Biodiversity restoration and conservation of inland water ecosystems for environmental and human well-being

BioReset

BiodivRestore-406

2020 - 2021 Joint Call

Joint COFUND Call on "Conservation and restoration of degraded ecosystems and their biodiversity, including a focus on aquatic systems"

Deliverable 1.1.2

Data from monitoring of pharmaceuticals in surface waters and WWTP effluents and influents

Lead Beneficiary	Work package	Delivery month
REQUIMTE	**1**	**37**

1. Executive Summary

BioReset proposes to advance treatment processes (chemical, physical, biological and their combination) to promote ecosystem recovery and conservation and to develop assessment strategies. Diatoms will be used to model ecosystem conservation and restoration since their communities show high levels of biodiversity. The diatoms will provide an expeditious method to compare different recovery strategies and water treatment processes, allowing to address timescale and key conservation/restoration questions. The full environmental, economic, and social viability of the upgraded and innovative treatment technologies will be assessed. Based on this knowledge, scale-up studies in geographically different sites (Portugal and Spain) will be performed to ascertain the technical and economic feasibility at a larger scale and recommended action guidelines will be issued.

BioReset also envisages the creation of a representative space-time picture of the presence of emerging contaminants in inland waters and its correlation to effects on diatom communities. For this, powerful analytical techniques, such as gas- and liquid chromatography, will be used. Besides these methods, and to obtain real-time information, miniaturized analytical platforms that can perform fast, and on-site monitoring will also be employed.

Deliverable 1.1.2 provides details about the monitoring of pharmaceuticals in streams, river and ocean waters, and wastewater treatment plant (WWTP) effluents and influents, developed in Work Package (WP) 1.

2. Task description

WP1 regards analytical methods to analyse emerging contaminants (EC, pharmaceuticals and microplastics) in inland waters using established and novel methods. Task 1.1 focuses on monitoring pharmaceuticals with ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) and microplastics with GC-Pyr-MS/MS. The methodology for pharmaceutical analysis, including extraction and analysis, is described in the team members' previous works [1-3]. It involved solid-phase extraction (SPE) followed by UHPLC-MS/MS. The target compounds include pharmaceuticals from various therapeutic groups, as well as their corresponding metabolites and one degradation product.

3. WP1 - Task 1.1 team members

The Team members in WP1, Task 1.1, regarding pharmaceutical analysis, are:

Name	Organization	Role	Name	Organization	Role
Cristina Delerue-Matos	REQUIMTE	Task coordinator	Magda Almeida	AdCL	Researcher
Manuela Correia	REQUIMTE	Researcher	Roberto Barbosa	AdCL	Researcher
Paula Paíga	REQUIMTE	Researcher	Ana Soares	AdCL	Researcher
Sandra Jorge	AdCL	Researcher			

4. Developed activities

Sampling Campaign 2024

In this sampling campaign, 30 target compounds were analyzed, including pharmaceuticals used for Alzheimer's and Parkinson's diseases, psychiatric drugs, and the stimulant caffeine. Five samples were collected from each source: streams, rivers, ocean water, and WWTP effluents and influents. A total of 25 samples were extracted using SPE and analyzed by UHPLC-MS/MS.

Sample treatment

Samples were collected was performed by the REQUIMTE team. Once received in the laboratory, samples were vacuum filtered through a 0.45 µm nylon membrane filter.

Pharmaceuticals under study

The analytes under study (pharmaceuticals, metabolites, and degradation products) were:

Caffeine	Fluoxetine	Rasagiline
Carbamazepine	Norfluoxetine	Amantadine
Citalopram	Paroxetine	Apormorphine
Citalopram N-oxide	Sertraline	Ropinirole
Citalopram propionic acid	Trazodone	Rivastigmine
Desmethylcitalopram	Venlafaxine	Selegiline
O-Desmethylvenlafaxine	Benserazide	Safinamide
Diazepam	Pramipexole	Donepezil
Didemethylcitalopram	Carbidopa	Rotigotine
10, 11-epoxycarbamazepine	Galantamine	Entacapone

Extraction and analysis

The analytes were extracted using SPE and analyzed by UHPLC-MS/MS on a triple-quadrupole mass spectrometer operating in electrospray ionization (ESI) mode. Lab Solutions LC-MS software (version 5.80, Shimadzu) was used for system control and data processing, with quantification performed by Multiple Reaction Monitoring (MRM). The extraction and chromatographic conditions - including column selection, program settings, elution mode, mobile phases, flow rate, oven temperature, source parameters, ionization mode, precursor ions, product ions, mass spectrometry conditions, and ion ratio - were optimized. The optimized SPE procedure developed and chromatographic conditions are detailed in the author's previous publications [1-3] and summarized in the following paragraph.

Target compounds were isolated using Strata-X SPE cartridges (200 mg, 3 mL). To each sample (50 mL of WWTP influent, 100 mL of WWTP effluent, or 250 mL of water sample), a suitable volume of 0.1 M ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2EDTA) solution was added to achieve a final concentration of 0.1% (g solute/g solution). The pH of each sample was adjusted to 2 using 37% HCl. Pre-treated samples were passed through SPE cartridges conditioned with 5 mL of methanol and equilibrated with 5 mL of ultrapure water, followed by 5 mL of ultrapure water adjusted to pH 2. For the clean-up step, 5 mL of ultrapure water was passed through each cartridge, after which cartridges were left under maximum vacuum pressure for 1 hour. Analytes were eluted with 10 mL of methanol, evaporated to dryness under nitrogen, and reconstituted in 500 µL of acetonitrile (30:70, v/v). Finally, 5 µL of the isotope labelled internal standard (ILIS) mixture was added to standards and samples.

Several chromatographic programs were used for the analysis of the target pharmaceuticals [1-3]. For the analysis in negative ionization mode, a Kinetex C18 column (2.6 × 150 mm i.d., 1.7 µm particle size) from Phenomenex (USA) was used, and chromatographic separation was achieved using ultrapure water and acetonitrile at a flow rate of 0.22 mL/min. For the analysis in positive ionization mode, the chromatographic separation was carried out in a Cortecs® UPLC C18+column (100 × 2.1mmi.d.; 1.6 µm particle size) from Waters (Milford, MA, USA), using 0.1% formic acid in ultrapure water and acetonitrile, at a flow rate of 0.3 mL/min. For both ionization modes, the injection volume was 5 µL, column oven was set at 30°C, and the auto sampler was operated at 4°C.

Analytical Method Validation: Ensuring Accuracy and Reliability

Linearity, method detection limits (MDLs), method quantification limits (MQLs), precision (intra- and inter-day), recovery, and matrix effects (ME) were included in the validation tests. For calibration curves, the area was plotted against analyte concentration using linear regression analysis. The MDLs and MQLs were determined using the minimum detectable amount of each analyte with signal-to-noise ratios of 3 and 10, respectively. Intra- and inter-day analyses were expressed as the relative standard deviation (RSD (%)). Recovery was calculated by comparing the areas of the quantification ion for samples spiked before solid-phase extraction (pre-spiked sample) with the areas of the quantification ion for samples spiked after solid-phase extraction (post-spiked sample). The matrix effect was assessed by comparing the area of a standard prepared in the matrix with the area of the standard prepared in solvent.

5. Results

Validation of the analytical method

Method linearity was confirmed graphically across a concentration range of 0.5 to 1000 µg/L (12 levels), with correlation coefficients (R) exceeding 0.997, indicating a strong linear relationship between the analyte concentration and the peak area. The method led to good precision values, with RSD (%) of intra- and inter-day analysis lower than 10%. Recovery tests involved three fortification levels per matrix, with two extractions per level. Results were consistent for all levels, showing RSD<10%. SPE extraction yielded recoveries higher than 90%. As expected, in general, higher matrix effects were observed in wastewaters when compared with surface waters. The MDL and MQL were determined for all matrices where pharmaceuticals were detected. The MDL ranged from 0.0200 to 21.1 ng/L in the surface water matrix and from 0.200 to 55.5 ng/L in the wastewater matrix. The lowest MDL values showed the method's high sensitivity and revealed qualities of UHPLC-MS/MS for accurate quantification and confirmation of trace levels of the pharmaceuticals, metabolites, and degradation products in environmental samples.

Analysis of the target compounds

From the 30 analysed compounds, a total of 22 were detected in at least one sample. Notably, didemethylcitalopram, norfluoxetine, apomorphine, benserazide, carbidopa, donepezil, galantamine, pramipexole, and safinamide were not detected in any of the analysed samples. Fluoxetine was the only compound detected in all 25 samples, followed closely by caffeine, which was detected in 24 out of 25 samples. Concentrations ranged from 29.8–656 ng/L for caffeine, <MDL to 381 ng/L for psychiatric drugs, and <MDL to 37.1 ng/L for Alzheimer's disease (AD) and Parkinson's disease (PD) pharmaceuticals in surface water. In wastewater, concentrations ranged from 140–76,991 ng/L for caffeine, <MDL to 5,227 ng/L for psychiatric drugs, and <MDL to 206 ng/L for AD and PD pharmaceuticals. The highest concentration observed was for caffeine, with a value of 76,991 ng/L. Significantly, 13 out of 15 psychiatric drugs (86.7%) were detected in at least one sample, highlighting their widespread presence. Additionally, 7 out of 14 compounds (50%) associated with AD and PD pharmaceuticals were also detected, underscoring the relevance of these compounds in the studied samples (Table 1). These findings provide valuable insights into pharmaceutical contamination trends in the river ecosystem and WWTP discharges, enhancing understanding of environmental and public health impacts.

Table 1. Concentrations of detected compounds in surface water (ocean, streams, and rivers) and WWTP effluents and influents (<MDL, below method detection limit; n.d.-not detected).

Compounds	Concentration (ng/L)																								
	AO1	AO2	AO3	AO4	AO5	S1	S2	S3	S4	S5	R1	R2	R3	R4	R5	E1	E2	E3	E4	E5	I1	I2	I3	I4	I5
Carbamazepine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**52.0**	**20.5**	**48.5**	**26.7**	**108**	**11.4**	**17.6**	**19.0**	**150**	**1,347**	**1,005**	**599**	**318**	**446**	**1,144**	**565**	**220**	**372**	**497**
RSD (%)							5.38	1.34	1.21	1.87	3.30	4.91	1.43	4.06	0.898	7.36	0.0341	1.55	1.32	2.98	0.618	2.91	2.34	6.12	9.41
Citalopram N-oxide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Citalopram propionic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**35.1**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	**<MDL**	**46.7**
RSD (%)															3.58										13.2
Citalopram	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	**38.6**	**<MDL**	**<MDL**	**<MDL**	**<MDL**	**<MDL**	**<MDL**	n.d.	n.d.	n.d.
RSD (%)																5.39									
Desmethylcitalopram	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	n.d.	**38.0**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																0.729									
***O*-Desmethylvenlafaxine**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**35.5**	n.d.	n.d.	n.d.	**381**	**44.9**	**20.6**	**21.2**	**203**	**5,227**	**4,143**	**3,583**	**1,850**	**2,938**	**4,027**	**1,949**	**821**	**1,506**	**2,609**
RSD (%)							1.27				11.3	11.1	1.13	2.78	1.50	3.04	6.94	5.41	8.66	12.4	0.174	3.05	6.50	18.2	2.32
Diazepam	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**20.6**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																			1.05						
Didemethylcitalopram	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
10,11-Epoxycarbamazepine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**25.7**	n.d.	n.d.	n.d.	n.d.	n.d.	**68.4**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)											10.2						1.23								
Fluoxetine	**5.62**	**15.7**	**5.55**	**5.35**	**5.67**	**5.64**	**5.60**	**5.63**	**6.02**	**5.77**	**5.82**	**5.54**	**5.79**	**6.16**	**8.06**	**17.1**	**14.3**	**60.3**	**20.2**	**14.2**	**58.5**	**99.0**	**28.9**	**29.6**	**29.7**
RSD (%)	0.203	9.65	2.76	6.12	0.604	0.723	1.72	1.16	3.09	4.86	3.42	9.40	3.89	5.00	1.86	4.22	10.1	12.1	3.52	10.5	12.2	1.91	0.206	1.58	2.12
Norfluoxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Paroxetine	n.d.	**<MDL**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Sertraline	n.d.	**4.28**	**<MDL**	n.d.	**6.41**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**27.4**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)		11.67			3.73													18.6							
Trazodone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	**<MDL**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	**<MDL**	**92.9**	**<MDL**	**<MDL**	**<MDL**	**<MDL**	**<MDL**	**<MDL**	**<MDL**
RSD (%)																		**9.0**							
Venlafaxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**10.9**	n.d.	n.d.	n.d.	**70.4**	**26.9**	**22.1**	**24.0**	**65.1**	**862**	**665**	**513**	**393**	**506**	**773**	**460**	**87.5**	**330**	**481**
RSD (%)							4.90				4.18	2.48	9.19	0.949	1.58	10.4	2.66	1.76	3.56	7.78	0.501	1.84	5.39	7.68	4.17
Amantadine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**9.75**	n.d.	n.d.	n.d.	n.d.	**149**	**95.9**	**97.5**	**109**	**49.0**	**55.4**	n.d.	n.d.	n.d.	**206**
RSD (%)											8.82					11.7	2.49	4.97	1.68	17.2	15.1				16.9
Apormorphine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Benserazide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Carbidopa	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Donepezil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Entacapone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**37.1**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)									0.944																
Galantamine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Pramipexole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Rasagiline	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Rivastigmine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**37.0**	**22.8**	**9.68**	**2.79**	**14.6**	n.d.	n.d.	n.d.	n.d.	**15.0**
RSD (%)																7.14	6.67	17.5	23.3	2.44					1.79
Ropinirole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	n.d.
RSD (%)																									
Rotigotine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	n.d.	n.d.	n.d.	
RSD (%)																									n.d.
Safinamide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Selegiline	**<MDL**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**<MDL**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RSD (%)																									
Caffeine	**72.3**	**44.8**	**29.8**	**85.7**	**34.3**	n.d.	**33.2**	**31.5**	**86.3**	**33.2**	**630**	**121**	**31.6**	**122**	**213**	**12,018**	**9,164**	**318**	**130**	**76,991**	**9,802**	**57,640**	**38,001**	**448**	**536**
RSD (%)	8.38	6.48	15.1	6.44	5.83		0.479	4.64	1.72	16.4	14.5	6.23	13.2	1.91	6.36	6.21	0.0249	2.12	1.49	4.90	3.37	7.91	7.70	17.0	0.638

6. Associated indicators

Publications

1. Paula Paíga, Cristina Delerue-Matos, Tracing Pharmaceuticals in Water Systems: Focus on Neurodegenerative and Psychiatric Treatments, Journal of Xenobiotics 14 (4) (2024) 1807-1825; https://doi.org/10.3390/jox14040096.

Communications

1. Cristina Delerue-Matos, Removal of pharmaceuticals from wastewaters: is the revised European Directive a solution or a headache?, Seminar "Urban Wastewater Treatment: Ready for Zero Pollution?", April 9, 2024, Universidade Católica Portuguesa (Porto, Portugal).
2. Manuela Correia, Surface Water and Wastewater Monitoring: Pharmaceuticals, Metabolites, and degradation products, "Biodiversity restoration and conservation of inland water ecosystems" conference, April 18 - 19, 2024, Instituto Superior de Engenharia do Porto (Porto, Portugal).

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[1] Paíga, P., Santos, L.H.M.L.M., Ramos, S., Jorge, S., Silva, J.G., Delerue-Matos, C. (2016). Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation. Science of the Total Environment 573, 164-177.

[2] Paíga, P., Santos, L.H.M.L.M., Delerue-Matos, C. (2017). Development of a multi-residue method for the analysis of human and veterinary pharmaceuticals and some of their metabolites in aqueous environmental matrices by SPE-UHPLC-MS/MS. Journal of Pharmaceutical and Biomedical Analysis 135, 75-86.

[3] Paíga, P., Correia, M., Fernandes, M.J., Silva, A., Carvalho, M., Vieira, J., Jorge, S., Silva, J.G., Freire, C., Delerue-Matos, C. (2019). Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: Hourly variation. Science of the Total Environment 648, 582-600.