Research Article

Method Development and Validation for Simultaneous Estimation of S-Adenosyl methionine and Metadoxine by RP-HPLC

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ABSTRACT

To develop a method for simultaneous estimation of s-adenosyl methionine and metadoxine by RP-HPLC and validate the developed method according to ICH guidelines2. The column used for method development of s-adenosyl methionine and metadoxine was shimadzu C18, 250× 4.6 mm, 5µm. Phosphate Buffer (pH 3.5) : Methanol were used in the ration of 92:8 for the method development. The developed method was validated for various parameters such as specificity, linearity, range, accuracy, precision, system suitability, robustness, ruggedness etc. The method was developed and optimized method was chosen and validated and the results were tabulated according to ICH guidelines6. The result obtained in this study demonstrated that the HPLC method described is specific, accurate, precise, linear, ruggedness, robustness and stability indicating for the simultaneous determination of s-adenosyl methionine and metadoxine.

Keywords: s-adenosyl methionine, metadoxine, method development, HPLC.

INTRODUCTION

S-Adenosylmethionine (SAMe) is a natural substance present in the cells of the body. It is a direct metabolite of the essential amino acid L-methionine[9]. Metadoxine is clinically used to treat acute and chronic alcoholic intoxication and alcoholic liver diseases due to its capabilities of speeding up the elimination of alcohol and aldehyde in the plasma[9]. There was no method available in literature for the estimation of the drug in the formulation at the time undertaking for study RP-HPLC method for the Simultaneous Estimation of S-Adenosyl Methionine and Metadoxine in bulk and pharmaceutical dosage form has been reported. Hence an attempt was made to develop a specific, precise, accurate, linear, simple, rapid, validated and cost effective RP-HPLC method for the estimation of S-Adenosyl Methionine and Metadoxine in dosage form[1].

MATERIALS AND METHODS

Chemicals and reagents

S-Adenosyl Methionine and Metadoxine was a gift sample from MPC division of CDRI. All chemicals used were analytical grade. Methanol (HPLC grade), Potassium dihydrogen phosphate (AR grade), Orthophosphoric acid (AR grade) in house mill Q water was used throughout the study.

Instrumentation

The RP-HPLC method was developed on a SHIMADZU LC-2010 with UV Detector, with spectral width of 1 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells. Analytical balance (Shimadz) was used for all weightings. pH meter (Elchem) was used. Sonicator (Saisonic) was used.

Selection of wavelength

A stock solution was prepared by dissolving 100 mg of the drug in100 ml of Methanol. From the stock solution, the range of 50 to 250 µg/ml was prepared. The λ Max of S-Adenosyl Methionine and Metadoxine was scanned from 200 to 400 nm in an UV visible spectrophotometer and the spectrum was recorded[3]. From the spectra, the detection wavelength 210 nm was identified[8].

Preparation of standard stock solution

The stock solution was prepared by weighing accurately about 25 mg of S-Adenosyl Methionine WRS into a 50 mL of volumetric flask add 25 mL of mobile phase for solubilising the drug and sonicated for about 10 min. Finally make up to volume with the mobile phase.

Standard preparation

The stock solution was prepared by weighing accurately 25 mg of Metadoxine into a 100 mL of volumetric flask dissolve in 50 mL of mobile phase for solubilising the drug and sonicated for about 10 min; add 10 mL of standard stock solution. Finally make up to the volume with mobile phase. The solution contains 250 µg/mL of S-Adenosyl Methionine and Metadoxine respectively.

Sample preparation

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 250 mg of the sample were transferred into 100 mL of volumetric flask dissolve in 50 mL of mobile phase for solubilizing the drug and sonicated for about 30 min. Finally cool and make up to the volume with mobile phase. Filter the above solution through 0.45 µm nylon membrane filter with discarding

10 mL of the filtrate. Further dilute the above solution to get final concentration of 250 µg/mL respectively.

Method Development for HPLC

An isocratic HPLC RP C_{18} Column (SHIMADZU C_{18}AR, 250×4.6 mm, packed with 5 µm particle size) was used. The mobile phase used was a mixture of phosphate buffer and methanol (pH 3.5) in the ratio of 92:8 v/v; it was filtered through a 0.22 µm membrane filter in a Millipore filtration assembly and pumped from the respective solvent reservoirs to the column at a flow rate of 1 ml/min[8]. The run time was set at 20 min and column was maintained at ambient temperature. The volume of injection is 10 µl. Prior to the injection of drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluent was monitored at 210 nm and data acquired was stored and analyzed with the software. The system suitability tests are parameters that confirm the validity of a well behaved chromatographic system. Instrument performance parameters such as peak area %RSD and USP tailing factor were established fig:2.

Validation[7]

Linearity

Five concentrations of the standard mixture, 50%, 75%, 100%, 125% and 150% were injected and chromatogram was recorded. A graph was plotted for the concentration of the corresponding drug versus Area. The Correlation coefficient(r) for each drug was calculate fig; 1.

Accuracy

To determine the accuracy in sample preparation method of standard additions was made for measuring the recovery of the drugs. To the known standard solution concentrations of the drug (50%, 75%, 100%, 125%, and 150%) was added. The accuracy was expressed as the percentage of the analytes recovery tab;2.

Method Precision

It is very important that the method developed should be precise. Six replicates of the sample prepared from the commercial tablets were injected and Assay was calculated to measure the repeatability of retention times and peak area of standard and sample.

Robustness

To verify the robustness of the method, the analysis was done under variable flow rates. The flow rate as per the developed method is 1.0 ml/min. This has been purposely changed to and the chromatogram was obtained.

Ruggedness

To test the ruggedness of the method, the analysis was done on different days and different chemists to check for any changes in the chromatograph. The percentage RSD for the retention time and area was calculated.

RESULT AND DISCUSSION

The Reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of s-adenosyl methionine and metadoxine in bulk drug and in combined dosage forms. The RP-HPLC separation was achieved on a C_{18} Column (SHIMADZU C_{18}AR, 250×4.6 mm, packed with 5 µm particle size) was used. The mobile phase used was a mixture of phosphate buffer and methanol (pH 3.5) in the ratio of 92:8 v/v; the flow rate was monitored at 1ml per min. The wavelength was selected for the detection was 210 nm. The percentage purity for S-Adenosyl Methionine was found to be 98.76% and Metadoxine was found to be 100.51%. So this method was suitable for analysing the marketed formulation. The % Recovery of the drug was found to be in the range of 99.6-101.7% for S-Adenosyl Methionine and Metadoxine. This indicates that the method was accurate. In the present study, the % RSD for S-Adenosyl Methionine it was found to be 0.63%, 0.33%, 0.26%, 0.37%, 0.41%, 0.32% and Metadoxine it was found to be 0.15%, 1.65%, 0.12%, 0.11%, 1.86%, 1.76% in AUC, The % RSD value indicates good degree of precision within the specified range. In the system Ruggedness % RSD was found 0.16% for S-Adenosyl Methionine and Metadoxine it was found 0.16% respectively. In Method Ruggedness the % RSD for S-Adenosyl Methionine for First Analyst was found 1.36% and Second Analyst was found 1.33% and Metadoxine was found 0.18%, 0.25% respectively.

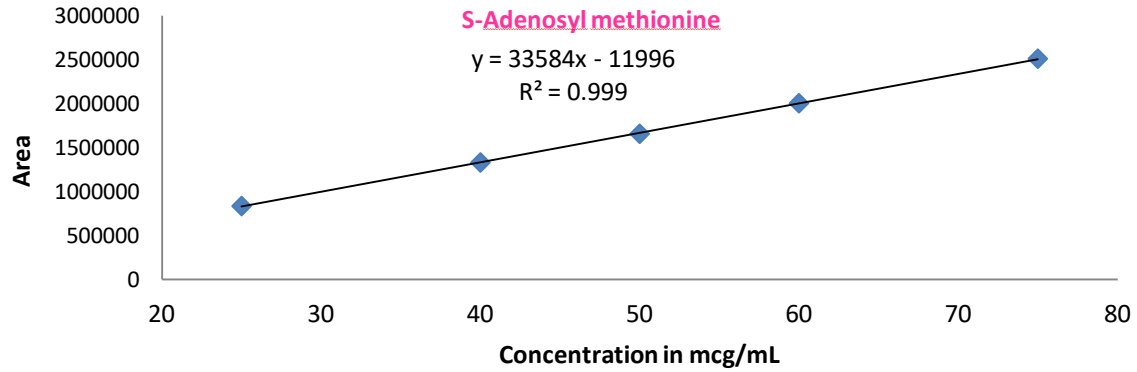
CONCLUSION

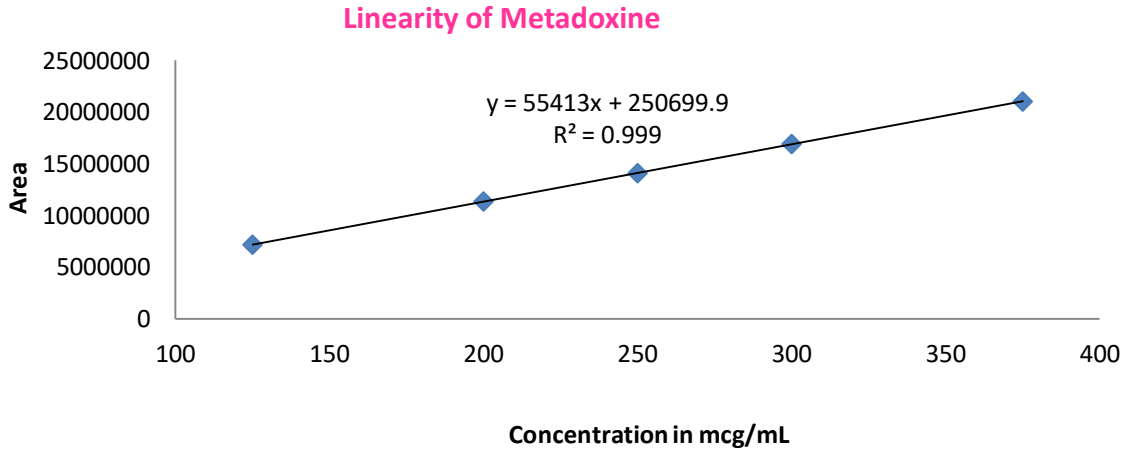
The results of the present study indicated that the developed method is simple, precise and cost effective for the simultaneous estimation of S-Adenosyl Methionine and Metadoxine in bulk and pharmaceutical formulation. The developed and validated RP-HPLC method outlined is very low cost, rapid easy to perform. When flow rate, mobile phase, pH was slight changed in robustness retention time had changed significantly. The validation study carried out for the determination of S-Adenosyl Methionine and Metadoxine in Livapt tablets show satisfactory results meeting the acceptance criteria, and hence the method stands validated. This method can be used for routine analysis and for stability sample analysis.

Table 1: Calibration curve for s-adenosyl methionine and metadoxine

S.No	S-Adenosyl Methionine				Metadoxine			
	Concentration (mcg/ml)	First peak	Second area	Total Area	Concentration (mcg/ml)	First peak	Second area	Total Area
1	25	606344	227283	833627	125	770867	6397572	7168439
2	40	963895	365933	1329828	200	1225335	10120892	11346227
3	50*	1198478	458013	1656491	250*	1521798	12563034	14084832
4	60	1452686	551798	2004484	300	1826133	15079649	16905782
5	75	1810207	701443	2511650	375	2271487	18742875	21014362

Fig:1





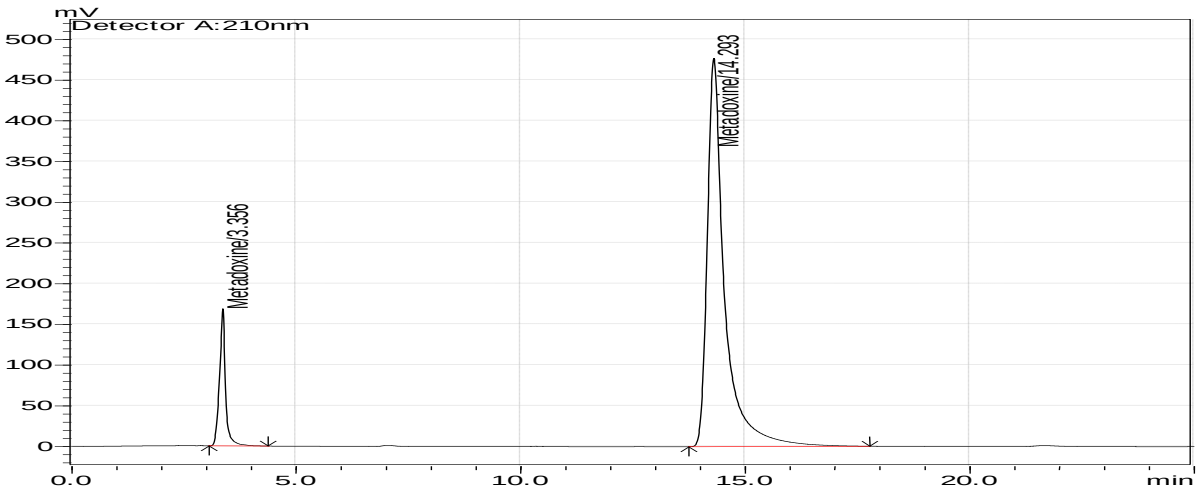


Figure 2: Chromatogram of s-adenosyl methionine and metadoxine

Table 2: Accuracy

S. No	Test Area	Amount of S-Adenosyl Methionine obtained by analysis		
		Obtained mcg/mL	Present mcg/mL	Recovery in percentage
1	2003876	63.13	62.56	100.91
2	2012600	63.40	62.56	101.34
3	1999195	62.98	62.56	100.67
Average:			**100.98%**	
RSD:			**0.34%**	
S. No	**Test Area**	**Amount of Metadoxine obtained by analysis**		
		Obtained mcg/mL	**Present mcg/mL**	**Recovery in percentage**
1	16943732	302.90	302.22	100.23
2	16900444	302.13	302.22	99.97
3	16995004	303.82	303.22	100.53
Average:			**100.24%**	
RSD:			**0.28%**	

Table 3: System Suitability

S.No	Parameters	S-Adenosyl Methonine	Metadoxine
1.	Theoretical plates	4250	4895
2.	Tailing factor	1.0	1.10
3.	Retention Time	7.0 & 10.4	3.3 & 14.2
4.	Resolution	4.12	5.02

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