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Stereospecific high-performance liquid chromatographic analysis of naringenin in urine

Jaime A. Yáñez, Neal M. Davies *

College of Pharmacy, Department of Pharmaceutical Sciences and Pharmacology and Toxicology Graduate Program, Washington State University, Pullman, WA 99164 6534, USA

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Abstract

A method of analysis of naringenin [(+/−)-4′,5,7-trihydroxyflavanone] in biological fluids is necessary to study the kinetics of in vitro and in vivo metabolism, tissue distribution in fruits and humans. A simple high-performance liquid chromatographic method was developed for simultaneous determination of naringenin enantiomers in rat and human urine. Urine (0.1 ml) was precipitated with cold acetonitrile after addition of the internal standard, daidzein. Separation was achieved on a Chiralcel OD-RH column with UV detection at 292 nm. The calibration curves were linear ranging from 0.5 to 100 μg/ml for each enantiomer. The mean extraction efficiency was >99%. Precision of the assay was <9.4% (CV), and was within 5.4% at the limit of quantitation (0.5 μg/ml). Bias of the assay was lower than 16%, and was within 15% at the limit of quantitation. The assay was applied successfully to the urinary excretion of naringenin in rats and humans.

Keywords: Reversed-phase HPLC; UV-detection; Stereospecific; Enantiomer; Flavonoid

1. Introduction

Naringin [(+/−)-4′,5,7-trihydroxyflavanone 7-rhamnoglucoside] (Fig. 1a) is a chiral flavanone-7-*O*-glycoside present in citrus fruits, tomatoes, cherries, oregano, beans, and cocoa [1–6]. After consumption, the neohesperidose sugar moiety is rapidly cleaved off the parent compound in the gastrointestinal tract and liver to leave the aglycone bioflavonoid naringenin [(+/−)-4′,5,7-trihydroxyflavanone] (Fig. 1b). The ratio between the amount of naringenin and naringin varies among different food products. For instance, citrus fruits contain higher amounts of the glycoside naringin, while tomatoes have higher amounts of the aglycone naringenin [3]. The proposed metabolism of naringenin in the gastrointestinal tract and liver is presented in Fig. 1.

Naringenin has been previously quantified utilizing a variety of methods including high-performance liquid chromatography with UV and photodiode-array detection [7–12], liquid chromatography coupled with mass spectrometry [13–16], gas chromatography coupled with mass spectrometry [17]. All of these methods have overlooked the fact that naringenin is a chiral compound. There are, however, a couple of reports demonstrating that micellar electrokinetic chromatography [18], and multidimensional liquid chromatography coupled with mass spectroscopy [15] can separate naringenin enantiomers. However, baseline resolution and separation was not evident [18], and quantification was not validated in biological matrices [15,18]. There is also a recent report showing the separation of the (−)-naringenin enantiomer using circular dichroism [19], however, the separated enantiomers were not applied to biological systems. There was a report by Geiser et al. in the Pittcon 2000 that a Chiralpak AD-RH under supercritical fluid chromatography (SFC) could separate the enantiomers of naringenin. In our laboratory using a Chiralpak AD-RH column with HPLC we failed to demonstrate baseline resolution for the analysis of naringenin in biological matrices.

Interestingly, the results from various scientific and epidemiological studies have suggested that tomato consump-

* Corresponding author. Tel.: +1 509 335 4754; fax: +1 509 335 5902.
E-mail address: ndavies@wsu.edu (N.M. Davies).

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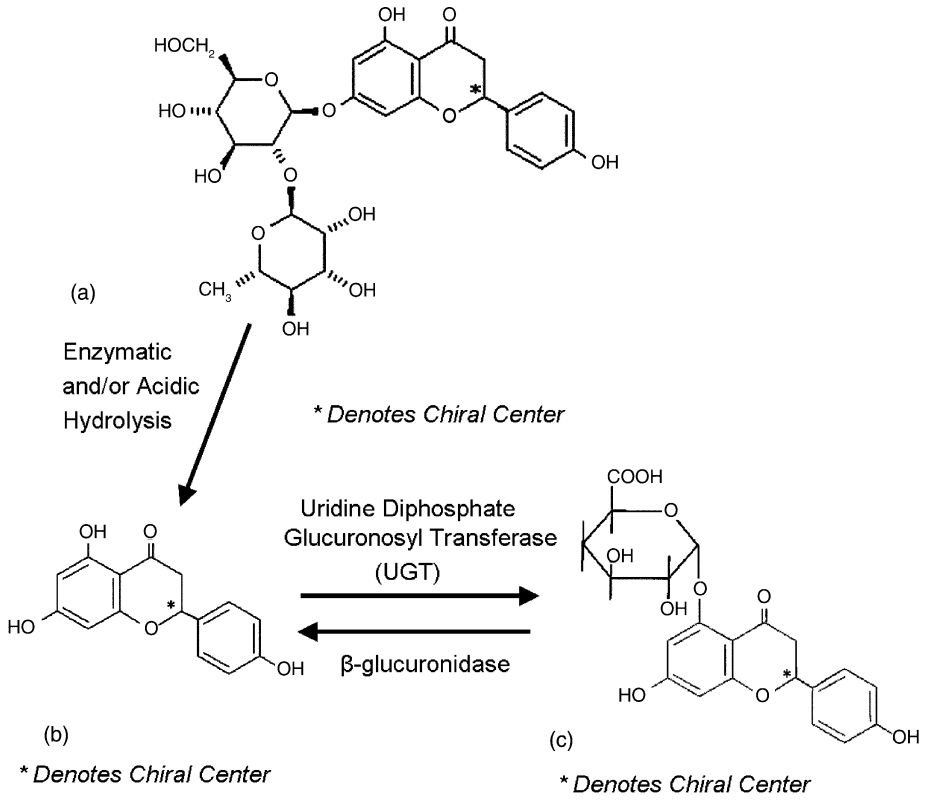

Fig. 1. Naringin metabolism. Structure of naringin (a), structure of naringenin (b), and structure of 5-*O*-naringenin glucoronidate (c). Asterisk (*) denotes Chiral Centre.

tion may prevent some chronic degenerative diseases, and have demonstrated that tomato and tomato-based product consumption may reduce the risk of different types of cancers [20]. Tomatoes are considered one of the most important sources of lycopene and it is generally thought that their potential anti-cancer properties are attributable to lycopene's antioxidant activity [20,21]. Although, plant phenols may contribute to the health protection ascribed to fruit and vegetable consumption, no studies of disposition of the enantiomers of naringenin in tomatoes or after tomato-based product intake have been published. Furthermore, a recent study showed that the co-administration of polyphenols enhances the antioxidants properties of lycopene. Therefore, it is possible that the tomato benefits could be attributed to a positive synergistic action between lycopene and other bioavailable tomato constituents, such as naringenin, rather than only lycopene [20]. Based on this, it is also possible that the tomato benefits could be attributed to one or both of the enantiomers of naringenin.

To our knowledge, no study has been published characterizing the separation of naringenin enantiomers in pharmacokinetic studies as there are no validated direct methods of stereospecific analysis of naringenin in the literature. The Chiralcel OD-RH column is a commercially available column, which can be utilized in the reverse phase. The present study describes a simple stereoselective, isocratic, reversed-phase high-performance liquid chromatography (HPLC) method for the determination of the enantiomers of naringenin and its application to in vivo kinetic studies.

2. Experimental

2.1. Chemicals and reagents

Racemic naringin, naringenin, daidzein, β-glucuronidase Type IX A and *H. pomatia* type-HP-2 were purchased from Sigma Chemicals (St. Louis, MO, USA). HPLC grade acetonitrile and water were purchased from J.T. Baker (Phillipsburg, NJ, USA). Phosphoric acid was from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA). Campbell's Tomato Juice® was purchased from a local grocery. Rats were obtained from Charles River Laboratories. Ethics approval for animal experiments was obtained from Washington State University. Human experiments were conducted with written informed consent according to the principles of the Declaration of Helsinki.

2.2. Chromatographic system and conditions

The HPLC system used was a Shimadzu HPLC (Kyoto, Japan), consisting of an LC-10AT VP pump, a SIL-10AF auto