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M.M. Arce, M.C. Ortiz, S. Sanllorente

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HPLC-DAD AND PARAFAC FOR THE DETERMINATION OF BISPHENOL-A AND ANOTHER FOUR BISPHENOLS MIGRATING FROM BPA-FREE POLYCARBONATE GLASSES

M.M. Arce[a], M.C. Ortiz[a,1], S. Sanllorente[a]

[a]*Department of Chemistry*

Faculty of Sciences, Universidad de Burgos

Plaza Misael Bañuelos s/n, 09001 Burgos (Spain)

Abbreviations[2]

Abstract

Bisphenol A (BPA) is considered an endocrine disrupting chemical as it affects the human hormonal system and is widely used in polycarbonate (PC) manufacture. As the population is continually exposed to BPA, European regulations have limited its industrial use in cosmetics, thermal paper, toys and food contact materials. Because of these restrictions, other bisphenols are replacing BPA, but they show similar harmful effects as BPA.

In this work, migration of five bisphenols (BPS, BPF, BPA, BPAF, BPZ) from BPA-free PC glasses into simulant B (3% acetic acid (w/v) in aqueous solution) has been determined by means of HPLC-DAD. For this task, twenty glasses were exposed to a migration test at 70°C for 24 hours, more severe conditions than the ones suggested in technical guides. Afterwards, PARAFAC (parallel factor analysis) decomposition, validation of PARAFAC models, and evaluation of CCα (decision limit) and CCβ (detection capability) were carried out.

A study was performed by comparing the population results (concentration), obtained for analytical blanks (n=40) *versus* the ones obtained for the migration test from BPA-free polycarbonate glasses (n=40) by means of the corresponding probability density functions fitted for both populations.

A statistically significant difference between both populations was only found for BPA. BPA migrated from PC glasses with concentration greater than 0.49 μg L^{-1}, for probabilities of false positive (α) and false negative (β) equal to 0.05 and 0.07, respectively. However, the maximum amount of BPA migrated from PC glasses was 5.60

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[1] Corresponding author. Telephone number: 34-947259571. *E-mail address*: mcortiz@ubu.es (M.C. Ortiz).

[2] Bisphenol A (BPA); bisphenol AF (BPAF); bisphenol F (BPF); bisphenol S (BPS); bisphenol Z (BPZ); decision limit (CCα); detection capability (CCβ); endocrine disrupting chemical (EDC); food contact material (FCM); parallel factor analysis (PARAFAC); polycarbonate (PC); specific migration limit (SML).

µg L^{-1}, a value lower than the established limit for the specific migration of a non-authorised substance (10 µg kg^{-1}).

Keywords: bisphenols; migration; BPA-free glass; HPLC-DAD; PARAFAC; Food Contact Materials

1. Introduction

From 2001, bisphenol A (BPA) is considered an endocrine disrupting chemical (EDC), which was classified in group 1 as a substance with evidence of endocrine disruption [1].

EDCs are compounds that can affect the human hormonal system, and in consequence, can cause serious detrimental health effects. These substances are contained in foodstuffs, cosmetics, personal care products, etc, all of them daily use [2].

Several authors have developed studies about the diverse exposure sources for BPA: i) clothing items such as pantyhose [3] and socks [4]; ii) a wide variety of foods that are part of a normal diet [5]; iii) food packaging made of different materials [6,7]; iv) personal care products [8]; v) ambient sources such as dust in the home [9] or air in the workplace [10]; vi) other types of samples such as: toys [11], liquid medicines (syrups and injectables) [12], tickets (thermal paper) [13], printing inks [14], dental material [15] or medical devices [16]. In addition, the exposure of animals to BPA should also be taken into account, since bioaccumulation along the food chain can represent an additional source of BPA for humans [17].

For decades, concern about the continuous exposure to EDCs (and more specifically to BPA) and the adverse effects derived from it has grown very quickly. Thus, European regulations have limited the industrial use of BPA in cosmetics [18], thermal paper [19], toys [20] and food contact materials (FCMs) [21]. In order to avoid these restrictions, other compounds are replacing BPA. Other bisphenols can be used in FCMs [22], although migration of bisphenol S (BPS) is limited since 2011 [23].

In the bibliography consulted, it is noteworthy the wide variety of BPA substituents which were determined in different samples: foodstuffs [5], FCMs [24], thermal paper [13], source water and drinking water [25], human blood serum [26]. Nevertheless, a large number of investigations [27,28,29,30] showed that other bisphenols display similar effects as BPA, considering bisphenol AF (BPAF) more toxic and BPS less detrimental in comparison to BPA. Moreover, the European Commission suggested in 2019 that substances with a similar structure should be assumed to have toxicological properties as harmful as those of the most toxic known substance in the group [31]. That document also explained that BPA is being replaced by other bisphenols, and demanded the ban of bisphenols in all FCMs.

BPA is a chemical compound widely used in the manufacture of epoxy resins and polycarbonate (PC). Due to the ban of BPA for PC infant feeding bottles in 2011 [32], nowadays different PC products are being marketing as BPA-free. Nevertheless, this thus not mean bisphenol-free or endocrine disruptor activity-free.

In this work, determination and quantification of BPA and another four bisphenols: BPS; bisphenol F (BPF); BPAF and bisphenol Z (BPZ) were carried out. BPA-free PC glasses, purchased in a local kitchen supply store, were subjected to a migration test.

Several analytical techniques have been used by other authors for the determination and quantification of BPA, such as molecular spectrofluorimetry from excitation-emission data matrices (EEMs) [33] or high performance liquid chromatography coupled to a fluorescence detector (HPLC-FLD) [11]. Simultaneous determination of BPA and other bisphenols has been carried out through gas chromatography-mass spectrometry (GC-MS) [34], ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) [25] or high performance liquid chromatography coupled to a diode array detector (HPLC-DAD) [13]. Regulation in force [35] suggests HPLC-DAD or HPLC-FLD in order to determine BPA, and that is why the previously optimized HPLC-DAD method [36] for the separation of the five selected bisphenols was chosen for the present work. With this fast method, the five bisphenols are eluted in less than 4 min, which allows saving time and reagents.

Once experimental chromatographic data were obtained, several three-way algorithms can be used with chromatographic signals. Multivariate curve resolution coupled to alternating least squares (MCR-ALS) has been widely applied in analytical chemistry and its related fields [37,38], and it has been used to solve coeluted compounds. Its major limitation in identifying and quantifying an analyte is the presence of rotational ambiguities and non-unique solutions. However, the non-uniqueness problem can be alleviated or totally avoided in some cases through the intelligent use of the data structure and appropriate constraints when MCR-ALS is used. On the other hand, previous works have demonstrated the usefulness of three-way calibrations based on the PARAFAC (parallel factor analysis) decomposition using chromatographic data obtained with different detectors that provide multivariate signals (mass spectrometers or diode array detectors) [37,39]. PARAFAC versus MCR-ALS respects the position of the data with respect to neighbouring data within the data cube. In addition, it is flexible for common problems in chromatography such as peak shift, using PARAFAC2 in the latter case. Another advantage facing other second-order algorithms is the unique solution that PARAFAC decomposition provides. For this reason, PARAFAC decomposition was used for the quantification and unequivocal identification of bisphenols, as well as for the detection of possible interferents that can be present in the migration samples.

As technical guidelines describe [40], glasses are considered kitchenware that can be used with cold, hot or room temperature foodstuff. Migration tests can be carried out in two different conditions: at 40°C for 24 hours or at 70°C during 2 hours, simulating different foodstuff temperatures. Moreover, the simulant to be selected for the migration test will be different depending on the foodstuff with which the glass is to be filled up [23]. Finally, migration tests were carried out with simulant B, acetic acid 3% (w/v), whose choice was based on a previous work of our investigation group which showed that a higher quantity of BPA migrated with simulant B than with D1 [41]. As for time and temperature of exposure, more severe conditions than the ones suggested in technical guides were chosen (70°C for 24 hours). Based on other migration experiments previously carried out for 2 hours (as is indicated in technical guidelines [40]) and 10 hours in which none bisphenol had been detected, severe conditions were imposed to know if in situations of higher temperatures or longer periods of time than those established by the regulations in force, the different bisphenols could migrate.

One of the purposes of this work was to check that the possible amount of migrated BPA did not exceed the established specific migration levels. Specific migration limit (SML) is set at 50 μg kg^{-1} [21], except for articles intended for children, in which case the use of BPA is banned and only a maximum migrated amount of 10 μg kg^{-1} is authorised [23]. On the other hand, knowing if BPS, BPF, BPAF and BPZ, the alternatives to BPA, were present in the analysed glasses would be useful. For this purpose, comparisons between populations (concentrations) obtained from analytical blanks and migration test samples were done.

2. Material and methods

2.1. Chemicals and reagents

4,4′-isopropylidenediphenol (BPA, CAS no. 80-05-7), 4,4′-methylenediphenol (BPF, CAS no. 620-92-8) and 4,4'-cyclohexylidenebisphenol (BPZ, CAS no. 843-55-0) were purchased from Sigma-Aldrich (Steinheim, Germany). 4,4'-(hexafluoroisopropylidene)diphenol (BPAF, CAS no. 1478-61-1) was supplied by Alfa Aesar (Kandel, Germany). 4,4′-sulfonyldiphenol (BPS, CAS no. 80-09-1), acetonitrile (CAS no. 75-05-8; LiChrosolv® isocratic grade for liquid chromatography) and methanol (CAS no. 67-56-1; LiChrosolv® isocratic grade for liquid chromatography) were obtained from Merck (Darmstadt, Germany). Glacial acetic acid (CAS no. 64-19-7; HiPerSolv Chromanorm for HPLC) was acquired in VWR Prolabo Chemicals (Fontenay-sous-Bois, France). Deionised water was obtained by using the Milli-Q gradient A10 water purification system from Millipore (Bedford, MA, USA).

2.2. Standard solutions and samples

Standard solutions. Individual standard stock solutions of 500 mg L^{-1} were prepared by dissolving each standard in methanol. For each bisphenol, solutions of 100 mg L^{-1} were prepared from their respective stock solution by dilution with methanol. Calibration standard solutions, which were mixtures of the five bisphenols at twelve different concentration levels in the range of 0 to 5 mg L^{-1} (0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, 4 and 5 mg L^{-1}), were prepared by dilution with methanol. All solutions were stored protected from light at 4°C.

Migration test samples. For migration test samples, a volume of 170 mL of simulant B (3% acetic acid (w/v) in aqueous solution) was used as food simulant in every glass, which was always preheated in an oven at 70°C. As technical guidelines state [42], simulant B reached up to approximately 0.5 cm from the edge of the glasses (articles intended to be filled). Twenty PC glasses were exposed to migration test at 70°C in the oven for 24 hours. The glasses were previously rinsed without soap or detergent in order to remove any traces of dust and dirt. Once the migration test has ended, migration extracts were collected after migration testing in 250 mL amber glass canisters and were stored at 4°C. No damage, breaks or cracks were observed in the visual inspection of the glasses after the migration procedure. Finally, each of the extracts was concentrated to dryness in a rotary evaporator at a pressure of 72 mbar and the residue was dissolved in 5 mL of methanol.

Analytical blanks. Twenty analytical blanks were prepared reproducing the whole procedure, without the migration test step, that is, volumes of 170 mL of simulant B were concentrated to dryness in the rotary evaporator and residues were dissolved in 5 mL of methanol.

Recovery. In order to calculate the recovery of the whole procedure, three mixture solutions (0.2, 0.3 and 0.4 mg L^{-1} of each bisphenol) were prepared by dilution with simulant B. As in the previous case, 170 mL of each solution were exposed to the pre-concentration procedure (rotary evaporator and dissolution in methanol). After the pre-concentration procedure, the collected extracts were diluted in methanol (volumes of 800, 500 and 200 µL in 5 mL flasks).

Hence, a total of 136 analyses were carried out: 20 analytical blanks, 20 migration test samples, 12 recovery samples and 12 calibration standards, all of them analysed in duplicate and also 8 control samples (methanol blanks).

2.3. Instrumental

Simulant B was preheated before extractions in a 200209 JP Selecta oven (Barcelona, Spain). Migration tests samples were obtained using a Heratherm OMS180 Thermo Fisher Scientific oven (Langenselbold, Germany). Sample pre-concentration was carried out using a rotary evaporator at a pressure of 72 mbar (ILMVAC, Ilmenau, Germany). An Ultrasonic Cleaner (VWR International BVBA, Leuven, Belgium) was employed for dissolving the residue obtained from the rotary evaporator.

Analysis of bisphenols was carried out using an Agilent 1260 Infinity HPLC chromatograph (Santa Clara, CA, USA) consisting of a quaternary pump (G1311C), a sampler (G1329B), a thermostatic column compartment (G1316A) and a diode array detector (G7117C). A Kinetex EVO-C18 column (150 mm × 4.6 mm, 5 µm) was used for the separation. Deionized water, methanol and acetonitrile were used as mobile phases.

The conditions for chromatographic analysis (flow and composition of the mobile phase) were optimized in Ref. [36]. Separation was carried out using an isocratic mobile phase of water (A) 22%, methanol (B) 58% and acetonitrile (C) 20%, at a flow rate of 0.66 mL min^{-1}. The temperature of the column compartment was fixed at 20°C and the injection volume was 10 µL. The diode array detector recorded the absorbance between 200 and 500 nm, each 2 nm, during the analysis, for 10 min. Fig. S1 of the Supplementary Material shows an example of the chromatogram obtained in HPLC-DAD analysis of a calibration standard recorded at a wavelength of 224 nm.

2.4. Software

The data were recorded through OpenLab CDS ChemStation software. PARAFAC decompositions were carried out using the PLS_Toolbox [43] for MATLAB [44]. Regression models and accuracy lines were fitted using STATGRAPHICS Centurion 18 [45], which was also employed for the fitting of the density function of the experimental populations (analytical blanks and migration test samples). Decision limit (CCα) and detection capability (CCβ) were determined using the DETARCHI program [46].

3. Theory and methodology

3.1. Parallel factor analysis (PARAFAC)

With a HPLC-DAD instrument, matrices of values of absorbance (x_{ijk}) are recorded at J wavelengths and I elution times for K samples. These matrices can be arranged in a three-way array ($I \times J \times K$), $\underline{\mathbf{X}}$, and decomposed with PARAFAC (parallel factor analysis) technique [47]. PARAFAC is a chemometric technique with the second-order advantage, which means that, under trilinearity of the data array, a PARAFAC decomposition of $\underline{\mathbf{X}}$ gives a unique estimation of the chromatographic, spectral and sample profiles. The trilinear PARAFAC model can be written as in Eq. (1) [48].

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk} \;, \quad i = 1, 2, \ldots, I; \quad j = 1, 2, \ldots, J; \quad k = 1, 2, \ldots, K \qquad (1)$$

where F is the number of factors; $\mathbf{a}_f$, $\mathbf{b}_f$ and $\mathbf{c}_f$ are the loading vectors of the chromatographic, spectral and sample profiles, respectively; and e_{ijk} is the residual of the fitting model.

The core consistency diagnostic, known as CORCONDIA index [49], measures the trilinearity degree of the experimental data array, being 100 the maximum value achievable. The uniqueness property makes possible the unequivocal identification of analytes, even if interferents that share retention time with the analytes of interest are present. In this work, the unequivocal identification was verified by calculating the correlation and similarity coefficients between the spectral profiles from PARAFAC decomposition and the spectra from a reference sample of each bisphenol. Moreover, in the chromatographic profile, analytes were identified by the coincidence of the experimental retention time of each bisphenol and the chromatographic profile obtained in the PARAFAC decomposition.

3.2. Comparison between populations

In order to ensure that the migrated quantity of the different bisphenols in the test samples were different from those obtained in analytical blanks, authors proceeded to compare populations (concentrations).

It would not be correct to compare the average values because of the concentration migrated of each polycarbonate glass was different from each other since each one must be subjected to the migration test. The aim was to ensure that the concentrations in a population of PC glasses were different from those found in another population of analytical blanks.

This comparison has been made, by means of the corresponding probability density functions fitted for both populations (analytical blanks and migration test samples) by using the concentration computed with the sample loadings of PARAFAC decomposition.

Using a hypothesis test whose null hypothesis was 'There is no bisphenol in the sample' *versus* the alternative hypothesis 'There is bisphenol in the sample', a critical value (region of rejection of H_0) has been established for the probability of false positive (α) equal to 0.05, and a probability of false negative (β) has been determined for each bisphenol. Values above this critical value can be considered different from the analytical blanks.

4. Results and discussion

4.1. PARAFAC models

The samples analysed were arranged in four arrays (one of them was jointly employed for the bisphenols BPF and BPA) of size ($I \times 151 \times 136$). I value corresponds to the number of recorded elution times for the selected window for each bisphenol (as can be seen in Table 1). On the other hand, 151 corresponds to the number of wavelengths (between 200 and 500 nm) and 136 are the analysed samples (described in Section 2.2.).

PARAFAC models were fitted with unimodality constraint in the chromatographic profile, non-negativity constraint in the spectral profile and without constraint in the sample profile. As can be seen in Table 1, models had at least a CORCONDIA index of 99% and the explained variance greater than 89.59% in the four cases. Chromatographic, spectral and sample profiles of the four PARAFAC models are shown in Figures 1 to 3.

As can be observed in Fig. 1, the interferents from the first and second PARAFAC models, which co-eluted with BPS and BPA respectively, had quite big chromatographic loadings. Utilization of PARAFAC technique allowed the detection of those substances and their differentiation from the analytes of interest, which involved avoiding over-quantification and therefore decreasing the probability of giving false positives (since if a univariate calibration had been used, the peak area of the corresponding bisphenol would have been increased).

Those factors from PARAFAC models, depicted in grey, did not correspond to any bisphenol. As can be seen in Fig. 2, their spectra were very different from the ones obtained for BPS, BPF, BPA, BPAF and BPZ.

Fig. 3 shows the sample loadings of the 136 analyses carried out. As can be observed, similar loadings were obtained for analytical blanks and migration test samples. In order to know if bisphenols really migrated from PC glasses, and if their samples could be distinguished from the analytical blanks, a statistical comparison between both populations (each of them formed by 40 data) was done.

4.2. Unequivocal identification

The unequivocal identification of the five bisphenols was guaranteed following two confirmatory criteria laid down for HPLC-DAD:

i) The retention time of the analyte, obtained from the chromatographic profile of PARAFAC model, shall be the same as that of the reference sample, within a margin of ± 2.5%.

ii) The spectrum of the analyte, obtained from the spectral profile of PARAFAC model, shall match with that of the reference sample, within a margin in the absorption maxima of ± 2 nm, as is indicated in Ref. [50].

The retention times of bisphenols in the reference samples matched with the ones from PARAFAC models (values shown in Table 2). In this work, the unequivocal identification in the spectral profile was made by means of: i) the correlation coefficient between the vector of spectral profile of a reference sample and the one obtained from the PARAFAC model for this profile and ii) the similarity coefficient calculated via the cosine of the angle between these two vectors [51,52]. Spectra of the reference samples are shown in Fig. 2. The correlation and similarity coefficients are shown in Table 2. Correlation coefficients were all very close to 1 and similarity coefficients were higher than 0.95, which implies that each pair of vectors can be considered equal [53]. Therefore, both criteria are fulfilled.

4.3. Performance criteria

4.3.1. Calibration and accuracy lines

Calibration lines "sample profile loadings *versus* true concentration" were fitted for each bisphenol with the seven standard solutions which were in the range from 0 to 1.5 mg L^{-1} (all concentration levels were replicated). Table 3 shows these regression models, which were significant (p-value was less than 10^{-4}, being the null hypothesis of this test H_0: Regression is not significant). Except for one model the p-value for the lack-of-fit test (H_0: There is not lack of fit in the model) were above 0.05, therefore there is no evidence to reject the H_0.

Trueness and precision were verified using the accuracy lines "predicted concentration *versus* true concentration", whose results are shown in Table 3. P-values for the elliptical joint confidence region test for intercept and slope of the accuracy lines were computed at 95% of confidence level [54] (H_0: Intercept equal to zero and slope equal to one) and are showed in row 10 in Table 3. As can be observed, all p-values are 1, that is, it is not possible to reject H_0 therefore it is possible to conclude that the procedure is unbiased. The precision of the method could be estimated from the residual standard deviations (s_{yx}) shown in row 9 in this table.

4.3.2. Decision limit and detection capability

Decision limit (CCα) and detection capability (CCβ), terms defined by European regulations concerning the performance of analytical methods [50,55], were determined with probabilities of false positive (α) and false negative (β) fixed at 0.05. The calculated values for this work are shown in rows 11 to 12 in Table 3. The analytical method enabled the quantification of BPS, BPF, BPA, BPAF and BPZ, above 92.3, 61.4, 44.0, 138.9 and 65.9 µg L^{-1} (for $\alpha = \beta = 0.05$) respectively.

4.3.3. Recovery of the procedure

Recovery of the procedure was only calculated in nine out of the twelve recovery samples analysed. The exclusion of the other three was because their sample loadings from PARAFAC models were outside the calibrated concentration range (as can be seen in Fig. 3).

For recovery, new calibration lines were fitted for each bisphenol with the ten standard solutions which were in the range from 0 to 3 mg L^{-1} (all concentration levels were replicated). It was found that one replicate of standard 3 mg L^{-1} BPAF solution was an outlier (studentized residual equal to -3.89) and the model for BPAF was redone without it. These new calibration lines were validated, in the same way as is described in Section 4.3.1.

Recovery results obtained for this work are shown in Table S1 of the Supplementary Material. These values were calculated for three dilutions (800, 500 and 200 µg L^{-1}) of three mixture solutions (0.2, 0.3 and 0.4 mg L^{-1} of each bisphenol), which were analysed in duplicate. With the pre-concentration procedure used, a good recovery was obtained for the five bisphenols, as their average values were all above 95%.

4.4. Migration testing

In order to quantify the amount of each bisphenol that migrates from the polycarbonate glasses, a migration test was carried out, as has already been mentioned, at 70ºC in an oven for 24 hours using simulant B. In this way, twenty migration test samples were obtained to which the pre-concentration procedure was applied. This same procedure was carried out with the twenty analytical blanks (all described in Section 2.2.).

Once the twenty migration test samples and the twenty analytical blanks were analysed in duplicate, sample loadings from PARAFAC decomposition were obtained (depicted in Fig. 3). With these loadings and the calibration lines explained in Section 4.3.1. the concentration of each bisphenol present in the pre-concentrated samples was obtained. The statistical summary of both populations (analytical blanks, n=40, and migration test, n=40) is shown in Table S2 of the Supplementary Material.

Afterwards, distributions that best fitted to the data for each bisphenol and for each population (analytical blanks and migration test samples) were searched. Columns 1 to 5 in Table S3 of the Supplementary Material show type and parameters that define the selected distributions. Those fittings between the chosen cumulative distributions (depicted by curves in blue and red) and the data of the populations (displayed by squares in blue and red) are shown in Fig. S2 of the Supplementary Material.

Once the distributions were defined, a hypothesis test was carried out. The null hypothesis of the test was posed in the population of analytical blanks (H_0: There is no bisphenol in the sample) *versus* the alternative hypothesis (H_a: There is bisphenol in the sample). For a probability of false positive (α) equal to 0.05, the critical value is obtained, then probability of false negative (β) was evaluated. The values obtained for both probabilities and the critical value for each distribution are shown in columns 6 to 8 in Table S3 of the Supplementary Material. These results can also be described in a graphical way as is shown in Fig. 4 (A-E).

From the five bisphenols analysed, a statistical difference between analytical blanks and migration test samples was only found for BPA, specifically for α equal 0.05, the critical value was 0.0162 mg L^{-1} and β equal to 0.07. In this case, it could be asserted that BPA truly migrated from glasses, for samples whose calculated concentration was above this critical value.

Applying the factors of pre-concentration and recovery, the critical value for BPA was 0.49 µg L^{-1} and minimum and maximum of the population of migration test samples for BPA were -0.51 and 5.60 µg L^{-1}, referring to the concentration in the original migration matrix. Therefore, the maximum quantity of migrated BPA, 5.60 µg L^{-1}, was much lower than the SML (50 µg kg^{-1}) [21]. In addition, considering that these glasses can be intended for children, in which case the use of BPA would be banned, 5.60 µg L^{-1} neither reached the 10 µg kg^{-1} limit established for a non-authorised substance [23].

However, confirmation of migration of BPS, BPF, BPAF or BPZ from the PC glasses could not be ensured since the fitted distributions for the concentrations found in the migration test samples were not statistically different from those of the analytical blanks. This can be seen in values of the probabilities of false negative β, which range from 0.64 to 1.00 (see Table S3 of the Supplementary Material) and in the overlapping of the distributions, shown in Fig. 4.

5. Conclusions

BPA-free polycarbonate glasses were subjected to severe migration conditions (at 70°C for 24 hours) in order to determine the migrated quantity of the five bisphenols considered. Samples were analysed by means of HPLC-DAD and PARAFAC decompositions were carried out to guarantee the unequivocal identification of analytes. The procedure applied for the determination of five bisphenols has saved time and money in each analysis.

A statistically significant difference between both populations was only found for BPA. BPA migrated from PC glasses with concentration greater than 0.49 µg L^{-1}, for probabilities of false positive (α) and false negative (β) equal to 0.05 and 0.07, respectively. The maximum quantity of BPA migrated from the PC glasses was 5.60 µg L^{-1}, an amount much lower than the established limit for the migration of a non-authorised substance, 10 µg kg^{-1}.

Conflict of interest

None.

Acknowledgments

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Table 1. Parameters of the models obtained by means of PARAFAC decomposition.

Analyte	Time window (min)	Size (*I* x *J* x *K*)	CORCONDIA (%)	Number of factors	Explained variance (%)
BPS	2.15 - 2.30	46 x 151 x 136	100	2	95.30
BPF and BPA	2.35 - 2.90	166 x 151 x 136	99	3	89.59
BPAF	3.15 - 3.40	76 x 151 x 136	100	2	93.62
BPZ	3.65 - 3.95	91 x 151 x 136	99	2	97.98

Table 2. Performance criteria for the unequivocal identification of bisphenols.

Analyte	Retention time (min)		Coefficients	
	Reference sample	PARAFAC	Correlation	Similarity
BPS	2.186	2.186	0.9997	0.9997
BPF	2.460	2.463	1.0000	1.0000
BPA	2.773	2.776	0.9992	0.9993
BPAF	3.263	3.263	1.0000	1.0000
BPZ	3.793	3.800	1.0000	1.0000

Table 3. Performance criteria of the analytical method. Parameters of calibration and accuracy lines (s_{yx} is the standard deviation of regression). Decision limit and detection capability (for $\alpha = \beta = 0.05$). Both CCα and CCβ expressed in μg L^{-1}.

		BPS	BPF	BPA	BPAF	BPZ
Calibration line	Intercept	0.002	-0.001	-0.002	-0.001	-0.001
	Slope	0.032	0.034	0.034	0.032	0.036
	Correlation coefficient	0.9985	0.9993	0.9996	0.9964	0.9992
	s_{yx}	0.001	0.001	0.001	0.001	0.001
	P-value (H_0: Regression is not significant)	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
	P-value (H_0: There is no lack-of-fit)	0.003	0.564	0.133	0.828	0.046
Accuracy line	Intercept	$-1.3\ 10^{-5}$	$-2.7\ 10^{-5}$	$1.8\ 10^{-6}$	$-3.6\ 10^{-6}$	$1.4\ 10^{-5}$
	Slope	1.000	1.000	1.000	1.000	1.000
	s_{yx}	0.030	0.020	0.015	0.046	0.022
	P-value (H_0: Intercept = 0 and slope = 1)	1.000	1.000	1.000	1.000	1.000
CCα		46.0	30.5	22.3	71.6	33.2
CCβ		92.3	61.4	44.0	138.9	65.9

FIGURE CAPTIONS

Figure 1. Loadings from the chromatographic profiles of the four PARAFAC models. BPS is represented in orange, BPF in yellow, BPA in purple, BPAF in blue, BPZ in green, and all the detected interferents in grey.

Figure 2. Loadings from the spectral profiles of the four PARAFAC models: A) for BPS, represented in orange, B) for BPF and BPA, in yellow and purple respectively, C) for BPAF, in blue, and D) for BPZ, in green. All the detected interferents are shown in grey. Additionally, spectra from the respective reference samples are depicted in the same colours.

Figure 3. Loadings from the sample profiles of the four PARAFAC models: A) for BPS, represented in orange, B) for BPF and BPA, in yellow and purple respectively, C) for BPAF, in blue, and D) for BPZ, in green. All the detected interferents are shown in grey.

Figure 4. Density functions, related to distributions described in Table S3 of the Supplementary Material, of the analytical blanks (blue colour) and the migration test samples (red colour): A) BPS, B) BPF, C) BPA, D) BPAF and E) BPZ. Data correspond to the concentration (mg L^{-1}) of bisphenol present in the pre-concentrated samples. Probability of false positive (α) equal to 0.05 (area coloured in blue), critical value (dashed vertical line) and probability of false negative (β, area coloured in red).

Determination of 5 bisphenols from BPA-free polycarbonate glasses using HPLC-DAD

There is statistical difference between blanks and migration test samples for BPA

BPA migrated from BPA-free PC glasses is lower than the specific migration limit

Another bisphenols as BPS, BPF, BPAF and BPZ were not found in BPA-free PC glasses

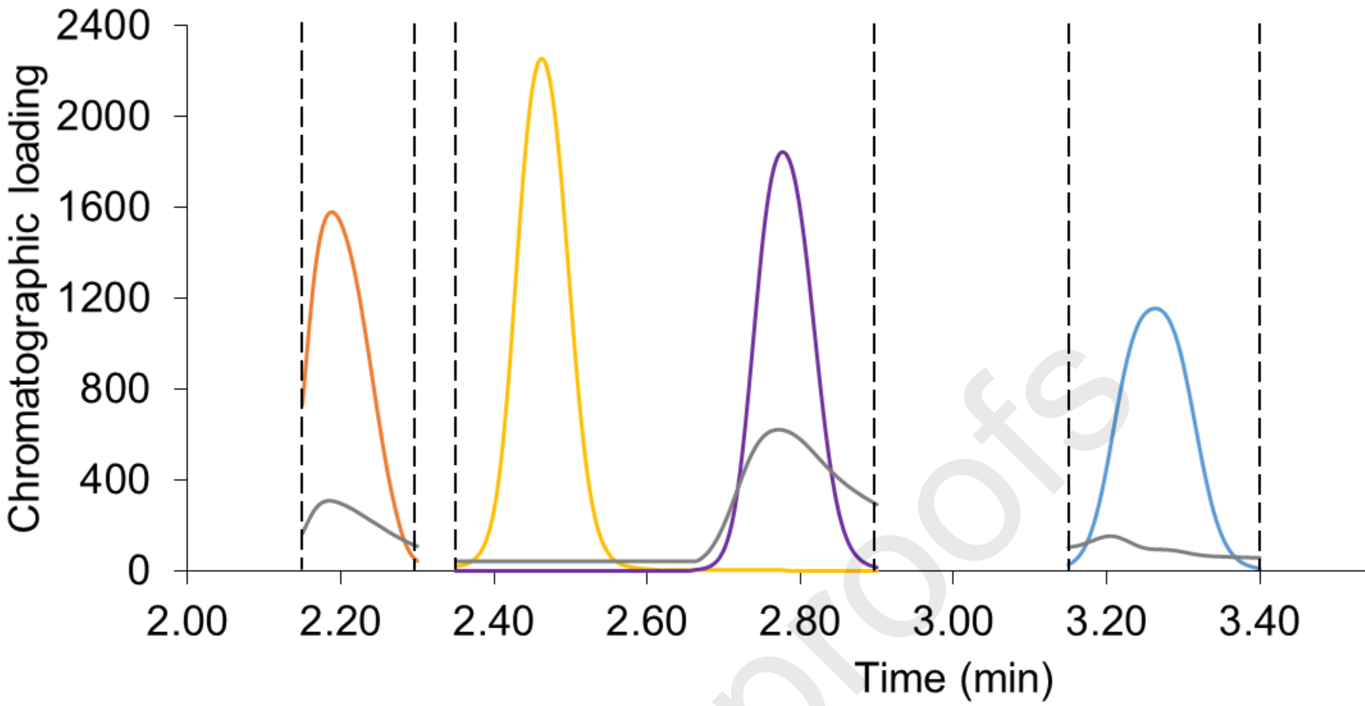
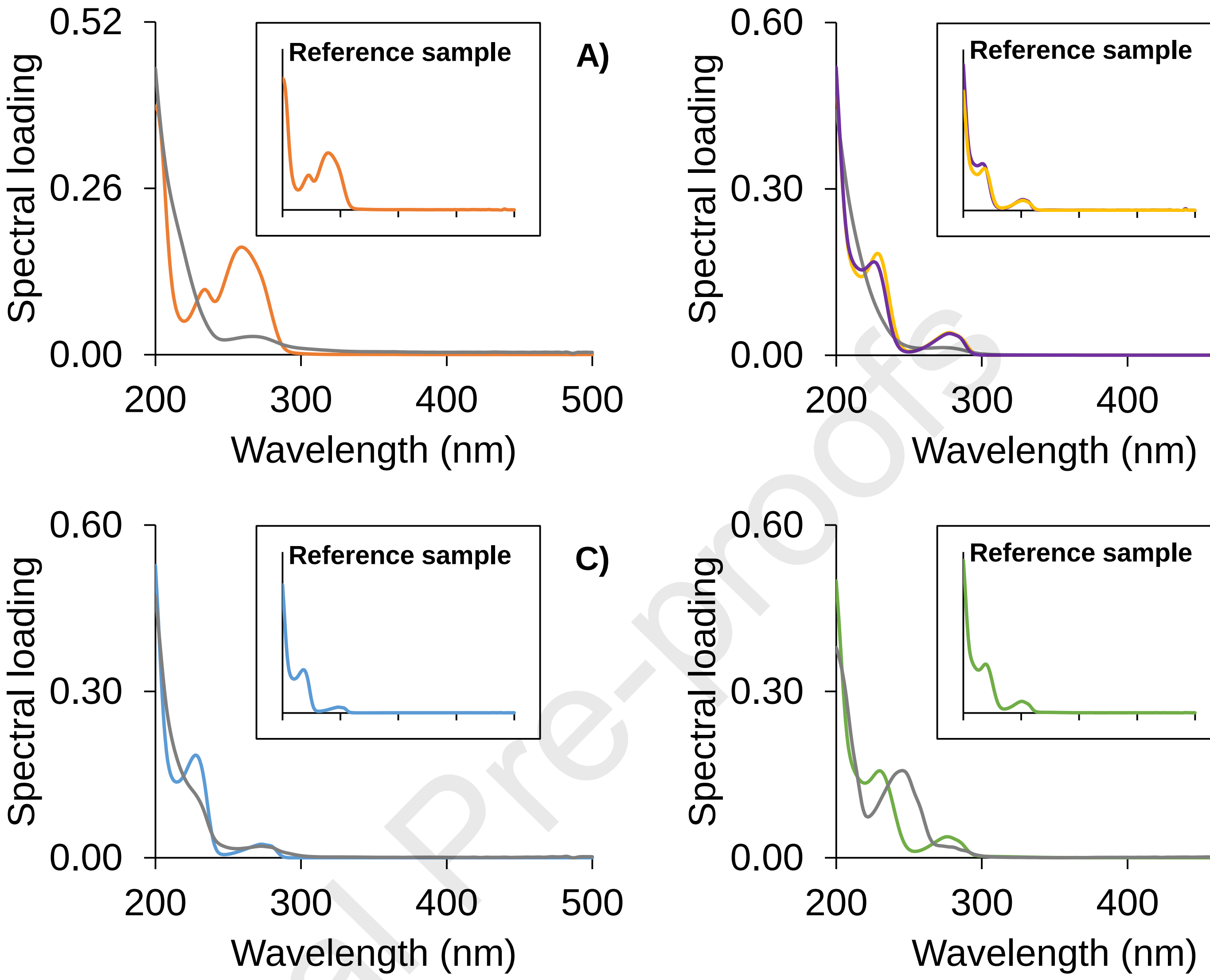

Chromatographic loading
2400
2000
1600
1200
800
400
0
2.00
2.20
2.40
2.60
2.80
3.00
3.20
3.40
Time (min)

Figure 1


0.52
0.26
0.00
Spectral loading
Reference sample
A)
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300
400
500
Wavelength (nm)
0.60
0.30
0.00
Spectral loading
Reference sample
200
300
400
Wavelength (nm)
0.60
0.30
0.00
Spectral loading
Reference sample
C)
200
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Wavelength (nm)
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0.30
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Spectral loading
Reference sample
200
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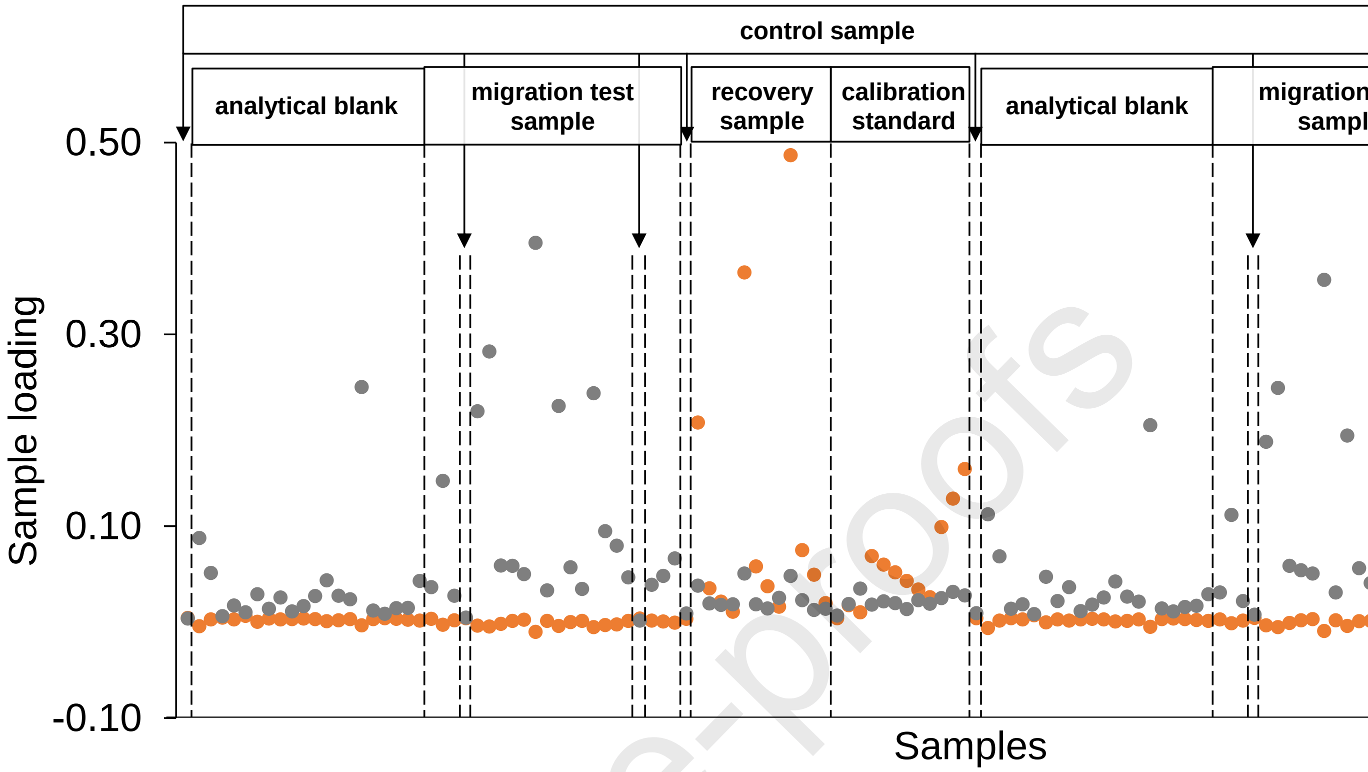
400
Wavelength (nm)

Figure 2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analytical blank
migration test sample
recovery sample
calibration standard
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Sample loading
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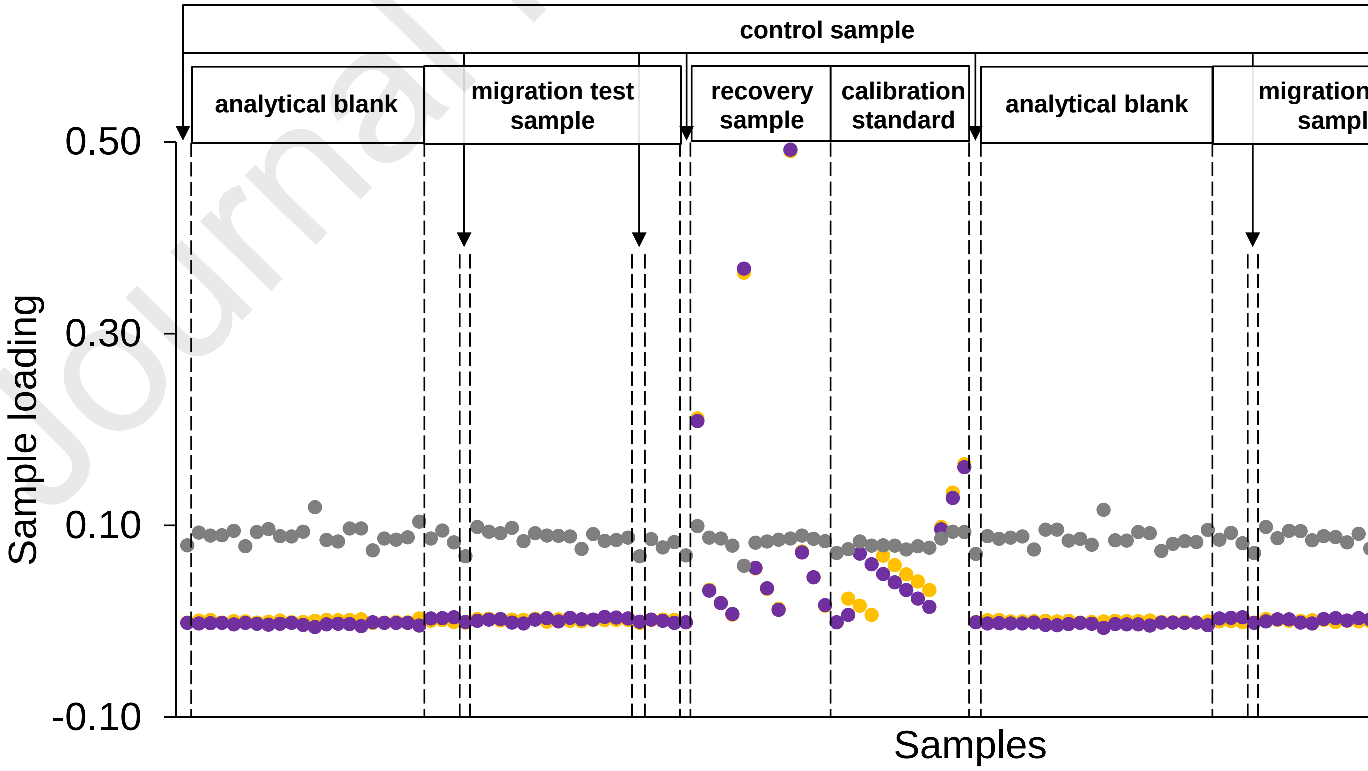
0.30
0.10
-0.10
Samples

Figure 3A


control sample
analytical blank
migration test sample
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migration
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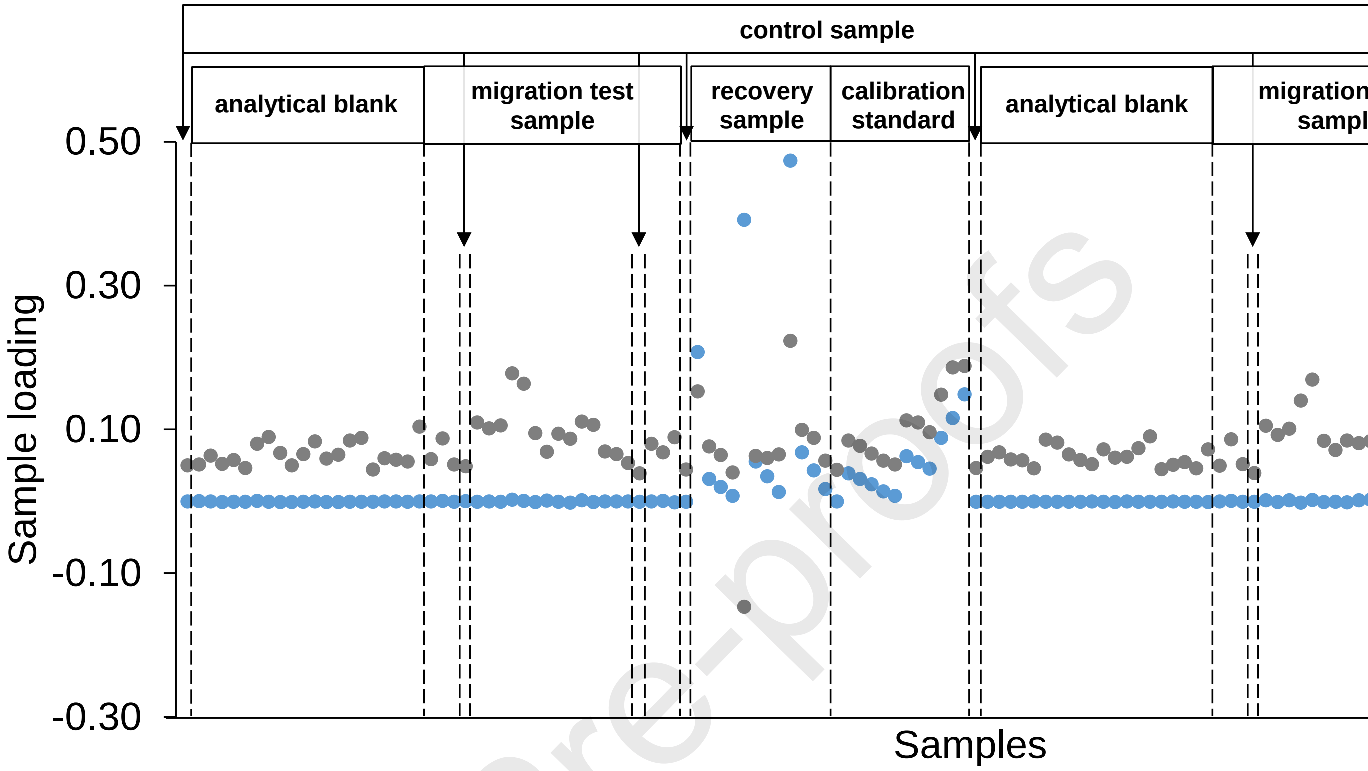
Samples

Figure 3B



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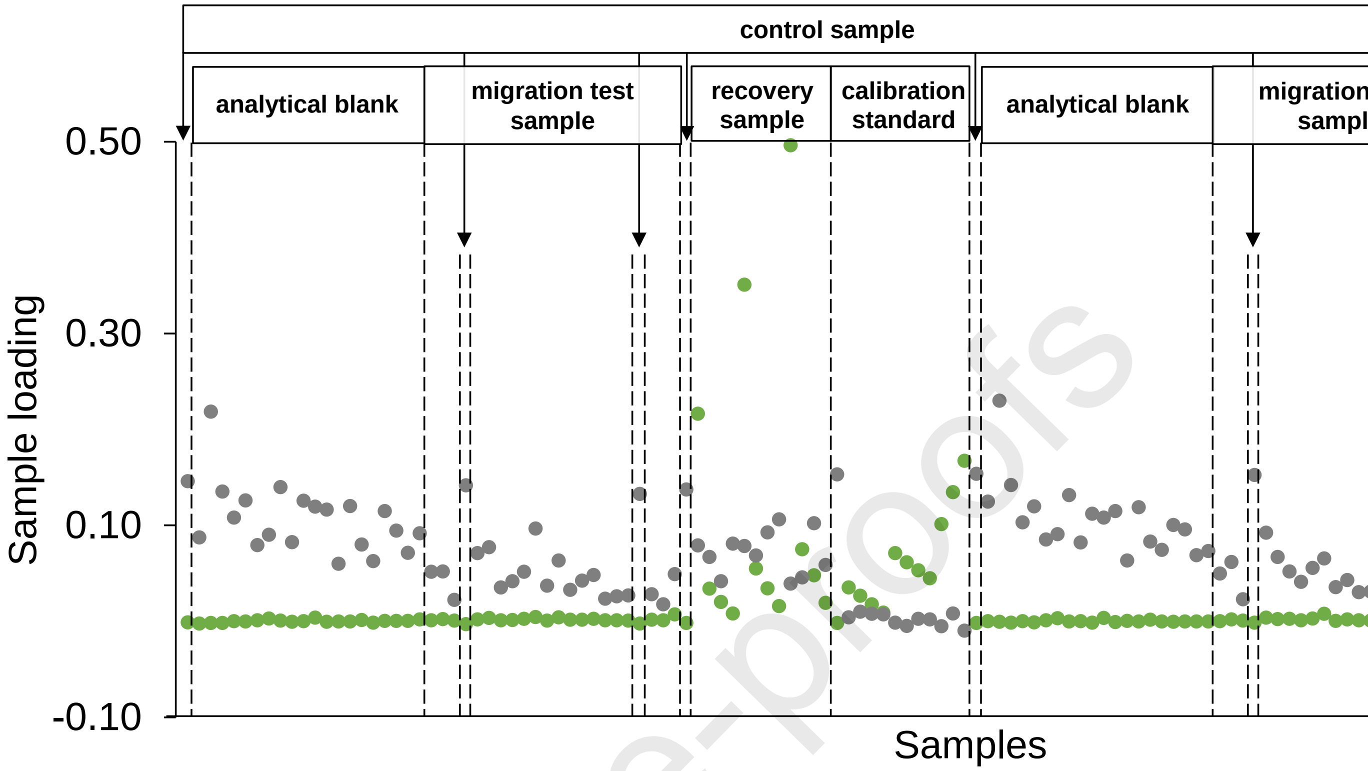

Figure 3D

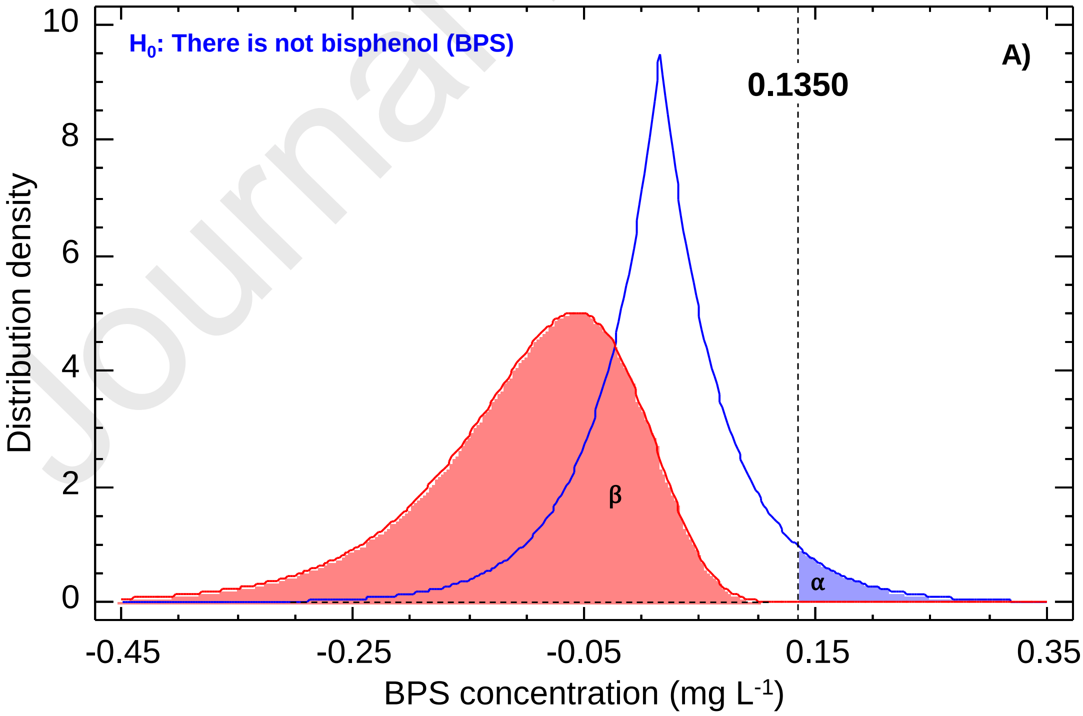

Figure 4A

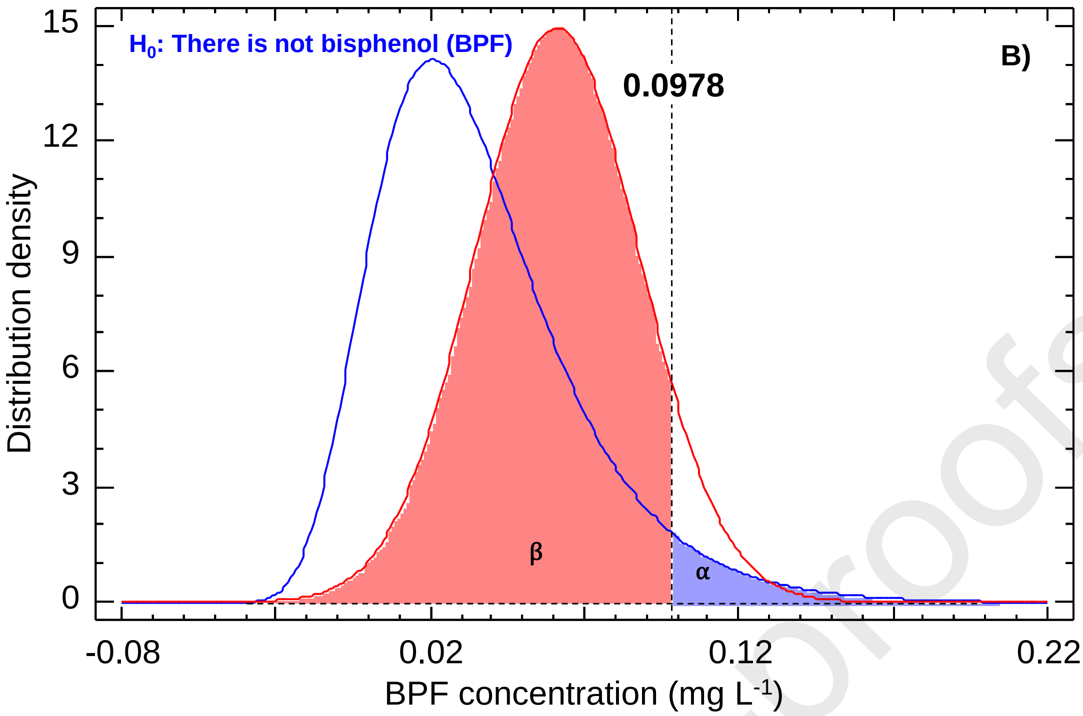

Figure 4B

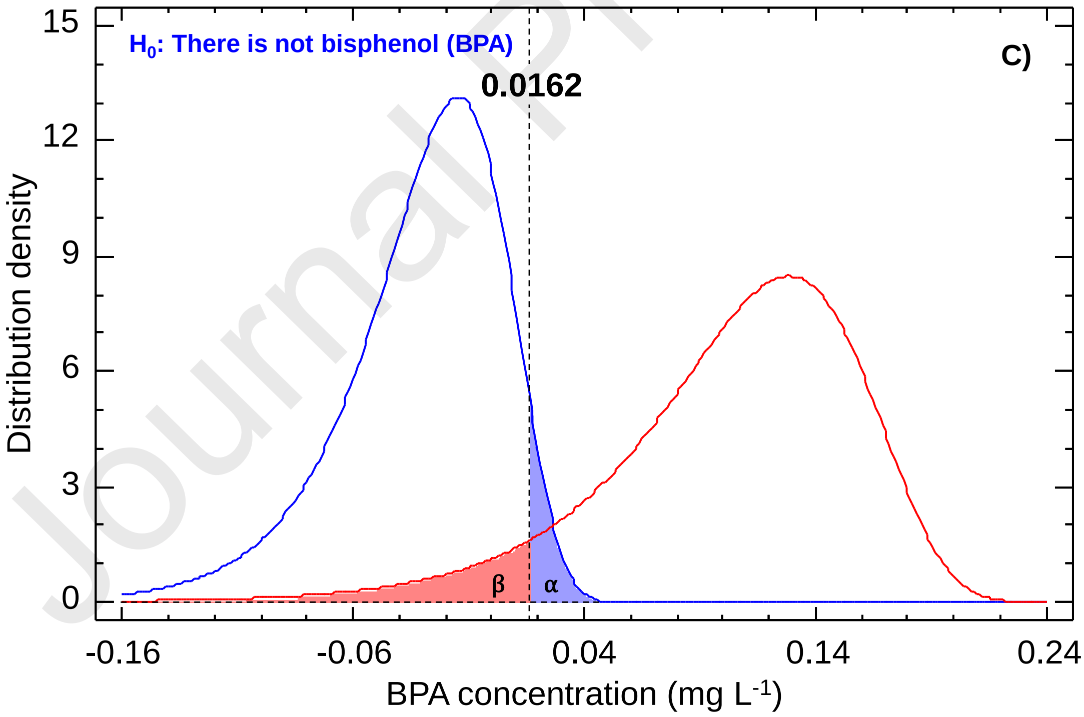

Figure 4C

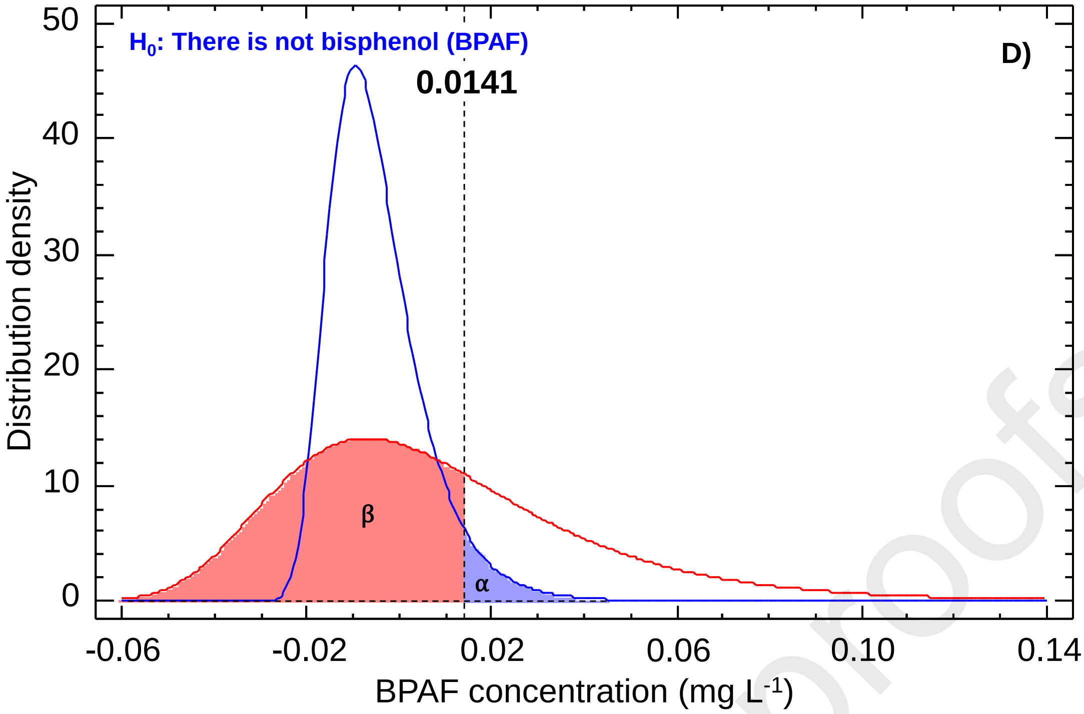

Figure 4D

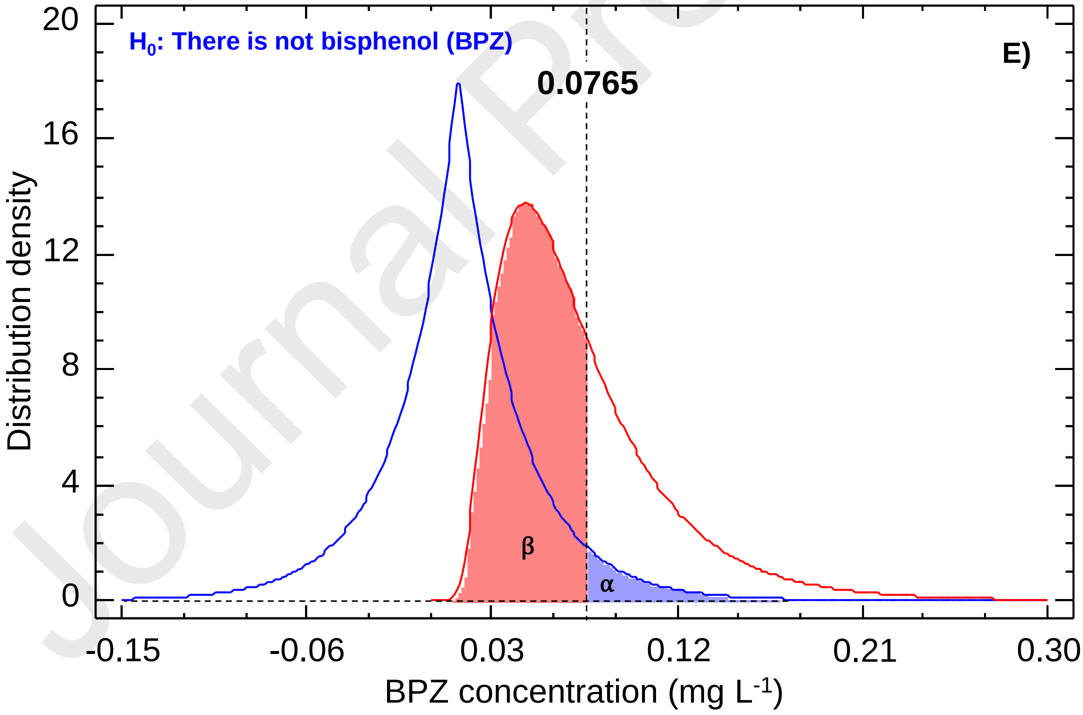

Figure 4E

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CREDIT

M. M. Arce,	
M.C. Ortiz	
S. Sanllorente	

AUTHOR STATEMENT:

M.M. Arce: Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing.

M.C. Ortiz: Conceptualization, Funding acquisition, Methodology, Supervision, Writing - review & editing.

S. Sanllorente: Conceptualization, Methodology, Supervision, Writing - review & editing.