Journal of Chromatography A, 1320 (2013) 96–102

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Liquid chromatography–full scan-high resolution mass spectrometry-based method towards the comprehensive analysis of migration of primary aromatic amines from food packaging

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ARTICLE INFO

Article history:
Received 9 August 2013
Received in revised form 16 October 2013
Accepted 19 October 2013
Available online 27 October 2013

Keywords:
High-resolution mass spectrometry
Primary aromatic amines
Packaging materials
Migration
Method validation

ABSTRACT

European Union legislation has established that plastic food contact materials shall not release primary aromatic amines (PAAs), which are toxic compounds and suspected human carcinogens. As valid alternative to existing methods for PAA determination, which are based on spectrophotometric test or targeted liquid chromatography–tandem mass spectrometry (LC–MS/MS) approaches, in this study a LC-Orbitrap–full scan-high resolution mass spectrometry (HRMS) method was devised and validated for the determination of migration levels of 22 PAAs from food contact materials, thus exploiting the specificity of accurate mass measurement. Direct injection of the simulant (acetic acid 3%, w/v) into the LC–MS system after migration, without any pre-treatment step, makes the developed method of great value for rapid screening analysis of a large number of amines. A very fast and efficient separation (<11 min) of PAAs was achieved. Detection limits in the 0.06–0.7 $\mu g\,kg^{-1}$ range were calculated for 17 out of 22 of the investigated PAAs, however obtaining values within 5.3 $\mu g\,kg^{-1}$ for the other 5 amines. Good dynamic linear ranges from two to four orders of magnitude ($r^2 \geq 0.990$) were obtained and satisfying results were achieved in terms of intra-day (RSDs < 10%) and inter-day repeatability (RSDs < 17%). Trueness values in the 70 ± 1–$131 \pm 5\%$ range proved reliability of the developed method for PAAs quantification also at very low concentration levels. Finally, the method was successfully applied to a range of different real plastic multilayer food packaging materials, noticing in all cases levels below the established limits of detection.

1. Introduction

The occurrence of primary aromatic amines into food may derive from the use of polyurethanic adhesives for the manufacture of plastic multilayer films [1]. In fact, these adhesives, which are the most exploited to couple together the different layers, may contain unreacted aromatic isocyanates coming from the incomplete polymerization process of polyurethane; after packing, water present into foods can hydrolyze residual aromatic isocyanates, thus leading to PAAs formation [2]. In addition, the migration of aromatic amines from food contact materials, printed or coloured with azo dyes, can also occur due to the possibility, under certain conditions, that the azo group is reduced to form PAAs. Unlike the aromatic amines originating from isocyanates in adhesives reacting with moisture, the aromatic amines originating from colourants may already be present as monomer impurities in the food packaging, since they are often used as starting compounds in the production of azo pigments [3].

Another possible source might be the packaging made from recycled paper due to the massive use of printed paper in the recycling process. Finally, several evidences concerning the migration of PAAs into food were associated to black nylon kitchen utensils for repeated use, as reported by Trier et al. [4].

Taking into account that many aromatic amines are classified as toxic compounds and/or suspected human carcinogens [5], their migration into foodstuff from food contact material is subjected to restrictions according to European Union Regulation 10/2011: plastic material and articles shall not release primary aromatic amines in detectable quantity in food or food simulants. The detection limit is set to 0.01 mg of substances per kg of food or food simulant and it applies to the sum of primary aromatic amines released [6].

Currently the accepted standard approach for the analysis of PAAs is a spectrophotometric method [7], which involves the use of aniline as standard compound for calibration curve, the results

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0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.10.063

being reported as aniline equivalents. Although good sensitivity of this method, the lack of selectivity and the high risk of false positive results have required the development of alternative analytical methods for individual identification and quantification of these compounds in accurate migration studies. For this reason and after the suggestion by different national standards bodies the colorimetric method should only be used for screening purposes [8], confirmatory and alternative methods have been devised based on gas chromatography-mass spectrometry (GC–MS) after derivatization [9] and on liquid chromatography and capillary electrophoresis with ultraviolet detection (LC–UV and CE–UV) [10,11]. Currently the most exploited technique is liquid chromatography coupled with mass spectrometry (LC–MS) or tandem mass spectrometry (LC–MS/MS) thanks to its high accuracy, reliability and selectivity [12–16].

In this context, in the study of Pezo at al. [13] the overestimation in the quantification of PAAs by the spectrophotometric method was explained by performing high resolution mass spectrometry-based analysis (HRMS) on the extracts; in fact, HRMS analysis allowed to identify non-intentionally added substances, impurities and other migrant compounds not discriminated by the colorimetric approach, and usually not investigated in the targeted MS/MS approach.

Most of the existing methods for the determination of PAAs in food simulants are based on a targeted LC–MS/MS technique [12–15] operating in multiple reaction monitoring (MRM) acquisition mode, thanks to its high sensitivity and selectivity. However, the intrinsic limitation of this approach is the impossibility to detect novel analytes for which no ion transitions are acquired. By contrast, the specificity of accurate mass and the full-spectrum acquisition in HRMS permits to perform both targeted as well as non-targeted analysis, with good performance not only for qualitative but also for quantitative purposes [13,17]. In fact, HRMS allows the determination of the accurate masses of all ionizable compounds eluting during the chromatographic run, reaching good sensitivity also in full scan acquisition mode, as proved in our recent study dealing with the determination of melamine migration from melamine tableware by Orbitrap-HRMS [18].

In the present study a LC-Orbitrap–full scan-HRMS method was developed and validated for the determination of migration levels of 22 PAAs in food simulants. In addition, the direct injection of the simulants without any pre-treatment step makes the devised method of great value for rapid screening analysis of a large number of PAAs. Finally, the developed method was exploited to assess the risk linked to PAAs migration from food contact material samples made of plastic laminates available on the market.

2. Material and methods

2.1. Chemicals

Methanol, acetone and glacial acetic acid were HPLC grade and purchased from VWR International, Ltd. (Poole, England); deionized water was prepared using a MilliQ system (Millipore, Bedford, MA, USA). Pentafluoropropionic acid (PFPA), aniline-2,3,4,5,6-d5 (aniline-d5, internal standard, IS) and all the analytical standards of primary aromatic amines, listed in Table 1, were from Sigma–Aldrich (St. Louis, MO, USA).

The food simulant was 3% acetic acid in water (w/v) (simulant B) and its density was conventionally set to 1.0 g cm^{-3}.

2.2. Standard solution preparation

A standard stock solution of each primary aromatic amine was prepared at a concentration of 500 mg kg^{-1} in methanol, with the exception of 4,4′-diaminodiphenyl ether which was dissolved in acetone.

The calibration standard solutions were obtained by dilution of an intermediate standard solution containing all the investigated amines at the concentration of 5 mg kg^{-1} in simulant B.

2.3. Samples

Plastic laminate materials were tested for the migration of PAAs. In particular, the most common laminates for food contact material were collected from different Italian manufacturers and analyzed within their usual job rotation period.

2.4. Sample preparation

The migration test was carried out following the conditions suggested in the European Standard EN13130-1:2004 [19]. A surface of 0.6 dm^2 of plastic laminate was previously cut into small pieces of about 1 cm^2 each and then immersed in 100 mL of simulant B. After, the solution was incubated at 70 °C for 2 h shaking continuously. An aliquot of the extract was filtered with a 0.45 μm glass microfiber filter (Whatman, GE Healthcare, Little Chalfont, UK), then aniline-d5 (IS) was added to the final concentration of 2 μg kg^{-1} prior to the LC–MS analysis.

The calibration curves for the quantification of PAAs released from the samples were built on standard solutions in simulant B at concentrations within the working range from LOQ of each amine to 150 μg kg^{-1} (IS at 2 μg kg^{-1}).

2.5. HPLC–HRMS analysis

LC separation was performed using a 100 mm × 2.1 mm inner diameter, 2.6 μm particle size, Kinetex C18 analytical column (Phenomenex, Torrance, CA, USA) thermostated at 40 °C on a Dionex UltiMate® 3000 Standard LC systems (Thermo Fisher Scientific Inc., Waltham, MA, USA). Gradient elution separation was carried out using PFPA as mobile phase modifier and a flow rate of 300 μL min^{-1} (solvent A: 4.7 mM PFPA in water; solvent B: 4.7 mM PFPA in methanol) under the following conditions: solvent B was initially set at 10%, then delivered by a linear gradient from 10–40% in 2 min, to 84% in 11 min and to 100% in 0.5 min; then solvent B was maintained at 100% for 1.5 min before column re-equilibration (8 min). The injection volume was 20 μL.

Mass spectrometer was a LTQ Orbitrap XL hybrid FTMS (Thermo Fisher Scientific Inc.) equipped with a pneumatically assisted electrospray (ESI) interface. The sheath gas (nitrogen, 99.9% purity), the auxiliary gas (nitrogen, 99.9% purity), and the sweep gas (nitrogen, 99.9% purity) were delivered at flow rates of 40, 20 and 20 arbitrary units, respectively. Source conditions, in positive ion mode, were as follows: ESI voltage, 4 kV; capillary voltage, 21 V; tube lens, 70 V; capillary temperature, 275 °C. Full-scan accurate mass spectra (mass range from 50 to 300 Da) were obtained at a mass resolution of 30,000 FWHM (m/z 400). Identification and quantification of target compounds was performed using the accurate mass of the protonated molecule within a mass window of 5 ppm. The identity of target compounds was confirmed based on the accurate mass of the protonated molecules in combination with the retention times (tolerance of 2.5% compared with those of reference standards), isotopic patterns and a minimum peak threshold of 10,000 units. The quantification was performed by using the extracted ion chromatograms (XIC) of the m/z value corresponding to the protonated molecules.