Spectrophotometric Determination of Citalopram in Pharmaceutical Formulations

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(Received 27th July 2007, revised 8th October 2007)

Summary: An accurate, simple and reproducible spectrophotometric method for the determination of citalopram hydrobromide either in pure form or pharmaceutical formulations has been developed. The drug was extracted from formulations with chloroform in an alkaline medium and reacted with 7, 7, 8, 8-tetracyanoquinodimethane (TCNQ) as π-acceptor to give a colored complex, which absorbs maximally at 570 nm. Beer's law was obeyed in the concentration range 8- 240 $\mu g\ ml^{-1}$ with molar absorptivity of 4.2×10^3 L $mole^{-1}$ cm^{-1}. Effect of different variables was studied and conditions were optimized. Results have also been validated statistically. The proposed method has been found to be sensitive, rapid, accurate and precise for the quantitative determination of citalopram hydrobromide in raw and dosage forms without interferences from commonly used excipients.

Introduction

Citalopram is a highly selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor (SSRI) that plays a significant role in learning and memory processes [1]. Due to the selective action on 5-HT uptake, citalopram seems to be a valuable tool in studying the role of central serotonergic system in experimental neuropharmacology [2]. Citalopram like other SSRIs showed antidepressant effects in animal modes of depression [3, 4]. It was effective in the treatment of alcoholism [5, 6], panic disorder and school phobia [7] and relieved the symptoms of diabetic neuropathy [8]. Patients with Alzheimer's disease or senile dementia of Alzheimer type treated with citalopram showed a significant improvement in emotional disturbances [9].

Very few analytical methods appeared in the literature for the determination of citalopram hydrobromide in tablets are generally based HPLC with UV detection [10], HPLC with fluorescence detection [11], capillary zone electrophoretic method [12], densitometric HPTLC, and videodensitometric HPTLC methods [13]. HPLC-electrospray ionization mass spectrometry (HPLC-MS/ESI) [14] and HPLC-positive ion electrospray mass spectrometry [15] method has been reported for the quantification of citalopram in human plasma. Officially assay of citalopram hydrobromide is not described in British and United States Pharmacopoeias. In this study, an attempt has been made to develop a simple, accurate and low cost spectrophotometric method for the quantitative determination of citalopram hydrobromide in pharmaceutical formulations without the interference of other constituents in the formulation.

Results and Discussion

The charge-transfer (CT) reactions have been widely studied recently. Many drugs are easy to be determined by spectrophotometry based on color charge-transfer (CT) complexes formed with electron acceptors [16, 17]. The charge-transfer (CT) complexes are formed between electron donors, having sufficiently low ionization potential, and acceptors, having sufficiently high electron affinity. The transfer of an electron from a donor to an acceptor is readily possible in the charge-transfer process [18].

Absorption Spectrum of Colored Complex

Citalopram base reacts with TCNQ when heated at 60 °C for ten minutes in water bath to give a colored complex, the absorption maximum of which, under optimum conditions, is 570 nm.

Reaction Mechanism

TCNQ has been used for quantitative determination of pharmaceutical drugs in dosage forms by charge-transfer complex formation [19, 20]. Interaction with TCNQ in acetonitrile solution was found to yield a deep color causing characteristic longer wavelength absorption band. The predominant chromagen with TCNQ is blue colored radical anion, which probably resulted through the dissociation of an original donor-acceptor complex with the drug. This complex is formed by the lone pair of electron donated by the citalopram base as n-donor and the charge transfer reagent as an electron

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acceptor, where a partial ionic bond (D^+ A) is assumed to be formed.

$$D + A \longrightarrow [D \longrightarrow A] \longrightarrow D^+ + A^+$$

Donor Acceptor — Donor-acceptor complex — Redical anion

The dissociation of the complex was promoted by the high ionizing power of acetonitrile solvent [21].

Stoichiometry of the complex

The application of Job's method of continuous variation revealed a 1: 1 molar ratio for the complex formed as show in Fig. 1.

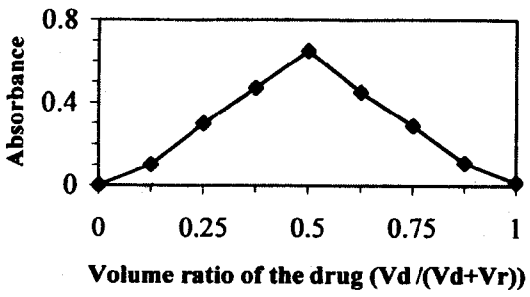

Fig. 1: Stoichiometric ratio of the complex.

Optimization of Reaction Conditions

The spectrophotometric properties of the colored species formed with TCNQ as well as different parameters affecting the color developpment, were extensively studied. The optimum conditions for the proposed procedure have been established by studying the reaction as function of the concentration of the reagent, the nature of the solvent, heating time and stability of the colored species.

Effect of Solvent

In order to select the most appropriate solvent, for optimum reaction development and dilution, different solvents were tested. Chloroform was found to be good solvent for charge transfer reaction in terms of color stability and absorption intensity. So chloroform was used throughout this research work because it gave highest absorption readings.

Effect of Color Producing Reagent

7,7,8,8-Tetracyanoquinodimethane (TCNQ) was used as color producing reagent. It was found that one ml of 1mg ml^{-1} solution of 7,7,8,8-tetracyanoquinodimethane (TCNQ) gave maximum color (Fig. 2), above and below this concentration the color intensity diminished and color becomes unstable. So one ml of 1mg ml^{-1} solution of TCNQ was considered the optimum value for maximum color development.

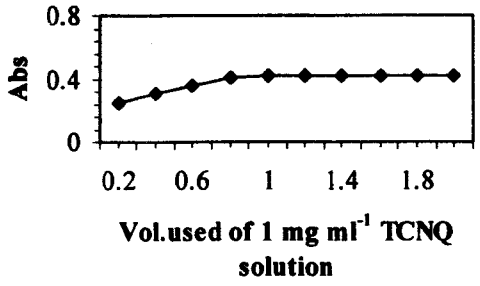

Fig.2: Effect of reagent concentration.

Effect of Temperature and Heating Time

The effect of temperature is shown in Fig. 3. The color starts developing at 40 °C and reaches its maximum intensity at 60 °C. As the temperature increases the complex stars dissociating, due to which the color intensity decreases. The effect of heating time on color intensity is shown in Fig. 4. It was found that heating for ten minutes at 60 °C gave maximum color and above and below these temperature and time the color intensity decreased and color becomes unstable.

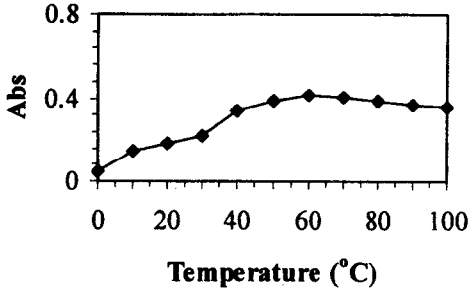

Fig. 3: Effect of temperature.

Sensitivity

The results for the determination of citalopram hydrobromide are shown in table 3,

Table-1: Determination of citalopram hydrobromide in the presence of excipients (For interferences studies, 40 μg ml^{-1} of citalopram was used).

Excipients	Amount taken (μg ml^{-1})	% Recovery ± RSD (n = 5)
Microcrystalline cellulose	300	101.5 ± 0.40
Lactose monohydrate	300	100.2 ± 0.35
Povidone	100	99.26 ± 0.40
Starch	200	102.7 ± 0.20
Magnesium Stearate	300	99.52 ± 0.48
Primojel	50	98.95 ± 0.30

Table-2: Analysis of citalopram hydrobromide in pharmaceutical formulations (n = 5) using proposed and reference method [10].

Dosage forms	% Found ± SD		*t-value*	*F-value*
(Tablets)	Proposed	Reference		
Cipramil	100.07 ± 0.65	99.88 ± 0.45	0.41	3.02
S-Pram	99.60 ± 0.48	99.23 ± 0.56	0.32	3.12
Pramcit	99.86 ± 0.52	100.65 ± 0.47	0.21	1.25
Cheer	98.53 ± 0.44	99.78 ± 0.52	0.42	4.01
Citopram	98.72 ± 0.41	99.85 ± 0.47	0.28	4.28

Table-3: Analytical parameters.

Parameters	Values
λ_{max}	570 nm
Beer's law limit (μg ml^{-1})	8-240
Molar absorptivity (L mole^{-1} cm^{-1})	4.266 × 10^3
Sandell's sensitivity (μg cm^{-2})	9.5 × 10^{-2}
Regression equation (Y*)	
Slope (b)	0.0105
Intercept (a)	0
Correlation coefficient (r)	0.9999
RSD** (%)	0.54
Limit of detection (μg ml^{-1})	4.14
Limit of quantitation (μg ml^{-1})	13.67

Y* = a + bC

Where C is the concentration of analyte (μg ml^{-1}) and Y is absorbance unit.

** =Calculated from five determinations.

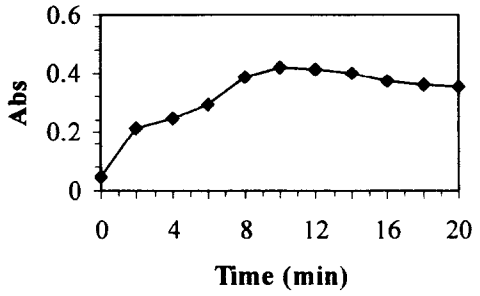

Fig. 4: Effect of heating time.

which shows the sensitivity, validity and reproducebility of the proposed method. It is also reasonably precise and accurate, as the amount taken from identical samples is known and the amount found does not exceed a relative standard deviation of 0.65 (n = 5). The calibration graph is linear in the range of 8-240 μg ml^{-1}. The apparent molar absorptivity calculated is 4.266 × 10^3 L mole^{-1} cm^{-1}. The correlation coefficient between absorbance and concentration is 0.999.

Interferences

The quantitative assessment of tolerable amounts of different excipients (w/v) under experimental conditions is given in Table-2. Various amounts of diverse interfering compounds were added to a fixed amount of citalopram hydrobromide (40 μg ml^{-1}) and proposed procedure for spectrophotometric determination was followed. Excipients up to the concentrations shown in the Table-2 do not interfere with the assay. In addition, recoveries in most cases were around 100 % and the lower values of the RSD indicate the good precision of the method.

Application

The applicability of the proposed method for the determination of citalopram in commercial dosage forms was examined by analyzing marketed products. The results of the proposed method were statistically compared with reference method [10] and summarized in the Table-3. Calculated *t-test* and *F-test* values [22] are less than the theoretical ones at 95 % confidence level, indicating no significant difference between the methods compared. The proposed method is simple, reproducible, sensitive, and accurate and is successfully applied for the quantitative determination of citalopram hydrobromide in quality control laboratories.

Experimental

A Hitachi U 1100, UV/ Vis spectrophotometer (Japan) with silica glass cell of 1 cm thickness was used. Officially calibrated Pyrex glassware was used throughout this study. Pure drug of Citalopram hydrobromide was supplied by Bio Fine Pharmaceuticals (Pvt.) Ltd. Multan, Pakistan. Commercial dosage forms of citalopram hydrobromide were purchased from the local market. All reagents and solvents used were of Analytical Reagent Grade.

1 mg ml^{-1} 7,7,8,8-tetracyanoquinodimethane (TCNQ), *Fluka,* Austria, was prepared in 1,4-dioxan. An aqueous solution of sodium carbonate (0.5 M, Merck, Germany) was prepared in doubly distilled water.

Preparation of Citalopram Base Solution

A citalopram base solution was prepared by transferring 100 ml of 0.5 % citalopram hydrobromide solution (aqueous) into 250 ml separating funnel, followed by 25 ml of 0.5 M sodium carbonate solution. The contents of separating funnel were mixed well, shaken for two minutes and then extracted with 100 ml chloroform. The two phases were allowed to separate and the chloroform layer was dried over anhydrous sodium sulphate.

Proposed Analytical Procedure

Aliquots of 8-240 μg ml^{-1} of the citalopram base solution were pipetted into a series of 10 ml standard volumetric flasks. Then, 1 ml TCNQ solution was added to each flask. Heated the reaction mixture in water bath at 60°C for ten minutes. The complete color develop was attained in this time period. Allowed the reaction mixture to attain the room temperature then made up the volume to the mark with chloroform. The absorbance was measured within the stability period of one hour after dilution at 570 nm against reagent blank.

Analysis of Pharmaceutical Formulations

An accurately weighed portion of powdered tablet equivalent to 250 mg of citalopram hydrobromide was stirred well with 20 ml distilled water and left standing for five minutes. The residue was filtered on Whatman filter paper No.42 and washed with distilled water. The filtrate and washings were diluted to the volume in 50 ml measuring flask with same solvent. The citalopram hydrobromide solution was converted into citalopram base following the procedure given under the head "preparation of citalopram base solution", and subjected to the proposed procedure for the determination.

Molar Ratio of Reactants in Complex

Job's method of continuous variation was employed, a 2×10^{-3} M standard solution of drug and reagent were used .A series of solutions were prepared in which the total volume of the drug and reagent was constant. The drug and reagent were mixed in various proportions and then diluted in a 10 ml calibrated flask with the optimum solvent. Measured the absorbance after treating the reagent at best time and temperature against reagent blank under the same conditions

Conclusion

It may be concluded that the newly developed spectrophotometric method for the determination of citalopram hydrobromide is simple, sensitive, accurate, rapid, and economical. The results are in good agreement with the reference method. The method can be successfully applied for the determination of citalopram in the pharmaceutical raw and dosage forms

Acknowledgements

One of us (Asad Raza) is thankful to Mr. Saadat Ali Ghouri, Managing Director, Bio Pharma, Multan, Pakistan for providing necessary research facilities to carry out this work. This work is a part of PhD dissertation of Mr. Asad Raza and is continuation of the previously reported work [23-35]. Authors are grateful to Prof. Dr. Muhammad Zafarullah, Vice-Chancellor, Bahauddin Zakariya University, Multan for providing research facilities to chemistry students.

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