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Determination of zinc pyrithione in shampoos by HPLC and HPLC-MS/MS

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Synopsis

Methods have been developed for the determination of zinc pyrithione (ZPT) in shampoos using high-performance liquid chromatography (HPLC) and high-performance liquid chromatography-mass spectrometry/mass spectrometry (HPLC-MS/MS). Samples were washed by water first to remove surfactant and water-soluble impurities, then ultrasonic-extracted by acetonitrile–methanol for 30 min, and finally analyzed by MG C_{18} column (250 mm × 4.6 mm, 5 μm) or RP-18e (100 mm × 3 mm, 2 μm) plus APCI-MS/MS. Limits of detection were determined as 0.015% (HPLC) and 0.003% (HPLC-MS/MS), with a limit of quantization of 0.05% and 0.01%, respectively. The recoveries were 85.8–104% (HPLC) and 87.6–107% (HPLC-MS/MS). A good linear relationship was obtained from 3.20 $\mu g \cdot ml^{-1}$ to 200 $\mu g \cdot ml^{-1}$ (HPLC) and 1.00 $\mu g \cdot ml^{-1}$ to 200 $\mu g \cdot ml^{-1}$ (HPLC-MS/MS). The proposed methods have been successfully applied to the analysis of ZPT in many shampoos. The established two methods were rapid and reproducible with low interference.

INTRODUCTION

Zinc pyrithione (ZPT) was the zinc chelate of 2-pyridinethiol 1-oxide, and it was used as a cosmetic preservatives in the late 1960s. Procter and Gamble Co. discovered the antidandruff properties of ZPT first, and it was known as an effective bactericide, fungicide, and algicide through various clinical studies (1,2). Such properties have led to ZPT being extensively used in cosmetics especially as an antidandruff agent in hair care products. Now, it is the most common active ingredient in antidandruff shampoo products.

The toxicity of pyrithione salts by various routes of exposure has been studied in several species of animals and has been described previously (3–5). On the basis of research data, the Economic Community (76/768/EEC, now Regulation [EC] No 1223/2009) Council Directive allowed ZPT to be used in cosmetics as a preservative in hair products at 1.0% and other rinse-off products at 0.5%. The ZPT was not allowed in oral hygiene products. For leave-on hair products, it is allowed at 0.1% in cosmetics. In China, Hygienic Standard for Cosmetics allowed ZPT to be used in cosmetics as a preservative in rinse-off

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products at 0.5% and as an antidandruff in rinse-off products at 1.5%. Also, ZPT was not allowed in oral hygiene products.

The use of ZPT in antidandruff preparations required analytical techniques for quality control during manufacture and market supervision. The direct HPLC analysis of ZPT was difficult in the past owing to the problematic interaction with reversed-phase packing materials (6). In recent years, attempts to avoid such unwanted interactions have focused upon the improvement of silica stationary phase. Several methods have been developed for detecting ZPT in many types of samples including cosmetics, water, and so on (7–14). For example, Bones *et al.* improved a method for the determination of ZPT in environmental water samples incorporating online extraction and preconcentration coupled with liquid chromatography atmospheric pressure chemical ionization mass spectrometry. Limits of detection was 18 $ng \cdot l^{-1}$, with a limit of quantitation of 62 $ng \cdot l^{-1}$. The percentage recoveries were $72 \pm 9\%$ (7). Thomas established a method to determine ZPT in water samples by copper chelate formation and high-performance liquid chromatography (HPLC)–atmospheric pressure chemical ionization mass spectrometry. The recoveries of the method were $77 \pm 17\%$, the limit of detection was 20 $ng \cdot l^{-1}$ (8). Nakajima reported a high-performance liquid chromatographic determination of ZPT in antidandruff preparations based on copper chelate formation. The calibration graph was linear from 0.1 to 0.5 μg for ZPT. The recoveries from four shampoos were 98.0–100.6% (10). However, the procedure of these methods was complicated and was not suitable for routine tests. Moreover, no high-performance liquid chromatography-mass spectrometry/mass spectrometry (HPLC-MS/MS) method for ZPT in shampoos has been reported yet. Since the formulations of shampoo were complex, HPLC method might lead to inaccurate results because of matrix interferences. In this article, both HPLC and HPLC-MS/MS methods for ZPT in shampoos were described, the HPLC was used for routine tests, and the HPLC-MS/MS was used for complex matrix samples. The sensitivity and selectivity of the developed methods were appropriate for the desired application, and the methods have been used for evaluating lots of different sources of shampoos.

EXPERIMENTAL

INSTRUMENTATION AND REAGENTS

HPLC method was carried out using a Waters 2695 system with DAD detector (Waters, Milford, MA). HPLC-APCI-MS/MS method was carried out using a Thermo TSQ Quantum Access system with an atmospheric pressure chemical ionization source (Thermo Scientific, West Palm Beach, FL).

Reagent water used throughout this study was obtained from a Millipore Milli-Q water purification system (Millipore, Bedford, MA). ZPT (95%) was received from Sigma-Aldrich (Steinheim, Germany). HPLC grade solvents (methanol and acetonitrile) were purchased from Merck (Darmstadt, Germany). Other reagents (ammonium acetate, potassium dihydrogen phosphate, EDTA-Na_2, and phosphoric acid) were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Samples were obtained from a local market.

PREPARATION OF STANDARDS

ZPT calibration standards were freshly made up at the start of each batch of analysis. Typically, the standard (20.0 mg) was suspended in 90 ml methanol–acetonitrile (1:9, v/v) and subjected to ultrasonic treatment in the dark. Then the solution was cooled to the room temperature, made up to 100 ml and passed through a 0.22 μm filter. A set of standard solutions were produced by diluting the mixture to 1.00(for HPLC-MS/MS method only), 3.20(for HPLC method only), 10, 50, and 100 $\mu g \cdot ml^{-1}$. ZPT standards were stored in the refrigerator in darkness when not in use.

HPLC ANALYSIS

HPLC method was carried out using a Waters 2695 system and Shiseido MG C_{18} column (250 mm × 4.6 mm, 5 μm). The mobile phase was 0.01 $mol \cdot l^{-1}$ potassium dihydrogen phosphate, adjusted 0.5 $mmol \cdot l^{-1}$ EDTA-Na_2 at pH 4.0 with phosphoric acid, methanol–acetonitrile gradient of run time of 25 min (Table I). Column temperature was maintained at 25°C, wavelength was 272 nm, flow rate was 1 $ml \cdot min^{-1}$, injection volume was 10 μl. The retention time of ZPT was 10.5 min. Figure 1 showed the ultraviolet spectrum of ZPT standard (210–400 nm).

HPLC-MS/MS ANALYSIS

HPLC-MS/MS method was carried out using a Thermo TSQ Quantum Access system operated under positive polarity. Tandem mass spectrometry was performed under the following conditions: discharge current: 4 μA, vaporizer temperature: 400°C, sheath gas pressure: 40 psi, aux gas pressure: 5 psi, capillary temperature 300°C. Multiple reaction monitoring mode (MRM) was performed using the fragmentation transitions of m/z 317→m/z 173.8, m/z 317→m/z 189.8 (Table II).

Gradient separations were performed on a Merck Chromolith Performance RP-18e column (100 mm × 3 mm, 2 μm). The mobile phase was 1$mmol \cdot l^{-1}$ ammonium acetate–methanol–acetonitrile run over a gradient (Table III). Column temperature was maintained at 30°C, flow rate was 0.25 $ml \cdot min^{-1}$, injection volume was 20 μl. The retention time of ZPT was about 5.0 min.

Table I
Gradient Elution Program (HPLC)

Time (min)	Potassium dihydrogen phosphate solution (%)	Acetonitrile (%)	Methanol (%)
0.0	85	5	10
5.0	85	5	10
8.0	40	50	10
18.0	10	80	10
19.0	10	80	10
20.0	85	5	10
25.0	85	5	10

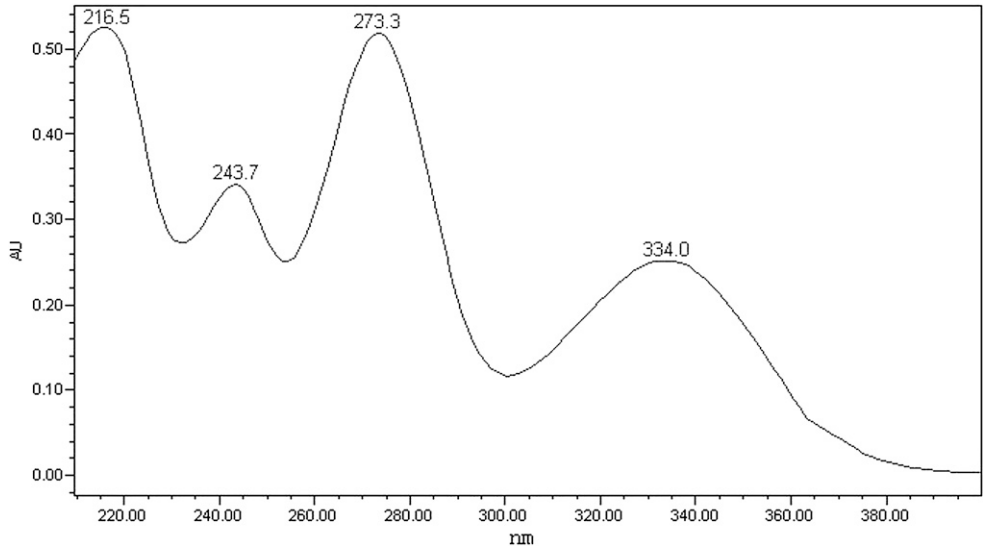

Figure 1. UV spectrum of ZPT standard.

SAMPLE EXTRACTION OF ZPT

A 0.500 g amount of sample was accurately weighed into a 50ml-stoppered centrifuge tube, 5–10 ml of water was added. The tube was shaken vigorously, and then centrifuged at 8000 rpm for 5 min. The supernatant was discarded, and the residue was mixed with 40 ml methanol–acetonitrile (1:9, v/v) by a vortex mixer. After ultrasonic extraction for 30 min, the solution was cooled to the room temperature, and diluted to 50 ml with methanol–acetonitrile and centrifuged at 8000 rpm for 5 min. An aliquot of the solution was filtered through a 0.22-μm membrane filter prior to analysis. The residue was extracted the second time by methanol–acetonitrile in the same way.

RESULTS AND DISCUSSION

SAMPLE EXTRACTION

Although the sample extracted by methanol–acetonitrile could analyze directly, it had much surfactants or impurities that might pollute the chromatography and mass spectrometry system. Since ZPT does not dissolve in water, the research used water to remove surfactant and water-soluble impurities in the sample. After washing the samples with water in the first step, the chromatography of the sample was much cleaner (Figure 2).

Table II
Mass Parameters of MRM Mode for ZPT Determination

Qualitative ion	Quantitative ion	Collision energy (V)
317 > 173.8	317 > 173.8	18
317 > 189.8		24

Table III
Gradient Elution Program (HPLC-APCI-MS/MS)

Time (min)	Ammonium acetate solution (%)	Acetonitrile (%)	Methanol (%)
0.00	70	15	15
5.00	5	45	50
6.00	5	45	50
7.10	70	15	15
10.0	70	15	15

Then, the content of ZPT in water, twice methanol–acetonitrile extract were analyzed and compared. Table IV showed that very little of ZPT was dissolved in the water rinse step and most ZPT was extracted in the first methanol–acetonitrile extraction step. Tiny ZPT was dissolved in the second extraction. Thus, in the routine tests, the samples only need to be extracted by methanol–acetonitrile once.

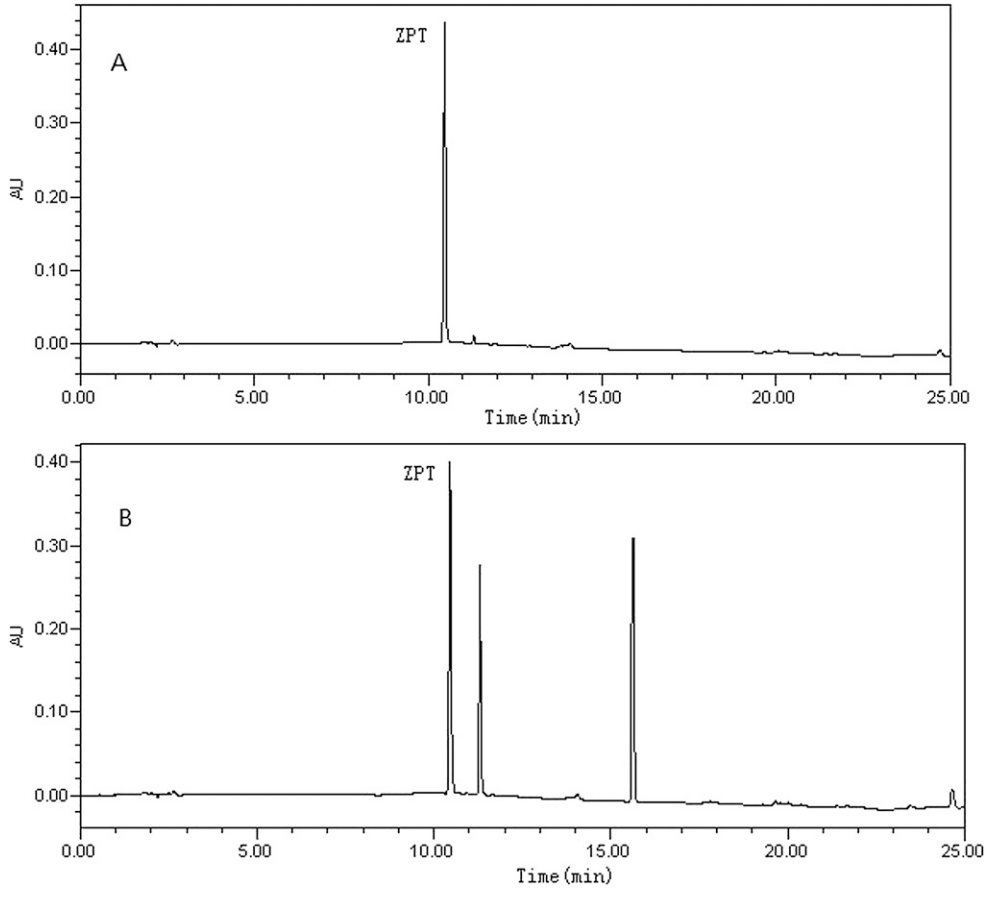

Figure 2. Chromatography of sample with (A) and without (B) water washing.

Table IV
Content of ZPT (%) in Water, Twice Methanol–Acetonitrile Extract

Extract	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Water	0.014	0.010	0.019	0.010	0.012	0.013	Not detected
First methanol–acetonitrile extraction	1.21	0.47	1.33	0.99	0.85	0.66	0.29
Second methanol–acetonitrile extraction	0.016	0.009	0.019	0.015	0.016	0.010	Not detected

Shampoo suppliers said that these samples contained about 1.3%, 0.5%, 1.4%, 1.0%, 0.9%, 0.7%, and 0.3% ZPT, respectively.

HPLC ANALYSIS

Initial work investigated a range of reversed-phase columns for the determination of ZPT in an attempt to identify a stationary phase that was essentially free from metallic impurities and also exhibited very low silanol activity. Most C_{18} column resulted in very short retention time, low column efficiency, or tailing peak. Fortunately, using a simple gradient elution as depicted in Table I, the peak for ZPT on Shiseido MG C_{18} column eluted as a small fronted peak because of the high purity monolithic silica substrate and also the superior level of endcapping. Ingredients in the shampoos did not show any interference in the determination of ZPT. Moreover, other common antidandruff agents (salicylic acid, ketoconazole, climbazole, and piroctone olamine) could be analyzed in the same chromatography condition.

HPLC-MS/MS ANALYSIS

Through several tests, the Chromolith Performance RP-18e monolithic column outperformed all HPLC-MS/MS particulate columns with a large sharp symmetrical peak, whereas many normal column leading to serious leading peak (Figure 3). For this reason, the Chromolith column was used for all further HPLC-MS/MS studies.

According to the former reports, the ionization and MS response of ZPT was low; thus, the research evaluates both positive and negative ionization modes of APCI. Poor ionization of ZPT was observed in the negative ion mode, whereas positive APCI produced both a quasi-molecular ion (m/z 317.0) and two characteristic fragments (m/z 173.8 and 189.8).

These two product ions were chosen to meet the requirement of Identification Points ≥ 4.0 by the Decision 2002/657/EC. The m/z 173.8 was chosen as quantitation ion and the m/z 189.8 as confirmation ion. The relative ion abundance was measured at various levels spiked in blank shampoo samples. Both relative ion abundance ratios of 317.0/173.8 and 317.0/189.8 met the requirements set by Decision 2002/657/EC.

To determine the optimum ionization, a standard ZPT solution was infused into the mass spectrometer to minimize fragmentation and maximize sensitivity. These parameters were automatically fine-tuned using the software and the collision energy for each daughter ion

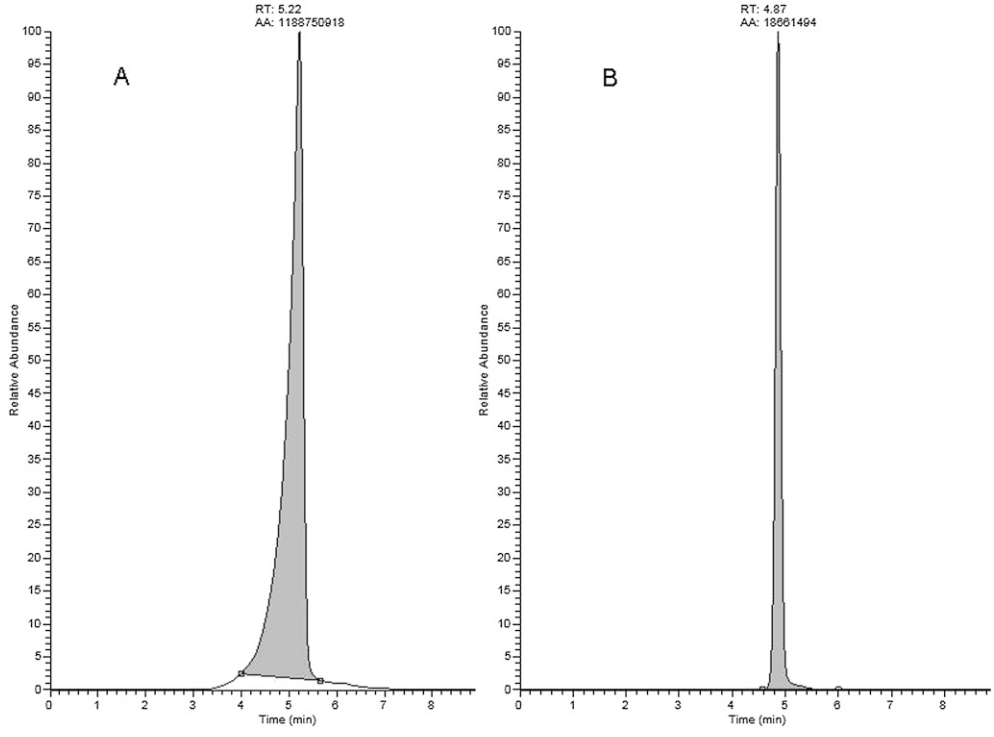

Figure 3. TIC (total ion chromatography) of ZPT in normal C18 (A) and Chromolith Performance RP-18e column (B).

was optimized in MRM mode to get the best sensitivity. The optimum settings were found to be: discharge current: 4 μA, vaporizer temperature: 400°C, sheath gas pressure: 40 psi, aux gas pressure: 5 psi, capillary temperature: 300°C. Under optimized conditions, the resultant APCI-MS-MS spectrum for ZPT depicted three significant ions, the quasi-molecular $(M + H)^+$ ion at m/z 317.0 and other two ions at m/z 173.8 and 189.8.

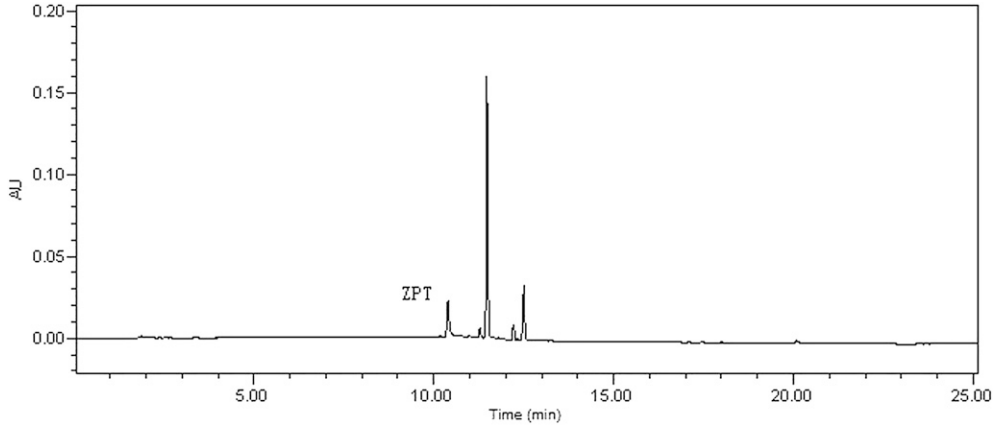

Figure 4. Chromatography of blank shampoo sample with 0.05% ZPT.

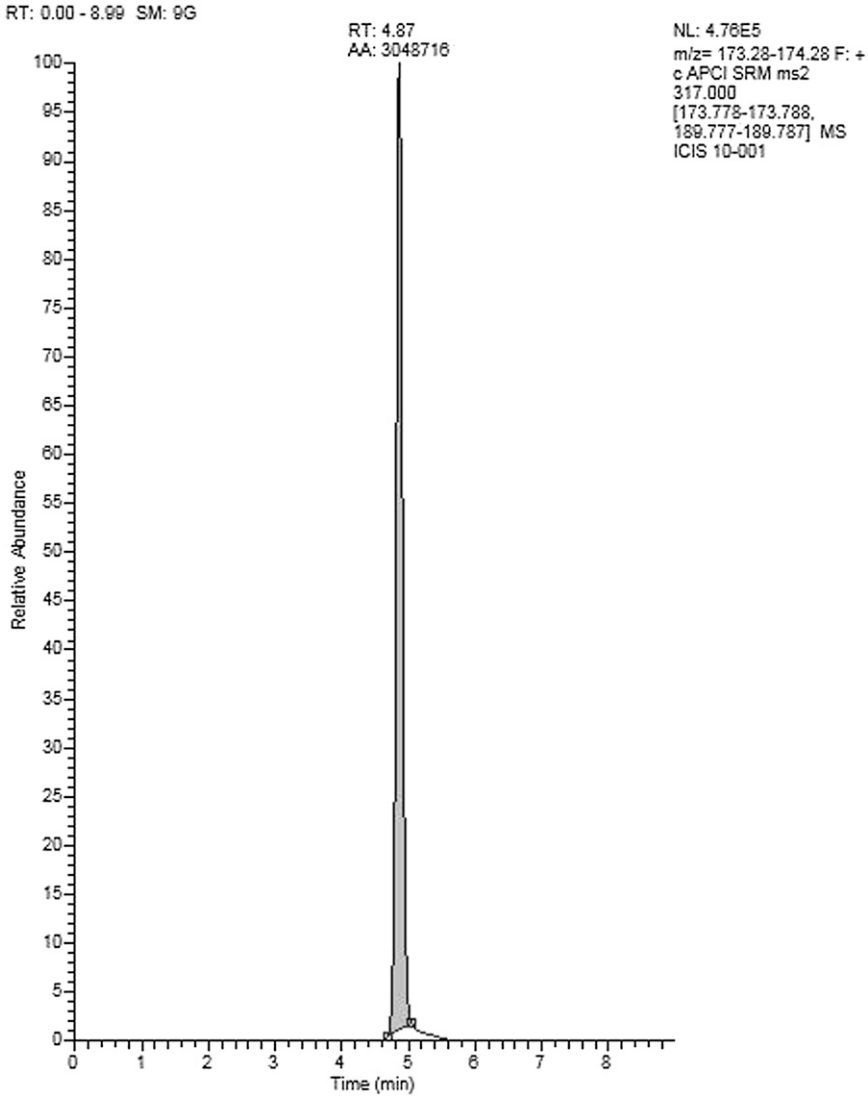

Figure 5. Chromatography of blank shampoo sample with 0.01% ZPT. XIC (extracted ion chromatography at m/z 173.8).

METHOD PERFORMANCE OF HPLC

Linearity was determined in the region of 3.2–200.0 $\mu g \cdot ml^{-1}$, and the calculated regression coefficient was R^2 = 0.9989. The limits of detection and quantization were calculated as three and ten times the standard deviation of the baseline noise for blank extractions of shampoo, respectively. The limits of detection and quantization were 0.015% and 0.05%, respectively. Although the detection limit of HPLC method was not low, it was enough for routine analysis of cosmetics, which was above 0.1% in most cases.

Precision and repeatability was evaluated at three levels (blank shampoo sample with 0.1%, 0.5%, and 2% ZPT). The relative standard deviation (RSD) of six injections was

evaluated and analyzed with different analysts and different apparatus. Precision tests demonstrated that the method yielded good precision (RSD < 4.53%). Reproducible injections were also satisfactory with RSD values ranging from 0.33% to 5.12%. (Figure 4).

The recovery experiments were performed in triplicate. The recovery data were determined by dividing the value obtained for the sample prepared with the added standard, by the amount added, and then multiplying by 100%. The percentage recoveries were found to be 85.8–104% (Table V), which were satisfactory in all cases. Therefore, quantification by external calibration can be effectively employed.

METHOD PERFORMANCE OF HPLC-MS/MS

Linearity was determined in the region of 1.0–200.0 $\mu g \cdot ml^{-1}$, and the calculated regression coefficient was $R^2 = 0.9991$. The limits of detection and quantization were 0.003% and 0.01%, respectively.

Table V
Recovery Tests

Sample	Method	ZPT added in the sample (%)	Analysis results (%)	Recovery (%)	CV (%)
1	HPLC	0.05	0.0436	87.2	2.36
		0.35	0.312	89.1	3.56
		1.06	0.909	85.8	2.13
		1.76	1.65	93.7	1.99
	HPLC-MS/MS	0.05	0.0438	87.6	4.21
		0.35	0.325	92.9	2.96
		1.06	0.996	94.0	3.12
		1.76	1.72	97.7	2.01
2	HPLC	0.010	—	—	—
		0.064	0.0589	92.0	3.68
		0.16	0.155	96.9	4.21
		1.60	1.67	104	2.89
	HPLC-MS/MS	0.010	0.00926	92.6	4.57
		0.064	0.0605	94.3	2.16
		0.16	0.171	107	1.57
		1.60	1.65	103	3.00
3	HPLC	0.05	0.0463	92.6%	2.68
		0.35	0.321	91.7%	2.01
		1.06	0.909	85.8%	3.66
		1.76	1.56	88.6%	1.95
	HPLC-MS/MS	0.05	0.0483	96.6%	3.21
		0.35	0.352	101%	1.96
		1.06	0.969	91.4%	2.13
		1.76	1.78	101%	3.26

Table V
Continued

Sample	Method	ZPT added in the sample (%)	Analysis results (%)	Recovery (%)	CV (%)
4	HPLC	0.010	—	—	—
		0.064	0.0590	92.2%	2.63
		0.16	0.165	103%	3.21
		1.60	1.60	100%	3.01
	HPLC-MS/MS	0.010	0.00946	94.6%	4.26
		0.064	0.0600	93.8%	1.29
		0.16	0.161	101%	1.86
		1.60	1.68	105%	2.67
5	HPLC	0.05	0.0446	89.2%	3.25
		0.35	0.322	92.0%	1.98
		1.06	0.999	94.2%	2.56
		1.76	1.67	94.9%	2.74
	HPLC-MS/MS	0.05	0.0478	95.6%	3.65
		0.35	0.315	90.0%	2.96
		1.06	0.986	93.0%	2.68
		1.76	1.75	99.4%	1.98
6	HPLC	0.010	—	—	—
		0.064	0.0598	93.4%	4.26
		0.16	0.159	99.4%	2.36
		1.60	1.60	100 %	1.98
	HPLC-MS/MS	0.010	0.00962	96.2%	3.58
		0.064	0.0650	102%	2.96
		0.16	0.170	106%	1.65
		1.60	1.56	97.5%	1.33
7	HPLC	0.05	0.0430	86.0%	4.36
		0.35	0.310	88.6%	2.86
		1.06	0.990	93.4%	2.98
		1.76	1.69	96.0%	2.01
	HPLC-MS/MS	0.05	0.0448	89.6%	4.99
		0.35	0.345	98.6%	1.58
		1.06	1.09	103%	2.38
		1.76	1.70	96.6%	3.00

Precision and repeatability was evaluated at three levels (blank shampoo sample with 0.02%, 0.1%, and 1% ZPT). The RSD of six injections was evaluated and analyzed with different analysts and different apparatus. Precision tests demonstrated that the method yielded good precision (RSD < 4.9%). Reproducible injections were also satisfactory with RSD values ranging from 4.2% to 6.8%.

The recovery experiments were performed in triplicate. The percentage recoveries were found to be 87.6–107% (Table V), which were satisfactory in all cases. Therefore, quantification by external calibration can be effectively employed. (Figure 5).

SAMPLE TESTS

The established HPLC and HPLC-MS/MS methods were used to test several same shampoos to investigate the consistency. Through *t*-test, these two methods showed no statistical difference ($p > 0.05$).

Research analyzed 102 batches of shampoos in China, especially antidandruff shampoo. Among all, 44 antidandruff samples contained ZPT ranged from 0.11% to 1.46% (all of them below the Chinese regulation). Most brands of antidandruff shampoo (52.6%) contained ZPT as the active ingredient.

CONCLUSION

The simple sample preparation procedure coupled with HPLC and HPLC-MS/MS detection was developed for the analysis of ZPT in shampoos. The pretreatment step (used water to wash the sample) could effectively remove water-soluble surfactants and impurities that might pollute the chromatography and mass spectrometry system. To our knowledge, no similar pretreatment step has been reported in other published methods. And other common antidandruff agents (salicylic acid, ketoconazole, climbazole, and piroctone olamine) also could be analyzed in the same chromatography condition.

These methods were validated in a real sample matrix and showed high sensitivity with acceptable recovery and reproducibility, while also yielding a limit of detection that was adequate for the detection of real samples. Limits of detection were determined as 0.015% (HPLC) and 0.003% (HPLC-MS/MS), with a limit of quantization of 0.05% and 0.01%, respectively. The recoveries were 85.8–104% (HPLC) and 87.6–107% (HPLC-MS/MS). The reproducibilities were 0.33–5.12% (HPLC) and 4.2–6.8% (HPLC-MS/MS). The HPLC method was suitable for high-throughput routine analysis, and the HPLC-MS/MS was suitable for complex matrix samples.

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