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Determination of aloenin, barbaloin and isobarbaloin in *Aloe* species by micellar electrokinetic chromatography

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Abstract

Aloenin, barbaloin and isobarbaloin in JP Aloe[1], *Aloe barbadensis* (*Aloe vera*) and *Aloe arborescens* Miller var. *natalensis* Berger (*Aloe arborescens* Miller) were determined by micellar electrokinetic chromatography (MEKC) with 50 m*M* sodium dodecyl sulfate. Aloenin, barbaloin and isobarbaloin were well separated by MEKC and as little as 5.5 pg/11 nl of the three compounds could be detected. The determination took around 14 min. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Aloenin; Barbaloin; Isobarbaloin

1. Introduction

The *Aloe* species used in pharmaceuticals, folk medicine, and food products have been mostly Cape Aloes, *Aloe vera*, and *Aloe arborescens*. Among the components of *Aloe*, the anthranoids barbaloin and isobarbaloin (Fig. 1, major components of the commercial product, aloin, are barbaloin and isobarbaloin), are the major low molecular mass components, and have been used as purgatives [1–3]. Besides these anthranoids, *Aloe arborescens* also contains aloenin (Fig. 1) as a major component in about the same quantity as the total of barbaloin and isobarbaloin. These compounds have been mostly analyzed by reversed-phase HPLC [4–7].

Capillary electrophoresis (CE), a relatively new separation technique, has been recently applied for the analysis of many compounds in plants, foods and

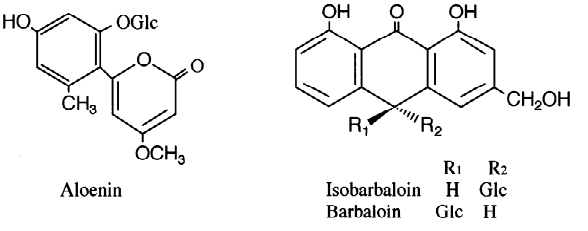

Fig. 1. Structures of aloenin, barbaloin and isobarbaloin.

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[1]JP Aloe means a product based on the Japanese Pharmacopeia and is the dried juice of *Aloe ferox* Miller or its hybrid with *Aloe africana* Miller or *Aloe spicata* Baker, which are the so-called Cape Aloes.

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drugs [8–22]. CE has high separation efficiency, requires only small amounts of samples and running buffers, and the time for analysis by CE may be shorter than that for HPLC. Furthermore, CE does not require organic solvents for running buffers, which could be a source of serious environmental pollution when disposed of, and its equipment is easy to operate and maintain. CE is therefore a good method for such uses as routine tests on materials and products for quality control.

Recently, CE has been employed for the analysis of sennosides, a kind of anthranoids, in commercial formulations [23,24] and some other plant components in drugs [25,26]. In the present study, we determined the aloenin, barbaloin and isobarbaloin in *Aloe* leaves, JP aloe and commercial health foods containing *Aloe arborescens* by means of micellar electrokinetic chromatography (MEKC) [27] using CE apparatus, and compared the results with those for HPLC.

2. Experimental

2.1. Chemicals and materials

The following chemicals were used: Sodium dodecyl sulfate (SDS) (Bio-Rad, Richmond, CA, USA); barbaloin (Sigma, St. Louis, MO, USA); isobarbaloin (purified from purchased aloin); aloenin (Aloe Pharmaceutical Co. Ltd., Shizuoka, Japan); JP Aloe (Suzu Pharmaceutical Co. Ltd., Osaka, Japan).

All other chemicals were of analytical reagent grade, and purchased from Wako Pure Chemical (Osaka, Japan). *Aloe arborescens* and *Aloe vera* plants were cultivated in the herb garden at our Institute.

The following health food products containing *Aloe arborescens* as their major constituent were analysed: Yurikaron (Yurika Co. Ltd., Hisai, Mie, Japan); Kidachi-Aloe-Matsu (Kowa Rimiteddo Co. Ltd., Tokyo, Japan); Kidachi-Aloe-Funmatsu (Sokensha, Yokohama, Kanagawa, Japan); Kidachi-Aloe-Ekisuryu (Minami Health Foods, Co. Ltd., Kumagaya, Saitama, Japan); Kidachi-Aloe-Ryu (Sapurikkusu, Co. Ltd., Fujioka, Gunma, Japan).

2.2. Apparatus

2.2.1. Capillary electrophoresis

Capillary electrophoresis was performed with a Capillary Electrophoresis Measuring System CAPI-3200 equipped with a multichannel UV–Vis detector (Otsuka Electronics, Osaka, Japan) and an uncoated fused-silica capillary with an internal diameter of 75 μm and length of 38 cm (to the detector. overall length, 50 cm. Otsuka Electronics, Osaka, Japan). Samples were introduced by syphoning for 20 s and the capillary was arranged so that there was a difference of 25 mm in height between the sampling and running buffer sides.

2.2.2. Analytical HPLC for determination

The HPLC system for the determination of the compounds of interest consisted of two Shimadzu (Kyoto, Japan) LO-10AT pumps equipped with a Shimadzu SCL-10A system controller connected to a Shimadzu column oven, CTO-10A, and a Shimadzu diode array detector, SPD-M10AV. Samples were injected with a Model 7125 (Rheodyne, Cotati, CA, USA) sample valve equipped with a 100-μl loop. Peak areas were calculated with a Shimadzu work station (CLASS-LO10/M10A). A degasser (Shimadzu DGU-4A) for mobile-phase buffers was attached to the pumps.

2.2.3. Preparative HPLC for isolation

The HPLC for the isolation of isobarbaloin was performed using a preparative HPLC system (Tosoh, Tokyo, Japan) consisting of a pump (CCPP-M) equipped with a system controller (SC-8020) connected to a column oven (CO-8020), a spectrophotometer (UV-8020) and a fraction collector (FC-8020). Samples were injected with a Model 7125 (Rheodyne, Cotati, CA, USA) sample valve equipped with a 500-μl loop. A degasser (Tosoh, SD-8022) for mobile-phase buffers was attached to the pump.

2.3. Conditions of determination

2.3.1. MEKC

MEKC was performed using a running buffer consisting of 50 m*M* SDS solution in 25 m*M* sodium

tetraborate, pH 9.4 (in most case, pH 9.42). The injection volume was 11 nl. Samples were electrophoresed at 37°C and 12 kV with an average current of 70 μA and absorbance at 293 nm was measured for the determination.

Before each run, the capillary was rinsed with a 1.0 *M* NaOH solution for 120 s, and pure water purified with a Milli-Q system (Millipore, Bedford, MA, USA) for 120 s. Thereafter the capillary was refilled with running buffer for more than 180 s. These solutions were introduced by syphoning as the same manner as described in Experimental.

2.3.2. Reversed-phase HPLC for determination

Reversed-phase HPLC was performed referring to the methods described by Okamura et al. [6], Zonta et al. [5] and Park et al. [7], according to the following conditions by the procedure mentioned below:

Column: Inertsil ODS-2 column (4.6×150 mm, GL. Sciences Inc.; Tokyo, Japan); mobile phase: water–methanol; elution profile: 0 min, 75:25; 10 min, 75:25; 25 min, 60:40; 35 min, 60:40; 45 min, 50:50, 65 min, 0:100; 75 min, 0:100. Chromatography was performed at 40°C with a flow-rate of 1.0 ml/min, and detection was at 293 nm. A total of 10 μl of samples were injected in the usual manner.

2.4. Preparative reversed-phase HPLC for isolation of isobarbaloin

Preparative reversed-phase HPLC was performed refering to methods described by Ishii et al. [28] as follows:

A methanol solution of aloin was injected onto a TSK-GEL ODS-8OTs column (21.5×300 mm, Tosoh, Tokyo, Japan) incorporated in a preparative HPLC system (Tosoh, Tokyo, Japan) and the separation was performed according to the following conditions by the procedure mentioned below: Mobile phase: methanol–water; elution profile: 0 min, 60:40; 25 min, 80:20; 26 min, 60:40; 40 min, 60:40; flow-rate: 5 ml/min; temperature: 40°C; detection: 293 nm. The isobarbaloin fraction was collected and rechromatographed after concentration of the fraction in the same manner as described above for further purification. A total of 500 μl of samples were injected in the usual manner.

2.5. Sample preparation

The fresh leaves of *Aloes* were homogenized using a Polytron (Kinematica, GmbH, Switzerland) without any additional solvents and filtered through a Whatman GF/A paper (Maidstone, Kent, UK). Then the filtrate was concentrated by an Amicon hollow fiber dialyzer-concentrator DC-10L (Grace Japan, Tokyo, Japan) equipped with an ultrafiltrating membrane with a rejection limit of over 10 000 molecular mass. The filtrate was lyophilized.

One ml of methanol was added to 100 mg of the lyophilized powder (*Aloe vera* and *Aloe arborescens*), JP Aloe and commercial health foods containing *Aloe arborescens* to extract components. The mixture was vortexed for 15 min and then centrifuged at 18 000 *g* for 10 min. The supernatant was filtered through a 0.22 μm-pore size membrane and the filtrate was diluted to one-one hundredth of the starting concentration with pure water prior to use.

3. Results and discussion

3.1. Separation profiles of standards

Standard solutions of aloenin, the isobarbaloin isolated by the procedure described in Experimental and barbaloin were prepared by dissolving these substances in methanol at a concentration of 5 mg/ml and filtering through a 0.22 μm-pore size membrane. The filtered solutions were diluted with pure water prior to use.

MEKC was conducted with SDS concentrations ranging between 25 and 100 m*M*. A concentration of 50 m*M* was adopted for this study, because good separation was not obtained at the 25 m*M* concentration, though the separation time was shorter at 25 m*M*.

The standard solutions were analyzed as authentic samples to observe the separation profile and typical results for MEKC and HPLC are shown in Figs. 2 and 3, respectively. Injection volumes were typically 11 nl for MEKC and 10 μl for HPLC. Good

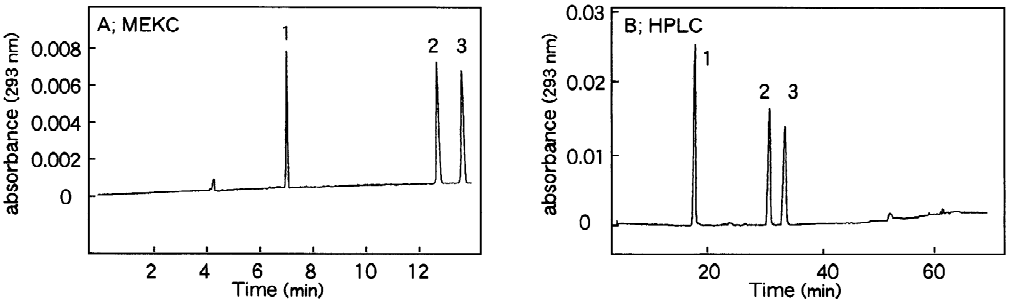

Fig. 2. Separation of standard compounds by MEKC(A) and HPLC(B). 1=aloenin, 2=isobarbaloin, 3=barbaloin. MEKC conditions: capillary, uncoated fused-silica (75 μm×50 cm); running buffer; 50 m*M* SDS solution in 25 m*M* sodium tetraborate; temperature, 37°C; applied voltage, 12 kV; detection at 293 nm. HPLC conditions: column, Inertsil ODS-2 column (4.6×150 mm, GL. Sciences Inc., Tokyo, Japan), mobile phase, water–methanol; elution profile, 0 min, 75:25; 10 min, 75:25; 25 min, 60:40; 35 min, 60:40; 45 min, 50:50, 65 min, 0:100; 75 min, 0:100. Chromatography was performed at 40°C with a flow-rate of 1.0 ml/min, and detection was at 293 nm.

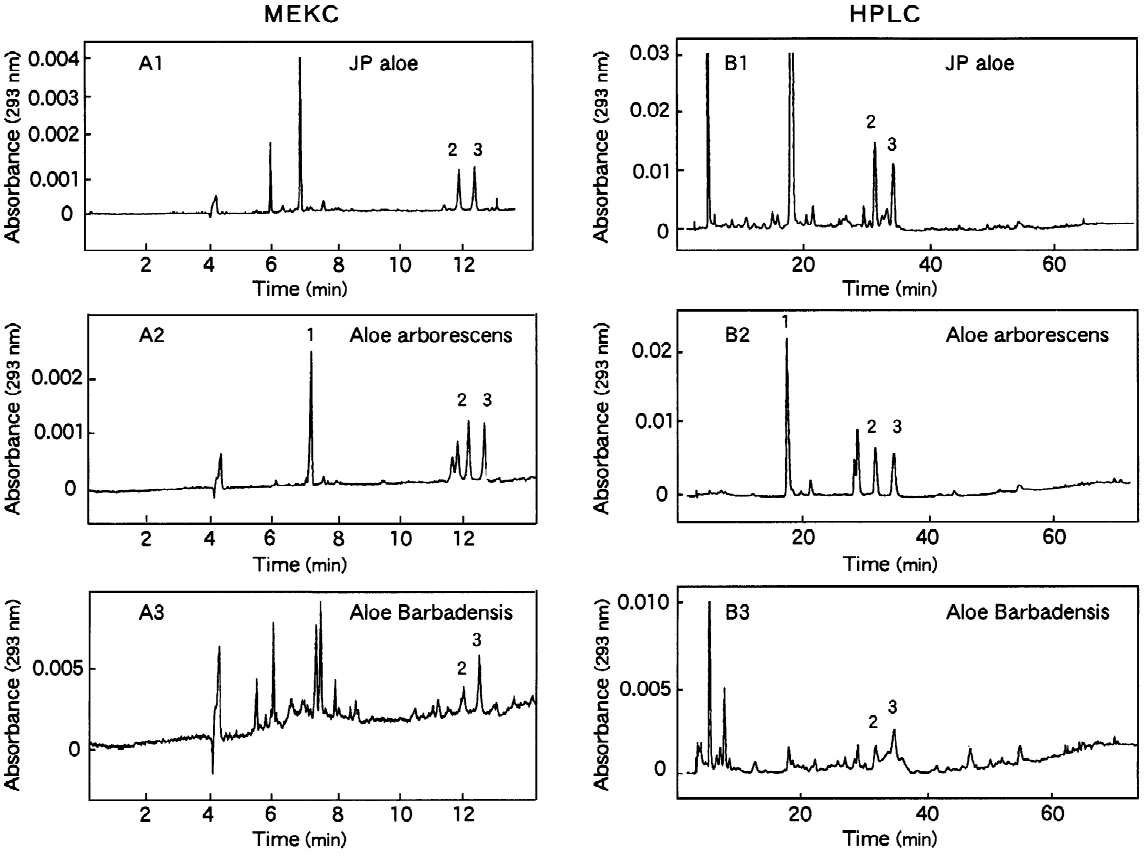

Fig. 3. Separation profiles of aloe components by MEKC (A1, A2, A3) and HPLC (B1, B2, B3). 1=aloenin, 2=isobarbaloin, 3=barbaloin. Conditions of MEKC and HPLC are the same as in Fig. 2.

separations were obtained by the methods described in Experimental. Aloenin, isobarbaloin and barbaloin were separated in this order by MEKC and HPLC. The separation time for barbaloin was about 14 min by MEKC and 35 min by reversed-phase HPLC, and amounts of each sample which could be detected were as little as 5.5 pg in 11 nl (0.55 μg/ml) and 2 ng in 10 μl (0.2 μg/ml) for MEKC and HPLC, respectively (S/N ratio $S=2/1$). Concentrations investigated by MEKC and HPLC ranged from 0.1 to 10 μg/ml and 0.125 to 5 μg/ml, respectively. The concentration of 0.1 μg/ml was not detected by MEKC and that of 0.125 μg/ml was not detected by HPLC. The absorbances increased linearly up to the highest concentration investigated by the two methods.

The apparent purity of the isobarbaloin isolated in this study was calculated as 82% from the areas of the chromatogram obtained by analytical HPLC.

3.2. Analysis of aloes

The results from MEKC and HPLC analyses of JP Aloe, *Aloe arborescens* and *Aloe vera* are shown in Fig. 3. In analysis by both MEKC and HPLC, isobarbaloin and barbaloin were detected in the three *Aloes*, while aloenin was only detected in *Aloe arborescens*. While the peak for JP Aloe had a retention time similar to that for aloenin in *Aloe arborescens*, the retention time was different from that of aloenin and so was the absorption spectrum by photodiode array analysis.

Fig. 3 shows that each *Aloe* has a different profile for both MEKC and HPLC analyses indicating a difference in constituents between them.

Reversed-phase HPLC analysis using acetonitrile as the mobile phase requires a shorter time for the determination of aloenin, isobarbaloin and barbaloin than that using methanol. However, acetonitrile is not applicable to the analysis of *Aloe arborescens*, because it contains some components whose retention times overlap with that of barbaloin (Data not shown).

3.3. Recovery of added standard samples

Lyophilized powders of *Aloe arborescens* (prepared according to the procedure described in Sample preparation for barbaloin and isobarbaloin) and Kidachi-Aloe-Matsu (for aloenin) were used to set the concentrations of these three components in aloes after extraction at about the same as their concentrations in the added standard samples.

A total of 100 μl of methanol solutions containing 500 μg/ml aloenin, barbaloin and isobarbaloin were separately added to 100 mg of the lyophilized powders of *Aloe arborescens* (for barbaloin and isobarbaloin) and of Kidachi-Aloe-Matsu (for aloenin). After addition of 900 μl of methanol, the mixtures were treated as described in Experimental. The final concentrations of the three added standards should be 0.5 μg/ml if recovery is 100%. According to concentration measurements that we carried out for five extractions, the average recovery for each standard was 99.1% or over (Table 1).

3.4. Comparison of determinations

The amounts of aloenin, isobarbaloin and barbaloin in the five commercial products containing *Aloe arborescens* and the lyophilized sample of *Aloe arborescens* prepared as described in Sample preparation were measured to investigate the relationship between determinations by MEKC and HPLC analyses (Fig. 4). The content of aloenin was estimated using aloenin as the standard and that of isobarbaloin and barbaloin using barbaloin as the standard on the assumption that their molecular absorbency coefficients are identical. The contents and ratios of the contents of aloenin, isobarbaloin and barbaloin were different for all six measured materials, but these were good correlation between the determinations by MEKC and HPLC (Fig. 4). Both coefficients of correlation and regression coefficients were over

Table 1
Recovery of added standard samples[a]

	MEKC	HPLC
	% C.V.	% C.V.
Aloenin	100.8±5.16	100.2±4.20
Isobarbaloin	101.5±1.54	100.4±5.46
Barbaloin	99.97±4.24	99.12±2.36

[a] Values are the mean of five determinations. C.V., coefficient of variation.

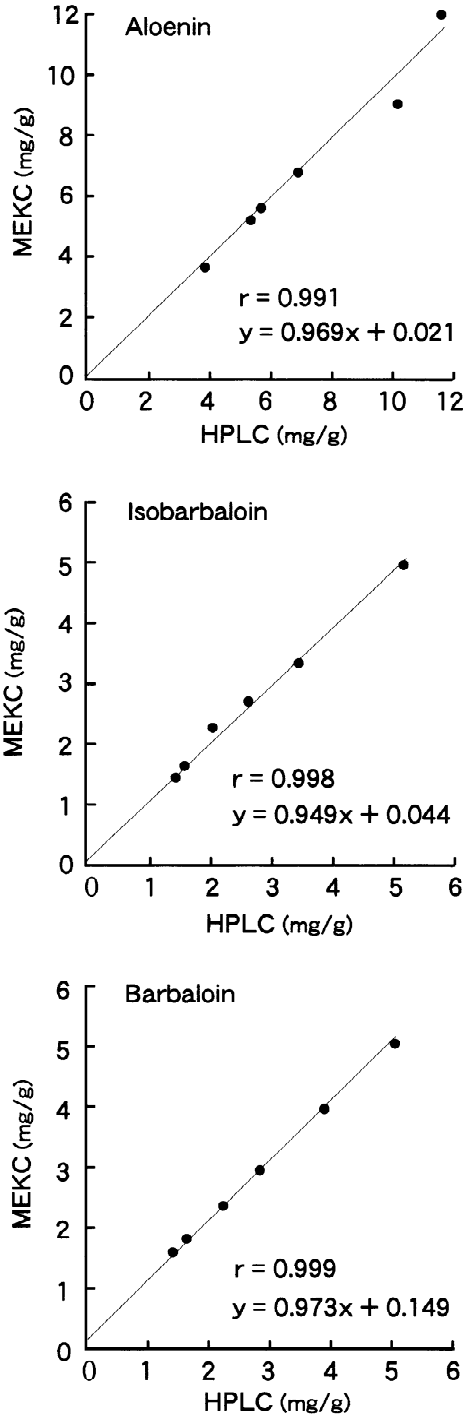

Fig. 4. Correlation between analyses by MEKC and HPLC. A= aloenin, B=isobarbaloin, C=barbaloin. The value of each point shows the mean of three determinations. *y*: regression coefficient.

0.95 for the three substances. The correlation and regression coefficients are indicated in Fig. 4.

Relative standard error (RSE) was calculated from three determinations of each commercial product and from lyophilized preparation of *Aloe arborescens*. The RSE values (minimum, maximum and mean) by MEKC for aloenin were 0.65%, 2.90% and 1.42%, for isobarbaloin were 0.23%, 3.21% and 1.55%, and for barbaloin were 1.26%, 3.91% and 2.06%, respectively, and the values by HPLC were for aloenin were 0.38%, 4.04% and 1.96%, for isobarbaloin were 1.55%, 3.54% and 2.43%, and for barbaloin were 0.72%, 2.86% and 1.92%, respectively.

4. Conclusions

MEKC is an alternative to HPLC for the determination of aloenin, isobarbaloin and barbaloin in *Aloes*. Aloenin, isobarbaloin and barbaloin were well separated by MEKC. MEKC has advantages over HPLC, requiring smaller amounts of samples and mobile phase, and a shorter time for analysis. The amounts of aloenin, isobarbaloin and barbaloin determined by MEKC were well correlated with those determined by reversed-phase HPLC analysis.

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