Development and validation of spectrophotometric method for estimation of anti-asthmatic drug doxofylline in bulk and pharmaceutical formulation

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A simple, sensitive and accurate UV spectrophotometric method has been developed for the determination of an anti-asthmatic drug, doxofylline, in raw material and in tablets. The drug showed maximum absorption at 272 nm in water. Beer’s law was obeyed in the concentration range 5-50 μg mL⁻¹ of drug with an apparent molar absorptivity and Sandell sensitivity of 6.2 × 10³ L.mol⁻¹cm⁻¹ and 0.0363 μg cm⁻²/0.001Å, respectively. The limits of detection and quantification were calculated to be 0.9623 and 2.9161 μg mL⁻¹, respectively. The method was successfully applied to the determination of doxofylline in tablets. Results were validated statistically as per ICH guidelines. It was found that the excipients present in the commercial formulation did not interfere with the method.

Keywords: Doxofylline, UV spectrophotometry, ANOVA, ICH guidelines

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Doxofylline (Fig. 1) is a novel methyl xanthine bronchodilator indicated for the treatment of bronchial asthma and chronic obstructive pulmonary disease (COPD) in adults¹. Chemically, doxofylline is 7-(1,3-dioxalan-2-ylmethyl) theophylline²³. The drug is not official in any pharmacopoeia. A survey of literature has not revealed any UV spectrophotometric method for the determination of the drug in pharmaceutical formulation whereas reports are available for the estimation of the drug in human serum and rat plasma by HPLC⁴⁻⁶, and in plasma by RP-HPLC⁷.

In the present study, a simple, precise and accurate analytical method for the estimation of doxofylline in pure form and in solid dosage form was developed. The results of the analysis were validated by statistical methods and recovery studies⁸⁹.

Notes

Experimental Procedure

Materials
Doxofylline reference substance was obtained from Mars Therapeutics & Chemicals Ltd. (Secunderabad, India). Tablets of brand Doxobid™ (Batch No. DB00406, Dr. Reddy’s Laboratories Ltd., India) containing 400 mg of doxofylline were procured from a local pharmacy. Double distilled water was used as the solvent for the experiment.

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Instrument
A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Precisa 310M, Switzerland).

Standard stock solution
The standard solution of doxofylline was prepared by dissolving accurately weighed 10 mg of the drug in water and diluted to 100 mL with water to obtain a final concentration of 100 μg mL⁻¹. This stock solution was used to prepare further dilutions of standard solution.

Calibrating curve
Aliquots (0.5-5 mL) of stock solution of doxofylline were transferred into a series of 10 mL volumetric flasks and volume was made up to the mark with water to produce the concentration ranging from 5-50 μg mL⁻¹. The absorbance of each solution was measured at 272 nm (Fig. 2) against water as blank. A calibration curve was prepared by plotting absorbance versus concentration.

Fig. 1—Structure of doxofylline
Estimation of doxofylline in tablets

For the analysis of drug in bulk, accurately weighed 10 mg sample was dissolved in 100 mL water in a volumetric flask. After suitable dilution, the absorbance of final sample was recorded against the blank at 272 nm.

For the analysis of the dosage form, twenty tablets of doxofylline (400 mg) were ground to fine powder and mixed thoroughly. A quantity of powder equivalent to 10 mg of the drug was transferred to a 100 mL volumetric flask and dissolved in about 40 mL distilled water by shaking on a rotary flask shaker for 2 h. The solution was filtered through Whatman filter paper (No. 41). The filter paper was washed with water. The washings were added to the filtrate and the final volume was made up to 100 mL. After suitable dilution, the absorbance of final sample corresponding to 20 μg mL⁻¹ was recorded at 272 nm against water blank.

Recovery studies

The data were analyzed by linear simple regression by the least-squares method. Pure drug at three levels was added to a fixed amount of drug in tablet powder and the total amount was determined, and hence the percentage recovery of the pure drug added was calculated.

The precision and accuracy of the assay as well as linearity of the calibration curve were determined for intra- and inter-day on three different days. The precision was expressed as the percent coefficient of variation of each curve. The statistical data were calculated by ANOVA.

Results and Discussion

The method was validated according to the guidelines of International Conference on Harmonisation (ICH)¹⁰. A standard calibration curve of the drug was constructed by plotting absorbance versus concentration. Linear absorbance versus concentration gave equation Y= 0.026x-0.0263 with a correlation coefficient 0.9997. Beer’s law is obeyed over the concentration range 5-50 μg mL⁻¹ with an apparent molar absorptivity (ε) and Sandell sensitivity of 6.2 × 10³ L mol⁻¹ cm⁻¹ and 0.0363 μg cm⁻²/0.001A, respectively. The limits of detection (LOD) and quantification (LOQ) are calculated to be 0.9623 and 2.9161 μg mL⁻¹. Percentage relative standard deviation of 0.6175 was observed for analysis of six replicate samples of brand Doxobid™. The linear regression equation with a correlation coefficient (r) of 0.9997 indicates a good linearity between absorbance and concentration in the range of 5-50 μg mL⁻¹. The value of percentage relative standard deviation less than 1% and low percentage range of error confirm the high degree of precision and accuracy of the proposed method. To examine the absence of either positive or negative interference of the excipients used in the formulation, recovery studies were carried out by addition of known quantities of standard drug solution to pre-analyzed sample at three different concentration levels and the determination was repeated. The assay result obtained by the proposed method was found to be 99.544 ± 0.473%, which is in good agreement with the labeled amounts.

The observed concentrations of doxofylline reference substance in the tablets were not significantly different from the stated concentrations confirmed by Student’s t test, P=0.05 (99.54%, n=6). The percentage recovery value (100.47 ± 0.277, n =3), which is close to 100%, indicates the accuracy of the method and absence of interference of the excipients present in the formulation. The ANOVA analysis showed there is no significant difference (Fₜₐₜ value < tabulated F value at p= 0.05) among the assay results obtained in three different days at different times.

Conclusion

The method is very simple, precise and sensitive. Since no UV-spectrophotometric method is reported for the estimation of doxofylline from pharmaceutical dosage forms the present method may be useful for routine analysis of doxofylline from tablets as well as bulk.

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References