Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 892–897
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

HPLC method for enantioselective analysis of cloprostenol

Květa Kalíková [a], Eva Tesařová [a,*], Zuzana Bosáková [b]

[a] *Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, 128 43 Prague 2, Albertov 2030, Czech Republic*
[b] *Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, 128 43 Prague 2, Albertov 2030, Czech Republic*

Received 2 March 2007; received in revised form 14 June 2007; accepted 15 June 2007
Available online 19 June 2007

Abstract

A new HPLC method for the separation and quantification of cloprostenol enantiomers was developed. The optimized separation system consisted of Chiralcel OD-RH column and acetonitrile–sodium dihydrogenphosphate (pH 3.0; 20 mM) (33:67, v/v) as the mobile phase. Baseline resolution of (±)-cloprostenol ($R = 2.16$) was achieved and the analysis time did not exceed 10 min. Limits of detection and quantification were units of μmol/l at 274 nm. The respective values decreased an order of magnitude at 210 nm. The R.S.D. values obtained for the retention factor, peak area and peak height of each enantiomer were less than 2%. Conditions for semipreparative separation of the enantiomers can be achieved easily just by a small adaptation of the mobile phase composition.

Keywords: Reversed phase HPLC; Enantioselective separation; Chiralcel OD-RH column; Validation; Prostaglandin analogue; Cloprostenol

1. Introduction

Prostaglandins are successfully employed in veterinary medicine. Reproductive management programs based on strategic use of prostaglandin F2α to induce and synchronize oestrus in post-partum dairy cows are widespread [1]. Repeated shortening of the oestrous cycle during early lactation in high-yielding dairy cows, however, could impair corpus luteum function and thus decrease fertility.

Cloprostenol is a synthetically prepared analogue of prostaglandin F2α. The preparation has ability to elicit luteolysis and uterine contraction; it is also used for synchronizations of rates and some other treatments. Chemical synthesis produces a racemic mixture of (±)-cloprostenol. However, (+)-enantiomer (see Fig. 1) was shown to have higher biological activity and only this enantiomer seems to exhibit luteolytic activity [2–4]. A chemoenzymatic synthesis of (+)-cloprostenol based on enantiospecific process catalyzed by the yeast *Kluyveromyces marxianus* has also been developed [5].

Repeated application of (+)-cloprostenol (sodium salt) affects progesterone concentrations that indicate function of corpus luteum in cows and influences milk production, parity and endometritis [1]. The positive impacts of cloprostenol on the induction of luteolysis but also its clinical side effects were observed in mares [6]. Cows with subclinical endometritis treated with cloprostenol (or cephapirin) had significantly increased relative pregnancy rate. The reproductive performance was improved just after a single treatment with these preparations [7]. The positive effect of cloprostenol on reproduction of cows diagnosed with endometritis seems to be the main reason for further investigations in this field nowadays [8].

Different effects of the racemic cloprostenol and its single enantiomer, (+)-cloprostenol, have been described by several authors [9–11] and are still a subject of extensive studies. In vitro investigations confirmed stereoselective binding of (+)-cloprostenol (and also of natural prostaglandin F2α) to be 150 times more potent than (±)-cloprostenol on corpus luteum cells and 10 times more potent on myometrium cells [12]. The significantly lower affinity of the racemic cloprostenol (as compared to (+)-enantiomer) to the tissue receptors was explained by a possible interference of the (−)-enantiomer from the racemate for the (+)-cloprostenol receptors [2].

In spite of the great interest in cloprostenol application and investigation of racemate versus pure enantiomer effects only few methods (HPLC) for its analysis can be found in the lit-

* Corresponding author. Tel.: +420 2 21951296; fax: +420 2 24919752.
E-mail address: tesarove@natur.cuni.cz (E. Tesařová).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.06.016

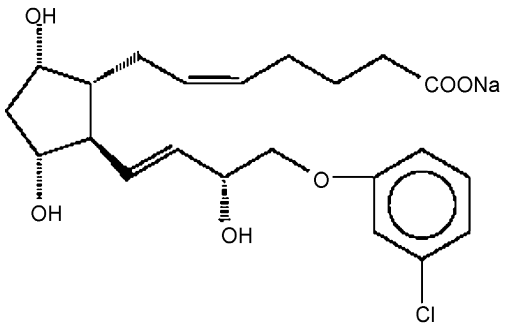

Fig. 1. Chemical structure of (+)-cloprostenol (sodium salt of [9α,11α,15*R*-trihydroxy-16-(3-chlorophenoxy)-17,18,19,20-tetranor-prosta-5*Z*,13*E*-dien-1-oic acid]).

erature. A laborious semipreparative method was designed that consisted of preparation of a diastereomeric mixture of methyl ester trismandelates of (±)-cloprostenol, their separation on a silica-column with trichloromethane–ethylacetate (2:1, v/v) and subsequent alkaline hydrolysis of the esters to obtain the enantiomers [13]. An achiral method was used to control cloprostenol content in pharmaceutical preparation (solution) using an Ultrasphere ODS column and methanol–phosphate buffer (pH 6.75; 0.02 M) (50:50, v/v) as mobile phase [14]. Separation of cloprostenol epimers (stereoisomers the configuration of which differs just in the 15 position) was described on silica gel (Zorbax SIL) stationary phase. Addition of small amount of water (less than 1%) to hexane-propan-2-ol mobile phase was shown to dramatically improve separation efficiency and in this way also resolution [15].

However, to the best of our knowledge a method for direct chiral separation of cloprostenol enantiomers (stereoisomers, which differ in configuration on all stereogenic centers) has not been described in the literature, yet. In view of the above-mentioned facts and extensive concern of pharmaceutical companies over chiral separation of the racemic mixture of cloprostenol, the aim of this work is to develop a simple analytical method for chromatographic separation and determination of cloprostenol enantiomers. The method is aimed for enantiomeric purity control but it should be easy switched to a semipreparative mode.

2. Experimental

2.1. Equipment

Measurements were performed with a liquid chromatograph (Pye Unicam, Cambridge, UK) consisting of a PU 4015 pump, a PU 4020 UV–Vis detector and a Rheodyne 7725 injector with a 20 μl loop (Cotati, CA, USA). CSW32 software provided by DataApex (Prague, Czech Republic) was used for the process control and data handling. Another liquid chromatograph (Waters, Milford, MA, USA) composed of a Waters 1525 binary HPLC pump, Waters 717 plus autosampler and Waters 2487 dual λ absorbance detector, with Breeze software, was used for study of reproducibility of the method. NCSS software (NCSS, Kaysville, UT, USA) was applied to perform one-way analysis of variance (ANOVA). Temperature was controlled *via* Mistral column thermostat (Spark, Emmen, The Netherlands).

The chiral stationary phases (CSPs) tested during the method development were Chirobiotic T (teicoplanin-bonded CSP), Chirobiotic TAG (teicoplanin aglycon-bonded CSP), Chirobiotic V (vancomycin-bonded CSP) and Chirobiotic R (ristocetin A-bonded CSP), all 250 mm × 4.6 mm i.d., particle size 5 μm. These macrocyclic antibiotic-based columns were manufactured by ASTEC (Whippany, NJ, USA). The flow rate used was 0.6 ml/min in reversed phase and polar-organic modes and 1 ml/min in normal-phase mode. Analyses were performed at laboratory temperature. Another type of chiral stationary phases used in our experiments was Chiralcel OD-RH column (column size 150 mm × 4.6 mm) with guard cartridge (cartridge size 10 mm × 4 mm) (Chiral Technologies Europe, France). The chiral stationary phase of both the column and the cartridge consisted of cellulose tris(3,5-dimethylphenylcarbamate) (see Fig. 2) coated on 5 μm silica gel. This column was thermostated at 20 °C. The flow rate was set to 0.7 ml/min. Detection was carried on at wavelength of the absorption maximum 274 nm. At this wavelength mobile phase constituents and potential impurities do not interfere. Nevertheless, detection sensitivity is usually higher at wavelengths' range of 190–215 nm where the interference takes place. Therefore, linearity, limit of detection (LOD) and limit of quantification (LOQ) were also determined at 210 nm.

2.2. Chemicals

Methanol (MeOH), *n*-hexane (hex), propan-2-ol (IPA) and acetonitrile (ACN) for HPLC were obtained from Sigma–Aldrich (Prague, Czech Republic). Triethylamine (TEA, purity > 99.5%) and glacial acetic acid (HAc, purity > 99%) were from Fluka (Prague, Czech Republic). Sodium dihydrogenphosphate dihydrate was purchased from Lachema (Brno, Czech Republic) and *ortho*-phosphoric acid (85%) from Fluka (Buchs, Switzerland). Water was prepared with a Milli-Q water purification system (Millipore, Milford, MA, USA).

(±)-Cloprostenol and (+)-cloprostenol, purity 99.8%, were obtained from Nerapharm (Neratovice, Czech Republic). Stock

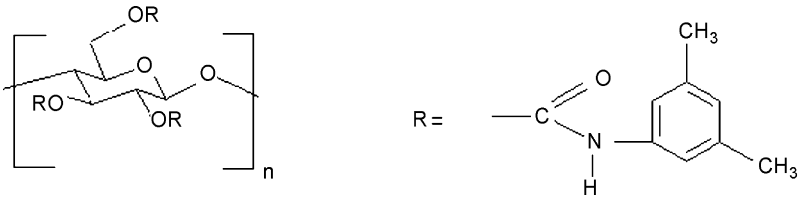

Fig. 2. Chemical structure of chiral stationary phase of Chiralcel OD-RH column.