INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

# A VALIDATED STABILITY INDICATING RP-UPLC METHOD FOR SIMULTANEOUS DETERMINATION OF WATER SOLUBLE VITAMINS, CAFFEINE AND PRESERVATIVES IN PHARMACEUTICAL FORMULATIONS

**G. Nagamallika[1*] and M. Aruna Devi[2]**

[1]QIS College of Pharmacy, Ongole, Andhra Pradesh, India.
[2]CVM College of Pharmacy, Karimnagar. Andhra Pradesh, India.

**ABSTRACT**

A novel, sensitive and selective stability-indicating gradient reverse phase ultra performance liquid chromatographic method was developed and validated for the quantitative determination of thiamine hydrochloride, riboflavin-5′-phosphate sodium, pyridoxine hydrochloride, Nicotinamide, D (+)-Panthenol, caffeine and two preservatives (Methylparaben and proylparaban) in multivitamin syrup preparation. The chromatographic separation was achieved on HSS T3 50mm × 2.1mm,1.7µm column by using mobile phase containing a gradient mixture of solvent A (0.1% Trifluoro acetic acid in water) and solvent B(50:50 v/v mixture of Acetonitrile and methanol at flow rate of 1.0 mL $min^{-1}$. Column temperature was maintained at 48°C and detection was carried out at 200, 254 and 290 nm. The described method shows excellent linearity and the correlation coefficient for thiamine hydrochloride, riboflavin-5′-phosphate sodium, pyridoxine hydrochloride, Nicotinamide, D (+)-Panthenol, caffeine, Methylparaben and proylparaban was more than 0.99. To establish stability-indicating capability of the method, drug product was subjected to the stress conditions of acid, base, oxidative, hydrolytic, thermal and photolytic degradation. The degradation products were well resolved from thiamine hydrochloride, riboflavin-5′-phosphate sodium, pyridoxine hydrochloride, Nicotinamide, D (+)-Panthenol, caffeine, Methylparaben and propylparaban. The developed method was validated as per ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

**Keywords:** Water-soluble vitamins, Preservatives, UPLC stability-indicating, Multivitamin syrup.

## 1.0 INTRODUCTION

Vitamins are biologically active compounds which act as controlling agents for an organism's normal health and growth. Vitamins are vital to human development and long-term health; therefore, infants are usually prescribed a vitamin supplement to ensure they receive the recommended daily allowance of each vitamin. Children under one year of age are usually given this supplement in liquid form. Vitamins are a chemically diverse set of compounds varying in size, structure, and other properties. They are generally classified by their water solubility, with the classifications of water-soluble and fat-soluble. A thorough literature survey has revealed many spectrophotometric and HPLC methods for the estimation of vitamin(s) in biological fluids and pharmaceutical formulations[1-6]. To the best of our knowledge, there is no stability-indicating UPLC method reported for the simultaneous estimation of water soluble vitamins, caffeine and preservatives in syrup formulation. Therefore, attempts were made in this study to develop a fast, sensitive, selective and stability-indicating reverse phase ultra-performance liquid chromatography (UPLC) method for the simultaneous determination of

water soluble vitamins, caffeine and preservatives in syrup formulation. The proposed method is able to separate all the vitamins, caffeine and preservatives with each other and from their impurities, degradation products and placebo components. The developed UPLC method was validated with respect to specificity, linearity, limit of detection and quantification, precision, accuracy and robustness.

## 2.0 MATERIALS AND METHODS

All the reagents were of analytical-reagent or LC grade unless stated otherwise. Milli-Q-water was used throughout the experiment. Working standards of Water soluble Vitamins (Pyridoxine Hydrochloride, Nicotinamide, D-Panthenol, Thiamine Hydrochloride, Riboflavin 5-Phosphate), Caffeine, Methyl hydroxy benzoate and Propyl hydroxy benzoate, Multivitamin syrup preparation, were obtained from M/S ADCOCK INGRAM, RD&I, Sabax Road, Aero ton, Johannesburg, 2013, South Africa. Sodium hydroxide, Hydrochloric acid, Hydrogen peroxide, Trifluoro acetic acid, acetonitrile and methanol were procured from Merck, Mumbai.

### 2.1 Apparatus

The UPLC system consisted of high pressure pump, Photo diode array detector and 10 µL capacity injector loops. The column used was HSS T3 50mm × 2.1mm, 1.7µm column. The output signal was monitored and processed using Empower software.

### 2.2 Chromatographic conditions

HSS T3, 50×2.1 mm, 1.7µm column was used for separation. Chromatographic separation was achieved using timed gradient. The mobile phase consisting of A: buffer (0.1% Trifluoro acetic acid in water) and B: a mixture of 50% acetonitrile and 50% methanol with a timed gradient programme was used. The gradient condition of the mobile phase was: 0 min 0% solvent B, 0.1 min 2% solvent B, 1.6 min 12% solvent B, 2.8 min 12% solvent B, 3 min 55% solvent B, 3.8 min 2% solvent B and 4 min 2% solvent B with further 2 min for system equilibration. The flow rate of the mobile phase was 1.0 mL/min with detection at 190-400 nm. The column temperature was kept at 48$^{o}$C and the injection volume was 10 µL.

### 2.3 solution preparation

#### 2.3.1 Standard stock solution

2.7mg/mL of Caffeine; 0.11mg/mL of Riboflavin-5'Phosphate; 0.09mg/mL of Pyridoxine Hydrochloride; 0.42mg/mL of Nicotinamide; 0.16mg/mL of Thiamine HCl; 0.11mg/mL of D-Panthenol and 0.27mg/mL of Methylparaben; 0.03mg/mL of Propylparaben in water.

#### 2.3.2 Sample solution

2.7mg/mL of Caffeine; 0.11mg/mL of Riboflavin-5'Phosphate; 0.09mg/mL of Pyridoxine Hydrochloride; 0.42mg/mL of Nicotinamide; 0.16mg/mL of Thiamine HCl; 0.11mg/mL of D-Panthenol and 0.27mg/mL of Methylparaben; 0.03mg/mL of Propylparaben in water from Multivitamin syrup preparation.

### 2.4 stress degradation study

To determine whether the analytical method was stability indicating, standard solution was stressed under various conditions includes photolytic degradation (Exposed to 1.2 billion Lux.), acid hydrolysis (1N Hydrochloric acid at 50$^{0}$C for 3 days), base hydrolysis (1N sodium hydroxide at 50$^{0}$C for 3 days), thermal degradation (at 60$^{0}$C for 3 days) and Oxidative degradation (3% Hydrogen peroxide at 50$^{0}$C for 3 days).

### 2.5 method validation

The method was validated according to International Conference on Harmonisation (ICH) guidelines for validation of analytical procedures. The validated parameters were system suitability, specificity, range and linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, ruggedness and robustness[7].

## 3.0 RESULT AND DISCUSSION

Different chromatographic conditions were tested to develop the stability-indicating LC method. The mobile phase was optimized through the evaluation of different buffers and organic solvents. The use of 0.1% Trifluoro acetic acid and acetonitrile in gradient mode resulted in better peak symmetry and good resolution and short run time. Due to the different absorption max of analytes, the elution was monitored at 200nm; 254nm and 290nm. These all polar analytes retained with in 4.0min runtime and maximum separation using high strength silica with reduced particle size Stationary phase. (Fig. 2).

### 3.1 Degradation Studies

There was no interference from sample placebo; degradants peaks and calculated %Mean Assay of individual analyte from each stress condition. It showed that Caffeine; Riboflavin-5'Phosphate; Pyridoxine Hydrochloride; Nicotinamide; Thiamine HCl; and Methylparaben; Propylparaben shows

acceptable degradation in Oxidation condition. The Riboflavin-5'Phosphate; Nicotinamide; Thiamine HCl and Methylparaben; Propylparaben shows Significant degradation observed in Acid and base hydrolysis. The Riboflavin-5'Phosphate shows Significant degradation in all stress condition (Table 1).

### 3.2 Method Validation

#### 3.2.1 System Suitability

The System suitability of the method was determined by complete separation of Caffeine; Riboflavin-5'Phosphate; Pyridoxine Hydrochloride; Nicotinamide; Thiamine HCl; D-Panthenol and Methylparaben; Propylparaben from standard solution using parameters like Retention time (TR); Tailing factor (T), Theoretical plate count; Resolution (Rs); and %RSD of replicate injections was less than 4.0% from the Table 2. The results will ensure transferability of the method and increase the reliability of the results obtained.

#### 3.2.2 Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of measuring analyte were added to placebo preparations and were subjected to the proposed UPLC method. Results of recovery studies are shown in Table 3.

#### 3.23 Precision

Method repeatability (intra-day precision) was evaluated by assaying six injections of sample solution. The mean % assay is well within the acceptance criterion that is; assay value should be between 97.0 and 103.0%. The intermediate precision (inter-day precision) was performed by assaying six injections of sample solution prepared by different analyst, different HPLC system in different days. The mean % assay is within the acceptance criteria. The results indicated the good precision of the developed method (Table 4).

#### 3.2.4 Robustness

To determine the robustness of the analytical method by estimating the assay of sample under deliberately modified chromatographic conditions. Deliberately modify the actual chromatographic conditions specified under the method like flow rate, solvent composition and column temperature and buffer strength on lower and higher side of the actual value. Determine the assay of analyte from Multivitamin syrup preparation under these deliberately modified chromatographic conditions. There was no significant change in the retention times of analytes and assay when the composition and flow rate of the mobile phase were changed.

The results are illustrated in Table 5 through Table 6.

#### 3.2.5 Linearity

The linearity of detector response to different concentrations of all the analytes was studied in the range of 10-150 µg $mL^{-1}$ at ten different levels. The results indicated good linearity. The results are given in Table 7.

#### 3.26 Stability of analyte solutions

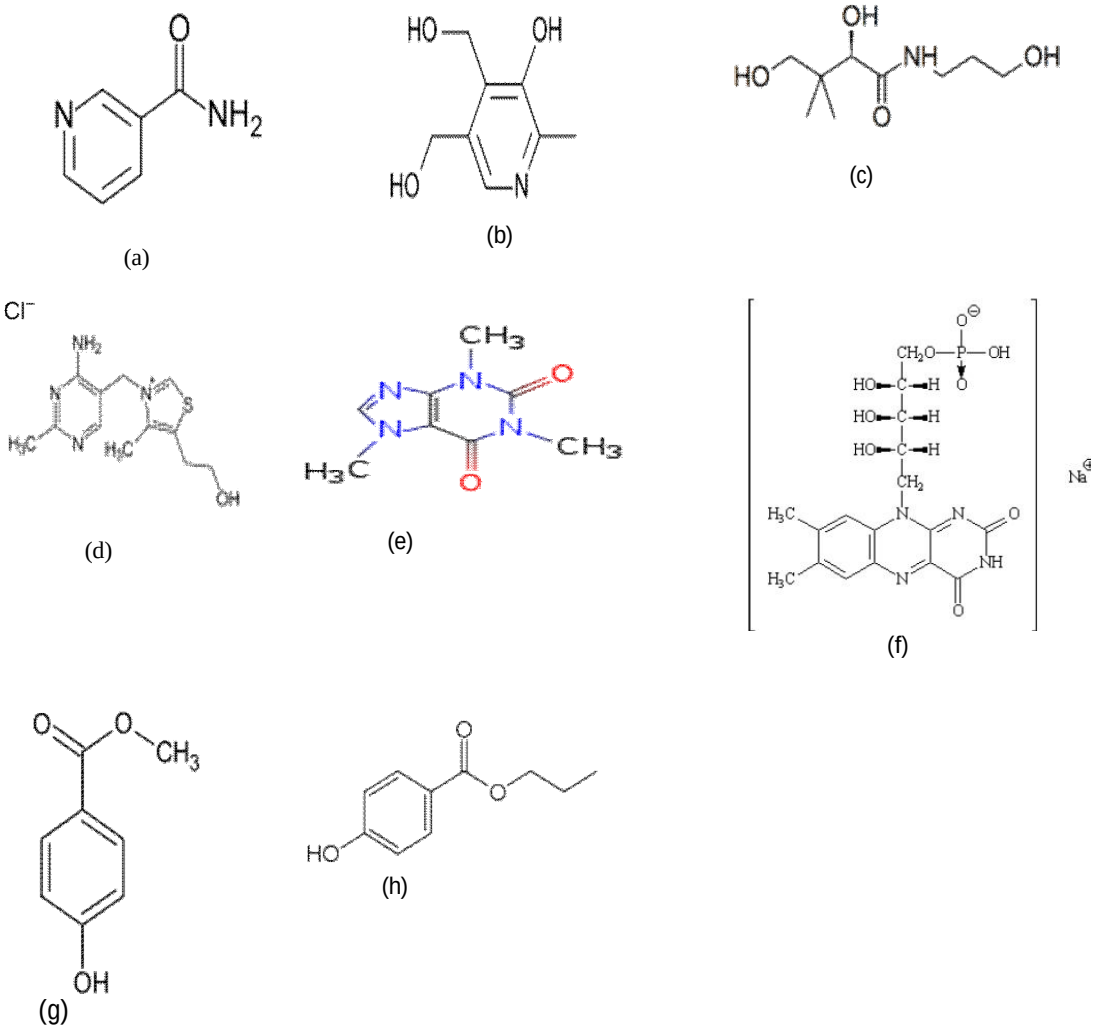
The R.S.D. of assay of standard stock solution during solution stability and mobile phase stability experiments was within 1%. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay determination was stable up to 72 h.

**Table 1: Stress Study Results of all the analytes in multivitamin syrup preparation**

| | %Assay | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Robust Condition** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** |
| Normal | 95.46 | 99.89 | 96.96 | 99.07 | 99.71 | 95.20 | 99.20 | 99.19 |
| 1N HCl, at 50°C for 3 days | 96.90 | #90.29 | 99.81 | 98.45 | 100.47 | #83.10 | #89.54 | #89.88 |
| 1N NaOH, at 50°C for 3 days | #85.99 | #85.75 | 100.83 | 98.68 | 98.25 | #77.11 | #85.80 | 95.58 |
| 3%$H_2O_2$, at 50°C for 3 days | #87.21 | #89.64 | #71.74 | 99.09 | #81.62 | #86.41 | 101.84 | 98.41 |
| at 50°C for 1 day | #82.17 | #60.05 | 99.52 | 99.80 | 98.90 | #89.75 | 101.27 | 98.15 |
| Exposure to 360nm for 3 days | 98.53 | #88.80 | 100.14 | 99.36 | 96.67 | 94.46 | 98.77 | 98.60 |
| At 60°C / 75%RH for 3 days | 97.02 | #87.49 | 100.14 | 98.99 | 97.43 | 103.81 | 99.67 | 99.87 |

* A: Nicotinamide; B: Thiamine Hydrochloride; C: Pyridoxine Hydrochloride; D: D-Panthenol; E: Caffeine anhydrous; F: Riboflavin-5'phosphate sodium; G: Methyl hydroxybenzoate; H: Propyl hydroxybenzoate

# Significant degradation




**(a) Nicotinamide**
**(b) Pyridoxine hydrochloride**
**(c) Panthenol**
**(d) Thiamine hydrochloride**
**(e) Caffeine**
**(f) Riboflavine-5-phophate sodium**
**(g) Methyl hydroxy benzoate**
**(h) Propyl hydroxy benzoate**

**Fig. 1: Chemical Structure of the Analytes**

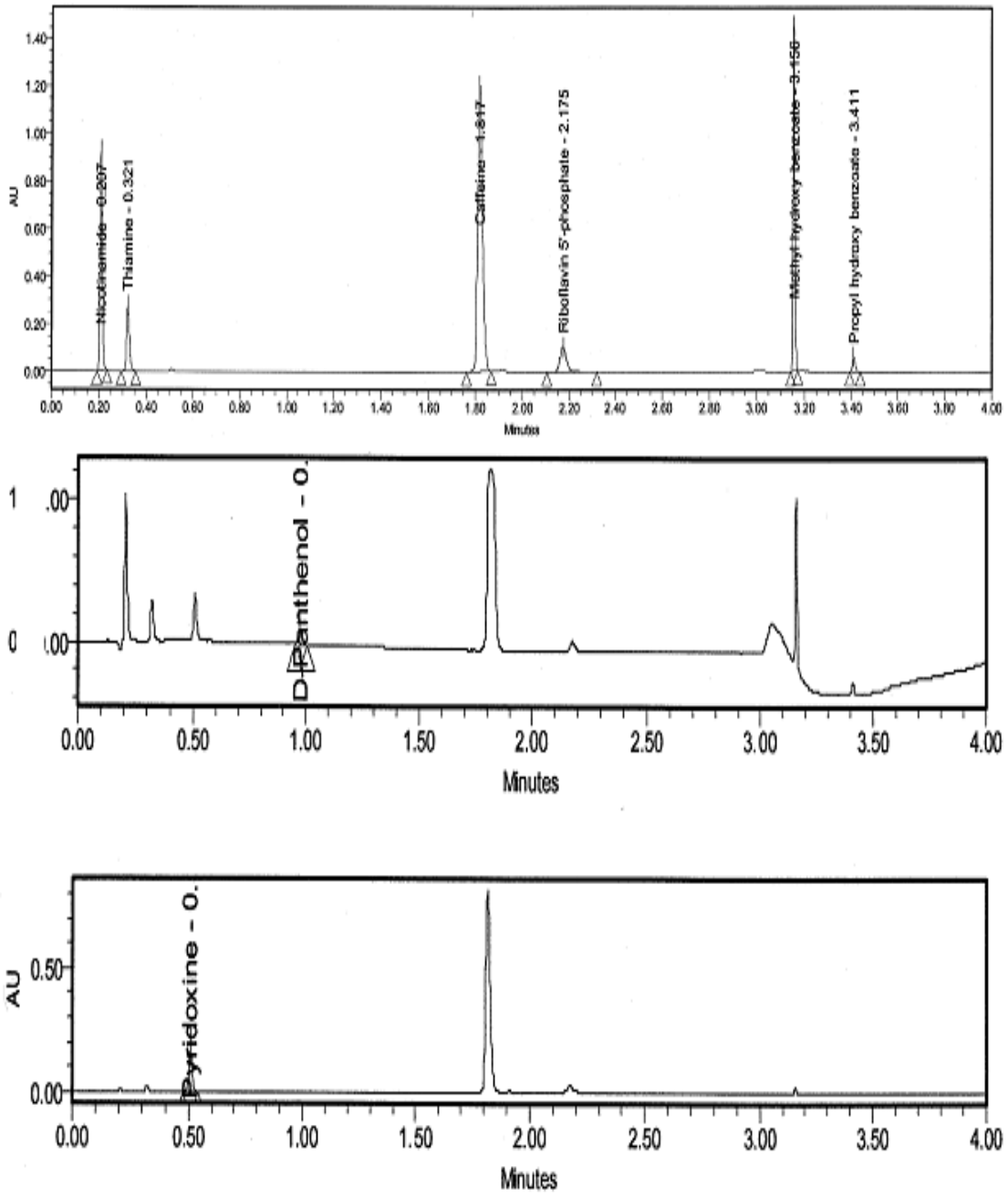



**Fig. 2: Chromatograms showing separation of analytes at 254 nm, 200nm and 290nm**

**Table 2: System suitability Results**

| Name of the Peak | λ-max nm | RT Min | K-Prime | Resolution | Tailing | Plate count | %RSD |
|---|---|---|---|---|---|---|---|
| Nicotinamide | 254 | 0.2 | 1.07 | - | 1.27 | 2149 | 0.04 |
| Thiamine | 254 | 0.3 | 2.21 | 5.51 | 1.31 | 2994 | 3.99 |
| Pyridoxine | 290 | 0.5 | 4.08 | - | 1.19 | 6791 | 0.19 |
| D-Panthenol | 210 | 1.0 | 8.72 | - | 1.10 | 20923 | 0.71 |
| Caffeine | 254 | 1.8 | 17.17 | 48.37 | 1.25 | 35802 | 0.05 |
| Riboflavin- 5'-Phosphate | 254 | 2.2 | 20.75 | 8.04 | 1.21 | 29787 | 0.49 |
| Methyl hydroxy benzoate | 254 | 3.2 | 30.56 | 30.25 | 1.04 | 757613 | 0.08 |
| Propyl hydroxy benzoate | 254 | 3.4 | 33.11 | 13.66 | 1.15 | 355450 | 0.23 |

**Table 3: Accuracy Results**

| Sample | %Recovery level | %Relative recovery ±SD |
|---|---|---|
| Nicotinamide | 80 | 100.87±2.65 |
| | 100 | 99.18±0.48 |
| | 120 | 98.21±0.12 |
| Thiamine Hydrochloride | 80 | 100.09±1.48 |
| | 100 | 98.59±0.20 |
| | 120 | 98.59±1.52 |
| Pyridoxine Hydrochloride | 80 | 96.12±1.49 |
| | 100 | 96.12±0.75 |
| | 120 | 103.44±1.82 |
| D-Panthenol | 80 | 101.35±2.80 |
| | 100 | 98.90±1.41 |
| | 120 | 98.29±0.59 |
| Caffeine anhydrous | 80 | 100.99±1.58 |
| | 100 | 99.15±0.87 |
| | 120 | 98.54±0.18 |
| Riboflavin- 5'phosphate sodium | 80 | 94.54±1.94 |
| | 100 | 94.01±0.29 |
| | 120 | 96.07±1.37 |
| Methyl hydroxybenzoate | 80 | 99.82±2.06 |
| | 100 | 99.68±1.37 |
| | 120 | 98.60±1.26 |
| Propyl hydroxybenzoate | 80 | 96.29±8.88 |
| | 100 | 96.49±7.02 |
| | 120 | 96.80±6.57 |

**Table 4: Precision Results**

| Name of the Analyte | Mean %Assay ± SEM* (Intraday) | Mean %Assay ± SEM* (Interday) |
|---|---|---|
| Nicotinamide | 97.97± 1.44 | 98.64± 1.12 |
| Thiamine Hydrochloride | 99.65± 0.53 | 99.05± 0.43 |
| Pyridoxine Hydrochloride | 98.02± 0.85 | 97.92± 0.84 |
| D-Panthenol | 99.20± 1.78 | 99.10± 1.68 |
| Caffeine anhydrous | 97.86± 0.83 | 97.88± 0.78 |
| Riboflavin-5'phosphate sodium | 99.08± 0.60 | 99.01± 0.57 |
| Methyl hydroxy benzoate | 97.85± 0.81 | 97.88± 0.61 |
| Propyl hydroxy benzoate | 98.64± 1.12 | 98.04± 1.01 |

**Table 5: Robustness Results for Retention time (in minutes)**

| Retention time (min) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Robust Condition** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** |
| Normal | 0.203 | 0.303 | 0.502 | 1.004 | 1.802 | 2.202 | 3.201 | 3.405 |
| Flow rate – 0.99ml/min | 0.206 | 0.306 | 0.506 | 1.011 | 1.808 | 2.208 | 3.202 | 3.402 |
| Flow rate – 1.01ml/min | 0.201 | 0.301 | 0.501 | 0.998 | 1.798 | 2.197 | 3.200 | 3.403 |
| Column temperature - 46ºC | 0.205 | 0.305 | 0.504 | 1.001 | 1.805 | 2.205 | 3.207 | 3.407 |
| Column temperature - 50ºC | 0.204 | 0.307 | 0.505 | 1.003 | 1.804 | 2.207 | 3.203 | 3.408 |
| 0.09% TFA in water | 0.202 | 0.306 | 0.506 | 1.008 | 1.809 | 2.209 | 3.205 | 3.402 |
| 0.11% TFA in water | 0.207 | 0.304 | 0.507 | 1.001 | 1.805 | 2.204 | 3.208 | 3.404 |
| **% Difference** | | | | | | | | |
| Flow rate – 0.99ml/min | 1.48 | 0.99 | 0.80 | 0.70 | 0.33 | 0.27 | 0.03 | 0.09 |
| Flow rate – 1.01ml/min | 0.99 | 0.66 | 0.20 | 0.60 | 0.22 | 0.23 | 0.03 | 0.06 |
| Column temperature - 46ºC | 0.99 | 0.66 | 0.40 | 0.30 | 0.17 | 0.14 | 0.19 | 0.06 |
| Column temperature - 50ºC | 0.49 | 1.32 | 0.60 | 0.10 | 0.11 | 0.23 | 0.06 | 0.09 |
| 0.09% TFA in water | 0.49 | 0.99 | 0.80 | 0.40 | 0.39 | 0.32 | 0.12 | 0.09 |
| 0.11% TFA in water | 1.97 | 0.33 | 1.00 | 0.30 | 0.17 | 0.09 | 0.22 | 0.03 |

* A: Nicotinamide; B: Thiamine Hydrochloride; C: Pyridoxine Hydrochloride;
D: D-Panthenol; E: Caffeine anhydrous; F: Riboflavin-5'phosphate sodium;
G: Methyl hydroxybenzoate; H: Propyl hydroxybenzoate

**Table 6: Robustness Results for %assay**

| % Assay | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Robust Condition** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** |
| Normal | 97.97 | 99.65 | 98.02 | 99.20 | 97.06 | 99.08 | 97.15 | 98.64 |
| Flow rate – 0.99ml/min | 98.11 | 99.11 | 98.43 | 99.43 | 97.13 | 99.12 | 97.88 | 98.22 |
| Flow rate – 1.01ml/min | 98.33 | 99.85 | 98.62 | 99.54 | 97.91 | 99.24 | 97.04 | 98.73 |
| Column temperature - 46ºC | 97.87 | 99.13 | 98.94 | 99.88 | 97.23 | 99.01 | 97.17 | 98.78 |
| Column temperature - 50ºC | 98.22 | 99.67 | 98.62 | 99.02 | 97.11 | 99.34 | 97.43 | 98.51 |
| 0.09% TFA in water | 98.17 | 99.22 | 98.67 | 99.04 | 97.24 | 99.53 | 97.88 | 98.11 |
| 0.11% TFA in water | 98.25 | 99.61 | 98.83 | 99.12 | 97.31 | 99.78 | 97.21 | 98.32 |
| **% Difference** | | | | | | | | |
| Flow rate – 0.99ml/min | 0.14 | 0.54 | 0.42 | 0.23 | 0.07 | 0.04 | 0.75 | 0.43 |
| Flow rate – 1.01ml/min | 0.37 | 0.20 | 0.61 | 0.34 | 0.88 | 0.16 | 0.11 | 0.09 |
| Column temperature - 46ºC | 0.10 | 0.52 | 0.94 | 0.69 | 0.18 | 0.07 | 0.02 | 0.14 |
| Column temperature - 50ºC | 0.26 | 0.02 | 0.61 | 0.18 | 0.05 | 0.26 | 0.29 | 0.13 |
| 0.09% TFA in water | 0.20 | 0.43 | 0.66 | 0.16 | 0.19 | 0.45 | 0.75 | 0.54 |
| 0.11% TFA in water | 0.29 | 0.04 | 0.83 | 0.08 | 0.26 | 0.71 | 0.06 | 0.32 |

* A: Nicotinamide; B: Thiamine Hydrochloride; C: Pyridoxine Hydrochloride;
D: D-Panthenol; E: Caffeine anhydrous; F: Riboflavin-5'phosphate sodium;
G: Methyl hydroxybenzoate; H: Propyl hydroxybenzoate

**Table 7: Linearity Results**

| Name of the Analyte | Slope | Intercept | %Y-Intercept | Correlation coefficient ($r^2$) |
|---|---|---|---|---|
| Nicotinamide | 6265 | 501.07 | 0.08 | 0.999984 |
| Thiamine Hydrochloride | 2735 | -16780.94 | -6.36 | 0.997383 |
| Pyridoxine Hydrochloride | 1635 | 57.42 | 0.04 | 0.999975 |
| D-Panthenol | 1022 | 938.55 | 0.90 | 0.997832 |
| Caffeine anhydrous | 17113 | 1268.35 | 0.07 | 0.999986 |
| Riboflavin-5'phosphate sodium | 2100 | -2143.02 | -1.03 | 0.999931 |
| Methyl hydroxyl benzoate | 7529 | 2288.45 | 0.30 | 0.999888 |
| Propyl hydroxyl benzoate | 604 | -572.00 | -0.98 | 0.997029 |

## 4.0 CONCLUSION

This UPLC method is accurate, precise, reproducible, specific, and stability-indicating. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of an economical and readily available mobile phase, UV detection, lack of extraction procedures, low tR and no internal standard. All these factors make this method suitable for quantification of all the analytes in pharmaceutical dosage forms. The method can be successfully used for routine analysis of multivitamin preparations without interference.

## ACKNOWLEDGEMENTS

The authors thank the management of Lee Pharma Limited; Hyderabad for providing the samples and necessary facilities for the completion of this work.

## REFERENCES

1. Stojanka Vidović, Biljana Stojanović, Jelena Veljković, Ljiljana Pražić-Arsić, Goran Roglić and Dragan Manojlović. Simultaneous determination of some water-soluble vitamins and preservatives in multivitamin syrup by validated stability-indicating high-performance liquid chromatography method. Journal of Chromatography A. 2008;1202(2):155-162.
2. Staroverov VM, Deineka VI, Grigor'Ev AM, Prokhoda EF, Pokrovskill MV and Ivanov VV. HPLC analysis of water-soluble vitamins in the multivitamin syrup Oligovit. Pharmaceutical chemistry journal. 2004;38(3):172-75.
3. Pavlos F. Chatzimichalakis, Victoria F. Samanidou, Robert Verpoorte and Loannis N. Papadoyannis. Development of a validated HPLC method for the determination of B-complex vitamins in pharmaceuticals and biological fluids after solid phase extraction. Journal of separation science. 2004;27(4):1181-1188.
4. Novi Yantih, Diah Widowati, Wartini and Tiwi Aryani. Validation of HPLC method for determination of thiamine hydrochloride, riboflavin, Nicotinamide, and pyridoxine hydrochloride in syrup preparation. Canadian Journal on Scientific and Industrial Research. 2011;2(7):269-78.
5. Farajzadeh MA and Nagizadeh S. A Simple and Reliable Spectrophotometric Method for the Determination of Ascorbic Acid in Pharmaceutical Preparations. Journal of Analytical Chemistry. 2003;58(10):927-932.

6. Mohamed A. Eldawy, Tawfik AS and Elshiabouri SR. Rapid, sensitive spectrophotometric method for the determination of ascorbic acid. Anal Chem. 1975;47(3):461-465.
7. International Conferences on Harmonization Q2 (R1), Validation of analytical Procedures: Text and methodology, 2005.