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Universitat de València | Universitat Politècnica de València

Universitat d'Alacant
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UNIVERSITAT JAUME I

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Universidad Católica de Valencia San Vicente Mártir

CSIC
Consejo Superior de Investigaciones Científicas

think in azul

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Descripción del Grupo de Trabajo

IUPA. 25 investigadores del ámbito de la Química Analítica. Técnicas Crom-MS avanzadas en Medio Ambiente, Seguridad Alimentaria, Toxicología..

Juan V Sancho Llopis (IP1)
Maria Ibáñez Martínez (IP2)
Tania Portolés Nicolau
Miriam González Hernández

UHPLC-ESI-IMS-QTOFMS
GC-EI-QOrbitrap MS (SPME, TDU)
Screening (Target, Suspect, Nontarget)
Metabolomics/Volatolomics

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Objetivos y tareas

- **Objetivo 5.1. (L.A2.13, L.A2.16)** Caracterizar materias primas para piensos, incluyendo fuentes de proteína alternativa, y los piensos formulados para dorada (*Sparus aurata*) de acuicultura. Evaluar el efecto de la alimentación con esos piensos a lo largo del ciclo completo de vida en la calidad nutricional, funcional y sensorial de dorada. Incluye identificar compuestos bioactivos y posibles contaminantes en las porciones comestible y vísceras (UMH1 metales pesados, UJI1 contaminantes orgánicos).

  - **Tarea 5.1.1 (M1-14) - Caracterización de materias primas y piensos formulados para dorada**

  - **Tarea 5.1.2 (M6-36) - Caracterización de doradas obtenidas de los diferentes sistemas de alimentación y en diferentes etapas del desarrollo**

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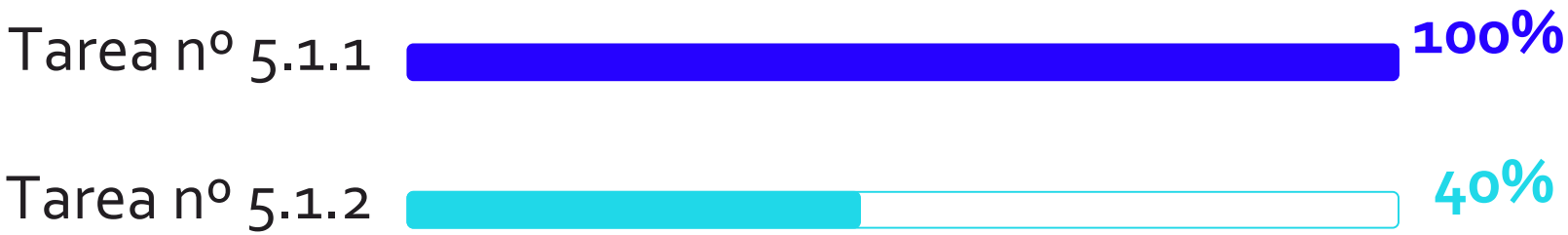
Grado de consecución de las tareas



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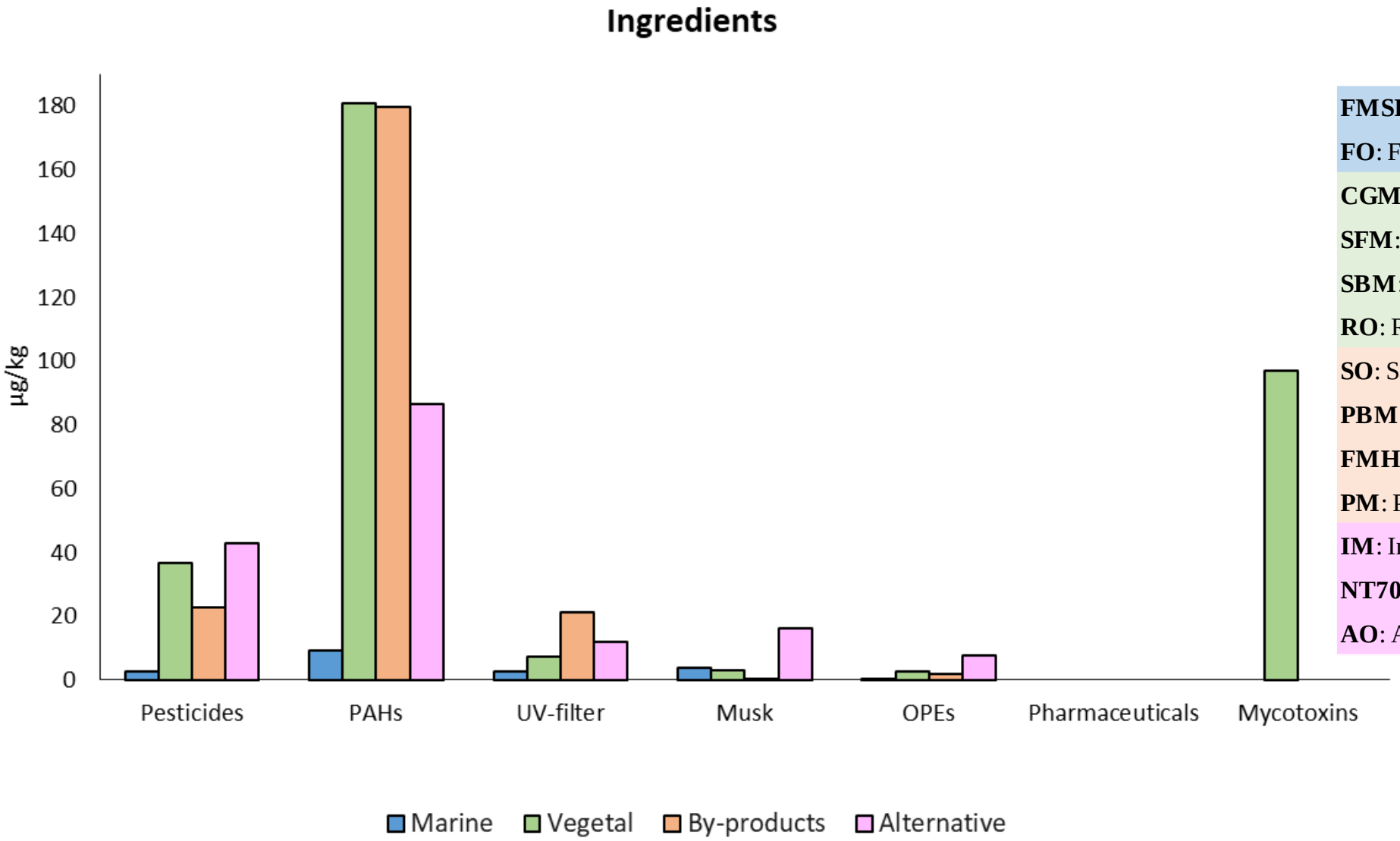
Resultados obtenidos hasta el momento (target-INGRED 6 mm)



FMSP: Fishmeal Super Prime

FO: Fish Oil

CGM: Corn Gluten Meal

SFM: Sunflower Meal

SBM: Soybean Meal

RO: Rapeseed Oil

SO: Salmon Oil

PBM: Porcine Blood Meal

FMH: Feathermeal Hydrolysate

PM: Poultry Meal

IM: Insect Meal

NT70: Aminopro NT70-C. Glutamicum

AO: Algae Oil

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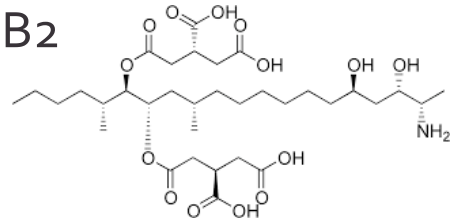
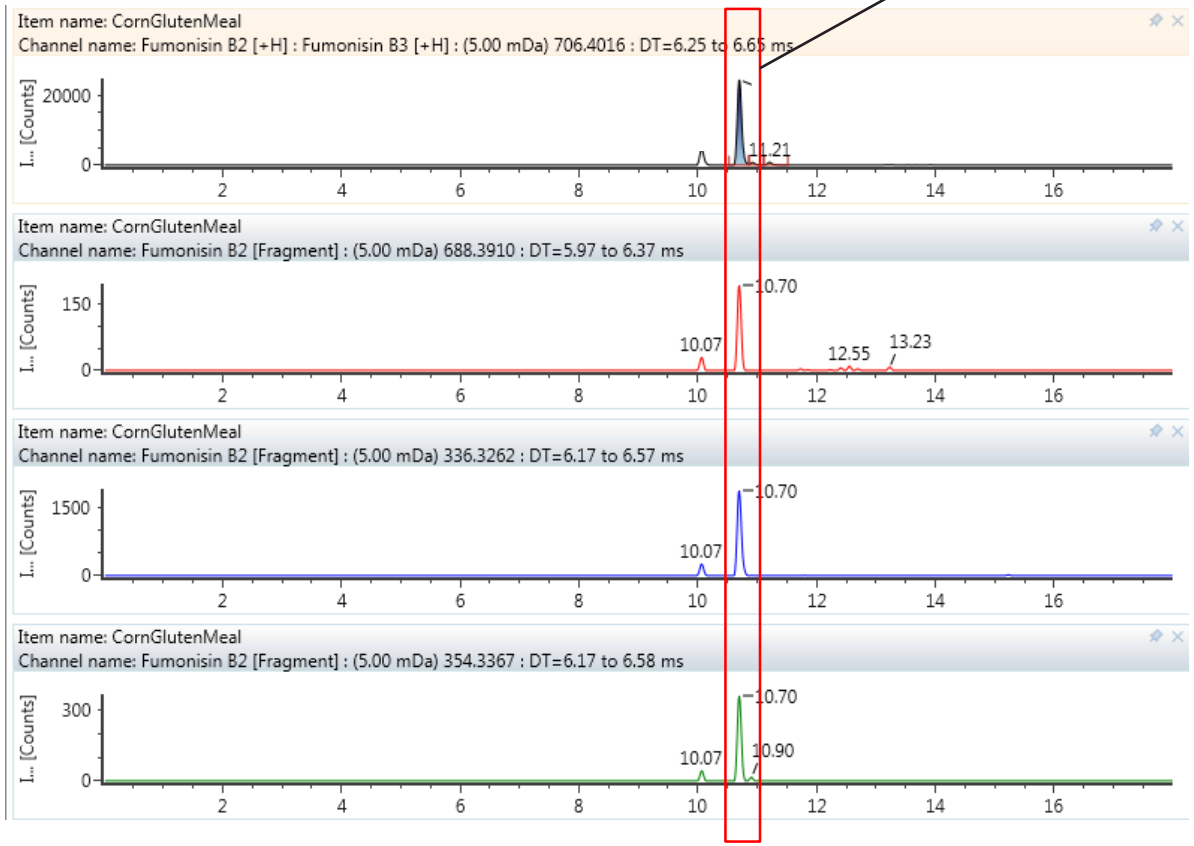
Resultados obtenidos hasta el momento (suspect-INGRED T2)

Fumonisin B2





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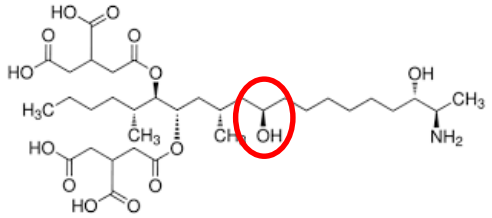
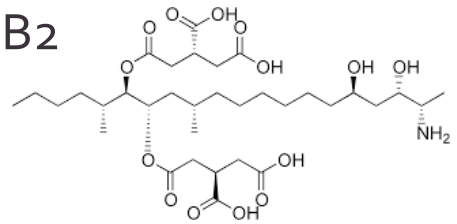
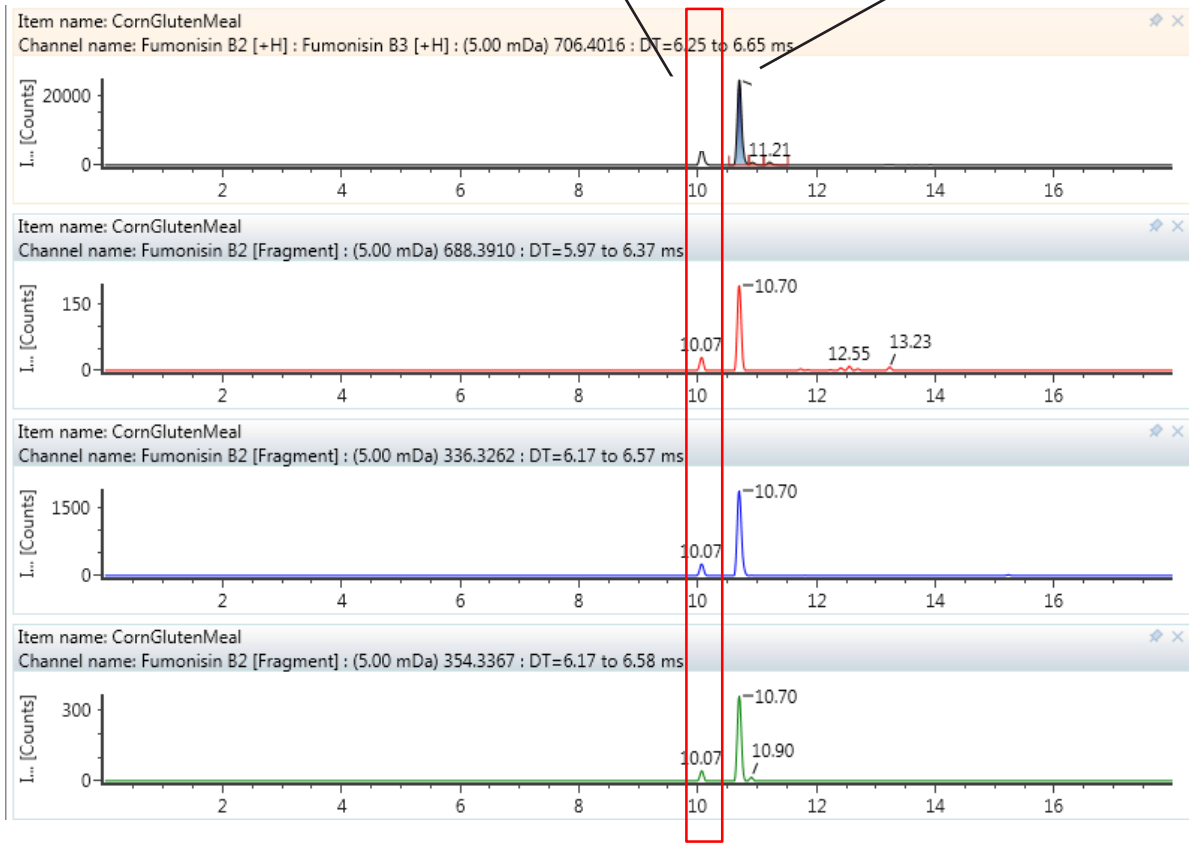
Resultados obtenidos hasta el momento (suspect-INGRED T2)

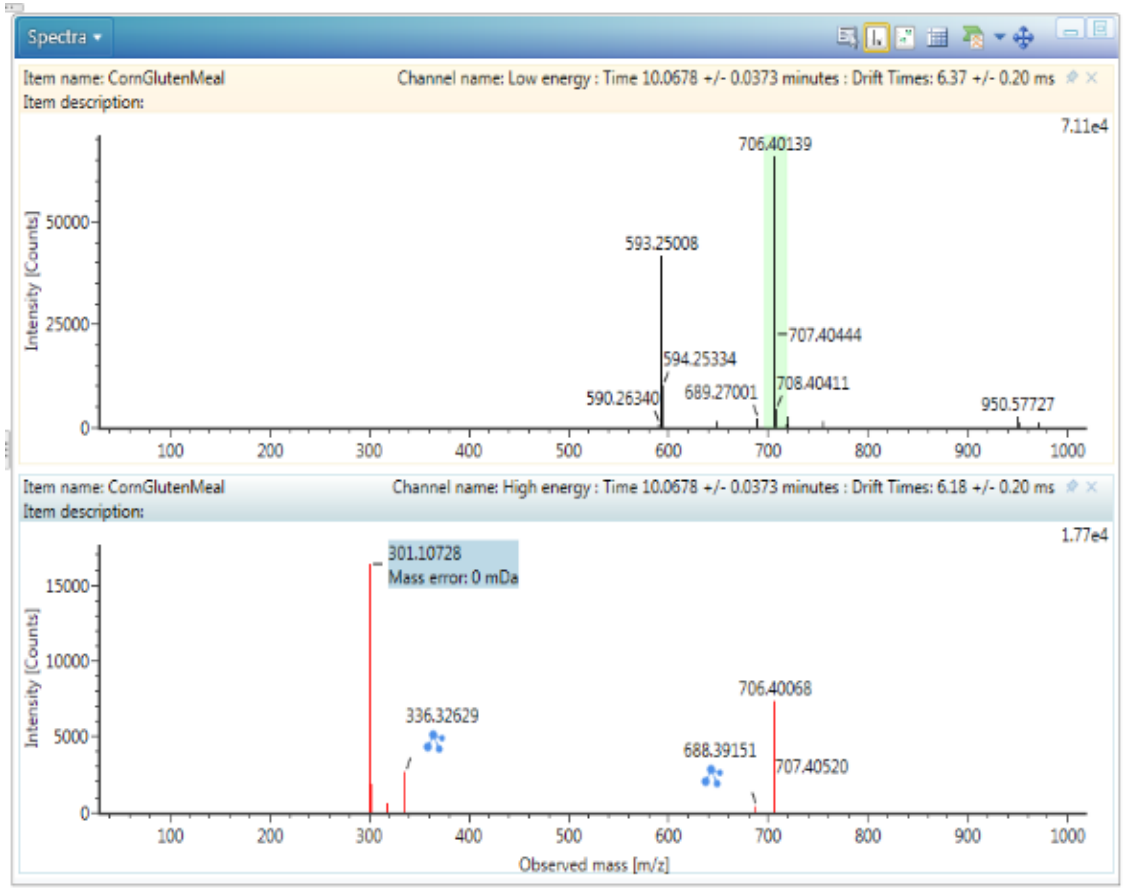


Fumonisin B3

Fumonisin B2







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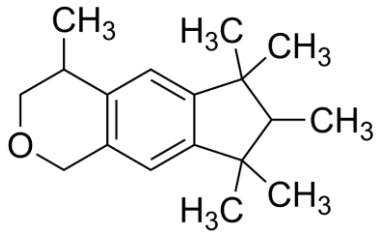
Resultados obtenidos hasta el momento (target-FEED 6 mm)

CHEMICAL FAMILY	COMPOUND	FEEDS 6mm		
		CTRL	PAP	ALT
PESTICIDES	DEET	0.3	0.4	0.5
	Diphenylamine	0.6	0.7	0.9
	Simazine		1.8	
PAHs	Acenaphthene	23.4	8.5	12.1
	Acenaphthylene	NQ		
	Anthracene	2.8	3.0	3.8
	Phenanthrene	2.8	3.0	3.8
MUSK	Galaxolide	51.8	6.3	
	Tonalid		4.6	
UV-FILTER	EHMC		0.3	0.2
	Octocrylene	72.2	32.2	17.6
OPEs	TBP H	0.3	0.3	0.4
MYCOTOXINS	Fumonisin B2	d	d	d

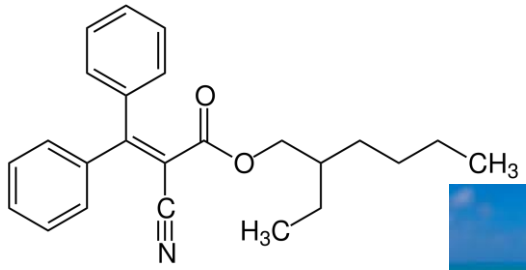
CTRL: Control diet

PAP: Processed Animal and Plant Protein

ALT: Alternative Protein Sources



Galaxolide



Octocrylene

d: no fragment ion

NQ: not quantified

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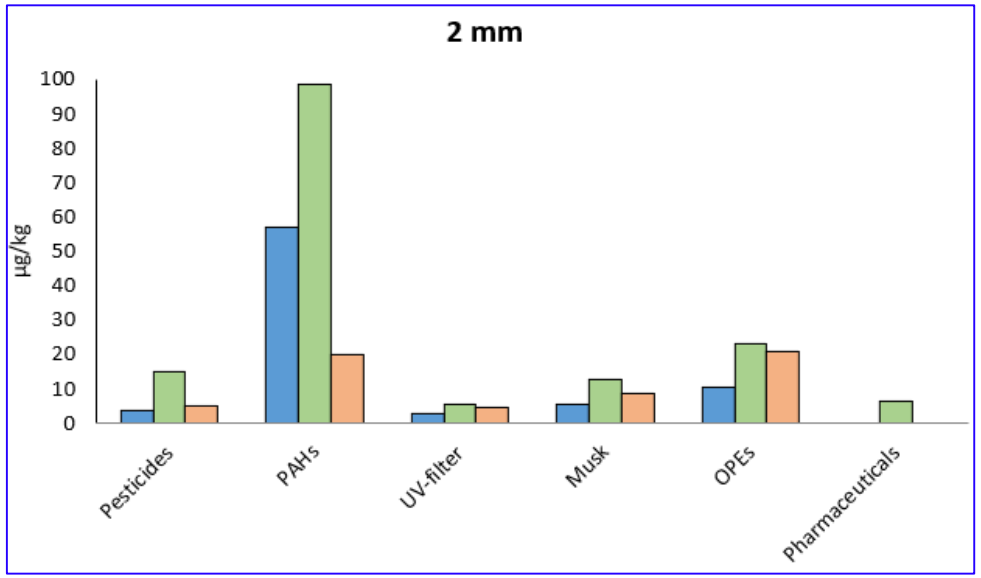
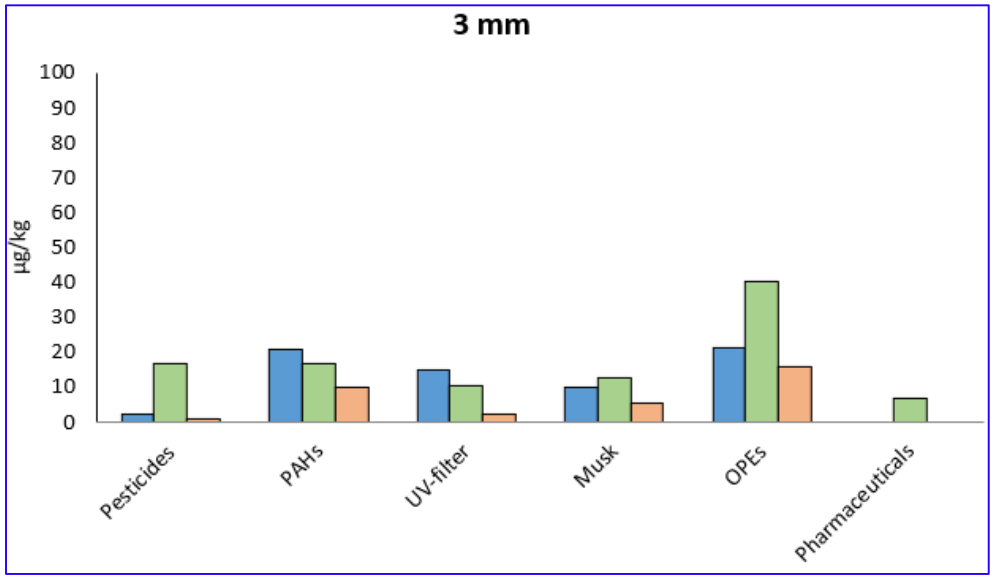
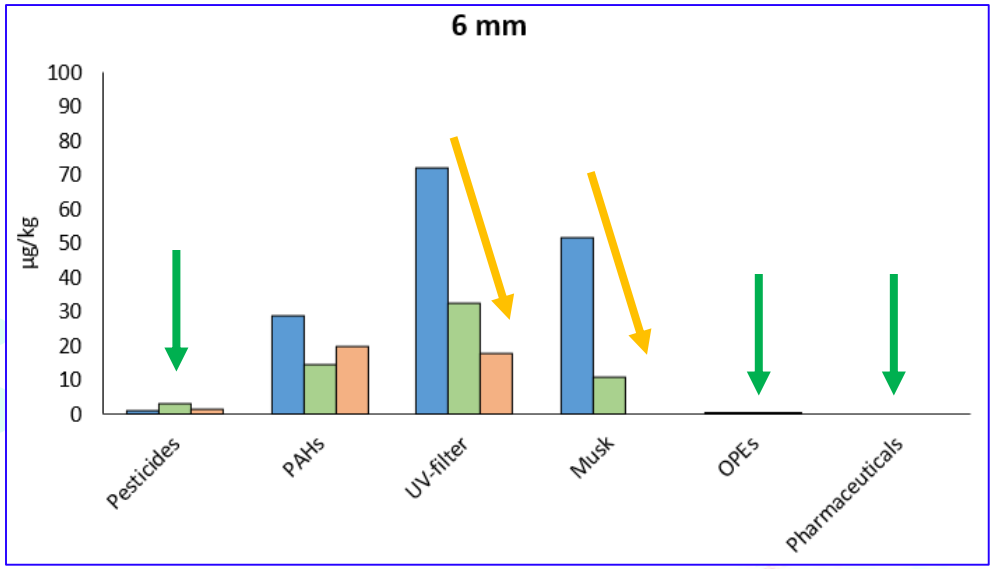
Resultados obtenidos hasta el momento (target-FEED)





CTRL PAP ALT



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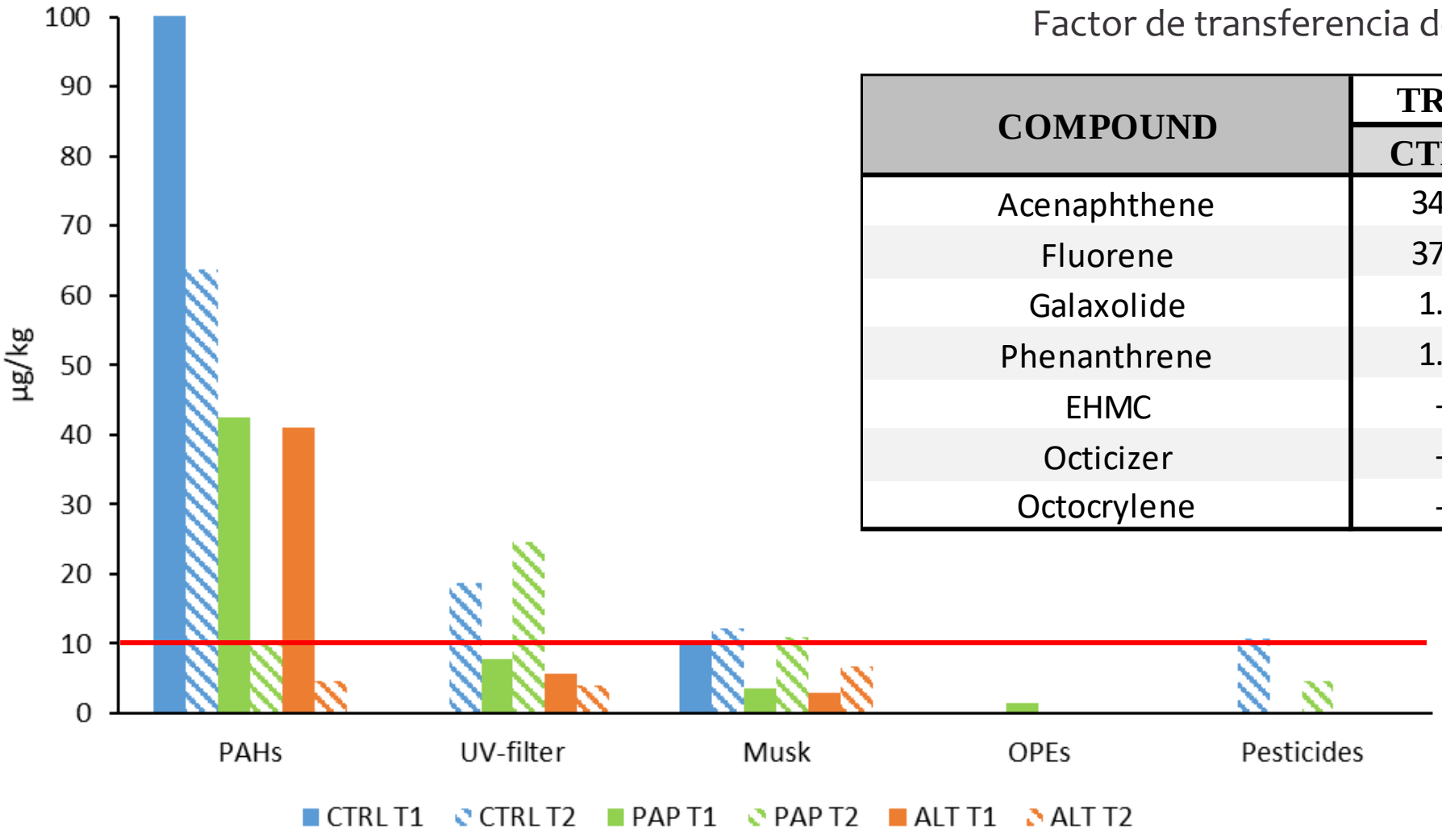
Resultados obtenidos hasta el momento (target-FILLET T1-T2)



Factor de transferencia de pienso a filete

COMPOUND	TRANSFER FACTOR T1		
	CTRL	PAP	ALT
Acenaphthene	34.8	-	-
Fluorene	37.0	0.8	3.2
Galaxolide	1.2	0.3	0.5
Phenanthrene	1.1	0.5	1.5
EHMC	-	0.5	-
Octicizer	-	0.1	-
Octocrylene	-	0.3	0.5

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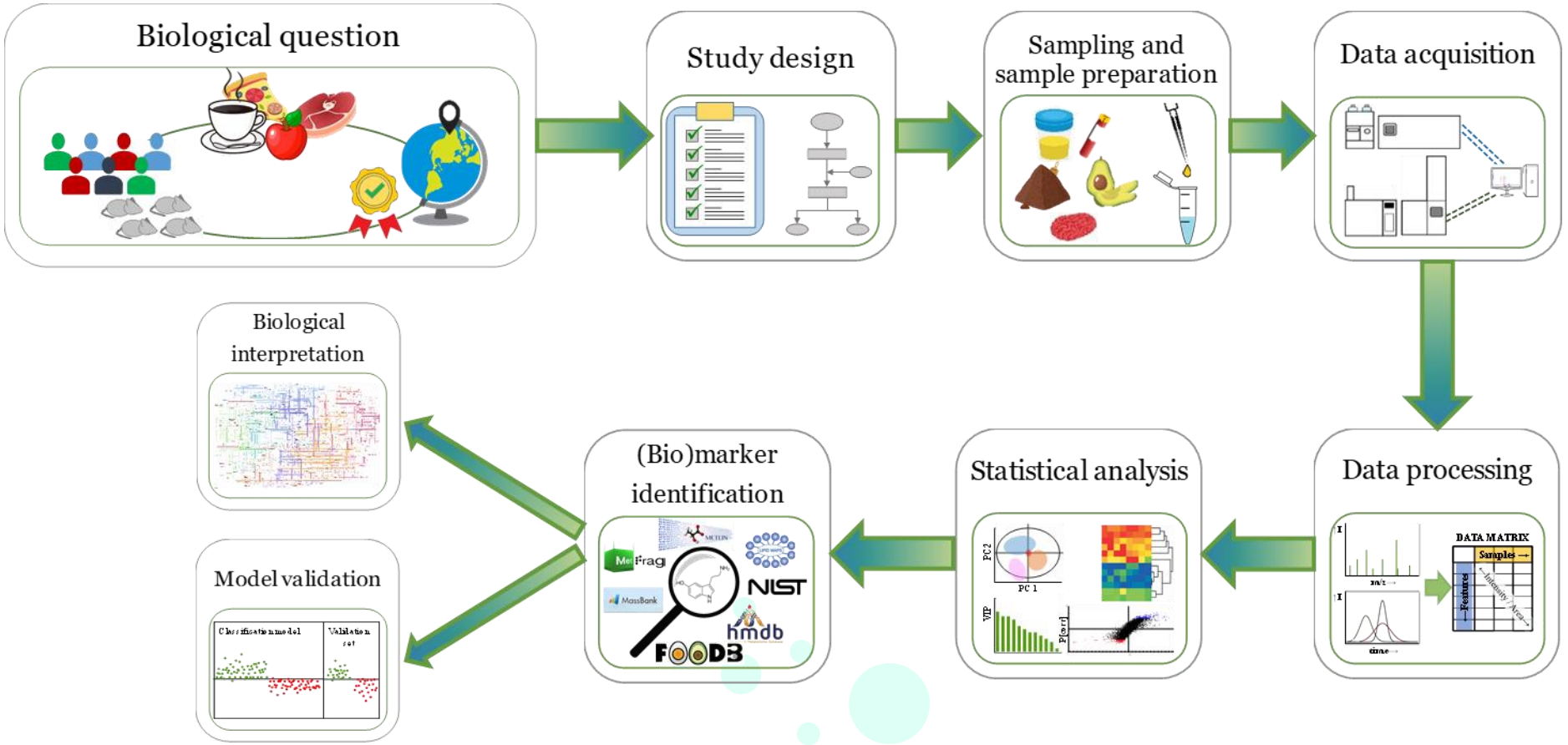
Resultados obtenidos hasta el momento (LIPIDÓMICA)

Flujo de trabajo para un estudio en metabolómica no dirigida



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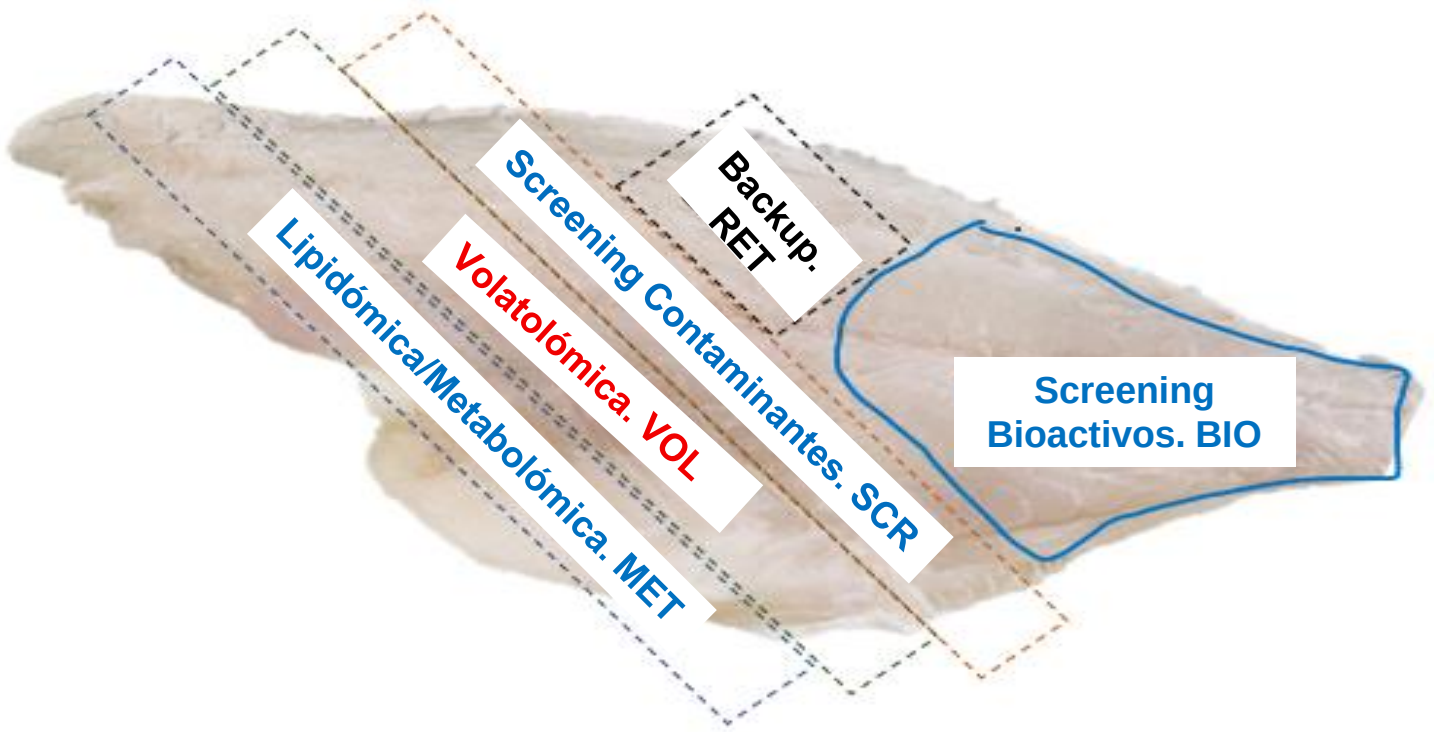
Resultados obtenidos hasta el momento (LIPIDÓMICA)



16 muestras x 3 dietas =
48 muestras analizadas

Patrones internos

- 15:0-18:1-d7-15:0-TG
- 15:0-18:1-d7-PC

Tratamiento de muestra

FILLET

Lyophilisation 16h

1 g

Extraction with 16 mL $CHCl_3$:MeOH (2:1)
+ 30 min at 4 °C
+ 4 mL H_2O
+ 10 min at 4 °c
+ Centrifugation 5 min, 2000 rpm

↓ Organic Layer (Lower phase) - $CHCl_3$

+ Solvent Exchange into 1 mL of isopropanol
+ 100 ppb Internal Standard

UHPLC-ESI-IMS-QTOFMS

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Resultados obtenidos hasta el momento (LIPIDÓMICA)

Método instrumental

- Instrumento: HPLC-ESI-IMS-QTOF
- Columna: RP C18
- Modos de ionización: ESI+/ESI-
- Fases móviles:

Fase A: ACN:H2O (60:40) + 0.1% HCOOH + 10 mM NH4HCOO

Fase B: IPA:ACN:H2O (90:8:2) + 0.1% HCOOH + 10 mM NH4HCOO

- Gradiente:

t (min)	A (%)	B (%)
0	80	20
22	20	98

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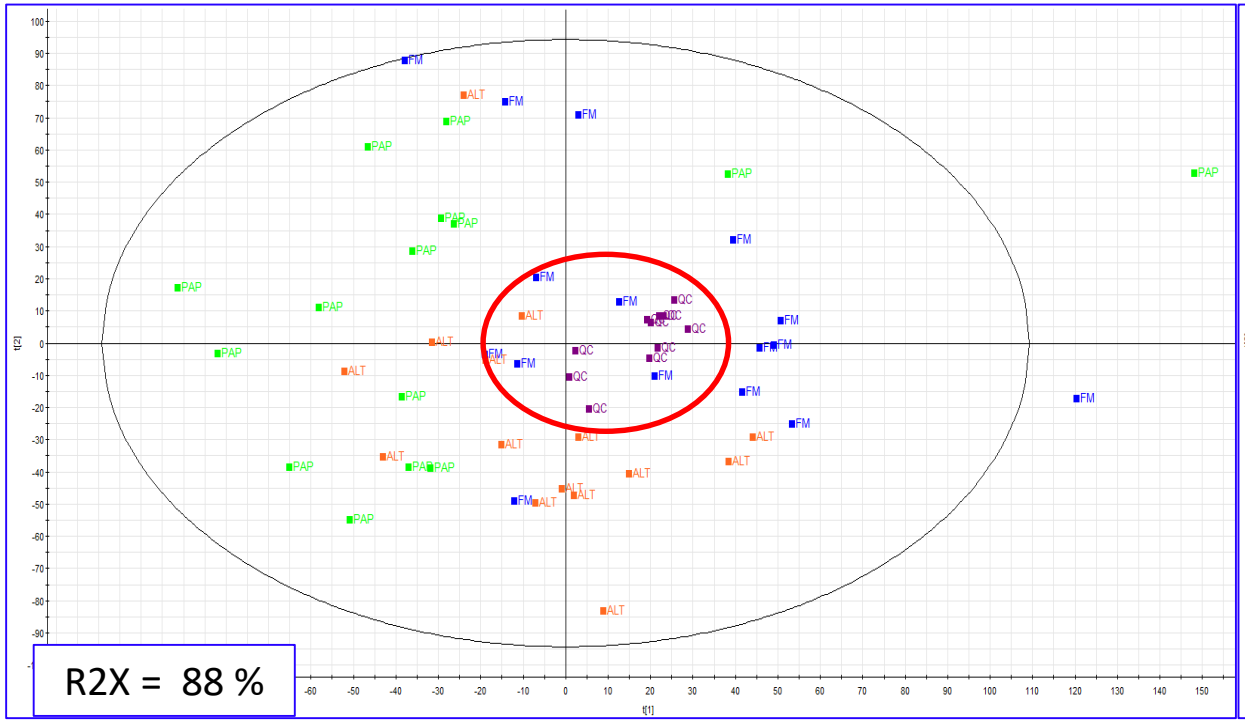
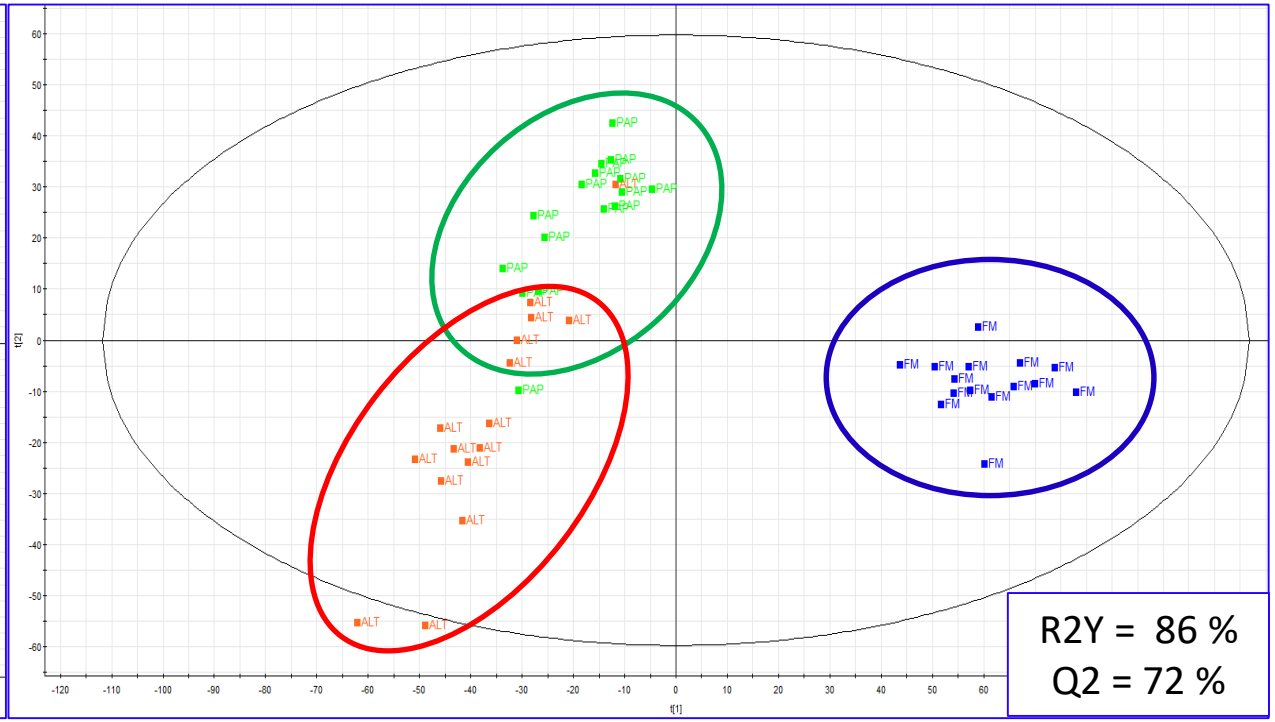
Resultados obtenidos hasta el momento (LIPIDÓMICA , RP+)

Tratamiento estadístico de los datos

1. PCA



2. PLS-DA



CTRL ALT PAP QC

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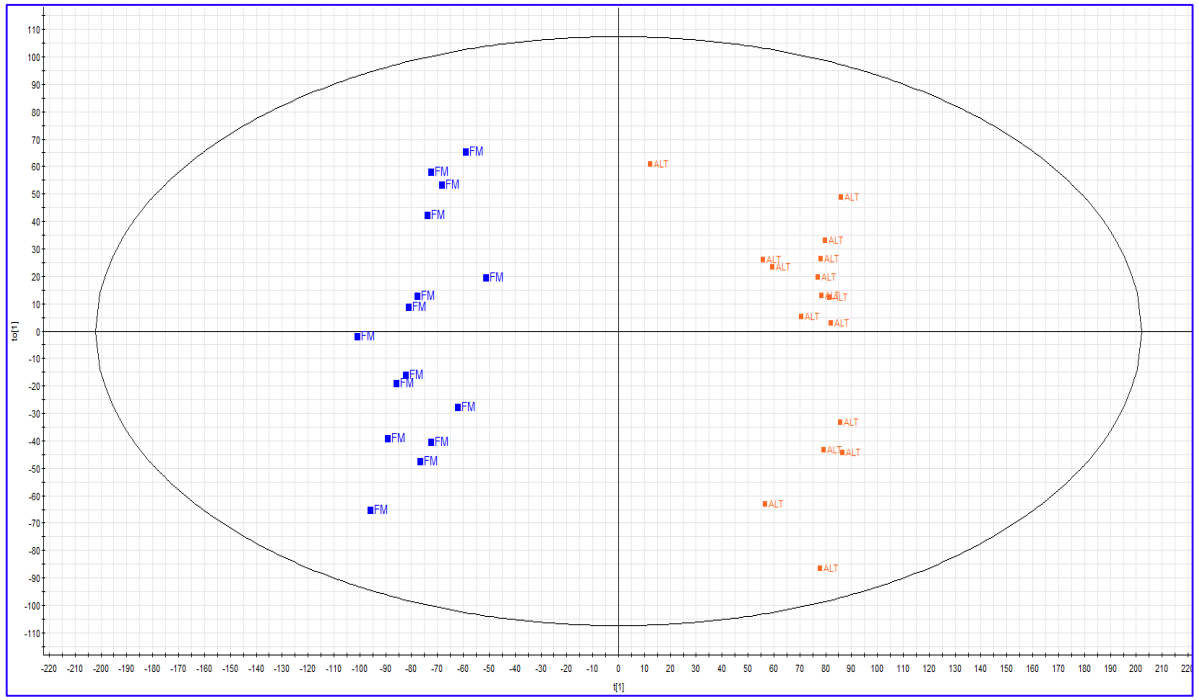
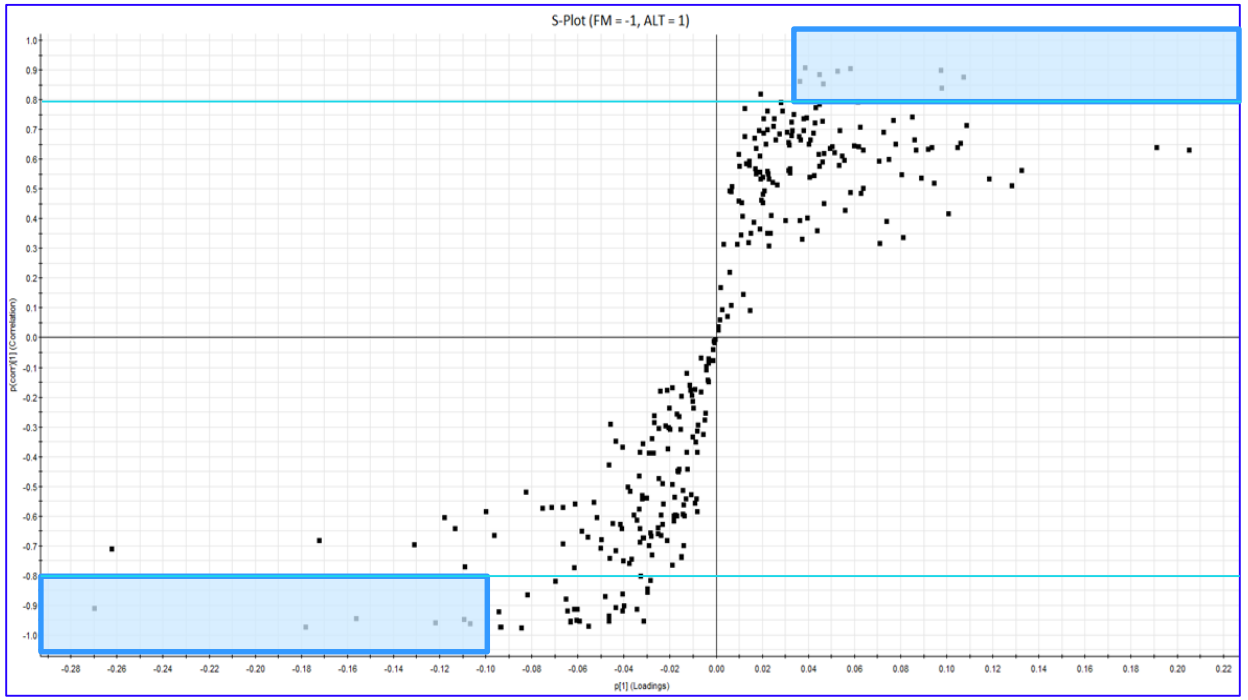
Resultados obtenidos hasta el momento (LIPIDÓMICA , RP+)

CTRL vs ALT

3. OPLS-DA



4. S-plot



CTRL ALT

p(corr) > ± 0.8 + p(Loading) > ± 0.1

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Resultados obtenidos hasta el momento (LIPIDÓMICA, RP+)

ALT diet							
p(corr)*	RT (min)	m/z	Formula	Fragments	Experimental CCS	Tentative Identification	CCS error (%)*
0.85	12.62	539.4675	C33H62O5	257.2110, 521.4561	249.54	DG(12:0/18:1)	0.4
0.85	1.66	570.3559	C30H52NO7P	533.2644, 552.3454	247.44	LysoPC(22:5)	4
0.84	0.97	590.3221	C30H50NO7P	184.0733	250.25	PC(22:6/0:0)	6
0.83	9.43	792.5471	C45H78NO8P	651.5352	306.75	PE(40:6)	7
0.82	11.27	533.4181	C31H58O5	257.2115	242.04	DG(12:0/16:1)	0.2
0.82	17.86	769.6322	C47H86O6	493.4243	304.20	TG	3
0.81	9.55	880.5846	C52H82NO8P	184.0736	327.38	PC(42:8)	7
0.80	10.92	584.4440	C37H60O5	257.2106, 567.4410	250.38	DG(12:0/22:6)	0.2
0.79	7.23	591.4025	C31H59O8P	257.2110	259.44	PA(16:1/12:0)	3
0.79	18.43	771.6478	C47H88O6	493.4249	306.97	TG	3

10 marcadores de la dieta ALT

7 marcadores de la dieta CTRL

CTRL diet							
p(corr)*	RT (min)	m/z	Formula	Fragments	Experimental CCS	Tentative Identification	CCS error (%)*
0.98	10.15	822.599	C47H84NO8P	806.569, 184.0737	323.47	PC(39:5)	7
0.97	10.18	776.5583	C45H78NO7P	385.2744	305.59	PE(P-18:0/22:6)	6
0.95	10.38	716.5555	C40H78NO7P	699.532	296.18	PE(P-35:1)	6
0.95	9.53	796.5838	C45H82NO8P	184.0737	323.99	PC(27:4)	9
0.95	11.12	848.6160	C49H86NO8P	311.2370, 367.2630	335.62	PE/PC	10
0.92	9.36	820.5856	C47H82NO8P	659.5016	311.10	PC(39:6)	4
0.88	9.65	790.5741	C46H80NO7P	625.5185	311.69	PC(P-16:0/22:6)	6

*p(corr) en valor absoluto
*Se calcula con una *predicted CCS*

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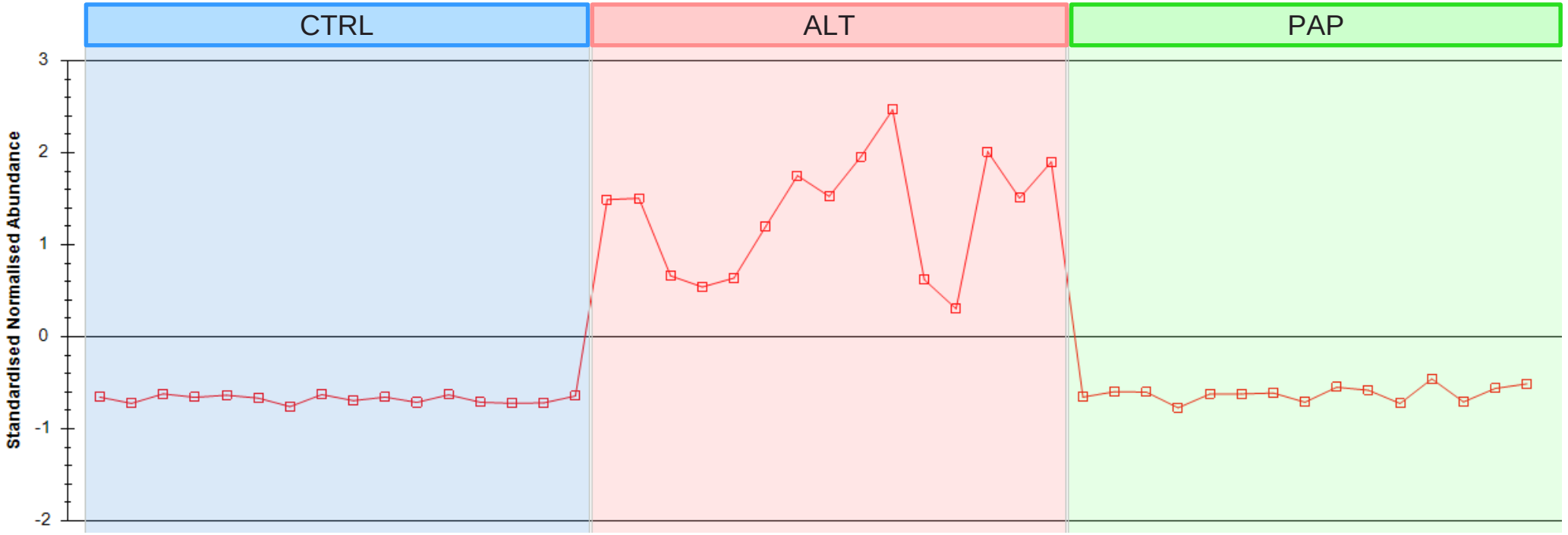
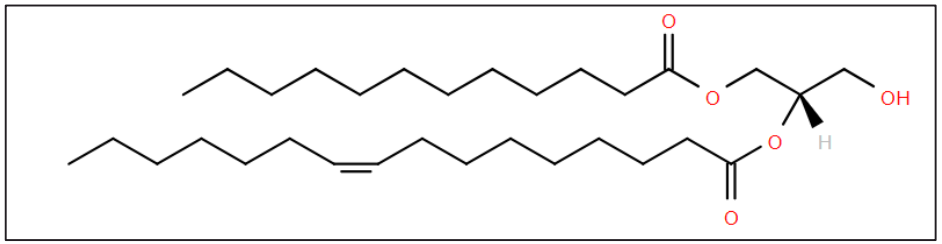
Resultados obtenidos hasta el momento (LIPIDÓMICA, RP+)

Feature 11.27_533.4181m/z_242.04



Dieta ALT



DG(12:0/16:1)

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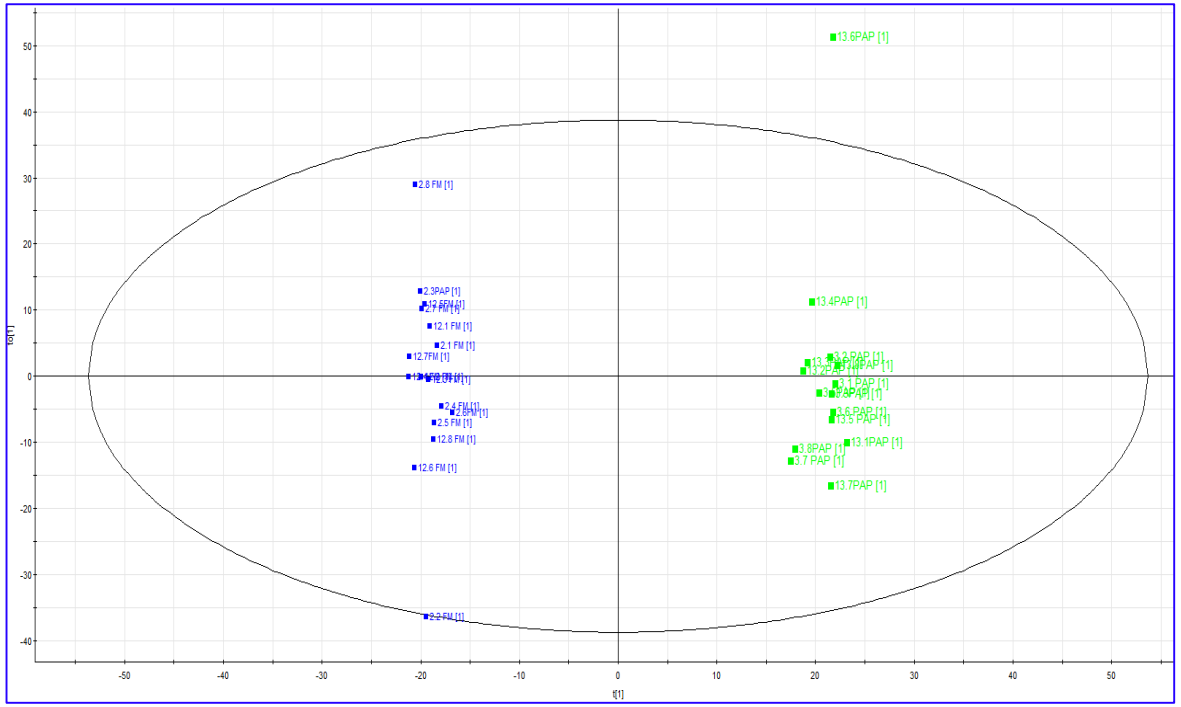
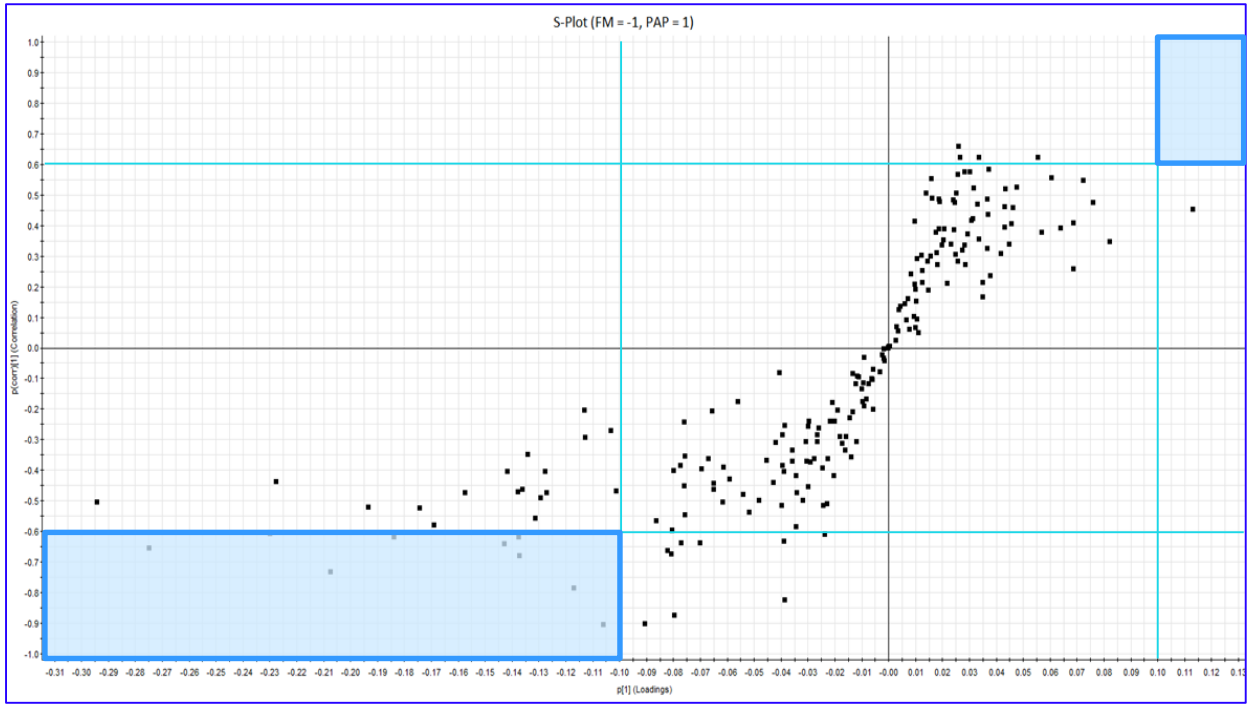
Resultados obtenidos hasta el momento (LIPIDÓMICA , RP-)

CTRL vs PAP

3. OPLS-DA



4. S-plot



CTRL PAP

p(corr) > ± 0.6 + p(Loading) > ± 0.1

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Resultados obtenidos hasta el momento (LIPIDÓMICA, RP-)

2 marcadores de la dieta ALT

ALT diet							
p(corr)*	RT (min)	m/z	Formula	Fragments	Experimental CCS	Tentative Identification	CCS error (%)*
0.66	8.53	817.501	C46H75O10P	327.2323	311.59	PG(18:2_22:6)	6
0.61	8.85	767.4859	C42H73O10P	255.2323	303.51	PG(16:0_20:5)	6

1 marcador de la dieta PAP

PAP diet							
p(corr)*	RT (min)	m/z	Formula	Fragments	Experimental CCS	Tentative Identification	CCS error (%)*
0.64	12.10	856.6060	C47H88NO10P	279.2324	333.36	PS(41:2)	9

1 marcador de la dieta CTRL

CTRL diet							
p(corr)*	RT (min)	m/z	Formula	Fragments	Experimental CCS	Tentative Identification	CCS error (%)*
0.68	9.91	790.5382	C45H78NO8P	506.3248	305.36	PE(20:1_20:5)	5

*p(corr) en valor absoluto
*Se calcula con una *predicted CCS*

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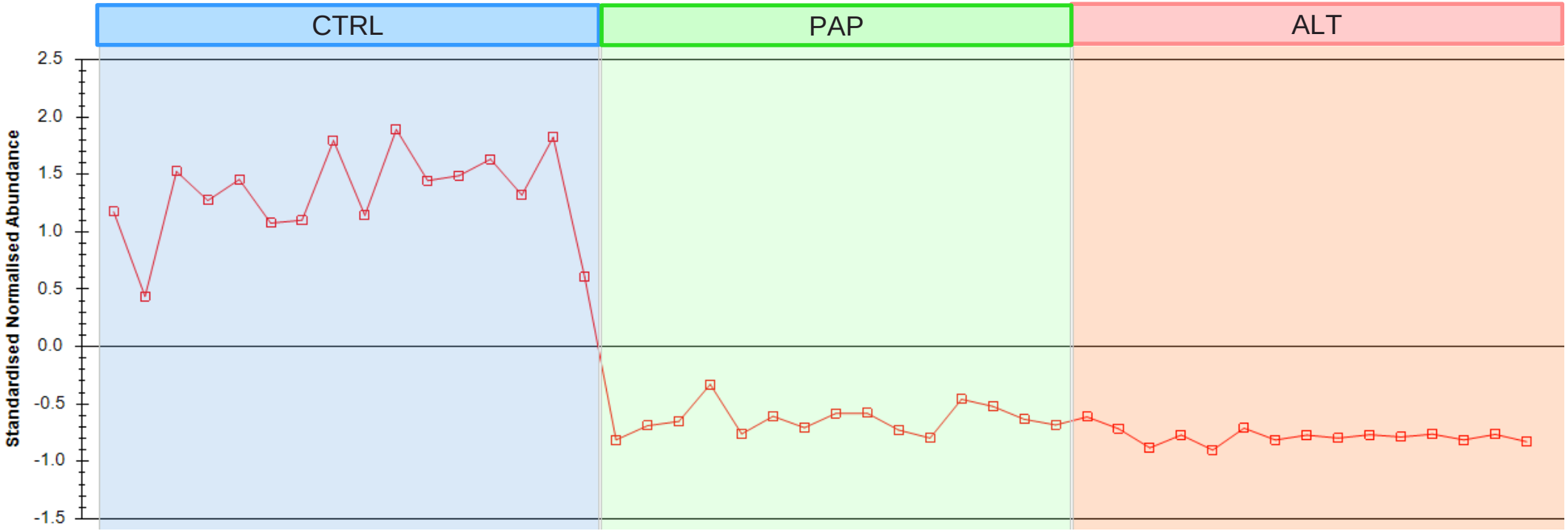
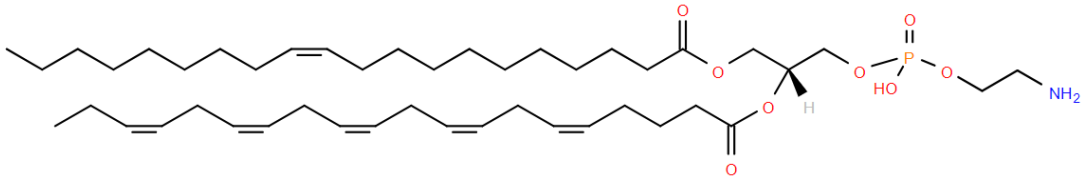
Resultados obtenidos hasta el momento (LIPIDÓMICA, RP-)

Feature 9.91_790.5382m/z_305.36



Dieta CONTROL



PE(20:1_20:5)

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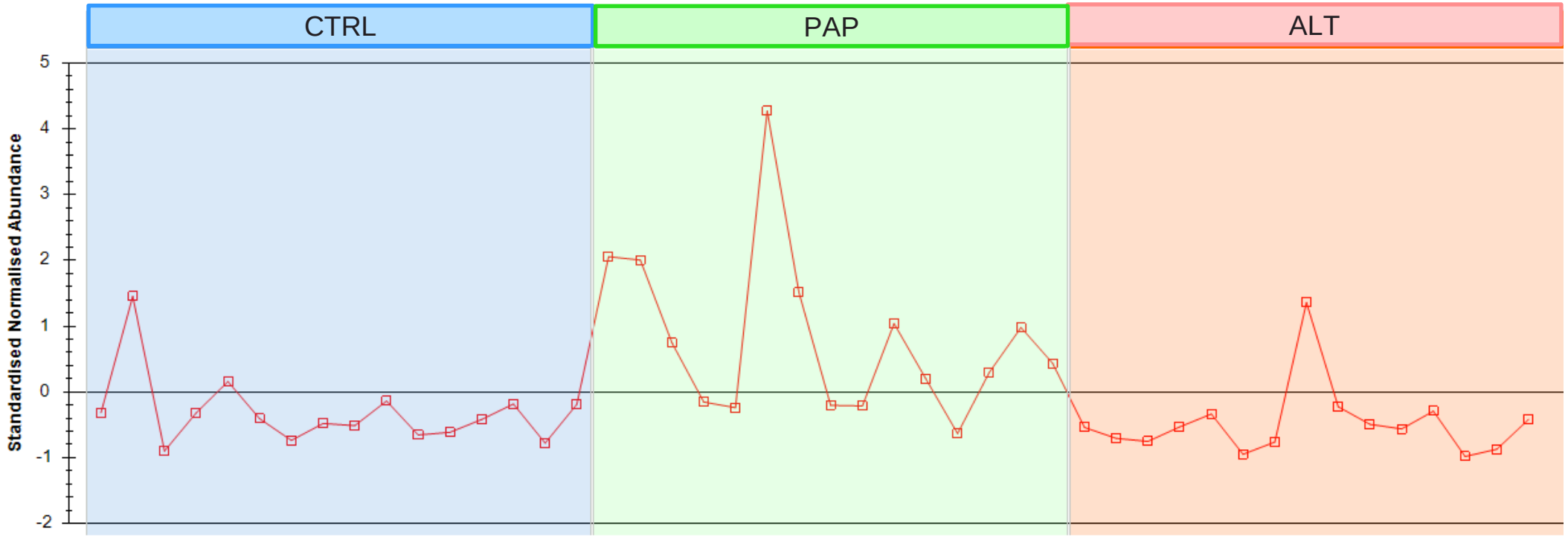
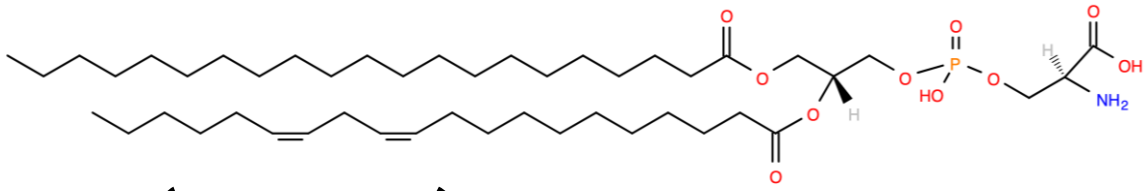
Resultados obtenidos hasta el momento (LIPIDÓMICA, RP-)

Feature 12.10_856.6060m/z_333.36



Dieta PAP



PS(41:2) **PS(21:0_20:2)**

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Hoja de ruta 6 próximos meses

- Tarea 5.1.2
  - Terminar elucidación de los marcadores encontrados en el estudio de lipidómica en filetes (tanda 1).
  - Correlacionar estos marcadores con perfiles de ácidos grasos (UMH1)
  - Validar en filetes de la tanda 2 los marcadores elucidados en la tanda 1
  - Empezar estudio de volatolómica en filetes
  - Analizar las muestras de plasma y mucus en RP+/RP- y HILIC+/HILIC-

azul

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Plan de Recuperación, Transformación y Resiliencia

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Thanks | Gràcies

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Juan Vicente Sancho Llopis

Universitat Jaume I