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Liquid chromatographic quantitation of the lactone and the total of lactone and carboxylate forms of 9-nitrocamptothecin in human plasma

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Abstract

Simple and sensitive high-performance liquid chromatography (HPLC) assays were developed and validated for the quantitation of the investigational anticancer drug 9-nitrocamptothecin (9-NC) as the lactone form and as the total of the lactone(I) and carboxylate(II) forms in human plasma. For the assay of lactone form (9NC-lac), the analytical method involved a protein precipitation step with adding a mixture of cold acetonitril–chloroform (5:1 (v/v), −20 °C) to plasma sample that stabilized the pH-dependent conversion of I to II. After evaporation under gentle stream of nitrogen gas (40 °C) the dry extract was dissolved in mobile phase (pH 5.5). For determination of the total of the lactone and carboxylate forms of the drug (9NC-tot), plasma samples were deproteinated with cold acetonitril (−20 °C) acidified with perchloric acid (5%), which resulted in the conversion of the carboxylate into the lactone form. After centrifugation the upper solvent was evaporated (nitrogen, 40 °C) and the dry extract was dissolved in mobile phase (pH 3.5). All separations were performed on a RP-C_8 column, using a mixture of acetonitril–water as eluent (pH 3.5 for total form and pH 5.5 for lactone form) and UV detection. The presented assay was linear over a concentration range of 25–1500 ng/ml with lower limit of quantitation of 25 ng/ml for both 9NC-tot and 9NC-lac. Within-run and between-run precision was always less than 7.5% in the concentration range of interest. The reported assay method showed good characteristics of linearity, sensitivity, selectivity and precision allowing applying in pharmacokinetic studies.

Keywords: 9-Nitrocamptothecin; HPLC; Lactone; Total; Plasma

1. Introduction

9-Nitrocamptothecin (9-NC, RFS2000, Fig. 1) is a novel, lipophilic analogue of the natural plant alkaloid camptothecin, which has demonstrated high antitumor activity against advanced pancreatic carcinoma, ovarian epithelial cancer and leukemia [1–5].

Camptothecins are an important class of anti-cancer drugs that exert their antitumor activity by specifically inhibiting of DNA unwinding enzyme Topoisomerase I through binding the enzyme, stabilising the "cleavable complex" along the DNA replication fork which results in an accumulation of single and double strands DNA breaks and ultimately cell death [6–9].

Chemically, the analogues share the common features of a planner aromatic five-ring system with a lactone moiety that is required for optimal inhibition of Topoisomerase I activity but all of these drugs undergo a rapid, reversible, non enzymatic hydrolysis from closed lactone form to the inactive hydroxy carboxylated form with loss of pharmacologic activity [10–12]. Both the lactone/carboxylate ratio at equilibrium and the rate of conversion between the two forms is affected by the pH. In acidic medium (pH <4) the lactone structure predominants, while at alkaline pHs, including physiological pH the formation of the carboxylate is favoured [13–15]. Fassberg and Stella [13] studied the kinetic and mechanism of the hydrolysis of camptothecin and some analogues in

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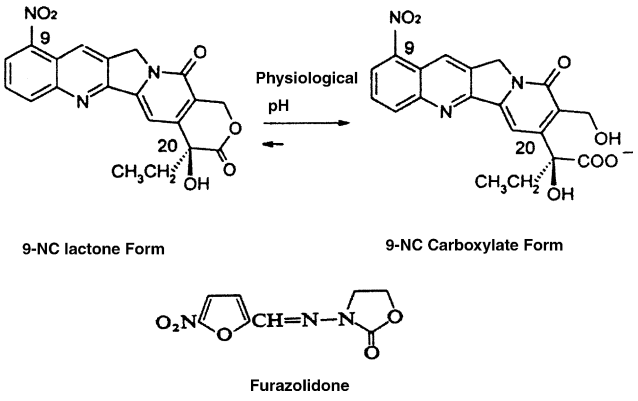

Fig. 1. Chemical structures and equilibrium reaction between the lactone and carboxylate forms 9-NC and chemical structure of furazolidone (I.S.).

aqueous solutions with different pHs. The results showed that at pH values ≤4 camptothecins were exclusively in lactone form and at pH values ≥8, they were exclusively in carboxylate form and the conversion was very fast in both directions [13].

Since an intact lactone form of camptothecins is of vital importance for the biological activity, several high-performance liquid chromatography (HPLC) methods have been developed for the analysis of lactone and total (lactone plus carboxylate) forms of camptothecin derivatives [16–23].

A few methods, which have been reported for the determination of 9-NC in plasma, analyze the drug only as the total (lactone plus carboxylate) form [24,25]. Since lactone moiety is the active form of this anticancer agent, it is important to design a sensitive analysis method for measurement of both lactone (9NC-lac) and total (9NC-tot) forms in plasma.

We therefore developed a sensitive and specific reversed-phase HPLC method with UV detection for the analysis of 9-NC as the lactone and total forms in human plasma.

2. Experimental

2.1. Chemicals and reagents

9-NC, 99.8% pure, was purchased from Yuanjian Pharmaceutical Technology Develop Co. (China). Furazolidone as the internal standard was obtained from Fluka.

HPLC grade acetonitril and the analytical grade dimethyl sulfoxide (DMSO), chloroform and perchloric acid (70%, w/v) were purchased from Merck (Darmstadt. Germany).

Drug-free human plasma was obtained from the Central Laboratory of the Blood Transfusion Service (Tehran, Iran).

2.2. Instruments

The HPLC system consisted of a model 1001 solvent delivery system and a model 2700 UV detector, equipped with Millennium chromatography manager for integration (all from Knauer, Germany).

Separations were achieved on a Nucleosil-100 C_8 column (250 mm × 4 mm i.d., 5 μm particle size) that protected by a Nucleosil-100 C_8 Encapped guard (4 mm × 4 mm i.d., 5 μm particle size) obtained from Knauer (Germany).

2.3. Chromatographic conditions

The mobile phase was a mixture of acetonitril–water (43:57, v/v) with the pH adjusted to 5.5 and 3.5 with perchloric acid (5%, v/v) for the assay of 9NC-lac and 9NC-tot, respectively.

The mobile phase was degassed by ultrasonication and delivered at a flow rate of 1.3 ml/min for both assays. The ultraviolet detector was set at wavelength of 370 nm.

2.4. Stock solutions and standards

9-Nitrocamptothecin stock solution (100 μg/ml) was prepared by dissolving the appropriate amount of the drug in DMSO and stored at −20 °C prior to further dilutions.

Working solutions were prepared daily at concentrations of 0.5, 1, 2, 5, 10, 20, 30 μg/ml by serial dilutions of stock solution with acetonitril and were stored at −20 °C prior to use.

Spiked plasma samples used as calibration standards were prepared daily by addition of appropriate amounts of the working solutions to drug-free human plasma, resulting in calibration standards of 25, 50, 100, 250, 500, 1000, 1500 ng/ml of 9-NC. In the case of the lactone form (9NC-lac) the prepared calibration standards were used immediately after preparation.

The internal standard solution was prepared by dissolving 20 mg of furazolidone in 100 ml acetonitril to give a final concentration of 200 μg/ml. This solution was diluted further by acetonitril yielding a final concentration of 40 μg/ml and stored at 2–8 °C prior to apply.

2.5. Sample preparation for the lactone form of 9-NC

To 250 μl of human plasma in a 2.0 ml polypropylene eppendorf cup a volume of 10 μl of I.S. solution in acetonitril (40 μg/ml), 500 μl of cold acetonitril (−20 °C) and 100 μl of cold chloroform were added. The mixture was vortex-mixed for 30 s and subsequently was centrifuged for 3 min at 14,000 rpm at ambient temperature. The supernatant was collected in a glass tube and evaporated at 40 °C under a gentle stream of nitrogen gas, until a completely dried residue was left over. One hundred and twenty five microliter of the mobile phase (prepared for the assay of lactone form) was added to the residue and after vortex mixing for 30 s a volume of 100 μl of the solution was injected into the HPLC system.

2.6. Sample preparation for total 9-NC

To 250 μl of human plasma a volume of 20 μl of IS solution in acetonitril (40 μg/ml), 500 μl of cold acetoni-