

Spectrofluorometric Determination of Citalopram HBr in Tablets

H. Eda ŞATANA*^o, Nusret ERTAŞ*, Nilgün Günden GÖĞER*

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Summary

Citalopram is a selective serotonin reuptake inhibitor that is used for the treatment of depression. A rapid, selective, sensitive, and simple fluorescence method has been developed for the direct determination of citalopram in pharmaceutical tablets. Standard citalopram and the tablets were dissolved in distilled water and excited at 240 nm, and fluorescence emission intensity was measured at 300 nm. Calibration curve was linear between 5.0×10^{-7} - 2.5×10^{-6} M citalopram. LOD and LOQ values were found as 8.0×10^{-9} and 2.6×10^{-8} M citalopram, respectively. The method has been applied for the determination of citalopram in tablets, and no interference effect was observed from excipients. Recovery values were in the range of 100.5-101.3%. The proposed method was compared with UV-VIS spectrophotometric method and it was found that there was no statistically significant difference between the two methods in terms of accuracy and precision.

Key Words: Citalopram, spectrofluorometry, pharmaceutical tablets.

Received □ : □07.04.2009

Revised □ : □25.05.2009

Accepted □ : □11.06.2009

Tabletlerdeki Sitalopram HBr'ün Spektroflorimetrik Yöntemle Tayini

Özet

Sitalopram seçici serotonin geri alım inhibitörüdür ve depresyon tedavisinde kullanılır. Farmasötik tabletlerdeki sitalopramın doğrudan tayini için hızlı, seçici, hassas ve basit bir spektroflorimetrik yöntem geliştirilmiştir. Standart sitalopram ve sitalopram içeren tabletler distile suda çözüldükten sonra 240 nm de uyarılmış ve 300 nm de floresans emisyon şiddetleri ölçülmüştür. Kalibrasyon eğrisi 5.0×10^{-7} - 2.5×10^{-6} M sitalopram derişimi aralığında doğrusaldır. LOD ve LOQ değerleri sırasıyla 8.0×10^{-9} ve 2.6×10^{-8} M sitalopram olarak bulunmuştur. Yöntem sitalopramın tabletlerden tayini için uygulanmış ve yardımcı maddelerden kaynaklanan herhangi bir girişim etkisi gözlenmemiştir. Geri kazanım değerleri %100.5-101.3 arasında bulunmuştur. Geliştirilen yöntem UV-VIS spektrofotometrik yöntemle karşılaştırılmış ve her iki yöntem arasında doğruluk ve kesinlik açısından istatistiksel olarak anlamlı bir fark bulunamamıştır.

Anahtar Kelimeler: Sitalopram, spektroflorimetri, farmasötik tablet.

INTRODUCTION

Citalopram (1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile) is a selective and potent serotonin reuptake inhibitor. Citalopram exhibits clinical efficacy comparable with classical tricyclic antidepressants but does not have the adverse anticholinergic and cardiovascular effects commonly associated with those drugs. The structure of citalopram is shown in Figure 1.

20-40 mg day⁻¹ of citalopram is used. Maximum plasma levels are reached after 4h and plasma half-life is 37h.

Citalopram is mainly metabolized in the liver by cytochrome P enzyme (1).

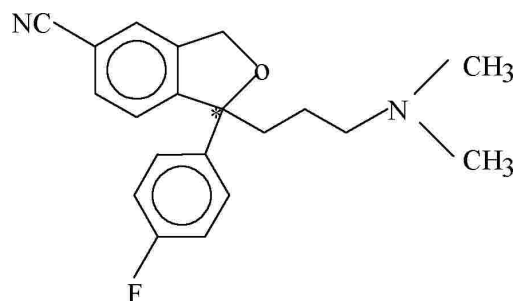


Figure 1 : □Chemical structure of citalopram.

* Gazi University, Faculty of Pharmacy, Department of Analytical Chemistry, Etiler - Ankara, Turkey

^o Corresponding author e-mail: eda@gazi.edu.tr

Several studies describing the determination of citalopram in pharmaceuticals and biological fluids can be found in the literature. Most of these studies use methods that are based on the use of high performance liquid chromatography (HPLC) with UV (2-5), fluorometric (6-8) and mass spectrometric (9-11) detectors. Gas chromatography (GC) (12,13) and gas chromatography with mass spectrometric detection (GC-MS) (14-16) were also used. Electrophoretic methods were also used for the determination of citalopram in pharmaceutical formulations (17-19) and biological fluids (20-22).

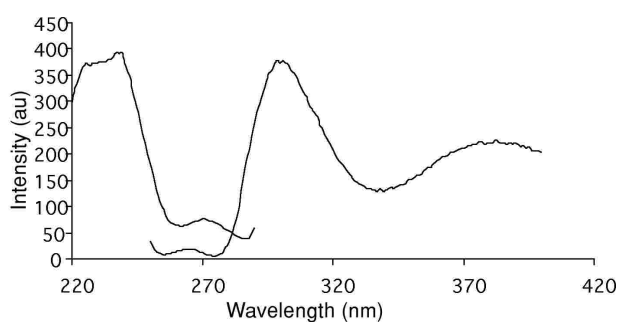


Figure 2 : Excitation and emission spectra of citalopram (240-300 nm)

Spectrofluorometry is considered as one of the most convenient analytical techniques because of its inherent simplicity, low cost, and wide availability in most laboratories. To the best of our knowledge, there is no spectrofluorometric method for the determination of citalopram. For these reasons, this study is presented to describe a simple, sensitive and economical spectrofluorometric method for the determination of citalopram in its pharmaceutical dosage form. Quantitative determination of citalopram was also performed using a UV-VIS spectrophotometric method, and the results were compared to those obtained by spectrofluorometric method.

EXPERIMENTAL

Spectrofluorometric Method

Apparatus

Spectrofluorometric analysis was performed with a Varian Cary Eclipse spectrofluorometer. Measurements were performed with 10 mm quartz cell. Excitation and emission wavelengths were 240 and 300 nm, respectively. Spectral band pass was 10 nm. Xenon flash lamp was used as light

source. Nüve, Fuge CN 090 type centrifuge and Bondelin Sonorex type sonicator were used for sample preparation throughout this study.

Preparation of Standard Solutions

Citalopram standards and its pharmaceutical preparation Cipram® film tablets were obtained from H. Lundbeck (Denmark). For the preparation of standard citalopram stock solution, 10 mg citalopram was accurately weighed and dissolved in distilled water in a 25.0 ml volumetric flask and then adjusted to 25.0 ml with distilled water. Standard solutions in the range of 5.0×10^{-7} - 2.5×10^{-6} M were prepared by appropriate dilution of the stock solution. Calibration curve was constructed by measuring analytical signals obtained for these concentrations at the optimized conditions.

Analysis of Tablets

Ten tablets were weighed and their average mass was recorded, and they were then powdered. An amount of tablet powder equivalent to 2 mg citalopram was weighed, transferred to a 50.0 ml volumetric flask and ultrasonicated for 10 min. The volume was then adjusted to the mark with distilled water. Then 0.1 ml of this solution was transferred to a 10.0 ml volumetric flask and diluted with distilled water. The fluorescence emission intensities of the solutions were measured at 300 nm. The amount of citalopram in pharmaceutical tablets was then calculated by using the calibration curve.

Spectrophotometric Method

Apparatus

A Shimadzu UV-160 double-beam UV-VIS spectrophotometer was used. Spectral band pass was 2 nm and scan speed was set at 480 nm min^{-1} . The spectra of the standard and sample solution were recorded in 1 cm quartz cells against blank over the range of 200-400 nm.

Preparation of Standard Solutions

For the preparation of standard citalopram stock solution, 20 mg of citalopram was accurately weighed and dissolved

in distilled water in a 50.0 ml volumetric flask and then adjusted to 50.0 ml with distilled water. Standard solutions in the range of 1.0×10^{-5} - 5.0×10^{-5} M citalopram were prepared by appropriate dilution of the stock solution. Their absorbance values were measured at 239 nm. The calibration equation was computed by regression of absorbance against concentration of citalopram. The equation was $A = 16990(C) + 0.0165$ ($r = 0.9999$), where A and C represent absorbance and molar concentration of citalopram, respectively (Table 1).

Table 1. Regression data of the calibration lines for spectrofluorometric and spectrophotometric methods

	Spectrofluorometry	UV-VIS Spectrophotometry
Linear Range (M)	5.0×10^{-7} - 2.5×10^{-6}	1.0×10^{-5} - 5.0×10^{-5}
Slope	1.8×10^8	16990
Intercept	6.64	0.0165
Correlation Coefficient	0.9999	0.9999
Standard Error of Slope	1.1×10^6	139.9
Standard Error of Intercept	1.79	4.64×10^{-3}
LOD ^a	8.0×10^{-9}	1.0×10^{-7}
LOQ ^b	2.6×10^{-8}	3.4×10^{-7}

^a LOD (Limit of Detection) = $3 \times S/m$

^b LOQ (Limit of Quantitation) = $10 \times S/m$

Analysis of Tablets

The average mass of 10 tablets was determined and the tablets were finely powdered in a mortar. An amount of the powder equivalent to 2.50 mg citalopram was weighed and transferred to a 25.0 ml volumetric flask. The powder was dissolved in distilled water and the solution was completed to the mark with distilled water. The sample solution was ultrasonicated for 10 min. The non-dissolved excipients were allowed to precipitate and 1 ml of the supernatant solution was transferred to a 10.0 ml volumetric flask and diluted with distilled water. The absorbance values of the sample solutions were measured at 239 nm. The citalopram content of tablets was calculated using the above-mentioned calibration curve.

RESULTS and DISCUSSION

In the present work, we described the application of spectrofluorometry for the determination of citalopram in pharmaceutical samples. Citalopram shows a native fluorescent character because of its isolated aromatic ring. Citalopram HBr is soluble in water and emits fluorescence

at 300 nm when it is excited at 240 nm (Fig 2.). The information about the maximum concentration at which fluorescence linearity may be expected (i.e., absorbance < 0.05) was obtained from the electronic absorption spectra. This limit was estimated up to approximately 2.7×10^{-6} M. Considering these data, the dynamic linear range was 5.0×10^{-7} - 2.5×10^{-6} M of citalopram. The equation of the calibration curve was $I = A + BC$, where I was the fluorescence intensity (in arbitrary units), and C was the concentration of citalopram in M. After least-squares linear fit of the fluorescence emission data, we obtained $A = 6.64$, $B = 1.8 \times 10^8$, $r = 0.9999$. The detection limit and quantification limit were 8.0×10^{-9} M and 2.6×10^{-8} M, respectively, calculated from $3 S/m$ and $10 S/m$, where S was the standard deviation of a set of 10 replicates of 5.0×10^{-7} M citalopram solution and m was the slope of the calibration curve (Table 1). Unknown aqueous samples of citalopram prepared from tablets were studied by applying the above procedure, and the results are shown in Table 2. The calculated results were in agreement with the declared citalopram content. In order to test the accuracy of the proposed method, a recovery study and a reference method were used. The recovery study was performed by the addition of a known amount of citalopram to a known concentration of the commercial tablets (standard addition method). The resulting mixtures were assayed and the results obtained were compared with the calculated results. Considering the spectra of the standard and sample solutions and the recovery values shown in Table 3, there was no interference effect

Table 2. Results for the determination of citalopram in tablets by spectrofluorometric and UV-VIS spectrophotometric methods

	Spectrofluorometric Method	UV-VIS Spectrophotometric Method
Mean (mg)	19.36	19.63
SD	0.22	0.35
RSD %	1.17	1.79
t.SD/n	0.27	0.43
CL	19.09-19.63	19.20-20.06

CL: Confidence limit

Mean: Mean of five samples

Table 3. Recovery studies of citalopram in tablets by spectrofluorometric and UV-VIS spectrophotometric methods

Sample No	Spectrofluorometric Method				UV-VIS Spectrophotometric Method			
	Added (M)	Found (M)	Recovery %	Bias %	Added (M)	Found (M)	Recovery %	Bias %
1	4.98×10^{-7}	5.00×10^{-7}	100.5	0.40	1.0×10^{-5}	9.8×10^{-6}	98.0	-2
2	9.97×10^{-7}	1.00×10^{-6}	101.2	0.30	1.50×10^{-5}	1.47×10^{-5}	98.0	-2
3	1.50×10^{-6}	1.52×10^{-6}	101.3	1.33	2.00×10^{-5}	2.01×10^{-5}	100.5	0.5

Table 4. Intra-day and inter-day precision of citalopram by spectrofluorometric and UV-VIS spectrophotometric methods

Spectrofluorometric Method					UV-VIS Spectrophotometric Method				
Theoretical Concentration (M)	Intra-day measured concentration (M) ^a		Inter-day measured concentration (M) ^b		Theoretical Concentration (M)	Intra-day measured concentration (M) ^a		Inter-day measured concentration (M) ^b	
	Mean	RSD %	Mean	RSD %		Mean	RSD %	Mean	RSD %
5.00x10 ⁻⁷	5.06x10 ⁻⁷	0.48	5.05x10 ⁻⁷	0.33	1x10 ⁻⁵	9.75x10 ⁻⁶	0.29	9.85x10 ⁻⁶	1.07
1.50x10 ⁻⁶	1.51x10 ⁻⁶	0.51	1.51x10 ⁻⁶	0.53	3x10 ⁻⁵	3.02x10 ⁻⁵	0.19	3.02x10 ⁻⁶	0.09
2.50x10 ⁻⁶	2.50x10 ⁻⁶	0.60	2.55x10 ⁻⁶	0.16	5x10 ⁻⁵	4.98x10 ⁻⁵	0.12	4.98x10 ⁻⁶	0.10

^aMean concentration represents five different measurements of citalopram

^bInter-day mean values were determined from five different runs over a one-week period

from additives in the tablets. Intra-day and inter-day precision were also studied. Intra-day precision was studied by analyzing repeatedly, on the same day, five replicates of three different concentration levels. Inter-day precision values were determined from five different runs over a one-week period. Their results are shown in Table 4.

The quantitative determination of citalopram in tablets was also carried out using a UV-VIS spectrophotometric method as a reference method. In this method, standard citalopram and tablets were dissolved in distilled water and their absorbance values were measured at 239 nm. Validation studies were also performed for the UV-VIS method. Linear equation, correlation coefficient and other validated data are shown in Table 1. The method was applied to the tablets for the determination of citalopram and the results are shown in Table 2. The recovery study was performed in order to check the accuracy of the method (Table 3). Intra-day and inter-day precision were also investigated and the results are given in Table 4. A comparison of the two methods shows that the results obtained from the UV-VIS spectrophotometric method were in good agreement with those obtained from the spectrofluorometric method. The results obtained from the two methods were compared statistically by Student's t-test and Fisher test. As shown in Table 5, the calculated t and F values were less than the

Table 5. Statistical comparison of the two methods in terms of accuracy and precision

Amount Labeled (20 mg/tablet) Amount Found (mg)	Spectrofluorometric Method	UV-VIS Spectrophotometric Method
		19.36
	t _{calculated} = 1.29	t _{theoretical} = 2.31 (p=0.05)
	F _{calculated} = 2.53	F _{theoretical} = 6.39 (p=0.05)

theoretical values, indicating no significant difference between the mean content of citalopram and the precision obtained by the two proposed methods.

CONCLUSION

Novel, rapid, sensitive, and selective spectrofluorometric and spectrophotometric methods were developed and validated for the determination of citalopram in tablets. High percentage recovery values were obtained from both. There was no significant difference between the two methods. It can be concluded that the proposed methods are simple, accurate, precise, and can be employed successfully for the routine determination of citalopram in tablet formulations.

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