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METHOD DEVELOPMENT AND METHOD VALIDATION FOR RELATED SUBSTANCES OF BISOPROLOL FUMARATE AND HYDROCHLOROTHIAZIDE BY RP-HPLC IN PHARMACEUTICAL DOSAGE FORM

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Keywords:

RP-HPLC Method Development, Bisoprolol fumarate, Hydrochlorothiazide, Precision, Accuracy and Cost effective

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ABSTRACT

The objective of the proposed method is to develop simple and accurate method for the estimation ofrelated substances in bisoprolol fumarate and hydrochlorothiazide pharmaceutical dosage forms by HPLC. Hence on the basis of literature survey it was thought to develop a precise, accurate, simple and reliable, less time consumingmethod forthe estimation of related substances.The developed chromatographic (RP-HPLC) method forBisoprolol fumarate, Hydrochlorothiazide and their related substances is said to be rapid, simple, precise, accurate, and cost effective that can be effectively applied for the routine analysis in research institution, quality control department in industries, approved testing laboratories, biopharmaceutical studies, clinical pharmacokinetic studiesand for determination of impurities in formulated products.From the overall results obtained it was concluded that the developed method was more accurate, precise, specific and robust with ±5ºC in temperature, ±0.2ml/min in flow rate, ±10% variation in organic phase.

1. INTRODUCTION

Pharmaceutical analysis plays a vital role in the pharmaceutical product development. Pharmaceutical analysis is a specialized branch of analytical chemistry. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis reveals the chemical identity of the sample. Quantitative analysis establishes the relative amount of one or more of these species or analytes in numerical terms.Chromatographic techniques are predominantly used in the pharmaceutical industry for a large variety of samples. HPLC is one of the chromatographic techniques is widely used for checking the purity of new drug candidates, monitoring changes or scale ups of synthetic procedures, evaluating new formulations, and scrutinizing quality control/assurance of final drug products[1,2].

High Performance Liquid Chromatography:

High performance liquid chromatography is basically a highly improved form of column chromatography.HPLC employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch.The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process if diffusion is minimized a faster and effective separation can be achieved. The technique of high performance liquid chromatography is so called because of its improved performance when compared to classical column chromatography. Advances in column technology high-pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed and highly resolvedmethod of separation[3,4].

The HPLC is the method of choice in the field of analytical chemistry, since this method is specific,robust, linear, precise and accurate and the limit of detection is low[5-7].

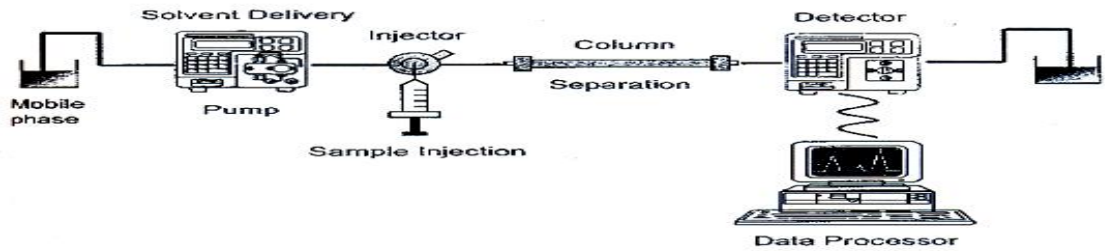
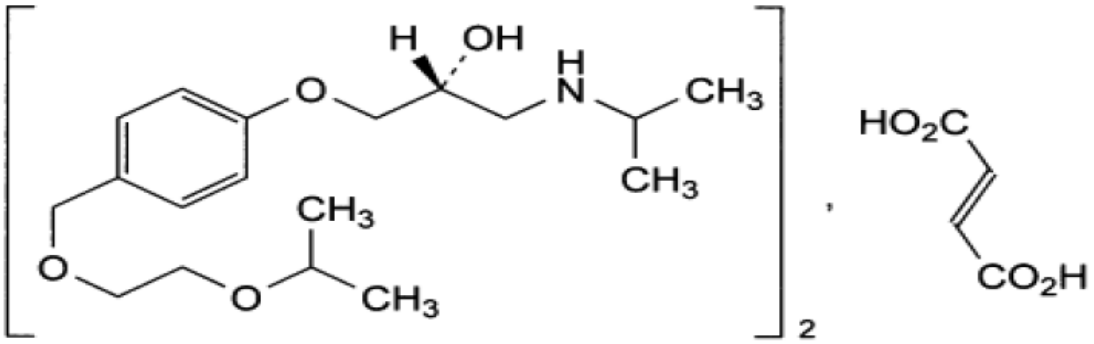


Fig No: 1 Block diagram of HPLC

2.DRUG PROFILES

2.1. Bisoprolol Fumarate



Bisoprolol fumarate chemical structure

Chemical Name :(RS)-1-[4-[[2-(1-Methylethoxy)ethoxy] methyl]phenoxy]-3-[(1-methylethyl)amino]propan-2-ol.

Molecular Formula : $C_{18}H_{31}NO_4$.

Molecular Mass : 325.443 g/mol.

pka : 3.5.

Color : White in color.

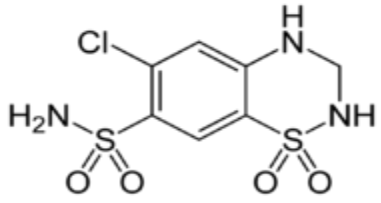
Nature : Slightly hygroscopic powder.

Solubility : Very soluble in water, freely soluble in methanol.

Class : Anti-hypertensive, β_2-bloker.

Melting point : 100^0c.

2.2. Hydrochlorothiazide



Hydrochlorothiazide chemical structure

Chemical Name **:** 6-Chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothia diazine-7-sulfonamide.

Molecular Formula **:** $C_7H_8ClN_3O_4S_2$

Molecular Mass **:** 297.74 g/mol.

pka ***: 7.9***.

Color **:** White powder.

Class : **Diuretic, Anti-Hypertensive.**

Melting point **:** 274^0C

3. MATERIALS AND METHODS

Method development by RP-HPLC method for Bisoprolol fumarate and Hydrochlorothiazide

Selection of suitable related impurities for the estimation

↓

From physicochemical properties select detector λmax

↓

Selection of suitable stationary phase

↓

Development of suitable mobile phase

↓

Perform forced degradation experiments to challenge method

↓

Separation of each related substance finely

↓

Validation of the developed method

Materials used for method development and validation

Chemicals used

Disodium hydrogen phosphate : Merck

Orthophosphoric Acid: Merck

Water : Milli-Q

Methanol, Acetonitrile : Rankem

Software used : Empower-2

Standards used

Bisoprolol fumarate Working Standard of known Potency

Hydrochlorothiazide Working Standard of known Potency

Impurities used

Bisoprolol fumarate known Impurities as per British pharmacopoeia

Hydrochlorothiazide known Impurities as per British pharmacopoeia

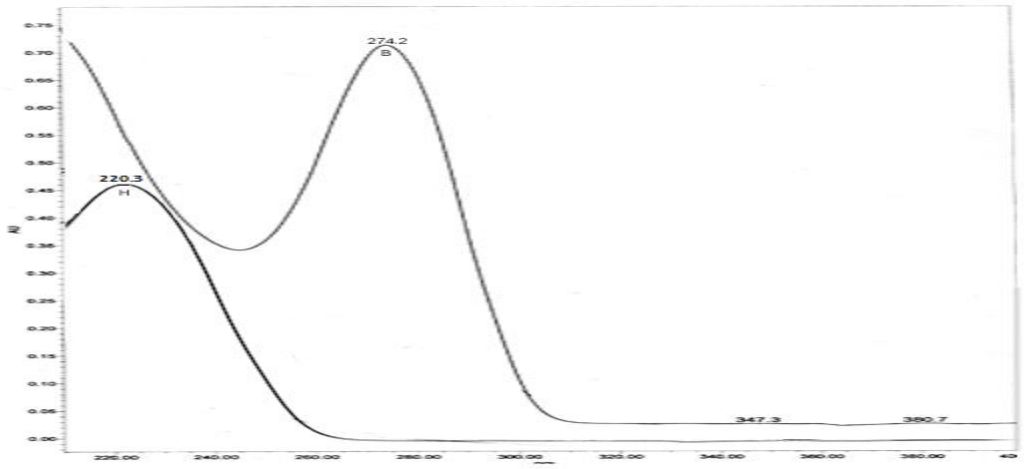


Fig No: 2- UV-spectrum of Bisoprolol fumarate and hydrochlorothiazide

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Table No-1: Gradient program

Time	MP-A%	MP-B %
0.01min	98.0 %	2.0 %
25.00 min	82.0 %	18.0 %
50.00min	55.0 %	45.0 %
55.00 min	55.0 %	45.0 %
57.00 min	98.0 %	2.0 %
65.00 min	98.0 %	2.0 %

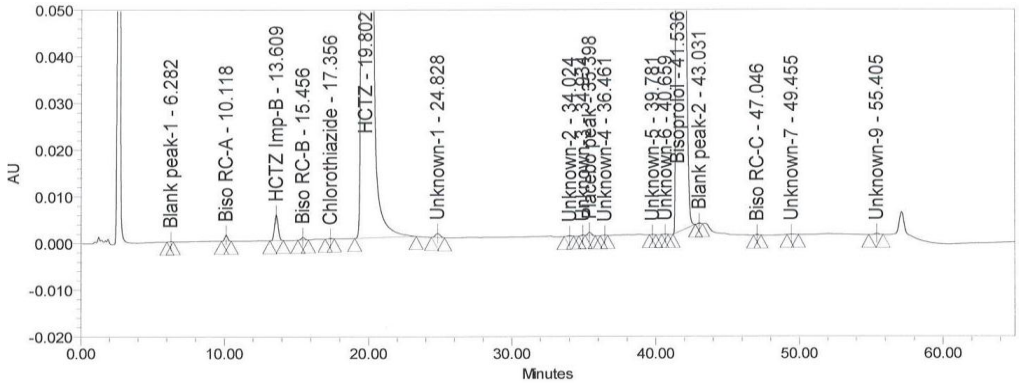


Fig No: 3- Final chromatogram for sample

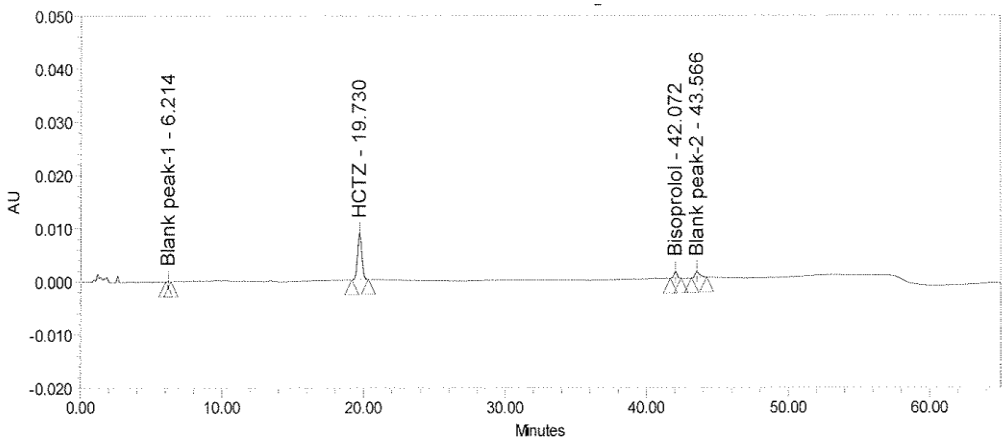


Fig No: 4- Final chromatogram for standards

4. RESULTS

Table No-2: Results of system suitability

System suitability parameters	Observed value	Acceptance limit
The %RSD for BisoprololFumarate and HCTZ from Six replicate injections of standard solution	Bisoprolol :1.3 HCTZ : 0.9	NMT 10.0%
The Resolution between Bisoprolol RC-B and HCTZ-RC-B in SST solution	4.0	NLT 1.8
The Resolution between HCTZ and Chlorothiazide peak in SST solution	4.2	NLT 2.0

Table No-3: Results of Interferences from degradation products for Bisoprololfumarate

Stress Condition	% Degradation	Peak Purity		Purity flag
		Purity Angle	Purity threshold	
Acid degradation	15.069	3.418	6.898	NO
Base degradation	1.144	11.806	31.359	NO
Peroxide degradation	1.135	4.953	11.717	NO
water degradation	0.862	5.683	14.014	NO
Sunlight degradation	0.613	0.430	0.576	NO
UV-light	1.048	0.452	0.599	NO
Heatdegradation	8.435	0.642	0.682	NO
Humidity	1.765	0.380	0.589	NO

Table No-4: Results of Interference from degradation products for HCTZ

Stress Condition	% Degradation	Peak Purity		Purity flag
		Purity Angle	Purity threshold	
Acid degradation	0.767	2.217	2.442	NO
Base degradation	7.527	2.655	6.001	NO
Peroxide degradation	2.860	2.263	3.940	NO
water degradation	3.434	2.275	3.817	NO
Sunlight degradation	0.076	1.423	3.583	NO
UV-light	0.110	1.424	3.554	NO
Heat degradation	1.361	1.367	3.428	NO
Humidity	0.441	1.778	3.564	NO

Table No-5: Results of Precision of impurity Bisoprolol RC-A, Bisoprolol RC-B and Bisoprolol RC-C

S.No	Bisoprolol RC-A		Bisoprolol RC-B		Bisoprolol RC-C	
	RRT	% Impurity	RRT	% Impurity	RRT	% Impurity
1	0.243	0.651	0.371	0.283	1.135	0.581
2	0.242	0.654	0.370	0.291	1.135	0.583
3	0.241	0.655	0.370	0.284	1.135	0.580
4	0.241	0.653	0.370	0.289	1.135	0.582
5	0.241	0.651	0.370	0.282	1.135	0.584
6	0.241	0.651	0.369	0.287	1.135	0.586
Avg	0.242	0.653	0.370	0.286	1.135	0.583
% rsd	0.3	0.3	0.2	1.3	0.0	0.4

Table No-6: Results of Precision of HCTZ IMP-B, Bisoprololfumarate and HCTZ

S.No	HCTZ IMP-B		Bisoprolol fumarate		HCTZ	
	RRT	% Impurity	RT	% Area	RT	%Area
1	0.687	0.456	41.421	19616	19.593	184529
2	0.688	0.460	41.406	20172	19.701	186792
3	0.688	0.461	41.407	18238	19.661	178799
4	0.687	0.463	41.384	17339	19.620	174813
5	0.687	0.465	41.389	17063	19.606	172203
6	0.687	0.471	41.375	17089	19.575	173896
Avg	0.687	0.463	41.390	18253	19.627	178505
%RSD	0.1	1.1	0.1	7.4	0.2	3.4

Table No-7: Results of accuracy of Bisoprolol fumarate

Sample No.	Spike level	'µg/mL' added	'µg/mL' found (recovered)	% recovery	Mean % recovery
1.	50%	0.6200	0.6298	101.6	101.7
2.	50%		0.6322	102.0	
3.	50%		0.6301	101.6	
1.	75%	0.9920	1.0382	104.7	105.7
2.	75%		1.0560	106.5	
3.	75%		1.0523	106.1	
1.	100%	1.2400	1.2669	102.2	102.0
2.	100%		1.2644	102.0	
3.	100%		1.2642	102.0	
1.	125%	1.6119	1.6289	101.1	100.8
2.	125%		1.6251	100.8	
3.	125%		1.6213	100.6	
1.	150%	1.8599	1.8799	101.1	101.3
2.	150%		1.8816	101.2	
3.	150%		1.8866	101.4	
4.	150%		1.8762	100.9	
5.	150%		1.8871	101.5	
6.	150%		1.8870	101.5	

Table No-8: Results of accuracy of HCTZ

Sample No.	Spike level	'µg/mL' added	'µg/mL' found (recovered)	% recovery	Mean % recovery
1.	50%	1.4868	1.5349	103.2	103.7
2.	50%		1.5473	104.1	
3.	50%		1.5410	103.6	
1.	75%	2.3789	2.3033	96.8	96.9
2.	75%		2.2911	96.3	
3.	75%		2.3223	97.6	
1.	100%	2.9736	2.8591	96.1	96.2
2.	100%		2.8561	96.0	
3.	100%		2.8682	96.5	
1.	125%	3.8657	3.7067	95.9	96.0
2.	125%		3.7045	95.8	
3.	125%		3.7164	96.1	
1.	150%	4.4604	4.3878	98.4	98.2
2.	150%		4.3494	97.5	
3.	150%		4.3966	98.6	
4.	150%		4.3691	98.0	
5.	150%		4.4024	98.7	
6.	150%		4.3765	98.1	

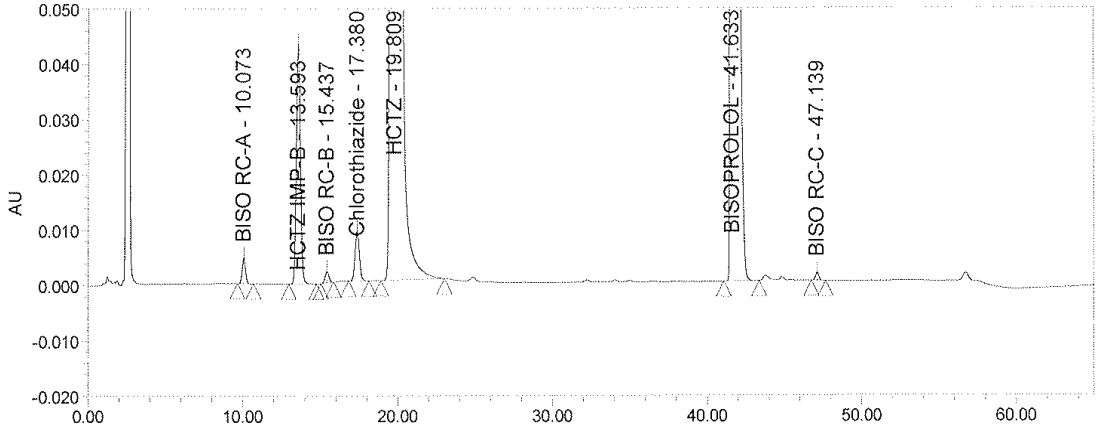


Fig No: 5- Chromatogram of system suitability preparation

Peak Results

	Name	RT	Area	USP Resolution	USP Tailing
1	BISO RC-A	10.073	71438		1.0
2	HCTZ IMP-B	13.593	792540	7.8	1.1
3	BISO RC-B	15.437	35798	3.9	1.0
4	Chlorothiazide	17.380	179507	3.9	1.1
5	HCTZ	19.809	77025404	4.1	1.3
6	BISOPROLOL	41.633	8293113	29.1	2.4
7	BISO RC-C	47.139	22671	8.7	1.1

Fig No: 6- Results of system suitability

	Name	Peak Label	Retention Time (min)
1	BISO RC-A		10.154
2	HCTZ IMP-B		13.633
3	BISO RC-B		15.546
4	Chlorothiazide		17.422
5	HCTZ		19.854
6	BISOPROLOL		41.740
7	BISO RC-C		47.254

Fig No: 7- Retention times for impurities and standard drug

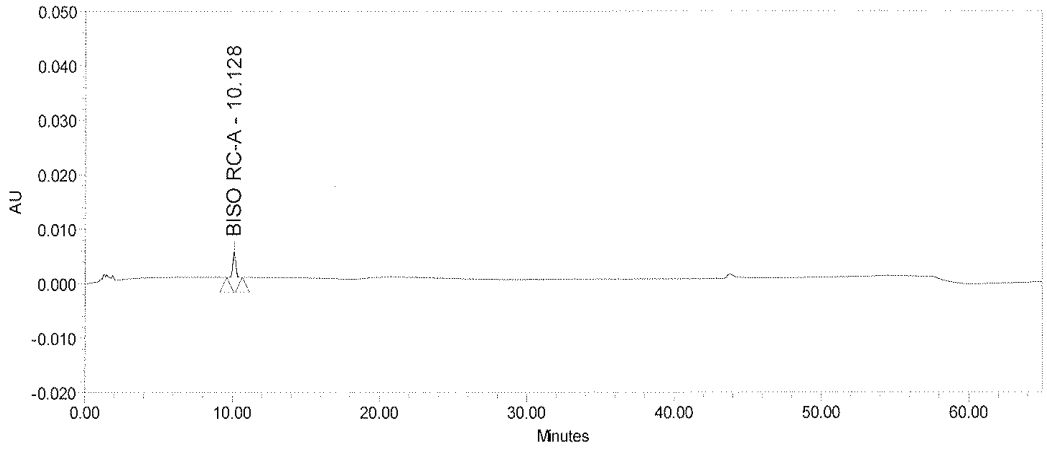


Fig No: 8- Chromatogram of Biso impurity-A

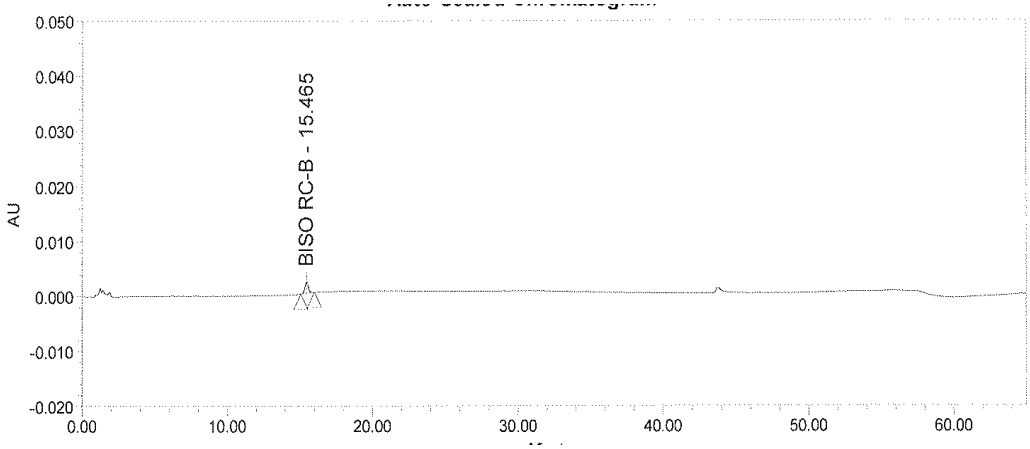


Fig No: 9- Chromatogram of Biso impurity-B

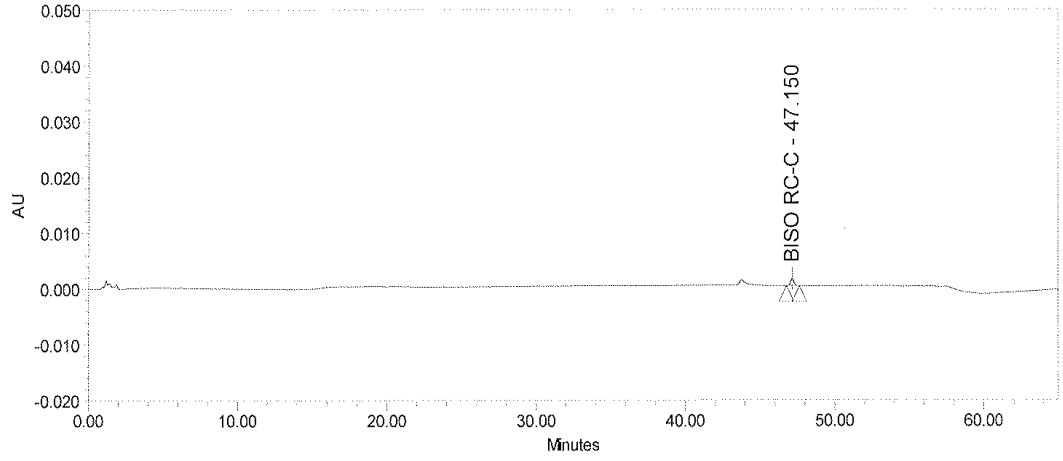


Fig No: 10- Chromatogram of Biso impurity-C

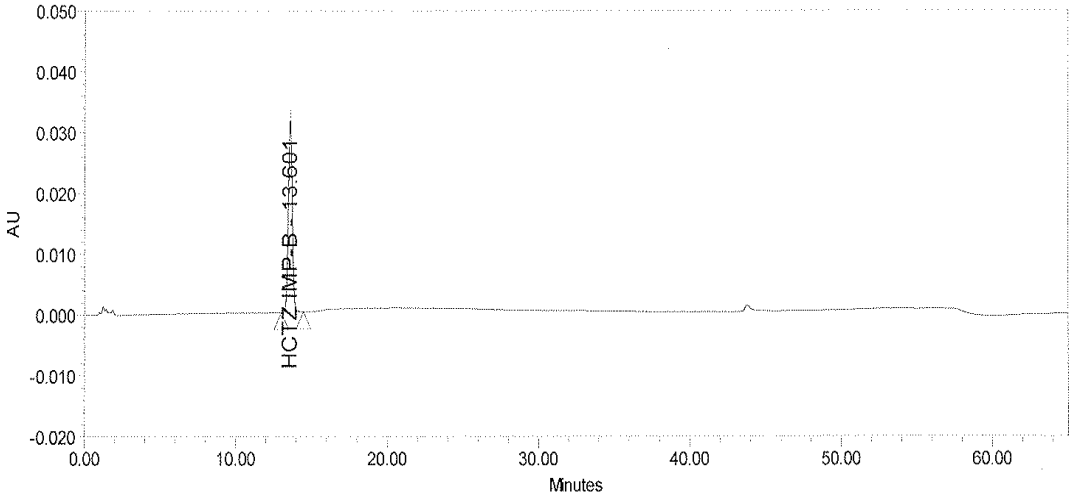


Fig No: 11- Chromatogram of HCTZ impurity-B

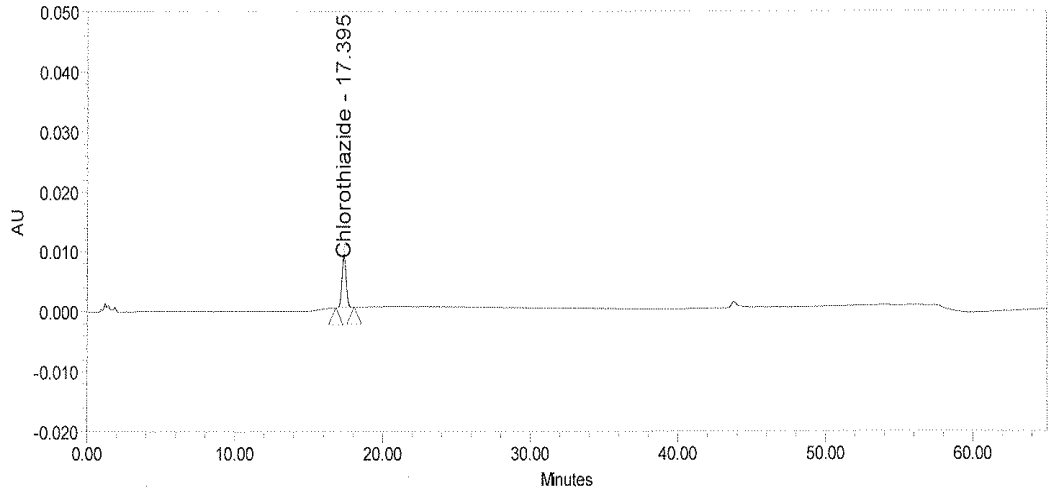


Fig No:12- Chromatogram of Chlorothiazide impurity

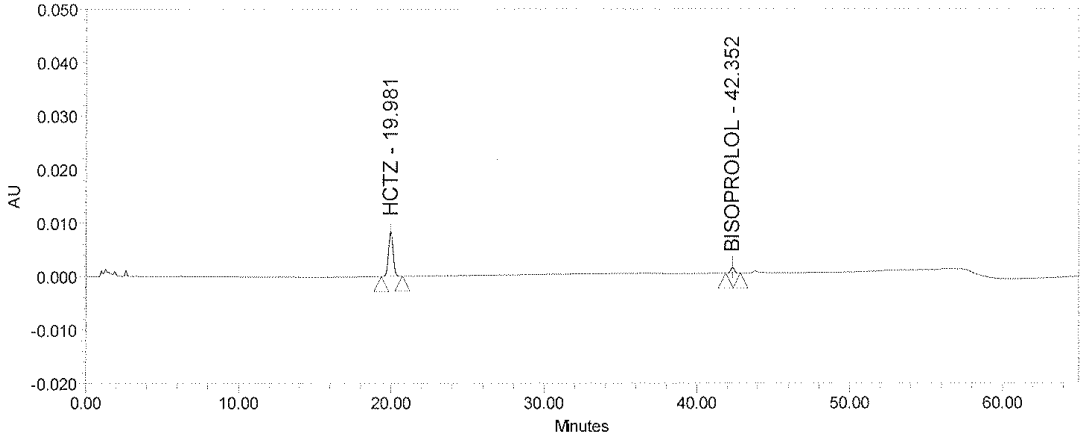


Fig No: 13- Standard chromatogram of Bisoprololfumarate and HCTZ

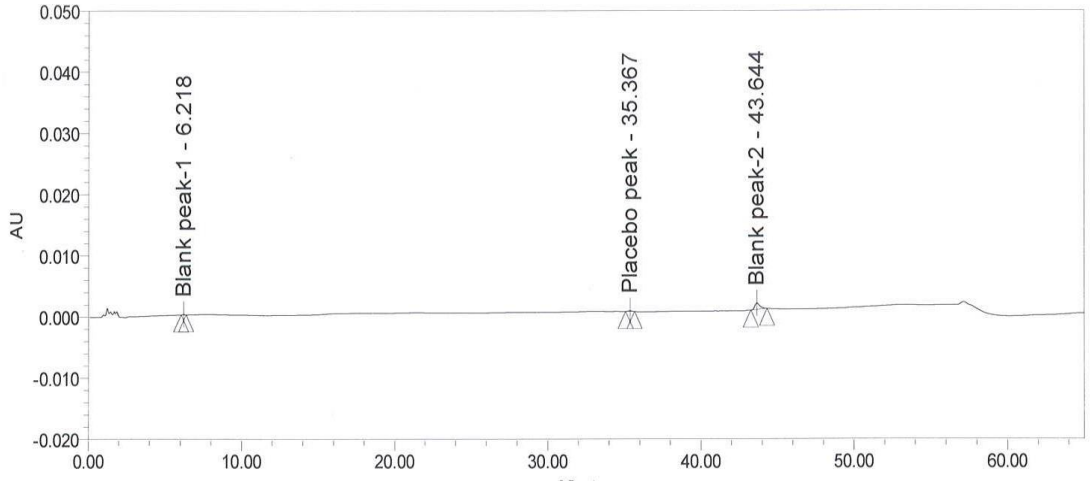


Fig No: 14- Placebo chromatogram of Bisoprololfumarate and HCTZ

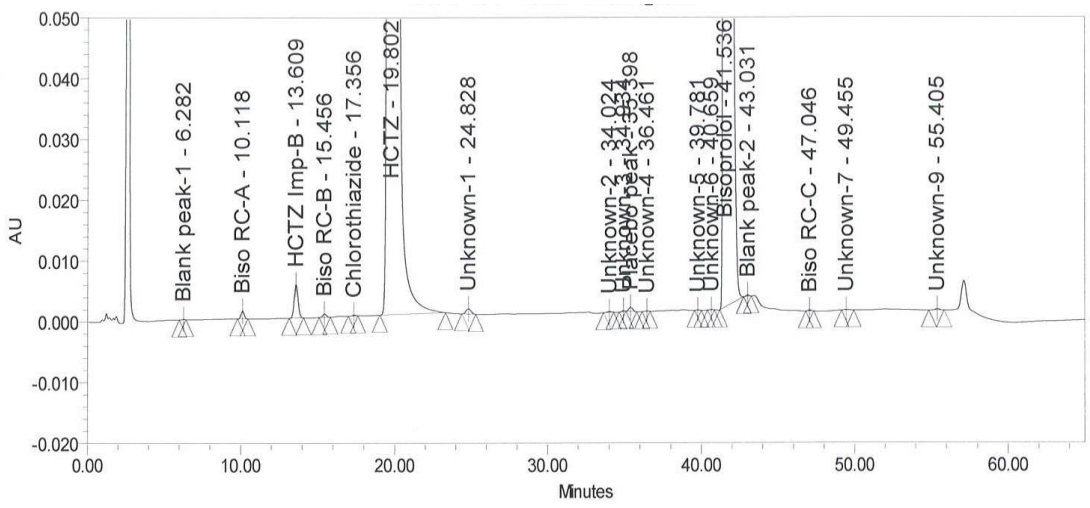


Fig No: 15- Precision Sample chromatogram of Bisoprololfumarate and HCTZ

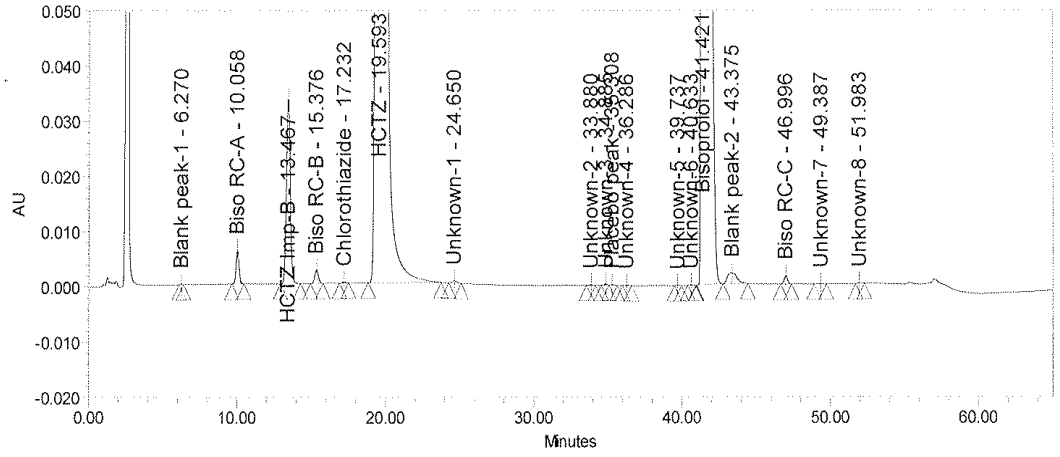


Fig No: 16- Precision sample chromatogram of Bisoprololfumarate and HCTZ

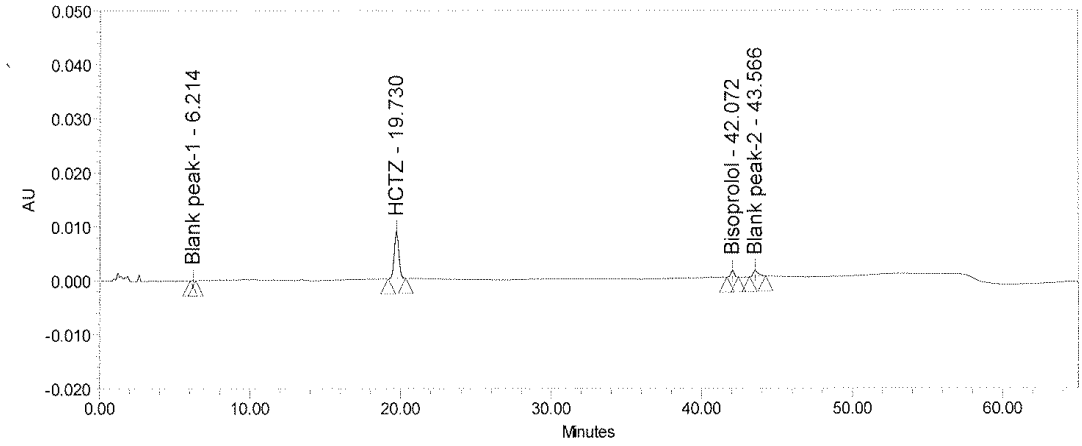


Fig No: 17- Precision standard chromatogram of Bisoprololfumarate and HCTZ

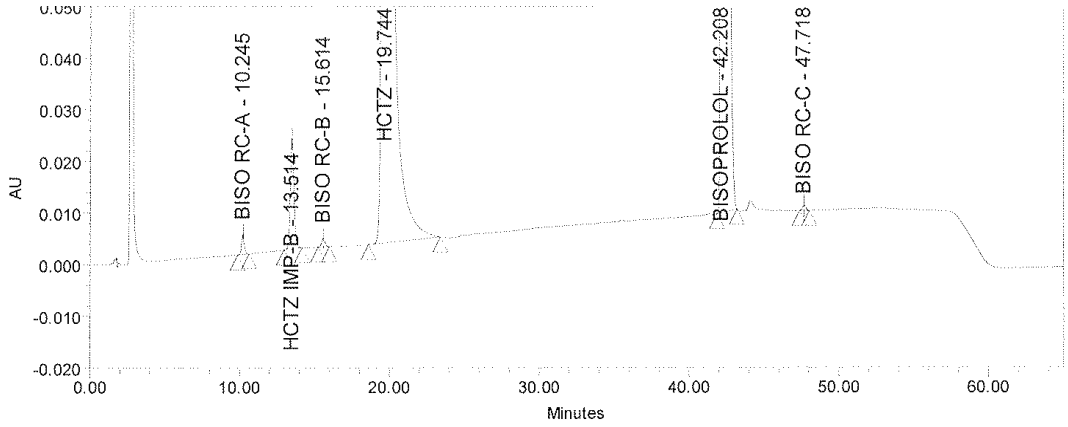


Fig No: 18- Recovery chromatogram for sample at 50% range

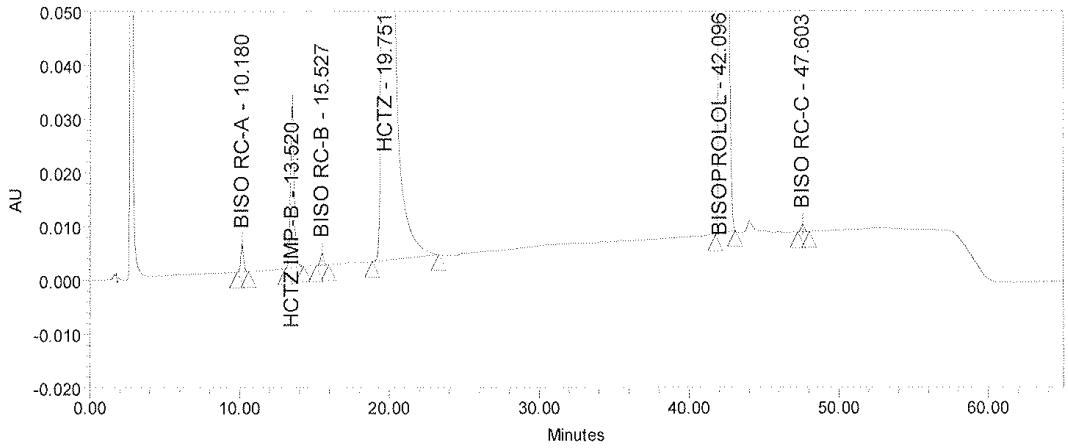


Fig No: 19- Recovery chromatogram for sample at 75% range

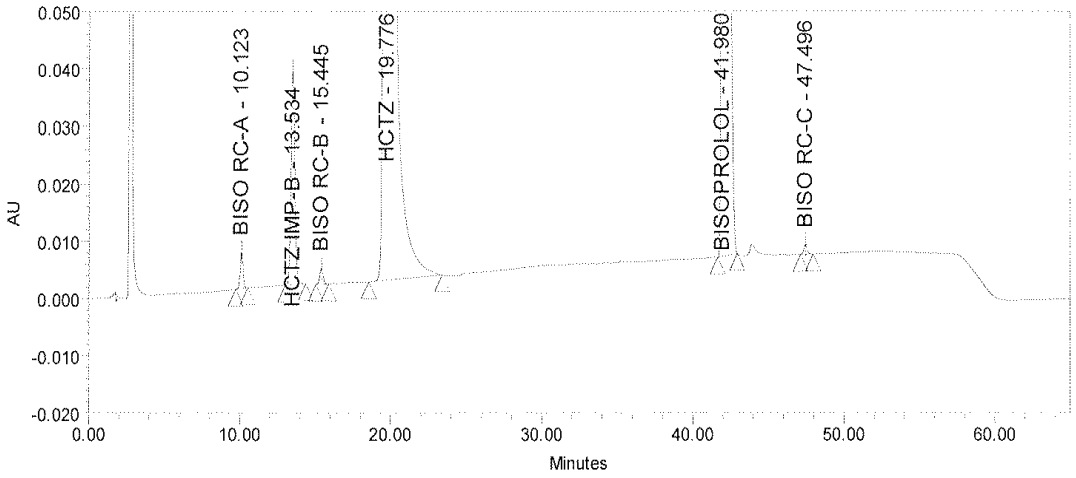


Fig No: 20- Recovery chromatogram for sample at 100% range

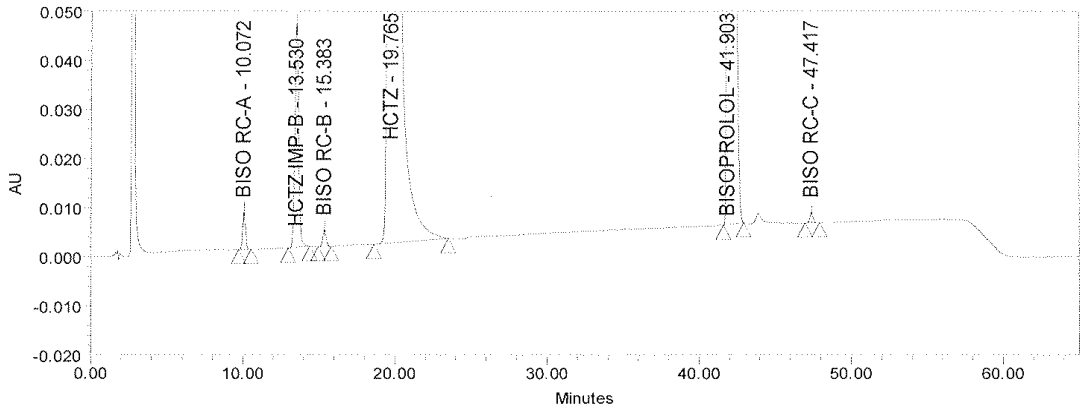


Fig No: 21- Recovery chromatogram for sample at 125% range

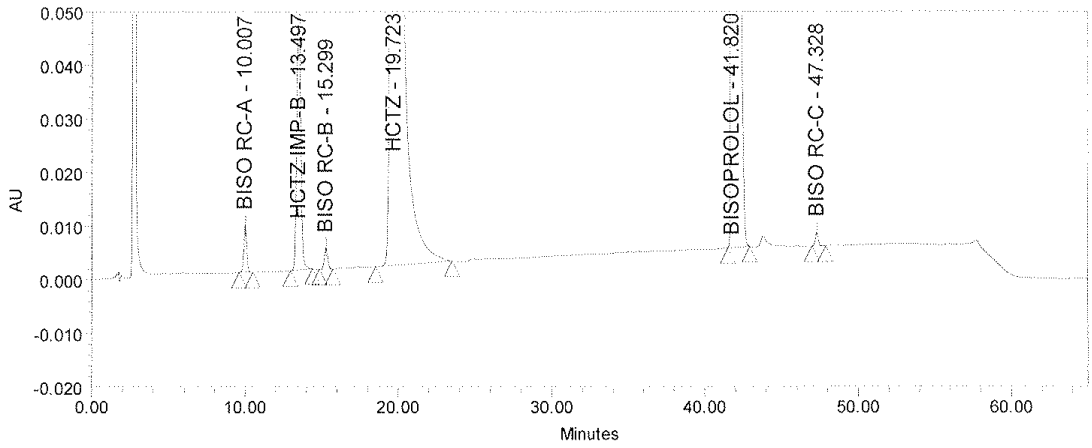


Fig No: 22- Recovery chromatogram for sample at 150% range

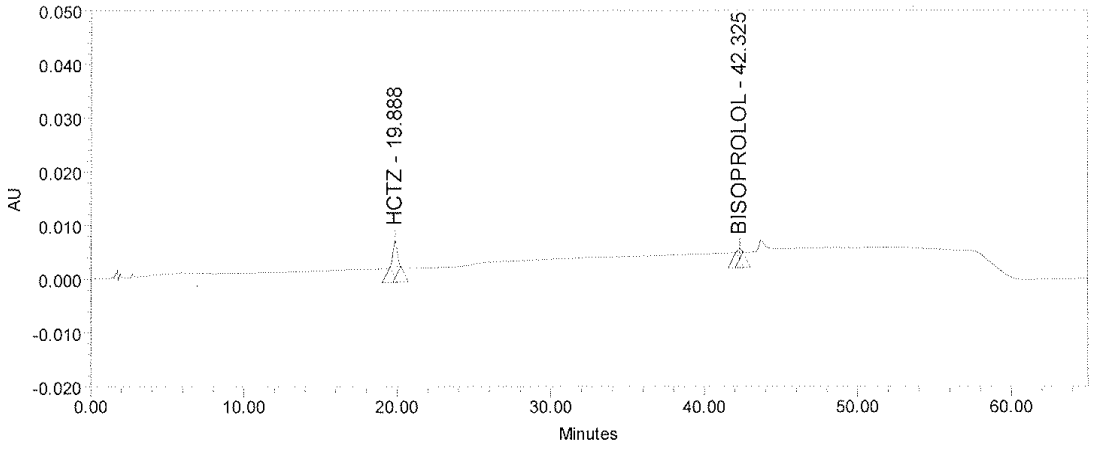


Fig No: 23- Recovery chromatogram for standard at 50% range

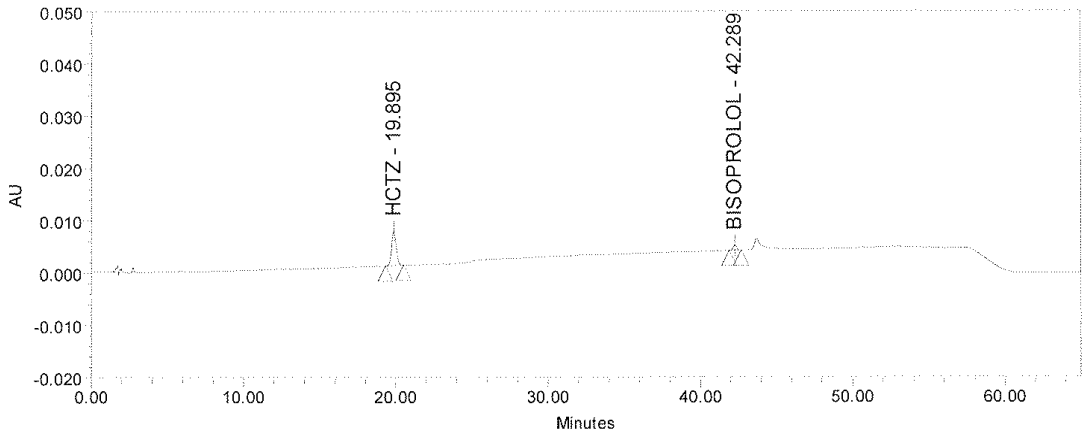


Fig No: 24- Recovery chromatogram for standard at 75% range

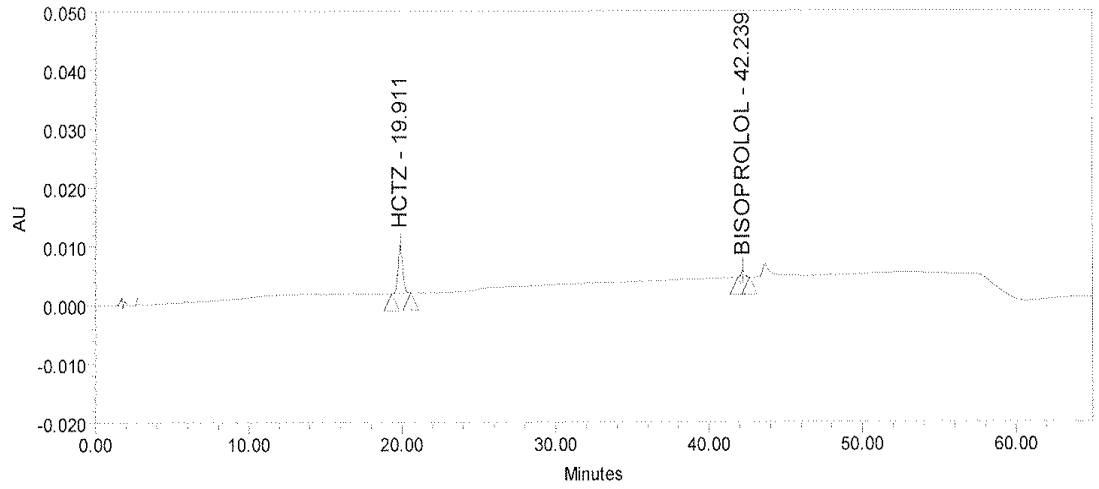


Fig No: 25- Recovery chromatogram for standard at 100% range

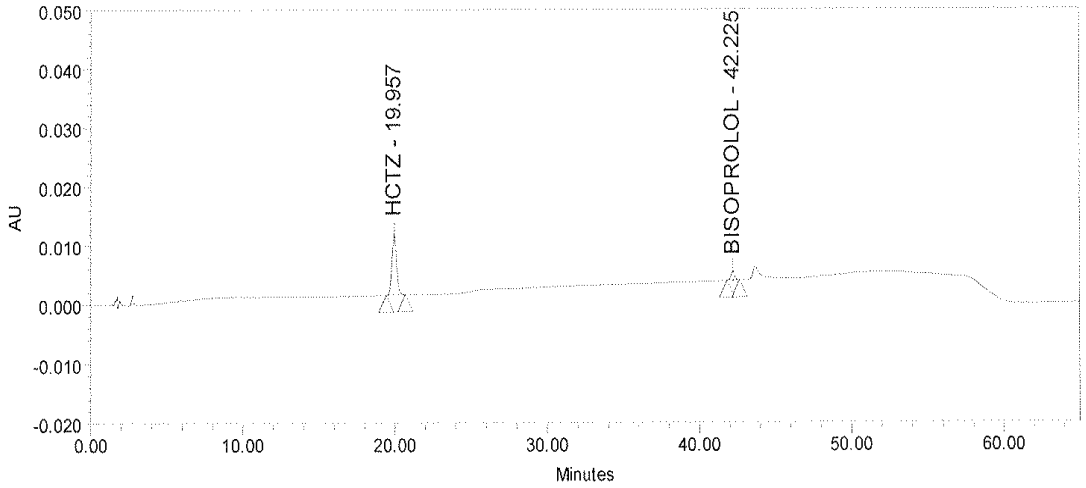


Fig No: 26- Recovery chromatogram for standard at 125% range

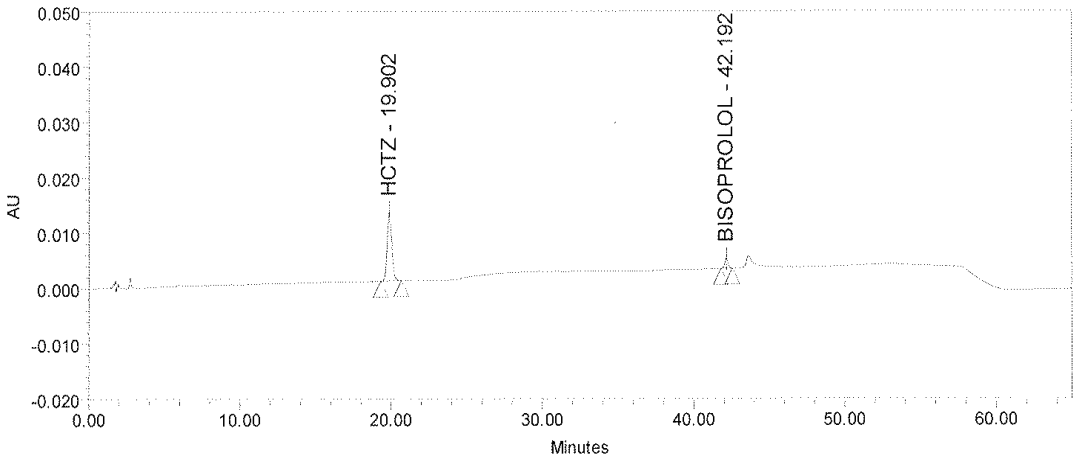


Fig No: 27- Recovery chromatogram for standard at 150% range

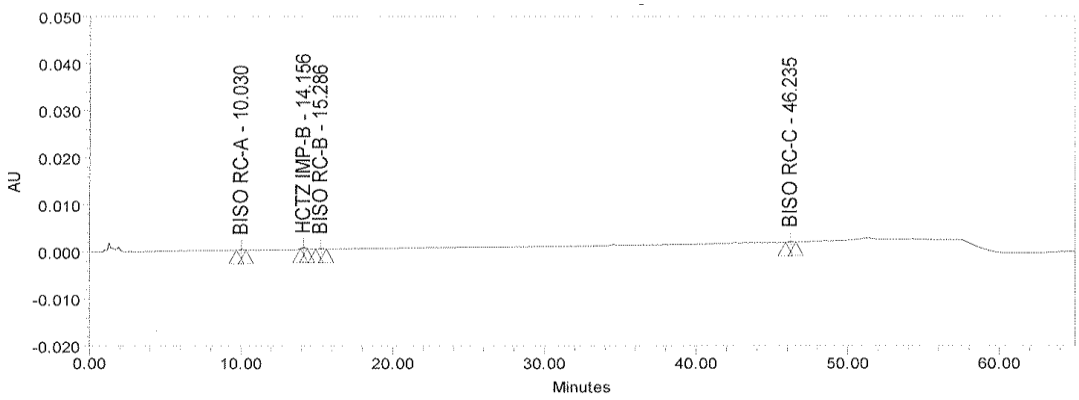


Fig No: 28- All the impurities at LOQ level

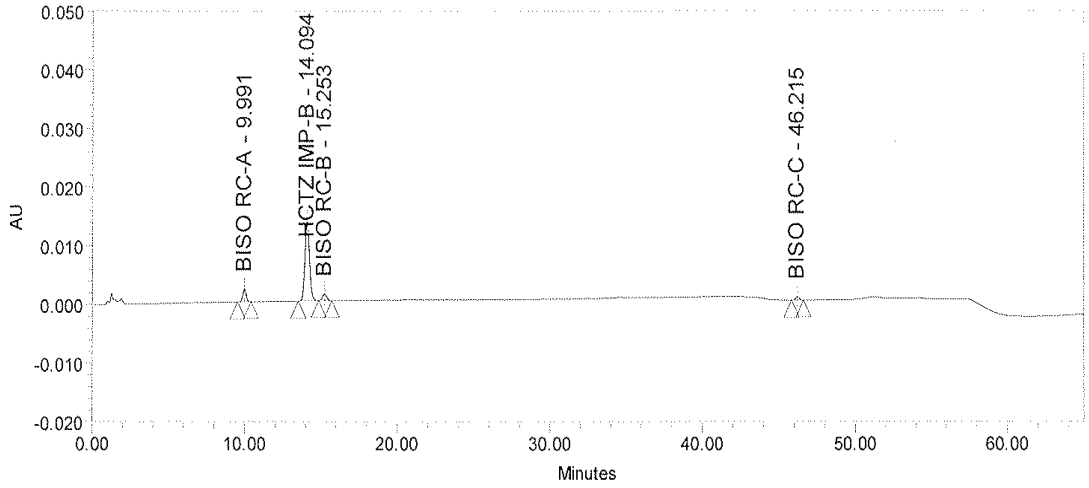


Fig No: 29- Linearity of impurities at- 50% level

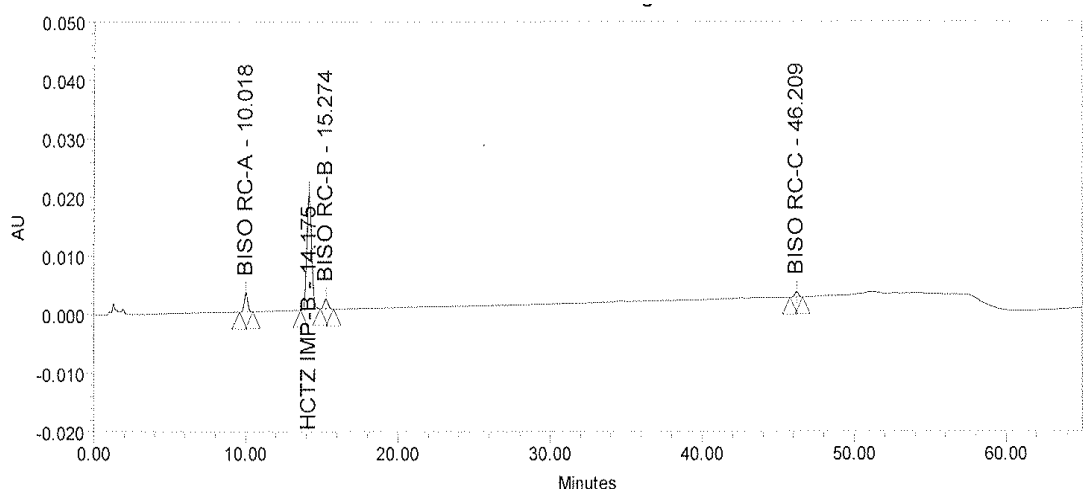


Fig No: 30- Linearity of impurities at- 75% level

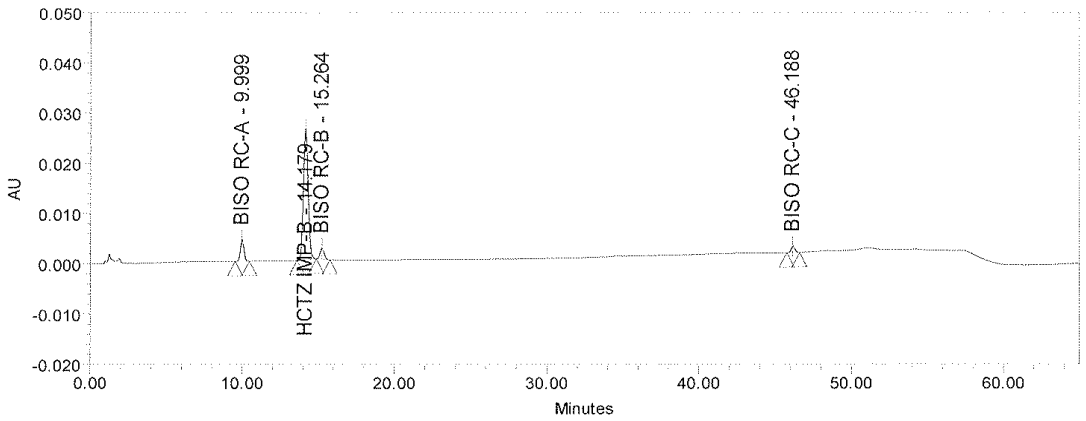


Fig No: 31- Linearity of impurities at- 100% level

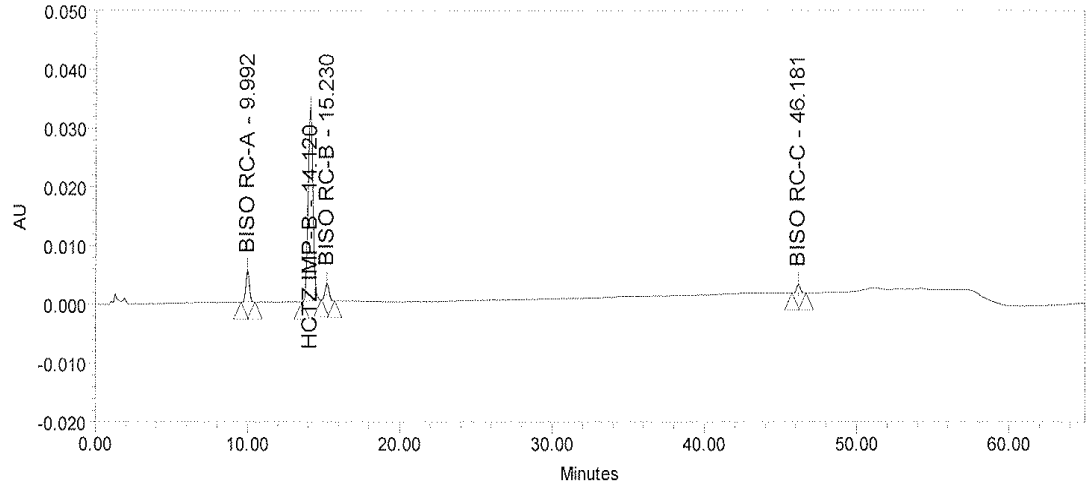


Fig No: 32- Linearity of impurities at- 125% level

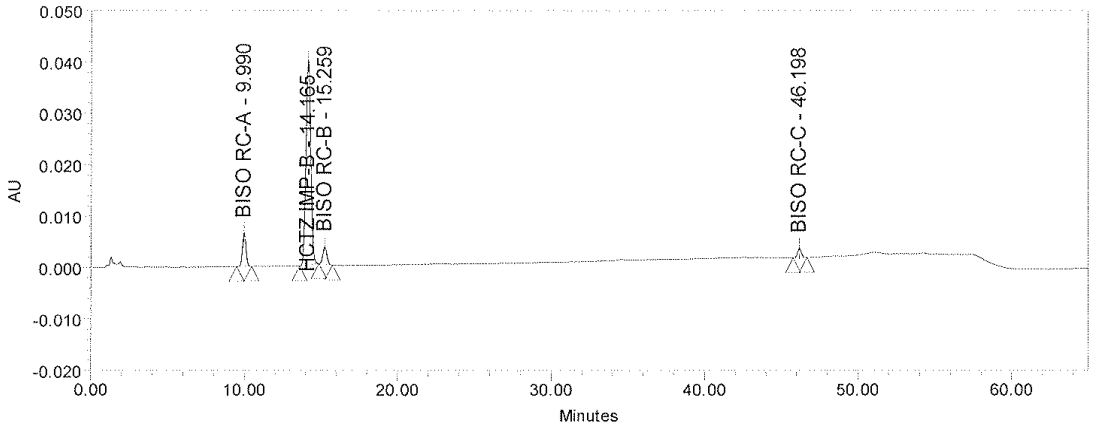


Fig No: 33- Linearity of impurities at- 150% level

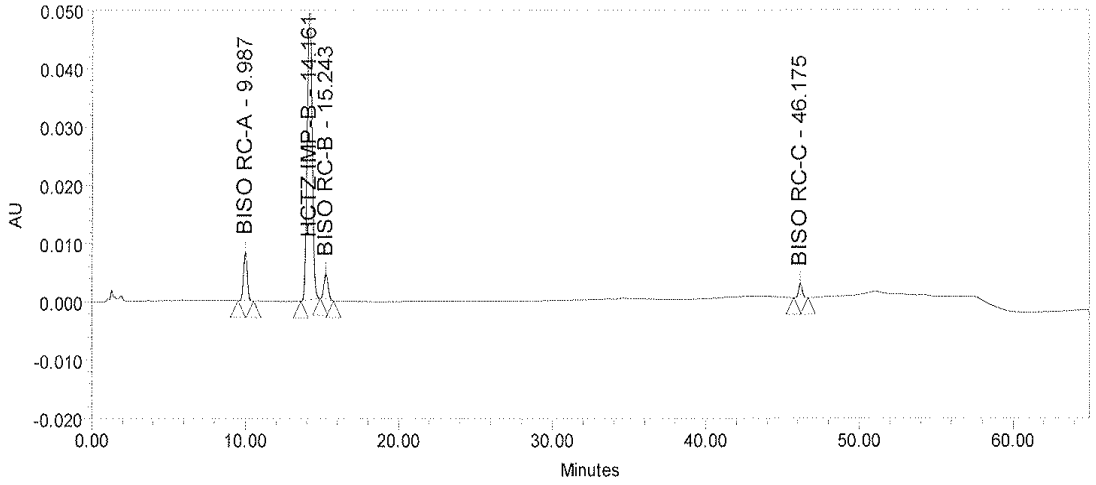


Fig No: 34- Linearity of impurities at- 200% level

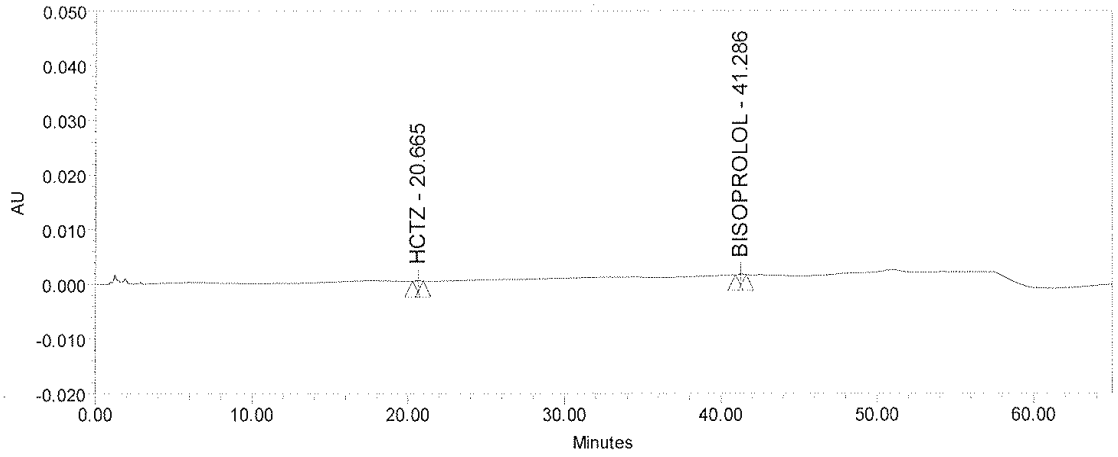


Fig No: 35- Standards at LOQ level

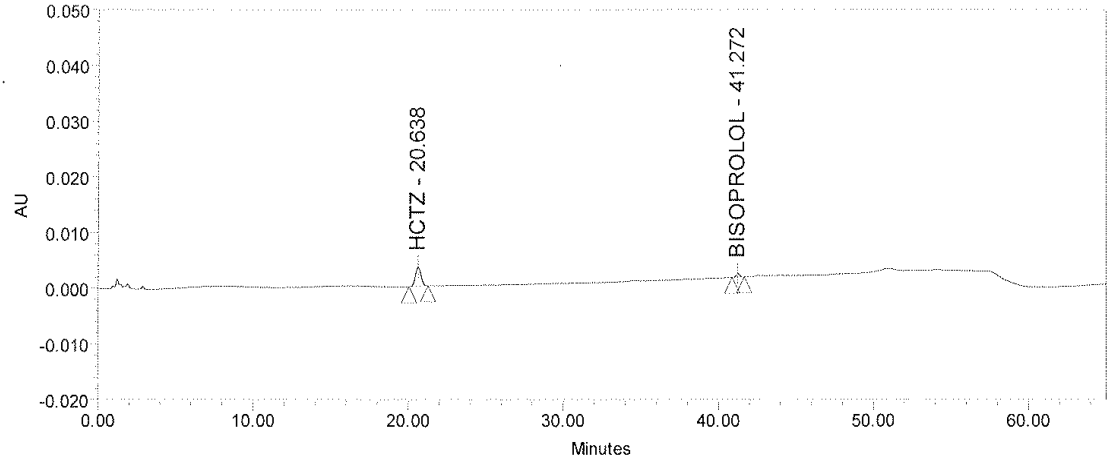


Fig No: 36- Linearity of standards at – 50% level

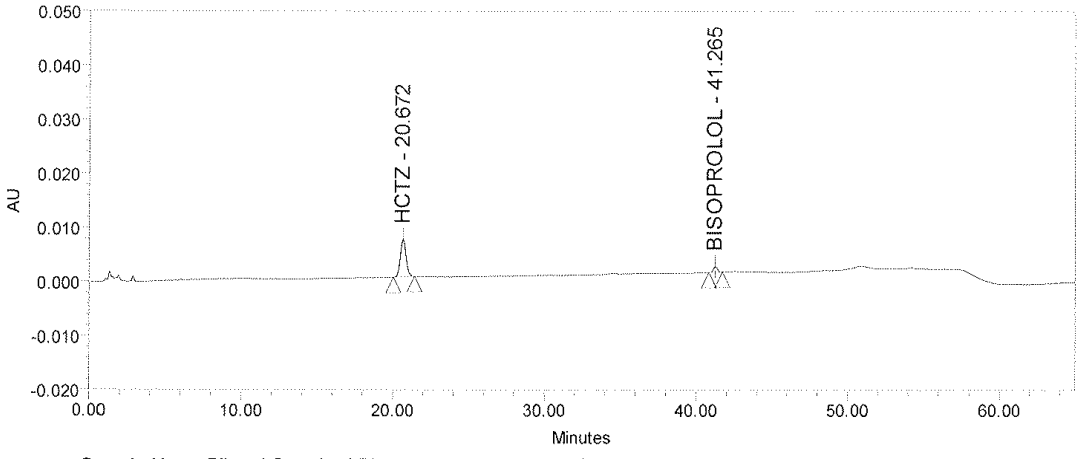


Fig No: 37- Linearity of standards at – 75% level

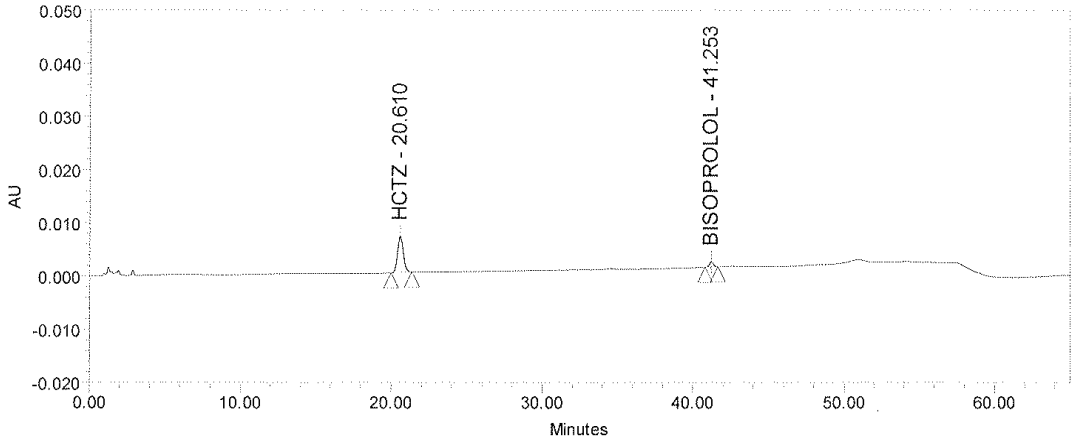


Fig No: 38- Linearity of standards at – 100% level

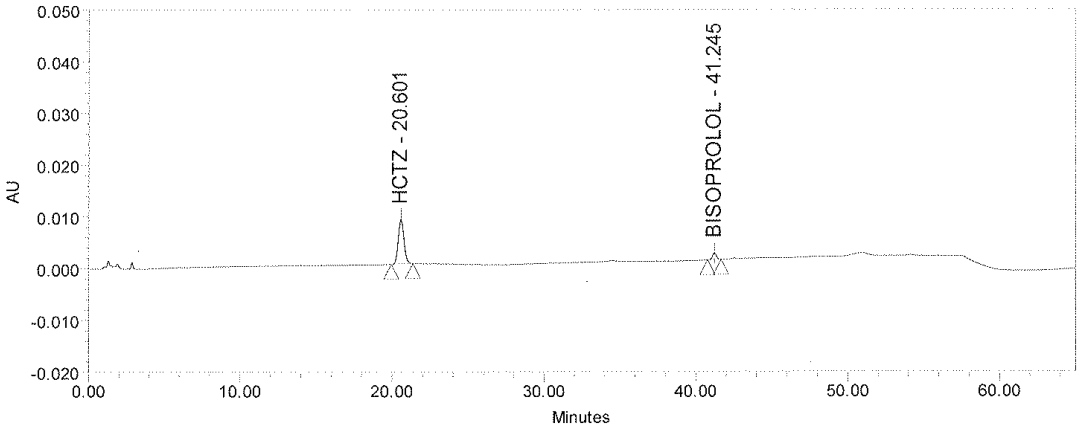


Fig No: 39- Linearity of standards at – 125% level

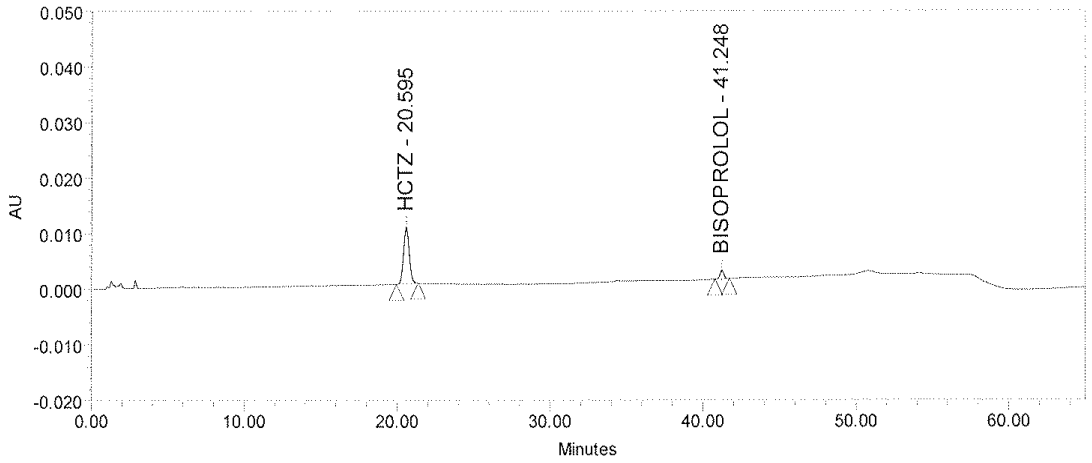


Fig No: 40- Linearity of standards at – 150% level

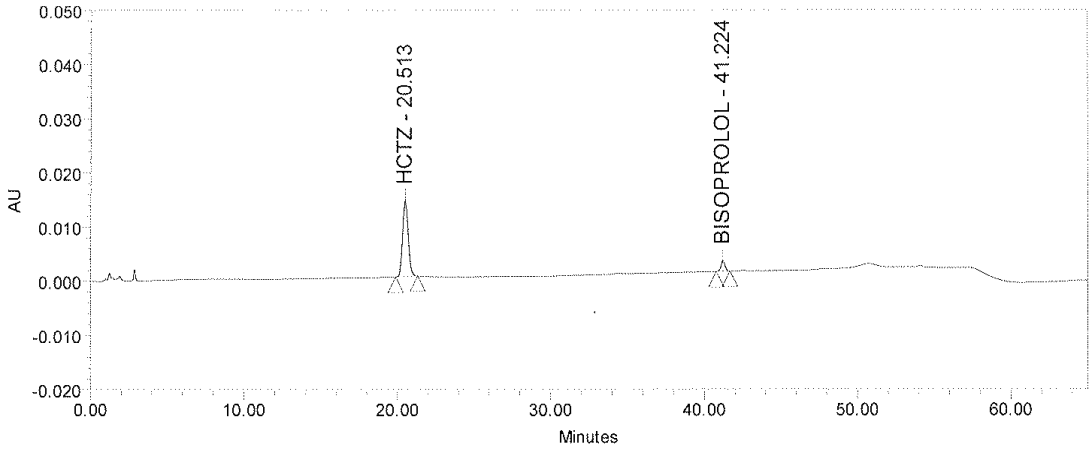


Fig No: 41- Linearity of standards at – 200% level

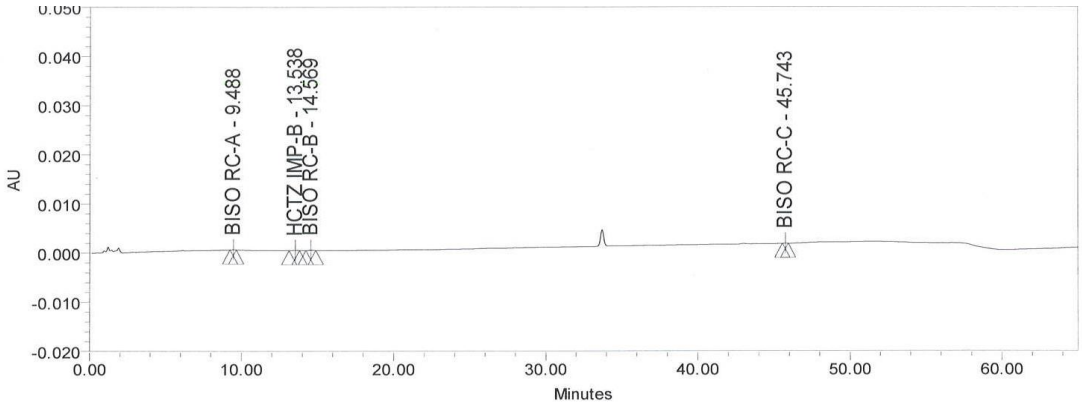


Fig No: 42- LOD of all impurities

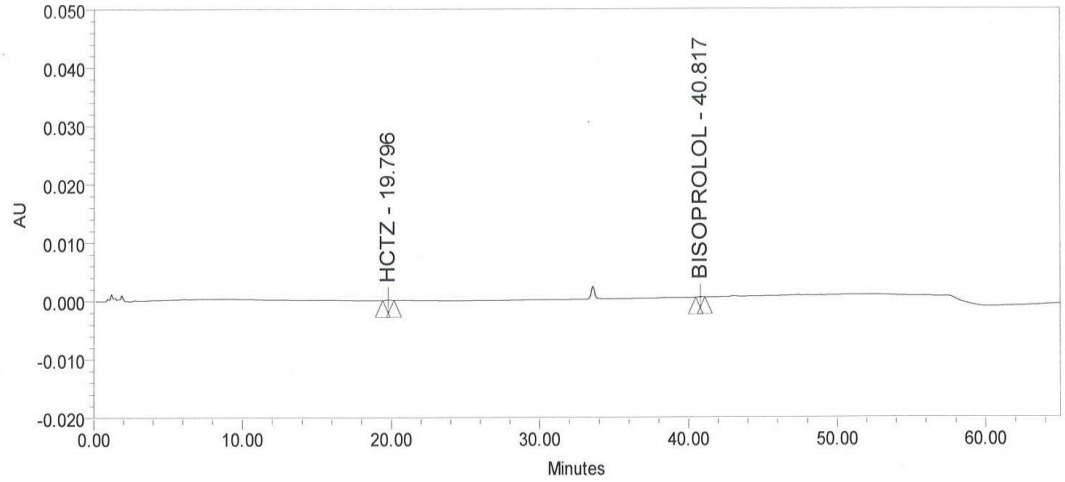


Fig No: 43- LOD of standards

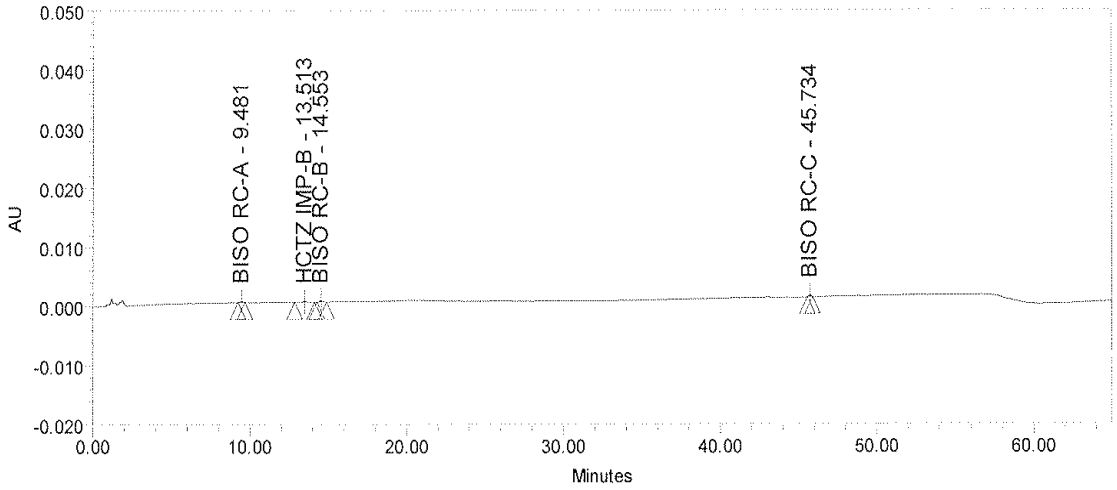


Fig No: 44- LOQ of all impurities

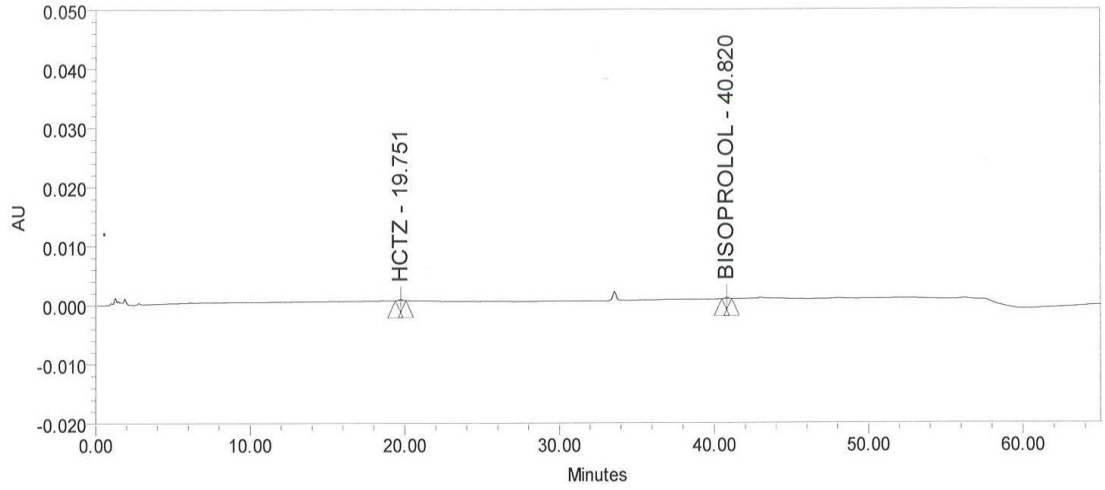


Fig No: 45- LOQ of standards

5. CONCLUSION

The developed chromatographic (RP-HPLC) method forBisoprolol fumarate, Hydrochlorothiazide and their related substances is said to be rapid, simple, precise, accurate, and cost effective that can be effectively applied for the routine analysis in research institution, quality control department in industries, approved testing laboratories, biopharmaceutical studies, clinical pharmacokinetic studiesand for determination of impurities in formulated products.From the overall results obtained it was concluded that the developed method was more accurate, precise, specific and robust with ±5ºC in temperature, ±0.2ml/min in flow rate, ±10% variation in organic phase.

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