IJPSR (2021), Volume 12, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 December 2020; received in revised form, 18 April 2021; accepted, 26 May 2021; published 01 November 2021

A VALIDATED STABILITY-INDICATING RP-HPLC METHOD FOR DETERMINATION OF ESMOLOL HYDROCHLORIDE AND ITS RELATED IMPURITIES

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Keywords:

Esmolol Hydrochloride, Impurities, Characterization, LC-method, Degradation studies

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ABSTRACT: Esmolol Hydrochloride belongs to the class II anti-arrhythmic drugs. Due to composite compositions and low reproducibility, challenges are still warranted for the systematic method development for related substances and identification of its acid degradation products. Development, validation and characterization of RP-HPLC stability-indicating method for the quantitative analysis of two impurities in Esmolol hydrochloride and its stress degradants. The validation of this method was achieved as per ICH Q2 (R1) guidelines with the optimized experimental conditions. To achieve the proposed method on C_{18} (250 mm × 4.6 mm, 5µ) column and the temperature was sustained at 30°C and run time was 12 min. The mobile phase consists of A–Acetonitrile, B–0.01N Potassium dihydrogen orthophosphate in water (pH=4.8). The injection volume of samples was 10µL, and UV detection was carried out at 221nm. Linearity ranges were covered 0.25-1.5 ppm for ASL-8123, ACC-9675 and Esmolol Hydrochloride. The newly developed method for separation of impurities along with the pure drugs was found to be capable of giving swift retention times while still preserving good resolution than that attained with conventional RP-HPLC. The proposed method has capable to separate these impurities with high resolution in bulk drug substance and hence this method can be used in the humdrum quality analysis in quality control samples and stability studies in bulk drugs.

INTRODUCTION: The chemical name of Esmolol Hydrochloride is methyl 3-(4-{2 -hydroxy-3-[(propan-2-yl) amino] propoxy} phenyl) propanoate hydrochloride. The metabolism of Esmolol can be decreased when combined with 1-(2-Phenylethyl)-4-phenyl-4-acetoxypiperidine. The chemical name of ASL-8123 is 3-[4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl] propanoate hydrochloride and the chemical name of ACC-9675 is methyl 3-[4-[2-hydroxy-3-[(ethylamino) propoxy) phenyl] propionate hydrochloride.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(11).6016-27

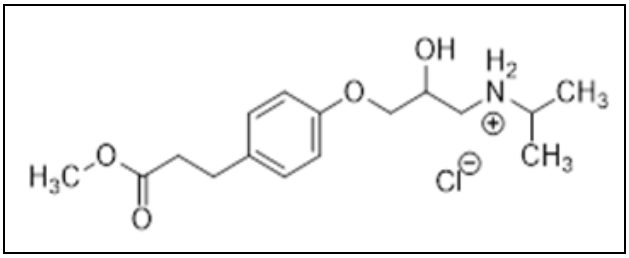
This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(11).6016-27

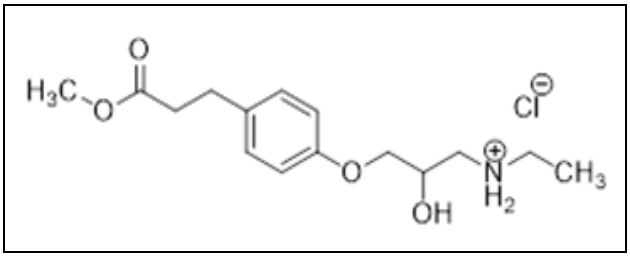
Esmolol, advertising with the trade name Brevibloc, is a cardioselective beta-1 receptor blocker. It has a quick onset but the thick duration of action without causing substantial intrinsic sympathomimetic at endorsed beneficial doses. The mechanism is completely based on blocking β-adrenergic receptors in the heart; it is also leading to the decreased force and pace of heart reductions. Esmolol averts the action of epinephrine and norepinephrine, substances transpire in nature [1]. It has a molecular formula of $C_{16}H_{25}NO_4$ with molecular weight 295.374 **Fig. 1**. Its nature is white or off-white crystalline powder, having a solubility of approximately 0.144 mg per mL in water at 25°C.

Esmolol Hydrochloride is the salt form of Esmolol, which belongs to the class II anti-arrhythmic drugs.

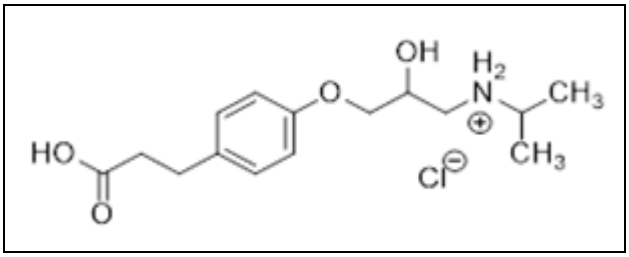
Esmolol hydrochloride blocks β-1 receptors in cardiac muscle and at higher doses, it will occlude β-2 receptors and vascular smooth muscle thus directing to its muscle relaxation [2, 3]. The purpose of this study is the organization of the standard analytical method for detection of Esmolol Hydrochloride impurities along with Active Pharmaceutical Ingredient. This method can be applied slickly for many diverse samples. Method validation was accomplished and upgraded successfully, and the method has proven to be more efficient compared to the other existing methods [4-7].



ESMOLOL HYDROCHLORIDE



ACC-9675



ASL-8123

FIG. 1: STRUCTURE OF ESMOLOL, IMPURITIES ACC-9675 AND ASL-8123

MATERIALS AND METHODS:

Drug Substance: Working standards Esmolol Hydrochloride (99.9%), ASL-8123 (99.4%) and ACC-9675 (99.9%) were procured from Spectrum Pharma Labs, Hyderabad, India.

Instrumentation: Waters-2695, High-Performance Liquid Chromatography fortified with PDA detector integrated with Empower 2 software, supplied by M/s. Waters Corporation, USA. Mettler-Toledo analytical balance, model AG-245 capable of weighing 0.01 mg, supplied by M/s. Mettler AG, Switzerland. Sonicator supplied by M/s. Serwell instrument, India. Digital pH meter supplied by M/s. Serwell instruments, India, UV-VIS spectrophotometer integrated with UV win 6 Software supplied by M/s. PG Instruments T60, Vacuum pump supplied by M/s. Crompton and Hot Air Oven supplied by M/s. Serwell instrument, India.

Chemicals and Reagents: HPLC grade Acetonitrile, HPLC grade Methanol and HPLC water were purchased from Merck chemical division, India. AR grade Potassium dihydrogen orthophosphate, TEA, Ortho-phosphoric acid and sodium dihydrogen Ortho phosphate supplied by M/s. Rankem, avantor performance material, India used for present study.

Preparation of Mobile Phase: Mobile Phase: A– Acetonitrile, B–0.01N Potassium dihydrogen orthophosphate in water (pH=4.8).

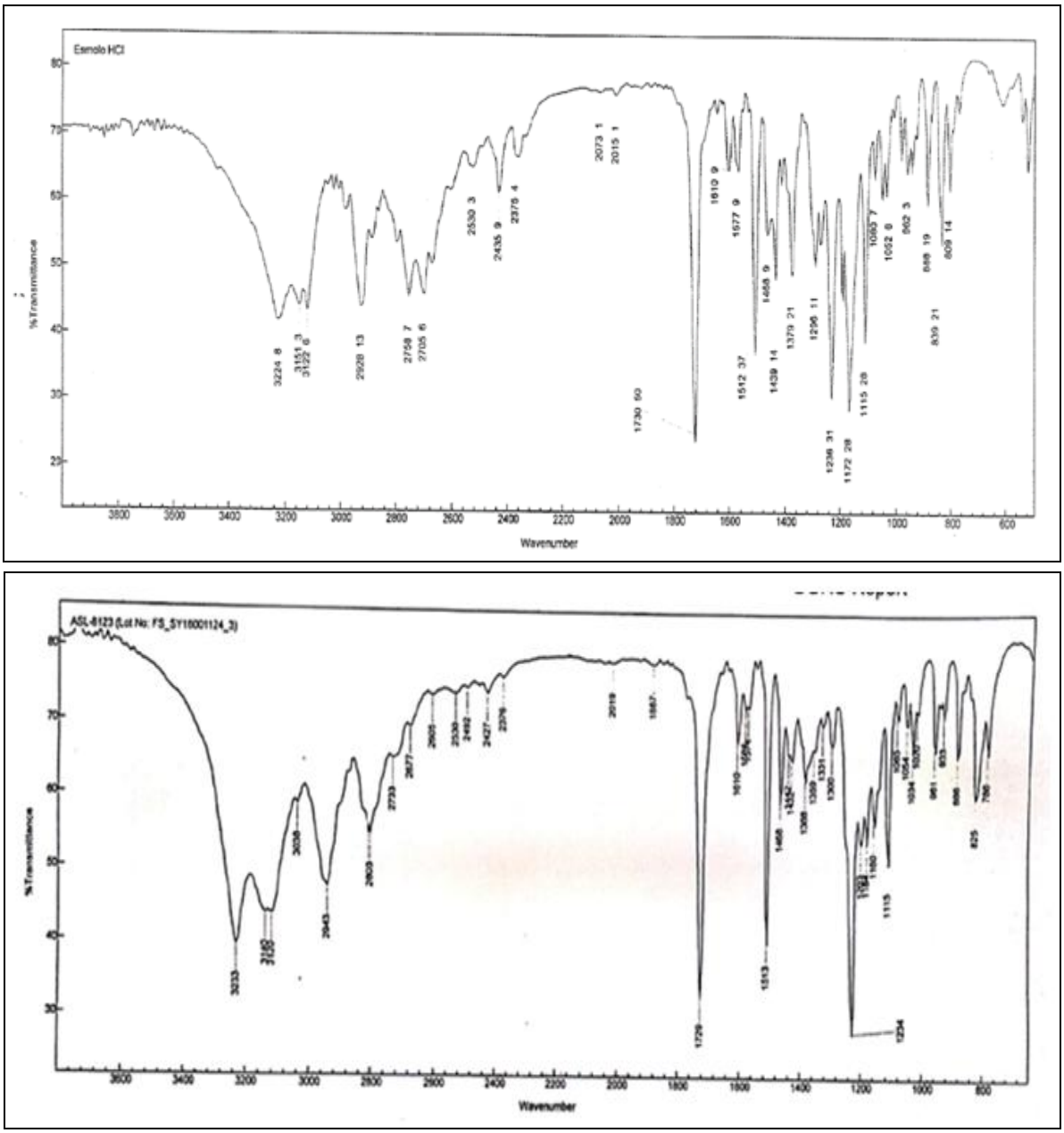
Preparation of Impurity Stock Solution: 0.5mg of Impurity A and B are separately weighed and transferred into a 10mL volumetric flask and were makeup with the diluent gives 50ppm solution of impurity.

Preparation of Standard Solution: Accurately weighed 0.5mg of Esmolol hydrochloride drug, transferred to 10mL volumetric flask, and dissolved in a small amount of diluent. The solution is sonicated for 5min and make up to the mark with diluent, which gives 50ppm solution. 0.2mL of the above solution was transferred to another 10 mL volumetric flask and was made up with the diluent to give 1ppm solution.

Chromatographic Conditions: The mobile phase was a combination of Mobile Phase: A– Acetonitrile, B–0.01N Potassium dihydrogen ortho phosphate in water (pH=4.8). The contents of the

mobile phase were filtered, before it was used, through a 0.45µm membrane filter, degassed for 10 min, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 mL/min, C_{18} (250 mm × 4.6 mm, 5µ). The column temperature was preserved at 30 °C and run time 12 min. The injection volume was maintained 10µL. UV detection was carried out using a UV-PDA detector at 221 nm; the diluent is water and acetonitrile (50:50).

Characterization of Esmolol and Impurities (ASL-8123 and ACC-9675): FT-IR Spectra recorded as KBr dispersions (1:150 w/w) for Esmolol Hydrochloride, ASL-8123, and ACC-9675 on FT-IR spectrophotometer (Perkin Elmer, USA) with Spectrum 10 software. Spectra were collected from 600 to 3800cm^{-1} with a resolution of 4 cm^{-1}, 32 scans/spectrum **Fig. 2**. Esmolol Hydrochloride, ASL-8123, and ACC-9675 have been identified by ^{1}H-NMR and ^{13}C-NMR[8] and full scan spectrum of Esmolol Hydrochloride, ACC-9675 and ASL-8123 increasing concentration shifted the chemical shifts (**Fig. 3** to **Fig. 8**), used line broadening of 0.3Hz for the ^{1}H spectrum and 1.0Hz for the ^{13}C spectrum. Characterization of NMR composed to chemical shifts and integral values for the proton signal and chemical shifts for the carbon signal (Bruker, USA).



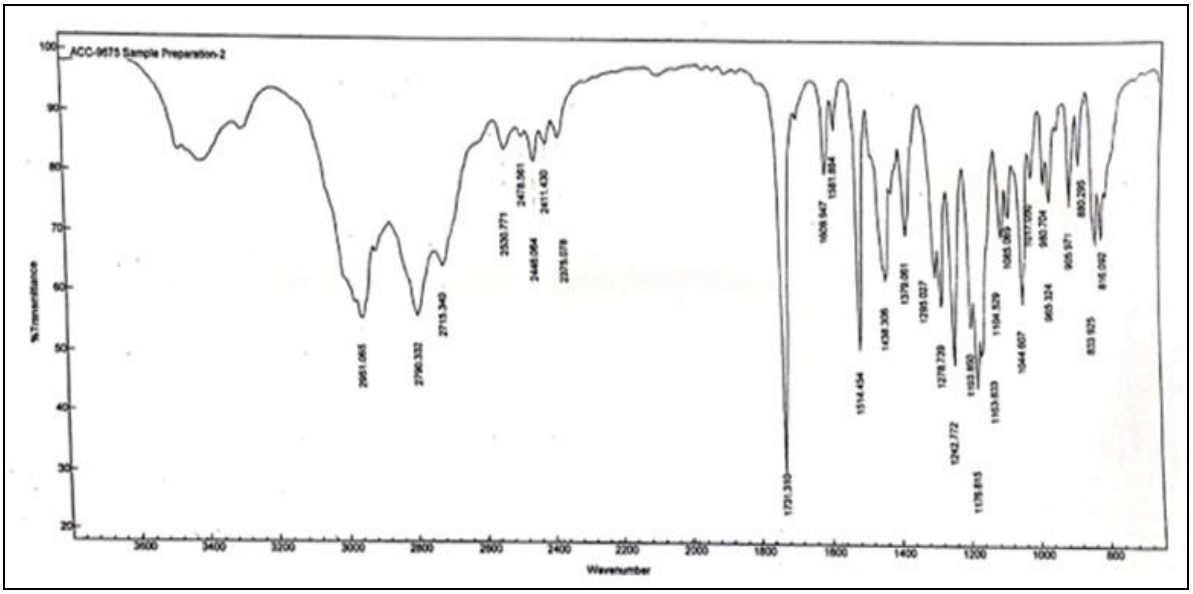


FIG. 2: FT-IR SPECTRUM OF ESMOLOL, ACC-9675 AND ASL-8123

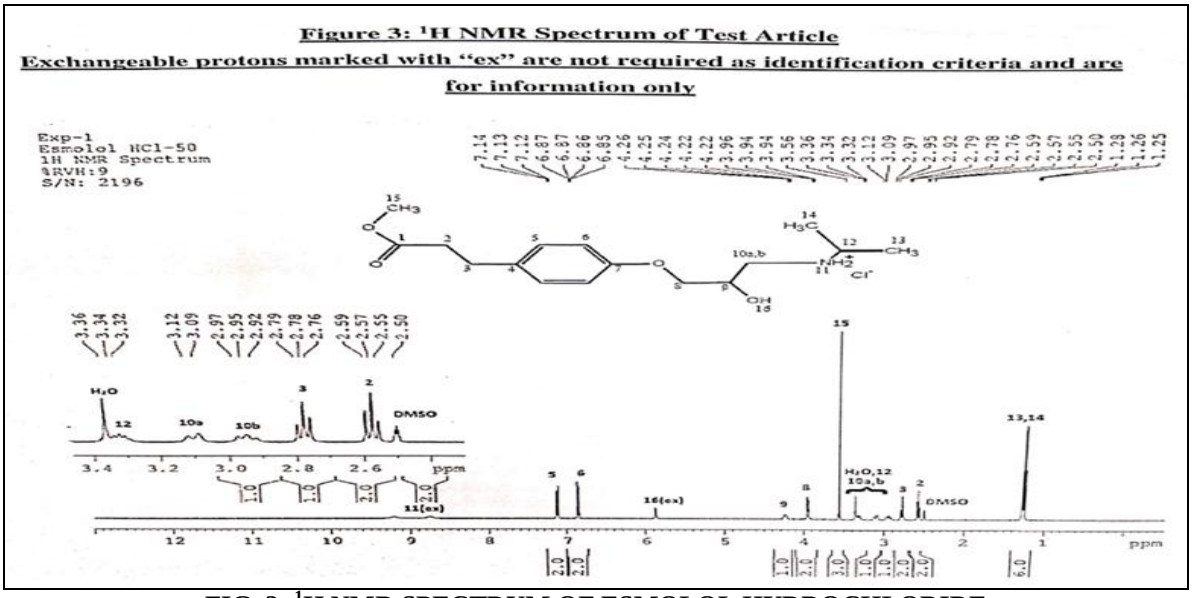


FIG. 3: ^{1}H NMR SPECTRUM OF ESMOLOL HYDROCHLORIDE

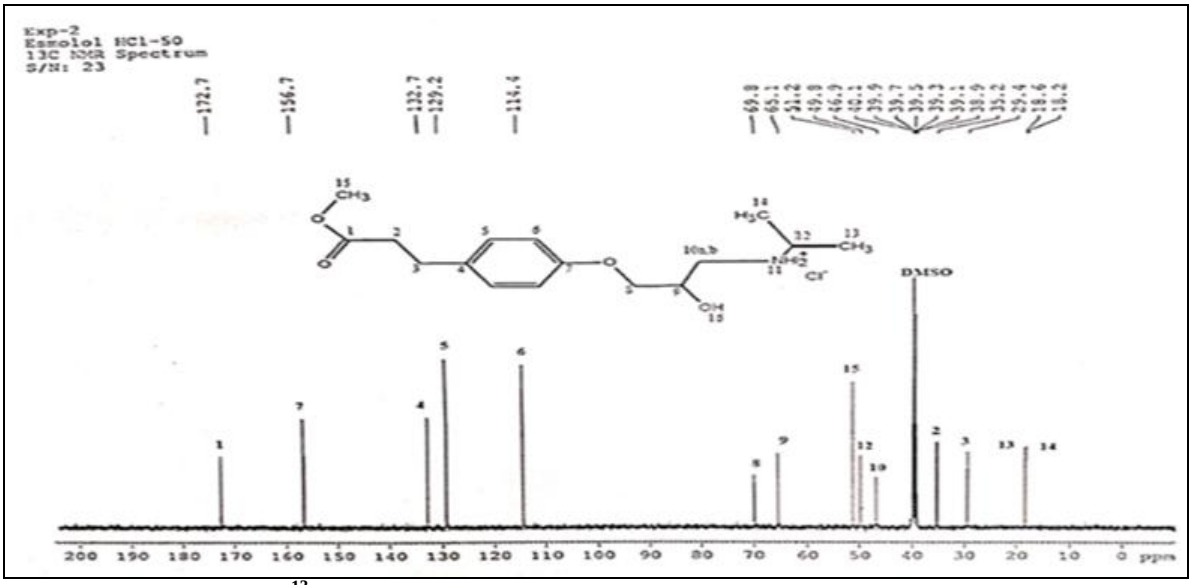


FIG. 4: ^{13}C NMR SPECTRUM OF ESMOLOL HYDROCHLORIDE

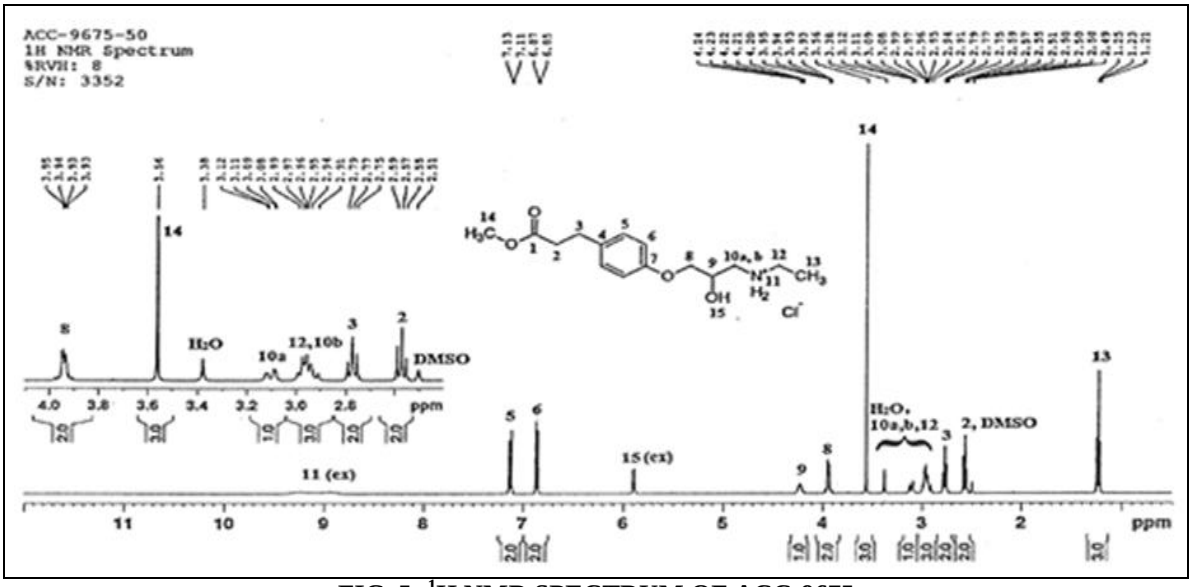


FIG. 5: ^{1}H NMR SPECTRUM OF ACC-9675

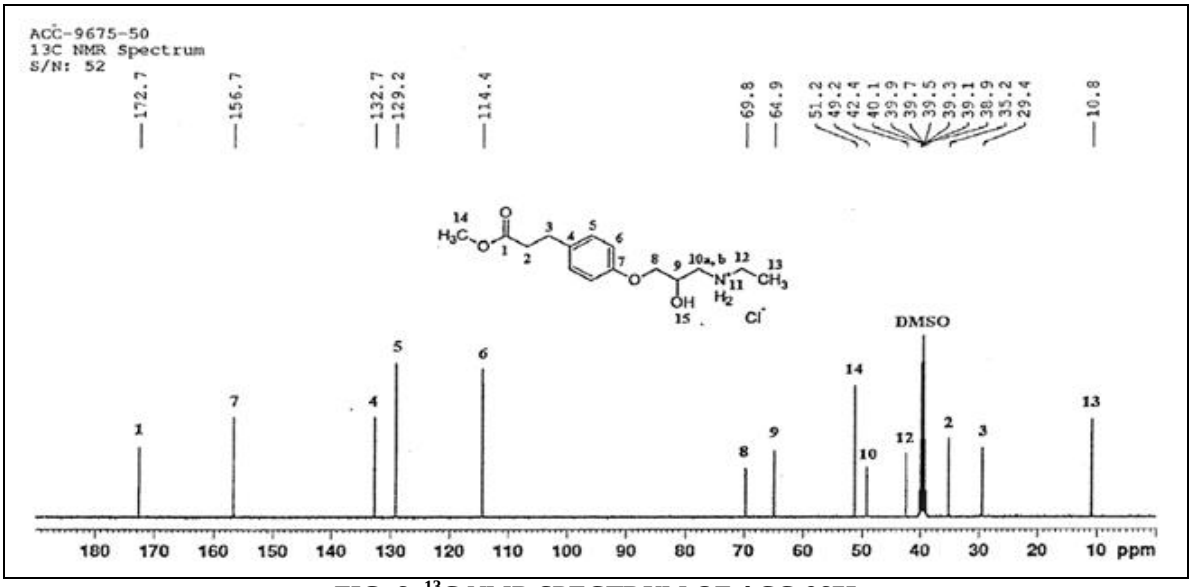


FIG. 6: ^{13}C NMR SPECTRUM OF ACC-9675

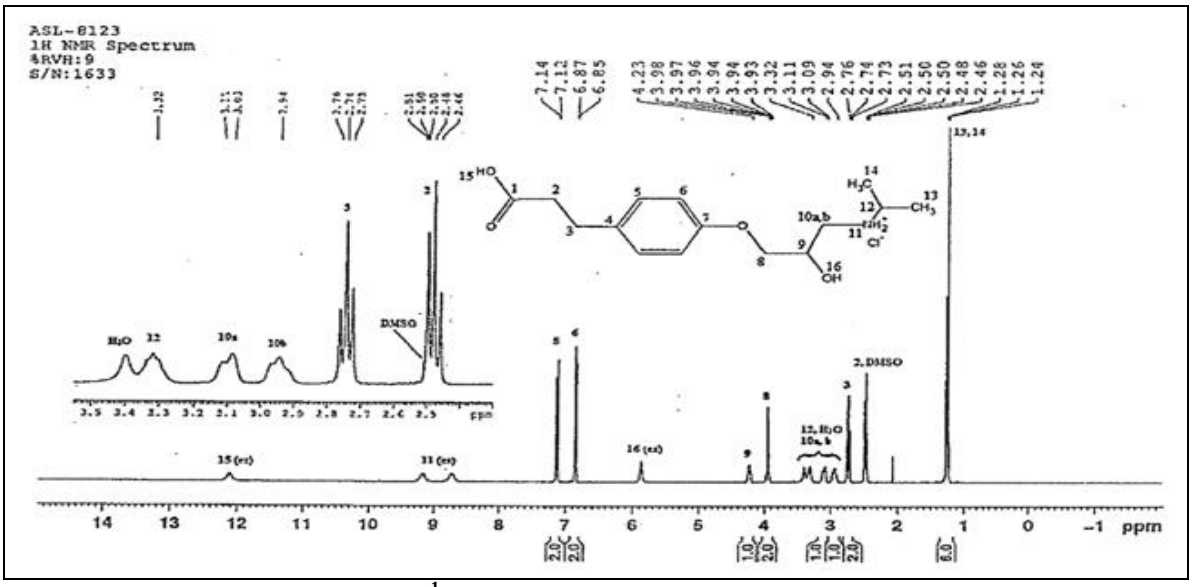


FIG. 7: ^{1}H NMR SPECTRUM OF ASL-8123

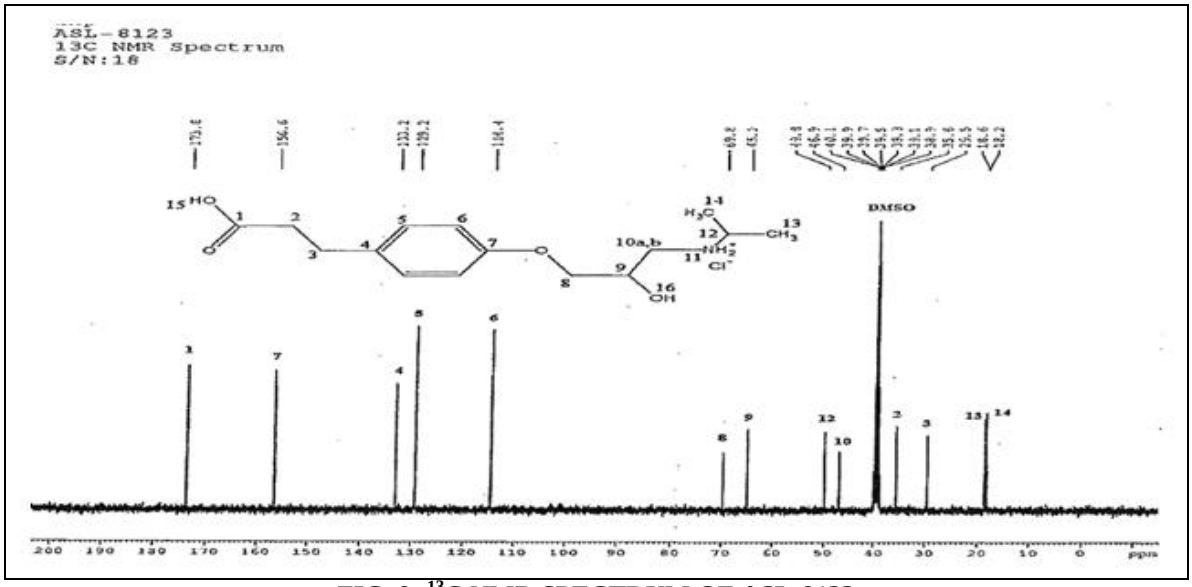


FIG. 8: ^{13}C NMR SPECTRUM OF ASL-8123

Method Development: After several trials, optimal chromatographic conditions were fixed for better separations [9, 10]. The separate standard calibration lines were constructed for drugs and impurities. A series of aliquots were prepared from the above stock solutions using mobile phase to get the concentrations 0.25–1.5 µg/mL for Esmolol Hydrochloride, ACC-9675 (Impurity-B), and ASL-8123 (Impurity-A). Each concentration was injected 3 times into the chromatographic system. Each time peak area and retention time are documented separately for the drug and impurities. Calibration curves were constructed by taking an average peak area on Y-axis and concentration on X-axis separately for drug and impurities. Regression equations were calculated from the calibration curves as shown in **Fig. 9, 10,** and **11**. These equations were used for the estimation of impurities in the bulk drug substance.

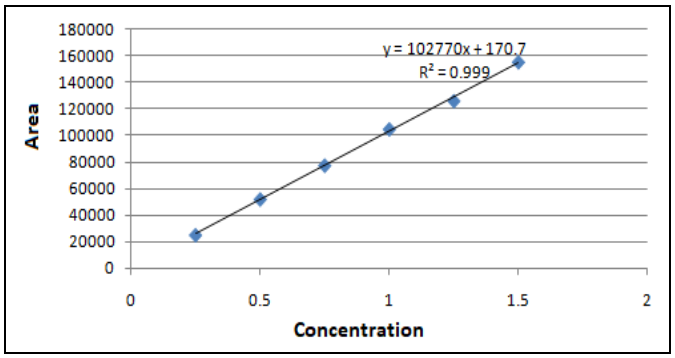


FIG. 9: CALIBRATION CURVE FOR ESMOLOL HYDROCHLORIDE

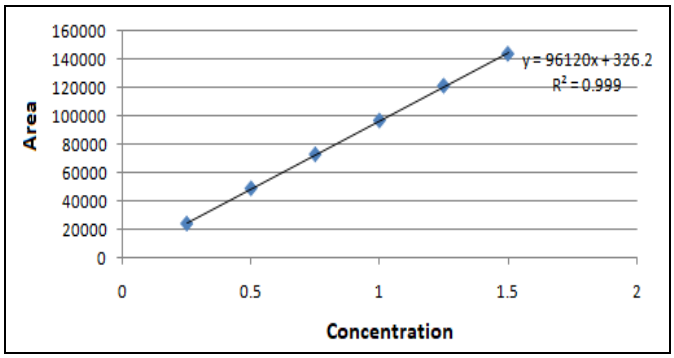


FIG. 10: CALIBRATION CURVE FOR ASL-8123

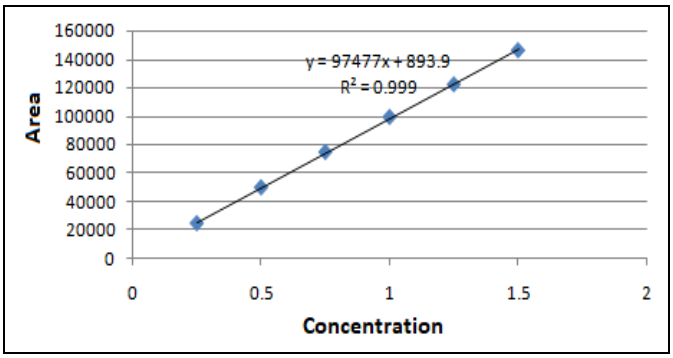


FIG. 11: CALIBRATION CURVE FOR ACC-9675

Estimation of Impurities in Esmolol Hydrochloride:

Preparation of Impurity Stock Solution: 0.5mg of Impurity A and B are accurately weighed and transferred into a 10 mL volumetric flask and makeup with the diluent gives 50ppm solution of impurity.

Spiking Impurity: Standard Esmolol Hydrochloride 10mg is weighed and transferred into a 10mL volumetric flask labelled precision spiked. 0.2 mL of solution for impurity stock is transferred to the volumetric flask labelled precision spiked and makeup to 10 ml with diluent. The results are shown in **Table 1**.

TABLE 1: ESTIMATION OF IMPURITIES IN ESMOLOL HYDROCHLORIDE

S. no.	Area of Esmolol Hydrochloride	Area of Impurity A	% of Impurity A (w/w)	Area of Impurity B	% of Impurity B (w/w)
1	104796	96841	0.994	99004	1.010
2	103923	96990	0.995	100934	1.020
3	104143	96652	0.992	99121	1.006
4	101810	97565	1.001	100970	1.025
5	102419	96980	0.995	99819	1.014
6	104308	96591	0.991	99435	1.010
% RSD	1.0	0.33	0.32	0.80	0.64

Method Validation: In the validation, repeatability and reproducibility demonstrated for analytical method and was consistently produced a result meeting its intended analytical applications [11-13]. The method was accomplished as per ICH guidelines. The developed method was corroborated by performing system suitability, linearity, limit of detection (LOD), the limit of quantification (LOQ), precision, accuracy, selectivity and robustness [14].

TABLE 2: SYSTEM SUITABILITY PARAMETERS

Parameter	Esmolol Hydrochloride	Impurity-A	Impurity-B
Retention time	8.037	6.383	5.014
USP Tailing	1.01	1.82	1.08
Plate count	15974	31815	15970
USP Resolution	7.4	7.7	-

System Suitability Parameters: The system suitability, six replicates of working standards samples of Esmolol Hydrochloride, Impurity-A, and Impurity-B were injected and studied the parameters like plate number (N), Tailing, and resolution. The results were revealed in **Table 2**.

Accuracy: Accuracy of impurities was covered in the range of 50, 100, and 150% with triplicate injections by adding a known amount of each Impurity standard to a pre-analyzed concentration of both impurities (Impurity A and Impurity B) and calculated the % of recoveries. The results were revealed in **Table 3** and **Table 4**.

TABLE 3: RESULTS OF THE RECOVERY STUDIES FOR IMPURITY-A

Average amount recovered (%w/w)	Amount added (%w/w)	Average amount added (%w/w)	% Recovery	Mean % Recovery	% RSD
0.498	0.4970	0.497	100.00	100.23	0.4
	0.4970		100.02		
	0.4970		100.67		
0.989	0.9940	0.994	99.66	99.48	0.2
	0.9940		99.30		
	0.9940		99.48		
1.480	1.4910	1.491	99.45	99.27	0.2
	1.4910		99.19		
	1.4910		99.17		

TABLE 4: RESULTS OF THE RECOVERY STUDIES FOR IMPURITY-B

Average amount recovered (%w/w)	Amount added (%w/w)	Average amount added (%w/w)	% Recovery	Mean % Recovery	% RSD
0.050	0.0500	0.050	99.56	99.62	1.9
	0.0500		97.71		
	0.0500		101.59		
0.101	0.0999	0.100	100.85	100.73	0.1
	0.0999		100.61		

	0.0999		100.72		
0.150	0.1499	0.150	99.99	99.82	0.2
	0.1499		99.81		
	0.1499		99.67		

Precision: The precision of the analytical method was demonstrated by analyzing six sets of sample solutions. The related substance of all six replicates sample preparations was determined and calculated the mean percentage of related substance. Standard deviation and percentage of the relative standard deviation for the same were calculated. The results were shown in **Table 5**. Precision is the level of repeatability of results as reported between impurities analyzed for the method.

To check the Precision 50 µg/mL each (Esmolol Hydrochloride, Impurity-A and Impurity-B) were taken. The precision of the proposed method, *i.e.*, the method variation in the peak area of the drug solutions, was calculated in terms of % RSD, and the results were reported in **Table 5**. Statistical results revealed that the relative standard deviation of each drug for 6 times was less than 1.0. The results are revealed in **Table 5**.

TABLE 5: METHOD PRECISION

S. no.	Area of Esmolol Hydrochloride	Area of Impurity A	% of Impurity A (w/w)	Area of Impurity B	% of Impurity B (w/w)
1	104796	96841	0.994	99004	1.010
2	103923	96990	0.995	100934	1.020
3	104143	96652	0.992	99121	1.006
4	101810	97565	1.001	100970	1.025
5	102419	96980	0.995	99819	1.014
6	104308	96591	0.991	99435	1.010
% RSD	1.0	0.33	0.32	0.80	0.64

Linearity: The linearity of the method was determined in the concentration range of 0.25–1.5µg/mL for Esmolol Hydrochloride, Impurity-A and Impurity-B. The peak area versus concentration data was analyzed with least-squares linear regression. The slope and intercept of the calibration curve were reported. The results were revealed in **Tables 6** and **7**.

TABLE 6: CALIBRATION DATA

% of Level	Concentration (ppm)	Area of Esmolol HCl	Area of Impurity-A	Area of Impurity-B
25	0.25	25142	23740	24412
50	0.5	52076	48704	49730
75	0.75	77463	72681	74689
100	1	104754	96621	99425
125	1.25	125970	121151	122436
150	1.5	155211	143694	146426

TABLE 7: OPTICAL CHARACTERISTICS OF ESMOLOL HYDROCHLORIDE, IMPURITY-A & IMPURITY-B

Parameters	Esmolol HCl	Impurity-A	Impurity-B
Linearity range (µg/mL)	0.25-1.5	0.25-1.5	0.25-1.5
Regression line equation	y = 102770x+170.7	y = 96120x+326.29	y = 97477x+893.98
Correlation coefficient (*r*)	0.999	0.9998	0.9997

LOD and LOQ: Limit of detection and Limit of quantification was traditionally based on the signal to noise ratio (S/N) 3:1 and 10:1 correspondingly. The results were revealed in **Table 8**.

TABLE 8: LOD & LOQ

Parameters	Esmolol HCl	Impurity-A	Impurity-B
LOD (µg/mL)	0.03	0.15	0.16
LOQ (µg/mL)	0.09	0.43	0.48

Robustness: The robustness of the related substance method was established by introducing trivial changes in the chromatographic condition, which included the temperature (27°C and 33°C), flow rate (0.8mL/min and 1.2mL/min) and a mobile phase (60Buffer: 40Acetonitile and 70 Buffer: 30Acetonitile). The results were exposed in **Table 9**.

TABLE 9: ROBUSTNESS STUDIES OF ESMOLOL HYDROCHLORIDE

Method parameters	Conditions	Retention Time (RT)
		Esmolol Hydrochloride
Flow Rate	0.8mL/min	8.868
Flow Rate	1.2mL/min	7063
Temperature	27°C	9.024
Temperature	33°C	7.743
Mobile Phase	60Buffer: 40Acetonitile	6.696
Mobile Phase	70Buffer: 30Acetonitile	10.815

Specificity and Selectivity: Specificity is the degree to which the procedure applies to a single analyte along with impurities and is checked in each analysis by groping samples and impurities for any interfering peaks. The specificity of the method was assessed with regard to interference. Different samples were injected and premeditated with respective impurities. The RP-HPLC chromatograms recorded for the drug substance (a mixture of the drug and impurities) showed no interfering peaks within the retention time ranges. The respective chromatogram for Esmolol Hydrochloride, Impurity-A, and Impurity-B has shown in **Fig. 12,** which shows that the selected drug and impurities were effectively separated. Thus, the RP-HPLC method proposed in this study was selective.

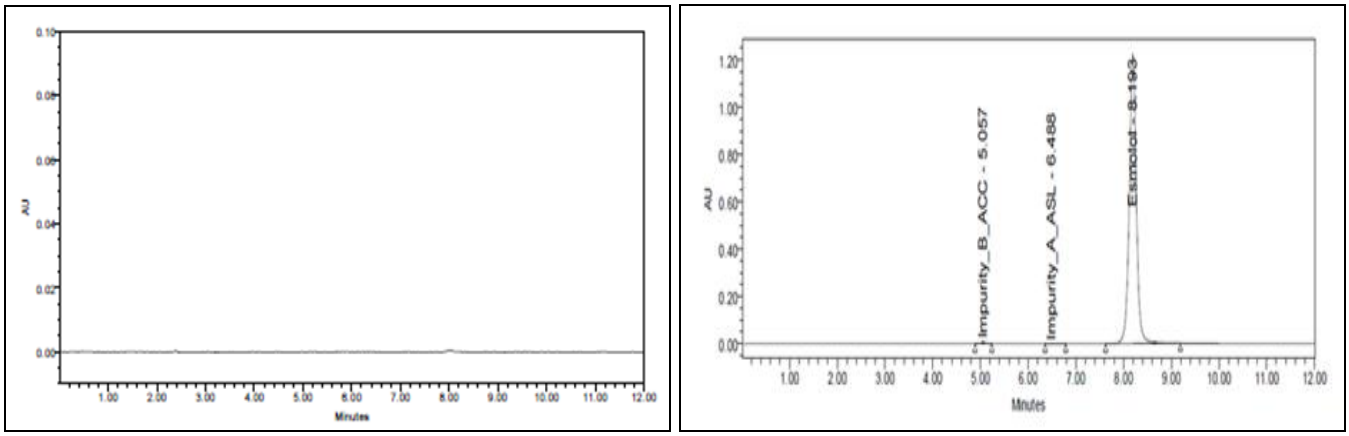


FIG. 12: TYPICAL RP-HPLC CHROMATOGRAM FOR BLANK, ESMOLOL HYDROCHLORIDE, ACC-9675 AND ASL-8123

Stress Degradation Studies: Weighed accurately 50mg of Esmolol Hydrochloride standard and transferred into a 50mL volumetric flask and makeup to the volume with diluent.

Acid Degradation: Transferred the 5.0 mL aliquot of above stock solution into a 25 mL round bottom flask, and 2.5 mL of 0.1 N HCl was added. The flask was heated at 60°C for 30 min using Buchirota evaporator, then cooled and neutralized with 0.1N NaOH solution. Make upto the mark with the mobile phase and percentage of degradation was calculated.

Alkali Degradation: Accurately, 5.0 mL aliquot of above stock solution was transferred into a 25 mL round bottom flask, and 2.5 mL of 0.1 N NaOH was added. The flask was heated at 60°C for 30 min using Buchirota evaporator, then cooled and neutralized with 0.1N HCl solution. Makeup to the mark with mobile phase and percentage of degradation was calculated.

Photolytic Condition: A 5 mL aliquot of the above solution was exposed to sunlight for about 6 h, and then the sample was diluted with 5 mL of mobile phase and the percentage of degradation was calculated.

Thermal Condition: Weighed Esmolol Hydrochloride 25mg and transferred into a Petri dish and kept in a hot air oven for 6 h at 105°C. The sample was shifted and placed in a desiccator till reaching room temperature. From this Petri dish, accurately weighed Esmolol Hydrochloride 10mg and transferred to a 10mL volumetric flask and makeup to the volume with diluent.

Photo Catalytic Degradation under UV: Accurately weighed Esmolol Hydrochloride 25mg into different Petri dishes and kept under UV light. From this Petri dish accurately weighed Esmolol Hydrochloride 10mg and transferred to a 10mL volumetric flask and makeup to 10mL with diluent.

RESULTS AND DISCUSSION:

Optimized Chromatographic Conditions: The optimized chromatographic conditions are stated above. The best peak shape and extreme separation were achieved with the mobile phase composition of: A–Acetonitrile, B–Potassium dihydrogen ortho phosphate in water. The best peak separation, peak symmetry and reproducibility were obtained on C_{18} (250 mm × 4.6 mm, 5µ). The optimum wavelength for detecting the analyte was found to be 221 nm with a flow rate of 1.0 mL/min. Most of the developed methods are reported HPLC till date use C_8 column or C_{18} columns. Most of these are carried out with complex mobile phase compositions. Hence challenges were directed towards the development of a simple and better method on a commonly used C_{18} column with good resolution between the impurities. Different logical amendments were trying to get good separation among the impurities and the active pharmaceutical ingredients. These changes included a change in mobile phase composition, column temperatures and ingredient mode on different C_{18} columns.

Accuracy: The percentage recovery of Impurity-A and Impurity-B in Esmolol Hydrochloride were attained in a range from 50.00% to 150.00% respectively. The related standard deviation value of replicated sets was less than 2.0%, which indicates that this method is highly accurate. The results are revealed in **Tables 3** and **4**.

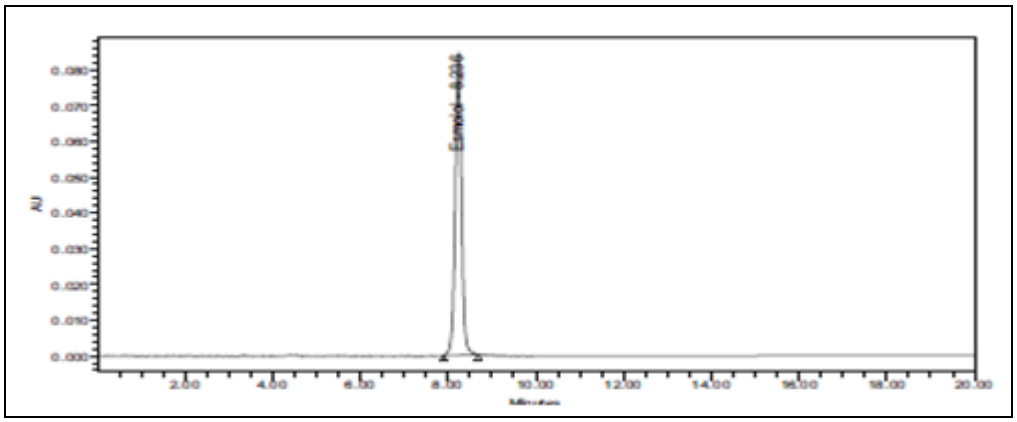
Precision: The precision of the method was determined by repeatability and intermediate precision of Esmolol Hydrochloride, Impurity-A, and Impurity-B standard solution. The obtained results of repeatability and intermediate precision were less than 2. The percentage of RSD value of replicated sets was less than 2.0, which indicates that this method is highly precise. The results are shown in **Table 5**.

Linearity: The calibration curve for the Esmolol Hydrochloride, Impurity-A, and Impurity-B were linear over each concentration range of 0.25-1.5µg/ml. The data for the peak area versus concentration were treated by linear regression analysis, and the correlation coefficient (r^2) was obtained (0.99). The calibration curve was calculated based on the regression equation. The statistical analysis revealed that the proposed method was linear, and the results were as shown in **Tables 6** and **7**.

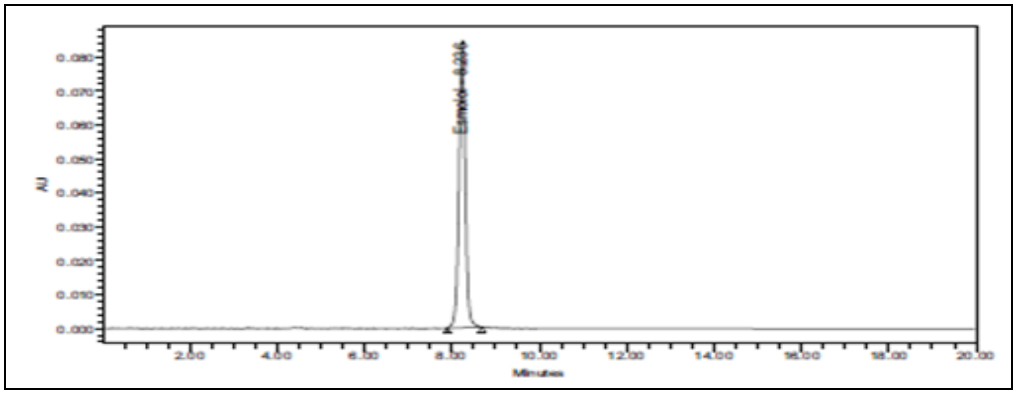
LOD and LOQ: The results of Limit of detection and Limit of quantitation data were within the acceptance criteria. The signal-to-noise ratio is well within the acceptance criteria. The results were as revealed in **Table 8**.

Robustness: The robustness of the related substance method was established by introducing minor changes in the chromatographic condition, which included the percentage of flow rate (0.8 and 1.2mL/min), Mobile Phase (60Buffer: 40Acetonitile and 70Buffer: 30Acetonitile), and temperature (27°C and 33°C). The developed method was unaffected by minor deliberated changes, which represents that the proposed method was robust. The results were shown in **Table 9**.

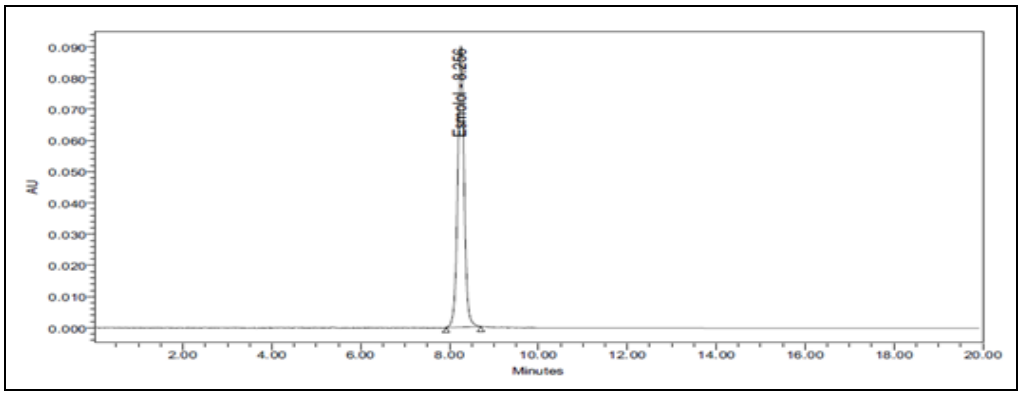
Degradation Studies: Upon performance of degradation studies, Esmolol Hydrochloride was active after degradation **Fig. 13**. The active present after degradation studies results are given in **Table 10**.



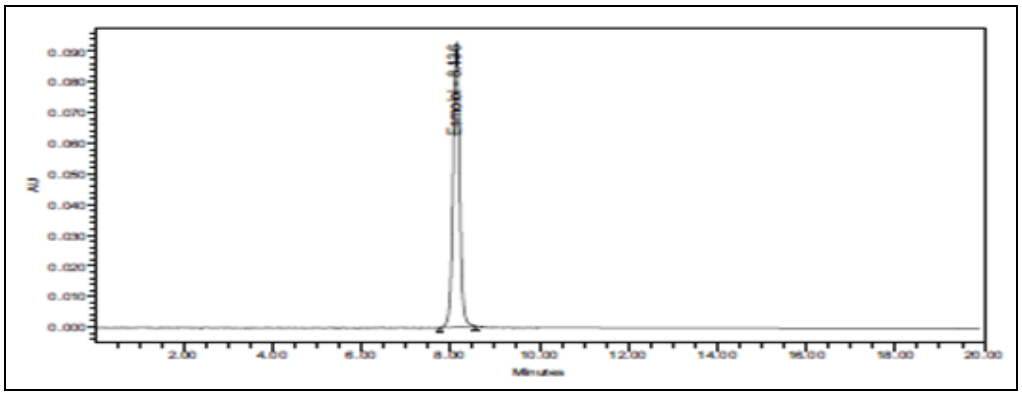
CHROMATOGRAM IN ACIDIC CONDITION



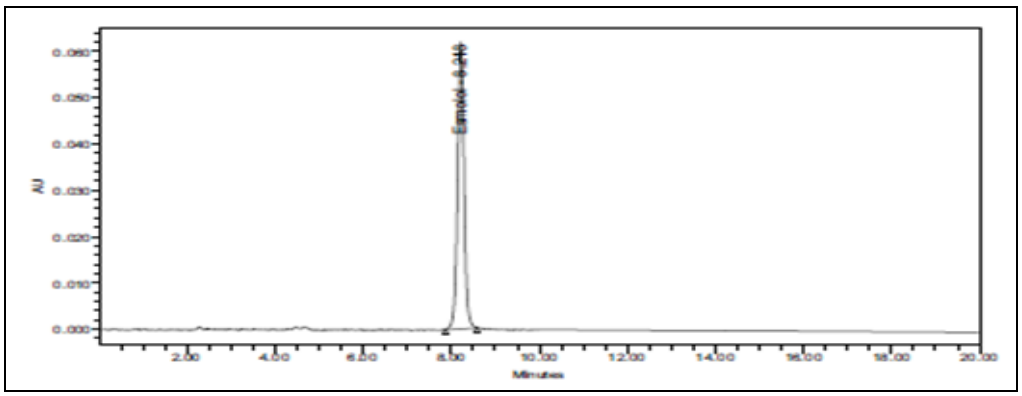
CHROMATOGRAM IN BASIC CONDITION



CHROMATOGRAM IN THERMAL CONDITION



CHROMATOGRAM IN UV CONDITION



CHROMATOGRAM IN PEROXIDE CONDITION

FIG. 13: CHROMATOGRAMS FOR DEGRADATION STUDIES

TABLE 10: RESULTS OF STRESS DEGRADATION STUDIES

Stress conditions	Acid	Base	Peroxide	Thermal	UV
Active present after degradation (%)	96.92%	97.88%	96.68%	97.00%	97.25%

CONCLUSION: In conclusion, stress testing (or forced degradation studies) is an important part of drug with the impurity development process, and the pharmaceutical industry always shows much interest in this area. A modest, rapid, accurate, and precise stability-indicating RP-HPLC analytical method has been developed and validated for the quantitative analysis of Esmolol Hydrochloride with related substance.

The proposed assay RP-HPLC method for separation of different types of degradation products along with the pure drugs was found to be capable of giving swift retention times with maintaining good resolution. The current method exhibited excellent performance in terms of sensitivity, selectivity, and speed. The stress testing followed as per ICH guidelines reveal that the method is more specific and stability-indicating.

The proposed method is capable of separating related substances from their degradation products and hence can be applied to the analysis of routine quality control samples and samples obtained from stability studies.

ACKNOWLEDGEMENT: Authors are thankful to VFSTR for constant support and encouragement. SK thankful to Spectrum laboratories, Hyderabad, India, for providing Esmolol Hydrochloride, ASL-8123, and ACC-9675 working standards as gift samples and thankful to Spectrum Laboratories, Hyderabad, for providing instrumental facilities to carry out research work.

CONFLICTS OF INTEREST: Authors have no conflict of interest.

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How to cite this article:
Kanithi S, Kumar CNSSP and Naidu CG: A validated stability indicating RP-HPLC method for determination of esmolol hydrochloride and its related impurities. Int J Pharm Sci & Res 2021; 12(11): 6016-27. doi: 10.13040/IJPSR.0975-8232.12(11).6016-27.