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ANALYSIS OF PHTHALIC ACID, BISPHENOL A AND BISPHENOL A DIMETHACRYLATE IN MEXICAN FOOD CANS BY HPLC WITH EVAPORATIVE LIGHT SCATTERING DETECTOR

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Bisphenol A (BPA), bisphenol A dimethacrylate (BisDMA) and phthalic acid (PA) endocrine disruptors can migrate from the plastic lining of cans to canned foods producing serious health problems when they exceed allowable concentration limits for consumption. In this work, a method was assessed for the determination of BPA, BisDMA and PA in vegetable food cans from Mexico using a food simulant. Those disruptors were determined by HPLC connected to an Evaporative Light Scattering Detector (ELSD), and simultaneous detection by UV-Vis detector was used for validation. The most frequently found disruptor in major concentration was PA over the range of 5.40 to 112.39 µg/L. The samples analyzed did not exceed the migration limit accepted by the US-FDA and US-EPA for bisphenols. Our results showed that HPLC-ELSD produces chromatograms with accurate signals and smaller detection limits than the UV-Vis detector for the substances analyzed here.

Keywords: Evaporative Light Scattering Detector, endocrine disruptors, xenostrogen migration, canned food.

Phthalic acid (PA) and bisphenol A (BPA), or its derivatives such as bisphenol A dimethacrylate (BisDMA), are used to strengthen the inner coatings of conserve food cans and in the manufacturing of different kinds of food containers. However, these substances are referred to as endocrine disruptors or xenoestrogens, since they have harsh effects on the reproductive and endocrine systems (MASUYAMA et al., 2000; HIROI et al., 2004; BONEFELD-JØRGENSEN et al., 2007; HALDEN, 2010; CHOI et al., 2012). Migration of substances with endocrine disruptor activity from the cans or plastic containers to the foods

at high temperatures has previously been reported (BROTONS et al., 1995; KANG et al., 2003). Therefore, it is important to analyze and monitor the content of those substances in the canned food. HPLC coupled with UV-Vis or fluorescence detectors is the technology most widely used for this purpose (PASEIRO et al., 1991; UEMATSU et al., 2003). The Evaporative Light Scattering Detector (ELSD) is a semiuniversal detector regarded as a valuable alternative instead of UV detection for liquid chromatographic analysis of substances that do not contain a chromophore. Another advantage is that the derivation steps are avoided (PETRITIS et al., 2002; VERVOOT et al., 2008; ALMELING & HOLZGRABE, 2010).

We describe a reversed-phase HPLC method with ELSD for the measurement of PA, BPA and BisDMA in vegetable cans from the Mexican market. To our knowledge, this method is innovative, since its use has not been reported previously for the analysis of both bisphenol and phtalate endocrine disruptors.

1. Materials and methods

1.1. Materials

1.1.1 Chemicals. All chemicals were purchased from Sigma-Aldrich. Bisphenol A (BPA), bisphenol A dimethacrylate (BisDMA), phthalic acid (PA) and chloroform had a purity above 99%. Methanol and acetonitrile were HPLC grade with a purity of 99.9%.

1.1.2. Samples. The analyzed samples were 10 cans of vegetables (2 sweet corn kernels, 2 jalapeno peppers, 1 mushroom, 3 peas and, 2 peas and carrots) bought in supermarkets in San Luis Potosi City, Mexico.

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72 *1.2. Methods*

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74 *1.2.1 Extraction.* The determination of bisphenols and phthalates in food cans was based on
75 methodology reported by BROTONS et al. (1995), using water as food simulating liquid as
76 follow: 300 mL of water (Milli-Q) were added to each sample and sterilized at 121°C for
77 15 min. An aliquot of 75 mL was taken and 20 mL of methanol were added. It was
78 vigorously agitated for 10 min in a decantation funnel, and then, 40 mL of chloroform were
79 added. Afterwards, it was agitated once again for 10 min and centrifuged at 1200 *g* for 15
80 min. The organic phase was taken and concentrated in a rotary evaporator at 40°C and
81 pressure was reduced to a final volume of 10 mL. The solvent was eliminated using a
82 nitrogen stream to a final volume of 1 mL. Then 0.5 mL of sulphuric acid was added and
83 centrifuged at 1200 *g* for 10 min, to eliminate organic remains. The organic phase was
84 aspired and dried through a stream of gaseous nitrogen. The dry remains were solubilized
85 in 1 mL of methanol and analyzed by HPLC as described below. Methods for pre-
86 concentrate or cleanup phtalate disruptors, such as solid-phase extraction or molecularly
87 imprinted polymer, (QI et al., 20011), were not necessary because the food simulating
88 liquid was not too complex.

89 *1.2.2 Chromatography*. The Waters 600 HPLC system consisted of a gradient
90 pump, degasser, column oven (at room temperature), UV-Vis 2487 detector (Waters), and
91 evaporative light scattering detector (ELSD; Eurosep instruments, France). The detectors
92 were connected in series for simultaneous detection of UV (absorption wavelength-270 nm)
93 and ELSD (at optimal temperature of nebulization and evaporation, as described below).

Samples were separated on a C-18 column (150 mm x 4.6 mm, 5µm) from Merck (Darmstadt, Germany). The solvents were acetonitrile at 50% in phase A and acetonitrile at 100% in phase B, at 1 mL/min of flow rate with a gradient 0-100% of B in 20 min, and a temperature of 25°C. All eluents were filtered through 0.45 µm filters before use. The injected sample volume was 20 µL. For the ELSD, optimal operation conditions were obtained by injecting 20 µL of BFA standard (0.228 µg/mL) and changing the temperatures of nebulization and evaporation in the range from 20 to 25ºC and 40 to 60ºC, respectively.

1.2.3. Calibration and validation. Stock solutions of BPA ($2.3x10^4$ µg/L), BisDMA ($3.6x10^4$ µg/L), and PA ($1.6x10^4$ µg/L) standards were prepared with methanol, aliquoted, and stored at -20ºC. The identification of analytes was based on the retention time of standards. To determine the reproducibility of method, 20 µL of the endocrine disruptor standards were injected ten times and retention times were determined. The arithmetic means of the obtained data were calculated. The accuracy and precision of method were determined by spike recovery experiments. For this, known concentrations of 60 µg/L, 100 µg/L and 6 µg/L of PA, BPA and BisDMA standard, respectively, were added to a sample with a known area in triplicate. Three calibration curves from endocrine disruptors stock solutions with five concentrations for each standard in triplicate were performed. For BPA the concentration range of 0.22 to 228 µg/L was covered. For BisDMA the concentrations were from 0.36 up to 18.22 µg/L; and for PA, the concentrations were from 1.66 up to 160 µg/L, to demonstrate the linearity of our method. The peak area/amount ratios of the analytes, were constructed from integrated chromatograms. The limit of detection (LOD) was determined by a signal-to-noise ratio of 3:1 and the limit of quantification (LOQ) was determined by a signal-to-noise ratio of 10:1, using the following equations X_B+3SD=LOD and X_B+10SD=LOQ, respectively, where X_B is the average of blank response value, and

SD, the standard deviation.

2. Results and discussion

Among the most important factors for the optimization of ELSD parameters are the nebulizer and evaporation temperature (ALMELING & HOLZGRABE, 2012). The effect of the nebulization and evaporation temperatures on the response of HPLC-ELSD to the injection of the BPA standard is shown in the Fig. 1. A maximum response was attained at a nebulization temperature of 23ºC (Fig. 1A), and the optimal temperature of evaporation was 45ºC (Fig. 1B). These values were used for subsequent analysis.

PA, BPA, and BisDMA were detected simultaneously using ELSD and UV-Vis detectors. The method provided excellent separation of the substances analyzed. Fig. 2 shows the separation of analytes in a vegetable sample. It can be observed that ELSD produced stable baselines during gradient elution chromatography. Typical tests such as accuracy, precision, reproducibility, the linearity and the limits of detection and quantification have been developed to demonstrate the validation of HPLC methods. An recovery area for PA of 85% was achieved; for BPA 90% was recovered; and for BisDMA 80% was recovered, thus demonstrating the accuracy and precision of method. The reproducibility of the chromatographic method was performed with ten injections of 20µL each from stock solutions of PA, BPA and BisDMA standard. The peaks matched to respective retention time for each endocrine disruptor and no interferences in the peaks were observed Also the areas corresponding to each standard showed minimal variation. The linearity of the detector was determined by means of the production of straight lines from the analysis of

five increasing concentrations of the analytes (THOMPSON et al., 2002). Table 1 and 2 show the retention times, detection and quantification limits for the ELSD and UV-Vis detector, respectively. The limits of detection and quantification were determined by means of the signal-to-noise method (MACDOUGALL & CRUMMETT, 1980; LONG & WINEFORDNER, 1983). For the analyzed substances the detection limit ranged from 0.009 to 0.18 µg/L for the ELSD, whereas for the UV-Vis detector, the limits were from 0.03 to 10 µg/L. Using the ELSD, the limit of quantification was 0.24 µg/L for PA, 0.04 µg/L for BPA and 0.02 µg/L for BisDMA. The calibration curves are shown in Table 3, and the coefficient of regression (R^2) for each analyte was >0.994, indicating a good fit for the ELSD detector used.

Our results showed that ELSD produces chromatograms with accurate signal and with smaller detection limits than the UV-Vis detector for the substances analyzed here.

The analysis of xenoestrogens in the samples of vegetable cans is shown in Table 4. PA was the most frequently found compound, and its concentration ranged from 5.40 to 112.39 µg/L for samples of jalapeno peppers and mushrooms, respectively. BPA at 83.14 µg/L was found in only one sample of jalapeno peppers, whereas BisDMA was found in two samples of peas and sweet corn kernel, and the concentrations detected were 5.60 and 9.60 µg/L, respectively. The concentration of bisphenols found in food cans is still within the safe limits of 50µg/kg/day according to the US-FDA and US-EPA (VOM SAAL & WHELSHONS, 2006). MUNGUIA et al., (2002) found 5.59 ± 2.43 µg/L of BPA in jalapeno pepper cans. KANG et al., 2003 found BPA up to 5 µg/L in food cans. BPA concentrations ranging from 29.9 to 80 µg/L were found in samples of canned vegetables from Brazil, France, Spain, Turkey, and the USA (BROTONS et al., 1995). The migration

of bisphenol derivatives obtained in our work, is similar to that migration found in food cans from Japan (SAJIKI et al., 2007; YONEKUBO et al., 2008).

3. Conclusion

The presence of bisphenols and phthalates in canned food simulant indicates that the concentration found could be hazardous, considering that concentrations of very low levels of these contaminants can accumulate in adipose tissue and later affect the endocrine system and metabolism (ELOBEID & ALLISON, 2008; BEN-JONATHAN et al., 2009). Our results showed that HPLC-ELSD produces chromatograms with accurate signals and smaller detection limits than the UV-Vis detector for the substances analyzed here.

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Figure captions

Fig. 1. Setup of the optimal ELSD parameters of A) nebulization and B) evaporation temperatures using a BPA standard.

Fig. 2. Typical chromatogram of a jalapeno pepper sample in a conserve food can using the optimal ELSD parameters (upper) and UV-Vis detector (lower). The peak corresponds to phthalic acid.