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Spectrochimica Acta Part A 68 (2007) 1220–1230
SPECTROCHIMICA ACTA PART A
www.elsevier.com/locate/saa

Application of spectrophotometric, densitometric, and HPLC techniques as stability indicating methods for determination of Zaleplon in pharmaceutical preparations

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Received 7 October 2006; received in revised form 14 January 2007; accepted 24 January 2007

Abstract

Spectrophotometric, spectrodensitometric and HPLC are stability indicating methods described for determination of Zaleplon in pure and dosage forms.

As Zaleplon is easily degradable, the proposed techniques in this manuscript are adopted for its determination in presence of its alkaline degradation product, namely *N*-[4-(3-cyano-pyrazolo[1,5a]pyridin-7-yl)-phenyl]-*N*-ethyl-acetamide. These approaches are successfully applied to quantify Zaleplon using the information included in the absorption spectra of appropriate solutions.

The second derivative (D_2) spectrophotometric method, allows determination of Zaleplon without interference of its degradate at 235.2 nm using 0.01N HCl as a solvent with obedience to Beer's law over a concentration range of 1–10 $\mu g\,ml^{-1}$ with mean percentage recovery $100.24 \pm 0.86\%$.

The first derivative of the ratio spectra (^1DD) based on the simultaneous use of (^1DD) and measurement at 241.8 nm using the same solvent and over the same concentration range as (D_2) spectrophotometric method, with mean percentage recovery $99.9 \pm 1.07\%$.

The spectrodensitometric analysis allows the separation and quantitation of Zaleplon from its degradate on silica gel plates using chloroform:acetone:ammonia solution (9:1:0.2 by volume) as a mobile phase. This method depends on quantitave densitometric evaluation of thin layer chromatogram of Zaleplon at 338 nm over a concentration range of 0.2–1 $\mu g\,band^{-1}$, with mean percentage recovery 99.73 ± 1.35.

Also a reversed-phase liquid chromatographic method using 5-C8 (22 cm × 4.6 mm i.d. 5 μm particle size) column was described and validated for quantitation of Zaleplon using acetonitrile:deionised water (35:65, v/v) as a mobile phase using Paracetamol as internal standard and a flow rate of 1.5 $ml\,min^{-1}$ with UV detection of the effluent at 232 nm at ambient temperature over a concentration range of 2–20 $\mu g\,ml^{-1}$ with mean percentage recovery $100.19 \pm 1.15\%$.

The insignificance difference of the proposed methods results with those of the reference one proved their accuracy and precision.

Keywords: Zaleplon; Derivative spectrophotometry; Derivative ratio spectrodensitometry; HPLC

1. Introduction

Zaleplon is *N*-[3-(3-cyanopyrazolo[1,5-a]pyrimidin-7-yl) phenyl]-*N*-ethyl acetamide [1]. It is a pyrazolopyrimidin derivative. Although not structurally related to the benzodiazepines, Zaleplon acts through binding to the γ-amino butyric acid ($GABA_A$)-benzodiazepine receptor complex producing sedative and hypnotic effects similar to those of benzodiazepines [2–4]. It is reported to have relative selectivity for the Ω_1-subtype of benzodiazepines binding site [5]. The chemical structure, molecular weight and molecular formula of Zaleplon are illustrated in Fig. 1.

The literature survey reveals several methods for determination of Zaleplon in plasma, namely, liquid chromatography–electro spray ionisation–mass spectrometry assay, high-performance liquid chromatography, chemical ionisation–mass spectrometry [6–8], and RP-HPLC with fluorescence detection [9]. A spectrofluorimetric technique is used for its determination in micellar medium [10]. Zaleplon was found among drugs screened in hair and oral fluids using LC–MS [11,12]. It was quantitatively screened in blood as silylated derivative by gas chromatography–selected ion monitoring mass spectrometry and gas chromatography electron capture detection [13]. A fast gas chromatography–negative-ion chemical ionization

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doi:10.1016/j.saa.2007.01.024

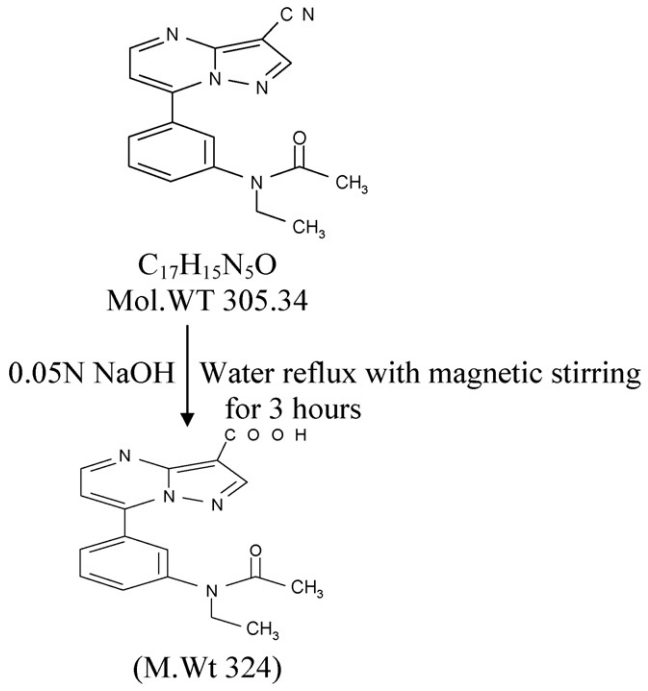

Fig. 1. The chemical structure, molecular weight and molecular formula of Zaleplon followed by the degradation pattern of Zaleplon in 0.05N NaOH.

mass spectrometry with micro scale volume samples preparation method was described for its determination in blood [14]. It was determined in capsules by voltammetric method [15]. A capillary electrophoresis with laser-induced fluorescence detection and liquid chromatography–mass spectrometry techniques were adopted for separation and identification of its metabolites in human urine [16].

The above literature revealed that up to the present time nothing has been published concerning the proposed methods for determination of Zaleplon in presence of its alkaline degradation product.

The proposed procedures were successfully applied to the routine and quality control analysis of Zaleplon in its pharmaceutical preparation.

2. Experimental

2.1. Apparatus

1- Shimadzu UV–vis 1601 PC spectrophotometer with 1 cm quartez cuvettes for spectrophotometric determinations (Kyoto, Japan).
   The following requirements are taken into consideration:
   - Scan speed: fast.
   - $\Delta\lambda$: 4 (for both D_2 and ^{1}DD methods).
   - Scaling factor: 100 (for D_2 method) and 10 (for ^{1}DD method).
2- UV lamp with short wavelength 254 nm (USA).
3- TLC Scanner 3 densitometer (Camage, Muttenz, Switzerland).
   The following requirements are taken into consideration:
   - Slite dimensions = 6.00 × 0.445, Micro.
   - Scanning speed = 20 mm s^{-1}.
   - Data resolution = 100 μm $step^{-1}$.
4- Sample applicator for TLC linomat IV with 100 μl syringe (Camage, Muttenz, Switzerland).
5- TLC plates (20 cm × 20 cm) coated with silica gel 60 F254 (Merck KgaA, Darmstad, Germany).
6- The HPLC system consisted of a Perkin Elmer system equipped with series 200 auto sampler, series 200 lc pump, series 200 UV/vis detector. The stationary phase was 5-C_8 (22 cm × 4.6 mm i.d. 5 μm particle size) column using acetonitrile:deionised water (35:65, v/v) as a mobile phase.

2.2. Materials

2.2.1. Authentic samples

1- Zaleplon (Batch No. 0550407) was kindly supplied by The Egyptian Co. for Pharmaceutical and Chemical Industries. S.A.E (EPCI), Industrial Zone, Bayad El-Arab, Beni Suef, Egypt. Its purity was reported to be 98.5% according to the company analysis certificate.
2- Paracetamol (Batch No. 34055) was kindly supplied by Memphis Co. for Pharm and Chemical Ind., Cairo, Egypt. Its purity reported to be 99% according to the company analysis certificate.

2.2.2. Dosage form

1- Siesta® capsules (Batch No. 50507), labeled to contain 10 mg Zaleplon is from SIGMA For Al Andalous Medical Company, Cairo, Egypt.
2- Zalocid® capsules (Batch No. 044060609/3), labeled to contain 10 mg Zaleplon is from The Egyptian Co. for Pharmaceutical and Chemical Industries. S.A.E. (EPCI).

2.2.3. Chemicals and solvents

1- Chloroform, hydrochloric acid, acetone, ammonia solution, NaOH and methanol all are from (El NASR Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt).
2- Acetonitrile HPLC grade (SDS, France).
3- Deionised water (SEDICO pharmaceuticals Co., Cairo, Egypt).

N.B.: All the chemicals were of analytical grade. The solvents used for the spectrophotometric and spectrodensitometric methods were of spectrophotometric grade and those used for HPLC were of HPLC grade.

2.2.4. Degraded samples

2.2.4.1. Preparation of pure degraded sample. 0.5 g Zaleplon powder was transferred to 250 ml stoppered flask, water refluxed with 100 ml 0.05N NaOH with magnetic stirring for 3 h. The solution was cooled, the collected precipitate contains a mixture of Zaleplon and its degradate, from which the degradate was separated in pure form by preparative normal phase column chromatography using silica gel as a stationary phase and chloro-