Journal of Pharmaceutical Chemistry
http://www.vensel.org/index.php/jphchem

VENSEL

Formulation, method development and validation of water soluble vitamins B_1, B_2 & B_6 in bulk and tablet dosage form by HPTLC method

[1]Devi Velmurugan, [2,*]Jambulingam Munusamy, [3]Ananda Thangadurai Subramaniam, [2]Anandakumar Karunakaran, [2]Abdul Latiff MKM, [1]Kamalakannan Dhanapal

[1]Department of Pharmaceutical Analysis, JKK Nataraja College of Pharmacy, Komarapalayam-638183, TN, India
[2]Department of Pharmaceutical Analysis, Swamy Vivekanandha College of Pharmacy, Elayampalayam-637205, TN, India
[3]Department of Pharmaceutical Chemistry, University of Tabuk, Kingdom of Saudi Arabia

Abstract: In the present study we are reporting dissolution, method development and validation of water soluble vitamins B_1, B_2 & B_6 in bulk and tablet dosage form by HPTLC method. The method is based on separation of the three vitamins using HPTLC. Thin layer chromatographic plates coated with silica gel $60F_{254}$ as the stationary phase and acetonitrile : water (6:4 v/v) as mobile phase. The chromatographic analysis was carried out in the reflectance and absorbance mode at 280 nm. The method was validated with respect to linearity, accuracy and precision, limit of detection and limit of quantitation. It was then applied for analysis of vitamins B_1, B_2 & B_6 in combined tablet dosage form. The above method developed was reproducible with good resolution and the results of analysis have been validated with correlation coefficient of 0.9990

Keywords: HPTLC; vitamin B_1; vitamin B_2; vitamin B_6; method development; validation

1. Introduction

Vitamin B_1 (Thiamine) is chemically known as 3-(4-amino-2-methyl-5-pyrimidinyl)methyl)-5-(2-hydroxyethyl)-4-methylthiazolium chloride (Figure 1.). It is used to treat vitamin B_1 deficiency and required for maintenance of normal growth and transmission of nerve impulses.[1-2] Thiamine, after it gets converted into thiamine pyrophosphate plays a role in carbohydrate and protein metabolism as co-enzyme. Deficiencies of vitamin B_1 result in Beriberi, characterized by gastro-intestinal (GI) manifestations, peripheral neuropathy and cerebral deficits. It is absorbed from GI tract by both diffusion and active transport mechanisms.[1]

Vitamin B_2 (Riboflavin) is chemically known as 7,8-dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl] benzo[g]pteridine-2,4-dione (Figure 1.). It also acts as coenzyme and necessary for the red-ox reactions in the body. It is also necessary in maintaining integrity of RBCs.[1] It is widely distributed into all body tissues and breast milk. Small amounts are stored in liver, spleen, kidneys and heart. It undergoes hepatic metabolism.[1]

Vitamin B_6 (Pyridoxine) is chemically known as 4, 5- bis (hydroxymethyl)-2-methylpyridin-3-ol (Figure 1.). It also functions as coenzyme in amino acid, carbohydrate and lipid metabolism. It is absorbed by passive diffusion in the jejunum to a lesser extent in the ileum. Undergoes metabolism in liver and converted to 4-pyridoxine acid metabolite. It is excreted mostly as 4-pyridoxic acid in the urine. [3]

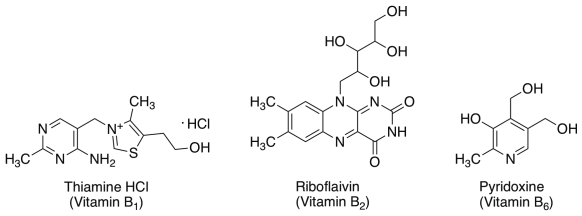

Figure 1. Structure of drugs

Literature survey revealed the availability of High-Performance Liquid Chromatographic (HPLC) method for the analysis of B_1, B_2 & B_6 in pharmaceutical formulations[4-8] and food materials[9-12]. Few reports on spectrophotometric methods are also available.[13-14] Till date there is no report available on High-Performance Thin-Layer Chromatographic (HPTLC) method for the estimation of vitamin B_1, B_2 and B_6

With this background the objective of the presented work is to develop a simple HPTLC method for the simultaneous estimation of Thiamine Hydrochloride, Riboflavin and Pyridoxine in bulk and formulated dosage form and to validate the method as per ICH guidelines.[15]

2. Result and Discussion

Various chromatographic separations were carried out.

Submitted on: 23 Nov, 2017
Revised on: 5 Feb, 2018
Accepted on: 31 Mar, 2018

*corresponding author: JM Tel: +91-9942655715 ; E-mail: jambulingam48@gmail.com

Best separation was achieved in mobile phase acetonitrile: water (6:4 v/v) saturated for 30 mins and UV detection was carried out at 280 nm. The R_f value for B_1, B_2 and B_6 were found to be 0.27, 0.44 and 0.63. respectively. Linearity relationship over the concentration range 0.5 µg/mL for the vitamins were observed from respective calibration curve with correlation coefficient of 0.999 The 3D-chromatogram of calibration concentration shows good correlation co-efficient. The accuracy of proposed method was determined by standard addition method at three level 80%, 100% & 120%. In precision study it was found that %RSD was less than 2%. Limit of detection was found to be 3.750278µg/mL, 3.744794 µg/mL and 3.775815 µg/mL for B_1, B_2 & B_6, respectively with calculated LOD of 3.3 σ/S. Limit of quantification was found to be 11.36448 µg/mL, 11.34786 µg/mL and 11.44186 µg/mL for B_1, B_2 & B_6, respectively with calculated LOQ of 10σ/S; where, S and σ are slope and standard deviation of the response, respectively.

3. Conclusions

A simple, precise and accurate HPTLC method was developed for the estimation of of water soluble vitamins B_1, B_2, & B_6 in fixed-dose combination of formulated tablets. The method was validated for linearity, precision, accuracy and LOD & LOQ. The method was found to be simple and accurate when compared to other existing methods found in literature and journal.

4. Experimental

Materials and methods: *Chemicals and Reagents*: Working standards of B_1 (Thiamine hydrochloride), B_2 and B_6 were obtained as gift sample from Saimirra Innopharm Pvt Ltd, Chennai, Tamilnadu, India. Magnesium sterate, talc and microcrystalline cellulose, acetonitrile (AR), ethyl acetate (AR), toluene (AR) were obtained from Loba Chemical Ltd. Mumbai, India. *Instruments*: UV Spectrophotometer (Perkin Elmer), data acquisition was made with Lambda 25 software. Chromatographic separation was achieved on Camag twin trough glass chamber (20x10 cm) and data acquisition was made with Wincat software.

4.1. Preparation of granule

Each tablet containing 250 mg of B_1, 40 mg of B_2 and 50 mg B_6 were prepared by wet granulation technique and the total weight was approximately 500 mg (Table 1.).

Table 1. Composition of tablets

Ingredients	Weight (mg)
B_1 (Thiamine Hydrochloride)	250
B_2	40
B_6	50
Starch	60
Magnesium Stearate	10
Talc	10
Microcrystalline cellulose	80

4.2. Dissolution procedure

The release rate of formulated tablet were determined using USP dissolution test apparatus type II (paddle type). The dissolution test was performed by using 900 mL of 0.1 N HCl at 50 rpm for 1 h at an ambient temperature. Aliquots of 10 mL were withdrawn for every five min over a period of 1 h. The samples were filtered through whatman filter paper (No. 45) by discarding 4 mL of the filtrate and were analysed at respective wavelengths. The amount of drug released was calculated from the cumulative data (Table 2.).

Table 2. Results for % drug release

Time (min)	% drug release		
	B_1	B_2	B_6
5	55.59	29.81	29.69
10	76.76	74.80	81.36
15	87.58	82.74	89.35
45	90.05	88.32	95.37
60	99.16	101.52	99.55

4.3. HPTLC method

Determination of Isobestic point

For determining the isobestic point the solution of B_1, B_2 & B_6 at the concentration of 50 µg/mL each between 200 and 800 nm. The overlaid spectrum was shown in Figure 2.

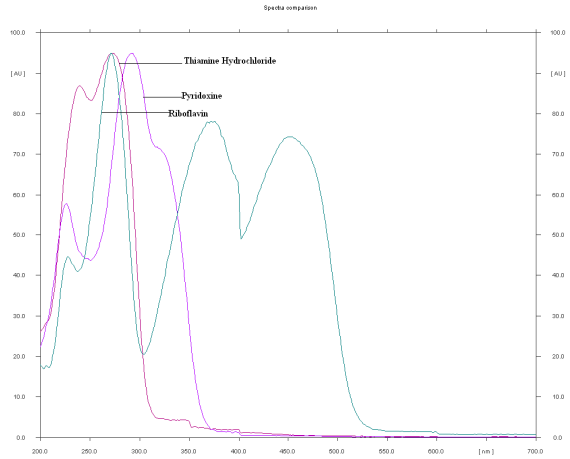

Figure 2. Overlaid spectrum of B_1, B_2 and B_6

Chromatographic conditions

Chromatographic separation was performed on 10×10 cm aluminium plates pre-coated with 100 µm layer of silica gel $60F_{254}$.The methanol pre-washed TLC plates were activated at 80 °C for 5 min before applying sample. The TLC plates were applied with sample leaving 15 mm from the bottom edge using Linomat V semi-automatic applicator. The TLC plates were then developed in twin trough chamber using acetonitrile: water in the ratio of 6:4 v/v as mobile phase. Scanned using TLC scanner IV at a speed of 20mm/sec and detection wavelength of 280 nm (Figure 3.). Win-CATS software was used for data acquisition and analysis.

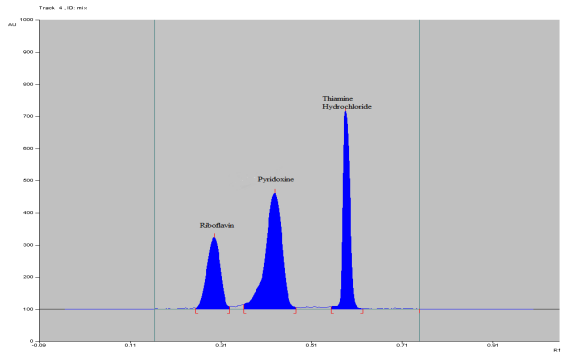

Figure 3. Chromatogram of B1, B2 & B6 for Optimization method

Assay method

Preparation of standard: An amount of 25 mg each of B_1, B_2 and B_6 was weighed and dissolved in a 5 mL of NaOH and 20 mL of methanol. It was then sonicated for 25 min and filtered through 0.45 µm nylon filter. The filtrate was used for assay.

Preparation of sample: Twenty formulated tablets were weighed and triturated. A powder equivalent to 250 mg was taken and dissolved in 5mL of NaOH and 45 mL of methanol. It was then sonicated for 25 min and filtered through 0.45 µm nylon filter.

Injection volume of 1.0, 1.5 & 2.0 µL of B_1, B_2 & B_6, respectively were applied as a spot for both sample and standard on stationary phase (Figure 4. & Table 3.)

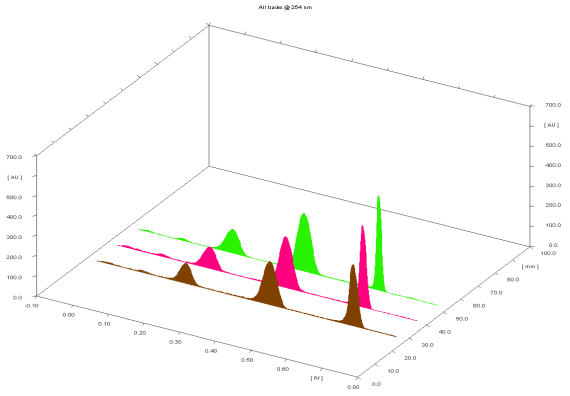

Figure 4. Chromatogram for Assay by HPTLC method

Table 3. Assay data by HPTLC method

Drug	Area		Amount (mg)		% purity
	Sample	Std	Label claim	Calc	
B_1	27676.9	28147.9	250	0.2490	99.5
B_6	15298.2	15505.2	50	0.0495	99.2
B_2	21623	22033	40	0.0398	99.3

4.4. Validation of HPTLC

Method developed was further validated as per ICH guidelines by evaluating linearity, accuracy and precision, limit of detection (LOD) and limit of quantification (LOQ).

Linearity studies: B_1, B_2 and B_6 were found to be linear in the concentration range of 0.5-3.0 µg/mL. The correlation coefficient, r was found to be 0.9990 for vitamins B_1, B_2 and B_6 respectively (Figure 5. & Table 4.).

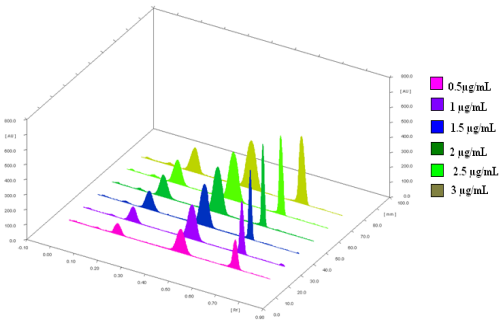

Figure 5. Linearity Chromatogram of vitamins B_1, B_2 & B_6 by HPTLC method

Table 4. Recovery studies by HPTLC method

Drug	% Recovery (n=6)	%RSD
B_1	100.06	0.513141
B_2	100.09	0.007956
B_6	99.85	0.002185

Accuracy studies: The accuracy of the method was confirmed by recovery studies. The %RSD values for recovery analysis were found to be less than 2. This indicates that there are no interferences due to the excipients used in the formulation. Hence the accuracy of the method was confirmed (Figure 6. & Table 5.).

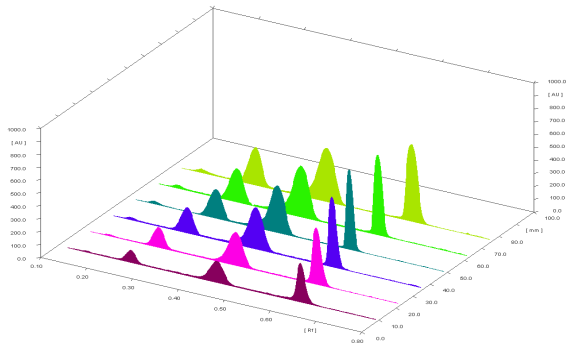

Figure 6. Chromatogram for accuracy

Table 5. Recovery Analysis by UV method

Drug	Label claim	Amount (µg/mL)			% mean recovery
		Present	added	recovered	
B_1	80	8.056	50.10	58.10	100.02
	100	10.190	50.10	50.42	100.80
	120	12.195	50.10	50.25	100.40
B_2	80	8.350	50.25	58.25	100.50
	100	10.190	50.25	50.32	100.64
	120	12.240	50.25	50.28	101.54
B_6	80	8.175	50.20	50.75	101.52
	100	10.285	50.20	50.28	100.36
	120	12.150	50.20	50.16	100.06

Precision studies: System precision for B_1, B_2 and B_6 was performed in a single day with five replicates of injections at a concentration 2.0 µg/mL (Figure 7. & Table 6a-6c.).

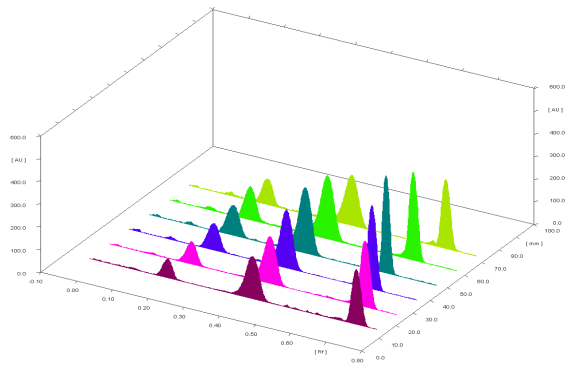

Figure 7. Chromatogram for Precision

Limit of Detection and Quantification: The approach based on the standard deviation of intercept value and the slope of the calibration graph was used for determining the limits of Detection (3.3 σ/S) and limits of Quantification (10 σ/S).

Table 6a. Intra-day Analysis by UV method

B_1		**4 (µg/mL)**	**10 (µg/mL)**
	Average(n=3)	1.099567	2.8393
	S.D	0.00034	0.000294
	%RSD	0.030915	0.010368
B_2		**6 (µg/mL)**	**8 (µg/mL)**
	Average(n=3)	1.964333	1.9879
	S.D	0.00034	0.000455
	%RSD	0.017305	0.022869
B_6		**2 (µg/mL)**	**6 (µg/mL)**
	Average(n=3)	0.399233	1.989433
	S.D	0.000287	0.000386
	%RSD	0.071824	0.019396

Table 6b. Inter-day analysis by UV-method (Analyst 1)

B_1		**4 (µg/mL)**	**10 (µg/mL)**
	Average(n=3)	1.097933	2.839333
	S.D	0.000702	0.000404
	%RSD	0.063973	0.014234
B_2		**6 (µg/mL)**	**8 (µg/mL)**
	Average(n=3)	1.969267	1.987833
	S.D	0.000493	0.000611
	%RSD	0.025049	0.030737
B_6		**2 (µg/mL)**	**6 (µg/mL)**
	Average(n=3)	0.395233	1.9875
	S.D	0.000252	0.0003
	%RSD	0.063674	0.015094

Table 6c. Inter-day analysis by UV-method (Analyst 2)

B_1		**4 (µg/mL)**	**10 (µg/mL)**
	Average(n=3)	1.098433	2.838167
	S.D	0.000503	0.000493
	%RSD	0.045822	0.017381
B_2		**6 (µg/mL)**	**8 (µg/mL)**
	Average(n=3)	1.9695	1.988733
	S.D	0.000436	0.000569
	%RSD	0.022132	0.028592
B_6		**2 (µg/mL)**	**6 (µg/mL)**
	Average(n=3)	0.395667	1.9875
	S.D	0.000208	0.0004
	%RSD	0.052612	0.020126

Acknowledgement

Authors acknowledge their respective institutes for availing the facility to conduct the research.

Reference

1. Bender, D. A.; Botham, K. M.; Granner, D. K.; Keeley, F. W.; Kennelly, P. J.; Mayes, P. A.; Murray, R. K.; Rand, M. L.; Rodwell, V. W.; Weil, P. A., Harper's Illustrated Biochemistry. Ed. 26; McGraw-Hill.: New Delhi, 2008; pp-2220-2226.
2. Wilson, C. O.; Beale, J. M.; Block, J. H., Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry. Ed. 12; Lippincott Williams & Wilkins: Baltimore, MD, 2011, pp-915-957.
3. A to Z drug facts. Ed. 7; Wolters Kluwer Health Inc. 2006, pp-1313-1387.
4. Amidžić, R.; Brborić, J. S.; Čudina, O. A.; Vladimirov, S. M. Rp-HPLC determination of vitamins, folic acid and B12 in multivitamin tablets. J Serb Chem Soc 2005**,** 70 (10), 1229-1235.
5. Amin, M.; Reusch, J. High-performance liquid chromatography of water-soluble vitamins: II. Simultaneous determinations of vitamins B1, B2, B6 and B12 in pharmaceutical preparations. J Chromatogr 1987**,** 390 (2), 448-453.
6. Chen, Z.; Chen, B.; Yao, S. High-performance liquid chromatography/electrospray ionization-mass spectrometry for simultaneous determination of taurine and 10 water-soluble vitamins in multivitamin tablets. Anal Chim Acta 2006**,** 569 (1-2), 169-175.
7. Khor, S.; Tee, E. S. Development of a HPLC method for the simultaneous determination of several B-vitamins and ascorbic acid. Malays J Nutr 1996**,** 2 (1), 49-65.
8. Kwok, R. P.; Rose, W.; Tabor, R.; Pattison, T. S. Simultaneous determination of vitamins b1, b2, b6, and niacinamide in multivitamin pharmaceutical preparations by paired-ion reversed-phase high-pressure liquid chromatography. J Pharm Sci 1981**,** 70 (9), 1014-1017.
9. Ekinci, R.; Kadakal, C. Determination of seven water-soluble vitamins in tarhana, a traditional Turkish cereal food, by high-performance liquid chromatography. Acta Chromatogr 2005**,** 15, 289.
10. Esteve, M.; Farré, R.; Frıgola, A.; Pilamunga, C. Contents of vitamins B1, B2, B6, and B12 in pork and meat products. Meat Sci 2002**,** 62 (1), 73-78.
11. Hasan, M. N.; Akhtaruzzaman, M.; Sultan, M. Z. Estimation of vitamins B-complex (B2, B3, B5 and B6) of some leafy vegetables indigenous to Bangladesh by HPLC method. J Anal Sci Methods Instrum 2013**,** 3 (03), 24.
12. Zafra-Gómez, A.; Garballo, A.; Morales, J. C.; García-Ayuso, L. E. Simultaneous determination of eight water-soluble vitamins in supplemented foods by liquid chromatography. J Agric Food Chem 2006**,** 54 (13), 4531-4536.
13. Issa, M. M.; Abu Shanab, A.; Stefan-van Staden, R.-I. Resolution of ternary mixture of aspirin, atorvastatin, and clopidogrel by chemometric-assisted UV spectroscopic and liquid chromatography methods. Int J Spectrosc 2013**,** 2013.
14. Rajput, G. K.; Kumar, A.; Kumar, A.; Srivastav, G. To develop a simple (UV-Vis spectrometric) method for the estimation of water soluble vitamins. Int J Drug Formul Res 2011**,** 2 (3).
15. ICH Steering Committee, ICH Q2A Text on Validation of Analytical Procedures (CPMP/ICH/381/95). European Agency for the Evaluation of Medicinal Products, International Commission on Harmonisation: London, 1994.