Pontificia Universidad JAVERIANA Bogotá

Univ. Sci. 25 (1): 95-111, 2020.
doi: 10.11144/Javeriana.SC25-1.vahp

ORIGINAL ARTICLE

Validating a High-performance Liquid Chromatography method for the quantification of gibberellic acid in germinating maize seeds

Juan D. Rivera[1, 2, 4, *], Javier Torres[1, 3], Yaned M. Correa-Navarro[1, 2, 4]

Edited by
Juan Carlos Salcedo-Reyes
(salcedo.juan@javeriana.edu.co)

1. Grupo de Investigación en Campos Electromagnéticos Medio Ambienta y Salud Publica.

2. Grupo de Investigación en Estudios Ambientales en Agua y Suelo.

3. Departamento de Física, Universidad de Caldas, Manizales, Colombia.

4. Departamento de Química, Universidad de Caldas, Manizales, Colombia.

* juan.rivera@ucaldas.edu.co

Received: 22-10-2018
Accepted: 29-01-2020
Published on line: 01-04-2020

Citation: Rivera JD, Torres J, Correa-Navarro YM. Validating a High-performance Liquid Chromatography method for the quantification of gibberellic acid in germinating maize seeds, *Universitas Scientiarum,* 25 (1): 95-111, 2020. doi: 10.11144/Javeriana.SC25-1.vahp

Funding:
N.A.

Electronic supplementary material:
N.A.

Abstract

Gibberellic acid is a phytohormone that triggers the germination of seeds in a state of dormancy. Through the quantification of this hormone, the physiological condition of seeds of economic importance can be studded. In this work we validated a High-Performance Liquid Chromatography method to quantify gibberellic acid in germinated maize (*Zea mays* L.) seeds. Chromatographic conditions included the use of a C-18 reversed-phase column, acetonitrile-formic acid (1:9 %) as the mobile phase, flow of 0.5 mL min^{-1}, and detection at 195 nm. We evaluated our method for seven analytical parameters. The method was linear for gibberellic acid concentrations from 1.0 $mg{\cdot}kg^{-1}$ to 50.0 $mg{\cdot}kg^{-1}$. The method's limits were 0.3 $mg{\cdot}kg^{-1}$ and 1.0 $mg{\cdot}kg^{-1}$ for detection and quantification, respectively. The method was highly precise; we obtained variable but low relative standard deviations (2.62 % - 12.66 %) for the studied gibberellic acid concentrations. We assessed accuracy through recovery percentages, ranging from 52.85 % - 63.68 %, for three gibberellic acid concentrations. We conclude that our analytical method can be used to measure gibberellic acid during the early stages of maize germination. In addition, the method could be used for the analysis of other types of plant matrices.

Keywords: High-Performance Liquid Chromatography; Dynamic sonication-assisted solvent extraction (DSASE); phytohormones; gibberellic acid; validation; concentration; quantification.

Introduction

Gibberellic acid (GA_3) is a phytohormone that triggers plant growth and development. It also promotes dormant seed germination and stem elongation [1]. GA_3 occurs in three chemical forms: free, conjugated, and bound. The free form is physiologically active, whereas the conjugated one is not. The bound form is either a reserve of gibberellic acid or a form of

transport of this phytohormone in the plant [2]. GA_3 quantification in seeds helps evaluating physiological activity during germination and can be carried out with new chromatographic methods.

Novel chromatographic methods require validation. which a series of standardized experimental tests, which allow establishing the method's operating characteristics and limitations. If the method to be validated is qualitative, it requires establishing its precision, sensitivity, and limit of detection (LOD). Whereas the validation of a quantitative analytical method requires determining its linearity, accuracy, recovery, uncertainty of measurement, and limit of quantification (LOQ) [3].

GA_3 has been quantified via high performance liquid chromatography (HPLC) in *Arabidopsis thaliana* [4], Chinese cabbage (*Brassica rapa*) [5], rice (*Oryza sativa*) [6], and fruits such as apples, oranges, peaches, pears, and grapes [7]. Thus far, no attempts have been made to quantify GA_3 in maize (*Zea mays* L.).

The objective of this study was to validate a chromatographic method for the quantification of GA_3 in germinated maize seeds. This validation was carried out in the laboratory of chromatography at Universidad de Caldas - Colombia.

Materials and Methods

Chemical reagents

The compounds employed were: GA_3 standard (St. Lois, MO, USA), Fisher Scientific acetonitrile chromatographic grade (Pittsburgh, PA, USA), and analytical grade formic acid (Scharlau, Spain). Water was purified in a Millipore Direct-Q system (Bedford, MA, USA).

Maize seeds

Commercial type Maize seeds (*Zea mays* L.) variety ICA-V305, produced by Semillas del Pacífico (Cartago, Colombia), were purchased. Seeds without visible damage and with uniform morphology were preselected and sequentially passed through sieves of mesh sizes 8.0 × 8.0 mm and 6.0 × 6.0 mm to narrow their size range. Seeds were separated into large, medium, and small. A total of 100 medium-sized seeds with an average mass of 0.3878 ± 0.0002 g and an average volume of 0.356 ± 0.0080 cm^3 were employed in this study.

Preparation of the seeds

Maize seeds were placed in (100 × 15) mm Petri dishes with absorbent paper moistened with 12.0 ± 0.1 mL of distilled water, as germination matrix. All seeds were then kept in an Incucell 222 L incubator (Planegg, Germany) without light at a temperature of 30.0 ± 0.1 °C. Humidity was kept at 59.00 ± 3.39 %. After 48 h, seeds were removed from the incubator and crushed in an electric mill until to a fine powder. The powder was stored in a hermetic seal bag at -20 °C for subsequent extraction [8].

Extraction of gibberellic acid

GA_3 was obtained from the seed powder by dynamic sonication-assisted solvent extraction (DSASE), according to the method described by Rivera *et al*. [9]. Acetonitrile-formic acid 5.0 % (8:2) was used as a solvent. A 3.0 mL stainless steel extraction cell was filled with 0.8000 ± 0.0001 g of seed powder and placed inside a Branson 5210 ultrasound bath (Hampton, NH, USA) with water temperature control, T < 35.0 °C. The solvent flow was controlled through the cell at 0.4 $mL \cdot min^{-1}$ with a peristaltic pump. The extraction was carried out during 25.0 min, obtaining 10.0 ± 0.1 mL of the extract.

Chromatographic conditions

GA_3 was quantified via high-performance liquid chromatography (HPLC) with a Shimadzu HPLC device (Marlborough, MA, USA). The device is equipped with a prominence degasser DGU-205 (Marlborough, MA, USA), a Shimadzu prominence SPD-M20A diode array detector (DAD) (Massachusetts, USA), a Shimadzu CTO-10AS VP oven (Marlborough, MA, USA), and a Shimadzu SIL-10AF auto sampler (Marlborough, MA, USA). The program used for the analysis of the data was the Shimadzu LC-Solution Software (Marlborough, MA, USA).

The HPLC procedure included the modifications proposed by Bhalla *et al*. [10]. A Supelcosil LC-18-DB 5.0 μm (150 × 4.6 mm) particle size column was used. The mobile phase was acetonitrile with 0.01 %formic acid with a linear gradient detailed as follows: acetonitrile with 0.01 %(1:9 %) formic acid for 5.0 min, (7:3 %) for 40.0 min, and (1:9 %) for 10.0 min at a flow of 0.5 $mL \cdot min^{-1}$ in all cases. An aliquot of 10 μL of each solution containing gibberellic acid was injected into the chromatograph and detected at a wavelength of 195 nm. Prior to chromatographic analysis, all samples were filtered through QLS membranes with 0.2 μm pores.

Calibration curve

A calibration curve was made with a standard of gibberellic acid at seven concentrations (1.0, 5.0, 10.0, 20.0, 30.0, 40.0, and 50.0) $mg \cdot kg^{-1}$. These solutions were prepared dissolving the standard in acetonitrile. To know the relationship between the chromatographic signal, in terms of area, and the concentration of GA_3, a simple linear regression was performed by the ordinary least squares method. The assumptions of normality, homoscedasticity, and independence were verified in order to validate the information provided by the parametric ANOVA of the regression. Four replicates were carried out for each pattern for a total of 28 data points.

Analytical parameters

To determine the power of the analytical method, the following parameters were determined: sensitivity, selectivity, linearity, precision, accuracy, and limits of detection and quantification. These variables were assessed following the guide for the validation of analytical methodologies and calibration equipment used for the analysis of illicit drugs in materials seized and biological specimens [3] and the validation guide and determination of measurement uncertainty [11].

Recovery

The concentration of the analyte was established in germinated maize seeds to quantify the percentage of recovery of the method. To this aim, doping was performed on the fine seed powder to adjust the concentration of the analyte at low, medium, and high levels in percentages of 30.0 %, 50.0 % and 70.0 %, respectively. A total of 0.8000 ± 0.0001 g of maize powder were added to volumes of a standard GA_3 at the concentrations shown in **Table 1**. The doped samples were subjected to ventilation to efficiently volatilize the solvent. Subsequently, the extractions of GA_3 by DSASE were carried out according to the proposed protocol.

Statistical analysis of the data

Statistical analyses were performed using the Statgraphics Centurion XI software in demo mode. A calibration curve with an external standard was made for the quantification of GA_3 present in the test samples. The assumptions of normality (Shapiro-Wilks), homoscedasticity (Bartlett), and independence (Durbin-Watson) were checked on this calibration curve (linear regression model) to ascertain the results generated in the curve.

Table 1. Amounts of standard gibberellic acid used for doping.

Increase in analyte concentration (%)	Doping concentration (mg kg^{-1})	Doping volume (µL)
30.0	9.2	420
50.0	15.8	400
70.0	18.4	480

Results and Discussion

We developed a HPLC-DAD method to quantify GA_3 in maize seeds and undertook its validation, trough the construction of a calibration curve and assessed the method's performance for a set of analytical parameters.

Calibration Curve

We performed a regression analysis to find the best linear adjustment of the GA_3 standard detection within a concentration range of (1.0 to 50.0) mg kg^{-1}. All replicates of each GA_3 concentration, and not their averages, were fed to the regression analysis. This was done in order to ensure that no relevant data were missing from the analysis of variance and subsequent validation parameters analyses. We took this approach accepting the caveat of possibly compromising the regression's determination coefficient.

The assumptions of normality (p-value = 0.243), independence (p-value = 0.791), and homoscedasticity (p-value = 0.097) were verified Thus, residuals were normally distributed without autocorrelation, and the chromatographic signal variances of the different concentration were equal. Based on results we can safely determine GA_3 concentrations in maize seeds within the assessed concentration range.

The analysis of variance revealed a significant relationship between the peak area and G_3 concentration at a significance level of 5.0 % (p-value < 0.001). The resulting linear model formula is $A = 21.2131C + 0.0002$, with parameter values presented in **Table 2**.

Table 2. Regression analysis of gibberellic acid.

Analyte	Intercept β_0 $(mUA \cdot min)$	Standard deviation of β_0 $(mUA \cdot min)$	Slope β_1 $\left(\frac{mUA \cdot min}{mg/kg}\right)$	Standard deviation of β_1 $\left(\frac{mUA \cdot min}{mg/kg}\right)$	Correlation coefficient R	Determination coefficient R^2
GA_3	0.00022	8.1543	21.2131	0.3051	0.9975	0.9951

Limit of detection (LOD) and Limit of Quantification (LOQ)

The LOD of the method was determined experimentally injecting low concentrations of the analyte. **Fig. 1** shows the set of GA_3 concentration with an integrable chromatographic signal was. The lowest GA_3 concentration detected was 0.3 $mg \cdot kg^{-1}$ at a retention time (Rt) of 16.65 min, characteristic of the studied analyte. Consequently, this value was considered as the LOD.

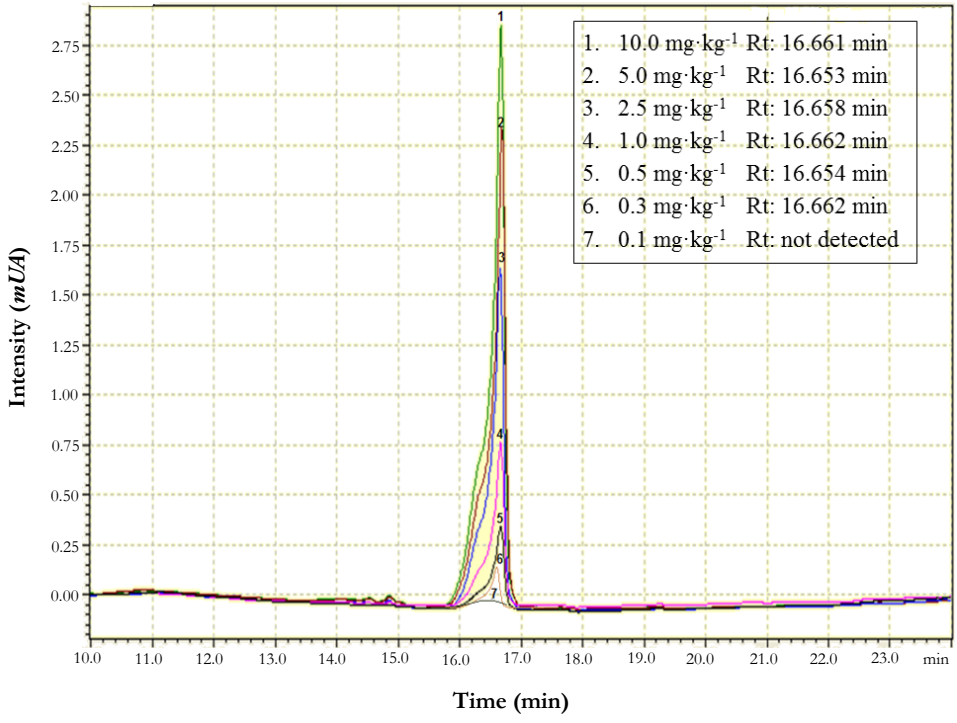

Figure 1. Chromatographic signal at seven low concentrations of gibberellic acid.

The LOQ was calculated from the experimental LOD. The signal/noise ratio (S/N) for the LOD is found at a S/N $\geq$ 3 proportion, and the S/N relation for LOQ has to be greater than or equal to ten [12]. Therefore, when relating both signals the resulting LOQ was equal to 10/3 LOD. Based on this equation, a limit of quantification of 1.0 mg·kg^{-1} was obtained.

Sensitivity

Calibration sensitivity

The calibration sensitivity of the method (*Cs*) corresponded to the slope of the calibration curve (β_1) used for quantification ($Cs = \beta_1$) [11]. A hypothesis test was performed to determine if there was a relationship between signal variation and analyte concentration. The null hypothesis was rejected, that is, the slope of the calibration line was different from zero with a significance of 5 % (p-value $<$ 0.001). Therefore, the magnitude of the signal varies as analyte concentration changes.

The confidence intervals for the slope of the calibration line were also determined. These revealed that for an increase in GA_3 concentration of 1.0 mg·kg^{-1}, the signal varied from 20.5834 mUA·min to 21.8427 mUA·min in the range of the calibration curve with a confidence level of 95 %.

Analytical sensitivity

An analytical sensitivity of the method (γ) of 0.7560 kg·mg^{-1} was determined. This analytical parameter related calibration sensitivity and instrumental noise (S_y) as follows ($\gamma = Cs/S_y$) [13]. The obtained analytical sensitivity value allowed comparisons between different methods of analysis. To facilitate this comparison, we worked with the inverse of the analytical sensitivity (γ^{-1}) [13] with a value of 1.3228 mg·kg^{-1}. This concentration is the minimum amount analyte that can be detected with our method in 1.0 kg of sample.

Linearity

Compliance with linearity was measured through a test of lack of adjustment, consisting of Fisher's test (F test). The experimental value of the F test is given by $F_{Exp} = (S_{y/x})^2/(S_y)^2$, where F_{Exp} corresponds to the expected F and refers to the lack of fitting of the model, and $S_{y/x}$ is the standard deviation due to the lack of adjustment in the regression model [14]. The variables of the F test were determined from the calibration curve. Because F_{Exp} (1.057) is smaller than F_{Table} (2.150), the null hypothesis could not be rejected (significance of 5 % and p-value = 0.3378). This is to say, there is no need for higher order

terms in the model and the relationship between analyte concertation (from 1.0 to 50.0 $mg \cdot kg^{-1}$) and its quantification value is solely linear.

Precision

The Precision of the method was determined as the relative standard deviation (RSD) at low, medium, and high analyte concentrations by doping samples. RSD values lower than 15 % and 20 % are acceptable at high and low analyte concentrations, respectively. According to this information, our RSD values lower than 15 % show that the quantitative method under test is precise. The obtained RSD for the low, medium, and high analyte concentrations were 12.66 %, 2.62 %, and 12.40 %, respectively. The standard deviation of the calibration curve was 28.8502 mUA·min.

Selectivity

The selectivity of the method was determined by studying the spectral purity of the GA_3 peak in the samples. For this purpose, the HPLC (LC-Solution) program was used. We determined that the absorption spectra of the GA_3 peak had a spectral purity of 99.18 %, concluding that there are no detectable interferences that produce an overlap to the analyte peak [14]. In addition, we compared the absorption spectra of the standard and the sample along their peak maximum ascending and descending portions and corroborated their identity.

Accuracy

The Method's accuracy was determined based on the systematic error of the calibration curve and on the recovery percentage at low, medium, and high analyte concentrations. To evaluate the systematic error, a hypothesis test was carried out to determine if the intercept (β_0) of the calibration curve was different from zero. An intercept different from zero implies that the equipment provides a signal when the analyte's concentration is equal to 0.0 $mg \cdot kg^{-1}$. The test result revealed that the intercept of the calibration curve was equal to zero and, therefore, the method did not exhibit a systematic error (5.0 % significance, p-value = 1.0000). In addition, the confidence intervals at the intercept of the calibration were obtained. For an analyte concentration of 0.0 $mg \cdot kg^{-1}$, the instrument's signal varied from -16.8295 mUA·min to 16.8299 mUA·min with a confidence level of 95.0 %.

The recovery percentage at different analyte concentrations was obtained from Equation 1, where S corresponds to the analyte concentration added to the sample, C is the concentration of analyte in the sample, and CS is the concentration of analyte after doping. The results obtained are presented in **Table 3**.

Table 3. Percentages of recovery for the employed doping levels.

Doping level	Recovery (%)	Standard deviation (%)
Low (30.0 %)	63.68	29.33
Medium (50.0 %)	56.49	4.23
High (70.0 %)	52.85	15.58

$$\% \text{ Recovery} = \frac{CS - C}{S} * 100\% \quad (1)$$

A simple parametric ANOVA was carried out, on the assumption that there were no significant differences between the three concentrations evaluated with a significance of 5 %. The assumptions of normality, independence, and homoscedasticity of the data were verified. The percentages of recovery obtained were all greater than the lowest level of doping, and at an intermediate doping level, the smallest standard deviation was obtained. Furthermore, since that percentage of recovery at this doping level did not differ significantly from those in the other two doping levels, doping at medium concentration levels is recommended for this type of analysis.

The percentages of recovery of GA_3 obtained by DSASE (52.00 % to 63.00 %) were similar to those reported by Urbanova *et al*. [4] The authors performed solid phase extractions with recovery percentages between 52.73 % and 74.14 %. This result is also similar to that reported by Wu *et al*. [6] (62.00 %) by means of hollow fiber liquid-liquid-liquid microextraction. The percentages of recovery obtained by the present work are acceptable given the presence of extraction-interfering molecules in the matrix analyzed. Solid phase extractions, prior to chromatography analyses, have thus become standard procedure [15, 16]. A summary of our validation results is shown in **Table 4**.

Table 4. HPLC-DAD method validation parameters.

Analyte	Calibration sensitivity (Cs) $(mUA \cdot min / \frac{mg}{kg})$	Analytical sensitivity (γ) ($kg \cdot mg^{-1}$)	Inverse of analytical sensitivity (γ^{-1}) ($mg \cdot kg^{-1}$)	Linear Range ($mg \cdot kg^{-1}$)
GA_3	21.2131	0.7560	1.3228	1.0 – 50.0

Sample analysis

The developed HPLC-DAD method was used for the quantification of GA_3 in samples of maize seeds at three different times after sowing 0 h, 24 h, and 48 h, seeking to test the sensitivity of the analytical method.

The peaks in the chromatograms of the GA_3 standard and a sample of germinated seeds had a retention time of 16.680 min (**Fig. 2**). For the maize sample, a peak spectral purity of 99.18 % was obtained, indicating that the excipients of the matrix did not generate signals that interfered with the peak of the analyte. Thus, the signal generated by the sample is pure.

We determined G_3 concentrations in seeds with three different sowing times using parametric ANOVA and Tukey's multiple range tests. We observed G_3 concentration differences among the three sowing times (5 % significance of; p-value < 0.0001). GA_3 concentration was significantly higher in seeds after 48 h of sowing (**Table 5**). Our method was thus sensitive to variations in analyte concentration and is able to detect concentration differences below 1.0 $mg \cdot kg^{-1}$. The uncertainty of the samples was calculated using the following equation:

$$S_x = S_{y/x}/(m.n^{1/2}) \quad (2)$$

where $S_{y/x}$ is the standard deviation due to the lack of fitting in the regression model, m is the slope of the regression and n is the number of replicates of the sample.

Table 5. Concentration of gibberellic acid in maize seeds at three different germination times.

Germination time (h)	Concentration GA_3 ($mg \cdot kg^{-1}$)	Uncertainty ($mg \cdot kg^{-1}$)
0	1.3	0.2
24	2.2	0.2
48	3.7	0.2

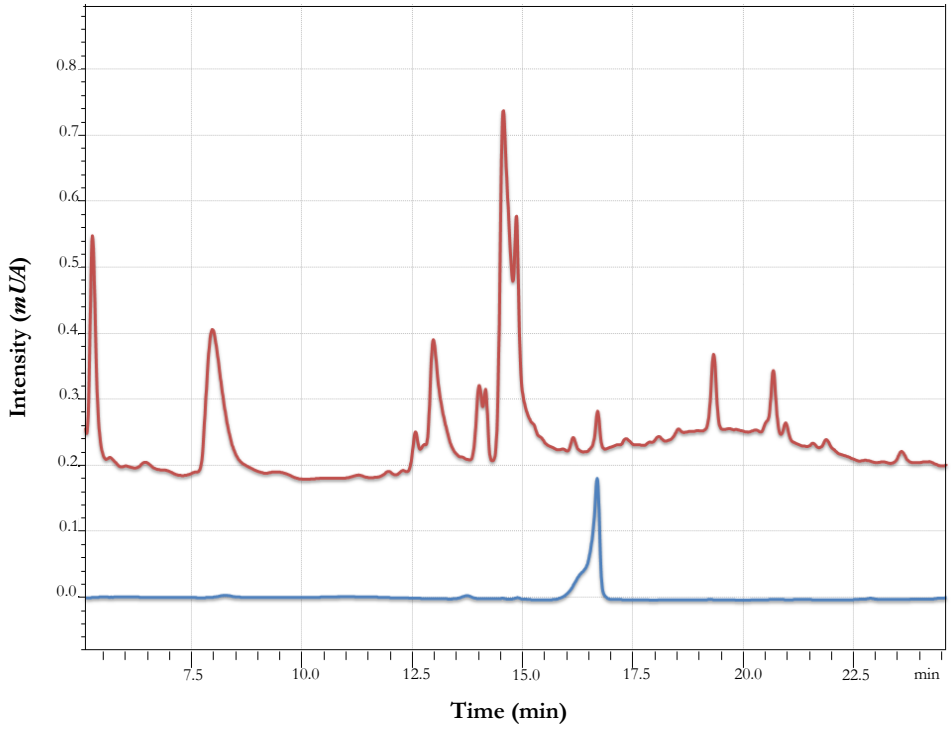

Figure 2. Standard chromatograms (blue) and maize seed samples (red) at 48 h after sowing.

Conclusion

We have validated an HPLC-DAD method for the determination of gibberellic acid in maize seeds during their early stages of germination. The set of tested validation parameters indicates that our method exhibits low systematic

and random errors and is highly sensitive, selective, accurate, and precise. This method can be safely used to quantify G_3 in maize seeds within the concertation ranges tested.

Acknowledgements

This work was conducted thanks to the research and graduate studies grant no. cd 0455216, by Universidad de Caldas. The grant is addressed to the project: Analysis of the influence of magnetic stimulation on the behavior of the concentration of hormones in maize seeds (*Zea mays* L.).

Conflict of interests

The authors sate having no conflict of interests

References

[1] Daviere JM, Achard P. Gibberellin signaling in plants. *Development*, 140: 1147-1151, 2013.

doi: 10.1242/dev.087650

[2] Miransari M, Smith DL. Plant hormones and seed germination. *Environmental and Experimental Botany*, 99: 110-121, 2014.

doi: 10.1016/j.envexpbot.2013.11.005

[3] UNODC, Directrices para la validación de métodos analíticos y la calibración del equipo utilizado para el análisis de drogas ilícitas en materiales incautados y especímenes biológicos. Naciones Unidas, New York, USA 2010.

[4] Urbanová T, Tarkowská D, Novák O, Hedden P, Strnad M. Analysis of gibberellins as free acids by ultra performance liquid chromatography-tandem mass spectrometry. *Talanta*, 112: 85-94, 2013.

doi: 10.1016/j.talanta.2013.03.068

[5] Hamayun M, Khan SA, Khan AL, Ahmad N, Nawaz Y, Sher H, Lee I. Gibberellin producing Neosartorya sp. CC8 reprograms Chinese cabbage to higher growth. *Scientia Horticulturae*, 129: 347-352, 2011.

doi: 10.1016/j.scienta.2011.03.046

[6] Wu Q, Wu D, Duan C, Shen Z, Guan Y. Hollow fiber-based liquid-liquid-liquid micro-extraction with osmosis: II. Application to quantification of endogenous gibberellins in rice plant. *Journal of Chromatography A*, 1265: 17-23, 2012.

doi: 10.1016/j.chroma.2012.09.066

[7] Xie W, Han C, Zheng Z, Chen X, Qian X, Ding H, Shi L, Lv C. Determination of Gibberellin A3 residue in fruit samples by liquid chromatography-tandem mass spectrometry. *Food Chemistry*, 127: 890-892, 2011.

doi: 10.1016/j.foodchem.2011.01.018

[8] Correa YM, Buriticá LM, Rivera JD, Penagos JP, Torres JI. Optimización del protocolo para la extracción y la cuantificación de proteínas totales en semillas germinadas de maíz (*Zea mays* L.). *Revista Facultad de. Ciencias Basicas*, 13(1): 60-64, 2017.

Retrieved from
https://revistas.unimilitar.edu.co/index.php/rfcb/article/view/2756

[9] Rivera JD, Correa YM, Penagos JP. Evaluación de métodos de extracción para la obtención del ácido giberélico en semillas germinadas de maíz (*Zea mays* L.). *Revista Colombiana de Química*, 46(2): 45-50, 2017.

doi: 10.15446/rev.colomb.quim.v46n2.63015

[10] Bhalla K, Singh SB, Agarwal R. Quantitative determination of gibberellins by high performance liquid chromatography from various gibberellins producing Fusarium strains. *Environmental Monitoring and Assessment*, 167: 515-520, 2010.

doi: 10.1007/s10661-009-1068-5

[11] Instituto de Salud Pública. Validación de métodos y determinación de la incertidumbre de la medición. Santiago, Chile 2010.

Retrieved from
https://www.academia.edu/24922817/Validaci%C3%B3n_de_m%C3%A9todos_y_determinaci%C3%B3n_de_la_incertidumbre_de_la_medici%C3%B3n_Aspectos_generales_sobre_la_validaci%C3%B3n_de_m%C3%A9todos_

[12] Miller JN, Miller JC. Estadística Y Quimiometría Para Química Analítica. Prentice Hall, Madrid, Spain 2002.

[13] Danzer K, Currie LA. Guideline for calibration in analytical chemistry. Part 1. Fundamentals and single component calibration. *Pure and Applied Chemistry*, 70(4): 993-1014, 1998.

doi: 324 10.1016/j.cell.2014.03.046

[14] Olivieri AC. Practical guidelines for reporting results in single- and multi-component analytical calibration: A tutorial. *Analytica Chimica Acta*, 868: 10-22, 2015.

doi: 10.1016/j.aca.2015.01.017

[15] Ge L, Peh CYC, Yong JWH, Tan SN, Hua L, Ong ES. Analyses of gibberellins by capillary electrophoresis-mass spectrometry combined with solid-phase extraction. *Journal of Chromatography A*, 1159: 242-249, 2007.

doi: 10.1016/j.chroma.2007.05.041

[16] Manzi M, Gómez A, Arbona V. Rapid and reproducible determination of active gibberellins in citrus tissues by UPLC/ESI-MS/MS. *Plant Physiology and Biochemistry*, 94: 1-9, 2015.

doi: 10.1016/j.plaphy.2015.04.015

Validación del método de Cromatografía Líquida de Alta Eficiencia para la cuantificación de ácido giberélico en semillas germinadas de maíz

Resumen: El ácido giberélico es una fitohormona que detona la germinación de semillas en estado de dormancia. A través de la cuantificación de esta hormona, se puede estudiar la condición fisiológica de las semillas de importancia económica. En este trabajo, se validó el método de Cromatografía Líquida de Alta Eficiencia para cuantificar el ácido giberélico en semillas germinadas de maíz (*Zea mays* L.). Las condiciones cromatográficas incluyeron el uso de una columna de fase inversa C-18, acetonitrilo-ácido fórmico (1:9 %) como fase móvil, flujo de 0.5 mL min^{-1} y detección a 195 nm. Se evaluó este método para siete parámetros analíticos. El método fue linear para las concentraciones de ácido giberélico entre 1.0 $mg \cdot kg^{-1}$ y 50.0 $mg \cdot kg^{-1}$. Los límites del método fueron 0.3 $mg \cdot kg^{-1}$ y 1.0 $mg \cdot kg^{-1}$ para detección y cuantificación, respectivamente. El método fue altamente preciso; se obtuvieron desviaciones estándar variables, pero relativamente bajas (2.62 % - 12.66 %) para las concentraciones de ácido giberélico estudiadas. Se determinó la exactitud a través de porcentajes de recuperación, cuyo rango estuvo entre 52.85 % y 63.68 % para las tres concentraciones de ácido giberélico. Se concluye que este método analítico puede usarse para medir ácido giberélico durante los primeros estadios de la germinación del maíz. Además, el método podría usarse para el análisis de otros tipos de matrices de plantas.

Palabras clave: Cromatografía Líquida de Alta Eficiencia; Extracción Dinámica con Solvente Asistida por Sonicación (DSASE); fitohormonas; ácido giberélico; validación; concentración; cuantificación.

Validação de um método por Cromatografia Líquida de Alta Eficiência para a quantificação de ácido giberélico em sementes germinadas de milho

Resumo: O ácido giberélico é um fitohormônio que dispara a germinação de sementes em estado de dormência. Por meio da quantificação deste hormônio, se pode estudar a condição fisiológica das sementes de importância econômica. Neste trabalho validou-se um método por Cromatografia Líquida de Alta Eficiência para quantificar o á ido giberélico em sementes germinadas de milho (*Zea mays* L.). As condições cromatográficas incluíram o uso de uma coluna de fase reversa C-18, acetonitrila-ácido fórmico (1:9 %) como fase móvel, fluxo de 0.5 $mL.min^{-1}$ e detecção a 195 nm. O método foi avaliado em sete parâmetros analíticos. O método foi linear para as concentrações de ácido giberélico entre 1.0 $mg.kg^{-1}$ e 50 $mg.kg^{-1}$. Os limites do método foram 0.3 $mg.kg^{-1}$ e 1.0 $mg.kg^{-1}$ para detecção e quantificação, respectivamente. O método foi altamente preciso; obtiveram-se desvios-padrões variáveis, mas relativamente baixos (2.62 % - 12.66 %) para as concentrações de ácido giberélico estudadas. Determinou-se a exatidão através de porcentagem de recuperação, variando entre 52.85 % e 63.68 % para as três concentrações de ácido giberélico. Conclui-se que este método analítico pode ser usado para a determinação de ácido giberélico durante os primeiros estágios da germinação do milho. Adicionalmente, o método poderia usar-se para a análises de outros tipos de matrizes de plantas.

Palavras-chave: Cromatografia Líquida de Alta Eficiencia; Extração dinâmica com solvente acelerada por sonicação (DSASE); fitohormônios; ácido giberélico; validação; concentração; quantificação.

Juan David Rivera Giraldo

Degree in Biology and Chemistry and master's in chemistry from the Universidad de Caldas (Colombia). Occasional professor of the chemistry department and member of the research group in electromagnetic fields, environment and public health and of the Research Group in Environmental Studies in Water and Soil (GEAAS) of the Universidad de Caldas.

ORCID: 0000-0003-1335-5620

Javier Torres Osorio

Is an associate professor attached from Universidad de Caldas. He has two master degrees, in Physics Instrumentation and Electrical Engineering (Bioelectronics) from Universidad Tecnológica de Pereira Also he is Ph. D (c) in Agronomical Science and leader of the Research Group on Electromagnetic Fields Environment and Public Health, Assigned to the Department of Physics, Faculty of Exact and Natural Sciences, Universidad de Caldas, Colombia.

ORCID: 0000-0002-9962-5769

Yaned Milena Correa Navarro

Chemical Technologist from the Universidad Tecnológica de Pereira, chemistry from the Universidad del Quindío, master's in chemical sciences from the Universidad Nacional Sede Medellín. Associate professor of the chemistry department and member of the research group in electromagnetic fields, environment and public health and of the Research Group in Environmental Studies in Water and Soil (GEAAS) of the Universidad de Caldas.

ORCID: 0000-0001-9236-209X