

Titrimetric and spectrophotometric determination of lamivudine in pharmaceuticals

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Three new methods are described for the assay of lamivudine in bulk drug and in tablet dosage forms using chloramine-T and two dyes, methyl orange and indigo carmine, as reagents. In titrimetry, aqueous solution of lamivudine is treated with a measured excess of chloramine-T in sulphuric acid medium and after the oxidation of lamivudine is judged to be complete, the unreacted oxidant is determined iodometrically. Spectrophotometric methods entail the addition of a known excess of chloramine-T to lamivudine in hydrochloric acid medium followed by determination of residual oxidant by reacting it with a fixed amount of either methyl orange and measuring the absorbance at 520 nm (Method A) or indigo carmine and measuring the absorbance at 610 nm (Method B). In all the methods, the amount of chloramine-T reacted corresponds to the amount of lamivudine. Titrimetric method is applicable over 3.5-10 mg range. In spectrophotometric methods, the systems obey Beer's law for 0.1-1.0 and 0.25-3.5 $\mu\text{g mL}^{-1}$ for method A and method B, respectively. Intra-day and inter-day precision and accuracy of the developed methods were evaluated. The methods were successfully applied to the assay of lamivudine in tablet formulations and the results were compared with those of a reference method by applying Student's t- and F-tests. No interference was observed from common tablet adjuvants.

Keywords: Lamivudine, Assay, Titrimetry, Spectrophotometry, Chloramine-T, Tablets

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Lamivudine (LMV), chemically, (2R-cis)-4-amino-1-(2-hydroxymethyl)-1,3-oxathiolan-5-yl}-2-(1H)-pyrimidinone(-)-2'-deoxy-3'-thiacytidine, is the active ingredient of many pharmaceutical preparations concerned with the treatment of human immuno deficiency virus infector HIV, the virus that causes AIDS¹⁻³. LMV is also active against hepatitis B virus. The drug is official in Martindale, the Extra Pharmacopoeia⁴. Literature survey revealed that not many methods are available for the assay of LMV and most of the methods found in the literature are applicable to the determination of the drug in body fluids. High performance liquid chromatography (HPLC) is the most widely used technique and has been applied for the determination of LMV in human plasma⁵⁻⁷, plasma, saliva and cerebrospinal fluid⁸, human serum⁹, blood cells¹⁰, and also for the determination of drug metabolites in urine¹¹, and blood plasma¹². LMV in human serum has also been determined by capillary electrophoresis¹³. Very recently, the drug in combination with stavudine has been assayed by HPTLC¹⁴ and with zidovudine by reversed phase HPLC¹⁵ in tablet dosage forms.

An UV-spectrophotometric method based on the measurement of the absorbance of the aqueous solution at 295 nm has been reported by Rajasekaran *et al.*¹⁶. The method is applicable over 10-60 $\mu\text{g mL}^{-1}$. A similar method but involving the measurement at 270 nm has been developed by Sankar *et al.*¹⁷. One of the first reports on the visible spectrophotometric determination of LMV is based on the measurement of coloured condensation products formed with three aromatic aldehydes¹⁸. But, in these procedures, the absorbance is measured at shorter wavelengths where the interference from the tablet excipients is more. Sarma *et al.*¹⁹ have recently reported three procedures based on redox and complexation reactions. The first two methods involve the oxidation of LMV by a measured excess of KMnO_4 or KIO_3 followed by the estimation of the unreacted oxidant by reacting with either fast green FCF or 3-methyl benzothiazolinone hydrazone. In the third method, iron(II) formed following the reaction of LMV with iron(III) chloride is complexed with ferricyanide and the blue coloured complex measured. The same authors²⁰ have proposed three more procedures based on the use of *N*-

bromosuccinimide-celestine blue, cobalt thiocyanate and molybdate as reagents. There is also a report on the development of assay methods²¹ based on diazocoupling, redox (Folin-Ciocalteu reagent) and redox-complexation (ferric chloride-orthophenanthroline) reactions. But, the reported methods suffer from one or the other disadvantage such as use of unstable reagents^{19,20}, expensive chemical¹⁹, low sensitivity²⁰, liquid-liquid extractions or heating step²⁰. Further, the reported HPTLC¹⁴ and HPLC¹⁵ methods are limited to assay of LMV in combined dosage forms and need sophisticated and expensive instrumentation. The low cost and the ease of operation make the titrimetric and the spectrophotometric techniques highly desirable alternatives for the assay of LMV in pharmaceuticals. For this reason and as a result of the therapeutic importance of LMV, it was of interest to investigate the application of chloramine-T as a titrimetric and spectrophotometric reagent for the assay of LMV. The proposed methods exhibit useful analytical characteristics such as sensitivity, speed, accuracy and precision for the assay of LMV in pure form in tablet dosage forms.

Experimental Procedure

Reagents and materials

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Chloramine-T solution (0.02 M) was prepared by dissolving 5.634 g of the chemical (Qualigens fine chem., Glaxo India Ltd., Mumbai) in water and diluting to 1 litre, and used for titrimetric work after iodometric standardization²². For spectrophotometric investigation, the above solution was diluted appropriately with water to get 150 and 600 $\mu\text{g mL}^{-1}$ chloramine-T solutions for method A and method B, respectively. To prepare 50 $\mu\text{g mL}^{-1}$ methyl orange for method A, first, a 500 $\mu\text{g mL}^{-1}$ dye solution was prepared by dissolving 59 mg of dye (s.d. fine-chem Ltd., Mumbai, assay 85%) in water and diluting to 100 mL in a calibrated flask, and filtered using glass wool. This was diluted ten-fold with water to get the required concentration. For method B, first, a 1000 $\mu\text{g mL}^{-1}$ indigo carmine solution was prepared by dissolving 112 mg of dye (s.d. fine-chem Ltd., Mumbai, 90% assay) in water and diluting to 100 mL, and filtered. This was appropriately diluted with water to get 200 $\mu\text{g mL}^{-1}$. Sulphuric acid (1 M) was

prepared by adding 14 mL of concentrated acid (s.d. fine-chem Ltd., Mumbai, Sp gr 1.82) to 236 mL of water with cooling. Hydrochloric acid (5 M) was prepared by diluting 111 mL of concentrated acid (s.d. fine chem., Mumbai, sp.gr. 1.18) to 250 mL with water. Sodium thiosulphate solution (0.02 M) was prepared by dissolving about 5 g of the chemical (SISCO Chem. Industries, Mumbai) in 1 litre of water and standardized with pure potassium dichromate iodometrically²³. Aqueous solutions of potassium iodide (10%) and starch indicator (1%) were prepared in the usual way. Pharmaceutical grade LMV (certified to be 99.7% pure) was procured from Aurobindo Pharma, Hyderabad, India, and was used as received. A 1 mg mL^{-1} solution of LMV was prepared by dissolving 250 mg of pure drug in water and diluting to 250 mL with water and used for assay by titrimetry. This stock solution (1000 $\mu\text{g mL}^{-1}$) was diluted with water to get working concentrations of 2 and 10 $\mu\text{g mL}^{-1}$ LMV for method A and method B, respectively.

Methods

Titrimetry

A 10 mL aliquot of pure drug solution equivalent to 3.5-10 mg of LMV was measured accurately and transferred into a 100 mL titration flask. Five mL of 1 M sulphuric acid followed by 10 mL of 0.02 M chloramine-T solution were added and kept aside for 15 min with occasional swirling. Then, 5 mL of 10% potassium iodide solution were added to the flask and the liberated iodine was titrated with 0.02 M sodium thiosulphate to a starch end point. A blank titration was run under same conditions. The drug content in the aliquot was calculated from:

$$\text{Amount (mg)} = \frac{(B-S)M_w R}{2} \quad \dots(2)$$

where B = volume of thiosulphate solution consumed in the blank titration, mL, S = volume of thiosulphate solution consumed in the sample titration, mL, M_w = relative molecular mass of the drug and R = strength of chloramine-T solution, mol L^{-1} .

Spectrophotometric method using methyl orange (Method A)

Aliquots of pure LMV solution (0.5 to 5.0 mL; 2 $\mu\text{g mL}^{-1}$) were transferred into a series of 10 mL calibrated flasks and the total volume was adjusted to 5 mL with water. To each flask were added 2 mL of

5 M hydrochloric acid followed by 1 mL of chloramine-T solution ($150 \mu\text{g mL}^{-1}$). The contents were mixed well and the flasks were set aside for 15 min with occasional shaking. Finally, 1 mL of $50 \mu\text{g mL}^{-1}$ methyl orange solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 520 nm against water blank after 5 min.

Spectrophotometry with indigo carmine (Method B)

Varying aliquots (0.25-3.5 mL) of standard $10 \mu\text{g mL}^{-1}$ LMV solution were delivered into a series of 10 mL calibrated flasks and the total volume was brought to 3.5 mL with water. To each flask were added 2 mL of 5 M hydrochloric acid and 1 mL of $600 \mu\text{g mL}^{-1}$ chloramine-T solution successively; the flasks were let stand for 15 min with occasional shaking. Then, 1 mL of $200 \mu\text{g mL}^{-1}$ indigo carmine solution was added to each flask, the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 610 nm against water blank after 5 min.

In either spectrophotometric method, the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

Assay of formulations

Twenty tablets were weighed and ground into a fine powder. Powder equivalent to 100 mg of LMV was weighed accurately into a 100 mL calibrated flask, 60 mL of water added and shaken for 20 min. Then, the volume was made up to the mark with water, mixed well, and filtered using a Whatman No 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (1 mg mL^{-1} LMV) was taken for assay by titrimetric procedure. The filtrate was diluted appropriately to get 2 and $10 \mu\text{g mL}^{-1}$ concentrations for analysis by spectrophotometric method A and method B, respectively.

Results and Discussion

The proposed methods are indirect and are based on the determination of surplus chloramine-T after allowing the reaction between LMV and oxidant to occur. In titrimetry, the unreacted chloramine-T is determined iodometrically, and in spectrophotometric methods, the same is determined by reacting with a fixed amount of either methyl orange or indigo carmine. The latter methods make use of the

bleaching action of chloramine-T on either dye, the decolouration being caused by the oxidative destruction of the dye.

Optimisation of experimental conditions

Titrimetry

Since the reaction stoichiometry was found to be 1:2, it is probable that the drug undergoes oxidation to sulphone, according to the scheme given in Fig. 1. Non-stoichiometric results were obtained in hydrochloric acid medium and a 0.2 M sulphuric acid concentration was found optimum although reaction stoichiometry was unaffected in the range 0.04-0.4 M H_2SO_4 . The oxidation reaction was found to be complete and quantitative in 15 min and contact times up to 20 min had no effect on the stoichiometry and the results. Beyond 20 min and up to 60 min a small amount of CAT was consumed but without resulting in any significant reaction stoichiometry. Hence, it is necessary to terminate the oxidation step at the end of 15 min to obtain accurate and precise results. A 10 mL aliquot of 0.02 M chloramine-T solution was found adequate for quantitative oxidation of LMV. Employing 0.02 M chloramine-T solution, 3.5-10 mg of LMV could be conveniently determined. The relation between the amount of drug and titration end point was examined. The linearity is apparent from the calculated correlation coefficient of -0.9954 and suggests that the reaction between LMV and chloramine-T proceeds stoichiometrically in the ratio 1:2.

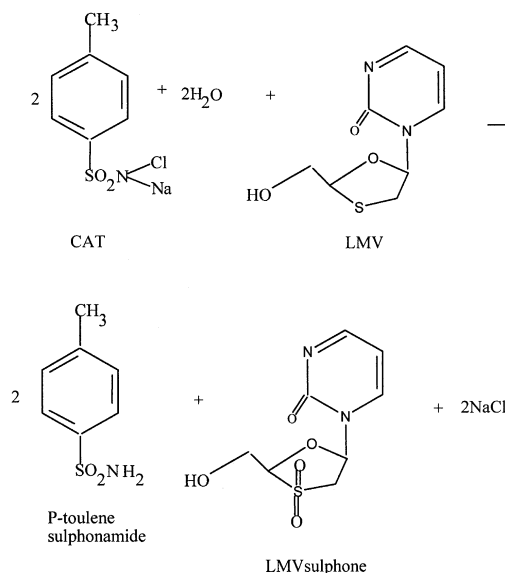


Fig. 1 — Probable reaction scheme

Spectrophotometry

In the proposed spectrophotometric methods, the ability of chloramine-T to effect oxidation of LMV and irreversibly destroy methyl orange or indigo carmine to colourless products in acid medium has been used. LMV when added in increasing concentrations to a fixed concentration of chloramine-T, consumes the latter and there will be a concomitant decrease in its concentration. When a fixed concentration of either dye is added to decreasing concentration of chloramine-T, a concomitant increase in the concentration of dye results. A proportional increase in the absorbance at the respective $\lambda_{\max}$ is observed with increasing concentration of LMV.

Preliminary experiments were performed to fix the upper limits of the dyes that could be determined spectrophotometrically, and these were found to be 5 and 20 $\mu\text{g mL}^{-1}$ for methyl orange and indigo carmine, respectively. A chloramine-T concentration of 15 $\mu\text{g mL}^{-1}$ was found to irreversibly destroy the red colour of 5 $\mu\text{g mL}^{-1}$ methyl orange whereas 60 $\mu\text{g mL}^{-1}$ chloramine-T was required to bleach the blue colour due to 20 $\mu\text{g mL}^{-1}$ indigo carmine. Hence, different amounts of LMV were reacted with 1 mL of 150 $\mu\text{g mL}^{-1}$ chloramine-T in method A and 1 mL of 600 $\mu\text{g mL}^{-1}$ chloramine-T in method B followed by determination of the residual oxidant as described under the respective procedures.

For both the steps, hydrochloric acid medium was found to be ideal. Two mL of 5 M HCl in a total volume of 8 mL was adequate for the oxidation step which, was complete in 15 min, and the same quantity of acid was employed for the estimation of the dye. Contact time of 15 min is not critical and any delay upto 30 min had no effect on the absorbance. A 5 min standing time was found necessary for the complete bleaching of the dye colour by chloramine-T. The absorbance of either dye colour was stable for several hours in the presence of reaction products. Since the reagent blanks in both methods were practically colourless, the absorbance of the dye colour was measured against a water blank.

Analytical data

A linear correlation was found between absorbance at $\lambda_{\max}$ and concentration of LMV. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values of both methods are given in Table 1. Regression analysis of Beer's law data using the method of least squares was

Table 1 — Analytical parameters of the spectrophotometric methods

Parameter	Method A	Method B
$\lambda_{\max}$, nm	520	610
Beer's Law limits, $\mu\text{g mL}^{-1}$	0.1-1.0	0.25-3.5
Molar absorptivity, l/mol/cm	1.39×10^5	4.60×10^4
Sandell sensitivity, $\mu\text{g/cm}^2$	1.64	4.98
Limit of detection, $\mu\text{g mL}^{-1}$	0.319	0.979
Limit of quantification, $\mu\text{g mL}^{-1}$	0.966	2.969
Regression equation (Y)*		
Intercept (a)	-0.0013	0.001
Slope (b)	0.6115	0.1987
S_a	0.004	0.0102
S_b	0.0045	0.003
Correlation co-efficient (R)	0.9998	0.9994

S_a = Standard deviation of intercept.

S_b = Standard deviation of slope.

made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 1. The graph showed negligible intercept as described by the regression equation $Y = a + bX$ where Y is the absorbance and X concentration in $\mu\text{g mL}^{-1}$. The limit of detection and quantification calculated according to ICH guidelines²⁴ are also given in Table 1 and reveal the very high sensitivity of the spectrophotometric methods.

Method validation

Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solution at three different levels (within the working limits) was analysed, each determination being repeated seven times. The relative error (%) and relative standard deviation (%) were less than 3.5 and indicate the high accuracy and precision for the methods (Table 2). Titration method with a relative error of less than 1% and relative standard deviation of less than 1% is the most accurate and precise compared to other two methods. For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which standard drug solution at three different levels was determined each day for five days with all solutions being prepared afresh each day. The day-to-day relative standard deviation values were in the range of 0.2-0.7% and represent the best appraisal of the methods in routine use.

Table 2 — Intra-day precision and accuracy of the methods

Titrimetry					Spectrophotometric method A					Spectrophotometric method B				
LMV taken mg	LMV found mg	Range mg	RE, %	SD, mg	RSD, %	CL	LMV taken $\mu\text{g mL}^{-1}$	LMV found $\mu\text{g mL}^{-1}$	Range $\mu\text{g mL}^{-1}$	RE, %	SD $\mu\text{g mL}^{-1}$	RSD, %	CL	
4.0	3.98	0.09	0.5	0.03	0.73	3.98 ± 0.03	0.2	0.21	0.004	3.5	0.0013	0.61	0.21 ± 0.0012	
6.0	6.05	0.09	0.8	0.03	0.57	6.05 ± 0.03	0.4	0.39	0.005	1.78	0.0022	0.57	0.39 ± 0.0021	
8.0	7.98	0.04	0.3	0.02	0.24	7.98 ± 0.02	0.6	0.61	0.006	1.17	0.0021	0.35	0.61 ± 0.0019	

RE, Relative error; SD, Standard deviation; RSD, Relative standard deviation and

CL, Confidence limits at 95% confidence level for six degrees of freedom.

Interference study

To investigate the effect of tablet fillers on the measurements involved in the methods, placebo analysis was carried out. A mixture containing lactose, starch, talc, magnesium stearate, sodium alginate and calcium gluconate in the ratio 80:7:2.5:0.5:1:9 was extracted with water and filtered using a quantitative filter paper. The filtrate was subjected to analysis by the proposed methods and it was found that a positive relative error of 1.5, 2.0 and 2.5% for method A, method B and method C, respectively, was obtained. From this study, it is apparent that the usual co-formulated substances seldom interfere in the methods.

Application to analysis of commercial samples

In order to check the validity of the proposed methods, LMV was determined in some commercial formulations. Table 3 gives the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically by a Student's t-test for accuracy and variance ratio F-test for precision with those of the reference method¹⁶ at 95% confidence level. The calculated t- and F-values (Table 3) did not exceed the tabulated values ($t=2.77$, $F=6.39$) for four degrees of freedom indicating that there was no significant difference between the proposed methods and the reference method in respect to accuracy and precision.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analysed tablet powder was spiked with pure LMV at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative (97.46-105.1%) and revealed that co-formulated substances such as talc, starch, gum acacia, lactose, sodium alginate, magnesium stearate, calcium carbonate, calcium gluconate and calcium dihydrogenorthophosphate did not interfere in the determination.

Conclusion

Three useful micro methods for the determination of lamivudine have been developed and validated. The methods are simple and rapid taking not more than 15-20 min for the assay. The titrimetric method which is applicable over 3.5-10 mg range and the spectrophotometric method using methyl orange with

Table 3 — Results of assay of tablets by the proposed methods

Brand name of tablet*	Nominal amount, mg	Reference method	Titrimetry	% found* $\pm$ SD	
				Spectrophotometric method A	Spectrophotometric method B
Lamidac	100	101.5 $\pm$ 0.92	102.6 $\pm$ 0.54 $t=2.38$ $F=2.90$	101.1 $\pm$ 1.15 $t=0.61$ $F=1.56$	100.8 $\pm$ 1.48 $t=0.92$ $F=2.59$
Heptavir	100	98.42 $\pm$ 1.18	99.24 $\pm$ 1.46 $t=0.98$ $F=1.53$	99.52 $\pm$ 1.61 $t=1.87$ $F=2.33$	100.1 $\pm$ 1.31 $t=1.45$ $F=5.15$
Lamivir	150	102.6 $\pm$ 1.54	100.6 $\pm$ 0.85 $t=2.64$ $F=3.28$	99.5 $\pm$ 1.30 $t=3.45$ $F=1.40$	101.1 $\pm$ 1.75 $t=1.44$ $F=1.29$

*Marketed by: a. Cipla India Ltd; b. Aurobindo; c. Genix

**Mean value of five determinations

Tabulated value of t at 95% confidence level is 2.77

Tabulated value of F at 95% confidence level is 6.39

a molar absorptivity of $1.39 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ are the most sensitive ever reported for lamivudine. The methods rely on the use of simple and cheap chemicals and techniques but provide a sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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