Spectrophotometric determination of citalopram hydrobromide in tablet dosage form using chloranil

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Abstract: A fast, sensitive and extraction free spectrophotometric method for the quantitative determination of citalopram hydrobromide in pharmaceutical raw and tablet formulations has been proposed. The newly proposed method is based on the charge transfer reaction between citalopram as electron donor and chloranil as electron acceptor. The charge transfer complex of citalopram and chloranil shows λ_{max} at 550 nm in methanol. The experimental conditions such as reaction time, temperature, stoichiometry of the colored complex have been optimized. The developed method allows the determination of citalopram hydrobromide over a concentration range of 1-25 µg/ ml. The proposed method is used to determine the citalopram in tablet dosage forms. The results of proposed method are compared to the official USP method. The newly developed method is accurate, reproducible and easy to perform. It does not require stringent experimental conditions. No interference has been observed for excipients and additives in tablet formulations.

Keywords: Spectrophotometric; Citalopram determination; Chloranil, Pharmaceutical preparations

INTRODUCTION

Citalopram [(RS)- 1- [3-(dimethyl amino)propyl]-1-(4-fluorophenyl)-1, 3-dihydroiso benzofuran-5-carbonitrile] (fig. 1) is a widely used antidepressant. It belongs to the class of selective serotonin reuptake inhibitors having broad spectrum of therapeutic activity against depressive disorders (Brunton *et al*., 2008). It has U.S. Food and Drug Administration approval to treat major depression, and is prescribed off label for a number of anxiety conditions. Citalopram is approved to treat the symptoms of major depression. Citalopram is frequently used to treat anxiety, panic disorder and body dysmorphic disorder. It has been found to greatly reduce the symptoms of diabetic neuropathy (Sindru *et al*., 1992) and premature ejaculation (Atmaca *et al*., 2002). The literature shows that citalopram hydrobromide is determined by various analytical methods including High Performance Liquid Chromatography (Menegola *et al*., 2008; Gandhi *et al*., 2008; Nageswara *et al*., 2006; Das *et al*., 2012), Micellar electrokinetic chromatographic method (Rodríguez *et al.,* 2008), Spectrofluorimetric method (Vasantharaju et al., 2008), LC-MS/MS method (Frison *et al.,* 2008; Cao *et al*., 2007), Fast fourier continuous cyclic voltammetry (Parviz *et al*., 2007), Capillary electrophoretic method (Şatana *et al*., 2006), Adsorptive stripping voltametric method (Nouwsa *et al*., 2006), Chiral capillary electrophoresis (CE) method (Andersen *et al.*, 2003), HPTLC (Saravanan *et al*., 2012) and UV-Visible Spectrophotometric method (Sagar *et al*., 2004; Raza., 2006; Raza *et al*., 2008; Badiadka *et al*., 2010) in Plasma and pharmaceutical dosage forms. Some of these methods involve costly equipment like LC-MS, HPLC etc. and the operating procedures are very tedious and time consuming. In most of the laboratories these equipment are not available and highly skilled staff is required to operate them. So the spectrophotometric method is a common choice that is available in almost every analytical laboratory. While the available spectrophotometric methods for the determination of citalopram have some disadvantages of sensitivity, interfering of excipients and involve extraction process that lead to decrease the accuracy of the methods. Therefore it is a need to develop simple, sensitive and extraction free spectrophotometric method for the estimation of citalopram hydrobromide in pharmaceutical tablet formulations. Literature review shows that the proposed reaction between citalopram and chloranil has never been exploited for spectrophotometric determination of citalopram hydrobromide.

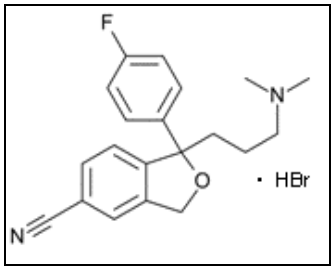

Fig. 1: Chemical structure of citalopram hydrobromide.

The purpose of the present study is to develop a sensitive and cost effective spectrophotometric method that rely on the use of cheap chemicals and simple technique but provides accuracy compared to costly and sophisticated technique like HPLC.

MATERIALS AND METHODS

Equipment

All absorption spectra were recorded using (U 1100 Hitachi, Japan) UV-visible spectrophotometer. Microsoft

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Excel 2007 was used to calculate the statistical parameters.

Materials and reagents

The following chemicals were obtained from their local suppliers and used without further purification: Pure grade citalopram hydrobromide reference standard (Bio Fine Pharmaceuticals (Pvt.) Ltd. Multan, Pakistan), its potency was 99.89% by HPLC method. Chloranil (Sigma-Aldrich, Germany), methanol (Analytical Reagent Grade, BDH, UK), 1,4-dioxane (Sigma-Aldrich, Germany) and sodium bicarbonate (Analytical Reagent Grade, BDH, UK). All the reagents and solvents used were of analytical grade. Three different formulations of citalopram namely S-pram (BioFine Pharmaceuticals Pvt. Ltd. Mutan, Pakistan) Cheer (Wilshire Laboratories Pvt. Ltd. Lahore, Pakistan) and Cipram (Lundbeck, Karachi Pakistan) were obtained from retail pharmacies. Each tablet was labeled to contain 20 mg citalopram base. The excipients present in the tablets are: microcrystalline cellulose, lactose monohydrate, povidone, magnesium stearate, starch, sodium starch glycolate and titanium dioixide.

Preparation of standard solutions

Citalopram hydrobromide standard solution (1mg/ml): This was prepared by weighing 100 mg accurately on an electronic weighing balance dissolved in enough methanol in 100 ml volumetric flask and made up to 100 ml with methanol.

Chloranil standard solution (0.25 mg/ml): A 25 mg quantity of chloranil was accurately weighed on an electronic weighing balance and dissolved in 1,4-dioxane and the volume was made up to 100 ml with 1,4-dioxane**.**

Sodium bicarbonate solution (2 mg/ml): A 200 mg quantity of sodium bicarbonate was accurately weighed on an electronic weighing balance and dissolved in distilled water and the volume was made up to 100 ml with water.

Procedure

Calibration Plot for Citalopram-Chloranil Complex

Aliquots of 1-25 µg/ml of pure drug solution were pipted into a series of 10 ml volumetric flasks. 2 ml sodium bicarbonate solution was added to each flask to basify the contents. Then sufficient volumes of chloranil solution were added to each of the test tubes according to the proposed stoichiometry. The contents were mixed well and left at room temperature for five minutes. Volume was made up to the mark with methanol. After which the absorbance of each of the samples was determined at 550 nm against reagent blank. Absorbance values were plotted against the concentration to obtain the calibration plot for citalopram hydrobromide.

Assay Procedure for the tablet formulations

Twenty tablets were accurately weighed and their average weight was determined. After grinding into fine powder quantity equivalent to 100 mg of pure drug was dissolved in 40 ml methanol in 100 ml volumetric flask. The contents were shaken manually for two minutes and volume was made to the mark with same solvent. The contents were filtered through Whatman filter paper No 42 to remove the excipients. Appropriate volume (about 1 ml) from this filtrate was taken in 100 ml measuring flask and added 2 ml of sodium bicarbonate solution and 1 ml of chloranil solution. The contents of mixture were kept for five minutes at room temperature. After the complete color development, the volume was made up to 100 ml in volumetric flask with methanol to get the final concentration of drug 10 µg/ml. The absorbance of the solution was measured at 550 nm against sample blank

Stoichiometry of the citalopram-chloranil complex

The Job's method of continuous variation (Basavaiah *et al*., 2005) was employed. Equal molarity solutions of the drug and chloranil were prepared. The final concentration of the drug solution was maintained 10 µg/ml. In 10 ml volumetric flask, made the solution of drug and reagent in the ratio of (0: 10, 1: 9, ……, 9: 1, 10: 0) respectively. The reaction was allowed to proceed as described in proposed method. Absorbance of the solutions was recorded at 550 nm against the reagent blank.

RESULTS

Absorption spectra

A solution of chloranil in 1-4 Dioxane had a reddish yellow color with λ_{max} at 430 nm. On reaction, the colorless drug solution of citalopram hydrobromide with chloranil solution, a violet red color was obtained. This suggested a charge-transfer complex formation was scanned in the visible range of 400 - 800 nm that showed a maximum absorption at 550 nm (fig. 2). The interaction between citalopram and chloranil is shown in Scheme 1. This complex was formed instantaneously and remained stable for one hour. The absorbance band of chloranil showed a bathochromic shift.

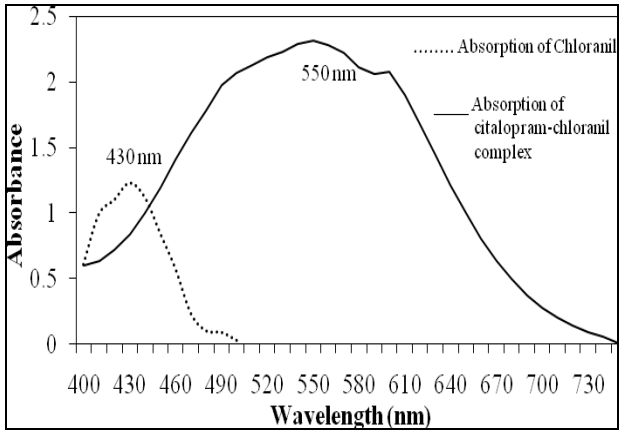
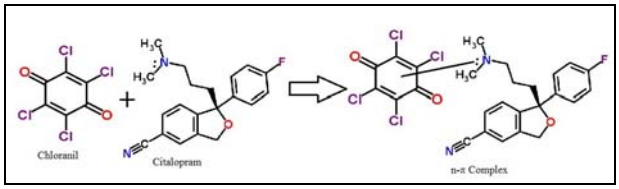


Fig. 2: Absorption spectra of chloranil and citalopram-chloranil complex.

Stoichiometry of the reaction

The molar ratio of the color complex formed between citalopram and chloranil reagent was determined by applying the mole ratio and continuous variation Job's method using a same concentration solution of the drug and the reagent. The result indicated that the complex was formed in the ratio of 1:1 (fig. 3). This finding supports that the interaction of citalopram and chloranil used takes place at one site, which was the more sterically free terminal basic amino group (tertiary amine). A possible reaction pathway between citalopram and chloranil is shown in the Scheme I.



Scheme 1: Proposed structure of citalopram-chloranil charge transfer complex

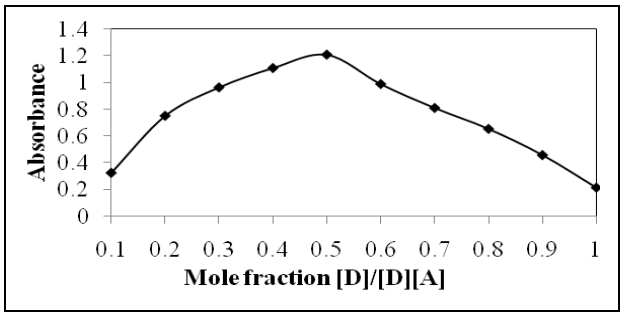


Fig. 3: Job's Method for citalopram-chloranil complex

Effect of variables

The various parameters that affect the color development and its stability were studied and optimized. These parameters included reagent concentration, reaction time, temperature and stability of the colored product. The effect of the concentration of chloranil was studied by adding different volumes of 0.25 mg/ml chloranil solution to a fixed concentration of citalopram hydrobromide (10µg/ml). The maximum absorbance was obtained with 1ml of 0.25mg/ml chloranil solution (fig. 4). The optimum reaction time was determined by observing the absorbance increment at the λ_{max} of the formed complex. The charge transfer complex between citalopram and chloranil solution developed within five minutes at room temperature (25°C) as shown in fig. 5. The colored product formed by the charge transfer reaction between citalopram and chloranil is stable for one hour that is suitable time for an analyst to perform analytical measurements. The absorbance and concentration plot was found to be linear over the concentration range of 1-25µg/ml. Calibration graph (n=5) at 550 nm revealed very small intercept (-0.002) and good linear relationship (r= 0.9999) between the absorbance and concentration of citalopram.

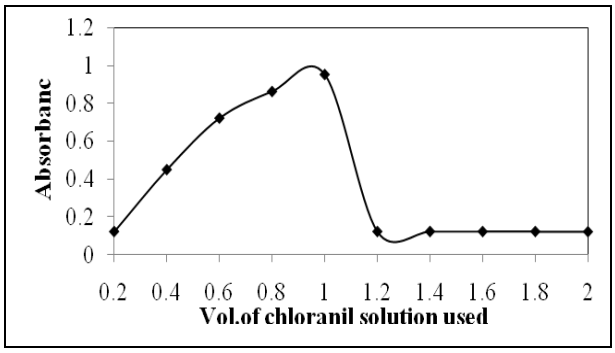


Fig. 4: Effect of reagent concentration on color formation.

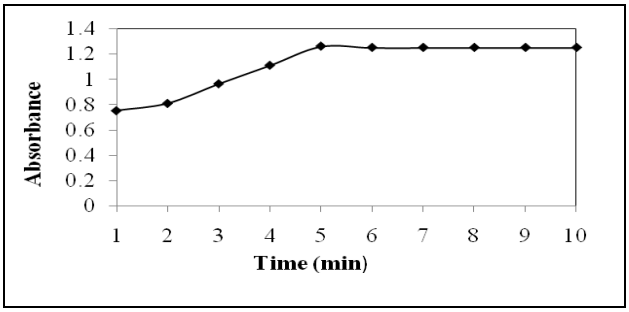


Fig. 5: Effect of time on color formation.

Interferences

More than 99 % recovery of citalopram hydrobromide was obtained in the presence of various excipients and additives used in tablet formulations such as microcrystalline cellulose, lactose monohydrous, povidone, magnesium stearate, starch and primojel. A known amount of drug (10µg/ml) was analyzed with stated excipients in different concentrations. The results of analysis are presented in table 2. Excipients up to the concentrations stated in the table 2 have no interference with the proposed procedure.

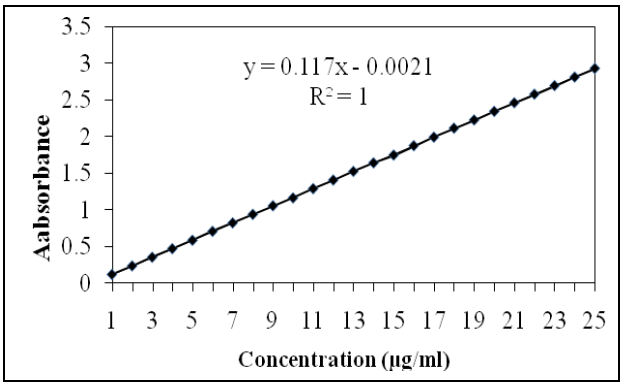


Fig. 6: Beer's law measuring range

Table 1: Statistical analysis of calibration graph and analytical data for the complex of citalopram with chloranil method (n=5)

Parameters	Values
λ_{max} (nm)	550
Beer's law measuring range (µg/ml)	1-25
Molar absorptivity (L $mole^{-1}cm^{-1}$)	4.7×10^4
Sandell's sensitivity(µg/ml)	8.6×10^{-3}
Limit of Detection (µg/ml)	1.05
Limit of Quantification (µg/ml)	3.46
Slope	0.117
Intercept	-0.002
Correlation coefficient	0.9999

Table 2: Percent recovery of citalopram hydrobromide in presence of excipients/additives

Excipient/additive	Amount taken (µg/ml)	Recovery [a] % ± S.D.
Microcrystalline cellulose	450	99.67 ±0.56
Lactose monohydrous	560	100.71 ±0.23
Povidone,	200	99.23 ±0.34
Magnesium stearate	300	99.78 ±0.67
Starch	400	99.87 ±0.87
Primojel	160	100.24 ±0.78
Titanium dioxide	90	99.89 ±0.56

[a] Average of five determinations

DISCUSSION

Some hydrochloride/ bromide salts of amines do not react with π-acceptors because they do not possess a lone pair of electrons. Similarly, citalopram hydrobromide, for the same reason, is unable to react with chloranil in its present form. Therefore, sodium bicarbonate is added to basify the solution. In previous studies (Raza., 2006 and Raza *et al*., 2008) the extraction process was used to extract the drug in basic form and then it reacted with certain π-acceptor to form the colored charge transfer complex. But in this work, the direct reaction of citalopram and chloranil took place in presence of sodium bicarbonate. Results are very good. So this is an extraction free, less time consuming and an easy method

Analytical applications

Pharmaceutical formulations (Cheer, S-pram, and cipram tablets) containing citalopram hydrobromide 20 mg/tab were analyzed by the proposed method and the accuracy was tested by the standard addition method in which variable amounts of pure drug were added. The results are shown in table 3. These results confirmed that the developed method is not apt to interference by tablet excipients and additives used in tablet formulations. The proposed method was applied to determine citalopram hydrobromide in pharmaceutical tablet preparations and the official U.S.P. method was used for comparison. The results are presented in table 4. The efficiency of the method was estimated by Student-*t* values and *F*-ratio tests.

Method validation

The linearity, slope and the intercepts were calculated using the regression equation. Precision and accuracy of the proposed method were tested by carrying out the determination of five replicates of pure and commercial samples of the drug, whose concentration was within Beer's law measuring range. Values of the standard deviation (SD), relative standard deviation (RSD) and range of error at 95% confidence level were calculated (Harvey *et al*., 2000). The two methods have been applied to various pharmaceutical formulations and recovery studies have been made by the standard-addition method. Intra-day precision and intra-day error of the methods were assessed from the results of five replicate analyses on the pure drug solution. The mean values and relative standard deviation values for replicate analysis at three different concentration levels were calculated. Accuracy of the methods was determined by recovery studies via the standard addition method. The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the current ICH guidelines (ICH Nov. 2005).

CONCLUSION

A simple, sensitive, selective and extraction free spectrophotometric method for the determination of citalopram hydrobromide has been developed. The developed method has the advantage of sensitivity compared with the existing spectrophotometric methods reported in the literature as shown in table 5. The proposed method is easy to perform and does not involve any stringent experimental variables which affect the reliability of the results. There is no interference from common additives and excipients. The method thus can be used in the determination of citalopram hydrobromide in pure and tablet formulations in quality control laboratories of pharmaceutical industries.

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Table 3: Determination of citalopram hydrobromide in pharmaceutical dosage forms applying the standard addition technique

Proposed method	Taken amount (μg/ml)	Added amount (μg/ml)	Proposed method recovery [a] (%)		
			Cheer tablets	S-Pram tablets	Cipram tablets
	10	-	100.28	99.89	100.08
		10	100.28	100.08	99.63
		20	99.96	99.58	99.25
		30	100.96	100.05	99.86
		40	99.87	100.12	100.21
Mean±S.D.			100.06±0.15	99.94±0.22	99.80±0.38

[a] Average of five determinations

Table 4: Determination of citalopram hydrobromide in pharmaceutical formulations

Sample	Recovery [a] ± S.D. %	
	Official method (USP 2009)	Proposed method
Cheer tablets [b]	100.25±0.36	100.12 ± 0.78
t		0.35
F		1.18
S-Pram tablets [b]	99.68±0.45	99.98 ± .56
t		0.25
F		1.30
Cipram tablets [b]	99.78±0.53	99.84 ± 0.21
t		0.69
F		1.16

[a]Mean ± standard deviation of five determinations.
[b]All tablets contain citalopram hydrobromide equivalent to 20 mg citalopram base
The student *t*-test and *F*-test were calculated using MS Excel 2007.

Table 5: Comparison of the literature's spectrophotometric methods with newly developed method

λ_{max} (nm)	Beer's law measuring range (μg/ml)	Molar absorptivity (L mole^{-1}cm^{-1})	Color producing Reagent	Remarks	Reference
240	4-40	1.04×10^2	UV method	Excipients may interfere	Raza (2006)
590	10-250	3.3×10^3	DDQ	Less sensitive	Raza (2006)
570	8-240	4.2×10^3	TCNQ	Less sensitive	Raza *et al* (2008)
480	1.0-7.0	2.10×10^4	Bromate-bromide mixture	Mixture of reagents use and involve multi steps	Badiadka *et al* (2010)
510	0.6-6.2	7.30×10^4	1,10-phenanthroline	Involve three reagents and require 20 min.	Basavaiah *et al* (2005)
550	1-25	4.7×10^4	Chloranil	Highly sensitive single step reaction and requires only 5 minutes to develop color	Present work

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