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NOTE

Development and Validation of Spectrophotometric Method for the Determination of Anti-HIV Drug

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A simple, sensitive and precise spectrophotometric method has been developed for the determination of one anti-HIV drug, lamivudine, in bulk and in its pharmaceutical formulations. Lamivudine gives blue coloured chromogen with phosphomolybdotungstic acid [Folin-Ciocalteu (FC) reagent] under alkaline conditions with an absorption maximum at 750 nm. The chromogen obeys Beer's law in the concentration range of 7.5–75 μg/mL. The proposed method is reproducible, statistically validated and recoveries range from 98.65 to 99.80%.

Key Words: Spectrophotometry, Lamivudine, F.C. reagent, Phosphomolybdotungstic acid,

Lamivudine[1] is chemically 4-amino-1-[2-(hydroxy methyl)-1,3-oxathiolan-5-yl]-2-pyrimidinone. It is used in the treatment of AIDS as an antiviral substance. It acts by inhibiting nucleoside reverse transcriptase. This drug is not yet official in any pharmacopoeia. Literature survey reveals that only few methods on spectrophotometry[2, 3] and HPLC[4–6] are reported for the estimation of lamivudine. Analytically important functional groups in lamivudine have not been exploited properly in developing visible spectrophotometric methods. So an attempt has been made in this direction in developing a visible spectrophotometric method using FC reagent and alkali. The method is based on the reduction of FC reagent to molybdenum blue (λ_{max} : 750 nm) by lamivudine under alkaline conditions.

A Systronics double-beam UV-Visible spectrophotometer (Model 2201) with 1 cm matched quartz cells was used for spectral and absorbance measurements. Aqueous solution of sodium carbonate (9.43×10^{-2} M) and commercially procured FC reagent (2 N) were used.

Preparation of standard and sample solution: About 100 mg of lamivudine (bulk or formulation) was accurately weighed and dissolved in 100 mL of distilled water. It was filtered and the filtrate was diluted with distilled water to get a working standard solution of 250 μg/mL.

Assay Procedure: Aliquots (0.5–2.5 mL, 250 μg/mL) of standard drug solution were transferred into a series of 25 mL graduated tubes. To each tube 1.5 mL of FC reagent followed by 7 mL of sodium carbonate solution were added. The

contents were mixed thoroughly and kept at room temperature for 30 min. The solutions were made up to 25 mL in each tube with distilled water. The absorbance values of the final coloured solution were measured at 750 nm against a reagent blank prepared in a similar manner. The amount of drug was computed from its calibration graph.

The optimum conditions for proposed method were established by varying one parameter at a time (OVAT)[7] and keeping the others fixed and observing the effect produced on the absorbance of the coloured species. Beer's law limit, molar absorptivity, Sandell's sensitivity[8] and regression characteristics of the proposed method are presented in Table-1. The relative standard deviation and % range of error at 95% confidence level are also given in Table-1. Commercial formulations (tablets) containing lamivudine were successfully analyzed by the proposed method. The results obtained by the proposed and reference methods for dosage forms were compared statistically by means of F- and t-tests and were found not to differ significantly at the 95% confidence level. Recovery studies were performed by adding a fixed amount of the drug to the preanalyzed formulations and the results are presented in Table-2 .

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHOD FOR LAMIVUDINE

Parameter	
λ_{max} (nm)	750
Beer's law limit (μg/mL)	7.5–75
Molar absorptivity (L mol^{-1} cm^{-1})	2.49×10^4
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.0249
Regression equation*	
Slope (b)	6.95×10^{-3}
Intercept (a)	3.33×10^{-4}
Correlation coefficient (r)	0.9999
Relative standard deviation† (%)	1.44
% Range of error (95% confidence limits)	1.51

*Y = a + bc, where c is the concentration of lamivudine in μg/mL and Y is the absorbance at 750 nm.
†Calculated from six determinations.

The interference studies in the determination of lamivudine in pharmaceutical formulation revealed that normally existing excipients and additives like starch, talc, stearic acid, boric acid, gelatin, magnesium carbonate and SLS were found not to interfere even when present in excess than anticipated amount.

Mechanism

The colour formation following reaction of FC reagent with lamivudine in this method may be explained in the following manner based on the analogy with reports of the earlier workers[9, 10]. The mixed acids in the FC preparation involve the following chemical species,

$3H_2O, P_2O_5, 13WO_3 \cdot 5MoO_3 \cdot 10H_2O$ and $3H_2O, P_2O_5, 14WO_3 \cdot 4MoO_3 \cdot 10H_2O$

Lamivudine probably affects a reduction of 1, 2 or 3 oxygen atoms from tungstate and/or molybdate in FC reagent (phosphomolybdotungstic acid), thereby producing one or more of the possible reduced species which have a characteristic intense blue color.

TABLE-2
ESTIMATION OF LAMIVUDINE IN PHARMACEUTICAL FORMULATIONS

Formulation (tablet)	Labelled amont (mg/tablet)	Amount found by proposed method*	Reference method†	% Recovery by proposed method‡
Lamidac	100	99.99 ± 0.1039 F = 3.00 t = 2.13	99.89 ± 0.18	98.65 ± 0.352
Lamivir	150	150.10 ± 0.16 F = 2.52 t = 1.88	148.92 ± 0.276	99.28 ± 0.58
Heptavir	100	99.44 ± 0.341 F = 3.91 t = 1.49	99.56 ± 0.321	99.80 ± 0.52

*Average ± s.d. of six determnations, 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.

†Developed in the laboratory using 0.1 N HCl (λ_{max} : 279 nm).

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

Conclusion

The proposed method is simple, sensitive and has reasonable precision and accuracy. The proposed method is useful for the determination of lamivudine in pure form and pharmaceutical formulations.

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