HPLC Determination of (+)-Pseudoephedrine and (−)-Ephedrine in Japanese Herbal Medicines Containing Ephedra Herb Using Solid-Phase Extraction

Makoto ICHIKAWA,* Manabu UDAYAMA, Kazuhiko IMAMURA, Sumihiro SHIRAISHI, and Hiromichi MATSUURA

Healthcare Research Institute, Wakunaga Pharmaceutical Co., Ltd.; 1624, Shimokotachi, Koda-cho, Takata-gun, Hiroshima 739–1195, Japan. Received January 14, 2003; accepted February 24, 2003

We developed a rapid and simple HPLC method combined with solid-phase extraction (SPE) for quantitative analysis of (+)-pseudoephedrine (PEP) and (−)-ephedrine (EP) in Japanese herbal (Kampo) medicines such as Kakkon-to, Sho-seiryu-to, Goshaku-san and Bofu-tsusho-san. SPE was performed on TOYOPAK IC-SP M containing propylsulfonic groups. Determination of PEP and EP was carried out using ion-pair reversed-phase HPLC with sodium dodecyl sulfate. *N*-Benzyldiethylamine was used as an internal standard. The analytical procedure was validated with regard to specificity, linearity, accuracy, and precision. These data suggest that the analytical method developed in this study is useful for quantitative analysis of PEP and EP in various formulations of Kampo medicine containing Ephedra herb.

Key words Ephedra herb; solid-phase extraction; Japanese herbal medicine; (+)-pseudoephedrine; (−)-ephedrine; quantitative analysis

Ephedra herb, the terrestrial stems of *Ephedra sinica* STAPF, *E. intermedia* SCHRENK et C.A. MEYER or *E. equisetina* BUNGE (Ephedraceae) defined in the Japanese Pharmacopoeia fourteenth edition (JP14),[1] is one of the most important crude drugs of Japanese herbal (Kampo) medicines which have been used as antitussive, expectorant, antipyretic analgesic, and bronchodilator agents. It has been reported that the biological properties of Ephedra herb are attributed to ephedrine-type alkaloids such as (+)-pseudoephedrine (PEP), (−)-ephedrine (EP), (−)-norephedrine, (+)-norpseudoephedrine, (−)-methylephedrine, and (+)-methylpseudoephedrine. In these alkaloids, PEP and EP are the main bioactive constituents in most *Ephedra* species and comprise 74—100% of the total alkaloids in the plants.[2,3] The total contents of PEP and EP are also regulated in the JP14.[1]

Several reports have been previously published on the quantitative determination of ephedrine-type alkaloids in Ephedra herb using HPLC,[1—10] GC,[11—14] and capillary electrophoresis.[15—18] JP14 adopted an ion-pair reversed-phase HPLC method using sodium dodecyl sulfate (SDS).[1]

The pretreatment of the quantitative analyses of ephedrine-type alkaloids in some Kampo medicines, which are simple formulas or consist of less than 9 kinds of crude drugs, can be completed with the simple extraction (*e.g.* 50% methanol).[4,8,10,18] On the other hand, it is difficult to apply the simple extraction for the pretreatment of the complex formulas such as Goshaku-san and Bofu-tsusho-san, which consist of 17 kinds of crude drugs. The complete extraction of the alkaloids requires the extraction procedure with chloroform under alkali condition.[3] However, the use of chloroform should be avoided from the viewpoint of environmental protection. Others have also reported using extraction solvents such as diethyl ether, but the methods are too complicated because they require at least 4 extractions.[5,9,19]

Recently, solid-phase extraction (SPE) has been used as an alternative to liquid-liquid extraction. Hurlbut *et al.*[7] used SPE for sample preparation of herbal products containing ephedrine-type alkaloids, however an internal standard was not used.

In this study, we developed a method for the quantitative analysis of PEP and EP in Kampo medicines such as Kakkon-to, Goshaku-san, Sho-seiryu-to, and Bofu-tsusho-san that uses pretreatment by cation-exchange SPE coupled with an ion-pair reversed-phase HPLC method. The method was also applied to Kampo medicine formulations such as fine granules and syrup.

Experimental

Materials and Reagents (+)-Pseudoephedrine hydrochloride, (±)-norephedrine (NE) hydrochloride and (±)-methylephedrine (ME) hydrochloride were purchased from Alps Pharmaceutical Industries Co., Ltd. (Gifu, Japan). (−)-Ephedrine hydrochloride was purchased from Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan). Crude drugs controlled by JP14[1] and The Japanese Standards for Herbal Medicines[20] were obtained from Matsuura Yakugyo Co., Ltd. (Nagoya, Japan). SDS (ion-pair reagent), acetonitrile (HPLC) and *N*-benzyldiethylamine (IS, internal standard) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). A TOYOPAK IC-SP M column was purchased from Tosoh Co., Ltd. (Tokyo). A Mega Bond Elut PRS column was purchased from Varian (CA, U.S.A.). The SPE column was first preconditioned with 10 ml each of methanol, followed by water, and finally a mixture of ethanol and 0.1% phosphoric acid (9 : 1, v/v; solvent A). An IS solution of 0.8 mg/ml as the internal standard solution was prepared in 1-propanol. The formulations of the commercial medicines evaluated in this study are listed in Table 1.

Preparation of Kampo Medicine Extracts Extraction was performed according to a previously described method.[21] The mixtures of the crude drugs were prepared based on the Kampo formulas shown in Table 2. Each mixture was placed in a bottle and then extracted with 20 times the volume of water at 100 °C for 1 h. The extracts were filtrated with gauze and lyophilized to obtain the dried extracts. Ephedra herb deficient extracts were also prepared in the same manner.

HPLC Systems An HPLC system (Shimadzu, Kyoto, Japan) consist of SIL-10ADVP auto-injector, SPD-M10AVP photodiode array detector, LC-10ADVP pump, DGU-14A degasser, and CBM-10A communications bus module was used. Separations were carried out with a TSK gel ODS-80TSQA (particle size of the packing 5 μm, 150 mm×4.6 mm i.d., Tosoh). The mobile phase was a mixture of water, acetonitrile and phosphoric acid (650 : 350 : 1, v/v/v) containing 0.5% SDS and delivered at a flow rate of 1 ml/min. The column temperature was maintained at 50 °C with CTO-10ACVP column oven (Shimadzu). The detection wavelength was set at 210 nm for quantitative determination and was set in the range of 190—350 nm for the evaluation of peak purity. Analytical data were acquired using

* To whom correspondence should be addressed. e-mail: ichikawa_m@wakunaga.co.jp

Table 1. Formulations of Commercial Medicines Evaluated

Product (Forms)	Ingredients
A (Fine granule)	Goshaku-san
B (Fine granule)	Kakkon-to, acetaminophen, anhydrous caffeine, noscapine hydrochloride, chlorpheniramine maleate, potassium guaiacolsulfonate
C (Syrup)	Kakkon-to, acetaminophen, anhydrous caffeine, noscapine hydrochloride, chlorpheniramine maleate, potassium guaiacolsulfonate

Table 2. Kampo Medicine Extracts Evaluated

Formula	Crude drugs[a)]
Kakkon-to	Pueraria Root (8), Ephedra Herb (4), Jujube (4), Cinnamon Bark (3), Peony Root (3), Glycyrrhiza (2), Ginger (1).
Goshaku-san	Atractylodes Lancea Rhizome (3), Citrus Unshiu Peel (2), Poria Sclerotium (2), Pinellia Tuber (2), Japanese Angelica Root (2), Magnolia Bark (1), Peony Root (1), Cnidium Rhizome (1), Angelica Dahurica Root (1), Immature Orange (1), Platycodon Root (1), Cinnamon Bark (1), Ephedra Herb (1), Jujube (1), Ginger (1), Dried Ginger (1), Glycyrrhiza (1).
Sho-seiryu-to	Ephedra Herb (3), Peony Root (3), Dried Ginger (3), Glycyrrhiza (3), Cinnamon Bark (3), Asiasarum Root (3), Schisandra Fruit (3), Pinellia Tuber (6).
Bofu-tsusho-san	Japanese Angelica Root (1.2), Peony Root (1.2), Cnidium Rhizome (1.2), Gardenia Fruit (1.2), Forsythia Fruit (1.2), Mentha Herb (1.2), Ginger (1.2), Schizonepeta Spike (1.2), Saposhnikovia Root (1.2), Ephedra Herb (1.2), Rhubarb (1.5), Glauber's salt (1.5), Platycodon Root (2), Scutellaria Root (2), Glycyrrhiza (2), Gypsum (2), Talc (3).

a) Ratios of the crude drugs are shown in parentheses.

CLASS-LC10 software (version 1.64, Shimadzu). The injection volume was 20 μl.

Evaluation of Extraction Method The extraction solvents were determined from extraction efficiency and peak separation of the mixture containing PEP, EP and IS on the chromatogram. Kampo medicine extracts were extracted by shaking for 20 min with 50% methanol or three kinds of solvent mixtures of ethanol and 0.1% phosphoric acid (1 : 1, 8 : 2 or 9 : 1; v/v). The extract solution was pretreated with a TOYOPAK IC-SP M column, and then analyzed by HPLC. Reflux was performed on a water bath at 90 °C, and extraction time was determined by the extraction efficiencies of PEP and EP in Kampo medicine extracts.

Evaluation of SPE Column for Pretreatment Two kinds of SPE columns, a TOYOPAK IC-SP M column and a Mega Bond Elut PRS column, were tested. The standard solution containing PEP, EP, IS, NE and ME was prepared with solvent A (0.04 mg/ml). Ten ml of this solution was slowly passed through an SPE column. After washing the column with 10 ml of solvent A, the analytes were eluted with 5 ml of 0.75 mol/l ammonium chloride. The eluate was further diluted to 10 ml with water and analyzed by HPLC. The recoveries of these compounds were calculated by comparison with standards with or without the SPE procedure.

SPE Conditions The standard solution containing PEP, EP and IS was prepared with solvent A (0.04 mg/ml). Ten ml of this solution was slowly passed through a TOYOPAK IC-SP M column, and washed with 10 ml of solvent A. In order to determine the ionic strength of the eluate, the analytes were eluted with 5 ml of three different concentrations of ammonium chloride solution (0.5 mol/l, 0.75 mol/l or 1.0 mol/l). To determine the volume of eluate, the analytes were eluted 5 times with 2 ml of 0.75 mol/l ammonium chloride. Each eluate was further diluted to 10 ml with water and analyzed by HPLC.

HPLC Conditions To optimize PEP, EP and IS separation, the concentrations of acetonitrile and SDS used were in the range of 33—37% and 0.4—0.6%, respectively, and column temperature was evaluated at 25, 35 or 50 °C.

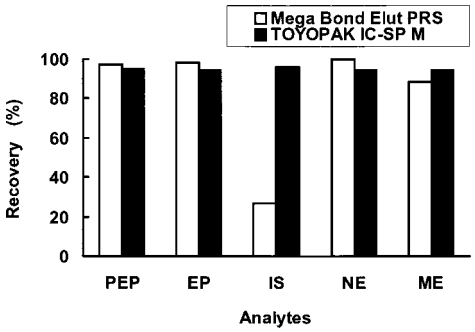

Fig. 1. Effects of TOYOPAK IC-SP M and Mega Bond Elut PRS on PEP, EP, IS, NE and ME Recoveries

PEP: (+)-pseudoephedrine, EP: (−)-ephedrine, IS: *N*-benzyldiethylamine, NE: (±)-norephedrine, ME: (±)-methylephedrine.

Assay Procedure Solid sample containing PEP (0.4—1.0 mg) was added to 2 ml of the internal standard solution and 50 ml of solvent A. The solution was heated under a reflux condenser on a water bath at 90 °C for 20 min, and centrifuged at 3000 rpm for 10 min. Ten ml of supernatant was slowly passed through the TOYOPAK IC-SP M column. The column was washed with 10 ml of solvent A and eluted with 10 ml of 0.75 mol/l ammonium chloride. In the case of a liquid sample (Product C in Table 1), 30 ml of sample was added to 2 ml of the internal standard solution and 20 ml of solvent A. The solution was briefly mixed, and centrifuged at 3000 rpm for 10 min. The supernatant was pretreated with the column in the same manner.

Results and Discussion

Evaluation of Extraction Method The extraction method was evaluated using 50% methanol as the extraction solvent, which has been previously used for analyzing ephedrine-type alkaloids in Ephedra herb.[1,2,18)] The results of the HPLC analysis indicated that the polar constituents in the Kampo medicine extracts interfered with the PEP, EP and IS peaks on the chromatogram. We evaluated the effects of the extraction solvents on the polarity using three kinds of solution mixtures of ethanol and 0.1% phosphoric acid (1 : 1, 8 : 2 or 9 : 1; v/v). An unsatisfactory background level on the chromatogram was observed with increasing polarity of the extraction solvents. Therefore, we decided to use a mixture of ethanol and 0.1% phosphoric acid (9 : 1, v/v; solvent A) as the extraction solvent. However, decreasing polarity caused solidification of the sample and resulted in low extraction efficiencies for PEP and EP. The complete extraction of PEP and EP from Kampo medicine extracts was improved by refluxing with solvent A for 20 min without any decomposition of PEP, EP or IS.

Evaluation of SPE Column for Pretreatment The results of the recovery test are shown in Fig. 1. There was no significant difference in the recoveries of the alkaloids, but the recovery of IS for Mega Bond Elut PRS was considerably lower than that of TOYOPAK IC-SP M. Therefore, TOYOPAK IC-SP M was selected as the pretreatment column. TOYOPAK IC-SP M columns are packed with resin based on polymers, whereas Mega Bond Elut PRS is based on silica gel. The interactions between IS and the silica surface may cause the low recovery seen with Mega Bond Elut PRS. Additionally, not only PEP and EP, but also NE and ME showed good recoveries when TOYOPAK IC-SP M was used. The results suggest that our method can be also useful for the minor constituents (−)-norephedrine and (−)-methylephedrine, as

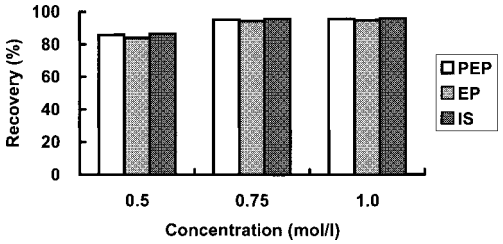

Fig. 2. Effects of Ammonium Chloride Concentration on PEP, EP and IS Elution

PEP: (+)-pseudoephedrine, EP: (−)-ephedrine, IS: *N*-benzyldiethylamine.

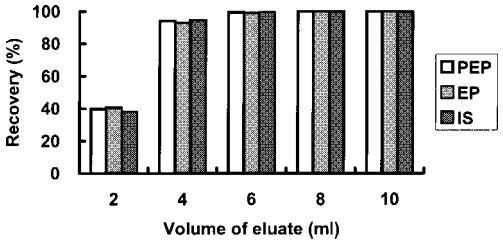

Fig. 3. Effects of Eluate Volume of Ammonium Chloride Solution on PEP, EP and IS Elution

PEP: (+)-pseudoephedrine, EP: (−)-ephedrine, IS: *N*-benzyldiethylamine.

well as PEP and EP in Ephedra herb.

SPE Conditions The recoveries of the alkaloids were about 85% in the case of 0.5 mol/l ammonium chloride, but both the usage of 0.75 mol/l and 1.0 mol/l ammonium chloride yielded good recoveries of about 95%, as shown in Fig. 2. As the eluate, 0.75 mol/l ammonium chloride was adopted to prevent excess elution of other constituents. The retained compounds were eluted completely by more than 8 ml of the eluate (Fig. 3). Therefore, 10 ml was chosen as an adequate volume.

HPLC Conditions A acetonitrile, diluted phosphoric acid, and SDS have been used as the mobile phase for HPLC analysis.[1—4] In this study, to find the optimum elution conditions, parameters including the concentrations of acetonitrile and SDS were examined. The peaks of PEP, EP and IS could not be completely separated when 37% acetonitrile was used, but 33% and 35% produced good separation among these compounds (Fig. 4a). Thirty five % was selected as an optimum concentration of acetonitrile in the mobile phase to shorten the analytical time. Figure 4b shows the effects of the SDS concentration on the retention time for PEP, EP and IS. The retention of all compounds was dependent on the SDS concentration, and complete separation was achieved in the concentration range of 0.4—0.6%. On the other hand, only 0.5% SDS showed good separation when Kampo medicine extracts were analyzed. Therefore, we decided to use 0.5% SDS as the optimum concentration for analysis of PEP and EP in the Kampo medicine extracts. With respect to column temperature, all of the conditions tested in this study showed good separation (Fig. 4c). Under these conditions, the optimum temperature to elute all constituents within 35 min was 50 °C.

Method Validation The proposed method for the quantitative analysis of PEP and EP in Kampo medicines was validated with regard to specificity, linearity, accuracy and precision.

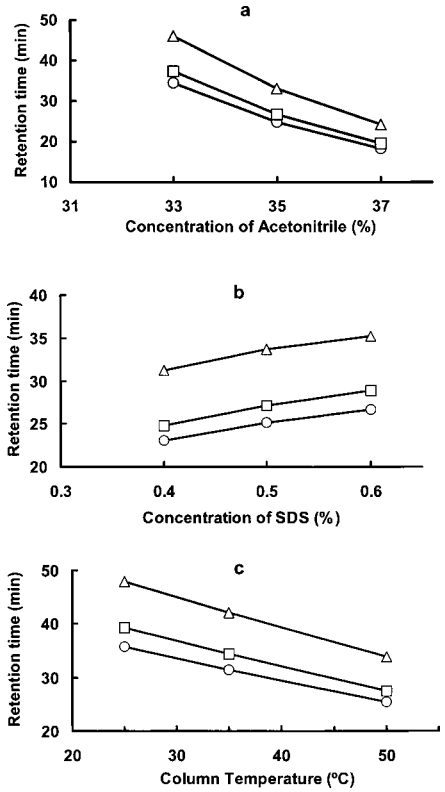

Fig. 4. Effects of Acetonitrile Concentration (a), SDS Concentration (b) and Column Temperature (c) on Elution Profiles of PEP, EP and IS

○: (+)-pseudoephedrine (PEP), □: (−)-ephedrine (EP), △: *N*-benzyldiethylamine (IS). HPLC conditions: column, TSK gel ODS-80TSQA (particle size of the packing 5 μm, 150 mm×4.6 mm i.d.); flow rate, 1 ml/min; wavelength, 210 nm. (a) Column temperature, 50 °C; mobile phase, mixture of water and acetonitrile containing 0.5% SDS and 0.1% phosphoric acid. (b) Column temperature, 50 °C; mobile phase, mixture of water, acetonitrile and phosphoric acid (650 : 350 : 1, v/v/v) containing SDS. (c) Mobile phase, mixture of water, acetonitrile and phosphoric acid (650 : 350 : 1, v/v/v) containing 0.5% SDS.

The specificity was confirmed by analyzing the Kampo medicine extracts and their Ephedra herb deficient extracts. HPLC analysis using Ephedra herb deficient extracts showed no interfering peaks (Fig. 5). The PEP, EP and IS peaks in the Kampo medicine extracts were identified with standards by inspection of their retention times and UV spectra.

Linearity was examined with standard samples prepared in the range of 2—70 μg/ml of PEP and EP containing IS (32 μg/ml). Linear relationships between the concentration (μg/ml, x-axis) and peak area ratio (PEP or EP/IS, y-axis) were expressed by the following equations: $y=0.0294x+0.0042$ for PEP and $y=0.0290x+0.0048$ for EP. The correlation coefficients were greater than 0.999. Both calibration curves yielded straight lines.

Accuracy tests were carried out as follows: (+)-pseudoephedrine hydrochloride and (−)-ephedrine hydrochloride at 3.2 mg/g (for Kakkon-to and Sho-seiryu-to) and 1.6 mg/g (for Goshaku-san and Bofu-tsusho-san) were added to Ephedra herb deficient extracts. The recoveries were 98.2—99.7% for PEP and 100.1—101.4% for EP, and the coefficients of variation (CV) values were 0.1—0.4% and 0.1—0.6%, respectively (Table 3).

Intra-day precision was investigated by analyzing three separately prepared samples on the same day. The CV values were 0.1—0.8% for Kampo medicine extracts (Table 4) and 0.1—0.4% for commercial medicines (Table 5). The repro-

ducibility of injection was evaluated by six replicate injections using the standard solution and the sample solutions of Kampo medicine extracts. The CV values were less than 0.4% for all tested solutions, as shown in Table 6.

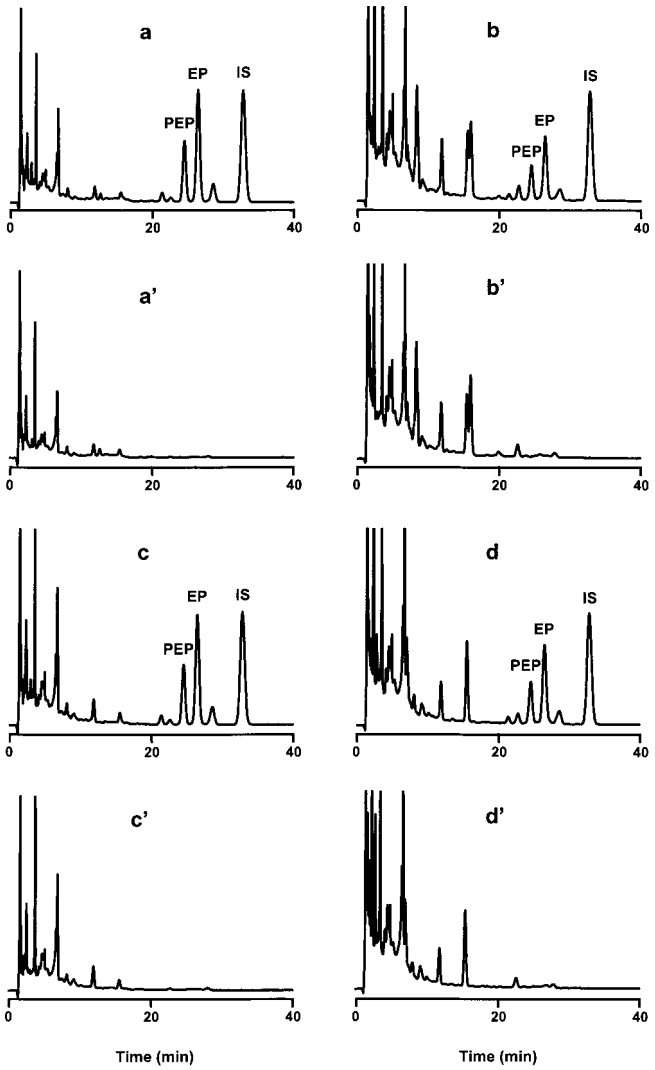

Fig. 5. HPLC Chromatogram of Kampo Medicine Extracts

(a) Kakkon-to, (a') Ephedra herb deficient Kakkon-to, (b) Goshaku-san, (b') Ephedra herb deficient Goshaku-san, (c) Sho-seiryu-to, (c') Ephedra herb deficient Sho-seiryu-to, (d) Bofu-tsusho-san, (d') Ephedra herb deficient Bofu-tsusho-san. Peaks: PEP=(+)-pseudoephedrine. EP=(−)-ephedrine. IS=*N*-benzyldiethylamine.

HPLC conditions: column, TSK gel ODS-80TSQA (particle size of the packing 5 μm, 150 mm×4.6 mm i.d.); flow rate, 1 ml/min; wavelength, 210 nm; column temperature, 50°C; mobile phase, mixture of water, acetonitrile and phosphoric acid (650 : 350 : 1, v/v/v) containing 0.5% SDS.

Assay of Kampo Medicines The contents of PEP and EP in the Kampo medicine extracts and commercial medicines are shown in Tables 4 and 5, respectively. Typical chromatograms obtained from commercial medicines are shown in Fig. 6. The peaks of PEP, EP and IS in the medicines tested were identified by inspection of their retention times and UV spectra. Our data indicated that the other ingredients and additives in these medicines had no influence on the PEP and EP analyses.

In conclusion, we have developed a rapid and simple HPLC method using SPE for the quantitative analysis of PEP and EP in Kampo medicines containing Ephedra herb such as Kakkon-to, Sho-seiryu-to, Goshaku-san, and Bofu-tsusho-

Table 3. Recoveries of PEP and EP Spiked to Ephedra Herb Deficient Extracts

Formula	PEP		EP	
	Recovery (%)	CV[a] (%)	Recovery (%)	CV[a] (%)
Kakkon-to	99.7	0.4	100.1	0.6
Goshaku-san	98.2	0.3	100.4	0.1
Sho-seiryu-to	99.7	0.1	100.7	0.3
Bofu-tsusho-san	98.4	0.4	101.4	0.3

a) Coefficient of variation (n=3), PEP: (+)-pseudoephedrine, EP: (−)-ephedrine.

Table 4. Contents of PEP and EP in Kampo Medicine Extracts

Formula	PEP		EP	
	Content (mg/g)	CV[a] (%)	Content (mg/g)	CV[a] (%)
Kakkon-to	1.36	0.4	2.72	0.2
Goshaku-san	0.42	0.8	0.85	0.2
Sho-seiryu-to	1.33	0.3	2.69	0.2
Bofu-tsusho-san	0.48	0.6	1.03	0.1

a) Coefficient of variation (n=3), PEP: (+)-pseudoephedrine, EP: (−)-ephedrine.

Table 5. Contents of PEP and EP in Commercial Medicines

Product	PEP		EP	
	Content (mg/daily dose)	CV[a] (%)	Content (mg/daily dose)	CV[a] (%)
A	0.86	0.4	1.81	0.2
B	2.91	0.3	5.17	0.2
C	0.49	0.2	1.05	0.1

a) Coefficient of variation (n=3), PEP: (+)-pseudoephedrine, EP: (−)-ephedrine.

Table 6. Reproducibility of Injection

	Formula	PEP		EP	
		Ratio of peak area[a]	CV[b] (%)	Ratio of peak area[a]	CV[b] (%)
Standard solution		0.5691	0.3	0.6045	0.1
Sample solution	Kakkon-to	0.3987	0.4	0.7844	0.4
	Goshaku-san	0.2341	0.4	0.4570	0.3
	Sho-seiryu-to	0.3891	0.4	0.7710	0.3
	Bofu-tsusho-san	0.2831	0.4	0.5943	0.1

a) Peak area ratio of PEP (or EP) to IS, *b*) Coefficient of variation (n=6), PEP: (+)-pseudoephedrine, EP: (−)-ephedrine.

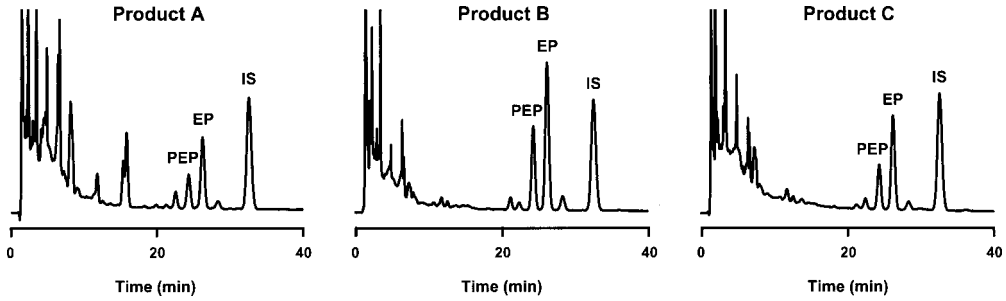

Fig. 6. HPLC Chromatogram of Commercial Medicines

HPLC conditions and peak signs are the same as Fig. 5.

san. The analytical method was validated with regard to its specificity, linearity, accuracy, and precision. The method could be applied to determine to contents of PEP and EP in Goshaku-san and Bofu-tsusho-san, which together contain 17 kinds of crude drugs, and various dosage forms such as fine granules and syrup.

References

1) The Japanese Pharmacopoeia fourteenth edition, published by the Society of Japanese Pharmacopoeia, Jiho, Tokyo, 2001, pp. 975—976.
2) Sagara K., Suto K., Ito Y., Nagai Y., Suzuki H., Satake M., *Iyakuhin Kenkyu*, **27**, 255—261 (1996).
3) Kamiya H., Abstracts of papers, 22th Symposium on Natural Drug Analysis, Osaka, on 12 November 1993, pp. 41—53.
4) Sagara K., Oshima T., Misaki T., *Chem. Pharm. Bull.*, **31**, 2359—2365 (1983).
5) Jian-Sheng Z., Zhen T., Zhi-Cen L., *Planta Med.*, **54**, 69—70 (1988).
6) Gurley B. J., Wang P., Gardner S. F., *J. Pharm. Sci.*, **87**, 1547—1553 (1998).
7) Hurlbut J. A., Carr J. R., Singleton E. R., Faul K. C., Madson M. R., Storey J. M., Thomas T. L., *J. AOAC Int.*, **81**, 1121—1127 (1998).
8) Okamura N., Miki H., Harada T., Yamashita S., Masaoka Y., Nakamoto Y., Tsuguma M., Yoshitomi H., Yagi A., *J. Pharm. Biomed. Anal.*, **20**, 363—372 (1999).
9) Noguchi M., Hosoda K., Suzuki H., *Yakugaku Zasshi*, **107**, 372—376 (1987).
10) Noguchi M., Kubo M., Naka Y., *Yakugaku Zasshi*, **98**, 923—928 (1978).
11) Yamasaki K., Fujita K., Sakamoto M., Okada K., Yoshida M., Tanaka O., *Chem. Pharm. Bull.*, **22**, 2898—2902 (1974).
12) Cui J. F., Niu C. Q., Zhang J. S., *Acta Pharm. Sin.*, **26**, 852—857 (1991).
13) Hansen L. B., *J. Pharm. Sci.*, **90**, 943—948 (2001).
14) Betz J. M., Gay M. L., Mossoba M. M., Adams S., Portz B. S., *J. AOAC Int.*, **80**, 303—315 (1997).
15) Liu Y.-M., Sheu S.-J., *J. Chromatogr.*, **600**, 370—372 (1992).
16) Liu Y.-M., Sheu S.-J., *J. Chromatogr.*, **637**, 219—223 (1993).
17) Flurer C. L., Lin L. A., Satzger R. D., Wolnik K. A., *J. Chromatogr. B.*, **669**, 133—139 (1995).
18) Isozaki T., Yamamoto K., Hitomi N., Kosaka N., Abstracts of papers, 24th Symposium on Natural Drug Analysis, Osaka, on 10 November 1995, pp. 10—19.
19) The Japanese Pharmacopoeia thirteenth edition, published by the Society of Japanese Pharmacopoeia, Dai-ichi Hoki, Tokyo, 1996, p. 390.
20) Standards for Nonpharmacopoeial Crude Drugs, Supervision by the Pharmaceutical Affairs Bureau, Second Examination Division, Ministry of Health and Welfare, Yakuji Nippo, Tokyo, 1989.
21) Ippanyo Kampo Shoho No Tebiki, Supervision by the Pharmaceutical and Supply Bureau of Health and Welfare Ministry of Japan (ed.), Yakugyo Jihosha, Tokyo, 1976.