Research Article **ISSN: 2395 – 5597**

International Journal of Engineering and Robot Technology

Journal home page: www.ijerobot.com

https://doi.org/10.36673/IJEROBOT.2021.v08.i02.A09

VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF URSODIOL IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A new sensitive, specific, linear, precise and accurate RP-HPLC method was developed and validated for estimation of Ursodiol in Bulk and Tablet dosage form. An isocratic, RP- HPLC method was developed. Shimadzu shim pack C18 (250mm x 4.5µm, x 5µ) column. Shimadzu Prominence-I LC-2030C plus equipped with Auto sampler as the instrument model. Mobile phase consists of mixture of Methanol: Ethyl acetate: Water in the ratio (50:30:20 v/v) at a flow rate of 1.0ml /min with injection volume of 10µL. UV detection was performed at 243 nm. The Linearity was established for Ursodiol in the range of 5-30µg/ml with correlation coefficient of 0.9995. LOD and LOQ were found to be 0.011µg/ml and 0.034µg/ml respectively. Retention time of Ursodiol were found to be 2.811 mins. The % Recovery was found to be 99.20-101.69 and %RSD was found with in ± 2. The method has been validated according to ICH guidelines for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed validated method was successfully applied for reliable quantification of Ursodiol in bulk and pharmaceutical dosage form.

KEYWORDS

Ursodiol, RP- HPLC, Validation and Pharmaceutical formulations.

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INTRODUCTION

Ursodeoxycholic acid (UDCA) also called as ursodiol and it is a secondary bile acid, produced in humans and most other species from metabolism by intestinal bacteria. It was synthesized in the liver in some species and it was first identified in bear bile, which is the derivation of its name Ursus. In purified form, it is used to treat or prevent several diseases of the liver or bile ducts[1].

Literature survey revealed that there were few analytical methods have been reported for the

determination of the Ursodiol in pure drug and pharmaceutical dosage form by using UV-Spectrophotometric[2-6], RP-HPLC[7-14] so far.

The aim of the present work is to develop and validate a novel, rapid, precise and specific Area under curve UV spectrophotometric method for estimation of Ursodiol in bulk and tablet dosage form.

MATERIAL AND METHODS

Material and reagents

The Ursodiol were obtained as a gift sample from Shilpa Medicare Ltd, Dabaspet, Bengaluru and its pharmaceutical dosage form Ursodiol 20 tablet labelled claim 150mg from local pharmacy manufactured by Synokem Pharma India Ltd. Methanol, Ethyl acetate anddistilled water were obtained Bharathi College of pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, India. All chemicals used are of HPLC grade. Distilled water was used throughout the experiment.

Instrumentation

Chromatographic separation was performed on a Shimadzu Prominence-i LC-2030 plus equipped with Auto sampler comprising a variable wavelength programmable UV detector. Shimadzu shim pack C18 (250mm x 4.5µm x 5µ) column is used.

Preparation of solutions Mobile phase preparation

The Mobile phase consisted of a mixture of Methanol (50%), Ethyl acetate (30%), Water (20%) in the ratio of 50:30:20 v/v, which was filtered through a membrane and degassed before use.

Preparation of sample Standard Solution

The formulation tablets of Ursodiol (Ursocad 150mg) were crushed to give finely powdered material. From the Powder prepared a 100mg of Ursodiol was accurately weighed, transferred in a 100ml volumetric flask, add 30ml of diluents and sonicate to dissolve and dilute to volume with diluent. Transfer 10mL of standard stock solution into 100ml volumetric flask and dilute to volume with diluent. And an appropriate concentration of sample was prepared at the time of analysis. 10µl of these solutions were injected in triplicate into HPLC system and the peak areas were recorded.

Preparation of Standard solution

10mg of Ursodiol was dissolved in 10ml of methanol in 10ml volumetric flask (1000µg/ml). Further dilution was made from above in such a way that the final concentration consists of 5, 10, 15, 20, 25, and 30µg/ml.

System suitability requirements from stock and standard solutions

Tailing factor: NMT 2.0

Theoretical Plates: NLT 2000

RESULTS AND DISCUSSION

Validation of the proposed method

The proposed method was validated as per ICH guidelines[15-17]. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity

From the chromatograms of blank and standard (Prepared from Formulation). It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention time. The specificity results are shown in Table No.2.

Linearity

The linearity of the response of the drug was verified at six concentration levels, ranging from 5-30µg/ ml of Ursodiol in each linearity level were prepared. 10µl of each concentration was injected into the HPLC system. The response was read at 243nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were presented in Table No.3.

Precision

Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision, six replicate standard solutions (20µg/ml) of Ursodiol were injected. % RSD was calculated and it was found to be 0.490 and interday precision done same as intraday, six replicate standard solutions (20µg/ml) of Ursodiol were injected. % RSD was calculated and it was found to be 0.429 which are well within the acceptable criteria of not

more than 2.0. Results of system precision studies are shown in Table No.4.

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (150 mg of Ursodiol). The recovery values for Ursodiol ranged from 98.0 to 102.0%. The average recoveries of three levels of Ursodiol were found to be 99.20-101.69%. The results are shown in the Table No.5.

Limit of detection and Limit of quantification

The limit of detection is an analytical method is the smallest amount of analyte in a sample which can be reliable detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of the analyte in sample which can be quantitatively determined. LOD and LOQ were calculated using formula LOD = 3.3(SD)/S and LOQ = 10(SD)/S. Results were shown in Table No.6.

Ruggedness

The ruggedness of test method was demonstrated by carrying out precision study in six preparations of sample on a single batch sample by different analysts, the results of the precision study are tabulated as below Table No.7. The % RSD values are less than 2.

Robustness

Robustness is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in the procedure. The robustness of the method was evaluated by analysing the system suitability standard and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate (±0.2ml/min), column temperature (±5C) and detection wavelength (±2nm) shown in Table No.8.

Acceptance criteria

System suitability should pass as per test method at variable conditions.

CHROMATOGRAPHIC CONDITIONS

Table No.1: HPLC method development parameters

S.No	HPLC method development parameters	
1	Column	C18, 250nm X 4.5µm, 5µ
2	Flow rate	1.0ml /min
3	Wavelength	243nm
4	Column temperature	Ambient
5	Injection volume	10µL
6	Run time	6minutes
7	Diluents	Mobile phase
8	Elution	Isocratic

Table No.2: Specificity of Ursodiol

S.No	Name of the solution	Retention time in min
1	Blank	0
2	Ursodiol (Standard)	2.811

Table No.3: Linearity of Ursodiol

S.No	Concentration (µg/ml)	Peak area* (mv)
1	5	25912
2	10	52996
3	15	78486
4	20	103841
5	25	128401
6	30	157356

*Average of six determinations

Table No.4: Results of precision of ursodiol

S.No	Intraday Names	Studies Peak area	Interday Names	Studies Peak area
1	Injection-1	103841	Injection-1	104016
2	Injection-2	103268	Injection-2	103422
3	Injection-3	102973	Injection-3	102794
4	Injection-4	104264	Injection-4	103264
5	Injection-5	103632	Injection-5	103818
6	Injection-6	104185	Injection-6	103185
7	AVG	103693.83	AVG	103416.5
8	STDEV	508.478	STDEV	443.844
9	%RSD	0.490	%RSD	0.429

Table No.5: Results of recovery of Ursodiol

S.No	Level of addition/ %	Amount added (µg/ml)	Amount found	%Recovery ±Standard deviation*	%RSD
1	50	10	30.49	101.69±0.208	0.204
			30.46		
			30.58		
2	100	20	39.96	99.99±0.329	0.329
			40.18		
			39.85		
3	150	30	49.61	99.20±0.660	0.665
			49.27		
			49.93		

*Average of three determinations

Table No.6: System suitability parameters

S.No	Parameters	Rosuvastatin calcium
1	Linearity	5-30µg/ml
2	Regression equation	y = 5193.1x +286.33
3	Correlation coefficient	R^2 = 0.9995
4	Retention time	2.811min
5	Run time	6min
6	Limit of detection (LOD)	0.011µg/ml
7	Limit of quantification (LOQ)	0.034µg/ml
8	Tailing factor	1.18
9	Theoretical Plate	5349

Table No.7: Results of ruggedness of ursodiol

I. By changing the Analysts

S.No	Concentration	T1	T2	Mean	SD	%RSD
1	5	25912	25648	25780	186.67	0.72
2	10	52394	52649	52521.5	180.31	0.34
3	15	78976	78524	78750	319.61	0.40
4	20	104426	104789	104607.5	256.67	0.24
5	25	128901	128614	128757.5	202.93	0.15
6	30	157556	157268	157412	203.64	0.12

*Average of three determinations

II. By changing the instrument

S.No	Concentration	T1	T2	Mean	SD	%RSD
1	5	25901	25648	25774.5	178.89	0.69
2	10	52550	52780	52665	162.63	0.30
3	15	78865	78435	78650	304.05	0.38
4	20	104516	104694	104605	125.86	0.12
5	25	128812	128523	128667.5	204.35	0.15
6	30	157646	157356	157501	205.06	0.13

* Average of three determinations

Table No.8: Robustness results for Ursodiol

S.No	Parameters	Conditions	Tailing Factor	% RSD
1	Column Temperature	Decreased (-5°C)	1.13	0.30
		Increased (+ 5°C)	1.011	
2	Flow rate (ml/min)	Decreased (-2min/min)	1.18	0.56
		Increased (+2 min/min)	1.012	
3	Wavelength	Decreased(-2nm)	1.016	0.98
		Decreased(+2nm)	1.17	

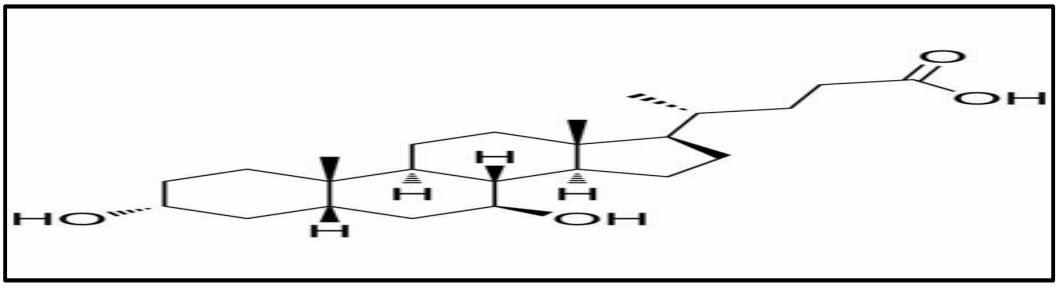

Figure No.1: Chemical structure of Ursodiol

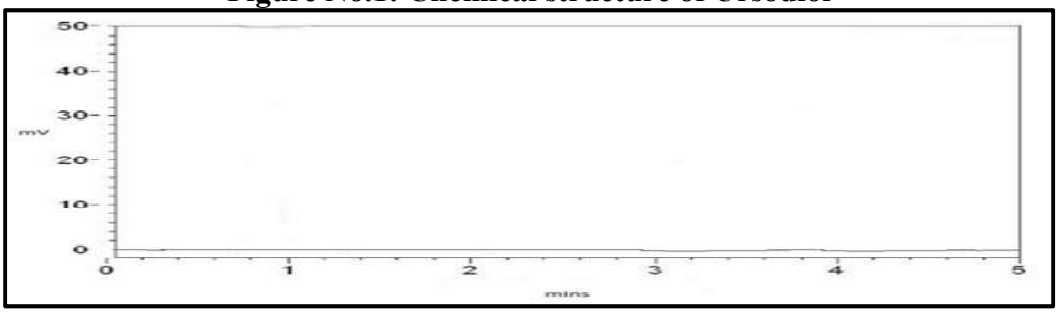

Figure No.2: Chromatogram of blank solution of Ursodiol

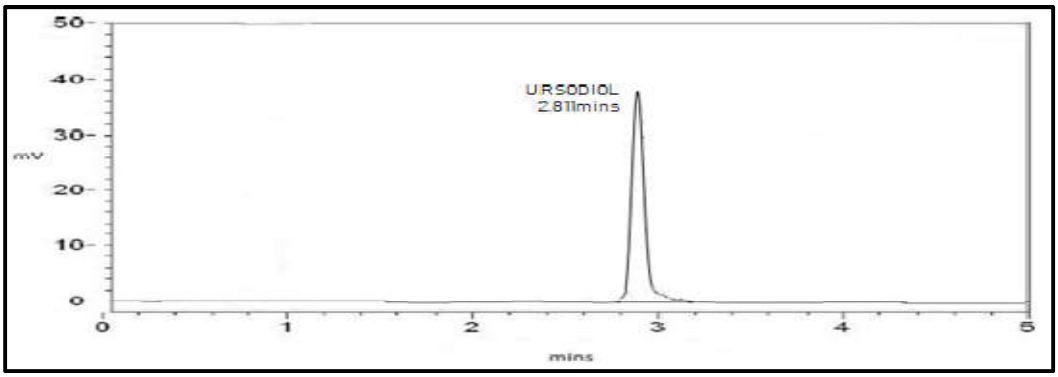

Figure No.3: Chromatogram of standard solution of Ursodiol

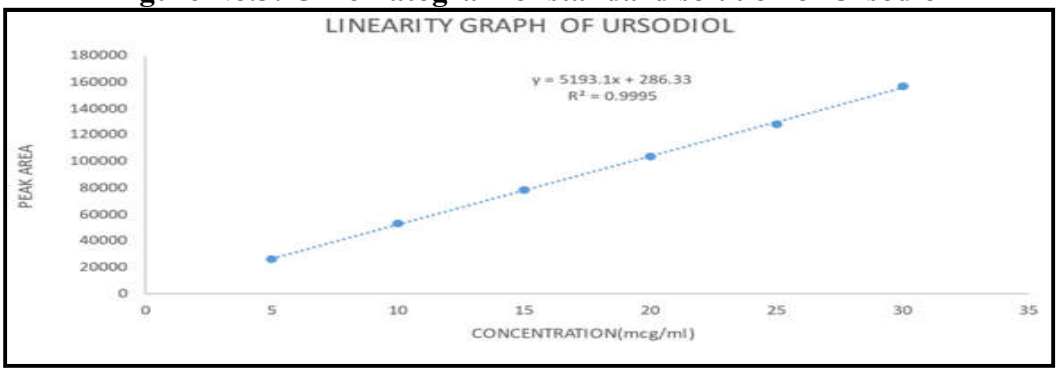

Figure No.4: Linearity of Ursodiol

CONCLUSION

The present analytical method was validated as per ICH guidelines and met the acceptance criteria. It was concluded that the developed analytical method was simple, accurate, economical and sensitive, and can be used for routine analysis of Ursodiolin bulk drug and pharmaceutical dosage forms.

ACKNOWLEDGEMENT

We authors wish to thank our management, principal of Pharmacy College for providing all facilities in the College.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Karthik N R *et al*. Validated RP-HPLC method for the estimation of ursodiol in bulk and tablet dosage form, *International Journal of Engineering and Robot Technology,* 8(2), 2021, 72-78.