Research Article

Simultaneous Estimation of DL-Methionine and Pyridoxine Hydrochloride in Tablet Dosage Form by RP-HPLC

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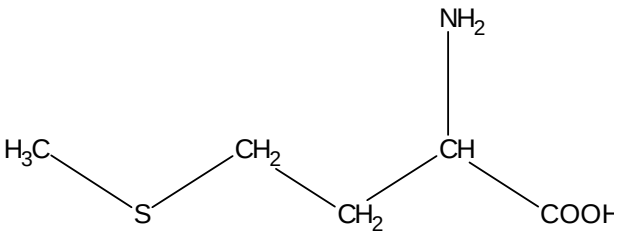
ABSTRACT
A simple, selective, rapid, precise and economical RP HPLC method has been developed for the determination of DL-Methionine and Pyridoxine Hydrochloride in tablet formulation. The analysis was resolved by using a mobile phase (Potassium dihydrogen orthophosphate) at a flow rate of 1ml/min on an isocratic consisting of Agilent 1200 HPLC system on variable wavelength UV detector using, Peerless basic C18 (4.6 mm x 15 cm, 5 µm) column at a wavelength of 210 nm. The retention time were found to be DL-Methionine (3.5min), Pyridoxine HCl (14 min). The percent recovery of DL-Methionine and Pyridoxine Hydrochloride were found to be in between 98% to 102%. The developed method was simple, precise, accurate and reproducible and therefore suitable for routine analysis of drugs in tablet dosage form.

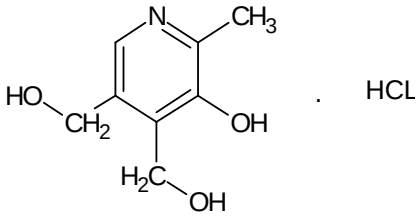
Keywords: Dl-Methionine, Pyridoxine Hydrochloride, Validation.

INTRODUCTION

DL-Methionine designed chemically as 2-amino-4-(methulthio)butanoic acid . It is used in amino deficiency . Pyridoxine Hydrochloride designed chemically as 5-hydroxy-6-methylpyridine-3,4-dimethanol hydrochloride. It is used in sideroblastic anaemia therapy. The objective of present study was to develop a simple, precise, accurate and economic HPLC analytical method with better detection range for the estimation of DL-Methionine and Pyridoxine Hydrochloride in bulk drugs. The developed method was validated as per ICH guidelines and suitable statistical tests were performed on validation data.



DL-Methionine



Pyridoxine HCl

EXPERIMENTAL

Apparatus

Instrument used in present study were Agilent 1200 and Shimadzu LC-2010. Liquid chromatographic system equipped with UV-Vis detector and data analysed by using Chromeleone 6.8 version software.

Materials

DL-Methionine and Pyridoxine hydrochloride working standard and marketed drugs formulation Gardian tablet were procured from Okasa Pharma Pvt. Ltd., Satara,(Maharashtra,India).

Water used was of HPLC grade. Potassium dihydrogen orthrophosphate was used in mobile phase and Potassium hydroxide was used for pH adjustment.

Chromatographic condition

Chromatographic separation was perfomed on Peerless basic C18, 4.6 mm X 15 cm, 5µm. column. The analysis was resolved by using a mobile phase (0.025 M potassium dihydrogen orthrophosphate ,adjusted pH to 7.0 potassium hydroxide) at a flow rate of 1.0 ml/min. The injection volume was 20 µl and ambient at temperature. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated. Analysis was perfomed at ambient temperature. Detection was carried out a 205 nm. Retention time were found to be for DL-Methionine (3.5 mins), Pyridoxine hydrochloride(14 mins) within run time of 25 mins.

Preparation of solutions

Diluent: Purified water

Standard preparations

Weighed accurately and transfered about 50 mg of DL-Methionine working standard and 25 mg of Pyridoxine Hydrochloride working standard into a 250 ml volumetric flask. Added 180 ml of diluent. Heated on water bath, which is maintained at 100 0C, for 10 minutes. Sonicated to dissolve, cool and diluted upto the volume with diluent. Futher diluted 5 ml to 50 ml with diluents.

Sample preparation

Weighed accurately and transfered 0.80 gm of sample into a 250 ml volumetric flask. Added sufficient amount of purified water. Heated on water bath which is maintained at 100 0C for 10 minutes. Sonicated for 20 mins, cooled and diluted upto the volume with diluent. Futher diluted 5 ml to 25 ml. Filtered through 0.45 µm Syringe filter.

RESULT AND DISCUSSION

Analytical method used for assay of DL-methionine and Pyridoxine hydrochloride used in Gardian Tablet by using High performance liquid chromatography techniques was validated. Validation was carried out on Agilent 1200 and Shimadzu LC 2010 HPLC system with Chromeleon software (6.8). The validation of the method was assessed by establishing validation criteria such as Specificity and System Suitability, Linearity and Range, Precision (repeatability & intermediate precision), Accuracy, Soltion Stability and Robustness study.

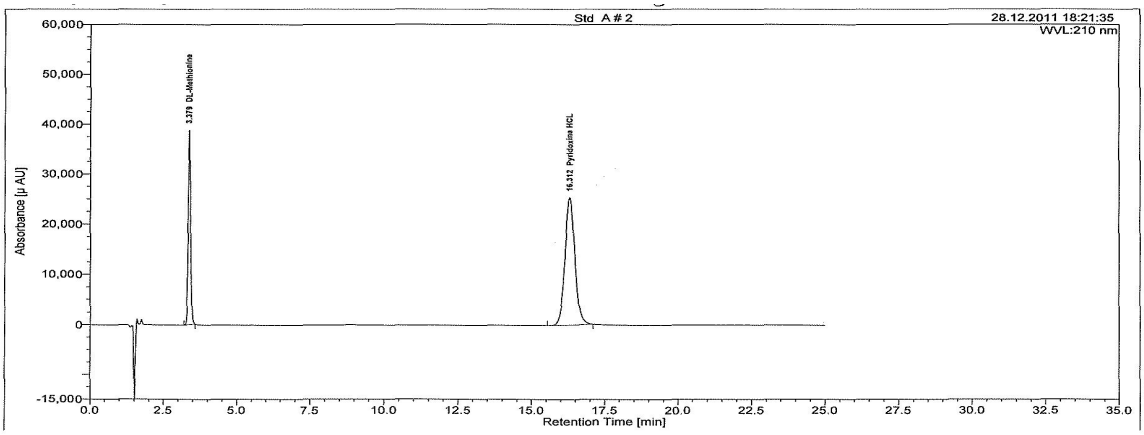


Fig. 1: A Typical chromatogram of Standard

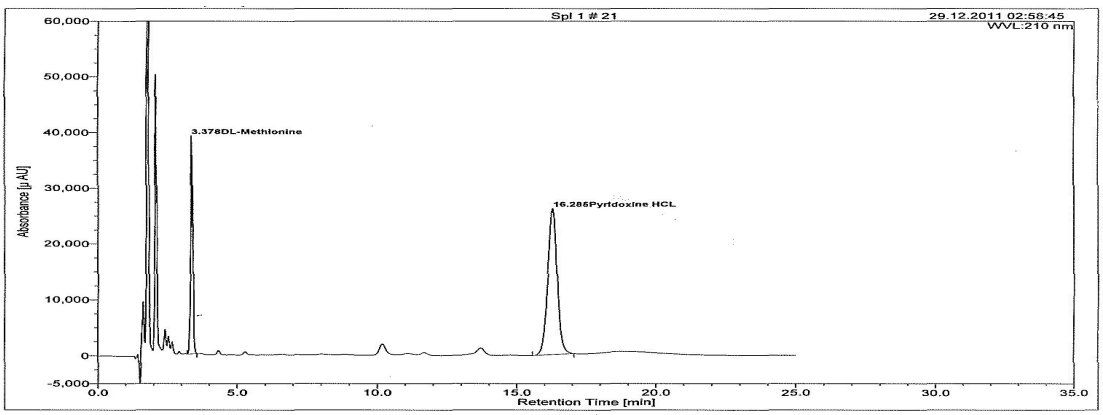


Fig. 2: A Typical Chromatogram of Sample

Validation of method

Specificity & System Suitability

Specificity was carried out to monitor interferences from blank and to monitor system suitability. Standard solutions were injected into the chromatpgram in six replicates. The % RSD for peak area response and retention were found within limit (Not more than 2.00% for peak area response and not more than 1.00% for retention time). The system suitability parameters like theoretical plates and tailing factor were found within limits.

Linearity

Linearity and Range were carried out over a range of 50 to 150 % of working level concentration. The linearity regression correlation coefficient, % Y intercept and % RSD for peak area response and retention time for lower and higher range were calculated. The linearity regression correlation coefficient for the component was found within limit (Not less than 0.999). The % Y intercept for the component was found within the limit. (Not more than ± 2.0). The % RSD for the peak area response and retention time for lower and higher range was found within limit. The % RSD for Response factor was found within limit the limit (NMT 5.00%).

Accuracy

Accuracy levels were prepared by 50,100, & 150% of working level concentration, prepared in triplicate for each levels and the percentage recovery were calculated for each levels separately. The percentage recoveries observed for the levels were found well within the limit set for the accuracy study (Not less than 98.0% and not more than 102.0%), shown that the content was recovered and hence is accurate.

Intermediate Precision (Ruggedness)

Sample was reanalysed by another analyst on another day by using another columns for six times. Results were calculated. The results of intermediate precision study along with repeatability study were compared and found well within the limit set for the intermediate precision study.

Table 1: Summary of System Suitability

Parameters	Theoretical Plates	Tailing Factor	Similarity Factor	% RSD of STD A for Area	% RSD of STD A for RT
DL-Methionine	8391	1.1	1.0	0.07	0.05
Pyridoxine Hydrochloride	11470	1.1	1.0	0.03	0.10

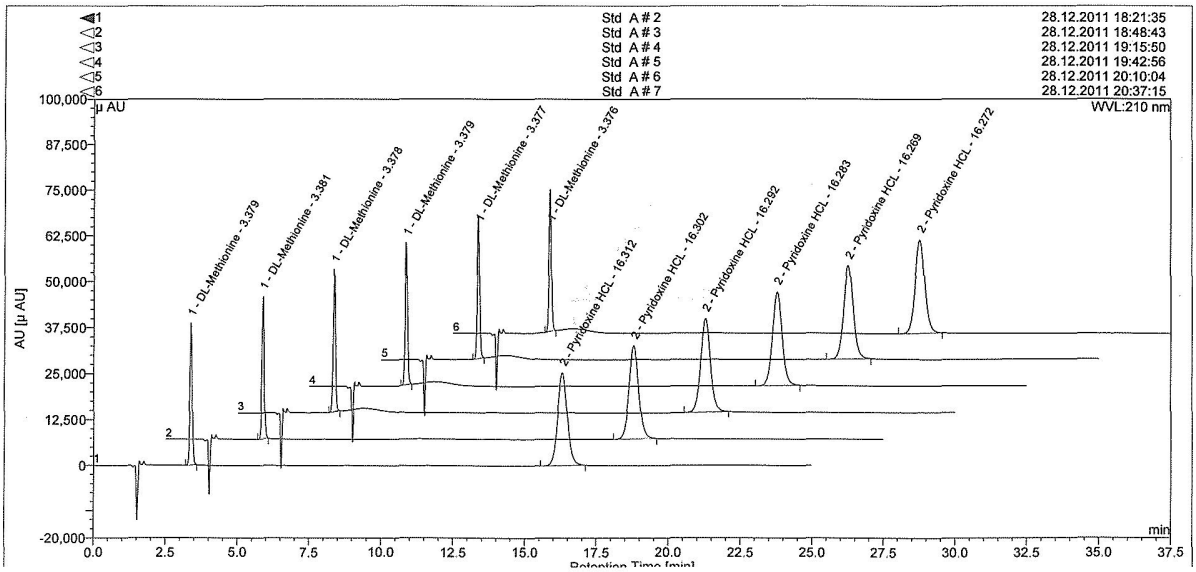


Fig. 3: Standard Mix. (6 replicates) for Specificity

Table 2: Summary of Linearity

Name	% Y Intercept	Correlation Coefficient	Response Factor (%RSD)
DL-Methionine	0.2	1.000	1.3%
Pyridoxine Hydrochloride	0.5	1.000	s0.3%

Peak Name DL-Methionine UV_VIS_1	o. of Calibratio Points DL-Methionine UV_VIS_1	Calibration Type L-Methionin UV_VIS_1	Slope	Y intercept	% Y Intercep	Corelation Coefficient	R Square
DL-Methionine	18	Lin	10516.101	449.025	0.2	1.000	0.99983

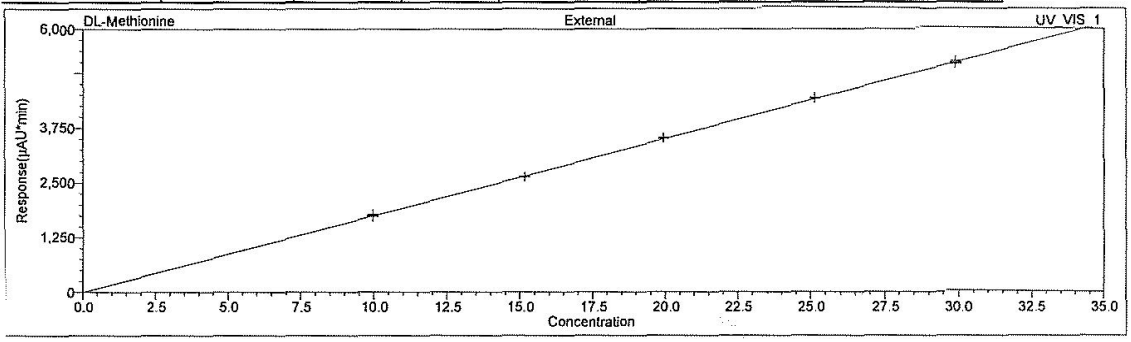


Fig. 3: Linearity of DL-Methionine

nearity of Standards.

Peak Name Pyridoxine Hcl UV_VIS_1	o. of Calibratio Points Pyridoxine Hcl UV_VIS_1	Calibration Type yridoxine H UV_VIS_1	Slope	Y intercept	% Y Intercep	Corelation Coefficient	R Square
Pyridoxine Hcl	18	Lin	56847.316	2844.804	0.5	1.000	0.99941

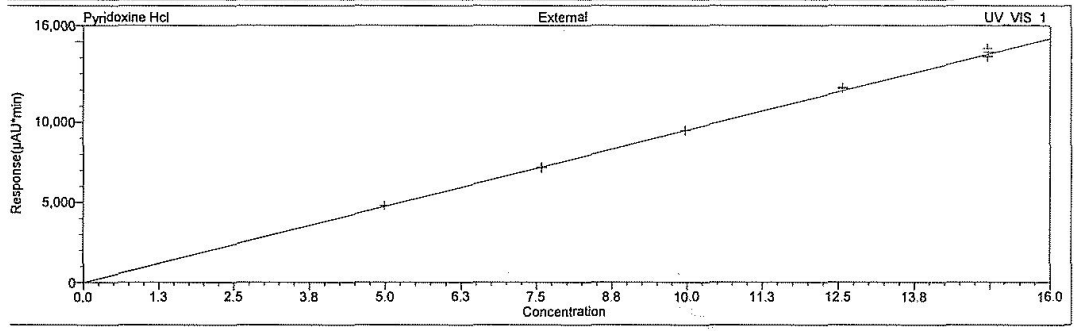


Fig. 4: Linearity of Pyridoxine Hydrochloride

Table 3: Summary of Accuracy

Parameters	Recovery (50%)	Recovery (100%)	Recovery (150%)	% RSD of Recovery
DL-Methionine	99.6	101.4	100.1	1.1
Pyridoxine HCl	101.4	99.7	101.2	1.2

Table 5: Precision

Parameters	% RSD of Assay	% Variation	Similarity Factor
DL-Methionine	1.6	0.9	1.0
Pyridoxine HCl	1.8	1.1	1.0

Table 5: Robstness

Parameter	Flow rate		Change in pH	
Change in parameter	0.9 ml/min	1.1 ml/min	Low pH (6.8)	High pH (7.2)
% LABLE CLAIM				
DL-Methionine	102.4	105.4	102.5	102.6
Pyridoxine HCl	107.5	109.2	107.6	107.5

The results of robustness study along with precision study were compared and found well within the limits set for the robustness study. Hence the method is robust.

CONCLUSION

The proposed method was found to be highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method was robust in the separation and quantification of DL-Methionine and Pyridoxine Hydrochloride. This method can be used for the routine analysis of production samples. The information presented herein could be very useful for quality monitoring of bulk samples and well employed to check the quality during stability studies.

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