

Screening and Quantitative Analysis for Sulfonylurea-Type Oral Antidiabetic Agents in Adulterated Health Food Using Thin-Layer Chromatography and High-Performance Liquid Chromatography

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(Received February 23, 2005; Accepted March 23, 2005)

Screening and quantitative analysis for six sulfonylurea-type oral antidiabetic agents (SU-OADs), tolbutamide (TOL), acetohexamide (ACE), chlorpropamide (CHL), gliclazide (GLC), glibenclamide (GLB), and glimepiride (GLM), in adulterated health food has been developed. The SU-OADs were extracted with acetone and then the extract was subjected to thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). In the TLC analysis, good separation was achieved with a mixture of *n*-butyl acetate containing 0.4% formic acid as a solvent. Ultraviolet irradiation at 254 nm was used to detect the SU-OADs. Specificity was obtained with Dragendorff's test solution, 10% phosphomolybdic acid methanol solution, and 30% sulfuric acid methanol solution. On the other hand, with a gradient reverse-phase HPLC system equipped with a photodiode array detector, we were able to detect the SU-OADs within 15 min using an ODS column and acetonitrile-ammonium acetate buffer as a mobile phase. Quantitative analysis based on HPLC was also performed with the absolute calibration curve method. Recoveries were 90.7–105.2% for the drugs tested. The intra- and interassay relative standard deviations were 0.2–8.1 and 0.6–7.2%, respectively. When the methods were applied to prescription glibenclamide tablets, health food, and spiked samples, good selectivity, separation, recovery, and precision were obtained.

Key words — sulfonylurea-type oral antidiabetic agent, screening, health food, thin-layer chromatography, high-performance liquid chromatography

INTRODUCTION

In recent years, with increasing of interest in health, the consumption of health food has been growing in Japan. The most commonly consumed health food may be vitamins and mineral products aimed at maintaining health. On the other hand, health food advertising weight loss, hypoglycemic effects, and the improvement of sexual dysfunction have a growing market share. Some products are adulterated with synthetic drugs,^{1–4)} although natu-

ral or herbal materials are stated to be the ingredients. Over the past few years in Japan, several cases have been reported concerning unexpected adverse effects when consumers have consumed health food adulterated with synthetic drugs.

An examination of distributed health food thus becomes an important issue to prevent the harmful effects of synthetic drugs as admixtures in health food from the standpoint of “proper distribution of medications” and “food safety.” As previously reported, glibenclamide, a sulfonylurea-type oral antidiabetic agent (SU-OAD), was detected in a health food product advertising a hypoglycemic effect.⁴⁾ However, there have been few reports concerning analytical methods for health food adulterated with synthetic drugs. For the analysis of SU-OADs, a number of reports have described analytical meth-

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ods for biosamples with high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC/MS).⁵⁻⁷⁾ The matrices of health food are diverse and different from those of biosamples. Therefore analytical methods differ based on the biosample. LC/MS is a valuable tool for the identification of constituents, although it is expensive and complicated. Recently, Ku *et al.* have reported an analytical method for antidiabetic drugs in adulterated traditional Chinese medicines using high-performance capillary electrophoresis (HPCE).⁸⁾ Although this method is applicable to the monitoring of the drugs in health food, HPCE is also a specialized method for most laboratories, including manufacturers of health food. Therefore simpler methods are required for the examination of drugs in products. In this study, the screening for and quantitative analysis of the SU-OADs tolbutamide (TOL), acetohexamide (ACE), chlorpropamide (CHL), gliclazide (GLC), glibenclamide (GLB), and glimepiride (GLM) in adulterated health food using thin-layer chromatography (TLC) and HPLC were developed.

MATERIALS AND METHODS

Chemicals and Reagents — TOL, GLC, and GLB were purchased from Sigma (St. Louis, MO, U.S.A.). CHL, ACE, and GLM were obtained from ICN Pharmaceuticals, Inc. (Bryan, OH, U.S.A.), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Toronto Research Chemicals, Inc. (North York, ON, Canada), respectively. Lactose was purchased from Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan). Potato starch was obtained from Yoshida Pharmaceutical Co., Ltd. (Tokyo, Japan). HPLC-grade acetonitrile and all other chemicals (analytical grade) were obtained from Wako Pure Chemical Industries, Ltd.

Sample Preparation — Teabag, capsule, tablet, and granulate health food was subjected to analysis in the present study. The content of teabags and tablets is powdered prior to the analysis. A 1-day or half-day dosage of each sample was weighed for analysis. For the teabags, one-quarter or one teabag was subjected to analysis. Each sample was mixed with 40 ml of acetone and shaken at 200 min⁻¹ for 1 hr with a reciprocating shaker (Recipro shaker, TAITEC Corporation, Saitama, Japan) before being centrifuged to obtain the supernatant. Then the extracts were evaporated until dried and reconstituted

with 2 ml of acetone for the TLC sample solutions. For HPLC analysis, each sample was extracted following the same procedure as used for the TLC samples and centrifuged. Then the supernatant was filtered using a 0.45- μ m syringe filter and the solution was used for the analysis.

TLC Analysis — Standard solutions of TOL (2 mg/ml), ACE (2 mg/ml), CHL (2 mg/ml), GLC (2 mg/ml), GLB (1 mg/ml), and GLM (500 μ g/ml) were prepared in methanol for TLC analysis. Silica gel 60 precoated HPTLC plates (thickness 0.25 mm) containing a fluorescent indicator at 254 nm (Merck, Darmstadt, Germany) were used. The plates were spotted, respectively, with 2 μ l of TOL, CHL, GLC, GLB, and GLM, and 1 μ l of ACE standard solutions at 20 mm from the bottom edge as the starting line and then every 8 mm along the line. The sealed chamber containing 100% *n*-butyl acetate or a mixture of *n*-butyl acetate containing 0.2, 0.4, or 2.0% formic acid was saturated for 1 hr following equilibration of the plates for 30 min in the chamber. Then the plates were developed to a distance of about 70 mm. After air-drying, the SU-OADs on the plates were examined under ultraviolet (UV) irradiation at 254 nm (main wavelength) and/or with spraying detection reagents, Dragendorff's test solution (DD), 10% phosphomolybdic acid methanol solution (PM), and 30% sulfuric acid methanol solution (SA). The DD solution for spraying was prepared according to the *Japanese Pharmacopoeia*.⁹⁾ Detection with SA was performed by spraying the solution following heating at 100°C for 1 min and UV irradiation at 254 nm.

For determination of the limit of detection (LOD), 2, 1, 0.5, and 0.2 μ l of TOL, CHL, GLC, GLB, and GLM standard solutions and 1, 0.5, 0.25, and 0.1 μ l of 0.2 mg/ml ACE solution were assayed. **HPLC Conditions** — Sample solutions were analyzed with an HPLC system consisting of a Waters Alliance 2695 separation module (Waters Corporation, Milford, MA, U.S.A.) equipped with photodiode array detector model 2996 (Waters Corporation). The samples were separated using a Cadenza CD-C18 (75 \times 4.6 mm, particle size 3 μ m) column coupled to the guard column from Imtakt Corporation (Kyoto, Japan) kept at 40°C during the run in the column oven. The mobile phase consisted of acetonitrile (eluent A) and ammonium acetate buffer (0.01 M, adjusted to pH 4.0 with acetic acid) (eluent B). The rapid gradient elution was started at 30% A held for 3 min, linearly increased to 50% A in 5 min, and held for 7 min. The running time was

15 min followed by 8 min to equilibrate the column. The flow rate was set at 1.0 ml/min. Injection volumes were 10 μ l/sample. The wavelength of the photodiode array detector for screening was set from 210 to 360 nm, and monitoring of chromatographic peaks was performed at 230 and 247 nm. Quantitative analysis was also performed at 230 nm for CHL, TOL, GLC, GLB, and GLM, and at 247 nm for ACE. Data storage and processing were performed with Empower software (Waters Corporation).

HPLC Analysis — The calibration standards with concentrations of 5, 10, 25, 50, and 100 μ g/ml of TOL, ACE, CHL, and GLC, and 1, 5, 10, 25, and 50 μ g/ml of GLB and GLM were made in volumetric flasks with methanol. The calibration standards were freshly prepared for each assay. To evaluate the linearity of the calibration curves, five calibration curves in each examination were separately prepared. To determine the LOD and limit of quantification (LOQ), 5 μ g/ml of the TOL, ACE, CHL, and GLC calibration standards and 1 μ g/ml of the GLB and GLM calibration standards were used. The LOD and LOQ were defined as averages of the amounts (ng) over 3 days at which the signal-to-noise ratio (SN) was 3 and 10, respectively.

Validation — The absolute recovery and precision of the quantitative analysis were determined over 3 days. Quality control (QC) samples were prepared at three doses (low, middle, and high) using a mixture of lactose and starch (1 : 1, w/w), both of which are used widely as excipients. Two grams of lactose and starch mixture were spiked with three doses of each of the SU-OADs as follows: CHL, ACE, TOL, and GLC, 0.2, 1, and 4 mg; GLB, 0.1, 1, and 2 mg; and GLM, 0.05, 0.2, and 0.5 mg. Six independent replicates were prepared at each QC sample concentration. Peak areas of QC samples were used to calculate analytical recoveries. The precision (relative standard deviation, RSD %) of the analytical procedure was evaluated by determining the intra- and interday coefficients of variation.

Applications — Four types of general prescription glibenclamide tablets (1.25 mg/tablet) were analyzed using the described TLC and HPLC methods ($n = 6$). In addition, 11 health food products advertising hypoglycemic effects were subjected to the described TLC method prior to HPLC analysis. Moreover, three representative types of health food, tablet, teabag, and capsule products were spiked with SU-OADs (CHL, ACE, TOL, GLC, and GLB, 1 mg; GLM, 0.2 mg) for assessment using HPLC methods ($n = 6$).

RESULTS AND DISCUSSION

TLC Analysis

The present study examined the optimal conditions for developing solvent mixtures and specific detection reagents for SU-OADs. Irradiation with UV rays at 254 nm was used for the assessment of the spot shape and separation of the SU-OADs. Several solvent mixtures are described in the *Japanese Pharmacopoeia*⁹⁾ and the *Japanese Pharmaceutical Codex*¹⁰⁾ for purity tests of SU-OADs. They are classified as acidic mixtures and basicity mixtures. For the development of TLC, an acidic mixture of *n*-butyl acetate, chloroform, and formic acid (3 : 2 : 1) was better than the basicity mixtures in terms of spot shape and separation because the basicity mixtures caused broader spots. The condition was optimized without chloroform to reduce chemical hazards to health and the environment. Four types of *n*-butyl acetate mixtures with a different ratio of formic acid (0, 0.2, 0.4, or 2%) were prepared and subjected to TLC analysis. Representative TLC chromatograms are shown in Fig. 1. With an increased concentration of formic acid in the mixture, the sensitivity and R_f value of GLC were improved under UV irradiation at 254 nm, although the separation of the SU-OADs tested worsened. The best separation of SU-OADs was achieved with the *n*-butyl acetate mixture containing 0.4% formic acid. Representative R_f values of TOL, ACE, CHL, GLC, GLB, and GLM in three examinations were 0.78, 0.58, 0.69, 0.48, 0.40, and 0.35, respectively.

In the examination of detection reagents for SU-OADs, the spraying of DD produced a yellow-red spot from the GLB standard solution and pale yellow-red spots from the GLC and GLM standard solutions. Only a dark-green spot from the GLC standard solution was detected with the spraying of PM. By spraying SA following heating at 100°C for 1 min and UV irradiation at 254 nm, a characteristic blue fluorescent spot was obtained from the GLB standard solution. These specific responses to detection reagents were a help in the detection of the drugs in the crude samples. On the other hand, specificity with detection reagents for TOL, ACE, and CHL remained unclear.

Each LOD of the SU-OAD on the TLC plate developed with the optimal solvent was determined visually (Table 1). The sensitivity of TOL with UV irradiation was inferior. Because a dosage of hundreds of milligrams of TOL is used for clinical treatment, it is considered that TOL is sufficiently sensi-

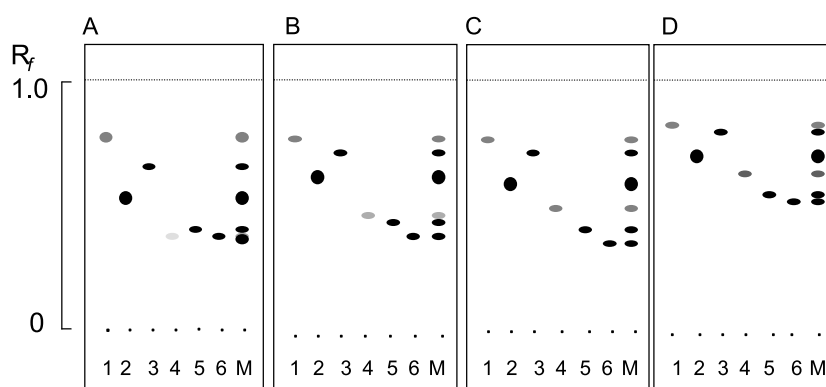


Fig. 1. Representative TLC Chromatograms of SU-OADs Developed with Formic Acid/*n*-Butyl Acetate Mixtures

Solvents used in the development of TLC were: A) 100% *n*-butyl acetate, B) 0.2% formic acid/*n*-butyl acetate mixture, C) 0.4% formic acid/*n*-butyl acetate mixture, and D) 2% formic acid/*n*-butyl acetate mixture. These spots were detected with UV irradiation at 254 nm. [◐] Pale black spot. [●] Black spot. Spot no. 1: tolbutamide; no. 2: acetoexamide; no. 3: chlorpropamide; no. 4: gliclidazide; no. 5: glibenclamide; no. 6: glimepiride; M: mixture of standard solutions.

Table 1. LODs of SU-OADs on TLC Developed with 0.4% Formic Acid/*n*-Butyl Acetate Mixture

Drug	UV (μg)	DD (μg)	PM (μg)	SA (μg)
TOL	2.0	—	—	—
ACE	0.01	—	—	—
CHL	0.5	—	—	—
GLC	0.5	2.0	0.25	—
GLB	0.5	0.5	—	0.25
GLM	0.25	1.0	—	—

Each LOD was determined visually with triplicate independent assays. Each value is shown as spot micrograms. UV: ultraviolet irradiation at 254 nm; DD: Dragendorff's test solution; PM: 10% phosphomolybdic acid methanol solution; SA: 30% sulfuric acid methanol solution; [—]: not detected.

tive to detect the agents in health food. In addition, ACE was the most sensitive of the SU-OADs tested. By spraying the detection reagents, the sensitivity and selectivity of GLC and GLB improved. Since the clinical dosage of GLB is in the order of milligrams, concentrations of GLB are estimated to be several milligrams or less of the daily dosage in samples. Therefore spraying of SA may be indispensable for the analysis of GLB using TLC.

As the results suggest, the detection of GLC, GLB, and GLM is achieved by comparing R_f values under UV irradiation and specific color and fluorescent spots using detection reagents.

HPLC Analysis

As shown in Fig. 2A and 2B, good separation of the tested SU-OADs was obtained using the HPLC conditions described above. Because the pK_a values of SU-OADs are weakly acidic, an acetate buffer

adjusted to pH 4.0 was employed as the mobile phase. The representative retention times of CHL, ACE, TOL, GLC, GLB, and GLM were about 6.1, 6.9, 7.5, 9.2, 11.4, and 12.5 min, respectively. The UV spectrum of each peak of the SU-OADs was also determined (Fig. 2C). UV absorption spectra of CHL, TOL, GLC, and GLM were a similar, which had a maximum of only approximately 230 nm. The ACE spectrum exhibited a maximum of 247 nm. The spectrum of GLB exhibited maxima of 230 and 301 nm. In addition, a slight maximum of 275 nm was also observed in the GLB spectrum (Fig. 3). The 275 and 301 nm maxima on the spectrum are employed for one method for the identification of GLB in the *Japanese Pharmacopoeia*.⁹⁾

Validation of Quantitative Analysis using HPLC

The linearity of the calibration curves of the SU-OADs is represented by the correlation coefficients (r), slope, and intercept with y -axis values. In the linear equation, the peak-area of the drugs can be expressed as the y -axis, while the x -axis is their concentration ($\mu\text{g}/\text{ml}$). The regression equations of five calibration curves and their correlation coefficients were: CHL, $y = 32315x + 5099$ ($r = 0.9999$); ACE, $y = 30137x + 306$ ($r = 0.9999$); TOL, $y = 29502x + 4478$ ($r = 0.9999$); GLC, $y = 25387x + 13513$ ($r = 0.9999$); GLB, $y = 32871x + 2542$ ($r = 0.9999$); GLM, $y = 31395x + 3196$ ($r = 0.9999$). The RSD values of the slopes of CHL, ACE, TOL, GLC, GLB, and GLM were 1.7, 0.5, 1.9, 1.5, 1.5, and 0.6%, respectively.

The LOD and LOQ values of the SU-OADs are shown in Table 2. The LOD values as amounts in-

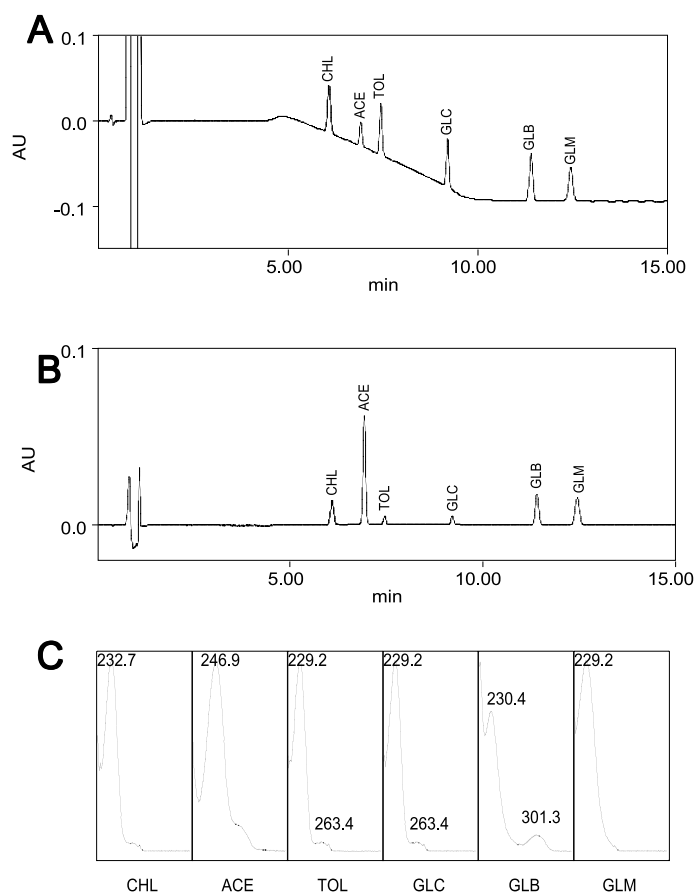


Fig. 2. Representative HPLC Chromatograms of Standard Solution (A, B) and UV Spectra of the Detected Peaks (C)

A, B) The standard solution contained 10 $\mu\text{g/ml}$ of tolbutamide, acetohexamide, chlorpropamide, gliclazide, glibenclamide, and glimepiride. The injection volume was 10 μl . Monitoring of chromatographic peaks was performed at 230 nm (A) and 247 nm (B). C) Each UV spectrum from 210 and 360 nm was obtained from detected peaks of the standard solution. CHL: chlorpropamide; ACE: acetohexamide; TOL: tolbutamide; GLC: gliclazide; GLB: glibenclamide; GLM: glimepiride.

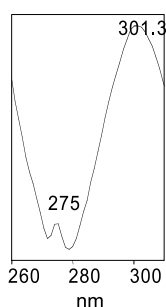


Fig. 3. Expanded UV Spectrum of the Glibenclamide Peak

The UV absorption spectrum of the glibenclamide peak on the HPLC chromatogram in Fig. 2A was expanded between 260 to 310 nm.

ected were less than 2 ng for each of the SU-OADs. These results suggest that the sensitivity of HPLC is 10–2000 times higher than that of TLC. When doubtful spots were detected in TLC analysis, sample extracts had to be analyzed using HPLC.

Intra- and interday variations of these assays for

Table 2. LOD and LOQ of SU-OADs in HPLC Analysis

Drug	LOD (ng)	LOQ (ng)
CHL	1.2	3.9
ACE	1.1	3.7
TOL	1.8	6.0
GLC	1.8	6.1
GLB	0.8	2.5
GLM	0.9	2.9

The LOD and LOQ values were determined with signal-to-noise ratios of 3 and 10, respectively. Monitoring of chromatographic peaks was performed at 230 nm for CHL, TOL, GLC, GLB, and GLM, and at 247 nm for ACE. Each value is in nanograms of injection (10 $\mu\text{l/sample}$).

three dosages of the QC samples were determined. QC samples were prepared using drug excipients. One health food may not represent all matrices of samples distributed on the market because of the difference in ingredients. Therefore excipients were used as model samples. No peak interfering with the

Table 3. Intra- ($n = 6$) and Interassay ($n = 18$, 3 days) Recovery Rates and Precision (RSD %) Data in HPLC Analysis

Drug		Low dose		Middle dose		High dose	
		Mean %	RSD %	Mean %	RSD %	Mean %	RSD %
CHL	Day 1	91.7	4.8	95.0	1.3	93.1	0.8
	Day 2	100.5	4.4	98.0	1.0	96.3	0.7
	Day 3	98.4	6.6	98.2	0.7	98.9	0.5
	Interassay	96.8	6.5	97.1	1.8	96.1	2.6
ACE	Day 1	94.6	1.0	96.6	1.3	97.7	0.4
	Day 2	98.1	3.1	97.0	1.4	100.6	0.3
	Day 3	90.7	1.8	97.5	1.4	105.2	0.4
	Interassay	94.4	3.9	97.0	1.3	101.2	3.2
TOL	Day 1	94.5	1.5	99.3	0.6	96.6	0.7
	Day 2	99.1	2.2	99.4	0.7	99.3	2.7
	Day 3	97.3	2.9	98.0	0.8	99.0	0.2
	Interassay	97.0	3.0	98.9	0.9	98.3	2.0
GLC	Day 1	99.0	3.4	98.0	0.9	96.8	1.1
	Day 2	103.3	2.8	98.7	0.5	98.6	0.8
	Day 3	100.7	3.5	97.3	0.7	97.5	0.6
	Interassay	100.9	3.6	98.0	0.9	97.6	1.1
GLB	Day 1	96.2	2.6	98.3	0.8	100.1	0.4
	Day 2	93.5	2.4	100.0	0.5	100.0	0.4
	Day 3	101.3	2.8	100.2	0.6	101.0	0.4
	Interassay	97.0	4.2	99.5	1.1	100.4	0.6
GLM	Day 1	96.0	8.1	99.4	3.1	101.4	1.3
	Day 2	101.8	5.8	100.8	1.0	100.1	0.9
	Day 3	97.2	7.6	100.5	2.0	102.7	0.7
	Interassay	98.3	7.2	100.2	2.1	101.4	1.4

Dosages: CHL, ACE, TOL and GLC: 0.2 mg (low), 1 mg (middle), and 4 mg (high); GLB: 0.1 mg (low), 1 mg (middle), and 2 mg (high), GLM: 0.05 mg (low), 0.2 mg (middle), and 0.5 mg (high). RSD % : precision.

SU-OADs was found in the HPLC chromatogram of the blank excipient. As shown in Table 3, the intra- and interday recovery rates ranged from 90.7 to 105.2%. The intra- and interday precisions (RSD) of these QC samples for each analyte were lower than 8.1. Although the precisions of the low-dosage drugs were slightly higher than the other QC samples, these results were acceptable.

Applications

This assay was also used for the screening and determination of GLB in four types of prescription GLB tablets as model samples. GLB is commonly used as an oral antidiabetic drug and is sometimes detected in health food.⁴⁾ Each powdered solution of GLB tablet was equivalent in weight to one tablet. These samples were independently analyzed with the TLC and HPLC methods. TLC analysis showed that a spot was detected at an R_f value of 0.38 by

UV irradiation with all of the sample solutions. The spots also showed a specific color or fluorescence with DD and SA. The properties of the spots corresponded to those of GLB. HPLC analysis also showed that the UV absorption spectrum of a peak at 11.5 min had maxima of 230 and 301 nm and a slight maximum of 275 nm, which corresponded to GLB. Quantitative analysis of these samples showed 92.5 to 100.2% content versus the indicated amount. Assay precisions were between 0.4 and 0.9%. In general, the content of the synthetic drugs ranged from 90 to 110% of the indicated amount. The true amount in the tablets was unclear, although these results satisfied the criterion.

The present TLC analysis was also applied to 11 types of health food, such as tablets (samples A–D, G, and H), teabags (samples E, I, and J), and capsule products (samples F and K) advertising hypoglycemic effects prior to HPLC analysis. The TLC plates

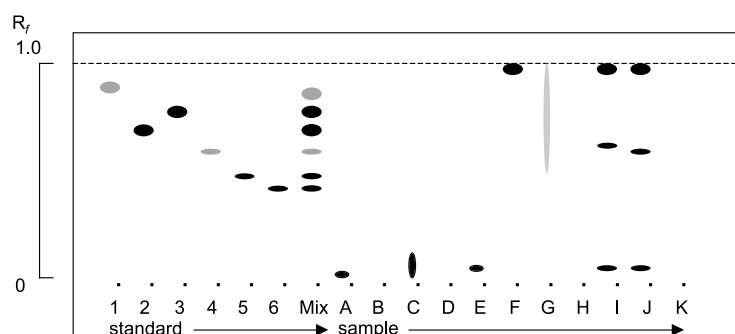


Fig. 4. TLC Chromatograms of 11 Types of Health Food Developed with 0.4% Formic Acid/*n*-Butyl Acetate Mixture

These spots were detected with UV irradiation at 254 nm. [○]: Pale black spot. [●]: Black spot. Standard no. 1: tolbutamide; no. 2: acetohexamide; no. 3: chlorpropamide; no. 4: gliclazide; no. 5: glibenclamide; no. 6: glimepiride; Mix: mixture of standard solutions. Samples A–D, G, H: tablet-type health food; E, I, J: teabag-type health food; F, K: capsule-type health food.

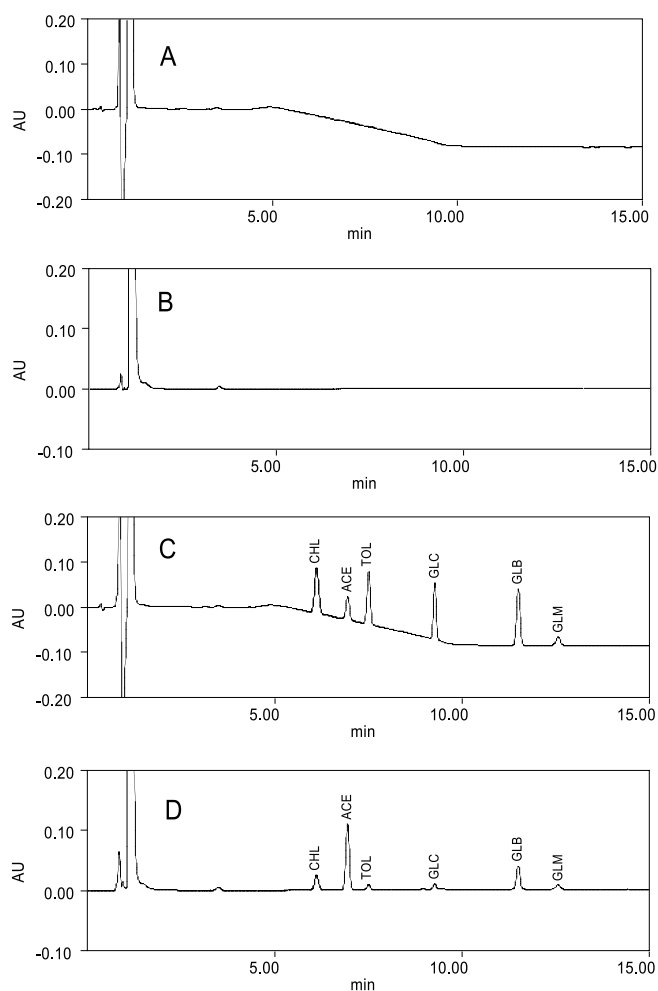


Fig. 5. Representative HPLC Chromatograms of Teabag-Type Health Food (A, B) and Spiked Health Food (C, D)

A, B) Teabag-type health food (corresponding to sample E in Fig. 4) advertising hypoglycemic effects. C, D) Health food spiked with SU-OADs. The monitoring of chromatographic peaks was performed at 230 nm (A, C) and 247 nm (B, D).

were spotted with 3 μ l of each sample solution. As shown in Fig. 4, a few impurity spots were detected among several samples with UV irradiation. Al-

though spots at R_f 0.59 and 0.57 resembled the R_f value of GLC in samples I and J, respectively, these spots were not detected using DD and PM reagents.

Table 4. Recovery Rates of Six Drugs in Three Types of Health Foods ($n = 6$)

Drug	Sample 1		Sample 2		Sample 3	
	Mean %	RSD %	Mean %	RSD %	Mean %	RSD %
CHL	101.2	0.7	101.3	1.6	98.0	1.0
ACE	93.2	2.3	101.3	1.4	99.5	0.3
TOL	99.5	0.6	100.2	0.9	97.1	0.9
GLC	100.2	0.6	96.3	0.9	96.2	1.1
GLB	97.6	1.2	96.8	0.6	95.1	0.8
GLM	99.3	1.3	99.0	1.5	95.6	1.9

Sample 1: tablet; sample 2: teabag; sample 3: capsule. Dosages: CHL, ACE, TOL, GLC, and GLB: 1 mg; GLM: 0.2 mg. RSD % : precision.

In addition, no peak that interfered with the SU-OADs was found in the HPLC chromatograms of any sample. Therefore these results indicate that the SU-OADs tested were not detected in all samples. Moreover, three tablet, teabag, and capsule products, corresponding to samples C, E, and K, respectively, were used as model samples for the recovery studies of real samples. A representative HPLC chromatogram of the teabag-type health food is shown in Fig. 5. The recoveries of these spiked samples are shown in Table 4. Assay precisions shown as RSD% were lower than 2.3%. All of these data indicate good accuracy of the methods.

In conclusion, these results suggest that the described methods can be applied to the analysis of SU-OADs and to determine their contents in adulterated health food with simple techniques. However, the present study aimed at the screening of SU-OADs, and therefore the final identification of detected constituents must be analyzed with infrared spectrometry and/or mass spectrometry. Finally, these reported methods are usable for examination not only by researchers but also by manufacturers. We expect increases in the number of opportunities for examination to lead to a decline in the harmful effects of health food.

Acknowledgements This study was supported by Grants-in-Aid for the Encouragement of Basic Science and Technology from the Kanagawa Prefectural Government. We thank Natsuko Matsuda and Yoriko Aso for technical assistance.

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