藥物食品檢驗局調查研究年報. 12：6－9. 1994.
Ann. Rept. NLFD Taiwan R.O.C. 12：6－9. 1994.

Short Communication

Determination of ascorbyl dipalmitate in cosmetic whitening powders by liquid chromatography

Mei-Hwa Lien, Bin-Chung Huang and Mei-Chich Hsu*

National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, 161-2, Kuen-Yang St., Nankang, Taipei 11513 (Taiwan)

(First received April 15th, 1993; revised manuscript received May 25th, 1993)

ABSTRACT

A reversed-phase column liquid chromatograpic method was developed for the assay of ascorbyl dipalmitate in whitening powders. The linear calibration range was 0.1–0.7 mg/ml ($r = 0.9999$), and recoveries were generally greater than 98.8%. The coefficient of variation was less than 0.64%. The method has been applied to whitening powders produced by four different cosmetics companies. The assay results were compared with those obtained from a sodium hydroxide titration assay of ascorbyl dipalmitate in commercial whitening powders. The method can be successfully used for routine quality control and offers advantages in speed, simplicity and reliability.

INTRODUCTION

Ascorbyl dipalmitate is a more stable form of ascorbic acid and is found in nail lacquers [1], lotions [2,3], skin powders [4–6], skin conditioners [7], sunscreen [8], freckle formulations [9], creams [10,11] and dentifrices [12]. It has also been shown to extend the stability of adhesive transdermal pharmaceuticals [13]. Ascorbic acid and ascorbic acid derivatives are thought to be produced by the inactivation of tyrosinase, an enzyme that mediates the early steps of the melanin biosynthetic pathway [14]. Owing to its skin-whitening action, ascorbyl dipalmitate has been used widely in skin-whitening preparations [15–23].

In order to ensure effectiveness, safety and quality, the manufacture and import of medicated cosmetics are strictly controlled. In Taiwan no-one can operate such a business without obtaining approval and a license from the Department of Health, Taiwan. Requirements for approval and license applications for medicated cosmetics are almost the same as those for drugs. Hence, it is imperative to be able to determine accurately the amount of ascorbyl dipalmitate in cosmetic whitening powders.

Few methods have been reported in the literature for the analysis of ascorbyl dipalmitate. Luckewicz and Saccro [24] reported a differential scanning colorimetric method for the determination of ascorbyl dipalmitate and Matsumoto and Shinozaki [25] and Yamaoka *et al.* [26] described LC procedures for the determination of ascorbyl dipalmitate in vitamin preparations and ointments. The present official assay method for the analysis of ascorbyl dipalmitate in whitening

* Corresponding author.

原載於 Journal of Chromatography. 1993. 645：362－365.

powder is a titration method [27]. The purpose of this investigation was to develop an HPLC method that would permit the accurate determination of ascorbyl dipalmitate in cosmetic whitening powders.

EXPERIMENTAL

Apparatus

A Waters Model 510 liquid chromatography pump (Waters Chromatography Division, Millipore, Milford, MA, USA), a Shimadzu SPD-6A UV detector and a Waters 745 data module were employed during the study. The mobile phase was pumped through a reversed-phase column (Spherisorb S5 ODS2, 15 cm × 4.6 mm I.D., 5 μm; Phase Separation) with an isocratic flow-rate of 1.0 ml/min. The detector was set at 240 nm. Chromatography was performed at room temperature. Injections of 20 μl of all solutions to be analysed were made.

Reagents and materials

Methanol (liquid chromatography grade) was obtained from ALPS Chemical, Taipei, Taiwan. Glacial acetic acid, sodium hydroxide solution (0.1 mol/l) and ethanol (reagent grade) were obtained from E. Merck, Darmstadt, Germany. Octyl dimethyl *p*-aminobenzoic acid, ascorbyl dipalmitate and phenolphthalein were obtained from Tokyo Kasei Kogyo, Tokyo, Japan.

Mobile phase

The mobile phase was methanol–40% glacial acetic acid (97:3, v/v). The mobile phase was filtered (0.45 μm pore size Millipore filter) and degassed with an ultrasonic bath prior to use.

Internal standard solutions

Internal standard octyl dimethyl *p*-aminobenzoic acid (100.0 mg) was dissolved in 1.0 l of ethanol and shielded from light.

Ascorbyl dipalmitate standard solutions

To form ascorbyl dipalmitate standard solution, internal standard solution was added to an accurately weighed amount of ascorbyl dipalmitate standard equivalent to 40.0 mg and the volume was brought up to 100.0 ml. The solution should be shielded from light.

Sample preparations

To form sample preparations, internal standard solution was added to an accurately weighed amount of whitening powder equivalent to 40.0 mg of ascorbyl dipalmitate and the volume was brought up to 100.0 ml. The solution should be shielded from light.

Solution for linearity response

Seven concentrations of ascorbyl dipalmitate, which ranged from 0.1 to 0.7 mg/ml, were prepared. Each concentration was chromatographed six times.

Solution for recovery studies

To an accurately weighed 40.0 mg of ascorbyl dipalmitate of sample composites of commercial preparations were added different amounts of ascorbyl dipalmitate standard. Each solution was made up to 100.0 ml with internal standard solution and was chromatographed in triplicate.

Sodium hydroxide titration

Whitening powder equivalent to *ca.* 300.0 mg of ascorbyl dipalmitate, accurately weighed, was transferred to a glass-stoppered, 50.0-ml conical flask, 30 ml of ethanol added, and the solution mixed. The solution was filtered and the flask and the filter were washed with small portions of ethanol. The washings were added to the filtrate. Two drops of phenolphthalein TS were added. The solution was titrated with 0.02 *M* sodium hydroxide until the entire mixture changed to a red colour. The red colour should persist for 30 s. Each millilitre of 0.02 *M* sodium hydroxide is equivalent to 13.06 mg of ascorbyl dipalmitate ($C_{18}H_{68}O_8$).

RESULTS AND DISCUSSION

Reproducibilities were determined by chromatographing seven standard solutions of ascorbyl dipalmitate ranging in concentration from 0.1 to 0.7 mg/ml in the presence of the internal standard octyl dimethyl *p*-aminobenzoic acid. Standard curves were obtained by plotting peak-area

ratios *versus* concentrations. The linear correlation coefficient was 0.9999 ($y = 0.1252x - 0.0027$). The coefficients of variation in the within-day assay were between 0.15 and 0.64% at the concentration of 0.4 mg/ml. The coefficient of variation in the between-day assay ($n = 5$) was 0.59% at the same concentration.

Standard addition recovery studies of ascorbyl dipalmitate from sample composites of commercial whitening powder were performed. The average recovery was 98.8–100.4% for four commercial compositions. These data indicate that the proposed HPLC method is relatively unaffected by the sample matrix.

Typical chromatograms of the ascorbyl dipalmitate in commercial whitening powder are shown in Fig. 1. The retention time was about 3 min for the internal standard and 8 min for ascorbyl dipalmitate. Excipients from commercial cosmetic formulations did not interfere. Furthermore, the HPLC method can separate compounds related to ascorbyl dipalmitate, *i.e.*, ascorbic acid and ascorbyl palmitate, which were eluted prior to ascorbyl dipalmitate (Fig. 1).

A study was initiated to ascertain the suitability of the proposed method for stability studies. Sample solutions of ascorbyl dipalmitate were stored in a cabinet at ambient temperature. Samples were taken from the cabinet periodically for LC assay. The resulting mixtures yielded chromatograms containing an additional

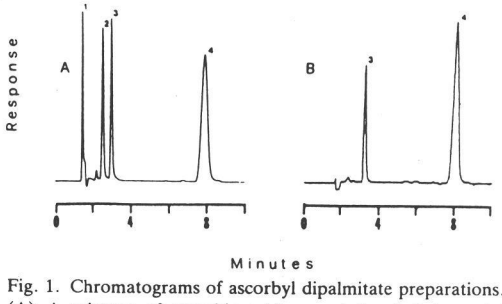

Fig. 1. Chromatograms of ascorbyl dipalmitate preparations. (A) A mixture of ascorbic acid, ascorbyl palmitate, octyl dimethyl *p*-aminobenzoic acid and ascorbyl dipalmitate. (B) A sample of cosmetic whitening powder. Peaks: 1 = ascorbic acid; 2 = ascorbyl palmitate; 3 = octyl dimethyl *p*-aminobenzoic acid; 4 = ascorbyl dipalmitate.

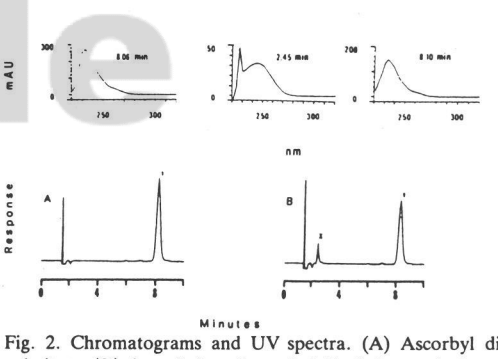

Fig. 2. Chromatograms and UV spectra. (A) Ascorbyl dipalmitate; (B) degradation of ascorbyl dipalmitate solution at ambient temperature for 5 days. Peaks: 1 = ascorbyl dipalmitate; x = degradation product.

peak, which did not interfere with the interpretation and measurement of the chromatographic peaks for ascorbyl dipalmitate and octyl dimethyl *p*-aminobenzoic acid, as shown in Fig. 2. In addition, ascorbyl dipalmitate disappeared and degradation product accumulated with increasing storage time. To examine the purity of the ascorbyl dipalmitate peak and identify the degradation product in the time-degraded samples, a UV photodiode array detector was used. The evaluation of chromatographic peak homogeneity was performed by absorbance ratios and a three-dimensional spectrochromatogram. The results presented good confirmation of the ascorbyl dipalmitate peak identity (data not shown). The peak obtained from the degradation of ascorbyl dipalmitate solution indicated that it may be ascorbyl palmitate, since its UV spectrum corresponds to the spectrum of ascorbyl palmitate (Fig. 2).

Four cosmetic whitening powders were analysed for ascorbyl dipalmitate content by HPLC. These samples were also assayed by the titration method. The results are shown in Table I. A *t*-test was applied to the data. The assay results obtained by the titration method were statistically significantly higher than those obtained by the proposed chromatographic method. These higher results are not unexpected, since the titration method is non-selective, and the end point of the titration is affected by other variables [28,29]. The skin-whitening powders contain ascorbyl dipalmitate, pyridoxine hydrochloride, mannitol,

TABLE I

COMPARISON OF TITRATION AND HPLC ASSAYS FOR ASCORBYL DIPALMITATE

Manufacturer	Declared (mg/ml)	Found (% of declared)	
		Titration[a]	HPLC[b]
A	250	113.1	98.0
B	250	110.0	98.5
C	250	112.6	97.6
D	250	120.1	92.6

[a] Average of two determinations.
[b] Average of triplicate determinations.

starch, etc. It is possible that the pyridoxine hydrochloride may be responsible for the bias of the titrimetic method described.

This study demonstrates the applicability of the proposed HPLC method for the determination of ascorbyl dipalmitate in cosmetic whitening powders. The method can be successfully used for routine quality control and offers advantages in speed, simplicity and reliability.

ACKNOWLEDGEMENTS

The authors thank Miss Hsiou Chuan Chung for her assistance in the preparation of this manuscript. This work was supported by the Division of Drug Chemistry, National Laboratories of Foods and Drugs, Department of Health.

REFERENCES

1 N. Ito, *Jpn. Kokai Tokkyo Koho JP 02235805 A2, Heisei*, September 18 (1990).
2 *Jpn. Kokai Tokkyo Koho JP 60116618 A2, Showa*, June 24 (1985).
3 A. Noda, M. Yamaguchi, M. Aizawa and Y. Kumano, *Eur. Pat. Appl. EP 316054 A1*, May 17 (1989).
4 G. Okuyama, S. Shinho and J. Shimoyama, *Jpn. Kokai Tokkyo Koho JP 61130206 A2, Showa*, June 18 (1986).
5 A. Imamura and T. Kondo, *Jpn. Kokai Tokkyo Koho JP 63280005 A2*, November 17 (1988).
6 A. Munakata, *Jpn. Kokai Tokkyo Koho JP 02169508 A2, Heisei*, June 29 (1990).
7 I. Koiso, N. Kanbe, M. Oyama, Y. Okaya and K. Kinoshita, *Jpn. Kokai Tokkyo Koho JP 62129212 A2, Showa*, June 11 (1987).
8 S. Tobe and K. Shimizu, *Jpn. Kokai Tokkyo Koho JP 61078715 A2, Showa*, April 22 (1986).
9 A. Yokozuka, T. Takayabu, *Jpn. Kokai Tokkyo Koho JP 61263906 A2, Showa*, November 21 (1986).
10 *Jpn. Kokai Tokkyo Koho JP 59073508 A2, Showa*, April 25 (1984).
11 H. Tamabuchi, *US Pat., 4 818 521 A*, April 4 (1989).
12 T. Shino, *Jpn. Kokai Tokkyo Koho JP 62273910 A2, Showa*, November 28 (1987).
13 *Jpn. Kokai Tokkyo Koho JP 59059616 A2, Showa*, April 5 (1984).
14 Y. Tomita, A. Hariu, C. Mizuno and M. Seiji, *J. Invest. Dermatol.*, 75 (1980) 379.
15 K. Masamoto, Y. Murakami and H. Tamabuchi, *Jpn. Kokai Tokkyo Koho JP 52079032 A2, Showa*, July 2 (1977).
16 *Jpn. Kokai Tokkyo Koho JP 59010505 A2, Showa*, January 20 (1984).
17 *Jpn. Kokai Tokkyo Koho JP 59065007 A2, Showa*, April 13 (1984).
18 *Jpn. Kokai Tokkyo Koho JP 59104308 A2, Showa*, June 16 (1984).
19 *Jpn. Kokai Tokkyo Koho JP 60078912 A2, Showa*, May 4 (1985).
20 R. Oomi and A. Matsumoto, *Jpn. Kokai Tokkyo Koho JP 61100509 A2, Showa*, May 19 (1986).
21 S. Yoshikawa, Y. Hiraki and T. Koyama, *Jpn. Kokai Tokkyo Koho JP 02304012 A2, Heisei*, December 17 (1990).
22 K. Omae and K. Ito, *Jpn. Kokai Tokkyo Koho JP 04077409 A2, Heisei*, March 11 (1992).
23 T. Hikima and M. Kondo, *Jpn. Kokai Tokkyo Koho JP 04091009 A2, Heisei*, March 24 (1992).
24 W. Luckewicz and R. Saccaro, *J. Soc. Cosmet. Chem.*, 41 (1990) 359.
25 K. Matsumoto and K. Shinozaki, *Jpn. Kokai Tokkyo Koho JP 61183217 A2, Showa*, August 15 (1986).
26 K. Yamaoka, M. Matsui, Y. Saito and T. Sato, *Byoin Yakugaku* 7 (1982) 355.
27 *Cosmetic Regulations for Raw Materials of Taiwan*, Department of Health, Executive Yuan, Taipei, 1988, p. 57.
28 G. Hoogewijs and D.L. Massart, *J. Chromatogr.*, 309 (1984) 329.
29 J.C. Kraak and J.P. Crombeen, *J. Liq. Chromatogr.*, 5 (Suppl. 2) (1982) 273.