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Study of forced degradation behavior of Eletriptan hydrobromide by LC and LC–MS and development of stability-indicating method

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ABSTRACT

The objective of the present study was to report the stability profile of novel antimigrain drug Eletriptan hydrobromide based on the information obtained from forced degradation studies. The drug was subjected to acid (0.1–1 mol L^{-1} HCl), neutral and base (0.1–1 mol L^{-1} NaOH) hydrolysis and to oxidative decomposition (3–15% (v/v) H_2O_2). Photolysis and thermo degradation at 75 °C were carried out in methanol solution and in solid state with both Eletriptan hydrobromide bulk drug and the tablet formulation. The products formed under different stress conditions were investigated by LC and LC–MS.

The experimental conditions for LC were chosen by employing experimental design and multicriteria decision making methodology. These powerful tools enabled the accomplishment of satisfactory resolution with the shortest possible analysis time. Analytes were separated on a C_{18} column (*XTerra*™, 150 mm × 3.9 mm, 5 μm) with the mobile phase composed of methanol–water solution of TEA (pH 6.52, 1%, v/v) (30:70, v/v) pumped at 1 mL min^{-1} flow rate. The column temperature was set at 50 °C and the detection at 225 nm using DAD detector. The LC method was suitably modified for LC–MS analysis which was further used to characterize the arisen degradation products. The possible degradation pathway was outlined based on the results.

The drug appeared to be instable towards every stress condition but oxidation. The stability was not jeopardized even under more exaggerated conditions such as increased temperature of the solutions to 75 °C, increased strength of acid/alkali solutions and prolonged testing period.

Validation of the LC-DAD method was carried out in accordance with ICH guideline. The method met all required criteria and was applied when testing the commercially available tablets.

1. Introduction

Forced degradation studies are usually part of the drug development strategy being undertaken to elucidate the intrinsic stability of the drug. Such studies are therefore conducted under more severe and exaggerated conditions than those usually used for long-term stability tests. The information gathered may help establishing the drug degradation pathway as well as development and validation of the suitable analytical procedures [1–3].

Eletriptan hydrobromide (EH) is a relatively novel serotonin 5-$HT_{1B/1D}$ receptor agonist used for the treatment of acute migraine headaches. Its pharmacological effects include the constriction of cerebral blood vessels and neuropeptides secretion blockade which eventually relieves the pain [4,5]. Chemically, EH is 3-{[(*R*)-1-methyl-2-pyrrolidinyl] methyl}-5-[2-(phenylsulfonyl) ethyl] indole hydrobromide (Fig. 1A). The literature revealed only one paper dealing with the determination of EH in biological matrixes [6] and a few studies of EH tablet formulations covering the assay of EH in the presence of related organic impurities [7,8]. Neither of the already published methods reports the physical and chemical stability of EH. Consequently, the main objective of this study was to carry out a comprehensive stress study on EH by subjecting it to various experimental conditions. The investigations involved acid, alkaline and neutral hydrolysis, oxidative and thermal decomposition and stability towards light. An integral aim of the study was to postulate possible degradation pathway of the drug.

The suitable stability-indicating LC-DAD method was developed for the analysis of stress samples. Since the chromatographic behavior of target substances may be influenced by various experimental parameters, the whole study was carried out by employing experimental design methodology. The investigations included the mutual changes of the mobile phase composition and the column temperature. The central composite design was used to obtain a predictive model which adequately represents changes in the chromatographic response within the zone of interest [9–13].

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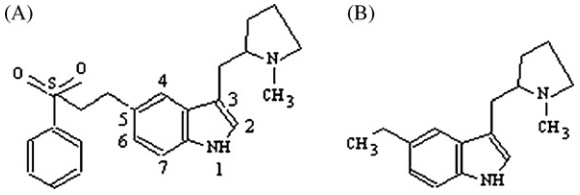

Fig. 1. Structures of Eletriptan (A) and UK 120.413 (B).

Furthermore, in order to achieve the best possible chromatographic performance of the method, it was necessary to evaluate several different target responses. In order to reach a compromise among them, the Derringer's desirability function was used. The desirability function was constructed for each individual response and afterwards the overall desirability function was established [13–17].

The developed stability-indicating LC-DAD method was validated and successfully applied for the analysis of the commercially available EH tablets.

2. Experimental

2.1. Drugs and reagents

The standard substances of Eletriptan hydrobromide, UK 120.413 and *Relpax*® tablets containing 20 mg of Eletriptan in a form of hydrobromide were obtained from *Pfizer H.C.P. Corporation*. Phenobarbiton USP reference standard, triethylamine (TEA) (*Merck*, Darmstadt, Germany), glacial acetic acid (*Zorka Pharma*, Šabac, Serbia), 85% formic acid (*Lach-ner*, Neratovice, Czech Republic), sodium hydroxide (*Centrohem*, Stara Pazova, Serbia), 36% hydrochloric acid (*Centrohem*, Stara Pazova, Serbia) and hydrogen peroxide (*Zorka Pharma*, Šabac, Serbia) were also used. All reagents were of analytical grade. Methanol-gradient grade (*Lab Scan*, Dublin, Ireland) and acetonitrile-HPLC grade (*Merck*, Darmstadt, Germany) were used for chromatography while water was obtained from *System Simplicity 185* purification systems (*Millipore*, Massachusetts, USA).

2.2. Equipment and experimental conditions

HPLC-UV analyses were done by using *Hewlett-Packard* 1200 series (Palo Alto, CA, USA) chromatographic system equipped with on-line degasser, binary pump, column oven and diode array detector. Sample injection was made through *Rheodyne* injector valve with a 20 μL sample loop. The data was acquired with *HP ChemStation* software. The method used C_{18} *XTerra*™ (5 μm, 150 mm × 3.9 mm) column (*Waters*, Massachusetts, USA). After being loaded onto the column, the sample eluted at the temperature of 50 °C at a flow rate of 1 mL min^{-1} with the mobile phase consisted of methanol–water solution of TEA (1%, v/v) (30:70, v/v) while pH of the water phase was adjusted to 6.52 with glacial acetic acid. Before use, the mobile phase was degassed and vacuum filtered through 0.45 μm nylon membranes (*Alltech Associates*, Lokeren, Belgium). The detection of analytes was performed at 225 nm.

HPLC–MS analyses were carried out on a system in which the HPLC part consisted of *Agilent technologies* 1200 series chromatographic system (Waldron, Germany) comprising of on-line degasser, binary pump, auto injector, column oven and diode array detector. The MS system consisted of *Agilent technologies* 6210 Time-Of-Flight LC–MS system (Waldron, Germany). The whole system operated using *Agilent technologies Mass-Hunter workstation* software. The separations were achieved on C_{18} *Zorbax Eclipse plus* (150 mm × 4.6 mm, 1.8 μm,) column (*Agilent Technologies*, Wilmington, DE, USA). The mobile phase was pumped at 1.4 mL min^{-1} flow rate and consisted of acetonitrile–water solution of formic acid (0.2%, v/v) in a gradient elution program. The initial mobile phase consisted of acetonitrile–water phase in the ratio 5:95 (v/v) and after 1.5 min the amount of acetonitrile begun to increase linearly to reach 95:5 (v/v) level achieved in 26th minute. For the next 9 min the mobile phase composition did not change, and from 35th minute to 40th minute the amount of acetonitrile begun to decrease to reach 5:95 (v/v) level. The post time was 5 min. The HPLC analyses were performed at 40 °C and the samples were detected in the range 190–450 nm. The mass spectrometer operated in the positive electrospray ionization mode with mass/charge (m/z) ratio in the range of 100–2000 m/z. The desolvation gas was nitrogen set at 12 L min^{-1} flow rate and desolvation temperature was 350 °C. The nebulizer pressure was 45 psig. Capillary voltage was set at 4000 V and fragmentor voltage at 140 V.

2.3. Solutions

A 1.00 mg mL^{-1}, 0.20 mg mL^{-1} and 0.50 mg mL^{-1} stock solutions of EH, UK 120.413 and phenobarbiton as the internal standard were prepared in methanol.

Working solutions for stress decomposition studies were containing 500.00 μg mL^{-1} of EH and 5.00 μg mL^{-1} of UK 120.413. 50.00 μg mL^{-1} solution of phenobarbiton was prepared as well. The sample preparation for assay of EH in tablets was done in the following way: the quantity of twenty tablets was accurately weighed, finely powdered and the equivalent to 25.00 mg of EH was transferred with 25 mL of methanol into a 50-mL volumetric flask. After sonicating and shaking the mixture for 25–30 min, it was made up to volume with the same solvent. The working concentration of EH in the prepared sample was 500.00 μg mL^{-1}, same as in the case of EH standard substance.

The concentrations of EH in the six solutions used for construction of the standard curve were in the range of 25.00–250.00 μg mL^{-1}. The same six solutions of UK 120.413 were prepared in the concentration range of 0.05–0.50 μg mL^{-1}. The concentration of phenobarbiton in all these solutions was 5.00 μg mL^{-1}. The sample of EH tablet formulation was used to prepare the test solutions containing EH in the concentration 50.00 μg mL^{-1}. The concentration of phenobarbiton in the same solutions was also 5.00 μg mL^{-1}.

The known quantities of EH and UK 120.413 standard substances were added to the finely powdered EH tablets for the method accuracy testing. The working solutions were prepared afterwards according to the similar procedure already described with EH tablets to attain concentrations at 80%, 100% and 120% of EH label claim. Three solutions were prepared for each of the following concentrations: 40.00 μg mL^{-1}, 50.00 μg mL^{-1} and 60.00 μg mL^{-1} for EH and 0.08 μg mL^{-1}, 0.10 μg mL^{-1} and 0.12 μg mL^{-1} for UK 120.413. The concentration of phenobarbiton in each solution was 5.00 μg mL^{-1}.

2.4. Stress decomposition studies

Forced degradation studies of bulk drug and drug formulation included appropriate solid state and solution state stress conditions in accordance with the ICH regulatory guidance [3]. Some helpful practical aspects on conducting and development of stability-indicating assays of specific drugs were also found in literature [1–2,18–26].

Stress decomposition studies were performed initially with EH working concentration of 500 μg mL^{-1} in methanol. Acid hydrolysis was performed by mixing 1 mL of EH working solution in three separate 5-mL volumetric flasks with 1 mL of 0.1 mol L^{-1}, 0.5 mol L^{-1} and 1.0 mol L^{-1} HCl solutions, respectively, and the mixtures were kept at room temperature for 8 h. The study in alkaline condition was carried out in a similar manner with 0.1 mol L^{-1}, 0.5 mol L^{-1} and 1.0 mol L^{-1} NaOH for 8 h. These experiments were repeated at