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Validated spectrophotometric method for analysis of dipyridamole and Lamivudine using eosin Y.

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Abstract: Validated, accurate, simple and reliable spectrophotometric method was described for the assay of dipyridamole and lamivudine in their different dosage forms. The suggested method depended on a binary reddish orange coloured complex formation between eosin Y and each one of the two analytes in presence of carboxy methyl cellulose and aqueous buffered medium for dipyridamole and lamivudine, respectively at pH 3.7 . The coloured binary complexes exhibited maximum absorbance at 554.0 nm for dipyridamole and lamivudine. The absorbance concentration graphs were linear over ranges of (1.0-10.0 µg/mL) and (2.0-20.0 µg/mL), with lower detection limits of 0.27 µg/mL and 0.46 µg/mL and lower quantitation limits of 0.82 µg/mL and 1.40 µg/mL for dipyridamole and lamivudine, respectively. The developed method was applicable to the assay of dipyridamole and lamivudine in their different dosage forms, and the obtained results were in acceptable agreement with all those provided by reference methods. The present method is appropriate for the assay of the two drugs on account of its cost effectiveness, simplicity and rapidity.

Keywords: Spectrophotometric; Dipyridamole; Lamivudine, Eosin Y; Dosage Forms.

1. INTRODUCTION

Dipyridamole (DPD) is in a class of drugs called antiplatelet agents, it works by preventing excessive blood clotting, Figure 1 illustrates its chemical structure. DPD is a pyramidopyrimidine compound that modestly decreases platelet activity by acting as a phosphodiesterase inhibitor. It also has significant endothelial activity and functions as a vasodilator. DPD prevents the endothelium attachment of platelets and it is an inhibitor of adenosine reuptake. It is used in thromboembolic disorders and in the management of stroke. Oral DPD is used for the prophylaxis of thromboembolism after cardiac valve replacement; it also has been used in the management of myocardial infarction[1] . DPD is indicated as, anti-anginal, anti-thrombotic for prostatic valves and also indicated in case of pulmonary hypertension. It is unlikely to lead to a lasting decrease in blood pressure and this may explain why strokes rather than coronary events may be avoided by this drug. For infants, DPD is often prescribed to treat a rare condition called Kawasaki disease[2]. By reviewing the literature review, DPD was determined either singly or in combination with other drugs in their dosage forms by spectrophotometric [3,4], spectrofluorimetric[5] electrochemical[6] and HPLC methods [7,8].

Lamivudine (LMV) is in a class of drugs known as reverse transcriptase nucleoside inhibitors. LMV must be intracellularly converted to its triphosphate form, which then competes for incorporation into the developing viral DNA strand with cytosine triphosphate. This results in chain termination and prevents the replication of viral DNA. It works by reducing the amount of hepatitis B virus and HIV in the blood, so it has activity against hepatitis B and human immunodeficiency viruses. In addition to, it has been used with other antiviral drugs for the treatment of human immunodeficiency virus infections [9]. Furthermore, LMV is active against a human immunodeficiency virus resistant to zidovudine.[10] Chemical structure of LMV is illustrated in Figure 1. By reviewing the literature review, LMV was determined either singly or in combination with other drugs in their dosage forms. Examples of the reported methods, spectrophotometric [11,12] , HPLC [13,14], and capillary electophoretic methods [15,16].

Eosin Y is a xanthene dye commonly used for assaying many pharmaceutical compounds. As it has a single carboxyl group, it is known as an acidic dye. It is able to form ion-pair complexes with basic drugs in acid medium, this resulted in a major bathochromic

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shift in the dye's original UV-VIS spectrum or even a quenching effect on its native fluorescence spectrum that was then used for analytical purposes. The complexes formed between numerous pharmaceutical components and eosin Y for their spectrofluorimetic or spectrophotometric assay has been always investigated [17-19]. The method described is designed to study the binary complex formation between DPD or LMV and eosin Y in a trial to develop an easy, sensitive, lower-cost and accurate spectrophotometric method for analysis of DPD and LMV in their dosage forms.

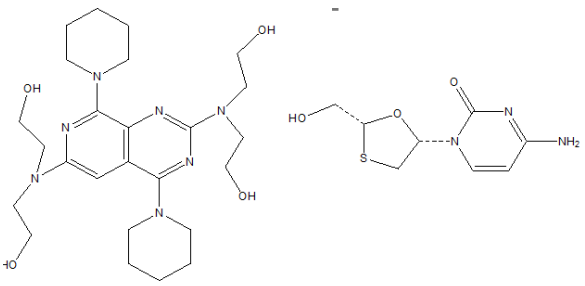

Figure (1): Chemical structure of Dipyridamole and Lamivudine, DPD; Chemical Formula: C24H40N8O4, Molecular Weight= 504.63. LMV; Chemical Formula: C8H11N3O3S, Molecular Weight = 229.3.

2. METHODS

2.1. Instrument

Shimadzu (Kyoto, Japan) UV-1601 PC, UV-Visible double-beam spectrophotometer was operated on a fast scan speed. For adjusting the pH, A HANNA pH-meter (Romania) was used.

2.2. Materials and reagents

Pure DPD was supplied by the Chemical Industries Development (CID) company, Giza, Egypt. (certified to have purity of 99.97%). Pure LMV was kindly provided by EVA PHARMA for Pharmaceuticals Medical Appliances S.A.E., Kafr El Gabal-Haram, Giza , Egypt. (certified to have a purity of 99.99%). DPD and LMV dosage forms were purchased from local pharmacies: Persantin® Tablets (batch # 103030), containing 75 mg of DPD/tablet. Lamidine® Tablets (batch # 402336), containing 150 mg of LMV/tablet. All reagents used were of analytical grade. During the work, distilled water was used and the solvents were of spectroscopic grade: Eosin Y was purchased from (Merck, Darmstadt, Germany). Solutions of Eosin ($2x10^{-3}$M) and (4×10^{-3} M) were prepared by dissolving 0.14gm or 0.28gm in 100mL distilled water, respectively. Anhydrous sodium acetate together with acetic acid were obtained from (Merck, Darmstadt, Germany). The required pH value was obtained through mixing appropriate volumes of (0.2 M) acetic acid and (0.2 M) sodium acetate solutions to prepare 0.2 M acetate buffer .[20] Surfactants: Citrimide, Sodium dodecyle sulphate (SDS), CMC, Tween 80, Concentrated sulfuric acid H_2SO_4 (99% purity) were obtained from El-Nasr Pharmaceutical Chemicals Company (ADWIC), Abu Zaabal, Egypt. 0.1%w/v aqueous solution of carboxy methyl cellulose (CMC) was prepared.

2.3. Standard solutions

Standard solutions (100.0 µg/mL) of DPD and LMV were prepared separately in 0.05M H_2SO_4 and distilled water for DPD and LMV, respectively and further dilution was made as appropriate using the same solvent.

2.4. Procedures

2.4.1. Construction of the calibration curves

For DPD, accurate volumes of the stock solution in concentration range (10.0-100.0 µg /mL) of drug were transferred into a set of volumetric flasks measures 10 mL. To each flask, 2 mL of (0.1%) CMC and 2mL of ($2x10^{-3}$ M) eosin Y were added and mixed well. Then, each flask was completed by the addition of (0.2 M) acetate buffer (pH 3.7).

For LMV, accurate volumes of the stock solution in concentration range (20.0-200.0µg/ml) of drug were transferred into a set of volumetric flasks measures 10 mL. To each flask, 4mL distilled water and1mL of (4x10-3 M) eosin Y were added and mixed well. Then, each flask was completed by the addition of (0.2 M) acetate buffer (pH 3.7).

For DPD and LMV, the absorbance of each concentration was measured at 554.0 nm against a suitable blank and absorbance was plotted versus the final drug concentration in µg /mL and the regression equations were derived.

2.4.2. Applications to dosage forms

Ten tablets (Persantine 75 mg or Lamidine 150 mg tablets) were weighted, finely powdered and thoroughly mixed well; then an exactly weighed quantity of the powder equivalent to 10.0 mg of DPD or LMV were transferred into100 mL volumetric flasks, and extracted with 75 mL of 0.05M H_2SO_4 or distilled water for DPD or LMV, respectively. The flasks were subjected to sonication for 30 min and completed with the suitable solvent then filtered. The obtained solutions claimed to contain 100 µg/mL of

each drug then analyzed adopting the procedures previously mentioned under " **Construction of the Calibration curves** ". From the corresponding regression equation, the nominal content of tablets was estimated.

3. RESULTS AND DISCUSSION:

The aim of this research was to develop an uncomplicated and estimable spectrophotometric method without the need of prior extraction for the estimation of DPD and LMV in their dosage forms.
DPD and LMV were found in this study to form an ion pair reddish orange complexes with eosin Y at pH 3.7 with λ max 554.0 nm for both drugs; Figure 2. The formed complexes are chiefly due to the electrostatic interaction under acidic pH between the cationic drugs and the anionic functional group of eosin.

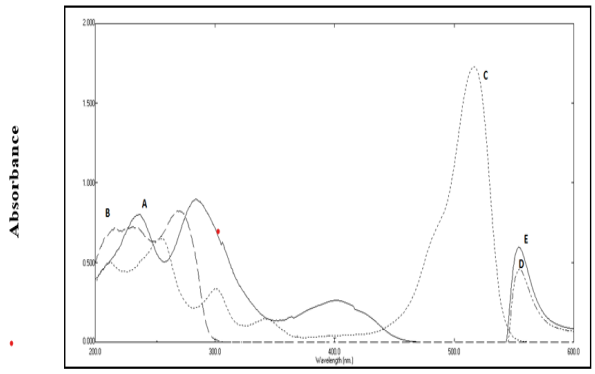

Figure (2): Absorption spectra of: (A) DPD in 0.05 MH_2SO_4 (5 µg/ mL). (B) LMV in water (8 µg /mL). (C) Blank eosin Y (4×10^{-3} M) in water. (D) Eosin Y binary complex with lamivudine (10 µg/mL) at pH 3.7. (E) Eosin Y binary complex with dipyridamole (7 µg/mL) at pH 3.7.

3.1. Optimization of the reaction conditions

pH: Over the pH range (3.6-5.5), the impact of pH on the absorbance intensity of the formed complexes was investigated. It was noticed that, the values of optimum absorbance for both drugs were achieved at pH 3.7.; Figure 3. **Volume of eosin Y reagent**: Various volumes of (2×10^{-3} M) and ($4x10^{-3}$ M) eosin Y solution were added to DPD and LMV respectively to assess the optimum reagent volume. 1.5-2.5 mL for DPD and 0.5-1.5mL for LMV were found to be sufficient to give maximum color intensity. Thus 2 mL and 1 mL of eosin solution were used for DPD and LMV, respectively. Lower volumes showed decreased absorbance; Figure 4.

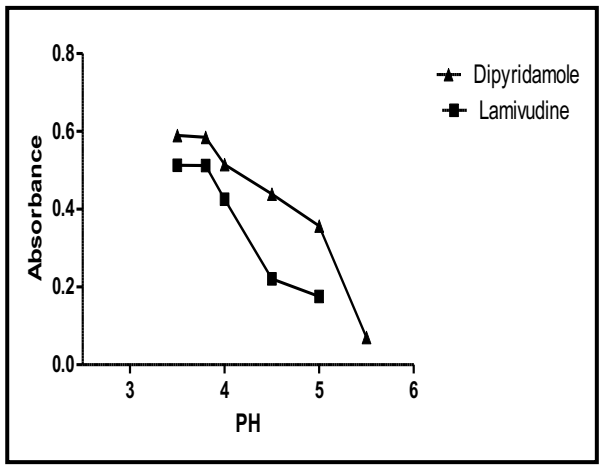

Figure (3): Effect of pH of 0.2 M acetate buffer on the absorbance value of 7µg/mL and10 µg/mL for Dipyridamole and Lamivudine with eosin Y (2×10^{-3} M) and (4×10^{-3} M) respectively.

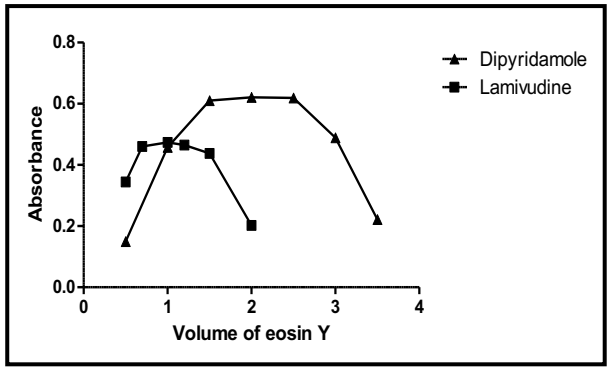

Figure (4): Effect of volume of eosin (2×10^{-3} M) and (4×10^{-3} M) on the absorbance value of product with 7.0 µg/mL and10 µg/mL for DPD and LMV respectively.

Effect of surfactant: The slight solubility of eosin Y complexes with drugs in aqueous acidic solutions amounted to either extraction with organic solvent [21] or addition of nonionic surfactant namely methyl cellulose, SDS, tween 80 and Cetrimide to stabilize and solubilize the resulted complex [22, 23] . Consequently, several trials have been carried out to solve this problem, where the previously mentioned surfactants were tried. SDS and Cetrimide showed decreased absorbance while tween 80 and methyl cellulose were attempted to prevent precipitation of the complex. Methyl cellulose was the best giving good reliability results, hence 0.1% of its aqueous solution was used where maximum absorbance was achieved using 2.0 ± 0.2 mL of 0.1% methyl cellulose for DPD; Figure 5.

However, the reproducibility was adversely affected in case of LMV. Consequently, the method reported by El-Brashy et al[24] was applied in case of LMV. This method was based on preserving the maximum dilution of the drug before adding the dye solution and mixing well before adding the acidic buffer. The complex stability was significantly improved by following this technique and prevention of precipitate formation was achieved.

Temperature: For both drugs, it was noticed that, at room temperature, the absorbance was optimum; increasing the temperature led to precipitate formation that could be due to the coagulation of the resulted complex.

Time:the influence of time on the development and sta bility of the resulted complex has been studied and it has been found that the complex is instantly formed and found to be stable for 2 hours and the complex did not precipitate.

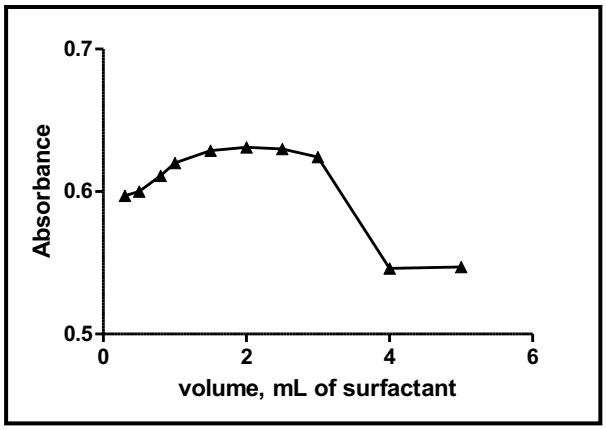

Figure (5): Effect of volume of methyl cellulose on the absorbance of the reaction product of 7.0 µg/mL DPD with Eosin Y (2×10^{-3} M).

3.2. Stoichiomery of the reaction

These complexes are formed due to electrostatic interaction between carboxylate anion of the dye and the most basic center (amino group) in the drug molecule. Using Job's Method[25] (Figure 6), the reaction was found to be in the ratio of 1:2 of the drug to eosin for DPD because it has two basic centers and 1:1 for LMV as it has one basic center. Figure 7 indicates the proposed reaction pathway mechanism.

3.3. Validation of the developed method

The suggested method was validated regarding the guidelines stated by International Conference of Harmonization (ICH) Q2 (R1) [26] .

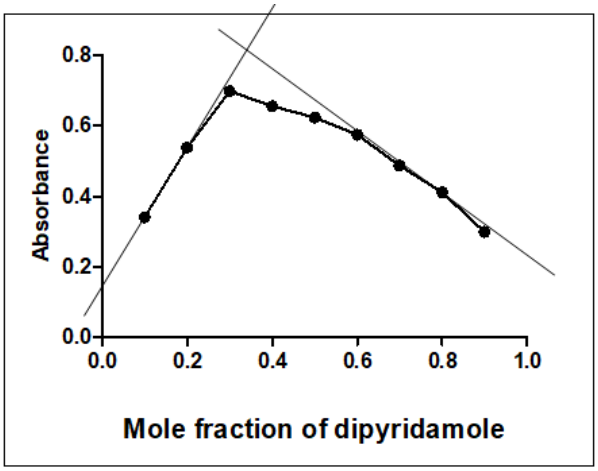

Figure (6) A: Stoichiometry of the reaction of (A) Dipyridamole with eosin Y (2.0×10^{-3} M) by Job's method.

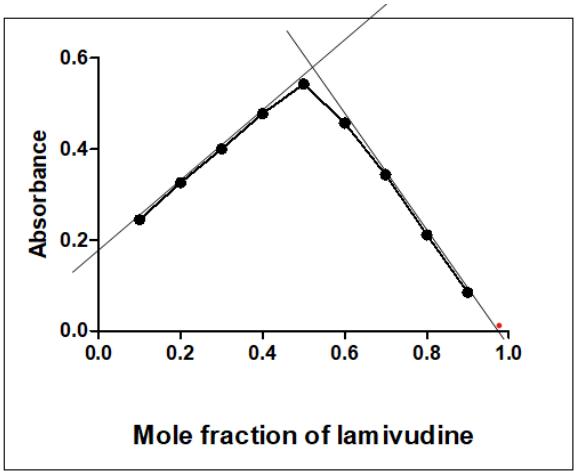

Figure (6) B: Stoichiometry of the reaction of (B) Lamivudine with eosin Y (4.0×10^{-3} M) by Job's method.

3.4. Linearity

Linear relationship was found to exist between the absorbance values and the corresponding drugs concentration over the range of (1.0-10.0) ug/ml and (2.0-20.0) ug /ml for DPD and LMV respectively; Table 1.

3.5. Limit of detection (LOD) and limit of quantification (LOQ):

LOD and LOQ were determined as stated by ICH Q2(R1) recommendations[26]. LOD was found to be (0.27 µg/mL) for DPD and (0.46 µg/mL) for LMV, while LOQ was found to be (0.82 µg/mL) and (1.40 µg/ mL) for DPD and LMV, respectively; Table 1.

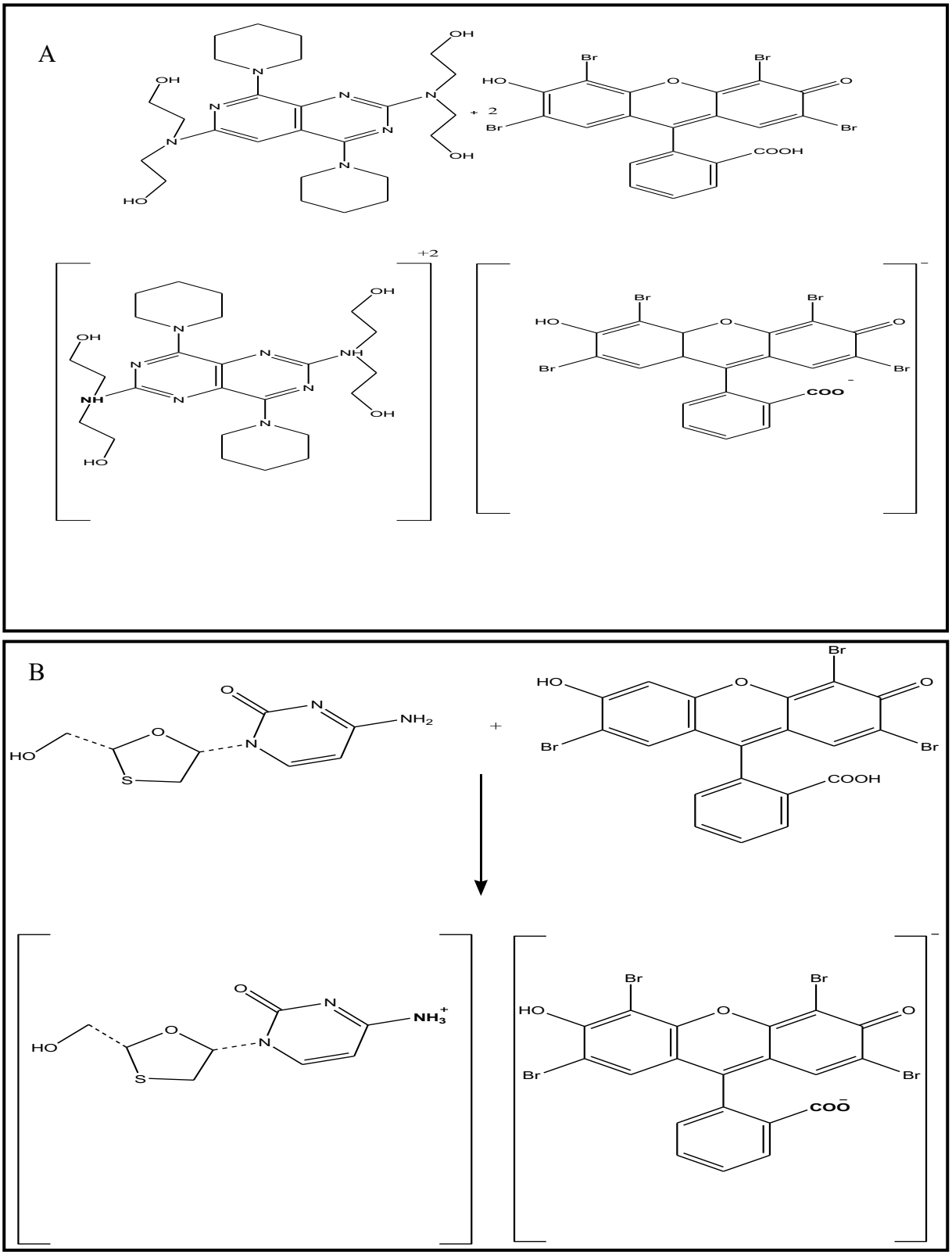

Figure (7): Proposed mechanisms for the reaction between eosin Y, (A) Dipyridamole, (B) Lamivudine

Table (1): Analytical performance data for the determination of the studied drugs by the proposed method.

Parameter	Dipyridamole	Lamivudine
Linearity range ($\mu g\ mL^{1}$)	(1.0-10.0)	(2.0-20.0)
Intercept ± S.D	0.022±7.120x10-3	0.027±6.265x10-3
Slope ± S.D	0.087±1.140x10-3	0.045±5.102x10-4
Correlation coefficient (r)	0.9997	0.9998
SD of residuals ($S_{y/x}$)	0.91x10-2	7.45x10-3
(LOD) ($\mu g\ mL^{-1)}$	0.27	0.46
(LOQ) ($\mu g\ mL^{-1}$)	0.82	1.40

3.6. Precision

Evaluation of the intra-day or interday precision of the present method was carried out by analyzing three concentrations of DPD and LMV in raw materials in the same day or over three consecutive days, respectively. The small values of (% RSD) ranged from 0.78 to 1.61% and 0.80 to 1.35 % for DPD and LMV respectively; indicate reasonable repeatability, high precision and accuracy of the present method, Table 2.

3.7. Accuracy

The results of the proposed method were compared to those obtained using the reference methods [27,28] to validate the accuracy of the proposed method, The first one[27] is the official HPLC method to determine DPD using C18 column , phosphate buffer : methanol (30:70) of pH 4.6 was used as mobile phase and UV detection at 288.0 nm .

The second one is spectrophotometric method to determine LMV which based on direct spectrophotometric measurement of LMV solution at 280.0 nm in water [28] . Statistical analysis [29] of the results using student's t-Test and variance ratio F-test showed no significant differences between them regarding accuracy and precision, respectively, Table 3.

3.7. Robustness

Method robustness was determined by the resistance of the proposed method to deliberate minor change in the parameters of the experiment such as the change of eosin Y volume (2.0 ± 0.2) and (1 ± 0.2) for DPD and LMV respectively. The absorption intensity was not affected by these minor changes, proved good robustness of the developed method.

Table (2): Precision data for the determination of the studied drugs by the proposed method.

Parameters		Dipyridamole (µg/mL)			Lamivudine(µg/mL)		
		2.0	8.0	10.0	2.0	10.0	20.0
Intraday[a]	%Found	99.01	97.09	98.58	98.43	99.42	97.16
		98.50	99.35	99.78	97.64	98.84	98.18
		97.03	98.54	100.11	99.21	97.38	99.43
	Mean	98.18	98.33	99.49	98.42	98.55	98.26
	%RSD	1.05	1.17	0.81	0.80	1.06	1.16
	%Error	0.61	0.67	0.47	0.46	0.61	0.67
Interday[b]	%Found	98.02	99.19	99.89	99.21	99.71	98.86
		100.49	98.87	98.47	100.79	98.55	97.50
		97.53	97.73	97.92	98.11	97.67	99.77
	Mean	98.68	98.60	98.76	99.37	98.64	98.71
	%RSD	1.61	0.78	1.03	1.35	1.04	1.16
	%Error	0.93	0.45	0.60	0.78	0.60	0.67

Each result is the average of three separate determinations.[a] Within the day [b] Three consecutive days.

3.8. Specificity

Method specificity was assessed by the absence of interference from the common excipients of the dosage forms during analysis; Table 4.

3.9. Applications

The developed method was successfully used to assay DPD and LMV in their different dosage forms. Statistical comparison of the obtained results to the reported methods[27, 28] was performed applying t-test and F-test[29]. The results obtained proved excellent accuracy and precision of the developed method; Table 4.

5. CONCLUSION

To determine DPD and LMV in their dosage forms, an accurate and simple visible spectophotometric method was developed through water soluble ion- pairing complex with eosin Y. In this method; no need to use organic solvents which makes the proposed method environmentally friendly.

The advantages of this method were its simplicity, time-saving and non- requiring various elaborate treatments or boring methods for extraction, which make the developed method appropriate in quality control laboratories for routine analysis.

Table (3): Assay results for the determination of Dipyridamole and Lamivudine in pure form by the proposed and comparison methods [27,28].

Ranges	Proposed Method			Comparison methods [27,28]		
	Taken (µg/mL)	Found (µg/mL)	% Recovery	Taken (µg /mL)	Found (µg/mL)	% Recovery
Dipyridamole	1.00	1.03	103.00	5.00	4.88	97.60
	2.00	2.02	101.00	10.00	10.24	102.40
	4.00	3.98	99.50	15.00	14.88	99.20
	7.00	6.98	99.71			
	8.00	7.85	98.13			
	10.00	10.13	101.30			
Mean %			100.44			99.73
± S.D.			1.57			2.43
t-test	0.470(2.360)					
F-test	2.41(5.79)					
Lamivudine	2.00	2.02	101.00	2.00	1.94	97.00
	5.00	5.07	101.40	4.00	4.09	102.25
	10.00	9.67	96.70	8.00	7.97	99.63
	15.00	14.9	99.33			
	20.00	19.9	99.50			
Mean %			99.59			99.62
± SD			1.86			2.56
t-test	0.019(2.447)					
F-test	1.88(6.94)					

Each result is the average of three separate determinations.

The values between parentheses are the tabulated t and F values at P=0.05.

Table (4): Assay results for the determination of Dipyridamole and Lamivudine in their different dosage forms by the proposed and Comparison methods. [27,28]

Ranges	Proposed Method			Comparison methods [27,28]		
	taken (µg/mL)	found (µg/mL)	% Recovery	taken (µg /mL)	found (µg/mL)	% Recovery
Persantine 75mgtablet	4.0	4.06	101.50	5.0	4.92	98.40
	8.0	7.92	99.00	10.0	10.15	101.50
	10.0	10.1	101.00	15.0	14.92	99.47
Mean %			100.50			99.79
± S.D.			1.35			1.55
t-test	0.650(2.776)					
F-test	1.32(19.00)					
Lamidine 150mg tablet	5.0	4.87	97.40	2.0	1.97	98.50
	10.0	10.13	101.30	4.0	4.04	101.00
	20.0	19.80	99.00	8.0	7.99	99.88
Mean %			99.23			99.79
± SD			2.0			1.26
t-test	0.410(2.776)					
F-test	2.51(19.00)					

Each result is the average of three separate determinations.
The values between parentheses are the tabulated t and F values at P=0.05.

Conflict of interest

The authors declare no conflict of interest Ethics

Ethics statement: NA

Author contribution

Authors FB and SA designed the study and wrote the protocol. SB performed the experimental work and statistical analysis. Authors FB and ME supervise the analyses of the study. Authors SA and SB wrote the first draft of the manuscript and managed literature searches. All authors read and approved the final manuscript

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