Near-infrared spectroscopy: a rapid alternative technique to reducing sugars determination in juice of sugarcane (*Saccharum officinarum* L.)

[Espectroscopia del infrarrojo cercano: una técnica rápida para la determinación de azúcares reductores en jugo de caña (*Saccharum officinarum* L.)]

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Abstract

Context: The work in the field, in the production of sugarcane (*Saccharum officinarum* L.), makes it necessary to have quick and simple methods of detecting different compounds.

Aims: To optimize the analysis of glucose and fructose in juice of sugarcane in field, we studied the possibility the use of Near-infrared spectroscopy (NIR) and their correlation with high-performance liquid chromatography (HPLC) (reference method) to reporting from the laboratory reliable in short time data.

Methods: Sugarcane samples were collected in the field. The extraction of cane juice was done by macerating pieces of sugarcane. The determination of glucose and fructose was carried out by reference method HPLC and by NIR in the transmittance mode. Through chemometrics and principal components analysis, the calibration model was developed.

Results: The statistical parameters of the regression method were obtained as the coefficient of determination of 0.9717 and 0.9965, and smallest calibration error of 0.0681 and 0.0229. The standard prediction error was predicted between 0.136 and 0.08 for glucose and fructose, respectively, using the regression model. The above statistical variables indicated the high correlation between HPLC and NIR.

Conclusions: These results allow to incorporate the NIR technique in the routine of analysis of samples, complementing the quality analysis of sugarcane juice (quality control field).

Keywords: fructose; glucose; high-performance liquid chromatography; near-infrared spectroscopy.

Resumen

Contexto: El trabajo en campo, en la producción de caña de azúcar (*Saccharum officinarum* L.), hace necesario contar con métodos rápidos y sencillos de detección de diferentes compuestos.

Objetivos: Optimizar el análisis de glucosa y fructosa en jugo de caña de azúcar en campo, estudiamos la posibilidad del uso de espectroscopía de infrarrojo cercano (NIR) y su correlación con cromatografía líquida de alta eficacia (HPLC) (método de referencia) para informar desde el laboratorio datos fiables en poco tiempo.

Métodos: Se recolectaron muestras de caña en campo. La extracción del jugo de caña se realizó mediante la maceración de trozos de caña de azúcar. La determinación de glucosa y fructosa se realizó por método primario y/o referencia HPLC y por NIR en el modo transmitancia. A través de la quimiometría y el análisis de componentes principales, se desarrolló el modelo de calibración.

Resultados: Se obtuvieron los parámetros estadísticos del método de regresión como: coeficiente de determinación de 0,9717 y 0,9965 y error estándar de calibración de 0,0681 y 0,0229. El modelo predictivo reportó el error estándar de predicción de 0,136 y 0,08 para la glucosa y fructosa, respectivamente. Las variables estadísticas anteriores indicaron la alta correlación entre HPLC y NIR.

Conclusiones: Estos resultados permiten incorporar la técnica NIR en la rutina de análisis de muestras, complementando el análisis de calidad de jugos de caña (control calidad campo).

Palabras Clave: cromatografía líquida de alta eficacia; espectrofotometría de infrarrojo cercano; fructosa; glucosa.

INTRODUCTION

In the sugar industry, the quality control laboratories report reliable and timely data from the analysis carried out on sugarcane juice, accompanying the knowledge of the maturational development of the crop and contributing to the decision-making process. Analytical methodologies are supported according to the International Commission for Uniform Methods of Sugar Analysis (ICUMSA). Among the parameters that are controlled is the content of inverted sugars (glucose and fructose), present in the various stages of the development of the sugarcane crop (*Saccharum officinarum* L., *Poaceae*) (Meade, 1967; Chen et al., 1991; ICUMSA, 2007).

One of the methods used in the quality control in the determination of inverted (or reducing) sugars is the Lane and Eynon method. The principle of this method is the reduction of Cu²⁺ present in the Felling solution by an aldehyde group. This method is applied daily in laboratories for practicality and simplicity. However, the accuracy is not adequate. It provides an approximation of the content of the reducing sugars, given that all reducing material present in the sugarcane juice, in addition to the aldehyde groups of glucose and fructose, reacts positively with Felling's reagent in copper reduction. You must also have a necessary amount of sample and a time of analysis required in the degree (ICUMSA, 2007; Herrera Zambrano, 2011).

Another widely used method with high-reliability standards is separation by HPLC. It is undoubtedly one of the most versatile and far-reaching techniques. It is selective for these sugars since it has the property of separation by cation exchange and exclusion by size. However, the high cost in equipment and reagents, previous preparation of the sample by successive filtrations, the time that should be available in the separation by chromatography, does not make it opportune in the delivery of results according to the scope of sampling in the field area (Quattrocchi et al., 1992; Skoog et al., 2000; ICUMSA, 2007; Herrera Zambrano, 2011).

Considering the aforementioned methodologies and taking into account the need to reduce analysis times, use methods and technologies to minimize environmental impact by decreasing the use of reagents, while maintaining high accuracy in the results, the near-infrared spectroscopy technique is proposed (NIR) as an alternative method, which meets these criteria, in the analysis of glucose and fructose in sugarcane juice. The complexity of the NIR signal that is obtained in the set of spectra makes it necessary to use chemometric techniques that allow modeling a large amount of information obtained. The set of spectra is subjected to a pre-treatment of data to minimize some spectral contributions that are not convenient, thus simplifying the calibration model and obtaining better results (Miller y Miller, 2002; Castillo, 2007; FOSS, 2012). The HPLC method was used as a method to evaluate the accuracy in comparison with the NIR technology, using the correlation of its results.

In the scientific literature there are several documents that present the application of near-infrared spectrophotometry in other compounds (such as in the quantification of sucrose) and in other raw materials (Larrahondo et al., 2001; Xie et al., 2009; Chen et al., 2014; Ramírez-Morales et al., 2016). For example, it has been applied in honey samples as support in the identification of adulteration (Contreras Ruiz, 2014). However, the results showed high dispersion using the Milkoscan/Foss instrument. It has also been applied in virgin cane honey (Herrera Zambrano, 2011), in raw materials such as fodder and animal feed, measuring protein content, humidity, starch (Chodak, 2008). Likewise, explorations are reported in the characterization of the soil, in the determination of Carbon and Nitrogen, where correlation coefficients R^2 of 0.93 were achieved (Jarquin-Sanchez et al., 2011). In areas such as medicine, the use of NIR radiation as a non-invasive diagnostic method is reported. In the measurement of oxygen saturation, oxygenation index for all tissues. In the processes of ischemia and blood flow measurement and the detection of tumors and the vascularization of extremities (Ramírez-García et al., 2012).

MATERIAL AND METHODS

Location

The samples were collected in the Mayagüez SA facilities, located in Candelaria, Valle del Cauca (3°24'00.5"N 76°19'37.2"W). The determination of inverted sugars (glucose-fructose) was carried out in the chromatography laboratory of CENICAÑA. The reading in NIR was developed in the soil laboratory of Ingenio Mayagüez S.A. (IMSA).

Sampling

The sampling procedure in the field was carried out according to internal manuals of the Quality Management System of the organization. The samples analyzed in this study (129 to 141 in the calibration phase and 66 in the prediction phase), were sampled in the period between January 2014 and March 2015. The population sample considered for this study is represented in the farms planted with sugarcane (*Saccharum officinarum* L.), selected randomly and with a range of ages between 8 to 14 months. For these ages under study, the concentration range of glucose and fructose ranges from 1.5 to 0.1% (each) (Meade, 1967; Chen et al., 1991; ICUMSA, 2007). In this group of samples, the representative varieties make up 80% of the planted area in general, which are represented in CC 85-92, CC 93-4181, CC 93-4418, (CC, CENICAÑA Colombia).

In the field process, the extraction of sugarcane juice was carried out by macerating pieces of sugarcane. The amount of juice extracted in each sample ranged from 100 mL to 150 mL. Once the juice was extracted, the plastic container was immediately identified according to the consecutive internal, relating the place where the sample of sugarcane juice was taken. For the conservation in the field, portable coolers and gel coolant bags (UTEK, -23°C, USA) were used. The samples were received in the laboratory, where they were refrigerated and/or frozen until analysis. Approximately 75 mL of juice sample was removed for the determination of glucose and fructose by the primary method and/or HPLC reference (ICUMSA, 2007). The remaining amount of the juice sample was analyzed by NIR in the transmittance mode, and in

the wavelength range between 400 nm and 2500 nm (FOSS, 2012). In this way, they were processed for the construction of the calibration model and the correlation study between these two instrumental techniques, between 129 to 141 samples in the calibration phase and 66 samples in the prediction phase.

Determination of glucose and fructose by HPLC

The determination of glucose and fructose was performed using an HPLC (WATERS, Breeze, USA), with a binary pump system with flow 0.6 mL/min, the autosampler with a temperature of 10°C, the heating chamber of the column at 90°C, the refractive index detector at 48°C. The separation column packed with sulfonated styrene-divinylbenzene resin with Ca^{2+} exchanger was used (Column Sugar pack I). The mobile phase: 0.05 M EDTA solution (analytical reagent, Fisher brand, USA). In the quantification, glucose standards (analytical reagent, Fisher, USA), fructose (analytical reagent, Merck, Germany) were used. The juice samples were taken to a dilution of 2 mL in 50 mL, flask type A, completing the volume with type I water. Subsequently, it was passed through a 0.22-micron filter and taken to the HPLC autosampler chamber, in the corresponding vial (Quattrocchi et al., 1992).

Analysis by near-infrared spectrophotometry

The analytical development was carried out using the near infrared absorption spectrophotometer (NIR, FOSS, XDS RLA model, Silver Spring, Maryland, USA), in a wavelength range from 400 nm to 2500 nm, with a Si/SPb transmittance detector (Silicon-Lead Sulfide) (Skoog et al., 2000). For the start of the reading in the NIR, the instrument was stabilized by turning on the instrument 45 minutes before the readings and 10 minutes the tungsten filament lamp. The temperature of the samples at the time of the analysis was between 20 to 22°C. A 1 mm wide quartz cell was used to take the sample in NIR. The cell was previously purged, the first time with distilled water and the two subsequent times with the sample of analysis. Subsequently, 1 mL of sugarcane juice sample was

transferred (with the help of the transfer pipette) in the quartz cell. A 400 mL plastic beaker was used to receive the waste. The readings of the NIR spectra were made according to the operating procedure of the ISIscan software (version 4.6.8, Foss Infrasoft International, USA). Each spectrum of sugarcane juice was stored in the corresponding software project. The results obtained for glucose (%) and fructose (%) by the HPLC reference method were recorded in the corresponding NIR spectrum. This set of spectra (samples) was then exported in the WinISIS program (version 4.6.8, Foss Infrasoft International, USA). The spectrum selection process was developed, the mathematical treatment of the group of spectra in the range of wavelength between 1100 nm and 2500 nm, as well as the statistical regression method of the calibration model.

Statistical analysis (chemometry)

In the development of the calibration model, the selection of samples was first taken into account by principal components analysis (PCA). PCA information was restricted by the company (IMSA). The PCA only uses spectral information, allowing to condense the variability of the data matrix into a smaller volume of information. Therefore, a more efficient calibration process is achieved. Likewise, it allows to find the structure of the data and identify logical groups and even "outlier" or unwanted samples. Then, the mathematical pre-treatment used is the second derivative, which solves problems of band overlap and the displacement of baselines, this type of derivative eliminates displacements that vary linearly with the wavelength (FOSS, 1998, 2012; Cozzolino, 2002; Miller and Miller, 2002; Castillo, 2007).

In the development and evaluation of the calibration model, different parameters involved in the calculation were taken as spectral interval, spectral pre-treatment, outliers, number of factors, errors concerning the calibration. For this process, the calibration model was developed by the modified partial least squares (MPLS) regression method. This model has as its principle the reduction of the number of measured variables. In the development of this model, through the selection of factors (factors in this model is 6 and 9 for glucose and fructose, respectively), we sought to incorporate all relevant information, eliminating noise. The selection of the number of factors was carried out through cross-validation. In this method, the group of samples is divided into segments. This process leaves a sample outside each time and uses the rest of the samples to calculate the model, repeating the process until all the samples are excluded and predicting the property to be determined (FOSS, 1998; Castillo, 2007) .

Simultaneously, with cross-validation, the criterion for selecting the number of factors with which the smallest calibration error (SCE) is calculated is used. The SCE is a statistical parameter that indicates the upper limit when a prediction occurs. When the calibration equation is applied to the training set itself (calibration), the SCE is calculated from f-residuals as:

$$SCE = \sqrt{\frac{\sum fi^2}{N - K - 1}} \tag{1}$$

Where N is the number of samples and number K of wavelengths or factor.

Subsequently, its prediction was evaluated in a new group of samples, independent of the calibration group, in the working concentration range of the calibration model and of known composition, by means of the analysis by the reference method. In this process, the prediction of the statistical model has new comparison variables such as the Standard Prediction Error (SPE), coefficient of determination (RSQ), which finally represent the variability of the calibration model developed for the NIR prediction. The SPE is calculated from f-residuals of the new set of samples as:

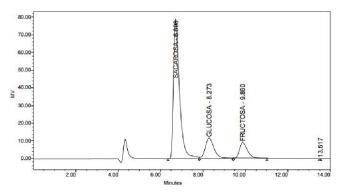$$SPE = \sqrt{\frac{\sum 'fi^2}{N - K - 1}} \tag{2}$$

Once the calibration model was developed using: 1) principal components analysis, PCA, 2) the model of mathematical pre-treatment of the spectra (samples): second derivative, 3) the regression method for the equation: PLS and MPLS , and, 4)

scatter analysis: scatter: Detrend, with the samples destined for this purpose (FOSS, 2012). The type of correlation presented by the comparison of the spectral data concerning the comparison of the instrumental techniques and the type of response of the constituents under study was observed.

RESULTS AND DISCUSSION

Determination of glucose and fructose by HPLC

The separation process of the sugars was carried out obtaining a series of chromatograms, similar to the one exposed below. Fig. 1 shows the chromatogram with the different retention times for some of the sugars present in the sugarcane juice. For this investigation, the elution of sucrose, glucose, and fructose, respectively, is shown. That is, the shorter retention time (minutes) corresponds to the lowest interaction with the stationary phase and greater affinity with the mobile phase is in that order for sucrose is 6.698, glucose 8.273 and fructose 9.860, respectively.

The principle of the chromatographic separation of sugars, such as glucose and fructose, is carried out by HPLC, which uses a separation column filled with a cation exchange resin with a calcium base, which exhibits properties such as ligand exchange and size exclusion. The diluted sample is previously filtered for injection into the column. Depending on the geometry of the hydroxyl of the different sugars, these interact with the cations of the resin by affinity, gradually varying the results of the elution times of the different species. The method is applied to molecules of low relative molecular weight, which can enter the pores of the resin and establish temporary bonds with the opposite ions. Long molecules do not easily enter

pores, and they elute more rapidly. The chromatogram of the separated components is obtained by refractometric differences of the eluent of the column. The exact estimation of peak height for sugar is obtained by an electronic integration system that then relates it to that obtained by a standard (Quattrocchi et al., 1992; ICUMSA, 2007; Herrera Zambrano, 2011; Skoog et al., 2014).

Figure 1. Glucose-fructose separation chromatogram in sugarcane juice.

HPLC-NIR correlation

Table 1 shows the statistical results of the proposed model for the calibration of the glucose and fructose parameter. According to the proposed regression method, the results of the RSQ for glucose and fructose stand out, which indicates that more than 97 and 99% of the constitutive variation in the calibration samples is explained by the model statistical (Miller and Miller, 2002; FOSS, 2012). Also, the value of the SEC is acceptable given that the average difference between the laboratory values (primary method/reference) and the NIR prediction values are below 0.1 at a confidence level of 95%.

Table 1. Statistical results of the calibration model: glucose and fructose parameters.

Components	N	SD	Est Min	Est Max	SEC	RSQ	SECV	1-VR
Glucose	141	0.4049	0.000	1.8162	0.0681	0.9717	0.0993	0.9428
Fructose	129	0.3862	0.000	1.7206	0.0229	0.9965	0.0424	0.9887

N: number of samples (spectra); Est Min- Est Max: concentration reading range; SD: standard deviation; SEC: standard calibration error; RSQ: coefficient of determination; SECV: standard error of cross validation; 1-VR: coefficient of determination in the cross validation.
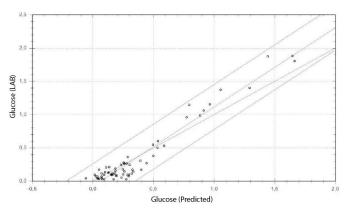
Complementing these results, the coefficient of determination in the cross-validation (1-VR), indicates that the constitutive variation in the calibration samples is explained by the statistical model in 94 and 98% for glucose and fructose, respectively. Therefore, gathering these parameters, it is presented that the statistical model developed is a source of high reliability in the prediction of said constituents.

The results obtained in the present study are similar to those reported by Larrahondo et al. (2001) in sugarcane where acceptable results of the correlation coefficients of R^2 0.80 were presented, in the analysis of sucrose, glucose, fructose, using HPLC reference method and a FOSS NIRSystems 6500. Also, in the determination of Brix (%) and Pol (%), apparent sucrose content, using refractometric and polymetric reference methods respectively, where the results were more conclusive, presenting correlation coefficients between 0.98 and 0.99 and standard errors of 0.13 to 0.14%. In a group of 128 samples in the Valle del Cauca region (Colombia).
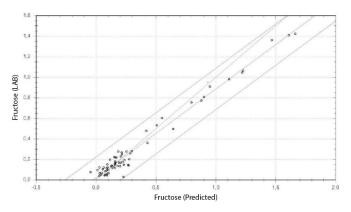
Zossi et al. (2010) report satisfactory results, using as reference method a digital polarimeter optical Activity, polar model 2001 and a FOSS NIR Systems 6500 in the quantification of Pol (%), the content of apparent sucrose, with correlation coefficient R^2 of 0.99 and standard error of 0.25, in a group of samples of 2 335 samples in the Tucumán region (Argentina). Also reported in studies of sugarcane (Clarke et al., 1992; Johnson, 2001; Madsen et al., 2003).

Fig. 2 shows the correlation between reference method and/or primary HPLC (glucose LAB) and the NIR prediction for the glucose parameter in sugarcane juice. Fig. 3 corresponds to the fructose parameter. Figs. 4 and 5 show the residue analysis of the glucose and fructose parameter, respectively.

In Figs. 2 and 3 the distribution regarding dispersion of the entire group of selected samples is observed. For the concentration range from 0.0 to 0.4%, the dispersion is lower, and the population

of samples in this area represents 70%. Between the concentration range of 0.5 to 1.8% where there is a greater dispersion, and the sample population is 30% of the group of selected samples. Figs. 4 and 5 also show this behavior. In the residual range, for concentrations from 0.0 to 0.4% the dispersion is between + 0.1 to - 0.2, and ± 0.15 for glucose and fructose respectively. On the other hand, in the residual range, for the concentrations from 0.5 to 1.83% the dispersion is between + 0.22 to + 0.44, and - 0.1 to -0.25 for the glucose and the fructose, respectively.

Figure 2. Correlation between data obtained from high-performance liquid chromatography (HPLC) standard method (glucose LAB) and data predicted from Near-infrared spectroscopy (NIR) for glucose in sugarcane juice.

Figure 3. Correlation between data obtained from high-performance liquid chromatography (HPLC) standard method (fructose LAB) and data predicted from Near-infrared spectroscopy (NIR) for fructose in sugarcane juice.
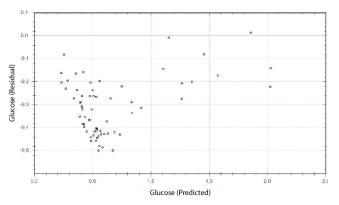
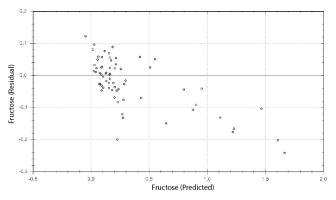**Figure 4.** Analysis of residuals vs. prediction, glucose parameter.

Figure 5. Analysis of residuals vs. prediction, fructose parameter.

Overall, the results show the convenience of increasing the population in the concentration range of the study, emphasizing concentrations greater than 0.5% in both parameters, with the aim of minimizing the graphic dispersion (positive bias for glucose and bias negative for fructose) and therefore consolidate the statistical model of calibration.

For the development of the prediction model phase, a group of 66 samples different from the initial calibration group was used. The results are presented in Tables 2 and 3. The SPE values describe the difference between the results of the samples analyzed by the reference method and the NIR calibration model prediction results, from a set of routine samples that were not used in the calibration. With this data, you can see how robust and efficient the calibration model developed is. The result of SPE for glucose was 0.13, and for fructose of 0.08, it states that the calibration model for the fructose parameter has greater robustness compared to the glucose parameter model. However, both have a high correlation represented in

the RSQ coefficient of determination (Miller and Miller, 2002).

In summary, with the data reported previously, the objective of comparing the two proposed instrumental techniques of HPLC and NIR, in the determinations of glucose and fructose in sugarcane juice presents a high correlation. It is evident that the statistical method developed has a low dispersion among its data which also makes the NIR technique a fast and reliable alternative method in the routine characterization of these sugars in sugarcane juice. However, the calibration model should be strengthened with a greater proportion of spectra (samples) throughout the concentration range.

For the glucose of structural name D-glucopyranose and the fructose of structural name D-fructopyranose, both of empirical formula $C_6H_{12}O_6$, the NIR technology is based on the spectral region that extends from the end of the highest wavelengths of the region visible (700 nm) up to 3000 nm and the dipole moment change of the molecules. The absorption in the near infrared corresponds to overtones and bands of a combination of the fundamental vibrations observed in the middle infrared. They occur in the region of 3000 to 17000 cm^{-1}. The participating links are, in general, C-H, N-H and O-H (Insuasty et al., 1993; Skoog et al., 2000; 2014; Castillo, 2007). The overtones, which correspond to transitions with $\Delta\upsilon \geq 2$, are approximately multiples of the fundamental vibrational frequency. Similarly, the combination bands are approximately the sum of different energies of vibration, whether fundamental or overtones (Insuasty et al., 1993; Castillo, 2007). The overtones and the combination bands are less likely than the fundamental transitions, which causes the absorption in the near region (NIR) to have less intensity in the middle region (MIR). The intensity of absorption in the NIR region depends on the anharmonicity of the link. The bond anharmonicity is proportional to the probability of overtones and combination bands. For this reason, the C-H, N-H, O-H bonds are very anharmonic because of the difference between their atomic weights of the link atoms (Castillo, 2007).

Table 2. Glucose constituent prediction statistics.

SEP	Bias	Slope	Bias Limit	Samples used	SD predicted
0.136	-0.008	1.191	0.600	66	0.383

Samples	LAB	ANL	Residual	Bias
13	0.382	0.499	-0.117	-0.109
14	0.171	0.326	-0.155	-0.147
15	1.801	1.664	0.137	0.145
16	1.870	1.443	0.427	0.435
17	1.885	1.648	0.237	0.245
18	0.990	0.885	0.105	0.113
19	0.134	0.100	0.034	0.042
20	0.603	0.539	0.064	0.072
21	0.045	0.072	-0.027	-0.019
22	0.103	0.249	-0.146	-0.138
23	0.075	0.295	-0.220	-0.212

LAB: data reference method, ANL: NIR prediction.

Table 3. Fructose constituent prediction statistics.

SEP	Bias	Slope	Bias Limit	Samples used	SD predicted
0.08	-0.022	0.864	0.600	66	0.401

Samples	LAB	ANL	Residual	Bias
13	0.029	0.229	-0.200	-0.177
14	0.168	0.163	0.005	0.027
15	1.362	1.466	-0.104	-0.081
16	1.407	1.610	-0.203	-0.181
17	1.423	1.665	-0.242	-0.220
18	0.810	0.902	-0.092	-0.069
19	0.136	0.181	-0.045	-0.023
20	0.601	0.550	0.051	0.073
21	0.066	0.104	-0.038	-0.016
22	0.140	0.132	0.008	0.030
23	0.095	0.015	0.080	0.103

LAB: data reference method, ANL: NIR prediction.

Considering the origin of spectral information that has absorption in the infrared region, the probability of NIR prediction is high in this type of samples given the organic character in the bonds present in the glucose and fructose molecules (CH, HOH, OH, C = O), which have absorption in the near infrared region (Castillo, 2007).

CONCLUSIONS

The organic character in the bonds present in the molecules of glucose and fructose (C-H, H-O-H, O-H, C = O), make these molecules optimal in the absorption and prediction by NIR. The results obtained by comparing the NIR technique with HPLC is very promising in the quantification of sugars such as glucose and fructose in the sugar industry, it can be used as a fast and high-confidence instrument alternative, once the calibration model has been developed. It is a non-destructive technique of the sample, which simplifies the analytical process, since it does not require prior preparation or use of reagents, therefore, the sample is analyzed directly as it reaches the laboratory. Once the predictive model has been developed, the analysis by NIR decreases the analysis time by 90%, concerning conventional methods such as Lane and Eynon titration and separation by HPLC. NIR, being a secondary technique, does not entirely displace the reference methods; they are permanent support in the control of the statistical model and the developed regression process.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTION:

Contribution	García Asprilla IS	Ramírez-Navas JS
Concepts or ideas	x	
Design	x	
Definition of intellectual content	x	x
Literature search	x	x
Experimental studies	x	
Data acquisition	x	
Data analysis	x	x
Statistical analysis	x	
Manuscript preparation	x	x
Manuscript editing	x	x
Manuscript review	x	x