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An improved validated HPLC method for separation of metoprolol and hydrochlorothiazide impurities in metoprolol and hydrochlorothiazide tablets

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ABSTRACT

A rapid, specific, sensitive high-performance liquid chromatographic method has been developed for determination of Metoprolol Succinate and Hydrochlorothiazide impurities and its degradation products in pharmaceuticals preparation. HPLC was performed on a C18 column with "mobile phase A" consisting of 95:5 v/v of pH 2.5 buffer solution and methanol; while "mobile phase B" consisted of 55:45 v/v methanol and acetonitrile. The mobile phase was pumped in a gradient manner at the flow-rate of 1.0 mL min^{-1}. Ultraviolet detection was performed at 225 nm. Metoprolol Succinate and Hydrochlorothiazide impurities and degradation products along with process impurities were chromatographed with a total run time of 60 minutes. Calibration showed that response of impurities was a linear function of concentration over the range LOQ to 300% of the target concentration ($r^2 \geq 0.999$) and the method was validated over this range for precision, accuracy, linearity and specificity. For precision study, percentage relative standard deviation of each impurity was <15% (n = 6). The method was found to be precise, accurate, linear and specific. The method was successfully employed for estimation of Metoprolol Succinate and Hydrochlorothiazide impurities and its degradation products in commercial available capsules and in-house developed formulations.

Keywords: HPLC – Metoprolol Succinate, Hydrochlorothiazide, Impurities and Method validation

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INTRODUCTION

Metoprolol succinate (METO) is chemically (RS)-1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2ol succinate, is a cardio selective β-blocker, used in the treatment of hypertension, angina pectoris, arrhythmia, myocardial infraction and heart failure. The drug is a lipophilic adrenoreceptor antagonist (b-blocker) with a short half-life. The chemical structure is shown in Figure 1A [1]. Hydrochlorothiazide (HCTZ), chemically, is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide1,1-dioxide, is a popular diuretic drug of the thiazide class. It is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and in the prevention of kidney stones. The chemical structure is shown in Figure 2B. It is used widely alone or in combination for the treatment of cardiovascular disorders, viz, hypertension, angina, and congestive cardiac failure [2-5].

Metoprolol Succinate and Hydrochlorothiazide drugs are official in the British Pharmacopoeia and United States Pharmacopoeia but their combination is not official. Based on the literature survey, no official method has yet been developed for their separation and its impurities [6-8].

Several methods have been reported using HPLC with UV and fluorescent detection for the determination of Metoprolol Succinate and Hydrochlorothiazide individually in pharmaceutical dosage forms as well as in biological

fluids [9-17]. A new drug application (NDA) has been submitted to FDA (U.S. Food and Drug Administration) by AstraZeneca, a multinational pharmaceutical firm, in respect of the above combination [18]. Thus, application of an HPLC method with high sensitivity and selectivity will find use for the determination of metoprolol succinate and hydrochlorothiazide impurities and its degradation products in pharmaceutical formulations.

MATERIALS AND METHODS

2.1 Chemicals and Reagents

Metoprolol Succinate and it impurities, Impurity A, Impurity-B, Impurity-C and Impurity-D and Hydrochlothiazide and its impurities, Chlorothizide and Benzothiazide impurities from USP. Acetonitrile (HPLC-grade from J.T. Baker, USA), and Potassium dihydrogen phosphate, Sodium Hydroxide, Hydrochloric Acid, Hydrogen Peroxide were from Merck (Darmstadt, Germany. Water was purified by a Millipore (Bedford, MA, USA) Milli-Q water-purification system and passed through a 0.22 µm membrane filter (Durapore; Millipore, Dublin, Ireland) before use.

2.2 Equipment

HPLC analysis was performed with a Waters Alliance system equipped with a quaternary solvent manager, sample manager, column-heating compartment, and Photodiode array detector. This system was controlled by Waters Empower software.

The specificity study was conducted by using heating oven, stability chamber and heating mantel (Thermo Lab, India).

2.3 Standard and Sample Preparation

The standard stock solution of Metoprolol Succinate and Hydrochlorothiazide was prepared by dissolving an accurately weighed amount of working standards in diluent, resulting in a concentration of 0.8mg/mL and 0.2mg/mL respectively. Above solution further diluted in diluent to get a concentration of 2.4 µg mL^{-1} and 0.6 µg mL^{-1} respectively.

The impurity stock solutions for Metoprolol impurity A, B, C and D and Chlorothizide and Benzothiazide impurities was prepared by dissolving an accurately weighed amount in diluent, resulting in a concentration of 2.4 µg $mL^{-1}$of each impurity of Metoprolol impurities and 0.6 µg mL^{-1} for HCTZ impurities.

The test solution was prepared by dissolving an accurately weighed portion of the powder, equivalent to 80 mg of Metoprolol (20mg of HCTZ) in 70mL diluent. After sonicating for around 30minutes, volume made up to 100mL.Above solution was filtered through 0.45µ PVDF filter to eliminate insoluble excipients. The clear liquid used for chromatographic analysis.

2.4 Chromatography

The analytes were separated on an Waters HPLC with ACE 5 C18 column (250 mm x 4.6 id, 5µ) at column oven temperature of 30^{o}C with a gradient run program at a flow-rate of 1.0 mL min^{-1} (Table 1). Before use, the mobile phase was filtered through a 0.45 µm Millipore filter. UV detection was performed at 225 nm. The sample injection volume was 10 µL in partial-loop mode.

2.5 Method Validation

The method was validated for specificity, precision, accuracy, sensitivity and linear range as per the International Conference on Harmonization (ICH) guidelines [19].

2.5.1 Specificity:

A study was conducted to demonstrate the interference from placebo. Sample solutions were prepared by taking the placebo equivalent to the amount present in the sample solution and analyzed as per test method. Chromatograms of placebo preparations are not showing any interference at the retention time of known impurities as well as analyte peaks.

A study was conducted to demonstrate the known impurities interference by spiking the sample solution with all the known impurities at 0.3% spike level and analyzed as per test method. It is found that all the known impurities are separated from each other and also from analyte peaks.

The known impurities of Metoprolol Succinate and Hydrochlorothiazide were injected individually to confirm the retention time.

A study was conducted to demonstrate the effective separation of degradants from Metoprolol Succinate and Hydrochlorothiazide peak. The drug product was subjected to hydrolysis by refluxing the test solution in 5 N Sodium hydroxide solution at 60ºC for 4 h. Similarly the acidic hydrolysis was performed by refluxing test solution in 5N Hydrochloric acid solution at 60ºC for 4 h. The neutral hydrolysis was done in water at refluxing temperature of 60ºC for 4 h. Oxidation studies were performed in 30 % Hydrogen Peroxide solution at Bench top for 4h. On photo stability study drug product was sufficiently spread on petri plates (1 mm thick layer) and exposed to sunlight and UV light at ambient conditions for 7 days. Humidity study was performed separately by exposing drug product to humidity at 25°C, 90% RH for 5 days. Thermal degradation study was performed by heating drug product at 105° C for 24 h.

Similarly placebo samples were prepared as like as drug product by exposing formulation matrices without drug substance.

Stressed samples were injected into the HPLC system with photo diode array detector by following test method conditions.

2.5.2 Precision:
The precision of test method was evaluated by using six samples spiked with known Impurities at 0.3% level and analyzed as per test method.

2.5.3 ACCURACY:
To confirm the accuracy of the method, recovery studies were carried out by standard addition technique. Samples were prepared in triplicate by spiking all known impurities in test preparation at the level of LOQ , 50%, 100%, 200% and 300% of the standard concentration and analyzed as per the test method.

2.5.4 Sensitivity:
Sensitivity of the method was established with respect to Limit of detection and limit of quantification for Metoprolol Succinate and Hydrochlorothiazide impurities. Series of concentration of drug solution and its impurities were injected, LOD and LOQ established by Signal to Noise ratio method.

Precision was performed at LOQ level for all the known impurities by injecting six replicate injection of each impurity at the concentration obtained from above method.

2.6 Linearity of Detector Response:
A series of solutions of all the known impurities in the concentration ranging from limit of quantification level LOQ to 300% of standard concentration were prepared and injected into the HPLC system.

Application of Developed Method:
The method suitability was verified by analyzing three different strengths of finished product of innovator and in-house formulated product. The content of 20 Tablets powder was accurately weighted and transfered equivalent to about 80 mg of Metoprolol succinate into a 100 mL volumetric flask, add about 70 mL of diluent and sonicate for about 15 minutes with intermittent shaking, dilute to volume with diluent and mix well. The solution was filtered through 0.45 µm PVDF filter and injected.

RESULTS AND DISCUSSION

Selectivity, sensitivity, resolution, and speed of chromatographic separation were optimized for the HPLC method. The retention times of Benzochlorothiazide at about 7.97, Chlorothiazide at about 10.17, impurity - A at 24.59, impurity-B at 29.06, impurity-C at 15.98, impurity-D at 39.58, Hydrochlorothiazide at about 11.48 and Metoprolol at 28.76 minutes respectively, under the chromatographic conditions described, and the total run time was 60 min. Chromatograms obtained from blank, diluted standard, controlled sample and Test sample spiked with impurities are shown in Figures 2A, 2B, 2C, 2D and 4E respectively.

HPLC system has been proved to be a promising tool for separation with shorter run time. Use of ACE 5 C18, 250 × 4.6 mm, 5 µm as stationary phase enabled optimization of HPLC for both peak selectivity and analysis speed. Hydrochlorothiazide, Metoprolol and its impurities were well separated with good peak shape and resolution. No interfering peaks were observed in blank & placebo, indicating that signal suppression or enhancement by the product matrices was negligible.

After satisfactory development of method it was subjected to method validation as per ICH guideline [19]. The method was validated to demonstrate that it is suitable for its intended purpose by standard procedure to evaluate adequate validation characteristics. The result of system suitability parameter was found to be complying acceptable suitability criteria: relative standard deviation of replicate injection is not more than 5.0% The result of specificity study ascertained the known impurities are separated from each other and also from Metoprolol and Hydrochlorothiazide peak and spectral purity of all exposed samples found spectrally pure [Table 3]. The % RSD of replicate determination was found to be <5 during precision study, which indicates that the method is precise and data of precision study are shown in Table 4. The result obtained in the recovery study were found within the range of 85% to 115% (LOQ to 300%), which indicates that method is accurate and data for the same is given in Table 5 and 6. Sensitivity of method was verified and method is found to be linear, accurate and precise at limit of quantification and data of LOD & LOQ study are given in Table 7 and 8. The calibration curve of all impurities were obtained by plotting the peak area of individual impurity versus concentration over the range of LOQ to 300% and were found to be linear (r = 0.999). The data of regression analysis of the calibration curves are shown in Table 9. The applicability of the method was verified by the determination of impurities in Dutoprolol (innovator) and In house formulation. The impurity content was found to be satisfactory in both formulations and data shown in Table 9.

Figure 1A: Chemical structure of Metoprolol Succinate

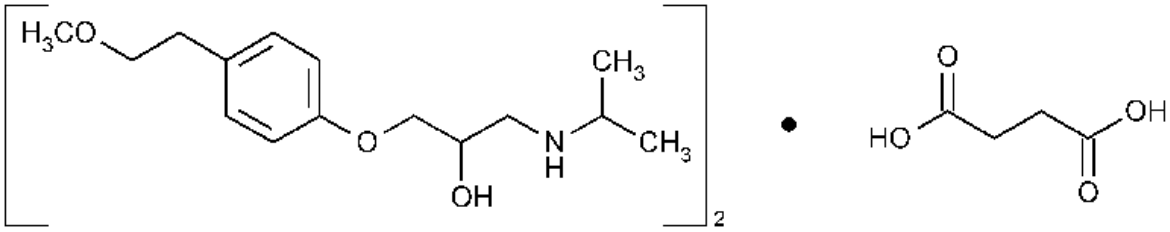


Figure 1B: Chemical structure of Hydrochlorothiazide

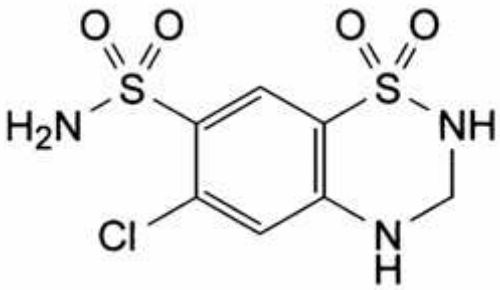


Table-1. Gradient program for elution of Metoprolol and Hydrochlorothiazide and impurities

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–15	95 → 85	5 → 15	linear gradient
15-40	85 → 40	15 → 60	linear gradient
40-50	40 → 30	60 → 70	linear gradient
50-53	30 → 95	70 → 5	Initial gradient
53–60	95	5	re-equilibration

Table-2. Chromatographic Conditions

LC Column	ACE 5 C18, 250 × 4.6 mm, 5 µm
Flow Rate	1.0mL/minutes
Run Time	460 minutes
Wavelength	225nm
Column oven Temperature	30°C

Table 3. Results of Specificity Study

S.No.	Stress conditions	% Degradation	Individual % Degradation					
			Metoprolol				Hydrochlorothiazide	
			% IMP-A	% IMP-B	% IMP-C	% IMP-D	%Benzo.	%Chloro
1	Treated with 5N HCl solution at 60°C temperature for about 4 hours	5.22	0.09	0.36	0.08	0.18	4.51	ND
2	Treated with 5N NaOH solution at 60°C temperature for about 4 hours	37.07	ND	ND	0.05	ND	36.82	ND
3	Treated with 30% H_2O_2 solution for about 4 hours on bench top.	3.46	0.57	0.27	0.18	ND	0.19	0.21
4	Treated with water at 60°C temperature for about 4 hours.	8.55	0.02	ND	ND	ND	8.32	0.03
5	Exposed to humidity at 25°C/90% RH for about 5 hours.	0.03	ND	ND	ND	ND	0.03	ND
6	Exposed to heat at 105°C temperature for about 24 hours.	2.83	ND	0.30	0.01	1.50	0.03	ND
7	Exposed to UV light for about 200 Watt hours/Square metre	0.03	ND	ND	ND	ND	0.03	ND
8	Exposed to White light for about 1.2 million lux hours.	0.03	ND	ND	ND	ND	ND	0.03

Table 4. Percentage of RSD of impurities in precision study

Nominal concentrations (µg mL^{-1})	Precision (RSD, %) (*n* = 6)
Benzochlorothiazide	0.3
Chlorothiazide	0.3
IMPURITY-A	0.7
IMPURITY-B	0.7
IMPURITY-C	0.6
IMPURITY- D	0.7

Table 5. Percentage Recovery of impurities at different level

Nominal concentrations	% Mean Recovery of (n = 3)					
	Benzochlorothiazide	Chlorothiazide	IMP-A	IMP-B	IMP-C	IMP-D
Low@50%	105.3	102.1	108.1	108.1	105.5	100.0
Midle@100%	103.1	102.0	105.5	105.9	105.4	102.7
High@300%	102.1	98.6	103.6	104.4	106.1	102.3

Table 6. Percentage of Recovery & precision at LOQ level

Nominal concentrations	Benzochlorothiazide	Chlorothiazide	IMP-A	IMP-B	IMP-C	IMP-D
% Recovery	99.4	102.4	99.8	102.3	103.4	98.0
% RSD on precision (n= 6)	0	0	0	0	8.2	11.1

Table 7. Limit of detection (LOD) and limit of Quantification (LOQ) of impurities in percentage

Nominal concentrations	Benzochlorothiazide	Chlorothiazide	IMP-A	IMP-B	IMP-C	IMP-D
LOD in %	0.002	0.002	0.002	0.001	0.002	0.001
LOQ in %	0.005	0.008	0.006	0.004	0.007	0.004

Table 8. Correlation Coefficient of impurities

Parameters (n=5)	Benzochlorothiazide	Chlorothiazide	IMP-A	IMP-B	IMP-C	IMP-D
Slope	187172	129331	36526	50836	43147	44063
Y intercept	760	645	1112	1710	431	4147
Correlation Coefficient	0.9999	0.9999	0.9998	0.9998	0.9999	0.9997

Table 9. Impurity profile of different formulation

Product Name	% of Benzothiadiazine	% of Chlorothiadiazine	% of IMP-A	% of IMP-B	% of IMP-C	% of IMP-D	% of Total Impurity
Dutoprolol 25mg/12.5mg	0.152	0.092	0.2	ND	ND	0.041	0.569
Dutoprolol 50mg/12.5mg	0.174	0.025	0.083	0.013	ND	0.042	0.384
Dutoprolol 100mg/12.5mg	0.165	0.032	0.045	0.007	ND	0.033	0.338
In house formulations	0.023	ND	ND	ND	ND	ND	ND

Figure 2A: Typical Chromatogram of Blank

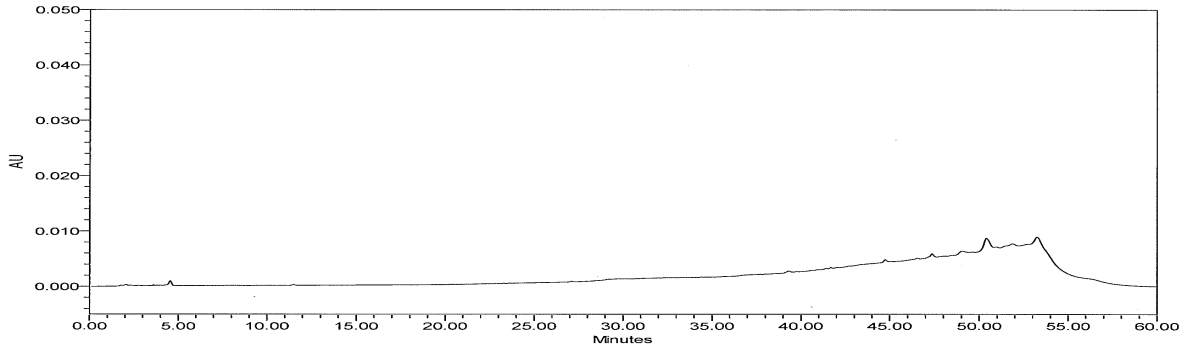


Figure 2B: Typical Chromatogram of Standard Solution

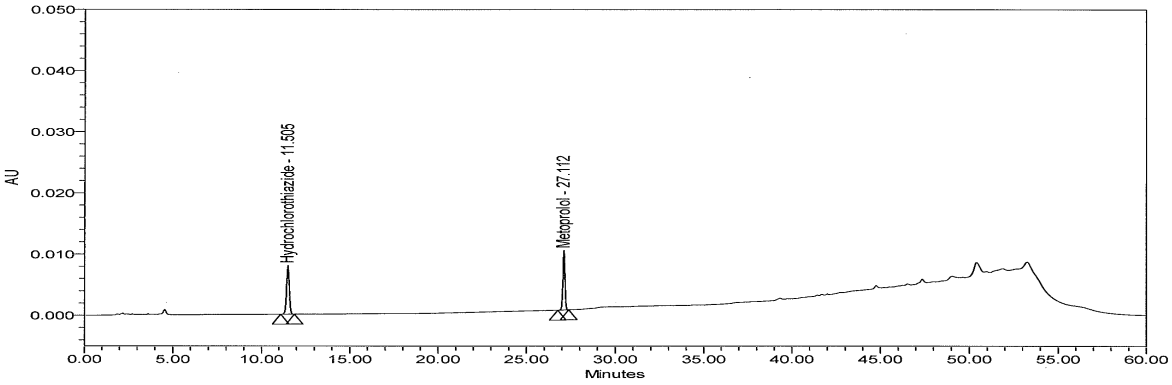


Figure 2C: Typical Chromatogram of Placebo Solution

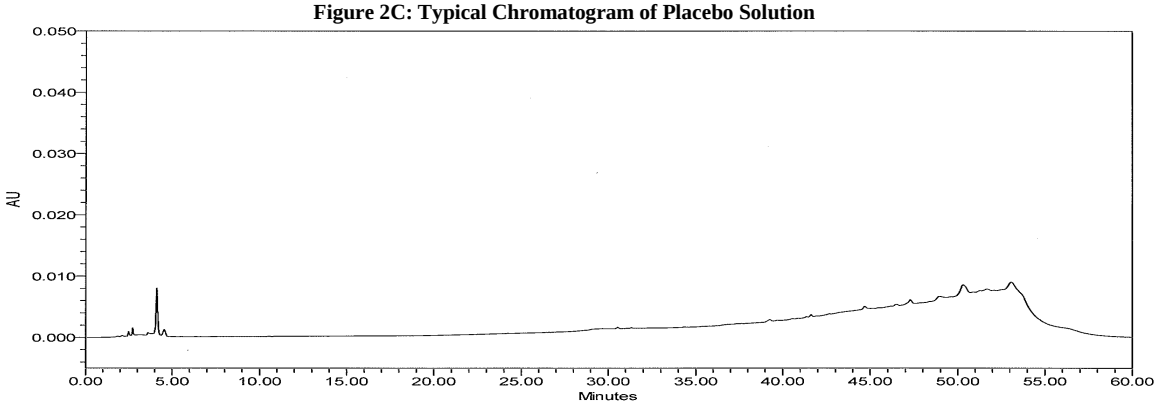


Figure 2D: Typical Chromatogram of Control Sample Solution

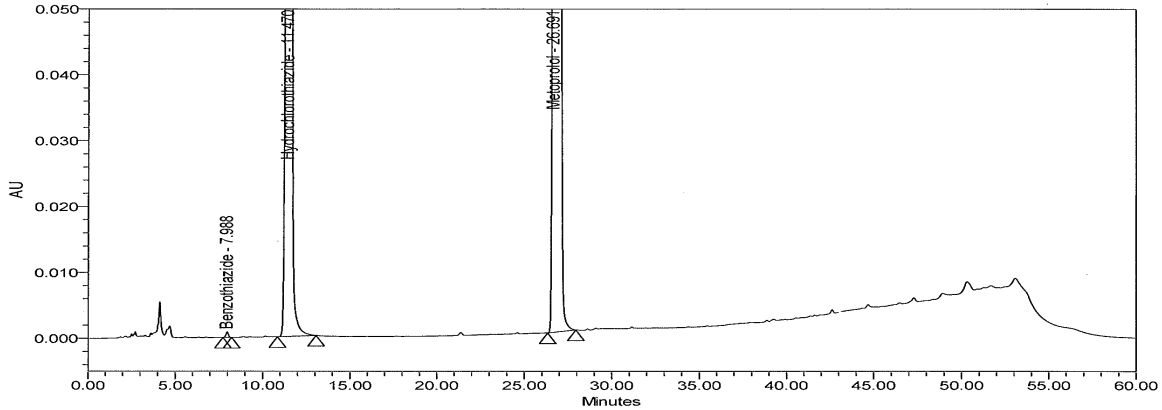
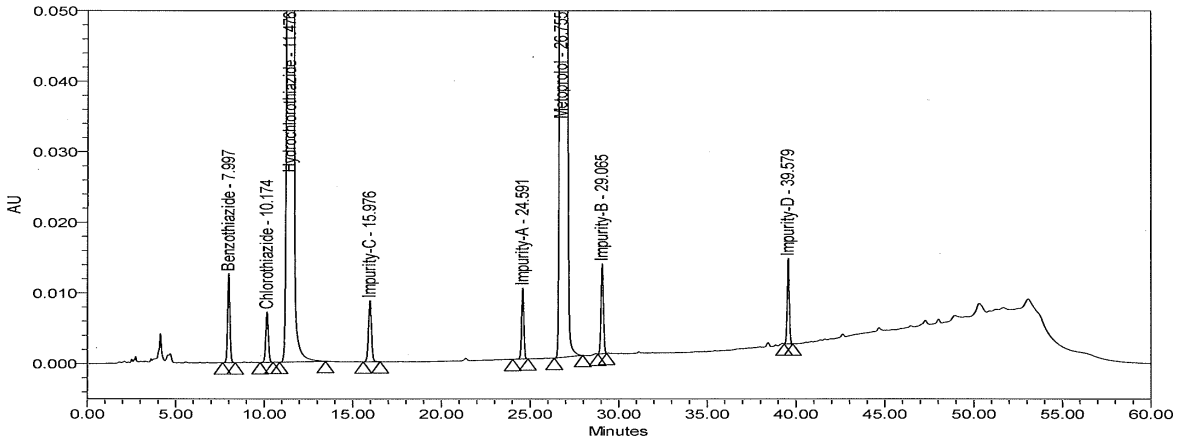


Figure 2E: Typical Chromatogram of Spiked Sample Solution



CONCLUSION

A rapid, specific, sensitive high-performance liquid chromatographic method has been developed for determination of Metoprolol Succinate and Hydrochlorothiazide impurities and its degradation products in pharmaceuticals preparation. A number of analytical approaches have been previously described to determine Metoprolol Succinate and Hydrochlorothiazide individually in pharmaceutical dosage forms as well as in biological, however, this is the first study reporting a validated reversed phase method for impurity quantification in Metoprolol Succinate and Hydrochlorothiazide formulation. Metoprolol Succinate and Hydrochlorothiazide drugs are official in the British Pharmacopoeia and United States Pharmacopoeia but their combination is not official. Based on the literature survey, no official method has yet been developed for their separation and its impurities The simple HPLC method developed in this study makes it suitable for separation and estimation of impurities without interference from excipients and other related substances present in the pharmaceutical matrices. The analytical performance and the result obtained from analysis of two different formulations demonstrated that the method is reliable and sufficiently robust. In conclusion, the high sensitivity, good selectivity, accuracy and reproducibility of the HPLC method developed in this study makes it suitable for quality control analysis of complex pharmaceutical preparation containing Metoprolol Succinate and Hydrochlorothiazide and its impurities.

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