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Journal of Chromatography B, 819 (2005) 191–196
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of lauroyl-indapamide in rat whole blood by high-performance liquid chromatography

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Received 7 July 2004; accepted 31 January 2005
Available online 11 February 2005

Abstract

A method based on a liquid–liquid extraction procedure followed by high-performance liquid chromatography (HPLC) coupled with UV–visible detection is described and validated for the determination of lauroyl-indapamide in rat whole blood. The blood sample was extracted with diethyl ether after the addition of 10% trifluoroacetic acid (aq.). The chromatographic separation was performed on a Chromasil ODS column, using methanol–acetonitrile–tetrahydrofuran–0.2% trifluoroacetic acid (170:20:15:38, v/v/v/v) as the mobile phase. The UV detection wavelength was set at 240 nm. The extraction recovery of lauroyl-indapamide was ranged from 76.5 to 82.6%, and the calibration curve had a good linearity in the range of 0.048–200 μg/ml ($r = 0.9976$). The method presents appropriate intra-day and inter-days repeatabilities, showing values below 7.4% in terms of the percentage of relative standard deviation (R.S.D.). The method proposed is simple, rapid and sensitive, being useful for pharmacokinetic studies in rats.

Keywords: Lauroyl-indapamide; HPLC; Rat whole blood; Pharmacokinetic study

1. Introduction

Lauroyl-indapamide, 3-(lauroylaminosulfanyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1H-indol-1-yl)benzamide (Fig. 1), is a new compound derived from indapamide with an increased liposolubility to obtain a longer action and to be suitable for liposomal preparations. It belongs to a well-known pharmacological active compound series classified as an antihypertensive agent with diuretic activity, which can significantly reduce rat blood pressure. A number of analytical methods have been reported concerning the determination of indapamide, the parent drug of lauroyl-indapamide, in dosage form [1,2] or in biological fluids including serum, plasma, urine and whole blood [3–11]. However, to the best of our knowledge, there is little information available in the literatures about lauroyl-indapamide, even less about its assay in the biological fluids, which made it considered necessary to develop an accurate assay to quantify it in vivo, so as to know exactly the characteristics of the substance in biological tests.

Therefore, an analytical method based on high-performance liquid chromatography (HPLC) to determine lauroyl-indapamide in biological fluids is reported here for the first time. Considering lauroyl-indapamide is preferentially bound to blood cells like indapamide [4,5], we selected rat whole blood as the biological sample. The method developed here was validated with parameters such as precision, linearity, selectivity, detection and quantitation limit, and was also successfully applied to following the pharmacokinetic behavior of lauroyl-indapamide in Wistar rats.

2. Experiments

2.1. Materials and reagents

Lauroyl-indapamide (purity ≥ 99%) was synthesized from indapamide, isolated and purified with silica-resin chro-

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doi:10.1016/j.jchromb.2005.01.032

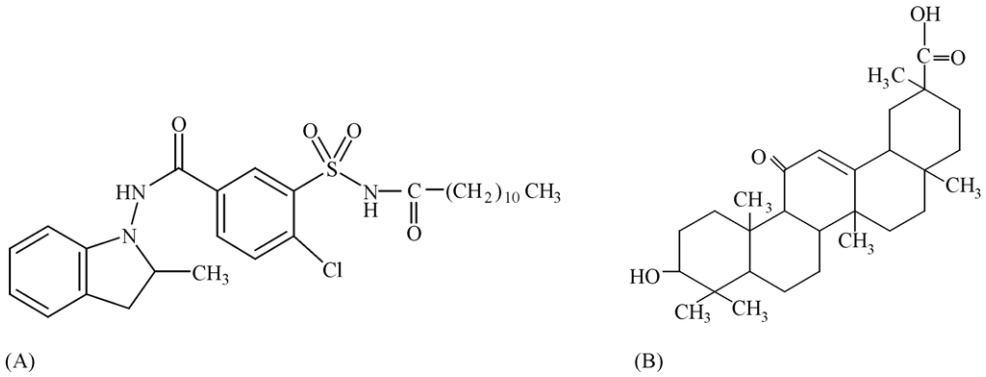

Fig. 1. The chemical structures of (A) lauroyl-indapamide and (B) glycyrrhetinic acid.

matography, and its identity and purity were documented by the means of HPLC, UV, NMR and IR. Glycyrrhetinic acid (internal standard, IS, see Fig. 1) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol, acetonitrile and tetrahydrofuran were of HPLC grade and purchased from Concord Technology Company (Tianjin, China), and other chemicals used were of analytical grade. Distilled water prepared from demineralized water was used throughout the study. Wistar rats were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University.

2.2. Instrumentations

The chromatographic system consisted of a Waters Model 510 pump (Waters Corporation, USA) and an SPD-10A UV–vis detector (Shimadzu, Japan). The signals from the detector were collected and analyzed with a computer equipped with N2000 Chromatography Data System (Zhejiang University, Zhejiang, China).

2.3. Chromatographic condition

Chromatographic separation was achieved on a Chromasil ODS column (250 mm × 4.6 mm, 5 μm) (Elete, Dalian, China) with a SecurityGuard column (10 mm × 4.6 mm, 5 μm) filled with the same materials. The temperature was maintained at ambient temperature. The mobile phase was consisted of methanol–acetonitrile–tetrahydrogen–0.2% trifluoroacetic acid (170:20:15:38; v/v/v/v), filtered with 0.45 μm cellulosic Millipore membrane (Xinya, Shanghai, China) and degassed by ultrasonic before use, and delivered at an isocratic flow rate of 0.8 ml/min. The detection wavelength was set at 240 nm, the injection volume was 20 μl.

2.4. Preparation of standard and quality control solutions

The stock solutions of lauroyl-indapamide (0.8 mg/ml) and glycyrrhetinic acid (0.5 mg/ml) were prepared with methanol. A series of standard working solutions with the concentration in the range of 0.096–400 μg/ml for lauroyl-indapamide were obtained by a further dilution of the stock solution with methanol. The internal standard working solution (0.05 mg/ml) was prepared by the dilution of the stock internal standard solution with methanol. Quality control (QC) working solutions were prepared at the concentrations of 1.0, 10 and 100 μg/ml with the same way as that of the standard working solutions. All the solutions were stored at 4 °C and brought to room temperature before use.

2.5. Sample preparation

To a 200 μl aliquot of rat whole blood, 50 μl of the internal standard working solution (0.05 mg/ml) and 50 μl of 10% trichloroacetic acid were added. The mixture was extracted with 1 ml of diethyl ether by vortex for 5 min, and then centrifuged at 3000 × *g* for 10 min in a microcentrifuge. The upper organic phase was transferred to another tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μl methanol (HPLC grade), and mixed by vortex for 30 s, a 20 μl aliquot of the solution was injected directly into the HPLC system for analysis.

2.6. Validation of the analytical method

2.6.1. Linear range

Calibration curve data were generated by spiking a series of 200 μl drug-free whole blood with 50 μl of a standard working solution (1.0, 10 and 100 μg/ml) and 50 μl of the internal standard working solution, the following extraction procedure and HPLC analysis were performed on the samples as described above. The calibration curve was constructed by plotting the peak area ratios of the analyte to the internal standard against the concentrations of the analyte in the whole blood and analyzed by linear regression analysis.

2.6.2. Precision and intra-day and inter-days repeatabilities

The precision was determined by analyzing the extracted quality control samples of different concentrations more than