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Determination of sunscreen agents by micellar electrokinetic chromatography

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Abstract: The separation of UV-A and UV-B sunscreens by micellar electrokinetic chromatography has been studied. The optimized method, which involves the presence of an anionic surfactant (sodium dodecyl sulphate) and an organic modifier in the background electrolyte, was applied to determine these sunscreens in cosmetic products. Identification was achieved by "on-line" UV spectra. Recovery was in the range of 88–92% and the lower limit of detection was 0.15 mg ml^{-1}.

Keywords: *UV-A and UV-B sunscreens; micellar electrokinetic chromatography; MEKC; diode-array detection (DAD).*

Introduction

Sunscreens are compounds that absorb selectively UV-B (280–320 nm) and/or UV-A (320–400 nm) rays; they play a significant role in preventing photobiological damage of the skin, that can lead to cutaneous disorders such as skin cancer and premature aging [1]. Among UV-A filters benzophenone- and propandione-derivatives are widely used whereas 2-phenyl-benzylimidazole-5-sulphonic acid represents a common UV-B absorber (Fig. 1).

The efficacy and safety of these synthetic sunscreens have been a matter of interest, in relation to the total formulation, instructions for use and sun protection claims. As an alternative, plant extracts are used to mitigate the deleterious effect of sunlight, because of the photoprotective action of their components that absorb radiation over a wide UV range (280–400 nm) [2].

Several methods have been proposed for the identification and determination of sunscreens in cosmetic products, involving mainly reversed-phase high-performance liquid chromatography (HPLC). However, this technique has been applied to separate either UV-A or UV-B filters and often requires gradient elution, with long analysis times [3].

Capillary electrophoresis (CE) in the micellar mode is known as micellar electrokinetic chromatography (MEKC) and combines the positive features of electrophoretic separation with those from hydrophobic interaction between analytes and micelles, thus allowing better resolution than reversed-phase HPLC [4, 5]. To confirm the potential of MEKC, the sunscreens reported in Fig. 1 (UV-A and UV-B) have been analysed both as standard mixtures and as components of cosmetic products. For such products a simplified clean-up procedure has been used [6] and the identity of the investigated sunscreens has been assessed by comparison of "on-line" UV spectra with standards. Finally, the UV absorbing properties of two sunscreens (III and IV) have been compared with those of two compounds, naringenin and cinnamic acid, which are commonly present in natural extracts.

Experimental

Materials

Sodium dodecyl sulphate (the Sigma Chemical Co.) was used as an electrophoresis reagent. Sodium tetraborate, sodium monohydrogen phosphate, potassium dihydrogen

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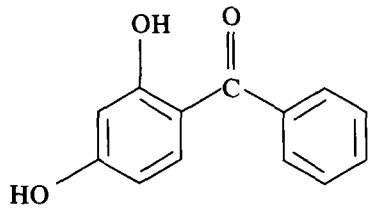
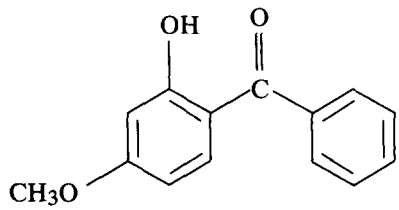


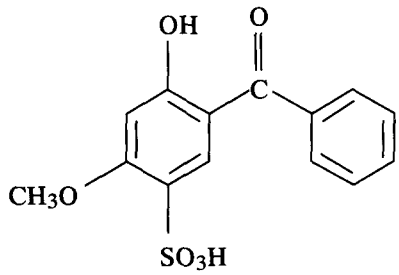
(I)

2,4-Dihydroxybenzophenone



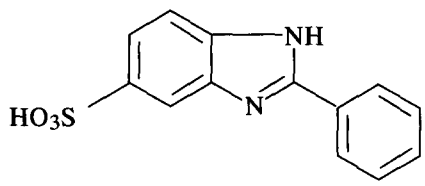
(II)

2-Hydroxy-4-methoxy-benzophenone



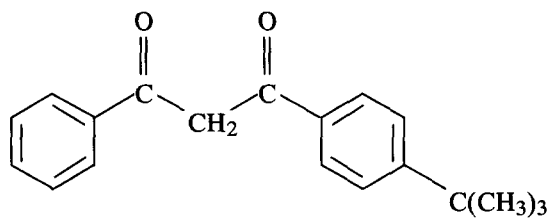
(III)

2-Hydroxy-4-methoxy-benzophenone-5-sulphonic acid



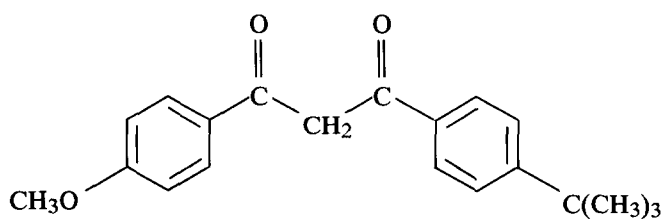
(IV)

2-Phenylbenzimidazole-5-sulphonic acid



(V)

1-(4-Tert-butylphenyl)-3-phenyl-1,3-propandione



(VI)

1-(4-Tert-butylphenyl)-3-(4-methoxyphenyl)-1,3-propandione

Figure 1
Structures of UV filter standards UV-A (I-VI-V) and UV-B (IV-II-III).

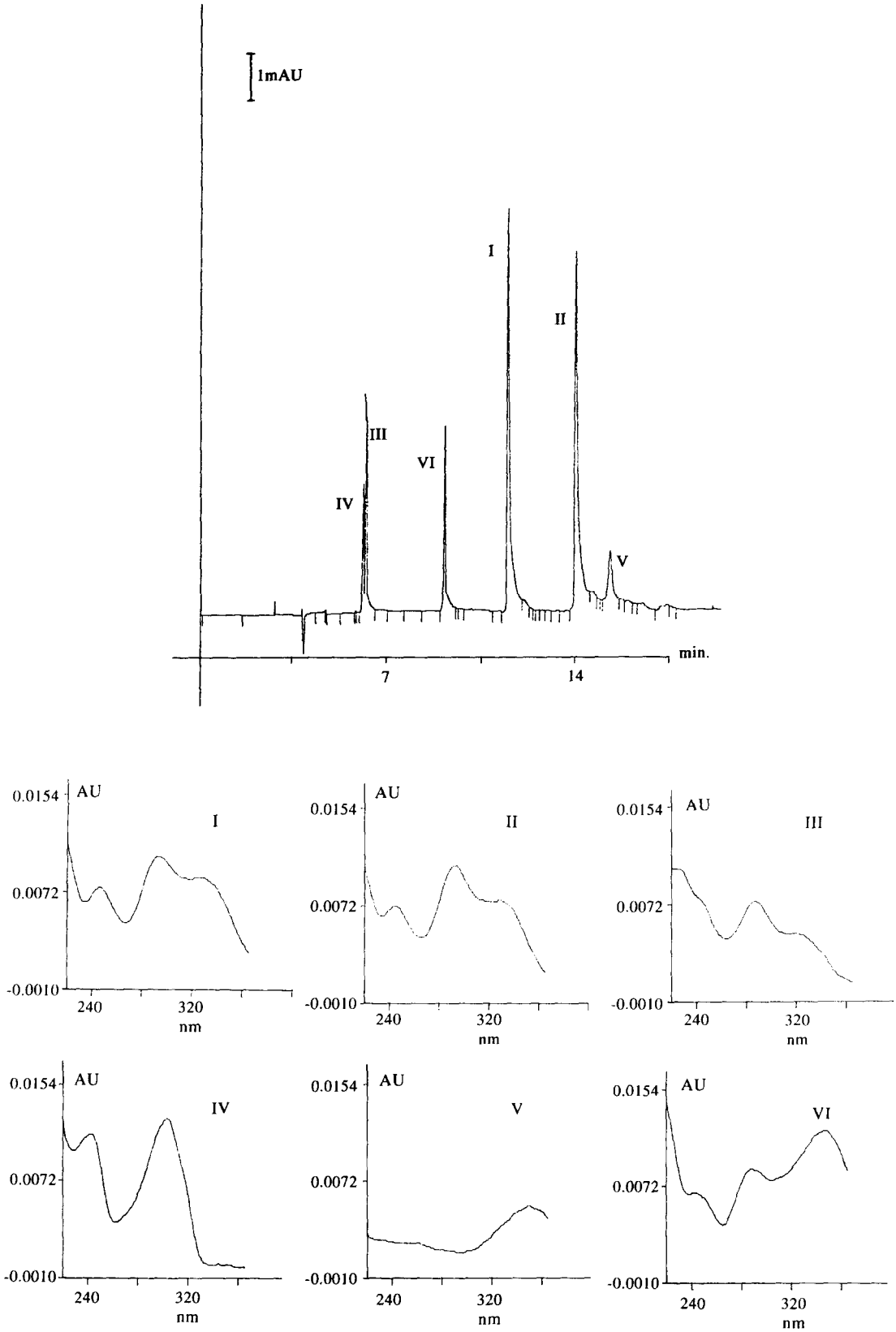


Figure 2
(A) MEKC separation of six standards. (B) "on-line" UV spectra. Buffer: 18 mM phosphate (pH 7) and 30 mM SDS with 2.5% (v/v) acetonitrile. Detection at 280 nm. Voltage 270 V cm^{-1}. For other MEKC conditions see Experimental. For peaks see Fig. 1.

phosphate, phosphoric acid and sodium hydroxide were reagent grade. Acetonitrile was HPLC grade solvent. Cosmetic products were purchased from different commercial sources.

Apparatus

Two capillary electrophoresis systems were employed: (1) A model 270A (Applied Biosystems, Inc., San Jose, CA, USA) apparatus equipped with a 50 cm (to detector) × 50 μm i.d. fused silica capillary and a Shimadzu C-R3A Chromatopac integrator; (2) A Biofocus 3000 (Bio-Rad Laboratories, Hercules, CA, USA) system equipped with a UV fast scanner, a Biofocus 50 cm × 50 μm i.d. capillary cartridge (uncoated) and a Dell 425s/L computer.

The analysis buffer was 18 mM phosphate (pH = 7) containing 30 mM SDS and 2.5% (v/v) of acetonitrile.

The voltage applied was in the range 250–300 V cm^{-1} and the temperature was 30°C. The injection was by aspiration (0.5 s) for the 270A apparatus and by pressure (10 s) for the Biofocus 3000 apparatus.

Sample preparation

About 1 g of cosmetic sample was exactly weighed into a centrifuge tube, then 2 M H_2SO_4 (0.25 ml) and methanol (10 ml) were added. The tube was stirred for about 5 min in an ultrasonic bath and then centrifuged at 1000 *g* for 10 min. The supernatant was diluted with 5 × 10^{-4} M H_2SO_4 and extracted with dichloromethane (2 × 20 ml). The organic extracts were dried over anhydrous Na_2SO_4 and filtered; the filtrate was evaporated to dryness under reduced pressure and the residue dissolved in methanol (20 ml). Aliquots of this methanolic solution were diluted with the buffer (1:2, v/v). Standards were dissolved in ethanol (4 mg ml^{-1}) and then diluted with the buffer (1:4, v/v).

Results and Discussion

To resolve the compounds I–VI (Fig. 1), it was necessary to carry out experiments under different conditions, involving two buffers (borate and phosphate) with different concentrations and pH values. The influence of the presence of a surfactant (sodium dodecyl sulphate, SDS) and organic modifiers (methanol and acetonitrile) was also examined. The best resolution has been achieved using 18 mM phosphate buffer (pH 7.0) and 30 mM SDS

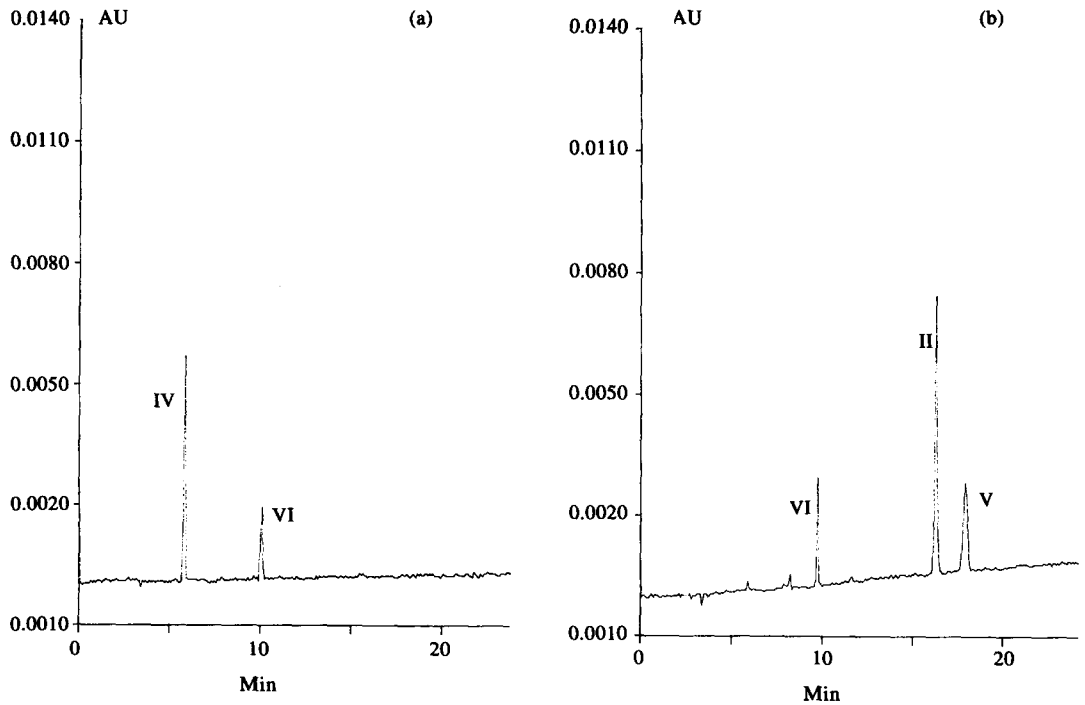


Figure 3
(A) Spiked sample in aqueous phase; (B) Spiked sample in organic phase. Voltage 240 V cm^{-1}. For MEKC conditions see Fig. 2; for peaks see Fig. 1.

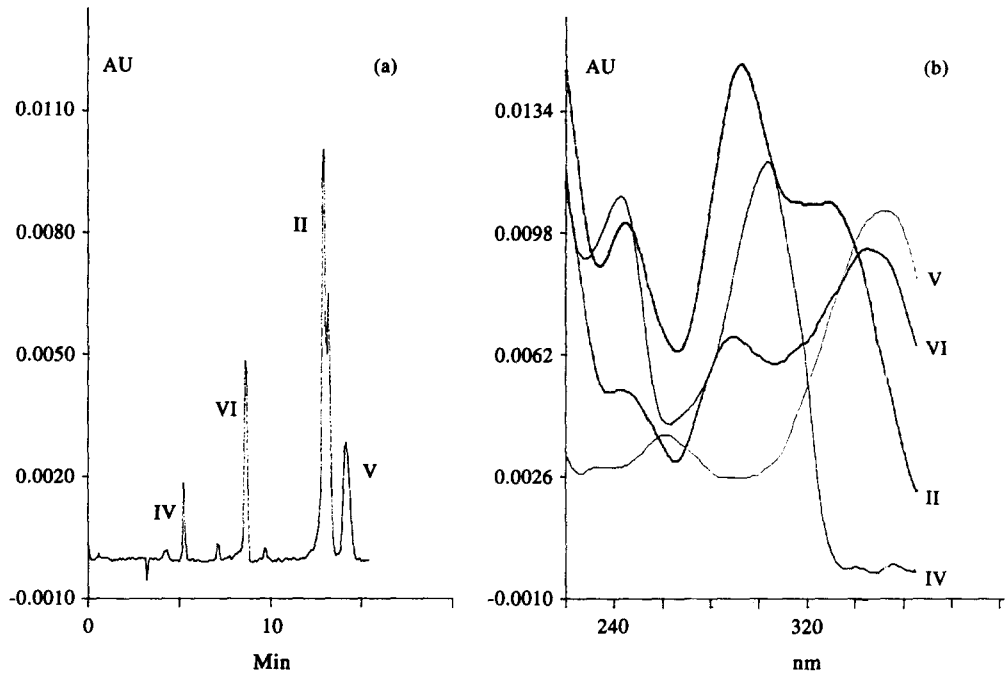


Figure 4
(A) Electropherogram of commercial cream (organic and aqueous phase 1:1, v/v); (B) "on-line" UV spectra. For MEKC conditions see Fig. 2. For peaks see Fig. 1.

with 2.5% (v/v) acetonitrile. As shown in Fig. 2 base-line separation was obtained in less than 16 min. This separation, which is very difficult by reversed-phase HPLC, represents positive evidence of the potential of MEKC in overcoming resolution problems.

In practice, commercial cosmetic products do not contain all these compounds simultaneously, and therefore analysis is normally less complicated. Nevertheless, the sample preparation still represents a difficult problem because of the different solubilities of the UV-filters. Thus, the hydrophilic substances IV and VI remain in water, whereas the others are easily extracted in organic solvents owing to their hydrophobic nature. Hence, organic and aqueous phases need to be analysed to determine both classes of sunscreens, unless the type is clearly specified. This approach has been verified by analysing cosmetic products spiked with known amounts of UV-A (V, VI) and UV-B (II, IV) filters. Figure 3 shows the separation of sunscreens present in the aqueous phase (A) and organic phase (B). Rectilinear responses between peak areas and concentrations (mg ml^{-1}) applied were obtained from five replicate analyses of each standard (II, IV, V, and VI) in the range 0.25–2 mg ml^{-1}, as indicated by the following equations

$y = 21.7x + 0.41$ $r = 0.997$ (II)
RSD = 1.44% ($n = 6$) at 1.0 mg ml^{-1}
$y = 11.2x + 1.12$ $r = 0.998$ (IV)
RSD = 1.84% ($n = 6$) at 1.0 mg ml^{-1}
$y = 7.4x + 0.52$ $r = 0.998$ (V)
RSD = 1.37% ($n = 6$) at 1.0 mg ml^{-1}

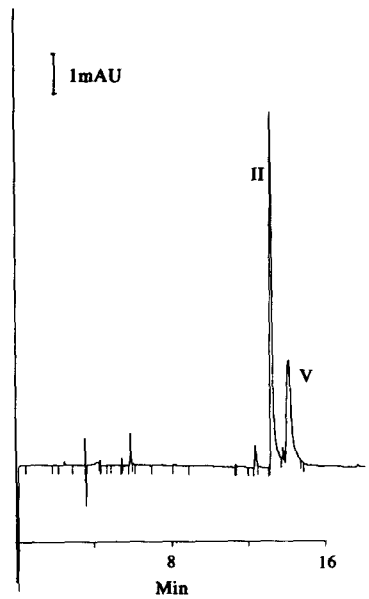


Figure 5
Electropherogram of commercial cream (organic phase) MEKC conditions as in Fig. 2. For peaks see Fig. 1.

Table 1
Content* of sunscreens II, IV, V and VI in two commercial creams

Cream	II	IV	V	VI
A	12.34 ± 0.21	0.62 ± 0.01	4.58 ± 0.08	2.25 ± 0.04
B	10.31 ± 0.18	—	2.13 ± 0.03	—

*Content expressed as mg g^{-1} ±SD (n = 6).

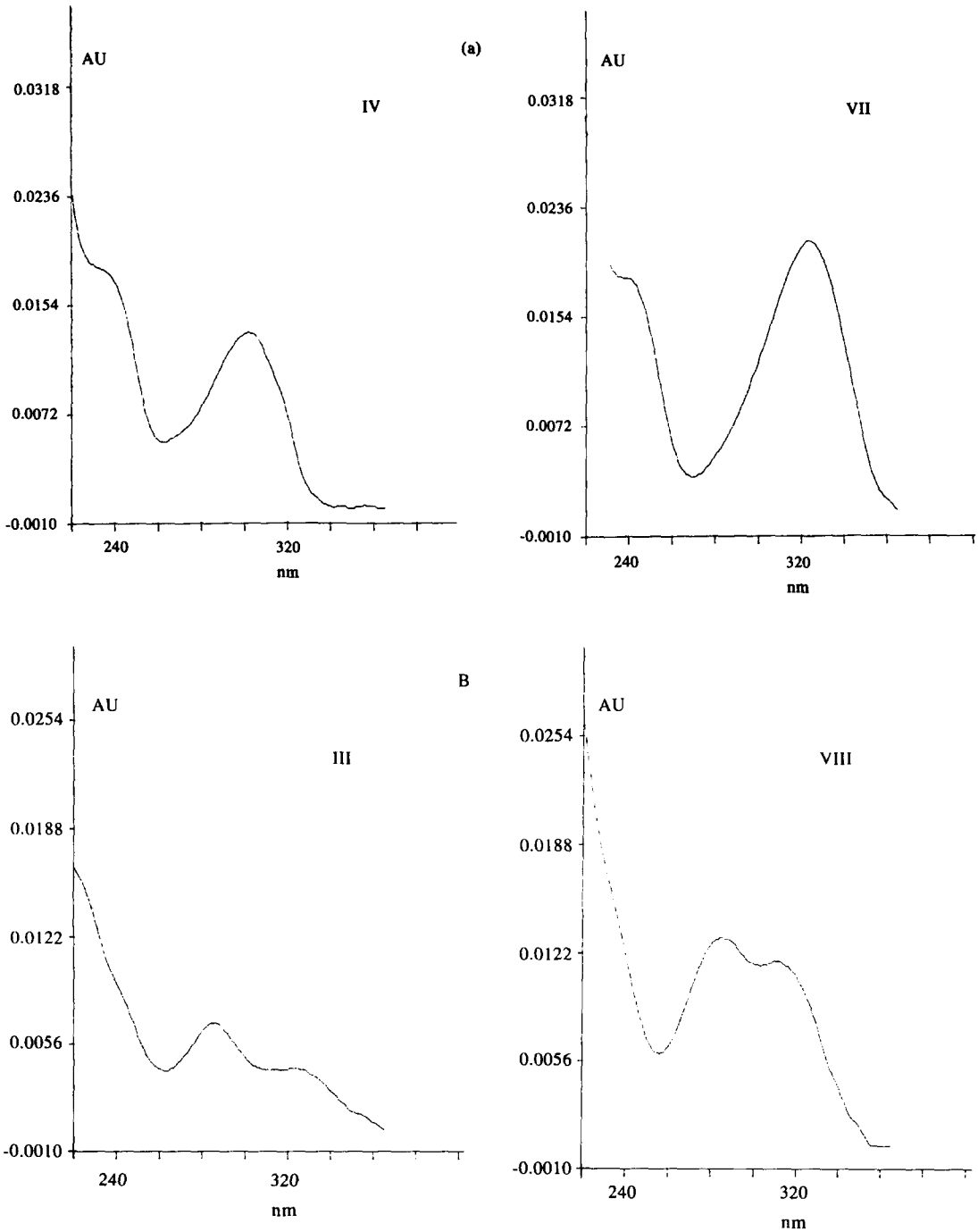


Figure 6
Comparison between equimolar solutions of: (A) 2-phenylbenzimidazole-5-sulphonic acid, IV and naringenin, VII; (B) 2-hydroxy-4-methoxy-benzophenone-5-sulphonic acid, III and cinnamic acid, VIII.

$y = 16.6x + 0.84$ $r = 0.999$ (VI)
RSD = 1.69% ($n = 6$) at 1.0 mg ml^{-1}

where y = peak area, x = mg ml^{-1}.

The recovery of the added sunscreens was 88–92% and the lower limit of detection was about 0.15 mg ml^{-1} at 280 nm. As already noted [7], the amount of the organic solvent (used to redissolve the extract) present in the injected sample influences the migration times, which may vary (2–4%). For this reason, the "on-line" UV spectra are important for definitive identification of the peaks.

Different commercial preparations were then analysed. An example is given in Fig. 4, which shows a typical electropherogram of combined aqueous and organic phases (1:1, v/v) obtained from a product containing the sunscreens II, IV, V, and VI. Figure 5 reports the electropherogram of the organic phase obtained from another commercial cream; the aqueous phase did not contain filters. Quantitation of II, IV, V and VI was achieved by external standardization ($n = 6$; RSD = 1.8%) and the results are reported in Table 1.

The spectra of the synthetic sunscreens (III and IV) were compared with two natural UV absorbing compounds, naringenin (VII) and cinnamic acid (VIII), with equimolar solutions. The results shown in Fig. 6 indicate that natural and synthetic compounds have analogous UV-absorbing properties and account for the use of some phytocosmetics as sunscreens.

It may be concluded that micellar electrokinetic chromatography allows the rapid analysis of common sunscreens. Moreover, the presence of an organic modifier in the background electrolyte permits a resolution not achievable by HPLC. Finally, the "on-line" UV spectra are of prime importance for definitive identification of the peaks.

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References

[1] J.J. Fuchs, M.E. Huflejt, L.M. Rothfuss, D.S. Wilson, J. Carcamo and L. Packer, *Photochem. Photobiol.* **50**, 739–744 (1989).
[2] G. Kindl and W. Raab, in *Licht und Haut*, p. 145. Govi-Verlag Pharmazeutischer Verlag Gmbh, Frankfurt (1993).
[3] L. Gagliardi, A. Amato, L. Turchetto and G. Cavazzutti, *Anal. Letts* **23**, 2123–2133 (1990).
[4] H. Nishi, T. Fukuyama, M. Matsno and S. Terabe, *J. Chromatogr.* **513**, 279–285 (1990).
[5] P.G. Pietta, P.L. Mauri, A. Rava and G. Sabbatini, *J. Chromatogr.* **549**, 367–373 (1991).
[6] L. Gagliardi, A. Amato, A. Basili and G. Cavazzutti, *J. Chromatogr.* **408**, 409 (1987).
[7] P.G. Pietta, P.L. Mauri, L. Zini and C. Gardana, *J. Chromatogr.* **680**, 175–179 (1994).

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