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RESEARCH ON THE DEVELOPMENT, VALIDATION ON SIMULTANEOUS QUANTIFICATION OF METOPROLOL SUCCINATE AND HYDROCHLOROTHIAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS USING RP-HPLC

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ABSTRACT

*A simple, accurate and precise High Performance Liquid Chromatographic (HPLC) method has been developed for simultaneous determination of Metoprolol Succinate and Hydrochlorothiazide in bulk and dosage form. The method has been validated as per the guidelines of ICH. The separation is achieved on 250*4.6mm C18, 5micron (Capcell) column with flow rate 1.0 mL/minute in isocratic mode using 0.1% OPA: Methanol (50:50) as mobile phase. Observations are recorded at 222 nm. The retention time of metoprolol succinate and hydrochlorothiazide were found at 3.25 min and 4.19 min respectively. The linearity range was found to be in the range of 10-50µg/ml for Metoprolol Succinate and Hydrochlorothiazide 5-25µg/ml. Correlation co-efficient for calibration curve of Metoprolol Succinate and Hydrochlorothiazide was found to be 0.999 and 0.990 respectively. The method is simple, accurate, reproducible and can be used for simultaneous analysis of Metoprolol Succinate and Hydrochlorothiazide.*

KEYWORDS: *Metoprolol Succinate, Hydrochlorothiazide, OPA, Methanol*

1.INTRODUCTION

METOPROLOL SUCCINATE

Metoprolol succinate is a beta-1 selective (cardio selective) adrenoceptor blocking agent that was first discovered and synthesized in **1969**. It was developed as an extended-release salt form of metoprolol and received its first U.S. patent in 1992, widely known by the brand name **Toprol XL**.

Unlike its counterpart, metoprolol tartrate, the succinate form is engineered to provide a controlled and predictable release of medication over a 24-hour period, making it suitable for once-daily administraion.

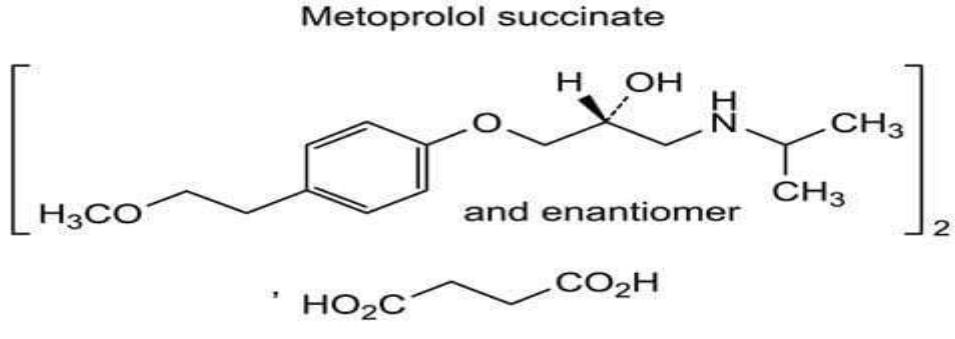


Fig 1:Structure of Metoprolol Succinate

IUPAC name: bis(1-[4-(2-methoxyethyl)phenoxy]-3-(propan-2- yl amino) propan-2-ol)

Mechanism of Action:- A powerful cardioselective β1-adrenergic receptor blocker, metoprolol succinate prevents catecholamines from attaching to cardiac myocytes. This reduces heart rate, myocardial contractility, and cardiac output, all of which reduce myocardial oxygen demand. Additionally, by inhibiting the kidneys' secretion of renin, it

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lowers blood pressure. Stable receptor blockage is provided for a full day by its extended-release formulation.

HYDROCHLOROTIAZIDE

Researchers at Merck & Co. and Ciba Specialty Chemicals (now a part of Novartis) created Hydrochlorothiazide (HCTZ), a thiazide diuretic (often referred to as a "water pill") in 1958 during a time of rapid advancement in renal pharmacology. It was designed to specifically target the distal convoluted tubule of the kidney, unlike loop diuretics that act on the loop of Henle. It was first approved by the U.S. FDA on February 12, 1959.

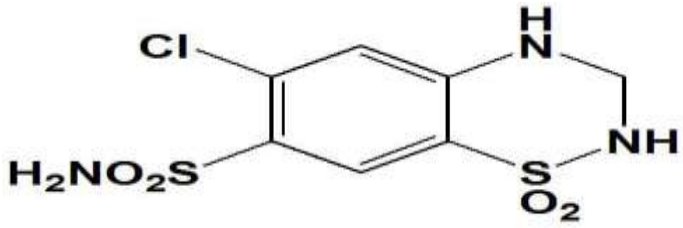


Fig 2: Structure of Hydrochlorothiazide

IUPAC Name: 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1- dioxide.

Mechanism of Hydrochlorothiazide:- Hydrochlorothiazide, a thiazide diuretic, inhibits the Na+/Cl-cotransporter in the kidney's distal convoluted tubule, preventing sodium and chloride reabsorption. This leads to increased water and salt excretion, lowering blood pressure by reducing cardiac output and total plasma volume. Long-term use results in vasodilation, decreasing peripheral vascular resistance. Additionally, the drug increases calcium reabsorption and causes potassium loss, making it effective for treating hypertension and edema by promoting fluid loss and relaxing vascular smooth muscle.

2.MATERIALS AND METHODS

Chemicals and Reagent: Orthophosphoric acid (Finar), and HPLC grade methanol (qualigens) were utilized for the investigation and filtered through 0.45µm membrane filters. The formulation of Metoprolol Succinate, Hydrochlorothiazide tablets was acquired from Met XL^{R}H25. (village-Konkona, Tehsil Nalagarh, D0ist.Solan, (H.P),India).

Instrumentation: UV-3092 LABINDIA double beam with UV win software, UV-Visible Spectrophotometer with 1 cm matched quartz cells. UFLC SHIMADZU Model: LC-20AD, UV-Visible dual absorbance detector with a manual injector. The results were observed and integrated by using Lab solutions software. A SHISEIDO C18 (250 x 4.6 mm I.D,5µm) column is used for separation.

Wavelength selection: The 200–400 nm range was scanned using a 10µg/ml solution. The UV Absorbance Spectra of Metoprolol Succinate and Hydrochlorothiazide demonstrated wavelengths of 222nm and 235 nm, respectively. The overlapping maximum absorbance wavelength for Metoprolol Succinate and Hydrochlorothiazide was determined to be 222nm.

Uv- visible graph

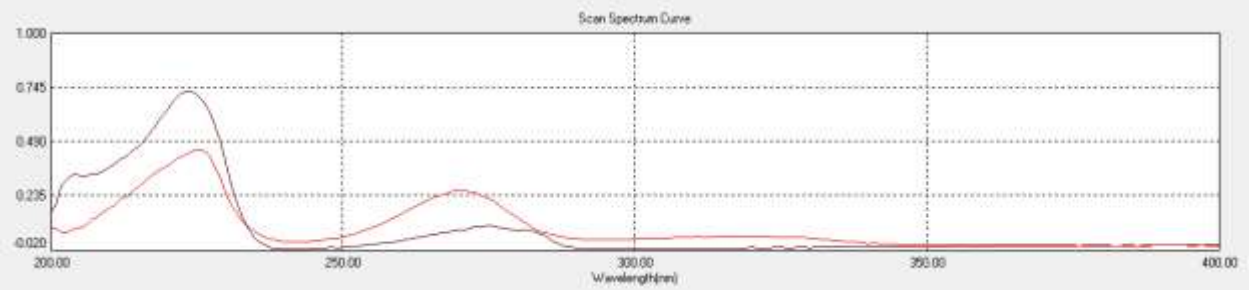


Fig 3:UV Spectrum of Metoprolol Succinate and Hydrochlorothiazide

Chromatographic Conditions

Orthophosphoric acid and methanol (50:50 v/v) made up the mobile phase, which had a flow rate of 1.0 ml/min and a detection wavelength of 222 nm.

Preparation of 0.1N Orthophosphoric acid: Accurately measure and take 0.1ml of orthophosphoric acid and make it up to 100 ml with HPLC grade water.

Preparation of Mobile Phase: Mobile phase was prepared by mixing 0.1% orthophosphoric acid and Methanol taken in the ratio 50:50. It was subjected to sonication to remove the dissolved gases which may interfere in the final chromatograms.

Preparation of Standard solution: Precisely weigh 10 milligrams of Metoprolol Succinate and Hydrochlorothiazide into a 10-milliliter volumetric flask, then adjust with mobile phase to reach 1000 µg/ml. Pour 1 milliliter of the stock solution into a 10-milliliter volumetric flask, then top it off with mobile phase to achieve 100 µg/ml.

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Preparation of Sample solution: Take 1 milliliter of the 100 µg/ml solution and use the mobile phase to make it equal to 10 µg/ml.

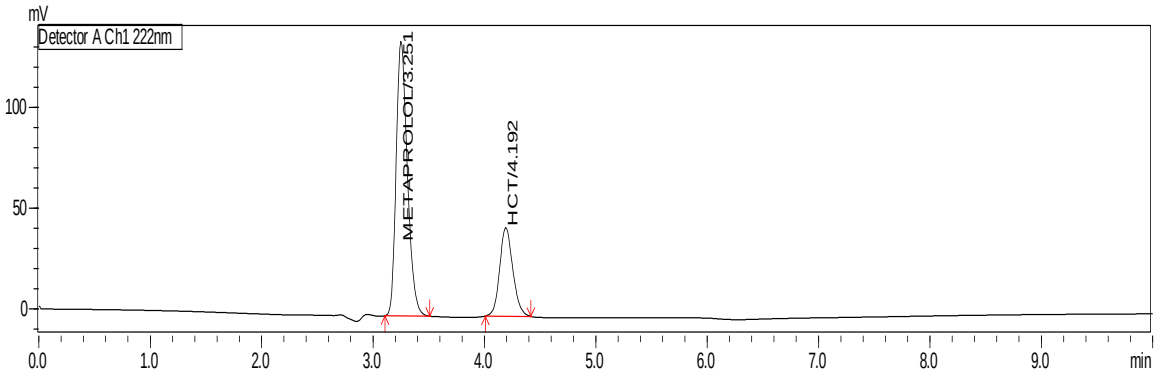
3.RESULTS AND DISCUSSION

To accomplish the required chromatographic separation, the HPLC method for Metoprolol Succinate and Hydrochlorothiazide was developed by carefully adjusting a number of parameters, including the stationary phase selection, flow rate, wavelength and mobile phase composition. In addition, the injection volume, detector, and gradient mode should be considered. For optimization, system suitability parameters need to be looked.

Method development and optimization of chromatographic conditions

We first attempted using the mobile phase with ratio of 60:40% v/v orthophosphoric acid and Acetonitrile at a flow rate of 1 ml/min. However, we found that the peak was not adequately eluted by this mobile phase. Next, the mobile phase using 65:35% v/v of 0.1% orthophosphoric acid and acetonitrile at a flow rate of 1 ml/min. The peak had moderate strength and low resolution when it eluted. After that, and 70:30 0.1% orthophosphoric acid and acetonitrile were used in the mobile phase; however, the results showed low resolution and retention duration. A mobile phase consisting of 50:50% 0.1% orthophosphoric acid and acetonitrile then produced low-resolution peaks and tailing at a flow rate of 1 milliliter per minute. A mobile phase consisting of 60:40 0.1%v/v orthophosphoric acid, and methanol produced peaks with reduced retention and resolution at the same flow rate. After that, the mobile phase was assessed using a ratio of 50:50 0.1% v/v orthophosphoric acid, and methanol at a flow rate of 1 ml/min. In the isocratic technique of elution, a sharp peak, good intensity, and good retention length were noted. After a review of the system's suitability, the findings were judged to be within acceptable bounds.

Optimization Method



b

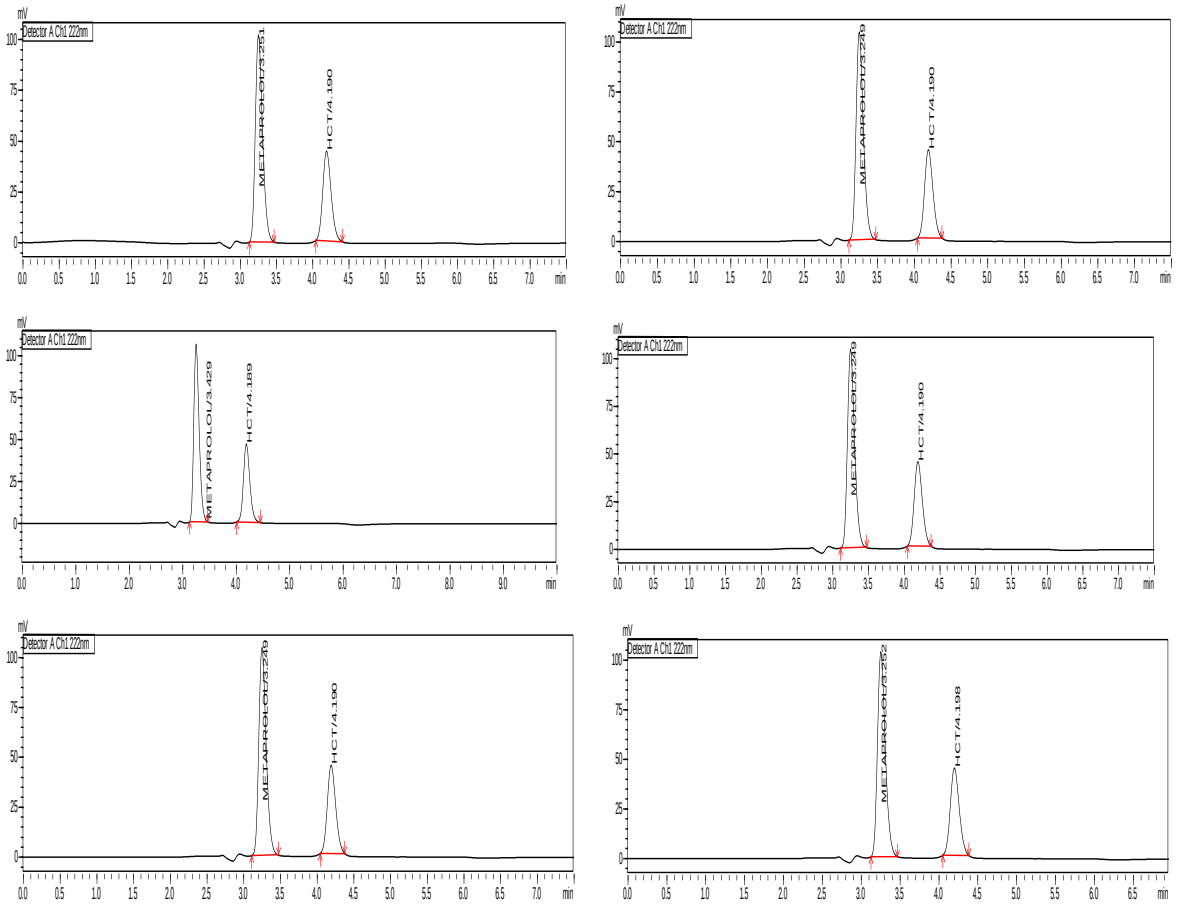
4.METHOD VALIDATION

SYSTEM SUITABILITY: The system suitability parameter indicates a very high degree of reproducibility of the developed chromatographic method. This novel method, at 10 µg/ml concentration which reflects high column efficiency, good precision, and better reproducibility. A 10µl solution was injected in six replicates run into the HPLC system and examined with an emphasis on retention time and their standard deviations and relative standard deviations was calculated.

Table1: System suitability of Metoprolol succinate and Hydrochlorothiazide

INJ NO	META	HCT
1	708251	304649
2	701050	303920
3	715817	302218
4	724354	301266
5	722411	310980
6	727378	300912
AVG	716543.5	303990.8
SD	10219.2	3723.802
RSD	1.426179	1.224972

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LINEARITY &RANGE

Linearity refers to the relationship between the analysis response and the concentration of analyte. It is a critical parameter in method validation, as it demonstrates the method ability to produce accurate and reliable results. Throughout the Metoprolol Succinate and Hydrochlorothiazide concentration ranges, the method was linear. Therefore, when a graph is plotted against concentration vs. area, as shown in figures 5 and 6, a nice linear relationship is seen.

Table 2: Linearity Metoprolol succinate and Hydrochlorothiazide

METOPROLOL SUCCINATE		HYDROCHLOROTIAZIDE	
CONCENTRATION	PEAK AREA	CONCENTRATION	PEAK AREA
10µg/ml	766062	5µg/ml	379759
20µg/ml	1562939	10µg/ml	760978
30µg/ml	2307968	15µg/ml	1162749
40µg/ml	2979403	20µg/ml	1460207
50µg/ml	3774020	25µg/ml	1793752

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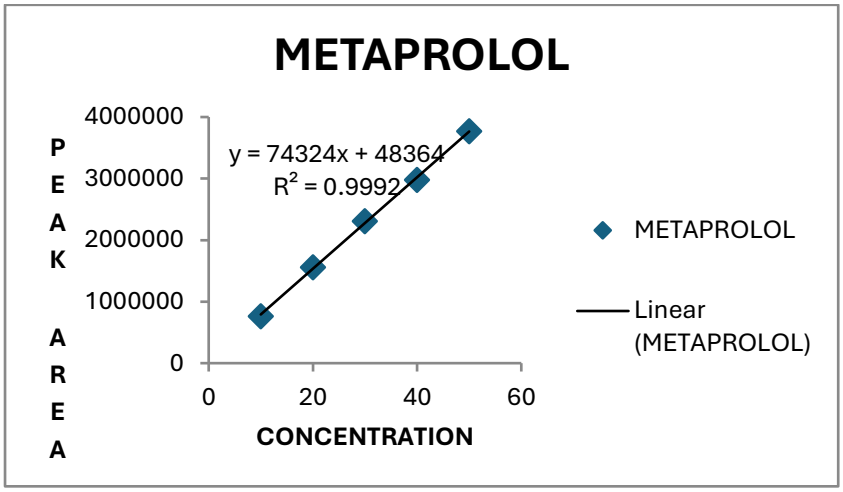


Fig 4: Calibration curve for linearity of Metoprolol succinate with peak area

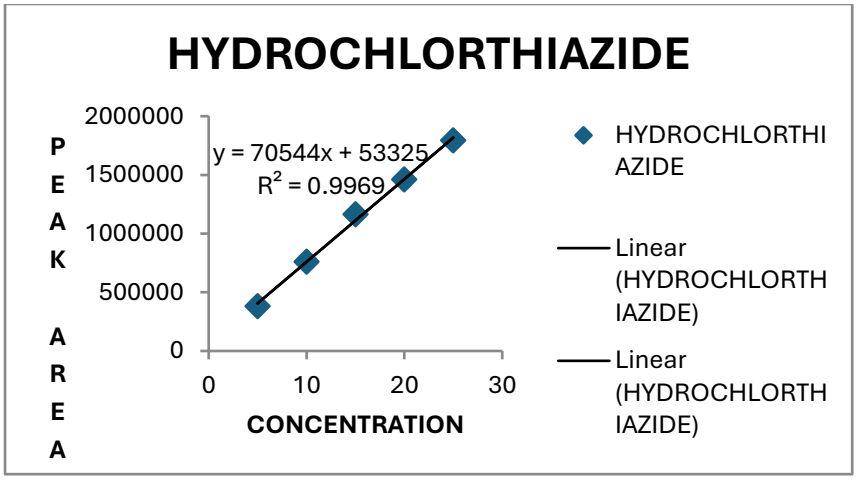
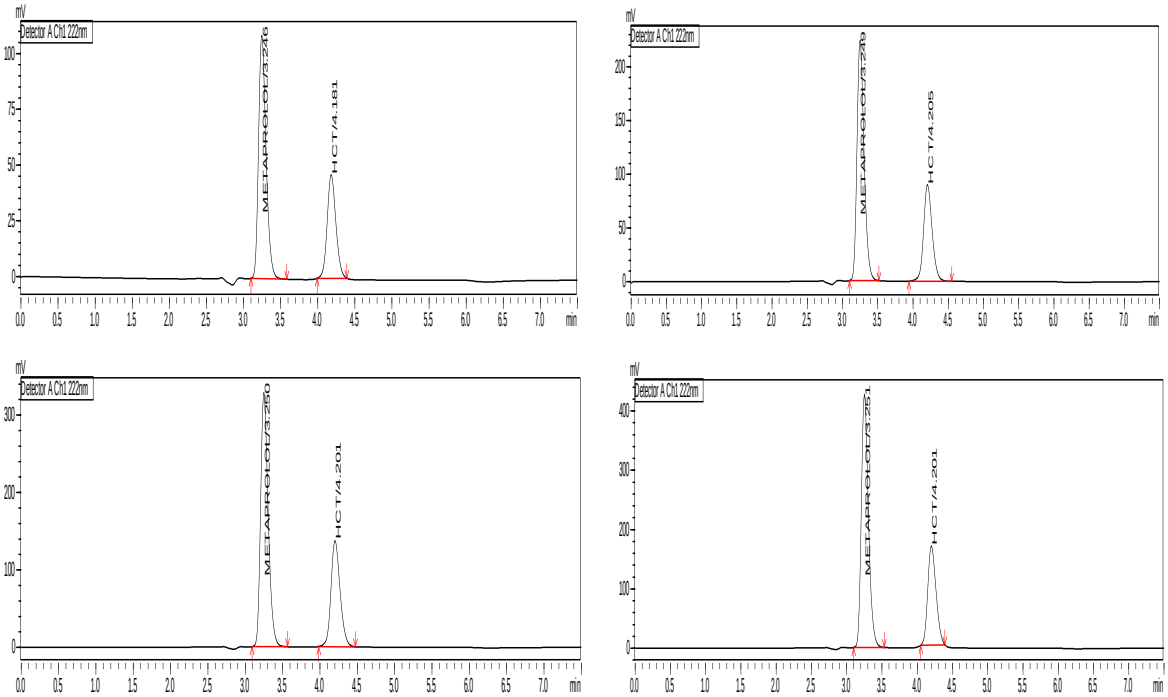
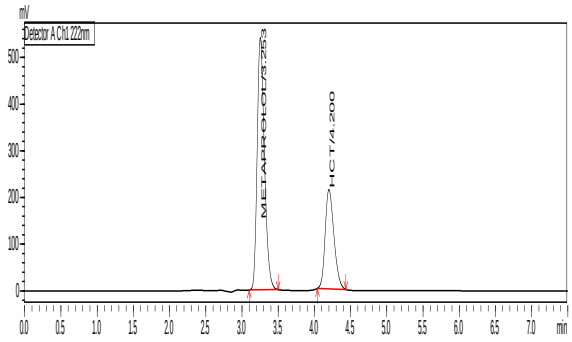


Fig 5:Calibration curve for linearity of Hydrochlorothiazide with peak area



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PRECISION

Precision is a critical parameter in method validation. Precision is the closeness of individual measurements of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of a sample. It is a measure of the variability or dispersion of the analytical results.

Types of Precision

1. Repeatability: precision attained when the analysis is carried out using the same tools, by the same analyst, and under the same circumstances.

2. Intermediate Precision: Precision obtained when the analysis at different conditions, such as different days, analysts, or equipment

3. Reproducibility: Precision obtained when the analysis is performed in different laboratories. To assess the repeatability of the suggested method, six duplicates of the Metoprolol Succinate and Hydrochlorothiazide mixed standard solution (10 µg/mL) were tested using the same apparatus in the same lab, and %RSD was calculated. Intermediate precision was verified at different times regularly and on three subsequent days in succession. The %RSD was calculated for all precision studies. According to ICH requirements, Six duplicates of 10µg/ml of hydrochlorothiazide and metoprolol succinate were used to measure the precision and repeatability of the evaluated High Performance Liquid Chromatography technology. The accuracy of the established method was discovered. This can be seen in table 3&4.

Table 3: Intra Precision of Metoprolol succinate and Hydrochlorothiazide

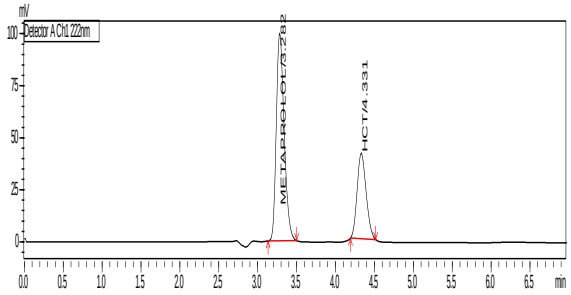
METAPROLOL SUCCINATE				HYDROCHLOROTIAZIDE		
S.NO	**L Q C**	**M Q C**	**H Q C**	**L Q C**	**M Q C**	**H Q C**
1	774082	2050675	2745521	323232	1030522	1752717
2	783117	2002726	2823581	324825	1058492	1808715
3	770563	2020480	2737721	332011	1068397	1772263
AVG	775920.67	2024627	2768941	326689.33	1052470.3	1777898.3
SD	6475.8204	24242.006	47480.072	4677.0198	19642.41	28421.149
RSD	0.8345983	1.1973567	1.7147376	1.4316414	1.8663148	1.5985813

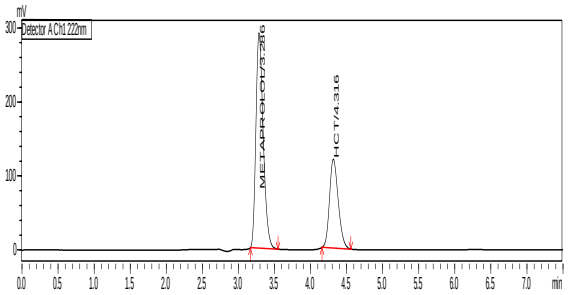
Table 4:Inter Precision of Metoprolol succinate and Hydrochlorothiazide

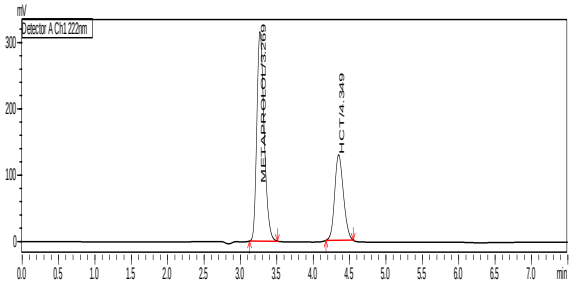
METAPROLOL SUCCINATE				HYDROCHLOROTIAZIDE		
S.NO	**L Q C**	**M Q C**	**H Q C**	**L Q C**	**M Q C**	**H Q C**
1	775920.7	2024627	2768941	326689.3	1052470	1777898
2	758180	2040191	2682631	337140	1036552	181846
3	773597	2025688	2676787	328550	1057811	1819635
AVG	769232.57	2030168.7	2709453	330793.1	1048944.3	180536
SD	9642.0598	8695.7923	51600.918	5574.7561	11059.341	23769.36
RSD	1.2534648	0.4283286	1.9044773	1.6852698	1.0543306	1.316617

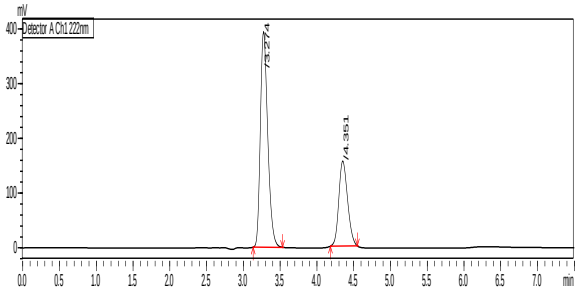
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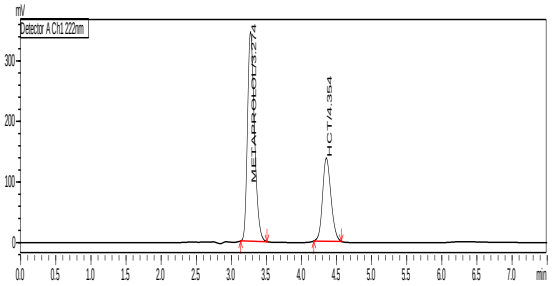
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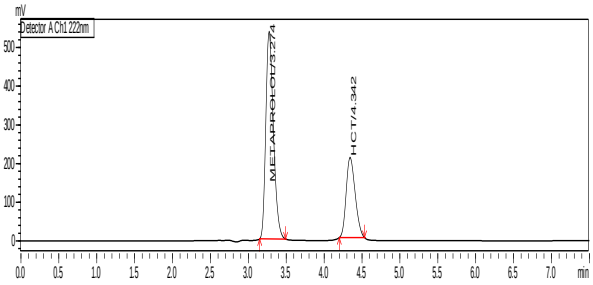




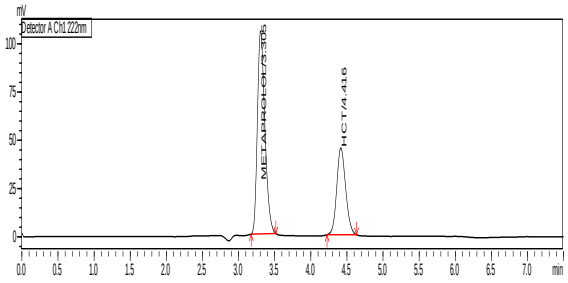


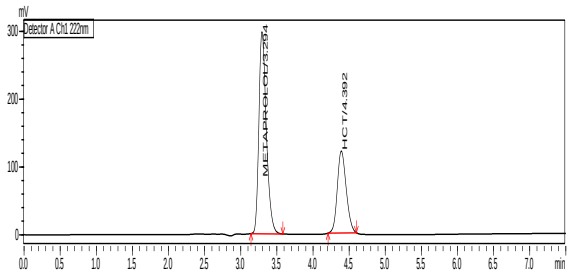


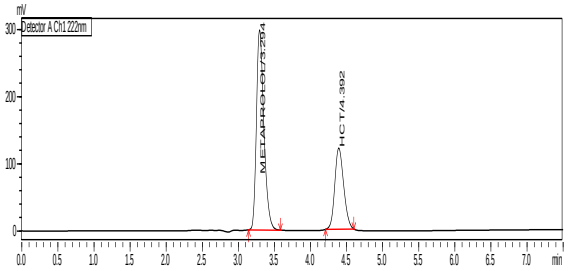


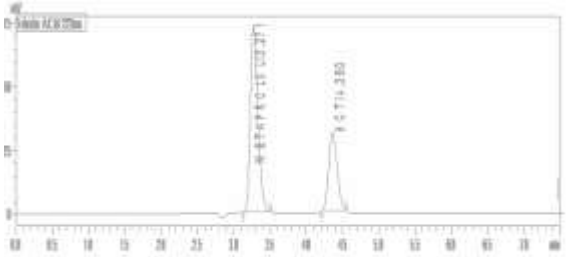


Inter precision:

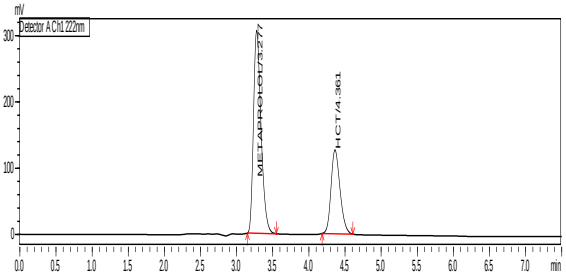


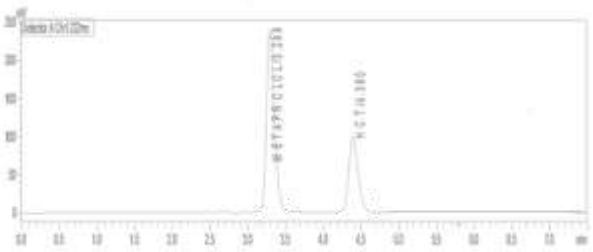






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SENSITIVITY

LOD &LOQ

Limit of Detection (LOD): The lowest concentration of an analyte that can be consistently detected, even though quantification might not be feasible, is known as the Limit of Detection (LOD).

Limit of Quantitation (LOQ): The lowest analyte concentration that can be quantitatively measured with a certain level of confidence is known as the Limit of Quantitation.

Limit of Detection and Limit of Quantitation demonstrate the method's sensitivity and ability to detect and quantify low levels of analytes.6 The Y-intercept (standard deviation: SD) and mean slope (S) values are replaced in the following equation: LOD = 3.3 X S.D. /S and LOQ-10 X S.D. /S S/N ratios were used to examine the LOD&LOQ. Results are outlined in table which are indicating enhanced sensitivity for detection and quantification.

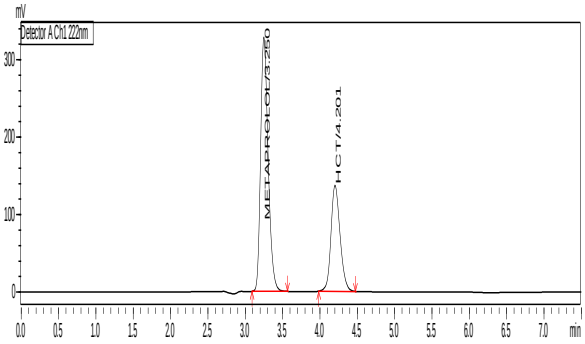
Table 5:Sensitivity of Metoprolol succinate and Hydrochlorothiazide

	METOPROLOL SUCCINATE	HYDROCHLOROTIAZIDE
LOD	1.00µg/ml	0.1µg/ml
LOQ	5.00µg/ml	1.00µg/ml

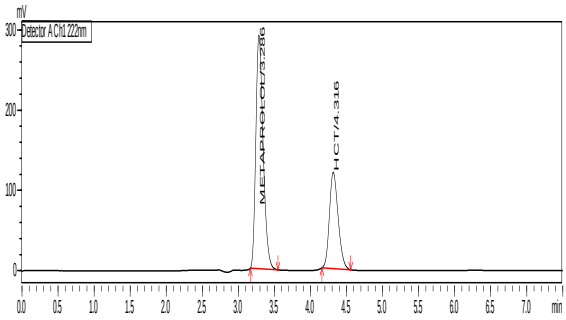
ROBUSTNESS

An analytical method is said to be robust if it can withstand minor, intentional adjustments to its parameters, such as pH, temperature, flow rate, and column type. Robustness guarantees that, even in the face of minor changes in experimental conditions, the analytical approach will continue to be valid.

The mobile phase ratio (OPA:MET = 50:50) and flow rate (1 ml/min) were intentionally altered by ± 2% in order to evaluate the durability of the approach. The robustness test was carried out twice at a 10µg/ml concentration. Four samples of hydrochlorothiazide and metoprolol succinate were analyzed under particular conditions, such as a change in mobile phase composition of ± 2% and a change in flow rate of ± 2%, with an emphasis on how these changes impacted the system suitability characteristics. The approach proved sufficiently resilient because the computed percentage RSD was within the ICH limit.



49:51 ratio of OPA and Methanol



flow rate :0.9ml/min

ASSAY

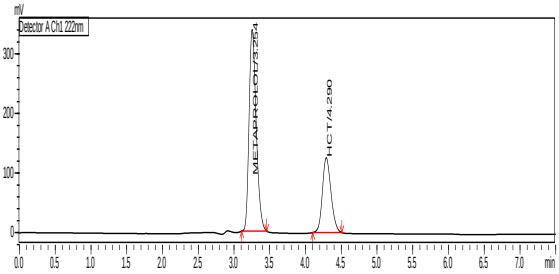
High-precision, specific analytical technique used to quantify the concentration or potency of an API (Active Pharmaceutical Ingredient) in a sample, generally ensuring quality control. It works by separating the active component from excipients and impurities using a liquid mobile phase pumped through a column, followed by detection

Table 6: Assay of Metoprolol succinate and Hydrochlorothiazide

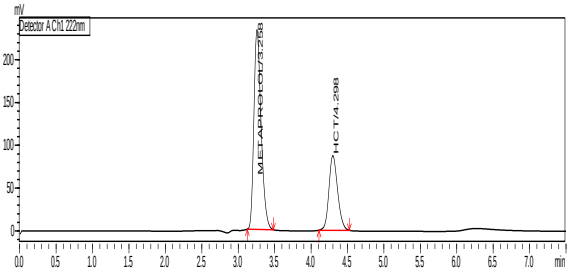
	METOPROLOL SUCCINATE	HYDROCHLOROTIAZIDE
Y	810791	408207
M	74324	70544
C	48364	53325
X	10.2585	5.030648

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Trail-1



Trail-2



5.CONCLUSION

The developed RP-HPLC method for simultaneous estimation of metoprolol and hydrochlorothiazide is simple, precise, accurate, and robust. Optimization of chromatographic conditions led to well-resolved peaks and good system suitability. Validation covered specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness, showing excellent linearity and minimal interference. The method displays low relative standard deviation values for precision, indicating consistency. The LOD and LOQ confirm adequate sensitivity, and robustness testing shows the method is unaffected by minor variations in conditions.

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