agar. The diameter of each cup was 10 mm. To these cups, 50 μl of the test solution was added with the help of a sterile micro pipette. The test solution was prepared by dissolving 5 mg compound in 5 ml of DMF. The drug solution was allowed to diffuse for about 2 h. The plates were incubated at 37° for 24 h and zones of inhibition in mm were recorded. Standard drugs used for testing were ampicillin, chloramphenicol, norfloxacin and greseofulvin. Results showing the highest activity of the compounds by cup plate method are given in Table 1.

All the compounds synthesised were screened for *in vitro* antimicrobial activity against Gram positive bacteria, *S. aureus* and *S. pyogens* and Gram negative bacteria, *E. coli* and *K. aerogens*. The fungal strain used for testing was *A. niger*. The standard drugs used for comparison were ampicillin, chloramphenicol, norfloxacin and greseofulvin. Compounds IIIb, IIIe, IIIf, IIIr, IIIy, IIIaa, IIIII, IIIrr displayed highest activity against above microbes. The other compounds show mild to moderate activity against these organisms. Table 1 represents the activity observed against the microbes investigated.

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Spectrophotometric Determination of Lamivudine and Stavudine

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A simple, sensitive spectrophotometric method has been developed for the determination of lamivudine and stavudine in pure and its pharmaceutical formulations. Lamivudine and stavudine give green coloured chromogens with 3-methyl-2-benzothiozolinone hydrazone hydrocloride and ferric chloride with absorption maximum at 660 nm and 630 nm, respectively. The chromogens obey Beer's law in the concentration ranges of 2.5 to 40 μg/ml and 2.5 to 12.5 μg/ml, respectively for lamivudine and stavudine.

Lamivudine (LMD) and stavudine (SVD) are antiHIV drugs. LMD is chemically 4-amino-1-[2-(hydroxy methyl)-1,3-oxathiolan-5-yl]-2-pyrimidinone[1] and it acts by inhibiting nucleoside reverse transcriptase, which selectively inhibits HIV1 replication[2]. SVD is chemically 2,3-didehyro-3-deoxy thymidine[1], which acts as a competitive inhibitor of deoxythymidine tri phosphate and incorporation causes termination of DNA chain elongation[2]. A few HPLC methods for

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the estimation of LMD[3-6] and SVD[3,7] in human plasma in combination with other antiHIV drugs and a spectrophotometric method for determination of LMD in pharmaceutical formulations[8] have been reported. In this paper, the authors report a simple, sensitive and reproducible spectrophotometric method for the determination of LMD and SVD in pure form as well as in phatmaceutical formulations. The drugs react with 3-methyl-2-benzothiozolinone hydrazone hydrochloride (MBTH) in presence of $FeCl_3$ and give a green coloured complex with absorption maximum at 660 nm for LMD and at 630 nm SVD.

All the chemicals used were of analytical grade. Ferric chloride and MBTH were procured from Loba Chemie. Commercially available formulations Lamidac-Zydus Cadila, Lamivir and Stavir-40, Cipla, Heptavir-100-Hetero Drugs Ltd., were procured from the local market. Spectral and absorbance measurements were made on a Systronics UV-Vis spectrophotometer model 117 with 10 mm-matched quartz cells.

Solutions of ferric chloride (0.033 M) and MBTH (0.2%) were prepared in distilled water. About 100 mg of LMD or SVD (pure or from formulation) was accurately weighed and dissolved in 50 ml of distilled water. To each of these solutions, 5 ml concentrated HCl was added and kept in a boiling water bath for 1 h. The volume was made up to 100 ml with distilled water. These stock solutions were further diluted to get a concentration of 500 μg/ml.

For the assay of LMD, aliquots of standard solution of LMD ranging from 0.1 to 0.5 ml (1 ml=500 μg) were transfered into a series of test tubes. To these, 1 ml of MBTH (0.2%) and 1 ml of $FeCl_3$ (0.033 M) were added successively. The solutions were heated on a boiling water bath for 10 min and cooled to room temperature. The reacted solutions were transferred to 10 ml volumetric flasks and the final volume was made up to the mask with distilled water. The absorbance of the green coloured complex formed was measured at 660 nm against the reagent blank and the amount of LMD present in the sample solution was computed from the calibration curve.

For the assay of SVD, aliquots of standard solution of SVD ranging from 0.5 to 2.5 ml (1ml=500 μg) were transfered in to a series of test tube. To that 1 ml of $FeCl_3$, (0.033 M) and 1 ml of MBTH (0.2%) were added successively. Then the test tubes were heated on a boiling water bath for 5 min and cooled to room temperature. The resulting solutions were transfered to 10 ml calibrated test tubes and the final volume was made up to the mark with distilled water. The absorbance of the green coloured species, formed was measured at 630 nm against the reagent blank and the amount of SVD present in the sample solution was computed from the calibration curve.

Hydrolyzed LMD and SVD in slightly acidic conditions react with MBTH in the presence of $FeCl_3$ to form a complex with a maximum absorption at 660 and 630 nm, respectively. The concentration of MBTH, $FeCl_3$, sequence of addition of reagents, the reaction temperature, time and the final dilution were optimized to get good sensitivity, stability and minimum blank reading. The absorbance of reaction product for LMD remains stable, for 1.5 h where as it remains stable for 3 h for SVD and absorbance must be measured in these times.

Under the experimental conditions described, the absorbance (A) was found to be a linear function of the concentration (C, μg/ml) for both drugs over the Beer's law range, given in Table 1. The Sandell's sensitivity, molar extinction coefficient, correlation coefficient, % relative standard deviation, (calculated from the eight measurements containing 3/4th of the amount of the upper Beer's law limits of LMD and SVD), % range of error (0.05 to 0.01 confidence limits)

TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION.

Parameters	Lamivudine	Stavudine
Beer's law limit (μg/ml)	5.0-25	25-125
Sandell's sensitivity ($\mu g/cm^2$/0.001 absorbance unit	0.0249	0.1572
Molar extinction coefficient (L/mole.cm)	1.560×10^4	0.2472×10^4
% Relative standard deviation	0.5778	0.9340
% Range of error		
0.05 confidence limits	±0.4831	±0.78096
0.01 confidence limits	±0.7149	±1.1554
Correlation coefficient	0.9998	0.9989
Regression equation (Y*)		
Slope (a)	0.03908	0.0086
Intercept (b)	0.0114	0.0060

Y*=b+aC, where "C" is concentration in μg/ml and Y is absorbance unit.

TABLE 2: ESTIMATION OF LAMIVUDINE AND STAVUDINE IN PHARMACEUTICAL FORMULATIONS.

Sample	Labelled amount (mg)	Amount found (mg) ± s.d.		% Recovery
		Reported method[9]	Proposed method	
Lamivudine				
Lamidac	100	99.8±0.29	99.9±0.88	99.9
Lamivir	150	148.9±0.08	150.1±1.64	100.0
Heptavir	100	99.4±1.25	99.9±1.74	99.3
Stavudine				
Stavir	40	39.7±0.56	39.8±0.95	99.5

were calculated and the results are included in the table.

The recovery values obtained for the determination of LMD and SVD in pharmaceutical formulations (tablets) by the proposed and reported method[9] are compared in Table 2. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparations and the mixtures were analyzed by the proposed methods. Interference studies revealed that the common excipients and other additives usually present in the dosage form did not interfere in the proposed methods.

It is postulated that the reaction of MBTH with hydrolyzed LMD and SVD in presence of $FeCl_3$ proceeds via oxidative coupling reaction, similar to that reported between phenols and MBTH[9,10]. MBTH on oxidation looses two electrons and a proton, forming the electrophilic intermediate, which is an active coupling species. It is probable that the intermediate undergoes electrophilic substitution with the investigated drugs to form a green coloured complex. From the data obtained it is evident that the proposed method is simple, sensitive precise and accurate and hence it can be used for the routine determination of LMD and SVD in bulk as well as in pharmaceutical formulations.

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