Black cohosh rhizome (*Cimicifuga racemosa* L. Nutt.)

1. Scope
This method identifies dried Black cohosh rhizome or root (*Cimicifuga racemosa* (L.) Nutt, syn. *Actaea racemosa* L.) by HPTLC fingerprint and discriminates the adulterants dried rhizome or roots of Yellow cohosh (*C. americana* Michx., syn.: *A. podocarpa* DC.), *C. foetida* L. (syn.: *A. cimicifuga* L.), *C. dahurica* (Turcz.) Maxim, and *C. heracleifolia* Kom.

2. Source of method
CAMAG MOA 005 (Pharmeuropa)

3. Procedure
Sample preparation: Mix 0.5 g of powdered sample with 10 mL of ethanol-water (1:1) and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants/filtrates as test solutions.

Reference substances: Dissolve 1 mg of actein, isoferulic acid in 10 mL of methanol.
Optional: Dissolve 23-epi-26-deoxyactein, cimifugin or norcimifugin in 10 mL of methanol.

Application: 2 µL of references, 2 µL of test solutions

Mobile phase: Toluene, ethyl formate, formic acid 50:30:20 (v/v/v)

Development:
- Saturated chamber
- Developing distance 70 mm from lower edge
- Relative humidity 1-5 %

Derivatization reagent: Sulfuric acid reagent
Preparation: 20 mL of sulfuric acid are mixed with 180 mL of ice-cooled methanol. Use: Dip (time 0, speed 5), heat at 100°C for 5 min

Documentation
1.) Clean plate, white RT
2.) Clean plate, UV 254 nm
3.) Developed, UV 254 nm
4.) Developed, UV 366 nm
5.) Sulfuric acid reagent, UV 366 nm
6.) Sulfuric acid reagent, white RT
4. Results

Fig. 1) UV 254 nm

Fig. 2) UV 366 nm

Fig. 3) Sulfuric acid reagent, UV 366 nm
**System suitability test**

Actein: a brown zone at Rf ~ 0.37 (white RT).
Isoferulic acid: a brown zone at Rf ~ 0.55 (white RT).

Cimifugin: a yellow zone at Rf ~ 0.24 (white RT).
23-epi-26-deoxyactein: a brown zone at Rf ~ 0.32 (white RT).
Norcimifugin: a blue fluorescent zone at Rf ~ 0.41 (366 nm after derivatization)

**Identification**

Compare result with reference images in *Image Comparison Viewer*. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.

Under white light a characteristic fingerprint is detected. Below the yellow zone of reference substance cimifugin there are two violet zones and just above the application position two brown zones are detected (blue arrow). There is a brown zone at the position of actein and one at the position of 23-epi-26-deoxyactein. The pattern in the marked region of the green arrow (triterpene glycoside) may vary (Fig. 5).

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### Table: Sample Preparation

<table>
<thead>
<tr>
<th>Track</th>
<th>Volume</th>
<th>Sample</th>
<th>Track</th>
<th>Volume</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 µL</td>
<td>Isoferulic acid</td>
<td>9</td>
<td>2 µL</td>
<td><em>C. heracleifolia</em></td>
</tr>
<tr>
<td>2</td>
<td>2 µL</td>
<td>Norcimifugin</td>
<td>10</td>
<td>2 µL</td>
<td><em>C. dahurica</em></td>
</tr>
<tr>
<td>3</td>
<td>2 µL</td>
<td>Actein</td>
<td>11</td>
<td>2 µL</td>
<td><em>C. americana, Yellow cohosh</em></td>
</tr>
<tr>
<td>4</td>
<td>2 µL</td>
<td>23-epi-26-Deoxyactein</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2 µL</td>
<td>Cimifugin</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2 µL</td>
<td>Black cohosh rhizome 1</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2 µL</td>
<td>Black cohosh rhizome 2</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2 µL</td>
<td><em>C. foetida</em></td>
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</table>
Test for adulteration

No dark zone is seen at the position of cimifugin or just above it under UV 254 nm.

For detection of ≥ 5% adulteration of black cohosh samples use the same procedure as for identification, but with application volumes of 20 μL of the test solutions. Apply the test solution twice, e.g.: application scheme: Cn = test samples. Track 7 is inactive; the plate will be cut after development. Left and right side of the plate are treated with different derivatization reagents.

<table>
<thead>
<tr>
<th>Track</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<th>6</th>
<th>7</th>
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<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
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<tbody>
<tr>
<td>Applic. vol. [µL]</td>
<td>2</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>2</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Test sample</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Actein</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>C. racemosa</td>
<td>C1</td>
<td>C2</td>
<td>C3</td>
<td>C4</td>
<td></td>
<td></td>
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<td></td>
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</table>

Detection A: (impurity: C. americana) examine under UV light 254 nm before derivatization.

Detection B: (impurity: C. foetida) derivatization with freshly prepared boric acid/oxalic acid reagent. Dissolve 4.5 g of boric acid in 150 mL of ethanol (solution A). Dissolve 5 g of oxalic acid in 50 mL of ethanol (solution B). Combine equal volumes of solutions A and B and mix well. Immerse the plate in the freshly prepared reagent for 1 s and then heat it at 120 °C for 5 min. Examine the plate under UV light 366 nm.

Detection C: (impurities: C. heracleifolia and C. dahurica) derivatization with antimony(III) chloride reagent. Dissolve 8 g of antimony(III) chloride in 200 mL of chloroform. Immerse the plate in the reagent for 1 s and then heat it at 120 °C for 10 min. Examine the plate under UV 366 nm.
Detection A: the chromatogram obtained with the test solution does not show any quenching zone at Rf ~ 0.30.

Detection B: the chromatogram obtained with the test solution neither shows a fluorescent zone at the position of cimifugin (Rf ~ 0.24) nor a fluorescent zone above the application position (Rf ~ 0.06).

Detection C: the chromatogram obtained with the test solution does not show any fluorescent zones just above the position of actein (Rf ~ 0.39).