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DEVELOPMENT AND VALIDATION OF UPLC METHOD FOR ESTIMATION OF PAROXETINE AND CLONAZEPAM

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ABSTRACT

Validation is a documented program that provides a high degree of assurance that a facility or operation will consistently produce product meeting a predetermined specification. The present paper illustrates about development and validation of a new, simple, precise and accurate UPLC method for the determination of the paroxetine and clonazepam in bulk and its dosage forms. Mobile phase composition is acetonitrile, methanol and buffer were mixed in the ratio of 10:50:40. Linearity of the method was established by analysis of mixed standard solution containing 250-750 µg/ml for paroxetine and 10-30 µg/ml for clonazepam. The calibration curves drawn by plotting the response versus concentration were found to be linear and their coefficient of correlations (R^2) values are 0.9993 and 0.9997 for paroxetine and clonazepam respectively. The good percentage recovery of the sample clearly indicates. The reproducibility and accuracy of the developed method. Similarly, the RSD value for precision was found to be within acceptable limit. The method can be useful for the day-to-day routine analysis in the quality control departments of bulk and pharmaceutical formulations industries.

Keywords: Paroxetine, clonazepam, benzodiazepine receptors, validation, linearity

INTRODUCTION

Analytical chemistry is defined as the science and art of developing sensitive, reliable and accurate method for determining the composition of materials in terms of elements or compounds which they contain. Pharmaceutical analysts in research and development (R & D) of pharmaceutical industry plays a very comprehensive role in

new drug development and follow up activities to assure that a new drug product meets the established standards, its stability and continued to meet the purported quality throughout its shelf life. Method validation is the process for establishing that performance characteristics of the analytical method are suitable for the intended application. Chromatographic methods need to be validation before first routine use **[1]**.

Paroxetine is a potent and highly selective inhibitor of neuronal serotonin reuptake. It 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. It is freely soluble in methanol and ethanol, sparingly soluble in dichloromethane and slightly soluble in water **[2, 3]**.

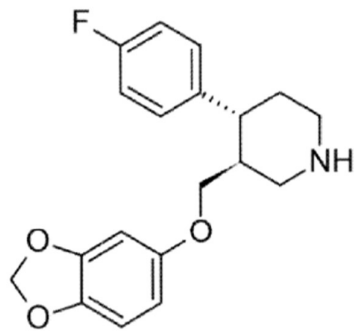

Figure 1: Chemical structure of paroxetine

Clonazepam is a chemically benzodiazepine analogue. 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. Benzodiazepines, in this way, block the cortical and limbic arousal that occurs following stimulation of the reticular pathways. It is practically insoluble in water, slightly soluble in alcohol and methanol and very slightly soluble in ether **[2-4]**.

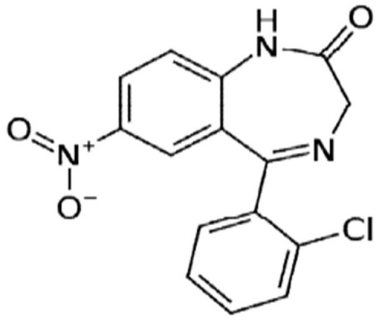

Figure 2: Chemical structure of clonazepam

The aim of the present research work is to develop a novel analytical method for the simultaneous estimation of paroxetine and clonazepam in tablet dosage form by Ultra Performance Liquid Chromatography (UPLC).

MATERIALS AND METHODS

Reagents

Paroxetine and clonazepam working reference standard procured from Sun Pharma (Sikkim, India). Potassium dihydrogen orthophosphate, ortho phosphoric acid, HPLC grade acetonitrile,

methanol, water are used as laboratory grade.

Instrumentation

Table 1: Instruments used

Instrument/Equipment's used	Makers
UV-Visible spectrometer	Shimadzu UV-2550
UPLC	Thermo Scientific
Sonicator	Remi Instrument Ltd
Analytical balance	Metler Toledo-AG204
Detector	Photodiode Array Detector
pH Meter	Micropro Grademate-DI707
Analytical column	Thermo Fischer scientific Hypercel C_{18} column (50 x 2.1mm, 1.8µm).

Method development

Selection of wavelength: The sensitivity of the UPLC method that uses PDA detection depends upon the proper selection of the wavelength. An ideal wavelength is one that gives good response for the drugs to be detected. In an entire UV visible region both the drugs were strongly absorbed at 265 nm. Hence, this wavelength was selected for further studies **[4, 5]**.

Mobile phase: Acetonitrile, methanol and buffer were mixed in the ratio of 10:50:40 and sonicated for 20 min, filtered with 0.45 µ membrane filter.

Diluent: Mobile phase **[6, 7]**.

Standard solution (Solution A): Weighed accurately 50 mg paroxetine working reference standard and transferred carefully in to a100ml volumetric flask sufficient mobile phase and sonicated for 10 min, cooled to room temperature and diluted makeup with 100ml volumetric flask. **(Solution B):** Weighed accurately 10 mg clonazepam working reference standard and transferred carefully in to a100ml volumetric flask sufficient mobile phase and sonicated for 10 min, cooled to room temperature and diluted makeup with 100ml volumetric flask.

Mixture standard solution: 2 ml was pipetted out from the stock solution (B) and transferred in to a 100 mL volumetric flask, (solution A) diluted up to the mark with mobile phase. Then the standard solution with the concentration of 500 µg/mL of paroxetine and 20µg/mL of clonazepam. Resulting solution was then filtered with 0.45 µ membrane filter.

Preparation of sample solutions: Twenty tablets were accurately weighed and finely powdered. A quantity of powder weight equivalent to 50 mg of paroxetine 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 500 µg/mL of paroxetine and 20 µg/mL of clonazepam.

0.45 µ membrane filter **[8-11]**. The assay % is calculated using the following formula:

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

RESULTS AND DISCUSSION

Several mobile phase compositions were tried to resolve the peaks of paroxetine and clonazepam. The optimum mobile phase containing Acetonitrile, methanol and buffer were mixed in the ratio of 10:50:40 was selected **(Table 2-4)**.

In an entire UV visible region both the drugs were strongly absorbed at 265 nm. Hence, this wavelength was selected for further studies is 265 nm **(Figure 3)**.

Robustness

Robustness was performed by analysing the samples at different wavelengths and varying flow rates. The results were shown in below **Tables 5-12**.

Accuracy

Accuracy results of paroxetine and clonazepam are depicted in below **Tables 13-24**. The good percentage recovery of the sample clearly indicates, the reproducibility and accuracy of the developed method. Similarly, the RSD value for precision was found to be within acceptable limit.

Table 2: Assay results for commercial formulation

Paroxetine		Clonazepam	
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**

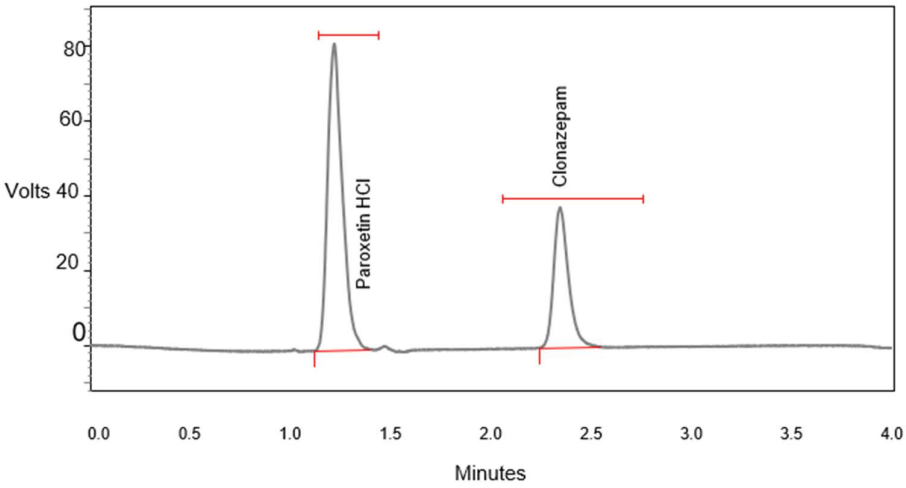

Figure 3: Assay of commercial formulation

Table 3: Evaluation of stability studies of paroxetine

Sample area	Standard area	Avg. wt.	Amount present	% Amount present
432157	433294	262.9	12.40486295	99.2389
435712	433294	262.9	12.50690755	100.0553
432178	433294	262.9	12.40546575	99.24373

Table 4: Evaluation of stability studies of clonazepam

Sample area	Std. area	Avg. wt.	Amount present	% Amount present
199876	199268	262.9	0.497513	99.50268
199873	199268	262.9	0.497506	99.50118
199876	199268	262.9	0.497513	99.50268

Table 5: Robustness (by changing the wavelength + 1 nm of paroxetine)

Sample area	Std area	Avg. wt.	Amount present	% Amount present
433625	433294	262.9	12.4470012	99.57601
433158	433294	262.9	12.43359618	99.46877
433691	433294	262.9	12.4488957	99.59117
		Avg.	12.44316436	99.54531
		SD	0.00834025	0.066722
		% RSD	0.067026765	0.067027

Table 6: Robustness (by changing the wavelength + 1 nm of clonazepam)

Sample area	Std area	Avg. wt.	Amount present	% Amount present
199865	199268	262.9	0.497486	99.4972
199766	199268	262.9	0.49724	99.44792
199872	199268	262.9	0.497503	99.50068
		Avg.	0.49741	99.48193
		SD	0.000148	0.029512
		% RSD	0.029665	0.029665

Table 7: Robustness (by changing the flow rate + 10µl of paroxetine)

Sample area	Std. area	Avg. wt.	Amount present	% Amount present
433871	433294	262.9	12.45406251	99.6325
433697	433294	262.9	12.44906793	99.59254
433965	433294	262.9	12.45676074	99.65409
		Avg.	12.45329706	99.62638
		SD	0.003903112	0.031225
		% RSD	0.031341999	0.031342

Table 8: Robustness (by changing the flow rate + 10µl of clonazepam)

Sample area	Std. area	Avg. wt.	Amount present	% Amount present
199876	199268	262.9	0.497513	99.50268
199832	199268	262.9	0.497404	99.48077
199887	199268	262.9	0.497541	99.50815
		Avg.	0.497486	99.4972
		SD	7.24E-05	0.014488
		% RSD	0.014561	0.014561

Table 9: Robustness (by changing pH + 0.05 of paroxetine)

Sample area	Std. area	Avg. wt.	Amount present	% Amount present
433258	433294	262.9	12.43646664	99.49173
433197	433294	262.9	12.43471566	99.47773
433175	433294	262.9	12.43408416	99.47267
		Avg.	12.43508882	99.48071
		SD	0.001234295	0.009874
		% RSD	0.009925902	0.009926

Table 10: Robustness (by changing pH + 0.05 of clonazepam)

Sample area	Std. area	Avg. wt.	Amount present	% Amount present
199365	199268	262.9	0.496241	99.24829
199866	199268	262.9	0.497488	99.4977
199357	199268	262.9	0.496222	99.24431
		Avg.	0.49665	99.3301
		SD	0.000726	0.14516
		% RSD	0.146139	0.146139

Table 11: Robustness (by changing mobile phase + 2% of paroxetine)

Sample area	Std area	Avg. wt.	Amount present	% Amount present
432157	433294	262.9	12.40486295	99.2389
435781	433294	262.9	12.50888816	100.0711
435175	433294	262.9	12.49149322	99.93195
		Avg.	12.46841478	99.74732
		SD	0.055720481	0.445764
		% RSD	0.446893064	0.446893

Table 12: Robustness (by changing mobile phase + 2% of clonazepam)

Sample area	Std. area	Avg. wt.	Amount present	% Amount present
199802	199268	262.9	0.497329	99.46584
199346	199268	262.9	0.496194	99.23883
199863	199268	262.9	0.497481	99.4962
		Avg.	0.497001	99.40029
		SD	0.000703	0.140651
		% RSD	0.141499	0.141499

Table 13: Accuracy of paroxetine 50%

Sample area	Std. area	Added amt	Amt Recovery	% Amt recovery
218362	433294	25	25.07191307	100.2877
219449	433294	25	25.19672036	100.7869
221846	433294	25	25.47193938	101.8878
		Avg.	25.2468576	100.9874
		SD	0.204671858	0.818687
		% RSD	0.810682507	0.810683

Table 14: Accuracy of paroxetine 100%

Sample area	Std. area	Added amt	Amt Recovery	% Amt recovery
433621	433294	50	49.78754552	99.57509
433527	433294	50	49.77675262	99.55351
433612	433294	50	49.78651216	99.57302
		Avg.	49.78360343	99.56721
		SD	0.005955435	0.011911
		% RSD	0.011962643	0.011963

Table 15: Accuracy of clonazepam 50%

Sample area	Std area	Added amt	Amt Recovery	% Amt recovery
99612	199268	5	4.958905	99.1781
99214	199268	5	4.939091	98.78183
99254	199268	5	4.941083	98.82166
		Avg.	4.94636	98.92719
		SD	0.01091	0.218198
		% RSD	0.220565	0.220565

Table 16: Accuracy of clonazepam 100%

Sample area	Std area	Added amt	Amt Recovery	% Amt recovery
199214	199268	10	9.917312	99.17312
199351	199268	10	9.924132	99.24132
199345	199268	10	9.923833	99.23833
		Avg.	9.921759	99.21759
		SD	0.003854	0.038543
		%RSD	0.038847	0.038847

Table 17: Evaluation of precision study (paroxetine 0 h)

Sample area	Std area	Avg wt.	Amt present	% Amt present
431257	433294	262.9	12.37902888	99.03223
435784	433294	262.9	12.50897428	100.0718
432175	433294	262.9	12.40537963	99.24304
435784	433294	262.9	12.50897428	100.0718
431278	433294	262.9	12.37963167	99.03705
432587	433294	262.9	12.4172059	99.33765
		Avg.	12.4331991	99.46559
		SD	0.060526594	0.484213
		% RSD	0.486814319	0.486814

Table 18: Evaluation of precision study (paroxetine 8 h)

Sample area	Std area	Avg wt.	Amt present	% Amt present
431257	433294	262.9	12.37902888	99.03223
432157	433294	262.9	12.40486295	99.2389
439815	433294	262.9	12.62468223	100.9975
433756	433294	262.9	12.45076149	99.60609
432150	433294	262.9	12.40466202	99.2373
432517	433294	262.9	12.41519658	99.32157
		Avg.	12.44653236	99.57226
		SD	0.090314316	0.722515
		% RSD	0.725618298	0.725618

Table 19: Evaluation of precision study (clonazepam 0 h)

Sample area	Std area	Avg. wt.	Amt present	% Amt present
199872	199268	262.9	0.497503	99.50068
199147	199268	262.9	0.495699	99.13976
199247	199268	262.9	0.495948	99.18955
199254	199268	262.9	0.495965	99.19303
199578	199268	262.9	0.496772	99.35432
199587	199268	262.9	0.496794	99.35881
		Avg.	0.496447	99.28936
		SD	0.00069	0.138074
		% RSD	0.139062	0.139062

Table 20: Evaluation of precision study (clonazepam 8 h)

Sample area	Std area	Avg. wt.	Amt present	% Amt present
199874	199268	262.9	0.497508	99.50168
199324	199268	262.9	0.496139	99.22788
199265	199268	262.9	0.495993	99.19851
199872	199268	262.9	0.497503	99.50068
199771	199268	262.9	0.497252	99.4504
199358	199268	262.9	0.496224	99.2448
		Avg.	0.49677	99.35399
		SD	0.000723	0.144657
		% RSD	0.145598	0.145598

Table 21: Evaluation of precision study paroxetine Lab 1

Sample area	Std area	Avg. wt.	Amt present	% Amt present
430217	433294	262.9	12.34917617	98.79341
435879	433294	262.9	12.51170121	100.0936
435712	433294	262.9	12.50690755	100.0553
432570	433294	262.9	12.41671792	99.33374
432470	433294	262.9	12.41384747	99.31078
432201	433294	262.9	12.40612595	99.24901
		Avg.	12.43407938	99.47264
		SD	0.063292639	0.506341
		% RSD	0.509025535	0.509026

Table 22: Evaluation of precision study clonazepam Lab 1

sample area	Std area	Avg. wt.	Amt present	% Amt present
199870	199268	262.9	0.497498	99.49969
199400	199268	262.9	0.496329	99.26571
199330	199268	262.9	0.496154	99.23086
199647	199268	262.9	0.496943	99.38867
199876	199268	262.9	0.497513	99.50268
199235	199268	262.9	0.495918	99.18357
		Avg.	0.496726	99.3452
		SD	0.000693	0.138614
		% RSD	0.139528	0.139528

Table 23: Evaluation of precision study paroxetine Lab 2

Sample area	Std area	Avg. wt.	Amt present	% Amt present
433628	433294	262.9	12.44708731	99.5767
433256	433294	262.9	12.43640923	99.49127
433158	433294	262.9	12.43359618	99.46877
433621	433294	262.9	12.44688638	99.57509
433281	433294	262.9	12.43712684	99.49701
433694	433294	262.9	12.44898181	99.59185
		Avg.	12.44168129	99.53345
		SD	0.006686139	0.053489
		% RSD	0.053739831	0.05374

Table 24: Evaluation of precision study clonazepam Lab 2

Sample area	Std area	Avg. wt.	Amt present	% Amt present
199025	199268	262.9	0.495395	99.07903
199878	199268	262.9	0.497518	99.50367
199644	199268	262.9	0.496936	99.38718
199847	199268	262.9	0.497441	99.48824
199735	199268	262.9	0.497162	99.43248
199358	199268	262.9	0.496224	99.2448
		Avg.	0.49678	99.3559
		SD	0.000822	0.164434
		% RSD	0.1655	0.1655

Linearity

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

CONCLUSION

The main objective of the present work is to develop a novel, simple UPLC method for simultaneous estimation of paroxetine and clonazepam combined dosage form. Linearity of the method was established by analysis of mixed standard solution containing 250-750 µg/ml for paroxetine and 10-30 µg/ml for clonazepam. The calibration curves drawn by plotting the response versus concentration were found to be linear and their coefficient of correlations (R^2) values are 0.9993 and 0.9997 for paroxetine and clonazepam respectively. The good percentage recovery of the sample clearly indicates, the reproducibility and accuracy of the developed method. Similarly, the RSD value for precision was found to be within acceptable limit. 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.

Conflicts of interest

None.

REFERENCES

[1] Erk N, Biryol I. Voltametric and HPLC techniques for the determination of paroxetine Hydrochloride. Pharmazie. 2003; 58:699–704.

[2] Aziz Unnisa, Santosh Kumar S, Yogesh Babu A, Siva Chaitanya K, Mrudula B. Development and validation of RP-HPLC-PDA method for the simultaneous estimation of clonazepam and paroxetine hydrochloride in bulk and tablet dosage forms. Journal of Pharmacy Research. 2014; 8(9):1212-1217.

[3] Bhagyasree T, Neelam I, Ajitha A, Uma Maheshwara Rao. Assay method development and validation for simultaneous estimation of paroxetine and clonazepam by RP-HPLC. International Journal of Pharmaceutical Research & Analysis. 2014; 4(8):421-427.

[4] Geetharam Yanamadala, Praveen Srikumar P. Development and validation of a stability-indicating HPLC method for the simultaneous determination of paroxetine hydrochloride and clonazepam in pharmaceutical dosage forms. Int J Pharm. 2014; 4(1):448-457.

[5] Thaidala Sriveni, Vanamala Naveen, Vemula Sai Rupa, Aeruva Renuka, Sunil Porika, M Akiful Haque, Vasudha Bakshi, Narender Boggula. Development and Validation of Dolutegravir in Bulk and Formulation: An Anti-Retroviral Drug Using UV-Spectroscopy. International Journal of Pharmaceutical Quality Assurance. 2021; 12(1):57-60.

[6] Tijare LK, Rangari NT, Mahajan UN. A review on bioanalytical method development and validation. Asian J Pharm Clin Res. 2016: 9:6-10.

[7] Lakshmi Surekha G, Kumaraswamy Gandla, 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(3):1768-1770.

[8] Bolla, S., Boggula, N. Quantitative determination of Nebivolol Hydrochloride and Rosuvastatin Calcium in laboratory prepared mixture by robust, high-performance liquid chromatographic method. Discov. Chem. 2025; 2:61.

[9] Srinivas Reddy G, Prasad Reddy SLN, Shiva Kumar Reddy L. Development and validation of a stability indicating liquid chromatographic method for the simultaneous estimation of paroxetine and clonazepam in bulk and its pharmaceutical formulations. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(10):397-402.

[10] Gope Edward Raju, Srikanth Pottendla, Suneetha Yaparthi. Novel Ultra-Performance Liquid Chromatography Method for Concurrent Estimation and Pharmacokinetic Analysis of Favipiravir and Molnupiravir in

Rat Plasma. Asian J Pharm Clin Res. 2025; 18(2):90-95.

[11] Sandeep Bolla, Narender Boggula. Stability-indicating liquid chromatographic method for the simultaneous estimation of colchicine and rosuvastatin in combination. Essential Chem. 2025; 2(1):1-11.