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Research Article

Extractive Spectrophotometric Method for the Determination of Lamivudine and Zidovudine in Pharmaceutical Preparations Using Bromocresol Purple

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A new spectrophotometric method has been established for the quantitation of lamivudine (LVD) and zidovudine (ZVD) in pharmaceutical preparations. The method is based on the reaction between the investigated drug substances and bromocresol purple (BCP) producing ion-pair complexes in acidic buffers which are suitable for chloroform extraction. The maximum absorbance of these complexes was measured at 424 nm in chloroform. All variables were studied to optimize the reaction conditions. Linearity ranges were found to be 25–250 μg mL^{-1} for LVD-BCP and 50–300 μg mL^{-1} for ZVD-BCP. The developed method was successfully applied for the determination of these drugs in pharmaceutical preparations. Excipients in pharmaceutical formulations did not interfere in the analysis. The results were compared statistically with those obtained by the HPLC method reported in the literature. According to the results, the proposed method can be recommended for quality control and routine analysis.

1. Introduction

Zidovudine (ZDV, 3′-azido-3′-deoxythymidine) and lamivudine (LVD, 2′-deoxy-3′-thiacytidine) (Figure 1) are synthetic nucleoside analog reverse transcriptase inhibitors (NRTIs) with activity against the human immunodeficiency virus (HIV) with a long history of use in HIV-infected patients [1, 2].

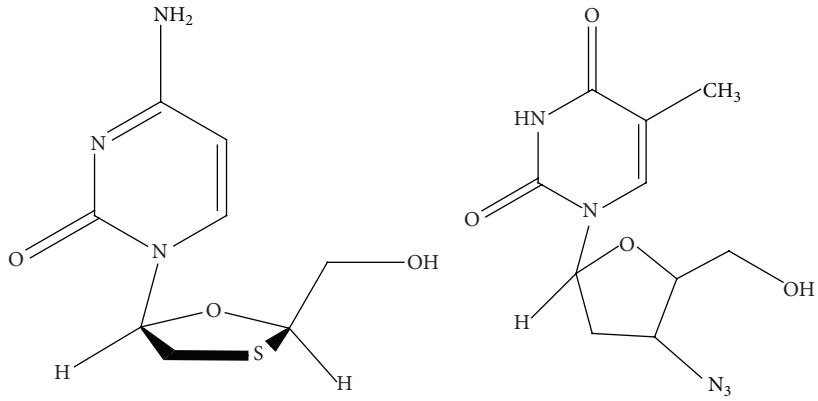
The literature reveals various methods for the determination of the mentioned drug substances in biological fluids and pharmaceutical preparations such as UV-visible spectrophotometry [3–5], high performance liquid chromatography (HPLC) [6–12], capillary zone electrophoresis [13] for LVD, UV-visible spectrophotometry [14, 15], HPLC [16–18], and high performance thin layer chromatography (HPTLC) [19, 20] for ZVD.

Simple, selective, and sensitive spectrophotometric methods are required for the determination of these substances. But lack of chromophore group in their structure makes direct UV detection inapplicable. In this case, formation of ion-pair complexes enables UV-visible detection. There are some studies in the literature for drug analysis depending on this complex composition by using different dyes such as bromophenol blue, bromocresol green, methyl orange, and methylene blue [15, 21–24]. One of them [15] is developed for ZVD but requires additional chemicals and more detailed reaction procedure than the proposed method.

The aim of the proposed study is to develop a spectrophotometric method by using bromocresol purple (BCP) for LVD and ZVD that can be used in routine laboratories without requiring high cost equipment and sophisticated operator such as HPLC. Moreover, the developed method is simple, rapid, accurate, precise, and sensitive for the determination of LVD and ZVD in bulk and pharmaceutical forms.

2. Experimental

2.1. Apparatus. Measurements were taken on a Hitachi spectrofluorimeter Model U-2900 equipped with Xenon lamp and 1 cm quartz cells. pH measurements were carried out with WTW pH 526 digital pH meter, calibrated with buffer solution (pH 7.0).



LVD
$C_8H_{11}N_3O_3S$
(a)

ZVD
$C_{10}H_{13}N_5O_4$
(b)

Figure 1: Chemical structures of LVD and ZDV.

2.2. Reagents and Solutions. All chemicals and reagents were of analytical-reagent grade. LVD, ZVD, and their pharmaceutical preparations Zeffix (containing 100 mg of LVD per tablet) and Retrovir (containing 250 mg of ZVD per capsule) were kindly supplied by GlaxoSmithKline Pharmaceutical Industry (Istanbul, Turkey). BCP was obtained from Merck (Darmstadt, Germany). Bidistilled water was used throughout the work.

BCP solution (0.05%) was prepared in 10% ethanol in water. The stock solutions of LVD and ZVD (1 mg mL^{-1}) were prepared in water.

2.3. Procedure. Various volumes from stock solutions of LVD and ZVD were transferred to stoppered glass tubes, and then the volume of LVD solution was completed to 1 mL and ZVD solution was competed to 0.5 mL by adding water. To each tube 2 mL phthalate buffer (pH 2.0 for LVD and pH 2.5 for ZVD) were added followed by the addition of 2 mL (for LVD) and 2.5 mL (for ZVD) of 0.05% BCP solution. Each reaction mixture, with 5 mL total volume, was extracted with 5 mL chloroform by mixing for 2 min. with a vortex mixer. The two phases were allowed to separate and the chloroform layers were passed through anhydrous sodium sulphate. The organic layers were completed to 5 mL with chloroform in volumetric flasks. The absorbances of the yellow-colored chloroformic extracts were measured at 424 nm at room temperature against blank solution, prepared similarly except addition of the drug substances.

2.4. Sample Preparation. Twenty capsules or tablets were weighed and an amount equivalent to one tablet (each tablet contains 100 mg LVD) and one capsule (each capsule contains 250 mg ZVD) was accurately weighed and transferred into 100 mL (for LVD) and 250 mL (for ZVD) calibrated flasks. 50 mL water was added to each flask, and then the mixtures were shaken mechanically and sonicated in an ultrasonic bath totally for 30 min and diluted with water, mixed, and filtered. A 10 mL of each filtrate was diluted to 50 mL and suitable aliquots were used for analysis using the procedure given above (Section 2.3).

3. Results and Discussion

3.1. Optimization of the Analytical Procedure. Optimum conditions necessary for rapid and quantitative formation of colored ion-pair complexes with maximum stability and sensitivity were established by a number of preliminary experiments.

3.1.1. Absorption Spectra. LVD and ZVD react with BCP in acidic buffer to give chloroform soluble yellow-colored ion-association complexes which exhibit absorption maximum at 424 nm. Under the experimental conditions, the reagent blank showed negligible absorbance as shown in Figure 2.

3.1.2. Effect of pH. The effect of pH was studied by extracting the colored complex in the presence of various buffers such as phosphate buffer (pH = 2.5–8.0), phthalate buffer (pH = 2.0–8.0), and acetate buffer (pH = 3.5–6.0). It was found that the maximum color intensity and constant absorbance were observed in phthalate buffer of pH 2.0 for LVD-BCP and pH 2.5 for ZVD-BCP (Figure 3).

3.1.3. Effect of Solvent. The effect of extraction solvent on the ion-pair complexes was examined. Chloroform, ethyl acetate, ether, dichloromethane, benzene, and methyl isobutyl ketone were tested as extraction solvents for effective extraction of colored species from aqueous phase. Chloroform was selected because of its slightly higher efficiency on color intensity and selective extraction of the drug-dye complex from the aqueous phase. It was observed that only one extraction with chloroform was adequate to achieve a quantitative recovery of the complex.

Table 1: Optical characteristics and statistical data of the regression equations for ZVD and LVD with BCP.

Parameter	LVD	ZVD
λ_{max} (nm)	414	414
Beer's law limit (μg mL^{-1})[b]	25–250	50–300
Molar absorptivity (l mol^{-1} cm^{-1})	3.68×10^3	3.047×10^3
Sandell's sensitivity (μg cm^{-2} per 0.001 absorbance unit)	1.604×10^{-5}	1.140×10^{-5}
Regression equation[a]		
Slope ± SD	0.0037 ± 0.00013	0.0036 ± 0.00026
Intercept ± SD	−0.0153 ± 0.000054	0.0772 ± 0.000050
Correlation coefficient, r	0.9995	0.9997
LOD (μg mL^{-1})	0.106	0.217
LOQ (μg mL^{-1})	0.352	0.722
Intra-day[b]	0.65	0.54
Inter-day[c]	0.87	0.71

[a] $A = a + bC$ (where C is the concentration of drug in μg mL^{-1}).
[b] Average of six determinations.
[c] Results of seven different days.

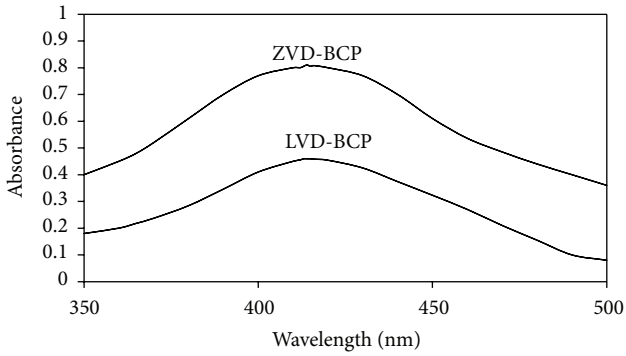


Figure 2: Absorption spectra of the ion pair complexes of LVD-BCP (100 μg mL^{-1} LVD) and ZVD-BCP (200 μg mL^{-1} ZVD).

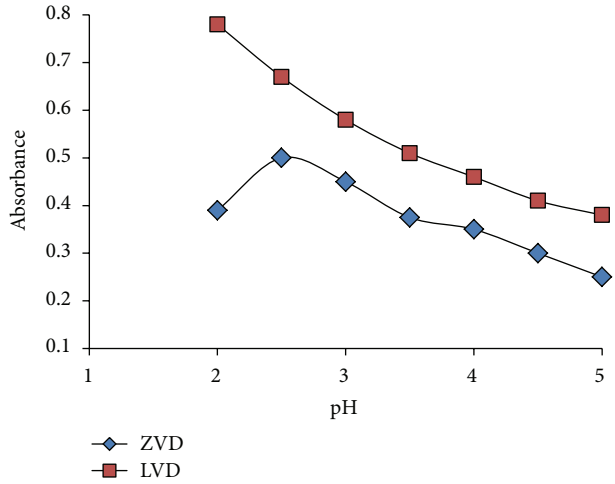


Figure 3: Effect of pH on the ion-pair complexes.

3.1.4. Effect of Reagent Concentration. The effect of dye concentration on the intensity of the color developed was tested using different volumes of the reagent. The results show that 2 mL (for LVD) and 2.5 mL (for ZVD) of BCP were found to be optimum and excess of dyes does not affect the color of the complex or the absorbance.

3.1.5. Effect of Temperature on the Colored Complexes. The effect of temperature on the colored complexes was studied at different temperatures. It was found that the colored complexes were stable up to 35°C. At higher temperature the drug concentration was found to increase due to volatile nature of chloroform. As a result, the absorbance of colored complexes increases. Besides, the resultant complexes were stable 48 h at 25 ± 1°C in the dark.

3.1.6. Effect of Shaking Form and Time. Ion-pair complexes were formed by using vortex mixer and shaking times ranging from 0.5 to 5 min were studied. Optimum shaking time as 2 min was selected for both methods.

3.1.7. Stoichiometric Relationship. In order to determine the molar ratio between the investigated drug substances and BCP, Job's method of continuous variation was used [25]. In this method, solutions of drugs and dyestuff with identical molar concentrations were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. These measurements showed that the stoichiometry of the reactions were found as 1 : 1.

3.2. Quantification

3.2.1. Linearity and Range. Beer's law range, molar absorptivity, Sandell's sensitivity, regression equation, and correlation coefficient are listed for each method in Table 1. A linear relationship was found between the absorbance at λ_{max} and the concentration of the drug in 25–250 μg mL^{-1} for LVD and 50–300 μg mL^{-1} for ZVD. Regression analysis of Beer's law

Table 2: Determination of LVD and ZVD in dosage forms by standard addition method.

	Amount of taken drug (μg)	Amount of pure drug added (μg)	Total amount found (μg)[a] (Mean ± S.D.[b])	RSD (%)	Recovery of pure drug added (%)
LVD	100	25	125.24 ± 1.28	1.02	100.96
	100	100	200.35 ± 1.01	0.50	100.35
	100	150	251.25 ± 2.21	0.88	100.83
ZVD	100	50	150.02 ± 1.02	0.68	100.04
	100	100	201.28 ± 1.24	0.62	101.28
	100	200	300.12 ± 2.08	0.69	100.06

[a]Five independent analyses.
[b]Standard deviation.

Table 3: Determination of LVD and ZVD in dosage forms by the proposed and reference methods.

Statistical value	LVD		ZVD	
	Proposed method[a]	Reference method [7]	Proposed method[b]	Reference method [6]
Mean[c]	100.12	100.13	249.57	249.93
Recovery (%)	100.12	100.13	99.83	99.97
RSD (%)	0.15	0.198	0.33	0.15
t-test of significance[d]	0.003		0.0088	
F-test of significance[d]	1.72		0.209	

[a]Zeffix (containing 100 mg of lamivudine per tablet).
[b]Retrovir (containing 250 mg of zidovudine) per capsules.
[c]Five independent analyses.
[d]$P = 0.05$, $t = 2.23$, $F = 5.05$.

plots at λ_{max} reveals a good correlation. The regression equations, $A = a + bC$ (where A is the absorbance of 1 cm layer, b is the slope, a is the intercept, and C is the concentration of the measured solution in $\mu g\,mL^{-1}$), were obtained by the least-squares method. High molar absorptivities of the resulting colored complexes indicate high sensitivity of the method.

3.2.2. Validation of the Method. The validity of the method for the analysis of LVD and ZVD in its pure state and in formulations were examined by analyzing the samples using the proposed procedure. The results obtained for pure drug are given in Table 1. The precision and accuracy of the method were tested by analyzing six replicates of the drug within Beer's law limits. The low values of relative standard deviation (RSD) indicate good precision and reproducibility of the method. The results of analysis of dosage forms are given in Table 1. The results were reproducible with low RSD values.

To study the accuracy of the method, recovery experiments were carried out by standard addition technique. For each drug, different amounts of pure sample solutions were added to two different concentrations of standard drug solution and assayed. The results are given in Table 2. The average percent recoveries were 100.04–101.28% for LVD and ZVD, respectively.

3.2.3. Analysis Pharmaceutical Preparations. The developed and validated method was applied to analyses of the drug substances in their pharmaceutical preparations. The results were compared statistically by Student's t-test and F-test with the results obtained by the HPLC methods described in the literature [6, 7]. The calculated t- and F-values did not exceed the tabulated values, indicating that there is no significant difference between the developed method and valid HPLC methods in the respect of mean values and standard deviations at 95% confidence level. Statistical results are given in Table 3.

4. Conclusion

A significant advantage of the extractive spectrophotometric method is its applicability for the determination of the compounds in a multicomponent mixture. Unlike the gas chromatography and HPLC procedures, the instrumentation is simple and is not of high cost. The sensitivity in terms of molar absorptivity and the precision in terms of RSD of the method are very suitable for the determination of LVD and ZVD in pure and dosage forms. The reagent utilized in the proposed method is cheaper and readily available, and procedures do not involve any critical reaction conditions or tedious sample preparation procedures. The commonly used additives such as magnesium trisilicate, magnesium stearate, lactose, starch, and carboxymethylcellulose do not interfere in the analysis. The proposed method is simple and rapid with high precision and accuracy when compared to many of the reported methods [3–5]. The proposed method can be used for analysis of these drug substances in quality control and research and routine laboratories.

Conflict of Interests

The author certifies that there is no conflict of interests with any financial organization regarding the investigated drug substances and used chemicals in the study.

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