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Determination of Aloesin and Aloeresin A for the Detection of Aloe in Beverages

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This work describes a sensitive high-performance liquid chromatography (HPLC) method for the quantification of aloesin and aloeresin A in alcoholic beverages containing aloe as a flavoring agent. The compounds were prepared from *Aloe ferox* juice. Sephadex LH20 and ion-exchange resin AG1X2 column chromatography were used for aloesin. Aloeresin A was obtained by Sephadex LH20 and silica gel column chromatography followed by purification on Discovery DSC-18 solid-phase extraction tubes. A 98 mg amount of aloesin (>99% purity) and 34 mg of aloeresin A (>98% purity) were recovered from 2.5 g of aloe juice. The HPLC method was validated, and intra- and interday performances were established. In-house validation was carried out by analyzing samples of beverages with and without aloe as a flavoring agent.

KEYWORDS: Aloesin; aloeresin A; *C*-glucosylchromones; aloe juice; hydroalcoholic beverages; *Aloe ferox*

INTRODUCTION

Aloe dried juice obtained from the leaves of several *Aloe* species (Liliaceae) is largely used in food and beverages for the aromatic and bitter taste. The pharmaceutical industry employes the dried juice for the laxative and cathartic effects of the anthraquinones. Other pharmacological activities have been attributed to *Aloe* spp., including a well-documented anti-inflammatory (*1*−*3*) immunomodulatory (*4*), and antigastric ulcer activities (*5*). In addition, *Aloe* spp. are largely used in foods and beverages as flavoring agents. Because of the adverse pharmacological effects of aloe constituents on consumers, the EEC listed aloin as a marker of *Aloe* occurrence in food and limited the amount of aloin to levels of 0.1 ppm in foods and beverages and 50 ppm in alcoholic beverages (European Community Directive 88/388).

The choice of aloin as a marker of aloe in beverages is inadequate since aloin is unstable in hydroalcoholic solutions, and the lack of a high-purity commercial standard may lead to controversial results when the concentration of aloin has to be determined (*6*). The degradation products include aloin dimers and trimers and aloe-emodin (*7*). Alternatively, aloe-emodin was suggested, but this choice is also inadequate due to the occurrence of the substance in other plant extracts used in the food and beverage industry, such as rhubarb (*8*, *9*). As alternative markers for the presence of aloe in alcoholic beverages, compounds of the group of 5-methylchromones aloesin and aloeresin A were suggested. These compounds were shown to be stable in hydroalcoholic solutions for at least 3 weeks, and they are specific to *Aloe* spp. (*6*).

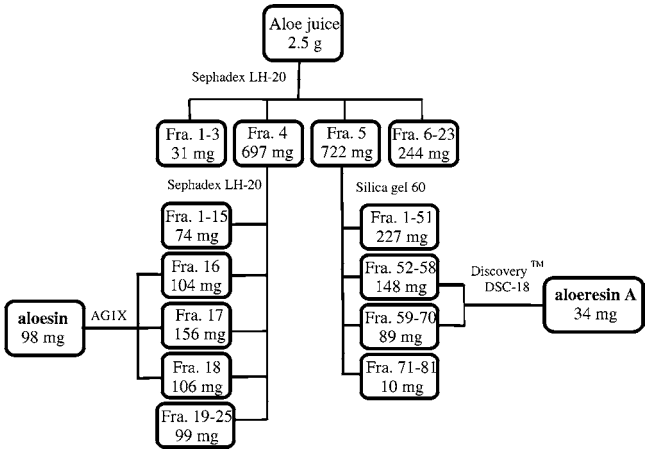

Figure 1. Scheme of the chromatography followed to isolate and purify aloesin and aloeresin A from aloe juice.

The present work describes the high-performance liquid chromatography (HPLC) method for quantification of aloesin (2-acetonyl-8-glucopyranosyl-7-hydroxy-5-methylchromone) and aloeresin A (*p*-coumaric ester of aloesin) in alcoholic beverages. The two compounds were not commercially available; hence, they were prepared in a pure form from *Aloe ferox* juice to serve

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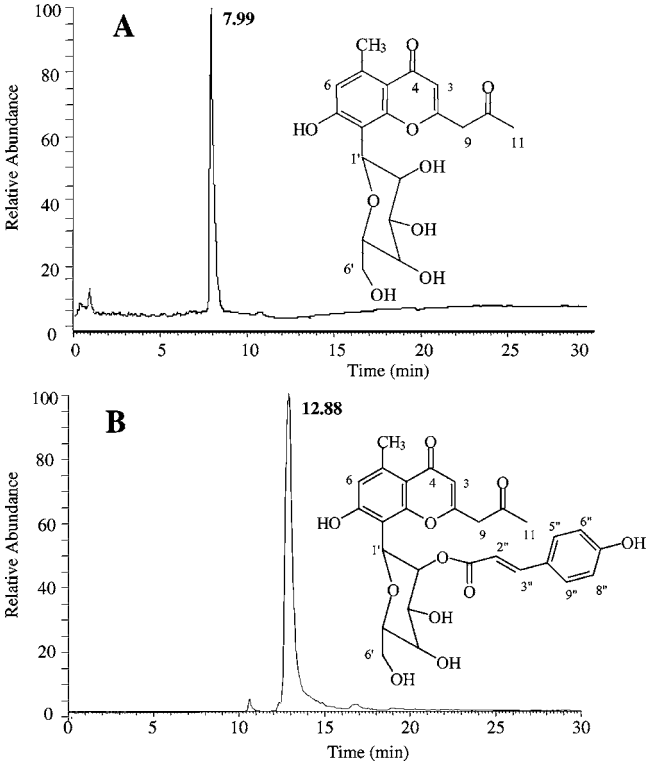

Figure 2. Total ion current and structure of aloesin (**A**) and aloeresin A (**B**).

Table 1. MS Data of Aloesin and Aloeresin A in Electrospray Ionization (ESI) Negative Mode

compound	molecular ion $[M-H]^-$	product ions
aloesin	393	273, 245, 231, 203
aloeresin A	539	393, 375, 273, 231

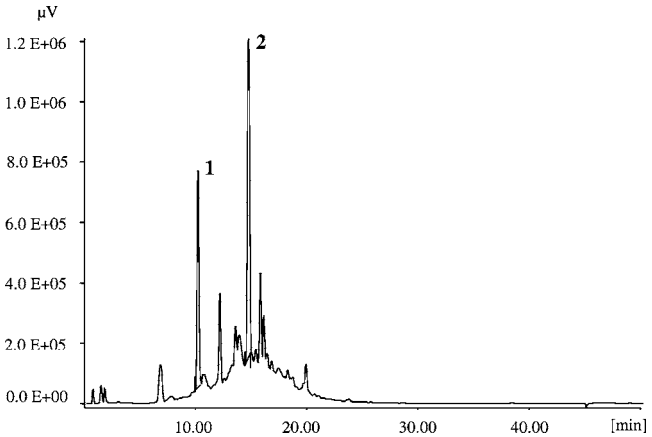

Figure 3. Representative HPLC-UV analysis of an alcoholic beverage containing aloe. Key: **1**, aloesin; **2**, aloeresin A.

as a standard for the calibration curves. For in-house validation of the method, the analytical procedure developed was applied to the determination of aloesin and aloeresin A in alcoholic beverages available on the Italian market.

MATERIALS AND METHODS

Materials. The juice of *A. ferox* from South Africa was supplied by Bauer Srl (Trieste, Italy). Commercial alcoholic beverages were commonly purchased from drugstores. Sephadex LH20 was from

Table 2. Intraday and Interday Precision and Accuracy of the HPLC Method for Aloesin Dissolved in Ethanol:Water 70:30

concentration prepared (ppm)	concentration found mean	CV (%)	error (%)
	intraday[a]		
5	5.09	2.3	1.9
10	9.70	1.3	–3.0
50	50.48	1.3	1.0
100	99.86	0.2	–0.1
	interday[b]		
5	5.15	0.8	3.0
10	9.84	1.4	–1.6
50	49.56	0.4	–0.9
100	100.22	0.1	0.2

[a] Values are the means of five replications/day. [b] Values are the means of five days of replications (one injection for each standard).

Table 3. Intraday and Interday Precision and Accuracy of the HPLC Method for Aloeresin A Dissolved in Ethanol:Water 70:30

concentration prepared (ppm)	concentration found mean	CV (%)	error (%)
	intraday[a]		
5	4.54	0.7	–9.2
10	9.75	0.3	–2.5
50	52.09	0.3	4.2
100	99.00	0.1	–1.0
	interday[b]		
5	4.77	4.9	–4.6
10	9.80	2.0	–2.0
50	51.22	2.3	2.4
100	99.42	0.6	–0.6

[a] Values are the means of five replications/day. [b] Values are the means of five days of replications (one injection for each standard).

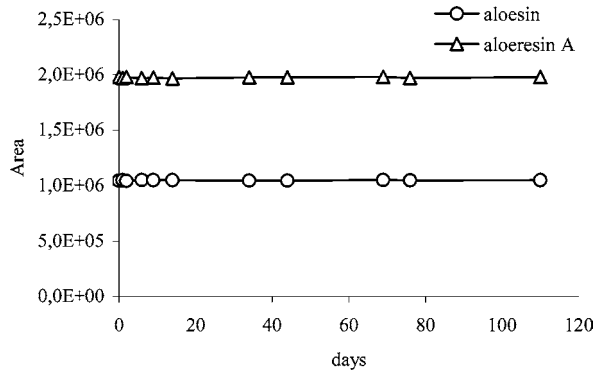

Figure 4. Peak areas of aloesin and aloeresin A in alcoholic solution at different time intervals.

Pharmacia (Uppsala, Sweden), and silica gel-60 (230−400 mesh), silica gel 60 F_{254} thin liquid chromatography (TLC) plates, and all solvents of analytical grade were from Merk (Darmstadt, Germany); HPLC grade water was produced using a Milli Q apparatus (Millipore, Billerica, MA). Anionic exchange resin AG1-X2 (200−400 mesh) was obtained from Bio-Rad (Hercules, CA), and Discovery DSC-18 tubes were from Supelco Inc. (Bellefonte, PA).

Isolation and Identification of Aloesin and Aloeresin A. The isolation of aloesin and aloeresin A was performed as described in **Figure 1**. A 2.5 g amount of aloe juice dissolved in 5 mL of methanol was loaded on Sephadex LH20 (200 g, column Ø 3 cm). Twenty-three fractions of 150 mL of methanol were eluted, taken to dryness under N_2, and analyzed by TLC on silica gel F254 plates, using ethyl acetate: methanol:water 70:13.5:10 (v:v:v) as the elution system. By following the blue fluorescence at 254 nm (*10*), aloesin (MW 394) and aloeresin

Table 4. Best Fit Values and Goodness of Fit of Interday Calibration Curves

standard	slope ± SD	IC 95% (%)	CV (%) slope	intercept ± SD	*r*	*F*	Sy.x
aloesin	12850 ± 21	12730–12980	0.2	−2297 ± 954	1.0000	111000	3269
aloeresin A	15182 ± 182	14710–15650	1.2	3346 ± 3837	0.9997	10630	12470

Table 5. Intraday and Interday Precision and Accuracy of the HPLC Method for Aloesin Added to Commercial Matrices

matrix	concentration prepared (ppm)	concentration found mean	CV (%)	error (%)
		intraday[a]		
1	5	5.06	6.3	1.3
	10	9.42	4.4	−5.8
	50	50.66	0.6	1.3
	100	99.72	0.2	−0.3
2	5	5.23	1.5	4.5
	10	9.61	0.5	−3.9
	50	49.78	0.9	−0.4
	100	100.10	0.2	0.1
		interday[b]		
1	5	5.43	6.0	8.6
	10	9.48	2.7	−5.2
	50	49.47	1.2	−1.1
	100	100.31	0.3	0.3
2	5	5.16	0.9	3.1
	10	9.54	0.8	−4.6
	50	50.23	0.7	0.5
	100	99.92	0.2	−0.1

[a] Values are the means of five replications/day. [b] Values are the means of five days of replications (one injection for each point).

Table 6. Intraday and Interday Precision and Accuracy of the HPLC Method for Aloeresin A Added to Commercial Matrices

matrix	ppm added	concentration found mean	CV (%)	error (%)
		intraday[a]		
1	5	4.61	0.3	−7.8
	10	9.80	0.3	−2.0
	50	51.70	0.2	3.4
	100	99.19	0.0	−0.8
2	5	4.83	6.1	−3.3
	10	10.10	1.7	1.0
	50	50.05	1.2	0.1
	100	100.01	0.3	0.0
		interday[b]		
1	5	5.05	5.0	1.0
	10	9.53	3.3	−4.7
	50	51.50	0.5	3.0
	100	99.30	0.1	−0.7
2	5	5.00	5.4	0.1
	10	10.01	1.4	0.1
	50	50.39	0.3	0.8
	100	99.81	0.1	−0.2

[a] Values are the means of five replications/day. [b] Values are the means of five days of replication (one injection for each point).

A (MW 540) were found predominantly in fractions 4 (696 mg) and 5 (722 mg), respectively. The presence of the two compounds was confirmed by HPLC−mass spectrometry (MS) analysis.

Fraction 4 was further fractionated on Sephadex LH20 (200 g, column Ø 3 cm), eluting with methanol. Twenty-five fractions were eluted and analyzed by TLC. Aloesin eluted in fractions 16 (103.5 mg), 17 (156 mg), and 18 (106 mg), as confirmed by HPLC-MS. Fractions 16−18 were collected, dissolved in 100 mL of distilled water, and added with 5 g of AG1X2 resin under stirring for 30 min. After centrifugation, aloesin was detected in the supernatant, which was taken to dryness and weighed. This step yielded 97.5 mg of aloesin. The purity (99%) of the compound was determined by TLC and HPLC-MS analysis. The total ion current (TIC) of aloesin is reported in **Figure 2A**. Fraction 5 obtained from the first Sephadex column was further fractionated on silica gel 60 column (250 g, column Ø 4 cm). Eighty-two Fractions were eluted with 50 mL of chloroform:methanol 9:1 (v:v). Fractions 52−58 (148 mg) and fractions 59−70 (89.1 mg), containing aloeresin A as shown by TLC and HPLC-MS analysis, were collected. Aloeresin A was further purified by chromatography on Discovery DSC-18 prepacked columns (5 g), eluting with a 5% stepwise gradient of methanol:water from 70:30 to 45:55. Aloeresin A eluted with methanol: water 40:60. This step yielded 34 mg of pure aloeresin A (98%), as checked by TLC and HPLC-MS analysis. The TIC of aloeresin A is reported in **Figure 2B**. Chemical identification of aloesin and aloeresin A was established by NMR and MS analysis. ^{1}H NMR (500 MHz) were recorded at 298 K with a Bruker AVANCE-DRX 500 spectrometer in MeOH-d_4 solutions. ^{1}H NMR data were consistent with the chemical shifts previously reported (*10*, *11*).

MS data giving molecular ions and *m*/*z* values of the major fragments are shown in **Table 1**. MS data were obtained using an LCQ mass spectrometer (Thermo Finnigan, San Jose, CA) equipped with an ES ion trap. Instrument control and data acquisition and processing were performed using a Microsoft Windows-NT-based LCQ Navigator software. The operating parameters were as follows: source voltage, 5 kV; capillary voltage, −20 V; capillary temperature, 200 °C; collision energy, 10% of 5 V. The instrument was interfaced with a P 4000 Thermo Separation Products HPLC System. The column and analytical conditions were the same used for the HPLC-UV analyses.

HPLC-UV Analysis of Aloesin and Aloeresin A. HPLC-UV was carried out with a Jasco instrument PU 980 equipped with a Jasco UV detector mod 870 (Jasco Europe, Cremella, Italy) and column furnace Jones chromatography (Pontypridd, United Kingdom). A reverse phase column Purosphere RP-18, 12.5 cm × 0.4 cm, 5 μm with a 0.4 × 0.4 i.d. (5 μm) with a precolumn Purosphere (Merck) was used. Analysis conditions were as follows: column temperature, 40 °C; flow, 1 mL/min; detection wavelength, 300 nm; mobile phase, gradient of water: acetonitrile from 99:1 to 1:99 in 36 min, 10 μL (1 μL for aloe infusions) of sample was injected using a model AS950 Jasco autosampler. Chromatographic data were acquired using a J.M.B.S. Borwin software version 1.21.60 software (Grenoble, France).

Validation of the HPLC Method for Aloesin and Aloeresin A Determination. Samples containing a linear range of concentrations of aloesin or aloeresin A dissolved in ethanol:water 70:30 (0−100 ppm) were analyzed five times a day and repeatedly for 5 days to determine the intra- and interday precisions [expressed as coefficient of variation (CV), %] and accuracy (error, %) of the calibration curves. Fresh solutions were prepared every day. Calibration curves were calculated by plotting ppm of aloesin/aloeresin A against the area of the corresponding peak. The calculation of the curves included origin. A one-way analysis of variance test was used for statistical analysis. The method was also validated by preparing calibration curves spiking two different commercial matrices containing aloesin or aloeresin A with 5−100 ppm of each compound. Samples were filtered on 0.45 μm filters before injection.

Evaluation of Aloesin and Aloeresin A Stability in Alcoholic Beverages. The stability of the two analytes in alcoholic beverages was established by keeping aliquots of an alcoholic beverage at 40 °C for 110 days and comparing the corresponding peak areas at different times against the area at time 0. The HPLC method and calibration curves using external standard were as described above.

Determination of Aloesin and Aloeresin A in Hydroalcoholic Infusions and Commercial Beverages. Alcoholic infusions were prepared from 12.5 g of dried aloe juice (seven batches) treated with 120 mL of hot distilled water; infusion lasted for 48 h; afterward, 300

Table 7. Best Fit Values and Goodness of Fit of Interday Calibration Curves in Commercial Matrices

standard	matrix	slope ± SD	CI 95% slope	CV (%) slope	intercept ± SD	r	F	Sy.x
aloesin	**1**	12874 ± 107	12600–13150	0.8	953116 ± 2344	0.9999	22580	7259
	2	12788 ± 72	12630–12950	0.5	85163 ± 1213	1.0000	65780	4225
aloeresin A	**1**	15466 ± 68	14870–16060	0.4	506476 ± 3551	0.9998	6763	15940
	2	15291 ± 135	18540–18690	0.9	2388379 ± 8203	0.9999	613500	2014

Table 8. Levels of Aloesin and Aloeresin A in Alcoholic Beverages[a]

alcoholic beverages	aloesin ppm (mean ± SD)	CV (%)	aloeresin A ppm (mean ± SD)	CV (%)
A ($n = 10$)	70.0 ± 6.2	8.9	67.6 ± 5.8	8.6
B ($n = 10$)	6.8 ± 0.6	8.8	7.4 ± 0.5	6.8
C ($n = 2$)	426.0		453.0	
D ($n = 2$)	852.0		804.6	
E ($n = 2$)	ND		ND	
F ($n = 2$)	ND		ND	

[a] A–D contain aloe; E and F do not contain aloe; and ND, not detectable.

mL of 96% ethanol was added, and the mixture was kept for 13 days at room temperature, stirring every 2 days. Samples were filtered on paper before analysis by HPLC. Six alcoholic beverages present on the Italian market were chosen according to the occurrence and absence of aloe in their compositions, and they were analyzed. A representative profile of the HPLC-UV chromatogram of an alcoholic beverage is reported in **Figure 3**.

RESULTS

Validation of the Analytical Procedure for the Determination of Aloesin and Aloeresin A. The HPLC procedure was validated to establish the intra- and interday accuracy (CV %) and precision (error %) for solutions of aloesin and aloeresin A made in ethanol:water, 70:30 (v/v). Results are shown in **Tables 2** and **3**. Best fit values and goodness of fits of interday calibration curves are shown in **Table 4**. The detection limit for aloesin and aloeresin A was 0.15 ppm, which represents the intensity of the signal three-fold higher than the background noise. The limit of quantification for both of the compounds was 0.5 ppm, calculated as the minimum amount that can be determined with a precision <20% (expressed as CV %, which was 9.4 and 8.5 for aloesin and aloeresin A, respectively).

Analyses were also carried out on samples prepared by adding known amounts (5−100 ppm) of aloesin and aloeresin A to beverages containing aloe infusions as flavoring agents (matrices 1 and 2). Results are shown in **Tables 5** and **6**. Best fit values and goodness of fits of interday calibration curves in commercial matrices are shown in **Table 7**.

Stability of Aloesin and Aloeresin A in Alcoholic Beverages. The stability for aloesin and aloeresin A was assessed as described in the Materials and Methods. The areas of the peaks at T_0 and T_{110} were 1041203−1045964 for aloesin and 1977435−1978284 for aloeresin A. From the peak areas, the values of ppm at T_0 and T_{110} were calculated (81 ppm of aloesin and 130 ppm of aloeresin A at both times). **Figure 4** shows the peak areas for both compounds at all time intervals. Therefore, no decay was observed under these conditions.

Levels of Aloesin and Aloeresin A in Alcoholic Infusions and Commercial Beverages. The content of aloesin and aloeresin A in seven batches of aloe infusion was 9267 ± 1162 ppm (mean ± SD, CV % = 12.5) for aloesin and 9341 ± 630 ppm (mean ± SD, CV % = 6.7) for aloeresin A.

The levels of aloesin and aloeresin A in different alcoholic beverages purchased from various drugstores are reported in **Table 8**. As expected, no trace of the two analytes was detected in the alcoholic beverages E and F not containing aloe.

DISCUSSION

The juice of *Aloe* spp. (Liliaceae) is widely used in the food and beverages industry for the bitter taste and its aromatic properties. According to our results, for the regulatory aspects, methylchromones aloesin and aloeresin A, specific for *Aloe* spp., can be reasonably suggested as markers alternative to aloin to detect the presence of aloe in foods and beverages. In comparison to aloin, according to our results, the amount of both substances in alcoholic solution at 40 °C is stable for more than 3 months. If the European Community (EC) considers the revision of the list of restricted compounds, a reliable method validated to measure aloesin and aloeresin A will become necessary. At this aim, we developed an HPLC method that can be employed for the identification of aloe extracts in drinks and for the quantitative evaluation of the compounds proposed as alternative markers.

Aloesin and aloeresin A are not commercially available; thus, they have been prepared from the plant extracts in a pure form to serve as a standard for the calibration curves. The HPLC-UV method reported in this work has good precision (error <10%), good accuracy, and adequate sensitivity (limit of quantification = 0.5 ppm) for the determination of the two substances in beverages.

As requested, the method is able to distinguish beverages with and without aloe in their composition. The content of aloesin and aloeresin A in four different alcoholic drinks containing aloe varied greatly from 7 to 800 ppm, whereas no traces of the two compounds were found in two beverages lacking aloe.

In conclusion, the present work suggests the use of aloesin and aloeresin A as new markers, specific and stable, for aloe determination in foods and beverages. An HPLC-UV method that can serve food control laboratories when monitoring these compounds might be required in the future and is also reported.

ABBREVIATIONS USED

EC, European Community; HPLC, high-performance liquid chromatography; TLC, thin-liquid chromatography; MS, mass spectrometry; ESI, electrospray ionization.

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LITERATURE CITED

(1) Langmead, L.; Makins, R. J.; Rampton, D. S. Anti-inflammatory effects of aloe vera gel in human colorectal mucosa in vitro. *Aliment Pharmacol. Ther.* **2004**, *19*, 521−527.

(2) Yagi, A.; Kabash, A.; Okamura, N.; Haraguchi, H.; Moustafa, S. M.; Khalifa, T. I. Antioxidant, free radical scavenging and anti-inflammatory effects of aloesin derivatives in Aloe vera. *Planta Med.* **2002**, *68*, 957−960.

(3) Speranza, G.; Morelli, C. F.; Tubaro, A.; Altinier, G.; Duri, L.; Manitto, P. Aloeresin I, an anti-inflammatory 5-methylchromone from cape aloe. *Planta Med.* **2005**, *71*, 79−81.
(4) t'Hart, L. A.; van den Berg, A. J.; Kuis, L.; van Dijk, H.; Labadie, R. P. An anti-complementary polysaccharide with immunological adjuvant activity from the leaf parenchyma gel of Aloe vera. *Planta Med.* **1989**, *55*, 509−512.
(5) Eamlamnam, K.; Patumraj, S.; Visedopas, N.; Thong-Ngam, D. Effects of Aloe vera and sucralfate on gastric microcirculatory changes, cytokine levels and gastric ulcer healing in rats. *World J. Gastroenterol.* **2006**, *12*, 2034−2039.
(6) Zonta, F.; Bogoni, P.; Masotti, P.; Micali, G. High-performance liquid chromatographic profiles of aloe constituents and determination of aloin in beverages, with reference to the EEC regulation for flavouring substances. *J. Chromatogr. A* **1995**, *718*, 99−106.
(7) Shindo, T.; Ushiyama, H.; Kan, K.; Uehara, S.; Yasuda, K.; Takano, I.; Saito, K. Contents of barbaloin-related compounds in aloe drinks and their change during storage. *Shokuhin Eiseigaku Zasshi* **2002**, *43*, 122−126.
(8) Komatsu, K.; Nagayama, Y.; Tanaka, K.; Ling, Y.; Basnet, P.; Meselhy, M. R. Development of a high performance liquid chromatographic method for systematic quantitative analysis of chemical constituents in rhubarb. *Chem. Pharm. Bull. (Tokyo)* **2006**, *54*, 941−947.
(9) Gong, Y. X.; Li, S. P.; Wang, Y. T.; Li, P.; Yang, F. Q. Simultaneous determination of anthraquinones in Rhubarb by pressurized liquid extraction and capillary zone electrophoresis. *Electrophoresis* **2005**, *26*, 1778−1782.
(10) Holdsworth, D. K. Chromones in aloe species. I. Aloesin—A C-glucosyl-7-hydroxychromone. *Planta Med.* **1971**, *19*, 322−325.
(11) Gramatica, P.; Monti, D.; Speranza, G.; Manitto, P. Aloe revisited. The structure of aloeresin A. *Tetrahedron Lett.* **1982**, *23*, 2423−2424.

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