Multi-Dimensional HPLC Analysis of Serine Containing Chiral Dipeptides in Japanese Traditional Amber Rice Vinegar

Nutchaya SEREEKITTIKUL1,2, Reiko KOGA1,3, Takeyuki AKITA1, Aogu FURUSHO1, Roland REISCHL4, Masashi MITA5, Akira FUJII6, Kazunori HASHIGUCHI6, Masanobu NAGANO6, Wolfgang LINDNER7, Kenji HAMASE*1

1Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
2Faculty of Pharmacy, Mahidol University, 447 Sri-Ayuthaya Road, Rajathevi, Bangkok 10400, Thailand
3Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan
4Department of Bioscience, University of Salzburg, Hellbrunnerstrasse 34, Salzburg 5020, Austria
5Shiseido Co., Ltd., 1-6-2 Higashi-shimbashi, Minato-ku, Tokyo 105-8310, Japan
6Sakamoto Kurozu, Inc., 21-15, Uenosono-cho, Kagoshima 890-0052, Japan
7Institute of Analytical Chemistry, University of Vienna, Waehringerstrasse 38, Vienna A-1090, Austria

Abstract
Several D-amino acids have been identified in higher animals including humans, and are now increasingly recognized as biologically active substances and/or biomarkers. Therefore, the presence of related compounds, such as dipeptides, containing D-amino acids in biological matrices is one of the recent topics of increasing interest. In the present study, on-line multi-dimensional HPLC systems combining a narrowbore reversed-phase column (KSAARP, 1.5 mm i.d. x 500 mm), a weak anion-exchange column (KSAAAX, 1.5 mm i.d. x 250 mm) and a Pirkle-type enantioselective column (KSAACSP-001S or R, 1.5 mm i.d. x 250 mm) have been designed for the chiral analysis of Ser-containing dipeptides in fermented products. As target analytes, Ser-Gly and Gly-Ser were selected. For the sensitive analysis, the dipeptides were pre-column derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) to form the NBD-dipeptides, which were detected upon their fluorescence. During the selective multi-dimensional analysis, the target dipeptides were separated from other substances by the reversed-phase and anion-exchange columns as their D/L scalemic mixtures and automatically introduced to the enantioselective column, where the D/L-forms were separated with selectivity / separation factor (α) and resolution (Rs) values higher than 1.07 and 0.71, respectively. The present 3D-HPLC system was well applicable to the analysis of Japanese traditional amber rice vinegar where relatively large amounts of the L-dipeptides and trace amount of D-Ser-Gly were observed.

Keywords: D-Amino acid; Dipeptide; Enantiomer separation; Black vinegar; Multi-dimensional HPLC

1. Introduction
Amino acids are vital components in all organisms, and all of the proteinogenic ones, except for glycine, have enantiomer (D- and L-forms). It had been believed for a long time that the amino acids in the higher animals are only the L-forms and that their D-forms are not present at least as functional molecules. However, along with the progression of analytical technologies, several D-amino acids can be found in mammals including humans and they are increasingly recognized as physiologically active...
substances and/or biomarkers [1-4]. Concerning D-Ser, it is currently well known as an endogenous co-agonist of the N-methyl-D-aspartate (NMDA)-type glutamate receptors that are essential for the synaptic transmission in the learning and memory pathways via long-term potentiation [5] and long-term depression [6]. D-Ser was also reported to be related with NMDA receptor-dependent pathological conditions [7,8], including schizophrenia [9], Alzheimer’s disease [10] and amyotrophic lateral sclerosis [11]. The progression of chronic kidney disease is also significantly associated with the levels of D-Ser in the plasma of patients [12].

Since some of the D-amino acids play significant roles in humans, the foods containing high levels of D-amino acids are thought to be valuable as their exogenous origin [1,13]. Based on the knowledge that various D-amino acids are produced by microorganisms [14-16], fermented products such as yogurt [17-19], cheese [20-22], alcoholic beverages [17,18,20,21,23-27] and vinegars [18,19,21,28,29] got investigated throughout the world. D-Ala, D-Asp and D-Glu are present in many fermented products [17-29] and their amounts were determined to be around 1 mM in soy sauces and around 5 mM in cheese [27], for instance. A relatively high concentration of D-Pro (higher than 0.5 mM besides 5 mM of L-Pro) was found in balsamic vinegars [28] and about 0.2 mM of D-Ser was detected in Japanese traditional black vinegars [29]. Considering the presence of high levels of D-amino acids in fermented products, dipeptides containing D-amino acids might also be present and the evaluation of their contents, especially those of the D-Ser containing dipeptides, is of interest.

The aim of the present study is to design a highly sensitive and selective method for the analysis of Ser-Gly and Gly-Ser (Gly is one of the most abundant amino acids) enantiomers and to evaluate their amounts in the fermented product. For the chiral analysis of amino acids and related compounds in biological matrices, various methods including CE-MS [30], CE-LIF [28], GC-MS [23,24,31], GC-FID [18,20,23], HPLC-FL [21,22,25-27], HPLC-MS/MS [17], 2D-GC [32] and 2D-HPLC [29,33-37] have been reported. Most of them use chiral derivatization by appropriate reagents and non-chiral RP columns [17,21,22,25-27] or enantioselective separations employing chiral stationary phases (columns) [18,20,23,24,29,31-37]. Among these methods, the 2D-HPLC method is one of the most powerful techniques to analyze trace amounts of the D-amino acids in real world samples. It was successfully applied to the determination of all the proteinogenic amino acids in the black vinegars [29] and also applicable to the determination of non-proteinogenic ones in various biological samples [35]. Considering that the amounts of D-amino acid-containing dipeptides might be extremely low, the highly sensitive and selective multi-dimensional HPLC might be a suitable and straightforward concept for the enantioselective analysis of chiral dipeptides in fermented products.

In the present study, on-line 2D and 3D-HPLC systems combining reversed-phase, anion exchange and enantioselective columns have been designed for the chiral analysis of Ser-Gly and Gly-Ser. These methods were used for the determination of the target chiral dipeptides in Japanese traditional amber rice vinegar (black vinegar), demonstrating that the designed/developed 3D-HPLC system is a powerful tool for the chiral metabolite analysis in complicated matrices like fermented products.

2. Experimental

2.1. Materials

D-Ser-Gly, L-Ser-Gly, Gly-D-Ser and Gly-L-Ser were products of Watanabe Chemical Industries (Hiroshima, Japan). 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Formic acid (FA), boric acid, trifluoroacetic acid (TFA) and methanol (MeOH) of HPLC grade were obtained from Wako Pure Chemical Industries (Osaka, Japan). Acetonitrile (MeCN) of HPLC grade was a product of Nacalai Tesque (Kyoto, Japan). Water was purified by a Milli-Q Integral 3 system (Merck Millipore, Darmstadt, Germany). The Japanese traditional amber rice vinegar was provided from Sakamoto Kurozu, Inc. (Kagoshima, Japan). All other reagents were of the highest reagent grade and were used without further purification.

2.2. Sample preparation

To the Japanese traditional amber rice vinegar, 100-fold volumes of 400 mM sodium borate buffer (pH 8.0) were added and 20 µL of the diluted solution was mixed with 5 µL of 40 mM NBD-F in MeCN. The mixture was heated at 60°C for 2 min, and an aqueous solution of 0.2% TFA (25 µL) was added. The reaction mixture (10 µL) was injected into the HPLC systems described in sections 2.3 and 2.4. The derivatization reaction of target dipeptides is shown in Fig. 1.

![Fig. 1. Fluorescence derivatization of Ser-Gly and Gly-Ser with NBD-F.](image)
2.3. 2D-HPLC system for the enantioselective analysis of Ser-Gly and Gly-Ser in Japanese traditional amber rice vinegar

The 2D-HPLC analysis of Ser-Gly and Gly-Ser was accomplished using a Nanospace SI-2 HPLC apparatus (Shiseido, Tokyo, Japan) with the EZChrom SI software. The first dimension of the 2D-HPLC system consisted of two pumps (3101), an auto sampler (3033), a column oven (3004) and a fluorescence detector (3213). A KSAARP column (1.5 mm i.d. x 500 mm, original C18 column produced by the collaboration with Shiseido) was used for the reversed-phase separation. The temperature of the column oven was set at 45ºC. In this dimension, the NBD-dipeptides were separated as D and L mixtures using an isocratic elution with aqueous 10% MeCN containing 0.05% TFA (75 µL/min). After the reversed-phase separation, the NBD-dipeptides were automatically fractionated and collected in a multi-loop valve. This valve was used as an injection valve to introduce the collected fraction to the second dimension of the 2D-HPLC system. A Pirkle-type enantioselective column (KSAACSP-001S, 1.5 mm i.d. x 250 mm, original column produced by the collaboration with Shiseido) was used for the chiral separation. In this dimension, the enantiomers of NBD-Ser-Gly and NBD-Gly-Ser were separated at 25ºC using an isocratic elution mode with 0.025% FA in the mixed solvents of MeOH/MeCN = 75/25 and MeOH/MeCN = 25/75, respectively. The flow rate of the mobile phase was 200 µL/min, and the detection of the NBD-dipeptides was carried out at 530 nm with excitation at 470 nm.

2.4. 3D-HPLC system for the enantioselective analysis of Ser-Gly and Gly-Ser in Japanese traditional amber rice vinegar

To analyze the Ser-Gly and Gly-Ser as NBD derivatives in a three-dimensional manner, a Nanospace SI-2 HPLC apparatus (Shiseido) was adopted, and the data processing was carried out by the EZChrom SI software. The present 3D-HPLC system was comprised of a degasser (3202), three gradient pump systems (3101 and 3201), an auto sampler (3023), two column ovens (3004 and 3014), two fraction collection multi-loop valves, and three fluorescence detectors (3213). The flow diagram is shown in Fig. 2. In the first dimension, the NBD-dipeptides were separated by the same conditions as those used in the 2D-HPLC system (described in Section 2.3). The fractions of the NBD-dipeptides were automatically collected and injected into the second dimension by appropriately switching of the multi-loop valve and high pressure valve (V1). In the second dimension, the NBD-dipeptides were separated again as their D and L mixtures by a non-chiral weak anion exchange column (KSAAAAX, 1.5 mm i.d. x 250 mm, original column made by the collaboration with Shiseido). The mobile phase was 0.025% FA in MeOH/MeCN = 75/25 at the flow rate of 150 µL/min. The column temperature was 10ºC (controlled by SSC-2120 column oven, Senshu Scientific, Tokyo, Japan). The NBD-dipeptides were then automatically collected again and injected into the third dimension by controlling the multi-loop valve (1,000 µL each) and the high pressure valve (V2). In the third dimension, the enantiomers of NBD-Ser-Gly and Gly-Ser were separated at 25ºC using the enantioselective column (KSAACSP-001S or KSAACSP-001R, 1.5 mm i.d. x 250 mm, original columns made by the collaboration with Shiseido). The mobile phase for NBD-Ser-Gly was 0.02% FA in MeOH/MeCN = 80/20, and that for NBD-Gly-Ser was 0.0375% FA in MeOH/MeCN = 25/75 (200 µL/min). The NBD-dipeptides were detected by their fluorescence at 530 nm with excitation at 470 nm.

3. Results and discussion

3.1. Reversed-phase separation and enantiomer separation of Ser-Gly and Gly-Ser as their NBD-derivatives

For the sensitive and selective determination of Ser-Gly and Gly-Ser, the dipeptides were pre-column derivatized with NBD-F and analyzed by the newly developed 2D and 3D-HPLC systems. Reversed-phase separation of the NBD-dipeptides in the first dimension and enantiomer separation in the final dimension are the essential steps, and
their separation conditions were investigated. In the first dimension of the multi-dimensional HPLC systems, the NBD-dipeptides were separated from other peptides, amino acids and interfering substances by their hydrophobicity differences using a reversed-phase column (KSAARP, 1.5 mm x 500 mm, an originally designed narrowbore-C18 column by the collaboration with Shiseido). In order to obtain a sufficient separation by the KSAARP column, the mixed solution containing Ser-Gly and Gly-Ser in addition to the amino acids, Ser and Gly was analyzed. The mobile phase conditions were investigated considering the MeCN and TFA concentrations. As a result, the NBD-dipeptides were nicely separated using the aqueous solution of 10% MeCN containing 0.05% TFA as the mobile phase within 50 min (NBD-Ser-Gly and NBD-Gly-Ser were eluted at 46 and 48 min, respectively, selectivity/separation factor (α) = 1.06 and resolution (Rs) = 1.96), and NBD-Ser and NBD-Gly were eluted at 57 and 83 min, respectively (Fig. 3).

It is worth mentioning that - unlike many other dipeptides - L-Ser-Gly/D-Ser-Gly and Gly-L-Ser/Gly-D-Ser are real enantiomeric pairs and thus can only be separated by using enantioselective chromatographic methods. For the enantiomer separations, equal amounts of the D and L forms of dipeptides were mixed and analyzed by a KSACSP-001S column (1.5 mm x 250 mm, an originally designed enantioselective column by the collaboration with Shiseido). The enantiomers of the NBD-dipeptides were separated by the isocratic elution using a mixture of MeOH and MeCN containing FA, and the ratio of MeOH/MeCN in addition to the FA concentration were investigated. As a result, 0.025% FA in the mixed solution of MeOH/MeCN = 75/25 (v/v) was selected, and the highest α and Rs values for the Ser-Gly enantiomers (1.14 and 1.32, respectively) were obtained. On the other hand, the suitable ratio of MeOH/MeCN for Gly-Ser enantiomers was MeOH/MeCN = 25/75 (v/v, containing 0.025% FA) with the α and Rs values of 1.19 and 1.69, respectively (Fig. 4). Concerning the elution order, the D-enantiomers of the NBD-dipeptides were faster than the L forms in both cases.

Until now, several papers have been published about the reversed-phase separation of NBD-amino acids including Ser and Gly using C18 columns. The columns of conventional size (4.6 mm x 250 mm), NBD-Ser and NBD-Gly were separated using aqueous solutions containing MeCN and TFA as the mobile phases (tetrahydrofuran and 2-propanol were occasionally used as the mobile phase additives) and the retention times of NBD-Ser and NBD-Gly were around 30 and 40 min, respectively [38-40]. A more sensitive and environmentally friendly HPLC system using a microbore reversed-phase column (0.53 mm x 1000 mm) has also been reported for the separation of the NBD-amino acids. Using the aqueous mobile phase containing 6% MeCN and 0.06% TFA, NBD-Ser and NBD-Gly were also nicely separated about 30 min [33]. Concerning the elution order, NBD-Ser always eluted faster than NBD-Gly. In the present study, a KSAARP column of 1.5 mm x 500 mm was used as the analytical column in which NBD-Ser and NBD-Gly were eluted at 57 and 83 min, respectively, by the aqueous solution of 10% MeCN containing 0.05% TFA. The retention profiles and elution order of the two NBD-amino acids were correlated well with the previous studies, where NBD-Ser consistently eluted faster than NBD-Gly.

The separations of various sequence isomers including Ser-Gly and Gly-Ser by the C18 columns have also been reported [41,42]. Using a 10 mM phosphate buffer solution (pH=2.10) as the mobile phase, separations of the non-derivatized dipeptides were tested by the Spherisorb-ODS (4.0 mm x 200 mm), YWG-ODS (4.6 mm x 250 mm) and Nucleosil-ODS (4.0 mm x 150 mm) columns. The retention factors of Ser-Gly and Gly-Ser were 0.02-0.12; both of them had very weak retention and
were not well separated [41]. The higher retention factors for Ser-Gly and Gly-Ser have also been reported using the Shandon ODS-Hypersil column (4.6 mm x 250 mm). The aqueous solutions of 0.01 M ammonium acetate containing 0, 10 and 30% MeOH were used as mobile phases and the retention factors around 0.4-0.7 were obtained. However, the retention factors of Ser-Gly and Gly-Ser were too low to achieve a sufficient separation [42]. In the present study, the dipeptides were derivatized with NBD and peptides in the black vinegar were used and the obtained α values for Gly fractions were higher than the 90 (min). The α values for Gly enantiomers were 1.69, respectively. These values were practically sufficient within 40 min and the Rs values were 1.32 and 1.69, respectively. In the present study, a KSAACSP 001S column (1.5 mm x 250 mm) was used in the multi-loop valve (NBD-Ser-Gly, 44.5-47.0 min, 187.5 µL; NBD-Gly-Ser, 47.0-49.5 min, 187.5 µL) and injected consecutively into the second dimension (KSAACSP-001S). In the second dimension, the same mobile phases as described in section 3.1 were used and the enantiomers of the NBD-dipeptides were nicely separated within 30 min (NBD-Ser-Gly, α=1.12, Rs=1.57; NBD-Gly-Ser, α=1.18, Rs=2.31). The present 2D-HPLC system was used to analyze the long-time fermented Japanese traditional amber rice vinegar and the obtained chromatograms are shown in Fig. 5. In the first dimension, the NBD-amino acids and peptides in the black vinegar were separated within 90 min. Although the peaks for the NBD-dipeptides were not clearly observed, the fractions were collected at the expected retention times and injected into a KSAACSP-001S column. In the second dimension, relatively large amounts of the L-enantiomers of both NBD-dipeptides were found, and trace peaks of possibly their d-enantiomers were also observed. However, several unknown peaks were eluted close to and also together with the peaks of the NBD-dipeptides, and a more selective HPLC system is essentially needed to clarify unquestionably the presence of the Ser-Gly and Gly-Ser enantiomers.

3.2. Multi-dimensional HPLC analysis of Ser-Gly and Gly-Ser in the Japanese traditional amber rice vinegar

According to the results of the reversed-phase and enantiomer separations obtained and discussed in section 3.1, an on-line 2D-HPLC system combining KSAARP and KSAACSP-001S columns has been constructed. In the first dimension, NBD-Ser-Gly and Gly-Ser were separated by a reversed-phase column within 50 min using an aqueous mobile phase containing 10% MeCN and 0.05% TFA (NBD-Ser-Gly and NBD-Gly-Ser were eluted at 46 and 48 min, respectively, α=1.06 and Rs=1.96). The fractions of the NBD-dipeptides were automatically collected in the multi-loop valve (NBD-Ser-Gly, 44.5-47.0 min, 187.5 µL; NBD-Gly-Ser, 47.0-49.5 min, 187.5 µL) and injected consecutively into the second dimension (KSAACSP-001S). In the second dimension, the same mobile phases as described in section 3.1 were used and the enantiomers of the NBD-dipeptides were nicely separated within 30 min (NBD-Ser-Gly, α=1.12, Rs=1.57; NBD-Gly-Ser, α=1.18, Rs=2.31). The present 2D-HPLC system was used to analyze the long-time fermented Japanese traditional amber rice vinegar and the obtained chromatograms are shown in Fig. 5. In the first dimension, the NBD-amino acids and peptides in the black vinegar were separated within 90 min. Although the peaks for the NBD-dipeptides were not clearly observed, the fractions were collected at the expected retention times and injected into a KSAACSP-001S column. In the second dimension, relatively large amounts of the L-enantiomers of both NBD-dipeptides were found, and trace peaks of possibly their d-enantiomers were also observed. However, several unknown peaks were eluted close to and also together with the peaks of the NBD-dipeptides, and a more selective HPLC system is essentially needed to clarify unquestionably the presence of the Ser-Gly and Gly-Ser enantiomers.

As a more selective analysis system, an on-line 3D-HPLC system combining KSAARP, KSAAX and KSAACSP-001S columns has been designed. In the first dimension, the same conditions as for the on-line 2D-HPLC system were employed and the NBD-dipeptides were separated and the fractions collected via V1 were automatically injected into the second column (KSAAX). For the second dimension, a mobile phase of MeOH/MeCN containing FA was selected, and the ratio of MeOH/MeCN as well as the FA concentration were investigated. The NBD-Ser-Gly and NBD-Gly-Ser were eluted within 60 min by the isocratic elution using 0.025% FA in MeOH/MeCN = 75/25. The fractions of the NBD-dipeptides were again automatically collected via V2 and injected into the
third dimension (KSAACSP-001S), where the enantiomers of NBD-Ser-Gly and NBD-Gly-Ser were separated. In order to optimize the enantiomer separations, the mobile phase conditions were also investigated. For Ser-Gly, the enantiomers were separated using 0.02% FA in 80% MeOH 20% MeCN as the mobile phase (α=1.09, Rs=1.04), and for the Gly-Ser enantiomers, the mixed solution of MeOH/MeCN (25/75, v/v) containing 0.0375% FA was used (α=1.07, Rs=0.71). The values of the lower limit of detection for the NBD-dipeptides were 10 fmol/injection.

The present 3D-HPLC system was successfully applied to the analysis of the black vinegar. Figure 6 shows the on-line 3D-HPLC chromatograms of the NBD-Ser-Gly and NBD-Gly-Ser enantiomers in the Japanese traditional amber rice vinegar. In the first dimension, the NBD-dipeptides in the black vinegar were separated, and the fractions were automatically collected. In the second dimension, several unknown peaks were found and were separated from the NBD-dipeptides again, and the fractions of the NBD-dipeptides were automatically collected and injected into the final enantiomer separation step. In the third dimension, a relatively large amount of L-Ser-Gly and small amount of D-Ser-Gly were found; the %D value (D/(D+L) x 100) was 15.5%. For Gly-Ser, only the L-enantiomer was found. To confirm the presence of the Ser-Gly and Gly-Ser enantiomers, a KSAACSP-001R column (1.5 mm x 250 mm, an enantioselective column having an opposite chiral center from KSAACSP-001S) was used. By using the KSAACSP-001R column, the elution order of the NBD-dipeptide enantiomers was converted (L-enantiomers eluted faster than D-forms) and the obtained %D values of the Ser-Gly and Gly-Ser were 15.3% and 0%, respectively. These values were quite similar to those obtained by the KSAACSP-001S column, indicating that the peaks of the NBD-Ser-Gly and NBD-Gly-Ser enantiomers are unbiased and genuine dipeptide enantiomers in the black vinegar.

Until now, the presence of various D-amino acids has been reported in the fermented products such as vinegar [18,19,21,28,29], cheese [20-22] and yogurt [17-19]. Concerning vinegar, various brands including balsamic, sherry, cider and white wine vinegars were analyzed, and D-Asp, D-Glu, D-Arg, D-Pro and D-Ala were found in all these products [28]. In the sherry vinegar, the presence of D-Ser and D-Leu was also reported [21]. In the Japanese black vinegar, various D-amino acids were found (Ala, Arg, Asp, Glu, Leu, allo-Ile, Met, Phe, Pro, Ser, allo-Thr and Val). Among them, the amounts of the D-Ala, D-Glu, D-Asp and D-Ser are relatively high (about 100 nmol/mL or higher) [19,29]. Based on the investigations of several commercially available Japanese black vinegar samples, the amounts of the D-amino acids were strongly affected by the production procedure and duration of the fermentation/aging processes. The relatively large amount of the D-amino acids including Ser were frequently observed in the black vinegars produced by the traditional procedure [29]. In the present study, a Japanese black vinegar sample produced by the traditional earthenware jar fermentation was analyzed by the 2D and 3D-HPLC systems. The L-enantiomers of both Ser-Gly and Gly-Ser, and interestingly also a non-negligible amount of D-Ser-Gly were found. To the best of our knowledge, this is the first report to show the presence of a dipeptide containing D-amino acid in the Japanese traditional black vinegar, and further applications of the present method to various real world samples are the matters of interest in the future.

4. Conclusion

In the present study, on-line 2D and 3D-HPLC systems have been developed for the chiral analysis of Ser-Gly and Gly-Ser, and were applied to the analysis of Japanese traditional amber rice vinegar. Using the 2D-HPLC system, the enantiomers of the target dipeptide were not completely separated from unknown substances. On the
other hand, the target dipeptides were nicely separated using the 3D-HPLC system from the intrinsic substances in the complicated matrix of the fermented black vinegar sample, and the peaks of the dipeptide enantiomers were clearly observed without interference. These results demonstrated that in some cases, the selectivity of a 2D-HPLC system is not sufficient and the 3D-HPLC system is a powerful technique for the analysis of chiral dipeptides in complicated matrices. The present results also clearly demonstrated the presence of D-Ser-Gly in the black vinegar using the 3D-HPLC system from the intrinsic substances in the complicated matrix of the fermented black vinegar and further studies focusing on their stereoselective analyses are ongoing.

Acknowledgements
This study was partly supported by JSPS KAKENHI Grant Number 15H05749 and 16H05080 Japan. The authors appreciate Shiseido Co., Ltd., for their technical support.

References
[1] Hamase, K.; Morikawa, A.; Zaitsu, K. J. Chromatogr. B 2002, 781, 73-91.
[2] Fujii, N.; Kaji, Y.; Fujii, N. J. Chromatogr. B 2011, 879, 3141-3147.
[3] Miyoshi, Y.; Koga, R.; Oyama, T.; Han, H.; Ueno, K.; Masuyama, K.; Itoh, Y.; Hamase, K. J. Pharm. Biomed. Anal. 2012, 69, 42-49.
[4] Miyoshi, Y.; Oyama, T.; Itoh, Y.; Hamase, K. Chromatography 2014, 35, 49-57.
[5] Henneberger, C.; Papouin, T.; Oliet, S. H. R.; Rusakov, D. A. Nature 2010, 463, 232-237.
[6] Kakegawa, W.; Miyoshi, Y.; Hamase, K.; Matsuda, S.; Matsuda, K.; Kohda, K.; Emi, K.; Motohashi, J.; Konno, R.; Zaitsu, K.; Yuzaki M. Nat. Neurosci. 2011, 14, 603-611.
[7] Guevara, C. M.; Mani, A. R. Eur. J. Pharmacol. 2016, 780, 216-223.
[8] Nishikawa, T. J. Chromatogr. B 2011, 879, 3169-3183.
[9] Bendikov, I.; Nadri, C.; Amar, S.; Panizzutti, R.; De Miranda, J.; Wolosker, H.; Agam, G. Schizophrenia. Res. 2007, 90, 41-51.
[10] Madeira, C.; Lourenco, M. V.; Vargas-Lopes, C.; Suemoto, C. K.; Brandão, O. C.; Reis, T.; Leite, R. E. P.; Laks, J.; Jacob-Filho, W.; Pasqualucci, C. A.; Grinberg, L. T.; Ferreira, S. T.; Panizzutti R. Transl. Psychiatry 2015, 5, e561.
[11] Sasabe, J.; Miyoshi, Y.; Suzuki, M.; Mit, M.; Konno, R.; Matsuoka, M.; Hamase, K.; Aiso, S. Proc. Natl. Acad. Sci. USA 2012, 109, 627-632.
[12] Kimura, T.; Hamase, K.; Miyoshi, Y.; Yamamoto, R.; Yasuda, K.; Mit, M.; Rakugi, H.; Hayashi, T.; Isaka, Y. Sci. Rep. 2016, 6, 26137.
[13] Morikawa, A.; Hamase, K.; Inoue, T.; Konno, R.; Zaitsu, K. Amino Acids 2007, 32, 13-20.
[14] Yoshimura, T.; Esaki, N. J. Biosci. Bioeng. 2003, 96, 103-109.
[15] Cava, F.; Lam, H.; de Pedro, M. A.; Waldor, M. K. Cell. Mol. Life Sci. 2011, 68, 817-831.
[16] Sasabe, J.; Miyoshi, Y.; Rakoff-Nahoum, S.; Zhang, T.; Mita, M.; Davis, B. M.; Hamase, K.; Waldor, M. K. Nat. Microbiol. 2016, 1, 16125.
[17] Jin, D.; Miyahara, T.; Oe, T.; Toyoioka, T. Anal. Biochem. 1999, 269, 124-132.
[18] Casal, S.; Oliveira, M. B.; Ferreira, M. A. J. Chromatogr. A 2000, 866, 221-230.
[19] Eto, S.; Yamaguchi, M.; Bounoshita, M.; Mizukoshi, T.; Miyano, H. J. Chromatogr. B 2011, 879, 3317-3325.
[20] Brückner, H.; Hausch, M. Chromatographia 1989, 28, 487-492.
[21] Brückner, H.; Langer, M.; Lüpké, M.; Westhauser, T.; Godel, H. J. Chromatogr. A 1995, 697, 229-245.
[22] Csapó, J.; Varga-Visi, É.; Lóki, K.; Albert, Cs. Amino Acids 2007, 32, 39-43.
[23] Erbe, T.; Brückner, H. J. Chromatogr. A 2000, 881, 81-91.
[24] Ali, H. S. M.; Pätzold, R.; Brückner, H. Amino Acids 2010, 38, 951-958.
[25] Kato, S.; Ishihara, T.; Henni, H.; Kobayashi, H.; Yoshimura, T. J. Biosci. Bioeng. 2011, 111, 104-108.
[26] Gogami, Y.; Okada, K.; Oikawa, T. J. Chromatogr. B 2011, 879, 3259-3267.
[27] Inoue, Y.; Katsumata, T.; Watanabe, H.; Hayase, F. Food Sci. Technol. Res. 2016, 22, 679-686.
[28] Carlavilla, D.; Moreno-Arribas, M. V.; Fanali, S.; Cifuentes, A. Electrophoresis 2006, 27, 2551-2557.
[29] Miyoshi, Y.; Nagano, M.; Ishigo, S.; Ito, Y.; Hashiguchi, K.; Hishida, N.; Mita, M.; Lindner, W.; Hamase, K. J. Chromatogr. B 2014, 966, 187-192.
[30] Simó, C.; Rizzi, A.; Barbas, C.; Cifuentes, A. Electrophoresis 2005, 26, 1432-1441.
[31] Pätzold, R.; Brückner, H. Amino Acids 2006, 31, 61-72.
[32] Waldhier, M. C.; Almstetter, M. F.; Nürnberger, N.; Gruber, M. A.; Dettmer, K.; Oefner, P. J. Chromatogr. A 2011, 1218, 4537-4544.
[33] Hamase, K.; Miyoshi, Y.; Ueno, K.; Han, H.; Hirano, J.; Morikawa, A.; Mita, M.; Kaneko, T.; Lindner, W.; Zaitsu, K. J. Chromatogr. A 2010, 1217, 1056-1062.
[34] Koga, R.; Miyoshi, Y.; Sato, Y.; Mita, M.; Konno, R.; Lindner, W.; Hamase, K. Chromatography 2016, 37, 15-22.
[35] Koga, R.; Miyoshi, Y.; Sato, Y.; Mita, M.; Konno, R.;
Lindner, W.; Hamase, K. *J. Chromatogr. A* **2016**, *1467*, 312-317.

[36] Szökő, É.; Vincze, I.; Tábi, T. *J. Pharm. Biomed. Anal.* **2016**, *130*, 100-109.

[37] Morikawa, A.; Fukuoka, H.; Uezono, K.; Mita, M.; Koyanagi, S.; Ohdo, S.; Zaitsu, K.; Hamase, K. *Chromatography* **2017**, *38*, 53-58.

[38] Hamase, K.; Homma, H.; Takigawa, Y.; Fukushima, T.; Santa, T.; Imai, K. *Biochim. Biophys. Acta* **1997**, *1334*, 214-222.

[39] Huang, Y.; Nishikawa, T.; Satoh, K.; Iwata, T.; Fukushima, T.; Santa, T.; Homma, H.; Imai, K. *Biol. Pharm. Bull.* **1998**, *21*, 156-162.

[40] Aoyama, C.; Santa, T.; Tsunoda, M.; Fukushima, T.; Kitada, C.; Imai, K. *Biomed. Chromatogr.* **2004**, *18*, 630-636.

[41] Zou, H.; Zhang, Y.; Hong, M.; Lu, P. *J. Liq. Chromatogr.* **1992**, *15*, 1797-1806.

[42] Lundanes, E.; Greibrokk, T. *J. Chromatogr.* **1978**, *149*, 241-254.

[43] Reischl, R. J.; Lindner, W. *J. Pharm. Biomed. Anal.* **2015**, *116*, 123-130.