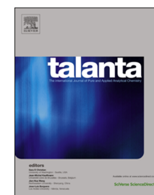
Talanta 131 (2015) 221–227

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta



Method validation using weighted linear regression models for quantification of UV filters in water samples

Claudia Pereira da Silva, Elissandro Soares Emídio, Mary Rosa Rodrigues de Marchi*

Analytical Chemistry Department, Institute of Chemistry, São Paulo State University (UNESP), Araraquara, SP 14801-970, Brazil

ARTICLE INFO

Article history:
Received 21 May 2014
Received in revised form
14 July 2014
Accepted 16 July 2014
Available online 30 July 2014

Keywords:
UV filters
Validation
Matrix effect
Heteroscedasticity
Weighted regression

ABSTRACT

This paper describes the validation of a method consisting of solid-phase extraction followed by gas chromatography–tandem mass spectrometry for the analysis of the ultraviolet (UV) filters benzophenone-3, ethylhexyl salicylate, ethylhexyl methoxycinnamate and octocrylene. The method validation criteria included evaluation of selectivity, analytical curve, trueness, precision, limits of detection and limits of quantification. The non-weighted linear regression model has traditionally been used for calibration, but it is not necessarily the optimal model in all cases. Because the assumption of homoscedasticity was not met for the analytical data in this work, a weighted least squares linear regression was used for the calibration method. The evaluated analytical parameters were satisfactory for the analytes and showed recoveries at four fortification levels between 62% and 107%, with relative standard deviations less than 14%. The detection limits ranged from 7.6 to 24.1 ng L^{-1}. The proposed method was used to determine the amount of UV filters in water samples from water treatment plants in Araraquara and Jau in São Paulo, Brazil.

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1. Introduction

Ultraviolet (UV) filters are compounds designed to absorb ultraviolet (UVA and UVB) radiation, and they are commonly used in sunscreen products to attenuate the negative effects of solar radiation. Because of their effectiveness against the harmful effects of ultraviolet radiation, these compounds have begun to be added not only to sunscreen products but also to the formulations of many everyday products, such as cosmetics, skin creams, body lotions, shampoos, sprays and hair dyes, among many other personal care products (PCPs).

In addition to their considerable use in PCPs, UV filters also have other smaller applications in textiles, household products, plastics, optical products and agricultural products [1]. Because of their wide use, UV filters are introduced into the environment in significant amounts, both directly, due to recreational activities (diffuse sources) and industrial wastewater discharges (point sources), and indirectly, due to the discharge of wastewater effluents [2].

Because of these significant inputs, there has been growing interest in the study of UV filters in various environmental compartments and of their negative effects with regard to human and animal life. Consequently, several methods have been developed and/or optimized for the detection and quantification of these compounds in environmental matrices. These methods have predominantly consisted of chromatographic techniques that typically require a clean-up step and/or concentration of analyte using, for example, SPE, SPME, DLMME or SBSE [3–7].

However, the use of an existing method or the development of a new method is not sufficient for proper quantification. To ensure that the method used for extraction and analysis provides reliable data with regard to quantification of the analytes, method validation is essential prior to its application [8].

Validation studies are an essential tool during the implementation and continuation of best practices in all analytical areas. Such studies are usually structured and contain reference guide documents. However, because several differences can exist between these documents, more than one document is typically used [9]. Despite the differences, it is necessary to have consensus that the method is validated for the intended purpose and that it meets all requirements for the intended analytical applications, thus ensuring the reliability of the results. To accomplish this goal, the analytical performance parameters must be verified [10].

One of the parameters that must be evaluated is the selection of the calibration curve to be used for quantification. Generally, linear regression by the method of ordinary least squares (OLS) is used. However, it is important to highlight that heteroscedasticity in the analytical curve should not be neglected, as it can lead to a significant loss of precision, especially at low concentrations of

* Corresponding author. Tel.: +55 16 33019610; fax: +55 16 33019692.
E-mail addresses: claudiaufs@yahoo.com.br (C.P. da Silva), elissandro_se@yahoo.com.br (E.S. Emídio), mssqam@iq.unesp.br, maryrmarchi@gmail.com (M.R.R. de Marchi).

http://dx.doi.org/10.1016/j.talanta.2014.07.041
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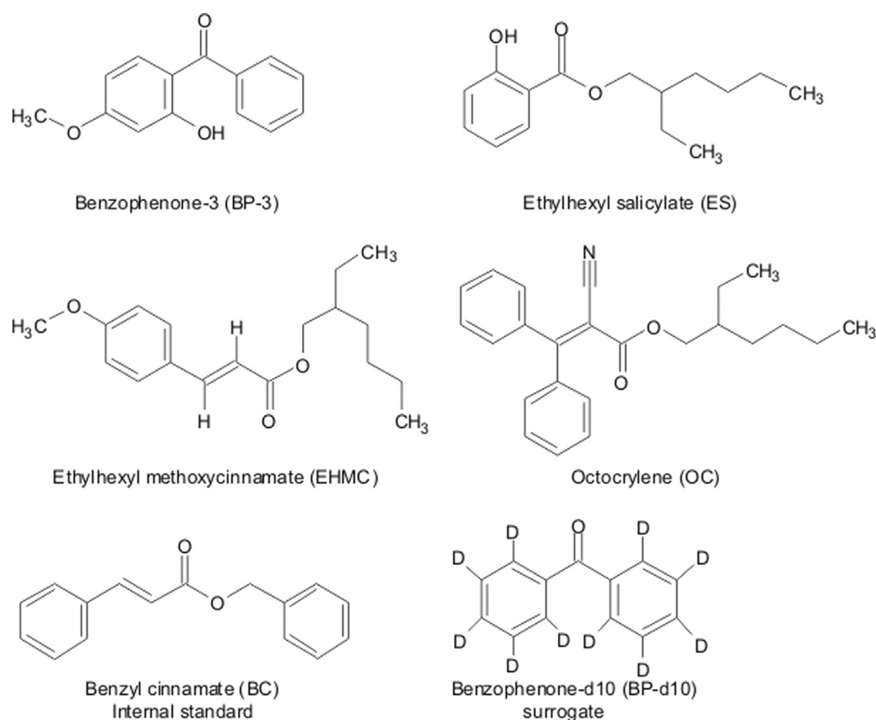

Fig. 1. Structures of UV filters.

the curve. It should be noted that UV filters are present at low concentrations in the environment; therefore, maintaining precision at the low end of the curve is especially important for UV filters. The wrong regression method can also contribute to systematic errors of measurement [11].

In this article, we validated a method for the determination of the UV filters benzophenone-3 (BP-3), ethylhexyl salicylate (ES), ethylhexyl methoxycinnamate (EHMC) and octocrylene (OC) (Fig. 1) in aqueous samples. The proposed method consisted of a solid-phase extraction (SPE) followed by gas chromatography coupled to tandem mass spectrometry (GC–MS/MS). Even, method validation be the subject of many discussions, there are still many inconsistencies between the guide documents, in some cases different terminology can be found throughout the same document. With this, much confusion is found in adequate definition of the criteria assessed in method validation. An example, error quite common in analytical chemistry, is the use of the correlation coefficient as a test for linearity and the negligence in the heteroscedasticity of the data in the analytical curve. There are few data in the literature addressing these criteria regression models. The validation method was performed to evaluate the following figures of merit: selectivity, analytical curve, precision, trueness, limit of detection and limit of quantification.

2. Experimental

2.1. Standards, solvents and sorbents

Benzophenone-3 98%, ethylhexyl salicylate 99%, ethylhexyl methoxycinnamate 98%, octocrylene 97%, benzophenone-d10 (surrogate), and the internal standard benzyl cinnamate (BC) 99%, were supplied by Sigma-Aldrich (St. Louis, MO, USA). Methanol (MeOH) and ethyl acetate (AcOEt) were HPLC grade and were obtained from Mallinckrodt Baker, Inc. (Paris, KY, USA). Hydrochloric acid (HCl, 37% m/v) was purchased from JT Baker Chemical Co. (Phillipsburg, NJ, USA) and was used to adjust the pH of the sample. The solid-phase extraction (SPE) cartridges (200 mg/6 mL) were acquired from Phenomenex (Torrance, CA, USA).

Individual standard solutions of each UV filter (1000 mg L^{-1}) were prepared in ethyl acetate and then diluted to mixtures with concentration of 5 mg L^{-1}. These solutions were stored in the dark at −20 °C. Further dilutions to working standard solutions, which were used to prepare the spiked water samples employed during validation, were also prepared in ethyl acetate daily.

2.2. Sample collection and pre-treatment

Water samples were collected in October 2012 and March 2013 at the water treatment plants (WTPs) in Araraquara and Jau (São Paulo, Brazil). The water samples were collected in 4 L capacity amber Pyrex glass bottles with screw caps and were transported to the laboratory under refrigeration at 4 °C (ice packs) and stored in the dark. Prior to extraction, the samples were filtered through a glass fiber filter (Macherey-Nagel GF3) with a 0.6 μm pore size.

Samples were extracted using SPE. The SPE cartridges were first conditioned with 5 mL of AcOEt, 5 mL of MeOH and 5 mL of deionized water. Sample volumes of 500 mL at pH 3 were passed through the cartridge at a flow rate of approximately 10 mL min^{-1}. Next, the cartridge was rinsed using 50 mL of a 5% MeOH/deionized water solution. The cartridge was then dried under total vacuum for 5 min and a total of 500 μL of MeOH was added to facilitate drying. The analytes were eluted with 3 × 2 mL of ethyl acetate. Then, 500 μL of the internal standard solution of 100 μg L^{-1} benzyl cinnamate was added to the eluate. The volume of the eluate was reduced to 1 mL under a gentle flow of nitrogen gas prior to analysis by GC–MS/MS.

2.3. Instrumentation

The analytes were analyzed by GC–MS/MS using a Varian CP-3800 gas chromatograph that was equipped with a Saturn 2000 ion trap mass spectrometer. The chromatography was performed under the following conditions. Helium was used as the carrier gas at a constant flow rate of 1.2 mL min^{-1}. The temperature of the injector was

maintained at 300 °C in splitless injection mode with a sampling time of 1 min. A fused-silica ZB-5MSi capillary column (30 m length × 0.25 mm i.d. and 0.25 μm film thickness) (Phenomenex, Torrance, CA, USA) was used for the separation. The column temperature program was as follows: initial temperature 60 °C, maintained for 1 min, then ramped at 25 °C min^{-1} to 160 °C, maintained for 1 min, ramped at 10 °C min^{-1} to 300 °C and held at this temperature for 2 min. The total run time was 22.0 min. The transfer line, trap and manifold temperatures were maintained at 300, 50 and 220 °C, respectively.

2.4. Optimization of operating conditions for SPE and GC–MS/MS

The detailed procedure for the extraction of the UV filters and the details of the chromatographic method can be found in previous reports published from our laboratory [7].

2.5. Validation procedure

Validation studies were planned and executed using reference guide documents published nationally and internationally and articles published in recent years. We demonstrated that the method was validated for the intended purpose by evaluating the following performance parameters: selectivity, analytical curve, precision, trueness, limit of detection and limit of quantification.

3. Results and discussion

3.1. Method validation

The optimization of the extraction and analysis parameters is performed using a method that enables the efficient quantification of analytes. For this purpose, the performance of the method was evaluated under optimized extraction and analytical conditions, and the method was validated in terms of selectivity, analytical curve, precision, trueness, limit of detection and limit of quantification.

3.1.1. Selectivity

The selectivity of this method was ensured through the use of MS/MS following the chromatographic separation. The investigation of nonspecific selectivity (also known as matrix effects) was conducted by investigating the presence of matrix interferents that altered the performance measurements. Calibration curves were prepared in the solvent and in the matrix extract. These curves, shown in Fig. 2, were prepared at the same concentrations as in [8,12,13]. Through visual analysis of the curves, it appeared that only ES exhibited no pronounced matrix effects. The BP-10, EHMC and OC exhibited matrix-induced decreased responses, while BP-3 showed an increased chromatographic response in the matrix extract.

Mathematically, the calculation to assess the matrix effects can be achieved using Eq. (1) [12].

$$\%\ \text{matrix effect} = \frac{X_2 - X_1}{X_2} \times 100 \quad (1)$$

where X_1 is the slope of the curve obtained by injection of the analytical solutions of each analyte prepared in the matrix, and X_2 is the slope of the curve obtained by injection of the analytical solutions of each analyte prepared in the solvent.

The matrix effect is not considered significant, i.e., it should not influence the quantitative analysis, at values $<20\%$ or $>-20\%$ [12]. The calculated matrix effect values for BP-10, ES, BP-3, EHMC and OC were 62%, 5%, −280%, 44% and 52%, respectively. These values confirmed the visual analysis that indicated four of the five UV filters under study were influenced by the matrix. Significant matrix effect values were observed for BP-3 (−280%), which was likely due to the high polarity of BP-3. The active sites of the chromatographic system were available to retain the more polar analytes in the pure solvent injection, and thus a smaller amount of BP-3 was detected. However, a competition existed between the matrix components and the more polar analytes for the active sites of the chromatographic system in the matrix extract, which led to an enhancement of the response [14].

For most analytes studied, the signals were influenced by the co-extracted substances. It was concluded that, to ensure the reliable quantification of analytes, it was necessary to prepare analytical curves from calibration standards in the matrix extract.

3.1.2. Analytical curve

The analytical curves were prepared in water samples prior to the SPE procedure from a minimum of six concentration levels from 20 to 4000 ng L^{-1} for BP-10, ES and BP-3 and from 50 to 4000 ng L^{-1} for EHMC and OC (curves shown in Table 1).

According to Table 1, all curves possessed correlation coefficients, "r", greater than or equal to 0.99. However, the value of r or r^2 is inappropriate as a test model fit [15–17]. Therefore, it was necessary to evaluate the fit of the model using an alternative approach, namely, by assessing the homoscedasticity of data [18].

The study of the homoscedasticity of the data was performed using the F-test and the residual plot. In the F-test, the data are assumed to be uniformly distributed when the tabulated F-value (F_{tab}) is greater than the experimental F-value (F_{tab}) ($F_{tab} > F_{exp}$). The F_{tab} is obtained from a table of F distribution critical values with confidence levels of 99% for $(n-1)$ degrees of freedom, and the F_{exp} is obtained using Eq. (2) [19,20].

$$F_{exp} = \frac{S_2^2}{S_1^2} \quad (2)$$

where the experimental F-value is expressed as the ratio between the variances obtained at the lowest (S_1^2) and highest (S_2^2) concentration level of the working range.

In the F-test, the results obtained for BP-10, ES, BP-3, EHMC and OC were 20009, 1173, 24583, 1178 and 8274, respectively. All of these values were greater than $F_{tab}=99$ (tabulated F-value obtained with a confidence level of 99% and $(n-1)$ degrees of freedom equal to 2). It was evident that the data were heteroscedastic, as the condition of homoscedasticity ($F_{tab} > F_{exp}$) was not realized for any of the analytes.

In the evaluation of homoscedasticity by residual graphs, the residual (R) is calculated by Eq. 3, which establishes the difference between the measured values (S_{exp}) and the calculated values from the regression equation (S_{int}) [21].

$$R = S_{exp} - S_{int} \quad (3)$$

where the (S_{exp}) is the experimental signal, and (S_{int}) is the interpolated signal from the regression equation.

After the residual calculations are performed, the graphs are plotted as the residuals vs. concentration (Fig. 3). From the analysis of the residual graphs, the residues were not randomly distributed around the axis of concentrations. Additionally, the variance was more pronounced at higher concentrations, confirming the heterogeneity of the data that was previously revealed by the F-test.

The heterogeneity of the data was confirmed, and the choice of a new, more appropriate calibration model to define the relationship between concentration and response of the analytes was necessary [18]. Often, reduces calibration range to improve the model. However, the goal was not to work with a narrower calibration range, as UV filters have been found in the environment from ng L^{-1} to low μg L^{-1} levels. Thus, a large calibration range was ideal for quantification. Therefore, we used a weighted least squares linear regression model. Weighted models are commonly adopted to minimize the greater

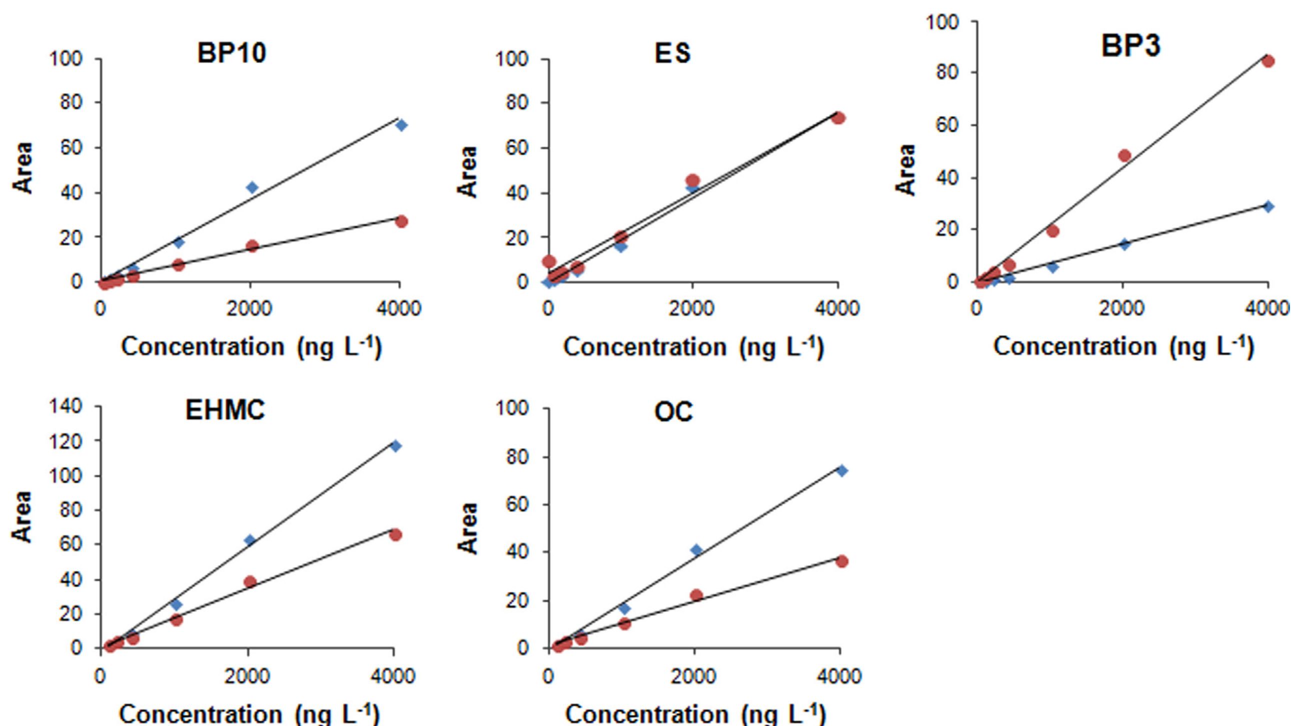

Fig. 2. Analytical curves (GC–MS/MS) in solvent (blue quadrade) and in the matrix extract (red circle) for the studied UV filters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Analytical curves (GC–MS/MS) for analytes in the matrix.

Analyte	Range (ng L^{-1})	Calibration equation($y=ax+b$)	r
BP-10	20–4000	$y=0.0078x+0.7028$	0.994
ES	20–4000	$y=0.0144x+2.2911$	0.991
BP-3	20–4000	$y=0.0241x-0.2487$	0.998
EHMC	50–4000	$y=0.0111x+1.2692$	0.991
OC	50–4000	$y=0.0067x+1.2121$	0.987

influence of the higher concentrations of the regression [20]. The residual plots are shown in Fig. 3.

An ideal weighted model will balance the regression line to generate an error that is uniformly distributed throughout the calibration range [19]. The appropriate weighting factors, w_i, can be calculated from the inverse of the variance (S_i^{-2}) using Eq. (4) [18].

$$w_i = \frac{S_i^{-2}}{\sum_i \frac{S_i^{-2}}{n}} \quad (4)$$

However, this weighting factor is generally impractical, because it requires several determinations for each calibration point and because a fresh calibration line should be performed each time the method is used. Other empirical weights based on the variable x (concentration) or variable y (response) can provide a simple approximation of the variance. The empirical weights (w_i) most widely used are $1/y^{0.5}$, $1/y$, $1/y^2$, $1/x^{0.5}$, $1/x$, $1/x^2$ [20].

Each of these weights can be applied to the linear regression equation. The conversion of a linear regression equation without weighting to a weighted linear regression is performed using the term w_i in the calculations of the parameters "a" and "b". The weighted coefficients "a" and "b" are calculated by Eqs. (5) and (6) [18].

$$a_w = \frac{\sum_i w_i\, x_i\, y_i - n\,\overline{X}_w\,\overline{Y}_w}{\sum_i w_i\, x_i^2 - n\,\overline{X}_w^2} \quad (5)$$

$$b_w = \overline{Y}_w - b\,\overline{X}_w \quad (6)$$

The weighted correlation coefficient (r_w) can be calculated by Eq. (7)

$$r = \frac{\sum w_i \sum w_i\, x_i\, y_i \; - \; \sum w_i\, x_i \sum w_i\, y_i}{\sqrt{\sum w_i \sum w_i\, x_i^2 - (\sum w_i\, x_i)^2}} \quad \sqrt{\sum w_i \sum w_i\, y_i^2 - (\sum w_i\, y_i)^2} \quad (7)$$

The choice of the weighting model must consider models that possess a small sum of the relative errors calculated by Eq. (8) in combination with a random distribution around the axis of concentrations. As an example, Table 2 reports the regression parameters of the calibration curve generated for each of the eight weighting models for BP-10 and the sums of the relative errors for each of the eight models.

$$\%\,\mathrm{RE} = \frac{C_{(\exp)} - C_{(nom)}}{C_{(nom)}} \times 100 \quad (8)$$

where the experimental concentration $C_{(exp)}$ is obtained from the weighted equation, and $C_{(nom)}$ is the theoretical or nominal concentration.

The models 2, 4 and 8 presented the smallest sums of the relative errors. While models 2, 4 and 8 all had low values for the sums of %RE, as shown in plots of %RE vs. concentration in Fig. 4, model 2 exhibited the best distribution of %*RE* around the axis of concentrations. Therefore, model 2 was selected to best define the correlation between the concentration and the response of BP-10.

The same approach was followed to define the best weighting factor for the other analytes (data not shown). The models chosen for each analyte in the study are shown in Table 3. Therefore, these new curves (weighted values of a and b) were used for the quantification of the UV filters in the water samples.

3.1.3. Precision and trueness

To evaluate the trueness of this method, recovery experiments were performed in triplicate at four fortification levels: LOQ, 200, 1000 and 2000 ng L^{-1}. The precision was evaluated by testing the

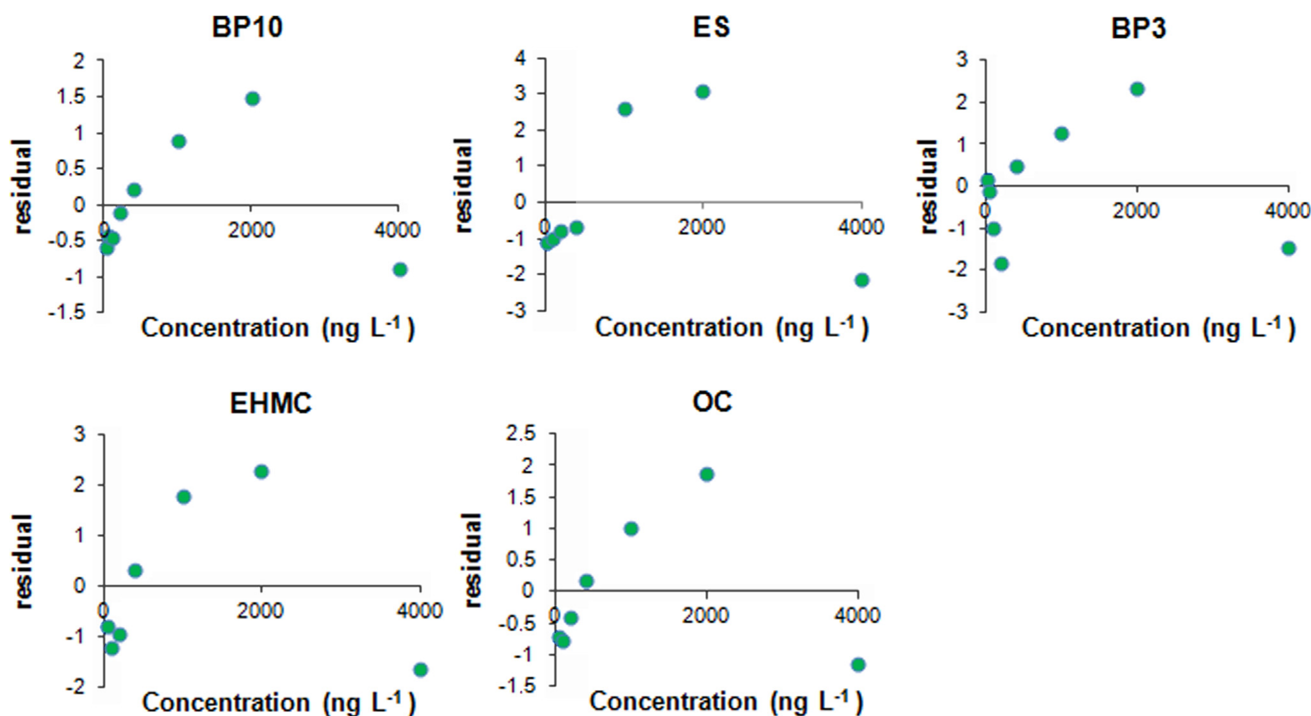

Fig. 3. Statistical residuals plotted against concentrations.

Table 2
Regression parameters of the analytical curve ($y = ax + b$) generated for each weight (w) and the respective sums of the relative errors ($\sum$ER%) for BP-10.

BP-10 Model	w_i	a[b]	b[c]	r[d]	$\sum$ER%
1	1[a]	0.0155	0.7028	0.9972	−538
2	$\frac{s_i^{-2}}{\sum_i s_i^{-2}/n}$*	0.0184	0.0887	0.9941	16
3	$\frac{1}{y^{0.5}}$	0.0161	0.2592	0.9968	−53
4	$\frac{1}{y}$	0.0165	0.1834	0.9958	11
5	$\frac{1}{y^2}$	0.0140	0.3854	0.9922	−103
6	$\frac{1}{x^{0.5}}$	0.0163	0.0334	0.9967	181
7	$\frac{1}{x}$	0.0170	0.0038	0.9955	176
8	$\frac{1}{x^2}$	0.0208	0.0001	0.9912	0

[a] Unweighted.
[b] Slope.
[c] y-Axis intercept.
[d] Correlation coefficient

repeatability of the recovery through calculations of coefficients of variation (CV). The recoveries and the coefficients of variation obtained for the UV filters are shown in Table 4.

As shown in Table 4, the proposed method was demonstrated to be suitable and reliable for the determination of UV filters because the recoveries and coefficients of variation obtained at the four levels of fortification were within the acceptable limits recommended by [22,23].

3.1.4. Limit of detection and limit of quantification

The detection limits of the UV filters were calculated from the parameters of the analytical curves. Specific calibration curves were used in the range of LOD of 1–100 ng L^{-1} for BP-10, ES and BP-3; 1–200 ng L^{-1} for EHMC; and 10–200 ng L^{-1} for OC. The corresponding LOD concentrations were calculated using both Eq. (9) and also an equation that is statistically more defensible, published by ISO 11843-2:2000 [24] (Eq. (10)) [25].

$$\mathrm{LOD} = \frac{3s_{y/x}}{a} \qquad (9)$$

$$\mathrm{LOD} = \frac{2t_{0.05,\, n-2}\ S_{y/x}}{a}\sqrt{\frac{1}{k} + \frac{1}{I \times J} + \frac{\bar{x}^2}{J\sum_i (x_i - \bar{x})^2}} \qquad (10)$$

Unlike the LODs, the limits of quantification must exhibit acceptable precision and trueness. Some authors, such as those in [3,26], have used Eq. (11) to calculate LOQs. However, the proposal that the LOQs must be calculated using this equation has not found great benefit [25] because a higher concentration must be reported for the LOQ to ensure greater precision. Alternatively, if concentrations lower than the calculated LOQ exhibit acceptable precision and trueness, a lower concentration can be reported for the LOQ. In summary, the LOQ calculated by Eq. (11) is only a rough estimate, and a good rule to follow is assumed to be a compromise between precision and trueness.

$$\mathrm{LOQ} = \frac{10\ S_{y/x}}{a} \qquad (11)$$

The values obtained for the LODs and LOQs of the method using Eqs. (9), (10) and (11) for the UV filters are shown in Table 5.

The LOD values obtained in this study were comparable to and sometimes better than those obtained in previous studies in which the extraction was performed by SPME [27–30], DLLME [3,26], SBSE [4,31] and SPE [32].

The recovery experiments performed with concentrations below the LOQs calculated by Eq. (11) (data shown in the second column of Table 4) showed acceptable precision and recovery. Therefore, the values of the method LOQs established as the first point of the calibration curve within the regression range (Table 4) were lower than the LOQs calculated by Eq. (11), except for ES. Therefore, according to the definition, the LOQs of the method were the lowest concentrations that were quantitatively determined with acceptable precision and trueness.

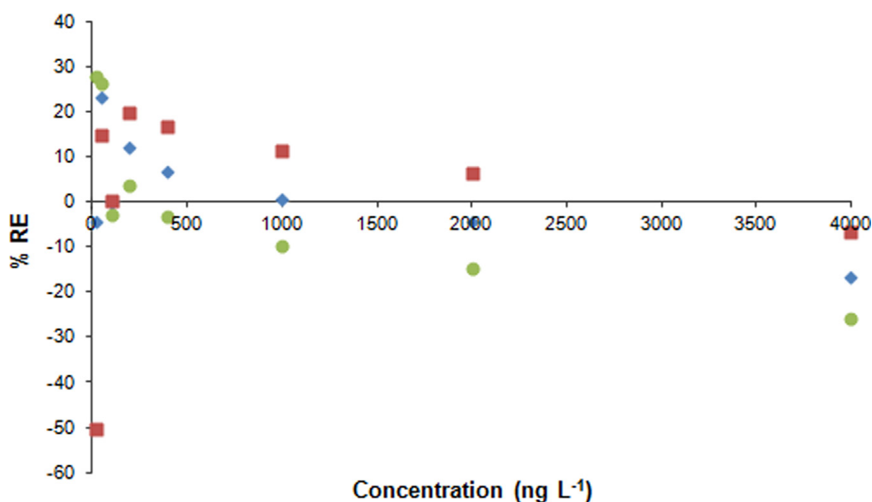

Fig. 4. Percentage of relative error (RE%) versus concentration, obtained for different regression models: model 2 (blue diamond), model 4 (red quadrate) and model 8 (green circle). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Regression parameters (optimized) of the analytical curve generated for each analyte and the respective sums of the relative errors (∑%RE).

Analyte	Model	w_i	a[a]	b[b]	r[c]	∑%RE
BP-10	2	$\frac{s_i^{-2}}{\sum_i s_i^{-2}/n}$	0.0184	0.0887	0.9941	16
ES	2	$\frac{s_i^{-2}}{\sum_i s_i^{-2}/n}$	0.0321	1.0877	0.9977	20
BP-3	3	$\frac{1}{y^{0.5}}$	0.0484	-0.3162	0.9985	−13
EHMC	3	$\frac{1}{y^{0.5}}$	0.0233	0.2815	0.9949	51
OC	2	$\frac{s_i^{-2}}{\sum_i s_i^{-2}/n}$	0.0163	0.4134	0.9912	−13

[a] Slope.
[b] y-Axis intercept.
[c] Correlation coefficient.

Table 4
Average recoveries and coefficients of variation (CV) for n=3.

Analyte	LOQ[a] ng L^{-1} (%CV)	200 ng L^{-} (%CV)	1000 ng L^{-1} (%CV)	2000 ng L^{-1} (%CV)
BP-10	107 (11)	96 (3)	98 (3)	105 (10)
ES	77 (10)	72 (5)	72 (5)	69 (5)
BP-3	91 (12)	85 (3)	95 (4)	94 (4)
EHMC	85 (7)	76 (5)	74 (5)	65 (10)
OC	62 (13)	76 (5)	70 (5)	63 (14)

[a] LOQ – limits of quantification obtained visually. Spiked at 10, 100, 10, 50 and 50 ng L^{-1} for BP-10, ES, BP-3, EHMC and OC, respectively. The internal standard BC was spiked at 50 μg L^{-1}.

Table 5
Limits of detection and quantification for the SPE and GC–MS/MS method.

Analyte	LOD[a] (ng L^{-1})	LOD[b] (ng L^{-1})	LOQ (ng L^{-1})
BP-10	9.3	10.0	30.9
ES	12.1	13.1	40.4
BP-3	7.1	7.6	23.5
EHMC	23.5	24.1	78.2
OC	19.3	20.8	64.4

[a] From Eq. (9).
[b] From Eq. (10).

Table 6
Concentrations of UV filters in natural, treated and chlorinated water (ng L^{-1}). BP-10 was used as a surrogate. Average recoveries and coefficients of variation (%CV) for n=3.

Analyte	Araraquara			Jau		
	Chlorinated water	Treated water	River water	Chlorinated water	Treated water	River water
BP-10[a]	110 (10%)	100 (2%)	102 (5%)	105 (6%)	100 (3%)	96 (5%)
ES	< LOQ	< LOQ	< LOQ	< LOD	< LOD	< LOD
BP-3	< LOQ	< LOQ	< LOQ	n.d.	< LOQ	< LOQ
EHMC	< LOD	< LOQ	< LOD	< LOD	< LOD	< LOQ
OC	< LOQ	< LOQ	< LOD	< LOQ	< LOQ	< LOD

LOD and LOQ from Table 5.
n.d.: not detected.
[a] BP-10 at 200 ng L^{-1}.

3.2. *Application of the method to the analysis of water samples*

The validated method was applied to the determination of UV filters in water samples. Water samples were collected (March 2013) at water treatment plants in Araraquara and Jau in São Paulo, Brazil. Three points of sampling were performed: at the entrance prior to treatment (river water), post-treatment without chlorination (treated water), and post-treatment with chlorination (chlorinated water). The samples were analyzed by SPE and GC–MS/MS, and the results of the measured concentrations of BP-3, ES, EHMC and OC are shown in Table 6. For BP-10, the use of the surrogate revealed the average recoveries and the coefficients of variation (n=3).

The low levels found did not indicate that these substances are not present in the environment because only water was analyzed. Furthermore, the analyses were performed at a time when there was likely minimal sunscreen usage.

From Table 6, the method performed very efficiently, as indicated by the recoveries and coefficients of variation of the surrogate. The results showed recoveries very close to 100% and coefficients of variation < 10%, indicating that the method demonstrated acceptable precision and trueness.

4. Conclusions

The analytical performance parameters evaluated were satisfactory in terms of selectivity, analytical curve, precision, trueness, limit of detection and limit of quantification. The specific selectivity was

verified through the use of detection by MS/MS. The nonspecific selectivity was confirmed from the observation of matrix effects (both overestimation and underestimation) for most analytes. Therefore, to minimize these effects, the analytical curves used for quantification were prepared in the matrix extract.

Due to the large concentration range of the analytical curve, homoscedasticity was not achieved. Therefore, weighted models were applied to obtain analytical curves with the best fits of the data and hence more reliable quantifications. Although these models were more complicated and laborious than linear regression by ordinary least squares, their implementation led to results with greater trueness, mainly because for large concentration ranges.

The potential of the method for the quantification of the UV filters BP-3, ES, EHMC and OC in water samples was further evidenced by the recoveries obtained, which confirmed the trueness and precision of the method. The recoveries for four fortification levels of the analytes ranged from 63% to 107%, with repeatability between 3% and 14%. The calculated limits of quantification ranged from 23.5 to 78.2 ng L^{-1}, with clear evidence that the lower values were quantified based on the lowest concentration of the calibration curve within the range of values validated with acceptable trueness and precision.

Acknowledgments

The authors are grateful for doctoral studentships from CAPES (Brazilian Coordination for the Improvement of Higher Education Personnel) and CNPq (Brazilian National Counsel of Technological and Scientific Development) for C.P. da Silva and E.S. Emídio, respectively. The research support provided by Foundation for Science, Technology and Education Support (FACTE; Grant no. 1018/2012) is also thanked.

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