Theophylline (1,3-dimethylxanthine), especially in sustained release preparations, is widely prescribed in the therapy of asthma [1]. Among the theophylline analogs recently introduced for such therapy is doxofylline (2-[7’-theophylline methyl]-1,3-dioxolane), which is claimed to retain the therapeutic properties of theophylline but have a lower incidence of side effects [2, 3]. It is given by mouth in doses of up to 1200 mg daily [4]. Although various bioanalytical methods for estimation of doxofylline in human serum [5–8] and spectrophotometric method for estimation of doxofylline in dosage form [9] have been reported in the literature, there is no HPLC method reported for the estimation of doxofylline in pharmaceutical formulations. The present work describes a simple, precise and accurate RP-HPLC method for estimation of doxofylline in commercial dosage form. The results of analysis were validated using International Conference on Harmonization (ICH) guidelines [10].

EXPERIMENTAL

Reagents. Acetonitrile (HPLC grade) was procured from Qualigens, India; Milli-Q-water was purchased from Rankem, India. Orthophosphoric acid was obtained from E. Merck, India. Reference standards of doxofylline were procured from Zydus Cadila, Ahmedabad, India.

Instrumentation. The HPLC system used in the study was Jasco (Japan), a rheodyne injection valve equipped with a 20 µL loop (Rheodyne, USA), a detector (Jasco, UV-2075 plus intelligent detector UV/Vis), LC-NET II/ADC integrator was used. Separation was accomplished on a Hiq Sil C 18 W column using a mobile phase of acetonitrile : buffer (50 : 50), pH 3, at a flow rate of 1 mL/min with detection of analyte at 272 nm. The separation was achieved within 3.1 ± 0.3 min for doxofylline sample. The method showed good linearity in the range of 10–80 µg/mL. The intra and inter day RSD ranged from 0.37–0.53%. The recovery (mean ± S.D.) of low, middle and high concentrations were 100.04 ± 0.80, 100.01 ± 0.20, 100.07 ± 0.30 respectively. Limit of detection and limit of quantification were 0.03 and 0.1 µg/mL, respectively.

Method development. To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. Various solvent systems were tried for the development of a suitable HPLC method for determination of Doxofylline in bulk drug and pharmaceutical formulations. Mobile phase tried for this purpose were acetonitrile : buffer (80 : 20), acetonitrile : buffer (50 : 50), methanol : water (50 : 50), methanol : water : acetonitrile (35 : 30 : 35). The condition that gave the best resolution and symmetry was selected. Same solvent system was used for the extraction of the drug from the formulation containing excipients which was used for quantification.

Calibration Curve. A stock solution of doxofylline (100 µg/mL) was prepared by dissolving 50 mg of drug in 100 mL of mobile phase, further 2 mL of this solution were transferred to a 10 mL volumetric flask and volume was made up to 10 mL to obtain 100 µg/mL solution. Different concentrations (1–80 µg/mL) were made for the preparation of calibration curve from the stock solution. The mobile phase after filtration was used. The separation was achieved on a Hiq Sil C 18 W column using a mobile phase of acetonitrile : buffer (50 : 50), pH 3, at a flow rate of 1 mL/min with detection of analyte at 272 nm. The separation was achieved within 3.1 ± 0.3 min for doxofylline sample. The method showed good linearity in the range of 10–80 µg/mL. The intra and inter day RSD ranged from 0.37–0.53%. The recovery (mean ± S.D.) of low, middle and high concentrations were 100.04 ± 0.80, 100.01 ± 0.20, 100.07 ± 0.30 respectively. Limit of detection and limit of quantification were 0.03 and 0.1 µg/mL, respectively.

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tion through a 0.45 μm membrane filter was delivered at 1.0 mL/min for column standardization, and baseline was continuously monitored during the process. The UV scan of doxofylline was performed between 200–400 nm and wavelength of detection was selected at 272 nm. The doxofylline UV spectrum is shown in Fig. 2. The prepared dilutions were injected serially and areas under the peaks were calculated for each dilution. The stability of drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after 48 h storage of drug solution at laboratory bench conditions and in the refrigerator.

Method Validation. Linearity. The concentrations of doxofylline within 0–80 μg/mL were prepared from stock solution (100 μg/mL) and areas under peak were calculated. The graph was plotted between concentration and area under peak for linearity.

Precision. Precision was considered at two levels, i.e., repeatability and intermediate precision. Repeatability of sample application was determined as intra-day variation whereas intermediate precision was determined by carrying out inter-day variation for the determination of doxofylline at three different concentration levels of 8, 6, and 32 μg/mL.

Accuracy as Recovery. Accuracy of the method was studied by recovery experiments. About 5.5 mg of placebo and 16, 32, and 48 mg of doxofylline were transferred into a 100 mL volumetric flask. About 50 mL of mobile phase were added, sonicated for 10 min and shaken for 5 min. The volume was made up to the mark with mobile phase and mixed. Solution was filtered through a 0.45 μm Millipore HVLP filter and the filtrate collected by discarding few mL of filtrate. Finally 5.0 mL of this solution were diluted to 50.0 mL with mobile phase. The final concentrations for 50, 100, and 150% accuracy were 16, 32, and 48 μg/mL of doxofylline, respectively.

Specificity. A synthetic mixture containing 16 mg of doxofylline and 30 mg each of starch, lactose, magnesium stearate, and avicel, which are present as excipients in the tablet dosage form, was accurately weighed and transferred to a 50 mL volumetric flask. The mixture was shaken well with 30 mL of methanol and then diluted to volume with methanol. After filtration, 5 mL of the filtrate were transferred to a 50 mL volumetric flask and diluted to volume with mobile phase, to furnish a final solution containing 32 μg/mL of doxofylline.

Robustness. Robustness was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions for the determination of doxofylline. Robustness of the method was determined by changing the flow rate (0.8 and 1.2 mL/min), mobile phase ratio (±10%), pH (±10%), and temperature (±10%).

Assay of Commercial Dosage Form. Accurately weighed quantity of tablets powder equivalent to about 80 mg of doxofylline in to a 250 mL volumetric flask was transferred. About 100 mL of mobile phase was added, the solution was sonicated for 20 min with continuous shaking at 30°C. Volume was made up with mobile phase. The solution was filtered through a 0.45 μm HVLP filter paper by discarding first few mL of the filtrate. A 5.0 mL aliquot of the solution was diluted to 50 mL with mobile phase to make final concentration 32 μg/mL of doxofylline. Standard solu-