

Comparative study for pharmaceutical quality among bland-name drug and generic drugs of compound glycyrrhizin injections in China

Abstract

The physicochemical properties (pH and osmolarity), ingredients, and impurities containing in compound glycyrrhizin injections (eight items) marketed in China were compared with those in bland-name drug (Stronger Neo-Minophagen C injection). Glycyrrhizin (GZ), glycine (Gly), and L-cysteine (CysH) as the ingredients, moreover, glycyrrhetic acid (GA), 3-monoglucuronyl-glycyrrhetic acid (MGGA), and L-cystine (CysS) as the impurity were determined by HPLC. The pH and osmolarity were different every each pharmaceutical product, but the variation between batch was very small. On the other hand, although the contents of GZ, Gly, and CysH in bland-name drug were approximately 100% of the label claim, the contents of GZ in generic drugs were the range of 91.8-100.9%, indicating the GZ contents in four products were clearly less than value indicated in label (<97%). The remarkable difference was not accepted by impurities content such as GA and MGGA. The contents of CysH in generic drugs were the range of 79.9-100.4%, and CysS was determined in all generic drugs, suggesting that CysH may decompose to be CysS depending on the pH of injections in generic drug only. Because the variation of the ingredient content was big and products with a little quantity for the ingredients were recognized, establishment of the preparation that can maintain the prescribed ingredient content and the severity of the assay will be required.

Key words:

Generic drug, glycyrrhetic acid, glycyrrhizae radix, glycyrrhizin, quality evaluation

Introduction

Glycyrrhizin (glycyrrhizin monoammonium; GZ) is a therapeutic drug for eczema, dermatitis, liver function improvement in the chronic hepatitis, which was approved in Japan in 1948. The GZ is a nature ingredient extracted from *Glycyrrhiza glabra* L. and is the glucoside that glucuronic acid of 2 molecules made ether binding with glycyrrhetic acid (GA). Except for the injections, oral formulations are marketed as the ethical drugs; however, the absorption of GZ from gastrointestinal tract is very poor.^[1-3] Therefore, therapeutic effect of intravenous administration of GZ is very beneficial compared with that of oral administration. The ingredient of bland-name drug of this injection is glycyrrhizin monoammonium 2.65 mg per 1 ml (2 mg as glycyrrhizic acid), glycine (Gly) 20 mg, and L-cysteine hydrochloride hydrate 1.1145 mg (1 mg as L-cysteine hydrochloride (CysH)), and this formulation was gotten the production approval as the

combination agent of GZ and amino acids. Recently, 21-kind generic drugs are marketed as same ingredients in Japan. On the other hand, the GZ formulation in foreign countries is commercially available in China, Korea, Taiwan, Mongolia, etc., and spreads as therapeutic drug with high safety and efficacy for chronic hepatitis patients. Especially, in China, several products of GZ are available as compound glycyrrhizin injections. Moreover, optical isomer of GZ, 18alpha-GZ, and glycyrrhizin diammonium are available as single ingredient without amino acid.

In such situation, Stronger Neo-Minophagen C[®] injection (SNMC, Minophagen Pharmaceutical Co. Ltd., Tokyo, Japan) was recognized as a bland-name drug among GZ formulations marketed in China in 2012. The quality evaluation among the bland-name drug and generic drugs are not investigated officially. For quality evaluation of GZ products in Japan, the differences

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Kenjiro Koga, Hironori Kikuchi

Department of Clinical Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3, Kanagawa-machi, Kanazawa, Japan

Address for correspondence:

Prof. Kenjiro Koga,
 Department of Clinical Pharmaceutics, Faculty of Pharmaceutical Sciences,
 Hokuriku University, Ho-3, Kanagawa-machi, Kanazawa, Japan
 E-mail: k-koga@hokuriku-u.ac.jp

of physicochemical properties and ingredient contents such as glycyrrhizic acid, Gly, and CysH were reported,^[4] indicating that these contents in several kind generic drugs were less than that of SNMC. And a remarkable difference was accepted by the content of impurities such as GA, 3-monoglucuronyl-glycyrrhetic acid (MGGA), and L-cystine (CysS). Namely, the quality evaluation as the differences between brand-name drug and generic drugs is needed for GZ products marketed in China. In this paper, pH, osmolarity ratio, contents of ingredients (GZ, Gly, and CysH), and contents of impurities (GA, MGGA, and CysS) were determined. Absolutely, this work will contribute to enhance the product quality of GZ injections.

Experimental

Chemical and reagents

Glycyrrhizic acid (GLYO605, 18beta type, purify 100%) was purchased from National Institute of Health Sciences (Tokyo, Japan). Glycyrrhizic acid (18alfa type, purify 96.2%) was a gift from Amato Pharmaceutical Co. Ltd. (Fukuchiyama, Japan). Glycyrrhetic acid (purify 100%), 3-monoglucuronyl glycyrrhetic acid (purify 100%), glycine (purify 100%), L-cysteine hydrochloride (purify 99.0%), L-cystine (purify 100%) were purchased from Wako Pure Chemical Industries Co. (Osaka, Japan). Saline was purchased from Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan). Other chemicals were of HPLC or reagent grade.

GZ Pharmaceutical products

Used commercial products of compound glycyrrhizin injections are shown in Table 1. The brand-name drug is SNMC (No.1). No. 2, 4, 5, 6, 7, 9 products have same ingredients as SNMC, however, No.3 and 8 products are compound glycyrrhizin injection having different composition, the ingredients of No. 3 and 8 products are glycyrrhizin diammonium and glycyrrhizin magnesium, respectively. These products were kept at 20-25°C in a dark room. All products were changed to a screw vial right before analyzing. Freeze-drying products, No. 5, 6, and 9 were dissolved with saline, then were adjusted to 10, 50, and 20 ml, respectively, using flasks of 10, 50, and 20 ml.

pH assay

The pH values of all products were measured three times for each batch sample using a pH meter (HM-7E, DKK-TOA Corporation, Tokyo). The calibration was carried out using pH6.80 and pH4.01 standard solutions.

Osmolarity assay

The osmolarity values of all products were measured three times for each batch sample using a Semi-micro Osmometer (K-7400, Herbert Knauer GmbH, Germany). The calibration was carried out using 0 and 400 mOsm standard solutions. The osmolarity of product No.6 was corrected by 1.25-fold for determination value, because No.6 had to dissolve with 40 ml, but there was not a 40-ml flask. The data were indicated as osmolarity ratio (300 mOsm = 1.0).

Table 1: Compound glycyrrhizin injections

Product	Lot number	Volume (ml)	GZ (mg)	L-Cys.HCl (mg)	Gly (mg)
SNMC	G1321	20	40	20	400
	G1331	20	40	20	400
	G1341	20	40	20	400
AI GAN BAO	100503	20	40	20	400
	110701	20	40	20	400
	110703	20	40	20	400
GAN LI XIN	1109171	10	50 ¹⁾	0	0
	111017104	10	50 ¹⁾	0	0
GAN YU	100801	20	40	20	400
	110402	20	40	20	400
	20110606	20	40	20	400
LONG DI TAI	11021901	freeze-drying	20	10	200
	11070301	freeze-drying	20	10	200
	11081902	freeze-drying	20	10	200
OU LI KANG	201012292	freeze-drying	80	40	800
	201106101	freeze-drying	80	40	800
	201109031	freeze-drying	80	40	800
PAI GAN NENG	101203	20	40	20	400
	110314	20	40	20	400
	110708	20	40	20	400
TIAN QING GAN MEI	1107132	10	50 ²⁾	0	0
	1107181	10	50 ²⁾	0	0
	1108242	10	50 ²⁾	0	0
WEI YI XING	110103D1	freeze-drying	40	20	400
	110103D2	freeze-drying	40	20	400
	110501A1	freeze-drying	40	20	400

SNMC is a brand-name drug. No.2, 4, 5, 6, 7, and 9 are generic drugs having same ingredients as SNMC. GZ (mg): content as glycyrrhizic acid, ¹⁾50 mg as glycyrrhizin diammonium, ²⁾50 mg as glycyrrhizin magnesium

GZ (18 α -GZ and 18 β -GZ) determination

GZ has both optical isomer as 18 α -GZ (α -GZ) and 18 β -GZ (β -GZ) by direction of hydrogen connected to 18-position carbon in structure. It is written in product information sheets that GZ of product No.1, 2, 4, 5, 6, 7, and 9 are β -GZ and GZ of No.8 is 18 α -GZ. The determination of both GZ by HPLC was carried out by the modification of previous report.^[5] Product No.1, 2, 4, 5, 6, 7, 9 were diluted 1000-fold with 100 mM phosphate buffered solution (pH7.4), and product No.3, 8 were diluted 2000-fold with 100 mM phosphate buffered solution (pH7.4). Each solution (10 μ l) was injected to HPLC system. The HPLC system was a semimicro type; it was equipped with a LC-10ADvp pump, SIL-20A autosampler, DGU-20As degassing apparatus, SPD-20A UV detector, CR7A-plus data processor (Shimadzu, Kyoto, Japan). Briefly, a Capcell Pak 5C18 UG column (1.5-mm inner diameter, 150-mm length, Shiseido, Tokyo, Japan) and a Capcell Pak 5C18 UG120 (1.5-mm inner diameter, 10-mm length, Shiseido) as the guard column was used 40°C during separation. Detection was performed at a UV wavelength of 254 nm. Acetonitrile/0.6% perchloric acid solution adjusted to pH8.0 with 25% ammonia solution (2:8, v/v) was used as the mobile phase; the flow rate was 0.08 ml/min. The detection limit was 200 ng/ml.

GA determination

All products were diluted 2-fold with 100 mM phosphate-buffered solution (pH7.4). Each solution (10 μ l) was injected to HPLC system. The HPLC system was the same as GZ determination except for mobile phase. Methanol/0.6% perchloric acid solution adjusted to pH8.0 with 25% ammonia solution (8:2, v/v) was used as the mobile phase.

MGGA determination

All products were diluted 2-fold with 100 mM phosphate-buffered solution (pH7.4). Each solution (10 μ l) was injected to HPLC system. The HPLC system was the same as GZ determination except for detection and mobile phase. The detection was performed at a UV wavelength of 251 nm, and methanol/0.6% perchloric acid solution adjusted to pH2.5 with 25% ammonia solution (78:22, v/v) was used as the mobile phase.

Gly determination

All products (0.5 ml) were put in a 50-ml flask and were adjusted with 0.2 M NaOH (sample solutions). Gly was dissolved with 0.2 M NaOH as the standard solution (0.2 mg/ml as the concentration of Gly). The standard or sample solution (80 μ l), 0.2 M NaOH (0.7 ml), and 3% phenyl isothiocyanate dissolved with 1-propanol (0.6 ml) were mixed, and then were incubated at 50°C for 30 min. After cooling the mixture solution, the mixture solution and benzene (0.2 ml) were mixed, and were shaken at 500 rpm for 5 min using a Vortex Shaker (VR-36D, Taitec, Saitama, Japan). After the mixture solution was centrifuged at 2500 \times g for 5 min, the supernatant was removed using a glass pipette. The lower layer (0.4 ml) and 8 M HCl (0.4 ml) were put in a 10-ml glass-stoppered centrifuge tube, and the mixture solution was incubated at 50°C for 60 min. After cooling the mixture solution, the mixture solution and ethyl acetate (4 ml) were mixed in the centrifuge glass tube, and then was shaken at 500 rpm for 15 min. After centrifuging the

mixture solution at 2500 \times g for 5 min, the supernatant (0.5 ml) was transferred to a 10-ml glass tube, and was evaporated to dryness under a continuous stream of nitrogen gas and on a block heater set to 80°C. After cooling the glass tube, 1.0 ml of methanol/20 mM sodium acetate (8:17, v/v) was added to the glass tube, and then was shaken for 1 min using a vortex mixer. The final solution was filtered with a Millipore membrane (LCR13-LH, pore size: 0.22 μ m, Japan Millipore, Tokyo, Japan). An aliquot (10 μ l) of the filtered solution was injected to HPLC system.

The HPLC system was the same as GZ determination except for mobile phase and flow rate. Methanol/20 mM sodium acetate (8:17, v/v) was used as the mobile phase, and the flow rate was set to 0.07 ml/min.

L-Cysteine and L-Cystine determination

CysH standard solution (2 μ g/ml as the concentration of L-cysteine hydrochloride) and CysS standard solution (2 μ g/ml as the concentration of L-cystine) were prepared by dissolving with mobile phase, acetonitrile/0.1% phosphoric acid with 3.5 mM sodium 1-heptanesulfonate (9:95, v/v). Each product solution was diluted 500-fold with the mobile phase. The HPLC system was the same as GZ determination except for detection, and mobile phase. The detection wavelength was set to 210 nm.

Statistical analysis

Statistical analysis using Dunnett multiple comparison test was performed for data of GZ, GA, MGGA, CysH, and Gly. The controlled group was set to bland-name drug (SNMC). A *P* value < 0.05 was considered to be statistically significant.

Results and discussion**Physicochemical properties**

Table 2 shows the pH values and osmolarity ratios in 9-kind products of glycyrrhizin compound injections containing bland-name drug (SNMC) and generic drugs. The pH values of SNMC were at the range of 6.05-6.19 (6.12 \pm 0.06) in three batches. The pH values of products from GE-A to GE-F with same ingredients were at the range of 5.15-7.10 as the mean, indicating that the pH level was varied by products. Although

Table 2: pH values and osmolarity ratios in nine products

Product	pH	Osmolarity ratio
SNMC	6.12 \pm 0.06	1.50 \pm 0.01
GE-A	7.10 \pm 0.24	1.41 \pm 0.06
GE-B	7.06 \pm 0.17	1.49 \pm 0.01
GE-C	6.49 \pm 0.12	0.96 \pm 0.00
GE-D	6.16 \pm 0.03	2.04 \pm 0.12
GE-E	5.15 \pm 0.07	1.94 \pm 0.04
GE-F	5.29 \pm 0.10	1.77 \pm 0.02
GE-G	6.98 \pm 0.03	0.96 \pm 0.02
GE-H	7.41 \pm 0.11	0.94 \pm 0.01

Data represent the mean \pm SD of two or three batches. The osmolarity of OU LIKANG dissolved with 50 ml of saline was calculated by 1.25-fold. GE name of product is different from the numerical order listed to Table 1 in deference to obligation of keeping secrecy.

the standard deviation (SD) of GE-A, GE-B, GE-C, GE-E, GE-F, and GE-H were bigger than that of SNMC, the values were all within the defined range. Namely, the variation indicates the retainment of quality management for batch-to-batch reproducibility.

Osmolarity ratios in 9-kind products were divided to three groups. Namely, the value was approximately 1.0, 1.5 or 2.0. The SD value in GE-D was big compared to those of other products. In similar to pH value, all osmolarity ratios were within the defined range in spite of the variability in batch-to-batch was observed.

On the whole, it is suggested that the physicochemical properties such as pH and osmolarity are managed within the value of standard every products.

Glycyrrhizic acid concentration in products

Although β -GZ is ingredient in SNMC and generic drugs with same composition, a very small amount of α type GZ is included in GZ bulk. Therefore, concomitant determination of both isomers was carried out. Table 3 shows the concentrations of α -GZ and β -GZ in all products. In product information sheet, TIAN QING GAN MEI (No.8 in Table 1) only indicates that α -GZ is contained as the ingredient. The content of β -GZ in SNMC was 101.2-102.0% ($101.6 \pm 0.3\%$) in three batches. The content of β -GZ in GE-D was similar to that of SNMC; however, the β -GZ contents in other products were less than 100%. Especially, the β -GZ contents in GE-A, GE-B, GE-H were lower than 95%, and GZ content in GE-B was significantly low. The α -GZ content in GE-G was over 100%. The sum of α -GZ and β -GZ in GE-H was 98.4%, indicating that GE-H was distinguishing formulation in comparison with other products. Moreover, the SD values in GE-B, GE-C, GE-D, GE-E, GE-F, and GE-H were remarkably larger than that of SNMC (0.3%). These results suggested that the lack of GZ content and the variation of SD value might be affected by the purity of GZ included in the bulk and a difference of the method for measurement of GZ in the quality of the products. In generic drugs, it is an important problem for product management that the content of GZ is clearly less than 100% in a comparison every batch. For the lack of GZ

content, β -GZ content in extract from *Glycyrrhiza glabra* L. is approximately 75% (detailed experiments are not shown). Namely, many minor constituents are obtained in GZ bulk powder. Interestingly, it was reported that detection as abnormal high peak area in HPLC determination of GZ was observed.^[6] As the cause, monomethyl glycyrrhizic acid which is a minor constituent of GZ containing the bulk powder was decomposed to GZ by hydrolysis. In this way, it is predictable that minor constituents (similar compound of GZ) during manufacturing process or after preparation may affect the content of final GZ in products. Therefore, the degree of purity of GZ (approximately 75%) is an important factor for the GZ content. Although normal manufacturing variations are expected, remarkable variation in batch-to-batch levels can indicate that the manufacturing process of the generic drugs was not adequately controlled and was not validated.

Glycyrrhetic acid concentration in products

Glycyrrhetic acid (GA) has the structure that 2 glucuronic acids dissociate from GZ. GA is originally contained in the extract from *Glycyrrhiza glabra* L., is one of main impurities. Interestingly, GA has pharmacological action as similar to GZ such as anti-inflammatory.^[7,8] Therefore, the GA content in product may affect therapeutic efficiency by GZ. Table 4 shows GA content and the rate of GA content for GZ content in all products. The GA content in SNMC was $0.52 \pm 0.01 \mu\text{g/ml}$, and the GA rate for GZ content was 0.026%. This result suggested that GA content in SNMC was very small. Therefore, the GA content is not a level to have an influence on the therapeutic effect of GZ. Among the generic drugs, GA contents in GE-A and GE-B products were approximately 4-fold high compared with that in SNMC (significant high level; $P < 0.05$ vs. SNMC). On the other hand, GA contents from GE-C to GE-H were similar or lower level as compared to that in SNMC. Interestingly enough, GA content in GE-G was zero. In any case, the GA rate for GZ content was too small ($<0.15\%$). Meanwhile it was reported that pseudo-aldosteronism was occurred by the large amount administration of GZ. Furthermore, it was reported that GA may strongly relate to the pseudo-aldosteronism as one of the factors to induce the adverse effect.^[9-11] Therefore, when a small amount of GA included in the GZ products is

Table 3: α -GZ and β -GZ contents in nine products

Product	α -GZ (%)	β -GZ (%)
SNMC	0	101.6 ± 0.3
GE-A	0	$91.8 \pm 0.3^*$
GE-B	0	94.2 ± 4.5
GE-C	0	97.3 ± 2.9
GE-D	0	100.9 ± 3.5
GE-E	0	96.5 ± 2.1
GE-F	0	95.4 ± 3.1
GE-G	$111.4 \pm 1.1^*$	0
GE-H	82.7 ± 5.6	15.7 ± 0

Data represent the mean \pm SD among batch. GE name of product is different from the numerical order listed to Table 1 in deference to obligation of keeping secrecy. Statistical analysis was performed by Dunnett multiple comparison test. Statistical analysis for GE-H was carried out using a sum total of α -GZ and β -GZ. * $P < 0.05$ vs. SNMC.

Table 4: GA and MGGA contents in nine products

Product	GA ($\mu\text{g/ml}$)	(%)	MGGA ($\mu\text{g/ml}$)	(%)
SNMC	0.52 ± 0.01	0.026	0.38 ± 0.04	0.019
GE-A	$1.97 \pm 0.32^*$	0.107	0.21 ± 0.04	0.011
GE-B	$2.19 \pm 1.29^*$	0.120	$0.05 \pm 0.04^*$	0.003
GE-C	0.30 ± 0.12	0.016	0.22 ± 0.01	0.011
GE-D	0.88 ± 0.27	0.043	0.22 ± 0.00	0.011
GE-E	0.22 ± 0.15	0.011	0.61 ± 0.16	0.031
GE-F	0.12 ± 0.10	0.007	0.48 ± 0.15	0.025
GE-G	0.00	0.000	0.00*	0.000
GE-H	0.15 ± 0.03	0.003	0.00*	0.000

Percentage (%) is the GA and MGGA content ratios for GZ content in each product. GE name of product is different from the numerical order listed to Table 1 in deference to obligation of keeping secrecy. Statistical analysis was performed by Dunnett multiple comparison test. * $P < 0.05$ vs. SNMC.

administered consecutively, the pharmaceutical attention is necessary for patients as the symptom in pseudo-aldosteronism.

3-monoglucuronyl-glycyrrhetic acid concentration in products

3-Monoglucuronyl-glycyrrhetic acid (MGGA) has the structure that one glucuronic acid dissociate from GZ contained two molecule glucuronic acids. As similar to the GA, MGGA is originally contained in the extract from *Glycyrrhiza glabra* L. Moreover, it was reported that MGGA is metabolized from GA in liver.^[12] Intriguingly, there are some reports that MGGA may cause pseudo-aldosteronism for chronic hepatitis patients.^[13,14] Therefore, the determination of MGGA is important for pharmaceutical quality in products. MGGA contents in products are shown in Table 4. MGGA contents in SNMC were the range of 0.34-0.43 µg/ml (0.38 ± 0.04 µg/ml), and the ratios for GZ content were 0.019%. This level did not have remarkable difference compared to GA content (%) for GZ content in SNMC. The MGGA contents in other products also had low level for GZ content. Particularly, MGGA content in GE-B among generic drugs with same ingredient was significantly low ($P < 0.05$ vs. SNMC). Because MGGA content does not have a predominant high level among all products, it is thought that the difference of the product will not be reflected to the incidence of adverse effect such as pseudo-aldosteronism. In addition, GE-G and GE-H among the generic drugs did not contain MGGA, suggesting that the extraction process from *Glycyrrhiza glabra* L. or refinement process of GZ is superior.

L-Cysteine, L-Cystine, and glycine contents in products

L-Cysteine hydrochloride (CysH) and glycine (Gly) are ingredients in SNMC and generic drugs (GE-A to GE-F). L-Cysteine is easily oxidized in aqueous solution, and is changed to L-cystine (CysS). Therefore, the determination of CysS was also carried out. Table 5 shows these contents in products. For CysH and Gly, the contents were represented as percentage for the content indicated in product sheet.

Table 5: Cystein.HCl, Cystine, and Glycine contents in seven products

Product	CysH (%)	CysS (mg/ml)	Gly (%)
SNMC	102.8 ± 5.2	0.0	100.2 ± 0.5
GE-A	90.3 ± 5.2	0.140 ± 0.031	94.6 ± 6.8
GE-B	89.2 ± 2.4	0.138 ± 0.006	96.7 ± 3.1
GE-C	91.3 ± 3.0	0.088 ± 0.047	98.2 ± 4.0
GE-D	91.1 ± 7.2	0.149 ± 0.046	97.9 ± 10.4
GE-E	100.4 ± 6.6	0.051 ± 0.072	105.0 ± 5.2
GE-F	79.9 ± 4.2*	0.006 ± 0.009	97.7 ± 6.7

Percentage of cystein. HCl (CysH) and glycine (Gly) represent for contents indicated product sheet. Data represent the mean ± SD of three determinations. GE name of product is different from the numerical order listed to Table 1 in deference to obligation of keeping secrecy. Statistical analysis was performed by Dunnett multiple comparison test.

* $P < 0.05$ vs. SNMC.

The content of CysH in SNMC was 102.8 ± 5.2%. On the other hand, the CysH contents in generic drugs were the range of 79.9-100.4%. Especially, the content in GE-F was significantly low compared to that in SNMC. The result indicated that CysH contents were not satisfied depending on a product. As the reason that determined CysH content was less than defined amount of CysH, two factors are considered. Namely, i) lack of content of CysH in the initial preparation process and ii) degradation of CysH after the preparation. As the latter factor, the measurement of CysS which is a decomposition product of CysH will contribute to the possibility definitely. In fact, the concentration of CysS in SNMC was zero, supporting that CysH in SNMC was filled as defined content (1 mg/ml as the concentration of L-cysteine hydrochloride), and did not decompose to CysS. On the other hand, all generic drugs (GE-A to GE-F) contained CysS. Especially, the CysS concentrations in GE-A, GE-B, and GE-D were high (0.138-0.149 mg/ml). Contrastingly, those in GE-E and GE-F were low, i.e., 0.051 and 0.006 mg/ml, respectively. From these results, it was guessed that the difference in quantity of CysS in generic drugs depended on the decomposition rate from CysH to CysS in the period from manufacture process to storage. Sodium sulfite is added to all products as an antioxidant in order to inhibit the decomposition of CysH. However, the different in antioxidant effect on CysH was observed. As the predicted factors, the temperature condition in manufacture process of each product and final pH of each product solution were assumed. Notably, the mechanism which CysH is easily changed to CysS by small amount of metal ion in neutral or alkaline solution may be tightly correlated with the latter predicted factor. In fact, the pH values of GE-A and GE-B were approximately 7, that is, high values compared to that of SNMC (pH6.12). Therefore, it is thought that high CysS contents in GE-A and GE-B depended on the pH in both products. Whereas, the pH values in GE-E and GE-F were 5.15 and 5.29, respectively. Namely, both pH values were low compared to that in SNMC. The results that CysS contents were low in GE-E and GE-F were identified as the low pH values. Therefore, it is logical to consider that CysS content was affected by the pH level in product. As the other factors, sterilization temperature in manufacture process, dissolved oxygen in product solution, etc. were also considered, however, the detail information was not obtained.

The relationship between pH and stability of CysH is a very important problem. Generally, many generic drugs are produced by condition coordinated with the physicochemical properties of bland-name drug.^[15] Therefore, the quality of generic drugs is secured. However, in generic drugs of GZ, there are the products which set a pH level in the acidic or basic direction than SNMC. The difference in pH level supply a characteristic property, however, the recognition of the problem for the stability may be necessary. Furthermore, in all generic drugs of GZ, it is thought that antioxidant provision is necessary as well as setting of this pH based on CysS having been detected.

Gly concentration in SNMC was 100.2%. On the other hand, Gly concentrations in generic drugs were the range of 94.6-105.0%. Moreover, the variation (standard deviation; SD) in Gly concentration among batches in each product was also remarkably high compared to that in SNMC. Especially, the

SD in GE-D was 10.4%. The results that SD levels in generic drugs were more than 3% will submit as an important quality control.

Conclusions

The physicochemical properties (pH and osmolarity), ingredient contents (GZ, CysH, and Gly), and impurity (GA, MGGA, and CysS) in a brand-name drug (SNMC), 6 kind generic drugs with same ingredients, and 2 kind generic drugs with optical isomer of GZ were investigated. For the physicochemical properties, the pH and osmolarity ratio depending on each product were different, however, these values were within the defined values. And the differences in variation (SD) of pH and osmolarity among the batches in each product were observed with acceptable level. Therefore, it was judged that there were not problems for the physicochemical properties. On the other hand, some characteristic differences were observed for the comparison with brand-name drug in ingredient and impurity assay. First, the GZ contents in three batches of SNMC were at the range of 101.2-102.0% ($101.6 \pm 0.3\%$). This precision in SNMC was superior to other products conspicuously. Especially, the products which was under 95% in GZ content is recognized, and the products which the variation between the batches has a big are also recognized. Second, an interesting result was provided in the content of the CysH. Although sulfite sodium as an antioxidant was included in all products, the product which CysH content was maintained $100 \pm 5\%$ was only SNMC. In generic drugs, CysS content was affected by the pH level of the products, indicating that products with high CysS content were set to high pH level. Third, variation (SD) of Gly content in generic drugs was high compared with that of SNMC.

In conclusion, it was assumed that management during the process of manufacture, the purity of the ingredient, the fixed-quantity of the ingredient after the product preparation, and the way of the quality control of the batch unit will be needed to consider.

References

1. Nakata T, Kato H, Yano S, Kanaoka M, Pharmacokinetics of glycyrrhizin and glycyrrhetic acid in healthy human after prolonged administration of glycyrrhizin formulation. *Wakan Iyakugaku Kaishi* (Japanese) 1986;3:278-9.
2. Koga K, Kawashima S, Shibata N, Takada K. Novel formulation of a

- liver protection drug glycyrrhizin. *Yakugaku Zasshi* 2007;127:1103-14.
3. Sakiya Y, Akada Y, Kawano S, Miyauchi Y. Rapid estimation of glycyrrhizin and glycyrrhetic acid in plasma by high-speed liquid chromatography. *Chem Pharm Bull* (Tokyo) 1979;27:1125-9.
4. Koga K, Ri S, Ohyanagi K, Takada K, Comparative study of physicochemical properties and constituents of glycyrrhizin injectable solutions. *Jpn J Pharm Health Care Sci* (Japanese) 2008;34:593-600.
5. Koga K, Ohmachi K, Kawashima S, Takada K, Murakami M. Determination of alpha-glycyrrhizin and 18beta-glycyrrhizin in dog plasma by high-performance liquid chromatography. *Chromatogr B Biomed Sci Appl* 2000;738:165-8.
6. Hayashi M, Kadowaki E, Takamatsu T, Matsuoka M. Elucidation on abnormal behavior of glycyrrhizin salts in high performance liquid chromatography determination for quasi-drug products. *Yakugaku Zasshi* 1992;112:496-502.
7. Sasaki H, Suzuki N, AlShwaimi E, Xu Y, Battaglini R, Morse L, *et al.* 18 β -Glycyrrhetic acid inhibits periodontitis via glucocorticoid-independent nuclear factor-kappa B inactivation in interleukin-10-deficient mice. *J Periodontol Res* 2010;45:757-63.
8. Ishida T, Mizushima Y, Yagi S, Irino Y, Nishiumi S, Miki I, *et al.* Inhibitory effects of glycyrrhetic acid on DNA polymerase and inflammatory activities. *Evid Based Complement Alternative Med* 2012;2012:650514.
9. Serra A, Uehlinger DE, Ferrari P, Dick B, Frey BM, Frey FJ, *et al.* Glycyrrhetic acid decreases plasma potassium concentrations in patients with anuria. *J Am Soc Nephrol* 2002;13:191-6.
10. Sugimoto T, Ogawa N, Yamamoto K, Uzu T, Nishio Y, Kashiwagi A. A hyperparathyroid state in a patient with glycyrrhetic acid-induced pseudoaldosteronism. *Intern Med* 2007;46:57-8.
11. van Rossum TG, De Jong FH, Hop WC, Boomsma F, Schalm SW. Chronic hepatitis C and portal hypertension: Diagnosis, treatment with interferon, glycyrrhizin or propranolol. *J Gastroenterol Hepatol* 2001;16:789-95.
12. Akao T, Akao T, Hattori M, Kanaoka M, Yamamoto K, Namba T, *et al.* Hydrolysis of glycyrrhizin to 18 beta-glycyrrhetyl monoglucuronide by lysosomal beta-D-glucuronidase of animal livers. *Biochem Pharm* 1991;41:1025-9.
13. Ohtake N, Kido A, Kubota K, Tsuchiya N, Morita T, Kase Y, *et al.* A possible involvement of 3-monoglucuronyl-glycyrrhetic acid, a metabolite of glycyrrhizin (GL), GL-induced pseudoaldosteronism. *Life Sci* 2007;80:1545-52.
14. Kato H, Kanaoka M, Yano S, Kobayashi M. 3-Monoglucuronyl-glycyrrhetic acid is a major metabolite that causes licorice-induced pseudoaldosteronism. *J Clin Endocrinol Metab* 1995;80:1929-33.
15. Lewek P, Kardas P. Generic drugs: the benefits and risks of making the switch. *J Fam Prac* 2010;59:634-40.

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