

Brachiaria plantaginea (Link) Hitchc. as a Potential (New) Source of**Shikimic Acid. Quantification by NIR and PLS Regression**

Maria Cristina A. Costa^{*1}(PQ), Marcus B. Matallo²(PQ), Márcia M. C. Ferreira¹(PQ), Sonia C. N. Queiroz³(PQ), Sydnei Dionísio B. de Almeida²(PQ) and Daniel A. de Siqueira Franco²(PQ)

¹Theoretical and Applied Chemometrics Laboratory (LQTA), Institute of Chemistry, University of Campinas-Unicamp, P.O. Box 6154, Campinas-SP, 13084-971, Brazil

²Instituto Biológico, R. Raul Teixeira Penteado, Km 3.5, Jardim das Palmeiras, Campinas - SP, 13092-543 Brazil

³Embrapa Meio Ambiente, Rodovia SP 340, km 127.5, Jaguariúna-SP, 13820-000 Brazil

Keywords: *Brachiaria plantaginea*, Shikimic acid, NIR spectroscopy, PLS regression, chemometric analysis

Abstract

Shikimic acid is a natural compound, precursor of the antiviral *Oseltamivir* used against influenza A. It is a scarce and expensive chemical, obtained mainly from seeds of shrubs natives in China and Japan. In this study we propose a fast and clean procedure for the quantification of shikimic acid in *B. plantaginea*, an alternative source of shikimic acid, using NIR spectroscopy combined with PLS regression.

Introduction

This study shows the results of shikimic acid accumulation in *Brachiaria plantaginea*, abundant grassy in Brazil and other countries in Africa and America, after glyphosate spraying at three different doses. Forty four samples of *B. plantaginea* were analyzed and shikimic acid was quantified by high performance liquid chromatography (HPLC). Although HPLC is quantitative and precise, it can also be time-consuming and costly, requiring large quantities of expensive and toxic organic solvents.¹

The main objectives of this study were investigate the glyphosate dose and exposure period of the *B. plantaginea* to the herbicide that would result in the greatest accumulation of shikimic acid; then, propose a fast and clean procedure for the quantification of shikimic acid in samples of *B. plantaginea* using NIR spectroscopy combined with PLS regression.

Results and Discussion

The herbicide glyphosate was sprayed at reduced doses of 36.0, 3.6 and 0.36 g acid equivalent (a.e.) of glyphosate ha⁻¹. The determination of shikimic acid in *B. plantaginea* leaves was performed at 3, 6, 9 and 12 days after treatments. The results show a higher accumulation of shikimic acid after 6 days of glyphosate application at the concentration of 36.0 g acid equivalent of glyphosate ha⁻¹, which are consistent with the literature.²

Spectra of 44 samples were obtained using diffuse reflectance mode in the range of 4000 to 10000 cm⁻¹ with 4 cm⁻¹ resolution. Different mathematical pretreatments were applied to the spectra, and the

data were mean-centered. The calibration model (7 factors) exhibited coefficient of determination, $R^2 = 0.9930$ ($SEC = 84.05$). For external validation, $R^2 = 0.9317$ and $SEP = 154.91$. For external validation, the mean prediction error was 10% and the range error ratio (RER) was 9.42, indicating that the model is qualified for screening calibration.

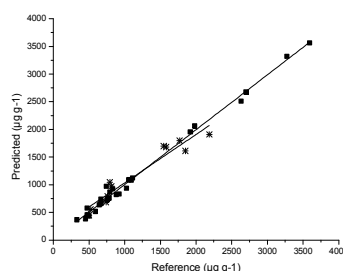


Figure 1. Plot of reference vs. predicted values for (■) calibration and (*) external validation of shikimic acid in *B. plantaginea* samples models (7 factors).

Conclusions

The reasonable agreement between reference vs. predicted values for calibration and external validation sets indicates that the final model can be used for an approximate prediction of new samples. The difficulty in getting a better model can be explained in part by the wide range of shikimic acid concentrations (333.9 to 3592.45 µg g⁻¹). Despite of the difficulty to establish a PLS model for this data set, the results were satisfactory, demonstrating that NIR spectroscopy associated to PLS regression is a possible alternative to quantify shikimic acid in *B. plantaginea*.

Acknowledgments

MMCF acknowledges the Brazilian Governmental Agency CNPq for financial support. MBM thanks CNPq for funding the project 431927/2013-9.

¹Dong, M. W. *LCGC*, **2013**, *16*, 3.

<http://www.chromatographyonline.com/essence-modern-hplc-advantages-limitations-fundamentals-and-opportunities>

²Martin, E.; Duke, J.; Pelkki, M.; Clausen, E. C.; Carrier, D. J. *Appl. Biochem.* **2010**, *162*, 1660.