Research Article CODEN: IJPRNK ISSN: 2277-8713
Sagar VLN, IJPRBS, 2013; Volume 2(6): 270-283 IJPRBS

INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

HPLC METHOD VALIDATION FOR THE DETERMINATION OF ASSAY CONTENT IN CHOLIC ACID

SAGAR V L N[1], SHARMA G V R[1], OMPRAKASH G[1], BHARAT KUMAR BUGATA[2], VASUDEVA RAO A[3]

1. Department of Chemistry, Gitam University, Rushikonda, Visakhapatnam, Andhra Pradesh, India.
2. Department of Biochemistry and Bioinformatics, Gitam University, Rushikonda, Visakhapatnam, Andhra Pradesh, India.
3. Senior Research Fellow-Council of Scientific and Industrial Research, New Delhi, India.

Accepted Date: 16/11/2013; Published Date: 27/12/2013

Abstract: High Performance Liquid Chromatography (HPLC) method validation was performed for the determination of assay content in cholic acid. The validation method utilizes an Luna C18, 150 x 4.6mm, 5µm column at ambient temperature, isocratic elution with aqueous sodium phosphate buffer at pH 3.0±0.05, acetonitrile and methanol (40:40:20) as the mobile phase. The mobile-phase flow rate was 1.0 mL min^{-1}. The linearity, range, system precision, method precision, method ruggedness are found to be satisfactory. Therefore the method is assumed to be selective for the determination of assay content in cholic acid by HPLC.

Keywords: Cholic acid, HPLC, Validation

PAPER-QR CODE

Corresponding Author: MR. SAGAR VLN

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How to Cite This Article:

Sagar VLN, IJPRBS, 2013; Volume 2(6): 270-283

INTRODUCTION

Synthesis of bile acid from cholesterol in liver is the major pathway of cholesterol catabolism. Thereby cholesterol is modified by oxidation, reduction of the side chain and subsequently conjugation by glycine and taurine, respectively. These amphiphatic molecules are essential to solubilize dietary lipids and vitamins to promote their absorption. The most abundant bile acids in humans comprise the 1^{o} primary bile acids (cholic acid (CA) and chenodeoxycholic acid (CDCA)) and the 2^{o} bile acids (deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA)) formed by deconjugation and dehydroxylation by gastro intestinal bacteria in the colon. Bile acids are reabsorbed and transported back to the liver to enter into enterohepatic flow [1,2].

In spite of its role in dietary lipid absorption and cholesterol homeostasis, bile acids metabolism increasingly recognized as an attractive pharmacological target for treatment of vascular and metabolic diseases such as obesity, type 2 diabetes and atherosclerosis. The understanding of bile acid functions requires methods which cover the difficulty of this structurally diverse group of molecules [3-11]. In recent past a number of methods using liquid chromatography were developed allowing analysis of free and conjugated bile acids without derivatization [12-19]. However, most of the protocols showed disadvantages with time consuming extraction procedures, long analysis times or lack of baseline separation of isobaric species [20-21]. In the present study we performed a method validation of cholic acid by HPLC.

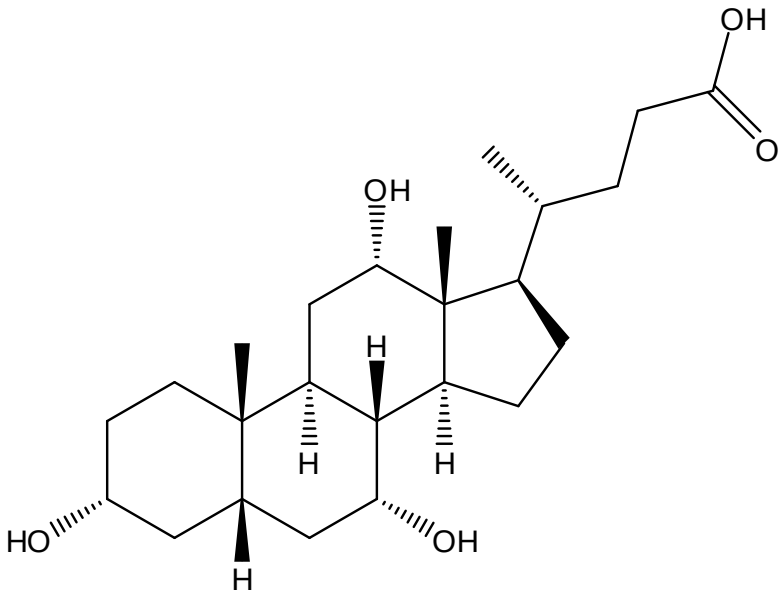


Figure 1. Structure of Cholic acid.

MATERIALS AND METHODS

Chemicals, Reagents and Samples

The chemicals, equipment and samples which were used in the present stduy was given in the following Tables 1-3.

Table 1: List of chemicals.

Name of the reagent/solvents	Grade	Make
Sodium dihydrogen phosphate	GR	Merck
Sodium dihydrogen phosphate	Suprapure	Merck Germany
Methanol	HPLC	Merck
Methanol	HPLC	Merck Germany
Orthophosphoric acid (85%)	AR	Merck
Acetonitrile	HPLC	Merck
Acetonitrile	HPLC	J.T.Baker
Water	-	Milli-Q

Table 2: List of equipments.

Name of the Instrument	I.D.Number	Make
HPLC	Waters-Alliance	2695 model pump with 2489 UV detector
Analytical balance	Stratories	CP224S
Microbalance	Stratories	CP2P
pH meter	Eutech	pH510

Table 3: List of the samples.

Name of the standard	Supplier	Grade	Lot No/ B.No
Dehydrocholic acid	Sigma-aldrich	Sigma	10144927
Chenodeoxy cholic acid	-	IH	GFH/A241/049
Deoxy cholic acid	Sigma-aldrich	Sigma	011M54161V
Cholic acid	-	IH	G1F063

Chromatographic conditions

The chromatographic condition used in the present study were given in the following Table 4.

Table 4: Chromatographic conditions.

Column	**Luna C18, 150 x 4.6mm, 5µm** **Phenomenex Part No: 00F-4252-E0**
Flow	1.0 mL/minute
Column oven temperature	Ambient
Detector wavelength	RI
Injection volume	20 µL
Run time	50 minutes
Elution	Isocratic
Diluent	Buffer : Acetonitrile : Methanol (40:40:20)

Buffer preparation

Dissolved 6.8 grams of sodium dihydrogenphosphate in water, diluted with water to 1000 ml and mixed, and then adjusted with dilute orthophosphoric acid to a pH of 3.0 (±0.05).

Mobile phase preparation

Mobile phase was prepared by mixing Buffer Solution, Acetonitrile and methanolin the ratio of (40:40:20) (v/v).

Preparation of Standard and System Suitability Solution

Weighed and transfered about 100.0 mg of Cholic acid into 50mL volumetric flask. Added to it about 30 mL diluent and sonicated the mixture for a few minutes until it is dissolved. Diluted to volume with the diluent and mixed well. (Label as standard stock solution)

Sample preparation

Weighed and transfered about 100.0 mg of Cholic acid into 50mL volumetric flask. Added to it about 30 mL diluent and sonicated the mixture for a few minutes until it is dissolved. Diluted to volume with the diluent and mixed well. (Label as sample preparation-1 & 2).

Procedure

If the system suitability passes, inject the sample preparations 1 and 2 (Order of injections as specified) and record the chromatograms. The approximate retention time is about 6.0 minutes.

Table 5: Order of Injection.

Name of the preparations	No. of injections	Purpose
Diluent (Mobile Phase)	1	System equilibration
Main standard solution [Standard I]	5	For system suitability and assay calculation purpose
Sample (Preparation-1)	1	Assay content in Sample analysis
Sample (Preparation-2)	1	

Calculations

The System Suitability must be calculated to have a % RSD of the peak area for five consecutive injections of the standard solution to be not more than 2%

$$\%Assay = \frac{\text{Peak area of cholic acid in sample prep}}{\text{---}} \times \frac{Cs}{\text{---}} \times 100$$

Avg Peak area of cholic acid in std prep Ct

Where,

Cs = Concentartion of cholic acid in standard preparation.

Ct = Concentartion of cholic acid in standard preparation.

RESULTS AND DISCUSSION

System Suitability Data

The standard solutions were prepared and analysed as per the proposed method, The % RSD of the peak area for the injections of the standard solution throughout the run standard solution of Cholic acidpeak to demonstrate system suitability for studying of each validation parameter. The system suitability results are as follows (Table 6).

Table 6: Summary of percentage relative standard deviation peak area of Cholic acid in standard solution.

Validation Parameter	The % RSD cholic acid peak in standard solution five replicate injection (Not more than 2.0)
Specificity	0.74
Linearity	0.55
Accuracy	1.16
Precision(System & Method Precision)	0.42
Intermediate precision	1.67

Selectivity/Specificity:

Each specified impurity solution was prepared individually (i.e., Dehydrocholic acid, Chenodeoxy cholic acid, Deoxy cholic acid and Cholic acid) and a solution of all known specified impurities spiked with the Cholic acid at 1.0% level and finally Cholic acidsample was also prepared. All these solutions were analyzed by using the RI detector. The acceptance criteria was based on the general recommendations such as 1) Peak should be homogeneous and there should be no coeluting peaks, 2) Peak purity of analyte should pass. For peak purity of

analyte, purity angle should be less than the purity threshold. 3) No blank interference should be at the retention time of analyte and 4) The difference of assay between unspiked and spiked samples should not be more than ± 1.5. From the data (Table 7) it was showed that Cholic acid peak was well resolved from the other known specified impurities, and there is no blank interference at the retention time of Cholic acid peak. Therefore the method is selective for the determination of assay of Cholic acid drug substances.

Table 7: Summary of retention time (RT), RT ratio for Cholic acidand its related impurities and the peak purity values of Cholic acidpeak.

Peak name	Retention time (minutes)	RT ratio	USP resolution
Dehydrocholic acid	6.35	0.426	-
Cholic acid	14.907	1.000	-
Cheno deoxy cholic acid	32.35	2.170	-
Deoxy cholic acid	36.749	2.465	-

* Individual Injection

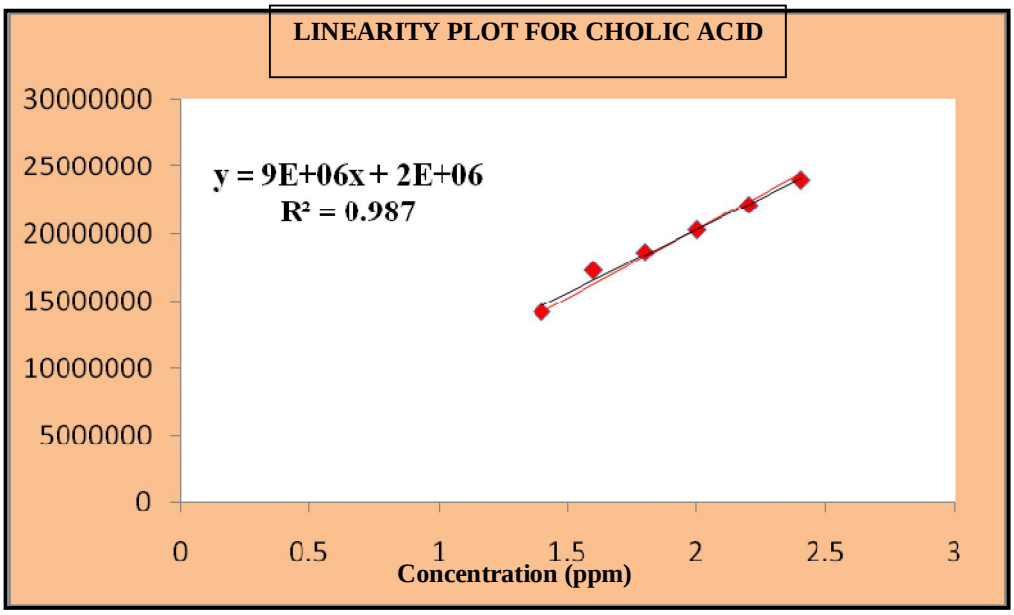
Linearity:

The linearity of the HPLC method was demonstrated for Cholic acidsolutions ranging from 1.4014 mg/mL to 2.4024 mg/mL, which is equivalent to 70.0 % to 120.0 % of the Cholic acidworking concentration. Cholic acid sample solutions at concentrations within the mentioned range were prepared and analyzed (duplicate) as per the method. The linearity results obtained are shown in Table 8 and Figure 2 shows the line of best fit for average peak area versus concentration of Cholic acid. The general acceptance guidelines which include a) No apparent non linearity should be observed graphically for Cholic acid, b) The correlation coefficient (R) should not be less than 0.99 and c) Report the slope and intercept values. As shown in the figure, the linearity results for Cholic acidin the specified concentration range were found satisfactory, with a correlation coefficient (R) greater than 0.990.

Table 8: Linearity for Cholic acid.

Level	Conc (mg/mL)	Peak area in injection	Average peak area of Cholic acid
70.0%	1.4014	14169570	14169570
80.0%	1.6016	17258208	17258208
90.0%	1.8018	18627188	18627188
100.0%	2.002	20286601	20286601
110.0%	2.2022	22129855	22129855
120.0%	2.4024	23951338	23951338

Figure 2: Linearity plot for Cholic acid.



Regression statistics

Slope (9302582.275), Intercept (1711212.105), Correlation coeffcient (R) (0.994) and Coefficient of determination (R^2) (0.987)

Accuracy

The accuracy of the method was determined by analyzing three solutions containing Cholic acid at approximately 80.0 %, 100.0 % and 120.0 % of the working strength. Each solution was analyzed in triplicate. The Percentage recovery results obtained are listed in Table 9. The recovery should be in the range of 98.0 % to 112.3 % and the percentage relative standard deviation should be less than 10.00. The percentage recovery values obtained were in the range of 98.29 to 112.23 and the percentage relative standard deviation obtained in the range of 0.16 to 6.71, which is within the acceptance criteria as mentioned in the protocol. The acceptance criterion was satisfied by the accuracy data.

Table 9: Accuracy of cholic acid

Level	Measured Conc. (mg/mL)	% Recovery	Avg	SD	%RSD
	1.5984	101.37	101.34	0.1617	0.16
80.0 %	1.5976	101.49			
	1.6008	101.17			
	2.018	112.23	104.51	7.0138	6.71
100.0 %	1.998	98.53			
	2.001	102.77			
	2.4012	98.40	98.36	0.0586	0.060
120.0 %	2.4096	98.29			
	2.4048	98.38			

Range

Range of the method was determined from the linearity and accuracy data. The range of the method was found to be between 0.0283 mg/mL (70.75 %) to 0.0482 mg/mL (120.50%), i.e. 70.0 % to 120.0 % level. The range should be about 70.0 % to 120.0 % with respect to working concentration..

Precision:

The repeatability expresses the precision under the same operating conditions over a short interval of time. It expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample.

System precision:

The System precision was performed by injecting a standard solution of Cholic acid at working concentration for six times. Result of peak area of the Cholic acidis summarized in Table 10. The percentage relative standard deviation for the peak area of Cholic acid should not be more than 0.73. The percentage relative standard deviation for the peak area of the Cholic acid was 0.53 at the working concentration. Therefore acceptance criteria was satisfied.

Table 10: Summary of peak area for in system precision solution.

Injection No.	Peak area of Cholic acid
1	20711763
2	20731292
3	20794773
4	20939372
5	20957400
6	20927420
Average peak area	20843670
SD	110929.3796
%RSD	0.53

Method precision

The method precision was performed by analyzing a sample solution of Cholic acidat working concentration six times (Six replicate sample preparation). Table 11 shows the percentage relative standard deviation of Cholic acidassay values of six replicate sample preparations. The percentage relative standard deviation for the assay values should not be more than 1.00. The

percentage relative standard deviation for the assay values (six preparations) of Cholic acidwas 0.40 at the working concentration.

Table 11: Summary of results for precision of the method.

Preparation No.	Assay (% w/w) as Cholic acid
1	99.51
2	99.86
3	100.35
4	100.05
5	100.67
6	100.11
Average Assay (% w/w) on dried basis	100.09
SD	0.3988
% RSD	0.40

Ruggedness (Intermediate precision):

Evaluating the variability of the results obtained with the analysis of six sample preparations of Cholic acidby different analysts on different days with different instruments and different columns in intermediate precision study. The results are summarized in Table 12. The acceptance criteria is based on the general rules such as a) The bias in assay determination for each parameter should not be more than ±1.5, b) The percentage relative standard deviation among the results obtained should not be more than 1.00, c) The overall percentage relative standard deviation for 12 preparations should not be more than 2.00 and d) The absolute difference between the average assay should not be more than ± 1.5. The bias in assay values obtained is in the range of 0.47 to +1.84. The percentage relative standard deviation of percentage assay value obtained was 0.40 and 0.64. The overall percentage relative standard deviation for 12 preparations of percentage assay values obtained is 0.25. The absolute difference between the average assay value obtained is 0.84. The acceptance criteria was sucessfully fulfilled.

Table 12: Results of Intermediate precision.

Sample ID	Analyst (1) /Day (1)/ Instrument(1)/Column (1) [(%w/w) of assay as Cholic acid	Analyst (2) /Day (2)/ Instrument(2)/Column (2) [(%w/w) of assay as Cholic acid	Bias
Sample-1	99.51	97.67	1.84
Sample-2	99.86	99.39	0.47
Sample-3	100.35	98.56	1.79
Sample-4	100.05	98.77	1.28
Sample-5	100.67	99.21	1.46
Sample-6	100.11	99.15	0.96
Average assay (%)	100.09	98.79	1.30
SD	0.3988	0.63	
%RSD	0.40	0.64	
Over all % RSD (12 preparations)	0.84		

CONCLUSION

In conclusion, the HPLC method validation study, presents a adequate procedure to obtain correct and reliable values for the determinatin of assay content in cholic acid.

REFERENCES

1. C. Thomas, R. Pellicciari, M. Pruzanski, J. Auwerx, K. Schoonjans, Nat. Rev. Drug Discov. 7 (2008) 678.

2. A.F. Hofmann, L.R. Hagey, Cell. Mol. Life Sci. 65 (2008) 2461.

3. P. Lefebvre, B. Cariou, F. Lien, F. Kuipers, B. Staels, Physiol. Rev. 89 (2009) 147.

4. S.M. Houten, M. Watanabe, J. Auwerx, EMBO J. 25 (2006) 1419.

5. L. Ye, S. Liu, M. Wang, Y. Shao, M. Ding, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 860 (2007) 10.

6. D. Tagliacozzi, A.F. Mozzi, B. Casetta, P. Bertucci, S. Bernardini, I.C. Di, A. Urbani, G. Federici, Clin. Chem. Lab. Med. 41 (2003) 1633.

7. M. Ando, T. Kaneko, R. Watanabe, S. Kikuchi, T. Goto, T. Iida, T. Hishinuma, N. Mano, J. Goto, J. Pharm. Biomed. Anal. 40 (2006) 1179.

8. I. Burkard, E.A. von, K.M. Rentsch, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 826 (2005) 147.

9. C. Eckers, A.P. New, P.B. East, N.J. Haskins, Rapid Commun. Mass Spectrom. 4 (1990) 449.

10. S. Ikegawa, N. Murao, T. Motoyama, T. Yanagihara, T. Niwa, J. Goto, Biomed. Chromatogr. 10 (1996) 313.

11. A.H. Bootsma, H. Overmars, R.A. van, A.E. van Lint, R.J. Wanders, A.H. van Gennip, P. Vreken, J. Inherit. Metab. Dis. 22 (1999) 307.

12. D.W. Johnson, H.J. ten Brink, R.C. Schuit, C. Jakobs, J. Lipid Res. 42 (2001) 9.

13. K.A. Mills, I. Mushtaq, A.W. Johnson, P.D. Whitfield, P.T. Clayton, Pediatr. Res. 43 (1998) 361.

14. A. Roda, A.M. Gioacchini, C. Cerre, M. Baraldini, J. Chromatogr. B: Biomed. Appl. 665 (1995) 281.

15. H. Sakakura, M. Suzuki, N. Kimura, H. Takeda, S. Nagata, M. Maeda, J. Chromatogr. 621 (1993) 123.

16. Y. Alnouti, I.L. Csanaky, C.D. Klaassen, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 873 (2008) 209.

17. E. Tessier, L. Neirinck, Z. Zhu, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 798 (2003) 295.

18. K. Bentayeb, R. Batlle, C. Sanchez, C. Nerin, C. Domeno, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 869 (2008) 1.

19. S. Perwaiz, B. Tuchweber, D. Mignault, T. Gilat, I.M. Yousef, J. Lipid Res. 42 (2001) 114.

20. U.S. Department of Health and Human Services Food and Drug Administration, 2001.

21. M. Scherer, C. Gnewuch, G. Schmitz, G. Liebisch, Journal of Chromatography B, 877 (2009) 3920–3925.