TCM Herbal Drugs – Decoction – Granules: is there a Phytoequivalence?

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Granules for prescription are present in the European market since many years. In some EU countries their market share exceeds the amount of genuine TCM herbal drugs. Besides single herbal drug granules, complex formulas are provided. Granules originating from Taiwan are commercialised as patent medicines, which are still illegal in the EU.
Decoction vs. Granules

**Decoctions**
- are often unpleasant herbal preparations for the patient
- need experience and a considerable time for preparation
- usually are prepared for a few days: stability is a problem
- prepared from different herbal drugs and mixed following the prescription of a TCM practitioner

**Granules**
- are easy to handle and have a better compliance
- anticipate the extensive process of decoction:
- they are prepared by decoction/extraction with water and addition of suitable excipients
- consist of one herbal drug and are mixed in a pharmacy for the patient
- are easily dissolved in water ready for use
Granule Preparation

decoction of TCM herbal drug(s)

concentration of aqueous extract

spray- or freeze drying

mixing with excipients

formulation of granules
TCM Granules: Problems

1. Proof of Identity: macroscopy, microscopy, TLC/HPTLC fingerprinting

2. non declared production and extraction process:
   - matrix components added (starch, dextrin)
   - herbal drug powder added
   - toxic solvents used for extraction
   - phytoequivalence not guaranteed

3. Pharmaceuticals by presentation:
   import license required
Problems with Constituents

- TCM Granules on the European Market normally do not have a declared minimum or maximum content or range of constituents (markers).
- TCM Granules mostly declare a Drug-Extract-Ratio of 5:1.
- Granules referring to the same herbal drug and the same application are often produced by different extraction procedures and different extraction solvents: Phytoequivalence not obvious.

Each Supplier claims to have the optimal quality.
Quality Requirements for Granules

- **Identity**
  - TLC/HPTLC fingerprint
  - HPLC-fingerprint
  - DNA-identification

- **Purity Tests**
  - Mycotoxins
  - Heavy metals
  - Pesticides
  - Microbiological contamination
  - Irradiation
  - Solvent residues

- **Assay**
  - Quantification of toxic constituents
  - Quantification of active markers
  - Labelling

TCM Granules should meet the Quality Requirements of the Ph Eur Extract Monograph
Granules as Ph Eur : Extracts

Definition:
Extracts are preparations of liquid, semi-solid or solid (dry extracts) consistency, obtained from herbal drugs.

Different *types of extract* may be distinguished:

- **Standardised extracts** are adjusted within an acceptable tolerance to a given content of constituents with known therapeutic activity; ....
- **Quantified extracts** are adjusted to a defined range of constituents; ...
- **Other extracts** are essentially defined by their production process.

Granules could be considered as a form of Other Extracts.
Example No 1: Siegesbeckiae herba

Ch P 2010

- *Siegesbeckia glabrescens* MAKINO
- *Siegesbeckia orientalis* L.
- *Siegesbeckia pubescens* MAKINO

Pinyin: Xixiancao

稀莶草
Siegesbeckiae herba

Chemical compounds identified:
Diterpenoids, fatty acids, flavonoids, steroids (β-sitosterol)
active compound: Kirenol?
- analgetic effects
- antiinflammatory effects

Kirenol
Kirenol

Kirenol was shown to be present in all three species: 
*Siegesbeckia glabrescens, S.pubescens, S.orientalis*

Content of Kirenol: higher in leaves compared to stem

Kirenol: Reference Substance according Ch P 2010
**HPTLC fingerprinting**

- Kirenol + β-Sitosterol are suitable markers
- good separation and precision
- wine processed herbal drug -> Kirenol not present
- not visible in HPTLC fingerprinting
  -> according to ChP 2010: same analytical requirements as genuine herbal drug material!
- Content of Kirenol in commercial granules for prescription?
New: TLC/ HPTLC Method

- extraction: 1.5 g powdered drug + 10 mL methanol, sonicate for 30 min
- reference: 1 mg Kirenol (K) + β-Sitosterol (S) per 10 mL methanol
- mobile phase: dichloromethane: methanol (5:1, V/V)
- distance: 6 cm from application line
- stationary phase: silica gel $F_{254}$

Chloroform (ChP 2010) could be replaced by dichloromethane!
Track 1: *reference standards (K, S)*
Track 2-4,6-9: *Siegesbeckiae herba* extracts *Chinese origin*
Track 5: *Siegesbeckiae herba* extract *German origin*
Track 10: *S. orientalis*; Track 11: *S. pubescens*;
Track 12: *S. glabrescens*;
Track 13: *S. orientalis* wine-processed
TCM granules – HPTLC Fingerprint

Apparent differences in fingerprint!

Track 1: reference substances (K, S)
Track 2, 3: Siegesbeckia granules extract
Track 4, 5: Siegesbeckiae herba extract
Track 6: Siegesbeckiae herba decoction
TCM granules/decoction – phytoequivalece ?

Track 1: reference substance (K)
Track 2, 4: Siegesbeckia granules H₂O-extract
Track 2, 5: Siegesbeckia granules MeOH-extracts
Track 6: Siegesbeckiae herba decoction
Track 7: Siegesbeckiae herba MeOH-extract

Kirenol not visible in granules but in decoction ➔ therapeutic eqivalence?
Summary: Siegesbeckiae herba

- New HPTLC method for Identification C established
- different Siegesbeckia species included in one monograph?
- differences in HPTLC fingerprinting
- which species have been used for the preparation of granules?
- further investigations on granules of different commercial samples are needed
Scrophulariae radix – ningpo figwort

- *Scrophularia ningpoensis* HEMSL. – Scrophulariaceae
- Pinyin: Xuanshen, 玄参
- Chemical constituents
  - Iridoid glycosides
  - Phenylpropanoid glycosides
  - Flavonoids
  - Volatile oil
  - Fatty acid
- Possible pharmacological active compounds
  → Iridoid- & phenylpropanoid glycosides, cinnamic acid
  → Harpagoside (antiinflammatory)
Identification: TLC/HPTLC - Ch P 2010

- Extraction:
  2 g powdered herbal drug + 25 mL methanol, macerate for 1 h, ultrasonic for 30 minutes and filter. Evaporate to dryness, dissolve residue in 25 mL of water. Extract by shaking with two 30 mL quantifies of n-butanol saturated with water, combine n-butanol solutions, evaporate to dryness, dissolve residue in 5 mL of methanol.

- Reference:
  1.0 mg harpagoside per 10 mL.

- Mobile phase:

- Migration distance:
  6 cm from application line.

- Stationary phase:
  silica gel F$_{254}$.

- Derivatization reagent:
  5% solution of vanillin in sulfuric acid.
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<th>1</th>
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<tbody>
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<td>Track 10, 11</td>
<td>reference standards <strong>harpagide</strong> and <strong>harpagoside</strong></td>
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<td>Scrophulariae radix extract of <strong>Chinese samples</strong></td>
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<td>Scrophulariae radix extract of <strong>German sample</strong></td>
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<td>Track 9</td>
<td><strong>Scrophularia nodosa</strong></td>
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**TLC/HPTLC – new method**

- **Extraction:**
  1.5 g powdered herbal drug + 10 mL 1-butanol, sonicate for 30 min, filtrate, evaporate filtrate to dryness and dissolve residue in 1 mL methanol.

- **Reference:**
  1.0 mg harpagide + 1.0 mg harpagoside per 1 mL methanol.

- **Mobile phase:**

- **Migration distance:**
  6 cm from application line.

- **Stationary phase:**
  silica gel F<sub>254</sub>.

- **Derivatization reagent:** vanillin-sulfuric acid reagent.
Identification C: TLC/ HPTLC - new method

Track 1,2 reference standards harpagide and harpagoside
Track 3-8, 10-11 Scrophulariae radix extract of Chinese origin
Track 9 Scrophulariae radix extract of German origin
TLC/ HPTLC, new method

- Harpagide and harpagoside suitable markers
- Good separation and precision
- Shortened extraction procedure
- Chloroform in mobile phase was replaced due to toxicity
- Mobile phase homogeneous
- Derivatization reagent less corrosive
Comparison: Scrophulariae radix TCM granules versus decocotion

→ Harpagide and harpagoside are both visible in granules and decoction
→ therapeutic equivalence, „Phytoequivalence“

Track 1,2 reference standards harpagide and harpagoside
Track 3 Scrophulariae radix, Chinese sample (Identification C)
Track 4 Scrophulariae granules (Identification C)
Track 5 Scrophulariae radix, Chinese sample decoction
Track 6 Scrophulariae granules water (1.5 g granules + 25 mL water)
Conclusion: Scrophulariae radix

- Suitable method for Identification C (HPTLC/ TLC).

- Experiments with different granules concerning therapeutic equivalence/ phytoequivalence resulted in "Phytoequivalence" in consideration of previous TLC experiment.
Xanthii fructus – siberian cocklebur fruit

• *Xanthium sibiricum* PATR. – Asteraceae

• Pinyin: Cang‘erzi, 蒼耳子 (蒼耳子)

• Chemical constituents
  
  • Fixed oil (e.g. linoelic acid)
  
  • Volatile oil (e.g. trans-caryophyllene)
  
  • Sesquiterpenes (e.g. xanthumin)
  
  • Triterpens (e.g. stigmasterol)
  
  • Organic & phenolic acids
  
  • Glycosides (e.g. *attractyloside, carboxyatractyloside*)
Identification: TLC/ HPTLC – Ch P 2010

• Extraction:
  2 g powder + 25 mL of methanol, heat under reflux for 20 minutes and filter. Concentrate to about 2 mL.

• Reference:
  reference drug.

• Mobile phase:
  \( n\)-butanol:glacial acetic acid:water (4:1:5, upper layer).

• Migration distance:
  6 cm from application line.

• Stationary phase:
  silica gel \( \text{F}_{254} \).

• Derivatization reagent:
  ammonia vapour (fluorescent spots).
Identification: TLC/ HPTLC – Ch P 2010

Track 1-7  Xanthii fructus extract of **Chinese origin** (processed and unprocessed)
Track 8  Xanthii fructus extract of **German origin** (unprocessed)
Track 10  Carboxyatractyloside
Identification TLC/HPTLC – new proposal

• Extraction
0.5 g powdered herbal drug + 5 mL methanol 70% (V/V), extraction for 15 min, ultrasonic treatment

• Reference: Carboxyatractyloside 1 mg/mL, Chlorogenic acid 1 mg/mL, 1,5-dicaffeoylquinic acid 1 mg/mL

• Mobile phase: $n$-butanol:glacial acetic acid:water (4:1:5, upper layer)

• Migration distance: 6 cm from application

• Stationary phase: silica gel $F_{254}$

• Derivatization reagent: anisaldehyde-reagent
Identification TLC/ HPTLC – new proposal

Track 1-8  Xanthii fructus extract (Chinese origin, processed and unprocessed)
Track 9   Xanthii fructus extract (German origin, unprocessed)
Track 10-12  Xanthii fructus granules

References:
Track 13  carboxyatractyloside (CATR)
Track 14  chlorogenic acid (CA)
Track 15  1,5-dicaffeoylquinic acid (DCQA)
**TCM Granules – sample 1**

Track 1  chlorogenic acid (CA)
Track 2  1,5-dicaffeoylquinic acid (DCQA)
Track 3  carboxyatractyloside (CATR)
Track 4  Xanthii fructus methanol 70% extract; Chinese sample
Track 5  Xanthii fructus decoction; Chinese sample
Track 6  Xanthii fructus granules water (1.5 g granules + 25 mL warm water)
TCM Granules – sample 2

→ Fingerprints are looking quite different
→ Carboxyatractyloside not visible in decoction and granules!
→ Atractyloside not visible in decoction but in granules!

→ Phytotherapeutic equivalence??

Track 1  chlorogenic acid (CA)
Track 2  1,5-dicaffeoylquinic acid (DCQA)
Track 3  carboxyatractyloside (CATR)
Track 4  Xanthii fructus methanol 70% extract; Chinese sample
Track 5  Xanthii fructus decoction; Chinese sample
Track 6  Xanthii fructus granules water (1.5 g granules + 25 mL warm water)
Summary: Xanthii fructus

The proof of phytoequivalence when testing commercial granules versus classical decoctions of the herbal drug Xanthii fructus demonstrated that the samples examined showed doubtful results for the presence of the respective marker compounds!
Final Conclusion

- TCM Granules vs. TCM herbal drugs have a rising share in the EU market (30-40% in Germany, Switzerland ca. 80%)

- The assurance of a comparable quality between Commercial Granules and the corresponding TCM herbal drugs is rather doubtful

- TCM Granules at the moment are unregulated products for pharmaceutical and medical use

- No specific quality monographs for Granules exist in the actual Ph Eur: Establishment of monographs for TCM Granules is absolutely necessary in order to follow the actual market situation in Europe