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Short communication

Sensitive and simultaneous quantification of zinc pyrithione and climbazole deposition from anti-dandruff shampoos onto human scalp

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ABSTRACT

A sensitive ultrahigh performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method has been developed and validated for simultaneous quantification of zinc pyrithione (ZPT) and climbazole (CBZ) deposited onto human scalp from anti-dandruff (AD) shampoos. Scrubbing with a buffer solution was used as the sampling method for the extraction of ZPT and CBZ from scalp. Derivatization of ZPT was carried out prior to UHPLC–MS/MS analysis. The identification of ZPT and CBZ was performed by examining ratios of selected multiple reaction monitoring (MRM) transitions in combination with UHPLC retention times. The limit of detection for ZPT and CBZ was established to be 1 and 2 ng/mL, respectively. This sensitivity enables the quantification of ZPT and CBZ at deposition levels in the low ng/cm^2 range. The method was successfully applied for the analysis of scalp buffer scrub samples from an *in vivo* study. The levels of ZPT and CBZ deposited on the scalp at different time points after application of the AD shampoo were measured. The results revealed that dual-active AD shampoo delivered more ZPT onto the scalp in a single wash than single active shampoo did. The amount of ZPT and CBZ retained on the scalp after AD shampoo application declined over 72 h.

1. Introduction

The use of an anti-dandruff (AD) shampoo is one of the most applied home remedies for the treatment of dandruff. The active ingredients of AD shampoos are anti-fungals, the two most common being zinc pyrithione (ZPT) and climbazole (CBZ). The combination of ZPT and CBZ, as dual actives in a shampoo formulation has been proven to be able to deliver superior antidandruff efficacy and desired end sensory benefits [1]. The amount of AD actives deposited onto the human scalp in the process of shampoo application and rinse-off is considered as one of the crucial factors which determine the AD shampoo efficacy [2,3]. Hence, methods for the measurement of AD actives deposited onto the scalp are required.

There are many analytical methods for the determination of ZPT and CBZ in shampoos [4–10]. The complicated matrices of shampoos can make the analysis challenging, but high sensitivities for the detection of ZPT and CBZ are not required due to their rather high levels in the AD shampoos. A number of more sensitive methods were developed for the determination of ZPT and CBZ in environmental matrices [11–14]. For *in vivo* studies monitoring ZPT and CBZ deposition onto the scalp, besides sensitive detection, efficient sampling and sample pre-treatment techniques are required. In a previous study [15], we developed a sensitive method for the determination of CBZ deposited on artificial skin and human scalp from AD shampoos. For ZPT analysis, unfortunately, sensitive and easy-to-use methods are still lacking. Due to the unwanted interactions of the compound with the silanol groups from silica-based liquid chromatography (LC) stationary phases [13] and the transchelation with other cations (*e.g.*, Fe, Cu) [6,13] during the analysis, poor peak shapes are often obtained, especially at ppb levels. A

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liquid chromatography–mass spectrometry (LC–MS) method for the direct analysis of ZPT was developed by Yamaguchi et al. in 2006 [17]. Unfortunately the sensitivity of this method was insufficient to ensure detection of ZPT at all relevant levels. Derivatization of the ZPT with fluorescing groups was reported to stabilize the pyrithione complex and improve the detection limits [18], but for the complex scalp samples the selectivity of fluorescence detection is not sufficient. Mass spectral confirmation is needed, while maintaining the excellent peak shape and sensitivity of the derivatization/fluorescence route. Moreover, simultaneous determination of ZPT and CBZ deposited on human scalp would be needed, for which, however, no method has been reported till now.

In the present contribution a method is described and validated for simultaneous quantification of ZPT and CBZ deposited on human scalp. Scrubbing the scalp with a buffer solution was applied as the sampling method for extraction of ZPT and CBZ from scalp [15] and an *in vivo* study was designed for method development and validation. An ultrahigh performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method was employed for the separation and detection of ZPT (after derivatization) and CBZ in scalp buffer scrub samples.

2. Materials and methods

2.1. Chemicals and reagents

All reagents and solvents used in the experiment were analytical grade or better. Pure water (18.2 MΩ) prepared using a Milli-Q® Advantage A10 Water Purification System (Millipore, Bedford, MA, USA) was used for preparation of solutions and dilutions. ZPT (95%), CBZ (99%), EDTA-2Na, 2, 2-dipyridyl disulfide (DPS) and phosphate buffered saline (PBS) tablets were supplied by Sigma–Aldrich (St. Louis, MO, USA). HPLC grade dimethyl sulfoxide (DMSO), acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). Ammonium acetate, Triton X-100 and Tween 80 were purchased from SCRC (Shanghai, China). A surfactant-modified PBS solution for the buffer scrub sampling method was prepared by adding four PBS tablets, 1.0 g of Triton X-100 and 5.0 g of Tween 80 to 1 L of Milli-Q water. Saturated EDTA-2Na solution was prepared by dissolving 25 g EDTA-2Na in 100 mL Milli-Q water, stirring at 60 °C for 1 h. DPS derivatization solution was prepared by dissolving 0.6 g DPS in 50 mL acetonitrile.

2.2. Test shampoos

The three test shampoos used in this study were purchased at a local supermarket in Shanghai, China. They were one dual-active AD shampoo (1% ZPT, 0.5% CBZ), one single active AD shampoo (1% ZPT) and one beauty shampoo (no AD actives).

2.3. In vivo study

The *in vivo* study was conducted in Shanghai, China at the Unilever internal facility. It was cleared by the Joint Research Ethics Committees, Shanghai, and all subjects gave their informed consent to participate. This was a single centre, randomized, half head, double-blind and single gender study. Each subject tested two products. Healthy male subjects aged 18–65 years (inclusive) not using anti-dandruff shampoo during the past 3 months were recruited and screened by the so-called Total Weighted Head Score (TWHS) system [16]. The study lasted for one week with five visits. A total of 27 subjects completed the whole study. The buffer scrub sampling method was applied for extraction of ZPT and CBZ during the whole study. More details on this method can be found elsewhere [15]. A surfactant-modified PBS solution was used as extraction fluid for extracting ZPT and CBZ after the scalp was gently massaged with a Teflon rod. For each sampling site, about 3.5 mL of the buffer solution was collected for quantitative analysis of ZPT and CBZ.

During the first visit the subjects washed their hair using the commercial beauty shampoo and they were reminded that no hair washing or wetting was allowed except during the visits. The second visit was three days later. During this visit the subjects washed their hair using the commercial AD Shampoos. Sampling was carried out before and after shampoo application to obtain baseline samples and 'right after wash' samples. During the third visit (twenty four hours after AD shampoo application), sampling was performed to obtain 24 h after washing samples. During the fourth visit (forty eight hours after AD shampoo application), sampling was performed to obtain the 48 h after washing samples. During the fifth visit (72 h after AD shampoo application), sampling was performed to obtain 72 h after washing samples. All the scalp buffer scrub samples were collected in Nunc 15 mL centrifuge tubes (Thermo Scientific, Waltham, Massachusetts USA) and were stored at −20 °C prior to analysis.

2.4. Sample treatment

The scalp buffer scrub samples (about 3.5 mL per sample) were first diluted with methanol (two-fold) to break the emulsion and precipitate proteins. For the DPS derivatization, 5 mL of the methanol extract of the sample was transferred into a 15 mL centrifuge tube. Next, 80 μL of a saturated solution of EDTA-2Na and 200 μL of DPS solution were added; the sample was thoroughly mixed by vortex and then placed in the dark for 1 hour. After the derivatization, the sample solutions were filtered over a 0.45 μm Nylon filter supplied by SCRC (Shanghai, China) prior to UHPLC–MS/MS analysis.

2.5. Standard solutions

A CBZ and ZPT mixed stock solution was prepared in DMSO at a concentration of 1000 mg/L for each analyte. It was stored at 4 °C in the dark prior to use. The working solutions were prepared freshly by the appropriate dilution of this stock solution with a mixture of 50% of mobile phase A and 50% mobile phase B. Prior to UHPLC–MS/MS analysis, the working solutions were subjected to the same DPS derivatization procedure as the buffer scrub samples.

2.6. UPLC–MS/MS analysis

A Waters ACQUITY UPLC system coupled to a Quattro Micro API mass spectrometer (Waters, Manchester, UK) was used for the sample analysis. Separation was carried out on a Waters ACQUITY UPLC BEH C18 column (2.1 mm × 50 mm × 1.7 μm). The mobile phase was composed of 20 mM ammonium acetate in water (A) and methanol (B) programmed in the linear gradient mode [time 0 min, 80% A, maintain 1 min; time 3.5 min, decrease immediately to 50% A; time 5.5 min, decrease immediately to 10% A; time 6.5 min, maintain 10% A; time 8 min, increase immediately to 80%]. Atmospheric pressure chemical ionization (APCI) in positive mode was used for all experiments. Optimum APCI performance was obtained at a corona current of 3.6 μA, a cone voltage of 32 V, an extractor voltage of 3 V, a source temperature of 100 °C, an APCI probe temperature of 300 °C, a desolvation gas (nitrogen) flow of 450 L h^{-1} and a cone gas (nitrogen) flow of 25 L h^{-1}.The multiple reaction monitoring (MRM) mode was used for the determination of CBZ and ZPT. The collision gas (argon) pressure was set at 3×10^{-3} mbar. The dwell time for each MRM transition was 0.20 s. ZPT derivative was analysed using the transitions of m/z 237.0 > 126.0 (collision energy 17 V) and 237.0 > 111.0 (collision energy 17 V) signals while