Quality Control Protocol for German Chamomile Authentication and Discrimination from Related Toxic Adulterants Using Near-Infrared Spectroscopy Coupled to Chemometrics. (PG-05)

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Introduction

German Chamomile is known to be one of the most widely used plants in the growing market of herbal drugs due to its vast range of medicinal and therapeutic properties. The high demand on the plant raw material makes it prone to deliberate as well as unintentional adulteration by other morphologically-resembling flowers. Adulteration by potentially toxic plant-based adulterants imposes a great risk considering that German chamomile is widely incorporated in infants' preparations.

Materials and Methods

- **Plant materials and sample preparation**

Samples from German chamomile (*Matricaria recutita* L.) and its proposed toxic adulterants; *Anthemis cotula* L., *Senecio desfontainei* Druce and *Senecio vulgaris* L. were prepared in addition to, their deliberately adulterated mixtures of German chamomile.

- **NIR spectroscopy measurement, data acquisition and Spectral data pre-treatment**

Samples were scanned three times using NIR and the spectra were mean centered. Weighted multiple scatter correction (WMSC) achieving scatter and baseline drift correction were performed using OPUS software.

Acquired raw NIR spectral data were trimmed and subjected to pre-processing based on1st order derivative transformation improving overlapped peaks and enhance their resolution, in addition to standard normal variate (SNV) transformation to eliminate errors that may arise between samples during NIR measurements. (Figure 1)

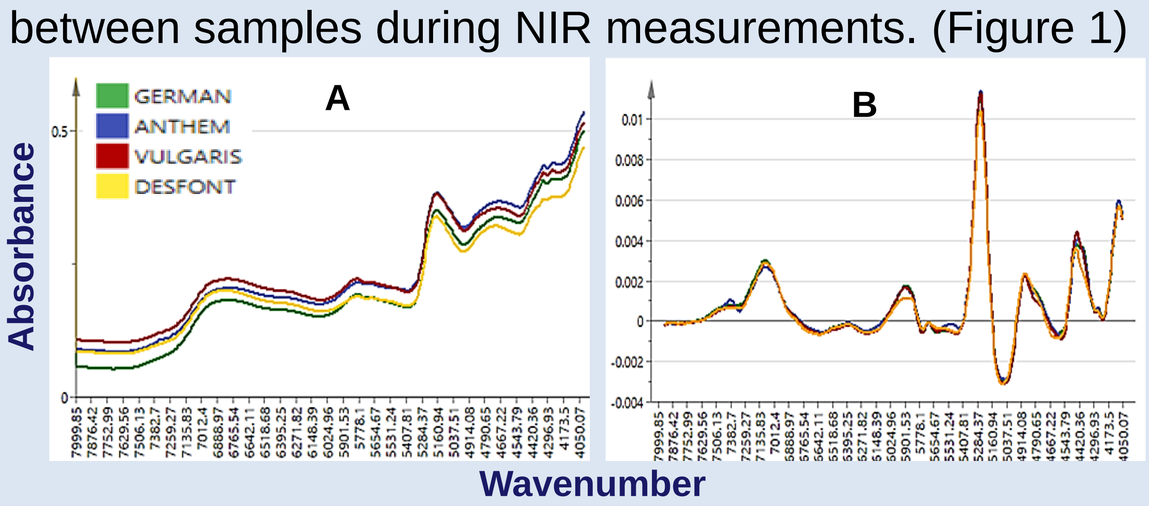

Figure 1: Overlay of (A) raw NIR spectra and (B) 1st derivative and SNV transformed NIR spectra of representative samples covering the range of 4000-8000 cm^{-1}

- **Multivariate statistical analysis**

The pre-processed spectra were then utilized for constructing different statistical models; soft independent modelling of class analogy (SIMCA), Orthogonal Projections to Latent Structures – Discriminant Analysis (OPLS-DA), Partial least squares regression (PLSR) as illustrated in (Figure 2).

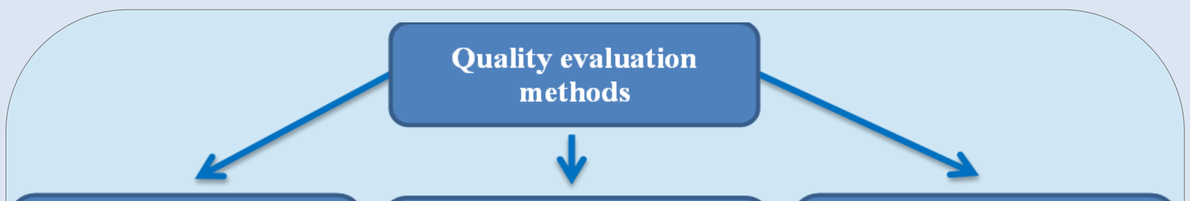

Figure 2: NIR spectroscopy combined with multivariate data analysis workflow

Results

SIMCA is a supervised pattern recognition method constructed based on similarities among samples for their classification to predefined classes. It was applied successfully for the authentication of both genuine German chamomile samples and its toxic adulterants without any misclassification (Figure 3A) with perfect specificity & sensitivity, confirmed via Cooman's plots. However, it fell short in discrimination of adulterated samples with < 2% adulteration levels. This problem was successfully resolved using OPLS-DA models based on the mutual differences among samples, which showed excellent specificity. (Figure 3B & 4B)

Also, **OPLS-DA** model revealed a great discriminatory power for German chamomile and its adulterants (Figure 4A), validated via permutation plots.

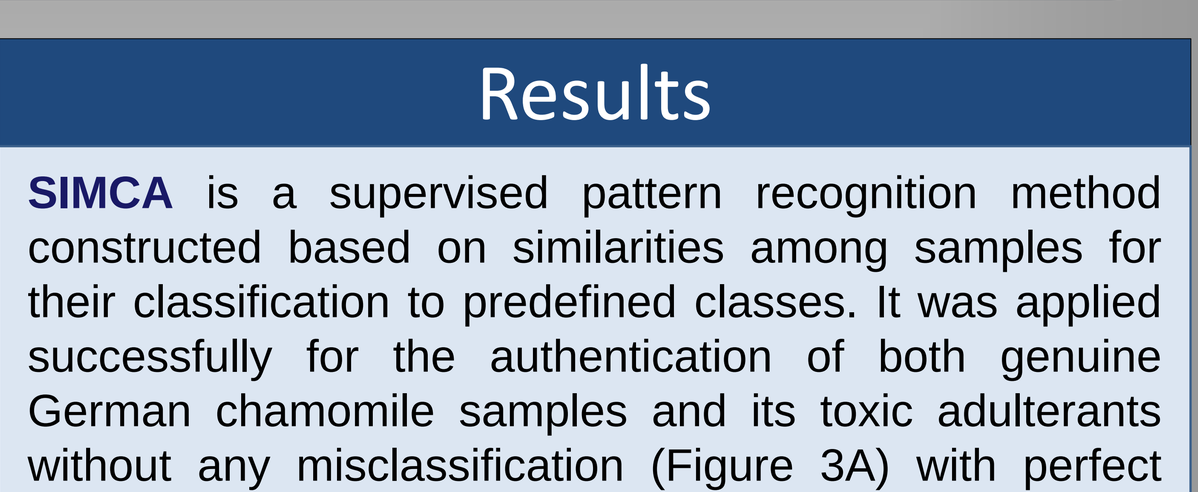

Figure 3: SIMCA score scatter plot for model comprising (A) genuine German samples and its proposed toxic adulterants, (B) unadulterated German samples and adulterated binary mixtures.

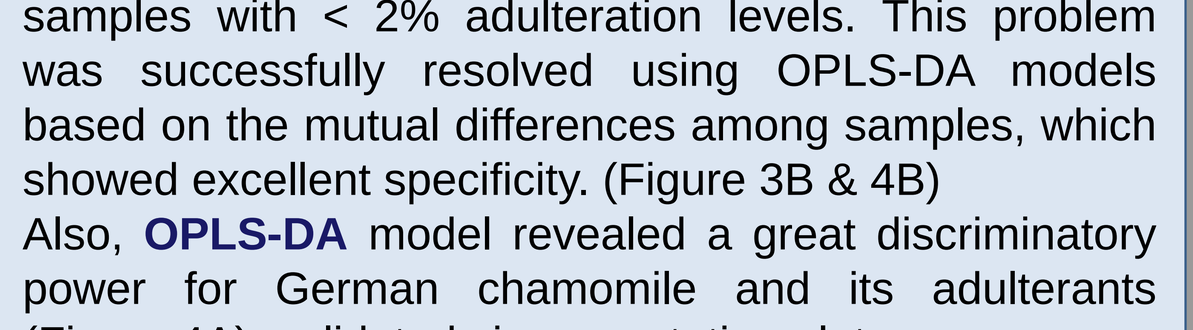

Figure 4: OPLS-DA score scatter plot of model comprising (A) genuine German samples and its proposed toxic adulterants, (B) unadulterated German samples and adulterated binary mixtures.

In addition, **PLSR** models were constructed for quantitation of each of the proposed toxic adulterants individually covering the concentration range of 0.5-50%. The constructed PLSR models were improved clearly after **variable selection** via omitting non-informative spectral variability arising from noise, baseline variation and chemical and physical interferents, and reflected in enhanced values of R_2, RMSEC, RMSECV, RMSEP and permutation intercepts.

Conclusions

- NIR combined to chemometrics was implemented **for the first time** to develop a fast and reliable analytical tool for authentication and detection of common toxic adulterants in the powdered material of German chamomile (*M. recutita* L.) flowers.
- The constructed SIMCA and OPLS-DA models were beneficial in unravelling authentication and discrimination problems.
- Meanwhile the constructed PLS regression models for deliberately adulterated samples exhibited limit of detection and quantitation (LOD & LOQ) below those reported to be toxic levels for the given adulterants.
- The designed chemometric tools guarantee the authenticity and purity of the studied samples with minimal sample pre-treatment. Consequently, the proposed methods displayed their potential for replacing other time- and solvent-consuming analytical methods.

References

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2. Quintelas, C., Mesquita, D. P., Ferreira, E. C., & Amaral, A. L. (2019). Quantification of pharmaceutical compounds in wastewater samples by near infrared spectroscopy (NIR). *Talanta*, *194,* 507–513. https://doi.org/10.1016/j.talanta.2018.10.0761.