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**DETERMINATION OF HYOSCYAMINE CONTENT**

**(BELLADONNA EXTRACT)**

(Method refer to CP 2015)

This product is extracted from plant belladonna Atropa belladonna L.

**Traits**: This product is brown liquid, smell slightly smelly.
**Relative density**: 0.892 ~ 1.090

**Identification:**

(1) take this product 1ml, add water 5ml, concentrated ammonia test solution 5ml, shake and extract with ether 3 times, each 10ml, combined ether solution, evaporated, the residue dissolve in 1ml ethyl acetate, as the test product solution. Also take the reference substance of atropine sulfate, add methanol to make 1ml solution containing 2mg atropine sulfate, as the reference solution. According to the thin layer chromatography test, absorb the test solution 1μl, the reference solution 5μl, respectively, points on the same silica gel G plate, ethyl acetate - methanol - concentrated ammonia(17: 2: 1) as developer , start, take out, dry, spray with dilute iodinated bismuth potassium solution. In the test product chromatography, chromatography with the reference substance at the corresponding position, was the same color spots.

(2) take the reserve filtrate from [content determination] as the test solution. Also take scopolamine hydrobromide reference substance, L-anisodamine reference substance and atropine sulfate reference substance amount, plus the mobile phase from [content determination] to make solution, per 1ml each containing 0.1mg, as the reference solution. According to the method from [content determination], test the test product chromatography showed same retention time chromatographic peak with scopolamine hydrobromide reference substance, L-anisodamine reference substance and sulfate atropine reference. The separation of the three chromatographic peaks and the other peaks shall not be less than 1.5; the sum of the peak areas of the remaining two chromatographic peaks, other than the sulfuric acid atropine peaks, shall not be less than 6.3% of the above three chromatographic peak areas.

**Examination**

Ethanol: 52% to 66%.

Total solid: measure accurately 10ml test product solution, move to evaporating dish dried to constant weight, evaporated, dried at 105 ℃ for 3 hours, move to the dryer, cooling for 30 minutes, quickly weighed. This product should not contain less than 1.7g total solids.

Other: Should comply with the provisions of the flow extract and the extract under the provisions.

Characteristic map: Determination by high performance liquid chromatography.

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**Chromatographic conditions and system suitability test**

With octadecyl silane bonded silica as filler. Methanol as the mobile phase A and 0.05% phosphoric acid solution as the mobile phase B. Gradient eluted by the following table. The detection wavelength was 344 nm. Calculated by scopolamine peak, the number of theoretical plates should not be less than 5000.

| Time (min) | mobile phase A (%) | mobile phase B (%) |
|---|---|---|
| 0~5 | 3~15 | 97~85 |
| 5~60 | 15~60 | 85~40 |

**Preparation of reference solution**

Take scopolamine reference substance amount, accurately weighed, plus 50% methanol made solution, 1ml containing 10μg.

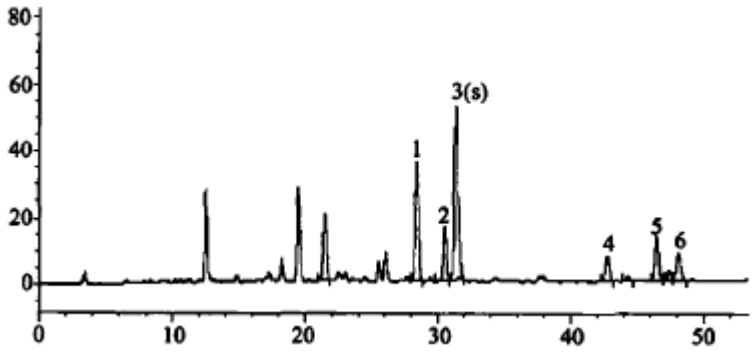
**Preparation of the test solution**

Precise weigh of this product 1ml, placed in 25ml volumetric flask, add 50% methanol dissolved and diluted to the mark, shake, filter, take continued filtrate

**Test Method**

Respectively, precisely measure the reference solution and the test solution 10μl, into the liquid chromatography, determination.

There shall be six characteristic peaks in the characteristic map of the test solution. The peak corresponding to the reference peak is the S peak, and the relative retention time of each characteristic peak and S peak shall be calculated. The relative retention time should be within ± 5% of specified value. The values are 0.897 (peak 1), 0.965 (peak 2), 1.000 [peak 3 (S)], 1.354 (peak 4), 1.473 (peak 5), 1.528 (peak 6). Calculate the relative peak area of peak 1, peak 5 and S peak, the relative peak area of peak 1 shall not be less than 0.30, and the relative peak area of peak 5 shall not be less than 0.10.




Reference characterization map

Peak 3 (S): Scopolamine

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Reference column: Asahi company XB-C18(250nm*4.6nm*5μm)

**Content determination**

According to high performance liquid chromatography

Chromatographic conditions and system suitability test:

Octadecyl silane bonded silica as a filler; take acetonitrile-phosphate buffer (6.8 g of potassium dihydrogen phosphate dissolved in 1000 ml of water, add 10 ml of triethylamine, with phosphoric acid adjust PH value to 2.8) (7:93) as the mobile phase; detection wavelength of 210nm. Based on the peak of atropine sulphate to calculate, theoretical plate number should be calculated not less than 4000.

**Preparation of reference substance solution**

Accurately weighed atropine sulphate (120 ℃ dry to constant weight) as reference substance, plus mobile phase to make solution 0.17mg per 1ml.

Preparation of the test solution:

Precisely weigh amount of this product 2ml, set split funnel, add ammonia test solution 15ml, shake, extracted with ethyl acetate five times, each 15ml, combined ethyl acetate extract, evaporated , Residual plus mobile phase dissolved and transferred to 10ml volumetric flask. Dilute with the mobile phase to the scale, shake, filtrate, take the filtration solution as preparation. Take the filtrate 1ml, set 10ml volumetric flask, take the mobile phase to dilute to mark, shake, filtrate, take the filtration solution.

**Determination method**

Respectively, precisely measure the reference solution and the test solution 10μl, into the liquid chromatography to test.

This product per 1ml containing alkaloids based on atropine sulfate [$(C_{17}H_{23}NO_3)_2 * H_2SO_4$], not less than 6.6mg