Chemometrics-assisted cyclodextrin–enhanced excitation–emission fluorescence spectroscopy for the simultaneous green determination of bisphenol A and nonylphenol in plastics

Rocío B. Pellegrino Vidal, Gabriela A. Ibañez,** Graciela M. Escandar*

Instituto de Química Rosario (CONICET-UNR), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531 (2000) Rosario, Argentina. E-mail: escandar@iquir-conicet.gov.ar

Abstract

The aim of this work was to quantify two relevant priority chemicals, bisphenol A (BPA) and 4-nonylphenol (NP), coupling the sensitivity of fluorescence in organized media and the selectivity of multivariate calibration, measuring excitation-emission fluorescence matrices in an aqueous methyl-β-cyclodextrin solution. The studied priority pollutants are two of the most frequently found xenoestrogens in the environment, and are therefore of public health concern.The data were successfully processed by applying unfolded partial least-squares coupled to residual bilinearization (U-PLS/RBL), which provided the required selectivity for overcoming the severe spectral overlapping among the analyte spectra and also those for the interferents present in real samples. A rigorous International Union of Pure and Applied Chemistry (IUPAC)-consistent approach was applied for the calculation of the limits of detection. Values in the ranges 1–2 and 4–14 ng mL^{-1} were obtained in validation samples for BPA and NP, respectively. On the other hand, low relative prediction errors between 3–8 % were achieved. The proposed method was successfully applied to the determination of BPA and NP in different plastics. In positive samples, after an easy treatment with a small volume of ethanol at 35 $^{\circ}$C, concentrations were found to range from 26 to 199 ng g^{-1} for BPA, and from 95 to 30,000 ng g^{-1} for NP.

1. Introduction

Bisphenol A (BPA) and nonylphenol (NP) are the most often detectable xenoestrogens in environmental samples [1], and have also been identified in a wide variety of other samples such as animal tissues, fish, milk, soft drinks, food containers, plastics, baby bottles, etc [2–5]. BPA is profusely used for the production of epoxy resins applied as protective coatings in food and beverage cans and polycarbonate plastics. The latter, in turn, is used in the manufacture of plastic food containers and water bottles [6]. It was reported that more than *ca*. 2,000 tonnes of BPA are annually released into the environment through domestic and industrial activities under normal conditions of use [7,8]. On the other hand, NP is a degradation product of nonylphenol ethoxylate, which is applied as non ionic surfactant in industrial and agricultural processes [9]. It was corroborated that NP gets into food through miscellaneous pathways and at different stages of food production. This includes as a potential source of contamination, the hydrolysis of the antioxidant *tris*(nonylphenyl)phosphate used as a heat stabilizer in the manufacture of many polymeric food-packaging materials [10]. Although NP is a common degradation product of alkylphenol ethoxylates used as dispersing or stabilizing agents in food-packaging plastics, it is not clear whether this is the source of NP in food [11].

The widespread BPA and NP human exposure is of high concern because these compounds could play a role in reproductive cancers, fertility and other endocrine related problems [4,12,13]. Although in recent years innovative methods based on sensors and biosensors have been reported [7], both liquid and gas chromatography-mass spectrometry (LC-MS and GC-MS) remain the most commonly applied methods for the determination of BPA and/or NP in different types of samples. Further, special attention is given to separation/extraction techniques prior to the chromatographic analysis [12,14–16].

Fluorescence detection for these compounds has been used in some chromatographic methods, either after derivatisation [17,18] or using a mobile phase of high organic content [16,19–22], which increase the sensitivity.

To the extent of our literature search, a direct spectrofluorimetric method for the simultaneous analysis of these relevant drugs in aqueous medium has not yet been reported. This may due to two main facts: 1) their low fluorescence intensities in aqueous solution and 2) the strong overlapping between their fluorescence spectra. Fluorescence spectroscopy is already known to be very useful for developing environmentally friendly analytical methodologies. Therefore, the aim of this work was to develop a new and reliable method for the simultaneous spectrofluorimetric determination of BPA and NP within the framework of green analytical chemistry [23].

As reported in a previous work [24], the fluorescence intensity of both analytes is significantly enhanced in water by the presence of methyl-β-cyclodextrin (M-β-CD), and hence this CD was used as auxiliary reagent for the present study. However, although the organized medium could significantly increase the sensitivity of the method, a selectivity issue arises due to the strong overlapping between the spectra of both compounds. The situation is even more critical if the presence of matrix interferences is considered. In this context, second-order multivariate calibration is a useful tool for improving the selectivity of analytical methods [25]. It allows one to obtain the so-called second-order advantage, an intrinsic property of second-order data which permits analyte quantitation in the presence of foreign components not present in the calibration set of samples.

Thus, the present quantitative analysis was carried out measuring excitation–emission fluorescence matrices (EEFMs) of BPA and NP under optimal working conditions. The tested algorithms were parallel factor analysis (PARAFAC) [26], unfolded partial least squares coupled to residual bilinearization (U-PLS/RBL) [27], and multidimensional PLS

[28] coupled to RBL (N-PLS/RBL). A comparison of these algorithms was carried out, because they are in principle appropriate for dealing with the evaluated data. Since BPA and NP are well-known packaging migrants and contaminants, the feasibility of the proposed methodology was demonstrated through the determination of these compounds in plastic materials of different origin.

2. Experimental

2.1. Reagents and solutions

All reagents were of high-purity grade and used as received. BPA and M-β-CD were purchased from Sigma-Aldrich (Milwaukee, WI, USA). 4-Nonylphenol (NP) was provided by Fluka (Buchs, Switzerland). Methanol was obtained from Merck (Darmstadt, Germany), ethanol was provided by Sintorgán (Bs. As., Argentina) and ethyl acetate by Carlo Erba (Milan, Italy).

Methanol stock solutions of BPA and NP of about 1.00 mg mL^{-1} were prepared and stored in dark flasks at 4 ºC. From these solutions, more diluted methanol solutions (0.050 mg mL^{-1}) were obtained. Working aqueous solutions were prepared immediately before their use by taking appropriate aliquots of methanol solutions, evaporating the organic solvent by use of dry nitrogen, and diluting with ultrapure water from a Millipore system (Molsheim, France) to the desired concentrations. Stock solutions of M-β-CD were prepared in ultrapure water.

2.2. Apparatus

EEFMs were measured on a PerkinElmer LS 55 luminescence spectrometer equipped with a xenon discharge lamp (equivalent to 20 kW for 8 µs duration) and connected to a PC microcomputer, using 1.00 cm quartz cells. Instrumental parameters were: excitation and emission slits 5 nm, photomultiplier voltage 850 V, scan rate 1500 nm min^{-1}. The temperature of the cell holder was regulated using a Lauda (Frankfurt, Germany) Alpha RA8 thermostatic bath.

HPLC was carried out on an Agilent 1200 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump operating at 0.7 mL min^{-1} and a fluorescence detector irradiating at 225 nm and measuring at 306 nm. A Rheodyne injector with a 20.0 µL loop was employed to spread the sample onto a Poroshell 120 EC C18 column (2.7 µm average particle size, 100 mm × 4.6 mm i.d.).

2.3. EEFM calibration and validation sets

A calibration set was constructed by preparing 10 calibration samples following a central composite design (see Table S1 in Supplementary Material). A validation set with 9 randomized validation samples was prepared with the concentrations of BPA and NP reported in Table S1.

Calibration and validation solutions were prepared as follows: aliquots of standard solutions of BPA and NP were simultaneously placed in a 5.00 mL volumetric flask. An appropriate amount of M-β-CD stock solution was added, and finally ultrapure water was added to the mark in order to obtain a final concentration of 1×10^{-3} mol L^{-1} of M-β-CD. EEFMs were collected in the following ranges: 215-285 nm each 0.5 nm (excitation

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wavelengths), and 295-365 nm each 2 nm (emission wavelengths), giving an arrangement of 131×35 data points. Data were saved in ASCII format, and transferred to a PC Sempron AMD microcomputer for subsequent computational treatment.

2.3. HPLC procedure

The proposed method was validated by HPLC, following a modified version of the procedure suggested by Zhou et al. [21]: the mobile phase consisted of ultrapure water (solvent A) and methanol (MeOH, solvent B). Prior to HPLC analysis, both solvents were filtered by vacuum through a 0.22 µm membrane filter (Millipore, Massachusetts, USA). In order to achieve a successful resolution of the analytes in the studied matrices, the following gradient elution program was employed: 0–5.5 min, isocratic elution of 40% solvent A-60% solvent B; 5.5–10 min, linear gradient from 40% solvent A-60% solvent B to 10% solvent A, 90% solvent B; 10–17 min, isocratic elution of 10% solvent A-90% solvent; 27–25 min, back to the initial condition of 40% solvent A-60% solvent B, for the subsequent injection.

2.4. Real samples

Different plastic sources (classified according to their composition) were purchased from local stores. The samples were cut into small pieces, washed five times with 50 mL of ultrapure water and dried. Then, 1 g of each plastic sample and 2.50 mL of ethanol were placed in a flask, and stirred at 35° C for 1 h under reflux. In the case of films wraps, 0.1 g were weighted and treated with 5.00 mL of ethanol, owing to their higher concentration of NP. Due to photosensibility of the analytes, all the procedure was developed in the darkness, by protecting the sample with aluminum foil, and under a nitrogen atmosphere. After cooling

at room temperature, the mixture was filtered with 0.45 µm nylon filter membrane. Then, two aliquots were separated in order to be analyzed by different techniques. For HPLC analysis, 125-250 µL of the solution were evaporated under reduced pressure and the resulting product was dissolved with 500 µL of mobile phase. This solution was then analyzed by liquid chromatography following the experimental conditions previously described. For fluorescence analysis, 1.00-2.00 mL of the extract solution were transferred to a 2 mL volumetric flask and evaporated under reduced pressure. The appropriate amount of stock solution of M-β-CD was added, and finally completed to the mark with ultrapure water (final concentration M-β-CD 1×10^{-3} mol L^{-1}). EEFMs were collected at the same conditions as the calibration and validation samples.

2.5. Chemometric algorithms and software

The theory of the applied algorithms (PARAFAC, U- and N-PLS/RBL) is well documented [25] and a brief description can be found in the Supplementary Material. The routines employed are written in MATLAB 7.0. All algorithms were implemented using the graphical interface of the MVC2 toolbox, which is available on the Internet [29].

3. Results and discussion

3.1. BPA and NP fluorescence behavior

As was demonstrated in a previous work, the low fluorescence intensities of both BPA and NP in water are significantly enhanced by β-CD and some of its derivatives, through the formation of inclusion complexes [24]. Specifically, it was established that the

use of M-β-CD at concentrations which ensure an almost complete complex formation (e.g. $C_{\text{M-β-CD}} > 1 \times 10^{-3}$ mol L^{-1}) represents a suitable strategy to determine both analytes at parts-per-billion levels [24]. In Fig. 1A the relative fluorescence intensities for BPA and NP in aqueous solution and in the presence of M-β-CD can be compared. In the specific case of BPA, it can be appreciated how a virtually non-fluorescent analyte develops a very strong signal in the organized medium.

It was also corroborated that a temperature decrease leads to a slight fluorescence enhancement for both analytes (more marked in the BPA system) while the blank signal is not modified. Therefore, the quantitative experiments were conducted at 5 °C.

As was previously stated, although the use of CD would allow the individual determination of the mentioned analytes at very low concentration levels, the strong overlapping among their excitation and emission spectra hinders their simultaneous fluorescence determination through a usual zeroth-order calibration. For a better visualization of this situation, the corresponding normalized spectra are shown in Fig. 1B. In addition, taking into account the high probability that real samples contain other constituents able to interfere in the fluorimetric analysis, a second-order calibration using EEFMs and algorithms which achieve the so-called second-order advantage was attempted [30].

3.2. Quantitative analysis

3.2.1. Synthetic samples

For building a second-order calibration model, EEFMs were recorded for the calibration samples. The final spectral ranges, selected after a suitable consideration of the regions with maximum signals for these analytes, were 215–280 nm (excitation) and 295-335 nm (emission). Subsequently, validation samples containing the studied analytes at

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concentrations different from those used for the calibration step were prepared and subjected to chemometric analysis (Table S1). It is important to remark that final concentrations included in the known linear fluorescence concentration ranges were: 0-50 ng mL^{-1} for BPA, and 0-150 ng mL^{-1} for the less fluorescent NP, and no attempts were made to establish the upper concentration of the linear ranges.

A group of EEFM data constitute a trilinear three-way array, and thus these matrices could in principle be successfully processed by PARAFAC, a friendly algorithm which provides physical interpretation of the fluorescence profiles of the sample constituents [25]. Nevertheless, the significant spectral similarity between BPA and NP precluded the successful decomposition of the present second-order data, resulting in poor PARAFAC predictions [31].

Therefore, algorithms based on latent variables (U- and N-PLS) were subsequently probed. In contrast to PARAFAC, U- and N-PLS do not render approximations to pure constituent profiles, but these algorithms are flexible enough to cope with systems showing a significant spectral overlapping [25]. The optimum number of factors for modeling the calibration set, obtained applying the cross-validation method described by Haaland and Thomas [27,32], was three and four for U- and N-PLS respectively. When three factors were used with N-PLS, bad results were obtained, showing that the number of latent variables needed by this algorithm was four.

Figure 2 shows the good prediction results corresponding to the application of U- and N-PLS to validation samples. In addition, a recommended test for checking the accuracy of an analytical method is based on the regression of predicted vs nominal concentrations, and the estimation of the so-called elliptical joint confidence region (EJCR) [33]. This test consists of: (1) plotting the elliptical region of mutual confidence (usually at a 95% confidence level) of the slope and intercept for the plot of predicted vs nominal

concentrations in the slope-intercept plane, and (2) checking if the theoretically expected values of slope equal to 1 and intercept equal to 0 are included within the ellipse. When the ideal point is included within the EJCR, this indicates accuracy of the used methodology. In the studied system the ideal (1, 0) point lies inside the EJCR surface when both U- and N-PLS are applied (Fig. 2), suggesting that these algorithms allow for a good prediction of BPA and NP concentrations in validation samples. However, the statistical results (Fig. 2 and Table 1) indicate that U-PLS renders predictions of slightly better quality than N-PLS/RBL. Notice that the limits of detection (LODs) were calculated according to a novel IUPAC-consistent estimator [34], which adopts the form of a detection interval, as shown in Table 1. Further, it is important to remark that these low values were achieved without a pre-concentration step.

When the LODs of the proposed approach are compared with those obtained using a zeroth-order calibration in the presence of M-β-CD ($LOD_{BPA} = 4$ ng mL^{-1} and $LOD_{NP} = 9$ ng mL^{-1}, ref. 24) we can conclude that the present method provides lower detection limits, even when the two analytes are simultaneously determined, highlighting the positive influence of second-order data in both sensitivity and selectivity [25].

3.2.2. Real samples analysis

The suitability of the proposed method was demonstrated through the quantification of BPA and NP in samples that are a source of potential exposure to humans such as food and beverage packages among others. Different procedures have been reported in the literature for the extraction of xenoestrogens from plastic materials. Total plastic dissolution with tetrahydrofurane, dichloromethane or chloroform and subsequent polymer reprecipitation with either ethanol or methanol, or extraction with NaOH have been proposed [18,19,35–37]. These procedures employ significant amounts of organic solvents, and it should be taken into

account that under relatively strong experimental conditions some plastics such as polycarbonates undergo hydrolysis yielding additional BPA amounts [19,35]. Less severe conditions have also been applied using methanol, ethanol, acetonitrile, *n*-heptane, and a cyclohexane/2-propanol mixture [38–44] as extracting solvents. We adopted this latter protocol.

In a first stage, BPA and NP were extracted during 2 hs with different solvents at 55 °C [41] from a polyethylene terephtalate (PET) material selected as a model, and then their concentrations were measured using an HPLC-fluorescence detection standard method [21]. Among the three evaluated solvents, namely methanol, ethyl acetate and ethanol, the latter one showed the best extractive power, which was manifested through the largest recovery. Once ethanol was selected as extractive solvent, the time and temperature of extraction were investigated through a factorial design. For the two assayed temperatures (35 and 55 °C), three extraction times (1, 2 and 3 hs) were probed. It was corroborated that 1 h extraction at 35 °C produced better results.

Table 2 shows the recovered concentrations of BPA and NP in the investigated material under optimal working conditions. Besides, a recovery study was also carried out adding increasing concentrations of both analytes into the sample and subjecting it to the extraction process described above (Table 2). The obtained results in the range of 96–117% suggest satisfactory recoveries, supporting the applied procedure.

Once the extraction process was established, different samples were investigated using the proposed spectrofluorimetric second-order method. The complexity of the real analyzed samples can be appreciated in Fig. 3, which shows EEFM plots for a typical calibration sample and for the extract of one of the investigated plastic materials after the treatment indicated above. The strong spectral interference from the matrix is evident.

However, the physical removal of these interferences is not necessary when using an appropriate second-order calibration methodology.

Preliminary studies showed that N-PLS/RBL did not render satisfactory results when applied to the presently complex samples. The fact that this behavior occurs with real samples is indicative that the problem lies in the matrix. Apparently the algorithm N-PLS/RBL confuses analyte and interference spectra, leading to inadequate predictions. This effect has also been observed in other complex systems [45–47]. Therefore, the chemometric treatment was carried out by applying U-PLS/RBL. In addition to the calibration latent variables, U-PLS required the RBL procedure with three unexpected components in most cases.

Table 3 summarizes the found concentration values of BPA and NP, in ng of analyte per gram of investigated sample, using the proposed method and a reference chromatographic one [21]. Both methods were compared through a paired Student's t-test, and the obtained values ($t = 0.46$ for BPA and $t = 0.16$ for NP, see Table 3) favorably compare with the tabulated values for $n - 1$ degrees of freedom and at a 95% significance level ($t_{\text{crit}(0.05,3)} = 2.35$ and $t_{\text{crit}(0.05,5)} = 2.01$], suggesting that the obtained values are statistically comparable to those provided by the reference method. The statistical equivalence among the obtained values demonstrates the capacity of U-PLS/RBL to cope with interferences from concomitants in the real samples.

The statistical values for the U-PLS/RBL results in real samples are shown in Table 1. The values of LOD, LOQ and RMSEP are expressed in both ng mL^{-1} and ng g^{-1} of solid material, and they show a good precision and an appropriate sensitivity. Nevertheless, sensitivity can be improved, if required, by employing a protocol that includes a higher sample amount and a small extraction volume.

In relation to the expected amount of BPA and/or NP in plastic materials, the reported values in the literature depend on the type of investigated material and also on the applied

extraction method (Table 4). As expected, significant levels of the studied analytes are reported when the working protocol includes a total dissolution of the material.

The BPA and NP levels found in the present work are similar to those reported following a similar extraction procedure, with BPA values not larger than about 200 ng g^{-1}, and high NP recovered concentrations from both polyethylene (PE) and polyvinyl chloride (PVC) films.

4. Conclusions

A sustainable spectrofluorimetric method, suitable for the simultaneous determination of BPA and NP has been proposed. The use of both M-β-CD and second-order calibration allowed these concern and widespread xenoestrogens to be quantified at part-per billion levels without the need of pre-concentration steps. The measured second-order data had a positive impact on the method sensitivity, and specifically the combination with the U-PLS/RBL algorithm was essential to achieve enough selectivity. This allowed their simultaneous determination, resolving the high degree of spectral overlapping of both analytes, and rendering excellent results, even in the presence of non-trivial amounts of interferences from non-targeted organic compounds present in real matrices. The coupling with the U-PLS/RBL algorithm as chemometric tool makes it unnecessary the chromatographic separation of the analytes and the use of clean-up steps for the removal of interfering compounds. As a result, a rapid quantitation is achieved with a non-sophisticated instrument, frequently found in routine laboratories, and avoiding environmentally dangerous organic solvents.

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Table 1

Statistical results for BPA and NP in validation and real samples using EEFMs and the indicated algorithms.

	U-PLS		N-PLS	
	BPA	NP	BPA	NP
Validation samples				
Calibration range (ng mL^{-1})[a]	0 – 50	0 – 150	0 – 50	0 – 150
γ (mL ng^{-1})	3.3	0.9	2.8	0.6
LOD ranges [min-max](ng mL^{-1})	1 – 2	4 – 14	1 – 2	6 – 11
LOQ ranges [min-max](ng mL^{-1})	3 – 6	11 – 41	4 – 7	17 – 34
RMSEP (ng mL^{-1})	3	2	1	7
REP (%)	8	3	4	9
	U-PLS/RBL			
Plastic samples				
γ (mL ng^{-1})	0.6	0.3		
LOD ranges [min-max](ng mL^{-1})	6 – 7	15 – 24		
LOD ranges [min-max](ng g^{-1})	15 – 18	35 – 50		
LOQ ranges [min-max](ng mL^{-1})	17 – 21	45 – 70		
LOQ ranges [min-max](ng g^{-1})	40 – 50	100 – 150		
RMSEP (ng mL^{-1})	2	4		
RMSEP (ng g^{-1})	7	16		
REP (%)	6	3		

[a] No attempts were made to establish the upper concentration of the linear ranges. γ, analytical sensitivity; LOD, limit of detection calculated according to ref. 34; LOQ, limit of quantification calculated as LOD $\times$ 3; RMSEP, root-mean-square error of prediction; REP, relative error of prediction.

Table 2
Extracted concentrations of BPA and NP from a plastic material and recovery (Rec) study.

	BPA			NP		
Added (ng mL^{-1})	HPLC (ng mL^{-1})	Rec (%)	Added (ng mL^{-1})	HPLC (ng mL^{-1})	Rec (%)
0	120	-	0	314	-
25	146	104	100	410	96
50	176	111	200	519	102
100	237	117	300	650	112

Table 3
Determination of BPA and NP in different plastic materials using EEFMs and U-PLS/RBL[a].

	Sample	Material[b]	BPA U-PLS/RBL	HPLC	t^c	NP U-PLS/RBL	HPLC	t^c
1	Water bottle	PET	130(2)	120(2)		325(10)	314(6)	
2	Soda bottle	PET	99(5)	106(2)		365(4)	368(7)	
3	Water bottle	PET	ND	ND		ND	ND	
4	Soda bottle	PET	ND	ND		ND	ND	
5	Ethanol bottle	PE	199(10)	195(6)		375(5)	413(8)	
6	Plastic food tray	PE	66(1)	68(2)		153(8)	162(3)	
7	Bleach bottle	PE	ND	ND		ND	ND	
8	Film wrap	PE	ND	ND		$8.9(3)\times10^2$	$8.8(3)\times10^2$	
9	Disposable spoon	PVC	ND	ND		ND	ND	
10	Water piping	PVC	ND	ND		272(5)	247(7)	
11	Toy	PVC	ND	ND		95(6)	93(3)	
12	Film wrap	PVC	ND	ND		$30(2)\times10^3$	$30(2)\times10^3$	
13	Plastic food tray	PP	ND	ND		ND	ND	
14	Bowl	PC	ND	ND		101(2)	99(4)	
15	Bowl	PC	26(1)	24(1)		115(8)	113(6)	
					0.46			0.16

[a] Concentrations are given ng g^{-1}; experimental standard deviations of duplicates are given between parentheses and correspond to the last significant figure; ND, not detected.
[b] PET, polyethylene terephtalate; PE, polyethylene; PVC, polyvinyl chloride; PP, polypropylene; PC, polycarbonate.
[c] Calculated values when a paired Student's t-test is applied.

Table 4
Selected examples of BPA and NP concentrations found in plastic materials using different extraction and determination methods.

Method	Extraction procedure	BPA	NP	Ref
GC-MS	Cyclohexane partial or total dissolution (PS and PVC) and methanol Soxhlet extraction 20 h		<30–287,000 ng g^{-1} Concentrations of up to 1,400 ng g^{-1} in 85% of the analyzed samples	11
HPLC-FD	THF total dissolution and reprecipitation with ethanol	2,900–20,000 ng g^{-1} (PC); 60,500–290,100 ng g^{-1} (PVC)	27,300 to 1,028,000 ng g^{-1} (PVC)	18
HPLC-FD	DCM total dissolution and reprecipitation with methanol	7,000–57,700 ng g^{-1} (PC baby bottles)		19
HPLC/UV/FD	DCM total dissolution and reprecipitation with methanol	30,000 ng g^{-1} (microwavable PC container)		35
ES	Chloroform total dissolution and NaOH extraction	2,333–9,041 ng mL^{-1}/bottle (baby bottles)		36
HPLC/FD	DCM total dissolution and reprecipitation with methanol	4,000–141,000 ng g^{-1} (PC baby milk bottles)		37
HPLC, GC-MS, LC-MS	Methanol	296 and 345 ng/casing (PC hemodialyzer casings)		38
ES	Methanol	187 ng mL^{-1} (PC drinking bottle); 176 ng mL^{-1} (PC ice bucket); ND in PS condiment box, PP water glass, PET snack box, PS fruit dish[a].		39
GC-MS	Boiling methanol 2 h		<5–1,720 ng g^{-1} (PVC films); <5 ng g^{-1} (PVC dishes)	40
ES	Ethanol, 4 h, 55 °C	40–79 ng mL^{-1} (PC food packages)[a]		41
HPLC/UV/FD	Acetonitrile, 24 h, 60°C	43,000–483,000 ng g^{-1} (PVC stretch films)		42
	n-Heptane 60 min		190–630 ng mL^{-1} (PVC food wraps); ND in PE, PO, nylon and PVDC films	43
HPLC/ED	Cyclohexane/2-propanol (1:1 v/v), overnight, room temperature		$<500,000$–3,300,000 ng g^{-1} (PVC films for food-wrapping)	44

[a] 1.0 gr of each plastic material. ED, electrochemical coulometric-array detection; ES, electrochemical sensor; DCM, dichloromethane; FD, fluorescent detection; ND, not detected; PE, polyethylene; PET, polyethylene terephtalate; PC, polycarbonate; PO, polyolefin; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride; PVDC, polyvinylidene chloride; THF, tetrahidrofurane.

Figure captions

Fig. 1. (A) Excitation and emission fluorescence spectra for BPA (red), NP (blue), and blank (black) in water (solid lines), in the presence of M-β-CD at 20 °C (dashed lines), and in the presence of M-β-CD at 5 °C (dash dot-dotted lines). (B) Normalized excitation and emission fluorescence spectra for BPA (red), NP (blue), and blank (black) in the presence of M-β-CD. $C_{BPA} = C_{NP} = 500$ ng mL^{-1}, $C_{M-\beta-CD} = 1 \times 10^{-3}$ mol L^{-1}.

Fig. 2. Plots for BPA (red) and NP (blue) predicted concentrations using U- and N-PLS in validation samples as a function of the nominal values (the solid lines are the perfect fits), and elliptical joint regions (at 95% confidence level) for the slope and intercept to the regression of the corresponding data. Black points mark the theoretical (intercept = 0, slope = 1) point.

Fig. 3. Three-dimensional plots and the corresponding contour plots of excitation-emission fluorescence matrices for (A) a calibration sample containing 43 and 128 ng mL^{-1} BPA and NP respectively, and (B) a plastic material (PE) after the treatment indicated in the experimental section (C_{BPA} found = 20 ng mL^{-1}, C_{NP} found = 38 ng mL^{-1}). In both samples the final $C_{M-\beta CD} = 1 \times 10^{-3}$ mol L^{-1}.

(A)

Fluorescence intensity

400

300

200

100

0

↑ M-β-CD (5ºC)
M-β-CD (20ºC)
Water

240   280   320   360

Wavelength (nm)

(B)

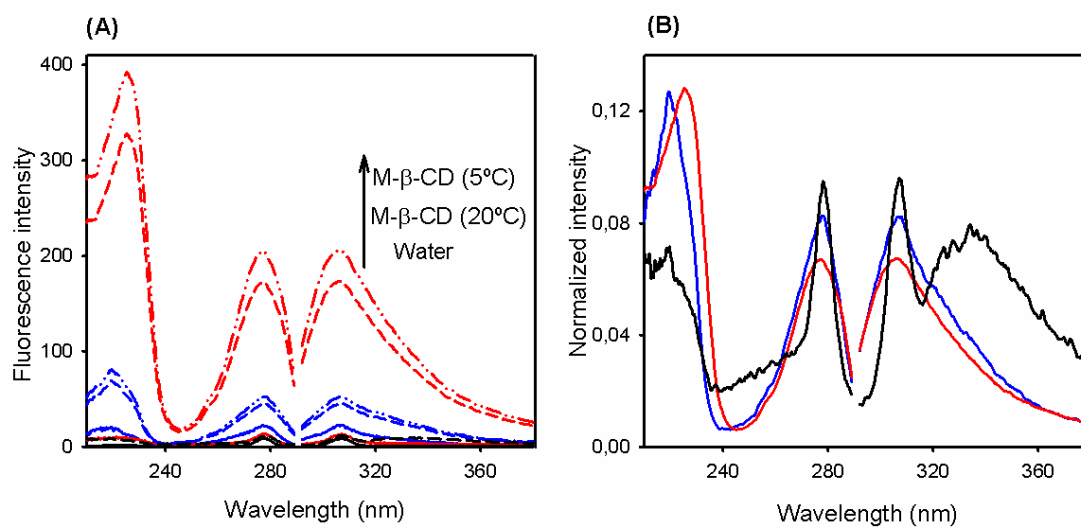Normalized intensity

0,12

0,08

0,04

0,00

240   280   320   360

Wavelength (nm)

Figure 1

Figure 2

Figure 3