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Research article

Stability indicating validated spectrometric method for simultaneous estimation of citalopram & clonazepam in marketed formulation

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ABSTRACT

Simple, sensitive, specific and economic spectrophotometric method was developed and validated for simultaneous quantitation of Citalopram & Clonazepam in tablet dosage form. New method based on the simultaneous estimation of drugs in a binary mixture without previous separation was developed. In simultaneous equation method, Citalopram & Clonazepam were quantified using their absorptivity values of at selected wavelengths, viz., 239 nm and 308 nm respectively. The accuracy and reproducibility of the proposed method was statistically validated by recovery studies. The simultaneous equation method permits simple, rapid and direct determination of Citalopram & Clonazepam in commercially available tablet dosage form without previous separations and can therefore be used for routine analysis of both drugs in quality control laboratories.

Keywords: Citalopram, Clonazepam, Validation Vierodt's method.

INTRODUCTION

Citalopram is a highly selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor (SSRI) with minimal effects on the neuronal reuptake of norepinephrine and dopamine. It is a bicyclic phthalane derivative (racemic) which is not related to the tricyclic or tetracyclic antidepressants. It is used in the treatment of major depressive disorder. Citalopram is primarily used to treat the symptoms of depression but can also be prescribed for social anxiety disorder, panic disorder or obsessive-compulsive disorder. Clonazepam an anticonvulsant used for several types of seizures, including myotonic or atonic seizures, photosensitive epilepsy, and absence seizures, although tolerance may develop. It is seldom effective in generalized tonic-clonic or partial seizures. The mechanism of action appears to involve the enhancement of gamma-aminobutyric acid receptor responses. Several spectroscopic methods are reported for estimation of Citalopram and Clonazepam from pharmaceutical formulations. Human plasma. Developed spectrophotometric method is simple methods of simultaneous analysis of Citalopram and Clonazepam from marketed tablet formulation. The developed method was found to be rapid, accurate, reproducible and economical. This method can be used successfully for quality control testing of the drug from combined tablet dosage form. The nonavailability of UV-Spectrophotometry method until now for the analysis of this component made it worthwhile objective to pursue the present research work. Therefore, in the proposed work, a successful attempt has been made to develop analytical method with due consideration of accuracy, sensitivity, rapidity, economy. Studies confirmed the accuracy of proposed method [1-7].

MATERIAL AND METHOD

Apparatus

A Thermospectronic model is Systronics-108 (Double beam) Spectrophotometer with 1cm. matched quartz cells.

Reagents and chemicals

Citalopram & Clonazepam were obtained from WINDLAS Biotech Ltd., Dehradun (INDIA) and were used as working standards. Methanol of analytical grade and double distilled water were used

throughout the analysis.

Commercial formulation

A commercial pharmaceutical preparation, Clonafit tablet (Citalopram 10mg and Clonazepam 5mg) was procured from the local market.

Preparation of standard stock solution

10 mg of Citalopram was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the diluent Methanol:Water (70:30), to give a stock solution of 1000 ppm of Citalopram and 10 mg of Clonazepam was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the diluent Methanol : Water (70:30) , to give a stock solution of 1000 ppm. Of Clonazepam.

Preparation of working standard solution

From stock solutions of Citalopram 1 ml was taken and diluted up to 10 ml. from this solution 1.0,2.0,3.0,4.0 and 5.0 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 10,20,30,40,50 and 60 µg/ml concentration and From stock solutions of Clonazepam 1 ml was taken and diluted up to 10 ml. from this solution 0.25,0.5,0.75,1.0,1.25, and 1.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 2.5,5,7.5,10,12.5 and 15 µg/ml concentration.

Estimation in the marked formulation

Twenty tablets were weighed and ground to a fine powder. Tablet powder equivalent to 200 mg Citalopram (10 mg Clonazepam) was weighed and transferred to a 100 ml volumetric flask and volume was made up to 50 ml with diluents to obtain concentration of 1000µg/ml. Resultant solution was filtered through Whattmann filter paper. 1 ml of filtrate was taken in 10 ml volumetric flask and volume was made up to 10 ml with diluents to obtain concentration of 100µg/ml. Further 2.5 ml of this solution was taken and diluted up to 10 ml obtain final concentration of 25µg/ml. Absorbance of the sample solutions at 239.0 nm and 308.0 nm was measured and from the absorbance values, the concentration of drugs in the sample solution was determined by using Vierodt's formula. The contents were calculated using the following equations,

$$C_X = (A_2\, ay_1 - A_1\, ay_2)/ (ax_2\, ay_1 - ax_1\, ay_2)$$

$$C_Y = (A_1\, ax_2 - A_2\, ax_1)/ (ax_2\, ay_1 - ax_1\, ay_2)$$

Where C_X and C_Y are the concentrations of Citalopram and Clonazepam respectively, ax_1 and ax_2 are the absorptivity values of Citalopram at 239.0 nm and at 308.0 nm respectively, ay_1 and ay_2 are the absorptivity values Clonazepam at 239.0 nm and at 308.0 nm respectively and A_1 and A_2 are the absorbances of the diluted sample at 239.0nm and at 308.0 nm respectively.

Absorptivity of Citalopram at 239.0 nm

Absorptivity of Citalopram at 308.0 nm

Absorptivity of Clonazepam at 239.0 nm

Absorptivity of Clonazepam at 308.0 nm

Where,

A_1= Absorbance of sample solution at 239.0 nm

A_2= Absorbance of sample solution at 308.0 nm

Cx= Concentration of Citalopram in g/liter in sample solution

Cy= Concentration of Clonazepam in g/liter in sample solution.

After obtaining the recorded absorbances, A_1 and A_2 and substituting the values concentration of each drug can be calculated (Table No. 2).

Table 1: Result of Optical Parameter of Citalopram and Clonazepam

Parameters	Observation of Citalopram	Observation of Clonazepam
λ_{max}	239	308
Beer's law limit (µg/mL)	10-50	2.5 -15
Regression equation	0.029x+0.017	0.042x -0.001
Correlation Coefficient (r^2)	0.999	0.999
Molar Absorptivity (L mol^{-1} cm^{-1})	$1.11*10^4$	$1.04*10^4$
Sandell's Sensitivity µg/mL 0.001 absorbance unit	$3.4*10^{-4}$	$3.3*10^{-4}$

Table 2: Result of Assay of Tablet Formulation

Brand Name	Citalopram		Clonazepam	
	Label Claim (mg)	% Purity	Label Claim (mg)	% Purity*
CLONAFIT	10 mg	97.8%	0.5 mg	97.55%

***Denotes average of five determination**

[a]RSD = Relative standard deviation

[b]n = Number of determination

[c]LOD = Limit of detection

[d]LOQ= Limit of quantitation

Table 3: Summary of validation parameters for Vierordt's method

Parameter		Citalopram	Clonazepam
Linearity range(µg/mL)		10-50	2.5 -15
Correlation coefficient(r^2)		0.999	0.999
Precision(RSD)[a]	Inter day(n=3)	0.046	0.034
	Intraday(n=3)	0.029	0.0015
	Analyst to analyst	0.109	0.510
Accuracy (%)	80	100.6	101.0
	(RSD)[a]	0.13	0.29
	100	100.9	100.3
	(RSD)[a]	0.12	0.13
	120	105.0	102.3
	(RSD)[a]	0.16	0.18
Repeatability(RSD, n[b]=3)		1.039	1.168
LOD[c]		1.2 µg/mL	1 µg/mL
OQ[d]		3.6 µg/mL	3 µg/mL

Precision and accuracy

The precision of the method was evaluated by inter day and intraday variation studies. In intraday studies, working solutions of standard and sample were analysed thrice in a day and percentage relative standard deviation (% RSD) was calculated. In the inter day variation studies, working solution of standard and sample were analysed on three consecutive days and percentage relative standard deviation (% RSD) was calculated. The data is shown in Table no.3.

Stress condition		Time (in hrs.)	Citalopram		Clonazepam	
			% Degradation	Degradation	% Degradation	Degradation
Acidic		2	22%	Yes	16%	Yes
Alkali		2	10%	Yes	29%	Yes
Oxidative		2	28%	Yes	$\lambda_{max\ Change}$	Yes
U.V.light		24	$\lambda_{max\ Change}$	Yes	13%	Yes
Thermal	Weat heat	After 3 hr	$\lambda_{max\ Change}$	Yes	Yes	Yes
	Weat heat	After 6 hr	$\lambda_{max\ Change}$	Yes	Yes	Yes

The accuracy of the method was determined by recovery studies. The recovery studies were performed by the standard addition method at 80%, 100% and 120% level and the percentage recoveries were calculated and are shown in Table no.3.

Limit of detection and limit of quantitation

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in Table no.3.

LOD = 3.3 (σ / S)

Where, S = slope of calibration curve**,** σ = standard deviation of the response .The Limit of Quantification (LOQ) is the smallest concentration **the** analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table no.3.

LOQ = 10 (σ / S)

Where, S = slope of calibration curve**,** σ = standard deviation of the response.

Forced degradation studies

Acidic degradation

5 ml of standard stock solution was transferred in 50 ml volumetric flask and 1ml concentrated HCl was added to it and kept for 2 hours at ambient temperature then diluted to 25 ml with diluent. The solution was finally diluted to 50 ml with diluent and UV spectra were taken.

Alkali degradation

5 ml of standard stock solution was transferred in 50 ml volumetric flask and 1ml 10 M NaOH was added to it and kept for 2 hrs then diluted to 25 ml with diluent. The solution was finally diluted to 50 ml with diluent and UV spectra was taken.

Oxidative degradation

5 ml of standard stock solution was transferred in 50 ml volumetric flask.1ml 30% H_2O_2 was added to it and kept for 2 hours. It was diluted till 25 ml with diluent. The solution was finally diluted to 50 ml with diluents and UV spectra were taken.

Degradation by UV light

5 ml standard stock solution was transferred in 50 ml volumetric flask in UV chamber for 24 hours then diluted up to the mark with diluents to produce 10 µg/ml and UV spectra was taken.

Thermal degradation

Wet heat: 5 ml standard stock solution was transferred to two different 50 ml volumetric flask and kept on water bath for three and six hours respectively at 80^0 C. It was diluted up to the mark with diluents to produce 10 µg/ml and UV spectra was taken.

RESULTS AND DISCUSSION

The overlain spectra of Cita and Clona exhibit λ_{max} of 239 and 308 nm for Cita and Clona respectively which are quite separated from each other. This wavelength was selected for simultaneous estimation of Cita and Clona and it is assume to be sensitive wavelength.Standard calibration curves for Cita and Clona were linear with correlation coefficients values in the range of 0.999-0.999 at all the selected wavelengths. Detection limits and quantitation limits of Citalopram and Clonazepam were found to be 1.2 and 1 µg/mL respectively, whereas quantification limits were 3.6 and 3 µg/mL respectively. Recovery of the drugs from the sample matrices was between 100-105% for Citalopram & 101-102 % for Clonazepam & RSD for Precision for both drugs from the sample matrices between 0.13-0.16 for Citalopram & 0.29-0.29% For Clonazepam and results were validated as per ICH guidelines [8-9].

CONCLUSION

The Vierordt's method permits simple, rapid and direct determination of Citalopram and Clonazepam in commercially available tablet dosage form without previous separation. The results of analysis of two drugs from tablet formulation using method was found close to 100%, Standard deviation was satisfactorily low indicating accuracy and reproducibility of the method. Recovery studies wsa satisfactory which showed that there is no interference of excipients.

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