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Spectrophotometric Assay of Pyridoxine Hydrochloride (Vitamin B_6) in Pharmaceutical Preparations and Serum Via Arsenazo III- Cerium (III) Reaction

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ABSTRACT

A simple, rapid, accurate and precise spectrophotometric method is proposed for determination of pyridoxine hydrochloride (Vitamin B_6) in pure form and in its pharmaceutical preparations and serum. The method is based on the oxidation-reduction reaction between vitamin B_6 and cerium (IV) ion (ceric ion), then the subsequent reaction of cerium (III) with arsenazo III reagent in acidic medium in the presence of the neutral surfactant (Triton X-100) to produce a green complex which is stable, water soluble and has a maximum absorption at 716 nm with a molar absorptivity of 1.12×10^5 $l.mol^{-1}.cm^{-1}$. Beer's law is obeyed in the concentration range of 1 to 14 μg of vitamin B_6 in a final volume of 25 ml. The proposed method has been applied successfully to the assay of pyridoxine hydrochloride in pharmaceutical preparations and serum.

التقدير الطيفي للبيروكسيدين هيدروكلورايد(فيتامين B_6) في مستحضرات صيدلانية ومصل الدم إعتماداً على تفاعل كاشف آرسين آزو III مع ايون السيريوم الثلاثي

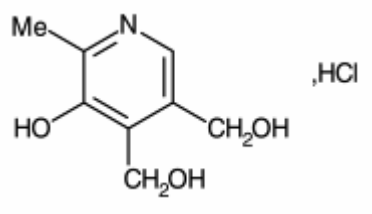
الملخص

تم اقتراح طريقة طيفية بسيطة، سريعة، دقيقة ومتوافقة لتقدير البيروكسيدين هيدروكلورايد (الفيتامين B_6) بشكله الحر وفي مستحضراته الدوائية وفي مصل الدم. اعتمدت الطريقة على تفاعل الأكسدة والاختزال بين فيتامين B_6 و أيون السيريوم الرباعي يتبعها مفاعلة ناتج التفاعل أيون السيريوم الثلاثي مع الكاشف آرسين آزو III في وسط حامضي بوجود العامل الخافض للشد السطحي المتعادل (Triton X-100) ليعطي معقداً ذا لون أخضر مستقر وذائب بالمحلول المائي تُقاس شدة امتصاصه عند الطول الموجي 716 نانوميتر وكانت قيمة الامتصاصية المولارية هي 1.12×10^5 لتر.مول$^{-1}$.سم$^{-1}$ وانطبق قانون بير في مدى التركيز بين 1 و14 مايكروغرام من الفيتامين B_6 في حجم نهائي 25 مللتر. تم تطبيق الطريقة المقترحة بنجاح في تقدير الفيتامين B_6 في مستحضراته الدوائية ومصل الدم.

INTRODUCTION

Vitamins can be defined as essential organic compounds required in minuscule amounts (referred to as micronutrients). Vitamins mainly function as catalysts for reactions within the body. They contain no useful energy, but as catalysts, they serve as essential links and regulators in metabolic reactions that release energy from food (Bender, 1992).Vitamins B6 and B2 are involved in the metabolism of homocysteine, vitamin B6 serves as cofactor for cystathionine ß-synthase and cystathioninelyase, which convert homocysteine to cystathionine and then to cysteine. Vitamin B6 exists in seven forms: pyridoxine (PN), pyridoxine 5'-phosphate (PNP), pyridoxal (PL), pyridoxal 5'-phosphate (PLP), pyridoxamine (PM), pyridoxamine 5'-phosphate (PMP), and the catabolite, 4-pyridoxic acid.The Pyridoxine form, is a water-soluble vitamin. It was discovered in 1934 by P. Gyorgy. (Midttun et al., 2005).

Pyridoxine hydrochloride is (5- hydroxy- 6- methyl pyridine -3,4- diyl) dimethanol hydro-chloride (British Pharmacopeia, 2000).



Pyridoxine Hydrochloride,

$C_8H_{11}NO_3$. HCl

M.Wt = 205.6 g/mol

Many analytical methods were used for the determination of pyridoxine hydrochloride (vitamin B_6), these methods included colorimetric, the method is based on the measurement of the blue product results from the reaction between pyridoxine hydrochloride and 2,6-dichloroquinonechloroimide (John, 1941). The diazotized p-aminoacetophenone (Elmer et al., 1945) also was described for colorimetric determination of pyridoxine hydrochloride. An orange species formed when pyridoxine hydrochloride is treated with diazotized dapsone and sulphanilamide in a mixture of trichloroacetic acid and sulphuric acid at room temperature (Sane et al., 1983).

A spectrophotometric method is also described for the determination of pyridoxine hydrochloride (vitamin B_6) by flow injection analysis depending on the reaction of vitamin B_6(pyridoxine) with N,N-diethyl-p-phenylenediamine after oxidation by potassium hexacyanoferrate (III) (Clezio and Orlando, 1999). Another spectrophotometric method is also described for the determination of pyridoxine hydrochloride based on the reaction between pyridoxine hydrochloride and 1,10-phenanthroline in the presence of ferric nitrate, the reddish brown colored product shows maximum absorption at 510 nm (Kuchekar et al., 2002).

A micellar reversed-phase liquid chromatographic procedure was also developed for the determination of B_6 group vitamins, i.e. pyridoxine, pyridoxal and pyridoxamine, in human serum (Josep et al., 2004).

Arsenazo III reagent gives sensitive and selective reactions with several cations, such as reaction with cerium (III) ion in the presence of cerium (IV) ion. This reaction can be used in the determination of some organic compounds which have the ability to undergo oxidation reaction with cerium (IV) ion (Al-Abdaly, 2005).

The aim of the present work is to evaluate simple, rapid, accurate and precise spectrophotometric method for the determination of pyridoxine hydrochloride (vitamin B_6) based on the oxidation – reduction reaction of vitamin B_6 with cerium (IV) ion, then the subsequent reaction of cerium (III) ion with arsenazo III reagent in acidic medium in presence of Triton X-100 (neutral surfactant) to yield a colored complex whose intensity is indirectly related to the concentration of vitamin B_6.

EXPERIMENTAL

Instruments

All spectrophotometric measurements are performed on SPECORD 200 – Analytikijena UV-Visible Recording Spectrophotometer by using 1-cm silica cells. pH meter type HANNA 301 pH - Ion meter is used for pH readings.

Reagents

All chemicals used are of analytical - reagent grade.

Standard Pyridoxine Hydrochloride (Vitamin B_6) solution, 100μg.ml^{-1}. This solution is prepared by dissolving 0.01 g of Pyridoxine hydrochloride (SDI- Iraq) in 5 ml of distilled water and the volume is diluted to 100 ml with distilled water in a volumetric flask. This solution is stable for at least one week.

Working Pyridoxine Hydrochloride (Vitamin B_6) solution, 10μg.ml^{-1}. This solution is prepared by appropriate dilution of standard solution.

Ammonium ceric sulphate [cerium(IV) ion solution], 4.4×10^{-5}M. This solution is prepared by dissolving 0.007g of ammonium ceric sulphate dihydrate (BDH) in 250 ml of distilled water in a volumetric flask, this solution is freshly prepared daily.

Arsenazo III reagent solution, 2×10^{-4}M. This solution is prepared by dissolving 0.0411 g of arsenazo III (Fluka) in 250 ml distilled water in a volumetric flask.

Sulphuric acid solution, 0.05 N, This solution is prepared by appropriate dilution of 1.4 ml of the concentrated sulphuric acid (35.5 N) solution to 1000 ml with distilled water in a volumetric flask.

Triton X-100, (1%, V/V). This solution is prepared by dilution of 1 ml of Triton X-100 to 100 ml with distilled water in a volumetric flask.

Serum Sample. Venous blood samples (5 ml) were drawn from healthy control groups , then transferred immediately to a clean dry plain tube .The blood sample was allowed to clot for at least 10-15 min at room temperature and then centrifuged for 10 min at 3000 Xg. Serum was collected, then the precipitation of protein was performed by using 10 % trichloroacetic acid (TCA 10%).(Tietz,1999). 0.5 ml and 1 ml of the serum were used in subsequent experiments.

Vitamin B_6 Tablets solution , 10μg.ml^{-1}. A 10 tablets (40mg B_6/tablet - Iran Hormone. Drug Company) were weighed and powdered and then dissolved in 50 ml of 0.1 M HCl, after that the solution is heated in a water bath for 15 minutes. The solution is cooled and diluted to 100 ml with distilled water and then filtration was performed(discarding the first 20 ml) (British Pharmacopeia, 2000) .
A 2.5 ml of the filtrate was diluted to 100 ml with distilled water to get a solution of 100 μg.ml^{-1} vitamin B_6. The tested solution is prepared by appropriate dilution .

Ampoule of Vitamin B_6 solution, 100μg.ml^{-1}. The 100 μg.ml^{-1} B_6 solution is prepared by diluting 1 ml of the ampoule (25mg B_6/ml- Panther Pharmaceutical Company, Ltd.) content to 100 ml with distilled water and then 40 ml of this solution is diluted to 100 ml with distilled water in a volumetric flask. Less dilute solution is prepared by appropriate dilution.

Procedure and calibration graph

To a series of 25-ml calibrated flasks, an increasing volume (0.1-1.8 ml) of 10 μg.ml^{-1} B_6 solution is transferred, followed by 7 ml of 4.4×10^{-5}M cerium (IV) ion solution and 0.2 ml of 0.05 N sulphuric acid solution, standing for 15 minutes, then 2 ml of 2×10^{-4}M arsenazo III reagent solution and 4 ml of Triton X-100 (1% v/v) are added. After dilution the flasks with distilled water, the absorbances are measured at 716 nm against the reagent blank. Beer's law is obeyed over the range of concentration 1 to 14 μg vitamin B_6 in 25 ml and a concentration above 14 μg in 25 ml gives a negative deviation (Fig. 1).The apparent molar absorptivity, referred to vitamin B_6, has been found to be 1.12×10^5 l.mol^{-1}.cm^{-1}.

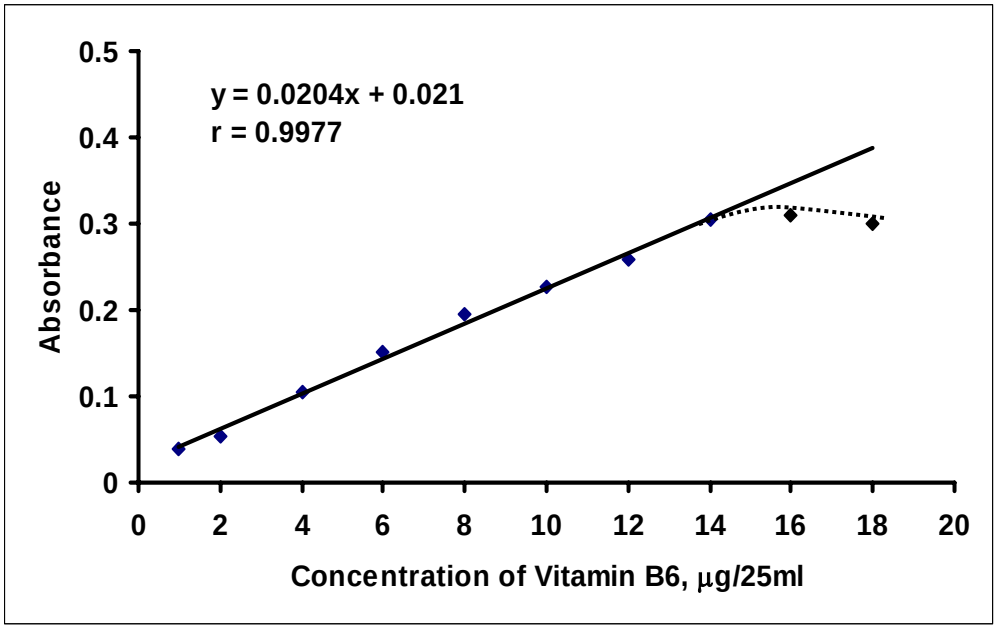


Fig. 1: The calibration graph for vitamin B_6 determination using the reaction between arsenazo III and cerium(III).

Results and Discussion

During the investigation , 10 μg of vitamin B_6 is taken and the final volumes are brought to 25 ml with distilled water.

Optimization of variables

The effect of various parameters on the absorption intensity of the colored complex is studied and the reaction conditions have been optimized. The preliminary investigations showed that the complex formed have a maximum absorption at 646 nm against reagent blank.

Effect of pH

The effect of pH on intensity of the colored complex is examined. Different volumes (0.05 – 0.5 ml) of 0.05 N sulphuric acid solution is added to an aliquot of solution containing 10 μg of vitamin B_6. The intensities of absorption are read against the reagent blank. The results are shown in Table (1).

Table 1: Effect of pH on absorbance.

ml of 0.05 N H_2SO_4	Absorbance	Final pH
0.05	0.093	3.51
0.10	0.177	3.29
0.15	0.200	3.10
0.20	0.214	3.02
0.25	0.201	2.88
0.30	0.192	2.83
0.40	0.180	2.70
0.50	0.110	2.55

The results shown in Table 1 indicate that the pH of 3.02(pH ≈ 3.0) is considered optimum. A pH 3.0 is selected for subsequent investigation because of good sensitivity. Four buffer solutions of pH 3.0 with different compositions have been tested, tartaric acid-NaOH (B_1), citric acid-NaOH (B_2), KH phthalate-HCl (B_3), and formic acid-NaOH (B_4), (Table 2).

Table 2: Effect of buffer solutions on absorbance.

ml of buffer solution	Absorbance/ml of buffer added			
	B_1	B_2	B_3	B_4
1	0.012	0.006	0.006	0.121
2	0.050	0.028	0.008	0.171
3	0.056	0.029	0.005	0.131
4	0.010	0.002	0.003	0.081
5	0.003	0.000	0.002	0.068
pH range	3.1 – 3.0	3.0 – 2.97	3.1 – 2.9	3.0 – 2.9

The results in Table (2) indicate that all types of buffer solutions decrease the intensity of absorption of the colored complex, so that the use of buffer solutions is not recommended. A 0.2 ml of 0.05 N sulphuric acid solution has been recommended for subsequent experiments.

Effect of oxidizing agent [cerium(IV) ion] amount

Different amounts of cerium (IV) ion solution are added and the optimum amount which gives higher intensity of colored complex and higher value of correlation coefficient (Table 3), has been selected.

Table 3: The effect of ceric ion amount on absorbance.

ml of 4.4×10^{-5}M cerium(IV) ion solution	Absorbance/μg Vitamin B_6 in 25ml				r*
	1	4	7	10	
3	0.051	0.090	0.128	0.146	0.9916
5	0.050	0.094	0.177	0.206	0.9868
7	0.051	0.132	0.201	0.239	0.9972
10	0.047	0.143	0.224	0.278	0.9926
* r = Correlation Coefficient					

The results shown in Table (3) indicate that the volume of 7 ml of 4.4×10^{-5}M cerium (IV) ion solution is an optimum amount because of highest value of correlation coefficient and therefore recommended for subsequent experiments.

Effect of time on reduction of cerium(IV) ion

The effect of time needed to complete the reduction of cerium (IV) ion to cerium (III) ion is studied by standing of the solutions after adding cerium(IV) ion solution for different times, then the other reagents are added and the absorbances measured against the reagent blank (Table 4).

Table 4: Effect of time on reduction process.

Time (min.)	0	5	10	15	20	25	30	35	40
Absorbance	0.034	0.152	0.197	0.231	0.221	0.120	0.080	0.020	0.020

The results indicate that complete reduction of cerium (IV) ion occurs after 15 minutes and the intensity decreased above 20 minutes because the intensity of reagent blank solutions increase. Therefore, the standing time 15 min. is recommended for the subsequent experiments.

Effect of arsenazo III reagent amount

The effect of the amount of arsenazo III reagent on maximum formation of the colored complex is investigated. The results is shown in Table (5).

Table 5: Effect of arsenazo III reagent amount on absorbance.

ml of 2×10^{-4}M arsenazo III reagent	Absorbance/μg Vitamin B_6 in 25ml				r
	1	4	7	10	
1	0.042	0.079	0.106	0.111	0.9551
2	0.059	0.127	0.186	0.232	0.9964
3	0.033	0.103	0.155	0.204	0.9962
4	0.030	0.082	0.110	0.162	0.9919
5	0.030	0.071	0.097	0.127	0.9952

The results shown in Table (5) indicate that 2 ml of arsenazo III reagent solution give, the higher sensitivity and higher value of correlation coefficient, therefore it has been selected for subsequent experiments.

Effect of surfactant

The effect of surfactant were studied by the addition of 3 ml of various types of surfactant (cationic, anionic and neutral) to the medium of reaction with different orders of addition. The results are shown in Table (6).

The selected surfactants are :

Cetyltrimethylammonium bromide (CTAB) (cationic)
Sodium dodecyl sulphate (SDS) (anionic)
iso-Octylphenoxypolyethoxyethanol (Triton X-100) (neutral)

Table 6: Effect of surfactants and the order of additions.

Surfactant solution	Absorbance /order* of addition				λmax, nm	Δλ**, nm
	I	II	III	IV		
CTAB 1×10^{-3}M	0.006	0.011	0.031	0.050	644	114
SDS 1×10^{-3}M	0.111	0.121	0.147	0.185	716	170
Triton X-100 1% (v/v)	0.075	0.061	0.094	0.237	716	170

The absorbance without surfactant = 0.232

* I. Vitamin B_6 + Surfactant (S)+ Ce^{+4}+ Sulphuric acid (H_2SO_4) + Reagent (AzIII)

II. Vitamin B_6 + Ce^{+4}+S+ H_2SO_4+ AzIII

III. Vitamin B_6 + Ce^{+4}+ H_2SO_4+S+ AzIII

IV. Vitamin B_6 + Ce^{+4}+ H_2SO_4+ AzIII +S

** $\Delta\lambda_{max}$ without surfactant = 116 nm

The results in Table (6) indicate that Triton X-100 had increased the intensity of absorbance for the formed complex with order of addition No. IV beside to red shift effect. Therefore, the effect of different amounts of the Triton X-100 solution on the intensity of formed complex has been studied and the results as shown in Table (7).

Table 7 : Effect of amount of Triton X-100.

ml of Triton X-100 (1%)	1	2	3	4	5
Absorbance	0.257	0.257	0.258	0.278	0.222

The results in Table (7) indicate that the addition of 4 ml of Triton X-100 solution with order of addition No. IV can be recommended for subsequent experiments.

Effect of Time

The effect of time on the development and stability of the colored complex for different amounts of vitamin B_6 is investigated under the optimum experimental conditions established. Complete color formation occurs immediately after all reaction mixtures are added and the absorbance of the complex remains constant for at least 20 minutes (Table 8).

Table 8: Effect of time on the absorbance of complex.

μg of Vitamin B_6 in 25 ml	Absorbance* / minutes standing time					
	5	10	15	20	25	30
1	0.052	0.052	0.052	0.053	0.053	0.053
3	0.074	0.074	0.076	0.076	0.076	0.074
7	0.198	0.201	0.200	0.200	0.195	0.161
10	0.256	0.274	0.272	0.272	0.257	0.231

*After 15 minutes reaction time of Vitamin B_6 with Ce(IV) ion

The results shown in Table (8) indicate that the stability period is sufficient to allow several measurements to be performed simultaneously.

Absorption spectra

Absorption spectra of the colored complex formed from the reaction between cerium(III) ion and arsenazo IIII reagent in acidic medium in presence of Triton X-100 against its corresponding reagent blank shows maximum absorption at 716 nm in contrast to the arsenazo reagent blank which have less absorption at the same wave length (Fig. 2).

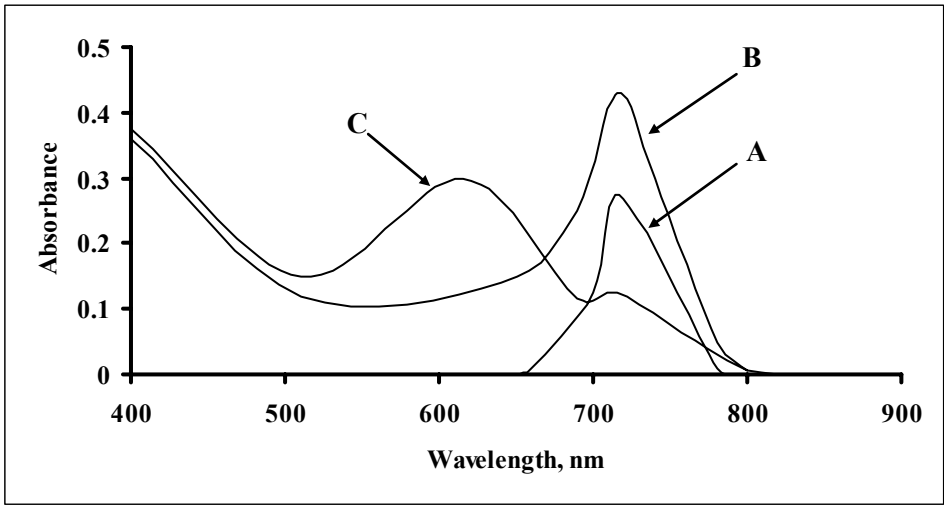


Fig.2: Absorption spectra of 10 μg Vitamin B_6 / 25 ml treated according to the optimum conditions and measured against (A) blank, (B) distilled water and (C) blank measured against distilled water.

Accuracy and precision

To check the accuracy and precision of the calibration graph, Vitamin B_6 is determined at three different concentrations. The results shown in Table (9) indicate that the calibration graph is satisfactory.

Table 9: Accuracy and precision.

Amount of Vitamin B_6 taken, µg/25 ml	Relative error*, %	Relative standard deviation*, %
3	+0.80	±2.76
7	- 0.67	±1.01
10	+1.20	±2.44

* Average of five determinations

Nature of the reaction between Vitamin B_6 and cerium(IV) ion

Job's method (Delevie, 1997) has been used in the determination of the reaction ratio of Vitamin B_6 with cerium(IV) ion. The obtained results (Fig. 3) showed that a 1:1 Vitamin B_6 to cerium(IV) ion ratio is obtained.

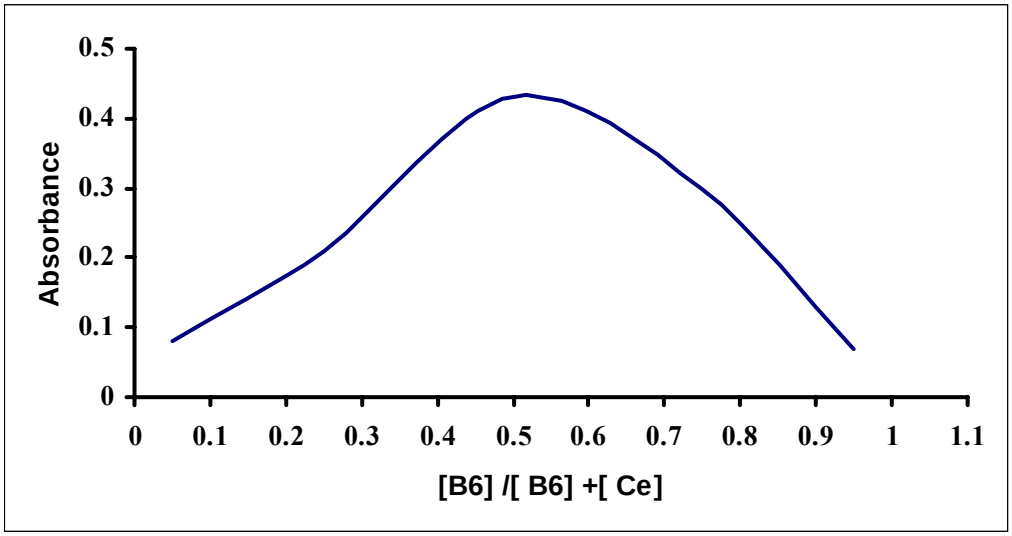


Fig.3: Job's plot for Vitamin B_6 – Cerium (IV) ion.

Nature of arsenazo III-cerium(III) ion complex

The stoichiometry of the reaction is investigated using the Job's method under the optimized conditions. The obtained results (Fig. 4) showed that a 1:1 arsenazo III to cerium(III) ion ratio is obtained. This result is identical with that in the literature (Sandell and Onishi, 1978)

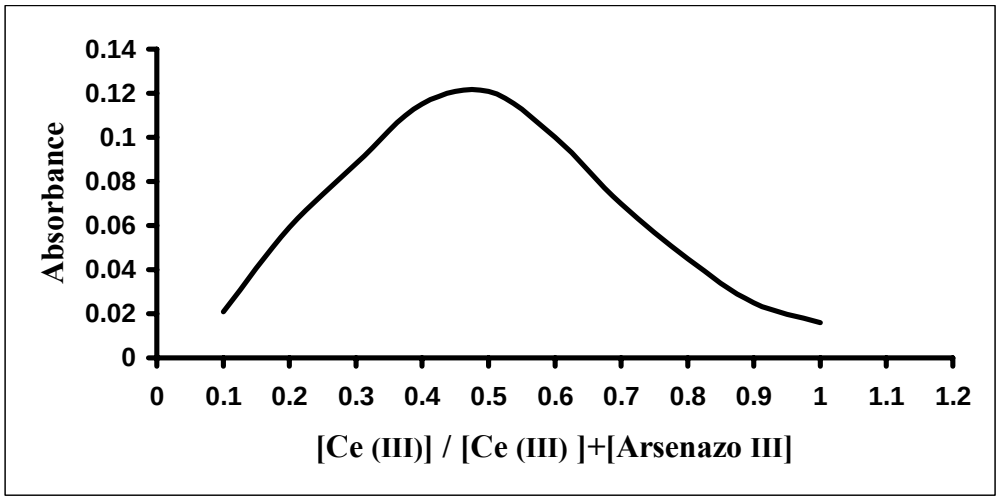
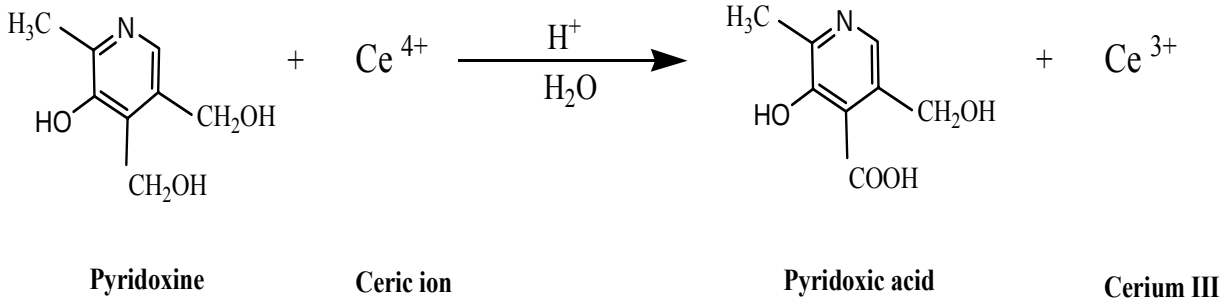


Fig. 4: Job's plot for Arsenazo III – Cerium(III) ion complex.

The probable mechanism of the reaction might be the following:



The above equation indicates a 1 : 1 of Vitamin B_6 to cerium(IV) reaction ratio.

Analytical applications

The proposed method is applied to determine Vitamin B_6 in serum and pharmaceutical preparations. On applying proposed procedure, good recovery is obtained as shown in Table 10 and 11.

Table 10: Determination of Vitamin B_6 in serum.

μg of standard Vitamin B_6 added	% Recovery / ml serum used	
	0.5	1.0
4	100.4	98.4
8	101.1	99.6
14	99.7	100.3

Table 11: Determination of Vitamin B_6 in pharmaceutical preparations.

Drug	Pharmaceutical preparation	Supplier	Certified value	µg B_6 present/ 25 ml	µg B_6 measured/25 ml	Recovery (%)
Vitamin B_6 (Pyridoxine HCl)	Tablet	Iran Hormone Drug Company	40 mg / tablet	4	3.40	85.00
				8	7.85	98.12
				12	12.10	100.80
Vitamin B_6	Injection Ampoule	Panther (London) Ltd.	25 mg / ml	3	3.05	101.16
				7	6.96	99.42
				10	9.90	99.00

The performance of the proposed method is assessed by calculating the student's t – test compared with the standard method (British Pharmacopeia, 2000). At the 95% confidence limit for four degree of freedom , the calculated t - values do not exceed the theoretical value 2.776 (Table 12), indicating that there is no significant difference between the proposed method and the standard method.

Table 12: Analysis of Vitamin B6 by proposed and standard method .

Drug	Drug content (µg / 25 ml)			Recovery* (%)		t-exp.
	Present	Found				
		Present method	Standard method	Present method	Standard method	
Vitamin B_6 Tablet	12	11.85	11.98	98.75	99.83	0.65
Vitamin B_6 Injection	12	12.27	12.06	102.25	100.50	0.98
*Average of five determinations						

Comparison of the methods

Table (13) shows the comparison between some of analytical variables obtained from the present method with that of the recent spectrophotometric methods.

Table 13: Comparison of the methods.

Analytical parameters	Present method	Literature method (Clezio and Orlando,1999)	Literature method (Kuchekar et al., 2002)
pH	3.02	7.00	3.60
Temperature ($^{\circ}$C)	Room temperature	Room temperature	Room temperature
Development time (minutes)	5	Direct measuring	-
λ_{max} (nm)	716	684	510
Medium of reaction	Aqueous	Aqueous	Aqueous
Reagent	Arsenazo III	N,N-diethyl-p-phenylenediamine	1,10-phenanthroline
Oxidizing reagent	Cerric ammonium sulphate	potassium hexacyanoferrate(III)	Ferric nitrate
Beer's law range (ppm)	0.04 – 0.56	0.5 – 6.0	12 – 32
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.12×10^5	-	-
RSD (%)	$\leq \pm 2.76$	$\leq \pm 2.00$	$\leq \pm 2.94$
Color of the dye	Green	Blue	Reddish brown
Application of the method	Determination of vitamin B_6 in tablet and injection ampoule	Determination of vitamin B_6 in solid drug and injection ampoule	. Determination of vitamin B_6 in presence of melatonin

The results in Table 13 shows that the suggested method for the determination of vitamin B_6 have a good sensitivity comparing with the other methods.

CONCLUSION

A simple, rapid, accurate and precise spectrophotometric method is evaluated for determination of pyridoxine hydrochloride (Vitamin B_6) in pure form and in its pharmaceutical preparations and serum. The method is based on oxidation-reduction reaction between vitamin B_6 and cerium (IV) ion (ceric ion), then the subsequent reaction of cerium (III) with arsenazo III reagent in acidic medium in presence of the neutral surfactant (Triton X-100) to produce a green complex which is stable, water soluble and has a maximum absorption at 716 nm with a molar absorptivity of 1.12×10^5 $l.mol^{-1}.cm^{-1}$. Beer's law is obeyed in the concentration range from 1 to 14μg of vitamin B_6 in a final volume of 25 ml. The proposed method has been applied successfully for the assay of Vitamin B_6 in pharmaceutical preparations and serum.

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