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High-performance liquid chromatographic determination of zinc pyrithione in antidandruff preparations based on copper chelate formation

KEIKO NAKAJIMA* and TOSHIKO YASUDA
Public Health Laboratory of Chiba Prefecture, 666-2, Nitona-cho, Chiba 280 (Japan)
and
HIROYUKI NAKAZAWA
Department of Pharmaceutical Sciences, National Institute of Public Health, 4-6-1, Shirokane-dai, Minato-ku, Tokyo 108 (Japan)
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SUMMARY

A simple and rapid method for the determination of zinc pyrithione (ZPT) in antidandruff preparations by high-performance liquid chromatography (HPLC) has been developed. ZPT in samples was converted into a stable copper(II) complex by mixing with cupric sulphate solution followed by extraction with chloroform. HPLC was carried out on a Nucleosil 5 C_{18} column (15 cm × 4.6 mm I.D.) using methanol–water (3:2) as the mobile phase with UV detection at 320 nm. The calibration graph was linear from 0.1–0.5 μg for ZPT. The recoveries from four shampoos were 98.0–100.6% with high accuracy.

INTRODUCTION

Zinc pyrithione (ZPT), the zinc chelate of 2-pyridinethiol 1-oxide is used as an antidandruff agent in shampoos, hair rinses and hair conditioners. A specific and rapid determination of ZPT is necessary for the quality control of the commercial antidandruff preparations. Several methods[1–5] have been developed for the determination of ZPT. Kondo and Takano[6] determined ZPT in commercial shampoos and the residues on human hair by high-performance liquid chromatography (HPLC) after pre-labelling with a fluorescent agent. Cheng and Gadde[7] determined ZPT in commercial shampoos directly by reversed-phase HPLC. However, direct analysis of coordination compounds is difficult owing to the interaction with reversed-phase packing materials[8] or stainless-steel components of the liquid chromatograph even if Zn^{2+} is added to the mobile phase. Fenn and Alexander[9] successfully determined ZPT in commercial products by transchelation to the Cu^{II} complex using normal-phase HPLC. The Cu^{II} complex is very stable and is considered also to be suitable for reversed-phase HPLC.

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This paper describes the reversed-phase HPLC determination of ZPT in commercial shampoos, hair rinses and hair conditioners by using the conversion of ZPT into the Cu^{II} complex.

EXPERIMENTAL

Materials

ZPT and 2-pyridinethiol 1-oxide were purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

Analytical-reagent grade copper(II) sulphate pentahydrate, citric acid, disodium hydrogenphosphate dodecahydrate, disodium ethylenediaminetetraacetate (EDTA), chloroform and methanol were obtained from Wako (Osaka, Japan).

Buffer solution (pH 5.0) was prepared by mixing 0.1 *M* citric acid and 0.2 *M* disodium hydrogenphosphate (97:103).

ZPT standard solutions were prepared by dissolving ZPT in chloroform saturated with water (0.02–0.10 mg/ml).

Apparatus and chromatographic conditions

HPLC was carried out using an NSP-800-9 pump (Nihon Seimitsu Kagaku, Tokyo, Japan), a KMT-60A-II autosampler (Kyowa Seimitsu, Tokyo, Japan) and an NS-310II UV detector (Nihon Seimitsu Kagaku, Tokyo, Japan) set at 320 nm. A stainless-steel column (15 cm × 4.6 mm I.D.) packed with Nucleosil $5C_{18}$ (particle size 5 μm) (Macherey, Nagel & Co., Düren, F.R.G.) was used at 25°C.

The mobile phase was methanol–water (3:2, v/v) at a flow-rate of 1.0 ml/min. The metallic portions of the chromatographic system were prewashed with 0.1% EDTA (at 0.5 ml/min) followed by water before the chromatography.

Integration of peak areas was accomplished with a Model 7000B integrator (System Instruments, Tokyo, Japan).

Sample preparation

A sample containing 10 mg of ZPT was weighed accurately into a 100-ml volumetric flask and diluted to volume with buffer solution (pH 5.0) saturated with chloroform. If the dilution resulted in separation into liquid and solid phases, water saturated with chloroform was used instead of buffer solution. After shaking well for a few minutes and sonication in an ultrasonic bath for a few minutes, 10.0 ml of chloroform saturated with water and 2.0 ml of 1 *M* copper(II) sulphate solution were added to 10.0 ml of the above solution. The mixture was shaken vigorously for 5 min and centrifuged for 5 min at 1500 *g*. A 5-μl portion of the lower layer was injected into the chromatograph.

Calibration graph

Portions of 10.0 ml of ZPT standard solution (0.02–0.10 mg/ml) were transferred into centrifuge tubes and to each tube 10.0 ml of buffer solution (pH 5.0) saturated with chloroform and 2.0 ml of 1 *M* copper(II) sulphate solution were added. The mixture was processed as described under *Sample preparation*. A calibration graph for ZPT was constructed using peak areas.

RESULTS AND DISCUSSION

Suitability of metal complexes for chromatographic analysis

The suitability of metal complexes of ZPT was studied using 2-pyridinethiol 1-oxide (PT), which was the ligand of ZPT, and the metal ions Zn^{II}, Fe^{III}, Sn^{II}, Co^{III}, Cu^{II} and Ni^{II}.

A chloroform solution of PT was shaken with aqueous solutions of various metal ions. Fig. 1 shows the UV absorption spectra of the various metal complexes of PT. The Cu^{II} complex had the greatest absorbance at 320 nm.

The chelate formation constant of the 1:1 Cu^{II} complex of PT is much greater than those of the Zn^{II}, Ni^{II}, Co^{II} and Mn^{II} complexes[10]. The Cu^{II} complex is so stable that it is expected not to exchange for other metals during chromatographic processing.

For these reasons the copper chelate formation procedure was applied to the determination of ZPT.

Structure of the Cu^{II} complex

The structure of the Cu^{II} complex was investigated by the continuous variation method[11], as shown in Fig. 2. The structure of the Cu^{II} complex is suggested to be as Fig. 3, in agreement with the results of elemental microanalysis[12]. The conversion of ZPT into the Cu^{II} complex occurred so rapidly that the shaking time required for the formation and extraction of the Cu^{II} complex in the organic phase was less than 5 min.

Chromatography and calibration graph of ZPT

ZPT itself gave a tailing peak when methanol–water was used as the mobile phase. Conversion of ZPT into the Cu^{II} complex eliminated this problem. The Cu^{II}

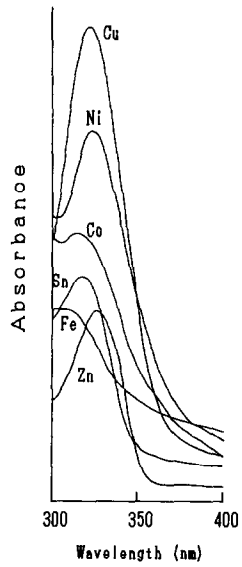
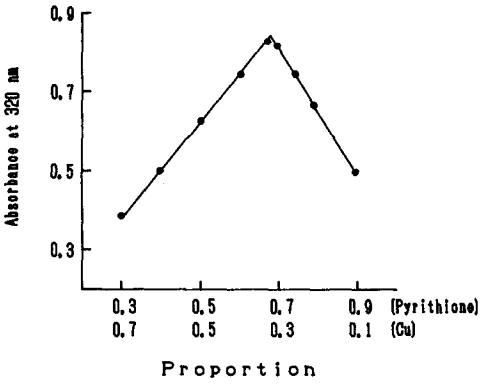


Fig. 1. UV absorption spectra of metal complexes of pyrithione.



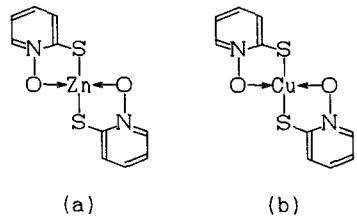


Fig. 2. Composition of Cu^{II} complex of pyrithione by continuous variation method.

Fig. 3. Structures of (a) zinc pyrithione and (b) Cu^{II} complex of pyrithione.

complex gave a good peak shape (as shown in Fig. 4) with a 1.07% relative standard deviation for the peak areas of five injections. The calibration graph was linear in the range 0.1–0.5 μg of ZPT.

Suitability of diluent for sample

ZPT is not dissolved in shampoos or other products but dispersed homogeneously. ZPT is insoluble in water and soluble in 1 *M* hydrochloric acid or

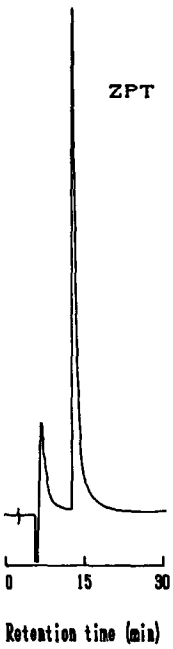


Fig. 4. Typical chromatogram obtained from shampoo. Conditions as given under Experimental.

ammonia–ammonium chloride buffer solution (pH 11.0). In 1 *M* hydrochloric acid ZPT changes to the free form which dimerizes under irradiation with UV light[5]. In our study, ZPT dissolved in 1 *M* hydrochloric acid showed a degradation of 10% immediately, 25% after 24 h and 93% after 4 days of storage at room temperature in a light-resistant container.

ZPT is stable in ammonia–ammonium chloride buffer solution (pH 11.0). However, this buffer solution, on addition of copper(II) sulphate solution, produces a deep-blue complex salt. Owing to the formation of this complex salt, the extractability of the Cu^{II} complex of ZPT in the organic phase decreased.

Based on these results, it was considered to be reasonable to dilute samples with buffer solution (pH 5.0) or water. By sonicating the aqueous sample solution, sufficient uniformity was obtained, with a 1.40% relative standard deviation for five determinations.

Validation of the method

Four commercial shampoos which were not antidandruff preparations and were

TABLE I

RECOVERY OF ZINC PYRITHIONE ADDED TO SHAMPOO

Results were obtained from five replicate analyses of shampoos containing 1.0% of zinc pyrithione.

Sample	*Recovery (%)*	*R.S.D. (%)*
A	98.0	2.0
B	99.5	0.8
C	100.6	1.3
D	98.9	1.5

TABLE II

ANALYSIS OF ZINC PYRITHIONE IN COMMERCIAL COSMETICS

Sample	*ZPT (%)*	
	Iodimetry	*Proposed method*
Shampoo 1	0.75	0.78
2	0.98	0.98
3	0.79	0.81
4	1.45	1.45
5	0.92	0.95
6	0.90	0.90
7	0.92	0.96
8	0.91	0.91
9	0.75	0.75
Hair rinse 1	0.28	0.29
2	0.45	0.46
3	0.44	0.43
Hair conditioner	0.48	0.49

spiked with 1.0% of ZPT were analysed according to the present method. The recoveries of ZPT for five determinations were 98.0–100.6% with relative standard deviations of 0.8–2.0%, as shown in Table I.

Analysis of commercial samples

Table II gives results obtained by the proposed method in comparison with those obtained by iodimetry[3]. The results agreed with each other.

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