Quantitative Determination of Octadecenedioic Acid in Human Skin and Transdermal Perfusates by Gas Chromatography–Mass Spectrometry

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Abstract

A gas chromatographic (GC) method with mass spectrometric (MS) detection is developed and validated for the accurate and precise determination of octadecenedioic acid (C18:1 DIOIC) in human skin samples and transdermal perfusates. C18:1 DIOIC is extracted using methanol. The saturated analogue 1,18-octadecanedioic acid (C18:0 DIOIC) is added as internal standard. Prior to analysis, both compounds are converted to their trimethylsilylated derivatives using *N,O-bis*(trimethylsilyl)trifluoroacetamide with 15% trimethylchlorosilane. Quantitation is performed in selected ion monitoring mode with a limit of quantitation of 250 ng/mL. Linearity with a correlation coefficient of 0.998 is obtained over a concentration range of 250–2000 ng/mL. Values for within-day accuracy range from 94.5% to 102.4%, and from 97.5% to 105.8% for between-day accuracy. Within- and between-day precision values are better than 5% and 7%, respectively. The recovery values from the various matrices vary from 92.6% to 104.0%. The GC–MS method is employed for the determination of C18:1 DIOIC after application of an emulsion containing the active ingredient onto human skin in vitro. The results demonstrate that the method is suitable for the determination of C18:1 DIOIC in human skin samples and transdermal perfusates.

Introduction

Octadecenedioic acid (C18:1 DIOIC) is used in various cosmetic and dermatological formulations as a skin whitening and anti-ageing active ingredient (1–3). Its mechanism of skin lightening was previously hypothesized to be reduction in tyrosinase formation by binding of C18:1 DIOIC to the peroxisome proliferator-activated receptor, resulting in a reduced expression of tyrosinase mRNA (4), and therefore in a lower production of tyrosinase — the key enzyme in the production of the pigment melanin. For C18:1 DIOIC to become effective as a skin whitener, it has to be delivered to the melanocytes, which are located in the basal layer of the epidermis. Therefore, it is of interest to investigate the transdermal and dermal delivery of C18:1 DIOIC into human skin. For the performance of these studies, a sensitive assay is required to detect low concentrations of C18:1 DIOIC in skin samples and transdermal perfusates.

Few reports exist in which C18:1 DIOIC was determined qualitatively and/or quantitatively. It was qualitatively detected in serum of patients with Reye syndrome by gas chromatography–mass spectrometry (GC–MS) analysis following liquid–liquid extraction and derivatization using triethylamine and *N,O-bis*(trimethylsilyl)trifluoroacetamide (BSTFA) (5). Tonsgard and Getz (6) analyzed long chain dicarboxylic acids in serum of Reye syndrome patients using GC with flame ionization detection (FID) after derivatization of the dicarboxylic acids with triethylamine and *N,O-bis*(trimethylsilyl)acetamide. Furthermore, C18:1 DIOIC, a monomer of cutin and suberin, was found in plant extracts after depolymerization and was identified by GC–MS, and quantitatively determined as dimethylester by using GC–FID (7–11).

To the best of our knowledge, no method has been described in literature for the quantitative determination of C18:1 DIOIC in skin samples and transdermal perfusates. Skin penetration studies with octadecenedioic acid (Arlatone Dioic DCA) have been performed using ^{14}C-labeled octadecenedioic acid (1,12). As strict regulations exist for handling radio-labeled compounds, the aim of our study was to develop a GC method for the quantitative determination of C18:1 DIOIC in human skin samples obtained after in vitro penetration experiments. GC with MS detection in selected ion monitoring (SIM) mode was chosen because it is a very sensitive and selective technique for determining low quantities of an analyte in the presence of various matrix components. Hence, time-consuming purification steps prior to analysis can be circumvented and the quantitation of the analyte can be carried out from different matrices. Trimethylsilylation utilizing BSTFA with trimethylchlorosilane

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(TMCS) was selected for derivatization in this study, as it is a commonly applied and simple derivatization procedure. The method was tested for linearity, specificity, accuracy, and precision. Furthermore, the analytical procedure was applied to the analysis of C18:1 DIOIC skin samples obtained from in vitro skin penetration experiments.

Experimental

Reagents and materials

Arlatone Dioic DCA, a mixture of dicarboxylic and monocarboxylic acids obtained by biofermentation of oleic acid (1), was kindly provided by Uniqema (Gouda, The Netherlands). According to Uniqema, the Arlatone Dioic DCA batch used for the preparation of the formulation contained 58.1% C18:1 DIOIC. The same batch was utilized for standard sample preparation. 1,18-Octadecanedioic acid (C18:0 DIOIC, 95.5% purity) was purchased from Dr. Ehrenstorfer-Schäfers (Augsburg, Germany) and was used as internal standard (IS). HPLC-grade methanol and ethanol were obtained from BDH (Dorset, UK) and Sigma Aldrich (Johannesburg, South Africa), respectively. BSTFA (99% purity) and TMCS (99.6% purity) were purchased from Supelco (Bellefonte, PA). Dried pyridine (max 0.0075% H_2O, ≥ 99.5% purity) was obtained from Riedel de Haën (Seelze, Germany). Deionized water from a Millipore Milli-Q purification system was employed. Glass screw-capped autosampler vials (1.5 mL) from Agilent (Palo Alto, CA) with 100 µL pulled point glass inserts (Agilent) were used for derivatization and sample analysis.

Instrumentation and conditions

The trimethylsilyl (TMS) derivatives of C18:1 DIOIC and C18:0 DIOIC were analyzed by GC–MS using an Agilent 6890^{Plus} GC equipped with an Agilent 5973 MS detector in electron-impact mode (70 eV). Aliquots of 1 µL of each sample were injected by means of a split/splitless injector with a single-tapered splitless glass inlet liner (900 µL) containing deactivated glass wool. All injections were performed with an Agilent 7673 auto sampler. The injector temperature was maintained at 270°C and operated in the splitless mode at 0.8 bar, with a purge flow-rate of 21.3 mL/min after 1.00 min. A 30 m length × 250 µm and 0.25 µm film thickness DB-5 column (Agilent) with a stationary phase of (5%-phenyl)-methylpolysiloxane was used for all analyses. Ultra-high purity helium (5.5) was utilized as carrier gas and the column flow was maintained at a constant flow of 1.2 mL/min. The initial oven temperature was set at 80°C with a 2.0 min hold period and was then ramped to 265°C at 20°C/min, and finally ramped from 265°C to 300°C at a rate of 30°C/min. The oven was maintained at 300°C for 2.0 min for a total run time of 32.42 min. The transfer line was set at 280°C, the source temperature at 230°C, and the quadrupole at 150°C. The MS was operated in the SIM mode. Ions monitored for the derivatized analyte and IS were *m/z* 441 and 443, respectively, measured with a dwell time of 100 ms. Quantitation was performed based on the ratio of the peak area of the active ingredient to the peak area of the IS.

Standard preparation

A 1.7 mg/mL stock solution of Arlatone Dioic DCA in methanol containing 1 mg/mL C18:1 DIOIC was prepared and diluted to 1 µg/mL and 500 ng/mL C18:1 DIOIC with methanol. A stock solution of C18:0 DIOIC (IS) with a concentration of 0.5 mg/mL was prepared in methanol. The C18:0 DIOIC stock solution was diluted to 1 µg/mL with methanol.

For the preparation of calibration samples, different volumes of C18:1 DIOIC standard solutions (25, 50, and 75 µL of 500 ng/mL and 50, 75, and 100 µL of 1 µg/mL) were pipetted into micro-inserts of autosampler vials. Additionally, 25 µL of 1 µg/mL C18:0 DIOIC IS solution were added to each calibration sample. The samples were carefully dried under a stream of nitrogen. Twenty-five microliters BSTFA containing 15% TMCS and 25 µL pyridine were added and the samples were heated for 1 h at 75ºC. The final concentrations of C18:1 DIOIC in the calibration samples after adding 50 µL of derivatization mixture were 250, 500, 750, 1000, 1500, and 2000 ng/mL with a C18:0 DIOIC concentration of 500 ng/mL. Aliquots of 1 µL of each sample were injected into the GC–MS.

Sample preparation

The project "In vitro transdermal delivery of drugs through human skin" was approved by the Ethics Committee of the North-West University (Potchefstroom, South Africa), and skin was obtained with informed consent of the donors (Ethics Committee reference number: 04D08).

The penetration study was conducted using Franz diffusion cells (Figure 1) with an exposed skin area of 1.13 cm^2 and a receptor volume of approximately 2 mL. White, human female abdominal skin was obtained from cosmetic surgery (Sunward Park Hospital, Boksburg, South Africa) and prepared within 24 h after removal. The skin was rinsed with deionized water and dried with paper tissue. The surface of the skin was wiped once with an ethanol-soaked cotton swab to remove possible fat residual from the subcutaneous fat layer. Afterwards, a skin layer with a thickness of 400 µm was dermatomed with a Zimmer electric dermatome (Zimmer Inc., Warsaw, IN). The prepared skin was placed dermal side down on filter paper and stored in aluminum foil at –20°C until use. One hour prior to the diffusion study, the skin was thawed at room temperature, cut into circular pieces, and placed epidermal side up between donor and receptor chamber. Prior to the penetration experiment, skin integrity was tested by measuring the electrical resistance across the skin using a Tinsley LCR Databridge

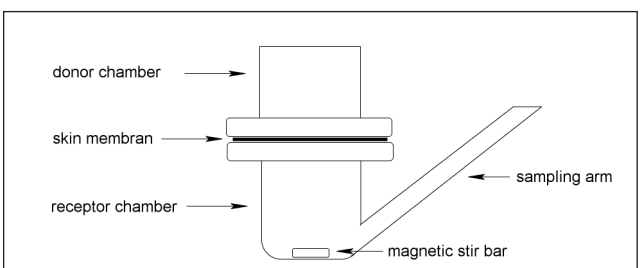

Figure 1. Schematic illustration of a Franz diffusion cell.

Model 6401 (Tinsley Precision Instruments, Croydon, UK). Donor and receptor chambers were filled with 0.9% aqueous sodium chloride and the Franz diffusion cells were placed in a preheated water bath at 37 ± 1°C. After an equilibration period of 30 min, the electrical resistance was measured. Afterwards, the aqueous sodium chloride was removed and the receptor compartments were filled with receptor fluid (water–ethanol 1:1 [v/v]). After an equilibration time of another 30 min in the water bath, 25 µL of an oil-in-water emulsion containing 1.8% Arlatone Dioic DCA were applied with a positive displacement pipette and spread evenly on the skin surface area with the tip of the pipette. The residual left on the tip of the pipette was also analyzed for C18:1 DIOIC after extraction with methanol, and the applied amount of C18:1 DIOIC could be calculated for each cell.

The duration of the study was 24 h. At specified time intervals (3, 6, 9, 12, 18, and 24 h), the entire volume of receptor fluid was withdrawn and replaced with fresh receptor media. After taking the last receptor sample (Re sample) at 24 h, the receptor chamber was rinsed two times with fresh receptor fluid. These solutions were subsequently added to the 24 h Re sample. After rinsing the receptor chamber, the donor residual left on the surface of the skin after conclusion of the permeation experiments was removed with cotton swabs (Do samples). The skin was removed from the cells and cut into smaller pieces to increase the surface area for extraction. C18:1 DIOIC was extracted from the Do and skin samples for at least 24 h with methanol.

A specified amount of IS was immediately added to all the samples. The amount of IS was calculated to correspond to a concentration of 500 ng/mL after appropriate dilution, evaporation of solvent, and adding 50 µL of derivatization mixture as utilized for the calibration samples. The samples were kept at –20°C until analysis. Prior to analysis, the samples were brought to room temperature, vortex mixed for 1 min, and diluted if necessary. A specified volume was than pipetted into micro-inserts of autosampler vials and carefully dried under a stream of nitrogen. Twenty-five microliters BSTFA containing 15% TMCS and 25 µL pyridine were added and the samples were derivatized for 1 h at 75°C. One microliter of each sample was injected into the GC–MS.

Recovery and specificity

The same procedure as described in the "Sample preparation" section, utilizing the same oil-in-water emulsion but without Arlatone Dioic DCA, was applied to obtain blank skin samples. The blank skin samples (Do, skin, and Re sample) were divided into two aliquots and one of the aliquots as well as the same volume of methanol (control sample) were spiked with a known amount of C18:1 DIOIC (2000 ng/mL) and C18:0 DIOIC (500 ng/mL). The other aliquot of the blank skin samples was used to test for interferences (specificity). The experiments were performed in duplicates with skin from two different skin donors. The recovery of C18:1 DIOIC from the various skin samples was determined by comparing the amount of C18:1 DIOIC analyzed from the skin samples to the amount of C18:1 DIOIC determined from the control samples (prepared in methanol).

Results and Discussion

Derivatization and chromatography

In this study, trimethylsilylation of the carboxyl groups of C18:1 DIOIC and its IS using BSTFA was chosen to convert the analytes into more volatile and less polar derivatives. TMCS was added as a silylation catalyst and pyridine as a proton acceptor. The first tests in scan mode illustrated that at least 10% TMCS and temperatures higher than 60°C were necessary to detect TMS-esters of C18:1 and C18:0 DIOIC. Different temperatures (65°C, 75°C, and 85°C) and incubation times (0.5, 1, and 1.5 h), as well as various amounts of catalyst (10% and 20%) were tested in SIM mode (*m/z* 441 $[M\text{-}CH_3]^+$ for C18:1 DIOIC and *m/z* 443 $[M\text{-}CH_3]^+$ for C18:0 DIOIC) to optimize the derivatization procedure. No significant differences in peak area were obtained between the various tested derivatization procedures. Furthermore, all derivatization procedures resulted in symmetrical, narrow peaks for C18:1 and C18:0 DIOIC-TMS. Therefore, it can be concluded that the incubation of the sample at 65°C for 1 h with a catalyst concentration of 10% TMCS in BSTFA yielded complete derivatization, as no increase in peak area and no change in peak symmetry occurred at higher temperatures, longer durations, and/or increased catalyst concentrations. For further analysis, the derivatization was conducted using BSTFA containing 15% TMCS for 1 h at 75°C to ensure complete derivatization of C18:1 DIOIC and its IS.

The mass spectra of C18:1 DIOIC-TMS (A) and C18:0 DIOIC-TMS (B) are shown in Figure 2. The molecular ions (*m/z* 456 for C18:1 DIOIC-TMS and *m/z* 458 for C18:0 DIOIC-TMS) were unobservable or very weak. Both mass spectra show clear mass fragments related to dicarboxylic acids which have been converted to their di-TMS esters. Furthermore, the mass fragment pattern of C18:0 DIOIC-TMS was confirmed by computer library (Nist 98), matching Figure 2C. On the other hand, no reference spectrum was available for C18:1 DIOIC-TMS. A mixture of dicarboxylic and monocarboxylic acids was used as a standard (because no authentic standard of C18:1 DIOIC was readily available) and therefore, C18:1 DIOIC-TMS was identified by comparison of its mass spectral pattern to the one of C18:0 DIOIC-TMS (the saturated analogue). Additionally, the mass spectra of both compounds were verified by comparison with data reported in literature (5). It should be noted that the described method neither determines the position of the double bond within the molecule nor distinguishes the *trans* from *cis* enantiomers.

The quantitative analysis of C18:1 DIOIC was performed in SIM mode (*m/z* 441 for C18:1 DIOIC-TMS and *m/z* 443 for C18:0 DIOIC-TMS) for enhanced sensitivity and specificity. The SIM chromatogram of a standard sample is shown in Figure 3A. According to Uniqema, Arlatone Dioic DCA also contained 0.54% C18:0 DIOIC (IS). This necessitated an additional test to assess whether the small amount of C18:0 DIOIC present in Arlatone Dioic DCA interfered with the analysis of the added amount of IS. Therefore, a standard sample with a high concentration of Arlatone Dioic DCA containing 3000 ng/mL C18:1 DIOIC (exceeding the highest concentration of the calibration curve) in methanol was tested in SIM mode

(*m/z* 443) for peaks at the retention time of C18:0 DIOIC. A minor peak occurred at the retention time of C18:0 DIOIC with a peak area less than 1.5% of the peak area of 500 ng/mL of C18:0 DIOIC (the added amount of IS). Therefore, the small amount of C18:0 DIOIC in Arlatone Dioic DCA was considered insignificant in the analysis of the added IS. An internal standard sample of 500 ng/mL C18:0 DIOIC in methanol was investigated in SIM mode (*m/z* 441) for possible interference with the analysis of C18:1 DIOIC. No peak was observed at the retention time of C18:1 DIOIC at *m/z* 441. Hence, it can be concluded that 500 ng/mL of the purchased C18:0 DIOIC could be employed as IS.

Calibration curve

The calibration curve of C18:1 DIOIC in methanol was obtained from three series of standard samples (250, 500, 750, 1000, 1500, and 2000 ng/mL) containing 500 ng/mL IS. The three series were prepared and analyzed on three different days. The calibration curve is shown in Figure 4. As the regression coefficient exceeds 0.99 (13), the correlation between the ratio of peak areas (analyte/IS) and concentration of analyte was found to be linear within the tested concentration range. Another set of calibration curves was generated in various matrices (blank Re sample, blank skin sample, and blank Do sample) and compared with the calibration curve prepared in methanol. No significant differences were observed between the various regression lines. As a result of the fact that no significant interference occurred from the impurities of the various

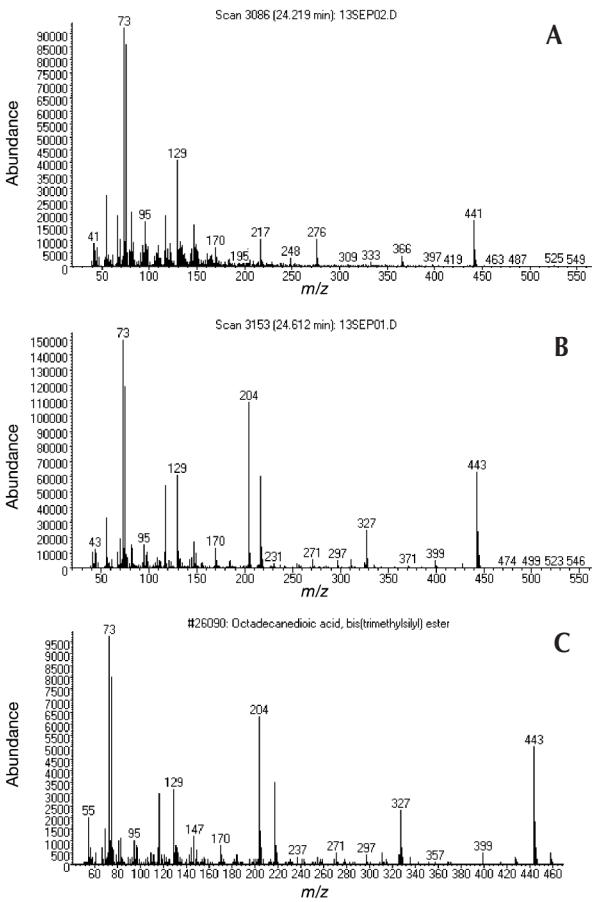

Figure 2. Mass spectra of standard C18:1 DIOIC-TMS (5 µg/mL) (A), standard C18:0 DIOIC-TMS (5 µg/mL) after derivatization and GC–MS analysis as described in the text (B); reference mass spectrum of C18:0 DIOIC-TMS, Nist 98 (C).

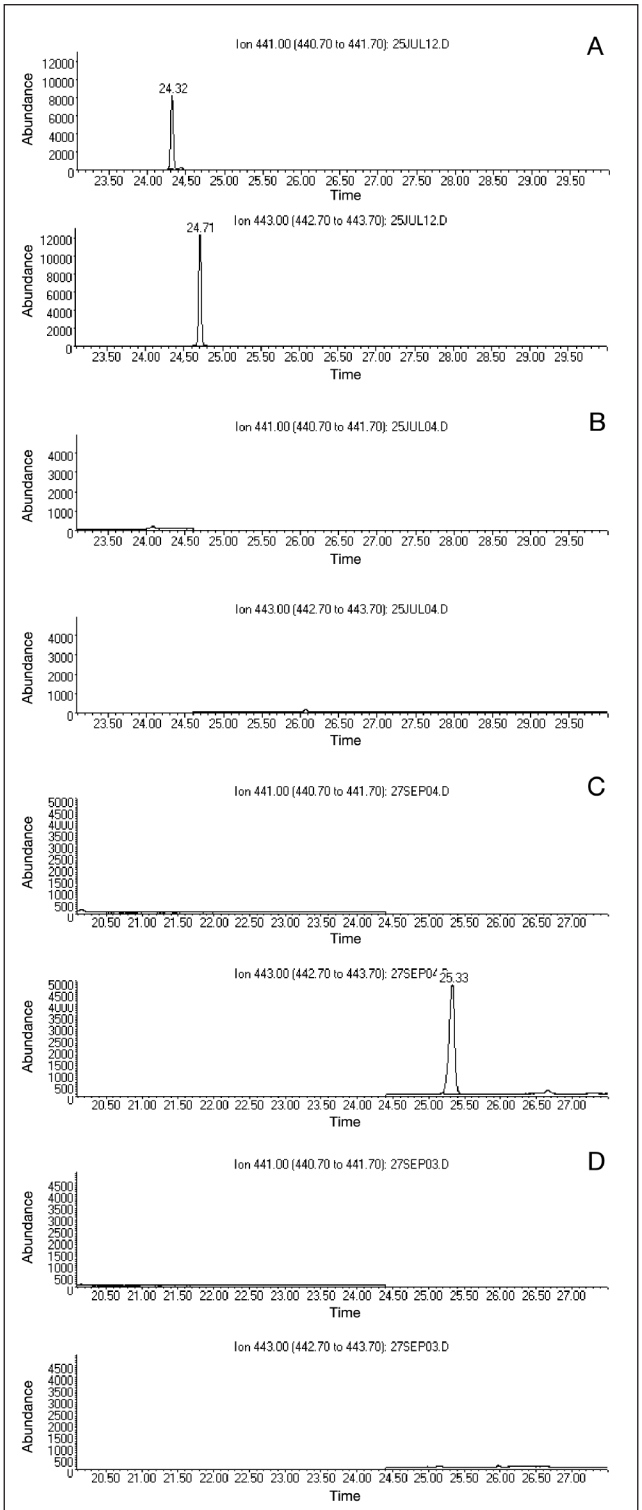

Figure 3. SIM chromatograms (*m/z* 441 and 443) of standard C18:1 DIOIC (1000 ng/mL) with C18:0 DIOIC (500 ng/mL) (A), blank Do sample (B), blank Re sample (C), and blank skin sample after derivatization and GC–MS analysis as described in the text (D).

matrices (as mentioned later), quantitative assessment of C18:1 DIOIC in the different matrices was performed using the calibration curve obtained in methanol.

Recovery

Recovery experiments were employed for two reasons: firstly, to determine the extent of derivatization of C18:1 DIOIC and IS in the presence of impurities; and secondly, to establish the degree of extraction of the active ingredient from the various matrices. The highest C18:1 DIOIC concentration (2000 ng/mL) of the calibration curve and 500 ng/mL IS were used to perform recovery experiments. The lowest dilution of skin samples was employed as to ensure inclusion of the highest amount of impurities as they also consumed BSTFA for derivatization. These samples were compared to unextracted samples prepared in methanol, and recovery values between 85–115% were considered acceptable. The recovery of C18:1 DIOIC from the different skin samples was as follows: 92.6% from the Do sample, 104.0% from the skin sample, and 101.0% from the Re sample. The values were within the acceptance range and therefore it was demonstrated that the extraction efficacy and derivatization of C18:1 DIOIC from various skin sample matrices were sufficient up to a concentration of 2000 ng/mL C18:1 DIOIC and 500 ng/mL IS.

Specificity

Blank skin samples obtained from two different skin donors were tested for interference. According to the guideline for bioanalytical method development from the US Food and Drug Administration (14), a minimum of six different skin donors should be employed to establish the specificity of the method. The availability of human skin is limited and therefore only two skin donors were used to test for interference. However, during diffusion studies a blank Re sample was collected from each skin donor employed in the experiments and analyzed for interference.

No peaks were detected in the SIM chromatograms at the retention time of C18:1 DIOIC (*m/z* 441) and C18:0 DIOIC (*m/z* 443) with the blank Do (Figure 3B), blank Re (Figure 3C), and blank skin samples (Figure 3D). An impurity peak occurred in the SIM chromatogram (*m/z* 443) of the blank Re sample at 25.3 min. However, this peak did not influence the analysis of C18:0 DIOIC. Consequently, it could be concluded that the impurities of the various blank skin samples did not interfere with the analysis of C18:1 DIOIC.

Accuracy and precision

Accuracy (percentage of measured concentration from nominal concentration) and precision (relative standard deviation) were established following the analysis of three standard samples at three different concentration levels (low, medium, and high) in triplicate on the same day (within-day) and on four consecutive days (between-day). As no differences were assessed between the calibration curve prepared in methanol and calibration curves generated in the various matrices (Do sample, skin sample, Re sample), accuracy and precision were determined using standard samples prepared in methanol. The acceptance criteria were set at 85–115% for accuracy and 15% for precision (13,14). The obtained values are within the acceptance criteria (Table I), and therefore, the analytical method can be considered accurate and precise for the determination of C18:1 DIOIC in the concentration range between 250–2000 ng/mL. The limit of quantitation (LOQ) of C18:1 DIOIC was set at 250 ng/mL, as it could be analyzed accurately and precisely.

Stability

The stability of C18:1 DIOIC and C18:0 DIOIC in methanol (stock solution) was tested at –20°C and 4°C. Furthermore, the stability of both compounds was investigated in the receptor medium (ethanol–water 1:1 [v/v]) for 24 h at 37°C (corresponding to the duration of the permeation study) and afterwards at –20°C and 4°C. C18:1 DIOIC and C18:0 DIOIC were stable in methanol and receptor medium for at least 3 weeks at 4°C and for at least 2 months at –20°C. Additionally, no degradation was observed for either compound after 24 h in the receptor medium at 37°C.

As TMS derivatives are moisture-sensitive and easily degradable, a stability test of both trimethylsilylated compounds in the derivatization mixture was performed. Therefore, the same sample after derivatization, left in the autosampler tray at room temperature (20ºC), was injected several times over a certain time period. It was found that the derivatized compounds were stable for at least 22 h. This suggested a single day loading of samples during analysis.

Quantitative analysis of C18:1 DIOIC in human skin and transdermal perfusates samples

The method was employed for the analysis of samples obtained from permeation experiments after application of an emulsion containing 1.8% Arlatone Dioic DCA onto human skin in vitro. Six replicates were performed and skin from three different skin donors was used to investigate for inter-

Table I. Determination of Within- and Between-Day Accuracy and Precision of the Method

	Within-day (n = 3)			Between-day (n = 4)		
Concentration added (ng/mL)	Concentration found (mean ± SD, ng/mL)	Accuracy (%)	Precision RSD (%)	Concentration found (mean ± SD, ng/mL)	Accuracy (%)	Precision RSD (%)
250	236.2 ± 11.6	94.5	4.9	243.7 ± 16.8	97.5	6.9
1000	1024.2 ± 3.2	102.4	0.3	993.0 ± 36.5	99.3	3.7
2000	1989.6 ± 13.1	99.5	0.7	2117.0 ± 70.4	105.8	3.3

individual variability (15). The recovered amounts of C18:1 DIOIC from the various skin samples and transdermal perfusates after 24 h permeation are given in Table II. The cumulative amount permeated versus time plots are shown in Figure 5. The steady-state flux (J_{ss}) could be obtained from the slope of the linear part of the cumulative amount permeated versus time plot. The steady-state flux values of C18:1 DIOIC are given in Table II, and the regression coefficients for the linear regressions exceeded 0.996. The applied amount of C18:1 DIOIC for all six cells was 423.9 ± 2.3 µg/cm^2 (mean ± SD). After the diffusion experiments (Do samples), 308.8 ± 31.6 µg/cm^2 was washed off and was considered as not absorbed by the skin.

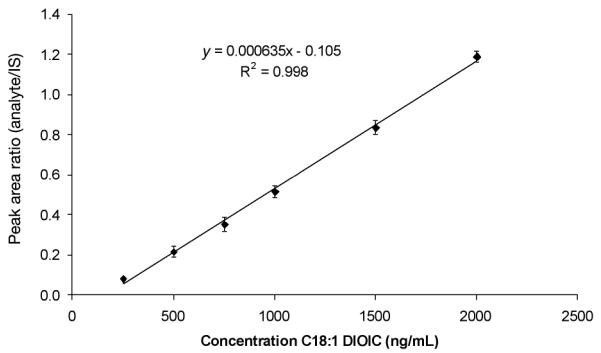

Figure 4. Calibration curve of C18:1 DIOIC in methanol (mean ± SD of *n* = 3 standard samples per concentration level analyzed on three different days).

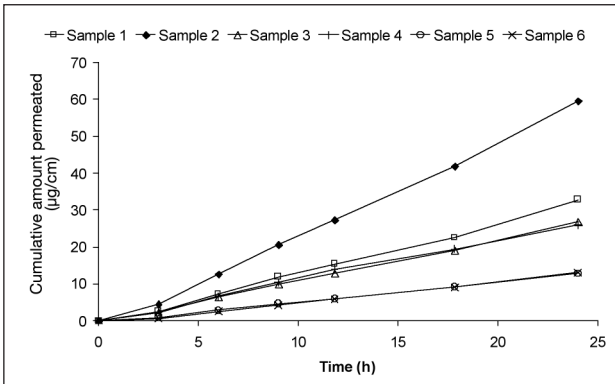

Figure 5. Cumulative amount permeated versus time plot of C18:1 DIOIC through human skin in vitro after permeation experiments as described in the text.

Only 8.1 ± 2.8 µg/cm^2 of C18:1 DIOIC was retained in the skin, and 28.5 ± 17.2 µg/cm^2 permeated the skin. It can also be seen from the results that differences between the various skin donors arose, as reported in literature (16,17). Skin donor one (sample 1 and 2) showed the highest permeability for C18:1 DIOIC, followed by skin donor two (samples 3 and 4) and skin donor three (samples 5 and 6). Furthermore, a high intra-individual variability occurred within skin donor one compared to skin donors two and three (Table II, Figure 5). The reason might be a difference in skin integrity as determined by electrical resistance measurements across skin. The electrical resistance of sample 1 (22.6 kΩ) was higher compared to sample 2 (17.9 kΩ), indicating that the higher flux value of sample 2 might be due to a reduced skin integrity. The other two skin donors exhibited lower intra-individual variability in electrical resistance (skin donor two: 14.7 kΩ [sample 3] and 14.6 kkΩ [sample 4]; skin donor three: 30.6 kΩ [sample 5] and 32.2 kΩ [sample 6]).

To elucidate the reliability of the penetration data, the total recovery of C18:1 DIOIC after completion of the penetration experiment was determined. Therefore, the sum of Do sample, skin sample, and all Re samples of each diffusion cell was calculated and referred to the applied amount of C18:1 DIOIC. The values of total recovery are also presented in Table II. According to Diembeck et al. (15), the total recovery of the compound is recommended to be 100 ± 15%. The values of total recovery were within 91.5% and 100.5%, with the exception of one cell with a recovery of only 73.2%. Additionally, a low value of the Do sample was obtained for the same cell compared to the other cells, indicating that an experimental error occurred during extraction of C18:1 DIOIC from the donor phase and/or washing off the residual donor phase after completion of the diffusion studies. Except for this one value, the recovery values confirmed that the extraction of C18:1 DIOIC from the different samples (Do, skin, and Re samples) and the derivatization were complete. Furthermore, the high recovery corroborates the stability of C18:1 DIOIC during diffusion experiments, sample handling, and storage, as well as the reliability of the analytical method.

Conclusion

In conclusion, the presented GC–MS method is an accurate, precise, and reliable technique for determining C18:1 DIOIC in human skin and transdermal perfusates with adequate recovery. It was shown that with the use of an MS detector in SIM mode, the sensitivity and specificity is sufficient to employ the method in percutaneous permeation studies for the quantitative assay of skin samples. Furthermore, the sensitivity of the method allows the assessment of low concentrations of C18:1 DIOIC in transdermal perfusates, and therefore the method can be employed for the determination of the flux of C18:1 DIOIC through human skin.

Table II. Skin Permeation Results of C18:1 DIOIC after 24 h Application of a 1.8% Arlatone Dioic DCA Emulsion

Sample	Donor (µg/cm^2)	Skin (µg/cm^2)	Re 24 h (µg/cm^2)	J_{ss} (µg/cm^2/h)	Total recovery (%)
1	334.7	12.2	32.8	1.40	100.5
2	284.3	9.7	59.5	2.58	95.1
3	321.7	8.4	26.9	1.14	95.0
4	332.0	8.1	26.0	1.10	97.6
5	324.2	5.3	12.8	0.56	91.5
6	256.2	4.8	13.1	0.58	73.2

Acknowledgments

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