visible (UV-Vis) spectroscopy is a simple instrument, cheap, easy operated, rapid response time, available in many laboratories and offers acceptable analytical figures of merit when dealing with trace levels of metals in different matrices. But due to its low detection power, extraction and preconcentration procedures are a must which can dramatically improve the detection limit as well as the selectivity of the technique.

Several extraction/pre-concentration procedures have been employed for this purpose depending on the availability of equipment and the specific characteristics of the

technique. The methods include evaporation, electro-deposition, surface adsorption, co-precipitation, ion-exchange, solid phase extraction (SPE), liquid-liquid extraction (LLE) and cloud-point extraction (CPE). Exclusively, all these procedures for the determination of Pb and Cd in different matrices by using atomic spectrometric techniques have been well reviewed[14,15]. Despite that each procedure has its merits and drawbacks, but the choice depends by virtue of analytical problem. Recently, CPE has appeared as a simple promising alternative methodology of extracting analytes (inorganic or organic) into stationary phase comprises of the nonionic micelles. Simply, in CPE, hydrophobic analytes is distributed between surfactant and aqueous phases and when the solution heated over a critical temperature of nonionic surfactant, called cloud point, the hydrophobic species are in a position to interact with the micelles thus being separated and concentrated in the small volume of surfactant-rich phase[16]. This phase acts as an organic solvent with the extracted analyte partitioned between this phase and the aqueous solution containing only very small amounts of the dissolved surfactant[17].

Most reports in chemical literatures for pre-concentration and detection of Pb and Cd in various matrices by CPE have relied on commercial organic reagents to form chelate (hydrophobic) with one or two metals at specific pH, apt to interact with surfactant in solution. These including, 1-(2-thiazolazo)-2-naphthol (TAN)[18], *O*,*O*-diethyldithiophosphate (DDTP)[19,20], ammonium pyrrolidinedithiocarbamate (APDC)[21], 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP)[22], 2-(2-thiazolylazo)-p-cresol (TAC)[23], 2-(5-bromo-2-pyridylazo)-5(diethylamino)phenol(5-Br-PADAP)[24], dithizone(HDz)[25]. bis((1H-benzo [d] imidazol-2yl)ethyl) sulfane (BIES)[26], pyridyl-azo-naphthol (PAN)[27], 4-(2-pyridylazo)-resorcinol (PAR)[28], 1-phenylthiosemicarbazide (1-PTSC)[29].

Recently, few authors have opened a new avenue for synthesis of a novel family of organic reagents as ligands for metal ions extraction in an attempt to improve the selectivity and broadening the analytical characteristics of CPE methodology. In this respect, the Schiff's base chelating ligand, bis(2-methoxy benzaldehyde) ethylene diimine, for preconcentration and speciation of chromium[30], N-salicylideneaniline (SA) for extraction of copper[31], phenanthraquinone monophenyl thiosemicarbazone, (PPT) for colorimetric determination of lead[32], Bis((1*H*-benzo[*d*]imidazol-2yl) methyl) sulfane [BHIS] for the determination of silver[33], N,N¢-bis[(1R)-1-ethyl-2-hydroxyethyl] ethanediamide (DAD1) and N,N¢-bis[(1S)-1-benzyl-2-hydroxyethyl]ethanediamide (DAD2) for the simultaneous preconcentration of both trace and toxic metals in water[34], 2-(((1*H*-benzo[*d*]imidazol-2-yl)methoxy)methyl)-1*H*-benzo[*d*]imidazole (BIMMBI) for the pre-concentration of Ag, Zn, and Pb ions in various samples were synthesized and used in CPE methodology[35].

In retrospection, among various commercially produced ligands used in extraction procedures, including cloud point extraction, thiazolylazo dye derivatives such as, TAN, PAN, PAR and 5-Br-PADAP were used because their metal complexes are highly stable and have rather limited solubility in aqueous solution but much greater solubility in organic systems[36].

In this piece of work, a new thiazolylazo dye reagent was synthesized and characterized, to investigate the cloud-point extraction process of Pb and Cd using 7-(6-methoxye 2-benzothiazoly azo) -8-hydroxyquinoline [7-(6-MBTA8HQ)] as a new complexing agent and Triton X-114 as non-ionic surfactant and their determination in the bee honey samples by UV-Vis spectrophotometry.

EXPERIMENTAL

Apparatus

A Shimadzu 1650 PC model double-beam UV-Vis spectrophotometer (Japan) equipped with 10-mm optical path cell were used for the scanning study of absorption spectra of the complexes formed, while absorbance measurements were carried out with spectrophotometer Sunny UV-7804C (China). The effect of temperature was investigated by using a water bath WB 710 model (OPTIMA, Japan). A microprocessor pH meter 211 model (Triup International Corp., Italy) with a combined electrode was used for pH measurements.

Reagents and Solutions

All the chemicals used were of analytical reagent grade, and were used without further purification. Distilled water was used for diluting the samples and reagents. 2-amino-6-methoxy benzothiazole, 8-hydroxyquinoline (RIEDEL-DE HAEN AG SEELZE-Germany), Sodium nitrite (BDH), hydrochloric acid (BDH), and ethanol were purchased from (GCC, England). Triton X-114 (Acros Organics, New Jersey, USA) stock solution (10% v/v) was prepared by dissolving 10 mL of concentrated solution in water. Stock solutions of Pb (II) and Cd (II) ions (1000 mg L^{-1}) were prepared by dissolving (0.1598 g) and (0.1630 g) of Pb $(NO_3)_2$ (BDH) and $CdCl_2$ (Merck) in distilled water, respectively. Working standard solutions of each metal ion were freshly prepared by appropriate dilutions of the stock standard solutions. A acetate buffer solution (0.1 mol L^{-1}) was prepared from acetic acid and sodium acetate.

Synthesis procedure of 7-(6-methoxye 2-benzothiazoly azo)-8 hydroxyquinoline (6-MBTA8HQ)

The synthesis of 7-(6-MBTA8HQ) was accomplished according to general procedure described elsewhere[37] with some modifications of starting materials as shown in Fig. 1.

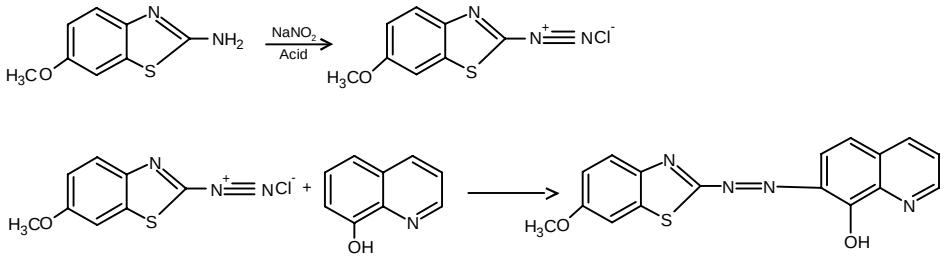

Fig. 1: Synthetic path of reagent 7-(6-MBTA8HQ)

A 2-amino-6-methoxy benzothiazole (3.6044 g, 0.02 mol) was dissolved in 25 mL of distilled water and 5 mL of concentrated hydrochloric acid and diazotized below 5^{o}C with (1.38 g, 0.02 mol) sodium nitrite. The resulting diazonium chloride solution was added dropwise with cooling to the solution of (2.903 g, 0.02 mol) 8-hydroxyquinoline dissolved in 50 mL of distilled water and the mixture left in the refrigerator overnight. The mixture was neutralized with dilute hydrochloric acid to (pH = 6.0). The solid product was filtered off, washed with cold distilled water, crystallized twice from hot ethanol and dried over $CaCl_2$ to give yield of 86 %, M.P. (232-234^{o}C) and Mw. (336.3673 g mol^{-1}) .

General procedure for CPE

Aliquots of 2.5 mL of a solution containing lead (II) or cadmium (II) in the analytical concentration range Pb (II) of 5 - 100 ng mL^{-1} or Cd (II) = 0.32-7.54 ng mL^{-1}, 1 mL of acetate buffer solution (pH = 4.0 or 5.0), 0.5 or 0.4 mL of Triton X-114 (10%) and 0.2 or 0.3 mL of 5.0 x 10^{-4} mol L^{-1} 7-(6MBTA8HQ) reagent solution were mixed in a 5 mL standard flask and diluted to mark with distilled water. The contents of the flask were transferred into a 10 mL centrifuging tube and the phase separation was induced by heating the contents in a water bath at 60^{o}C for 20 min. Separation of the phases was accelerated by centrifuging at 4000 rpm for 20 min. Without cooling, the surfactant-rich phases became viscous. Then, the aqueous phase could be separated by using a syringe. Subsequently, 3 mL of ethanol was added to the surfactant-rich phase in order to decrease its viscosity and make the final volume feasible to transfer into the optical cell of 10 mm for the measurement of each metal ion spectrophotometrically at the respective absorption maxima against a reagent blank prepared under similar conditions.

Preparation of honey samples

The honey samples were slightly heated in a water bath at 40°C 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_3 and 0.5 mL of H_2O_2 were added and the mixture allowed to stand for 12 h. Subsequently, the flasks were closed with screw caps and heated to 100°C 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 lead and cadmium content according to the prescribed general procedure for CPE.

Statistical analysis

All mathematical and statistical computations were made using Excel 2007 (Microsoft Office) and Minitab version 14 (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Absorption spectra and characteristics of the complex

The absorption spectra of 7-(6-MBTA8HQ)$_2$Pb and 7-(6-MBTA8HQ)$_2$Cd complexes were recorded in the presence of surfactants against a reagent blank prepared under the

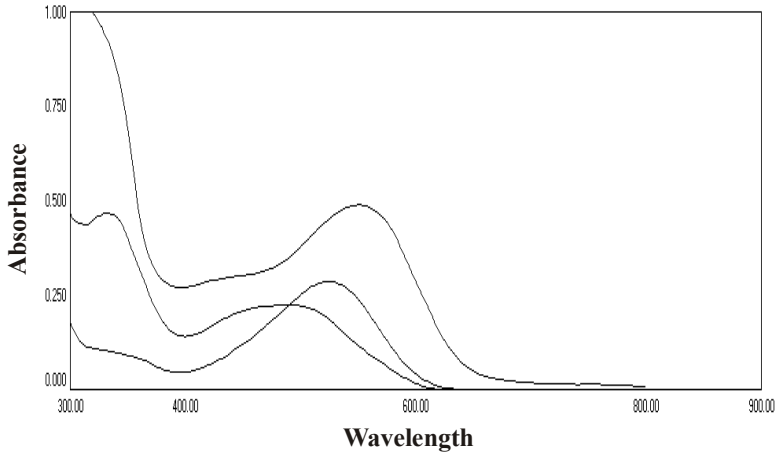

Fig. 2: Absorption spectra (a) Reagent 7-(6-MBTA8HQ) = 5 x 10^{-4} M (b) Pb (II)-7-(6-MBTA8HQ) complex, Pb (II) = 40 ng mL^{-1}, 0.2 mL of 7-(6-MBTA8HQ) = 5 x 10^{-4} M, Buffer pH = 4 (1 mL), 0.5 mL of 10 % (v/v) Triton X-114 (c) Cd (II))-7-(6-MBTA8HQ) complex, Cd (II) = 7.5 ng mL^{-1}, 0.3 mL of 7-(6-MBTA8HQ) = 5 x 10^{-4} M, Buffer pH = 5 (1 mL), 0.4 mL of 10 % (v/v) Triton X-114

identical conditions. The spectra of Pb (II) and Cd (II) complexes show the absorption maxima of 524 and 550 nm with molar absorptivities (ε) of 2 x 10^6 L mol^{-1} cm^{-1} and 1 x 10^7 L mol^{-1} cm^{-1} obtained respectively while the ligand 7-(6-MBTA8HQ gave the absorption maxima of 490 nm as depicted in Fig. 2.

The stoichiometry of the Pb (II)-7-(6-MBTA8HQ) and Cd (II)-7-(6 MBTA8HQ) complexes were studied, under the established experimental conditions, by Job's and mole ratio methods. The obtained results indicated that the composition of the complex was 1 : 2. In additions these complexes were characterized by using spectroscopic techniques and the related chemical structure as shown in Fig. 3.

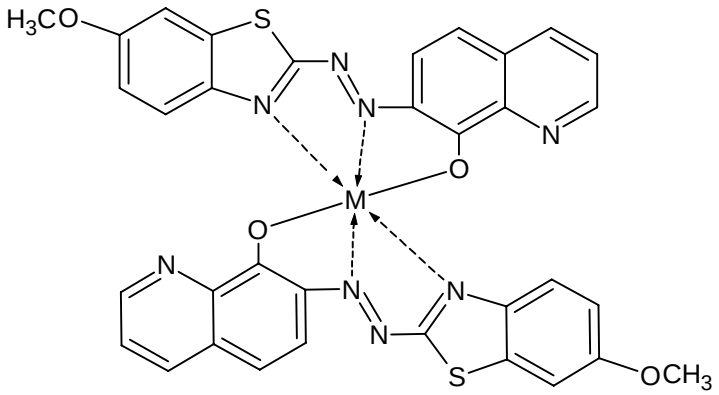

Fig. 3: The chemical structure of 7-(6-MBTA8HQ) $_2$M, where M = Pb^{2+} and Cd^{2+}

Optimization of CPE Procedure

The effects of several experimental parameters which impact the CPE efficiency were carried out by classical optimization (one-factor-at-a-time). In this approach, we observe the effect of one factor at a time (OFAT) on an experimental response. While only one factor is changed, others are kept at a constant level. Although, the "optimization" performed by OFAT does not ensure at all that the real optimum will be conformed, but it would be valid only if the variables to be optimized would be totally independent from each other (i.e. no interactive effects among the variables)[38]. Nevertheless, the classical optimization certainly leads at least to an improvement of the analytical method. In as much as the extraction efficiency of the CPE depends on dual factors, some of regarding the prior formation of a complex with sufficient hydrophobicity and the other for the formation of micelles to obtain the desired separation and preconcentration. Consequently, the effects of pH, concentration of ligand, nonionic surfactant Triton X-114 concentration and equilibration temperature and time centrifugation time were selected in this study.

Effect of pH

The effect of pH on formation of the- M (II)7-(6-MBTA8HQ) complexes in Triton X-114 medium was determined by recording their absorbance signals at λ_{max} , over the range of 3-9, using different pH acetate buffer solutions. The experiment was performed with 5 x 10^{-4} M 7-(6-MBTA8HQ) and 10% (v/v) Triton X-114. The results are shown in Fig. 4.

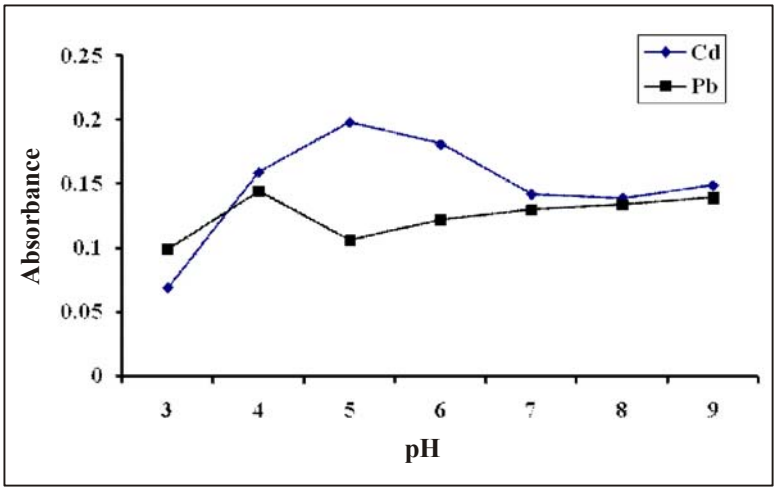

Fig. 4: Effect of pH on the formation of 7-(6-MBTA8HQ)-M (II) complexes formed with Pb (II) and Cd (II). Conditions: Pb (II) = 14 ng mL^{-1}, 0.2 mL of 7-(6-MBTA8HQ) = 5 x 10^{-4} M, 0.5 mL of 10 % (v/v) Triton X-114 and Cd (II) = 2.7 ng mL^{-1}, 0.3 mL of 7-(6-MBTA8HQ) = 5 x 10^{-4} M, 0.4 mL of 10 % (v/v) Triton X-114

As can be seen in Fig. 4, the absorbance first increased with increasing pH and reached a maximum at pH 4.0 and 5.0 for Pb (II) and Cd (II) complexes, respectively. The absorbance gradually decreased because of partial dissociation of the complexes at higher pH, which may result in incomplete extraction of both complexes. Therefore, pH 4.0 and 5.0 were selected as the optimum pH's for complete formation of for Pb (II) and Cd (II) complexes respectively.

Effect of 7-(6MBTA8HQ) concentration

The effect of the 7-(6MBTA8HQ) concentration was investigated by measuring the absorbance signal according to the general CPE procedure of solution containing 14.7 ng mL^{-1} Pb or 1.8 ng mL^{-1} Cd, 10% (v/v) of Triton X-114 and various amounts of the 6-MBTA8HQ (0.1-0.5 mL) of 5 x 10^{-4} mol L^{-1}. In both cases (Pb(II) or Cd(II)), the

analytical responses increase rapidly as the concentration of 7-(6MBTA8HQ) increases and decrease slightly with further increase in the chelating agent (Fig. 5). Consequently, 0.2 or 0.3 mL of 5 x 10^{-4} mol L^{-1} of 7-(6MBTA8HQ) was chosen as optimum for Pb (II) or Cd (II). The slight difference in the contact of chelating agent with Pb (II) or Cd (II) may be attributed to differences in the stability constants of complexes formation in the micellar medium[19].

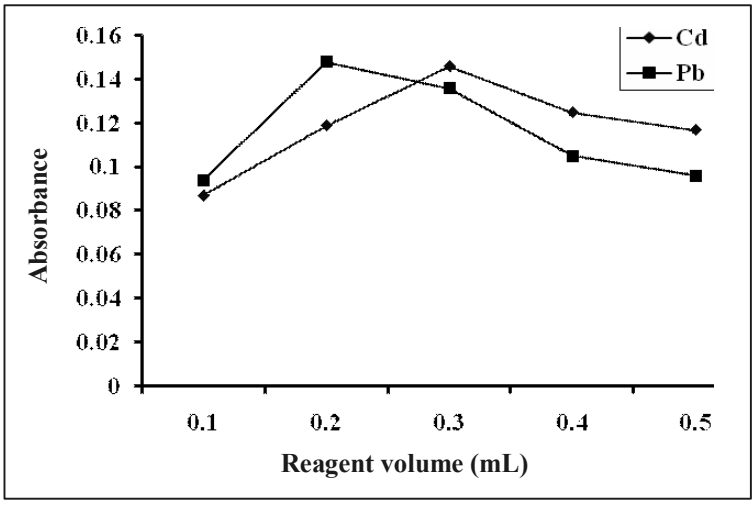

Fig. 5: Effect of concentration of 7-(6MBTA8HQ) on the determination of Pb (II) and Cd (II) Conditions: Pb (II) = 14.7 ng mL^{-1}, Buffer pH = 4 (1 mL), 0.5 mL of 10% (v/v) Triton X-114. and Cd (II) = 1.8 ng mL^{-1}, Buffer pH = 5 (1 mL), 0.4 mL of 10% (v/v) Triton X-114

Effect of triton X-114 amount

Fig. 6 depicts the effect of variation of Triton X-114 amount on the absorbance signal for the determination of Pb (II) and Cd (II) ion. Different volumes of Triton X-114 (10% v/v) ranging from 0.1-1 mL were used in this study at previously optimum conditions.

As shown in Fig. 6, the absorbance for both ions increased by increasing the Triton X-114 concentration up to 0.5 and 0.4 mL of 10% (v/v) for Pb (II) and Cd (II) respectively, and then suddenly decreased at higher amounts. Therefore, 0.5 and 0.5 mL of 10% (v/v) Triton X-114 was used as the optimum concentration for lead and cadmium, respectively.

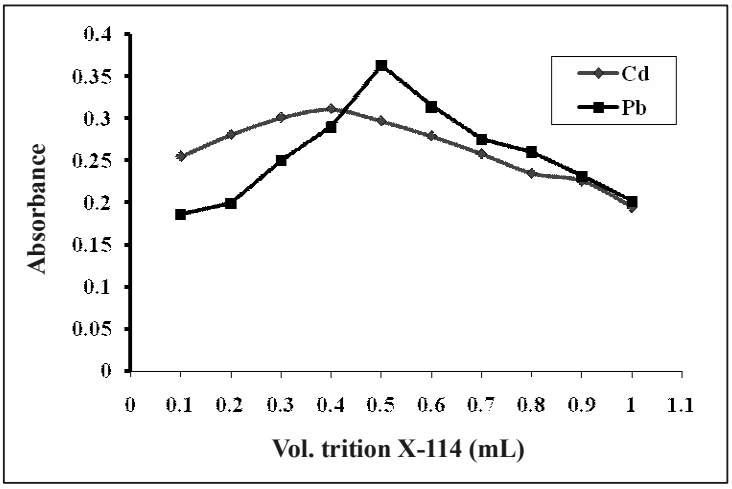

Fig. 6: Effect of Triton X-114 concentration on the analytical signal. Conditions: Pb (II) = 50 ng mL^{-1}, 0.2 mL of 7-(6MBTA8HQ) = 5 x 10^{-4} M, Buffer pH = 4 (1 mL), Cd (II) = 4.7 ng mL^{-1}, 0.3 mL of 7-(6MBTA8HQ) = 5 x 10^{-4} M, Buffer pH = 5 (1 mL)

Effect of the equilibrium temperature and the incubation time

The effects of the equilibrium temperature and the incubation time were examined due to their importance for the reaction completion and efficient separation of the phases, which reflect certainly the magnitude of preconcentration factor of an analyte. Consequently, a study was carried out to choose the range of temperature that enhances higher absorbance signals for Pb (II) and Cd (II) ions. The temperature was varied from 25°C to 80°C in a search of optimum value. It can be seen from Fig. 7 that the highest absorbance signals were achieved when the temperature at 60°C.

It was also observed that the incubation time of 20 min is sufficient for the maximum absorbance of both ions (Fig. 8). Thus, the temperature of 60°C for 20 min was selected to fulfill efficient separation conditions. The effect of centrifugation rate and time also was investigated on extraction efficiency. A centrifuge time of 20 min at 4000 rpm was selected for the entire procedure as being optimum and beyond this time no confirmation was observed for improving extraction efficiency.

Method validation

Under the optimized conditions, the calibration graphs were constructed by plotting the absorbance signal against the concentrations of each analyte subjected according to the general procedure for CPE. The solutions were transferred into the optical cell of 10 mm for

the measurement of each metal ion spectrophotometrically at the respective absorption maxima against a reagent blank prepared under similar conditions. The calibration data are summarized in Table 1.

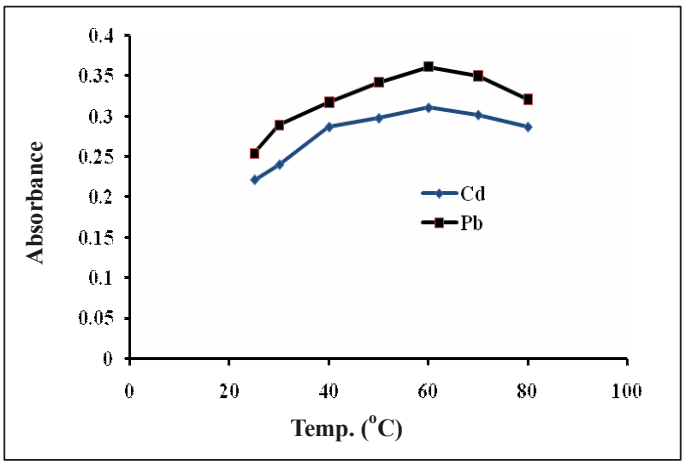

Fig. 7: Effect of the temperature on the absorbance for Pb (II) and Cd (II) complexes (Conditions: Pb (II) = 50 ng mL^{-1}, 0.2 mL of 7-(6-MBTA8HQ) = 5 x 10^{-4} M, Buffer pH = 4 (1 mL), 0.5 mL of 10 % (v/v) Triton X-114. Cd (II) = 4.7 ng mL^{-1}, 0.3 mL of 7-(6-MBTA8HQ) = 5 x 10^{-4} M , Buffer pH= 5 (1 mL), 0.4 mL of 10 % (v/v) Triton X-114.)

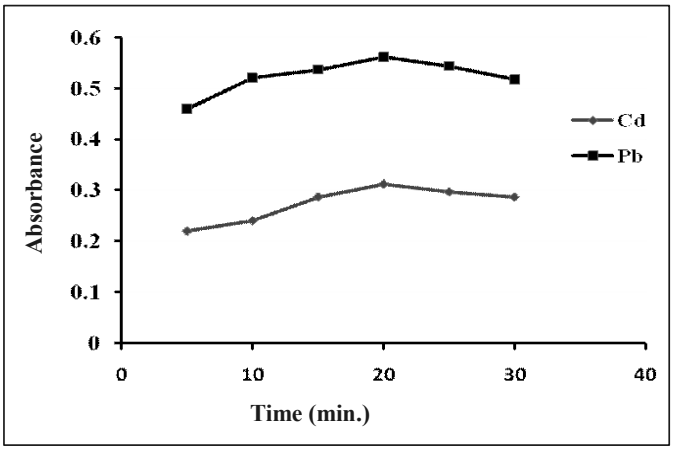

Fig. 8: Effect of the incubation time on the absorbance for Pb (II) and Cd (II) complexes Conditions: Pb (II) = 80 ng mL^{-1}, 0.2 mL of 7-(6-MBTA8HQ) = 5 x 10^{-4} M, Buffer pH = 4 (1mL), 0.5 mL of 10 % (v/v) Triton X-114. Cd (II) = 4.7 ng mL^{-1}, 0.3 mL of 7-(6-MBTA8HQ) = 5 x 10^{-4} M, buffer pH= 5 (1 mL), 0.4 mL of 10 % (v/v) Triton X-114.)

Table 1: Method validation of the spectrophotometric determination of Pb (II) and Cd (II) using CPE procedure

Parameter	Pb (II)	Cd (II)
λ_{max} (nm)	524	550
Regression equation	A= 6.1494 C + 0.0575	A = 60.501C + 0.0316
Correlation coefficient(r)	0.9993	0.9994
C.L. for the slope (b ± tsb*)* at 95%	6.1494 ± 0.3080	60.501 ± 1.5603
C.L. for the intercept (a ± tsb*)* at 95%	0.0575 ± 0.0187	0.0316 ± 0.0056
Concentration range (ng mL^{-1})	5-100	0.32-7.50
Limit of detection (ng mL^{-1})	3.9	0.19
Limit of quantitation (ng mL^{-1})	13.1	0.64
Sandell's sensitivity ($\mu g.cm^{-2}$)	1.04×10^{-4}	1.12×10^{-5}
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	2×10^{6}	1×10^{7}
Composition of complex (M: L)*	1 : 2	1 : 2
RSD% (n=7) at 10 ng Pb mL^{-1} and 0.94 ng Cd mL^{-1}	1.12%	1.79%
Recovery%	96.27 ± 3.53	97.57 ± 3.48
Preconcentration factor	71	91
Enrichment factor	114	84

*Job's and mole ratio methods used

The results showed that a linear calibration curve was obtained in the range of 5-100 ng mL^{-1} lead and the linear regression equation was;

A = 6.1494 conc. + 0.0575 (r = 0.9993)

A linear calibration curve was obtained in the range of 0.32-7.50 ng mL^{-1} cadmium and the linear regression equation was:

A = 60.501 conc. + 0.0316 (r = 0.9994)

These regression lines have a coefficient of determination (R^2) of 99.87% and 99.88% for lead and cadmium respectively, which suggest they are statistically valid.

The molar absorptivity, Sandell's sensitivity with composition of complex is also given in Table 1. The limit of detections obtained for both metal ions by the proposed

method were generally much better than that obtained by ICP-OES and FAAS techniques (Table 2). However, they were worse than that obtained by ETAAS using platform atomization as shown in Table 2.

Table 2: Comparison of the proposed method with reported methods for the pre-concentration and CPE of lead and cadmium

Sample	Reagent	Surfactant	LOD (ng mL^{-1})	EF	Detection	Ref.
Human hair	DDTP	Triton X-114	Pb (2.68) Cd (0.62)	Pb (43) Cd (22)	FAAS	1
Vegetable hair, urine	BISE	Triton X-114	Pb (2.8) Cd (0.0014)	Pb (39) Cd (48)	FAAS	4
Drinking water	TAC	Triton X-114	Pb (1.05) Cd (0.077)	Pb (58) Cd (22)	FAAS	8
Water	TAN	Triton X-114	Pb (4.5) Cd (0.75)	Between 15.1-20.2	FI-AAS	3
Tobacco	Br-PADAP	Triton X-114	Pb (13) Cd (4)	Pb (158) Cd (62)	TS-FF-AAS	6
Urine	ADDTP	Triton X-114	Pb (0.040) Cd (0.002)	Pb (16) Cd (16)	ETAAS (platform)	2
Blood	DDTP	Triton X-114	Pb (0.08) Cd (0.02)	Pb (34) Cd (71)	ETAAS	7
Biological	DDTP	Triton X-114	Pb (0.04) Cd (0.006)	Pb (18) Cd (129)	ETAAS	9
Calcium-rich	5-Br-PADAP	Triton X-114	Pb (40) Cd (5.3)	---------- ----------	ICP-OES	5
Honey bee	6MBTA-8HQ	Triton X-114	Pb (3.9) Cd (0.19)	Pb (114) Cd (84)	UV-Vis SP	This work

LOD; Limit of detection, EF; Enrichement factor, FAAS : Flame atomic absorption spectrometry, ETAAS: Electrothermal atomic absorption spectrometry, FI-FAAS: Flow

injection FAAS, TS-FF-AAS; Thermospray AAS, SP: Spectrophotometry, DDTP: *O,O*-Diethyldithiophosphate, ADDTP: Ammonium O,O-diethyldithiophosphate, TAN: 1-(2-thiazolylazo)-2-naphthol, BISE: bis((1H-benzo [d] imidazol-2yl)ethyl) sulfane, 5-Br-PADAP: 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol, Br- PADAP: 2-(bromo-2-pyridylazo)-5-diethyl-amino-phenol, TAC : 2-(2-thiazolylazo)-p-cresol, 6MBTA-8HQ : 7-(6-Methoxy 2-benzothiazolyl azo)-8-Hydroxyquinoline.

The enrichment factor which is defined as the ratio of the slope of calibration curves obtained with and without CPE found to be of 114 and 84 for lead and cadmium, respectively. This revealed that the prepared ligand beside CPE-spectrophotometry gave satisfactory analytical figures of merit which is more comparable with those obtained by previous studies using commercial ligands and atomic spectrophotometric techniques (Table 2).

Precision and Accuracy

Seven replicate analyses of 10 ng mL^{-1} lead and 0.94 ng mL^{-1} cadmium solutions following the general CPE procedure gave repeatability in term of relative standard deviation (RSD) of 1.12% and 1.79%, respectively (Table 1).

Because the commercial certified reference material for honey was not available and in order to investigate if the proposed method is subjected to systematic error, the accuracy in term of recovery percent was studied by spiking of 10, 30 and 50 ng mL^{-1} lead and 0.5, 1.0 and 3.0 ng mL^{-1} cadmium to an appropriate amount of honey sample solution prepared previously and then the same steps were followed with the general CPE procedure. The results were tabulated in Table 3, indicating that there is no highly significant systematic error in case of the presence of other constituents in honey matrix.

Table 3: Accuracy of the proposed method

Amount metal ion taken (ng mL^{-1})		Amount metal ion taken (ng mL^{-1})	Rec. (%)	E_{rel} (%)	Mean Rec. % ± t.s/√n
Pb	10	9.50	95.0	-5.0	96.27 ± 3.53
	30	28.8	96.0	-4.0	
	50	48.9	97.8	-2.2	
Cd	0.5	0.48	96.0	-4.0	97.57 ± 3.48
	1.0	0.98	98.0	-2.0	
	3.0	2.96	98.7	-1.3	

Interference Study

The effect of most diverse ions expected in the honey matrix on the determination of 100 ng mL^{-1} Pb (II) and 7.5 ng mL^{-1} Cd (II) solutions were studied following the general CPE 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 indicate that some of metal ions like, Ca (II), Na (I), K (I), As (III) and Sb (II) have no appreciable effect on the Pb and Cd responses, while the other metal ions such as Mg (II), Fe (III), Co (II) and Ni (II) have exceeded the allowable limits of interferences for both Pb (II) and Cd (II) as shown in Table 4 and 5.

Several masking agents such as oxalic acid, citric acid, tartaric acid, 5-sulphosalicylic acid, 1-10 phenathroline, sodium fluoride and ascorbic acid individually or mixture were tested to control the interferences of Fe (III), Co (II) and Ni (II). The experiments have shown that the interference effect of the above ions on Pb absorbance signal was held efficiently by adding of 0.4 mL of 0.01 M 5-sulphosalicylic acid or 0.4 mL of 0.01 M mixture of (oxalic acid + citric acid + sodium fluoride + tartaric acid + 1-10, phenanthroline) without any appreciable masking of Pb ions. Whilst ascorbic acid has substantially decreased the absorbance of Pb due to its competing with chelating agent used thereby it ruled out.

Table 4: Effect of divers ions on the absorption signal of Pb (100 ng mL^{-1} and 0.663 absobance unit) by CPE-spectrophotometry

Interferent	Interferent/Pb (II) ratio	A unit	AΔ	E_{rel} (%)
Ca (II)	1000	0.676	0.013	1.90
Mg (II)	500	0.700	0.037	5.50
Na (I)	1000	0.662	-0.001	-0.15
K (I)	1000	0.668	0.006	0.90
Fe (III)	500	0.720	0.057	8.50
As (VI)	1000	0.650	-0.013	-1.90
Sb (II)	1000	0.650	-0.013	-1.90
Co (II)	500	0.724	0.061	9.20
Ni (II)	500	0.708	0.045	6.70

Other experiments have also been carried out in an attempt to eliminate the interference effects on Cd ions (Table 5).

Table 5: Effect of divers ions on the absorption signal of Cd (7.5 ng mL^{-1} and 0.485 absobance unit) by CPE-spectrophotometry

Interferent	Interferent/Cd(II) ratio	A unit	AΔ	E_{rel} (%)
Ca (II)	2000	0.490	0.005	1.03
Mg (II)	2000	0.527	0.042	8.60
Na (I)	2000	0.479	-0.006	-1.20
K (I)	2000	0.491	0.006	1.20
Fe (III)	500	0.544	0.059	12.2
As (VI)	2000	0.493	0.008	1.60
Sb (II)	2000	0.496	0.011	2.26
Co (II)	2000	0.510	0.025	5.15
Ni (II)	2000	0.514	0.029	5.97

It has shown that ascorbic acid and 1,10 phenanthroline have a high power for masking cadmium ions leading to decrease the absorbance signal significantly, therefore they are excluded. An efficient control of the interfering effects on Cd ions was achieved by using either 0.2 mL of 0.01 M 5-sulphosalicylic acid or 0.6 mL of 0.01 M mixture of oxalic acid, citric acid, sodium fluoride, and tartaric acid without any appreciable masking of Cd ions. Consequently, the presence of interferents had no explicit effect on the target metals with adding the selected masking agents as shown in Table 6.

Table 6: Limits of interfering ions in the determination of 100 ng mL^{-1} Pb (II) and 7.5 ng mL^{-1} Cd (II) by the proposed method

Interfering ion	Interferent/Pb(II)	$\%E_{rel}$[a]	Interferent/Cd(II)	$\%E_{rel}$[a]
Ca (II)	1000	1.20	2000	1.60
Mg (II)	500	-0.60	2000	1.20
Na (I)	1000	0.00	2000	-0.80
K (I)	1000	-1.30	2000	-0.40
Fe (III)	500	1.20	500	1.60
As (VI)	1000	-1.35	2000	1.00
Sb (II)	1000	-1.35	2000	1.20
Co (II)	500	1.50	2000	0.40
Ni (II)	500	0.75	2000	0.80

a: after addition masking agents

Determination of Cd and Pb in Bee honey samples

The developed method was applied to the lead and cadmium determination in three Iraqi apiaries and seven foreign honey samples purchased from local markets. The results of the combined CPE-Spectrophotometry were compared with electrothermal atomic absorption spectrometric method in our laboratory as displayed in Table 7.

Table 7: Spectrophotometric determination of Pb (II) and Cd (II) in honey samples using CPE

Bee honey samples	Concentration of Pb (μg g^{-1})		Concentration of Cd (μg g^{-1})	
	Present method[a]	GF-AAS[b]	Present method[a]	GF-AAS[b]
Iraqi 1*	0.173 ± 0.0035	0.178 ± 0.0028	0.098 ± 0.0036	0.095 ± 0.0007
Iraqi 2*	0.112 ± 0.0025	0.116 ± 0.0010	0.075 ± 0.0027	0.068 ± 0.0007
Iraqi 3*	0.173 ± 0.0035	0.174 ± 0.0012	0.115 ± 0.0035	0.121 ± 0.0007
Iranian 1	0.067 ± 0.0033	0.066 ± 0.0010	0.020 ± 0.0021	0.025 ± 0.0001
Iranian 2	0.145 ± 0.0046	0.146 ± 0.0015	0.039 ± 0.0019	0.045 ± 0.0001
Saudi Arabia 1	0.280 ± 0.0041	0.282 ± 0.0026	0.106 ± 0.0030	0.112 ± 0.0010
Saudi Arabia 2	0.138 ± 0.0035	0.146 ± 0.0020	0.033 ± 0.0029	0.028 ± 0.0001
Germany 1	0.288 ± 0.0037	0.291 ± 0.0010	0.286 ± 0.0033	0.290 ± 0.0010
Germany 2	0.221 ± 0.0036	0.221 ± 0.0010	0.156 ± 0.0030	0.160 ± 0.0020
Spain	0.184 ± 0.0027	0.189 ± 0.0017	0.128 ± 0.0034	0.129 ± 0.0012

[a] mean ± standard deviation; n = 5
[b]mean ± standard deviation; n = 3
*from Al-Diwaniyah city, Al-Qadyisia Governorate

The student t-test was applied to evaluate the significance level of results obtained between the proposed method and the sensitive ETAAS for further checking of the applicability and reliability of CPE-spectrophotometric method. The statistical analysis of the results shown in Table 7 proved that there was no significant difference at 95% confidence level for the determination of Pb and Cd by both methods. The calculated experimental values $|t|$ were 0.09 and 0.05 for Pb and Cd results, respectively. These values are less than the critical $t_{0.05,16}$ =2.101(two-tailed) indicating that no difference between the two means (Ho, $\mu_1=\mu_2$) and the resultant p values [P(T<=t) two-tailed] were 0.930 and 0.971

for both Pb and Cd, respectively, higher than 0.05 which also lead to the same statistical conclusion. For the assessment the precision of the measurements by both methods, F-test analysis provided evidence that the variability in the proposed method and ETAAS were not significantly different at 95% confidence level as the test statistic F of 1.00 and 0.97 in Pb and Cd measurements obtained by both methods were less than the critical $F_{0.05, 9,9} = 4.03$ two-tailed.

The results in Table 7 revealed that mean values obtained of Pb in all the selected samples were considerably below the permissible criteria i.e < 0.3 μg g^{-1} and ranged from 0.067 to 0.288 μg g^{-1} according to the norms established by the Codex Alimentarius Commission of the Food Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations[39]. Whereas Cd concentration exceeded the permissible level for the most selected bee honey according to Codex Alimentarius Commission norms which should be at 0.03 μg g^{-1}. However, Cd concentration in most samples (Table 7) gave below the permissible levels according to European Union Standards[40] i.e.<. 0.1 μg g^{-1} and ranged from 0.02 to 0.098 μg g^{-1}.

CONCLUSION

Since its establishment, the works reported by using the bride CPE methodology in combination with UV-Vis spectrophotometric techniques are so scanty compared with other spectrometric techniques. Thus, the present study offers a new avenue of improving conventional analytical methods toward easy, simple and inexpensive routine analyses instead of using expensive instrumentations in this field such as FI-AAS, TS-FF-AAS ETAAS, ICP-OES (Table 2). The simple synthesized ligand used in this work increases the sensitivity, limit of detection, selectivity and popularity of UV-Vis Spectrophtometry after CPE beside the solvent-free extraction of toxic metals from its matrix. The proposed method was successfully applied for the determination of Pb and Cd in the presence of each other in honey matrix from different sourses and can be used for the determination of toxic metals in any food matrices as an alternative to other spectrometric techniques.

REFERENCES

1. G. Celli and B. Maccagnani, B. Insectol., **56**, 137 (2003).
2. P. Przybylowski and A. Wilczynska, Food Chem., **74**, 289 (2001).
3. C. Fredes and G. Montenegro, Cien. Inv. Agr., **33**, 50 (2006).
4. F. Kummrow, F. F. Silva, R. Kuno, A. L. Souza and P. V. Oliveira, Talanta, **75**, 246 (2008).

5. V. A. Lemos and A. L. de Carvalho, A Review, Environ. Monit. Assess., **171**, 255 (2010).

6. D. W. Ball, J. Chem. Educ., **84**, 1643 (2007).

7. M. D. Ioannidou, G. A. Zachariadis, A. N. Anthemidis and J. A. Stratis, Talanta, **65**, 92 (2005).

8. P. Pawel, Trends in Anal. Chem., **28**, 117 (2009).

9. M. Madejczyk and D. Baralkiewicz, Anal. Chim. Acta, **617**, 11 (2008).

10. T. Golob, U. Dobersek, P. Kump and M. Necemer, Food Chem., **91**, 593 (2005).

11. A. Khuder, M. Ahmad, R. Hasan and G. Saour, Microchem. J., **95**, 152 (2010).

12. G. Sanna, M. I. Pilo, P. C. Pin, A. Tapparo and R. Seeber, Anal. Chim. Acta, **415**, 165 (2000).

13. P. Buldini, S. Cavalli, A. Mevoli and J. Lal Sharma, Food Chem., **73**, 487 (2001).

14. M. Das Grac, A. K. Jailson, B. De Andrade, D. S. De Jesus, V. A. Lemosc, L. S. F. Bandeira, W. N. L. Dos Santos, M. A. Bezerra, F. A. C. Amorim, A. S. Souza and S. L. C. Ferreira, A Review, Talanta, **69**, 16 (2006).

15. S. L. C. Ferreira, J. B. De Andrade, M. Das Grac, A. Korn, M. G. Pereira, V. A. Lemos, W. N. L. Dos Santos, F. M. Rodrigues, A. S. Souza, H. S. Ferreira and E. G. P. Da Silva, Review, J. Hazard. Materials, **145**, 358 (2007).

16. J. L. Manzoori and A. Bavili-Tabrizi, Microchem. J., **72**, 1 (2002).

17. J. Szymanowski and J. Radioanal, and Nucl. Chem., **246**, 635 (2000).

18. J. Chen and K. C. Toe, Anal. Chim. Acta, **450**, 215 (2001).

19. J. L. Manzoori and A. Bavili-Tabrizi, Anal Chim. Acta, **470**, 215 (2002).

20. T. A. Maranhão, E. Martendal, D. L. G. Borges, E. Carasek, B. Welz and A. J. Curtius, Spectrochim. Acta, Part B, **62**, 1019 (2007).

21. H. Han, Y. Xu and C. Zhang, Communications in Soil Science and Plant Analysis, **42**, 1739 (2011).

22. J. L. Manzoori and H. Abodlmohammad-Zadeh, Acta Chim. Slov., **54**, 378 (2007).

23. L. A. Portugal, H. S. Ferreia, W. N. L. Dos Santos and S. L. C. Ferreia, Microchem. J., **87**, 77 (2007).

24. J. Chen, A. Xiao, X. Wu, K. Fang and W. Lui, Talanta, **67**, 992 (2005).

25. M. A. Bezerra, S. M. Do Nascimento Maêda, E. P. Oliveira and M. De Carvalho and R. E. Santelli, Spectrochim Acta, Part B, **62**, 985 (2007).

26. M. Ghaedi, A. Shokrollahi, K. Niknamb, E. Niknam, A. Najibi and M. Soylak, J. Hazard. Mater., 168, 1022 (2009).

27. H. C. Rezende, C. C. Nascentes and N. M. M. Coelho, Microchem. J., **97**, 118 (2011).

28. E. L. Silva, P. S. Roldan and M. F. Giné, J. Hazard. Maters., **171**, 1133 (2009).

29. D. Citak and M. Tuzen, Food and Chem. Toxicology, **48**, 1399 (2010).

30. F. Shemrani, S. D. Abkenar and A. S. Mirroshandel, Anal. Sci., **19**, 1453 (2003).

31. N. H. Youcef, T. H. Benabdallah and H. Ilikti, Can. J. Anal. Sci. Spect., **51**, 267 (2006).

32. M. A. Akl, Anal. Sci., **22**, 1227 (2006).

33. F. Ahmad, K. Niknam, I. E. Niknam, S. Delavari and A. Khanmohammadi, E-J. Chem., **8**, 435 (2011).

34. E. Kilinc, A. Cetin, M. Togrul and H. Hosgoren, Anal. Sci., **24**, 763 (2008).

35. G. Mehrorang, S. Ardeshir, N. Khodabakhsh, E. Niknam, D. Derki, D. Somayyeh and S. Mustafa, J. AOAC Int., **92**, 907 (2009).

36. V. A. Lemos, E. S. Santos, M. S. Santos and R. T. Yamaki, Microchim. Acta, **158**, 189 (2007).

37. M. G. A. Korn, A. C. Ferreira, L. S. G. Teixeira and A. C. S. Costa, J. Braz. Chem. Soc., **10**, 46 (1999).

38. R. Leardi, Anal. Chim. Acta, **652**, 161 (2009).

39. Anonymous Codex Alimentarius Commission Joint FAO/WHO Food Standards Programme Recommended European-Regional Standard, Agenda Item 5 CX/PFV 00/4 Add, 1 July (2000).

40. D. Byrne, EC Commission Decision (draft) Amending Annex II to Council Directive 92/118/EEC (2000).

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