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ORIGINAL ARTICLE

Validated stability-indicating methods for the determination of zafirlukast in the presence of its alkaline hydrolysis degradation product

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Abstract Three simple stability-indicating methods for the analysis of Zafirlukast (ZAF) in the presence of its alkaline degradation products were developed and validated as per the International Conference on Harmonization (ICH) guidelines to evaluate the stability-indicating power of the proposed methods. The developed high-performance liquid chromatographic technique was achieved on ZORBAX–ODS (5 μm, 150 × 4.6 mm, i.d.) by isocratic elution with a mixture of acetonitrile/0.05 M phosphate buffer, pH 5.0, (50:50; v/v) as a mobile phase at flow rate of 1.0 mL min^{-1}, followed by UV detection at 240 nm. The method could determine ZAF in the range of 2–40 μg mL^{-1} with a mean percentage recovery of 99.73 ± 0.903. The proposed HPLC method was utilized to investigate the kinetics of alkaline degradation of ZAF. First derivative of the ratio spectra (^{1}DD) method was applied to analyze the drug under investigation without any interference from its degradation product with a linearity range of 4–32 μg mL^{-1} and with a mean percentage recovery of 99.85 ± 0.608. A chemometric method was also developed using the partial least squares (PLS) model for selective determination of ZAF in the range of 4–40 μg mL^{-1}, the mean percentage recovery was found to be 100.00 ± 0.336.

1. Introduction

Zafirlukast (ZAF), chemically [4-(5-cyclopentyloxy-carbonylamino-1-methyl-indol-3-ylmethyl)-3-methoxy-*n*-*o*-tolylsulfonylbenzamide] is a novel selective peptide leukotriene receptor antagonist,[1] used as an antiasthmatic drug in the prophylaxis and treatment of mild-to-moderate chronic asthma in adults and children.[2] ZAF is a competitive orally administered inhibitor of the cysteinyl leukotriene LTC_4, LTD_4 and LTE_4 in respiratory tracts.[3] By using zafirlukast as a single dose, bronchoconstriction caused by foreign allergens is inhibited and also decreases the bronchial hyper responsiveness to the inhaled histamine. Bioavailability is reduced when administrated after a high fat or protein meal. It is highly bounded to plasma proteins especially albumin (99%). It is metabolized in the liver by the cytochrome P450 enzyme. Its half life is about 10 h and the onset of action was seen in 1 h and takes

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3 h to reach peak plasma concentration. ZAF is eliminated mainly through feces (90%) and 10% only in the urine.[4,5]

Till date there are only few analytical methods reported for the estimation of ZAF in pharmaceutical preparations and in biological fluids. These methods include high performance liquid chromatography,[6–10] capillary electrophoresis,[11] spectrophotometry,[7,12] and electrochemical methods.[13,14]

Nowadays, investigation on the chemical stability and kinetics of decomposition of drugs is an essential matter to the quality control of pharmaceuticals and to understand the degradation pathway of drugs which is important to evaluate the product's shelf-life period. Up to now no literature review is reported for kinetic studies of the degradation process of ZAF, so the presented work is concerned to investigate the kinetics of the alkaline degradation process of the cited drug using the proposed HPLC method.

The aim of the present work is to develop simple, sensitive and selective stability-indicating methods for the quantitative determination of Zafirlukast in the presence of its alkaline degradation product and in pharmaceutical formulations. This was achieved by developing different techniques including HPLC, first derivative of the ratio spectra and PLS mathematic methods.

2. Experimental

2.1. Instrumentation

High performance liquid chromatography composed of a quaternary pump (1200 series, G 1311A) with an ultraviolet variable wavelength detector, 1200 series (Agilent Technologies, Waldbronn, Germany) and equipped with a 20-μl injector loop manual injector (model 7725 I USA), Dual-beam UV–visible spectrophotometer, (UVProbe 1800 version 2.32 Shimadzu, Kyoto, Japan) with matched 1-cm quartz cells, connected to an IBM compatible personal computer (PC) and a HP-600 inkjet printer. Bundled, UV-PC personal spectroscopy software version 3.7, was used to process the absorption and the derivative spectra. The data were then exported into MICROSOFT EXCEL program. The chemometric calculations were performed in Matlab for Windows™ version 7 Mathworks Inc. 2004. The PLS procedure was taken from PLS Toolbox 2.1, Eigenvector Research Inc. 2001 created by B.M. Wise, N.B. Gallagher for use with Matlab.

2.2. Materials and reagents

Pure samples of Zafirlukast were kindly supplied by DELTA PHARMA S.A.E, Tenth of Ramadan City, A.R.E.

The purity of the samples was found to be 100.43 ± 0.735% ($n = 5$) according to the reported method.[7]

Ventair® tablets, Batch No. 06093, labeled to contain 20 mg of Zafirlukast per tablet; manufactured by DELTA PHARMA S.A.E, Tenth of Ramadan City, Egypt.

Acetonitrile and methanol (HiPer Solv®, HPLC grade, E. Merck, Darmstadt, FRG).

All other chemicals were of analytical grade.

2.3. Standard solutions for the drug

Standard stock solutions of ZAF (800 μg mL^{-1}) were prepared by dissolving the pure sample in acetonitrile.

For the HPLC-method, a working standard solution (80 μg mL^{-1}) was prepared in the described mobile phase by 10-times dilution of the stock solution. While, other working standard solutions (80 μg mL^{-1} and 40 μg mL^{-1}) were prepared in acetonitrile for the first derivative of ratio spectra method (^{1}DD) and chemometric method, respectively.

2.4. Preparation of the alkali-induced degradation product

Accurately weighed 80 mg of ZAF was dissolved in 20 mL acetonitrile. Subsequently, 25 ml 1 M sodium hydroxide was added and the solution was heated in a temperature controlled oven at 100 °C for 2.5 h. The solution was concentrated nearly to dryness under vacuum, cooled to room temperature (~25 °C), then quantitatively transferred into a 100-mL measuring flask and the volume was completed with acetonitrile. Complete alkaline degradation of the studied drug was confirmed by the proposed HPLC method, where no peaks corresponding to intact drug were detected in case of the degraded samples.

Structural elucidation of the obtained degradation product was achieved by IR and Mass spectrophotometry.

2.5. Analytical techniques

2.5.1. Solution stability

The solution stability of zafirlukast was evaluated by leaving the standard solutions (20 μg mL^{-1} in acetonitrile) in tightly capped volumetric flasks, protected from light on a laboratory bench and in the refrigerator. The stability of studied compound solutions was checked by the proposed HPLC method.

2.5.2. Calibration curve for HPLC method

A series of standard solutions containing 2–40 μg mL^{-1} ZAF, were prepared by suitably diluting aliquots of the working standard solution of ZAF using acetonitrile/water (50:50; v/v).

The chromatographic separation was carried out at ambient temperature on a ZORBAX–ODS (150 × 4.6 mm, i.d.), particle size (5 μm), (Agilent Technologies, Waldbronn, Germany), isocratically at 1.0 mL min^{-1} with a mobile phase consisting of a mixture of acetonitrile/0.05 M phosphate buffer, pH 5.0, (50:50; v/v). The mobile phase was filtered through a 0.45 μm Millipore membrane filter and was degassed for ~15 min. in an ultrasonic bath prior to use. To reach equilibrium, the analysis was usually started after the passage of 50–60 mL of the mobile phase. The eluted analytes were detected at 240 nm, with a sensitivity of 0.001 AUFS (Absorbance Unit Full Scale).

Triplicate 20-μL injections were made for each solution and the peak area ratios of the cited drug to 12 μg mL^{-1} ZAF as an external standard were plotted against the corresponding concentrations to obtain the calibration graph.

2.5.3. Calibration curve for first derivative of ratio spectra method (^{1}DD)

Accurately measured volumes of ZAF stock solution (0.5–4 mL) were transferred separately into 10-mL calibrated flasks, diluted to volume with acetonitrile/water (50:50; v/v) to reach the concentration range of 4–32 μg mL^{-1}.The zero-order spectra of ZAF standard solutions were recorded using the same solvent as a blank and divided by the 8 μg ml^{-1} spectrum of the completely degraded drug substance. The first derivative