Determination of valuable alkaloids in poppy capsules by near infrared reflectance spectroscopy

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Introduction

In Germany the goal of poppy (*Papaver somniferum* L.) breeding and cultivation is, besides the extraction of alkaloids for pharmaceutical products, the use of poppy oil and seeds in food. Especially in connection with biofarming, in the near future poppy products will get a special importance. At present, the cultivation of poppy is limited by restrictions of the German Federal Health Agency which fixed the maximal morphine contents in *Papaver somniferum* at 0.01 g/100 g in the dry capsule. Therefore, since 1992 some activities have been started to generate poppy forms with low morphine contents succeeding the development of three new low-morphine poppy lines.[1] Furthermore, research activities were focused on the development of genetically characterised poppy lines varying with regard to their alkaloid spectrum and alkaloid content.[2,3] Because usual analysis methods to determine the individual alkaloids are very time-consuming and expensive, a near-infrared reflectance spectroscopic method for the simultaneous prediction of morphine, codeine, papaverine, thebaine and noscapine in ripe capsules of poppy (*Papaver somniferum* L.) was developed.

Sample material and reference analysis

Five different poppy varieties ('Cosmos', 'Riesenmohn', 'Quedlinburger', 'Soma', 'Przemko') as well as two F_2 populations obtained from crosses between low-morphine and high-morphine poppy plants were cultivated in the experimental garden of the Federal Centre for Breeding Research on Cultivated Plants (BAZ) in Quedlinburg (Germany). The dry capsule of each plant was hand-harvested, removed from the stigmatic tissue, seeds and stems and powdered by means of a grinding mill.

The isolation of alkaloids from the poppy capsules was performed according to a procedure described earlier.[4] HPLC separation of the alkaloids was carried out on an Agilent 1100 system using a Luna 5μ C18 reversed phase column (250 × 4.6 mm i.d., Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of water (containing 0.1% triethylamine) and methanol. Flow rate was 1 mL min^{-1}; UV-detection was set at 283 nm. Morphine pentahydrate, codeine base, papaverine hydrochloride, thebaine base and noscapine hydrochloride (Sigma–Aldrich, St. Louis, USA) were used as standard substances. For quantification of the individual alkaloids the external standard method was applied.

Near infrared measurements and chemometrics

A set of 100 single plant samples were measured on a dispersive near infrared spectrometer (NIRSystem 5000, Foss Instruments Inc., Hamburg, Germany). The powdered poppy capsules were transferred into a rectangular cup (51 x 64 mm, 11 mm depth) and measured twice with 32 scans each time. Development of appropriate chemometric methods was carried out with the commercial statistic programme GRAMS (Galactic Ind., Salem, USA). A partial least square (PLS) algorithm was used with an optimum number of PLS factors. The calibration accuracy was described by the multiple coefficient of determination (R^2) and the overall error between modelled and reference HPLC values (standard error of cross-validation, *SECV*). All data in the calibration set were checked carefully to detect and eliminate outlier samples.
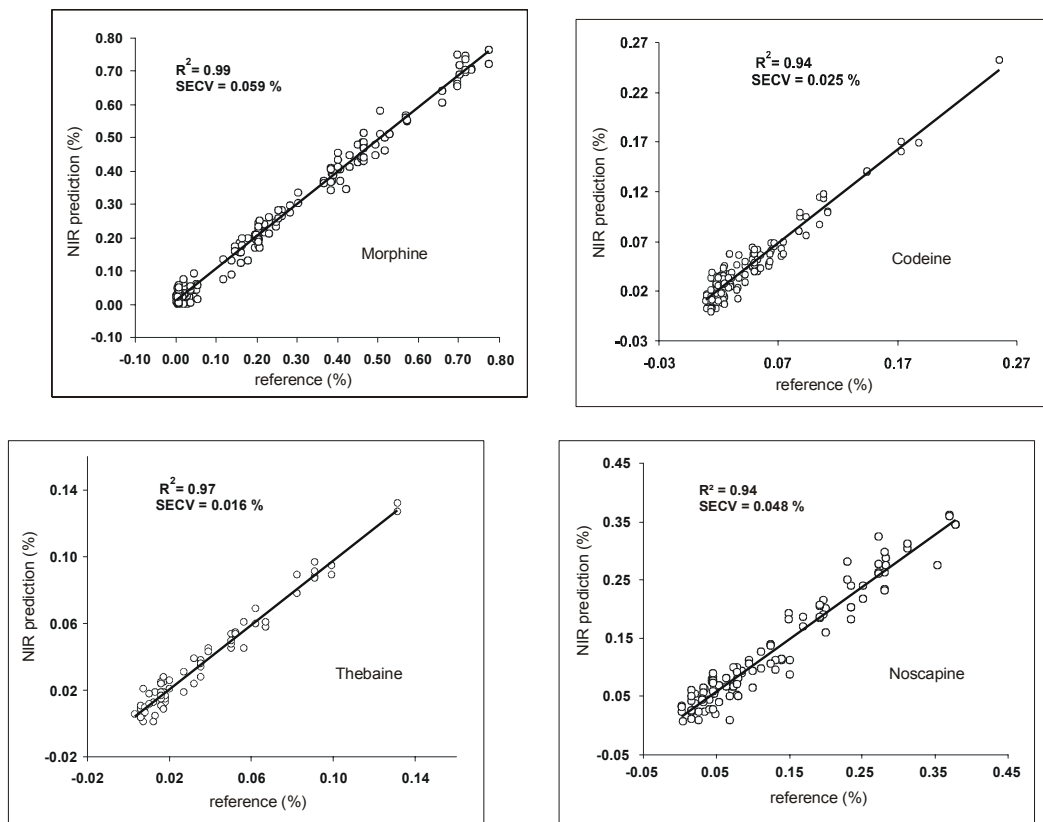
Results

Generally, for all alkaloids analysed in this study (morphine, codeine, papaverine, thebaine and noscapine) the observed values for correlation coefficients between the second derivatives of the Near infrared (NIR) function and the reference HPLC data were > 0.93 (Table 1 and Figure 1).

Table 1. NIR calibration statistics performed at dried homogenised poppy capsules (*results are expressed in % dry matter).

analyte	range*	mean*	R^2	SECV*
morphine	0.001 - 1.108	0.282	0.986	0.059
codeine	0.009 - 0.608	0.084	0.937	0.025
papaverine	0.010 - 0.260	0.048	0.951	0.030
thebaine	0.003 - 0.131	0.037	0.966	0.016
noscapine	0.002 – 0.370	0.050	0.934	0.048

Besides morphine, also the other alkaloids occurring in lower amounts could be determined with acceptable prediction quality. The high correlation quality is mainly related to the fact that overtones and combination bands of specific C–N– fundamental vibrations, caused by the alkaloid substances mentioned, present characteristic absorptions which stand out against the spectral background of the cellulose matrix.

The described method is very useful for the evaluation of poppy breeding material, for quality control of incoming raw materials in the food and pharmaceutical industry (for example, poppy extracts), for rapid on-site control of poppy field-cultivation and in the area of forensic analysis.

Figure 1. Reference values vs NIR predictions of the individual alkaloid content in dry powdered poppy capsules (results are expressed in % dry matter).

Acknowledgements

References

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