DISCUSSION
6. DISCUSSION

- Five simple HPTLC methods were developed and validated for the quantitative estimation of Abacavir, Tenofovir, Emtricitabine, Efavirenz and Nelfinavir from bulk and its formulation (Tablets and capsules).

- The instrument used in the present study was Camag Linnomat V- automatic sample applicator, Hamilton syringe (100µl), Camag TLC scanner 3, Camag Twin through chamber of appropriate size, Camag Wincats software was used for acquisition, evaluation and storage of chromatographic data.

- The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis.

- The analysis of Abacavir, Tenofovir, Emtricitabine, Efavirenz and Nelfinavir was carried out on TLC aluminum plates precoated with silica gel 60 F 254 as the stationary phase. The mobile phase used were a mixture of (Chloroform: Methanol 9: 1 v/v), (Chloroform: Methanol 8.5: 1.5v/v), (Chloroform: Methanol 8.5: 1.5v/v), Toluene: Ethyl acetate: Formic acid (10: 3: 1 v/v) and n-Butanol: Ethyl acetate: Diethyl ether (5: 4: 1 v/v) respectively.

- Abacavir, Tenofovir, Emtricitabine, Efavirenz and Nelfinavir have absorption maxima at 284nm, 270nm, 275nm, 254nm and 254nm respectively. Therefore, the wavelengths were chosen as 284nm, 270nm, 275nm, 254nm and 254nm respectively for the estimation.
The Limit of detection was found to be 17.068ng/ml while quantification limit was 51.723ng/ml for Abacavir, 4.123ng/ml and 12.494ng/ml for Tenofovir, 4.320ng/ml and 13.092ng/ml for Emtricitabine, 8.190ng/ml and 24.819ng/ml for Efavirenz and 12.658ng/ml and 38.358ng/ml for Nelfinavir respectively.

The R.S.D value for the assay is 0.714% for Abacavir, 0.793% for Tenofovir, 0.346% for Emtricitabine, 0.484% for Efavirenz and 0.884% for Nelfinavir respectively which indicates that the method is precise and reproducible (According to ICH guidelines).

Correlation coefficient ($r^2$) for Abacavir was found to be 0.9992, indicating the linearity of the method between the concentrations of 400-2400ng/ml with Rf value 0.81±0.02 (Fig 6.1)
Correlation coefficient ($r^2$) for Tenofovir was found to be 0.9994, indicating the linearity of the method between the concentrations of 200-1200ng/ml with Rf value 0.54±0.01 (Fig 6.2)

Correlation coefficient ($r^2$) for Emtricitabine was found to be 0.9992, indicating the linearity of the method between the concentrations of 200-2200ng/ml with Rf value 0.56±0.02 (Fig 6.3)
Correlation coefficient ($r^2$) for Efavirenz was found to be 0.9991, indicating the linearity of the method between the concentrations of 300-1800ng/ml with Rf value 0.41±0.01 (Fig 6.4)

Correlation coefficient ($r^2$) for Nelfinavir was found to be 0.9993, indicating the linearity of the method between the concentrations of 400-2400ng/ml with Rf value 0.55±0.01 (Fig 6.5)

The percentage recovery of bulk drug and formulation (Tablets and capsules) was found to be 99.49 to 99.79 %, 98.39 to 101.19 %, 98.79 to 99.61 %, 99.38 to 99.68
% and 99.44 to 100.52 % for Abacavir, Tenofovir, Emtricitabine, Efavirenz and Nelfinavir respectively, This demonstrating that the developed HPTLC method is simple, accurate, precise and reproducible.

- The method was validated in terms of Linearity, Accuracy, Precision, Specificity, Limit of detection, Limit of quantification and Range.

- The peaks in the chromatograms indicating that the methods are free from interferences. The repeatability and reproducibility indicated that the proposed methods are precise and reproducible.

- The developed HPTLC methods for quantitative estimation of Abacavir, Tenofovir, Emtricitabine, Efavirenz and Nelfinavir were accurate, precise, linear, specific, simple, stable, rapid and within the range. Hence the present HPTLC methods are suitable for routine assay of these drugs in raw materials and in pharmaceutical formulations in the quality control laboratories.