Agricultural Research & Technology
Open Access Journal
ISSN: 2471-6774

Juniper Publishers
key to the Researchers

Research Article
Volume 25 Issue 2 - October 2020
DOI: 10.19080/ARTOAJ.2020.25.556296

Agri Res & Tech: Open Access J

FT-NIRs assisted Machine and Deep learning for Determination of Acteosides, Aucubin and Catalpol Contents of *Plantago lanceolate*

Cedric Camps[1] and Xavier Simonnet[2]

[1]*Agroscope, Institute for Plant Production Sciences IPS, Switzerland*

[2]*Mediplant, Swiss Research Center on Medicinal and Aromatic Plants, CH-1964 Conthey, Switzerland*

Submission: September 30, 2020; **Published:** October 05, 2020

***Corresponding author:** Cédric Camps, Agroscope, Institute for Plant Production Sciences IPS, Route des Eterpys 18, 1964 Conthey, Switzerland

Abstract

Plantago lanceolate is important for animal feed as it improves the nutritional value of forages. Aucubin, acteosids and catalpol are important compunds present in this plant but their determination is time consuming, costly and needs chemicals. In the present study we develop a method based on FT-NIR spectroscopy to determine aucubin, acteosides and catalpol from dry powders of plantago lanceolate. FT-NIR spectra were processed in PLS- and deep learning methods and the accuracies of models have been calculated and compared. Aucubine, acteosides and catalpol contents have been predicted with a root mean square error of 0.56, 0.25 and 0.18%, respectively by using PLS method. Deep learning did not allowed to improve the accuracies but allowed obtaining similar accuracies. FT-NIR based method showed interesting results and is promising to develop a fully usable method for selection programs of plantain with high level contents of biomolecules.

Keywords: Plantago Lanceolate, FT-NIR Spectroscopy, PLS, Deep Learning, Aucubin, Acteosids, Catalpol

Introduction

Plantago lanceolate is known for its medicinal properties on animals such as bovids and ovids [1]. As animal feed, it also has effects on the quality of the meat supplied as well as on the development of organs and the carcass of animals [2]. Among medicinal properties, the inhibition of Trichostrongylus colubriformis larvaes development in bovids and ovids is important for farmers to the economic maintenance of their animal breeding farm. Medicinal properties of Plantago lanceolate are commonly attributed to molecules present in the plant leaves and stems: Aucubin, acteoside and catalpol. Navarrete, Kemp, Pain and Back [3] showed that Plantago lanceolate with natural concentrations of bioactives (aucubine) allowed decreasing NH3-gas production due to fermentation compared to the control. The level of bioactive contents in Plantago lanceolate is genetically determined but it can affect by various environmental or agronomic factors such the plant density or relative humidity [4,5]. Such bioactive components are currently quantified by expensive analytical methods needing chemicals, time, high competences and know-how. High performance liquid chromatography (HPLC) is often used to determine series of sample for bioactive contents in the framework of variety selection programs or to control new varieties [6]. Variety selection programs need a rapid and low-cost method to determine bioactive contents of series of thousands samples. FT-NIRs (Fourier-transform Near-Infrared-Spectroscopy) supported by chemometric analysis could be an asset to reduce the time and cost of such analyses. Such a method as been successfully develop to quantify bioactive compounds in other plant species [7,8]. Thus, the present paper presents the possibility of measuring bioactive contents of different varieties of Plantago lanceolate by FT-NIRs and chemometrics.

Material and Methods

Plant material

Five varieties from different origins of *Plantago lanceolate* have been used in the present experiment (Table 1). Among these varieties, two were from Germany, one from new-Zealand, one from Canada and one from Switzerland.

Table 1: Varieties of Plantago lanceolate and their origins.

Code	Variety	Country	Supplier
PL1	Noflor	CH	Mediseeds
PL2	UnKnown	CA	Richters
PL3	Libor	DE	Pharmasaat
PL4	Arterner	DE	Pharmasaat
PL5	KRC7877	NZ	PGG Wrightson seeds

HPTLC analyses

Extraction: 500mg of a dried and crushed sample leaves are taken and added with 45mL of a water-ethanol solution (50-50). The mixture is homogenized with a vortex for 10s, placed in an ultrasonic bath for 10min at 60**°C** and finally homogenized again using a vortex for 10s. The homogenate is filtered and the filtrate is collected in a 50mL flask using 5ml of the water-ethanol solution (50-50). The flask is completed to 50ml with the water-ethanol solution (50-50). 1mL is taken from this solution for HPTLC analysis.

Phenylpropanoids: Standards of actoside, aucubine and catalpol are prepared at a concentration of 1 mg / ml. A range of 5 volumes of each standard solution (12**µ**L, 9**µ**L, 7**µ**L, 4**µ**L, 2**µ**L) is deposited for HPTLC analysis. For the analysis of the actosides the migration solution used is composed of ethyl acetate (25mL), ultrapure water (6.75mL), formic acid (2.75mL) and acetic acid (2.75mL). An aliquot of 10mL of the sample is analyzed. The silica plate is scanned at 330 nm for determination of acteoside concentration.

Iridoïds: For quantitation of aucubin and catalpol, the migration solution is composed of chloroform (35mL), methanol (20mL) and 5mL of a solution of ammonia (1mL of trifluoroacetic acid in 50mL of ammonia at 25%). A developing reagent is used (10% sulfuric acid in methanol) in which the silica plate is dipped at a speed of 5m / sec for 1s. The plate is then heated at 110 **°C** for 3 min and then scanned at 450 nm for the estimation of concentrations of aucubine and catalpol.

FT-NIR spectroscopy

Chemometric

Partial least square regression: Spectra were gathered in a matrix Xn,p where n is the number of spectra and p the number of wavenumber steps. The reference-values (Aucubine, Acteoside and Catalpol) were gathered in column vectors yn,1. Samples were separated in a calibration set and a validation set. The accuracy and goodness of models has been evaluated according to several indicators: the coefficient of determination (R2), root mean square errors (RMSE), the ratio ratio of prediction to deviation (RPD) [9] and the range error ratio (RER). The values of the beta-coefficient of the first latent variable will be plotted to evaluate the weight of wavelength absorbance of each model. All data analyses were performed with OPUS software and Matlab R2019b (The MathWorks, Inc., Natick, MA, USA).

Deep learning

Deep learning approach has been performed using the "nntraintool" of Matlab R2019b® (The MathWorks, Inc., Natick, MA, USA). To predict the values of chemicals, the trainlm algorithm which is a network training function that updates weight and bias values according to Levenberg-Marquardt optimization has been computed. Then, data were divided into a calibration data set and a validation data set for each chemical compound to be predict (Table 2). The results will be summarize on the basis of correlation values of calibration and validation steps (Rcal and Rval), the root mean square error of calibration and validation (RMSEC and RMSECV), the ratio performance to deviation (RPD), the ratio range to deviation (RER), the overall presentation of spectral data, the plot of actual versus predicted values of the chemical contents and the plot of residuals regardless of the standard deviation of the chemical contents values.

Table 2: PLS-values of Aucubine, Acteoside and Catalpol content.

PLS Parameters		Aucubine	Acteoside	Catalpol
n	CAL/VAL	192/48	193/55	155/25
Wavenumber ranges (nm)	CAL/VAL	1332-1836	1332-1836 2172-2357	1332-2175
LV	CAL/VAL	11	5	15
Corr. Coef. (R)	CAL/VAL	0.88/0.88	0.73/0.77	0.80/0.65
RMSE	CAL/VAL	0.54/0.56	0.26/0.25	0.19/0.18
RPD	CAL/VAL	2.08/2.15	1.47/1.61	1.41/1.51
RER	CAL/VAL	9.11/9.16	6.85/7.32	8.05/6.50
Offset	CAL/VAL	0.55/0.75	0.63/0.67	0.30/0.35

How to cite this article: Cedric C, Xavier S. FT-NIRs assisted Machine and Deep learning for Determination of Acteosides, Aucubin and Catalpol Contents of Plantago lanceolate. Agri Res & Tech: Open Access J. 2020; 25 (4): 556296. DOI: 10.19080/ARTOAJ.2020.25.556296

slope	CAL/VAL	0.78/0.69	0.53/0.53	0.55/0.47
Pre-treatment of spectra		MSC	D1(17) + SS	D1(17)
Content (% FW)	CAL/VAL	[0.6 - 5.52] / [0.4 - 5.53]	[0.66 - 2.44] / [0.88 - 2.71]	[0.08 - 1.61] / [0.28 - 1.45]

CAL: calibration, VAL: validation, n: number of spectra samples, LV: the number of latent variables, R: correlation coefficient, RMSE: root mean square error, RPD: ratio performance to deviation, RER: range error ratio. MSC: multi-scatter corrections, SS: straight line subtraction, D1(n): first derivative with a stepwise of 17.

Results and discussion

Chemical composition

Chemical contents of the five varieties have been measured for aucubin, acteoside and catalpol compunds (Figure 1). Aucubin content is from less than 1% to more than 5% for the first and the fifth varieties, respectively. The contents of the other three varieties being intermediate to the two previously mentioned. Values of acteoside contents are ranged from 0.5% to 2.6% depending on the varieties with highest values for the variety 5. Finally, catalpol contents are generally very low, under 1% and the levels are quite similar for all varieties.

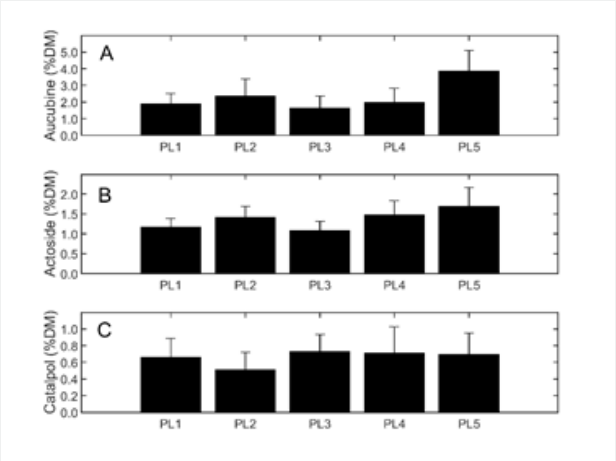

Figure 1: Aucubine (A), Acteoside (B) and Catalpol (C) content of the 5 Plantago lanceolate varieties (PL1-5).

PLS prediction of chemical composition

Partial least square regressions (PLS) have been carried out to attempt to model the prediction of aucubine, acteoside and catalpol contents par FT-NIR spectroscopy. The table 2 gathers the statistics of the models. Aucubine and Acteosides contents were the most accurately predicted with R-values of 0.88 and 0.77, respectively. The accuracies of these two models were around 0.56 %DW and 0.25%DW, respectively. Such results are visually confirmed by the plot of actual vs predicted values (figure 2A and 2B). The models were built on the wavelength region 1332-1836nm for both models plus 2175-2357nm for Acteoside model. Loading-values of both models have been plotted on figure 3. Prediction models of aucubine and acteoside rely on 2 common absorption bands in the vicinity of 1420nm and 1660-1680nm. Prediction of catalpol content was less accurate with root mean square error around 0.2%DW and correlation coefficient in validation step of 0.65 (Table 2). As expected, the very low range of catalpol values, sometime less than 0.1% did not allowed to establish a correct prediction. The model rely on one common wavelength absorbance around 1420nm but other parts of the spectra were less relevant in the prediction compared to the two other models (aucubine and acteoside) (Figure 3).

Deep learning

An approach based on deep-learning analyses has been attempted with the aim of improving the results obtained with the partial least square method. In this approach, the data set has also been divided into a calibration and a validation set as previously and the entire spectra have been processed in the analyses. The table 3 shows the values of the models for each chemical content. The accuracy of acteosides and aucubin models remained correct with root mean square error of 0.24%DW and 0.47%DW. RPD-values show correct levels for selection programs but values too low for analytical prediction. RER-values are promising and show that overall range of chemical values is suitable for modelling. The Figure 4 presents the plots issue from the deep-learning analysis for the prediction of aucubin-content. The spectral collection does not present visual abnormal spectra, so spectra observation did not allow to select samples as outlayer. However, the analysis

How to cite this article: Cedric C, Xavier S. FT-NIRs assisted Machine and Deep learning for Determination of Acteosides, Aucubin and Catalpol Contents of Plantago lanceolate. Agri Res & Tech: Open Access J. 2020; 25 (2): 556296. DOI: 10.19080/ARTOAJ.2020.25.556296

of the residues show that several samples go outside the limit values set at +/- 2SD (Figure 4C). On this basis, a few spectra have been downgraded as outlayers. Finally, Figure 4B expresses the predicted values as a function of the measured values. This plot confirms the promising results given by the relatively low values of quadratic error (RMSECV = 0.47). Similar deep learning approach has been conducted for determination of acteosides and catalpol contents and determination of outlayers (plots no shown).

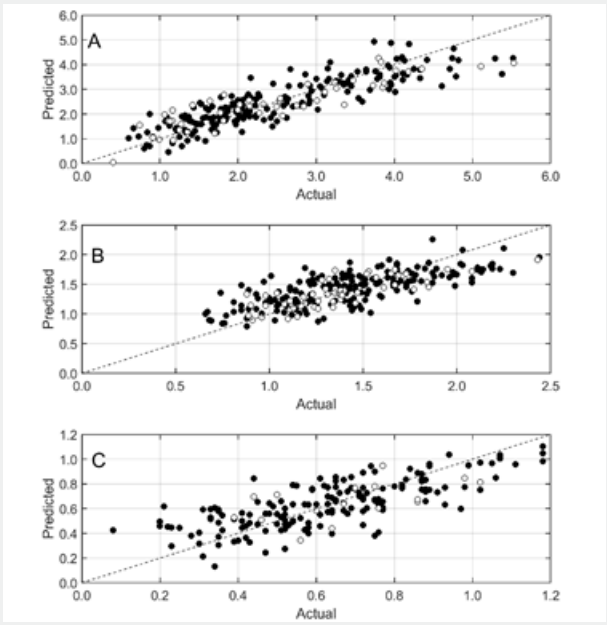

Figure 2: Scatter plot of actual vs PLS-predicted values of chemical contents of plantain leaves. Calibration (•), validation (○). A: Aucubine, B: Acteosids, C: Catalpol. Units are expressed in % of Dry matter.

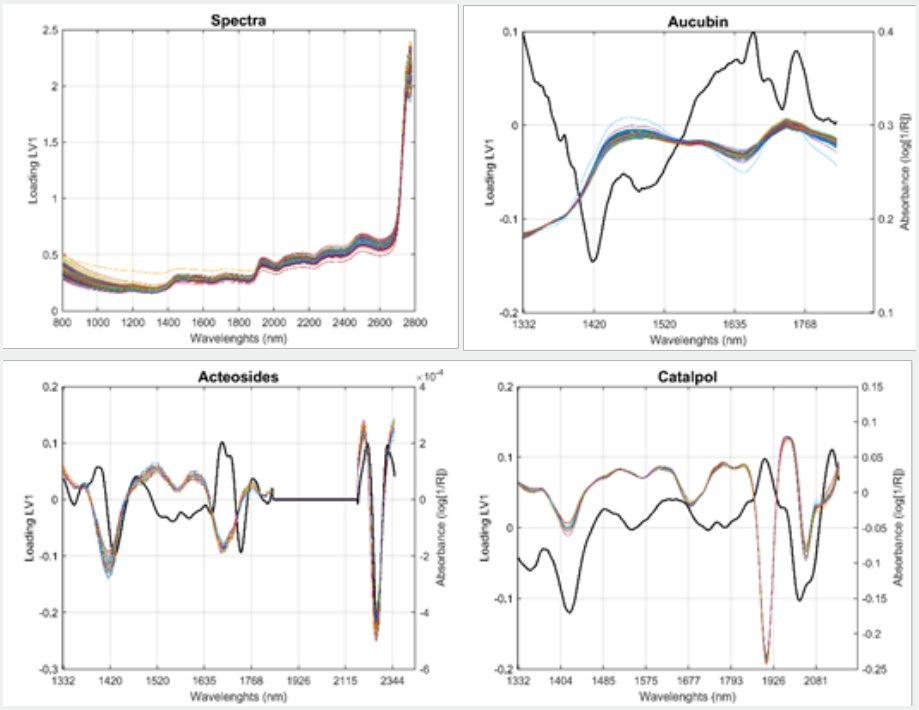

Figure 3: FT-NIR data collection (A), pre-processed FT-NIR spectra (colored lines) and beta-coefficients of the first latent variable (Black line) of the PLS regression models for Acteoside (B), Aucubine (C) and Catalpol (D) predictions

How to cite this article: Cedric C, Xavier S. FT-NIRs assisted Machine and Deep learning for Determination of Acteosides, Aucubin and Catalpol Contents of Plantago lanceolate. Agri Res & Tech: Open Access J. 2020; 25 (4): 556296. DOI: 10.19080/ARTOAJ.2020.25.556296

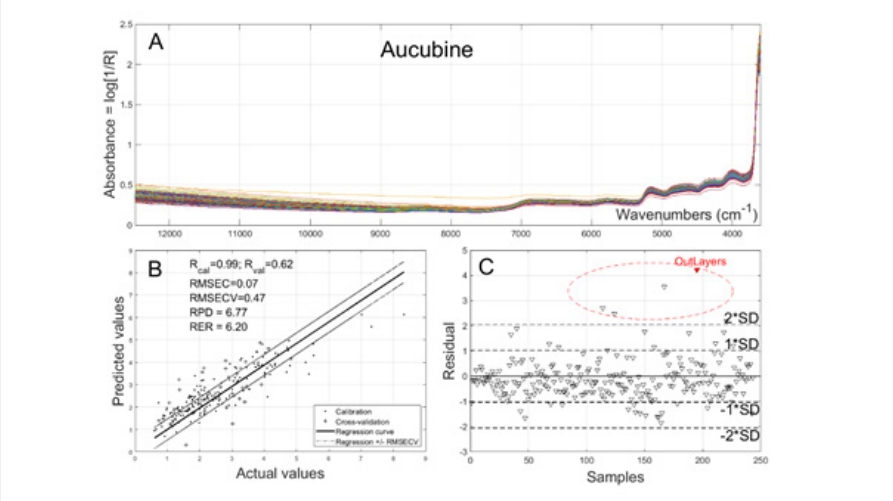

Figure 4: Prediction of Aucubine by Deep learning approach. FT-NIR Spectral data set (A), Regression plot (B) and Residual (C).

Conclusion

The present study aimed at evaluating the possibility to develop a rapid and non-invasive method to determine the aucubin, acteosides and catalpol contents of *Plantago lanceolate*. The interest of this approach is to be able to assay the chemical compounds of several hundred or thousands of samples in a short period of time and at a lower cost. Based on FT-NIR spectra, obtained prediction levels were correct whether with one (PLS) or the other method (Deep learning) of data analysis. Consequently, deep learning approach did not allowed to significantly improve the prediction accuracies. Classic PLS-regression would be the method to be recommended to determine aucubin, acteosids and catalpol contents. In further trials, models will be improved by adding more variability with including more varieties of *plantago lanceolate*.

References

1. W Rumball, RG Keogh, GE Lane, JE Miller, RB Claydon (1997) Grasslands Lancelot' plantain (Plantago lanceolata L.). New Zealand Journal of Agricultural Research 40 : 373-377.
2. TJ Fraser, SM Scott, JS Rowarth (1996) Pasture species effects on carcass and meat quality. Proceedings of the New Zealand Grassland Association 58 : 63-66.
3. S Navarrete, PD Kemp, SJ Pain, PJ Back (2016) Bioactive compounds, aucubin and acteoside, in plantain (Plantago lanceolata L.) and their effect on in vitro rumen fermentation. Animal Feed Science and Technology 222 : 158-167.
4. E Osinska, W Roslon, A Geszprych, A Morawska, L Gontar (2014) Effect of plant density on growth, development and quality of Ribwort Plantain (Plantago Lanceolata L.). Journal of International Scientific Publications: Agriculture and Food 2 : 267-277.
5. S Gonda (2012) Effect of High Relative Humidity on Dried Plantago lanceolata L. Leaves during Long-term Storage: Effects on Chemical Composition, Colour and Microbiological Quality. Phytochemical Analysis 23: 88-93.
6. M Al-Mamun, D ABE, H KOFUJITA, Y TAMURA, H SANO (2008) Comparison of the bioactive components of the ecotypes and cultivars of plantain (Plantago lanceolata L.) herbs. Animal Science Journal 79 : 83-88.
7. C Camps 2014) Prediction of essential oil content of oregano by hand-held and Fourier transform NIR spectroscopy. Journal of the Science of Food and Agriculture 94: 1397-1402.
8. C Camps, M Toussirot, M Quennoz, X Simonnetb (2011) Determination of artemisinin and moisture content of Artemisia annua L. dry powder using a hand-held near infrared spectroscopy device. Journal of Near Infrared Spectroscopy 19 : 191-198.
9. P Williams, D Sobering (1993) Comparison of commercial near infrared transmittance and reflectance instruments for analysis of whole grains and seeds. Journal of Near Infrared Spectroscopy 1: 25-32.

How to cite this article: Cedric C, Xavier S. FT-NIRs assisted Machine and Deep learning for Determination of Acteosides, Aucubin and Catalpol Contents of Plantago lanceolate. Agri Res & Tech: Open Access J. 2020; 25 (2): 556296. DOI: 10.19080/ARTOAJ.2020.25.556296

DOI:10.19080/ARTOAJ.2020.25.556296

How to cite this article: Cedric C, Xavier S. FT-NIRs assisted Machine and Deep learning for Determination of Acteosides, Aucubin and Catalpol Contents of Plantago lanceolate. Agri Res & Tech: Open Access J. 2020; 25 (4): 556296. DOI: 10.19080/ARTOAJ.2020.25.556296