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Chromatographic Method Development and Validation for Quantitative Determination of Ursodeoxycholic Acid in Ursodeoxycholic Acid Tablets

T.B. SAWANT* and D.V. MANE

Department of Chemistry, Shri Chhatrapati Shivaji College, Omerga-413 606, India

*Corresponding author: E-mail: tbsawant_916@rediffmail.com

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An HPLC assay method is developed for quantitative determination of ursodeoxycholic acid in ursodeoxycholic acid tablets. The developed method is a simple, accurate, rugged, precise and stability indicating chromatographic method. The stationary phase used in separation of known impurities and ursodeoxycholic acid drug was C_{18}, 250 mm × 4.6 mm, 5 µm. The mobile phase was prepared by mixing of buffer and acetonitrile in the ratio of 50:50 v/v pumped at a flow rate of 1.5mL/min. The developed chromatographic method is validated. The method is accurate, precise and linear over the concentration range of 50-150 %. Mean recovery of ursodeoxycholic acid tablets is found 99.7 ± 0.4. The method is simple and stability indicating, and hence can be used by common laboratories for the determination of ursodeoxycholic acid (assay) in ursodeoxycholic acid ER tablets.

Keywords: Ursodeoxycholic acid, Impurity, Forced degradation.

INTRODUCTION

Ursodeoxycholic acid (Fig. 1) is a naturally occurring bile acid with multiple hepatoprotective activities, improves liver condition in patients with a wide range of chronic liver diseases [1]. Ursodeoxycholic acid is increasingly used for the treatment of cholestatic liver diseases [1]. Studies done on ursodeoxycholic acid suggests different mechanisms of action like: (1) protection of cholangiocytes against cytotoxicity of hydrophobic bile acids, resulting from modulation of the composition of mixed phospholipid-rich micelles, reduction of bile acid cytotoxicity of bile and, possibly, decrease of the concentration of hydrophobic bile acids in the cholangiocytes; (2) stimulation of hepatobiliary secretion, putatively *via* Ca^{2+}- and protein kinase Ca-dependent mechanisms and/or activation of p38MAPK and extracellular signal-regulated kinases (Erk) resulting in insertion of transporter molecules (*e.g.*, bile salt export pump and conjugate export pump) into the canalicular membrane of hepatocyte and possibly, activation of inserted carriers; (3) protection of hepatocytes against bile acid-induced apoptosis, involving inhibition of mitochondrial membrane permeability transition (IMPT) and possibly, stimulation of a survival pathway [2].

Since ursodeoxycholic acid shows moderate absorption only in short wavelength region *i.e.* 200-210 nm, quantification of the amount of ursodeoxycholic acid in its formulations along with excipients and solvents by simple UV spectrophotometry is hampered by possible interferences [3]. High performance

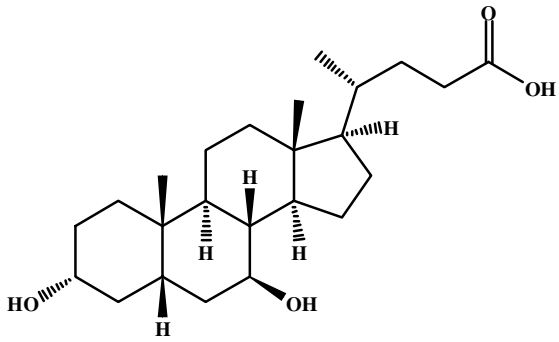

Fig. 1. Structure of ursodeoxycholic acid

liquid chromatography (HPLC) of its assay utilizing refractive index (RI) detector has been reported in United States Pharmacopoeia [4]. Moreover, there are few analytical methods for the quantification of ursodeoxycholic acid in biological fluids and few pharmaceutical dosage forms have been developed [5-8]. Most of them described HPLC methods coupled with MS and evaporative light scattering mass detection in biological matrix [5-8].

Ursodeoxycholic acid is commercially available as tablets and capsules. Stability of the such formulations is one of the important criteria to decide the expiry of drug product. To determine the assay of the drug during the storage period, stability indicating analytical method play an important role. There are few analytical methods developed by HPLC for the estimation

of ursodeoxycholic acid in ursodeoxycholic acid tablets and capsules [9-13]. From the literature survey, it was observed that there is no stability indicating analytical method available for determination of drug quantity during stability studies. The analytical methods used for stability studies require stability indicating analytical method which need to be proved by peak purity during forced degradation study [14,15]. Hence this work was focused to develop stability indicating, precise, accurate, specific and robust chromatographic method for quantification of ursodeoxycholic acid in ursodeoxycholic acid tablets in its pharmaceutical dosage forms. The wavelength selected in proposed method (210 nm) and additional validation parameters like forced degradation studies and solution stability of sample and standard are the advantages over current published methods [3,5-8]. The developed method is validated as per International Conference on Harmonization (ICH) Q2 (R1) and United State of Pharmacopoeia (USP) [16-18]. The method found to be specific, precise, accurate, robust and stability indicating. The method validation is compliant with validation criteria of ICH Q2 (R1) and USP guidelines. This method can be used in the quality control or in research laboratories of pharmaceutical companies for assay determination of ursodeoxycholic acid tablets during its release as well as for stability studies.

EXPERIMENTAL

Sodium dihydrogen orthophosphate dihydrate, disodium hydrogen orthophosphate dihydrate, tetrabutylammonium hydroxide (40 %-HPLC grade), tetrabutylammonium hydrogen sulphate and acetonitrile were procured from Merck, India. The ultrapure water was generated from milli-Q water purifier.

Chromatographic parameters: The chromatographic column used was ODS (C18), 250 mm × 4.6 mm, 5 µm (Devlosil), which was maintained at 45 ºC. The mobile phase was prepared by mixture of buffer and acetonitrile in the ratio of 50:50 v/v. The flow of the mobile phase was 1.5 mL/min. The injection volume was 10.0 µL. The column effluents were monitored by UV detector at 210 nm.

Buffer solution: Dissolved about 12 g of sodium dihydrogen orthophosphate dihydrate, 4 g of disodium hydrogen orthophosphate dihydrate and 11 g of tetrabutyl ammonium hydrogen sulphate in 1000 mL of HPLC grade water. Sonicated for degassing and filtered through 0.45 µm membrane filter.

Diluent: Dissolved 6 g of sodium dihydrogen orthophosphate dihydrate, 2 g of disodium hydrogen orthophosphate dihydrate in 535 mL of water and added 65 mL of 40 % tetrabutylammonium hydroxide to it. Mixed the solution with 400 mL of acetonitrile.

Standard preparation: Weighed accurately about 300mg of ursodeoxycholic acid as working standard and transferred to 20 mL volumetric flask. The contents of the flask was dissolved in diluent with sonication and intermittent shaking and volume was made up to the mark with diluent to nominal concentration about 15000 µg/mL.

Sample preparation: Weighed equivalent to powdered 20 tablets to 300 mg of ursodeoxycholic acid in 20 mL volumetric flask and dissolved in diluent with sonication and intermittent shaking (15000 µg/mL). Filtered through 0.45 µm nylon syringe filter.

Method validation: Developed analytical method is validated for parameters as suggested by ICH Q2 (R1) and United States Pharmacopoeia.

System suitability: The system suitability test was performed in accordance with United States Pharmacopoeia [18].

Specificity: The specifity of developed LC method for all impurities was carried out by injecting placebo, known impurities of ursodeoxycholic acid (chenodeoxycholic acid, cholic acid and lithocholic acid). The diluent (blank), placebo solution, individual impurities and standard drug solution (15000 µg/mL) were injected in sequence for evaluation of specificity of proposed method. The chromatograms were monitored for any peak eluted at the retention time of drug.

Forced degradation: The forced degradation studies in acidic, alkali, oxidation and thermal condition were carried out during development. No degradation was observed which confirmed that ursodeoxycholic acid is stable at all conditions. This study also confirmed that there is no co-elution of blank, placebo or other substance with the principle peak.

Precision: Method precision was evaluated by six sample preparations as per above mentioned procedure for sample preparation of same homogeneous powdered sample of ursodeoxycholic acid tablets and calculated % recovery for ursodeoxycholic acid in each sample preparation. The % RSD for set of six preparations was calculated. The intermediate precision of the method was also evaluated using different analyst and a different instrument in the same laboratory by carrying out six sample preparations of tablets and calculated % recovery for ursodeoxycholic acid in each preparation. Calculated the % RSD for 12 results. The acceptance criteria for % RSD was not more than 2 % and the absolute difference between results by two analysts was not more than 2 %.

Recovery (accuracy): In order to evaluate the accuracy of the proposed method, recovery test was performed by adding known amount of standard solution to the placebo formulation sample, followed by analysis using the proposed chromatographic method. The recovery studies were done at three different levels at 50, 100 and 150 % with three determinations of working level concentration using standard spiking method in placebo.

All the above solutions were prepared in triplicate and were analyzed using proposed chromatographic condition. The recovery at each level was calculated by using the theoretical value from exact weight taken for spiking. The % recovery was calculated with respect to amount added. The recovery at each level should be in the range of 98 to 102 % and overall % RSD of nine results should be less than 2 %.

Linearity: The linearity plot was constructed for ursodeoxycholic acid in the concentration range of 50 to 150 % of sample concentration (15000 µg/mL). The primary stock solution of ursodeoxycholic acid working standard was prepared. From the primary stock solution, appropriate dilutions were made to get concentration of 7500, 12000, 15000, 18000 and 22500 µg/mL. The calibration curve was plotted as concentration of the respective drug solutions verses the peak area at each level. The results were statistically evaluated and correlation coefficient determination, slope and y-intercept values were calculated.

Robustness: For robustness study, the sample concentration 15000 µg/mL of ursodeoxycholic acid was used. Three

TABLE-1
FORCED DEGRADATION STUDY RESULTS

Condition	Assay (%)	Degradation (%)	Peak purity
Normal conditions	98.5	Not applicable	Pass
Acid degradation: 5 mL of 0.1 N HCl solution, heating at 70 °C for 2 h on water bath	97.0	No degradation	Pass
Alkali degradation: 5 mL of 0.1 N NaOH solution heating at 70 °C for 2 h on water bath	98.5	No degradation	Pass
Peroxide degradation: 2 mL 3 % H_2O_2 solution, heating at 60 °C for 1 h on water bath	98.1	No degradation	Pass
Thermal degradation: heating at 80 °C for 24 h	98.0	No degradation	Pass

chromatographic parameters were considered for the robustness study *viz*., (a) effect of mobile phase composition (± 2 %), (b) effect of flow rate (± 0.1 mL/min) and (c) column oven temperature (± 2 ºC).

Standard and sample solutions stability in diluent: Solution stability was carried out for sample solution (15000 g/mL) in a tightly capped volumetric flask at ambient temperature for 72 h. The sample and standard solution were injected immediately after preparation in the HPLC system considering it as an initial (0 h) as baseline.

RESULTS AND DISCUSSION

Method development: The solubility of ursodeoxycholic acid in water was found to be as 20 mg/L. The drug solution was scanned on PDA and the spectra of ursodeoxycholic acid was recorded. The wavelength 210 nm was selected which permitted the detection of ursodeoxycholic acid with adequate sensitivity. In this study, the chromatographic method optimization was carried out by utilizing different stationary phase (C8 and C18) to achieve the resolution of potential impurities from main drug.

Several different compositions of organic modifier with buffer were tried for better resolution of impurities at different pH mobile phase. Individual drug solution and known impurities was injected into column and elution pattern of ursodeoxycholic acid and its impurities was injected into column and elution pattern was studied.

Specificity: The chromatograms during specificity study (Figs. 2-4) of diluent, placebo, standard and sample solution shows that there is no co elution of any peak with ursodeoxycholic acid.

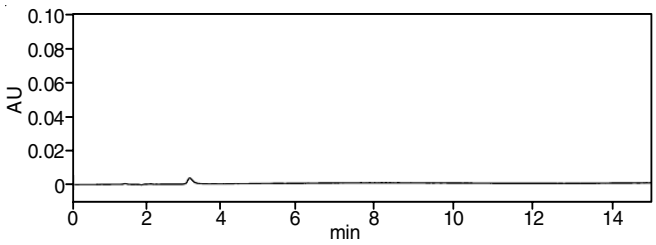

Fig. 2. Blank chromatogram

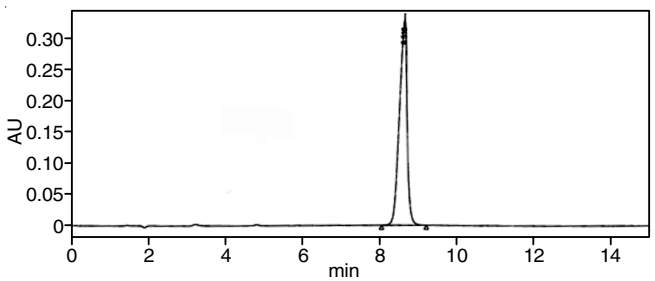

Fig. 3. Standard chromatogram

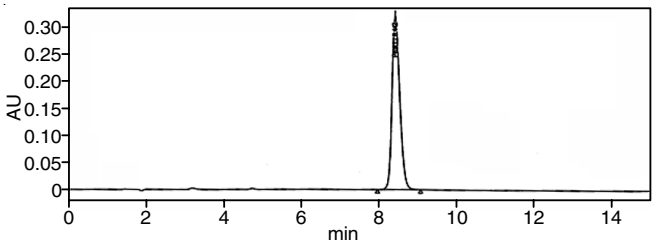

Fig. 4. Sample chromatogram

Forced degradation: During method development, forced degradation study was done to check the co-elution of any degradants along with drug peak. The study was done in different stressed conditions like acidic (0.1 M HCl), basic (0.1 N NaOH), oxidation (3 % H_2O_2) and thermal (directly exposed to heat at 80 ºC for 48 h) and the peak purity of UDCA was monitored. Peak purity of ursodeoxycholic acid peak was found to fulfill all the conditions (Table-1). The developed chromatographic method was also found to be highly specific and stability indicating for quantitative determination of ursodeoxycholic acid in in ursodeoxycholic acid tablets.

Precision: The intraday precision was evaluated by performing six (n = 6) assay determinations on same homogeneous sample of ursodeoxycholic acid tablets and the % RSD was found to be 0.44 %. The % RSD for inter-day precision for two sets (n = 12) for their % recovery was found to be 0.46 %. The absolute difference between results for intermediate precision was found 0.5 % (Table-2).

TABLE-2
PRECISION RESULTS

Sample No.	% Assay (Precision)	% Assay (intermediate precision)
1	98.9	99.9
2	99.3	100.2
3	99.2	100.1
4	99.8	99.5
5	100.1	100.5
6	99.6	99.6
Mean	99.5	100.0
% RSD	0.44	0.38
Overall mean		99.7
Over all % RSD		0.46
Absolute difference of assay results		0.5%

Accuracy: The % recovery at 50, 100 and 150 % was found to be 99.8 ± 0.3, 99.6 ± 0.45 and 99.8 ± 0.45 %, respectively (Table-3). The overall mean recovery was found to be 99.7 ± 0.4 %. The recovery results were found within acceptance criteria, thus, developed method found to be accurate for determination of ursodeoxycholic acid in ursodeoxycholic acid tablets.

TABLE-3
ACCURACY

Level	Sample No.	Recovery (%)	Mean	RSD (%)
50 %	1	99.5	99.8	0.3
	2	99.8		
	3	100.1		
100 %	1	99.1	99.6	0.5
	2	100.0		
	3	99.6		
150 %	1	99.3	99.8	0.5
	2	99.8		
	3	100.2		
Over all mean			99.7	0.4

Linearity: Linearity of the response of ursodeoxycholic acid drug against concentration at five different level was performed in the range of 7500-22500 µg/mL and response for UDCA found to be linear. The correlation coefficient (r^2) was found to be 0.9999. The linearity was found with in acceptance criteria.

Robustness: The robustness of the method was verified by making the deliberate changes in the critical chromatographic parameters. The results are presented in Table-4 and found to be within the acceptance criteria.

TABLE-4

HPLC parameter		Results (%)
Initial assay results	As per actual method	98.9
Organic modifier (acetonitrile) composition in mobile phase (± 2 %)	+2 % (52 %)	99.2
	-2 % (48 %)	99.5
Pump flow rate ± 0.1 mL/min	1.6 mL/min	98.9
	1.4 mL/min	99.0
Column oven temperature ± 2 °C	43 °C	99.1
	47 °C	99.0

Stability of sample and standard solutions: The ursodeoxycholic acid sample found to be stable up to 72 h in diluents at ambient temperature. The results after 72 h were found to be 99.8 and 99.9% for standard and sample solutions, respectively.

Application of developed method: The proposed chromatographic method was used for determination assay of ursodeoxycholic acid tablets and the results were found within the specification.

Conclusion

The proposed HPLC method is accurate, linear, precise, stability indicating and rugged methodology for quantitative determination (assay) of ursodeoxycholic acid in ursodeoxycholic acid tablets of different strengths. The results of the method validation study show that the method is accurate and linear in the range of 7500-22500 µg/mL of ursodeoxycholic acid. The analytical method validation was done as per ICH Q2(R1) and USP guidelines. This method can be used by quality control or research laboratories for quantitative determination of the content (assay) of ursodeoxycholic acid tablets of different concentration.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. G. Paumgartner and B. Ulrich, *Hepatology*, **36**, 525 (2002); https://doi.org/10.1053/jhep.2002.36088.
2. P. Angulo, *Curr. Gastroenterol. Rep.*, **4**, 37 (2002); https://doi.org/10.1007/s11894-002-0036-9.
3. N. Anderson, D. Johnston and P. Vojvodic, *J. Pharm. Biomed. Anal.*, **8**, 987 (1990); https://doi.org/10.1016/0731-7085(90)80156-J.
4. Anonymous, United States Pharmacopoeia 30–National Formulary 25 (USP 30–NF 25), United States Pharmacopoeial Convention Inc., Rockville, MD, USA, pp. 1787-1788 (2007).
5. Y. Alnouti, I. Csanaky and C. Klassen, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **873**, 209 (2008); https://doi.org/10.1016/j.jchromb.2008.08.018.
6. M. Scherer, C. Gnewuch, G. Schmitz and G. Liebisch, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*. **877**, 3920 (2009); https://doi.org/10.1016/j.jchromb.2009.09.038.
7. X. Xiang, Y. Han, M. Neuvonen and M. Niemi, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*. **878**, 51 (2010). https://doi.org/10.1016/j.jchromb.2009.11.019.
8. A. Roda, C. Cerre, P. Simonini, C Vaccari and A Pistollo, *J. Lipid Res.*, **33**, 1393 (1992).
9. M. Lu, *China Pharmacy*, **30**, 033 (2008).
10. S. Varinder, P. Kumar, S. Gurjeet, G. Shah and R. Dhawan, *J. Pharm. Res.*, **9**, 46 (2015).
11. A. Peepliwal, C.G. Bonde and K.G. Bothara, *J. Pharm. Biomed. Anal.*, **54**, 845 (2011); https://doi.org/10.1016/j.jpba.2010.10.001.
12. J. Mukherjee and T. Pal, *Int. J. Pharm. Sci. Res.*, **2**, 73 (2011); https://doi.org/10.13040/IJPSR.0975-8232.2(1).73-78.
13. O. Boscolo, S. Flor, C. Dobercky, L. Salvo, V. Tripodi, S. Lucangioli. *IOSR J. Pharm.*, **7**, 111 (2017).
14. M. Bakshi and S. Singh, *J. Pharm. Biomed. Anal.*, **28**, 1011 (2002); https://doi.org/10.1016/S0731-7085(02)00047-X.
15. M. Blessy, R.D. Patel, P.N. Prajapati and Y.K. Agrawal, *J. Pharm. Anal.*, **4**, 159 (2014); https://doi.org/10.1016/j.jpha.2013.09.003.
16. International Conference on Harmonization Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1) (2005).
17. United States of Pharmacopoeia 38 NF–33, United States Pharmacopeial Convention General chapter <1225>, Validation of Compendial Procedures, pp. 1445-1450 (2015).
18. United States of Pharmacopoeia 38 NF–33, United States Pharmacopeial Convention General Chapter <621>, Chromatography, pp. 424-434 (2015).