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ORIGINAL ARTICLE / ÖZGÜN MAKALE

SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF FLUOXETINE AND OLANZAPINE GREENNES ASSESSMENT

FLUOKSETİN VE OLANZAPİN'İN EŞ ZAMANLI SPEKTROFOTOMETRİK TAYİNİ VE YEŞİL KİMYA UYGULAMALARI

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ABSTRACT

Objective: *In our study, the simultaneous determination of fluoxetine (FLX) and olanzapine (OLZ) was performed by absorbance subtraction and absorbance correction spectrophotometric methods.*
Material and Method: *The active substances were determined by choosing the isosbestic point of 232 nm in the absorbance subtraction method and 255 nm and 245 nm wavelengths in the absorbance correction method. The accuracy of the methods was determined by applying the percentage recovery studies to the laboratory mixtures. The percent recovery values were found in the range of 98.1-100.2 for OLZ and 96.8-105.3 for FLX. The concentration range studied was 3.12-15.62 and 3.45-17.28 µg/ml for OLZ and FLX, respectively.*
Result and Discussion: *In the study, two active substances used in antidepressant treatment were determined simultaneously. Today, these active substances used in the treatment have started to be used in combination in order to achieve a better effect of the treatment. Therefore, simultaneous analysis of two active substances becomes important. Two different spectrophotometric methods were used for analysis. The methods have been successfully applied and validated for the simultaneous determination of antidepressant active substances. Since the applied methods do not require pre-separation and can be applied directly, the amount of waste generated is reduced. Environmentally sensitive methods have been applied.*
Keywords: *Determination, fluoxetine, olanzapine, spectrophotometry*

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ÖZ

Amaç: *Çalışmada fluoksetin (FLX) ve olanzapinin (OLZ) etken maddelerinin aynı anda tayini absorbans çıkarma ve absorbans düzeltme spektrofotometrik yöntemleri uygulanarak yapılmıştır.*
Gereç ve Yöntem: *Etken maddeler absorbans çıkarma yönteminde isosbestik nokta olan 232 nm ve absorbans düzeltme yönteminde 255 nm, 245 nm dalga boyları seçilerek tayin edilmiştir. Yüzde geri kazanım çalışmaları laboratuvar karışımlarına uygulanarak yöntemlerin doğruluğu yapılmıştır. Yüzde geri kazanım değerleri OLZ için 98.1-100.2, FLX için 96.8-105.3 aralığında bulunmuştur. Çalışılan konsantrasyon aralığı, OLZ ve FLX için sırasıyla 3.12-15.62 ve 3.45-17.28 µg/ml dir.*
Sonuç ve Tartışma: *Çalışmada antidepresan tedavisinde kullanılan iki etken madde aynı anda tayin edilmiştir. Günümüzde tedavide kulanılan bu etken maddeler tedavinin etkisinin daha iyi sağlanabilmesi için kombinasyon şeklinde kullanıma sunulmaya başlanmıştır. Bu nedenle iki etken maddenin aynı anda analizi önem kazanmaktadır. Analiz için iki farklı spektrofotometrik yöntem uygulanmıştır. Yöntemler antidepresan etken maddelerin aynı anda tayini için başarıyla uygulanmış ve valide edilmiştir. Uygulanan yöntemler ön ayırma gerektirmediği ve direkt olarak uygulanabilmesi sayesinde oluşan atık miktarı azalmaktadır. Çevreye hassas olarak yöntemler uygulanmıştır.*

Anahtar Kelimeler: *Fluoksetin, kantitatif belirleme, olanzapin, spektrofotometri*

INTRODUCTION

Low mood is an indicator of depression, an illness that involves abnormalities in numerous brain networks. Research has shown that one of the most significant disorders affecting human health and longevity is depression. Antidepressants are frequently used to treat various forms of depression, bipolar affective disorder, anxiety [1,2].

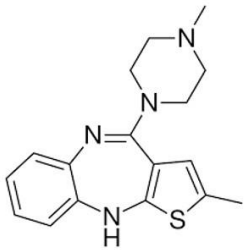


Figure 1. Chemical structure of OLZ

The combined use of the active ingredients of olanzapine (OLZ), an atypical antipsychotic, and fluoxetine (FLX), a selective serotonin reuptake inhibitor, is more effective in alleviating bipolar I depression than the use of OLZ alone [3]. OLZ chemical name is 2-methyl-4-(4-methylpiperazin-1-yl)-10*H*-thieno[2,3-b][1,5]benzodiazepine (Figure 1). FLX, *N*-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propan-1-amine (Figure 2) is used as an antidepressant.

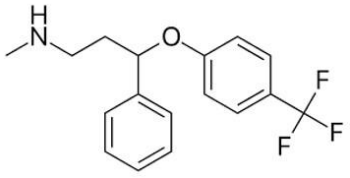


Figure 2. Chemical structure of FLX

In green chemistry applications, it is important to reduce the amount of waste generated, to use less harmful solvents, to reduce the amount of solvent used, to make direct determination without pre-preparation steps and to reduce energy use [4]. Methods are being developed to reduce the damage to

the environment due to the analysis. The method we have developed has also been applied in an environmentally friendly manner. To apply the experimental used device was consumed lower energy. Complex devices such as HPLC, LC-MS consume more energy. To apply the experimental used device was consumed lower energy. Also, no pre-preparation, extraction or derivatization process was applied in the proposed method. Experiments were carried out by dissolving the active ingredients directly in a single solvent. The effects of waste on the environment have been minimized due to their direct and indirect effects on our health.

The use of active substances in combination is increasing. therefore, it is important to be able to analyze drugs simultaneously. There are several studies in the literature conducted with different methods. In the literature, two active substances were determined simultaneously by the HPLC method [5,6]. Liquid-liquid extraction was analyzed by GC-MS, HPLC-MS/MS and LC-MS/MS methods [7-10]. Analysis was performed in LC-MS/MS plasma with a protein precipitation process [11], and in another study, analysis was performed from plasma by applying a microextraction process [12] in human plasma with solid phase extraction and LC-MS/MS methods [13,14]. These analyzes are made using expensive devices, the energy consumption is high and the sample preparation processes are laborious.

There are also several derivative spectrophotometric methods when the literature is searched. First derivative or ratio derivative methods are used in the methods [15,16]. The active ingredient of OLZ was determined by the spectrophotometric method during sample preparation [17,18]. FLX was quantified by spectrophotometric or spectrofluorimetric method [19,20].

The use of combined drugs in the treatment of depression is increasing. For this reason, it is important to analyze drugs simultaneously. Expensive devices and many chemicals were used in previous methods. Quantitative determination was made simply and easily with the new spectrophotometric methods applied.

The applied analysis method is simple, fast, can be applied in routine analysis laboratories without requiring preliminary preparation and expensive programs. In this study, the simultaneous determination of the active ingredients of OLZ and FLX was analyzed using two spectrophotometric methods. The first method is absorbance subtraction, and the second method is the absorbance correction method. The methods were applied directly for the determination of active substances. Validation studies have been carried out. The method was applied without the need for complex devices and was applied directly. No additional chemical agents were added. Because of these features, the developed method is sensitive to the environment. Our aim in this study is to analyze two active substances, which are important to analyze simultaneously, simply, using simple devices, in accordance with green chemistry.

MATERIAL AND METHOD

Instrument and Software

The spectra of the active substances were taken in the spectrophotometer device, in the UV region, between 200-400 nm. A quartz cuvette was used for measurements. The brand and model of the dual-beam spectrophotometer device used is Shimadzu UV 1800. UV probe 2.52 was used as software for the spectra. Excel program was used to create calibration curves and apply data.

Used Chemicals

Used all materials were of analytical grade. Reference standards were kindly supplied by Abdi Ibrahim Pharmaceutical Industry, Turkey. Liquid chromatography grade methanol was purchased from Merck (Darmstadt, Germany).

Preparation of Standard Solutions

By transferring the 10.0 mg of active substances into 50.0 ml volumetric flasks containing methanol, standard stock solutions of each of the two analytes, equal to (0.2 mg/ml), of OLP and FLX were prepared separately. Active substances solutions containing 3.12-15.62 µg/ml of OLP, 3.45-17.29 µg/ml of FLX were solved separately in methanol. Laboratory mixtures were prepared three replicates in certain proportions and measurements were made and percent recovery values were calculated.

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RESULT AND DISCUSSION

In the study, two active substances were analyzed by absorbance subtraction and absorbance correction methods. The spectra of OLZ and FLX between 200-300 nm are given in Figure 3. The methods are applied direct prepared laboratory mixture.

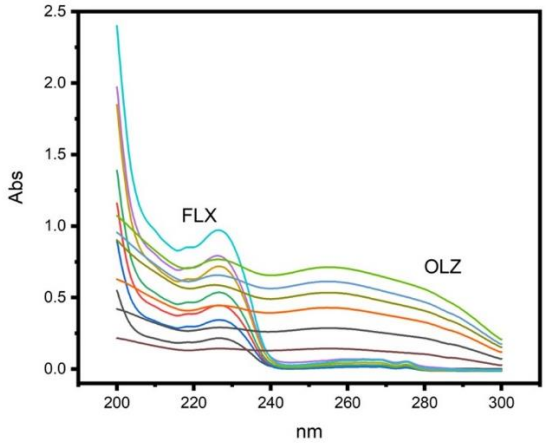


Figure 3. Zero order spectrum of active ingredient

Absorbance Subtraction Method

The first of the applied methods is the absorbance subtraction method. This method is based on the isoabsorption point spectrophotometric method. At the point of isoabsorption, the drug mixture acts as a single ingredient as they exhibit equal absorptive value. Thus, by measuring the absorbance value at the selected isoabsorption point (λiso), the total concentration of both drugs (λiso) can be calculated using the absorbance [21].

The isosbestic point of 232 nm was chosen to apply the method. The isosbestic points of the active ingredients are given in Figure 4. 280 nm was chosen as the second wavelength. The absorbance factor value for OLZ was calculated by dividing the absorbance at the isosbestic point by the absorbance at 280 nm and its value was found to be 1.29.

To determine OLZ from the mixture, the absorbance of the mixture at 280 nm was multiplied by the absorbance factor value. The concentration was calculated with the help of the calibration curve created according to the concentration versus the absorbance at the isosbestic point. The parameters of the method are given in Table 1.

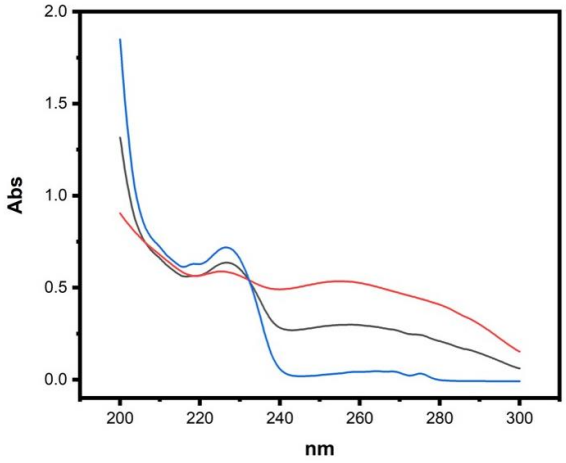


Figure 4. Isosbestic point of active ingredient

Table 1. The parameter of the absorbance subtraction method

Parameters	OLZ	FLX
Concentration range (µg/ml)	3.12-15.62	3.45-17.28
Wavelength, nm	232	232
Intercept value	-0.0053	-0.0053
Slope value	0.0461	0.0461
Correlation coefficient, R^2	0.9965	0.9965

Absorbance Correction Method

Two wavelengths, 245 and 255 nm, were chosen to apply the absorbance correction method. To calculate the absorbance correction factor value, the absorbance value of OLZ at 255 nm was mathematically divided by the absorbance value at 245 nm. The wavelength of the mixture at 245 nm was multiplied by the absorbance correction factor value. In order to calculate the FLX concentration, this calculated value from the absorbance value of the mixture at 255 nm was subtracted. The concentration of OLZ in the mixture was calculated. Absorbance at 255 nm versus concentration was plotted to generate the calibration curve for the method. The calibration curve parameters are given in Table 2.

Absorbance correction factor $F_{OLZ} = Abs_{255\ nm} / Abs_{245\ nm}$

Table 2. The calibration curve parameter of the absorbance correction method

Parameters	OLZ	FLX
Concentration range (µg/ml)	3.12-15.62	3.45-17.28
Wavelength, nm	255	255
Intercept value	0.0027	-0.0154
Slope value	0.0464	0.0036
Correlation coefficient, R^2	0.9965	0.9965

Recovery Results for Methods

Recovery studies were calculated for the laboratory mixture. These mixtures were prepared in different concentration series. The recovery results were shown in Table 3.

Table 3. The recovery results of absorbance correction and subtraction method

Sample	Added concentration		Recovery %	
	OLZ	FLX	OLZ	FLX
Absorbance correction method				
Sample I	6.25	6.91	100.2	105.3
Sample II	9.37	6.91	100.3	96.7
Sample III	6.25	10.37	103.2	97.4
Absorbance subtraction method				
Sample I	6.25	6.91	97.2	96.9
Sample II	9.37	6.91	98.4	96.8
Sample III	6.25	10.37	98.1	99.2

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Precision Results for Methods

Precision studies were carried out to demonstrate the reproducibility of the methods. The calculated results are given in Table 4.

Table 4. The precision results of absorbance correction and subtraction method

Sample	Added concentration		Found concentration %	
	OLZ	FLX	OLZ	FLX
Absorbance correction method				
1. day	10.93	10.37	102.2	104.3
2. day	10.93	10.37	100.9	98.7
3. day	10.93	10.37	103.4	99.4
Absorbance subtraction method				
1. day	10.93	10.37	97.9	97.9
2. day	10.93	10.37	99.4	99.8
3. day	10.93	10.37	98.8	101.2

In the method applied, no separation process was applied and no additional chemical was used, and it was determined directly (Table 5).

Table 5. Comparison table with other studies

Analyte	Reagent	Linear range (µg/ml)	Correlation coefficent	Recovery %	Reference
FLX and OLZ	-	3.45-17.28 and 3.12-15.62	0.9965	97.4-100.2	Proposed method (Absorbance correction)
FLX and OLZ	-	3.45-17.28 and 3.12-15.62	0.9965	99.2-98.1	Proposed method (Absorbance subtraction)
FLX and OLZ	-	100.00-600.00 and 5.00-17.50	0.9998 and 0.9999	100.78 and 100.28	15
FLX and OLZ	HCl	8.00-80.00 and 2.00-20.00	0.9991 and 0.9997	98.9 and 95.2	16
OLZ	HCl and phosphate buffer saline	3.00-18.00	0.9999 and 0.9998	99.8-101.5	17
FLX	HCl and 3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid sodium salt	9.00-54.00	0.9995	100.5	19

Assessment of Greenness for Methods

When the method is evaluated for the environment, it is superior to other applied methods in that it does not use a complex device, does not have complex pre-preparation processes and does not have an extraction step.

A green analytical procedure index (GAPI) evaluation was made for the method. According to

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the GAPI, the methods are examined according to 15 different parameters such as sample preparation, solvents used, and energy consumption [22]. The applied method was examined according to the GAPI and the GAPI results for the method we applied are shown in Figure 5.

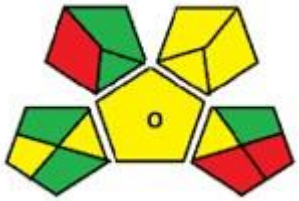


Figure 5. GAPI result of applied methods

In conclusion, two spectrophotometric method was applied active ingredient of FLX and OLZ. The method applied as simple, practically and without pre-preparation process the comparison of the other method. The active ingredients were solved simple and were measured directly. Thanks to the applied methods two substances were determined simultaneously without expensive devices. Therefore, the energy consumption is reduced. The chemical reagents were not used because of pretreatment procedure. The applied methods were greenness for environmental for this reason. These methods can be applied routine analysis laboratory.

AUTHOR CONTRIBUTIONS

Concept: G.T., N.E.; Design: G.T., N.E.; Control: G.T., E.O., N.E.; Sources: G.T., E.O., N.E.; Materials: G.T., E.O., N.E.; Data Collection and/or Processing: G.T., E.O., N.E.; Analysis and/or Interpretation: G.T., E.O., N.E.; Literature Review: G.T., E.O., N.E.; Manuscript Writing: G.T., N.E.; Critical Review: G.T., N.E.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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