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Univariate data analysis *versus* multivariate approach in liquid chromatography. An application for melamine migration from food contact materials

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ABSTRACT

The aim of this work is focused on the melamine migration from food contact materials (FCMs), considering data obtained from univariate analysis *versus* that obtained from multivariate approach in liquid chromatography coupled to diode array detector.

Plastic food contact materials are made from monomers and additives. Moreover, non-intentionally added substances (NIAS) can be part of the composition of the FCM: raw material impurities or process by-products, inks or adhesives.

Any compound present within a FCM can migrate to foodstuff. Specific migration of some substances from plastic FCMs to food/simulant is limited by European legislation in force (Commission Regulation No 10/2011).

Quantification of analytes in migration samples through a univariate analysis could lead to erroneous results. As an example, in liquid chromatography NIAS can interfere when coeluting with analytes or when they have close retention time. In that case, an overestimation would happen and the verification of the compliance of the specific migration limit (SML) of a substance would be incorrect.

A solution to the problem can be found in the application of a chemometric tool with the second-order advantage, which allows the unequivocal identification of analytes. Specifically, for this work, PARAFAC/PARAFAC2 decomposition technique along with tensors arranged from HPLC-DAD data of migration (test and kinetics) samples were used for the identification and quantification of melamine.

Results of melamine quantity found in migration samples from five types of melaware by means of a multivariate approach were compared to results obtained with a univariate data analysis carried out with values of chromatographic peak area as response. The comparison reveals that in test samples, univariate analysis supposes an overestimation in the quantity of melamine of 30 % on average, with respect of the concentration obtained from the multivariate approach. Besides, in kinetics samples it is remarkable that for one migration cycle the melamine found was 10 times above the one that obtained with PARAFAC decomposition.

Summing up, multivariate data analysis of migration samples supposes a great advantage in order to comply with the established regulation about migrants and to decrease the false non-compliant results.

1. Introduction

The aim of this work resides in the comparison of the results obtained by means of a conventional univariate analysis and the ones obtained from a multi-way technique (PARAFAC). The correct use of PARAFAC, when the data are trilinear, allows the unequivocal identification of the substances of interest, even if interferents that coelute with the analyte exist. The advantage that multivariate analysis of samples with complex matrix is remarkable, in order to comply with stablished regulations and to reduce the false non-compliant results. The above approach will be applied to the analytical procedure to determine melamine by means of liquid chromatography with diode array detector (HPLC-DAD) in samples obtained from food contact materials (FCM).

The materials that are directly or indirectly in contact with daily-consumed foodstuff are more and more numerous. Thus, nowadays these materials are at the centre of attention of the agencies responsible for citizens healthcare. Food contact materials are a cause of great concern, especially due to the possibility of some compounds migrating

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from these materials to foodstuff. However, these materials are also used in order to avoid food contamination and to extend the lifespan of the food. The demand for takeaway food is another application of these materials that is increasing exponentially. This inevitably implies greater contact between foodstuff and package. Besides, manufacturers try to make packaging more durable, as well as, easier and lighter to transport. All this leads production companies to develop new materials or to incorporate new substances (additives) in order to achieve this aims [1].

Food contact materials can have multiple formulas both for the type of components that are employed and the proportion in which they intervene. These materials can be group in 17 different categories [2]: active and intelligent materials and articles, adhesives, ceramics, cork, rubbers, glass, ion-exchange resins, metals and alloys, paper and board, plastics, printing inks, regenerated cellulose, silicones, textiles, varnishes and coatings, waxes, and wood.

Without any doubt, one of the most important groups as regards production and worldwide consumption is plastics. In addition to plastic FCMs themselves, many paper and board packages have inner plastic laminates and also the majority of metallic packages are coated with polymeric materials [3].

These plastics are made of a polymeric base (macromolecule composed of repeated units of monomers), which can be a homopolymer if these units are of a single type or a copolymer when there are two or more different units [4]. For manufacturing additives are employed in order to obtain specific final properties of the product and also to simplify and cheapen the production process. Some of these additives are: plasticizers, lubricants, colorants, stabilisers, fillers, flame retardants, foaming agents, impact modifiers, antistatic agents and antimicrobials [5].

In addition to monomers and additives, inks or adhesives used for the presentation to market and/or consumer are also present in the final product. Likewise, other unknown substances whose origin is in raw material impurities or by-products formed during the manufacturing process can be found in the final product, named as non-intentionally added substances (NIAS) [3].

Any compound that forms part of a FCM can migrate to foodstuff. Specific migration of certain substances from plastic materials to foodstuff/food simulant is regulated by Commission Regulation No 10/2011 [6]. Therefore, the verification of the compliance of specific migration limits (SML) in the materials of which components are known is not a problem. This verification is performed as follows: i) a method is developed for the analyte(s) of interest, ii) migration samples are obtained, iii) by means of a conventional univariate data analysis the method is validated and also migrated analyte(s) is(are) quantified, and iv) results of the concentration found are compared with the SML.

The problem arises when one of the NIAS interferes in the univariate analysis, for example, in liquid chromatography this interference can happen when coeluting with the analyte or when their retention times are very close. In this situation, the quantification of analyte in migration samples is erroneous.

A solution when migration samples (complex matrix because one does not know all the components) are been worked with is the employment of n-way techniques. These techniques present the second-order advantage, which means that: from three-way data sets, to which an adequate decomposition using chemometric tools is applied, it is possible to separately identify the analyte and possible interferents present in samples as long as the data are trilinear [7].

Several chemometric techniques can be applied to analyse multidimensional data, three-way (or N-way) analysis of data cubes (or higher-order data arrays), in order to unequivocally identify and quantify the analyte(s) in complex samples. The most common ones are generalized rank annihilation method (GRAM), multivariate curve resolution by alternating least squares (MCR-ALS), parallel factor analysis (PARAFAC/PARAFAC2), direct trilinear decomposition (DTLD), TUCKER3, and N-way partial least squares and unfolded partial least squares which later require residual trilinearization (N-PLS/RTL and U-PLS/RTL) [7–9]. Among them, some authors prefer PARAFAC as a chemometric tool for their analyses of pharmaceuticals [8] and of food matrices [10,11] due to the easier interpretation of the results obtained from higher order datasets, to the simple and fast quantitative estimation, and to the better values of performance criteria.

Therefore, the possibility of applying the advantages that PARAFAC provided as a decomposition technique could be considered for this work. In this investigation migration (test and kinetics) samples, obtained from five types of kitchenware made of melamine–formaldehyde resin, were analysed by means of HPLC-DAD. For each injection, the software provides the peak area value, integrated at the selected wavelength for melamine. However, for the multivariate analysis a three-way data set is obtained with the full spectrum recorded, considering the elution times around the retention time of melamine.

The comparison between univariate *versus* multivariate data analysis shows that interferents are present in the sample matrix, these interferents coeluting with the analyte of interest, melamine. That is, carrying out the univariate analysis through peak areas, whose integration is also taking into account part of the corresponding area related to the interferent, means overestimating the analyte which implies the surpass of the SML for melamine in plastic materials, fixed at 2.5 mg kg^{-1} by European regulation [12].

2. Material and methods

2.1. Chemicals and reagents

Melamine was purchased from Alfa Aesar (Kandel, Germany). Acetonitrile (LiChrosolv® isocratic grade for liquid chromatography) was obtained by Merck (Darmstadt, Germany). Glacial acetic acid (HiPerSolv Chromanorm for HPLC) was supplied by 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. Instrumental

An Ultrasonic Cleaner (VWR International BVBA, Leuven, Belgium), a 200209 JP Selecta oven (Barcelona, Spain), and a water bath equipped with an immersion thermostat Digiterm 200 (JP Selecta S.A., Barcelona, Spain) were employed. For the determination of melamine an Agilent 1260 Infinity HPLC chromatograph (Santa Clara, CA, USA) was used which consists of a quaternary pump (G1311C), a sampler (G1329B), a thermostatic column compartment (G1316A), and a diode array detector (G7117C). For the separation, a Kinetex EVO-C18 column (150 × 4.6 mm, 5 μm) and acetonitrile and deionized water as solvents of the mobile phase were selected. The chromatographic conditions are summarized in: injection volume 20 μL, isocratic mobile phase acetonitrile:water (15:85, v/v), flow rate 0.6 mL min^{-1}, and column temperature 20 °C. For each injection, the peak area value for melamine, integrated at the selected wavelength of 230 nm, is given by the software. Moreover, software also records for each elution time around the retention time, the absorbance in the spectral range between 200 and 500 nm (each 2 nm), obtaining a three-way data set (one data cube).

2.3. Simulant

Testing should be performed under standardized test conditions (time, temperature and food simulant) representing the worst foreseeable conditions of use of the plastic kitchenware [6], being simulant B (3 % acetic acid in aqueous solution (w/v)) the worst case for melamine kitchenware testing [13] which also has been asserted by other authors [14]. In general, simulant B is used for hydrophilic foods which have a pH below 4.5, and is specifically assigned for: beverages such as water, juice, coffee, tea, beer, among others; fresh fruit and vegetables or in

form of purée, jams, compote, pastes, preserves or similar products; animal products as meat or fish and other sea foods; milk products such as yoghurt, cream and cheese; and miscellaneous products like vinegar or sauces [6].

2.4. Standard solutions and samples

Melamine standard stock solution of 500 mg L^{-1} was prepared by dissolving the standard in water. Melamine 50 mg L^{-1} solution was prepared by dilution with simulant B. Twelve calibration standards between 0 and 10 mg L^{-1} were daily prepared by dilution with simulant B. All solutions were stored at 4 °C.

Migration test samples were obtained from five types of kitchenware (glass, mug, cutlery, big cup and bowl) following the technical guidelines [13]. Three articles (A, B, C) of each type of kitchenware were exposed to three migration tests and were analysed in triplicate. Each migration test consists of an exposure of 2 h at 70 °C with preheated simulant B.

Migration kinetic samples were obtained by subjecting one new article of each type of kitchenware to successive migrations, each one of 30 min at 70 °C with preheated simulant B, and besides migration cycles of 1 h were carried out on another new mug. In migration kinetics the response considered is the amount of melamine accumulated during these cycles.

2.5. Software

OpenLab CDS ChemStation software was used for acquiring data. PARAFAC and PARAFAC2 decompositions were carried out with the PLS_Toolbox [15] for MATLAB [16]. Regression models and accuracy lines were fitted and validated using STATGRAPHICS Centurion 18 [17].

3. Theory

3.1. Parallel factor analysis (PARAFAC/PARAFAC2)

Data arrays employed for this work contain values of absorbance (x_{ijk}) recorded with a HPLC-DAD instrument at I elution times and J wavelengths for K samples. Thus, each three-way array ($\underline{\mathbf{X}}$) of size (I × J × K) can be decomposed with PARAFAC [18]. This chemometric technique has 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. Eq. (1) shows in a generalized way a trilinear PARAFAC model [19].

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

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.

Two characteristics that define the goodness of a fitted PARAFAC model are the CORCONDIA index [20] and the number of factors (F) [18], both related to each other. The CORCONDIA index, that is core consistency diagnostic, measures the trilinearity degree of the experimental data array when F ≥ 2, 100 % being the maximum value achievable. In this way, the unique PARAFAC model that was sought is obtained when the tensor is trilinear and the appropriate number of factors are chosen.

Sometimes, a low value for the CORCONDIA index can be due to deviations from trilinearity when shifts in the retention time of the analytes from sample to sample appear in the chromatogram [21,22]. In that case, PARAFAC2 models are used to correct the problem, allowing the chromatographic profile to vary from one matrix to another. The structure of a PARAFAC2 model is shown in Eq. (2).

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

Moreover, for the migration kinetic samples, which were obtained in order to study the tendency of the accumulated concentration of melamine migrated after several consecutive migration cycles, the PARAFAC/PARAFAC2 model structure differs slightly (see Eq. (3)). The chromatographic and spectral profiles of the F factors are the same from one cycle to another since the model is trilinear. However, in the sample profile the following happens: the matrices used in the arranging of the three-way array were calculated as the sum of the HPLC-matrix data of each cycle and the HPLC-matrices of the previous cycles (e.g. the matrix of the cycle number five contained in the tensor consists of the HPLC-matrix of the cycle number five together with the HPLC-matrix of the four previous cycles). Therefore, the sample profile is composed of the cumulative sum up to c of the sample profiles of the c migration cycles.

$$\sum_{k=1}^{c}\left(\sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}\right) = \sum_{f=1}^{F} a_{if} b_{jf}\left(\sum_{k=1}^{c} c_{kf}\right) + e'_{ijk}\ i = 1,2,\ldots,I; j = 1,2,\ldots,J; c = 1,2,\ldots,K, \quad (3)$$

Previously to the fitting of PARAFAC/PARAFAC2 models, it is necessary to define if any constraint needs to be imposed or not in either of the three profiles. In general, applying any of these constraints (unimodality, non-negativity, etc.) leads to models that explain more variance of the data tensor and that with a better CORCONDIA index [23].

The imposition of any constraint always has to be justified. Hence, considering the positivity of the spectra as well as of the concentrations, the application of the non-negativity constraint in the spectral and sample profiles (for this work J and K dimensions respectively) seems to be coherent. In turn, for the chromatographic profile (I dimension), the unimodality constraint makes sense since it forces the presence of a unique maximum for each (F) factor. That means, a chromatographic peak for each compound (identified by means of the model) present in the matrix of the sample [24].

Furthermore, the uniqueness property of the PARAFAC decomposition technique 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 coefficient between the spectral profiles from PARAFAC/PARAFAC2 decompositions and the spectra from a reference sample of melamine.

4. Results and discussion

4.1. PARAFAC/PARAFAC2 models

Decomposition PARAFAC/PARAFAC2 models for the determination of melamine were fitted for each kitchenware and migration type (testing or kinetics). Characteristics of these models are shown in Table 1.

As can be observed, column 3 in Table 1 shows the size (I × J × K) of the employed experimental data tensor. For migration test (part a) in Table 1), in all cases except the mug, tensors with size 76 × 151 × 41 were arranged, corresponding to data recorded between 1.7 and 2.2 min (I = 76), at the 151 wavelengths between 200 and 500 nm, for 41 samples which are spread out in 14 calibration standards (2 of them are replicates) and 27 samples obtained from migration test (3 articles exposed 3 times and analysed in triplicate). In the decomposition of the mug tensor an anomalous behaviour of two samples was observed, specifically two replicates of the third exposure of the mug B. These samples were deleted and the model was fitted again with the tensor of size 76 × 151 × 39.

Table 1
Characteristics of the PARAFAC/PARAFAC2 decomposition models obtained for the determination of melamine in migration samples: a) test and b) kinetics.

Migration	Kitchenware	I × J × K	Model	Constraint [a]			Number of factors	CORCONDIA	Variance X (%)	Correlation coefficient (n = 25)
				P1	P2	P3				
a) Test	Glass	76 × 151 × 41	PARAFAC	U	N	N	2	99	96.95	0.9997
	Mug	76 × 151 × 39	PARAFAC	U	N	N	2	98	97.26	0.9995
	Cutlery	76 × 151 × 41	PARAFAC2	N	N	N	2	100	97.76	0.9991
	Big cup	76 × 151 × 41	PARAFAC	UN	N	N	2	97	97.02	0.9997
	Bowl	76 × 151 × 41	PARAFAC2	N	N	N	2	100	97.46	0.9991
b) Kinetics	Glass	31 × 151 × 30	PARAFAC	U	N	N	2	96	91.75	0.9998
	Mug	76 × 151 × 86	PARAFAC	U	N	N	2	99	83.29	0.9966
	Cutlery	31 × 151 × 29	PARAFAC2	UN	N	N	2	100	99.06	0.9997
	Big cup	76 × 151 × 30	PARAFAC2	U	N	N	3	100	93.93	0.9999
	Bowl	76 × 151 × 30	PARAFAC	N	N	N	3	97	84.27	0.9999
	Mug 1 h	76 × 151 × 35	PARAFAC2	U	N	N	3	100	77.84	0.9999

[a] Constraints used for chromatographic (P1), spectral (P2) and sample (P3) profiles are codified as: (N) non-negativity, (U) unimodality, (UN) unimodality and non-negativity.

For migration kinetics (part b) in Table 1), the I dimension changes in glass and cutlery, since the time window was reduced between 2.0 and 2.2 min (31 scans). Moreover, the sample profile size differs from one type of kitchenware to another, 14 calibration standards along with: 15 migration samples for cutlery (n = 29); 16 for the glass, big cup and bowl (n = 30); 21 for the mug exposed to 1-hour cycles (n = 35). The biggest difference resides in the sample profile size for the mug (n = 86), since the data tensor was arranged with 3 calibration sets (3 × 14) and 44 migration samples.

Columns 4 to 7 in Table 1 detail if correction of chromatographic shift between samples using PARAFAC2 has been necessary or not, as well as the imposed constraints to tensor profiles (P1, P2, P3). The

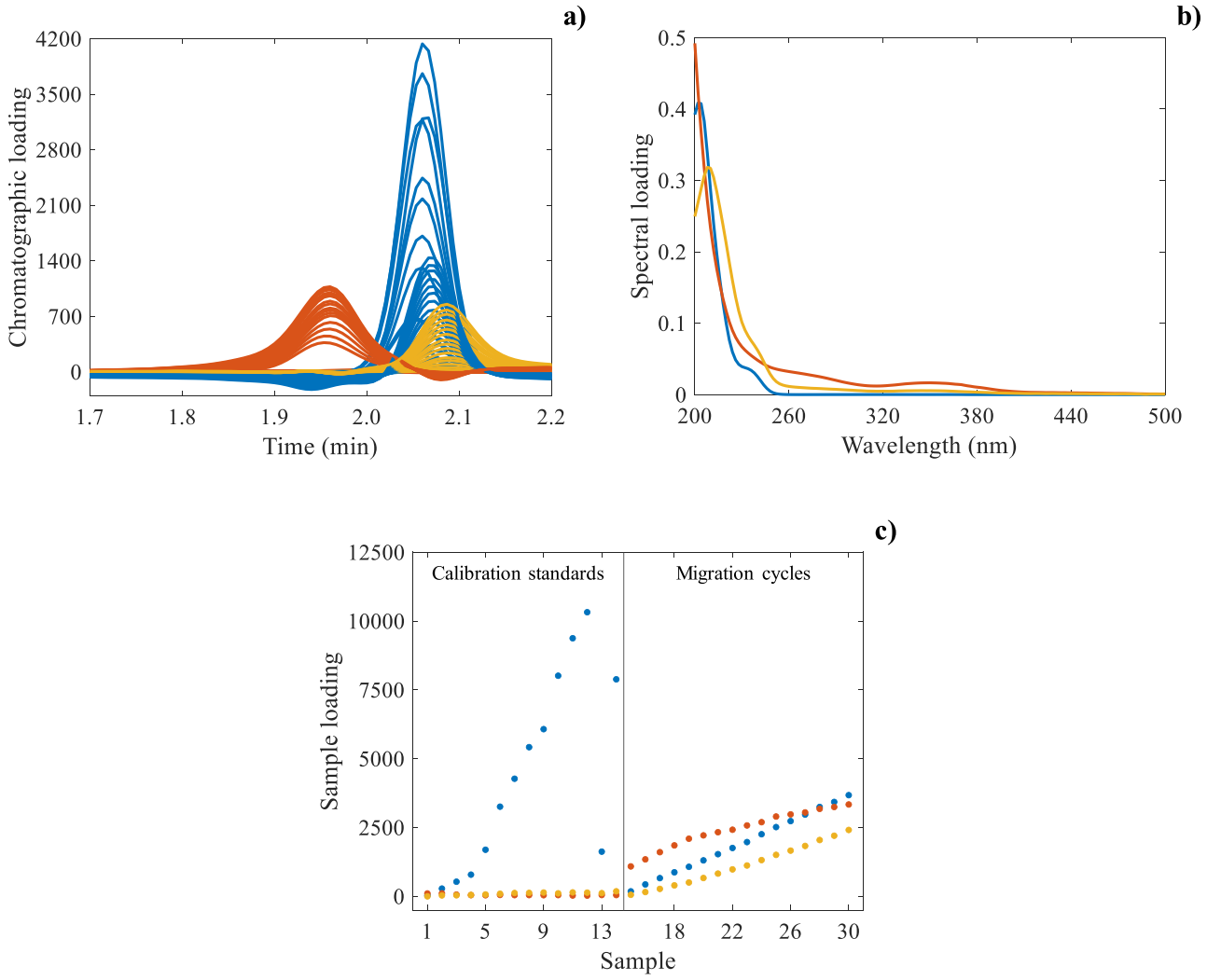

Fig. 1. Loadings of the PARAFAC2 model obtained for big cup migration kinetics: a) chromatographic, b) spectral and c) sample profiles. The blue factor is related to melamine, the orange one to interferent 1 and the yellow one to interferent 2.

factors needed for the model fitting (F), the CORCONDIA index and the variance of tensor that model explains are shown in columns 8 to 10 in the same table. Values between 96 and 100 % were obtained for CORCONDIA and the explained variance was between 77.84 and 99.06 %.

Fig. 1 shows an example from among Table 1 models, specifically the PARAFAC2 decomposition for migration kinetics from the big cup. The model was fitted with three factors, where the blue one is considered the analyte of interest and the other two are interferents present in samples (from here on out the orange factor will be named interferent 1 and the yellow factor as interferent 2). As can be observed in the chromatographic profile in Fig. 1a), interferents coelute with melamine, especially the interferent 2 which appears below the analyte peak depicted in blue. Fig. 1b) shows the spectral loadings of the three factors, where the blue peak ones would be employed in order to unequivocally identify melamine (Section 4.2.) and the orange and yellow peaks ones would be employed in the discussion about interferents (Section 4.3.). Once an analyte is assigned with the corresponding factor, sample loadings from Fig. 1c) would be employed in calibration and prediction of melamine in samples. That means, the data tensor is formed by data matrices recorded during the analysis of known (calibration) and unknown (migration) concentration samples.

4.2. Unequivocal identification

The spectral loadings obtained from each PARAFAC/PARAFAC2 decomposition allow for the unequivocal identification of the analyte, melamine in this case. In fact, the unequivocal identification was made by means of the correlation coefficient between the spectral profile (wavelengths between 200 and 248 nm) of a reference sample and the one obtained (for the same spectrum range) from the decomposition model for this profile. The correlation coefficients are shown in column 11 of Table 1, and as can be observed, all of them are very close to 1. Thus, guaranteeing that the factor initially associated with melamine had been correctly assigned.

4.3. Discussion about interferents

The final aim of this work is to show a procedure in order to avoid the overestimation in the determination of migrated melamine, an aim that is reflected in this paper with the utilization of PARAFAC/PARAFAC2 as decomposition technique, independently of which type of compound the interferent(s) is(are). However, a study of the interferents found in migration samples when using PARAFAC/PARAFAC2 decomposition was carried out.

For this task, spectra obtained from each PARAFAC/PARAFAC2 model of Table 1 by means of correlation coefficients were compared. The correlation coefficients calculated are shown in Table 2. Table 2A) contains the results for melamine factor, and as can be observed, its identification is guaranteed in the 11 models, all the values being greater than or equal to 0.9956 (marked in bold in this table).

As can be observed in Table 1, decomposition models were fitted with two or three factors depending on the type of kitchenware and the type of migration samples (test or kinetics) analysed. For the 3 models of three factors (migration kinetics of big cup, bowl and mug 1 h), the same two interferents in orange and yellow were obtained (spectra of interferent 1 and interferent 2 can be seen in Fig. 1b). For the remaining 8 models (fitted with two factors), the interferent obtained was compared with interferent 1 in Table 2B) and with interferent 2 in Table 2C).

In Table 2B) the high correlation (marked in bold without brackets) between interferent 1 from three-factor models (rows 1, 2 and 3) and the interferent of 4 out of the 8 two-factor models (migration testing of glass, mug and big cup, and migration kinetics of mug) can be seen in columns 1, 2, 4 and 7 respectively. It is possible to associate the interferent of these 4 models with interferent 1. In the case of migration kinetics of glass (column 6) although good values of correlation were obtained for interferent 1, no association can be asserted because of the good values in column 6 in Table 2C). Furthermore, the interferent of the other 3 two-factor models (migration testing of cutlery and bowl, and migration kinetics of cutlery) can in no way be associated with interferent 1 (columns 3, 5 and 8 in Table 2B)). Neither does the interferent of these 3 models present good correlation coefficients with the interferent 2 as can be seen in columns 3, 5 and 8 in Table 2C). Because of that, and only for the purpose of trying to identify the interferents of the two-factor models, 4 new three-factor decomposition models were fitted.

PARAFAC/PARAFAC2 decomposition is a chemometric technique based on least squares which is highly affected by the magnitude difference between target analyte and interferent(s). Due to the fact that some calibration standards and some migration samples contain high melamine concentration, the PARAFAC/PARAFAC2 decomposition model not be able to identify the possible interferent(s) present as another factor, so data tensors without the matrices of those samples were arranged for the fitting of the 4 new models. This possible size effect in chromatographic and sample loadings between melamine and interferents has already been seen in other investigations between analyte and internal standard [25].

The correlation coefficients (marked in bold and in brackets) between these 4 new models (migration testing of cutlery and bowl, and migration kinetics of glass and cutlery in columns 3, 5, 6 and 8 respectively) and the previously fitted 3 three-factor models (rows 1, 2 and 3) are shown in Table 2B) for the interferent 1 and in Table 2C) for the interferent 2. In all cases these correlation values have been improved in relation with the ones previously calculated. Fig. 2 shows the spectral profile of the 4 new three-factor decomposition models: a) migration testing of cutlery, b) migration testing of bowl, c) migration kinetics of glass and d) migration kinetics of cutlery.

Table 2A
Correlation coefficients between spectra related to the factors obtained from the PARAFAC/PARAFAC2 decomposition models in Table 1. A) Values calculated by means of 25 wavelengths (range between 200 and 248 nm) for factors associated with melamine.

	Migration	a) Test					b) Kinetics					
Migration	Kitchenware	Glass	Mug	Cutlery	Big cup	Bowl	Glass	Mug	Cutlery	Big cup	Bowl	Mug 1 h
a) Test	Glass											
	Mug	1.0000										
	Cutlery	1.0000	0.9999									
	Big cup	1.0000	1.0000	0.9999								
	Bowl	0.9999	0.9998	1.0000	0.9998							
b) Kinetics	Glass	0.9999	0.9998	0.9999	0.9997	0.9999						
	Mug	0.9974	0.9976	0.9967	0.9977	0.9961	0.9961					
	Cutlery	0.9998	0.9998	0.9999	0.9997	0.9999	1.0000	0.9961				
	Big cup	0.9998	0.9997	0.9999	0.9996	0.9999	1.0000	0.9957	1.0000			
	Bowl	0.9998	0.9997	0.9999	0.9996	1.0000	1.0000	**0.9956**	0.9999	1.0000		
	Mug 1 h	0.9999	0.9999	1.0000	0.9998	0.9999	0.9999	0.9966	0.9999	0.9999	0.9999	

Table 2B
Correlation coefficients between spectra related to the factors obtained from the PARAFAC/PARAFAC2 decomposition models in Table 1. B) Values calculated by means of 101 wavelengths (range between 200 and 400 nm) for factors associated with interferent 1 (orange factor in Fig. 1).

	Migration	a) Test					b) Kinetics					
Migration	Kitchenware	Glass	Mug	Cutlery	Big cup	Bowl	Glass	Mug	Cutlery	Big cup	Bowl	Mug 1 h
b) Kinetics	Big cup	**0.9971**	**0.9939**	0.5295	**0.9929**	0.4433	0.9726	**0.9961**	0.3914			
				(0.9903)		**(0.9960)**	**(0.9981)**		**(0.9851)**			
	Bowl	**0.9857**	**0.9720**	0.4422	**0.9821**	0.3625	0.9281	**0.9753**	0.3410	**0.9846**		
				(0.9544)		**(0.9744)**	**(0.9908)**		**(0.9974)**			
	Mug 1 h	**0.9966**	**0.9936**	0.5072	**0.9947**	0.4167	0.9679	**0.9933**	0.3494	**0.9971**	**0.9764**	
				(0.9921)		**(0.9917)**	**(0.9931)**		**(0.9752)**			

Table 2C
Correlation coefficients between spectra related to the factors obtained from the PARAFAC/PARAFAC2 decomposition models in Table 1. C) Values calculated by means of 31 wavelengths (range between 200 and 260 nm) for factors associated with interferent 2 (yellow factor in Fig. 1).

	Migration	a) Test					b) Kinetics					
Migration	Kitchenware	Glass	Mug	Cutlery	Big cup	Bowl	Glass	Mug	Cutlery	Big cup	Bowl	Mug 1 h
b) Kinetics	Big cup	0.7807	0.8301	0.7274	0.7700	0.6078	0.9377	0.8435	0.3973			
				(0.9543)		**(0.9843)**	**(0.9920)**		**(0.9940)**			
	Bowl	0.8099	0.8552	0.6874	0.7997	0.5619	0.9548	0.8681	0.3446	**0.9983**		
				(0.9365)		**(0.9925)**	**(0.9969)**		**(0.9938)**			
	Mug 1 h	0.8387	0.8805	0.6497	0.8290	0.5196	0.9688	0.8923	0.2988	**0.9941**	**0.9985**	
				(0.9199)		**(0.9975)**	**(0.9969)**		**(0.9891)**			

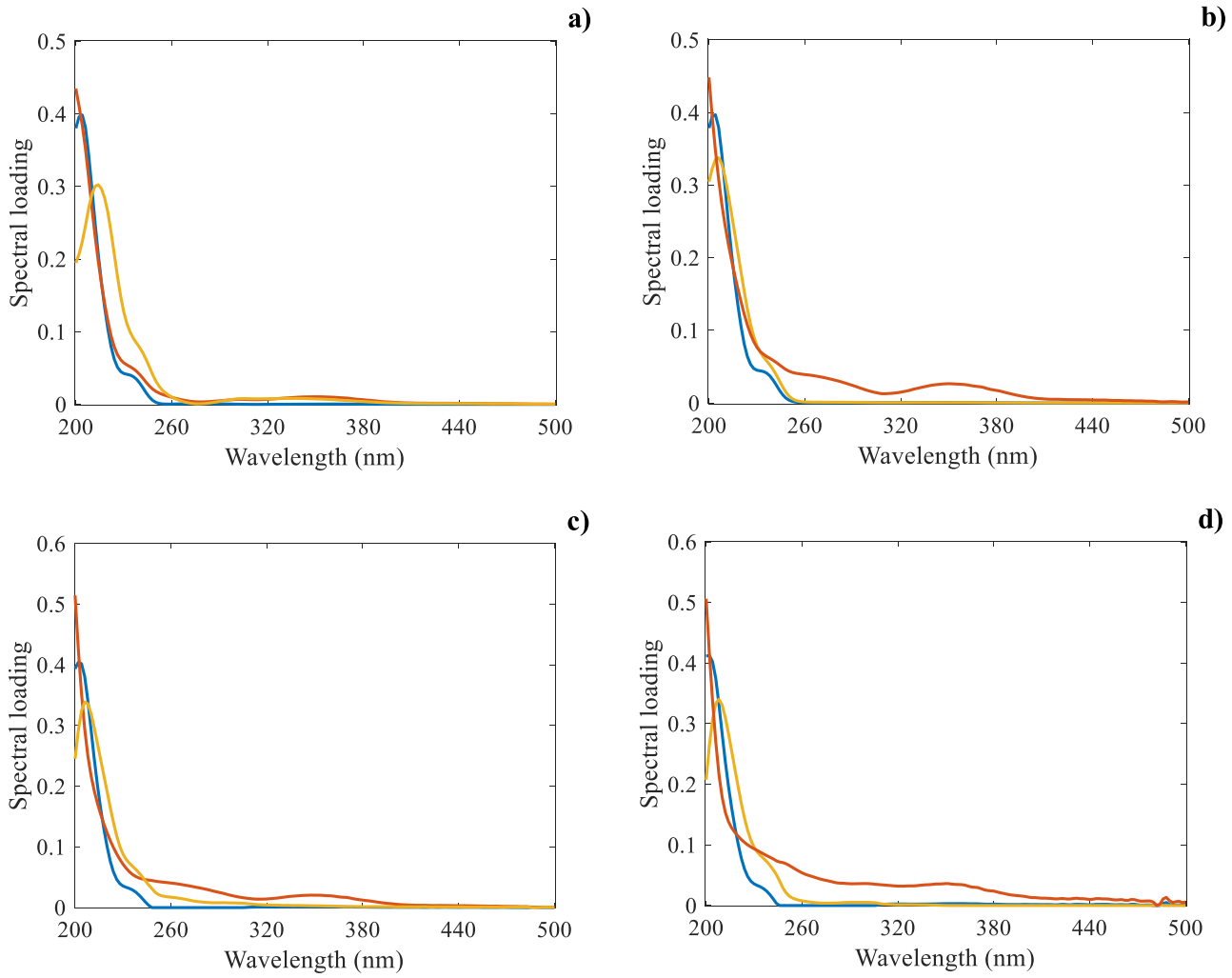

Fig. 2. Spectral loadings of the 4 new three-factor PARAFAC/PARAFAC2 models obtained for: a) migration testing of cutlery, b) migration testing of bowl, c) migration kinetics of glass and d) migration kinetics of cutlery. The blue factor is related to melamine, the orange one to interferent 1 and the yellow one to interferent 2.

4.4. Performance criteria

4.4.1. Calibration and accuracy lines

Calibration lines for each kitchenware and type of migration were fitted from the sample loadings obtained by means of each PARAFAC/PARAFAC2 decomposition model in Table 1. The fitting of these regression models, sample profile loadings *versus* true concentration, was carried out for 14 points which correspond to 12 concentration levels, 2 of them are replicates. Exceptionally, three calibration sets (n = 42) were used for the calibration line of the migration kinetics in the mug. Besides, for the migration test in the bowl, an outlier datum with studentized residue equal to −4.43 was found, so the calibration line was fitted with 13 data. Rows 1 to 5 and 10 to 14 in Table S1 in the Supplementary Material show the parameters of all regression models for the melamine.

Validation of calibration lines was done in terms of trueness and precision. Precision was evaluated through the standard deviation (s_{yx}) shown in rows 4 and 13 in Table S1 in the Supplementary Material. However, trueness was checked by means of intercept and slope of the accuracy line (predicted concentration *versus* true concentration) the

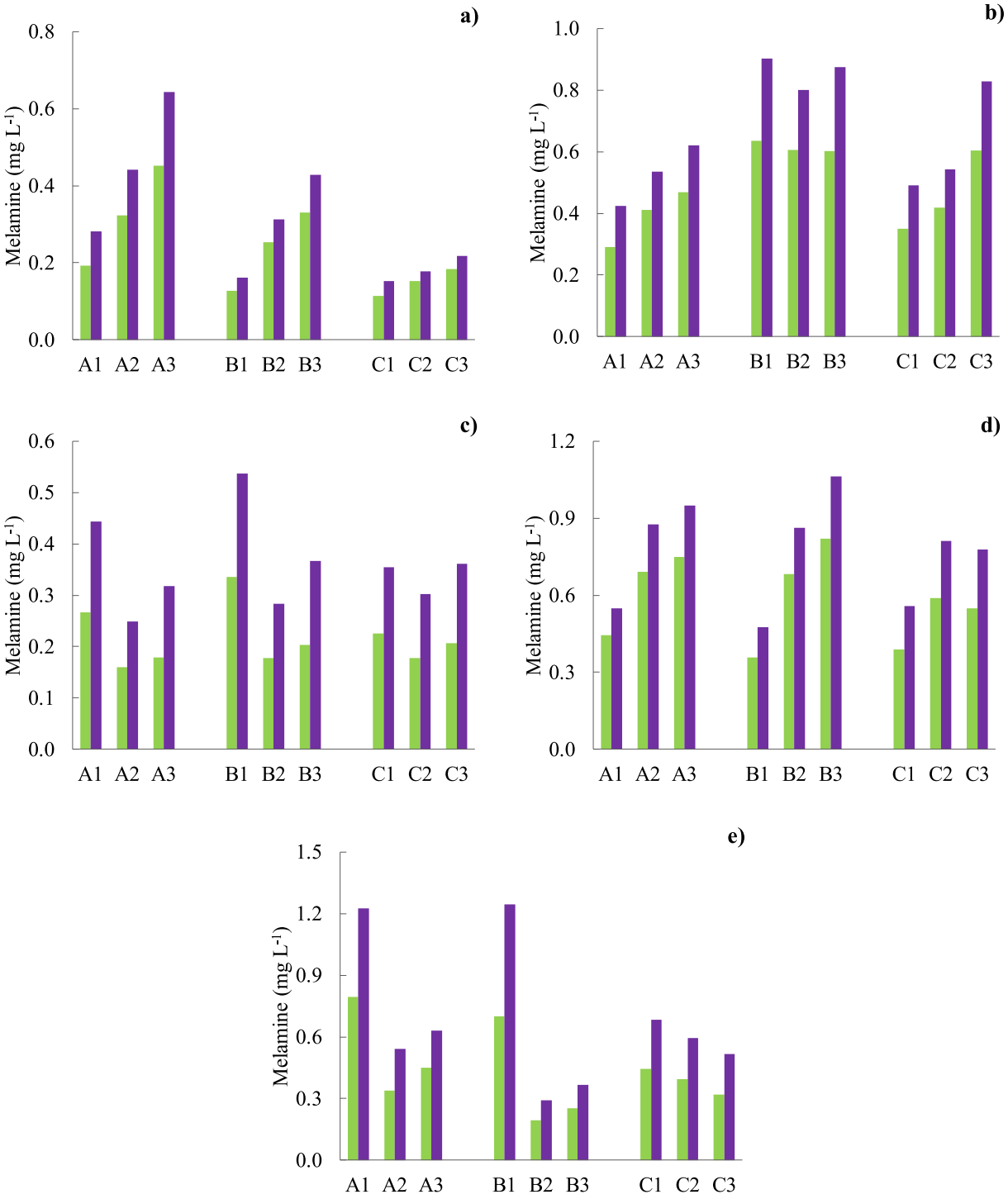

Fig. 3. Comparison of the melamine concentration (in mg L^{-1}) obtained for migration testing from different types of kitchenware: a) glass, b) mug, c) cutlery, d) big cup and e) bowl. Calculated values by means of a univariate conventional analysis (in purple) and by means of PARAFAC or PARAFAC2 decomposition (in green).

null hypotheses being to test H_0: Intercept equal to zero and slope equal to one [26]. In all cases, p-values were above 0.05, which means there is no evidence to reject the null hypotheses. The parameters of accuracy lines for melamine are shown in rows 6 to 9 and 15 to 18 in Table S1 in the Supplementary Material. It can be concluded that the method is unbiased and does not have constant or proportional error.

4.5. Migration samples

Once calibration lines had been validated, prediction of concentration of melamine found in migration test and migration kinetics samples was done. By means of sample loadings obtained from PARAFAC/PARAFAC2 models of Table 1 and along with the regression models, results with a multivariate approach were obtained. Afterwards, these results were compared with the ones obtained through a univariate data analysis, carried out with values of the chromatographic peak area [27].

4.5.1. Migration testing

Fig. 3 shows the comparison of the results obtained for migration test samples: a) glass, b) mug, c) cutlery, d) big cup and e) bowl. In these bar graphs, green refers to multivariate analysis and purple to univariate. Concentration of migrated melamine (in mg L^{-1}) is displayed in the ordinate axis. The abscissa axis is subdivided in articles A, B and C, and each of them in turn in exposures 1, 2 and 3. Each bar shows the average value of 3 instrumental replicates.

As can easily be seen in the five graphs in Fig. 3, the concentration of melamine present in the samples is higher when techniques with the second-order advantage (e.g. PARAFAC or PARAFAC2 decomposition) are not apply. The non-application leads to an overestimation in the concentration of analyte found due to the fact that the coeluting interferent(s) inevitably intervene in the melamine peak area integration. This provokes the overestimation and may derive in false non-compliant results when the calculated concentration exceeds the specific migration limit (SML for melamine is 2.5 mg kg^{-1} [12]). As can be observed, the allowed limit is not exceeded in any case.

In migration test samples from each type of kitchenware, univariate analysis supposes an increase of more than 30 % in the quantity of melamine found with respect to that obtained by means of PARAFAC/PARAFAC2 decomposition. Specifically, percentages were 31, 37, 67, 33 y 55 % for glass, mug, cutlery, big cup and bowl respectively. By observing these results, it is noteworthy that the highest percentages (cutlery and bowl) correspond to kitchenware which had decorative elements on the surface that comes into contact with the simulant (analysed kitchenware can be seen in Ref. [27]).

4.5.2. Migration kinetics

Fig. 4 shows the comparison of results obtained for migration kinetics samples: 4a) glass, 4b) cutlery, 4c) mug, 4d) mug exposed to 1-

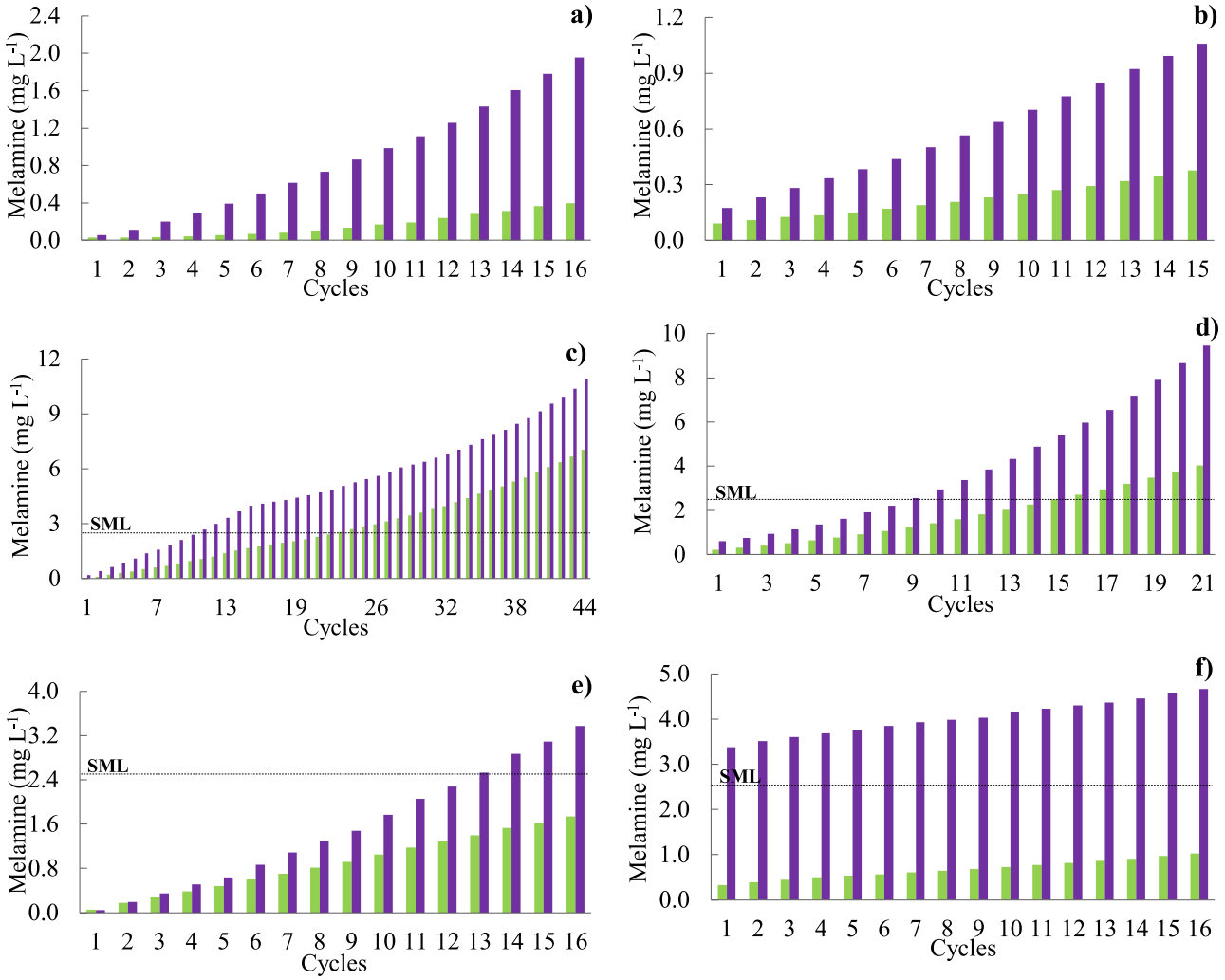

Fig. 4. Comparison of the melamine concentration (in mg L^{-1}) obtained for migration kinetics from a) glass, b) cutlery, c) mug, d) mug exposed to 1-hour cycles, e) big cup and f) bowl. Calculated values by means of a univariate conventional analysis (in purple) and by means of PARAFAC or PARAFAC2 decomposition (in green). The black dashed horizontal line indicates the melamine SML.

hour cycles, 4e) big cup and 4f) bowl. As for migration testing, the green colour refers to the multivariate analysis and purple to univariate one. However, on ordinate axis accumulated concentration of migrated melamine is displayed (in mg L^{-1}), that is, the quantity found in each migration cycle was added to that quantified on the previous cycles. Hence the increasing trend seen in all bar graphs in Fig. 4. In this case, the abscissa axis displays the number of migration cycles which each kitchenware has been exposed to. In Fig. 4c-f, the SML for the substance has been shown with a black dashed horizontal line, above all to indicate the differences between giving a false non-compliant result or not depending on the experimental data type analysis applied.

Comparison for glass and cutlery is shown in Fig. 4a) and b) respectively. Despite the overestimation committed with univariate analysis, for the number of analysed cycles, any of the compared data treatments did not exceed the SML for melamine (2.5 mg kg^{-1}) [12].

For the rest of the kitchenware, in which the SML was exceeded with univariate analysis, the number of cycles after which that level is exceeded is now (multivariate analysis) higher. The examples of mug for cycles both of 30 min and 1 h are shown in Fig. 4c) and d), increasing from 11 to 23 and from 9 to 16, respectively, the number of cycles needed to exceed the SML.

For the big cup and bowl (Fig. 4e) and f)), the SML is not even exceeded after the cycles carried-out with multivariate analysis. However, univariate analysis led to the conclusion that this happened for the big cup after 13 cycles, shown in Fig. 4e).

One of the more remarkable points of this work resides in the comparison for bowl kinetics. One of the two unidentified substances (remember that the PARAFAC model had 3 factors, see row 10 in Table 1), that has close retention times to that for melamine, is present in the matrix of the sample in a concentration of such magnitude that entails that the estimation with univariate analysis be 10 times higher for the first cycle than that the found with PARAFAC. That is, the quantity of melamine found for the bowl in cycle one was 3.379 mg L^{-1} applying univariate analysis and 0.324 mg L^{-1} by means of PARAFAC decomposition.

5. Conclusions

This work presents a study of a case where the quantification of the analyte through a conventional univariate data analysis leads to results which overestimate the quantity of melamine found, since non-intentionally added substances (NIAS) can be present in migration samples and occasionally can share their retention time with analytes of interest (or be very close each another).

From the comparison of the results obtained from a univariate analysis and from a multivariate approach, using the same data, this paper reveals the impact of the employment of n-way techniques with the second-order advantage (e.g. PARAFAC/PARAFAC2 decomposition) when migration samples are involved. Choosing properly the number of factors of the model, PARAFAC/PARAFAC2 trilinear decomposition estimates unique profiles for each factor. That allows the unequivocal identification of the analyte (melamine identified from the spectral profile) and also avoids the overestimation (associated with false non-compliant results).

CRediT authorship contribution statement

M.M. Arce: Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **M.C. Ortiz:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. **S. Sanllorente:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2022.107648.

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