Journal of Pharmaceutical & Biomedical Analysis,
Vol. 1, No. 2, pp. 229–233, 1983
Printed in Great Britain

0731–7085/83 $3.00+0.00
Pergamon Press Ltd.

Short Communication

Determination of coumarin and 6-methylcoumarin in cosmetics by high-performance liquid chromatography

A. BETTERO* and C. A. BENASSI

Institute of Pharmaceutical Chemistry, Padua University, Padova, Italy

Keywords: *Reversed phase HPLC; coumarin; 6-methylcoumarin; stopped-flow technique; gradient elution.*

Introduction

Coumarin, found in many plants and essential oils [1, 2], is a lactone obtained by the cyclization of *o*-hydroxycinnamic acid, which gives coumarin its particular flavour; it is used in the cosmetic and food industries to fix or strengthen the fragrance and scent of many chemicals [3]. Both fragrance and toxicity can be radically modified by the presence of different moieties in the molecule [4]. Coumarin and 6-methylcoumarin are used at concentrations of 0.03–0.8% and 0.01–0.4% respectively, depending on the product formulations [5, 6].

Histological evidence of hepatotoxicity [7, 8] and of carcinoma in the bile duct [9] has been observed in animals after long-term administration of coumarin in the diet. In 1974, the Council of Europe permitted its presence in food in quantities up to 5 ppm [3]. The urinary excretion of coumarin metabolites [10] following percutaneous absorption is similar in pattern to that found after oral administration. 6-Methylcoumarin in suntan cosmetics has been identified as a photocontact allergen [11]. Blistering of the skin and systemic effects can be caused by continued use of suntan products containing 6-methylcoumarin in a wide segment of the population not already allergic to the substance [12]. The Federal Drug Administration has proposed prohibition of the use of 6-methylcoumarin in cosmetics and topical preparations of drugs [11].

A simple, specific and sensitive method for the separation and estimation of coumarin and 6-methylcoumarin in cosmetics has not yet been reported. The present work is devoted to establishing an analytical method which could be extended to the largest possible number of cosmetic formulations with minimal manipulation of the samples. It

* To whom correspondence should be addressed.

is based on reversed-phase high performance liquid chromatography and UV spectroscopy [13]. Many cosmetics have been similarly analysed by modifying the eluent gradient of the chromatographic mobile phase [14].

Experimental

A Perkin–Elmer Series 3B liquid chromatograph equipped with a Rheodyne 7125 injection valve was used. Detection of coumarin and 6-methylcoumarin was carried out with a Perkin–Elmer LC-75 variable wavelength detector provided with the autocontrol accessory and a model 561 potentiometric recorder. Separation was achieved with a Merck RP-8 HPLC column (10 μm C-8 bonded phase), length 25 cm. Standard solutions of coumarin and 6-methylcoumarin (supplied by Farmitalia, Milan, Italy) were prepared by dissolving 1 mg of each in methanol to a concentration of 10 μg/ml. Samples (1 g) of each randomly selected commercial product, accurately weighed, were diluted with methanol, filtered through a 0.45 μm Millipore filter and diluted to 10 ml with methanol before analysis. Chromatography was carried out with various gradient profiles (Fig. 1). The mobile phase, acetonitrile–water, was delivered at a flow rate of 1.0 ml/min.

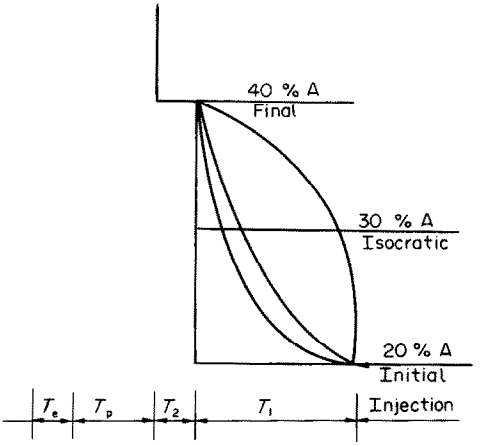

Figure 1
HPLC gradient profiles obtained using different values of n in the equation:

$$A_t = A_i + (A_f - A_i)\cdot\left(\frac{t}{T_1}\right)^n$$

where A_t = % A at time t; A_i = % A initially; A_f = % A finally; T_1 = total gradient time; t = elapsed time; n = 0.2, 2.0, 3.0. Solvent programme: A, % acetonitrile in water; T_1 = 20 min; T_2 = 5 min; T_p = 10 min (purge); T_e = 5 min (equil.).

Results and Discussion

The solvent programme for the separation of coumarin and 6-methylcoumarin from interfering substances was chosen from among the gradient curvatures provided by Series 3B LC apparatus. Good detection sensitivity was obtained at 220 nm. Satisfactory separation of a peak with a retention time equal to that of the reference standard was obtained in a commercial perfume by changing the gradient profile of the mobile phase (Fig. 2). The flow was stopped with the sample in the detector cell and its UV spectrum

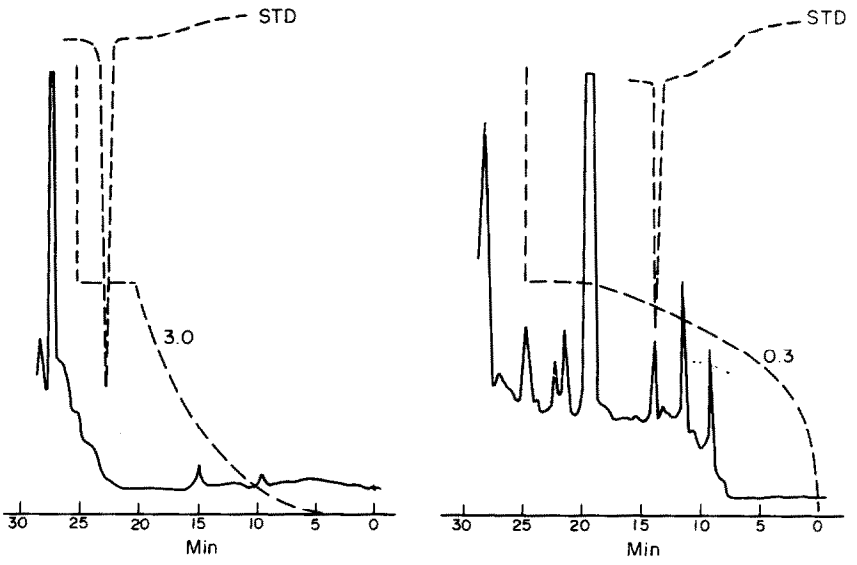

Figure 2
Separation and identification of coumarin in commercial perfume obtained using $n = 3.0$ and $n = 0.3$.

obtained. To confirm the identification and purity of coumarin, absorbance ratios were calculated. Figure 3 illustrates a good separation of coumarin in a commercial eau de cologne sample. Similar experiments were carried out in an effort to detect 6-methylcoumarin in fragrance and suntan products. Figure 4 shows the chromatograms of a commercial perfume compared with that of a 6-methylcoumarin reference standard. Absorbance ratios did not confirm the presence of 6-methylcoumarin, but good separation of 6-methylcoumarin added to the same commercial perfume was obtained using a modified gradient profile (Fig. 5).

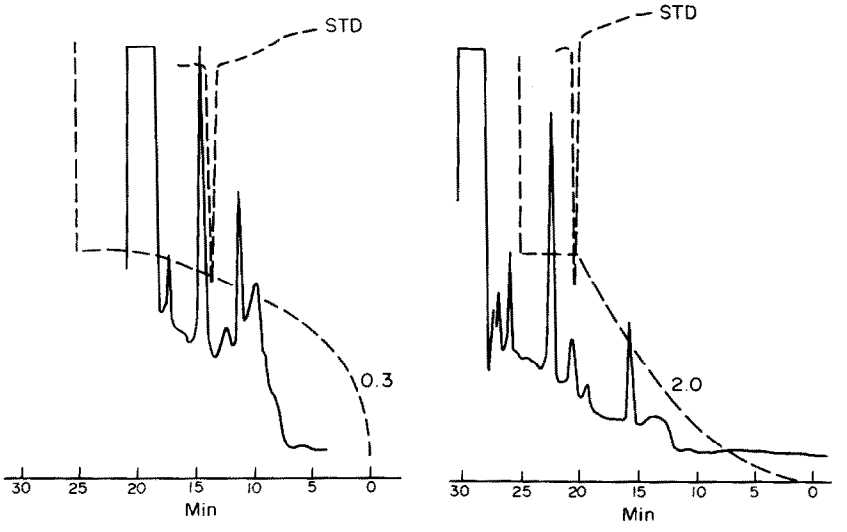

Figure 3
Separation of coumarin in commercial eau de cologne using $n = 0.3$ and $n = 2.0$.

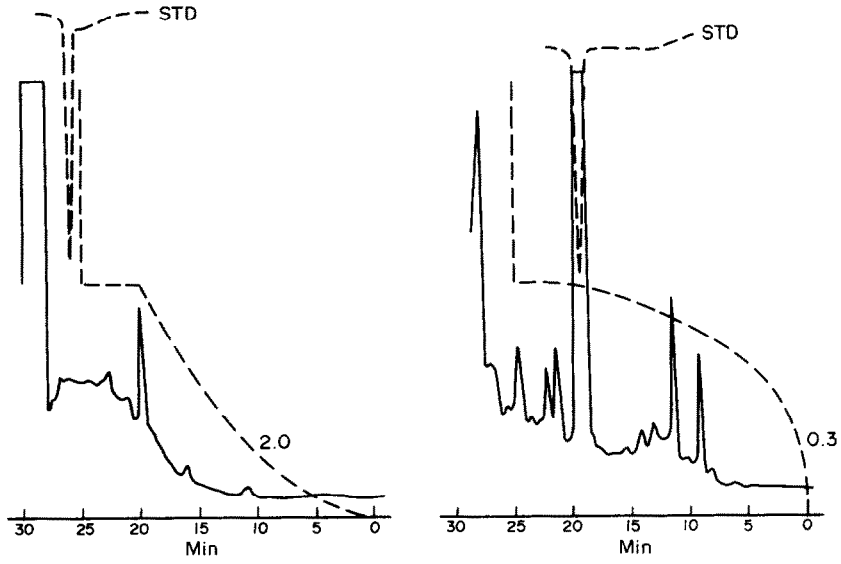

Figure 4
Chromatograms of a commercial perfume compared with 6-methylcoumarin reference standard (n = 2.0 and n = 0.3).

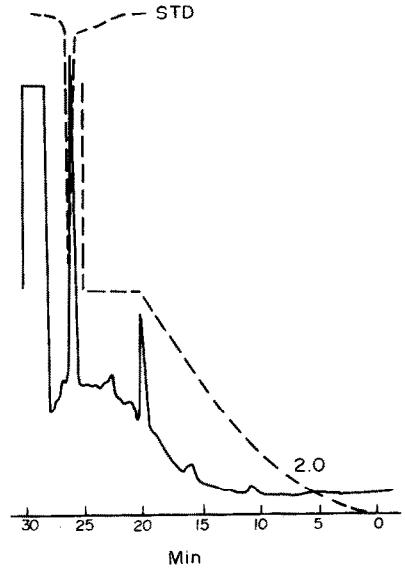

Figure 5
Separation of 6-methylcoumarin standard (10 ppm) added to commercial perfume (n = 2.0).

Table 1 lists the results obtained from cosmetics analysed by the gradient HPLC and stopped-flow technique. Coumarin was detected in all commercial samples, whereas the presence of 6-methylcoumarin was not confirmed. Recovery of added reference standard (10 ppm) was found to be 97–99% (n = 5). The detection limits at twice the noise level were approximately 0.1 ppm, determined in each case by injecting known quantities of reference standard solutions added to the sample.

Table 1
Determination and recovery of coumarin and 6-methylcoumarin in cosmetics by variable gradient HPLC

Sample	n*	Coumarin		6-Methylcoumarin
		Concentration (mg/100 ml)	Recovery (%)	Recovery (%)
Perfume	0.3	3.870	101 ± 1 (n = 5)	99 ± 1 (n = 5)
Eau de cologne	2.0	1.073	98 ± 1	98 ± 2
Eau de cologne	2.0	1.740	99 ± 2	98 ± 2
After shave	2.0	2.784	99 ± 1	98 ± 2
Suntan oil	2.0	—	97 ± 2	98 ± 3
Suntan cream	2.0	—	97 ± 3	96 ± 3

* n = Gradient curvature (see Fig. 1).

The results confirmed the usefulness of reversed-phase HPLC with variable profile gradient elution [14] and the stopped-flow technique [15] for the rapid screening of coumarin and 6-methylcoumarin in cosmetic preparations as an alternative to other methods [16, 17]. The combination of column separation and UV spectroscopy allowed detection irrespective of the complexity of the cosmetics analysed, as confirmed by recovery experiments (Table 1).

Cosmetics contain a variety of components which are incompletely separated by a single mobile phase; the key to the proposed method was the ability to elute all the components from the column. A convex gradient was used when a number of components which were difficult to separate were grouped together toward the end of the chromatogram, while a concave gradient facilitated the separation of each peak. The HPLC method proposed allows the rapid and reliable detection of coumarin and 6-methylcoumarin in cosmetic preparations; it can be carried out routinely. Moreover, it seems to be suitable for field monitoring of these compounds in a variety of cosmetics.

References

[1] E. Späth, *Ber.* **70A**, 83–117 (1937).
[2] E. Gildemeister and F. Hoffman, *Die Atherischen Ole*. Vol. IIId, p.591. Akademie Verlag, Berlin (1966).
[3] A. J. Cohen, *Food Cosmet. Toxicol.* **17**, 277–289 (1979).
[4] R. Selleri, C. Bottré and G. Orzalesi, *Chimica e Tecnologia dei Prodotti Cosmetici*, p. 132. Conti Tipocolor, Firenze (1977).
[5] D. L. J. Opdyke, *Food Cosmet. Toxicol.* **12**, 385–388 (1974).
[6] D. L. J. Opdyke, *Food Cosmet. Toxicol.* **14**, 605 (1976).
[7] L. W. Hazleton, T. W. Tusing *et al.*, *J. Pharmacol. Exp. Ther.* **118**, 348–359 (1956).
[8] E. C. Hagan, W. H. Hansen *et al.*, *Food Cosmet. Toxicol.* **5**, 141–157 (1967).
[9] F. U. Bär and F. Griepentrog, *Med. Ernähr.* **8**, 244 (1967).
[10] I. Pekker and E. A. Schäfer, *Arzneim.-Forsch.* **19**, 1744–1745 (1969).
[11] H. J. Heirmann, *Drug Cosmet. Ind.* **124**, 104–105 (1979).
[12] J. A. Wenninger, *Cosmet. Technol.* December, 37–38 (1979).
[13] A. Bettero and C. A. Benassi, *Farmaco Ed. Prat.* **36**, 140–147 (1981).
[14] R. W. Yost, J. Stoveken and V. M. Maclean, *J. Chromatogr.* **134**, 73–82 (1977).
[15] R. W. Yost, L. S. Ettre and R. D. Conlon, *Practical Liquid Chromatography*, p. 154. Perkin–Elmer, U.S.A. (1980).
[16] K. Gundermann, H. Fuchs and P. Baecher, *Brau und Weinwirtschaft* **118**, 418–419 (1978).
[17] P. A. P. Liddle and P. De Smedt, *Ann. Nutr. Aliment.* **32**, 931–939 (1978).

[Received for review 14 July 1982; revised manuscript received 17 December 1982]