

Stability-Indicating HPLC Methods for the Quantification of Cyproheptadine Hydrochloride, Vitamins B₁, B₅ And B₆ in Tablets using RP-HPLC with UV Detection

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Abstract

The availability of reliable and fast analytical method is crucial for the quality control of multi-API in a single dosage form. Herein, two methods have been developed and validated for the determination of Cyproheptadine hydrochloride (CH), Thiamine mononitrate (Vit. B₁), Calcium pantothenate (Vit. B₅) and Pyridoxine hydrochloride (Vit. B₆) in uncoated tablets. To assay the vitamins, a mobile phase combination of Phosphate buffer pH 3.5 and Methanol in the ratio 93:7, using Eurospher ODS (150 x 4.5mm) as the stationary phase with a flow rate set at 1.0 mL/min at ambient temperature conditions was adopted. Wavelength of detection was 270 nm with a run time of 0.00 to 5.50 minutes for Thiamine mononitrate and Pyridoxine hydrochloride, and 205 nm from 5.50 to 11.00 minutes for Calcium pantothenate. A good resolution and a short run time of 11 minutes were achieved with the validated conditions. The retention times of Thiamine mononitrate, Pyridoxine hydrochloride and Calcium pantothenate were 2.823±0.020, 4.184±0.007 and 10.025±0.015 respectively.

The mobile phase for Cyproheptadine HCl was methanol and an ion-pairing solution (70:30), set at a flow rate of 1mL/min, a runtime of 8 minutes, an injection volume of 20 µL and a wavelength of 285 nm using Eurospher ODS (150 x 4.5 mm) as the stationary phase. The retention time for Cyproheptadine HCl was 4.961±0.006. Both methods were found to be specific, robust, accurate and precise over the concentration ranges of 0.0192 mg/mL–0.0288 mg/mL for Thiamine mononitrate, 0.0128–0.0192 mg/mL for Pyridoxine hydrochloride, 0.032–0.048 mg/mL for Calcium pantothenate and 0.032-0.048 mg/mL for Cyproheptadine hydrochloride. The Correlation Coefficient (r²) for Cyproheptadine hydrochloride, Thiamine mononitrate, Pyridoxine hydrochloride and Calcium pantothenate were greater than 0.999.

The purpose of this study was to develop and validate simple HPLC methods for the estimation of Cyproheptadine hydrochloride, Vitamins B₁, B₅ and B₆ in combined dosage forms. The proposed methods were found to be precise, specific, accurate and robust for the estimation of Cyproheptadine hydrochloride, Vitamins B₁, B₅ and B₆.

Keywords: Cyproheptadine hydrochloride, Thiamine mononitrate, Pyridoxine hydrochloride, Calcium pantothenate, RP-HPLC, Validation

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1. Introduction

Cyproheptadine hydrochloride is the hydrochloride salt of a synthetic methyl-piperidine derivative with antihistaminic and anti-serotonergic properties. Cyproheptadine competes with free histamine (HA) for binding at HA-receptor sites, thereby competitively antagonizing histamine stimulation of HA-receptors in the gastrointestinal tract, large blood vessels, and bronchial smooth muscle. This agent also competes with free serotonin for binding at serotonin receptor sites. Cyproheptadine exhibits anticholinergic and sedative properties and has been shown to stimulate appetite and weight gain. Cyproheptadine has a molecular formula of C₂₁H₂₂CIN (National Center for Biotechnology Information 2020a). Thiamine mononitrate has a molecular formula of C₁₂H₁₇N₅O₄S. It plays a key role in intracellular glucose metabolism and it is thought that thiamine inhibits the effect of glucose and insulin on arterial smooth muscle cell proliferation. Thiamine plays an important role in

helping the body convert carbohydrates and fat into energy. It is essential for normal growth and development and helps to maintain proper functioning of the heart and the nervous and digestive systems (Wishart *et al.* 2018). Pyridoxine hydrochloride has a molecular formula of $C_8H_{12}ClNO_3$. It is the hydrochloride salt form of pyridoxine, a water-soluble vitamin B. Pyridoxine hydrochloride is converted into the active form pyridoxal 5'-phosphate (PLP), an essential cofactor in many enzymatic activities including synthesis of amino acids, neurotransmitters, and sphingolipids. This vitamin is essential to red blood cell, nervous system, and immune systems functions and helps maintain normal blood glucose levels (National Center for Biotechnology Information 2020b). Calcium pantothenate has a molecular formula of $C_{18}H_{32}CaN_2O_{10}$. It is the calcium salt of the water-soluble vitamin B₅, ubiquitously found in plants and animal tissues with antioxidant property. Pantothenate is a component of coenzyme A (CoA) and a part of the vitamin B₂ complex. Vitamin B₅ is a growth factor and is essential for various metabolic functions, including the metabolism of carbohydrates, proteins, and fatty acids. This vitamin is also involved in the synthesis of cholesterol, lipids, neurotransmitters, steroid hormones, and hemoglobin (National Center for Biotechnology Information 2020c). Several chromatographic methods for the determination of Cyproheptadine hydrochloride, Thiamine mononitrate, Pyridoxine hydrochloride and Calcium pantothenate have been reported (Shino *et al.* 2008; Hasimoglu and Ghodke 2018; Yantih *et al.* 2011; Staroverov *et al.* 2004). The aim of this study is to develop simple RP-HPLC methods. This study was done in accordance with the International Conference on Harmonization (ICH) guidelines (Q2(R1) 2005).

2. Materials and Methods

2.1 Equipment and materials

1-Hexanesulphonic acid sodium salt for HPLC (VWR Prolabo chemicals 10E050001), Glacial Acetic acid (BN CC2C620145), Acetonitrile (HPLC grade (Thermo Fisher Scientific India, PVT Ltd, 99.8%, 4353)), Syringe-driven filter unit (Make: Millipore Millex, Lot Number: ROAAB2861), pH Meter (Make: Labinda Model: SAB 5000), Millipore vacuum pump (Make: Millipore, Model: WP6122050), 0.2 μ m Mobile phase filter (Make: Millipore Model: GNWP); Potassium dihydrogen orthophosphate, Phosphoric acid, Methanol (HPLC Grade (VWR Chemicals; 100.0%, BN 14K240503)), Distilled water, Volumetric pipettes, Knauer HPLC system with Autosampler and UV detection, Measuring cylinder, ODS Column (Eurospher-150 x 4.6) mm column; particle size 5 μ m (Make: Knauer (Germany)), Analytical balance (Make: Kern and Sohn GmbH, Model: ABJ 120-4M).

2.2 Instrumentation and Chromatographic conditions

A High Performance Liquid Chromatographic (HPLC) system, controlled by Knauer Advanced Scientific Instrument data handling system (Knauer 2000) and coupled with an autosampler was used for the analysis. The data was recorded using Euro Chrome software. For the analysis of the Vitamins, a stainless steel column 150 mm long, 4.5 mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5 μ m diameter (Make: Knauer Advanced Scientific, Germany) was used with the mobile phase composed of Phosphate buffer pH 3.5 and Methanol (93:7) at ambient temperature. The mobile phase was pumped through the column at a flow rate of 1 mL/minute. The sample injection volume was 20 μ L. The UV detector was set at a wavelength of 270 nm from 0.00 to 5.50 minutes for the detection of Thiamine mononitrate and Pyridoxine hydrochloride, and 205 nm from 5.50 to 11.00 minutes for the detection of Calcium pantothenate. The run time was 11 minutes. For Cyproheptadine, same column as for the vitamins was used. The mobile phase consisted of an ion pairing reagent prepared separately and mixed with methanol in the ratio (30:70). The wavelength was 285 nm, injection volume of 20 μ L, flow rate of 1 mL/min and Runtime of 8 minutes.

Model tablets containing 1.5 mg Thiamine mononitrate, 1 mg Pyridoxine hydrochloride and 2.5 mg Calcium pantothenate were prepared with the following excipients; Lactose, Dicalcium phosphate granules, Microcrystalline cellulose Avicel 12, Sodium starch glycolate, Talc, Magnesium stearate and Cab-O-sil. Samples were analysed using the developed RP-HPLC method. The results are reported as means \pm SEM (standard error of the mean). Results were statistically analysed for significant differences.

2.3 Preparation of solutions

2.3.1 Analytical Solutions for Analysis of Cyproheptadine HCl

2.3.1.1 Diluent

Acetonitrile, Glacial Acetic acid and Water (5:1:94)

2.3.1.2 Preparation of Solution A

Approximately 700 mg of 1-Hexanesulphonic acid sodium salt was weighed and transferred into a 500 mL volumetric flask containing 250 mL of distilled water, dissolved, added 5 mL of Glacial acetic acid and made to volume with distilled water.

2.3.1.3 Preparation of Mobile phase

Into a 500 mL volumetric flask, about 350 mL of methanol (HPLC grade) was transferred and mixed with 150 mL of Solution A.

2.3.1.4 Preparation of Standard solution

This was prepared by accurately weighing and transferring about 40 mg of Cyproheptadine HCl working standard into a 100 mL volumetric flask and dissolved to the mark with the diluent with intermittent shaking for 30 minutes. 5 mL of the resultant solution was dissolved into a 50 mL volumetric flask and diluted to the mark with the diluent.

2.3.1.5 Preparation of Sample Solution

About 240 mg of the synthetic blend was weighed into a 100 mL volumetric flask and dissolved with the diluent for 30 minutes to the mark.

2.3.2 Preparation of Analytical Solutions for Analysis of Vitamins B₁, B₅ and B₆.

2.3.2.1 Preparation of Buffer solution

About 500 mg of monobasic potassium phosphate was dissolved in 1000 mL of water and adjusted with phosphoric acid to a pH of 3.5.

2.3.2.2 Preparation of Mobile phase

Into a 1000 mL volumetric flask, 900 mL of the phosphate buffer was transferred, and 100 mL of Methanol HPLC grade was added with thorough mixing.

2.3.2.3 Preparation of Standard solution

About 24 mg of Vit. B₁ working standard, 40 mg of Vit. B₅ working standard and 16 mg of Vit. B₆ working standard were accurately weighed and transferred into a 100 mL volumetric flask. They were dissolved and diluted to the mark with distilled water. 5 mL was transferred into a 50 mL volumetric flask and diluted to the mark with distilled water.

2.3.2.4 Preparation of Sample solution

About 960 mg of the synthetic blend was weighed and transferred into a 250 mL volumetric flask. 10 mL of methanol was added and swirled to disperse the specimen. Distilled water was added to volume, mixed and filtered.

2.4 Method Development

In order to develop a relatively cheap chromatographic system for Vitamins B₁, B₅ and B₆, Methanol (HPLC grade) was chosen as the only organic solvent during the developmental stages. In order to obtain the best resolution possible, various mobile phase ratios were utilized. A mobile phase ratio of Phosphate buffer pH 3.5 and Methanol (93:7) was found appropriate at a flow rate of 1 mL/min with wavelength of 270 nm from 0.00 to 5.50 minutes for the detection of Thiamine mononitrate and Pyridoxine hydrochloride, and 205 nm from 5.50 to 11.00 minutes for the detection of Calcium pantothenate. Eurospher ODS (150 x 4.5mm) was the column used at an injection volume of 20 µL and Run time of 11 minutes. Column temperature was ambient.

The method for Cyproheptadine hydrochloride consisted of an Ion pairing reagent (1-Hexanesulphonic acid sodium salt) used in the mobile phase to enhance the separation of the vitamins and Cyproheptadine hydrochloride on the reversed phase column. A mobile phase composition of Methanol and ion pairing solution (70:30) was used with a flow rate of 1 mL/min, runtime of 8 minutes, injection volume of 20 µL and a wavelength of 285 nm.

3. Results and Discussions

3.1 Specificity and specificity by forced degradation

In evaluating specificity, there were no interferences. Injections of the placebo and the mobile phase (diluent) gave no peaks. The order of elution is as follows; Thiamine mononitrate followed by Pyridoxine hydrochloride and then Calcium pantothenate.

Table 1. Retention times of Peaks obtained from Placebo, Active ingredients and Diluent

Solutions	Retention time (minute)
Diluent for Cyproheptadine hydrochloride	No peak
Diluent for Vitamins B ₁ , B ₅ and B ₆	No peak
Placebo for Cyproheptadine hydrochloride	No peak
Placebo for Vitamins B ₁ , B ₅ and B ₆	No peak
Synthetic blend containing Cyproheptadine hydrochloride, Vitamins B ₁ , B ₅ and B ₆ (Method used for analyzing Cyproheptadine HCl)	5.128 ± 0.005
Synthetic blend containing Cyproheptadine hydrochloride, Vitamins B ₁ , B ₅ and B ₆ (Method used for analyzing Vitamins B ₁ , B ₅ and B ₆)	2.823 ± 0.020; 4.184 ± 0.007; 11.025 ± 0.015
Cyproheptadine hydrochloride working standard solution	4.961 ± 0.006
Thiamine mononitrate working standard solution	3.144 ± 0.004
Pyridoxine hydrochloride working standard solution	4.233 ± 0.007
Calcium pantothenate working standard solution	10.303 ± 0.014

3.1.1 Representative chromatograms justifying specificity of the two methods

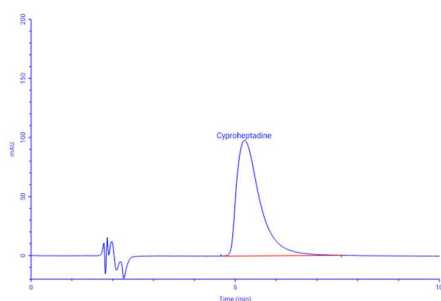


Figure 1. Standard solution (CH)

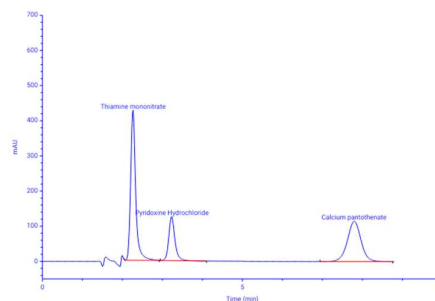


Figure 2. Standard solution (Vitamins B₁, B₅ and B₆)

Table 2. Results obtained from thermally stressed (dry heat in oven at 80 °C for 4 hours) samples

No.	Solutions	Retention time (minute)
1	Thermal stressed synthetic blend solution for Vitamins B ₁ , B ₅ and B ₆	Vit B ₁ = 2.033 min, Vit. B ₆ = 4.083 min, Vit. B ₅ = No peak
2	Thermal stressed placebo for Vitamins B ₁ , B ₅ and B ₆	No peaks
3	Thermal stressed synthetic blend for Cyproheptadine Hydrochloride	4.961 min
4	Thermal stressed placebo for Cyproheptadine Hydrochloride	No peaks

Table 3. Results of System suitability test

Parameter	CH	Vit. B ₁	Vit. B ₆	Vit. B ₅
Retention Time (min)	4.961±0.006	3.144±0.004	4.233±0.007	10.303±0.014
PA (mAu*min)	63.5857±0.149	11.9722±0.075	5.0195±0.038	6.9625±0.013
RSD of RT (min)	0.28	0.28	0.43	0.33
RSD of PA (%)	0.57	1.54	1.83	0.44
Tailing Factors	1.010	1.024	1.069	1.016
	Resolution			
		Vit. B ₁ and Vit. B ₆	Vit. B ₆ and Vit. B ₅	Vit. B ₁ and Vit. B ₅
		1.763±0.719	5.168±2.109	6.931±2.829

The system was found suitable as per predetermined acceptance criterion prior to the generation of all other data from the execution of other method performance characteristics.

3.2 Precision

3.2.1 Repeatability

Six sample preparations of the synthetic blend were analysed as per the protocol. The percentage contents and the % RSD were determined. RSD for the sample preparation of Cyproheptadine was 1.39% That of Vit. B₁, Vit. B₅ and Vit. B₆ were 0.90%, 1.01% and 1.51% respectively.

3.2.2 Intermediate Precision

3.2.2.1 Inter-day Precision

Analysis conducted on Day 1 and Day 2 were compared. For Cyproheptadine, RSD for Day 1 was 1.39% and that for Day 2 was 1.80%. For the Vit. B₁, RSD for Day 1 was 0.90% and Day 2 was 1.53%. For Vit. B₆, Day 1 was 1.51% and Day 2 was 1.47%. For Vit. B₅, Day 1 was 1.01% and Day 2 was 1.05%. Results obtained from Day 1 and Day 2 were statistically analyzed to determine any significant differences. For acceptance of the null hypothesis, F-calculated is not more than F-critical value of 5.05 at 95% confidence level (Olaniyi 2005). CH gave an F-calculated value of 1.49. Vit. B₁ was 2.66, Vit. B₆ was 2.56 and Vit. B₅ was 1.88. Hence, it was concluded that, there was no significant differences between data obtained on Day 1 and Day 2 for all the actives.

3.2.2.2 Intraday Precision

For CH, RSD for Analyst A was 1.39% and Analyst B was 1.81%. For Vit. B₁, RSD for Analyst A was 0.90% and Analyst B was 1.40%. Vit. B₆ had an RSD of 1.51% for Analyst A and 1.53% for Analyst B. For Vit. B₅, Analyst A had 1.01% and Analyst B had 1.12%. There were no significant differences between both Analysts as CH gave an F-calculated value of 1.55. Vit. B₁ was 2.45, Vit. B₆ was 1.03 and Vit. B₅ was 1.16 which were less than the F-critical value of 5.05 at 95% confidence level.

3.3 Linearity and Range

Table 4. Results of Linearity Study

	Vit. B ₁	Vit. B ₆	Vit. B ₅	CH
Concentration Range (mg/mL)	0.0192 to 0.0288	0.0128 to 0.0192	0.0320 to 0.0480	0.0320 to 0.0480
Slope	63221	17655	19991	62223
Intercept	1.4534	2.9225	-3.5730	5.0216
Intercept %	0.09 %	0.01 %	0.44 %	0.20 %
Correlation coefficient	0.999580	0.999004	0.999574	0.999783
Regression Coefficient	0.999159	0.998009	0.999148	0.999566

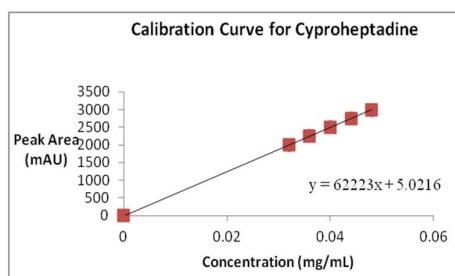


Figure 3. Calibration curve for CH

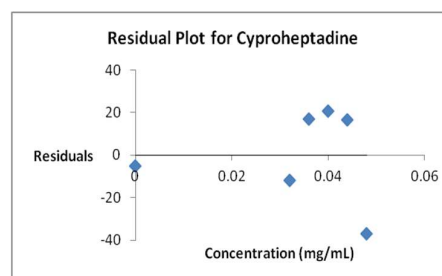


Figure 4. Residual plot for CH

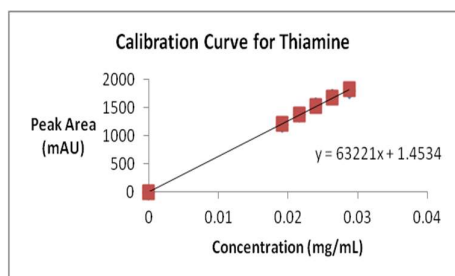


Figure 5. Calibration Curve for Vit. B₁

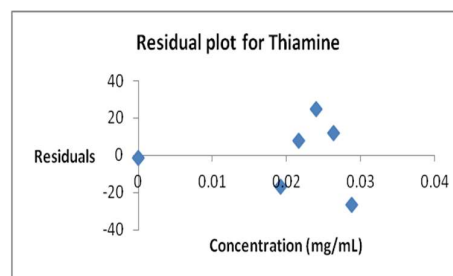


Figure 6. Residual plot for Vit. B₁

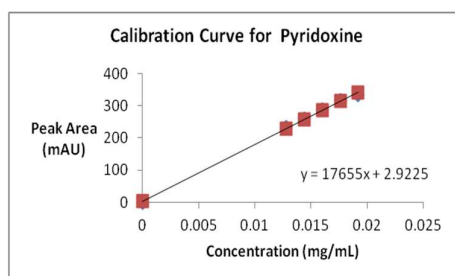


Figure 7. Calibration for Vit. B₆

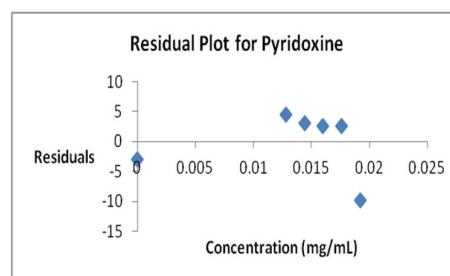


Figure 8. Residual plot for Vit. B₆

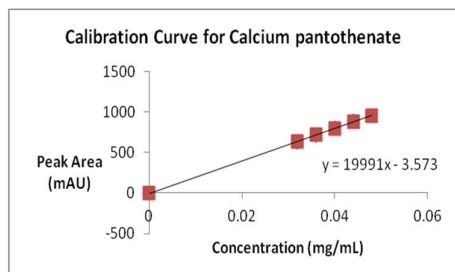


Figure 9. Calibration curve for Vit. B₅

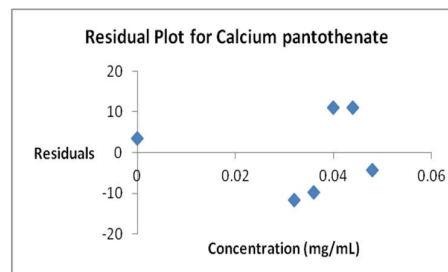


Figure 10. Residual plot for Vit. B₅

Table 5. Regression coefficient table for CH, Vitamins B₁, B₅ and Vit. B₆

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Vit. B ₁						
Intercept	1.453429	20.28788	0.07164	0.946327	-54.8747	57.78161
Vit. B ₆						
Intercept	2.922482	5.816094	0.502482	0.641731	-13.2256	19.07055
Vit. B ₅						
Intercept	-3.57302	10.76416	-0.33194	0.756602	-33.4591	26.31307
CH						
Intercept	5.021553571	23.9132	0.209991	0.843937	-61.3721	71.41523

Table 6. Residual output for CH and Vit. B₁

Observation	CH		Vit. B ₁	
	Predicted Y	Residuals	Predicted Y	Residuals
1	1996.169182	-12.0852	1215.292	-17.0041
2	2245.062636	17.03236	1367.022	7.987114
3	2493.956089	20.56091	1518.752	24.63629
4	2742.849543	16.38046	1670.482	12.22346
5	2991.742996	-36.867	1822.211	-26.3894
6	5.021553571	-5.02155	1.453429	-1.45343

Table 7. Residual output for Vit. B₆ and Vit. B₅

Observation	Vit. B ₆		Vit. B ₅	
	Predicted Y	Residuals	Predicted Y	Residuals
1	228.9095	4.517461	636.1372	-11.7652
2	257.1579	3.101079	716.101	-9.70102
3	285.4063	2.554696	796.0648	11.1322
4	313.6547	2.587314	876.0286	11.02341
5	341.9031	-9.83807	955.9924	-4.26237
6	2.922482	-2.92248	-3.57302	3.573018

From the statistical treatment of the linearity data of Cyproheptadine, the response of Cyproheptadine was linear

between 80.0 % to 120.0 % level. The correlation coefficient is greater than 0.998. In addition, the analysis of residuals shows that, the values are randomly scattered around zero, which fits, and well within the linear model. To evaluate whether the y-intercepts are significantly different from zero, the P-value was determined. If P value is > 0.05 , then intercept is statistically equal to zero. For Cyproheptadine, P value is 0.843937. Hence, it is statistically equal to zero. In addition, the origin is within the lower and the upper limit of the 95 % confidence level that gives a high degree of confidence to the values obtained for intercept. Moreover, the value of the intercept is less than 5 % of the area response at 100 % level.

From the statistical treatment of the linearity data of Thiamine mononitrate, Pyridoxine hydrochloride and Calcium pantothenate, the responses of Thiamine mononitrate, Pyridoxine hydrochloride and Calcium pantothenate were linear between 80.0 % to 120.0 % level. The correlation coefficients are greater than 0.998. In addition, the analysis of residuals shows that, the values are randomly scattered around zero, which fits, and well within the linear model. To evaluate whether the y-intercepts are significantly different from zero, the P-values were determined. If P value is > 0.05 then intercept is statistically equal to zero. For Thiamine mononitrate, Pyridoxine hydrochloride and Calcium pantothenate, P values were 0.946327, 0.641731 and 0.756602. Hence, it is statistically equal to zero. In addition, the origin is within the lower and the upper limit of the 95 % confidence level that gives a high degree of confidence to the values obtained for intercept. Moreover, the value of the intercept is less than 5 % of the area response at 100 % level.

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined. For Cyproheptadine HCl, LOD was 0.0019 mg/mL and LOQ was 0.0058 mg/mL. Vitamin B₁ had LOD of 0.0027 mg/mL and LOQ of 0.0083 mg/mL. Vitamin B₅ had LOD of 0.0014 mg/mL and LOQ of 0.0042 mg/mL. Vitamin B₆ had LOD of 0.0014 mg/mL and LOQ of 0.0043 mg/mL.

3.4 Robustness

Changes in flow rate (± 0.2 mL/min) and wavelength (± 2) were effected to determine the robustness of the method. Irrespective of the changes in chromatographic conditions, all the system suitability parameters remained unaffected and results conformed to acceptance criteria indicating robustness of the methods.

Table 8. Resolution factors between analytes

	0.8 mL/min			1.0 mL/min			1.2 mL/min		
	Vit. B ₁	Vit. B ₆	Vit. B ₁	Vit. B ₁	Vit. B ₆	Vit. B ₁	Vit. B ₁	Vit. B ₆	Vit. B ₁
	and Vit. B ₆	and Vit. B ₅	and Vit. B ₅	and Vit. B ₆	and Vit. B ₅	and Vit. B ₅	and Vit. B ₆	and Vit. B ₅	and Vit. B ₅
Mean	2.654	10.366	13.019	2.636	10.071	12.707	2.758	9.835	12.593
\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
SEM	0.011	0.013	0.009	0.013	0.027	0.027	0.015	0.011	0.021

3.5 Accuracy

Recovery studies ensures the quantification of target compounds. It is therefore very important in determining the accuracy of an analytical method. The percentage recoveries were found to be in the range of 98.60%-100.58% for CH, 98.98-100.44% for Vit. B₁, 100.00-101.63% for Vit. B₆ and 100.17-101.08% for Vit. B₅. There were not significant differences between the results obtained between the various concentration levels as T-values were less than 4.303 at 95 % confidence level.

Table 9. Results from Study of Accuracy

Vit. B ₁						
Level	50%	100%	50%	150%	100%	150%
Mean % Recovery	100.44	99.45	100.44	98.98	99.45	98.98
T-calculated	1.349		3.977		0.677	
Vit. B ₆						
Level	50%	100%	50%	150%	100%	150%
Mean % Recovery	101.63	100.02	101.63	100.00	100.02	100.00
T-calculated	1.559		3.436		0.024	
Vit. B ₅						
Level	50%	100%	50%	150%	100%	150%
Mean % Recovery	100.17	101.08	100.17	100.83	101.08	100.83
T-calculated	0.939		0.797		0.316	
CH						
Level	80%	100%	80%	120%	100%	120%
Mean % Recovery	100.58	99.92	100.58	98.60	99.92	98.60
T-calculated	1.031		2.908		3.531	
T-critical	4.303 at 95 % probability level					

3.6 Stability in Analytical Solution

The standard and sample solutions were injected within a period of 24 hours and was concluded that, standard and sample solutions of Thiamine mononitrate, Pyridoxine hydrochloride and Calcium pantothenate can be assayed within 24 hours after preparation.

4. Conclusion

The validation of the proposed isocratic RP-HPLC methods for the estimation of Cyproheptadine hydrochloride, Thiamine mononitrate, Pyridoxine hydrochloride and Calcium pantothenate in model tablets were carried out as per ICH guidelines. The methods were found to be specific, robust, accurate and precise for the estimation of Cyproheptadine hydrochloride, Thiamine mononitrate, Pyridoxine hydrochloride and Calcium pantothenate in fixed dosage forms over the concentration ranges of 0.0192 mg/mL–0.0288 mg/mL for Thiamine mononitrate, 0.0128–0.0192 mg/mL for Pyridoxine hydrochloride, 0.0320–0.0480 mg/mL for Calcium pantothenate and 0.0320–0.0480 mg/mL for Cyproheptadine hydrochloride.

Thus, the methods stand validated for the estimation of Cyproheptadine hydrochloride, Thiamine mononitrate, Pyridoxine hydrochloride and Calcium pantothenate in fixed dosage forms and may be used to certify in-process and finished products as well as employed in Stability studies.

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