QUANTIFICATION OF BISPHENOL RELATED COMPOUNDS IN CANNED FOOD BY HPLC-FLD

Antía Lestido Cardama*, Ana Rodríguez Bernaldo de Quirós*, Juana Bustos**, Mª Isabel Santillana**, Perfecto Paseiro Losada*, Raquel Sendón*

*Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy. University of Santiago de Compostela, Spain

** National Food Center, Spanish Agency for Food Safety and Nutrition, Majadahonda, Spain

INTRODUCTION

Polymeric coatings are commonly used in metal food cans to protect food from corrosion. Major types of interior can coatings are made from synthetic polymers known as epoxy-based resins. These resins usually contain among their components bisphenol related compounds such as bisphenol A (BPA) or bisphenol A diglycidyl ether (BADGE), which can be released as well as oligomers and/or derivatives (hydrolyzed or chlorinated) and reach the food. Although, there is no specific European legislation for can coatings, there are specific migration limits (SML) for some substances that are known to migrate.

It is important to develop analytical tools for the identification of these potential migrants into the food with the ultimate objective of ensure the consumer safety. For this, a multi-target method based on liquid chromatography with fluorescence detection (HPLC-FLD) was applied to quantify thirteen bisphenol related compounds in canned food. In addition, a liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method was optimized for confirmation purposes.

A total of twelve food cans that cover several types of food including fish (tuna, sardines), seafood (clams, mussels), vegetables (corn, olives, asparagus, tomato) and fruit (peach) were taken as study samples.

EXPERIMENTAL

Code	Type of sample	Fat content	pH
ES	White asparagus buds	0.1g/100g (Satur.: 0.0g)	5.0
TO1	Fried tomato	3.3g/100g (Satur.: 0.4g)	4.0
TO2	Fried tomato (home style)	7.0g/100g (Satur.: 0.9g)	4.0
AH	Chamomile olive with bone	18g/100g (Satur.: 3.3g)	3.7
AL	Natural clams	2.7g/100g (Satur.: 0.9g)	6.2
AA	Light tuna in extra virgin olive oil	33g/100g (Satur.: 4.8g)	5.5
ME	Pickled mussels	6.2g/100g (Satur.: 1.6g)	4.7
SR	Sardines in olive oil	28g/100g (Satur.: 4.2g)	5.9
AN	Natural light tuna	1.4g/100g (Satur.: 0.3g)	5.7
AR	Olives stuffed with anchovy pasta	16.9g/100g (Satur.: 3.0g)	4.1
MA	Peaches in syrup	0g/100g (Satur.: 0.0g)	3.8
MZ	Naturally sweet corn, without salt	1.7g/100g (Satur.: 0.4g)	6.5

Table 1: Information about the samples included in the study.

EXTRACTION PROCEDURE

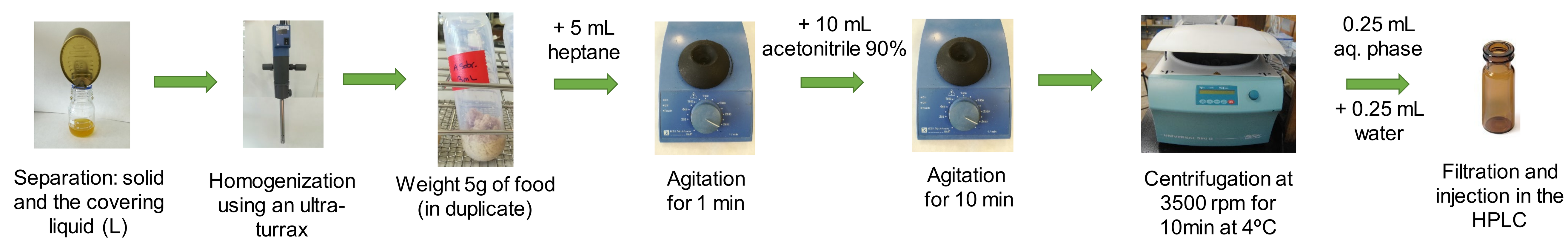

Figure 1: HPLC Agilent Technologies.

Column	Phenosphere 80A ODS (150 mm × 3.2 mm, 3 µm)
Column Tª	30ºC
Mobile phase	MeOH: ACN (50:50, v/v) and water
Flow rate	0.5 mL/min
Injection volume	10 µL
Gradiente elution	55% water and 45% MeOH:ACN for 2 min, MeOH:ACN was increased up to 75% in14 min, then up to 100% MeOH:ACN in 7 min
Fluorescence detection	Excitation: 225 nm Emission: 305 nm
MS Data acquisition	Selected reaction monitoring (SRM)
Source	Positive and negative atmospheric pressure chemical ionisation (APCI)
Vaporizer Tª	400ºC
Capillary Tª	350ºC

Table 2: Experimental condition of HPLC-FLD and LC-MS/MS methods.

Figure 2: LC-MS/MS Thermo Scientific.

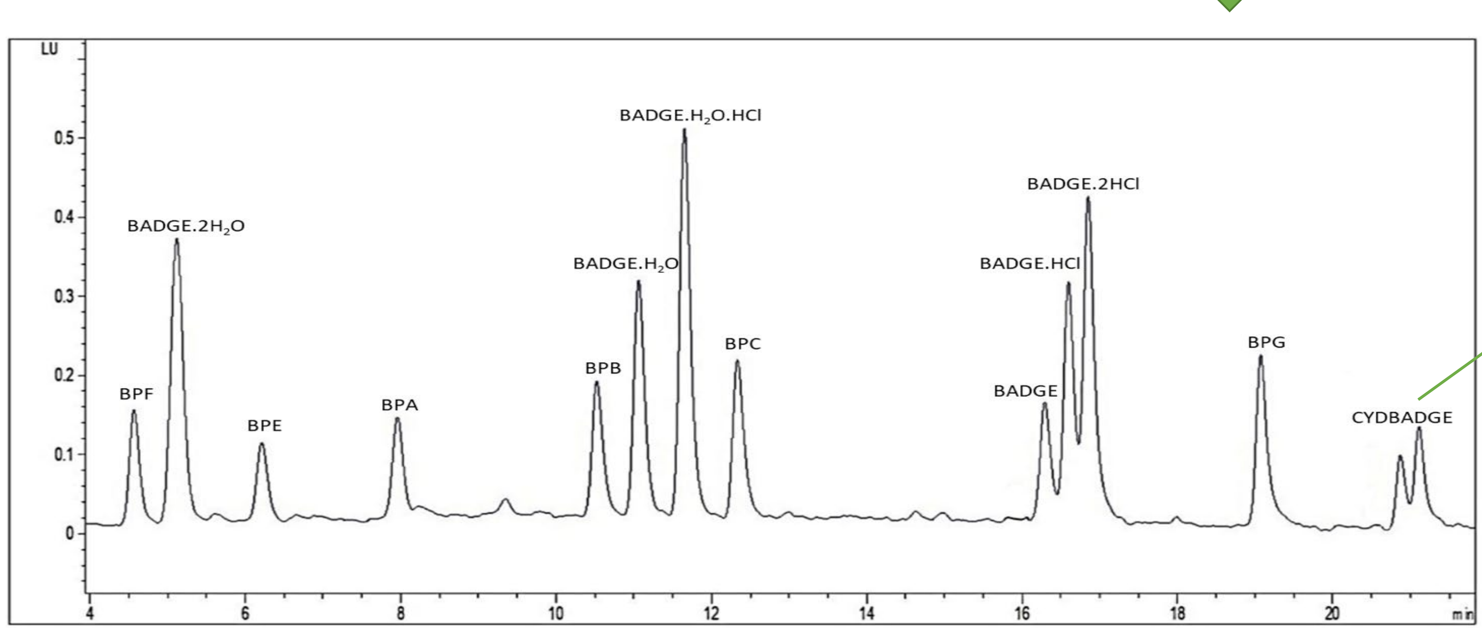

Figure 3: A HPLC-FLD chromatogram corresponding to a mix solution

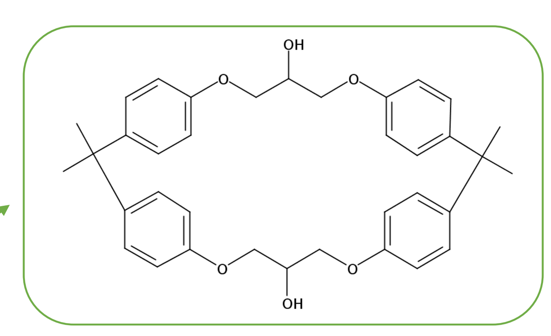

Figure 4: Chemical structure of cyclo-di-BADGE.

Compound	Formula	CAS N°	Molecular Weight (g/mol)	APCI mode	Parent ion	Product ions
BPA	$C_{15}H_{16}O_2$	80-05-7	228.29	-	226.9	133.0, 211.8
BPB	$C_{16}H_{18}O_2$	77-40-7	242.31	-	240.9	210.7, 211.8
BPC	$C_{17}H_{20}O_2$	79-97-0	256.34	-	254.9	146.9, 239.8
BPE	$C_{14}H_{14}O_2$	2081-08-5	214.26	-	212.9	196.8, 197.8
BPF	$C_{13}H_{12}O_2$	620-92-8	200.23	-	198.9	93.0, 105.0
BPG	$C_{21}H_{28}O_2$	127-54-8	312.45	-	311.0	174.9, 294.9
BADGE	$C_{21}H_{24}O_4$	1675-54-3	340.41	+	381.9	134.9, 190.8
BADGE.H_2O	$C_{21}H_{26}O_5$	76002-91-0	358.43	+	399.9	106.9, 134.8
BADGE.2H_2O	$C_{21}H_{28}O_6$	5581-32-8	376.44	-	374.8	226.8, 300.6
BADGE.HCl	$C_{21}H_{25}ClO_4$	13836-48-1	376.87	+	417.9	106.9, 134.9
BADGE.2HCl	$C_{21}H_{26}C_{l2}O_4$	4809-35-2	413.33	+	382.2	191.1, 135.2
BADGE.H_2O.HCl	$C_{21}H_{27}ClO_5$	227947-06-0	394.89	-	283.0	211.0, 226.0
CYDBADGE	$C_{36}H_{40}O_6$	20583-87-3	568.71	+	569.0	134.8, 106.9

Table 3: Compounds analyzed in this work with their MS/MS conditions.

RESULTS AND DISCUSSION

The HPLC-FLD method developed to determine the migrants in the food samples was validated showing an adequate linearity ($R^2 \geq 0.9994$), low detection levels (LOD = 0.005 mg/L), good repeatability (RSD % < 12) and acceptable recoveries (>70 %) determined by spiking experiments on food samples at three concentrations (0.05, 0.1 and 0.2 µg/g) by duplicated during three consecutive days (n=6).

Among the target compounds, BPA, BADGE.2H_2O, BADGE.H_2O.HCl and CYDBADGE were detected, while no presence of BADGE, BADGE.H_2O, BADGE.HCl, BADGE.2HCl and other bisphenol analogues were detected in any of the analyzed samples.

BPA was detected in a concentration range that goes from 0.066 to 0.202 µg/g in the sample of mussels (ME). Recently, a specific migration limit of 0.05 mg/kg of food from varnishes or coatings was established for BPA. CYDBADGE was other of the most detected compounds and the highest concentration was found in the sample of mussel, both solid food (ME) and pickled sauce (MEL), with values of 1.43 and 10.77 µg/g respectively. These results were confirmed by LC-MS/MS.

There was no significant relationship between the concentrations of contaminants and the type of food, the pH values or the content of fat.

With this concentration data and consumer data, the next step in this research would be the estimation of the exposure to these analytes through diet.

Compound	Range of linearity (mg/L)	Equation	R^2	Repeatability (RSD %)
BPF	0.0125-0.25	y=51.5x+0.0238	0.9994	6
BADGE.2H_2O	0.0125-0.25	y=131.73x+0.1333	0.9998	6
BPE	0.0125-0.25	y=41.553x+0.0121	0.9998	5
BPA	0.0125-0.25	y=53.627x+0.0917	0.9998	4
BPB	0.0125-0.25	y=73.127x+0.1154	0.9999	5
BADGE.H_2O	0.0125-0.25	y=138.9x+0.0863	0.9999	5
BADGE.H_2O.HCl	0.0125-0.25	y=134.5x-0.0687	0.9999	5
BPC	0.0125-0.25	y=91.033x-0.0054	0.9998	6
BADGE	0.0125-0.25	y=163.1x+0.1088	0.9999	2
BADGE.HCl	0.0125-0.25	y=119.9x+0.0887	0.9998	4
BADGE.2HCl	0.0125-0.25	y=129.83x-0.0604	0.9999	3
BPG	0.0125-0.25	y=111.03x-0.0754	0.9999	5
CYDBADGE	0.0125-0.25	y=85.333x+0.6533	0.9997	10

Table 4: Validation results by HPLC-FLD.

Compound	Concentration in the samples (µg/g)				
	AL	AN	ME	MEL	SRL
BADGE.2H_2O	0.607	0.513	0.724		
BPA	0.121	0.066	0.202	0.131	
BADGE.H_2O.HCl			0.189		
CYDBADGE	0.578	0.342	1.43	10.77	0.134

Table 4: Concentrations obtained in the positive samples.

Acknowledgement

The study was financially supported by the Ministerio de Ciencia, Innovación y Universidades, by Fondo Europeo de Desarrollo Regional (FEDER), and by Agencia Estatal de Investigación Ref.No. PGC2018-094518-B-I00 "MIGRACOATING" (MINECO/FEDER, UE). Antía Lestido is grateful for her grant "Programa de axudas á etapa predoutoral" da Xunta de Galicia (Consellería de Cultura, Educación e Ordenación Universitaria).