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Development and validation of an analytical method for the stability of duloxetine hydrochloride

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Abstract

The objective of the study was to develop and validate an analytical method for estimating the stability of duloxetine hydrochloride. The drug was subjected to the stress conditions prescribed by the International Conference on Harmonization, including hydrolysis, oxidation, photolysis and dry heat. Five degradation products were formed, which were separated by high-performance liquid chromatography on a Kromasil C18 (150 mm × 4.6 mm, 5 μm) column in a gradient elution programme. The flow rate was 1 ml/min, and the detection wavelength was set to 225 nm. The retention time of the drug was 35.7 min, and analysis was completed within 40 min. The method was validated with respect to linearity, precision, accuracy, robustness and limits of detection and quantification as per the International Conference on Harmonization. The results were linear ($r^2 = 0.999$) over the range 50–400 μg/ml and accurate over the range 99.41–102.98. The method was robust and rugged, as there was insignificant variation in the results of analysis with changes in flow rate and temperature separately.

Keywords: Duloxetine hydrochloride; Stability; Validation; Degradation product

1. Introduction

Duloxetine ((+)-(*s*)-*N*-methyl-3-(1-naphthyloxy)-3-(thiophen-2-yl)-propan-1-amine) is a selective serotonin and norepinephrine reuptake inhibitor used primarily in the treatment of major depressive disorders and stress urinary incontinence [1]. Duloxetine is also used to treat pain and tingling in diabetic neuropathy. Duloxetine, also known as LY248686 [2,3], is a potent dual inhibitor of reuptake of serotonin (5-hydroxytryptamine) and norepinephrine; its effect depends on binding to human serum albumin. It is approved by the United States Food and Drug Administration for the treatment of diabetic neuropathic pain.

Duloxetine hydrochloride is a highly lipophilic compound with strong base properties and a pK_a of 9.5. Its structure is shown in Fig. 1. Phenolic impurities in duloxetine hydrochloride samples have been identified by mass spectrometry, nuclear magnetic resonance spectroscopy and X-ray analysis [4–6].

The purity of a drug product is determined on the basis of the percentage of the labelled amount of active pharmaceutical ingredient found in it by a suitable analytical method, which provides evidence for specificity, linearity range, accuracy, precision, detection limit, quantification limit, ruggedness and robustness of the method for regulatory purposes. High-performance liquid chromatography (HPLC) is the established technique for separating non-volatile organic compounds, drugs, metabolites and toxic residues by isocratic and gradient elution. Reverse-phase, ion-pair, ion and ion exchange

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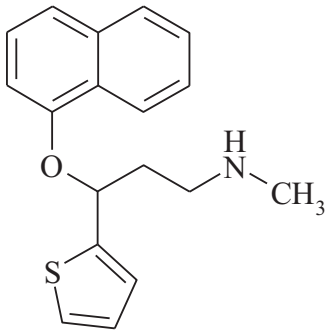

Fig. 1. Structure of duloxetine hydrochloride.

HPLC and sometimes size exclusion chromatography are used conventionally. If the mobile phase remains constant throughout HPLC separation, the separation is deemed to be isocratic, while if the sample contains components of a wide range of polarities, gradient elution is the only way to elute all the compounds in the sample in a reasonable time while maintaining peak resolution by changing the ratio of polar to non-polar compounds in the mobile phase during the sample run. For a reverse-phase gradient, the solvent is initially relatively polar and slowly becomes more non-polar. A sample containing compounds with a wide range of polarities can be separated by gradient elution in a shorter time without loss of resolution in the earlier peaks or excessive broadening of later peaks; it is more difficult to maintain a constant flow rate with continuous changes in mobile phase composition. Gradient elution is used in preparative and large-scale chromatography for separation.

The parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH) suggests that stress studies be carried out on a drug to establish its inherent stability [7], i.e. the extent to which a product retains the same properties and characteristics it had at the time of packaging, within the specified limits, throughout storage and use [8,9]. Stability testing thus indicates the effect of environmental factors on the quality of a drug or a formulated product and is used to predict its shelf life, determine the proper storage conditions and suggest labelling instructions. Various analytical methods have been reported namely, HPLC [10–17], high-performance thin-layer chromatography [18,19], ultra-performance liquid chromatography [20] and liquid chromatography–tandem mass spectrometry [21–23]. Degradation studies have also been reported [24–27] under thermal, acidic, alkaline, neutral hydrolysis and oxidative photolytic stress conditions.

2. Experimental

2.1. Materials

Pure duloxetine hydrochloride was donated by Unimark Remedies Ltd., Mumbai, India. Acetonitrile (HPLC grade) was purchased from Thomas Baker, Mumbai. Other chemicals used were of analytical grade. Ultrapure (double-distilled) water was obtained from a water purification unit.

2.2. Instrumentation

The HPLC instrument used was a Shimadzu LC-20 Prominence (Shimadzu, Kyoto, Japan) system equipped with an LC-20AD binary pump, a SPD-M20A photodiode array detector and a rheodyne injector. The output signal was monitored and processed with liquid chromatography solution software (Shimadzu, Kyoto, Japan). The injection volume was 20 μl, and chromatographic separation was achieved on a Kromasil C18 (150 mm × 4.6 mm), 5 μm particle size column.

2.3. Preparation of standard stock solution

A standard stock solution of the drug was prepared by dissolving pure drug in the mobile phase, i.e. 100 mg duloxetine hydrochloride in 100 ml methanol. The solution was sonicated and filtered through Whatman filter paper, and the resulting solution was further diluted with the mobile phase to a concentration of 1000 μg/ml.

2.4. Preparation of mobile phase

Solvent A: Potassium dihydrogen phosphate (0.68 g) was dissolved in 500 ml water, 0.1% triethylamine was added, and the pH was adjusted to 3.5 with orthophosphoric acid. Triethylamine was used to reduce tailing of the analyte. Methanol was added in proportions of 8.5:1.5. The solution was filtered through a 0.45-μm nylon 66 membrane filter and was further degassed in a sonicator for about 15 min. Solvent B was Methanol.

2.5. Degradation studies

All stress decomposition studies were performed at an initial drug concentration of 1000 μg/ml in methanol. Acid hydrolysis was performed in 0.5 N HCl at 80 °C for 9 h. The study under alkaline conditions was carried out in 1 N NaOH at 80 °C for 4 h. For the study under neutral conditions, drug dissolved in water was heated at 80 °C for 4 h. Oxidative stress studies were carried out at room