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ARAŞTIRMA MAKALESİ /**RESEARCH ARTICLE**

A SIMPLE FLOW INJECTION ANALYSIS FOR THE DETERMINATION OF CITALOPRAM IN TABLETS USING UV DETECTION

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ABSTRACT

Citalopram is chemically a bicyclic phthalate and a selective serotonin reuptake inhibitor (SSRI). Citalopram is a racemic drug used for the treatment of depression associated with mood disorders. It is also used on occasion in the treatment of body dysmorphic disorder and anxiety. In the previous studies, simple, rapid, and accurate analytical methods for the determination of citalopram in tablets have been developed. In the present study, Flow Injection Analysis of citalopram hydrobromide using UV detection was described. It was found that the optimum concentration of methanol in view of peak morphology, was 10 % (v/v) and the best flow rate was found to be 1.3 ml.min^{-1}. Active material was detected at 239.5 nm. The calibration equation was linear in the range 1.11x10^{-7}-5.55x10^{-7} M. Limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 3.8x10^{-8} and 1.11x10^{-8} M, respectively. The proposed method was applied to determine citalopram hydrobromide in pharmaceutical preparations. The results were compared with UV-spectrophotometry.

Keywords: Citalopram, Flow injection analysis, UV- spectrophotometry.

TABLETLERDE SİTALOPRAM TAYİNİ İÇİN UV DETEKSİYON KULLANILARAK GELİŞTİRİLMİŞ BASİT BİR AKIŞ ENJEKSİYONU ANALİZİ

ÖZ

Sitalopram kimyasal yapı bakımından bisiklik bir ftalat olup seçici serotonin geri alım inhibitörüdür (SSRI). Davranış bozukluklarıyla ilişkili depresyonun tedavisinde kullanılan rasemik bir ilaçtır. Ayrıca zaman zaman beden dismorfik bozukluğu ve anksiyete tedavisinde de kullanılır. Daha önceki araştırmalarda, sitalopram içeren tabletlerin tayini için kolay, hızlı ve kesin analitik metodlar geliştirilmiştir. Bu çalışmada, UV deteksiyonu kullanılarak sitalopram hidrobromür için akış enjeksiyonu analizi tanımlanmıştır. Pik morfolojisi açısından en elverişli metanol konsantrasyonunun %10 (h/h) ve en iyi akış hızının ise 1.3 ml.dak^{-1} olduğu bulunmuştur. Aktif materyal 239.5 nm' de detekte edilmiştir. Kalibrasyon denklemi 1.11x10^{-7}-5.55x10^{-7} M aralığında doğrusal olarak bulunmuştur. Sitalopram için saptama sınırı ve tayin sınırı sırasıyla 3.8x10^{-8} ve 1.11x10^{-8} M olarak hesaplanmıştır. Önerilen metod farmasötik preparatlardaki sitalopramı tayin etmek için geliştirilmiştir. Sonuçlar UV-spektrofotometri ile karşılaştırılmıştır.

Anahtar Kelimeler: Sitalopram, Akış enjeksiyonu analizi, UV- spektrofotometri.

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1. INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are a class of antidepressants used for the treatment of depression, anxiety, panic disorder, social phobia, obsessive compulsive disorder, post-traumatic disorder and pre-menstrual dysphoric disorder (Kristoffersen et al., 1999; Molander et al., 2001; Unceta et al., 2008; Hashimoto et al., 2009). SSRIs have a better tolerated adverse effect profile when compared to the other antidepressants such as tricyclic antidepressants with approximately equivalent antidepressant efficacy (Kristoffersen et al., 1999; Tournel et al., 2001; Molander et al., 2001; Juan et al., 2005; Hayes et al., 2009).

Citalopram (CTL); (1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile) is a bicyclic benzofurazan derivative that belongs to SSRIs which increase 5-HT transmission by inhibiting 5-HT uptake with a low affinity for noradrenaline uptake (Kristoffersen et al., 1999; Unceta et al., 2008; Bagheria et al., 2008; Renerio et al., 2009). (Fig. 1) It is administered as a rasemic mixture; containing both (−)-R- and (+)-S-enantiomers (Macek et al., 2001; Greiner et al., 2007; Bagheria et al., 2008; Hayes et al., 2009). It is the most selective and potent SSRI drug for the inhibition of serotonin reuptake which has no interactions with cytochrome P450 unlikely to most of drugs and has low potential for drug–drug interactions (Unceta et al., 2008; Bagheria et al., 2008). The advantages of this drug's therapeutic profile have led to an increasing use of it in treatment of depressed patients and combined drug therapies (Unceta et al., 2008; Bagheria et al., 2008; Hashimoto et al., 2009; Renerio et al., 2009; Hayes et al., 2009).

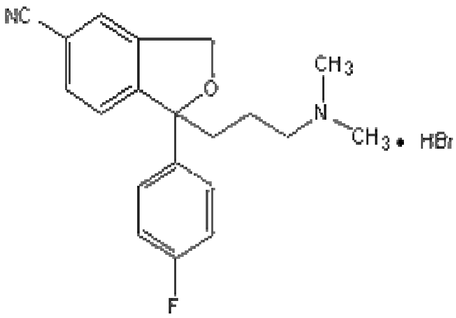

Figure 1. Chemical structure of CTL

Several analytical methods have been developed for the analysis of CTL. Most of these methods are based on reversed phase high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection and a few studies with fluorescence detection in plasma and serum samples (Kristoffersen et al., 1999; Macek et al., 2001; Juan et al., 2005; Unceta et al., 2008). A HPLC coupled with mass spectrometry (MS) quantification of CTL is also published (Juan et al., 2005). There are also studies carried out in biological matrices such as plasma, serum, blood, urine and hair using liquid chromatography–tandem mass spectrometry (LC–MS) (Unceta et al., 2008). Gas chromatography (GC) and thin-layer chromatography (TLC) are also applied in CTL quantification (Kristoffersen et al., 1999; Molander et al., 2001; Tournel et al., 2001).

Flow-injection analysis (FIA), which is essentially the introduction of a sample into a solution stream continuously passing through a detector, is a very important methodological application in analytical chemistry which has a simple chemical processes, low cost apparatus, easy application and ability in leading to results that are usually of good quality. To use of a flow-injection system to determine active materials in pharmaceutical preparations allows good quality results in a rapid process (Altıokka et al., 2001; Dal et al., 2005; Can et al., 2008).

In the present study, it was aimed to develop a new FIA method for determination of CTL and also to determine CTL in pharmaceutical preparations with the developed method.

2. EXPERIMENTAL

2.1 Materials and Reagents

CTL (Cipla, India) standart was used. Other chemicals were of analytical grade and provided from Merck Com. (Darmstadt, Germany). The commercial preparation of CTL, Citara® tablets each containing 20mg CTL, Fako Actavis (Iceland), was purchased from a local pharmacy.

2.2 Apparatus

The FIA system consisting of a Model Spectra System SCM 1000 degasser, Spectra System P1000 isocratic pump, Spectra System SN4000 connector, Spectra System UV6000LP diode array detector (Thermo Finnigan, USA).

A Shimadzu spectrophotometer (Model UV 2401 PC, Japan) was used to measure the absorbance in batch-wise operations. The absorbance of the effluent was monitored at 239.5 nm. The flow-rate was 1.3 ml.min^{-1}.

2.3 Preparation of Solutions

Standard CTL solution was prepared by dissolving in methanol and made up to 100 ml. This stock solution was employed for the preparation of other dilutions. Mobile phase was an aqueous solution of methanol (10%, v/v).

For the quantification of CTL, 1 Citara® tablet consisting of 20 mg CTL was dissolved in methanol. It was sonicated for 30 minutes and it was made up to 100 ml by methanol.

Flow-injection analysis was performed in a supporting solution consists of methanol. The signals were detected at 239.5 nm where monochromatic light is absorbed maximum. Standard and sample solutions were injected to a 15 µl fix volume of loop. The variation of flow–rate was examined in a wide range of 0.8-1.6 ml.min^{-1}.

3. RESULTS AND DISCUSSION

3.1 Optimization of FIA Method

Analytical parameters were considered compromising the component of the solvent system. Solvent system must dissolve CTL, it must be cheap and easily provided. It was decided that 10 percent methanol is very suitable solvent for these purpose.

UV spectrum of CTL was obtained in the range of 200-400 nm to decide the flow-injection detection wavelength. A 1.11x10^{-5} M CTL solution was prepared, diluting with methanol from the stock solution. A maximum appeared at 239.5nm.

The effect of flow-rate on the peak area and peak height of CTL (3.33x10^{-5} M) in the range of 0.8-1.6 ml.min^{-1} was investigated. Bigger peak areas were appeared by pumping through flow-rate values lower than 1.0 ml.min^{-1}. Asymmetric peaks with lower peak areas were observed with a flow-rate higher than 1.4 ml.min^{-1}.

Suitable flow rate was chosen as 1.3 ml.min^{-1} to perform the quantification studies and peak area response was used, in the rest of experiments. (Table 1)

3.2 Repeatability and Intermediate Precision

Repeatability and intermediate precision were tested using 3.33x10^{-7} M CTL solution in methanol at 1.3 ml.min^{-1} of flow-rate and 239.5 nm of detection wavelength in three operating days with 6 samples. The results were evaluated using the response of CTL involving peak area and peak height as can be seen in Table 2. Very low variation coefficients below 2 % of relative standard deviation (RSD) were obtained showing the method is sufficiently precise. (Table 2)

3.3 Linearity

The calibration line of response of peak area as a function of the concentration was constructed in the concentration range of 1.11x10^{-7} – 5.55x10^{-7} M of CTL solution at three operating days. The detailed statistical results are shown in Table 3. (Table 3)

Statistically evaluated data show acceptable linearity with high regression coefficients and intercepts close to the origin, in the studied range for FIA. (Fig. 2)

3.4 Detection and Determination Limits

The detection limit of the FIA method was found to be 3.8x10^{-8} M according to the criteria of signal-to-noise (S/N = 3) and the determination limit was calculated to be 1.11x10^{-8} M accepting the signal-to-noise (S/N = 10).

3.5 Application of the Method to the Commercial Tablets

The developed method for the determination of CTL was applied to the commercial tablets, which contain 20 mg CTL each, employing optimum FIA conditions.

As a standart method, UV-spectrophotometry, was used to compare with the method accuracy of flow-injection analysis because of its validity.

A good linear relation between absorbance and concentration of CTL was obtained in the range of 1.11x10^{-5} – 5.55x10^{-5} M at 239.5 nm of detection wavelength using methanol as blank. It fits to the equation of [A = 16801,80 C (M) - 0,0077; r = 0.9997].

The results of the commercial tablets assayed by FIA and UV spectrophotometry as described in the experimental section are presented in Table 4. (Table 4)

FIA has many advantages such as simplicity, short analysis time, high sampling frequency, and low expense of reagents and samples, making the technique suitable for quality control and routine analysis in many fields such

Table 1. Flow Rate

Flow Rate	Mean	SD	%RSD
0.8	150553	2565.42	1.70
1	130243	1908.94	1.47
1.2	104899	1556.67	1.48
1.3	96633	402.28	0.42
1.6	73614	1749.55	2.38

Table 2. The repeatability and intermediate precision tests of CTL (3.33x10-7 M)

	Repeatability			Intermediate Precision (n=18)
	Day 1 (n=6)	Day 2 (n=6)	Day 3 (n=6)	
	Area	Area	Area	Area
Mean	60192.2	60206.6	60265.6	60221.47
SD	179.70	102.50	125.07	133.33
RSD %	0.29	0.17	0.21	0.22

Table 3. The linearity results of CTL peak area signals in the concentration range of 1.11x10-7-5.55x10-7 M with 1.3 ml.min-1 flow-rate and at 239.5 nm detection wavelength.

	Intra-day			Inter-day
	Day 1 (n=6)	Day 2 (n=6)	Day 3 (n=6)	Whole Days (n=18)
Slope	$1.33x10^{11}$	$1.36x10^{11}$	$1.36x10^{11}$	$1.35x10^{11}$
Intercept	16169.20	15287.10	15316.80	15591.03
Correlation Coefficient	0.9995	0.9999	0.9999	0.9997

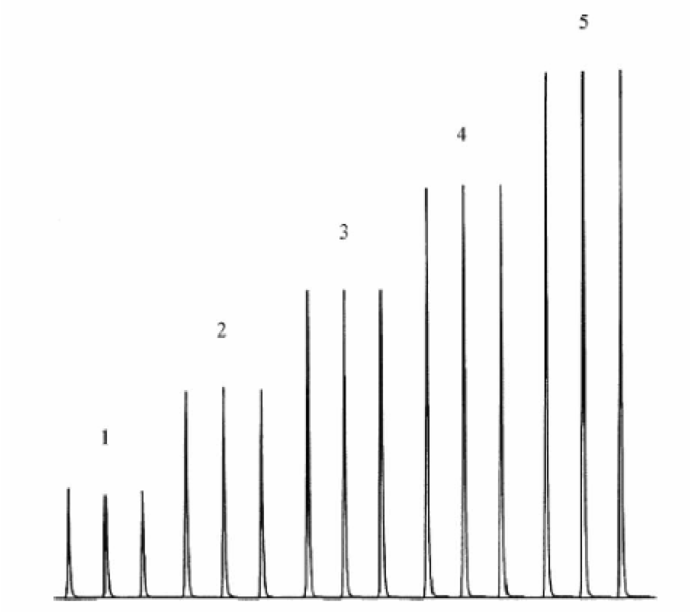

Figure 2. UV signal concentration of CTL (1, 1.11x10-7; 2, 2.22x10-7; 3, 3.33x10-7; 4, 4.44x10-7; 5, 5.55x10-7 M)

	FIA	UV
Mean (n=6)	20.15	20.03
SD	0.14	0.11
RSD %	0.7	0.53

Table 4. The determination results of CTL in commercial tablets (Declared amounts of tablets = 20 mg)

as environmental, food analysis, and biosensor technology. FIA methods were also successfully applied in pharmaceutical assays carried out in the past. This method has time saving, simple instrumentation and lower running cost which is preferable for routine analysis when it is compared to the general chromatographic methods (Can et al, 2008).

In our study, the results of statistical analysis show no significant difference between the proposed method and standard method. As a result, we concluded that the developed method here is simple, accurate, precise and rapid and it can be used in routine analysis of CTL.

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