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Spectrophotometric and Reversed-Phase High-Performance Liquid Chromatographic Method for the Determination of Doxophylline in Pharmaceutical Formulations

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ABSTRACT

Two methods are described for determination of Doxophylline in a solid dosage form. The first method was based on ultraviolet (UV)-spectrophotometric determination of the drug. It involves absorbance measurement at 274 nm (λ_{max} of Doxophylline) in 0.1 N hydrochloric acid. The calibration curve was linear, with the correlation coefficient between 0.99 and 1.0 over a concentration range of 0.20–30 mg/ml for the drug. The second method was based on high-performance liquid chromatography (HPLC) separation of the drug in reverse-phase mode using the Hypersil ODS C_{18} column (250 X 4.6 mm, 5 mm). The mobile phase constituted of buffer acetonitrile (80:20) and pH adjusted to 3.0, with dilute orthophosphoric acid delivered at a flow rate 1.0 ml/min. Detection was performed at 210 nm. Separation was completed within 7 min. The calibration curve was linear, with the correlation coefficient between 0.99 and 1.0 over a concentration range of 0.165–30 mg/ml for the drug. The relative standard deviation was found to be <2.0% for the UV-spectrophotometry and HPLC methods. Both these methods have been successively applied to the solid dosage pharmaceutical formulation, and were fully validated according to ICH guidelines.

Key words: Doxophylline, HPLC, reversed-phase, UV-spectrophotometry

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INTRODUCTION

Doxophylline is chemically designated as 7(1, 3 dioxolone-2-yl methyl) theophylline. Presence of a dioxolane group in position 7 differentiates it from theophylline.[1] The chemical structure of Doxophylline is provided herewith [Figure 1].[2]

It is a new antibronchospastic drug recently introduced in therapy, with pharmacological properties like theophylline, a potent adenosine receptor antagonist. Doxophylline does not affect gastric acid secretion, either *in vivo* or *in vitro,* unlike theophylline. The lack of side-effects with doxophylline indicates that the drug can be used safely and effectively in the treatment of chronic obstructive pulmonary disease (COPD).[3] Doxophylline inhibits phosphodiesterase (PDE IV) activities with the consequent increase of cyclic AMP, which determines relaxation of the smooth musculature. Doxophylline appears to have decreased affinities toward adenosine A1 and A2 receptors, which may account for the better safety profile of the drug. Doxophylline does not interfere with calcium influx into the cells or antagonize calcium channel blockers.[4] Unlike aminophylline, it has low secretagogue activity and is suitable for asthmatic patients with peptic ulcer disease.[5]

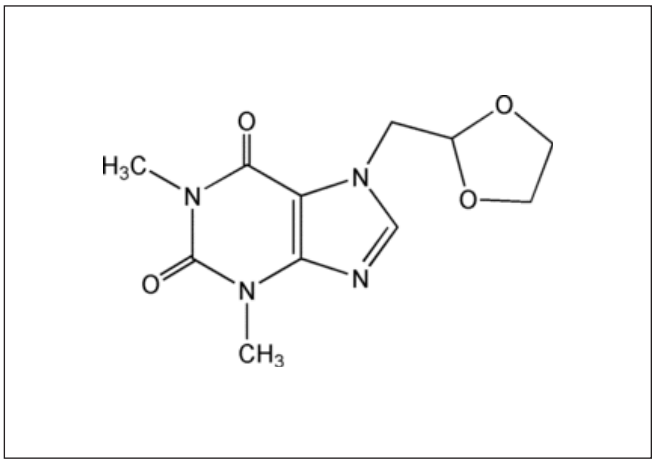

Figure 1: Structure of Doxophylline

Doxophylline is indicated for the treatment of bronchial asthma and COPD.[6]

Some analytical methods for quantitative determination of Doxophylline in pharmaceutical formulations are described in the literature, like ultraviolet (UV)-spectrophotometry[7] and LC-MS (Liquid Chromatography-Mass Spectroscopy).[8-10] At present, no high-performance liquid chromatography (HPLC) and UV-spectrophotometric methods are reported for the estimation of Doxophylline in a tablet dosage form. The purpose of this work is to develop and validate the proposed methods for routine analysis in a quality control laboratory.

EXPERIMENTAL PROCEDURE

Instrument and condition

1. UV-visible spectrophotometer - Model UV-1700 (Shimadzu, Tokyo, Japan).
2. HPLC system - Shimadzu LC 2010C integrated system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C column oven and 2010C programmable auto sampler controlled by CLASS-VP software. (SHIMADZU USA Manufacturing Inc, 1900, SE 4th Ave, Canby, OR, 97013-4348, North America, USA)
3. Analytical column - Hypersil ODS C_{18} (250 X 4.6 mm, 5 mm particle size), (Weber Consulting, Attila u. 38/b. H-2132 Göd, Hungary)
4. Detector - UV visible
5. Chromatographic parameters- Detection at 210 nm, flow rate 1.0 ml/min.
6. Mobile phase - Potassium dihydrogen phosphate (pH 3.0 ± 0.2 adjusted with orthophosporic acid)–acetonitrile (80:20, v/v).
7. Diluent - 0.1 N hydrochloric acid.

Reagents

1. Doxophylline reference standard - Assigned purity 99.24% (Cadila Healthcare Limited, Ankleshwar, Gujarat, India).
2. Acetonitrile - AR grade (Spectrochem), Spectrochem Private Limited, Office 221, 2nd Floor, Anand Bhuvan, 17, Babu Genu Road, Princess Street, MUMBAI - 400 002.
3. Orthophosphoric acid - AR grade (E-Merck Limited), E-Merck (India) Ltd, Shiv Sagar Estate, `A', Dr. A B Road, Worli, Mumbai, 400018, India
4. Commercially available Doxophylline tablet - Claimed to contain 800 mg of the drug. Procured from Zydus Cadila, Ahmedabad, Gujarat, India.

Standard preparation

For UV-spectrophotometric and HPLC methods

Standard stock solution of 400 μg/ml was prepared by dissolving 40 mg working standard of Doxophylline in 100 ml of diluent. The working standard solution of Doxophylline had a final concentration of 20 μg/ml and was prepared by appropriate dilution from the stock solution.

Sample preparation

For UV-spectrophotometric and HPLC methods

Twenty tablets were weighed and crushed into fine powder. An accurately weighed quantity of powder equivalent to about 125 mg of Doxophylline was transferred into a 250 ml volumetric flask. Add 100 ml of diluent and sonicate it for 30 min with continuous shaking. Make the volume up to the mark with 0.1 N HCl. This solution was filtered through a 0.45 μm HVLP nylon filter. Make an appropriate dilution to get the final concentration of Doxophylline 20 μg/ml. Appropriated aliquots were subjected to the above methods and the amount of Doxophylline was determined.

UV-spectrophotometric method

Construction of the calibration curve

λ_{max} of Doxophylline (20 μg/ml) was determined by scanning the drug solution in diluent and was found to be at 274 nm. To construct Beer's plot for Doxophylline, dilutions were made in diluent using stock solution at different concentration (4, 12, 16, 20, 24, and 30 μg/ml) levels. The drug followed linearity within the concentration range of 4–30 μg/ml.

Assay of the tablet formulation

Twenty tablets were weighed and crushed into fine powder.