Table 3 Comparative Table on Assay Conditions for Crude Drugs in CP, JP, KP and VP

No.	Latin name	Assay (↑: Not less than)	1) method	2) developed solvent	3) detection
1	*Aconitum carmichaeli* **Debeaux** (ブシ)				
	JP PROCESSI ACONTI RADIX	Total Alkaloids 0.7~1.5% (Type 1), 0.1~0.6% (Type 2), 0.5~0.9% (Type 3)	Titration		
2	*Anemarrhena asphodeloides* **Bunge** (チモ)				
	CP RHIZOMA ANEMARRHENAE	Diosgenin ↑ 1.0%	HPLC (ODS column)	methanol / water (95 : 5)	Evaporative Light Scattering method
3	*Angelica dahurica* **Bentham et Hooker fil** (ビャクシ)				
	CP RADIX ANGELICA DAHURICAE	Imperatorin ↑ 0.080%	HPLC (ODS column)	methanol / water (55 : 45)	UV 300 nm
4	*Astragalus membranaceus* **Bunge** (オウギ)				
	CP RADIX ASTRAGALI	Astragaroside IV ↑ 0.04%	HPLC (ODS column)	acetonitrile / water (32 : 68)	Evaporative Light Scattering method
5	*Bupleurum scorzonerifolium* **Willd.** (サイコ)				
	JP BUPLEURI RADIX	Saikosaponin a + d ↑ 0.35%	HPLC (ODS column, I.D. 4.6 mm x 15 cm, 5 mm)	1) acetonitrile / water (2 : 3) 2) 50' 3) adjust flow rate to elute Saikosaponin d at ca. 8 min	UV 206 nm
	KP BUPLEURI RADIX	Saikosaponin a ↑ 0.3%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) acetonitrile / water (35 : 65) 2) 20' 3) 0.8 mL/min	UV 203 nm
6	*Carthamus tinctorius* **Linne** (コウカ)				
	CP FLOS CARTHAMI	Hydroxysafflor A ↑ 1.0%, Kaempferide ↑ 0.05%	HPLC (ODS column)	Hydroxysafflor A [methanol / acetonitrile / 0.7% phosphoric acid (26 : 2 : 72)], Kaempferide [methanol / 0.4% phosphoric acid (52 : 48)]	Hydroxysafflor A (UV 403 nm), Kaempferide (UV 367 nm)
7	*Cimicifuga heracleifolia* **Komarov** (ショウマ)				
	CP RHIZOMA CIMICIFUGAE	Ferulic acid ↑ 0.1%	HPLC (ODS column)	acetonitrile / 0.1% phosphoric acid solution (13 : 87)	UV 316 nm
8	*Cinnamomum cassia* **Blume** (ケイヒ)				
	CP CORTEX CINNAMOMI	Cinnamic acid ↑ 1.5%	HPLC (ODS column)	acetonitrile / water (35 : 75)	UV 290 nm
	KP CINNAMOMI CORTEX	Cinnamic acid ↑ 0.03%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) methanol / water / glacial acetic acid (12 : 88 : 1) 2) 20' 3) 2.0 mL/min	UV 280 nm
9	*Cornus officinalis* **Siebold et Zuccarini** (サンシュユ)				
	CP FRUCTUS CORNI	Loganin ↑ 0.60%	HPLC (ODS column)	acetonitrile / water (15 : 85)	UV 240 nm
	KP CORNI FRUCTUS	Loganin ↑ 0.5%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) methanol / water (30 : 70) 2) 20' 3) 1.0 mL/min	UV 240 nm
10	*Curcuma longa* **Linne** (ウコン)				
	CP RHIZOMA CURUCUMAE LONGAE	Curcumin ↑ 1.0%	HPLC (ODS column)	acetonitrile / 4% glacial acetic acid solution (48 : 52)	UV 430 nm
11	*Ephedra sinica* **Stapf** (マオウ)				
	CP HERBA EPHEDRAE	Ephedorine hydrochloride ↑ 1.0%	HPLC (ODS column)	acetonitrile / 0.1% phosphoric acid solution (9 : 87)	UV 207 nm
	JP EPHEDRAE HERBA	Total alkaroids ↑ 0.7%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) sodium lauryl sulfate (1 in 128) / acetonitrile / phosphoric acid (640 : 360 : 1) 2) 45' 3) adjust flow rate to elute ephedrine at ca. 14 min	UV 210 nm
	KP EPHEDRAE HERBA	Total alkaroids (Ephedrine+ Pseudoephedrine) ↑ 0.7%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) sodium lauryl sulfate (1 in 128) / acetonitrile / phosphoric acid (640 : 360 : 1) 2) 45' 3) adjust flow rate to elute ephedrine at ca. 14 min	UV 210 nm
	VP HERBA EPHEDRAE	Total alkaroids ↑ 0.8%	Titration		
12	*Epimedium koreanum* **Nakai** (インヨウカク)				
	CP HERBA EPIMEDII	Total flavonoids ↑ 5.0%, Icariine ↑ 0.50%	Total flavonoids (Absorption) Icariine [HPLC (ODS column)]	Total flavonoids (methanol), Icariine [acetonitrile / water (30 : 70)]	UV 270 nm
13	*Eucommia ulmoides* **Oliver** (トチュウ)				
	CP CORTEX EUCOMMIAE	Pinoresinol-di-glucopyranoside ↑ 0.1%	HPLC (ODS column)	methanol / water (25 : 75)	UV 277 nm
14	*Evodia rutaecarpa* **Bentham** (ゴシュユ)				
	CP FRUCTUS EVODIAE	Evodiamine+Rutaecarpine ↑ 0.15%	HPLC (ODS column)	acetonitrile / 0.04% octanesulfonic acid sodium salt (43 : 57)	UV 225 nm
15	*Forsythia suspensa* **Vahl** (レンギョウ)				
	CP FRUCTUS FORSYTHIAE	Forsythin ↑ 0.15%	HPLC (ODS column)	acetonitrile / water (25 : 75)	UV 277 nm
16	*Fritillaria thunbergii* **Miq.** (バイモ)				
	CP BULBUS FRITILLAIAE THUNBERGII	Peimine + Peimisine ↑ 0.080%	HPLC (ODS column)	acetonitrile / water/ethylenediamine (70 : 30 : 0.3)	Evaporative Light Scattering method
17	*Gardenia jasminoides* **Ellis** (サンシシ)				
	CP FRUCTUS GARDENIAE	Geniposide ↑ 1.8%	HPLC (ODS column)	acetonitrile / water (15 : 85)	UV 238 nm
	JP GARDENIAE FRUCTUS	Geniposide ↑ 3.0%	HPLC (ODS column, I.D. 6 mm x 15 cm, 5 mm)	1) water / acetonitrile (22 : 3) 2) 30' 3) adjust flow rate to elute Geniposide at ca.15 min	UV 240 nm

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18	*Glycyrrhiza uralensis* **Fisher,** *G. glabra* **Linne (カンゾウ)**				
	CP RADIX GLYCYRRHIZAE	Glycyrrhizinic acid ↑ 2.0%, Liquiritin ↑ 1.0%	HPLC (ODS column)	Glycyrrhizinic acid [methanol / 0.2 mol/L ammonium acetate / glacial acetic acid (67 : 33 : 1)], Liquiritin [acetonitrile / 0.5%gracial acetic acid (1 : 4)]	Glycyrrhizinic acid (UV 250 nm), Liquiritin (UV 276 nm)
	JP GLYCYRRHIZAE RADIX	Glycyrrhizinic acid ↑ 2.5%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) dilute acetic acid / acetonitrile (3 : 2) 2) 20˚ 3) adjust flow rate to elute glycyrrhizic acid at ca.10 min	UV 254 nm
	KP GLYCYRRHIZAE RADIX	Glycyrrhizinic acid ↑ 2.5%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) dilute acetic acid / acetonitrile (3 : 2) 2) 20˚ 3) adjust flow rate to elute glycyrrhizic acid at ca.10 min	UV 254 nm
	VP RADIX GLYCYRRHIZAE	Glycyrrhetic acid ↑ 6.0%	Weight		
19	*Leonurus japonicus* **Houtt. (ヤクモソウ、純外)**				
	CP HERBA LEONURI	Stachydrine ↑ 0.50%	TLC (Silica gel TLC)	ethyl acetate / 1-butanol / hydrochloric acid (1 : 8 : 3)	1) 105˚ 2) UV 510 nm
20	*Lonicera japonica* **Thunberg (キンギンカ、純外)**				
	CP FLOS LONICERAE	Chlorogenic acid ↑ 1.5%	HPLC (ODS column)	acetonitrile / 0.4% phosphoric acid solution (13 : 87)	UV 327 nm
21	*Magnolia officinalis* **Rehder et Wilson var.** *biloba* **Rehder et Wilson (コウボク)**				
	CP CORTEX MAGNOLIAE OFFICINALIS	Magnolol+Honokiol ↑ 2.0%	HPLC (ODS column)	methanol / water (78 : 22)	UV 294 nm
	JP MAGNOLIAE CORTEX	Magnolol ↑ 0.8%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) water / acetonitrile / acetic acid (50 : 50 : 1) 2) 20˚ 3) adjust flow rate to elute magnolol at ca.14 min	UV 289 nm
	KP MAGNOLIAE CORTEX	Magnolol ↑ 0.8%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) water / acetonitrile / acetic acid (50 : 50 : 1) 2) 20˚ 3) adjust flow rate to elute magnolol at ca.14 min	UV 289 nm
22	*Paeonia lactiflora* **Pallas (シャクヤク)**				
	CP RADIX PAEONIAE ALBA	Paeoniflorin ↑ 1.6%	HPLC (ODS column)	acetonitrile / 0.1% phosphoric acid solution (14 : 86)	UV 230 nm
	JP PAEONIAE RADIX	Paeoniflorin ↑ 2.0%	HPLC (ODS column, I.D. 4-6 mm x 15 cm, 5 mm)	1) water / acetonitrile / phosphoric acid (850 : 150 : 1) 2) 20˚ 3) adjust flow rate to elute paeoniflorin at ca.10 min	UV 232 nm
	KP PAEONIAE RADIX	Paeoniflorin ↑ 2.0%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) water / acetonitrile (4 : 1) 2) 20˚ 3) adjust flow rate to elute paeoniflorin at ca.10 min	UV 230 nm
23	*Paeonia suffruticosa* **Andrews (ボタンピ)**				
	CP CORTEX MOUTAN	Paeonol ↑ 1.2%	HPLC (ODS column)	methanol / water (45 : 55)	UV 274 nm
	JP MOUTAN CORTEX	Paeonol ↑ 1.0%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) water / acetonitrile / acetic acid (100) (65 : 35 : 2) 2) 20˚ 3) adjust flow rate to elute paeonol at ca.14 min	UV 274 nm
	KP MOUTAN CORTEX RADICIS	Paeonol ↑ 1.0%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) water / acetonitrile / acetic acid (100) (65 : 35 : 2) 2) 20˚ 3) adjust flow rate to elute paeonol at ca.14 min	UV 274 nm
	VP CORTEX PAEONIA SUFFRUTICOSAE	Paeonol ↑ 1.0%	Absorption	water	UV 274 nm
24	*Panax ginseng* **C. A. Meyer (ニンジン)**				
	CP RADIX ET RHIZOMA GINSENG	Ginsenoside Rg1+Re ↑ 0.30%, Ginsenoside Rb1 ↑ 0.20%	HPLC (ODS column)	Solution A: acetonitrile, Solution B: water, 0-35 min (A 19 : B 81), 35-55 min (A 19-29 : B 81-71), 55-70 min (A 29 : B 71), 70-100 min (A 29-40 : B 71-60)	UV 203 nm
	JP GINSENG RADIX	Ginsenoside Rg1 ↑ 0.10%, Ginsenoside Rb1 ↑ 0.20%	HPLC (ODS column, I.D. 4-6 mm x 15 cm, 5 mm)	1) water / acetonitrile (7 : 3) 2) 40˚ 3) adjust flow rate to elute Ginsenoside Rb1 at ca.20 min	UV 203 nm
25	*Platycodon grandiflorum* **A. De Candolle (キキョウ)**				
	CP RADIX PLATYCODI	Total saponin ↑ 6.0%	Dry weight	methanol	Dry weight (105˚)
26	*Pogostemon cablin* **Bentham (カッコウ、純外)**				
	CP HERBA POGOSTEMONIS	Patchouli alcohol ↑ 0.10%	GC		
27	*Polygonatum sibiricum* **Redoute (オウセイ、純外)**				
	CP RHIZOMA POLYGONATI	Glucose ↑ 7.0%	Absorption	80% ethanol	UV 582 nm
28	*Prunella vulgaris* **Linne var.** *lilacina* **Nakai (カゴソウ)**				
	CP SPICA PRUNELLAE	Ursolic acid ↑ 0.12%	HPLC (ODS column)	methanol / water (88 : 12)	UV 210 nm
29	*Prunus armeniaca* **Linne,** *P. armeniaca* **Linne var.** *ansu* **Maximowicz (キョウニン)**				
	CP SEMEN ARMENIACAE AMARUM	Amygdalin ↑ 3.0%	Titration	silver nitrate solution (0.1 mol/L)	
	KP ARMENIACAE SEMEN	Amygdalin ↑ 3.0%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) methanol / water (20 : 80) 2) 20˚ 3) 1.0 mL/min	UV 214 nm
	VP SEMEN ARMENIACAE AMARUM	Amygdalin ↑ 3.0%	Titration		
30	*Prunus persica* **Batsch,** *P. persica* **Batsch var.** *davidiana* **Maximowicz (トウニン)**				
	KP PERSICAE SEMEN	Amygdalin ↑ 0.5%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) methanol / water (20 : 80) 2) 20˚ 3) 1.0 mL/min	UV 214 nm
31	*Rheum palmatum* **Linne (ダイオウ)**				
	CP RADIX ET RHIZOMA RHEI	Aloeemodin+Rhein+Emodin+Chrysophanol+Physcion ↑ 1.5%	HPLC (ODS column)	methanol / 0.1% phosphoric acid solution (85 : 15)	UV 254 nm
	JP RHEI RHIZOMA	Sennoside A ↑ 0.25%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) dilute acetic acid (100) (1 in 80) / acetonitrile (4 : 1) 2) 40˚ 3) adjust flow rate to elute sennoside A at ca.15 min	UV 340 nm
	KP RHEI RHIZOMA	Sennoside A ↑ 0.25%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) dilute acetic acid (100) (1 in 80) / acetonitrile (4 : 1) 2) 40˚ 3) adjust flow rate to elute sennoside A at ca.15 min	UV 340 nm

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32	*Schizandra chinensis* **Baillon** (ゴミシ)				
	CP FRUCTUS SCHISANDRAE CHINENSIS	Schisandrin ↑ 0.40%	HPLC (ODS column)	methanol / water (13 : 7)	UV 250 nm
33	*Scutellaria baicalensis* **Georgi** (オウゴン)				
	CP RADIX SCUTELLARIAE	Baicalin ↑ 9.0%	HPLC (ODS column)	methanol / water / phosphoric acid (47 : 53 : 0.2)	UV 280 nm
	JP SCUTELLARIAE RADIX	Baicalin ↑ 10.0%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) dilute phosphoric acid (1 in 146) / acetonitrile (18 : 7) 2) 50˚ 3) adjust flow rate to elute baicalin at ca. 6 min	UV 277 nm
	KP SCUTELLARIAE RADIX	Baicalin ↑ 10.0%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) dilute phosphoric acid (1 in 146) / acetonitrile (18 : 7) 2) 50˚ 3) adjust flow rate to elute baicalin at ca. 6 min	UV 277 nm
	VP RADIX SCUTELLARIAE	Flavonoid calculate as Baicalin ↑ 4.0%	Absorption	ethanol	UV 279 nm
34	*Strychnos nux-vomica* **Linne** (ホミカ)				
	CP SEMEN STRYCHNI	Strychnine 1.20-2.20%	HPLC (ODS column)	acetonitrile / 0.01 mol/L heptanesulfonic acid sodium salt and 0.02 mol/L potassium dihydrogen phosphate (21 : 79)	UV 260 nm
	JP STRYCHNI SEMEN	Strychnine ↑ 1.07%	HPLC (ODS column, I.D. ca. 4 mm x ca. 15 cm, 5-10 mm)	1) 6.8 g of monobasic potassium phosphate in water to 1000 mL / acetonitrile / triethylamine (45 : 5 : 1), adjust to a pH of 3.0 2) room tempurature 3) adjust flow rate to elute strychnine at ca. 17 min	UV 210 nm
	KP STRYCHNI SEMEN	Strychnine ↑ 1.05%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) 6.8 g of monobasic potassium phosphate in water to 1000 mL / acetonitrile / triethylamine (45 : 5 : 1), adjust to a pH of 3.0 2) room tempurature 3) adjust flow rate to elute strychnine at ca. 17 min	UV 210 nm
	VP SEMEN STRYCHNI	Strychnine ↑ 1.2%	Absorption	0.5 mol sulphuric acid	UV 262, 300 nm
35	*Syzygium aromaticum* **Merrill et Perry** (チョウジ)				
	CP FLOS CARYOPHYLLI	Eugenol ↑ 11.0%	GC (10% polyethylene glycol-20M)		
36	*Zingiber officinale* **Roscoe** (シュウキョウ)				
	KP ZINGIBERIS RHIZOMA	6-Gingerol ↑ 0.4%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) acetonitrile / water (45 : 55) 2) 20˚ 3) adjust flow rate to elute 6-gingerol at ca. 7 min	UV 280 nm
37	*Aloe ferox* **Miller** (アロエ)				
	CP ALOE	Barbaloin ↑ 6.0%	HPLC (ODS column)	acetonitrile / water (25 : 75)	UV 355 nm
	JP ALOE	Barbaloin ↑ 4.0%	HPLC (ODS column, I.D. ca. 6 mm x ca.15 cm, 5 mm)	1) water / acetonitrile / acetic acid (100) (74 : 26 : 1) 2) 30˚ 3) adjust flow rate to elute barbaloin at ca.12 min	UV 360 nm
	VP ALOE	Hydroxyanthracen ↑ 28.0%	Absorption	0.5% magnesium acetate in methanol	UV 512 nm
38	*Alpinia officinarum* **Hance** (リョウキョウ)				
	CP RHIZOMA ALPINAE OFFICINARUM	Cineol ↑ 0.15%	GC		
39	*Angelica pubescens* **Maximowicz** (トウドッカツ、局外)				
	CP RADIX ANGELICAE PUBESCENTIS	Osthol ↑ 0.50%	HPLC (ODS column)	acetonitrile / water (60 : 40)	UV 322 nm
40	*Arctium lappa* **Linne** (ゴボウシ)				
	CP FRUCTUS ARCTII	Arctiin ↑ 5.0%	HPLC (ODS column)	methanol / water (1 : 1.1)	UV 280 nm
41	*Areca catechu* **Linne** (ビンロウジ)				
	CP SEMEN ARECAE	Arecoline ↑ 0.30%	Titration		
42	*Aster tataricus* **Linne fil.** (シオン、局外)				
	CP RADIX ET RHIZOMA ASTERIS	Shionone ↑ 0.10%	HPLC (ODS column)	1)acetonitrile / water (96 : 4) 2) 40˚	1) 10% sulfuric acid in ethanol 2) 110˚ 3) UV 390 and 650 nm
43	*Cassia angustifolia* **Vahl**, *C. acutifolia* **Delile** (センナ)				
	CP FOLIUM SENNAE	Sennoside B ↑ 2.5%	Absorption	0.5% magnesium acetate in methanol	UV 515 nm
	JP SENNAE FOLIUM	Total Sennosides (sennoside A and sennoside B) ↑ 1.0%	HPLC (ODS column, I.D. 4-6 mm x 15 cm, 5 mm)	1) 2.45 g of tetra-*n*-heptylammonium bromide in 1000 mL of a mixture of dilute 1 mol/L acetic acid-sodium acetate buffer pH 5.0 (1 in 10) / acetonitrile (17 : 8) 2) 50˚ 3) adjust flow rate to elute sennoside A at ca. 26 min	UV 340 nm
	KP SENNAE FOLIUM	Total Sennosides (sennoside A and sennoside B) ↑ 1.0%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) 2.45 g of tetra-*n*-heptylammonium bromide in 1000 mL of a mixture of dilute 1 mol/L acetic acid-sodium acetate buffer pH 5.0 (1 in 10) / acetonitrile (17 : 8) 2) 50˚ 3) adjust flow rate to elute sennoside A at ca. 26 min	UV 340 nm
44	*Cassia obtusifolia* **Linne**, *C. tora* **Linne** (ケツメイシ)				
	CP SEMEN CASSIAE	Cryosophanol ↑ 0.080%	HPLC (ODS column)	methanol / 0.1% phosphoric acid solution (85 : 15)	UV 254 nm
45	*Chrysanthemum indicum* **Linne** (キクカ)				
	CP FLOS CHRYSANTHEMI INDICI	Buddoleoside ↑ 0.80%	HPLC (ODS column)	methanol / water / glacial acetic acid (26 : 23 : 1)	UV 334 nm
46	*Citrus aurantium* **Linne** (キジツ)				
	CP FRUCTUS AURANRII IMMATURUS	Synephrine ↑ 0.30%	HPLC (ODS column)	methanol / potassium dihydrogen phosphate solution (50 : 50)	UV 275 nm
47	*Cnidium monnieri* **Cusson** (ジャショウシ)				
	CP FRUCTUS CNIDII	Osthol ↑ 1.0%	HPLC (ODS column)	acetonitrile / water (65 : 35)	UV 322 nm
48	*Coix lacryma-jobi* **Linne var. *ma-yuen* Stapf** (ヨクイニン)				
	CP SEMEN COICIS	Glycerin trioleate ↑ 0.50%	HPLC (ODS column)	acetonitrile / dichloromethane (65 : 35)	Evaporative Light Scattering method
49	*Crataegus pinnatifida* **Bunge var. *typica* Schneider** (サンザシ、局外)				
	CP FRUCTUS CRATAEGI	Citric acid ↑ 5.0%	Titration		

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50	*Crocus sativus* **Linne** (サフラン)				
	CP STIGMA CROCI	Crocin I+II ↑ 10.0%	HPLC (ODS column)	methanol / water (45 : 55)	UV 440 nm
	JP CROCUS	Crocin (Content of active principle)	Absorption	0.098 g of carbazochrome sodium sulfonate in water to 100 mL	UV 438 nm
51	*Gentiana scabra* **Bunge** (リュウタン)				
	CP RADIX ET RHIZOMA GENTIANAE	Gentiopicrin ↑ 1.0%	HPLC (ODS column)	methanol / water (3 : 7)	UV 270 nm
52	*Lindera aggregata* **(Sims) Kosterm.** (ウヤク)				
	CP RADIX LINDERAE	Linderane ↑ 0.030%	HPLC (ODS column)	acetonitrile / water (56 : 44)	UV 235 nm
53	*Lycium barbarum* **Linne**, *L. chinense* **Miller** (クコシ)				
	CP FRUCTUS LYCII	Glucose ↑ 1.8%, Betaine ↑ 0.30%	Glucose (Absorption), Betaine [TLC (Silica gel TLC)]	Glucose (80% ethanol), Betaine [acetone / absolute ethanol / hidrochloric acid (10 : 6 : 1)]	Glucose (UV 490 nm), Betain (UV 515, 590 nm)
	KP LYCII FRUCTUS	Betaine ↑ 0.5%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) acetonitrile / water (85 : 15) 2) 20゜ 3) 1.0 mL/min	UV 210 nm
54	*Peucedanum praeruptorum* **Dunn**, *P. decursivum* **Maxim.** (ゼンコ、純外)				
	CP RADIX PEUCEDANI	Praeruptorin A ↑ 0.90%	HPLC (ODS column)	methanol / water (75 : 25)	UV 321 nm
55	*Pharbitis nil* **Choisy** (ケンゴシ)				
	CP SEMEN PHARBITIDIS	Caffeic acid+Caffeic acid ethyl ester ↑ 0.20%	HPLC (ODS column)	Solution A: acetonitrile, Solution B: 0.04% phosphoric acid solution , 0-12 min (A 13 : B 87), 12-13 min (A 13-54 : B 87-46), 13-19 min (A 54 : B 46)	UV 325 nm
56	*Phellodendron amurense* **Ruprecht**, *P. chinense* **Schneider** (オウバク)				
	CP CORTEX PHELLODENDRI AMURENSIS	Berberine ↑ 0.6%	HPLC (ODS column)	acetonitrile / 0.1% phosphoric acid solution (50 : 50)	UV 265 nm
	CP CORTEX PHELLODENDRI CHINENSIS	Berberine ↑ 3.0%	HPLC (ODS-column)	acetonitrile / 0.1% phosphoric acid solution (50 : 50)	UV 265 nm
	JP PHELLODENDRI CORTEX	Berberine ↑ 1.2%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) 3.4 g of monobasic potassium phosphate and 1.7 g of sodium lauryl sulfate in water to 1000 mL / acetonitrile (1 : 1) 2) 40゜ 3) adjust flow rate to elute berberine at ca. 10 min	UV 345 nm
	KP PHELLODENDRI CORTEX	Berberine ↑ 0.6%	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) 3.4 g of monobasic potassium phosphate and 1.7 g of sodium lauryl sulfate in water to 1000 mL / acetonitrile (1 : 1) 2) 40゜ 3) adjust flow rate to elute berberine at ca. 10 min	UV 345 nm
57	*Piper nigrum* **Linne** (コショウ)				
	CP FRUCTUS PIPERIS	Piperine ↑ 3.0%	HPLC (ODS column)	methanol / water (77 : 23)	UV 343 nm
58	*Polygala tenuifolia* **Willdenow** (オンジ)				
	CP RADIX POLYGALAE	Polygalic acid ↑ 0.70% (HPLC)	HPLC (ODS column)	methanol / 0.2% phosphoric acid solution (70 : 30)	UV 210 nm
59	*Prunus mume* **Siebold et Zuccarini** (ウバイ、純外)				
	CP FRUCTUS MUME	Citric acid ↑ 15.0%	Titration		
60	*Pueraria lobata* **Ohwi** (カッコン)				
	CP RADIX PUERARIAE LOBATAE	Puerarin ↑ 2.4% (HPLC)	HPLC (ODS column)	methanol / water (25 : 75)	UV 250 nm
	JP PUERARIAE RADIX	Puerarin ↑ 2.0% (HPLC)	HPLC (ODS column, I.D. 4-6 mm x 15 cm, 5 mm)	1) 0.05 mol/L sodium dihydrogen phosphate TS / acetonitrile (9 : 1) 2) 40゜ 3) adjust flow rate to elute puerarin at ca.15 min	UV 250 nm
	KP PUERARIAE RADIX	Puerarin ↑ 2.0% (HPLC)	HPLC (ODS column, I.D. 4-6 mm x 15-25 cm, 5-10 mm)	1) methanol / water (25 : 75) 2) 15-25゜ 3) 1.0 mL/min	UV 254 nm
61	*Rehmannia glutinosa* **Liboschitz** (ジオウ)				
	CP RADIX REHMANNIAE	Catalpol ↑ 0.20% (HPLC)	HPLC (ODS column)	acetonitrile / 0.1% phosphoric acid solution (1 : 99)	UV 210 nm
62	*Salvia miltiorrhiza* **Bunge** (タンジン)				
	CP RADIX ET RHIZOMA SALVIAE MILTIORRHIZAE	Tanshinone IIA ↑ 0.20%, Salvinolic acid B ↑ x.0%	HPLC (ODS column)	Tanshinone IIA: [methanol / water (75 : 25)], Salvinolic acid B [methanol / acetonitrile / formic acid / water (30 : 10 : 1 : 59)]	Tanshinone IIA (UV 270 nm), Salvinolic acid B (UV 286 nm)
63	*Saposhnikovia divaricata* **Schiskin** (ボウフウ)				
	CP RADIX SAPOSHNIKOVIAE	Cimicifugoside+5-Methoxyviminol ↑ 0.24%	HPLC (ODS column)	methanol / water (40 : 60)	UV 254 nm
64	*Saussurea lappa* **Clarke** (モッコウ)				
	CP RADIX AUCKLANDIAE	Costunolide +Dehydrocostuslactone ↑ 1.8%	HPLC (ODS column)	methanol / water (65 : 35)	UV 225 nm
65	*Schizonepeta tenuifolia* **Briquet** (ケイガイ)				
	CP SPICA SCHIZONEPETAE	Pulegone ↑ 0.080%	HPLC (ODS column)	methanol / water (80 : 20)	UV 252 nm
66	*Scrophularia ningpoensis* **Hemsley**, *S. buergeriana* **Miquel** (ゲンジン、純外)				
	CP RADIX SCROPHULARIAE	Harpagoside ↑ 0.050%	HPLC (ODS column)	Solution A: acetonitrile, Solution B: 1% acetic acid solution, 0-20 min (A 20-50 : B 80-50)	UV 278 nm
67	*Sophora flavescens* **Aiton** (クジン)				
	CP RADIX SOPHORAE FLAVESCENTIS	Matrine+Oxymatrine ↑ 1.2%	HPLC (ODS column)	acetonitrile / absolute ethanol / 3% phosphoric acid solution (80 : 10 : 10)	UV 220 nm
68	*Sophora japonica* **Linne** (カイカ、純外)				
	CP FLOS SOPHORAE	Total flavonoids ↑ 8.0%, Rutin ↑ 6.0%	Total flavonoids (Absorption) Rutin [HPLC (ODS column)]	Total flavonoids (methanol), Rutin [methanol / 1% glacial acetic acid solution (32 : 68)]	Total flavonoids (UV 500 nm), Rutin (UV 257 nm)
69	*Vitex trifolia* **Linne** (マンケイシ、純外)				
	CP FRUCTUS VITICIS	Viteticarpin ↑ 0.030%	HPLC (ODS column)	methanol / 0.4% phosphoric acid solution (60 : 40)	UV 258 nm

びショウキョウは KP のみ，サンシシ，ニンジン及びサフランは CP と JP のみ，さらにブシは JP のみ定量法が設定されていた．全般的に JP と KP はほぼ同一の分析条件が設定されていた．

考察

今回の比較表作成より，東アジア地区 4 カ国の生薬に関する確認試験法及び定量法（成分含量測定法）の共通点，相違点が明らかとなった．特に前報でも報告したが，局方作成に当り，VP は CP を KP は JP をそれぞれ参考にして作成されているため，CP と VP，また JP と KP との間にはそれぞれ共通点が多く認められた．また確認試験における TLC 条件に関して CP 及び VP では TLC 法に使用する溶媒の種類が非常に多く，かつ多成分系の条件が設定されているのが特徴であると考えられた．

TLC の展開溶媒に関しては，例えばサイコでは CP 及び VP において有害試薬が使用されていないのに対し，JP 及び KP ではクロロホルムが使用されていた．他方，マオウでは逆に JP 及び KP では有害試薬が使用されていないのに対し，CP 及び VP ではクロロホルムが使用されていた．このように生薬により各国における有害試薬の使用状況が明らかに異なっていた．今後はクリーンアナリシスにおける国際調和の観点から，我が国も含め有害試薬を使用している国は，今回の比較表をもとに，他国の有害試薬を使用しない試験法を参考として自国の試験法を変更する努力を行うことが重要と考えられた．

定量法に関しては，VP では未だに HPLC による分析法が確立されておらず，また定量法が設定されている生薬も少なかった．しかし，FHH 会議では，次の VP 改正第 4 版において HPLC 法の導入を含め，多くの点で変更が行われる旨，報告がなされている．CP 2005 年版では 2000 年版と比較して HPLC 法を設定した生薬が飛躍的に増加しており，さらに ELSD 法等，新たな検出機器の導入が認められ，中国政府の生薬の規格設定に関する強い意気込みが感じられた．また KP ではケイヒ，サンシュユ，キョウニン，トウニン，ショウキョウ，クコシに関して HPLC を用いた試験法が設定されているのに対し，JP では未だに設定がなされていない状況であった．今後 JP では，KP に収載されている上記 6 生薬の定量法の検討並びに CP において導入された ELSD 法等，新規検出法の検討が重要な課題と考えられた．

本報では前報と同様，各国薬局方の運用状況等については考慮せず，一元的に比較表を作成し，検討を行った．しかし，各国それぞれの法制度下では薬局方の運用状況が異なるため，各国における運用面の調査も必要と考えられた．

結論

前報に引き続き，将来的な国際調和を踏まえ，各国の薬局方について共通点と相違点を認識することを目的として，特に確認試験法における TLC 条件並びに定量法（成分含量測定法）における HPLC 条件等の各種分析条件の詳細について比較表の作成を試みた．

この結果，TLC 法を用いた確認試験が設定されている生薬は 106 種の共通生薬のうち 89 種で，定量法が設定されている生薬は 69 種であった．TLC 法を用いた確認試験が設定されている 89 種のうち 4 カ国局方すべてにおいて設定されている生薬は 15 種であり，1 種を除きすべての局方においてほぼ同一の TLC 条件が設定されていた．また展開溶媒に有害試薬を使用している生薬が 43 種存在し，クリーンアナリシスにおける国際調和の必要性が示唆された．

定量法に関しては，CP において HPLC 法が設定されている生薬の増加が顕著であった．さらに 4 種の生薬について HPLC 法の検出機器として，JP 並びに KP では未だに導入されていない ELSD 検出器が設定されていたのが特徴的であった．

今後は，4 カ国局方に収載されている生薬に関連する一般試験法を精査し，各国の生薬試験法（試料の採取，異物，分析用試料の作成，乾燥減量，灰分，酸不溶性灰分，エキス含量，精油含量，鏡検等）の各項目について，試験法の設定の有無，試験方法についても比較表の作成を行う予定である．

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保等に関する研究」によった．

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ORIGINAL PAPER

Physicochemical quality evaluation of natural compounds isolated from crude drugs

Standard compounds for the official specification and testing method of "Processed Aconite Root" and "Powdered Processed Aconite Root" in the Japanese Pharmacopoeia

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Abstract Aconite root has high toxicity caused by diester alkaloids, thus it was necessary to define the limiting value of diester alkaloids used in medicine formulation. To give the quality of "Processed Aconite Root" and "Powdered Processed Aconite Root" in the Japanese Pharmacopoeia (14th edn, supplement II), we established the official specification and evaluation methods of standard substances. High qualitative grade diester alkaloids, aconitine, hypaconitine, jesaconitine and mesaconitine, which were useful to evaluate the purity of processed aconite root and powdered processed aconite root, were prepared and evaluated for their stability. We studied the physicochemical specification and evaluation methods of these alkaloids. In addition, an "Aconitum diester alkaloids standard solution for purity", which was used for the purity test, was prepared, and we also studied its physicochemical specification and evaluation methods. In addition, to evaluate the quality of processed aconite root and powdered processed aconite root, a TLC identification test was established. A monoester alkaloid of benzoylmesaconine hydrochloride was used as the reference standard in the latter test, and we also investigated its physicochemical specification and evaluation methods.

Keywords Processed aconite root · Powdered processed aconite root · Japanese Pharmacopoeia · Standard substance · Aconitum diester alkaloids · Benzoylmesaconine hydrochloride

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Introduction

A component limiting test to prescribe the content of toxic ingredients that may be included in crude drugs and herbal medicines (Kampo medicines) was defined by the Japanese Pharmacopoeia (JP). Previously, we produced an official specification and method for evaluating aristolochic acids [1, 2] when the component limiting test to examine the non-detection of aristolochic acid I in the Asiasari Radix was published.

Here, we establish official specification and evaluation methods of standard substances for "Processed Aconite Root" and "Powdered Processed Aconite Root" for the Japanese Pharmacopoeia 14th edn supplement II (JP14-II) [3]. The raw material of aconite root has high toxicity, which is generally attenuated by a variety of procedures such as immersion into salt water, coating with lime or ash, and/or some heat treatment (e.g., autoclaving) prior to medicinal use. Hikino [4–6] reported that the toxicity is caused by diesteralkaloids, and was diminished by processing as above because diesteralkaloids were decomposed into monoester alkaloids or further decomposed products. The total amount of diesteralkaloids remaining therefore requires tight regulation. High qualitative grade diester alkaloids (aconitine, hypaconitine, jesaconitine and mesaconitine) were prepared for quantitative purity tests. The physicochemical specification, evaluation methods and stability of these alkaloids were studied. In addition, an "Aconitum diester alkaloids standard solution for purity" was also prepared and evaluated.

To evaluate the quality of processed aconite root and powdered processed aconite root, a TLC-based identification test was developed. Benzoylmesaconine hydrochloride was used as the reference standard, and we investigated its physicochemical specification and evaluation methods.

Experimental

Apparatus and reagents

NMR spectra were measured on an AM400 (Brucker Biospin, Ibaraki, Japan), MS spectra were measured on a CONCEPT 1H (Kratos Analytical, Manchester, UK).

IR and UV spectra were recorded on an FT/IR-430 (Jasco, Tokyo, Japan) and U-3000 (Hitachi, Tokyo, Japan, pre-calibrated); data were analyzed with UV Solution software (Hitachi).

Water contents were acquired with a Karl Fischer Moisture Titrator (Coulometric Method) MKC-610-DT (pre-calibrated; Kyoto Electronics Manufacturing, Kyoto, Japan), catholytes and anolytes were equipped with HYDRANAL- Coulomat CG-K and HYDRANAL-Coulomat AK (Sigma-Aldrich, Sydney, Australia).

HPLC chromatograms were taken on a LC-10ADvp high pressure gradient system (Shimadzu, Kyoto, Japan; pre-calibrated), data were analyzed with CLASS-VP software (Ver. 6.12 SP2; Shimadzu), and HPLC was performed on TSK gel ODS-80TM (ϕ 4.6 × 150 mm, 5 μm) (Tosoh, Tokyo, Japan).

The scales used were CP225D (Sartorius, Goettingen, Germany; pre-calibrated) and M5P-F (Sartorius, pre-calibrated).

To prepare the aconitum diester alkaloids, the mixture solution was dispensed with a preparative pipetter (Dispensette ORGANIC EASY CALIBRATION; BrandTech Scientific, Essex, CT) and a lure lock-type syringe Gastight #1005, (Hamilton, Reno, NV). Column chromatography was carried out on Silica gel 60 (Merck, Darmstadt, Germany), Aluminum oxide 90 standardized activity II-III (70–230 mesh ASTM) (Merck) and Chromatorex Chromatography Silica Gel NH (size 100–200 mesh) (Fuji Silysia Chemical, Kasugai, Aichi Japan).

TLC was performed on pre-coated Silicagel 60 F_{254} glass plates (Merck).

Preparation of aconitum diester alkaloid standards

Dried tubers of *Aconitum* species were extracted with diethylether and ammonia solution, and we collected the diethylether fraction. This fraction was subjected to silica gel column chromatography with MeOH/$CHCl_3$ to obtain the alkaloid fraction, which was successively subjected to silica gel column chromatography with EtOAc/cyclohexane/Et_2NH or NH silica gel column chromatography with EtOAc/hexane to obtain aconitum diester alkaloids, aconitine, hypaconitine, jesaconitine and mesaconitine. These aconitum diester alkaloids were repeatedly recrystallized with EtOH(99.5), dried for not less than 12 h in a desiccator [reduced pressure not exceeding 0.67 kPa, phosphorous(V) oxide, 40°C] to prepare the "aconitum diester alkaloid standards". Commercial reagents were also used in the preparation. Three lots of each "Aconitum diester alkaloid standard" were prepared.

Evaluation of aconitum diester alkaloids

The following tests were performed according to JP:

Description A 10 mg sample of each compound was weighed, placed on white paper, and observed with the naked eye

Solubility	A 10 mg sample of each compound was weighed, and tested for solubility to comply with JP
Melting point	The melting point was measured according to "Melting Point Determination" as described in JP
IR spectrum	IR spectra were measured by the KBr disk method according to "Infrared spectrophotometry" as described in JP
UV spectrum	UV spectra were measured following "Ultraviolet-visible Spectrophotometry" in JP. Samples (5 mg) were weighed and dissolved in EtOH(99.5) to exactly 200 ml, and used as the sample solution. The specific absorption, $E^{1\%}_{1\,cm}$, was calculated at 230 nm for aconitine, hypaconitine and mesaconitine, and at 258 nm for jesaconitine

Purity

TLC method

The sample (5.0 mg) was weighed exactly and dissolved in 2 ml acetonitrile, and used as the sample solution, which was pipetted as 1 ml exactly, acetonitrile was added to exactly 50 ml, and used as the standard solution. These solutions were tested as directed under TLC. Solutions of samples and standards (20 μl each) were spotted on a TLC plate, and developed with a mixture of EtOAc/EtOH(99.5)/ammonia water(28) (40:3:2) to a distance of 10 cm, and the plate was air-dried. The plate was sprayed evenly with Dragendorff's test solution (TS), air-dried, then sprayed evenly with sodium nitrate TS (acceptance criteria: spots other than the principal spot obtained from the sample solution were not more intense than the principal spot with the standard solution).

HPLC method

Conditions were chosen to correspond to those applied in the testing method for the crude drug (processed aconite root).

Exactly 5.0 mg sample was weighed and dissolved in 5 ml acetonitrile, and used as the sample solution, which was pipetted as exactly 1 ml, acetonitrile was added to exactly 50 ml, and used as the standard solution. Aliquots of 10 μl of sample solution and standard solution were subjected to the HPLC test according to the following operating conditions, and we determined each peak area using the automatic integration method:

Detector	A UV absorption detector (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine)
Column	A stainless steel column of 4.6 mm internal diameter and 150 mm length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm particle diameter)
Column temperature	A constant temperature of about 40°C
Mobile phase	A mixture of phosphate buffer solution (19.3 g disodium hydrogen phosphate dodecahydrate was dissolved in 3,660 ml water and 12.7 g phosphoric acid added) and tetrahydrofuran (9:1)
Flow rate	The flow rate was adjusted so that the retention time of aconitine was about 26 min, hypaconitine was about 23 min, mesaconitine was about 19 min and jesaconitine was about 36 min
Time span of measurement	About three times as long as the retention time of each alkaloid
Acceptance criteria	The total area of peaks other than those of each aconitum diester alkaloid and the solvent obtained from the sample solution not larger than the peak area of corresponding aconitum diester alkaloid with the standard solution

System suitability test

We required analytical validation for the JP testing methods, so we constructed validation items for the HPLC method. The following three items were tested with this method.

Test for required detectability Each standard solution was pipetted as 1 ml, and acetonitrile was added to exactly 20 ml. This solution was run as 10 μl under the above operating conditions, and we confirmed that the peak area of aconitum diester alkaloids obtained from 10 μl of this solution was equivalent to 3.5–6.5% of that obtained from 10 μl of each standard solution.

System performance Samples of 1 mg each of aconitine, hypaconitine and mesaconitine, and 8 mg jesaconitine were dissolved in 200 ml acetonitrile, and run as 10 µl of this solution under the above operating conditions, and we confirmed that mesaconitine, hypaconitine, aconitine and jesaconitine were eluted in this order, and each resolution value between their peaks was not less than 1.5, respectively.

System repeatability The test was repeated six times with 10 µl of each standard solution under the above operating conditions, confirming that the relative standard deviation of the peak area of each aconitum diester alkaloid was not more than 1.5%.

Moisture content

The moisture content was measured with the Karl Fischer moisture titrating method (coulometric method); however, the sample was dried for not less than 12 h in a desiccator [reduced pressure not exceeding 0.67 kPa, phosphorous(V) oxide, 40°C]. Each sample was weighed accurately at 5 mg and the test was performed.

Stability of diester alkaloids in solvent

Forced degradation test of aconitine

To confirm the stability of aconitine in MeOH, the solution of aconitine was refluxed and the production of impurities was monitored by HPLC.

Aconitine was weighed at about 5 mg, dissolved in 10 ml MeOH, and 1 ml was pipetted exactly, MeOH was added to exactly 10 ml, heated under a reflux condenser on a water bath for 2 h and used as the sample solution.

Temporal stability

To confirm the temporal stability of diester alkaloids in solvents, MeOH, CH_3CN and 0.05 mol/l phosphoric acid buffer (pH 2.7), solutions of each diester alkaloid in these solvents were stored and the production of impurities was monitored by HPLC. About 20 mg of each sample was weighed and dissolved in 100 ml of each solvent as identified above, respectively. Solutions were stored in the following three conditions: light shielding at under 5°C, light shielding at room temperature and light exposure at room temperature. The sampling periods were 3 days, 1 week, 2 weeks, 1 month, 2 months, 3 months and 6 months.

Sampling of the aconitum diester alkaloid mixture standard solution

The aconitum diester alkaloids, aconitine, hypaconitine, jesaconitine and mesaconitine that complied to the specification were weighed at 10, 30, 10 and 20 mg, respectively, then a mixture of phosphate buffer solution and CH_3CN (1:1) or CH_2Cl_2 was added to make accurately 1,000 ml.

Evaluation of the aconitum diester alkaloid mixture standard solution by HPLC

The analysis proceeded as for the testing method for the crude drug (processed aconite root) except for the mobile phase and flow rate:

Mobile phase	A mixture of phosphate buffer solution (19.3 g disodium hydrogen phosphate dodecahydrate was dissolved in 3,660 ml water and 12.7 g phosphoric acid added) and tetrahydrofuran (183:17)
Flow rate	The flow rate was adjusted so that the retention time of mesaconitine was about 31 min

Sampling of the aconitum diester alkaloid mixture standard

A accurately measured 5 ml sample of aconitum diester alkaloids standard solution, dissolved in CH_2Cl_2, was placed in a brown glass bottle, the solvent was evaporated, and further dried in vacuo at room temperature. The air was exchanged for nitrogen then the bottles were capped air-tight.

Stability of the aconitum diester alkaloid mixture standard

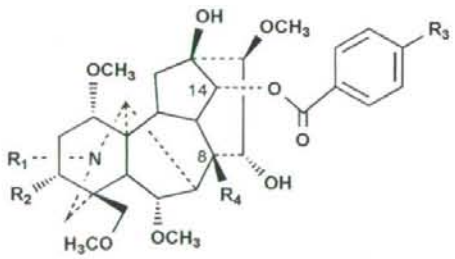
The bottle of aconitum diester alkaloid mixture standard was opened, and exactly 5 ml of a mixture of phosphate buffer solution and CH_3CN (1:1) was added. The bottle was then capped and vigorously shaken, dissolved using ultrasonic waves, and used as the sample solution. It was stored with light shielding under 5°C. The sampling periods were the initial timepoint and at 1 year.

Results and discussion

Preparation and evaluation of aconitum diester alkaloids

Aconitum diester alkaloids were prepared at sufficiently high quality to be useful for quantitative limiting tests, using the HPLC method, as the reagent for component determination listed in JP14-II. Purity was defined as not less than 98.0 or 99.0% by HPLC relative area. Each evaluation method was established according to the guidelines for the official specification and testing method for medicinal raw materials [7]. The structures of the alkaloids obtained are shown in Fig. 1, and were identified by comparing NMR, MS, UV and IR data with references [8–13].

Specification values were established by actual measurements of in-house products and/or commercial products. We carried out the following tests with three lots of each alkaloid, and three replications, respectively.



	R_1	R_2	R_3	R_4
aconitine	C_2H_5	OH	H	$OCOCH_3$
jesaconitine	C_2H_5	OH	OCH_3	$OCOCH_3$
mesaconitine	CH_3	OH	H	$OCOCH_3$
hypaconitine	CH_3	H	H	$OCOCH_3$
benzoylmesaconine	CH_3	OH	H	OH

Fig. 1 Structures of aconitum diester alkaloids

Description, solubility and melting point

The description and melting point of each alkaloid are shown in Table 1. Aconitine, hypaconitine and mesaconitine were crystalline; however, jesaconitine was an amorphous powder. Table 2 lists the solubility of the samples.

Identification

Aconitum diester alkaloids were identified by examining their IR spectra in comparison with the authentic data. The IR spectra are shown in Fig. 2, and each diester alkaloid was distinct. The specification values of IR were determined and are shown in Table 3.

UV absorbance

The absorption maximum of jesaconitine under UV was different from that of the other three diester alkaloids. Therefore, aconitine, hypaconitine and mesaconitine were measured at 230 nm, while jesaconitine was measured at 258 nm. The UV spectra of the diester alkaloids are shown in Fig. 3. The results and specification values are shown in Table 4. Specification values were calculated at the mean ± 3SD (standard deviation).

Moisture content

All the diester alkaloids had less than 1.0% moisture, so did not require calculation of the anhydrous basis of standard substances for quantitative component analysis with HPLC in the following purity test.

Purity

Aconitum diester alkaloids were produced from crude drugs, so the purity test was required to detect the ingredients projected to remain throughout the production process. In general, impurities were identified by TLC and HPLC.

Table 1 Description of aconitum alkaloids

Standard	Color	Shape	Melting point[a]
Aconitine	White	Crystals or crystalline powder	About 185°C (with decomposition)
Hypaconitine	White	Crystals or crystalline powder	About 175°C (with decomposition)
Jesaconitine	White	Powder	-
Mesaconitine	White	Crystals or crystalline powder	About 190°C (with decomposition)
Benzoylmesaconine HCl[b]	White	Crystals or crystalline powder	About 250°C (with decomposition)

[a]Melting point was shown as the mean value (n=9)

[b]Benzoylmesaconine HCl: benzoylmesaconine hydrochloride

Table 2 Solubility of aconitum alkaloids

Standard	Solubility in solvent[a]			
	Acetonitrile	Ethanol (99.5)	Diethyl ether	Water
Aconitine	Sparingly soluble	Sparingly soluble	Slightly soluble	Practically insoluble
Hypaconitine	Soluble	Sparingly soluble	Sparingly soluble	Practically insoluble
Jesaconitine	Freely soluble	Freely soluble	Freely soluble	Practically insoluble
Mesaconitine	Slightly soluble	Slightly soluble	Very slightly soluble	Practically insoluble
Benzoylmesaconine HCl	Soluble	Soluble	Practically insoluble	Soluble

[a]Solubilities are expressed by General Notices in Japanese Pharmacopoeia (JP)

[b]Benzoylmesaconine HCl: benzoylmesaconine hydrochloride

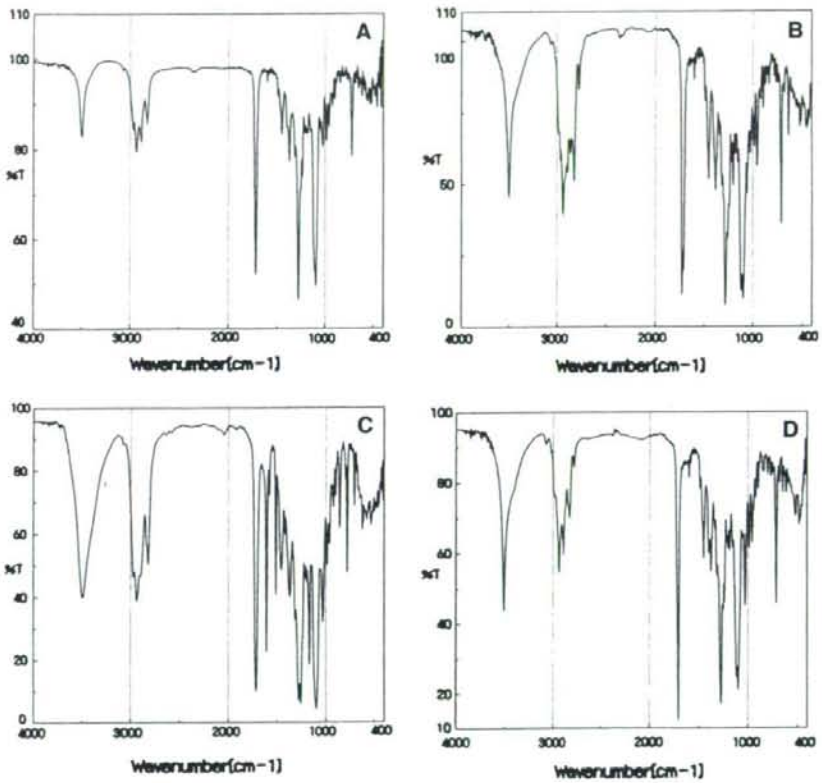


Fig. 2 IR spectra of diester alkaloids for identification. **a** Aconitine, **b** hypaconitine, **c** jesaconitine, **d** mesaconitine

Thin layer chromatography To detect the alkaloids specifically, we adopted the following detection conditions. Dragendorff's TS was sprayed evenly on the plate, which was then air-dried and sprayed evenly with sodium nitrite TS. Sodium nitrite TS intensified the sensitivity of aconitum alkaloids more than Dragendorff's TS alone. The result is shown in Fig. 4. The diester alkaloids were separated from each other, and detected clearly, even at less than 0.1 μg/ml, and each diester alkaloid complied with the acceptance criteria mentioned in the experimental section. Additionally, monoester alkaloids could be analyzed using the same method. We show samples of processed aconite root and neoline in Fig. 4 for reference. A spot detected at

Table 3 IR specification values of diester alkaloids

Standard	Specification values[a] (ν_{max} cm^{-1})
Aconitine	3,500, 1,718, 1,278, 1,111, 1,097, 717
Hypaconitine	3,500, 1,728, 1,712, 1,278, 1,118, 1,099, 714
Jesaconitine	3,500, 1,715, 1,607, 1,281, 1,259, 1,099, 772
Mesaconitine	3,510, 1,713, 1,277, 1,116, 1,098, 717

[a]Specification values were shown as the mean value (n=9)

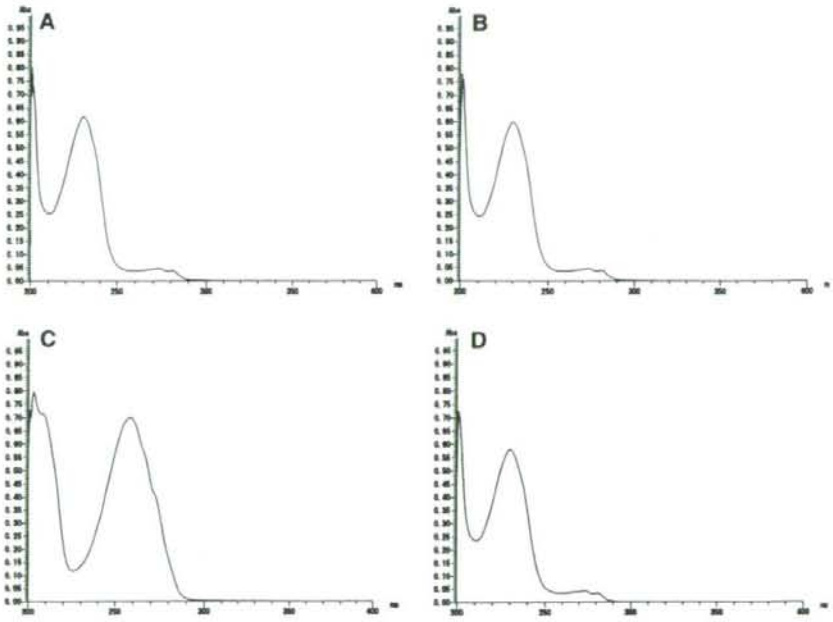


Fig. 3 UV spectra of diester alkaloids for identification. **a** Aconitine, **b** hypaconitine, **c** jesaconitine, **d** mesaconitine

Table 4 UV specification values of diester alkaloids

Standard	Wavelength (nm)	Specific absorption[a]		
		Average	SD	Specification value
Aconitine	230	227.3	5.2	211–243
Hypaconitine	230	234.7	5.6	217–252
Jesaconitine	258	280.1	3.3	270–291
Mesaconitine	230	229.1	5.8	211–247

[a]Specification values were shown as the mean ± 3 SD (n=9)

about Rf 0.3 in processed aconite root was neoline of the chasmanine-type alkaloid.

High performance liquid chromatography HPLC analysis conditions were optimized to separate each peak of four diester alkaloids and their related substances. HPLC chromatograms of the alkaloids are shown in Fig. 5. The total area of the peaks other than the peak of each alkaloid and solvent obtained with the sample solution was not larger than the peak area of each alkaloid with the standard solution, respectively. Thus, each diester alkaloid complied with the acceptance criteria mentioned in the experimental section.

Stability of diester alkaloids

The temporal stability of four diester alkaloids was tested over 6 months and a forced degradation test of aconitine was performed. The temporal stability of four diester alkaloids was evaluated under three

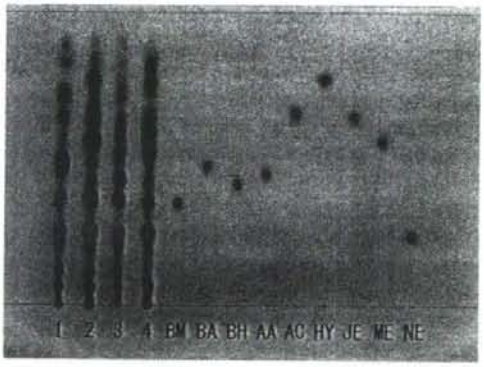


Fig. 4 TLC of aconitum alkaloids and commercial samples for purity. Samples: *1–4* (see Table 5), *BM* benzoylmesaconine, *BA* benzoylaconine, *BH* benzoylhypaconine, *AA* 14-anisoylaconine, *AC* aconitine, *HY* hypaconitine, *JE* jesaconitine, *ME* mesaconitine, *NE* neoline

conditions to examine the effects of light exposure and temperature. The results of the temporal stability test are shown in Fig. 6. All four alkaloids degraded faster in MeOH than in the other solutions, and temperature had little effect on degradation, while methanolysis at C-8 of aconitine was observed in the forced degradation test of aconitine in MeOH.

This proved that MeOH was not a suitable solvent for use under the testing conditions. Consequently, we concluded that a mixture of phosphate buffer

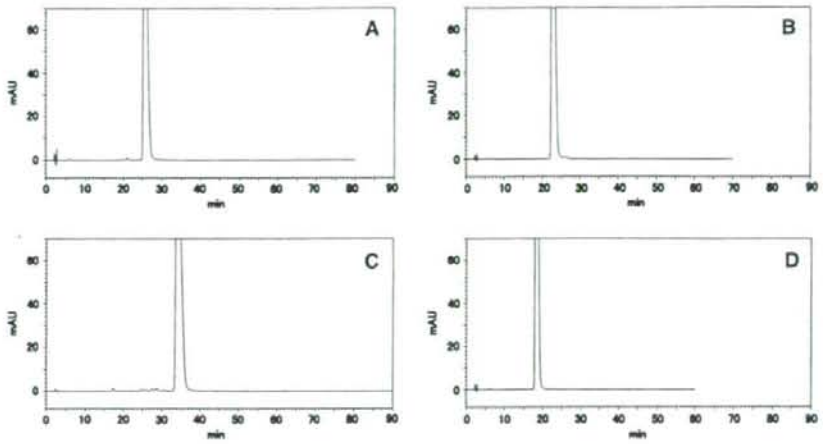


Fig. 5 HPLC of diester alkaloids for purity. **a** Aconitine, **b** hypaconitine, **c** jesaconitine, **d** mesaconitine. Each chart was magnified about 2^2 on the y-axis

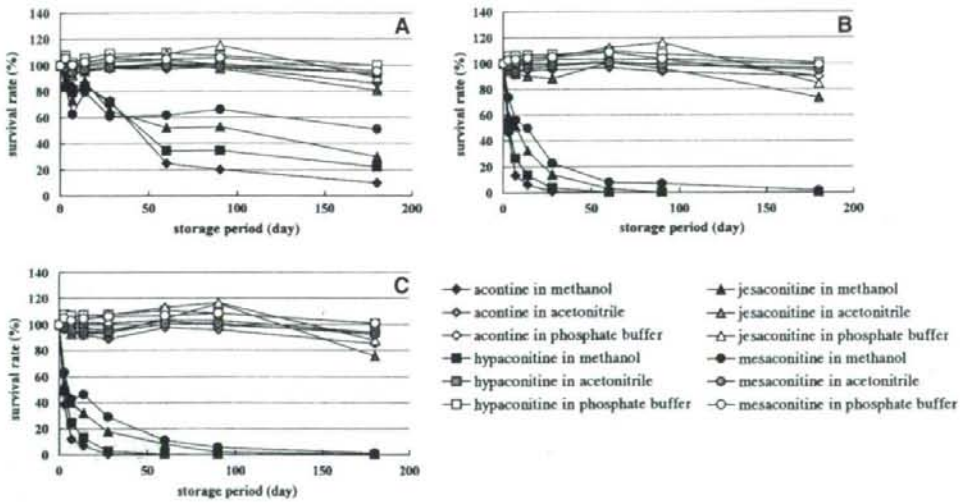


Fig. 6 Temporal stability of diester alkaloids in solution. **a** Stored with light shielding under 5°C, **b** Stored with light shielding at room temperature. **c** Stored with light exposure at room temperature. Survival rate (%) indicates the ratio of the peak area of stored solution versus that of the initial solution

and acetonitrile could be used as the solvent for HPLC.

Official specification and evaluation method for aconitum diester alkaloid mixture solution

The following three conditions were required to construct official specification and evaluation methods with HPLC:

1. The peaks of diester alkaloids must not overlap and their peak areas should be different for discrimination.
2. The range of the calibration curve of a standard solution with 1-point calibration must cover the content of diester alkaloids in crude drugs. Incidentally, the component limiting values are as follows, processed aconite root (1 g): aconitine, jesaconitine, hypaconitine and mesaconitine are 60, 60, 280 and 140 μg respectively, and their total amount is not more than 450 μg. Powdered processed aconite root (1 g): aconitine, jesaconitine, hypaconitine and mesaconitine are 55, 40, 55 and 120 μg, respectively, and their total amount is not more than 230 μg.

3. Each diester alkaloid in a solution must be stable.

Aconitum diester alkaloids, aconitine, jesaconitine, hypaconitine and mesaconitine were weighed at 10, 10, 30 and 20 mg, respectively, then we added a mixture of phosphate buffer solution and CH_3CN (1:1) to accurately make 1,000 ml. In this case, the ratio of the peak heights of aconitine, jesaconitine, hypaconitine and mesaconitine in HPLC were about 10:1:35:30 at 231 nm, and about 2:8:7:6 at 254 nm. The HPLC chromatogram of the aconitum diester alkaloid mixture solution is shown in Fig. 7.

Stability of the aconitum diester alkaloid mixture solution

In JP14-II, the HPLC peak height method was used for the component limiting test of diester alkaloids; however, the retention time, peak shape and peak height varied with small changes in the solvent composition. Consequently, we evaluated the stability of the alkaloids by comparing the HPLC peak area. After storage for 1 year, the peak area of each diester alkaloid showed scarcely any change. The survival ratios (%) of stored sample versus the initial sample were 99.3% (aconitine), 100.0% (jesaconitine), 99.3% (hypaconitine), and 100.2% (mesaconitine), respectively, evaluated by the HPLC peak area of each diester alkaloid.

Official specification and evaluation method for "Benzoylmesaconine hydrochloride with thin-layer chromatography"

"Processed Aconite Root" is listed as the tuberous root of *Aconitum carmichaeli* Debeaux or *Aconitum japonicum* (Thunberg). We adopted benzoylmesaconine hydrochloride for reference, because it is present at a relatively high abundance in both sources. We carried out the following tests to evaluate the quality of benzoylmesaconine hydrochloride.

Description, solubility and melting point

Benzoylmesaconine hydrochloride was a white crystal or crystalline powder (see Table 1).

Purity

Impurities were identified by TLC. Benzoylmesaconine was separated clearly, and detected sensitively even at less than 0.1 μg/ml. Sample solutions were prepared at 5 mg/2 ml acetonitrile solution, which was detected at about 100 times the detection limit. Regarding the stability of benzoylmesaconine hydrochloride in acetonitrile, no impurities such as a degradation product were detected with TLC after 6 months' storage. Additionally, several commercial crude drugs were subjected to the same method, and the results are shown in Fig. 8. The trial samples are

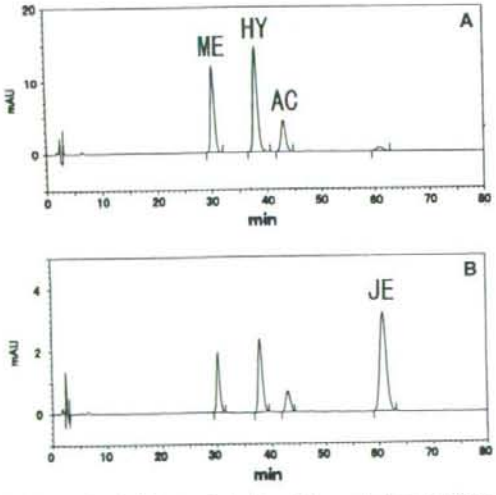


Fig. 7 HPLC of diester alkaloid mixture solution. **a** Detected with UV wavelength 231 nm, **b** detected with UV wavelength 254 nm. *AC* Aconitine, *HY* hypaconitine, *ME* mesaconitine, *JE* jesaconitine

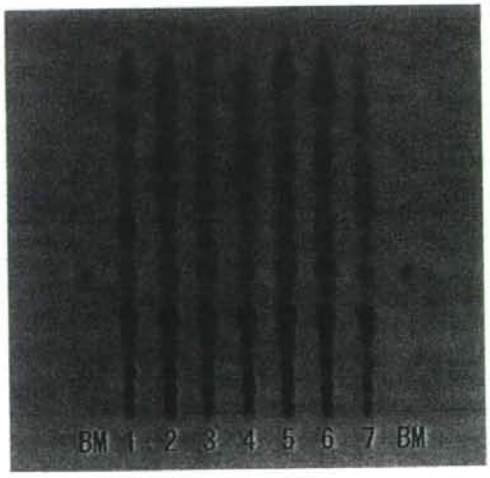


Fig. 8 TLC of benzoylmesaconine hydrochloride and commercial samples. Samples: *1–7* (see Table 5), *BM* benzoylmesaconine hydrochloride

Table 5 Samples for the identification of benzoylmesaconine in Fig. 8

No.	Sample name*	Preparing and process
1	Processed Aconite Root 1	Autoclaved
2	Processed Aconite Root 1	Autoclaved
3	Processed Aconite Root 2	Heated or autoclaved after rinsed in salt or rock salt water
4	Processed Aconite Root 3	Treated with lime after rinsed in salt water
5	Powdered Processed Aconite Root 1	Autoclaved
6	Powdered Processed Aconite Root 1	Autoclaved
7	Powdered Processed Aconite Root 2	Heated or autoclaved after rinsed in salt or rock salt water

*These samples are based on their preparation process in JP14 supplement II

shown in Table 5. Benzoylmesaconine was detected in every sample.

Conclusion

In conclusion, we prepared high purity standard substances useful for purity tests. Aconitine, hypaconitine and mesaconitine were not less than 99.0%, and jesaconitine was not less than 98.0% by HPLC relative area. We also prepared benzoylmesaconine hydrochloride that was not less than 98.0% by TLC. Next, we verified the purity of these substances, and established their official specification and evaluation methods. Our study was reflected in JP14-II, which went into effect in January 2005. Their listed names are "Aconitine for purity", "Hypaconitine for purity", "Mesaconitine for purity", "Jesaconitine for purity", "Benzoylmesaconine hydrochloride for thin-layer chromatography" and "Aconitum diester alkaloids standard solution for purity". Finally, we stress that it is essential to maintain the experimental and analytical environment to acquire credible data.

Acknowledgements We are most grateful to the JP members concerned for supporting this work.

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味認識装置による漢方処方の味の評価に関する研究（第3報）苓桂朮甘湯に関する検討

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Evaluation of the Taste of a Kampo Formula by a Taste-Sensing System (3) [1,2]: the Taste of Ryokeijutsukanto

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In the course of our studies to evaluate objectively the taste of Kampo formulae by a taste-sensing system, we investigated the characteristic taste and taste factors of Ryokeijutsukanto, of which the component crude drugs are Poria Sclerotium, Glycyrrhiza, Cinnamon Bark and Atractylodes rhizome or Atractylodes Lancea Rhizome.

The results suggested the following facts. 1) Glycyrrhiza mainly contributes to the saltiness of Ryokeijutsukanto and Poria Sclerotium decreases it. 2) Atractylodes Rhizome contributes to the anionic bitterness which shows up in Atractylodes Rhizome containing Ryokeijutsukanto, while Cinnamon Bark contributed to it in the case of Atractylodes Lancea Rhizome containing Ryokeijutsukanto. 3) The taste-sensing system does not recognize a difference between the taste of Ryokeijutsukanto and that of the mixed preparations consisting of the corresponding four single decoctions of the component crude drugs. 4) No component crude drugs solely expresses the Ryokeijutsukanto-like taste in the decoction. In other words, the combination of the tastes of the component drugs expresses that of the Ryokeijutsukanto decoction.

Keywords: taste evaluation, Kampo formula, Ryokeijutsukanto, taste-sensing system

はじめに

日本薬局方生薬総則で規定される生薬の性状の項では，“味”は適否の判定基準とされている．従って生薬や生薬から調製される漢方処方における味の表現は，化学的合成医薬品と比べてより重要であり，客観的であることが望まれる．我々は，漢方処方の品質評価の一環として，漢方処方の味の客観的な評価に関する味認識装置の有用性について検討を行っている．これまでに，本装置による測定により漢方処方の味の数値化が可能であり，また，葛根湯においては，その構成生薬の一つであるマオウが葛根湯独自の味に大きく寄与していることなどを明らかにした[1,2]．今回は，繁用処方のひとつであり，第十五改正日本薬局方[3]にエキスが新規収載された苓桂朮甘湯について，味認識装置を用いた味測定並びにヒトによる味覚試験を行い，処方の味を特徴付けている味要素についての検討を行った．また，苓桂朮甘湯の構成生薬は，ブクリョウ，ケイヒ，カンゾウ，ジュツの4種類であるが，日本薬局方に収載された本処方エキスのジュツに関しては，ビャクジュツ及びソウジュツいずれも使用が認められている．そのため本研究では，ジュツとしてビャクジュツ及びソウジュツそれぞれを使用し，比較検討を行った．更に，漢方薬は，その煎じ方により抽出される成分含量やエキスの収量が異なるという報告はあるが[4-7]，味の違いについての詳細な報告は無い．そこで今回，苓桂

Table 1 Components of test samples based on Ryokeijutsukanto

No.	Samples	Component crude drugs				
		Poria Sclerotium	Glycyrrhiza	Cinnamon Bark	Atractylodes Rhizome	Atractylodes Lancea Rhizome
1.	Ryokeijutsukanto (A)*	○	○	○	○	
2.	Ryokeijutsukanto (AL)*	○	○	○		○
3.	Poria Sclerotium removing Ryokeijutsukanto (A)		○	○	○	
4.	Poria Sclerotium removing Ryokeijutsukanto (AL)		○	○		○
5.	Glycyrrhiza removing Ryokeijutsukanto (A)	○		○	○	
6.	Glycyrrhiza removing Ryokeijutsukanto (AL)	○		○		○
7.	Cinnamon Bark removing Ryokeijutsukanto (A)	○	○		○	
8.	Cinnamon Bark removing Ryokeijutsukanto (AL)	○	○			○
9.	Atractylodes Rhizome removing or Atractylodes Lancea Rhizome removing Ryokeijutsukanto	○	○	○		
10.	Poria Sclerotium	○				
11.	Glycyrrhiza		○			
12.	Cinnamon Bark			○		
13.	Atractylodes Rhizome				○	
14.	Atractylodes Lancea Rhizome					○
15.	Separately decocted mixture 1	○	○	○	○	
16.	Separately decocted mixture 2	○	○	○		○
17.	Separately decocted mixture 3		○	○	○	
18.	Separately decocted mixture 4		○	○		○
19.	Separately decocted mixture 5	○		○	○	
20.	Separately decocted mixture 6	○		○		○
21.	Separately decocted mixture 7	○	○		○	
22.	Separately decocted mixture 8	○	○			○
23.	Separately decocted mixture 9	○	○	○		

* (A) and (AL) indicate that the corresponding is prepared samples with Atractylodes Rhizome and Atractylodes Lancea Rhizome, respectively.

朮甘湯の各構成生薬の単味煎出液を混合して再構成した苓桂朮甘湯煎出液を作製し，再構成した場合と本来の処方との味の比較を行った．また，構成生薬から一味ずつ除いて煎出した液についても味の比較を行った．本報では，これらの検討により得られた知見について報告する．

材料及び方法

1. 実験材料

本研究で使用した生薬（日本薬局方適合品）は，株式会社ウチダ和漢薬より入手し，残余は国立医薬品食品衛生研究所生薬部に保管されている．使用した生薬及びロット番号は以下の通りである．ブクリョウ (Lot No. US263116)，ケイヒ (Lot No. US453102)，カンゾウ (Lot No. US452804)，ビャクジュツ (Lot No. SU262825)，ソウジュツ (Lot No. US262921)．

2. 装置

味の測定には，味認識装置 SA402B（株式会社インテリジェントセンサーテクノロジー）を用いた．また，苓桂朮甘湯などの煎出には，自動煎じ器煎治（株式会社ウチダ和漢薬）を用いた．

3. 試薬・試液

塩化カリウム (30 mM) と酒石酸 (0.3 mM) を溶解した水溶液を基準液とした．基準液は，無味に近く，かつ味認識装置で使用する脂質膜センサープローブで安定した出力値が得られる溶液である．塩化カリウム及び酒石酸は和光純薬工業株式会社より入手した．また，水は EQS-10L システム（日本ミリポア株式会社）により精製した超純水を使用した．

4. 試料の調製

苓桂朮甘湯関連の各試料の構成生薬を Table 1 に示す．

4-1. 苓桂朮甘湯

苓桂朮甘湯一日分量（構成生薬：日局ブクリョウ 6 g，日局ケイヒ 4 g，日局カンゾウ 2 g，日局ビャクジュツもしくは日局ソウジュツ 3 g，総量 15 g）に，水 600 mL を加え，自動煎じ器にて，約 300 mL になるまで煎出した．

煎出液をろ過し，水で容量を300 mLに合わせ，3,000 rpmで10分間遠心分離した．遠心分離した上清を，ヒトによる味覚試験用試料液とした．また，遠心分離した上清に，塩化カリウム (10 mM) と酒石酸 (0.1 mM) を添加した液を味認識装置測定用試料液とした (Table 1, No. 1, 2).

4-2. 各一味抜き苓桂朮甘湯の煎出液（同時煎一味抜き苓桂朮甘湯）

苓桂朮甘湯の構成生薬から一種類を除き，40 mL/g 生薬の割合で水を加え，自動煎じ器にて，約半量になるまで煎出した．煎出液をろ過し，水を加えて容量をはじめに加えた水の半量に合わせ，3,000 rpm で 10 分間遠心分離した．遠心分離した上清を，ヒトによる味覚試験用試料液とした．また，遠心分離した上清に，塩化カリウム (10 mM) と酒石酸 (0.1 mM) を添加した液を味認識装置測定用試料液とした (Table 1, No. 3 ~ 9).

4-3. 単味生薬煎出液

苓桂朮甘湯の各構成生薬 15 g に，水 600 mL を加え，自動煎じ器にて，約 300 mL になるまで煎出した．煎出液をろ過し，水で容量を 300 mL に合わせ，3,000 rpm で 10 分間遠心分離した．遠心分離した上清を，ヒトによる味覚試験用試料液とした．また，遠心分離した上清に，塩化カリウム (10 mM) と酒石酸 (0.1 mM) を添加した液を味認識装置測定用試料液とした (Table 1, No. 10 ~ 14).

4-4. 苓桂朮甘湯各構成生薬煎出液の混合液（別煎混合苓桂朮甘湯）

4-3 の各単味生薬煎出液について，ブクリョウ煎出液 60 mL，ケイヒ煎出液 40 mL，ビャクジュツもしくはソウジュツ煎出液 30 mL，カンゾウ煎出液 20 mL の割合で混合した液を，ヒトによる味覚試験用試料液とした．また，この液に塩化カリウム (10 mM) と酒石酸 (0.1 mM) を添加した液を味認識装置測定用試料液とした (Table 1, No. 15 ~ 23).

5. 測定方法

5-1. 味認識装置を用いた味測定

味認識装置を用いて，既報[1,2]と同様に 4-1 から 4-4 の各試料液について味の測定を行い，ウェーバーの法則[8-10]に基づいてヒトが感じる味強度の違いを推定し，得られた推定値を各味要素の数値とした．今回，本装置を用いて推定した味の要素は，酸性苦味，酸性苦味後味，旨味，旨味後味，塩味，酸味，渋味，渋味後味である．既報[1,2]にて測定を行った塩基性苦味及び甘味に関しては，センサの安定が得られなかったため，評価には用いなかった．

5-2. ヒトによる味覚試験

4-1 から 4-4 の，ヒトによる味覚試験用試料液について，本研究室所属の5名（男性2名，女性3名，年齢21 ~ 44 歳）により，味の評価を行った．各試料液について，試料液約 5 mL を数秒間口に含み，味を評価した．1 回毎に口内を市販のミネラルウォーターで漱ぎ，各試料液について上記試験を複数回行い，第十五改正日本薬局方原案作成要領[11]に基づいて味の表現を決定した．

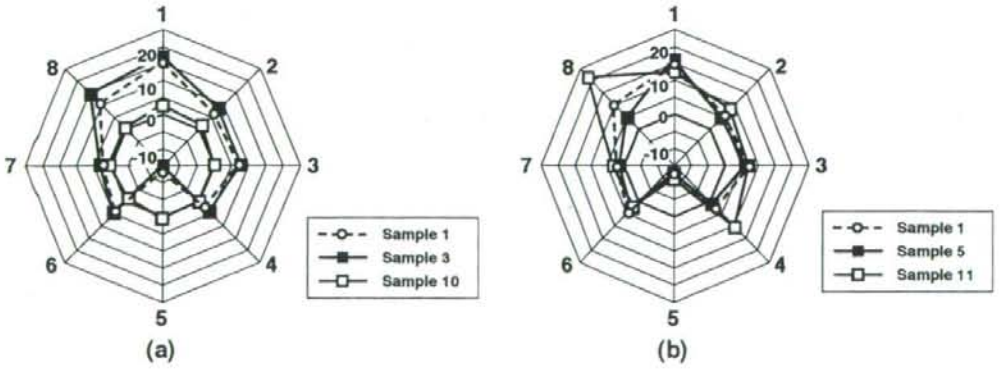

Fig. 1 Change of taste patterns by removing Poria Sclerotium (a) or Glycyrrhiza (b) from the Ryokeijutsukanto formula
The data were obtained by the taste-sensing system. The numbers (1 ~8) on circumference of graphs show the taste factors of anionic bitterness, after taste of anionic bitterness, umami, after taste of umami, sourness, astringency, after taste of astringency and saltiness, respectively. Sample numbers on each legends as described in Table 1.

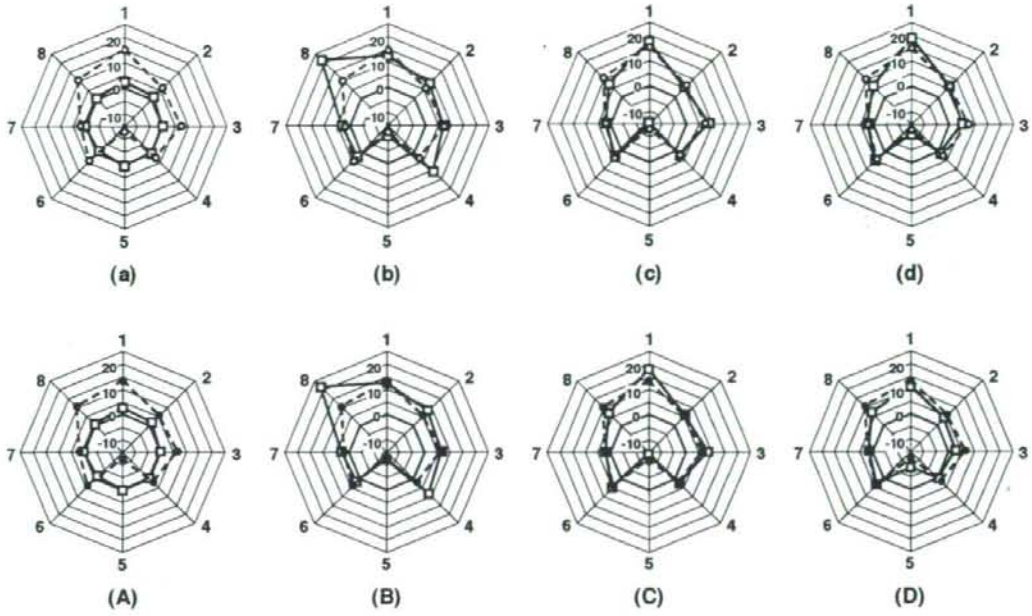

Fig. 2 Taste patterns of Ryokeijutsukanto and the decoctions of the respective crude drugs which are the components of the Ryokeijutsukanto formula

The data were obtained by the taste-sensing system. The dashed line with open circle in graphs of (a) ~ (d) shows the taste value of Atractylodes Rhizome used Ryokeijutsukanto. The dashed line with closed circle in graphs of (A) ~ (D) shows the taste value of Atractylodes Lancea Rhizome used Ryokeijutsukanto. The solid line with open square in each graphs shows the taste value of Poria Sclerotium (a, A), Glycyrrhiza (b, B), Cinnamon Bark (c, C), Atractylodes Rhizome (d) and Atractylodes Lancea Rhizome (D) decoctions, respectively. The numbers on circumference of graphs are as described in Fig. 1.

結果及び考察

1. 苓桂朮甘湯の味を特徴付けている味要素について

今回検討を行った苓桂朮甘湯の味認識装置による測定において，評価を行った味要素のうち，塩味と酸性苦味について，特徴が認められた．

1-1. 塩味

味認識装置による測定において，苓桂朮甘湯と同時煎一味抜き苓桂朮甘湯を比較すると，ビャクジュツ使用，ソウジュツ使用，また，同時煎，別煎混合の別に関係なく，構成生薬のうちブクリョウを除いた場合，塩味の数値が大きくなった．一方，構成生薬のうちカンゾウを除いた場合，塩味の数値が小さくなった．例として，ビャクジュツ使用の苓桂朮甘湯とブクリョウ (Fig. 1a) もしくはカンゾウ (Fig. 1b) を除いた煎出液及びそれぞれの単味煎出液の比較チャートを示す．更に，苓桂朮甘湯と比較して，カンゾウの単味煎出液が非常に大きな塩味の数値を示したのに対し，カンゾウ以外の構成生薬の単味煎出液はすべて，苓桂朮甘湯より塩味の数値が小さかった (Fig. 2)．従って，苓桂朮甘湯が示す塩味に大きく寄与しているのはカンゾウであり，カンゾウが示す強い塩味を主にブクリョウが低下させているものと考えられる．また，カンゾウは甘味が特徴的であるが，本研究においてもセンサの安定性が得られず，甘味の測定はできなかった．しかし今回の結果から，甘味だけでなく塩味にもカンゾウの特徴が現れることが示された．しかし，ヒトによる味覚試験では，塩味については特に言及されなかった．塩味に応答特性を持つセンサは，ナトリウムイオンだけでなく，カリウムイオンにも強く応答する性質を有している．一方，ヒトにとって標準的な塩味は，塩化ナトリウムであると考えられる．カンゾウの代表的な含有成分であるグリチルリチンは，生薬内では通常カリウム塩として存在し，水抽出の際にはカリウム塩として抽出されることが知られている [12]．従って，味認識装置はこのカリウムイオンに強く反応したのに対し，ヒトはカリウムイオンを典型的な塩味として認識しなかったものと考えると，本研究結果を説明することが可能となる．