A SELECTIVE AND HIGHLY SENSITIVE ANALYTICAL METHODOLOGY FOR DETERMINATION OF BISPHENOL A IN CANNED TOMATOES

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Abstract - A selective and highly sensitive analytical methodology for determination of Bisphenol A (BPA) in canned tomatoes was developed and validated. The method was based on selective liquid/solid extraction, combined with liquid chromatography–electrospray ionization tandem mass spectrometry in the multiple reaction monitoring (MRM) mode and negative ionization. The linearity of the detector response was verified for canned tomato samples and the limit of quantification of the method (LOQ) was calculated to be 0.02 µg/Kg. The method was in-house validated evaluating specificity, trueness, within-day and between-days precision. The mean recoveries of BPA from tomato samples were higher than 94% and good reproducibility was observed. The matrix effect was studied and compensated using deuterated labeled standard (d_6-BPA). To assess the BPA migration from epoxy-coating cans, 16 samples belonging to six commercial brands, retailed in Italian markets, were tested. All the tomato samples exhibited migration levels below 4 µg/Kg. In any case no sample contained BPA exceeding the European Union limit for migration (600 µg/Kg of food).

Keywords: Bisphenol A, canned tomatoes, liquid chromatography-tandem mass spectrometry, migration.

1. *Introduction*

Metallic cans for food packaging are made with a thin coating film on the inner surface to avoid electrochemical corrosion and contamination of the product by migration of metal ions. Common coating materials are organosols or epoxy resins, condensation products of Bisphenol A (BPA) with epichlorhydin [1]. BPA may remain unreacted when polymerization conditions or the curing process is insufficient and a significant amount can migrate in food packaged in containers lined with these plastics. The attention to BPA presence in food was due to the documented association between human exposure to this endocrine disruptor and some related pathological disorders, including thyroid hormone disruption [2], cancer [3], liver damage [4], metabolic sindrome [5]. The levels of BPA in several canned foods are reported in the literature; although in all the single food these levels are below the concentration limits recommended to avoid health risks, there is the possibility of a synergic effect by eating different commodities contaminated by BPA. Therefore, it is important to monitor BPA presence in packaged foods and drinks, because a little attention has been previously directed to this contamination, considering the assumption rate as a negligible risk

[6]. In order to protect the consumer from potential food hazards and to perform adequate risk assessment, highly reliable extraction and accurate quantification methods have be developed to measure this compound in foods. The aim of this paper is to develop and validate a selective and highly sensitive analytical methodology based on chromatography–electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) for determination of BPA in two types of canned tomatoes products (peeled and cherry), retailed in Italian markets. Several studies reported the data about canned vegetables (carrots, beans, and peas), canned fruits, drinks, baby foods, soups and tuna. On the contrary a few data have been published about BPA concentrations in canned tomatoes.

2 Materials and Methods

2.1 Materials and reagents

BPA (purity grade 98%) and d_6-BPA, used as internal standard (IS), were purchased from Sigma Aldrich (Sigma-Aldrich, Milano, Italy). HPLC grade reagents, including water, methanol (MeOH) and acetonitrile (ACN) were purchased from Romil (ROMIL Ltd, UK). The MIP cartridges purchased from Polyntell (Polyntell SA, Paris, France) were AFFINIMIP® SPE Bisphenol A in glass tube. Peeled and cherry tomatoes in commercial size of 400 gr (diameter 7.5 cm and height 10.5 cm) were investigated. In particular, for each tomato type of different brand, 16 samples (four cans, with the same batch number, from four production lots with an expire date between 18 and 14 months later) were purchased from different local supermarkets in Naples, Italy.

2.2 Preparation of standard reference materials and quality control samples

A BPA stock standard solution at 1 mg/mL was prepared in MeOH. In a similar manner, a stock standard solution of d_6-BPA, used as internal standard (IS), was prepared at 1 mg/mL in MeOH. Both standard stock solutions were stored in dark glass vials at −20 °C until use. The intermediate standard solutions were prepared from the stock solutions by serial dilution with MeOH/water (1/1, v/v). In a concentration range from 0.1 to 100 ng/mL, eight-point calibration curve standards were prepared fresh daily. Four levels of quality control samples (QCs) in tomato samples containing 0.10 ng/mL (LLOQ-QC, lowest level quality control) 1.0 ng/mL (L-QC low quality control), 10 ng/mL (M-QC, middle quality control), and 100 ng/mL (H-QC, high quality control) of BPA were prepared by spiking blank tomato samples with standard solutions for determination of trueness (mean recoveries) and within-day and between-days precision. The standard solution volume not exceeds the 2% total volume of blank tomato sample.

2.3 Extraction and clean up of tomato samples

The contents of each canned tomato sample were macroscopically homogenized in a kitchen mixer. Then a 5.00 ± 0.01 g of sample were added with 5 mL of methanol and 100 µL intermediate IS solution at 1 µg/mL and homogenized (3 min at 15000 rpm) using a Diax 900 homogenizer (Heidolph Instruments Gmbh &Co.KG). The homogenate was stirred (30 min) at room temperature and then centrifuged (10 min at 3500 rpm) at 24°C. The supernatant was recovered and loaded onto AFFINIMIP cartridge [7]. In each working session, a method blank containing 5 mL of a mix 1:1 MeOH/water (v/v) added with 100 µL intermediate IS solution at 1 µg/mL was also introduced and processed. Calibration curve standards were also incubated under the same conditions of the tomato samples.

2.4 LC/ESI-MS/MS analysis

A Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific Inc, Italy) was used in this study. The chromatographic separation was carried out using a 100 × 4.6 mm Kinetex 2.6µm F5 stainless steel HPLC column, equipped with a guard column (Phenomenex, Italy). The mobile phase consisted of water (A) and methanol (B). Chromatography was run at 0.3 mL/min by linear gradient elution. The HPLC system was coupled to a triple quadrupole instrument (API 2000; AB Sciex, Germany) equipped with a TurboIon electrospray source. The analytes were quantified in multiple reaction monitoring (MRM) mode. The following ion transitions were monitored: *m/z* 227.1 →*m/z* 212.1 (quantifier) and *m/z* 227.1→ *m/z* 133.2 (qualifier) for BPA; *m/z* 233.1→ *m/z* 215.0 (quantifier) and *m/z* 233.1→ *m/z* 138.2 (qualifier) for d_6-BPA. The data were handled by the Analyst™ software version 1.5.1 (ABI Sciex).

BPA identification was based the retention time of both quantifier and qualifier product ions. The peak area ratios of BPA and IS were reported vs BPA standard concentrations, and calibration curves were calculated by linear regression; BPA concentration in tomato samples was calculated by interpolation of calibration curves, as well as the measured concentrations of standards and quality control samples (QCs).

3 Results and Discussion

3.1 Optimization and validation of BPA analysis in tomato

By injecting standard solutions (20 μL) at concentrations from 0.1 to 100 ng/mL, corresponding to about 0.02-20 μg BPA/Kg tomato, a linear calibration curve (R=0.992) was obtained by measurement of peak areas versus concentration. The limit of detection (LOD) of the detectors is 0.01 ng/mL. The limit of quantification (LOQ) of the HPLC method is 0.02 μg BPA/Kg of canned tomato. The method was in-house validated by evaluating detector response linearity, within-day and between days precision, trueness by mean recoveries, and ruggedness for slight changes, using quality control samples (QCs). Mean recoveries and possible matrix effects were evaluated using canned tomatoes spiked at three different concentrations of BPA: 1.0 ng/mL (L-QC low quality control), 10 ng/mL (M-QC, middle quality control), and 100 ng/mL (H-QC, high quality control) in six replicates. Mean percentage recovery was calculated by comparing the mean area response of samples spiked before extraction to that of samples spiked at the same levels after extraction, at each concentration tomato samples. The effective mean recoveries of BPA, without considering compensation due to the labeled IS, were observed in the range from 94.7%-102.6% for peeled tomato and 92.2%-98.5% for cherry samples. Figure 1 show a chromatograms obtained from LC/ESI–MS/MS analysis of tomato samples. In particular Figure 1a refers to a peeled tomato sample spiked with a known concentration of our target analyte (1 ng/mL of BPA) prior to extraction procedure; while Figure 1b refers to an peeled tomato sample spiked with a 1 ng/mL of BPA after the extraction procedure. The retention time of BPA was approximately 3.52 minutes. By using MIP cartridges, a lower baseline noise was observed, and no more peaks from the matrix were present in the chromatograms.

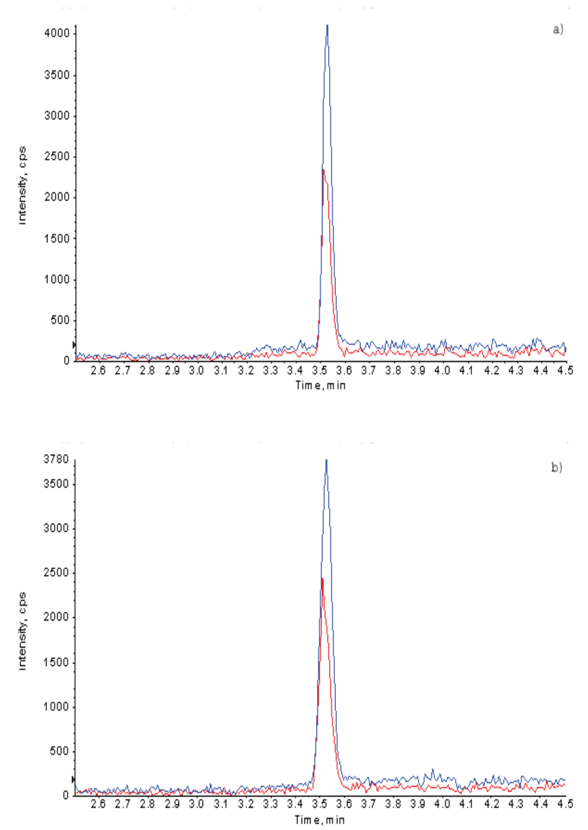

Fig. 1 Chromatograms obtained from analysis of samples spiked before extraction procedure (a) and after extraction procedure (b) at BPA concentration of 1 ng/mL. The blue line refers to the transition m/z 227.1→m/z 212.1 (quantifier), while the red line to m/z 227.1→m/z 133.2 (qualifier).

Ion suppression in the BPA responses was observed and a matrix effect was calculated in a range 83.4%-89.0% for peeled tomato samples and 75.2–80.7% for cherry tomato samples. In any case, the use of IS allows to correct the matrix effect. The within day precision was found within a 5.2-9.1% %RSD range. The values of within day trueness were between 98.5% and 101.3%. The between days precision (%RSD) was ≤ 9.2%. The values of between days trueness were in a range 96.5- 102.7%.
Method ruggedness for minor changes was tested by evaluating the performances of different analysts using different HPLC column lots. The % RSD was ≤ 7% and the percentage of nominal values ranged from 94.8% to 103.0%.

3.2 Determination of BPA concentration in canned tomato products

Table 1 describes the size and pH of the tomato products selected for the study. All canned tomatoes were produced in Italy and retailed in four supermarkets in Naples (Italy). Peeled and cherry tomatoes were in commercial size of 400 g, thus the surface directly in contact with the food was equal. For privacy the names of the manufacturers were not reported. The six brands were listed as 1-6, according to the order of BPA levels measured in each brand. In fact, by testing at room temperature (T=25°C) quadruplicate cans for each tomato product, BPA was detected in all the samples, with mean concentrations ranging from 0.31 ± 0.05 µg/Kg to 3.80 ± 0.10 µg/Kg in peeled tomatoes, from 0.90 ± 0.08 µg/Kg to 2.21 ± 0.11 µg/Kg in cherry tomatoes. No sample contained BPA exceeding the limit set by the EU Commission at 600 µg/Kg (Regulation 2004/19/CE). Furthermore, samples from different production lots were found to have nearly the same BPA content.
By comparing the two commodities of tomato products for each brand, the measured differences in BPA concentration are not relevant in comparison with the European Union limit and can be due to different ratios between the solid and aqueous portion.
In Table 1, it is also reported the average cost per kilogram for each product. This is a very important parameter to determine whether solely economic reasons may influence the choice of a product or whether the risk hazard is inversely proportional to the cost. The most expensive brand 1 has the lowest levels. For the other brands, no significant relationship was observed between the product's cost and the BPA content.

Table 1. Characteristics of the investigated canned tomato products

	Peeled Tomatoes				Cherry Tomatoes			
	Contents (g)	pH	Cost (€/Kg)	BPA (µg/Kg)	Contents (g)	pH	Cost (€/Kg)	BPA (µg/Kg)
Brand 1	400	4.20	2.25	0.31±0.08	400	4.26	2.50	0.87±0.09
Brand 2	400	4.40	1.25	0.65±0.12	400	4.32	1.45	1.05±0.11
Brand 3	400	4.30	1.20	1.45±0.18	400	4.30	1.15	1.36±0.22
Brand 4	400	4.36	1.10	2.72±0.27	400	4.28	1.80	1.62±0.15
Brand 5	400	4.30	1.23	3.44±0.48	400	4.26	1.00	1.91±0.34
Brand 6	400	4.30	1.25	3.77±0.35	400	4.28	1.70	2.21±0.32

4 Conclusions

This study confirmed that BPA migration from epoxy-coated cans into tomato products occurs. The migration of BPA in canned tomato (from 0.30 to 3.80 µg/Kg), stored at T=25°C, could be due to the differences in can coatings (type, amount, (type, amount, thickness, etc.) and to the sterilization conditions (temperature and duration) used by the different companies. The measured BPA resulted well below the present European Union legislation limit at 600 µg/Kg (Regulation 2004/19/CE). Thus, exposure to BPA through consumption of canned tomato is much lower than the Tolerable Daily Intake (TDI) for BPA at 0.05 mg/Kg body weight established by European Food Safety Authority (EFSA) (EFSA-Q-2005-100, 2006).

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