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ORIGINAL ARTICLE

Selective separation, detection of zotepine and mass spectral characterization of degradants by LC–MS/MS/QTOF

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Abstract A simple, precise, accurate stability-indicating gradient reversed-phase high-performance liquid chromatographic (RP–HPLC) method was developed for the quantitative determination of zotepine (ZTP) in bulk and pharmaceutical dosage forms in the presence of its degradation products (DPs). The method was developed using Phenomenex C_{18} column (250 mm × 4.6 mm i.d., 5 µm) with a mobile phase containing a gradient mixture of solvents, A (0.05% trifluoroacetic acid (TFA), pH = 3.0) and B (acetonitrile). The eluted compounds were monitored at 254 nm; the run time was within 20.0 min, in which ZTP and its DPs were well separated, with a resolution of >1.5. The stress testing of ZTP was carried out under acidic, alkaline, neutral hydrolysis, oxidative, photolytic and thermal stress conditions. ZTP was found to degrade significantly in acidic, photolytic, thermal and oxidative stress conditions and remain stable in basic and neutral conditions. The developed method was validated with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness as per ICH guidelines. This method was also suitable for the assay determination of ZTP in pharmaceutical dosage forms. The DPs were characterized by LC–MS/MS and their fragmentation pathways were proposed.

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1. Introduction

The parent drug stability test guidelines Q1A (R2) issued by International Conference on Harmonization (ICH) [1–4] requires the stress stability studies to be done on a drug to establish its inherent stability characteristics. This helps to identify the likely degradation pathways and degradation products (DPs) of the drug. It is a prerequisite that analytical test procedures should be stability indicating and fully validated. Accordingly, the aim of the present study was to establish inherent stability of zotepine (ZTP) and to develop a stability-indicating assay method through stress studies under a variety of ICH recommended test conditions [1,5–8]. The chemical name of

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ZTP is 2-((8-chlorodibenzo[b,f]thiepin-10-yl)oxy)-N,N-dimethylethanamine (Fig. 1). It is an atypical antipsychotic drug which is highly effective in acute exacerbation of schizophrenia. It has fewer adverse effects than conventional antipsychotics [9]. Green et al. [10] reviewed research studies on ZTP and its adverse reactions related to metabolic effects and movement disorders. A thorough literature search revealed that few LC and LC–MS methods are available for determination of ZTP in plasma, serum and other biological matrices [11–13]. A liquid chromatography quadrupole time-of-flight mass spectrometry (LC–QTOF–MS) method was reported for the analysis of ZTP in 77 blood samples [14]. Nozaki et al. [15] investigated the electrochemical oxidation behavior of ZTP and its fragmentation using electrospray ionization–mass spectrometry (ESI–MS) coupled with a microflow electrolytic cell. Capillary electrophoresis method was reported for determination of ZTP and its metabolite in human plasma [16]. GC and GC–MS methods were also reported for quantification of ZTP in biofluids [9,17,18]. As there are no reports available on the degradation behavior, identification and characterization of DPs of ZTP formed under various stress conditions, the present work has been undertaken on development of an HPLC-UV stability indicating assay method for separation and determination of ZTP in the presence of DPs and characterization of degradants by using LC–QTOF–MS.

2. Experimental

2.1. Materials and stability equipments

ZTP was obtained as a gift sample by Symed Laboratory Limited, Hyderabad, India. Acetonitrile (HPLC grade) was purchased from Merck (Lichrospher, Darmstadt, Germany). Water was purified by using a Milli-Q Gradient ultrapure water system (Billerica, MA 01821, USA). Analytical reagent grade trifluoroacetic acid (TFA), hydrochloric acid (HCl), sodium hydroxide (NaOH), and hydrogen peroxide (H_2O_2) used in the present study were purchased from S.D Fine chemicals (Mumbai, India). Photo stability studies were carried out in a OSWORLD (model JRIC-11C) photo stability chamber with humidity and temperature control. The chamber was equipped with an illumination bank made of light sources, viz., a cool white fluorescent lamp designed for emitting significant radiation same as that specified in ISO 10977 (1993) 320 nm and a near UV fluorescent lamp with a maximum energy emission between 350 nm and 370 nm [19] for providing an overall illumination of not less than 1.2 million lux hours and irradiation density of not less than 200 W/m^2. Thermal stability studies were performed in a dry air oven (Osworld Scientific Equipments Pvt. Ltd., Mumbai, India).

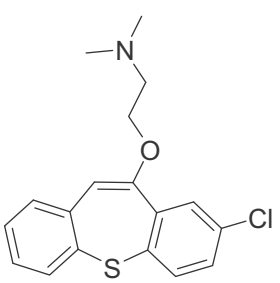

Fig. 1 Chemical structure of zotepine.

2.2. Instrumentation

The analysis was carried out using an Agilent 1200 series HPLC instrument (Agilent Technologies, USA) coupled to a quadrupole time-of-flight (Q-TOF) mass spectrometer (Q-TOF LC/MS 6510 series classic G6510A, Agilent Technologies, USA) equipped with an ESI source. The data acquisition was under the control of Mass Hunter workstation software. The chromatographic data were recorded using a computer system with chemstation data acquiring software. The HPLC-UV data were used for quantitative determination of ZTP and the same chromatographic conditions were used for mass spectral identification and characterization of its degradants.

2.3. LC–MS conditions

The chromatographic separations were carried out on a reversed-phase Phenomenex Luna C18 (250 mm × 4.6 mm i.d.) column with particle size of 5 µm (Phenomenex, Hyderabad, India) and the column was maintained at an ambient temperature (30 °C). HPLC separation was achieved with gradient elution (Table 1) using 0.05% TFA buffer (pH adjusted to 3.0), and acetonitrile as mobile phase. The mobile phase was filtered through 0.45 µm nylon membrane and degassed by using an ultra sonicator before use. The injection volume was 20 µL and the mobile phase flow rate was at 1 mL/min. A splitter was placed before the ESI source, allowing entry of only 35% of the eluent. The typical operating source conditions for MS scan of ZTP in positive ESI mode were optimized as follows: the fragmentor voltage was set at 80 V; the capillary at 3000 V; the skimmer at 60 V; nitrogen was used as the drying (300 °C; 9 L/min) and nebulizing (45 psi) gas. Ultra high pure nitrogen was used as collision gas. All the spectra were recorded under identical experimental conditions, and are an average of 25 scans.

2.4. Stress degradation studies

All stress decomposition studies were performed with an initial drug concentration of 1 mg/mL in methanol and water (8:2). Acid hydrolysis was performed in 1 M HCl at room temperature for 18 h. The study in alkaline condition was carried out in 5 M NaOH at 70 °C for 5 days under reflux. For neutral degradation study, the drug was dissolved in a mixture of methanol and water (8:2) and was heated at 60 °C for 7 days under reflux. Oxidative studies

Table 1 The optimized gradient elution program for ZTP and its DPs.

Time (min)	Mobile phase	
	A (%)	B (%)
0.01	75	25
2.50	50	50
10.0	40	60
12.5	0	100
20.0	0	100
22.5	75	25
30.0	75	25

A: 0.05%TFA (trifluoroacetic acid, pH 3); B: acetonitrile.