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Original Article

Development and Application and Validation of Q – absorbance spectrophotmetric method for Paroxetine hydrochloride and Clonazepam in combined dosage form

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ABSTRACT

A simple, sensitive, accurate and precise Q – Absorbance spectrophotometric method have been developed for simultaneous determination of Paroxetine hydrochloride and Clonazepam in bulk and tablet dosage form. Q – Absorbance ratio method involves Q – Absorbance at isobastic point (239.65 nm) and λ_{max} (271.23 nm) in methanol. Both the drugs obeyed the Beer's law in conc. range 5-30 µg/ml, coefficient correlation (r^2<1). The method was validated statistically and recovery studies were carried out to confirm the accuracy. Commercially tablet formulation was successfully analysed using the developed methods.

Keywords: Clonazepam, Paroxetine hydrochloride, Q – absorbance

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INTRODUCTION

Clonazepam (CLO; 5 –(o – chlorophenyl)- 1,3 dihydro – 7 – nitro – 2 H – 1,4 – benzodizepin with prominent anticonvulsant, anxiolytic properties. It has been most effective in the treatment of typical and atypical absence, myoclonic, akinetic seizures, and infantile spasms. Co- administration of barbiturate may exacerbate the drowsiness caused by clonazepam. Clonazepam (CLZ) 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-minobutyric 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.[1,2]

Paroxetine hydrochloride (PH) [(-)- Trans – 4R – (4'- flurophenyl)-3S-[3',4' – methylenedioxyphenoxy) methyl] Piperidine hydrochloride] is a selective serotonin (5 – hydroxy - tryptamine, 5HT) reuptake inhibitor (SSRI) and potentiates 5 – HT in the CNS. PH is indicated for the treatment of major depressive disorder, social anxiety disorder, obsessive – compulsive disorder, panic disorder, generalized anxiety disorder, and posttraumatic stress disorder.[3,4]

Depression and anxiety disorders are distinct illnesses that often coexist. Patients with combined depression and anxiety are more debilitated than patients with either condition alone. Mixed anxiety depression is gaining recognition as a separate diagnosis has been included in the 10th edition of the International Classification of Diseases. Currently, a fixed dose combination of an antidepressant, such as Paroxetine, and an anti-anxiety drug, such as Clonazepam, is an available option for the treatment of co-morbid depression and anxiety.[5]

According to literature, Clonazepam and Paroxetine are official in IP, USP and BP when used individually[6], but the combination of Clonazepam and Paroxetine is not official in any Pharmacopoeia. Various analytical methods, such as Spectrophotometry , spectrofluorimetry, HPLC[7] and HPTLC[8], have been reported to detect Clonazepam and Paroxetine alone and in combination with other drugs in Pharmaceutical dosage forms. Spectrophotometric methods and the stability indicating HPLC method have been reported for the estimation of Clonazepam and Paroxetine in combined pharmaceutical formulations[9,10]. However development and validation of Paroxetine and Clonazepam in Pharmaceutical formulations by method has not been yet reported.

Hence, this manuscript is the first to describe the development and validation of Vierodt's or simultaneous estimation method as per the ICH guidelines ICH Q2 (R1) for the Q – absorbance ratio methodof Clonazepam and Paroxetine

MATERIALS AND METHODS

Pure drugs of PXT HCl and CLZ were obtained as gift samples from Torrent Pharma Pvt. Ltd., Ahemdabad, Gujarat. A.R. grade methanol for preparing solutions was issued from the college. Commercially available (PARI CR Plus, IPCA Lab.) containing 12.5 mg of PXT HCl and 0.5 mg CLZ per tablet, was randomly selected for the study and were procured from the local market. The solution of 0.1 N HCl was prepared in double distilled water as per IP 1996 procedure. A Shimadzu UV/Vis 1601 double beam spectrophotometer with a fixed slit width (2 mm) and 1 cm matched quartz cells was used for all the spectral measurements.

Preparation of standard stock solution: Standard stock solutions (100 µg/ml) of PXT HCl and CLZ were prepared separately by dissolving 10 mg of PXT HCl and CLZ respectively in 100 ml methanol. Suitable aliquot of standard stock solutions were diluted with 0.1 N HCl to obtain solutions of PXT HCl (12.5 µg/ml) and CLZ (0.5 µg/ml). The resulting solutions were scanned in the range of 200 -400 nm in 1 cm cells against solvent as blank. The UV absorption overlain spectrum of PXT HCl and CLZ is depicted in fig. 1.

From the overlain spectra the wavelengths 239.65 nm (iso- absorptive point) and 271.23 nm (λ_{max} of CLZ) were selected for Simultaneous equation method. Standard stock solutions of PXT HCl and CLZ were diluted with 0.1 N HCl to obtain concentration range of 5 – 30 µg/ml and absorbances were measured at selected wavelengths. The concentration of drug against absorbance was plotted to obtain calibration curves were found to be linear in the concentration range under study. The absorptivity values of PXT HCl at 239.65 nm and 271.23 nm were 9.19 and 10.41 while respective values for CLZ were 334.84 and 679.38.

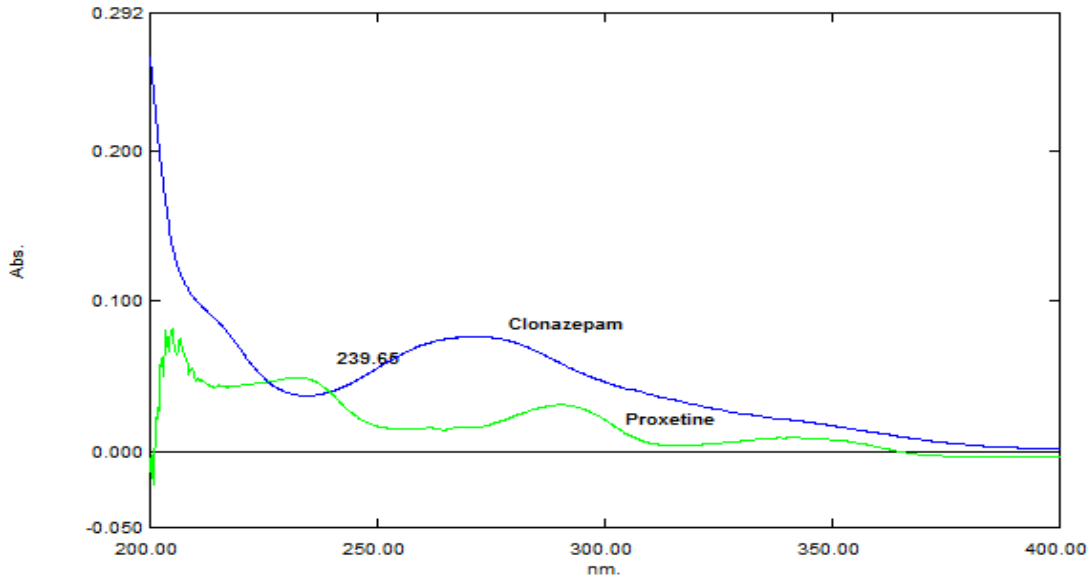

Fig.1 : Overlain spectra of Paroxetine(12.5 ug/ml) and Clonazepam (0.5 ug/ml)

Amount of each drug was estimated by substituting the absorbance and absorptivity values in the following equations:
C_{PXT} = (Qm – Qy)/(Qx – Q y) × A_1/ax_1 and C_{CLZ} = (Qm – Qx)/ (Qx – Qy) ×A_1/ay_1, where A_1 and A_2 are the absorbances of mixtures at 239.65 nm and 271.23 nm. ax_1 is absorptivity value of PXT HCl at 239.65 nm, ax_2 is absorptivity value of PXT HCl at 271.23 nm, ay_1 is absorptivity value of CLZ at 239.65 nm and ay_2 is absorptivity value of CLZ at 271.23 nm and Qm = A_2/A_1, Qy = ay_2/ay_1 and Qx = ax_2/ax_1.

For analysis of both PXT HCl and CLZ in tablets, twenty tablets were accurately weighed and average weight was calculated. Tablets were finely powdered and mixed thoroughly. Quantity of tablet powder equivalent to 12.5 mg of PXT HCl was weighed accurately, dissolved in 100 ml methanol and sonicated for 15 min. the solution was filtered through Whatman filter paper and transferred to volumetric flask. The aliquot portion of filterate was further diluted with 0.1 N HCl to get final concentration of about 12.5 µg/ml of PXT HCl and 0.5 µg/ml of CLZ. Tablet sample solutions prepared were analysed by scanning at respective set of wavelengths and absorbance difference values were noted and concentration of each drug was calculated from respective calibration curve.

Method validation

Method was validated statistically as per ICH/USP 16 guidelines for all the parameters like accuracy, linearity, precision, ruggedness. To study accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80, 100 and 120 % of the test concentration). A known amount of drug was added to pre analysed tablet powder and percentage recovery was calculated. The results of recovery studies were satisfactory and are presented in (Table 1) . Linearity was constructed in the range of 5 – 30 µg/ml. PXT HCl and CLZ in tablets were found to be linear in the range ± 20% of test concentration. Precision was studied by analyzing three replicates of sample solutions and concentrations were calculated. Ruggedness was established by carrying out experiment at different conditions like interday, intraday and by different analyst. There was no interference of the excipients present in the formulation. By observing validation parameters (Table 3) the method described were found to be specific, accurate, precise and economical and can be successfully applied to analyze commercially available tablets containing PXT HCl and CLZ. The results obtained are in good agreement with the labeled content, summarized in Table 4. Due to high sensitivity and simple sample preparation, the methods described can be used for undergraduate studies. Hence, simple and economical methods always have a role in pharmaceutical analysis.

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TABLE 1: STATASTICAL DATA OF CALIBRATION CURVES FOR PXT HCl AND CLZ

Parametrs	For PXT HCl	For CLZ
Wavelength (nm)	239.65 nm	271.23 nm
Beer's Law limit (µg/ml)	5 – 30	5 - 30
Correlation coefficient (r^2<1)	0.997	0.998
Regression equation	Y = 0.026 x + 0.018	Y = 0.016 + 0.016

TABLE 2: RECOVERY STATUS DATA

Level of standard addition (%)	% Recovery ± SD*	
	PXT HCl	CLZ
80	99.95 ± 0.41	98.47 ± 0.53
100	99.23 ± 0.48	98.95 ± 0.63
120	99.93 ± 0.61	99.88 ± 0.47

*****Mean of three estimations, SD is standard deviation

TABLE 3 : SUMMARY OF VALIDATION PARAMETERS

Parameters	PXT HCl	CLZ
Linearity and range	± 20 % of test concentration	± 20 % of test concentration
Beer's Law limit (µg/ml)	5 - 30	5 – 30
Precision ± SD*(n = 3)	99.47 ± 0.86	99.16 ± 1.066
Ruggedness, % Label Claim(n = 3)		
Intraday	99.49	99.28
Interday	100.53	99.87
Different analyst	99.83	99.64

(n = 3) results are mean of three deviations, SD is standard deviation

TABLE 4 : RESULTS OF TABLET FORMULATION ANALYSIS

Drug	% Label Claim*	± SD*
PXT HCl	99.47	± 0.8608
CLZ	99.16	± 1.066

*Results are mean of three deviations, SD is standard deviation

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