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Simple Oxidimetric Methods for Determination of Stavudine or Lamivudine

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Three simple and sensitive visible spectrophotometric methods (A, B and C) have been described for the estimation of stavudine (STU) or lamivudine (LAM) in pure state and in unit dosage forms. These are based on the oxidation of STU or LAM with oxidant (potassium permanganate, MnO_4^-, method-A; potassium permanganate-sodium metaperiodate, MnO_4^--IO_4^-, method-B; ferric chloride, Fe(III), method-C), followed by the estimation of unreacted oxidant with fast green FCF (FG FCF, method-A) or product formed [aldehyde with 3-methyl benzothiazolinone hydrazone (MBTH), method-B; Fe(II) with potassium ferricyanide $[Fe(CN)_6]^{3-}$, method-C. The variable parameters of all these methods have been optimised and the chemical reactions involved are presented. The results obtained in these methods are statistically validated and recoveries range from 99.07 to 99.95%

Key words: Oxidimetric, Methods, Determination, Stavudine, Lamivudine.

INTRODUCTION

Stavudine (STU), [1-(2,3-dideoxy-β-O-glycero-pent-2-enofuranosyl)thymine], is a nucleoside reverse transcriptase inhibitor related to thymidine with activity against retro-viruses including HIV. Lamivudine (LAM) [2(1H)-pyrimidinone, 4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-, (2R cis)] is an antiviral medication, which is highly active against hepatitis-B virus and human immuno deficiency virus (HIV). They are available in capsules, tablets and lotion form for oral administration. Literature survey reveals that only a few methods based on UV[1] and HPLC[2, 3] are reported for these drugs, while no visible spectrophotometric method is reported for stavudine. The sole visible spectrophotometric method for lamivudine is based on condensation with aromatic aldehydes[4]. During the course of our efforts to develop simple, sensitive and selective procedures for various drugs, it was observed that the structural features of STU or LAM have not been fully exploited for designing such procedures. This paper presents three such analytical methods based on the reactions of STU

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or LAM with MnO_4^--FG FCF (A), MnO_4^--IO_4^- (B) and Fe(III)-$[Fe(CN)_6]^{3-}$(C). All these methods are applicable for the determination of STU or LAM in bulk form and formulations.

EXPERIMENTAL

A Milton Roy Spectronic 1201 and Systronics 106 digital spectrophotometer with 1 cm matched quartz cells were used for spectral and absorbance measurements.

All the chemicals were of analytical reagent grade and all the solutions were prepared with double distilled water. Freshly prepared solutions were always used.

Aqueous solutions of $KMnO_4$ (BDH, 1.89×10^{-3}M in 2M H_2SO_4), fast green FCF (FG FCF) (Chroma, 1.236×10^{-4} M in 1 M H_2SO_4), Na_2SO_4 (BDH, 1 M) were prepared for method A. Aqueous solutions of $KMnO_4$ (BDH, 6.32×10^{-4} M in 2 M H_2SO_4), $NaIO_4$ (BDH, 2.33×10^{-3} M), MBTH (Fluka, 8.56×10^{-3}) were prepared for method B. Aqueous solutions of $FeCl_3$ (Wilson Lab, 1.1085×10^{-2} M), $K_3[Fe(CN)_6]$ (BDH, 3.024×10^{-3}M), HCl (1 M) were prepared for method C.

Preparation of standard drug solution: A 1 mg/mL solution was prepared by dissolving 100 mg of drug (STU or LAM) in 100 mL distilled water and the stock solution was further diluted with distilled water to obtain the working standard solution of concentration 50 μg/mL or 80 μg/mL for STU or LAM respectively for method-A and 25 μg/mL for both drugs in method-B and 400 μg/mL or 600 μg/mL for STU or LAM respectively for method-C.

Sample solution: An accurately weighed or measured amount of sample (tablet or capsule or lotion) equivalent to 100 mg of the drug (STU or LAM) was extracted with chloroform (2×15 mL) and filtered through a pledget of cotton containing 2 g of anhydrous sodium sulphate. The combined filtrate was evaporated to dryness and the residue was dissolved in 100 mL of distilled water to achieve a concentration of 1 mg/mL. This solution was further diluted stepwise with distilled water to get working standard solutions for methods A, B and C for STU or LAM as described in the standard drug solution and analysed as per procedure described for bulk samples.

Analysis of bulk samples

Method A: To a series of 25 mL graduated test tubes containing list volumes (0.5–2.5 mL) of STU 50 μg/mL or LAM 80 μg/mL, 0.5 mL of $KMnO_4$ solution was added. The total volume in each tube was brought to 10 mL with distilled water and kept aside for 10 min to oxidise the drug. Subsequently 4.0 mL each of FG FCF solution and sodium sulphate solution were added and mixed thoroughly. After 5 min, the volume was brought to 25 mL with distilled water and absorbances were measured at 640 nm against distilled water. Blank was prepared similarly omitting the drug and its absorbance was measured against distilled water. The decrease in absorbance corresponding to consumed $KMnO_4$ and, in turn, to the drug concentration, was obtained by subtracting the absorbance

of the blank solution from that of the test solution. The calibration graph was drawn by plotting the decrease in the absorbance of dye (FG FCF) against the amount of drug (STU or LAM). The STU or LAM concentration was determined with appropriate Beer's law plot.

Method B: Into a series of 25 mL graduated test tubes aliquots of STU or LAM solution ranging from 1.0–6.0 mL, 25 μg/mL were taken and then 0.5 mL of $KMnO_4$ and 1.0 mL of $NaIO_4$ were added and kept in a boiling water bath for 10 min. After that 1.0 mL of MBTH solution was added and heated further for 3 min. The solutions were cooled to room temperature and the volume in each tube was made up to mark with distilled water. The absorbances were measured at 620 nm against a similar reagent blank. The coloured species was stable for 1 h. The amount of STU or LAM was computed from the calibration curve.

Method C: Into a series of 10 mL calibrated tubes containing drug solution of volumes (0.25 mL–1.5 mL) of 400 μg mL^{-1} of STU or of 600 μg mL^{-1} of LAM was added 1 mL of $FeCl_3$ and stoppered immediately and shaken well for 5 min. Then 0.5 mL of potassium ferricyanide solution was added into each tube and stoppered immediately. After 5 min, 1 mL of 1 N HCl was added and made up to 10 mL with double distilled water. The absorbance of each solution was measured at 740 nm against reagent blank prepared in similar way within the stability period of 40 min. The drug content was determined with a standard plot.

RESULTS AND DISCUSSION

The optimum conditions for the development of the methods A–C were established by varying the parameter one at a time[5] and keeping others fixed and observing the effect produced on the absorbance of the coloured species.

Analytical data: The Beer's law limits, molar absorptivity, Sandell's sensitivity, detection limits[6], regression equation and correlation coefficients obtained by least squares treatment of these results are given in Table-1. The relative standard deviation and percentage range of error at 95% confidence level of each method are given in Table-1. Recovery studies were carried out by addition of known standard drug solution to preanalysed sample solution. Results of recovery studies are reported in Table-2. The precision of each method was tested by analysing six replicate samples containing 3 μg mL^{-1}, 4 μg mL^{-1} and 40 μg ml^{-1} for methods A, B and C respectively to STU or 4.8 μg mL^{-1}, 4 μg mL^{-1} and 45 μg mL^{-1} for methods A, B and C respectively to LAM.

The interference studies in the determination of STU or LAM in pharmaceutical formulation revealed that the normally existing excipients and additives like starch, talc, stearic acid, boric acid, gelatin, magnesium carbonate and sodium lauryl sulphate were found not to interfere even when present in excess than the anticipated amount. However, a preliminary clean up procedure with $CHCl_3$ is necessary to avoid interference due to the presence of reducing sugars like lactose if present, prior to the estimation of STU or LAM in formulations.

TABLE- 1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS OF LAM AND STU

Parameter	LAM		
	Method A	Method B	Method C
Beer's law limits ($\mu g\ mL^{-1}$), C	1–8	0.6–6.0	9–75
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	1.289×10^4	2.023×10^4	1.238×10^3
Sandell's sensitivity ($\mu g\ cm^{-2}$/0.001 absorbance unit)	1.77×10^{-2}	1.133×10^{-2}	1.852×10^{-1}
Detection limit	3.534×10^{-2}	3.312×10^{-2}	5.74×10^{-1}
Regression equation* (Y = a + bc) slope (b)	5.625×10^{-2}	8.840×10^{-2}	5.426×10^{-3}
Intercept (a)	-6.0×10^{-4}	-1.33×10^{-3}	-6.0×10^{-4}
Correlation coefficient (r)	0.9999	0.9999	0.9998
Relative standard deviation (%)†	0.1454	0.8710	0.9390
% Range of error (95% confidence limits)	0.1526	0.9150	0.9860
Parameter	STU		
	Method A	Method B	Method C
Beer's law limits ($\mu g\ mL^{-1}$), C	0.5–5.0	0.5–60	6.5–50
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	2.589×10^4	2.242×10^4	1.704×10^4
Sandell's sensitivity ($\mu g\ cm^{-2}$/0.001 absorbance unit)	8.658×10^{-3}	1.0×10^{-2}	1.315×10^{-1}
Detection limit	3.603×10^{-2}	4.725×10^{-2}	1.405
Regression equation* (Y = a + bc) slope (b)	1.164×10^{-1}	1.003×10^{-1}	7.383×10^{-3}
Intercept (a)	-2.6×10^{-3}	-1.125×10^{-3}	1.933×10^{-3}
Correlation coefficient (r)	0.9999	0.9999	0.9997
Relative standard deviation (%)†	0.5420	0.7760	0.3490
% Range of error (95% confidence limits)	0.5680	0.8140	0.3670

*Y = a + bC, where C is concentration.

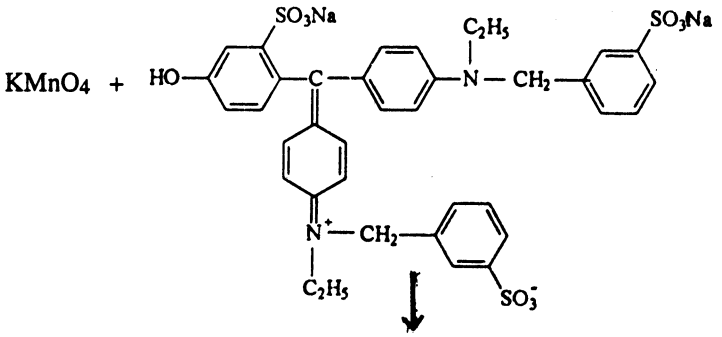
†Calculated from six determinations.

Chemistry of coloured species

Method A: Gordon[7] reported an analytical method for the determination of sorbic acid using MnO_4^--FG FCF reagent. The method A is based on the oxidation of STU or LAM with excess $KMnO_4$ to form oxidation products (probably mixture) but reproducible under proposed experimental conditions (step 1). In step 2, the unreacted $KMnO_4$ was determined with FG FCF. The reaction in acid

medium is a complex reduction of Mn(VII) to Mn(II). Approximately 1.8 permanganate molecules are reduced to Mn(II) by one dye molecule (a 9 electron reaction). The sequence of reaction is shown in Scheme-1.

STU or LAM + $KMnO_4$ → Mixture of carboxylic acids (non- stoichiometric as several alternate pathways possible) + $KMnO_4$ + Mn(II) (unreacted)



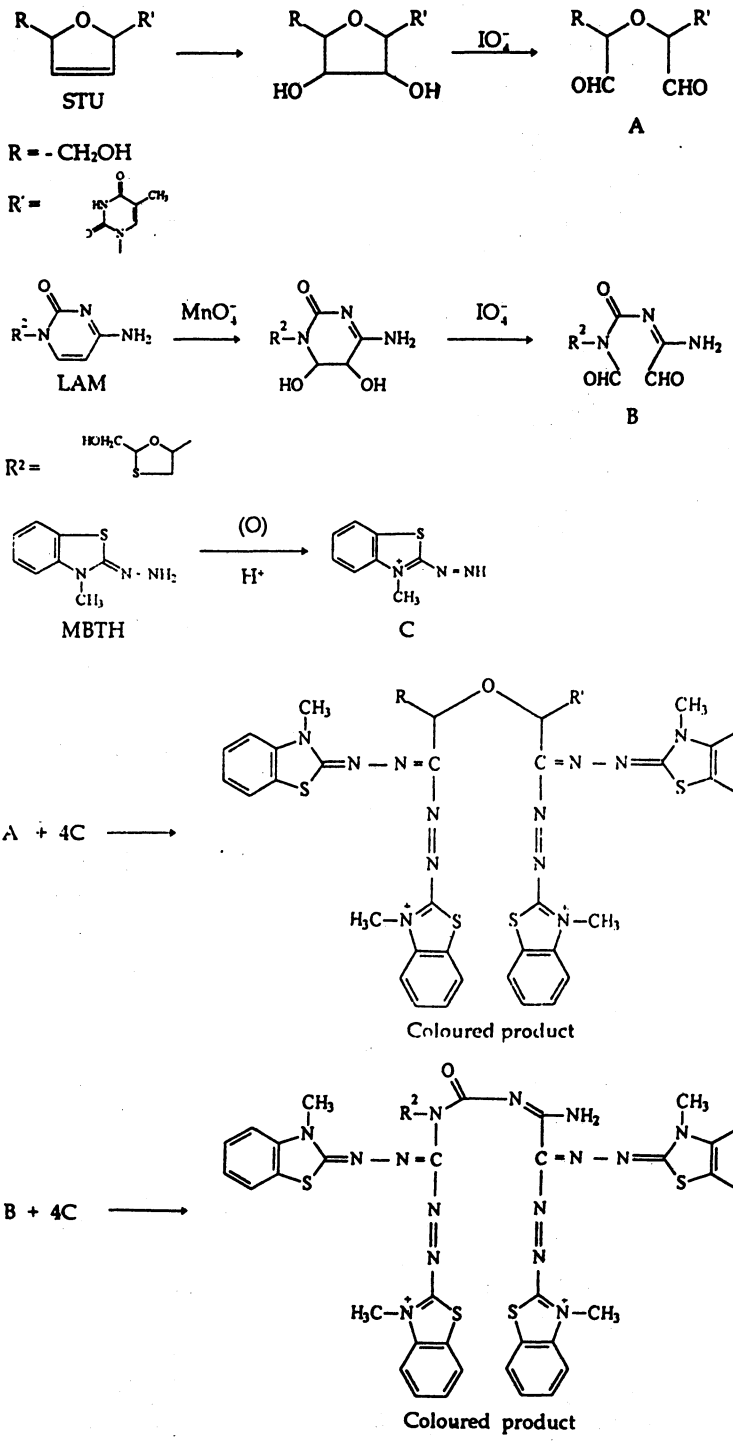
Mn(II) + Dye unreacted (coloured) + Mixture of compounds with the rupture of conjugate system (corresponds to dye reacted, colourless not stoichiometric: as several alternate pathways possible)

Scheme-1

Method B: Lemieux reagent (aqueous solution of MnO_4^- + IO_4^-) was used for the oxidation of double bond (RCH=CHR[1]) to produce aldehydes[8]. MBTH on oxidation with excess of oxidant loses two electrons and a proton forming an electrophile intermediate which then condenses with aldehydes to give blue coloured cationic dye as illustrated by Sawicki *et al.*[9]

Since STU or LAM possess double bond, the author developed a reaction with MnO_4^--IO_4^-/MBTH. This method involves two steps. In the first step MnO_4^- oxidises initially through the involvement of double bond in it to a diol accompanied by further oxidation with periodate yielding aldehyde. The probable sequence of reaction based on analogy is presented in Scheme-2.

Method C: Two drugs namely STU and LAM exhibit high reducing character and so they are capable to reduce Fe(III) to Fe(II). In the present investigations, the drug (STU or LAM) was treated with excess of Fe(III) and the reduced product (Fe(II)) which corresponds to the drug concentration in the usual way by giving prussian blue colour with $Fe(CN)_6^{3-}$ as given in Scheme-3.


STU
A
R = - CH2OH
R' =
LAM
B
R2 =
MBTH
C
A + 4C
Coloured product
B + 4C
Coloured product

Scheme-2

TABLE 2
ESTIMATION OF LAMIVUDINE AND STAVUDINE IN PHARMACEUTICAL FORMULATIONS

Formulations*	Labelled amount (mg)	Amount found by proposed methods†			Reference method**	% Recovery by proposed methods‡		
		A	B	C		A	B	C
Lamivudine								
Tablet I	150	149.14 ± 0.72 F = 1.65, t = 0.359	149.84 ± 1.218 F = 4.73, t = 2.02	149.06 ± 0.78 F = 1.94, t = 0.042	149.09 ± 0.560	99.49 ± 0.48	99.89 ± 0.85	99.70 ± 0.52
Tablet II	150	149.81 ± 0.568 F = 1.19, t = 0.12	149.56 ± 0.76 F = 2.14, t = 1.733	149.33 ± 0.628 F = 1.45, t = 0.39	148.99 ± 0.520	99.28 ± 0.94	99.56 ± 0.62	99.50 ± 0.42
Tablet III	100	99.42 ± 0.56 F = 2.6, t = 0.59	99.82 ± 0.75 F = 4.67, t = 0.06	99.53 ± 0.73 F = 4.425, t = 0.63	99.32 ± 0.347	99.66 ± 0.38	99.89 ± 0.89	99.53 ± 0.73
Lotion IV	50 mg/mL	49.83 ± 0.192 F = 1.043, t = 0.307	49.97 ± 0.187 F = 1.01, t = 1.615	49.53 ± 0.397 F = 4.46, t = 0.57	49.69 ± 0.188	99.07 ± 1.08	99.95 ± 0.37	99.77 ± 0.21
Stavudine								
Tablet I	30	29.85 ± 0.14 F = 1.65, t = 2.12	29.89 ± 0.089 F = 4.09, t = 1.77	29.7 ± 0.188 F = 1.09, t = 1.18	29.17 ± 0.180	99.51 ± 0.447	99.66 ± 0.29	99.25 ± 0.15
Tablet II	40	39.85 ± 0.183 F = 1.926, t = 1.23	39.9 ± 0.126 F = 4.06, t = 0.459	39.58 ± 0.567 F = 4.98, t = 0.108	39.60 ± 0.254	99.65 ± 0.450	99.77 ± 0.26	98.97 ± 0.15
Tablet III	30	29.67 ± 0.235 F = 1.65, t = 0.524	29.87 ± 0.117 F = 2.58, t = 1.7	29.85 ± 0.136 F = 1.91, t = 0.94	29.70 ± 0.188	99.14 ± 0.787	99.41 ± 0.39	99.50 ± 0.45
Tablet IV	4	39.67 ± 0.335 F = 1.74, t = 0.91	39.71 ± 0.135 F = 3.3, t = 1.4	39.61 ± 0.338 F = 1.67, t = 0.708	39.7 ± 0.261	99.19 ± 0.828	99.29 ± 0.34	99.28 ± 0.66

*Different batches from two different pharmaceutical companies

†Average ± standard deviation of six determinations, the t- and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit. F = 5.05, t = 2.57.

‡Recovery of 10 mg added to the preanalysed pharmaceutical formulations (average of three deteminations).

**Developed in the laboratory for STU (λ_{max} 264 nm) or LAM (λ_{max} 271 nm) using 2-propanol solvent.

$$\text{STU or LAM} + \text{Fe(III)} \longrightarrow \text{Oxidised STU or LAM} + \text{Fe(II)}$$

$$3Fe^{2+} + 2[Fe(CN)_6]^{3-} \longrightarrow Fe_3[Fe(CN)_6]_2$$

Scheme-3

Conclusion

All the proposed methods have higher λ_{max} values and sensitivity. This is a decisive advantage since the interference from the associated ingredients will be less at higher wavelengths than at lower wavelength. The sensitivity orders of methods for STU and LAM are A > B > C and B > A > C respectively. The λ_{max} order of the coloured species is C > A > B for STU or LAM. The proposed methods are simple, rapid and have reasonable precision and accuracy. All the proposed methods are useful for the determination of STU or LAM and provide a wide choice, depending on the needs of the specific situation.

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