the nature network

PhytoLab

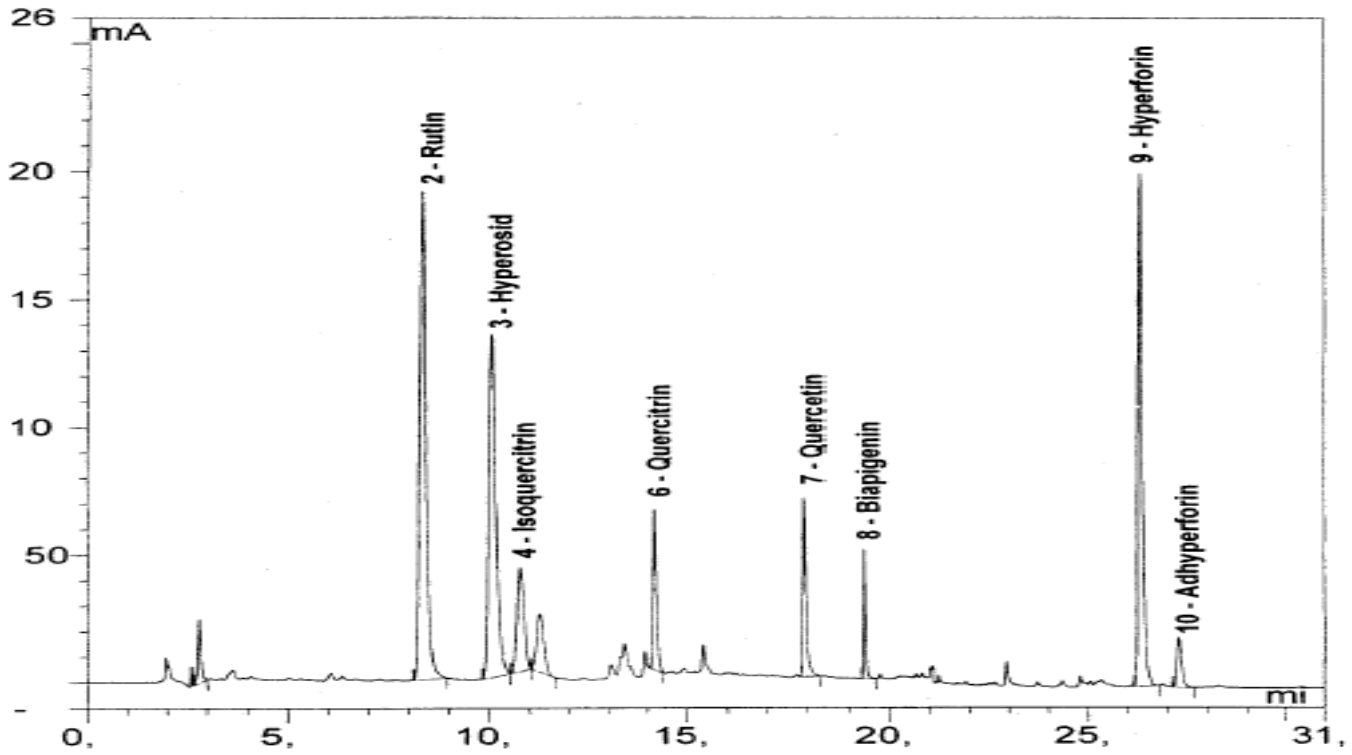
How much of Quality and Identity do we need?

Dr. Klaus Reif
PhytoLab GmbH & Co. KG
91487 Vestenbergsgreuth
Germany

Outline

- Introduction
- Definitions
- Reference standards (Characterisation, documentation)
- Conclusions

Hyperici herbae siccum extractum quantificatum



The problem – collaborative study

Determination of hyperforin in St. John‘s Wort extracts

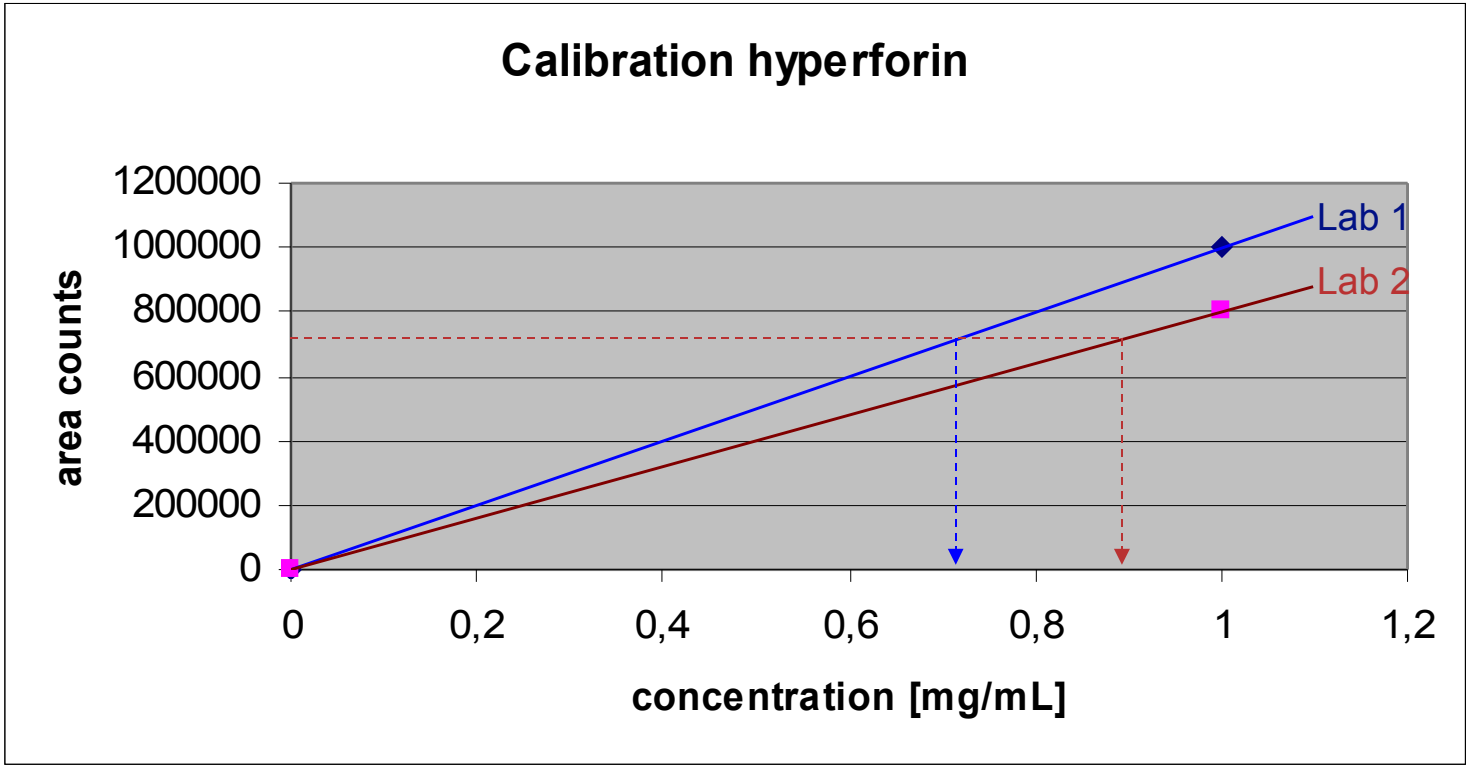
- Result Lab 1: 1.80 mg/g total hyperforin
- Result Lab 2: 2.25 mg/g total hyperforin

→ 25 % difference !!! and now???

is it a problem of the method (sample preparation etc.)?

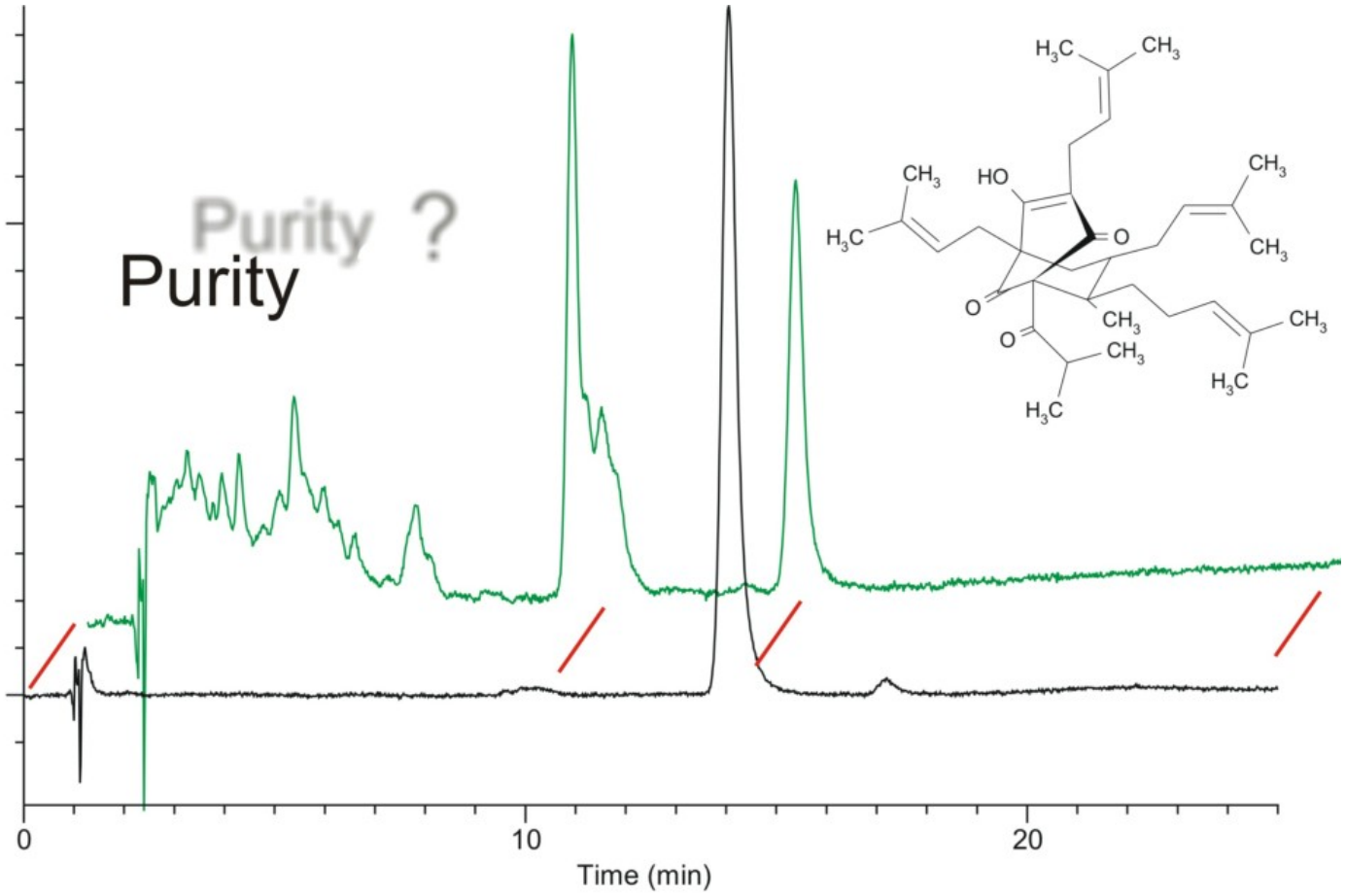
The solution

sample: 720,000 sample weight: 2 g sample: dilution factor 5



Lab 1: 1.80 mg/g Lab 2: 2.25 mg/g

Comparing Hyperforin supplied by two different companies



How much quality do we need?

For both identification and quantification of substances with known therapeutic activity or markers respectively purified and well defined reference substances are essential.

The absolute content of a quantitative reference standard must be evaluated by appropriate methods!

Definitions

- Primary Reference Substance
- Secondary Reference Substance („content standard“)

Reference standard (CPMP/QWP/2819/00)

- „A **reference standard**, or reference material, is a substance prepared for use as the standard in an assay, identification, or purity test.
- In the case of herbal medicinal products, the reference standard may be a botanical sample of herbal drug, a sample of the herbal drug preparation e.g. extract or tincture or a chemically defined substance e.g. a known active constituent, **a marker substance** or a known impurity.
- The reference standard has a quality appropriate to its use.
- The composition of reference standards of herbal drugs and herbal drug preparations intended for use in assays should be adaequately controlled and the purity of a standard should be measured by validated quantitative procedures.“

Requirements of the BfArM

„The quality of all reference standards, also used for identity and purity tests, have to be conform with attachment 6 of the commentaries of the BfArM for the registration of medicinal products. Information on source/synthesis/isolation have to be provided.“

„For in-house standards the proof of the chemical structure is required (H-NMR, C-NMR, GC-MS, UV/Vis, IR, elemental analysis) and an exact assay is required.“

„If this is not possible, the exact content has to be measured by the combination of two independent, ***complementary*** *analytical procedures. The content of water, residual solvents, ash (!) and other impurities has to be taken into account“.*

Documentation (Primary Reference Standard)

- name (trivial, IUPAC, synonyms)
- CAS-Nr.
- empirical formula and chemical structure
- source (synthesis or isolation)
- short description of the synthesis or isolation
- physicochemical properties
- certificate of analysis
- analytical methods and validation data

Documentation (Primary Reference Standard)
Identity

- appearance, color, odor
- IR
- NMR (^{1}H, ^{13}C, 2-D NMR) ⟶ residual solvents
- MS (GC-MS-MS, HPLC-MS-MS etc.)
- UV-Vis (spectrum and peak purity measurements)
- HPTLC

Interpretation and/or comparison with data from reference literature!

Documentation (Primary Reference Standard)
purity

- elemental analysis (organic and inorganic impurities*, water, residual solvents)
- ICP-MS (inorganic impurities*)
- water content (microscale Karl-Fischer titration)
- residual solvents (microscale headspace GC - technique)
- TLC
- HPLC; GC (method of normalization, „100%-method“)

* Determination of ash is not practicable (1g weighed quantity acc. to Ph.Eur.): 1 g hyperforin = 41,500 €

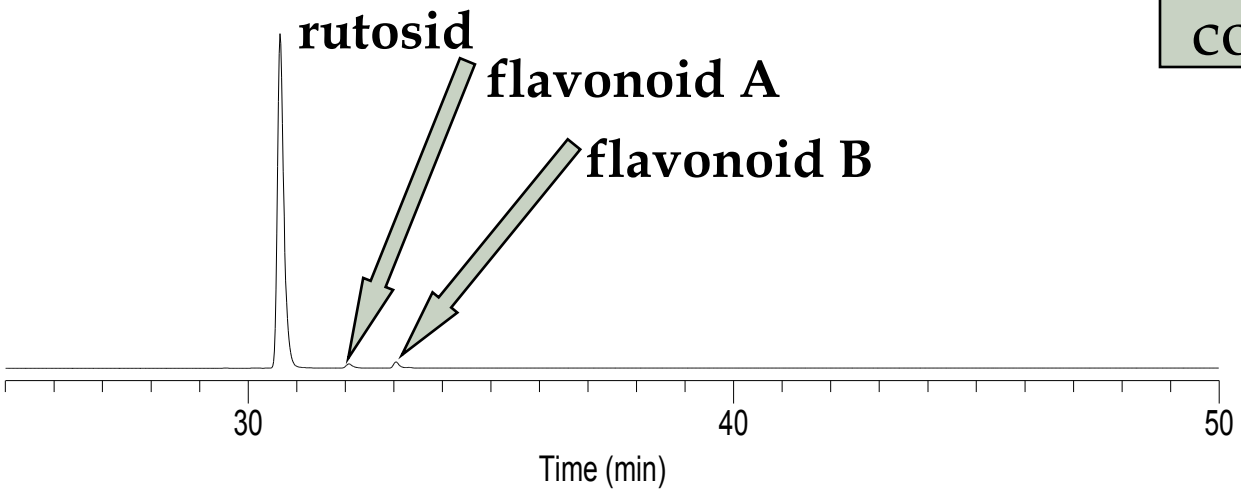
Documentation (Primary Reference Standard) content

- any suitable, validated method for quantitation (HPLC, UV-Vis, GC, HPTLC)
- 2 different methods are used with variation of the stationary and the mobile phase to achieve different selectivity
  - C18 vs. C8 AND MeOH vs. ACN
- one of these methods may be the same used for the finished herbal product
- comparison with CRS/USP-standard (if available)
- assay by an absolute method (volumetric titration):

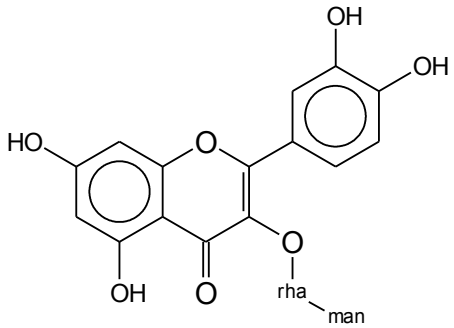
  → high risk of false results!!!

Documentation (Primary Reference Standard)
content

method 1: HPLC



content: 99.09 %



method 2: titration acc. Ph. Eur.

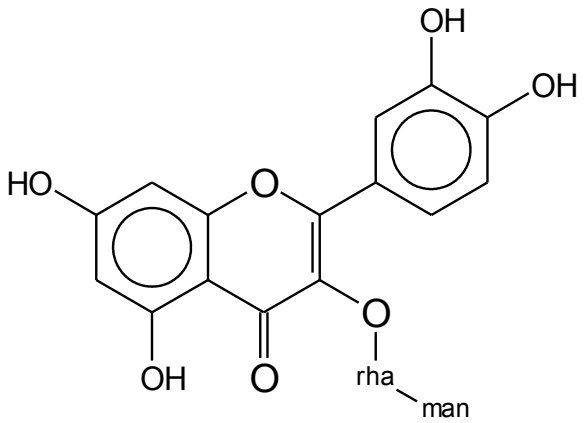
content: 101,7 %

Documentation (Primary Reference Standard) content

titration of 4‘ -und 7-OH-Gruppe:

it is not possible to distinguish between rutosid and its impurities (quercitrin and quercetin)!

The result of method 1 (HPLC) is more realistic!!



phyproof reference standards ("content standard")

- *is a primary chemical reference substance without a comprehensive proof of identity (e.g NMR etc.)*
- *at least one test on identy (HPLC-MS)*
- *its content being assigned without comparison to another substance (e.g. to a primary standard)*
- *the content is calculated by mass balance including chromatographic profile, water, residual solvents and inorganic impurity*
- *each batch delivered with complete CoA*
- *actual retest*

phyproof reference standards, „content standard“ calculation – mass balance

Defined content = [100 – (%water + %inorganic impurities + %residual solvents)] · % HPLC

100 %

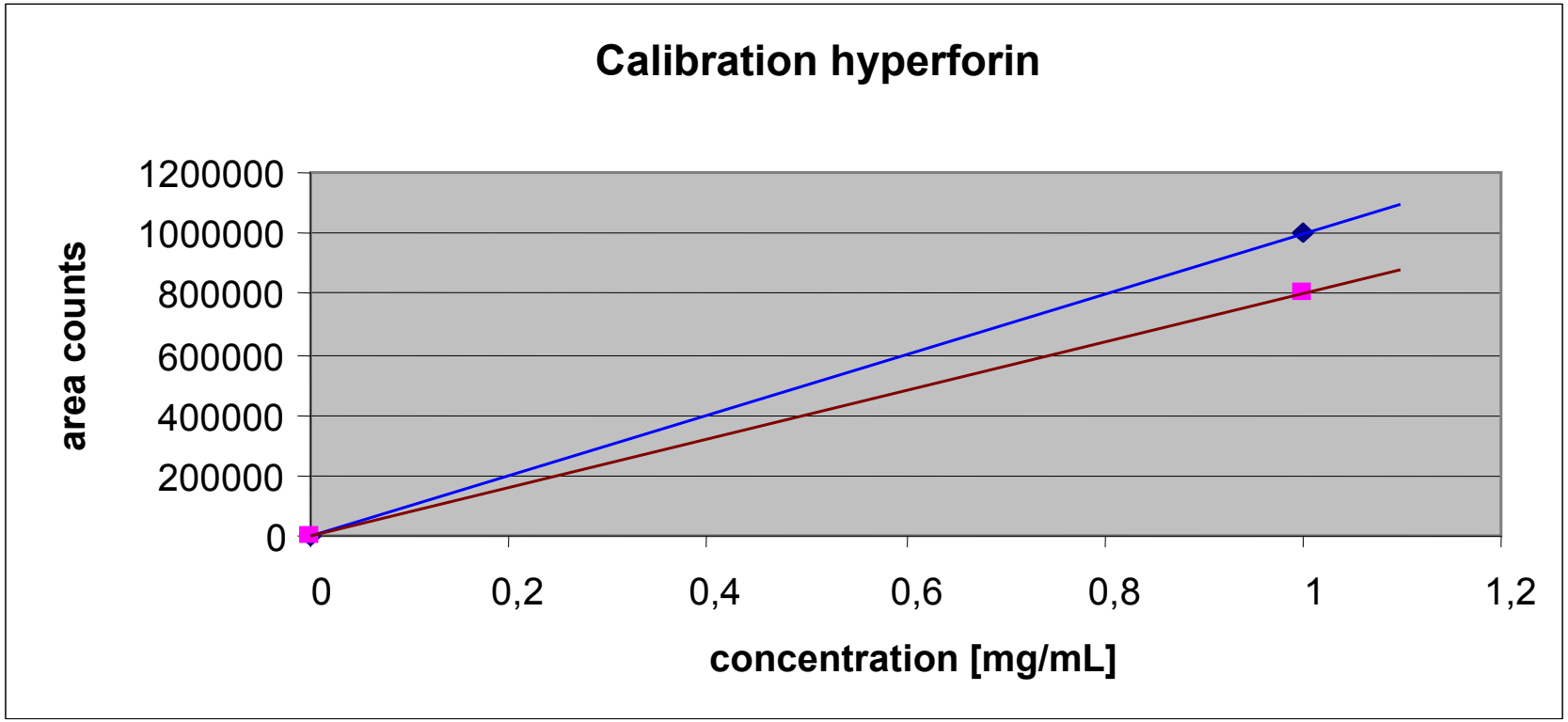
- 2.5 % water
- 0.1 % SiO_2
- 0.4 % methanol

- 97.0 %

97.0 % · 0.9909 =

96.1 %

Secondary reference substance ("working standard")



Primary standard: 96.1 %

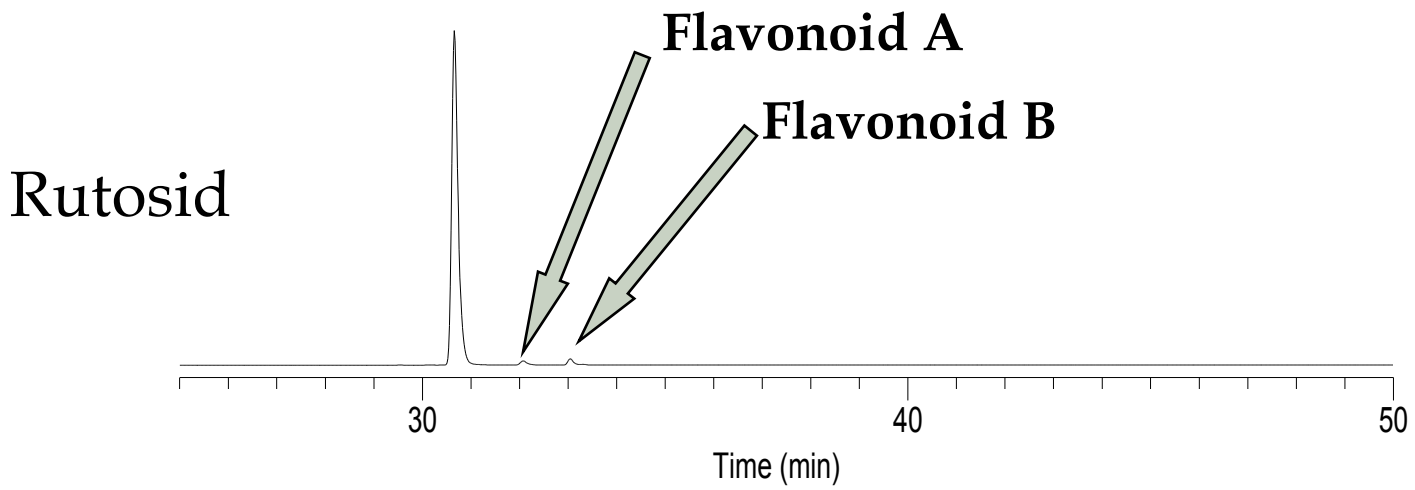
Secondary standard: 76.9 %

Documentation (Primary Reference Standard)
validation of the assay

- Specificity
  - UV-Vis-Spectrum
  - Peak purity
  - chromatogram of diluent and eluent
- repeatability (N>6)
- intermediate precision
- accuracy
- linearity, linear range
- (robustness)??

Requirements of the BfArM

„For the completion of the validation documentation of the assay of the reference substance exhaustive measurements of the robustness of the method are essential“

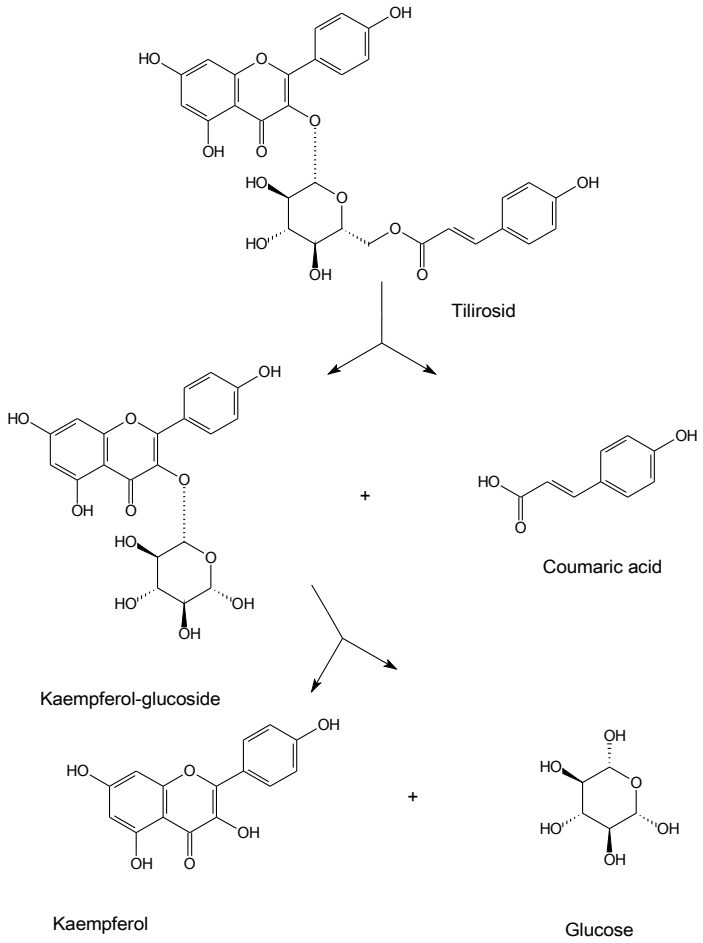


If the robustness has been shown for the product it is not necessary to prove it for the standard! (Accepted)

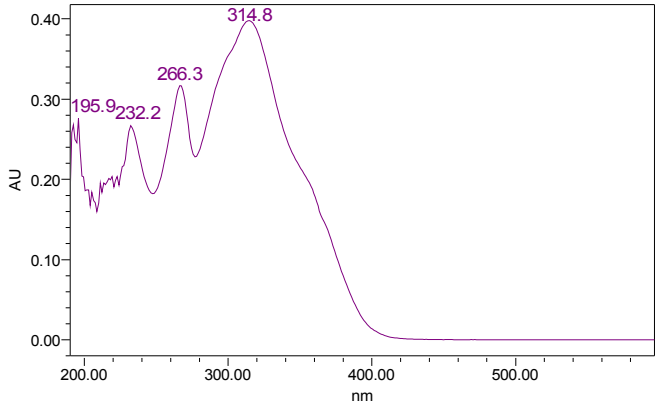
Requirements of the BfArM

„For an assay using the method of normalization (100 % - method) a detection wavelength of 260 nm or higher is not suitable for the detection of impurities like monomeric sugars“

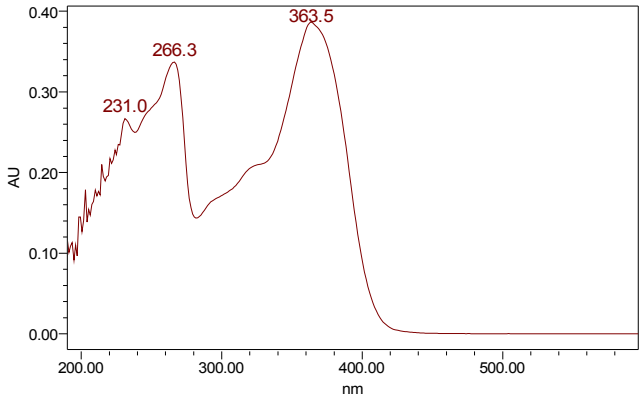
Degradation pathway of tilirosid



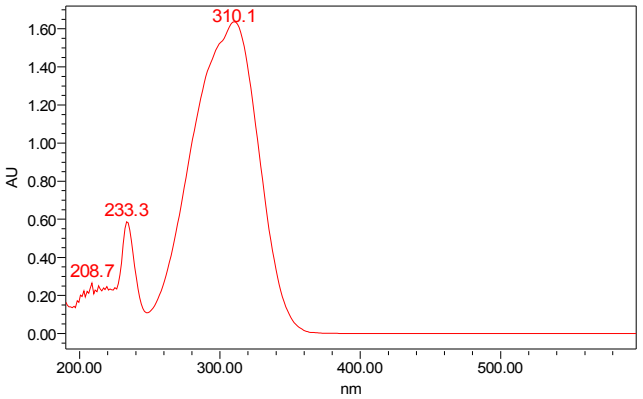
UV-Vis-Spectra of Tilirosid and impurities



Tilirosid

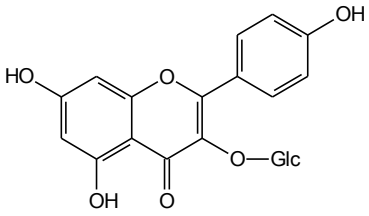


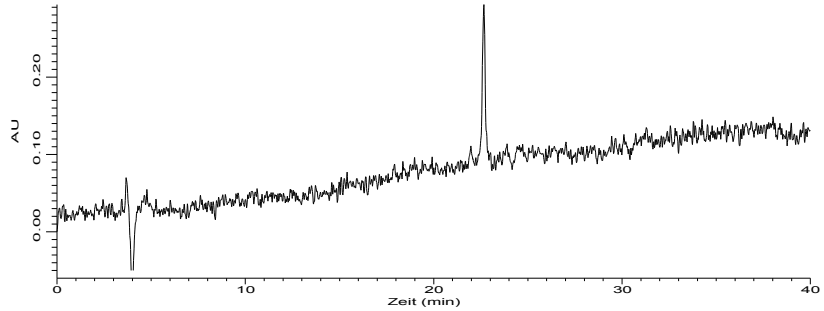
Kaempferol (-glucoside)

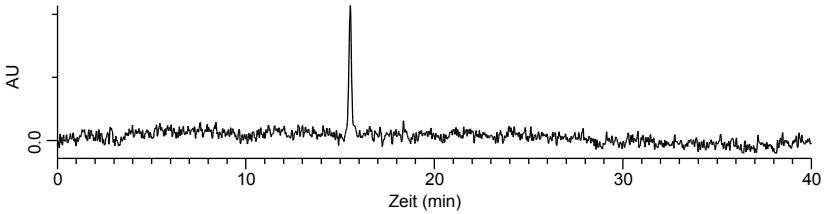


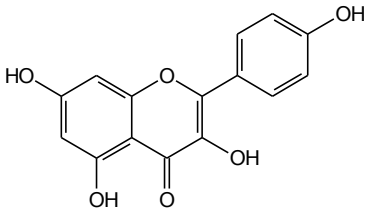
p-Coumaric acid

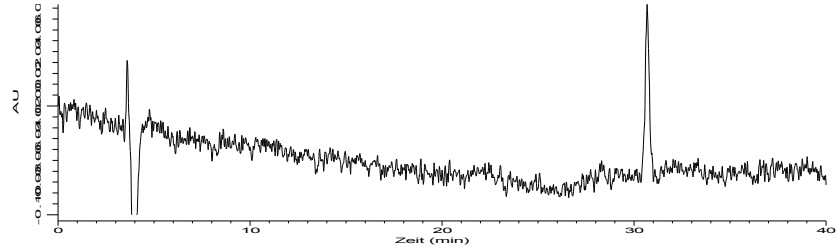
Tilirosid and degradation products at 200 nm



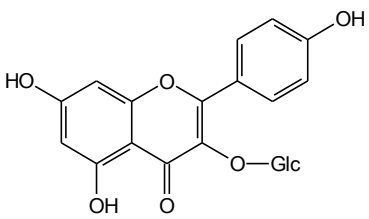


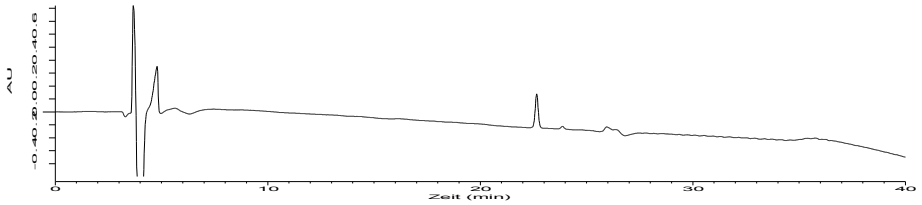


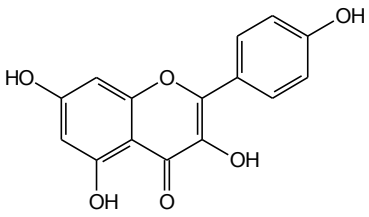


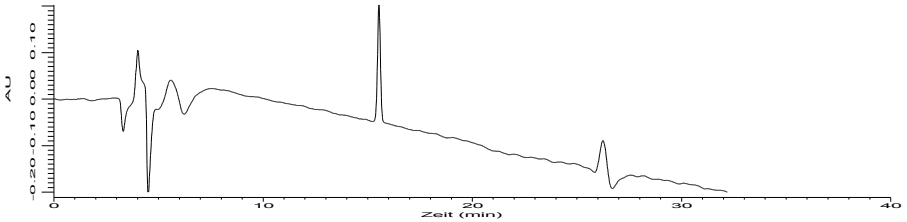


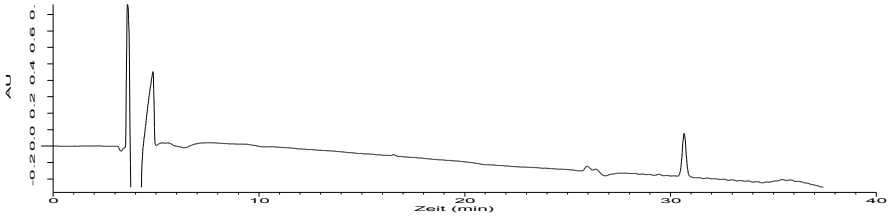
Tilirosid and degradation products at 233 nm



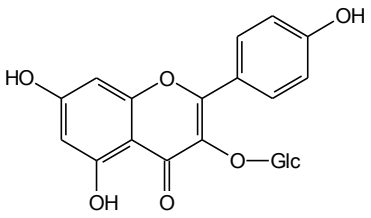


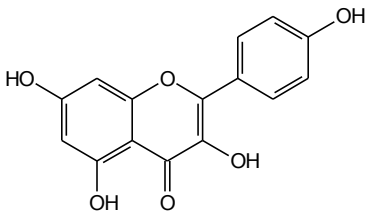


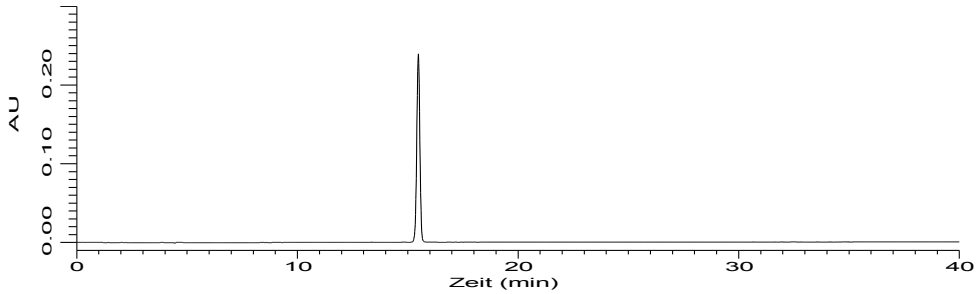


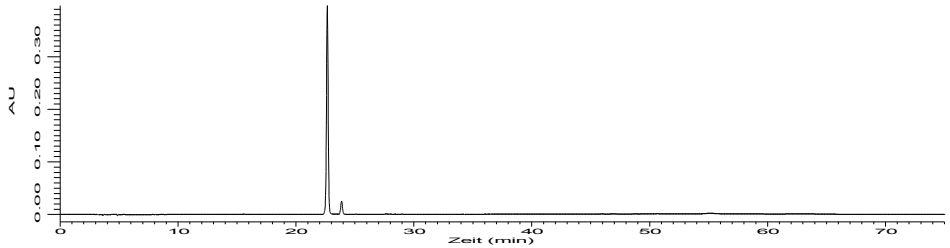


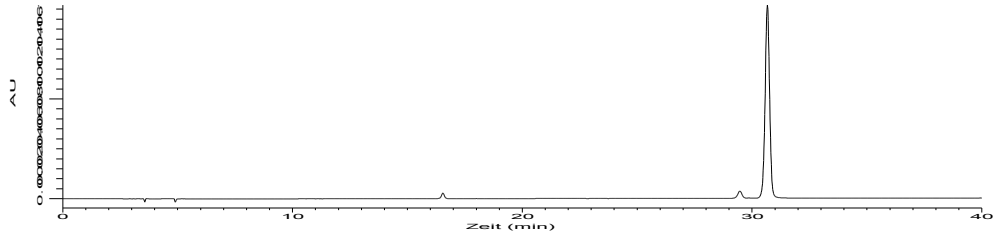
Tilirosid and degradation products at 314 nm



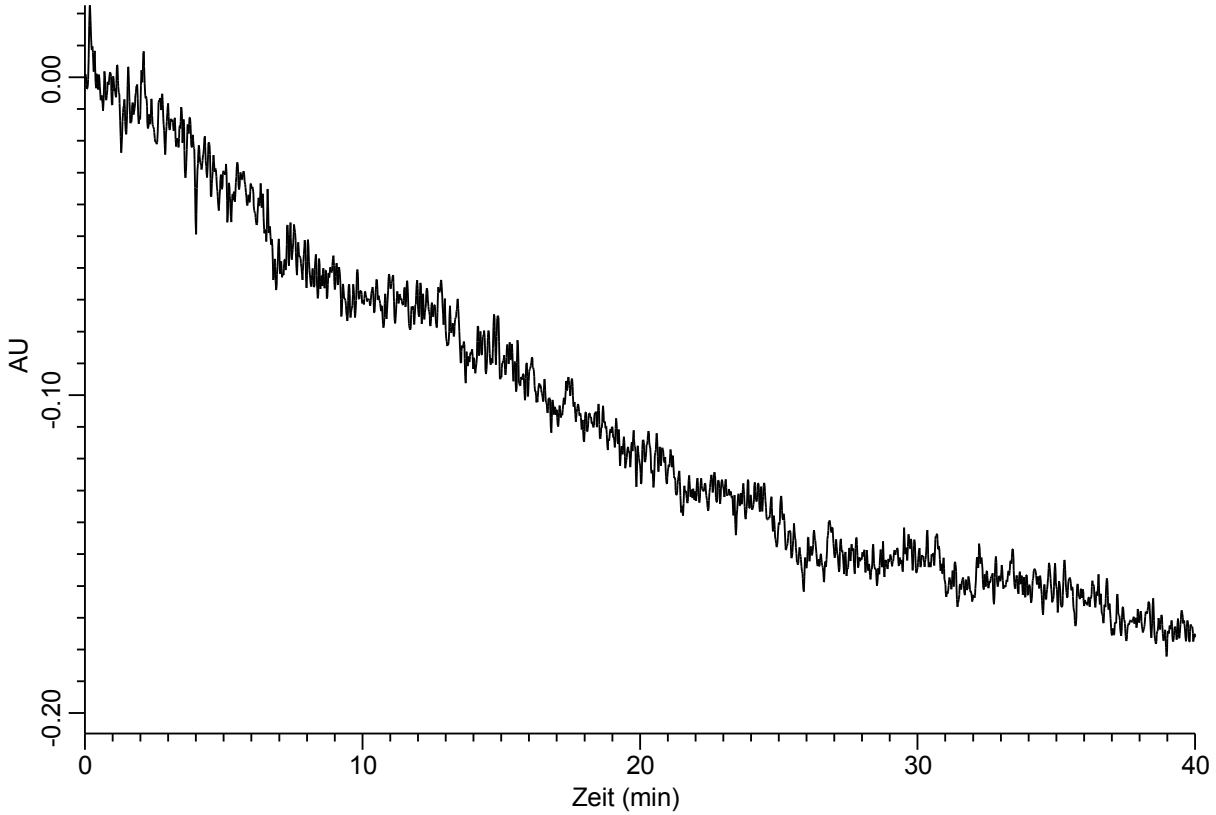








Glucose at 200 nm



Requirements of the BfArM

„For an assay using the method of normalization (100 % - method) a detection wavelength of 260 nm or higher is not suitable for the detection of impurities like monomeric sugars“

It makes no sense to measure monomeric sugars!

- *Monomeric sugars can not be detected at 200 nm with high sensitivity*
- *Bad Signal-to-Noise ratio at 200 nm* → *low sensitivity*
- *all other degradation products can be detected at the same wavelength like Tilirosid (314 nm)!*
- *excellent Signal-to-Noise-ratio at 314 nm with high sensitivity for degradation products*

Reference standards – where to buy?

- USP-standards
- CRS-Standards (EDQM, Strasbourg, France)
- PhytoLab (**phyproof** reference substance)
  - Diamond standards (= Primary Reference Standard) incl. a complete quality dossier
  - content standard (= Secondary Reference Standard) with CoA
  - Identity standard (= qualitative standard)
- ChromaDex, Carl Roth, Extrasynthese, etc.

Conclusions

- not every requirement of the registration authorities make sense from a scientific point of view (e.g. ash, robustness, selectivity of the columns, detection wavelength)
- PHARMEUROPA Vol. 18, April 2006:
  - *„…assigned value via a mass balance.“*
  - *„…it would be preferable to have a designated primary standard even if the content … (of the standard)… had an assigned value als low as 80 %“*
- For most of the herbal products we use only markers without therapeutic activity used for bach control releas! Why should the quality the same like for chemical active substances?
- You should know the quality of all reference standards, but their quality is not essential for the quality of the herbal product

Thank you for your attention !