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Target analysis of primary aromatic amines combined with a comprehensive screening of migrating substances in kitchen utensils by liquid chromatography–high resolution mass spectrometry

Yovana Sanchis [a,b], Clara Coscollà [a,b], Marta Roca [a,b], Vicent Yusà [a,b,c,*]

[a] Foundation for the Promotion of the Health and Biomedical Research in the Valencia Region (FISABIO-Public Health), 21, Avenida Catalunya, 46020 Valencia, Spain
[b] Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain
[c] Analytical Chemistry Department, University of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100 Burjassot, Spain

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ABSTRACT

An analytical strategy including both the quantitative target analysis of 8 regulated primary aromatic amines (PAAs), as well as a comprehensive post-run target screening of 77 migrating substances, was developed for nylon utensils, using liquid chromatography–orbitrap-high resolution mass spectrometry (LC–HRMS) operating in full scan mode. The accurate mass data were acquired with a resolving power of 50,000 FWHM (scan speed, 2 Hz), and by alternating two acquisition events, ESI+ with and without fragmentation. The target method was validated after statistical optimization of the main ionization and fragmentation parameters. The quantitative method presented appropriate performance to be used in official monitoring with recoveries ranging from 78% to 112%, precision in terms of Relative Standard Deviation (RSD) was less than 15%, and the limits of quantification were between 2 and 2.5 μg kg^{-1}. For post-target screening, a customized theoretical database was built for food contact material migrants, including bisphenols, phthalates, and other amines. For identification purposes, accurate exact mass (< 5 ppm) and some diagnostic ions including fragments were used. The strategy was applied to 10 real samples collected from different retailers in the Valencian Region (Spain) during 2014. Six out of eight target PAAs were detected in at least one sample in the target analysis. The most frequently detected compounds were 4,4′-methylenedianiline and aniline, with concentrations ranging from 2.4 to 19,715 μg kg^{-1} and 2.5 to 283 μg kg^{-1}, respectively. Two phthalates were identified and confirmed in the post-run target screening analysis.

1. Introduction

Primary aromatic amines (PAAs) appear in food products mainly from food contact materials (FCMs). These amines are formed by hydrolysis of aromatic isocyanates in polyurethane adhesives, and by degradation of azodyes used as colourants in nylon kitchen utensils and other plastic materials [1]. Evidence suggests that some PAAs are carcinogenic pollutants [2], consequently their migration into foodstuff is subjected to regulations and restrictions. The European Union Regulation 10/2011 has established a migration limit of 0.01 mg kg^{-1} for the sum of the regulated primary amines [3].

Brede et al. [1] identified the polyamide cooking utensils as a common source of PAAs. Likewise, Mortensen et al. [4] also detected the migration of several PAAs, especially aniline (ANL) and 4,4′-methylenedianiline (MDA) from black polyamide cooking utensils, which exceeded the limits permitted by the EU legislation. From these early studies onwards, numerous alerts have been issued by the rapid alert system for food and feed (RASFF) for excessive concentrations of PAAs migrating from FCMs [5].

The need to carry out a continuous surveillance and the frequently detected violations of the migration limits has led to the development of different analytical methods. The most commonly used technique for determining PAAs has been gas chromatography coupled to mass spectrometry (GC–MS), after derivatization [1]. Additionally, liquid chromatography–tandem mass spectrometry (LC–MS/MS) [3,6–8] has currently become a very sensitive and selective analytical tool for the quantitative determination of these polar compounds. However, LC–MS/MS presents some limitations such as the impossibility to conduct a retrospective analysis of the

* Corresponding author at: Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain. Tel.: +34 961925865; fax: +34 961925704.
E-mail address: yusa_vic@gva.es (V. Yusà).

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samples for the post-target identification of other relevant compounds not included in the initial scope.

In some fields such as pesticide and veterinary drug analysis, LC coupled to high-resolution mass spectrometry (HRMS), using Orbitrap and time-of-flight (TOF) analysers has increasingly become more popular [11–13] owing to its capacity of using the full-scan acquisition mode with high sensitivity, combined with high resolving power (> 50,000 FWHM) and accurate mass measurements (1–5 ppm). This powerful tool enables the development of both a quantitative analysis of the priority regulated substances (target analysis) and a post-target screening of suspected compounds. Mattarozzi et al. [9] have recently developed and validated an innovative LC–HRMS method for the comprehensive determination of PAAs in plastic multilayer food-packaging. However, the authors do not include the retrospective analysis of other possible substances migrating from the plastic material.

In the present study we have developed an analytical strategy that combined the quantitative target analysis of the 8 regulated PAAs, with the post-target screening (identification) of 77 specific migration substances from nylon kitchen utensils, using a standardized extraction protocol with acetic acid (simulant) and direct injection into an LC–HRMS without clean-up. The analytical strategy was applied to samples collected in an official monitoring programme in the Valencia Region, Spain.

2. Experimental

2.1. Chemicals and reagents

High purity standard amines, 2,6-toluenediamine (98%) (2,6-TDA); 2,4 toluenediamine (98%) (2,4-TDA); aniline (98%) (ANL); 1,5-naphthalenediamine (99%) (1,5-DAN); 1,3-phenylenediamine (99%) (m-PDA); 4,4′-methylenedianiline (98.5%) (4,4′-MDA); 4,4′-oxydianilina (99%) (4,4′-DPE); and 3,3′-dimethylbenzidine (98%) (3,3′-DMB) were supplied by Sigma Aldrich. The food simulant was 3% acetic acid in water (w/v) and its density was conventionally set to 1.0 g cm^{-3}.

Individual stock standards were prepared weighting 25 mg of pure standard using a 5-decimal analytical balance and dissolving it in methanol. Mix working solutions at 5 μg mL^{-1} were prepared with methanol and at 100 ng mL^{-1} were prepared with acetic acid (AcH, 3%). Calibration solutions (1.5 or 2, 5, 10, 15 and 20 ng mL^{-1}) were prepared by adding variable volumes of the mix working solutions to the simulant (3% AcH in water, w/v).

HPLC-grade methanol was supplied by Scharlau (Barcelona, Spain). HPLC-grade water was purchased from Merck (Darmstadt, Germany). Acetic acid (Reag. Ph. Eur.) and ammonium hydroxide solution 25% were provided by Panreac (Barcelona, Spain).

Statistical data manipulation and numerical analysis of data resulting from the experimental design were carried out using the statistical package MINITAB for Windows, Release 14 (Minitab Inc., Birmingham, UK).

2.2. Samples

Ten black and grey nylon kitchen utensils were sampled from different retailers by the Food Safety Department of the Valencian Region (Spain) during 2014. Each sample consisted of 3 replicated for the migration test and another one for the surface calculation.

2.3. Sample preparation

The migration test was carried out following the European Standard EN 13130-1:2004 [10,14]. In short, each sample was placed in a beaker which was in turn filled with a volume of simulant (AcH, 3%) enough to cover the piece of utensil used for extraction. The beaker was covered with aluminium foil to avoid light exposure, and transferred to a preheated boiler. The top of the beaker was also covered with aluminium foil to reduce simulant loss by evaporation. After 2 h at 100 °C, test specimens were removed from the simulant and it was cooled down to room temperature. Then, the extract was placed in an Erlenmeyer flask with a glass stopper and stored at 4 °C until analysis. The volume of the acetic acid added into the beaker before and after the extraction was measured and recorded for the expression of results.

1 ml of extract was neutralized with 250 μl of ammonium hydroxide solution and filtered using a 0.45 μm microfiber filter (Whatman, GE Healthcare, Little Chalfont, UK) before injection in the LC–HRMS. Each sample was analysed in triplicate.

2.4. LC–HRMS

The chromatographic separation was performed on an Accela liquid chromatography UHPLC system equipped with a Phenyl hexil column (100 mm × 2.1 mm, 2.1 μm), both from ThermoFisher Scientific (Bremen, Germany). The flow rate used was 400 μl min^{-1} and the injection volume was 10 μl. Separations were performed using a binary gradient. The mobile phase was composed of H_2O (A) and methanol (B) and the binary gradient conditions were as follows: 0–8 min, linear from 2% to 50% B; 8–10 min, linear from 50% to 80% B; 10–11 min, linear from 80% to 95% B; 11–13 min isocratic 95% B; 13–15 min, linear from 95% to 2%. The total run time was 17 min.

Mass spectrometric analysis was performed on a single-stage Orbitrap MS (Exactive™, Thermofisher Scientific, Bremen, Germany). The system was equipped with a heated electrospray ionization interface (HESI-II). The detection was carried out in positive ionization mode (ESI+) using the following optimized operational parameters: spray voltage, 4.4 kV; sheath gas (N_2, > 95%), 50 arbitrary units (a.u.); skimmer voltage, 50 V; capillary voltage, 50 V; heater temperature, 500 °C; and capillary temperature, 123 °C. The mass spectra was acquired using two alternating acquisition functions (i) full scan MS without fragmentation, ESI+; mass resolving power=50,000 FWHM; scan range=80–800 Da; scan time=0.5 s (2 Hz); (ii) the same parameters but with full scan MS all ions fragmentation (the higher collision cell (HCD) was switched on at a collision energy of 20 eV). The automatic gain control (AGC) was set to 1×10^6 ions. The external mass calibration of the spectrometer was performed using a ready-to-use calibration mixtures (Mas Cal5 (+) from Supelco (USA). Data acquisition and processing was performed using Thermo Scientific TraceFinder™ software version 3.1. Extracted ion chromatograms (XIC) for individual compounds were reconstructed from the full-scan data with a mass tolerance of 5 ppm.

2.5. Analytical method validation and identification criteria

Samples were analysed under quality assurance protocols following the ISO 17025. Quality control procedures to check the method performance were implemented in each batch of samples, including reagent blanks, matrix blanks and spiked blank samples. Market samples were stored at −4 °C until analysis.

As there is no specific guideline prescribed for validation of methods concerning migrating substances from food contact materials, the quantitative method was validated using the SANCO/125771/2013 guideline [15], defining the following performance criteria: recoveries within 70–120%; repeatability RSD ≤ 20 and LOQ lower enough to be used for regulatory purposes [3]. There is no reference material available on primary amines in contact-food materials, so the accuracy of the method was carried out using a