Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1031 (2004) 319–324
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Development and validation of a non-aqueous reversed-phase high-performance liquid chromatographic method for the determination of four chemical UV filters in suncare formulations

C.G. Smyrniotakis, Helen A. Archontaki*

Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece

Abstract

A non-aqueous reversed-phase high performance liquid chromatographic method (RP–HPLC) with UV detection at 313 nm was developed and validated for simultaneous determination of methylene bis-benzotriazolyl tetramethylphenol (Tinosorb M) along with three other chemical UV filters, octocrylene (Eusolex OCR), octyl methoxycinnamate (Eusolex 2292) and octyl salicylate (Eusolex OS) in suncare products. An isocratic elution was performed on a Hypersil BDS RP-C_{18} column (250mm × 4.6 mm), 5 μm particle size, using a mobile phase consisted of methanol–acetonitrile (90:10, v/v) with a flow-rate of 1.5 ml/min. The determination of the four UV filters was not interfered by the excipients in the products. The method of external standard, as well as the standard addition method was used for the determination. The external standard calibration curves were linear for Eusolex OCR, Eusolex 2292, Eusolex OS, and Tinosorb M in the concentration ranges of 0.5–100 μM, 0.5–100 μM, 0.5–200 μM, and 0.2–100 μM, respectively. Day-to-day relative standard deviation of the determination was within 3%. Limits of detection and quantitation of the above compounds were found equal to 36 and 110 nM, 220 and 660 nM, 170 and 520 nM, 44s and 130 nM, respectively. The recovery of these four chemical UV filters from the spiked samples was 96–103%.

Keywords: Sunscreens; Validation; Non-aqueous liquid chromatography; Methylene bis-benzotriazolyl tetramethylphenol; Octocrylene; Octyl methoxycinnamate; Octyl salicylate

1. Introduction

Progressive and continuous damage of the stratospheric ozone layer has been the cause of an increase in erythema, burning, dehydration, photodermatoses, photoaging and skin cancer in recent years.

Chemical sunscreens are compounds that absorb or reflect deleterious UV rays and prevent or minimize the harmful effects of the solar radiation on the skin. Among these, Tinosorb M (Fig. 1) is, according to the manufacturer, a highly efficient sun filter due to its triple action: UV absorption by a photostable organic molecule, light scattering and light reflection by its microfine structure. It has been proved to be extremely photostable. It shows synergistic effect with other organic UV filters and a stabilizing effect on them.

Several methods have been developed to determine UV filters in suncare products [1–8]. Organic filters have been determined by NMR [5], Raman [6] and UV absorption spectroscopy [7,8], gas chromatography [9], high performance thin layer chromatography [10], and especially high performance liquid chromatography [1–4]. However, no method has been reported in the literature for the determination of Tinosorb M in any kind of sample.

The purpose of the present work was to develop a simple, fast, sensitive, and reproducible reversed-phase HPLC method for the simultaneous determination of Tinosorb M, Eusolex OCR, Eusolex 2292, and Eusolex OS in suncare products.

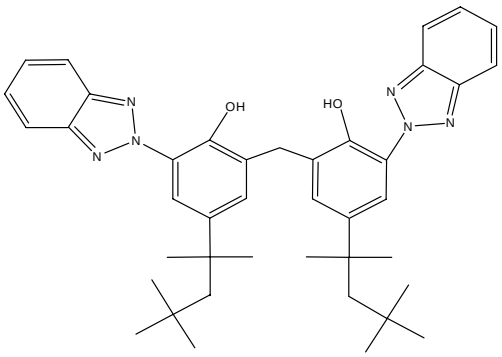
2. Experimental

2.1. Instrumentation

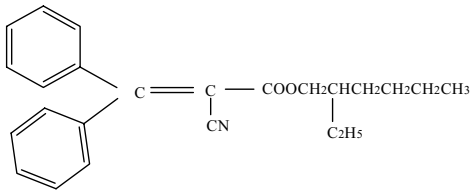
The chromatographic system used, consisted of a Waters 600E multisolvent delivery system and a Waters 486 UV

* Corresponding author. Tel.: +30-210-7274756; fax: +30-210-7274750.
E-mail address: archontaki@chem.uoa.gr (H.A. Archontaki).

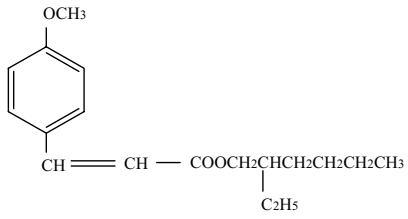
0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.08.078



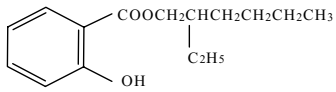
Tinosorb M



Eusolex OCR



Eusolex OM



Eusolex OS

Fig. 1. Chemical structures of Tinosorb M, Eusolex OCR, Eusolex OM, and Eusolex OS.

detector (Waters, Milford, MA, USA). The above system was controlled using Millennium 2010 software (Waters).

2.2. Chemicals and reagents

All chemicals were of analytical purity grade. Highly purified water with a Milli-Q RG water purification system (Millipore, Bedford, MA, USA) was used in all procedures. Acetonitrile (ACN) and methanol (MeOH) of HPLC grade were purchased from Merck (Darmstadt, Germany). The four chemical UV filters of technical purity grade, suncare products (creams and spray) and their excipients of analytical purity grade were kindly donated by the pharmaceutical company Lavipharm (Peania, Attica, Greece).

2.3. Chromatographic conditions

A reversed-phase Hypersil (England) BDS RP-C_{18} column (250mm × 4.6 mm), 5 μm particle size, was used. The mobile phase, methanol–acetonitrile (90:10, v/v), was degassed with Helium at a rate of 20 ml/min. The flow-rate of the mobile phase was 1.5 ml/min. Injection volume was 20 μl. Experiments were performed at ambient temperature. Absorption was measured at 313 nm. The total elution time was less than 15 min.

2.4. Solution preparation

2.4.1. Standard solutions

Stock standard solutions of the four UV filters were prepared daily. These solutions of Eusolex OCR, Eusolex 2292, and Eusolex OS were prepared in mobile phase and their concentration was about 1.00 mM. Stock solutions of Tinosorb M with concentration of 0.10 mM, were prepared dissolving 0.001–0.002 g in 25 ml of dimethyl formamide, shaken in an ultrasonic bath for 30 min and then diluted to 100 ml with mobile phase.

In order to construct the corresponding calibration curves and evaluate the precision of the proposed method, working standard solutions of Tinosorb M, Eusolex OCR, Eusolex 2292, and Eusolex OS were prepared in the concentration range of 10–100 μM. In order to establish the linearity range and calculate the limits of detection and quantitation, working standard solutions of the above compounds were prepared in the concentration range of 0.2–100 μM for Tinosorb M, 0.5–100 μM for Eusolex OCR, 0.5–100 μM for Eusolex 2292, and 0.5–200 μM for Eusolex OS.

2.4.2. Sample preparation

Determination of Tinosorb M, Eusolex OCR, Eusolex 2292 and Eusolex OS in suncare products was performed using calibration curves. Stock solutions of these products were prepared following the same procedure as that for the preparation of stock standard solutions of Tinosorb M. A volume of 1–2 ml of stock solutions of these samples was diluted to 10 ml with mobile phase so that the final expected concentration of the chemical UV filters in the injected solutions was approximately 30–100 μM. Recovery studies of Tinosorb M, Eusolex OCR, Eusolex 2292, and Eusolex OS were performed in four different kinds of suncare products (creams and spray) using the method of standard addition. A series of four solutions was prepared. The first solution was prepared as described in this paragraph. The other three solutions contained, increasing amounts of standard solutions of them, 20–60 μM, 12–54 μM, 11–43 μM, and 22–98 μM, respectively. The prepared solutions were then injected to the HPLC system.

2.5. Data analysis

Calibration curves of Tinosorb M, Eusolex OCR, Eusolex 2292, and Eusolex OS were constructed for their determina-

tion in suncare products. Regression equations were obtained through unweighed least squares linear regression analysis, using peak areas as a function of their concentration.

3. Results and discussion

3.1. Mobile phase

Several mixtures of solvents were tried in order to achieve the elution of Tinosorb M. This was accomplished only when water was not present in the eluent mixtures. This fact led to the use of a non-aqueous reversed-phase HPLC method. Further optimization of the mobile phase showed that increasing the ratio MeOH–ACN, elution time of Tinosorb M decreased, while no significant change was observed in the elution time of the other three compounds. Finally, the most acceptable optimized mobile phase was MeOH–ACN (90:10, v/v). With this mobile phase the capacity factor k', the selectivity a and the peak asymmetry factor T (10%) for Eusolex OCR were 1.4, 1.3, 1.3; for Eusolex 2292 were 1.8, 1.3, 1.4; for Eusolex OS were 2.1, 1.1, 1.4; and for Tinosorb M were 14, 6.6, 1.2, respectively.

3.2. Selectivity

A typical chromatogram of a cream sample is shown in Fig. 2. Each sample, in addition to the UV filters, contained several excipients. Each excipient was dissolved in dimethylformamide, diluted with mobile phase, filtered and then injected in the chromatographic system. It was proved that none of the excipients interfered with the determination of the four UV filters under the experimental conditions used. Retention times observed for Eusolex OCR, Eusolex 2292, Eusolex OS and Tinosorb M were 2.4, 2.8, 3.0, and 14.8 min, respectively. Good resolution between the examined chromatographic peaks was assured by the values of R_s (1.9–31.2).

3.3. Calibration curves of UV filters

Under the experimental conditions described in Sections 2.3 and 2.4.1 linear calibration curves were constructed every day in the concentration range of 10–100 μM for the UV filters. Regression analysis revealed that the calibration curves of Tinosorb M, Eusolex OCR, Eusolex 2292, and Eusolex OS were linear in the investigated concentration range. The linearity was also assured by a log area/log C diagram where slope was 1, throughout the whole concentration range. Calibration curves were also constructed using the method of standard additions. Their slopes were statistically identical with the above ones, which was an additional proof that there was no interference from the excipients of the samples. The analytical parameters of representative calibration curves are summarised in Table 1.

3.4. Precision and accuracy

To verify the precision of the proposed HPLC method, within-day and between-days precision in measurements, of the four chemical UV filters in both standard solutions and suncare products was obtained. Within-day and between-days relative standard deviations (R.S.D.s) were found less than 3% in all the above cases.

The accuracy of the developed method was examined by recovery studies conducted as described in Section 2.4.2. The mean recovery of the above UV filters from the spiked samples was calculated and results are shown in Table 2.

Table 1
Analytical parameters of calibration curves of the four chemical UV filters

Calibration method	UV filter	Concentration range (μM)	Regression equation[a]		
			Intercept ($a \pm$ S.D.)[b]	Slope ($b \pm$ S.D.)[b] $\times 10^{-10}$	$r(n)$[c]
External standard calibration	Eusolex OCR	10–100	−10607 ± 7666	1.01 ± 0.01	0.9997(5)
	Eusolex 2292	10–100	−3887 ± 11096	2.01 ± 0.02	0.9998(5)
	Eusolex OS	10–100	−20670 ± 9127	0.326 ± 0.008	0.9994(5)
	Tinosorb M	10–100	−2230 ± 4058	1.20 ± 0.008	0.9999(5)
Method of Standard addition[d]	Eusolex OCR	12–54	420711 ± 1082	0.994 ± 0.006	0.9999(4)
	Eusolex 2292	11–43	777181 ± 1754	2.000 ± 0.008	0.9999(4)
	Eusolex OS	22–98	204682 ± 2468	0.324 ± 0.004	0.9999(4)
	Tinosorb M	20–60	359739 ± 6138	1.21 ± 0.02	0.9999(4)

The chromatographic conditions: BDS-C_{18} column, mobile phase MeOH–ACN (90:10, v/v), flow-rate 1.5 ml/min, detection wavelength 313 nm and room temperature.

[a] Linear unweighed regression analysis, with a regression equation $y = a + bx$, where x is concentration in moles.

[b] S.D. is the standard deviation of intercept and slope.

[c] r is the correlation coefficient and n is the number of points in each calibration curve; each point is the mean of three experimental measurements.

[d] In the standard addition method the nominal concentrations of the UV filters in the injected sample solutions were: Eusolex OCR 30 μM, Eusolex 2292 50 μM, Eusolex OS 50 μM, and Tinosorb M 40 μM.

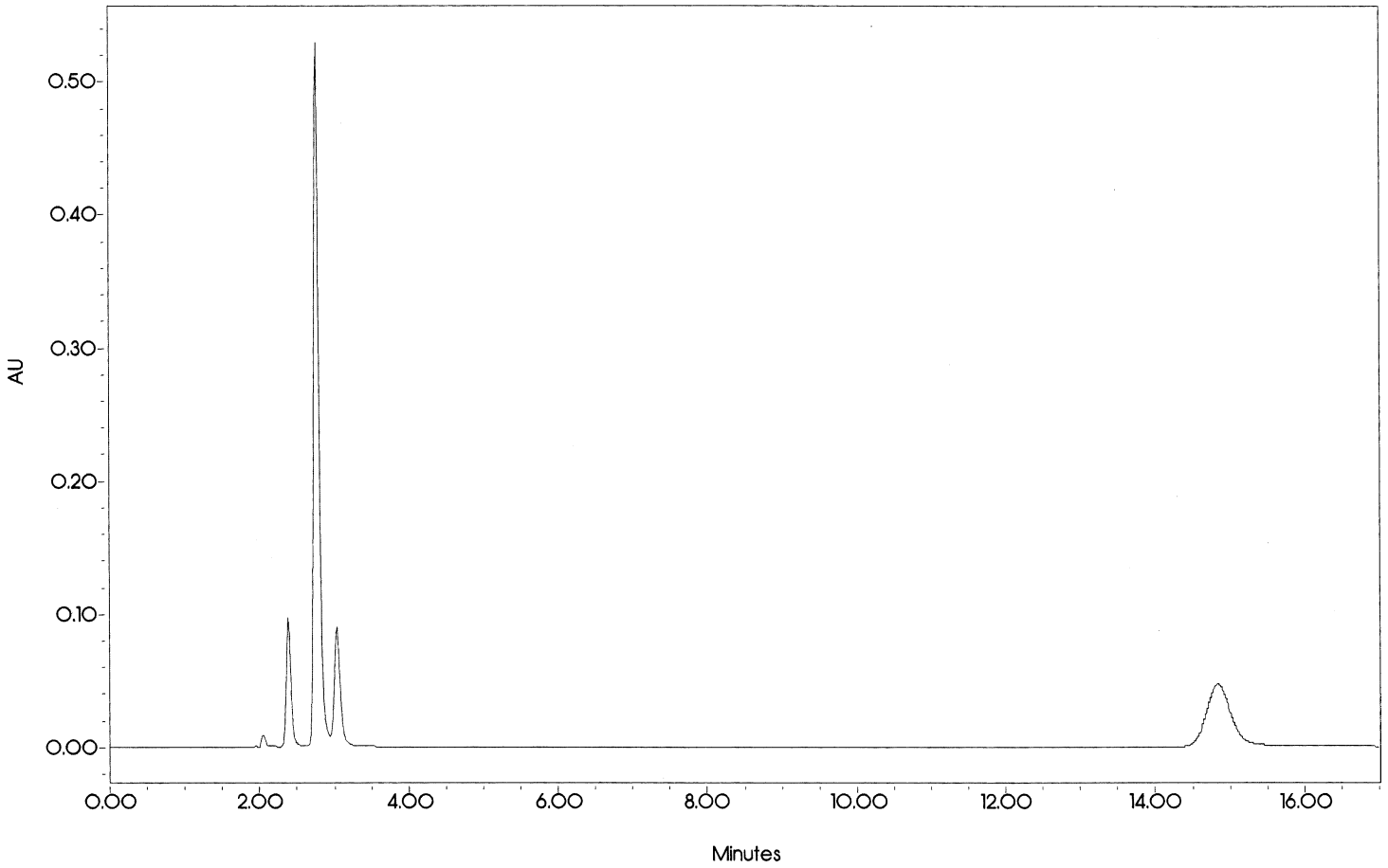


Fig. 2. A typical chromatogram of a sample (cream 2), in which the nominal concentrations of the UV filters were: Eusolex OCR 30 μM, Eusolex 2292 50 μM, Eusolex OS 50 μM, and Tinosorb M 40 μM. The chromatographic conditions used were: BDS-C_{18} column, mobile phase MeOH–ACN (90:10, v/v), flow-rate 1.5 ml/min, detection wavelength 313 nm and room temperature. The peaks correspond to Eusolex OCR, Eusolex 2292, Eusolex OS, and Tinosorb M in the order that they are eluted.

Table 2
Recovery studies for the determination of the four UV filters

UV filters	Spiked concentration (μM)[a]	Mean recovery ± S.D.(%)[b]
Eusolex OCR	12	103.1 ± 6.2
	24	101.2 ± 3.0
	54	99.0 ± 2.3
Eusolex 2292	11	102.1 ± 2.2
	25	98.8 ± 1.7
	43	99.8 ± 1.2
Eusolex OS	22	96.2 ± 2.1
	60	98.5 ± 2.4
	98	99.2 ± 1.5
Tinosorb M	20	100.5 ± 1.3
	40	100.0 ± 1.4
	60	100.8 ± 1.5

The chromatographic conditions: BDS-C_{18} column, mobile phase MeOH–ACN (90:10, v/v), flow-rate 1.5 ml/min, detection wavelength 313 nm and room temperature.

[a] The nominal concentrations of the UV filters in the injected sample solutions were: Eusolex OCR 30 μM, Eusolex 2292 50 μM, Eusolex OS 50 μM, and Tinosorb M 40 μM.

[b] S.D. is the standard deviation of the mean recovery.

3.5. Limits of detection (LOD) and quantitation (LOQ)

The LOD was defined as the analyte concentration that gives a signal equal to $y_b + 3.3s_b$, where y_b is the signal of the blank and s_b is its standard deviation. Similarly, the LOQ was defined as $y_b + 10s_b$. In the unweighed least-squares method is quite suitable in practice to use $s_{y/x}$ [11] instead of s_b and the value of the calculated intercept a instead of y_b. Thus

$$\text{LOD} = \frac{3.3s_{y/x}}{b} \quad \text{and} \quad \text{LOQ} = \frac{10s_{y/x}}{b}$$

where b is the slope of the regression line. Based on the above equations, the calculated LOD values for Eusolex OCR, Eusolex 2292, Eusolex OS, and Tinosorb M were 36, 220, 170, and 44 nM, respectively. The calculated LOQ values were 110, 660, 520, and 130 nM, respectively.

3.6. Determination of UV filters in suncare products

Preparing the samples according to the instructions shown in Section 2.4.2, UV filters were determined in the suncare products provided.

Since there was no official or other method described in the literature for the determination of all four chemical filters in similar samples, the method of standard additions was applied to all suncare products. As already mentioned, the slopes of the latter calibration curves were statistically identical to those obtained with the method of the external standard. Using both methods, the percentage of the chemical UV filters in the analyzed samples was found the same, within the experimental error. The results are tabulated in Table 3.

Table 3
Concentrations (%, w/w) of the four chemical UV filters in the suncare products analyzed

Suncare products	UV filter	Nominal concentration (%, w/w)	Concentration found ± SD[a] (%, w/w)		
			External standard calibration		Method of standard addition, Between-days (3)[b]
			Within-day (3)[b]	Between-days (4)[b]	
Cream 1	Eusolex OCR	10.00	9.98 ± 0.04	10.12 ± 0.06	10.11 ± 0.08
	Eusolex 2292	7.50	7.43 ± 0.07	7.44 ± 0.04	7.35 ± 0.05
	Eusolex OS	5.00	5.38 ± 0.03	5.29 ± 0.09	5.19 ± 0.04
	Tinosorb M	15.00	14.52 ± 0.15	14.10 ± 0.11	14.04 ± 0.07
Cream 2	Eusolex OCR	2.00	2.15 ± 0.02	2.11 ± 0.02	2.12 ± 0.04
	Eusolex 2292	5.00	4.98 ± 0.04	4.94 ± 0.05	4.91 ± 0.06
	Eusolex OS	5.00	5.19 ± 0.05	5.28 ± 0.07	4.91 ± 0.05
	Tinosorb M	8.00	7.54 ± 0.18	7.62 ± 0.07	7.30 ± 0.11
Cream 3	Eusolex OCR	2.00	2.14 ± 0.02	2.14 ± 0.04	2.16 ± 0.03
	Eusolex 2292	5.00	4.93 ± 0.07	4.95 ± 0.04	5.00 ± 0.02
	Eusolex OS	5.00	5.18 ± 0.07	5.22 ± 0.12	5.27 ± 0.07
	Tinosorb M	8.00	7.60 ± 0.09	7.59 ± 0.20	7.16 ± 0.09
Spray	Eusolex OCR	–	–	–	–
	Eusolex 2292	6.00	6.00 ± 0.07	5.92 ± 0.04	6.05 ± 0.06
	Eusolex OS	3.00	3.30 ± 0.04	3.27 ± 0.04	3.02 ± 0.05
	Tinosorb M	5.50	5.27 ± 0.06	5.03 ± 0.09	5.08 ± 0.08

The chromatographic conditions used were: BDS-C_{18} column, mobile phase MeOH–ACN (90:10, v/v), flow-rate 1.5 ml/min, detection wavelength 313 nm and room temperature.

[a] SD is the standard deviation of the mean (%, w/w) concentration found.

[b] The number in parenthesis shows the number of different samples of the same suncare product that were used for the determination of the four chemical UV filters.

3.7. Robustness and ruggedness

Robustness of the proposed method was assessed with respect to small deliberate alterations in several experimental parameters. Determining the four chemical UV filters in cream 2, change of the content of the mobile phase from MeOH–ACN (90:10, v/v) to (93:7, v/v) and (87:13, v/v) did not change the results for Eusolex OCR more than 1.4%, for Eusolex 2292 more than 1.2%, for Eusolex OS more than 1.4%, and for Tinosorb M more than 3.2%. These changes were comparable with the R.S.D. of the method. During these changes the parameters t_R, a and R_s remained statistically the same. Similar observations were made, changing the amount of dimethylformamide, during the preparation of the samples, from 25 to 22 and 28 ml and the shaking time in the ultrasonic bath from 30 to 28 and 32 min. However, changing the flow-rate from 1.5 to 1.4 and 1.6 ml/min, the parameters t_R, a and R_s of the chromatographic peaks remained statistically the same while changes up to 7% were observed during the determination of Eusolex OCR, Eusolex 2292 and Eusolex OS which jumped to 15% in the determination of Tinosorb M. These changes may be due to the specific mechanism of the non-aqueous RP–HPLC, approach that has not been yet fully explored [12,13].

Ruggedness of the developed method was indicated by the between days precision because it included changes in reagents, chemicals, and solvents. Moreover, using different columns of the same company (Hypersil BDS RP-C_{18}), the parameters t_R, a and R_s of the chromatographic peaks remained statistically the same. At the same time, in the determination of Eusolex OCR, Eusolex 2292, and Eusolex OS changes of the results up to 3% were noticed, which increased up to 10% in the determination of Tinosorb M.

4. Conclusions

In spite of the diverse chemical behavior, which becomes obvious looking at the different chemical structures (Fig. 1) of the examined four UV filters, a simple, fast and reliable non-aqueous RP–HPLC method was developed, optimized and validated for their determination in four suncare products. The big advantage of the proposed method is that Tinosorb M can be determined along with Eusolex OCR, Eusolex 2292, and Eusolex OS in a single analysis. The other alternative would have been to analyze the same sample twice, with two different methods, one for the determination of Eusolex OCR, Eusolex 2292, and Eusolex OS and the other for the determination of the extremely hydrophobic compound Tinosorb M.

Acknowledgements

The authors are very grateful for the financial support given by the Special Research Account of Athens University, Athens, Greece. They also thank sincerely the pharmaceutical company Lavipharm that assigned this project to them and kindly donated the chemical UV filters of technical purity grade, excipients of analytical purity grade and suncare product formulations. They also deeply thank the PhD candidate M. Vertzoni for her useful suggestions.

References

[1] A. Chisvert, M.C. Pascual-Marti, A. Salvador, Fresenius J. Anal. Chem. 369 (2001) 638–641.
[2] S. Santo, J. Chromatogr. A 870 (2000) 199–205.
[3] A. Chisvert, M.C. Pascual-Marti, A. Salvador, J. Chromatogr. A 921 (2001) 207–215.
[4] X. Yao, X. Zheng, X. Qin, Q. Qi, Se Pu 16 (1998) 223–225.
[5] K. Mori, K. Itoh, S. Susuki, H. Nakamura, Jpn. J. Toxicol. Environ. Health 42 (1996) 60–66.
[6] J. Cheng, Y.S. Li, R.L. Roberts, G. Walker, Talanta 44 (1997) 1807–1813.
[7] A. Chisvert, A. Salvador, M.C. Pascual-Marti, Anal. Chim. Acta 428 (2000) 183–190.
[8] A. Chisvert, A. Salvador, M.C. Pascual-Marti, J.G. March, Fresenius J. Anal. Chem. 369 (2001).
[9] T. Felix, B.J. Hall, J.S. Broodbelt, Anal. Chim. Acta 371 (1998) 195–203.
[10] B. Musial, J. Sherma, J. Planar Chromatogr.-Mod. TLC 10 (1997) 368–371.
[11] J.C. Miller, J.N Miller, Statistics for Analytical Chemistry, Wiley, New York, 1984 (Chapter 4).
[12] Csaba Horvath, High Performance Liquid Chromatography–Advances and Perspectives, vol. 2, Academic Press, New York, 1980 (Chapter 1).
[13] Lloyd R. Snyder, Joseph J. Kirkland, Joseph L. Glajch, Practical HPLC Method Development, second ed., Wiley, New York, 1992 (Chapter 6).