Research Article www.ijcps.com

International Journal of
Chemical and Pharmaceutical Sciences
2014, Dec., Vol. 5 (4)

ISSN: 0976-9390
IJCPS

Development and validation of a rapid UPLC Assay method for the simultaneous estimation of paroxetine and clonazepam in tablet dosage form

Umadurai M* and Vijaya Nagarajan.

Department of Pharmaceutical Analysis, C.L. Baid Metha College of Pharmacy, Chennai, Tamilnadu, India.

***Corresponding Author:** E-Mail: umaduraimd@gmail.com

Received: 02 Dec 2014, Revised and Accepted: 06 Dec 2014

ABSTRACT

A simple, precise, rapid, specific and accurate reverse phase Ultra performance liquid chromatography (UPLC) method was developed for simultaneous estimation of Paroxetine and Clonazepam in pharmaceutical dosage form. Chromatographic separation was performed on Thermo fischer scientific Hypercel C18 column (50x 2.1mm, 1.8µm) column with mobile phase comprising of mixture of Acetonitrile: Methanol: Potassium di hydrogen orthophosphate buffer (8:52:40)buffer (pH 3, adjusted with Ortho phosphoric acid) at the flow rate 0.5 ml/min. The detection was carried out at 265 nm. The retention times of paroxetine and clonazepam were found to be 1.28 and 2.45 mins respectively with a run time of 4 mins, theoretical levels for paroxetine and clonazepam were 4144and 5067 respectively, with a resolution of 8.09. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness.

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

1. INTRODUCTION

Paroxetine hydrochloride (PAR) Chemically, (3S, 4R)-3-[(2H-1,3 benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl) piperidine hydrochloride hemihydrate (Figure 1) belong to a class of antidepressant agents known as selective serotonin-reuptake inhibitor (SSRIs). It is used to treat major depressive disorder (MDD). Paroxetine likely inhibits the Reuptake of serotonin at the neuronal membrane, enhances serotonergic neurotransmission by reducing turnover of the neurotransmitter, therefore it prolongs its activity at synaptic receptor sites and Potentiates 5-HT in the CNS [1].

Clonazepam (CLP) chemically, 5-(2-chlorophenyl)-7-nitro-2, 3-dihydro1H-1,4-benzodiazepin-2-one (Fig 2) belongs to the drug class benzodiazepines. It is prescribed for the treatment of anxiety and seizure disorders. Mechanism of action involves allosteric interactions between central benzodiazepine receptors and gamma amino butyric acid (GABA) receptors potentiate the effects of GABA.As GABA is an inhibitory neurotransmitter, this results in increased inhibition of the ascending reticular activating system [2].

Literature survey states that Paroxetine hydrochloride and Clonazepam [3] are official in IP [4], BP [5] and USP [6] with HPLC methods for their assay individually .Combined dosage form of Paroxetine and clonazepam is not official in any monographs. Numerous UV[7], spectrophotometric [8], HPLC [9-21], GCMS [22], HPTLC [23-24] based methods have been reported for estimation of these drugs alone or in combination with other drugs in pharmaceutical dosage forms and biological fluids.

The combination of these two drugs is not official in any pharmacopoeia; Only one RP-HPLC method was reported for the estimation of Paroxetine hydrochloride and Clonazepam combination. But it has the excessive run time. So the present study was designed to develop a simple, precise, and rapid analytical RP-UPLC method, which can be used for the analysis of assay method for simultaneous estimation of paroxetine and clonazepam. The developed method was validated in accordance with ICH [25] guidelines.

2. Experimental

2.1. Materials

Working standards of Paroxetine and Clonazepam were donated by Sun Pharma (Sikkim, India). The pharmaceutical Panazep® tablet containing Paroxetine 12.5 mg and Clonazepam 0.5mg were procured from local market.

The reagents, potassium dihydrogen ortho phosphate and Phosphoric acid were of analytical-reagent grade supplied by M/S SD Fine chemicals (Mumbai, India).

The solvents, acetonitrile (MeCN) and methanol (MeOH) were of HPLC grade supplied by M/S SD Fine chemicals (Mumbai, India). HPLC grade water was obtained following distillation in glass and passage through a Milli-Q® Academic system (Millipore, Bangalore, India) and was used to prepare all solutions.

2.1. Instrumentation

An Ultra High performance liquid chromatography PDA detector and data-handling system Chrom Quest and all pH measurements were performed on a pH meter (Metrohm, model 654 Herisau)

2.2. Chromatographic condition

The mobile phase consisted of a mixed and degassed solution containing mixture of Acetonitrile: Methanol: Potassium di hydrogen orthophosphate buffer(8:52:40)buffer (pH 3, adjusted with Ortho phosphoric acid) at the flow rate 0.5 ml/min. The peak separations were achieved on Thermo fischer scientific Hypercel C18 column (50x 2.1mm, 1.8μm) column. Quantification was achieved at 265 with PDA detection.

2.3. Preparation of solutions

2.3.1. Preparation of Phosphate Buffer Solution

272.19 mg of Potassium di hydrogen orthophosphate (2 mM) was dissolved in sufficient water (HPLC grade) with aid of sonicator and the volume was made up to 1000ml with water. Finally pH was adjusted to 3 with ortho phosphoric acid.

2.3.2. Preparation of mobile phase

Acetonitrile, Methanol and Buffer were mixed in the ratio of 8:52:40 and sonicated for 20minutes, Filtered with 0.45 μ membrane filter.

2.3.3. Preparation of working Standard solutions

50 mg of Paroxetine and 10 mg of Clonazepam were weighed accurately, transferred in to a 100 volumetric flask separately and sufficient mobile phase was added to dissolve it. Then the solution was sonicated for 10 minutes. Then made the volume up to the mark with mobile phase. 2 mL was pipetted out from the clonazepam stock solution and transferred in to a 10 mL volumetric flask, diluted up to the mark with mobile phase. Then the standard solution with the concentration of 500μg/mL of paraxetine and 20μg/mL of Clonazepam. Resulting solution was then filtered with 0.45 μ membrane filter.

2.3.4. Preparation of Sample solutions

Twenty tablets were accurately weighed and finely powdered. A quantity of powder weight equivalent to 50mg of paoxetine and 2 mg of clonazepam were weighed and transferred to a 100 mL volumetric flask and sufficient mobile phase was added to dissolve it. Then the solution was sonicated for 10 min. Final volume was adjusted with the mobile phase and filtered with 0.45 μ membrane filter. Then the sample solution with the concentration of 500μg/mL of paraxetine and 20μg/mL of Clonazepam. Resulting solution was then filtered with 0.45 μ membrane filter.

2.3.5. Validation

The developed method was validated according to ICH guidelines. The method was validated in terms of specificity, system suitability, linearity, precision, accuracy, robustness, LOD and LOQ.

3. RESULTS AND DISCUSSIONS

3.1. Specificity

Specificity of the method was established by injecting the blank and placebo (synthetic mixtures). No interferrence was observed between the placebo and blank with principal peaks and hence the method was specific for these two drugs.

3.2. System suitability

System performance was determined by system suitability parameters such as retention time, theoretical plates, asymmetric factor and resolution were calculated and percentage RSD was found to be less than 2 % indicating good performance of the system.

3.3. Linearity

Linearity of the method was established by analysis of mixed standard solution containing 250-750 μg/ml for Paroxetine and 10-30μg/ml for Clonazepam. The calibration curves drawn by plotting the response versus concentration were found to be linear and their coefficients of correlations (R^2) values are 0.9993 and 0.9997 for paroxetine and Clonazepam respectively. The calibration graph was presented in figure 1 and 2 respectively.

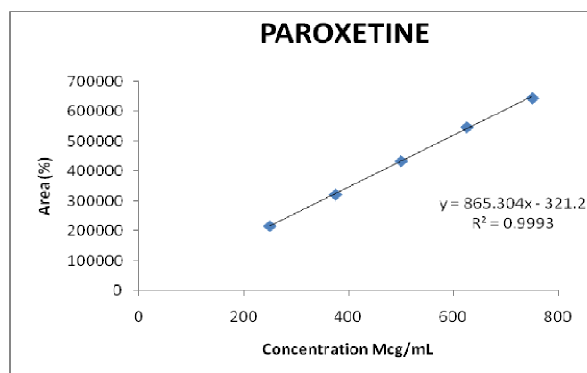

Figure - 1: Calibration graph of Paroxetine.

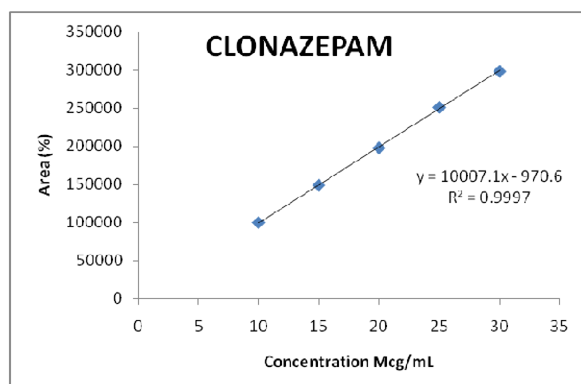

Figure - 2: Calibration graph of Clonazepam.

3.4. Accuracy

Accuracy of the method was checked by recovery studies at the level of 50%, 100% and 150% of known amount of Paroxetine and clonazepam were added to the placebo from the label claim. Paroxetine and Clonazepam recovered in all the levels were found to be close to 100%, indicates that the accuracy of the proposed method. The results are presented in table 1.

3.5. Precision

Precision study was established by injecting the sample solution (multiple sampling of the same homogeneous sample) without changing the assay procedure and the results were presented in table 2. The low % RSD (< 2 %) for Paroxetine and Clonazepam indicated that the method is precise.

3.6. Ruggedness

A study was conducted to determine the effect of variation in analyst to analyst, lab to lab and instrument to instrument in triplicate measurement as per the assay method. Percentage RSD was calculated for each condition and results are presented in table 3.

3.7. Robustness study

The degree of reproducibility obtained as a result of small deliberate variations in the method parameters has proven that the method is robust and the data are summarized in table 4.

Table - 1: Evaluation of accuracy

Conc	Added amount		Amount recovered		Percentage recovered (%)	
	PAR	CLO	PAR	CLO	PAR	CLO
50	25	5	25.24	4.94	100.98	98.92
100	50	10	49.78	9.92	99.56	99.21
150	75	15	75.35	14.83	100.37	98.89
Mean % Recovery					100.30	99.00
% RSD					0.57	0.14

Table - 2: Evaluation of precision

Parameter	Sampling interval	PAR			CLO		
		Amount Present (mg)	Amount Present (%)	% RSD	Amount Present (mg)	Amount Present (%)	% RSD
Within-day	0 hrs	12.43	99.46	0.48	0.496	99.28	0.13
	8 hrs	12.44	99.57	0.72	0.496	99.35	0.14
	16 hrs	12.44	99.54	0.47	0.495	99.06	0.32
Between day	1st day	12.41	99.30	0.42	0.49	99.10	0.45
	2nd day	12.39	99.16	0.18	0.495	99.11	0.35
	3rd day	12.49	99.93	0.53	0.494	98.99	0.11

Table - 3: Evaluation of Ruggedness						
Parameter	PAR			CLO		
	(gm)	(%)	%RSD	(gm)	(%)/	%RSD
Analyst 1	12.44	99.52	0.61	0.495	99.10	0.62
Analyst 2	12.46	99.75	0.51	0.495	99.10	0.20
Instrument 1	12.49	99.50	0.40	0.495	99.00	0.53
Instrument 2	12.50	100.07	0.45	0.495	99.03	0.16
Lab1	12.43	99.47	0.50	0.496	99.34	0.13
Lab 2	12.44	99.53	0.11	0.496	99.35	0.16

Table - 4: Evaluation of Robustness study							
Parameter	Sampling interval	PAR			CLO		
		Amount Present (mg)	Amount Present (%)	% RSD	Amount Present (mg)	Amount Present (%)	% RSD
Wavelength	-1nm	12.44	99.52	0.08	0.497	99.43	0.073
	+1nm	12.44	99.54	0.06	0.497	99.48	0.02
Mobile Phase	-2%	12.43	99.50	0.37	0.494	98.98	0.085
	+2%	12.46	99.74	0.44	0.497	99.40	0.14
Flow rate	-0.1ml	12.43	99.45	0.16	0.495	99.12	0.089
	+0.1ml	12.45	99.62	0.03	0.497	99.49	0.0144
pH	-0.05	12.43	99.50	0.034	0.495	99.16	0.20
	+ 0.05	12.43	99.48	0.009	0.496	99.33	0.146

Table - 5: Evaluation of Solution stability				
DAY	PAR		CLO	
	% Assay for test preparation solution at room temperature			
	Amount present (mg)	(%)	Amount present (mg)	(%)
Initial	12.404	99.23	0.4971	100.26
1	12.506	100.05	0.4978	99.56
2	12.405	99.24	0.4971	100.28
3	12.502	99.87	0.4969	99.71

3.8. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on the standard deviation of the response (50% concentration solution) and the slope of calibration graph. LOD and LOQ were found to be 138.36 ng/ml and 419.29ng/ml for paroxetine and 74.81ng/ml and 226.72ng/ml for clonazepam.

3.9. Stability

Standard and sample Solutions stability were checked up to 3 days at room temperature and the responses was measured on one time at each day. Results revealed that, there was no degradation of paroxetine and clonazepam during this period. The results are presented in table 5.

3.10. Method application to the marketed dosage form

Assay was performed on marketed dosage form as per the above described procedure. Six replicate injections of sample solutions were given in to HPLC system without changing the proposed method conditions and the amount paroxetine and clonazepam present in each tablet was calculated and the results are presented in table 6.

Table - 6: Assay results for commercial formulation

PAR		CLO	
Amount present (mg)	**Percentage (%)**	**Amount present (mg)**	**Percentage (%)**
12.47	99.76	0.501	100.00
12.52	100.00	0.492	98.40
12.44	99.52	0.489	97.80
12.43	99.44	0.489	97.80
12.49	99.92	0.494	98.80
12.54	100.32	0.499	99.80
SD	0.2972	SD	0.8749
% RSD	0.29	RSD	0.88

4. CONCLUSION

Thus, to summarize, the proposed UPLC method of analysis was found to be accurate and precise, as depicted by the statistical data of analysis. The developed method is non tedious, with a very simple phase composition extremely small flow rate and relatively short run time. All these factors enable rapid quantification and simultaneous analysis of two drugs in bulk and pharmaceutical formulation without any excipient interference. It can therefore be concluded that the reported method could find practical application as an economical and rapid quality control tool for simultaneous analysis of the cited drugs from their combined dosage forms in both research and industrial quality control laboratories

5. REFERENCES

1. MedicineNet is an online, healthcare media publishing company. http://www.medicinenet.com/paroxetine/article.htm.
2. Drugs.com is the comprehensive and up-to-date source of drug information online. http://www.drugs.com/clonazepam.html.
3. The DrugBank database is a bioinformatics and cheminformatics resource that combines detailed drug data with comprehensive drug target information. http://www.drugbank.ca/drugs.
4. The Indian Pharmacopoeia. Government of India, Delhi, 2014; 2: 1435 and 2439.
5. British pharmacopoeia. Stationary office, **London Medicines and Health care products regulatory agency,** 2014; (2&3): 506-508 and 587-588.
6. **The United states Pharmacopoeia,** USP 36, NF 31, 5: 3052 &4695.
7. Vinay Patel B, Jayant Dave B, Falguni Patel M and Chhaganbhai Patel N. UV Spectrophotometric method for Identification and Estimation of Clonazepam in Tablet dosage form. **International Journal of Pharmaceutical Research and bioscience,** 2012; 1: 62-70.
8. Kakde RB and Satone DD. Spectrophotometric Method for Simultaneous Estimation of Escitalopram Oxalate and Clonazepam in Tablet Dosage Form. **Indian Journal Pharm Sci,** 2009; 71: 702–705.
9. Christopher J and James TS. Analysis of Clonazepam in a tablet dosage form using small bore HPLC. **Journal of Pharmaceutical and Biomedical Analysis,** 1998; 18: 453–460.
10. Meghana D, Lahari K, Shantha Kumari K and Prakash K. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Clonazepam and Propranolol Hydrochloride in Bulk and Pharmaceutical Dosage Forms. **Inventi Rapid: Pharm Analysis & Quality Assurance,** 2012; 2: 1-4.
11. Gandhi SV, Dhavale ND, Jadhav YV and Sabnis SS. Spectrophotometric and reversed-phase high-performance liquid chromatographic methods for simultaneous determination of Escitalopram and Clonazepam in combined tablet dosage form. **Journal of AOAC International,** 2008; 91: 33-38.
12. Chusena Narasimharaju B, Devala Rao G and Usha P. Development and validation of an RP-HPLC method for the simultaneous determination of Escitalopram Oxalate and Clonazepam in bulk and its pharmaceutical formulations. **International Current Pharmaceutical Journal,** 2012; 1: 193-198.
13. Sai T, Annapurneswari K, Shilpa S, Bala Truipura Sundari C and Jayathirtha rao V. Development and validation of a RP-HPLC method for simultaneous estimation of Propranolol HCl and Clonazepam in bulk and pharmaceutical dosage forms. 2012; 218-222.
14. Chakole RD, Charde MS, Bhavsar N and Marathe RP. Simultaneous Estimation of Escitalopram and Clonazepam by RPHPLC in pharmaceutical formulations. **International Journal of Phytopharmacy,** 2012; 2: 25-29.
15. Isabelle Forfar B, Fabienne P and Christian J. Development of a rapid RP–HPLC method for the determination of Clonazepam in human

plasma. **Journal of Pharmaceutical and Biomedical Analysis,** 2004; 36: 865-869.

16. Mistsuhrio N, FukavaK, Tadashi S and Yoshihiro K. Highperformance Liquid Chromatographic Assay of Clonazepam in Human Plasma Using a Non-porous Silica Column. **Biol. Pharm. Bull,** 2004; 27: 893-895.
17. Bares IF, Pehourcq F and Jarry C. Development of a rapid RP-HPLC method for the determination of Clonazepam in human plasma and Urine. **Journal of pharma and Biomed Analysis,** 2004; 19(36): 865-869.
18. Sallusto, Benedetta C, Kassapidis, Chryssanthi and Morris Raymond G. High performance Liquid chromatography Determination of Clonazepam in plasma Using Solid-phase Extraction. **Therapeutic Drug Monitoring,** 1994; 16: 174-172.
19. Taylor EH, Sloniewsky D and Gadsden RH. Automated extraction and high performance liquid chromatographic determination Serum Clonazepam. The drug monitoring, 1984; 6: 474-477.
20. Zhang Y, Cheng Yi-Nong and Xing-Zhong, Simultaneous determination of Diazepam and Clonazepam in human serum by solid phase extraction RPHPLC. **Chinese Pharmaceutical Journal,** 2002; 22: 337-339.
21. Lakshmi Surekha M, Kumaraswamy G, Ashwini GL, A Validated RP-HPLC method for the estimation of Paroxetine Hydrochloride in bulk and tablet dosage form. **Journal of Pharmacy Research,** 2012; 5: 1768-1770.
22. Min BH and Garland WA. Determination of Clonazepam and its 7-Amino Metabolite in Plasma and Blood by Gas Chromatography-Chemical Ionization Mass Spectrometry. **Journal of Chromatography,** 1977; 139: 121-133.
23. Dhavale N, Gandhi S, Sabnis S and Bothara K, Simultaneous HPTLC determination of Escitalopram and Clonazepam incombined tablets, **Chromatographia,** 2008; 67: 487-490.
24. Kakade R, Satone D and Bawane N. HPTLC method for simultaneous analysis of Escitalopram and Clonazepam in pharmaceutical preparation. **J. Planar Chromatography,** 2009; 22(6): 417-420.
25. **ICH Harmonised Tripartite Guideline,** Text on validation of analytical Procedures Q2(R1), 2005.