Direct Immersion−Solid-Phase Microextraction Coupled to Gas Chromatography−Mass Spectrometry and Response Surface Methodology for Nontarget Screening of (Semi-) Volatile Migrants from Food Contact Materials

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Abstract

ABSTRACT: Toward a more rigorous inspection of food contact materials, the importance of sample preparation for nontarget screening should be addressed. Direct immersion−solid-phase microextraction coupled to gas chromatography mass spectrometry (DI-SPME-GC-MS) was optimized for nontarget screening of migrants in 3% acetic acid, 10% ethanol, and 95% ethanol food simulants by response surface methodology (RSM) in the present study. Optimum conditions were DVB/CAR/PDMS fiber, no pH adjustment for 10% and 95% ethanol simulant but pH adjustment to 7 for 3% acetic acid simulant, no salt addition, 5 min preincubation, 55 min extraction at 70 °C, and 8 min desorption at 250 °C. In addition, 9.5 times dilution of 95% ethanol samples prior to extraction was required. pH modification of 3% acetic acid samples was found to be critical for the extraction of amines. The proposed methodology was then evaluated by determining the limit of detection (LOD) as well as repeatability of 35 food contact materials related substances. Except for those amines and diols which have a relatively high LOD, the LODs of the rest of the substances were 0.1−14.1 μg/kg with a precision of 1.9−23.0% in 10% ethanol and were 0.1−20.2 μg/kg with a precision of 2.5−19.6% in 3% acetic acid simulant. The LOD and precision in 95% ethanol simulant were 0.7−163.7 μg/kg and 1.4−26.8%, respectively. The proposed method can be applied for an overall screening of migrants from these three simulants at even trace levels, though attention should be paid to some specific analytes, e.g., diols and amines, which could have a high LOD and toxicity.

Introduction

Food contact materials (FCMs) are manufactured from raw materials and so-called intentionally added substances (IASs), such as antioxidants, lubricants, UV stabilizers, and so on. However, nonintentionally added substances (NIASs) can be present in FCMs as well, e.g., reaction byproducts, oligomers, degradation and/or impurities of raw materials, etc.Both of them could migrate into the contacting foodstuffs from FCMs and therefore post potential risks to human health. Target analysis is a conventional way to

check the compliance of FCMs with legislation. However, this strategy does not work for NIASs since we do not even know what they are. To further ensure consumers' health, nontarget screening (NTS) has drawn increasing attention in recent years.[1,3] Nevertheless, to the best of our knowledge, most of the publications about NTS of migrants mainly focus on the strength of different techniques, especially the use of high resolution mass spectrometry, in the qualification of unknown compounds.[4−9] Little attention has been concentrated on the potential of various sample preparations in NTS. Sample preparation is one of the important aspects of NTS because it determines the capacity of the screening methodology, i.e., the number of substances that can be detected and their limit of the detection (LOD). Liquid chromatography (LC) coupled to high-resolution mass spectrometry (HRMS) is powerful and commonly used for structure elucidation of unknown migrants. However, it is still time-consuming, laborious, and sophisticated, posing a great challenge to analysts since no FCM-related library is publicly available to date.[10] Interestingly, 47 out of 89 FCM-related chemicals (<500 Da) tentatively identified by LC-Q-HRMS in 10 scientific articles[5−8,11−16] were found in the NIST 14 library for GC-MS (Table S1). The fact suggests that the power of GCMS with libraries can be further explored in terms of NTS. Moreover, the retention index is well established to assist identification in GC-MS, increasing the reliability of identification when no reference standard is available, which is quite common for NIAS. Then, developing a sensitive GC-MS method toward a wide range of analytes can release the burden of structure annotation of many compounds using HRMS and help us focus on truly unknown analytes. Migration testing using different types of food simulants (3% acetic acid, 10% ethanol, 95% ethanol, etc.)[17] is widely applied to assess the safety of FCMs because they are much simpler than foodstuffs, helping us focus more on those components coming out from FCM. Various strategies have been applied to extract migrants from aqueous simulants prior to GC analysis, for example, liquid−liquid extraction (LLE),[18] rotatory evaporation and redissolution with GC-amenable solvents,[19] and headspace− solid-phase microextraction (HS-SPME).[20] To our knowledge, only LLE has been optimized for NTS of migrants from 50% ethanol (a food simulant for milk);[21] however, in comparison to LLE, SPME is simpler, solvent-free, and available in autosampler. It is a versatile and nonexhaustive sample preparation tool and has been successfully applied in a wide variety of fields, e.g., flavor and fragrance investigations, environmental studies, and diverse bioanalytical applications.[22] In light of these advantages, the present work aims to develop and optimize direct immersion

(DI)−SPME for the extraction of migrants from different food simulants regarding untargeted screening. As far as we know, it is the first attempt to optimize DI-SPME for untargeted migrants screening purposes. Experimental conditions that would affect the extraction efficiency of DI-SPME were first optimized using a central composite design (CCD) and response surface methodology (RSM). The optimization was conducted using migration solutions from recycled polyolefin samples instead of only a few selected standards since it would contain many polyolefin-related chemicals and would be more representative of a screen polyolefin's migrants. In addition, the power of the optimized DI-SPME for untargeted screening of migrants from other FCMs was also evaluated by determining the LOD and repeatability of 35 reference standards which are commonly found in FCMs. The present study is part of our work to assess the potential of using recycled polyolefins for food contact purposes and will offer us convenience, reliability, and robustness to comprehensively investigate potential humanhealth- related compounds that are present in recycled polyolefins.

■ MATERIALS AND METHODS

Reagents and Samples. Standards were purchased from Sigma-Aldrich (Madrid, Spain): triethylamine (121−44−8), pxylene (106−42−3), caprolactam (105−60−2), α-methylstyrene (98−83−9), 2,6-diaminotoluene (823−40−5), allyl methacrylate (96−05−9), naphthalene (91−20−3), 2-naphthylamine (91−59−8), dipropylene glycol monomethyl ether (34590−94−8), eugenol (97−53−0), 1-dodecene (112−41−4), diphenyl ether (101−84−8), benzophenone (119−61−9), 2-ethylhexyl acrylate (103−11−7), dimethyl isophthalate (1459−93−4), ethylene glycol dimethacrylate (97−90−5), 2,6-diisopropylnaphthalene (24157−81−1), o-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA, 72915−12−9), diphenyl carbonate (102−09−0), 2,4,7,9-tetramethyl-5-decyne-4,7-diol (126−86−3), bisphenol A (80−05−7), diethyl sebacate (110−40−7), stearamide (124−26−5), dibutyl sebacate (109−43−3), Cyasorb UV 12 (131−54−4), Tinuvin 326 (3896−11−5), Chimassorb 81 (1843−05−6), glyceryl monostearate (123−94−4), octocrylene (6197−30−4), bis(2-ethylhexyl)adipate (DEHA, 103−23−1), dioctyl terephthalate (4654−26−6), tributyl acetylcitrate (77−90−7), dinonyl phthalate (84−76−4), dihexyl sebacate (122−62−3), and 2,5-bis(5-tert-butyl-2-benzoxazolyl)thiophene (7128−64−5). Stock solutions (more than 1000 μg/g) of each standard were prepared in methanol or ethanol except for Tinuvin 326 and 2,5-bis(5-tert-butyl-2-benzoxazolyl)thiophene, which were prepared in dichloromethane and hexane,

respectively. They were then grouped (ca. 8 standards per group), mixed, and diluted to 10 μg/g in ethanol as working solutions. A tiny amount (0.018g) of working solutions was spiked into different food simulants (18 mL), that is, 10 ng/mL in simulants, to evaluate the performance of the developed method. For less sensitive analytes, higher concentrations were prepared accordingly. All standards and solutions were under gravimetric control. Recycled polyolefin pellets (cylinder-like, d = 5 mm, h = 2 mm, density = 971 kg/m3) were supplied by a European company. Postconsumer polyolefin was collected, washed, and extruded without applying a superclean process according to the company. Twenty grams of sample was immersed in 100 mL of food simulant (3% acetic acid and 95% ethanol) for migration at 70 °C for 2 h. Afterward, they were filtered (0.2 μm hydrophilic polypropylene filter) at room temperature and stored in the fridge at −25 and 4 °C for 95% ethanol and 3% acetic acid, respectively, to minimize any change over time. They were then used for optimization by DI-SPME-GC-MS. Blanks were simultaneously prepared to remove sample-irrelative features. GC-MS Analysis. A 6500 CTC autosampler mounted gas chromatograph (6890N) coupled to a mass spectrometer (5975) was from Agilent (California, USA). The separation was performed on a DB-5 MScolumn from Agilent (30m× 0.25 mm id, 0.25 μm film thicknesses). Agilent ultrainert liners (id = 0.75 and 4mmfor SPME and liquid injection, respectively) were used. The inlet temperature was set at 250 °C, and the carrier gas flow (He) was 1.0 mL/min. A scan mode with a mass range from 40 to 700 Da was applied. A spitless mode and 5 min solvent delay were employed. Two microliters of injection volume was applied for liquid injection. The ramp of temperature was as follows: held 50 °C for 5 min, increased to 300 °C at a rate of 8 °C/min, and held for 10 min. Grob mixture was used for quality control of the GC-MS. Sample Treatment for 95% Ethanol Samples. Two strategies were applied to process 95% ethanol samples. One was to concentrate 5 mL of sample into 1 mL using a nitrogen concentrator (Techne DB-3; Staffordshire, UK) at 40 °C and directly injected into GC-MS. Another one was to use DISPME-GC-MS, which was to transfer an aliquot of 1.9 mL sample into an 18 mL glass vial followed by adding 16.1 mL of water (that is 10% ethanol). The obtained solution was mixed, extracted by DI-SPME (1 cm 50/30 μm DVB/CAR/PDMS fiber), and finally injected into GC-MS. The DI-SPME was accomplished using a 6500 CTC autosampler, and the conditions were a 600 rpm stirring rate, 5 min preincubation, 30 min extraction at both 40 and 80 °C, and 8 min desorption in the GC inlet. Following desorption, the fiber was cleaned at 270 °C in a fiber cleaner for 2 min. Selection of Fiber Coating. The extraction efficiencies of 5 SPME fibers were

compared using the DI-SPME process described above except for the extraction temperature that was fixed at 80 °C. One centimeter long fibers including 50/30 μm DVB/CAR/PDMS, 100 μmPDMS, 85 μmCAR/PDMS, 85 μm polyacrylate (PA), and 65 μm PDMS/DVB were purchased from Supelco (PA, USA). All fibers were conditioned prior to use according to the manufacturer's guide. Identification of the Most Relevant DI-SPME Factors. The selected fiber was then used for finding out the most important DI-SPME factors. Influences of salt (5% and 10% NaCl and 5% Na2SO4) and pH (pH = 5, 7 (original), and 9) were first examined. It is worth mentioning that all DI-SPME optimizations including RSM were conducted using a diluted 95% ethanol migration sample, though the final optimized parameters were applied for 3% acetic acid samples as well. The only exception was that the effect of pH (pH = 2 (original), 7, and 9) on the extraction efficiency of migrants from 3% acetic acid was examined independently since the pH of 3% acetic acid is very low and would have a significant influence on the extraction. NaOH pellets were first used to neutralize 3% acetic acid solutions on a large scale up to ca. 6.5 because of its high acidity. NaOH and acetic acid water solutions (both high and low concentrations) were then utilized for fine adjustment. Moreover, each group of comparative experiment was processed in the same batch to minimize any change (if there was) of the sample. Response Surface Methodology: Central Composite Design. Once the most relevant factors were identified, a response surface methodology was employed to optimize the best conditions. A blocked CCD including 14 experiments (7 for each block) was used. The first block consisted of four factorial points (−1, 1) and three central points (0, 0); the second block included four rotatable axis (star) points (−1.414, 1.414) and three central points. The studied factors and their levels are shown in Table S2. The RSM and all statistical analyses across the study were processed by R programming,23 using the rsm24 and desirability25 packages. Evaluation of the Strength of the Proposed DI-SPMEGC- MS Method for Nontarget Screening of FCM Migrants. The potential of the developed nontarget screening method was evaluated by determining the LOD and repeatability of 35 reference standards that are possibly present in food contact materials. The LOD was calculated as the concentration that has a signal-to-noise ratio (S/N) of 3 using the least rather than the most abundantion. Regarding NTS, detection of the least instead of the most abundant ion is the basis of a reliable library match. The repeatability was calculated under 10 μg/kg when possible; if not, higher concentrations were used. The 35 standards(MW< 500 Da) were selected from the following lists considering their availability in the authors'laboratory as well: (1) analytes

that were detected in our recycled polyolefin sample and had a Cramer III level; (2) chemicals that are potentially present in FCM;26 (3) substances that were identified in FCM by LC-QTOF-MS but are present in the NIST 14 library; (4) substances that have a specific migration limit lower than 0.05 mg/kg food in the Commission Regulation EU 10/2011.17 The long list of standards for validation covers a wide range of molecular weights and structures regarding FCMs.

■ RESULTS AND DISCUSSION

Sample Treatment for 95% Ethanol Migration Samples. For 95% ethanol simulant, it is convenient to inject it directly into GC-MS; however, higher sensitivity is welcome for NTS of migrants regarding human health. A simple way to achieve higher sensitivity is to concentrate the sample by evaporating the solvent, although some volatile compounds could be vented as well. Another convenient way is to use SPME, since it is highly automated and well-connected to GC-MS thanks to the CTC autosampler. Unlike 3% acetic acid and 10% ethanol simulants, 95% ethanol samples cannot be extracted directly by DI-SPME because high ethanol content would damage or shorten the lifespan of the fiber. One of the compromises is to dilute it, and then it can be extracted by DISPME. The dilution decreases the concentration, though higher sensitivity could be obtained by DI-SPME thanks to its powerful extractability. This way, the loss of volatile compounds can be avoided. In order to see the capability of these two methods (solvent evaporation and DI-SPME), 95% ethanol migration from recycled polyolefin sample was used instead of selecting a few standards because the results of the mentioned strategy could vary a lot depending on the standards selected. What is more, recycled polyolefins were thought to be much more complex than the virgin one, and thus, it can be a good representative sample for developing a nontarget migrant screening method for recycled polyolefin FCM. The performance of these two strategies is shown in Figure 1. As can be seen, liquid injection had much better performance than DI-SPME at 40 °C in terms of the height of peaks at the right side of the chromatogram (more than ca. 24 min, relatively big molecules), while it turned out to be the opposite regarding peaks on the left-hand side (Figure 1A). The reason could be that those small molecules (on the left-hand side) were lost during the concentration process. However, Figure S1 depicts liquid injection without concentration having a much lower peak height across the whole chromatogram meaning that the concentration step did not have significant negative effects on volatile substances. Another reason for worse performance for high molecular weight substances

by DI-SPME could be that the used conditions were not appropriate to extract them. Considering that extraction temperature is a critical parameter for DI-SPME, higher temperature (80 °C) for DI-SPME was applied. Figure 1B demonstrates that DI-SPME at 80 °C had higher efficiency for almost all peaks. The results suggest that DISPME applying a high extraction temperature has higher potential for nontarget screening of migrants in 95% etanol simulant than liquid injection. Another advantage of using this strategy is that only one calibration curve is needed for each compound when quantifying migrants in 10% and 95% ethanol. Selection of SPME Fiber. Fiber coating plays an important role in SPME because the physicochemical properties of the coating greatly affect the distribution of analytes between the sample and the coating. As depicted in Figure 2, PAfiber showed a much lower performance than the DVB/CAR/PDMS one across the whole chromatogram. The result was in agreement with a previous study where different fibers were compared to extract volatile compounds in plain sufu by HS-SPME.27 As a polar coating, PA fiber was observed to extract much less of the least polar analytes.28 From an NTS point of view, migrants could be both polar and nonpolar. As such, PA fiber was not suggested for NTS. As for the nonpolar PDMS fiber, it was better than the PA one (Figure S2A), though when compared to the DVB/CAR/PDMS fiber, its limitation was apparent mainly for those components located on the left-hand side of the chromatogram in Figure S2B. However, the discrepancies among DVB/CAR/PDMS, CAR/PDMS, and PDMS/DVB fibers were not visually obvious (Figure S2C and D), and the number of peaks (S/N > 10 and with clear spectrum) detected were the same (140). Nevertheless, the number of substances extracted from a commercial plain sufu by these three fibers differed significantly in the research by Chen et al.27 probably because they used HS-SPME instead of DI-SPME. The HS-SPME conditions applied, which were not specified in their study, might have an effect as well, since the conditions would influence their performances. Further examination was done in terms of peak area. Regarding total peak area, no significant difference was observed among them (ANOVA, $p = 0.197$). Nonetheless, the peaks were divided into two groups according to the order of magnitude of their peak areas (Table S3), namely group 1 (47 peaks) and group 2 (93 peaks), respectively. The total peak area of each group was then calculated, and multivariate analysis of variance (MANOVA) showed significant difference ($p = 0.01939$) among the three fibers. Univariate one-way ANOVAs depicted that the distinction mainly came from the group 1 substances ($p = 0.005047$). Figure 3 shows the pairwise mean comparisons regarding group 1 (Tukey HSD multiple comparisons). As can be seen, both

DVB/CAR/PDMS and CAR/PDMS fibers had better performances than the PDMS/DVB fiber with respect to group 1 substances. It is noteworthy that the total peak area of group 1 substances accounted for only ca. 2.5% of the total peak area of all peaks, and it was even smaller than the standard deviation of that of group 2. Therefore, the distinctions in group 1 were hidden when using the total peak area of all peaks as a measure. In addition, most of the group 1 compounds had a retention time lower than 20 min, which means that most of the group 1 compounds are small sized substances or more volatile compounds. This fact suggests that the DVB/CAR/PDMS and CAR/PDMS fibers had better extractability over small molecules than the PDMS/DVB fiber. The result can be explained by the fact that carboxen (CAR) has a much higher percentage of micropores, which are good for small molecule extraction, than the divinylbenzene (DVB). As for CAR/PDMS and DVB/CAR/PDMS fibers, no significant difference was found, though DVB/CAR/PDMS was selected for NTS of migrants from recycled polyolefins considering its smaller standard deviation over the two groups. Identification of the Most Relevant Factors. There are many factors that could affect the efficacy of DI-SPME including the stirring rate of the agitator, preincubation time, addition of organic solvent, dilution of samples, addition of salt, sample pH, extraction temperature, and extraction time. The agitator stirring rate was found to be positive for all classes of analytes in the research by Zhang et al.29 because it enhances mass transport of the analytes. As such, 600 rpm was chosen herein according to their research to enable fast agitation without causing mechanical damage to the fiber. Preincubation time was deemed to be more important for HS-SPME, since equilibrium between the sample and its headspace is critical, while for DISPME, preincubation is employed to control sample temperature prior to extraction.29 Thereby, a short preincubation time (5 min) was applied in the present study. The addition of organic solvent may promote the release of analytes bound to the matrix. However, considering the simplicity of food simulants, organic solvent addition will not be beneficial but will act as a competitor of analytes. Therefore, no organic solvent was added herein. Sample dilution with water can minimize the matrix effect and increase the release of analytes bound to the matrix.30 Again, food simulants are simple, and dilution would not be profitable but decrease the concentration of analytes (Figure S3). It is necessary to dilute 95% ethanol samples as mentioned above, and 10% ethanol food simulant was well tested and found not to negatively impact the life span of SPME fibers. Therefore, 95% ethanol samples were diluted 9.5 times, which is 10% ethanol. Salt addition was found to promote the extraction of certain analytes thanks to

the salting-out effect.29 In addition, Na2SO4 was reported to offer better extraction efficiency as well as better repeatability as compared to NaCl.30 Surprisingly, 5% and 10% NaCl and 5% Na2SO4 did not improve extraction efficiency but negatively affected the baseline of the chromatograms in the present study (Figure 4). As a result, no salt was added in the present study. Sample pH is critical for some kinds of analytes, since only the nonionized form of analytes is extracted by SPME fibers. However, no significant differences were found u nder pH 5, 7, and 9 (ANOVA, p = 0.36 and 0.26 for group 1 and group 2 substances, respectively). Chromatograms are available in Figure S4 as well. From an NTS perspective, the pKa of migrants would vary a lot, and pH 7 could be a good balance. Therefore, no pH modification (pH 7) was made for 10% or diluted 95% ethanol samples. Regarding 3% acetic acid samples, pH modification did greatly impact the extraction. After modifying the sample to pH 7 and 9 (the original was 2), there were many emerging peaks (cycled in red) across the chromatograms (Figure 5). Most of them were found to be amines as shown in the figure. As is known, amines are bases, and the nitrogen lone pair of amines can take a hydrogen ion from a hydroxonium ion and form ammonium ions.31 As a consequence, they are difficult to extract by DI-SPME under an acidic environment, and neutralization of pH is necessary. Some compounds showed higher peaks in the original sample, though the number of them was limited and they could be detected in the pH 7 sample as well with slightly lower intensities. When the pH was further increased to 9, some peaks disappeared; for example, stearic acid (cycled in blue), which is very common in FCM as a slip agent, was totally absent in the pH 9 sample. This behavior could be expected, as at pH 9, stearic acid (pKa = 10.15) is in dissociated (anionic) mode, while at pH 7 it is in its molecular form. Thus, 3% acetic acid was modified to pH 7 prior to extraction. Extraction temperature is critical for DI-SPME. On the one hand, a higher temperature increases analyte diffusivity in the sample and thus increases the extracted amount under preequilibrium conditions. On the other hand, increasing extraction temperature has a negative effect on the partition coefficient between the fiber coating (stationary phase) and the sample.30 As such, extraction temperature was further optimized using RSM. Extraction time did significantly influence the extraction as well (Figure S5). Considering the possible interactions between extraction temperature and time, extraction time was selected for further optimization as well. Response Surface Methodology: Central Composite Design. Unlike the one-variable-at-a-time method, response surface methodology enables us to evaluate not only the individual influence of significant factors but also their interactive effects. By

applying proper experimental designs, a suitable prediction mathematic model can be obtained based on the fit of a polynomial equation to the experimental data, which allows us to determine the outcome inside the range studied for each factor.29,32 For this purpose, a central composite design (CCD) was employed to optimize the extraction temperature and extraction time. From a nontarget screening point of view, lowering the limit of detection across the whole chromatogram is preferred; that is, the higher all the peaks, the better the outcome. Total peak area seems to be a good measure of the yield; however, using total peak area would bury the information from those small peaks as mentioned above. It is important to balance the outcome of these two groups of analytes. From this perspective, dividing them into two groups as described above is a good compromise. Doing so, the size of analytes as well as magnitudes of peak areas were taken into account. To maximize the overall outcome of these two groups, the prediction mathematic models of each group were first built through RSM. A multiple-response approach, namely Derringer and Suich's desirability function, was then applied to obtain the optimized set of values for each factor that has the maximum overall desirability. The overall desirability was calculated by the geometric mean of the desirability of each group, and the scale for each group can be set according to their relative importance to the overall desirability. Group 2 had a higher weight (double) than group 1 because the number of peaks in group 2 was twice that in group 1 (93 vs 47). The response surface plots for group 1, group 2, and overall desirability are shown in Figure 6. The determination coefficients (R2) for group 1 and group 2 were 0.9299 and 0.9304, respectively, and the lacks of fit were 0.7896 and 0.1303, respectively, indicating a good fit for the two groups. As can be seen, temperature negatively affected the extraction of group 1 substances but positively influenced the group 2 chemicals. Increasing temperature enhances the mobility of chemicals but also decreases the partition of them between the fiber and the simulant. Group 1 might reach equilibrium easily since the compounds were relatively small and had low intensities as mentioned above. As a result, a high temperature would reduce the amount of group 1 chemicals attached to the fiber. On the other hand, extraction time was beneficial for both groups, especially for group 2 whose total peak area increased remarkably over time. Therefore, a compromised temperature and longer time would give the highest throughput as is evidenced by the overall desirability response surface plot. The optimum conditions to have the highest overall desirability were determined as a 70 °C extraction temperature and 55 min extraction time. Experimental responses for group 1 and group 2 under the optimum

conditions matched the predicted values attained by the mathematical models well (t test, $n = 4$, $p > 0.05$). Evaluation of the Strength of the Proposed DI-SPMEGC-MS Method for Nontarget Screening of FCM Migrants. As evidenced above, the optimal DI-SPME conditions gave the best extraction of migrants from migration samples of recycled polyolefins. However, the capability of the proposed method for a more generic nontarget screening toward different FCMs was evaluated by determining LOD and a repeatability of 35 standards covering a wide variety of molecular weights and structures. Among the 35 standards evaluated, most of them had a very low level of LODs (Table 1, < 10 μg/kg in 10% ethanol and 3% acetic acid but a little bit higher in 95% ethanol) suggesting that the proposed method is powerful for nontarget screening of most of the analytes at even a trace level. However, there were also exceptions. Some chemicals, e.g., triethylamine, 2-naphthylamine, and BPA, had a relatively high LOD (60.6, 217.2, and 319.1 μg/kg, respectively, in 10% EtOH) in comparison to others, but they are still at the parts per billion level. In addition, there were four analytes that could not be detected even at 1 mg/kg. Among them, 2-naphthylamine, triethylamine, caprolactam, and 2,6-diaminotoluene are amines. As was previously reported by Ning et al.,33 most of the aliphatic and heterocyclic amines can be strongly adsorbed on the column and injector during GC analysis; hence, low concentration cannot be detected without derivatization. Interestingly, many amines were detected in the 3% acetic acid migration sample after pH modification, which suggests that their concentration could be high. Their high concentration in 3% acetic acid simulant could be expected due to the alkaline nature of amines, which will be protonized and, thus, increase the migration from the plastic. For the other three (dipropylene glycol monomethyl ether, BPA, and Cyasorb UV12), they are diols or diol ether. As was pointed out, substances containing more than one alcohol functional group could have low volatility, thus derivatization may be needed to promote volatility.34 The main difficulty in untargeted screening analysis when using chromatography is to select the peaks to be identified. A common proposal is to focus the effort only on the highest peaks and neglect all those below a certain size, assuming that they correspond to a very low concentration level.3 However, this is not necessarily true, because the response of analytes varies a lot. For example, many of the amine and diol analytes could have relatively low responses in GC-MS analysis. Their peaks could be small even in relatively high concentrations, which would be of high human health concerns. In addition, many of them are included in the NIST 14 library. Once they are detected in GCMS, they can be easily identified with the help of libraries. Maybe they

can be readily detected in LC, with or without previous concentration, but the identification is still challenging regarding untargeted screening. As such, when conducting untargeted screening, many of the small peaks can be easily checked if they are amines or diols with the help of libraries, while in LC-MS analysis without the library, this task will be more challenging.

■ CONCLUSIONS

For the first time, direct immersion−solid-phase microextraction coupled to gas chromatography mass spectrometry has been optimized for untargeted screening of volatile and semivolatile migrants from 3% acetic acid, 10% ethanol, and 95% ethanol food simulants by response surface methodology together with central composite design. The optimization was based on the recycled polyolefin samples, though it is thought to be suitable for virgin polyolefins and other types of food contact materials considering the complexity of postconsumer recycled polyolefins as well as the method evaluation, which assessed the LOD and repeatability of 35 chemicals that could come from different types of FCM. The proposed method can extract most of the tested analytes at very low concentrations (<10 μg/kg, which is the specific migration limit (SML) for the nonlisted substances in the Regulation 10/2011/EU). However, many amine and diol compounds were found to have a relatively high LOD or even not detected at 1 mg/kg even though they are included in the NIST library. The fact could be due to their GC-unamenable properties rather than the DI-SPME process and demonstrates once again that the size of peaks in GC-MS is not always indicative of a low concentration, and this criterion cannot be applied to any migrant. As such, we recommend doing NTS based on qualifiable features instead of on size of peaks. For 3% acetic simulant samples, pH adjustment to 7 is of great importance to detect many amine substances. It is quite difficult, if not impossible, to develop an analytical method for all types of analytes. Nontarget screening does not necessarily mean comprehensive because of the limitation of the analytical approach applied. In this sense, knowledge and experience about the strength of the employed analytical method in untargeted screening as well as information about the sample would be helpful for comprehensive FCM safety assessment. For example, knowing the low response of many amine and diol analytes in can be easily identified as amine or diol chemicals with the help of a library search.

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Table 1. LOD and Repeatability (n = 3) of the 35 Analytes in 10% Ethanol, 95% Ethanol, and 3% Acetic Acid Food Simulants

					10% ethanol (μg/kg)		95% ethanol (μg/kg)		3% acetic acid (μg/kg)	
chemicals	CAS	MF	XlogP	mass	LOD	RSD(%)	LOD	RSD(%)	LOD	RSD(%)
triethylamine	121−44−8	$C_6H_{15}N$	1.4	101	60.6	20.9	711.9	n.a.	13.1	10.2
p-xylene	106−42−3	C_8H_{10}	3.2	51	2.1	11.4	24.9	9.4	2.8	12.4
caprolactam	105−60−2	$C_6H_{11}NO$	−0.1	42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-methylstyrene	98−83−9	C_9H_{10}	3.5	51	1.2	12.8	14.2	13.2	1.0	13.4
2,6-diaminotoluene (2,6-TDA)	823−40−5	$C_7H_{10}N_2$	0.9	77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
allyl methacrylate	96−05−9	$C_7H_{10}O_2$	1.7	111	14.1	6.9	163.7	14.8	20.2	17.5
naphthalene	91−20−3	$C_{10}H_8$	3.3	102	0.4	13.6	5.2	7.0	0.4	7.1
2-naphthylamine	91−59−8	$C_{10}H_9N$	2.3	89	217.2	7.7	2524.0	n.a.	21.2	14.8
dipropylene glycol monomethyl ether	34590−94−8	$C_7H_{16}O_3$	0.7	104	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-methoxy-4-(prop-2-en-1-yl)phenol (eugenol)	97−53−0	$C_{10}H_{12}O_2$	2.0	55	4.6	9.9	53.0	4.1	2.5	9.6
1-dodecene	112−41−4	$C_{12}H_{24}$	6.8	168	0.8	11.3	9.0	9.5	0.7	10.3
diphenyl ether	101−84−8	$C_{12}H_{10}O$	4.2	65	0.4	11.1	4.7	7.3	0.4	5.5
benzophenone	119−61−9	$C_{13}H_{10}O$	3.4	51	0.7	5.6	8.1	8.0	1.0	12.7
2-ethylhexyl acrylate	103−11−7	$C_{11}H_{20}O_2$	3.8	112	0.3	17.7	3.5	14.0	0.1	12.0
dimethyl isophthalate	1459−93−4	$C_{10}H_{10}O_4$	2.2	50	2.0	12.0	22.9	17.9	1.3	3.7
ethylene glycol dimethacrylate	97−90−5	$C_{10}H_{14}O_4$	1.9	113	1.6	12.8	18.2	11.9	0.6	11.7
2,6-diisopropylnaphthalene	24157−81−1	$C_{16}H_{20}$	5.8	141	0.1	6.2	0.7	3.3	0.2	2.5
o-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA)	72915−12−9	$C_7H_4F_5NO$	1.3	117	10.6	8.0	123.1	6.3	8.5	14.5
diphenyl carbonate	102−09−0	$C_{13}H_{10}O_3$	3.3	94	4.7	7.9	54.2	16.1	2.2	18.5
2,4,7,9-tetramethyl-5-decyne-4,7-diol	126−86−3	$C_{14}H_{26}O_2$	2.7	169	22.4	17.8	259.8	2.9	5.9	17.7
4,4′-(propane-2,2-diyl)diphenol (bisphenol A)	80−05−7	$C_{15}H_{16}O_2$	3.3	119	319.1	13.5	3707.8	7.8	81.0	19.6
diethyl sebacate	110−40−7	$C_{14}H_{26}O_4$	3.5	158	2.8	15.6	32.8	14.6	0.3	10.4
bis(2-hydroxy-4-methoxyphenyl) methanone (cyasorb UV12)	131−54−4	$C_{15}H_{14}O_5$	3.3	124	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
stearamide	124−26−5	$C_{18}H_{37}NO$	6.8	283	1.3	14.6	14.9	9.7	1.6	5.1
dibutyl sebacate	109−43−3	$C_{18}H_{34}O_4$	5.3	214	0.8	12.9	9.2	12.4	0.6	4.6
2-tert-butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol (tinuvin 326)	3896−11−5	$C_{17}H_{18}ClN_3O$	5.6	91	0.4	14.2	4.8	14.9	0.6	16.9
[2-hydroxy-4-(octyloxy)phenyl](phenyl) methanone (chimassorb 81)	1843−05−6	$C_{21}H_{26}O_3$	6.8	197	5.9	18.1	68.7	26.8	4.0	13.9
glyceryl monostearate	123−94−4	$C_{21}H_{42}O_4$	7.4	327	11.0	23.0	127.9	7.9	31.7	3.8
2-ethylhexyl 2-cyano-3,3-diphenylprop-2-enoate (octocrylene)	6197−30−4	$C_{24}H_{27}NO_2$	7.1	165	3.0	15.8	34.5	1.4	1.0	6.3
bis(2-ethylhexyl) adipate	103−23−1	$C_{22}H_{42}O_4$	6.8	241	0.2	8.6	2.0	16.4	0.4	12.1
dioctyl terephthalate	4654−26−6	$C_{24}H_{38}O_4$	9.9	57	2.1	18.7	24.8	13.7	1.1	15.7
tributyl acetylcitrate	77−90−7	$C_{20}H_{34}O_8$	3.3	329	2.0	1.9	23.4	12.7	0.6	12.4
dinonyl phthalate	84−76−4	$C_{26}H_{42}O_4$	10.1	167	2.9	14.4	33.7	18.4	3.8	16.8
diheryl sebacate	122−62−3	$C_{26}H_{50}O_4$	9.0	297	1.8	10.5	20.7	21.6	0.8	10.3
2,5-bis(5-tert-butyl-2-benzoxazolyl) thiophene	7128−64−5	$C_{26}H_{26}N_2O_2S$	8.0	105	132.0	12.6	1533.7	n.a.	194.4	9.7

[a]Note: Mass is the least abundant ion used for calculating LOD. RSD represents repeatability calculated under 10 μg/kg when possible; for less sensitive compounds, higher concentrations were used accordingly.

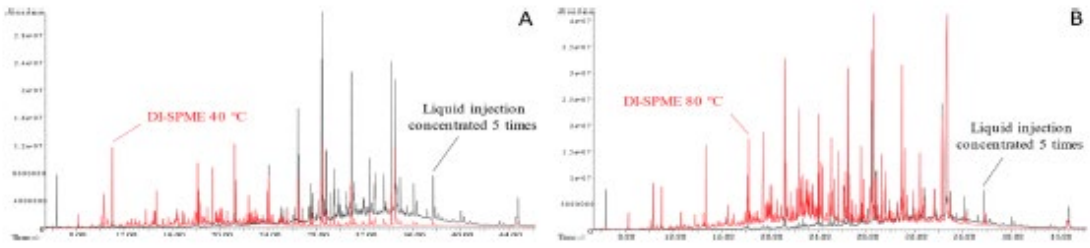

Figure 1. Comparison of liquid injection with DI-SPME at 40 °C (A) and with DI-SPME at 80 °C (B) using DVB/CAR/PDMS fiber.

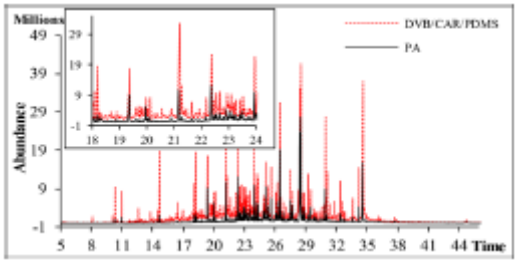

Figure 2. Comparison of DVB/CAR/PDMS and PA fibers

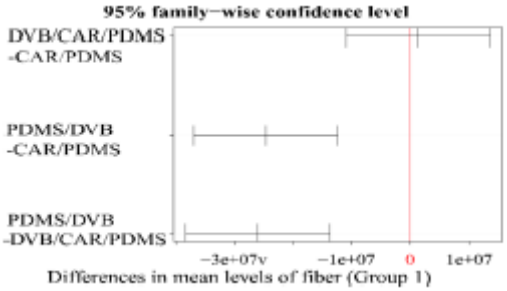

Figure 3. Tukey HSD pairwise mean comparisons regarding group 1 substances.

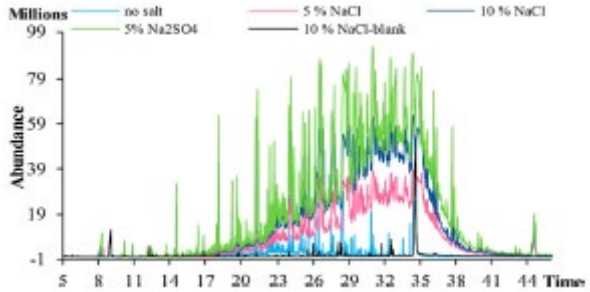

Figure 4. Effect of salt addition on the extraction efficiency from diluted 95% ethanol samples.

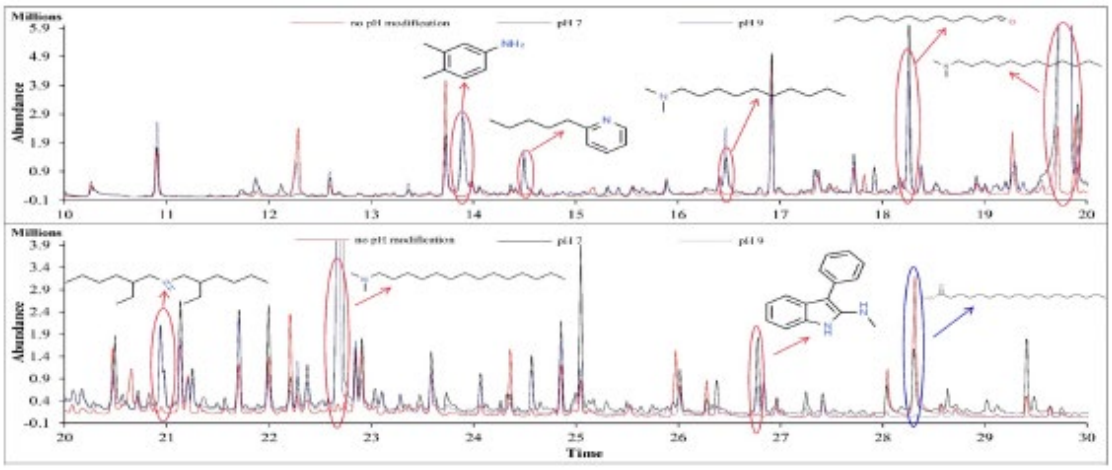

Figure 5. Effect of pH modification on the extraction efficiency from 3% acetic acid sample.

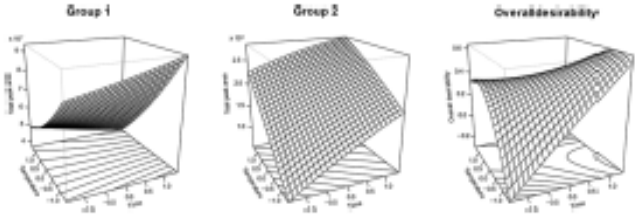

Figure 6. Response surface plots for group 1, group 2, and overall desirability.