



the nature network

PhytoLab

Current Problems with Identification of Herbal Drugs

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Overview of topics

Introduction

Equiseti herba (Ph.Eur.)

Cimicifugae racemosae rhizoma (Ph.Eur. draft)

Plantaginis ovatae seminis tegumentum (Ph.Eur.)

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Conclusion

Introduction

Unprocessed, whole, comminuted or powdered plants or parts of plants usually in a dried state but sometimes fresh, which are used for medicinal purposes, are normally described as "Herbal Drugs". Herbal drugs are obtained from cultivated or wild plants. They vary more or less in composition and properties depending on the habitat, climate zone and annual variations. Raw products of herbal origin are naturally subject to considerable variation in terms of their composition and constituents.

Introduction

Identification and purity tests are normally done by macroscopic, microscopic tests and thin layer chromatography. Sometimes GC- or HPLC-methods are used. In a herbal monograph of the European Pharmacopoeia (Ph.Eur.) under "IDENTIFICATION" and "TESTS" methods are described.

As chemically defined constituents contributing to therapeutic activity of a plant material are frequently not known, markers are generally employed to characterise the herbal drug. For this purpose the botanical species must be clearly defined considering the existence of chemical races of plants. A characterisation of herbal material by specific fingerprints is necessary to maintain permanently an equivalent quality.

Introduction

Occasionally herbal drugs are adulterated with foreign or even toxic plants. This applies especially to plants from collection in wild habitats, where confusion with similar plants as well as poorly qualified personnel presents a risk for the quality of the herbal drug itself. Specific methods are used to detect known adulterations.

On the basis of some examples of herbal drugs current problems using identification and purity tests in practice will be shown.

Equisetum Stem (Ph.Eur.)

01/2005:1825

EQUISETUM STEM

Equiseti herba

DEFINITION

Whole or cut, dried sterile aerial parts of
Equisetum arvense L.

Content: minimum 0.3 per cent of total flavonoids expressed
as isoquercitroside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried drug).

CHARACTERS

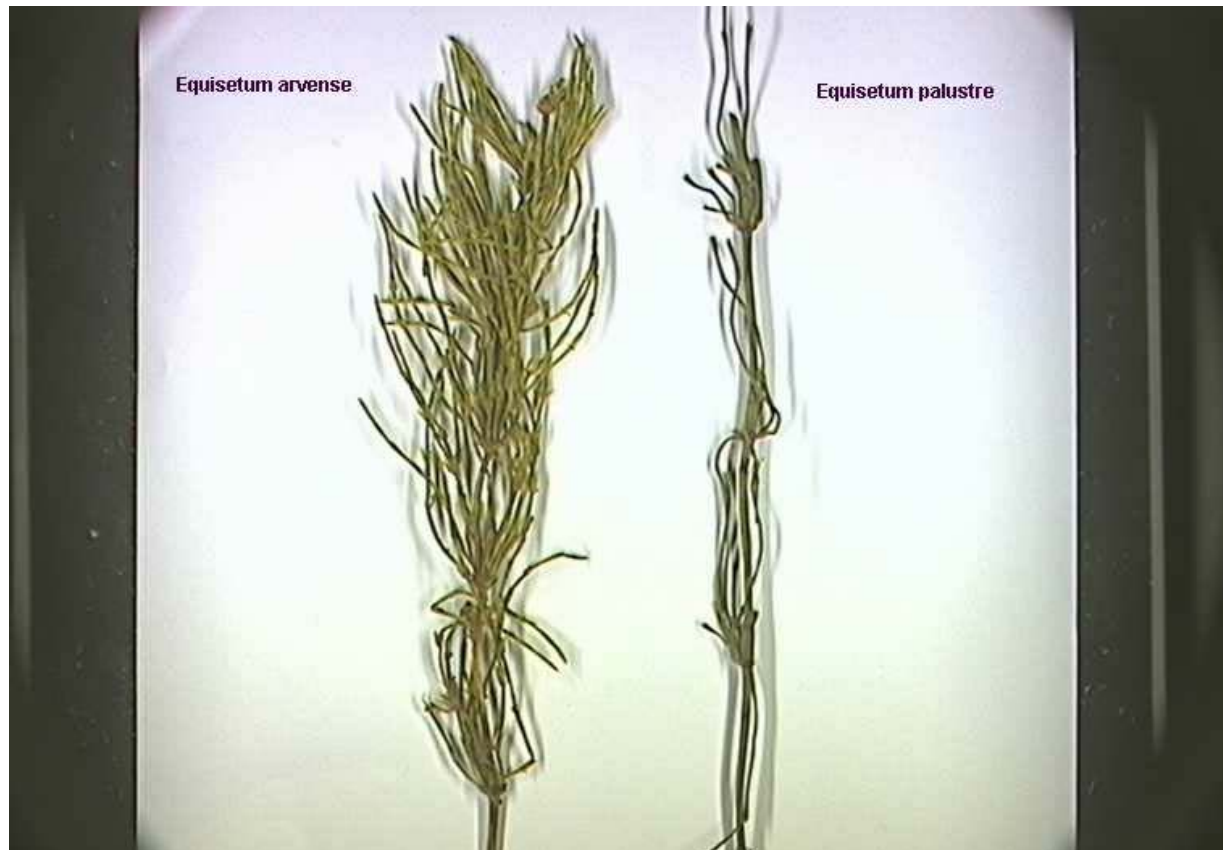
Macroscopic and microscopic characters described under
identification tests A and B.

Equisetum Stem (Ph.Eur.)

There are a lot of subspecies and hybrids of *Equisetum arvense* described in literature. Other *Equisetum* species and hybrids including alkaloid containing species like *Equisetum palustre* have to be detected.

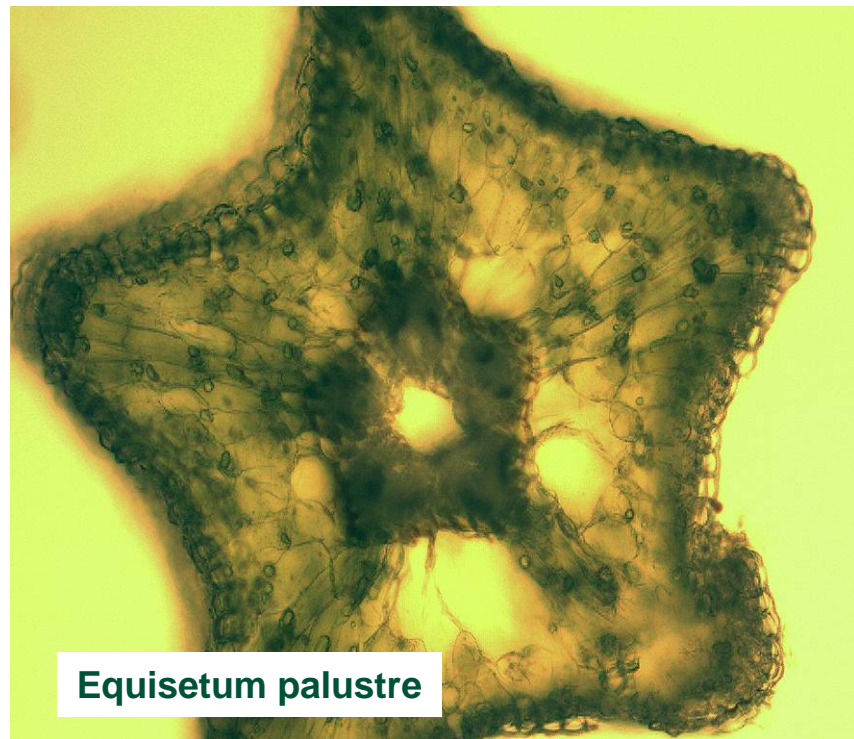
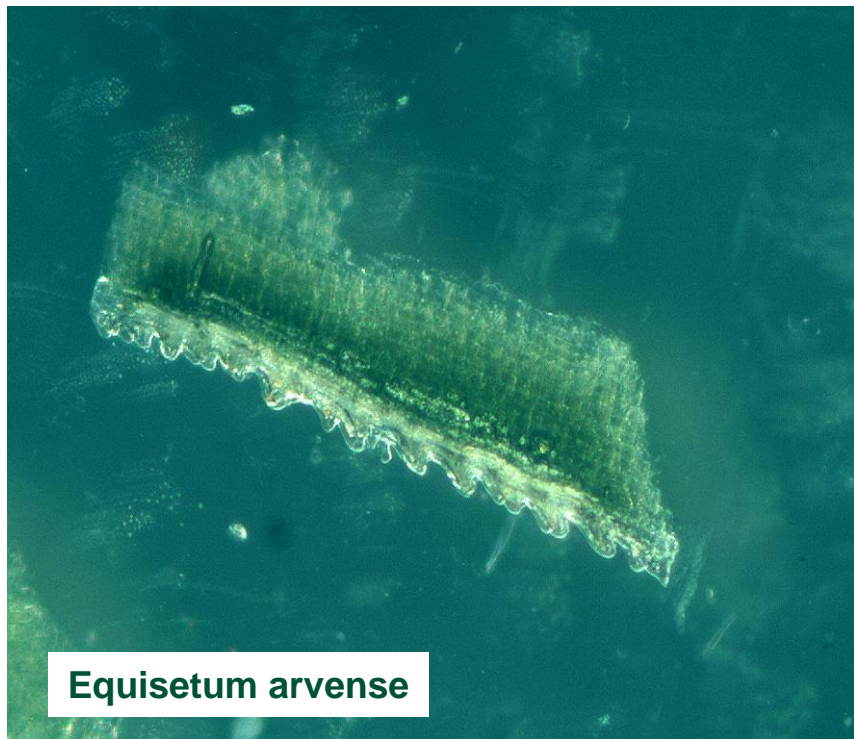
How could this problem be solved using the tests of Ph.Eur. Monograph?

Macroscopic picture of the whole dried herb



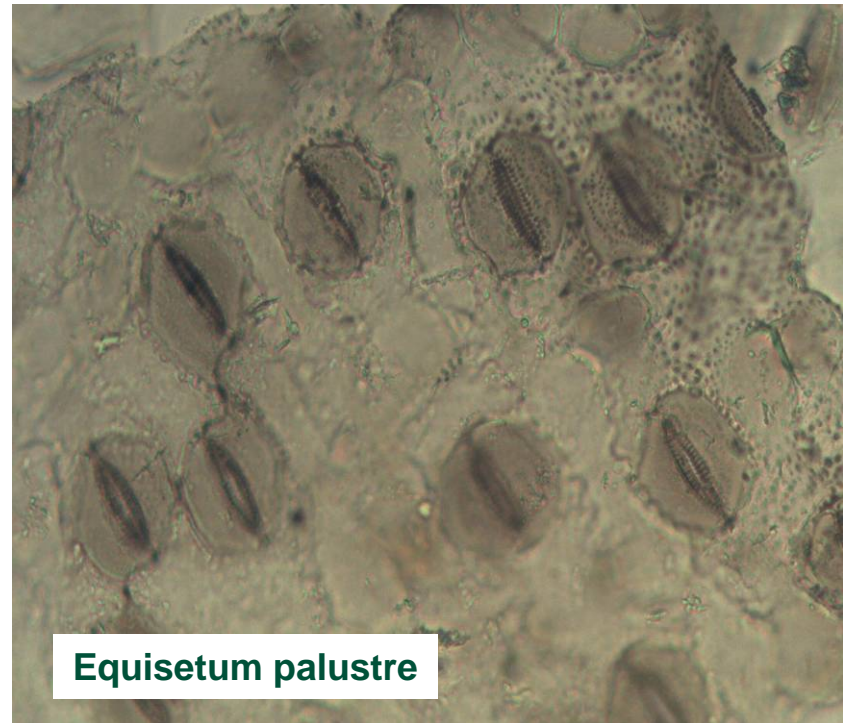
Whole herbal drug (short spears)

Microscopic picture



Different looking epidermal protuberances

Microscopic picture



Paracytic stomata with „teeth“ or „zip“

Equisetum Stem (Ph.Eur.)

Under IDENTIFICATION C and under TESTS there is a TLC test described.

TESTS

Foreign matter (2.8.2): maximum 5 per cent of stems from other *Equisetum* species and hybrids and maximum 2 per cent of other foreign matter.

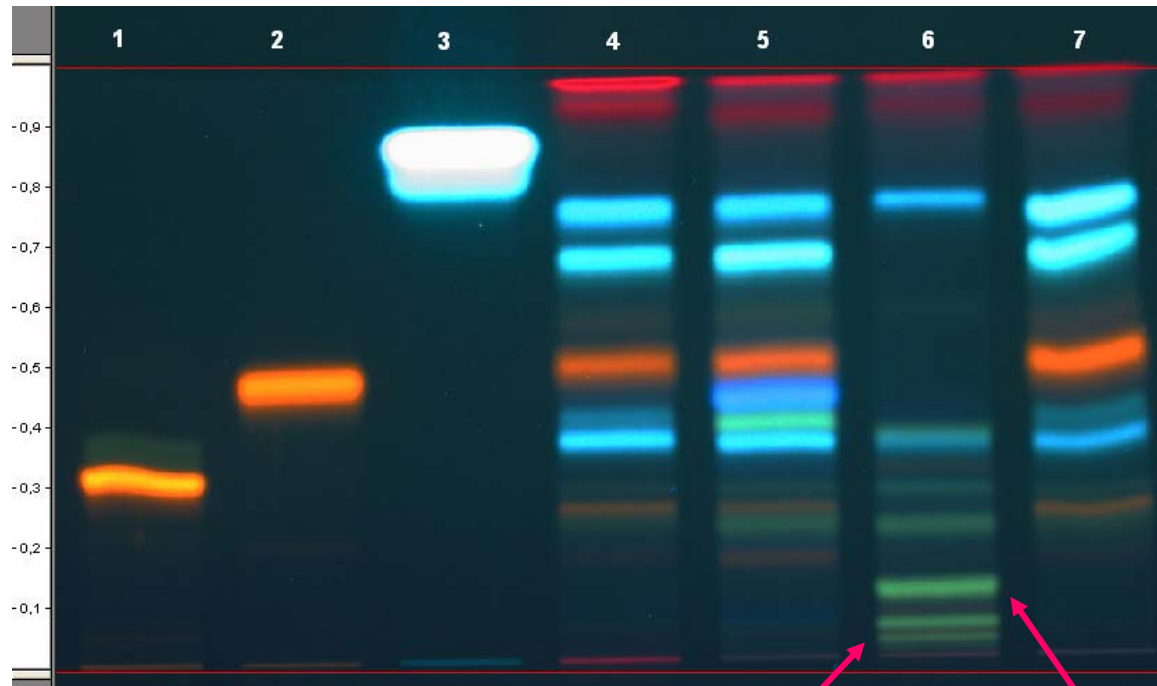
Other *Equisetum* species and hybrids. Thin-layer chromatography (2.2.27).

Equisetum Stem (Ph.Eur.)

| Top of the plate | |
|--|--|
| <p>Caffeic acid: a greenish-blue fluorescent zone</p> <hr/> <p>Hyperoside: an orange fluorescent zone</p> <hr/> <p>Rutin: an orange fluorescent zone</p> | <p>2 red fluorescent zones</p> <p>2 greenish-blue fluorescent zones</p> <hr/> <p>An orange fluorescent zone</p> <p>2 greenish-blue fluorescent zones</p> <hr/> |
| Reference solution | Test solution |

Results: the chromatogram obtained with the test solution shows no yellow or greenish-yellow fluorescent zone shortly above the starting line.

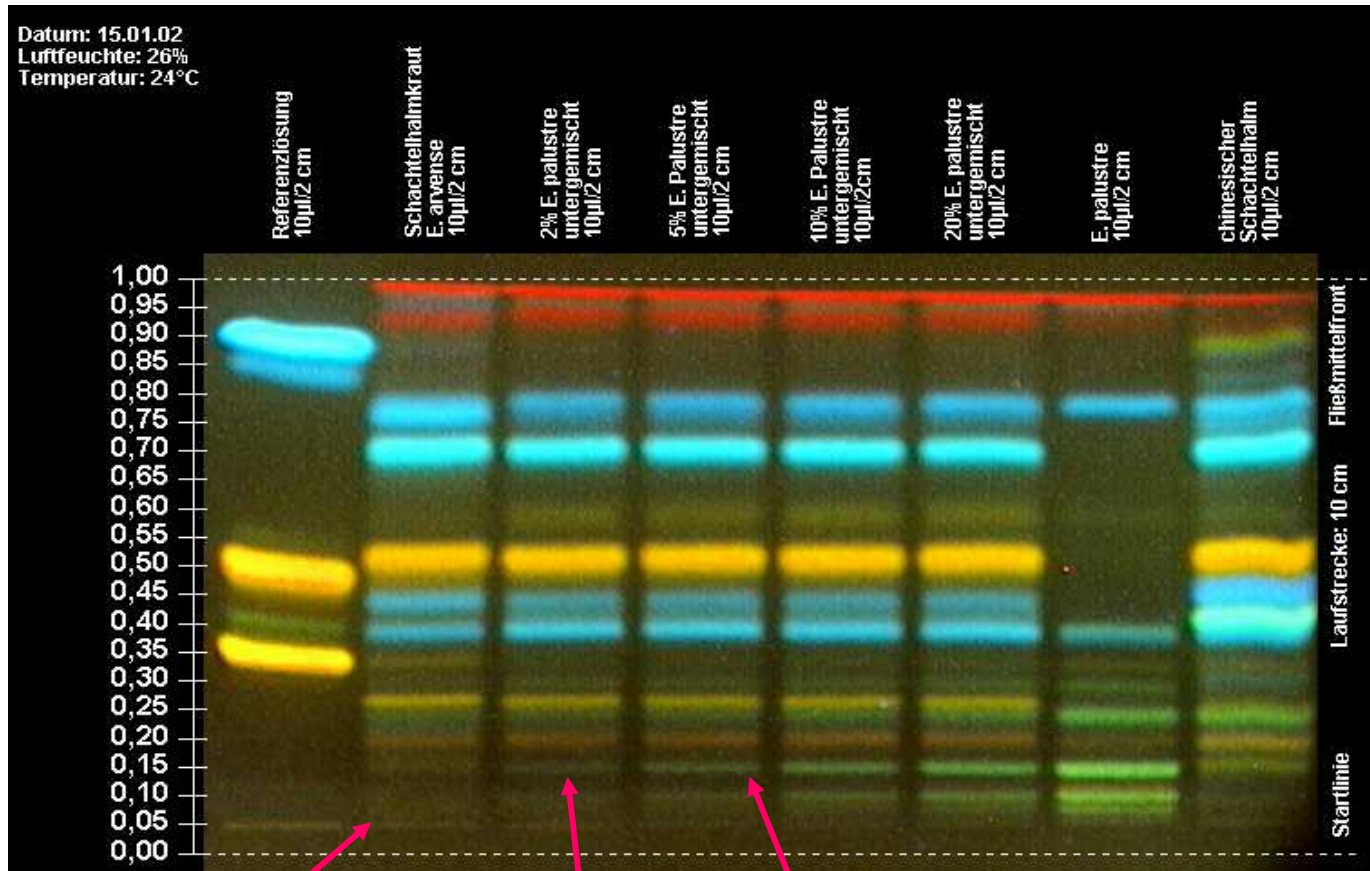
E. arvense and E. palustre



- 1 Rutoside
- 2 Hyperoside
- 3 Caffeic acid
- 4 *E. arvense*
- 5 *E. arvense* (China)
- 6 *E. palustre*
- 7 *E. arvense*

yellow or greenish-yellow zones

E. arvense and E. palustre



E. arvense

2% and 5% of E. palustre

Cimicifugae racemosae rhizoma (Ph.Eur. draft)

There are different Cimicifuga or Actaea species on the market indicated as Rhizoma Cimicifugae, but only a few of them are the real „Black cohosh“ (*C. racemosa*). Pharm.Chin. monograph Rhiz. Cimicifugae describes *C. heracleifolia*, *C. dahurica* and *C. foedita*.

It is difficult to describe Identification and Purity tests and to find out specific characteristics of cimicifuga racemosa.

Cimicifugae racemosae rhizoma (Ph.Eur. draft)

Phytochemical research has led to the isolation of 42 triterpene glycosides, hydroxycinnamic acid derivatives and chromones from black cohosh.

Macroscopic and microscopic tests of the dried, cut rhizome could not distinguish between the different species.

Thin layer chromatography is feasible for identification test but not for purity test because the fingerprints are quite similar.

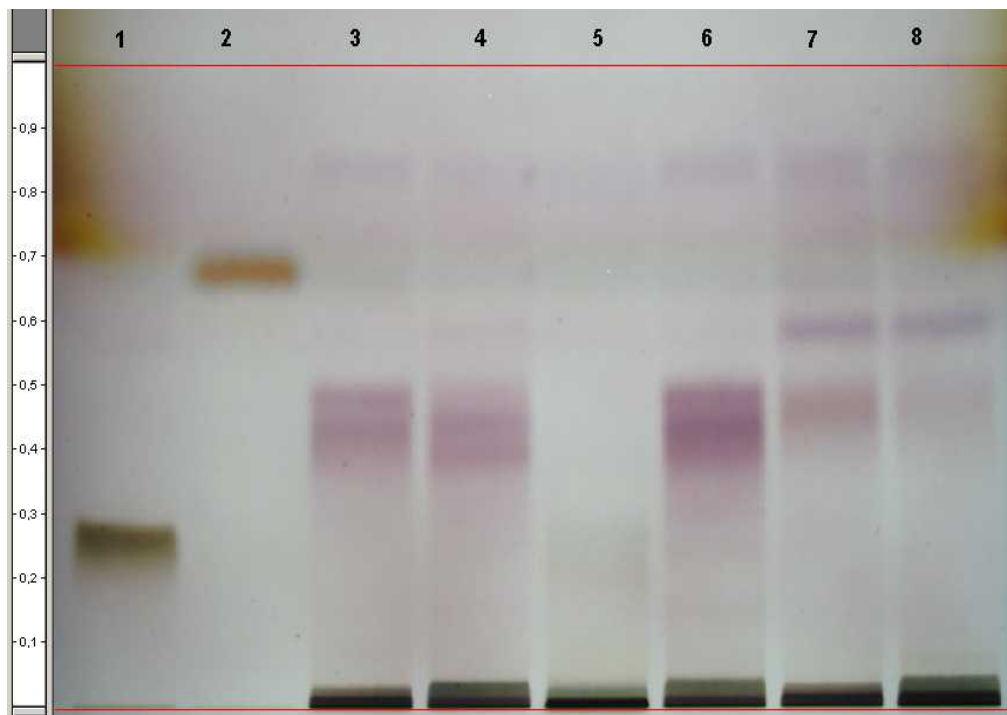
Cimicifugae racemosae rhizoma (Ph.Eur. draft)

There is also a need for authentic samples of all adulterations.

Adulteration with *cimicifuga foedita* could be found by detecting the compound cimifugin. The chromone *cimifugin* is present in *C. foedita* and in some other of the adulterated „Black Cohosh“ products but not in *C. racemosa*.

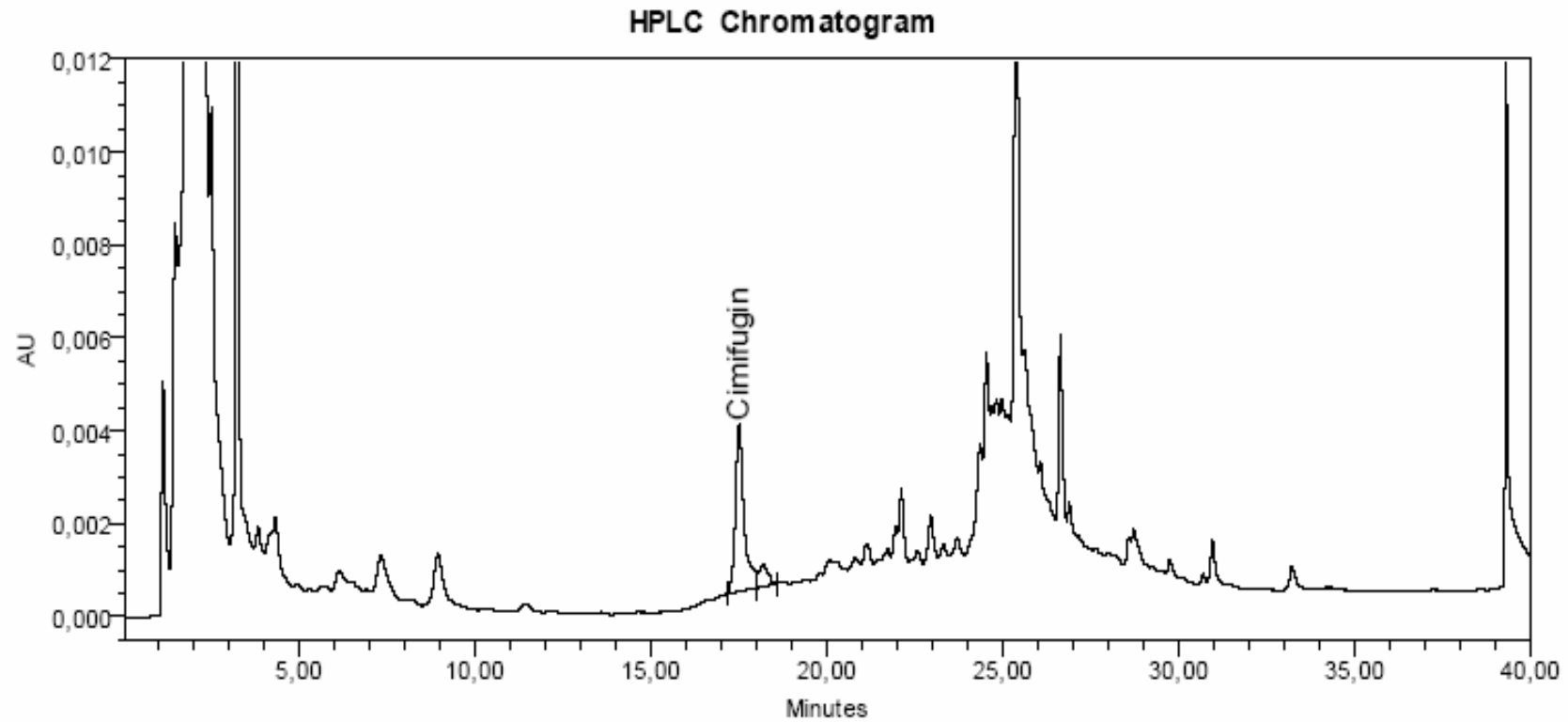
Therefore, detection of cimifugin proves a product is not Black Cohosh. There is a HPLC/UV-assay described to analyse cimifugin.

Cimicifugae racemosae rhizoma (Ph.Eur. draft)



| | |
|---|------------------------------------|
| 1 | Arbutin |
| 2 | Hydrochinon |
| 3 | <i>C. racemosae</i> rhizoma |
| 4 | <i>C. racemosae</i> rhizoma |
| 5 | <i>Caulophyllum thalictroides</i> |
| 6 | <i>C. racemosae</i> radix |
| 7 | <i>C. foetidae</i> radix |
| 8 | <i>Cimicifugae</i> rhizoma (China) |

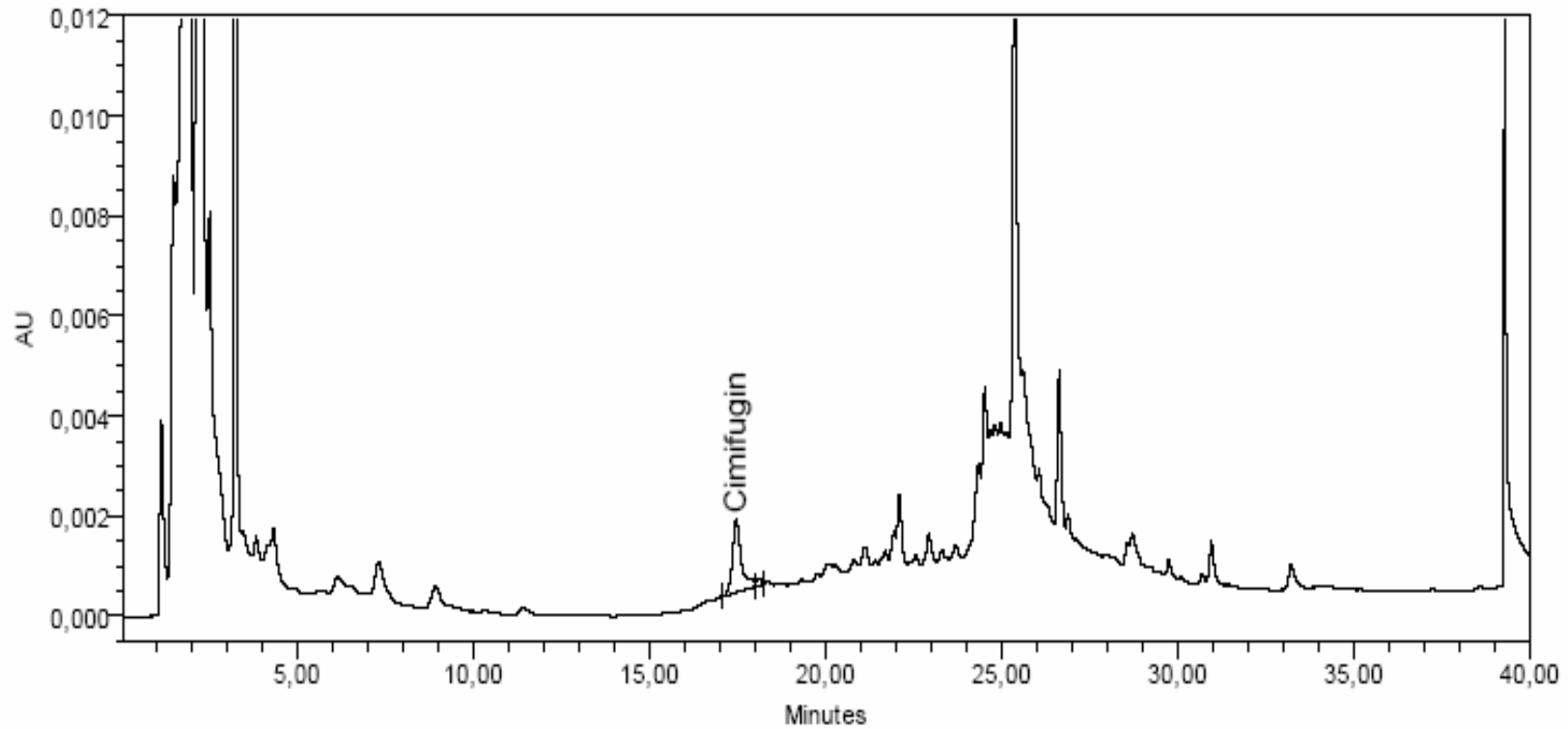
5% *Cimicifuga foedita* in *C. racemosa*



2,5% *Cimicifuga foedita* in *C. racemosa*

Sample Name: Test solution 2,5%

HPLC Chromatogram

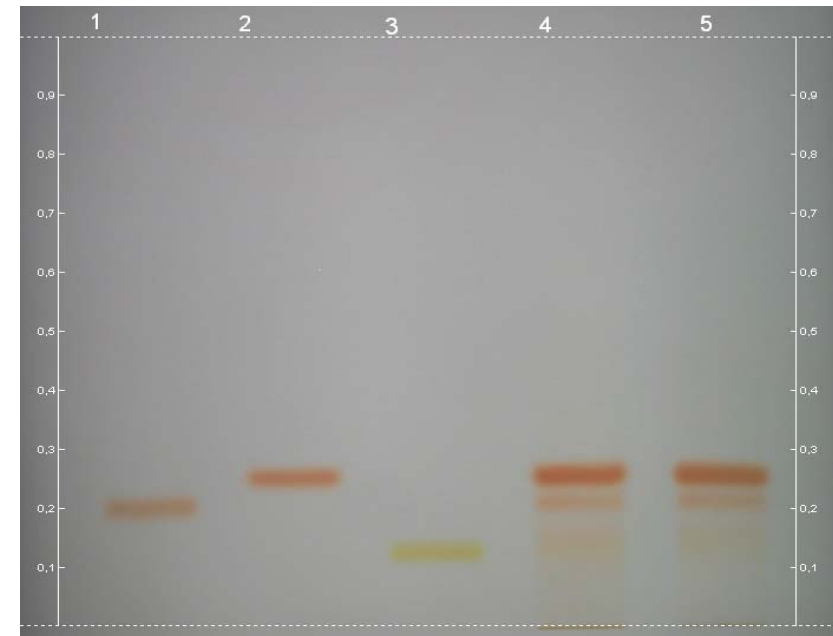
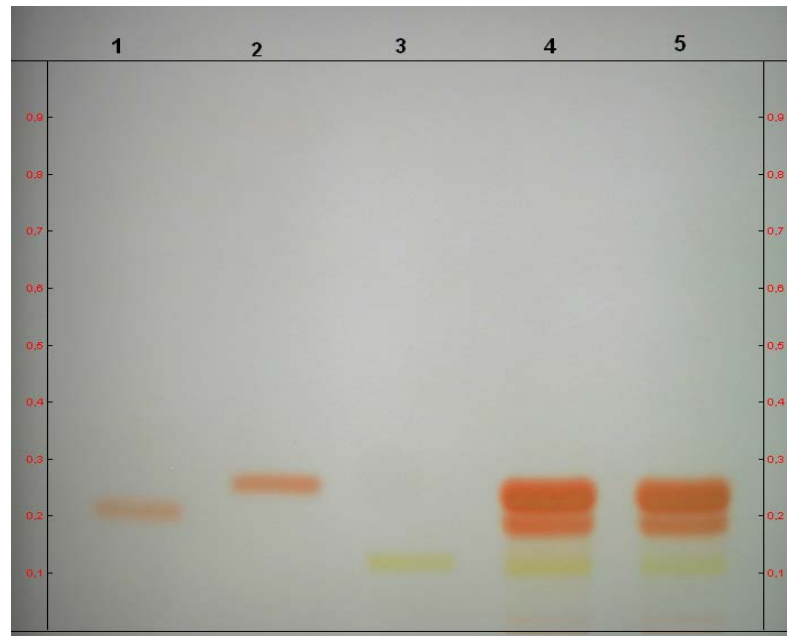


Isphagula Husk (Ph.Eur.)

Identification C describes a TLC analysis of *Plantaginis ovatae seminis tegumentum*. The weight of the powdered drug is 10mg.

Results: the chromatogram obtained with the test solution shows 2 orange-pink zones (arabinose and xylose) and a yellow zone (galactose) similar in position and colour to the zones in the chromatograms obtained with the reference solutions.

Isphagula Husk (Ph.Eur.)



50mg in place of 10mg of powdered drug

Results: the chromatogram obtained with the test solution shows 2 orange-pink zones (arabinose and xylose) and a yellow zone (galactose) similar in position and colour to the zones in the chromatograms obtained with the reference solutions.

Betulae folium (Ph.Eur.)

Identification C describes TLC analysis. There should be detected:

Quercetin (brownish yellow zone)

Other yellow brownish zones

Hyperoside (intense zone)

Chlorogenic acid (blue zone)

Rutin (faint zone)

Betulae folium (Ph.Eur.)



| | |
|-----|------------------------------------|
| 1 | Caffeic acid |
| 2 | Hyperoside |
| 3 | Chlorogensäure |
| 4 | Rutoside |
| 5-8 | Betulae folium of different origin |

„The zone corresponding to rutin is very faint or could be absent and the zone corresponding to hyperoside is intense“

Passiflorae Herba (Ph.Eur.)

Identification C describes a TLC analysis. There should be detected:

vitexin (green fluorescence above)

orientin (brownish-yellow fluorescence above hyperoside)

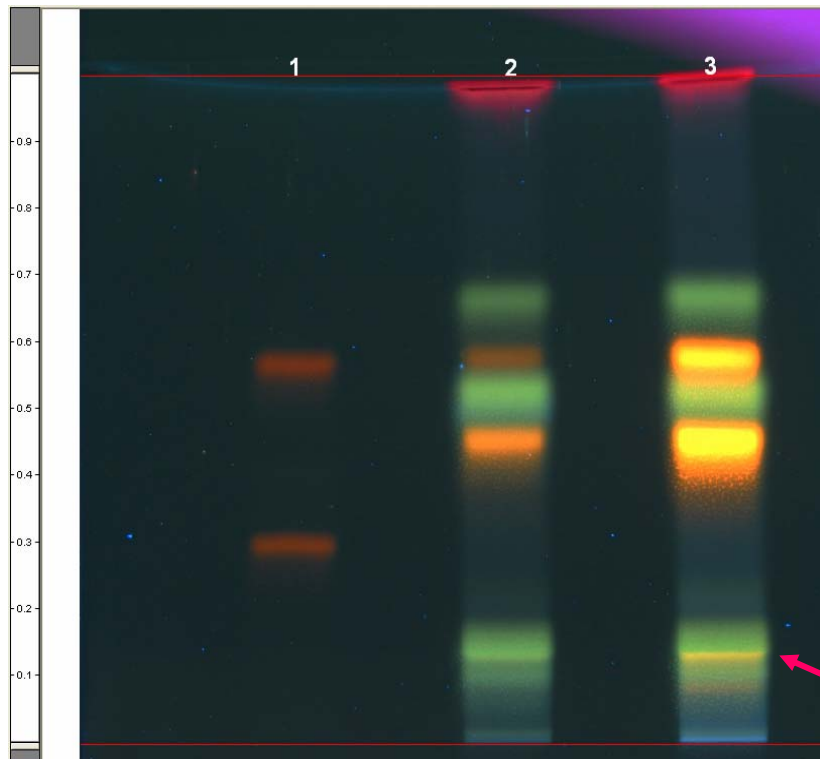
isovitexin (green fluorescence above)

iso-orientin (yellow fluorescence below hyperoside)

diglycosylflavone (green fluorescence above)

intense yellow fluorescence (below rutin)

Passiflorae Herba (Ph.Eur.)



| | |
|---|----------------------|
| 1 | Rutoside, Hyperoside |
| 2 | Passiflorae herba |
| 3 | Passiflorae herba |

intense yellow zone is missing in some batches

Conclusion

Herbal drugs are obtained from cultivated or wild plants. They vary more or less in composition and properties depending on the habitat, climate zone and annual variations. This causes problems in the identification and purity tests of herbal drugs.

Sometimes samples could not comply with the description in the Ph.Eur. monograph in 100%. In some cases the monograph should be revised (isphagula husk, betula, passiflora).

On the other hand some test must be validated in the laboratory to get the right results (equisetum).

New monographs have to consider all possible adulterations using the best satisfying method (cimicifuga).



Thank you for
your interest!