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Formulation, Method Development of Chemometric Assisted UV Spectrophotometric Method for the Estimation of Vitamins B_1, B_2 & B_6

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Abstract: ***Chemometric methods are the development of quantitative structure activity relationships or the Evaluation of analytical–chemical data. The data flood generated by modern analytical instrumentation is one reason, which analytical chemists in particular develop for applications of Chemometric methods. In this study, chemometric assisted UV spectrophotometric method was developed and validated for estimation of Vitamins B_1, B_2& B_6. It involves absorbance measurement at 264nm λ max of Thiamine Hydrochloride, 223 nm λ max of Riboflavin and 219nm λ max of Pyridoxine in Double distilled water. Linearity was obtained in the range of 2 – 10 µg/ ml for the three drugs with correlation coefficient 0.9990 for three vitamins. The methods were validated as per International Conference on Harmonization Q2 (R1) (ICH) guidelines. These methods are simple, rapid and can be easily used as an alternative analysis tool in the quality control as well as in process control of drugs and formulation.***

Keywords: Vitamins B_1, B_2& B_6,UV spectroscopy, chemometric method, ICH guidelines

1. Introduction

Vitamin B_1 (Thiamine) is water –soluble vitamin (Fig no: 1(a)). It is absorbed by both diffusion and active transport mechanisms. Absorption following IM administration is rapid and complete Thiamine, after conversion to thiamine pyrophosphate, function with adenosine triphosphate (ATP) in carbohydrate metabolism. Deficiencies result in beriberi, characterized by GI manifestations, peripheral neuropathy, and cerebral deficits.

Vitamin B_2 (Riboflavin) a yellow to Orange yellow Crystalline Powder, Soluble in water, practically insoluble in Chloroform in ethanol and ether(Fig no: 1(b)). It is converted in body to coenzyme necessary in oxidation reduction. Also necessary in maintaining integrity of RBC_s

Vitamin B_6 (Pyridoxine) a white or almost white crystalline powder(Fig no: 1(c)), Soluble in water,sparingly soluble in acetone in ether and chloroform. It is absorbed by passive diffusion in the jejunum and to a lesser extent in the ileum. Primarily stored in the liver, lesser amount in the muscle and brain, Metabolized in the liver and converted to 4-pyridoxine acid metabolite, Excreted mostly as 4-pyridoxic acid in the urine.[1-5]

The extensive literature survey carried out and revealed that there are very few methods reported for the estimation of these drugs in other combinations. Hence an attempt was made to develop a specific, precise, accurate, linear, simple, rapid, validated and cost effective UV method for estimation of Thiamine Hydrochloride, Riboflavin and Pyridoxine in combined dosage forms.[5-8]

The specific aim of the research was to develop a UV Spectrophotometric method for the estimation of Thiamine Hydrochloride, Riboflavin and Pyridoxine in bulk and formulated dosage form and to validate the proposed methods in accordance with ICH guidelines for the intended analytical application.

2. Materials and Methods

Chemicals and Reagents
The working standard Thiamine Hydrochloride, Riboflavin and Pyridoxine was obtained from Saimirra innopharm pvt Ltd, Chennai, Tamilnadu, India. Double distilled water (AR grade) was obtained from Loba cheme (India) Ltd; Starch, Magnesium Stearate, Talc and Microcrystalline cellulose were obtained from Loba cheme (India).

Instruments
The analysis was performed by using the UV Spectrophotometric instrument Perkin Elmer with equipped UV detector. Data acquisition was made with lambda 25 software and Analytical balance (Schimadzu) was used for the weighing purpose. Statistical data acquisition was made with Graphic pad.

Determination of λ max:
The quantity containing 100 mg of Thiamine Hydrochloride, Riboflavin and Pyridoxine were taken in 100 ml volumetric flask Separately, and volume was made up to the mark with Double distilled water to obtain 1000µg/ml from which 5ml of solution was taken from each volumetric flask, and diluted to 100 ml and made up to volume to obtain 50µg/ml concentration of Thiamine Hydrochloride, Riboflavin and Pyridoxine respectively. The above solution were scanned over range of 200-400nm.The results were shown in the fig no: 2(a-c).

Formulation of Tablet

Preparation of granule:
Each tablet containing 250 mg of (Vitamin B1), 40 mg of (Vitamin B2) and 50 mg (Vitamin B6) was prepared by wet granulation technique. In each formulation, the amount of pure drug was 250 mg of vitamin B_1, 40 mg of vitamin B_2 and 50 mg of vitamin B_6 and the total weight of a tablet was approximately 500 mg. [Table no: 1].

Dissolution procedure
The release rate of formulated tablet was determined by using USP dissolution test apparatus type II (paddle type). The dissolution test was performed using 900ml of 0.1 N HCI at 50rpm for 1 hrs at ambient temperature. Aliquot of 10 ml were withdrawn at an interval of 5 min, 10 min 15 min, 30 min, 45 min, and 60 min. The samples were filtered through what man filter paper (No. 45) by discarding 4ml of the filtrate and analyzed at 264 nm, 223 nm and 219 nm respectively. From the cumulative data, the amount of drug released was calculated. (Table no: 2).

3. Method Development by UV Spectroscopy

Chemometric method

Preparation of standard stock solution
Accurately weighed 100mg of vitamins B_1,B_2& B_6were taken separately into 100 ml clean volumetric flask and volume was made up to the mark with Double distilled water to obtain 1000µg/ml. 0.5 ml of resultant solution was taken and diluted to 10 ml to obtain 50µg/ml concentration respectively.(Table no: 3).

Analysis of Formulated tablets
Twenty tablets were weighed and triturated into fine powder. The amount of powder equivalent to 500 mg of formulated Tablet was accurately weighed and transferred into 100 ml clean, dry volumetric flask and volume made up to mark with solvent (5000 µg/ml). From this 1 ml was pipetted out into a 100 ml dry, clean volumetric flask and the volume was made up to mark with Double distilled water to obtain 50µg/ml concentration.

Cramer's Matrix Calculation Method
Molar Absorptivity (ε) values were calculated by using the absorbance measured at 264nm, 223nm, and 219 nm for each compound in the ternary mixture and moreover the matrix method was used greatly to simplify and easily solve. By using (ε) values, a system of equations with three unknowns can be written for the compounds in the ternary mixture as follows,

This matrix can be solved and each compound was determined by solving the following operations;

$$\begin{pmatrix} \varepsilon_{Thi264} & \varepsilon_{Ribo223} & \varepsilon_{Pyri219} \\ \varepsilon_{Thi264} & \varepsilon_{Ribo223} & \varepsilon_{Pyri219} \\ \varepsilon_{Thi264} & \varepsilon_{Ribo223} & \varepsilon_{Pyri219} \end{pmatrix} \begin{pmatrix} Thi\ 0.6535 \\ Ribo\ 0.8521 \\ Pyri\ 0.7920 \end{pmatrix} = \begin{pmatrix} Thi_{264} \\ Ribo_{223} \\ Pyri_{219} \end{pmatrix}$$

Where,

$$\Delta = \begin{pmatrix} \varepsilon_{Thi264} & \varepsilon_{Ribo223} & \varepsilon_{Pyri219} \\ \varepsilon_{Thi264} & \varepsilon_{Ribo223} & \varepsilon_{Pyri219} \\ \varepsilon_{Thi264} & \varepsilon_{Ribo223} & \varepsilon_{Pyri219} \end{pmatrix}$$

$$\Delta_1 = \begin{pmatrix} A_{m264} & \varepsilon_{Ribo223} & \varepsilon_{Pyri219} \\ A_{m223} & \varepsilon_{Ribo223} & \varepsilon_{Pyri219} \\ A_{m219} & \varepsilon_{Ribo223} & \varepsilon_{Pyri219} \end{pmatrix}$$

$$\Delta_2 = \begin{pmatrix} \varepsilon_{Thi264} & A_{m264} & \varepsilon_{Pyri219} \\ \varepsilon_{Thi264} & A_{m223} & \varepsilon_{Pyri219} \\ \varepsilon_{Thi264} & A_{m219} & \varepsilon_{Pyri219} \\ \varepsilon_{Thi264} & \varepsilon_{Ribo223} & A_{m264} \end{pmatrix}$$

$$\Delta_3 = \begin{pmatrix} \varepsilon_{Thi264} & \varepsilon_{Ribo223} & A_{m223} \\ \varepsilon_{Thi264} & \varepsilon_{Ribo223} & A_{m219} \\ C_{Thi} = \frac{\Delta 1}{\Delta}, & C_{Ribo} = \frac{\Delta 2}{\Delta}, & C_{Pyri} = \frac{\Delta 3}{\Delta} \end{pmatrix}$$

Validation of UV Spectroscopy
This method validated by evaluating linearity, accuracy method and limit of detection (LOD) and limit of quantification (LOQ) were performed according with ICH guidelines.

Linearity studies
The linearity of measurement was evaluated by analysing different concentration of Standard solution of vitamins B1, B2 & B6. The Beer's law was obeyed in concentration range of 2 to 10 µg/ml for three vitamins. The correlation coefficient was found to be 0.9990 for vitamins B1, B2 & B6 respectively. [Fig no: 3(a-c), Table no: 4].

Accuracy studies
To ascertain the accuracy of proposed method recovery studies were carried out by standard addition method at three different levels (80% 100%, 120%) percent recovery for vitamins B1,B2 & B6 was found in the range of 100.02 - 101.52% [Table no : 5].

Precision studies:
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed condition. The % RSD is not more than 2%. [Table no: 6, 7 (a&b)].

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 (3.3xslope/S.D.) and limits of Quantification (10xslope/S.D).

4. Results & Discussion

Absorbance spectra of Thiamine Hydrochloride, Riboflavin and Pyridoxine shown in the [fig no 2(a- c)] the three wavelength 264nm, 223nm & 219nm were chosen determination of water soluble vitamins respectively. Various mixture of composition of Thiamine Hydrochloride, Riboflavin and pyridoxine were prepared at different concentration which obeys the Beer's Lambert's law from 2-10 µg/mL for three water soluble vitamins. With co-relation coefficient of 0.999 for three vitamins respectively. By using matrix calculations the concentrations of the amount was found to 0.2480mg for Thiamine Hydrochloride, 0. 0396 mg for Riboflavin and 0.0497 mg for Pyridoxine respectively. Mean recovery and Relative standard deviation of the proposed

method where found to be 0.005072, 0.006138 & 0.01021 respectively. In precision study it was found that %RSD was less than 2%. Limit of Detection were value was found to be10.44µg/mL, 13.04 µg/mL and 10.43 µg/mL respectively were calculated LOD= 3.3 σ/S. Limit of Quantification of were values was found to be 31.64µg/mL, 39.54 µg/mL and 31.62µg/mL for Vitamins B_1, B_2& B_6 respectively were calculated LOQ= 10σ/S where, S- Slope, σ- Standard deviation of the response.

5. Figures & Tables

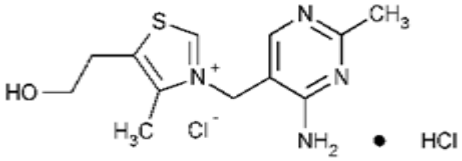

Figure 1(a): Structure of Thiamine Hydrochloride

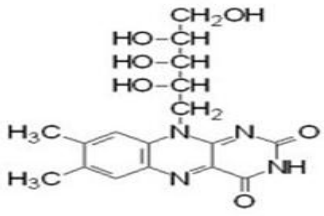

Figure 1(b): Structure of Riboflavin

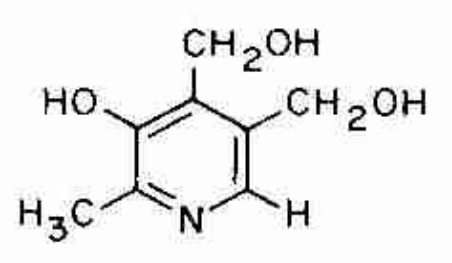

Figure 1(c): Structure of Pyridoxine

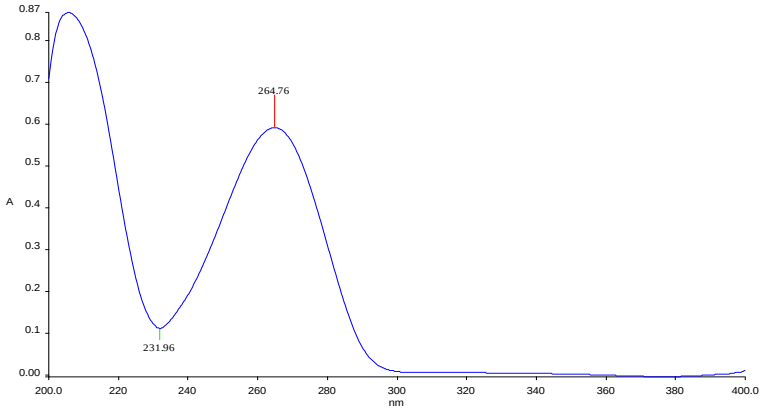

Figure 2(a): Spectrum for Thiamine Hydrochloride

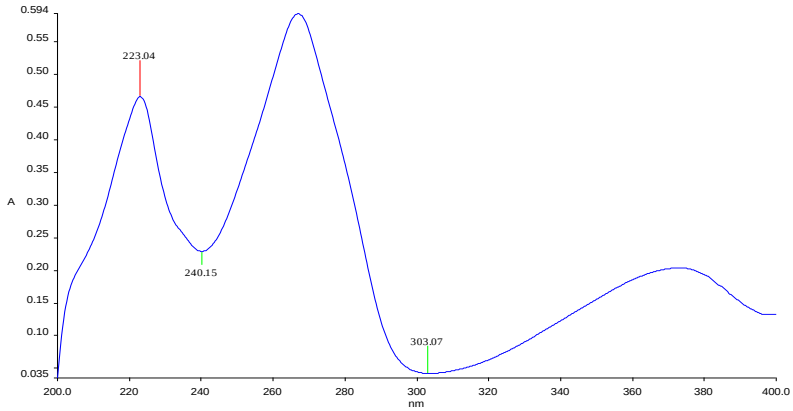

Figure 2(b): Spectrum for Riboflavin

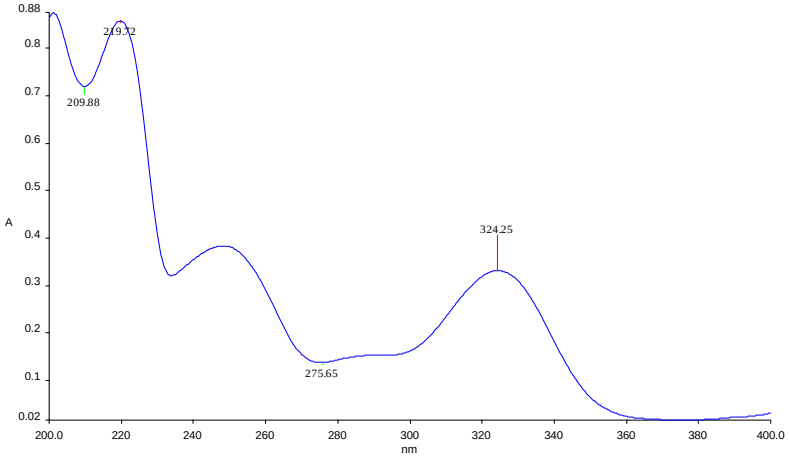

Figure 2(c): Spectrum for Pyridoxine

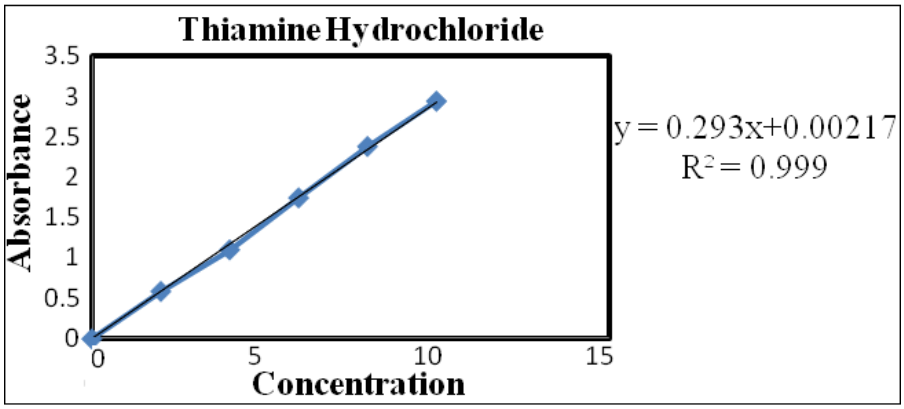

Figure 3(a): Calibration curve of Thiamine Hydrochloride by UV method

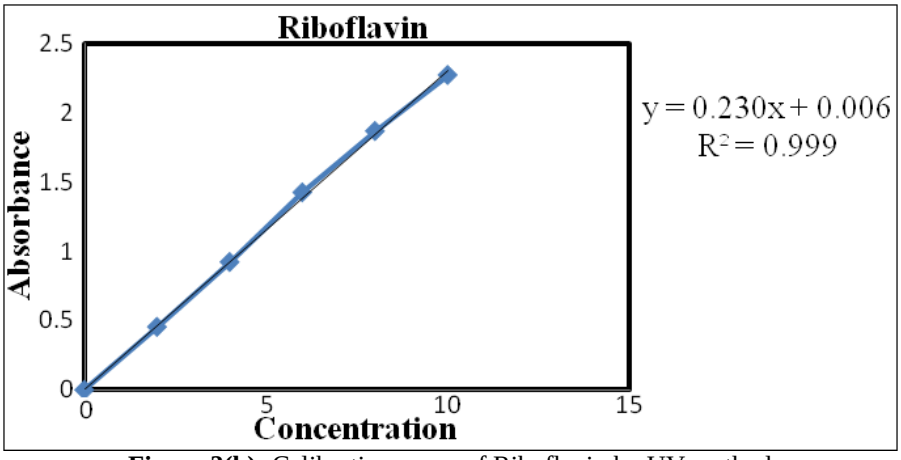

Figure 3(b): Calibration curve of Riboflavin by UV method

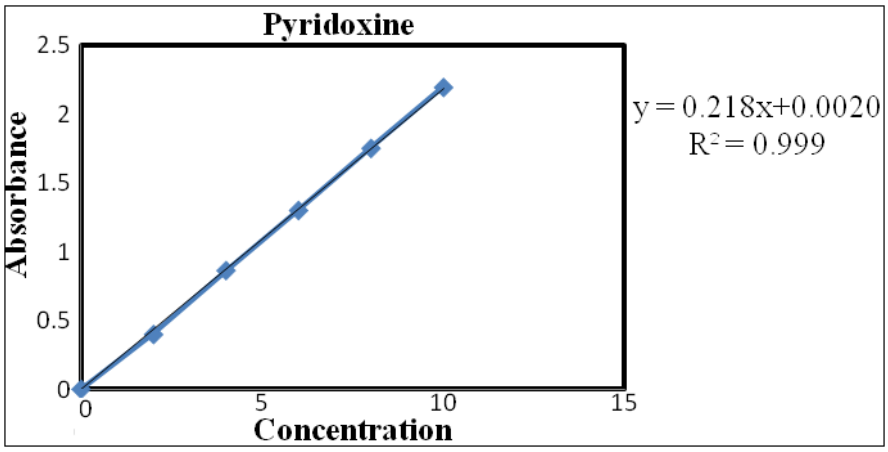

Figure 3(c): Calibration curve of Pyridoxine by UV method

Table 1: Composition of tablets

S.No	Ingridents	Weight (Mg)
1.	Thiamine Hydrochloride	250
2.	Riboflavin	40
3.	Pyridoxine	50
4.	Starch	60
5.	Magnesium Sterate	10
6.	Talc	10
7.	Microcrystalline cellulose	80

Table 2: Results for % Drug Release

S.No	Time (hr)	% drug release		
		Thiamine Hydrochloride	Riboflavin	Pyridoxine
1.	5mins	55.59	29.81	29.69%
2.	10mins	76.76	74.80	81.36%
3.	15mins	87.58	82.74	89.35%
4.	45mins	90.05	88.32	95.37%
5.	60mins	99.16	101.52	99.55%

Table 3: Result for Chemometric method

Drug	$E^{1\%}_{cm}$	Absorbance for formulation drug	Amount present(mg)	% purity
Thiamine Hydrochloride	3028.59 2062.50 1285.692	0.0353	0.2480	99.2
Riboflavin	4060.61 3974.46 1610.49	0.0552	0.0396	99
Pyridoxine	431.69 4087.37 1840.56	0.0492	0.0497	99.4

Table 4: Calibration Parameters for UV method

Parameters	Thiamine Hydrochloride	Riboflavin	Pyridoxine
Linearity range(µg/mL)	2-10µg/mL	2-10µg/mL	2-10µg/mL
Linearity equation	0.293x+0.00217	0.230x + 0.006	0.218x+0.0020
Co- relation coefficient(r)	0.999	0.999	0.999
Slope	0.296950	0.230066	0.22041
Sandell's sensitivity	0.0033	0.0043	0.0044
SD (σ)	0.9468	0.6921	0.7091
LOD (3.3 × σ / Slope)	10.44µg/mL	13.04µg/mL	10.43 µg/mL
LOQ (10 × σ / Slope)	31.64µg/mL	39.54 µg/mL	31.62 µg/mL

Table 5: Recovery Analysis by UV method

Levels of % recovery	Amount present (µg/mL)			Amount added (µg/mL)			Amount recovered (µg/mL)			%Mean Recovery		
	B1	**B2**	**B6**	**B1**	**B2**	**B6**	**B1**	**B2**	**B6**	**B1**	**B2**	**B6**
80	8.056	8.350	8.175	50.10	50.25	50.20	58.10	58.25	50.75	100.02	100.50	101.52
100	10.190	10.190	10.285	50.10	50.25	50.20	50.42	50.32	50.28	100.80	100.64	100.36
120	12.195	12.240	12.150	50.10	50.25	50.20	50.25	50.28	50.16	100.40	101.54	100.06

Table 6: Intraday Analysis by UV method

	Thiamine Hydrochloride		Riboflavin		Pyridoxine	
	4(µg/ml)	10(µg/ml)	6(µg/ml)	8(µg/ml)	2(µg/ml)	6(µg/ml)
Average(n=3)	1.099567	2.8393	1.964333	1.9879	0.399233	1.989433
S.D	0.00034	0.000294	0.00034	0.000455	0.000287	0.000386
%RSD	0.030915	0.010368	0.017305	0.022869	0.071824	0.019396

Table 7 (a): Interday Analysis by UV method (Analyst 1)

	Thiamine Hydrochloride Analyst 1		Riboflavin Analyst 1		Pyridoxine Analyst 1	
	4(µg/mL)	10(µg/mL)	6(µg/mL)	8(µg/mL)	2(µg/mL)	6(µg/mL)
Average(n=3)	1.097933	2.839333	1.969267	1.987833	0.395233	1.9875
S.D	0.000702	0.000404	0.000493	0.000611	0.000252	0.0003
%RSD	0.063973	0.014234	0.025049	0.030737	0.063674	0.015094

Table 7(b): Interday Analysis by UV method (Analyst 2)

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

6. Conclusions

A simple, precise and Accurate method was developed by HPTLC and UV Spectroscopy method have been developed for analysing 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. Both the methods were found to be simple and accurate when compared to other existing methods found in literature and journal.

References

[1] Indian Pharmacopeia, Published by the Indian Pharmacopoeia commission Ghaziabad.2014; 3: 2856-2602).
[2] Sathyanarayana U, Chakrapani U. Text book of Biochemistry, Uppala author publishers, Vijayawada.2007; 3:135-146.
[3] Robert K, Murray, Darya K, and Granner. Harper's Illustrated Biochemistry, Lange medical books/Mc graw – Hill publishers, New Delhi.2008; 26-2220-226.
[4] Ac. Deb. Fundamentals of Biochemistry. New central book agency, Calcutta.2001; 7:172-194.
[5] A to Z drug facts, Published by Wolters kluwer health, inc. 2006; 7:1313-1387.
[6] Zakir Sultan M.D, Nazmul Hasan M, Akhtaruzzaman M.D et al .Estimation of Vitamins B-Complex B_2, B_3, B_5 and B_6 of Some Leafy Vegetables Indigenous to Bangladesh by HPLC Method. Journal of Analytical Sciences, Methods and Instrumentation 2013; 3: 24-29.
[7] Bo Chen, Zhi Chen, Shouzhuo Yao. Simultaneous determination of taurine and 10 water-soluble vitamins in multivitamin tablets by High-performance liquid chromatography/electrospray ionization-mass spectrometry method. *Analytica Chimica Acta.* 2006; 169-175.
[8] Behailu Urgessa, Abdel-Maaboud, Ismail Mohamed, Fathy Kandeel El-Fiky et al. Determination of vitamin B1, B6 and B12 in tablet and injection form by high-performance thin-layer chromatographic (HPTLC) method. 2008; 1:115-119.