Istituto Zooprofilattico Sperimentale Umbria Marche

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XXII International Mass Spectrometry Conference

August 26 - 31, 2018 – Florence, Italy

IMSC FLORENCE 2018

LC-MS/MS versus GC-MS/MS for Hexabromocyclododecanes (HBCDs) analysis in food

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INTRODUCTION

HBCDs are used as additives in plastic polymers to reduce flammability; they can be released in the environment and accumulate in food chain. Technical mixture contain mainly diastereomers α, β, and γ [1]. Both gas-chromatography (GC) and liquid chromatography (LC) are used for HBCDs analysis, but GC does not resolve the diastereomers. The aim of this study was to set up the best analytical method for HBCDs in food comparing GC-MS/MS and LC-MS/MS.

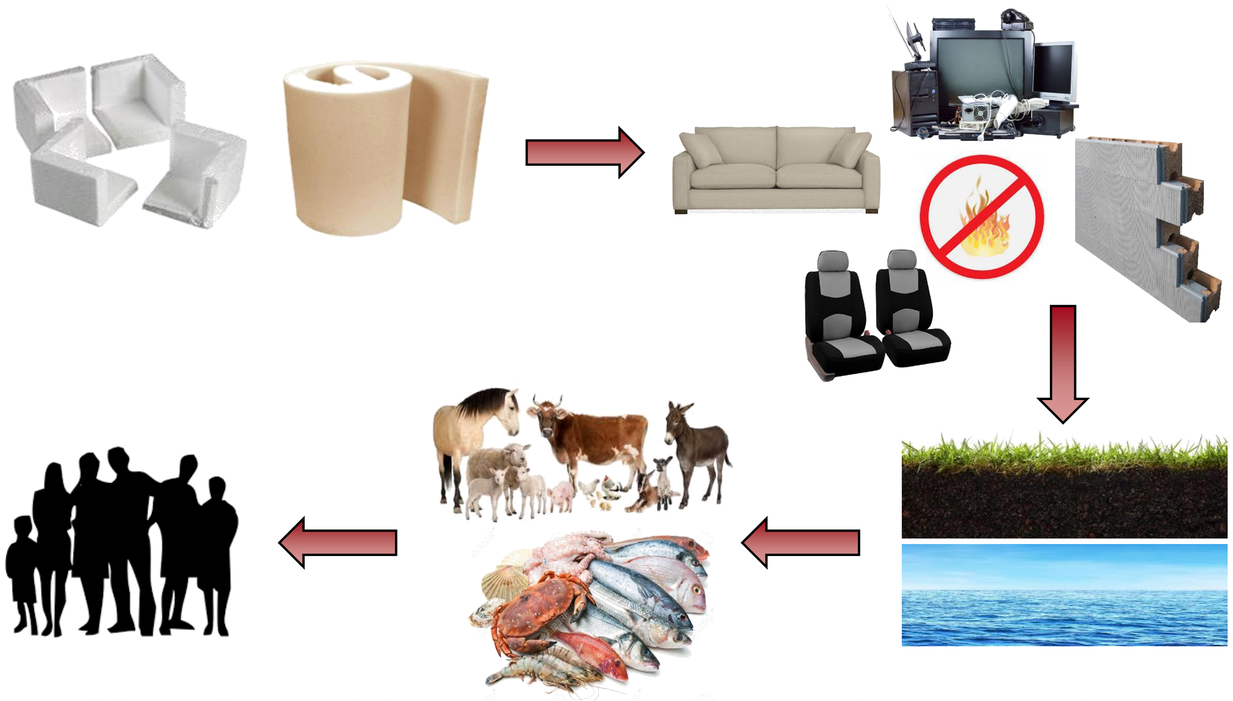

Figure 1. *HBCDs are additives not chemically bounded to the polymers, therefore they can easily leach out into the environment during production, use and disposal of the products. Because of their persistence, they can be found in the different environmental compartments, such air, sediment and water and accumulate in food and biological matrices.*

METHODS

α, β, and γ-HBCD were analyzed in isotopic dilution after QuEChERS-like extraction followed by acidic Extrelut-NT3®/Si-SPE and GPC clean up. Instrumental analysis was performed in GC-MS/MS on a RTX-1614 (15m x 250µm x 0.10µm) column and PTV injection (10 µL) [2]. A LC-MS/MS method was also developed, using a Kinetex XB-C18-100Å column (2.6 µm, 100X2.10mm) and MeOH (phase A) and 2 mM CH_3COONH_4/MeOH (50:50) (phase B) as mobile phase [3]. The method was validated in muscle at 10, 20, 50 and 100 pg/g in intra-laboratory reproducibility conditions.

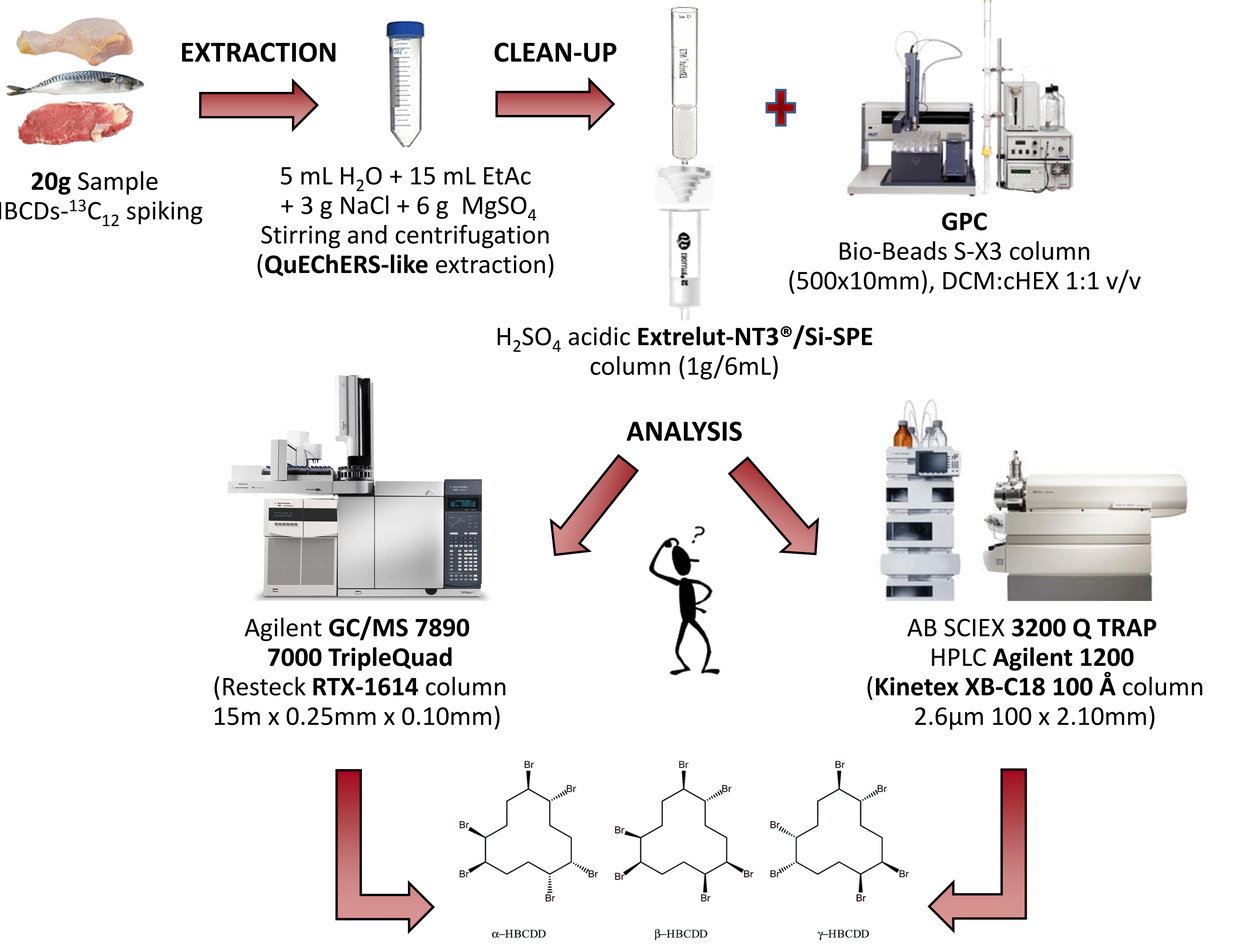

Figure 2. *Analytical methods flow diagram: which is the best technique for HBCDs determination?*

RESULTS

Literature reports that GC does not enable the resolution of α, β, and γ isomers: HBCDs can be quantified only as sum. Furthermore, the development of the GC method is extremely tricky because the 3 isomers inter-convers at T>160 °C and decompose at T> 240 °C or in dirty GC systems [4]. The performances of the GC-MS/MS method were not satisfactory, because of the impossibility to separate the three isomers yielding in a not completely accurate quantification. During method set up, LC-MS/MS turned out to be the method of choice for HBCDs analysis, because of the absence of thermal degradation and of its resolution. The analytical performances were systematically studied in LC-MS/MS. Accuracies and precision, in intra-laboratory reproducibility conditions, were satisfactory for all the isomers: apparent recoveries were between 92 and 107% and precisions between 9 and 22%. Recoveries of the surrogate labelled standards were higher than 70%. No laboratory background contamination was detected.

GC-MS/MS

METHOD

GC PARAMETER			
INJECTION MODE		**COLUMN**	
Mode	Solvent vent PTV	**Type**	Agilent RTX-1614 (15 m x 0,25 mm x 0,1 µm)
Injection Program	50 °C (0.5 min) 600 °C/min to 325 °C	**Flow**	1 ml/min
Vent time	0,5 min	**Oven Program**	50 °C
Flow	50 mL/min		30 °C/min to 140 °C (1 min)
Pressure	5 psi		20 °C/min to 285 °C
Purge flow to split vent	50 mL/min at 2 min		40 °C/min to 325 °C (1 min)
Injection Volume	10 µL		

MS PARAMETER										
Ion Source			**Quadrupole**				**Interface**			
230 °C			150 °C				320 °C			
Time segment	**Scan Type**	**Delta EMV (V)**	**Compound**	**Transition**	**Precursor ion (m/z)**	**MS1 res**	**Product ion (m/z)**	**MS2 res**	**Dwell**	**CE (V)**
1	MRM	600	α-, β-, γ-HBCDD	q	560.8	Widest	157.2	Wide	60	25
				tg	318.9		157			10
				q	239		159			6

Table 1. *GC-MS/MS method.*

CHROMATOGRAM

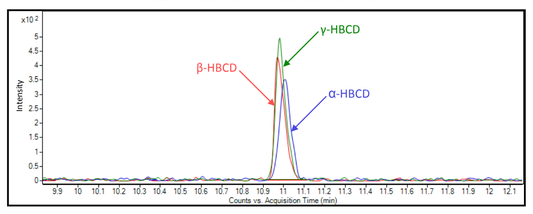

Figure 3. *α-, β- and γ-HBCD at 500 pg/mL each (TIC mode). The isomers overlaps completely.*

NO ISOMERS SEPARATION
ONLY HBCDs SUM QUANTIFICATION

LC-MS/MS

METHOD

HPLC Agilent 1200			
Column	Kinetex XB-C18 2.6 µm 100Å da 100 x 2.10 mm		
Flow rate	0,2 mL/min		
Injection volume	20 µL		
Column temperature	25°C		
	Time (min)	**Mobile phase A (%)**	**Mobile phase B (%)**
Gradient	0	50	50
	0,5	50	50
	3,5	100	0
	8,5	100	0
	11	65	35
	16,5	65	35
	17,5	50	50
	30	50	50

Applied Biosystem 3200 QTRAP TripleQuad									
Source temperature	500°C								
Curtain Gas (CUR)	20 psi								
Collision Gas (CAD)	High								
Ion Source GAS 1 (GS1)	40 psi								
Ion Source GAS 2 (GS2)	20 psi								
Ion Spray Voltage (IS)	-4500 V								
Analyte		**Precursor ion**	**Product ion**	**Dwell Time**	**DP (V)**	**EP (V)**	**CE (V)**	**CEP (V)**	**CXP (V)**
α-, β-, γ-HBCD	tg	640,7	79,0	200	-35	-5	-47	-20	-8
	q	640,7	81,0						
α-, β-, γ-HBCD-$^{13}C_{12}$	tg	652,7	79,0	100					
	q	652,7	81,0						

Table 2. *LC-MS/MS method.*

CHROMATOGRAM

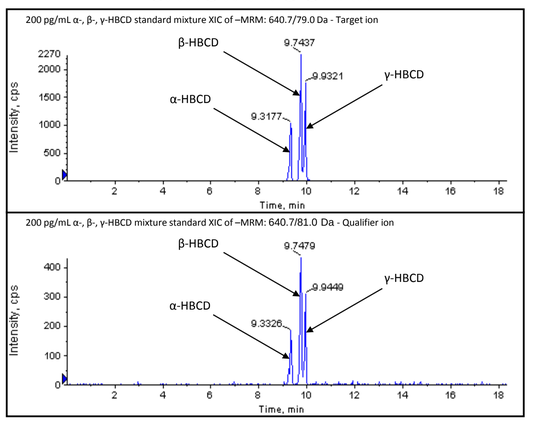

Figure 4. *α-, β- and γ-HBCD standard mix at 200 pg/mL. The three isomers are completely resolved.*

α, β, γ-HBCDs SEPARATION

VALIDATION RESULTS

	Mean (pg/g)	n	sr (pg/g)	RSD$_r$ (%)	sR (pg/g)	RSD$_R$ (%)	Repeatability (pg/g)	Apparent R% mean ± ds	Internal standard R% HBCD -13C12 mean ± ds
10 pg/g LEVEL									
α-	9,9	14	1,8	18	2,2	22	5,5	92 ± 22	α- 91 ± 16
β-	9,8	14	1,7	17	1,6	16	5,1	98 ± 16	
γ-	9,7	14	1,1	11	1,3	13	3,4	96 ± 10	γ- 76 ± 13
20 pg/g LEVEL									
α-	19	13	2,2	11	2,3	11	6,7	93 ± 11	α- 92 ± 19
β-	21	13	2,5	12	2,2	11	7,7	105 ± 11	
γ-	20	13	2,7	13	2,6	13	8,3	101 ± 12	γ- 72 ± 13
50 pg/g LEVEL									
α-	51	13	4,8	10	6,5	13	15	100 ± 11	α- 93 ± 12
β-	52	13	3,4	7	5,7	11	11	103 ± 9	
γ-	50	13	4,2	8	5,2	10	13	99 ± 9	γ- 77 ± 9
100 pg/g LEVEL									
α-	102	13	7,4	7	9,1	9	23	103 ± 9	α- 97 ± 17
β-	106	13	8,1	8	12,4	12	25	107 ± 13	
γ-	101	13	6,6	7	9,5	9	20	101 ± 10	γ- 77 ± 12

Table 4. *Mean, replicates (n), repeatability and reproducibility standard deviation (s_r and s_R), repeatability and reproducibility relative standard deviation (RSD$_r$ and RSD$_R$), recovery (R%) of native HBCD and labelled analogues.*

VALIDATION PLAN

HBCDs level (pg /g)	SD conc. (ng/mL)	Added volume (µL)	C (ng /g) HBCDs-$^{13}C_{12}$	N. of samples	N. total samples	N. procedural blank
10	10	20	0.5	14	53	8
20	10	40		13		
50	10	100		13		
100	10	200		13		

Table 3. *Analytical method validation plan.*

CONCLUSIONS

The results confirmed LC-MS/MS as the method of choice for HBCDs analysis enabling to reach good performances also at 10 pg/g, which was set as method LOQ. The procedure performs well in fish products and lean terrestrial muscles. The applicability to other food matrices should be further tested.

NOVEL ASPECT

Few data are available in Italy about HBCDs in food, therefore this method enables an extensive monitoring at very low contamination levels.

Acknowledgements

Work funded by the Italian Ministry of Health (project code IZSUM RC0052015)

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