METABOLOMICS ANALYSIS IN PLASMA OF *ANGUILLA ANGUILLA* SPECIES EXPOSED TO A MIXTURE OF PHARMACEUTICALS

4th INTERNATIONAL CONFERENCE ON RISK ASSESSMENT OF PHARMACEUTICALS IN THE ENVIRONMENT
Barcelona, 9 - 10 October 2023

Dyana Vitale*[1], Rodrigo Álvarez-Ruiz[2], Vicente Andreu[1] and Yolanda Picó[1],
[1]Food and Environmental Safety Research Group of the University of Valencia (SAMA-UV), Desertification Research Centre (CIDE) CSIC-GV-UV, Valencia, Spain; [2]Purdue University, Department of Agronomy, West Lafayette, USA; *dyana.vitale@uv.es

Background

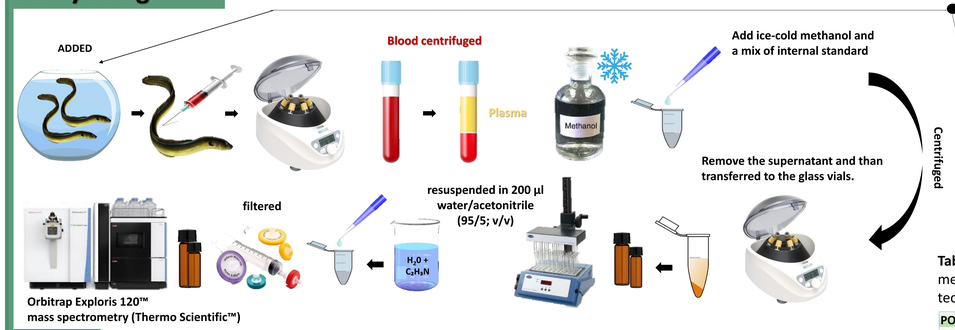
The **European eel** has been shown to be a reliable **bioindicator** for assessing the ecological quality of aquatic ecosystems.

To assess the impact of the **pharmaceuticals** on fish and determine whether **altered molecules in plasma** could serve as **biomarkers for fish** in future monitoring, a **bioaccumulation study** was conducted in ***Anguilla anguilla.***

The aim of the study was been applied an untargeted metabolomics studies, using **Compound Discoverer v3.2. (Thermo Scientific™) and MetaboAnalyst 5.0 software.**

The data were subjected to a variety of statistical techniques. Based on the pathway results, a total of **31 most significantly expressed metabolites** were identified in different time of exposure (**7th, 28th and 30th day**).

Study design



Pharmaceuticals ID (positive compounds)
Etoricoxib
Vildagliptin
Atenolol
Metformin

Pharmaceuticals ID (negative compounds)
Salicylic acid
Triclosan
Naproxen
Ibuprofen

Results

The data were also subjected to a variety of **statistical techniques** using the Compound Discoverer 3.2 software, including **one-way ANOVA (*p*-value < 0.05) followed by Post hoc Tukey's *t*-test**.

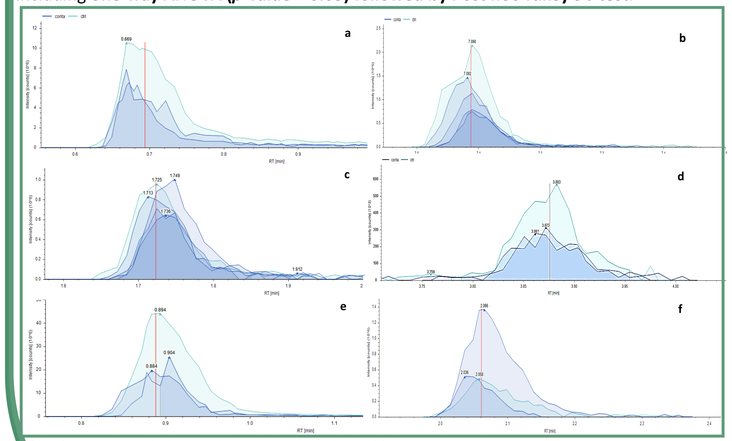


Figure 1: Chromatograms peak illustration of some metabolites detected comparing the group treated with pharmaceuticals and the control group, resulting as up and down regulated metabolites. L-Tyrosine **(a);** Decanoylcarnitine **(b);** L-Phenylalanine **(c);** 2-(1H-indol-3-yl)acetic acid **(d);** Aspartame **(e);** 2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid **(f)**.

***Conta:** samples with the addition of pharmaceuticals; **Ctrl:** control samples.

Table 1: Up regulated and down regulated metabolites determination using the statistical techniques in both ion mode.

POSITIVE ION MODE		
FC ≥ 1.5	***P-value less or equal to 0.05***	***Up-regulated***
Adipic acid		
Decanoylcarnitine		
Benzylbutylphthalate		
Thriphenyl phosphate		
FC ≤ -1.5	***P-value less or equal to 0.05***	***Down-regulated***
2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid		
2-(1H-indol-3-yl) acetic acid		
3-(2-methylpropyl)-octahydropyrrolo[1,2-a] pyrazine-1,4-dione		
Aspartame		
Atenolol		
L-Tryptophan		
Proline		
Quinoline		
N-Acetyldopamine		
L-Phenylalanine		
Valine		
L-(-)-Methionine		
L-Tyrosine		
Isoleucine		

NEGATIVE ION MODE		
FC ≥ 1.5	***P-value less or equal to 0.05***	***Up-regulated***
Salicylic acid		
Eicosenoic acid		
Cannabinodiol		
FC ≤ -1.5	***P-value less or equal to 0.05***	***Down-regulated***
Triclosan		
Bisphenol A		
L-Phenylalanine		
L-Tyrosine		
Clofibric acid		
Furosemide		
Chloramphenicol		
Thiamphenicol		
4-hydroxybenzoic acid		

Conclusions

The last days of exposure (**28th and 30th**), several pathways involving amino acids metabolism indicating a possible oxidative damage, so that the developed approach serves as a significant tool for toxicity assessment, specially for ***A. Anguilla*** species.

A total of 86 metabolites in ESI+ and 36 in ESI- ion modes were observed in plasma of the ***A. anguilla*** species. Some of these metabolites were selected for hierarchical clustering based on statistical significances.

Metabolite profiling

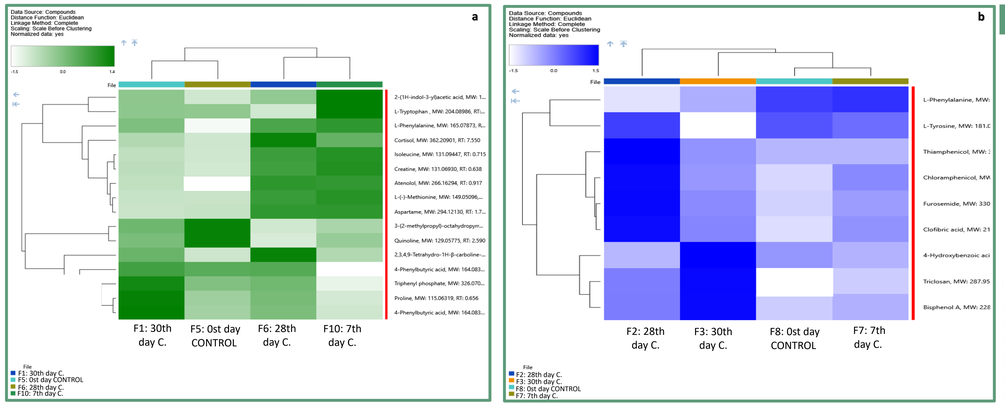


Figure 2: Hierarchical clustering analysis of the significantly different metabolites determinates in positive ion mode **(a)** and in negative ion mode **(b)**. Color denotes the abundance of metabolites, from the highest (green or blue) to the lowest (white).

***C:** sample with the addition of pharmaceuticals; **CONTROL:** without addition of pharmaceuticals

The most abundance of metabolites were found at **7th and 28th days** of the pharmaceutical exposure experiment in the plasma samples **(Fig.2 a) in the positive ion mode.** While**, in negative ion mode** were found at **28th** and **30th days** of the exposure **(Fig.2 b)**. Some added pharmaceuticals were found in the samples and not in the control groups **(see Fig. 2).**

Metabolic pathways

PATHWAY RESULTS	METABOLITES ASSOCIATED	FDR
Biosynthesis of unsaturated fatty acids	Hexadecanoic acid; Octadecanoic acid; Octadecenoic acid; Linoleate;(4Z,7Z,10Z,13Z,16Z,19Z)-Docosahexaenoic acid	9.19e-04
Aminoacyl-tRNA biosynthesis	L-Phenylalanine; L-Methionine; L-Isoleucine; L-Tyrosine; L-Proline	2.26e-03
Phenylalanine, tyrosine and tryptophan biosynthesis	L-Phenylalanine; L-Tyrosine;	1.47e-02
Phenylalanine metabolism	L-Phenylalanine; L-Tyrosine;	5.02e-02
Linoleic acid metabolism	Linoleate;	6.46e-01
Arginine and proline metabolism	Creatine; L-Proline	7.10e-01
Valine, leucine and isoleucine biosynthesis	L-Isoleucine	7.89e-01
Ubiquinone and other terpenoid-quinone biosynthesis	L-Tyrosine;	7.89e-01
Vitamin B6 metabolism	Pyridoxamine	7.89e-01
Sphingolipid metabolism	Phytosphingosine;	1.00e+00
Glycine, serine and threonine metabolism	Creatine	1.00e+00
Cysteine and methionine metabolism	L-Methionine	1.00e+00
Fatty acid degradation	Hexadecanoic acid	1.00e+00
Fatty acid elongation	Hexadecanoic acid	1.00e+00
Valine, leucine and isoleucine degradation	L-Isoleucine	1.00e+00
Tyrosine metabolism	L-Tyrosine	1.00e+00
Fatty acid biosynthesis	Hexadecanoic acid	1.00e+00
Steroid hormone biosynthesis	Cortisol	1.00e+00

Table 2: Detailed results from the pathway analysis and metabolites associated obtained with MetaboAnalyst 5.0 platform.
FDR p is the **p value** adjusted using False Discovery Rate

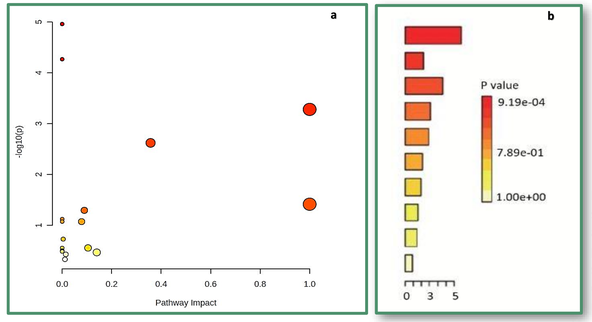


Figure 4: Metabolome view map of significant metabolic pathways altered after 30 days of pharmaceuticals exposure in plasma samples (**a**) and metabolite set enrichment analysis (MSEA), (**b**) represented also in the **table 2**.

Acknowledgments:

This study forms part of the **ThinkInAzul programme** and was supported by **MCIN** with funding from the **European Union next Generation EU(PRTR-C17.I1)** and the **Generalitat Valenciana**.