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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ANALYSIS OF ABACAVIR LAMIVUDINE AND ZIDOVUDINE BY SPECTROPHOTOMETRIC METHOD IN TRIPLE MIXTURE

ABAKAVİR, LAMİVUDİN VE ZİDOVUDİN'İN ÜÇLÜ KARIŞIMDA SPEKTROFOTOMETRİK ANALİZİ

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ABSTRACT

Objective: *The simultaneous determination of abacavir (ABV), lamivudine (LMV) and zidovudine (ZDV) were applied by dual amplitude difference method coupled with ratio difference spectrophotometric methods.*
Material and Method: *The LMV was quantified by selected the 226.0 nm and 235.0 nm in the dual amplitude difference method. For ratio difference method 297.0 nm and 268.0 nm wavelengths and 266.0 nm, 245 nm wavelengths were chosen to quantify respectively ABV and ZDV. Accuracy studies have been carried out with percent recovery.*
Result and Discussion: *The proposed study, three active substances used in Human immunodeficiency virus (HIV) treatment were quantified. These active ingredients are used in combination to provide effective treatment. With the applied methods, firstly LMV was determined by dual amplitut difference method, then ABV and ZDV were determined by ratio difference. The three active ingredients were studied in the concentration range of 3-21 µg/ml. Correlation coefficients were found to be between 0.9985 and 0.9996. Recovery results range from 95.2 to 106.2. In the method, it was only dissolved in the solvent and measured, and the analysis was carried out without pre-preparation and expensive equipment.*
Keywords: *Abacavir, determination, lamivudine, zidovudine*

ÖZ

Amaç: *Çalışmada abacavir (ABV), lamivudine (LMV) ve zidovudine (ZDV) etken maddelerinin aynı*

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anda tayini ikili amplitüd farkı ile birlikte oran farkı spektrofotometrik yöntemleri uygulanarak yapılmıştır.

Gereç ve Yöntem: *İkili amplitüd farkı yönteminde 226.0 nm ve 235.0 nm seçilerek LMV için miktar tayini yapılmıştır. Oran farkı yöntemi için 297.0 nm ve 268.0 nm dalga boyları ve 266.0 nm, 245 nm dalga boyları sırasıyla ABV ve ZDV'nin miktar tayini için seçilmiştir. Yöntemlerin doğruluğu laboratuvar karışımlarına yüzde geri kazanım çalışmaları uygulanarak belirlenmiştir.*

Sonuç ve Tartışma: *Önerilen çalışmada, HIV tedavisinde kullanılan üç farklı etken maddenin aynı anda miktar tayini yapılmıştır. Yöntemler antiviral ilaçların aynı anda tayini için başarı ile uygulanmıştır. Bu etken maddeler, etkili tedavinin sağlanması için kombinasyon olarak kullanılmaktadır. Uygulanan yöntemler ile, ilk olarak dual amplitut difference yöntemi ile LMV tayin edilmiş sonrasında oran farkı ile ABV ve ZDV tayin edilmiştir. Üç etken madde 3-21 µg/ml konsantrasyon aralığında çalışılmıştır. Korelasyon katsayısı 0.9985 ile 0.9996 olarak bulunmuştur. Geri kazanım sonuçları 95.2 ile 106.2 arasındadır. Yöntemde sadece çözücüde çözünüp ölçülmüştür ön hazırlama işlemi ve pahalı cihazlar olmadan analiz yapılmıştır.*

Anahtar Kelimeler: *Abacavir, kantitatif belirleme, lamivudin, zidovudin*

INTRODUCTION

Viruses are the infectious agents that have a simple structure and can only multiply in their more developed cells in human, animal, plant, bacteria and similar organisms. Viruses have to use the biochemical mechanisms of the cells they enter to synthesize their own genetic material and new viral proteins. Human immunodeficiency virus (HIV) is an RNA virus of the retrovirus type. It retains T lymphocytes in blood and lymphoid tissue.

There are many different classes of antiretroviral used in HIV treatment. Antiretroviral drugs are classified as nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors according to their mechanism of action. The purpose of these active substances used in combination is to prevent HIV replication.

Abacavir (ABV), (4-(2-amino-6-(cycloproylamino)-9*H*-purin-9-yl)cyclopent-2-enylmethanol (Figure 1A) is an antiretroviral drug used orally. It is a guanosine nucleoside reverse transcriptase inhibitor that can be used in combination with other antiretroviral active substances. Lamivudine (LMV), 4-amino-1-((2R,5S-2-(hydroxymethyl)-1,3-oxathiolan-5-yl) pyrimidin-2-(1*H*)-one (Figure 1B) is an antiviral drug used in combination with other drugs in the treatment of HIV infection. Zidovudine (ZDV), 3-azido-3-deoxythymidine (Figure 1C) is used in the treatment of HIV infection in adults and children [1].

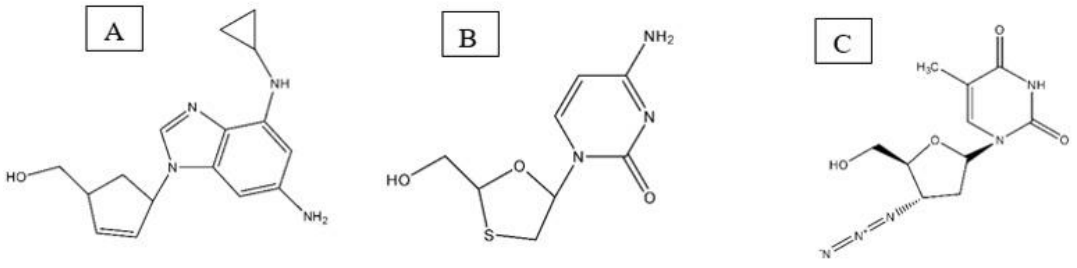

Figure 1. Chemical structure of ABV, LMV and ZDV

In literature, the drugs have been determined using different techniques. Several techniques have been studied quantification for three drug, ABV, LMV and ZDV, chemometric method [2], LC-MS/MS [3-5], high-performance liquid chromatography (HPLC) [6-11]. Analyzes were made in different matrices, including pharmaceutical preparation, wastewater, and plasma.

Due to the role of drugs in the form of combination in therapy, simultaneous quantification of the active substances is important and there is a lack of such a spectrophotometric method in the literature.

In this study, we present a simple, not need a special program, cost reduced, and accuracy validated analytical method to determine ABV, LMV and ZDV in prepared laboratory solution by novel spectrophotometric methods without any pretreatment step. The method is dual amplitude difference method coupled with ratio difference spectrophotometric method. The method can be applied in quality control laboratories for the simultaneous quantification of three active substances. The goal we want to reach in this study is to analyze simultaneously three active substances, which are important to analyze simultaneously, simply, using simple devices. To apply the experimental used device was consumed lower energy. 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. Applied experiment, three active substances used in the treatment of HIV were determined simultaneously. Spectrophotometer, which can be found in every laboratory, and software were used in the quantitative determination study. This allowed the method to be applied easily and economically.

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 15.0 mg of active substances into 50.0 ml volumetric flasks containing methanol, standard stock solutions of each of the three analytes, equal to (0.3 mg/ml), of ABV, LMV and ZDV were prepared separately. Active substances solutions containing 3.0-21.0 µg/ml of ABV, LMV and ZDV were solved separately in methanol. Laboratory mixtures were prepared three replicates in certain proportions and measurements were made and percent recovery values were calculated.

RESULT AND DISCUSSION

Three active ingredients were analyzed by dual amplitude difference method coupled with ratio difference spectrophotometric technique. The spectrum of ABV, LMV and ZDV between 200-400 nm are given in Figure 2. The methods are applied direct prepared laboratory mixture.

Dual Amplitude Difference Method Coupled with Ratio Difference

In the dual amplitude method, two wavelengths were selected and a factor spectrum was constructed [12].

Using ZDV as a divisor, the dual amplitude difference method enables the elimination of the interfering component. ZDV will thus cancel out when the subtract between two chosen wavelengths is obtained since it is a constant whereas the ratio of ABV to ZDV has the same amplitudes. This enables the easy measurement of LMV in different synthetic-prepared combinations with varied ratios of ABV and ZID.

The spectrum of the laboratory mixture was divided by the ZDV' spectra (6.0 µg/ml) as a divisor in the proposed approach to obtain a ratio spectra. For the LMV's finding of a zero amplitude difference for ABV and ZID, the amplitudes at 226.0 and 235.0 nm were selected, Figure 3.

The ternary mixture is divided into the ZDV spectrum. Amplitude difference was found in the obtained ratio spectrum. The amplitude difference (226.0 and 235.0 nm) was multiplied by the factorized

spectra. The ratio spectra of LMV to ZDV was constructed. To obtain the factorize spectrum, the LMV spectrum was divided ZDV spectrum and the obtained spectrum was divided into selected wavelengths (226.0 and 235.0 nm) difference. The constructed of factorize ratio spectrum is shown in Figure 4.

The zero order spectrum of LMV were created by multiplying the resulting ratio spectra by the ZDV' (divisor) (6.0 µg/ml). Thus, the LMV compound was determined at its maximum absorbance. The maximum absorbance of LMV was determined at 272.0 nm over the 3.0–21.0 µg/ml calibration range.

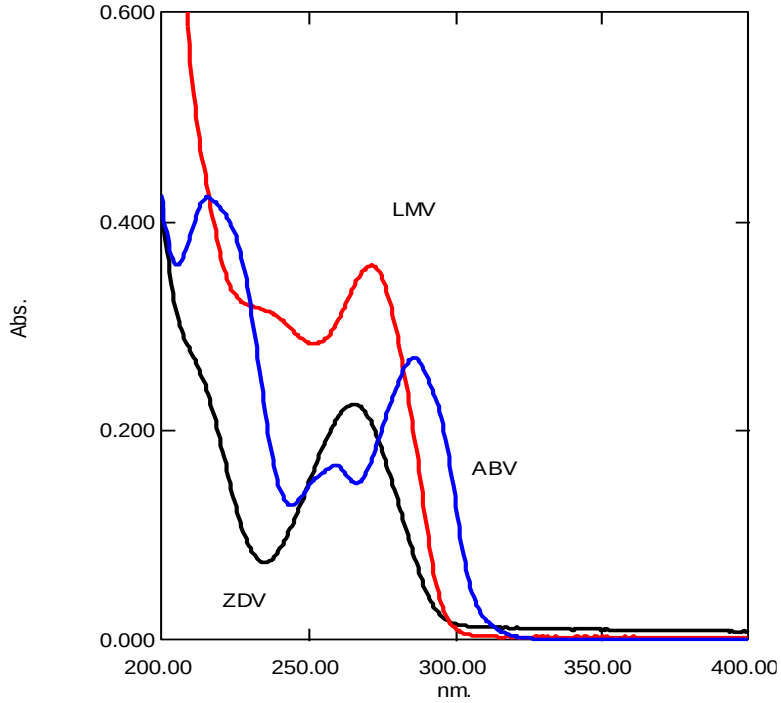

Figure 2. Zero order spectrum of ABV, LMV and ZDV

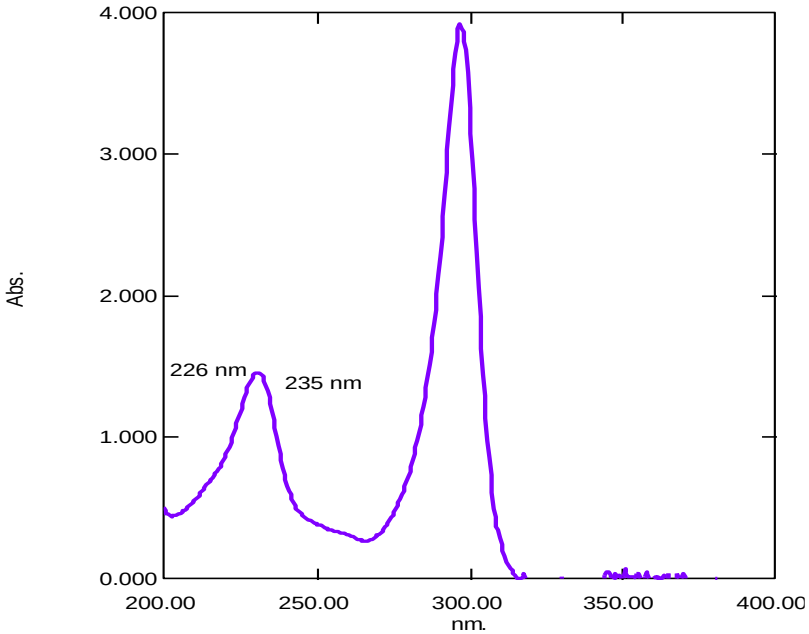

Figure 3. Ratio spectrum of ABV (3.0 µg/ml) to ZDV (6.0 µg/ml)

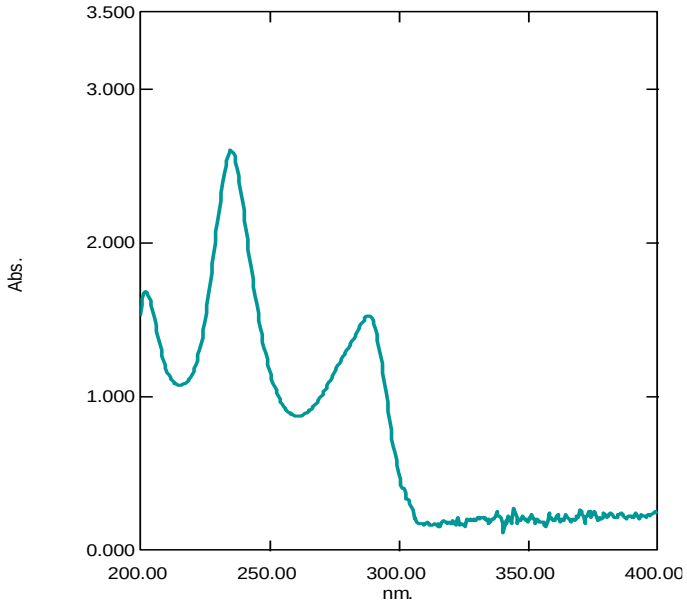

Figure 4. The obtain factor spectrum of LMV

ABV and ZDV mixture was obtained by subtracting the LMV spectra (D^0) from the relevant mixture.

For a mixture of ABV and ZDV was applied ratio difference method. For this method different ZDV's spectrums were divided by constant ABV's spectrum. Two different wavelengths were selected to obtain the ratio spectrum. A calibration graph was drawn by plotting the difference between the selected wavelengths (266.0 nm and 255.0 nm) against the concentration. To determine the ABV, a ratio spectrum was constructed. While creating the ratio spectrum, ABV spectrums were divided into ZDV spectrums. The selected wavelengths were 297.0 and 268.0 nm. This ratio spectrums were shown in Figure 5.

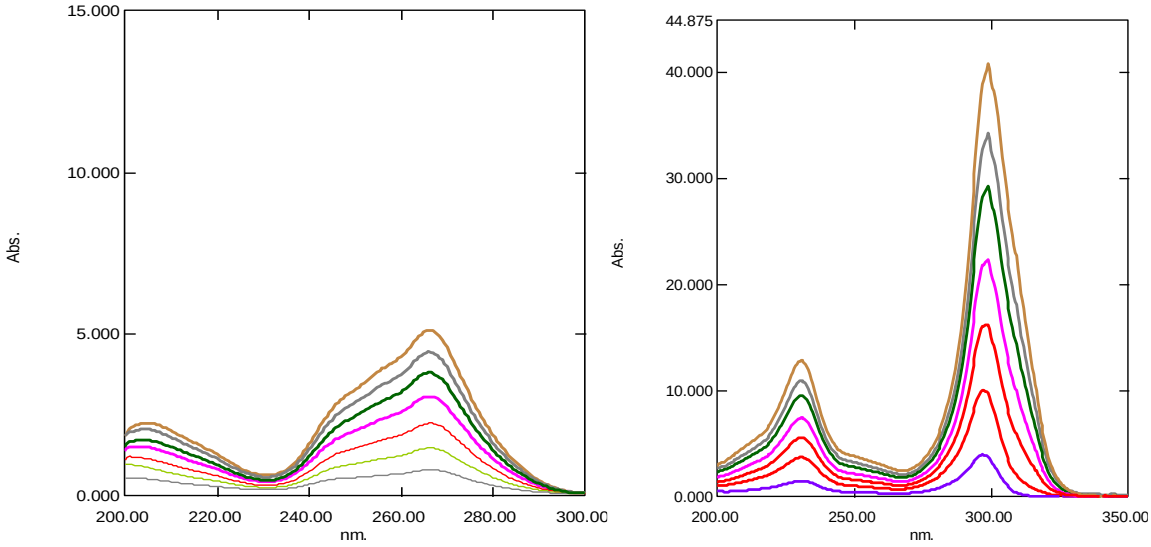

Figure 5. The ratio spectrum of ZDV and ABV

A calibration graph was drawn against concentration versus difference wavelengths for ABV and ZDV. Calibration graph parameters were given in Table 1.

Table 1. The parameter of the spectrophotometric method

Parameters	ABV	LMV	ZDV
Concentration range (µg/ml)	3.0-21.0	3.0-21.0	3.0-21.0
Wavelength, nm	297.0-268.0	272.0	266.0-255.0
Intercept value	-1.531	-0.012	-0.009
Slope value	1.808	0.040	0.062
Correlation coefficient, R^2	0.9996	0.9987	0.9985

Recovery Results for Methods

Recovery experiments were made from the laboratory mixture. These mixtures were prepared in different concentration series. The recovery values were illustrated in table 2.

Table 2. The recovery results of spectrophotometric method

Sample	Added concentration			Recovery %		
	ABV	LMV	ZDV	ABV	LMV	ZDV
Dual amplitude difference coupled with ratio difference method						
Sample I	3.0	3.0	6.0	106.2	102.3	96.4
Sample II	3.0	3.0	12.0	104.3	98.7	95.2
Sample III	3.0	6.0	3.0	103.2	99.4	97.2
Sample IV	9.0	9.0	18.0	95.4	97.2	96.1
Sample V	18.0	9.0	18.0	97.2	98.9	98.2

Precision Results for Methods

Precision studies were carried out to demonstrate the reproducibility of the methods. The calculated results are given in Table 3.

Table 3. The precision results of dual amplitude difference coupled with ratio difference method

Sample	Added concentration			Found concentration %		
	ABV	LMV	ZDV	ABV	LMV	ZDV
1. day	12.0	12.0	12.0	101.2	103.3	100.5
2. day	12.0	12.0	12.0	100.4	97.8	104.1
3. day	12.0	12.0	12.0	102.4	99.2	103.2

In the study was applied directly by spectrophotometer and without derivatization procedure (Table 4).

Table 4. The comparison of other methods

Analyte	Linear range µg/ml	Correlation coefficient	Recovery %	Reference
ABA, LAM and ZID	3.0-21.0	0.9996, 0.9987 and 0.9985	95.2-106.2	Proposed method
ABA, LAM and ZID	0.015-5.0	-	92.0-102.0	8
ABA, LAM and ZID	4.9-306.0	0.9999, 0.9999 and 0.9997	98.7-103.7	9

Assessment of Greenness for Methods

When the method is evaluated for the environment, it is superior to other applied methods in that it was used a simple device, easy processes and did not have an extraction step.

A green analytical procedure index (GAPI) evaluation was made for the method. According to the GAPI, the methods are examined according to 15 different parameters such as sample preparation, solvents used, and energy consumption [13]. The applied method was examined according to the GAPI and the GAPI results for the method we applied are shown in Figure 7.

Figure 7. GAPI result of applied methods

In conclusion, the applied spectrophotometric method was applied active ingredients of ABV, LMV and ZDV. The spectrophotometric method is dual amplitude difference method coupled with ratio difference. The method was applied to the active ingredients in two steps. First, LMV was obtained by dual amplitude difference method and then ABA and ZID were determined by ratio difference method. The method applied as fast, practically and without pre-preparation step the comparison of the other method. The active ingredients were solved simple and were measured directly. Thanks to the applied methods three drugs 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 easily for determination of three active substances and it is an easily applicable method in the laboratory.

AUTHOR CONTRIBUTIONS

Concept: G.T., N.E.; Design: G.T., N.E.; Control: G.T., N.E.; Sources: G.T., N.E.; Materials: G.T., N.E.; Data Collection and/or Processing: G.T., N.E.; Analysis and/or Interpretation: G.T., N.E.; Literature Review: G.T., 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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