European Pharmacopoeia Reference Standards for Herbal Drugs and Preparations

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Content of the presentation

- Definitions and guidelines
- Previous and new policy in monographs on herbal drugs
- Testing
- Examples
- Assignment and use
Definitions and Guidelines (2)

• General Chapter 5. 12.


«Reference standards are also established for the determination of the content of components of herbal drugs and herbal drug preparations. These may be: the active principles themselves, marker constituents used for quantification, or extracts. Reference standards consisting of extracts are established using well-characterised samples of active principles or markers»
Previous policy

- In case of HPLC assays description of a reagent of minimum purity as “reference substance”. Purity had to be determined by the user.
New policy

- Previous system was considered insufficient because of:
  - Inconsistency with policy applied in monographs for substances for pharmaceutical use
  - Assignment to be done by the user
  - Quality of the plant material not controlled in a harmonised manner
  - Availability and costs of reagents
New policy

- In future reference substances with assigned contents will be described in monographs for herbal drugs which may be either:
  - the “active principle” or a marker substance
  - a standardized extract

The choice depends on availability, price, stability
Active ingredients or marker substances

- Boldine CRS - Monograph boldo leaf
- Coumarin CRS - Monograph melilot
- Ruscogenins CRS - Monograph butcher’s broom
- Chlorogenic acid CRS – Monograph nettle leaf, purple coneflower root and herb
- Verbenalin CRS - monograph verbena herb
Active ingredients or marker substances

- Salicin CRS - monograph willow bark
- Capsaicin CRS - monographs for standardized capsicum tincture, capsicum oleoresin
- Nonivamide CRS – see capsaicin
- Harpagoside - monograph Devil’s claw root
- Oleuropein – monograph olive dry extract
- Aescin – monograph horse chestnut dry extract
Active ingredients or marker substances

- Rhein CRS – draft monograph rhubarb
- Rosmarinic acid – melissa leaf and extract, peppermint leaf and extract
Establishment of active ingredients or markers

- Structure elucidation by IR, NMR, MS
- Purity determination by HPLC
- Determination of water and solvents and/or LOD/TGA
- Inorganic impurities
- DSC or other absolute methods such as titration
Establishment of active ingredients or markers

- Content assignment on the “as is” basis
  \[ 100 - (\text{water+solvents}) \times \text{chromatographic purity/100} \]

- Lyophilized substance (verbenalin): assignment on a mg/vial basis (ex. 0.97 mg of verbenalin/vial for verbenalin CRS 1)
Standardized extracts

Option chosen when the “active principle” or marker substance are not available in sufficient amounts:

- Milk thistle standardized dry extract
- Ivy leaf standardized tincture
- Valerian standardized dry extract
- Agnus castus fruit standardized dry extract
Standardized extracts

Example: Valerian standardized extract and Valerenic acid
Standardized extracts

Example: Valerian standardized extract and Valerenic acid
Standardized extracts

Example: *Hederacoside* and *Hedera helix* tincture
Standardized extracts

Example: Hederacoside and *Hedera helix* tincture
Standardized extracts (monitoring)

Example: Hedera helix tincture (addition of ethylparaben)
Standardized extracts

Example: Agnus Castus dry extract

Casticin test solution
Standardized extracts

Example: Agnus Castus dry extract
Casticin reference solution
Standardized extracts

Example: Agnus Castus dry extract

Test solution
Use of a marker as CRS

- **Problem**: neither active principle nor extract are available:

  **Example**: Monograph for Lemon verbena leaf

  **Definition**: Content minimum 2.5 % of acteoside

  **HPLC assay**: resolution requirement of 3.5 for the separation between acteoside and internal standard (ferulic acid R)
Use of a marker as CRS

Test solution with addition of ferulic acid
Use of a marker as CRS

- **Solution:**
  - Determination of response factor of acteoside/ferulic acid
  - Use of ferulic acid as CRS with assigned content
CRS in the test for related substances: Qualitative use for peak identification
Monograph Anise oil, GC-test for foeniculin
Thank you for your attention and patience