EW: “**Mass spectrometry in support of the environment, food, and health interaction and disease**”

Characterization of non-intentionally added substances in food packaging nanofilms by analytical approaches based on HRMS

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

INIA Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

María Dolores Hernando Guil

ERANET SUSFOOD: Sustainable food production

Title: Improved resource efficiency throughout the post-harvest chain of fresh-cut fruits and vegetables

The Fruit and Vegetable Sector is an important segment of the European Agroindustry, with a weight of about 18% of the value of EU agricultural production

Fresh cut products:
This sector is a major user of water; about 70% of the water consumption is for cleaning and decontamination

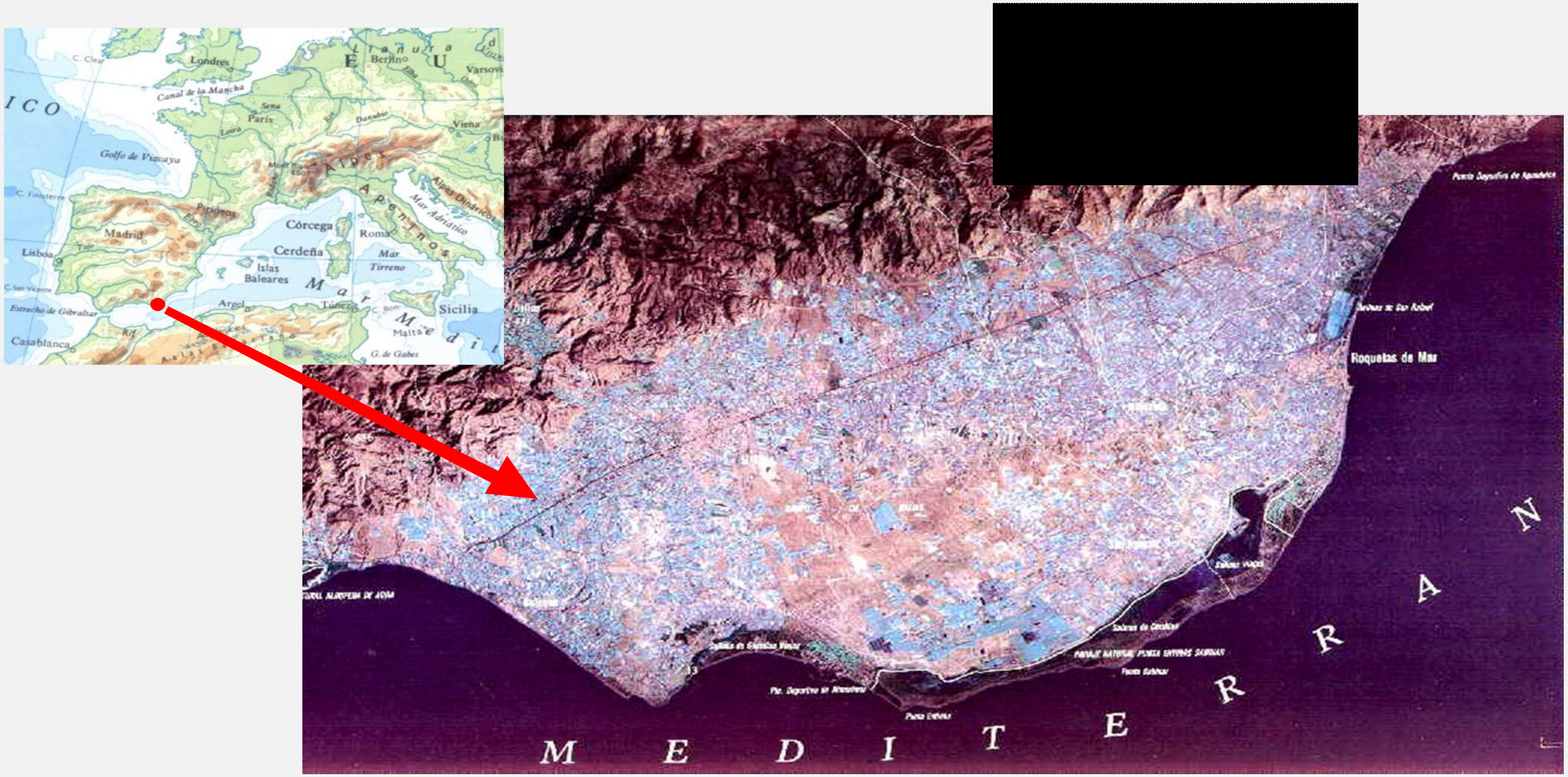
Proper disinfection technology is required to ensure microbial safety avoiding at the same time the formation of disinfection by-products such as chlorinated chemicals

Growth of microorganisms is favoured by cutting/slicing, which remove the natural barrier

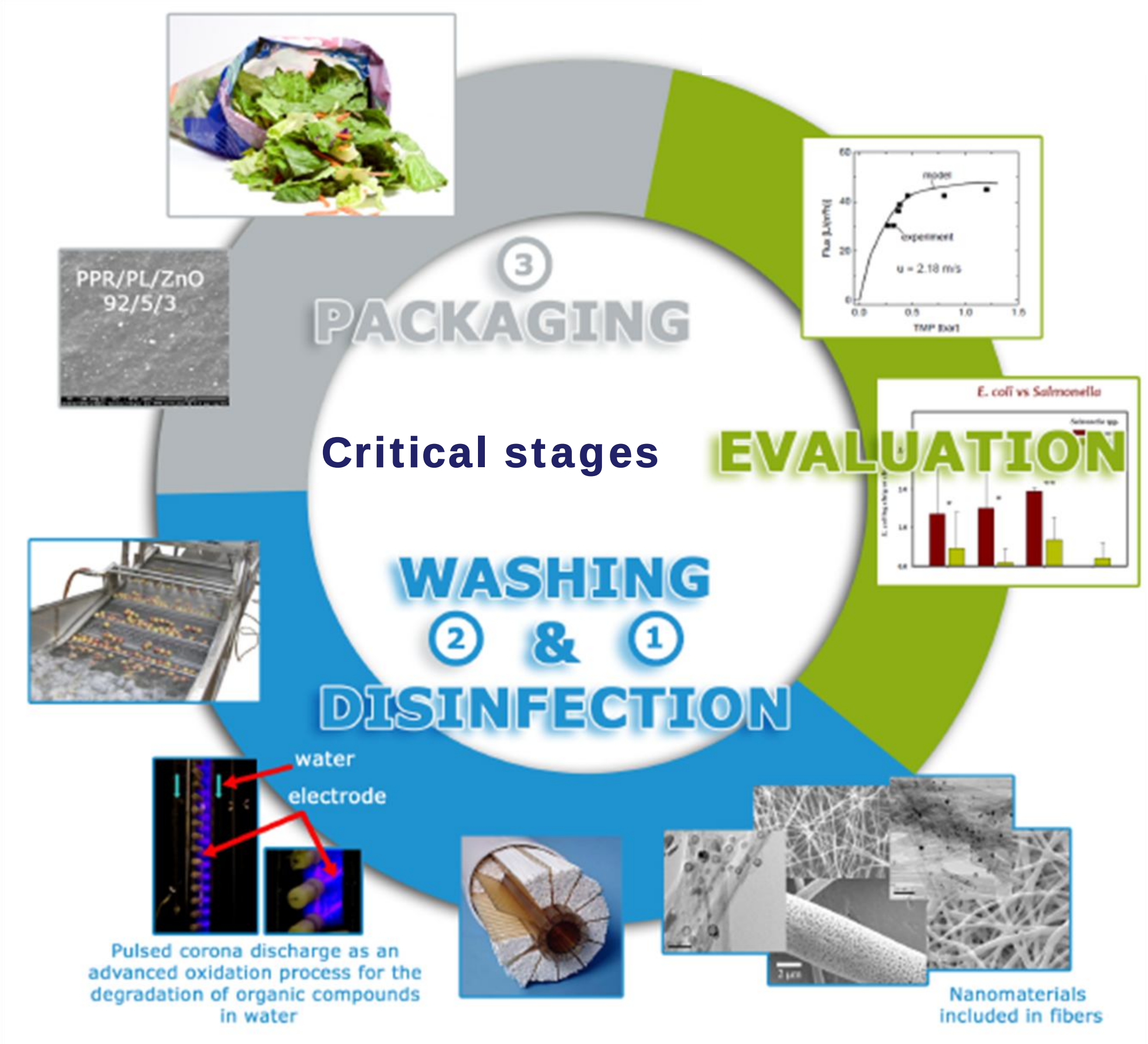
The quality of fresh-cut products also depends critically on packaging technology, which has to preserve good appearance and flavour, as well as meet safety requirement

Context

Exporters of food products.
Water stressed areas, Mediterranean basin.



washing technologies and packaging materials



Resource-efficient food processing:

- Water savings
- Reduce use of chemicals
- Valorization of food residues

Food quality & safety:

- Extension of shelf-life
- Avoid harmful disinfection by-products
- Good appearance and flavour

These goals are to be achieved by the combination of the following **nanotechnology-based solutions for fresh-cut products**:

Washing & desinfection:

Introduction of **new membranes with biocidal functionality** to allow operating in closed loop and the reduction or elimination of chemical disinfectants.

Development of a **hybrid technology** in which the use of **ozone** combined with **ultrafiltration** using ceramic membranes with (eventually) active surface.

Shelf-life improvement:

Development of **packaging materials with antimicrobial** components, to increase shelf life and, to reduce wastes.

CEREAL

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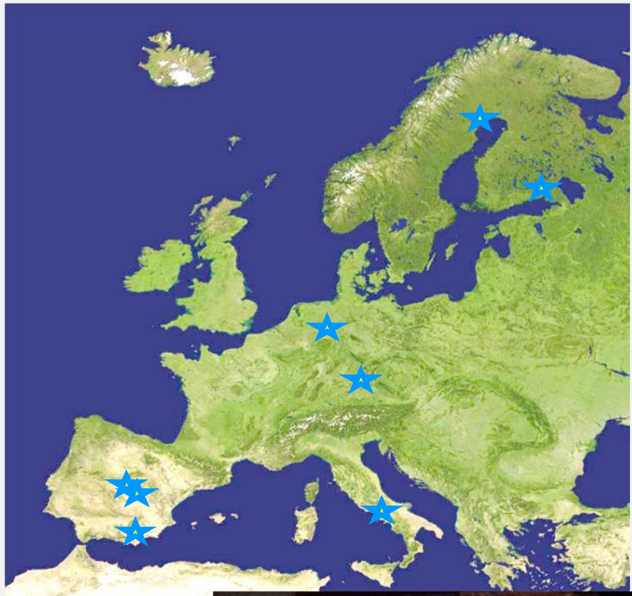

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LULEÅ UNIVERSITY OF TECHNOLOGY

Fraunhofer IVV
Institute for Process Engineering and Packaging
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Partners

CEREAL approach

To design the new packaging materials mainly monolayers by directing the structure and properties of the polymer matrix during crystallisation taking into consideration the material characteristics, the nanoparticles shape (rod shape and plate-like), the conditions of the manufacturing packaging process (extrusion and blowing).

To develop materials having improved physical, mechanical, barrier and antibacterial activity properties that contribute to extend shelf-life and quality of the food

Development of industrial scale processes for the synthesis and the post-treatments of metal oxide nano-powders, and fabrication of nanocomposites by melt processing.

Packaging materials

New Food Packaging Nanomaterials (FPN)

- **Selection** Matrices/Nanoparticles
- **Optimization** Processing
- **Assessment** Packaging properties
- **Assessment** Quality/Safety of Food; : Recyclibility/Degradability Properties

Consiglio Nazionale delle Ricerche

1. **Development**
2. **Optimization of the processing conditions**
3. **Assessment of properties of the materials :**
   filler/matrix interactions, thermal stability and rheological behaviour of the nanocomposites, optical properties, UV- absorption and degradation upon illumination, thermal and mechanical properties and processability
4. **Evaluation of new materials**
   - *Migration of substances*
   - *Antimicrobial activity, shelf life prolongation and impact on fresh-cut produce quality. Relevant test strains: Listeria monocytogenes, E. coli, Pseudomonas fluorescens, Bacillus subtilis (spores) and Aspergillus niger (spores).*

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Polypropylene –based nanocomposites

(PP)-based nanocomposites functionalized with zinc oxide nanoparticles (ZnO NPs) and polylimonene (PL)

PPR3221 (wt%)	PL (wt%)	ZnONPs (wt%)	Composition
100	0	0	PPR
95	5	0	PPR/PL 95/5
97	0	3	PPR/ZnO 97/3
92	5	3	PPR/PL/ZnO 92/5/3

Biopolymer metal oxide nanocomposites

Nanocomposites :Poly(lactic acid) (PLA), PL, ZnO NPs, and ZnONPs coated with stearic acid

PLA (wt%)	PL (wt%)	ZnONPs (wt%)	mZnONPs (wt%)	Code
100	-	-	-	PLA
97	-	3	-	PLA/ZnO3%
95	-	5	-	PLA/ZnO5%
93	-	-	3	PLA/mZnO3%
95	-	-	5	PLA/mZnO5%
85	10	5	-	PLA/PL/ZnO

Packaging materials

Non-intentionally added substances (NIAS) are compounds **present in food contact materials** (FCM), **not added** for a technical purpose during manufacture.

Relevant issue for the **food packaging industry**

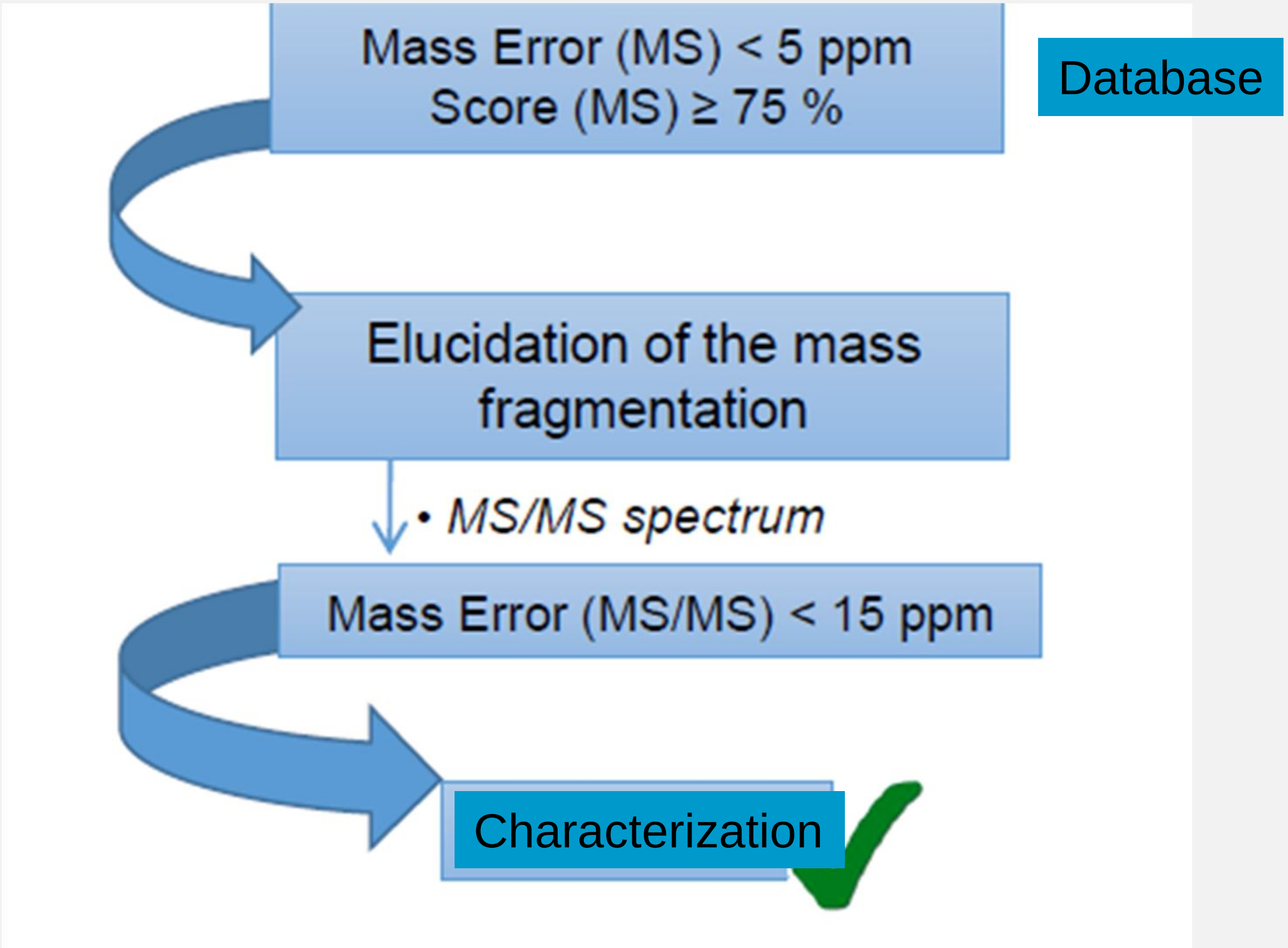
Impurities - bulk materials

Reaction intermediates formed during manufacture

Degradation of products.

Contaminants

LC-HRMS



Mass Error (MS) < 5 ppm
Score (MS) ≥ 75 %
Database
Elucidation of the mass fragmentation
• MS/MS spectrum
Mass Error (MS/MS) < 15 ppm
Characterization

Test conditions for overall migration

Test	Expected contact with food	Migration test conditions
OM1	Contact with frozen and cooled food	10 days @ 20°C
OM2	Long storage at room temperature + short heat-ing	10 days @ 40°C
OM3	Short heating	2 hours @ 70°C
OM4	High temperature use	1 hour @ 100°C
OM5	High temperature use (up to 121°C)	2 hours @ 100°C Or 1 hour refluxing @ 121°C
OM6	Use of simulant A,B or C at a temperature of more than 40°C	4 hours @ 100°C or 4 hours refluxing
OM7	Use of fatty food at a high temperature	2 hours @ 175°C

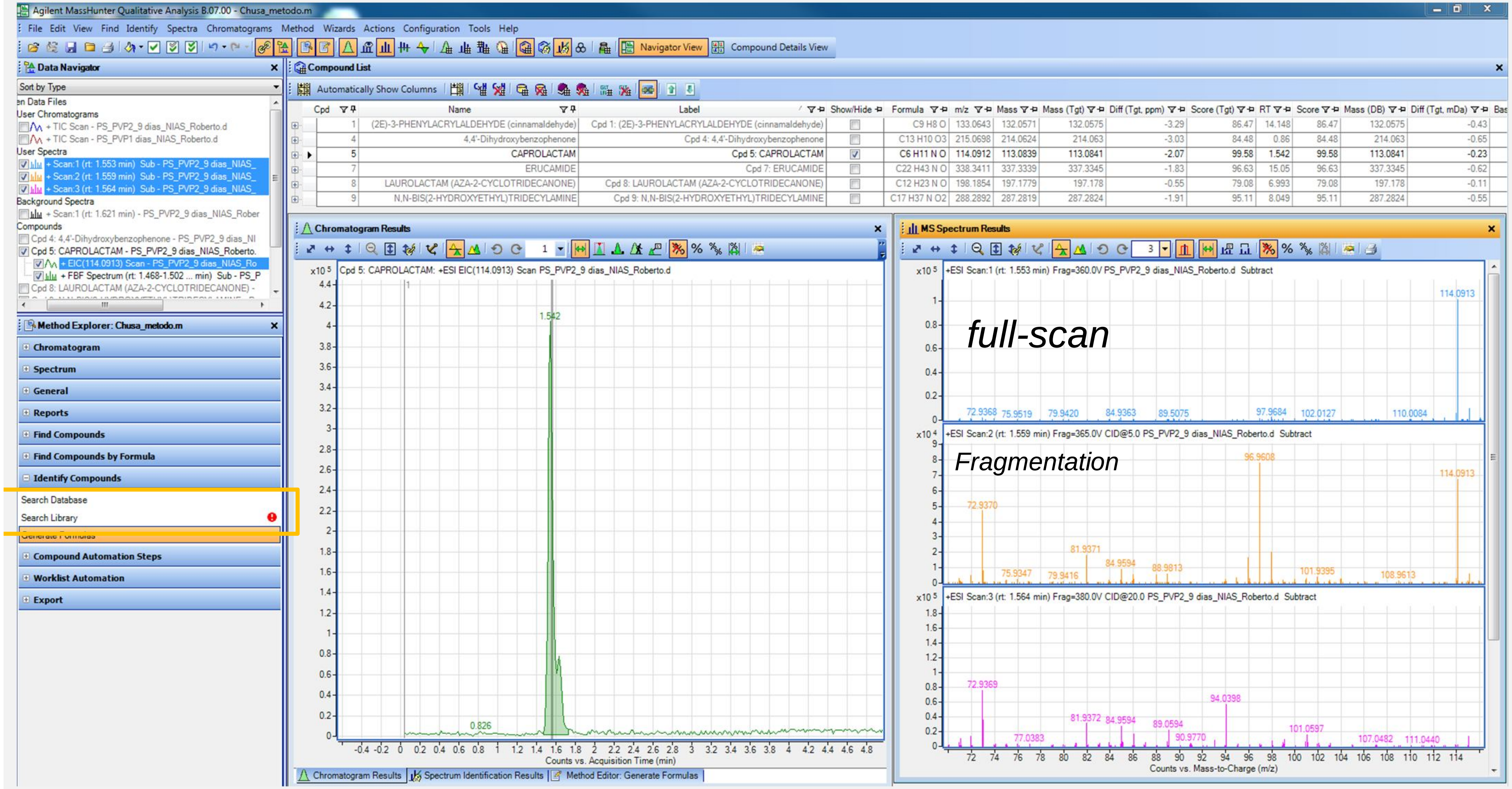
Simulant	Abbreviation
Ethanol 10% (v/v)	Simulant A
Acetic acid 3% (w/v)	Simulant B
Ethanol 20% (v/v)	Simulant C
Ethanol 50% (v/v)	Simulant D1
Vegetable Oil	Simulant D2
Modified polyphenylene oxides, particle size 60-80 mesh, pore size 200 nm	Simulant E for dry foods

- Framework Regulation (EC) No 1935/2004
  - (EC) No 2023/2006 Good Manufacturing Practice
  - 2007/42/EC Regenerated cellulose film
  - 84/500/EEC Ceramics, as amended
  - 1895/2005/EC BADGE/BFDGE/ NOGE
  - Plastics
    - (EU) No 321/2011 restriction of use of Bisphenol A in plastic infant feeding bottles
    - (EU) No 10/2011 Plastics Implementation Measure, as amended/corrected
    - (EC) No 282/2008 Recycled plastics
    - (EU) No 284/2011 polyamide and melamine plastic kitchenware
  - (EC) No 450/2009 Active and intelligent materials
  - 93/11/EEC Nitrosamines and nitrosatable substances

EU legislation

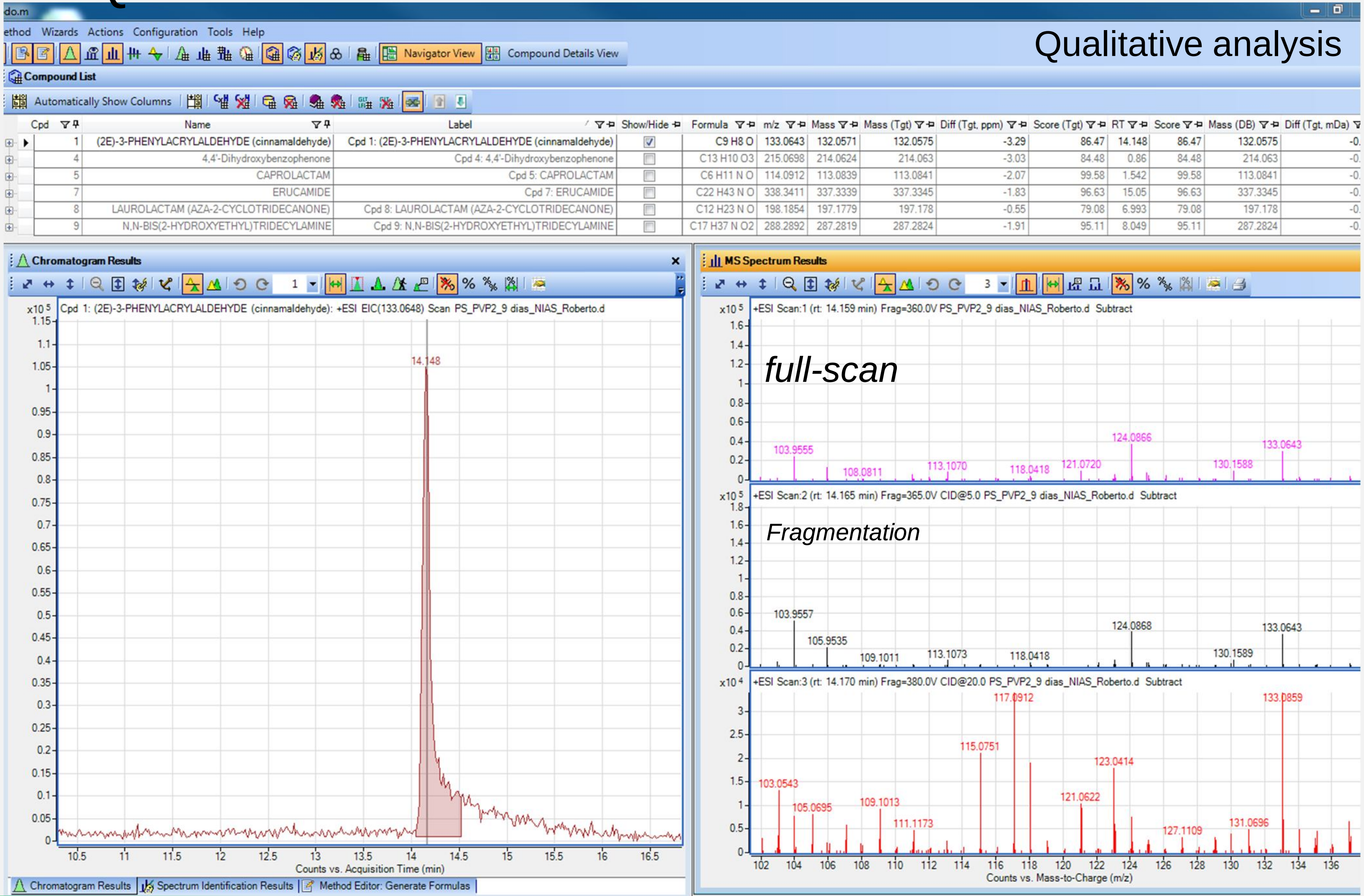
LC-QTOF-MS

Qualitative analysis



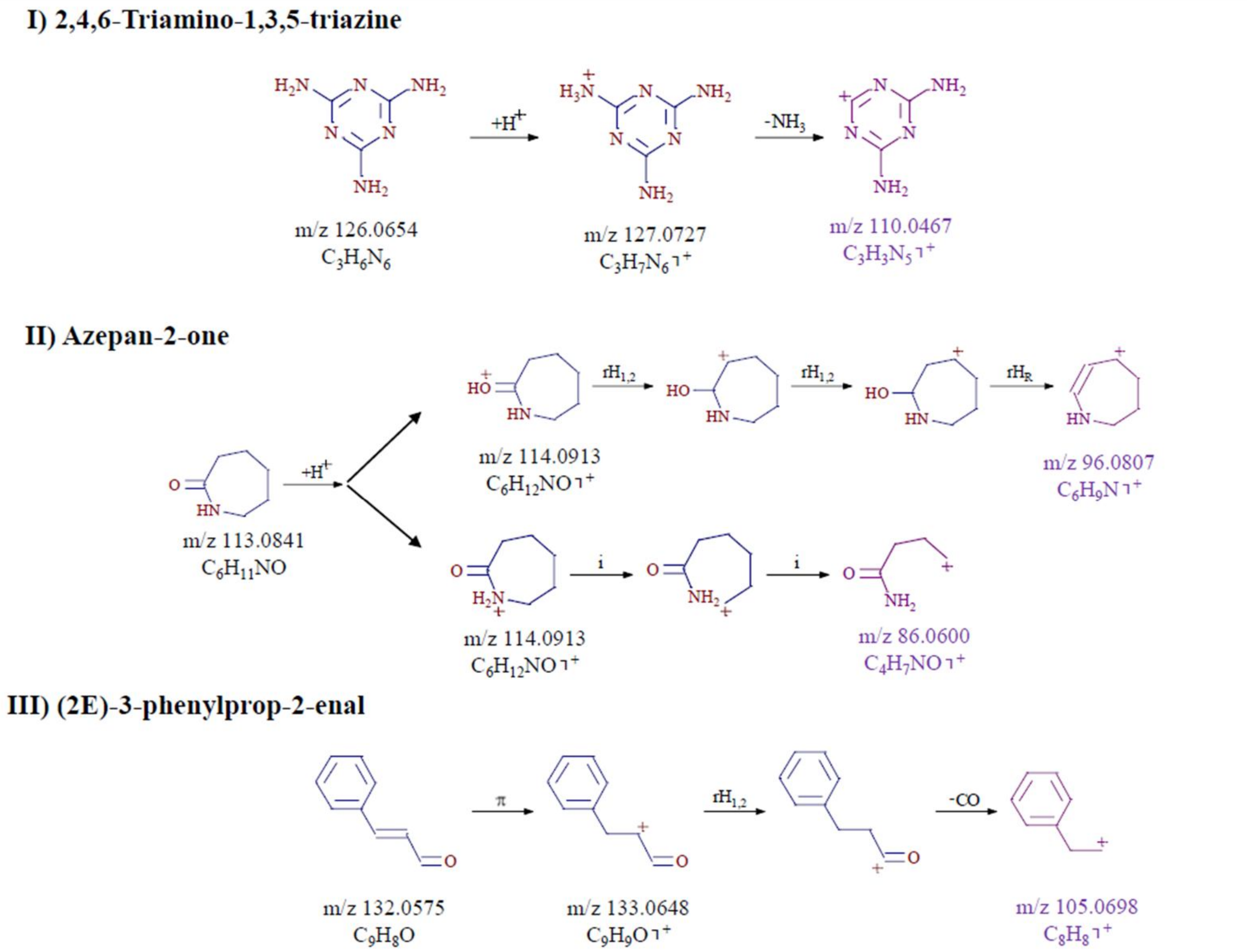
Screening analysis

LC-QTOF-MS



Screening analysis

Simulation of fragmentation



***rH:** Charge-Site Rearrangement; **i:** inductive cleavages; **π:** π-Bond Dissociation*

Tentative assignation of fragment ions

LC-QTOF-MS

Polypropylene –films

RT	Molecular ion	Accurate mass [H$^+$] (m/z)	Error (ppm), scope (%)	Product ion (loss)	Accurate mass [H$^+$] (m/z)	Error (ppm)	NIAS (tentative identification)
Simulant A							
0.8	$C_3H_6N_6$	127.0727	−0.82 ppm, >86%	$C_3H_3N_5$ (–NH_3)	110.0467	−2.7	2,4,6-Triamino-1,3,5-triazine
1,5	$C_6H_{11}NO$	114.0913	−0.70 ppm, >98%	C_6H_9N (–H_2O)	96.0807	−8.9	Azepan-2-one
				C_4H_7NO (–C_2H_4)	86.0600	−8.0	
8,9	C_9H_8O	133.0648	1.50 ppm, >76%	C_8H_8 (–CO)	105.0698	5.0	(2*E*)-3-Phenylprop-2-enal
Simulant B							
0.8	$C_3H_6N_6$	127.0727	2.5 ppm, >65%	$C_3H_3N_5$ (–NH_3)	110.0467	9.0	2,4,6-Triamino-1,3,5-triazine
1,5	$C_6H_{11}NO$	114.0913	−1.35 ppm, >85%	C_6H_9N (–H_2O)	96. 0807	−9.7	Azepan-2-one
				C_4H_7NO (–C_2H_4)	86.0600	7.6	
8,9	C_9H_8O	133.0648	0.3 ppm, >76%	C_8H_8 (–CO)	105.0698	7.9	(2*E*)-3-Phenylprop-2-enal

Data processing LC-QOrbitrap-MS

Compounds | Compounds per File | Merged Features | Features | Custom Explanations | ChemSpider Results | Mass List Search Results

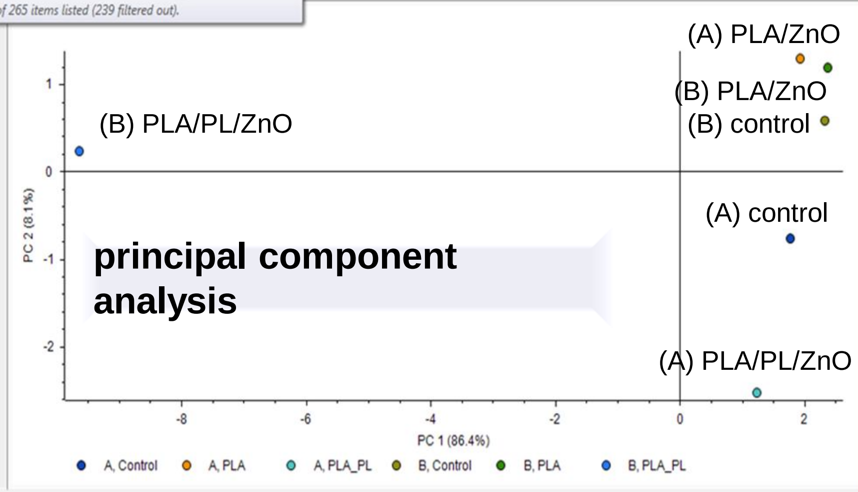
	Checked	Predicted Formula	Molecular Weight	RT [min]	# ChemSpider Results	Area (Max.)	Group Areas: Control, F1, A	PLA, F3, A	PLA_PL, F4, A	PLA, F5, B	PLA_PL, F6, B*	Control, F7, B	Log2 Fold Char: (Control, F1, A) / (Control, F7, B)	(PLA_PL, F4, A) / (PLA, F3, A)	(PLA_PL, F6, B) / (PLA, F5, B)*
1		C11 H21 N4 P S3	336.06740	9.799	3	119314	1.06e4	1.17e4	1.49e4	1.10e4	1.19e5	8.55e3	0.31	0.35	3.43
2		C17 H35 N O	269.27156	11.975	4	97521	2.05e4	2.10e4	2.64e4	9.71e3	9.75e4	2.13e4	-0.05	0.33	3.33
3		C9 H18 S3	222.05626	9.799	2	285715	2.62e4	3.44e4	2.94e4	2.99e4	2.86e5	2.66e4	-0.03	-0.23	3.26
4		C6 H16 Cl N2 O5 P	262.04869	8.322	4	228734	2.00e4	2.10e4	2.21e4	2.66e4	2.29e5	1.86e4	0.10	0.07	3.10
5		C9 H18 S3	222.05626	8.318	2	461044	4.05e4	4.44e4	4.67e4	5.64e4	4.61e5	3.31e4	0.29	0.07	3.03
6		C6 H17 N8 P S2	296.07494	9.803	2	164875	1.77e4	2.00e4	2.17e4	2.02e4	1.65e5	1.48e4	0.26	0.12	3.03
7		C22 H43 N O2	353.32916	11.837	9	212010	2.75e4	3.08e4	2.64e4	2.73e4	2.12e5	2.47e4	0.16	-0.22	2.96
8		C20 H37 N7 O2	407.30125	12.217	0	771023	1.49e5	1.44e5	1.09e5	1.04e5	7.71e5	1.25e5	0.25	-0.39	2.90
9		C6 H17 N8 P S2	296.07494	10.778	2	180076	3.16e4	2.58e4	3.13e4	2.48e4	1.80e5	3.60e4	-0.19	0.28	2.86
10		C14 H29 N O	227.22481	11.027	9	234816	2.98e4	2.91e4	3.34e4	3.25e4	2.35e5	3.24e4	-0.12	0.20	2.86
11		C11 H20 N10 S3	388.10417	10.779	1	225895	3.50e4	3.38e4	3.71e4	3.18e4	2.26e5	3.11e4	0.17	0.13	2.83

Show Related Tables

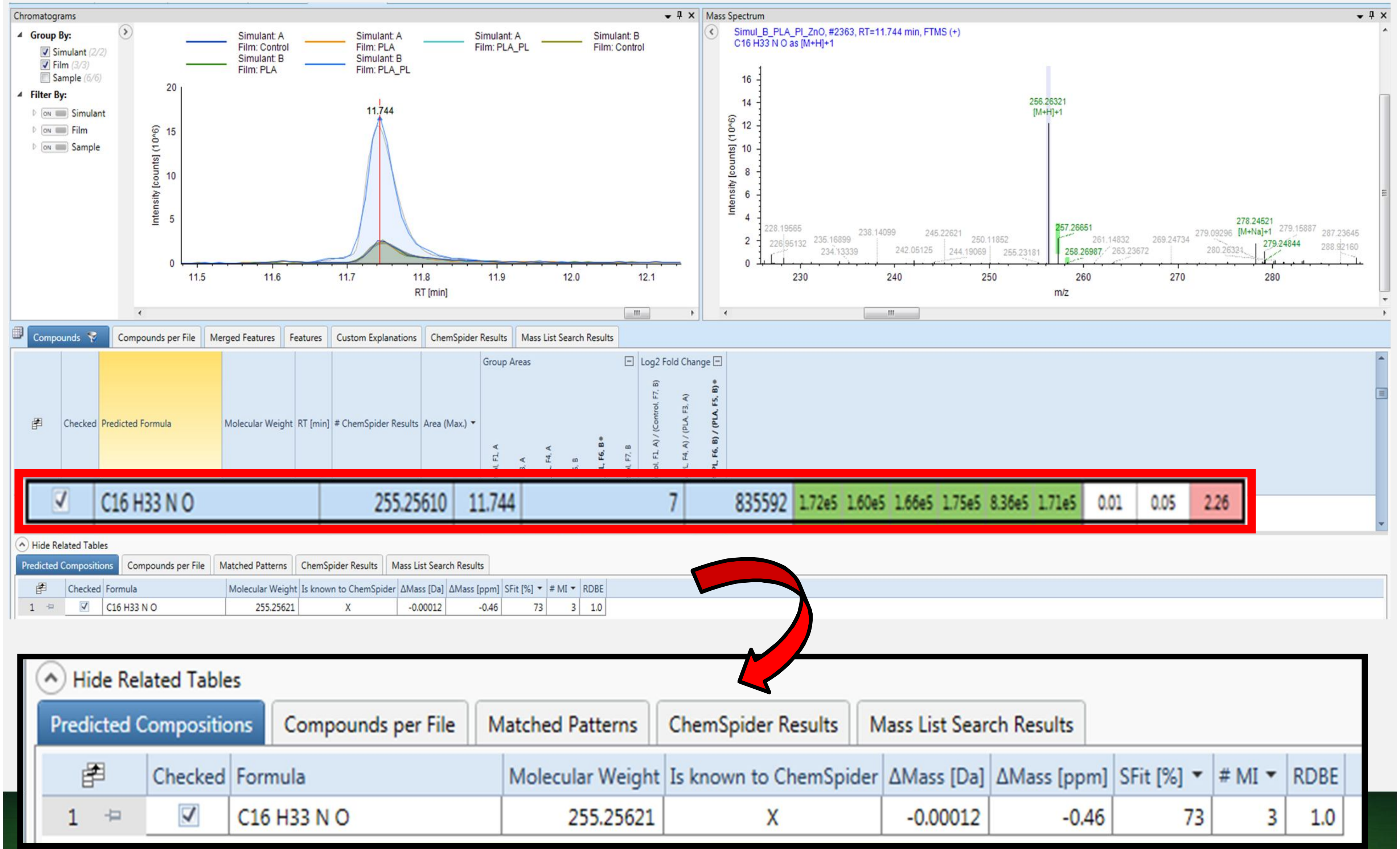
Principal Component Analysis

Data Source: Compounds X Data: PC 1 Y Data: PC 2 Center and scale Use normalized areas

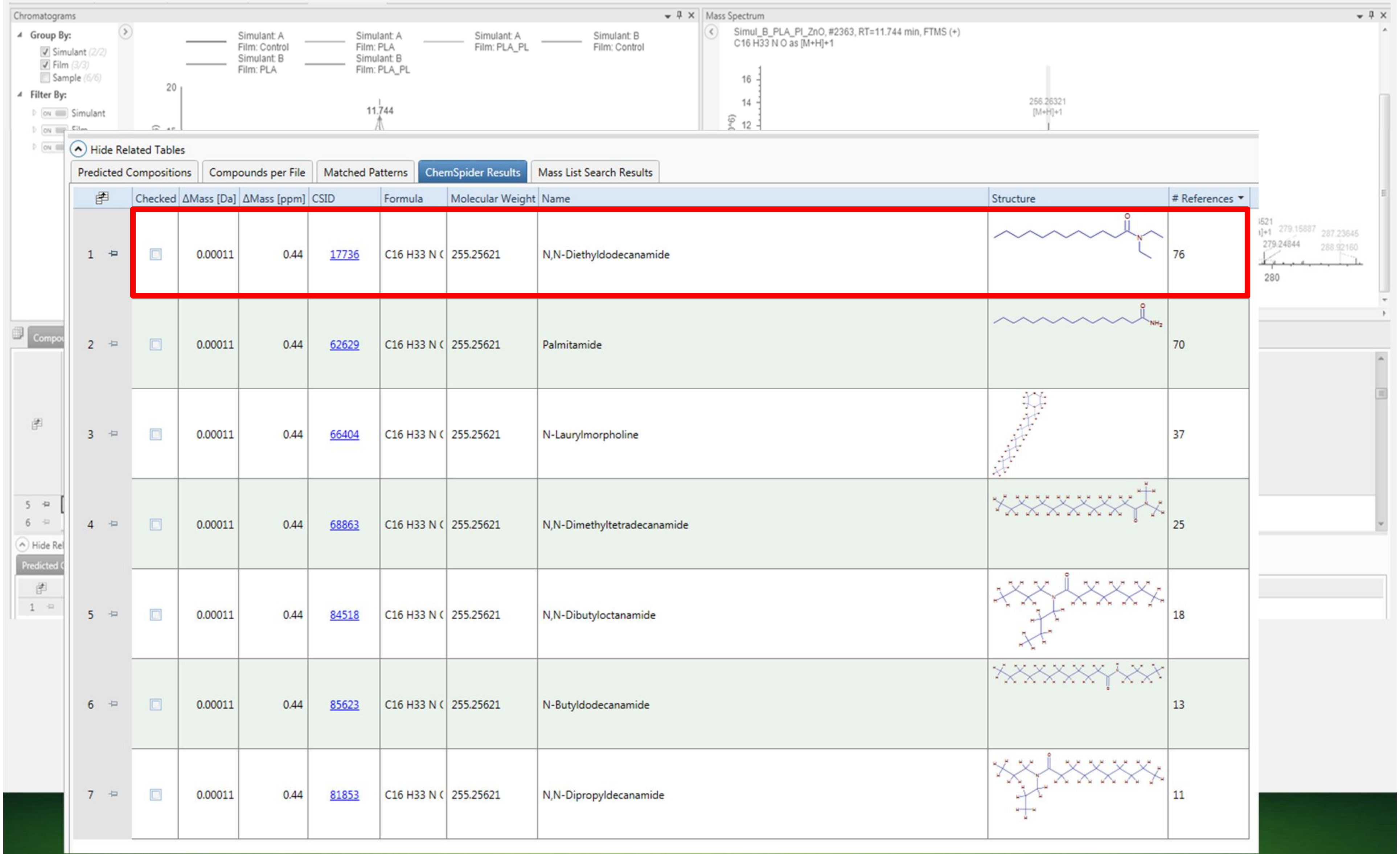
Compounds grouped by molecular weight and retention time
26 of 265 items listed (239 filtered out).



structural elucidation



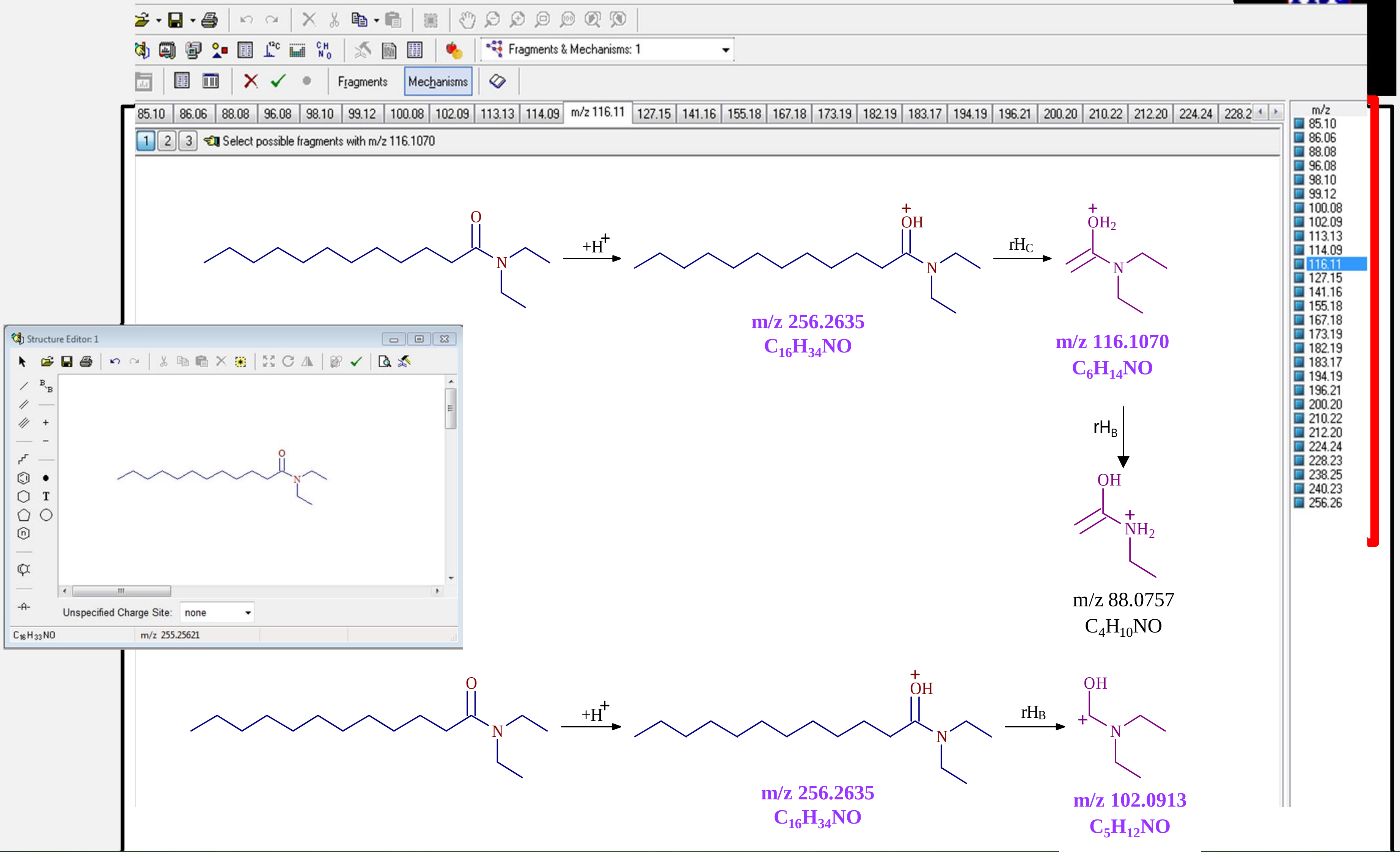
N,N-Diethyldodecanamide



	Checked	ΔMass [Da]	ΔMass [ppm]	CSID	Formula	Molecular Weight	Name	Structure	# References
1	☐	0.00011	0.44	17736	C16 H33 N (	255.25621	N,N-Diethyldodecanamide		76
2	☐	0.00011	0.44	62629	C16 H33 N (	255.25621	Palmitamide		70
3	☐	0.00011	0.44	66404	C16 H33 N (	255.25621	N-Laurylmorpholine		37
4	☐	0.00011	0.44	68863	C16 H33 N (	255.25621	N,N-Dimethyltetradecanamide		25
5	☐	0.00011	0.44	84518	C16 H33 N (	255.25621	N,N-Dibutyloctanamide		18
6	☐	0.00011	0.44	85623	C16 H33 N (	255.25621	N-Butyldodecanamide		13
7	☐	0.00011	0.44	81853	C16 H33 N (	255.25621	N,N-Dipropyldecanamide		11

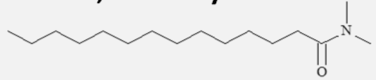
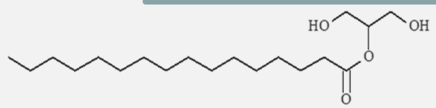
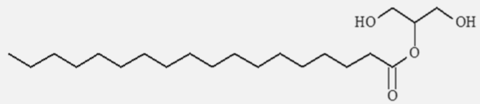
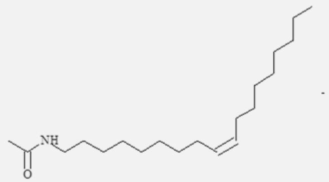
Tentative fragmentation pathway

N,N-Diethyldodecanamide



LC-QOrbitrap-MS

Biopolymer metal oxide nanocomposites

PRECURSOR ION				FRAGMENT IONS			
Rt	accurate mass value $[M+H]^+$	formula proposed $[M+H]^+$	mass deviation* (ppm)	accurate mass value (*m/z*)	formula proposed	mass deviation* (ppm/mDa)	Candidate compounds
11.7	256.2635	$C_{16}H_{34}NO$	-1.2	116.1070 102.0913	$C_6H_{14}NO$ $C_5H_{12}NO$	-2.6 / 0.3 -1.4 / 0.1	**N,N-Diethyldodecanamide** 
11.9	331.2843	$C_{19}H_{39}O_4$	-1.0	313.2737 99.0441	$C_{19}H_{37}O_3$ $C_5H_7O_2$	-2.3 / 0.7 -5.0 / 0.5	**1-Palmitoylglycerol** 
12.2	359.3156	$C_{21}H_{43}O_4$	-0.7	341.3050 285.2788	$C_{21}H_{41}O_3$ $C_{18}H_{37}O_2$	-0.6 / 0.2 -2.0 / 0.6	**Glycerol stearate** 
12.5	310.3104	$C_{20}H_{40}NO$	-0.9	268.2999 210.1852	$C_{18}H_{38}N$ $C_{13}H_{24}NO$	0.2 / 0.1 -3.4 / 0.7	**N-[(9Z)-9-Octadecen-1-yl]acetamide** 

Characterization of NIAS; use of standards. Simulant B: 2.7 – 7.6 $ng.g^{-1}$

GC-HRMS

Deconvolution

- Min. intensity signals: 2e6
- Mass Error (MS) : 5 ppm
- Signal to noise: 10
- Ion overlap window: 98
- RT Alignment: 10 sec

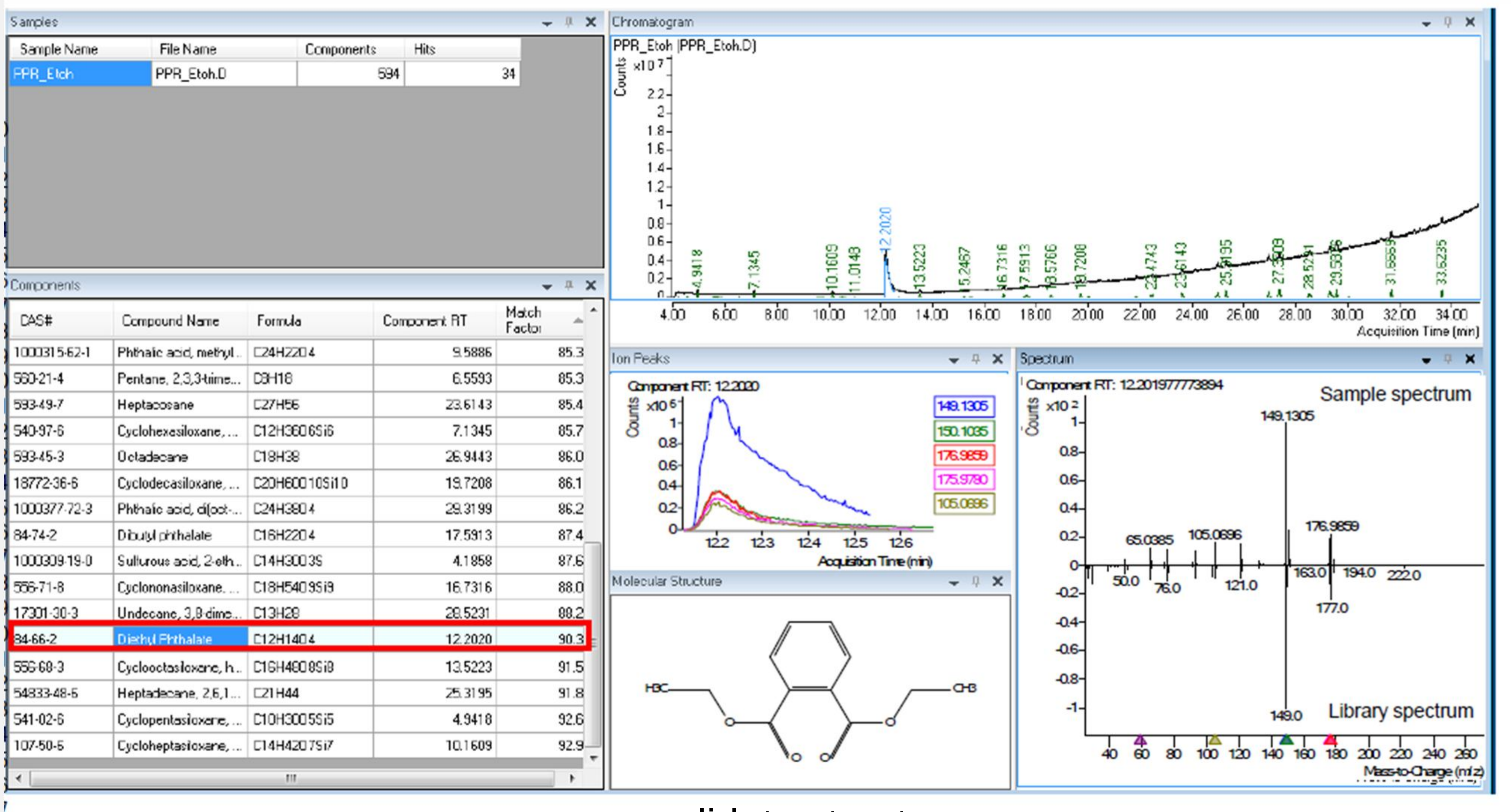
Mass spectral library
(250.000 chemicals)

- # Top matches: 4
- **SI Threshold: 800**
- **Score Threshold: 95**

Characterization

GC-QTOF-MS

Characterization NIAS in PP films

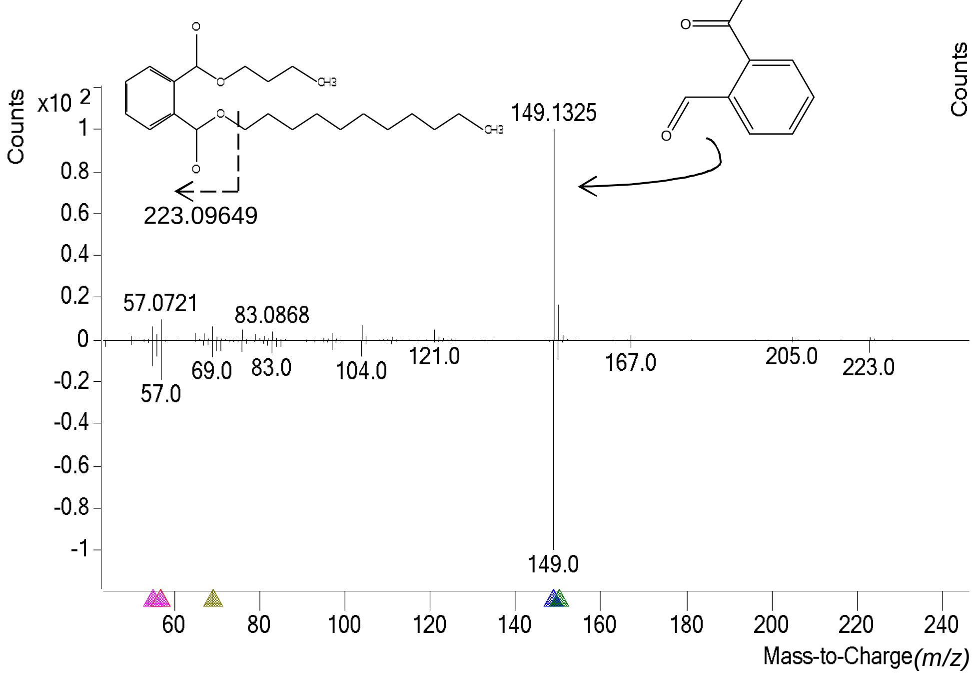


candidate structure
of the parent compound

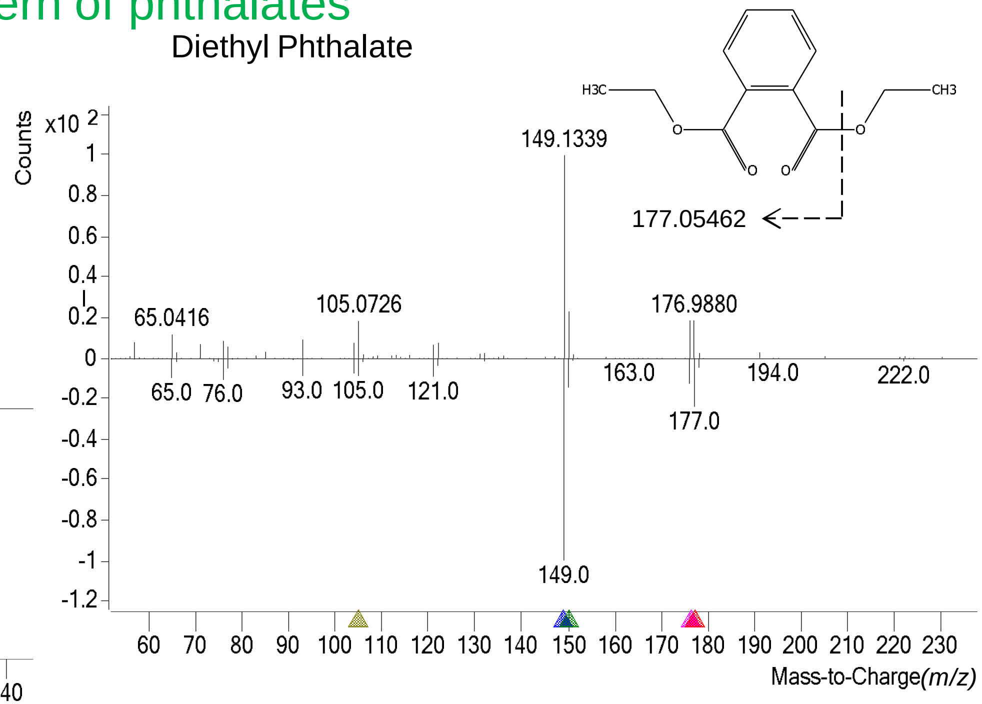
Phthalates

Fragmentation pattern of phthalates

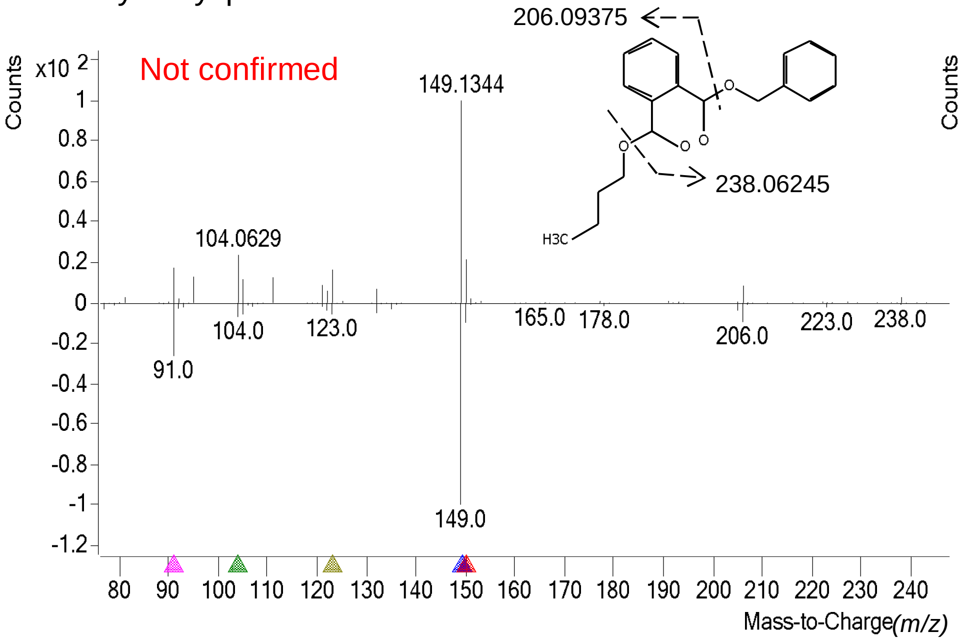
Phthalic acid, butyl undecyl ester



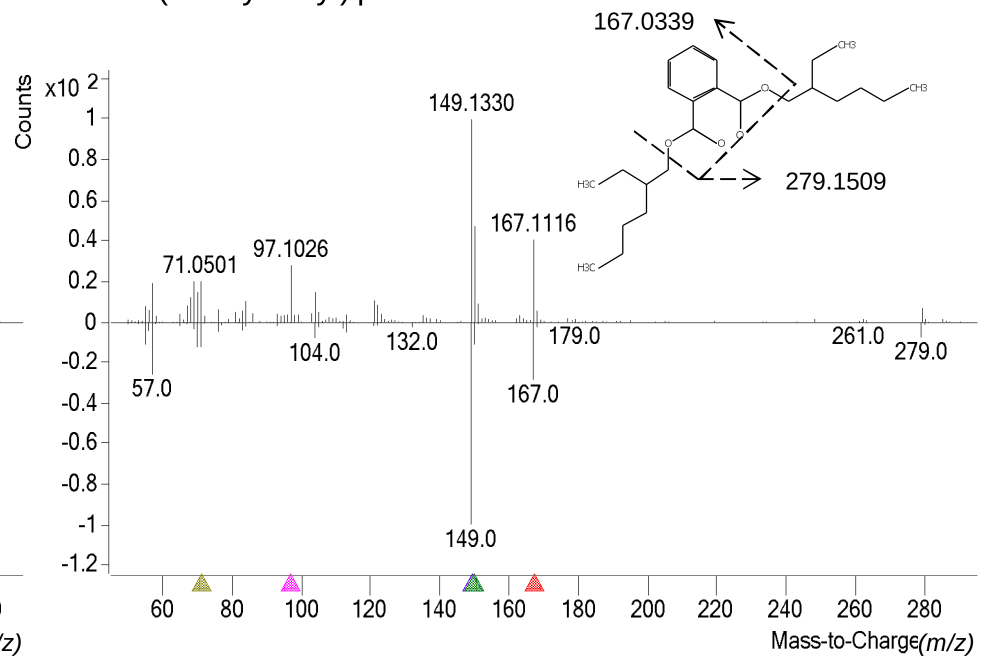
Diethyl Phthalate



Benzyl butyl phthalate

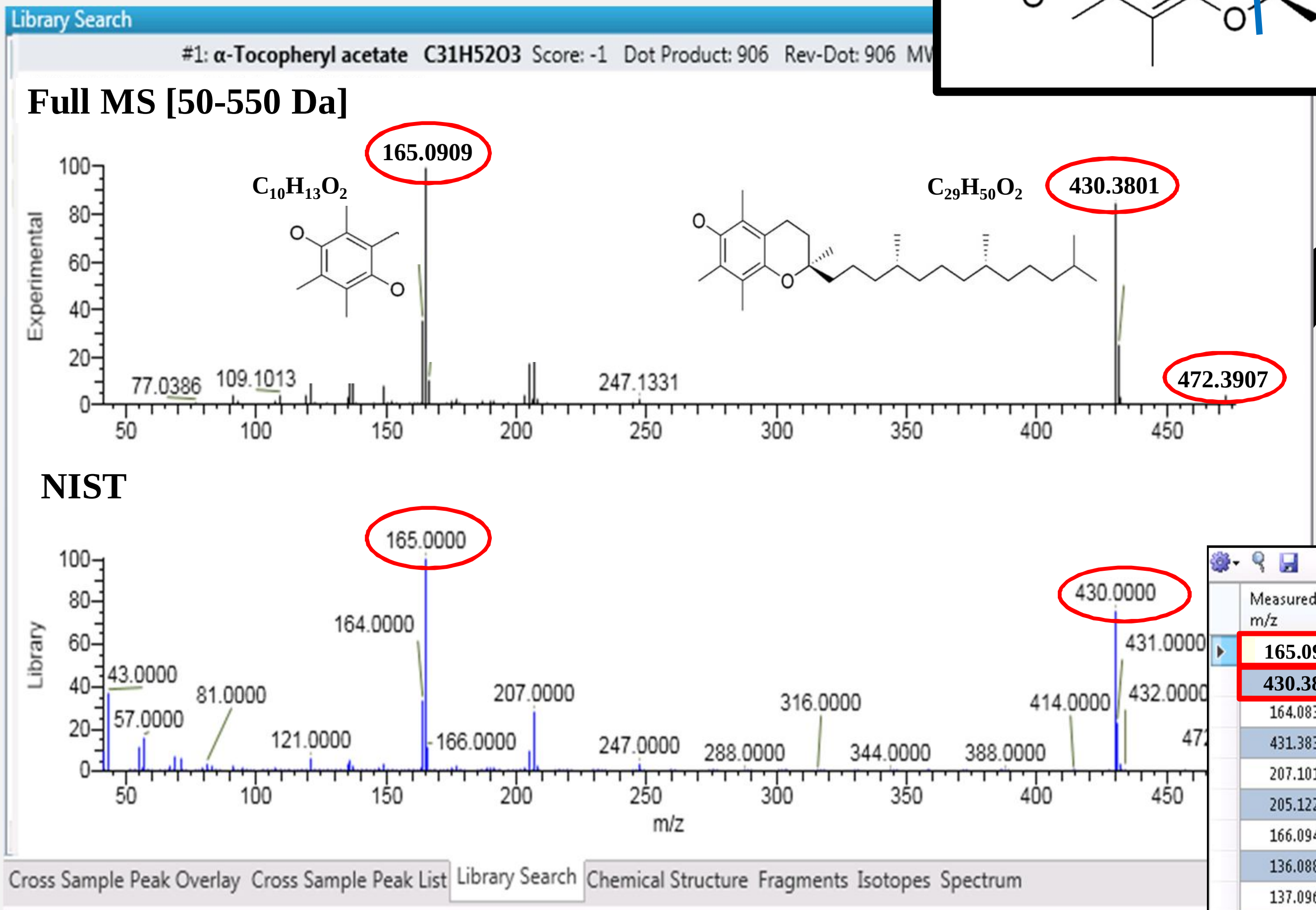


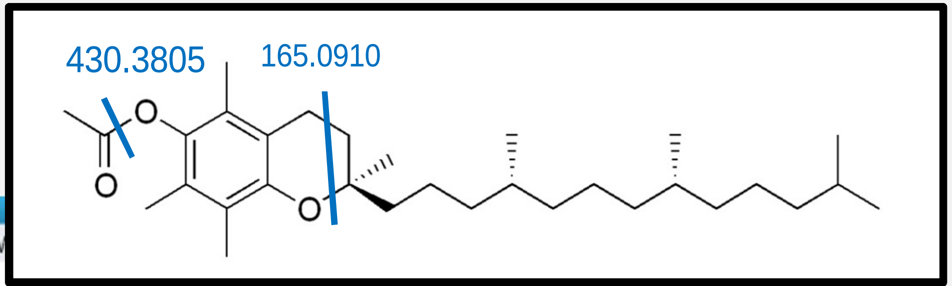
Bis(2-ethylhexyl) phthalate



GC-QOrbitrap-MS

α-Tocopherol acetate

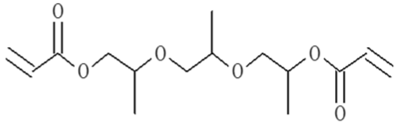
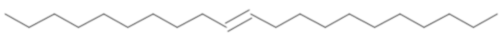
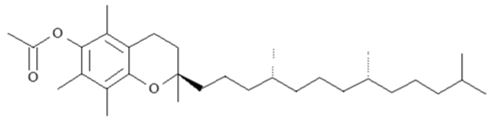




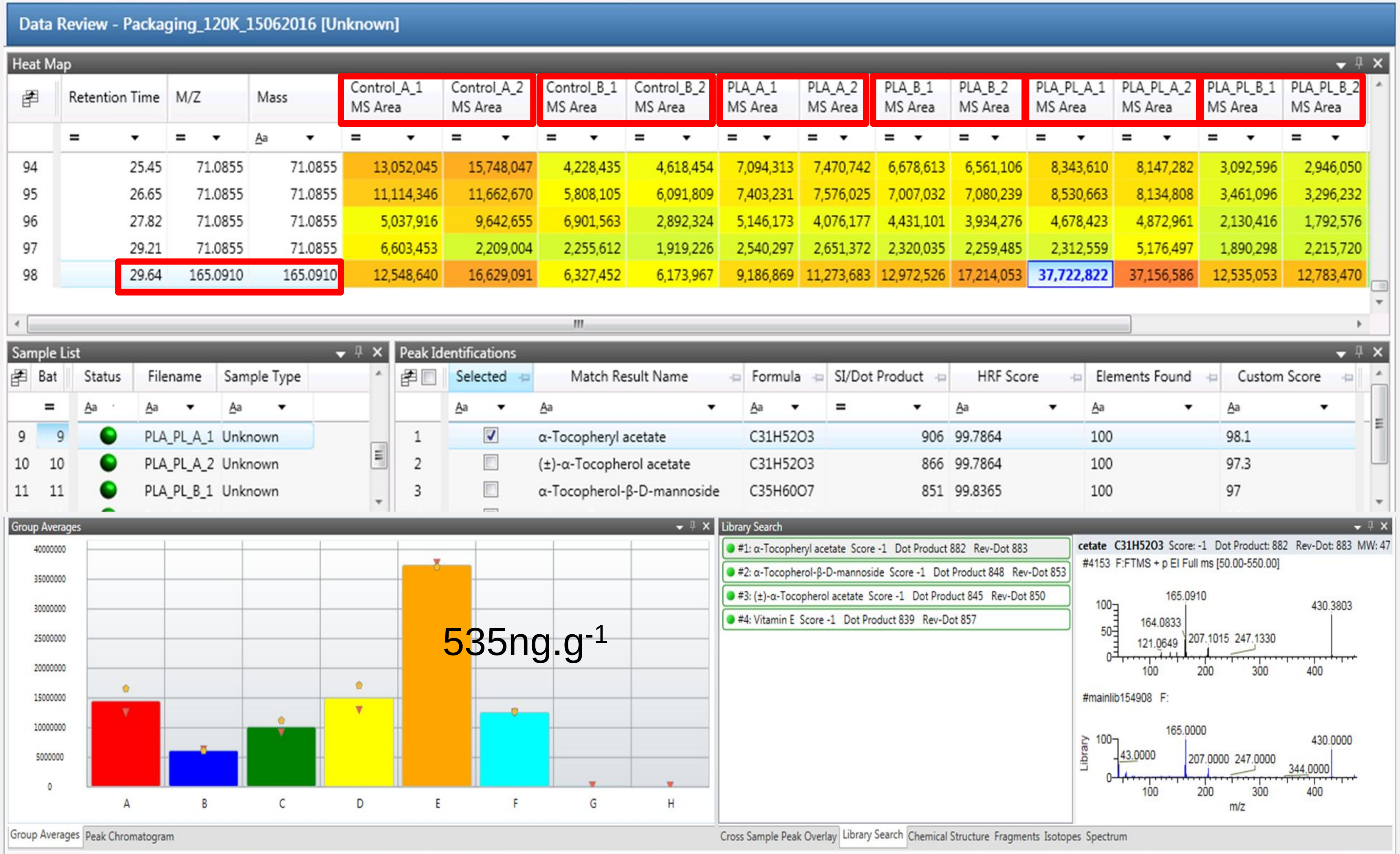
Measured m/z	Area	Height	Fragment ID	Theo m/z	Mass error (ppm)
165.0909	37836558	8750640	C(12)10 H13O2	165.091006	**0.109**
430.3801	31673161	7420216	C(12)29 H50O2	430.380532	**0.871**
164.083267	13554781	3110982	C(12)10 H12O2	164.08318	0.530219
431.383667	9556090	2225774	C(12)28 C(13)1 ...	431.383887	0.509523
207.101486	7495847	1764741	C(12)12 H15O3	207.10157	0.405598
205.122345	6666276	1556613	C(12)13 H17O2	205.122306	0.19013
166.094376	3863970	911630	C(12)9 C(13)1 H...	166.094361	0.091514
136.088409	3858639	878135	C(12)9 H12O	136.088266	1.050788
137.096222	3664361	844437	C(12)9 H13O	137.096091	0.955533
121.064934	3545749	822305	C(12)8 H9O	121.064791	1.181184

GC-QOrbitrap-MS

PLA films

PRECURSOR ION						FRAGMENT IONS			
Rt	accurate mass value (m/z)	formula proposed	SI*	score*	HRF*	accurate mass value (*m/z*)	formula proposed	mass deviation* (ppm/mDa)	candidate compounds
9.8	300.1567	$C_{15}H_{24}O_6$	827	96.6	98.0	113.0597 55.0178	$C_6H_9O_2$ C_3H_3O	1.4 / 0.2 1.6 / 0.1	**tripropylene glycol diacrylate** 
11.0	294.3281	$C_{21}H_{42}$	820	96.4	99.0	69.0699 83.0855	C_5H_9 C_6H_{11}	0.5 / 0.03 0.3 / 0.02	**10-Heneicosene** 
29.6	472.3911	$C_{31}H_{52}O_3$	898	97.5	99.8	430.3801 165.0909	$C_{29}H_{50}O_2$ $C_{10}H_{13}O_2$	0.9 / 0.4 0.8 / 0.1	**alpha-Tocopherol acetate** 

α-Tocopherol acetate



	Retention Time	M/Z	Mass	Control_A_1 MS Area	Control_A_2 MS Area	Control_B_1 MS Area	Control_B_2 MS Area	PLA_A_1 MS Area	PLA_A_2 MS Area	PLA_B_1 MS Area	PLA_B_2 MS Area	PLA_PL_A_1 MS Area	PLA_PL_A_2 MS Area	PLA_PL_B_1 MS Area	PLA_PL_B_2 MS Area
94	25.45	71.0855	71.0855	13,052,045	15,748,047	4,228,435	4,618,454	7,094,313	7,470,742	6,678,613	6,561,106	8,343,610	8,147,282	3,092,596	2,946,050
95	26.65	71.0855	71.0855	11,114,346	11,662,670	5,808,105	6,091,809	7,403,231	7,576,025	7,007,032	7,080,239	8,530,663	8,134,808	3,461,096	3,296,232
96	27.82	71.0855	71.0855	5,037,916	9,642,655	6,901,563	2,892,324	5,146,173	4,076,177	4,431,101	3,934,276	4,678,423	4,872,961	2,130,416	1,792,576
97	29.21	71.0855	71.0855	6,603,453	2,209,004	2,255,612	1,919,226	2,540,297	2,651,372	2,320,035	2,259,485	2,312,559	5,176,497	1,890,298	2,215,720
98	29.64	165.0910	165.0910	12,548,640	16,629,091	6,327,452	6,173,967	9,186,869	11,273,683	12,972,526	17,214,053	37,722,822	37,156,586	12,535,053	12,783,470

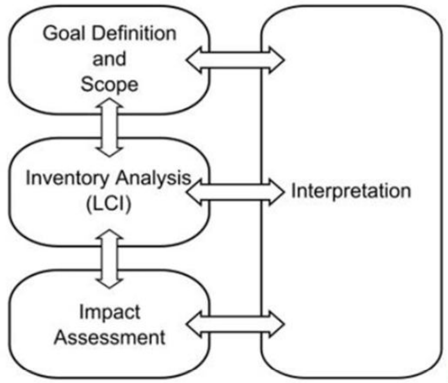
	Bat	Status	Filename	Sample Type
9	9		PLA_PL_A_1	Unknown
10	10		PLA_PL_A_2	Unknown
11	11		PLA_PL_B_1	Unknown

	Selected	Match Result Name	Formula	SI/Dot Product	HRF Score	Elements Found	Custom Score
1	☑	α-Tocopheryl acetate	C31H52O3	906	99.7864	100	98.1
2	☐	(±)-α-Tocopherol acetate	C31H52O3	866	99.7864	100	97.3
3	☐	α-Tocopherol-β-D-mannoside	C35H60O7	851	99.8365	100	97

- **Control,** simulant A
- **Control,** simulant B
- **PLA**, simulant A
- **PLA**, simulant B
- **PLA/PL,** simulant A
- **PLA/PL,** simulant B

Use of standards: Identification, quantification

Intro to LCA



- ISO 14040, 14044
- Functional Unit
- Cut-off criteria
- Foreground vs Background data

> LCA Philosophy

Environmental improvements are quantified by **comparing** the damages caused by a complete system using the newly developed technologies **against** reference systems **representative** from current-use technologies

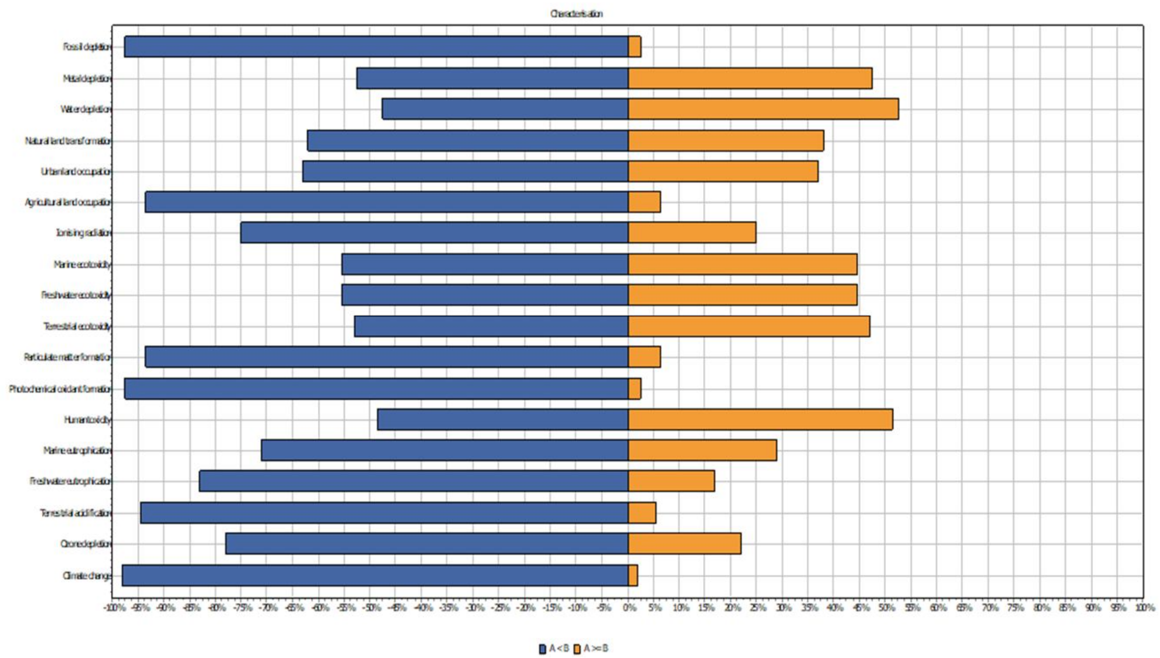
ENV. IMPACT CURRENT-USE TECHNOLOGIES − ENV. IMPACT NEW TECHNOLOGIES = NET ENVIRONMENTAL IMPROVEMENT

Reference scenarios:
Ecoinvent database
European reference Life Cycle Database ELCD (European Commission's Joint Research Centre)
Data provided by the CEREAL partners

Universidad de Oviedo

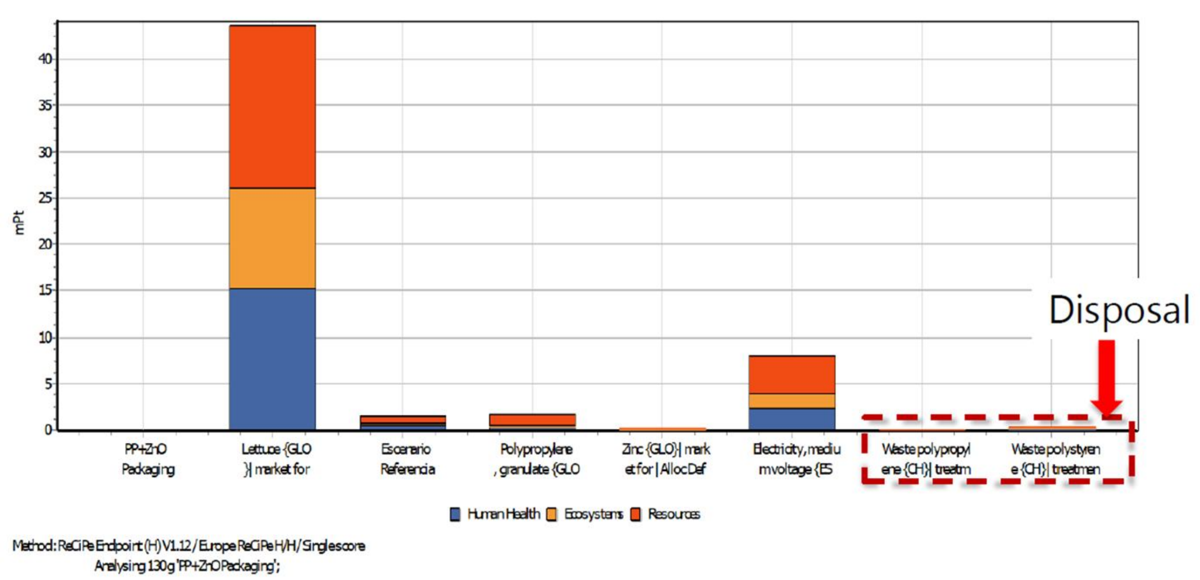
Montecarlo analysis *PLA/ZnO vs Ref.*

Midpoints show better performance in most cases for proposed package

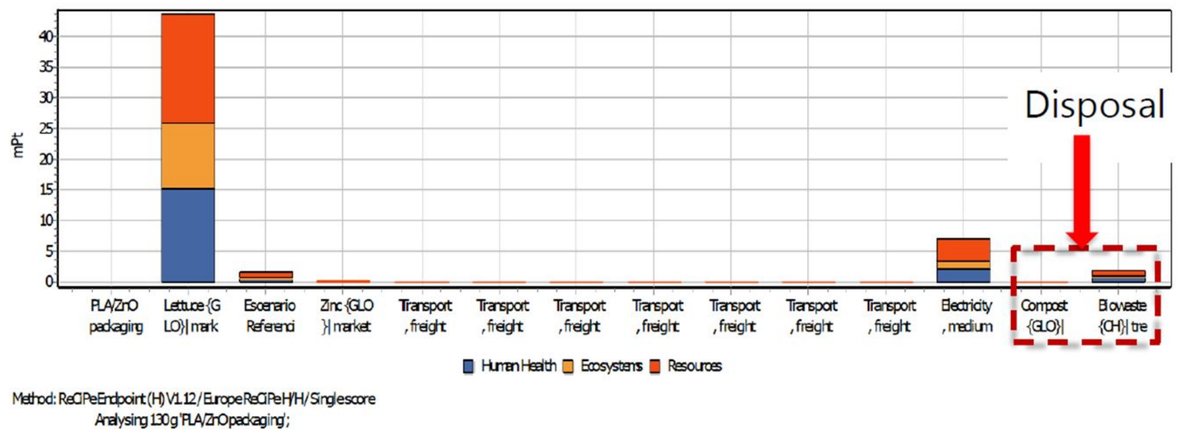


Single score ReCiPe (H/H) shows that the use of PLA+ZnONP reduces the environmental impact of lettuce consumption by 10% while the package made from PP+ZnONP entails reductions of a 9%.

PP/ZnO
Hot Spots



PLA/ZnO
EndPoints



The contribution of packaging to the whole environmental impact, including disposal stage, is not relevant

The production stage is by far the most environmental damaging

HRMS approach is useful for the optimization process and development of films

Migration of NIAS from PP based films in both simulants A and B, and from PLA films in simulant A and B.

NIAS detected are not included in the candidate EU list of chemicals to be evaluated for risks

Based on ZnONPs release test (ICP-MS), the nanopolymers composed of PP/PL/ZnONPs, 92/5/3, and PLA/PL/ZnONPs provided the most suitable contact material with improved functionality

ZnONPs release test (ICP-MS): solubilization in the form of Zn^{2+} is below SML (25 $mg.kg^{-1}$)

PL additive appears to hinder Zn^{2+} release

Conclusions

EW: “**Mass spectrometry in support of the environment, food, and health interaction and disease**”

Thanks