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Stress degradation studies on etamsylate using stability-indicating chromatographic methods

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Abstract

Two sensitive and reproducible methods are described for the quantitative determination of etamsylate in the presence of its degradation products. The first method was based on high-performance liquid chromatographic (LC) separation of the drug from its degradation products on the reversed phase, kromasil column [C_{18} (5-μm, 25 cm × 4.6 mm, i.d.)] at ambient temperature using a mobile phase consisting of methanol and water (50:50, v/v). Flow rate was 0.6 ml min^{-1} with an average operating pressure of 180 kg cm^{-2} and retention (t_R) time was found to be 2.93 ± 0.05 min. Quantitation was achieved with UV detection at 305 nm based on peak area with linear calibration curves at concentration range 10–100 μg ml^{-1}. The second method was based on high-performance thin layer chromatographic (HPTLC) separation followed by densitometric measurement of spots at 305 nm. The separation was carried out on Merck HPTLC aluminium sheets of silica gel 60 F_{254} using toluene:methanol:chloroform (8.0:4.5:6.0, v/v/v) as mobile phase. This system was found to give compact spots for etamsylate after double development (retention factor, R_f value of 0.23 ± 0.02). The second order polynomial regression analysis data was used for the regression line in the range of 500–6000 ng $spot^{-1}$. Both methods have been successively applied to pharmaceutical formulation. No chromatographic interference from the tablet excipients was found. Both methods were validated in terms of precision, robustness, recovery and limits of detection and quantitation. The analysis of variance (ANOVA) and Student's *t*-test were applied to correlate the results of etamsylate determination in dosage form by means of HPTLC and LC method. Drug was subjected to acid and alkali hydrolysis, oxidation, dry heat, wet heat treatment and photo-degradation. As the proposed methods could effectively separate the drug from its degradation products, they can be employed as stability indicating one. Moreover, the proposed LC method was utilized to investigate the kinetics of the acidic, alkaline and oxidative degradation processes at different temperatures and the apparent pseudo first order rate constant, half-life and activation energy was calculated. In addition the pH-rate profile of degradation of etamsylate in constant ionic strength buffer solutions with in the pH range 2–11 was studied.

Keywords: Etamsylate; High-performance liquid chromatography; Reversed phase; High-performance thin layer chromatography; Method validation; Quantitative analysis; Student's *t*-test; Kinetics of degradation; pH-rate profile; Arrhenius plot; Activation energy

1. Introduction

Etamsylate (Fig. 1a) chemically diethylamine 2,5-dihydroxybenzenesulphonate is official in British Pharmacopoeia [1]. It is a white or almost white, crystalline powder, very soluble in water, freely soluble in methanol, soluble in ethanol, practically insoluble in methylene chloride. Etamsylate possesses antihemorrhagic properties and also enhances P-selectin membrane expression in human platelets and cultured endothelial cells [2–9].

Literature survey reveals few analytical methods reported for the quantitative estimation of etamsylate. Thin layer chromatographic method has been reported in British Pharmacopoeia for quantitative estimation of etamsylate and its known impurity (benzene-1,4-diol) (Fig. 1b). Yang et al. [10] have reported chemiluminescence method for the determination of etamsylate in pharmaceutical preparation.

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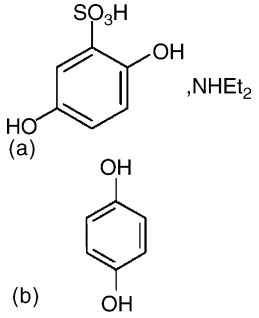

Fig. 1. (a) Structure of etamsylate. (b) Structure of hydroquinone (benzene-1,4-diol), a known impurity of etamsylate.

To our knowledge, no article related to the stability-indicating chromatographic determination of etamsylate in pharmaceutical dosage form has been reported in literature. The International Conference on Harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance [11]. Acidic, alkaline, oxidative and photolytic stability are required. An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradation products. Consequently, the implementation of an analytic methodology to determine etamsylate in pharmaceutical dosage form in presence of its degradation products is a pending challenge of the pharmaceutical analysis. Therefore, it was thought necessary to study the stability of etamsylate towards acidic, alkaline, oxidative, UV and photodegradation processes. The aim of this work was to develop stability-indicating chromatographic methods for determination of etamsylate in presence of its degradation products and related impurities for assessment of purity of bulk drug and stability of its bulk dosage forms using LC and HPTLC-densitometry. The two methods are simple, accurate, specific, repeatable, stability indicating, reduces the duration of the analysis and suitable for routine determination of etamsylate in tablet dosage form. Both the proposed methods were validated in compliance with ICH guidelines [12,13] and its updated international convention [14]. Furthermore, the developed LC method was used to investigate the kinetics of the acidic, alkaline and oxidative degradation processes by quantitation of drug at different temperatures, and to calculate the activation energy and half-life for etamsylate degradation. The proposed LC method was also utilized for pH-rate profile study of degradation of etamsylate in constant ionic strength buffer solutions with in the pH range 2–11.

2. Experimental

2.1. Materials

Pharmaceutical grade of etamsylate (batch no: et-S4220) was kindly supplied as a gift sample by Finecure Pharma Ltd., Gujarat, India, used without further purification and certified to contain 99.65% (w/w) on dried basis. All chemicals and reagents used were of LC grade and were purchased from Merck Chemicals, India.

2.2. Instrumentation and chromatographic conditions

2.2.1. For LC method

The LC system consisted of a pump (model jasco PU 1580, intelligent LC pump) with auto injecting facility (AS-1555 sampler) programmed at 20 μl capacity per injection was used. The detector consisted of a UV–vis (Jasco UV 1575) model operated at a wavelength of 305 nm. The software used was jasco borwin version 1.5, LC-Net II/ADC system. The columns used were Kromasil C-18 (250 mm × 4.6 mm, 5.0 μm) Flexit Jour Laborarories Pvt. Ltd., Pune, India and Finepak SIL-5, C-18 (250 mm × 4.6 mm, 5.0 μm) Jasco Corporation, Japan. Different mobile phases were tested in order to find the best conditions for separation of etamsylate in presence of its degradation products. The optimal composition of the mobile phase was determined to be methanol:water (50:50, v/v). The flow rate was set to 0.6 ml min^{-1} and UV detection was carried out at 305 nm. The mobile phase and samples were filtered using 0.45 μm membrane filter. Mobile phase was degassed by ultrasonic vibrations prior to use. All determinations were performed at ambient temperature.

2.2.2. For HPTLC densitometry

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on precoated silica gel aluminium Plate 60 F_{254}, (20 cm × 10 cm) with 250 μm thickness; (E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat IV (Switzerland, supplied by Anchrom Technologists, Mumbai). The plates were prewashed by methanol and activated at 110 °C for 5 min prior to chromatography. A constant application rate of 0.1 μl s^{-1} was employed and space between two bands was 6 mm. The slit dimension was kept at 5 mm × 0.45 mm and 10 mm s^{-1} scanning speed was employed. The monochromator bandwidth was set at 20 nm with K 320 cut off filter, each track was scanned thrice and baseline correction was used. The mobile phase consisted of toluene:methanol:chloroform (8.0:4.5:6.0, v/v/v) and 15 ml of mobile phase was used per chromatography. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). Dimensions: length × width × height = 12 cm × 4.7 cm × 12.5 cm. It was saturated (lined on the two bigger sides with filter paper that had been soaked thoroughly with the mobile phase) and the chromatoplate double development was carried out in dark with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 °C ± 2) at relative humidity of 60% ± 5. The length