4. EXPERIMENTAL

Apparatus and software

Apparatus and software given below is common for all proposed RP-HPLC, spectrophotometric and HPTLC methods.

4.1 HPLC instrumentation

The Shimadzu HPLC system consisting of gradient pump (LC-10AT vp pump), mixer (SUS vp Assy (new)), rheodyne injector, UV-VIS dual wavelength detector SPD -10A vp) hamilton syringe (25 µL) and analytical weighing balance (AUX 200) (all from Shimadzu, Kyoto, Japan) were used. The separations were achieved on a Phenomenex Luna C–18 (2) (250 × 4.6 mm, 5 µm) column (UK) with UV detection. Sonicator (SONICA 2200MH), vacuum pump (model XI 5522050) and Millipore filtration kit for solvents and sample filtration (Millipore, Bangalore, KRN, India) were used throughout the experiment. The Spinchrom CFR software-single channel was used for acquisition, evaluation and storage of chromatographic data.

4.2 Spectrophotometric instrumentation

Shimadzu UV-1700 and UV-1601 double beam spectrophotometer connected to a computer loaded with Shimadzu UVPC software (Shimadzu, Kyoto Japan) was used for all the spectrophotometric measurements. The spectral bandwidth was 1 nm and the wavelength scanning speed[1] was 2800 nm min^{-1}. The absorption spectra of the reference and test solutions were carried out in a 1 cm quartz cells over the range of 200-350 nm. The chemometric calculations on the resulting data were carried out in R-software environment (version 2.1.1) (www.r-project.org (website for the R software environment)) which is a GNU implementation of S^2 language and environment which was developed at Bell Laboratories.

4.3 HPTLC instrumentation

The samples were spotted in the form of bands of width 6mm with a Camag microliter syringe (25 µL) on precoated silica gel aluminium plate 60F-254

(20cm×10cm with 0.2mm thickness, E. Merck, Germany) using a Camag Linomat automatic TLC sampler IV (Switzerland). A constant application rate of 100 nL sec^{-1} was employed and space between tracks was 13 mm. The slit dimension was kept 5mm×0.45mm micro, 20 mm sec^{-1} scanning speed was employed. Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for mobile phase was 10 min at room temperature. The length of chromatogram run was approximately 80 mm. Subsequent to the development; TLC plate was dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III with winCATS software (slit-micro, 6 x 0.45 mm) in the absorbance mode. The source of radiation utilized was deuterium lamp.

4.4 Pure standards and reagents

ATOR was donated by Biocon, Ltd (Bangalore, KRN, India) FENO and RAMP Dr. Reddy's Laboratories Limited (Hyderabad, AP, India) AMLO and EZET from Torrent Pharmaceuticals limited (Ahamedabad, GUJ, India) HCTZ and NEB from M/s Micro labs limited, (Bangalore, KRN, India) were certified to contain 99.6% and 99.8% range of purity. The drugs were used without further purification. All the solvents used in analysis were of spectroscopic and HPLC grade and water of ultra pure grade of 18 MΩhm resistance was obtained in-house to use in analysis.

Reference

(1) El-Gindy, A. *Il Farmaco* 2005, *60*, 745-753.

(2) Richard, A.; Becker; John, M. C.; Allan, R. W. *The New S Language*; Chapman & Hall: New York, 1988.