Talanta 85 (2011) 2175–2181

Contents lists available at ScienceDirect

Talanta

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

Fast liquid chromatography-diode array detection assisted by chemometrics for quantification of seven ultraviolet filters in effluent wastewater

Maryam Vosough*, Nahal Rahimdoost Mojdehi

Chemistry and Chemical Engineering Research Center of Iran, P.O. Box 14335-186 Tehran, Iran

ARTICLE INFO

Article history:
Received 30 April 2011
Received in revised form 18 July 2011
Accepted 19 July 2011
Available online 27 July 2011

Keywords:
Ultraviolet filters
Liquid chromatography-diode array detection
Solid-phase extraction
Wastewater
Second-order calibration

ABSTRACT

A fast chromatographic method is presented for simultaneous quantification of seven organic ultraviolet (UV) filters (benzophenone-3,4-methylbenzilidene camphor, octocrylene, 1-(4-tert-butylphenyl)-3-(4-methyoxyphenyl)1,3-propanedione), ethylhexyl methoxy cinnamate, ethylhexyl salicylate and homosalate) in effluent wastewater samples. The UV filters were pre-concentrated by Bond Elut-ENV cartridges and separated on an ODS column (15 cm × 0.46 cm, 5 μm) in less than 2.5 min using a non-aqueous mobile phase of methanol–acetonitrile (50:50, v/v) with flow-rate of 1.5 mL min^{-1}. Appropriate baseline correction through asymmetric least squares was applied to reduce the matrix of background signals in three way data. Then, second-order calibration based on multivariate curve resolution-alternating least squares (MCR-ALS) was implemented on the unfolded three-way data obtained from liquid chromatography with diode array detection (LC-DAD) through standard addition calibration method for handling co-eluted peaks, systematic and proportional errors. Recoveries ranging from 76% to 130% and %RSD values less than 11.2 for all UV filter shows the accuracy and precision of the proposed method in wastewater samples. In addition, statistical *t*-test as well as computed elliptical joint confidence region (EJCR) confirms the accuracy of the proposed method and indicates the absence of both constant and proportional errors in the predicted concentrations. This study demonstrates that coupling of the fast HPLC-DAD method with powerful algorithm of MCR-ALS can be considered as an efficient method for quantification of UV filters in highly contaminated samples of wastewaters where both time and cost per each analysis can be reduced significantly.

1. Introduction

Organic UV filters such as 4-methylbenzilidene camphor (4-MBC), ethylhexyl methoxycinnamate (EMC), octylcrylene (OCR), and benzophenone-3 (BPN) are used in sunscreen creams, lotions and other personal care products. The crucial effect of these chemical compounds against some skin damages such as photo-induced immune suppression and carcinogenesis is well known [1]. Since these compounds are designed to mostly remain on the outer surface of the skin, therefore they are easily washed off during any contact with water like bathing, swimming, and entered into natural water resources through direct or indirect pathways [2]. Extensive usage of UV filters in sunscreens and other cosmetics along with increasing their shelf life and with continuous transfer to environment has produced a class of emerging contaminants during last decade [3]. So, screening of UV-filters has been received much attention in environmental studies. In addition, monitoring of these chemical compounds in the influent and effluent wastewater samples is a useful way to study the removal efficiency of wastewater treatment plants [4].

The different drawbacks of organic UV filters to environment have been discussed previously [2]. The lipophilic character and relative persistency of some of these compounds which lead to their bio-accumulation, as well as their harmful effects, such as low birth weight, cellular damages, hormonal activity, and different allergies, have raised attentions to their environmental analysis [5–7].

For many years, most of the relevant analytical methods were focused on the determination of these compounds in commercial formulation of personal care products (PCPs) to attenuate the negative effects from sunlight exposure and to control the quality of procedures. Reverse-phase high performance liquid chromatography (RP-HPLC) with UV–vis detection was the method of choice for the identification and determination of UV filters in sunscreens in several studies [8–11]. According to high concentration of UV-filter compounds in such matrixes (at least at mg g^{-1} level), most of the sample preparation methods applied for this purpose, were considered as clean-up techniques and were carried out without any need for sample enrichment. During the last few years, environmental impact of UV-filters as a class of emerging contaminants has

* Corresponding author. Tel.: +98 214 458 0720; fax: +98 214 458 0762.
E-mail address: vosough@ccerci.ac.ir (M. Vosough).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.07.068

been studied and various analytical procedures have been developed. In fact, because of bioaccumulation properties of UV filters, even low contamination level of UV-filters during an enough period of time, can lead to considerable health and environmental damages and so the trace analysis techniques seems to be inevitable [2]. Many extraction and clean-up procedures have been reported to obtain satisfactory results in different environmental samples [12–21]. HPLC with UV detector and diode array detection (DAD), gas chromatography–mass spectrometry (GC–MS) and liquid chromatography with tandem mass spectrometry (LC–MS) detection were chosen for qualitative and quantitative analyses of UV-filter compounds. In the LC separation of these compounds, some authors proposed the use of surfactant-modified hydro-organic eluents for satisfactory resolution of overlapping peaks [13,21]. In a review published in 2007, Giokas et al. explained all analytical methods for measuring UV filters in biological and environmental samples [2]. Also, in 2009, Richardson published a review on developments in water analysis for emerging environmental contaminants. One class of these compounds were devoted to UV filters and newly developed analytical methods were reviewed [3].

In the chromatographic analysis of complex environmental samples such as river and wastewaters, poor chromatographic resolution or partial peak separation between matrix constituents and the compounds of interest often occurs. In these cases, the analytes of interest can be quantified through univariate calibration by changing the experimental conditions, i.e., adding a reagent such as sodium sulfite to the sample before pre-concentration [22], optimization of mobile phase condition to longer run times or using an organic modifier in the mobile phase [23]. However these involve spending time and resources and strongly depend on the sample matrix. Also there is no guarantee to ensure that the separation will be complete. Second-order calibration methods that process three-way data are today useful alternatives to solve these types of problems. In fact, a large number of reports on the analysis of complex samples were presented using hyphenated chromatographic data and second-order calibration methods [24]. There are several second-order models with different trilinearity assumptions for multiway analysis, such as generalized rank annihilation method (GRAM) [25], alternating trilinear decomposition (ATLD) [26], self-weighted alternating trilinear decomposition (SWATLD) [27], parallel factor analysis (PARAFAC) [28], PARAFAC2 [29], multivariate curve resolution alternating least squares (MCR-ALS) [30,31], bilinear least squares (BLLS) [32], and unfolded partial least squares/residual bilinearization (U-PLS/RBL) [33]. Among these algorithms, PARAFAC2 and MCR-ALS allow deviations to the trilinearity of three-dimensional data. MCR-ALS is an excellent tool for modelling of LC-DAD data in case of retention time shift between chromatograms and also for exploiting the second-order advantage [34]. This method has been used for the analysis of various samples through recent years [35–39]. In fast LC methodology coupled with MCR-ALS modelling, we can imply studies such as the analysis of biocide mixture using short columns [40], determination of phenolic acids in strawberry samples using an organic modifier [41] and determination of dyes in beverages using a dramatic change in the mobile phase condition [42]. Recently, a fast chromatographic method using PARAFAC2 was proposed by the author for simultaneous quantification of four aflatoxins in pistachio samples [43]. The most important points in this field can be notified as reducing the time of analysis, the amount of consumed solvents and finally cost per analysis.

The major challenge in determination of UV filters is to provide reliable and matrix-free analytical methods for determination of these compounds in biological and environmental samples at low concentration levels [2]. In the present study, we proposed a new strategy based on a non-aqueous and fast LC with MCR-ALS modelling in combination with standard addition method for quantification of seven UV filters among the mostly found compounds in wastewater samples. Fig. 1(a) shows the chromatogram of a real effluent wastewater sample, in multiple wavelengths, from a sewage treatment plant which is studied in this paper. The appearance of a high band at the beginning of the chromatogram (due to the organic components of the sample) and the co-eluted analytes is clear as shown in this figure. For better observation of the analytes, the chromatogram of a spiked sample is inserted in a new window and monitored at 305 nm. Chemical and trade name as well as abbreviated names of the UV filters which are used in this paper are given in Table 1.

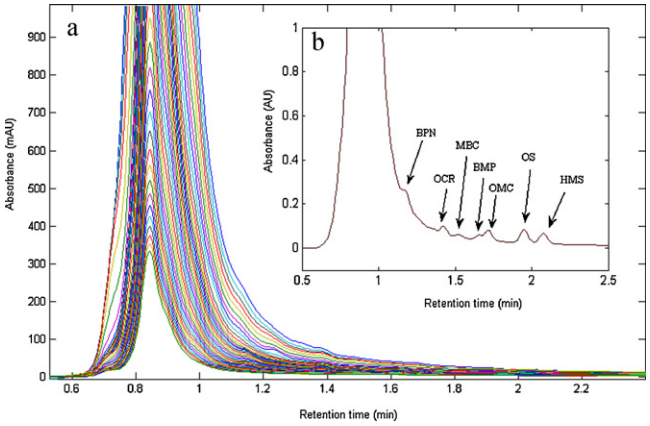

Fig. 1. Chromatographic profile of a typical wastewater sample monitored at multiple wavelengths (every 3rd wavelength has been shown for more clarity) (a) and chromatogram belongs to the same sample spiked with seven UV filters (w-s6) measured at 305 nm (b). The analytes of interest are indicated.

2. Experimental

2.1. Chemicals and solvents

Benzophenone-3,4-methylbenzilidene camphor, octocrylene, 1-(4-tert-butylphenyl)-3-(4-methyoxyphenyl)1,3-propanedione, octyl methoxy cinnamate, octyl salicylate and homosalate were all obtained from Merck (Germany) in their pure form. HPLC-grade methanol (MeOH), acetonitrile (ACN), ethyl acetate (EA) and dichloromethane (DCM) were from Merck. Sodium chloride and hydrochloric acid (32%) which were used in sample preparation and pH adjustment, respectively, were of analytical reagent quality from Merck. Ultrapure water was obtained from a Milli-Q water purification system from Millipore (USA). Filter reservoirs and polystyrene-divinylbenzene cartridges (Bond Elut-ENV) (6 mL, containing 500 mg of sorbent) were purchased from Varian (USA).

2.2. Instrumentation and software

An Agilent 1200 Series system equipped with a Rheodyne 7725 manual injector with a 20-μL injection loop, a degasser system, a quaternary pump, a column oven, a Hewlett-Packard

Table 1
Selected target UV-filters in the present study.

Abbreviation	Chemical name	Trade name	CAS reg. no.
BPN	Benzophenone-3	Eusolex® 4360	131-57-7
OCR	Octocrylene	Eusolex® OCR	6197-30-4
MBC	4-Methylbenzilidene camphor	Eusolex® 6300	38102-62-4
BMP	Methoxydibenzoylmetane	Eusolex® 9020	70356-09-1
OMC	Octyl methoxy cinnamate	Eusolex® 2292	5466-77-3
OS	Octyl salicylate	Eusolex® OS	118-60-5
HMS	Homosalate	Eusolex® HMS	118-56-9

Table 2
Nominal concentrations of the seven UV filters in spiked effluent wastewater samples.

Analyte	Spiked concentration in validation samples ($\mu g\,L^{-1}$)					
	w-s1	w-s2	w-s3	w-s4	w-s5	w-s6
BPN	4.0	4.0	1.0	3.0	15.0	2.0
OCR	5.0	8.0	0.7	2.0	12.0	3.0
MBC	0.5	1.5	15.0	2.0	10.0	5.0
BMP	10.0	12.0	1.0	3.0	20.0	5.0
OMC	15.0	20.0	3.0	2.0	0.0	10.0
OS	18.0	15.0	5.0	8.0	20.0	10.0
HMS	20.0	15.0	5.0	8.0	20.0	10.0

1200 series photo diode-array detector (DAD) with an ODS column (15 cm × 0.46 cm, 5 μm particle size) were used for quantitative analysis of mixture of UV filters. The whole chromatographic system was controlled by Chemstation software for LC 3D ver. B.03.01 running on a personal computer. HPLC instrument set up and software were from Agilent technologies Inc. (USA). A non-aqueous isocratic mobile phase composition consisting methanol–acetonitrile (50:50, v/v) was optimized for all analysis. The flow-rate of the mobile phase and injection volume were 1.5 mL min^{-1} and 20 μL, respectively. The solvents were filtered daily through a 0.45 μm Nylon membrane filter before use. The total run time was less than 3 min. The column oven temperature was set at 30 °C. Photometric detection using DAD detector was recorded between 220 and 400 nm with the spectral resolution of 1.5 nm and integration period of 0.4 s per spectrum. Extraction and clean-up steps were performed using a Visiprep™ solid phase extraction manifold (Supelco, USA) connected to a vacuum mini pump (Aldrich, France), using the 500 mg Bond Elut-ENV cartridges (6 mL).

HPLC-DAD data were exported as Microsoft Excel® file format using Chemstation software Version B.03.01. Baseline correction routines based on an adaptation of the method described in Ref. [44] for data matrices were kindly provided by H. C. Goicoechea (Ciudad Universitaria, Santa Fe, Argentina). Routines for MCR-ALS are available at http://www.ub.edu/mcr/welcome.htm and all algorithms were written in MATLAB (version 7.2.0.232 R2006a, The Mathworks, Natick, MA).

2.3. Preparation of standards, spiked samples and standard additions

Adequate standard solutions of each individual compound were prepared by dissolving the certain weights in 10 mL of methanol. 1000 mg L^{-1} of stock standard solutions was renewed weekly and kept in dark at −18 °C. All required working standard solutions were prepared daily by diluting the standard solutions with MeOH:ACN (50:50, v/v). All working solutions were stored in the dark at 4 °C and were filtered through membrane PTFE filters (0.45 μm pore size) before injection into the chromatographic system.

Wastewater effluent sample was collected from a sewage treatment plant in Tehran (Iran). Before analysis, it was vacuum-filtered through a 1 μm micro fibre glass filter and then a 0.45 μm membrane filter. The sample was then acidified with hydrochloric acid (pH 3) and finally was kept at 4 °C in a refrigerator in order to minimize microbial degradation.

Seven wastewater aliquots of the sample were spiked at different concentration levels of seven UV filters (containing a non-spike sample) for simulating the real samples (see Table 2). Then, 0.0, 15.0, 25.0, 35.0 and 45.0 μg L^{-1} of BPN, OCR, MBC, OMC, BMP and 0.0, 20.0, 40.0, 60.0 and 80.0 μg L^{-1} of OS and HMS, respectively, were added to five aliquot of each previously fortified sample for calibration using the standard addition strategy. All concentration levels were corresponded to values in the linear range of 0.1–100 μg L^{-1} for BPN, OCR, BMP and OMC, 0.02–80 μg L^{-1} for MBC and 0.4–200 μg L^{-1} for OS and HMS, respectively.

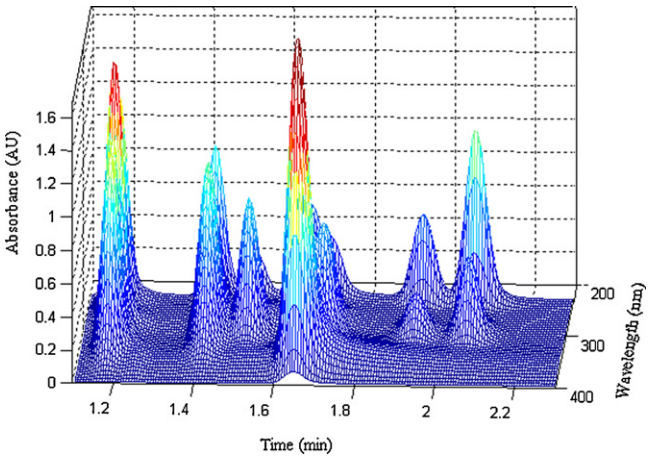

Fig. 2. 3D representation of LC-DAD chromatograms (subset between 1.1 and 2.3 min) of a mixture of UV filters at concentration values of (1) 110, (2) 100, (3) 50, (4) 140, (5) 95, (6) and (7) 200 mg L^{-1} (corresponding to the numbers in Table 3) recorded between 220 and 400 nm.

All samples were shaken vigorously to ensure homogenization and were subjected to the following procedure. Unspiked and one of the spiked samples (w-s6) were prepared and analysed three times to check the repeatability of the analysis.

2.4. SPE procedure

Predetermined weights of NaCl were added to all previous wastewater samples to obtain 5% (w/v) salt content, and samples were filtered through a Nylon membrane filter (0.45 μm) to remove any suspended particulate matter.

Before extraction, the cartridges were conditioned by passing a gentle flow of 5 mL ethyl acetate/dichloromethane (1:1, v/v), followed by 5 mL of methanol and then the same volume of HPLC water. Next, predetermined volume (500 mL) of the real or spiked wastewater sample was passed through the cartridge, at the rate of 6 mL min^{-1}. The sorbent was cleaned up by passing 5 mL of HPLC water and then was dried under vacuum for 5 min. Desorption step was carried out by eluting the sorbent with 3 mL of dichloromethane and 5 mL of methanol consequently. The extracted sample was dried under a gentle stream of nitrogen and re-dissolved in 1.0 mL of mobile phase before HPLC analysis.

3. Results and discussion

3.1. Chromatographic analysis

To obtain a fast chromatographic run, different elution programs were tested for separation of the analytes on the C18 column. Finally a non-aqueous and isocratic composition, methanol–acetonitrile (50:50, v/v), was found to produce a run time 2.5 min. Fig. 2 shows a three dimensional view of the chromatographic run recorded at multiple wavelengths, corresponding to a standard mixture of seven UV filters (in the concentration range between 50 and 200 mg L^{-1} for different analytes). As can be seen from Fig. 2, the analysis time was very short, but it produced a number of overlapping between some of the analytes and so originated data could be conveniently processed using multivariate algorithms.

In the next step, different parameters were considered for preconcentration through SPE. The first was the pH of wastewater samples. There are some reports on increasing extraction efficiency

Table 3
Regions in which chromatographic data were divided.

Analytes (assigned number)	Region	Sensors (data points)	Time region (min)	Retention time (min)
BPN (1)	1	165–195	0.90–1.35	1.17
OCR (2)	2	205–270	1.35–1.80	1.42
MBC (3)	2			1.51
BMP (4)	2			1.65
OMC (5)	2			1.70
OS (6)	3	271–330	1.80–2.18	1.92
HMS (7)	3			2.06

of the SPE procedures for UV-filters using acidic solutions [21]. Comparison of the extraction proceeding using acidic and non-acidic solutions which is supposed to be percolated through the cartridge, acidic ones showed the better recovery values. It can be as a result of prevention of the hydrolysis process and ionization of analytes that reduces their retention on the selected sorbent. So a pH of 3 was maintained throughout the whole work.

Then, the breakthrough volume was studied in order to establish the optimum volume of the water samples that can be passed through the SPE cartridge without significant loss of target analytes. So the same concentration (5 $\mu g\,L^{-1}$) of UV filters in different volumes of Milli-Q water (between 100 and 700 mL) was made and passed through the cartridge under the conditions in Section 2.4. The variation of analyte signals (peak areas at 355 nm for BMP and 305 nm for the rest of the analytes, respectively) along with increasing the sample volume passed through SPE cartridge revealed that there was not any significant curvature until 700 mL of the sample percolation (figure was not shown). Thus, 500 mL were selected as a compromise between the necessary sensitivity (for all analytes) required achieving the UV filter levels in wastewaters and the extraction time, as a critical step in the quantification process.

3.2. Quantification of UV filters in wastewater using second-order calibration

Fig. 1(b) shows chromatogram of an extracted wastewater sample spiked with different amounts of seven analytes (wastewater sample 7, w-s6), recorded at 305 nm. Each analyte is indicated in this figure. Each injection in the specified elution program and spectral detection in the 220–400 nm (with spectral resolution of 1.5 nm) produces a data matrix containing 211 rows (according to 0.9 and 2.3 min) and 121 columns (number of wavelengths).

In order to simplify the analysis, total chromatographic data registered for each sample was partitioned in three regions. As can be seen from Table 3, three regions r-1, r-2 and r-3 with the following compositions can be specified: r-1 contains only the analyte BPN, r-2 contains co-eluted peaks of OCR, MBC, BMP and OMC and r-3 contains co-eluted peaks of OS and HMS, respectively.

3.2.1. Application of background correction

Fig. 1 shows the high complexity of sample matrix and baseline drift. The SPE condition and also the HPLC elution program set in this study could not completely discriminate between analytes and mentioned contaminants of the real sample. This made a large peak at the beginning of chromatogram. The appearance of the baseline drift and co-elution problem between analytes and interferents led to this fact that neither identification nor quantification could be performed using classical univariate calibration. This point is more highlighted with employing fast analysis in which some overlapping between analytes may be appeared.

It has been shown [36,38,43] that background correction is a necessary step before many multivariate data processing methods, since its potential for reducing the complexity of the signals obtained from the real sample is well known. Among the different methods for baseline correction, we used the strategy proposed by Eilers et al. [44] for background elimination in two-dimensional signals based on asymmetric least squares splines regression approach. This method consists of estimating a two dimensional background matrix B (with the same dimensions as standards and sample matrices) using spline basis functions. As a compromise between the speed of calculation and accuracy of results, 10 basis functions, with a single regularization parameter with the value equal to one were used in this study. Details of the implementation of the mentioned method can be found in the literature [44,45].

Since the basis of the mentioned algorithm is a two-dimensional background correction through the estimating a background matrix (with the same dimensions of sample matrix), the effect of this correction is shown in Fig. 3(a) and (b), in the time window of 0.90–1.35 min (the steepest part of the baseline drift) for a

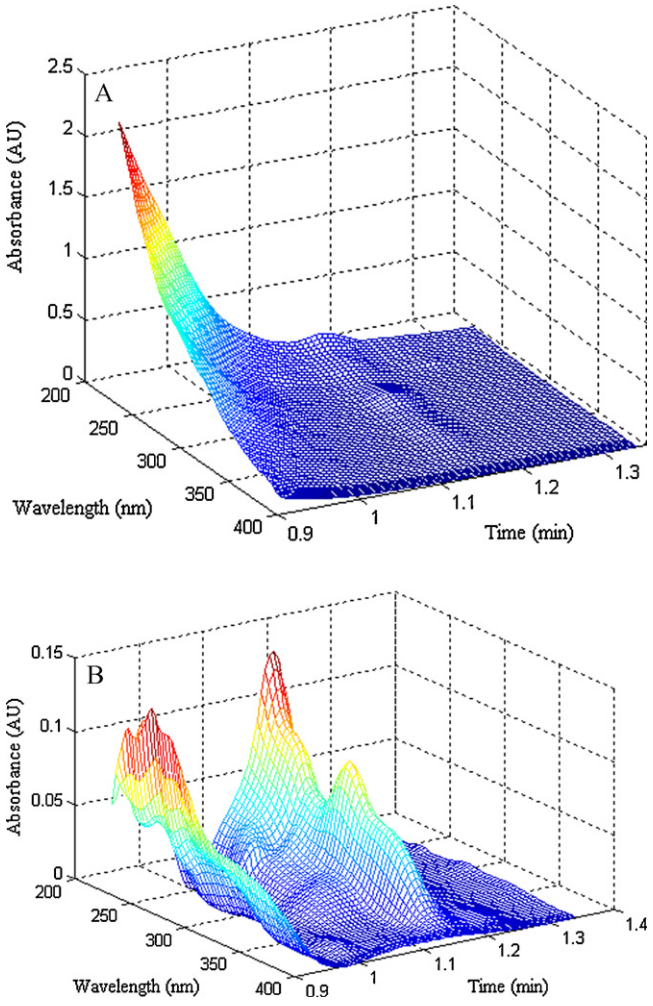

Fig. 3. 3D plots of first sample subset (r-1 in Table 3), corresponding to BPN in (a) spiked wastewater sample (w-s6 in Table 2) and (b) wastewater sample after correcting the background matrix.

landscape corresponding to wastewater sample 7 (w-s6). As it is clear, estimation and elimination of baseline signal has a significant effect on reducing matrix signal complexity. Figures show how important is to estimate a second-order background signal to simplify the signal appearance and consequently further data analysis.

3.2.2. MCR-ALS modelling and the standard addition samples

Presence of matrix effect was statistically confirmed (p-values <0.05) in regions r-1 and r-2 by comparing the slope and intercept of univariate calibration curve built with standards prepared in Milli-Q and wastewater samples. Appearance of unknown interfering compounds in the elution time region of analytes, hinders their direct determination by univariate calibration. Second-order multivariate calibration is known to be a suitable alternative in these cases. So second-order standard addition methodology combined with MCR-ALS [30,31] was implemented in the presented work, in such a way that matrix effect and co-elution problems could be effectively handled. In addition, MCR-ALS is a well-known method for processing of non-trilinear data set, such as three-way data obtained through different runs of HPLC system. Before the application of MCR-ALS, the number of components was estimated by principal component analysis of each matrix region. The algorithm also required an initial estimate of spectral profiles for each sample component. Among different multivariate curve resolution methods, we chose SIMPLISMA (simple interactive self-modelling mixture) methodology which is a pure variable base method and extracts all pure component spectra from a series of mixtures with varying compositions [46].

The column-wise augmented matrix **D** is decomposed through ALS algorithm considering the predefined number of components and a spectral matrix of initial estimate according to the following expression:

$$\mathbf{D} = \mathbf{C} \cdot \mathbf{S}^{\mathbf{T}} + \mathbf{E} \quad (1)$$

The matrix **D** has a number of rows equal to the total number of recorded elution time in different chromatographic runs for training samples ($I \times K$) and the number of columns equals to the considered number of wavelengths (J). The matrices **C** and **S** contain the time and spectral profiles of the N components involved in the process and **E** is a matrix of residuals not fitted by the model. According to standard addition methodology which was considered in the present study, the size of each global data matrix submitted to MCR-ALS analysis was $(I+1)K \times J$. During the ALS fitting, constraints of non-negativity in spectral profiles and non-negativity and unimodality in chromatographic profiles were imposed.

Six wastewater samples (w-s1 to w-s6) were spiked with seven UV filters in different concentration levels corresponding to Table 2 and were used for recovery studies. Then, 0.0, 15.0, 25.0, 35.0 and 45.0 $\mu g\,L^{-1}$ of BPN, OCR, MBC, OMC, BMP and 0.0, 20.0, 40.0, 60.0 and 80.0 $\mu g\,L^{-1}$ of OS and HMS, respectively, were added to five aliquots of each previously fortified sample for calibration using the standard addition method. In order to check whether the analytes were present in this sample, the quantification process was repeated for wastewater sample 1 without spiking the analytes (w-s0). In this way, three non-spiked wastewater aliquots together with four standard addition samples (with the same added concentrations as mentioned above) were prepared and analysed. After partitioning each data matrix into three regions, each region in the test matrix was augmented to the corresponding four standard addition matrices and then submitted to MCR-ALS. In order to check the repeatability, three replicate analyses of this sample were performed accordingly. Finally, the mean predicted concentrations and standard deviations of predicted concentrations were calculated.

The estimated elution profiles of the components eluted in region r-1 (BPN together with two interferences) in spiked test sample 7 (w-s6) and four related standard addition matrices are shown in Fig. 4. As it is clear, there are two matrix components which present sever overlapping with analyte BPN in the selected retention time region. The result of MCR-ALS decomposition in retention time direction indicates an acceptable increasing profile of BPN in the presence of nearly constant profiles of two interferences. In this manner, the quantification of the isolated analyte can be performed through estimated relative peak areas for the ascribed analyte and so a pseudo-univariate standard addition curve can be built.

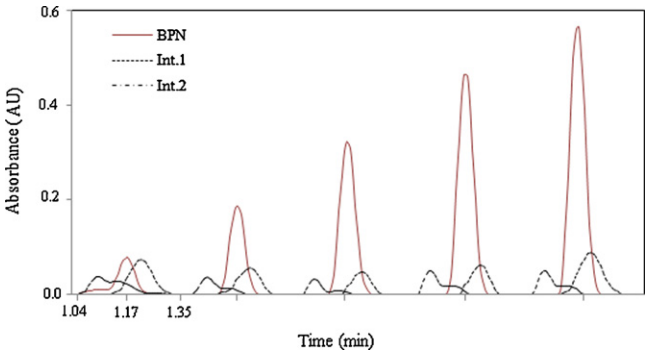

Fig. 4. Successive elution time profiles retrieved by MCR-ALS analysis for region 1 which includes analyte BPN (red solid line) and two interferences (dashed and dash dotted black lines) on sample w-s6 and corresponding four standard addition matrices. The remaining four profiles correspond to four standard addition calibration samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The process was repeated for the other regions and also for all other data sets. The number of estimated components for three regions of r-1, r-2 and r-3 were 3, 5 and 2, respectively. Fig. 5 shows the estimated chromatographic profiles of four analytes eluted in region r-2 and in the presence of one interfering compound for the above mentioned spiked sample. It is necessary to note that the MCR-ALS analysis was performed on the raw data matrices (without applying background correction) and very similar results (statistically confirmed) were obtained for the second and third regions (r-2 and r-3) with 6 and 3 components, respectively. But it was not the case for the region r-1, so that the incorrect results were obtained in this region without eliminating background signals. This fact proves that using a second-order strategy can be a convenient option for quantification purposes in both cases (when it is necessary to eliminate the background signal and when it is not).

The extracted spectral profiles for all analytes are presented in Fig. 6. High correlation coefficient values between real

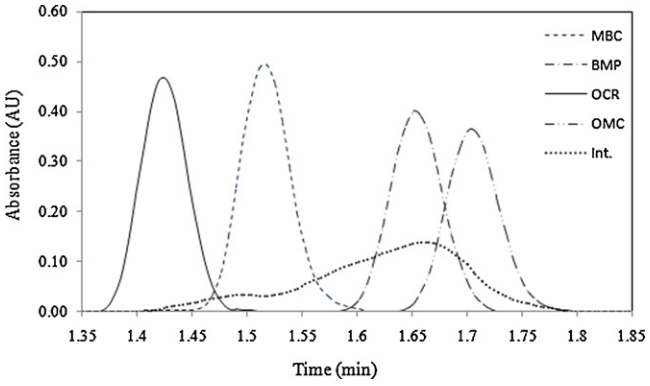

Fig. 5. Estimated elution time profiles retrieved by MCR-ALS analysis for region 2 which includes OCR (solid line), MBC (long dash), BMP (long dash dotted), OMC (long dash dot dotted) and interfering compound (dotted) on sample w-s6.

Table 4
Predicted concentrations using MCR-ALS on a real effluent domestic wastewater spiked with different amounts of UV filters.[a]

Analyte	Predicted concentrations ($\mu g\,L^{-1}$)							
	w-s0[b]	w-s1	w-s2	w-s3	w-s4	w-s5	w-s6	t_{calc} (t-test)[e]
BPN	1.56 [0.19][c]	5.61 (112.2)	3.98 (99.5)	0.76 (76.3)	2.73 (91.0)	14.96 (99.7)	1.87 [11.2][d] (93.5)	$0.95 < t_{crit}$
OCR	2.77 [0.02]	5.68 (113.6)	8.28 (103.5)	0.91 (129.7)	1.57 (78.5)	11.80 (98.3)	2.96 [8.4] (98.6)	$0.53 < t_{crit}$
MBC	6.41 [0.32]	0.56 (112.0)	1.20 (80.0)	15.6 (104.0)	1.95 (97.5)	9.85 (98.5)	5.33 [9.5] (106.6)	$0.05 < t_{crit}$
BMP	7.12 [0.16]	9.58 (95.8)	12.00 (100.0)	1.11 (111.0)	2.67 (89.0)	20.98 (104.9)	5.2 [0.9] (104.0)	$0.25 < t_{crit}$
OMC	10.41 [0.28]	15.6 (104.0)	20.98 (104.9)	2.86 (95.5)	1.57 (78.6)	10.75 –	10.66 [7.3] (106.6)	$0.39 < t_{crit}$
OS	2.33 [0.31]	16.5 (91.6)	14.79 (98.6)	3.91 (78.3)	8.15 (101.8)	15.60 (78.0)	9.80 [4.7] (98.0)	$2.08 < t_{crit}$
HMS	0.43 [0.07]	19.58 (97.8)	15.21 (101.4)	4.94 (98.8)	7.91 (98.9)	16.60 (83.0)	9.93 [3.7] (99.3)	$1.26 < t_{crit}$

[a] Recoveries in parenthesis.
[b] Unspiked wastewater sample.
[c] Pooled standard deviation obtained through concentration prediction of analytes using MCR/ALS modelling of three replicates of non-spiked waste with six series of standard addition matrices.
[d] RSD (%) for three replicates of Sp-6 in square brackets.
[e] $t_{calc} = (\bar{x} - \mu_0)/s/\sqrt{n}$, $\bar{x}$ is the average recovery, μ_0 is 100%, n is the number of measurements and in confidence level of 95%, $t_{crit} = 2.58$ (for OMC, $t_{crit} = 2.78$).

normalized spectral profiles and estimated ones (more than 0.995) for all analytes, confirm the high quality of deconvolution process. Table 4 contains the final results of analysis for seven UV filters in real effluent wastewater sample. Also, the nominal and predicted concentration results corresponding to application of MCR-ALS for determination of seven UV filters in validation samples are summarized in Table 4. As can be seen, there is a good agreement with the nominal concentration values. The recovery values were calculated taking into account the nominal concentration spiked in the wastewater samples and net predicted concentration of each analyte. These values for BPN, OCR, MBC, BMP, OMC, OS and HMS were 76.3–112.2%, 78.5–129.7%, 80.0–112.0%, 89.0–111.0%, 78.6–106.6%, 78.0–101.8% and 83.0–101.4%, respectively. Fig. 7 shows a good visualization of the recovery values computed using the results presented in Table 4. A t-test ($p = 0.05$) was carried out to compare the mean recoveries of seven analytes with the ideal value of 100%. As it is shown in Table 4, all t-values were less than tabulated critical values, so the results are satisfactory and prove that the algorithm could provide accurate results for validation samples. In addition, if elliptical joint confidence region (EJCR) [47] is calculated for the slope and intercept of the plot of predicted vs. nominal concentrations, it can be concluded that the computed ellipse for the global data set (at 95% confidence level) includes the theoretical expected point (1, 0). This fact confirms the absence of both constant and proportional error.

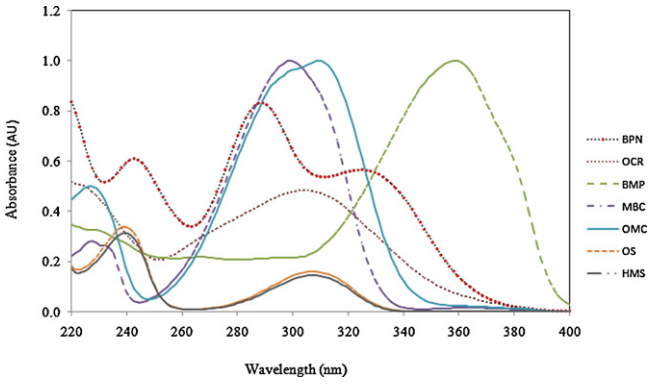

Fig. 6. Spectral profiles retrieved by MCR-ALS for BPN (dotted with red squares), OCR (dotted), MBC (dash dotted), BMP (long dash), OMC (solid line), OS (square dotted) and HMS (long dash dot dotted), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

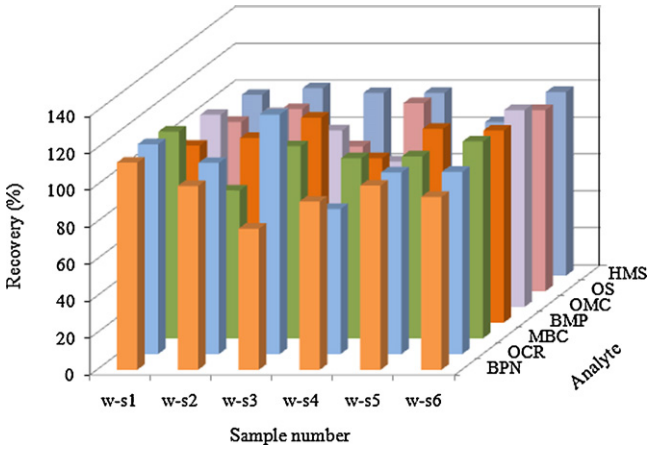

Fig. 7. Bar chart showing the recoveries computed for the seven analytes present in the six validation samples using the results presented in Table 4.

Finally, the calculated relative standard deviations (RSD%) of predicted concentration values for three replicates of sample w-s6 were lower than or equal to 11.2% which can be considered acceptable regarding the complexity of the analytical problems and low concentration values of analysed sample.

4. Conclusion

In the present study we proposed a new methodology for combining solid phase extraction with fast HPLC through defining a non-aqueous and surfactant-free organic mobile phase for effectively quantification of seven UV filters in highly drifted and contaminated domestic wastewater samples.

MCR-ALS algorithm combined with standard addition strategy was used as a powerful second-order algorithm for single and multi-target determination in the presence of matrix constituents by analysing pre-processed and unfolded three-dimensional arrays obtained from HPLC-DAD. As the retention time shift correction of all analytes through different HPLC injections is a hard task and sometimes impossible, applying MCR-ALS algorithm with its main feature in this field can be very efficient for qualitative and

quantitative purposes. The proposed method provided acceptable results for determination of these compounds at low concentrations ($\mu g\,L^{-1}$) in wastewater samples and in the presence of unknown overlapping interferences. Also the figures of merit can be easily calculated, by provision of a blank wastewater sample in a suitable sampling time or region. Considering UV filters as an important class of emerging pollutants, the proposed methodology can be a very promising and valid procedure for effectively quantification purposes in different environmental samples.

Acknowledgments

The authors gratefully acknowledge Dr. Amir Salemi for his useful suggestions during this work. Thanks are extended to Prof. Romá Tauler for providing access to the MATLAB routine for MCR-ALS on the internet.

References

[1] G.J. Nohynek, H. Schaefer, Regul. Toxicol. Pharm. 33 (2001) 285.
[2] D.L. Giokas, A. Salvador, A. Chisvert, Trends Anal. Chem. 26 (2007) 360.
[3] S.D. Richardson, Anal. Chem. 81 (2009) 4645.
[4] H.R. Buser, M.E. Balmer, P. Schmid, M. Kohler, Environ. Sci. Technol. 40 (2006) 1427.
[5] W. Li, T. Li, J. Liu, R. Bai, D.G. Ohandja, F.S. Wong, Water Res. 41 (2007) 3506.
[6] N. Negreira, I. Rodríguez, E. Rubí, R. Cela, J. Chromatogr. A 1216 (2009) 5895.
[7] A. Zenker, H. Schmutz, K. Fent, J. Chromatogr. A 1202 (2008) 64.
[8] J.E. Dinunzio, R.R. Gadde, J. Chromatogr. 519 (1990) 117.
[9] J. Meijer, M. Lodén, J. Liq. Chromatogr. 18 (1995) 1821.
[10] S.C. Rastogi, G.H. Jensen, J. Chromatogr. A 828 (1998) 311.
[11] C.G. Smyrniotakis, H.A. Archontaki, Anal. Chim. Acta 1031 (2004) 319.
[12] T. Kupper, C. Plagellat, R.C. Brändli, L.F. de Alencastro, D. Grandjean, J. Tarradellas, Water Res. 40 (2006) 2603.
[13] D.L. Giokas, V.A. Sakkas, T.A. Albanis, D.A. Lampropoulou, J. Chromatogr. A 1077 (2005) 19.
[14] D.A. Lambropoulou, D.L. Giokas, V.A. Sakkas, T.A. Albanis, M.I. Karayannis, J. Chromatogr. A 967 (2002) 243.
[15] V.A. Sakkas, D.L. Giokas, D.A. Lambropoulou, T.A. Albanis, J. Chromatogr. A 1016 (2003) 211.
[16] M.E. Balmer, H.R. Buser, M.D. Muller, T. Poiger, Sci. Technol. 39 (2005) 953.
[17] T. Poiger, H.-R. Buser, M.E. Balmer, P.-A. Bergqvist, M.D. Müller, Chemosphere 55 (2004) 951.
[18] R. Rodil, M. Moeder, J. Chromatogr. A 1179 (2008) 81.
[19] R. Rodil, M. Moeder, R. Altenburger, M. Schmitt-Jansen, Anal. Bioanal. Chem. 395 (2009) 1513.
[20] W. Buchberger, P. Zaborsky, Acta Chim. Slov. 54 (2007) 1.
[21] D.L. Giokas, V.A. Sakkas, T.A. Albanis, J. Chromatogr. A 1026 (2004) 289.
[22] N. Masqué, R.M. Marcé, F. Borrull, Chromatographia 48 (1998) 231.
[23] E. Comas, R.A. Gimeno, J. Ferré, R.M. Marcé, F. Borrull, F.X. Rius, J. Chromatogr. A 1035 (2004) 195.
[24] V. Gómez, M.P. Callao, Anal. Chim. Acta 627 (2008) 169.
[25] E. Sanchez, B.R. Kowalski, Anal. Chem. 58 (1986) 496.
[26] H.L. Wu, M. Shibukawa, K. Oguma, J. Chemom. 12 (1998) 1.
[27] Z.P. Chen, H.L. Wu, J.H. Jiang, Y. Li, R.Q. Yu, Chemom. Intell. Lab. Syst. 52 (2000) 75.
[28] R. Bro, Chemom. Intell. Lab. Syst. 38 (1997) 149.
[29] H.A.L. Kiers, J.M.F. Ten Berge, R. Bro, J. Chemom. 13 (1999) 275.
[30] R. Tauler, Chemom. Intell. Lab. Syst. 30 (1995) 133.
[31] A. De Juan, R. Tauler, J. Chromatogr. A 1158 (2007) 184.
[32] M. Linder, R. Sundberg, Chemom. Intell. Lab. Syst. 42 (1998) 159.
[33] A.C. Olivieri, J. Chemom. 19 (2005) 253.
[34] K.S. Booksh, B.R. Kowalski, Anal. Chem. 66 (1994) A782.
[35] E. Peré-Trepat, S. Lacorte, R. Tauler, Anal. Chim. Acta 595 (2007) 228.
[36] M.M. De Zan, M.D. Gil García, M.J. Culzoni, R.G. Siano, H.C. Goicoechea, M. Martínez Galera, J. Chromatogr. A 1179 (2008) 106.
[37] M.D. Gil García, M.J. Culzoni, M.M. De Zan, R. Santiago Valverde, M. Martínez Galera, H.C. Goicoechea, J. Chromatogr. A 1179 (2008) 115.
[38] M.D. Gil García, F. Canada, M.J. Canada, L. Culzoni, G.G. Vera-Candioti, H.C. Siano, M. Goicoechea, Martínez Galera, J. Chromatogr. A 1216 (2009) 5489.
[39] M.M. Galera, M.D. Gil García, M.J. Culzoni, H.C. Goicoechea, J. Chromatogr. A 1217 (2010) 2042.
[40] E. Peré-Trepat, A. Hildebrandt, D. Barceló, S. Lacorte, R. Tauler, Chemom. Intell. Lab. Syst. 74 (2004) 293.
[41] S. Mas, G. Fonrodona, R. Tauler, J. Barbosa, Talanta 71 (2007) 1455.
[42] M.J. Culzoni, A.V. Schenone, N.E. Llamas, M. Garrido, M.S. Di Nezio, B.S. Fernández Band, H.C. Goicoechea, J. Chromatogr. A 1216 (2009) 7063.
[43] M. Vosough, A. Salemi, Food Chem. 127 (2011) 827.
[44] P.H.C. Eilers, I.D. Currie, M. Durbán, Comp. Stat. Data Anal. 50 (2006) 61.
[45] M. Daszykowski, B. Walczak, Trends Anal. Chem. 25 (2006) 1081.
[46] W. Windig, J. Guilment, Anal. Chem. 63 (1991) 1425.
[47] A.G. González, M.A. Herrador, A.G. Asuero, Talanta 48 (1999) 729.