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Validation of HPLC method for quantitative determination of Tinosorb®S and three other sunscreens in a high protection cosmetic product

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Synopsis

A chromatographic method (high performance liquid chromatography) with a diode array detector was developed for simultaneous assay of Tinosorb®S (bis-ethylhexyloxyphenol methoxyphenyl triazine) with three other sunscreen agents [benzophenone-3, butyl methoxydibenzoylmethane (avobenzone) and ethylhexyl methoxycinnamate] in high protection sunscreen. Separations were performed on a RP-18 Nucleodur®Gravity®column (150 × 4.6 mm, 5 μm) eluted with a ternary gradient mixture constituted of tetrahydrofuran, acetonitrile and an aqueous solution of acetic acid. The quantitative analysis was achieved with internal calibration performed with octyl dimethyl para-aminobenzoate (PABA) at 330 nm. In accordance with the analytical references (SFSTP, ICH, ISO…), the accuracy of the method was evaluated using a statistical approach of the validation parameters (specificity, response function, linearity, precision and trueness). For each studied ultraviolet filter, an accuracy profile was determined on a predicted range. These profiles show a graphical representation of the recovery percentage and confidence limits centred on 100%. The method is validated and can be used for analysis in cosmetic sunscreen products.

Résumé

Une méthode chromatographique (CLHP) avec un détecteur à barrette de diodes a été mise au point pour le dosage simultané du Tinosorb®S (bis-éthylhexyloxyphénol méthoxyphényl triazine) en présence de trois autres filtres solaires [benzophénone-3, butyl méthoxydibenzoylméthane (avobenzone) et éthylhexyl méthoxycinnamate)] dans une émulsion solaire haute protection. La séparation est réalisée en gradient sur une colonne octadécylsilice Nucleodur®Gravity®(150 × 4.6 mm, 5 μm) avec un mélange éluant ternaire constitué de tétrahydrofurane, d'acétonitrile et d'une solution aqueuse d'acide acétique. L'analyse quantitative est obtenue grâce à un étalonnage interne réalisé avec l'octyl diméthyl PABA à 330 nm. Conformément aux référentiels analytiques en vigueur (SFSTP, ICH, ISO…) l'exactitude de la méthode est évaluée grâce à une approche statistique des critères de validation (spécificité, fonction de réponse, linéarité, fidélité et justesse). Pour chaque filtre UV étudié, un profil d'exactitude est établi sur un intervalle de dosage défini. Ces profils permettent de visualiser des pourcentages de recouvrement et des limites de confiance unilatérales centrés sur 100%. La méthode validée est applicable à l'analyse de lots industriels d'une émulsion solaire haute protection.

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Introduction

Radiations emitted by the sun and more particularly by the ultraviolet (UV) rays provoke harmful effects on the skin. The use of high sunscreen products can help to prevent or minimize these harmful effects on human health [1]. The use of more protective products [2] against radiations UV B (290–320 nm) and UV A (320–400 nm) has led the cosmetic industry to develop formulations containing many ultraviolet (UV) organic sunscreens [benzophenones, methoxycinnamates, methoxydibenzoyl methanes…] in association with chemical structures that more complex (derivatives of benzotriazole, camphor and triazines). The recent use of derivatives from triazines [Tinosorb®M and Tinosorb®S (Ciba, Basel, Switzerland)) allow the formulation of solar emulsions with spectrum filters of broader action (290–400 nm), ensuring a better cutaneous protection [3]. The studied cosmetic matrix is a solar emulsion of high protection (IP 50 UVB/27; UVA+++) which contains four UV filters: benzophenone-3 (BZ-3, 4.9%), avobenzone (BDM, 5.0%), ethylhexyl methoxycinnamate (EMC, 8.4%) and Tinosorb®S (EMT, 5.0%).

The maximum authorized concentrations of the substances present in the studied cosmetic product are regulated by the authorities (Table I) and it is essential to have a reliable analysis method to guarantee quality, integrity and the effectiveness of the product. The analytical aim is to obtain a precise and true method with a simultaneous separation of these very different sunscreens (polarity, solubility, steric configuration…).

The proposed quantitative method is carried out according to an internal calibration with octyl dimethyl para-aminobenzoate (PABA) (ODP) on a RP-18 Nucleodur®Gravity®column (150 × 4.6 mm, 5 μm) (Machery-Nagel, Düren, Germany) eluted with a ternary gradient mixture constituting of tetrahydrofuran, acetonitrile and an aqueous solution of acetic acid (0.1%).

For each studied solar sunscreen, the reliability of the quantitative method is evaluated by the determination of criteria of validation (specificity, response function, linearity, precision and trueness). Finally, achievement of accuracy profile allows visualizing the total efficiency of the method.

Materials and methods

Sunscreens

Benzophenone-3, avobenzone (BDM), EMC and ODP were purchased from Merck (Darmstadt, Germany). Tinosorb®S (EMT, bis-ethylhexyloxyphenol methoxyphenyl triazine) was purchased from Ciba. The chemical structures of these substances are showed in Fig. 1. The cosmetic sunscreen products were provided by ASEPTA Laboratories (Monaco).

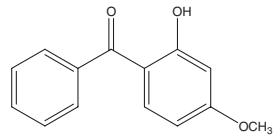
Standard solutions without matrix

Main solutions of BZ-3, avobenzone, EMC and ODP (internal standard) were prepared in absolute ethanol. Tinosorb®S was prepared in acetone. A solution containing all the sunscreens with internal standard was prepared in 50 mL of absolute ethanol. The concentrations of the analysed solutions are given in Table II.

Table I Maximum authorized concentrations of studied sunscreens

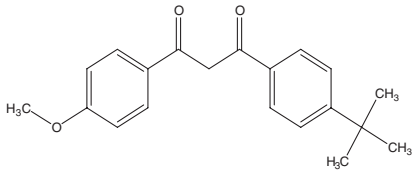
CAS	INCI name[a]	Usual name	C_{max}[b] (%)	Abbreviation
131-57-7	Benzophenone-3	Benzophenone-3	10	BZ3
70356-09-1	Butyl methoxydibenzoylmethane	Avobenzone	5	BDM
5466-77-3	Ethylhexyl methoxycinnamate	Ethylhexyl methoxycinnamate	10	EMC
187393-00-6	Bis-ethylhexyloxyphenol methoxyphenyl triazine	Tinosorb®S	10	EMT

[a]International Nomenclature for cosmetic ingredient.
[b]Maximum authorized concentration in cosmetic product according to the French order of 6 February, 2001.



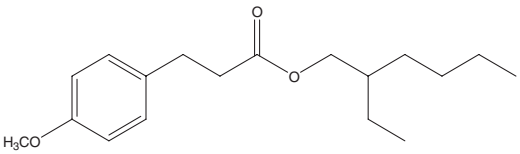
Benzophenone-3 (BZ3)

$C_{14}H_{12}O_3$ / 228.2 g mol^{-1}



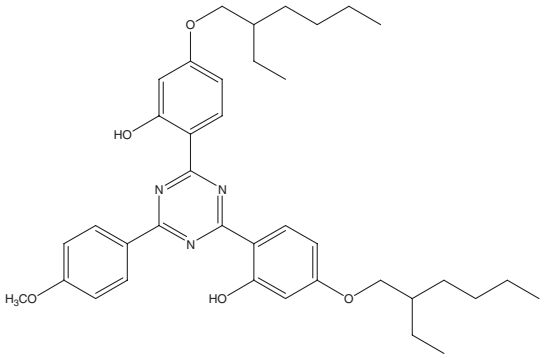
Avobenzone (BDM)

$C_{20}H_{22}O_3$ / 310.4 g mol^{-1}



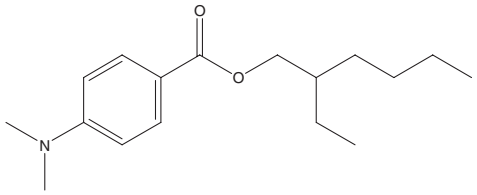
Ethylhexyl methoxycinnamate (EMC)

$C_{18}H_{26}O_3$ / 290.4 g mol^{-1}



Tinosorb® S (EMT)

$C_{38}H_{49}N_3O_5$ / 627.8 g mol^{-1}



Octyl dimethyl para-aminobenzoate (ODP)

$C_{17}H_{27}NO_2$ / 277.4 g mol^{-1}

Figure 1 Structures of studied sunscreens.

Simulated matrix standard solutions

The simulated matrixes of cosmetic sunscreen products for the validation were prepared at the laboratory (ASEPTA Laboratories, Monaco). The reconstitution of the simulated matrixes is carried out to 80%, 100% and 120% of the nominal concentration of each sunscreen present in the cosmetic product. The solutions for analysis were prepared by addition of 150 mg of simulated matrix and a constant volume of internal standard in 50 mL of absolute ethanol. The concentrations of the analysed solutions are given in Table III.

Blank matrix

The blank matrix of cosmetic sunscreen product without any sunscreen was prepared at the laboratory (ASEPTA Laboratories) according to the same protocol as the one used for the manufacture of cosmetic sunscreen product.

Cosmetic sunscreen products

The studied cosmetic sunscreen emulsion contains 4.9% (w/w) of BZ-3, 5.0% (w/w) of avobenzone, 8.4% (w/w) of EMC and 5.0% (w/w) of Tinosorb®S. Solutions of analysis were prepared by addition of

Table II Field of concentration of standard solutions without matrix

Concentration [μg mL^{-1}]	BZ3	BDM	EMC	EMT	ODP
Low level (80%)	118.7	120.2	202.2	120.9	122.0
Middle level (100%)	149.7	150.5	253.5	150.6	122.0
High level (120%)	179.2	181.2	303.1	181.0	122.0

BZ3, benzophenone-3; BDM, butyl methoxydibenzoylmethane; EMC, ethylhexyl methoxycinnamate; EMT, bis-ethylhexyloxyphenol methoxyphenyl triazine; ODP, octyl dimethyl; PABA, para-aminobenzoate.

Table III Field of concentration of simulated matrix solutions

Concentration [μg mL^{-1}]	BZ3	BDM	EMC	EMT	ODP
Low level (80%)	119.3	121.2	203.4	120.3	120.2
Middle level (100%)	148.8	150.1	254.6	150.2	–
High level (120%)	178.2	180.0	305.0	181.1	–

BZ3, benzophenone-3; BDM, butyl methoxydibenzoylmethane; EMC, ethylhexyl methoxycinnamate; EMT, bis-ethylhexyloxyphenol methoxyphenyl triazine; ODP, octyl dimethyl; PABA, para-aminobenzoate.

150 mg of cosmetic sunscreen product and a constant volume of internal standard in 50 mL of absolute ethanol.

Solvents

All solvents were of analytical grade or of HPLC grade. Acetonitrile, tetrahydrofuran and water were purchased from Sigma (Steinheim, Germany); absolute ethanol, acetic acid and acetone were purchased from VWR BDH Prolabo (Fontenay-sous-bois, France).

Equipment

A high performance liquid chromatographic (HPLC) system consisted of an Alliance 2695 Waters (Milford, MA, U.S.A) featuring an automatic injector, a column oven and a diode array detector (DAD) 2996. The analysis was performed at 25°C on a RP-18 Nucleodur®Gravity®column Macherey Nagel®(150 × 4.6 mm, 5 μm) provided with a guard column RP-18 Nucleodur® Gravity®5 μm Macherey Nagel®. The acquisition of the chromatograms is carried out in 3D between 210 and 400 nm, extractions were performed at 330 nm. The injection volume was 10 μL. All solutions of injection were filtered through filter membrane in PTFE, pore size 0.45 μm (Chromafil, Macherey Nagel®). The analysis is carried out with a constant flow rate of 1 mL min^{-1}, the gradient of elution used is given in Table IV.

Table IV Gradient profile to carry out the sunscreens analysis

Time (min)	Flow rate (mL min^{-1})	CH_3CN (%)	THF (%)	H_2O/CH_3COOH (%)	Curve[a]
0	1	3	56	41	6
13.0	1	3	56	41	6
33.0	1	20	0	80	6
33.1	1	3	56	41	6

[a]The curve indicates that the change of gradient is linear.

Results and discussion

Chromatographic analysis

The aim of this research was to determine the optimal experimental conditions allowing the chromatographic separation of Tinosorb®S and three other sunscreens (BZ-3, avobenzone and EMC) to realize a quantitative analysis in a complex cosmetic matrix. HPLC is the most used chromatographic technique for simultaneous qualitative and quantitative determination of several sunscreen agents in cosmetic products [4, 5]. A bibliographical study shows that separations are generally carried out on reversed phase columns using silica gels C18 [6–8] connected with an UV detector or a DAD. The other supports (octyl, phenyl, cyanopropyl…) are less used.

The mobile phase is generally made up of binary mixtures (methanol-water, acetonitrile-water) [9, 10] or ternary (methanol–acetonitrile–water), but avobenzone and EMC are partially co-eluted. The addition of tetrahydrofuran as organic modifier and acetic acid as additive in the aqueous phase leads to an improvement of the separation of sunscreens. Quaternary mixtures (methanol–acetonitrile–tetrahydrofuran–water) were also used

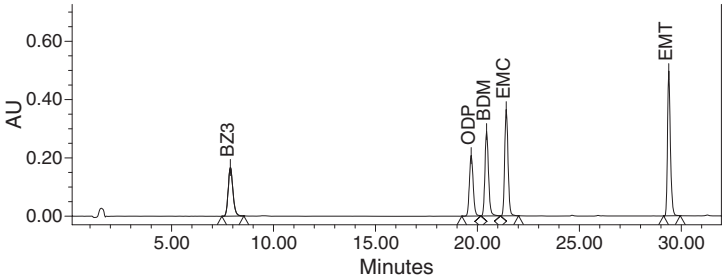


Figure 2 High performance liquid chromatogram showing separation of sunscreens in emulsion with octyl dimethyl para-aminobenzoate as an internal standard. RP-18 Nucleodur® Gravity® column (150 × 4.6 mm, 5 μm), gradient with THF–CH_3CN–water with 0.1% (v/v) of CH_3COOH. Column temperature 25°C, injection volume 10 μL, extraction at 330 nm.

on cyanopropyl [11] and phenyl [12] reversed phase.

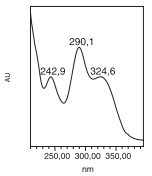
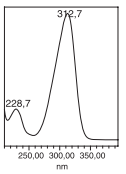
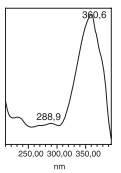
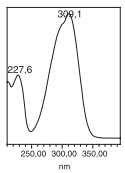
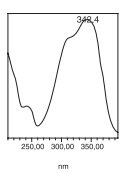
In this study, the whole resolution of Tinosorb®S (EMT), BZ-3, BDM, EMC and ODP was obtained with a RP-18 Nucleodur®Gravity®column using a ternary gradient, acetonitrile–tetrahydrofuran–water, with 0.1% of acetic acid (v/v). The gradient of elution was specifically optimized for the separation of four sunscreens and ODP (Table IV). The chromatogram of cosmetic sunscreen product in solution with the internal standard is given in Fig. 2. The extraction of the chromatogram at 330 nm shows that the obtained peaks are symmetrical and well resolved. The identifications of each peak were confirmed with commercial standards by comparisons of retention times and by extraction of their UV spectrum. A study of repeatability was carried out on 10 injections of a standard solution without matrix. The relative standard deviations obtained are very weak and lower than 0.2% (Table V).

Validation study

The validation was achieved according to the harmonization of approaches about validation of quantitative analytical procedure, established by the French company of Sciences and Pharmaceutical Technology – SFSTP [13, 14]. These documents used statistical calculation standardized protocols: ISO 5725 (precision and accuracy), ISO 11095 and ISO 8466 (linearity) [15].

The experimental design used for the validation implemented 25 standard solutions prepared at the laboratory which led to 63 independent analyses. To carry out this study, three levels of concentration within the range 80–120% of the working concentration of sunscreens were used. The

Table V Chromatographic characteristics of studied compounds

Studied compound	BZ 3	ODP	BDM	EMC	EMT
Retention time (min)	7.9	19.7	20.4	21.4	29.4
Extracted UV spectrum (nm)					
RSD (n=10)	0.08	---	0.05	0.06	0.18

BZ3, benzophenone-3; BDM, butyl methoxydibenzoylmethane; EMC, ethylhexyl methoxycinnamate; EMT, bis-ethylhexyloxyphenol methoxyphenyl triazine; ODP, octyl dimethyl para-aminobenzoate; UV, ultraviolet; RSD, relative standard deviation.

Table VI Statistical data and results obtained during the validation

Criteria	Benzophenone-3 (BZ3)			Avobenzone (BDM)			Ethylhexyl methoxycinnamate (EMC)			Tinosorb® S (EMT)		
Levels of concentration (%)	80	100	120	80	100	120	80	100	120	80	100	120
Concentration ($\mu g\ mL^{-1}$)	119.3	148.8	178.2	121.2	150.1	180.0	203.4	254.6	305.0	120.3	150.2	181.1
Slope	1.181			1.658			1.104			2.211		
y-intercept	0.087			0.073			0.091			0.161		
Correlation coefficient	0.993			0.996			0.995			0.996		
CV(r) %	0.21	0.10	0.22	0.15	0.07	0.15	0.16	0.08	0.15	1.32	0.13	0.23
CV(FI) %	1.98	1.60	1.46	1.16	1.40	1.60	3.02	1.97	1.28	1.32	0.80	1.15
Average recovery (%)	101.1	103.0	103.3	99.2	100.6	100.6	104.0	104.8	104.7	99.3	101.8	100.7

CV(r): repeatability coefficient of variation.
CV(FI): intermediate precision coefficient of variation.

injections were carried out in a random way; each chromatogram gives the analytical answers (area) of the four studied sunscreens and of the internal standard. Criteria of validation of quantitative method [16] (specificity, response function, linearity, precision, trueness and accuracy) are given for each analyte. This work leads to the realization of four various validations and permits the establishment of four accuracy profiles. The main obtained criteria of performance are given in Table VI.

Specificity – selectivity

The specificity was determined by comparing the results obtained from the analysis of blank matrix solution with those of simulated matrix solutions (Fig. 2) and a standard solution without matrix (Fig. 3). The chromatogram of the blank matrix clearly indicates that there is no response (signal) at the retention times of sunscreens and internal standard. Furthermore, the selectivity of the method is shown by a satisfactory resolution greater than 1.25 for each peak.

Response function

The analysis of 10 independent standard solutions permits to obtain 24 pairs of data to determine response functions of each sunscreen. The absence of aberrant values was shown at first by

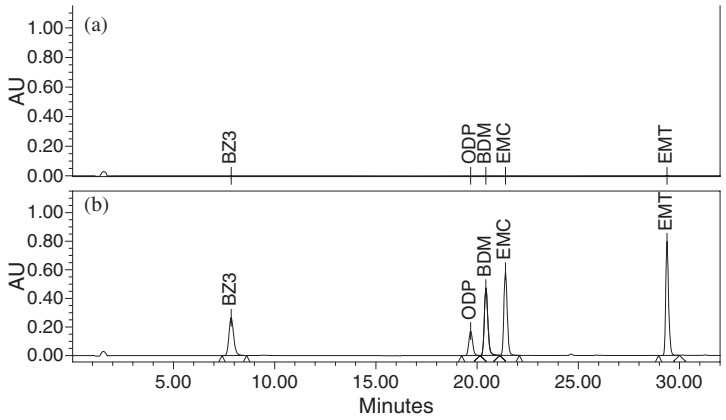


Figure 3 Specificity of the quantitative method. Chromatograms of blank matrix solution (a) and of standard solution without matrix (b). RP-18 Nucleodur® Gravity® column (150 × 4.6 mm, 5 μm), gradient with THF–CH_3CN–water with 0.1% (v/v) of CH_3COOH. Column temperature 25°C, injection volume 10 μL, extraction at 330 nm.

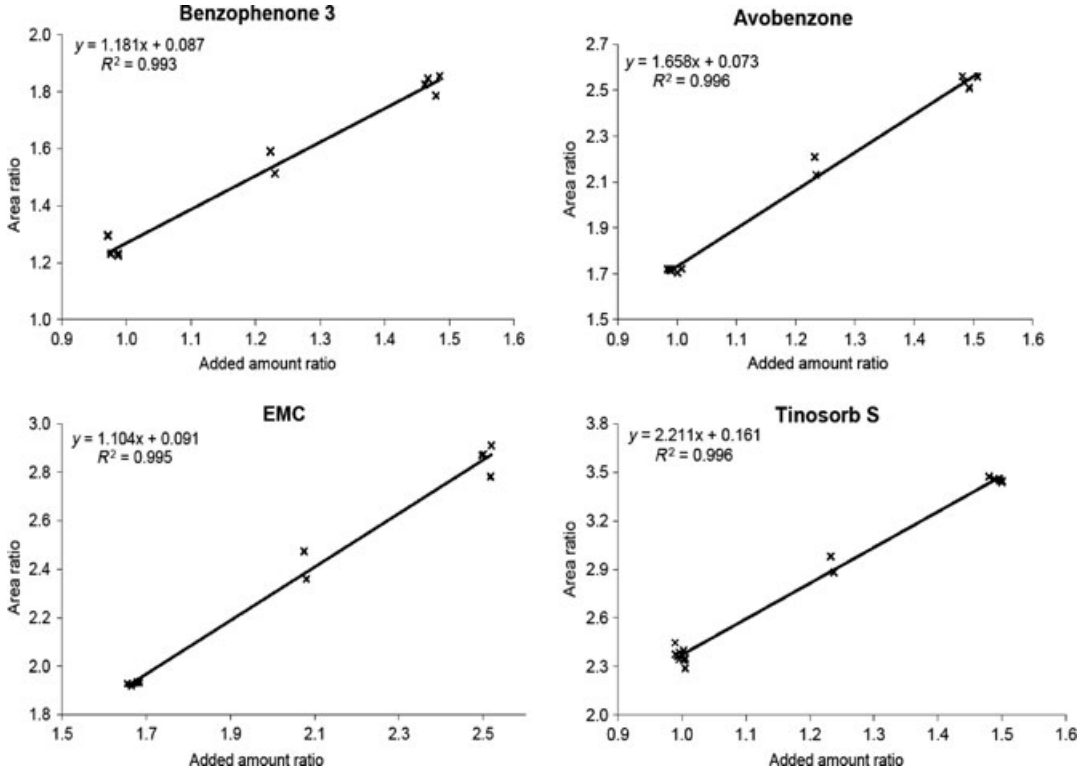


Figure 4 Response function of the four studied sunscreens.

statistical tests of Dixon, Cochran and by graphical observation of the residues on the studied range [17]. The most adapted response functions to describe the relations existing between amount ratio and area ratio are linear functions: $y = ax + b$. The straight lines obtained and the regression parameters of the equations are given in Fig. 4.

Precision

The precision of the method is evaluated on the whole range with the study of nine independent solutions (simulated matrix standard solutions) which permits to obtain 27 pairs of data. A precision profile characterizes the dispension of the method by drawing on a graph the coefficients of variation (CV) (relative standard deviation RSD) of repeatability coefficient of variation [CV(r)] and intermediate precision CV(FI) according to the concentration [18]. The precision profiles of the four studied sunscreens are given in Fig. 5. The CVs obtained are lower than 3%, which attests of a weak dispersion of the obtained values.

Linearity

The linearity of the method is established on the studied range with the pairs of data used for the determination of precision. The linearity of the method is graphically checked according to the rules of proportionality existing between the introduced concentrations and the found concentrations (Fig. 6). The linearity of the four studied sunscreens leads to linear models with slopes close to the unit and *y*-intercept comparable to a zero value. The linearity is required for the evaluation of the accuracy.

Trueness

The Trueness is given for each sunscreen by the calculation of the recovery percentage on the levels of concentration tested at 80%, 100% and 120% of the nominal concentration. The 27 pairs of values (three levels, three series, three repetitions) obtained with the simulated matrix standard solutions enable us to calculate the bias of the quantitative method expressed in percentage of recovery (Table VI). For

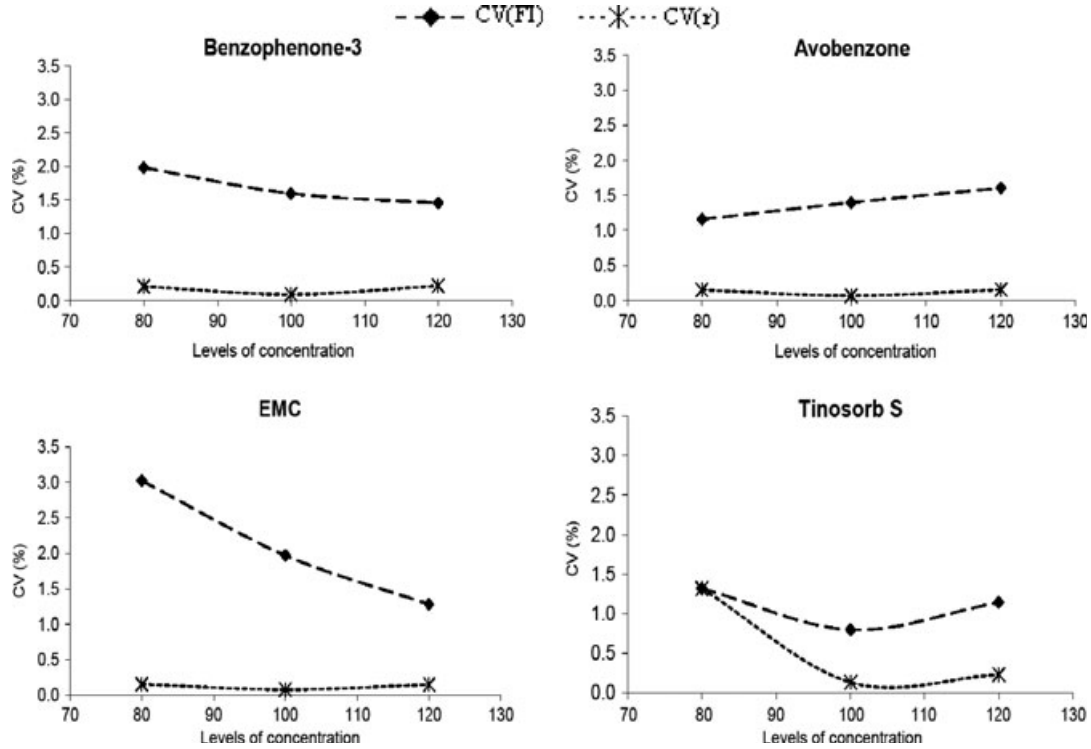


Figure 5 Precision profiles of the four studied sunscreens.

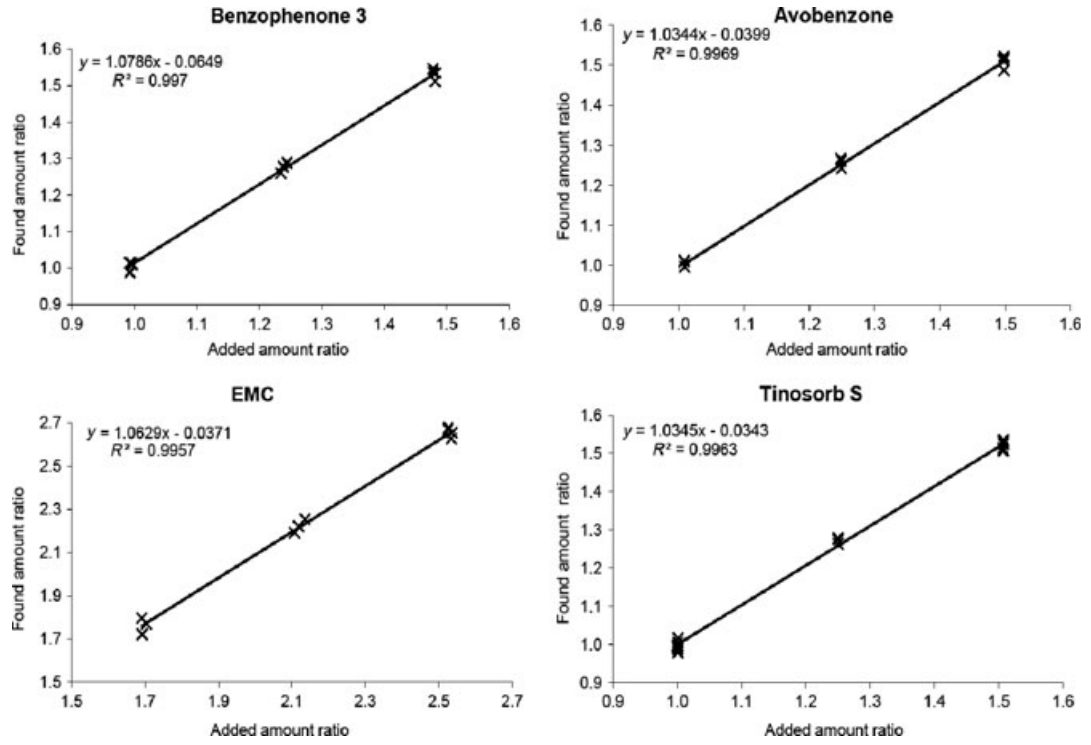


Figure 6 Linearity of the quantitative method of the four studied sunscreens.

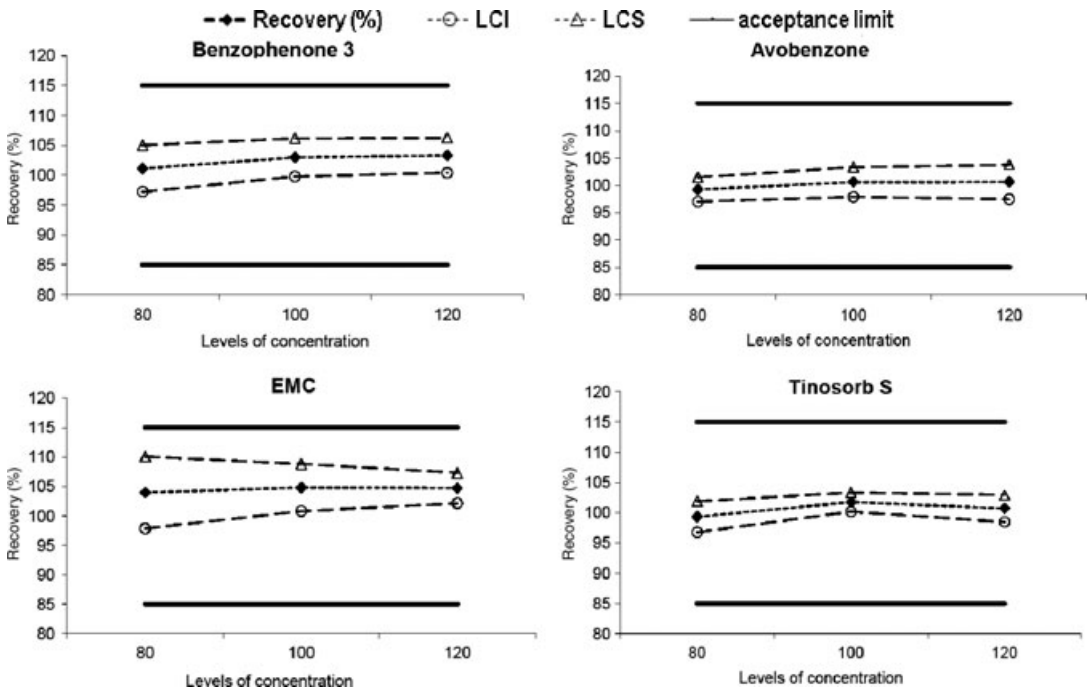


Figure 7 Accuracy profiles of the four studied sunscreens.

each sunscreen, recoveries are centred on 100%, which attests a weak bias of the method.

Accuracy

The accuracy (total error) is the expression of the sum of the trueness (bias) and precision (standard deviation). The total error of the quantitative method is evaluated for each sunscreen with four accuracy profiles (Fig. 7). These accuracy profiles show the trueness and the precision of the quantitative method for each analyte. The trueness is represented by the recovery percentage (Recovery %) and the precision is symbolized by the unilateral confidence limits (P = 95%) LCI (inferior confidence limit) and LCS (superior confidence limit) calculated with CV(FI).

The accuracy profiles obtained for Tinosorb®S (EMT), BZ-3, avobenzone and EMC are included within the acceptance limits of ± 15% which are the usually fixed for cosmetic specialties.

Conclusion

The proposed HPLC method enables the simultaneous quantitative determination of Tinosorb®S and three other sunscreens (BZ-3, avobenzone and EMC) contained in high protection cosmetic product in less than 30 minutes. A quantitative approach carried out with ODP as internal standard is permitted to determine the various criteria of validation necessary to obtain accuracy profiles. The specificity of the method has been proved. The response functions of the four sunscreens were linear models with correlation coefficients close to the unit. The weak dispersion of the values is confirmed by CV(FI) lower than 3%. The precision of quantitative method was satisfactory for each studied compound. The recovery percentages are centered on 100%, the trueness of the method is demonstrated. On the tested range, the quantitative method is precise and true, thus accurate.

The performances of the method are summarized on the accuracy profiles of the four sunscreens. These profiles are in accordance with the commonly allowed rules of acceptance for the cosmetic specialties. The quantitative method is validated and can be applied to the study of industrial batches.

Acknowledgements

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