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Analytical method development and validation of simultaneous estimation of dosulepin and methylcobalamin in tablet dosage form by RP-HPLC

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ABSTRACT

A sensitive, selective and precise high performance liquid chromatographic method has been developed and validated for the simultaneous determination of Dosulepin and Methylcobalamin in tablet dosage form. The method employed waters xterra C8 column (150 x 4.6 mm , 5 µm particle size) as the stationary phase while Acetonitrile, 0.1 M H3PO4 (70:30,v/v) was used as mobile phase. The Retention time of Dosulepin and Methylcobalamin were observed to be 1.89and 2.85 minutes, respectively. The flow rate was found to be 1ml/min and effluents were monitored at 222 nm. The linear regression analysis data for the calibration plots showed a good linear relationship for Dosulepin and Methylcobalamin over a concentration range of 150-450 µg/ml and 3-9 µg/ml respectively with correlation co-efficient of 0.999 for Dosulepin and 0.999 for methylcobalamin. The LOQ was found to be 9.23 and 9.57 µg/ml respectively for Dosulepin and Methylcobalamin. The method was validated as per ICH guideline and it was found to be accurate, precise and robust. Marketed formulation was analyzed successfully.

Keywords: Dosulepin (DOS), Methylcobalamin (MCA) or mecobalamin, HPLC, Validation.

INTRODUCTION

Dosulepin (IUPAC NAME "-3--N,N-dimethyl propan -1-amine") is a Tricyclic antidepressant (TCA). Dosulepin blocks the reuptake of serotonin and nor epinephrine in the brain, thereby increasing their levels. It is believed that this action is responsible for its mood-elevating effects. Dosulepin is relatively mild and is used for low-level anxiety, depression and similar disorders, as well as the treatment of chronic and ongoing pain disorders, particularly where insomnia and/or loss of appetite are present. The structure of dosulepin is shown in Fig. 1.

Dosulepin works by preventing serotonin and nor adrenaline from being reabsorbed back into the nerve cells in the brain. This helps prolong the mood lightening effect of any released noradrenaline and serotonin. In this way, dosulepin helps relieve depression 2. The dosage of Dosulepin in adults is 75 mg/day in divided doses or as a single dose at night, increasing to 150 mg/day 3. In certain circumstances, e.g. in hospital use or unresponsive patients, dosages up to 300 mg daily have been used[1-2].

Mecobalamin(Carbanide;cobalt(3+);[5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl)oxolan-3-yl] 1-[3-[(4Z,9Z,14Z)-2,13,18-tris(2-amino-2-oxoethyl)-7,12,17-tris(3-amino-3-oxopropyl)-3,5,8,8,13,15,18,19-octamethyl-2,7,12,17-tetrahydro-1H-corrin-21-id-3-yl]propanoylamino]propan-2-yl phosphate) is the neurologically active form of vitamin B12 and occurs as a water-soluble vitamin in the body, so it is a nutritional supplement. It is often given to people suffering from nervous disorder, because such conditions can be caused or aggravated by a deficiency in this vital nutrient. The liver does not convert cyanocobalamin, the commonly available form of vitamin B12, into adequate amounts of methylcobalamin, which the body uses to treat or correct neurological defects[3-4]. The structure of methylcobalamin is shown in Fig. 2.

MATERIALS AND METHODS

Instrumentation: The separation was carried out on HPLCsystem with Waters 2695 alliance with binary HPLC pump,Waters 2998 PDA detector, Waters Empower2 software and c8 xterra RP8 column (150mmx4.6mm, particle size5µm).

Chemicals: Prothiaden M (75 mg DOS and 1.5 mg MCA) manufactured by Abbott India Ltd. All chemicals and reagents used were of AR grade. Standard sample was taken from rainbow pharma training lab.

HPLC Conditions: The mobile phase consisting of orthophosphoric acid and acetonitrile(HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 70:30v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 222nm and the run time was 8min. The volume of injection loop was 5µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

Preparation of standard solution: Accurately weigh 300mg of dosulepin and 6mg of methylcobalamin into a 50ml of volumetric flask and dissolve the sample using water and sonicate it for 15min then finally make up the volume to 50ml.Now pipette out 1ml of this solution into 25ml of volumetric flask and make up the volume upto mark using mobile phase as shown in figure 3.

Preparation of sample solution: Accurately weighed 5 tablets and calculated average weight of those tablets and crushed. Transfer the tablet powder weigh about 1279.36mg of sample into 50ml of volumetric flask added with acetonitrile and water and sonicated for 30mins and make up the volume with water and filtered through the0.45µm millipore filter paper Transfer above solution 5ml into 25ml volumetric flask and make up the volume with mobile phase chromatogram is shown in figure 4.

METHOD VALIDATION

(a). System Suitability Studies: The column efficiency,resolution and peak asymmetry were calculated for the standard solutions The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method show in table 1.

(b). Specificity: Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both DOS and MCA from impurities.

(c). Accuracy: Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained in added recoveries of standard drugs were found to be accurate as shown in table 2(a) & 2(b).

(d). Precision: Method Precision was determined by injecting six replicates of drug sample solution. The retention times and peak areas of six replicates are recorded. The precision is expressed as the % RSD of Peak areas and it should not be more than 2% shown in table 3.

e). Linearity: Linearity of the method was determined by constructing calibration curves.Standard solutions of dosulepin and mecobalaminat different concentration level(50%,100%,125%, and 150%)were used for this purpose. Each measurement was carried out in six repicates to verify the reproducibility of the detector response at each concentration level.The peak areas of the chromatograms were plotted against the concentration of DOS and MCA to obtain the calibration curves.The five concentrations of the standrad were subjected to regression analysis to calculate equation and correlation coefficients as shown in Fig 5 & Fig 6.

(f). Limit of detection and limit of quantitation: Limit of detection and limit of quantitation represent the concentration of analyte that would yield signal to noise ratio of 3 for LOD and 10 for LOQ respectively. To determine LOQ and LOD serial dilutions of mixed standard solution of Dosulepin and Mecobalamin was made from standard solution. The samples were injected in the system and measured signal from the samples was compared with those of blank samples. LOD and LOQ was calculated from linear curve using formulae,

LOD= 3.3 * σ / slope, LOQ= 10 * σ / slope (Where σ = the standard deviation of the response and S = Slope of calibration curve) shown in table 4.

(g). Robustness: Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed are rugged and robust
shown in table 5(a) and 5(b).

RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig:3 and fig:4 those figures are Dosulepin and Methylcobalamin standard chromatogram and other one is formulation, they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method accuracy passed for both Dosulepin and Methylcobalamin evaluated by recovery studies and the percentage mean recovery was found to be 100.21% and 99.72% for dosulepin and methylcobalamin respectively. The method precision was passed for both the drugs given in table 3. Linearity calibration curve was given below fig: 5&6 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation coefficient.Linear correlation was found to be Y= 38829x+0 for Dosulepin and y = 44159x+0 for Methylcobalamin. LOD and LOQ values were 2.77µg/mL and 2.87 µg/mL for Dosulepin and 9.23µg/mL and 9.57µg/mL for Mecobalamin (Table-4). Method robustness results were given by table5 the result obtained implies method is robust for routine qualitative analysis.

CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of Dosulepin and Methylcobalamin using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific. Hence, this method can easily and conveniently adopt for routine quality control analysis of Dosulepin and Methylcobalamin in its pharmaceutical dosage forms.

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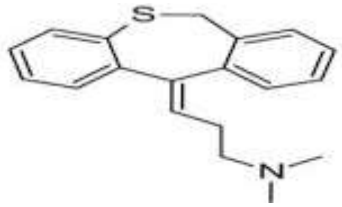

Fig. 1: Structure of Dosulepin

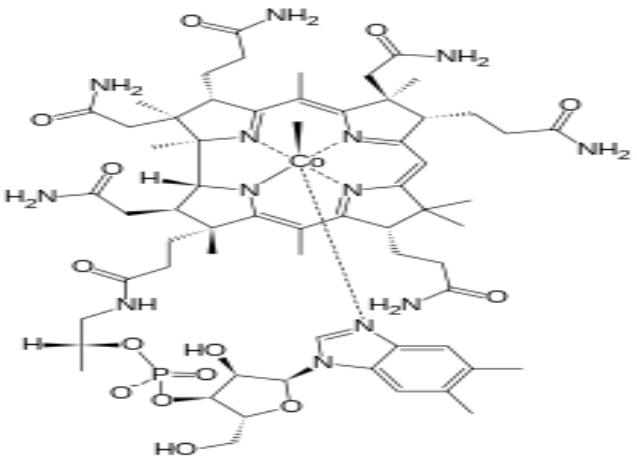

Fig. 2: Structure of Methylcobalamin

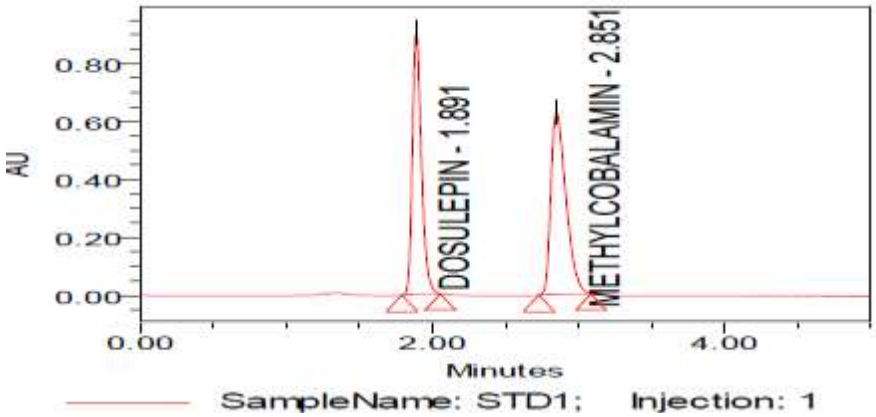

Fig. 3: Chromatogram of standard preparation

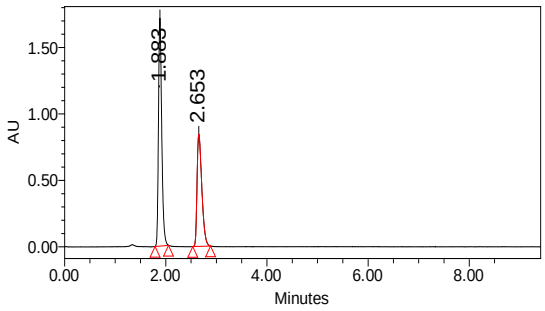

Fig. 4: Chromatogram of sample preparation

Component Summary Table
Name: DOSULEPIN

	SampleName	Inj	Name	RT	Area	USP Resolution	USP Tailing	USP Plate Count	s/n
1	STD1	1	DOSULEPIN	1.891	3914258		1.305	4576	325
Mean					3914258				
% RSD									

Component Summary Table
Name: METHYLCOBALAMIN

	SampleName	Inj	Name	RT	Area	USP Resolution	USP Tailing	USP Plate Count	s/n
1	STD1	1	METHYLCOBALAMIN	2.851	4513175	6.197	1.548	3552	6.27
Mean					4513175				
% RSD									

Parameter	Dosulepin	Methylcobalamin
Correlation Coefficient	0.999	0.999
Regression Equation	Y=38829X+0	Y=44159X+0
LOD	2.77	2.87
LOQ	9.23	9.57
Theoretical plates	4576	3552
Tailing	1.305	1.548

Table 1: System suitability parameters

ACCURACY OF DOSULEPINE						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	639.68	1948862	148.501	149.23	100.49	100.29
50%	639.68	1941133	148.501	148.63	100.09	
50%	639.68	1949927	148.501	149.31	100.54	
50%	639.68	1944840	148.501	148.92	100.28	
50%	639.68	1943827	148.501	148.84	100.23	
50%	639.68	1941026	148.501	148.63	100.08	
100%	1279.36	3887775	297.002	297.69	100.23	100.24
100%	1279.36	3888059	297.002	297.71	100.24	
100%	1279.36	3887192	297.002	297.65	100.22	
150%	1919.00	5820129	445.494	445.65	100.04	100.12
150%	1919.00	5826194	445.494	446.12	100.14	
150%	1919.00	5828611	445.494	446.30	100.18	
150%	1919.00	5822928	445.494	445.87	100.13	
150%	1919.00	5825696	445.494	446.08	100.16	
150%	1919.00	5827322	445.494	446.20	100	

Table 2(a): Recoveries of dosulepin drugs

ACCURACY OF METHYLCOBALAMIN				
Sample Area	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
2204470	3.000	2.99	99.60	99.64
2204572	3.000	2.99	99.60	
2200219	3.000	2.98	99.41	
2209917	3.000	3.00	99.84	
2208267	3.000	2.99	99.77	
2205079	3.000	2.99	99.62	
4411843.00	6.000	5.98	99.66	99.77
4418020.00	6.000	5.99	99.81	
4419829.00	6.000	5.99	99.84	
6627390	9.000	8.98	99.81	99.77
6628122	9.000	8.98	99.82	
6626994	9.000	8.98	99.80	
6623427	9.000	8.98	99.75	
6622014	9.000	8.98	99.73	
6620908	9.000	8.97	99.71	

Table 2(b): Recoveries of methylcobalamin drugs

S.No	**Sample Weight**	**Sample Area-1**	**Sample Area-2**	**% Assay**	**% Assay**
1	1279.36	3883090	4415132	99.11	99.74
2	1279.36	3888697	4419302	99.25	99.83
3	1279.36	3886731	4416680	99.20	99.77
4	1279.36	3880901	4419174	99.05	99.83
5	1279.36	3880007	4414889	99.03	99.73
6	1279.36	3885082	4414961	99.16	99.73
Average				99.13	99.77
STD				0.09	0.05
% RSD				0.09	0.05

Table-3: Precision studies of DOS & MCA

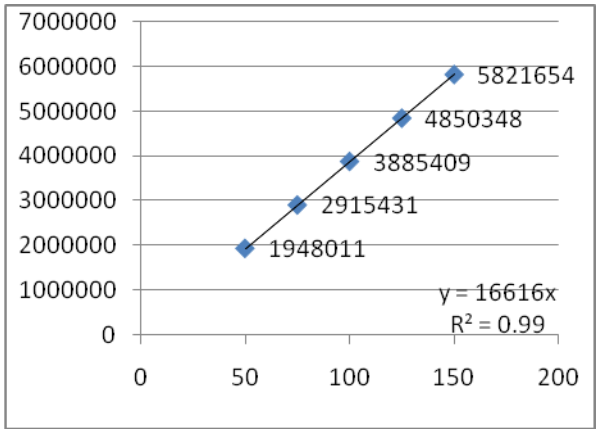

Fig 5: Linearity curve of dosulepin

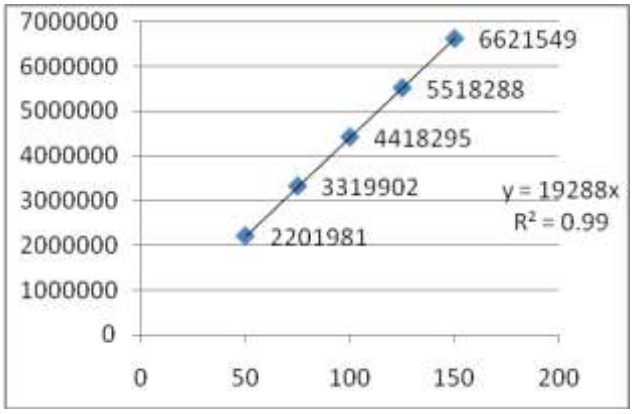

Fig 6: Linearity curve of mecobalamin

DRUG	LOD(**µg/ml**)	LOQ(**µg/ml**)
Dosulepin	2.77	9.23
Methylcobalamin	2.87	9.57

Table 4: LOD and LOQ of Dosulepin and Mecobalamin

	SampleName	Inj	Name	RT	Area	USP Resolution	USP Tailing	USP Plate Count
1	TEMP2	1	DOSULEPIN	1.867	3904831		1.366	4604
2	FLOW2	1	DOSULEPIN	1.562	3230432		1.232	4239
3	TEMP1	1	DOSULEPIN	1.872	3911066		1.358	4487
4	FLOW1	1	DOSULEPIN	2.335	4817098		1.328	5487

Table 5(a): Robustness of Dosulepin

	SampleName	Inj	Name	RT	Area	USP Resolution	USP Tailing	USP Plate Count
1	TEMP1	1	METHYLCOBALAMIN	2.521	4400640	4.560	1.572	3678
2	TEMP2	1	METHYLCOBALAMIN	2.528	4427439	4.825	1.567	4156
3	FLOW1	1	METHYLCOBALAMIN	3.154	5443143	5.088	1.722	4618
4	FLOW2	1	METHYLCOBALAMIN	2.151	3684827	4.927	1.420	3827

Table 5(b): Robustness of Methylcobalamin

REFERENCES

1. Indian Pharmacopoeia; Indian Pharmacopoeia commission, Ghaziabad, volume- II, p. 1250; 2010.
2. British Pharmacopoeia; British Pharmacopoeia Commission Office, London, volume- I, p. 758- 759; 2011.
3. Martindale- The Extra Pharmacopoeia; 31st Edn; The Royal Pharmaceutical Society, London, p. 310; 1996.
4. The Merck Index- An Encyclopedia of Chemicals, Drugs and Biological; 14th Edn; Merck Co. Inc., whitehouse, p. 3429; 2001.
5. The Merck Index- An Encyclopedia of Chemicals, Drugs and Biological; 14th Edn; Merck Co. Inc., Whitehouse, p. 6047; 2001.
6. Hardman JG., and Limbird LE. Goodman & Gilman's Manual of Pharmacology and Therapeutics; 10th Edn; McGraw-Hill, New Delhi, p. 1505-1510; 2001.
7. Tripathi KD. Essentials of Medical Pharmacology; 6th Edn; Jaypee Brothers Medical Publishers Ltd, New Delhi, chapter- 33, p. 441.
8. Instrumental method of chemical analysis B.K.Sharma Pg no 286-370
9. Gurdeep R. Chatwal, Sham K. Anand; Instrumental Methods of Chemical Analysis; 5th edition, Himalaya Publishing House; 2002.
10. ICH–Guidelines, Validation of Analytical Procedures: Methodology; ICH Harmonized Tripartite Guidelines, Q2(R1), Geneva, Switzerland, p. 5-17; 2005.
11. Journal on Liquid Chromatographic Estimation of Dosulepin HCl and Methylcobalamin in Pharmaceutical Formulation by Dhara B Desai, Dimal A Shah, Falgun A Mehta, Usmangani K Chhalotiya and Kashyap K Bhatt.