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Development Of A Rp-Hplc Method For The Estimation Of Diclofenac Sodium, Vitamin B_1, Vitamin $B_6$and Vitamin B_{12} Insoft Gelatin Capsule Dosage Form

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Research Article

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Abstract

Objective: A simple, efficient and reproducible Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for determination of Diclofenac sodium,Vitamin B_1,Vitamin B_6, and Vitamin B_{12} in soft gelatin capsule pharmaceutical formulation has been developed.

Method & Result: The Diclofenac Sodium, the separation was carried out in Phenomenex ODS C_{18} column (150 x 4.6mm; 5µm) using mobile phase consisting of mixture of 400mL potassium hydrogen phosphate (pH 3.0) and 600mL of Acetonitrile. The flow rate was 1.0mL/min and effluent was detected at 254nm. The retention time of Diclofenac sodium was 5.4 min. The percentage recovery was within the range between 100.00% and 101.86% for Diclofenac Sodium. The linearity range was found to be 20-400µg/mL (r^2 =1) for Diclofenac Sodium. The Vitamin B_1 & Vitamin B_6, the separation was carried out in Inertsil ODS C_{18} column (250 x 4.6mm; 5µm) using mobile phase consisting of mixture of 1000mL of Sodium 1-hexane sulfonic acid solution , 730mL of water , 270mL glacial acetic acid and 10ml of methanol The flow rate was 1.5mL/min and effluent was detected at 280nm. The retention time of Vitamin B_1 & Vitamin B_6 were 3.70 min & 7.74 min respectively. The percentage recovery was within the range between 99.46% and 101.03% & 99.35 and 101.78 respectively for Vitamin B_1 & Vitamin B_6. The linearity range was found to be 10-200µg/mL (r^2 =1) for Vitamin B_1 and for Vitamin B_6 the linearity range was found to be 10.5-210µg/mL. For Vitamin B_{12}, the separation was carried out in Phenomenex ODS C_{18} column (150 x 4.6mm; 5µm) using mobile phase consisting of mixture of .25mL trifluroacetic acid, 870mL of water and 130mL of Acetonitrile. The flow rate was 1.2mL/min and effluent was detected at 361nm.The retention time of Vitamin B_{12} was 6.75 min. The percentage recovery was within the range between 98.86% and 101.94% for Vitamin B_{12} .The linearity range was found to be 1-20µg/mL (r^2 =0.9999) for Vitamin B_{12}. The obtained chromatograms showed good resolutions between the peaks which helps to quantify the drugs in well-defined manner.

Conclusion: The Proposed study describes new and simple RP-HPLC method for the estimation of Diclofenac Sodium,Vitamin B_1,Vitamin B_6 and Vitamin B_{12} in soft gelatin capsule dosage form. The method was validated as per ICH guidelines and found to be simple, sensitive, accurate and precise. Therefore the proposed method can be successfully used for the routine lab analysis of estimation of Diclofenac Sodium,Vitamin B_1,Vitamin B_6, and Vitamin B_{12} in soft gelatin capsule dosage form.

Keywords: *Diclofenac Sodium,Vitamin B_1,Vitamin B_6 and Vitamin B_{12}.*

Introduction

The analgesic action of Non-Steroidal Anti-Inflammatory drugs (NSAIDs) has been explained on the basis of their inhibition of the enzymes that synthesize Prostaglandins. However, it is clear that NSAIDs exert their analgesic effect not only through peripheral inhibition of prostaglandin synthesis but also through a variety of other peripheral and central mechanisms. It is now known that there are 2 structurally distinct forms of the Cyclo-oxygenase enzyme (COX-1 and COX-2). COX-1 is a constitutive member of normal cells and COX-2 is induced in inflammatory cells. Inhibition of COX-2 activity represents the most likely mechanism of action for NSAID-mediated analgesia, while the ratio of inhibition of COX-1 to COX-2 by NSAIDs should determine the chances of adverse effects. In addition, some NSAIDs inhibit the Lipoxygenase pathway, which may itself result in the production of algogenic metabolites. Interference with G-protein-

mediated signals transduction by NSAIDs may form the basis of an analgesic mechanism unrelated to inhibition of prostaglandin synthesis. These are increasing evidence that NSAIDs have a central mechanism of action that augments the peripheral mechanism. This effect may be the result of interference with the formation of prostaglandins within the CNS. Alternatively, the central action may be mediated by endogenous Opioid peptides or blockade of the release of serotonin (5-hydroxytryptamine; 5-HT). A mechanism involving inhibition of excitatory amino acids of N-methyl-D-aspartate receptor activation has also been proposed[1-3].

An organic chemical compound (or related set of compounds) is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet. Thus, the term is conditional both on the circumstances and on the particular organism. For example, ascorbic acid is a vitamin for humans, but not for most other animals, and biotin and vitamin D are required in the human diet only in certain circumstances. Vitamins are classified by their biological and chemical activity, not their structure. Thus, each "vitamin" refers to a number of *vitamer* compounds that all show the biological activity associated with a particular vitamin[4].

In isocratic HPLC the analyte is forced through a column of the stationary phase(usually a tube packed with small round particles with a certain surface chemistry) by pumping a liquid (mobile phase) at high pressure through the column. The sample to be analyzed is introduced in a small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time and is considered a reasonably unique identifying characteristic of a given analyte. The use of pressure increases the linear velocity (speed) giving the components less time to diffuse within the column, leading to improved resolution in the resulting chromatogram. Common solvents used include any miscible combinations of water or various organic liquids.

Diclofenac sodium ischemically known as, 2[(2,6Dichlorophenyl) amino]benzene acetic acid sodium salt Diclofenac Sodiumis a non-steroid anti-inflammatory agent and shows a high degree of anti-inflammatory, analgesic, and antipyretic activity.It inhibits prostaglandin biosynthesis in vitro and in vivo. It is marketed as Voltaren among other brand names. Diclofenac Sodium is usually given by the oral route for direct effect on inflammation. In these forms of delivery, the maximal effect of Diclofenac can take place within five to 20 minutes of dosing, though some relief is immediately seen[5-7].

Vitamin B_1 chemically, Thiazolium, 3- [(4-amino-2-methyl 5 pyrimidinyl) methyl] - 5 -(2-hydroxyethyl)-4-methyl-,chloride,monohydrochloride is a type of B complex vitamins. Vitamin B_1 is also referred as Thiamine. The particular indication of this vitamin is neuritis of pregnancy if vomiting is severe.VitaminB_6chemically,5-Hydroxy-6-methyl3,4pyridinedimethanolhydrochloride is a type of B complex Vitamins. Vitamin B_6 is also referred as Pyridoxine. The particular indication of this vitamin isdrug-induced causes (eg, Isoniazid, Hydralazine, oral contraceptives) or inborn errors of metabolism.

Vitamin B_{12}chemically,α-(5,6-dimethylbenzimidazolyl)cobamidcyanide, is a type B complex vitamins. Vitamin B_{12} is also referred asCyanocobalamine.The particular indication of this vitamin is formation of blood in the body.Generally,route ofadministration by oral or nasal.

When Diclofenac Sodium is administered to women during menopause, physical weakness may be observed as a side effect.Inorder to overcome the side effects, Vitamin B_1,,Vitamin B_6, and Vitamin B_{12} are administered along with Diclofenac Sodium in soft gelatin capsules.

The literature survey reveals that a few analytical methods have been reported for the estimation of these drugs individually or in combination with other drugs by Spectrophotometric, high performance liquid chromatography[8], gas liquid chromatography and capillary electrophoresis.A simple, rapid, precise, accurate RP-HPLC method for the estimation of Diclofenac Sodium,Vitamin B_1, VitaminB_6 and Vitamin B_{12}in soft gelatin capsule dosage form has been developed and validated.

Material and Method

EXPERIMENTAL WORK

Chemicals

Diclofenac Sodium, Vitamin B_1,Vitamin B_6 and Vitamin B_{12} and Sample capsule was gifted by Central Drug Research Institute, (Lucknow). Acetonitrile, Methanol, Sodium 1-heanes sulphonicacid, Glacial acetic acid, ortho-phosphoric acid, potassium dihydrogen phosphate and tripple distil water were procured from Merck Laboratories.

DICLOFENAC SODIUM[9]

Instrumentation and Chromatographic Condition

The chromatographic separation was carried out on HPLC system (Agilent 1200 series, Germany) with UV-Variable wavelength dectector, PhenomenexODS C_{18} column (150 x 4.6mm; 5µm).The mobile phase consisting of mixture of potassium hydrogen phosphate in solution 400ml of (pH 3.0) and 600mL of Acetonitrile were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in isocratic condition into the column at a flow rate of 1.0mL/min. The detection was monitored at 254nm.The column temperature was ambient. The volume of injection loop was 20µLprior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system.

Preparation of Standard solutions

Dissolve accurately 20mg of Diclofenac Sodium RS in 100mLvolumetric flask, add 50mL of Mobile phase to dissolve completely, dilute tovolume with diluent, and mix filter through 0.2µm finer porosity membrane filter.

Analysis of Sample Preparation

Take 10 soft gelatin capsules and empty the contents into a small dish and transfer an accurately weighed portion of the mass equivalent to about 20 mg of Diclofenac to a 100mL volumetric flask, add 50mL of diluent to volume, and mix. Pass a portion of this solution through a filter having a 0.2µm finer porosity, discarding the first 10 mL of thefiltrate.Use the clear filtrate as the sample preparation.

Amount of Diclofenac Sodium in soft gelatin capsule

Sample area X Standard weight X 100 X Purity (as is) X 100

= ----------X Average weight

Standard area X 100 X Sample weight X 100 X Label claim

(Limit: 90.0% to 110.0%)

VITAMIN B_1 & VITAMIN B_6 [10]

Instrumentation and chromatographic condition

The chromatographic separation was carried out on HPLC system (Agilent 1200 series, Germany) with UV-Variable wavelength detector, Inertsil ODS C_{18} column (250 x 4.6mm; 5µm).The mobile phase consisting of a mixture of 1000mL of Sodium 1-hexane sulfonic acid solution , 730mL of water,270mL glacial acetic acid of and 10ml of methanol were mixed and filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in isocratic condition into the column at a flow rate of 1.5mL/min. A mixture of water, Acetonitrile and Glacial acetic acid (94:5: 1) was used as the diluent. The detection was monitored at 280nm.The column temperature was ambient. The volume of injection loop was 20µL prior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system.

Preparation of Standard solutions

Dissolve accurately 105mg of Vitamin B_1&100mg of Vitamin B_6RS in 100mLvolumetric flask. Add 50mL of diluent and sonicate to dissolve completely, dilute to volume with diluent, and further dilute 5mLto 50mL. Filter through 0.2µm finer porosity membrane filter.

Analysis of Sample Preparation

Take 10 soft gelatin capsules and empty the contents into a small dish and transfer 3mL of the sample in a 100mL volumetric flask, dilute to volume with diluent. Filter through 0.45µm Nylon filter. Take 5mL of the filtrate in a 50ml volumetric flask, adjust to volume with diluent.

Amount of Vitamin B_1& Vitamin B_6 in soft gelatin capsule

Sample area X Standard dilution X Purity (as is) X 3 X 100

= ----------

Standard area X Sample dilution X 100 X Label claim

(Limit: NLT 90.0%)

VITAMIN B_{12} [11]

Instrumentation and chromatographic condition

The chromatographic separation was carried out on HPLC system (Agilent 1200 series, Germany) with UV-Variable wavelength detector, PhenomenexODS C_{18} column (150 x 4.6mm; 5µm).The mobile phase consisting of a mixture of0.25mLtrifluroaceticacid, 870mL of water, 130mL of Acetonitrile were filtered through 0.45µm membrane filter before use, degassed and were pumped from the solvent reservoir in isocratic condition into the column at a flow rate of 1.2 mL/min. The detection was monitored at 361nm.The temperature was ambient. The volume of injection loop was 500µL prior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system.

Preparation of Standard solution

Dissolve accurately 25mg of Vitamin B_{12}RS in 100mLvolumetric flask, add 50mL of mobile phase to dissolve completely, Dilute to volume with mobile phase and further dilute 5mLto 50mL. Filter through 0.2µm finer porosity membrane filter.

Analysis of Sample Preparation

Take 10 soft gelatin capsules and empty the contents into a small dish and transfer an accurately weighed portion of the mass equivalent to about 0.2 mg of Vitamin $B_{12}$to a 20mL volumetric flask, add 10mL of mobile phase and sonicate, then make up the volume to 20mL, and mix. Pass a portion of this solution through a filter having a 0.2µm finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the sample preparation.

Amount of Vitamin B_{12}in soft gelatin capsule

Sample area X Standard weight X 2 X 20 X Purity (as is) X 100

= ----------X Average weight

Standard area X 100 X20 Sample weight X 100 X Label claim

(Limit: 90.0% to 110.0%)

Results and Discussion

DICLOFENAC SODIUM

System Suitability

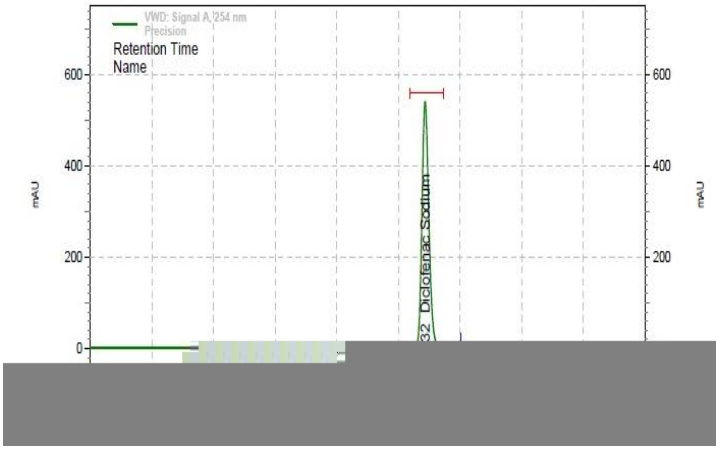
Table 1: System suitability for Diclofenac Sodium

S.No	Retention time	Area	Theoretical Plates
1	5.432	70395513	11330
2	5.428	70400159	11276
3	5.428	70476190	11288
4	5.428	70436657	11316
5	5.428	70407211	11317
6	5.423	70472993	11226
Average	**5.428**	**70431454**	**11292**
RSD (%)	**0.05**	**0.05**	**0.34**

Table 2: System suitability parameters for Diclofenac Sodium

S.No	System suitability parameters	Diclofenac Sodium
1.	Retention time	5.428
2.	Peak area response	70431454
3.	Theoretical plate	11292

Figure: 1 Chromatogram for the study of system suitability of Diclofenac Sodium



Specificity

Table 3: Specificity for Diclofenac Sodium

S.No	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	70431454
4.	Sample	1	71348903

Linearity and Range

Table 4: Linearity of response for Diclofenac Sodium

S.No	Concentration (µg/mL)	Area
1.	20	7356664
2.	40	14770602
3.	80	29585298
4.	120	44447056
5.	160	59287556
6.	200	74241051
7.	240	89244490
8.	280	104317924
9.	320	119560410
10.	360	134889015
11.	400	150279915

Figure: 2 Linearity of response for Diclofenac Sodium

Figure: 3 Chromatogram for the study of linearity of Diclofenac Sodium(20% of operating concentrations)

Figure: 4 Chromatogram for the study of linearity of Diclofenac Sodium (400% of operating concentrations)

ACCURACY

Table: 5 Accuracy of response for Diclofenac Sodium

S.No	Quantity added (Known) µg/mL	Area	% Recovery
1	140	54677470	100.00
2	168	65903546	100.44
3	196	77214247	100.87
4	224	88631052	101.31
5	252	100258439	101.87
6	280	111392035	101.86

Table 11: System suitability parameters for Vitamin B_1

S.No	System suitability parameters	Vitamin B_1
1.	Retention time	3.701min
2.	Peak area response	286657752
3.	Theoretical plate	8967

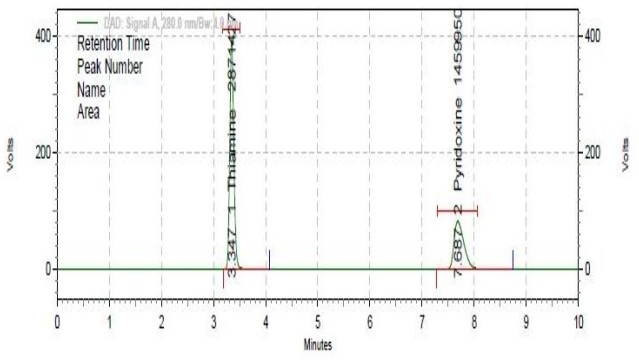
Table 12: System suitability for Vitamin B_6

S.No	Replicates	Retention time	Area	Theoretical Plates
1.	Area-1	7.687	145995078	7489
2.	Area-2	7.720	145652695	7546
3.	Area-3	7.727	145345304	7535
4.	Area-4	7.733	145436109	7527
5.	Area-5	7.740	145348939	7521
6.	Area-6	7.740	145583122	7510
	Mean	**7.7245**	**145560207**	**7521**
	RSD (%)	**0.25**	**0.17**	**0.27**

Table 13: System suitability parameters for Vitamin B_6

S.No	System suitability parameters	Vitamin B_6
1.	Retention time	7.72
2.	Peak area response	145560207
3.	Theoretical plate	7521

Figure: 9 Chromatogram for the study of system suitability of Vitamin B_1& Vitamin B_6



SPECIFICITY

Table 14: Specificity for Vitamin B_1

S.No	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	286657752
4.	Sample	1	370892469

Table 15: Specificity for Vitamin B_6

S.No	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	145560207
4.	Sample	1	186541390

Figure: 10 Chromatogram for the study of specificity of blank

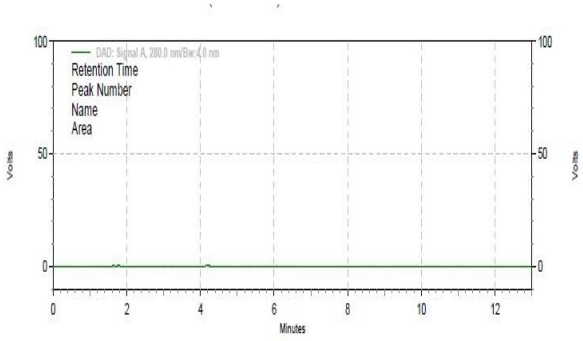


Figure: 11 Chromatogram for the study of specificity of placebo

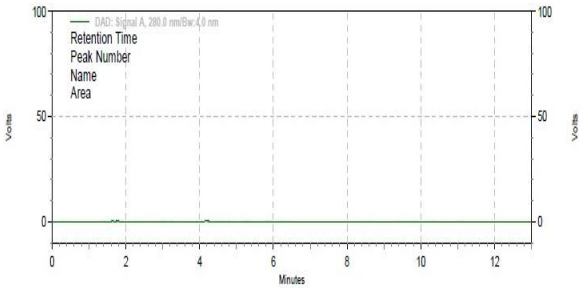


Figure: 12 Chromatogram for the study of specificity of Standard

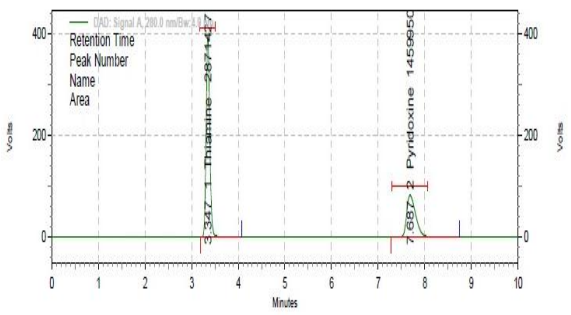


Figure: 13 Chromatogram for the study of specificity of sample

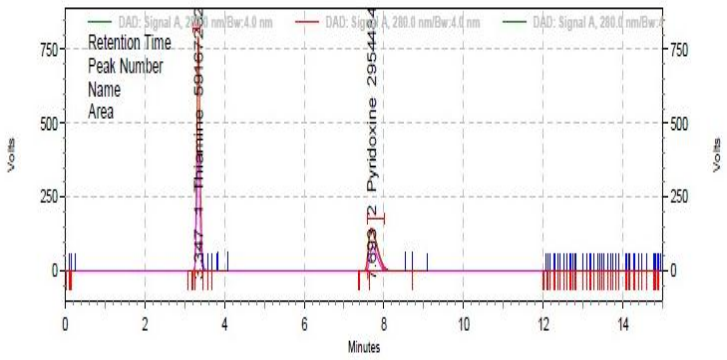
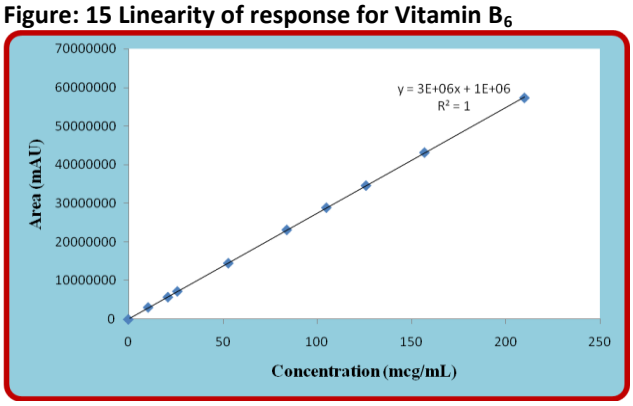


Figure: 15 Linearity of response for Vitamin B_6



LINEARITY AND RANGE

Table 16: Linearity of response for Vitamin B_1

S.No	Concentration (µg/mL)	Mean
1	10	15496625
2	20	36350800
3	25	36350800
4	50	73448730
5	80	117657537
6	100	146541126
7	120	175739070
8	150	218070820
9	200	286649512

Table: 17 Linearity of response for Vitamin B_6

S.No	Concentration (µg/mL)	Mean
1.	10.5	30935468
2.	21	57529086
3.	26	72481997
4.	53	145414044
5.	84	231775015
6.	105	289194386
7.	126	346432047
8.	157	431886748
9.	210	573479050

Figure: 14 Linearity of response for Vitamin B_1

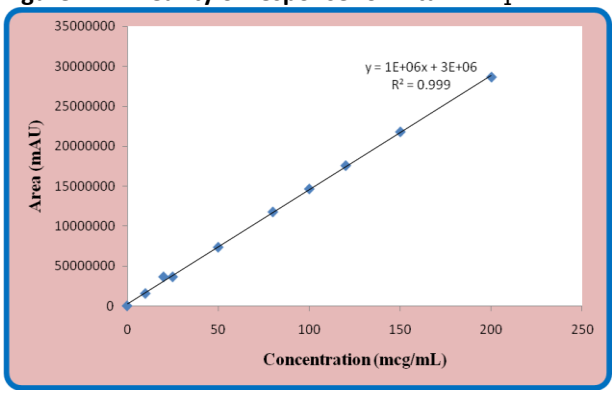


Figure: 16 Chromatogram for the study of linearity of Vitamin B_1 & Vitamin B_6(10% operating concentration)

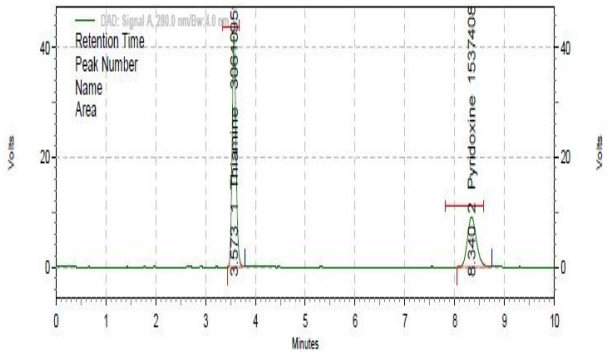
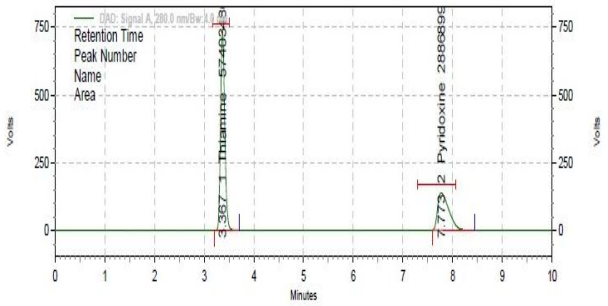


Figure: 17 Chromatogram for the study of linearity of Vitamin B_1 & Vitamin B_6(200% operating concentration)



.ACCURACY

Table 18: Accuracy for Vitamin B_1

S.No	Quantity added (Known) µg/mL	Area	% Recovery
1	100	203663196	100.00
2	115	232939824	99.46
3	130	262035522	98.97
4	140	290875524	102.02
5	160	329221160	101.03
6	170	348327318	100.61
7	185	377110935	100.09
8	200	407033426	99.93

Table 19: Accuracy for Vitamin B_6

S.No	Quantity added (Known) µg/mL	Area	% Recovery
1	100	101774521	100
2	115	116283316	99.35
3	130	130735288	98.81
4	140	145020887	101.78
5	160	159445650	98.92
6	170	173727762	100.41
7	185	188080328	99.89
8	200	202508127	99.49

Figure: 18 Chromatogram for the study of accuracy of Vitamin B_1 & Vitamin B_6

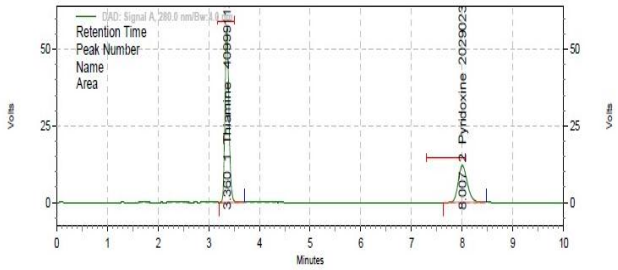
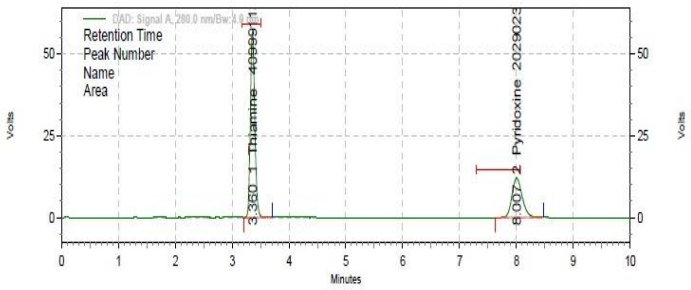


Figure: 19 Chromatogram for the study of accuracy of Vitamin B_1 & Vitamin B_6



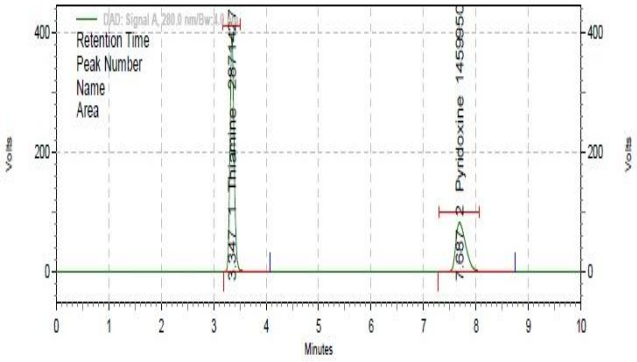
PRECISION

Reproducibility

Table :20 Precision - Reproducibility for Vitamin B_1&Vitamin B_6

S.No	Replicates	Area of Vitamin B_1	Area of Vitamin B_6
1	Sample-1	287142717	145995078
2	Sample-2	287490457	145652695
3	Sample-3	285551325	145345304
4	Sample-4	285987577	145436109
5	Sample-5	285907247	145348939
6	Sample-6	287867187	145583122
Average		**286657752**	**145560208**
Standard deviation		**962070.1**	**246742.7**
RSD (%)		**0.34**	**0.17**

Figure: 20 Chromatogram for the study of reproducibility of Vitamin B_1 & Vitamin B_6



Repeatability

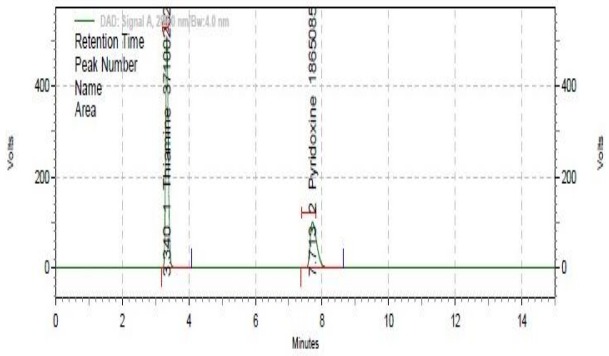
Table: 21 Precision - Repeatability for Vitamin B_1

S.No	Replicates	Area
1	Sample-1	371002525
2	Sample-2	370560488
3	Sample-3	370875528
4	Sample-4	370901911
5	Sample-5	370877530
6	Sample-6	371136833
Average		**370892469**
Standard Deviation		**191121.3**
RSD %		**0.05**

Table: 22 Precision - Repeatability for Vitamin B_6

S.No	Replicates	Area
1	Sample-1	186508549
2	Sample-2	186292798
3	Sample-3	186556221
4	Sample-4	186623789
5	Sample-5	186600426
6	Sample-6	
Average		**186541390**
Standard Deviation		**133444.6**
RSD %		**0.07**

Figure: 19 Chromatogram for the study of repeatability of Vitamin B_1 & Vitamin B_6

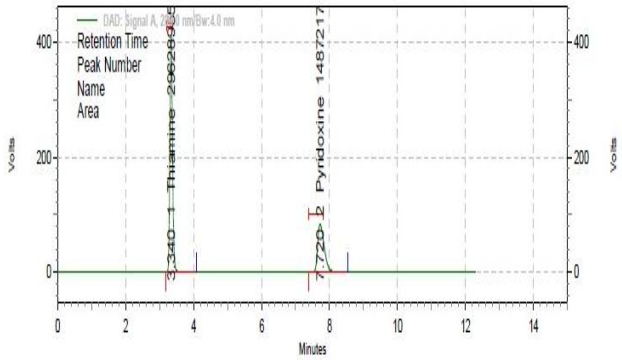


RUGGEDNESS

Table 23: Ruggedness data for Vitamin B_1& Vitamin B_6 - Change of analyst

S.No	Replicates	Area of Vitamin B_1	Area of Vitamin B_6
1	Sample-1	296289559	148721741
2	Sample-2	296545840	149010606
3	Sample-3	296324710	148921465
4	Sample-4	296445701	148786843
5	Sample-5	296461388	148782386
6	Sample-6	296465287	148817069
Average		**296422081**	**148840018**
Standard Deviation		**96259.2**	**106206.4**
RSD (%)		**0.03**	**0.07**

Figure: 20 Chromatogram for the study of ruggedness of Vitamin B_1 & Vitamin B_6



ROBUSTNESS

Table 24: Robustness - Change in the ratio of solvents in the mobile phase (- 2%) for Vitamin B_1& Vitamin B_6

S.No	Sample Name	Water:acetic acid (71:29)	Water:acetic acid (71:29)
		Area for Vitamin B_1	Area for Vitamin B_6
1.	Sample -1	296465287	148721741
2.	Sample -2	296324770	149016061
Mean		**296395029**	**148868901**
Standard deviation		**99360.524**	**208115.67**
RSD in %		**0.34**	**0.14**

VITAMIN B_{12}

SYSTEM SUITABILITY

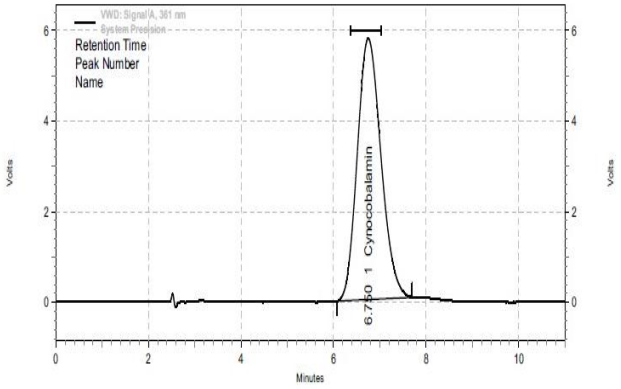
Table 25: System suitability for Vitamin B_{12}

S.No	Replicates	Retention time	Area	Theoretical Plates
1	Standard-1	6.75	3437555	797
2	Standard-2	6.75	3467728	795
3	Standard-3	6.76	3469258	793
4	Standard-4	6.76	3445447	795
5	Standard-5	6.77	3413547	793
6	Standard-6	6.77	3434737	789
Average		**6.76**	**3444712**	**794**
RSD (%)		**0.11**	**0.62**	**0.34**

Table 26:suitability System parameters for Vitamin B_{12}

S.No.	System suitability parameters	Vitamin B 12
1.	Retention time	6.76min
2.	Peak area response	3444712
3.	Number of theoretical plate	794

Figure: 21 Chromatogram for the study of system suitability of Vitamin B_{12}



SPECIFICITY

Table 27: Specificity for Vitamin B_{12}

S.No	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	3444712
4.	Sample	1	4643599

LINEARITY AND RANGE

Table 28: Linearity of response for Vitamin B_{12}

S.No	Concentration (µg/mL)	Area
1	1	347374
2	2	683822
3	2.5	864185
4	5	1742247
5	7.5	2573925
6	10	3449895
7	12	4163753
8	15	5130808
9	20	6829632

Figure: 22 Linearity of response for Vitamin B_{12}

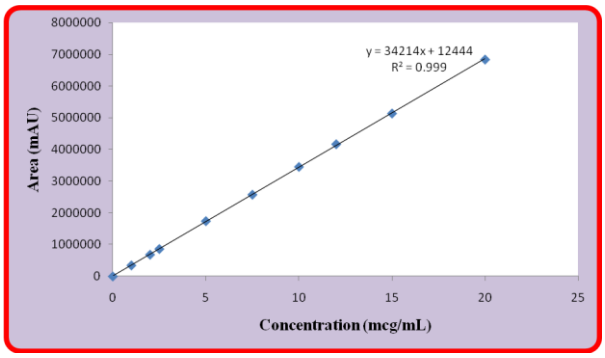


Figure: 23 Chromatogram for the study of linearity of Vitamin B_{12}(10% operating concentrations)

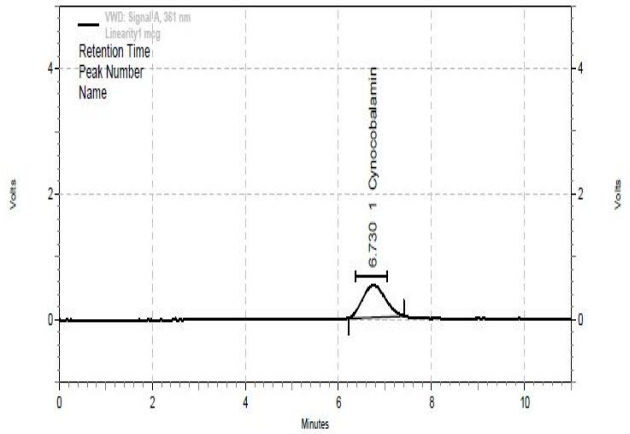
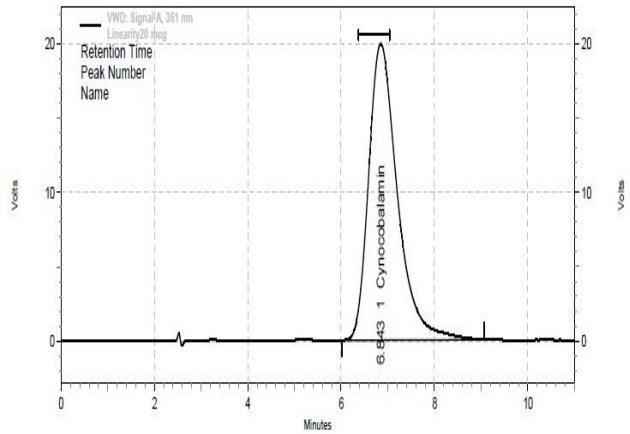


Figure: 24 Chromatogram for the study of linearity of Vitamin B_{12}(200% operating concentrations)



ACCURACY

Table: 29 Accuracy for Vitamin B_{12}

S.No	Concentration added (µg/mL)	Area	% Recovery
1	7	5738752	100.00
2	8	6505746	98.86
3	9	7389618	99.18
4	10	8513664	99.03
5	11	9281849	101.27
6	12	10305111	99.93
7	13	10978493	100.94
8	14	11670252	101.93

Figure: 25 Chromatogram for the study of accuracy of Vitamin B_{12}(100% recovery)

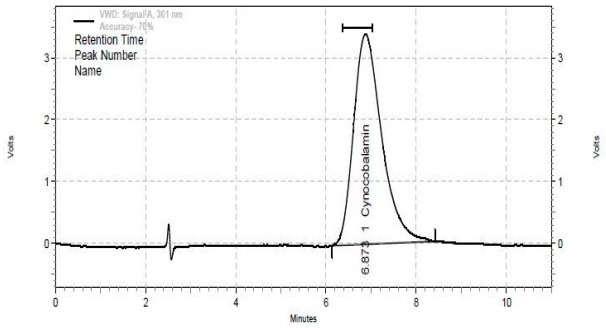
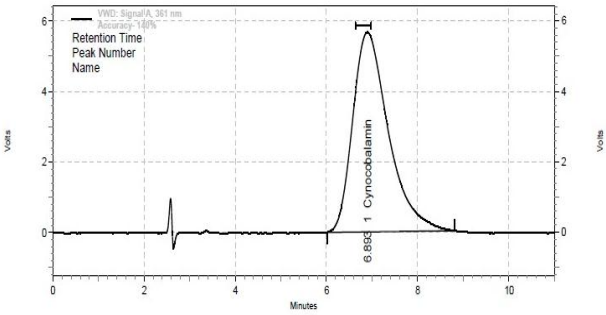


Figure: 26 Chromatogram for the study of accuracy of Vitamin B_{12}(200% recovery)



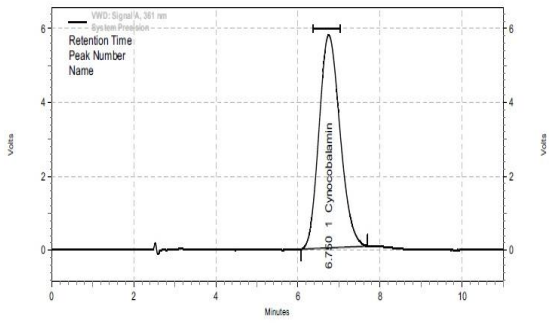
PRECISION

Reproducibility

Table 30: Precision - Reproducibility for Vitamin B_{12}

S.No	Sample	Area
1	Sample-1	3437555
2	Sample-2	3467728
3	Sample-3	3469258
4	Sample-4	3445447
5	Sample-5	3413547
6	Sample-6	3434737
Mean		**3444712**
Standard deviation		**21235.6**
RSD (%)		**0.62**

Figure: 27 Chromatogram for the study of reproducibility of Vitamin B_{12}

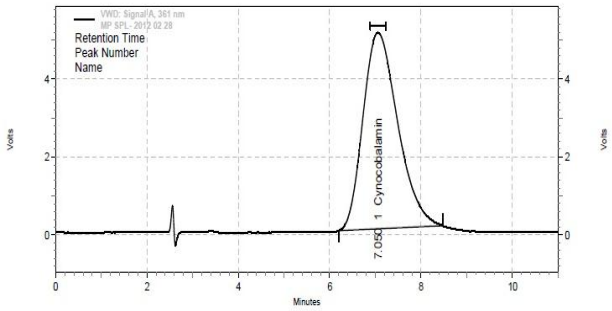


Repeatability

Table 31: Precision - Repeatability for Vitamin B_{12}

S.No	Sample	Area
1	Sample-1	4666015
2	Sample-2	4616369
3	Sample-3	4636558
4	Sample-4	4673274
5	Sample-5	4630055
6	Sample-6	4639325
Mean		**4643599**
Standard deviation		**21798.3**
RSD %		**0.47**

Figure: 28 Chromatogram for the study of repeatability of Vitamin B_{12}

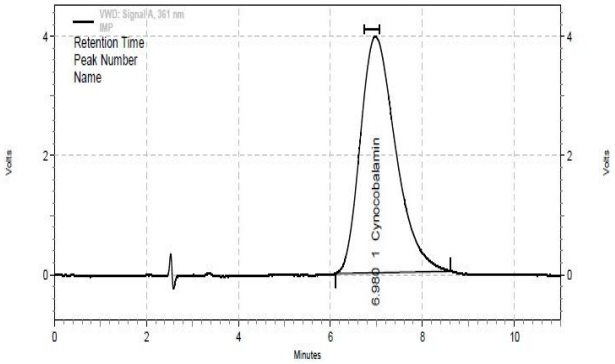


RUGGEDNESS

Table 32: Ruggedness data for Vitamin eB_{12}-Change of analyst

S.No	Sample name	Area
1	Sample-1	3550107
2	Sample-2	3596384
3	Sample-3	3560557
4	Sample-4	3556322
5	Sample-5	3534743
6	Sample-6	3566471
Mean		**3560764**
Standard deviation		**20551.4**
RSD (%)		**0.58**

Figure: 29 Chromatogram for the study of ruggedness of Vitamin B_{12}



ROBUSTNESS

Table 33: Robustness - Change in the ratio of solvents in the mobile phase (- 2%) for Vitamin B_{12}

S.No	Sample Name	Buffer:Aceto nitrile (85:15)
		Area
1.	Sample -1	6095632
2.	Sample -2	6052871
Mean		**6052871**
Standard deviation		**60473.89**
RSD %		**0.99**

Summary

A simple, efficient and reproducible Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for determination of Diclofenac sodium, Vitamin B_1,Vitamin B_6, and Vitamin B_{12} in soft gelatin capsule pharmaceutical formulation has been developed. The Diclofenac Sodium, the separation was carried out in Phenomenex ODS C_{18} column (150 x 4.6mm; 5µm) using mobile phase consisting of mixture of 400mL potassium hydrogen phosphate (pH 3.0) and 600mL of Acetonitrile. The flow rate was 1.0mL/min and effluent was detected at 254nm. The retention time of Diclofenac sodium was 5.4 min. The percentage recovery was within the range between 100.00% and 101.86% for Diclofenac Sodium. The linearity range was found to be 20-400µg/mL (r^2 =1) for Diclofenac Sodium. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. The Vitamin B_1 & Vitamin B_6, the separation was carried out in Inertial ODS C_{18} column (250 x 4.6mm; 5µm) using mobile phase consisting of mixture of 1000mL of Sodium 1-hexane sulfonic acid solution , 730mL of water , 270mL glacial acetic acid and 10ml of methanol The flow rate was 1.5mL/min and effluent was detected at 280nm. The retention time of Vitamin B_1 & Vitamin B_6 was 3.70 min & 7.74 min respectively. The percentage recovery was within the range between 99.46% and 101.03% & 99.35 and 101.78 respectively for Vitamin B_1 & Vitamin B_6. The linearity range was found to be 10-200µg/mL (r^2 =1) for Vitamin B_1 and for Vitamin $B_{6\ the}$ linearity range was found to be 10.5-210µg/mL. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. The Vitamin $B_{12,\ the}$ separation was carried out in Phenomenex ODS C_{18} column (150 x 4.6mm; 5µm) using mobile phase consisting of mixture of .25mL trifluroacetic acid, 870mL of water and 130mL of Acetonitrile. The flow rate was 1.2mL/min and effluent was detected at 361nm.The retention time of Vitamin B_{12} was 6.75 min. The percentage recovery was within the range between 98.86% and 101.94% for Vitamin B_{12} .The linearity range was

found to be 1-20µg/mL (r^2 =0.9999) for Vitamin B_{12}. The percentage relative standard deviation for accuracy and precision was found to be less than 2%.

Conclusion

The Proposed study describes new and simple RP-HPLC method for the estimation of Diclofenac Sodium, Vitamin B_1,Vitamin B_6 and Vitamin B_{12} in soft gelatin capsule dosage form. The method was validated as per ICH guidelines and found to be simple, sensitive, accurate and precise. Therefore the proposed method can be successfully used for the routine lab analysis of estimation of Diclofenac Sodium, Vitamin B_1, Vitamin B_6, and Vitamin B_{12} in soft gelatin capsule dosage form. Furthermore the test specificity proved that this method can be applied for estimation of all drug contents without any excipients interference. The obtained chromatograms showed good resolutions between the peaks which helps to quantify the drugs in well-defined manner.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.