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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 zero order derivative spectroscopic method has been developed and validated for the estimation of Ursodiol (Ursodeoxycholic acid) in 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 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 0.9998=b and it has showed good linearity, precision during this concentration range. The % recovery was found to be 98.50-100.66. The LOD and LOQ were found to be 0.023 and 0.07µg/ml. The percentage relative standard deviation is found to be less than 2. According to ICH guidelines the technique has been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for routine quantification of Ursodiol in bulk and pharmaceutical dosage form.

KEYWORDS: Ursodiol, zero order derivative spectroscopy, validation, pharmaceutical formulations.

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]

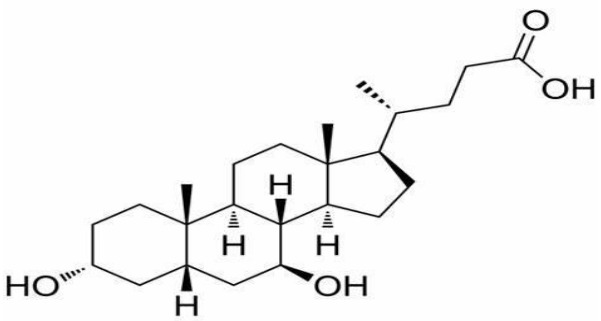

Fig.1: Chemical structure of Ursodiol.

Literature survey revealed that there were few analytical methods have been reported for the determination of Ursodiol in pure drug and 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 Zero order derivative UV Spectrophotometric method for estimation of Ursodiol in bulk and tablet dosage form.

MATERIALS AND METHODS

Instrument: UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) along with UV probe software. All weights were taken in analytical balance.

Chemicals: Ursodiol pure drug was 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.

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. The absorption spectra obtained and show maximum absorbance at 240nm, as the wavelength for detection (Fig-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 pipetted out 0.2, 0.4, 0.6, 0.8, and 1ml, into 10ml individual volumetric flask and diluted in Methanol: Acetonitrile:O-phosphoric acid(40:40:20)up to the mark and this gives 2, 4, 6, 8,and 10µg/ml concentration.

Preparation of sample solution: 10 tablets of Ursodiol marketed formulations was 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-phosphoric acid(40:40:20) and made up to the mark.

METHOD AND VALIDATION

The method was validated according to the ICH guidelines.

RESULTS AND DISCUSSION

Method: Zero order derivative spectroscopy.

Linearity: The linearity of an analytical method is its dimension 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 was measured at 240nm and absorbance values are shown in table-1. The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in Fig-3. Statistical variables like slope, intercept, regression equation, correlation coefficient and sandell's sensitivity were determined. (table-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 was established by intra-day and inter-day studies. 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-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 holds it constant and varied pure drug concentration. (table-4).

Ruggedness: The ruggedness is defined as the reliability of results when the method is performed under the variation in conditions. This includes distinct analyst, laboratories, instruments, temperature etc. Ruggedness was determined between distinct analyst, the value of %RSD was found to be less than 2. (table-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.

$$LOD = 3.3(SD)/S \text{ and } LOQ = 3(LOD)$$

LOD and LOQ value of were found Ursodiol be 0.023 and 0.07µg/ml.

Table 1: Results of calibration curve at 240nm by zero order spectroscopy.

SL NO	Concentration in µg/ml	Absorbance ± Standard deviation*
1	0	0
2	2	0.142±0.00095
3	4	0.294±0.001067
4	6	0.441±0.001067
5	8	0.594±0.00095
6	10	0.749±0.001106

**Average of six determinations.*

Table 2: Regression parameter Ursodiol for by zero order spectroscopy.

Regression parameter	Results
Range(µg/ml)	2-10
λ_{max}(nm)	240
Regression Equation	Y= 0.075x+0.0049
Slope(b)	0.075
Intercept(a)	0.0049
Correlation coefficient(r^2)	0.9998
Sandell's equation	0.013
Limit of detection(µg/ml)	0.023
Limit of quantitation(µg/ml)	0.07

Table 3: Determination of precision results for Ursodiol at 240nm by zero order spectroscopy.

Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
2	0.142±0.0020	1.40	0.141±0.013	0.92
4	0.287±0.0018	0.62	0.287±0.0016	0.55
6	0.441±0.0016	0.36	0.424±0.0012	0.28
8	0.595±0.0026	0.43	0.595±0.0024	0.40
10	0.750±0.00025	0.18	0.749±0.0014	0.18

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

Table 4: Determination of Accuracy results for Ursodiol at 240nm by Zero order spectroscopy.

Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
50	6	3	9.02	100.20 ±0.326	0.325
100	6	6	11.85	98.76 ±0.205	0.207
150	6	9	15.04	99.83 ±0.286	0.286

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

Table 5: Determination of Ruggedness results for Ursodiol at 240nm by Zero order spectroscopy.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.441	0.440
±Standard deviation*	0.0010	0.0017
%RSD	0.266	0.386

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

FIGURES

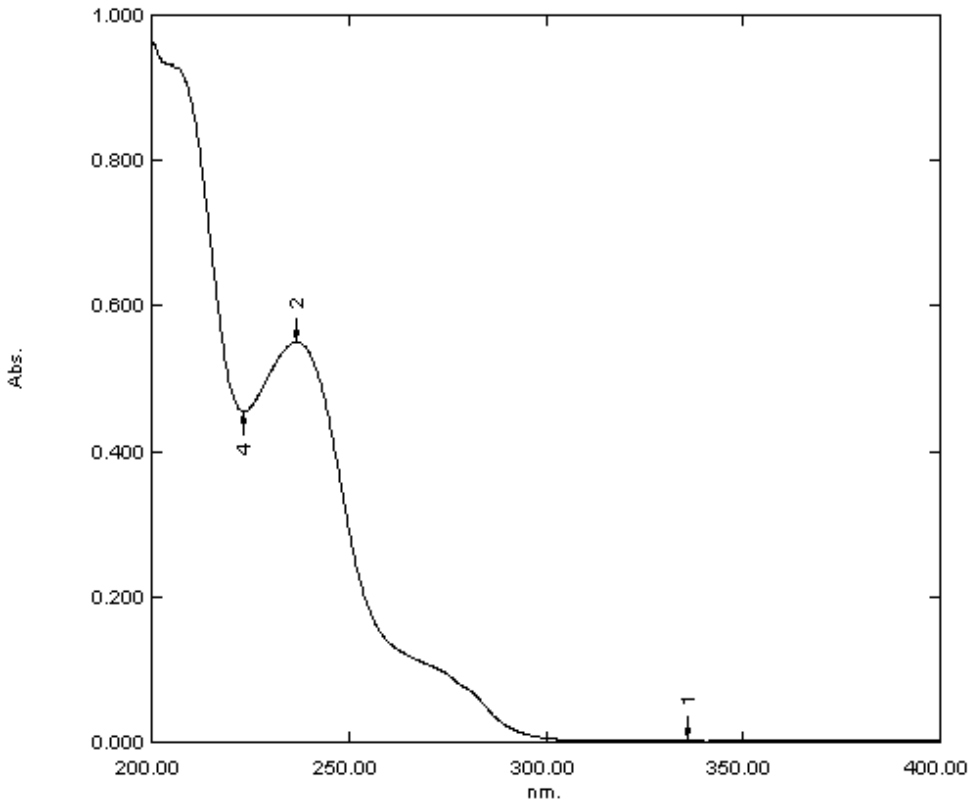

Fig. 2: Zero order spectrum of Ursodiol at 240nm.

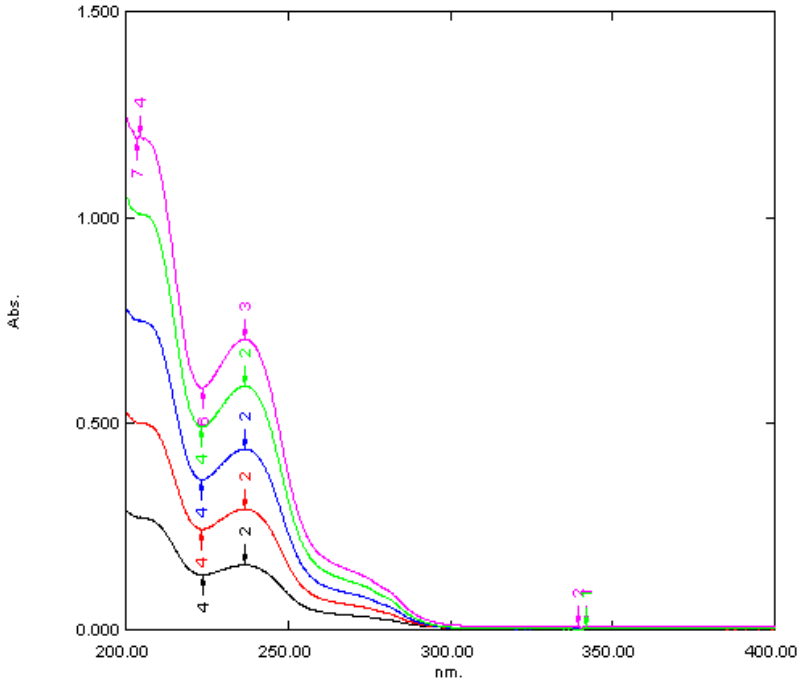

Fig. 3: Zero order overlain spectra of Ursodiol showing absorbance at 240nm.

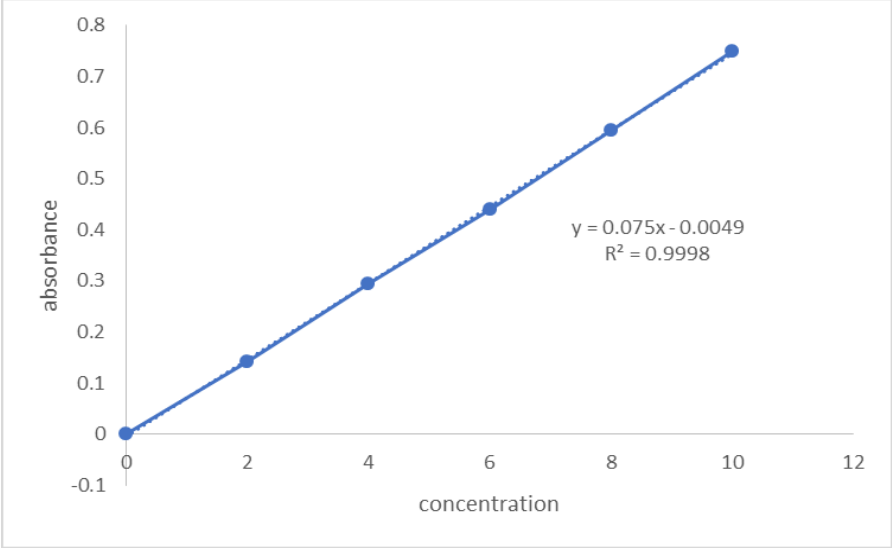

Fig.4: Calibration curve of Ursodiol by zero order spectroscopy.

CONCLUSION

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

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