**Application of Near-Infrared Spectroscopy and Chemometrics for In-line real-time monitoring during a chlorophyll extraction process**

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**Highlights**

* NIRS and Chemometrics applied to extraction process.
* Highly accurate prediction model. (R2 = 0.967, SEP = 2.823 mg/L;  = 5.04 %.
* The technical feasibility of the methodology was proved successfully.

**1. Introduction**

The applications and the importance of In-line real-time monitoring using Near-Infrared Spectroscopy (NIRS) and Chemometrics, as a Process Analytical Technology (PAT) in the Pharmaceutical and Food Industries, have increased through the years, due to their advantages and the results obtained (Scarff *et al*., 2006). Another interesting application for this technology is the chemical process of extraction. Liquid extraction using organic solvents is a very widespread technique for obtain molecules of industrial interest from biomass, *e.g.*, chlorophyll, that uses as colorant and additive. It is therefore interesting to search new sources of this molecule, one of this sources could be the pine needles, that left behind the forest exploitation, and have no apparent use. For this reason, the application of NIRS and Chemometrics for the real-time monitoring of chlorophyll extraction from pine needles is an alternative to improve the process and generate applicative knowledge.

**2. Methods**

*Chlorophyll extraction process.* Extractions were carried out according to the Lichtenthaler method (2001). Extraction conditions were optimizing with a Box-Behnken design, variables were: temperature (50 ºC), ethanol concentration (95 %) and time (240 min).

*Reference method.* Analyses were performed on an Agilent 1,100 liquid chromatographic system equipped with a diode-array UV/ vis detector. A 250 mm x 4.6 mm i.d., 5 μm particle size Inertsil ODS 2 column (Sugelabor) was used. Elution was performed at a flow rate of 1.0 mL/min at room temperature, using as the mobile phase a mixture (8:2 v/v) of methanol/water containing 0.025 % ammonium acetate and 0.05 % triethylamine as phase A and methanol/acetone (1:1 v/v) as phase B. Concentration is expressed in milligrams of chlorophyll by liter of extract.

*NIRS measurements.* Spectra were acquired with a Near-Infrared Spectrophotometer XDS Process Analytics (Foss- NIRSystems, Silver Spring, USA) using an in-situ fiber optic transflection probe, 3 mm pathlength. The samples were scanned in duplicate over the whole NIR range (800–2,200 nm) every 20 min until the kinetic extraction was ended. The spectra were then averaged and derived (second derivative) in order to reduce the signal/noise ratio (Corro-Herrera *et al*., 2016).

*Model development and validation.* Linear models were constructed using Partial Least Squares Regression (PLSR 1). Ten extraction batches were used to collect the samples spectra. Development of all PLS models was carried out by Vision software (v 3.5, Foss-NIRSystems, Sylver Spring, USA). To avoid overfitting due to many factors or latent variables (LVs), the software predicts residual error sum of squares (PRESS) calculated from random subsets cross validation; a lower PRESS value indicates an adequate number of factors in the model. To assess the prediction capacity of the models, the coefficient of determination (R2), standard error of calibration (SEC), standard error of prediction (SEP) and error (%) were used as quality parameters.

**3. Results and discussion**

Model quality parameters for calibration are R2 = 0.974, SEC = 2.453 mg/L and for validation R2 = 0.967, SEP = 2,823 mg/L;  = 5.04 %. LVs = 4 (Figure 1a and 1b, respectively). Considering the SEC and SEP as first quality parameter, they are consistent according to the same magnitude order of the value. For R2 of both, the values are close to the unit, showing the good correlation between the data and for validation.



Figure 1. a) Calibration curve and b) Validation curve: (⚫) laboratory reference, (⭘) NIRS prediction. c) Real-time monitoring of chlorophyll concentration during the extraction, using the prediction model. (⚫) Experimental data, (—) prediction of the model.

Once the model was validated, it was now used to the real-time monitoring during the extraction process. 10 batches were carried out for collect the data shows in the Figure 1c. Fitting of the model is quite good with a R2 =0.987. Chemometric model has the sufficient robustness to predict the concentration of chlorophyll throughout the process. Furthermore, this model fits to the variability of the process, and in addition to expresses the process kinetic behavior.

**4. Conclusions**

The technical feasibility of real-time monitoring through an immersion transflection probe, of chlorophyll extraction matrix, employing NIRS and Chemometrics, has been demonstrated. This assertion is based on the generation of a functional and robust prediction model for chlorophyll concentration, with R2 values close to 1 and low SEC and SEP.

**References**

1. M. Scarff, S.A. Arnold, L.M. Harvey, B. McNeil, Crit. Rev. Biotechnol. 26 (2006) 17-39.
2. H. K. Lichtenthaler, C. Buschmann, Current Protocols in Food Analytical Chemistry. (2001) F4.3.1-F4.3.8.
3. V. A. Corro-Herrera, J. Gómez-Rodríguez, P.M. Hayward-Jones, D.M. Barradas-Dermitz, M.G. Aguilar-Uscanga, Biotechnol. Prog. 32 (2016) 510-517.