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Powdered Cat's Claw

DEFINITION

Powdered Cat's Claw is Cat's Claw reduced to a powder or very fine powder. It contains NLT 0.3% of pentacyclic oxindole alkaloids as isopteropodine, calculated on the dried basis, as the sum of speciophylline, uncarine F, mitraphylline, isomitraphylline, pteropodine, and isopteropodine.

IDENTIFICATION

• **A. [THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST \(201\)](#).**

Standard solution: 100 mg of USP Powdered Cat's Claw Extract RS in 2 mL of methanol. Sonicate for 5 min, shaking occasionally, heat in a water bath at 60° for 15 min, cool, and centrifuge.

Sample solution: 5 g of Powdered Cat's Claw in 10 mL of methanol. Sonicate for 5 min, shaking occasionally. Heat the mixture in a water bath at 60° for 15 min, cool, and filter.

Adsorbent: Chromatographic silica gel mixture with an average particle size of 10–15 µm (TLC plates)

Application volume: 20 µL, as bands that are 1 cm in length

Developing solvent system: Ethyl acetate and hexane (95:5)

Derivatization reagent A: Dissolve 0.85 g of basic bismuth nitrate in 10 mL of glacial acetic acid and 40 mL of water by heating. Filter if necessary (Solution A). Dissolve 8 g of potassium iodide in 30 mL of water (Solution B). Mix *Solution A* and *Solution B* (1:1) to obtain a stock solution. Dilute 1 mL of the stock solution with 2 mL of glacial acetic acid and 10 mL of water. [NOTE—Use freshly mixed *Solution A* and *Solution B*.]

Derivatization reagent B: Use a 10% solution of sodium nitrite in water.

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram to a length of NLT 12 cm, and dry the plate in a current of air.

Acceptance criteria: Examine the plate under short-wave UV light (254 nm). The *Sample solution* chromatogram shows multiple zones that correspond in R_f values to those observed in the *Standard solution* chromatogram. Other zones of varying intensities may be observed in the *Sample solution*. Treat the plate with *Derivatization reagent A* followed by *Derivatization reagent B*, and examine the plate under white light. The *Sample solution* chromatogram shows multiple orange-brown zones that correspond in color and R_f values to those observed in the *Standard solution* chromatogram. Other colored zones of varying intensities may be observed in the *Sample solution*.

• **B.** The *Sample solution* exhibits peaks for speciophylline, uncarine F, mitraphylline, isomitraphylline, pteropodine, and isopteropodine at retention times that correspond to those in *Standard solution A*, as obtained in the test for *Content of Pentacyclic Oxindole Alkaloids and Limit of Tetracyclic Oxindoles*.

COMPOSITION

• **CONTENT OF PENTACYCLIC OXINDOLE ALKALOIDS AND LIMIT OF TETRACYCLIC OXINDOLES**

Standard solution A: Dissolve an accurately weighed quantity of USP Powdered Cat's Claw Extract RS in methanol, shaking for 1 min. Dilute with methanol to obtain a solution having a known concentration of about 0.5 mg of the labeled amount of total oxindole alkaloids per mL. Pass through a filter of 0.45-µm or finer pore size.

Standard solution B: 0.1 mg/mL of [USP Isopteropodine RS](#) in methanol. Pass through a nylon filter of 0.45-µm or finer pore size.

Sample solution: Accurately weigh approximately 750 mg of Powdered Cat's Claw, and place in a 10-mL centrifuge tube. Sonicate with 2.5 mL of methanol for 10 min. Centrifuge, and transfer this solution to a 10-mL volumetric flask. Repeat the extraction three additional times combining the extracts in the 10-mL volumetric flask, and dilute with methanol to volume. Transfer about 3 mL of the solution to a test tube containing 300 mg of polyamide powder, and shake for 1 min. Pass through a nylon filter of 0.45-µm or finer pore size, discarding the first part of the filtrate.

Solution A: Prepare a filtered and degassed 10 mM pH 7.0 phosphate buffer by mixing 6 mL of 1 N sodium hydroxide, 10 mL of 1 M monobasic potassium phosphate, and sufficient water to make 1000 mL. Adjust to a pH of 7.0 ± 0.1 by adding more of either solution.

Solution B: Acetonitrile

Solution C: Methanol and glacial acetic acid (99:1)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	65	35	0
17	65	35	0
25	50	50	0
30	50	50	0
31	0	0	100
36	0	0	100
39	65	35	0
49	65	35	0

Chromatographic system(See [Chromatography \(621\), System Suitability](#).)**Mode:** LC**Detector:** UV 245 nm**Column:** 4.6-mm × 10-cm; end-capped 3-μm packing L1**Flow rate:** 0.75 mL/min**Injection volume:** 10 μL**System suitability****Samples:** *Standard solution A* and *Standard solution B***Suitability requirements****Chromatogram similarity:** The chromatogram obtained using *Standard solution A* is similar to the reference chromatogram provided with the USP Powdered Cat's Claw Extract RS.**Tailing factor:** NMT 2.0 for the isopteropodine peak, *Standard solution B***Relative standard deviation:** NMT 2.0% for the isopteropodine peak in repeated injections, *Standard solution B***Analysis****Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Measure the areas of the analyte peaks. Identify the retention times of the peaks corresponding to speciophylline, uncarine F, mitraphylline, isomitraphylline, rhynchophylline, isorhynchophylline, pteropodine, and isopteropodine by comparison of the chromatogram of *Standard solution A* with the reference chromatogram provided with the lot of the USP Powdered Cat's Claw Extract RS used.

Separately calculate the percentages of speciophylline, uncarine F, mitraphylline, isomitraphylline, rhynchophylline, isorhynchophylline, pteropodine, and isopteropodine, as isopteropodine, in the portion of Powdered Cat's Claw taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response for each relevant alkaloid from the *Sample solution*

r_S = peak response for isopteropodine from *Standard solution B*

C_S = concentration of [USP Isopteropodine RS](#) in *Standard solution B* (mg/mL)

C_U = concentration of Powdered Cat's Claw in the *Sample solution* (mg/mL)

Calculate the content of pentacyclic oxindole alkaloids by adding the percentages of speciophylline, uncarine F, mitraphylline, isomitraphylline, pteropodine, and isopteropodine.

Calculate the content of tetracyclic oxindole alkaloids by adding the individual percentages of rhynchophylline and isorhynchophylline.

Acceptance criteria**Pentacyclic oxindole alkaloids:** NLT 0.3%**Tetracyclic oxindole alkaloids:** NMT 0.05%**CONTAMINANTS**

- [ARTICLES OF BOTANICAL ORIGIN, Limits of Elemental Impurities \(561\)](#): Meets the requirements

- [ARTICLES OF BOTANICAL ORIGIN, Pesticide Residue Analysis \(561\)](#): Meets the requirements

- [MICROBIAL ENUMERATION TESTS \(2021\)](#): The total aerobic microbial count does not exceed 10^5 /g; the total combined molds and yeasts count does not exceed 10^3 /g; and the bile-tolerant Gram-negative bacteria do not exceed 10^3 /g.