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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD TO DETERMINE URSODEOXYCHOLIC ACID IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

This study describes development and subsequent validation of a reversed phase high performance liquid chromatographic (RP-HPLC) method for the estimation of ursodeoxycholic acid used to solubilize cholesterol gallstones, in conventional tablet dosage formulation and in prepared dosage form. The chromatographic system was achieved in a BDS Hypersil C8 column (Thermo, 250mm x 4.6 mm, 5 μ) with an isocratic mobile phase comprising of methanol, water and phosphoric acid (77:23:0.6 v/v). Since the drug shows moderate absorption only in the short UV wavelength region (200-210 nm), quantification of the amount released from the pharmaceutical preparations by simple UV spectrophotometry is hampered by possible interferences from formulation excipients and dissolution medium components. A new method has been developed utilizing the above mobile phase and detection in refractive index (RI) detector. In this chromatographic condition ursodeoxycholic acid was eluted about 3.0 min with no interfering peaks of excipients used for the preparation of dosage forms. The method was linear over the range from 240-360 μ g/mL in raw drug ($r^2 = 0.995$). The intra-day and inter-day precision values were in the range of 0.144 - 0.332 % and 0.166 - 0.292 % respectively. Limit of detection (LOD) and limit of quantitation (LOQ) were 0.6 μ g/mL and 2 μ g/mL respectively. Results were validated according to International Conference on Harmonization (ICH) guidelines in marketed and prepared tablet formulation. The validated HPLC method is simple, sensitive, precise, accurate and reproducible.

Keywords:

Water insoluble drug,
Ursodeoxycholic acid,
Refractive index,
HPLC determination

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INTRODUCTION: Ursodeoxycholic acid (UDCA) is a white, odorless, crystalline powder with a bitter taste. Chemically it is 3 α , 7 β -dihydroxy-5-cholan-24-oic acid (**Fig. 1**). It is a water insoluble drug used as a drug for the dissolution of cholesterol gallstones^{1, 2, 3} because it reduces the cholesterol saturation of bile⁴. The use of UDCA for the treatment of other liver diseases, such as primary biliary cirrhosis, chronic hepatitis and biliary pains has been demonstrated^{5, 6, 7}. However in vivo studies have shown that intestinal absorption and consequently the bioavailability of the drug are generally poor and erratic both among different subjects, and within the same subject⁸. More than 50% is lost in the stool⁹ after a single oral dose of 300 mg.

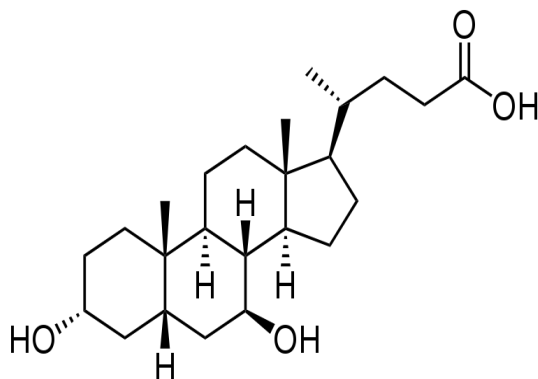


FIG. 1: URSODEOXYCHOLIC ACID (CAS NUMBER 128-13-2)

UDCA is commercially available as tablets and capsules. Since UDCA shows moderate absorption only in short UV wavelength region i.e. 200-210¹⁰, quantification of the amount released from the pharmaceutical preparations by simple UV spectrophotometry is hampered by possible interferences from formulation excipients and dissolution medium components¹¹. High Performance Liquid Chromatography (HPLC) method of its assay utilizing refractive index detector has been reported in United States Pharmacopoeia¹² using acetonitrile and water as mobile phase. Analytical determination of this drug was carried out by Giunchedi¹³ by modifying the HPLC method described earlier by Scalia¹⁴. HPLC determination of UDCA has also been reported with

Electron Capture Detector (ECD) detector¹⁵. No paper has been found to report development and validation of this drug using refractive index detector.

The objective of the present study was to develop a simple, precise, accurate and validated RP-HPLC method for the estimation and routine analysis of UDCA in raw drug and in commercially available dosage forms. This proposed HPLC assay for determination of UDCA has not yet been reported and therefore, this study claims to be a novel one. The results of the analysis were validated according to ICH guidelines¹⁶.

EXPERIMENTAL:

Chemical and Reagents: UDCA was obtained from Albert David Ltd., Kolkata, West Bengal, India. Tablets of brand Ursocol (300 mg) (Batch No. SK 93072), manufactured by Sun Pharmaceutical Industries Ltd., Silvassa, India, were procured from the local pharmacy. The tablet excipients like microcrystalline cellulose (MCC), sodium starch glycolate (SSG), Polyethylene glycol 6000 (PEG), Povidone K-30, Colloidal silicon dioxide, Magnesium stearate, Talc, ethyl cellulose, hydroxyl propyl methyl cellulose and titanium dioxide were procured from Stadmed Ltd. Kolkata. Methanol and all other chemicals of analytical grade were purchased from Merck, India. Milli-Q water was used to prepare the mobile phase preparation.

Preparation and characterization of UDCA tablets:

Tablets preparation: The immediate release UDCA tablet was done by wet granulation method. The required amount of the drug, MCC (diluent) and SSG (super disintegrant) were mixed in geometrical order. Then the binders Povidone and PEG 6000 were added above to prepare granules. These granules were dried in the oven to obtain the loss on drying value as 3.2-3.6 % and then sieved through 22# sized mesh. The granules after treating

with magnesium stearate (antiadherent) and talc (lubricant) were then compressed in CIP, 8 station, single rotary, "D"-Tooling machine of punch size 11.9 mm. The tablets were then film coated with ethyl cellulose (EC) and hydroxypropyl methyl cellulose (HPMC) mixed with the plasticizer PEG 6000 and titanium dioxide as opacifier.

Tablets characterization: The tablets obtained were convex shaped with an upper punch break line. The average weight of the tablets varied from 530 ± 7.5 % mg with thickness 4.6-4.7 mm. The disintegration test value was less than 15 minutes. Friability test data was not more than 1%.

Instrumentation and Chromatography: The HPLC system was of a Waters (USA), consisting of a solvent delivery pump (Model No. 515), a refractive index detector of Waters (Model No. 2414) with Empower 2 software for integration. Separation was achieved using a BDS Hypersil C8 column (Thermo, 250 mm x 4.6 mm, 5 μ). The isocratic mobile phase pumped at a flow rate of 1 mL/min consisted of methanol, water and phosphoric acid (77:23:0.6 v/v). The freshly prepared mobile phase was filtered through 0.45 μ m filter (Millipore, Milford, MA, and U.S.A) and degassed by sonication for 15 min. The injection volume was 25 μ L and all the separations were performed at room temperature using refractive index detector set at temperature 40°C with sensitivity 64.

Standard and Sample preparation:

Standard preparation: Stock solution (1mg/mL) of UDCA was prepared in methanol. Nine standard solutions of UDCA between 240-360 μ g/mL were prepared from the stock by subsequent dilution using the mobile phase.

Sample preparation for assay and recovery study: For the analysis of the dosage form, 10 Ursocol tablets containing 300 mg of UDCA was purchased. The tablets were weighed to calculate the average

weight of the tablet. Then the tablets were crushed and then an amount equivalent to 300 mg was taken in a 100 mL volumetric flask. Methanol (analytical grade) was added and shaken thoroughly to extract the drug from the excipients. The solution was filtered through Whatmann filter paper (No. 41). A volume of 1 mL was pipetted out and the final volume was made up to 10 mL with the mobile phase to prepare a concentration of 300 μ g/mL. The same treatment was carried out with prepared tablet dosage form. After suitable dilution, the drug concentration with six replicates for the marketed and prepared tablet dosage form was determined by HPLC using the calibration curve. The recoveries were determined by adding known amounts of UDCA reference substance (240, 300 and 360 μ g) to the formulated samples. A recovery exercise was then performed.

Method validation: The method was validated according to the ICH guidelines for the validation of analytical procedures. The parameters which were used to validate the method of analysis were linearity range, limit of detection (LOD), limit of quantification (LOQ), specificity, accuracy, precision and recovery.

RESULTS AND DISCUSSION: The chromatographic conditions were optimized and separation was performed on a BDS Hypersil C8 (Thermo) column using a mobile phase consisting of methanol, water and phosphoric acid. The proposed mobile phase composition allowed suitable retention time of UDCA and achieved good selectivity towards interference from the excipients of the formulation. Under the chromatographic conditions described, UDCA was eluted about 3.0 min. Good baselines and well shaped peak can be observed (**Fig. 3**). Calibration curve was constructed using standard UDCA solutions in the range of 240-360 μ g/mL. The linearity of the calibration curve (**Fig. 2**) was validated by high value of correlation coefficient ($r^2 = 0.995$).

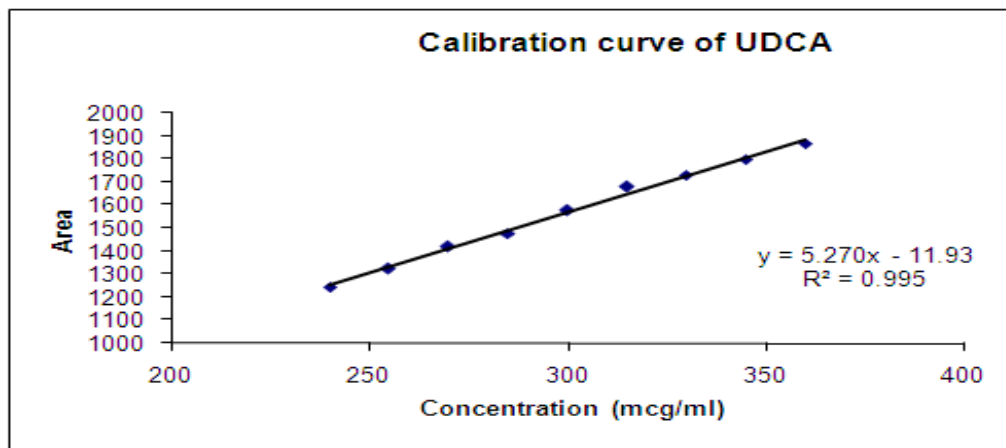


FIG. 2: LINEARITY CURVE OF URSODEOXYCHOLIC ACID

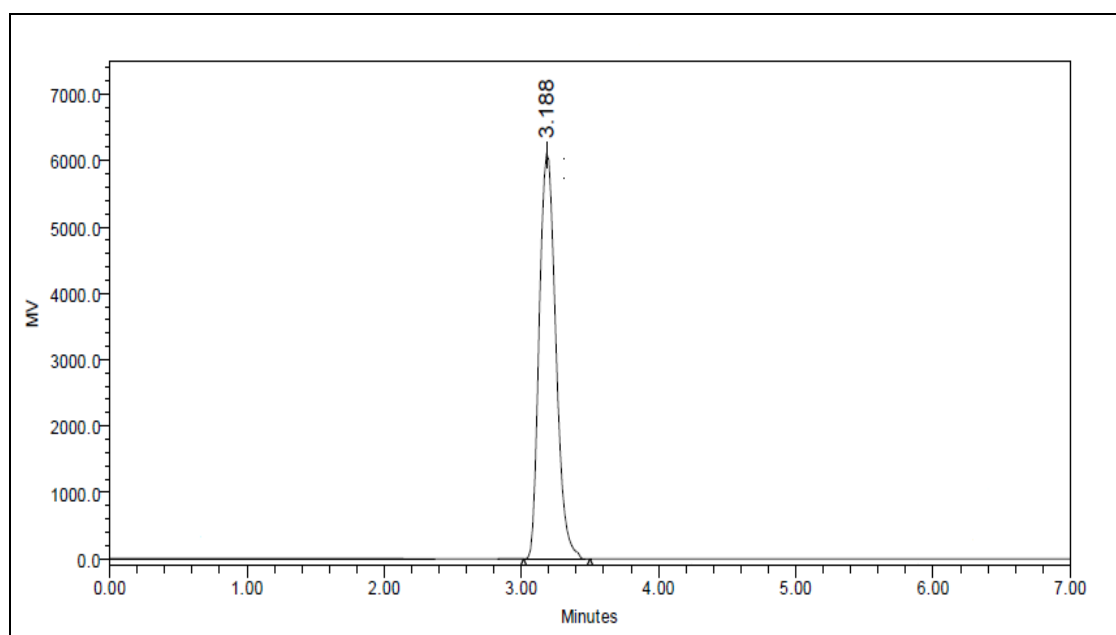


FIG. 3: CHROMATOGRAM OF URSODEOXYCHOLIC ACID IN FORMULATION WITH RT ABOUT 3.188 MINUTES

The limit of detection (LOD) and the limit of quantitation (LOQ) were 0.6 μ g/mL and 2 μ g/mL respectively. Low value of LOD and LOQ indicate the method is sensitive. The specificity of the proposed method demonstrated that the excipients present in marketed formulation and in the prepared formulation do not interfere with the drug peak. Furthermore, well-resolved peaks indicate the specificity of the method (Fig 3). Thus the proposed method is useful to quantify UDCA in different pharmaceutical formulations. The precision was determined by analyzing nine

different concentrations of the bulk drug on three different days. The intraday and interday precisions were calculated as 0.144-0.332 % and 0.166 - 0.292 % whereas the accuracy were calculated as 100.097-100.448 % and 100.073 - 100.346 % as shown in **Table 1**. The accuracy of the formulation was tested by assaying the marketed formulations and the prepared tablet dosage form on the same day and three different days. The results of formulation assay are shown in **Table 2**. Recovery results exceeded 100% for the tested formulations.

TABLE 1: INTRA DAY AND INTERDAY ACCURACY AND PRECISION

Concentration (µg/ml)	Mean (n = 6)	± S.D.*	Accuracy (%)	Precision (% R.S.D.) **	Relative error
Intra-day					
240	241.076	0.800	100.448	0.332	0.448
270	270.706	0.533	100.261	0.197	0.261
300	300.492	0.500	100.164	0.166	0.169
330	330.992	0.478	100.300	0.144	0.300
360	360.350	0.852	100.097	0.236	0.097
Inter-day					
240	240.830	0.703	100.346	0.292	0.346
270	270.540	0.650	100.200	0.240	0.200
300	300.610	0.505	100.203	0.168	0.203
330	330.897	0.549	100.272	0.166	0.272
360	360.265	0.830	100.073	0.230	0.073

* Standard Deviation ; ** Relative Standard Deviation

TABLE 2: ASSAY OF PHARMACEUTICAL FORMULATIONS

Sample (n=6)	Assay (%)	± S.D.*	R.S.D.**
Ursocol Tablets (300 mg)	102.084	1.504	1.473
Formulated Tablets (300 mg)	100.707	1.290	1.281

* Standard Deviation ; ** Relative Standard Deviation

TABLE 3: RECOVERY STUDIES IN DIFFERENT FORMULATIONS

Dosage form	Mean Recovery (n=6)	± S.D.*	R.S.D.**
Ursocol Tablets (300mg)	101.116 %	0.995	0.984
Formulated Tablets (300mg)	100.014 %	0.939	0.939

* Standard Deviation ; ** Relative Standard Deviation

CONCLUSION: The described HPLC method provides simple, sensitive, precise, accurate and reproducible quantitative method for routine analysis of UDCA in tablet dosage form. Simple sample preparation procedure and a retention time less than 5 minutes make this method unique and suitable for analyzing a number of samples in a short period. The formulations assays were within the acceptable limit. High percentage of recovery (Table 3) ensures the method to be free from interference of excipients and additives, used in the formulations.

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