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Determination of Vitamin B_6 (pyridoxine hydrochloride) in Pharmaceutical Preparations Using High Performance Liquid Chromatography

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Abstract

Determination of vitamin B_6 (pyridoxine hydrochloride) was described using high performance liquid chromatographic method. The analysis was achieved by cosmos IL $5C_{18}$-MS-II column (250 mm x 4.6 mm i. d., 5µm particle size) at room temperature. The mobile phase used was Acetonitrile, buffer solution (Citric acid, Na_2HPO_4 pH4) buffer solution in the ratio (70:30) (V: V). the flow rate was set to 1.25 $mL.min^{-1}$ and the retention time 1.82 min with UV-detection at 282 nm. Beer's law was obeyed over the concentration range 10-1250 $\mu g.mL^{-1}$. The method was accurate (relative error % less than 0.05%), precise (RSD better than ±1.05%), average recovery 100.05%, with a limit of detection and quantification of 2.2$\mu g.ml^{-1}$, and 7.34$\mu g.mL^{-1}$ respectively. The proposed method was successfully applied to determine the pyridoxine hydrochloride in pharmaceutical preparations in both forms of tablet and injection.

Keywords: vitamin B_6 (pyridoxine hydrochloride), HPLC, pharmaceutical preparations.

تقدير فيتامين B_6 (هيدروكلوريد البيريدوكسين) بتقنية كروماتوغرافيا السائل عالية الأداء

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الخلاصة

تم تقدير فيتامين B_6 (هيدروكلوريد البيريدوكسين) بتقنية كروماتوغرافيا السائل عالي الأداء باستخدام عمود من نوع cosmos IL $5C_{18}$–MS–II (5µm ,250 mm x 4.6 mm i. d.) ومكشاف UV عند طول موجي 282 نانوميتر، واستخدام الطور المتحرك المتكون من الاسيتونتريل: والمحلول المنظم حامض الستريك، فوسفات ثنائي الصوديوم الهيدروجينية Na_2HPO_4 (pH 4) كطور متحرك بنسبة (30:70) (V: V) وبمعدل سرعة جريان 1.25 مللتر.دقيقة$^{-1}$. وكان زمن الاحتجاز 1.82 دقيقة بازاحة ايزوكراتية وبدرجة حرارة الغرفة. وكان المنحني القياسي خطيا لمدى من التراكيز 10– 1250 مايكروغرام.مللتر$^{-1}$. وكان معدل نسبة الاسترجاع 100.05%، والانحراف القياسي النسبي أفضل من 1.05±% ونسبة الخطأ 0.05%، وأن الحد الادنى للكشف هو 2.2 مايكروغرام. مللتر$^{-1}$ وجد التقدير الكمي هو 7.34 مايكروغرام. مللتر$^{-1}$. تم تطبيق الطريقة المقترحة بنجاح لتقدير هيدروكلوريد البايرودوكسي في المستحضرات الصيدلانية.

Introduction

Pyridoxine hydrochloride (vitamin B_6) is a 5-hydroxy-6-methyl-3, 4-pyridinedimethanol hydrochloride with the chemical formula $C_8H_{11}NO_3.HCl$ [1, 2]. It is a white powder, soluble in water, slightly soluble in acetone, and insoluble in chloroform and ether [3]. The determination of vitamin content in food and in nutritional supplements is important in quality, labeling, and marketing [4].

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Pyridoxine is used to prevent low levels of vitamin B_6 which plays an important role in the health of nerves, skin and red blood cells [5].

Many spectrophotometric methods for the determination of pyridoxine hydrochloride were described, using diazotization reaction [6, 7], or using oxidation reduction reaction [8]; such methods were leaked to sensitivity [9-11], others were proposed derivative spectrophotometric procedures [12-14]. On the other side, such chromatographic methods need a special detection tools like fluorometer [15, 16], or mass spectrometer [17].

In this paper, pyridoxine hydrochloride (vitamin B_6) was determined by chromatographic method using cosmosil $5C_{18}$-MS-column and UV-detection at 282nm with high sensitivity and wide applicability.

Exprerimental Apparatus

A (shimadzue, LC-2010A, Japan) HPLC instrument was used with a pump (model LC-2010, high flow rate mode) and auto injector sampler. The detector was UV LC-2010 with D_2 lamp (800mv minimum energy). Separation was achieved using a cosmosil $5C_{18}$-MS-II column (4.6 mm I.D. x 250 mm, 5 µm particle size, Japan).

A shimadzue UV-1650 PC double beam spectrophotometer with 1cm quartz cells have been used for scanning spectra. pH measurement has beam done by pH-meter (HANNA pH 211, microprocessor pH meter, Mauritius), balance (Sartorius BL 20 S, Germany) has been used for weight measurements.

Materials

All chemicals and reagents are of analytical grade. Water was double distilled and filtered. Acetonitril (ACN), dichloromethane (DCM), methanol, and ethanol (Loba. Chemie) are of HPLC.

Pyridoxine hydrochloride (2000 µg.mL^{-1}): this solution was prepared by dissolving 0.10 gm of pure pyridoxine hydrochloride (from BDH) in double distilled water and filtered using 0.45 µm filter, the volume was completed to 50 ml in a volumetric flask, further dilution was followed to prepare 500 µg.mL^{-1}working solution.

Citric acid (0.1 M) solution: this solution was prepared by dissolving 1.92 g of citric acid ($C_6H_8O_7$) (from BDH) in 100 mL of double distilled water and filtered using 0.45 µm filter**.**

Disodium hydrogen phosphate (0.2 M) solution: this solution was prepared by dissolving 1.42 g of (Na_2HPO_4) (from Sigma) in 50 mL of double distilled water and filtered using 0.45 µm filter**.**

Buffer (pH 4) solution: this solution was prepared by mixing 61.9 mL of citric acid (0.1 M) with 38.1 mL of (0.2 M) Na_2HPO_4.

Pharmaceutical preparations

Pyridoxine hydrochloride (Smamvit-B6 tablet) 1000 µg.mL^{-1}solution: this solution was prepared by weighing 5 tablets (40 mg SDI), grinding to fine particles and mixing; a weight of this pharmaceutical powder (0.0457 g) equivalent to 0.01 g of pure pyridoxine hydrochloric acid was dissolved in solvent mixture (CAN: citric acid, Na_2HPO_4) (70: 30), then filtered using 0.45 µm filter, the volume was completed to 10 mL with the same solvent in a volumetric flask.

Pyridoxine hydrochloride (Meho B6 tablet) 1000 µg.mL^{-1} solution: this solution was prepared by weighing 5 tablets (40 mg Bijing company), grinding to fine particles and mixing; a weight of this pharmaceutical powder (0.042 g) equivalent to 0.01 g of pure pyridoxine hydrochloric acid was dissolved in solvent mixture (CAN: citric acid, Na_2HPO_4) (70: 30), then filtered using 0.45 µm filter, the volume was completed to 10 mL with the same solvent in a volumetric flask.

Pyridoxine hydrochloride (Mason natural-B6 tablet) 1000 µg.mL^{-1}solution: this solution was prepared by weighing 5 tablets (50 mg mason natural company), grinding to fine particles and mixing; a weight of this pharmaceutical powder (0.070 g) equivalent to 0.01 g of pure pyridoxine hydrochloric acid was dissolved in solvent mixture (CAN: citric acid, Na_2HPO_4) (70: 30), then filtered using 0.45 µm filter, the volume was completed to 10 ml with the same solvent in a volumetric flask.

Pyridoxine hydrochloride (Mefron injection) 1000 µg.mL^{-1} solution: this solution was prepared by taking 0.5 mL of injection solution (100mg pyridoxine hydrochloric acid /2ml) (Shanohai) and diluted to 25 mL using solvent mixture (CAN: citric acid, Na_2HPO_4) (70: 30), then filtered using 0.45 µm filter.

Recommended Procedure and Calibration Graph

20 µL of pyridoxine hydrochloride in the concentration range between (10 to 1250 µg.mL^{-1}) was injected under the optimum condition of analysis acetonitrile, buffer solution (citric acid, Na_2HPO_4 pH4) in the ratio (70:30)(V:V). The flow rate was set to 1.25 mL. min^{-1}, which exhibits a retention

time of 1.82 min using UV-detection at 282 nm. The sample was isocratically eluted through cosmosil $5C_{18}$-MS-II column (4.6 mm I.D. x 250 mm, 5 µm particle size). Figure-1 shows the calibration graph of pyridoxine hydrochloride, and according to Figure-1 Beer's law is obeyed over the concentration range 10-1250 µg.mL^{-1}.

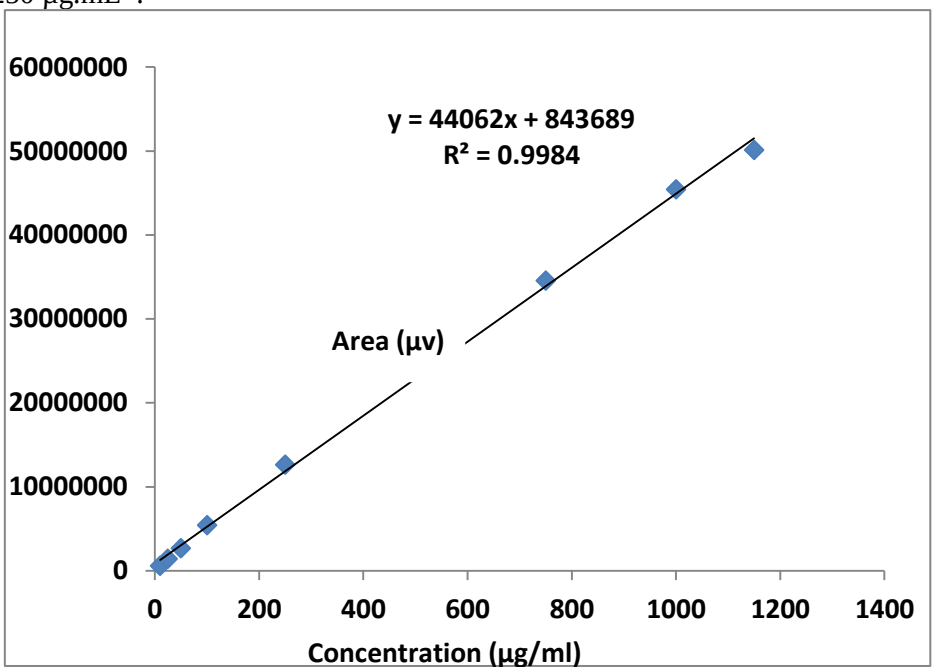

Figure 1-Calibration graph of pyridoxine hydrochloride

Figure-1 shows the linear relation between the area under the curve with the concentration of the of pyridoxine hydrochloride with 0.998 as a determination coefficient and 0.999 as a correlation coefficient.

Optimization of conditions

Selection of dissolution solvent and analytical wavelength

Figure-2 shows the absorption spectrum (at the range190-400 nm) of 100 µg.mL^{-1} of pyridoxine hydrochloride in different dissolution solvents against blank solution (dissolution solvent).The Figure exhibits higher absorption intensity at 282 ±2 nm., and acetonitrile: buffer gave the maximum one.

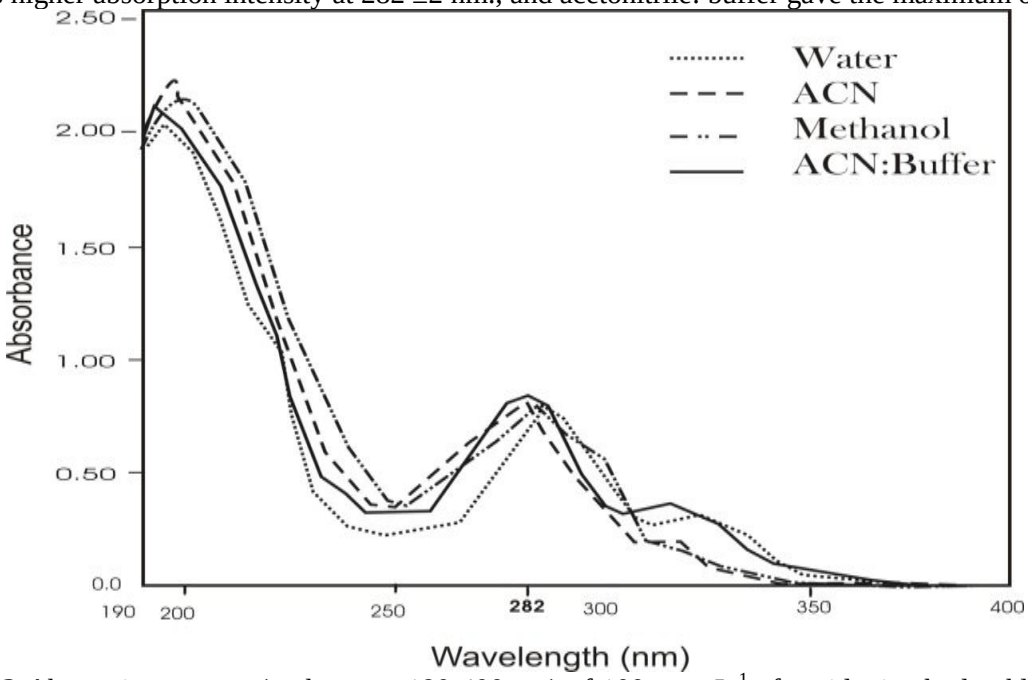

Figure 2-Absorption spectra (at the range190-400 nm) of 100 µg.mL^{-1} of pyridoxine hydrochloride in different dissolution solvents

Selection of mobile phase

Many solvents in many compositions and ratios were used as a mobile phase for is ocratically elution of sample after injection of 20 µl of 500 µg.ml^{-1} of the standard solution. The measurements were done at 282 nm at room temperature and using 1 ml min^{-1} as a flow rate.

Table 1-Selection of mobile phase

No.	Mobile phase	% Ratio	t_R (min)	k`	As.	Notes
1	Methanol: Water	90:10	-	-	-	N0 peak
2		70:30	2.21	1.30	3.78	Tailing
4		50:50	2.25	1.43	2.41	Tailing
5	Ethanol: Water	90:10	-	-	-	N0 peak
6		70:30	2.72	2.62	2.93	Tailing
7		50:50	2.70	2.38	3.16	Two peaks
8		40:60	2.69	3.11	2.51	Two peaks
9	**ACN: Water**	90:10	2.40	1.43	1.66	Tailing
10		**70:30**	**2.34**	**1.64**	**1.22**	**sharp peak**
11		50:50	2.33	1.55	1.56	Sharp peak
12		40:60	2.32	2.30	1.84	Tailing
13	ACN:Water:DCM	70:20:10	2.41	1.19	2.37	Tailing
14		60:30:20	2.38	1.22	3.14	Tailing

From Table-1 ACN: water (70:30)(V:V) was selected as a mobile phase of pyridoxine hydrochloride because of the symmetric chromatogram and the ideal capacity factor (k`); therefore, it was selected in subsequent experiments.

Selection of the analysis media

Many different weak acidic and weak basic media have been used to enhance the symmetry and to get a specific chromatogram of 20 µl of 500 µg.mL^{-1}of standard solutions; the measurement was done at 282 nm at room temperature and using 1 mL min^{-1}as a flow rate.

Tablet 2-Selection of the analysis media

No.	Mobile phase	%Ratio	pH	t_R (min)	k`	As.
1	ACN: Water: 1% Sod. bicarbonate	70:25:5	11	2.34	3.43	2.35
2	ACN: Water: 1% K_2HPO_4	70:25:5	9.5	2.41	4.38	2.78
3	ACN: Water: 1% Sod.acetate	70:25:5	8.6	2.35	0.65	3.51
4	ACN: Water: Phosphate buffer	70:15:15	7	2.24	3.85	1.65
5	ACN: Water: 1% Ammonium acetate	70:25:5	6.5	2.46	3.31	2.51
6	ACN: Water: Phosphate buffer	70:25:5	6	2.22	2.43	1.91
7	ACN: Water: KH_2PO_4	70:25:5	5.0	2.27	4.21	2.83
8	ACN: Water: 1%KHphthalate	70:25:5	4.1	2.25	2.07	2.25
9	ACN: Buffer solution (Sodium acetate, acetic acid)	70:30	4.0	2.20	0.98	2.41
10	**ACN: Buffer solution (Citric acid, Na_2HPO_4)**	**70:30**	**4.0**	**2.24**	**2.29**	**1.18**
11	ACN: Buffer solution(Tartaric acid, NaOH)	70:30	3.8	2.26	2.95	2.84
12	ACN: Buffer solution(Glycine, HCl)	70:30	3.8	2.23	2.17	2.42
13	ACN:H_3PO_4	70:30	3.5	2.24	3.55	1.88

Table-2 The best symmetry resulting from using ACN: buffer pH 4 solution (Citric acid, Na_2HPO_4) in the ratio (70:30) with the retention time 2.24 min, Figure-3.

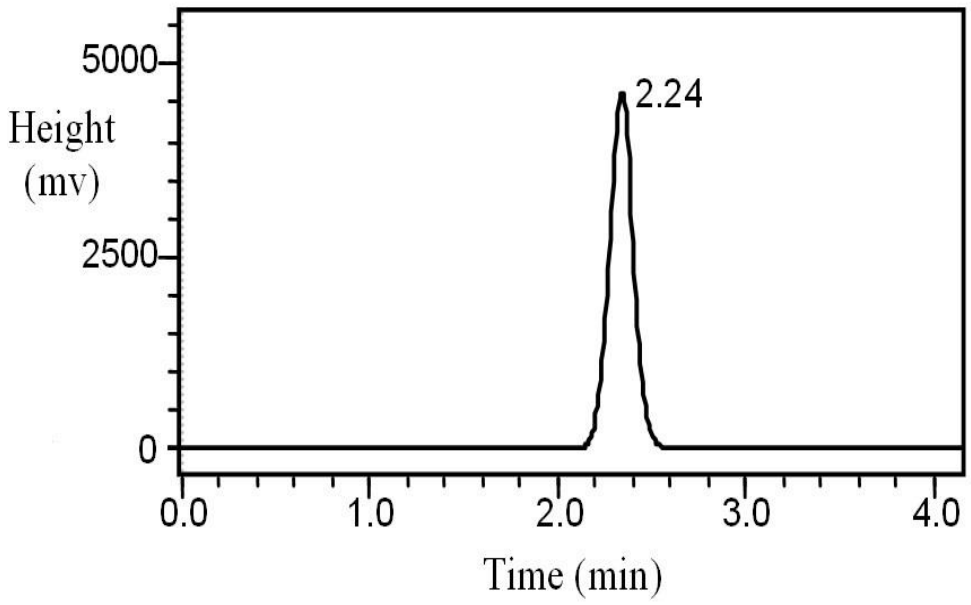

Figure 3-Chromatogram of pyridoxine hydrochloride (20 µl of 500 µg.mL^{-1}) using ACN:bufferpH 4 (Citric acid, Na_2HPO_4) (70:30) as a selected medium for the analysis

Effect of flow rate

Between 0.5 to 1.5 mL.min^{-1} a flow rate for the elution of pyridoxine hydrochloride has been followed. Table-3 shows that 1.25 mL.min.$^{-1}$ gave a good result in which it reduces the time of analysis and gave the best symmetry; therefore, it was used in subsequent experiments.

Table 3-Effect of Flow Rate

No.	Flow rate (mL. min^{-1})	t_R (min)	k´	As.	Pressure (Mpa)
1	0.5	4.46	1.89	1.22	4.8
2	0.75	2.99	1.49	1.19	6.8
3	1.0	2.24	2.29	1.20	9.5
4	**1.25**	**1.82**	**1.16**	**1.10**	**11.5**
5	1.5	1.51	2.31	1.22	13.5

Effect of temperature

15, room temperature, 30, and 35 ºC as a temperature of the column has been adjusted to follow its effect on the performance of analysis. Table-4 indicates good results at all temperatures, the room temperature was selected. Figure-4 shows the chromatogram at room temperature.

Table 4-Effect of Temperature

No.	Temperature (ºC)	t_R (min)	k´	As.
1	15	1.78	2.12	1.18
2	**25**	**1.82**	**1.16**	**1.10**
3	30	1.85	1.31	1.18
4	35	1.85	1.36	1.17

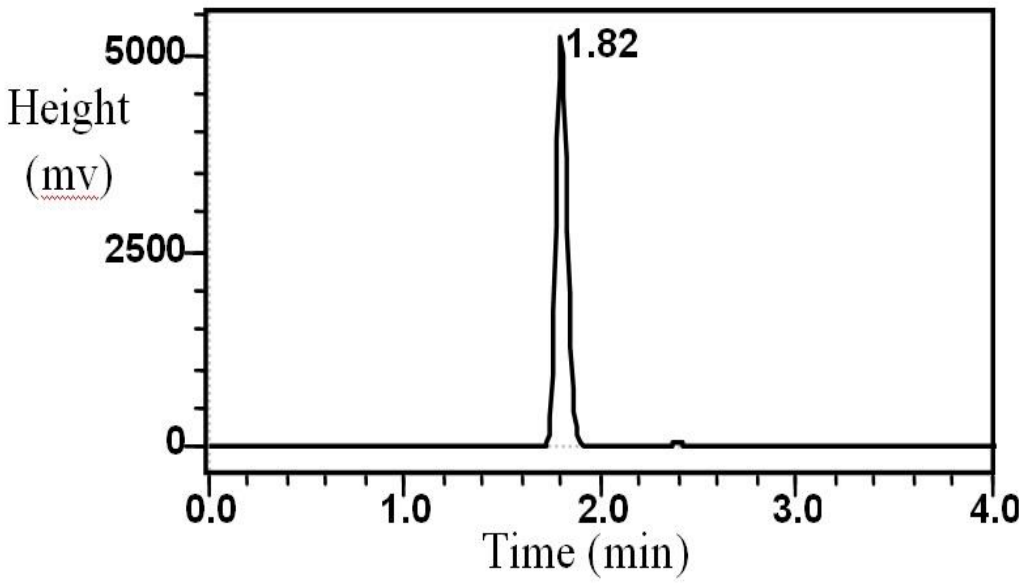

Figure 4-The final chromatogram of pyridoxine hydrochloride (20 µL of 500 µg.mL^{-1}) using ACN: buffer pH 4(Citric acid, Na_2HPO_4) (70:30) as a mobile phase and 1.25 ml.min^{-1} as a flow rate at room temperature

Accuracy and precision

Table-5 shows the accuracy and precision of three different concentrations of pyridoxine hydrochloride (50, 250, and 500 µg.mL^{-1}) in the form of recovery %, relative error RE %, and relative standard deviation RSD %.

Table 5-Accuracy and precision of the calibration graph

Pyridoxine Hydrochloride (µg.ml^{-1})	**Recovery %***	**Relative error,%**	**Relative standard deviation,%**
50	100.15	0.15	±1.35
250	100	0	±0.96
500	100	0	±0.84
Average	**100.05**	**0.05**	±**1.05**

*****Average of five determinations

Table-5 shows that the proposed method provides a good accuracy (average R.E. % is 0.05) and a good precision (average of RSD% is better than ±1.05).

Effect of ingredients

The recovery % of 10 µg.ml^{-1} of pyridoxine hydrochloride in the presence of the same amount of expected ingredient has been followed under the optimum analysis conditions. Table-6 shows the results.

Table 6-Effect of ingredients

Ingredient	**Recovery % of 10µg Pyridoxine HCl /10 µg ingredient**
Starch	102
Fructose	101
$CaSO_4$	104
Sucrose	107
Glucose	109
Arabic Gum	122

Table-6 shows that only Arabic gum is seriously and positively interfered in the analysis of pyridoxine hydrochloride.

Application of the method

Using the proposed chromatographic method, assay of pyridoxine hydrochloride in its pharmaceutical preparations, (Samavit B_6 (Tablet) 40mg SDI-Iraq), (Mason natural B_6 (Tablet) 50mg USA),(Meheco B_6 (Tablet) 40mg Beijing-China), and(MefronB_6 (Injection) 100mg/2ml Shanohai-China) has been followed under the optimum analysis conditions. The results are listed in Table-7.

Table 7-Application of the method

Pharmaceutical preparations	Pyridoxine HCl(μg.mL^{-1})	t_R (min)		Peak area		Recovery %
		Drug	Standard	Drug	Standard	
Samavit B_6 (Tablet) 40mg SDI-Iraq	25	1.83	1.84	11851223	12632548	93.81
	50	1.83	1.84	21955184	23704571	92.62
	750	1.84	1.84	31626701	34560441	91.51
	1000	1.84	1.84	41716705	45414788	91.85
Mason natural B_6 (Tablet) 50mg USA	25	1.87	1.83	13185678	12632548	104.37
	50	1.87	1.83	24535660	23704571	103.50
	750	1.88	1.84	36323016	34560441	105.09
	1000	1.89	1.84	46800306	45414788	103.30
MehecoB_6 (Tablet) 40mg Beijing –China	25	1.83	1.83	13046867	12632548	103.27
	50	1.83	1.83	24257703	23704571	102.33
	750	1.84	1.84	33745565	34560441	97.64
	1000	1.84	1.84	43906323	45414788	96.67
MefronB_6 (Injection) 100mg/2ml Shanohai - China	25	1.82	1.84	12671315	12632548	100.30
	50	1.82	1.84	22909665	23704571	96.64
	750	1.82	1.84	34034303	34560441	98.47
	1000	1.83	1.84	44410757	45414788	97.78

Table-7 shows a good applicability of pyridoxine hydrochloride in its pharmaceutical preparations in the both forms tablets and injections.

Experimental t-test

The table of t-test (at 95% confidence and for four degrees of freedom [18]) shows good trustability, according to Table (8) there is no significant difference between the proposed method and the literature method [19].

Table 8-Experimental t-test

Pharmaceutical preparations	Recovery % *		t-exp
	Present method	Literature method [19]	
Samavit B_6 (Tablet) 40mg SDI – Iraq	92.44	91.84	1.37
Mason natural B_6 (Tablet) 50 mg – USA	104.06	103.41	1.64
Meheco (Tablet) 40 mg Beijing – China	99.97	98.54	1.11
MefronB_6 (Injection) 100 mg/2ml Shanohai – China	98.29	97.57	1.01

*****Average of five determinations

Comparison of the method

A comparison of the proposed method with the literature methods [15,16] (Table-9) shows that the three methods are applicable. Moreover, the present method is more rapid, simple, and precise.

Table 9-Comparison of the method

Parameter	Present method	Litruturemethod [16]	Litruturemethod[15]
Column	Cosmosil $5C_{18}$-MS-II (250 mm x 4.6 mm i.d., 5µm particle size)	Spherisort OSD C_{18}(250 mm x 4.6 mm i.d., 5µm particle size m)	Phenomenex OSD C_{18} (250 mm x 3.2 mm i.d., 5µm particle size m)
Mobile phase	ACN: buffer solution (Citric acid, Na_2HPO_4) (70:30)(V:V)	Methanol: Sodium acetate : water (50:40:50)(V:V:V)	ACN: 0.05 M TEA and phosphate buffer (12:88) (V:V)
Ttemperature	Room temperature	Room temperature	Room temperature
Detection	UV-detection at 282 nm	UV-detection	Fluorescence λEx = 330, λEm = 420
Retention time(min)	1.82	3.03	7.35
Flow rate (ml.min^{-1})	1.25	1.0	0.4
Linearity(µg.ml^{-1})	0.2-22	0.4-150	-
Recovery %	100.05	102.52	105-97.8
RSD %	1.05	3.0	-
Application	Pharmaceutical Preparations	Pharmaceutical Preparations	Cinchona bark

Conclusion

A simple, precise, and accurate HPLC method for the determination of vitamin B6 (pyridoxine hydrochloride)in different pharmaceutical preparations using the proposed procedure without pre separation steps and/or adjustment of temperature has been reported in this paper.

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