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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CLONAZEPAM AND PAROXETINE IN TABLET DOSAGE FORMS

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ABSTRACT

A simple accurate, precise rapid isocratic RP-HPLC method development for the simultaneous estimation of Paroxetine and Clonazepam in tablet dosage forms. The chromatographic system was carried on INERTSIL (250x4.6mm, 5µ) column using mobile phase of phosphate buffer: acetonitrile: methanol in the ratio of 30:30:40 v/v at a flow rate of 1.0 ml/min. the eluents was detected at 268nm. The retention time of paroxetine was found to be 4.867 min and retention time for clonazepam was found to be 2.367 calibration curve was linear over the concentration range of paroxetine is 60-140 µg/ml and concentration range of clonazepam is 2.4-5.6 µg/ml the correlation coefficient for both peak was found to be 0.999 and 0.998 respectively. All the analytical validation parameters were determined and found in the limit as per ICH guidelines.

KEYWORDS: Paroxetine, Clonazepam, RP-HPLC, Validation, Phosphate buffer.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase, i.e., the sorbent. RP-HPLC is a very powerful technique for the analysis of peptides and proteins.[1] Paxil (paroxetine hydrochloride) is a selective serotonin reuptake inhibitor (SSRI) antidepressant used to treat depression, panic attacks, obsessive-compulsive disorder (OCD), anxiety disorders, post-traumatic stress disorder, and a severe form of premenstrual syndrome (premenstrual dysphoric disorder).

Paxil is available as a generic drug.[2] Paroxetine, also known by trade names including Paxil and Seroxat among others, is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class. It is used to treat major depressive disorder, obsess

Clonazepam, sold under the brand name **Klonopin** among others, is a medication used to prevent and treat seizures, panic disorder, and for the movement disorder known as akathisia. It is a tranquilizer of the benzodiazepine class. It is taken by mouth. It begins having an effect within an hour and lasts between six and 12 hours.[4] [5][6] Common side effects include sleepiness, poor coordination, and agitation. Long-term use may result in tolerance, dependence, and withdrawal symptoms if stopped abruptly. Dependence occurs in one-third of people who take clonazepam for longer than four weeks. It may increase risk of suicide in people who are depressed. If used during pregnancy it may result in harm to the baby.[7] It binds to $GABA_A$ receptors and increases the effect of the neurotransmitter GABA. Validation is the process of establishing documentary evidence demonstrating that a procedure, process, or activity carried out in testing and then production maintains the desired level of compliance at all stages. In the pharmaceutical industry, it is very important that in addition to final testing and compliance of products, it is also assured that the process will consistently produce the expected results.

Drug profile

Paroxetine is a potent highly selective serotonin reuptake inhibitor (SSRI), **Clonazepam** Allosteric interactions between gamma-amino butyric acid (GABA) receptors and central Benzodiazepine receptor potentiate the property of GABA.

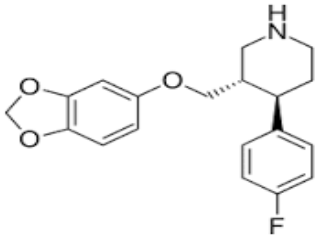


Fig:1: Structure for Paroxetine

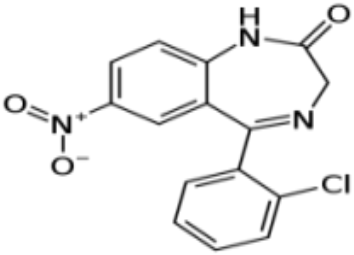


Fig:2: Structure for Clonazepam

MATERIALS & METHODS

INSTRUMENTATION

INSTRUMENT	MADE
• pH Meter	Thermo Electron Corporation
• HPLC	Shimadzu
• HPLC	Agilent
• Column	Inertsil ODS (250×4.6× 5µ) column

REAGENTS AND CHEMICALS

Water	-	HPLC Grade
Sodium di hydrogen ortho phosphate	-	AR Grade
Methanol	-	HPLC Grade
Potassium Di hydrogen ortho Phosphate	-	AR Grade
Acetonitrile	-	HPLC Grade
Di potassium hydrogen ortho phosphate	-	AR Grade

WORKING/REFERENCE STANDARDS:

Paroxetine and clonazepam bulk drugs are Gift Samples obtained from Finosopharma, Hyd. Stugil, (Paroxetine 100 mg and Clonazepam 4mg label claims) Obtained from local pharmacy.

MATERIALS & METHODS[8][9][10]

PREPARATION OF STANDARD SOLUTION OF PAROXETINE

10 mg of Paroxetine is dissolved in 100ml of Methanol. Pipette out 1ml of this solution and make up to 10 ml with methanol. The resulting solution has the concentration of 10µg /ml.

PREPARATION OF STANDARD SOLUTION OF CLONAZEPAM

10 mg of Clonazepam is dissolved 100 ml of Methanol. Pipette out 1ml of this solution and make up to 10 ml with methanol. The resulting solution having the concentration of 10 µg /ml.

PREPARATION OF TEST SOLUTION

20 tablets were weighed (each tablet contains 100 mg of Paroxetine and 4 mg of Clonazepam) and powdered. Take powder equivalent to 100 mg of Paroxetine and 4 mg of Clonazepam and dissolved in sufficient mobile phase and filtered. Further dilutions are prepared in 5 replicates of 100µg/ml of Paroxetine and 4µg/ml of Clonazepam was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Chromatographic conditions.[11][12]

S.No	Parameters	
1.	Mobile phase	Phosphate buffer (KH_2PO_4): Acetonitrile: Methanol
2.	Ratio	30:30:40
3.	Column	INERTSIL column (250×4.6mm× 5µ)
4.	Wavelength	224 nm
5.	Flow rate	1.0ml/min
6.	pH	4.0

RESULT AND DISCUSSION

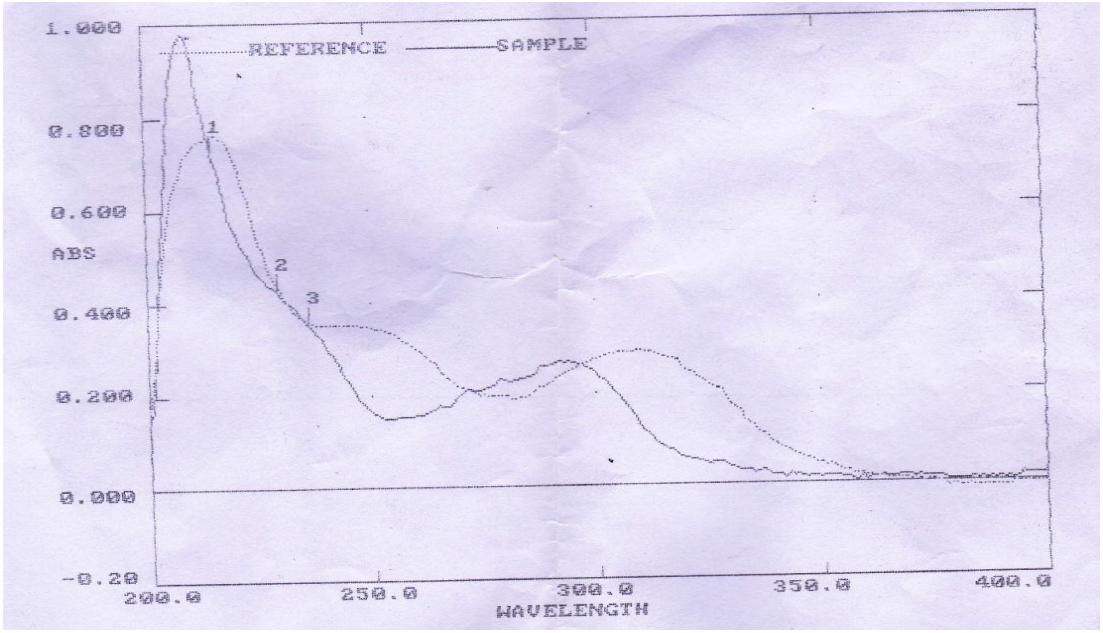


Fig:3: Chromatogram for determination of working wavelength.

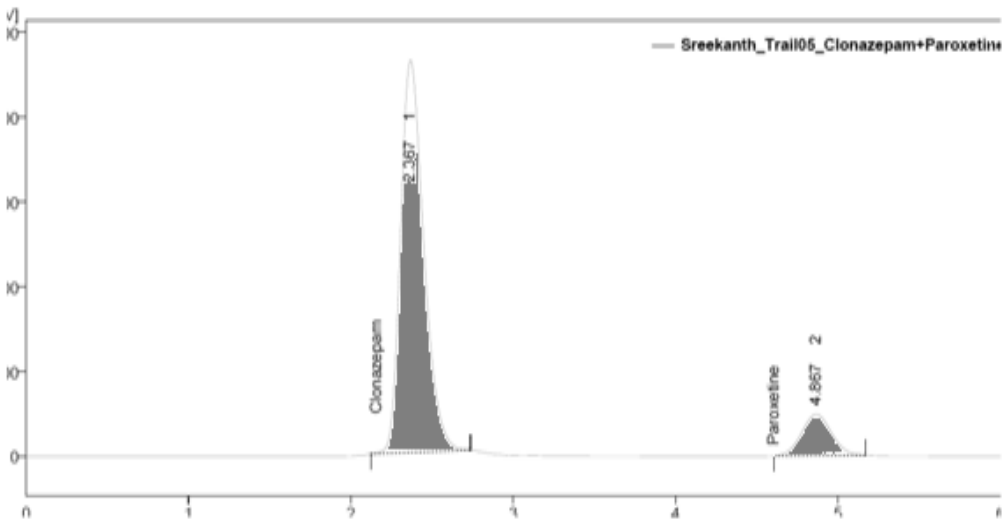


Fig 4: Chromatogram for optimized concentration.

ASSAY

S.No.	Name	Rt (min)	Peak Area	Asymmetry	Efficiency	Resolution
1	CLONAZEPAM	2.367	3156.483	1.400	2630	-
2	PAROXETINE	4.867	978.285	1.457	3407	7.926

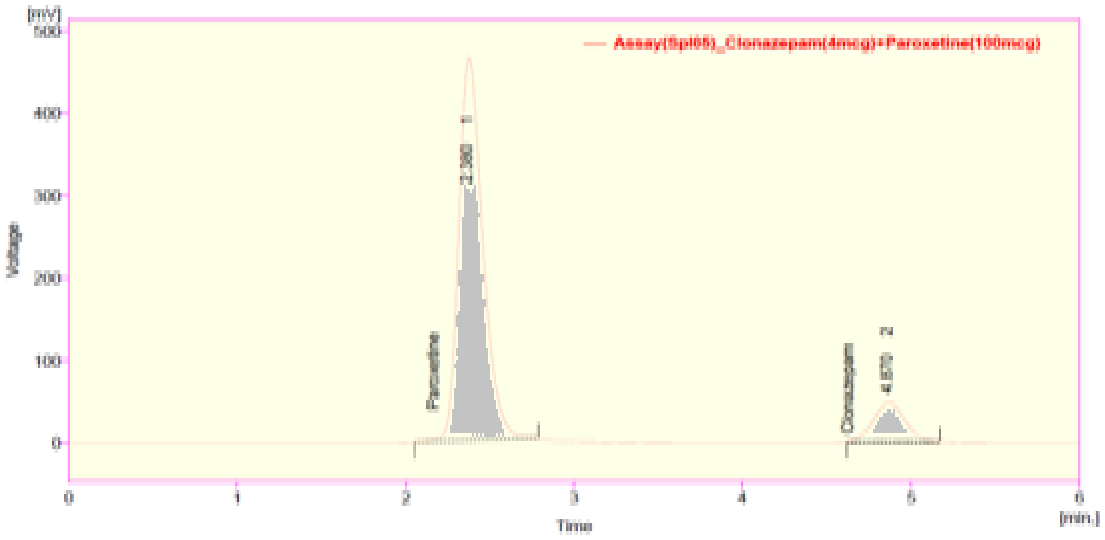


Fig 5: Chromatogram of Assay sample preparation.

Assay Results

PAROXETINE			CLONAZEPAM	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	4595.201	4586.227	620.260	625.861
Injection-2	4581.503	4572.126	622.927	622.854
Injection-3	4537.227	4517.674	625.206	634.200
Injection-4	4568.499	4600.157	625.778	616.708
Injection-5	4575.589	4594.241	626.022	624.567
Average Area	4571.604	4574.085	624.0386	625.438
Tablet average weight	300.23		300.23	
Standard weight	100.09		4.05	
Sample weight	301.45		301.45	
Label amount	100		4	
std. purity	96.2		96.3	
Amount found in mg	98.94		4.01	
Assay(%purity)	98.94		100.36	

Accuracy

Recovey level	**Accuracy PAROXETINE**					**Average% Recovey**
	Amount taken (mcg/ml)	**Area**	**Average area**	**Amount recovered (mcg/ml)**	**% Recovery**	
80%	100	4498.527	4463.661	98.98	98.98	100.08%
	100	4401.474				
	100	4490.981				
100%	120	5907.547	5676.783	121.61	101.34	
	120	5626.602				
	120	5496.201				
120%	140	6460.984	6438.544	136.88	96.92	
	140	6536.224				
	140	6318.425				

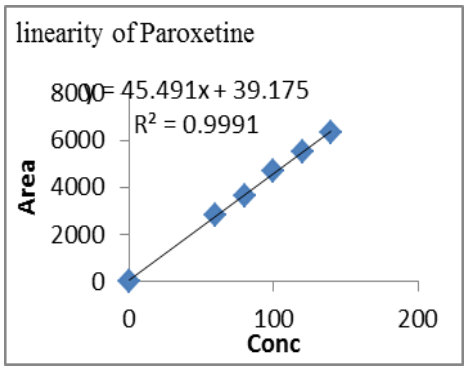
Recovery results for Clonazepam

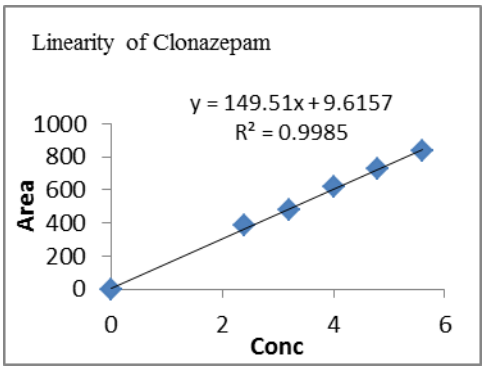
Recovery level	Accuracy CLONAZEPAM					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	% Recovery	
80%	4	586.024	591.806	3.93	98.15	96.89%
	4	577.759				
	4	611.634				
100%	4.8	758.345	765.676	4.93	102.80	
	4.8	776.958				
	4.8	758.725				
120%	5.6	836.26	836.923	5.53	98.70	
	5.6	831.205				
	5.6	840.437				

Precision

PAROXETINE			CLONAZEPAM		
S.No.	Rt	Area	S.No.	Rt	Area
1	2.427	4583.034	1	4.890	626.697
2	2.403	4584.532	2	4.927	627.874
3	2.397	4598.577	3	4.913	632.997
4	2.400	4570.390	4	4.887	627.37
5	2.387	4546.120	5	4.893	626.795
6	2.340	4530.777	6	4.820	628.228
Avg	2.3923	4568.905	avg	4.888	626.327
Stdev	0.0289	25.688	stdev	0.037	2.048
%RSD	1.21	0.56	**%RSD**	0.75	0.33

Linearity





Robustness

Result of Robustness study

Parameter	PAROXETINE		CLONAZEPAM	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.8 ml/min	3.253	1.529	5.927	0.982
1.2 ml/min	2.157	1.438	4.765	1.540
Wavelength				
222nm	2.400	1.441	4.753	1.038
226nm	2.350	1.545	4.743	1.137

Ruggedness

Results for Ruggedness

PAROXETINE	%Assay	CLONAZEPAM	%Assay
Analyst 01	96.53	Analyst 01	96.44
Anaylst 02	96.67	Anaylst 02	98.64
%RSD	0.036%	%RSD	0.054%

DISCUSSION

Assay

The amount of Paroxetine and Clonazepam present in the taken dosage form was found to be 98.94 % and 100.36 % respectively.

Accuracy[13]

The percentage mean recovery of Paroxetine and Clonazepam is 100.08% and 96.89% respectively. %.

System suitability

The % RSD for the retention times and peak area of Paroxetine and Clonazepam were found to be less than 2%.

Linearity and range[14]

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Paroxetine and Clonazepam is 0.999 and 0.9985.

Precision

Test results for Clonazepam and Paroxetine are showing that the %RSD of Assay results are within limits.

Robustness[15]

The system suitability parameters were within limit at all variable conditions.

Ruggedness

The % RSD between two analysts Assay values not greater than 2.0%, hence the method was rugge.

CONCLUSION

The validated method is found to be Specific, Linear, Precise, Accurate, Robust and Rugged for the estimation of Paroxetine and Clonazepam in tablet dosage form. Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy and specificity for routine analysis as well as for stability analysis.

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