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Research article ***HPLC***

Simultaneous estimation of new analytical rp-hplc method development and validation of propranolol and clonazepam

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ABSTRACT

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Clonazepam and Propranolol, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Zorbax C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Water (70:30) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 265 nm. The retention time of the Clonazepam and Propranolol was 2.061, 2.462 ±0.02min respectively. The method produce linear responses in the concentration range of 3-15µg/ml of Clonazepam and 60-300µg/ml of Propranolol. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Clonazepam, Propranolol, RP-HPLC, validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs

may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 10]

DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.
E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture.
E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.
E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [15]

MATERIALS AND METHODS

Clonazepam(Pure) from Sura labs, Propranolol(Pure) from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck,

HPLC METHOD DEVELOPMENT TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Clonazepam and Propranolol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 0.09ml of Clonazepam and 1.8ml of Propranolol from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Water in proportion 70:30v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Symmetry C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

PTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used and PDA	:	Waters HPLC with auto sampler Detector 996 model.
Temperature	:	35ºC
Column	:	Zorbax C18 (4.6×150mm, 5µ)
Mobile phase	:	Methanol: Water (70:30v/v)
Flow rate	:	1ml/min
Wavelength	:	265nm
Injection volume	:	10 µl
Run time	:	8 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of mobile phase

Accurately measured 700 ml (70%) of Methanol and 300 ml of Water (30%) a were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Water (70:30)
Column : Zorbax C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 265 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 8minutes

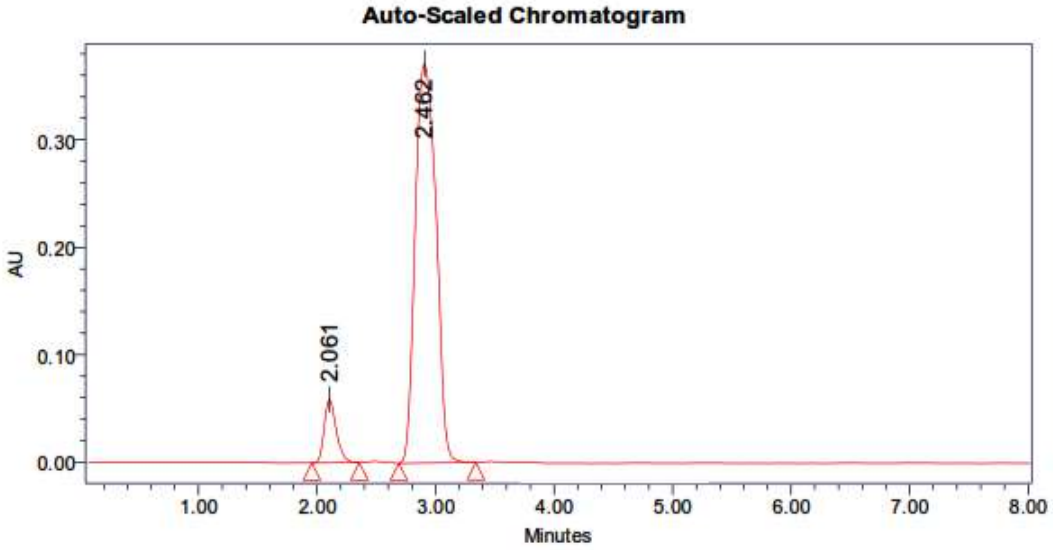

Fig 1: Optimized Chromatogram

Table 1: peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Clonazepam	2.061	247392	58952	1.2	7243
2	Propranolol	2.462	3530866	371748	1.1	3389

From the above chromatogram it was observed that the Clonazepam and Propranolol peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

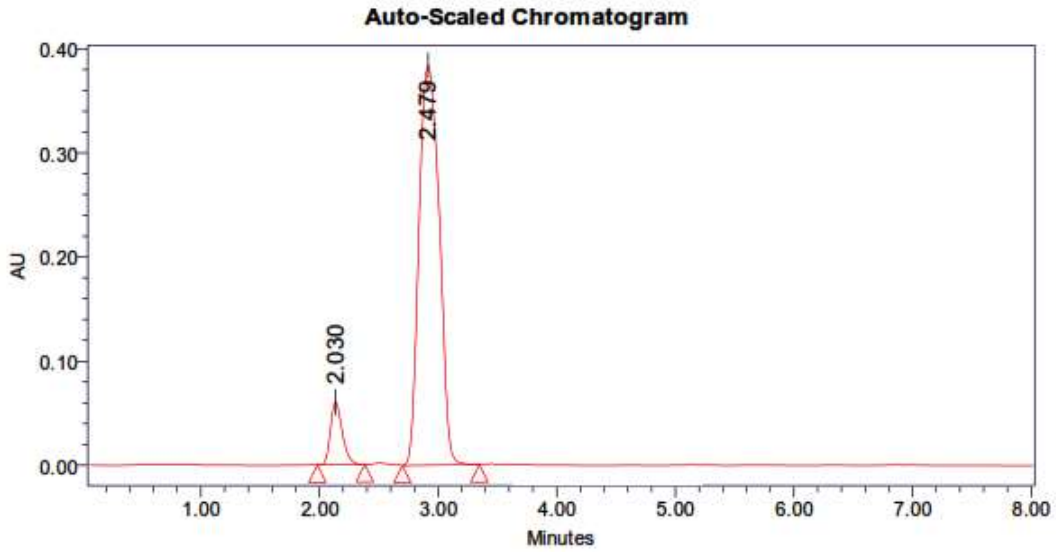

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Clonazepam	2.030	240019	60878	1.2	7246
2	Propranolol	2.479	3544380	384304	1.1	3375

- *Theoretical plates must be not less than 2000*
- *Tailing factor must be not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Assay (Standard)

Table 3: Results of system suitability for Clonazepam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Clonazepam	2.048	246713	73455	11318	1.1
2	Clonazepam	2.074	245617	78152	7105	1.2
3	Clonazepam	2.071	245830	78146	8974	1.2
4	Clonazepam	2.069	240552	78242	7087	1.2
5	Clonazepam	2.070	245725	77705	5124	1.2
Mean			244887.4			
Std. Dev			2462.26			
% RSD			1.005466			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Propranolol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Propranolol	2.446	3363754	636862	8484	1.1
2	Propranolol	2.490	3326434	641486	7889	1.0
3	Propranolol	2.489	3345949	638081	7846	0.9
4	Propranolol	2.488	3336621	617725	6772	0.9
5	Propranolol	2.490	3355244	631710	6884	0.9
Mean			3345600			
Std. Dev			14753.43			
% RSD			0.44098			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Assay (Sample)

Table 5: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Tailing	USP plate count
1	Clonazepam	2.068	244102	89282	1.2	5949
2	Propranolol	2.489	3357566	576562	1.0	6866
3	Clonazepam	2.070	240052	88021	1.2	5861
4	Propranolol	2.491	3371663	576999	1.0	6808
5	Clonazepam	2.067	243230	88882	1.2	5879
6	Propranolol	2.489	3364001	570315	1.0	6823

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=3364410/3345600×10/180×180/0.173×99.6/100×0.173/10×100
= 100.2%
The % purity of Clonazepam and Propranolol in pharmaceutical dosage form was found to be 100.2 %.

Linearity

Chromatographic data for linearity study

Clonazepam

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	3	88442
66.6	6	165724
100	9	242754
133.3	12	315906
166.6	15	396371

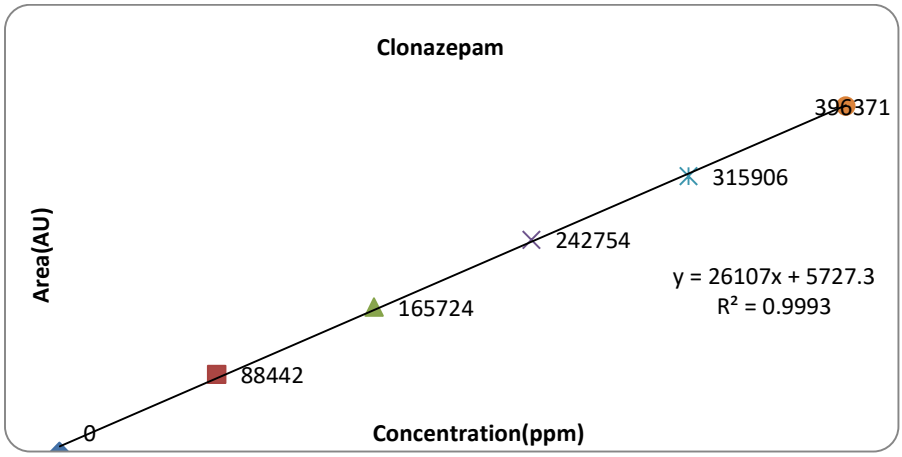

Fig 2: calibration graph for Clonazepam

Table 6: Chromatographic Data for Linearity Study Propranolol

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	60	1131032
66	120	2345302
100	180	3355282
133	240	4429382
166	300	5623754

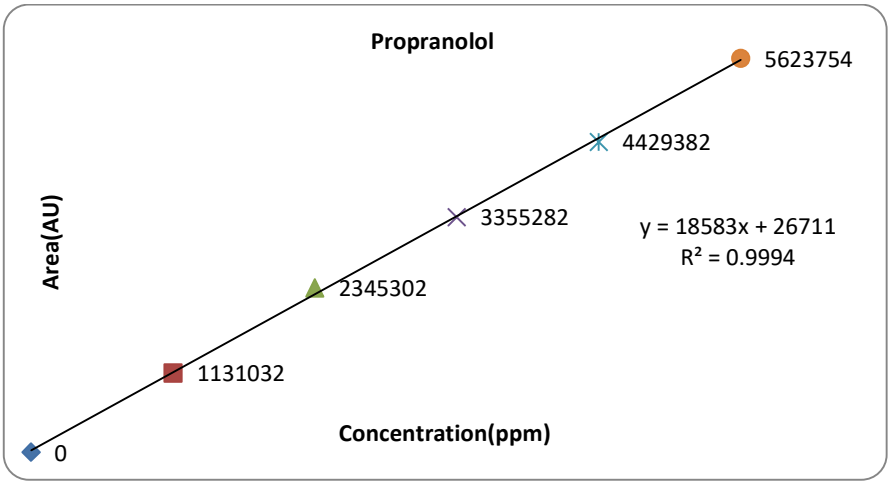

Fig 3: calibration graph for Propranolol

Repeatability

Table 7: Results of repeatability for Clonazepam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Clonazepam	2.065	249684	12079	5343	1.0
2	Clonazepam	2.064	249696	12068	5473	1.2
3	Clonazepam	2.064	246325	11949	5473	1.1
4	Clonazepam	2.065	249816	11811	5389	1.1
5	Clonazepam	2.067	249892	11735	5180	1.0
Mean			249082.6			
Std. Dev			1543.964			
% RSD			0.61986			

- *%RSD for sample should be NMT 2*

Table 8: Results of method precession for Propranolol

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Propranolol	2.486	3233700	59095	6654	1.2
2	Propranolol	2.484	3241323	57552	6524	1.3
3	Propranolol	2.482	3245927	57213	6440	1.3
4	Propranolol	2.483	3245927	57096	6411	1.4
5	Propranolol	2.483	3222194	54363	6260	1.4
Mean			3237814			
Std. Dev			10060.62			
% RSD			0.310722			

- %RSD for sample should be NMT 2

Intermediate precision

Table 9: Results of Intermediate precision Day 1 for Clonazepam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Clonazepam	2.066	242721	11323	5272	1.21
2	Clonazepam	2.066	240155	11564	5168	1.16
3	Clonazepam	2.066	240945	11887	5310	1.14
4	Clonazepam	2.065	240385	11938	5275	1.19
5	Clonazepam	2.069	249920	11652	5078	1.10
6	Clonazepam	2.067	240820	11750	5225	1.17
Mean			243991			
Std. Dev			4641.97			
% RSD			1.5			

- *%RSD of five different sample solutions should not more than 2*

Table 10: Results of Intermediate precision Day 1 for Propranolol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Propranolol	2.477	3325309	54143	6149	1.25
2	Propranolol	2.478	3323780	53740	6127	1.21
3	Propranolol	2.483	3328190	54791	6607	1.28
4	Propranolol	2.486	3329035	55098	6769	1.28
5	Propranolol	2.489	3325968	52379	6709	1.30
6	Propranolol	2.483	3327725	54779	6756	1.36
Mean			3326668			

Std. Dev	1985.641
% RSD	0.059689

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Table 11: Results of Intermediate precision Day 2 for Clonazepam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Clonazepam	2.067	249499	11594	5240	1.2
2	Clonazepam	2.069	240991	11357	5130	1.2
3	Clonazepam	2.068	240431	11878	5136	1.2
4	Clonazepam	2.069	241330	11748	5267	1.2
5	Clonazepam	2.067	240519	11830	5222	1.2
6	Clonazepam	2.067	240470	11475	5982	1.2
Mean			242206.7			
Std. Dev			3590.034			
% RSD			1.48222			

- *%RSD of six different sample solutions should not more than 2*

Table 12: Results of Intermediate precision Day 2 for Propranolol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Propranolol	2.485	3426979	53353	6700	1.3
2	Propranolol	2.484	3446641	54454	6563	1.3
3	Propranolol	2.496	3430606	53532	6855	1.3
4	Propranolol	2.484	3430952	55157	6864	1.3
5	Propranolol	2.490	3431676	56223	6942	1.3
6	Propranolol	2.490	3429187	58578	6644	1.3
Mean			3433812			
Std. Dev			7041.409			
% RSD			0.205061			

- *%RSD of six different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

Table 13: The accuracy results for Clonazepam

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	124675.7	4.5	4.56	101%	100.4%
100%	242006.3	9	9.0	100%	
150%	357449	13.5	13.4	99.7%	

Table 14: The accuracy results for Propranolol

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	1696259	90	89.8	99.8%	99.2%
100%	3351661	180	179.7	99.8%	
150%	4975094	270	267	98.6%	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Clonazepam

Table 15: Results For Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	247392	2.061	7243	1.2
Less Flow rate of 0.9 mL/min	69214	2.267	4713	1.3
More Flow rate of 1.1 mL/min	388838	1.864	4740	1.2
Less organic phase	445628	2.165	4709	1.2
More organic phase	69404	1.967	5590	1.4

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Propranolol

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3530866	2.462	3389	1.1
Less Flow rate of 0.9 mL/min	527373	2.690	5275	1.0
More Flow rate of 1.1 mL/min	4363129	2.284	5611	1.0
Less organic phase	3965572	2.590	5550	1.0
More organic phase	527708	2.390	6273	1.0

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Clonazepam and Propranolol in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Clonazepam and Propranolol was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: Water (70:30) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Clonazepam and Propranolol in bulk drug and in Pharmaceutical dosage forms.

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