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DETERMINATION OF ZINC PYRITHIONE IN COSMETIC PRODUCTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH PRE-LABELLING

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SUMMARY

A high-performance liquid chromatographic (HPLC) method has been developed for the determination of zinc pyrithione (Zpt) in commercial cosmetic products. Zpt was pre-labelled with N-dansylaziridine and determined by reversed-phase HPLC. Ingredients in cosmetic products did not interfere in the pre-labelling reaction. The calibration graph for Zpt was linear in the range 1.4–66 ng. The coefficient of variation was 0.9% for 17-ng injections. Using the proposed method, Zpt in commercial cosmetic products was determined with high sensitivity and high selectivity. The proposed method is also applicable to the determination of trace amounts of Zpt adsorbed on human hair.

INTRODUCTION

Many kinds of anti-dandruff agents are now commercially available, and zinc pyrithione (Zpt) is one of the most widely used. Zpt has been determined by polarography[1], iodometry[2], chelate exchange[3], thin-layer chromatography[4] and high-performance liquid chromatography (HPLC)[5]. Iodometry is the most frequently used method, but it lacks selectivity and sensitivity. Cheng and Gradde[5] determined Zpt in commercial shampoos by a reversed-phase HPLC method, which also lacks sufficient sensitivity to determine trace amounts of Zpt.

To determine Zpt selectively and sensitively, HPLC analysis after pre-labelling of the thiol groups with a fluorescent pre-labelling agent is considered to be most suitable. However, only a few reagents are available for the pre-labelling of a thiol group. Of these, N-dansylaziridine[6,7] was used in this study as it was superior to others in terms of selectivity, sensitivity and simplicity of the pre-labelling procedure.

This paper describes the optimization of the pre-labelling reaction and the chromatographic separation, and also describes the determination of Zpt in commercial cosmetic products with high sensitivity and high selectivity.

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EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of two LC-6A reciprocating piston pumps, an SCL-6A system controller, a SIL-6A autoinjector, a CTO-6A column oven (Shimadzu, Kyoto, Japan) and a 650-10LC fluorescence detector (Hitachi, Tokyo, Japan). Chromatograms, peak areas and retention times were obtained by using a Chromatopack C-R3A data processor (Shimadzu). ^{1}H NMR spectra were measured with a JEOL (Tokyo, Japan) GX-270 NMR spectrometer.

Reagents

Develosil C8-3 (Nomura Chemical, Seto, Japan), a porous spherical octylsilanized silica gel of average diameter 3 μm, was used as the stationary phase. Zpt was obtained from Tokyo Chemical Industry (Tokyo, Japan), N-Dansylaziridine from Sigma (St. Louis, MO, U.S.A.) and acetonitrile of HPLC grade from Kanto Chemical (Tokyo, Japan). Other reagents were of analytical-reagent grade.

Zpt standard solutions were prepared by dissolving 100 mg of Zpt in 200 ml of 10 m*M* Na_4EDTA solution and diluting with water as required. Sodium tetraborate–hydrochloric acid buffer (pH 8.5) was prepared by mixing 0.05 *M* sodium tetraborate and 0.1 *M* hydrochloric acid.

Calibration procedure

A 2.0-ml portion of Zpt standard solution (0.2–10 mg/l) was transferred into a reaction tube fitted with a screw-cap. To the reaction tube, 2.0 ml of sodium tetraborate–hydrochloric acid buffer and 2 ml of 5 m*M* N-dansylaziridine solution in ethanol were added and the the mixture was heated at 70°C for 90 min. A 20-μl portion of the resulting solution was injected into the HPLC system.

Analytical procedure for cosmetic products

To a sample containing 0.2–10 mg of Zpt, 10 ml of 10 m*M* Na_4EDTA solution were added and the resulting solution was diluted to 100 ml with water. A 2.0-ml portion of the solution was transferred into a reaction tube fitted with a screw-cap. The subsequent procedure was the same as that for the calibration procedure. The concentration of Zpt in the sample was calculated from the calibration graph.

Analytical procedure for human hair

Well chopped human hairs (*ca.* 10 mg) were placed in a reaction tube fitted with a screw-cap, 1.0 ml of 0.05 *M* potassium hydroxide was added and the mixture was heated at 70°C for 20 min. After cooling to room temperature, 1.0 ml of sodium tetraborate–hydrochloric acid buffer and 1.0 ml of 5 m*M* N-dansylaziridine solution in ethanol were added and the mixture was heated at 70°C for 90 min. A 20-μl portion of the resulting solution was injected into the HPLC system.

Chromatographic conditions

A stainless-steel column (150 mm × 4.6 mm I.D.) packed with Develosil C8-3 was used at 40°C. The mobile phases used were 0.1 *M* sodium perchlorate in acetonitrile–water (45:55) (solvent A) and acetonitrile (solvent B) at a flow-rate of

1.0 ml/min. For the determination of Zpt in cosmetic products, solvent A was used as the mobile phase. The determination of Zpt on human hairs was conducted according to the following programme: the starting condition was solvent A, maintained for 7 min, then the mobile phase was changed to solvent B for 10 min and again changed to solvent A, which was maintained for 8 min for reconditioning. The column effluent was monitored with excitation at 333 nm and emission at 540 nm.

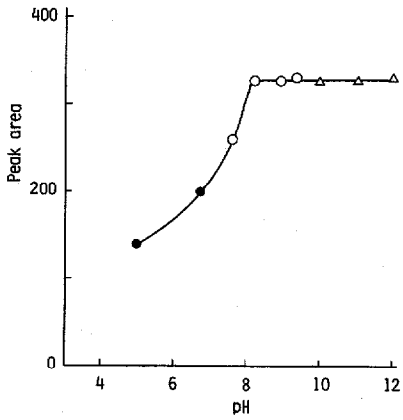
RESULTS AND DISCUSSION

Optimization of pre-labelling reaction

Pre-labelling of a thiol group in proteins with N-dansylaziridine was carried out selectively in a mixture of ethanol and water under neutral or weakly alkaline conditions[6,7]. The reaction of Zpt with N-dansylaziridine was investigated in various solvents. The reaction proceeded most smoothly in water; Zpt was dissolved in water by adding 10 m*M* Na_4EDTA solution, as the solubility of Zpt in water is extremely low. Fig. 1 shows the effect of the pH of the pre-labelling reaction on the peak area of Zpt. The peak area increases with an increase of pH and remains nearly constant above pH 8.2. Therefore, the pH of the pre-labelling reaction was chosen as 8.5. The effect of the concentration of N-dansylaziridine on the peak area of Zpt is shown in Fig. 2. The peak area increases rapidly up to a concentration of 2 m*M* and remains constant above 3 m*M*. The concentration of N-dansylaziridine was therefore fixed at 5 m*M*. The effects of reaction temperature and time on the peak area of Zpt were investigated (Fig. 3). As the peak area remained almost constant above 70°C for 60 min, the reaction temperature and time were fixed at 70°C and 90 min, respectively. On the basis of these results, the pre-labelling procedure was carried out as described under Experimental.

Reaction products of N-dansylaziridine with Zpt

Lankmayr *et al.*[7] suggested that the reaction of N-dansylaziridine with a com-



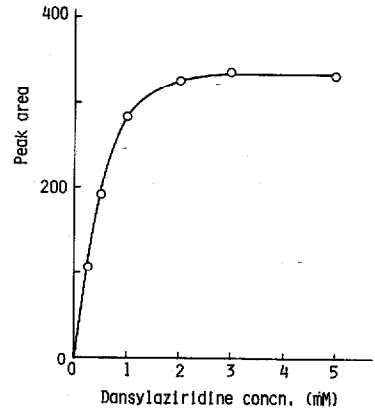


Fig. 1. Efect of pH of the pre-labelling reaction on the peak area of Zpt. ●, acetic acid–sodium acetate buffer; ○, sodium borate–hydrochloric acid buffer; △, sodium borate–sodium hydroxide buffer.

Fig. 2. Effect of concentration of N-dansylaziridine on the peak area of Zpt.

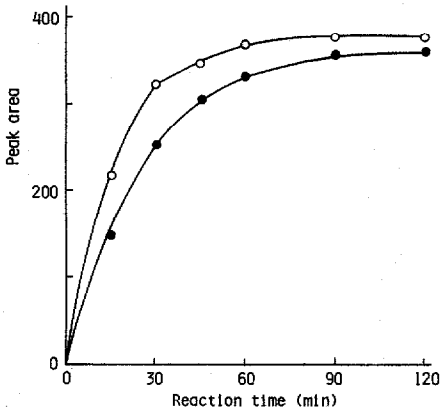
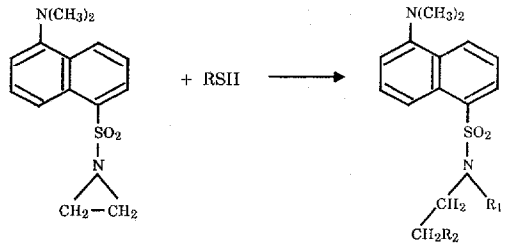


Fig. 3. Effect of reaction temperature and time on the peak area of Zpt. Reaction temperature: ●, 60°C; ○, 70°C.

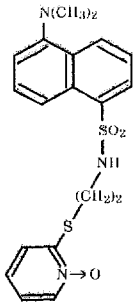
pound having a thiol group proceeds as shown below to afford compound I. However, it seems reasonable to consider that the reaction product is compound II.



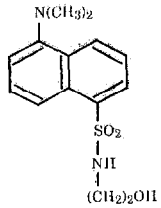
(I) $R_1 = SR, R_2 = H$
(II) $R_1 = H, R_2 = SR$

In order to confirm this reaction, the reaction product of N-dansylaziridine with Zpt was isolated by preparative HPLC. In the ^1H NMR spectrum, two triplets were observed at δ 3.1 (2H) and δ 3.3 (2H) ppm, which were assigned to the methylene groups adjacent to the N and S atoms, respectively, instead of the disappearance of a singlet (δ 2.4 ppm, 4H) assigned to the aziridine ring protons of N-dansylaziridine. Moreover, no triplet assigned to a methyl group appeared. Therefore, it was concluded that the thiol group of Zpt attacked at the methylene carbon of the aziridine ring of N-dansylaziridine to afford compound III.

N-Dansylaziridine also reacts with water, as will be discussed later. The structure of the reaction product was determined in the same manner as that of Zpt. Two triplets appeared at δ 3.1 (2H) and δ 3.6 (2H) ppm, which were assigned to the methylene groups adjacent to N and O atoms, respectively, and the triplet assigned to a methyl group was not observed. These results indicate that the structure of the reaction product is IV. Hence it was demonstrated that the reaction of N-dansylaziridine with a compound having a thiol group proceeded as shown above to afford compound II.



(III)



(IV)

Optimization of separation

Under the optimum reaction conditions, N-dansylaziridine also reacts with water to give compound IV as described above. Compound IV indicated a retention behaviour similar to that of Zpt (compound III) in reversed-phase HPLC. The separation of the two peaks was accomplished with only two HPLC packings, Develosil C8-3 and Develosil ODS-3. Satisfactory separation was obtained using Develosil C8-3 with acetonitrile–water (45:55) containing 0.1 *M* sodium perchlorate as a mobile phase. A typical chromatogram is shown in Fig. 4.

The calibration graph for Zpt was linear in the range 1.6–66 ng. The coefficient of variation for five measurements was 0.9% for 17 ng of Zpt.

Determination of Zpt in cosmetic products

The effect of the ingredients in cosmetic products such as shampoos and hair

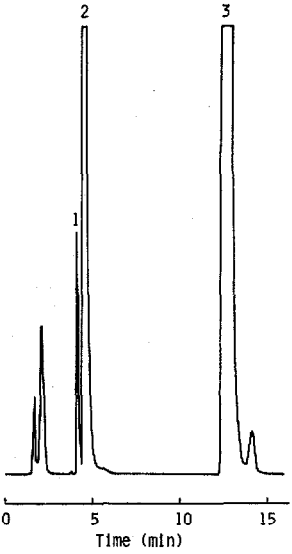


Fig. 4. Typical chromatogram of Zpt. 1, Zpt (compound III); 2, compound IV; 3, N-dansylaziridine.

TABLE I

EFFECT OF INGREDIENTS OF COSMETIC PRODUCTS ON THE PRE-LABELLING REACTION OF Zpt

Ingredient	*Ingredient added (μg)*	*Zpt*		*Recovery (%)*
		Present (μg)	*Found* (μg)*	
Sodium alkylsulphate	1000	10.00	9.87	98.7
Triethanolammonium alkylsulphate	1000	10.00	9.89	98.9
Sodium poly(oxyethylene) alkyl ethers sulphate	1000	10.00	9.86	98.6
Triethanolammonium poly(oxyethylene) alkyl ether sulphate	1000	10.00	9.96	99.6
Sodium α-olefin sulophonate	1000	10.00	10.00	100.0
Soap (Na)	1000	10.00	9.84	98.4
Carboxybetaine-type amphoterics	500	10.00	9.90	99.0
Imidazolinium-type amphoterics	1000	10.00	9.96	99.6
Poly(oxyethylene) alkyl ether	1000	10.00	9.83	98.3
Poly(oxyethylene) glycol	500	10.00	9.96	99.6
Lauroyldiethanolamide	500	10.00	9.93	99.3
Alkyltrimethylammonium chloride	600	10.00	9.85	98.5
Dialkyldimethylammonium chloride	300	10.00	9.95	99.5
Cetyl alcohol	200	10.00	10.00	100.0
Monoethanolamine	500	10.00	9.92	99.2
Diethanolamine	500	10.00	9.90	99.0
Triethanolamine	500	10.00	10.12	101.2
Ammonia	500	10.00	9.95	99.5

* Means of duplicate analyses.

TABLE II

RECOVERY OF Zpt ADDED TO COMMERCIAL SHAMPOOS AND RINSES

Sample	*Added (%)*	*Found* (%)*	*Recovery (%)*
Shampoo A	0.267	0.264	99.1
Shampoo B	0.237	0.237	100.0
Shampoo C	0.227	0.222	97.5
Rinse A	0.235	0.236	100.5
Rinse B	0.231	0.234	101.1

* Means of duplicate analyses.

TABLE III

COMPARISON OF RESULTS OF DETERMINATION OF Zpt IN COMMERCIAL SHAMPOOS AND RINSES BY THE PROPOSED METHOD WITH THOSE BY IODOMETRY[2]

Sample	*Zpt* (%)*	
	Proposed method	*Iodometry*
Shampoo A	0.55	0.54
Shampoo B	0.92	0.90
Shampoo C	1.50	1.44
Shampoo D	1.01	1.01
Rinse A	0.30	0.32

* Means of duplicate analyses.

rinses on the pre-labelling reaction was investigated. As shown in Table I, no interference effects were observed.

The accuracy of the proposed method was tested by adding known amounts of Zpt to commercial shampoos and rinses without Zpt. As shown in Table II, the recoveries were satisfactory. Comparison of the results of the determination of Zpt in commercial shampoos and rinses by the proposed method with those obtained by iodometry[2] showed satisfactory agreement (Table III). No interfering peaks were observed. Therefore, the proposed method should be applicable to the determination of Zpt in cosmetic products.

Fig. 5. Typical chromatogram of Zpt adsorbed on human hairs. 1, Zpt (compound III); 2, compound IV; 3, N-dansylaziridine; 4, unknown.

Determination of Zpt on human hairs

The determination of trace amounts of Zpt adsorbed on human hairs was investigated in order to evaluate the anti-dandruff effect of Zpt, as the sensitivity of the proposed method was high. There are many unknown compounds with thiol groups in human hairs that also react with N-dansylaziridine. These compounds appeared on the HPLC trace after the peak of Zpt and made the analysis time longer under isocratic conditions. Therefore, the eluent was changed to acetonitrile (solvent B) in order to elute these compounds after the elution of Zpt as described under Experimental. Extraction of Zpt from human hairs was carried out by heating at 70°C in 0.05 *M* potassium hydroxide solution for 20 min according to Watanabe[8]. Fig. 5 shows a typical chromatogram of Zpt adsorbed on human hairs; no interfering peak is observed.

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