

UV Spectrophotometric Determination of Some Anti-HIV Drugs

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A simple and sensitive UV spectrophotometric method for the determination of four anti-HIV drugs, lamivudine, stavudine, nelfinavir mesylate and nevirapine was developed with λ_{max} 270 nm, 265 nm, 253 nm and 284 nm respectively and these methods are extended to pharmaceutical preparations. There is no interference from any common pharmaceutical additives and diluents.

Key words: Spectrophotometric, determination, anti-HIV drugs.

INTRODUCTION

Lamivudine (LMD), stavudine (SVD), nelfinavir mesylate (NM) and nevirapine (NEV) are anti-HIV drugs^{1,2}. Chemically LMD is 4-amino-1-[2-(hydroxy methyl)-1,3-oxathiolan-5-yl]-2-pyrimidinone; SVD is 2,3-didehydro-3-deoxy thymidine, NM is N-(1,1-dimethyl ethyl) decahydro-2-[2-hydroxy-3-[(3-hydroxy-2-methyl benzoyl) amino]-4-(phenylthio)butyl]-3-isoquinoline carbaxamide mono-methane sulphonate and NEV is 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido-[1,4]-diazepine 6-one. LMD is nucleoside reverse transcriptase inhibitor that selectively inhibits HIV1 replication. SVD acts as a competitive inhibitor of deoxy thymidine triphosphate and incorporation causes termination of DNA chain elongation. NM is an inhibitor of HIV1 protease. NEV is a non-nucleoside reverse transcriptase inhibitor, which selectively inhibits HIV1 replication. Few HPLC methods were reported for the estimation of LMD³⁻⁶, SVD^{3,7}, NM^{3,8-11} and NEV^{3,11-13} in human plasma. The present investigation has been undertaken to develop a UV spectrophotometric method for the determination of anti-HIV drugs such as LMD, SVD, NM, NEV, which exhibit absorption maxima at 270 nm, 265 nm, 253 nm and 284 nm respectively.

EXPERIMENTAL

Spectral and absorbance measurements were made on Systronics UV-Visible spectrophotometer 117 with 10 mm matched quartz cells.

Preparation of standard solutions: Accurately weighed 100 mg of LMD and SVD was dissolved in 100 ml of distilled water and the solutions were diluted quantitatively with water to obtain a final concentration of 50 $\mu\text{g}/\text{mL}$, whereas for NM and NEV, 100 mg was weighed and dissolved in 100 mL of methanol

and the solutions were diluted quantitatively with methanol to obtain working standard solution of 100 $\mu\text{g/mL}$.

Preparation of sample solutions: Twenty tablets/capsules were accurately weighed and finely powdered. The 100 mg powder of each of LMV and SVD was dissolved in distilled water, whereas that of NM and NEV was dissolved in methanol. These solutions were filtered. The residue was washed with corresponding medium. The filtrates were diluted to 100 mL with distilled water for LMV and SVD, whereas with methanol for NM and NEV.

Method for LMD and SVD: Aliquots of working standard solutions of LMD or SVD ranging from 0.25 to 2.0 mL (1 mL containing 50 μg) were transferred into a series of 10-mL volumetric flasks and the volume was brought to 10 mL with distilled water. The absorbance was measured at 270 nm for LMD and at 265 nm for SVD against distilled water. The amount of LMD or SVD present in the sample solution was computed from its calibration curve.

Method for NM and NEV: To a series of 10 mL volumetric flasks aliquot samples of NM ranging from 0.5 to 2.5 mL (1 mL containing 100 μg) of NEV ranging from 0.25 to 1.5 mL (1 mL containing 100 μg) were transferred. Then the final volume was brought to 10 mL with distilled water. The absorbance was measured at 253 nm for NM and 284 nm for NEV against methanol as blank. The amount of NM or NEV present in the sample solution was computed from its calibration curve.

RESULTS AND DISCUSSION

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar extinction coefficient, per cent relative standard deviation, (calculated from the eight measurements containing 3/4th of the amount of the upper Beer's law

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION OF THE PROPOSED METHODS

Parameters	Lamivudine	Stavudine	Nelfinavir mesylate	Nevirapine
Beer's law limit ($\mu\text{g/mL}$)	1.5–10.0	12.5–10.0	5.0–25.0	2.5–15.0
Sandell's sensitivity ($\mu\text{g/cm}^2/0.001$ absorbance unit)	0.1436	0.01179	0.3802	0.1992
Molar extinction coefficient ($1 \text{ mole}^{-1} \text{ cm}^{-1}$)	2.706×10^4	3.297×10^4	1.0225×10^4	1.992×10^4
% Relative standard deviation	0.0387	0.4045	0.5066	0.7536
%Range of error:				
0.05 confidence limits	± 0.03237	± 0.3383	± 0.4235	± 0.6301
0.01 confidence limits	± 0.0479	± 0.5005	± 0.6267	± 0.9323
Correlation coefficient	0.9984	0.9997	0.9999	0.9998
Regression equation (Y^*):				
Slope (a)	0.0741	0.0839	0.0263	0.0494
Intercept (b)	-0.0130	0.0054	-0.0419	0.0056

$Y^* = b + aC$, where "C" is concentration in $\mu\text{g/mL}$ and Y is absorbance unit.

limits for all the drugs), per cent range of error (0.05 to 0.01 confidence limits) were calculated and the results are summarized in Table-1. The methods were applied for the analysis of the drugs in their pharmaceutical formulations. 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 proposed methods and the results are presented in Table-2. Interference studies revealed that the common excipients and other additives usually present in the dosage form did not interfere in the proposed methods.

TABLE-2
ESTIMATION OF LMD, SVD, NM AND NEV IN
PHARMACEUTICAL FORMULATIONS

Sample	Labelled amount (mg)	Amount found (mg) Proposed method	Recovery (%)
LMD Tablet			
1	100	99.97	99.97
2	150	149.74	99.82
3	100	99.36	99.36
SVD Capsules			
1	30	29.38	97.93
2	40	39.54	98.85
NM Tablets			
1	250	248.86	99.54
2	250	249.12	99.64
NEV Tablets			
1	200	199.73	99.86
2	200	198.31	99.40
3	200	199.23	99.61

In conclusion the proposed methods are most economic, simple, sensitive and accurate and can be used for the routine determination of LMD, SVD, NM and NEV in bulk as well as in its pharmaceutical preparations.

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