Journal of Chromatography, 291 (1984) 275–282
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,609

ASSAY OF MIXTURES OF PADIMATE-O AND OXYBENZONE IN SUNSCREEN FORMULATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

HENRY S. I. TAN*, RAHMAWATI SIH, SHARON E. MOSELEY and J. LEON LICHTIN
College of Pharmacy, University of Cincinnati Medical Center, Cincinnati, OH 45267 (U.S.A.)
(Received January 27th, 1984)

SUMMARY

A simple assay method for the quality control of some sunscreen products containing padimate-O and oxybenzone has been developed. A methanolic extract of the product containing sulfathiazole internal standard was subjected to reversed-phase high-performance liquid chromatography on a 10-μm Partisil ODS-2 column with methanol–acetonitrile (90:10, v/v) mobile phase. The drug–sulfathiazole peak height ratio was linear between 0.04–2.68 μg of padimate-O (r = 1.0003) and 0.02–1.05 μg of oxybenzone (r = 0.9997) injected. All peaks were well-resolved. Approximate retention times for sulfathiazole, oxybenzone and padimate-O were 3.9, 5.7 and 7.4 min., respectively. The height equivalent to a theoretical plate (± S.D.) were (n = 10) 0.79 ± 0.07, 0.53 ± 0.06 and 0.26 ± 0.04 mm, for sulfathiazole, oxybenzone and padimate-O, respectively. Average percent recoveries (± S.D.) (n = 3) from simulated lotions containing 7% padimate-O and 3% oxybenzone were: padimate-O, 101.4 ± 1.5%; oxybenzone 99.9 ± 1.9%; from simulated lipsticks containing (a) 7% padimate-O and 3% oxybenzone: 103.8 ± 1.2% and 100.1 ± 0.9%, respectively; and (b) 7% padimate-O and 0.5% oxybenzone: 99.4 ± 0.6% and 99.3 ± 2.4%, respectively. The method was successfully applied to marketed products.

INTRODUCTION

An increasing number of over-the-counter (OTC) sunscreen preparations containing mixtures of padimate-O (2-ethylhexyl *p*-dimethylaminobenzoate; Escalol 507) and oxybenzone (2-hydroxy-4-methoxybenzophenone; Uvinul M 40) are now available in the market. Many of these products contain 7% padimate-O and 3% oxybenzone because this composition provides the most protection from UV light[1] and has a sun protection factor (SPF) value of $\geqslant$ 15. The FDA review panel on sun protection products has determined that these sunscreen agents are safe in the dosage ranges used (1.4–8.0% for padimate-O and 2–6% oxybenzone[2]. However, continuous

* Presented at the 131st Annual Meeting of the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, Montreal, Canada, May 1984.

0021-9673/84/$03.00

applications of these products at higher concentrations may cause irritations and other problems. A simple and reliable assay method to aid in the quality control of these OTC products is, therefore, necessary.

Oxybenzone has been separated from other sunscreen agents by programmed temperature gas chromatography[3,4] and thin-layer chromatography[5]. High-performance liquid chromatography (HPLC) was also utilized for the analysis of oxybenzone, both by normal[4,6] as well as reversed-phase HPLC[4,7] in mixtures with other UV-absorbers. In all of the above papers, oxybenzone was not in a mixture with padimate-O and no recovery data were given.

Only one assay method was found in the literature for padimate-O. Cumpelik[8] assayed padimate-O by programmed temperature gas chromatography after silanization of the sample. In addition, the author assayed a mixture of 3% padimate-O and 3% oxybenzone in a commercial sunscreen product by the same method and found the mixture to contain 4% of padimate-O and 3.3% of oxybenzone; an accuracy of 85–90% was expected for the method.

This paper reports a simple reversed-phase HPLC method for the assay of padimate-O and oxybenzone in lotions and lipsticks using sulphathiazole as internal standard.

EXPERIMENTAL

Apparatus

The following were used: an Altex Model 330 liquid chromatograph with a Model 210 sampling injection valve (20-μl loop), Model 110A pump and a Model 153 fixed-wavelength UV detector (254 nm) (Beckman, Fullerton, CA, U.S.A.) and a Kipp & Zonen BD 40 strip chart recorder (Kipp & Zonen, Delft, The Netherlands).

Reagents and materials

The following materials and reagents were used: padimate-O (courtesy Van Dyk & Co., Belleville, NJ, U.S.A.), oxybenzone (courtesy BASF Wyandotte, Parsippany, NJ, U.S.A.), sulfathiazole (Sigma, St. Louis, MO, U.S.A.). All other chemicals used were analytical grade.

HPLC conditions

A 25 cm × 4.6 mm I.D. 10-μm Partisil PXS ODS-2 column (Whatman, Clifton, NJ, U.S.A.) was used at ambient temperature. A Beckman 4 cm × 4.6 mm I.D. guard column, packed with 25–37 μm Whatman Co:Pell ODS preceded the analytical column. An isocratic mobile phase system of acetonitrile–methanol (10:90, v/v) was delivered at the rate of 0.7 ml/min (*ca.* 200 p.s.i.). This mobile phase was filtered through a 0.45-μm membrane filter (Nylon-66; Rainin Instruments, Woburn, MA, U.S.A.) and degassed before use. The detector was attenuated to 0.16 a.u.f.s.

Internal standard solution

A solution of about 25 mg of sulfathiazole in 25.0 ml of methanol; 5.0 ml of this solution was diluted to 25.0 ml with methanol to give an approximately 0.20 mg/ml solution.

Standard solution

About 400 mg of padimate-O and about 100 mg of oxybenzone were transferred into a 50-ml volumetric flask, dissolved in methanol and the solution was diluted to volume with methanol. A volume of 1 ml of the solution was diluted to 50.0 ml in a volumetric flask. From the resulting solution, 5.0 ml was pipetted into a 10-ml volumetric flask and, after addition of 1.0 ml of internal standard solution, the solution was diluted to volume with methanol.

Sample solution

Lotions. An amount of lotion equivalent to about 90 mg of oxybenzone was weighed accurately in a 25-ml beaker. After the sample was made more fluid with the addition of about 3 ml of water, it was transferred into a 100-ml volumetric flask. The beaker was rinsed twice with 2-ml portions of water and the rinsings were combined in the volumetric flask. The sample was diluted to volume with methanol which was added slowly with vigorous shaking. Exactly, 10.0 ml of the mixture was pipetted into a 100-ml volumetric flask and diluted to volume with methanol. The mixture was filtered through a dry, fluted filter paper, discarding the first 5 ml of filtrate. A volume of 4 ml of the filtrate and 1.0 ml of the internal standard solution were pipetted into a 10-ml volumetric flask and diluted to volume with methanol. An aliquot of the solution was filtered through a 0.45 μm membrane filter prior to HPLC analysis.

Lipsticks. An amount equivalent to about 90 mg or 25 mg of oxybenzone (for preparations containing 3% or 0.5% oxybenzone, respectively) was weighed accurately in a 25-ml beaker, diluted with about 5 ml of chloroform and transferred into a 100-ml volumetric flask. The beaker was rinsed with two 2.5-ml portions of chloroform and the rinsings were combined to the 100-ml volumetric flask. The sample was diluted with methanol to volume under vigorous shaking and then subjected to the same procedure as described for the lotion sample.

Chromatographic procedure

By means of the injection valve, 20 μl of the prepared sample solution or standard solution were chromatographed under the operating conditions described above. Quantitation was based on relating the compound–internal standard peak height ratio of the sample to that of the standard.

RESULTS AND DISCUSSION

Padimate-O and oxybenzone are active ingredients in sunscreen products because of their relatively high molar absorptivities in the ultraviolet range of the electromagnetic radiation. In ethanol or methanol padimate-O and oxybenzone have λ_{max} values of 310 and 290 nm, respectively[9]. In chloroform oxybenzone has a molar absorptivity of 23,500 l g^{-1} cm^{-1} at λ_{max} 287 nm[4]. Since the isoamyl ester analog of padimate-O has a molar absorptivity of 24,000 l g^{-1} cm^{-1} in chloroform at 309 nm[4] it can be assumed that the molar absorptivity of padimate-O is greater than 20,000 l g^{-1} cm^{-1} in chloroform at this wavelength. UV detection appears, therefore, to be a suitable detection method. The analytical wavelength of 254 nm was, however, selected as this is the more common wavelength used in fixed-wavelength UV detec-

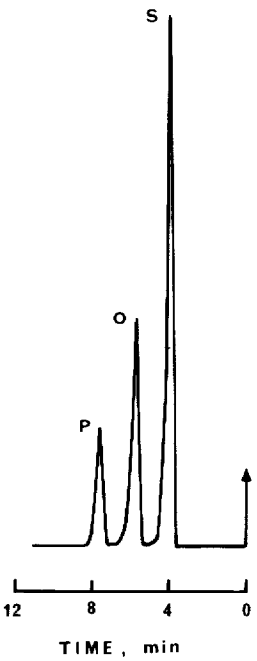

Fig. 1. Liquid chromatogram of a standard solution run under conditions described in the text. Peaks: S = sulfathiazole; O = oxybenzone; and P = padimate-O.

tors. The absorbances for both compounds are still substantial at 254 nm.

Under the experimental conditions described, sulfathiazole, oxybenzone and padimate-O were eluted as fairly symmetrical peaks and were well-resolved from one another (Fig. 1). The retention times were *ca.* 3.9, 5.7 and 7.4 min, respectively. The average height equivalent to a theoretical plate (HETP) of the column (± S.D.) was 0.79 ± 0.07, 0.53 ± 0.06, and 0.26 ± 0.04 mm, for sulfathiazole, oxybenzone and padimate-O, respectively (n = 10).

Quantitation was based on the compound–internal standard peak height ratio. With these ratios the linearity between detector response at 254 nm and amount of compound injected was established. Linearity was obtained between the investigated range of 0.04–2.68 μg of padimate-O and 0.02–1.05 μg of oxybenzone injected. Typical regression equations were $H = 0.097C + 0.004$ for padimate-O (r = 1.0003) and $H = 0.795C + 0.010$ for oxybenzone (r = 0.9997), where H = compound–internal standard peak height ratio and C = μg of compound injected. A linear relationship existed also between compound–internal standard peak area ratio and amount of compound injected, but the correlation coefficients r were only 0.9950 for padimate-O and 0.9914 for oxybenzone for the same concentration range. This and the fact that with some products an additional peak was observed that partially overlapped with the internal standard peak (see below) made quantitation by peak area ratio undesirable.

The described method was validated using simulated lotions and lipsticks formulations. Recovery studies were performed on these spiked lotion and lipstick placebos. The lotion placebo contained white petrolatum, light mineral oil, lanolin al-

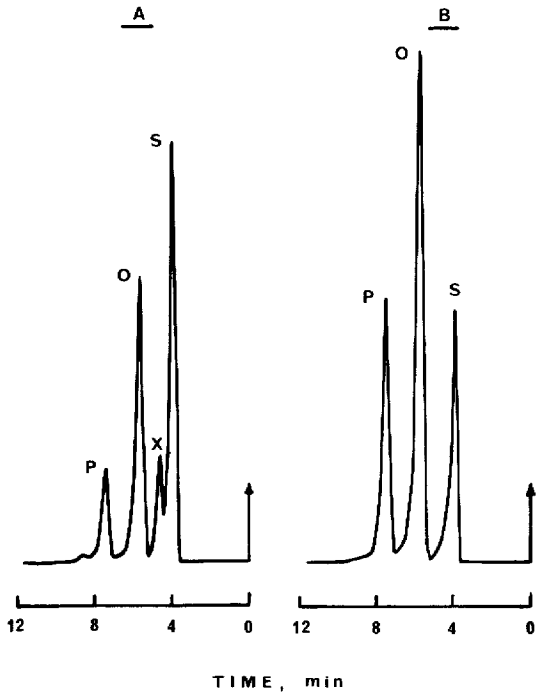

Fig. 2. Liquid chromatograms of an extract from a simulated lipstick (A) and lotion (B) formulation run under conditions described in the text. Peaks as in Fig. 1; X = propylparaben.

cohol, cetearyl alcohol, sucrose monostearate, propylene glycol, methyl- and propylparaben, and D & C Red No. 6 dye. The lipstick placebo was formulated with sucrose distearate, carnauba wax, ceresine wax, lanolin, paraffin wax, microcrystalline wax, castor oil, cetyl alcohol, oleyl alcohol, octyl palmitate, squalane, propylparaben and butylated hydroxyanisole. Typical chromatograms from these simulated lotion and lipstick formulations are shown in Fig. 2. For comparison, the liquid

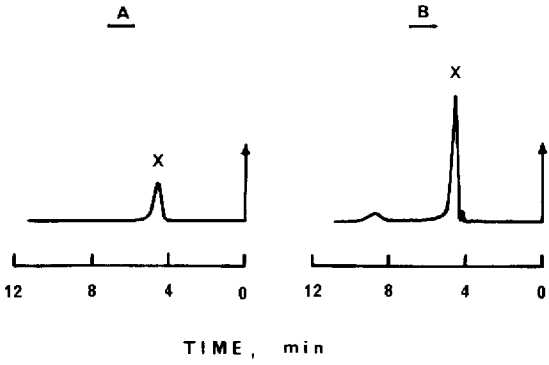

Fig. 3. Liquid chromatograms of an extract from a lotion (A) and lipstick (B) placebo run under conditions described in the text. Peaks: X = methyl- and/or propylparaben.

chromatograms of lotion and lipstick placebos, in the same amounts as used in recovery studies, are shown in Fig. 3. The lotion placebo showed a small peak with a retention time of *ca.* 4.4 min at 0.04 a.u.f.s. (Fig. 3A). However, at 0.16 a.u.f.s. this peak was negligible. This peak was identified as that of the parabens. The concentration of propylparaben in the lipstick formulation is higher than in the lotion, resulting in a fair size peak at *ca.* 4.4 min at 0.16 a.u.f.s. (Fig. 3B). Although this propylparaben peak overlapped with the sulfathiazole peak good recoveries were obtained when peak height ratios rather than peak area ratios were applied to the quantitation of padimate-O and oxybenzone (Table I) because generally, peak height measurements require less resolution of components[10]. Fig. 3B shows also a small peak at *ca.* 8.4 min but this peak did not interfere with the assay because it appeared after the elution of the active ingredients. Table I shows the recovery data from simulated preparations. The average percent recovery (± S.D.) from simulated lotions containing 7% padimate-O and 3% oxybenzone were (n = 3) 101.4 ± 1.5% for padimate-O and 99.9 ± 1.9% for oxybenzone. The simulated lipstick preparation gave average percent recoveries (± S.D.) (n = 3) of padimate-O, 103.8 ± 1.2% and oxybenzone, 100.1 ± 0.9% from a preparation containing 7% padimate-O and 3% oxybenzone. Simulated lipstick preparations containing 7% padimate-O and 0.5% oxybenzone resulted in average percent recoveries (± S.D.) of padimate-O, 99.4 ± 0.6% and oxybenzone, 99.3 ± 2.4% (n = 3). From the above results the overall

TABLE I

RECOVERY DATA FROM SIMULATED FORMULATIONS

Sample	*Amount (mg/g sample)*		*Amount found (mg/g sample)**		*Recovery (%)*	
	Padimate-O	*Oxybenzone*	*Padimate-O*	*Oxybenzone*	*Padimate-O*	*Oxybenzone*
A						
Lotion A1	70.3	30.2	71.1	29.7	101.1	98.3
Lotion A2	75.4	29.6	77.7	30.2	103.1	102.0
Lotion A3	72.2	30.1	72.3	29.9	100.1	99.3
Average recovery (%)					101.4	99.9
Coefficient of variation (%)					1.5	1.9
B						
Lipstick B1	71.6	30.0	74.4	30.0	103.9	100.0
Lipstick B2	71.8	28.1	75.4	27.9	105.0	99.3
Lipstick B3	72.2	28.5	74.1	28.8	102.6	101.1
Average recovery (%)					103.8	100.1
Coefficient of variation (%)					1.2	0.9
C						
Lipstick C1	71.5	5.1	71.4	5.2	99.9	102.0
Lipstick C2	71.3	5.0	71.1	4.9	99.7	98.0
Lipstick C3	68.4	4.5	67.5	4.4	98.7	97.8
Average recovery (%)					99.4	99.3
Coefficient of variation (%)					0.6	2.4

* Average from duplicate runs.

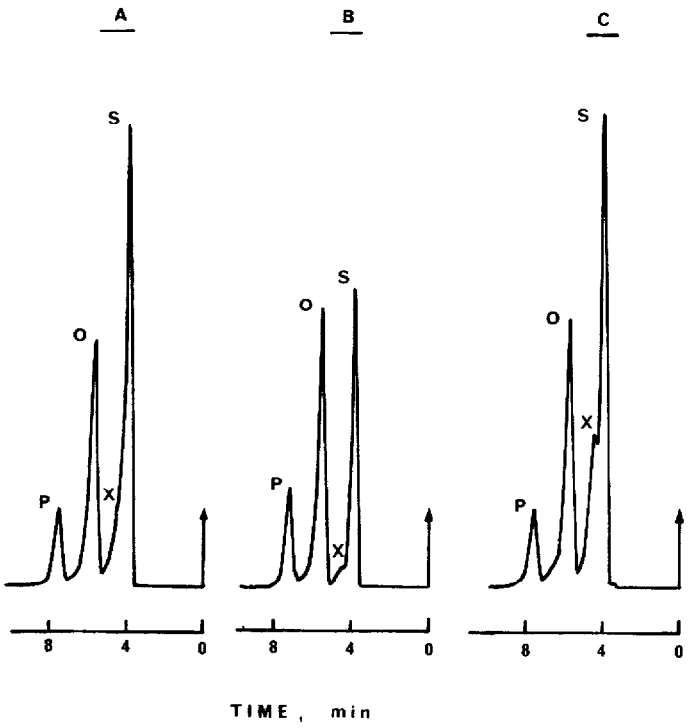

Fig. 4. Liquid chromatograms of (A) an extract from commercial lipstick C, (B) lotion A, and (C) lotion B, run under conditions described in the text. Peaks as in Figs. 1 and 3.

percent recoveries (± S.D.) were thus 101.6 ± 2.2% for padimate-O and 99.8 ± 1.6% for oxybenzone (n = 9).

The method was applied to the assay of the two compounds in some commercial sunscreen lotions and lipsticks. Typical chromatograms from these commercial products are shown in Fig. 4. Lotion A showed also an extraneous peak at *ca.* 4.4 min which presumably was due to the presence of propylparaben. Apparently, lotion B and lipstick A contained also propylparaben but in smaller quantities and therefore, gave a sulfathiazole peak with only a shoulder at *ca.* 4.4 min. The recovery data from these commercial products are tabulated in Table II. The table shows that the overall percent recoveries are similar to those of simulated preparations.

The proposed assay procedure allows for a simple and reliable quantitation of padimate-O and oxybenzone. With the above composition of padimate-O and oxybenzone the analytical sample amounted to about 3 g of lotion and 3–5 g of lipstick. The calculated theoretical total volume of methanol used in the two dilution steps was about 1100 ml per 3–5 g of sample. Experimentally, a two-step dilution procedure was performed so that the actual volume of methanol used is small. This large methanol–sample ratio caused the precipitation of most of the lotion and lipstick ingredients resulting in clean chromatograms and longer column life. Although a larger volume of water (for lotions) or chloroform (for lipsticks) will facilitate transfer of the sample from the weighing beaker to the volumetric flask, a larger volume of

TABLE II

RECOVERY DATA FROM COMMERCIAL SUNSCREEN PRODUCTS

Product	*Label claim (mg/g)*		*Amount found (mg/g)**		*Label claim (%)*	
	Padimate-O	*Oxybenzone*	*Padimate-O*	*Oxybenzone*	*Padimate-O*	*Oxybenzone*
Lotion A	70.0	30.0	70.8	31.3	101.1	104.3
	70.0	30.0	71.4	31.4	102.0	104.7
Lotion B	80.0	30.0	87.2	31.5	109.0	105.0
	80.0	30.0	87.3	31.7	109.1	105.7
Lipstick C	70.0	30.0	68.1	28.7	97.3	95.7
	70.0	30.0	68.2	28.3	97.4	94.3

* Average of duplicate assays.

water gave a cloudy filtrate during the filtration step whereas a larger volume of cloroform kept a substantial amount of the lipstick ingredients in solution. The chromatographic run can be completed within 8 min.

ACKNOWLEDGEMENTS

The authors thank the Van Dyk & Co., Belleville, NJ, U.S.A., and BASF Wyandotte Corp., Parsippany, NJ, U.S.A., for supplying the padimate-O and oxybenzone, respectively.

REFERENCES

1 R. M. Sayre, P. P. Agin, G. J. LeVee and E. Marlowe, *Photochem. Photobiol.*, 29 (1979) 559.
2 Food and Drug Administration, *Fed. Reg.*, 43 (1979) 38264.
3 J. Horacek, *Plasty Kauc.*, 13 (1976) 274; *C.A.*, 86 (1977) 6081z.
4 F. Eiden and C. Tittel, *Dtsch. Apoth.-Ztg.*, 121 (1981) 1874.
5 F. Eiden and C. Tittel, *Dtsch. Apoth.-Ztg.*, 121 (1981) 2693.
6 H. König and R. Ryschka, *Z. Anal. Chem.*, 315 (1983) 434.
7 G. Chiavari, V. Concialini and P. Vitali, *J. Chromatogr.*, 249 (1982) 385.
8 B. M. Cumpelik, *Cosmet. Toiletries*, 97 (1982) 67.
9 Food and Drug Administration, *Fed. Reg.*, 43 (1978) 38239, 38243.
10 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, NY, 2nd ed., 1979, p. 556 *ff.*