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Determination of Gibberellic Acid in Fermentation Broth and Commercial Products by Micellar Electrokinetic Chromatography

THUMNOON NHUJAK,*,†,‡ MONPICHAR SRISA-ART,† KANYARAT KALAMPAKORN,† VASANA TOLIENG,‡ AND AMORN PETSOM‡,§

Chromatography and Separation Research Group and Research Centre for Bioorganic Chemistry, Department of Chemistry, Faculty of Science, and The Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok 10330, Thailand

Micellar electrokinetic chromatography (MEKC) was developed as a method for quantitative determination of gibberellic acid (GA_3) in fermentation broth and commercial products, using 25 mM disodium tetraborate as a buffer at pH 9.2 and 100 mM sodium dodecyl sulfate as a micellar phase. The baseline resolution (R_s) of GA_3 from other compounds in fermentation broth was achieved with $R_s > 2.5$. The addition of methanol or acetonitrile in the MEKC buffer did not give a better resolution. Advantages of this MEKC method include high accuracy and precision and no sample preparation except for dilution and filtration.

KEYWORDS: Gibberellic acid; fermentation broth; capillary electrophoresis; micellar electrokinetic chromatography; high-performance liquid chromatography

INTRODUCTION

Gibberellic acid (GA_3) is a diterpenoid compound in a class of gibberellins, and its structure is shown in **Figure 1**. GA_3 is a plant hormone that promotes seed germination, stem elongation, premature flowering, and cone production and retards leaves and fruit senescence. GA_3 is also a metabolic product of the fungus *Gibberella fujikuroi* (*1*−*3*). Traditionally, GA_3 is produced by submerged fermentation by employing *G. fujikuroi*. Conventional techniques used for determination of GA_3 and other plant hormones include spectrophotometry (*3*), high-performance thin layer liquid chromatography (*4*), gas chromatography (*5*, *6*), and high-performance liquid chromatography (HPLC) (*7*−*9*).

Capillary electrophoresis (CE) is a powerful analytical tool and an alternative technique to HPLC. Advantages of CE include high speed, high efficiency and resolution, low or no consumption of organic solvent, small amount of sample, and advanced automation. Two CE modes used for the determination of GA_3 and other plant regulators have been reported to be capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). Although CE was developed for the separation of GA_3 and eight other plant growth regulators, using a phosphate borate buffer at pH 8.09, baseline resolution of some plant hormones was not achieved (*10*). The resolution was improved using cyclodextrins and cholic acid as buffer additives.

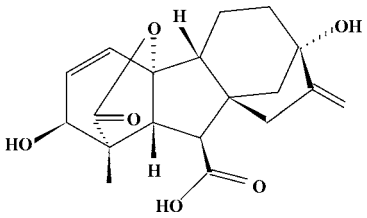

Figure 1. Structure of GA_3.

In addition, CZE was used for the determination of GA_3 and other plant regulators in plant tissues such as *Chara vulgaris* thallus (*11*−*13*) and banana leaves (*14*). The amount of GA_3 in *C. vulgaris* determined by both CZE and bioassay was found to be in good agreement. MEKC using borate as a running buffer and sodium dodecyl sulfate (SDS) as a micellar phase was developed for the determination of GA_3 and other plant hormones in tobacco flowers (*15*). The detection sensitivity was improved by using a bubble cell capillary to increase the cell path length or large volume sample stacking as an on-line preconcentration.

A method used for quantitative determination of GA_3 in fermentation broth has been reported to be HPLC (*16*). Because of the fermentation broth containing substrates, products, metabolite byproducts, and cells of microorganisms, liquid−liquid extraction with ethyl acetate was used to remove unwanted substances that may be deposited in an HPLC column. Disadvantages of the liquid−liquid extraction include the consumption of organic solvent and time and GA_3 loss during extraction.

* To whom correspondence should be addressed. Tel: 66-2-2187609. Fax: 66-2-2187598. E-mail: thumnoon.n@chula.ac.th.
† Chromatography and Separation Research Group.
‡ The Institute of Biotechnology and Genetic Engineering.
§ Research Centre for Bioorganic Chemistry.

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The CE method has not been previously reported for the determination of GA_3 in fermentation broth and commercial products. The aims of this work are to develop CE as a method for quantitative determination of GA_3 in fermentation broth and commercial products and to compare CE with HPLC for the determination of GA_3. MEKC separation was performed using a pH 9.2 $Na_2B_4O_7$ solution as a buffer and SDS as a pseudo stationary phase. MEKC optimization was carried out by variation of the concentration of SDS and the types and concentrations of organic additives.

MATERIALS AND METHODS

Chemicals. GA_3 and SDS were purchased from Fluka (Buchs, Switzerland), and gibberellin A_4 (GA_4) was purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Disodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) was supplied by Riedel-de Haën (Seeize, Germany). 3-Amino-4-methylbenzoic acid (AMBA) used as an internal standard in CE for the analysis of GA_3 was obtained from Fluka. An MEKC buffer used in CE was prepared by dissolving the desired amounts of $Na_2B_4O_7 \cdot 10H_2O$ and SDS with ultrapure water. Ethyl acetate (analytical grade) and methanol (HPLC grade) were supplied from Merck (Darmstadt, Germany). All solutions were prepared using ultrapure water and filtered through 0.45 μm filters prior to analysis.

Fermentation Broth. *G. fujikuroi* N9-34 for production of GA_3 was obtained from stock culture of the Institute of Biotechnology and Genetic Engineering (IBGE), Chulalongkorn University. The strain was maintained in a potato dextrose agar slant at 25 °C. One liter of a production medium containing the substances was prepared as follows: 120 g of sucrose, 2.39 g of $(NH_4)_2SO_4$, acid-hydrolyzed cotton seed hull with a nitrogen content of 1.14 g, 5.0 g of KH_2PO_4, 1.0 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of Al_2O_3, 2.0 mL of soybean oil, and water. An inoculum of *G. fujikuroi* was added to a 250 mL Erlenmeyer flask containing 50 mL of the production medium. The fermentation was carried out at 25 °C with 250 rpm rotating agitation for a desired day. The culture was filtered, and the fermentation broth was kept in a fridge at 0 °C prior to analysis of GA_3.

CE Analysis. All CE experiments were carried out on a 5010 Beckman CE instrument (Beckman Coulter, Inc., CA). An uncoated fused silica capillary (Polymicro Technologies, AZ) used was 57 cm in length (50 cm to detector) × 50 μm i.d., thermostated at 25 °C. Before doing the experiment each day, the capillary was rinsed with 0.1 M NaOH for 15 min and then with the MEKC buffer for 15 min. Prior to each injection, the capillary was rinsed with 0.1 M NaOH for 3 min and then with the MEKC buffer for 3 min. Sample and standard solutions were introduced by 0.5 psi pressure injection for 4 s. The voltage of +30 kV was applied during separation. UV detection was set at 214 nm. Each experiment was run in triplicate. No sample preparation, except for dilution and filtration, was required for the CE analysis of GA_3 in fermentation broth.

HPLC Analysis. All HPLC experiments were performed on a Beckman HPLC instrument (System Gold Nouveau). HPLC conditions in this work were used for routine analysis of GA_3 in the fermentation broth at IBGE. An analytical column used was 5 μm Betasil C_8 250 mm × 4.6 mm i.d. The mobile phase contained 35% methanol and 65% water adjusted to pH 3.0 with phosphoric acid. The flow rate was controlled at 1.0 mL min^{-1}. All sample solutions were injected using a 10 μL loop. Before introduction of samples into the HPLC column, the sample preparation of GA_3 in the broth was carried out using the procedure previously reported (*17*). The fermentation broth was filtered through a no. 42 membrane filter and adjusted to pH 3.0 with 0.1 M hydrochloric acid. Then, 3.0 mL of the pH 3.0 broth was transferred to a 10 mL glass tube. Four milliliters of ethyl acetate was added to the tube. The mixture was shaken using a vortex for 4 min to extract GA_3 into an ethyl acetate layer. The ethyl acetate solution in the upper layer was transferred into another tube containing anhydrous sodium sulfate in order to remove water. The tube was shaken again using a vortex for 1 min and then was centrifuged for 2 min. The solution was transferred into another tube and then evaporated using a vacuum rotary evaporator at 35 °C to remove all ethyl acetate. An aliquot of 3.0 mL of mobile phase was added into the tube to dissolve GA_3 and other residues. The sample solution was filtered with a 0.45 μm PTFE membrane filter. Approximately 30 min was used for the sample preparation prior to HPLC analysis.

RESULTS AND DISCUSSION

MEKC Optimization. To investigate suitable CE conditions for separation of GA_3 from other compounds in the broth, the 10 day fermentation broth diluted 10 times with water was used as a test sample solution. In initial work, separation was carried out by CZE using the buffer as 25 mM $Na_2B_4O_7$ at pH 9.2, and the obtained result is shown in **Figure 2a**. By spiking standard GA_3 into the diluted fermentation broth, the peak at a migration time of 4.177 min was identified to be GA_3. A small unknown peak A was found close to the GA_3 peak. The peak at a migration time of 5.325 min in **Figure 2a** belongs to AMBA, which is spiked as an internal standard for quantitative analysis in the following section. An increase in the concentration of $Na_2B_4O_7$ from 25 to 50 mM resulted in better resolution (R_s) of GA_3 and unknown A (R_s = 1.0 and 2.4, respectively), possibly due to a decrease in electromigration dispersion with an increase in the buffer ionic strength (*18*).

To compare with CZE, MEKC for separation of GA_3 and other compounds was carried out using 20−150 mM SDS as the micellar phase in 25 mM $Na_2B_4O_7$ as the buffer. **Figure 2b−d** shows an example of MEKC electropherograms of the fermentation broth diluted 10 times, using 20, 40, and 100 mM SDS. It can be seen in **Figures 2** and **3a** that an increase in the SDS concentration results in the long retention time of analytes due to a decrease in electroosmotic mobility (μ_{eo}) and an increase in the electrophoretic mobility (μ) of analytes partitioning into the SDS micellar phase (*19*). It should be noted that μ_{eo} and μ can be calculated from data of migration times in **Figure 2**. From **Figure 3b**, the better resolution of GA_3 and unknown peak A was obtained with increasing the SDS concentration. However, at 40 mM SDS concentration as shown in **Figure 2c**, another unknown peak B separating from the GA_3 peak was observed, and their resolution increased with increasing SDS concentration. An achieved baseline resolution (R_s > 1.5) of GA_3 and unknown B was obtained at a SDS concentration above 100 mM (**Figure 3b**). Above 100 up to 150 mM SDS, no any new unknown peak separating from GA_3 peak was found. It should be noted that 50 mM $Na_2B_4O_7$ in CZE gave an acheived baseline resolution (R_s > 1.5) for GA_3: unknown A, while 25 mM $Na_2B_4O_7$ in MEKC was chosen. This is because the low concentration of the $Na_2B_4O_7$ solution in MEKC can reduce Joule heating and allow the use of high concentrations of SDS in MEKC. The higher concentration of the $Na_2B_4O_7$ solution neither gave resolution of GA_3:unknown B in CZE nor improved resolution of GA_3:unknown B in MEKC. In addition, a longer analysis time was obtained with the high concentration of the $Na_2B_4O_7$ solution due to reduction of EOF. Therefore, 100 mM SDS in 25 mM $Na_2B_4O_7$ was selected as the MEKC buffer system for investigation on the effect of organic solvents on MEKC separation of GA_3 from unknown compounds in the fermentation broth.

Organic modifiers such as methanol and acetronitrile have been widely used for improving the resolution in MEKC. Therefore, methanol and acetronitrile 0−20% v/v were separately added in the MEKC buffer containing 25 mM $Na_2B_4O_7$ and 100 mM SDS. A longer migration time of analytes (**Figure 4**) was obtained with increasing the concentration of either methanol or acetonitrile mainly due to the reduction of μ_{eo} (*20*). **Figure 5** shows that improvement of resolution was not obtained

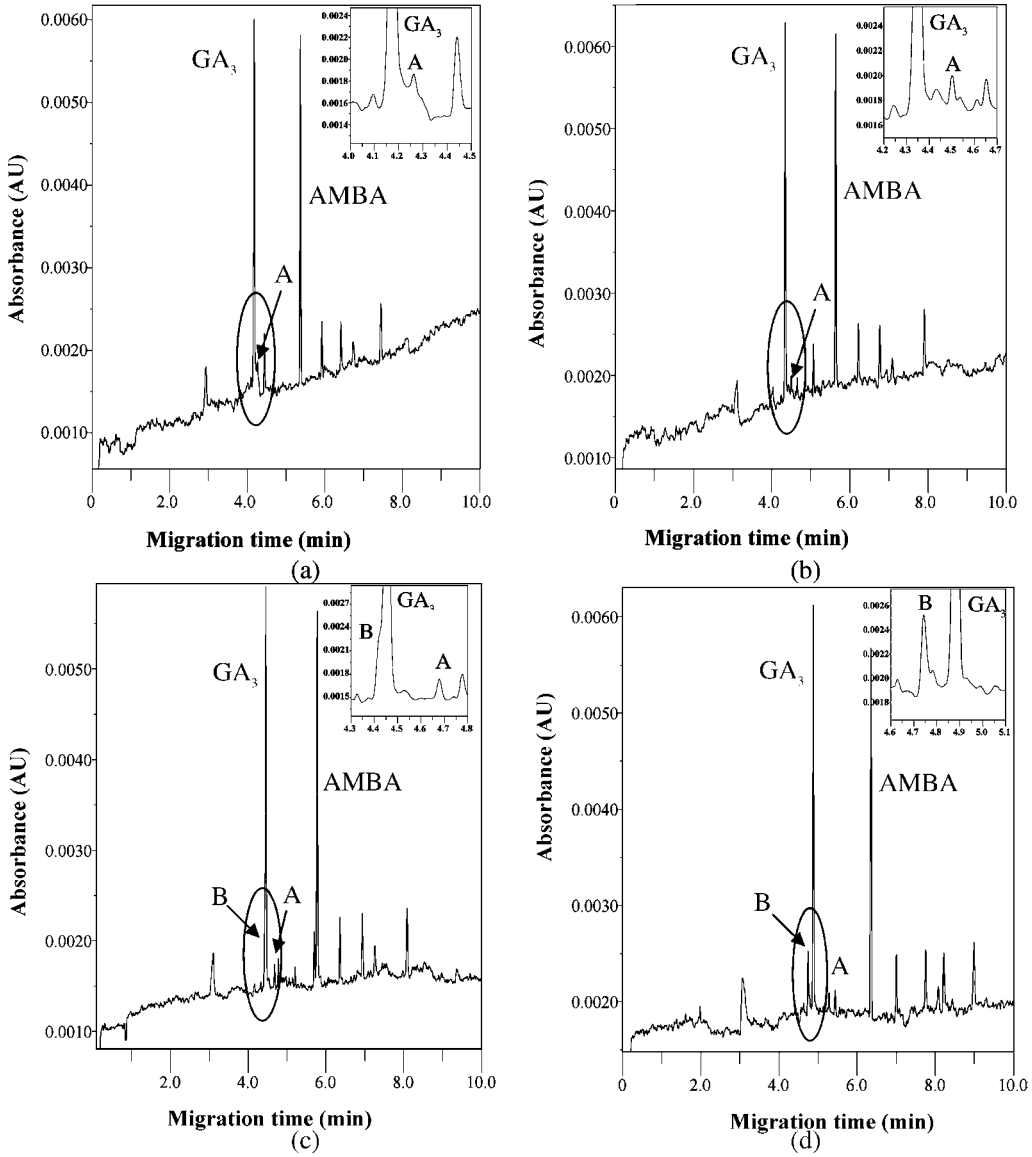

Figure 2. Example of MEKC electropherograms of the 10 day fermentation broth diluted 10 times using 25 mM $Na_2B_4O_7$ and the following SDS concentrations: (**a**) 0, (**b**) 20, (**c**) 40, and (**d**) 100 mM. CE conditions: uncoated fused silica capillary 50 μm i.d. × 57 cm (50 cm to detector); separation voltage, +30 kV; temperature, 25 °C; 0.5 psi pressure injection for 4 s; UV detection at 214 nm. GA_3, gibberellic acid; AMBA, 3-amino-4-methylbezoic acid; A, unknown; and B, unknown.

with the addition of either methanol or acetonitile, indicating a similar solubility of these compounds. This may be due to their similar structures of GA_3 and unknown B. It is thought that unknown B might be isoGA_3, a positional isomer of GA_3, obtained from isomeric hydrolysis of GA_3 (*21*). To confirm this, a standard solution of GA_3 was kept in a basic buffer for 2 days, and an unknown peak before GA_3 was found. By spiking this standard solution into a fermentation broth, the peak of unknown B with a greater peak area was obtained, indicating that these two unknowns should be the same compound.

Other peaks in the electropherogram of the broth may be GA_3 intermediates, byproducts, and compounds in media, hydrolyzed cotton seed hull and soybean oil. In comparison with the electropherogram of a GA_3 standard solution, peaks of the original compounds in food did not interfere with the GA_3 peak. For acidic fermentation with *G. fujikuroi*, the pathway for GA_3 biosynthesis from GA_{12}-aldehyde has been reported to be the following: GA_{12}-aldehyde → GA_{14}-aldehyde → GA_{14} → GA_4 → GA_7 → GA_3 (*1*, *2*). By spiking a GA_4 standard in the broth and using CE conditions as shown in **Figure 2d**, MEKC analysis showed that GA_4 completely separated from GA_3 and eluted before AMBA with high-resolution values of 21.5 and 9.0 for GA_3:GA_4 and GA_4:AMBA, respectively. In addition, GA_4 was not observed in the electropherogram of the broth. Because standards of other GA_3 intermediates and byproducts are not available, their migration times cannot be determined. However, these GA_3 intermediates and byproducts have different charges or structures with those of GA_3; therefore, they should have different retentions in MEKC. MEKC analysis of GA_3 in the broth was optimized by variation of the SDS concentration, type, and amount of organic solvent; therefore, we believe that GA_3 should be separated from other compounds in the broth. Because GA_3 is our main target, other components in the broth are not identified in this work. Identification and characterization of other components may be carried out using other techniques such as CE-MS, CE-MS-MS, and CE-NMR.

In MEKC, the resolution may be improved by variation of the buffer pH and the separation temperature or addition of other organic solvents. However, we prefer MEKC separation using a $Na_2B_4O_7$ solution, which is a buffer without the use of any

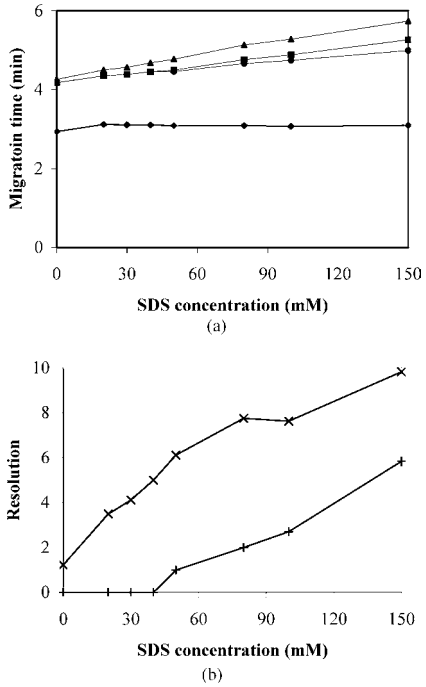

Figure 3. Effect of SDS concentration on (**a**) migration time of an EOF marker (◆), GA_3 (■), unknown A (▲), and unknown B (●) and on (**b**) resolution of GA_3:unknown A (×) and GA_3:unknown B (+). Other CE conditions are as shown in **Figure 2d**.

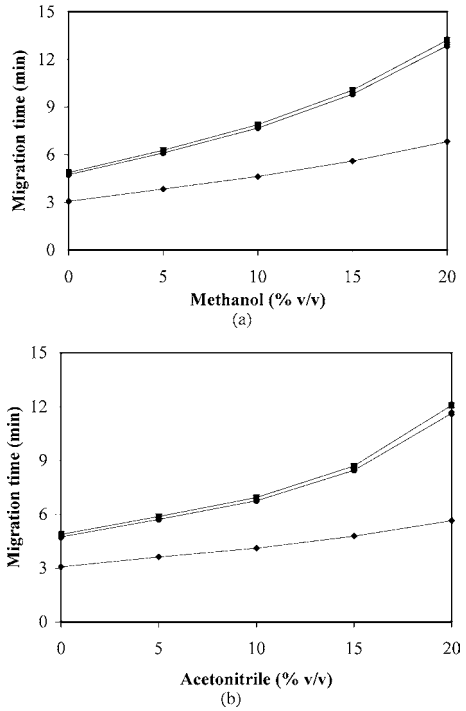

Figure 4. Effect of (**a**) methanol and (**b**) acetonitrile added in the MEKC buffer on migration time of an EOF marker (◆), GA_3 (■), and unknown B (●). Other CE conditions are as shown in **Figure 2d**.

reagent for adjusting pH, separation temperature of 25 °C near room temperature, and no organic solvent in the buffer. A decrease in the applied voltage from 30 to 25 kV for separation resulted in a longer analysis time and did not improve the resolution of GA_3 and unknown B. The baseline resolution of the GA_3 peak and unknown B obtained was 2.7, which is sufficient for quantitative analysis. Therefore, the following MEKC conditions were selected for determination of GA_3 in the fermentation broth: 25 mM $Na_2B_4O_7$ solution containing 100 mM SDS without any organic solvent.

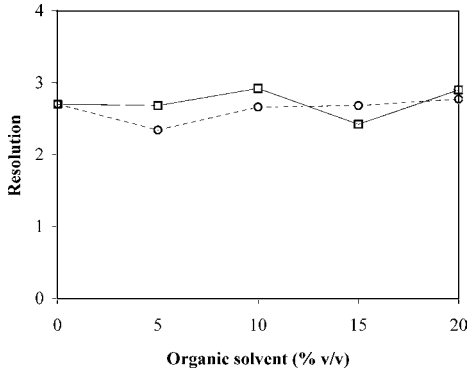

Figure 5. Effect of methanol (□) and acetonitrile (○) added in the MEKC buffer on resolution of GA_3 and unknown B. Other CE conditions are as shown in **Figure 2d**.

Table 1. CE Analysis of the Determined and Actual Amounts of GA_3 Spiked in Water and Fermentation Broths Diluted 10 Times

	amount of GA_3 (ppm)			
matrix	spiked	determined[a]	recovery (%)	RSD (%)
water	50.0	47.6 ± 1.9	95.1 ± 4.0	4.0
	140.0	136.0 ± 2.2	97.1 ± 1.5	1.6
diluted 5 days broth	50.0	48.8 ± 2.2	97.6 ± 4.4	4.5
diluted 10 days broth	50.0	47.6 ± 2.0	95.2 ± 4.1	4.3

[a] The average and standard deviation from 10 runs.

Validation of Method. The limit of detection (LOD), which is defined as the analyte concentration at a signal-to-noise ratio of 3 for GA_3, was found to be 5.0 ppm. The LOD may be improved by using a bubble cell capillary to increase the cell path length or large volume sample stacking as an on-line preconcentration (*15*). However, it is not necessary for this work where the amount of GA_3 in the fermentation broth is greater than 20 ppm. For a calibration plot, five standard solutions with concentration ranges of 20−200 ppm GA_3 in water were used, with 10 ppm AMBA as the internal standard. A calibration plot was constructed between the ratio of corrected peak area of GA_3 to AMBA, $A_{corr,ratio}$ (y) and the GA_3 concentration (x), where the corrected peak area is defined as the peak area divided by the migration time (*22*, *23*). The linear relationship gave the equation $y = 0.010x + 0.032$, with a high value of $r^2 = 0.9990$.

To determine accuracy and precision of the method and investigate the effect of the sample matrix on accuracy and precision, the known amounts of GA_3 spiked in water and fermentation broth diluted 10 times were separately prepared and determined by MEKC. Results of the amounts of GA_3 spiked in the water and diluted broths are listed in **Table 1**. The determined amount of GA_3 in water was directly obtained from the measurement of $A_{corr,ratio}$ and the calibration equation. The determined amount of GA_3 spiked in the diluted broth is obtained from the total determined amounts of GA_3 after spiking GA_3 subtracting the determined original amount of GA_3 in the diluted broth. From **Table 1**, the values of the recovery of 95−98% and relative standard deviation (RSD) < 5% indicate that a high accuracy and precision of the MEKC method are obtained, respectively. In addition, the sample matrix was found to cause no effect on the accuracy and precision of the method due to the same ranges of the recovery and RSD for GA_3 spiked in the water and the diluted broth.

Table 2. RSD of Migration Time (t_m), Electrophoretic Mobility (μ), and Corrected Peak Area of 100 ppm GA_3 (A_{corr})[a]

		t_m	μ	A_{corr}
% RSD	within day[b]	0.04 (4.879)	0.45 (–1.914)	1.54 (24.31)
(average)	between day[c]	0.65 (4.855)	0.56 (–1.914)	3.88 (25.22)

[a] The average values are in parentheses, and the units are min, 10^{-8} m^2 V^{-1} s^{-1}, and μAU for t_m, μ, and A_{corr}, respectively. [b] $n = 10$ runs. [c] $n = 5$ days.

Table 3. Amount of GA_3 in 10 Day Broth after Extraction

	amount of GA_3 (ppm) determined by		extraction recovery (%) determined by	
batch	MEKC[b]	HPLC[b]	MEKC	HPLC
1	495 ± 6	507 ± 2	55.4 ± 1.0	56.8 ± 0.9
2	488 ± 1	492 ± 3	54.6 ± 0.8	55.1 ± 0.9
3	481 ± 3	490 ± 3	53.9 ± 0.8	54.9 ± 0.9
average	488 ± 7	496 ± 8	54.7 ± 0.8	55.6 ± 0.9

[a] The amount of GA_3 in 10 day broth before extraction was 893 ± 13 ppm, determined by MEKC. [b] The average and standard deviation from three runs.

A GA_3 standard solution at 140 ppm was used to determine within day and between day precision of migration time, electrophoretic mobility, and corrected peak areas of GA_3. Results are shown in **Table 2**. For within day, each value was obtained from the 10 runs, while each value for between day was obtained from the average for triplicate runs for 5 days. For both within day and between day, the RSD values of t_m and μ were found to be less than 1.0%, indicating very high precision in the migration time and the electrophoretic mobility of GA_3 in MEKC. This is a benefit from the use of $Na_2B_4O_7$ as the buffer, which provides high capacity buffer and constant pH leading to stable EOF and electrophoretic mobility of analyte. A high precision of the values of A_{corr} within a day was obtained, with RSD < 2.0%. However, a poorer RSD (3.88%) for A_{corr} between day was observed, possibly because a fresh standard solution was used each day and variation of noise occurred. It should be noted that GA_3 can decompose; therefore, a fresh standard solution must be prepared for use each day.

A comparison of MEKC and HPLC was studied for determination of GA_3 in fermentation broth. As previously mentioned, HPLC analysis of GA_3 in the fermentation broth requires sample preparation by liquid extraction using ethyl acetate (*17*). To determine the recovery of GA_3 extraction, the amounts of GA_3 in 10 day fermentation broth before and after extraction were determined. The amount of GA_3 in 10 day fermentation broth before extraction was found to be 893 ± 13 ppm, determined by MEKC in triplicate runs. After that, the 10 day fermentation broth was divided into three batches and extracted with ethyl acetate using the procedure as described in section 2.4. After extraction, each solution was analyzed by HPLC and MEKC in triplicate runs. The amount of GA_3 was obtained by measuring peak area of GA_3 and using a calibration plot between the GA_3 peak area (y) as a function of the concentration of GA_3 standard solutions in the range of 200−1200 ppm, $y = 21191x + 3965$ with $r^2 = 0.9999$. **Table 3** shows a comparison of the amount of GA_3 in the broth after extraction, determined by HPLC and MEKC. Good agreement was obtained between the amount of GA_3 determined by these two separation methods. The extraction recovery was calculated to be ∼55%, obtained from the average of the amount of GA_3 determined by HPLC and MEKC. This indicates a loss of ∼45% GA_3 during the extraction. Therefore, direct MEKC analysis of GA_3 in the broth without any extraction should give the correct amount, in comparison with HPLC analysis via extraction.

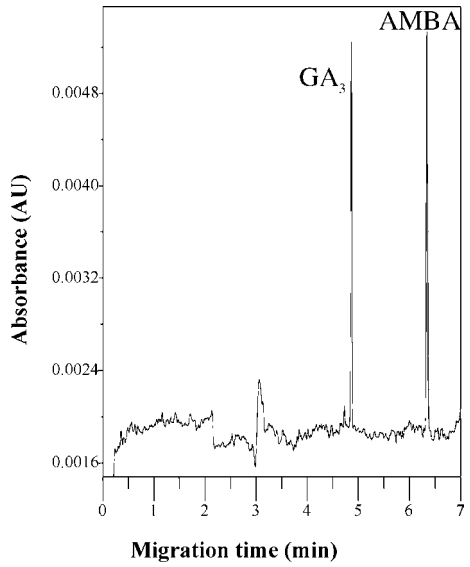

Figure 6. MEKC electropherogram of a solution of a CU Gibb tablet spiked 10 ppm AMBA. Other CE conditions are as shown in **Figure 2d**.

Table 4. GA_3 Content in Fermentation Broths and Commercial Products Determined by MEKC

sample	net weight or volume of products	GA_3 content unit	labeled	determined[a]
	(a) fermentation broth			
3 days		ppm		384 ± 21
5 days		ppm		697 ± 11
7 days		ppm		781 ± 13
10 days		ppm		893 ± 13
	(b) solid products of GA_3			
CU Gibb	1.43 g/tube	mg/tube	50	56.6 ± 2.0
CU Gibb	0.96 g/tablet	mg/tablet	100	106.4 ± 1.8
Nanto	1.6 g/tube	mg/tube	50	66.2 ± 1.0
Vimo Green	5.0 g/tablet	% w/w	1.34	0.97 ± 0.02
	(c) liquid products of GA_3			
Long Gibb	50 mL/bottle	% w/v	2.0	1.91 ± 0.05
Gibb Tree	150 mL/bottle	% w/v	2.0	1.75 ± 0.01
Siam Gibb	15 mL/bottle	% w/v	2.0	2.13 ± 0.01

[a] The average and standard deviation from three runs.

Application to Real World Samples. MEKC was also used to determine GA_3 in commercial products and to monitor the amount of GA_3 in fermentation broth for 3, 5, 7, and 10 days, using conditions shown in **Figure 2d**. The broth samples were filtered and diluted 10 times with water. For commercial products of GA_3, the appropriate amounts of samples were weighted and pipetted for solid and liquid products, respectively, and then dissolved in water. The appropriate dilution of sample solutions was performed. Each solution contained 10 ppm AMBA. **Figure 6** shows an example of an electropherogram of commercial products of GA_3 from a tablet of CU Gibb. The determined amounts of GA_3 in fermentation broths and commercial products are listed in **Table 4**. For the broth samples, the GA_3 content was found to increase ∼80% from 3 to 5 fermentation days and ∼15% from 5 to 7 and 7 to 10 fermentation days. The peak area and peak height of unknowns A, B, and others were found to increase with the longer duration of fermentation.

For samples of commercial GA_3 products, the determined amounts of GA_3 were mostly found to be in agreement with

the labeled amounts within ±15%. For our CU Gibb products, the determined amounts were obtained slightly higher than labeled amounts. This is due to the fact that the actual amount of GA_3 in CU Gibb products is typically 5−10% higher than the labeled amount in order to compensate for the decomposition of GA_3 during production and storage. For Vimo Green and Gibb Tree, the determined amounts are ∼26 and 13% less than the labeled amounts, respectively. This is possibly due to their actual amounts in these samples or decomposition of GA_3, which is consistent with the Vimo Green electropherogram containing a small peak of unknown B and another two unknown peaks. The solid sample of the CU Gibb tablet was found to contain unknown B, while no peak near the GA_3 peak for CU Gibb tube was seen. It should be noted that high temperature is used in the process of production of the CU Gibb tablets. This may be a reason for the decomposition of GA_3 in this sample.

In conclusion, MEKC has been shown to be an excellent method for the determination of GA_3 in the fermentation broth without any sample preparation except for filtration and dilution. For analysis of GA_3 by HPLC, sample preparation using solvent extraction is required, leading to ∼45% loss of GA_3. Another disadvantage of HPLC analysis is long time consumption, which is 30 min for sample preparation and 15 min for an HPLC run. In addition, advantages of the MEKC method include high accuracy and precision, short analysis time within 7 min, and low consumption of a buffer. Therefore, MEKC can be used as an alternative technique to HPLC for the determination of GA_3 in fermentation broth, in which the presence of the large amounts of ions and nutrients could add complication to quantitative analysis. Currently, the developed MEKC method is used to monitor GA_3 content in fermentation broths and to control the quality of our CU Gibb products.

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