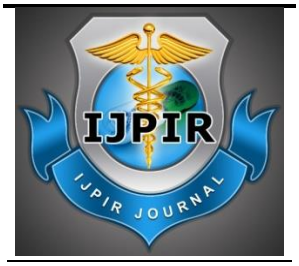
Research Article



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Validation of simple isocratic RP-HPLC method for clonazepam and propranolol HCL determination and it's application in the study of stress degradation

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ABSTRACT

A easy, exact, precise technique was developed for the synchronous estimation of the clonazepam and propranolol hcl in pharmaceutical measurement frame. Chromatographic conditions utilized are standard BDS C8 Column(150 x 4.6mm, 5µm) utilizing a mobile phase comtain of buffer 0.01N KH_2PO_4 (4.8pH): Acetonitrile taken in the ratio of 60:40 v/v at a flow rate of 1ml/min with UV recognition at238nm. Buffer utilized in this technique was 0.01N KH_2PO_4. Temperature was kept up at 30^oC. Retention time of propranolol hcl and clonazepam were observed to be 2.267min and 2.983min correspondingly. %RSD of the propranolol hcl and clonazepam were observed to be 0.5 and 0.correspondingly. %Recovery was get as 100.08% and 100.64% for propranolol hcl and clonazepam respectively. LOD, LOQ values get regression equations of propranolol hcl and clonazepam were 0.04, 0.11 and 0.02, 0.07 correspondingly. Regression equation of propranolol hcl is y = 39145x + 5375, and y = 79887x + 1421 of clonazepam. The developed method was approved in terms of precision, linearity, exactness, LOD, LOQ and specificity are in acknowledgment criteria, so the technique developed was easy and economical that can be adopted in regular Quality control tests in ventures.

Keywords: Propranolol HCL, Clonazepam, RP-HPLC technique, 0.01N KH_2PO_4.

INTRODUCTION

High Performance Liquid Chromatography (HPLC) is the fastest growing analytical technique for examination of drugs. Its simplicity, high specificity and large range of sensitivity make it ideal for the examination of many drugs in both dosage forms and biological fluids.

Propranolol HCL

A generally utilized non-cardio particular beta-adrenergic antagonist. Propranolol is utilized in the

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treatment or anticipation of numerous scatters including exceptional myocardial limited necrosis, arrhythmias, angina pectoris, hypertension, hypertensive crises, hyperthyroidism, headache, pheochromocytoma, menopause and uneasiness.

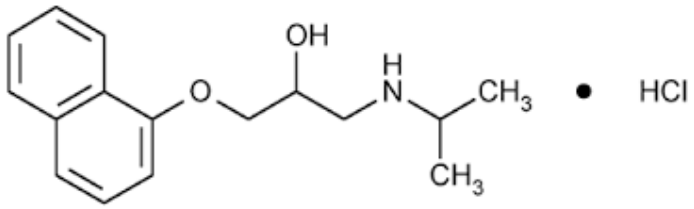

Fig no. 1 Chemical structure of propranolol HCL

CAS Number	**525-66-6**
Purity	>=98%
Molecular weight	259.3434
Molecular formula	$C_{16}H_{22}CLNO_2$
Physical state	Solid
Solubility	Soluble in water (50mg/ml), ethanol (10mg/ml), DMSO (<14.5mg/ml), and methanol.
Storage	Store at -20^oC
Melting point	163-165^o C

Clonazepam

Clobazam has a place with the 1,5-benzodiazepine class of medicine and is required to have a superior symptoms profile appeared differently in relation to more prepared 1,4-benzodiazepines.

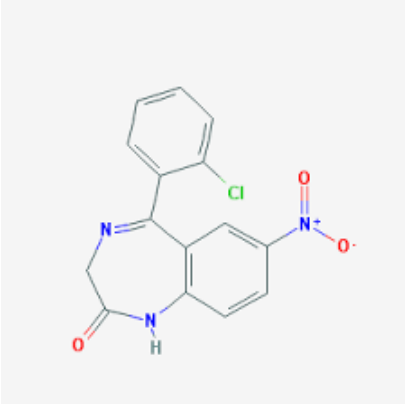

Fig no.2 structure of clonazepam

CAS Number	**1622-61-3**
Molecular weight	315.711
Molecular formula	$C_{15}H_{10}CLN_3O_3$
Physical state	Solid
Solubility	Soluble in chloroform, methylene chloride and water
Storage	Store at -20^oC

MATERIALS AND METHODS

Clonazepam and propranolol HCL pure drugs (API), blend propranolol hcl and clonazepam tablets (**Clonapax P**), Distilled water, Acetonitrile, Phosphate buffer, Methanol, OPA. All the above chemicals and solvents are from Rankem.

Instruments

Electronics Balance-Denver, p^H meter –BVK enterprises, India. Ultrasonicator-BVK enterprises. WATERS HPLC 2695 SYSTEM outfitted with quaternary pumps, PDA detector and auto sampler included with Empower 2 Software. UV-VIS spectrophotometer PG instruments T60 with extraordinary transmission capacity of 2 mm and 10 mm coordinated quartz cells included with UV win 6 software was utilized for estimating absorbance of propranolol hcl & clonazepam solutions.

Diluents

Based up on the solubility of the compounds, diluent was selected, Acetonitrile**:**water(50:50v/v).

Preparation of buffer

0.1% OPA Buffer: 1ml of conc. Ortho Phosphoric acid was diluted to 1000ml with water.

0.01N Potassium dihydrogen ortho phosphate buffer

Exactly weighed 1.36gm of potassium dihydrogen ortho phosphate in a 1000ml of volumetric flask add about900ml of milli-Q water added and degas to sonicate and lastly make up the volume with water.

Chromatographic Conditions

Mobile phase utilized are 0.01N KH_2PO_4**:** Acetonitrile (60:40 v/v), flow rate 1ml/min, column utilized are BDS C8 (4.6x 150mm, 5µm), Detector wave length was 238nm, column temperature 30^oC, Injection volume was 10µl, Run time 5min, Diluent utilizedwas water and Acetonitrile in the ration of 50:50.

RESULTS AND DISCUSSIONS

Optimized method

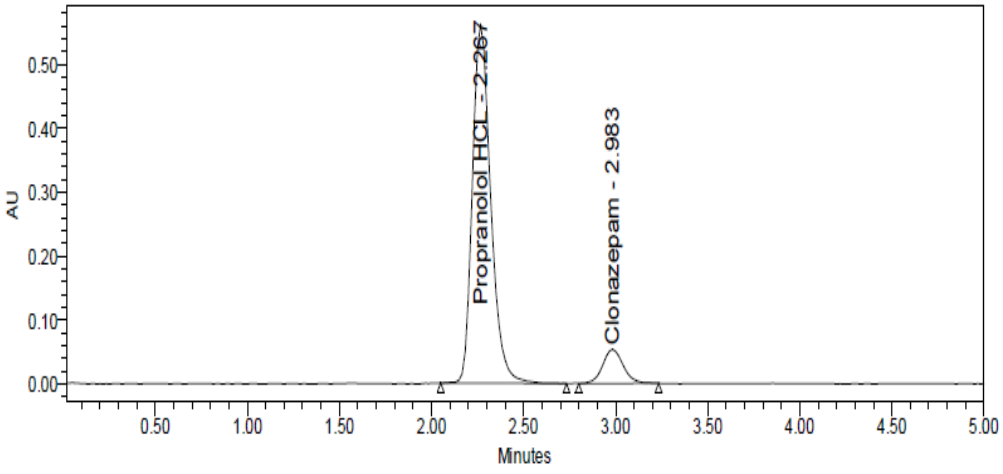

Fig no.3 Optimized chromatogram

System suitability

All the system suitability parameters were in the range and acceptable as per ICH guidelines.

Table no. 2 System suitability parameters for propranolol hcl and clonazepam

S no	Propranolol			Clonazepam			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.254	2474	1.26	2.972	3548	1.10	3.7
2	2.255	2418	1.24	2.973	3449	1.10	3.6
3	2.256	2492	1.26	2.975	3568	1.08	3.7
4	2.257	2450	1.25	2.976	3365	1.09	3.7
5	2.267	2573	1.26	2.983	3546	1.06	3.7
6	2.270	2479	1.24	2.989	3493	1.07	3.7

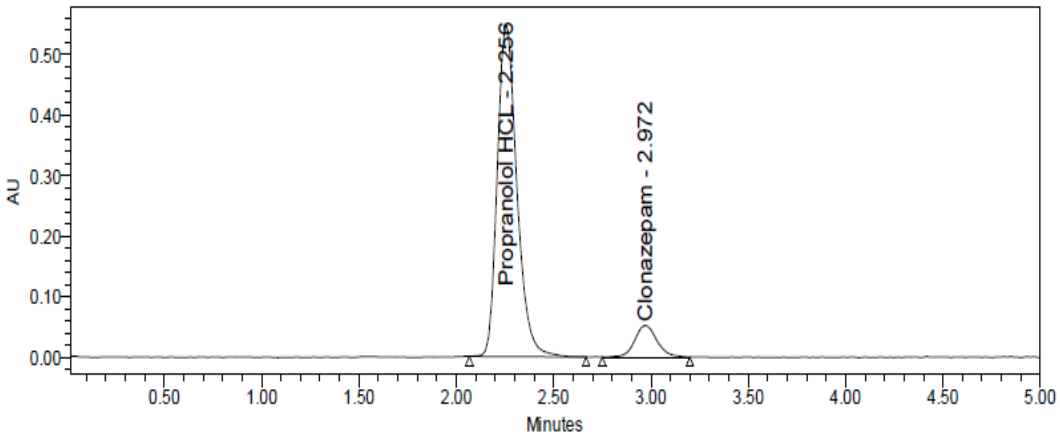

Fig no.4 system suitability chromatogram

Specificity

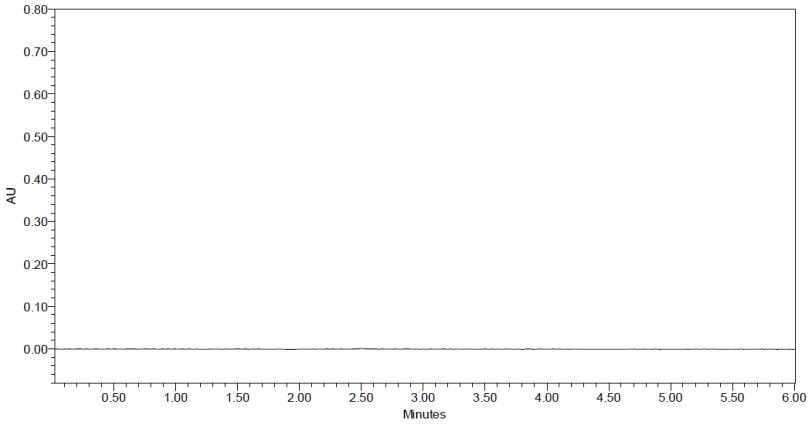

Fig no.5 Chromatogram of blank

Linearity

Table no.3 linearity table for propranolol hcl and clonazepam

Propranolol		Clonazepam	
Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
0	0	0	0
25	947107	1.25	99738

50	2025894	2.5	203119
75	2969284	3.75	306584
100	3874288	5	399372
125	4869287	6.25	495255
150	5902932	7.5	602922

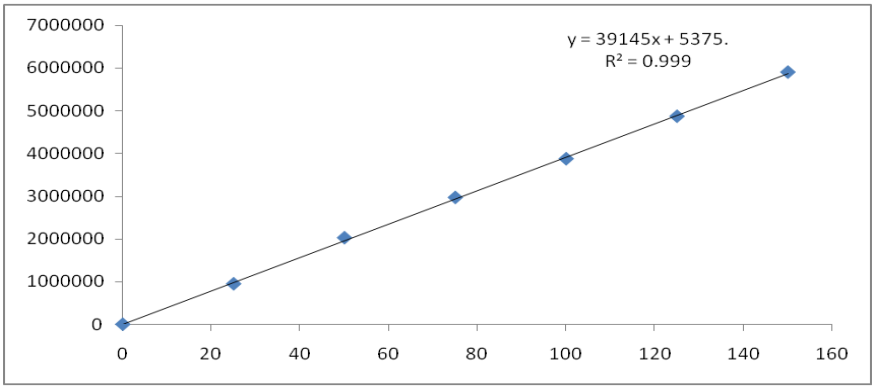

Fig no. 6 linearity chromatogram for propranolol hcl

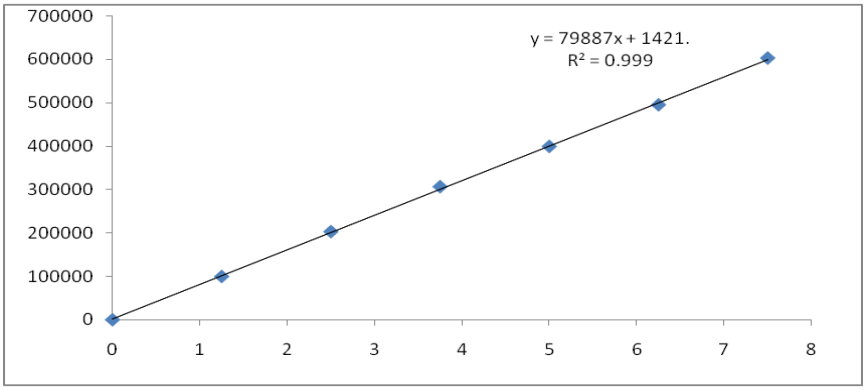

Fig no.7 linearity chromatogram for clonazepam

Precision: System precision

Table no. 6 system precision table for propranolol hcl and clonazepam

S. No	Area of Propranolol	Area of Clonazepam
1.	3848069	391081
2.	3875997	397876
3.	3832034	392739
4.	3847191	398538
5.	3881918	393257
6.	3804421	391835
Mean	3848272	394221
S.D	28595.5	3183.5
%RSD	0.7	0.8

Repeatability

Table no. 7 repeatability precision table for clonazepam and propranolol HCL

S. No	Area of Propranolol	Area of Clonazepam
1.	3849796	390940
2.	3800624	392691
3.	3839140	393239
4.	3832353	394407
5.	3847733	393008
6.	3850877	393871
Mean	3836754	393026
S.D	19081.6	1194.2
%RSD	0.5	0.3

Intermediate precision

Table no. 8 intermediate precision table for clonazepam and propranolol HCL

S. No	Area of Propranolol	Area of Clonazepam
1.	3735852	386075
2.	3795130	383276
3.	3726161	384337
4.	3781936	383121
5.	3731247	386818
6.	3724366	381085
Mean	3749115	384119
S.D	31077.9	2100.9
%RSD	0.8	0.5

Accuracy

Table no.9 accuracy table for propranolol HCL

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	50.61359	101.23	100.08%
	50	49.51156	99.02	
	50	50.19068	100.38	
100%	100	99.65022	99.65	
	100	99.43995	99.44	
	100	99.90461	99.90	
150%	150	150.89329	100.60	
	150	150.30499	100.20	
	150	150.44205	100.29	

Table no. 10 accuracy table for clonazepam

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	2.5	2.4995306	99.98	100.64%

	2.5	2.5144141	100.58
	2.5	2.5080301	100.32
100%	5	4.9255073	98.51
	5	4.9659018	99.32
	5	4.9119631	98.24
150%	7.5	7.5582761	100.78
	7.5	7.5478864	99.98
	7.5	7.5689787	100.58

Sensitivity

Table no. 11 sensitivity table for LOD and LOQ

Molecule	LOD	LOQ
Propranolol	0.04	0.11
Clonazepam	0.02	0.07

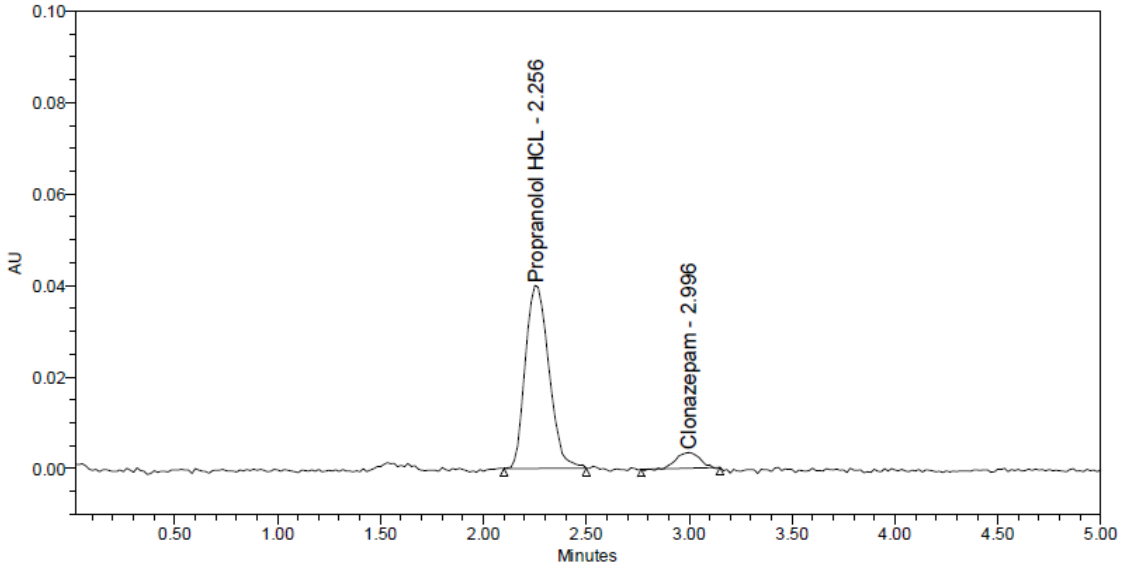

Fig no. 8 standard chromatogram for LOD

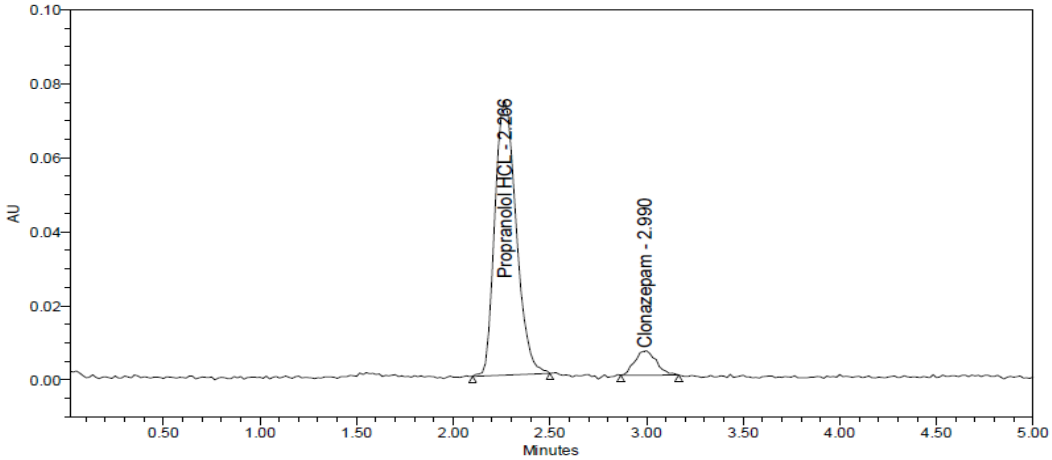

Fig no. 9 standard chromatogram for LOQ

Robustness

Table no. 12 robustness data of propranolol hcl and clonazepam

S.no	Condition	%RSD of Propranolol	%RSD of Clonazepam
1	Flow rate (-) 0.7ml/min	0.3	0.4
2	Flow rate (+) 0.9ml/min	1.1	1.0
3	Mobile phase (-) 55B:45A	0.3	0.4
4	Mobile phase (+) 65B:35A	1.0	0.4
5	Temperature (-) 25°C	0.1	0.5
6	Temperature (+) 35°C	0.6	1.0

Assay

Table no. 13 Assay data of propranolol HCL

S.no	Standard Area	Sample area	% Assay
1	3848069	3849796	99.94
2	3875997	3800624	98.66
3	3832034	3839140	99.66
4	3847191	3832353	99.49
5	3881918	3847733	99.89
6	3804421	3850877	99.97
Avg	3848272	3836754	99.60
Stdev	28595.5	19081.6	0.50
%RSD	0.7	0.5	0.5

Table no. 14 Assay data of clonazepam

S.no	Standard Area	Sample area	% Assay
1	391081	390940	99.07
2	397876	392691	99.51
3	392739	393239	99.65
4	398538	394407	99.95
5	393257	393008	99.59
6	391835	393871	99.81
Avg	394221	393026	99.60
Stdev	3183.5	1194.2	0.30
%RSD	0.8	0.3	0.3

Degradation studies

Table no. 15 Degradation studies table of propranolol hcl and clonazepam

Type of degradation	Propranolol			Clonazepam		
	AREA	%RECOVERED	% DEGRADED	AREA	% RECOVERED	% DEGRADED
Acid	3681441	95.57	4.43	376776	95.48	4.52
Base	3755097	97.48	2.52	384027	97.32	2.68
Peroxide	3779743	98.12	1.88	388694	98.50	1.50
Thermal	3824452	99.28	0.72	391049	99.10	0.90
UV	3822038	99.22	0.78	392806	99.54	0.46
Hydrolytic	3832656	99.22	0.78	391888	99.31	0.69

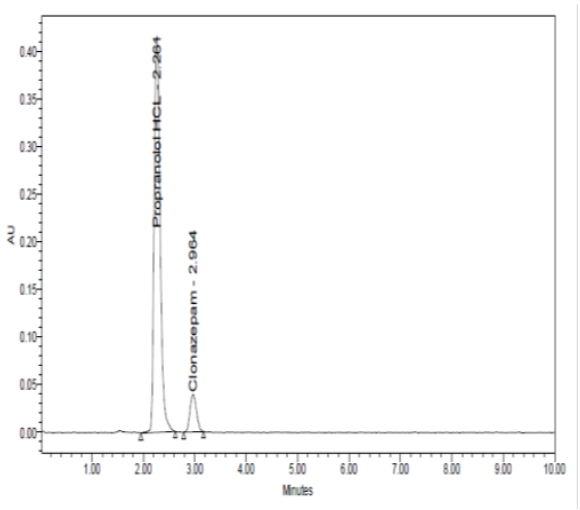

Fig no.10 Acid chromatogram

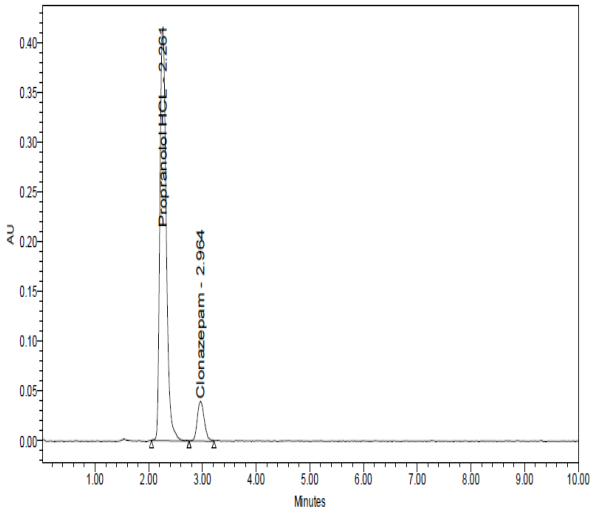

Fig no.11 Base chromatogram

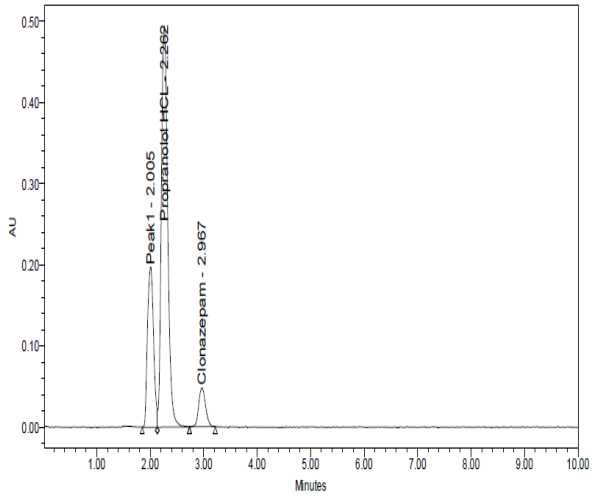

Fig no.12 Peroxide Degradation chromatogram

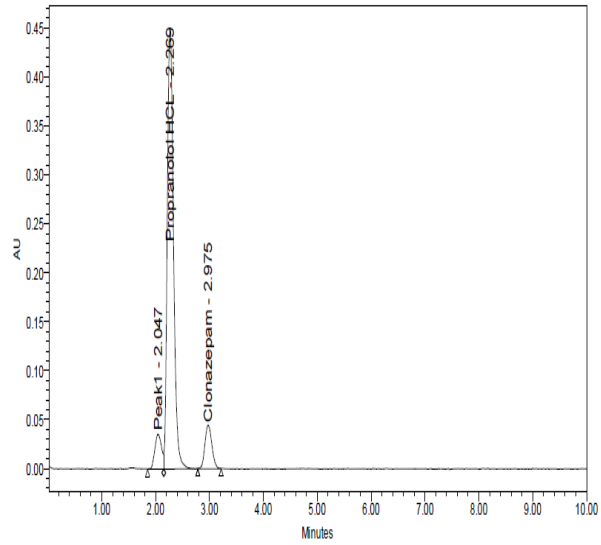

Fig no.13 Thermal degradation chromatogram

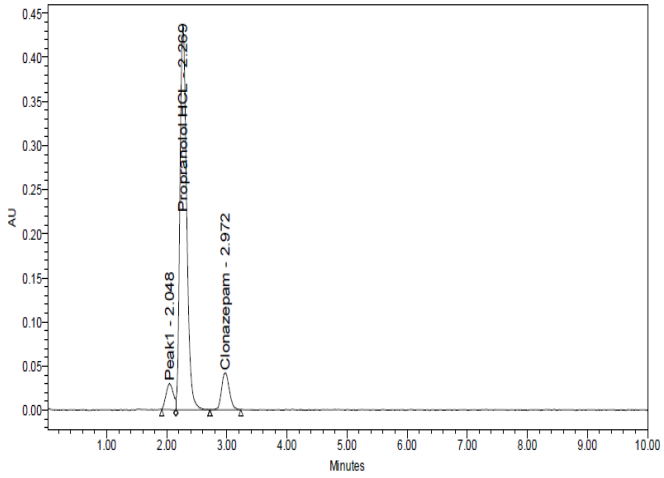

Fig no. 14 UV degradation chromatogram

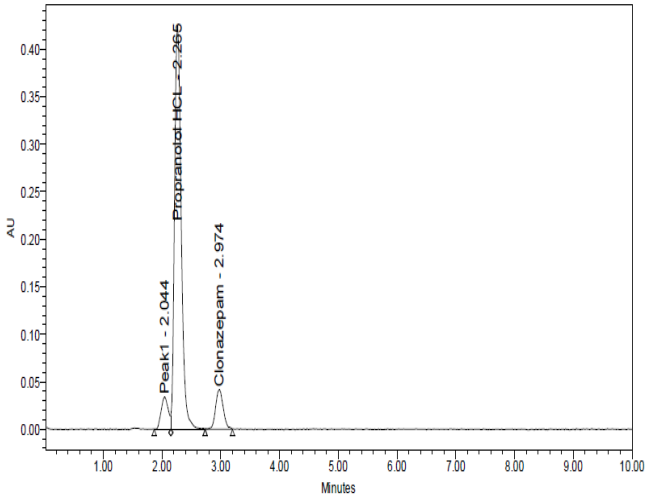

Fig no. Hyrolytic degradation chromatogram

CONCLUSION

The proposed HPLC technique for the purpose of clonazepam and propranolol HCL in dosage form was observe to be precise, exact, specific, straightforward rapid and economical. Hence, the current RP-HPLC technique is applied for routine examination in laboratories and is suitable for the quality control of the raw materials, formulations and can be further employed for bioequivalence studies.

Acknowledgement

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