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Method Development and Validation for Estimation of Ursodeoxycholic Acid in Tablet Dosage form by HPLC

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Authors' contributions

This work was carried out in collaboration among all authors. Author SS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MS and AK managed the analyses of the study. Authors KM and SJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Ursodeoxycholic acid (UDCA) is a pharmaceutical ingredient widely used in clinics. As bile acid it solubilizes cholesterol gallstones and improves the liver function in case of cholestatic diseases. The HPLC method for determination of assay of ursodeoxycholic acid tablet had been validated for precision, accuracy (recovery) & Linearity. In the present study, an attempt was made to provide a newer, simple, sensitive, precise, accurate stability and low cost HPLC method for the effective quantitative determination of ursodeoxycholic acid as an active pharmaceutical ingredient as well as in pharmaceutical preparations without the interferences of other constituent in the formulations. HPLC method is developed and validated for various parameters as per ICH guidelines. The validated method was effectively useful to the commercially accessible pharmaceutical dosage form, yielding extremely good and reproducible result.

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Keywords: Ursodeoxycholic acid; HPLC; precision; linearity and accuracy.

1. INTRODUCTION

Nature has been a source of medicinal agents for thousands of year and an impressive number of modern drug have been isolated from natural sources, many based on their use in traditional medicine [1,2]. HPLC is extensively considered to be a technique chiefly for biotechnological, biomedical and biochemical research as well as for the pharmaceutical industry, these fields currently comprise only about 50% of HPLC users. At present HPLC is used by a variety of fields including cosmetics, energy, food, and environmental industries [3]. New methods including Reverse Phase Liquid Chromatography allowed for improved separation between very analogous compounds. Innovative techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the ease of HPLC. Improvements in type of columns and thus reproducibility were made as terms such as micro-column, affinity columns and fast HPLC began to come out [4-8]. The objective of our research is to develop a method and validated that is more precise, accurate, specific and sensitive, had shortest retention time and give better resolution. The result of validation method was effectively useful to the commercially. The aim of our research is to develop dissolution and an assay analytical method for the estimation of ursodeoxycholic acid tablets by HPLC that is more precise, accurate and specific and give better resolution results.

Chart 1. Description of ursodeoxycholic acid [9,10]

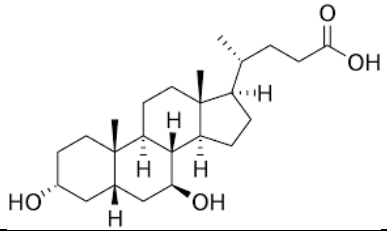
S. no.	Description	
1.	**Synonym**	3α, 7β-Dihydroxy-5 β-cholan-24-oic acid.
2.	**Molecular formula**	C_{24} H_{40} O_4
3.	**Molecular weight**	392.6
4.	**Structure**	
5.	**Category**	Hepatoprotective
6.	**Therapeutic indication**	Biliary cirrhosis (PBC)
7.	**Pharmacology**	Mechanisms of action of UDCA, changes in bile acid pool composition, hepatocyte membrane protection, effects and bicarbonate-rich hypercholeresis have been extensively studied. However, recent evidence indicate that UDCA is a potent intracellular signalling agent that counterbalances impaired biliary secretion, inhibits hepatocyte apoptosis and protects injured cholangiocytes against toxic effects of bile acids. It is clear that the relative contribution of these mechanisms to the anticholestatic action of UDCA depends on the type and stage of the liver injury.

Table 1. Instrument Required

S. no.	Intstrument	Make	Model
1	HPLC equipped with pump, injector and UV/ PDA detector	SHIMADZU	LC-2010 AHT
2	UPLC equipped with pump, injector and PDA detector	SHIMANZU	ACQUITY UPLC
3	Balance	METTLER	AB204-5
4	PH meter	Eutech	PH meter
4	PH meter	Eutech	-
5	Sonicator	Power Sonic 410	-
6	Analytical Balance	Sartorius	-

Table 2. Reagent and Chemicals Required

S.No.	Reagent/chemicals	Grade
1	Methanol	HPLC
2	Acetonitrile	HPLC
3	Water	Milli-Q
4	Orthophosphoric acid	AR
5	Formic acid	HPLC

2. EXPERIMENTAL SECTION

2.1 Instrument and Chemicals

For the estimation of ursodeoxycholic acid (URI DOX tablet) various chemical and instrument required are tabulated in Tables 1,2.

2.1.1 Mobile phase

Mix 500 volume of acetonitrile and 500 volume of a solution containing 1.2% w/v of sodium dehydrogenate orthophosphate dihydrate, 0.4% w/v of disodium dehydrogenate orthophosphate dihydrate and 1.08% w/v of tetrabutylammonium hydrogen sulphate in water.

2.1.2 Solvent A

Prepare a solution containing 535 ml of water, 6.0 g of sodium Dehydrogenate orthophosphate dehydrate 65 ml dihydrogen orthophosphate dihydrate solution (20%) and 400 ml of acetronitrile.

2.1.3 Reference preparation

Weigh accurately about 300 mg of Ursodeoxycholic acid working standard, transfer in a 20 ml volumetric flask and add about 10 ml solvent A , sonicate for 15 minute and make the volume 20 ml with solvent A. Filter with 0.45 ul micro nylon membrane filter. (Concentration: 15000 mcg/ml.)

2.1.4 Test solution

Break 20 tablets, weight accurately equivalent to 300 mg of Ursodeoxycholic acid transfer in a 20 ml volumetric flask and add about 10 ml solvent A sonicate for 15 min and make the volume 20 ml with solvent A, filter with 0.45 u micro nylon membrane filter (Concentration: 15000 mcg/ml.) Use suitable high performance liquid Chromatography equipped with UV Detector.

Calculation of Ursodeoxycholic acid (mg/tablet) A $= \frac{AT}{AS} \times \frac{WS}{20} \times \frac{20}{WT} \frac{A}{100}$ avg. Wt.

Ursodeoxycholic acid (%)= $A = \frac{}{300} \times 100$

Where,

AT = Average area of test solution
AS= Average area of reference solution Ws.= Weight of reference standard
P= % policy of the reference/ working standard

Methods validation is the procedure of demonstrating that analytical procedures are suitable for their intended use. The methods validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to sustain analytical procedures. The following are typical analytical performance characteristics which may be tested during methods validation [11-12].

2.2 Accuracy

The accuracy of a measurement is defined as the closeness of the measured value to the true value. In a process with high accuracy, a sample (whose —true value is known) is analyzed and the measured value is identical to the true value. Typically, accuracy is represented and determined by recovery studies, but there are three ways to determine accuracy: comparison to a reference standard recovery of the analyte spiked into blank matrix, or Standard addition of the analyte [13-16].

2.3 Precision

The precision of a logical procedure articulate the proximity of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the identical homogeneous sample under the prescribed conditions. Precision may be considered at three levels; Repeatability, Intermediate precision and Reproducibility. Precision should be obtained preferably using authentic samples. As parameters, the standard deviation, the relative standard deviation (coefficient of variation) and the assurance interval should be calculated for each level of precision [17-20].

Table 3. Chromatographic parameters

Column	Acquity UPLC BEHC18, 2.1x100mm, 1.7 m (make : water)
Column Temperature	40˚C
Flow rate	1.0 ml/min
Wave length	288 nm
Injection volume	2 ul
Run Time	10 minute

2.4 Linearity

The linearity of an analytical procedure is its aptitude (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. It may be demonstrated directly on the analyte, or on spiked samples using at least five concentrations over the whole working range. Moreover a visual evaluation of the analyte signal as a function of the concentration, suitable statistical calculations are recommended, such as a linear regression. The parameters slope and intercept, residual sum of squares and the coefficient of correlation should be reported.

2.5 Limit of Detection

The detection limit (DL) or limit of detection (LOD) of an individual procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. In analytical procedures such as HPLC that reveal baseline noise, the LOD can be based on a signal- to-noise (S/N) ratio (3:1), which is usually expressed as the concentration (e.g., percentage, parts per billion) of analyte in the sample.

2.6 Limit of Quantitation

The quantitation limit (QL) or limit of quantitation (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. It is usually expressed as the concentration (e.g., percentage, parts per million) of analyte in the sample. For analytical procedures such as HPLC that demonstrate baseline noise, the LOQ is generally estimated from a determination of S / N ratio (10:1) and is usually confirmed by an acceptable percent relative standard deviations (% RSD)

2.7 Range

The variety of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

2.8 Robustness

Robustness is defined as the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameters (e.g., pH, mobile-phase composition, warmth, and instrument settings) and provides an indication of its reliability during normal usage.

2.9 Specificity

Selectivity is defined as the ability of the method to separate the analyte from other components

Table 4. Proposed use acceptance criteria for the different parameters specified by ICH

Parameters	Proposed acceptance criteria
Linearity	r> 0.99, similar response ration
Precision- system	RSD < 2%
Precision- method	RSD < 2%
Precision-repeatability/reproducibility	%R & R<20%
Accuracy	FDA 98-102%,EPA 50-150%
Specifity	No interference
Detection limit	>2 times baseline
Quantitation limit	Signal –to-noise ratio=10:1
Range	Conc. Where data can be reliably determined

that may be present in the sample, including impurities. Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as impurities, degradation products and excipients. To determine specificity during the validation blanks, sample matrix (placebo), and known related impurities are analyzed to determine whether interferences occur.

2.10 System Suitability Determination

System suitability is the assessment of the components of an analytical system to show that the performance of a system meets the standards required by a method. For chromatographic assays, these may include tailing factors, resolution, and precision of standard peak areas, and comparison to a confirmation standard, capacity factors, retention times, and theoretical plates.

3. RESULTS AND DISCUSSION

3.1 Precision Method

The precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of a series of measurements.

3.2 Acceptance Criteria

The % relative standard deviation (RSD) for % assay of ursodeoxycholic acid per the six samples should not be more than 2.

3.3 Linearity

For linearity, standard, solution was prepared in the range of 70 % to 130% of the test concentration. A graph was plotted with injection volume on X axis and response (area) of the analytical on Y axis and the co-relation co-efficient was determined. The result obtained is summarized in the table.

The method linear from the injection volume 1.4 µl to 2.6 µl for the estimating of Ursodeoxycholic Acid. The co-relation co-efficient (CC) value should be less than 0.999.

3.4 Accuracy (Recovery)

The accuracy of an analytical procedure express the closeness of agreement between the volumes which is accepted either as a conventional true value or an accepted reference value and the value found. For known amount of ursodeoxycholic Acid raw material was spiked in the placebo in triplicate at 80% 100% & 120% of

Table 5. Method Precision

Sample No.	% Assay of Ursodeoxycholic Acid
1	98.61
2	98.61
3	101.80
4	99.97
5	98.92
6	98.56
Mean	99.41
SD	1.286
RSD (%)	1.294

Table 6. Linearity for Ursodeoxycholic Acid

S.No	Injection value (ul)	Response (area)
1	1.4	187124
2	1.6	216280
3	1.8	245030
4	2.0	271745
5	2.2	299774
6	2.4	356373
7	2.6	356373
Slope		**140834**
Intercept		**-9473.4**
Co-relation co-efficient		**0.99993**

test concentration. The amount of ursodeoxycholic Acid is quantified as per the test method. The percentage recovery is calculated from the amount found and actual added and the results are summarized in the table.

3.5 For Ursodeoxycholic Acid

The percentage recovery is calculated from the amount and accurate amount added and the results are summarized in the table.

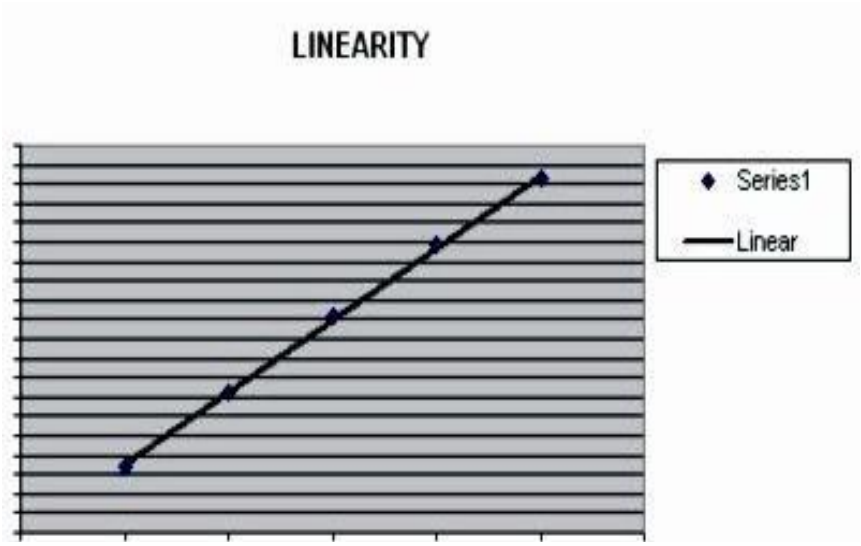


Fig. 1. Linearity for Ursodeoxycholic Acid

Table 7. Accuracy (Recovery)

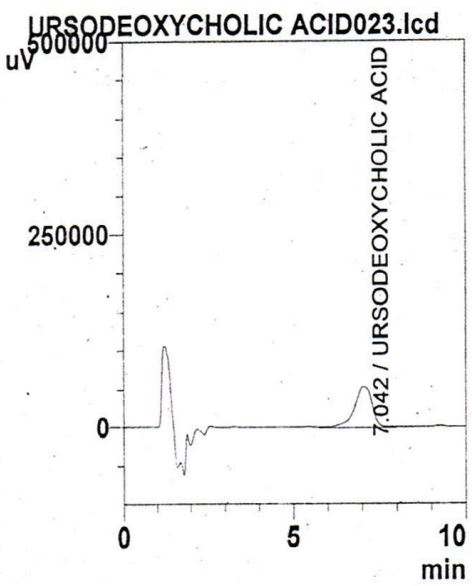
S. no.		Known amount added in Placebo in %	Recovery		% of recovery
			Individual value	Average values	
1	1	80%	79.40	80.25	79.95	100.69
	2	80%	79.40	79.87		
	3	80%	79.40	79.72		
	1	100%	99.25	97.25	97.67	98.41
	2	100%	99.25	97.63		
	3	100%	99.25	98.13		
2	1	120%	119.10	119.06	118.75	99.70
	2	120%	119.10	118.81		
	3	120%	119.10	118.37		

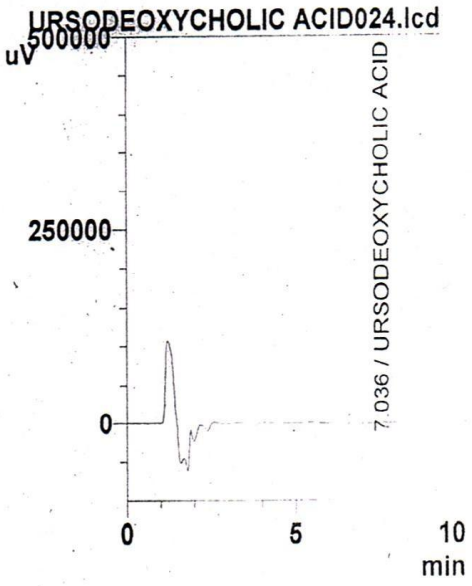
Table 8. Accuracy (Recovery) ursodeoxycholic acid

Validation parameters	Observation	Acceptance criteria
Precision		
Method precision	1.294%	RSD: NMT 2.0% co-relation co-efficient : NLT 0.999
Linearity	0.99962	98% to 102%

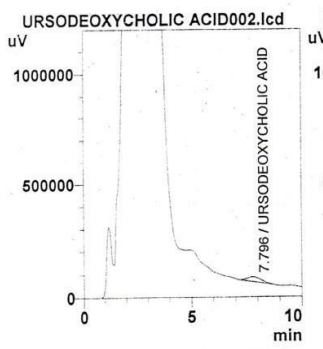
Table 9. Summary of validation parameters

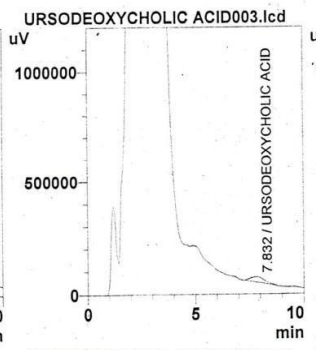
Validation parameters	Observation	Acceptance criteria
Precision		
Method precision	0687%	RSD:NMT 20% correlation coefficient : NLT 0.999
Linearity	0.99993	
Accuracy (Recovery)	98.61% to 101.19 %	98% to 102%

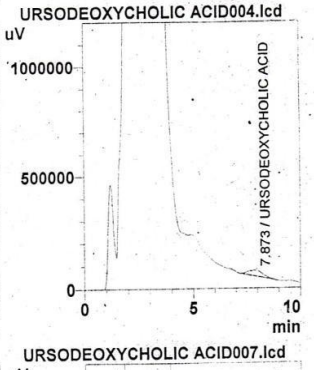


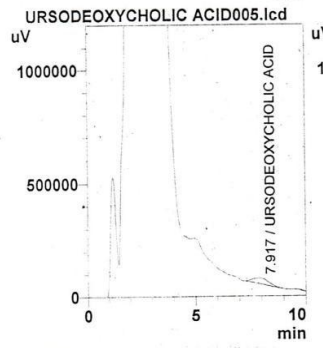


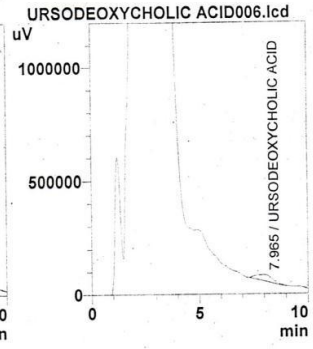
Recovery Sample 120%, Injection Volume : 20 ul, Run Time : 12.00 m

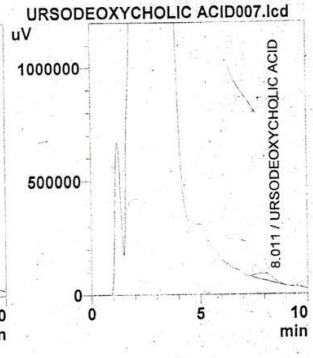


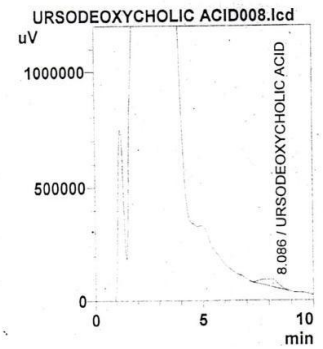




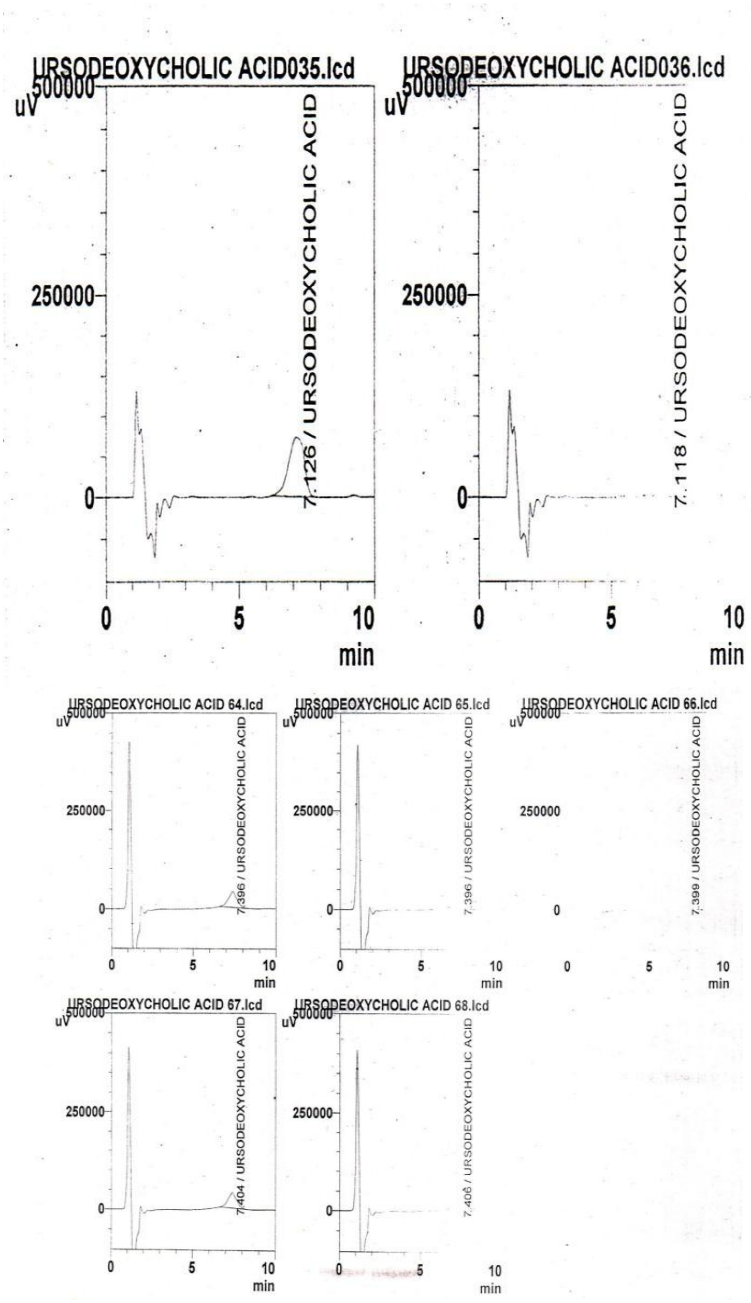








Precision method: Standard, Injection Volume: 20 ul, Run Time: 12:00 min.



Precision Method: Sample, Injection Volume: 20 ul, Run Time: 12:00 min.

Linearity : Blank, injection Volume: 2.00 ul, Run Time: 12:00 min.

Fig. 2. Summary of Validation Parameters

The analytical method convene the pre-established acceptance criteria for recovery study as per protocol, hence the method is accurate.

4. CONCLUSION

Ursodeoxycholic Acid Ursodeoxycholic acid is a bile acid which is produced rationally by the body. It works by reducing the amount of cholesterol released by your liver and by slowly dispersing the cholesterol. This breaks up the stones. Some ursodeoxycholic acid preparations can also help to treat primary biliary cholangitis. From the reported literature, there was no validation method developed by HPLC for the determination of ursodeoxycholic acid. So in the present study we develop and validated a chromatographic method by HPLC for the estimation of ursodeoxycholic acid tablet dosage form. For the optimization of Chromatography a number of preliminary trials were conducted and the effects of mobile phase, flow rate, solvent ratio were studied to check the retention time,

shape, resolution, and other chromatographic parameters. From these trials the mobile phase selected was mixture of phosphate buffer methanol and acetonitrile in the ratio of 50:35:15. Orthophosphoric acid this was found to be ideal mobile phase [21-25]. The flow rate was optimized at 1.5 mL/ min. and the column temperature is 400C detection was carried out at 288 nm for Ursodeoxycholic Acid. This system produced symmetric peak shape, good resolution for ursodeoxycholic Acid. The telling factor is not more than 2.0 and the RSD of Replicate injection is not more than 2.0%. In the present study, an attempt was made to provide a newer, simple, sensitive, precise, accurate and stability. HPLC method for the effective quantitative determination of ursodeoxycholic Acid as an active pharmaceutical ingredient as well as in pharmaceutical preparations [26] without the interferences of other constituent in the formulations. HPLC method is developed and validated for various parameters as per ICH guidelines.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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