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Note

Gas chromatographic and mass spectral determination of aloenin in skin-care cosmetics

HIROSHI NAKAMURA*
Tokyo Metropolitan Research Laboratory of Public Health, 3-24-1, Hyakunincho, Shinjuku-ku, Tokyo 169 (Japan)
and
TORU OKUYAMA
Department of Pharmacognosy and Phytochemistry, Meiji College of Pharmacy, 1-35-23 Nozawa, Setagaya-ku, Tokyo 154 (Japan)
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Cosmetics generally contain a wide variety of ingredients (*e.g.*, oils, waxes, alcohols, surfactants, fragrances, dyes, preservatives and plant extracts, and their identification and determination are of importance for quality control.

Various kinds of extracts derived from plants, especially aloe extracts, are used in skin-care cosmetics. Among many kinds of aloe species, the following three species are commonly used: *Aloe barbadensis* Miller (*A. vera* Linne), *A. ferox* Miller and *A. arborescens* Miller var. *natalensis* Rerger (Japanese name: Kidachi-Aloe) (Liliaceae)[1]. These three species contain anthrone glycosides, barbaloin, aloinosides A, B, etc., and their aglycone[2]. In general, barbaloin as a glucoside is easily hydrolysed, and therefore cannot be detected in commercial cosmetics, although it is present at high concentrations in aloe extracts[3].

Aloe arborescens Miller var. *natalensis* Rerger (Kidachi-Aloe) contains aloenin (O-glucoside) and barbaloin (C-glucoside); the former compound is very stable in comparison with the latter, even in water or methanol solution. Consequently, we tried to monitor the Kidachi-Aloe extracts in cosmetics by the determination of their aloenin contents.

There have been some reports of the detection of aloenin in aloe leaves[4], foods[4] and skin lotions[5] using thin-layer (TLC) and high-performance liquid chromatography (HPLC). However, the verification of aloenin in cosmetics by TLC and HPLC is not easy as interferences from the matrix substances occur. We have previously reported[3] that the aloenin and barbaloin in cosmetics such as cream and lotion could be measured by gas chromatography–mass spectrometery (GC–MS) as the trimethylsilyl derivatives. However, barbaloin in cosmetics cannot be detected because it is hydrolysed. The detection limit of aloenin in cosmetics was about 2 $\mu g/g$[3].

We report here a highly sensitive method using GC with mass fragmentography (GC–MF) to detect low concentrations of aloenin in cosmetics. This method was

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successfully applied to the determination of aloenin in several commercial skin-care cosmetics.

EXPERIMENTAL

Reagents

Fresh leaves (*ca.* 500 g) of *Aloe arborescens* Miller var. *natalensis* Rerger (Kidachi-Aloe), cut into small pieces, were extracted for 60 min with 500 ml of water–methanol (1:1) in an ultrasonic bath. The extract was concentrated with a vacuum evaporator (Büchi-Sibata RE-111A; Sibata, Tokyo, Japan) at 40°C to about 200 ml and centrifuged at 1600 *g* for 20 min. Aloenin in the supernatant was purified three times by the use of a fully automatic preparative high-performance liquid chromatograph (HLC-837; Tosoh, Tokyo, Japan) connected to a preparative column (300 × 55 mm I.D.) packed with octadecylsilica gel (ODS), and subsequently recrystallized from methanol to yeild aloenin, which was identified by its m.p. and NMR, mass and UV spectra[6].

A 100 μg/ml stock standard solution of aloenin in acetonitrile was prepared. Working standard solutions were prepared by suitable dilution of the stock solution with acetonitrile.

N,O-Bis(trimethylsilyl)acetamide (BSA) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and an ODS cartridge (Sep-Pak C_{18}) from Waters Assoc. (Milford, MA, U.S.A.). Other reagents (methanol, ethanol and acetonitrile) were of special grade reagent from Wako (Osaka, Japan).

Instrumentation

GC–MS system was a JOEL (Tokyo, Japan) JMS D-300 mass spectrometer equipped with a Hewlett-Packard (Palo Alto, CA, U.S.A.) 7710A gas chromatograph and a JOEL MS-MIDO3 multi-ion detector. GC was performed using a glass column (1.0 m × 2 mm I.D.) packed with 2% OV-17 on Chromosorb W AW DMCS (60–80 mesh) with helium as the carrier gas (flow-rate 30 ml/min) with temperature programming (250°C for 2 min, increased at 16°C/min to 280°C, held for 4 min). The injection port temperature was 300°C and the injection volume was 2 μl. The operating conditions for the mass spectrometer equipped with the multi-ion detector were follows: electron impact (EI) mode; ionization voltage, 70 eV; ionization current, 300 μA; ion source temperature, 250°C; ion multiplier 100–220 (−1.0 to −2.2 kV); gain of multi-ion detector 1 or 2; monitoring ion, *m/z* 392.

Analytical procedure

For aqueous and alcoholic cosmetics (lotion and hair tonic), a mixture of each sample (0.5 g) in ethanol (*ca.* 10 ml) was evaporated to dryness under reduced pressure at 40°C. The residue was dissolved in 20 ml of water, followed by passage through an ODS cartridge (previously rinsed with 10 ml of methanol and 10 ml of water) at a rate of about 8 ml/min. After washing with 10 ml of water, 5 ml of methanol were passed through the cartridge to elute aloenin. The eluate was evaporated to dryness *in vacuo* at 40°C, a 200-μl volume of acetonitrile was added to the residue and the mixture was transferred into a 2-ml mini-vial. A 200-μl volume of BSA was added and the vial was stoppered tightly. The mixture was held at 90°C in a water-bath for 60 min. After cooling, a 2-μl volume of the mixture was injected into the GC–MF apparatus.

For oil-rich cosmetics (cream, milky lotions, etc.), a 0.5-g sample was dissolved in ethanol (*ca.* 20 ml) and the mixture was filtered with a filter-paper (No. 2; Toyo Roshi, Tokyo, Japan), followed by evaporation to dryness. After addition of water (*ca.* 20 ml), the solution was passed through an ODS cartridge (previously rinsed as above) at a rate of 8 ml/min. After washing with 10 ml of water, 5 ml of methanol were passed through the cartridge to elute aloenin. The eluate was evaporated to dryness and the residue was dissolved in 200 μl of acetonitrile. The mixture was transferred into a 2-ml mini-vial, 200 μl of BSA were added and the vial was stoppered tightly. The mixture in the mini-vial was held at 90°C in a water-bath for 60 min. After cooling, a 2-μl volume of the mixture was injected into the GC–MF apparatus.

The analytical procedure is summarized schematically in Fig. 1.

RESULTS AND DISCUSSION

Pretreatment by ODS cartridge

For the detection of trace aloenin in skin-care cosmetics containing large

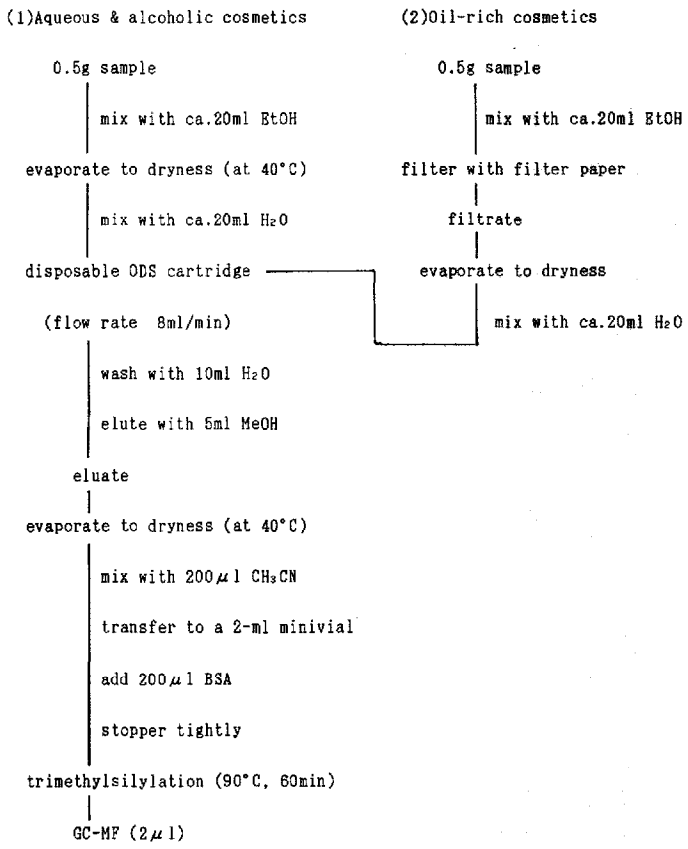

Fig. 1. Analytical procedure for the determination of aloenin in cosmetics. EtOH = Ethanol; MeOH = methanol.

amounts of interfering substances such as surfactants, preservatives or oily constituents, an effective clean-up was necessary.

After removing the volatile substances in a cosmetic sample *in vacuo*, a mixture of the residue in water was passed through a disposable ODS cartridge and the aloenin held on the ODS cartridge was eluted with methanol. Although the very slight content of matrix substances (*e.g.*, surfactants, preservatives) in the resulting solution was confirmed by HPLC with an ODS column of TSKgel ODS-80TM (15 cm × 4.6 mm I.D.) (Tosoh), using water–methanol (1:1) as the mobile phase, these substances did not interfere in the subsequent trimethylsilylation and the detection by GC–MF. These procedures with 0.5-g cosmetic sample resulted in a recovery of nearly 100% in all instances.

Trimethylsilylation

As aloenin is non-volatile, a derivatization procedure was essential. Of the derivatization reactions generally used in the GC determination of substances with hydroxy groups, we chose trimethylsilylation with BSA because of its expediency and sensitivity.

Trimethylsilylation was effected at 90°C for 60 min using BSA with acetonitrile. Trimethylsilyl (TMS) groups were introduced on all five hydroxy groups and as a result, a pentatrimethylsilyl derivative of aloenin was formed. A typical mass spectrum of the derivative is shown in Fig. 2. Ions such as those of m/z 755 (M − 15), 665 (M − 15 − 90) and 575 (M − 15 − 90 − 90) are recognized as the characteristic fragment ions of a TMS derivative, and the base peak (m/z 392) is presumed to be a di-TMS derivative fragment of the aglycone, which arose from cleavage of the TMS-sugar residue from the parent molecule followed by further trimethylsilylation of the resulting aglycone fragment. This phenomenon was observed in the mass spectrum of the penta-TMS derivative of arbutin (4-hydroxyphenyl-β-D-glucopyranoside), one of O-glucosides, which had a base peak (m/z 254) corresponding to a di-TMS derivative fragment of the aglycone[3].

Calibration graphs and recoveries

Calibration graphs obtained by plotting the peak height (ion intensity at m/z 392) against the concentration of aloenin showed good linearity over the ranges 0.2–2.0 and 2–20 μg/g, the regression equations being $y = 1.30 + 11.70x$ ($r = 0.999$)

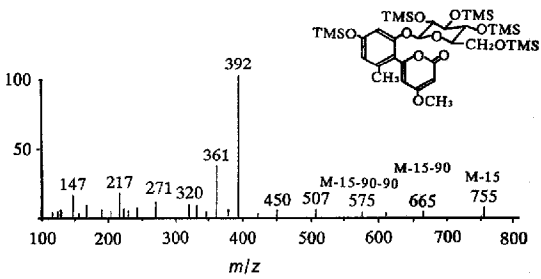

Fig. 2. Typical mass spectrum of the TMS derivative of aloenin obtained by GC–MS using a VG Analytical (Manchester, U.K.) Model 70s mass spectrometer equipped with a Hewlett-Packard 5890A gas chromatograph with a Megabore CB-1 fused-silica capillary column, the same as reported in our previous paper[3]. Vertical axis: intensity.

TABLE I

RECOVERIES OF ALOENIN FROM COSMETICS

Sample	*Added (μg/g)*	*Recovery (%)*	*R.S.D. (%) (n=5)*
Lotion	0.25	95	7.0
	2.5	97	3.9
Cream	0.25	94	7.3
	2.5	98	5.2

and $y = 1.78 + 1.64x$ ($r = 0.999$), respectively (y = peak height, x = aloenin concentration). The determination of aloenin concentration over wide ranges was possible by transforming the ion multiplier voltage of the mass spectrometer and the gain of the multi-ion detector.

Average recoveries of five determinations with addition of aloenin standards to a commercial lotion and a commercial cream are given in Table I. The average recoveries were more than 94% with relative standard deviations of 4–7%. The detection limit was 0.02 μg/g for the standard solutions, 0.05 ng per injection, on the basis of a signal-to-noise ratio of 1:5. This method has a 100-fold higher sensitivity than the previous GC method[3], which was carried out with a Megabore CB-1 fused-silica capillary column (15 m × 0.53 mm I.D.) with flame ionization detection, after treatment with an ODS cartridge and trimethylsilylation with BSA in the present method.

TABLE II

ALOENIN CONTENTS IN COMMERCIAL COSMETIC PRODUCTS

Sample	*Kidachi-Aloe extracts content combined into the products (%)*[a]	*Aloenin content (μg/g)*[b]
Lotions:		
1	1.30	42
2	0.15	1.2
3	0.01	0.80
Milky lotions:		
1	0.25	1.7
2	0.01	0.96
Creams:		
1	0.15	2.3
2	0.30	3.5
3	0.20	4.0
4	0.39	0.25
5	0.25	1.8
Face packs:		
1	0.20	2.3
2	0.50	4.8
Hair rinse	0.25	1.6
Hair tonic	1.50	6.2

[a] Reported by the manufactures.
[b] Detection limit 0.02 μg/g.

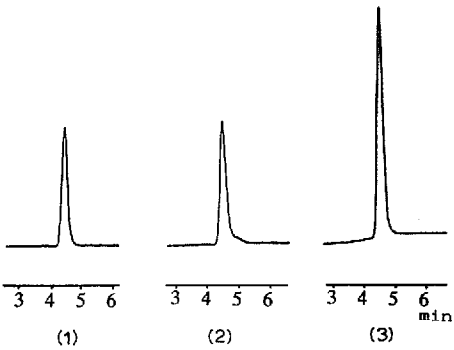

Fig. 3. Typical GC-MF at m/z 392 of commercial skin-care cosmetics. The retention time of each peak is 4.35 min, and the peak height of each sample was obtained with the following ion multiplier voltages (IMV) of the mass spectrometer and gain (G) of the multi-ion detector: (1) cream, IMV = −1.2 kV, G = 1; (2) face pack, IMV = −1.4 kV, G = 1; (3) milky lotion, IMV = −1.6 kV, G = 1. Other GC–MF conditions as under Experimental.

Aloenin content in commercial aloe cosmetics

The aloenin contents of several commercial cosmetics containing 0.01–1.5% of Kidachi-Aloe extracts was determined by the present method. The resulting contents were in the range 0.25–42 μg/g, as shown in Table II. Trace amounts of aloenin in commercial cosmetics containing Kidachi-Aloe extracts were successfully determined with less interferences by the present method. Typical GC–MF results for aloenin in commercial skin-care cosmetics are shown in Fig. 3.

CONCLUSION

Trace amounts of aloenin in commercial cosmetics containing Kidachi-Aloe extracts were determined by GC–MF as the trimethylsilyl derivative. The average recoveries were more than 94% with a relative standard deviation of 4–7% and a detection limit of 0.02 μg/g. This method is suitable for the verification of Kidachi-Aloe extracts in commercial skin-care cosmetics.

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