Goshajinkigan Extract

牛車腎気丸エキス

Change the Assay (3) Total alkaloids as follows:

Assay

(3) Total alkaloids (Benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride)—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. Centrifuge this solution, remove the diethyl ether layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether layer. To the aqueous layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, then centrifuge, and take the diethyl ether layer. To the aqueous layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the extracts, and evaporate the solvent under low pressure (in vacuo). Dissolve the residue in a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of benzoic acid for assay, and dissolve in a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*>according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_M, A_H and A_A, in the sample solution and the peak area of benzoic acid, A_S, in the standard solution.

Amount (mg) of benzoylmesaconine hydrochloride
$= M_S \times A_M/A_S \times 1/100 \times 4.19$

Amount (mg) of benzoylhypaconine hydrochloride
$= M_S \times A_H/A_S \times 1/100 \times 4.06$

Amount (mg) of 14-anisoylaconine hydrochloride
$= M_S \times A_A/A_S \times 1/100 \times 3.69$

M_S: Amount (mg) of benzoic acid for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhypaconine; 254 nm for 14-anisoylaconine).

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 μm in particle diameter).

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

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard solution TS for resolution check under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order, and the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benzoic acid is not more than 1.5%.

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