Oriental Journal of Chemistry Vol. **25**(4), 969-974 (2009)

Separation of thaiamine mononitrate ascorbic acid calcium-D-pantothenate, Niacinamide. pyridoxine hydrochloride and riboflavin by using propyl hydroxy β-cyclodextrin as an ion-pairing reagent

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(Received: June 19, 2009; Accepted: August 04, 2009)

ABSTRACT

The present communication dipics the development of a reversed phase H.P.L C. for the simultaneous estimation of acidic and basic vitamins from solid dosage of polyvitamin and vitamin - B-complex tablets of therapeutic and prophylactic N.F.I. The seperation of vitamins was determined at 280nm. The proposed method is advantageous over reported method, the introduction of propyl hydroxy β-cyclodextrin as an ion-pairing reagent used and found to be profound effect over other ion-pairing reagent. The proposed methods are linear accurate and precise and applicable for daily quality control routine analysis

Key words: β-Cyclodextrin, Vitamin - B-complex, Ion-pairing reagent, separation of vitamins.

INTRODUCTION

The thiamine mononitrate, ascorbic acid. calcium D-Pantothenate, Niacinamide, Pyridoxine Hydrochloride and Riboflavin are the water soluble vitamins[1]. A number of ingredients such as excipients vehicles, preservatives, included in the tablet formulation, which do not interfere in the assay of drug, A buffered Mobile phase of P^H 3.0 is selected in order to achieve the good separation of acidic and basic vitamins When only mobile phase having a mixture of water methanol and GAA. and p^H adjusted 3.0 by Triethyl amine run there was merging of all the vitamins with each other and was eluted earlier, while when 75-80 mg/ 100mL. Propyl hydroxy β-Cyclodextin[2-4] is added Thiamine Mononitrate, Pyridoxime hydrochloride, Ascorbic acid, Calcium-D-Pantothenate, Niacinamide, Riboflavin with 01 baseline and eluted slowly and slowly and for complete separation. it takes about 20.53 min. at flow rate 1ml/min at 280 nm, and sensitivity was kept 0.1 AUFS.

No official pharmacopeal method is reported for the separation of above mentioned vitamins by using P. H. B. C. D. The objective of this investigation was to introduce a chief and economical propyl hydroxy β-Cyclodextrin as an ion-pairing reagent and it is of anionic in nature.

The above method was developed by considering the following formulation of N.F.I[8-11]

Vitamin-B-Complex Therapeutic Prophylactic Tablet NFI		
a) Thiamine Mononitrate (Vit-B_1.	5mg	2mg
b) Ascorbic Acid (Vit-C.	200mg	80mg
c) Calcium D-Pantothenic	2mg	1mg

acid (Vit - B_5)		
d) Niacinamide (Vit - B_3.	50mg	25mg
e) Pyridoxine Hydrochloride (Vit - B_6.	2mg	0.5mg
f) Riboflavin (Vit - B_2.	5mg	2mg

Polyvitamin Tablet NFI	Therapeutic	Prophylactic
a) Thiamine Mononitrate (Vit-B_1)	5mg	2mg
b) Ascorbic Acid (Vit-C.	150mg	50mg
c) Calcium D-Pantotheric acid (Vit - B_5)	2mg	1mg
d) Niacinamide (Vit - B_3.	50mg	25mg
e) Pyridoxine Hydrochloride (Vit - B_6.	2mg	0.5mg
f) Riboflavin (Vit - B_2.	5mg	2mg

EXPERIMENTAL

Materials

De-ionized distilled water, Methanol (HPLC-grade) Acetonitrile (HPLC-grade). Glacial acetic acid (GR-grade), Triethylamine (GR-grade) Propyl-Hydroxy, β-Cyclodextrin.

Reference standard of Thiamine Mononitrate, Riboflorin Pyridoxine Hydrochloride, Niacinamide, Vitamin-C, Calcium-D-pantothenate were used.

Chromatography

Isocratic pump system, spectra physics series, P-100 with variable wavelength absorbance detector. Spectra series UV-100, operated at 280 nm and chromoto recorder SP-4600 spectra physics[12-13-14].

Column L_1(RP-18) C18 Column[15-16], Svoboda et.al (62) Shandon-ODS (5mμ 250 x 4.6mm) was used throughout ambient temprature. SP-4600 spectraphysics chromoto recorder 1.0 cm/m chart speed was used & 0.1AUFS sensitivity is used.

The ' Mobile Phase ' consisted of water, Methanol, Glacial Acetic acid (73:27:1) V/V, β-Cyclodextrin reagent was used as an ion-pairing reagent 75 mg/per 100 ml. of mobile phase.

Before analysis the mobile phase was filtered & degassed through 0.47 Micron filtre paper flow rate was 1.0 ml/min. with an average operating pressure of about 2000±5 PSI, column was washed with methanol at the end of each session.

The " DILUENT " consisted of water, Acetonitrile : Glacial acetic acid (95:5:1) pH adjusted 3.0 by Triethylamine.

MATERIAL AND METHODS

Preparation of standard stock solution

a. Weigh accurately 100mg of Thiamine mononitrate, pyridoxine Hydrochloride, Riboflavin, calcium D-pantothenate in 100ml. Volumetric Flask Separately dissolve it by diluent and make up the volume.
b. Weigh accurately 1gm of Ascorbic acid and Niacinamide in each 100ml Volumetric Flask dissolve completely by diluent and make up the volume.
c. Riboflavin dissolve by gentle heat on keeping on water bath.

Preparation of Standard solution

1. 8 mg/ml. of Thiamine Mononitrate with diluent.
2. 200 mg/ml. of Ascorbic acid with diluent
3. 80 mg/ml. of Niacinamide with diluent
4. 8 mg/ml. of Calcium D-pantothenate with diluent.
5. 4 mg/ml. of Pyridoxine hydrochloride with diluent.
6. 8 mg/ml. of Riboflavin with diluent.

Preparation of Standard Stock solution

Weigh accurately 100 mg of thiamine monitrate or thiamine hydrochloride, pyridoxine hydrochloride, calcium-D-pantethenate, riboflavin each 100 ml Volumetric Flask separately while 1000 mg of Ascrobic acid, Niacinamide in each 100 ml. Volumetric Flask Separately and dissolve completely with diluent and make up to 100 ml. with diluent.

Preparation of standard working solution

Pippete out 20 ml. of thiamine mononitrate, Riboflavin cal-pantothenate, Niacinamide while 50ml. of Ascorbic acid, 10 to 25 mL. with diluent

pippete out 5mL. in 50 mL. Volumetric Flask dilute and make up to 50 mL. with diluent.

Sample preparation[17-18]

Vitamin - B-Complex tablets

(Therapeutic & Prophylactic) NFI

Weigh not less than 20 tablets crushed into fine powders weigh accurately powder, equivalent to 20 mg of thiamine mononitrate in a 250 ml. add 150 mL. diluent. Volumetric flask shake for about 15 min. and then kept on water bath for Half an hour with continous shaking at 60-70^{0}C, cool and make up the volume with diluent.

Centrifuge the solution in order to settle the insoluble excepients of the tablets pippete 5ml of clear solution to 50ml. volumetric flask dilute to 50ml. with diluent.

Polyvitamin Tablets

[Therapeutic & Prophylactic] NFI

Weigh not less than 20 tablets. crushed into fine powder pass through 100 mesh completely.

Weigh accurately powder equivalent to 20mg of thiamine mononitrate in 250 mL. volumetric flask add 150 ml. diluent shake for 15 min. then kept on water bath for half an hour with contineous shaking at 60-70^{0}C, cool and make up the volume to 250 ml. with diluent centifuge the solution pippete 5ml in 50ml. volumetric flask dilute and make up the volume with diluent.

Calibration

50 µl of the working standard solution are injected at a time interval of 15 minutes evaluation is performed with uv detector at 280 nm. The Retention time is found to be around 2.25 minutes for Thiamine mononitrate, 2.89 minutes for Pyridoxine hydrochloride, 4.61 minutes for Ascorbic Acid, 6-92 for calcium-D-pantothenate, 14.61 minutes for Niacinamide and 20.53 minutes for Riboflavin peack area are recorded and calibration graph is obtained by ploting peak area against concentration in µg/ml. Fig (1)

Procedure

20 ml of standard & sample working solution separately was injected into the chomatographic system the amount of each drug/ tablet was calculated by compairing the peak area per sample and for the standard[19].

Recovery study

To study the accuracy, reproducibility and precise of the above method recovery experiments were carried out.

The recovery of added standard was studied at there different levels the fixed amount of the pre-analysed samples, standard drug were added three different levels each level was repeated seven times[20]. The recovery was estimated to be more than 99%.

RESULTS AND DISCUSSION

The present communication dipics the development of a reversed phase HPLC for the analysis of polyvitamin tablet prophylactic and therapeutic NFI and Vitamin B-Complex tablets. Therapeatic and Prophylactic NFI Analytical method is specific sensitive, accurate and precise.

A Number of ingredients such as excipients vehicles preservative, included in the tablet formation do not interfere in the assay of drug.

Linearity

The linearity of Thiamine Mononitrate (Vitamin-B_1), Pyridoxine Hydrochloride (Vitamin-B_6), Ascorbic acid (Vitamin-C), Calcium D-Pantothenate (Vitamin-B_5), Niacinamide (Vitamin-B_3) and Riboflavin (Vitamin-B_2) are established by plotting a graph of peak area of standard solution versus concentration in mg/ml. The linearity is found as fig (1)

a) Thiamine Mononitrate	-	2-12 µg/ml.
b) Pyridoxine Hydrochloride	-	1-7 µg/ml
c) Ascorbic Acid	-	40-320 µg/ml
d) Calcium D-Pantothenate	-	2-6 µg/ml
e) Niacinamide	-	20-100 µg/ml
f) Riboflavin	-	2-14 µg/ml

The proposed method is advantageous over the reported method with respect to the following points.

a. The proposed method incorporates use of a simple mobile phase a combination of water, methanol, acetonitrile & triethylamine.

CHROMATOGRAPHIC CONDITION

Column	:	L_1 (R_P-18) SHANDON - ODS (5 - 4) (250 x 4.6 mm)
Eluent	:	Water : Methanol : GAA 73 : 27 : 1
Flow rate	:	1.0 ml/min.
Inj. Volumes	:	50µl
pH	:	3.0
Sensitivity	:	0.1 AUFS
Wavelength	:	280 nm

Peaks		Conc/ml	Retention time	Peak Area	Base Line
1)	Thiamine Mononitrate	8 µg/ml	2.25 min	17276	01
2)	Pyridoxine HCL	4 µg/ml	2.89 min	31705	01
3)	Ascorbic acid	200 µg/ml	4.61 min	108403	01
4)	Calcium D-Pantothenate	4 µg/ml	6.92 min	54462	01
5)	Niacinamide	80 µg/ml	14.61 min	69861	01
6)	Riboflavin	8 µg/ml	20.53 min	99809	01

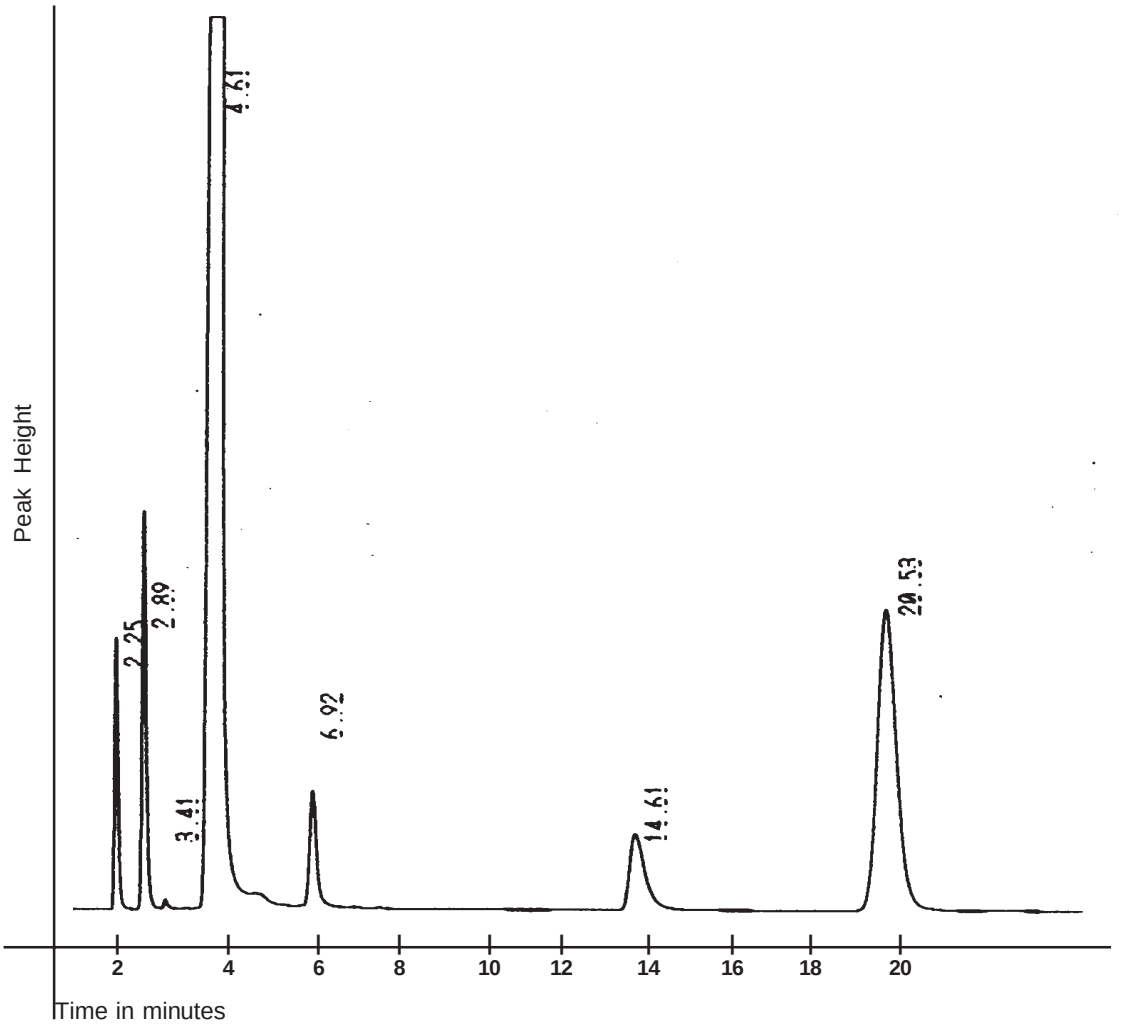

Fig. 1: Chromatogram showing separation of vitamins from Vitamin B-Complex tablets NFI (Therapeutic and prophyl lactic)

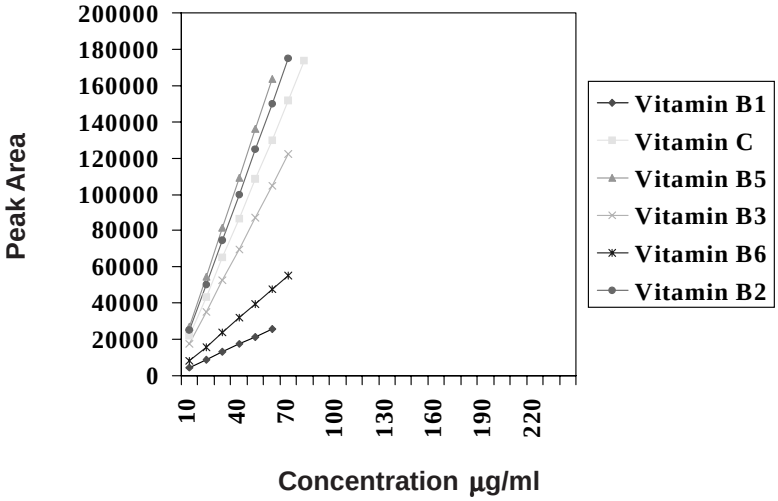

Fig. 2: Linearity Curve of Vitamin B1, Vitamin C, Vitamin B5, Vitamin B3, Vitamin B6, Vitamin B2 Plotted concentration in m**g / ml versus peak area**

b. In this proposed method the time of analysis is short as the total elution of the drug take place within 20.53 min. this results in reducing the time & cost of analysis which is required for routine qualtity control analysis.

c. 4.0 pH selected by conducting the experiment at different pH 2.5 to 5.0 keeping all the chromatographic condition same only 4.0 pH mobile phase used for good resolution of drugs. Also study conducted at different wavelength from 200 to 290 nm and found to be suitable 280 nm for better resolution of peaks of the drugs.

d. It is observed from the resolution of the different drugs that acidic durgs are eluted first followed by basic drugs. Such types of resolution of drugs or the basis of molecular characterstics indicates the affinity of the acidic and basic drugs with the stationary phase i.e. silanol (Si-OH bonding)[21,22,23,24]

e. Ion-pairing reagent reacts with the basic drugs because it is an anionic in nature and dealy its elution this is the reason why acidic drugs are eluated first.However n-Hexane sulphonic acid (sodium. salt) reported as an ion-pairing reagent in U.S.P. does not interact with thiamine mononitrate or hydrochloride because of this (-SH) group present in it that's why it is eluted first because of it is highly water soluble and having greater molecular weight than Ascorbic acid (Vit-C) and pantothenic acid.

f. Further, earliar elution of Vit. B_1 is confirm by Chromatogram with mobile phase having no ion-pairing reagent it is observed that the resolution of peak and retention time remains same, without and with ion-pairing reagent.From this it is clear that ion-pairing reagent does not react with Thiamine mononitrate or Thiamine hydrochloride.

ACKNOWLEDGMENTS

The authors are very thankful to Honorable Dr. Shamshuddin (R& D Manager), Vijay Fudke (Q/C Manager), Honorable Anwar S. Daud Director Zim Pharmacuetical Laboratories Ltd., Nagpur, and Dr. M.M. Wankede, Principal A.C.S. College, Chandrapur for constant encouragement and providing necessary facilities.

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