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ORIGINAL ARTICLE

The study of distribution of ingested terbinafine on skin with ambient ionization tandem mass spectrometry

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Abstract

We aim to develop an efficient and non-invasive strategy for monitoring of drugs and their metabolites via human skin. Probe sampling was combined with thermal desorption-electrospray ionization tandem mass spectrometry (TD-ESI/MS/MS) to characterize trace terbinafine, which was secreted on patient's skin after ingesting terbinafine tablets. The terbinafine ion signals were directly detected in the samples collected from different skin regions and the signals were monitored for 8 weeks. The detection and location of terbinafine via the skin suggest that the methods are useful in rapidly and noninvasively collecting the molecular information of the ingested drug on skin for pharmacokinetic studies.

Keywords: Epidermis, Skin, Terbinafine, Thermal desorption-electrospray ionization tandem mass spectrometry

1. Introduction

Skin is the largest human organ and plays a crucial role in protecting the body against numerous pathogens present in the external environment. Many metabolites are also secreted out onto skin through subcutaneous tissues like sweat glands and sebaceous glands. Since the secreted compounds on skin are mainly metabolic products or byproducts from various biological

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systems (e.g. circulatory, immune, lymphatic, etc.), changes in skin metabolite profiles can grant insight into pathophysiological changes of the studied individuals [1]. For this reason, research on skin metabolites has been a topic of interest over the last several decades [2−6]. Skin metabolites are therefore of clinical interest to the areas of medical diagnosis, organ pathophysiology, forensics, toxicology, exposomics, and anti-doping [7−13].

Although sebum or sweat samples can be non-invasively collected from the surface of skin, trace amounts of these secretions complicate sampling, detection and analysis. Thus, a sensitive analytical platform is needed to efficiently sample and detect trace metabolites in sebum. The approaches for efficiently collecting sebum include skin contact sampling and headspace sampling [1]. For the skin contact approach, sampling devices, strips and patches are placed on skin for a certain period of time to collect enough semi-volatile and non-volatile analytes. Liquid-absorbing materials are widely used to make sampling devices and patches, including semi-permeable sweat patches, isopropyl alcohol swabs, cotton pads, textile material probes, agarose hydrogel, and polydimethylsiloxane (PDMS) devices [14,15]. A headspace approach used a solid-phase microextraction (SPME) fiber for concentrating volatiles and semi-volatile metabolites on skin [2,16−18]. Although skin metabolites are successfully detected by the contact sampling approaches, the analytical processes including sampling, extraction, concentration and characterization are usually time and labor-consuming.

There have been substantial efforts to develop and integrate *in vivo* and *in situ* skin sampling techniques with state-of-the-art analytical instruments [2]. Gas chromatography/mass spectrometry (GC/MS) is the most widely used technique for characterizing sebum analytes including volatile organic compounds (VOCs), metabolites, disease biomarkers, aroma compounds, and lipid molecules [19−23]. Although GC/MS is highly regarded for its sensitive, qualitative and quantitative capabilities for sebum analysis, the analytical throughput is still low for its requirement of extensive sample preparation processes. On the other hand, liquid chromatography/mass spectrometry (LC-MS) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) were used to characterize specific epidermal compounds and created molecular topographical maps [24−28]. Cotton swabs or skin tape strips were used to perform sampling. However time-consuming and labor-intensive sample treatment such as solvents extractions, concentration, chromatographic analysis, and matrix deposition are still needed for both approaches.

Alternatively, AIMS has been demonstrated to be useful for rapidly characterizing samples from the skin [18,29−33]. Ambient ionization mass spectrometry (AIMS) encompasses the features of traditional mass spectrometric techniques but operates under ambient conditions and require minimal or no sample pretreatment, making AIMS highly applicable for *in situ* and real-time sample analysis [34−36]. In this study, we used thermal desorption-electrospray ionization/mass spectrometry (TD-ESI/MS) to conduct a non-invasive drug monitoring on human skin. TD-ESI/MS uses a metallic probe to sweep sample surfaces to collect analytes prior to their thermal desorption, electrospray ionization, and mass spectrometric detection. Since analyte desorption and ionization are restricted within a very small area, the ionization efficiency of the technique is extremely high [37]. The detection limits of TD-ESI/MS for common organic compounds are within sub-ppm levels in full scan mode, and ppb levels in multiple reaction monitoring (MRM) mode [37]. A TD-ESI/MS/MS analysis is typically completed in less than 15 s, enabling high-throughput screening of a large number of samples [38−48]. Applications of TD-ESI/MS/MS in biomedicine include the detection of (1) semi-volatile chemical compounds such as ingested pesticides and drugs in biological fluids for emergency care [42−46], (2) active ingredients in pharmaceutical products [38], and (3) drugs in plasma for pharmacokinetic studies [47,48]. In our previous study, TD-ESI/MS has been used to rapidly characterize sebaceous lipids [34]. Compared to the other AIMS approaches which were used to analyze skin surface lipids [30−33], the lipid profile of TD-ESI/MS is unique. All major classes of sebaceous lipids, including the unsaturated hydrocarbons (SQ) and nonpolar lipids (e.g. cholesterol), can be directly and rapidly detected without any sample treatment [34]. It is likely due the high temperature makes charged electrospray droplets dry faster, gas-phase ion/molecule reactions play a role in TD-ESI source for the ionization of thermal desorbed analytes [33].

Since the sampling and analysis for TD-ESI/MS and TD-ESI/MS/MS is non-invasive, sensitive, rapid, and capable of polar/nonpolar compounds, trace medicine secreted on skin can then be rapidly collected and analyzed. Herein, the capability of TD-ESI/MS/MS to detect the anti-fungal agent - terbinafine on skin of a patient who orally ingested terbinafine tablets for 12 weeks was first examined. Terbinafine is an allylamine-class agent that selectively inhibits the fungal squalene epoxidase and

has broad-spectrum activity against yeasts, fungi, molds, and dermatophytes. Terbinafine is clinically used for oral or topical treatment of mycoses of the skin such as onychomycosis, ringworm, and tinea capitis, where treatment duration depends on infection type and severity [49−53]. Terbinafine is highly lipophilic compound and tends to accumulate in sebum, hair, nail, and stratum corneum to play its primarily fungicidal action. Generally, pharmacokinetic studies of an oral drug could show that each drug administered will achieve its therapeutic concentration in the blood. In the case of terbinafine, directly detecting the drug on the skin surface can provide physicians more accurate and rapid information of the target skin area for treatment. A modification of the dosage of the drug can then be made promptly if there are any clinical concerns. In previous study, terbinafine in sebum, hair, *etc*. samples was determined by high-performance liquid chromatography methods. In this study, probe sampling was combined with TD-ESI/MS/MS to characterize trace terbinafine on different skin areas, which was secreted on patient's skin after ingesting terbinafine tablets. The distribution of terbinafine on the skin was visualized using a commercially available molecular mapping software. In addition, terbinafine ion signals on skin samples were monitored for 8 weeks after the test subject stopped taking the terbinafine tablets.

2. Experimental

2.1. Reagents and standards

Methanol (HPLC-grade) and glacial acetic acid (reagent-grade) were purchased from Merck (Darmstadt, Germany) and J.T. Baker (Phillipsburg, NJ, U.S.A.), respectively. Distilled deionized water (purified with a PURELAB Classic UV from ELGA, Marlow, U.K.) was used to prepare the electrospray solution and standard solutions. All reagents and solvents were used without further purification. Pure terbinafine standard was purchased from Sigma−Aldrich (St. Louis, U.S.A.). Stock solution of the terbinafine was prepared in methanol at an initial concentration of 10 mg/mL and then serially diluted to 0.1 ng/mL prior to further analysis.

2.2. Instrumentation

The TD-ESI mass spectrometric system was set up as described in our previous publication [37]. In brief, TD-ESI/MS is comprised of direct sampling probes, a thermal desorption unit, an electrospray ionization unit, and a triple quadrupole mass analyzer (Ultivo, Agilent, Santa Clara, U.S.A.). The probe used to collect samples was comprised of a stainless steel inoculating loop (diameter 2 mm, length 60 mm; Ching Ming Medical Device Co., Ltd. Taipei, Taiwan) which was screw-fastened to a probe holder by a pin clasp embedded in the holder. The temperature in the TD-ESI source was set at 280 °C — the optimized desorption temperature for terbinafine analysis — using a temperature controller (ANLY AT-502, Taipei, Taiwan). A hot nitrogen stream flowed at a pressure of 3 L/min from the top of the TD unit to deliver the thermally desorbed analytes into the ESI plume. The ESI solution comprised of methanol/water (1:1, *v/v*) with 0.1% acetic acid (*v/v*) was flown through a fused-silica capillary (inner diameter 100 μm) at a flow rate of 120 μL/h. A high voltage (+5 kV) was applied to the ESI capillary to induce ESI of the solution flowing out of the capillary via solution conduction. No additional nebulizing gas was used to assist with electrospray in this study. MS and MS/MS analyses were performed in positive ion mode to obtain mass spectra of molecular and precursor-product ions, respectively. MS analysis was conducted in full scan mode, while MS/MS analysis was performed in MRM mode using a normalized collision energy of 15 V.

2.3. Sebum sampling and analysis

Sebum samples were collected from a 22-year-old male volunteer who had diagnosed with onychomycosis and orally ingested terbinafine tablets for 12 weeks and a 22-year-old male volunteer with healthy skin, the latter of whom served as the healthy control. Samples were obtained after ethical review and approval by an institutional review board (KMUHIRB-E(I)-20160174), with informed consent given by both volunteers before commencing the study. To treat his onychomycosis, the onychomycosis patient was prescribed Lamisil® Tablets (250 mg/day of terbinafine hydrochloride).

Figure 1a shows the process of *in situ* sampling of the skin surface using a sampling probe. The skin was cleaned with an Alcohol Prep Pad (China Surgical Dressings Center Co. Ltd., Changhua, Taiwan) prior to sampling. The sebum samples were collected by gently sweeping the inoculating loop for 2 cm across the skin. Liquid samples were collected by dipping the inoculating loop into terbinafine standard solutions to collect 2 μL of the solution on the loop. After sampling, the probe was inserted into the TD-ESI source for subsequent MS analysis (Fig. 1b).

To explore the optimal mass spectrometric parameters and limit of detection (LOD) for

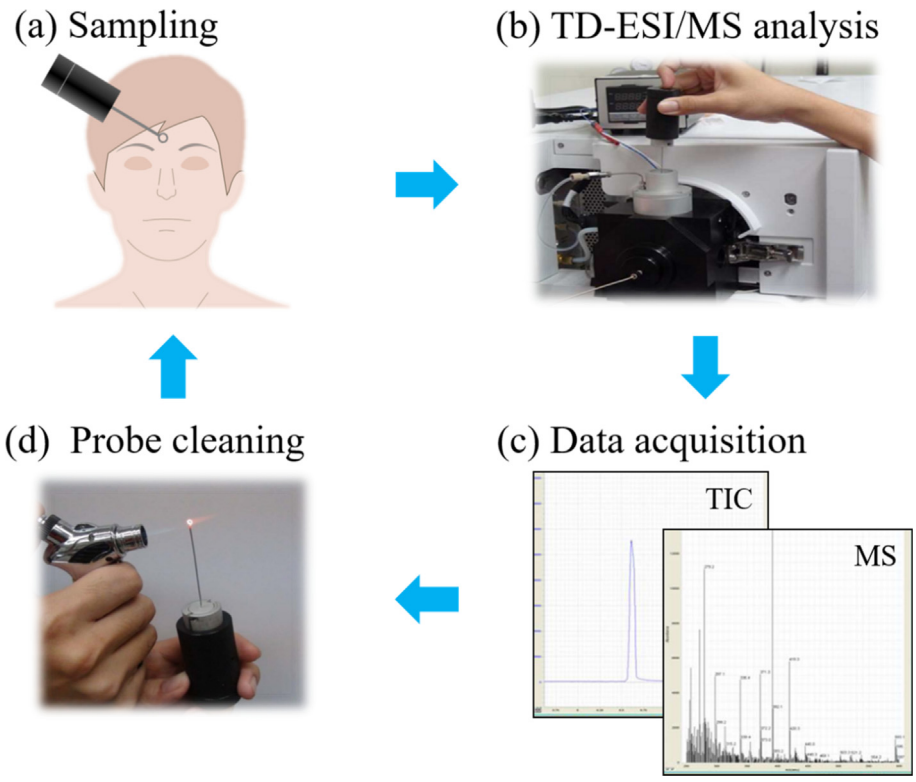

Fig. 1. Workflow of sebum analysis using TD-ESI/MS/MS, which involves (a) sampling by sweeping the sampling probe for 2 cm across the skin surface (e.g. the forehead) after cleaning with Alcohol Prep Pad 1 h, (b) inserting the probe into the TD-ESI unit, (c) obtaining the MS and MS/MS mass spectra, and (d) cleaning the probe with a flame.

terbinafine, liquid standards were analyzed using TD-ESI/MS (full scan mode) and TD-ESI/MS/MS (MRM mode), respectively (Fig. 1c). A terbinafine calibration curve was constructed by analyzing standards with concentrations ranging from 0.5 to 100 ng/mL in MeOH. The capability of TD-ESI/MS for characterizing terbinafine that is directly applied on skin was also explored. The skin of the hand was first cleaned with an alcohol prep pad. Immediately, a terbinafine standard solution (2 μL, 100 ng/mL) was spread evenly over an area of 2 cm × 2 cm on the back of the hand of the healthy control (for whom no baseline terbinafine ion signals were detected) and allowed to air-dry. Sampling was then performed by gently sweeping the probe for 2 cm across the terbinafine-smeared region of skin. Ten consecutive analyses of the samples collected from this region were performed, and the relative standard deviation (RSD) of the ten analysis was calculated.

To analyze the levels of secreted terbinafine with TD-ESI/MS/MS, sebum samples were collected after the onychomycosis patient had ingested Lamisil® Tablets daily for 2 weeks to allow internal terbinafine concentrations to reach homeostasis. Skin sampling was performed by gently sweeping the probe for 2 cm across the patient's alcohol-cleaned forehead, after which the probe was inserted into the TD-ESI source for analysis. TD-ESI/MS analysis was used to analyze the degree of accumulation of terbinafine secreted on the skin by collecting sebum samples before and after thoroughly washing the skin, as well as to explore the decrease in terbinafine secreted onto the skin. After taking a terbinafine tablet (250 mg) daily for 12 weeks, the onychomycosis patient stopped the medication, and sebum samples were collected and analyzed each week for 8 weeks overall.

After sample analysis, the probe loop was cleaned by burning it for 3–4 s with a high-temperature flame from a handheld butane torch to remove any residual organic compounds (Fig. 1d). Analysis of a sample — including sampling, thermal desorption, electrospray ionization, detection, and probe cleaning — was typically completed in approximately 30 s.

Various skin regions on both the onychomycosis patient (who self-ingested terbinafine for 2 weeks) and the healthy control were sampled and analyzed. Sebum samples were collected using multiple probes, where the inoculating loop from each probe was removed from the probe holder and inserted on

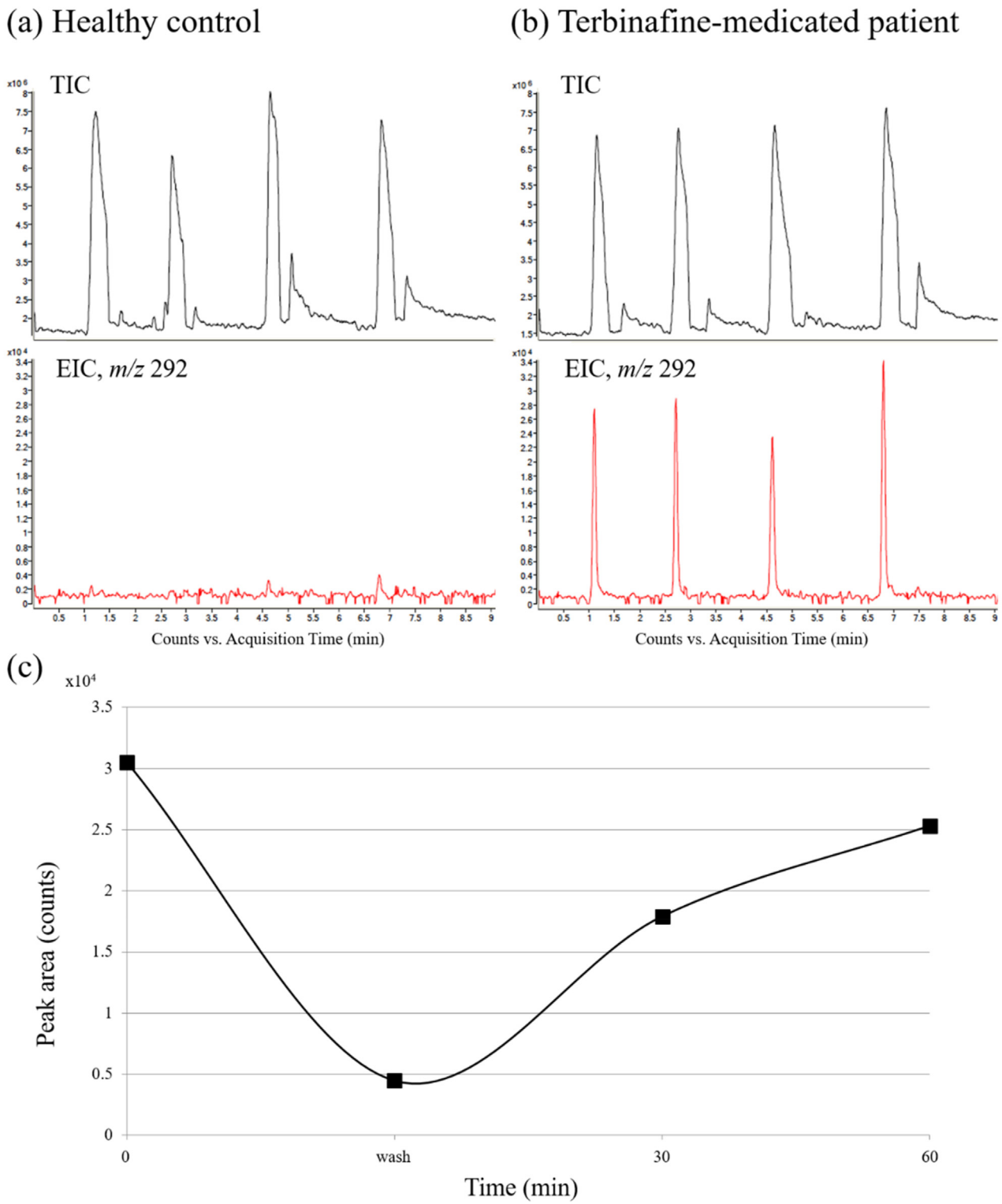

Fig. 2. TIC and EIC of terbinafine (m/z 292) for four consecutive analyses of sebum samples from (a) the healthy control who did not ingest terbinafine, and (b) the onychomycosis patient who ingested terbinafine for 2 weeks. (c) Peak areas of terbinafine (MRM for m/z 292→141) of sebum samples collected from the face of the onychomycosis patient before (Time = 0) and after washing the skin.

a Styrofoam plate covered with a cardboard box, with each plate holding up to 66 probes [36]. In this study, 168 probes were used to collect samples from 28 locations on the body (i.e. triplicate analysis for each sample location for two volunteers). Both the patient and healthy control were sampled after

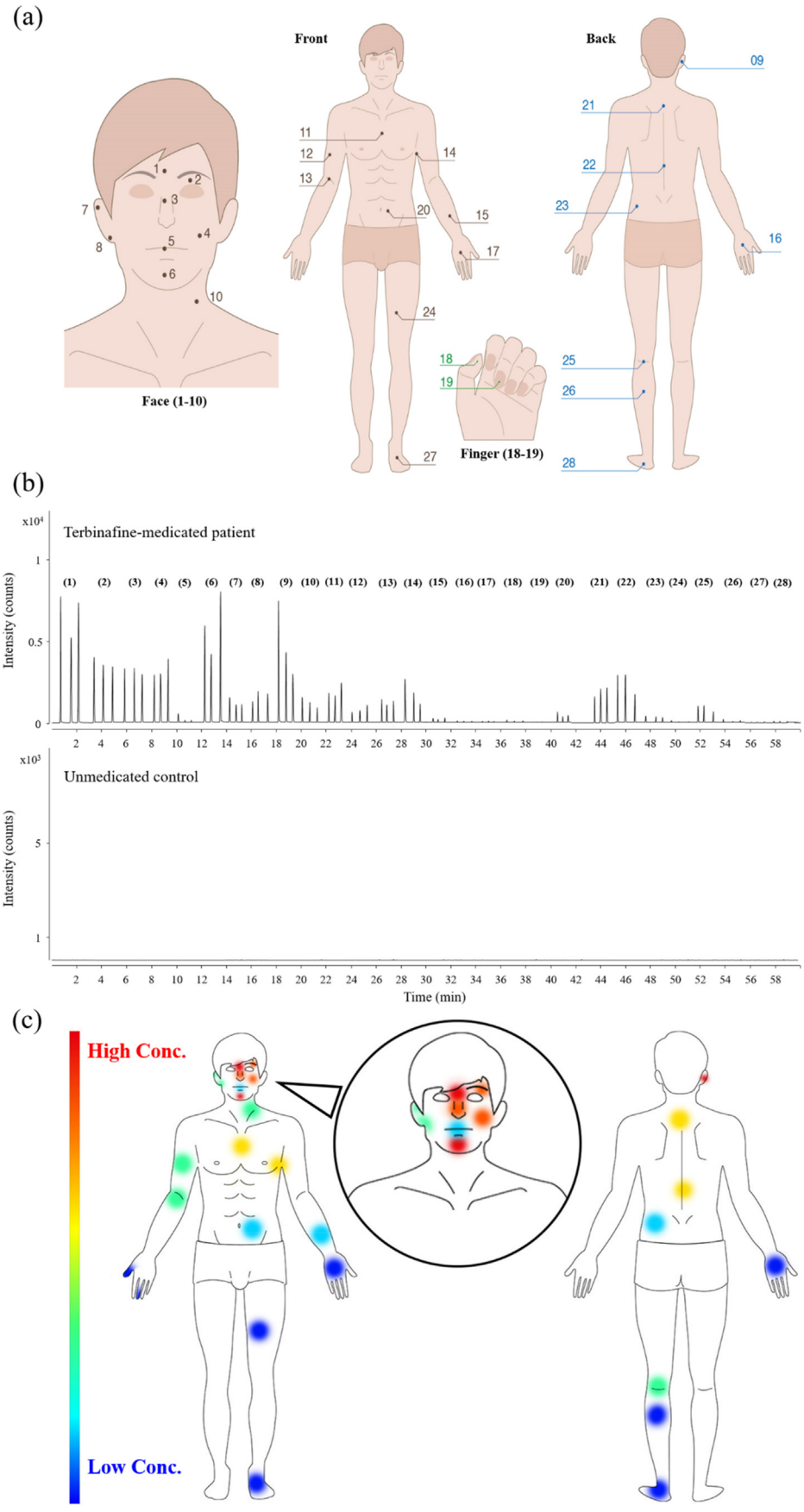
(a)
Front
Back
Face (1-10)
Finger (18-19)
(b)
Terbinafine-medicated patient
Unmedicated control
Intensity (counts)
Time (min)
(c)
High Conc.
Low Conc.

Fig. 3. (a) Sketch map of 28 sampling locations of the onychomycosis patient: 1) forehead, 2) eyelid, 3) nose bridge, 4) cheek, 5) lips 6) chin, 7) ear [inside], 8) earlobe, 9) ear [back], 10) neck, 11) chest, 12) upper arm, 13) cubital fossa, 14) armpit, 15) forearm, 16) hand [back], 17) palm, 18) fingertip, 19) fingernail, 20) abdomen, 21) upper back, 22) lower back, 23) waist, 24) thigh, 25) popliteal fossa, 26) calf, 27) instep, and 28) sole; (b) TIC of terbinafine (MRM for m/z 292→141) for sebum samples from different body regions of the terbinafine-medicated patient and the unmedicated control. Numbers above each current indicate the sampled skin regions as designated by Fig. 3a; (c) whole-body molecular mapping of terbinafine detected on the skin of front, face, and back side.

taking a bath and waiting 1 h for sebum secretion, where the entire sampling process was completed within 30 min. TD-ESI/MS/MS analysis of the sebum samples collected on each probe was conducted immediately after samples were collected and transferred to the MS laboratory.

3. Results and discussion

The LOD for the detection of terbinafine with TD-ESI/MS and TD-ESI/MS/MS was examined by analyzing standard solutions at different concentrations. Two microliters of the sample solution was deposited on the sampling probe with a micropipette. The probe was then inserted into the TD-ESI source for analysis. Figure S1a shows the TD-ESI mass spectra of terbinafine (*m/z* 292, MH^+) recorded from the aforementioned standard solutions with terbinafine concentrations of 0.1, 0.5, and 5 μg/mL in MeOH, respectively. No terbinafine ion signal (S/N<3) was detected for the standard solution of 0.1 μg/mL, but was detected from the solutions containing 0.5 and 5 μg/mL terbinafine standards. The LOD of terbinafine was then estimated to be between 0.1 and 0.5 μg/mL by using TD-ESI/MS (full scan mode). Figure S1b shows the total ion current (TIC) of terbinafine detected from the solutions containing 1, 10, and 100 ng/mL terbinafine standards with TD-ESI/MS/MS. An ion transition of *m/z* 292→141 for protonated terbinafine ion and its product ion was monitored during TD-ESI/MS/MS analysis. The results indicated that the LOD of terbinafine for TD-ESI/MS/MS was between 1 ng/mL and 10 ng/mL, and that the detection sensitivity of terbinafine ions with TD-ESI/MS/MS operated in MRM mode is at least 10 times better than that of TD-ESI/MS operated in full scan mode. Figure S1c shows the calibration curve for terbinafine with TD-ESI/MS/MS, where a good linearity for quantifying terbinafine with concentrations between 1 and 100 ng/mL ($R^2 = 0.9913$) was obtained.

To simulate skin sampling, the solution (2 μL) containing terbinafine standard (100 ng/mL) was applied on the back of the healthy control's palm and smeared for ca. 2 cm × 2 cm. After the solution was allowed to air-dry, a metallic probe was gently swept along the skin for 2 cm to collect a sample for TD-ESI/MS/MS analysis. Figure S2 shows the terbinafine ion signal (MRM mode for the transition of *m/z* 292→141) recorded from ten consecutive analyses of the samples collected from the terbinafine-smeared region. The relative standard deviation (RSD) of the ten analysis was calculated to be 16.38%, indicating that probe sampling combined with TD-ESI/MS/MS analysis shows a reasonably good repeatability for sebum sample analysis.

Figure 2 presents TIC and extracted an ion current (EIC) of terbinafine ion (*m/z* 292, MH^+, full scan mode) from quadruplicate analyses of sebum samples collected from the forehead skin of the healthy control (Fig. 2a) and terbinafine-medicated patients, respectively (Fig. 2b). Results of the analyses showed that terbinafine was not detected on the forehead skin of the healthy control but was detected on that of the terbinafine-medicated patient (EIC mode). The results indicate that TD-ESI/MS full scan mode is sensitive enough to detect trace terbinafine ingested by the onychomycosis patient. Assuming that 50% of the ingested terbinafine (250 mg) is secreted out of body through the skin (ca. 2 m^2), there is approximately 6.25 μg/cm^2 of terbinafine on the skin. Assuming 10% of the skin terbinafine on 1 cm^2 is collected by probe sampling, this means that 625 ng of terbinafine is collected on the probe during each sampling, which is far above the LOD of TD-ESI/MS and TD-ESI/MS/MS (Fig. S1). Figure 2c shows the peak area of terbinafine ion signals (MRM for *m/z* 292→141) detected in the sebum samples, which were collected before (Time = 0) and after washing the onychomycosis patient's face with facial cleanser first. As can be seen, terbinafine on the skin was dramatically removed by the facial cleanser (Time = wash), but its ion signal increased again and reached nearly the same level as it was in its pre-washed state 60 min later. Therefore, pre-washed state 60 min was served as the subsequent sebum sampling through this study.

To study the distribution of terbinafine on skin all over the body, a total of 28 skin regions of the body (10 on the face, 8 on the front body, 8 on the back body, and 2 on the finger) were selected for triplicate sampling (Fig. 3a). A total of 168 samples was collected for further TD-ESI/MS/MS analysis from two volunteers. We think sebaceous compounds are symmetrically distributed in right and left body, we

choose one side of different regions to perform sampling and analysis. Figure 3b shows the terbinafine ion signal (MRM mode for the transition of *m/z* 292→141) from the sebum samples collected from different skin regions of the terbinafine-medicated patient and the unmedicated healthy control, respectively. The numbers (#1−28) represent where the samples were collected (Fig. 3a and b). Terbinafine ion signals were detected in most of the sebum samples collected from the terbinafine-medicated patient; in contrast, no terbinafine ion signals were detected in sebum samples collected from the healthy control (Fig. 3b). In addition, terbinafine ion signals varied among the samples collected from different skin regions of the terbinafine-medicated patient, indicating regional variations in terbinafine secretion.

As is generally known, metabolites inside the body are secreted from the body through either sebaceous or sweat glands in the dermis. The possible mechanisms of metabolite secretion include the metabolites which circulate in the blood vessels, reach the capillaries, and diffuse into the dermis; they subsequently enter the glands and secrete from the skin. Sweat glands secrete mostly water and polar metabolites, while sebaceous glands secrete primarily less polar or non-polar metabolites [54]. Since terbinafine is a non-polar drug, it will be secreted to the skin mostly through sebaceous glands. Indeed, sebaceous glands are distributed all over the human body except for the palms of the hands and soles of the feet, which explains why lower terbinafine ion signals were detected in samples collected from those skin regions (#16-19 in Fig. 3b). On the other hand, the face and scalp have the highest density of sebaceous glands (400-900 glands/cm^2), as well as pilosebaceous units and meibomian glands (on the eyelids), and much higher terbinafine ion signals were detected in the samples collected from those regions (#1-9 in Fig. 3b). Most sebaceous glands are attached to hair follicles, which with the arrector pili muscle comprise the pilosebaceous unit. Figure 3c shows the distribution of terbinafine on skin throughout the body, where higher levels of terbinafine were detected in the samples collected from sebaceous-gland-rich areas (e.g. the face) than those from gland-sparse areas (e.g. the limbs). The highest terbinafine ion signals were detected in the samples collected from the forehead and chin (#1−9 in Fig. 3), while the lowest terbinafine ion signals were detected in the samples collected from the lips. The vermilion border of the lips is the only part of the face where the oral mucosa is continually exposed to the external environment. The skin of the lips has no hair follicles, no sweat glands, and no sebaceous glands. Therefore, very low terbinafine ion signals were detected on the lips, suggesting that the terbinafine secreted onto the skin around the lips may have been detected because the subject pursed his lips. In addition to the face, the ears, neck, chest, armpits, and back are also rich in sebaceous glands and consequently have higher terbinafine levels. These findings were in agreement with previous studies (skin patch sampling) which found that ingested terbinafine was mostly delivered to the stratum corneum, nails, and hair through sebaceous glands, and to a minor extent by direct diffusion through the dermis to the epidermis [50,54−57]. In these studies, the highest concentrations of terbinafine were accumulated in sebum and found to be 15%, 20%, 25% in the stratum corneum after 7, 12, and 14 days of oral doses (250 mg/day), respectively. Conversely, terbinafine concentrations were comparably lower in the hair and nails, while the highly hydrophobic terbinafine was not found in sweat [50]. Moreover, previous studies also indicated that relative lower terbinafine ion signals were detected in the peripheral nails after several days of medication [50].

It has been reported that the terbinafine persisted in plasma and peripheral tissue samples for prolonged periods after administration of the last dose. The present study was undertaken to characterize terbinafine during an 8-week washout phase following the last dose. Figure 4 shows the terbinafine ion signals in sebum samples collected from different skin regions of the onychomycosis patient during the last week of medication and each week after stopping medication up to 8 weeks. The numbers at the top indicate the skin regions where samples were collected (as designated in Fig. 3a and b). Even up to 2 weeks after stopping terbinafine medication, strong terbinafine ion signals were still detected in the sebum samples collected from the face of the patient (Regions #1−10). The terbinafine ion signals seem fluctuate through the washout phase. The possible reasons for this phenomenon include (1) sebum production fluctuates in response to changing hormone levels, the consumption of dietary fat or carbohydrate, body temperature, *etc*. Since the composition and amount of sebum can be influenced, secretion of sebaceous terbinafine may also quite dynamic; (2) the deviations of probe-sweeping sampling on human skin cannot be eliminated; (3) other environment factors, such as room temperature and humidity, may cause different skin conditions and affect the samples collecting. Although the terbinafine ion signals fluctuate through the washout phase, the results of

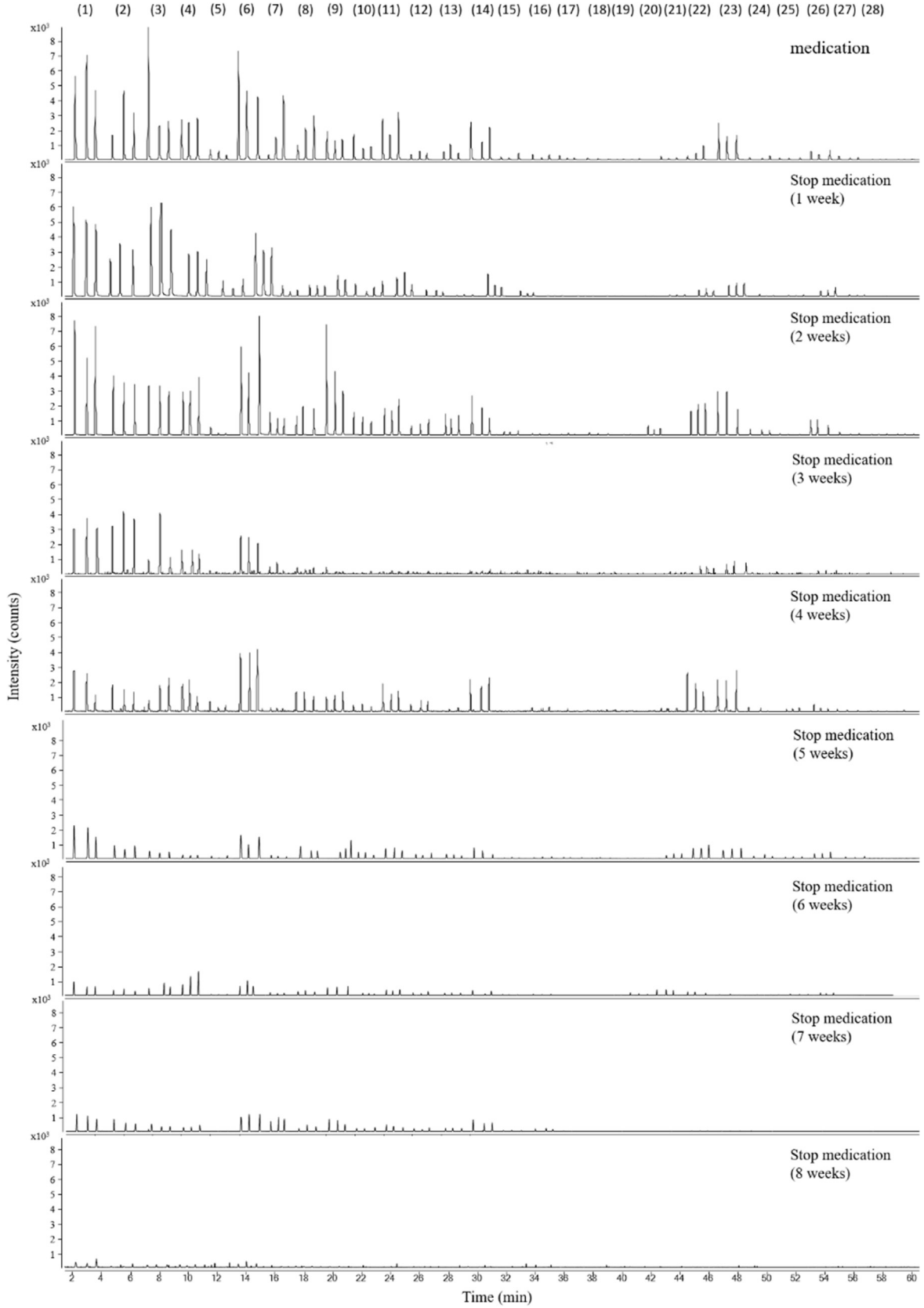

Fig. 4. Triplicate TD-ESI/MS/MS analyses of sebum samples collected from different skin regions of the onychomycosis patient after 12 weeks of terbinafine medication and each week after stopping medication up to 8 weeks. Numbers on each MRM current (terbinafine, m/z 292→141) indicate where the sebum samples sampled skin regions as designated by Fig. 3a.

this study are consistent with previous pharmacokinetic studies, which indicated that a single dose of terbinafine has a half-life of 30 h in blood, where the half-life is extended with an increase in dosage; the final half-life of terbinafine after reaching equilibrium is about 16.5 days [49]. Terbinafine ion signals started to decrease after the third week of stopping medication, and decreased significantly after the fifth week as terbinafine ion signals were only detected in the sebum samples collected from the upper body. No terbinafine ion signals (S/N<3) were detected in the sebum samples after stopping medication for 8 weeks.

A directly and rapidly AIMS approach for detecting ingested drugs via human skin has alternatively been developed, drug distribution of body locations on the skin was efficiently studied and the drug ion signal in different time after medication was accessibly monitored.

4. Conclusions

Skin and sebum profiles provide conveniently accessible information about the underlying drug metabolisms of the human body. In this study, probe sampling combined with TD-ESI/MS/MS analyses was used to rapidly detect the anti-fungal agent terbinafine in the sebum samples collected from different skin regions. Since sampling was easy and no sample preparation was required (e.g. solvent extraction, concentration, or matrix application), each analysis was completed within 30 s. Replicate analyses of terbinafine yielded satisfactory repeatability (RSD = 16.38%) and sensitivity (0.1 ng/mL). The terbinafine ion signals detected at different skin regions indicated that terbinafine levels were higher in the samples collected from sebaceous-gland-rich areas, especially in facial regions like the forehead, nose, cheeks, and chin; low terbinafine ion signals were detected in sweat-gland-rich areas such as the inner thighs, abdomen, and back. The terbinafine ion signal on patient's skin was monitored for 8 weeks after medication was terminated. Multi-drugs on skin can also be characterized after drug-medication. The novelty and significance of this work are concluded as two point: (1) directly and rapidly detecting ingested drugs via human skin by Ambient ionization mass spectrometry (AIMS). (2) using such a high-throughput approach, drug distribution of body locations on the skin was efficiently studied and the drug ion signal in different time after medication was accessibly monitored. The results suggested the potential for the use of TD-ESI/MS/MS to rapidly characterize crucial drug for pharmacokinetic studies.

Detection of the ingested terbinafine on skin is only a representative instance to demonstrate the capability for applying ambient ionization mass spectrometry in medical research. The developed analytical platform has an additional potential to help personalized medicine (precision medicine). For example, previous study showed the final elimination half-life is changed in patients with impaired liver or renal function, this will make the clearance rate of terbinafine be decreased. Monitoring of terbinafine on skin can give more effective medical decisions, practices, or interventions to the individual patient. Since the changes in skin metabolite profiles can grant insight into pathophysiological changes of studied individuals, monitoring the ion signals of specific ingredient secreted on skin instantly can give valuable information for optimal treatments or cures.

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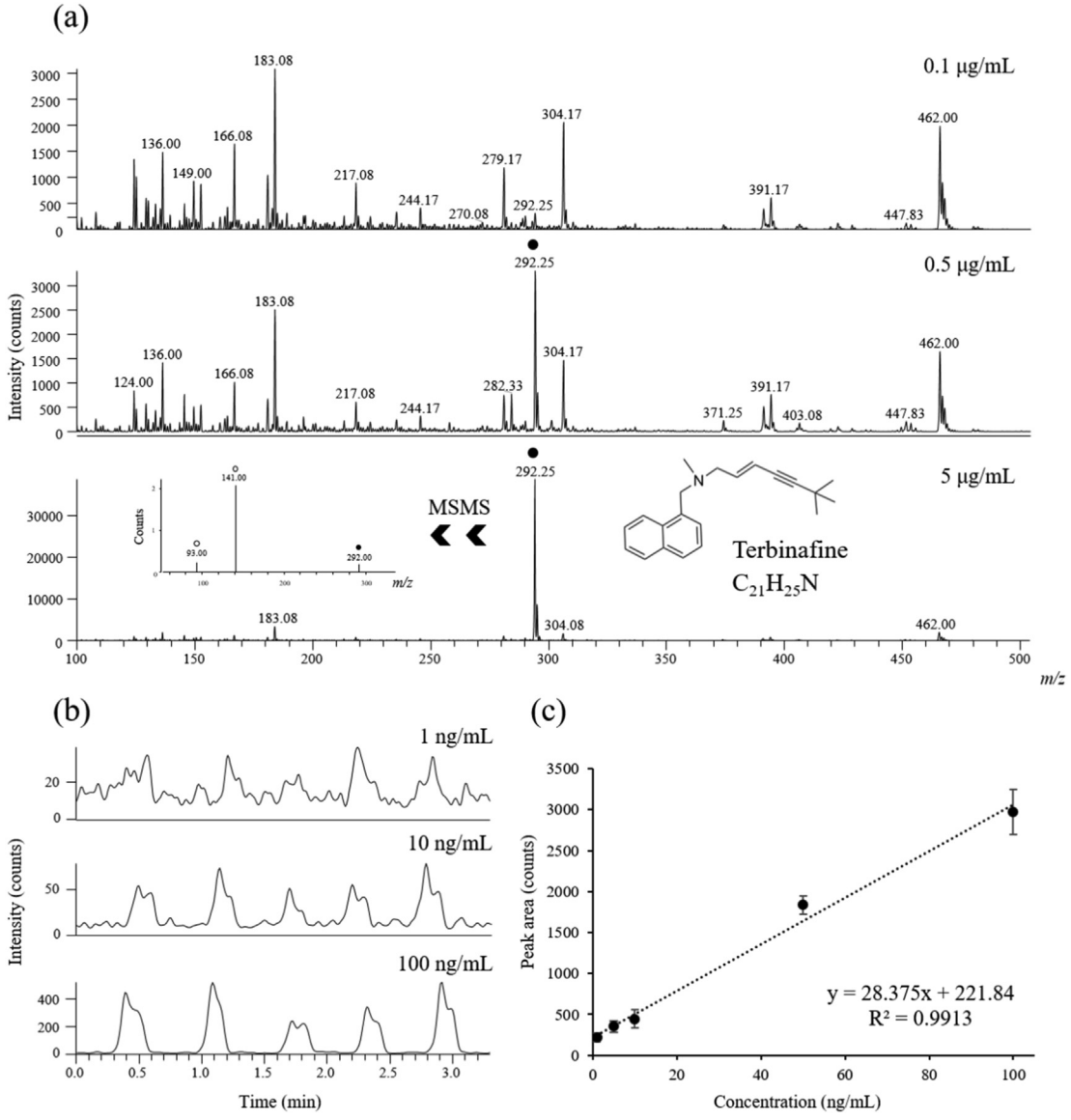

Fig. S1. (a) TD-ESI full scan mode mass spectra for terbinafine standards at concentrations of 0.1, 0.5, and 5 μg/mL (Inset: MS/MS analysis of terbinafine). (b) TIC of terbinafine standards (MRM for m/z 292→141) at concentrations of 1, 10, and 100 ng/mL. (c) Calibration curve of terbinafine standards ranges from 1 to 100 ng/mL.

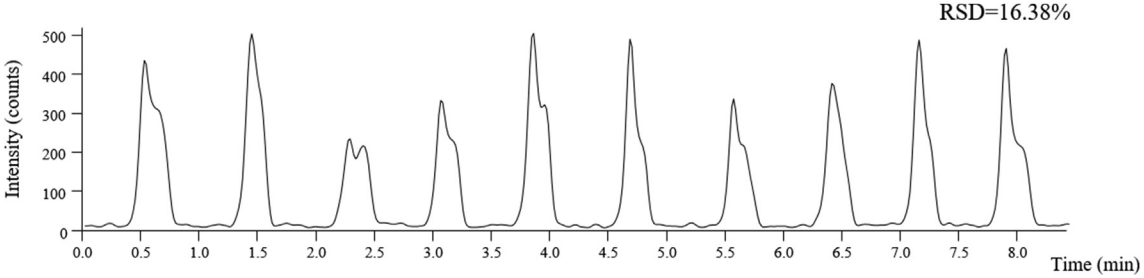

Fig. S2. TIC of terbinafine (MRM for m/z 292→141) from ten consecutive analyses of a terbinafine-applied region (ca. 2 cm × 2 cm) on the back of the hand of the unmedicated control. The sampled area was smeared with terbinafine standard solution (2 μL, 100 ng/mL) and allowed to air-dry.

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