Non-destructive prediction of quality parameters in chamomile flowers using near infrared spectroscopy

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Introduction

Chamomile (*Chamomilla recutita* L.) is one of the most important medicinal plants worldwide; the annual consumption of chamomile flowers in Germany alone is more than 4000 tonnes and the area under cultivation is estimated at 890 ha in 2001. Preparations of the drug find mainly uses in pharmaceutical, food and cosmetic industries but also other uses, such as for insect repellents, biological control and dyeing are known. Consumers' demand for high-quality chamomile products has stimulated breeding activities especially with the aim of increasing the essential oil and the bisabolol content in the drug. Chamomile oil is obtained by hydro-distillation of the air-dried flowers and stalks of the plant. The deep blue oil has a strong, characteristic odour and a bitter-aromatic taste. Responsible for the blue colour is chamazulene which is built from matricin during the distillation process. It is well known that several chamomile chemotypes can be distinguished based on the individual composition of their essential oils.[1,2] In this context the chemotypes are usually called according to the dominating bisaboloid substance in the oil fraction; it has been found that the natural occurrence of the individual chemotypes differs with respect to the geographical location (*bisabolol type*: Nord-East of Spain, *bisabolol oxide A type*: Egypt as well as Central, South and East Europe, *bisabolon oxide A type*: Albania and Turkey, *bisabolol oxide B type*: South America).

Intensive breeding experiments with chamomile performed in Germany resulted in several well-known diploid and tetraploid cultivars (for example, 'Degumille', 'Manzana', 'Bodegold'). Today, cultivation of tetraploids is preferred in Europe because they produce higher amounts of essential oil and they contain mostly high bisabolol and low bisabolol oxide contents in the volatile fraction. Furthermore, natural crossings between tetraploid cultivars and wild plants can be avoided. Nevertheless, the cultivars mentioned above segregate building partly chamomile progenies with low bisabolol content. Therefore continuous evaluation and selection of single-plants is necessary to maintain the high quality standard of the drug.

Because usual GC and HPLC methods to determine the contents of the above mentioned secondary substances in chamomile flowers are very time-consuming and expensive,[3–5] a new near infrared (NIR) method was developed for that purpose.

Sample material and reference material

The chamomile samples were cultivated in the experimental garden of the Federal Centre for Breeding Research on Cultivated Plants (BAZ) in Quedlinburg (Germany). The plant material was harvested at the beginning of the flowering period of 2000 and 2001. The drug was hydro-distilled according to the standard method described in the European Pharmacopoeia.[6] The isolated essential oils were analysed by GC/FID using a Hewlett-Packard chromatograph 6890 series, fitted with a HP-5, 50 m × 0.32 mm fused silica column (film thickness: 0.52 µm). Detector and injector

temperatures were set at 280°C and 250°C, respectively. The oven temperature was programmed from 100°C to 220°C at 8°C/min. Carrier gas was nitrogen at a constant flow of 1 mL min^{-1} (split 1:40). GC-MS analyses of the isolated essential oils were performed using a Hewlett Packard MSD 5972/HP 5890 series plus 2, equipped with a 15 m × 0.25 mm Permabond OV-1-DF fused silica column (film thickness: 0.25 μm). The ionisation energy was set at 70 eV. Pure standard substances (α-bisabolol, chamazulene, matricin) were purchased from Roth (Karlsruhe, Germany), Sigma–Aldrich (Taufkirchen, Germany) and Phytolab (Vestenbergsgreuth, Germany), respectively. The other analytes were tentatively identified by using the NBS75K and Wiley 138 library data bases of the GC-MS system. The percentage composition was computed from the GC peak areas without using any correction factors.

For determination of the individual matricin content approx. 200 mg of the air-dried and homogenised chamomile flowers were extracted with 25 mL acetonitrile/dichloromethane (2:1, v/v), centrifuged and subsequently filled up with the eluent to 25.0 mL. The resulting extract was filtered through a micro filter (0.45 μm) and, finally, an aliquot of 3 μL was used for the subsequent HPLC analysis (HP-System 1100 (Agilent, Waldbronn, Germany) with DAD (λ=244 nm). The separation was performed using a 3.5 μm Zorbax Eclipse XDB-C18 (150 x 3.0 mm) reversed phase column kept at 35°C. The eluent consisted of water and methanol (35:65, v/v); the flow rate was 0.5 mL min^{-1} (isocratic). For quantification of matricin the external standard method was applied using a pure standard substance (purity > 99%).

NIR measurements and chemometrics

The chamomile flowers were measured with a dispersive NIR spectrometer (NIRSystems 5000, Foss Instruments Inc., Hamburg, Germany). Development of appropriate chemometric methods was carried out with the commercial statistic programme WINISI (Infrasoft Intern. Inc., Port Matilda, USA). A partial least square (PLS) algorithm was applied with an optimum number of PLS factors covering the whole wavelength range (1100–2500 nm). The calibration accuracy was described by the multiple coefficient of determination (R^2), the standard error of calibration and the overall error between modelled and reference values (standard error of cross-validation, *SECV*). All data in the calibration set were checked carefully to detect and eliminate outlier samples. The optimum number of PLS factors for each component was determined applying the predictive residual error sum of squares (*PRESS*) calibration.

Results

Based on all chromatographic and spectral data obtained, a PLS algorithm was applied to develop a chemometric equation from 175 single plants for each quality parameter (Table 1). Whereas calibrations of fresh chamomile samples lack in prediction quality, the air-dried and homogenised samples present very good chemometric results (GC volatiles: R^2 = 0.99, *SECV* = 0.06; bisabolol: R^2 = 0.94, *SECV* = 0.04; matricin: R^2 = 0.93, *SECV* = 0.04). This is related to the fact that the concentration of the analyte increases significantly during the drying process. Furthermore, homogenisation of the sample causes more reliable As shown in Figure 1 the spectrum of fresh chamomile flowers is dominated by strong water bands occurring at 1430 and 1940 nm; in the NIR spectrum obtained from dried chamomile these bands have considerably less intensity. Furthermore the characteristic absorptions of bisabolol and other terpenoids can be observed between 2300 and 2380 nm. According to earlier measurements performed on essential oils these bands are mainly characterised by overtones or different combinations of CH-stretching and bending vibrations[7,8] (for example; 3x ν(CH), 2x ν(CH) + δ(CH), 2x ν(CH), ν(CH) + δ(CH), 3x δ(CH), ν(CH) + ν(CC)).

Table 1. NIRS calibrations performed at fresh, air-dried and powdered chamomile flowers (*results expressed in mg/100 g drug).

	fresh				
	sample number	calibration range*	SEC*	R^2	SECV*
bisabolol	340	0.02- 0.68	0.071	0.573	0.091
matricin	328	1.81 – 8.11	0.551	0.652	0.697
GC volatiles	334	0.60 – 2.32	0.132	0.861	0.151
	air-dried				
bisabolol	347	0.02- 0.68	0.036	0.902	0.053
matricin	338	1.81 – 8.11	0.380	0.852	0.517
GC volatiles	343	0.60 – 2.32	0.099	0.924	0.126
	air-dried and powdered				
bisabolol	346	0.02- 0.68	0.027	0.942	0.042
matricin	329	1.81 – 8.11	0.292	0.929	0.397
GC volatiles	340	0.60 – 2.32	0.040	0.988	0.059

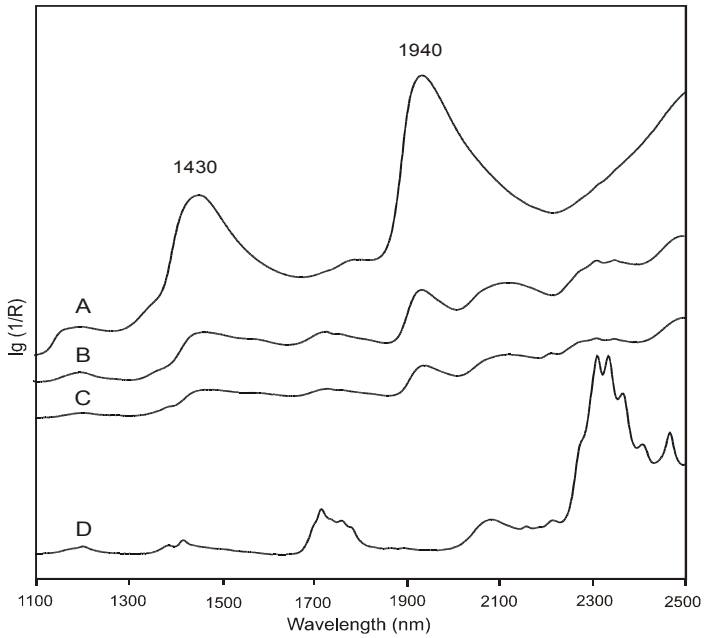

Figure 1. NIR spectra of fresh (A), air-dried (B) and powdered chamomile flowers (C) as well as a pure bisabolol standard (D).

Figure 2 demonstrates exemplarily the NIRS calibration for the total amount of GC volatile substances of dried and powdered chamomile flowers.

It has been found that also other chamomile components such as bisabolol oxide A and B, *cis*- and *trans*-spiroether as well as farnesene can be reliably predicted by NIRS. The newly developed NIRS methods can be successfully applied not only during breeding (selection of suitable single-plants

with high essential oil and bisabolol content) but also for efficient quality control purposes in the phytopharmaceutical industry.

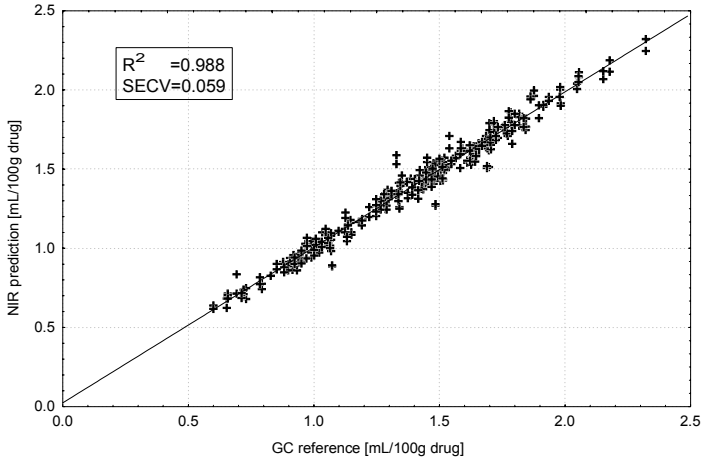

Figure 2. Reference values vs NIR prediction of the total amount of GC volatile substances occurring in powdered chamomile drug (*N* = 175).

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