INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

# RRLC METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF VITAMIN B1, B2, B3 AND B6 IN SOFT GELATIN CAPSULE FORMULATION

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**ABSTRACT**

A simple RRLC methods was developed for the determination of vitamin B1, B2, B3, and B6 present in soft gelatin capsule dosage form. Extended – C18 (50x4.6mm, 1.8 µm column in gradient mode with mobile phase (A) and mobile phase (B), the flow rate was 1.0 ml/min and UV detection at 280nm. The proposed method was also validated. The developed method was validated for linearity, accuracy, precision, specificity, robustness; the proposed method can be used for estimation of these drugs in combined dosage forms in soft gelatin capsule.

**Keywords**: RRLC, Vitamin B1, B2, B3 and B6.

## INTRODUCTION[1-6]

Thiamine Mononitrate is most important ester of thiamine is the diphosphate ester, thiamine pyrophosphate (TPP).It is also known as co-carboxylase. It functions as a coenzyme in metabolic reactions involving decarbozylation of certain α – ketoglutaric acid. TPP also serves as coenzyme in the transketolation reaction which occurs in the direct oxidative pathway of carbohydrate metabolism.TPP is an essential coenzyme of the enzyme system which helps synthesis of fats from carbohydrates and proteins.

Riboflavin is readily absorbed in the intestines after phosphorylation to riboflavin phosphate. It is present in blood and all tissue cells mostly as FMN and FAD. It is secreted in the milk and excreted in urine and faeces. A major portion of riboflavin is metabolized in the body to unknown compounds.

Nicotinic acid and nictinamide are readily absorbed from the intestines; they are present in blood, mostly in the erythrocytes. Niacin is excreted in urine either as niacinamide or as nicotinuric acid which is the glycine conjugate of nicotinic acid. The largest portion is excreted as methyl derivative, viz., N – methyl – nicotinamide. The methylation is done in the liver by the labile methyl groups supplied by methionine.

Synthesis of vitamin B6 by intestinal bacteria in man is limited. Therefore, it is necessary that the vitamin is adequately supplied in the diet. Pyridoxol, pyridoxal and pyridoxamine are nutritionally interconvertible in man. Among these, the phosphorylated derivatives of pyridoxal and pyridoxamine are functionally active. The major metabolic product of pyridoxine is 4 – pyridoxal acid, which is excreted in urine.

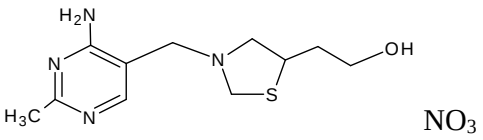


**Fig. 1: Chemical structure of Thiamine Mononitrate (B1)**

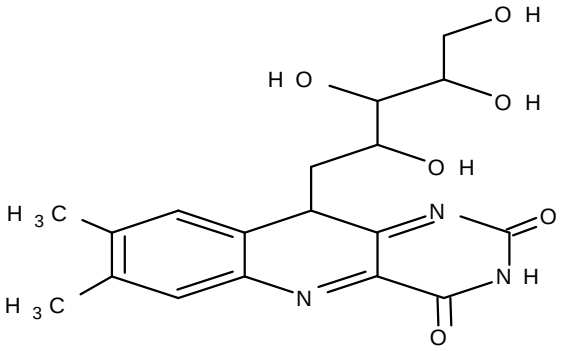

**Fig.2: Chemical structure of Riboflavin (B2)**

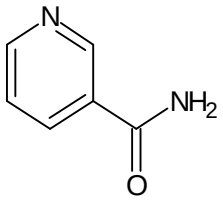

**Fig. 3: Chemical structure of Nicotinamide (B3)**

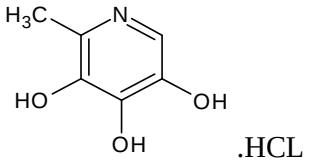

**Fig. 4:Chemical structure of Pyridoxine Hydrochloride (B6)**

A recent literature survey revealed that vitamins B1, B2, B3 & B6 in pharmaceutical dosage form. methods were available for the determination this involved RP-HPLC method method[7-15]. Although various analytical methods have been developed for the determination vitamins B1, B2 , B3 & B6 ,There has been no report in literature on the simultaneous estimation of vitamins B1,B2, B3 & B6 from the soft gelatin capsule, The present work describes the development of validated RRLC method, which can quantify these components simultaneously from a combined dosage form. The present RRLC method was validated as per the ICH guidelines.[16-17]

## EXPERIMENTAL

The separation was carried out on AGILENT RRLC 1200 system with a double pump, an auto injecting device, UV-visible detector, with chemstation software. Sample of vitamins B1, B2, B3 & B6 was received from Geltec pvt ltd Bangalore. Respectively and soft gelatin capsule are purchased from the local market, HPLC grade Methanol ,Glacial Acetic acid, Orthophosphoric , Triethylamin,1 – Pentane sulfonic acid sodium salt monohydrate AR Grade were procured from Merck specialties Pvt. Ltd., Mumbai,

### Mobile phase A

Dissolved 1.9g of pentane sulfonic acid sodium salt monohydrate in 1000 ml of water add 1.0 ml glacial acetic acid and 1.0 ml of triethylamine mix well and adjust pH at 4.0 with diluted ortho phosphoric acid and filter through 0.45µm membrane.

**Mobile phase B**
Methanol

**Diluents**
Mix 800 ml mobile phase A and 200 ml of methanol adjust the pH at 4.0 with diluted Orthophosphoric acid and filter through o.45µm membrane.

## Chromatographic Conditions

| Parameters | Method |
|---|---|
| Stationary phase(column) | extended – C18 (50x4.6mm, 1.8 µm) |
| pH | 4 |
| Flow rate | 1.0 (ml / min) |
| Column temperature | Ambient |
| Volume of injection loop | 20µl |
| Detection wavelength | 280nm |
| Runtime | 20 min |

**Gradient program**

| Time | mobile phase A | mobile phase B |
|---|---|---|
| 0.0 | 92.0 | 8.0 |
| 8.0 | 92.0 | 8.0 |
| 9.0 | 75.0 | 25.0 |
| 15.0 | 75.0 | 25.0 |
| 16.0 | 92.0 | 8.0 |

**Standard stock solution**
Weigh accurately about 10.0 mg of thiamine Mononitrate, 10.0 mg of riboflavin 150.0 mg of Nicotinamide, and 5.0 mg of pyridoxine Hydrochloride AWS and transfer into a clean dry 100ml volumetric flask, add 5 ml of acetic acid and heat on a water – bath ;maintained at 60°C for 10 minutes. Cool, add 70 ml of diluents, mix well, sonicate for 2 minutes and make up to volume with diluents. Filter through, Whatman No.01, discard initial 10 ml filtrate. Further dilute 5ml with diluents.

**Standard solution**
Transfer 5 ml of standard stock solution into a clean dry 50 ml volumetric flask, and make up to volume with diluents.

**Sample solution**
Cut 20 soft gelatin capsules, weigh accurately bout 8.5 g of the well mixed paste into a clean dry 100ml volumetric flask, add 5ml of acetic acid and heat on a water – bath maintained at 60°C for 10 minutes. Cool add 70 ml of diluents, mix well, sonicate for 2 min and make up to volume with diluents. Filter through Whatman No.01, discard initial 10ml filtrate. Further dilute 5ml to 50ml with diluents.

**Procedure**
Set the RRLC as per operating conditions. Inject 20µl of the standard solution in 5 times. Subsequently inject 20 µl of the sample solution in duplicate. Compare the peak areas obtained from the standard and test solutions and calculate the content in mg/average fill weight .

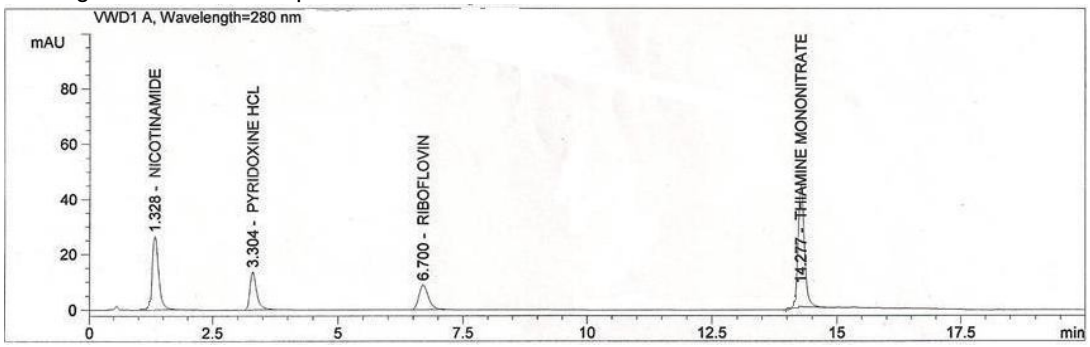


**Fig. 5: Typical Chromatogram of vitamin B1.B2, B3, and B6**

**Linearity and Range**

The result of the method was found to be linear in the range of 50µg/ml to 150 µg/ml of vitamin B1, B2, B3, and B6 peak areas recorded for all peaks and plotted peak *vs.* concentration. Coefficient of correlation for of Thiamine Mononitrate (0.9999), Riboflavin (0.9999), Nicotinamide (0.9998) and Pyridoxine Hydrochloride (0.9999)

**Linearity curve**

**Fig. 6**

**Fig. 7**

**Fig. 8**

**Fig. 9**

**Accuracy**

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100, and 120 % and the percentage recovery was calculated and presented in Table no 1. Recovery was within the range of 100 ± 2 % which indicates accuracy of the method.

**Table 1: Recovery of Pure Added to Formulation**

| Drug's name | Amount of drug added (mg) | Amount of drug recovered (mg) | Recovery % | Mean recovery % |
|---|---|---|---|---|
| thiamine Mononitrate | 10.45<br>10.3<br>10.1 | 10.30<br>10.29<br>10.25 | 98.56<br>99.90<br>101.48 | 99.98 |
| Riboflavin | 10.85<br>10.84<br>10.83 | 10.81<br>10.79<br>10.85 | 99.63<br>99.53<br>100.18 | 99.78 |
| Nicotinamide | 160.17<br>160.26<br>160.31 | 160.28<br>160.28<br>160.27 | 100.06<br>100.01<br>99.97 | 100.01 |
| pyridoxine Hydrochloride | 5.56<br>5.54<br>5.50 | 5.53<br>5.50<br>5.51 | 99.46<br>99.27<br>100.18 | 99.63 |

**Precision**

Analyze the same lot of sample which is used in method precision .the samples will be prepared as per method and analyzed by different analyst, on different day. Calculate the content of Nicotinamide, Pyridoxine HCL, Riboflavin and Thiamine Mononitrate in mg/Avg. fill wt and report % relative standard deviation between results and the response of the factor of drug peaks and percentage RSD were calculated and found sample are Nicotinamide (%RSD=0.36), Pyridoxine HCL(%RSD=0.68), (%RSD=0.7), and Thiamine Mononitrate(%RSD=0.53),

**Robustness**

A) Change in wavelength: The standard and samples will be prepared and analyzed as per method 3.0 at different wavelength ±2nm i.e.278 nm and 282 nm. Calculate the content of, Nicotinamide; Pyridoxine HCL, Riboflavin and Thiamine Mononitrate in Mg / Avg fill wt and % variation were found to be less than 2%.The result show table no 2 and 3.

b) Change in pH of buffer of mobile phase: the standard and samples will be prepared and analyzed as per method 3.0 by using different pH buffer of mobile phase ±0.2 nm i.e. pH 3.8 and pH 4.2 calculate the content of, Nicotinamide, pyridoxine HCL, Riboflavin and Thiamine Mononitrate in Mg / Avg fill wt and % variation were found to be less than 2%. The result show table no 4 and 5

**Table 2: Wavelength 278nm**

| S.No | Drug Name | Assay Mg / Avg fill wt | S D | % R S D |
|---|---|---|---|---|
| 1 | Nicotinamide | 19.79 | 0.04 | 0.20 |
| 2 | Pyridoxine HCL | 0.78 | 0.004 | 0.59 |
| 3 | Thiamine Mononitrate | 1.43 | 0.0078 | 0.55 |
| 4 | Riboflavin | 1.14 | 0.0089 | 0.78 |

**Table 3: Wavelength 282nm**

| S.No | Drugs Name | Assay Mg / Avg fill wt | S D | % R S D |
|---|---|---|---|---|
| 1 | Nicotinamide | 19.97 | 0.1353 | 0.68 |
| 2 | Pyridoxine HCL | 0.79 | 0.0072 | 0.91 |
| 3 | Thiamine Mononitrate | 1.41 | 0.0182 | 1.30 |
| 4 | Riboflavin | 1.11 | 0.0078 | 0.73 |

**Table 4: PH 3.8**

| S.No | Drugs Name | Assay Mg / Avg fill wt | S D | % R S D |
|---|---|---|---|---|
| 1 | Nicotinamide | 19.63 | 0.1654 | 0.84 |
| 2 | Pyridoxine HCL | 0.78 | 0.0074 | 0.95 |
| 3 | Thiamine Mononitrate | 1.40 | 0.0175 | 1.25 |
| 4 | Riboflavin | 1.13 | 0.0112 | 0.99 |

**Table 5: PH 4.2**

| S.No | Drugs Name | Assay Mg / Avg fill wt | S D | % R S D |
|---|---|---|---|---|
| 1 | Nicotinamide | 19.79 | 0.1977 | 1.00 |
| 2 | Pyridoxine HCL | 0.79 | 0.0041 | 0.51 |
| 3 | Thiamine Mononitrate | 1.40 | 0.0161 | 1.15 |
| 4 | Riboflavin | 1.14 | 0.0057 | 0.50 |

**Specificity**

**Placebo preparation**

Weigh accurately about 8.5g of placebo into a clean dry 100ml volumetric flask add 5 ml of acetic acid and heat on water – bath maintained at 60°C for 10 minutes. Cool, add 70 ml of diluents, mix well sonicate for 2min. and make up to volume with diluents. Filter through Whatman No.01, discard initial 10 ml filtrate. Further dilute 5 ml to 50 ml with diluents and analyzed as per method.

**Table 6**

| Wt. of placebo gm | Area response with respect to | Assay mg/avg fill wt. | Acceptance |
|---|---|---|---|
| 8.5526 | Nicotinamide | 0.0 | There should not be any interference to corresponding peak |
| | Pyridoxine HCL | 0.0 | |
| | Thiamine Mononitrate | 0.0 | |
| | Riboflavin | 0.0 | |

## CONCLUSION

The evaluation of obtained values suggests that the proposed HPLC methods provide simple, precise, rapid and robust quantitative analytical method for determination of vitamins B1, B2, B3 and B6 in soft gelatin capsule. The mobile phase is simple to prepare and economical and better precision accuracy was achieved than the reported method. thus, the proposed method id precise, accurate, and simple to perform . After validating proposed method as per ICH guidelines and correlating obtained values with the standard values, satisfactory results were obtained

## ACKNOWLEDGEMENT

The authors are thankful to Head, Department of Pharmaceutical Analysis, Nandha College of Pharmacy, Erode, and Tamilnadu. For providing facilities for the research work. Geltec Pvt Ltd, Bangalore. For providing the gift sample of vitamin B1, B2, B3 and B6.

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