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HPLC separation of naringin, neohesperidin and their C-2 epimers in commercial samples and herbal medicines

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Abstract

Flavanone glycosides, such as naringin and neohesperidin, are distributed in some *Citrus* species and have a chiral center in the C-2 position of the flavanone moiety. Naringin and neohesperidin (2*S*-form) were separated from the corresponding C-2 epimers (2*R*-*epi*-form) by normal-phase HPLC using a polysaccaride-derived chiral stationary phases (CSPs), CHIRALPAK® IB. The analyses of commercial samples of naringin revealed that the relative ratios of naringin to the C-2 epimer were 29–89%. In the case of a commercial sample of neohesperidin, the relative ratio of the neohesperidin (2*S*-form) is 84%.

The HPLC application to *Citrus* species used as crude drugs in Japan (Kijitsu, Kikoku and Tohi) showed that the relative ratios of naringin to the C-2 epimer were 75–93% in Kijitsu, 74–79% in Kikoku and 54–64% in Tohi. However, there is a quite small ratio of the (2*R*)-*epi*-neohesperidin in *Citrus*. This result suggested that the averages of relative ratio of (2*S*)-naringin in *Citrus* species reduced according to the maturity of fruits (Kijitsu < Kikoku < Tohi). Since the relative ratios of (2*S*)-naringin of dry extracts of 5 Kampo formulations (including Kijitsu or Kikoku) decreased to 42–54%, the conversion from naringin to the (2*R*)-epimer might be enhanced during the decoction process of the formulations.

Keywords: Naringin; Neohesperidin; Diastereomeric separation; Chiral stationary phase; *Citrus* species; Kampo formulations

1. Introduction

Flavanone glycosides are distributed in some *Citrus* species, and they exist as diastereomers (epimers) due to a chiral center in the C-2 position of the flavanone moiety. Naringin and neohesperidin (2*S*-form, Fig. 1) are contained as the main flavanone 7-*O*-glycosides in sour or bitter oranges (*Citrus* species) [1–3]. In Japanese Pharmacopoeia (JP), the well-dried immature fruits of *Citrus aurantium* L. var. *daidai* Makino, *C. aurantium* L. and *C. natsudaidai* Hayata are used as a crude drugs, AURANTII FRUCTUS IMMATURUS (Japanese names: Kijitsu or Kikoku), and the well-dried peels of *C. aurantium* L. and *C. aurantium* L. var. *daidai* Makino are used as a crude drug, AURANTII PERICARPIUM (Japanese name: Tohi). The crude drugs (AURANTII FRUCTUS IMMATURUS and AURANTII PERICARPIUM) are used as components in Kampo formulae and their quality are controlled by JP [4]. In the course of our continuous studies to improve the monographs of crude drugs in JP, we are progressing to establish the quantitative HPLC analysis of crude drugs. Naringin and neohesperidin were reported to have effects, such as the suppression of infection-induced endotoxin shock in mice [5], anti-allergic activity [6] and central nervous system depressant action [7]. Since diastereomers of flavanone glycosides differ from each other in biological activities [7,8], it is important to selectively quantity the C-2 diastereomers of naringin and neohesperidin by HPLC to ensure the quality of these crude drugs and formulae.

The diastereomeric separation of five flavanone glycosides (naringin, neohesperidin, hesperidin, narirutin and prunin) was reported by using a reversed-phase (RP) gradient HPLC on a β-cyclodextrin-bonded stationary phase, but those diastereomers were not well separated each other [2]. Krause and Galensa reported the diastereomeric separation of naringin with normal-phase gradient HPLC using a cellulose diol stationary phase, but this system did not provide complete separation between the diastereomers [9]. Aturki et al. reported

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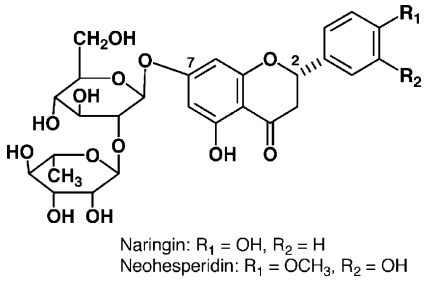

Fig. 1. Chemical structures of flavanone 7-*O*-glycosides.

good diastereomeric separation of four flavanone glycosides (naringin, hesperidin, narirutin and eriocitrin), but they used non-simple two-dimensional HPLC with a RP column and β-cyclodextrin-bonded column [10]. Caccamese et al. also reported that the normal-phase HPLC, using a cellulose-type CSP, CHIRALCEL® OD, provide good diastereomeric separation of naringin, although other flavanone 7-*O*-glycoside were not separated well [3].

Recently, our paper revealed that hesperidin, narirutin and neohesperidin, flavanone 7-*O*-glycosides (2*S*-form) were well separated from the corresponding C-2 epimers (2*R*-*epi*-form) by simple normal-phase HPLC with amylose-type chiral stationary phases (CSPs), CHIRALPAK® IA (amylose tris-3,5-dimethylphenylcarbamate immobilized on a 5 μm silica gel), using an online coupling CD detector [11]. This system was also applied to *Citrus* extracts and Kampo formulations and the results suggested that hesperidin was partly converted to the C-2 epimer during the decoction of Kampo formulae, although naturally occurring hesperidin in *Citrus* species has the 2*S* configuration [12]. However, this system provided poor separation of naringin and its C-2 epimer [11].

In this study, we performed the simultaneous diastereomeric separation of naringin and neohesperidin by normal-phase HPLC with a cellulose-type CPS, CHIRALPAK® IB (cellulose tris-3,5-dimethylphenylcarbamate immobilized on a 5 μm silica gel). In addition, its application to *Citrus* extracts and Kampo formulations was also described.

2. Experimental

2.1. Apparatus

A JASCO Gulliver Series consisting of two PU-980 pumps, an AS-950 autosampler, a CO-970 column oven and a UV-970 spectrophotometer operating at 284 nm were used for HPLC

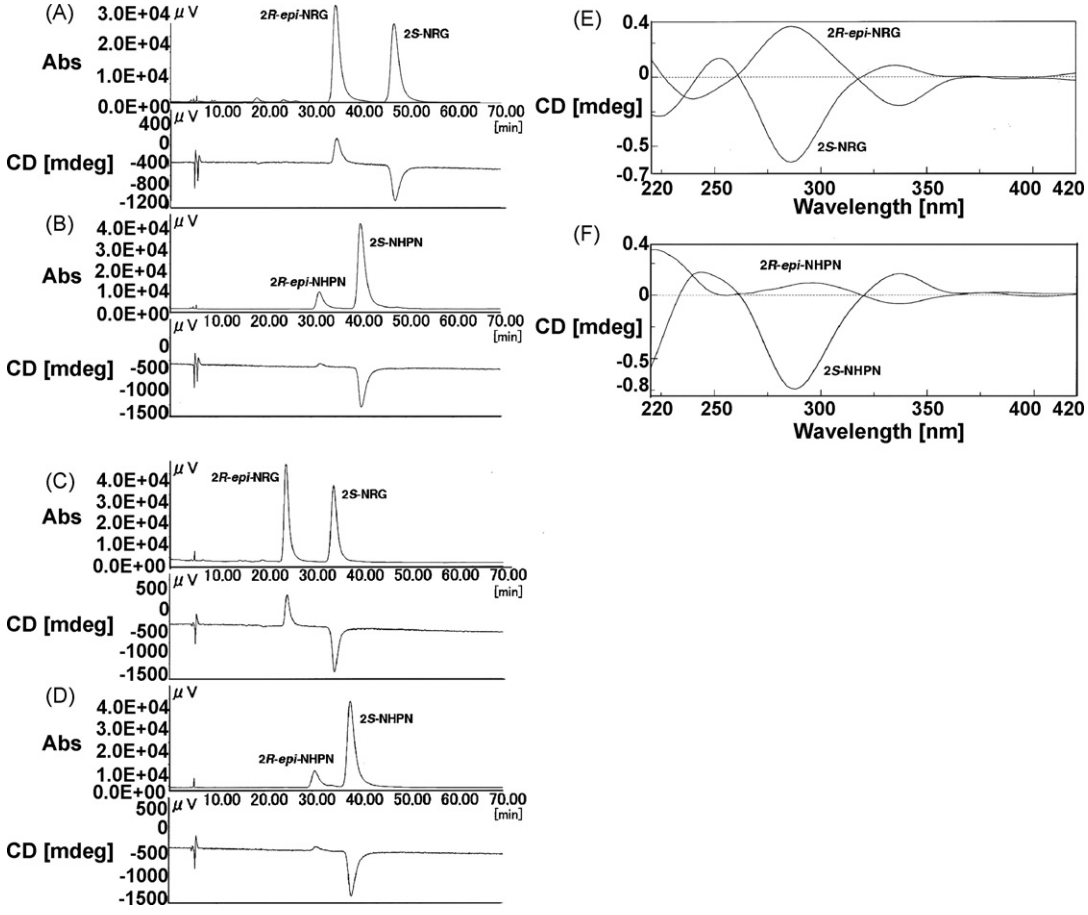

Fig. 2. HPLC separation of flavanone-7-*O*-glycoside C-2 diastereomers in commercial samples. The compounds of (2*S*)- and (2*R*)-*epi*-naringin (NRG) (A and C) and (2*S*)- and (2*R*)-*epi*-neohesperidin (NHPN) (B and D) were analyzed under two mobile phases. *Conditions*: Column, Chiralpak IB; mobile phases, *n*-hexane:$CHCl_3$:ethanol doped with 0.15%TFA (63:21:16, v/v/v) (A and B) and *n*-hexane:2-propanol:ethanol doped with 0.15%TFA (62:25:13, v/v/v) (C and D); flow rate, 0.7 ml/min. Upper and lower traces: UV and CD signal at 284 nm (A–D). Right traces: CD spectrum of the peaks at the stopped-flow mode. Data of (2*S*)- and (2*R*)-*epi*-naringin (NRG) (E) and (2*S*)- and (2*R*)-*epi*-neohesperidin (NHPN) (F) are shown.

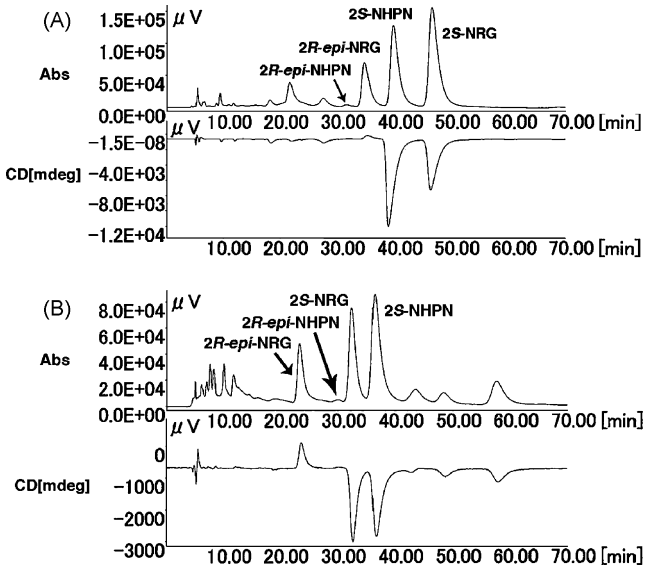

Fig. 3. HPLC separation of the MeOH extracts of (2*S*)- and (2*R*)-*epi*-naringin (NRG) and (2*S*)- and (2*R*)-*epi*-neohesperidin (NHPN) from Kijitsu (sample I, A) and Tohi (sample P, B). *Conditions*: Same as Fig. 2A and B (A) and Fig. 2C and D (B). Upper and lower traces: UV and CD signal at 284 nm.

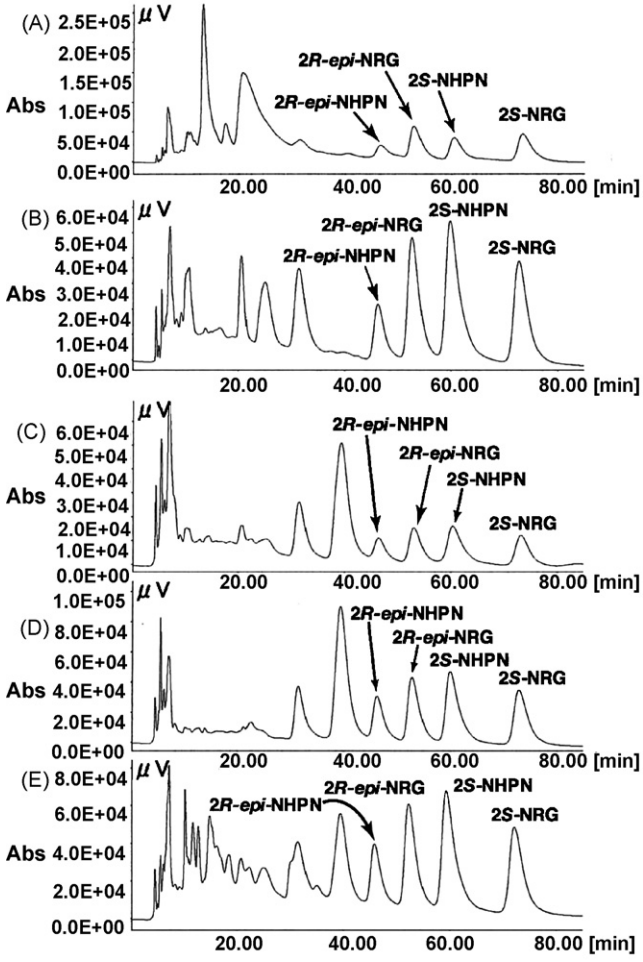

Fig. 4. HPLC separation of the MeOH extracts of (2*S*)- and (2*R*)-*epi*-naringin (NRG) and (2*S*)- and (2*R*)-*epi*-neohesperidin (NHPN) from Kampo formulations. Daisaikoto (sample A, A), Hainosankyuto (sample G, B), Gosekisan (sample L, C), Bukuryoin (sample Q, D) and Tudosan (sample S, E) are analyzed. *Conditions*: Column, Chiralpak IB; mobile phase, *n*-hexane:$CHCl_3$:ethanol doped with 0.13%TFA (67:19:14, v/v/v); flow rate, 0.7 ml/min; UV signal at 284 nm.

analyses. The peak area was calculated by using system analysis software, JASCO BORWIN (JASCO). Direct interfacing CD spectra were recorded on a JASCO CD-2095 spectropolarimeter. Separation were performed on a CHIRALPAK® IB [(cellulose tris-3,5-dimethylphenylcarbamate) column (250 mm × 4.6 mm) immobilized to 5 μm silica gel] which was purchased from Daicel Chemical Industries Ltd. (Tokyo, Japan). The mobile phases were *n*-hexane:$CHCl_3$:ethanol doped with 0.15% Trifluoroacetic acid (TFA) (63:21:16, v/v/v) (Figs. 2A, B and 3A), *n*-hexane:2-propanol:ethanol doped with 0.15%TFA (62:25:13, v/v/v) (Figs. 2C, D and 3B) and *n*-hexane:$CHCl_3$:ethanol doped with 0.13%TFA (67:19:14, v/v/v) (Fig. 4A–E). The flow rate was at 0.7 ml/min and the column temperature was 40 °C.

2.2. Chemicals

TFA was purchased from Wako Pure Ind. Ltd. (Osaka, Japan). All solvents were HPLC grade and purchased from Wako Pure Ind. Ltd. and Nacalai Tesque Inc. (Kyoto, Japan). Commercial naringin samples were purchased from Sigma (St. Louis, MO, USA, Lot No. 073K1006, sample A), Acros Organics (Geel, Belgium, Lot No. A018664101, sample B), Wako Pure Ind. Ltd. (Lot No. CEE0231, sample C), Kanto Chemical Co., Inc. (Tokyo, Japan, Lot No. 606F1259, sample D), Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan, Lot No. GK01, sample E), Funakoshi (Tokyo, Japan, Lot Nos. 04010607 and 06022807, sample F and I), Sigma and Aldrich (St. Louis, MO, USA, Lot No. 01721DD, sample G), Nacalai Tesque Inc. (Lot No. 14221-234, sample H). Neohesperidin was purchased from Funakoshi (Lot No. 04080306).

2.3. Sample preparation

Samples of naringin and neohesperidin were diluted in MeOH to a concentration of 0.5 mg/ml. Samples of AURANTII FRUCTUS IMMATURUS (Japanese name: Kijitsu or Kikoku, which are the well-dried immature fruits of *C. aurantium* L. var. *daidai* Makino, *C. aurantium* L. and *C. natsudaidai* Hayata) and AURANTII PERICARPIUM (Japanese name: Tohi, which is the well-dried peels of *C. aurantium* L. and *C. aurantium* L. var. *daidai* Makino) were obtained from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan), Uchida Wakanyaku Co., Ltd. (Tokyo, Japan), Mitsuboshi Pharmaceutical Co., Ltd. (Nara, Japan), Fukuda Ryu Co., Ltd. (Osaka, Japan) and Matsuura Yakugyo Co., Ltd. (Nagoya, Japan). Those crude drugs were cut into small pieces and a 100 mg aliquot of the crude drug was extracted by sonication for 15 min with MeOH (4 ml) at room temperature. Then, the solution was centrifuged at 2200 × *g* for 10 min to collect the supernatant and it was adjusted to 4 ml by MeOH as the HPLC sample solution.

The dry extracts for the commercial Kampo formulations, Daisaikoto, Hainosankyuto, Gosekisan, Bukuryoin and Tudosan formulations, were kindly provided by the Japan Kampo Medicine Manufacturers Association. These samples

were diluted with MeOH to a concentration of 25 mg/ml. Then, the aliquot (4 ml) of the solution was applied onto a sep-pak C-18 column and the MeOH eluate was used as the HPLC sample solution.

The Kijitsu sample K (3 g) was kept in boiling water (200 ml) for 15, 30, 45 and 60 min. Then, the aliquots (5 ml) of the solutions were evaporated and the residue was dissolved with 1 ml of MeOH as HPLC sample solutions.

All of the prepared HPLC sample solutions were filtered through a non-sterile 0.45 μm PTEE syringe filter, after which 5 μl of the commercial samples, 10 μl of the *Citrus* extracts and the boiled Kijitsu samples and 20 μl of the Kampo formulations were analyzed.

3. Results and discussion

3.1. Diastereomeric separation of commercial samples

Our previous HPLC analyses revealed that commercial flavanone glycoside samples contained C-2 diastereomers [11]. Thus, the commercial samples of naringin and neohesperidin were analyzed first by using CHIRALPAK® IB, which is a cellulose-type CSP under two different mobile phases. As shown in Fig. 2A–D, both commercial samples of naringin and neohesperidin were separated as two peaks under both mobile phases, and this suggested that those were diastereomeric mixtures. In addition, the online coupling of HPLC/CD provided for identification of the C-2 stereochemistry of the elution peaks. The second of the two peaks from the commercial naringin and neohesperidin samples are identified as (2*S*)-naringin and (2*S*)-neohesperidin, respectively, by their CD spectra (Fig. 2E and F) obtained by the stopped-flow mode, since the signs at 280–290 nm ($\pi \rightarrow \pi^*$ transition) and at 330–340 nm ($n \rightarrow \pi^*$ transition) are related to the absolute configuration by an extension of the Snatzke rule [12]. The relative ratios of flavanone glycoside C-2 diastereomers in the commercial samples are summarized in Table 1. It is revealed that the commercial naringin samples contained (2*S*)-naringin and the (2*R*)-epimer from the ratio of 89:11 to that of 29:71. In the case of a commercial neohesperidin sample, the relative ratio of the diastereomers is 84:16. The relative ratios of the (2*S*)-naringin and the (2*R*)-epimer in commercial samples varied greatly. Especially, relative ratios of (2*R*)-*epi*-naringin in commercial samples B, C and I were significantly high (69.3, 71.0 and 69.6%, respectively). As described in our previous paper [11], this phenomenon was also shown in the case of the commercial samples of hesperidin. Usually, commercial flavanone 7-*O*-glycosides were prepared by extraction of *Citrus* species with boiling water. But, the extraction procedure may be somewhat differ among the reagent companies. There is a possibility that *Citrus* species are extracted with a diluted aqueous alkaline to obtain flavanone 7-*O*-glycosides effeciently. In addition, flavanone 7-*O*-glycosides may be converted to the corresponding chalcones during the purification process because of the higher solubility of the chalcones. As a result, the conversion from the (2*S*)-forms to the (2*R*)-epimers of the commercial samples may increase during the extraction and purification process, comparing to the case of crude drug decoction with boiling water. Additionally, since the purity of commercial chemical reagents is generally checked by RP-HPLC, the reagent companies probably were not aware of the existence of the (2*R*)-epimer.

Table 1
Relative ratios of naringin and neohesperidin C-2 diastereomers in commercial samples

Sample	2*S* (%) : 2*R*-*epi* (%)
Naringin	
A	48.6 : 51.4
B	30.7 : 69.3
C	29.0 : 71.0
D	87.5 : 12.5
E	80.0 : 20.0
F	89.1 : 10.9
G	46.1 : 53.9
H	68.8 : 31.2
I	30.4 : 69.6
Neohesperidin	84.0 : 16.0

It is noted that hesperidin and narirutin, which have the different sugar moiety (rutinose) from that of naringin and neohesperidin (neohesperidose), are not separated from the corresponding C-2 epimers under this condition (data not shown), although the separations of these diastereomers have been successful on CHIRALPAK® IA as reported [11]. Polysaccaride-derived CSPs, such as phenylcarbamates of cellulose and amylose have been recognized as useful packing materials for the chromatographic separation of enantiomers and those CSPs are suitable for different applications. Okamoto and Yashima reported that the polar carbamate groups of the CSPs are preferably located inside, and hydrophobic aromatic groups of the CSPs are placed outside the polymer chain so that each solute may interact with the carbamate residues in the helical groove through hydrogen-bond formation, and amylose derivatives posses a wider and more compact helix [13]. Since sugar moieties of the flavanone glycosides can interact differently with the amylose and cellulose derivatives CSPs, selective separation may be conceivable.

3.2. Application to crude drugs, Citrus species

Next, we applied the online coupling of HPLC/CD to the analyses of the *Citrus* extracts. As shown in Fig. 3A, four constituents of naringin, neohesperidin and the corresponding C-2 epimers in Kijitsu and Kikoku extracts were simultaneously separated from each other under the same condition of those of the commercial samples as shown Fig. 2A and B. However, the application to Tohi extracts showed that the peaks of naringin, neohesperidin and the corresponding C-2 epimers were not completely separated. After examining the mobile phase, the four peaks of Tohi extracts were completely separated by switching from *n*-hexane/$CHCl_3$/ethanol to *n*-hexane/2-propanol/ethanol as mobile phase (Figs. 2C, D and 3B). Since CHIRALPAK® IB can be compatible with the various solvents, this CSP was considered useful for the analysis of actual samples.

The relative ratios of the C-2 diastereomers of samples of crude drugs are summarized in Table 2. The Kijitsu and Kikoku

Table 2
Relative ratios of naringin and neohesperidin C-2 diastereomers in crude drugs

Sample	Crude drugs	Naringin 2*S* (%) : 2*R*-*epi* (%)	Neohesperidin 2*S* (%) : 2*R*-*epi* (%)
A	Kijitsu	79.3 : 20.7	98.1 : 1.9
B	Kijitsu	83.9 : 16.1	98.1 : 1.9
C	Kijitsu	88.4 : 11.6	99.1 : 0.9
D	Kijitsu	84.5 : 15.5	99.1 : 0.9
E	Kijitsu	87.3 : 12.7	99.3 : 0.7
F	Kijitsu	74.7 : 25.3	95.5 : 4.5
G	Kijitsu	79.1 : 20.9	98.2 : 1.8
H	Kijitsu	87.3 : 12.7	98.0 : 2.0
I	Kijitsu	74.7 : 25.3	98.4 : 1.6
J	Kijitsu	92.8 : 7.2	99.0 : 1.0
K	Kijitsu	86.1 : 13.9	98.9 : 1.1
L	Kikoku	73.9 : 26.1	98.6 : 1.4
M	Kikoku	77.3 : 22.7	98.7 : 1.3
N	Kikoku	78.6 : 21.4	98.7 : 1.3
O	Kikoku	77.4 : 22.6	98.4 : 1.6
P	Tohi	58.5 : 41.5	95.7 : 4.3
Q	Tohi	63.5 : 36.5	96.7 : 3.3
R	Tohi	56.5 : 43.5	93.9 : 6.1
S	Tohi	60.9 : 39.1	94.6 : 5.4
T	Tohi	63.2 : 36.8	95.5 : 4.5
U	Tohi	58.9 : 41.1	94.4 : 5.6
V	Tohi	55.2 : 44.8	94.3 : 5.7
W	Tohi	54.3 : 45.7	94.1 : 5.9
X	Tohi	61.6 : 38.4	95.1 : 4.9

extracts contained about 7–26% of the (2*R*)-*epi*-naringin, and the Tohi extracts contained about 37–46% of the (2*R*)-*epi*-naringin. Meanwhile (2*R*)-*epi*-neohesperidin existed as only 1–6% in both extracts. This suggested that naturally occurring neohesperidin in *Citrus* species has a *2S* configuration.

Caccamese et al. reported that the relative ratio of the C-2 diastereomers of naringin undergoes a marked change with increasing maturity of the grapefruit (*C. paradisi*). Although naringin has mostly the *2S* configuration in quite small grapefruits (diameter 1.5 cm), according to the maturity of fruits, the (2*R*)-epimer increases until the relative ratio of the naringin and the (2*R*)-epimer is about 57:43 [3]. The averages of the relative ratios of (2*R*)-*epi*-naringin in Kijitsu, Kikoku and Tohi were 16.5, 23.2 and 40.8%, respectively (Table 2). Since the relative ratios of (2*R*)-*epi*-naringin in *Citrus* species increased according to the maturity of fruits (Kijitsu < Kikoku < Tohi), this result supported the finding of Caccamese et al. [3]. Flavanone (2*S*-form) in *Citrus* species is biologically synthesized via cyclization of chalcone by the catalyzing of the enzyme chalcone isomerase (CHI). Moriguchi et al. reported that the transcript level of CHI was high in young active tissues, such as young fruitlets of *Citrus* species, decreasing and disappearing toward maturation of the fruits [14], it follows that there was a decrease in the supply of flavanone (2*S*-form). Meanwhile, flavanone (2*S*-form) stored in the fruit vesicles might be converted non-enzymatically to the 2*R*-epimer. Thus, considering that the relative ratio of 2*R*-*epi*-naringin increased according to the maturity of *Citrus* species, the phenomenon mentioned above may be conceivable.

It is known that flavanone glycosides are easily converted to the corresponding chalcones [15]. In our previous paper, it was suggested that hesperidin in commercial samples and *Citrus* species was partly converted to the (2*R*)-epimer under boiling condition [11]. Also, Krause and Galensa have reported that naringin is converted to the (2*R*)-epimer under heating condition in an aqueous methanolic solution, though neohesperidin is not converted to the (2*R*)-epimer under the same condition [2]. Thus, the conversion of naringin and neohesperidin to the (2*R*)-epimers were tested with boiling water by using the Kijitsu sample K in this study (Table 3). The relative amount of naringin decreases time-dependently and the final ratio of naringin to its (2*R*)-epimer was 51:49, however, neohesperidin changed much slower under the same condition and the final ratio of neohesperidin to its (2*R*)-epimer was 91:9.

Table 3
Conversions of relative ratios of naringin and neohesperidin C-2 diastereomers in Kijitsu sample K in boiling water

Time (min)	Naringin 2*S* (%) : 2*R*-*epi* (%)	Neohesperidin 2*S* (%) : 2*R*-*epi* (%)
0	86.1 : 13.9	98.9 : 1.1
15	55.2 : 44.8	97.7 : 2.3
30	51.7 : 48.3	95.5 : 4.5
45	51.7 : 48.3	91.4 : 8.6
60	50.7 : 49.3	91.4 : 8.6

As reported previously, the 4-hydroxy group at the B-ring of flavanones containing a blocked 7-hydroxy group, such as narirutin are known to be susceptible to racemize at the C-2 position, compared to the 4-methoxy group, such as hesperidin, because of the formation of the quinoyl group [12]. Thus, the fact that the relative ratio of (2*R*)-*epi*-naringin to naringin, which possesse the 4-hydroxy groups, is higher than that of (2*R*)-*epi*-neohesperidin to neohesperidin in the *Citrus* extracts (Table 2) is accountable. In addition, the results of some commercial samples of naringin, which showed high conversion ratios (Table 1) could be explained by the same reason.

3.3. Application to Kampo formulations

Daisaikoto, Hainosankyuto, Gosekisan, Bukuryoin and Tudosan, which are Kampo formulae, contain the crude drug, Kijitsu (or Kikoku), namely the well-dried immature fruits of the *Citrus* species as described before. Therefore, naringin was analyzed as the marker constituent of the crude drug in the Kampo formulae. The peaks of naringin, neohesperidin, and the corresponding C-2 epimers were not completely separated at the same condition as that for the crude drug because of the chromatogram complication by increased components. Therefore the mobile phase were a little modified to *n*-hexane:$CHCl_3$:ethanol doped with 0.13%TFA (67:19:14, v/v/v).

As shown in Fig. 4A–E, naringin is well separated from other peaks in the chromatograms of those formulations. The (2*R*)-*epi*-naringin is detected in all chromatograms. The relative ratios of (2*S*)-naringin and the (2*R*)-epimer are from 54:46 to 42:58, while the relative ratios of neohesperidin C-2 diastereomers are from 91:9 to 57:43 (Table 4). As mentioned in our previous paper, when we compare the relative ratio of (2*R*)-*epi*-flavanone glycosides in Kampo formulations (Table 4) to those in crude drugs, the former is higher than the latter (Table 2). The com-

Table 4
Relative ratios of naringin and neohesperidin C-2 diastereomers in Kampo formulations

Sample	Kampo formulations	Naringin 2*S* (%) : 2*R-epi* (%)	Neohesperidin 2*S* (%) : 2*R-epi* (%)
A	Daisaikoto	42.1 : 57.9	57.1 : 42.9
B	Daisaikoto	53.0 : 47.0	91.9 : 8.1
C	Daisaikoto	49.4 : 50.6	NS[a] : NS
D	Daisaikoto	48.1 : 51.9	NS : NS
E	Daisaikoto	47.7 : 52.3	NS : NS
F	Daisaikoto	48.3 : 51.7	NS : NS
G	Hainosankyuto	50.2 : 49.8	77.6 : 22.4
H	Hainosankyuto	49.9 : 50.1	80.7 : 19.3
I	Hainosankyuto	50.3 : 49.7	87.1 : 12.9
J	Hainosankyuto	49.5 : 50.5	87.7 : 12.3
K	Hainosankyuto	50.3 : 49.7	84.3 : 15.7
L	Gosekisan	52.4 : 47.6	64.7 : 35.3
M	Gosekisan	53.0 : 47.0	79.3 : 20.7
N	Gosekisan	52.5 : 47.5	NS : NS
O	Gosekisan	54.4 : 45.6	90.9 : 9.1
P	Gosekisan	52.0 : 48.0	90.9 : 9.1
Q	Bukuryoin	52.5 : 47.5	66.3 : 33.7
R	Bukuryoin	50.7 : 49.3	78.2 : 21.8
S	Tudosan	47.1 : 52.9	66.4 : 33.6

[a] Not separated completely.

mercial Kampo formulations are usually prepared by decocting and subsequently spray-drying. It is thought that naringin and neohesperidin might be partly converted to the epimers during the processes of the decoction and spray-drying of the formulae.

4. Conclusion

This paper reported the normal-phase HPLC analysis for the diastereomeric separation of narigin and neohesperidin from the corresponding C-2 epimers simultaneously using the cellulose derivative-based CSP, CHIRALPAK® IB and an online coupling CD detector. This method was applied to the diastereomeic separation of naringin and neohesperidin contained in commercial samples, crude drugs and Kampo formulations. The results suggested that considerable amounts of the (2*R*)-naringin are contained in each sample, comparing to that of (2*R*)-neohesperigin, and that the relative ratios of (2*R*)-*epi*-naringin in *Citrus* species is increased according to the maturity of fruits (Kijitsu < Kikoku < Tohi). Since diastereomers differ from each other in physico-chemical and biological activities, this HPLC analysis using polysaccaride-derived CSPs could be applied to the diastereomeric separation of other compounds, especially flavonoid glycosides.

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