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Development and Validation of the UV Spectrophotometric Method of Ursodeoxycholic Acid in Methanol

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Abstract
New developments have been taking place in the field of Development and validation of analytical method for the estimation of Ursodeoxychlic acid by using Uv spectroscopy. A simple, rapid, accurate and economical UV-spectroscopic method has been developed for the estimation of Ursodeoxycholic. The absorption maxima for Ursodeoxycholic acid is simulated liver fluid was found to be 232 nm. The drug follows linearity in the concentration ranges 1-10 µg/ml at 232nm with a correlation coefficient value of 0.998. The proposed method was applied to liver disease pharmaceutical formulation and % drug estimated was found to be in the range of 99.99% that is good agreement with the lable claim. The accuracy of the method was checked by recovery experiment performed at three levels i.e., 80%, 100%, 120%. The percent recovery was found to be in the range of 90-120%. The low values of % RSD are indicative of accuracy and reproducibility of method. The % RSD < 2 indicates that method is precise.
Keywords: *UV- spectroscopy, Ursodeoxycholic acid, Method development, Accuracy, Precision.*

1 Introduction

Ursodeoxycholic acid (UDCA) is a white, odourless, crystalline powder with a bitter taste. Chemically it is 3, 7-dihydroxy-5-cholan-24-oic acid. It is a water insoluble drug used as a drug for the dissolution of cholesterol gallstones because it reduces the cholesterol saturation of bile. The use of UDCA for the treatment of liver disease, such as primary biliary, biliary cirrhosis, chronic hepatitis and biliary pain has been demonstrated. However in vivo studies it shown the intestinal absorption and as a result the bioavailability of the drug are generally poor and unusual both among different subjects, and within the same subject. More than 50% is lost in the stool 9 after a single oral dose of 300 mg

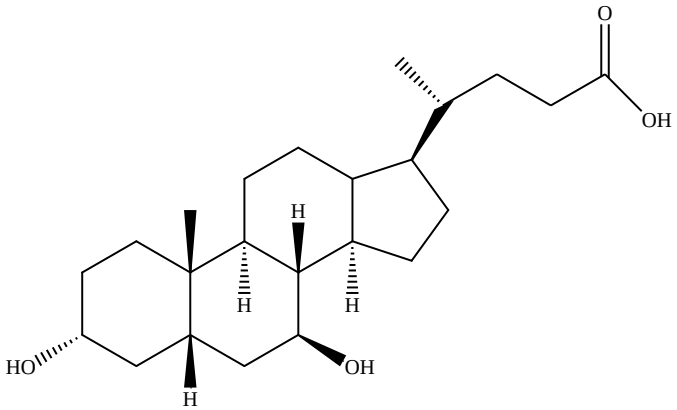

Fig.1 Ursodeoxycholic acids

2 Materials and Methods

2.1 Materials

Ursodeoxycholic acid standard powder was kindly supplied from Morepen Laoratories Limited, India. All chemical and reagent used were

obtained from Research Laboratory, Abhilashi University and were of analytical grade.

2.2 Preparation of standard stock solution

Accurately weighed 10 mg of Ursodeoxycholic acid was transferred to a 10 ml volumetric flask, dissolve in 5 ml simulated abdominal fluid by shaking and volume was adjusted with the same up to mark to give strength 1000µg/ml.

2.3 Preparation of working standard

From the above standard stock solution 5 ml was further diluted to 50 ml with SVF followed by sonication for 5-10 minute. The final strength was 100µg/ml. This stock was used to prepare various concentrations from 1-10 µg/ml by dilution with SVF.

2.4 Selection of wavelength for analysis of Ursodeoxycholic acid

Appropriate volume 1.2 ml of working stock solution of Ursodeoxycholic acid was transferred into a 10 ml volumetric flask, diluted with SVF up to the mark to give a concentration 12 µg/ml. The resulting solution was scanned between 200-400 nm using SVF as blank. The spectrum showed the absorbance maxims at 232 nm. This maxima was further used to get calibration curve.

3 Validation of Method

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result, or a product meeting was validated for different parameter like Linearity, Accuracy, Precision, Specificity, Robustness, Limit of detection (LOD), and Limit of Quantification (LOQ).

3.1 Linearity study

Stock solutions of Ursodeoxycholic acid (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 ml) were pipetted, into a series of ten 50 ml volumetric flask. The volume in each volumetric flask was made up to the mark with 50%v/v aqueous methanol and the content was mixed so as to obtain a final concentration in the range of 2 to 20µg/ml. The absorbance of the solution is measured at 232 nm against 50% v/v aqueous methanol used as a blank.

3.2 Accuracy

To the pre analysed sample solution, a known amount of standard stock solution at three different levels (80,100 and 120 %). The sample was reanalysed by the proposed method. The solutions were prepared in triplicate and the accuracy was indicated in %.

3.3 Precision

The term precision id defined by the ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH as the closeness of agreement between quantity values obtained by replicate measurement of a quantity under specific condition. Assessing the precision implies expressing numerically the random error or the degree of dispersion of a set of individual measurements by means of the standard deviation, the variance, or the coefficient of variation.

3.3.1 Precision of Repeatability

It is the concordance of a series of measurements of the same quantity when the experiments are conducted under same condition (analyst, apparatus, instrument, and day) in a rapid succession. For this experiment, standard solution of Ursodeoxycholic acid at 290 (10 µg/ml) was prepared and analysed six times as per the proposed method.

3.3.2 Intermediate Precision

It is the concordance of a series of measurement of the same quantity when the experiments are conducted within the same laboratory under different condition (analyte, apparatus, instrument, and day). Standard solution of Ursodeoxycholic acid at 290 (10µg/ml) was prepared and analyzed as per the proposed method

3.4 Sensitivity

The sensitivity of the measurement of Ursodeoxycholic acid was estimated in the term of the limit of detection (LOD) and limit of quantification (LOQ) by aid of proposed method. The LOD and LOQ of the proposed method were determined by using the equation:

$$LOD= 3.3\sigma/ S, \qquad LOQ= 10\sigma/S$$

Where σ is the standard deviation of the response (Y intercept) and S is the slop of the calibration curve.

3.5 System suitability

A system suitability test of the spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation were taken and % RSD of standard reading were taken for same. Acceptance criteria foe system suitability, % RSD of standard reading not more than 2.0%, were full fill during all validation parameter.

4. Conclusion

The present analytical method was validated as pet ICH Q2 (R1) guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was specific, precision, linear, and accurate while estimating the commercial formulation without interference of the excipients and other additive. Hence the present analytical method can be used for the routine determination of Ursodeoxycholic acid in pure pharmaceutical formulation at the minimum cost.

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