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DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF DULOXETINE AND MECOBALAMIN IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

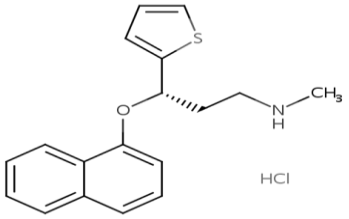
The main objective of this work is to develop a simple, fast, accurate, precise, rugged and linear Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for simultaneous estimation of Duloxetine and Mecobalamin in tablets and validate as per ICH guidelines. The optimized method uses a reverse phase column, Inertsil-ODS C_{18}(250 x 4.6 mm, 5µ) a mobile phase of potassium dihydrogen methanol: phosphate buffer (pH 4): in the proportion of 80:20v/v, flow rate of 1.0/min and a detection wavelength of 254 nm using a Photo diode array detector(2996). The developed method resulted in 2.956min for Duloxetine and 3.538 for Mecobalamin, having a run time of 10 minutes. Duloxetine and Mecobalamin exhibits linearity 50µg/ml-150µg/ml. % Relative standard deviations of system, intraday and ruggedness were found to be less than 2 for both the drugs. Percentage Mean recoveries were found to be in the range of 90-110, during accuracy studies by absolute method. A simple, fast, accurate, precise, linear and rugged RP-HPLC method was developed for simultaneous quantitative estimation of Duloxetine and Mecobalamin in capsules and validated as per ICH guidelines. Hence it can be used for the routine analysis of Duloxetine and Mecobalamin tablets in various pharmaceutical industries.

INTRODUCTION:

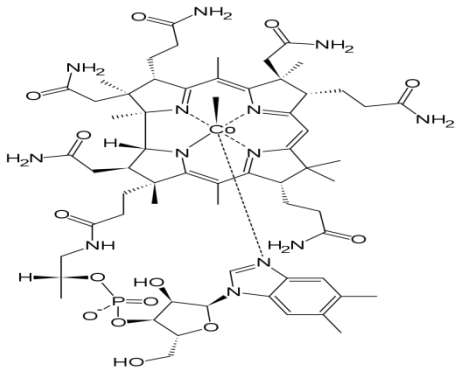
Analytical chemistry is a science deals with the identification, characterization and estimation of the compounds in the sample. The primary interest of an analytical chemist is to develop experimental methods of measurement to obtain information about the qualitative and quantitative tests for a given sample[1,2]. Before selecting the method the technical approach to solving the problem requires the analyst to consider the analytical information, required level of accuracy, cost, timing and availability of instruments and facilities[3].

High performance liquid chromatography (HPLC) is a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip a column under gravity, it is forced through under high pressure of up to 400 atmospheres[4,5]. That makes it much faster. All chromatographic separations, including HPLC operate under the same basic principle. Separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation[6].

Methods are developed for new products when no official methods are available[7]. Alternate methods for existing (non-pharmacopoeia) products are developed to reduce the cost and time for better precision and ruggedness. Trail runs are conducted, method is optimized and validated[8]. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use[9]. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice[10].



DULOXETINE



MECOBALAMIN

Materials and methods:

All chemicals and reagents used were of high quality, purity procured from various sources, phosphate buffer (as per IP), Methanol Merck (HPLC-Grade), Duloxetine and Mecobalamin Reputed pharmaceutical company, Duloxetine and Mecobalamin tablets containing 20/1.5mg, are Purchased from local market Waters - 2690/5, HPLC series with PDA, Inertsil -C18, BDS column, Detector wavelength 254nm, Colum Température is ambiant The Optimized chromatographic conditions are listed in Table No 1

Preparation of duloxetine standard solution: Weigh down 10mg's of Duloxetine and dissolved in 10ml of Mobile phase taken in 10ml of volumetric flasks and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from above solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase.

Preparation of Mecobalamin standard solution: Weigh down 10mg is of Mecobalamin and dissolved in 10ml of Mobile phase taken in 10ml of volumetric flask separately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from above solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase.

Preparation of potassium dihydrogen phosphate buffer:

Solution 1: Weigh accurately 13.61 gm of potassium dihydrogen phosphate dissolved in 1000 ml of HPLC grade water, then the pH is adjusted to 5.5.the solution sonicated for 10 min and filter through o.45µm membrane filter.

Solution 2: Weigh accurately 35.81 gm of di sodium hydrogen phosphate dissolved in 1000 ml of HPLC grade water.

Mix 96.4mL of solution 1 and 3.6 mL of solution 2

Validation of the Method

The method was validated in terms of system precision, linearity, precision, and specificity of the sample applications. The linearity of the method was investigated with correlation coefficient of Duloxetine and Mecobalamin was found to be 0.999 Precision was found to be lower than 1%. Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions, Placebo interference Sample was prepared by taking the placebo equivalent to about the weight in portion of test preparation as per the test method and blank interference mobile phase was prepared and injected and into the HPLC system, are in Fig No: 1-3

Accuracy: Accuracy of the method was expressed in terms of recovery of added compound at 50%, 100% and 150% level of sample. Mean % recovery and % RSD were calculated and were summarized in Table 2-3.The result shown that best recoveries (99.77±0.04) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

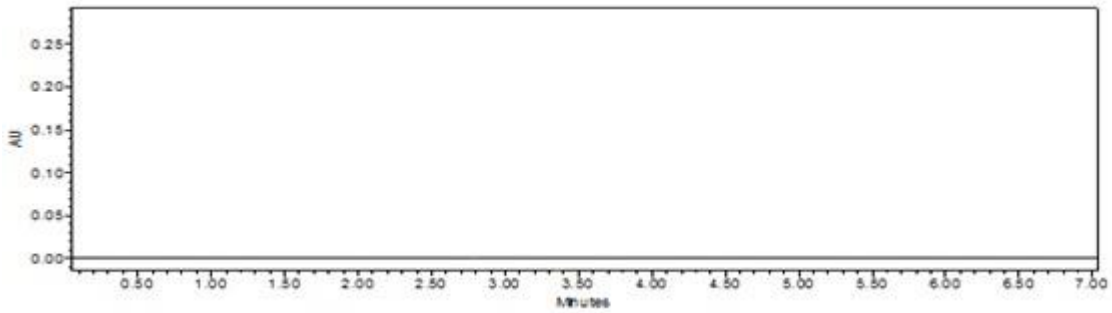


Fig No.1: Placebo chromatogram

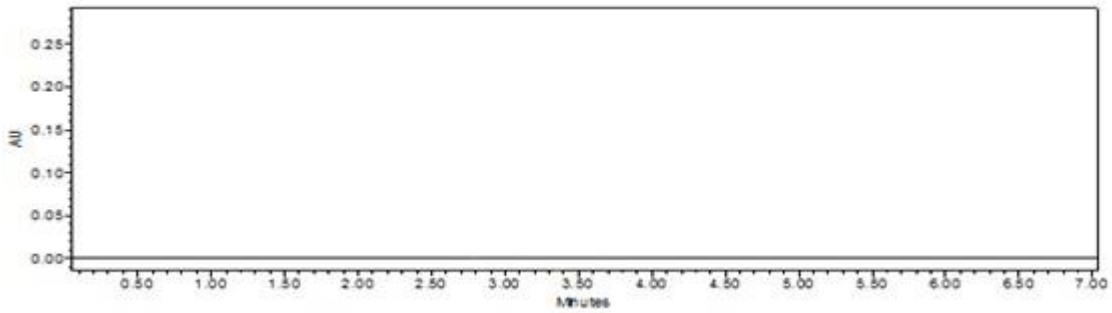


Fig No.2: Blank chromatogram

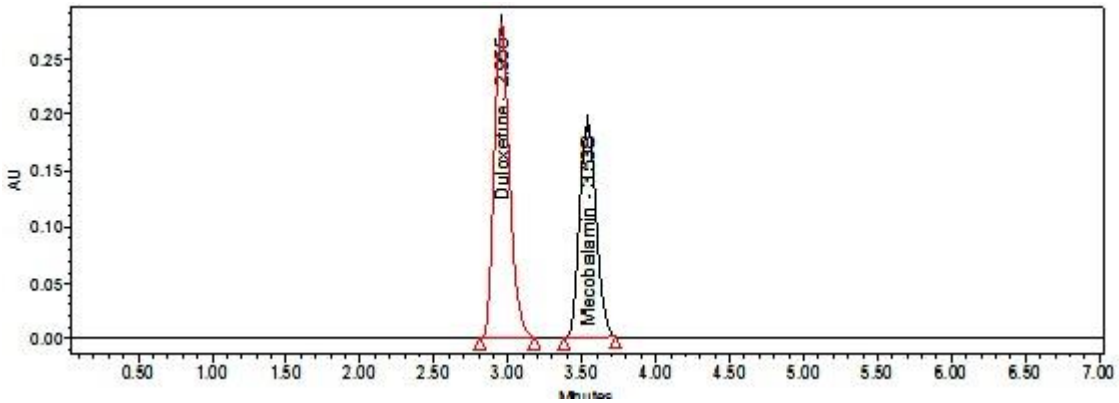


Fig No.3: chromatogram of Duloxetine and Mecobalamin

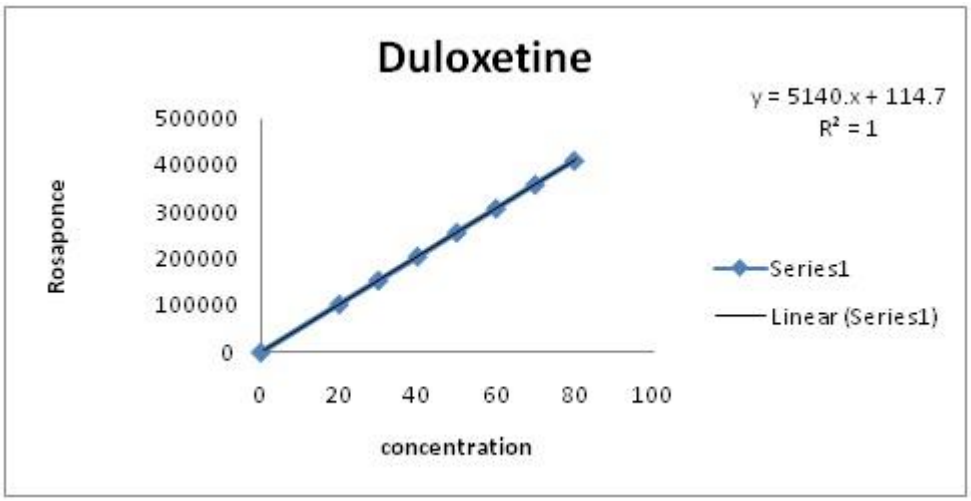


Fig No 4: Duloxetine Calibration curve

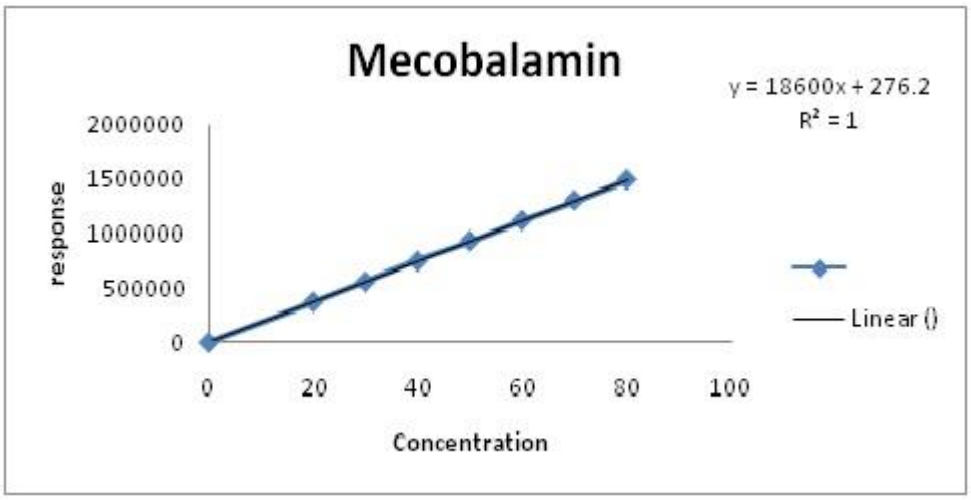


Fig No 5: Mecobalamin Calibration curve

Table No.1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil-ODS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : potassium dihydrogen phosphate (pH 4) (80:20)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	254nm
Drug RT (min)	2.956min for DT and 3.538 for MB.

Table No.2: Data of Accuracy for Duloxetine

Concentration % of spiked level	Amount added (ppm)	Peak area	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
					MEAN	%RSD
50% Injection 1	20	11984	20.15	100.75	99.69333	0.92
50% Injection 2	20	11941	19.86	99.31		
50% Injection 3	20	11909	19.80	99.02		
100 %Injection 1	40	12961	39.88	99.70	99.83333	0.41
100 % Injection 2	40	12974	40.12	100.30		
100% Injection 3	40	12952	39.80	99.50		
150% Injection 1	60	13103	60.12	100.21	99.97333	0.17
150% Injection 2	60	13679	59.76	99.61		
150% Injection 3	60	13081	60.06	100.10		

Table No.3: Data of Accuracy for Mecobalamin

Concentration % of spiked level	Amount added (ppm)	Peak area	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
					MEAN	%RSD
50% Injection 1	20	63381	20.04	100.22	100.06	0.18
50% Injection 2	20	63159	19.97	99.85		
50% Injection 3	20	63317	20.02	100.11		
100 % Injection 1	40	69459	40.01	100.02	100.04	0.091
100 % Injection 2	40	69526	40.05	100.14		
100% Injection 3	40	69405	39.98	99.96		
150% Injection 1	60	91811	60.08	100.14	100.02	0.09
150% Injection 2	60	912614	59.97	99.96		
150% Injection 3	60	91719	59.98	99.98		

Table 4: Data of Repeatability (System precision) for Duloxetine and Mecobalamin

	Injection	Peak Areas of Duloxetine		Peak Areas of mecobalamin	
		Peak area	%Assay	Peak area	%Assay
Concentration 100ppm	1	1218805	99.95	674753	98.66
	2	1214014	100.24	674261	99.30
	3	1215474	100.06	675298	101.53
	4	1227655	99.30	679221	100.53
	5	1267019	100.00	688636	99.98
Statistical Analysis	Mean	1228593	99.91	678433.8	100.00
	SD	22124.07	0.35819	6031.135	1.107678
	% RSD	1.800764	0.35	0.888979	1.10

Table.No:5 Data of Repeatability (Method precision) for Duloxetine and Mecobalamin

	Injection	Peak Areas of Duloxetine		Peak Areas of mecobalamin	
		Peak area	%Assay	Peak area	%Assay
Concentration 100ppm	1	1202110	98.6	633495	98.55
	2	1203700	99.02	635992	98.88
	3	1201851	98.12	639828	99.40
	4	1202255	98.31	639098	99.30
	5	1203283	98.81	648289	100.53
	6	1202349	98.36	631322	98.28
Statistical Analysis	Mean	1202687.6	98.48	637312	99.278
	SD	771.5483	0.352647	5988.879	0.827236
	% RSD	0.1358	0.35	0.0891	0.83

Table 6 Data of Intermediate precision (Analyst 1&II) for Duloxetine and Mecobalamin

	Injection	Peak Areas of Duloxetine		Peak Areas of mecobalamin	
		Peak area	%Assay	Peak area	%Assay
Concentration 100ppm	1	1202110	98.6	633495	98.55
	2	1203700	99.02	635992	98.88
	3	1201851	98.12	639828	99.40
	4	1202255	98.31	639098	99.30
	5	1203283	98.81	648289	100.53
	6	1202349	98.36	631322	98.28
Statistical Analysis	Mean	1206333.5	100.19	631322	98.28
	SD	771.5483	0.352647	5988.879	0.827236
	% RSD	0.1358	0.35	0.0891	0.84

Table No.7: Data of Linearity (Duloxetine and Mecobalamin)

Concentration (ppm)	Average area of duloxetine	Average area of mecobalamin
0	0	0
20	1202965	632546
30	1254371	658296
40	1295856	694400
50	1297167	730308
60	1308577	916282
70	1359903	9402046
80	139905	9788277
Slope	5140	18600
Y-intercept	114.7	276.2
Correlation coefficient	1	1

Table No.8: Data of system to system variability (Duloxetine and Mecobalamin)

S. no	Duloxetine		Mecobalamin	
	Peak area	Assay %	Peak area	Assay %
1	1203625	99.98	634360	98.65
2	1202225	99.30	634098	98.63
3	1202840	98.60	635696	98.86
4	1204283	99.30	633289	98.52
5	1202735	98.55	634147	98.63
6	1203110	98.73	633495	98.55
Mean	1203136.3	99.07667	634180.8	98.64
%RSD	1.35	0.56	0.019	0.12

Table No. 9: Data for Effect of variation in flow rate (Duloxetine)

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	1273707	1.362089		1206349	1.280574		1266195	1.285372
	1273211	1.352617		1205267	1.279932		1265885	1.299385
	1273948	1.376926		1205625	1.261721		1266303	1.308063
	1273465	1.345752		1205840	1.276089		1267243	1.274662
	1273862	1.374925		1205735	1.250640		1265762	1.267630
Avg	1273638.6	1.362462	**Avg**	1205763.2	1.269791	**Avg**	166277.6	1.287022
SD	3301.369	0.013609	**SD**	392.1635	0.01314	**SD**	582.9758	0.016786
%RSD	1.041	0.99	**%RSD**	0.19	1.03	**%RSD**	0.35	1.3

Table No.10: Data for Effect of variation in flow rate (Mecobalamin)

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	620286	1.322089		634322	1.604878		602077	1.285372
	619282	1.331920		635792	1.584354		601854	1.319385
	621337	1.296438		634360	1.543805		602403	1.292055
	620456	1.315454		635696	1.568590		603421	1.304561
	620765	1.326551		633147	1.559986		602465	1.294621
Avg	620425	1.31849	**Avg**	634663.4	1.572323	**Avg**	602444	1.299199
SD	754.0018	0.013728	**SD**	1100.917	0.023367	**SD**	599.8833	0.013223
%RSD	0.086	1.04	**%RSD**	0.184	1.48	**%RSD**	0.09	1.01

Repeatability

The % Relative standard deviations of Duloxetine and Mecobalamin for Repeatability was found to be 0.35 and 1.10 .Hence the %RSD values indicate a good degree of precision within the specified range. The results are tabulated in Table No 4

Method precision

Precision of the assay method was determined by injecting, six (6) individual samples, in duplicate, of Duloxetine and Mecobalamin.The results are tabulated in Table No 5.

Intermediate precession:

The % Relative standard deviations of Duloxetine and Mecobalamin for Intermediate precession was found to be 0.35 and 0.83.Hence the %RSD values indicate a good degree of precision within the specified range. The results are tabulated in Table No 6.

System Precision Ruggedness

The standard and sample solutions prepared by analyst-1 and analyst-2 are injected in different HPLC systems, on different day, using a different column. The system suitability parameters calculated by analyst -2 can be compared with those of Analyst -1. The results were tabulated in Table 8. These results indicated that the developed method is rugged.

Linearity

The linearity range of Duloxetine and Mecobalamin was evaluated by varying concentrations of standard solutions were injected into HPLC system. The linearity graph was plotted from (Fig:4-5). A calibration curve was constructed for each sample by plotting the peak area obtained the concentration. The correlation coefficient for the data was calculated as 0.999. The regression line were observed to be in the form of y = 5140x – 114.7. The linearity data for Duloxetine. The regression line were observed to be in the form of y = 18600x – 276.2 and Mecobalamin are presented in Table 7.

Robustness

Small changes in flow rate, composition of mobile phase and temperature, performed the robustness of method. Robustness was studied using three replicates of concentration level at 100%. The % RSD in robustness study was less than 2%, his indicates that the method is precise, accurate and robust, the results are tabulated in 9-10.

CONCLUSION

The present proposed RP-HPLC method for the assay of Duloxetine and Mecobalamin in tablet formulation was validated as per ICH Q2(R1) guideline and it meets to specific acceptance criteria. It is concluded that the developed method was specific, precise, linear, accurate, robust, cost effective and it proves all validation characteristics and it can be effectively applied for routine analysis in research institutions, quality control department in industries.

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