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Research Article

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Vitamins B_1, B_3, B_5 and B_6 in Multi Vitamin Injection

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Abstract

A simple, efficient and reproducible RP-HPLC method for simultaneous determination of vitamins B_1 (Thiamine hydrochloride), B_3 (Nicotinamide), B_5 (Dexpanthenol) and B_6 (Pyridoxine hydrochloride) in a combined multivitamin injection dosage form has been developed and validated. The Chromatographic Separation was carried out on Water C18 (250 × 4.6mm; 5µm) column using the mobile phase consists of buffer (pH 3.5) and methanol in the ratio 95:5. The mobile phase was flowed at the rate of 1.5 ml/min and effluent was detected at 210 nm. The retention times of Thiamine hydrochloride, Nicotinamide, Dexpanthenol and Pyridoxine hydrochloride were 2.492 min, 6.748 min, 20.084 min and 4.077 min respectively. The method was validated according to ICH guidelines and the acceptance criteria for system suitability, specificity, linearity, accuracy, precision and ruggedness were met in all cases. The method was linear in the range of 10-200 µg/ml for Thiamine hydrochloride (r^2 = 0.9992), 40-800 µg/ml for Nicotinamide (r^2 = 0.9994) 10-200 µg/ml for Dexpanthenol (r^2 = 0.9993) and 8-160 µg/ml for Pyridoxine hydrochloride (r^2 = 0.9991). The percentage relative standard deviation for precision was found to be less than 2.0%. Hence, the method could be successfully applied for routine analysis of B_1 (Thiamine hydrochloride), B_3 (Nicotinamide), B_5 (Dexpanthenol) and B_6 (Pyridoxine hydrochloride) from multivitamin injections.

1. INTRODUCTION

Thiamine hydrochloride (Fig. 1) chemically, 2-[3-[(4-amino-2-methylpyrimidin-5-yl) methyl]-4-methyl-1, 3-thiazol-3-ium-5-yl] ethanol hydrochloride, used to treat ulcerative colitis and persistent diarrhea [1-7]. Nicotinamide (Fig.2) chemically, pyridine-3-carboxamide, used to treat skin disorders, anxiety, Alzheimer's disease [2,8-12]. Dexpanthenol (Fig.3) chemically, 2,4-Dihydroxy-N-(3- hydroxypropyl)-3,3-dimethylbutanamide, used to minimize paralytic ileus; treatment of postoperative distention; topical to relieve itching and to aid healing of minor dermatoses[3,13-17] . Pyridoxine hydrochloride (Fig. 4) Chemicaly, 4, 5-Bis (hydroxymethyl)-2-methylpyridin-3-ol hydrochloride, used to treat nausea and vomiting in early pregnancy [4,18-22].

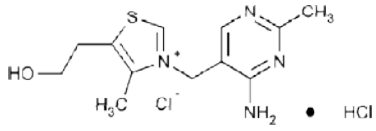

Figure 1: Structure of Thiamine Hydrochloride

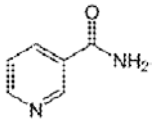

Figure 2: Structure of Nicotinamide

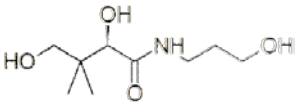

Figure 3: Structure of Dexpanthenol

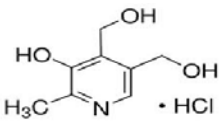

Figure 4: Structure of Pyridoxine HCL

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A literature survey revealed that a few analytical methods have been reported for the estimation of these vitamins individually or in combination with other vitamins by UV Spectrophotometry [5,23-26], High- Performance Liquid Chromatography [6,27], Electrospray ionization- mass spectrometry [7,28], reversed-phase ion-pair high performance liquid chromatography [8,29]. In this present work, an attempt was made to develop a simple, feasible and simultaneous determination of vitamins B_1, B_3, B_5 and B_6 in combined multivitamin injection by RP-HPLC. The proposed method was validated in accordance with International Conference on Harmonization (ICH) guidelines[9].

2. MATERIALS AND METHODS

2.1 Experimental

2.1.1 Chemicals and reagents

Methanol of HPLC grade, Potassium di hydrogen phosphate and Phosphoric acid were purchased from E.Merck (India) Ltd., Mumbai. Vitamins B_1, B_3 B_5 and B_6 were a gift sample by Caplin Point Laboratories Ltd., Gummidipoondi Taluk, Chennai – 601 201, Tamil Nadu, India. The commercially available multivitamin injection containing B1 (Thiamine hydrochloride), B3 (Nicotinamide), B5 (Dexpanthenol) and B6 (Pyridoxine hydrochloride) were procured from the local market.

2.1.2 Instrumentation and chromatographic conditions

The Chromatographic Separation was carried out on Water C18 (250 × 4.6mm; 5µm) column using the mobile phase consists of buffer (pH 3.5) and methanol in the ratio 95:5. The mobile phase was flowed at the rate of 1.5 ml/min and effluent was detected at 210 nm. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 15 min. with the mobile phase following through the system.

2.1.3 Preparation of Standard and Sample Preparations

a) Standard Preparation

Weighed accurately about 50 mg of Thiamine hydrochloride, 200 mg of Nicotinamide WS 50mg of Dexpanthenol and 40 mg of Pyridoxine hydrochloride transferred into a 50 ml volumetric flask. Added 20 ml of mobile phase used as diluent and Sonication was done for 5 minutes. Dissolved and diluted up to the volume with mobile phase. Transferred 5 ml of the above solution through a pipette into a 50 ml volumetric flask and diluted up to the volume with mobile phase. Filtered the solution through 0.22 µm Nylon filter and collected the solution in a HPLC vial after discarding the first 2 ml of filtrate.

b) Sample Preparation

Transferred 2 ml of the sample (which is equivalent to 10 mg of Thiamine Hydrochloride, 40 mg of Nicotinamide, 10 mg of Dexpanthenol and 8 mg of Pyridoxine Hydrochloride) through a pipette into a 100 ml volumetric flask. Added 20 ml of Diluent and Sonication was done for 5 minutes. Dissolved and diluted up to the volume with diluent. Filtered the solution through 0.22 µm Nylon filter and collected the solution into a HPLC vial after discarding the first 2 ml of filtrate.

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines.

3. RESULTS AND DISCUSSION

3.1 System Suitability

It is essential for the assurance of the quality performance of chromatographic system. Five injections of standard drug solutions, Vitamins B_1 (Thiamine hydrochloride), B_3 (Nicotinamide), B_5 (Dexpanthenol) and B_6 (Pyridoxine hydrochloride) were given separately to the system. The system suitability parameters such as retention time, peak area response, Tailing factor and number of theoretical plates and their Mean, Standard deviation & %RSD were also be calculated for the standard drug solutions and mentioned in Table 1 - 4. It was observed that all the values are with in the limits.

Table 1: System suitability for Vitamin B_1 (Thiamine hydrochloride)

S. No.	Standard	System suitability parameters			
		Peak area response	Number of theoretical plates	Tailing factor	Retention time (min)
1.	Standard -1	1506.9836	6397	1.28	2.492
2.	Standard -2	1509.3949	6127	1.29	2.483
3.	Standard -3	1510.0985	6331	1.3	2.479
4.	Standard -4	1516.7497	6281	1.29	2.469
5.	Standard -5	1507.4094	6034	1.31	2.457
Mean					**2.471**
Standard deviation					**0.018**
RSD in %					**0.728**

Table 2: System suitability for Vitamin B_3 (Nicotinamide)

S. No.	Standard	System suitability parameters			
		Peak area response	Number of theoretical plates	Tailing factor	Retention time (min)
1.	Standard -1	18462.30	14675	1.14	6.748
2.	Standard -2	18685.10	14686	1.11	6.407
3.	Standard -3	18320.90	14840	1.09	6.211
4.	Standard -4	18419.50	16212	1.20	6.399
5.	Standard -5	18629.32	16355	1.14	6.628
Mean					**6.541**
Standard deviation					**0.243**
RSD in %					**3.715**

Table 3: System suitability for Vitamin B_5 (Dexpanthenol)

S. No.	Standard	System suitability parameters			
		Peak area response	Number of theoretical plates	Tailing factor	Retention time (min)
1.	Standard -1	666.79993	18558	0.85	20.064
2.	Standard -2	664.86530	19569	0.86	18.821
3.	Standard -3	669.37195	18836	0.86	18.270
4.	Standard -4	668.22229	19315	0.87	18.895
5.	Standard -5	667.49945	18754	0.87	19.782
Mean					**19.398**
Standard deviation					**0.871**
RSD in %					**4.49**

Table 4: System suitability for Vitamin B_6 (Pyridoxine hydrochloride)

S. No.	Standard	System suitability parameters			
		Peak area response	Number of theoretical plates	Tailing factor	Retention time (min)
1.	Standard -1	4036.2522	12915	0.79	4.077
2.	Standard -2	4014.3444	13096	0.81	3.890
3.	Standard -3	4024.7307	13906	0.82	3.785
4.	Standard -4	4021.0183	12934	0.83	3.865
5.	Standard -5	4033.6352	12592	0.83	3.973
Mean					**3.945**
Standard deviation					**0.12**
RSD in %					**3.042**

3.2 Specificity

The specificity of the HPLC method is illustrated in Fig. 5, where a complete separation of Vitamins B_1, B_3, B_5, and B_6 were noticed in presence of other inactive excipients used in injections. In addition, there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the Table 5 -8.

Table 5: Specificity for Vitamin B_1 (Thiamine hydrochloride)

S. No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	1496.62610
4.	Sample	1	1453.84509

Table 6: Specificity for Vitamin B_3 (Nicotinamide)

S. No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	618.68878
4.	Sample	1	689.46204

Table 7: Specificity for Vitamin B_5 (Dexpanthenol)

S. No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	18.8249
4.	Sample	1	18.1719

Table 8: Specificity for Vitamin B_6 (Pyridoxine hydrochloride)

S. No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	3758.68530
4.	Sample	1	3687.21362

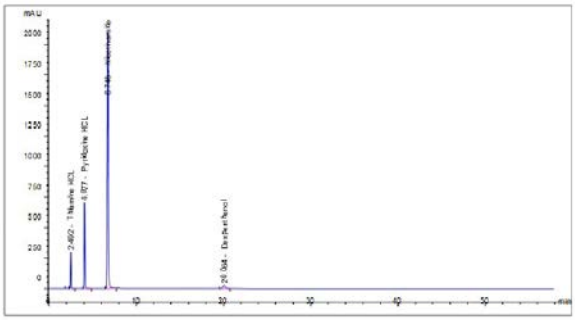

Figure 5: Typical HPLC Chromatogram of Sample Injection (Vitamins B_1, B_3, B_5, and B_6)

3.3 Linearity and Range

The Linearity of this method was determined at five levels from 10%– 200% of operating concentrations for Vitamins B_1, B_3, B_5, & B_6 and it was shown in Table 9. The plots of peak area of each sample against respective concentrations of Vitamins B_1, B_3, B_5, and B_6 were found to be linear (Fig.6 – 9) in the range of 10%– 200% of operating concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The linear regression equations and correlation coefficient were found. It observed that correlation coefficient and regression analysis were with in the limits.

Table 9: Linearity of response for Vitamins B_1, B_3, B_5 and B_6

Target level %	Concentration of (µg/ml)				Area obtained			
	Vit. B_1	Vit. B_6	Vit.B_5	Vit.B_3	Vit. B_1	Vit. B_6	Vit.B_5	Vit.B_3
10	10	8	10	40	174.75247	447.88669	65.7784	2158.47339
20	20	16	20	80	317.44849	814.6073	117.89529	3372.2998
50	50	40	50	200	736.44342	1879.89197	276.66666	8007.56055
100*	100	80	100	400	1599.89001	3750.21069	569.1496	16595.6000
120	120	96	120	480	1843.60242	4672.05469	690.27618	20226.8000
160	160	128	160	640	2536.22559	6347.97607	941.20715	26773.0000
200	200	160	200	800	3173.59131	7779.61475	1180.0033	32823.5000

***** Operating concentration

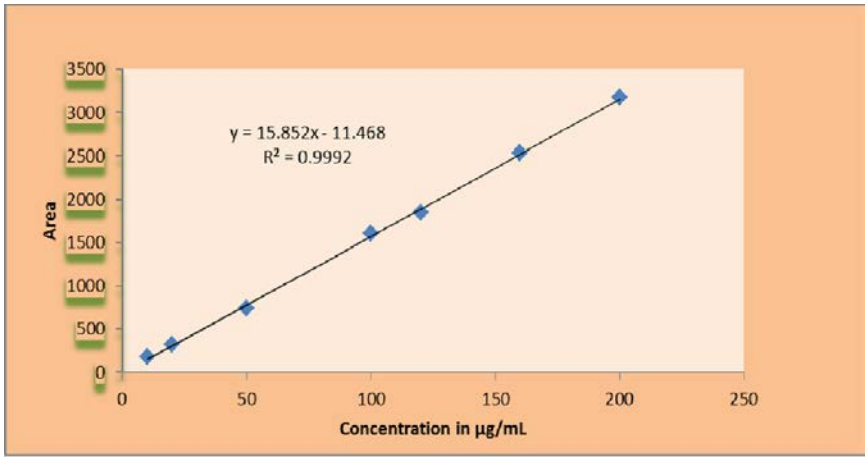

Figure 6: Linearity curve for Vitamin B_1

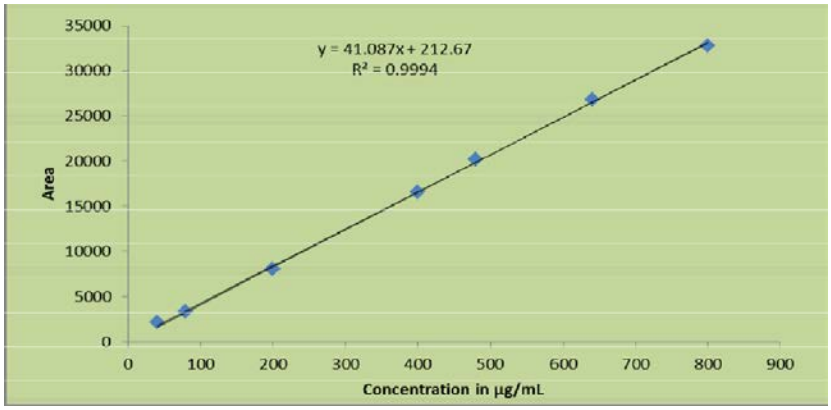

Figure 7: Linearity curve for Vitamin B_3

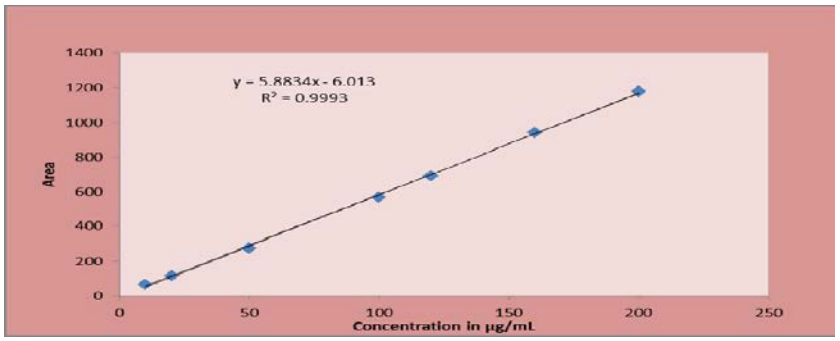

Figure 8: Linearity curve for Vitamin B_5

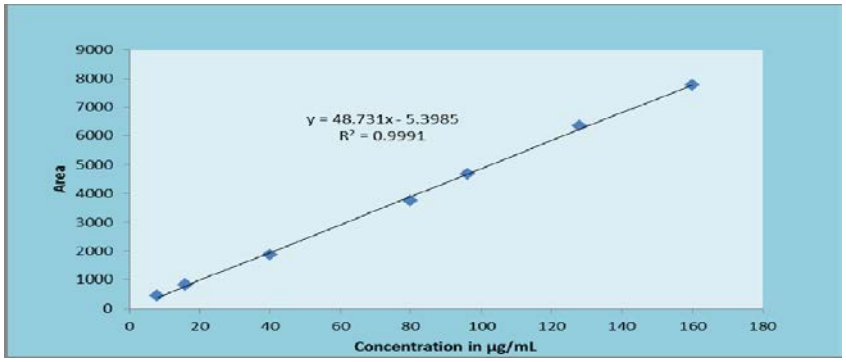

Figure 9: Linearity curve for Vitamin B_6

3.4 Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standards, Vitamins B_1, B_3, B_5, & B_6 were added to pre-analysed samples at a level from 80% up to 120% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in Table 10 -13. It was observed that the mean percentage recoveries were found to be for Vitamins B_1, B_3, B_5, & B_6 which demonstrated that the method was highly accurate.

Table 10: Accuracy for Vitamin B_1

S. No.	Target level	Vitamin B_1 added (mg)	Vitamin B_1 recovered (mg)	Drug Recovery (%)
1.	80%	0.08072	0.081959	101.53
2.	80%	0.08256	0.081978	99.29
3.	80%	0.08372	0.082925	99.05
4.	100%	0.0973	0.097017	99.71
5.	100%	0.09936	0.098721	99.36
6.	100%	0.1033	0.102717	99.44
7.	120%	0.1204	0.119976	99.65
8.	120%	0.1193	0.11935	100.04
9.	120%	0.1191	0.11957	100.39
Mean				**99.82**
Standard deviation				**0.75**
RSD in %				**0.72**

Table 11: Accuracy for Vitamin B_3

S. No.	Target level	Vitamin B_3 added (mg)	Vitamin B_3 recovered (mg)	Drug Recovery (%)
1.	80%	0.32796	0.332321	101.33
2.	80%	0.32766	0.329363	100.52
3.	80%	0.3287	0.331873	100.97
4.	100%	0.39264	0.388955	99.06
5.	100%	0.39712	0.393651	99.13
6.	100%	0.3982	0.405913	101.94
7.	120%	0.47402	0.46955	99.06
8.	120%	0.47522	0.470682	99.05
9.	120%	0.47412	0.469398	99.00
Mean				**100.00**
Standard deviation				**1.18**
RSD in %				**1.18**

Table 12: Accuracy for Vitamin B_5

S. No.	Target level	Vitamin B_5 added (mg)	Vitamin B_5 recovered (mg)	Drug Recovery (%)
1.	80%	0.11900	0.119094	100.08
2.	80%	0.11792	0.118972	100.89
3.	80%	0.11972	0.119929	100.17
4.	100%	0.1121	0.11252	100.37
5.	100%	0.1139	0.113625	99.76
6.	100%	0.11736	0.117723	100.31
7.	120%	0.1193	0.119013	99.76
8.	120%	0.12046	0.12075	100.24
9.	120%	0.12042	0.120043	99.69
			Mean	**100.14**
			Standard deviation	**0.37**
			RSD in %	**0.38**

Table 13: Accuracy for Vitamin B_6

S. No.	Target level	Vitamin B_6 added (mg)	Vitamin B_6 recovered (mg)	Drug Recovery (%)
1.	80%	0.06494	0.065414	100.73
2.	80%	0.06378	0.064843	101.67
3.	80%	0.06604	0.065963	99.88
4.	100%	0.0773	0.07709	99.73
5.	100%	0.07802	0.078437	100.53
6.	100%	0.0807	0.081433	100.91
7.	120%	0.0945	0.093819	99.28
8.	120%	0.09428	0.093354	99.02
9.	120%	0.09378	0.092894	99.06
Mean				**100.09**
Standard deviation				**0.92**
RSD in %				**0.92**

3.5 Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

3.6 Repeatability

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. Repeatability data for Vitamins B_1, B_3, B_5, & B_6 were shown in Table 14 -17. This indicated that method was highly precise.

Table 14: Precision – Repeatability for Vitamin B_1

S. No.	Sample Name	Area	Amount of drug present (mg)	Drug Recovery (%)
1.	Sample -1	1835.636	5.57	111.40
2.	Sample -2	1874.873	5.68	113.60
3.	Sample -3	1968.618	5.57	111.40
4.	Sample -4	2002.918	5.67	113.40
5.	Sample -5	2047.996	5.68	113.60
6.	Sample -6	2026.000	5.59	111.80
Mean				**112.53**
Standard deviation				**1.10**
RSD in %				**0.98**

Table 15: Precision – Repeatability for Vitamin B_3

S. No.	Sample Name	Area	Amount of drug present (mg)	Drug Recovery (%)
1.	Sample -1	21801.40	21.35	106.75
2.	Sample -2	21013.05	20.55	102.75
3.	Sample -3	22437.25	20.50	102.50
4.	Sample -4	22522.50	20.57	102.85
5.	Sample -5	23131.00	20.69	103.45
6.	Sample -6	23301.10	20.74	103.70
Mean				**103.66**
Standard deviation				**1.57**
RSD in %				**1.52**

Table 16: Precision – Repeatability for Vitamin B_5

S. No.	Sample Name	Area	Amount of drug present (mg)	Drug Recovery (%)
1.	Sample -1	676.740	5.35	107.00
2.	Sample -2	665.323	5.25	105.00
3.	Sample -3	727.344	5.37	107.40
4.	Sample -4	736.088	5.43	108.60
5.	Sample -5	725.891	5.24	104.80
6.	Sample -6	734.753	5.28	105.60
			Mean	**106.4**
			Standard deviation	**1.50**
			RSD in %	**1.42**

Table 17: Precision – Repeatability for Vitamin B_6

S. No.	Sample Name	Area	Amount of drug present (mg)	Drug Recovery (%)
1.	Sample -1	4514.453	4.27	106.75
2.	Sample -2	4581.609	4.33	108.25
3.	Sample -3	4761.943	4.20	105.00
4.	Sample -4	4845.079	4.27	106.75
5.	Sample -5	5010.323	4.33	108.25
6.	Sample -6	5064.331	4.35	108.75
Mean				**107.29**
Standard deviation				**1.40**
RSD in %				**1.30**

3.7 Ruggedness

Six sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The robustness data for Vitamins B_1, B_3, B_5, & B_6 were shown in Table 18. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was ruggedness.

Table 18: Ruggedness data for Vitamins B_1, B_3, B_5, & B_6

S. No	Replicate Samples	Area of Vitamin B_1	Area of Vitamin B_3	Area of Vitamin B_5	Area of Vitamin B_6
1	Sample-1	1748.40869	20.27674	656.66705	4042.02734
2	Sample-2	1755.36145	20.56924	654.39532	4041.56006
3	Sample-3	1739.27173	19.83864	650.99624	4104.12305
4	Sample-4	1745.68652	19.84414	659.71875	4096.19873
5	Sample-5	1745.64014	19.73074	653.00732	4100.67236
6	Sample-6	1742.56311	19.40354	657.24158	4085.91895
Mean		1746.15	19.94	655.33	4078.41
Standard Deviation		0.310	1.348	0.938	0.411
RSD (%)		0.278	1.338	0.888	0.393

4. CONCLUSION

The Proposed study describes a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the quantitative determination of vitamins B_1, B_3, B_5 and B_6 in a combined multivitamin injection dosage form. The method was validated as per ICH guidelines and found to be simple, specific, linear and precise. Therefore the proposed method can be successfully used for the routine analysis of vitamins B_1, B_3, B_5 and B_6 in pharmaceutical dosage form without interference.

5. ACKNOWLEDGEMENTS

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