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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE ESTIMATION OF LEDIPASVIR IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: The objective of this study was to develop a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of ledipasvir (LDP) in bulk and tablet formulation.

Methods: Stability-indicating RP-HPLC method was developed and validated for the estimation of LDP in bulk and tablet formulation. RP-HPLC was carried out on HiQ SiL C18 columns (250 mm × 4.6 mm, 5 µ particle size) using mobile phase acetonitrile:1 mM ammonium acetate buffer in the ratio of 90:10 v/v at a flow rate of 1 ml/min. The analytes were monitored using MD 2010 PDA detector at 333 nm.

Results: The retention time was found to be 3.843 min. The proposed method was found to be having linearity in the concentration range of 5–30 µg/ml. The number of theoretical plates obtained was 4236.50 which indicate the efficient performance of the column. The limit of detection was 0.305 µg/ml and limit of quantification was 0.923 µg/ml, which indicate the sensitivity of the method; the high percentage recovery indicates that the proposed method is highly accurate. The developed method has been validated according to the ICH guidelines and found to be simple, specific, precise, and accurate.

Conclusion: The proposed method is precise, accurate, and stability indicating. Therefore, the proposed method can be used for routine quality control and analysis of LDP during stability studies in bulk samples and tablet dosage forms.

Keywords: Method development, Stability indicating, Reverse-phase high-performance liquid chromatography, Ledipasvir, ICH.

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INTRODUCTION

Ledipasvir (LDP) is chemically methyl N-[(2S)-1-[(6S)-6-[5-[9,9-difluoro-7-[2-[(1S,2S,4R)-3-[(2S)-2-(methoxycarbonylamino)-3-methylbutanoyl]-3-azabicyclo[2.2.1]heptan-2-yl]-3H-benzimidazol-5-yl]fluoren-2-yl]-1H-imidazol-2-yl]-5-azaspiro[2.4]heptan-5-yl]-3-methyl-1-oxobutan-2-yl]carbamate. It is a potent inhibitor of HCV NS5A, a viral phosphoprotein that plays an important role in viral replication, assembly, and secretion. LDP in combination with sofosbuvir is used for the treatment of chronic hepatitis C, genotypes 1-6, usually in combination with other medications depending on the specific genotype [1,2]. A literature review revealed few spectrophotometric, reverse-phase high-performance liquid chromatography (RP-HPLC), stability-indicating RP-HPLC, and UPLC-ESI MS/MS [3-13] methods for the estimation of LDP. The purpose of this work was to develop a simple basic rapid and economic stability-indicating RP-HPLC method for the determination of LDP in its bulk and pharmaceutical dosage form so as to provide better scope for further research on the drug (Fig. 1).

METHODS

Chemical and reagents

The reagents used in this work were methanol (HPLC grade), ammonium acetate (AR) Grade, HCl (AR), NaOH (AR), and hydrogen peroxide (3%, m/v) (AR), which were procured from Merck, India. Distilled water (HPLC grade) was also used. LDP API was procured from Cipla.

Equipment

The instruments used in the study were HPLC (Jasco, PU 2080 Plus pump) integrated using Empower software, Photostability Chamber (Newtronic), Hot Air Oven (Kumar Laboratory Oven), electronic balance (Shimadzu balance), sonicator, digital pH meter, and calibrated glasswares.

Preparation of standard stock solution

Standard stock solution of LDP was prepared by dissolving 10 mg of drug in 10 ml of acetonitrile to get a concentration of 1000 µg/ml. From the standard stock solution, 1 ml was further diluted to 10 ml with mobile phase to get 100 µg/ml solution of LDP.

Analysis of formulations

Blend equivalent to 90 mg of LDP was prepared to satisfy dose/tablet of marketed formulation. Blend containing 90 mg LDP was prepared by spiking drug into excipients (20 mg starch, 4 mg magnesium stearate and lactose [q.s upto 200 mg]). Mixing was done by geometric addition method.

Preparation of test solutions

Blend equivalent to 10 mg of LDP was weighed and transferred to 10 ml volumetric flask and was diluted with methanol. It was sonicated for 10 min and filtered so as to get solution having a concentration of 1000 µg/ml. 1 ml of filtrate was further diluted with methanol, of which 1 ml was diluted with mobile phase to get the final concentration of 10 µg/ml LDP. Six determinations were carried out from homogenous sample to determine percentage assay.

Preparation of mobile phase

Mobile phase was prepared by mixing acetonitrile and 1 mM ammonium acetate buffer in the ratio of 90:10 v/v. It was then filtered through 0.45-µm membrane filter paper using filtration assembly and then sonicated on ultrasonic water bath for 10 min.

HPLC method development

After trying several permutation and combinations, it was found that mixture of acetonitrile and 1 mM ammonium acetate buffer in the ratio of 90:10 v/v gave a sharp peak of LDP and this system was optimized. It was observed that the developed chromatographic condition provides better separation of LDP (3.843 min) as shown in Fig. 2.

Stress degradation studies of bulk drug [14]

Degradation studies of the drug substance can help to identify the likely degradation products which can assist to establish the degradation pathways and the stability of the molecule. LDP was subjected under different condition of acid, base, neutral hydrolysis, oxidation, dry heat, and photolysis. For each study, two samples were prepared (Blank and of LDP drug solution). The blank subjected to stress in the same manner as the drug solution. Dry heat and photolytic degradation were carried out in a solid state.

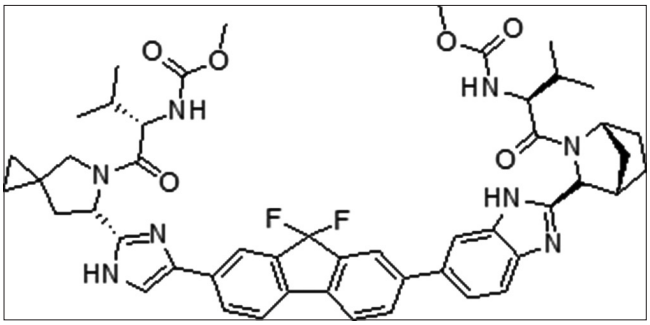

Fig. 1: Structure of ledipasvir

API degradation

Alkaline hydrolysis

About 1 ml working standard solution of LDP (1000 µg/ml) was mixed with 1 ml of 1 N methanolic NaOH and 8 ml of methanol. The solution was kept for 24 h in dark place. The resulting solution was neutralised and 2 ml was diluted with mobile phase to 10 ml and was injected (20 µg/ml). The chromatogram of LDP after alkaline hydrolysis shows 68.49% recovery, RT 3.849, and RT of degradant 5.91 and 6.58 (Fig. 3).

Acidic hydrolysis

About 1 ml working standard solution of LDP (1000 µg/ml) was mixed with 1 ml of 1 N methanolic HCl and 8 ml of methanol. The solution was kept for 24 h in the dark place. The resulting solution was neutralised, and 2 ml was diluted with mobile phase to 10 ml and was injected (20 µg/ml). The chromatogram of LDP after acid degradation shows 94.175% recovery, RT 3.84, and RT of degradant 6.54 (Fig. 4).

Neutral hydrolysis

About 1 ml working standard solution of LDP (1000 µg/ml) was mixed with 1 ml of distilled water and 8 ml of methanol. The solution was kept for 24 h in the dark place. 2 ml of the resulting solution was diluted with mobile phase to 10 ml and was injected (20 µg/ml). The chromatogram of LDP after neutral hydrolysis shows 87.38% recovery, RT 3.84, and RT of degradant 2.52 and 3.04 (Fig. 5).

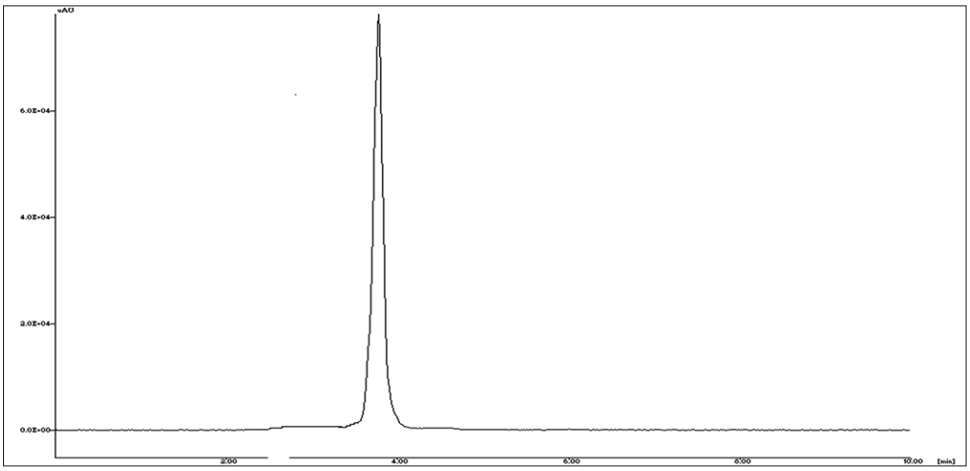

Fig. 2: Optimized chromatogram of standard ledipasvir (10 µg/ml)

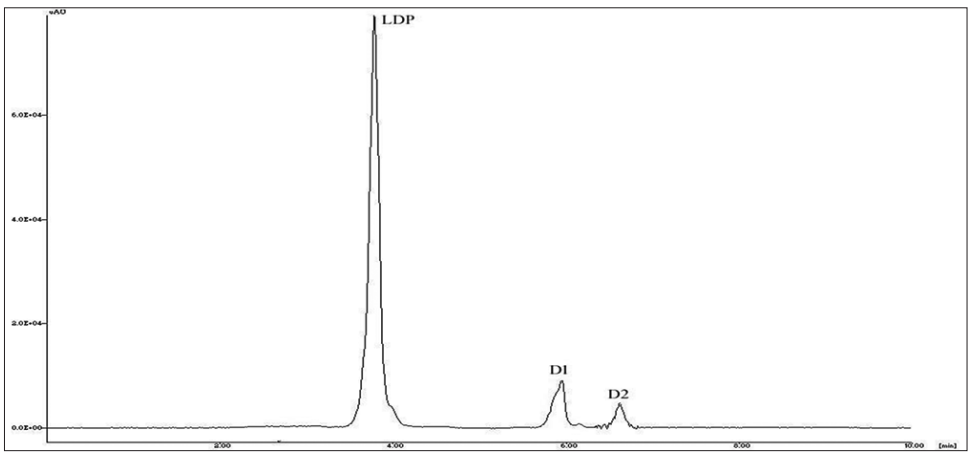

Fig. 3: Chromatogram of blank NaOH and ledipasvir (20 µg/ml) after alkaline hydrolysis

Oxidation

About 1 ml standard solution of LDP (1000 µg/ml) was mixed with 1 ml of 30% H_2O_2 solution and 8 ml of methanol. The solution was kept for 24 h in the dark place. 2 ml of the resulting solution was diluted with mobile phase to 10 ml and was injected (20 µg/ml). The chromatogram of LDP after oxidation shows 97.07% recovery, RT 3.84, and RT of degradant 5.93 (Fig. 6).

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (80°C) for 24 h. Sample was withdrawn after 24 h and processed as per standard solution preparation procedure mentioned under 1.5 to get 20 µg/ml as final concentration and was injected. The chromatogram of LDP after exposing to dry heat shows 99.781% recovery and RT 3.84 (Fig. 7).

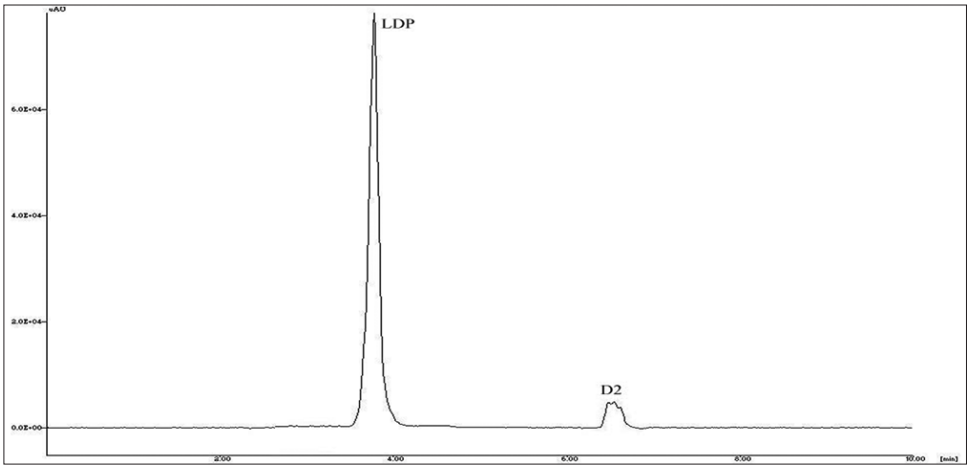

Fig. 4: Chromatogram of blank HCl and ledipasvir (20 µg/ml) after acid degradation

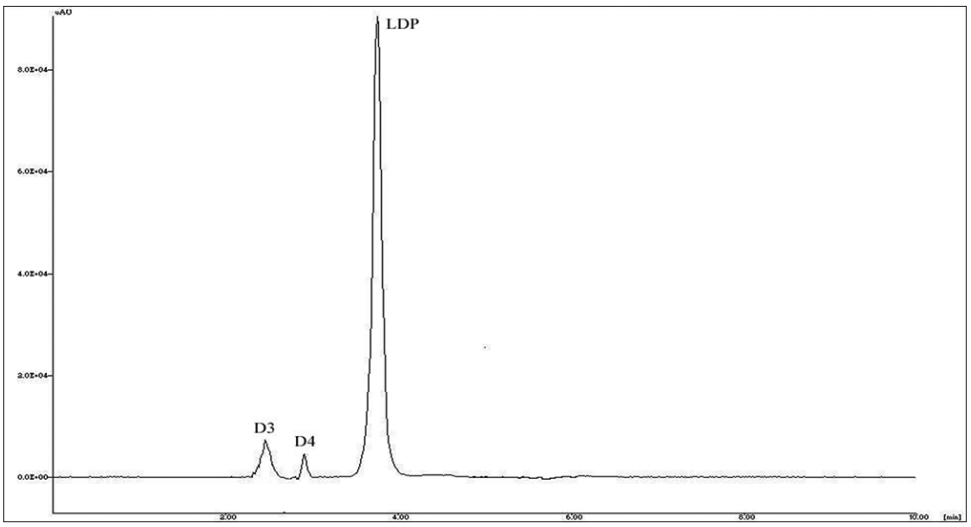

Fig. 5: Chromatogram of blank and ledipasvir (20 µg/ml) after neutral hydrolysis

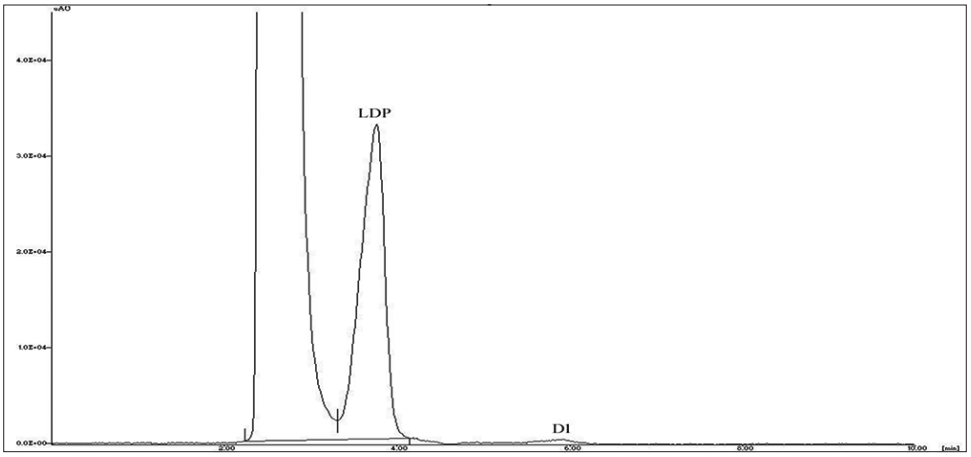

Fig. 6: Chromatogram of blank H_2O_2 and ledipasvir (20 µg/ml) after oxidation

Photodegradation studies

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt-h/m^2 and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux.Hr. A sample was withdrawn after exposure and processed as per standard solution preparation procedure mentioned under 1.5 to get 20 µg/ml as final concentration and was injected. The chromatogram of LDP after photodegradation shows 98.71% recovery (Fig. 8).

RESULTS AND DISCUSSION

Linearity and range

From the standard stock solution (100 µg/ml) of LDP, further dilutions were made with mobile phase to obtain a range of solution containing six different concentrations. Five replicates per concentration were injected. The linearity (relationship between peak area and concentration) was determined over the concentration range of 5–30 µg/ml as shown in Table 1.

Precision

The precision of the method was demonstrated by intra- and inter-day variation studies. In the intraday studies, three replicates of three different concentrations (10, 20, and 30 µg/ml) of LDP were analyzed in a day, and percentage RSD was calculated. For the interday variation studies, three replicates of different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated as shown in Table 2.

Accuracy

To check the accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 50,

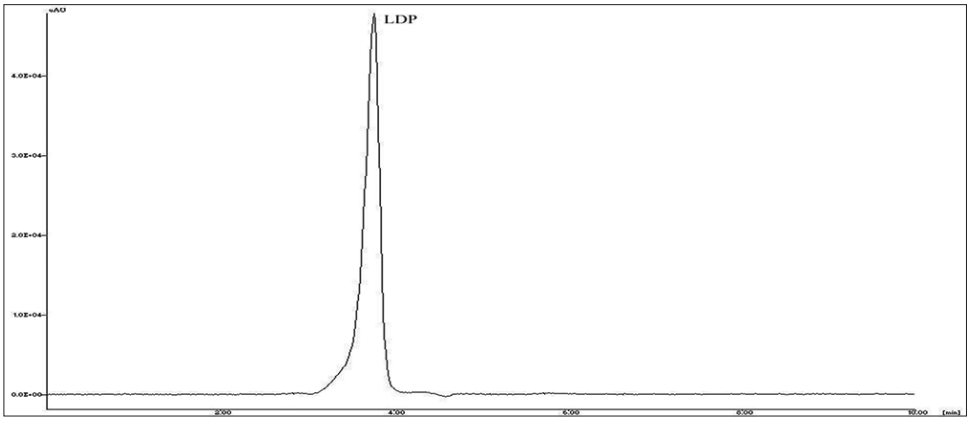

Fig. 7: Chromatogram of ledipasvir (20 µg/ml) after exposing to dry heat

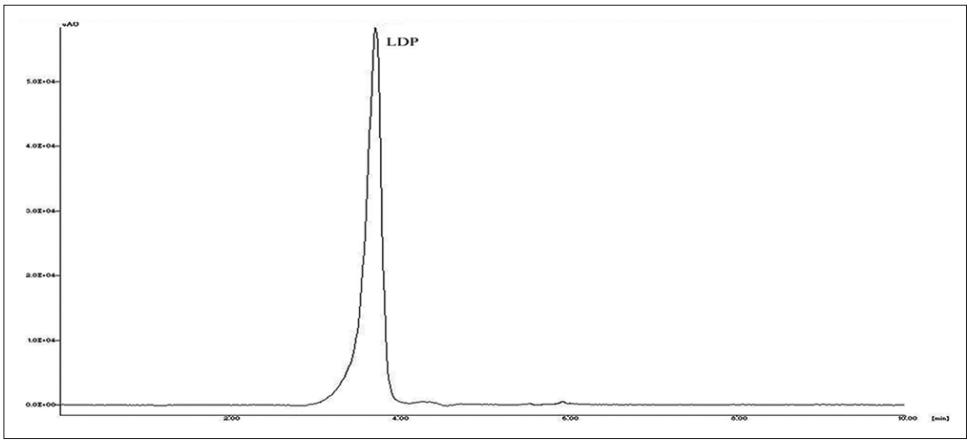

Fig. 8: Chromatogram of ledipasvir (20 µg/ml) after photodegradation

Table 1: Linearity study of LDP

Replicates	Concentrations of LDP					
	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	25 µg/ml	30 µg/ml
	Peak area					
1	151972.1	255990.9	367001.2	469512.7	590079.1	710045.9
2	149580.2	255500.5	366714.9	470997.9	591457.7	719094.6
3	150306.9	255866.2	363674.1	471719.4	589971.6	717073.1
4	151639.5	256245.6	362299.1	488759.8	597698.1	720149.2
5	150123.1	253683.1	358676.1	483872.2	591721.1	712947.7
Mean	150724.4	255457.3	363673.1	476972.4	592185.5	715862.1
SD	1029.481	1027.496	3434.636	8739.073	3180.901	4260.782
%RSD	0.683	0.402	0.944	1.832	0.537	0.595

Table 2: Precision study of LDP

Intraday precision			Interday precision		
Concentration(µg/ml)	**% Recovery**	**SD**	**Concentration (µg/ml)**	**% Recovery**	**SD**
10	98.611	0.236	10	98.410	0.257
20	98.544	0.257	20	98.929	0.355
30	101.023	1.273	30	100.772	0.851

Table 3: Recovery study of LDP

Level (%)	Concentration (µg/ml)		Area	% Recovery	Mean	% RSD
	Sample	Standard				
50	10	5	369546	100.043	99.591	0.401
			367516	99.443		
			366987	99.287		
100	10	10	477687	98.992	98.977	0.469
			475487	98.505		
			479678	99.433		
150	10	15	589254	98.969	98.945	0.461
			591614	99.387		
			586478	98.477		

100, and 150%. Basic concentration of sample chosen was 10 µg/ml of LDP from tablet solution. These solutions were injected in stabilized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of LDP were calculated using linearity equation of LDP as shown in Table 3.

Limit of detection (LOD)

LOD is calculated from the following formula:

$$LOD = 3.3\ \sigma/S$$

Where,

σ = standard deviation of response for the lowest concentration in the range.

S = slope of the calibration curve.

Limit of quantification (LOQ)

The quantitation limit is expressed as follows:

$$LOQ = 10\ \sigma/S$$

Where,

σ = standard deviation of response for the lowest concentration in the range.

S = slope of the calibration curve.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which flow rate and wavelengths were altered and the effects on the area were noted as indicated in Table 4. The summary of validation parameters is included in Table 5.

DISCUSSION

Stability-indicating RP-HPLC method for the determination of LDP was developed. Linearity for LDP was found in the range of 5–30 µg/ml with regression coefficient (r^2) = 0.999. LOD and LOQ values were 0.30 µg/ml and 0.92 µg/ml, respectively. The RSD values for intra- and inter-day precision studies were found to be <2%.

Degradation of LDP was found to occur under acidic condition (1N HCL, 24 h), alkaline condition (1N NaOH, 24 h), oxidative condition (30% H_2O_2, 24 h), and neutral (24 h) stress. LDP was considerably stable in dry heat and photostability stress testing.

Table 4: Robustness study of LDP

Drug	% RSD found for robustness study					
	Flow rate (1 ml/min)			Wavelength (nm)		
	0.9	1.0	1.1	332	333	334
LDP	0.269	0.220	0.252	0.314	0.260	0.212

Table 5: Summary of validation study

Validation parameter	LDP
Linearity equation	y=22567x+30895
Range	5–30 µg/ml
Assay (mean ± % RSD)	98.587±0.628
Precision	
A. Intraday precision	99.396±1.418
B. Interday precision	99.370±1.250
Accuracy (%)	
50	99.591±0.401
100	98.977±0.469
150	98.945±0.461
LOD	0.305
LOQ	0.923

CONCLUSION

In the present work, stability-indicating RP-HPLC method for the estimation of LDP was developed and validated as per the ICH guidelines. The standard deviation and % RSD (<2%) are within limit, indicating a high degree of precision of the methods.

From the above discussion, it can be concluded that the proposed method is precise, accurate, and stability indicating. Therefore, the proposed method can be used for routine quality control and analysis of LDP during stability studies in bulk samples and in tablet dosage forms.

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AUTHORS' CONTRIBUTIONS

The work was carried out by Amoldeep Shinde, under the guidance of Dr. Mrs. Minal Ghante and Dr. Sanjay Sawant. Both, Dr. Vandana Nikam

and Mrs. Shital Godse have contributed in writing the manuscript and proofreading of the same.

CONFLICTS OF INTEREST

The authors do not have any conflicts with any organization.

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