Contract n° 018486

NORMAN

Network of reference laboratories and related organisations for monitoring and bio-monitoring of emerging environmental pollutants

Co-ordination action

Priority 6.3 – Global Change and Ecosystems

Deliverable: C.2.2.

NSAID RESIDUES ANALYSIS IN WATER

Report on the 1st interlaboratory study (including statistical evaluation of the results and critical evaluation of the methodology used)

Due date of deliverable: April 2007

Actual submission date: April 2007

Start date of the project: **1st September 2005** **Duration: 3 years**

Work package leader: **CSIC**

Revision: final version

Dissemination level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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Sub-priority 1.1.6.3 Global Change and Ecosystems
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FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

Contents

I. Participant Laboratories: In alphabetical order

- Environmental Institute, Kos, Slovak Republic
- Institute for Environment and Sustainability, JRC, Ispra, Italy
- Jozef Stefan Institute, Ljubljana, Slovenia
- CNRS, LPTC - Université Bordeaux 1,Talence – France
- Eawag, Environmental Chemistry, Duebendorf, Switzerland,
  EPF Lausanne, Switzerland
- Europa Fachhochschule Fresenius, University of Applied Science, Idstein, Germany
- Federal Institute of Hydrology (BFG), Kablenz, Germany
- IIQAB-CSIC, Barcelona, Spain
- Mario Negri Institute for Pharmacological Research, Milan, Italy
- Pesticide Residues Laboratory, General Chemical State Laboratory, Athens, Greece
- Umweltbundesamt GmbH, Abt. Umweltwirksame Stoffe und Metaboliten, Wien, Austria.
- Universidade de Santiago de Compostela, Spain
- Universita "La Sapienza" di Roma, Italy

II. Objectives

The main objectives were:
To carry out the first validation level of chromatographic methods of analysis of Ketoprofen, Naproxen, Ibuprofen and Diclofenac.
To evaluate the main sources of variation
To evaluate the possible significant differences between methods based on Liquid chromatography and gas chromatography.
To assess the influence of sample matrix on the different chromatographic approaches
To assess stability of samples

III. General Information

NSAID compounds selected in this study were: Ketoprofen, Naproxen, Ibuprofen and Diclofenac.
Participants were requested to analyse these compounds in each sample.
Each participant received three batches of samples and each batch was composed by 3 samples:

- a natural sample of wastewater
- a fortified river sample
- spiked MilliQ water

A total number of 162 samples were distributed to 17 laboratories distributed along 11 European Countries (Austria, France, Germany, Greece, Italy, Norway, Slovak Republic, Slovenia, Spain, Switzerland, and UK) that initially took part on the exercise. A final number of 14 participations concluded the ring exercise.

In order to minimize the sources of variation all samples were collected transported, homogenised and prepared at the same time in a central laboratory (Environmental Chemistry Department, IIQAB-CSIC, Barcelona).
Samples were collected and prepared during the first week of September 2006.
All water samples were filtered through 0.7 and 0.45 µm glass micro-fibre filters to remove suspended matter and they were homogenized in a polyethylene bucked.
The samples were sent refrigerated.

Each participant received approximately 1,1 L of each sample. Participants were requested to measure and annotate the temperature of samples at reception. Most of participant received theirs samples in the range of 15-20 ºC. Participant were also requested to keep the samples under freezing conditions until the extraction of each batch, according to the dates indicated below.
Deadline for chromatographic analysis was November 17th, and the deadline for sending the results November 24th.

Samples were sent codified according to the table, but the wastewater samples were indicated to the participants.

	SPE Dates	SAMPLE CODES		
Batch 1	25-29 Sep 2006	A1 Fortified river water	B1 Wastewater	C1 Fortified Milli Q water
Batch 2	9-3 Oct 2006	A2 Wastewater	B2 Fortified Milli Q water	C2 Fortified river water
Batch 3	23-27 Oct 2006	A3 Fortified Milli Q water	B3 Fortified river water	C3 Wastewater

In order to maintain the anonymous character of each participant, an identification number was provided to each laboratory and they were requested to use it for results presentation.

IV. Analytical Protocols for NORMAN participants

Validation

Norman participants were requested to use one of the follow methods.

LC-MS Analytical Protocol

Extraction and Pre-treatment
Neutral pH
Extraction Volumes: 500 mL of MilliQ water and river samples
200 mL of wastewater effluent
SPE using Oasis HLB (60 mg, 3mL) polymeric cartridges.
Elution of cartridges with methanol
Reconstitution of extracts: 1mL of methanol-water (25:75, v/v).

LC-ESI-tandem MS analysis
Analysis of extracts: LC-ESI-tandem MS
Chromatographic separation: RP-18 column.
Analysis under NI mode, using as eluent A methanol and water as eluent B.
2 transitions, one for identification and one for quantification

GC-MS Analytical Protocol

Int. std.: d3 ibuprofen
SPE: Oasis HLB/60 mg
Elution: EtAc
Derivatisation: MTBSTFA (MSTFA)
SIM ions (IB:263, NP:287, KT:311, DF:352&354)
GC column: HP-5MS, 30m, 0.25mm, 0,25microm
GC oven: 65º (2min), rate 30º/min to 180º, rate 5º/min to 300 (hold 12 min)

V. Results

A total number of 126 samples were analyzed along the ring exercise and 486 results were collected corresponding to 14 participations in 13 laboratories.

Total No. Results = 486
Total No. Outliers = 23 (4.7%)

No. of participations using Liquid Chromatography = 7 (50%)
No. of participations using Gas Chromatography = 7 (50%)

No. of outliers using Liquid Chromatography = 8 (3.3% of results)
No. of outliers using Gas Chromatography = 15 (6.1 % of results)

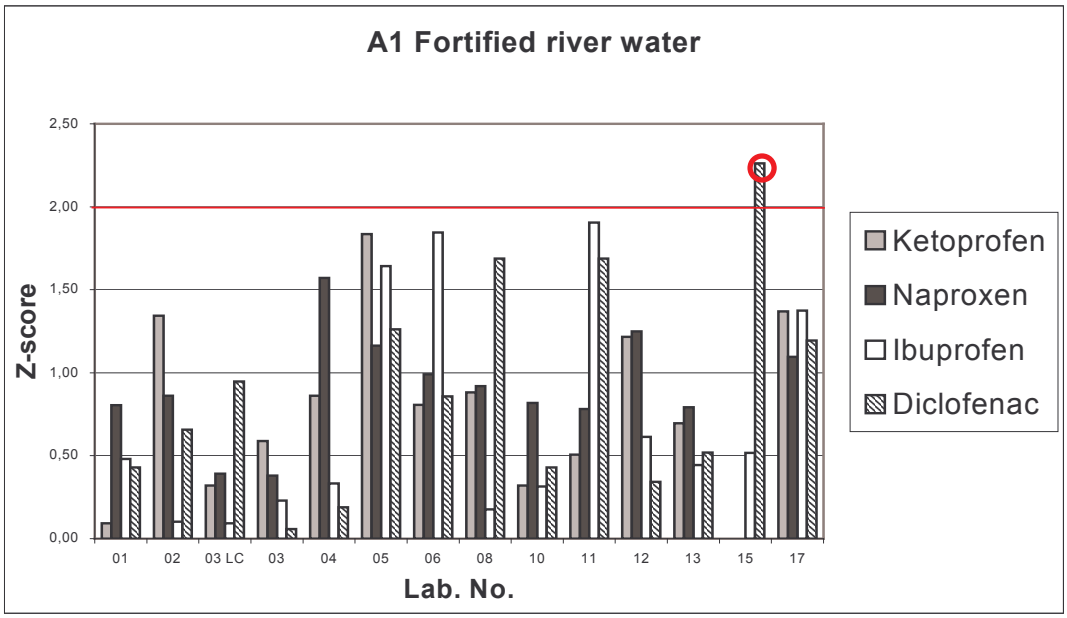
For each series of samples (batch 1, batch2, and batch3) the initial mean value (Xi), the initial standard deviation (σi), the upper warning limit (UWL), and the lower warning limit (LWL) values were calculated.

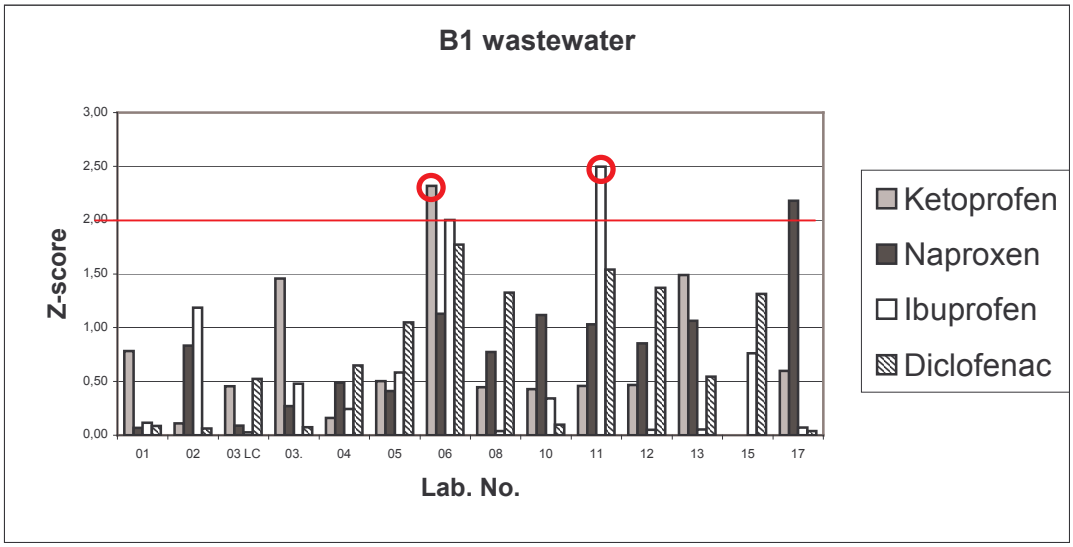
The limits were calculated as: UWL = (Xi+2 σi), and LWL = (Xi-2 σi)

As acceptance criteria for each result was used the *Z-score* function according to the Laboratory Accreditation & Audit Protocol: Food Inspection Directorate":
$Z = (X_{lab}-Xi) / \sigma i$
Where X_{lab} is a result, Xi is the initial mean value and the σi the initial standard deviation.
The results whose *Z*-value was over 3 was directly excluded and when the *Z-score* value was between 2 and 3 was applied the Dixon test with a 5% of significance level.

On Figure 1 are presented the *Z-score* values for each result of each participant.

In a second step the outlier values were excluded of data treatment the statistical parameters (mean value (Xi), standard deviation (σi), variance (σ^2), coefficient of variation (%CV) were calculated


A1 Fortified river water
Z-score
2,50
2,00
1,50
1,00
0,50
0,00
01
02
03 LC
03
04
05
06
08
10
11
12
13
15
17
Lab. No.
Ketoprofen
Naproxen
Ibuprofen
Diclofenac


B1 wastewater
Z-score
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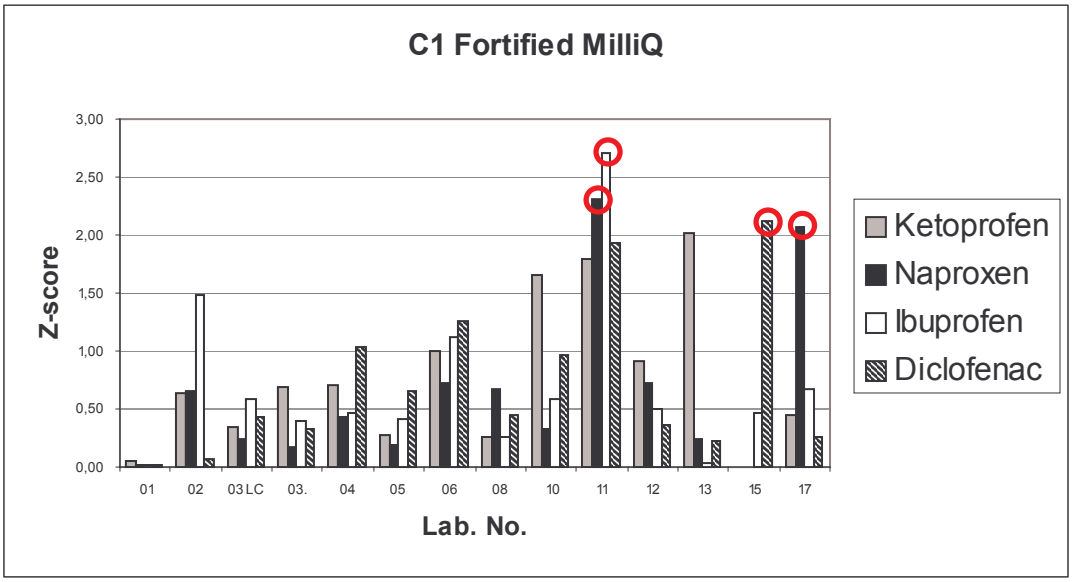
2,00
1,50
1,00
0,50
0,00
01
02
03 LC
03.
04
05
06
08
10
11
12
13
15
17
Lab. No.
Ketoprofen
Naproxen
Ibuprofen
Diclofenac


C1 Fortified MilliQ
Z-score
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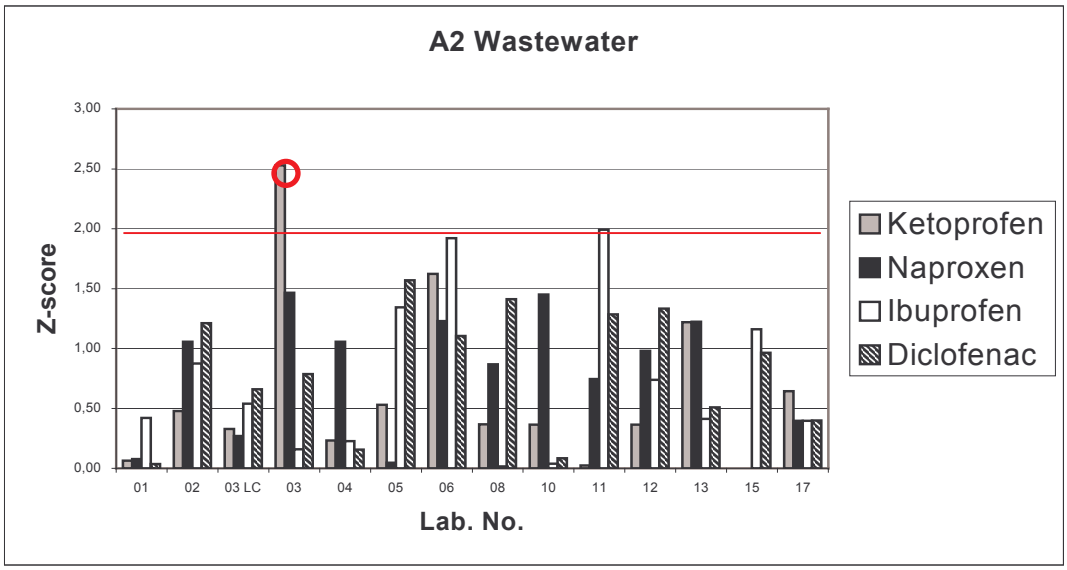
2,50
2,00
1,50
1,00
0,50
0,00
01 02 03 LC 03. 04 05 06 08 10 11 12 13 15 17
Lab. No.
Ketoprofen
Naproxen
Ibuprofen
Diclofenac


A2 Wastewater
Z-score
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2,50
2,00
1,50
1,00
0,50
0,00
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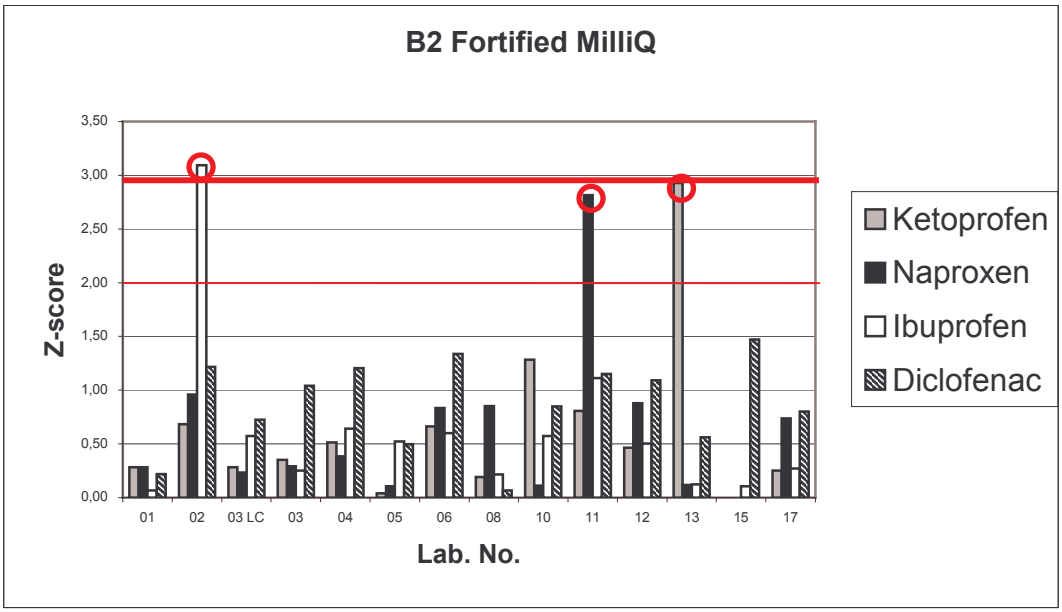
Lab. No.
Ketoprofen
Naproxen
Ibuprofen
Diclofenac


B2 Fortified MilliQ
Z-score
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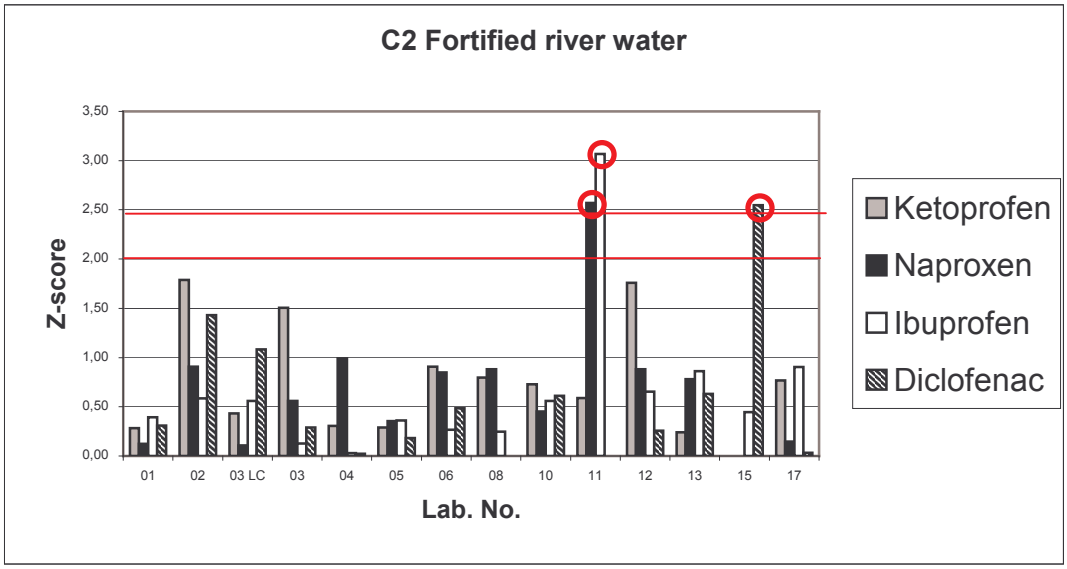
3,00
2,50
2,00
1,50
1,00
0,50
0,00
01 02 03 LC 03 04 05 06 08 10 11 12 13 15 17
Lab. No.
Ketoprofen
Naproxen
Ibuprofen
Diclofenac


C2 Fortified river water
Z-score
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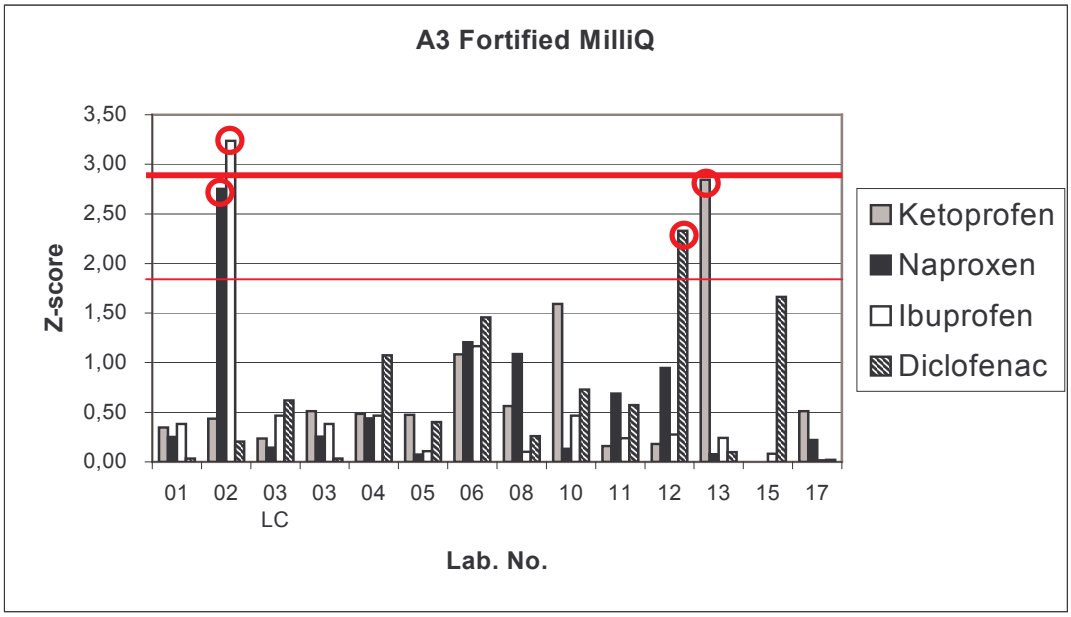
3,00
2,50
2,00
1,50
1,00
0,50
0,00
01
02
03 LC
03
04
05
06
08
10
11
12
13
15
17
Lab. No.
Ketoprofen
Naproxen
Ibuprofen
Diclofenac


A3 Fortified MilliQ
Z-score
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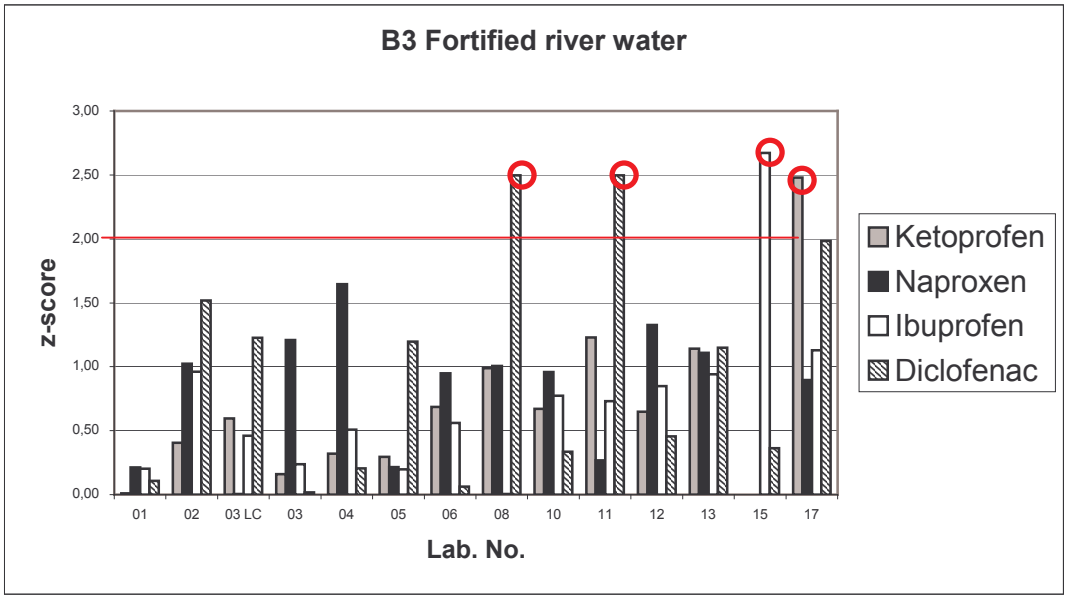
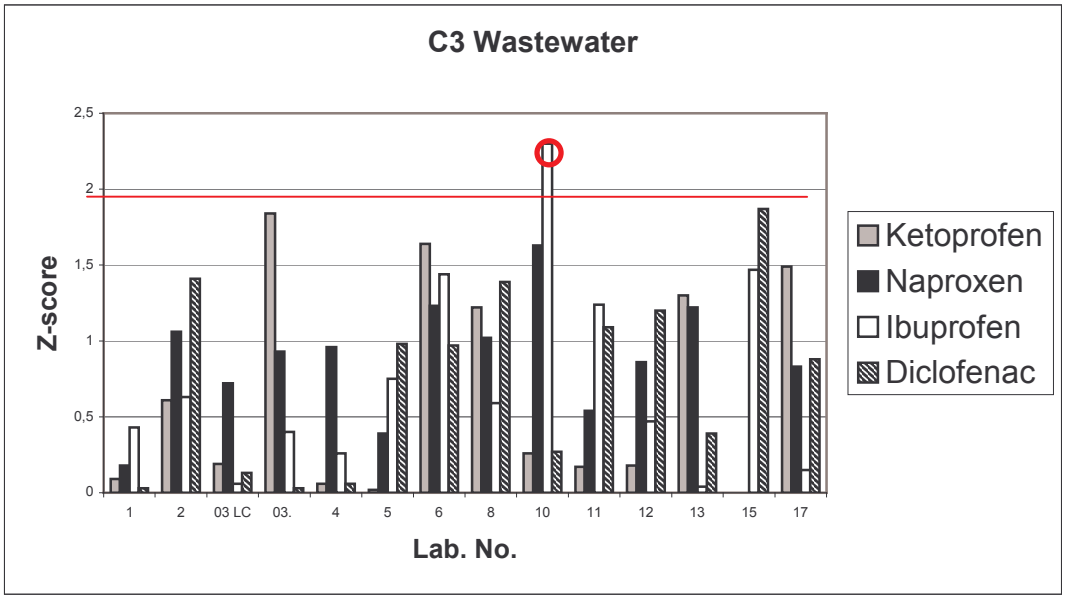
3,00
2,50
2,00
1,50
1,00
0,50
0,00
01
02
03 LC
03
04
05
06
08
10
11
12
13
15
17
Lab. No.
Ketoprofen
Naproxen
Ibuprofen
Diclofenac


B3 Fortified river water
z-score
3,00
2,50
2,00
1,50
1,00
0,50
0,00
01
02
03 LC
03
04
05
06
08
10
11
12
13
15
17
Lab. No.
Ketoprofen
Naproxen
Ibuprofen
Diclofenac


C3 Wastewater
Z-score
2,5
2
1,5
1
0,5
0
1
2
03 LC
03.
4
5
6
8
10
11
12
13
15
17
Lab. No.
Ketoprofen
Naproxen
Ibuprofen
Diclofenac

On Table 1 are summarized the corrected results (after outlier exclusion) obtained for each compound in the different types of samples along the exercise.

Ketoprofen

Wastewater	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
	Batch 1	13	678.77	282.23	78.27	800.00	25	1090	508.21	849.33	0
	Batch 2	12	601.08	235.24	67.91	620.95	112	897	451.61	750.54	1
	Batch 3	13	642.55	303.61	84.21	662.00	144	1200	459.06	826.03	1

Fortified river water	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
Fortification 290 ng/L	Batch 1	13	238.73	121.08	33.58	200	73	461	165.56	311.90	0
	Batch 2	13	297.92	134.13	37.20	260	62	538	216.85	378.98	0
	Batch 3	12	241.39	92.317	26.65	230	106.2	420	182.74	300.05	1

Fortified MilliQ water	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
Fortification 83 ng/L	Batch 1	12	164.24	99.52	28.73	133.5	64	365	101	227.47	0
	Batch 2	11	101.71	40.52	12.22	100.0	60	208.8	74.49	128.93	1
	Batch 3	11	97.35	33.96	10.24	91.00	37	151	74.54	120.17	0

Naproxen

Wastewater	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
	Batch 1	12	913.18	744.55	214.93	848.00	18	2140	440.11	1386.3	1
	Batch 2	13	858.17	635.68	176.31	887.00	77	1790	474	1242.3	0
	Batch 3	13	818.54	603.27	167.32	930.00	77	1800	153.95	1183.1	0

Fortified river water	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
Fortification 1124 ng/L	Batch 1	13	1109.0	845.51	234.50	1430.0	53	24.37	598.01	1620	0
	Batch 2	12	1088.9	746.59	215.52	1047.5	308	2446	614.51	15.63	1
	Batch 3	13	1066.6	764.33	211.99	1230.0	53.5	2325	604.69	1528	0

Fortified MilliQ water	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
Fortification 266 ng/L	Batch 1	11	174.32	96.214	29.010	200.00	51	330	109.68	238.95	2
	Batch 2	12	158.83	96.254	27.786	165.00	33.5	327	97.68	219.99	1
	Batch 3	12	201.96	106.87	30.850	225.00	24	369	134.06	269.86	1

Table 1 (1/2)

Ibuprofen

Wastewater	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
	Batch 1	13	1834.2	640.75	177.71	1980.0	233	2680	1446.9	2221.4	1
	Batch 2	14	1777.6	589.47	157.54	1715.5	645	2951.5	1437.3	2117.9	0
	Batch 3	13	1918.3	385.03	106.79	1904.0	1124	1685.5	1685.6	2151.0	1

Fortified river water	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
Fortification 675 ng/L	Batch 1	14	536.04	278.79	74.510	508.75	22	1067.1	375.1	699.0	0
	Batch 2	13	523.73	156.46	43.395	484.00	310	875.0	429.2	618.3	1
	Batch 3	13	542.74	190.58	52.859	603.00	278	811.1	427.6	657.9	1

Fortified MilliQ water	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
Fortification 225 ng/L	Batch 1	13	260.46	139.51	38.693	215.00	66	621	176.15	344.8	1
	Batch 2	13	253.15	102.87	28.532	246.00	165	547	190.97	315.3	1
	Batch 3	13	206.12	45.461	12.609	203.00	96	265	178.65	233.6	1

Diclofenac

Wastewater	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
	Batch 1	14	1482.2	784.75	209.73	1545.0	92	2557	1029.2	1935.2	0
	Batch 2	14	1476.4	919.93	245.86	1564.5	177	2920	945.29	2007.4	0
	Batch 3	14	1503.8	982.03	262.46	1457.0	177	3343	936.84	2070.7	0

Fortified river water	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
Fortification 398 ng/L	Batch 1	11	400.36	198.83	59.951	340.00	133	767	266.79	533.93	1
	Batch 2	11	408.27	145.78	43.955	457.00	129	609	310.34	506.21	1
	Batch 3	12	392.88	157.37	45.428	400.00	154	705	292.89	492.86	2

Fortified MilliQ water	Group	No. accepted results	Mean	Standard deviation	Standard error of mean	Median	Minimum value	Maximum value	95% Confidence Interval		No. of outliers
									From	To	
Fortification 200 ng/L	Batch 1	13	205.71	109.18	30.280	193.00	61	483.2	139.73	271.69	1
	Batch 2	14	167.43	79.237	21.177	156.00	61.4	284	121.69	213.17	0
	Batch 3	14	150.51	71.030	19.700	148.00	33	320	107.58	193.43	1

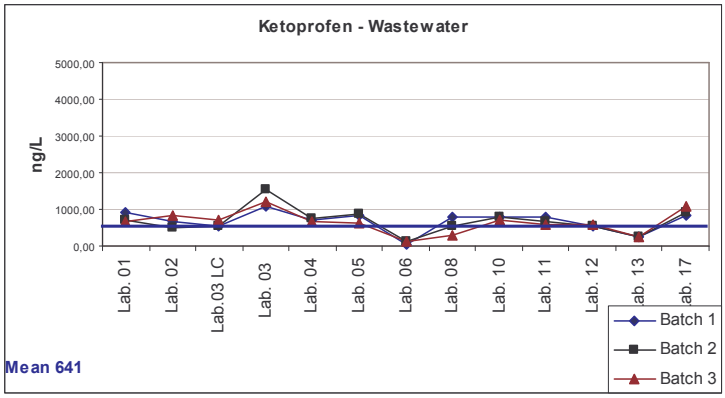
Table 1 (2/2)

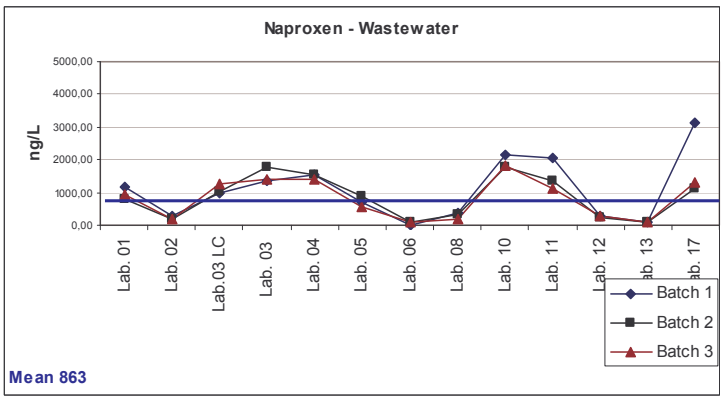
On table 2 are summarized the mean of each compound in each type of water samples.

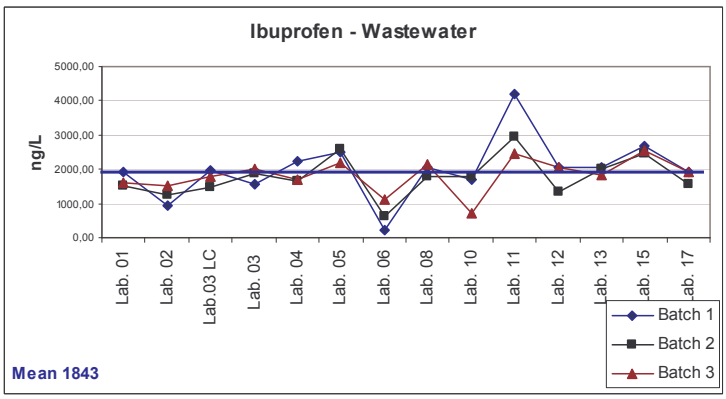
	Mean of batches	**Standard deviation of batch**
Wastewater		
Ketoprofen	640.8	38.9
Naproxen	863.3	47.5
Ibuprofen	1843.4	70.8
Diclofenac	1487.4	14.4
Fortified river water		
Ketoprofen	259.3	33.4
Naproxen	1088.2	21.2
Ibuprofen	534.2	9.6
Diclofenac	400.5	7.7
Fortifiend MilliQ water		
Ketoprofen	121.1	37.4
Naproxen	178.4	21.8
Ibuprofen	239.9	29.5
Diclofenac	174.55	28.3

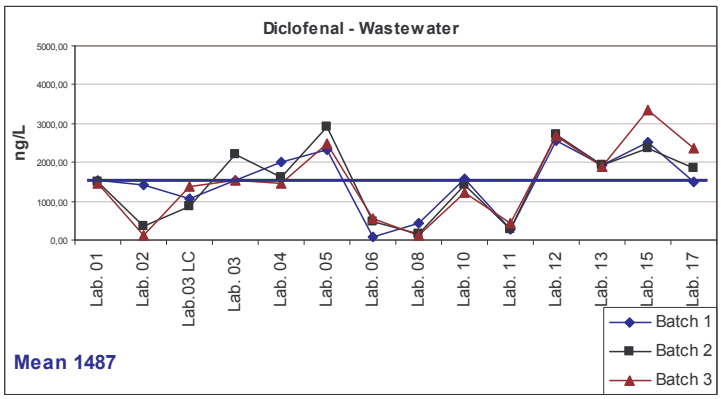
VI. Summary of participant's results

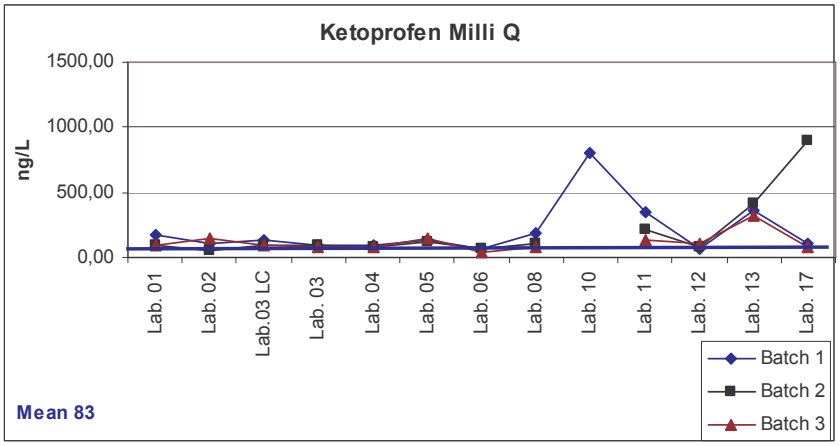
The summary of the results obtained for each participant in front of the mean value of the results is summarized on Figure










Ketoprofen Milli Q
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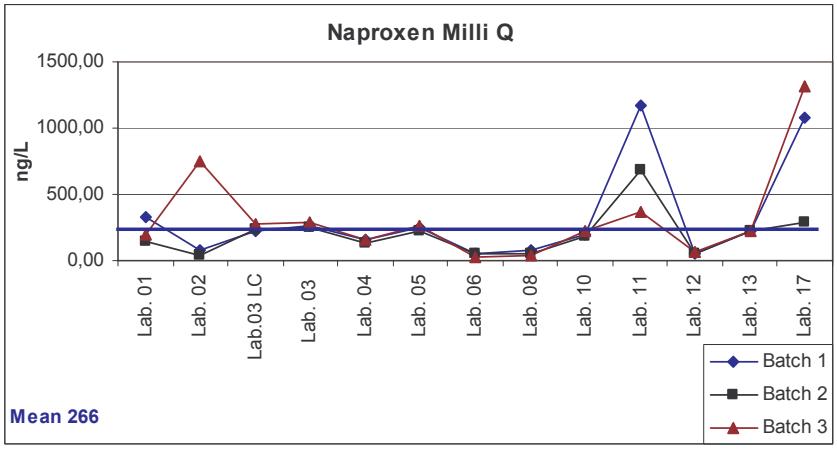
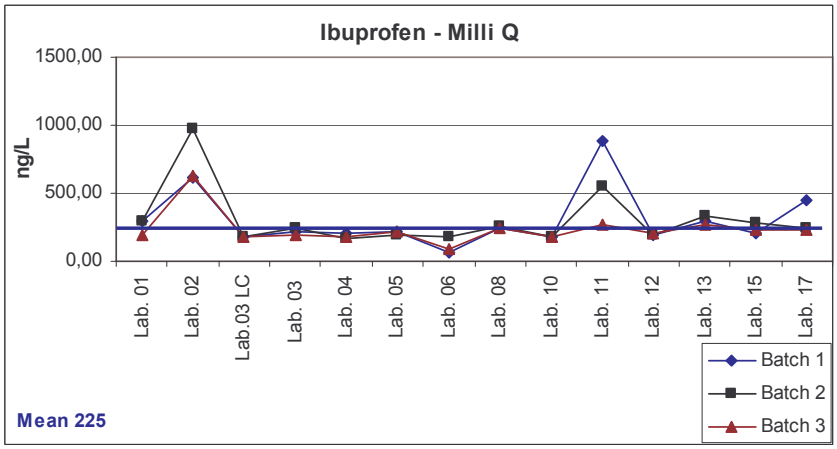
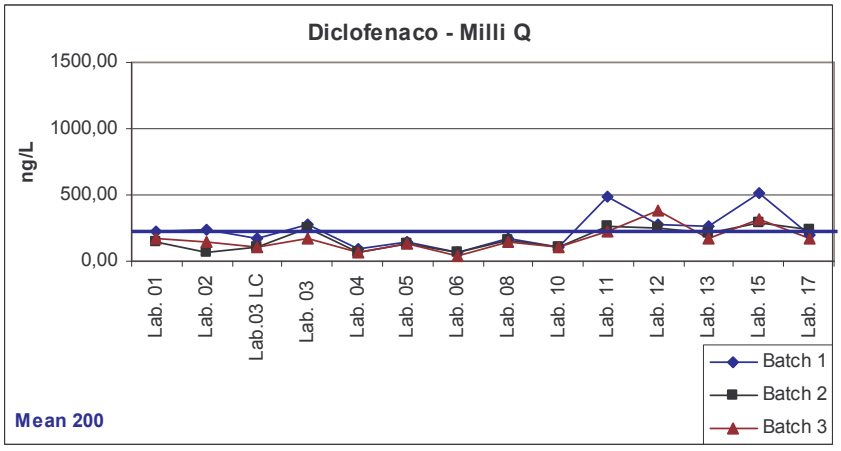
1000,00
500,00
0,00
ng/L
Lab. 01
Lab. 02
Lab.03 LC
Lab. 03
Lab. 04
Lab. 05
Lab. 06
Lab. 08
Lab. 10
Lab. 11
Lab. 12
Lab. 13
Lab. 17
Batch 1
Batch 2
Batch 3
Mean 83


Naproxen Milli Q
1500,00
1000,00
500,00
0,00
ng/L
Lab. 01
Lab. 02
Lab.03 LC
Lab. 03
Lab. 04
Lab. 05
Lab. 06
Lab. 08
Lab. 10
Lab. 11
Lab. 12
Lab. 13
Lab. 17
Batch 1
Batch 2
Batch 3
Mean 266


Ibuprofen - Milli Q
1500,00
1000,00
500,00
0,00
ng/L
Lab. 01
Lab. 02
Lab.03 LC
Lab. 03
Lab. 04
Lab. 05
Lab. 06
Lab. 08
Lab. 10
Lab. 11
Lab. 12
Lab. 13
Lab. 15
Lab. 17
Batch 1
Batch 2
Batch 3
Mean 225


Diclofenaco - Milli Q
1500,00
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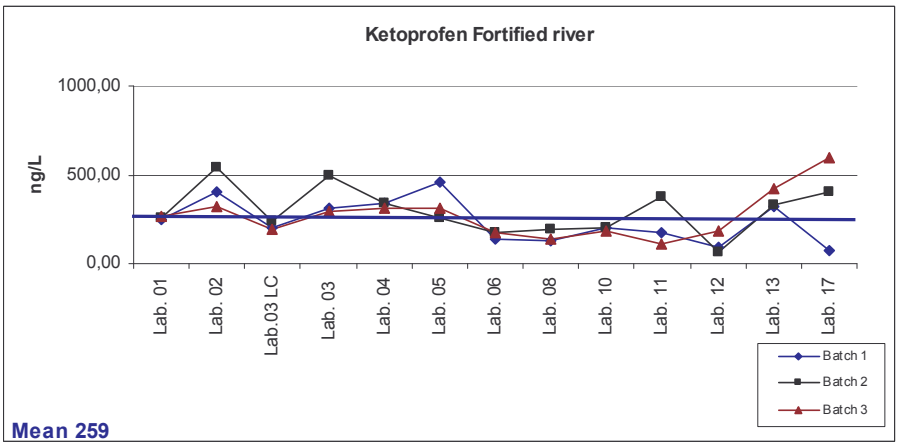
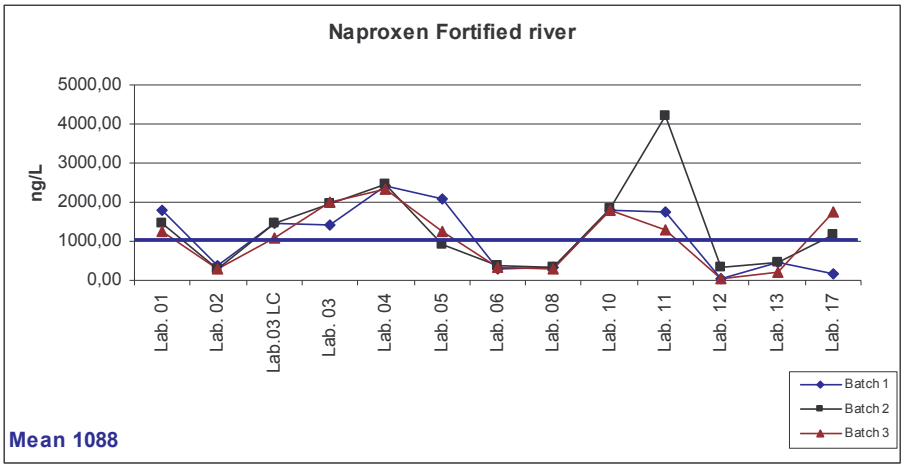
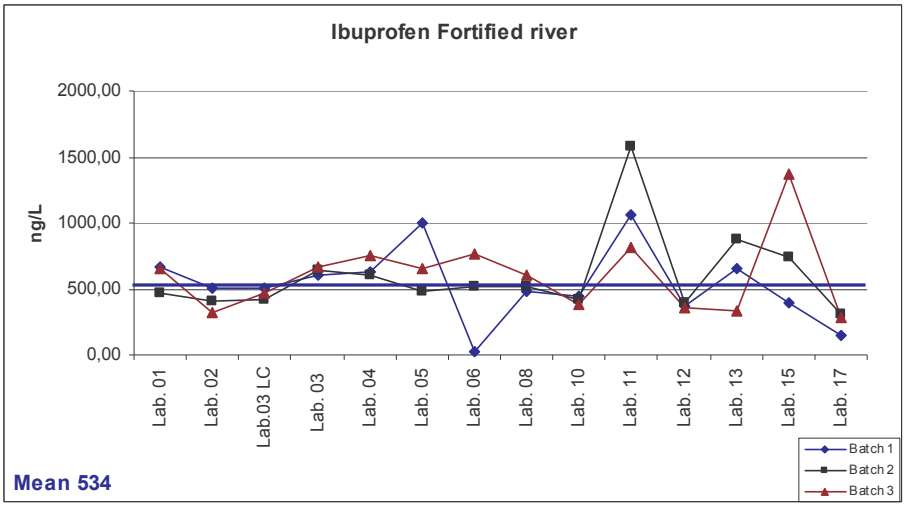
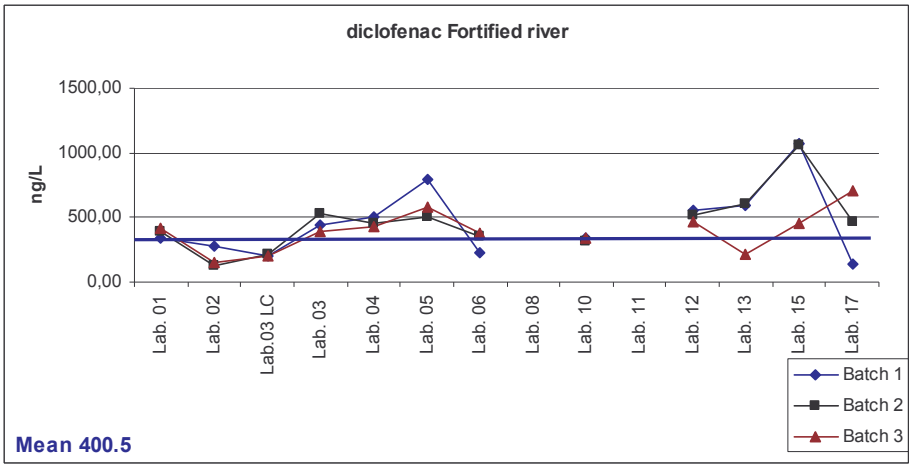
500,00
0,00
ng/L
Lab. 01
Lab. 02
Lab.03 LC
Lab. 03
Lab. 04
Lab. 05
Lab. 06
Lab. 08
Lab. 10
Lab. 11
Lab. 12
Lab. 13
Lab. 15
Lab. 17
Batch 1
Batch 2
Batch 3
Mean 200


Ketoprofen Fortified river
1000,00
500,00
0,00
ng/L
Lab. 01
Lab. 02
Lab.03 LC
Lab. 03
Lab. 04
Lab. 05
Lab. 06
Lab. 08
Lab. 10
Lab. 11
Lab. 12
Lab. 13
Lab. 17
Batch 1
Batch 2
Batch 3
Mean 259

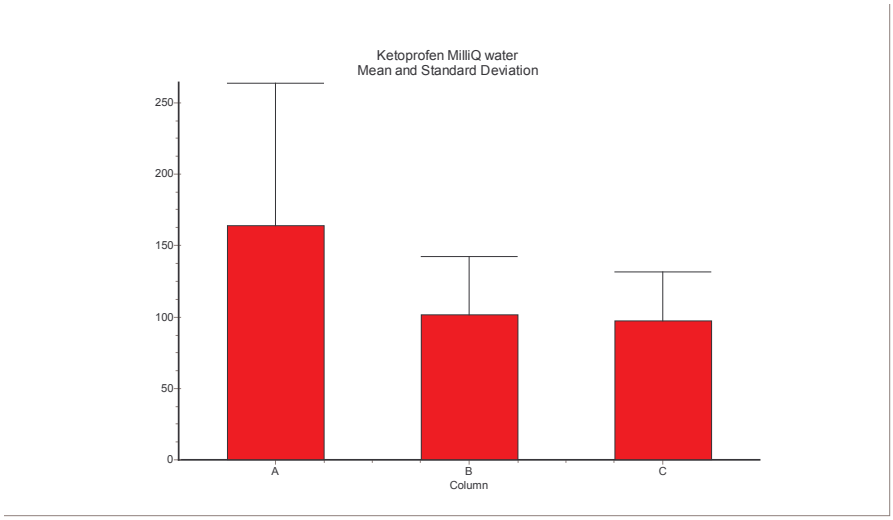

Naproxen Fortified river
5000,00
4000,00
3000,00
2000,00
1000,00
0,00
ng/L
Lab. 01
Lab. 02
Lab.03 LC
Lab. 03
Lab. 04
Lab. 05
Lab. 06
Lab. 08
Lab. 10
Lab. 11
Lab. 12
Lab. 13
Lab. 17
Batch 1
Batch 2
Batch 3
Mean 1088

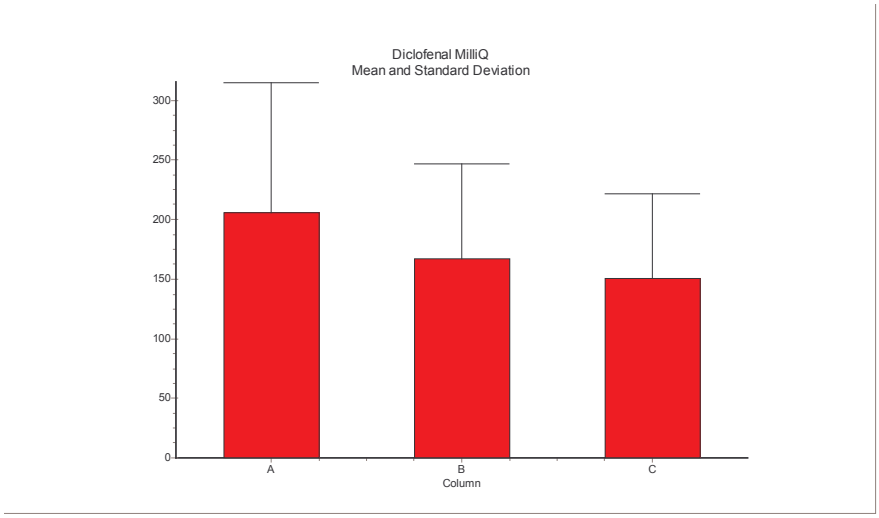

Ibuprofen Fortified river
2000,00
1500,00
1000,00
500,00
0,00
ng/L
Lab. 01
Lab. 02
Lab.03 LC
Lab. 03
Lab. 04
Lab. 05
Lab. 06
Lab. 08
Lab. 10
Lab. 11
Lab. 12
Lab. 13
Lab. 15
Lab. 17
Batch 1
Batch 2
Batch 3
Mean 534


diclofenac Fortified river
1500,00
1000,00
500,00
0,00
ng/L
Lab. 01
Lab. 02
Lab.03 LC
Lab. 03
Lab. 04
Lab. 05
Lab. 06
Lab. 08
Lab. 10
Lab. 11
Lab. 12
Lab. 13
Lab. 15
Lab. 17
Batch 1
Batch 2
Batch 3
Mean 400.5

VII. Stability of the samples

In order to establish the stability of samples along this intercomparison test, differences between variances obtained for every type of sample at different intervals were evaluated.
The Analysis of Variance for each compound is presented in Annex I.
In all cases the distribution of results followed a Gaussian distribution.
The results from the normality and Bartlett's tests are presented in Annex I.
In two cases, results from Bartlett's test suggest that the differences among standard deviation were significant along the exercise. These cases were Ketoprofen and Ibuprofen (see figure 3) in Milli Q water. This sample has a basic pH, and that probably influenced on the stability of these compounds.
For the rest of the cases good stability was obtained along the exercise.





Reproducibility and repeatability:

The measurement of precision of each laboratory to repeat the measurements on a sample at different intervals (batch): reproducibility (R) was calculated as:

$$R = \frac{\Sigma r_{lab}}{N}$$

Where

$r_{lab} = \Sigma (2 \cdot 2^{1/2})\ \sigma_{lab}$

N = number of samples (only results for stable samples were accounted)

σ_{lab} *is the standard deviation between results from the same laboratory on a stable sample at different intervals.*

Repeatability values obtained for the four measurements (Ketoprofen, naproxen, ibuprofen, and diclofenac) for river water and wastewater are summarized on tables 3 and 4. On Figure

Table 3: Repeatability (r) and reproducibility (R) values of each laboratory for the analysis of Ketoprofen, naproxen, ibuprofen and diclofenac in wastewater.

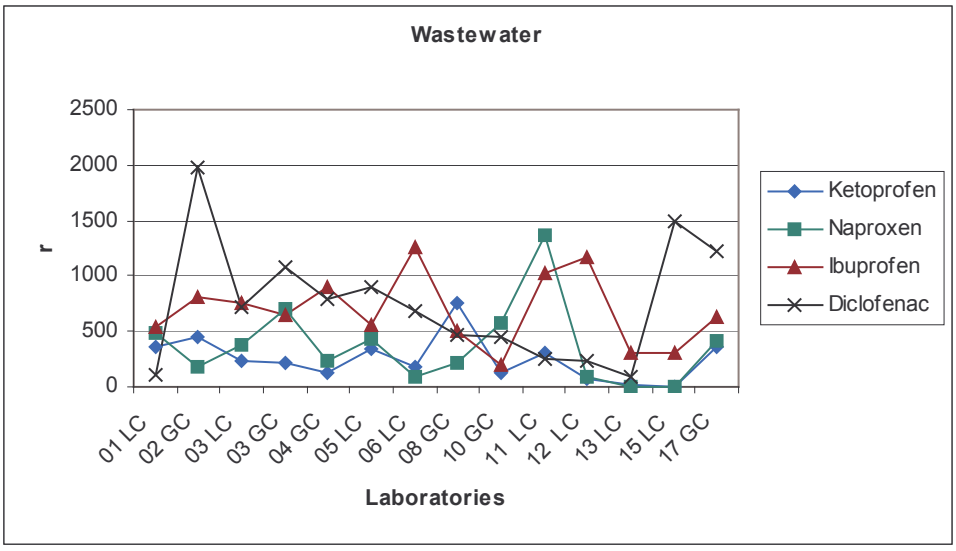
	Ketoprofen	Naproxen	Ibuprofen	Diclofenac
01 LC	356	488	546	113
02 GC	455	186	805	1978
03 LC	237	386	747	727
03 GC	220	704	645	1086
04 GC	133	233	898	792
05 LC	335	436	565	900
06 LC	174	96	1261	684
08 GC	752	217	495	469
10 GC	131	572	200	453
11 LC	311	1366	1018	253
12 LC	66	94	1166	226
13 LC	14	0	307	85
15 LC	0	0	309	1492
17 GC	368	412	621	1218
R	254	371	684	748

Table 4: Repeatability (r) and reproducibility (R) values of each laboratory for the analysis of Ketoprofen, naproxen, ibuprofen and diclofenac in river water.

	Ketoprofen	Naproxen	Ibuprofen	Diclofenac
01 LC	28	795	314	888
02 GC	308	143	257	726
03 LC	75	613	128	361
03 GC	328	891	99	281
04 GC	49	191	211	597
05 LC	298	1704	733	2074
06 LC	59	149	1065	3013
08 GC	92	61	169	478
10 GC	33	65	97	273
11 LC	397	997	512	1448
12 LC	178	462	47	133
13 LC	153	368	773	2188
15 LC			700	1980
17 GC	656	2242	235	664
R	204	668	381	1079

When “r” is superior to the Upper Warning Limit the variation coefficient for this laboratory should be considered significant. This coefficient is inversely proportional to the precision, which means a repeatability level significant lower than the rest.

On Figure 4 the repeatability coefficients (r) and coefficients of variation intra-laboratories are shown for the most complex matrix. Must be said that for ketoprofen one laboratory presented a significant superior variation coefficient and lower precision, laboratory number 8. For Naproxen a significant lower precision was showed by participant number 11, and for diclofenac laboratory number 2 showed a significant degree of variation..



VIII. Hierarchical cluster analysis

The possible interrelation between results was studied using the hierarchical cluster analysis. So as to all the results for each type of water were studied together and the results for each participant were grouped first according the Average Linkage Cluster method, and second according to the Nearest Neighbor Cluster method. In both cases the measure interval between results was the squared Euclidean distance.
Both cluster analysis concluded that the results of participating laboratories are independent of the analytical method used for the analysis of the samples (LC or GC).
On the other hand, no relation was obtained with the results and the temperature of the samples at reception in the range of temperatures studied.
On figure 4 Dendograms corresponding to the cluster analysis using the single linkage method are represented.

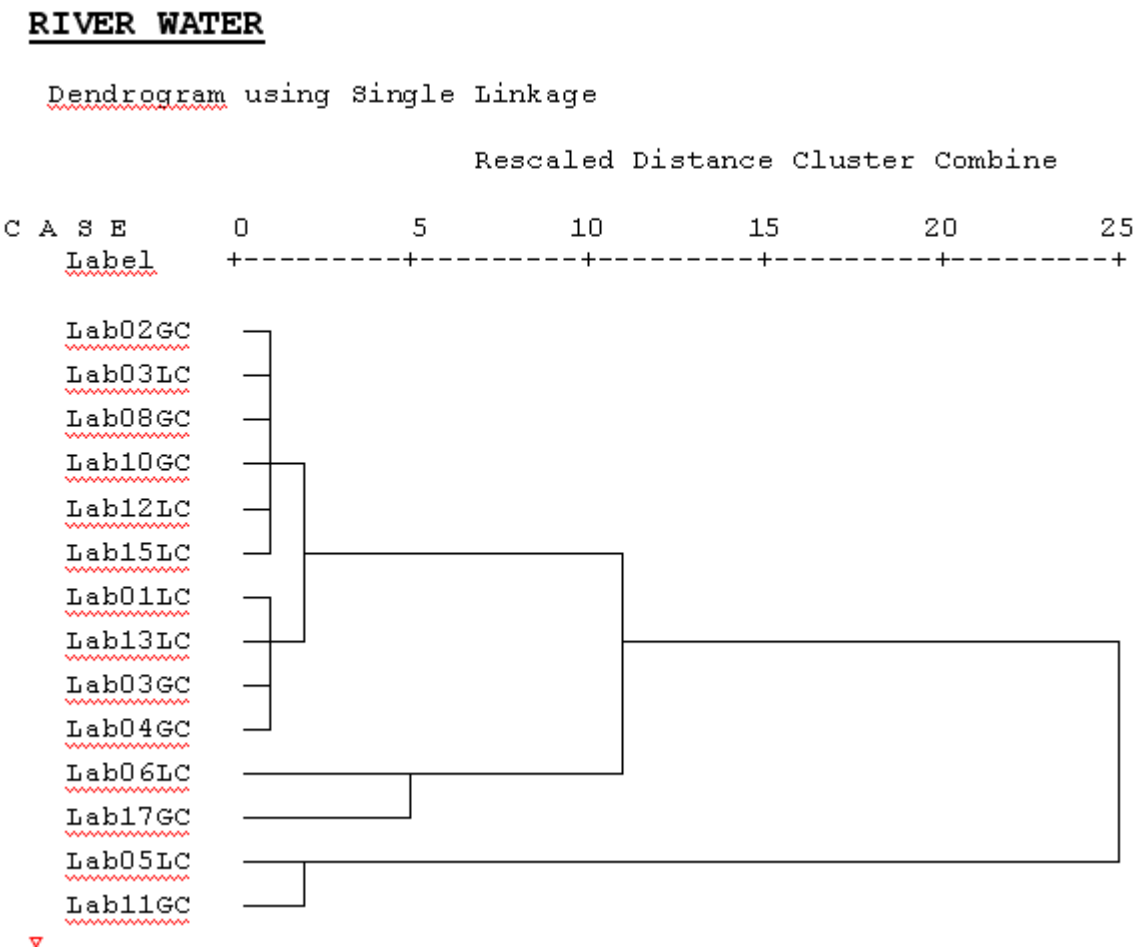
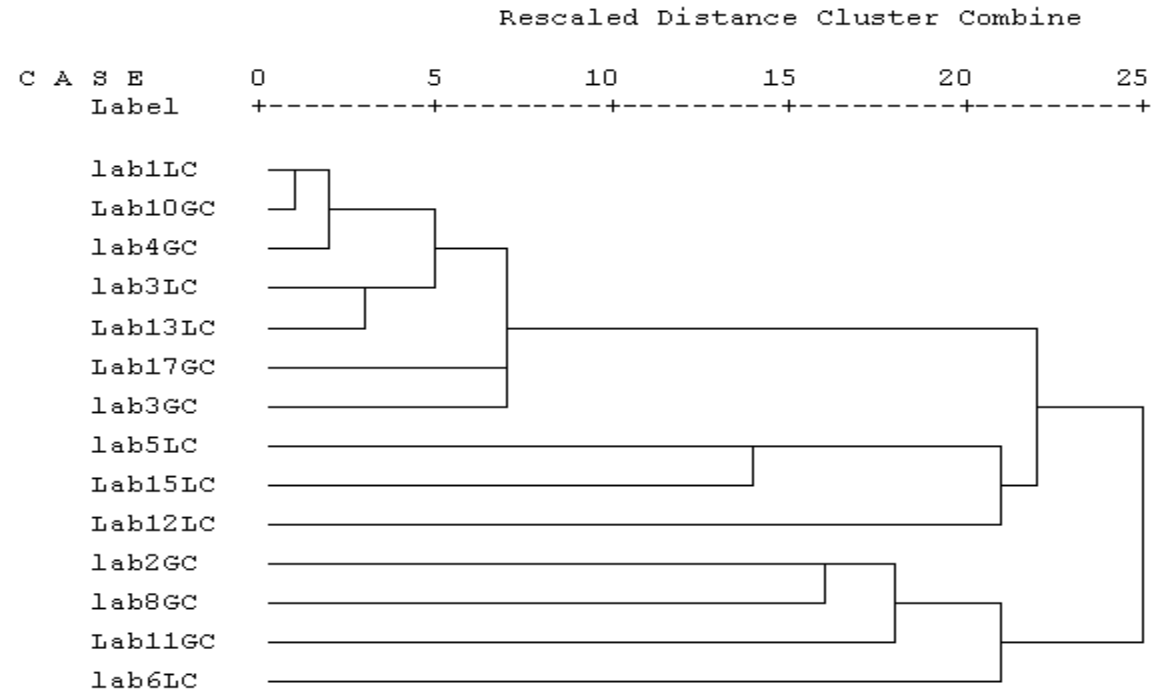
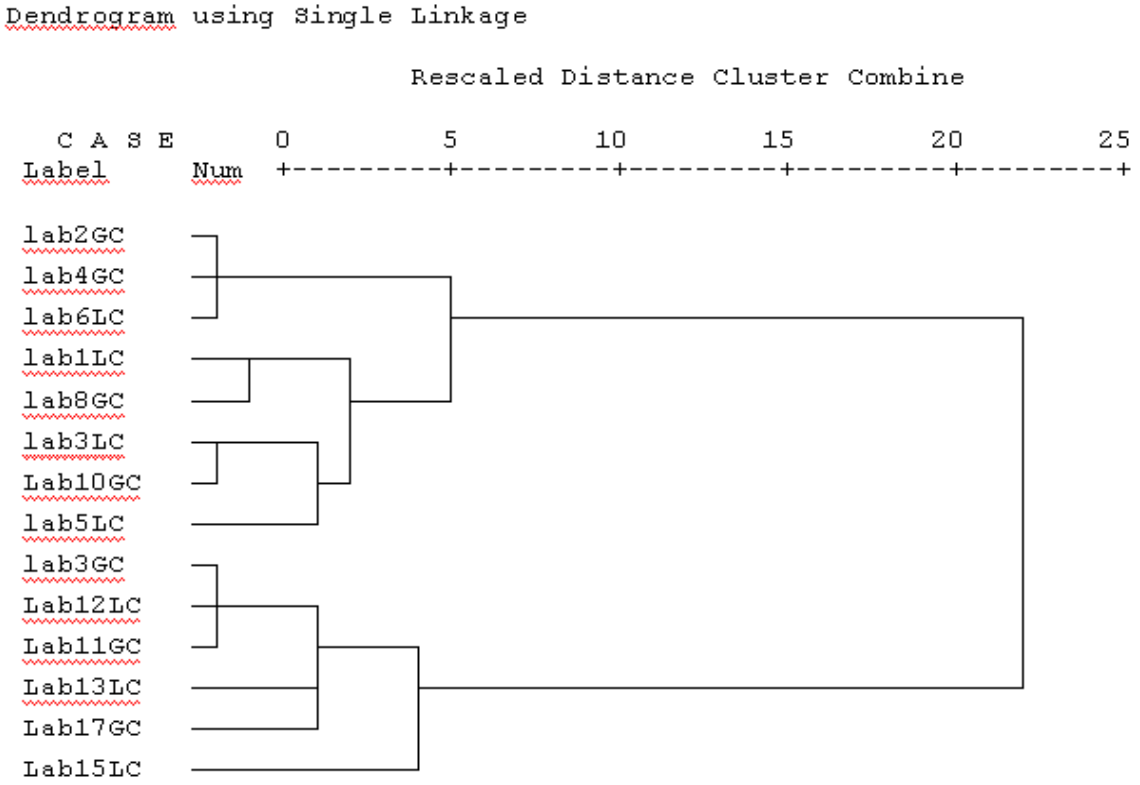


Figure 4(1/2)

Wastewater



Milli-Q



IX. Conclusions:

The number of participants that initiated this interlaboratory exercise was 17, and the final number of participants was 13 (77%).

The final number of results collected was 486 and 23 values were outliers (4,7%), and were discarded.

The number of outlier values by liquid chromatography was 8 (3,3% of results), whereas the number of outlier using gas chromatography was superior 15 (6,1 %), as expected because of the necessary additional step (derivatization).
Four laboratories obtained the higher number of outlier values: laboratory 11, 15, 17, and 2 with 7, 4, 3 and 3 outliers respectively.
The sample with higher number of outlier was the fortified MilliQ water, because the level of concentration was lower.
The second sample with more outlier values was wastewater due to the complexity of the matrix. Also for this sample were obtained the higher levels of variability.
The stability of samples were followed along the exercise by means of the Analysis of variance. In all cases ANOVA showed good stability for the NSAID selected in this study, and the variations among means were not significantly greater than expected by chance, with exception of Ketoprofen and Ibuprofen in the Milli-Q water, probably due to the basic pH of this sample, and the low level of concentrations.
A general good agreement was obtained between the concentrations of fortification and the mean values recorded by the participants. However, the precision of individual participants was low along the exercise, and that means a necessary protocol of sample treatment including (manipulations, how to defreeze the samples and during how long, etc…) in order to minimize sources of variation in the second ring.
About reproducibility of values recorded for the analyzed compound in the different types of samples was as well low, but that was expected due the high number of small different methods involved in the present edition.
The hierarchical cluster analysis concluded that no relation can be found between the results and if the samples were analyzed following a GC or a LC based method.
In addition no relation was obtained between the results and the temperature at reception.

ANNEX

Ketoprofen wastewater

One-way Analysis of Variance (ANOVA)

The P value is 0.7826, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 0.7237
The P value is 0.6964.
Bartlett's test suggests that the difference among the SDs is not significant.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
batch 1	0.2047	>0.10	Yes
batch 2	0.1795	>0.10	Yes
batch 3	0.1979	>0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	37677	18838
Residuals (within columns)	35	2670699	76306
Total	37	2708376	

F = 0.2469 =(MStreatment/MSresidual)

River water - Ketoprofen

One-way Analysis of Variance (ANOVA)

The P value is 0.3658, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 1.521
The P value is 0.4675.
Bartlett's test suggests that the differences among the SDs is not significant.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch1	0.1639	>0.10	Yes
Batch2	0.1497	>0.10	Yes
Batch3	0.2111	>0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	28723	14362
Residuals (within columns)	35	485565	13873
Total	37	514289	

F = 1.035 =(MStreatment/MSresidual)

Ketoprofen MilliQ water

One-way Analysis of Variance (ANOVA)

The P value is 0.0364, considered significant.
Variation among column means is significantly greater than expected by chance.

Tukey-Kramer Multiple Comparisons Test
If the value of q is greater than 3.482 then the P value is less than 0.05.

Comparison	Mean Difference	q		P value
Batch 1 vs Batch 2	62.533	3.188	ns	P>0.05
Batch 1 vs Batch 3	66.887	3.410	ns	P>0.05
Batch 2 vs Batch 3	4.355	0.2173	ns	P>0.05

Difference	Mean Difference	95% Confidence Interval From	To
Batch 1 - Batch 2	62.533	-5.756	130.82
Batch 1 - Batch 3	66.887	-1.401	135.18
Batch 2 - Batch 3	4.355	-65.402	74.112

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 13.838
The P value is 0.0010.
Bartlett's test suggests that the differences among the SDs is extremely significant.
Since ANOVA assumes populations with equal SDs, you should consider transforming your data (reciprocal or log) or selecting a nonparametric test.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch 1	0.1912	>0.10	Yes
Batch 2	0.2468	>0.10	Yes
Batch 3	0.1643	>0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	32618	16309
Residuals (within columns)	31	136890	4415.8
Total	33	169508	

F = 3.693 =(MStreatment/MSresidual)

Naproxen - Wastewater

One-way Analysis of Variance (ANOVA)

The P value is 0.9379, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 0.5417
The P value is 0.7627.
Bartlett's test suggests that the differences among the SDs is not significant.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch 1	0.1903	>0.10	Yes
Batch 2	0.1916	>0.10	Yes
Batch 3	0.1896	>0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	56183	28092
Residuals (within columns)	35	1.531E+07	437552
Total	37	1.537E+07	

F = 0.06420 =(MStreatment/MSresidual)

Naproxen - river

One-way Analysis of Variance (ANOVA)

The P value is 0.9906, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 0.2009
The P value is 0.9044.
Bartlett's test suggests that the differences among the SDs is not significant.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch1	0.2471 >	0.10	Yes
Batch2	0.2206 >	0.10	Yes
Batch3	0.2130 >	0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	11686	5843.2
Residuals (within columns)	35	2.172E+07	620586
Total	37	2.173E+07	

F = 0.009416 =(MStreatment/MSresidual)

Naproxen - MilliQ water

One-way Analysis of Variance (ANOVA)

The P value is 0.5702, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 0.1553
The P value is 0.9253.
Bartlett's test suggests that the differences among the SDs is not significant.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch1	0.2014 >	0.10	Yes
Batch2	0.1930 >	0.10	Yes
Batch3	0.1519 >	0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	11437	5718.6
Residuals (within columns)	32	320109	10003
Total	34	331546	

F = 0.5717 =(MStreatment/MSresidual)

Wastewater - Ibuprofen

One-way Analysis of Variance (ANOVA)

The P value is 0.8021, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 3.080
The P value is 0.2143.
Bartlett's test suggests that the differences among the SDs is not significant.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch1	0.2332 >	0.10	Yes
Batch2	0.1520 >	0.10	Yes
Batch3	0.07681 >	0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	134590	67295
Residuals (within columns)	37	1.122E+07	303322
Total	39	1.135E+07	

F = 0.2219 =(MStreatment/MSresidual)

Ibuprofen-river

One-way Analysis of Variance (ANOVA)

The P value is 0.9746, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 4.249
The P value is 0.1195.
Bartlett's test suggests that the differences among the SDs is not significant.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch1	0.1726	>0.10	Yes
Batch2	0.2018	>0.10	Yes
Batch3	0.1889	>0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	2420.2	1210.1
Residuals (within columns)	37	1740036	47028
Total	39	1742456	

F = 0.02573 =(MStreatment/MSresidual

Ibuprofen - Milliq water

One-way Analysis of Variance (ANOVA)

The P value is 0.3584, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 12.245
The P value is 0.0022.
Bartlett's test suggests that the differences among the SDs is very significant.
Since ANOVA assumes populations with equal SDs, you should consider transforming your data (reciprocal or log) or selecting a nonparametric test.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch1	0.2346	>0.10	Yes
Batch2	0.2062	>0.10	Yes
Batch3	0.2059	>0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	22609	11304
Residuals (within columns)	36	385352	10704
Total	38	407961	

F = 1.056 =(MStreatment/MSresidual)

Wastewater-diclofenac

One-way Analysis of Variance (ANOVA)

The P value is 0.9964, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 0.6469
The P value is 0.7237.
Bartlett's test suggests that the differences among the SDs is not significant.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch1	0.1890	>0.10	Yes
Batch2	0.1508	>0.10	Yes
Batch3	0.1322	>0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	5829.4	2914.7
Residuals (within columns)	39	3.154E+07	808826
Total	41	3.155E+07	

F = 0.003604 =(MStreatment/MSresidual)

Diclofenac -river

One-way Analysis of Variance (ANOVA)

The P value is 0.9763, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 1.049
The P value is 0.5920.
Bartlett's test suggests that the differences among the SDs is not significant.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch1	0.1647	>0.10	Yes
Batch2	0.1763	>0.10	Yes
Batch3	0.1578	>0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	1360.8	680.40
Residuals (within columns)	31	880291	28396
Total	33	881652	

F = 0.02396 =(MStreatment/MSresidual)

Diclofenal MilliQ

One-way Analysis of Variance (ANOVA)

The P value is 0.2712, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Post tests were not calculated because the P value was greater than 0.05.

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 2.434
The P value is 0.2962.
Bartlett's test suggests that the differences among the SDs is not significant.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
Batch1	0.1844	>0.10	Yes
Batch2	0.1459	>0.10	Yes
Batch3	0.2060	>0.10	Yes

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	20844	10422
Residuals (within columns)	37	285199	7708.1
Total	39	306043	

F = 1.352 =(MStreatment/MSresidual)