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## Chromatographic Method Development for Rapid Simultaneous Estimation of Calcium Pantothenate and Biotin in Pure and Tablet Dosage Form Using PDA Detector

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### ABSTRACT

A simple, precise and reliable RP-HPLC (Reverse phase high-performance liquid chromatography method) was developed and validated for the simultaneous estimation of calcium pantothenate (CP) and biotin (BI) in pure and their tablet dosage form. The chromatographic separation is being achieved by using Cosmosil C<sub>18</sub> column (250 mm × 4.6 mm i.d, 5 μm) and mobile phase consisting of buffer (Mono basic potassium dihydrogen orthophosphate with 10% orthophosphoric acid, pH adjusted to 2.20 ± 0.05) and methanol (70:30% v/v) with the flow rate of 1.0 ml/min. The PDA detector was used as detector, measuring the response at 210 nm. The retention time was found to be 4.5 and 9.8 min for CP and BI respectively. The linearity of the drugs was acquired in the range of 50-150 μg/ml and 5-15 μg/ml for CP and BI respectively. ICH guidelines were used for the validation of the results obtained.

**Keywords:** Calcium pantothenate, Biotin, HPLC, Simultaneous estimation, PDA detector.

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### INTRODUCTION

Pantothenic acid (Vitamin B5) is chemically Calcium; (((2R)-2,4 dihydroxy-3,3 dimethoxybutanoyl) amino) Propionate. It is a water-soluble vitamin that was identified in 1993, isolated and extracted from the liver in

1938 and first synthesized in 1940 [1]. Only D pantothenic acid possesses biological activity, but it is relatively unstable, a calcium pantothenate is a stable form it is water soluble crystalline and white in colour. Pantothenic acid is used in CoA (CoA is an essential cofactor in fatty acid oxidation, lipid elongation, and fatty acid synthesis) and in acyl carrier proteins (ACP). It also involved in the synthesis of neurotransmitter, steroid hormones and hemoglobin. Tired of fatigue (including apathy and malaise), headache and weakness is the most common symptoms in deficiency of pantothenic acid [2].

Biotin is chemically 5((3a S, 4S, 6aR)-2 oxohexahydro-<sup>1</sup>H-thieno (3,4-d) imidazol-4yl) pentanoic acid. It also is known as vitamin H or B7, it is a water-soluble vitamin, it synthesis by enteric bacteria, hence deficiency may occur in intestinal disorder. It acts as coenzyme in supporting the function of carboxylase. Disturbing the energy metabolism and various physiological functions are identified as biotin deficiency [3].

The literature review revealed that very few analytical methods have been reported for estimation of calcium pantothenate either individually or in combination with other B complex vitamin [4-15]. Hence, we developed a new, rapid, accurate and reproducible RP-HPLC procedure for simultaneous estimation of biotin and calcium pantothenate. The method was carried out with the guidelines given by ICH.

## EXPERIMENTAL PROCEDURE

### *Reagents and chemicals*

HPLC grade methanol was procured from RANKEM, RFCL (India) Ltd., Monobasic Potassium dihydrogen orthophosphate, and Orthophosphoric acid were purchased from FINAR Chemical (India) Ltd. Millipore water was used and it obtain from Milli-Q RO water system. CP and BI standards were used and it obtained from Fourrts (India) Laboratories Ltd. The marketed tablet dosage form used in this study was Hairgrow<sup>®</sup> labeled to contain 10 mg of BI and 100 mg of CP.

### *RP-HPLC method*

#### **Instrumentation**

The separation was carried out on SHIMADZU liquid chromatographic system, LC 2010 AT equipped with a quaternary pump, SHIMADZU SPD-M30A Photodiode Array Detector, an auto injector. The data was collected and processed using the software LC solution. Weighing was done on SHIMADZU AY-120 Balance.

#### **Buffer preparation**

A weighed quantity of 4.1 g of monobasic potassium dihydrogen orthophosphate was dissolved in 1000 ml of water and pH adjusted to  $2.20 \pm 0.05$  with 10% orthophosphoric acid. The same was filtered through 0.45  $\mu$  membrane filter.

**Chromatographic condition**

The chromatographic condition was achieved on Cosmosil C<sub>18</sub> (250 mm × 4.6 mm 5 μm.) column. The mobile phase consisting potassium dihydrogen orthophosphate buffer and methanol (70:30% v/v) adjusted to pH 2.20 ± 0.05 with 10% ortho phosphoric acid and filtered through 0.45 μ membrane filter (Millipore) and degassed prior to use. The flow rate was fixed at 1.0 ml/minute. Separation was achieved with an ambient temperature (25°C) and detection was attaining at 210 nm. The injection volume was 20 μL with a run time of 20 minutes.

**Preparation of standard solution**

Accurately weighed the quantity of 10 mg of BI and 100 mg of CP were transferred in to a 100 ml volumetric flask and dissolved in 25 ml of water and 0.2 ml of ammonia solution. Volume made up to 100 ml with water. From this 5 ml of above is transferred to a 50 ml volumetric flask and diluted to 50 ml with water to get 10 μg/ml of BI and 100 μg/ml of CP.

**Preparation of sample solution**

For the estimation of a drug in tablet formulation, twenty tablets were weighed and their average weight was determined. The tablets were finely powdered. A quantity of powdered tablet equivalent to 10 mg of BI and 100 mg of CP and was accurately weighed and transferred in to a 100 ml volumetric flask; 25 ml of water and 0.2 ml of Ammonia solution were added and sonicated for 10 minutes and filtered through Whatman filter No.41 paper. The volume was made to 100 ml with water. From this 5 ml of the solution is transferred to 50 ml volumetric flask and made up to the volume with water.

**Assay**

The column was calibrated with mobile phase for sufficient time until to obtain a stable baseline. A single injection of blank preparation, five replicates of standard solution and duplicate of sample preparation were made and peak area responses were recorded. The relevant data is shown in Table 1.

***Method validation*****System suitability**

For system suitability, six replicates of standard solutions were injected and parameters studied were a number of theoretical plates, peak area, resolution, retention time and tailing factor. The relevant data is shown in Table 2.

**Accuracy**

Recovery technique was employed in establishing the accuracy of the experiments, i.e. by external standard addition method. The result of recovery analysis is presented in Table 3. All the results were found to be in the acceptance criteria.

**Linearity and range**

During linearity study, it was observed that the peak response values of CP and BI in the marketed formulation were linear in the concentration range of 50-150% of the test concentration with a correlation coefficient close to one for this method of analysis.

**Precision**

System precision and method precision was used for verifying the validation of the proposed method. The system precision was evaluated by measuring the peak area responses of CP and BI for six replicate injections of the standard solutions. The method precision was determined by quantifying the sample solutions as per the proposed method, which yielded quite concurrent results, indicating the reliability of the method. All the data resided within the prescribed limit of 2% SD and RSD, indicating very good precision of the method.

**Robustness**

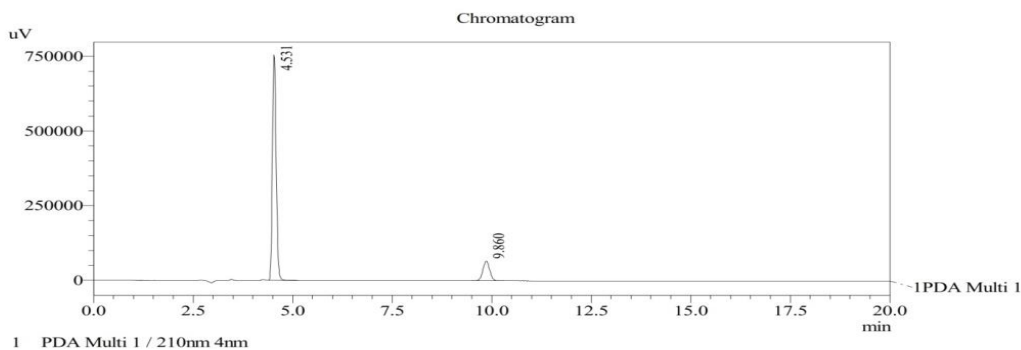
Robustness was performed by deliberately changing the chromatographic conditions. Flow rate of mobile phase was changed from 1.0 ml/min to 0.9 ml/min and 1.1 ml/min. Column temperature as changed from 30°C to 28°C and 32°C. Buffer pH was changed from 2.20 to 2.10 and 2.30. And the ratio of the mobile phase was changed from 70:30 (Buffer: methanol) to 72:30 (Buffer: methanol) and 70:32 (Buffer: methanol). Respective peak areas, dilution factors, sample and standard weight are taken into account to quantitative the amount of both drugs in mg per tablet.

**RESULT AND DISCUSSION**

Estimation of CP and BI in tablet dosage form by RP-HPLC method was carried out using optimized chromatographic conditions. The typical chromatogram of CP and BI is shown in Figure 1. The retention time for CP and BI were found to around be  $4.32 \pm 0.01$  and  $9.80 \pm 0.03$  minutes. The quantitative work produced satisfactory results with the resolution value of 2 and the high-resolution value obtained indicate the complete separation of the drugs. The linearity was studied in the concentration range from 49.60 µg/ml to 149.40 µg/ml for CP and 5.20 µg/ml to 15.00 µg/ml for BI. The regression coefficient ( $R^2$ ) value for CP and BI were found to be 0.9976 and 0.9997, respectively. The mean recovery for CP was 99.0 to 100.0% and 100.2 to 101.51% for BI, which is largely within the 90-110% range that is considered acceptable and it reveals that the method is accurate. System precision and method precision was used for verifying the validation of the proposed method. The % RSD was

found to be less than 2 for both drugs indicate the proposed method is precise. The specificity of the method was confirmed by injecting the placebo and observed that there was no interference due to placebo.

Sample Name : Standard  
 Sample ID : Specificity  
 Method Filename : HAIRGRO TAB.lcm  
 Vial : 5  
 Injection Volume : 20  $\mu$ L



Quantitative Results

ID#	Name	Ret. Time	Area
1	Calcium Pantothenate	4.531	4991449
2	Biotin	9.860	779360

**Figure 1.** Chromatogram of CP and BI.

The data for the ruggedness of CP and BI were found to be within the acceptance limit. Different validation parameters for the proposed HPLC method for determining CP and BI were summarized in Table 3. The results of the analysis were shown that the amounts of drugs were in good agreement with the label claim of the formulation.

**Table 1:** Assay of CP and BI.

Drug name (mg/ml)	Label claim (mg/ml)	Amount found $\pm$ SD	% Label claim
CP	100	98.69 $\pm$ 0.15	98.7
BI	10	9.93 $\pm$ 0.02	99.4

**Table 2:** System suitability parameters of CP and BI.

S. no.	Parameters	CP	BI
01.	Theoretical plates	8638	14392
02.	Tailing factor	1.23	1.05
03.	%RSD of RT	0.012	0.031

**Table 3:** Summary of validation parameters of CP and BI.

S. no	Parameters	CP	BI
01.	Linearity range	49.60-149.4 µg/ml	5.20-15.00 µg/ml
02.	Correlation co-efficient	0.9976	0.9997
03.	LOD	0.15 µg/ml	0.21 µg/ml
04.	LOQ	0.3 µg/ml	0.65 µg/ml
05.	System precision (%RSD)	0.24%	0.60%
06.	Method precision (%RSD)	1.13%	1.58%
07.	Robustness (% RSD)	0.06-1.14%	0.32-0.73%
08.	Ruggedness (% RSD)	0.89%	1.29%

### CONCLUSION

The developed method is simple, accurate, cost effective, less time consuming and the statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of CP and BI as bulk drugs and in combined pharmaceutical dosage forms without any interference from the excipients. The developed method under research will be an effective tool in the routine analysis of calcium pantothenate and biotin in pharmaceutical industry.

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