

Spectrophotometric Determination of Bismuth(III) with Arsenazo(III) Reagent in Water samples and Veterinary Preparation

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Abstract:

A simple , sensitive and accurate spectrophotometric method for the trace determination of bismuth (III) has been developed .This method is based on the reaction of bismuth (III) with arsenazo(III) in acid solution (pH=1.9) to form a blue water soluble complex which exhibits maximum absorption at 612nm .Beer's law is obeyed over the concentration range of 2-85 μg bismuth (III) in a final volume of 20 mL(i.e. 0.1 – 4.25 $\mu\text{g.mL}^{-1}$) with a correlation coefficient of (0.9981) and molar absorptivity $1.9 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$. The limit of detection (LOD) and the limit of quantification (LOQ) are 0.0633 and 0.0847 $\mu\text{g.mL}^{-1}$, respectively . Under optimum conditions,the stoichiometry of the reaction between bismuth (III) and arsenazo(III) reagent was found to be 1:2. The recoveries were obtained in the range of 98.9 - 100.0% and a relative standard deviation of ± 0.59 to $\pm 2.73\%$ depending on the concentration level of bismuth. The effect of interferences by a number of common cations and anions in the presence of composite mixture has been studied .The proposed method has been applied successfully for determination of bismuth in water samples and veterinary preparation .

Keywords: bismuth(III) , ArsenazoIII, spectrophotometry .

Introduction:

disorders especially for colitis , diarrhea and peptic ulcers . They were and still are used for burn bandage dressings ,antiseptic powders , and in the treatment of venereal diseases [4,5] . Bismuth forms low-melting alloys which are extensively used for safety devices in fire detection and extinguishing systems [6] . Bismuth is the least toxic among the heavy metals[1] . A number of toxic effects in humans have been attributed to bismuth compounds, such as nephrotoxic, neurotoxic, kidney damage symptoms nephropathy, osteoarthritis, hepatitis and neuropathology.

Bismuth is a strategic element , thus its identification and determination are very important [1]. It is found in the earth crust up to 0.0002% and it is widely found in the form of oxides , carbonates and sulfides in nature .Bismuth is usually obtained as sub-product in lead , copper , tin and gold ores [1,2].During the industrial metallurgical process of these ores , leaching stages with H_2SO_4 , HCl , and HNO_3 are involved, and highly acidic solutions with base metals and bismuth were obtained [3] Bismuth and its compounds are used in semiconductors , cosmic preparations , medicine for the treatment of syphilis and gastric

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AZIII as a chelating agent for determination of bismuth(III) in water samples and veterinary preparation.

Materials and Methods:

Apparatus

All absorption spectra and absorbance measurements were carried out by a Shimadzu UV-160 double beam UV-visible spectrophotometer (Japan) with matched 1 cm quartz cells. While all pH measurements were recorded by using HANNA 211 pH meter.

Reagents

All chemicals used are of highest purity available.

Stock bismuth (III) solution (1000 $\mu\text{g}\cdot\text{mL}^{-1}$) : It is prepared by dissolving 0.2312g of $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ (Fluka) in 3mL of 5M nitric acid and diluted to the mark with distilled water in a 100 mL volumetric flask [7].

Working bismuth solution (100 $\mu\text{g}\cdot\text{mL}^{-1}$) : This solution is prepared by diluting 10 mL of the stock solution of bismuth to 100 mL with distilled water in a volumetric flask.

Arsenazo III (AZIII) ($5 \times 10^{-4}\text{M}$) : This reagent is prepared by dissolving 0.0388g of AZIII (Fluke) in 100mL distilled water using a volumetric flask. The solution is then transferred to a brown bottle and remained stable for at least one month.

Nitric acid solution (0.1M) : It is prepared by diluting 2.25 mL of concentrated HNO_3 (11.2M) to 100 mL of distilled water in a volumetric flask.

Composite mixture solution(I)(0.01M) : This solution is prepared by dissolving 0.7505g of tartaric acid (BDH) with 0.2100g of sodium fluoride (BDH) in about 400 mL distilled water. The pH of the resulting mixture is adjusted to 1.9 with 0.1M HNO_3 solution and the volume is then completed to 500mL.

As the uses of bismuth in medicine increases, it has spread in the environment and the chance of exposure of organisms to bismuth has increased. Therefore, determination of bismuth in environmental and biological samples is important[7]. Several different techniques have been used for determination of bismuth. These include: hydride generation-atomic fluorescence spectrometry (HG-AFS) [8], Inductively coupled plasma atomic emission spectrometry (ICP-AES) [9], resonance light scattering (RLS) [10], stripping voltammetry [11,17], graphite furnace atomic absorption spectrometry (GFAAS) [5], square wave voltammetry (SWV) [12] and amperometry [13]. A large number of spectrophotometric methods have been also used for determination of bismuth in various samples due to their simplicity, rapidity, low costs and wide applications, for this purpose widely used reagents were xylenol orange [14], 1,2-diaminocyclohexaneN,N,N,N-tetraacetic acid(DACT) [15], 4-(2-benzothiazolylazo)-2,2-biphenyl diol (BTABD) [16], di-(hydrogenated tallow alkyl) dimethylammonium chloride [4], 1-amino-4,4,6-trimethyl(1H,4H) pyrimidine-2-thiol [1], bromopyrogallol red in the presence of triton X-114 [7], methyl thymol blue [18], and pyrocatechol violet (PCV) [19]. Some of these methods suffer from several disadvantages, such as, use of heating step, low range of determination, critical working conditions, no applications, time consuming and poor selectivity. Other methods are typically less sensitive, relatively complicated, or require ion exchange, solvent extraction and expensive instrumentation. These deficiencies have encouraged the authors to develop a simple, selective, sensitive and inexpensive method for the analysis of bismuth. The present work describes the application of

diluted drug solution is then treated as done in a recommended procedure.

Results and Discussion:

The preliminary investigation showed that on mixing 50 µg/20 mL of bismuth (III) with excess of 5×10^{-4} M AZIII reagent and distilled water in volumetric flask a blue binary water soluble complex [Bi(III)-AZIII] was observed and showed a maximum absorption at 613nm against the corresponding reagent blank solution . This observation led us to the development of a sensitive method for determination of bismuth based on a binary complex formation . The effect of various parameters on the absorption intensity of the coloured complex was studied and reaction conditions have been optimized .

Effect of pH

The effect of pH on the colour intensity of [Bi(III)-AZIII] complex is investigated by adding different amounts of 0.1M HNO₃ and 0.1M NaOH solutions .The complex is showed pH dependent absorption maximum at 612 nm, whereas the reagent blank solution showed maximum absorption at 534 nm . The optimum pH range for complex formation is 1.9-1.96 (Fig. 1). pH 1.9 is considered the optimum because of the high absorbance intensity and good colour contrast.

with distilled water using a volumetric flask.

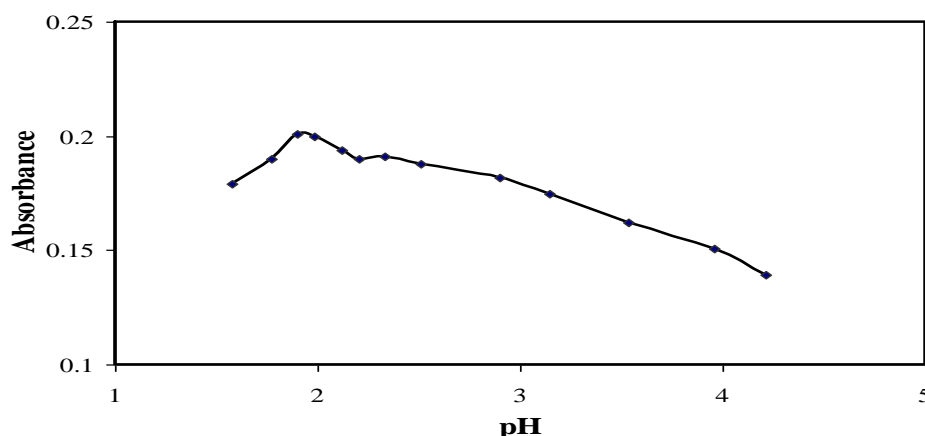
Composite mixture solution (II): It is prepared by dissolving 0.8805g of ascorbic acid (BDH) in 40mL of composite mixture (I) .The pH of the solution is adjusted to 1.9 with 0.1M HNO₃ and the volume is then completed to the mark with distilled water using a 50 mL volumetric flask .This solution is freshly prepared .

Recommended procedure

Aliquots of standard solution (2-100) µg of Bi(III) is transferred into a series of 20 mL volumetric flasks. To each flask, 3 mL of 0.1M nitric acid solution ,2 mL of composite mixture solution(II) and 1.5 mL of 5×10^{-4} M AZIII reagent are added .The contents are then completed to the mark with distilled water and mixed well . The absorbance of the formed coloured complexes are measured at 612 nm against the corresponding reagent blank .

Procedure for dosage form (Veterinary sample)

The content of 3 sachets of Diaclean containing 2000 mg bismuth subnitrate are weighed. A quantity of powder equivalent to 0.01 g of bismuth is weighed and dissolved in 10 mL of 2.5M nitric acid .The solution is then shaken thoroughly ,filtered and diluted with distilled water to 100 mL in a volumetric flask . An aliquot of the



Fig(1). Effect of apparent pH on absorbance of [Bi(III)-AZIII] complex of (50 µg/20ml Bi(III) ; 1.5 mL of 5×10^{-4} M AZIII and 0-5 mL 0.1M HNO₃ and 0.1M NaOH solutions)

formation of binary complex is investigated .The experimental results indicated that a 1.5 mL of 5×10^{-4} M AZIII reagent can be considered optimum because of its highest colour sensitivity and lowest corresponding blank value .

Effect of Masking agent on absorbance

To evaluate the applicability of AZIII reagent to the determination of bismuth , the effect of various masking agents which are usually used for interfering metal ions are studied .The results are shown in table 1.

Different amounts (1-4 mL) of 0.1M solutions of various acids (HNO₃ , HCl , H₂SO₄ and HClO₄) have been examined for the purpose of producing intense coloured complex with a strong colour contrast and lower blank value .The results indicate that HNO₃ is still the best and gives maximum absorbance and good colour contrast at pH1.9 . Therefore , 3 mL of 0.1M HNO₃ has been recommended for the subsequent experiments.

Effect of reagent amount

The influence of different amounts of the AZIII reagent with respect to bismuth(III) on the

Table (1) . Effect of masking agent on absorbance of 50 µg/ 20 mL Bi(III)

Masking agents*	Absorbance/ml. of masking agent			
	0.0	0.5	1.0	2.0
Ascorbic acid (0.1M)	0.267	0.268	0.264	0.262
Tartaric acid (0.01M)		0.269	0.265	0.271
Succinic acid (0.01M)		0.251	0.232	0.206
Sulphosalicylic acid (0.01M)		0.248	0.233	0.220
NaF (0.01M)		0.269	0.267	0.268
EDTA (0.01M)		0.009	0.007	0.007
Nitrilotriacetic acid(NTA) (0.01M)		0.011	0.006	0.009

* The pH of solutions are adjusted to 1.9 ,

The results in Table 1. show that ascorbic acid , tartaric acid and NaF solutions have no effect on the absorbance of [Bi(III)-AZIII] complex , while other masking agents exhibit decreasing in the absorbance owing to their complexing action with bismuth. Therefore , composite mixture solution(II) is prepared by dissolving 0.8507g of ascorbic acid (BDH) in 40 mL of composite mixture (I) and the volume is completed to 50 mL with distilled water in a volumetric flask and its effect on the absorbance of coloured complex is then examined

.The experimental results showed that 2mL of the composite mixture solution(II) was optimum and it was recommended for the subsequent experiment. The order of addition on the absorbance is also investigated . The experiments showed that the order of (Bi(III) + HNO₃ + composite mixture (II) + AZIII) at 612 nm is the optimum because of its high absorbance value .

Effect of surfactants

The presence of surfactants in a coloured reaction mixture solution may frequently lead to an increase in the absorbance intensity and a shift in the wavelength to higher values. In this respect, sodium dodecyl sulphate (SDS) (anionic surfactant), cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC) (cationic surfactants) and Triton X-100 (non-ionic surfactant) have been introduced. The results indicated that addition of surfactants show no useful effect. Therefore, they were omitted in this study.

Effect of time on colour development

To test the effect of time on the absorbance of the coloured complex at 612 nm, the Bi(III) complex has been prepared from different amounts (25 , 50 and 75 μg) of Bi(III) under the optimal experimental conditions, and the absorbance is measured at different time intervals up to 120 min . Table.2 indicates that the colour of the complex develops immediately and the absorbance remains maximum and constant for at least 120 minutes.

Table (2). Effect of time on colour development of Bi(III) complex, (AZIII : 5×10^{-4} M ; pH = 1.9)

μg of Bi(III)	Absorbance / min.										
	Imm.*	5	10	15	20	25	30	40	50	60	120
25	0.133	0.133	0.132	0.133	0.132	0.132	0.131	0.131	0.130	0.131	0.129
50	0.268	0.265	0.263	0.263	0.262	0.262	0.261	0.262	0.260	0.260	0.261
75	0.323	0.324	0.321	0.321	0.321	0.320	0.320	0.318	0.318	0.318	0.316

*Immediately : after dilution to the mark with distilled water using a 20 mL volumetric flask.

Calibration curve

Sandell's sensitivity have been found to be $1.9 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$ and $0.0109 \mu\text{g}.\text{cm}^{-2}$, respectively . The limit of detection (LOD) is $0.0633 \mu\text{g}.\text{mL}^{-1}$ and the limit of quantification (LOQ) is $0.0847 \mu\text{g}.\text{mL}^{-1}$.

A linear calibration curve passes through the origin is obtained over the concentration range of 2- 85 μg of Bi(III) in a final volume of 20 mL (i.e. $0.1 - 4.25 \mu\text{g}.\text{mL}^{-1}$). Higher concentrations show a negative deviation from Beer's law (Fig.2). The apparent molar absorptivity and

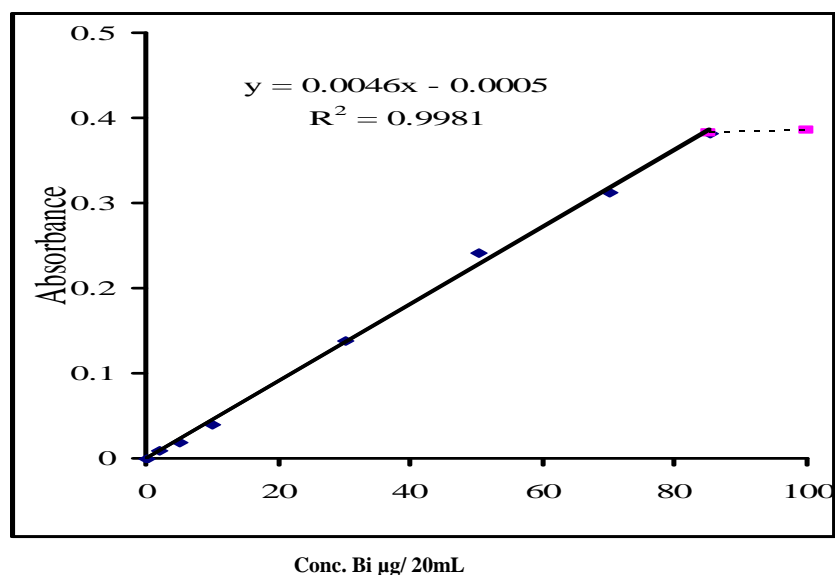


Fig (2). Calibration curve for bismuth determination with 5×10^{-4} M AZIII reagent at pH1.9 and in the presence of 2mL of composite mixture solution(II)

Final absorption spectra

solution(II). The coloured complex exhibits maximum absorption at 612 nm against the reagent blank solution (Fig.3).

Under the above established optimized conditions, bismuth ion forms a blue water soluble complex with AZIII reagent at pH1.9 in the presence of composite mixture

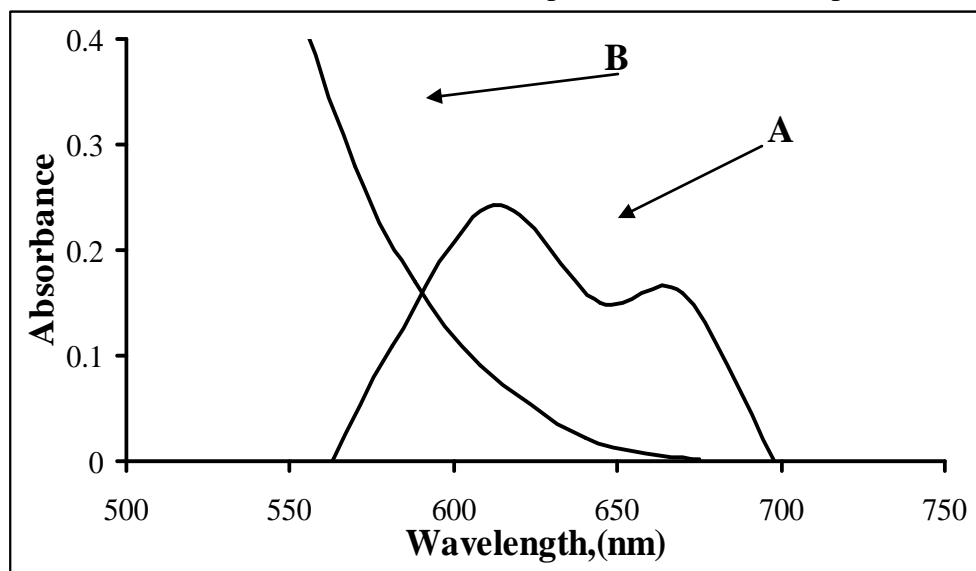


Fig (3). The absorption spectra (A) of 50 µg Bi(III) /20mL treated according to the recommended procedure (1.5 mL of 5×10^{-4} M AZIII and 3 mL of 0.1M HNO_3) measured against blank and (B) blank against distilled water.

Composition of the complex

The stoichiometry of the complex was studied under the established conditions by applying the continuous variation method (Job's method) and mole-ratio method [20]. The experiment results in both methods

(Fig. 4) show that the molar ratio of Bi(III) to AZIII in the complex is found to be 1:2 . The stability constant of the coloured complex is also studied [21] and it was found to be $4.48 \times 10^{11} \text{ M}^{-2}$.

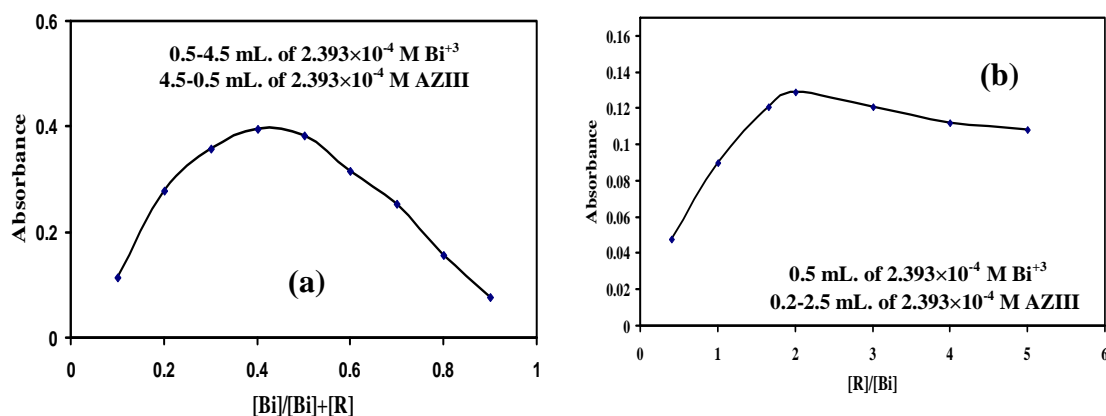


Fig (4) . (a) Continuous variation and (b) mole-ratio plots for Bi(III) – AZIII complex

Effect of Interference

tolerable. As shown in Table 3. The largest interfering species were found for Al^{3+} , Cu^{2+} , Ni^{2+} , Pb^{2+} , Mn^{2+} , Th^{4+} and PO_4^{3-} ions .

Effect of interfering species on determination of 50 $\mu\text{g}/20\text{mL}$ bismuth was studied under optimum conditions with proposed method . An error of $\pm 5\%$ in absorbance is considered

Table (3) . Individual tolerance limit of foreign ions on the determination of 50 μg of Bi (III)

Foreign ions added	Tolerance limit , μg
K^+ , Na^+ , Li^+ , Ag^+ , Fe^{2+} , Br^- , NO_3^-	2000 – 3000
Hg^{2+} , Mg^{2+} , SO_4^{2-}	1000
SO_3^{2-} , Cd^{2+} , Ca^{2+} , Ba^{2+} , Zn^{2+} , NH_4^+	500
Fe^{3+} , Co^{2+} , La^{3+} , Cr^{2+}	250
Cu^{2+} , Ni^{2+}	150
PO_4^{3-} , Pb^{2+} , Mn^{2+}	50
Al^{3+} , Cu^{2+}	25
Th^{4+}	<10

Also the effect of some foreign substances (e.g., glucose, lactose, starch and gum arabic), that usually present in dosage forms were studied by adding different amounts of foreign

substances to 50 μg of bismuth . It is observed that the studied foreign species did show any interfere with the proposed method (Table 4).

Table (4) . Effect of additives and excipients on the determination of 50 µg of Bi(III)

Interferences	Recovery(%) of 50 µg Bi(III)/µg of interferences		
	250	500	1000
Glucose	99.2	98.0	98.0
Lactose	102.4	100.8	100.4
Starch	100.0	100.4	101.6
Gum Arabic	99.6	98.0	98.0

Determination of Bi (III) in water samples

The proposed method has been successfully applied to the determination of bismuth(III) at three different concentrations added to

appropriate volumes of tap , river and sea water samples. The results are compiled in Table 5 and showed that the proposed method is suitable for determining bismuth with satisfactory recovery.

Table (5) . Determination of Bi (III) in water samples

Sample	ml. of sample	Bi(III) added,(µg)	Recovery*, (%)
Tap water	2	25	98.3
		50	98.8
		75	100.8
	5	25	96.7
		50	97.6
		75	99.2
	10	25	97.5
		50	98.4
		75	101.8
River water (Tigris river)	2	25	101.6
		50	100.8
		75	100
	5	25	100.8
		50	100.4
		75	99.5
	10	25	99.2
		50	101.2
		75	100.8
Sea water**	2	25	98.2
		50	97.6
		75	98.4
	5	25	88.4
		50	91.9
		75	94.0
	10	25	85.7
		50	89.1
		75	91.4

*average of three determinations

** Synthetic sea water was prepared according to the formula given in [22]

Determination of Bi (III) in a Veterinary preparation:

The present method has been also applied to the determination of Bi (III) in veterinary preparation .The results

are listed in Table 6 , from which it can be concluded that the method is suitable for determining bismuth in the veterinary preparation sample with satisfactory recovery.

Table (6) . Determination of Bi (III) in veterinary preparation

Veterinary Preparation	Bi(III) amount (µg)	Recovery, (%)*
Diaclean 2000 mg Bi ₅ O(OH) ₉ (NO ₃) ₄ /Sachet Avico , Jorden	25	98.1
	50	97.9
	75	100.4

*Average of five determinations.

The performance of the proposed method was assessed by calculating the student's t-test compared with the literature method [23]. The results in Table 7 show that the calculated values of t do not exceed

the theoretical values at the 95% confidence level[24] indicating that there is no significant difference between the proposed method and the reported method.

Table (7) . Determination of bismuth by the proposed and literature methods

Veterinary Preparation	Bismuth amount , µg	Recovery , % *		t.exp**
		Present method	Reported Method[24]	
Diaclean 2000 mg Bi ₅ O(OH) ₉ (NO ₃) ₄ /Sachet Avico , Jorden	25	98.1	98.6	0.27
	50	97.9	99.3	1.49
	75	100.4	98.9	1.54

*Average of five determinations.

**Tabulated t-value at 95% confidence level is 2.31 for (n= 10)

Conclusion:

A simple , sensitive and accurate spectrophotometric method has been developed for determination of bismuth(III) in aqueous solution , using arsenazoIII (AZIII) as chelating agent at pH1.9 . The molar absorptivity is $1.9 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$ at 612 nm. Beer's law is obeyed over the concentration range 0.1 – 4.25 µg.mL⁻¹ . The method has been applied successfully to the determination of Bi(III) in water samples and veterinary preparation .

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التقدير الطيفي للزموث مع الكاشف ارسين آزو (III) في نماذج مائية ومستحضر بيظري

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الخلاصة:

تم تطوير طريقة طيفية بسيطة وحساسة ودقيقة لتقدير الزموث (III) تعتمد الطريقة على تفاعل الزموث مع كاشف الارسين آزو (III) في وسط حامضي عند (pH=1.9) لتكوين معقد أزرق اللون ذائب في الماء يعطى أعلى شدة امتصاص عند الطول الموجي 612 نانوميتر. وقد تمت دراسة تأثير عدد من المتغيرات للحصول على الظروف المثلى للتفاعل . كانت حدود قانون بير تنطبق ضمن مدى التركيز 2- 85 مايكروغرام من الزموث (III) في حجم نهائي 20 مللتر وكان معامل الارتباط 0.9981 مايكروغرام. مللتر⁻¹ . بلغت قيمة حد الكشف (LOD) وقيمة حد التقدير الكمي (LOQ) للطريقة 0.0633 و 0.0847 مايكروغرام. مللتر⁻¹ على التوالي، بينما كانت قيمة معامل الامتصاص المولاري 1.9×10^4 لتر.مول.سم⁻¹ والانحراف القياسي النسبي بين (±0.59 to ±2.73%) اعتمادا على مستوى التركيز للزموث وان نسبة التفاعل بين الزموث والارسين آزو (III) في المعقد المتكون هي 2:1 . كذلك تمت دراسة تأثير العديد من الايونات الموجبة والسالبة الشائعة على تقدير الزموث في محلوله المائي وبوجود مزيج من عوامل الحجب ، وطبقت الطريقة بنجاح على تقدير الزموث في نماذج مائية وفي مستحضر بيظري .