Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1119 (2006) 216–223
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of estradiol and its degradation products by liquid chromatography

Lucie Havlíková [a], Lucie Nováková [a], Ludmila Matysová [a], Jan Šícha [b], Petr Solich [a,*]

[a] *Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic*
[b] *Bochemie Group, Herbacos-Bofarma Ltd., Štrossova 239, 530 02 Pardubice, Czech Republic*

Available online 8 February 2006

Abstract

A novel HPLC method for simultaneous determination of estradiol and its seven degradation products in topical gel was developed. Zorbax SB-CN (150 mm × 4.6 mm, 5 μm) analytical column and mobile phase composed of acetonitrile, phosphoric acid 0.085%, and tetrahydrofurane (27:63:10, v/v/v) at flow-rate 1.0 ml min^{-1} were used for the chromatographic separation using UV detection at 225 nm. The active substance estradiol was separated from all its known degradation products successfully. Two degradation products estrone and $\Delta^{9(11)}$-estrone were not separated sufficiently, their peaks were evaluated as a sum of two components. The method was validated according to ICH guideline recommendations and thereafter it was successfully applied for stability tests of topical cream Estrogel HBF in the quality control laboratory. Limits of detection for degradation products ranged from 1.03×10^{-5} to 1.14×10^{-4} mg ml^{-1}, limits of quantitation for degradation products were in the range 3.43×10^{-5} to 3.81×10^{-4} mg ml^{-1}. The developed method is selective, precise, accurate and sensitive enough for determination of estradiol and its known degradation products.

Keywords: HPLC; Estradiol; Degradation products; Pharmaceuticals; Stability studies; Simultaneous determination

1. Introduction

Nowadays, growing emphasis is placed on testing of purity and stability of active substances especially to assure the high quality of drug products in connection with safe medical care. Impurity is described as any component of the drug substance or drug product that is not the chemical entity defined as the drug substance or other additives to the drug product. This term is broad enough to include degradation products as impurities [1]. In ICH guidance degradation product is defined as an impurity resulting from a chemical change in the drug substance during manufacture and/or storage of the new drug product [2].

Degradation products are often present in very low levels. It is necessary not only to identify degradation products but also to determine their amount present in drug substances and drug products. HPLC method is one of the most widely used methods in Ph. Eur. 4 (European Pharmacopoiea) and in USP 28 for the assay of bulk drug material [3]. Such a method enables to perform analyses with high specificity, sensitivity, sufficient precision, and reproducibility. The great advantage of HPLC method is the possibility to get qualitative and quantitative information about component content in the sample during one-step analysis.

Estradiol, chemically1,3,5(10)-estratrien-3, 17β-diol is the most potent estrogen of a group of endogenous estrogen steroids which includes estrone and estriol. Estradiol is responsible for the growth of breast and reproductive epithelia, maturation of long bones, and development of secondary sexual characteristics [4]. Estradiol and its semi-synthetic esters are primarily used as menopausal hormones. Estradiol may also be used as replacement therapy for female hypogonadism or primary ovarian failure. The decrease of estradiol at menopause is often accompanied by vascular instability and rise in incidence of heart disease and an increasing risk of osteoporosis [5]. Estrogens are used for alleviation of menopausal symptoms or for prophylaxis of heart disease and osteoporosis.

According to pharmacopoeial regulations, the only degradation product to be monitored together with estradiol is estrone (chemically 1,3,5(10)-estratrien-3-ol-17-one). Further known degradation products of estradiol considered in our study are: 17α estradiol (chemically 17α estradiol hemihydrate), ethynylestradiol (chemically 17α-ethynyl-1,3,5(10)-estratriene-

* Corresponding author. Tel.: +420 495067294; fax: +420 495518718.
E-mail address: solich@faf.cuni.cz (P. Solich).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.01.085

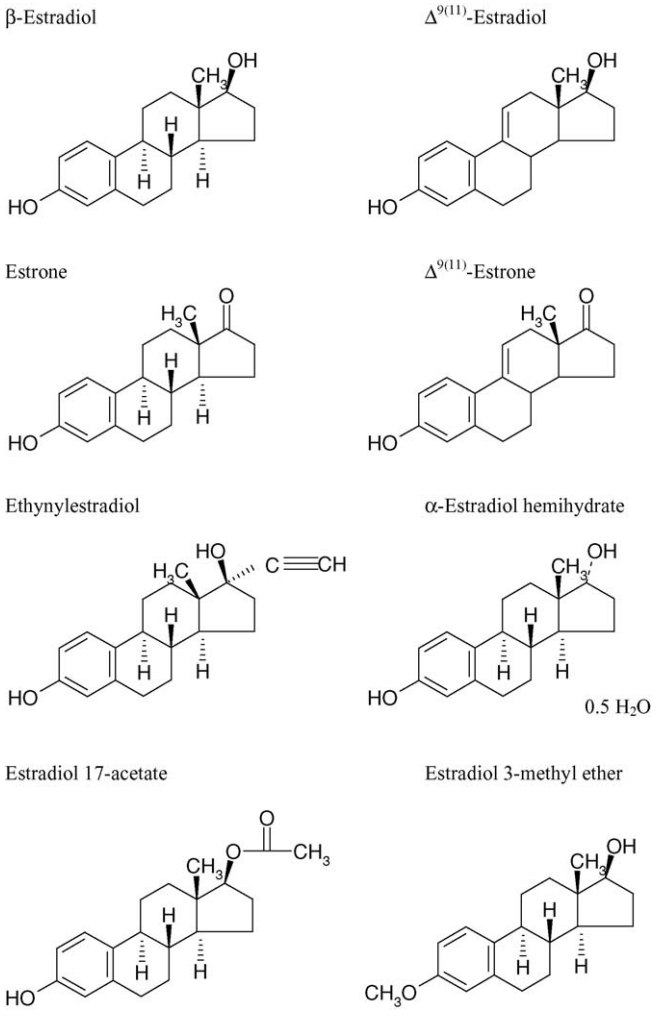

Fig. 1. Chemical structures—estradiol and its degradation products.

3,17β –diol), estradiol 3-methyl ether (chemically 17β hydroxy-3-methoxyestra -1,3,5(10)-triene), estradiol 17-acetate (chemically 1,3,5(10)-estratrien-3,17β-diol 17-acetate), $\Delta^{9(11)}$-estrone (1,3,5(10)-estratrien-3-ol-17-one), and $\Delta^{9(11)}$-estradiol (1,3,5(10),9(11)-estratetraen-3,17β-diol) (Fig. 1). Considered intermediates of estradiol are $\Delta^{9(11)}$-estrone, and $\Delta^{9(11)}$-estradiol.

The aim of this work was to develop a novel stability indicating HPLC method for simultaneous determination of estradiol and its known degradation products in topical gel.

The basic method for determination of estradiol and its degradation product estrone in topical pharmaceutical preparations is described in USP 28 [6] (mobile phase of acetonitrile and water 1:1). Recently, there has been found a number of reports dealing with determination of estradiol by liquid chromatography [7–9]. An HPLC method using mobile phase acetonitrile–methanol–water 23:24:53 was used for determination of estradiol and estrone in topical gel [10]. Zhao et al. [11] developed a method performed on X-Terra RP18 column for analysis of seven sexual hormones in cosmetics. A mixture of water-methanol-acetonitrile (50:32:18, v/v/v) was used as mobile phase. The seven sexual hormones were detected at 230 nm. Estradiol, 17α estradiol, and ethynylestradiol were determined by HPLC method in urine using gradient elution [12]. The temperature effect on retention and separation of estrogens in reversed phase high performance liquid chromatography has also been studied [13]. An HPLC-radioimmunoassay (HPLC-RIA) method was developed for analysis of steroid hormones [14]. The effect of formamide concentration in extraction solvent on the peak shape and recovery of degradation products of estradiol in transdermal formulation was studied. An Intersil ODS column and acetonitrile/water gradient elution were used for chromatography [15].

Several studies used liquid chromatography–mass spectrometry for monitoring of estrogen hormones in environmental water [16], in natural water and drinking water [17], or in river water [18]. Estrogen metabolites in human urine were determined using HPLC-EI/MS method [19].

To our best knowledge, no information dealing with simultaneous HPLC analysis of estradiol (active substance), $\Delta^{9(11)}$-estradiol, 17α estradiol, estrone, $\Delta^{9(11)}$-estrone, ethynylestradiol, estradiol 3-methyl ether, and estradiol 17-acetate was published yet.

2. Experimental

2.1. Chemicals and reagents

Working standards of estradiol, its seven degradation products, and flurbiprofen as internal standard were used for the purpose of this study. The standards of estradiol (active substance), estrone, ethynylestradiol, estradiol 3-methyl ether, 17α estradiol, and estradiol 17-acetate were obtained from Sigma-Aldrich (Prague, Czech Republic). $\Delta^{9(11)}$-Estrone and $\Delta^{9(11)}$-estradiol were obtained from Steraloids, Inc. (Newport, USA). Flurbiprofen was purchased from Sigma-Aldrich (Prague, Czech Republic).

Acetonitrile Chromasolv® for HPLC gradient grade and Tetrahydrofuran were provided by Sigma-Aldrich (Prague, Czech Republic). Phosphoric acid 85% p.a. was obtained from Merck (Darmstadt, Germany). HPLC grade water was prepared by Milli-Q reverse osmosis Millipore (Bedford, MA, USA) and it met all European Pharmacopoeia requirements.

2.2. Chromatographic system

A Shimadzu LC-2010 C system (Shimadzu, Kyoto, Japan) with built-in UV–VIS detector and built-in auto sampler (conditioned at 25 °C) was used to perform the analyses. Chromatographic software Class VP 6.12 was used for data collection and processing.

The selectivity of several columns with different stationary phases for analysis of estradiol and its known degradation products were tested. Supelco Discovery C18 (125 mm × 4.0 mm, 5 μm), Discovery RP Amide C16 (250 mm × 3 mm, 5 μm), and Discovery ZR-CARBON C18 (75 mm × 4.6 mm, 3.5 μm) columns were bought from Sigma-Aldrich (Prague, Czech Republic). The separation was tested also using Zorbax columns obtained from Agilent Technologies (Prague, Czech Repub-