

Philipps  Universität  
Marburg

## Homoeopathic monograph case study

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## Types of monographs

- Chemically defined compounds
- Minerals
- Non-toxic plant materials
- Toxic plant materials with a relatively stable toxic principle (e.g., *Atropa belladonna*)
- Toxic plant materials with a relatively instable toxic principle (e.g., *Rhus toxicodendron*)
- Others (mushrooms, animal materials)

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## Current problems (1)

- ❑ **Chemically defined compounds:** usually no problems
- ❑ **Minerals:** can be handled similar to chemically defined compounds, but usually mixture of a relatively large number of chemical elements. A precise and more or less simple method for determination of crystal structure is under discussion.
- ❑ **Non-toxic plant materials:** “Priority Zero”, because assay-question is unresolved

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## Current problems (2)

- ❑ **Toxic plant materials with a relatively stable toxic principle:** can be handled similar as raw materials used in phytotherapy. In addition to the starting material, also the mother tincture has to be described. The assay is usually on the toxic principle.
- ❑ **Toxic plant materials with relatively instable toxic principle:** serious problems, because a CRS of the toxic principle can not be established
- ❑ **Others:** Because of microbial concerns, no animal materials. Mushrooms were handled analogously to herbal starting materials.

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## Case study

### Monograph 2290: *Agaricus Phalloides* for homoeopathic preparations

- ❑ **Be careful:** the name of the monograph is rather confusing (reason of tradition), it is **not** a champignon.
- ❑ **Definition:** Whole, fresh mushroom (fruiting body) *Amanita phalloides* (Vaill. ex Fr.) Link.
- ❑ **No cultivation,** hand-picked in the wild
- ❑ **Question:** Identity of the material?



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## *Amanita phalloides*?



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## Amanita phalloides?



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## Monograph requirements

- ❑ Production of the mother tincture
- ❑ Characters of the mother tincture
- ❑ Identification (mainly by TLC)
- ❑ Test on adulterations (in this case on *A. muscaria*, because this is the only mother tincture, which is also prepared from a mushroom. Therefore, names can be mixed up.)
- ❑ Assay (difficulty: amanitin not available as CRS. The assay has to be established with a stable surrogate.)

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## Mother tincture

- ❑ The **mother tincture** complies with the requirements of the general monograph “Mother tinctures for homeopathic preparations” (2029).
- ❑ **Definition:** Content: **0.001** per cent m/m to **0.010** per cent m/m for the sum of **α-amanitin** and **β-amanitin** ( $C_{39}H_{54}N_{10}O_{14}S$ ;  $M_r$  919 for α-amanitin).
- ❑ **Production:** The mother tincture is prepared according to the following methods as prescribed in the monograph “Methods of preparation of homeopathic stocks and potentisation (2371)”: **method 1.1.5** or **method 1.1.10**, using 5 parts of the cut drug for 100 parts of ethanol (45 per cent V/V) and maceration for 3 weeks.
- ❑ **Characters:** Appearance: brownish-yellow, yellowish or green liquid.

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## TLC

- ❑ Because of the lack of low-molecular weight secondary metabolites, **more or less unspecific on polar compounds**
- ❑ **Mobile phase:** glacial acetic acid R, water R, butanol R (17:17:66 V/V/V)
- ❑ **Detection A:** examine in ultraviolet light at 254 nm
- ❑ **Detection B:** treat immediately with a 1 per cent V/V solution of *cinnamic aldehyde* R in methanol R and allow to dry; treat with *hydrochloric acid* R; examine in daylight

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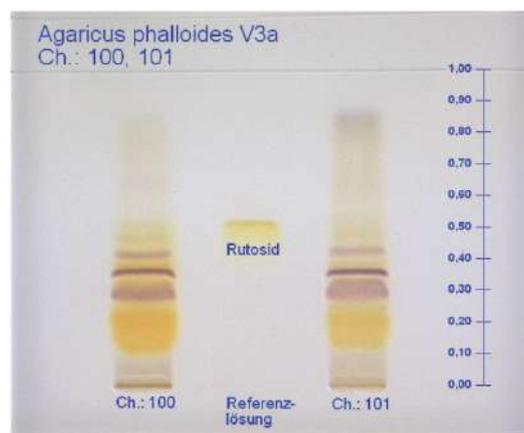


## TLC-Results

Top of the plate		
Rutoside: a quenching zone	Rutoside: a yellow zone	A violet zone
Sennoside B: a quenching zone		A violet zone
		A violet zone
		2 faint greyish-violet zones may be present
Reference solution (detection A)	Reference solution (detection B)	Test solution (detection B)

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## TLC-Results (2)



**Detection B:** Is the result convincing? The violet zones should be amanitins

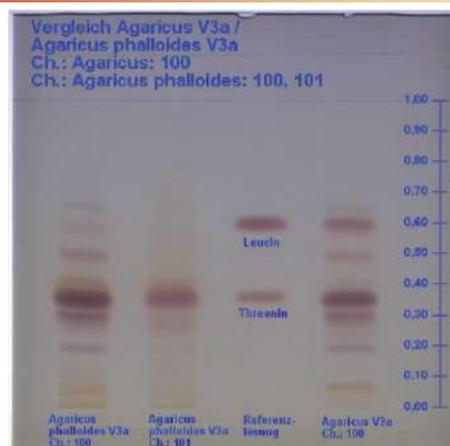
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## Adulteration

- ❑ **Mobile phase:** *glacial acetic acid R, water R, acetone R, butanol R* (10:20:35:35 V/V/V/V; mobile phase for amino acids).
- ❑ **Detection:** treat with a 1 g/L solution of *ninhydrin R* in butanol R and heat at 105 °C for 5-10 min; examine in daylight. **This is a TLC on amino acids.**
- ❑ **Results:** the presence of noticeable zones in the chromatogram obtained with the test solution, in the same position as the zones due to leucine and threonine in the chromatogram obtained with the reference solution, indicates adulteration with mother tincture of *Agaricus muscarius*.

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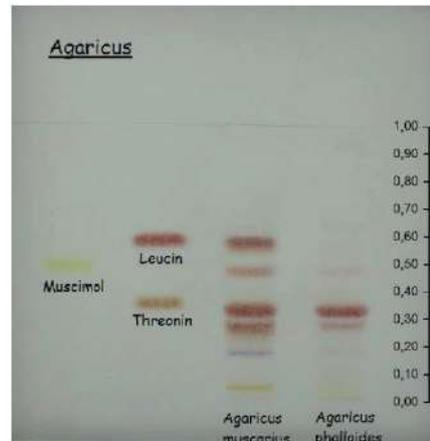
## TLC-Results (3)



**Detection with ninhydrin:** *Is the result convincing? The reddish-violet-brownish zones are amino acids*

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## TLC-Results (4)



**Detection with ninhydrin: Is the result convincing? The reddish-violet-brownish zones are amino acids**

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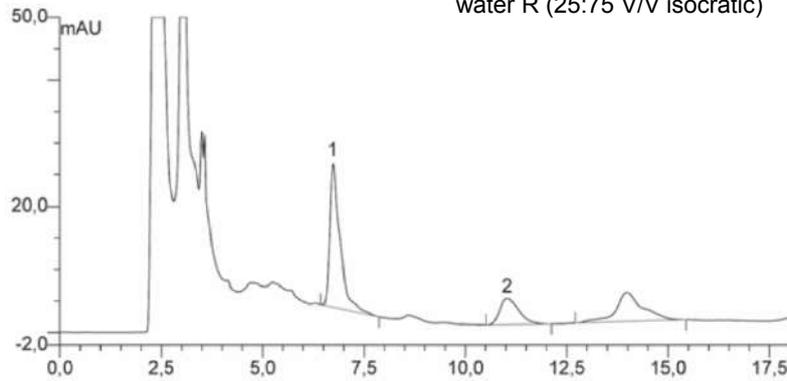
## Assay

- HPLC-Assay with the amino acid tryptophan as surrogate.
- Calculation of the amount of amanitin by a response factor, which has to be determined experimentally.

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## HPLC-Assay-1<sup>st</sup> attempt

**Mobile phase:** methanol R,  
water R (25:75 V/V isocratic)



1 Tryptophan    2  $\alpha$ -Amanitin

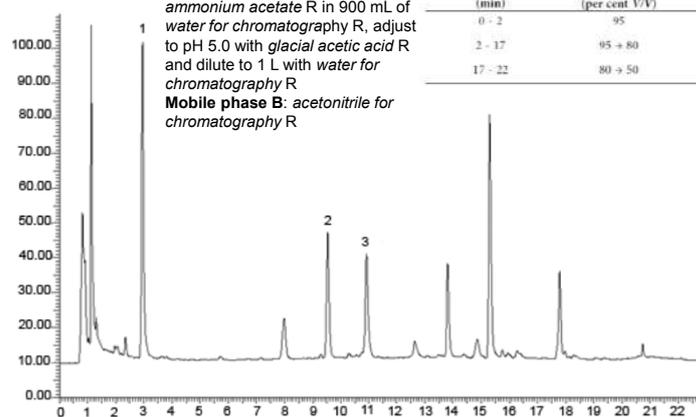
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## HPLC-Assay-2<sup>nd</sup> attempt

**Mobile phase A:** dissolve 1.54 g of ammonium acetate R in 900 mL of water for chromatography R, adjust to pH 5.0 with glacial acetic acid R and dilute to 1 L with water for chromatography R  
**Mobile phase B:** acetonitrile for chromatography R

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 2	95	5
2 - 17	95 → 80	5 → 20
17 - 22	80 → 50	20 → 50



1 Tryptophan    2  $\beta$ -Amanitin    3  $\alpha$ -Amanitin

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## Knowledge Database

Search Database online: Knowledge Database

Detailed view of Amanita phalloides ad preparations homoeopathica.

<b>Monograph Number</b>	52295												
<b>English Name</b>	Agriculture phalloides for homoeopathic preparations												
<b>French Name</b>	Agriculture bulbeuse pour préparations homoeopathiques												
<b>Latin Name</b>	Amanita phalloides ad preparaciones homoeopathicas												
<b>Phyto Name</b>													
<b>Botanical Name</b>													
<b>Pharmacopoeia</b>	27.1												
<b>Published in English Supplement</b>	9.0												
<b>Published in French Supplement</b>	9.0												
<b>Published in French Supplement</b>	9.0												
<b>Category</b>	Revision												
<b>State of matter</b>	0												
<b>Pharmaceutical</b>	To check the possibility to insert HPTLC conditions for Identification A (mother tincture) and to discuss the necessity of the TLC for mother tincture of Amanita muscaria (test for adulteration).												
<b>Pharmacology</b>	Available												
<b>Additional information</b>	Not available												
<b>History</b>	None history												
<b>International Pharmacopoeia (ICH_Q4A)</b>	NO												
<b>International Pharmacopoeia (European)</b>	NO												
<b>Reference standards</b>	<table border="1"> <thead> <tr> <th>Available since</th> <th>Exp. No.</th> <th>Name</th> <th>Batch No.</th> <th>Batch Quantity</th> <th>Price</th> </tr> </thead> <tbody> <tr> <td>2015/01</td> <td>1</td> <td>Reference Standard</td> <td></td> <td>100 mg</td> <td>70,00 €</td> </tr> </tbody> </table>	Available since	Exp. No.	Name	Batch No.	Batch Quantity	Price	2015/01	1	Reference Standard		100 mg	70,00 €
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<b>Physical information</b>	<table border="1"> <thead> <tr> <th>NAME</th> <th>Brand Name/Reference</th> </tr> </thead> <tbody> <tr> <td>Assay volume</td> <td>Amount 0.1g</td> </tr> <tr> <td>Assay volume</td> <td>From 0.1-1000µg C:10</td> </tr> <tr> <td>Assay time</td> <td>From 0 to 1000 µg/ml used for development of the method = 0.8 mL</td> </tr> </tbody> </table>	NAME	Brand Name/Reference	Assay volume	Amount 0.1g	Assay volume	From 0.1-1000µg C:10	Assay time	From 0 to 1000 µg/ml used for development of the method = 0.8 mL				
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View Search      Save

Other in Knowledge Database

Sample chromatogram with exact chromatographic conditions

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## Discussion

- ❑ **Assay on instable toxic compounds:** Broad and relatively low concentration range between 0.001 % m/m and 0.010 % m/m. Even with this broad concentration range, the mother tincture has a very limited shelf time. Unfortunately, the mushroom is available only once a year.
- ❑ **TLC identification:** Acceptable pattern of spots, but these spots are mostly diffuse
- ❑ **TLC-test on adulteration:** Amino acid pattern not very characteristic and seems to show relatively high variations. Test on muscimol is very specific for *A. muscaria*, but unluckily, muscimol is also relatively instable.

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