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ASSAY METHOD DEVELOPMENT AND VALIDATION FOR S IMULTANEOUS ESTIMATION OF PAROXETINE AND CLONAZEPAM BY RP- HPLC

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ABSTRACT

A simple, precise, rapid, specific and accurate reverse phase high performance liquid chromatography method was developed for simultaneous estimation of Paroxetine and Clonazepam in pharmaceutical dosage form. Chromatographic separation was performed on INERTSIL ODS 3V column, C18(250x4.6 ID) column, with mobile phase comprising of mixture of buffer (pH7, adjusted with ammonium acetate), methanol, acetonitrile in the ratio of 3:2:5, at the flow rate 1.0 ml/min. The detection was carried out at 224 nm. The retention times of paroxetine and clonazepam were found to be 2.367 and 4.867 mins respectively with a run time of 6 mins, theoretical levels for paroxetine and clonazepam were 2320 and 3211 respectively, with a resolution of 8.249. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness. Linearity of paroxetine was found in the range of 60-140µg/mL and that for clonazepam was found to be 2.4-5.6µg/mL.

KEYWORDS: Paroxetine, Clonazepam, RP-HPLC, Method development, Validation.

INTRODUCTION

Paroxetine is chemically a (3S,4R)-3-[(2H-1,3-benzodioxol -5 -yloxy) methyl]- 4- (4 fluorophenyl) piperidine. It is an antidepressant in a group of drugs called selective serotonin reuptake inhibitors (SSRIs).[1-5] SSRIs are primarily classified as antidepressants. The mechanism action of Paroxetine affects neurotransmitters, the chemicals that nerves within the brain use to communicate with each other. Neurotransmitters are manufactured and released by nerves and then travel and attach to nearby nerves.[6-10] Paroxetine works by preventing the reuptake of one neurotransmitter, serotonin, by nerve cells after it has been released. Since reuptake is an important mechanism for removing released neurotransmitters and terminating their actions on adjacent nerves, the reduced uptake caused by paroxetine increases free serotonin that stimulates nerve cells in the brain.[11-16]

Clonazepam is a benzodiazepine drug having anxiolytic, anticonvulsant. Clonazepam is chemically5-(2-chlorophenyl)- 1, 3- dihydro -7- nitro-2 #-1, 4-benzodiazepinone. Clonazepam is approved by the Food and Drug Administration for treatment of epilepsy and Panic Disorder. Clonazepam is a chlorinated derivative of nitrazepamand therefore a chloro-nitrobenzodiazepine. Clonazepam's primary mechanism of action is the modulation of GABA function in the brain, by the benzodiazepine receptor, located on GABAA receptors, which, in turn, leads to enhanced GABAergic inhibition of neuronal firing. Clonazepam exerts its action by binding to the benzodiazepine site of the GABA receptors, which causes an enhancement of the electric effect of GABA binding on neurons, resulting in an increased influx of chloride ions into the neurons. This results in an inhibition of synaptic transmission across the central nervous system.[16]

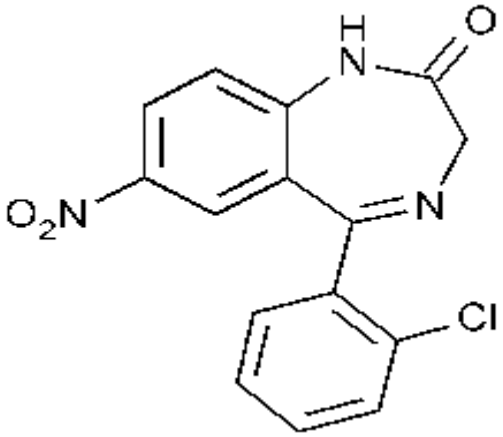

Figure 1: Molecular Structure of paroxetine.

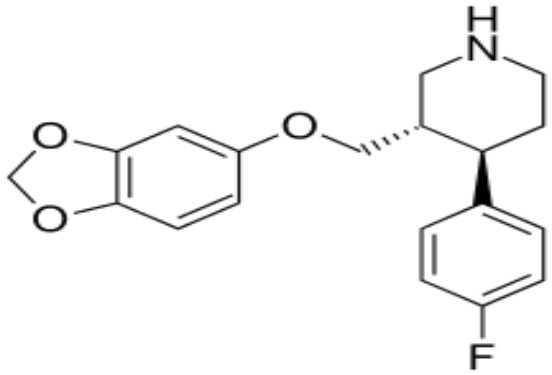

Figure 2: Molecular structure of clonazepam.

Literature survey revealed that few analytical techniques are available for estimation of paroxetinealone as well as in combine dosage form such as UV, HPLC.[4-8] Similarly few analytical methods are available for estimation of clonazepam alone and its combination with drugs such as UV and HPLC, colorimetry[1-8] but there is few methods for their simultaneous estimation using RP-HPLC. The present study was designed to develop a simple, precise, and rapid analytical RP-HPLC procedure, which can be used for the analysis of assay method for simultaneous estimation of paroxetine and clonazepam as there was only individual methods reported for both drugs. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of these two drugs in their combined dosage forms. Various validation aspects of the analysis accuracy, precision, recovery, the limits of detection and quantification etc have been measured as per ICH guidelines.[15]

MATERIALSANDMETHODS

Equipment

Chromatographic separation was performed on HPLC system-Water's, PDA Detector 2998 module, equipped with a solvent delivery pump, sample injector and column thermostats. CLASSVP Chromatographic system software was applied for data collecting and processing.

Chemicals and reagents

Methanol, Acetonitrile (HPLC grade)was used. Buffer used waspH-7(pH adjusted with ammonium acetate). (Referencestandards Paroxetine and Clonazepam were obtained from chandra Lab. paroxetine (100mg)and Clonazepam (4mg) manufactured by Johnson and johnson ltd, India.were procured from local market.

Preparation of Standard Solution

Standard stock solutions of Paroxetine and Clonazepam (µg/ml) were prepared by dissolving 100 mg of Paroxetine and 4 mg of Clonazepam dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5min and dilute to 100 ml with mobile phase. 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.

Preparation of Sample Solution

20 tablets (each tablet contains 100 mg of Paroxetine and 4 mg of Clonazepam) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Paroxetine and Clonazepam (µg/ml) were prepared by dissolving weight equivalent to 100 mg of Paroxetine and4 mg of Clonazepam and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 100ml with mobile phase. 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.

Preparation of Buffer

6.8gm of potassium di hydrogen phosphate (KH2PO4) was weighed and dissolved in 100ml of water and volume was made up to 1000 ml with water. Adjust the pH to 3.5 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of Mobile Phase

A mixture of 30 volumes of 50 mMPhosphate buffer(KH2PO4) pH4.0, 20 volumes of Methano l,and 50 volumes of Acetonitrile. The mobile phase was sonicated for 10 min to remove gases.

Optimized chromatographic conditions are as follows

- Column : INERTSIL ODS 3V column, C18 (4.6mm×250mm ×5µ)
- Mobile Phase : phosphate buffer: methanol: acetonitrile (3:2:5)
- Flow Rate : 1.0ml/min
- Volume : 5ul
- Column Temperature : 30^0c
- Sample temperature : 25^0c
- Detector wavelength : 224 nm (PDA)
- Run Mode : Isocratic
- Instrument : schimadzu with CLSSVP Software

METHODVALIDATION

Linearity

Standard stock solutions of Paroxetine and Clonazepam (µg/ml) were prepared by dissolving 100 mg of Paroxetine and 4 mg of Clonazepam in 100 ml of mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min. and dilute 100ml with mobile phase and further dilutions.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (preanalyzed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and

percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80%, 100%, 120%.

Precision

Intraday and interday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of An analytical method is usually expressed as the standard deviation correlative standard deviation (coefficient of variation) of seriesof measurements.

Robustness

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min), temperature.

Limit of detection (LOD) and Limit of quantification (LOQ) LOD and LOQ was calculated from linear curve using formulae.

LOD=3.3*σ/slope, LOQ=10*σ/slope

(Where σ=the standard deviation of the response and S= Slope of calibration curve).

Specificity

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both paroxetine and clonazepam from impurities.

RESULT AND DISCUSSION

Several mobile phase compositions were tried to resolve the peak of paroxetine and clonazepam. The mobile phase containing buffer: Ammonium acetate: Acetonitrile in proportion of 82:18v/v was found ideal to resolve the peak of paroxetine and clonazepam. Retention time of paroxetine and clonazepam were 2.1 and 3.3 min respectively (Figure 3). Result of assay is shown in Table- 2. The proposed method was found to be linear in concentration range 100-300µg/ml for paroxetine and 2- 6µg/ml for clonazepam. The data was shown in Table-3 and Figure-4&5. System suitability parameters were evaluated and results shown in (Table-4), which were within acceptance criteria. The mean percentage recovery for paroxetine and clonazepam was found to be 99.9 % and 100% respectively, which are well within the limit and hence the method was found to be accurate (Table-5). LOD and LOQ values were 2.34µg/mL and 7.10 µg/mL for paroxetine and 0.03 µg/mL and 0.09 µg/mL for clonazepam (Table-6). Results of intraday and interday precision were shown in the (Table-7a&7b). The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and temperature. The result obtained implies method is robust for routine qualitative analysis (Table-8).

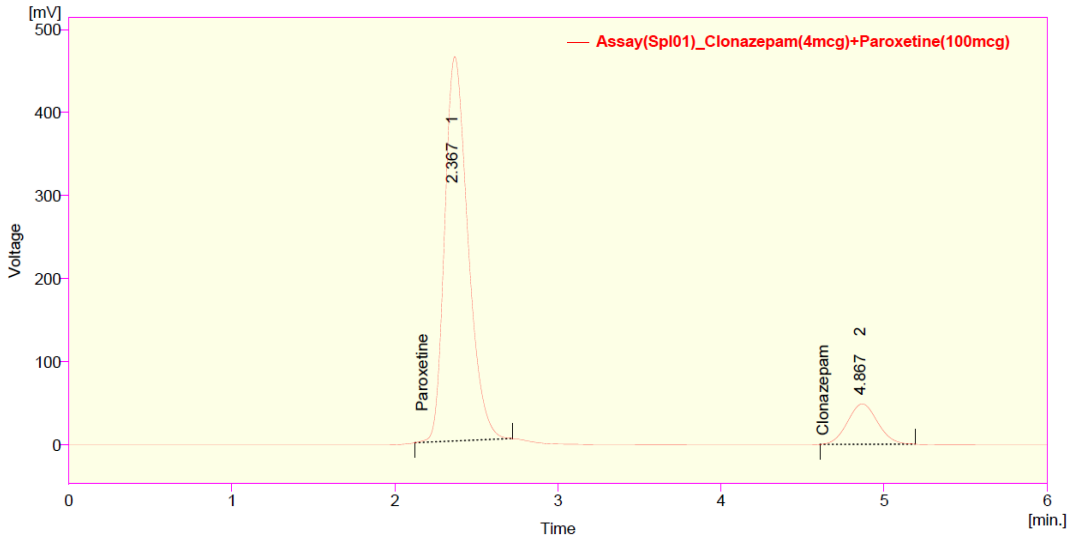

Figure 3: Chromatogram of sample.

Table 1. Observations of sample Chromatogram.

Sl.No	Name	Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
1	Paroxetine	2.367	2360112		1.20	2320
2	Clonazepam	4.867	6610849	8.247	1.34	3211

Table 2.Analysisdataofformulation.

Injection	Label claim(mg)	Assay (%)
Paroxetine	100	99.5
Clonazepam	4	100

Table 3: Linearity of Paroxetine and Clonazepam.

	Paroxetine		Clonazepam	
S.No.	Conc.(µg/ml)	Area	Conc.(µg/ml)	Area
1	60	2844.012	2.4	383.121
2	80	3607.702	3.2	482.379
3	100	4668.147	4	620.664
4	120	5523.646	4.8	726.780
5	140	6337.242	5.6	834.914

Table 4: System suitability parameters.

Parameters	Paroxetine	Clonazepam	Acceptance criteria
Theoretical plates	2320	3211	Not less than 2000
Tailing factor	1.20	1.34	Not more than 2
Resolution	-	8.247	Not less than 2

Table 5.Recoverystudies for Paroxetine and Clonazepam

Drug	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery	Mean recovery
	80	100	98.98	98.98	
Paroxetine	100	120	121.61	101.34	100
	120	140	139.88	99.92	
	80	4	3.93	98.15	
Clonazepam	100	4.8	4.93	102.80	99.89
	120	5.6	5.53	98.70	

Table 6. LOD and LOQ for Paroxetine and Clonazepam.

Drug	LOD (µg/ml)	LOQ (µg/ml)
Paroxetine	2.34	7.10
Clonazepam	0.03	0.09

Table 7(a).Resultsof Precision.

S.No	Sample Weight	Sample Area-1	Sample Area-2	% Assay
1	434.00	4583.034	629.697	99
2	434.00	4584.532	627.874	99
3	434.00	4598.577	632.997	99
4	434.00	4570.390	627.37	99
5	434.00	4546.120	629.795	99
6	434.00	4530.777	628.228	99
Average Assay:				99
STD				0.12
%RSD				0.13

n- Number of replicate injections.

Table 7(b). Results of precision.

Drug	Conc. (µg/ml)	Peak area (n=6)	% RSD
Paroxetine	100	4568.905	0.56
Clonazepam	4	629.327	0.33

Table 8: Results of Robustness study

S.no	Parameter	Condition	Theoretical levels		Tailing factor		Retention time	
			PARA	CNZ	paroxetine	clonazepam	PARA	CNZ
1.	Flow rate1	0.8 ml/min	2335	3212	1.529	0.982	3.253	5.927
	Flow rate2	1.0ml/min	2025	3115	1.432	1.134	2.413	5.054
	Flow rate3	1.2 ml/min	2425	3215	1.438	1.540	2.157	4.765
2.	Wave length	222 nM	2334	3214	1.441	1.038	2.400	4.753
		224 nM	2025	3213	1.440	1.031	2.413	5.041
		226 nM	2435	3222	1.545	1.137	2.350	4.743

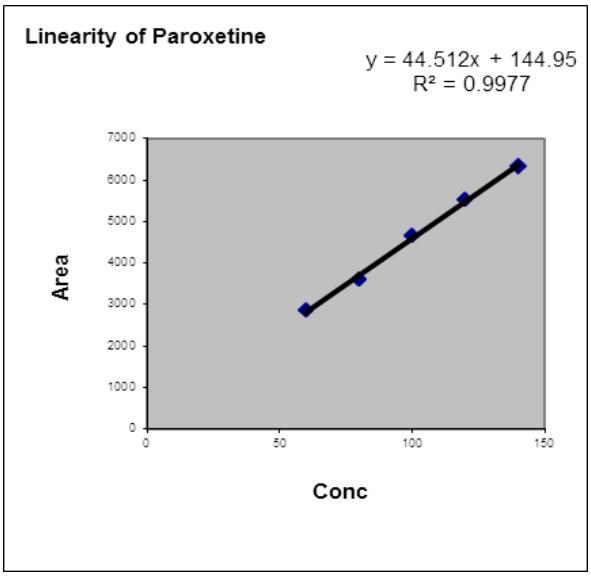

Fig: 4: Linearity graph of Paroxetine.

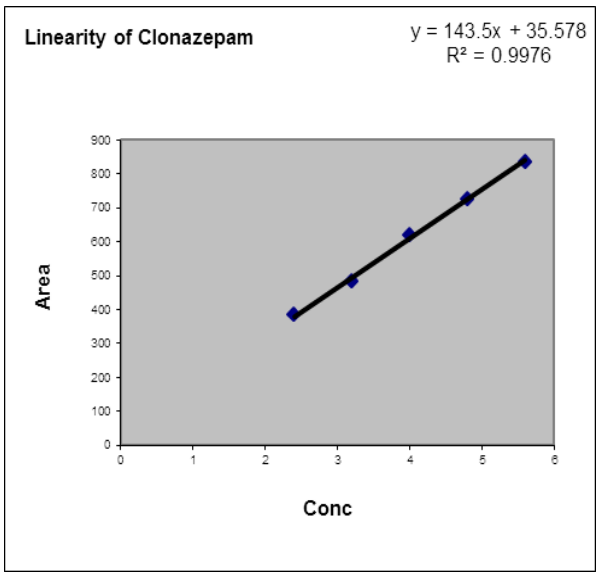

Fig 5: Linearity graph of Clonazepzm.

CONCLUSION

The proposed RP-HPLC method was used for the simultaneous estimation of paroxetine and clonazepam was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials, in vitro dissolution study of combinational dosage formulations containing paroxetine and clonazepam.

REFERENCES

1. Hapse SA, Wagh VS, Kadaskar PT, Dokhe MD and Shirsath AS. Spectrophotometric estimation and validation of hydrochlorothiazide in tablet dosage forms by using different solvents. *Der. Pharma. Chemica*, 2012; 4(1): 10-14.
2. Abd El Kawy M, El Gindy AE, Hegazy M, Shokry ES. Novel Spectrophotometric Method for Simultaneous Determination of Two Binary Mixtures Containing Hydrochlorothiazide by Ratio Subtraction. *Journal of Applied Sciences Research*, 2010; 6(8): 918-926.
3. Alagar Raja M and Selvakumar D. Method development and validation of hydrochlorothiazide in tablet dosage form by UV spectroscopy. *International Journal of Research in Pharmaceutical Sciences*, 2010; 1(3): 369-371.
4. Mashru RC, Sutariya VB, Thakkera J. High performance liquid chromatographic method for simultaneous determination of fosinopril sodium and hydrochlorothiazide in tablets formulation. *Ars.pharmaceutica*, 2006; 47: 375-383.
5. Rajashree M, Sutariya VB, Thakkera AJ. Spectrophotometric analysis of fosinopril sodium in pure form and tablets. *Indian Journal of Pharmaceutical Sciences*, 2006; 68(5): 643-645.
6. Erk N. Simultaneous determination of fosinopril and hydrochlorothiazide in pharmaceutical formulations by spectrophotometric methods. *Journal of pharmaceutical and biomedical analysis*, 2002; 27(6): 901-912.
7. Sibel A. Ozkan, CemalAkay, Şemsettin Cevheroglu, Zuhre Şenturk, Rapid and Accurate Simultaneous Determination of Fosinopril Sodium and Hydrochlorothiazide in Tablets by HPLC. *Journal of Liquid Chromatography & Related Technologies*, 2001; 24(7): 983-991.
8. Serap S, Olcay S, Sedef A, Lale E. Simultaneous determination of Fosinopril and Hydrochlorothiazide in tablets by derivative spectrophotometric and high-performance liquid Chromatographic methods. *Analytica Chimica Acta*, 2001; 427(2): 253-257.
9. Indian Pharmacopoeia. Government of India, Ministry of health and welfare published by Indian Pharmacopoeia Commission, 2007; 2: 576, 578.
10. United States of Pharmacopoeia, United States Pharmacopoeia Convention, Rockville, 2009; 2231.
11. Snyder R, Kirkland J, Glajch L. Practical HPLC method development, II ed, A Wiley International

publication, 1997; 235,266-268,351-353.653-600.686-695.
12. Skoog DA, Holler J, Nieman TA. Principle of Instrumental Analysis, 5th ed., 778-787.
13. Sharma BK. Instrumental Methods of Chemical Analysis, GOEL Publication House, Meerut, 133-161, 68-80, 114-165, 286-320.
14. ICH guidelines, validation of analytical procedure: methodology Q2B; I.C.H. Hormonized Tripartite Guidelines, 1996.
15. Anonymous 1. Drug profile of Paroxetine and Clonazepam [Online] Available at :http://www.rxlist.com/monopril-drug.htm.
16. Anonymous 2. http:// www. Drugs.com/ Paroxetine and Clonazepam.