Journal of Chromatographic Science, 2017, Vol. 55, No. 3, 251–257
doi: 10.1093/chromsci/bmw179
Advance Access Publication Date: 24 November 2016
Article

Article

An HPLC Procedure for the Quantification of Aloin in Latex and Gel from *Aloe barbadensis* Leaves

Dalia I. Sánchez-Machado[1], Jaime López-Cervantes[1,*], María F. Mariscal-Domínguez[1], Paola Cruz-Flores[1], Olga N. Campas-Baypoli[1], Ernesto U. Cantú-Soto[1], and Ana Sanches-Silva[2]

[1]Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, 85000 Ciudad Obregón, Sonora, México and [2]Department of Food and Nutrition, National Institute of Health Dr. Ricardo Jorge, I.P., Av. Padre Cruz, 1649-016 Lisbon, Portugal

[*]Author to whom correspondence should be addressed. Email: jaime.lopez@itson.edu.mx

Received 26 May 2016; Revised 27 September 2016; Editorial Decision 27 October 2016

Abstract

Aloin is an anthraquinone-C-glycoside present in *Aloe vera*. This compound is extremely variable among different species and highly depends on the growing conditions of the plants. The quantification of aloin in different extraction preparations has been a frequent problem due to the high instability of the compound. The aim of the present study is to develop and validated an analytical method for aloin detection in fresh and dry samples of *Aloe barbadensis* gel and latex using high performance liquid chromatography coupled to a diode array detector (HPLC-DAD). Phosphate buffered saline (pH 3) was selected as the extraction solvent. The aloin was separated using a Zorbax Eclipse AAA column (4.6 × 150 mm) at 35°C, and water and acetonitrile were used as the mobile phase at a flow rate of 0.9 mL/min. The linearity was satisfactory with a correlation coefficient greater than 0.999. Under these conditions, the method precision (relative standard deviation) was 3.71% for FL, 4.41% for dry latex, 0.81% for fresh gel and 4.42% for dry gel samples. Aloe latex was determined to have a greater amount of aloin than aloe gel. The method validation was satisfactory and exhibited adequate linearity, repeatability and accuracy.

Introduction

For hundreds of years, plants have been used to treat illnesses, making them potential sources of medicinal substances. In recent years, these sources have been exploited and used to derive a large number of available drugs (1).

Aloe barbadensis (syn. *Aloe vera*) has been reported throughout history as the most widely accepted specie of aloe used in ethnic medicine because of its anticarcinogenic, anti-inflammatory, antiviral and laxative properties (2). Thus, aloe has become one of the most commercially important plants cultivated worldwide. Aloe is a succulent perennial plant from the Liliaceae family, and it is found in hot and dry climates (3). There are more than one hundred reported constituents found in the leaves and gels. Aloe pulp has been shown to contain protein, lipids, amino acids, vitamins, enzymes, carbohydrates and inorganic compounds. The yellow exudate contains 1,8 dihydroxyanthraquinone derivatives and their glycosides (4). Aloin, an anthraquinone-C-glycoside, naturally occurs as a mixture of diastereoisomers aloin A and aloin B (1). The amount of aloin is also extremely variable among different species and highly depends on the growing conditions (5). The concentrations of these analytes also depend on the location of the leaf within the aloe plant as well as the site within the leaf, i.e., leaf margins, pulp center, etc. (6). Even with this variability, this compound has been reported to represent up to 30% of the dry weight of aloe latex (7). Considering the large percentage of aloin found in plants along with its multiple pharmacological properties, this constituent has been considered an

important indicator for quality control in pharmaceuticals and aloe-based products (8).

The most commonly used method for quantification of aloin is by high performance liquid chromatography (HPLC) with C_{18} columns as the stationary phase, UV detection (8–13) and capillary electrophoresis (14). In addition, mass spectrometry (MS) has been used to identify aloin in commercial aloe-based products and in *Aloe secundiflora* latex samples (12, 15, 16).

Currently, HPLC analysis requires aloin extraction by procedures using various solvents. The most commonly used solvents are methanol (17), ethanol (13), ethyl acetate (18) isopropyl alcohol and water (9). Most recently an ultrasensitive ionic liquid microextraction procedure has been used (19). Methanol is the most frequently used solvent for the extraction of anthraquinones; however, it is known to have high toxicity, and several studies have suggested that aloin and other anthraquinones degrade faster in methanol solutions (11). The stability of aloin is highly important for accurate validation of a method. Therefore, the search for solvents capable of maintaining aloin stability is crucial for accurate quantification of the compound. Previous studies have tested the stability of a standard solutions of aloin in phosphate buffers at different pH values, suggesting that lower pH values are preferable for aloin conservation (8). To our knowledge, leaf samples have not been tested in phosphate buffers. Therefore, the aim of this study was to develop and validate a method for the detection of aloin in fresh and dry samples of aloe gel and latex. Validation parameters such as linearity, precision, recovery, detection and quantification limits were determined. Additionally, the presence of aloin in the commercial samples was confirmed by HPLC-MS.

Experimental

Chemicals and reagents

HPLC-grade acetonitrile was purchased from J.T. Baker (Center Valley, PA, USA), and acetic acid and methanol were obtained from Sigma (St. Louis, MO, USA). The standard aloin with purity ≥97% ($C_{21}H_{22}O_9$, CAS 1415-73-2) was supplied by Santa Cruz Biotechnology (Dallas, TX, USA). Analytical-grade methanol and phosphate buffered saline (PBS) were purchased from Sigma (St. Louis, MO, USA). All aqueous solutions were prepared with ultrapure water purified with a NaNopure Diamond UV system (Barnstead International, Dubuque, IA, USA).

An aloin standard solution (924 µg/mL) was prepared in PBS (adjusted to pH 3 with 1 N hydrochloric acid) by dissolving an accurately weighed amount in a volumetric flask. The solution was stored at 4°C protected from light. Working solutions of aloin were prepared from the stock solution and diluted with PBS (pH 3). Two calibration curves were prepared. The first was prepared with volumes of 4, 3, 2 and 1 mL of standard solution filled to 5 mL, and the second was prepared with volumes of 30, 50, 250, 500 µL and 1 mL of standard solution filled to 10 mL. The stock solution and working solutions were prepared on the same day that they were analyzed. For quantification, peak heights were correlated with the concentration according to the calibration curve. All samples were analyzed in duplicate. Aloin content are cited as means ± standard deviations (SDs).

Samples preparation

Aloe barbadensis leaves were harvested from an intensive cultivation area in South Sonora, Mexico in plastic bags and washed with drinking water. The leaves were cut at the base and allowed to drain for 30 min to obtain fresh latex (FL). For fresh gel (FG), the leaf skin was removed, the inner and middle layer were scraped, which contained the vascular bundles transporting latex (20), and then these were homogenized in a blender. For dry samples, FG was dried at 40°C (24 h) in an oven while FL was dried at room temperature (22°C) for 24 h. Many leaf samples from commercial producers were received, but the tests shown discrepancies in the data. Thus, we decided to evaluate the distribution of aloin within different parts of the leaves and between the leaves of one plant. For the first study, each leaf was divided into four different sections: basal, terminal, center and leaf borders. For the second study, five leaves were collected from one plant and the analysis was performed for homogenized FG from all the leaf. The commercial samples including a juice concentrate and *A. barbadensis* capsules were obtained from herbal medicine stores.

The water content of FG, FL, dry gel and dry latex was eliminated by drying to constant weight in an oven at 70°C.

About 5 mg of dry latex and 10 mg of FL were dissolved in PBS (pH 3) to 5 mL. A total of 50 mg of dry gel, 2 g of FG and 100 mg of *A. barbadensis* capsules were all prepared by dissolving in PBS (pH 3) to 10 mL. The commercial aloe juice concentrate was injected directly without dilution. All samples were sonicated for 10 min and filtered through 0.45 µm pore size nylon membrane filters (GVS Fitter Technologies, Indianapolis, IN, USA) prior to HPLC analysis.

Equipment and chromatographic conditions

An Agilent series 1220 Infinity LC HPLC system (Agilent Technologies, Palo Alto, CA, USA) was equipped with a dual gradient pump, an auto-sampler and a diode array detector (DAD) LC 1260. Chromatographic analysis was performed using an analytical scale (4.6 × 150 mm) Zorbax Eclipse AAA column with a 5 µm particle size. Aloin was quantified using the peak heights after HPLC analysis. The analysis was conducted according to the HPLC conditions described in Table I.

Liquid chromatography with MS (LC–MS) was performed using an Agilent 1260 Infinity HPLC system (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 6230 TOF equipped with an ESI source. All acquisitions were performed in negative ion mode with a capillary voltage of +3,500 V. Nitrogen was used as the nebulizer gas (30 psig), and the dry gas temperature was 300°C at 10 L/min. The mobile phase was ultrapure water with 0.1% acetic acid (A) and acetonitrile with 0.1% acetic acid (B).

Results

The popularity of commercial aloe-based products has increased significantly over the years especially in the food and beverage

Table I. Conditions of Chromatographic Analysis

Parameter	Condition
Mobile phase	A: MilliQ water; B: Acetonitrile
Gradient program	(Min/A%:B%): 0/80:20, 23/20:80, 24/80:20
Flow rate	0.9 mL/min
Detector	254 nm
Temperature	35°C
Analysis time	24 min
Injection volume	10 and 20 µL

industry. Aloin is known to be have laxative properties; therefore, methods to quantify this analyte concentration are important in order to give consumers product safety. The great majority of studies on the extraction of aloin are done with acidified solvents or methanol, although the aloin is highly unstable when dissolved in methanol. We present an extraction method using PBS solution, where the aloin demonstrated to be stable at acidic pH. In addition, for measurement of aloin this HPLC method was validated and has appropriate analytical characteristics.

Aloin extraction

For the purpose of this study, different solvents (methanol, methanol:HCl, ethanol, ethanol:HCl and ethyl acetate) were tested in fresh aloe gel samples. The height of the aloin peaks in the samples extracted with methanol showed approximately double the height of those extracted with ethyl acetate and ethanol. However, aloin was highly unstable with methanol:HCl when were performed repeated analysis of the extracts during 12 h. Thus, the extraction solvent was changed to PBS buffered saline (PBS), a non-toxic solution, to improve the stability the aloin. And the aloin content was measured at two distinct pH values (3 and 7) over 25 h. The results showed higher stability at pH 3. There was an 80% decrease in the aloin peak height between 0 and 25 h at pH 7, while at pH 3, the height showed only a slight variation (0.02%).

HPLC separation and identification

In the current study, a preliminary trial was performed with standard aloin to determine the optimal chromatographic conditions for aloin detection. The optimal conditions for the separation of aloin in all samples (dry latex, FG, FL, dry gel and commercial products) are shown in Table I. Figure 1 shows the chromatograms obtained for the aloin standard, dry latex and FG samples. The peak for aloin was observed at 7.93 ± 0.02 min after an average of eight injections. Aloin was identified by comparing its retention time with the standard and using characteristic spectra obtained from the DAD.

To confirm the identification of the peaks, an LC–MS analysis of the commercial products was performed. First, to optimize the LC–MS conditions, a standard solution of 7.44 μg/mL of aloin was analyzed in negative ionization dual ESI mode, with a drying gas temperature

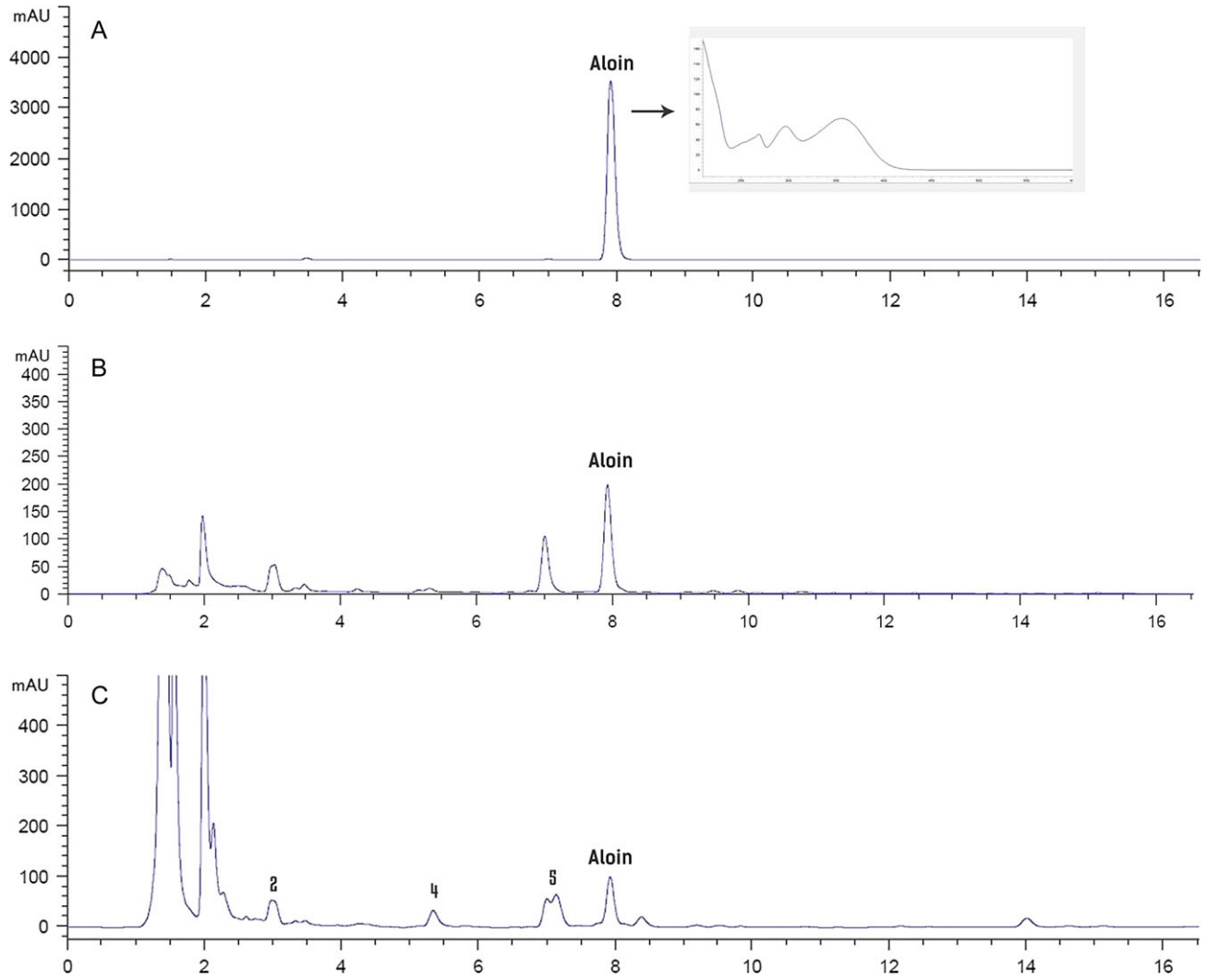

Figure 1. HPLC chromatograms of aloin standard with 554 μg/mL and UV absorption spectrum of the HPLC-DAD peak (A), dry latex (B) and dry gel (C); Unidentified compounds from anthraquinone group: 1, 2, 3, 4, 5 (aloin B).

Table II. Linearity for Aloin Analyzed

Sample	Range (μg/mL)	Equation	r^2	SD intercept	SD slope
Latex	162.5–3551.8	$y = 6.342x + 61.4494$	0.999	36.21	0.13
Gel	41.7–2454.8	$y = 13.294x + 9.5949$	0.999	7.92	0.09

x, amount (μg/mL); y, peak height; r^2, determination coefficient.

of 300–350°C, the nebulizing gas set between 30 and 60 psig and the fragmentor at 100–170 V. Optimal results were obtained with a drying temperature of 300°C, the nebulizing gas at 30 psig and the fragmentor at 170 V.

Method validation

The linearity of the standard curves (Table II) was expressed in terms of the determination coefficient (r^2) from plots of the integrated peak heights versus the concentrations of the standard (μg/mL). The method was linear between 162.5 and 3551.8 μg/mL for the latex samples and between 41.7 and 2454.8 μg/mL for the gel samples. The correlation coefficients are satisfactorily greater than 0.999. Table III shows the precision results, where the relative standard deviation (RSD) was found to be between 0.81% and 4.42% for repeatability. The reproducibility of total aloin in the samples was measured by analyzing the same sample on a total of 5 days, and the RSD values for the samples were found to be less than 4.31%.

Determination of the detection limit for aloin on the basis of signal to-nose ratios (3:1) was 3.6 ng/mL, and the quantification limit based on the signal to-nose ratio (10:1) was 11.8 ng/mL, as per American Chemical Society guidelines (21).

Accuracy was estimated using recovery assays for dry gel and dry latex samples. For evaluation of aloin recovery, two spiking levels were quadrupled in each sample prior to extraction and quantitation. The average recovery for dry gel was 90.4% (0.06% RSD) and 89.6% (0.06% RSD) for dry latex.

Aloin content in gel and latex of *A. barbadensis* leaves

The aloin contents of fresh and dry gel, as well as fresh and dry latex samples, were determined in different preparations of PBS buffer extracts. The analyses were conducted with leaf samples from commercial producers finding great variability in the aloin content as observed in Table IV, the FL samples quantified greater aloin amounts, with an average of 199.76 ± 0.74 mg aloin. For dry latex, the average aloin content was 176.26 ± 0.16 mg, 7.87 ± 0.05 mg for FG and 5.11 ± 0.12 mg for dry gel samples. These results show a percentage between 17% and 20% for fresh and dry exudate, which is very close to the previous study. For FG samples, the results show a range from 4.04 to 13.41 mg aloin/g dry weight. We found that the dry samples may contain less aloin due to rapid degradation by temperature (8).

Aloin content in different leaves from the same *A. barbadensis* plant

The practical applicability of the proposed analytical method was assessed by determining aloin content in different sections of the leaves. All extracts from the same leaf were homogenized to compare the aloin content between different leaves.

An *A. barbadensis* plant was selected for the determination of aloin in different sections. The selected leaves represent different levels of the plant, starting from the center and reaching the base.

Table III. Precision of the Method for the Determination of Aloin in Aloe Samples

Sample	Repeatability ($n = 8$)		Reproducibility ($n = 3$)	
	Mean ± SD[a]	RSD (%)	Mean ± SD[a]	RSD (%)
Fresh gel	4.10 ± 0.03	0.81	4.03 ± 0.09	2.44
Dry gel	3.86 ± 0.17	4.42	1.26 ± 0.04	3.87
Fresh latex	422.69 ± 15.68	3.71	353.06 ± 8.42	2.38
Dry latex	316.25 ± 13.95	4.41	303.87 ± 13.12	4.31

[a]Results expressed as mg/g dry mass.

Table IV. Variability of Aloin Content (mg aloin/g dry weight) in Samples of *A. barbadensis*

Sample	Dry latex	Dry gel	Fresh latex	Fresh gel
1	110.67 ± 0.04[a]	3.79 ± 0.02	212.56 ± 0.84	9.42 ± 0.10
2	159.27 ± 0.45	7.99 ± 0.32	217.88 ± 1.86	4.23 ± 0.12
3	291.16 ± 0.09	3.76 ± 0.05	224.98 ± 0.15	4.04 ± 0.01
4	225.74 ± 0.20	8.63 ± 0.01	247.73 ± 1.18	9.04 ± 0.11
5	108.97 ± 0.07	2.23 ± 0.02	199.15 ± 0.08	13.41 ± 0.02
6	161.76 ± 0.01	4.25 ± 0.01	96.27 ± 0.02	7.08 ± 0.07

[a]Means ± SDs ($n = 2$).

Starting from the largest leaf at the base and increases at each level until reaching the center. The aloe offshoot is found at the base of the aloe plant. Leaves lower, middle lower and middle upper represent the largest leaves, 67.5, 67.1 and 66.5 cm, respectively. The middle upper leaf originated from the middle section of the plant measured 20 cm, while the aloe leaf offshoot measured 21.1 cm. Figure 2 shows the aloin content of each leaf, where the leaves that showed a greater amount of aloin were of the upper leaf and the aloe offshoot.

Aloin content in different section of an aloe leaf

From the four sections previously described the highest mean was found in the terminal section of the leaf (22.28 mg aloin/g gel dry weight), while the basal section had minor content (1.08 mg aloin/g gel dry weight). The results show lower aloin quantification in the aloe margins (1.81 mg aloin/g gel dry weight) than in the center (4.40 mg aloin/g gel dry weight). The basal section was found to contain the lowest amount of aloin.

Aloin content in commercial aloe products

The practical applicability of the proposed analytical method was also assessed by determining the aloin content in three different aloe-based commercial products. The aloe capsules contained 2.97 μg/mL aloin. In the juice concentrate, the aloin could not be quantified because the chromatographic peak was very small. The aloin was not detected in the homeopathic aloe product.

All commercial products were also analyzed by LC–MS, where the presence of aloin was confirmed for the aloe capsules. For these analyses, 30 µL of the juice concentrate and of the aloe capsule extract were injected. Specifically, the ESI-MS analysis showed a molecular ion at *m/z* 417.1202 for aloe capsule (Figure 3). The mass calculator gave a value of *m/z* 417.1191 for the aloin compound, which was comparable to the molecular ions detected in the commercial product. Other aloe-based products such as the juice concentrate did not show a molecular ion comparable to aloin.

Discussion

The aloin extraction results are in agreement with the conclusions made by Jawade and Chavan (9), who suggested that a maximum extraction yield is achieved with methanol, making this solvent the most frequently utilized to extract anthraquinones. In the preliminary assays to validate the method, our results showed a gradual decrease in aloin content and a production of aloe-emodin, similarly to Chang *et al.* (20) indicating poor stability of aloin when dissolved in methanol. According to Ding (8), pH plays a critical role in stabilizing aloin, and aloin has been found to show a less rapid decline at pH 3 than at alkaline pH. Zonta *et al.* (22) have also reported that the decomposition of aloin is most rapid at basic pH values. These findings led to the use of PBS buffer at pH 3 for the following assays.

During the optimization of the chromatography conditions, various assays were conducted with a gradient of water:methanol as the mobile phase; flow rates of (0.6, 0.9, 1.0 and 1.5 mL/min) and column temperatures of (25°C, 30°C and 35°C) (23). The best peak resolution was obtained with a flow rate of 1.0 mL/min and a

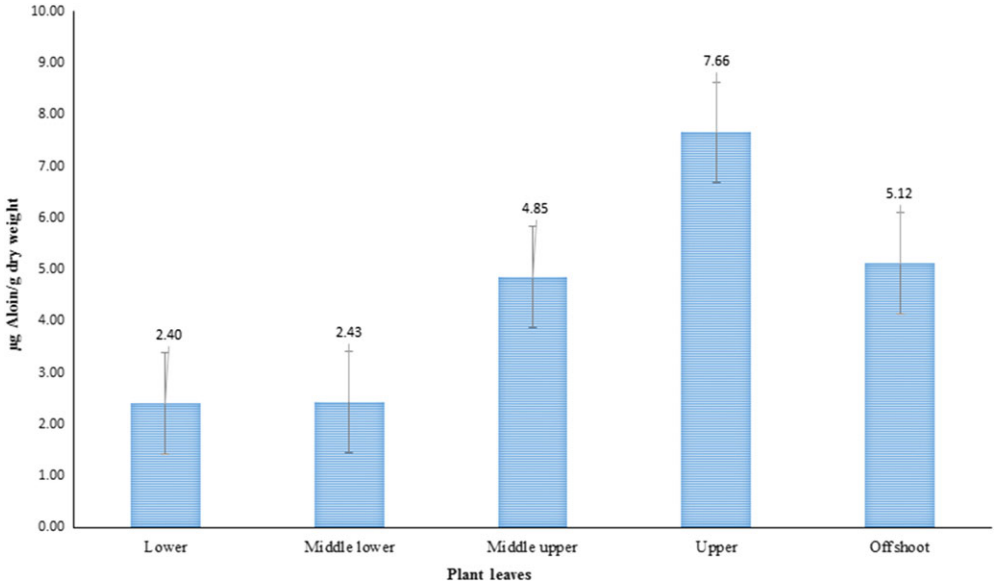

Figure 2. Aloin content of different aloe leaves belonging to the same *A. barbadensis* plant.

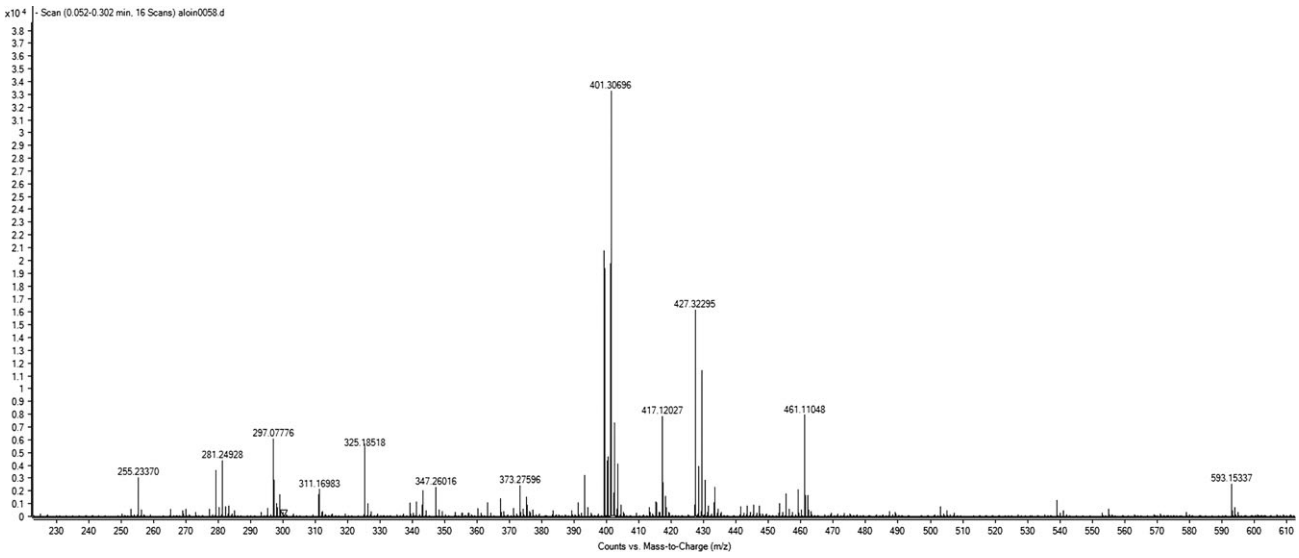

Figure 3. EI-MS chromatogram of aloin from the aloe concentrate commercial product.

column temperature of 35°C at a 45 min run time. Nonetheless, many authors have used acetonitrile:water as a mobile phase, in which the variation in the proportion of these solvents is high (9). The results showed that using the acetonitrile:water gradient shortened the total run time. The assays were performed at 254 nm, within the range of wavelengths (220–290 nm) used to detect aloin, in previous studies (12, 13).

For the HPLC method, the precision study was comprised of repeatability and reproducibility studies. A total of eight replicates of each sample were performed to assess repeatability, and three replicate analyses of each sample were conducted on different days to determine reproducibility. For the most part, the authors have reported RSD values lower than 5%; Chiang *et al.* (17) reported values between 0.3% and 0.6%, Elsohly and Gul (12) achieved values between 3.47 and 4.33 and Okamura *et al.* (24) reported 1.46%. However, Fanali *et al.* (1) reported a higher RSD value of 12.1% for aloin peak areas using a nano LC–MS method. The recovery values are similar to those reported by Logaranjan *et al.* (13). These results indicate that the present method can be used for quantitative analysis of aloin obtained from phosphate buffer extracts.

With reference to the aloin contents in gel and latex, the differences could be explained by variations between young and old leaves and growing conditions of the aloe plants. Aloin constitutes 30% of the aloe plant dry exudate (7). Park *et al.* (10) have previously reported an aloin content in freeze dried ethanol extracts of 1.14 ± 0.39 mg/g. Lucini *et al.* (25) have reported an aloin content of approximately 24.41 mg/g dry weight of aloe gel, a value that is between the observed ranges. To the best of our knowledge, there are few publications of aloin values in dry aloe latex, probably due to the difficult manipulation of the samples. Specifically, Paez *et al.* (26) have reported the aloin presence in yellow leaf latex from *A. vera*, and Ding *et al.* (8) have reported that aloin constitutes up to 30% of leaf exudates.

The leaves that showed the least amount of aloin originated from the base of the plant. Our results contradict the results from Beppu *et al.* (27) who found that the leaves from the middle positions had the lowest concentrations. The small aloe shoot reported the second highest amount of aloin, revealing that the younger leaves contained a higher amount of aloin than the older leaves located at the base. These results are in agreement with the findings of Gutterman and Chauser-Volfson (6) and Groom and Reynolds (28), who found higher aloin contents in younger leaves than in older leaves, mentioning that aloin is located within cell vacuoles, which causes the content to vary in different locations of the leaf. In agreement with Beppu *et al.* (27) the concentration of aloin was found to be greater in the leaves that originated from the center of the plant than those from the base.

Previous studies have found that the terminal section of the aloe leaf contains the highest content of aloin and secondary metabolites (6), which is consistent with our results. The studies by Gutterman and Chauser-Volfson (6) also found that the center parts of aloe leaves contain lower aloin content in comparison with the leaf margins. According to Gutterman and Chauser-Volfson (6) the leaf orientation has an effect on the aloin content.

Conclusion

The HPLC method presented in this study is simple, accurate and allows rapid quantitative determination of aloin in samples of *A. barbadensis* and its products. This method could be applied to the quality control of products containing aloin to prevent rapid degradation of the analyte with the use of a phosphate buffer as the extraction solution.

Acknowledgments

M.F.M.-D. gratefully acknowledges a Master grant scholarship from the National Council of Science and Technology (CONACYT: 548666).

Funding

This research was financed by project PROFAPI no. 2016-0025 from Instituto Tecnológico de Sonora.

References

1. Fanali, S., Aturki, Z., D'orazio, G., Rocco, A., Ferranti, A., Mercolini, L., *et al.*; Analysis of Aloe-based phytotherapeutic products by using nano-LC-MS; *Journal of Separation Science*, (2010); 33: 2663–2670.
2. Himesh, S., Sarvesh, S., Kaushelendra, M., Singhai, A.K., Neelesh, C.; Qualitative and quantitative profile of aloin isolated from *Aloe vera*; *International Research Journal of Pharmacy*, (2011); 2: 121–122.
3. Choche, T., Shende, S., Kadu, P.; Extraction and identification of bioactive components from *Aloe barbadensis* Miller; *Research & Reviews Journal of Pharmacognosy and Phytochemistry*, (2014); 2: 14–23.
4. Pal, S., Sahrawat, A., Prakash, D.; *Aloe vera*: composition, processing and medicinal properties; *International journal of current discoveries and innovations*, (2013); 2: 106–122.
5. Wamer, W.G., Vath, P., Falvey, D.E.; In vitro studies on the photobiological properties of Aloe emodina and Aloin A; *Free Radical Biology & Medicine*, (2003); 34: 233–242.
6. Gutterman, Y., Chauser-Volfson, E.; Peripheral defence strategy; variation of barbaloin content in the succulent leaf parts of *Aloe arborescens* Miller (Liliaceae); *Botanical Journal of the Linnean Society*, (2000); 132: 385–395.
7. Rebecca, W., Kayser, O., Hagels, H., Zessin, K., Madundo, M., Gamba, N.; The phytochemical profile and identification of main phenolic compounds from the leaf exudate of *Aloe secundiflora* by high-performance liquid chromatography-mass spectroscopy; *Phytochemical Analysis*, (2003); 14: 83–86.
8. Ding, W.J., Wu, X.F., Zhong, J.S., Wan, J.Z.; Effects of temperature, pH and light on the stability of Aloin A and characterization of its major degradation products; *International Journal of Food Science & Technology*, (2014); 49: 1773–1779.
9. Jawade, N., Chavan, A.; Ultrasonic-assisted extraction of aloin from *Aloe vera* gel; *Procedia Engineering*, (2013); 51: 487–493.
10. Park, M.K., Park, J.H., Kim, N.Y., Shin, Y.G., Choi, Y.S., Lee, J.G., *et al.*; Analysis of 13 phenolic compounds in *Aloe* species by high performance liquid chromatography; *Phytochemical Analysis*, (1998); 9: 186–191.
11. Kispotta, A., Srivastava, M.K., Dutta, M.; Free radical scavenging activity of ethanolic extracts and determination of aloin from *Aloe vera* L. leaf extract; *International Journal of Medicinal Aromatic Plants*, (2012); 2: 612–618.
12. Elsohly, M.A., Gul, W.; Determination of the antraquinones aloe-emodin and aloin-A by liquid chromatography with mass spectrometric and diode array detection; *Journal of AOAC International*, (2007); 90: 28–42.
13. Longaranjan, K., Devasena, T., Pandian, K.; Quantitative detection of aloin and related compounds present in herbal products and *Aloe vera* plant extract using HPLC method; *American Journal of Analytical Chemistry*, (2013); 4: 600–605.
14. Yang, Y., Wang, H., Guo, L., Chen, Y.; Determination of three compounds in *Aloe vera* by capillary electrophoresis; *Biomedical Chromatography*, (2004); 18: 112–116.
15. Wang, P., Zhou, W., Wamer, W., Krynitsky, A.J., Rader, J.I.; Simultaneous determination of aloin A and aloe-emodin in products containing *Aloe vera* by ultra-performance liquid chromatography with tandem mass spectrometry; *Analytical Methods*, (2012); 4: 3612–3619.

16. Kanama, S.K., Viljoen, A.M., Kamatou, G.P., Chen, W., Sandasi, M., Adhami, H.R., *et al.*; Simultaneous quantification of anthrones and chromones in *Aloe ferox* ("Cape aloes") using UHPLC-MS; *Phytochemistry Letters*, (2015); 13: 85–90.
17. Chiang, H.M., Lin, Y.T., Hsiao, P.L., Su, Y.H., Tsao, H.T.; Determination of marked components—aloin and aloe-emodin—in *Aloe vera* before and after hydrolysis; *Journal of Food and Drug Analysis*, (2012); 20(3): 646–652.
18. Pellizzoni, M., Molinari, G.P., Lucini, L.; Stability of the main Aloe fractions and Aloe-based commercial products under different storage conditions; *Agrochimica*, (2011); 55(5): 288–296.
19. Yan, Y., Chen, X., Hu, S., Tian, J., Bai, X.; Simultaneous preconcentration and analysis of anthraquinones based on ultrasound emulsification ionic liquid microextraction; *Journal of Chromatographic Science*, (2013); 52: 218–225.
20. Chang, X.L., Wang, C., Feng, Y., Liu, Z.; Effect of heat treatments on the stabilities of polysaccharides substances and barbaloin in gel juice from *Aloe vera* Miller; *Journal of Food Engineering*, (2006); 75: 245–251.
21. American Chemical society guidelines (ACS); Subcommittee of environmental analytical chemistry; *Analytical Chemistry*, (1980); 52: 2242.
22. Zonta, F., Bogoni, P., Masotti, P., Micali, G.; High-performance liquid chromatography profiles of aloe constituents and determination of aloin in beverages, with reference to the EEC regulation for flavouring substances; *Journal of Chromatography A*, (1995); 718: 99–106.
23. Azaroual, L., Liazid, A., Barbero, G.F., Brigui, J., Palma, M., Barroso, C.G.; Improved chromatographic methods for determination of bioactive compounds from *Aloe vera* leaves; *ISRN Chromatography*, (2012). DOI:10.5402/2012/609095.
24. Okamura, N., Asai, M., Hine, N., Yagi, A.; High-performance liquid chromatography determination of phenolic compounds in Aloe species; *Journal of Chromatography A*, (1996); 746: 225–231.
25. Lucini, L., Pellizzoni, M., Molinari, G.P.; Anthraquinones and β-polysaccharides content and distribution in aloe plants grown under different light intensities; *Biochemical Systematics and Ecology*, (2013); 51: 264–268.
26. Paez, A., Gebre, G.M., Gonzales, M., Tschaplinski, T.; Growth, soluble carbohydrates, and aloin concentration of *Aloe vera* plants exposed to three irradiance levels; *Environmental and Experimental Botany*, (2000); 44: 133–139.
27. Beppu, H., Kawai, K., Shimpo, K., Chihara, T., Tamai, I., Ida, C., *et al.*; Studies on the components of *Aloe arborescens* from Japan—monthly variation and differences due to part and position of the leaf; *Biochemical Systematics and Ecology*, (2004); 32: 783–795.
28. Groom, Q.J., Reynolds, T.; Barbaloin in Aloe species; *Planta Medica*, (1987); 53: 345–348.