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DEVELOPMENT AND VALIDATION OF RP-LC-UV METHOD FOR DETERMINATION OF URSODEOXYCHOLIC ACID IN DRUG SUBSTANCE AND DRUG PRODUCT

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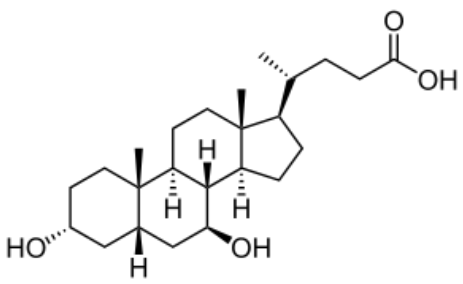
ABSTRACT

The present work is undertaken with an aim to develop and validate an accurate, precise and rapid method for the estimation of Ursodeoxycholic Acid in tablet and bulk form. The method is simple, accurate and sensitive. The separation was achieved on high pressure liquid chromatography shimadzu HPLC LC-2010with UV detector, a Reverse Phase C-18 Column (25 cm x 4.6 mm) i.e., particle size 5 µm) was used The HPLC system was equipped with the software LC solutions. Flow Rate: 1.0 ml/min Injection Volume: 50µl, Run Time: 10 min, coloum temp: 40ºC. The method was validated for specificity, precision, linearity, range, accuracy and robustness. The recovery range for Ursodeoxycholic acid is in the range of 99.94–100.0% and the method can be successfully applied for the routine analysis of the drug substance.

INTRODUCTION:

Ursodeoxycholic acid also known as ursodiol and the abbreviation is UDCA. Ursodeoxycholic acid, (UDCA) is a naturally occurring bile acid found in small quantities in normal human bile and in larger quantities in the biles of certain species of bears. It is a bitter-tasting white powder consisting of crystalline particles freely soluble in ethanol and glacial acetic acid, slightly soluble in chloroform, sparingly soluble in ether, and practically insoluble in water. The chemical name of ursodiol is 3a, 7ß-dihydroxy-5ß-cholan-24-oic ($C_{24}H_{40}O_4$). Ursodiol has a molecular weight of 392.56. (a) Its structure is shown below (b) IUPAC: 3a, 7ß-dihydroxy-5ß-cholan-24-oic
(c) Appearance: It is a bitter-tasting white powder (d) Molecular weight: 392.56 (e) Molecular formula: $C_{24}H_{40}O_4$ (f) Solubility:-freely soluble in ethanol and glacial acetic acid, slightly soluble in chloroform, sparingly soluble in ether, and practically insoluble in water (g) Melting point range: 203ºC – 204ºC (h) P ka: 4.66 (i) Heat of fusion: - 36.9 kJ/mol (j) Storage: Store in a tightly closed container. Store in a cool, dry area away from incompatible substances (k) Stability: Stable under normal temperatures and pressures. (l) Dose: Primary Biliary Cirrhosis Adult: 10- 15 mg/ Kg daily in 2-4 divided doses Prevention of gallstones adult: 300 mg B.I.D (m) Hepatic impairment: Chronic liver disease (except primary biliary cirrhosis): use with caution. (n) Administration should be taken with food.



2.0 EXPERIMENTAL:

O-Phosphoric acid:AR grade, Methanol: HPLC grade , Acetonitrile:HPLC grade Water: HPLC or equivalent grade

2.1 Standards and Samples:

Ursodeoxycholic acid, Ursodiol Capsules

2.2 Reagent preparation:

2.2.1Preparation of 0.1% ortho phosphoric acid: Transfer 1mL of ortho phosphoric acid (85%) into 1000mL volumetric flask containing about 300 mL of water, dilute to volume with water and mix well.

2.2.2Preparation of mobile phase: Mix 550mL of acetonitrile and 450mL of 0.1% ortho phosphoric acid mixed. Filter if necessary and degas.

2.2.3Preparation of diluent/blank: Methanol and water mixed in the ratio of 700:300

2.3Chromatographic conditions: Column : Waters symmetry 5µ C18, 25cm×4.6mm.
Detector: 205 nm
Flow rate: 1.0 mL/ minute
Injection volume: 50 µL
Column oven temperature: 40^0C
Sampler temperature: 25^0C
Run time: 10 minutes

2.4Preparation of Standard solution:

Weigh accurately and transfer 100mg of Ursodeoxycholic acid working standard into a 200 mL volumetric flask. Add 70mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix.

2.5Preparation of Sample solution:

Weigh accurately and transfer powder equivalent to 100mg of Ursodeoxycholic acid into a 200 mL volumetric flask. Add 70mL of diluent and sonicate for 20 minutes with intermediate shaking then cool to room temperature and dilute to volume with diluent and mix then filter the solution by using Nylon filter.

3.0 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, specificity, accuracy, precision and recovery, solution stability.

3.1 System suitability: To verify that analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set.

3.2 Specificity: Specificity is the ability of analytical method to assess unequivocally the analyte in the presence of component that may be expected to be present, such as impurities,degradation products and matrix components. Performed the specificity parameter of the method by injecting Blank (Diluent), placebo, standard preparation and sample preparation into the HPLC system. Record the retention times of Blank (Diluent), placebo, standard preparation and sample preparation.

3.2.1 Specificity by degradation studies: Force Degradation of Ursodiol capsules 300mg shall be carried out, to confirm that during stability study or throughout the shelf life, any degradation product if found should not interfere with the main peak of Ursodiol. In addition, the forced degradation study will help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, water hydrolysis and dry heat) for each of the degradants.

3.2.2 Sample preparation: Weighed and transferred 100mgequivalent of sample fill into a 200 mL volumetric flask, added about 70mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.2.3 Placebo preparation: Weighed and transferred 100mgequivalent of sample fill into a 200 mL volumetric flask, added about 70mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

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3.2.4 Acid stressed sample preparation (1.0 N HCL): Weighed and transferred 100mg equivalent of sample fill into a 200 mL volumetric flask, added 5 mL of 1.0 N HCL kept on water bath at 80^0C for 4 hours. Neutralized with 5mL of 1.0N NaOH. Added about 70 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

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3.2.5 Alkali stressed sample preparation (1.0N NaOH): Weighed and transferred 100mg equivalent of sample fill into a 200 mL volumetric flask, added 5 mL of 1.0N NaOHkept on water bath at 80^0C for 4 hours. Neutralized with 5 mL of 1.0 N HCl Added about 70 mL of diluent, sonicated to dissolve for 20 minutes

with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.2.6 Peroxide stressed sample preparation (3.0% v/v H_2O_2): Weighed and transferred 100mg equivalent of sample fill into a 200 mL volumetric flask, added 5 mL of 3.0% v/v H_2O_2kept on water bath at 80^0C for 2 hours. Added about 70 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.2.7 Neutral stressed sample preparation: Weighed and transferred 100mg equivalent of sample fill into a 200 mL volumetric flask, added 5 mL of water kept on water bath at 80^0C for 2 hours. Added about 70 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.2.8 Thermal stressed (Dry heat) sample preparation: Weighed and transferred 100mg equivalent of sample fill into a 200 mL volumetric flask heated in hot air oven for 4 hours at 80ºC. Removed and cooled to room temperature. Added about 70 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well then filter the solution by using Nylon filter.

3.3 PRECISION: The precision of an analytical method is the degree of agreement among individual test result when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation(coefficient of variation) of series of measurements.

3.3.1 System precision:
The system precision is checked by using standard chemical substance to ensure that the analytical system is working properly. The retention time and area of six determination should be measured and calculate relative standard deviation. Injected Blank, Standard preparation six times into the chromatograph. Record the chromatograph. Calculate the relative standard deviation.

3.3.2 Method precision:
In method precision, a homogenous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results of a single batch. Analyzed the sample of Ursodiol Capsules 300mg six times of a same batch as per analytical procedure. Calculated the % Assay of Ursodiol with respect to standard preparation.

3.3.3 Intermediate precision: The intermediate precision was carried out to ensure that the analytical results will remain unaffected with change in instrument, analyst, column and day. Repeated the method precision set by different analyst by using different instrument and different column on different day at different location.

3.4 Accuracy: The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Performed accuracy in different levels, at each level in triplicate ursodiol at 50%, 100%, and 150%. Analysed these samples in triplicate for each level and calculated the % recovery.

3.5 Linearity: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Performed the linearity using ursodiol standard in the range of 50% to 150% concentration.

3.6 Range: The range of analytical method is the interval between the upper and lower levels of analyte that has been demonstrated with a suitability and linearity.

3.7 Robustness: The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

3.7.1Robustness parameters:
Change in column temperature $\pm5^0$C
Change in flow rate ±0.2 mL/min
Change in organic phase ±5%

Figure 1: Linearity graph of Ursodeoxycholic acid standard

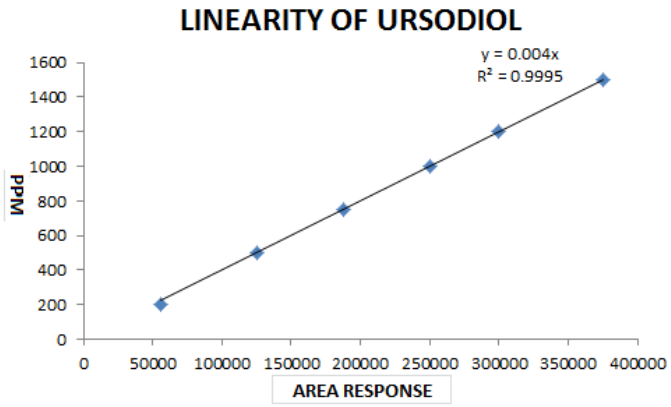

Table 1: Results of Accuracy (% Recovery)

S.no	Levels	Mg added	Mg recovered	% Recovery	Mean %	% RSD
1	50%	50.12	50.13	100.02	99.94	0.03
2		50.49	50.42	99.86		
3		50.35	50.32	99.94		
1	100%	100.25	100.22	99.97	100.00	0.01
2		100.19	100.29	100.09		
3		100.89	100.82	99.93		
1	150%	150.78	150.56	99.98	99.99	0.04
2		150.10	150.12	100.01		
3		150.46	150.42	99.97		

Table 2: Results of Method precision and intermediate precision results:

Parameter	Sample	% Assay of Ursodiol
Method precision	1	99.1
	2	99.9
	3	100.5
	4	98.9
	5	99.7
	6	98.7
Intermediate precision	1	100.2
	2	98.5
	3	98.9
	4	100.7
	5	99.8
	6	99.6
Mean of 12 determinations		99.5
RSD for 12 determinations		0.6%

Table 3: Assay of stressed sample:

Ursodiol Capsules USP 300 mg	
Stressed condition	%Assay
Sample as such	99.9
Acid (1.0N HCL) Stressed sample	89.23
Alkali (1.0N NaOH) Stressed sample	86.25
3% v/v peroxide Stressed sample	97.53
Neutral Stressed sample	98.56
Thermal Stressed sample	98.65

Table 4: Results of Robustness:

S.no	Parameter	Tailing factor	Theoretical plates	%RSD
1	Original condition	1.01	21045	0.1
2	Increase in flow rate	1.10	21096	0.2
3	Decrease in flow rate	0.99	20458	0.4
4	Increase in column temperature	0.99	21563	0.2
5	Decrease in column temperature	1.05	21789	0.6
6	Increase in organic phase ratio	1.02	20458	0.2
7	Decrease in organic phase ratio	1.03	21532	0.4

Table 5: Results of Filter compatibility:

Filters	%Difference	% Assay
Centrifuged sample	---	99.9
0.45µ Nylon filter	-0.9	100.2
0.45µ Nylon filter + prefilter	1.0	98.9
Millipore	0.8	99.1
PVDF filter (MDI)	-0.1	100.0
PVDF filter (PALL)	-0.2	100.2

Table 6: Results of Mobile phase stability:

S.no	Acceptance criteria	Day-1	Day-2	Day-3
1	The %RSD for 5 replicate injections of standard solution should be not more than 2.0%	0.5%	0.7%	0.6%
2	The tailing factor should not be more than 2.0	1.0	1.02	1.03
3	The theoretical plates should not be less than 2000	21025	21562	20986

Table 7: Results of Stability in analytical solution:

Sl.No	Time in Hours	% Difference in standard solution	% Difference in sample solution
1	1	0.1	0.2
2	2	0.1	0.1
3	4	0.2	0.3
4	8	0.1	0.3
5	16	0.3	0.5
6	20	0.4	0.7
7	24	0.6	1.0
8	26	0.4	1.8
9	28	0.5	2.1
10	30	0.9	2.4

3.8 ADDITIONAL PARAMETERS:

3.8.1 Filter compatibility studies:

3.8.1.1 Sample preparation: Weighed and transferred 100mg equivalent of sample fill into a 200 mL volumetric flask, added about 70 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well. Centrifuged a portion of this mixture at 4000 rpm for 5 minutes.

3.8.1.2 Placebo preparation: Weighed and transferred 100mg equivalent of placebo into a 200 mL volumetric flask, added about 70 mL of diluent, sonicated to dissolve for 20 minutes with intermittent shaking, cooled the sample solution to room temperature, diluted to volume with diluent and mixed well. Centrifuged a portion of this mixture at 4000 rpm for 5 minutes.

3.8.1.3 Centrifuged sample and placebo: Centrifuged sample and placebo preparation at 4000 rpm for 5 minutes. Collected the supernatant sample and placebo preparation in separate test tubes.

3.8.1.4 Sample and placebo filtered through 0.45µm Nylon filter: Filtered sample and placebo centrifuged solution through 0.45µm Nylon filter. Collected the sample and placebo preparation in separate vials.

3.8.1.5Sample and placebo filtered through Nylon + pre filter: Filtered sample and placebo centrifuged solution through Nylon + pre filter. Collected the sample and placebo preparation in separate vials.

3.8.1.6 Sample and placebo filtered through Millipore filter: Filtered sample and placebo centrifuged solution through Millipore filter. Collected the sample and placebo preparation in separate vials.

3.8.1.7 Sample and placebo filtered through PVDF filter (MDI): Filtered sample and placebo-centrifuged solution through PVDF filter (MDI). Collected the sample and placebo preparation in separate vials.

3.8.1.8 Sample and placebo filtered through PVDF filter (PALL): Filtered sample and placebo-centrifuged solution through PVDF filter (PALL). Collected the sample and placebo preparation in separate vials.

3.9 Stability in analytical solution: Evaluated the stability in analytical solution by injecting the standard solution and sample solution at regular interval.

4.0 Mobile phase stability: Evaluated the stability of mobile phase by injecting the standard preparation in conjunctive days and checked the system suitability parameters.

5.0 RESULTS AND DISCUSSION: The chromatographic conditions were optimized and separation was performed on a Waters symmetry 5µ C18, 25cm×4.6mm column using a mobile phase consisting 550mL of acetonitrile and 450mL of 0.1% ortho 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 4.0 min. Good baselines and well-shaped peak can be observed. Calibration curve was constructed using standard UDCA solutions in the range of 50-150 % of test concentration. The linearity of the calibration curve was validated by high value of correlation coefficient (r2 = 0.9995).

6.0 CONCLUSION

The proposed new HPLC method described in this paper provides a simple, convenient and reproducible approach for the identification and quantification of UDCA in bulk, and pharmaceutical formulations with good presentation. Analytical results are specific, accurate and precise values. In short, the developed method is simple, sensitive, easy and efficient having small chromatographic time and can be used for routine analysis.

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