ISSN: 2306-6091

International Journal of Pharmaceuticals and Health care Research (IJPHR)

IJPHR |Vol.13 | Issue 2 | Apr - Jun -2025

www.ijphr.com

DOI : https://doi.org/10.61096/ijphr.v13.iss2.2025.210-218

Research

A rp-hplc method developemnet for the simultaneous analysis of duloxetine hcl and methylcobalamine in pharmaceutical dosages forms

Kommu China Venkanna[1*], Ryali Durga Praveen[2], Sanaboina Venkata Abhiram[2], Yerra. Manga Devi[2], Gotikala. Sailu[2], Vegiraju Sri Saranya[2]

[1]*Associate Professor, Department of Pharmaceutical Analysis, Lydia College of Pharmacy, Ravulapalem, East Godavari, Andhra Pradesh-533238.*
[2]*Department of Pharmaceutical Analysis, Lydia College of Pharmacy, Ravulapalem, East Godavari, Andhra Pradesh-533238.*

*Author for Correspondence: Kommu China Venkanna
Email: kommu.chinavenkanna@gmail.com

Check for updates

Published on:19 Feb 2025

Published by:
DrSriram Publications

2025| All rights reserved.

Creative Commons Attribution 4.0 International License.

Abstract:

A simple, accurate and precise method for simultaneous estimation of Methylcobalamin and Duloxetine by RP-HPLC method has been developed. The chromatographic conditions were successfully developed by using inertsil ODS C18 5µm (4.6*250mm) column, the flow rate was 1ml/min, the mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The retention times were found to be 3.594 min and 5.328 min. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The suggested RP-HPLC method can be used for routine analysis of Methylcobalamin and Duloxetine in API and Pharmaceutical dosage form.

Keywords: Duloxetine, Methylcobalamin, RP-HPLC, Phosphate buffer, orthophosphoric acid.

INTRODUCTION

Analytical chemistry is a branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative analysis or detection of compounds and quantitative analysis of the compounds. A qualitative method yields information about the identity of atomic or molecular species or functional groups in the sample. A quantitative method, in contrast provides numerical information as to the relative amount of one or more of these components.

Classification Of Analytical Methods

Analytical methods are classified into two categories; they are classical methods and instrumental methods.

Classical methods

Analysis of substances was carried out by separating the components of interest in a sample by precipitation, extraction or distillation. For qualitative analysis, the separated components were then treated with reagents that yielded products that could be recognized by their colors, their boiling or melting points, their solubilities in a series of solvents, their optical activities, their odors or their refractive indexes. For quantitative analysis, the amount of analyte was determined by gravimetric or by volumetric measurements. In gravimetric measurements, the mass of the analyte or some compound produced from the analyte was determined. In volumetric measurements, also called titrimetric analysis, the volume or mass of a standard reagent required to react completely with the analyte is measured.

Instrumental methods

Measurement of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, fluorescence, mass-to-ratio began to be used for quantitative analysis of various inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative and quantitative determination. These newer methods for separating and determining chemical species are collectively known as instrumental methods of analysis. Most of the instrumental methods fit into one of the following three categories viz.., spectroscopy, electrochemistry and chromatography.

METHOD DEVELOPMENT

Method development for simultaneous estimation of Duloxetine Hcl and Methylcobalamine in Pharmaceutical dosage forms includes the following steps:

1. Selection of Detection wavelength

10 mg of Duloxetine Hcl and Methylcobalamine was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Duloxetine Hcl and Methylcobalamine. The isobestic point was taken as detection wavelength. The overlay spectrums are shown in Figs. 4.1, 4.2, & 4.3.

2. Selection of column

Column is selected based on solubility, polarity and chemical differences among Analytes [Column: Inertsil C18 (4.6 x 250mm, 5µm, Make: Waters)]

3. Selection of mobile phase

Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) has been selected as mobile phase. Buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved.

4. Selection of flow rate

Flow rate selected was 1ml/min

Flow rate is selected based on

1. Retention time
2. Column back pressure
3. Peak symmetry
4. Separation of impurities

5. Preparations and procedures

Preparation of Phosphate buffer :(PH: 4.6)

Weighed 6.8 grams of KH_2PO_4 was taken into a1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

Preparation of mobile phase

A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of ACN (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

Diluant Preparation

Mobile phase is used as Diluant.

Preparation of the individual Duloxetine Hcl standard preparation
10mg of Duloxetine Hcl working standard was accurately weighed and transferred into a10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of the individual Methylcobalamine standard preparation
10mg of Methylcobalamine working standard was accurately weighed and transferred into a
10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of Sample Solution (Tablet)
Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Methylcobalamine and Duloxetine Hcl (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluant.

Procedure
20µL of the standard, sample are injected into the chromatographic system and the areas for Methylcobalamine and Duloxetine Hcl peaks are measured and the %Assay are calculated by using the formulae.

System Suitability
Tailing factor for the peaks due to Methylcobalamine and Duloxetine Hcl in Standard solution should not be more than 2.0. Theoretical plates for the Methylcobalamine and Duloxetine Hcl peaks in Standard solution should not be less than 2000.

Assay calculation

$$Assay\,\% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where,
P = Percentage purity of working standard
Lc = LABEL CLAIM OF drug in mg/ml.

RESULTS AND DISCUSSION

Wavelength Detection
The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Duloxetine and Methylcobalamine. Was obtained and the isobestic point of Duloxetine and Methylcobalamine showed absorbance's maxima at 260 nm. The spectrums are shown in Fig 1-3.

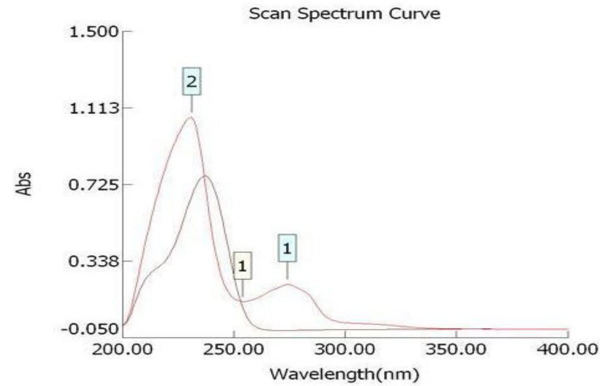


Fig 1: Overlay spectrum of Duloxetine and Methylcobalamine

The UV spectra of individual drugs are as follows:

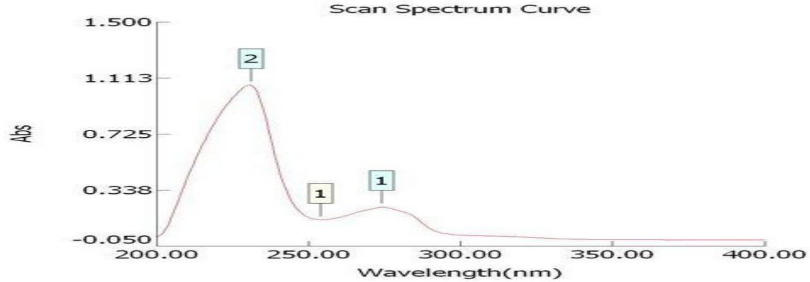


Fig 2: Spectrum of Duloxetine

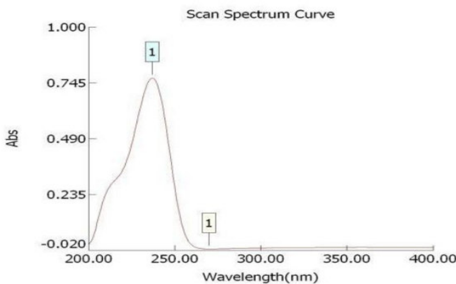


Fig 3: Spectrum of Methylcobalamine

METHOD DEVELOPMENT

The chromatographic method development for the simultaneous estimation of Duloxetine and Methylcobalamine were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the optimized chromatographic method was selected for the separation and quantification of Duloxetine and Methylcobalamine in API and pharmaceutical dosage form by RP-HPLC method.

Trial-1:

Chromatographic conditions.

Column : Agilent C18 (4.6*150mm) 5µm
Mobile phase rati : Water: Methanol (40:60%v/v)
Detection wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 10µl Column
temperature : Ambient
Auto sampler temperature : Ambient

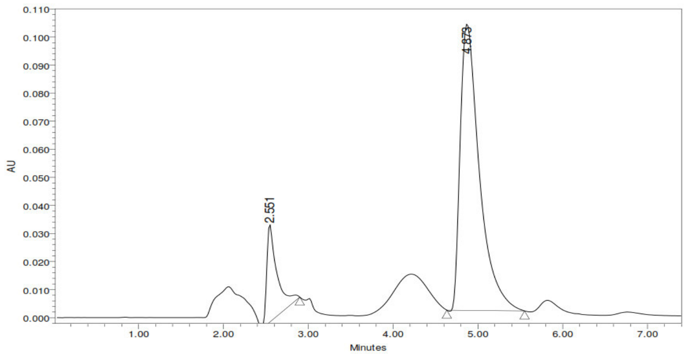


Fig 4: Chromatogram of Trial-1

Table 1: Details of Trial-1

S.No	Peak name	R_t	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Methylcobalamine	2.551	8671924	460798	745	2.19	
2	Duloxetine amineme	4.879	2283694	179357	1911	2.79	1.45

Methylcobalamine and Duloxetine were separated and two individual peaks are displayed. But they are not clear.

Trial-2:

Chromatographic conditions:

Column : Thermosil C18 (4.6*150mm) 5µm
Mobile phase ratio : Water: Methanol (40:60%v/v)
Detection wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 10µl Column
temperature : 40°
Auto sampler temperature : Ambient

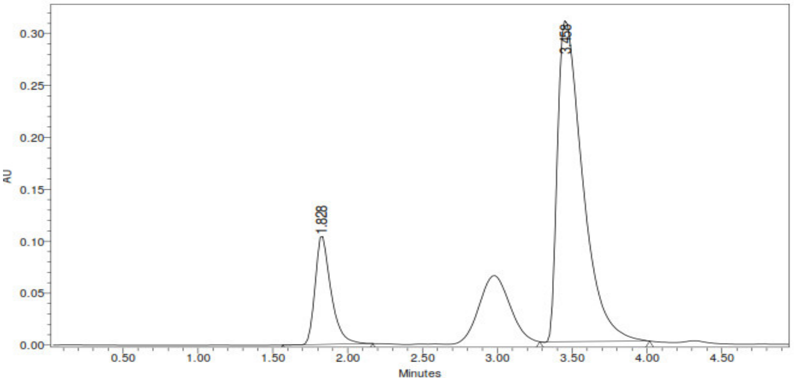


Fig 5: Chromatogram of Trial-2

Table 2: Details of Trial-2

S.No	Peak name	R_t	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Methylcobalamine	1.828	7913799	394185	722	2.21	
2	Duloxetine amineme	3.458	1853381	162758	2614	2.85	1.52

Peaks symmetry is being improved when compared to the previous trial. Further trials are conducted for better resolution.

Trial-3

Chromatographic conditions

Column : Agilent C18 5µm (4.6*250mm)
Mobile phase ratio : Phosphate buffer (0.05m) pH 5.0: Methanol(50:50%v/v)
Detection wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 10µl

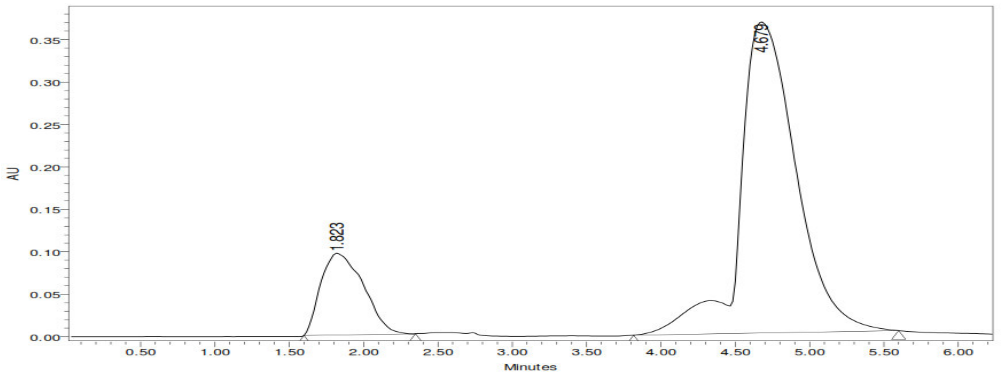


Fig 6: Chromatogram of Trial-3

Table 3: Details of Trail-3

S.No	Peak name	R_t	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Methylcobalamine	1.823	9849287	482363	198	1.97	
2	Duloxetine amineme	4.679	3272312	356630	5036	1.15	4.23

There is noticeable improvement in resolution. But peak symmetry is not achieved.

Trial-4

Chromatographic conditions:

Column : Inertsil ODS C18 5µm (4.6*250mm)
Mobile phase ratio : Phosphate buffer (0.05M) pH 4.6: MeOH
Detection wavelength : 255nm
Flow rate : 1ml/min Injection
volume : 20µl
Auto sampler temperature : Ambient

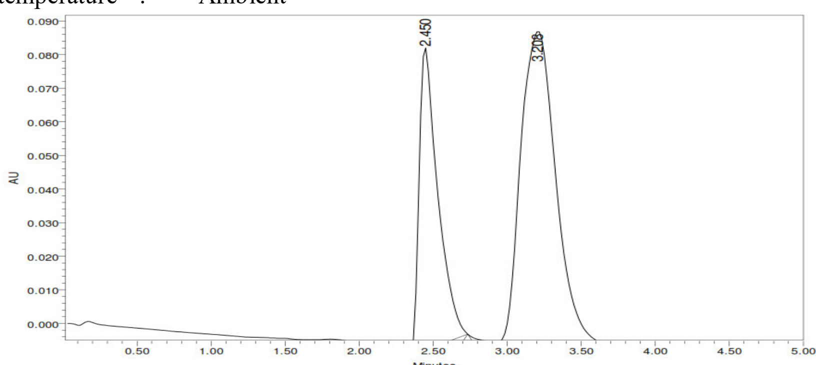


Fig 7: Chromatogram of Trial-4

Table 4: Details of Trial-4

S.No	Peak name	R_t	Area	Height	Plate count	Tailing	Resolution
1	Methylcobalamine	3.191	11286305	813690	1587	1.46	
2	Duloxetine amineme	3.945	3443649	160557	616	1.80	1.46

The tailing factor is within the limit. But the other parameters are not within the limit.

Trial-5

Chromatographic conditions

Column : Inertsil C18 5µm (4.6*250mm)
Mobile phase ratio : hosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) Detection
wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 20µl
Column temperature : Ambient

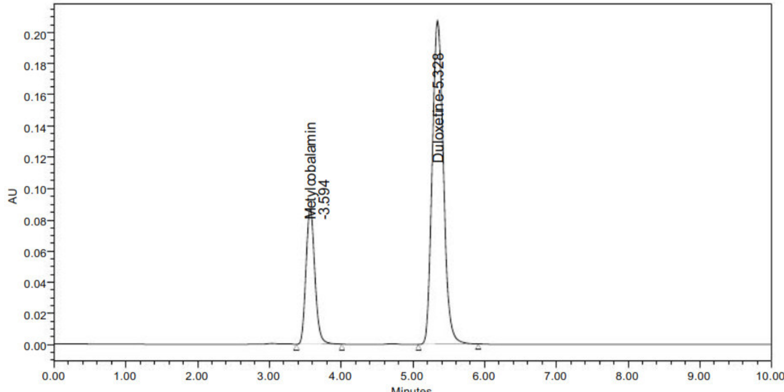


Fig 8: Chromatogram of Trail-5

Table 5: Details of Trail-5

	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Metylcobalamin	3.561	752347	87454	25.05		1.10	4087
2	Duloxetine	5.341	2255474	208319	74.95	6.75	1.11	5661

The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

ASSAY CALCULATIONS FOR DULOXETINE AND METHYLCOBALAMINE

The assay study was performed for the Duloxetine and Methylcobalamine. Each three injections of sample and standard was inject into chromatographic system.

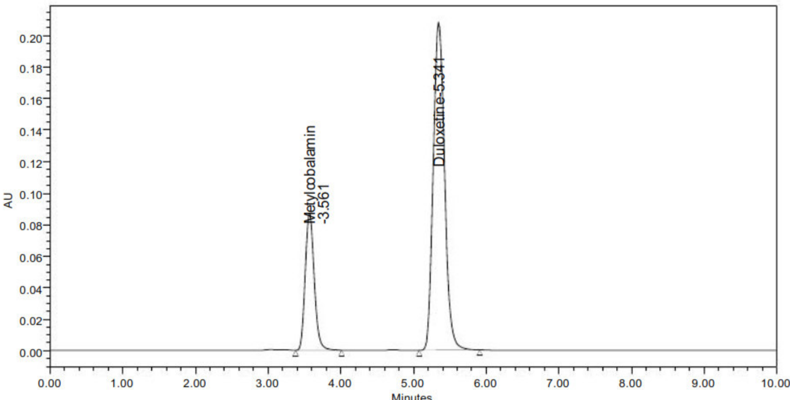
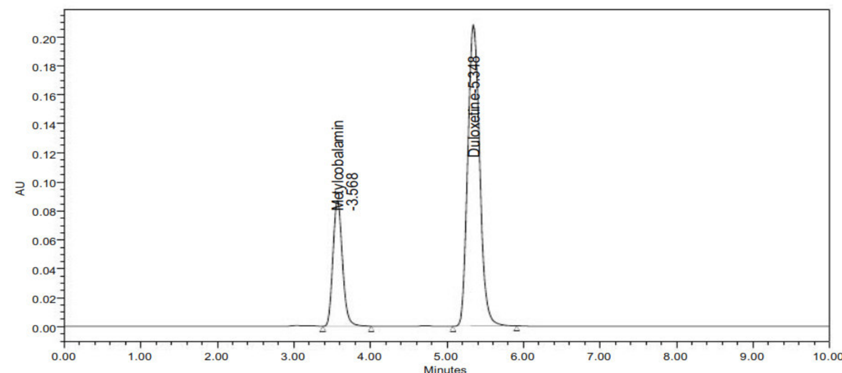


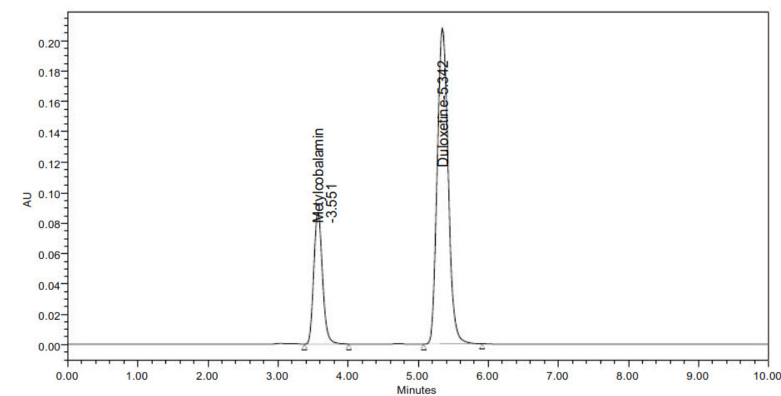
Fig 9: Chromatogram showing sample injection-1

S.No	Peak name	R_t	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Methylcobalamine	3.594	946124	155429	5105	1.3	8.1
2	Duloxetine Methylcobalamine	5.328	111541	13239	3788	1.4	



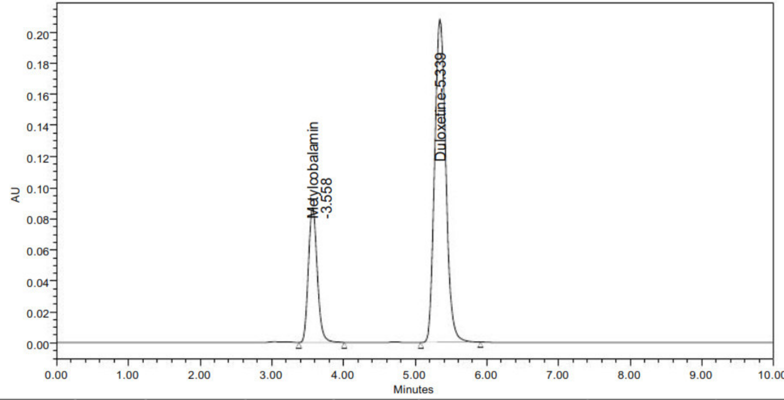
	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Metylcobalamin	3.568	752367	87488	25.02		1.12	4065
2	Duloxetine	5.348	2255489	208299	74.98	6.48	1.14	5698

Fig 10: Chromatogram showing sample injection-2



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Metylcobalamin	3.551	752354	87424	25.02		1.13	4069
2	Duloxetine	5.342	2255264	208299	74.98	6.67	1.12	5647

Fig 11: Chromatogram showing standard injection-1



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Metylcobalamin	3.558	752297	87468	25.07		1.11	4019
2	Duloxetine	5.339	2255315	208328	74.93	6.87	1.13	5663

Fig 12: Chromatogram showing standard injection-2

Calculations

Methylcobalamine

Wt of 10 tablets : 668 g

Avgas wt. : 0.668 g

$$Assay\,\% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

$$\frac{776673.9 \times 10 \times 0.5 \times 100 \times 10 \times 99.8 \times 0.668 \times 100}{771716.1 \times 10 \times 10 \times 458 \times 0.33 \times 100 \times 500}$$

= 101.4

Duloxetine	
Wt of 10 tablets	458 g.
Avgas wt	:0.458 g.

Assay% =100.7%

Tailing factor Obtained from the standard injection of Duloxetine and Methylcobalamine are 1.13 & 1.11. Theoretical Plates Obtained from the standard injection of Duloxetine and Methylcobalamine are 5663 & 4019. The system suitability parameters for Duloxetine and Methylcobalamine such as theoretical plates and tailing factor were found to be 5663, 1.13 and 4019, 1.11. Resolution was 6.8. The % purity of Duloxetine and Methylcobalaminein pharmaceutical dosage form was found to be 100.7% and 101.4% respectively.

CONCLUSION

A new method was established for simultaneous estimation of Methylcobalamine and Duloxetine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Methylcobalamine and Duloxetine by using inertsil ODS C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 3.594 mins and 5.328 mins. The % purity of Methylcobalamine and Duloxetine was found to be 100.7% and 101.4% respectively. The system suitability parameters for Methylcobalamine and Duloxetine such as theoretical plates and tailing factor were found to be 1.13, 4019 and 1.12, 5533 the resolution was found to be 6.8.

BIBILOGRAPHY

1. G.R.Chatwal, S.K.Anand. Text book of Instrumental Methods of Chemicaln Analysis, Himalaya Publishing House,5th Ed, 2002, p.2.566-2.570.
2. G.W.Ewing, Text book of Instrumental Methods of Chemical Analysis, Mc Graw-Hill Book Company, 5th ed, p.375-385.
3. B.K. Sharma, T e x t b o o k o f Instrumental Methods of Chemical Analysis, GOEL publishing house, Meerut, 23rd ed, p.288-289.
4. G.Vidyasagar, Textbook of Instrumental Methods of Drug Analysis, Pharmamed Press, 2009, p.106-120.
5. H. H Willard, L.L Merritt, J.A Dean, F.A. Settle, Textbook of Instrumental Methods of Analysis, CBS publishers and distributors, New Delhi, 7th Ed,1986, p.592-596.
6. H.H.Tackett, J.A.Cripe, G.Dyson, Positive displacement reciprocating pump fundamentals-power and direct acting types, Proceedings of the twenty-fourth international pump user's symposium,2008, p.45-58.
7. D.A.Skoog, F.J.Holler, S.R.Crouch, Textbook of Instrumental Analysis,Brooks/Cole, Cengage Learning India Private Limited, 2007, p.900-906.
8. R. E. Schirmer, Textbook of Modern Methods of Pharmaceuticals, CRC press,2nd Ed, P.242-244.
9. LR.Snyder, JJ Kirkland, LG.Joseph, Practical HPLC Method Development, Wiley Inter Science, New York, 2nd Ed, 1997, p. 1-56.
10. Ranjith singh, HPLC Method Development and Validation- an Overview, J Pharm. Educ. Res.4 (2013) 26-33.
11. ICH: Q2B, Analytical Validation – Methodology (1996)