Recent Progress in Manufacturing Technology of Crude Drug Preparations

Takayoshi Kimura
Department of Development of Manufacturing Technology, Tsumura Juntendo, Inc., Tokyo 102, Japan

한방엑스제제의 제조에 관한 기술의 진보

木村孝良
津村順天堂(株) 생산기술개발부

Please allow me to present our technical progresses on the quality and process control of manufacturing kampo extract medicine in Japan.

Firstly, let me state some characteristics of the classical kampo medicine in Japan. There are some different types of pharmaceutical preparations in the classical kampo medicine such as decoctions, pills and powder. Among them, decoctions are most popularly used in therapy.

Drawbacks of Decoctions

When patients take decoctions, they have to make their own decoctions by themselves, using a mixture of chopped crude drug in accordance with a proper prescription. Making the decoction by oneself, however, is not so easy. Especially, in modern-day therapy, there are drawbacks in the form of decoctions.

1. Changes in the quality of the crude drug itself and variations in the quality of the decoction occur when patients prepare it by themselves.
2. Preservation is difficult.
3. Preparation is tedious.
4. Portability is inconvenient.
5. Unpleasant feeling when taking the decoction.

Quality Difference of Decoctions

I would kike to show you the various differences in the quality of decoctions which have been prepared by using different grades of crude drugs and by different people. For this study, decoction of Mao-To was taken as an example.

Mao-To consists of 4 crude drugs named, Ephedrae Herba, Armeniacae Semen, Cinnamomi Cortex and Glycyrrhizae Radix mixed in the ratios as shown in Table I.

Table II shows the 2 experimental groups. I used different grades of Ephedrae Herba and Glycyrrhizae Radix. In the case of group A, the Ephedrae Herba and Glycyrrhizae Radix contained 0.83% *l*-ephedrine and 5.37% glycyrrhizin, respectively. And in group B, contents of *l*-ephedrine and glycyrrhizin were 0.36% and 3.31%, respectively. The other crude drugs were same grade in each group.

40 people as a monitor were divided into 2 groups. All the people had to prepare the decoction as follows: Add about 500 m*l* of distilled water to the amount of crude drug taken per day. Decoct it to half its volume and remove the residue while it is still hot.

Glycyrrhizin in Decoctions—The quality of the decoction was assessed by analyzing the amount

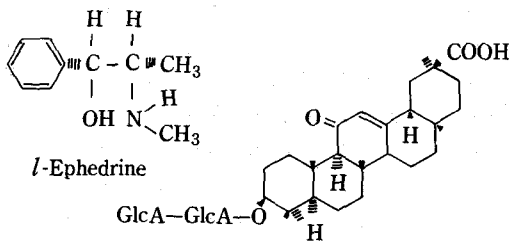
Table I—*Ingredient Composition of Mao-To.*

JP	Ephedrae Herba	5.0g
JP	Armeniacae Semen	5.0g
JP	Cinnamoni Cortex	4.0g
JP	Glycyrrhizae Radix	1.5g

하계 심포지움—생약제제 개발과 품질평가(1988년 8월 26일, 제약회관)에서 발표된 내용의 일부임.

Table II—*Used Crude Drugs.*

Experimental No.	Crude Drug	Contents of Index Component (%)
A	Ephedrae Herba Armeniacae Semen Cinnamomi Cortex	*l*-Ephedrine 0.83%
	Glycyrrhizae Radix	Glycyrrhizin 5.37%
B	Ephedrae Herba Armeniacae Semen Cinnamomi Cortex	*l*-Ephedrine 0.36%
	Glycyrrhizae Radix	Glycyrrhizin 3.31%



l-Ephedrine

Glycyrrhizin

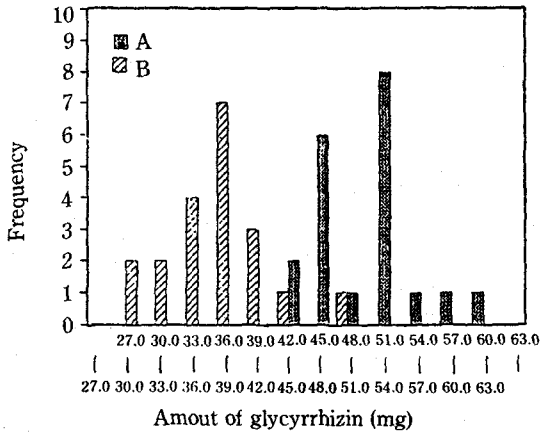


Figure 1—Frequency distribution on amount of glycyrrhizin.

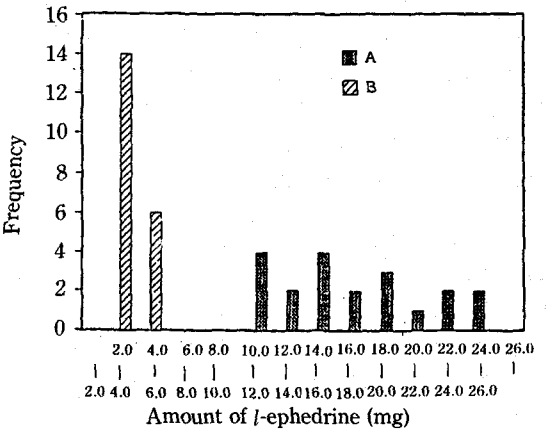


Figure 2—Frequency distribution on amount of l-ephedrine.

of glycyrrhizin as an indicator of this experiment. In Fig. 1, shaded columns show group B while solid columns represent group A. Amounts of glycrrhizin in the decoctions varied from 29 to 50 mg in Group B, which was prepared by low grade of Glycyrrhizae Radix. On the other hand, the amount of glycyrrhizin ranged between 45 to 60 mg for Group A. Hence, the distribution range of the amount of glycyrrhizin in Group A was found to be much higher than that in Group B. These results showed that the content of glycyrrhizin in the decoction was dependent on the grade of Glycyrrhizae Radix which was used.

***l*-Ephedrine in Decoctions**—Similarly, we used l-ephedrine as an indicator as shown in Fig. 2. For Group B, the amount of l-ephedrine varied between 0.1-5.8 mg. On the other had, the distribution range of *l*-ephedrine content for Group A varied widely between 10.2-25.6 mg. These results suggest that the quality of the decoction depends on the grade of crude drugs and the individual differences in preparations. Hence, we thought that the decoction is not a suitable form in the modern-day therapy.

Then, in order to adopt the kampo medicine into the modern-day therapy, kampo extract medicine which has the same efficacy of the decoction has been developed as a drug form like a western medicine. To complete the kampo extract medicine, the following items are required:

1. Fixation of the quality of the crude drugs.
2. To maintain a manufacturing method for production of the drugs which are equivalent quality to the decoction.
3. Assurance of reproducibility of the manufacturing conditions.
4. Development of evaluation methods to guarantee the quality of the kampo extract medicine and control of quality by these methods.

Recent Technical Progress of Manufacture in Kampo Extract Medicines

Next, I would like to talk about some works concerning with recent technical progress on manufacturing kampo extract medicines.

Among the manufacturing technologies for kampo extract medicines, drying to keep the drug quality is a key technology through the whole process for drying up the hot water extract of crude drugs.

Spray dry and freeze dry were adopted for manufacturing kampo extracts. Here, I will tell you about the study concerning with spray dry which is widely used in this field much more than freeze dry.

Spray Drying

Schematic diagram of spray dryer is shown in Fig. 3. Spray drying involves atomization of feed into a spray, and contact between spray and drying air heated from a1 resulting in moisture evaporation. The drying of spray proceeds until the desired moisture content in the dried product that is obtained in this area of the spray dryer, and the product is then recovered from the air by use of cyclone.

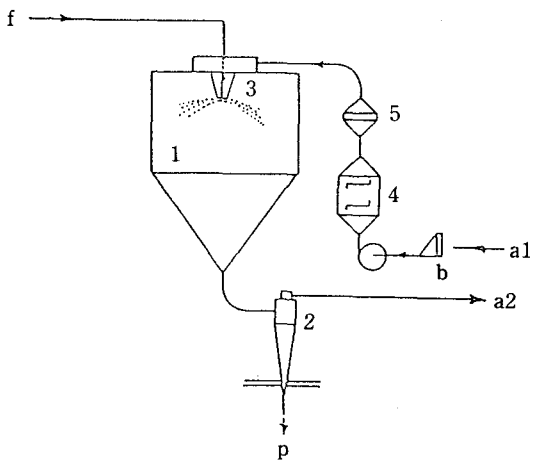


Figure 3—Drying process flow sheet.

a1: Inlet air
a2: Outlet air
f: Feed
b: Prefilter for drying air
p: Dried product
1: Spray-dryer chamber
2: Cyclone
3: Atomizer
4: Indirect air heater
5: HEPA filters

The advantage of this dryer is as follows:

1. Rates of evaporation are very rapid due to the vast surface area of droplets in a spray. A cubic metre of liquid forms approximately 2×10^{12} uniform 100 micrometer droplets. A total surface area of these droplets is over 60,000 m^2.
2. Residence time in the dryer is short and dried particles stay in the dryer for only 20 to 60 seconds.
3. The dried product does not rise much over a low wetbulb temperature of outlet air.

It is pointed out that kampo drugs contain not only stable ingredients but also unstable chemicals. Therefore, it is very important for us to keep the same quality of the dried extract to the original through the whole manufacturing process.

Stability of Sennosides—One of the well-known unstable chemical constituents in the crude drugs used in kampo medicines is sennoside in Rhei Rhizoma. Fig. 4 shows the stability of sen-

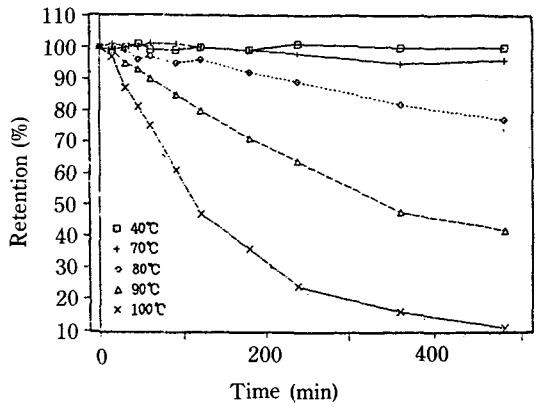


Figure 4—Time versus retention of purified sennoside A concentration in distilled water (initial concentration: about 0.04%).

Retention = $\frac{\text{sennoside A concentration at each time of duration}}{\text{initial concentration of sennoside A}} \times 100(\%)$

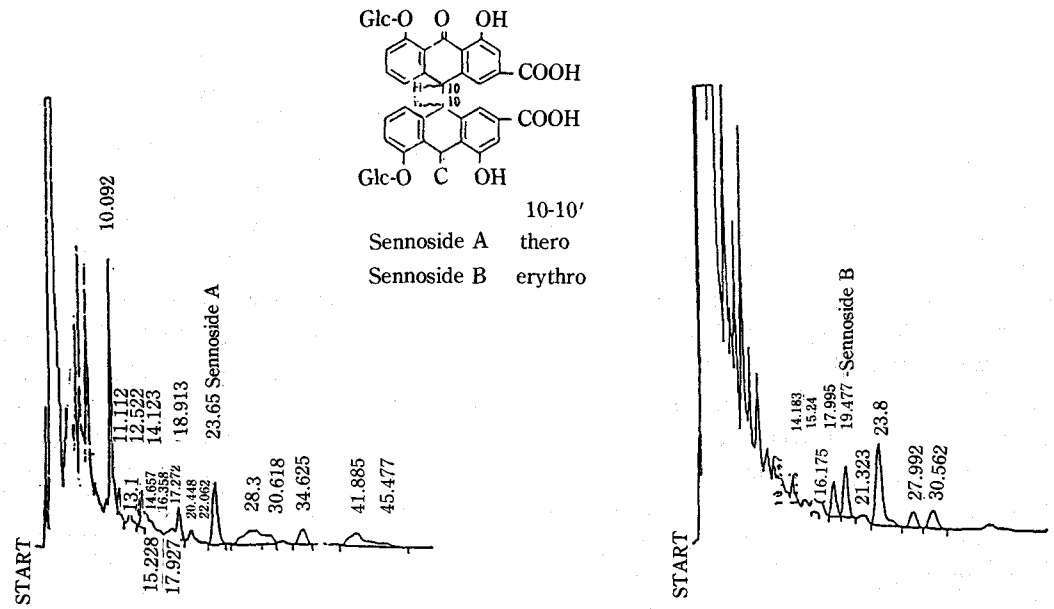


Figure 5—Chromatograms of extracted liquid of Rhei Rhizoma.

noside A in water at temperature varying from 40 °C to 100 °C.

As shown in this figure, sennoside A concentration in water decreased rapidly when the water temperature rose from 70 °C to 100 °C. Similar changes were also found for sennoside B.

Furthermore, in order to get some information about spray drying condition of hot air, we studied the influence of the drying of hot air, we studied the influence of the drying temperature on the recovery of the heat-sensitive ingredients in the extract. The recovery of sennoside A and B were taken as examples of the heat-sensitive chemicals.

Determination of sennoside A and B was carried out by HPLC. Fig. 5 shows the chromatograms of sennoside A and B in the extract prepared from Rhei Rhizoma.

Fig. 6 shows the changes of concentrations of sennosides in the dried extract prepared from Rhei Rhizoma. In this case, the temperature of the outlet air was fixed at 75 °C and temperature of the inlet air for drying was varied from 115 °C to 250 °C. When the temperature of the inlet air changed from 115 °C to 200 °C, there were no observable changes in concentration of sennoside A and B in the dried product. However, when the inlet air was at 250 °C, the concentration of sennoside A slightly decreased.

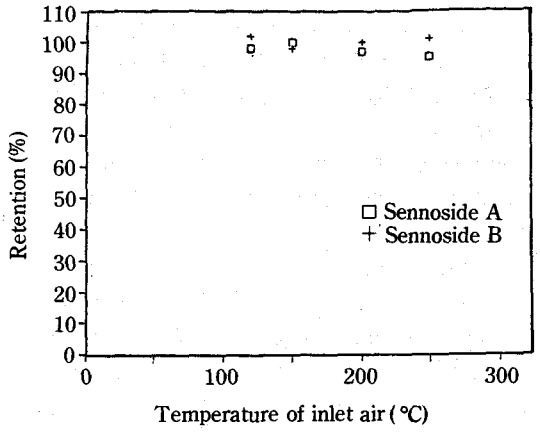


Figure 6—Effect of drying conditions on contents of sennoside A and sennoside B in solid.
Temperature of outlet air was 75 °C
Retention(%) =

$$\frac{\text{contents of sennoside A and sennoside B in dried product}}{\text{initial contents of sennoside A and sennoside B}} \times 100$$

Fig. 7 shows the results obtained under the condition of outlet air temperature at 115 °C. As shown in this figure, the retention of sennoside A

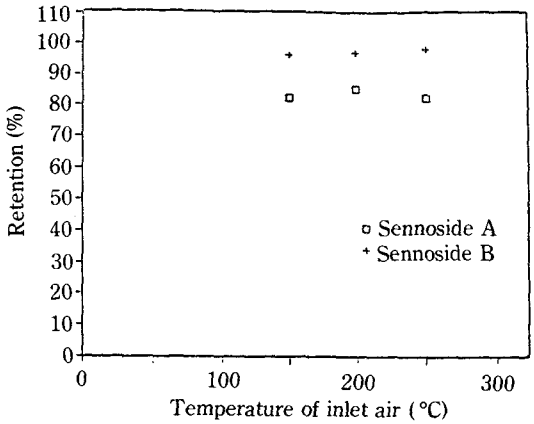


Figure 7—Effect of drying conditions on contents of sennoside A and sennoside B in solid. Temperature of outlet air was 115 °C.

Retention (%) = $\frac{\text{contents of sennoside A and sennoside B in dried product}}{\text{initial contents of sennoside A and sennoside B}} \times 100$

was 80-90% at any inlet air temperature operated.

From these results, we were able to understand that it is very important for us to fix the optimum conditions to produce kampo extract medicines which are of a constant quality. You can probably understand that the manufacture of kampo extract medicines is based on a lot of research and strict practice.

Furthermore, it is neccessary for us to assure the quality by fixation of the crude drug quality and reproducibility of manufacturing conditions.

Quantitative Analytical Methods for Crude Drugs

Now, I would like to talk about the recent progress of quantitative analyses for chemicals in kampo medicine as a reference of the quality.

For the drug analysis, chromatographical methods are most effectively applied. The types of chromatography are currently used as follows:

1. Open column chromatography
2. Gas chromatography (GC)
3. Thin layer chromatography-densitometry (TLCDM)
4. High performance liquid chromatography (HPLC)

High performance liquid chromatography is considered to be the most suitable to analyze the crude drugs and the kampo extract medicines.

Table III—*Assayable Compounds in Crude Drugs (in 1980).*

Compound (crude drug)	JP XI (1986)
Ephedrine (Ephedrae Herba)	titration method
Berberine (Coptidis Rhizoma)	weighing method
Berberine (Phellodendri Cortex)	weighing method
Glycyrrhizin (Glycyrrhizae Radix)	—
Sennoside A (Rhei Rhizoma)	—

Table IV— *Assayable Compounds in Crude Drugs (in 1985).*

by K. Noguchi, Gekkanyakuji, Vol. 27 No. 9 (1985), p. 136

Crude drug	Compound
Corydalis Tuber	corydaline
Scutellariae Radix	baicalin
Phellodendri Cortex	berberine
Coptidis Rhizoma	berberine
Puerariae Radix	puerarine
Glycyrrhizae Radix	glycyrrhizin
Armeniacae Semen	amygdalin
Cinnamomi Cortex	cinnamic aldehyde (cinnamic acid)
Ginseng Radix Rubra	ginsenoside Rg_1
Evodiae Fructus	evodiamine
Schisandrae Fructus	schizandrin
Bupleuri Radix	saikosaponin a
Gardeniae Fructus	geniposide
Corni Fructus	loganin
Rehmanniae Radix	catalpol
Paeoniae Radix	paeoniflorin
Rhei Rhizoma	sennoside A
Aurantii Nobilis Pericarpium	hesperidin
Zizyphi Fructus	cyclic AMP
Ginseng Radix	ginsenoside Rg_1
Sinomeni Caulis et Rhizoma	sinomenine
Moutan Cortex	paeonol
	paeoniflorin
Ephedrae Herba	ephedrine
Gentianae Scabrae Radix	gentiopicroside
Sophorae Radix	matrine
Uncariae Ramulus et Uncus	rhynchophylline

Assayable Compounds in Crude Drugs

As shown in Table III, according to the Japanese Pharmacopaeia in 1980, ephedrine, berbe-

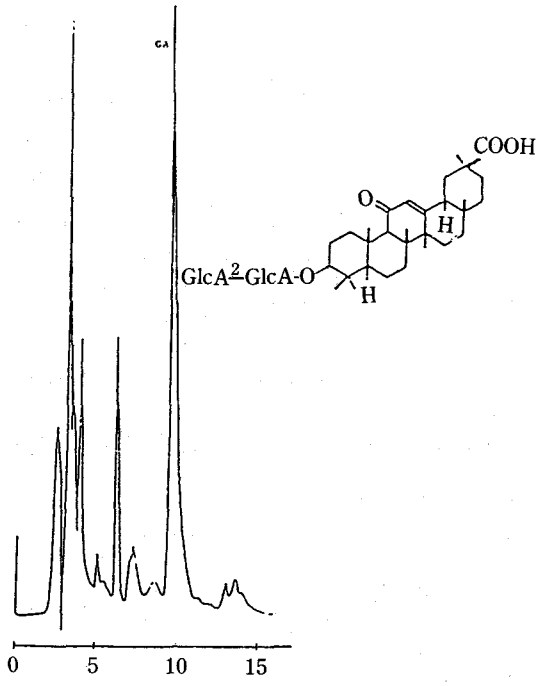


Figure 8—HPLC chromatogram for glycyrrhizin (Glycyrrhizae Radix).
(HPLC conditions)
Column : Nucleosil 5 C_{18} (4 × 250mm)
Column temp. : r.t.
Mobile phase : CH_3CN:H_2O:AcOH(40:60:1)
Detection : UV 250nm
Flow rate : 0.8 m*l*/min

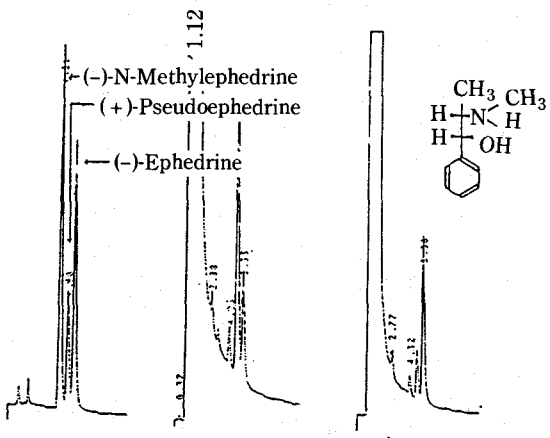
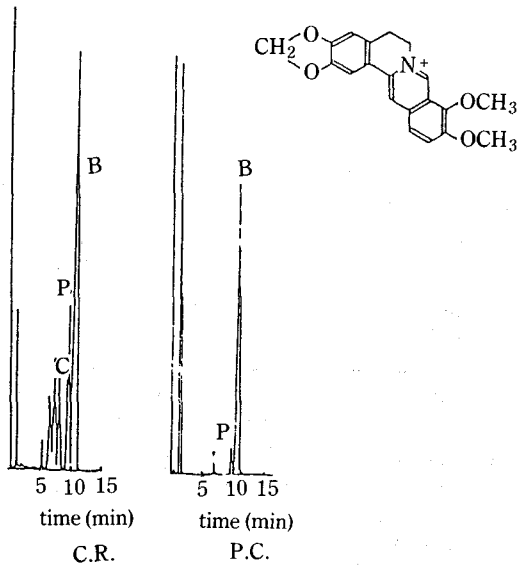


Figure 9—HPLC chromatograms for ephedrine (Ephedrae Herba).
(HPLC conditions)
Column : Nucleosil 50-5 (4 × 250mm)
Column temp. : 25 °C
Mobile phase : CH_3OH:NH_4OH (200:1)
Detection : UV 220nm
Flow rate : 1.5 m*l*/min



C: Coptisine, P: Palmatine, B: Berberine

Figure 10—HPLC chromatograms for berberine (Coptidis Rhizoma and Phellodendri Cortex).
(HPLC conditions)
Column : TSK GEL LS-410 (5μm) (4 × 150mm)
Column temp. : r.t.
Mobile phase : 0.1N·$C_4H_6O_6$:CH_3CN:MeOH:SDS (49.5:40:10:0.5)
Detection : UV 345nm
Flow rate : 1.5 m*l*/min

rine, glycyrrhizin and sennoside A were described as assayable compounds in the crude drugs. Also, in 1986, titration method for ephedrine and weighing method for berberine were supplemented.

Recently, Dr. Noguchi reported other assayable chemicals in some crude drugs as summarized in Table IV.

Hereafter, I would like to show you the individual analytical results of each ingredient in the crude drugs by HPLC. Each figure will show the chromatograms of the crude drugs. Furthermore, it wil feature upon the main ingredients which have been separated under the specified HPLC conditions.

Glycyrrhizin—Fig. 8 features upon glycyrrhizin of Glycyrrhizae Radix.

Ephedrine—Fig. 9 shows the chromatogram of the different grades of Ephedrae Herba. The

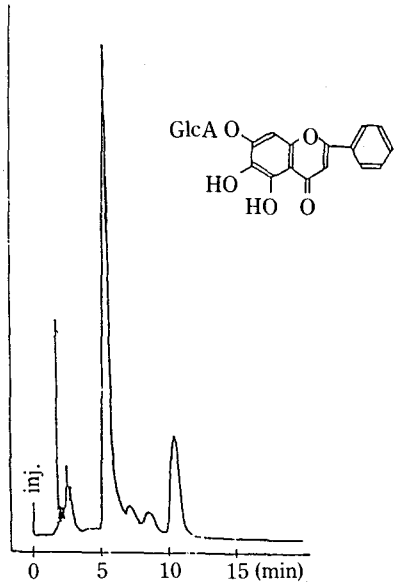


Figure 11— HPLC chromatogram for baicalin (Scutellariae Radix).
(HPLC conditions)
Column : Nucleosil 4 C_{18} (4 × 250mm)
Column temp. : 35 °C
Mobile phase : CH_3CN:H_2O:AcOH (28:71:1)
Detection : UV 273nm
Flow rate : 1.4 ml/min

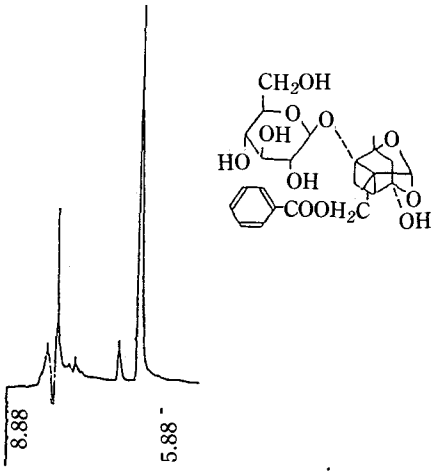


Figure 12—HPLC chromatogram for paeoniflorin (Paeoniae Radix and Moutan Cortex).
(HPLC conditions)
Column : TSK GEL LS-410 5μm (4 × 150mm)
Column temp. : 50 °C
Mobile phase : CH_3CN:H_2O:AcOH (12:88:1)
Detection : UV 230nm
Flow rate : 1.0 ml/min

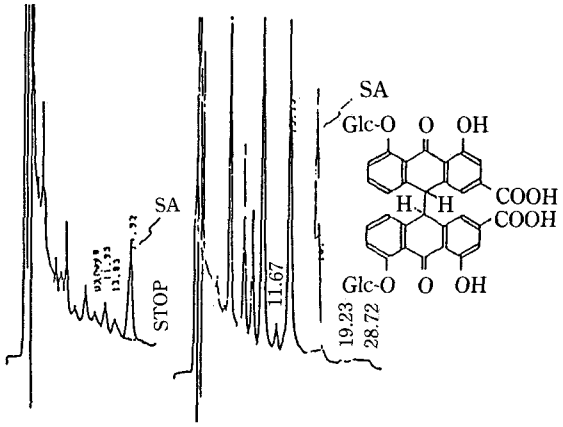


Figure 13—HPLC chromatogram for sennoside A (Rhei Rhizoma).
(HPLC conditions)
Column : Cosmosil 5 C_{18} (4.6×250mm)
Column temp. : r.t.
Mobile phase : H_2O:CH_3CN:AcOH (80:20:1)
Detection : UV 340nm
Flow rate : 1.0 ml/min

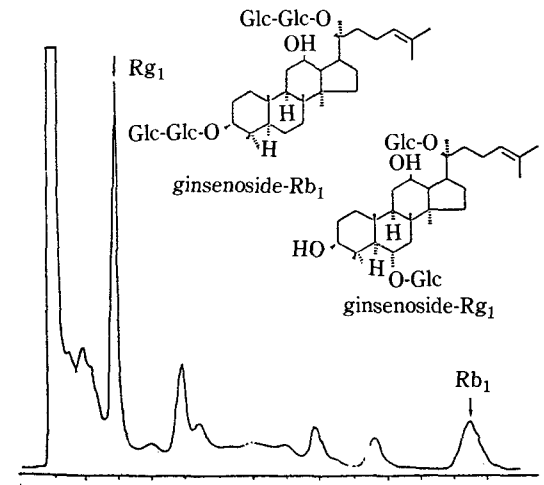


Figure 14—HPLC chromatogram for ginsenosides (Ginseng Radix).
(HPLC conditions)
Column : μ-Bondapak C_{18}
Column temp. : r.t.
Mobile phase : CH_3CN:H_2O (70%—82%)
Detection : UV 202nm
Flow rate : ml/min

composition of *l*-epnedrine and pseudoephedrine were remarkably different between these grades of Ephedrae Herba.

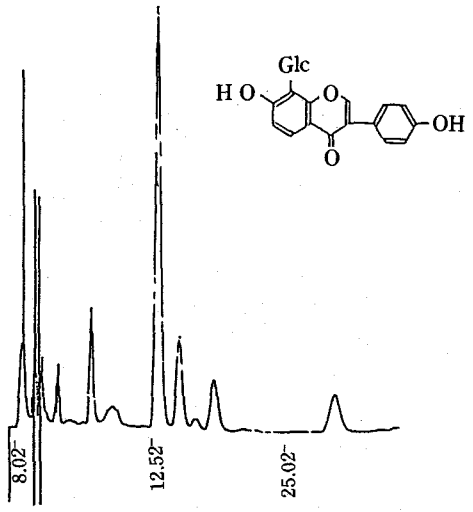


Figure 15—HPLC chromatogram for puerarin (Puerariae Radix).
(HPLC conditions)
Column : LS-120A
Column temp. : r.t.
Mobile phase : CH_3CN:H_2O (36:260)
Detection : UV 235nm
Flow rate : 1.5 ml/min

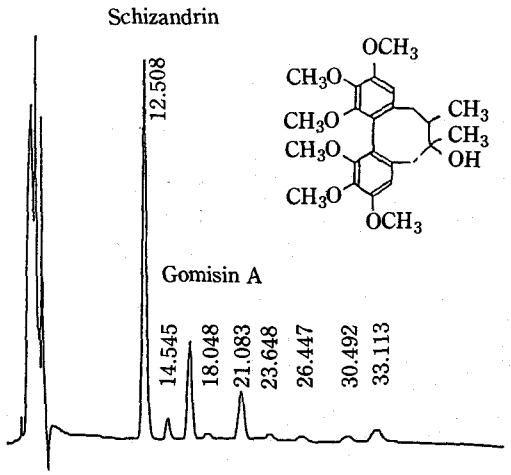


Figure 17—HPLC chromatogram for schizandrin (Schisandrae Fructus).
(HPLC conditions)
Column : LS-120A (4.6 × 250mm, 10um)
Column temp. : 25 °C
Mobile phase : CH_3CN:H_2O (1:1)
Detection : UV 252nm
Flow rate : 1.0 ml/min

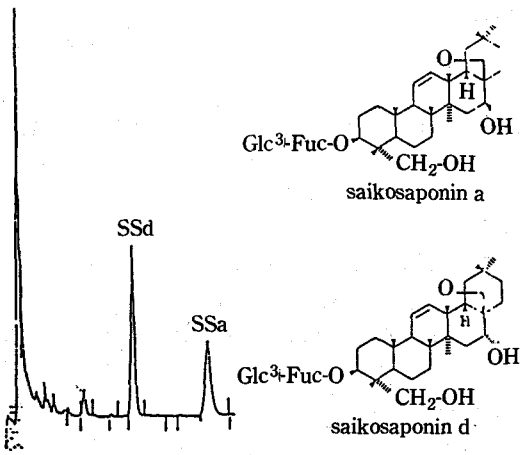


Figure 16—HPLC chromatogram for saikosaponins (Bupleuri Radix).
(HPLC conditions)
Column : ODS (4.6 × 250mm, 5um)
Column temp. : r.t.
Mobile phase : H_2O:CH_3CN (60:40)
Detection : UV 254nm
Flow rate : 0.8 ml/min

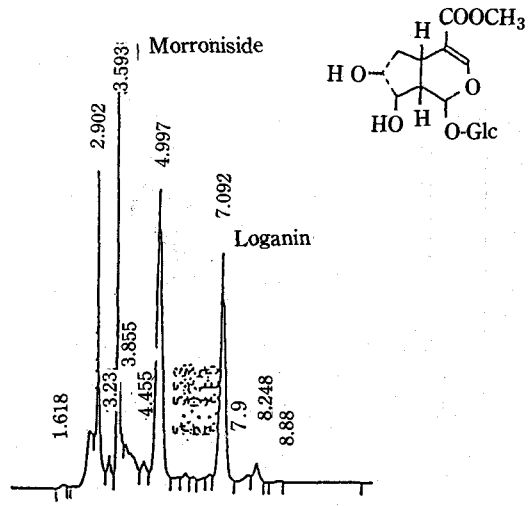


Figure 18—HPLC chromatogram for loganin (Corni Fructus).
(HPLC conditions)
Column : YMC-ODS (6 × 150mm)
Column temp. : 40 °C
Mobile phase : 0.05M·NaH_2PO_4:CH_3CN (6:1)
Detection : UV 240nm
Flow rate : 1.0 ml/min

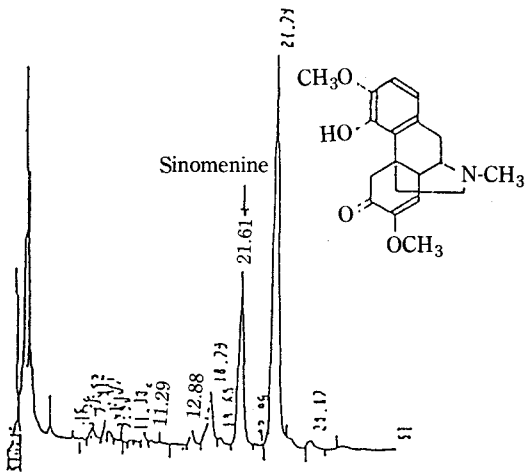


Figure 19—HPLC chromatogram for sinomenine (Sinomeni Caulis et Rhizoma).
(HPLC conditions)
Column : Develosil C_8 (4.6 × 150mm)
Column temp. : r.t.
Mobile phase : CH_3CN:H_2O:$H_2C_2O_4$:SDS (320 m*l*:680 m*l*:0.5 g:2 g)
Detection : UV 261nm
Flow rate : 1.2 m*l*/min

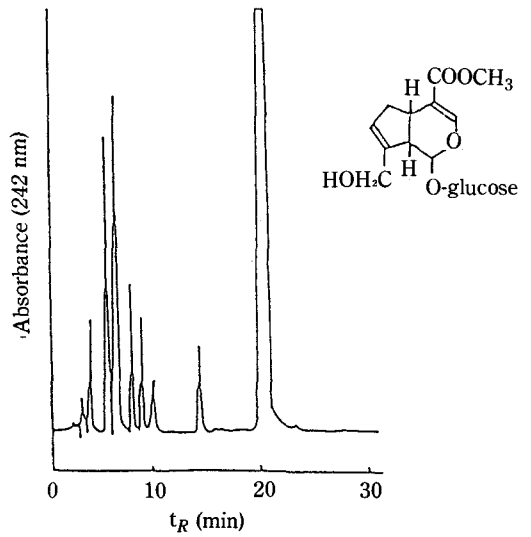


Figure 20—HPLC chromatogram for geniposide (Gardeniae Fructus).
(HPLC conditions)
Column : Microsorb C_8 (4.6 × 150mm)
Column temp. : r.t.
Mobile phase : 10 mM·H_2PO_4 Buffer (pH 3.0):MeOH (5:1)
Detection : UV 242nm
Flow rate : 1.0 m*l*/min

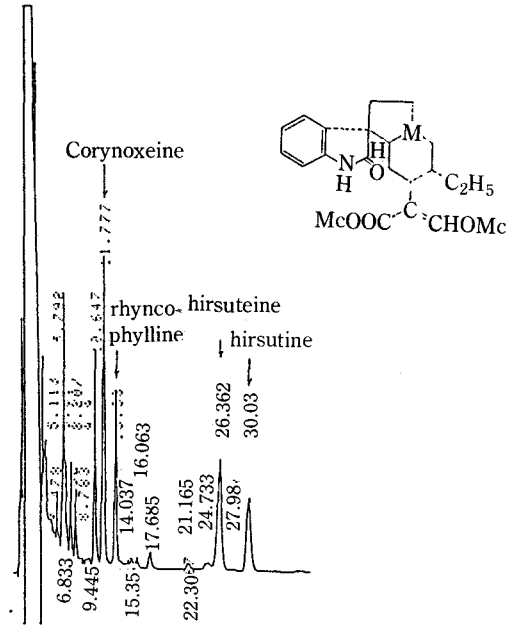


Figure 21—HPLC chromatogram for rhynchophylline (Uncariae Ramulus et uncus).
(HPLC conditions)
Column : Develosil C_8, 5 μm (4.6 × 150 mm)
Column temp. : r.t.
Mobile phase : CH_3CN : H_2O : $H_2C_2O_4$: SDS (450 m*l*:50 m*l*:0.5g:2g)
Detection : UV 250nm
Flow rate : 1.0 m*l*/min

Berberine—Fig. 10 shows the chromatographic profiles of Coptidis Rhizoma and Phellodendri Crotex. Berberine was separated by HPLC under these conditions.

Baicalin—Fig. 11 features upon baicalin of Scutellariae Radix.

Paeoniflorine—Fig. 12 features upon paeoniflorine of Paeoniae Radix.

Sennoside A—Fig. 13 features upon sennoside A of Rhei Rhizoma.

Ginsenosides—The chromatogram of Ginsenoside Rg of Ginseng Radix is shown in Fig. 14.

Puerarin—Puerarin of Puerariae Radix is featured upon in Fig. 15.

Saikosaponin—Similarly, saikosaponin a and d of Bupheuri Radix is shown in Fig. 16.

Schizandrin—Fig. 17 features on schizandrin and gomisin A of Schisandrae Fructus.

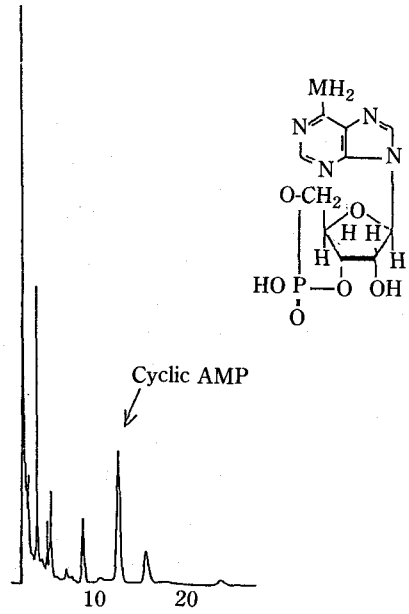


Figure 22—HPLC chromatogram for cyclic AMP (Zizyphi Fructus).
(HPLC conditions)
Column : Develosil ODS-5 (4.6 × 150 mm)
Column temp. : r.t.
Mobile phase : 0.05M·KH_2PO_4:MeOH (95:5)
Detection : UV 260 nm
Flow rate : 1.0 ml/min

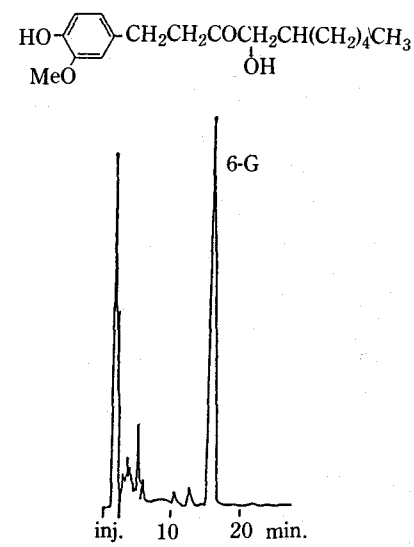
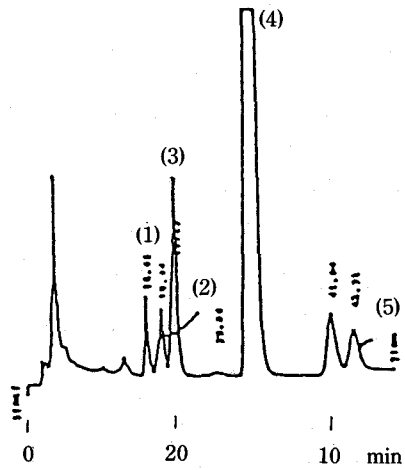
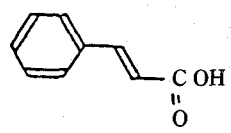


Figure 23—HPLC chromatogram for 6-gingerol (Zingiberis Rhizoma).
(HPLC conditions)
Column : LichroCART RP-18 (4 × 250mm)
Column temp. : r.t.
Mobile phase : CH_3CN:H_2O (38:62)
Detection : UV 280nm
Flow rate : 1.2 ml/min





(HPLC conditions)
Column : Lichrosorb Rp-18 (4.6 × 250mm)
Column templ : r.t.
Mobile phase : 30% CH_3CN:AcOH (100:1)
Detection : UV 254nm
Flow rate : 0.8 ml/min

1) Coumarin 2) *t*-Cinnamyl alcohol 3) *t*-Cinnamic acid
4) *t*-Cinnamic aldehyde 5) *o*-Methoxycinnamic aldehyde

Figure 24—HPLC chromatogram for cinnamic acid (Cinnamomi Cortex).

Table V—*Application of HPLC Method to Analyse Final Products.*

1. Influence of prescribed each crude drugs
pre-treatment: cleaning by the sep-pack column
change of mobile phase
change of column
change of Column temp.
2. Influence of additives
recovery after the pre-treatment
3. Standard chemicals
purity
water content
stability (in solid, in fluid)

Loganin—Loganin and morroniside of Corni Fructus is shown in Fig. 18.

Sinomenine—Sinomenine of Sinomeni Caulis is featured in Fig. 19.

Geniposide—Fig. 20 features upon geniposide in Gardenia Fructus.

Rhyncophylline—Corynoxeine, rhyncophylline, hirsuterine, and hirsutine of Uncariae Ramulus is shown here in Fig. 21.

Cyclic AMP—Cyclic AMP of Zizyphi Fructus is shown in Fig. 22.

6-Gingerol—6-Gingerol of Zingiberis Rhizoma is featured in Fig. 23.

Cinnamic Acid—Cinnamic acid of Cinnamomi Cortex is featured in Fig. 24.

This HPLC method can also be applied to analyse kampo extract medicines. When we analyse the kampo extract medicine, we need to take precautions as summarized in Table V.

Firstly, this analytical method may be influenced by some chemical compounds in each prescribed crude drug. Hence, further studies on the separation conditions and pretreatment of the medicine is required.

The second factor is the influence of additives in the medicine. Therefore, we have to check the recovery of chemical substances in the process of pretreatment.

The third factor involves standard chemicals. In quantitative analysis, standard chemicals are very important. We have to examine the water content and purity of the standard chemicals. We also have to check its stability for preservation in the solution or solid form.

My speech today only covers a part of our recent works. However, I am convinced that you would have realized how difficult it is to produce kampo extract medicines, how hard it is to standardize the manufacturing process and how severe to assure the quality of crude drugs and kampo extract medicines.