Research Article

Simultaneous Estimation of Loratadine and Ambroxol Hydrochloride from Tablet Dosage Form by HPLC Method

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Received: 11-06-2017; **Revised:** 02-08-2017; **Accepted:** 16-08-2017.

ABSTRACT

A simple, precise, accurate and rapid RP-HPLC method has been developed for simultaneous estimation of Ambroxol Hydrochloride and Loratadine in tablets dosage form. The method was carried out on a C18 column (25cm x4.6 mm i.d.x5micron particle size) with a mobile phase consisting of Water : Acetonitrile :Glacial Acetic acid in 65:35:05 ratio. The flow rate was adjusted to 1.2 ml / minute and detection was carried out at 254nm. The retention time obtained for Ambroxol Hydrochloride and Loratadine was 2.12 and 11.54 minutes respectively. The calibration areas were linear in the concentration range of 40-70 mg/ml for Ambroxol Hydrochloride and 8-13mg/ml for Loratadine. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification, solution stability. The proposed method can be used for simultaneous estimation of these two drugs in tablet dosage form.

Keywords: Ambroxol Hydrochloride, Loratadine, RP-HPLC, Validation, Tablet dosage form.

INTRODUCTION

Loratadine is chemically (Fig.1) Ethyl4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylate with a potent antihistaminic activity used in the treatment of utricaria and allergic rhinitis .

Ambroxol Hydrochloride is chemically (Fig.2) 1({[2-4 Amino-3, 5 dibromophenyl] methyl} amino) cyclohexanol, monohydrochloride, which is a semi synthetic derivative of vasicine obtained from the Indian shrub "Adhatoda Vasica". It is an expectorant and mucolytic agent which is used in the treatment of Bronchial asthma and Chronic Bronchitis. Ambroxol Hydrochloride also been reported to show cough suppressing and anti-inflammatory properties. Recently the inhibition of nitric oxide dependent activities of soluble guanylate cyclase was suggested as one of the molecular mechanisms of the therapeutic action of Ambroxol Hydrochloride, also used in pulmonary alveolar proteinosis in pulmonary distress and infant respiratory distress syndrome.

Literature survey showed that very few analytical methods have been reported for the estimation of Loratadine and Ambroxol Hydrochloride individually and in combination with other drugs using UV-VIS spectrophotometer, liquid chromatography, LC-MS, RP-LC, Capillary electrophoresis, HPLC with potentiometric detection etc.

Fixed combination containing Ambroxol Hydrochloride (60mg) and Loratadine (5mg) is available in the tablet dosage form in the market. Only one method was available for estimation but there was lot of scope for improvement. So efforts were taken to make available simultaneously evaluating, optimized, simple and cost effective HPLC method for estimation of Loratadine and Ambroxol Hydrochloride in tablet dosage form as per guidelines of International Conference on Harmonisation (ICH) [9].

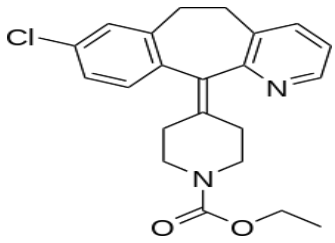
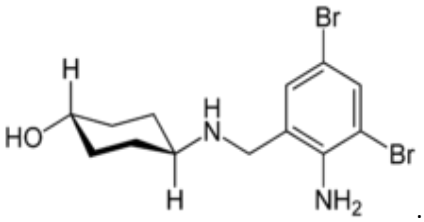


Figure 1: Loratadine



. **HCL**

Figure 2: Ambroxol Hydrochloride

MATERIALS AND METHODS

Acetonitrile, HPLC grade was procured from Merck (India) Limited. Glacial acetic acid was procured from S.D Fine Chemicals, India. Water HPLC grade was obtained from Milli-Q-RO water purification system. Reference standard of Ambroxol Hydrochloride and Loratadine were procured from Wallace Pharmaceuticals Pvt. Ltd, Goa ,India.

Chromatographic separation was performed on HPLC system with following details:

System: Thermo Fischer Ultimate 3000 **Column:** C18 (250 x 4.6 mm x 5 µm) Cosmosil

Electronic Balance: LCGC **Column:** Thermostat column compartment

Sonicator: Spectra Physics **Detector:** DAD-300 Diode Array Detector

pH Meter: Digisun AS220/X **Software :** Chromeleon **Injector:** Auto sampler

Chromatographic conditions

A Cosmosil C18 (250 mmX 4.6 mm X 5µm) column was used at ambient temperature. Mixed 650ml of water, 300ml of Acetonitrile and 50 ml of Glacial Acetic Acid to make 1000ml of mobile phase. This was filtered through glass fiber filter (0.45 µ). This was degassed. Flow rate was maintained at 1.2ml /minute. The elution was observed at 254nm. Some trials were carried out w. r. t change in the ratio of constituents of the mobile phase like 50:40:10/50: 50:00/ 45:50:05/70:25:5 etc. of Water: Acetonitrile: Glacial Acetic Acid. Injection volume and runtime were 20µl and 20 minutes respectively. In the ratio of 65:30:05 retention times for Ambroxol Hydrochloride and Loratadine observed to be 2.12 minutes and 11.54 minutes respectively. The two peaks were well resolved with good, sharp shape and symmetry was obtained.

Preparation of Mobile Phase

Mixed 650ml of HPLC grade water, 300ml of Acetonitrile and 50ml of Glacial Acetic Acid. The solution was filtered and degassed.

Preparation of standard stock solution

A. Ambroxol Hydrochloride standard solution (Solution "A"): Weighed accurately about 120mg of Ambroxol Hydrochloride working standard and transferred to a 100ml volumetric flask . Added 50ml of mobile phase and dissolved it completely. Made up volume with additional mobile phase. Mixed well.

B. Loratadine standard solution (Solution 'B'): Weighed accurately about 50mg of Loratadine. Made up volume with additional mobile phase and mixed well. Working standard and transferred to a100ml volumetric flask. Added 50ml of mobile phase and dissolved completely

C. Standard solution: Pipette out 10ml of standard solution (Solution B) and 50 ml of Ambroxol Hydrochloride standard solution (Solution A) to a 100ml volumetric flask. Added 50ml of mobile phase and mixed for 15 minutes. Made up the volume with additional mobile phase and mixed. Filtered through glass fiber filter paper.

D. Preparation of sample solution: Weighed powdered 20 tablets. Weighed accurately powder equivalent to 60 mg of Ambroxol Hydrochloride and 5 mg of Loratadine and transferred to a 100ml volumetric flask. Diluted to the mark with mobile phase and mixed well. Filtered through glass fiber filter paper.

Analysis of a Marketed formulation

To determine the content of Ambroxol hydrochloride and Loratadine in conventional tablet (Brand name: Pulmolor /Marketed product), Label claim: 60 mg Ambroxol Hydrochloride and 5 mg Loratadine per tablet. Twenty tablets were weighed .Their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 60 mg of Ambroxol Hydrochloride and 5 mg of Loratadine was transferred into a 100 ml volumetric flask containing 70 ml diluent, sonicated for 30 minutes and diluted up to 100 ml with diluent. Taken 2.5 ml from above solution in 25 ml volumetric flask and made up volume to 25 ml with diluent and made concentrations of 60 µg of Ambroxol Hydrochloride and 5 µg of Loratadine respectively. A 20- µl volume of sample solution was injected into HPLC system under the conditions described above.

RESULTS AND DISCUSSION

Method Development

1) Solubility: To arrive at right choice of Mobile Phase and diluents solubility of each compound was checked in all HPLC compatible solvents. Since target of Method Development was to estimate two compounds simultaneously, it was necessary to find a common solvent or diluents in which all compounds will have satisfactory solubility. Additionally the selected diluents should be capable toextract both compounds from tablets dosage form. A detailed and thorough suitability study narrowed down to Water, Acetonitrile and Glacial Acetic Acid in the ratio of 65:35:05.

2) Selection of UV detection wavelength: A detailed review of UV spectrum of two compounds suggested that 254nm is the most suitable wavelength, which can be employed for detecting all these components.

3) Selection of working pH range for mobile phase: Since two compounds are present in sample matrix, pH of mobile phase plays very vital role in separation. pKa values of all these two compounds were studied to select proper pH of mobile phase. From the study, conclusion was acidic pH would be better choice for separation of two actives.

4) Selection of Column: In a reverse phase chromatographic method development, selection of proper column is one of the key factors of Method Development. In reverse phase chromatographic separation wide range of columns like C8,C18,Cyano, Phenyl etc. of different make are available, which can be used for separation. Extensive literature survey revealed that in general 150 or 250 mm columns having diameter 4.6 mm and particle size 5 µm have been used for method development. Trials were taken on various columns and came to the conclusion for C18 column with 4.6 mm diameter with 5um particle size, which was finalized.

Final Method optimization

Final optimization was done to fix the remaining method parameters like flow rate of mobile phase, column oven temperature, concentration of each compound in standard and sample preparation. Effect of each individual parameter on separation was studied. Typical chromatogram showing separations between two compounds are shown in Fig.5.

Method Validation

The developed method was validated as per ICH (International Conference on Harmonization) guidelines with respect to System suitability, Precision; Specificity, Linearity, Accuracy, Limit of Detection, Limit of quantification, Ruggedness and Robustness.

Specificity

Specificity is the ability of Analytical Method to indentify and quantify the compounds of interest, without any interface in the presence of impurities or degradants which are likely to be present. Interferences may be either from blank or from placebo with the retention times of Ambroxol Hydrochloride and Loratadine. Identification of Ambroxol Hydrochloride, Loratadine from sample solution was done by comparing retention time of standard solution of individual components. Peak purity of both actives in sample solution was checked to confirm uniformity of all these peaks using Photo Diode Array Detector (PDA). Compliance of the method of the requirement for blank interference, identification and peak purity tests indicate that method is specific.

Linearity

Linearity shows proportionate response of analyte against concentration of analyte. Linearity of the method was estimated by using five concentrations of each compound within the 80% to 120% range of working concentration. For linearity experiments 42.29 mg to 63.29 mg for Ambroxol Hydrochloride and 8.7 mg to13.0 mg for Loratadine were used. Linearity curves for Loratadine and Ambroxol Hydrochloride are as shown in fig. 3 & Fig.4 respectively.

Linearity- Ambroxol HCl	
80%	42.2963 mg/ml
90%	47.3341 mg/ml
100%	52.7023 mg/ml
110%	57.4036 mg/ml
120%	63.2970 mg/ml
Linearity- Loratadine	
80%	80%
90%	90%
100%	100%
110%	110%
120%	120%

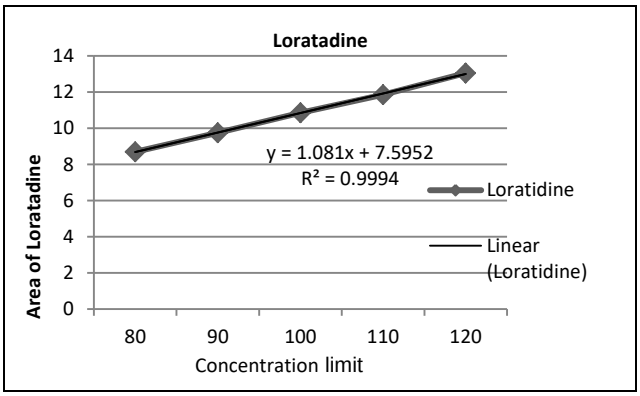


Figure 3: Linearity curve for Loratadine

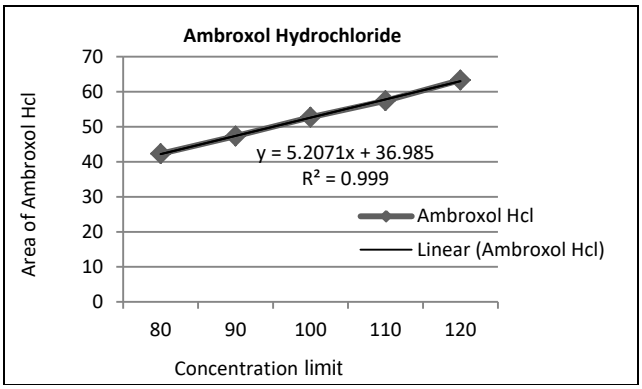


Figure 4: Linearity curve for Ambroxol HCl

Accuracy

Accuracy of a method is the closeness of observed values obtained using the method to the true value. Estimation of recovery by standard addition is a sound approach to demonstrate accuracy of the method. During recovery experiment, known amount of reference standard of each compound were spiked into the placebo of the sample at three different levels i.e.80%, 100% and 120% of sample concentration and prepared three samples of each level. These spiked samples along with one control sample were analysed. The experimental value of each compound obtained for each level was calculated and compared with actual added amount of respective component. Mean accuracy in percentage was calculated for all the three levels.

Precision

Precision of the method was demonstrated by repeatability (Intra- assay Precision) and intermediate Precision (Inter- assay) .Six different sample solutions of same concentration were prepared from same uniform sample and analysed against working standard solution. Assay values for each component were calculated and relative standard deviations (RSD) of assay values were evaluated. Very low RSD values indicate closeness of the results. Percent RSD of assay values from six samples were less than 1.0% for each compound indicates that the method is precise or repeatable.

Ruggedness

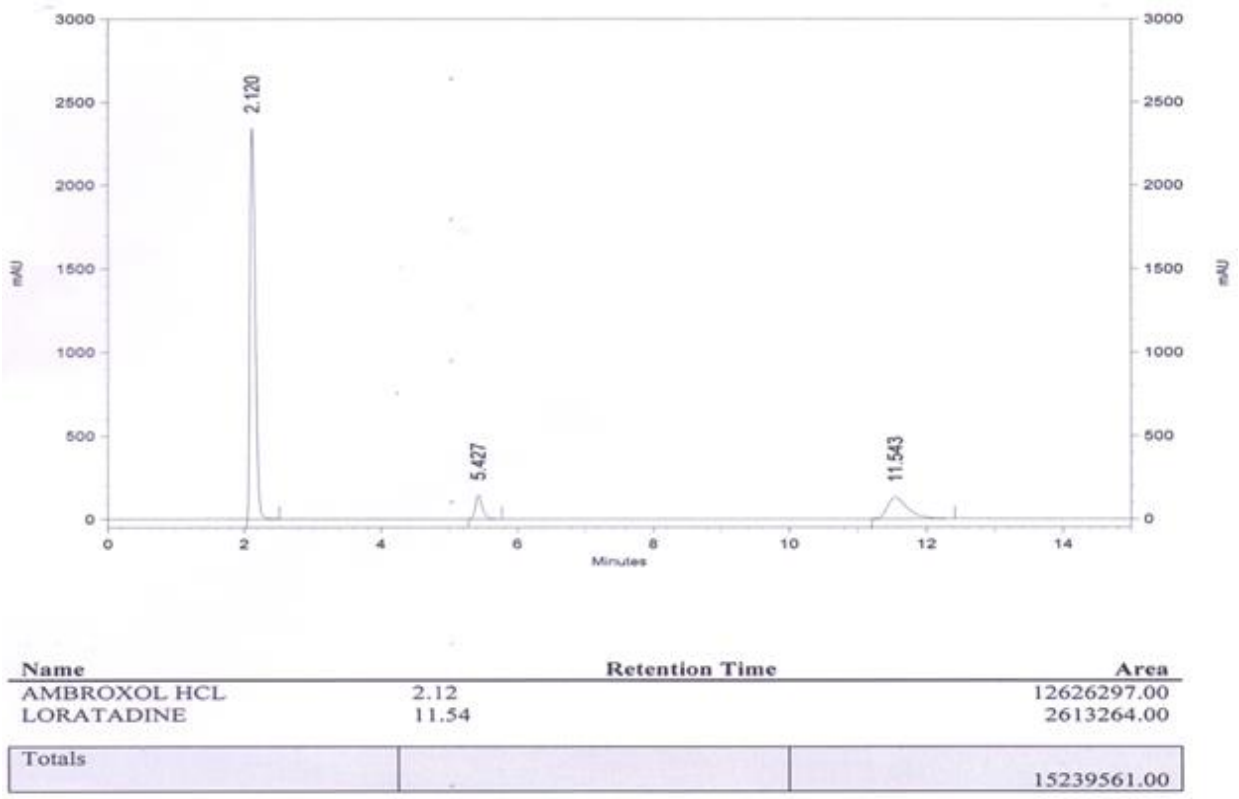
The intermediate precision was evaluated by preparing six different sample solution of same concentration as prepared in method precision and anlaysed on different days. Percent cumulative RSD of assay results for twelve samples were done. Six samples for method precision and six for intermediate precision were calculated. Percent RSD of assay values of each compound from twelve samples were less than 1.0%. The closeness of assay results and percent RSD values demonstrated that the method is rugged.

Robustness

Robustness is a validation parameter, which shows ability of analytical method to remain unaffected by slight but deliberate changes in method parameters. Robustness was demonstrated by making slight changes in parameters like flow rate (±5%), column temperature (± 2°C) and mobile phase composition (± 5%). Robustness study demonstrates that by making slight but deliberate changes in method parameters, method remains unchanged and gives consistent results. Results of original conditions and altered conditions are comparable.

Solution Stability

The Solution Stability of sample solution was evaluated by comparison of assay value of freshly prepared samples at room temperature for 24 hours. Standard solution and sample solution were prepared as mentioned in chromatographic conditions. Sample solution was analyzed and assay value was calculated against standard solution. Both the solutions were kept at room temperature for 24 hours were reanalyzed against freshly prepared standard solution and assay values were compared. Assay value of stored samples was compared with initial assay value. Difference between these two assays was less than 2.0% for both actives. Study demonstrated that sample solutions were stable up to24 Hours.



Name	Retention Time	Area
AMBROXOL HCL	2.12	12626297.00
LORATADINE	11.54	2613264.00
Totals		15239561.00

Figure 5: HPLC Chromatogram showing retention time of Ambroxol HCl and Loratadine

No	Name	Ret.Time (detected)	Asymmetry (EP)	Resolution (EP)	The.Plates (EP)
1	Ambroxol Hcl	2.12	1.29	40.17	9276
2	Loratadine	11.54	1.29	N.A	15232

Table 1: Precision of Ambroxol HCl and Loratadine

Sr. No.	Ambroxol Hydrochloride standard solution	Ambroxol Hydrochloride test solution	Loratadine standard solution	Loratadine test solution
1	12679839.0	12626297.0	2438483.0	2613264.0
2	12362613.0	12646715.0	2419163.0	2615747.0
3	12271203.0	12689661.0	2416706.0	2632258.0
4	12319535.0	12618778.0	2443130.0	2624952.0
5	12385948.0	12594918.0	2450641.0	2631571.0
Average	**12403827.6**	**12635273.8**	**2433624.6**	**2623558.4**
RSD in %	**1.29**	**0.28**	**0.62**	**0.33**

Table 2: Accuracy of Ambroxol HCl and Loratadine.

Sr. No	Conc. in %	% w/w Recovery Ambroxol HCl	% w/w Recovery Loratadine	Average recovery % Ambroxol HCl	Average recovery % Loratadine	Mean recovery in % Ambroxol HCl	Mean recovery in % Loratadine
1	80 %	81.59	80.31	101.99	100.38		
2	100 %	101.75	99.42	101.75	99.42	101.46%	100.05%
3	120 %	120.77	120.42	100.64	100.35		

Table 3: Robustness of Ambroxol HCl and Loratadine.

Change in instrument				
No of Injections	**Standard area of Ambroxol HCl**	**Test area of Ambroxol HCl**	**Standard area of Loratadine**	**Test area of Loratadine**
1	204.7	205.6	21.8	23.3
2	204.4	205.3	21.7	23.4
3	204.6	205.3	21.8	23.3
4	204.8	205.4	21.8	23.4
5	204.7	205.3	21.8	23.3
Average	**204.7**	205.4	**21.8**	**23.4**
RSD %	**0.1242**	**0.0524**	**0.0801**	**0.0436**
Assay of 1) Ambroxol HCl= 60.199 mg 2) Loratadine= 5.29 mg				
Change in extraction time from 15 minutes to 20 minutes				
No of Injections	**Standard area of Ambroxol HCl**	**Test area of Ambroxol HCl**	**Standard area of Loratadine**	**Test area of Loratadine**
1	12329910.0	12592363.0	2489979.0	2657431.0
2	12289347.0	12475184.0	2491848.0	2623256.0
Average	**12309628.5**	**12533773.5**	**2490913.5**	**2640343.5**
RSD %	**0.23**	**0.66**	**0.05**	**0.92**
Assay of 1) Ambroxol HCl= 61.07 mg : 2) Loratadine= 5.22 mg				

Table 4: Ruggedness of Ambroxol HCl and Loratadine.

Chemist A				
Sr. No.	**Standard area of Ambroxol HCl**	**Standard area of Loratadine**	**Test area of Ambroxol HCl**	**Test area of Loratadine**
1	12162833.0	2425711.0	12403158.0	2591978.0
2	12107572.0	2413717.0	12451316.0	2618706.0
3	12096949.0	2421877.0	12481790.0	2614289.0
4	12093289.0	2415082.0	12522946.0	2623726.0
Average	**12115160.8**	**2419096.8**	**12464802.5**	**2612174.8**
RSD %	**0.27**	**0.23**	**0.41**	**0.54**
Assay: Ambroxol Hydrochloride: 61.7 mg, Loratadine: 5.32 mg				
Chemist B				
Sr. No.	**Standard area of Ambroxol HCl**	**Standard area of Loratadine**	**Test area of Ambroxol HCl**	**Test area of Loratadine**
1	12187796.0	2455559.0	12505255.0	2625844.0
2	12231273.0	2447572.0	12503534.0	2620099.0
3	12291623.0	2454607.0	12595529.0	2625737.0
4	12231704.0	2453642.0	12521483.0	2604181.0
Average	**12235599.0**	**2452845.0**	**12531450.3**	**2618965.3**
RSD %	**0.35**	**0.15**	**0.35**	**0.39**

Table 5: Method validation of assay results of Ambroxol HCl and Loratadine.

Results of method validation of assay of Ambroxol Hydrochloride and Loratadine at each stage:

Validation Parameter	Acceptance Criteria	observation		
Specificity	Analyte Ambroxol Hydrochloride and Loratadine chromatographic peak should be specific, pure and distinct from each other	**Content**	Ambroxol Hydrochloride	Loratadine
		Retention time	About 2.1 min	About 11.5 min
		No apparent interference observed between the peaks, Ambroxol Hydrochloride and Loratadine peak is selectively separated from formulation matrix.		
Precision	The relative standard deviation should not be more than 2% for test solution and standard solution.	**Content**	**RSD**	
		Ambroxol Hydrochloride	Std =1.29% :Test =0.28%	
		Loratadine	Std =0.62% :Test =0.33%	
		It is observed that the precision of five replicate injections of homogeneous test solution assay for Ambroxol Hydrochloride and Loratadine & its standard solution results are within specified RSD limit.		
Accuracy	Recovery should be 98% to 102% with respect to the added percentage	**Content**	**Average Mean recovery**	
		Ambroxol Hydrochloride	101.46%	
		Loratadine	100.05 %	
Linearity	The test results with respect to test concentration should be linear and co-relation coefficient should not be less than 0.998.	**Content**	**Correlation coefficient**	**Slope**
		Ambroxol Hydrochloride	0.9998	1.021
		Loratadine	0.9996	0.9965
Ruggedness	The analytical result should be reproducible	**Content**	**Chemist A**	**Chemist B**
		Ambroxol Hydrochloride	61.7 mg	61.43 mg
		Loratadine	5.32 mg	5.26 mg
Robustness	The analytical result should be reproducible	**Content**	**Change in instrument**	
		Ambroxol Hydrochloride	60.199 mg/tab	
		Loratadine	5.29 mg /Tab	
		Content	**Change in extraction time 15 mins to 20 mins**	
		Ambroxol Hydrochloride	61.07 mg	
		Loratadine	5.22 mg	

CONCLUSION

The developed method is accurate, simple, rapid and selective for the simultaneous estimation of Ambroxol Hydrochloride and Loratadine in tablet dosage form. The sample preparation is simple, analysis time is short and elution is by isocratic method. The retention time of Ambroxol HCl and Loratadine are found to be 2.12 & 11.54 minutes respectively. The excipients of these commercial sample analysed did not interfere in the analysis, which proves excellent specificity of the method for these drugs analysis. Hence the proposed method can be conveniently adopted for the routine quality control analysis for this combination in tablet dosage form.

Acknowledgement: Authors would like to thank, Wallace Pharmaceuticals Private Limited, Goa, India for providing resources for formulation and subsequent analysis.

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Source of Support: Nil, **Conflict of Interest:** None.