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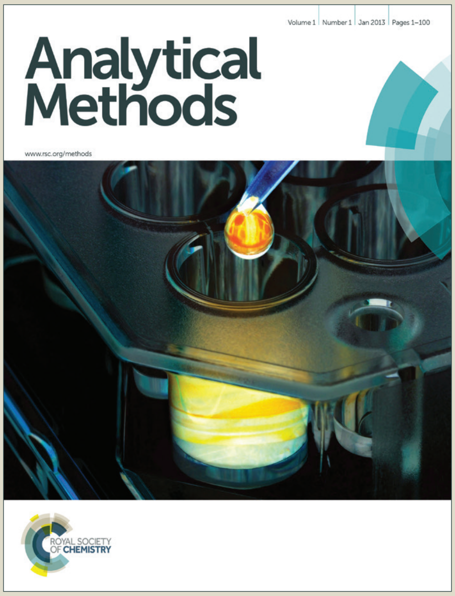

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Dispersive liquid-liquid microextraction for microvolume spectrophotometric determination of bismuth in pharmaceutical formulation and human serum

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Abstract

A new dispersive liquid–liquid microextraction (DLLME) method coupled with microvolume UV-vis spectrophotometry was developed for the determination of trace amounts of bismuth. The method is based on the complex formation of Bi(III) with iodide (BiI_4^-) and extraction into carbon tetrachloride as an ion pair assisted by methyltrioctylammonium chloride, which also acts as a disperser agent. The effect of important parameters, such as concentration of sulfuric acid and iodide in the sample solution, amount of methyltrioctylammonium chloride, type and volume of extraction solvent were investigated and optimized. The presented method is capable of determining bismuth in the range of 5 to 400 ng mL^{-1} with a limit of detection 1.6 ng mL^{-1}. The relative standard deviation for eight replicate measurements of Bi(III) at 200 and 75 ng mL^{-1} concentration levels was calculated to be 1.14 and 2.66 %, respectively. The developed method was successfully applied to the determination of bismuth in bismuth subcitrate tablet and human serum samples.

Keywords: Bismuth; Dispersive liquid–liquid microextraction; Microvolume spectrophotometry; Bismuth subcitrate; Human serum

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1. Introduction

Bismuth has been associated with medicine for more than two century, because of its demulcent properties in the gastrointestinal disorders.[1] Its pharmaceutical uses have expanded to antiacids, peptic ulcer treatments, radiotherapeutic agents and topical dermatological creams by some of its colloidal salts (like subcitrate and subgallate).[2] However, a number of toxic effects in human beings have been reported for the use of bismuth-containing pharmaceuticals, such as nephrotoxic, neurotoxic, kidney damage symptoms nephropathy.[3] Bismuth and its compounds are also used in the cosmetic industry, semiconductors, fuel carrier, alloys and metallurgical additives, fabrication of catalysts and in the preparation and recycling of uranium nuclear fuels.[4] As the use of bismuth and its compounds in different areas of life increased, its spread in the environment and the chance of exposure of organisms to bismuth has increased. Therefore, due to the presence of bismuth in environmental and biological samples at low levels, the demand for sensitive, low cost, rapid and reliable analytical methods for the preconcentration and determination of this element has increased.

A variety of techniques have been developed for the determination of bismuth in different samples. These include spectrophotometry,[5] flame atomic absorption spectrometry,[6, 7] electrothermal atomic absorption spectrometry,[8] hydride generation atomic absorption spectrometry,[9] X-ray fluorescence,[10,11] inductively-coupled plasma mass spectrometry,[12,13] inductively-coupled plasma optical emission spectrometry [14,15] and voltammetry.[16,17] Among the above mentioned techniques, UV-vis spectrophotometry is simplest and cost-effective technique for routine analysis and is also available in many laboratories. However, direct determination of bismuth is seldom carried out due to the relatively poor sensitivity of this approach. Thus, a preconcentration step before the detection of bismuth by UV-vis spectrophotometry is often required. The main purpose of the sample preparation is to isolate and concentrate the analytes of interest. Among the pretreatment methods, liquid–liquid extraction (LLE)[1] and solid phase extraction (SPE) are often used. Although these methods show good extraction and preconcentration efficiency, SPE is time consuming and labor intensive, and LLE require large volumes of poisonous organic solvent. Thus miniaturized, solvent free, rapid and environmental friendly sample preparation techniques are needed to overcome these limitations. Recent research has been oriented towards the miniaturized LLE method, which consequently led to the development of dispersive liquid–liquid microextraction (DLLME) by Assadi and his co-workers.[18] DLLME is based on a ternary component solvent system similar to homogeneous liquid–liquid extraction and cloud point extraction. In this technique an appropriate mixture of a water-immiscible extraction solvent and a water-miscible dispersive solvent is injected into an aqueous sample solution, forming a cloudy solution which markedly increases the contact surface between phases and reduces the extraction time with the increasing enrichment factors. The advantages of DLLME are simplicity and rapidity of operation, low cost, high recovery and enrichment factor and short extraction time. This method has been applied for the determination of trace organic compounds and metal ions in the different samples.[19-25]

The aim of this work was to develop a new method for the determination of bismuth using DLLME in combination with microvolume UV–vis spectrophotometry. The presented methodology is based on the microextraction of bismuth as an ion pair formed between BiI_4^- and cationic counter ion provided by methyltrioctylammonium chloride which also acts as dispersive agent. The parameters affecting the extraction efficiency were studied and optimized.

2. Experimental

2.1 Apparatus

Recording the spectra was performed by a GBC UV– vis spectrophotometer model Cintra 101 (Australia) and the absorbance measurements were made by a Jenway UV-vis spectrophotometer model 6320 (England) using quartz microcells with capacity of 350 µL. A Metrohm 632 (Switzerland) pH-meter was used to measure pH with a combined glass electrode. A model BHG HERMLE centrifuge (Germany) was used for the phase separation.

2.2 Reagents

All chemicals were of analytical grade and double distilled water was used throughout. A stock solution of 100 µg mL^{-1} of Bi(III) was prepared by dissolving 0.0232 g of bismuth nitrate pentahydrate (Merck, Germany) in 5 mL of concentrated sulfuric acid and diluting to 100 mL in a volumetric flask with distilled water. Working standard solutions were prepared daily by successive dilutions of this stock solution. A 0.10 M solution of potassium iodide was prepared by dissolving 1.660 g of potassium iodide (Merck) and diluting to 100 mL. Sulfuric acid solution was prepared by appropriate dilution of concentrated sulfuric acid (Fluka, 98 %, d=1.84 g mL^{-1}). Methyltrioctylammonium chloride was purchased from Merck.

2.3 Dispersive liquid–liquid microextraction procedure

An aliquot of Bi(III) solution, 1.5 mL of 0.05 M of sulfuric acid and 2 mL of 0.1 M potassium iodide were placed in a 10 mL volumetric flask and diluted to the mark with water and mixed thoroughly. The resulting solution was transferred to a glass test tube with a conical bottom, and subsequently 160 µL of carbon tetrachloride containing 1.6 mg of methyltrioctylammonium chloride was rapidly injected into the sample solution by a microsyringe to induce the formation of a cloudy solution, and the mixture was shaken manually. At this step, ion pair of BiI_4^- and methyltrioctylammonium cation was extracted into the fine droplets of carbon tetrachloride. The mixture was centrifuged at 5000 rpm for 5 min after which the dispersed fine droplets of the extraction phase were settled as an orange-yellow color at the bottom of the conical test tube (155 ± 5 µL). The settled phase was removed using a microsyringe and placed into the quartz microcell and its absorbance was measured at 492 nm against the blank. A blank solution was also run under the same procedure without adding any Bi(III).

2.4 Preparation of samples

For preparation of pharmaceutical sample, a bismuth subcitrate tablet was powdered and dissolved in 5 mL of concentrated nitric acid. The mixture was heated to dryness. Three sequential additions of 10 mL distilled water were then made and each times the solution was evaporated almost to dryness to eliminate the excess acid. The residue was dissolved in distilled water and filtered using a filter paper. The filtrate solution was diluted to 100 mL with distilled water in a volumetric flask [3]. An aliquot of this solution was analyzed for determination of bismuth using the DLLME procedure.

The human serum samples were collected from Blood Transfusion Organization (Ahvaz, Iran). 0.5 g of each sample was acidified with 1 mL of 20 % (w/v) trichloroacetic acid solution to precipitate the proteins. The mixture was diluted to 5 mL, then stirred for 5 min and centrifuged for 10 min at 5000 rpm. The supernatant was decanted and filtered through a filter paper into a 25 mL volumetric flask, afterward diluted to the mark with water [26]. An aliquot of this solution was subjected to the proposed DLLME procedure.

3. Results and discussion

The developed DLLME technique is based on the formation of BiI_4^- in aqueous solution and its extraction into organic phase as an ion pair assisted by methyltrioctylammonium chloride which allows the microvolume spectrophotometric detection of bismuth in the enrichment phase. Unlike the conventional DLLME, in this extraction method it is not required to use a dispersive solvent because methyltrioctylammonium chloride also plays the role of a disperser agent which is injected along with extraction solvent into the aqueous solution. In order to find the appropriate conditions for DLLME, different experimental parameters were studied and optimized.

3.1 Wavelength selection

In order to perform the quantification analysis by UV–vis spectrophotometry, the maximum absorption wavelength was established. Therefore, the sample solutions containing different concentrations of Bi(III) were examined according to the recommended procedure of DLLME and the corresponding spectra of sedimented phase were recorded in the range of 400-650 nm. As is evident in Fig. 1, the maximum absorption wavelength is at 492 nm for sedimanted phase, and upon increasing of Bi(III) concentration the absorbance at this wavelength is increased. Therefore, 492 nm was selected for measuring the absorbance of the extracted phase throughout this study.

3.2 Effect of sulfuric acid concentration

The preliminary experiments showed that the highest extraction efficiency of bismuth was achieved in acidic medium. It is well known that, Bi(III) can only exist in very acidic media and it is hydrolyzed at pH values higher than 2.5. The obtained results also showed that, the extraction efficiency was decreased at pH values higher than 2.5 which could be due to the decrease in the concentration of Bi(III) by conversion to $BiOH^{2+}$, BiO^+ or other species [27]. To achieve suitable acidic media, the effect of different concentrations of sulfuric acid was investigated. The results revealed that, the maximum absorbance was obtained in the range of 0.005- 0.010 M of sulfuric acid. Thus, a concentration of 0.007 M of sulfuric acid in the final solution was used as the optimum for further studies.

3.3 Effect of iodide concentration

Bi(III) forms a negatively charged complex, BiI_4^- with iodide which can be extracted to sedimanted phase along with methyltrioctylammonium as a counter ion. Therefore, the presence of iodide ion in the appropriate concentration is important on the extraction efficiency. For this means, the influence of iodide concentration in the range of 0.01-0.06 M on the DLLME of Bi(III) was investigated. Fig. 2 shows that the maximum value in the absorbance of organic phase was obtained over the range of 0.02-0.04 M of iodide and above this value it was decreased. Therefore, a concentration of 0.02 M of iodide was chosen as optimum.

3.4 Nature of the extraction solvent and disperser solvent

The selection of a suitable extraction solvent in DLLME is an essential consideration for efficient extraction. It should have several characteristics including low volatility, low water solubility, higher density than water and high capability for extracting target compound. Based on these requirements, carbon tetrachloride, trichloromethane and dichloromethane were chosen as extraction solvent. Therefore, the investigation was carried out using different volumes of these extraction solvents containing a constant amount of methyltrioctylammonium chloride, to achieve 160 µL of sedimented phase. The cloudy solution and two-phase system appeared by applying carbon tetrachloride and trichloromethane whereas this event was not occurred using dichloromethane. However, carbon tetrachloride was chosen, because in this case the highest and reproducible signal was observed, in addition smaller volume of extraction solvent was needed in the injection step.

In comparison with conventional DLLME method, the addition of methyltrioctylammonium chloride accelerates the formation of fine droplets of the extraction solvent in aqueous sample and increases the extraction efficiency. Although, methyltrioctylammonium chloride acts as disperser agent, the influence of several conventional disperser solvents such as methanol, ethanol, acetonitrile and acetone was studied. For this reason, supplementary experiments were performed via injection of a mixture containing appropriate amount of carbon tetrachloride, methyltrioctylammonium chloride and mentioned disperser solvents. The results showed that carbon tetrachloride is partially dissolved in disperser solvent and migrates into aqueous phase and the dissolved carbon tetrachloride is not sedimented down well and consequently the extraction efficiency is decreased. Finally, the suggested method was carried out only by injecting the extraction solvent (without disperser solvent) containing appropriate amount of methyltrioctylammonium chloride to the sample solution. Moreover unlike the routine disperser solvents, methyltrioctylammonium chloride could dissolve the smallest volume of carbon tetrachloride in aqueous solution, therefore volume of sedimented phase almost equal to the volume of injected carbon tetrachloride, which is an advantage for the proposed method.

3.5 Effect of methyltrioctylammonium chloride amount

As mentioned previously, injection of the methyltrioctylammonium chloride along with extraction solvent made a stable cloudy solution, without the aid of conventional disperser solvent. Consequently, the fast equilibrium (less than 30 s) was achieved due to the formation of very fine solvent droplets, which greatly enlarged the contact area between the extraction solvent and aqueous phase. According to this process the quick transition of analyte was occurred from aqueous phase to extraction solvent. Therefore, the study of the amount of methyltrioctylammonium chloride on the extraction of Bi(III) is very important because it plays both as an ion pair assisted and disperser agent. For this purpose, 160 μL of carbon tetrachloride containing different amounts of the methyltrioctylammonium chloride over the range of 0.0 to 3.6 mg was injected into the sample solutions. The experimental results are shown in Fig. 3. It is obvious that the absorbance increased up to 1.6 mg of methyltrioctylammonium chloride and then remained constant. Thus, 1.6 mg of methyltrioctylammonium chloride was chosen for the purpose of this study.

3.6 Effect of extraction solvent volume

The volume of the extraction solvent has strong effect on the enrichment factor and sensitivity due to its influence on volume of sedimented phase. For that reason a series of experiments were carried out with different volumes of carbon tetrachloride as extraction solvent in the range of 160 to 350 μL containing 1.6 mg of methyltrioctylammonium chloride. Using injection volumes less than 160 μL of the mixture, low volume of organic phase was obtained, so that the absorption signal could not be measured by spectrophotometer fitted with microcells. The obtained results showed that the absorbance of organic phase was decreased by increasing the carbon tetrachloride volume. This could be due to the dilution effect which decreases the concentration of the extracted species in sedimented phase. Thus, in order to achieve high enrichment factor and low detection limit, 160 μL of carbon tetrachloride containing 1.6 mg of methyltrioctylammonium chloride was selected as optimum volume of extraction solvent.

3.7 Effect of extraction time

Extraction time is usually a key parameter in DLLME due to its time-dependent nature. This term is defined as the time interval between injection of the mixture of solvents and the start of centrifugation [18]. Therefore, its effect was studied in the range of 0.5-5 min and the obtained results indicated that the extraction time does not have a significant influence on the extraction efficiency of Bi(III). This reveals that the extraction process is time-independent and very fast, due to the large surface of contact between the extraction solvent and the aqueous phase. This is the most important advantage of the DLLME technique.

The influence of centrifugation time was also studied. The results indicated that for a complete separation of organic and aqueous phase the mixture should be centrifuged for 5 min at 5000 rpm.

3.8 Interference studies

The effect of various ions on the determination of Bi(III) using the proposed DLLME method was studied. In these experiments, different amounts of other species were added to a solution containing 100 ng mL^{-1} of Bi(III) and the recommended procedure was applied. An error of ± 5 % in the absorbance reading was considered tolerable. The results presented in Table 1 show the good selectivity of the method.

3.9 Analytical features of the method

Under the optimized experimental conditions, analytical parameters (i.e., linear range, repeatability, limit of detection and preconcentration factor) were investigated. The corresponding calibration curve was linear in the range of 5–400 ng mL^{-1} of Bi(III) with regression equation A=0.0025C (ng mL^{-1}) + 0.0186 (Fig. 4). The results are summarized in Table 2. The preconcentration factor for the suggested DLLME method is calculated by the ratio of the sample volume (10 mL) and the final volume (160 μL). Thus, a preconcentration factor of about 62.5 was achieved using presented work. A comparison between the presented approach and some of the previously reported preconcentration methods for the determination of Bi (III) are given in Table 3. As can be seen in table the LOD, enrichment factor and precision of suggested method is better or comparable to many of the reported techniques.

3.10 Application to real samples

3.10.1 Analysis of bismuth in bismuth subcitrate tablet

The accuracy of the proposed method was evaluated by the analysis a commercial tablet of bismuth subcitrate containing 120 mg Bi_2O_3. According to the obtained result by the presented method the amount of Bi_2O_3 is 120.8 ± 1.4 mg which is in good agreement with the reported value.

3.10.2 Human serum samples

The present method was also utilized for the determination of Bi(III) in different human serum samples. The analyzed results are shown in Table 4. It was found that except for one sample, the amount of bismuth in some human serum samples was below the detection limit. Serum samples were spiked at two concentration levels, 50 and 100 ng mL^{-1} and good recoveries were obtained.

4. Conclusions

In the present study, a new method based on DLLME coupled to microvolume UV–vis spectrophotometry has been developed for sensitive determination of Bi(III) in pharmaceutical and human serum samples. Miniaturization of toxic organic solvent using dispersive liquid–liquid microextraction combined with microvolume UV–vis spectrophotometry allows the development of a green method which is environment-friendly. Besides simplicity of operation, rapidity, low sample volume, low cost and high preconcentration factor are some advantages of the suggested method. In this method, there is no need for additional dispersive solvent because methyltrioctylammonium chloride, as providing ion pair with BiI_4^-, acts as a disperser too. Some liquid phase microextraction (LPME) coupled with complex instrumental techniques for sensitive determination of Bi(III) have been reported,[13, 31-33] but the presented microvolume spectrophotometric method does not require sophisticated instruments. Furthermore, the application of spectrophotometric detection has merits of simplicity, cheapness and portability.

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Table 1 Effect of interfering ions on determination of 100 ng mL^{-1} Bi(III) using DLLME.

Ions	Tolerance ratio (w/w)
Na^+, K^+, Mg^{2+}, Ni^{2+}, Ca^{2+}, Pb^{2+}, Mn^{2+}, Cl^-, NO_3^-, CO_3^{2-}, HCO_3^-, Acetate	1000
NH_4^+, Ag^+, Cd^{2+}	800
Br^-, Zn^{2+}, Al^{3+}, Cr^{3+}, F^-, SO_4^{2-}, PO_4^{3-}	500
Hg^{2+}, Cu^{2+}	100
Au^{3+}, Sb^{3+}, Fe^{3+}	10
Pd^{2+}	5

Table 2 Analytical characteristics of the presented DLLME method for determination of Bi(III).

Parameter	Analytical features
Linear range (ng mL^{-1}) (n=10)	5 - 400
Correlation coefficient (r)	0.9993
Detection limit (ng mL^{-1}) (3σ, n=7)	1.6
Precision (RSD %)(200 and 75 ng mL^{-1}, n=8)	1.14, 2.66
Preconcentration factor (PF)	62.5

Table 3 Comparison of the proposed method with some of the previously reported methods for the preconcentration and determination of bismuth.

Enrichment procedure	Analytical technique	Enrichment factor	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	RSD (%)	Ref.
IP-DLLME	FAAS	28.6	30-1700	3.0	NG [a]	2
SPE	FAAS	333	4.0-300	2.3	2.3	3
RS-CPE	FAAS	43	Up to 40000	4.0	4.2	6
Anion-exchange resin	HG-AAS	NG	NG	0.225	1.55	9
CPE	FI-ICP-AES	81	Up to 50	0.12	2.3	15
CPE	UV–vis	20	4.6-120	2.0	2.41	28
SPE- anion exchanger	UV–vis	NG	40-400	30	< 20	29
N_2-MIP	AES	NG	300-30000	110	NG	30
DLLME	UV-vis	62.5	5-400	1.6	< 3	This work

[a] Not given

Table 4 Determination of Bi(III) in human serum samples by proposed method.

Sample No.	Added (ng mL^{-1})	Found [a] (ng mL^{-1})	Recovery (%)
1[b]	0	46.9 ± 1.2	-
	50	99.4 ± 2.6	105.0
	100	143.4 ± 3.8	96.5
2	0	N. D [c]	-
	50	49.4 ± 1.3	98.8
	100	94.8 ± 2.5	94.8
3	0	N. D	-
	50	50.6 ± 1.3	101.2
	100	96.6 ± 2.6	96.6
4	0	N. D	-
	50	47.8 ± 1.3	95.6
	100	95.2 ± 2.5	95.2
5	0	N. D	-
	50	51.6 ± 1.4	103.2
	100	99.8 ± 2.6	99.8
6	0	N. D	-
	50	51.4 ± 1.4	102.8
	100	101.7 ± 2.7	101.7

[a] Mean ± standard deviation (n = 3)

[b] Amount of Bi(III) was 23.3 μg g^{-1}

[c] Not detected.

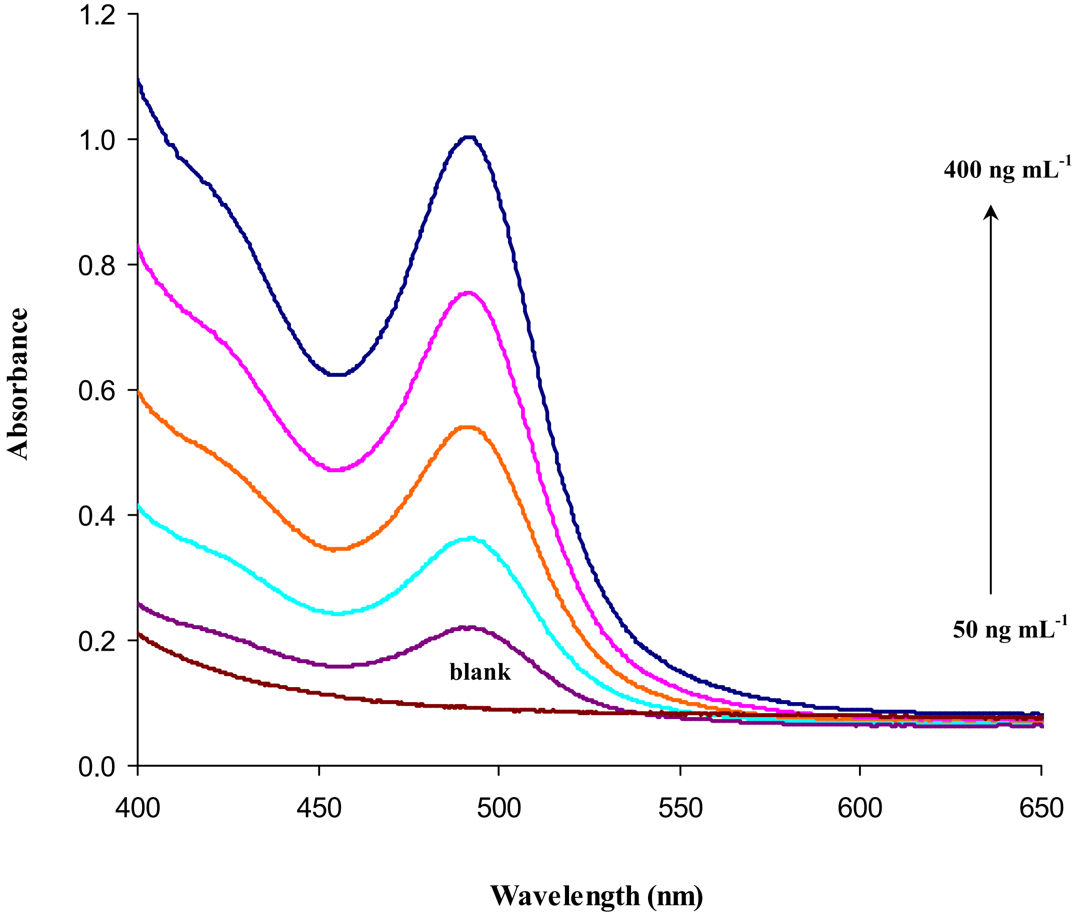
Figure Captions

Fig. 1. UV-visible absorbance spectra of sedimented phase in the absence (blank) and in the presence of 50, 100, 200, 300 and 400 ng mL^{-1} of Bi(III).

Fig. 2. Effect of iodide concentration in DLLME of Bi(III), each point refers to the mean of triplicate measurements. Extraction conditions: aqueous sample volume, 10 mL; sulfuric acid concentration, 0.007 M; amount of methyltrioctylammonium chloride, 1.6 mg ; volume of carbon tetrachloride, 160 µL; centrifugation time, 5 min.

Fig. 3. Effect of methyltrioctylammonium chloride amount in DLLME of Bi(III), each point refers to the mean of triplicate measurements. Extraction conditions: aqueous sample volume, 10 mL; sulfuric acid concentration, 0.007 M; iodide ion concentration, 0.02 M ; volume of carbon tetrachloride, 160 µL; centrifugation time, 5 min.

Fig. 4 Calibration curve for determination of bismuth using DLLME method. Extraction conditions: aqueous sample volume, 10 mL; sulfuric acid concentration, 0.007 M; amount of methyltrioctylammonium chloride, 1.6 mg; iodide ion concentration, 0.02 M ; volume of carbon tetrachloride, 160 µL; centrifugation time, 5 min.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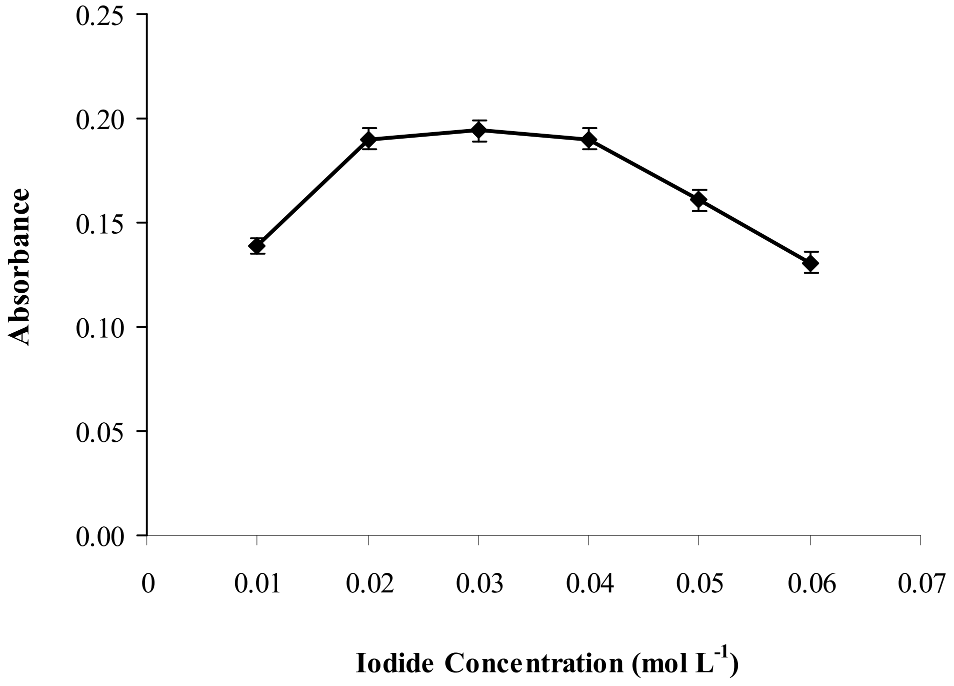
1.0
0.8
0.6
0.4
0.2
0.0
400
450
500
550
600
650
Wavelength (nm)
400 ng mL-1
50 ng mL-1
blank

Fig. 1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0.20
0.15
0.10
0.05
0.00
Absorbance
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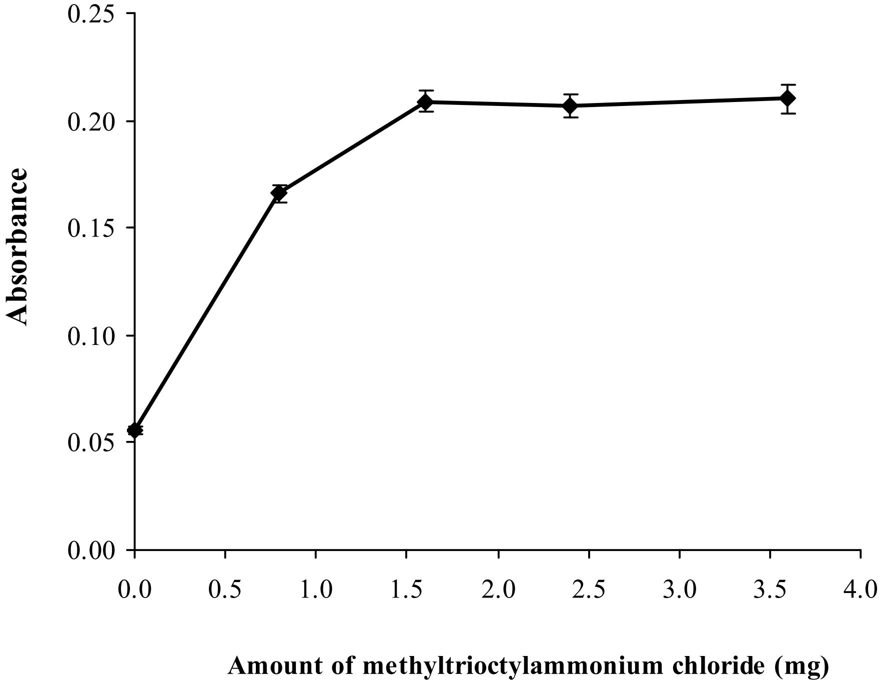
0.02
0.03
0.04
0.05
0.06
0.07
Iodide Concentration (mol L-1)

Fig. 2


Absorbance
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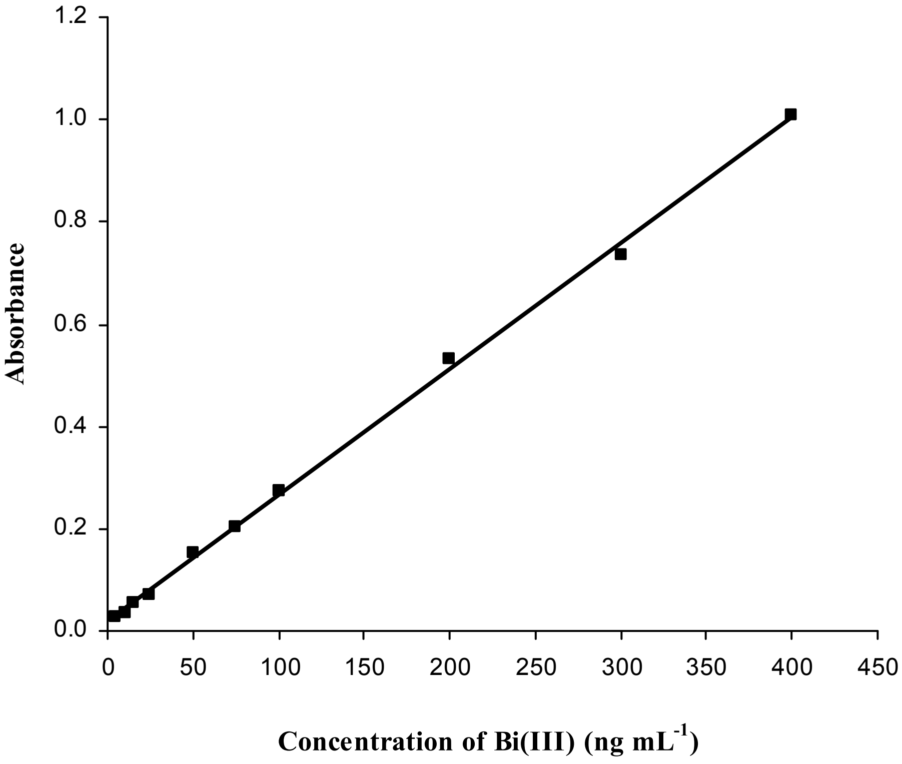
0.10
0.05
0.00
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Amount of methyltrioctylammonium chloride (mg)

Fig. 3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1.0
0.8
0.6
0.4
0.2
0.0
Absorbance
0
50
100
150
200
250
300
350
400
450
Concentration of Bi(III) (ng mL-1)

Fig. 4

Dispersive liquid-liquid microextraction for microvolume spectrophotometric determination of bismuth in pharmaceutical formulation and human serum

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