

# Suppl I, JP XVII (2017)

# Suppl I, JP XVII (2017)

## Tokishakuyakusan Extract

当帰芍薬散エキス

### Change the Identification as follows:

**Identification** (1) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 15 mL of water and 5 mL of 0.1 mol/L hydrochloric acid TS, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (Z)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography

<2.03>. Spot 10  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and Rf value with the blue-white fluorescent spot from the standard solution (Japanese Angelica Root and Cnidium Rhizome).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and Rf value with the purple spot from the standard solution (Peony Root).

(3) For preparation prescribed Atractylodes Rhizome—Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and Rf value with the blue-white fluorescent spot from the standard solution (Atractylodes Rhizome).

(4) For preparation prescribed Atractylodes Lancea Rhizome—Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the layer under reduced pressure, add 0.5 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20  $\mu$ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light

(main wavelength: 254 nm): a dark purple spot is observed at an  $R_f$  value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylamino-benzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (*Atractylodes Lancea* Rhizome).

(5) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 20 mL of water and 2 mL of ammonia solution (28), add 20 mL of a mixture of hexane and ethyl acetate (20:1), shake, and centrifuge. Separate the upper layer, evaporate the layer under reduced pressure, add 2 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of alisol A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10  $\mu$ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl formate, water and formic acid (30:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid TS on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and  $R_f$  value with the yellow fluorescent spot from the standard solution (*Alisma Tuber*).

*Suppl I,  
JP XVII  
(2017)*

*Suppl I,  
JP XVII  
(2017)*

*Suppl I,  
JP XVII  
(2017)*

*Suppl I,  
JP XVII  
(2017)*