Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 293–297

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Simultaneous determination of chondroitin sulfate sodium, allantoin and pyridoxine hydrochloride in pharmaceutical eye drops by an ion-pair high-performance liquid chromatography

Pengfei Jin*, Jie Ma, Xuejun Wu, Ding Zou, Chunhua Sun, Xin Hu

Department of Pharmaceutical Science, Beijing Hospital, No. 1 Dahua Road, Dongcheng District, Beijing 100730, China

ARTICLE INFO

Article history:
Received 3 February 2009
Received in revised form 23 April 2009
Accepted 23 April 2009
Available online 9 May 2009

Keywords:
Chondroitin sulfate sodium
Allantoin
Pyridoxine hydrochloride
HPLC
Eye drops
Pharmaceutical preparation

ABSTRACT

An ion-pair high-performance liquid chromatography (HPLC) method has been developed for the simultaneous determination of chondroitin sulfate sodium (CSS), allantoin and pyridoxine hydrochloride (VB_6) in a commercial eye drops dosage form. An Alltima C_{18} column (250 mm × 4.6 mm i.d., 5 μm) was used for the separation at room temperature, with 25 mM ammonium dihydrogen phosphate (containing 0.01% heptanesulfonic acid sodium salt) and acetonitrile (95:5, v/v) as the mobile phase at the flow rate of 0.5 mL min^{-1}. The detection wavelength for CSS, allantoin and VB_6 was 195 nm, 215 nm and 291 nm, respectively. The method showed good linearity for CSS, allantoin and VB_6, with correlation coefficients greater than 0.9996, in the range of 203.96–815.84 mg L^{-1}, 371.16–1488.64 mg L^{-1}, and 23.32–93.28 mg L^{-1}, respectively. The instrumental and method precisions were adequate with all relative standard deviations lower than 2.0%. The accuracy of this method, measured by the recovery of three compounds from spiked placebo solutions, was from 99.01% to 101.92%. The three components, CSS, allantoin and VB_6 were well separated from other ingredients and degradation products. This method is fast, simple, and can be used for direct and simultaneous determination of CSS, allantoin and VB_6 in the pharmaceutical preparation.

1. Introduction

Chondroitin sulfate sodium (CSS) is a sodium salt of a sulfated linear glycosaminoglycan which has disaccharide repeating unit formed by sulfate ester of N-acetylchondrosamine (2-acetamido-2-deoxy-β-D-galactopyronose) and D-glucuronic acid [1]. The chondrosamine moieties in the glycosaminoglycan are monosulfated primarily on position C-4 and less on position C-6 (Fig. 1(A)). These biological polymers act as the flexible connecting matrix in cartilage and tissues [2], and are using as active ingredients in many dietary supplements and pharmaceutical preparations. A photometric titration method using cetylpyridinium chloride has been the standard approach listed in USP for the assay of CSS raw materials and CSS containing tablets [3]. Other methods involving spectrophotometric assay [4,5], high performance capillary electrophoresis (HPCE) assay [6,7], size-exclusion HPLC assay [8], and strong anion-exchange HPLC assay following chondroitinase ABC enzyme digestion [9], have also been reported.

Allantoin (Fig. 1(B)), a purine metabolite, is an astringent and has been reported keratolytic. It is frequently used as a vulnerary to stimulate tissue repair in suppurating wounds, resistant ulcers, acne, seborrhea, cold sores, psoriasis, hemorrhoid, and other anorectal disorders [10,11]. Nowadays, a potentiometric titration assay method is commonly used for the determination of allantoin raw materials in USP [12]. In addition, assays by colorimetry [13,14], hydrophilic interaction chromatography (HILIC) [15], RP-HPLC [16–21], LC–MS/MS [22], capillary electrophoresis (CE) [23,24], and GC–MS [25], have been reported for the determination of allantoin in various types of matrix materials.

Pyridoxine hydrochloride (VB_6) (Fig. 1(C)) is a water-soluble vitamin and is involved primarily in the metabolism of amino acid, carbohydrate, and fat [26]. Many technique, including spectrophotometric, polarographic, fluorimetric, enzymatic, and microbiological approaches have been employed for the determinations of pyridoxine hydrochloride and other water-soluble vitamins. Most of these methods, however, are time-consuming and less accurate. In recent years, micellar electrokinetic capillary chromatography (MEKC) [27], calixarene based potentiometric sensor [28], and HPLC [29–32], have been reported for the analysis of VB_6 from pharmaceutical preparations with improved speed and accuracy. Further, an ion-pairing HPLC method was described [33] in USP.

The purpose of this study was to report, for the first time, a simple HPLC method for the direct and simultaneous determination of CSS, allantoin and VB_6 in a commercial eye drops product.

* Corresponding author. Tel.: +86 10 85133621; fax: +86 10 65283696 5.
E-mail address: j790101@sohu.com (P. Jin).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.04.037

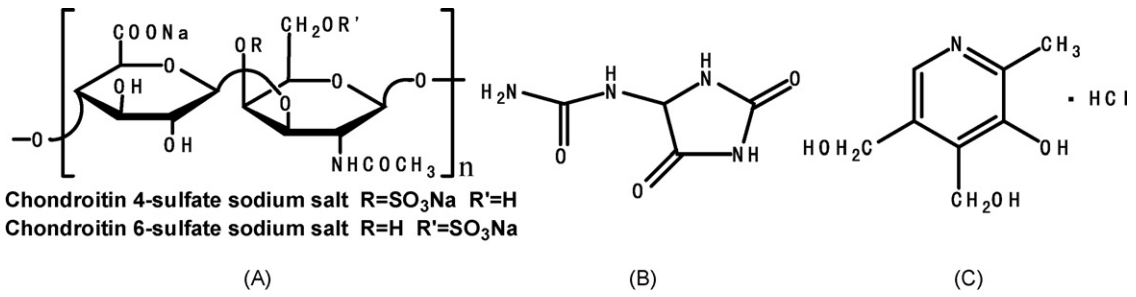

Fig. 1. Chemical structures for disaccharide unit of CSS (A), allantoin (B), and VB_6 (C).

2. Experimental

2.1. Reagents and chemicals

CSS standard raw materials (chondroitin 4-sulfate sodium salt from bovine trachea, lot & filling code: 346459/1 & 33701, HPLC determination purity 93.79%) were purchased from Fluka (Steinheim, Germany). Allantoin and VB_6 reference standards were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Heptanesulfonic acid sodium salt was purchased from Acros (New Jersey, USA). Analytical grade ammonium dihydrogen phosphate, hydrochloric acid, sodium hydroxide, and 30% hydrogen dioxide were obtained from Beijing Reagent Company (Beijing, China). HPLC grade acetonitrile was purchased from Fisher (Fair Lawn, NJ, USA). Redistilled water was prepared by Milli-Q system (Millipore, Bedford, MA, USA). All solvents were filtrated through 0.45-μm PTFE filters (HPLC Technology, Cheshire, UK) before use.

The eye drop sample, compound allantoin vitamin B_6-E and aminoethysulfonic acid eye drops (Eye charm V® eye drops), were made in Japan and obtained commercially. The active ingredients were labeled (per 100 mL) as 100 mg of CSS, 200 mg of allantoin, 10 mg of pyridoxine hydrochloride, 10 mg of tocopherol acetate, and 200 mg of aminoethylsulfonic acid.

2.2. Instrumentation and chromatographic conditions

The study was performed on a Waters 2695 quaternary pump system. A Waters 2696 photodiode array detector and an Empore professional® software were used for data acquisition and processing. The chromatographic separations were carried out on an Alltima C_{18} column (250 mm × 4.6 mm i.d., 5 μm, 100 Å, Alltech Associates Inc., Deerfield, IL, USA). The mobile phase composed of 25 mM ammonium dihydrogen phosphate (containing 0.01% heptanesulfonic acid sodium salt) and acetonitrile (95:5, v/v) was delivered at a flow rate of 0.5 mL min^{-1}. The selected detection wavelengths for CSS, allantoin and VB_6 were 195 nm, 215 nm, and 291 nm, respectively. The injection volume was 10 μL. All analysis was conducted at room temperature.

2.3. Preparation of standard solutions

A stock solution containing 2039.6 mg L^{-1} CSS, 3721.6 mg L^{-1} allantoin, and 233.2 mg L^{-1} VB_6 was prepared by dissolving accurately weighed amounts of standards in the mobile phase. Sequential dilutions were then made using the mobile phase, to reach final concentrations of 203.96 mg L^{-1}, 305.94 mg L^{-1}, 407.92 mg L^{-1}, 509.90 mg L^{-1}, and 815.84 mg L^{-1} for CSS, 371.16 mg L^{-1}, 558.24 mg L^{-1}, 744.32 mg L^{-1}, 930.40 mg L^{-1}, and 1488.64 mg L^{-1} for allantoin, and 23.32 mg L^{-1}, 34.98 mg L^{-1}, 46.64 mg L^{-1}, 58.30 mg L^{-1}, and 93.28 mg L^{-1} for VB_6. The mid-point concentration in these dilutions was used as working standard solution for system suitability studies.

2.4. Preparation of analytical samples

2 mL of the Eye charm V® eye drops were transferred into a 5 mL volumetric flask, made up to 5 mL with mobile phase and vortexed for 5 min. The resulting solutions (sample solutions) were then analyzed by HPLC after filtration through 0.20-μm PTFE membrane syringe filter (Anachem, Cheshire, UK).

2.5. Validation procedure

The developed method was validated for system suitability, specificity, linearity, precision, accuracy and LOD, following ICH recommendations [34,35].

2.5.1. System suitability

The key system suitability parameters, including theoretical plates and asymmetry factors for chromatographic peaks of CSS, allantoin, and VB_6 were calculated as European Pharmacopoeia [36] described.

2.5.2. Specificity

To assess the method specificity, a reconstituted eye drop placebo without CSS, allantoin, and VB_6 was prepared. The placebo solution was prepared using the same procedure as in preparing for the analytical samples (Section 2.4), and then subjected to HPLC analysis to evaluate the potential interferences from other ingredients (i.e. aminoethylsulfonic acid, tocopherol acetate, isoosmotic adjusting agent, etc.).

Forced degradation studies of CSS standard, allantoin standard, VB_6 standard, reconstituted placebo, and eye drop sample, under different stress conditions (heat, light, oxidation, acid and base), were conducted to evaluate the potential interferences of degradation products.

For preparing heat degradation products, 5 mL of placebo solution, 5 mL of analytical sample solution, 5 mL of CSS standard solution (400 mg L^{-1}, approximately), 5 mL of allantoin standard solution (800 mg L^{-1}, approximately), and 5 mL of VB_6 standard solution (40 mg L^{-1}, approximately), were heated at 80 °C for 24 h in dark, and then cooled to room temperature. To study light induced degradation products, these solutions were accumulatively exposed to directed sunlight for more than 48 h.

For preparing oxidation resulted degradation products, 1 mL of 9.0% hydrogen peroxide (v/v) was added to 2 mL of reconstituted placebo and 2 mL of eye drop sample, while 1 mL of 3.0% hydrogen peroxide (v/v) was added to 20 mg of CSS standard, 40 mg of allantoin standard, and 2 mg of VB_6 standard. After stored in dark for more than 24 h, the degraded placebo and eye drop sample were transferred into a 5 mL volumetric flask and brought to volume with the mobile phase, while the degraded standards were dissolved