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Spectrophotometric Determination of Lamivudine

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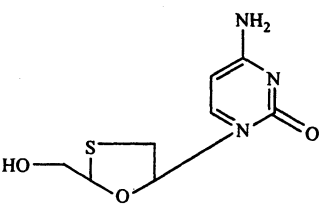
Three simple and sensitive spectrophotometric methods for the quantitative estimation of lamivudine in bulk drug and pharmaceutical formulations have been developed. The first method (Method I) is based on its diazotization with nitrous acid and subsequent coupling with phloroglucinol to form a yellow coloured species exhibiting absorption maximum at 445 nm and obeys Beer's law in the concentration range 2–10 g/mL. In the second method (Method II), the drug solution is reacted with Folin-Ciocalteau reagent in the presence of sodium carbonate solution to form a blue colour chromogen which has maximum absorption at 684 nm and obeys Beer's law in the concentration range 2–10 μg/mL. The third method (Method III) is based on the complex formation with 1,10-phenanthroline and Fe(III) exhibiting absorption maximum at 472 nm and obeys Beer's law in the concentration range 3–15 μg/mL. All coloured species are stable for more than 3 h. The results of analysis for all the three methods have been validated statistically and by recovery studies. The results of the above method are compared with the results of UV method which exhibited absorption maximum at 271 nm in alcohol.

Key words: Spectrophotometric, determination, lamivudine.

INTRODUCTION

Lamivudine is an anti-HIV agent. Lamivudine (2′-deoxy-3′-thiacyitidine) is a nucleoside analogue structurally related to zalcitabine and is a potent inhibitor of HIV-1 and HIV-2 *in vitro*. It belongs to the class of anti-HIV agents called the nucleoside reverse transcriptase inhibitors. Lamivudine is also currently under investigation for the treatment of hepatitis infection. There are no analytical reports for the quantitative estimation of lamivudine from bulk drug and formulations in literature except three from our laboratory earlier[2]. In continuation of



Lamivudine

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earlier work, in the present investigation, the authors have developed another method.

EXPERIMENTAL

All the chemicals used were of analytical grade reagents.

1. Aqueous solutions of phloroglucinol (0.5% w/v, always freshly prepared), sodium nitrite (0.1% w/v), ammonium sulphamate (0.5% w/v) and hydrochloric acid (5 M).
2. Folin-Ciocalteau reagent (F.C. reagent): Commercially available FC reagent (2 N) was suitably diluted to give 0.5 N with distilled water.
3. Sodium carbonate solution (20% w/v in water).
4. Aqueous solutions of ferric chloride (0.03 M), 1,10-phenanthroline (0.01 M) and phosphoric acid (0.2 M) were prepared. To avoid photochemical reduction, ferric chloride solution was prepared freshly and stored in an amber coloured bottle.

All spectral measurements were made on Systronics 119 spectrophotometer.

Standard and sample solutions: About 100 mg of (pure or equivalent formulation) lamivudine was accurately weighed and dissolved in 20 mL of absolute alcohol in a 100 mL volumetric flask and diluted up to the mark with absolute alcohol (solution I, 1 mg/mL); from solution I, 10 mL was pipetted out into a 100 mL volumetric flask and made up to the volume with alcohol (solution II, 100 μg/mL).

Assay

Method I: Into a series of 10 mL volumetric flasks, aliquots of lamivudine (solution II, 100 μg/mL) ranging from 0.2 to 1.0 mL were taken. To each flask hydrochloric acid (2 mL) and sodium nitrite (3 mL) were added and kept aside for 10 min. Then aqueous solutions of ammonium sulphamate (5 mL) and phloroglucinol (5 mL) were added to each flask at 3 min time interval and diluted to the mark with distilled water. The absorbance was measured at 445 nm against a reagent blank after 15 min and before 3 h. The amount of lamivudine was computed from the calibration curve.

Method II: Into a series of 10 mL volumetric flasks, aliquots of Lamivudine (solution II, 100 μg/mL) ranging from 0.2 to 1.0 mL were taken and added 2 mL of sodium carbonate solution followed by 2 mL of FC reagent and allowed to react for 10 min. The solution was then made up to volume with distilled water and the absorbance was measured at 684 nm against reagent blank. The amount of lamivudine was computed from the calibration curve. Colour was stable for more than 3 h.

Method III: Aliquots of lamivudine (solution II, 100 μg/mL) ranging from 0.3 to 1.5 mL were transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of ferric chloride (0.03 M) and 1 mL of 1,10-phenanthroline (0.01 M) were added and heated on boiling water bath for 15 min. Then the flasks were cooled to room temperature and 2 mL of orthophosphoric acid (0.2 M) was added to each flask. The volume was made up to the mark with distilled water. The absorbance of blood red coloured species was measured at 472 nm against reagent blank. The amount of lamivudine was computed from the calibration curve.

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table-1. The % RSD and per cent range of error (0.05 level confidence limit) calculated from six measurements containing 3/4 amount upper Beer's law limit of lamivudine are given in Table-1. The results show that the methods have reasonable precision. Comparison of the results obtained with the proposed and the reference methods for dosage forms (Table-2) confirm the suitability of these methods for the pharmaceutical dosage forms.

In order to justify the reliability and suitability of the proposed methods, known quantities of pure lamivudine were added to its preanalysed formulations and the mixtures were analysed by the proposed methods. The results of recovery experiments are also summarized in Table-2.

TABLE-1:
OPTICAL CHARACTERISTICS AND PRECISION

Parameter	Method I Phloroglucinol	Method II Folin-Ciocalteau	Method III 1,10-phenanthroline
λ_{max}(nm)	445	684	472
Beer's law limit (μg/mL)	2–10	2–10	3–15
Sandell's sensitivity (μm/cm^2/0.001 abs. unit)	0.021	0.120	0.089
Molar absorptivity	2.318×10^5	3.312×10^4	1.390×10^5
Regression equation (Y*) (Slope b)	2.6×10^{-3}	9×10^{-4}	2.4×10^{-3}
Intercept (a)	4.2×10^{-3}	6×10^{-3}	5.2×10^{-3}
Correlation coefficient (r)	0.9981	0.9912	0.9962
(%) RSD	1.5100	2.1400	1.8800
(%) Range of error (0.05 level confidence limit)	1.2620	1.7890	1.6230

*Y = a + bX, where X is the concentration of lamivudine in μg/mL and Y is the absorbance at the respective λ_{max}.

TABLE-2
DETERMINATION OF LAMIVUDINE IN PHARMACEUTICAL PREPARATIONS

Sample (tablets)	Labelled amount (mg)	Amount obtained by proposed method* (mg)		Reference method† (UV)	Percentage recovery*
T1	100	I.	99.48	99.01	99.25
		II.	99.34		99.54
		III.	99.05		99.30

*Results are average of eight measurements.
†As the drug is not official in any pharmacopoeia, official method is not available for comparison. UV method (λ_{max}: 271 nm in alcohol) developed in our laboratory was selected.

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