TROZ Centre for Agriculture in the Tropics and Subtropics

Universität Hohenheim

Can We Use Mid Infrared Spectroscopy (MIRS) for Quantifying Artemisinin, an Antimalarial Compound of *Artemisia annua*?

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Introduction

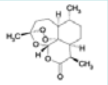
Artemisia annua is an alternative option for malaria treatment. Artemisinin, a sesquiterpen lactone, is one of its various active agents and is effective against drug-resistent plasmodium strains. It is part of the World Health Organisation (WHO) artemisinin based combination therapy (ACT), recommended since 2001.

A treatment with tea based on artemisia leaves has also proofed to be successful in 80% of treated cases according to ANAMED (Action for Natural Medicine). For quality control a rapid method to detect artemisinin content is highly desired as artemisinin content strongly varies within the plant and among varieties. In the past various methods have been developed. Most of them need an extraction or derivation of the compound artemisinin which is both time and cost consuming.

This study tested the quantification of artemisinin with Fourier transform mid infrared reflectance spectroscopy (FT MIRS), a method measuring the intensity of light absorbtion by a sample.

Artemisia annua
Family: *Asteraceae*
Names: sweet annie, qinghao

A.annua is an aromatic, annual herb. In China, it is a well-known medicinal plant for more than 2000 years. In the traditional Chinese medicine it isused to treat fever.
Artemisia annua Anamed (A-3) is a hybrid with more leaves and higher artemisinin content (up to 1,4%). artemisinin, produced in globular trichomes.



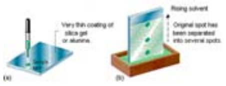
Structure of artemisinin

Objectives

- Identification of significant peak areas for artemisinin,
- Development of a quantification model with MIRS,
- Testing the reliability of MIRS for quantifying artemisinin content in *A. annua*.

Material and Methods

Thin layer chromatography (TLC)

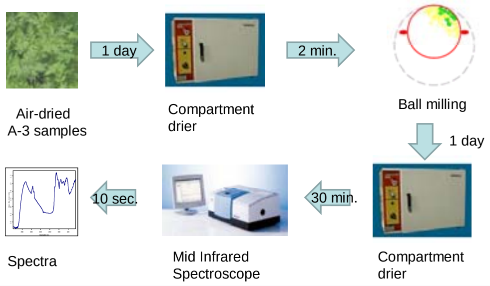


Source: chemed.chem.purdue.edu/.../bp/ch15/separate.php

Mid Infrared Spectroscopy (MIRS)

DRIFTS = Diffuse Reflectance Infrared Fourier Transform Spectroscopy

Set up of TLC and MIRS methodology

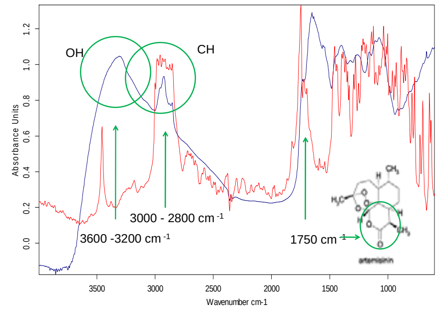


Sample preparation and procedure for artemisinin determination with MIRS

- A-3 leaf samples used in this study were collected within a study on A-3 grwoth performance in SW Ethiopia carried out at Boreda, Bonke and Chencha, Gamo Gafo Highlands. Sites varied reagrding altitude (2,200 – 2,900 m a.s.l. and climate (rainfall: 481 - 1,068 mm during the cropping season; mean average temperature during the cropping period: 13.3 – 21.1°C
- The artemisinin content of 41 leaf samples were detemined with Thin Layer Chromatography (TLC) with high pressure and Fourier transform mid infrared reflectance spectroscopy (FT MIRS).
- Based on these results, a prediction model by using OPUS Quant (Bruker Optics, Germany) was developed to determine leaf artemisinin content.
- FT-MIRS was carried at Hohenheim University by using a Bruker Tensor T27 with a Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) chamber.
- TLC was done by Médiplant, Couthey, Switzerland.

Conclusions

- Artemisinin can be identified by a significant peak at wavenumber 1760 cm^{-1}, the lactone peak.
- A reliable prediction model based on 41 leaf samples was developed (R^2 = 86 %; 92%).
- It is recommended to increase the sample number required the prediction model, e.g. 50 samples.
- Artemisinin detection below values of 0.24% is not possible by using TLC; therefore an alternative method suitable at lower ranges is recommended to improve the OPUS QUANT prediction model.



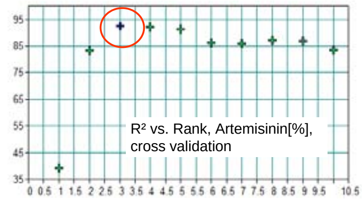
MIRS spectra of pure artemisinin and *A. annua* Anamed with a typical peak at wavenumber 1750cm^{-1}: Red spectra pure artemisinin (96%); Blue spectra *Artemisia annua* cv. Anamed.

Results

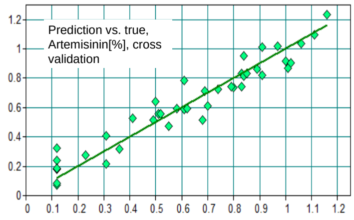
Prediction model 1 using all values

Spectral range	Rank	RPD	RMSECV	R^2	Preprocessing
1754.9 - 943	3	3.62	0.085	92.37	second derivation
1754.9 - 943	4	3.57	0.0862	92.03	second derivation
3018.1 - 2821.4	7	3.37	0.0915	91.16	min/max normalization
3018.1 - 2821.4	6	2.95	0.104	88.53	no preprocessing

RPD = Ratio of standard variation to standard error of prediction, RMSECV = Root mean square error of cross validation, Rank = Factors of partial least square model



Suggested rank: 3, R^2=92.37, RMSECV=0.085

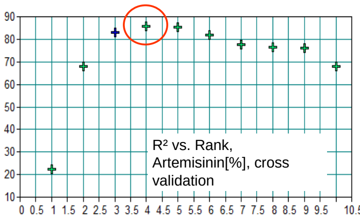


Rank: 3, R^2=92.37, RMSECV=0.085, BIAS: -0.00353, RPD: 3.62

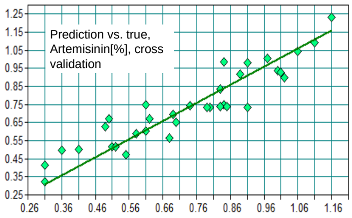
Prediction model 2 without low values

Spectral range	Rank	RPD	RMSECV	R^2	Preprocessing
3016.2 - 2815.6	8	2.7	0.085	86.23	min/max normalization
3021.9 - 2815.6	6	2.65	0.0866	85.72	MSC
1754.9 - 943	4	2.63	0.087	85.57	second derivation
1754.9 - 943	3	2.43	0.0945	82.96	second derivation
3550.3 - 3212.9	6	1.18	0.194	28.37	straight line subtraction

RPD = Ratio of standard variation to standard error of prediction, RMSECV = Root mean square error of cross validation, Rank = Factors of partial least square model, MSC = Mulitplicative scatter correction



Suggested rank: 3, R^2=82.96, RMSECV=0.0945



Rank: 4, R^2=85.57, RMSECV=0.087, BIAS: -0.00254, RPD: 2.63

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