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VALIDATED SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATION OF URSODIOL IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Simple, precise and accurate area under curve spectroscopic method has been developed and validated for the estimation of Ursodiolin bulk and pharmaceutical dosage form. The drug shows maximum absorption (λ_{max}) at 240nm in Methanol: Acetonitrile: O-phosphoric acid (40:40:20) solution and Area under Curve [AUC] in absorption spectra were measured between the wavelength range 235 to 245nm which obeys Beer's law in the concentration range of 2-10µg/ml. The linearity study was carried out and regression coefficient was found to be 0.9999 and it has showed good linearity, precision during this concentration range. The % recovery was found to be 98.25-99.55. The LOD and LOQ were found to be 0.154 and 0.469µg/ml. The percentage relative standard deviation is found to be less than 2. According to ICH guidelines the method have been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for routine quantitation of Ursodiolin bulk and pharmaceutical dosage form.

KEYWORDS

Ursodiol, Area under curve spectroscopy, 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 Ursodiol in pure drugand

pharmaceutical dosage forms by using UV spectrophotometric[2-6] and HPLC[7-14] so far.

The aim of present work is to develop and validate a novel, rapid, simple, precise and specific Area under curve Spectrophotometric method for estimation of Ursodiolin bulk and tablet dosage form.

MATERIAL AND METHODS

Instrument

Ultraviolent-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken on weighing balance.

Chemicals

Ursodiolpure drug was obtained as a gift sample fromShilpa Medicare Ltd Dabaspet, Bengaluru and its pharmaceutical dosage form Ursodiol 20 tablet labelled claim 150mg from local pharmacy manufactured by Synokem Pharma India Ltd.

Solvent

Methanol: Acetonitrile: O-Phosphoric acid (40:40:20) used as a solvent.

Selection of analytical wavelength

Appropriate dilutions of Ursodiol were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400nm. Area under Curve [AUC] in absorption spectra were measured between the wavelength range 235 to 245nm as the wavelength for detection (Figure No.2).

Preparation of 1M O-Phosphoric acid

68ml of o-phosphoric acid is transferred into 1000ml volumetric flask and make up the volume up to the mark with distilled water.

Preparation of standard stock solution:100mg of Ursodiol was weighed accurately and transferred in to 100ml volumetric flask and diluted in Methanol: Acetonitrile: O-Phosphoric acid (40:40:20) up to mark. From this, the solution was further diluted into 100µg/ml and pipette out 0.2, 0.4, 0.6, 0.8 and 1.0ml into 10ml individual volumetric flask and diluted in Methanol: Acetonitrile: O-phosphoric acid (40:40:20) up to mark, this gives 2, 4, 6, 8 and 10µg/ml concentration.

Preparation of sample solution

20 tablets of Ursodiol marketed formulations were weighed and powdered. A quantity of tablet powder equivalent to 100mg of Ursodiol was transferred into a 100ml of volumetric flask then it was diluted with Methanol: Acetonitrile: O-Phosphoricacid (40:40:20)and made up to the mark.

Method and validation

The method was validated according to ICH guidelines.

RESULTS AND DISCUSSION

Method

Area under curve spectroscopy.

Linearity

The linearity of an analytical method is its dimensions to show the test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 2-10µg/ml and Area under Curve [AUC] in absorption spectra were measured between the wavelength of 235 to 245nm as absorbance values are shown in Table No.1 (Figure No.3). The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in (Figure No.4). Statistical variables like slope, intercept, regression equation, correlation coefficient and Sandell's sensitivity were determined. (Table No.2).

Precision

The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the equivalent sample. Precision is determined by intra-day and inter-day study. Intra-day precision was determined by analysing the same concentration for six times in a same day. Inter-day precision was determined by analysing the same concentration daily for six days. (Table No.3).

Accuracy

The accuracy of an analytical method says that closeness of test results obtained by that method to the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels as 50%, 100% and

150%. In which the formulation concentration hold constant and varied pure drug concentration. (Table No.4).

Ruggedness

The ruggedness is defined as the reproducibility of results when the method is performed under the different in conditions. This includes distinct analyst, laboratories, instruments, temperature etc. Ruggedness was determined between different analyst; the value of %RSD was found to be less than 2. (Table No.5).

LOD and LOQ

The limit of detection is an individual analytical method is the smallest amount of analyte in a sample which can be reliably detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of analyte in a sample which can be quantitatively determined. LOD and LOQ was calculated by using following formula.

LOD = 3.3(SD)/S and LOQ = 3(LOD)

LOD and LOQ value of were found Rosuvastatin calcium be 0.154 and 0.469µg/ml.

Table No.1: Results of calibration curve at 235-245nm by area under curve method

S.No	Concentration in µg/ml	Absorbance ± Standard deviation*
1	0	0
2	2	0.144±0.000687
3	4	0.296±0.000898
4	6	0.442±0.00249
5	8	0.596±0.00124
6	10	0.747±0.00242

*Average of six determinations.

Table No.2: Regression parameter for Ursodiol at 235-245nm by area under curve method

S.No	Regression parameter	Results
1	Range (µg/ml)	2-10
2	Detection Wavelengths (nm)	235/245
3	Regression Equation	Y= 0.00748x+0.0032
4	Slope (b)	0.00748
5	Intercept (a)	0.0032
6	Correlation Coefficient (r^2)	0.9999
7	Sandell's equation	0.013
8	Limit of detection (µg/ml)	0.154
9	Limit of quantitation (µg/ml)	0.469

Table No.3: Determination of precision results for Ursodiol at 235-245nm by area under curve method

S.No	Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD **	Inter-day Absorbance ±Standard deviation*	%RSD **
1	2	0.144±0.0017	1.18	0.144±0.0013	0.9
2	4	0.295±0.0017	0.57	0.296±0.001	0.33
3	6	0.442±0.0017	0.38	0.442±0.0021	0.47
4	8	0.596±0.0013	0.22	0.596±0.0016	0.26
5	10	0.749±0.0016	0.22	0.745±0.0013	0.17

*Average of six determinations, **percentage relative standard deviation.

Table No.4: Determination of accuracy results for Ursodiol at 235-245nm by area under curve method

S.No	Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
1	50	6	3	8.91	98.95 ±0.377	0.381
2	100	6	6	11.84	98.68 ±0.397	0.402
3	150	6	9	14.8	98.69 ±0.234	0.237

*Average of six determinations, **percentage relative standard deviation.

Table No.5: Determination of Ruggedness results for Ursodiol at 235-245nm by area under curve method

S.No	Analysts	Analyst 1	Analyst 2
1	Mean absorbance	0.445	0.444
2	±Standard deviation*	0.002115	0.002672
3	%RSD	0.475	0.601

*Average of six determinations, **percentage relative standard deviation.

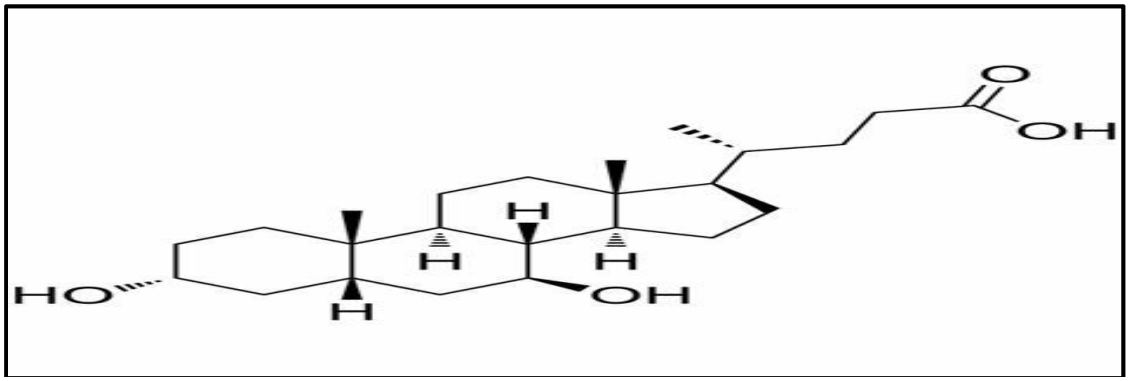

Figure No.1: Chemical structure of Ursodiol

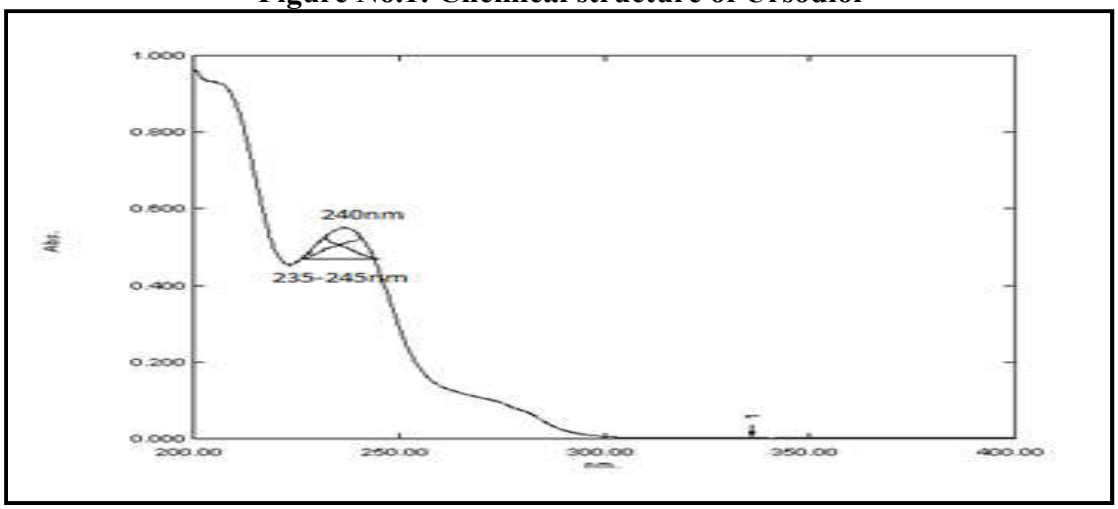

Figure No.2: Area under curve spectrum of ursodiol at 237-247nm

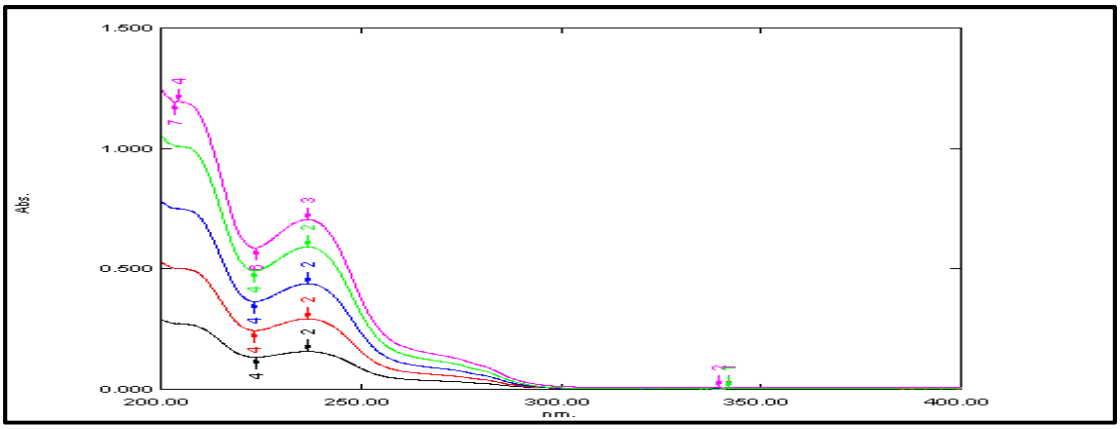

Figure No.3: Area under curve overlain spectra of ursodiol showing absorbance at235-245nm

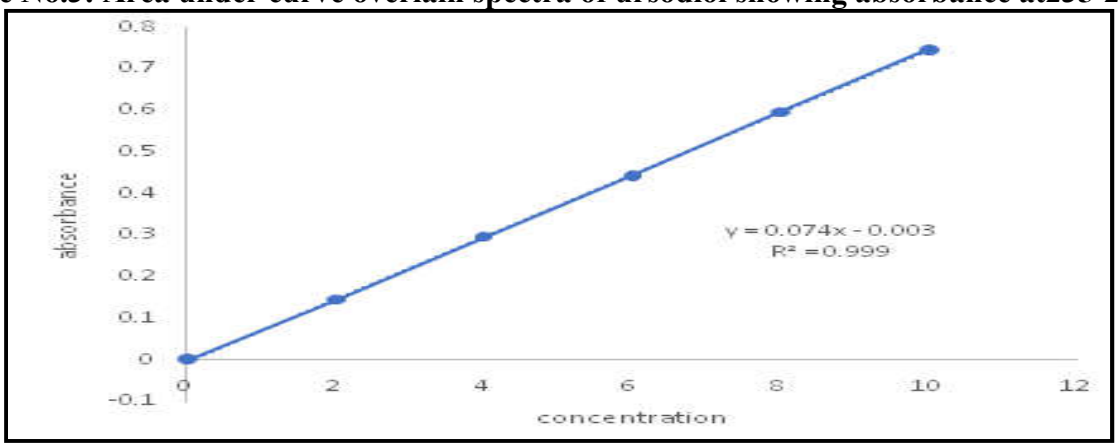

Figure No.4: Calibration curve of ursodiol at 235-245nm by area under curve

CONCLUSION

As per ICH guidelines, the developed analytical method meets the acceptance criteria. It was concluded that the method is simple, specific, accurate, economical, sensitive and can be used for routine analysis of ursodiol in bulk drug and in pharmaceutical dosage forms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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