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KINETIC MODELS OF MIGRATION OF MELAMINE AND FORMALDEHYDE FROM MELAMINE KITCHENWARE WITH DATA OF LIQUID CHROMATOGRAPHY

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Highlights

- Kinetic of migration testing for formaldehyde and melamine in food contact materials
- A procedure is proposed to analyze formaldehyde and melamine by HPLC-DAD
- The process of migration is related with the manufacturing process of kitchenware
- The accumulated amount of formaldehyde after repeated uses exceeds the SML

Abstract

European legislation has established a specific migration limit (SML) of 15 mg kg^{-1} for formaldehyde and 2.5 mg kg^{-1} for melamine. Formaldehyde resins are used in the manufacture of melamine kitchenware. Formaldehyde is listed in group 1 of the IARC list of carcinogenic compounds. To determine the quantity of formaldehyde and melamine as potential migrants from different types of melamine kitchenware (glass, mug, cutlery, big cup and bowl), a HPLC-DAD method has been implemented. This method is an alternative to the ones proposed in technical guidelines to determine formaldehyde by UV-visible

spectrophotometry and melamine by HPLC. The final objective was to fit the migration kinetic curves of these two analytes in melamine kitchenware.

After the method was validated, decision limit (CCα) and detection capability (CCβ) were calculated for both analytes, when the probabilities of false positive (α) and false negative (β) were fixed at 0.05; being CCβ 0.269 mg L^{-1} and 0.311 mg L^{-1} for melamine and formaldehyde respectively. CCα and CCβ were also calculated at the SML of both analytes.

The migration testing were conducted with simulant B (3% acetic acid (w/v) in aqueous solution), the conditions of each exposure being 70°C for 2 hours. The quantities of melamine and formaldehyde found in the third exposure of the total kitchenware analysed were between 0.21 and 1.09 mg L^{-1} and between 0.55 and 3.86 mg L^{-1}, respectively.

Migration kinetic curves were built for each type of kitchenware with the data of sixteen consecutive migration cycles (70°C each 30 minutes). The SML for melamine was surpassed in the mug, in the big cup and in the bowl after eleven, thirteen and one cycles, respectively.

$^{-1}$When more cycles were carried out in the mug, the values of the accumulated quantity of formaldehyde and melamine were 15.30 and 6.79 mg L^{-1}, respectively, after thirty-two cycles. Both concentrations exceeded the corresponding SML.

Keywords: melamine, formaldehyde, kitchenware, HPLC-DAD, migration kinetic curve, specific migration limit

1. Introduction

Formaldehyde is a widely used chemical compound. It is used in the manufacture of various insulating materials, as a fungicide, a germicide and an industrial disinfectant and as a preservative in the health industry. It is also used in different industrial chemical processes: the manufacture of varnishes, paints, glues, sizings for textiles and as a preservative in cosmetics [1].

Its main use is in the production of resins (phenol-formaldehyde, urea-formaldehyde, polyacetals, melamine-formaldehyde) due to the ease with which it polymerises. In particular, melamine-formaldehyde resin is widely used in kitchenware, and articles made of this polymer are commonly known as ‘melaware’. These resins are used as glues, adhesives and binders in the wood, pulp and paper industries, in synthetic glass fibres, plastics, coatings and textile finishes [1].

Until a few years ago, formaldehyde was classified in category 2A in the IARC list of carcinogenic compounds [2]. In June, 2004 in Lyon, an IARC international working group of experts in chemical carcinogenesis and related fields carried out the necessary tests and obtained enough evidence to conclude, in a monographic publication [3], that formaldehyde should be classified as a category 1 carcinogenic for humans, in which it has been included since 2012 [4]. The way of exposure to formaldehyde can be different: dermic, oral or

inhalation. For this reason, attempts are being made to eliminate or at least reduce its presence as well as to increase the requirements and the number of controls of its exposure.

Despite it being a carcinogenic for humans, it can be found as a natural product in most living systems and in the environment. It is produced in fruits and other foods, it is formed in mammals, including humans, through oxidative metabolic processes and is formed in the first stages of plant residue decomposition [1].

Other sources which give rise to formaldehyde in the environment are forest fires, smoke from tobacco and combustion processes. The hydrocarbons emitted by these processes (vehicle emissions, incinerators, refineries, wood burners, etc.) undergo a photochemical oxidation which releases formaldehyde into the environment. Fortunately, formaldehyde has a short life span in the environment as it is eliminated from the air by photochemical processes and by rain and biodegradation [1].

Melamine (2,4,6-triamine-1,3,5-triazine) is an organic compound produced from urea. In 2010, EFSA [5] reported that melamine and its salts are not expected to be accumulative. It is used mainly in the synthesis of melamine-formaldehyde resin, the applications of which include the manufacture of kitchenware and as an adhesive for making agglomerated boards and plywood in furniture manufacture [6,7,8].

When this kitchenware comes into contact with food, migration of these two substances (melamine and formaldehyde) to the food make take place [7], perhaps exposing people, mainly young children and babies, the main users of these types of kitchenware, to these agents.

European legislation establishes maximum migration levels known as specific migration levels (SML) for the two substances, specifically for migration from plastic materials in contact with foods [9,10] being 15 and 2.5 mg kg^{-1} for formaldehyde and melamine, respectively.

Due to the existing concerns over the migration of these compounds and in order to verify compliance with the legislation, various authors have published studies which quantify the migration of formaldehyde and melamine from kitchenware [8,11,12,13,14,15,168,].

The technical guidelines [17] indicates that the analytical process for the determination and quantification of melamine is high performance liquid chromatography coupled to an ultraviolet detector (HPLC-UV) at 230 nm. Formaldehyde requires prior derivatization, either with chromotropic acid in the presence of sulphuric acid, or with 2,4-pentanedione in the presence of ammonium acetate. Its determination and quantification is carried out by spectrophotometry, at 574 and 410 nm respectively [18].

The most widely used analytical technique for the determination of formaldehyde is spectrophotometry, with prior derivatization using chromotropic acid [12,13,14,16] or using 2,4-pentanedione [11,15]. Other authors use HPLC coupled to a diode array detector (DAD) derivatizing with 2,4-dinitrophenylhydrazine (DNPH) [8].

For the determination of melamine, the technique used is HPLC-UV [15,16], HPLC-DAD [8,13] and ultra high performance liquid chromatography coupled to a diode array detector (UHPLC-DAD) [12].

Apart from the determination of formaldehyde in migration testing, this analyte can also be determined by HPLC-DAD using a derivatizing agent such as DNPH in: environmental samples [19,20,21,22], wine [23], electronic cigarette vapour [24] or cigarette smoke [25], cosmetics and hair products [26,27], drug substance [28], nail polish remover and analytical-grade acetone [29]. Formaldehyde has also been determined by HPLC-UV with DNPH as derivatizing agent in shampoo [30]. Burini and Coli [31] use ethyl acetoacetate as derivatizing agent and HPLC-DAD for the determination of formaldehyde in spirits. Liquid chromatography coupled to a fluorescence detector (HPLC-FLD) and ampicillin as derivatizing agent were selected to determine formaldehyde in blood plasma [32].

Spectrophotometric techniques have been used to analyse rain water, mainstream smoke and tips of smoked cigarettes, using N,N-diethyl-p-phenylenediamine [33]; and paper and cardboard food packaging materials [34] and fish samples [35], derivatizing with 2,4-pentanedione. Other analytical techniques such as excitation-emission matrix fluorescence spectroscopy (EEM) have been chosen by different authors for the analysis of food samples, using as derivatizing agent pyronine in the presence of phosphoric acid and sodium iodate [36] or with cyclohexane-1,3-dione [37] or with n-propylamine [38]; and blood plasma samples with pyronine in the presence of sulphuric acid and potassium bromate [39].

Despite the existence of ample bibliography regarding ways in which to determine formaldehyde and melamine, there is no information as to the migration kinetic curves for these two migrants. Recently efforts have been made by the European Commission to elaborate technical reports in support of Regulation (EU) No 10/2011 of plastic food contact material [40]. This report brings together practical guidelines on the application of migration modelling for the estimation of specific migration. This research project validated the diffusion model, the mathematical equations to be applied, the estimation procedure for the mass transfer coefficients and the conditions for their appropriate application with special focus on monolayer polyolefin plastics. The research looks at the problem of building these models based on physical fundamentals but, despite being an extensive and thorough piece of work, cannot cover every type of plastic-food behaviour. Specifically, melamine-formaldehyde resin is not included in the research.

This work performs the determination, quantification and migration kinetics of melamine and formaldehyde in different kitchenware (glass, mug, cutlery, big cup and bowl), all purchased in a local shop. . Those two analytes were chosen for the determination because utensils were made of melamine-formaldehyde resin (melaware).

The determination and quantification of melamine and formaldehyde was carried out by means of high performance liquid chromatography coupled to a diode array detector (HPLC-DAD). The derivatization of the formaldehyde was done with 2,4-pentanedione in the presence of ammonium acetate and acetic acid. For both analytes, the decision limit (CCα)

and detection capability (CCβ) were determined at a concentration equal to zero [41] and at their SMLs [42].

Firstly, migration test samples were analysed according to Regulation 10/2011 [9] which indicates that three extractions are required and that only the third one should be analysed. Testing should be performed under standardized test conditions (time, temperature and food simulant) representing the worst foreseeable conditions of use of the plastic kitchenware. In addition, ref. [18] establishes that the migration conditions should be 2 hours at 70°C in food simulant (acetic acid 3% w/v) for articles that could not foreseeably be used for cooking. However, in this work the results are shown for the three exposures in order to be able to evaluate the effect of changing the article and the migration exposure. For this task, three articles (A, B, C) of each type of kitchenware were exposed to three migration tests and were analysed in triplicate.

In this study, the mathematical model of the migration kinetics which best explains the experimental data was fitted and validated and thus one can observe the tendency shown by the concentration migrated from these objects after their repeated use [43]. Taking into account that these utensils are used in a continuous way, the behaviour of the migrants with time and temperature is important from the point of view of human protection. For the five types of kitchenware, sixteen consecutive migrations were performed at 70°C every 30 minutes. In order to test whether the quantity of formaldehyde migrated was above the SML in any case, several additional cycles were done on the mug and the bowl. Finally, a complete migration kinetic curve was fitted using the experimental values obtained after twenty-one migration cycles at 70ºC for 1 hour on a new mug.

2. Material and methods

2.1. Chemicals and reagents

Formaldehyde (minimum 37% (w/v) and stabilized with about 10% methanol), ammonium acetate and 2,4-pentanedione were purchased from Merck (Darmstadt, Germany). Melamine was obtained by Alfa Aesar (Kandel, Germany). Acetonitrile (LiChrosolv® isocratic grade for liquid chromatography) was also supplied by Merck (Darmstadt, Germany). Glacial acetic acid (HiPerSolv Chromanorm for HPLC) was purchased from 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).

Other chemicals were employed. Sodium thiosulfate pentahydrate (pro analysis) was supplied by Merck (Darmstadt, Germany). Iodine and potassium iodate were obtained by Sigma-Aldrich (Madrid, Spain). Sodium hydroxide was purchased from Ercros (Barcelona, Spain). Hydrochloric acid of 37% purity (AnalaR Normapur) was obtained by VWR BDH Chemicals (Fontenay-sous-Bois, France). Potassium iodide was acquired in Labken (Barcelona, Spain).

2.2. Instrumental

An Ultrasonic Cleaner (VWR International BVBA, Leuven, Belgium) was employed for dissolving melamine standard stock solution. Simulant B was preheated before extractions in a 200209 JP Selecta oven (Barcelona, Spain).

Migration tests and migration kinetic curves samples were obtained using a water bath equipped with an immersion thermostat Digiterm 200 (JP Selecta S.A., Barcelona, Spain). The thermostatic bath was also employed in the derivatization process.

Quantification of melamine and formaldehyde 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), a diode array detector (G7117C) and OpenLab CDS ChemStation software. A Kinetex EVO-C18 column (150 mm × 4.6 mm, 5 μm) was used for the separation. Acetonitrile (solvent A) and deionized water (solvent B) were used as mobile phases.

The diode array detector was programmed to measure the absorbance at 230 nm and 410 nm for melamine and formaldehyde respectively. Retention times were 2.1 and 2.8 minutes, respectively. Figure 1a shows an example of chromatograms obtained in HPLC-DAD analysis of a sample. A and B are melamine and formaldehyde peaks, respectively, whose integrated areas were employed in the calculation of concentration of both analytes. Figure 1b shows the chromatogram of calibration standard solution that contained 0.25 mg L^{-1} of melamine (the lowest concentration level in calibration).

2.3. Standard solutions and samples

First, titration of formaldehyde commercial solution was carried out. Sodium thiosulfate pentahydrate and iodine solutions were employed in titration of formaldehyde. Iodine solution was titrated with sodium thiosulfate pentahydrate solution, and this, in turn, was titrated with potassium iodate and potassium iodide.

Individual standard stock solutions (melamine 500 mg L^{-1} and formaldehyde 1500 mg L^{-1}) were prepared by dissolving each standard in water and storing at 4°C. Formaldehyde 150 mg L^{-1} was prepared from standard stock solution by dilution with water. Melamine and formaldehyde 50 mg L^{-1} were prepared from their respective more concentrate solution by dilution with simulant B (3% acetic acid (w/v) in aqueous solution). Calibration standard solutions were prepared from each 50 mg L^{-1} solution by dilution with simulant B, in the range of 0 to 10 mg L^{-1} for each analyte. Solutions were stored at 4°C. Twelve calibration standards were used to build calibration lines (0, 0.25, 0.50, 0.75, 1.5, 3, 4, 5, 6, 7.5, 9 and 10 mg L^{-1}).

In all samples, simulant B is used as aqueous food simulant, which is always preheated in an oven at 70°C. As the technical guidelines [18] state, simulant B reaches up to approximately 0.5 cm from the edge of articles tested by filling (glass, mug, big cup and bowl). However, cutlery was tested by immersion and placed in a beaker. The simulant B covered cutlery up to 1 cm from the end of the handle so the functional part of the cutlery that comes into contact with the foodstuff was entirely covered. The simulant was collected after migration testing for the samples of glass and cutlery in a 250-mL volumetric flask, whereas a 500-mL volumetric flask was used for samples of mug, big cup and bowl and in both cases completed to the mark. Therefore, the dilution factors that must be applied to obtain the final result were different, depending on the type of kitchenware analysed.

In this work, migration testing was carried out first. Three articles of each type of kitchenware (glass, mug, cutlery, big cup and bowl, see Figure 2) were used in migration testing. Every article went through three exposures (70±0.2°C for 2 hours) as is indicated in ref. [18], each of which was analysed in triplicate. Secondly, the corresponding migration kinetics for both analytes have also been carried out. One new article of each type of kitchenware was used in this last case. Every article was subjected to successive migrations (70±0.2°C for 30 minutes) and their extracts were stored at 4°C. In all cases, simulant B was preheated in an oven at 70ºC. Then, it was poured inside the utensil. After being at 70ºC (during the time exposure) in a thermostatic bath, the total volume of simulant employed was collected and the article was left empty. These actions were repeated in every migration procedure. The utensils were not washed or rinsed between cycles. No traces were observed in the simulant in any of the cycles. In the visual inspection, no damage, break, crack or loss of the drawings (decoration of the utensils) were observed in the kitchenware after the migration procedure.

The chromatographic analysis was carried out in different conditions for each of the analytes: melamine and formaldehyde. Formaldehyde analysis needs a prior derivatization procedure, which consists of adding 16 mL of deionised water and 4 ml of a reactive solution, in this order, to a 4 mL aliquot of sample (calibration standard or test sample) in a 25-ml flask, completed to the mark with deionised water. Reactive solution was prepared by dissolving 15 g of ammonium acetate in 80 mL of water, approximately, adding 0.3 and 0.2 mL of glacial acetic acid and 2,4-pentanedione respectively, and completing to the mark in a 100-mL flask. The above prepared solutions were placed in a 60°C bath for 10 minutes, and then in an ice bath for 10 minutes.

The conditions for the melamine chromatographic analysis were an isocratic mobile phase consisting of acetonitrile/water (15:85, v/v) at a flow rate of 0.6 mL min^{-1}, while the conditions for the formaldehyde analysis were acetonitrile/water (20:80, v/v) at 1.0 mL min^{-1}. In both analyses, the temperature of the column compartment was 20°C, the injection volume was 20 µL, and the time of the entire analysis was 4 minutes.

2.4. Software

The regression models and the analysis of variance (ANOVA) were carried out using STATGRAPHICS Centurion XVII [44]. Decision limit (CCα) and detection capability (CCβ) were determined using the DETARCHI program [41] and CCα and CCβ at the specific migration limit [42] were estimated using NWAYDET (a program written in-house that evaluates the probabilities of false non-compliance and false compliance).

3. Results and discussion

3.1. Performance criteria

3.1.1. Calibration and accuracy lines

Two calibrations were done for melamine and formaldehyde with twelve standard solutions in a range from 0 to 10 mg L^{-1} for both analytes as was indicated in Section 2.3 (two concentration levels were replicated). It was found that standard 9 mg L^{-1} melamine solution was an outlier datum (studentized residual equal to -4.83) and the model for melamine was redone without it. Rows 1 to 6 in Table 1 show both regression models, which are significant at a 95% confidence level because the p-value is less than 10^{-4} (the null hypothesis of this test H_0: the model does not explain the variability of the response). Moreover, both models appear to be adequate for the experimental data, because the p-value for lack-of-fit test is greater or equal to 0.05 (the null hypothesis of this test H_0: the model is not biased).

In addition, rows 7 to 9 in Table 1 show accuracy line results for melamine and formaldehyde. The intercept of these lines 'estimated concentration *versus* true concentration' were not different from 0 and their slopes were not different from 1 at a significance level of 5%. Therefore, trueness was verified at 95% confidence level for both procedures. The precision of the method can be estimated from the residual standard deviation of the accuracy lines shown in row 9 in Table 1.

3.1.2. Decision limit and detection capability

CCα and CCβ were used to evaluate the detection limits in order to ensure the risks of false positive and false negative probabilities that in this work were fixed at 0.05. ISO 11843 [45] and several EU regulation [46] define the decision limit (CCα) as "the value of the net concentration the exceeding of which leads, for a given error probability α, to the decision that the concentration of the analyte in the analysed material is larger than that in the blank material". That is, decision limit (CCα) means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant. And the detection capability (CCβ) for a given probability of false positive, α, as "the true net concentration of the analyte in the material to be analysed, which will lead, with probability 1-β, to the correct conclusion that the concentration in the analysed material is larger than that in the blank material".

CCα and CCβ values of the procedure at a concentration equal to zero were calculated through the calibration lines and are shown in rows 10 to 11 in Table 1 [41]. [1,1] The procedure enables the determination of CCβ with probabilities of false positive (α) and false negative (β) equal to 0.05, being 0.269 and 0.311 mg L^{-1} for melamine and formaldehyde, respectively.

In the case of substances with an SML, CCβ means the smallest content of the substance that may be detected, identified or quantified in a sample with an error probability of β (false compliant), that is the concentration at which the method is able to detect the specific migration limit concentrations with a statistical certainty of 1 – β [46]. The values of CCβ when the probabilities of false non-compliant (α) and false compliant (β) were fixed at 0.05, were 2.690 and 17.25 mg L^{-1} for melamine and formaldehyde, respectively.

3.1.3. Unequivocal identification of analytes

The confirmatory criteria for HPLC-DAD laid down in [46] were followed in this work to guarantee the unequivocal identification of every analyte. These performance criteria are: i) The retention time of the analyte, shall be the same as that of the calibration standard in the appropriate matrix, within a margin of ± 2.5 %. ii) Verify that the spectrum matches with that of a reference standard, one of the main requirements is that the absorption maxima in the spectrum of every analyte shall be at the same wavelengths as those of the calibration standard within a margin determined by the resolution of the detection system. For diode array detection (DAD), this is typically within ± 2 nm.

The retention times of both analytes in the calibration standards match the ones in test samples, being 2.080 and 2.078 min for melamine and 2.826 and 2.805 min for formaldehyde, respectively.

Emission spectra between 200 and 248 nm for melamine and between 320 and 500 nm for formaldehyde, each 2 nm, were recorded to carry out the above requirements. Figure 3 shows emission spectra of a blank sample, a calibration standard and a test sample. As can be observed, the previous requirement was satisfied for both analytes. Moreover, correlation coefficients between the calibration standard sample and the test sample spectra were calculated, these being 0.993 and 1.000 for melamine and formaldehyde respectively.

3.2. Migration testing

In the migration testing, three articles were analysed (A, B, C) of each of the five types of kitchenware selected described in Section 2.3 (glass, mug, cutlery, big cup and bowl). Every article went through three migration exposures, each of which was analysed in triplicate.

The amount of melamine and formaldehyde which migrates from the kitchenware, obtained after carrying out the chromatographic analysis, is shown in Tables 2 and 3.

As can be seen, in no case is the SML surpassed [9,10]. For the majority of the kitchenware, the concentration found in the third exposure is greater than in the previous exposures. In this work, the amounts of melamine and formaldehyde migrated in the third exposure [18] were always below 1.09 mg L^{-1} (big cup) for the melamine and 3.86 mg L^{-1} (bowl) for the formaldehyde.

It can be concluded that the maximum amount found both of melamine and of formaldehyde for the total of the migration tests was 1.28 and 4.46 mg L^{-1}, respectively, in the first exposure in the bowl. In addition a certain degree of variability can be seen in the concentration found for the three articles for the same type of kitchenware and the same exposure. This leads us to believe that the migration process of the analytes from the material to the simulant is affected by the quality of the finished product, that is, that the conditions of the manufacturing process cause the migration to vary.

From an inferential point of view, a 2-way ANOVA with interaction was performed with the data in Tables 2 and 3 and the model for two fixed factors described in Eq. 1. The first factor considered was the article (at 3 levels: A, B, C) and the second one the migration exposure (at 3 levels: 1, 2, 3). The results of the 2-way fixed-level ANOVA are detailed in Table 4 for melamine and Table 5 for formaldehyde. The first 4 columns in these tables show the Mean Square (MS); from columns 5 to 7 the F calculated in the ANOVA; and the following columns 8 to 11 show the variance expressed as percentage. These results show that both factors and their interaction have a statistically significant effect at 95% confidence level because the p-value is less than 10^{-4} (the null hypothesis of this test H_0: there is not effect of factors (or interaction)).

$$X_{ij} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + \varepsilon_{ijk} \qquad [1]$$

It can be seen that the breakdown of the total variance in this ANOVA expressed as a percentage is distributed as follows: the lowest percentage is due to the residual error (σ^2 (ε)) being below 2.2% and 4.6% for melamine and formaldehyde respectively; the second, in general, is due to the interaction (σ^2 ($\tau\beta$)). A great difference has been observed in the percentages found, for the kitchenware analysed, for both factors (σ^2 (τ), σ^2 (β)).

From the analysis of variance, it can be deduced that migration procedure of both analytes depend strongly on articles and exposures, that is, that it depends on the industrial production process of each analysed article.

3.3. Migration kinetic curves

In order to build the migration kinetic curves, the amount of melamine and formaldehyde in each of the extracts obtained in each cycle was determined as described in Section 2.3. Migration kinetic models were built using the accumulated amount of melamine and formaldehyde, that is, by adding up the amount found in each of the migrations to the previous ones (sixteen cycles at 70°C for 30 minutes). Those mathematical models which best

explain the experimental data were fitted and statistically validated for the migration kinetic curves of each type of kitchenware.

All the models, included in Table 6, explain at least 99.31% of the response variability (milligram of analyte migrated per litre of simulant). The SML for melamine was surpassed in the mug, in the big cup and in the bowl, being 2.68, 2.54 and 3.38 mg L^{-1} the accumulated amount of melamine found after eleven, thirteen and one cycles, whereas the values for formaldehyde were below the SML. After sixteen cycles, the maximum amount found was 4.67 mg L^{-1} of melamine (in the case of the bowl) and 8.22 mg L^{-1} of formaldehyde (in the mug). Table 6 shows the models and the variance explained by them. Figures 4a and 4b show migration kinetic curves fitting for all the utensils analysed, in Fig. 4a for melamine and in Fig. 4b for formaldehyde. In these figures the glass is represented in purple, the mug in orange, the cutlery in green, the big cup in yellow and the bowl in blue. In both graphs one can clearly see the different behaviour shown by the melamine migration for the bowl compared with the rest of the fitted models.

In addition, an estimate was made of the number of theoretical consecutive migration cycles to which each type of kitchenware would have to be exposed in order to surpass the SML (2.5 and 15 mg L^{-1} for melamine and formaldehyde, respectively). The values calculated from the prediction in the models in Table 6 are shown in Table 7.

From the predictions obtained in the fitted models, it was decided to carry out thirteen further cycles, at 70°C for 30 minutes, in the mug and the bowl. The objective was to check whether one would really reach the limit for the formaldehyde in the mug and whether the new experimental points fitted the models proposed in the first study of the migration kinetics . The accumulated amount found after twenty-nine cycles was 6.07 and 13.62 mg L^{-1} of melamine and of formaldehyde respectively in the mug, and 6.07 and 6.83 mg L^{-1} in the bowl. In both utensils, the accumulated quantity of melamine migrated was more than twice the SML. On the contrary, the SML was not reached for the formaldehyde.

For the third time, several more cycles were performed on the mug. It was proven experimentally that with thirty-two cycles, an accumulated amount of formaldehyde migrated from the mug was reached which surpassed the SML, 15.30 mg L^{-1}. In addition, the accumulated quantity of melamine found after forty-three cycles was 10.39 mg L^{-1}, which quadruplicates the SML.

In order to perform the chromatographic analyses in a single session, in addition a complete migration kinetic curve was built using the experimental values obtained after twenty-one cycles at 70 ºC every 1 hour in a new mug. The mathematical new models for the melamine ($Y = (0.595 + 0.116x)^2$ with R^2 = 99.82%) and for the formaldehyde ($Y = (-0.257 + 0.856x^{0.5})^2$ with R^2= 99.98%) were fitted and statistically validated. The representation of these curves is shown in Figure 5.

Although the migration cycles carried out on the new mug were of 1 hour, instead of 30 minutes as in the previous study, after twenty one cycles the amount of formaldehyde

accumulated reached 13.36 mg L^{-1}, below the SML. This result again highlights what was observed in Section 3.2, those utensils of the same type of kitchenware made by the same manufacturer show different behaviour or migration tendencies, almost certainly due to the manufacturing process for each article.

The accumulated quantity of melamine migrated from the mug is nearly four times the SML, 9.46 mg L^{-1}. This quantity was found after twenty-one cycles (70°C for 1 hour) and this result is comparable with the one obtained in the previous study (10.39 mg L^{-1} of melamine was found after forty-three cycles at 70°C each 30 minutes). Therefore, similar amounts of melamine migrated when the number of consecutive cycles was the half, but the time of exposure was twice.

4. Conclusions

A fast method for the determination of melamine and formaldehyde by means of HPLC-DAD has been developed and the unequivocal identification of both analytes through their absorption spectra has been carried out.

The amount of melamine and formaldehyde , when any of the kitchenware analysed is used , has not exceeded the specific migration limit (SML) for both analytes after three exposures at 70ºC for 2 hours.

However, the migration kinetic curves built for the different kitchenware and for both analytes show that the accumulated amount of melamine and formaldehyde reached the specific migration limits even when the number of cycles performed was not high. For this reason, it seems reasonable to advise that those melamine-formaldehyde utensils, most commonly used by children, should be used with caution bearing in mind the carcinogenic properties of formaldehyde. The amount of melamine found in this work was not relevant since, according to the EFSA studies, melamine and its salts are not expected to be accumulative.

In addition, the analysis of variance has shown that the process of migration of the analytes from the material to the simulant is related with the articles and the exposures, and therefore with the manufacturing process of each article, which obviously depends to a large extend on the production batch.

The results obtained in this work are related to the analysis performed in five types of utensils made of melamine which have been produced by the same manufacturer. The intention of the authors with the conclusions obtained from this work was not to generalize about all the utensils made of melamine-formaldehyde resin available in the market.

Conflict of interest

Declarations of interest: none.**Acknowledgments**

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References

[1] M. Qu, J. Lu, R. He, Formaldehyde from environment, in: R. He (Eds.), Formaldehyde and Cognition, Springer, 2017, pp. 1-13.

[2] International Agency for Research on Cancer (IARC), Formaldehyde. Summary of data reported and evaluation, in: IARC (Eds.), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Wood dust and formaldehyde, IARC, Lyon, 1995, volume 62, pp. 332-336.

[3] International Agency for Research on Cancer (IARC), Formaldehyde. Summary of data reported and evaluation, in: IARC (Eds.), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol, IARC, Lyon, 2006, volume 88, pp. 273-280.

[4] International Agency for Research on Cancer (IARC), Formaldehyde. Evaluation, in: IARC (Eds.), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Chemical agents and related occupations. A review of human carcinogens, IARC, Lyon, 2012, volume 100 F, pp. 430.

[5] European Food Safety Authority (EFSA), Scientific opinion on melamine in food and feed, EFSA Journal 8 (2010) 1573.

[6] Y.-C. Tyan, M.-H. Yang, S.-B. Jong, C.-K. Wang, J. Shiea, Melamine contamination, Analytical and Bioanalytical Chemistry 395 (2009) 729-735.

[7] Y.-F. Hsu, K.-T. Chen, Y.-W. Liu, S.-H. Hsieh, H.-Y. Huang, Determination of melamine and related triazine by-products ammeline, ammelide, and cyanuric acid by micellar electrokinetic chromatography, Analytica Chimica Acta 673 (2010) 206-211.

[8] V. García Ibarra, A. Rodríguez Bernaldo de Quirós, R. Sendón, Study of melamine and formaldehyde migration from melamine tableware, European Food Research and Technology 242 (2016) 1187-1199.

[9] Commission Regulation (EU) No 10/2011 of 14 January 2011. Plastic materials and articles intended to come into contact with food, 2011.

[10] Commission Regulation (EU) No 1282/2011 of 28 November 2011 amending and correcting Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food, 2011.

[11] N. Poovarodom, K. Junsrisuriyawong, R. Sangmahamad, P. Tangmongkollert, Effects of microwave heating on the migration of substances from melamine formaldehyde tableware, Food Additives and Contaminants 31 (2014) 1616-1624.

[12] V. Mannoni, G. Padula, O. Panico, A. Maggio, C. Arena, M.-R. Milana, Migration of formaldehyde and melamine from melaware and other amino resin tableware in real life service, Food Additives and Contaminants 34 (2017) 113-125.

[13] K.H. Lund, J.H. Petersen, Migration of formaldehyde and melamine monomers from kitchen- and tableware made of melamine plastic, Food Additives and Contaminants 23 (2006) 948-955.

[14] E.L.J. Potter, E.L. Bradley, C.R. Davies, K.A. Barnes, L. Castle, Migration of formaldehyde from melamine-ware: UK 2008 survey results, Food Additives and Contaminants 27 (2010) 879-883.

[15] R.E. Martin, C.B. Hizo, A.M. Ong, O.M. Alba, H. Ishiwata, Release of formaldehyde and melamine from melamine tableware manufactured in the Philippines, Journal of Food Protection 55 (1992) 632-635.

[16] E.L. Bradley, V. Boughtflower, T.L. Smith, D.R. Speck, L. Castle, Survey of the migration of melamine and formaldehyde from melamine food contact articles available on the UK market, Food Additives and Contaminants 22 (2005) 597-606.

[17] UNE-CEN/TS 13130-27. Materials and articles in contact with foodstuffs. Plastics substances subject to limitation. Determination of 2,4,6-triamino-1,3,5-triazine in food simulants, 2006.

[18] EUR 24815 EN. Technical guidelines on testing the migration of primary aromatic amines from polyamide kitchenware and of formaldehyde from melamine kitchenware, 2011.

[19] F. Villanueva, A. Tapia, A. Notario, J. Albaladejo, E. Martínez, Ambient levels and temporal trends of VOCs, including carbonyl compounds, and ozone at Cabañeros National Park border, Spain, Atmospheric Environment 85 (2014) 256-265.

[20] A. Levart, M. Veber, Determination of aldehydes and ketones in air samples using cryotrapping sampling, Chemosphere 44 (2001) 701-708.

[21] D. Custódio, C.S. Guimarães, L. Varandas, G. Arbilla, Pattern of volatile aldehydes and aromatic hydrocarbons in the largest urban rainforest in the Americas, Chemosphere 79 (2010) 1064-1069.

[22] M.C. Prieto-Blanco, M.P. Iglesias, P. López-Mahía, S.M. Lorenzo, D.P. Rodríguez, Simultaneous determination of carbonyl compounds and polycyclic aromatic hydrocarbons in atmospheric particulate matter by liquid chromatography–diode array detection–fluorescence detection, Talanta 80 (2010) 2083-2092.

[23] R.J. Elias, V.F. Laurie, S.E. Ebeler, J.W. Wong, A.L. Waterhouse, Analysis of selected carbonyl oxidation products in wine by liquid chromatography with diode array detection, Analytica Chimica Acta 626 (2008) 104-110.

[24] L. Kosmider, A. Sobczak, M. Fik, J. Knysak, M. Zaciera, J. Kurek, M.L. Goniewicz, Carbonyl compounds in electronic cigarette vapors: Effects of nicotine solvent and battery output voltage, Nicotine & Tobacco Research 16 (2014) 1319-1326.

[25] T.L. Wang, H.W. Tong, X.Y. Yan, L.Q. Sheng, J. Yang, S.M. Liu, Determination of volatile carbonyl compounds in cigarette smoke by LC-DAD, Chromatographia 62 (2005) 631-636.

[26] E.N. Oiye, M.F.M. Ribeiro, L.L. Okumura, A. A. Saczk, P. Ciancaglini, M.F. de Oliveira, Forensic investigation of formaldehyde in illicit products for hair treatment by DAD-HPLC: A case study, Journal of Forensic Sciences 61 (2016) 1122-1125.

[27] P.-W. Wu, C.-C. Chang, S.-S. Chou, Determination of formaldehyde in cosmetics by HPLC method and acetylacetone method, Journal of Food and Drug Analysis 11 (2003) 8-15.

[28] A. Soman, Y. Qiu, L.Q. Chan, HPLC-UV method development and validation for the determination of low level formaldehyde in a drug substance, Journal of Chromatographic Science 46 (2008) 461-465.

[29] X.H.H. Huang, H.S.S. Ip, J.Z. Yu, Determination of trace amounts of formaldehyde in acetone, Analytica Chimica Acta 604 (2007) 134-138.

[30] A.N.M. Nguyet, L. Tallieu, J. Plaizier-Vercammen, D.L. Massart, Y.V. Heyden, Validation of an HPLC method on short columns to assay ketoconazole and formaldehyde in shampoo, Journal of Pharmaceutical and Biomedical Analysis 32 (2003) 1-19.

[31] G. Burini, R. Coli, Determination of formaldehyde in spirits by high-performance liquid chromatography with diode-array detection after derivatization, Analytica Chimica Acta 511 (2004) 155-158.

[32] W. Luo, H. Li, Y. Zhang, C.Y.W. Ang, Determination of formaldehyde in blood plasma by high-performance liquid chromatography with fluorescence detection, Journal of Chromatography B 753 (2001) 253-257.

[33] A.A. Mohamed, A.T. Mubarak, Z.M.H. Marestani, K.F. Fawy, Highly sensitive and selective catalytic determination of formaldehyde and acetaldehyde, Talanta 74 (2008) 578-585.

[34] C. Ekinci Dogan, R. Sancı, Formaldehyde migration in aqueous extracts from paper and cardboard food packaging materials in Turkey, Food Additives and Contaminants 8 (2015) 221-226.

[35] Y. Shen, W.F. Wang, Y.Y. Wu, A Method for Identification of Exogenous Formaldehyde in Bombay Duck (Harpodon nehereus), Food Analytical Methods 8 (2015) 2474-2481.

[36] G. Li, L. Han, Determination of formaldehyde in aquatic products by a sensitive catalytic fluorescence method, Analytical Methods 6 (2014) 426-432.

[37] X.-Q. Zhao, Z.-Q. Zhang, Microwave-assisted on-line derivatization for sensitive flow injection fluorometric determination of formaldehyde in some foods, Talanta 80 (2009) 242-245.

[38] S.P. Aubourg, Fluorescence detection in aldehyde containing model systems: Relationship with fish deterioration, Grasas y Aceites 49 (1998) 419-424.

[39] Y.-S. Wang, X. Tan, J.-H. Xue, G.-R. Li, L.-F. Shi, H.-M. Yang, L. Liu, B. Zhou, X.-L. Xiao, Determination of trace formaldehyde in blood plasma by resonance fluorescence technology, Analytica Chimica Acta 690 (2011) 234-239.

[40] EUR 27529 EN. Practical guidelines on the application of migration modelling for the estimation of specific migration, 2015.

[41] L.A. Sarabia, M.C. Ortiz, DETARCHI: a program for detection limits with specified assurance probabilities and characteristic curves of detection, TrAC Trends in Analytical Chemistry 13 (1994) 1-6.

[42] M.C. Ortiz, L.A. Sarabia, I. García, D. Giménez, E. Meléndez, Capability of detection and three-way data, Analytica Chimica Acta 559 (2006) 124-136.

[43] S. Sanllorente, L.A. Sarabia, M.C. Ortiz, Migration kinetics of primary aromatic amines from polyamide kitchenware: Easy and fast screening procedure using fluorescence, Talanta 160 (2016) 46-55.

[44] STATGRAPHICS Centurion XVII Versión 17.2.02 (64 bit). Statpoint Technologies, Inc. 2016.

[45] International Organization for Standardization, ISO 11843, Capability of detection, Part 1: Terms and definitions and Part 2: methodology in the linear calibration case, Genève, Switzerland, 2000.

[46] Commission Decision (EC) No 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002.

FIGURE CAPTIONS

Figure 1. a) Chromatograms of a sample, at 230 and 410 nm, for melamine (orange) and for formaldehyde (blue) respectively and b) chromatogram of a 0.25 mg L^{-1} melamine calibration standard solution at 230 nm.

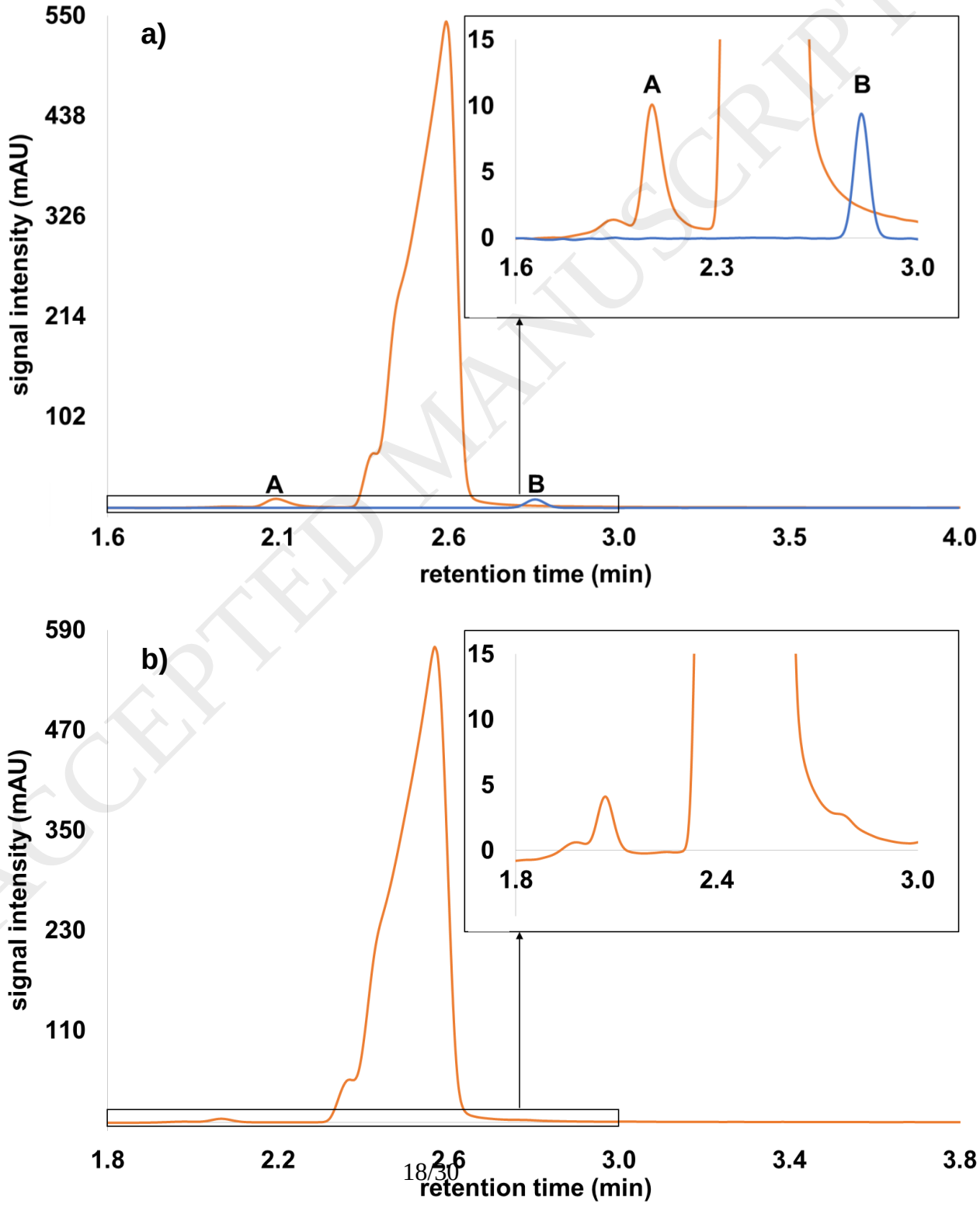

Figure 1.

Figure 2. Types of melamine kitchenware analysed: a) glass, b) mug, c) cutlery, d) big cup and e) bowl.

Figure 2.

Figure 3. Emission spectra for melamine and for formaldehyde recorded in HPLC-DAD analysis. Blank sample is represented in yellow, calibration standard solution in green and test sample in purple.

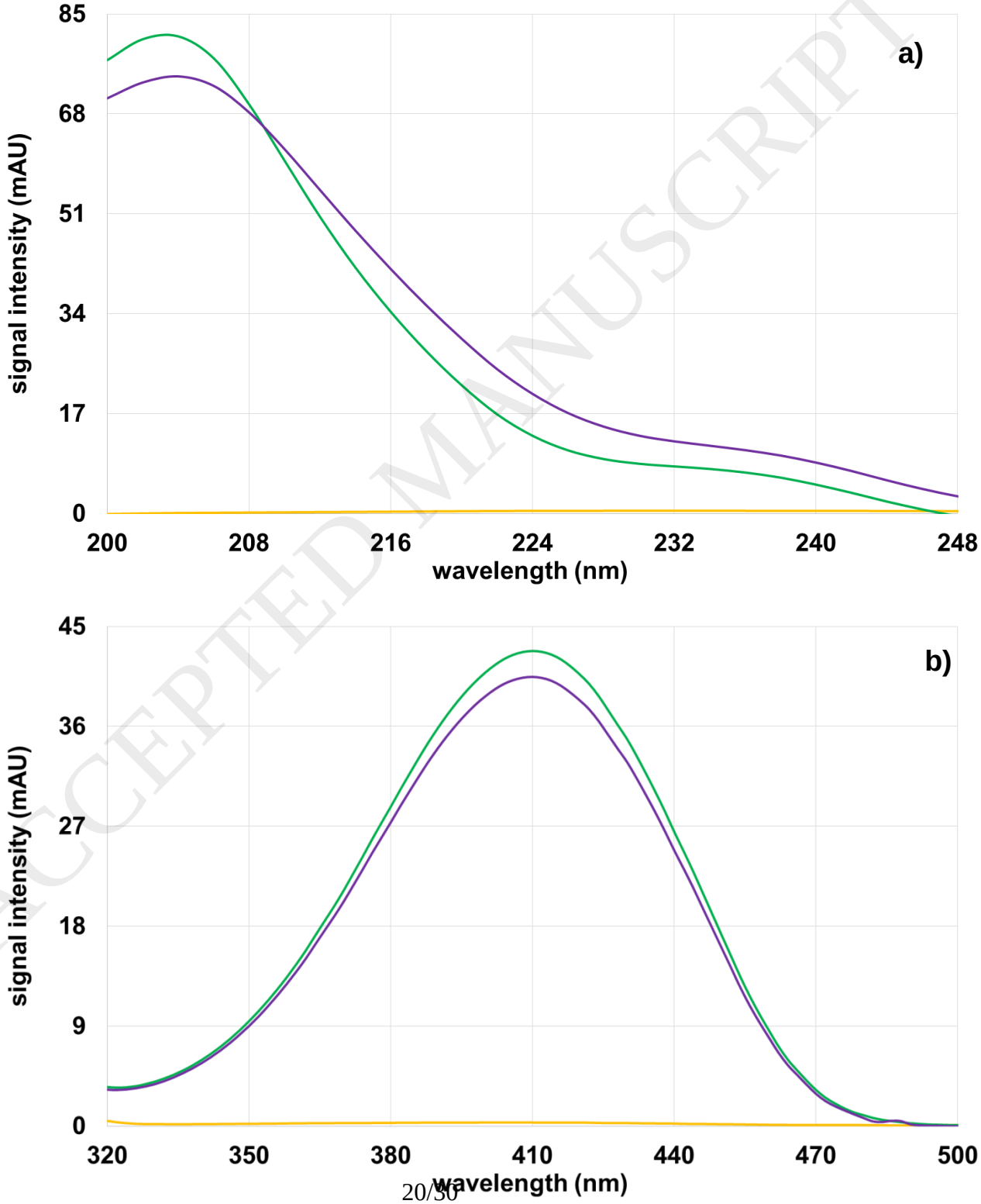

Figure 3.

Figure 4. Migration kinetic curves fitting: a) for melamine and b) for formaldehyde. Glass is represented in purple, mug in orange, cutlery in green, big cup in yellow and bowl in blue.

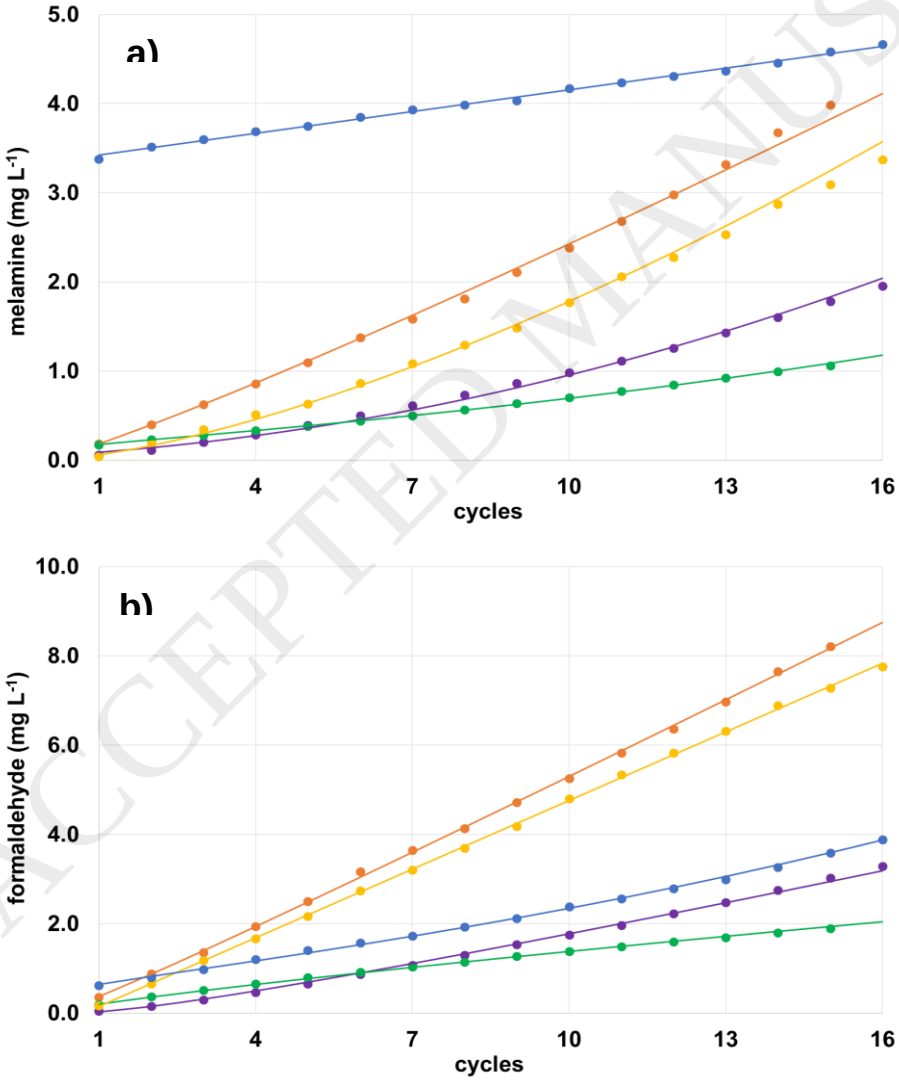

Figure 4.

Figure 5. Migration kinetic curves fitting for 21 consecutive migrations 70°C for 1 hour. Melamine is represented in orange and formaldehyde in blue.

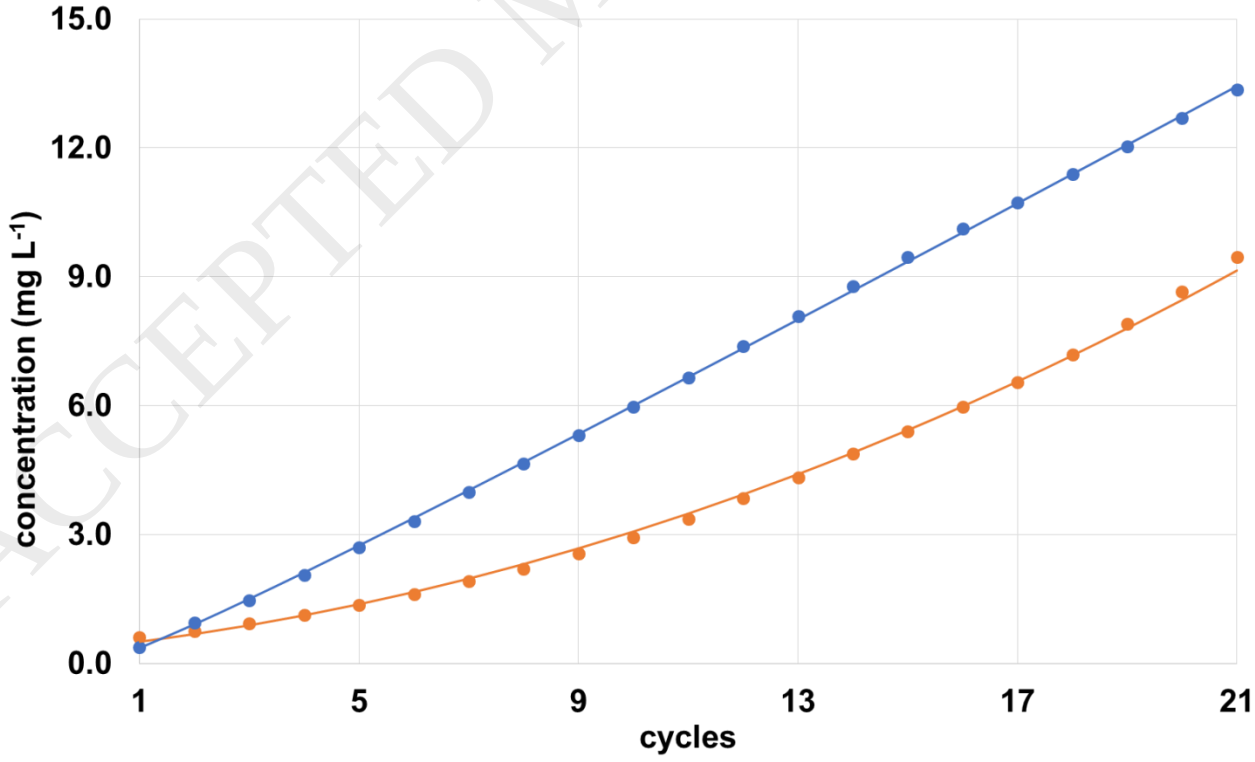

Figure 5.

Table 1. 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 $\alpha = \beta = 0.05$).

		Melamine	Formaldehyde
Calibration line	Intercept	2.126	-0.032
	Slope	65.311	22.773
	Correlation coefficient	0.999	0.999
	s_{yx}	4.606	1.870
	P-value (significance of regression)	$<10^{-4}$	$<10^{-4}$
	P-value (lack-of-fit test)	0.537	0.527
Accuracy line	Intercept	$-2.41 \cdot 10^{-6}$	-0.007
	P-value	0.999	0.835
	Slope	1.000	1.004
	P-value	0.999	0.574
	s_{yx}	0.071	0.079
CCα (x_0=0) mg L^{-1}		0.134	0.159
CCβ (x_0=0) mg L^{-1}		0.269	0.311
CCα (x=SML[a]) mg L^{-1}		2.597	16.15
CCβ (x=SML[a]) mg L^{-1}		2.690	17.25

[a] SML for melamine equal to 2.5 mg kg^{-1} and for formaldehyde equal to 15 mg kg^{-1}.

Table 2. Melamine migration test results from kitchenware, expressed in milligram of analyte per litre of simulant (mg L^{-1}). Three articles (A, B, C) of each type of kitchenware were analysed. Each of the exposures of every article were analysed by triplicate.

Melamine		Exposure 1			Exposure 2			Exposure 3		
Test sample		n=3			n=3			n=3		
Glass	A	0.271	0.284	0.289	0.421	0.445	0.459	0.618	0.640	0.675
	B	0.157	0.160	0.165	0.293	0.314	0.333	0.410	0.425	0.450
	C	0.156	0.153	0.150	0.162	0.176	0.192	0.218	0.223	0.210
Mug	A	0.419	0.429	0.425	0.549	0.524	0.533	0.608	0.623	0.628
	B	0.866	0.898	0.944	0.802	0.797	0.802	0.884	0.855	0.885
	C	0.476	0.500	0.498	0.546	0.537	0.543	0.820	0.829	0.840
Cutlery	A	0.441	0.440	0.449	0.249	0.250	0.250	0.315	0.316	0.323
	B	0.539	0.531	0.540	0.283	0.283	0.284	0.364	0.364	0.373
	C	0.368	0.358	0.337	0.309	0.310	0.288	0.361	0.361	0.361
Big cup	A	0.542	0.541	0.564	0.846	0.875	0.910	0.921	0.881	1.048
	B	0.473	0.474	0.481	0.861	0.847	0.884	1.033	1.068	1.087
	C	0.593	0.551	0.532	0.803	0.812	0.821	0.768	0.780	0.787
Bowl	A	1.262	1.227	1.190	0.552	0.537	0.534	0.624	0.632	0.638
	B	1.279	1.225	1.229	0.289	0.288	0.297	0.359	0.369	0.368
	C	0.692	0.672	0.685	0.589	0.594	0.601	0.516	0.514	0.516

Table 3. Formaldehyde migration test results from kitchenware, expressed in milligram of analyte per litre of simulant (mg L^{-1}). Three articles (A, B, C) of each type of kitchenware were analysed. Each of the exposures of every article were analysed by triplicate.

Formaldehyde		Exposure 1			Exposure 2			Exposure 3		
Test sample		n=3			n=3			n=3		
Glass	A	1.065	1.124	1.132	1.441	1.477	1.483	1.606	1.636	1.644
	B	1.079	1.129	1.143	1.380	1.398	1.384	1.636	1.674	1.695
	C	0.561	0.591	0.606	0.779	0.808	0.819	0.862	0.861	0.854
Mug	A	1.191	1.277	1.333	1.695	1.796	1.869	1.581	1.643	1.693
	B	1.542	1.637	1.699	1.841	1.925	1.986	1.652	1.717	1.764
	C	1.402	1.493	1.564	1.562	1.622	1.678	2.111	2.186	2.240
Cutlery	A	0.727	0.756	0.769	0.624	0.643	0.652	0.557	0.567	0.563
	B	0.772	0.804	0.823	0.590	0.605	0.610	0.551	0.568	0.576
	C	0.734	0.761	0.781	0.646	0.670	0.682	0.592	0.598	0.590
Big cup	A	1.591	1.689	1.722	2.005	2.082	2.156	1.916	1.969	1.998
	B	1.469	1.545	1.591	2.120	2.198	2.283	2.169	2.245	2.276
	C	1.409	1.482	1.565	1.718	1.771	1.807	1.393	1.438	1.467
Bowl	A	4.232	4.381	4.456	3.201	3.289	3.332	3.756	3.852	3.861
	B	1.240	1.300	1.324	1.060	1.100	1.127	1.405	1.420	1.416
	C	2.656	2.780	2.800	3.718	3.821	3.872	2.409	2.460	2.480

Table 4. ANOVA for melamine of two factors (τ: article, β: exposure) and their interaction (τβ) involved in the migration test (Eq. 1)

Melamine	MS calculated				F calculated			Variance estimated (%)			
	MS (τ)	MS (β)	MS (τβ)	MS (ε)	F (τ)	F (β)	F (τβ)	σ^2 (τ)	σ^2 (β)	σ^2 (τβ)	σ^2 (ε)
Glass	$1.69 \cdot 10^{-1}$	$1.21 \cdot 10^{-1}$	$1.79 \cdot 10^{-2}$	$2.65 \cdot 10^{-4}$	639.31	455.52	67.62	49.0	34.9	15.4	0.7
Mug	$2.65 \cdot 10^{-1}$	$7.64 \cdot 10^{-2}$	$3.03 \cdot 10^{-2}$	$2.70 \cdot 10^{-4}$	980.71	283.14	112.42	61.0	17.6	20.8	0.6
Cutlery	$9.95 \cdot 10^{-3}$	$6.28 \cdot 10^{-2}$	$9.63 \cdot 10^{-3}$	$5.56 \cdot 10^{-5}$	179.08	1129.55	173.40	9.7	61.6	28.2	0.5
Big cup	$1.94 \cdot 10^{-2}$	$4.09 \cdot 10^{-1}$	$2.59 \cdot 10^{-2}$	$1.23 \cdot 10^{-3}$	15.80	332.66	21.05	3.6	79.8	14.5	2.2
Bowl	$1.04 \cdot 10^{-1}$	$9.47 \cdot 10^{-1}$	$1.66 \cdot 10^{-1}$	$2.82 \cdot 10^{-4}$	369.88	3357.38	589.98	6.7	61.0	32.1	0.2

Upper significance levels of the F-distribution: $F(\tau) = F(\beta) = F_{0.05,2,18} = 3.55$; $F(\tau\beta) = F_{0.05,4,18} = 2.93$

Table 5. ANOVA for formaldehyde of two factors (τ: article, β: exposure) and their interaction involved in the migration test (Eq.1)

Formaldehyde	MS calculated				F calculated			Variance estimated (%)			
	MS (τ)	MS (β)	MS (τβ)	MS (ε)	F (τ)	F (β)	F (τβ)	σ^2 (τ)	σ^2 (β)	σ^2 (τβ)	σ^2 (ε)
Glass	1.26	$4.63 \cdot 10^{-1}$	$2.06 \cdot 10^{-2}$	$5.94 \cdot 10^{-4}$	2115.03	779.80	34.71	70.4	25.9	3.4	0.3
Mug	$1.11 \cdot 10^{-1}$	$3.76 \cdot 10^{-1}$	$1.56 \cdot 10^{-1}$	$4.97 \cdot 10^{-3}$	22.40	75.62	31.26	10.9	38.1	46.4	4.6
Cutlery	$1.19 \cdot 10^{-3}$	$9.04 \cdot 10^{-2}$	$2.45 \cdot 10^{-3}$	$2.82 \cdot 10^{-4}$	4.21	320.60	8.71	0.9	90.0	6.5	2.5
Big cup	$4.60 \cdot 10^{-1}$	$4.84 \cdot 10^{-1}$	$1.06 \cdot 10^{-1}$	$3.89 \cdot 10^{-3}$	118.25	124.25	27.16	35.7	37.6	23.9	2.7
Bowl	$1.53 \cdot 10^{1}$	$1.30 \cdot 10^{-1}$	1.17	$4.17 \cdot 10^{-3}$	3668.23	31.15	281.58	80.6	0.7	18.5	0.2

Upper significance levels of the F-distribution: $F(\tau) = F(\beta) = F_{0.05,2,18} = 3.55$; $F(\tau\beta) = F_{0.05,4,18} = 2.93$

Table 6. Migration kinetic curves for each kitchenware used: regression models and variance explained (R^2).

	Melamine		Formaldehyde	
	Model	R^2 (%)	Model	R^2 (%)
Glass	$Y = (0.225 + 0.075x)^2$	99.42	$Y = (-0.374 + 0.540x^{0.5})^2$	99.79
Mug	$Y = \exp(-1.700 + 1.123 \ln x)$	99.93	$Y = (-0.173 + 0.783x^{0.5})^2$	99.96
Cutlery	$Y = \exp(-2.359 + 0.631x^{0.5})$	99.96	$Y = \exp(-1.602 + 0.836 \ln x)$	99.88
Big cup	$Y = \exp(-2.831 + 1.481 \ln x)$	99.31	$Y = -0.362 + 0.512x$	99.97
Bowl	$Y = 3.343 + 0.081x$	99.64	$Y = \exp(-1.041 + 0.599x^{0.5})$	99.85

Table 7. Number of consecutive migrations or cycles (70°C for 30 minutes), predicted from migration kinetic curves fitting models (in Table 6), after what SML would have been reached.

	Melamine (2.5 mg L^{-1})		Formaldehyde (15 mg L^{-1})	
	x value	cycles	x value	cycles
Glass	18.02	19	61.87	62
Mug	10.27	11	26.72	27
Cutlery	26.94	27	173.26	174
Big cup	12.57	13	29.98	30
Bowl	*	1	39.16	40

* SML for melamine migrated from bowl was reached in the first cycle.