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Determination of bergamottin in human plasma after grapefruit juice ingestion by an UPLC/MS/MS method

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Bergamottin was identified as a cause of pharmacokinetic interaction with grapefruit juice intake and as a physiologically active substance involved in lipolysis. However, the quantification method on concentrations of bergamottin in systemic circulation has not been well established. The aim of this study was to develop a simple, sensitive and high-throughput determination system for bergamottin in human plasma using an ultra performance liquid chromatography (UPLC)-MS-MS method. The UPLC system equipped with a UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm) and an ESI prove was appropriate for detection of bergamottin. As a result, a primary product ion (m/z = 203) from precursor ion of bergamottin (m/z = 339) was observed. Plasma from a human volunteer who consumed grapefruit juice one hour before the time of blood sampling, was measured with the UPLC/MS/MS system. The determination of plasma-bergamottin was performed with the highest sensitivity presently available. In conclusion, we succeeded high performance bergamottin-determination in human plasma after grapefruit juice ingestion. The procedure can be useful to clarify pharmacokinetic and pharmacodynamic characteristics of bergamottin.

1. Introduction

Concomitant intake of grapefruit juice causes significant elevation of bioavailabilities of various drugs such as felodipine (Bailey 1991), lovastatin (Kantola 1998) and carbamazepine (Garg 1998). This fact is recognized as one of the most important pharmacokinetic interactions between drugs and food in clinical practice. Grapefruit components, including bergamottin, have inhibitory effects on intestinal CYP3A and P-glycoprotein activities related to the first pass metabolism of drugs (He 1998; Wang 2001). It was also reported that bergamottin induced CYP3A and CYP1A1/2 proteins and mRNAs (He et al. 1998; Wang et al. 2001). These findings confirm the findings of our study in which long-term ingestion of grapefruit juice induced hepatic cytochrome P450 in rats (Mohri 2000). Furanocoumarin derivatives are also involved in lipolysis and hypolipidemia (Kimura 1982; Yasuda 1984). Bergamottin has been marketed in Europe and the United States in weight-reducing supplements. It has been reported that bergamottin is a potent inhibitor of tumor initiation (Cai 1997). Pharmacokinetic and pharmacodynamic knowledge of bergamottin after consumption of grapefruit juice and the supplements including bergamottin are useful to understand the biological activities of these substances. We attempted to establish a quantitative determination system of the plasma bergamottin level using high-throughput, highly sensitive and high-resolution UPLC/MS/MS method.

2. Investigations, results and discussion

In the UPLC/MS/MS detection on 100 nM bergamottin standard solution in acetonitrile, a product ion scan was performed of the precursor ion (m/z 339), over a mass range of 100 to 350 m/z (Fig. 1). The fragmentation pattern was compared with the predicted structure. Major product ion detected in m/z of 203 was estimated to be the bergaptol residue with a proton. Retention time of bergamottin was 2.1 min. The coefficients of variation of the peak areas were 8.2, 1.8, and 2.1% at 0.03, 3, and 30 nM, respectively (n = 6).

Figure 2 shows the results for bergamottin in human plasma using the product ion. No interference peak around the appearance time of the bergamottin peak was observed in the plasma sample. The bergamottin concentration in plasma was obtained from the calibration curves for standard bergamottin concentrations (0.1–100 nM). Each test was performed in triplicate. Calibration curves showed linearity [calibration curve: bergamottin (nM) = 0.0433 × peak area + 0.374, r = 1.000]. Detection limit of the peak was estimated to be 0.025 nM (s/n = 3) of plasma bergamottin. The sensitivity of the present method is extraordinarily higher than that of the reported detection procedure using HPLC/PDA (detection limit: 300 nM) (Uesawa and Mohri, 2005) and HPLC/MS/MS (quantification limit: 0.5 nM) (Goosen 2004). Furthermore, bergamottin concentration in plasma of a human volunteer administered grapefruit juice was estimated using the UPLC system. As a result, an ob-

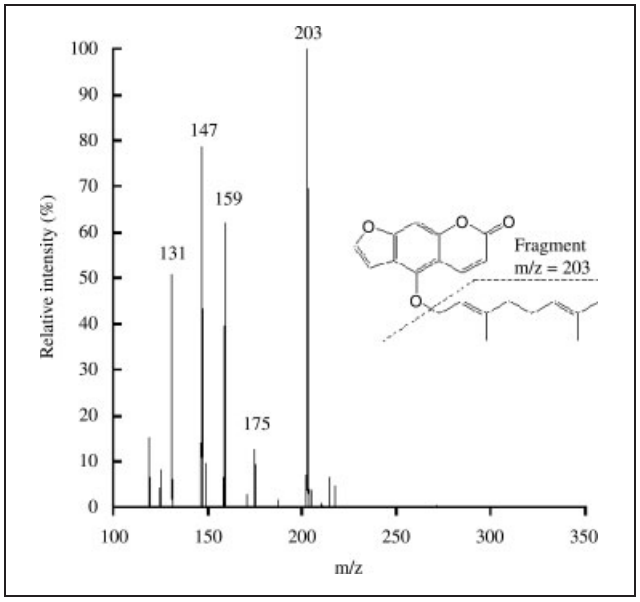

Fig. 1: Mass spectrum of bergamottin runs from 100–350 m/z in the positive ion mode; the spectra was derived from a product ion scan at the bergamottin mass (m/z 339). Peak corresponded to bergaptol (m/z 203)

vious peak was observed at the same retention time (Fig. 2E). The concentration of plasma bergamottin was determined as 2.31 ± 0.26 nM (mean $\pm$ S.D., in triplicate). This result indicates that the UPLC system developed in the present study is applicable to pharmacokinetic studies of bergamottin because of the high sensitivity with 0.025 nM of detection limit.

In conclusion, we succeeded with the method described to determine plasma bergamottin levels in a human volunteer who consumed one glass of grapefruit juice. The UPLC/MS/MS system shown the highest sensitivity of the methods known for bergamottin determination. The procedure established in the present study can be useful to demonstrate the pharmacokinetics and physiological activities of bergamottin.

3. Experimental

3.1. Materials

Bergamottin and formic acid were purchased from Daiichi Pure Chemicals Co. Ltd. (Tokyo, Japan) and Wako Pure Chemical Industries Ltd. (Osaka, Japan), respectively. Grapefruit juice was obtained from Pokka Corporation Ltd. (Aichi, Japan). The detection of bergamottin in grapefruit juice was performed according to a previously described method (Uesawa and Mohri 2005); including 29.1 μM of bergamottin in the juice. Acetonitrile and water HPLC grade were used (Kanto Chemical Co. Inc., Tokyo, Japan).

3.2. UPLC/MS/MS conditions

The ultra performance liquid chromatography (UPLC) system (Waters Acquity UPLC, Waters Corporation, Milford, MA, USA) was equipped with a Tandem mass spectrometer (Waters Quattro micro API, Waters) and a reverse-phase analytical Waters UPLC BEH C18 column [2.1 mm (inside diameter) × 50 mm; particle size 1.7 μm (Waters)]. Column temperature was set at 40 °C. A mobile phase consisting of 0.1% of formic acid aqueous solution (buffer A)/0.1% of formic acid acetonitrile solution (buffer B) was pumped through the column at a speed of 0.3 mL/min with a gradient of 0 to 1.5 min, from 30–95% (v/v) of buffer B and subsequently 95% (v/v) of buffer B in 1.5 min. The bergamottin peak from the UPLC column was analyzed using a multiple reaction monitoring (MRM) method in positive ion mode. The $[M + H]^+$ ion was recorded (m/z 339) at a cone voltage of 20 V and collision energy of 12 eV with cone and desolvation gas set at 800 L/h (at 350 °C) and 50 L/h, respectively. The ion source heater was set at 120 °C.

3.3. Human plasma sampling and sample preparation

A Japanese male volunteer (43 years old) gave written informed consent before entering the study as approved by the Ethics Committee for Human Research of Meiji Pharmaceutical University. The subject received 200 mL grapefruit juice. Blood samples were taken before and one hour after grapefruit juice administration. Blood samples were immediately centrifuged at 16,000 × g for 15 min and 4 °C. The plasma was then separated. Plasma samples including bergamottin for calibration was prepared with the subject's plasma before grapefruit juice administration. At this point 180 μL of acetonitrile was added to 20 μL of the plasma in a 2 mL plastic tube. After being shaken vigorously, the sample was centrifuged at 16,000 × g and 4 °C for 10 min; 5 μL of the supernatant was injected directly into the UPLC system. Calibration curves (0.1 to 100 nM of bergamottin in plasma) were drawn by linear least-squares regression analysis.

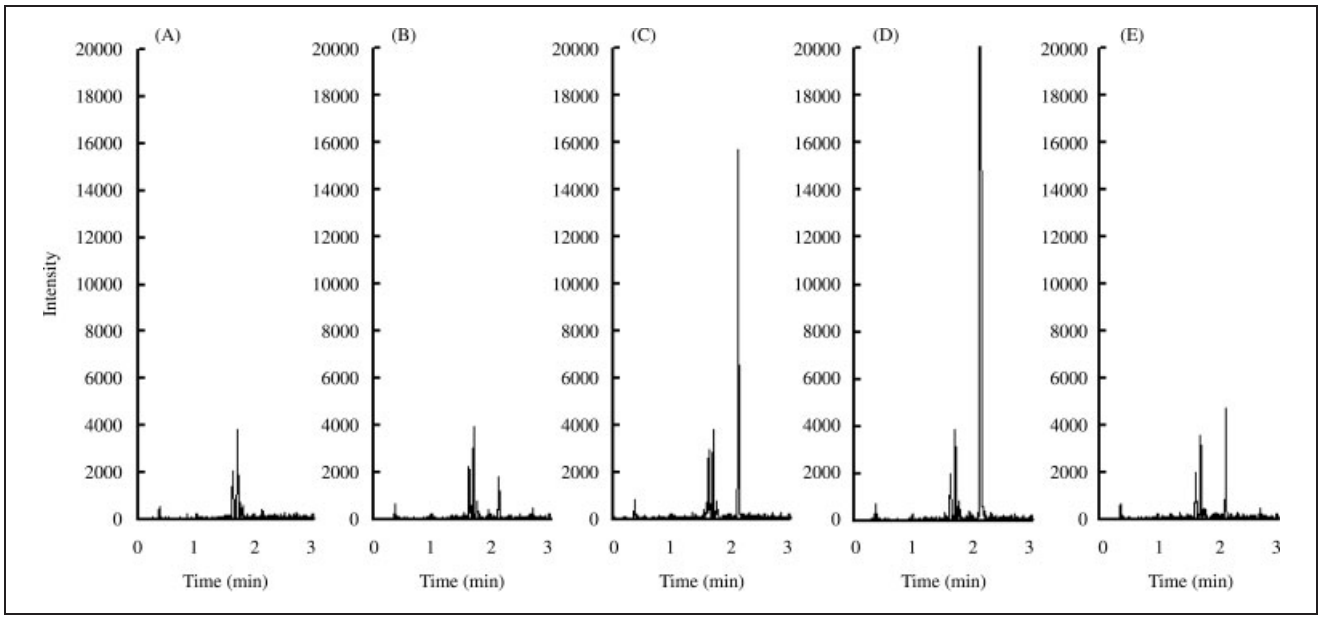

Fig. 2: UPLC/MS/MS chromatograms of bergamottin extracts from human plasma samples are shown; control plasma (A), plasma containing 1, 10, and 100 nM bergamottin (B, C, and D, respectively), and plasma after administration of grapefruit juice (E). Acetonitrile (180 μL) was added to 20 μL of the plasma; 5 μL of the supernatant was injected directly into the UPLC system

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