Journal of Chromatography, 291 (1984) 434–438
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,581

Note

Analysis of zinc pyrithione in shampoos by reversed-phase high-performance liquid chromatography

HAIYUNG CHENG and R. RAO GADDE*

Bristol-Myers Company, Pharmaceutical Research and Development Division, 100 Forest Avenue, Buffalo, NY 14213 (U.S.A.)

(First received December 13th, 1983; revised manuscript received January 17th, 1984)

Zinc pyrithione (ZPT), the zinc chelate of 2-pyridinethiol-1-oxide, is used as an antidandruff agent in commercial shampoos. It has been assayed by titration with Ti(III) ion[1], polarography[2], chelate-exchange[3] and thin-layer chromatography (TLC)[4]. However, titration analysis suffers from interferences from degradation products of zinc pyrithione. Direct analysis of zinc pyrithione by using polarography is difficult because of poor solubility of zinc pyrithione in aqueous system. Chelate-exchange analysis has a tedious procedure. TLC analysis is not very accurate or precise. This paper describes a reversed-phase liquid chromatographic method which utilizes zinc ions in the eluent to improve the chromatographic peak symmetry.

EXPERIMENTAL

Reagent and solvents

Zinc pyrithione and 2'2'-dithio-bis-pyridine-1-oxide were purchased from Olin Corporation. Thionicotinamide was purchased from Aldrich (Milwaukee, WI, U.S.A.). ACS grade zinc sulfate (heptahydrate) and methanol were purchased from Fisher Scientific (Pittsburg, PA, U.S.A.). HPLC grade chloroform was purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). Sebulon® shampoo (Westwood Pharmaceuticals) and its placebo were used in validating the method.

High-performance liquid chromatography

Reversed-phase chromatography was performed using a Waters Assoc. (Milford, MA, U.S.A.) Model 204 chromatograph with a M6000A pump, a U6K injector and a UV detector (Model 440), set at 313 nm. A 30 cm × 3.9 mm μBondapak C_{18} (Waters Assoc.) column was used for analysis. Output was monitored using an OmniScribe recorder (Houston Instruments) and integrator (Hewlett-Packard Model 3352B). The mobile phase was aqueous zinc sulfate (0.4%, w/v) in methanol–water (10:90), the flow-rate was 2.0 ml/min and the injection volume was 15 μl.

Unless otherwise mentioned, peak areas were used in the calculations.

Procedures

A methanolic solution of thionicotinamide (0.6 mg/ml) was used as internal

standard solution. Chloroform–methanol (95:5, v/v) was used as the diluent in preparing the standard and sample solutions for high-performance liquid chromatographic (HPLC) analysis.

Standard solution. A 25-ml aliquot of zinc pyrithione stock solution (1.90–2.10 mg/ml) in diluent was mixed with 1.6 ml of water and 25 ml of diluent in a 50 ml erlenmeyer flask. The mixture was shaken vigorously for 5 min and let set for 10 min for the two layers to separate. After discarding the upper layer, the lower layer was clarified by centrifuging. A 5-ml aliquot of this clear solution and 5 ml of the internal standard solution were pipetted into a 50-ml volumetric flask and made to volume with methanol.

Sample solution. A known amount of shampoo (*ca.* 2.5 g) was shaken vigorously with a 50-ml aliquot of diluent for 5 min, followed by sonication for 5 min in an ultrasonic bath and let set for 10 min. The lower layer was separated and processed exactly the same way as the lower layer in the standard solution preparation.

RESULTS AND DISCUSSION

Chromatography

Using methanol–water mixture as eluent, ZPT was found to give either a single or double tailing peak. Even with 90% methanol, the peak appeared at the solvent front but with noticeable tailing. Addition of Zn(II) to the eluent was found to eliminate these problems. The best peak shape and reasonable retention time for ZPT was observed using methanol–water (10:90) containing 0.4% (w/v) zinc sulfate as the eluent. At lower zinc-ion concentrations, shorter and broader peak resulted, thus lowering the sensitivity of detection. Thionicotinamide was selected as an internal standard due to its similarity to pyrithione and also the optimum retention time and peak shape. A typical chromatogram of a ZPT–thionicotinamide mixture is shown in Fig. 1.

Sample and standard preparation

Several problems arose in preparing the shampoo samples for HPLC analysis. Extraction with chloroform alone led to too much foaming and did not permit clear separation of the two layers. Further, the cloudy chloroform layer showed slight decrease in volume and was hard to clarify by centrifugation or filtration. Using chloroform–methanol (95:5, v/v) instead of chloroform minimized the foaming and clarification problems. To minimize the phase volume change effects due to redistribution of water and methanol into the two phases, water (equivalent in amount, present in the sample) was added to the standard and processed the same way as the sample.

The internal standard, thionicotinamide, undergoes partial distribution into the aqueous phase during extraction and the extent of the distribution could be affected by shampoo excipients. In order to make the procedure to be applicable to different shampoo products and also eliminate the error due to this distribution, the addition of internal standard was thus incorporated into the method after the extraction step.

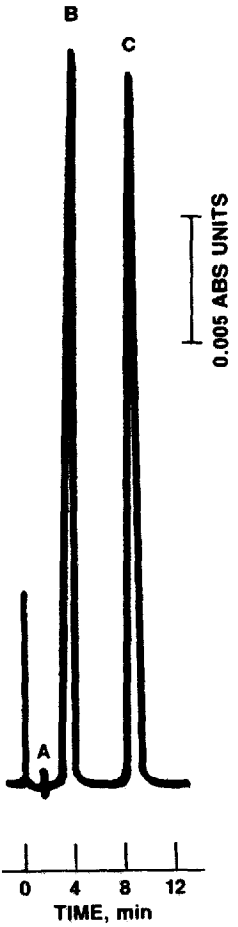

Fig. 1. Typical chromatogram of zinc pyrithione shampoo. Peaks: A = solvent front; B = ZPT; C = thionicotinamide.

Calibration plots

Five ZPT standards in chloroform–methanol diluent, in the concentration range 0.0382–0.153 mg/ml were chromatographed. Linear regression analysis of the peak area data gave a correlation coefficient of 0.9998 and a *y*-intercept equivalent to 3.6% of the normal response (2% ZPT shampoo). Peak height data however gave 0.991 and 23% respectively. Repeating this linearity study we found that the peak areas always give a better linear plot over that of peak heights. However the *y*-intercept is a function of the concentration range used in the study and leads to higher *y*-intercept as the range is shifted to higher concentrations. Apparently the ZPT equilibra and the interaction with the stationary phase is reducing the response at lower concentrations.

The slight non-linearity of the ZPT calibration plots and non-zero *y*-intercept do not lead to any significant errors if bracketed standards are used in the sample analysis (see method validation data below).

TABLE I

ACCURACY DATA

Spiked sample		*Zinc pyrithione found (mg)*	*Recovery (%)*
Placebo (mg)	*Zinc pyrithione* (mg)*		
2080	52.23	51.97	99.5
2130	52.23	52.65	100.8
2175	52.23	52.07	99.7
2515	52.23	51.50	98.6
2530	52.23	52.07	99.7
2630	52.23	52.28	100.1
3500	52.23	52.65	100.8
3490	52.23	53.12	101.7
3575	52.23	52.86	101.2

* Zinc pyrithione added to placebo as its solution (2.089 mg/ml) in chloroform–methanol (95:5, v/v).

Method validation

Chromatograms of shampoo placebo without ZPT showed no peaks corresponding to ZPT or thionicotinamide. Shampoo placebo spiked with known amounts of ZPT, representing shampoo containing 1.5–2.5% ZPT, were prepared and each analyzed in total. The results (Table I) show that the method is accurate with an average recovery of 100.2% (R.S.D. ± 1.0%).

The recovery data in Table I show a slight increasing trend in assay results with increase in placebo. Apparently this is due to the fixed amount of water in standard (1.6 ml) while the water content of sample increased from 1.3 to 2.0 ml with increase in placebo. The effects of differences in ZPT and water level between the samples and standards were further investigated by analyzing the marketed product using standards with different ZPT and water levels.

TABLE II

PRECISION DATA

Shampoo sample (mg)	*ZPT (%)*		
	Single standard	*Three standards (ZPT matched)*	*Three standards (ZPT & water matched)*
2038	2.01	2.04	2.08
2009	1.98	2.02	2.06
2016	2.01	2.05	2.08
2532	2.05	2.04	2.07
2513	2.04	2.04	2.06
2541	2.06	2.05	2.07
3534	2.14	2.08	2.08
3519	2.14	2.07	2.08
3510	2.14	2.07	2.08
Average (% R.S.D.)	2.06 (2.3)	2.05 (0.9)	2.07 (0.5)

The data in column 2 (Table II) were obtained using single standard which has ZPT and water concentrations matching 2.5 g sample. Column 3 data were obtained by using matched ZPT concentration but keeping water in all standards at 1.6 ml. The data in column 4 were obtained by matching ZPT and water concentrations between samples and standards. These data clearly demonstrate that matching the ZPT levels in samples and standards (or the use of bracketed standards) is necessary. Matching the water content between samples and standards is not very critical although desirable for very accurate assays. Addition of a fixed but larger amount of water to both sample and standard to minimize this effect due to water was not feasible since it led to excessive frothing of samples during extraction. Addition of internal standard in the extraction step did not correct this problem (see Sample and standard preparation).

It is known that ZPT is light sensitive but thermally stable[5]. Our studies confirmed this. Both ZPT solid and methanolic solution (0.1 mg/ml) were force degraded in light and by heat and analyzed by the HPLC procedure. In light (*ca.* 1000 footcandles, 33°C) the solution showed a degradation of 10.4% in 6 h; solid ZPT showed 15.2% degradation in 13 days. Both the solid and solution lost about 5% upon storage at 55°C for 45 days. None of these force degraded samples showed any peaks interfering with the ZPT or the internal standard peak. The ZPT peak in the chromatograms of the degraded samples was found to be homogeneous by comparing the absorbance ratio of these peaks with that of an undegraded standard. One of the possible degradation products of ZPT[5], 2,2'-dithio-bis-pyridine-1-oxide, was found not to interfere in this assay as it is retained by the column and does not elute easily.

The HPLC method described above for the ZPT assay is shown to be specific. With good precision and accuracy, this method offers decreased analysis time compared to other methods in literature.

ACKNOWLEDGEMENTS

We are thankful to Dr. Kenneth Tramposch and Mr. Ricky Krazmien for their assistance during this investigation and to Mr. John Daley for providing the shampoo and placebo.

REFERENCES

1 R. T. Brooks and P. D. Stemglanz, *Anal. Chem.*, 31 (1959) 561.
2 A. F. Krivis, E. S. Gazda, T. R. Supp and M. A. Robinson, *Anal. Chem.*, 35 (1963) 966.
3 B. L. Kabacoff and C. M. Fairchild, *J. Soc. Cosmet. Chem.*, 26 (1975) 453.
4 M. D. Seymour and D. L. Bailey, *J. Chromatogr.*, 206 (1981) 301.
5 *Zinc Omadine and Sodium Omadine, Technical Bulletin*, Olin Corporation, Stamford, CT, 1979.