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Development and validation of RP-HPLC method for simultaneous estimation of propranolol and clonazepam

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ABSTRACT

A reversed phase high performance liquid chromatographic method for routine analysis of propranolol and clonazepam has been developed using Discovery C18 column (4.6X150mm, 5µm) dimensions at ambient temperature .The mobile phase used for this study was mixture of potassium hydrogen phosphate and Acetonitrile at 45:55 ratio. The Mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1 ml/min for 6min. The elution was monitored at 238nm. Propranolol and Clonazepam were eluted at 2.191 min and 2.935 min respectively with good resolution. The optimized method was then validated according to the ICH guidelines. %RSD of the Propranolol and Clonazepam were and found to be 0.5 and 0.4 respectively. %Recovery was obtained as 99.53% and 99.38% for Propranolol and Clonazepam respectively. LOD, LOQ values obtained from regression equations of Propranolol and Clonazepam were 0.18, 0.54 and 0.004, 0.014 respectively. Therefore, a sensitive, robust, accurate was developed.

Keywords: RP-HPLC, Propranolol, Clonazepam, Simultaneous estimation, Validation.

INTRODUCTION

RP-HPLC employs mainly dispersive forces (hydrophobic or vanderwal's interactions). The polarities of mobile and stationary phases are reversed, such that the surface of the stationary phase in RP-HPLC is hydrophobic and mobile phase is polar, where mainly water-based solutions are employed [1]. RP-HPLC is by far the most popular mode of chromatography. Almost 90 % of all analyses of low-molecular-weight samples are carried out using RP-HPLC [2]. Dispersive forces employed in this separation mode are the weakest intermolecular forces, thereby making the overall background interaction energy in the chromatographic system very low compared to other separation techniques [3]. This low background energy allows for distinguishing very small differences in molecular interactions of closely related analytes. Adsorbents employed in this mode of chromatography are porous rigid materials with hydrophobic surfaces [4].

MATERIALS AND METHODS

Materials

Propranolol and Clonazepam pure drugs (API), Combination Propranolol and Clonazepam tablets (clonapax P), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

Instruments

Electronics Balance, p^H meter, Ultrasonicator**,** HPLC, UV-VIS spectrophotometer for measuring absorbance's of Propranolol and Clonazepam solutions.

Methods

Preparation of Standard stock solutions

Accurately weighed 10mg of Propranolol, 0.5mg of Clonazepam and transferred to 10ml flasks and 3/4 th of diluents was added to this flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1000µg/ml of Propranolol and 50µg/ml Clonazepam)

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipette out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml of Propranolol and 5µg/ml of Clonazepam)

Preparation of Sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10ml volumetric flask, 5 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000µg/ml of Propranolol and 50µg/ml of Clonazepam)

Preparation of Sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of Propranolol and 5µg/ml of Clonazepam)

Preparation of buffer

0.01N KH_2PO_4 Buffer

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5.4 with dil. Orthophosphoric acid solution.

RP-HPLC optimised chromatographic condition

The mobile phase used for this study was mixture of potassium hydrogen phosphate and Acetonitrile at 45:55 ratio. Stationary phase was Discovery C18 column (4.6X150mm, 5µm) dimensions at ambient temperature. The Mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1 ml/min for 6min. The elution was monitored at 238nm. Propranolol and Clonazepam were eluted at 2.191 min and 2.935 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated [5].

RESULTS AND DISCUSSIONS

Validation

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2[6]. All the system suitable parameters were passed and were within the limits. The results are depicted in Table 1 and the Fig. 1 shows System suitability Chromatogram

Table 1 System suitability parameters for Propranolol and Clonazepam

S no	Propranolol			Clonazepam			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.181	2827	1.20	2.903	3705	1.16	4.1
2	2.181	2799	1.20	2.905	3567	1.16	3.9
3	2.182	2891	1.21	2.905	3609	1.16	4.0
4	2.182	2769	1.20	2.908	3528	1.15	3.9
5	2.185	2836	1.20	2.913	3598	1.15	3.9
6	2.185	2832	1.21	2.913	3667	1.14	4.0

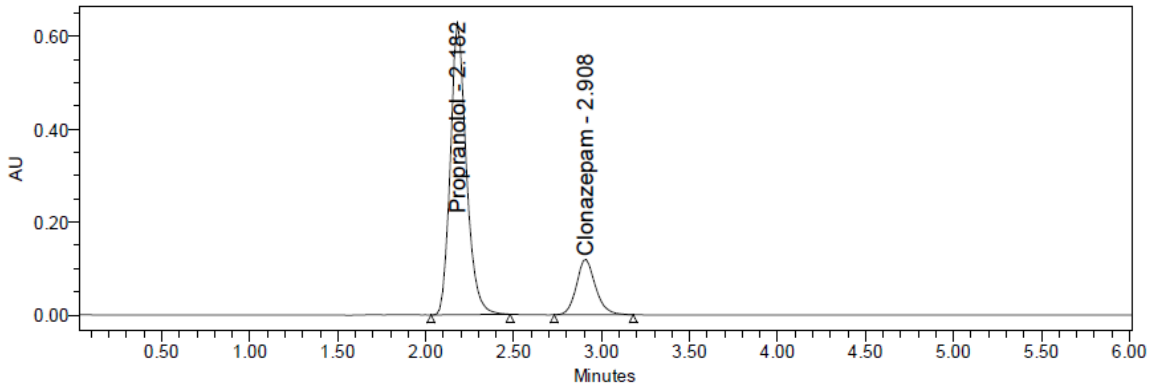

Fig. 1 System suitability Chromatogram

Specificity

Retention times of Propranolol and Clonazepam were 2.185 min and 2.913 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific. Fig.2, Fig.3, Fig.4 shows Blank, placebo and typical chromatogram.

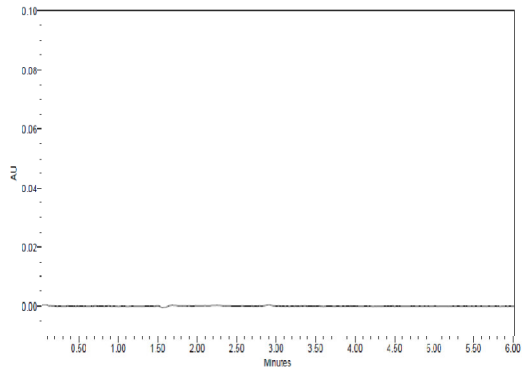

Fig. 2 Chromatogram of blank.

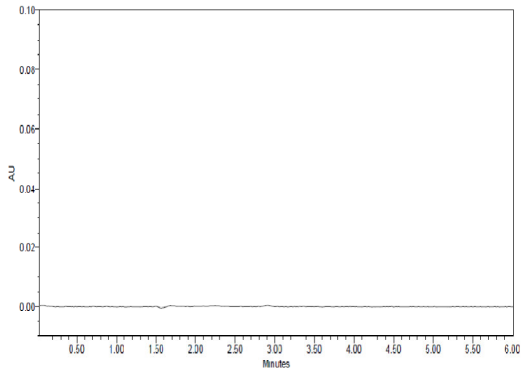

Fig. 3 Chromatogram of placebo

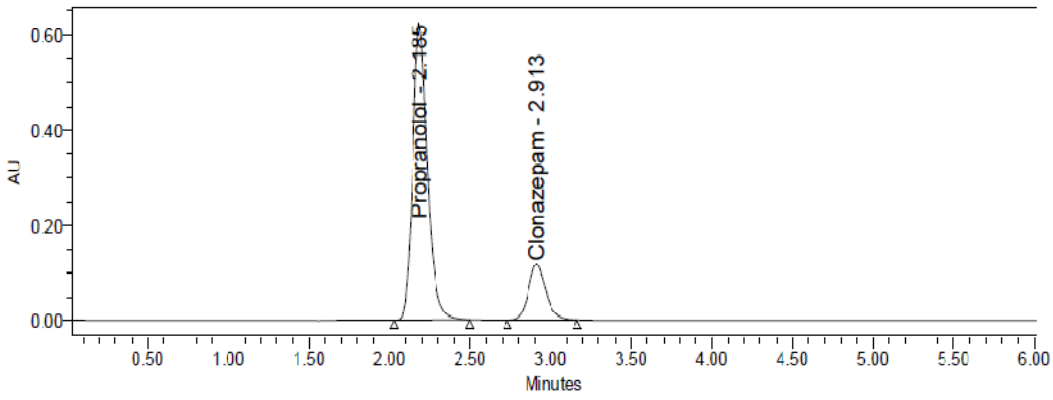

Fig. 4 Typical Chromatogram

Linearity

Six linear concentrations of Propranolol (25-150µg/ml) and Clonazepam (1.25- 7.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Propranolol was y = 4039.x + 1863 and of Clonazepam was y = 17295.x + 1761 Correlation coefficient obtained was 0.999 for the two drugs. Table 2 shows Linearity table for Propranolol and Clonazepam. Fig. 5 and Fig.6 represents calibration curve of propranolol and clonazepam. Fig. 7, 8, 9,10,11,12 shows 25%, 50%, 75%, 100%, 125% and 150% of linearity chromatogram of propranolol and clonazepam.

Table 2: Linearity table for Propranolol and Clonazepam.

Propranolol		Clonazepam	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**

0	0	0	0
25	102584	1.25	23385
50	207007	2.5	46111
75	302983	3.75	67931
100	410632	5	89408
125	502824	6.25	108127
150	607924	7.5	130433

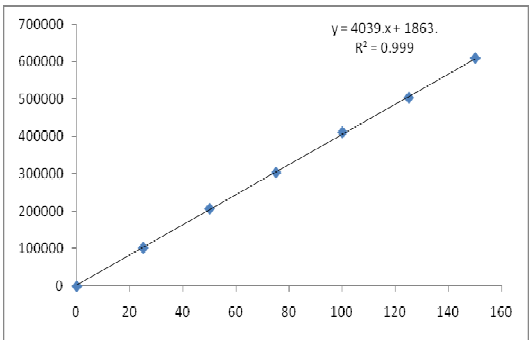

Fig. 5 Calibration curve of Propranolol

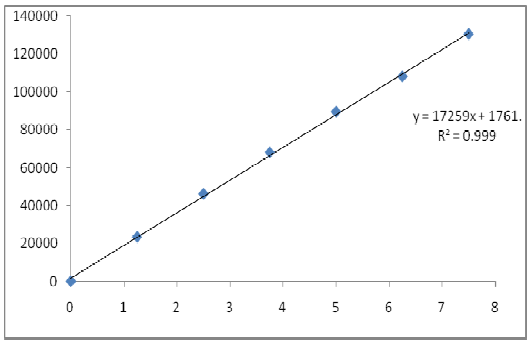

Fig. 6 Calibration curve of Clonazepam

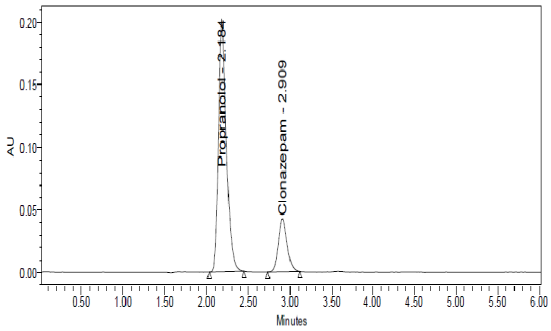

Fig. 7 Linearity 25% Chromatogram of Propranolol and Clonazepam

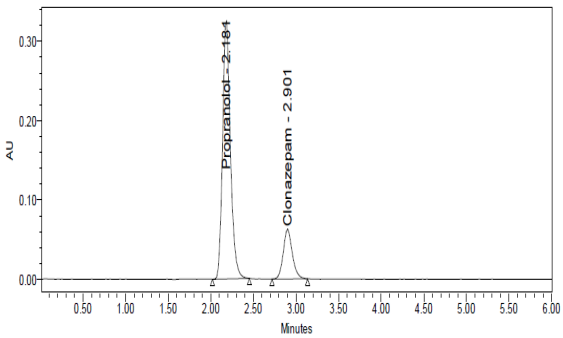

Fig. 8 Linearity 50% Chromatogram of Propranolol and Clonazepam

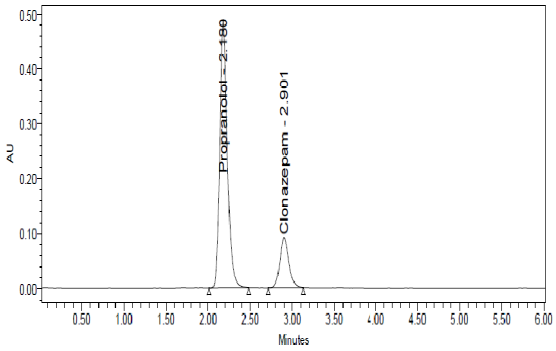

Fig. 9 Linearity 75% Chromatogram of Propranolol and Clonazepam

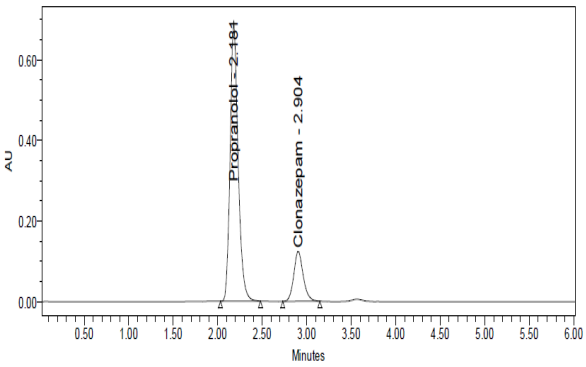

Fig. 10 Linearity 100% Chromatogram of Propranolol and Clonazepam

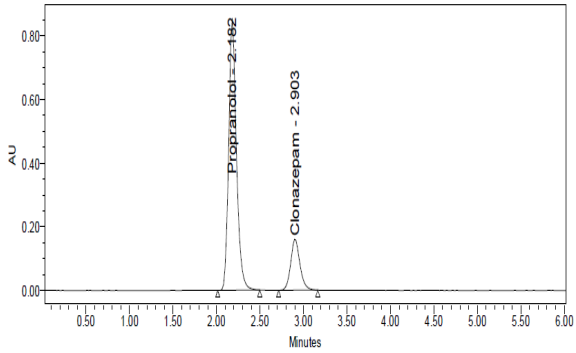

Fig. 11 Linearity 125% Chromatogram of Propranolol and Clonazepam

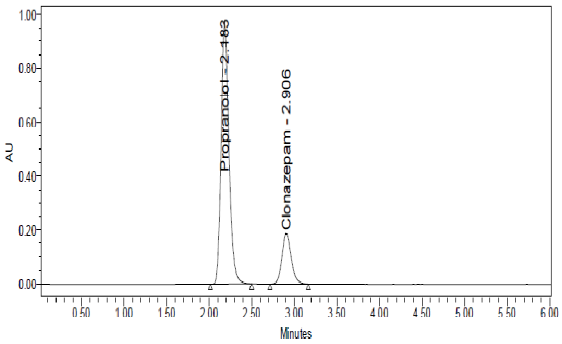

Fig. 12 Linearity 150% Chromatogram of Propranolol and Clonazepam

Precision

System precision

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.5% and 0.4% respectively for Propranolol and Clonazepam .As the limit of Precision was less than "2" the system precision was passed in this method [7]**.** The results are depicted in Table 3 and the Fig. 13 shows System precision Chromatogram

Table 3: System precision table of Propranolol and Clonazepam

S. No	Area of Propranolol	Area of Clonazepam
1.	414827	89478
2.	419177	89792
3.	418158	89417
4.	414812	89438
5.	417369	88997
6.	414103	89935
Mean	416408	89510
S.D	2098.2	328.1
%RSD	0.5	0.4

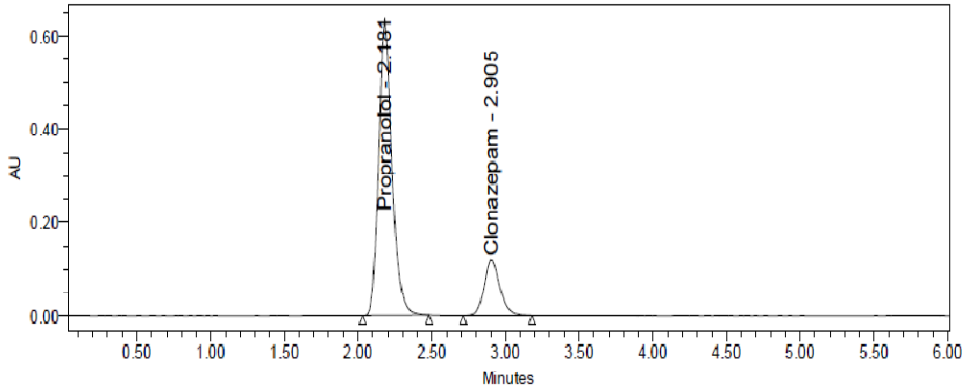

Fig. 13 System precision chromatogram

Repeatability

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.7% and 0.4% respectively for Propranolol and Clonazepam. As the limit of Precision was less than "2" the system precision was passed in this method [8]. The results are depicted in Table 4 and the Fig. 14 shows Repeatability Chromatogram

Table 4 Repeatability table of Propranolol and Clonazepam

S. No	Area of Propranolol	Area of Clonazepam
1.	416723	89303

2.	417882	89468
3.	419009	89988
4.	410946	89404
5.	413652	89954
6.	415856	89007
Mean	415678	89521
S.D	2952.1	383.1
%RSD	0.7	0.4

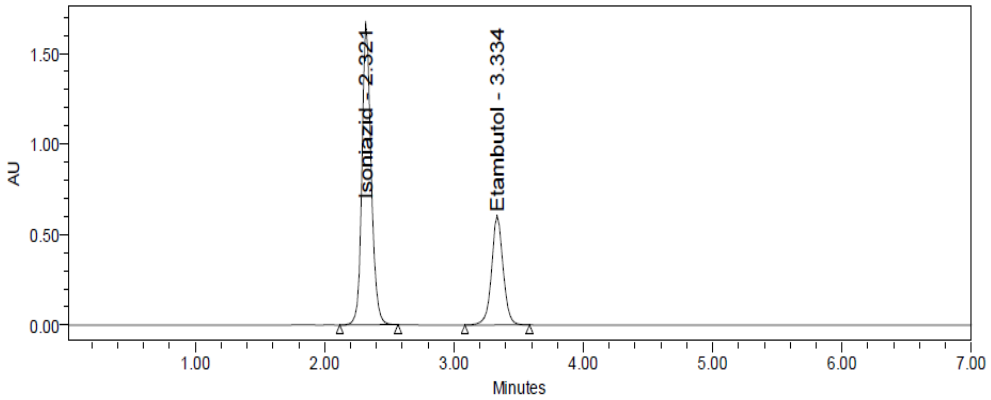

Fig. 14 Repeatability chromatogram

Intermediate precision (day_ day precision)

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.8% and 0.4% respectively for Propranolol and Clonazepam. As the limit of Precision was less than "2" the system precision was passed in this method [9]. The results are depicted in Table 5 and the Fig. 15 shows Inter Day precision Chromatogram

Table 5 Intermediate precision table of Propranolol and Clonazepam

S. No	Area of Propranolol	Area of Clonazepam
1.	406369	90940
2.	400788	90960
3.	402949	90661
4.	408979	90952
5.	407692	90693
6.	407668	90039
Mean	405741	90708
S.D	3185.2	354.1
%RSD	0.8	0.4

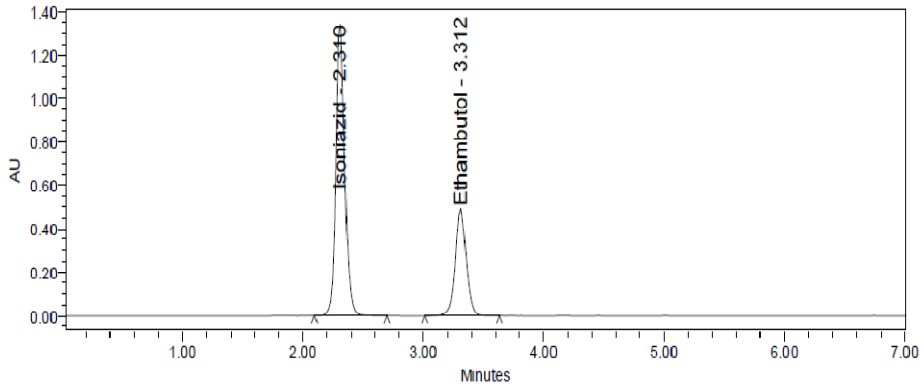

Fig. 15 Inter Day precision Chromatogram

Accuracy

Three levels of Accuracy samples were prepared by standard addition method [10]. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.53% and 99.38% for Propranolol and Clonazepam respectively. The results are depicted in Table 6 and 7 and the Fig. 16, 17 and 18 shows Accuracy 50%, 100% and 150% Chromatogram of propranolol and clonazepam

Table 6: Accuracy table of Propranolol

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	49.66	99.31	99.53%
	50	49.92	99.85	
	50	49.56	99.13	
100%	100	99.50	99.50	
	100	99.18	99.18	
	100	99.52	99.52	
150%	150	149.42	99.61	
	150	149.82	99.88	
	150	149.66	99.77	

Table 7 Accuracy table of Clonazepam

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	2.5	2.484	99.37	99.38%
	2.5	2.465	98.59	
	2.5	2.466	98.65	
100%	5	4.971	99.42	
	5	4.965	99.30	
	5	5.004	100.09	
150%	7.5	7.461	99.48	
	7.5	7.520	100.26	
	7.5	7.445	99.27	

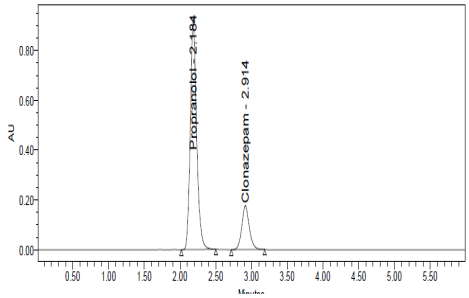

Fig. 16 Accuracy 50% Chromatogram of Propranolol and clonazepam

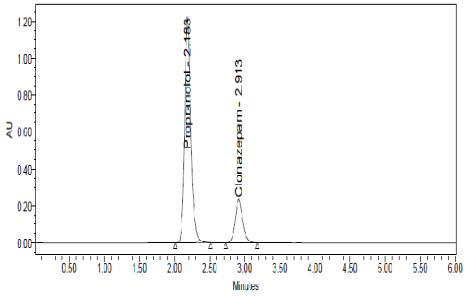

Fig. 17 Accuracy 100% Chromatogram of Propranolol and Clonazepam

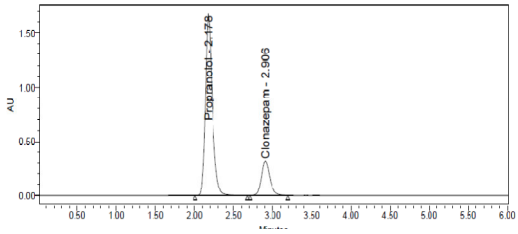

Fig. 18 Accuracy 150% Chromatogram of Propranolol and Clonazepam

SENSITIVITY: LOD and LOQ values are depicted in Table 8. Fig.19 and Fig.20 represents LOD and LOQ chromatograms.

Table 8 Sensitivity table of Propranolol and Clonazepam

Molecule	LOD	LOQ
Propranolol	0.18	0.54
Clonazepam	0.004	0.014

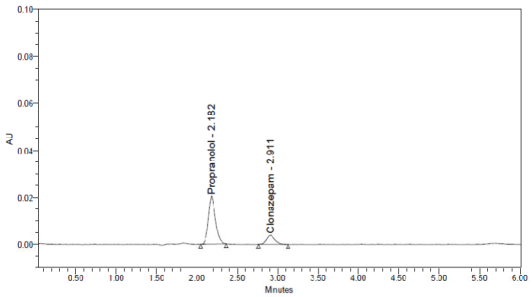

Fig. 19 LOD Chromatogram of Standard

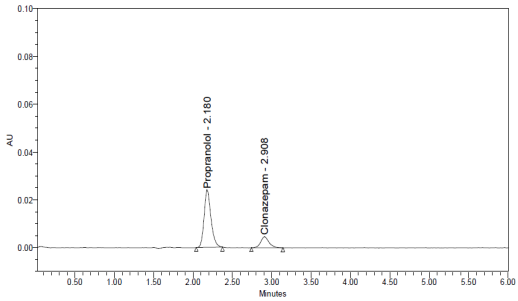

Fig. 20 LOQ Chromatogram of Standard

Robustness

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (40B:60A), mobile phase plus (50B:50A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner [11]. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. The results are depicted in Table 9 and the Fig. 21, 22, 23,24,25,26 Robustness Chromatograms of propranolol and clonazepam

Table 9 Robustness data for Propranolol and Clonazepam.

S.no	Condition	%RSD of Propranolol	%RSD of Clonazepam
1	Flow rate (-) 1.1ml/min	0.2	0.5
2	Flow rate (+) 1.3ml/min	0.8	1.2
3	Mobile phase (-) 35B:65A	0.1	0.2
4	Mobile phase (+) 45B:55A	0.4	0.7
5	Temperature (-) 25°C	0.5	0.6
6	Temperature (+) 35°C	0.1	0.2

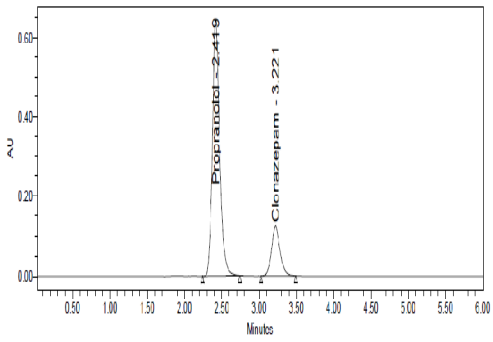

Fig. 21 Flow minus Chromatogram of Propranolol and Clonazepam.

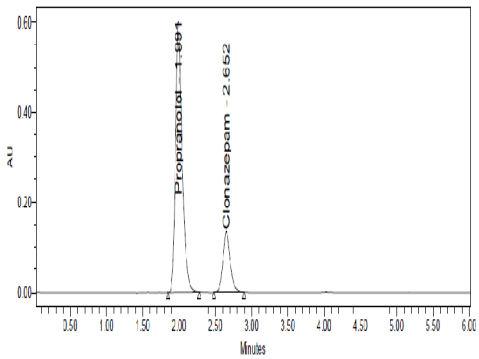

Fig. 22 Flow plus Chromatogram of Propranolol and Clonazepam.

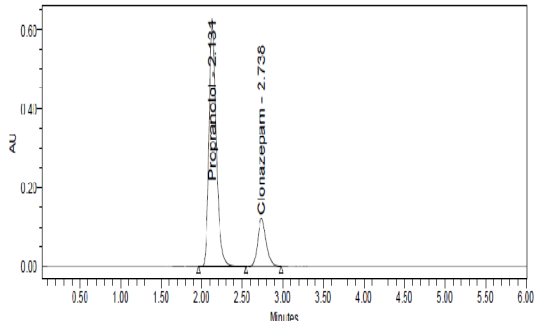

Fig. 23 Mobile phase minus Chromatogram of Propranolol and Clonazepam.

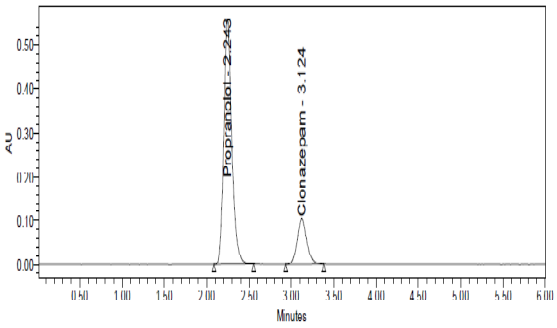

Fig. 24 Mobile phase Plus Chromatogram of Propranolol and Clonazepam.

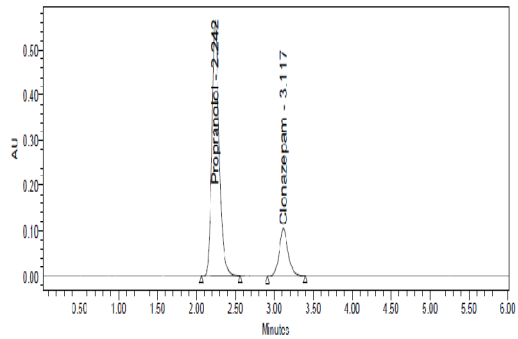

Fig. 25 Temperature minus Chromatogram of Propranolol and Clonazepam

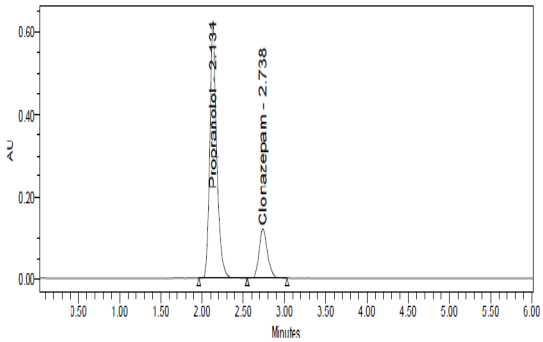

Fig. 26 Temperature plus Chromatogram of Propranolol and Clonazepam

Assay

Rhodes pharmaceuticals, bearing the label claim Propranolol 300mg, Clonazepam 150mg. Assay was performed with the above formulation. Average % Assay for Propranolol and Clonazepam obtained was 99.72% and 99.91% respectively. The results are depicted in Table 10, 11 and the Fig. 27 and 28 shows Chromatogram of working standard and sample solution

Table 10Assay Data of Propranolol

S.no	Standard Area	Sample area	% Assay
1	414827	416723	99.98
2	419177	417882	100.25
3	418158	419009	100.52
4	414812	410946	98.59
5	417369	413652	99.24
6	414103	415856	99.77
Avg	416408	415678	99.72
Stdev	2098.2	2952.1	0.71
%RSD	0.5	0.7	0.71

.

Table 11 Assay Data of Clonazepam

S.no	Standard Area	Sample area	% Assay
1	89478	89303	99.67
2	89792	89468	99.85
3	89417	89988	100.43
4	89438	89404	99.78
5	88997	89954	100.40
6	89935	89007	99.34
Avg	89510	89521	99.91
Stdev	328.1	383.1	0.4276
%RSD	0.4	0.4	0.4

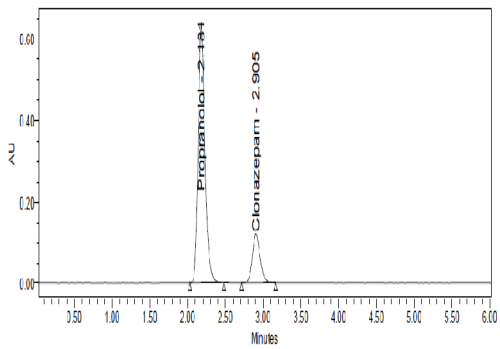

Fig. 27 Chromatogram of working standard solution

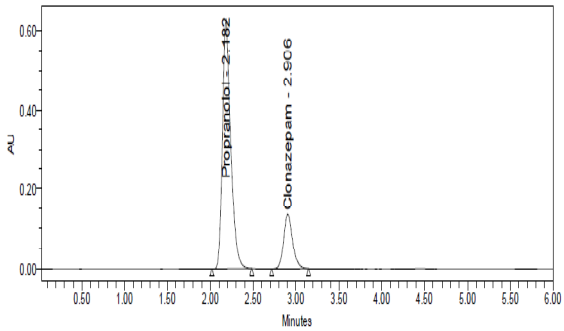

Fig. 28 Chromatogram of working sample solution

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Propranolol and Clonazepam in Tablet dosage form. Retention time of Propranolol and Clonazepam were found to be 2.191 min and 2.938. %RSD of the Propranolol and Clonazepam were and found to be 0.5 and 0.4 respectively. %Recovery was obtained as 99.53% and 99.38% for Propranolol and Clonazepam respectively. LOD, LOQ values obtained from regression equations of Propranolol and Clonazepam were 0.18, 0.54 and 0.004, 0.014 respectively. Regression equation of Propranolol is y = 4039.x + 1863, and y = 17259.x + 1761 of Clonazepam. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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REFERENCES

1. R.S.Satoskar, S.D.Bhandarkar and S.S.Ainapure. “Pharmacology and Pharmacotherapeutics”, 17th edition, Popular Prakashan, Mumbai, India, 2001.
2. “Burger’s Medicinal Chemistry and drug discovery”, 6 th edition, Wiley Interscience, New Jersey, 2007.
3. “Wilson and Gisvold’s Textbook of Organic Medicinal and Pharmaceutical Chemistry”, 11th edition,
4. A. Korolkovas. “Essentials of Medicinal Chemistry”, 2nd edition, Wiley Interscience, New Jersey, 1988.
5. “Goodman and Gilman’s The Pharmacological Basis of Therapeutics”, 9th edition, McGraw-Hill health professions division, New York, 1996.
6. Foye’s “Principles of Medicinal Chemistry”, 6th edition, Lippincott Williams & Wilkins, New york, 2008.
7. “Martindale - The Extra Pharmacopoeia”, 33rd edition, The Pharmaceutical Press, London, 2002. 7
8. A. H. Beckett and J. B. Stenlake. “Practical Pharmaceutical Chemistry”, Volume I and II, CBS Publishers & Distributors, New Delhi, India, 2000.
9. P. D. Sethi. “Quantitative Analysis of Drugs in Pharmaceutical Formulations”. 3 rd edition, CBS Publishers & Distributors, New Delhi, India, 1997.
10. H. H. Willard, L. L. Merrit, J. A. Dean and F. A. Settle. “Instrumental Method of Analysis”, 7th edition, CBS Publishers & Distributors, New Delhi, India, 1986.
11. R. A. Day and A. L. Underwood. “Quantitative Analysis”, 6th edition, PHI learning private limited, New Delhi, India, 2009