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Quantitative determination of 22 primary aromatic amines by cation-exchange solid-phase extraction and liquid chromatography–mass spectrometry

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ABSTRACT

Primary aromatic amines (PAAs) have been broadly studied due to their high toxicity. In this work a method for the analysis of 22 PAAs in aqueous simulants has been developed. The method is based on a solid-phase extraction step using cation-exchange cartridges and the subsequent analysis of the extracts by ultra-high-performance liquid chromatography with mass spectrometric detection. The recoveries obtained for all the amines analyzed ranged between 81 and 109%, linear range was between 0.03 and 75 μg L^{-1}, with the RSD values between 4.5 and 13.4% and an average value of 7.5% and limits of detection at μg L^{-1} level. The method has been applied to two real samples obtained from migration experiments of polyurethane based laminates to simulant B (water with 3% (w/v) acetic acid) which represents the worst case for the migration of aromatic amines. The main amines found in both samples were methylenedianiline isomers, obtained from the corresponding residual diisocyanates used during polyurethane adhesive polymerization. The total amine concentration found was 26 and 6.3 μg of aniline equivalents per kg of food simulant.

1. Introduction

Primary aromatic amines (PAAs), which are suspected to be carcinogenic compounds [1], could be present in a wide variety of samples. For this reason, their presence in different matrices has been broadly studied, especially in those materials that have been in contact with food [2,3].

PAAs can be produced by the decomposition of azo dyes used in printing inks, as the chromophoric azo group under certain conditions can be reduced to form aromatic PAAs. Since the azo dyes are extensively used in commercial articles such as textiles, cosmetics or plastics and also as food colorants [4,5], the determination of aromatic PAAs obtained from these dyes has been carried out previously by different methods of analysis, such as by GC–MS after derivatization with isobutyl chloroformate in hair dyes samples [6], by HPLC with on-line post-column photochemical derivatization in industrial dyes [7], by HPLC with electrospray ionization (ESI) MS/MS detection in industrial dyes [8] or by μ-HPLC with electrochemical detection [9] in some food colorants.

PAAs as well as neoformed compounds appear from polyurethane (PU) adhesives, commonly used for preparing multilayer laminates, which will be further used as food contact materials. PU adhesives are formed by the polymerization of polyols and diisocyanate monomers. In case the adhesive has not been properly cured or if the ingredients have been mixed wrongly, the polymerization reaction will not be efficient and the remaining unpolymerized aromatic isocyanates will come into contact with water producing primary aromatic amines. Although this risk is real and well known, PU adhesives have been extensively used for different purposes. Apart from its use in laminates of food packagings, it has been used in the manufacture of biocompatible prosthesis and medical devices [10] or in household and filling materials (PU foams). As part of the ongoing investigations about the degradation of PU adhesives, several methods have been developed to isolate and chemically analyze their degradation products such as the primary aromatic amines. In biomaterials, the analysis has been focused on toluenediamine (TDA) isomers, which have been carried out by HPLC–MS [11] and also by GC–MS after derivatization with pentafluoropropionic anhydride (PFPA) [12,13]. A comparison of different methods of analysis of aromatic amines obtained from toluendiisocyanate (TDI)-based PU foams has been developed by Marand et al. [14] that found a better sensitivity when the samples were derivatized with PFPA and analyzed by GC–MS than when the samples were analyzed by LC–MS. PU laminates used in food packagings are multilayered films cured with polyurethane adhesives. Papers about the analysis of amines in aqueous simulants in contact with food contact laminates have

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been previously published, derivatizated with GC–MS [15] and LC–MS [16].

According to the European Union (EU) regulations for food contact materials (Commission Directive 2002/72/EC [17] and Directive 19/2007/EC [18]), the limit of PAAs released by the packaging material (expressed as aniline) must be below 10 μg aniline equivalents per kg of food or food simulant (10 ppb). The German Federal Institute of Consumers Health Protection and Veterinary Medicine (BgVV) established a spectrophotometric method to determine the total content of PAAs in food packaging materials some years ago ("Photometric determination of primary aromatic amines in food simulants"; CEN/TC194/SC1/TG9). In this method the primary aromatic amines are derivatized to an azo dye and quantified as aniline hydrochloride equivalents. Although this colorimetric method reaches the required sensitivity, it has important drawbacks, the most critical one is the absence of selectivity that does not allow the individual quantification of the amine. This lack of selectivity can also lead to false positive results in the presence of other colorants likely present in the sample. For this reason it was considered interesting to develop a method with low detection limits and a good selectivity in order to be able to determine which individual PAAs are migrating from the packaging to the foodstuff and in which concentrations. The method proposed in this paper combines the solid-phase extraction (SPE) of the sample with the analysis of the extract by ultra-high-performance liquid chromatography (UHPLC)–MS. The SPE step allows the concentration of the analytes and the elimination of residual matrix components that could induce ionic suppression effects during the MS detection. Advantages of UHPLC in terms of resolution and speed have been broadly proved [19–21]. The combination of the SPE extraction with the UHPLC technology coupled to MS, offers a sensitive and accurate method for PAA quantification.

2. Materials and methods

2.1. Reagents and solutions

DSC-SCX cation-exchange cartridges (500 mg/3 mL) were purchased from Supelco (Bellefonte, PA, USA) and the manifold system was from Waters (Milford, MA, USA).

PAAs were bought from Sigma–Aldrich (St. Louis, MO, USA) and all of them were of analytical quality.

Supergradient HPLC-grade methanol, HPLC-grade acetic acid and ammonia (solution 32%) were purchased from Scharlau Chemie (Sentmenat, Spain), HPLC-grade acetone was acquired from Merk (Darmstadt, Germany) and water purified with a Milli-Q 185 Plus system (Millipore, Bedford, MA, USA) was used.

The PAAs selected for this study are shown in Table 1. Individual solutions of around 1000 mg L^{-1} were prepared in methanol, except 4,4-DPE that was prepared in acetone. The solutions of *p*-PDA, 4MmPDA, 2,6-TDA and 1,5-DAN were prepared monthly because of possible amine degradation processes.

2.2. UHPLC separation

Chromatography was carried out in an Acquity system using an Acquity UPLC BEH C18 column of 1.7 μm particle size (100 mm × 2.1 mm), both from Waters.

UHPLC conditions were optimized in order to achieve a good chromatographic resolution and sensitivity. Several parameters such as the mobile phase composition or the possibility of a post-column addition were tested. Chromatography was carried out at 0.3 mL min^{-1} column flow and at 45 °C column temperature. Methanol and water were used as mobile phases and the UHPLC gradient is shown in Table 2.

2.3. Mass spectrometry detection

A Quattro micro-atmospheric pressure ionization (API) tandem quadrupole mass spectrometer with an electrospray probe in positive mode (ESI+) from Micromass (Beverly, MA, USA) was used. Acquisitions were carried out in SIR (selected ion recording) and MRM (multiple reaction monitoring) modes.

For SIR detection, [MH^+] ions were monitored. Cone voltages were optimized between 20 and 50 V. For MRM detection, transitions to daughter ions were monitored and collision energies were optimized from 15 to 30 V. Voltage optimization was carried out by the direct perfusion into the MS at 10 μL min^{-1} of individual solutions of 10 mg L^{-1} of each amine. Table 1 shows [MH^+] ions, daughter ions, cone voltages and collision energies selected for each amine.

For the optimization of the tune MS parameters, 4 different desolvation gas temperatures ranging from 300 to 450 °C and 4 different desolvation gas flows ranging from 450 to 700 L/h were tested. Optimization was carried out by the injection of a mixture of 2 mg L^{-1} of the 23 amines. Final MS parameters are shown in Table 2.

2.4. SPE

For the SPE, the loading and the elution volumes were optimized. The loading solution contained around 100 μg L^{-1} of the 22 selected PAAs and it was prepared in purified water 3% (w/v) acetic acid. Following the cartridge indications, the elution solvent was methanol 5% (v/v) NH_3. For the optimization of the loading and the elution volumes, sample volumes ranging from 20 to 100 mL were passed through the cartridges, and then, the cartridges were eluted. Consecutive 1 mL aliquots of the elution solvent were collected and analyzed by UHPLC–MS. The water dilution factor of the extract collected was also optimized.

SPE final extraction protocol was as follows: the cartridges were conditioned with 2 mL of methanol and equilibrated with 2 mL of purified water containing 3% (w/v) acetic acid. Afterwards, 80 mL of sample solution was passed through the cartridges at around 1–1.5 mL min^{-1}. The cartridges were cleaned with 2 mL of purified water with 3% (w/v) acetic acid and dried with vacuum. Finally, they were eluted with methanol containing 5% (v/v) NH_3. The first milliliter of the eluate was discarded and the second one was collected for the UHPLC–MS analysis. Before the analysis, the SPE extract collected (1 mL) was diluted 1/1.6 with water, and a 15 μL aliquot was injected into the UHPLC system.

To check the reproducibility of the system, 4-aminoazobenzol was added as internal standard to the extract before the analysis.

2.5. Calibration curves and analytical parameters

For building the calibration curves, solutions of the 22 PAAs with concentrations ranging between 0 and 75 μg L^{-1} were prepared in purified water with 3% (w/v) acetic acid. Solutions were extracted following the SPE extraction protocol and the extracts were analyzed by UHPLC–MS under the conditions previously optimized.

To calculate the reproducibility of the method, 3 solutions with the same concentration (around 30 μg L^{-1}) were analyzed on different days and the results were compared.

Recovery of the method was checked by comparing the results obtained when a 30 μg L^{-1} PAAs solution was passed through the SPE cartridge to those obtained when the equivalent solution was directly analyzed by UHPLC–MS.

2.6. Sample analysis

Two different laminates used in food packaging were analyzed. Materials used for laminates elaboration are shown in Table 4. The