IMPIEGO DELLA SPETTROMETRIA DI MASSA NEL FOOD CONTACT MATERIALS

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Food contact materials

Equipment during food farming and processing (temperature/time)

Storage/Packaging

- Employement of Additives in polymers: antioxidants, UV absorbers, processing stabilizer (0.1-1%)
- Possible degradation at light/high temperature
- Non-intentionally added substances (NIAS)
- Possible migration of substances (known/unknown)

Re-usable plastic containers: AGEING and Mechanical Damage

Temperature

Ultraviolet and visible light

Chemical ageing

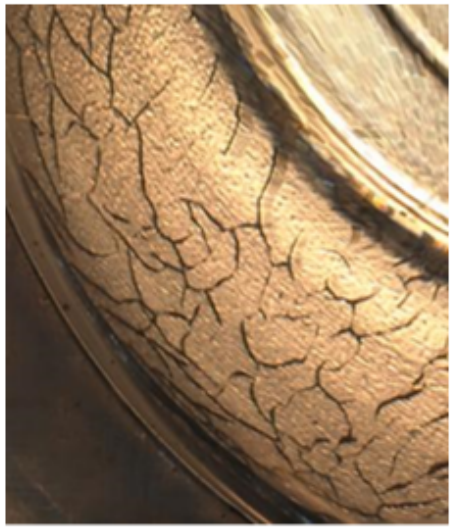
Physical ageing

Humidity

Atmospheric components

Contact with liquids (MeOH, amine groups)

Mechanical damage



POLYCARBONATE

- Formation of tiny scratches or small cracks
- Surface becomes opaque and foggy
- Formation of microcavities

→ Release of polymer components?

Most important regulations governing the plastic materials intented for contact with food

European Union

1935/2004/CE
2023/2006/CE
10/2011/UE

PIM
(Plastic Implementation Measure)

1.

Exclusive use of the substances listed:

- Polymerisation production aids
- Monomers
- Additives (no colorants)

COLORANTS
Decree 1973
NO MIGRATION

2.

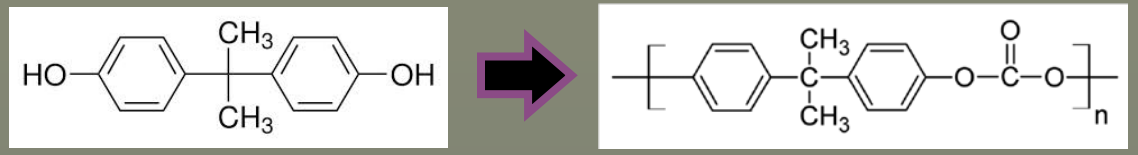
Overall Migration Limit (OML)
Specific Migration Limit (SML)

3.

Declaration of compliance

Bisphenol A-polycarbonate

Bisphenol A (BPA) is used as monomer in the fabrication of polycarbonate



BPA: well-known endocrine disrupter interfering with hormone signalling

BPA
Specific Migration Limit
0.6 mg/Kg

The European Commission (EC) published a new Directive 2011/8/EU to restrict **Bisphenol A** in feeding bottles that are intended for use by infants under the age of 12 months.

Recently, the French parliament has banned the use of polycarbonate in any food containers starting from January 2014

Oca M.L. et al., Talanta, 2013, *106*, 266-280.

EVALUATE POSSIBLE RELEASE FROM POLYCARBONATE:

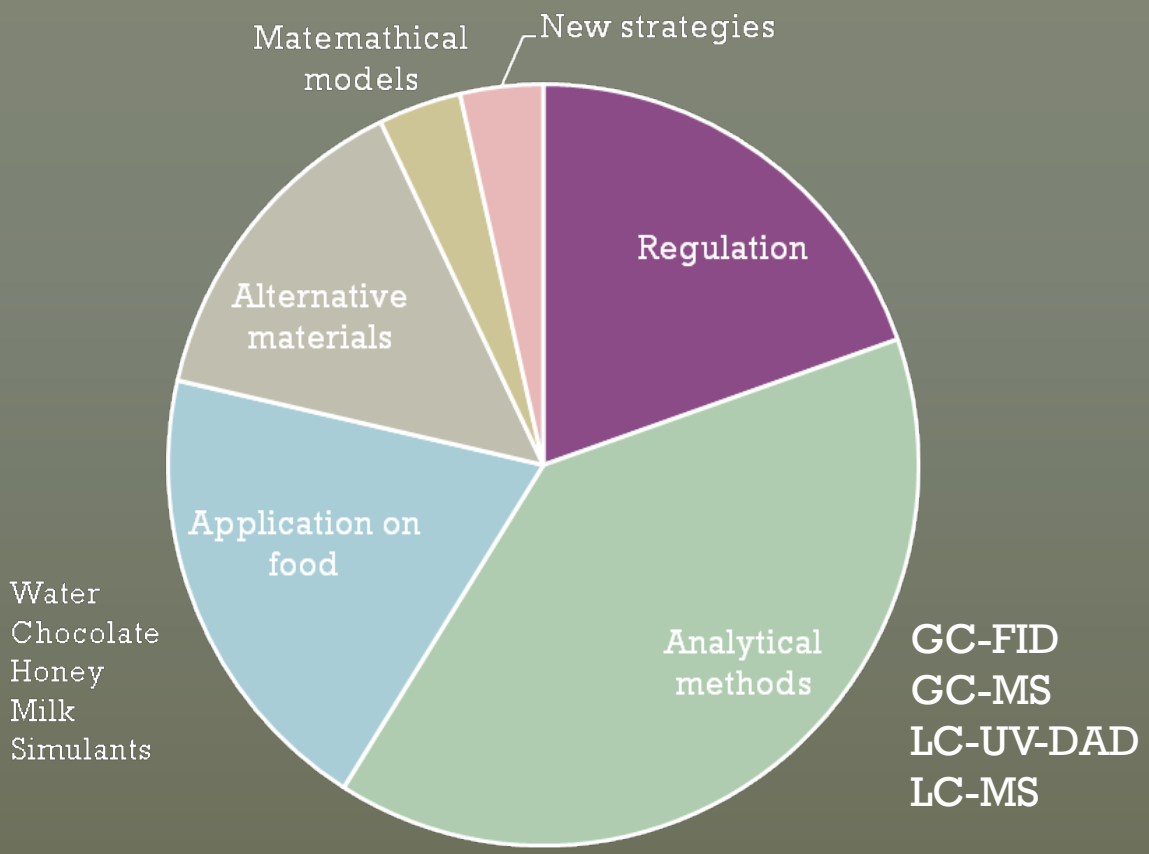
- **BISPHENOL A**
- **ADDITIVES**
- **Non Intentionally Added Substances (NIAS)**

- **INFLUENCE OF AGEING/DAMAGE ON MIGRATION??**

Migration of substances to food products

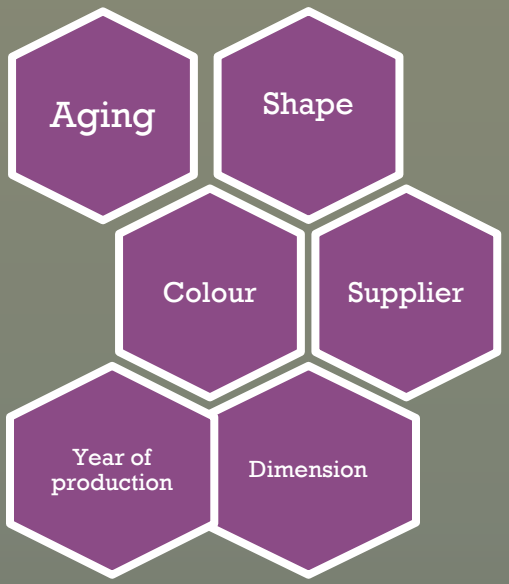
The development of suitable analytical methods to determine low concentrations of substances in foodstuffs and simulants appears essential

Literature overview



MATERIAL AND METHODS-Samples

Samples:polycarbonate



Information on product and repeated use:

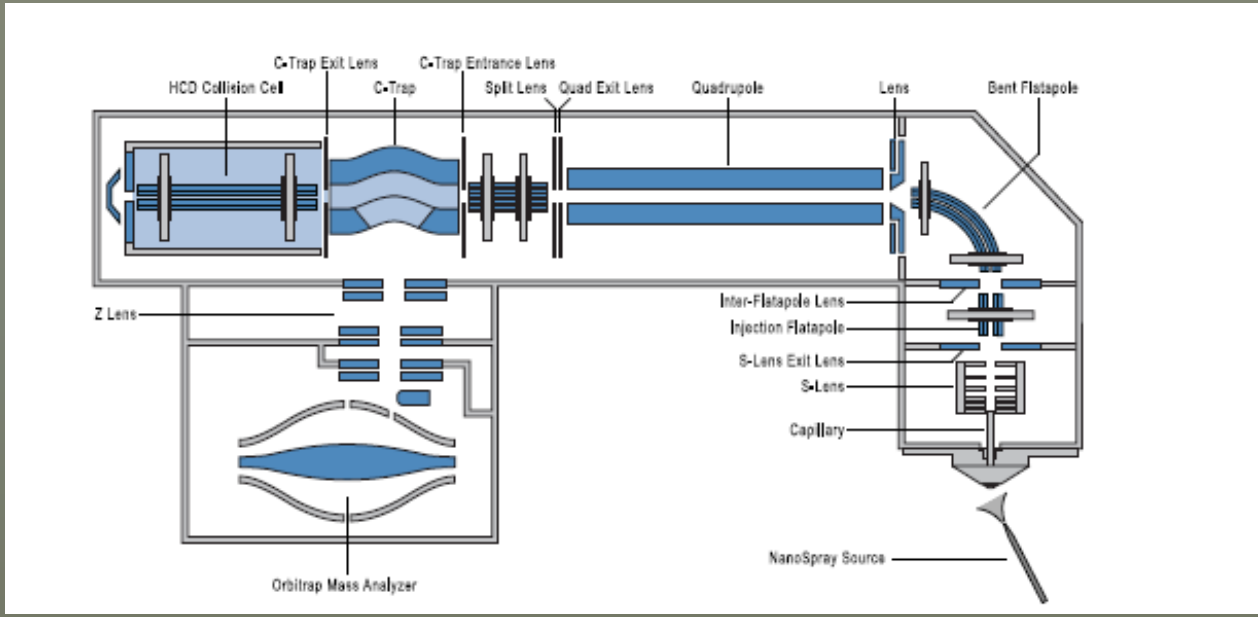
- Temperature (cycles)
- Time of contact
- Eventual mechanical stress
- Washing steps/temperature
- Detergents

Search for: additives (and dyes)
Monomers eventually released
Other unknown substances (NIAS)

Benchtop Orbitrap mass spectrometer: Q-Exactive

The quadrupole-orbitrap (Thermo Scientific™ Q Exactive™ MS) hybrid instrument was first introduced in 2011 and only few works have been reported in the field of proteomics and drug testing.

Presence of a mass selective quadrupole analyzer between the ion source and the C-trap



Construction details of the Q-Exactive

Combination of a quadrupole mass filter with an Orbitrap analyzer: fast switching times of quadrupole and high-resolving *m/z* analysis at ppm-accuracy

-Michalsky A. et al., Molecular and Cellular Proteomics 2011, *50*, 1-10.
-Kumar P. et al., Analytica Chimica Acta, 2013, 65-73.

MATERIALS AND METHODS-Analytical conditions

UHPLC analytical conditions

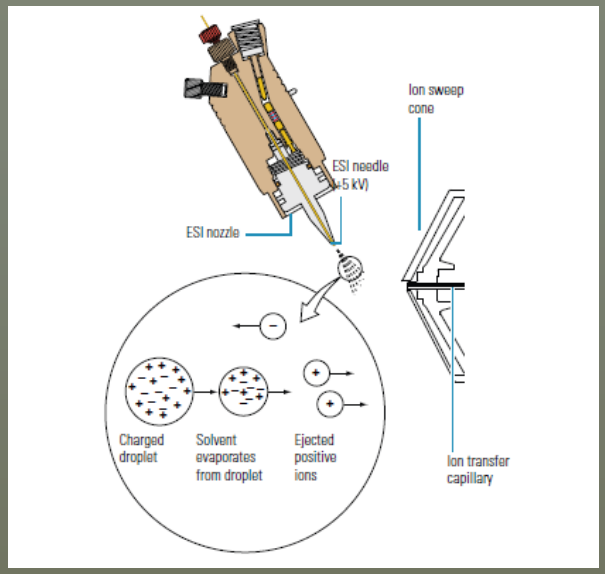
C18 capillary column (0.3 mm ID, 2.0 µm, 150 mm)
Flow rate: 10 µLmin^{-1}
Injection volume: 1 µL
Temperature: 35°C

Mobile phase

A) 1mM ammonium formate in 10 : 90 (v/v) methanol : water
B) 1mM ammonium formate in methanol

Optimization of **ESI source parameters** and **MS/MS conditions** by direct infusion of a standard mix solution

Sheat gas: 6
Auxiliary gas: 6
Sweep gas: 0
Capillary Temp: 320°C
Spray Voltage: +3.00, -2.70 kV
S-lens RF level: 50 V



Q-Exactive: acquisition mode

MATERIAL AND METHODS-Q-Exactive parameters

Data-Dependent Experiment

Positive and negative mode

Targeted approach

Full-MS/dd-MS² **with** the inclusion list including the masses of each precurson ion and their specifical collisional energy

Identification by:

1. Exact mass
2. Isotopic pattern
3. Retention time
4. Fragmentation spectrum

Full MS: 90-1200 *m/z*
Res: 70000
AGC target:1e^6
dd-MS/MS
Res: 35000
AGC target: 2e^5
TopN: 8

Untargeted approach

Full-MS/dd-MS² **without** the inclusion list

Identification by:

1. Exact mass
2. Isotopic pattern
3. Intepretation of mass spectrum

Full MS: possibility of retrospective analysis

MATERIAL AND METHODS-Sample preparation

1. Sample pieces of polycarbonate (1,5 g)
2. Dissolution with chloroform
3. Precipitation and extraction with methanol
4. Resuspension of the final extract

Determination of recovery by adding standards not present in the samples before sample treatment

FIRST METHOD

Dissolution with $CHCl_3$ (12 mL)
(Approx. 40 min)

↓ with MeOH (18 + 10mL)
(Immediate)

Resuspended with MeOH (2 mL)

FINAL METHOD

Dissolution with $CHCl_3$ (12 mL)

↓ with MeOH
(18 + 10 mL)

disruption of the precipitate

1st MeOH wash on (2 x 10 mL)

2nd Acetone wash on (2 x 10 mL)

3rd Acetone wash on (2 x 10 mL)

Recovery

BPA D_{16}=98±6
TINUVIN 326=42±3%
UVITEX OB=51±4%

Resuspended with MeOH
(2 mL)

Recovery

BPA D_{16} = 95±8
TINUVIN 326=102±7
UVITEX OB=84±3%

Resuspended with Acetone
(2 mL)

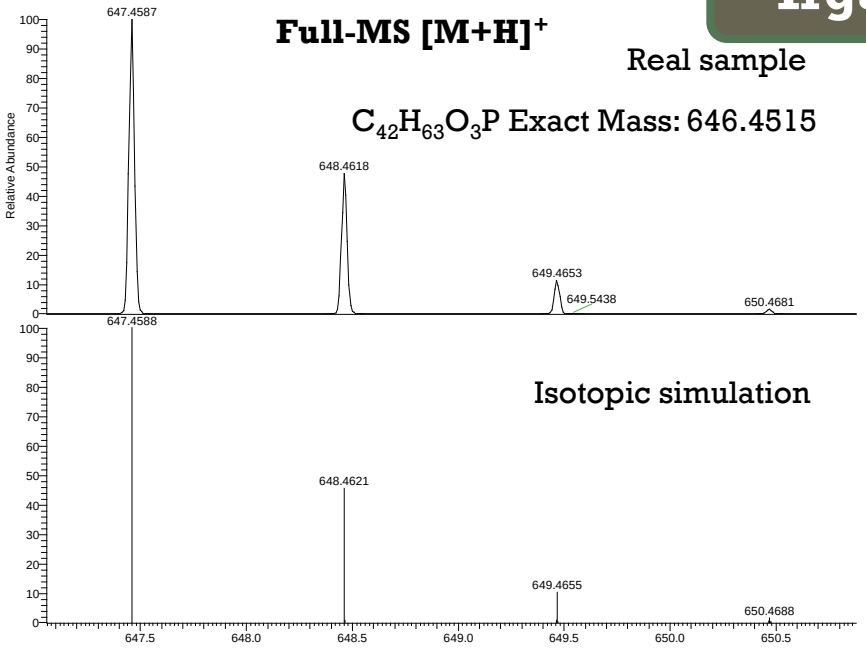
RESULTS-TARGETED ANALYSIS

Irgafos 168

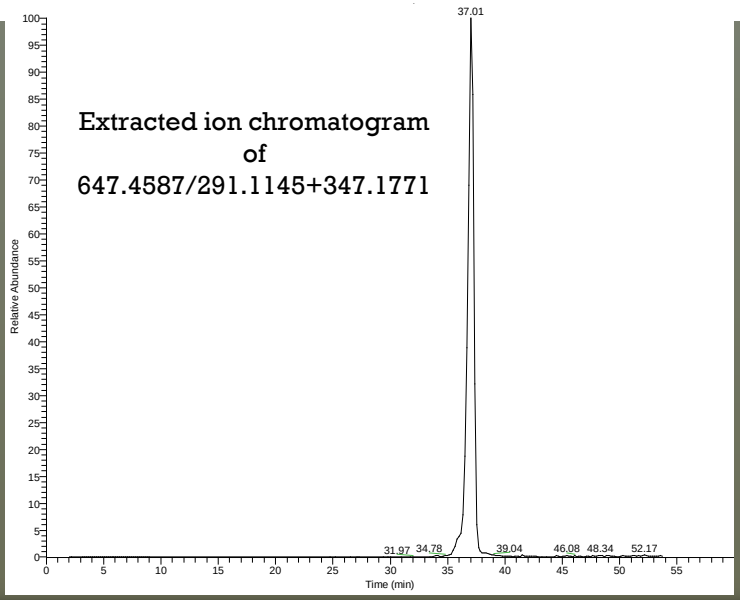
Full-MS $[M+H]^+$

Real sample

$C_{42}H_{63}O_3P$ Exact Mass: 646.4515

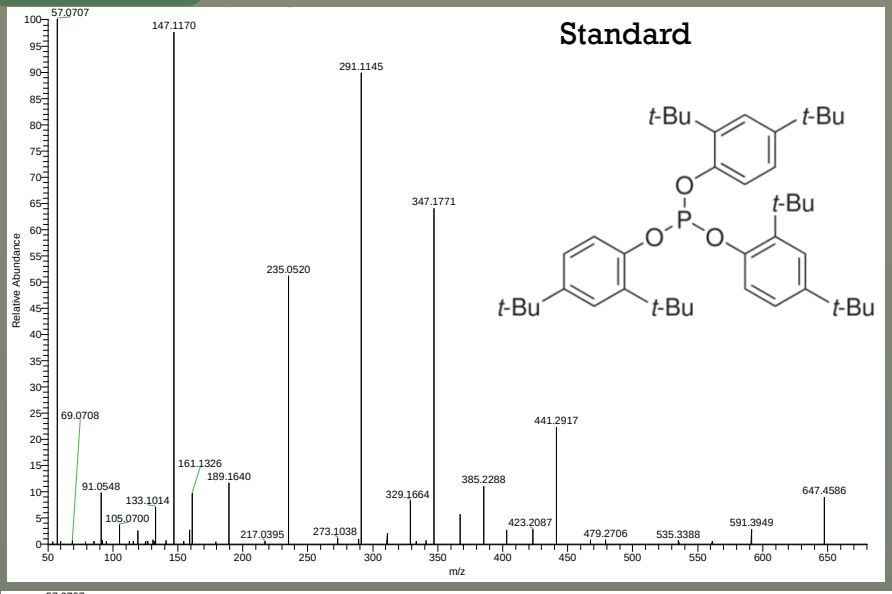


Extracted ion chromatogram of 647.4587/291.1145+347.1771

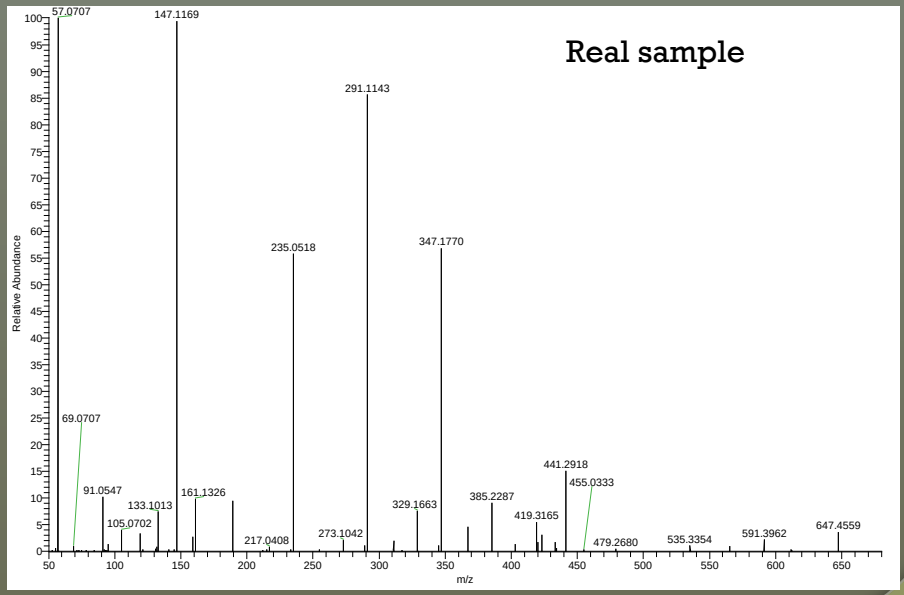


ESI-MS/MS spectrum

Standard



Real sample

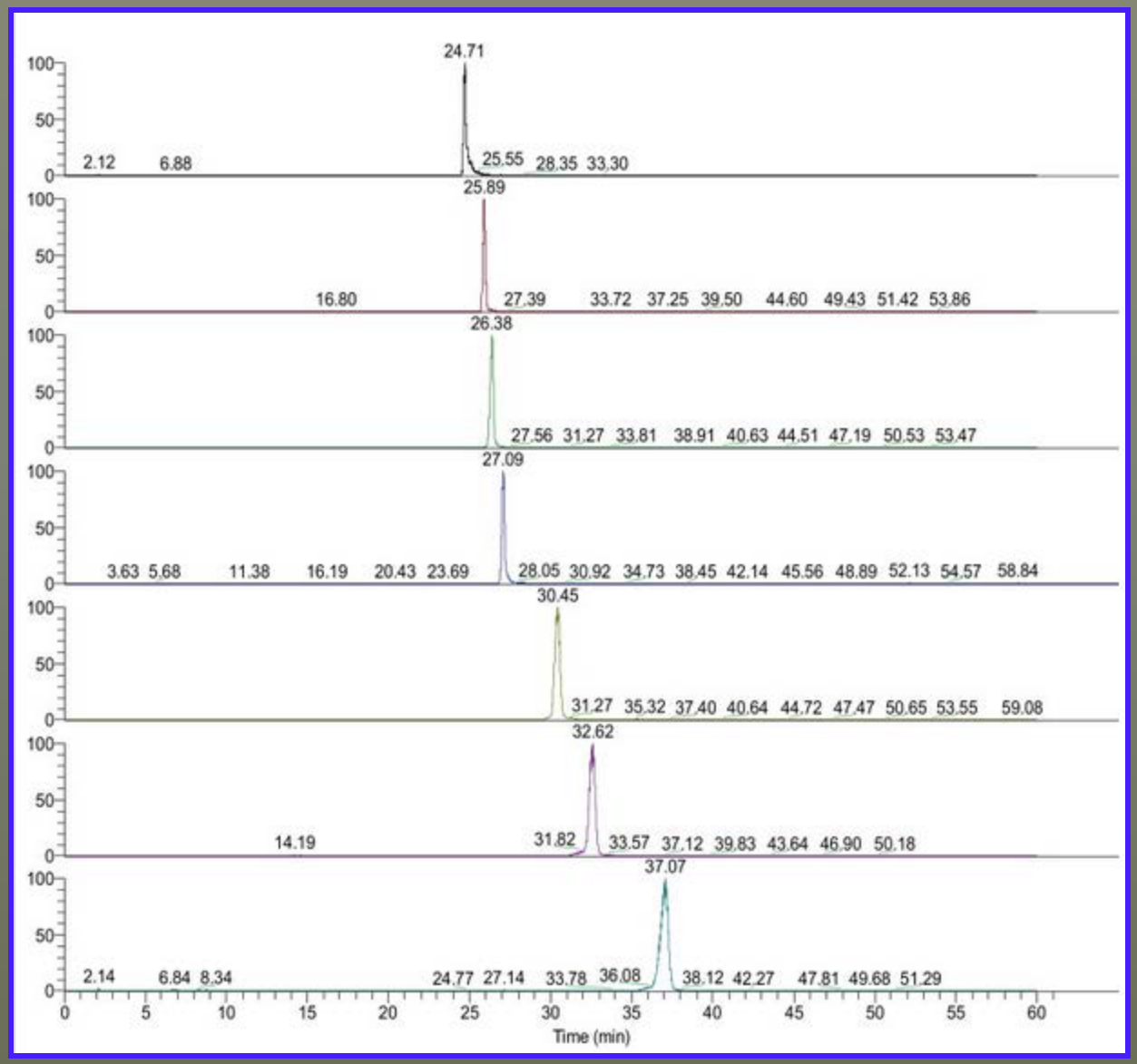


List of additives selected with molecular formula, detected ion and the two fragments selected for the identification with the collision induced dissociation energy.

Compounds	Molecular formula	Molecular ion	Products ions
BPA	$C_{15}H_{16}O_2$	227.1078 $[M - H]^{-1}$	211.0763, 133.0651 (hcd 65)
BHT	$C_{15}H_{24}O$	219.1754 $[M - H]^{-1}$	203.1437, 163.1119 (hcd 70)
BHA	$C_{11}H_{16}O_2$	179.1078 $[M - H]^{-1}$	164.0834, 149.0599 (hcd 52)
Tinuvin 234	$C_{30}H_{29}N_3O$	448.2383 $[M + H]^{+1}$	370.1917, 119.0856 (hcd 30)
Tinuvin 326	$C_{17}H_{18}ClN_3O$	316.1211 $[M + H]^{+1}$	260.0583, 107.0494 (hcd 48)
Tinuvin 327	$C_{20}H_{24}ClN_3O$	358.1681 $[M + H]^{+1}$	302.1057, 246.0431 (hcd 45)
Tinuvin 328	$C_{22}H_{29}N_3O$	352.2383 $[M + H]^{+1}$	282.1603, 212.0820 (hcd 50)
Cyasorb UV 9	$C_{14}H_{12}O_3$	229.0859 $[M + H]^{+1}$	151.0388, 105.0338 (hcd 50)
Cyasorb UV 12	$C_{15}H_{14}O_5$	275.0914 $[M + H]^{+1}$	169.0492, 151.0388 (hcd 30)
Cyasorb UV 24	$C_{14}H_{12}O_4$	245.0808 $[M + H]^{+1}$	151.0388, 121.0285 (hcd 35)
Cyasorb UV 5411	$C_{20}H_{25}N_3O$	324.2070 $[M + H]^{+1}$	212.0820, 92.0501 (hcd 50)
Irgafos 168	$C_{42}H_{63}O_3P$	647.4588 $[M + H]^{+1}$	347.1769, 291.1143 (hcd 40)
Advastab 800	$C_{30}H_{58}O_4S$	532.4394 $[M + NH_4]^{+1}$	329.2144, 143.0162 (hcd 20)
Uvinul 400	$C_{13}H_{10}O_3$	215.0703 $[M + H]^{+1}$	137.0232, 105.0338 (hcd 45)
Cyanox 2246	$C_{23}H_{32}O_2$	339.2330 $[M - H]^{-1}$	163.1119 (hcd 45)
Chimassorb 81	$C_{21}H_{26}O_3$	327.1955 $[M + H]^{+1}$	215.0703, 137.0232 (hcd 40)
Uvitex OB	$C_{26}H_{26}N_2O_2S$	431.1788 $[M + H]^{+1}$	415.1470, 401.1316 (hcd 62)
BADGE	$C_{21}H_{24}O_4$	358.2013 $[M + NH_4]^{+1}$	229.1221, 191.1065 (hcd 20)
Irganox 1076	$C_{35}H_{62}O_3$	548.5048 $[M + NH_4]^{+1}$	475.4143, 419.3515 (hcd 12)
Irganox 1010	$C_{73}H_{108}O_{12}$	1194.8179 $[M + NH_4]^{+1}$	729.2902, 563.2272 (hcd 20)
Irganox 1330	$C_{54}H_{78}O_3$	792.6289 $[M + NH_4]^{+1}$	569.4344, 219.1745 (hcd 20)
Irganox 1081	$C_{22}H_{30}O_2S$	357.1894 $[M - H]^{-1}$	194.0760, 163.1119 (hcd 35)

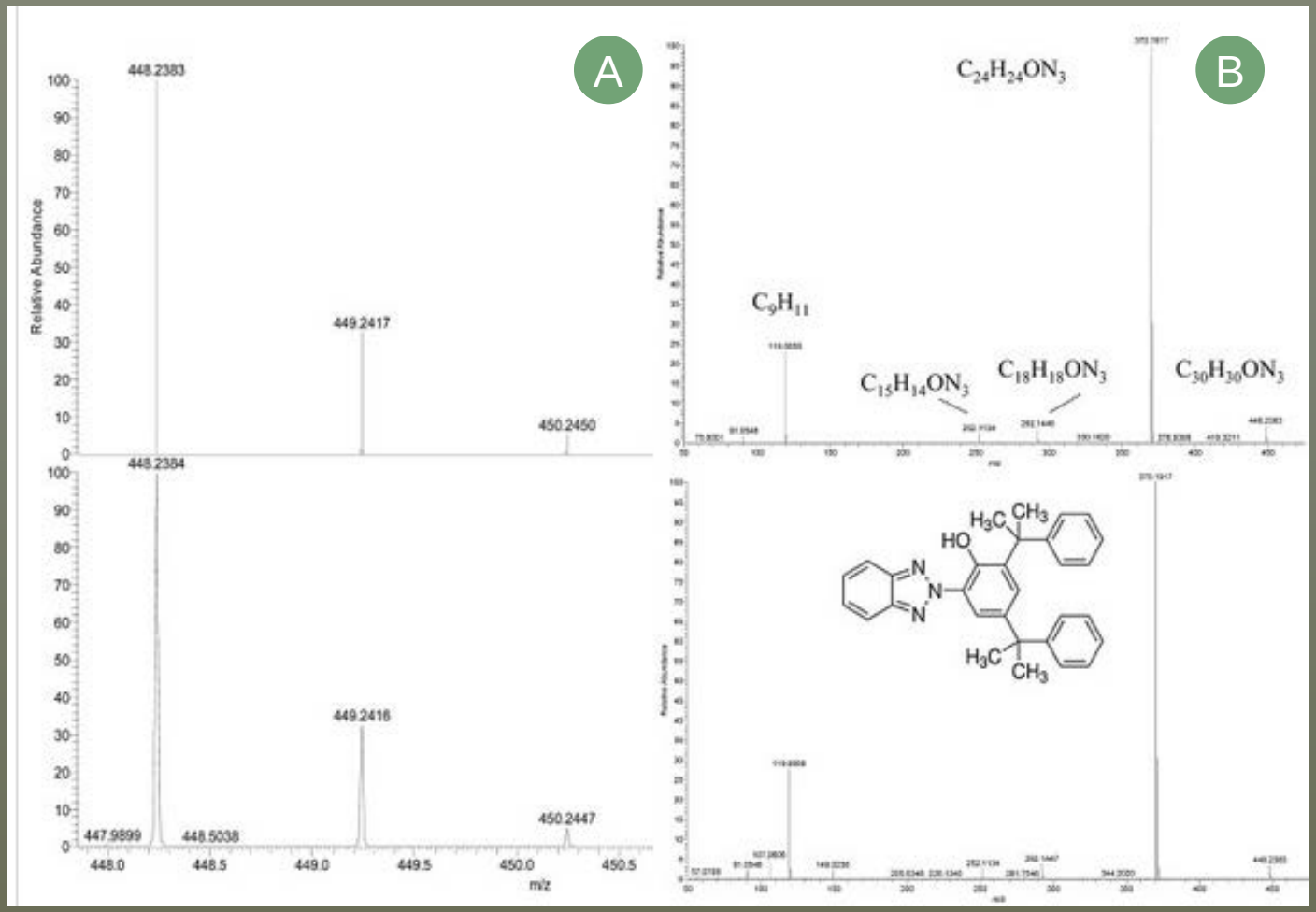
Bignardi et al. J. Chromatogr. A, 1372(2014)133

LC–ESI–HRMS chromatograms of the seven plastic additives found in polycarbonate extracts. From above Cyasorb UV5411, Tinuvin 234, Uvitex OB, Tinuvin 327, Advastab 800, Irganox 1076 and Irgafos 168.

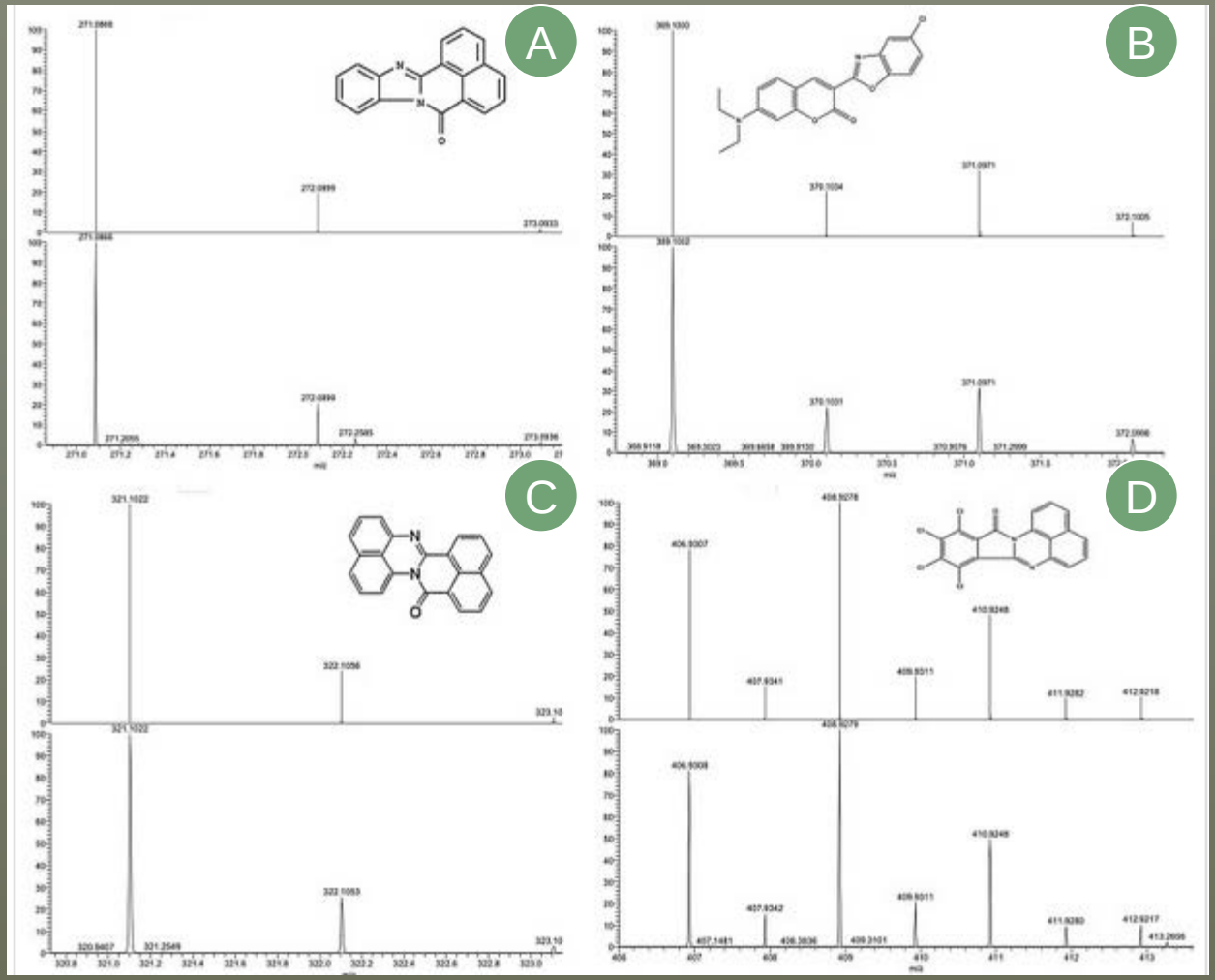


Panel A: representation of the isotopic pattern simulated by the software by setting the molecular formula of Tinuvin 234 (above) and that one found in the PC extract (below).

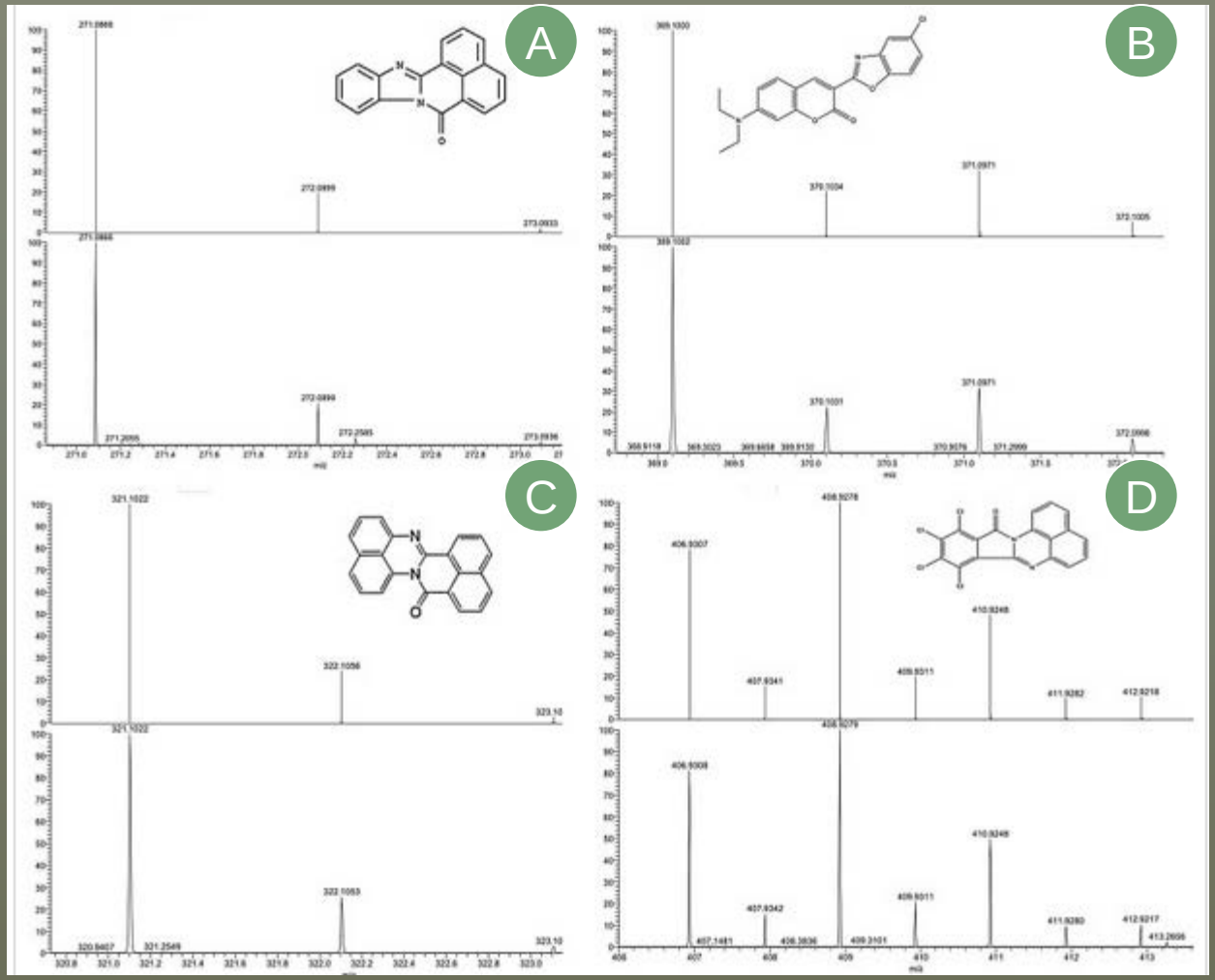
Panel B: comparison between fragmentation spectrum obtained by injecting a standard solution of Tinuvin 234 (above) and that found in the real sample (below). Insert: molecular structure of Tinuvin 234.



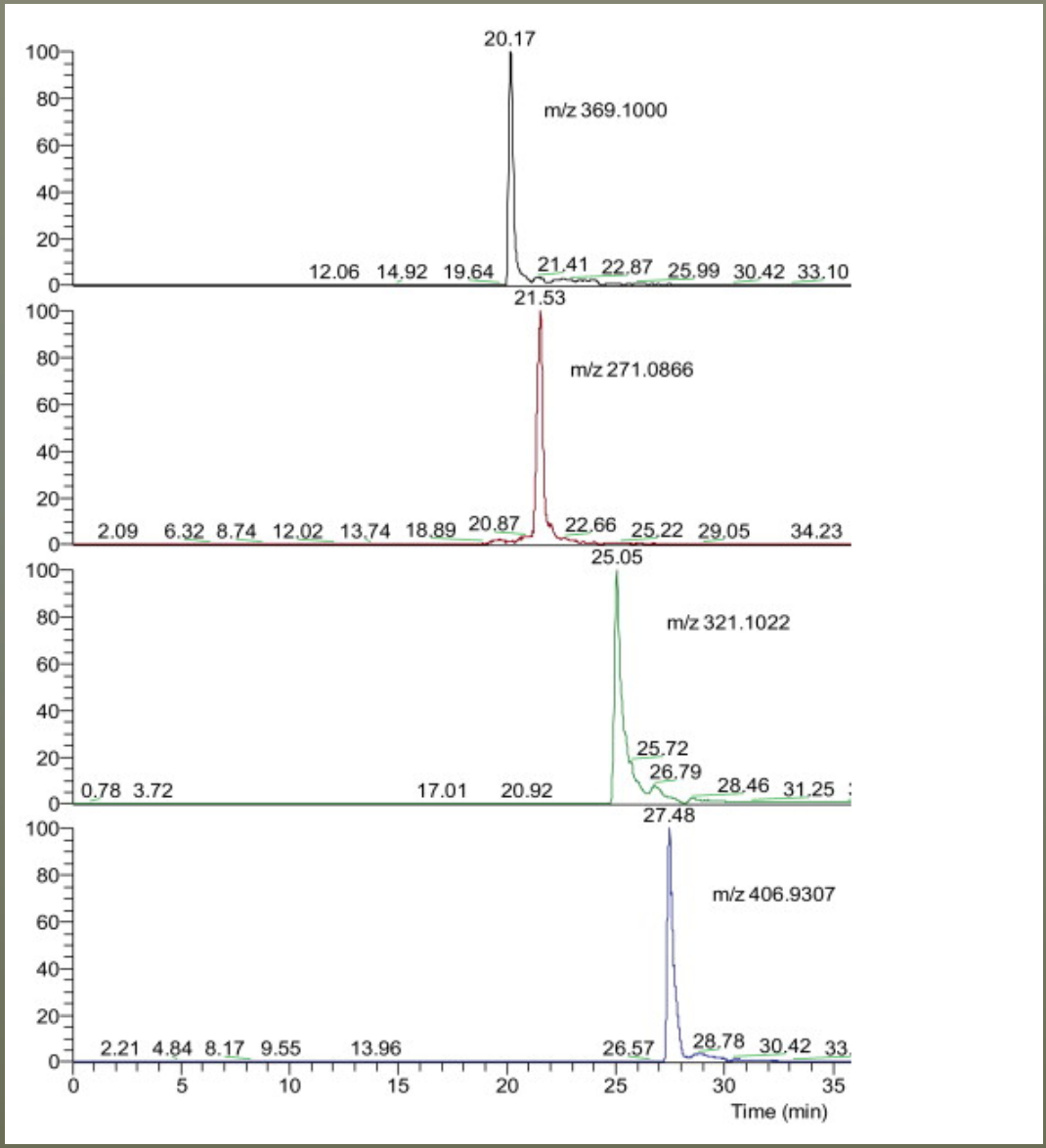
Panel A: colorant *Solvent Yellow 184* identified in the PC orange sample. Panel B: colorant *Solvent Yellow 232* identified in the PC yellow sample. Panel C: colorant *Solvent Red 179* identified in the PC red sample. Panel D: colorant *Solvent Red 135* found in the PC pink and orange sample.



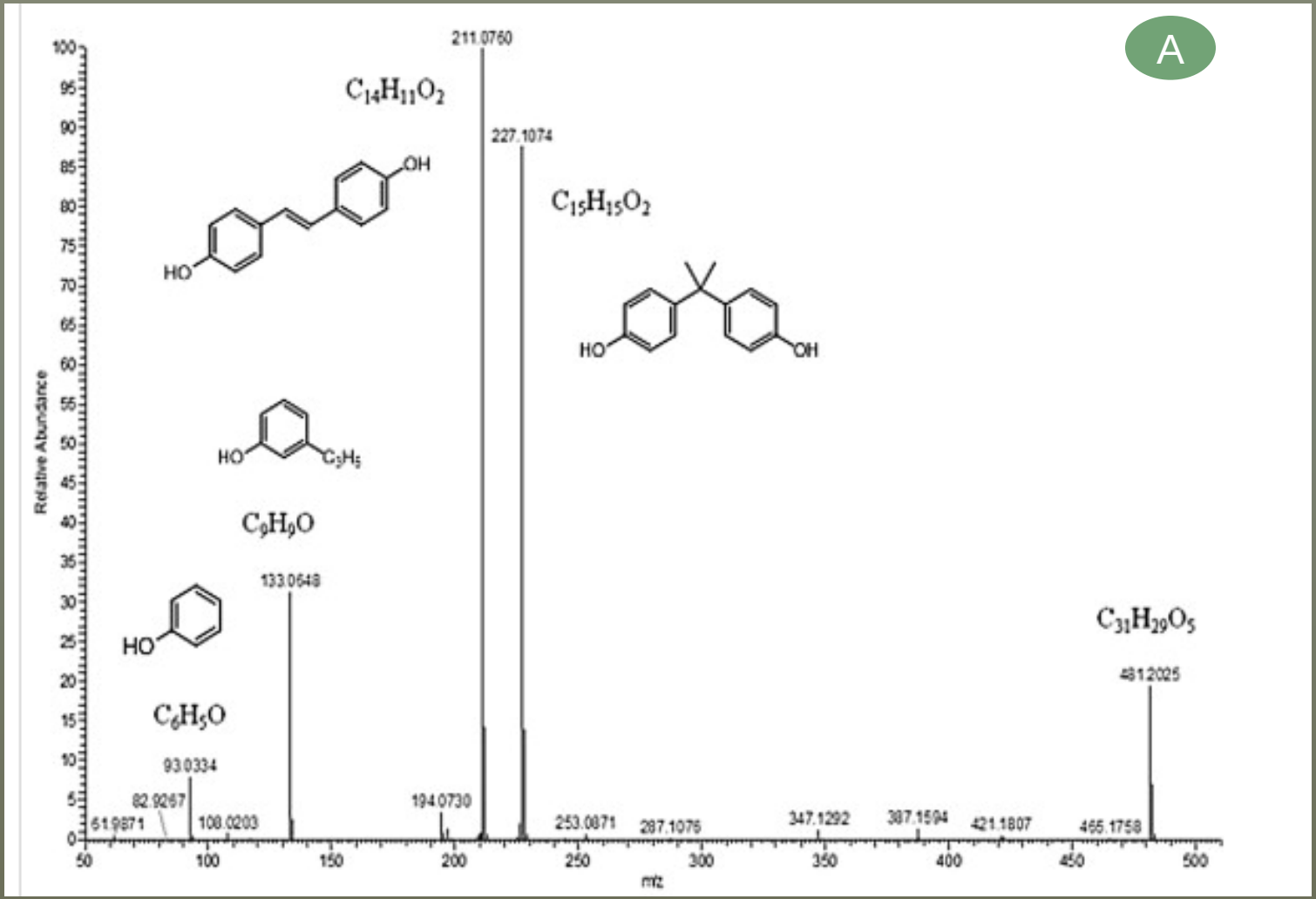
Panel A: colorant *Solvent Yellow 184* identified in the PC orange sample. Panel B: colorant *Solvent Yellow 232* identified in the PC yellow sample. Panel C: colorant *Solvent Red 179* identified in the PC red sample. Panel D: colorant *Solvent Red 135* found in the PC pink and orange sample.



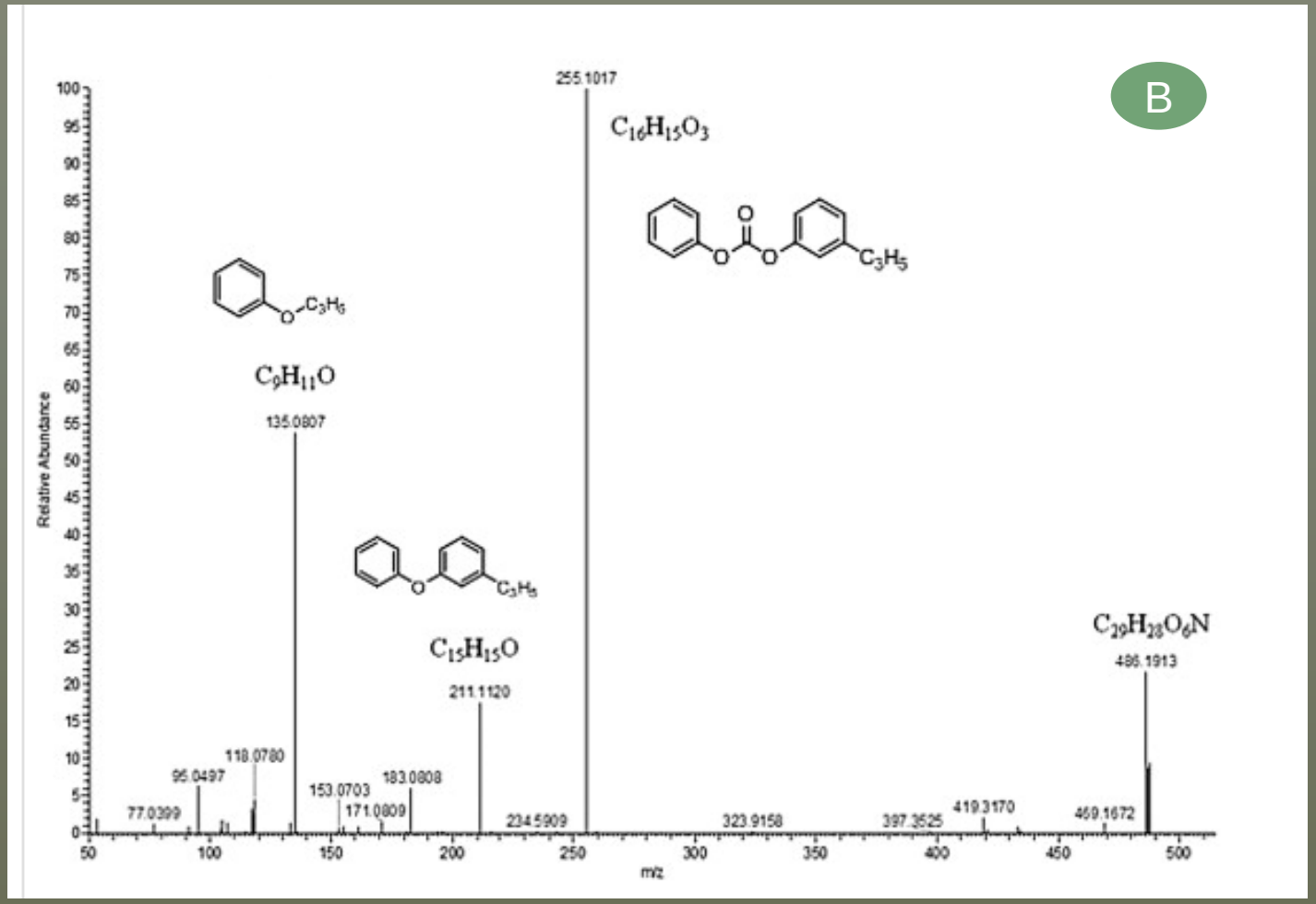
LC–ESI–HRMS chromatograms of the four organic colorants identified in the orange, yellow, pink and red PC samples, respectively. From above: *Solvent Yellow 232, Solvent Yellow 184, Solvent Red 179, Solvent Red 135.*



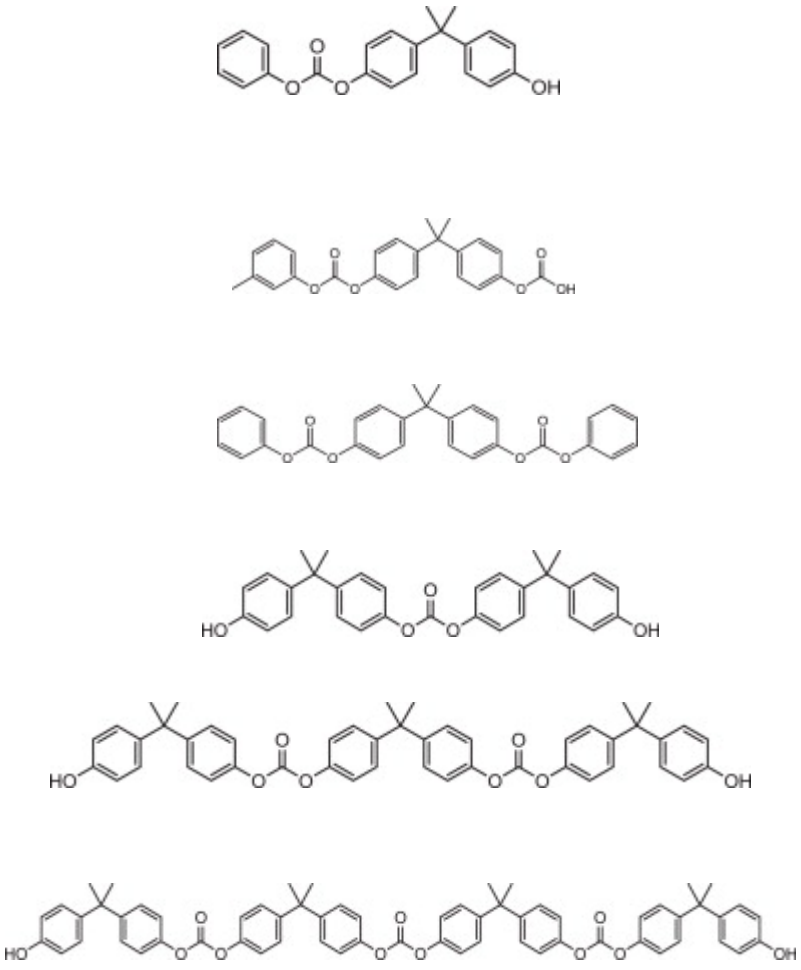
fragmentation spectrum of molecular ion at *m/z* 481.2022 detected in negative mode that provides as product ions bisphenol A (*m/z* 227.1078) and its fragments (*m/z* 211.0763, *m/z* 133.0651 and *m/z* 93.0335).

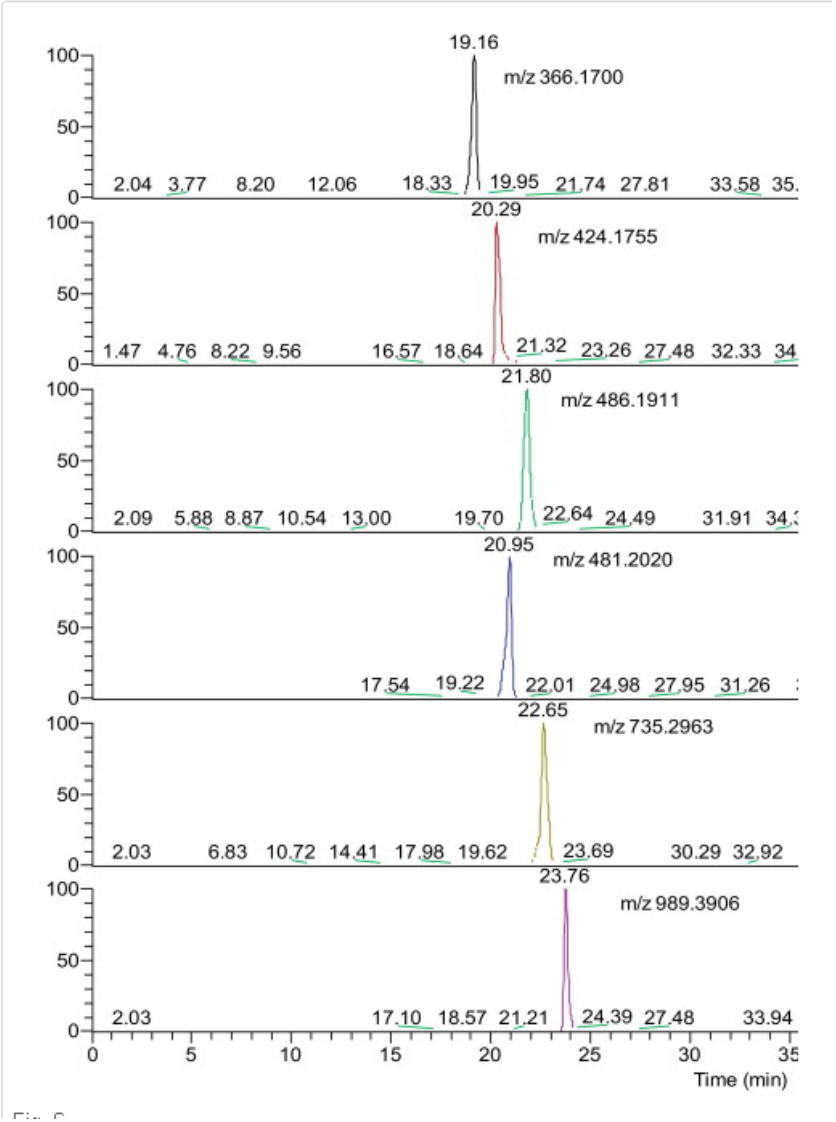


Fragmentation pathway of molecular ion at *m/z* 486.1913 detected in positive mode.

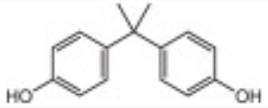
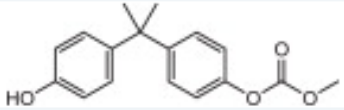
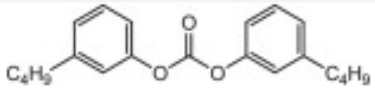
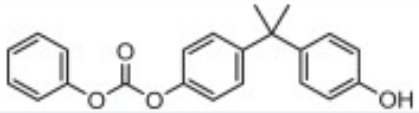
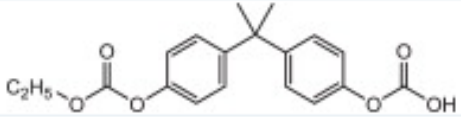
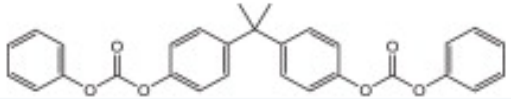
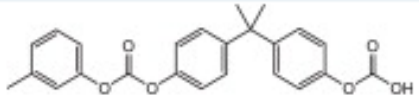
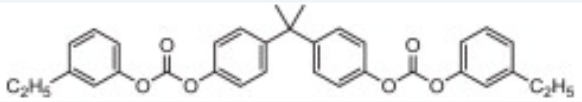
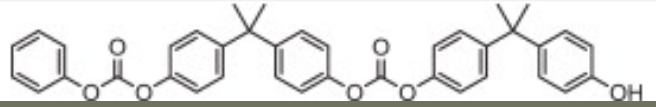


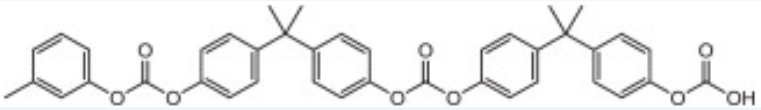
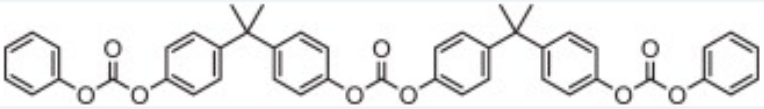
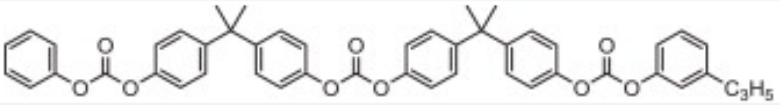
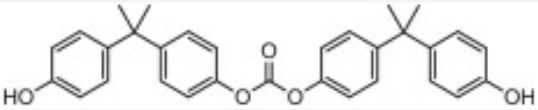
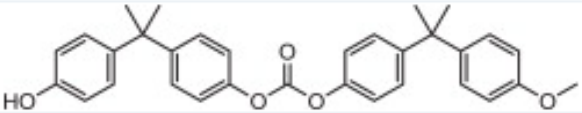
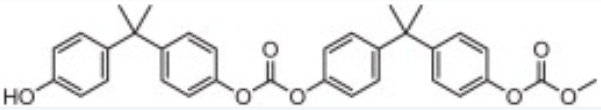
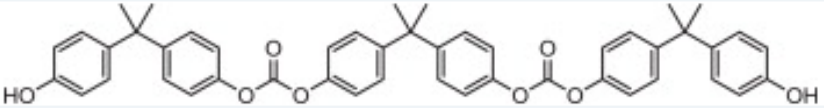
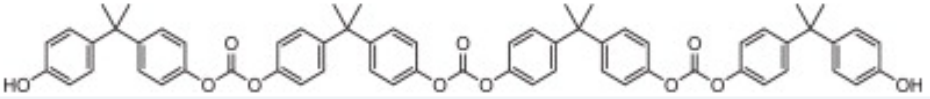
LC–ESI–HRMS chromatograms of the most representative potential BPA-polycarbonate degradation products found in all the samples analyzed.





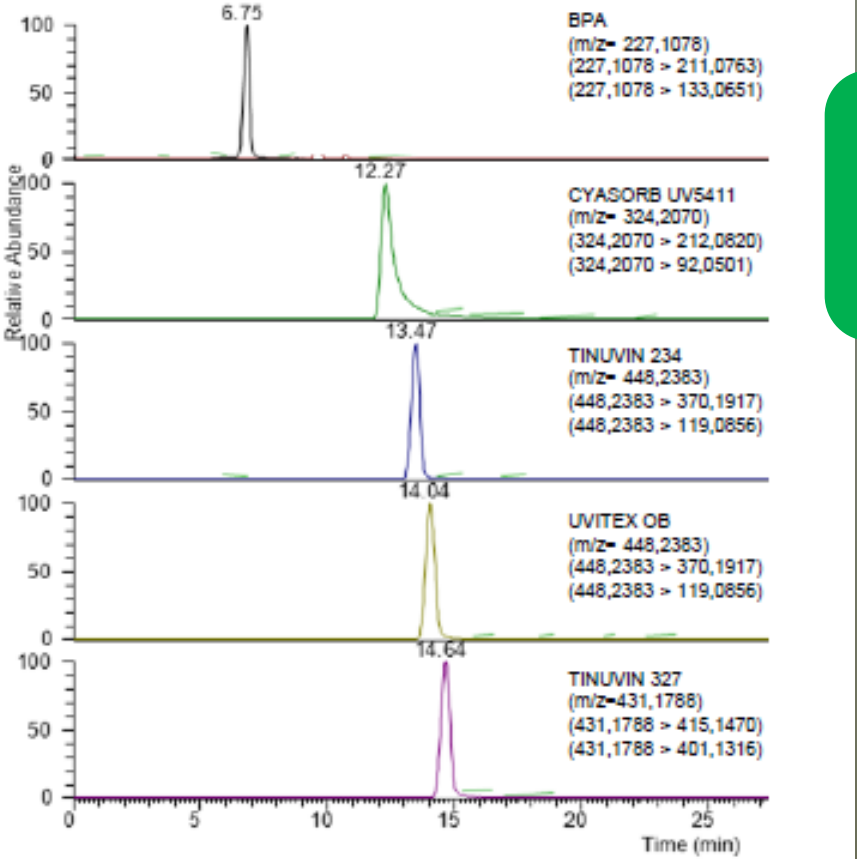
List of potential BPA-polycarbonate degradation products detected in PC samples.

Chemical Structure	Formula	Ion detected
	$C_{15}H_{16}O_2$ bisphenol A	227.1078 $[M - H]^{-1}$
	$C_{17}H_{18}O_4$	304.1543 $[M + NH_4]^{+1}$
	$C_{21}H_{26}O_3$	344.2221 $[M + NH_4]^{+1}$
	$C_{22}H_{20}O_4$	366.1700 $[M + NH_4]^{+1}$
	$C_{19}H_{20}O_6$	362.1601 $[M + NH_4]^{+1}$
	$C_{29}H_{24}O_6$	486.1911 $[M + NH_4]^{+1}$
	$C_{24}H_{22}O_6$	424.1755 $[M + NH_4]^{+1}$
	$C_{33}O_6H_{32}$	542.2537 $[M + NH_4]^{+1}$
	$C_{38}O_7H_{34}$	620.2642 $[M + NH_4]^{+1}$

Chemical Structure	Formula	Ion detected
	$C_{40}O_9H_{36}$	678.2698 $[M + NH_4]^{+1}$
	$C_{45}O_9H_{38}$	740.2854 $[M + NH_4]^{+1}$
	$C_{48}O_9H_{42}$	780.3167 $[M + NH_4]^{+1}$
	$C_{31}O_5H_{30}$	481.2020 $[M - H]^{-1}$
	$C_{32}O_5H_{32}$	495.2177 $[M - H]^{-1}$
	$C_{33}O_7H_{32}$	539.2075 $[M - H]^{-1}$
	$C_{47}O_8H_{44}$	735.2963 $[M - H]^{-1}$
	$C_{63}O_{11}H_{58}$	989.3906 $[M - H]^{-1}$

Migration experiments: procedure

Carried out in a climatic chamber at 40 °C for 1 h.
Tests were repeated three times according to the procedure described in legislation*.
Solvent was evaporated and resuspended in 1 mL of ethanol for LC and GC analysis.
Migration values were expressed in µg Kg-1 and ng Kg-1 on the basis of a surface/volume ratio of 6 dm2 per Kg of food simulant*



SIMULANTS:

- Ethanol 95% (v/v)
- Isoctane

*Commission Regulation (EU) No 10/2011
Hoextra E.J and Simoneau C. Release of BPA from PC – a review. Crit Rev Food Sci and Nutr., 53 (2013) 386-402

Bisphenol A (µg Kg-1)

Sample	Migration	Ethanol 95%	Isooctane
Red 2012	1st	0.74±0.06	0.16±0.001
	2nd	0.24±0.05	0.06±0.003
	3rd	< LOD	0.07±0.001
Blue 2010	1st	1.20±0.08	1.02±0.05
	2nd	0.58±0.01	0.09±0.003
	3rd	0.13±0.02	< LOQ
Orange 2009	1st	5.81±0.24	0.53±0.01
	2nd	0.66±0.01	0.15±0.01
	3rd	0.11±0.01	0.02±0.001
Yellow 2007	1st	1.08±0.08	0.32±0.01
	2nd	0.12±0.001	< LOQ
	3rd	0.53±0.03	< LOQ
Transparent 2000	1st	7.39±0.37	0.35±0.03
	2nd	3.45±0.36	< LOQ
	3rd	2.62±0.08	< LOQ

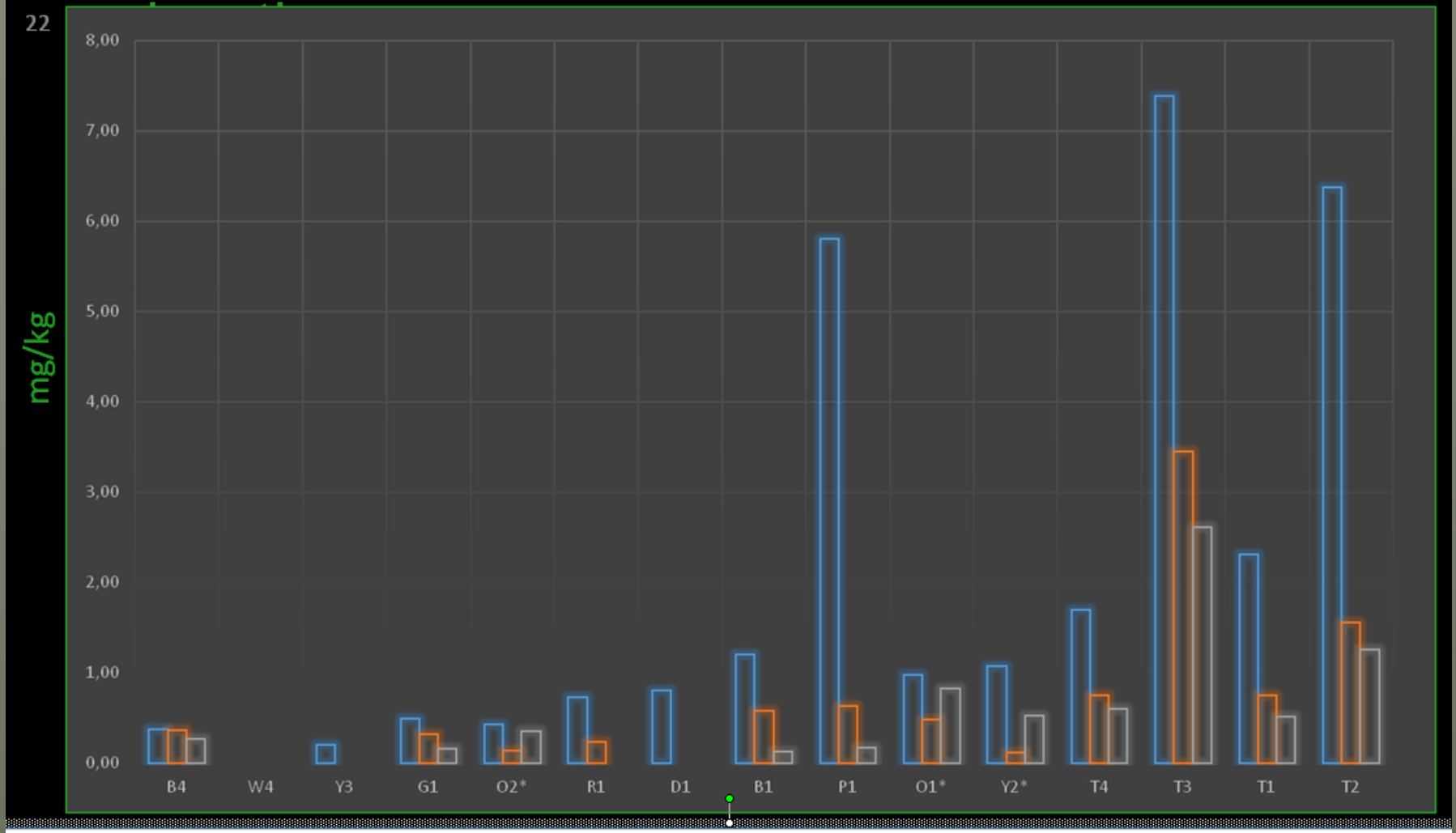
EFFECT OF THE SOLVENT: Ethanol vs. Isoctane

Additives (ng Kg-1)

Additive	Migration	Ethanol 95%	Isooctane
Cyasorb UV5411	1st	16644±666	13981±30
	2nd	2309±270	1324±66
	3rd	43±0.4	271±7
Tinuvin 234	1st	23004±277	17488±716
	2nd	3970±150	1271±19
	3rd	84±8	275±13
Tinuvin 327	1st	4463±91	2776±150
	2nd	681±45	199±5
	3rd	11±0.3	44±1
Uvitex OB	1st	4186±82	2989±141
	2nd	674±51	240±5
	3rd	15±1	53±1

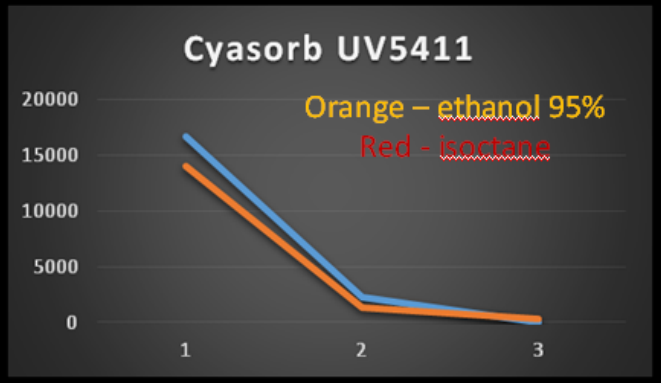
Amounts released in the two solvents during three consecutive migration experiments

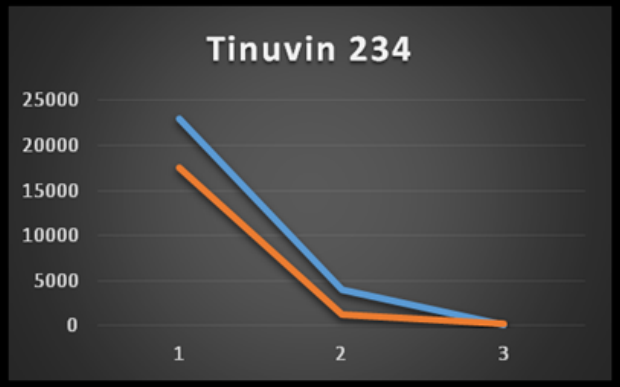
Decrease in BPA release from 1st to 3rd

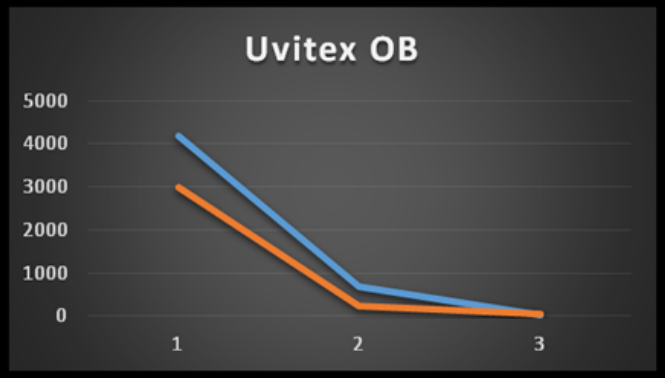


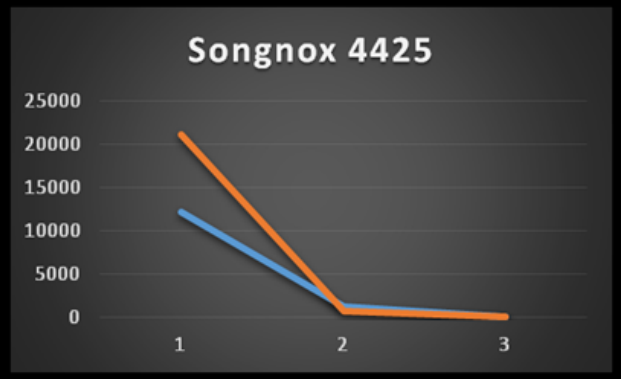
Bignardi et al., Anal. Bioanal. Chem. (2015) 407: 7917-7924

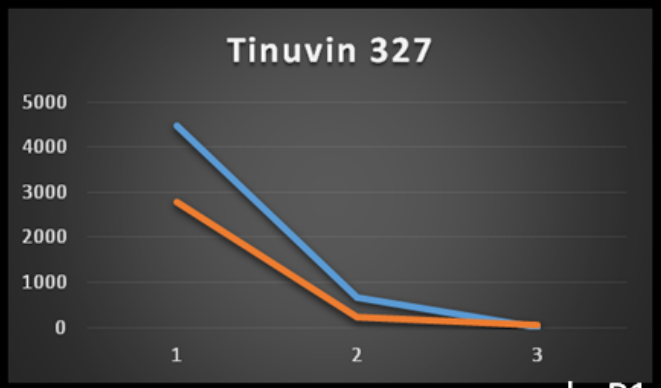
Additives: progressive decrease in 3 migrations











- Ethanol extracts more amount than isoctane for all compounds, except Songnox 4425.
- Isoctane shows always higher amount than ethanol in the third extraction!

No correlation with age

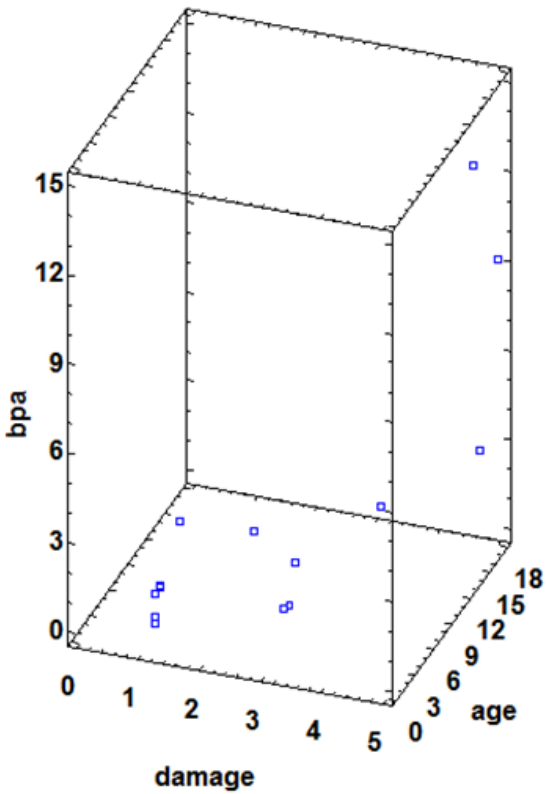
sample P1

Bisphenol A

Sample	1st migration	2nd migration	3rd migration	Damage level
White 2013	< LOD	< LOD	< LOD	1
Yellow 2013	0.20±0.01	< LOD	< LOD	1
Blue 2013	0.38±0.02	0.37±0.02	0.26±0.01	1
Green 2012	0.49±0.03	0.32±0.02	0.17±0.01	1
Orange 2012	0.43±0.01	0.14±0.01	0.35±0.03	1
Red 2012	0.74±0.06	0.24±0.05	< LOD	3
Dark 2011	0.81±0.07	< LOQ	< LOD	3
Blue 2010	1.20±0.08	0.58±0.01	0.13±0.02	3
Orange 2009	0.97±0.01	0.49±0.02	0.82±0.04	1
Yellow 2007	1.08±0.08	0.12±0.001	0.53±0.03	2
Transparent 2006	1.70±0.07	0.75±0.05	0.60±0.04	4
Transparent 2000	7.39±0.37	3.45±0.36	2.62±0.08	5
Transparent 1999	2.31±0.06	0.76±0.04	0.51±0.02	5
Transparent 1996	6.37±0.18	1.56±0.08	1.25±0.06	5

Newer samples, damaged surface

Older samples, not damaged surface



Correlations

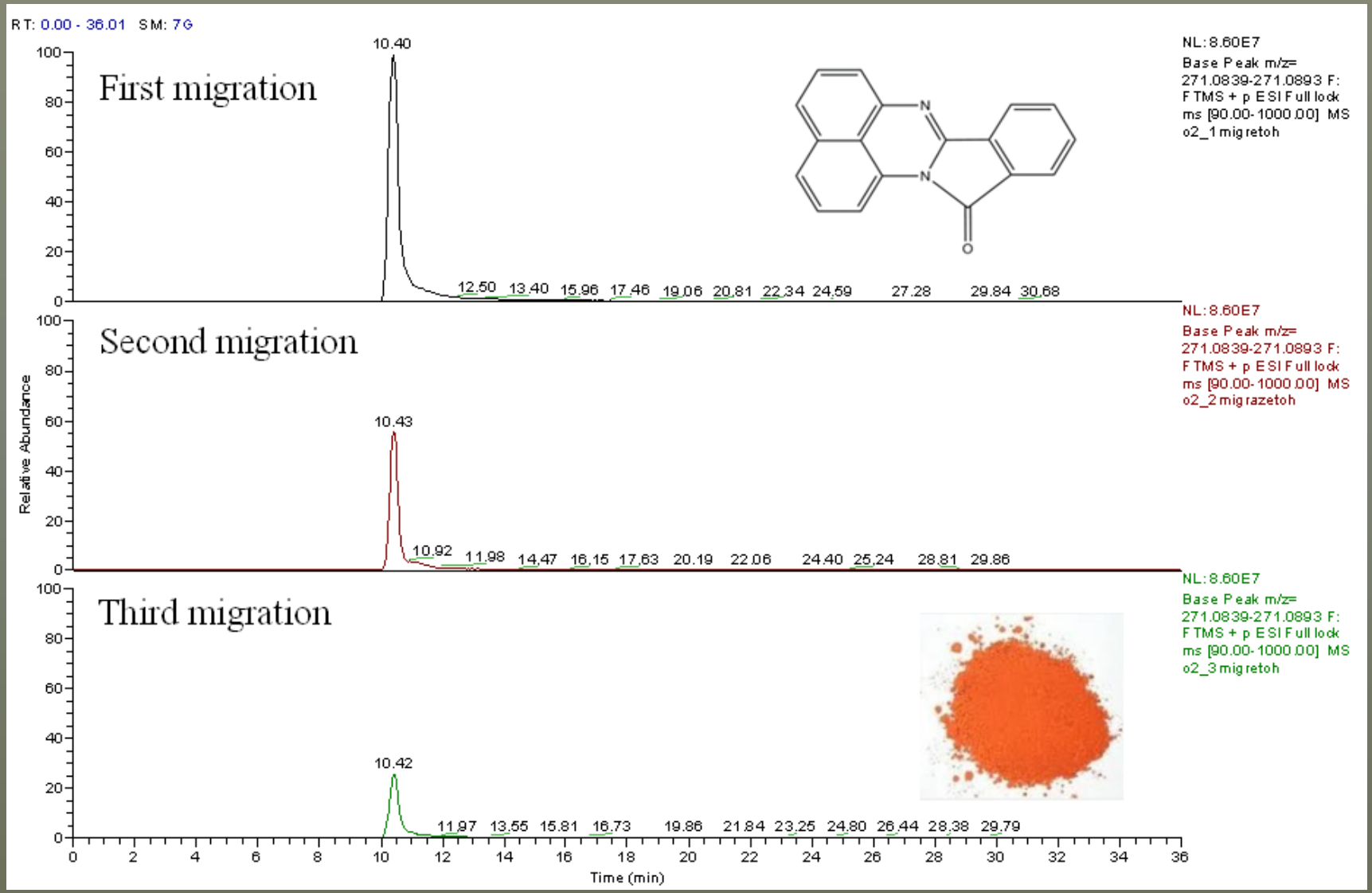
BPA-Age = 0.862

Damage-BPA = 0.786

Damage-Age = 0.863

BPA release seems to be more connected to age than to surface damage

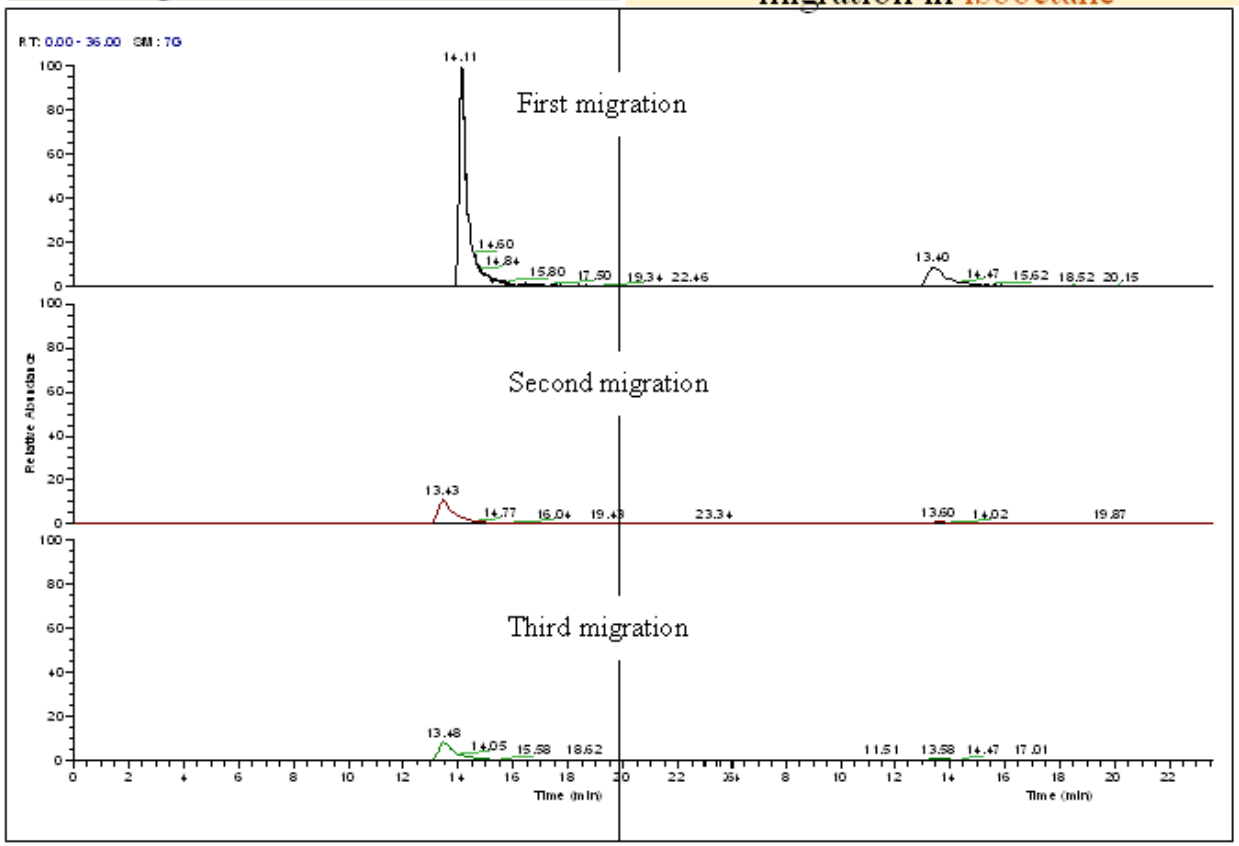
colorants


RT: 0.00 - 36.01 SM: 7G
First migration
10.40
12.50 13.40 15.96 17.46 19.06 20.81 22.34 24.59 27.28 29.84 30.68
NL: 8.60E7
Base Peak m/z= 271.0839-271.0893 F: FTMS + p ESI Full lock ms [90.00-1000.00] MS o2_1 migretoh
Second migration
10.43
10.92 11.98 14.47 16.15 17.63 20.19 22.06 24.40 25.24 28.81 29.86
NL: 8.60E7
Base Peak m/z= 271.0839-271.0893 F: FTMS + p ESI Full lock ms [90.00-1000.00] MS o2_2 migrazetoh
Third migration
10.42
11.97 13.55 15.81 16.73 19.86 21.84 23.25 24.80 26.44 28.38 29.79
NL: 8.60E7
Base Peak m/z= 271.0839-271.0893 F: FTMS + p ESI Full lock ms [90.00-1000.00] MS o2_3 migretoh
Relative Abundance
Time (min)

Effect of solvent: ethanol vs. isoctane

Red R1
solvent Red 179
migration in ethanol 95%

Red R1
solvent Red 179
migration in isooctane



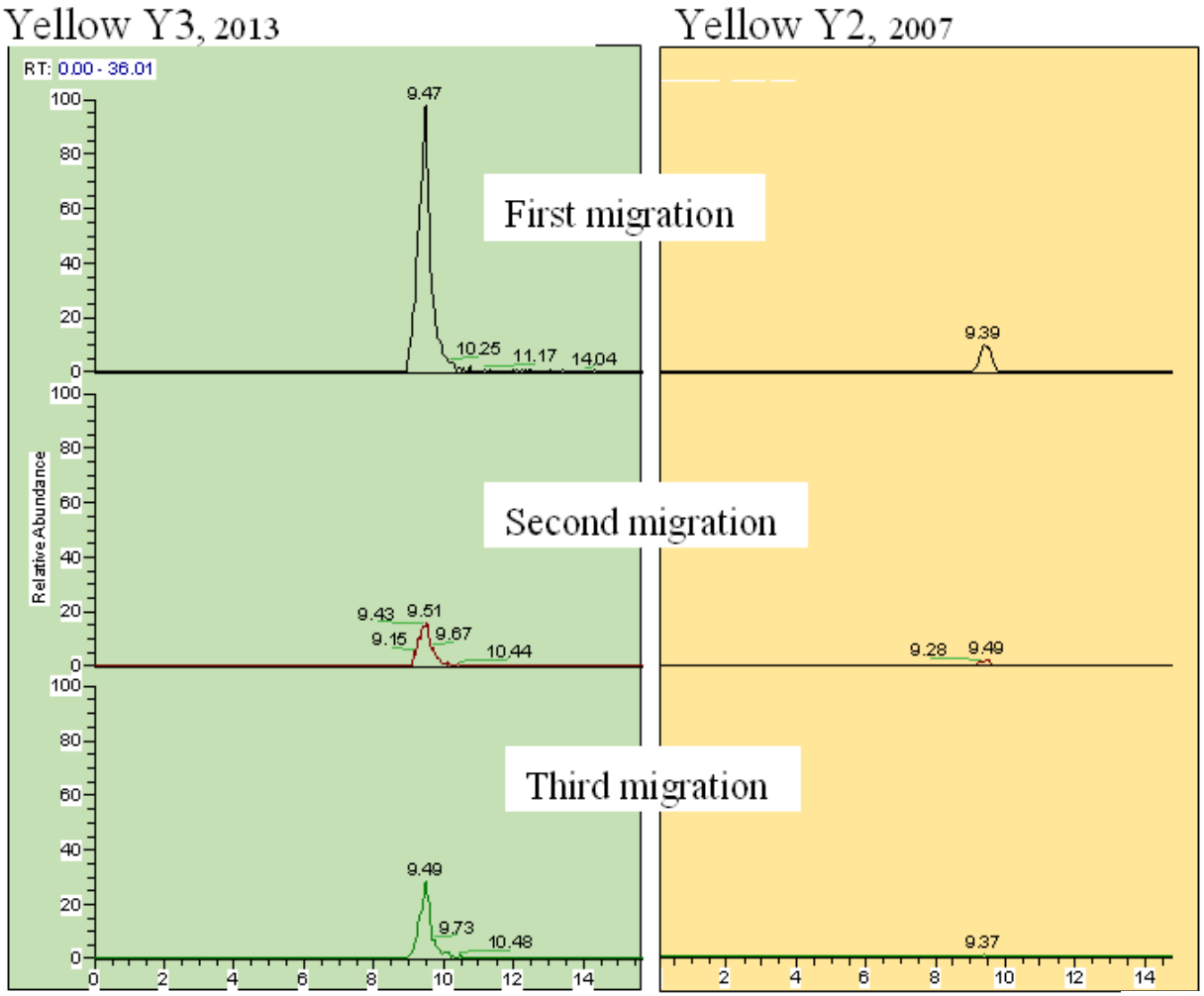
Higher migration in ethanol 95%

Dyes were identified in previous experiments of untargeted analysis

MIGRATION: EFFECT OF AGE

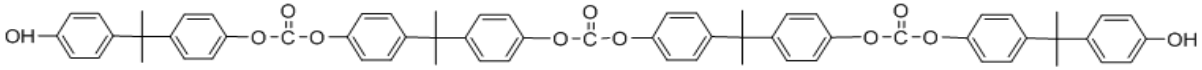
Experiments performed on two samples of same shape and colour but different age

New samples release more colouring agent

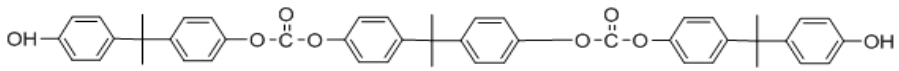


Products of degradation of PC

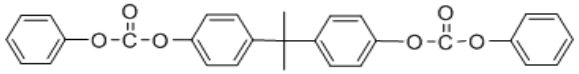
[M-H]⁻ 989.3906
$C_{63}H_{58}O_{11}$



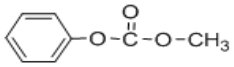
[M-H]⁻ 735.2963
$C_{47}H_{44}O_8$



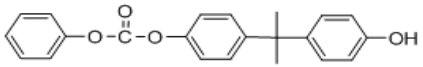
[M+NH₄]⁺ 486.1911
$C_{29}O_6H_{27}N$



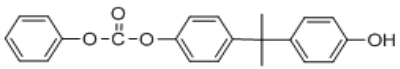
[M+H]⁺ 153.0546 $C_8H_8O_3$



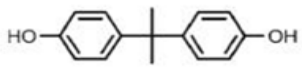
[M-H]⁻ 347.1289
$C_{22}H_{20}O_4$



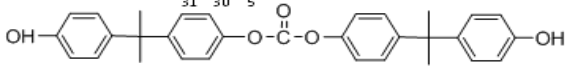
[M+NH₄]⁺ 366.1700 $C_{22}O_4H_{23}N$



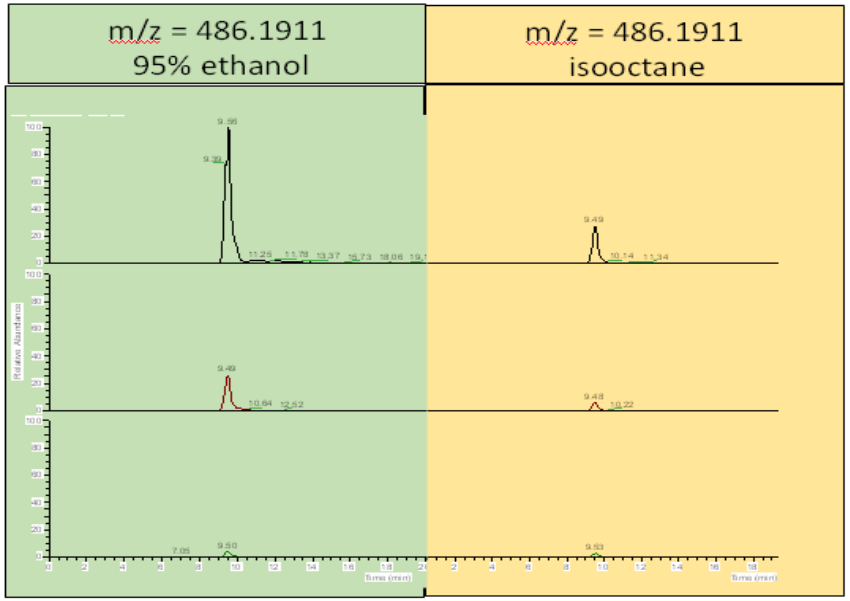
[M-H]⁻ 227.1078 $C_{15}H_{16}O_2$



[M-H]⁻ 481.2020
$C_{31}H_{30}O_5$

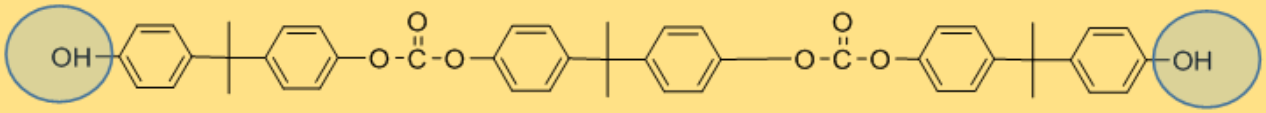


Sample: yellow Y2, year 2007

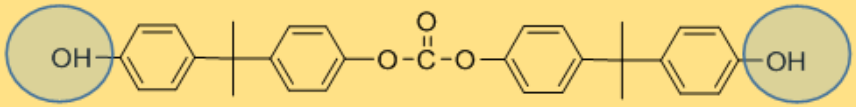


Compounds identified in experiments of untargeted analysis

PC degradation products

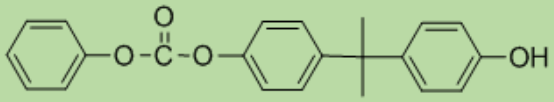


[M-H]⁻ 735.2963 $C_{47}H_{44}O_8$

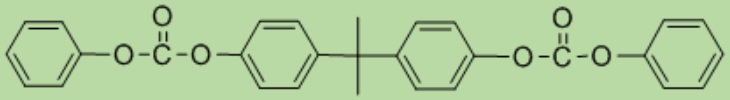


[M-H]⁻ 481.2020 $C_{31}H_{30}O_5$

Correlation with age, damage and BPA



[M+NH_4]⁺ 366.1700 $C_{22}O_4H_{23}N$



[M+NH_4]⁺ 486.1911 $C_{29}O_6H_{27}N$

Correlations

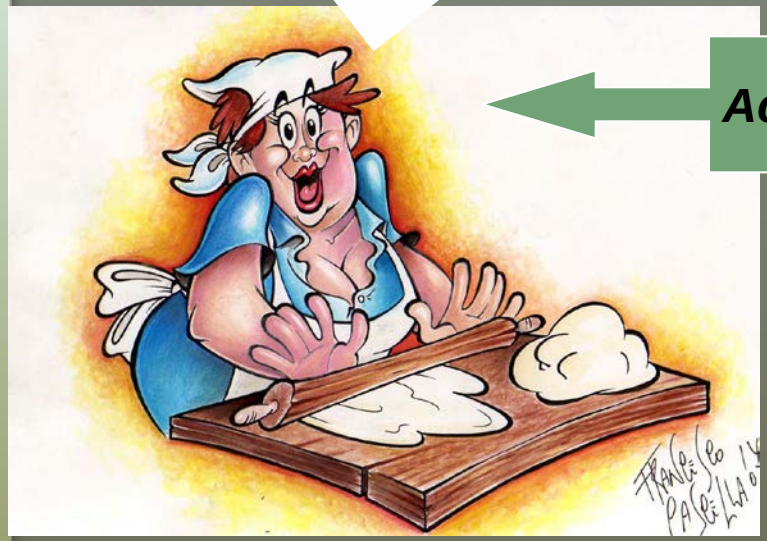
	AGE	SCRATCHES	AREA 481	AREA 486	AREA 366	AREA 735
AREA 481	0,861	0,744				
AREA 486	-0,312	-0,044	-0,347			
AREA 366	-0,494	-0,323	-0,449	0,776		
AREA 735	0,87	0,814	0,822	-0,288	-0,43	
uG/KG BPA	0,773	0,706	0,877	-0,235	-0,316	0,861

CONCLUSIONS

- Set up of an innovative analytical method suitable for the identification of additives, colorants and BPA-polycarbonate degradation products
- All additives found in the samples are approved for food-contact materials
- BPA Higher correlation with age of samples than with damage level
- ADDITIVES no correlation with age
- COLORANTS New samples release higher amount than old samples
- PRODUCTS OF DEGRADATION Different pattern of compounds

'I don't want any chemicals anywhere near my food.'

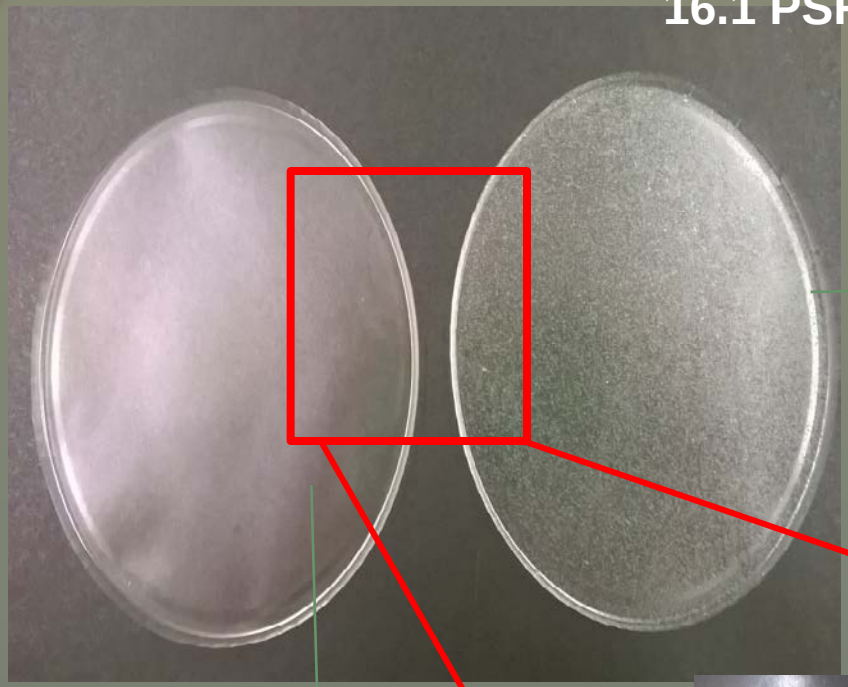
'I think it is a good thing. If oxygen is removed from food packs, the food will have more vitamins in it and that is better for you.'



***Active* Packaging?**

Ipotesi costituzione Gruppo Operativo - Misura 16.1 PSR

Realizzazione di film edibili utiilizzabili nel packaging alimentare



Film ottimizzato

Packagi ng Attivo

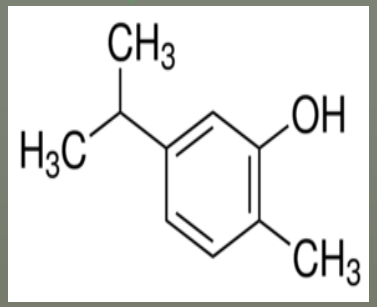
Film “modello”

Ipotesi costituzione Gruppo Operativo - Misura 16.1 PSR

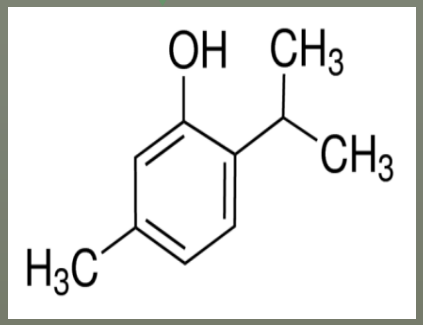
Olio essenziale di origano

Olio essenziale di timo

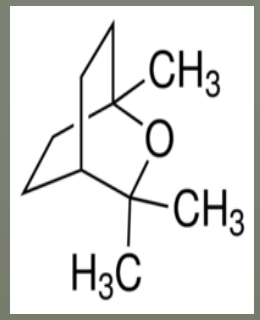
Olio essenziale di rosmarino



Carvacrolo



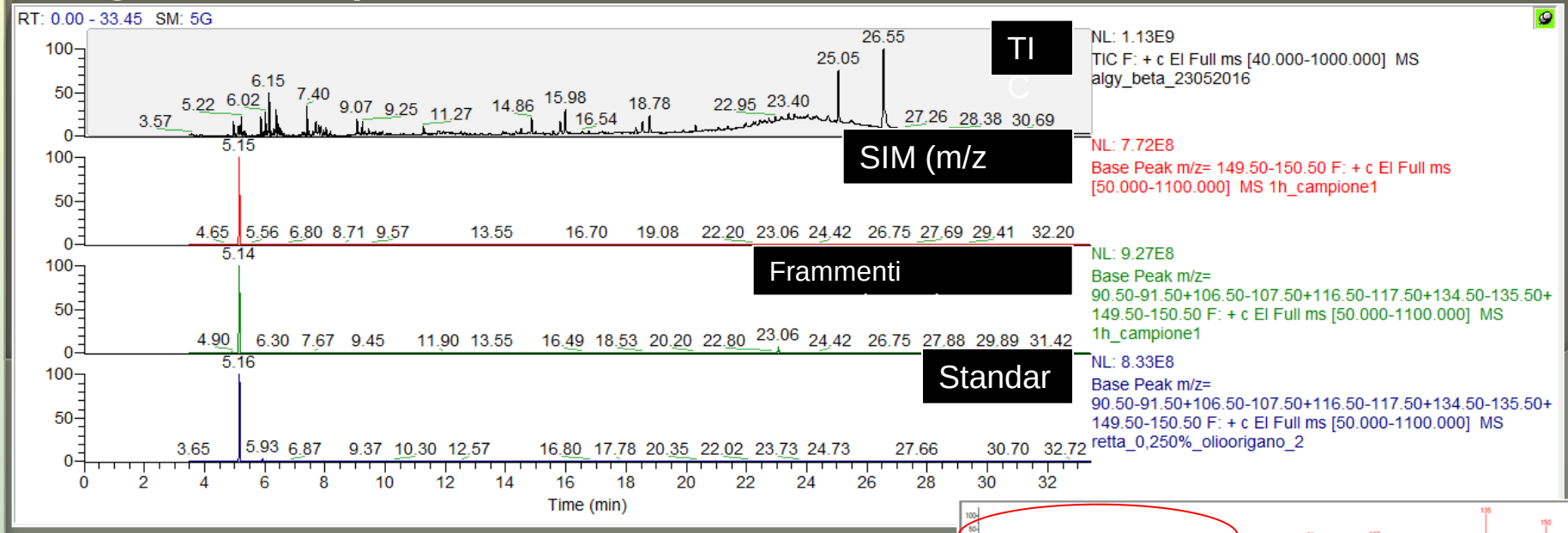
Timolo

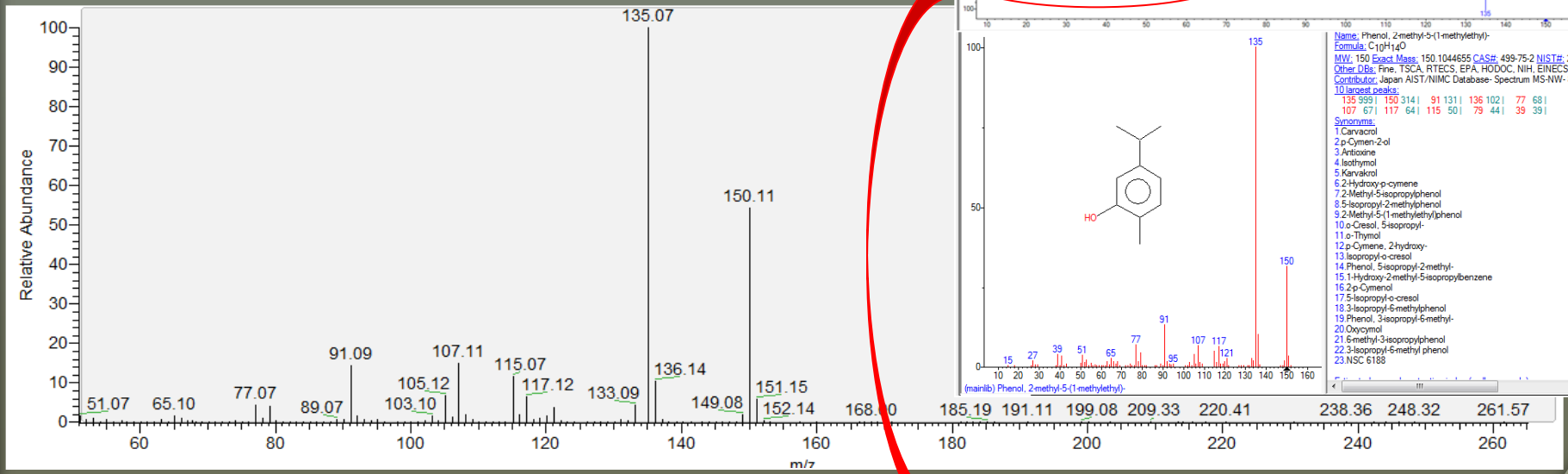


1,8 cineolo

Packagi ng Attivo

Carvacrolo rilasciato da un film edibile a contatto con un alimento (pasta sfoglia all'uovo)





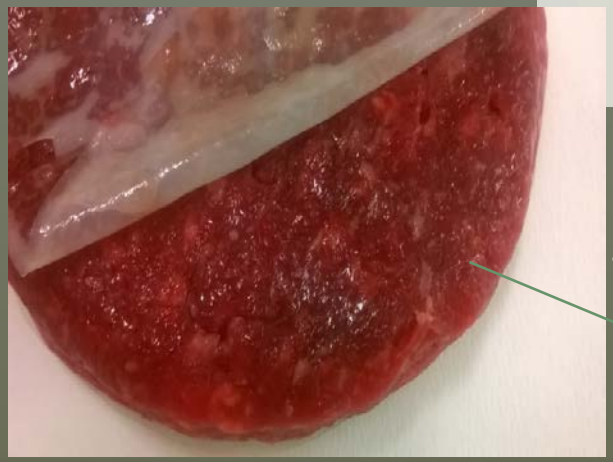
#	L.	M...	R.Match	Prob. (%)	Name
⊞1	M.	905	905	23.0	Phenol, 2-methyl-5-(1-methylethyl)-

Ipotesi costituzione Gruppo Operativo - Misura 16.1 PSR

Cellophan

Film edibile ottimizzato

Film edibile ottimizzato: differente ossidazione della carne ed effetto swelling del film



Confronto dei due film dopo 78 ore di conservazione in un frigorifero domestico

Porzione di carne sottostante il film

Ipotesi costituzione Gruppo Operativo - Misura 16.1 PSR

Packaging Attivo

Ipotesi costituzione Gruppo Operativo - Misura 16.1 PSR

Packaging Attivo

HMI High Materials Innovation Spin-off Parma University

Grazie per l'attenzione !

Cipack Centro Interdipartimentale per il Packaging