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SIMULTANEOUS DETERMINATION OF LAMIVUDINE AND ZIDOVUDINE USING Π-ACCEPTORS AS ANALYTICAL REAGENTS: A SPECTROPHOTOMETRIC STUDY

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ABSTRACT

Based on a new concept of AUC (Area Under Curve), two new sensitive and precise spectrophotometric methods have been proposed and developed for the simultaneous estimation of Lamivudine and Zidovudine in pure mixture and in pharmaceutical binary dosage forms using analytical reagents, DDQ (2,3-Dichloro-5,6-dicyano-1,4-benzoquinone) and *p*-CA (*p*-Chloranilic acid: 2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone). Method 1 involves the use of DDQ as analytical reagent and the AUC between 390 nm and 690 nm for DDQ was used for determination. Method 2 involves the use of *p*-CA as an analytical reagent and the AUC between 400 nm and 700 nm for *p*-CA was used for determination. The methods developed and construction of calibration curves using two analytical reagents viz., DDQ and *p*-CA are described. Optical and analytical parameters for the individual and simultaneous determination of Lamivudine and Zidovudine using AUC are tabulated. The methods have been validated and compared with HPLC methods in terms of standard deviation, t-tests and F-tests.

KEYWORDS: Spectrophotometry; Simultaneous estimation; AUC; Lamivudine; Zidovudine; Combivir tablet; DDQ, *p*-CA; CT- Complex; Validation.

INTRODUCTION

In continuation of our work in the study of simultaneous determination of individual drugs in their binary dosage forms, the present study is aimed at the development of two sensitive and simple spectrophotometric methods for the simultaneous determination of Lamivudine and Zidovudine in pure mixture and in pharmaceutical binary dosage forms using π-acceptors *viz.*, DDQ (2,3-Dichloro-5,6-dicyano-1,4-benzoquinone) and *p*-CA (*p*-Chloranilic acid: 2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone) as analytical reagents.

Lamivudine

Lamivudine (Fig. 1) is chemically known as 4-amino-1-[(2R, 5S)-2-(hydroxy- methyl)-1,3-oxathiolan-5-yl] primidin-2-(1H)-one. From the measurement of polymerase chain reactions[1], it is confirmed that the combination therapy of Lamivudine with Zidovudine is associated with substantial persistent increase in 4CD cell counts and decrease in HIV RNA. Lamivudine is an anti-retroviral drug belonging to the class of NRTIs (nucleoside reverse transcriptase inhibitors) and exhibits potent antiretroviral activity.[2]

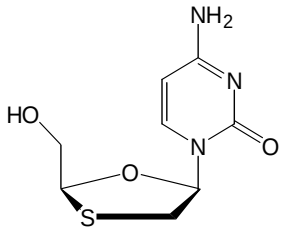


Fig. 1: Structure of Lamivudine.

Literature on the quantification of Lamivudine (LAM) in pharmaceutical forms and in human plasma, saliva, serum, urine and blood plasma has been reviewed thoroughly. UV-spectrophotometric methods based on the measuring the absorbance[3,4] and on the formation of colored product by condensation of LAM with aldehydes[5] are available for the determination of LAM. The quantitative methods involving redox and complexation[6], using N-bromosuccinimide-celestine blue, cobalt thiocyanate and ammonium molybdate as reagents were reported.[7] HPLC techniques for the

determination of LAM in human plasma[8-10], human serum[11], urine[12], cerebrospinal fluid[13], blood plasma[14] and blood cells[15] were used and reported. Capillary zone electrophoresis[16], titrimetric methods[17] and HPTLC method[18] were used for the determination of LAM in pharmaceutical forms. HPLC methods[19-21] and UV-spectrophotometric methods[22-24] have been reported recently for the determination of LAM in combination with other drugs. LAM was also estimated using $NaNO_2$-phloroglucinol, Fe (III)-phenanthroline, $KBrO_3$-KBr methyl orange and $KBrO_3$-KBr indigo carmine.[25] RP-HPLC method[26] to determine LAM in tablet dosage forms in combination with Zidovudine is available in the literature.

Zidovudine

Zidovudine (Fig. 2) is chemically 1-[(2R,4S,5S)-4azido-5- (hydroxymethyl) tetrahydrofuran-2-yl]-5-methylprimidine-2,4(1H,3H0-dione and used as an antiretroviral activity.[27,28] Zidovudine (ZID) is official in British pharmacopoeia and European pharmacopoeia.[29,30] It is an antiretroviral drug, the first approved for treatment of HIV and AIDS. Like other reverse transcriptase inhibitors, ZID works by inhibiting the action of reverse transcriptase, the enzyme that HIV uses to make a DNA copy of its RNA. The viral double-standard DNA is subsequently spliced into the DNA of a target cell, where it is called a provirus. More severe side effects include anemia and bone marrow suppression. These unwanted side effects might be caused by the sensitivity of the γ-DNA polymerase in the cell mitochondria. HPLC methods[31], spectrophotometric methods[32,33] and titrimetric methods[34] have been reported for the quantification of of ZID in pharmaceutical formulations.

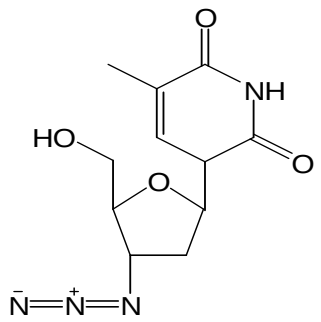


Fig. 2: Structure of Zidovudine.

Analytical methods like spectrophotometric[35], HPLC[36] and HPLC–UV[37] for the determination of Zidovudine in combination with Lamivudine are available in the literature. A stability indicating RP-HPLC method development and validation for simultaneous determination of Lamivudine and Zidovudine in combined dosage form[38] and simultaneous determination of Lamivudine, Zidovudine and Nevirapine in tablet dosage forms by RP-HPLC method[39,40] were reported.

Recently the green HPLC quantification method of Lamivudine, Zidovudine and Nevirapine with identification of related substances in tablets[41] and the comparative study to access the greenness of four analytical methods for simultaneous estimation of Lamivudine, Zidovudine and Nevirapine in pure form and pharmaceuticals using HPLC[42] have been reported. Simultaneous spectrophotometric estimation of Levofloxacin and Azithrmycin in their binary dosage form was reported from our laboratory.[43] In the present study, two new methods have been developed for the simultaneous estimation of Lamivudine and Zidovudine in their binary dosage forms.

MATERIALS AND METHODS

Instruments

The UV-Vis spectra of the study have been recorded on SHIMADZU 140 double beam spectrophotometer and also on ELICO SL 210 UV-Visible double beam spectrophotometer using quartz cells of 10 mm path length. An Elico model Li-120 *p*H meter was used for *p*H measurement.

Materials

DDQ (2,3-Dichloro-5,6-dicyano-p-benzoquinone) was obtained from SD Fine Chemicals. It was recrystallized twice from 3:1 mixture of chloroform and benzene. p-CA (P-Chloranilic acid) supplied by Rolex, Mumbai was used without further purification. HPLC grade acetonitrile was used throughout the work. The drugs Lamivudine, Zidovudine and drug mixture analysed were procured from Dr. Reddy's laboratories and Hetero Drugs Private Ltd, Hyderabad.

Methods and Calibration

Method 1 - DDQ

This method is developed for the simultaneous estimation of drugs in a binary mixture using DDQ (2,3-Dichloro-5,6-dicyano-1,4-benzoquinone) as an analytical reagent. Into a series of 10ml of flasks, different aliquots (1-9ml) of Lamivudine were taken and 1ml of DDQ was added, remaining volume was made up with solvent (Acetonitrile). The contents were shaken well and UV–Vis spectra were recorded. The OD at 480, 540 and 580nm for DDQ anion were noted. The areas under the curve (AUC) between 390nm and 690nm for DDQ were determined from the spectra. AUC_x was plotted against concentration of Lamivudine. From the slope of the plot K_x was determined. Similarly, analogous experiments were repeated for determination of K_y for Zidovudine.

Stock solution of mixture of Lamivudine and Zidovudine was prepared with same ratio as in tablet formulations. Form the stock, 1-9 ml of mixture of drugs were taken into series of standard flasks and 1 ml of reagent, DDQ was added. Remaining volume was made up with solvent (Acetonitrile). The contents were shaken well. UV-Vis spectra were recorded. The OD at 480,540 & 580 for DDQ anion were noted. AUC_{mix} was plotted against either Cx or Cy for calibration.

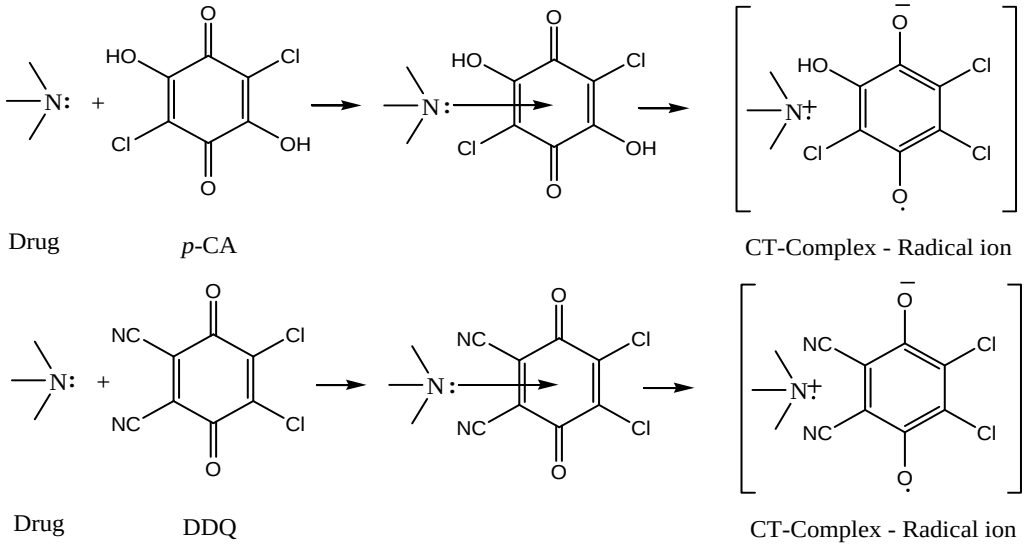
Method 2 - *p*-CA

This method is developed for the simultaneous estimation of drugs in a binary mixture using *p*-CA (*p*-Chloranilic acid: 2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone) as an analytical reagent. Into a series of 10ml of flasks, different aliquots (1-9ml) of Lamivudine were taken and 1 ml of *p*-CA was added, remaining volume was made up with solvent (Acetonitrile). The contents were shaken well and UV–Vis spectra were recorded. The OD at 540nm for *p*-CA anion were noted. The areas under the curve (AUC) between 400nm and 700nm for *p*-CA were determined from the spectra. AUC_x is plotted against the concentration of drug. From the slope of the plot K_x was determined. Similarly, analogous experiments were repeated for determination of K_y for Zidovudine.

Stock solution of mixture of Lamivudine and Zidovudine was prepared with same ratio as in tablet formulations. Form the stock, 1-9 ml of mixture of drugs were taken into series of standard flasks and 1ml of reagent, *p*-CA was added. Remaining volume was made up with solvent (Acetonitrile). The contents were shaken well. UV-Visible spectra were recorded. The OD at 540nm for *p*-CA anion was noted. AUC_{mix} was plotted against either Cx or Cy for calibration.

RESULTS AND DISCUSSION

The Charge Transfer (CT) complexes are formed by the molecular interaction between electron donors and electron acceptors. These electron donor-acceptor interactions can be studied spectrophotometrically for the determination of the drugs since these interactions are generally associated with the formation of intensely coloured charge transfer complexes, which absorb radiation in the visible region. The absorption bands of these complexes can be used for the quantification of electron donor drug molecules (Scheme 1).



Scheme 1: The molecular structures of π-Acceptors and Charge Transfer complex between Drug and π-Acceptors.

p-CA for example, is an analytical reagent and produces a band at 540nm for *p*-CA anion and is independent of the drug. It is also expected to interact with both the drugs in a binary mixture and exhibits band at 540 nm. As the extent of interaction is different in mixture, it is possible to analyze the concentration of each although the analytical wavelength is same. This prompted the author to give a thought in these lines. For the quantification, generally optical density at λ_{max} is measured against concentration of drug for calibration purpose. The authors thought area under curve (AUC) is more appropriate than the optical density. The authors proposed to measure the area under the curve for individual drugs as well as the mixture in a constant ratio of concentration as in the formulations.

AUC (Area under curve in mixture) = $AUC_X + AUC_Y$

Where X and Y are two drugs in the binary mixture

but AUC of X α C_X

and AUC of Y α C_Y

$AUC_X = K_XC_X$

$AUC_Y = K_YC_Y$

$AUC_{mix} = K_XC_X + K_YC_Y$ …… (1)

Dividing both sides of equation by K_XC_X

$$\frac{AUC_{mix}}{K_xC_x} = 1 + \frac{K_YC_Y}{K_xC_x}$$

But $\frac{K_YC_Y}{K_xC_x}$ = K (Constant)

$$\frac{AUC_{mix}}{K_xC_x} = 1 + K$$

$AUC_{mix} = (1 + K)K_XC_X$

$AUC_{mix} = (K_X + K, K_X)C_X$ ……(2)

Similarly

$$AUC_{mix} = K_X C_X + K_Y C_Y$$

Dividing both sides with $K_Y C_Y$

$$\frac{AUC_{mix}}{K_Y C_Y} = 1 + \frac{K_x C_x}{K_Y C_Y}$$

$$\frac{K_x C_x}{K_Y C_Y} = K \text{ (Constant)}$$

$$AUC_{mix} = (1 + K) K_Y C_Y \quad \text{.....(3)}$$

$$AUC_{mix} = (K_Y + K.\ K_Y) C_Y \quad \text{.....(4)}$$

The equations 2 and 4 imply that AUC_{mix} is either proportional to C_x or C_Y

By determining the AUC_{mix} for a mixture of drugs having constant ratio it is possible to construct the calibration curves to find the individual concentrations of drugs in a binary mixture.

Into a series of 10 ml of flasks, different aliquots (1-9ml) of drug Lamivudine were taken and 1ml of DDQ or p-CA was added, remaining volume was made up with solvent acetonitrile. The contents were shaken well and UV–Vis spectra were recorded. The OD at 540nm for *p*-CA anion and 480, 540 and 580nm for DDQ anion were noted. The area under the curve (AUC) between 390nm and 650nm for DDQ and between 400nm and 700nm for *p*-CA were determined from the spectra (Fig. 3 and 4).

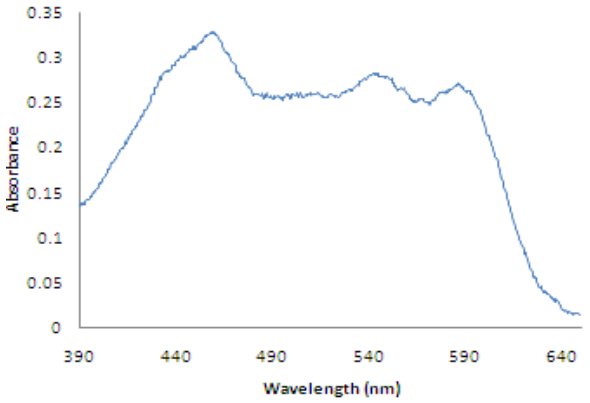


Fig. 3: Charge transfer spectrum of Lamivudine with DDQ.

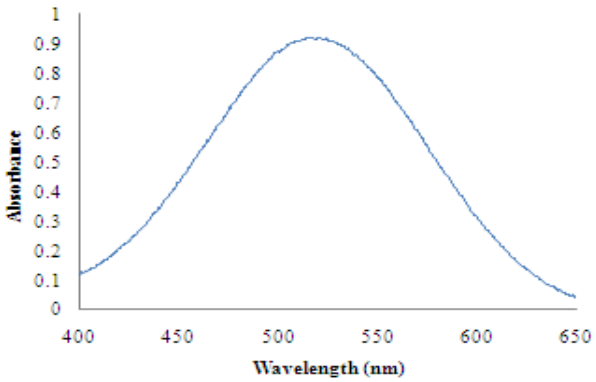


Fig. 4: Charge transfer spectrum of Lamivudine with p-CA.

The plots of AUC_x vs concentration of Lamivudine with DDQ and *p*-CA are shown in Fig. 5 and 6.

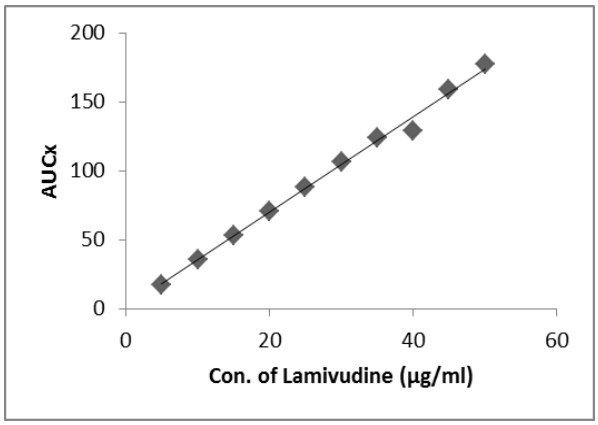


Fig. 5: Plot of AUC vs Con. of Lamivudine-DDQ.

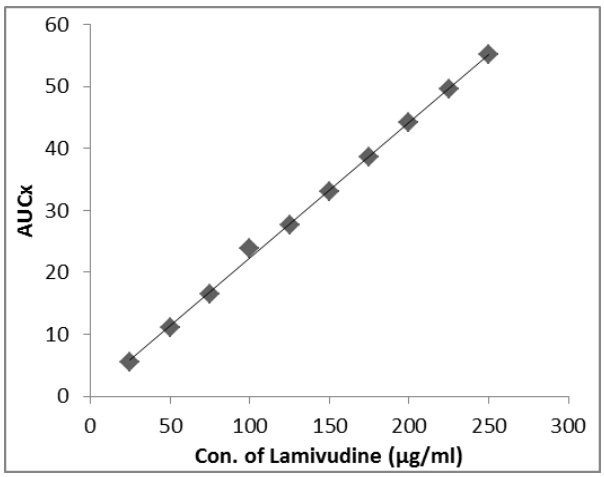


Fig. 6: Plot of AUC vs Con. of Lamivudine -p-CA.

From the slope of the plots K_x was determined. In the same way, analogous experiments were repeated for determination of K_y for Zidovudine (Fig. 7, 8, 9 and 10).

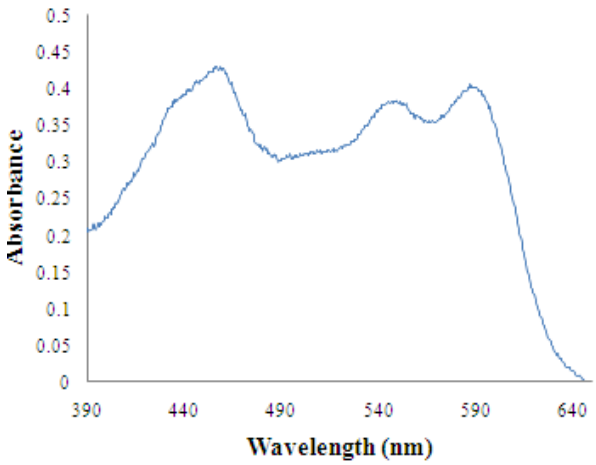


Fig. 7: Charge transfer spectrum of Zidovudine with DDQ.

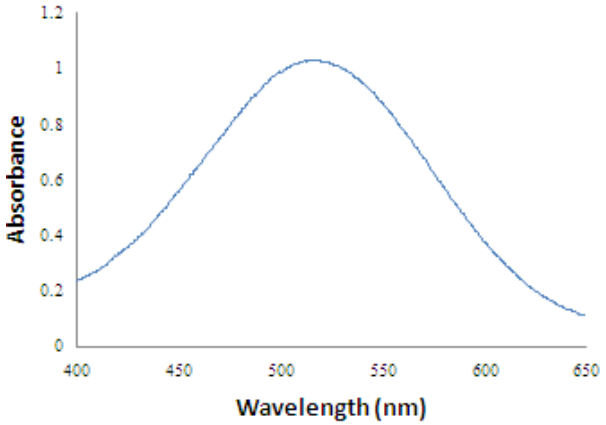


Fig. 8: Charge transfer spectrum of Zidovudine with p-CA

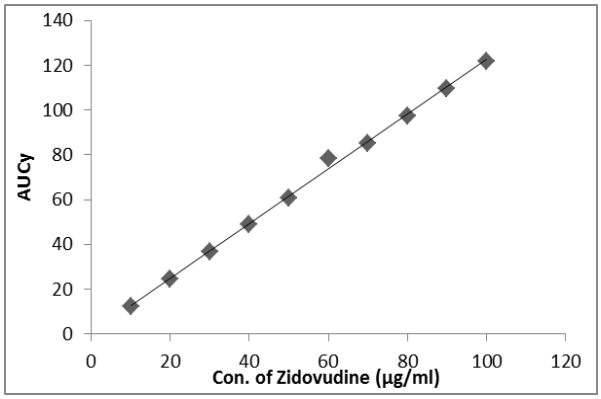


Fig. 9: Plot of AUC vs Con. of Zidovudine-DDQ.

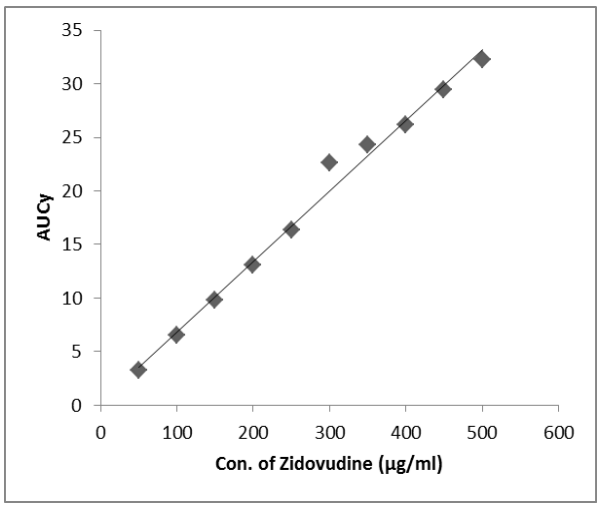


Fig. 10: Plot of AUC vs Con. of Zidovudine- p-CA.

Stock solution of mixture of drugs (Lamivudine and Zidovudine) was prepared with same ratio as in tablet formulations. From the stock 1-9ml of mixture of drugs were taken into series of standard flasks and 1ml of reagent DDQ or *p*-CA was added. Remaining volume was made up with solvent (Acetonitrile). The contents were shaken well. UV-Visible spectra were recorded (Figures 11 and 12). The OD at 540nm for *p*-CA anion and 480, 540 & 580 for DDQ anion were noted. AUC_{mix} was plotted either Cx or Cy (Figures 13 and 14).

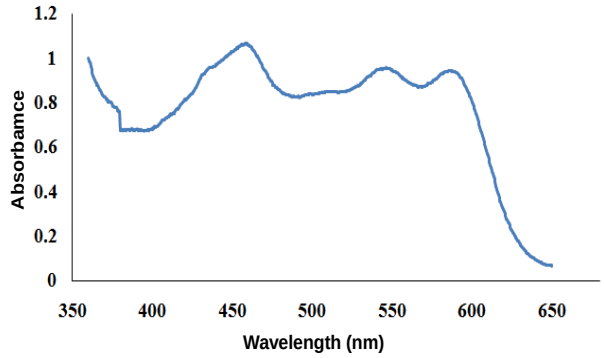


Fig. 11: Charge transfer spectrum of LAM + ZID with DDQ in pure form.

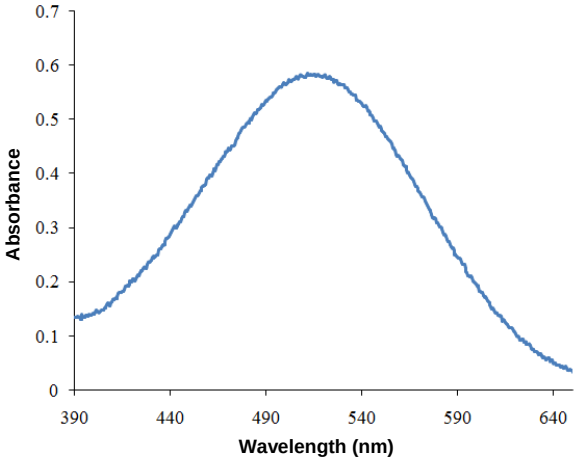


Fig. 12: Charge transfer spectrum of LAM + ZID with p-CA in pure form.

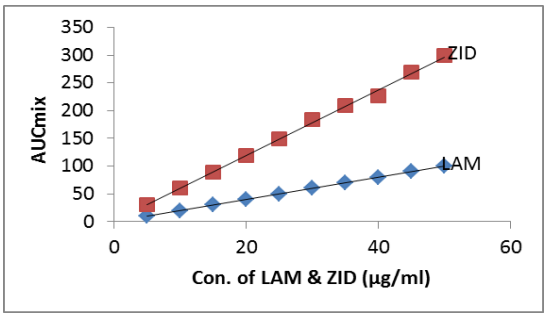


Fig. 13: Plot of AUCmix vs Con. of LAM & ZID-DDQ in pure form.

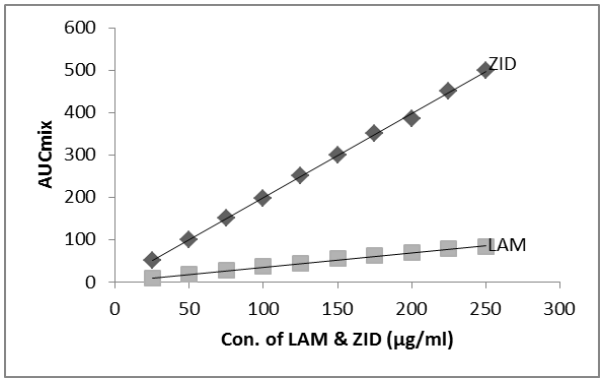


Fig. 14: Plot of AUCmix vs Con. of LAM & ZID -p-CA in pure form.

The optical characteristics and statistical data for the regression equation of the proposed method for the determination of individual drugs (Lamivudine and Zidovudine) are presented in Table 1 and in synthetic mixture in the ratio of 1:2 (Lamivudine and Zidovudine) of drugs as in tablets using area under curve (AUC) are presented in Table 2.

Table 1: Optical and analytical parameters for the individual estimation of Lamivudine and Zidovudine using area under curve.

Parameters	**DDQ**		***p*-CA**	
λ Lower and λ Higher for AUC	390-650		400-700	
Range of concentrations of drugs ($mgmL^{-1}$)	**Lamivudine**	**Zidovudine**	**Lamivudine**	**Zidovudine**
	5-60	10-120	25-300	50-600
Slope	3.556	1.212	0.218	0.064
Intercept	-0.300	0.433	0.255	0.296
Correlation coefficient	0.999	0.999	0.997	0.999
Residual intercept	0.3090	0.3672	0.1651	0.6190
LOD	0.7	1	2.5	1.5
LOQ	2.31	3.3	8.25	4.95

Table 2: Optical and analytical parameters for the simultaneous estimation of Lamivudine and Zidovudine in synthetic mixture in the ratio of 1:2 of drugs as in tablet using area under curve.

Parameters	**DDQ**		***p*-CA**	
λ Lower and λ Higher for AUC	390-650		400-700	
Range of concentrations of drugs (μgmL^{-1})	**Lamivudine**	**Zidovudine**	**Lamivudine**	**Zidovudine**
	5-100	5-100	25-500	25-500
Slope	1.991	5.88	2.013	0.341
Intercept	0.421	2.416	-2.619	1.132
Correlation coefficient	0.999	0.997	0.999	0.994
Residual intercept	0.3016	0.8912	1.525	0.2583
LOD	0.5	0.5	2.5	2.5
LOQ	1.65	1.65	8.25	8.25

Five different solutions of pure drug mixture in the range of calibration curve were selected and the recovery experiments were performed. The recoveries and their relative standard deviations are tabulated in Table 3.

Table 3: Application of proposed methods for the simultaneous estimation of Lamivudine and Zidovudine in the mixture in the ratio of 1:2 of drugs in pure form using area under curve.

Taken ($mg\ ml^{-1}$)				**Found ($mg\ ml^{-1}$)**				**Recovery (%)**			
Lamivudine		**Zidovudine**		**Lamivudine**		**Zidovudine**		**Lamivudine**		**Zidovudine**	
DDQ	***p*-CA**	**DDQ**	***p*-CA**	**DDQ**	***p*-CA**	**DDQ**	***p*-CA**	**DDQ**	***p*-CA**	**DDQ**	***p*-CA**
5	25	10	50	4.91	25.65	10.02	50.24	98.21	102.6	100.20	100.48
10	50	20	100	10.35	50.26	20.06	100.63	103.50	100.52	100.30	100.63
15	75	30	150	15.15	75.14	30.19	150.12	101.01	101.18	100.63	100.08
20	100	40	200	19.95	99.83	40.86	199.82	99.75	99.75	102.15	99.91
25	125	50	250	25.14	125.04	50.04	250.06	100.56	100.03	100.08	100.02
30	150	60	300	30.08	149.94	59.91	300.15	100.26	99.96	99.85	100.05

SD Proposed method				**SD Reference method**			
Lamivudine		**Zidovudine**		**Lamivudine**		**Zidovudine**	
DDQ	***p*-CA**	**DDQ**	***p*-CA**	**DDQ**	***p*-CA**	**DDQ**	***p*-CA**
1.7386	1.0740	0.4613	0.2886	1.7645	1.0132	0.3912	0.2996

t-Test				**F-test**			
Lamivudine		**Zidovudine**		**Lamivudine**		**Zidovudine**	
DDQ	***p*-CA**	**DDQ**	***p*-CA**	**DDQ**	***p*-CA**	**DDQ**	***p*-CA**
0.0229	0.0896	0.2498	0.0581	1.0300	0.8899	0.7991	1.0776

Similarly, different solutions of Combivir tablets (Lamivudine: Zedovudine 1:2) in the range of calibration curve were chosen and the assay was estimated using the calibration curve (Figures 15 and 16). The results of the recovery experiments are tabulated in Table 4.

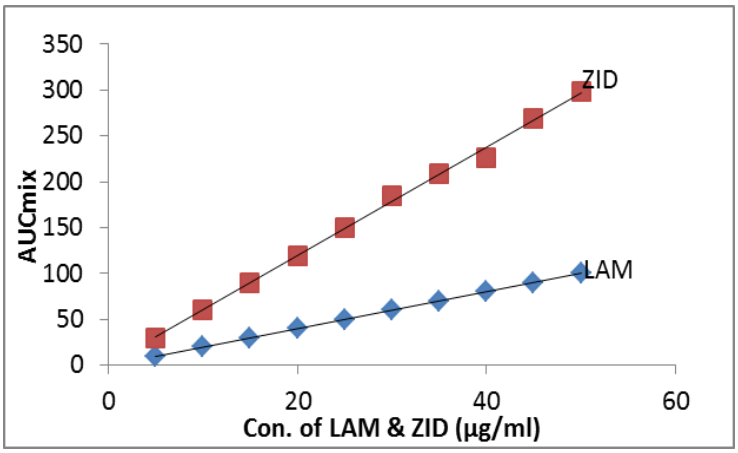


Fig. 15: Plot of AUCmix vs Con. of LAM & ZID-DDQ in dosage form.

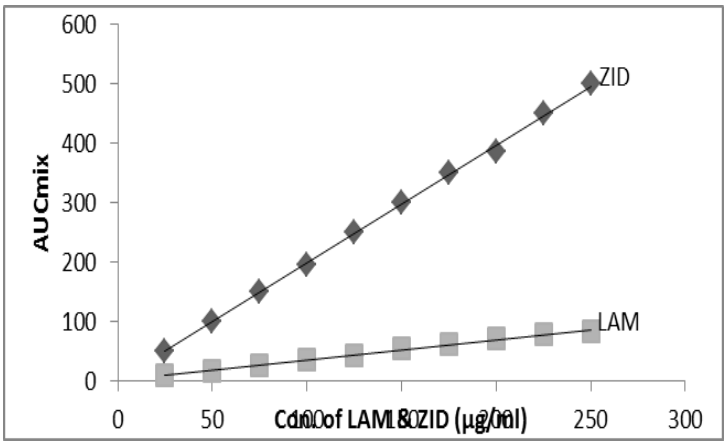


Fig. 16: Plot of AUCmix vs Con. of LAM & ZID-p-CA in dosage form.

Table 4: Application of proposed methods for the simultaneous estimation of Lamivudine and Zidovudine in the mixture in the ratio of 1:2 of drugs in Pharmaceutical form (Combivir tablets) using area under curve.

Taken (mg ml^{-1})				Found (mg ml^{-1})				Recovery (%)			
Lamivudine		Zidovudine		Lamivudine		Zidovudine		Lamivudine		Zidovudine	
DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA
5	25	10	50	5.10	25.22	10.05	49.06	102.00	100.88	100.50	98.12
10	50	20	100	10.24	49.94	20.37	99.85	102.40	99.88	101.85	99.85
15	75	30	150	14.78	75.06	30.01	150.26	98.51	100.08	100.03	100.17
20	100	40	200	19.96	100.24	39.46	200.24	99.86	100.24	98.65	100.12
25	125	50	250	25.43	124.92	50.12	250.46	101.72	99.93	100.24	100.18
30	150	60	300	30.26	150.05	59.46	300.16	100.86	100.03	99.12	100.05

SD Proposed method				SD Reference method			
Lamivudine		Zidovudine		Lamivudine		Zidovudine	
DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA
1.4783	0.3683	1.1227	0.8068	1.3682	0.3984	0.9836	0.7067

t-Test				F-test			
Lamivudine		Zidovudine		Lamivudine		Zidovudine	
DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA
0.1188	0.1225	0.2015	0.2018	0.8565	1.1701	0.7675	0.7672

CONCLUSION

Two new sensitive and precise methods are proposed for the simultaneous determination of Lamivudine and Zidovudine in a binary mixture using π – acceptors, DDQ and *p*-CA. These methods are based on the concept of area under curve (AUC). These methods are tested and validated as per guidelines of the ICH and can be applied for the simultaneous determination of Lamivudine and Zidovudine in a binary mixture in pharmaceutical laboratories.

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REFERENCES

1. Aquila RTD, Hughes MD, Johnson VA, Fischi MA. Nevirapine, Zidovudine and Didanosine compared with Zidovudine and Didanosine in patients with HIV-1 infection. A Randomized Double Blind placebo-controlled trial. Ann of Internal Med., 1996; 124(12): 1019-1030.
2. Mast ST, Gerberdin JC. Potency of Lamivudine. Clinical Research, 1991.
3. Rajasekara A, Murugesan S, Rajagopal KA, Blessing AGI, Chakravarthy TN, Pusparaj DSI. Spectrophotometric Determination of Lamivudine in Pharmaceutical Preparations. Oriental J Chem., 2000; 16(3): 563.
4. Sanker D.G, Reddy MVVN, Kumar JMR, Murthy TK. UV Spectro-photometric determination of some anti-HIV drugs. Asian J Chem., 2002; 14(1): 433-436.
5. Baig MV, Kapse GS, Raju SA. Spectrophotometric Determination of Lamivudine. Asian J Chem., 2001; 13: 185-189.
6. Sarma CSN, Sastry CK, Sastr, CSP. Simple osidative determination of Stavudine or Lamivudine. Asian J Chem., 2002; 14(2): 683-690.
7. Sharma CSN, Kamala Sastry C, Sastry CSP. Determination of stavudine and lamivudine by visible spectrophotometry. Acta Ciencia Indica Chem., 2002; 28: 221-225.
8. Aymard G, Legrand M, Trichereau N, Diquet B. Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl., 2000; 744: 227-240.
9. Pereira AS, Kenney KB, Cohen MS, Hall JE, Eron J J, Tidwell RR, Dunn JA. Simultaneous determination of lamivudine and zidovudine concentrations in human seminal plasma using high-performance liquid chromatography and tandem mass spectrometry. J Chromatogr B Biomed Sci Appl., 2000; 742: 173-183.
10. Fan B, Stewart JT. Determination of zidovudine/lamivudine/nevirapine in human plasma using ion-pair HPLC. J Phar Biomed. Anal, 2002; 28: 903-908.
11. Ozkan SA, Uslu B. Rapid HPLC assay for Lamivudine in pharmaceuticals and human serum. J Liq Chromatogr Rel Technol, 2002; 25: 1447-1456.
12. Plumb RS, Gray RDM, Hasker AJ, Taylor S. High performance liquid chromatographic assay for the sulphoxide metabolite of Lamivudine in human urine. J Chromatogr B Biomed Sci Appl., 1996; 687: 457-461.
13. Hoetelmanur RMW, Profit M, Mecnhorst PI, Mulder JW, Beijnen JH. Quantitative determination of (−)-2′-deoxy-3′-thiacytidine (lamivudine) in human plasma, saliva and cerebrospinal fluid by high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Sci Appl., 1998; 713: 387-394.
14. Zheng JJ, Wu ST, Emm TA. High-performance liquid chromatographic assay for the determination of 2′-deoxy-3′-thiacytidine (lamivudine) in human plasma. J Chromatogr B Biomed Sci Appl., 2001; 761: 195-201.
15. Moore JD, Valette G, Dorque A, Zhon J, Sommadossi JP. Simultaneous quantitation of triphophate metabolites of zidovudine, lamivudine and stavudine in peripheral mononuclear blood cells of HIV infected patients by high performance liquid chromatography-tandem mass spectrometry. J Am Soc Mass Spect., 2000; 11: 1134-1143.
16. Fan B, Stewart JT. Determination of lamivudine/didanosine/saquinavir in human serum using capillary zone electrophoresis. J Liq Chromato. Rel Technol., 2002; 25: 241-249.
17. Basavaiah K, Somashekar BC. Sensitive titrimetric and spectrophotometric methods for the assay of lamivudine in pharmaceuticals. J Sci Indust Res., 2006; 65: 349-354.
18. Wankede S.B, Gupta KR, Wadodkar SG. Simultaneous High Performance Thin Layer Chromatographic Estimation of Lamivudine and Stavudine in Tablet Dosage forms. Indian J Pharm Sci., 2005; 67: 96-97.
19. Nagulwar VP, Bhusari KP. A validated UV spectrophotometric method for the simultaneous estimation of Lamivudine, Nevirapine and Zidovudine in combined tablet dosage form. J Pharm Res., 2009; 2(4): 666-669.
20. Mainardes RM, Maria PD. Reversed phase HPLC determination of zidovudine in rat plasma and its pharmacokinetics after a single intranasal dose administration. Biol Res., 2009; 42: 357-364.
21. Notari S, Mancone C, Alonzi T, Tripod M, Narciso P, Ascenzi P. Determination of abacavir, amprenavir, didanosine, efavirenz, nevirapine, and stavudine concentration in human plasma by

MALDI-TOF/TOF. J Chromatogr B., 2008; 2(1): 249-257.
22. Devmurari VP. Simultaneous Spectrophotometric Determination of Lamivudine and Abacavir in the Mixture. Int J Pharm Sci Res., 2010; 1(7): 82-86.
23. Kenneth CM, Plus OU, Anthony AA. Spectrophotometric Determination of Lamivudine Using Chloranilic Acid and 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). Am J Anal Chem., 2011; 2(7): 849-856.
24. Biksham Babu A, Ramu G, Murali Krishna Ch, Brahma Reddy S, Rambabu. Spectrophotometric Determination of Lamivudine in Pure and Tablet Forms. C E- Journal of Chem., 2012; 9(2): 569-575.
25. Appalaraju S, Karadi AB, Kamalapurkar GS, Sarasambia PS. Spectro-photometric determination of Lamivudine. Asian J Chem., 2002; 14: 475-478.
26. Pallad MS, Rajesh PMN, Chatter M, Bhatt AR. RP-HPLC determination of zidovudine and lamivudine in tab dosage form. Indian Pharm. Sci., 2005; 67: 110-112.
27. U S P 28, N F 23, The United state pharmacopeial Convention, Asian Edition, 2005.
28. U S P 28, N F 23, The United state pharmacopeial Convention, Asian Edition, 2008.
29. British Pharmacopoeia, HM Stationery Press, 2002; 1817.
30. European Pharmacopoeia III, European Pharmacopoeia Commission, 2003; 5: 2724.
31. Dunge A, Sharda N, Singh B, Singh S. Validated specific High Performance Liquid Chromatography method for determination of Zidovudine during stability studies. Journal of Pharm Bio Anal, 2005; 37(5): 1109-1114.
32. Basavaiah K, Anil Kumar UR. Spectrophotometric determination of Zidovudine in pharmaceuticals based on Charge-transfer complexation involving N-Bromosuccinimide, Metol and Sulphuric acid as reagents. E - J Chem., 2007; 4(2): 173-179.
33. Basavaiah K, Anil Kumar UR. Simple spectrophotometric methods for the determination of Zidovudine using Chloramine-T, Methylene blue and Rhodamine-B as reagents. E-J Chem., 2006; 3(3): 173-181.
34. Basavaiah, K, Anil Kumar UR. Titrimetric and spectrophotometric determination of zidovudine in pharmaceuticals using chloramine-T and two dyes. Indian J Chem Tech., 2007; 14(2): 200-203.
35. Erk N. Spectrophotometric determination of indinavir in bulk and pharmaceutical formulations using bromocresol purple and bromothymol blue. Die Pharmazie, 2004; 59: 183-186.
36. Geetha R, Hemanthkumar A.K, Kumaraswami V, Soumya S. Simple liquid chromatography method for simultaneous determination of zidovudine and NVP in plasma. J Chromatogr B., 2006; 843(2): 339-344.
37. Uslu B, Özkan SA. Determination of Lamivudine and Zidovudine in Binary Mixture Using First Derivative Spectrophotometric, First Derivative of the Ratio Spectra, and HPLC-UV Methods. Anal Chim Acta., 2002; 466: 175-185.
38. Santosh kumar, Venkateshwar Rao J. A stability indicating RP-HPLC method development and validation for simultaneous determination of lamivudine and zidovudine in combined dosage form. Indo Am J Pharmaceut Res., 2014; 4(3): 1289-1297.
39. Anantha Kumar D, Naveen Babu M V, Seshagiri Rao JVLN, Jayathirtha Rao V. Simultaneous determination of lamivudine, zidovudine and nevirapine in tablet dosage forms by RP-HPLC Method. Rasayan J Chem., 2010; 3(1): 94-99.
40. Vamshi Krishna P V, Vinod Kumar K, Ramalingam P, Ramesh N, Harish Kumar Raju C, Sreeram B. Simultaneous Determination of Lamivudine, Zidovudine and Nevirapine in Tablet Dosage Forms by RP-HPLC. Am J Pharm Tech Res., 2012; 2(4): 894-901.
41. Ludivina VS, Mercedes Q, Qusmane D, Oliver M. Green HPLC quantification method of lamivudine, zidovudine and nevirapine with identification of related substances in tablets. Green Chem Lett Rev., 2022; 15(3): 695-704. Available from https:// doi.org/10.1080/17518253.2022.2129463.
42. Dibya SP, Patro SK, Nasser HA, Ibrahim AN. Comparative study to access the greenness of four analytical methods for simultaneous estimation of Lamivudine, Zidovudine and Nevirapine in pure form and pharmaceuticals using HPLC. Acta Pol Pharm Drug Res., 2022; 79(1): 41-48. Available from DOI: 10.32383/appdr/146883.
43. Sayanna Vittal S, Veeraiah T, Venkata Ramana Reddy Ch. Simultaneous spectrophotometric determination of Levofloxacin and Azithromycin using π–acceptors as analytical reagents. IOSR Journal of Pharmacy, 2019; 9(1): 50-61.