Effect of Combined Exposure to EDTA and Zinc Pyrithione on Pyrithione Absorption in Rats

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Abstract

Zinc pyrithione (ZnPT) is a coordination complex of zinc and has been used widely as an anti-dandruff agent in shampoos. Many shampoos contain both ZnPT and EDTA, a chelating agent speculated to increase ZnPT absorption, thereby raising concerns about neurotoxicity. Here, we investigated the effect of EDTA on ZnPT absorption by direct comparison of ZnPT and pyrithione (PT) concentrations in shampoo formulations, and by pharmacokinetic analysis of ZnPT, PT, and 2-methanesulfonylpyridine (MSP), the main ZnPT metabolite, in rat plasma or urine following exposure to shampoo containing ZnPT alone or a combination of ZnPT and EDTA. Approximately 17.3% of ZnPT was converted to PT by the addition of EDTA in the shampoo formulation. Plasma ZnPT and PT concentrations were not measured up to 24 hr after treatment with shampoo containing 1% ZnPT or 1% ZnPT + 2% EDTA in all rats. However, PT amount in 24-hr urine sample, MSP concentration in plasma, and MSP amount in 24-hr urine sample were approximately 4-, 2.6-, and 2.7-fold higher, respectively, in the 1% ZnPT + 2% EDTA shampoo group than in the 1% ZnPT shampoo group. As confirmed by the formulation analysis and in vivo pharmacokinetic analysis, the exposure of ZnPT could be increased by the absorption of PT due to partial dissociation of ZnPT into PT.

Key words: Zinc pyrithione, Pyrithione, Pharmacokinetic, Shampoo

INTRODUCTION

Zinc pyrithione (ZnPT) is a coordination complex of zinc (Fig. 1), and its use has been widely allowed in the European Union as an anti-dandruff agent in hair dressing formulations and shampoos, as a preservative at a concentration of 0.5% in cosmetic rinse-off hair-care products, and at a concentration of 1% in rinse-off antifouling hair-care products (1). It has also been intensively used worldwide as an antifouling agent in painting formulations.

The safety of ZnPT or pyrithione (PT) derivatives, such as sodium pyrithione (NaPT) and copper pyrithione (CuPT), has been studied in several animal species through different routes of administration. Dogs showed ocular damage...
involving the tapetum lucidum after treatment with oral ZnPT at 6-12 mg/kg/day for 6 days (2,3). Rats and rabbits showed hindlimb weakness or paralysis after subchronic and chronic administration of ZnPT (3,4). ZnPT can transform into CuPT by transchelation with copper ion in both laboratory and natural conditions (5,6). The environmental toxicity of ZnPT or CuPT and their degradation products on microalgae, macrophytes, crustaceans, fish, sea urchin, and other organisms have been studied (7-17). More than 200 shampoos contain both ZnPT and EDTA, a chelating agent. EDTA is useful to improve stability in shampoo formulations. ZnPT has been reported to show low penetration, whereas NaPT, with high water solubility, was shown to be absorbed through the skin in much greater amount than ZnPT (18). Thus, NaPT was prohibited as an ingredient in shampoo formulations. However, the current situation has been left without any criteria with respect to the formulation of ZnPT and EDTA at home and abroad.

These compositions are speculated to increase ZnPT absorption by EDTA, and the resulting toxicity is concerning (Fig. 2). ZnPT is absorbed in small quantities through the skin and is rapidly metabolized into its metabolites. Among the metabolites, 2-methanesulfonylpyridine (MSP) has been identified as a major serum metabolite of ZnPT (19). Thus, in this study, we evaluated the effect of EDTA on systemic ZnPT exposure by measuring ZnPT, PT, and MSP contents in rat plasma and urine following treatment with shampoos containing ZnPT alone or a combination of ZnPT and EDTA.

**MATERIALS AND METHODS**

*Chemicals.* ZnPT, PT, MSP, EDTA, imipramine HCl (an internal standard for MSP analysis), and d<sub>5</sub>-5-Nitro-5'-hydroxy-indirubin-3'-oxime (d<sub>5</sub>-AGM130; an internal standard for ZnPT and PT analyses) were purchased from Sigma-Aldrich Chemical Corporation (Milwaukee, WI, USA). Water was purified with a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals and reagents were of analytical grade and used without further purification.

*Preparation of shampoo formulations.* Shampoo formulations were prepared by adding ZnPT alone or ZnPT and EDTA in a commercial shampoo product (Head & Shoulder, Cincinnati, OH, USA) to yield a final concentration of 5% ZnPT or 5% ZnPT + 10% EDTA, respectively. The shampoo formulations were mixed thoroughly, then diluted 5 times with distilled water. The final diluted shampoo formulations (1% ZnPT or 1% ZnPT + 2% EDTA) were used in experiments.

*Animal experiments.* Male Sprague-Dawley rats (approximately 8 weeks old, n = 12) (Central Lab. Animal Inc., Seoul, Korea) were used for experiments. The rats were kept under controlled conditions (ambient temperature of 23 ± 2°C, humidity of 50 ± 10%, 12-hr light/dark cycle). Food (Central Lab. Animal Inc.) and water were supplied *ad libitum*. The rats were acclimated to the laboratory conditions for 7 days. All experimental procedures were approved by Dankook University’s Institutional Animal Care and Use Committee (DUIACUC), which adheres to the guidelines issued by the Institution of Laboratory of Animal Resources (ILAR). The rats were fasted for at least 12 hr prior to the start of experiment. Each group consisted of 6 males. The animals were approximately 10 weeks old at the study date. Individual body weights ranged from 270 to 350 g. The animals were then assigned to two groups. Group 1 was treated with shampoo containing 1% ZnPT, whereas group 2 was treated with shampoo containing 1% ZnPT + 2% EDTA. The rats were anesthetized with zoletil 50 (5 mg/kg) administered intramuscularly, and then cannulated using a polyethylene tube into the jugular vein. The distal end of the cannula was then tunneled subcutaneously to the back of the neck, where it was exteriorized. Blood coagulation in the cannula was prevented through introduction of 0.2 mL of heparinized normal saline (20 units/mL). After all cannulated rats completely recovered from anesthetization, they were partially anesthetized with zoletil 50 (3 mg/kg) administered intramuscularly, and then cannulated using a polyethylene tube into the jugular vein. The distal end of the cannula was then tunneled subcutaneously to the back of the neck, where it was exteriorized. Blood coagulation in the cannula was prevented through introduction of 0.2 mL of heparinized normal saline (20 units/mL). After all cannulated rats completely recovered from anesthetization, they were partially anesthetized with zoletil 50 (3 mg/kg) administered intramuscularly, and exposed to shampoo solutions. Six cannulated rats of each group were immersed below the cannulated part in each shampoo solution by holding for 10 sec, dried by wiping, and then placed in metallic cages. Blood samples were collected from the cannulated rats via the jugular vein before shampoo treatment and at 0.5, 1, 2, 3, 4, 6, 8, and 24 hr after shampoo treatment. Approximately 0.3 mL of blood samples were collected using heparin-coated disposable syringes. Immediately after collection, blood samples were centrifuged for 1-2 min at 10,000-13,000 rpm. The obtained
plasma samples were stored at −70°C until analysis. Urine samples were collected for 0-24 hr. After urine collection at 24 hr, the cages were washed with 10 mL distilled water, and the washings were mixed into the 24-hr urine sample. The final urine volume of each animal was reported, and each 1 mL of urine sample was stored at −70°C until analysis.

Determination of ZnPT and PT in rat plasma and urine. Chloroform:MeOH (2:1, v/v) (150 μL) was added to plasma and urine samples (50 μL), and the mixtures were mixed thoroughly for 30 sec. After centrifugation at 4°C and 13,000 rpm for 5 min, the chloroform layers (50 μL) were diluted with MeOH (50 μL). The processed samples (5 μL) were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). An Agilent 1260 (Santa Clara, CA, USA) HPLC system coupled to an API4000 Q-trap (AB Sciex, Framingham, MA, USA) quadrupole tandem mass spectrometer was used to determine ZnPT and PT concentrations in plasma and urine. The HPLC column was a Capcell PAK MG (3 mm × 35 mm, 3 μm particle size) connected with a guard column (SecuryGuard C18 4 × 2 mm, 3 μm; Phenomenex, CA, USA). The separation was carried out with a mobile phase consisting of MeOH (0.1% formic acid)/20 mM ammonium acetate (90/10, v/v), with the following conditions: flow rate, 350 μL/min; injection volume, 5 μL; column oven temperature, 40°C; run time, 1.5 min. The Q-Trap mass spectrometer was operated in the positive ion mode using an electrospray ionization source. High-purity nitrogen gas was used for the nebulizer and curtain gases. The source temperature was set at 400°C with a curtain gas flow of 50 L/min. The ion spray voltage was set at 5,000 V and the collision energy was 35 V for MSP, respectively. The following MRM transitions of the respective [M+H]+ ions were used to quantify MSP: m/z 158.2 → 78.0 and imipramine (IS): m/z 281.2 → 86.2. Calibration curves exhibited excellent linearity over a range of 5–1,000 ng/mL in rat plasma (Supplementary Fig. 3) and of 20–1,000 ng/mL in rat urine. The intra- and inter-precision values (%) in rat plasma were in a range of 97.2% to 99.9%, whereas the intra- and inter-precision values (CV, %) were less than 10% (Supplementary Table 3).

Data analysis. All chromatographic peaks were reviewed, and any integration corrections were made manually when necessary. Calibration curves were prepared using a linear regression with 1/x or 1/x² weighting. All standard and sample concentrations were determined using internal standard areas versus analyte areas. Individual plasma concentration-time data of MSP were analyzed using non-compartmental method of the WinNonlin 2.1 pharmacokinetic program. Nominal sample times were used. The area under the concentration-time curve (AUC) was calculated using the linear log-linear trapezoidal rule. The maximum plasma concentration (Cmax) and the time at which it was observed (Tmax) were directly determined from the plasma concentration-time profiles. All data were expressed as mean ± SD.

RESULTS

Analysis of shampoo formulations. ZnPT and PT concentrations in the shampoo formulations prepared in laboratory (Supplementary Fig. 4, Supplementary Table 4) were analyzed by LC-MS/MS, and the results are presented in Table 1. In the shampoo containing 1% ZnPT
alone, ZnPT and PT concentrations were 9.79 and 0.001 mg/mL, respectively, whereas in that containing 1% ZnPT + 2% EDTA, ZnPT and PT concentrations were 5.82 and 1.73 mg/mL, respectively. Recovery rates (%) of ZnPT in shampoo containing 1% ZnPT or 1% ZnPT + 2% EDTA were 97.9 % and 58.2 %, respectively. Moreover, approximately 17.3% of ZnPT was converted into PT in the shampoo containing 1% ZnPT + 2% EDTA (Table 1).

ZnPT and PT in rat plasma and urine. Analytical methods of ZnPT and PT concentrations in rat plasma and urine were established and validated (Supplementary Fig. 1, 2, Supplementary Table 1, 2). Two rats, 1 from each group, died during the sampling procedure after catheterization. Therefore, samples from five rats of each group were analyzed. ZnPT and PT concentrations in rat plasma and 24-hr urine sample after treatment with shampoo containing 1% ZnPT or 1% ZnPT + 2% EDTA are shown in Table 2. Plasma ZnPT and PT concentrations were not measured up to 24 hr after treatment with shampoo containing 1% ZnPT or 1% ZnPT + 2% EDTA in all rats. ZnPT amount was measurable in the 24-hr urine sample of most rats of the 1% ZnPT + 2% EDTA shampoo group. However, there was no significant difference in urine ZnPT amount between the two groups, and most of urine ZnPT must have originated from the washing water of metabolic cages, which were contaminated with ZnPT from wiped rat hair. PT amount in 24-hr urine was approximately 4-fold higher in the 1% ZnPT + 2% EDTA shampoo group than in the 1% ZnPT shampoo group.

**Table 2.** ZnPT and PT concentrations in rat plasma and urine after shampoo treatment

| Group                  | Plasma (ng/mL, n = 5) | Urine (µg, mean ± SD, n = 5) |
|------------------------|-----------------------|-----------------------------|
|                        | ZnPT                  | PT                          |
| 1% ZnPT                | BLQ                   | BLQ                         |
| 1% ZnPT + 2% EDTA      | BLQ                   | 1.0 ± 0.2                   |
|                        |                       | 4.7 ± 1.7                   |

BLQ: Below the lower limit of quantitation (50 ng/mL).

**Table 3.** Plasma MSP concentrations (ng/mL, mean ± SD) in rats after treatment with shampoo containing 1% ZnPT or 1% ZnPT + 2% EDTA

| Time (hr) | 1% ZnPT (n = 5) | 1% ZnPT + 2% EDTA (n = 5) |
|-----------|----------------|---------------------------|
| Blank     | BLQ            | BLQ                       |
| 0.5       | BLQ            | BLQ                       |
| 1         | 9.5 ± 10.3     |                            |
| 2         | 38.5 ± 26.8    |                            |
| 3         | 7.6 ± 1.8      | 91.3 ± 51.7               |
| 4         | 11.7 ± 2.6     | 138 ± 68.1                |
| 6         | 23.4 ± 5.5     | 225 ± 100                 |
| 8         | 35.9 ± 12.9    | 286 ± 122                 |
| 24        | 565 ± 435      | 1175 ± 269                |

BLQ: Below the lower limit of quantitation (5 ng/mL).

**Table 4.** Pharmacokinetic parameters of MSP in rat plasma after treatment with shampoo containing 1% ZnPT or 1% ZnPT + 2% EDTA

| Parameters | 1% ZnPT (µg/mL) | 1% ZnPT + 2% EDTA (µg/mL) |
|------------|-----------------|---------------------------|
| Cmax       | 0.57 ± 0.44     | 1.18 ± 0.27               |
| AUClast    | 4.9 ± 3.4       | 12.8 ± 1.4                |

Data are presented as mean ± SD.

**Table 5.** MSP amount (µg) in rat urine at 24 hr after shampoo treatment

| Group                  | Urine (µg, mean ± SD) |
|------------------------|-----------------------|
| 1% ZnPT (n = 5)        | 1.8 ± 1.2             |
| 1% ZnPT + 2% EDTA (n = 5) | 4.9 ± 1.2             |
DISCUSSION

Zinc pyrithione (ZnPT) has been widely used as an antifungal agent in hair dressing formulations and shampoos, and as an antifouling agent in painting formulations. The toxicity of ZnPT after oral, dermal, and inhalation administration has been examined in repeated toxicity studies using several animal species. A NOAEL of 500 μg/kg per day obtained from a chronic oral study (SCCS 2014) of ZnPT based on paralysis and hindlimb weakness, has been derived. The percutaneous absorption of ZnPT through the skin is very low with approximately 0.03-3.4%, compared with that of NaPT. Compared with ZnPT, NaPT showed much higher water solubility and is absorbed through the skin in much greater amounts (18). Therefore, NaPT was prohibited as an ingredient in shampoo formulations.

EDTA, a chelating agent, has been used as a component in shampoo formulations to improve stability. We investigated the effect of EDTA on ZnPT absorption in shampoo formulations. PT and MSP, among ZnPT metabolites (19,20), are used as indicators of systemic ZnPT exposure. In this study, a direct LC-MS/MS method was developed for quantitative determination of ZnPT, PT, and MSP concentrations in rat plasma and urine. Analytical results showed significant differences in ZnPT and PT concentrations between the two shampoo formulations tested in this study. Compared to those in the shampoo formulation without EDTA, ZnPT concentration decreased by approximately 59%, whereas PT concentration increased in the shampoo formulation with EDTA. These results indicated that ZnPT was partially dissociated to PT ion by chelating zinc ion of EDTA.

Plasma ZnPT concentrations were below the LLOQ in all samples from the 1% ZnPT and 1% ZnPT + 2% EDTA shampoo groups. Furthermore, there were no differences in PT concentration in plasma samples and ZnPT amount in 24-hr urine samples between the 1% ZnPT and 1% ZnPT + 2% EDTA shampoo groups. However, PT amount in 24-hr urine sample was approximately 4-fold higher in the 1% ZnPT + 2% EDTA shampoo group than in the 1% ZnPT shampoo group. As confirmed by the formulation analysis, this could be caused by increased absorption of PT due to partial dissociation of ZnPT into PT by EDTA. Systemic exposure to ZnPT could be increased by EDTA in cosmetic rinse-off hair-care products or antifungal hair-care products, and chronic use of these products may result in unexpected neurotoxicity. The current situation has been left without any criteria with respect to ZnPT and EDTA formulations at home and abroad. The effect of EDTA on systemic exposure to ZnPT should be confirmed through further studies, such as stability test of ZnPT in shampoos containing EDTA or patch test. However, rinse-off hair care products containing 1% ZnPT and EDTA are acceptable for safe use, according to risk assessment of ZnPT products including EDTA performed by the Ministry of Food and Drug Safety of Korea (MFDS).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Scientific Committee on Consumer Safety (SCCS) (2014) Opinion on Zinc Pyrithione (Colipa P81).
2. Cloyd, G.G., Wyman, M., Shadduck, J.A., Winrow, M.J. and Johnson, GR. (1978) Ocular toxicity studies with zinc pyri-
3. Snyder, F.H., Buehler, E.V. and Winek, C.L. (1965) Safety evaluation of zinc 2-pyridinethiol 1-oxide in a shampoo formulation. Toxicol. Appl. Pharmacol., 4, 425-437.

4. Sahenk, Z. and Mendell, J.R. (1977) Studies on the dying-back process of peripheral nerves using bis(N-oxopyridine-2-thionato)zinc(II). Neurology, 27, 393.

5. Grunnet, K.S. and Dahllof, I. (2005) Environmental fate of the antifouling compound zinc pyrithione in seawater. Environ. Toxicol. Chem., 24, 3001-3006.

6. Thomas, K.V. (1999) Determination of the antifouling agent zinc pyrithione in water samples by copper chelate formation and high-performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry. J. Chromatogr. A, 833, 105-109.

7. Bellas, J., Granmo, K. and Beiras, R. (2005) Embryotoxicity of the antifouling biocide zinc pyrithione to sea urchin (Paracentrotus lividus) and mussel (Mytilus edulis). Mar. Pollut. Bull., 50, 1382-1385.

8. Mochida, K., Amano, H., Onduka, T., Kakuno, A. and Fujii, K. (2009) Embryotoxicity of zinc pyrithione, an antifouling chemical, in fish. Environ. Res., 81, 81-83.

9. Koutsafis, A. and Aoyama, I. (2006) The interactive effects of binary mixtures of three antifouling biocides and three heavy metals against the marine algae Chaetoceros gracilis. Environ. Toxicol., 21, 432-439.

10. Gibson, W.B., Jeffcoat, A.R., Turan, T.S., Wendt, R.H., Hughes, P.F. and Twine, M.E. (1982) Zinc pyrithione: Serum metabolites of zinc pyrithione in rabbits, rats, monkeys, and dogs after oral dosing. Toxicol. Appl. Pharmacol., 62, 237-250.

11. Mochida, K., Ito, K., Harino, H., Onduka, T., Kakuno, A. and Fujii, K. (2006) Acute toxicity of pyrithione antifouling biocides and joint toxicity with copper to red sea bream (Pagrus major) and toy shrimp (Heptacarpus futilirostris). Environ. Toxicol. Chem., 25, 3058-3064.

12. Mochida, K., Ito, K., Harino, H., Onduka, T., Kakuno, A. and Fujii, K. (2008) Early life-stage toxicity test for copper pyrithione and induction of skeletal anomaly in a teleost, the mummichog (Fundulus heteroclitus). Environ. Toxicol. Chem., 27, 367-374.

13. Mochida, K., Amano, H., Onduka, T., Kakuno, A. and Fujii, K. (2011) Toxicity and metabolism of copper pyrithione and its degradation product, 2,2’-dipyridyldisulfide in a marine polychaete. Chemosphere, 82, 390-397.

14. Mochida, K., Ito, K., Harino, H., Tanaka, H., Onduka, T., Kakuno, A. and Fujii, K. (2009) Inhibition of acetylcholinesterase by metabolites of copper pyrithione (CuPT) and its possible involvement in vertebral deformity of a CuPT-exposed marine teleostean fish. Comp. Biochem. Physiol. C Toxicol. Pharmacol., 149, 624-630.

15. Okamura, H., Watanabe, T., Aoyama, I. and Hasobe, M. (2002) Toxicity evaluation of new antifouling compounds using suspension-cultured fish cells. Chemosphere, 46, 945-951.

16. Howes, D. and Black, J.G. (1975) Comparative percutaneous absorption of pyrithiones. Toxicology, 5, 209-220.

17. Gibson, W.B., Jeffcoat, A.R., Turan, T.S., Wendt, R.H., Hughes, P.F. and Twine, M.E. (1980) Zinc pyrithione: Urinary metabolites of zinc pyrithione in rabbits, rats, monkeys, and dogs after oral dosing. Toxicol. Appl. Pharmacol., 62, 237-250.

18. Jeffcoat, A.R., Gibson, W.B., Rodriguez, P.A., Turan, T.S., Hughes, P.F. and Twine, M.E. (1980) Zinc pyrithione: urinary metabolites of zinc pyrithione in rabbits, rats, monkeys, and dogs after oral dosing. Toxicol. Appl. Pharmacol., 56, 141-154.
**Supplementary Fig. 1.** Representative chromatograms for selected extracted samples (left for PT; right for IS) at double blank (A), blank sample (B), LLOQ (50 ng/mL, C), HLOQ (2,000 ng/mL, D), and real sample (E) in rat plasma.

**Supplementary Table 1.** Intra- and Inter-day accuracy and precision for the analysis of ZnPT and PT in rat plasma

| Plasma | Concentration (ng/mL) | Intra-day (n = 3) | Inter-day (n = 3) |
|--------|------------------------|-------------------|------------------|
|        |                        | Precision (CV, %) | Accuracy (%)     | Precision (CV, %) | Accuracy (%)     |
| ZnPT   | 50                     | 7.4               | 105.7            | 6.7               | 102.1            |
|        | 100                    | 3.5               | 102.0            | 4.1               | 98.6             |
|        | 500                    | 4.5               | 104.8            | 3.1               | 99.5             |
|        | 1600                   | 1.9               | 102.3            | 6.5               | 100.4            |
| PT     | 50                     | 11.8              | 108.9            | 11.5              | 112.7            |
|        | 100                    | 1.0               | 97.8             | 2.0               | 103.3            |
|        | 500                    | 2.5               | 97.1             | 3.3               | 102.1            |
|        | 1600                   | 2.0               | 111.5            | 6.0               | 105.1            |
Supplementary Fig. 2. Representative chromatograms for selected extracted samples (left for ZnPT; right for IS) at double blank (A), blank sample (B), LLOQ (50 ng/mL, C), HLOQ (2,000 ng/mL, D), and real sample (E) in rat urine.

Supplementary Table 2. Intra- and Inter-day accuracy and precision for the analysis of ZnPT and PT in rat urine

| Urine | Concentration (ng/mL) | Intra-day (n = 3) | Inter-day (n = 3) |
|-------|-----------------------|-------------------|-------------------|
|       |                       | Precision (CV, %) | Accuracy (%)      | Precision (CV, %) | Accuracy (%) |
| ZnPT  | 50                    | 4.9               | 110.5             | 6.0               | 107.7        |
|       | 100                   | 3.0               | 98.3              | 4.7               | 99.6         |
|       | 500                   | 3.1               | 108.6             | 5.1               | 105.8        |
|       | 1600                  | 6.7               | 102.1             | 4.7               | 103.7        |
| PT    | 100                   | 2.4               | 109.0             | 2.5               | 106.0        |
|       | 200                   | 6.5               | 96.8              | 2.9               | 95.5         |
|       | 500                   | 3.5               | 106.9             | 6.7               | 105.3        |
|       | 1600                  | 5.6               | 106.9             | 6.3               | 112.9        |
Supplementary Fig. 3. Representative chromatograms for selected extracted samples (left for MSP; right for IS) at double blank (A), blank sample (B), LLOQ (5 ng/mL, C), and HLOQ (1,000 ng/mL, D) in rat plasma.

Supplementary Table 3. Intra- and inter-day accuracy and precision for the analysis of MSP in rat plasma

| Plasma | Concentration (ng/mL) | Intra-day (n = 5) | Inter-day (n = 3) |
|--------|------------------------|-------------------|-------------------|
|        |                        | Precision (CV, %) | Accuracy (%)      | Precision (CV, %) | Accuracy (%)      |
| MSP    | 5                      | 8.8               | 99.6              | 9.5               | 98.7              |
|        | 15                     | 1.7               | 98.4              | 3.0               | 98.7              |
|        | 100                    | 2.0               | 99.7              | 2.3               | 99.9              |
|        | 800                    | 2.4               | 97.9              | 2.2               | 97.2              |
**Supplementary Fig. 4.** Representative chromatograms for selected extracted samples (left for ZnPT; right for IS) at double blank (A), blank sample (B), LLOQ (50 ng/mL, C), and HLOQ (2,000 ng/mL, D) in shampoo solution.

**Supplementary Table 4.** Intra- and Inter-day accuracy and precision for the analysis of ZnPT and PT in shampoo solutions (n = 3)

| Shampoo | Concentration (ng/mL) | Intra-day (n = 3) | Inter-day (n = 3) |
|---------|-----------------------|-------------------|-------------------|
|         |                       | Precision (CV, %) | Accuracy (%)      | Precision (CV, %) | Accuracy (%)      |
| ZnPT    | 50                    | 10.5              | 96.4              | 10.1              | 101.2             |
|         | 100                   | 3.7               | 101.9             | 2.4               | 108.2             |
|         | 500                   | 2.5               | 104.7             | 2.0               | 95.9              |
|         | 1600                  | 2.3               | 103.4             | 1.8               | 106.3             |
| PT      | 100                   | 13.9              | 107.3             | 12.5              | 110.4             |
|         | 200                   | 4.6               | 94.9              | 6.7               | 103.1             |
|         | 500                   | 0.8               | 102.4             | 2.3               | 97.3              |
|         | 1600                  | 3.4               | 107.6             | 1.4               | 105.5             |