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Condensed phase membrane introduction mass spectrometry: A new frontier for the real-time monitoring of hazardous chemical migration from food contact materials

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ABSTRACT

In this study, the application framework of Condensed Phase Membrane Introduction Mass Spectrometry (CP-MIMS), a direct mass spectrometry technique, is extended for real-time monitoring of migration processes from food contact materials (FCMs) with a focus on Bisphenol A (BPA) as re-emerging contaminant. The whole instrumental system was properly designed to meet important requirements in terms of signal stability, low noise and ease of handling. A dedicated MATLAB APP was developed for semi-automated processing of instrumental output. A full factorial experimental design was applied to optimize five response variables by varying the acceptor phase flow-rate and composition, stirring, temperature, and membrane length. The CP-MIMS method was validated in tap water and food simulants, obtaining detection limits in the 0.8–6 μg/kg range. Considering the great advantage of real-time analysis of BPA migration from FCMs, not yet explored in literature, its high sample throughput and compliance with the green analytical chemistry principles, the CP-MIMS method has proven to be suitable for the determination of BPA below the specific migration limit established by the EU (0.05 mg/kg). The applicability of the method was demonstrated by performing migration tests on plastic articles, acquiring the migration profile of BPA over time for samples that showed detectable release of BPA. Excellent trueness was proved by comparison with a confirmatory liquid chromatography-high resolution mass spectrometry method. This study provides important insights into the role of CP-MIMS in scientific research to achieve valuable temporal resolution in the study of dynamic processes, such as the release of compounds from FCMs.

1. Introduction

Within the continuous evolution of analytical chemistry, during the last two decades, considerable efforts have been devoted to improving analytical performance [1], reducing the environmental fingerprint [2], and minimizing analytical times [3]. Technological advances have led to high-throughput workflows that follow the principles of Green Analytical Chemistry (GAC), offering selectivity and sensitivity with minimal or no sample treatment and without chromatographic separation.

Chemical sensors and biosensors as well as direct mass spectrometry (MS) techniques meet these criteria. Chemical sensing relies on selective and high-affinity receptors [4]; on the other hand, direct ionization MS techniques rely mainly on unrivalled selectivity of MS detection. In this context, ambient ionization MS encompasses different and evolving techniques that enable direct analysis of samples in their native state, greatly reducing the analysis time [3]. However, ambient-MS are prone to matrix effect and does not offer the possibility of continuous real-time analysis in liquid phase, useful for investigation of dynamic processes and on-line monitoring. Condensed Phase Membrane Introduction Mass Spectrometry (CP-MIMS) fills this gap, being also suitable for on-line/on-site measurements [5,6]. CP-MIMS is a valid alternative to other direct ionization MS strategies: it relies on steady-state mass transfer between the sample and a liquid (condensed) acceptor phase (AP) through a semi-permeable hollow fiber membrane, such as

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polydimethylsiloxane (PDMS), which acts as interface between the sample and the mass spectrometer. This membrane is mounted on a probe and directly immersed into the sample, defined as "donor phase". After diffusion of the target analytes through the membrane, the constant flow of the AP through the membrane continuously conveys the permeated molecules to the mass spectrometer for ionization and MS analysis. Thus, CP-MIMS permits direct analysis in complex matrices by reducing the permeation of interfering compounds. Gills' group developed a variant of CP-MIMS consisting in the use of polymer inclusion membrane (PIM) systems: the use of a linear alkane cosolvent in a methanol AP in combination with a PDMS membrane generates an *in-situ* PIM system, leading to reduced response times and improved sensitivity due to the increased diffusivity and partitioning of the analytes across the membrane [7].

To date, different applications based on CP-MIMS have been described, especially in the environmental field [5,6]; in addition, CP-MIMS has been also exploited for the calculation of the dissociation constants of trace organic compounds [8] and for the real-time monitoring of reagents, intermediates and products involved in an organic synthesis process [9,10]. However, by contrast, the potential of CP-MIMS for food safety and quality assessment has been poorly explored [11].

A phenomenon of great concern for food safety is the migration of potentially hazardous chemicals from food contact materials (FCMs) to foods, which represents an issue that analytical chemistry has to face with particular attention to emerging or re-emerging contaminants [12].

In the European Union plastic materials to be intended as FCMs must comply with the EU Regulation 10/2011 [13] and subsequent amendments, the latest of which was published in August 2023 [14]. This regulation establishes compositional requirements, and testing requirements in terms of overall and specific migration, including guidelines on the selection of simulants and testing conditions. Bisphenol A (BPA), widely used to produce plastics, epoxy resins and paints, is a restricted substance under EU Regulation 10/2011 due to its endocrine-disrupting character and carcinogenic potential. In 2023 the European Food Safety Authority reduced the tolerable daily intake of BPA from 4 to 0.0002 μg/kg body weight (bw) per day, thus renewing interest in evaluating its release from FCMs (current specific migration limit, SML: 0.05 mg/kg) [15]. Considering the new TDI value, the European Commission (EC) is working on the proposal to ban BPA in the manufacture of FCMs, including plastic and coated packaging. In this context, the EC draft proposal states that "it is neither practical nor proportionate to prohibit the unintentional presence of BPA in recycled materials", therefore the monitoring of the presence of BPA in recycled materials by business operators remains an important issue that should be established by the European Commission.

The determination of trace levels of BPA in matrices of different nature is mainly carried out by hyphenated MS techniques, reaching detection limits (LODs) down to sub-ppb levels [16,17]. However, liquid chromatography (LC)- and gas chromatography (GC)-based methods are unsuitable for online monitoring purpose and in contrast with the GAC principles. Regarding the use of ambient MS techniques, Chen et al. proposed the use of Paper Spray MS for the rapid screening and simultaneous determination of BPA and its analogues in food contact materials [18]. Although this approach is high-throughput and more effective than conventional LC-MS-based techniques, it lacks adequate sensitivity (LODs in the 0.1–0.3 μg/mL range), making this application unsuitable for the assessment of material compliance within the current and future regulatory context. In the current literature, an analytical approach suitable for direct-online monitoring of BPA migration from food contact materials has not yet been proposed; the strategy would lead to unexplored levels of material characterization, also in association with different types of simulants.

To fill this knowledge gap, the present study focused on the development and validation of a highly sensitive CP-MIMS-based method for real-time measurement of BPA released by FCMs in water and food simulants. An innovative aspect of the present study is the development of a user-friendly software interface for semi-automatic signal processing for signal pretreatment, optimization and speed up of the subsequent parameterization of CP-MIMS output signals, as well as for noise measurement at both background and steady-state levels. CP-MIMS performance is generally evaluated based on three main parameters: the ability to discriminate analytes, the ability to detect trace concentrations, and the rapid measurement of the response time, which are related to selectivity, sensitivity and $t_{10\text{-}90}$ signal response time, respectively [19]. Therefore, these properties were carefully investigated by varying the acceptor phase composition, acceptor phase flow-rate, temperature and donor phase stirring according to a full factorial design of experiment (DoE) coupled with desirability functions to integrate the target responses of interest. Finally, the applicability of the CP-MIMS method was assessed by analyzing various commercial FCMs, including 25-year-old plastic items. This study incorporates the principles of green analytical chemistry, using the Analytical GREEness (AGREE) metric approach as an evaluation tool.

2. Materials and methods

2.1. Chemicals, materials and samples

A description of chemicals, solutions and samples is provided in Section S1 of the Supplementary material.

2.2. CP-MIMS system

The CP-MIMS apparatus was composed of four principal components: the acceptor phase (AP) delivery system, the CP-MIMS probe, the MS detector, and the magnetic stirrer hotplate (Fig. 1).

A high-performance liquid chromatography UltiMate™ 3000 Basic Automated LC System (Thermo Fisher Scientific, San Jose, CA) was used to deliver AP (25 % (v/v) heptane in methanol) at 70 μL/min. Just after the pump, a short LC chromatographic column (C18, 5 cm x 2.1 mm x 5 μm, Teknokroma, Spain) was placed upstream of the CP-MIMS probe, with the sole function of back-pressure and flow stabilization, that was necessary to reduce background and signal noise. The CP-MIMS probe - the core part of the system - was hand-made in our lab (Fig. S1, Section S2 of the Supplementary material). A Viper® capillary (Thermo Fisher Scientific) with zero-dead volume connections was folded into a "U-shape" and cut using a tube cutter. After a careful refining of the edges,

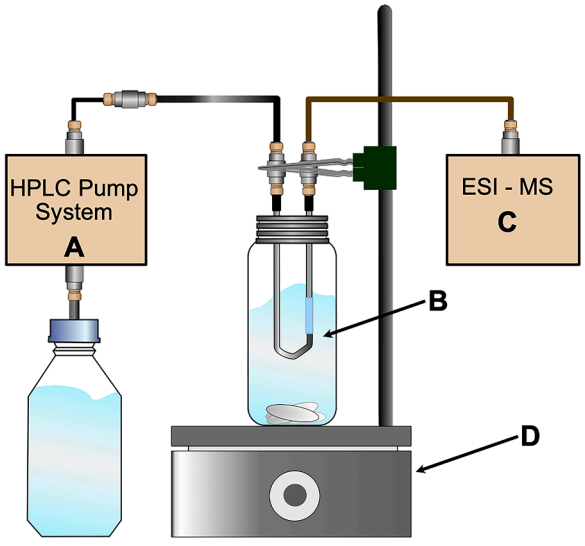

Fig. 1. Scheme of the CP-MIMS apparatus composed of: A) AP delivery system; B) CP-MIMS probe; C) MS detector; D) magnetic stirrer hotplate.

the two ends of the metal capillary were connected by fixing the PDMS hollow fiber (the CP-MIMS membrane) between them. To do this, the membrane had to be first washed with pure heptane for a few seconds to make it swell and temporarily increase dimensions and elasticity. After the insertion of the metal tubes into the membrane (approximately 5 mm each side), the heptane evaporates within a few seconds and the membrane shrinks, remaining stably fixed. The membrane working length was set to 3 cm. Then, the two sides of the capillary were both inserted into a 26 mm PTFE septum of a plastic cap: one was connected to the LC system for AP delivery, whereas the other was connected to the MS ionization source. The CP-MIMS probe was stored maintaining the membrane totally immersed in methanol and preventing any interaction with the air. An AREX F20500162 hot plate stirrer (Velp Scientifica, Usmate Velate, Italy) equipped with a digital VTF thermoregulator was used to heat the water bath to maintain the donor phase at 70 °C; in addition, the actual temperature of the donor phase (water and simulants) was regularly monitored by a glass thermometer immersed in it.

For MS detection, an LTQ-XL linear ion trap mass spectrometer (Thermo Fisher Scientific) equipped with an electrospray (ESI) ionization source was used. The system was operated in negative tandem mass spectrometry mode (MS/MS) with collision induced dissociation (CID), acquiring the signal in product ion scan mode. The sheath, auxiliary and sweep gas (nitrogen, 99.99 % purity) were delivered at flow-rates of 2, 0 and 0 arbitrary units, respectively. The other MS tune parameters were capillary temperature, 275 °C; spray voltage, −3.5 kV; capillary voltage, −45 V; tube lens, −90 V. The selection of the experimental parameters for ESI tune and MS/MS acquisition was performed by separately infusing BPA and β-naphthol (IS) into a continuous flow of the acceptor phase. For BPA, two MS/MS transitions - a quantifier (Q) and a qualifier (q) - were selected, being the former the most intense one. A pseudo-MS/MS transition was used for β-naphthol, since this compound is characterized by a weak CID fragmentation. Finally, an unidentified tracing signal derived from the acceptor phase was acquired as well, with the sole purpose of system monitoring and diagnostics. The extracted MS/MS transitions are reported in Table 1.

2.3. CP-MIMS analysis

The first step involves conditioning the system by continuously flowing the acceptor phase while keeping the membrane immersed in methanol. During this period, the signal of the tracing MS/MS transition was monitored to provide valuable information on the system stability. For method development and validation experiments, the membrane was fully immersed into 50 mL of pre-heated water or simulant (donor phase) contained in a glass beaker and fortified with the IS (10 μg/kg). Then, spiking experiments were performed by adding BPA to the donor phase. The acquisition time was set to 10 min starting from the BPA spike.

Finally, the probe was immersed in fresh methanol to wash out the analyte and re-establish the background level. During the experiments, the time of all crucial steps (e.g. membrane exposure to the donor phase, membrane removal from the donor phase, membrane rinsing, BPA addition in spiking experiments) was noted.

Table 1
Extracted MS/MS transitions for Bisphenol A, the internal standard (β-naphthol) and the tracing signal derived from the acceptor phase.

	Precursor ion (*m/z*)	Normalized collision energy	Product ions (*m/z*)
Bisphenol A	227.5	29	212.0[a]; 133.0[b]
β-naphthol	143.0	22	143.0[c]
Tracer	255.0	37	237.0

[a] Quantifier (Q) MS/MS transition.
[b] Qualifier (q) MS/MS transition.
[c] Pseudo MS/MS transition.

2.4. Data acquisition and signal processing

Due to the absence of chromatographic separation, the CP-MIMS output is a chronogram; it is described in and illustrated in Fig. S2, Section S2 of the Supplementary material. MS/MS raw files were extracted after a 15-points boxcar signal smoothing for further processing. A user-friendly MATLAB APP was developed for the parameterization of CP-MIMS output, allowing for faster semi-automated signal processing with a graphical user interface, as illustrated in Fig. S3, Section S2 of the Supplementary material. This tool reads the text file exported from the analytical instrument software, provides the user with the chronogram of CP-MIMS analysis, which is used to select the critical times for parameter calculation, and returns the experiment batch parameters in a matrix format that is suitable for the subsequent data analysis. This APP is freely available for download at the Milano Chemometrics and QSAR Research Group website [20]. The calculated parameters are briefly described below.

The corrected steady-state signal ($\overline{Y}_{steady}$) was calculated by averaging the steady-state signal intensity in a time range of 60 s and subtracting the average signal at the background ($\overline{Y}_0$) in a time range of 60 s after the complete equilibration of the membrane within the donor phase. The signal response time ($t_{10\text{-}90}$) is the time range required for the analytical signal to rise from 10 to 90 % of its steady-state value after membrane exposition to the donor phase containing analyte. The time dependent signal $Y(t)$ was first scaled into the range 0–100 as the following:

$$Y'(t) = \frac{Y(t) - \overline{Y}_0}{Y_{max} - \overline{Y}_0} \cdot 100 \quad (1)$$

where $\overline{Y}_0$ is the average signal at the background level, while Y_{max} is the highest observed value as the signal reaches the steady state. Then, the signal response time ($t_{10\text{-}90}$) was calculated as the difference between the time corresponding to the scaled signal value equal to 90 ($Y' = 90$) and the time corresponding to the scaled signal value equal to 10 ($Y' = 10$). To evaluate the effects of experimental noise on the response, the standard deviation of the signal at steady-state (s_{steady}) and at the background level (s_0) were measured considering the same time intervals used to calculate the corresponding average signals. Finally, since each experiment was carried out by five independent analyte spikes into freshly fortified donor solutions, all the considered parameters were estimated as the average over the instrumental replicates. For the corrected steady-state signal, the relative standard deviation (RSD%) was also calculated as the ratio of the standard deviation of the replicated $\overline{Y}_{steady}$ to its average value to evaluate the signal reproducibility and stability. The developed software interface allows the CP-MIMS signal processing of both the analyte and the internal standard, with the automatic calculation of steady-state signals normalized to the internal standard signal. Signals corrected for instrument drift were used for calibration and method validation.

2.5. Design of experiment (DoE)

To understand the way in which experimental conditions, hereinafter called factors, affect CP-MIMS performance, we applied the experimental design methodology. In particular, the effects of the acceptor phase composition using a co-solvent modified membrane system with different percentages of heptane to increase diffusivity (% Hept) and flow-rate (Flow), the temperature (T) and stirring (Stir) of the donor phase and the length of the membrane exposed to donor phase (L) were studied by a full factorial design [21]. Each of the operating factors was analyzed at two coded levels (–1, +1), which correspond to the minimum and maximum value, respectively, of the selected range of variation for the factor (Table S1, Section S4 of the Supplementary material). A total of 32 permeation experiments (Table S2, Section S4 of the Supplementary material) were then carried out under the different

specific combinations of the operating factors using a standard solution of BPA at a concentration of 100 μg/kg. For each experiment, an average response was derived from five replicated fortifications of the BPA standard solution into the donor phase. Three additional experiments were also carried out at the center of the experimental domain to estimate the pure experimental error. The 35 experimental runs were randomized to avoid potential bias.

The results from the full factorial design were evaluated by multilinear regression, fitting the mathematical relationship between the experimental factors and the modelled response y by the following linear model:

$$y = b_0 + \sum_{k=1}^{K} b_k x_k + \sum_{k=1}^{K-1} \sum_{j=k+1}^{K} b_{kj} x_k x_j \quad (2)$$

where b_0 is the intercept, x_k and x_j the coded values of the kth and jth factor, respectively, b_k the regression coefficient associated with the kth factor, which represents the main effect of the factor on the response, b_{kj} the coefficient associated to the interaction between factors k and j; K indicates the number of factors. Five relevant CP-MIMS parameters were considered: the corrected steady-state signal intensity ($\overline{Y}_{steady}$), the signal response time ($t_{10\text{-}90}$), the standard deviation of 60 s signal at steady-state (s_{steady}), the standard deviation of 60 s signal at the background (s_0), and the RSD of the corrected steady-state signal. To integrate these 5 parameters of interest and avoid the potential distortions due to correlation between responses, Principal Component Analysis (PCA) was used to calculate macro-variables (i.e., the principal components) as linear combinations of the 5 measured parameters [22]. PCA scores were then used as the response y in multilinear regression to derive the main and interaction effects of the operating factors and to finally estimate the optimal operating conditions.

2.6. Method validation

Method validation was performed according to the Eurachem guidelines [23]. Tap water and food simulants A and B contained in a glass beaker were spiked with IS at 10 μg/kg and used as blank matrices. For quantitative calculations, the BPA signal was normalized with respect to the IS acquired over a time interval of 60 s. Detection (LOD) and quantitation (LOQ) limits were calculated as 3.3 s/slope and 10 s/slope (ISO 11843-2) [24], respectively, where s was the standard deviation of the blank signal obtained from 10 independent blank measurements; in particular, LOD calculation was based on BPA qualifier transition, while for LOQ the BPA Q transition was considered; data normalized by the IS response were used in all cases. Precision was calculated as RSD% of 10 independent replicates at two concentration levels (10 and 100 μg/kg, respectively) and analyzed within the same day (repeatability) or for two weeks (one replicate per level per working day, intermediate precision).

Calibration curves were built using signal normalization by IS (at 10 μg/kg), exploring the LOQ-4000 μg/kg concentration range (7 concentration levels, 3 replicates for each concentration level), in ultrapure water, tap water, simulant A and simulant B. Homoscedasticity was verified by applying the Bartlett test and Mandel's fitting test was performed to check the linearity. The significance of the intercept (significance level 5 %) was established running a Student's t-test. The influence of the matrix on the combined effect of membrane permeation and ESI ionization was assessed by a t-test ($\alpha = 0.05$, two-tailed) between the curve slopes calculated in tap water, simulant A, simulant B with that calculated using ultrapure water. Trueness was assessed both by recovery experiments using water and simulants spiked at 1.5 × LOQs, 25 and 75 μg/kg, and by comparison with quantitative results obtained with a confirmatory LC-HRMS method, as the percent ratio between calculated and reference BPA concentration.

2.7. CP-MIMS real-time analysis on FCM items

The developed CP-MIMS probe was used for real-time monitoring of BPA release from commercial FCMs into water and food simulants (A and B) previously spiked with IS at 10 μg/kg and pre-heated at the experimental temperature. Migration tests were performed by exposing the CP-MIMS probe in water or simulant for 120 min at 70 °C, using the conditions reported in EU Regulation 10/2011 [13]; the chronogram was recorded throughout the experiment, where each concentration value was the average calculated over 1 min time frame centered on the 120th minute. In addition, after 120 min, an aliquot was collected for subsequent LC-HRMS analysis (confirmatory method) and a BPA spike at 100 μg/kg in the donor phase was performed to verify the CP-MIMS response. Since the aim of these experiments was not to verify the regulatory compliance of the investigated items but to evaluate the applicability of the CP-MIMS method using the regulated experimental conditions (migration media, temperature and time), measurements were conducted directly on the first migration. Migration tests on items of recent production were conducted using simulant A, simulant B or tap water considering the same number of samples for each medium. Conversely, due to the reduced availability of old plastic samples, migration tests with different migration media were performed on the same item. The standard deviation of the quantitative results was calculated based on the variation of the signal over the time range within which the average signal was evaluated. QC samples (10 μg/kg and 100 μg/kg in all matrices investigated) were regularly acquired (every ten migration experiments) to assess and monitor the stability of the BPA/IS ratio over time.

2.8. LC-HRMS confirmatory analysis

For confirmation purposes, an aliquot of water or simulants from migration tests was also analyzed using the UHPLC-Orbitrap technique. A detailed description of the method used is reported in Section S3 of the Supplementary material.

2.9. Greenness assessment of the proposed method

The compliance with the Green Analytical Chemistry principles was calculated using the freely available software developed by Pena-Pereira et al. [25].

3. Results and discussion

For the first time, the present study investigated and expanded the application framework of the CP-MIMS technique not only for the determination of BPA, but also for its real-time monitoring during migration tests in FCMs. This approach allows to obtain valuable temporal resolution in the study of dynamic processes, such as the release of compounds from FCMs [19].

The whole instrumental system, especially the membrane probe, was properly designed to meet important requirements in terms of signal stability, low noise and ease of handling. Starting from the state-of-the-art of the CP-MIMS configuration recently reported by Monaghan et al. [26], some modifications were implemented to increase the analytical stability and expand the applicability of the system. For this purpose, a short LC column was placed between the AP delivery system and the CP-MIMS probe to dampen the pressure fluctuations resulting from the mechanical action of the pump, thus allowing a reduction of the signal noise and simultaneously an increase in the method reliability. Since an unidentified impurity in the acceptor phase was observed, its MS/MS transition was used as a further improvement to monitor the system fluidic and diagnose problems in real-time for timely corrective action.

3.1. Design of experiments for CP-MIMS optimization

In addition to the instrumental improvements previously described, preliminary experiments were carried out to define which parameters and the corresponding experimental domain should be considered for method optimization by means of a design of experiments. Indeed, the experimental set-up strongly influences the CP-MIMS response profile in the chronogram, which is characterized by background noise, response noise, response intensity, ad signal response time (Fig. S2 in Section S2 of the Supplementary material). The parameters most related to the efficiency of this process were membrane length, donor phase, stirring, and AP flow-rate.

It is highlighted that the temperature of the donor phase and the percentage of a co-solvent in the AP are the main parameters that affect the analyte diffusivity through the membrane and the response time [6]. In particular, the co-solvent, commonly hexane or heptane, acts as a swelling agent of PDMS hollow fiber membrane, generating a PIM with related advantages in terms of analyte diffusivity [27]. As for the membrane, its length allows the regulation of the surface exposed to both the donor and acceptor phase, while the flow-rate and sample stirring affect the liquid-to-membrane mass transfer [28].

As a first step, since the membrane is simply adhered to the metal tubes, fluidic experiments allowed to determine the maximum flow-rate achievable before detachment, resulting in 120 μL/min. Temperature can also interfere with membrane adhesion as well, with 85 °C being the upper limit. Both the working range of the membrane length and stirring speed were selected based on previous studies dealing with CP-MIMS to obtain the best compromise between speed, efficiency of permeation and signal stability [6,19]; in fact, although high stirring speeds and membrane length are known to improve mass transfer, they also make the membrane more susceptible to vibrations, resulting in noise and mechanical weakness.

As for the acceptor phase, various solvents have been proposed in the literature based on the polarity of the analytes investigated [29,30]. Being BPA the target analyte, in this study the performance of both methanol and acetonitrile were tested, with the former providing the best results in terms of signal intensity according to the analyte polarity [27]. Hexane and heptane were tested as co-solvents, providing satisfactory results with no differences in terms of response. Therefore, heptane was selected due to its best score in the green solvent ranking [31]. It is worth mentioning that the experimental range explored was selected to preserve the integrity and efficiency of the membrane. This is one of the main advantages of PDMS, which can preserve its properties in a wide range of extraction conditions unlike Nafion™, the only other membrane material proposed for CP-MIMS [5]. The volume of the donor phase was set at 50 mL as it was the smallest volume for the complete immersion of the membrane even at high stirring speed.

After these preliminary evaluations, the experimental domain for the DoE optimization was defined as follows: AP flow-rate (70–100 μL/min); AP composition (5–25 % v/v heptane in methanol); membrane length (1–3 cm); temperature (25–70 °C); stirring (200–800 rpm).

For optimization purposes, a principal component analysis (PCA) was performed on the five CP-MIMS parameters ($\overline{Y}_{steady}$, $t_{10\text{-}90}$, s_{steady}, s_0, RSD), which were measured for 35 experiments (32 factorial + 3 experiments at the center of the experimental domain) as the average resulting from five different injections of the standard solution into the donor phase (Table S2, Section S4 of the Supplementary material). Due to extreme operating conditions, permeation did not occur for experiments 1, 5, 12, 21, so the corresponding parameters were missing (i.e., code NaN, Not A Number, in Table S2, Section S4 of the Supplementary material). These experiments were excluded from the statistical analysis. The PCA results are shown in Fig. 2.

PC1 explains >50 % of the total variability and is mainly influenced by the steady-state signal. More specifically, PC1 can be interpreted as the contrast between the steady-state signal $\overline{Y}_{steady}$, which must be high to achieve satisfactory sensitivity, and its RSD, which must be low to achieve high repeatability (Fig. 2b). PC2, which explains about 25 % of the data variability, is mainly determined by the response time $t_{10\text{-}90}$. The standard deviation of the 60-seconds signal s_{steady} is correlated to the steady-state signal, the higher the signal intensity, the higher the noise on the steady-state signal, while the standard deviation of the 60-seconds signal at background s_0 contributes equally to both PC1 and PC2. Considering the experiment projection in the space of PC2 vs PC1 (Fig. 2a), it can be noted that the experiments on the right side are characterized by high temperature (dark circles) and high percentage of heptane in the acceptor phase (big circles), while those on the bottom left side are obtained when low temperature (white circles) and high percentage of heptane in the acceptor phase are applied. Furthermore, the upper left side of the plot shows a group of experiments performed with different combinations of temperature and percentage of heptane. Finally, the three experimental replicates (grey circles 33, 34 and 35) are quite close to each other, which means that good repeatability is

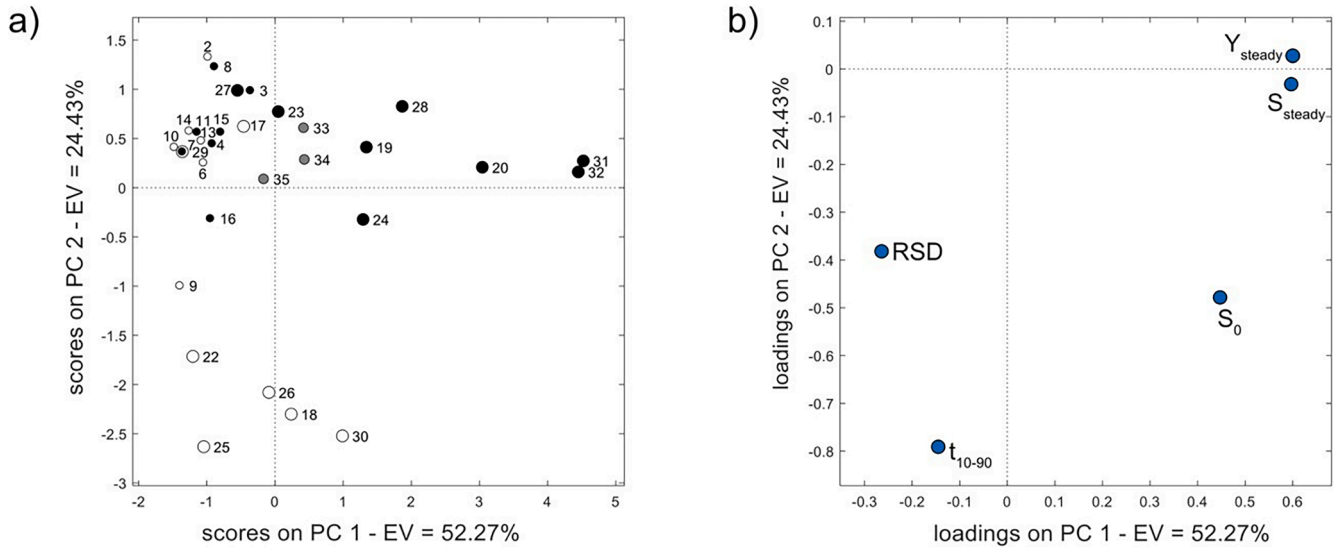

Fig. 2. Scores a) and loadings b) of the first two principal components (PC2 vs PC1) of the CP-MIMS parameters measured for 31 permeation experiments. Experiments are coloured with a greyscale based on temperature levels (the darker the colour, the higher the temperature) and their size is proportional to the percentage of heptane in the acceptor phase (the higher the size, the higher the %Hept).

achieved in the permeation experiments.

Since the most desirable experimental conditions are those in which the steady-state signal is as high as possible with minimum RSD and minimum response time $t_{10\text{-}90}$, PC1, which is the best combination of parameters encoding the desirability criteria, was selected as the independent variable *y* for the multilinear regression. The fitted model was satisfactory both in terms of R^2 equal to 85.9 % and ANOVA tests (*p*-value of 0.001 when testing model and residual sum of squares and 0.130 when testing lack of fit). The model coefficients associated with the factors and their interactions are shown in Fig. S4 (Section S4 of the Supplementary material). They represent the effects on the response and are useful to determine the most important operating parameters for tuning the experimental settings of CP-MIMS analysis. The temperature (T) and composition of the acceptor phase (%Hept) were found to have positive effect on the modelled response, as well as their interaction (T* %Hept), the interaction between donor phase stirring and membrane length (Stir*L) and, at a lower extent, the interaction between composition and flow-rate of the acceptor phase (Flow*%Hept). From these results it can be concluded that T and %Hept are the two most important operating factors, which positively contribute to increase $\overline{Y}_{steady}$ and reduce RSD; however, they also influence the signal noise, which increases under these conditions. Furthermore, to obtain a higher steady-state signal, longer membranes could be used but would require a high sample stirring speed to partially compensate for the naturally observed longer risetime ($t_{10\text{-}90}$). Although the acceptor flow-rate is not among the significant factors, it shows a moderate positive effect for the interaction with %Hept. Hence, increasing %Hept would require a high flow-rate; however, it was decided to keep it at the lower level both for the limited benefit of increasing it to the higher level [5] and to minimize the dilution of the permeant in the acceptor phase.

The optimal experimental conditions that were found to maximize the modelled response (PC1) were the following: 1) donor phase temperature: 70 °C; 2) acceptor phase composition: 25 % heptane; 3) acceptor phase flow-rate: 70 μL/min; 4) sample stirring: 800 rpm; 5) length of the membrane exposed to sample equal to 3 cm. It should be noted that these conditions were determined specifically for BPA analysis and cannot be generalized to other analytes.

3.2. Validation of CP-MIMS method

After DoE optimization, the CP-MIMS method was validated in tap water and in the simulants (A and B) to cover the application range of the EU Regulation 10/2011 [13]. Satisfactory results were obtained in terms of sensitivity, precision and trueness as summarized in Table S3 (Section S5 of the Supplementary material). The developed CP-MIMS method was found to be suitable for compliance assessment of FCM items in terms of migration of BPA into food, covering applications based on simulants A and B where it reaches LOD values at low μg/kg. Although the obtained LOD are about 100 times higher than those generally reported in literature by conventional HPLC-MS-based hyphenated techniques combined with laborious multistage sample treatment techniques [32], the CP-MIMS method has proven well-suited to detect and quantify BPA below the SML as established by the EU (0.05 mg/kg), considering the great advantage of direct and real-time analysis of BPA migration from FCMs, still unexplored in the literature.

Linearity was proved over 3 orders of magnitude, from LOQ to 4000 μg/kg. Good precision was obtained for all the investigated matrices, obtaining RSD % lower than 10 and 23 % for repeatability and intermediate precision, respectively. Trueness calculated on spiked samples resulted in the 78 ± 9 %–99 ± 4 % range ($n = 3$).

As for the assessment of matrix effect, the slopes of the calibration curves for standard solutions using ultrapure water were compared to those of matrix-matched solutions using tap water and the simulants (A and B); in this case, since the whole method is a one-step process, both analyte permeation through the membrane and ESI-ionization affected the signal intensity. The results showed that the slopes of the calibration curves calculated using tap water and simulant B were not significantly different from that obtained using ultrapure water. On the other hand, a slope reduction of 37 % was observed when matrix-matched solutions based on simulant A were used: this behavior can be ascribed to the ethanol content affecting the permeation efficiency of BPA.

3.3. Migration tests on commercial FCM samples

Finally, the applicability of the CP-MIMS method was demonstrated by subjecting commercial FCM items to migration tests and assessing the release of BPA in real time. Recently produced samples, such as coated metal cans, plastic jugs, graduated glasses and commercial flasks showed no detectable amounts of BPA throughout the experimental period. In fact, all these FCMs were produced after the entry into force of the EU Regulation 10/2011 and its amendment by the EU Regulation 2018/213 [33]. On the other hand, the developed CP-MIMS method allowed to quantify the BPA released from the 25-years old items in all migration media, in concentration even higher than the current SML. The same aliquots after 120 min of migration were analyzed with a confirmatory LC-HRMS method (a BPA high-resolution spectrum is reported in Fig. S5 in the Section S5 of the Supplementary material) obtaining an excellent agreement–higher than 97 %–for all samples, thus supporting the reliability and transferability of the method for the analysis of FCMs. Results obtained by both CP-MIMS and LC-HRMS techniques are reported in Table S4 (Section S5 of the Supplementary material). The CP-MIMS results converted into $\mu g/dm^2$ through the calculation of the area exposed to the migration media are reported in Table S5 (Section S5 of the Supplementary material). As shown in Fig. 3, it is worth mentioning that the valuable potential of CP-MIMS is the possibility of real-time monitoring of BPA migration over the time of the migration test. In fact, the analyte signal is continuously recorded over time allowing the conversion–by inverse prediction using the calibration curve of the recorded chronogram into a graph representing the variation of the concentration of BPA released from the material during the migration test.

The concentration trend also provided some key information on migration kinetics; for example, in the case of the baby bottle in simulant A, immediately after the plastic object came into contact with the simulant, the concentration of BPA increased rapidly, exceeding the SML after five minutes. Thereafter, the concentration continued to increase at a slower rate.

Our findings demonstrate that the CP-MIMS method could be used to assess the point at which BPA concentration overcomes the SML without performing the entire migration test, thus allowing a strong reduction in analysis time.

Information on the time it takes for a material to exceed a specific migration limit can be of fundamental importance for its formulation and characterization. To the best of our knowledge this is the first time ever that the release of substances from food contact material was monitored in real time.

3.4. Greenness assessment of the proposed method

To assess the environmental impact of the developed method, we used the Analytical GREEnness (AGREE) tool, which is the only metric technique that applies all 12 components/principles of Green Analytical Chemistry for the assessment of sustainability. The overall AGREE scale for the CP-MIMS-based method is shown in Fig. 4. Taking into account that the GAC metric operates on the principle of assigning a total score of 100 points for an ideal green analysis, the proposed method exhibited a score of 0.81, with a predominantly green hue [34]. A score above 0.75 indicates an excellent level of greenness in the assay.

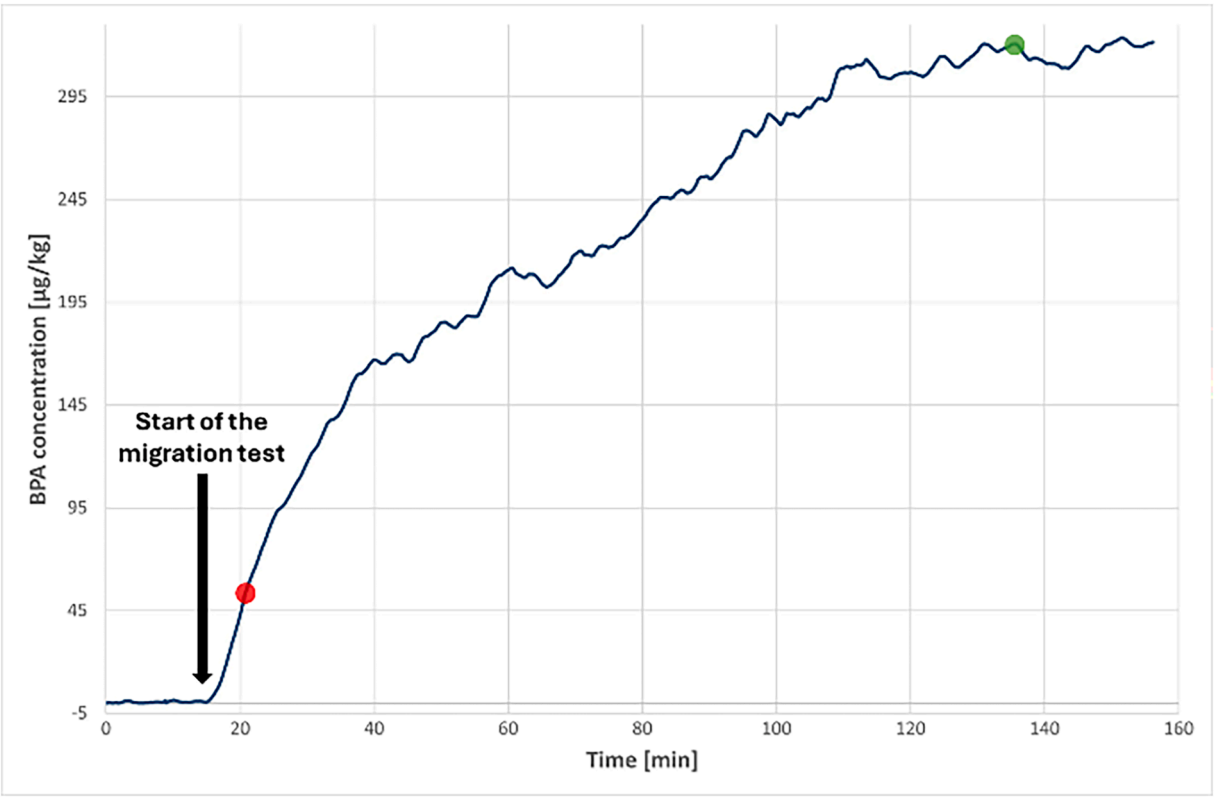

Fig. 3. Trend of the BPA concentration released from the "baby bottle" sample in simulant A. Red dot: the point where the SML was exceeded. Green dot: the value measured after 120 min of migration.

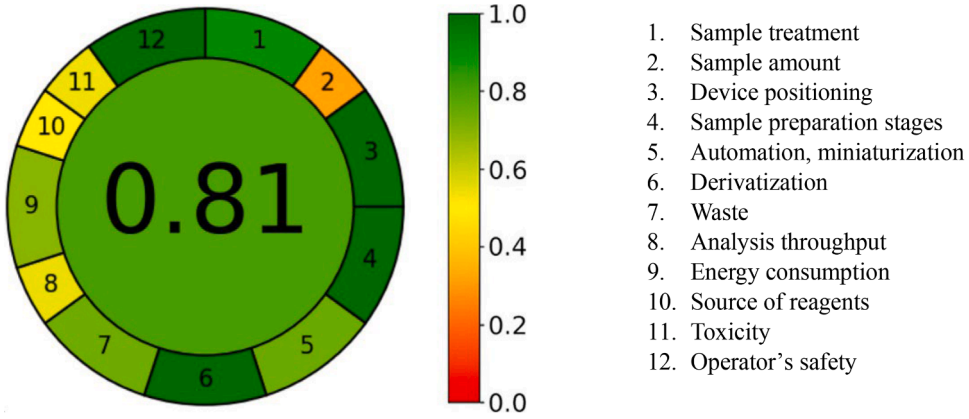

Fig. 4. Analytical GREEnness score for the developed CP-MIMS method. Scores: (1) 0.9, (2) 0.32, (3) 1.0, (4) 1.0, (5) 0.75, (6) 1.0, (7) 0.74, (8) 0.55, (9) 0.7, (10) 0.5, (11) 0.55, (12) 1.0.

4. Conclusions

For the first time, the CP-MIMS technique was proposed for the real-time monitoring of compounds released from food contact materials. The focus was on BPA, a re-emerging contaminant for which the legislation has rapidly evolved in recent years. The most innovative aspects of the study are: (i) the design of a robust set-up for the CP-MIMS system, paying particular attention to the membrane probe assembly, (ii) the multi-response optimization of experimental factors by DoE, and (iii) the creation and implementation of a user-friendly MATLAB APP for the parameterization of CP-MIMS output, allowing for semi-automatic signal processing with a graphical user interface. Furthermore, this is the first study in which the effects of experimental factors on the noise magnitude were estimated. Method validation proved that the developed CP-MIMS method is fit for purpose, allowing for an accurate BPA determination at low µg/kg levels in tap water, simulant A and simulant B. The results indicated an excellent greenness of the method. The application of the method by continuously recording the BPA signal during the migration tests has allowed to reach an unexplored information level on the migration phenomena, mainly related to the release kinetics. Further research will be focused on real-time monitoring of BPA and its analogs as hazardous chemical migrants from FCMs into food and beverages, as well as direct analysis in real samples, exploiting the MATLAB APP developed in this work for semi-automatic processing of the instrumental output.

Glossary

GAC, Green Analytical Chemistry; CP-MIMS, Condensed Phase Membrane Introduction Mass Spectrometry; AP, Acceptor Phase; PIM, Polymer Inclusion Membrane; PDMS, Polydimethylsiloxane; FCM, Food Contact Material; BPA, Bisphenol A; DoE, Design of Experiments; PCA, Principal Component Analysis; LC-HRMS, Liquid Chromatography High Resolution Mass Spectrometry; IS, Internal Standard.

Data availability

Data will be made available on request.

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CRediT authorship contribution statement

Maurizio Piergiovanni: Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Veronica Termopoli:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Conceptualization. **Cristian Maffezzoni:** Investigation, Formal analysis. **Nicolò Riboni:** Writing – review & editing, Visualization. **Viviana Consonni:** Writing – original draft, Methodology, Data curation. **Federica Bianchi:** Writing – review & editing, Validation, Formal analysis. **Monica Mattarozzi:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Davide Ballabio:** Writing – review & editing, Software, Methodology. **Maria Careri:** Writing – review & editing, Validation, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no competing financial interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.greeac.2024.100199.

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