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## Tokishakuyakusan Extract

当帰芍薬散エキス

### Change the Assay (1), (3) as follows:

#### Assay

(1) (*E*)-Ferulic acid—Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-ferulic acid for assay, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 2 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas,  $A_T$  and  $A_S$ , of (*E*)-ferulic acid in each solution.

$$\text{Amount (mg) of (E)-ferulic acid} = M_S \times A_T/A_S \times 1/50$$

$M_S$ : Amount (mg) of (*E*)-ferulic acid for assay taken, calculated on the basis of the content obtained by qNMR

#### Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 320 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and add 2 mL of phosphoric acid. To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: 1.0 mL per minute [the retention time of (*E*)-ferulic acid is about 10 minutes].

#### System suitability—

System performance: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (*E*)-ferulic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (*E*)-ferulic acid is not more than 1.5%.

(3) Atractylenolide III—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15

minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of atractylenolide III for assay, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas,  $A_T$  and  $A_S$ , of atractylenolide III in each solution.

Amount (mg) of atractylenolide III =  $M_S \times A_T / A_S \times 1/40$

$M_S$ : Amount (mg) of atractylenolide III for assay taken

*Operating conditions—*

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (550:450:1).

Flow rate: 1.0 mL per minute (the retention time of atractylenolide III is about 10 minutes).

*System suitability—*

System performance: When the procedure is run with 10  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atractylenolide III are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atractylenolide III is not more than 1.5%.

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