SECOND INTERLABORATORY EXERCISE ON DETERMINATION OF NSAIDs IN WATER SAMPLES

Report

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Network of reference laboratories for monitoring of emerging pollutants: NORMAN, Contract No. 018486

I. Participant Laboratories

- Ecole Polytechnique Fédérale de Lausanne (EPFL), Institut des sciences et technologies de l'environnement (ISTE), Lausanne, Switzerland
- European Commission - DG Joint Research Centre, Institute for Environment and Sustainability (IES), Ispra (VA), Italy
- General Chemical State Laboratory, Pesticide Residues Laboratory, Athens, Greece
- Institute of Chemical and Environmental Research (IIQAB-CSIC), The Department of Environmental Chemistry, Barcelona, Spain
- "Jožef Stefan" Institute (JSI), Department of Environmental Sciences, Ljubljana, Slovenia
- Mario Negri Institute, Department of Environmental Health Sciences, Milan, Italy
- Norwegian Institute for Water Research (NIVA), Oslo, Norway
- Umweltbundesamt GmbH (Austrian Federal Environment Agency), Vienna, Austria.
- Université Bordeaux 1, Institut des Sciences Moléculaires, Groupe de Physico et Toxico-Chimie, Talence, France
- University of A Coruña, University Institute of Environment (IUMA), Department of Analytical Chemistry, A Coruña, Spain
- University of Rome "La Sapienza", Department of Chemistry, Roma Italy

II. General information

1. *Sample preparation and transport*

Four nonsteroidal anti-inflammatory drugs (NSAIDs) were selected for the analysis in the Interlaboratory exercise: ibuprofen (IP), ketoprofen (KP), naproxen (NP) and diclofenac (DF). Three batches of samples were prepared for each laboratory; each consisting of 3 samples, where each batch was prepared from one of the following water matrices:

- three wastewater samples,
- three river water samples,
- three deionised water samples.

In order to minimize the sources of variation, the samples were collected, homogenized and prepared at IIQAB-CSIC, Department of Environmental Chemistry, Barcelona, Spain. The two matrices, wastewater treatment plant effluent and river water, were collected and transported to the laboratory on Friday, 15th June 2007, where upon they were filtered through 2.7 µm and 0.5 µm glass micro-fibre filters. Deionised water was not filtered. Afterwards, all samples were homogenized, spiked where specified and sub-sampled for homogeneity and stability testing. The samples were then transferred into 1 L polyethylene bottles (approx. 900 mL of each sample) and frozen overnight. The frozen samples were shipped on dry-ice to the participant laboratories on 19th and 20th June 2007. The total number of 117 samples was sent to 13 participants in 12 laboratories, distributed in 9 European countries: Norway, Greece, Switzerland, Italy, Spain, Slovakia, Austria, Slovenia and France. The samples arrived to participant laboratories in 24 to 72 hrs in frozen state.

Separately 1.5 mL of standard mixture in methanol was sent, with the following concentrations of NSAIDs: ibuprofen 42.80 mg/L, naproxen 40.00 mg/L, ketoprofen 56.40 mg/L, diclofenac 42.80 mg/L. The standard mixture was not sent on dry ice.

The samples were encoded as illustrated in Table 1. Wastewater samples were additionally labelled as for their extraction a different volume was requested than for the other two matrices.

Table 1: Sample matrices and encoding

SAMPLE CODES		
A1 Natural wastewater	**B1** Natural river water	**C1** Spiked deionised water
A2 Fortified wastewater	**B2** Fortified river water	**C2** Spiked deionised water
A3 Fortified wastewater	**B3** Fortified river water	**C3** Spiked deionised water

2. *Homogeneity of samples*

To assure and confirm the quality of sample preparation homogeneity of spiked samples from each batch was tested. Thus A2 & A3, B2 & B3 and C2 & C3 (Table 1), were subsampled after the spiking and homogenisation, where five samples per batch were taken from different layers in the polyethylene container. Two parallels were analysed per each sample, in total 10 samples were analysed per each batch. The homogeneity was statistically evaluated using x^2-*test*, proposing the H0 hypothesis that the homogeneity of mixing is achieved, when samples are only affected by random error. x^2-*test* was performed by Equation 1,

$$x^2 = \sum \frac{(O_i - E_i)^2}{E_i} \qquad \textit{Equation 1}$$

where O_i is an average of two parallels and E_i the mean of each batch containing 5 samples. For each tested batch (A, B and C) the homogeneity was confirmed by $x^2_{\text{exp.}} \langle x^2_{crit.}$ at five degrees of freedom and α=5%.

3. *Stability of samples*

Stability studies of NSAIDs in different matrices were not performed as this was one of the goals of the 1st NORMAN Interlaboratory Exercise. In addition, the participants were asked to perform the extraction immediately after the sample receipt; therefore the stability of the

NSAIDs in water matrices was not a relevant issue. Instead, the stability of NSAIDs in frozen cartridges was tested within three months after the sample extraction.

4. *Analytical protocols for NORMAN participants and NORMAN Validation*

The participants were asked to extract the samples within 48 hrs upon sample receiving and to keep the dried cartridges frozen until the analysis. The analysis deadline was three months from the extraction date.

With respect to laboratory equipment, two analytical protocols were predetermined at NORMAN Interlaboratory meeting in Ljubljana, April 2007 and are described below.

LC-MS Analytical protocol

- Neutral pH
- Internal standard d3 ibuprofen (when additional filtration was required, internal standard should be added after filtration and prior to SPE)
- Extraction volumes
    - 400 mL of deionised water and river samples
    - 200 mL of wastewater effluent
    - total volume of each sample: 900 mL
- SPE using Oasis HLB (60 mg, 3mL) polymeric cartridges
- Cartridge elution: 8 mL methanol
- Extract reconstitution: 1 mL of methanol-water (25:75, v/v)
- Extract analysis: LC-ESI-tandem MS
- Chromatographic separation: RP-18 column.
- Mode: NI
- Mobile phases
    - Mobile phase A: methanol with 5 mM NH_4 acetate
    - Mobile phase B: water with 5 mM NH_4 acetate
- 2 transitions when possible (one for identification and one for quantification)

GC-MS Analytical protocol

- Internal standard d3 ibuprofen (when additional filtration was required, internal standard should be added after filtration and prior to SPE)
- Extraction volumes
    - 400 mL of deionised water and river samples
    - 200 mL of wastewater effluent
    - total volume of each sample: 900 mL
- No acidification prior to analysis
- SPE using Oasis HLB (60 mg, 3mL) polymeric cartridges
- Cartridge elution: 2 ml ethylacetate
- Derivatisation: MTBSTFA 60°C, 1h
- SIM ions – 2 ions when possible
    - IB:263
    - NP:287
    - KT:311
    - DF:352 and 354
- GC column: HP-5MS, 30m, 0.25mm, 0,25µm
- GC oven: 65º (2min), rate 30º/min to 180º, rate 5º/min to 300 (hold 12 min)

5. *Data collection*

A total number of 108 samples were analysed in the NORMAN 2nd Interlaboratory Exercise by 12 participations from 11 different institutions. 7 LC and 5 GC (Table 2) laboratories took part in the ring test and submitted 773 results, including parallel and < LOD determinations. Among these, 428 values were subjected to subsequent data mining process, where 15 (3.5 %) or 18 (4,2 %) , in the classical and robust approach respectively, of them were excluded from the further calculation as outliers.

Table 2: Summary of analytical protocols used by each participating laboratory

Lab ID	1	2	3	4	5	6	7	8	10	11	12	13
Analytical protocol	LC	LC	GC	LC	LC	LC	LC	GC	LC	GC	GC	GC

III. Results

6. *Determination of outliers*

As an acceptance criterion for each result the z-score value was calculated using the following equation:

$$z = \frac{x_{lab} - x_0}{\sigma_0}$$

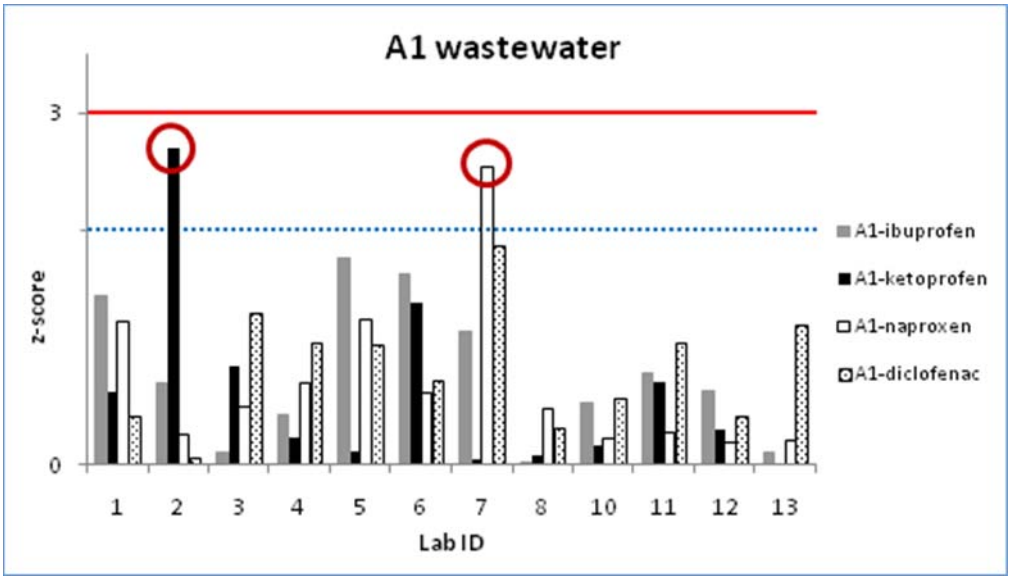
Equation 2,

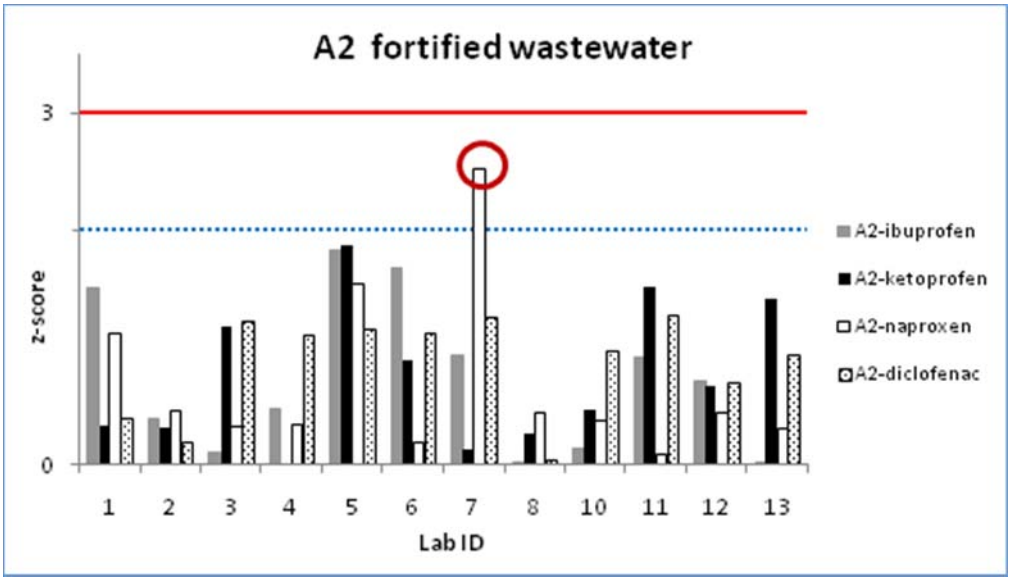
where x_{lab} is a laboratory mean, x_0 an initial mean and σ_0 an initial standard deviation of laboratory results. When z-score was higher than 3.0 [1], the result was automatically determined as an outlier (e.g. naproxen in A3, Table 3), while for the values ranging between $2.0 < |z| < 3.0$, i.e. suspect outliers, the Dixon test [2] was applied in order to accept or exclude them from the further data analysis. Thus, the data were first ranked in ascending order, and then based on the sample size the tau (τ) value for each suspect outlier was calculated [3]. Having the τ-value higher then the critical value at 5 % significance level for a given number of observations, the H0 hypothesis was rejected, thus concluding the extreme value was an outlier. The results of Dixon test are shown in Table 3.

Table 3: Results of the Dixon test on determination of outliers

sample-compound	τ	sample size	outlier	Lab ID
A1-ketoprofen	0.733	12	**YES**	2
A1-naproxen	0.548	12	**YES**	7
A2-naproxen	0.575	12	**YES**	7
A3-ketoprofen	0.515	12	**NO**	
A3-naproxen		12	**YES (z = 3.1)**	7
Total outliers (A)			**4**	
sample-compound	**τ**	**sample size**	**outlier**	**Lab ID**
B1-ibuprofen	0.576	12	**YES**	5
B1-naproxen	0.503	12	**NO**	
B2-diclofenac	0.422	12	**NO**	
B2-ibuprofen	0.469	12	**NO**	
B2-ketoprofen	0.634	12	**YES**	13
B3-ibuprofen	0.579	12	**YES**	5
B3-naproxen	0.792	12	**YES**	7
Total outliers (B)			**4**	
sample-compound	**τ**	**sample size**	**outlier**	**Lab ID**
C1-ibuprofen	0.509	12	**NO**	
C1-diclofenac	0.537	12	**NO**	
C1-ketoprofen	0.687	12	**YES**	5
C1-naproxen	0.865	11	**YES**	5
C2-ibuprofen	0.575	12	**YES**	2
C2-naproxen	0.540	12	**NO**	
C2-ketoprofen	0.606	12	**YES**	13
C3-diclofenac	0.564	12	**YES**	1
C3-naproxen	0.626	12	**YES**	5
C3-ketoprofen	0.743	12	**YES**	13
Total outliers (C)			**7**	

Z-score values for ibuprofen, ketoprofen, naproxen and diclofenac were calculated for each of the 9 samples (A1, A2, A3, B1, B2, B3, C1, C2, C3), analysed in participant laboratories (Lab ID 1-13). The bar-charts in Figure 1 illustrate the candidate outlier values between dotted (z = 2) and solid (z = 3) line. The outlier values determined by Dixon test are marked with circles.


A1 wastewater
3
0
z-score
1
2
3
4
5
6
7
8
10
11
12
13
Lab ID
A1-ibuprofen
A1-ketoprofen
A1-naproxen
A1-diclofenac


A2 fortified wastewater
3
0
z-score
1
2
3
4
5
6
7
8
10
11
12
13
Lab ID
A2-ibuprofen
A2-ketoprofen
A2-naproxen
A2-diclofenac

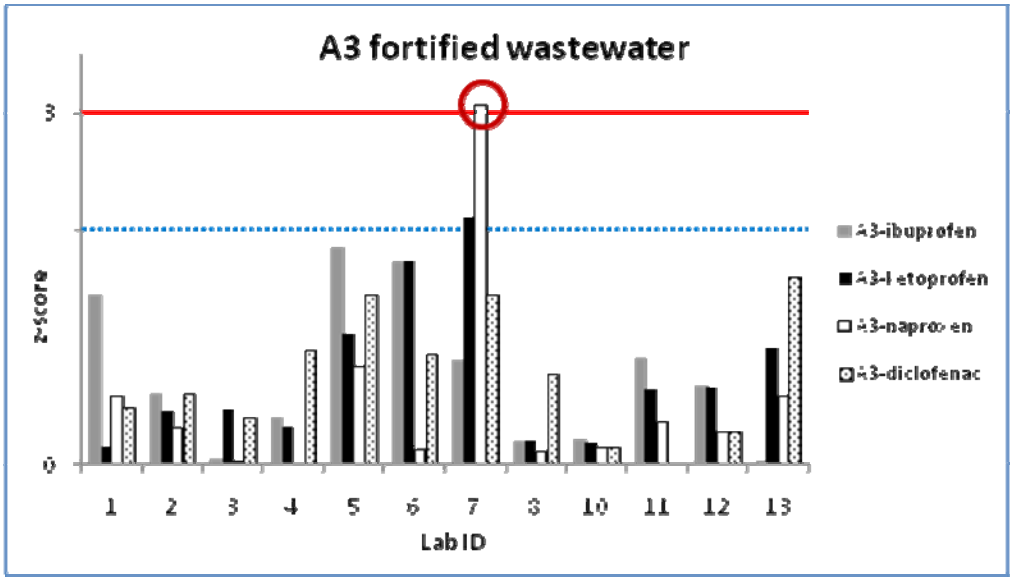
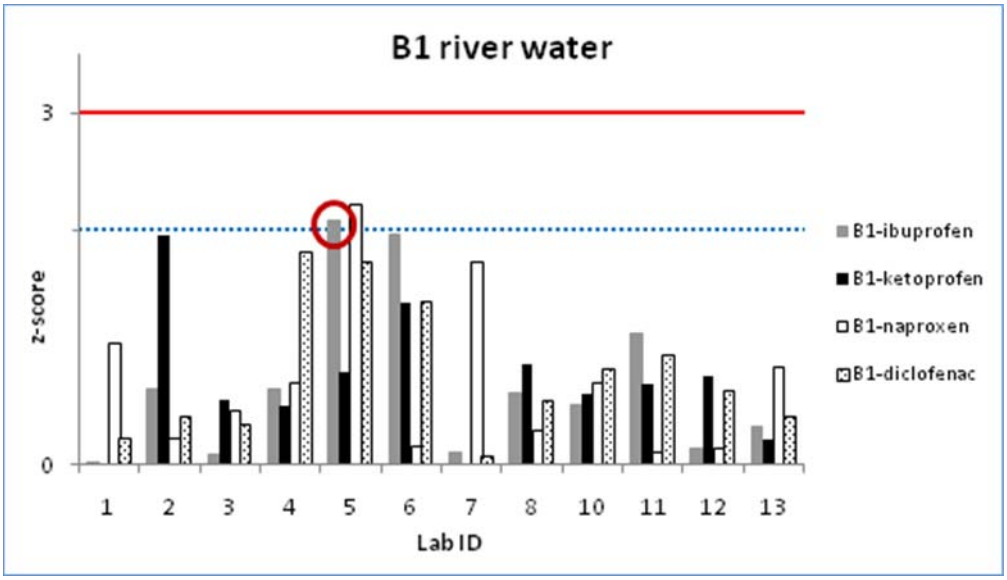
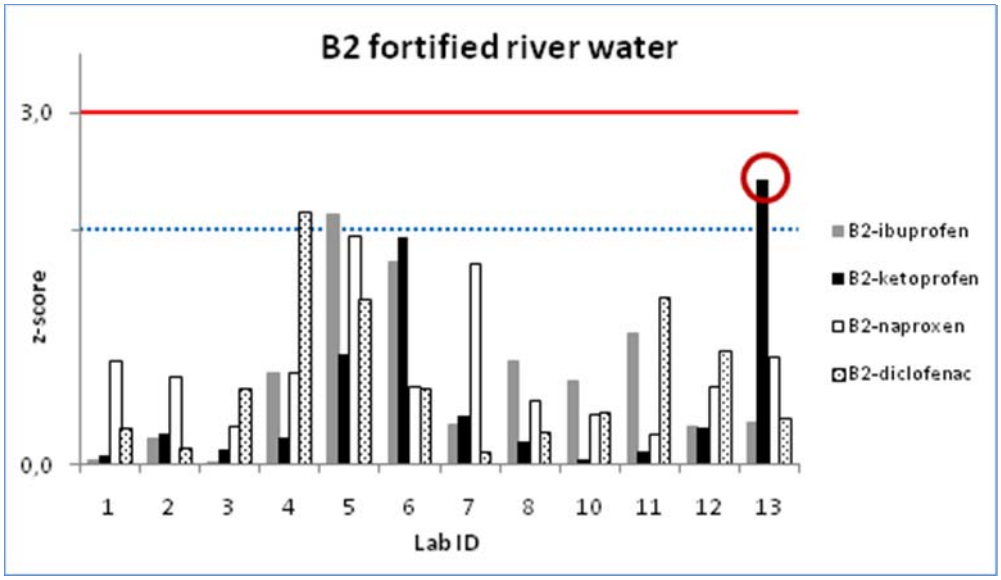

A3 fortified wastewater
3
0
z-score
1
2
3
4
5
6
7
8
10
11
12
13
Lab ID
A3-ibuprofen
A3-ketoprofen
A3-naproxen
A3-diclofenac

Figure 1 (1/3)


B1 river water
3
0
z-score
1
2
3
4
5
6
7
8
10
11
12
13
Lab ID
B1-ibuprofen
B1-ketoprofen
B1-naproxen
B1-diclofenac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3,0
0,0
z-score
1
2
3
4
5
6
7
8
10
11
12
13
Lab ID
B2-ibuprofen
B2-ketoprofen
B2-naproxen
B2-diclofenac

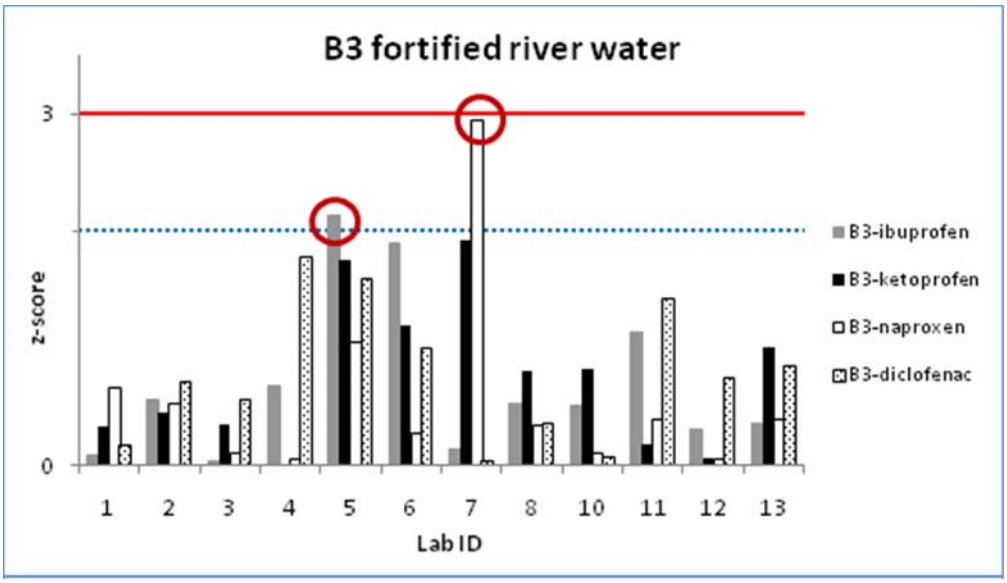

B3 fortified river water
3
0
z-score
1
2
3
4
5
6
7
8
10
11
12
13
Lab ID
B3-ibuprofen
B3-ketoprofen
B3-naproxen
B3-diclofenac

Figure 1 (2/3)

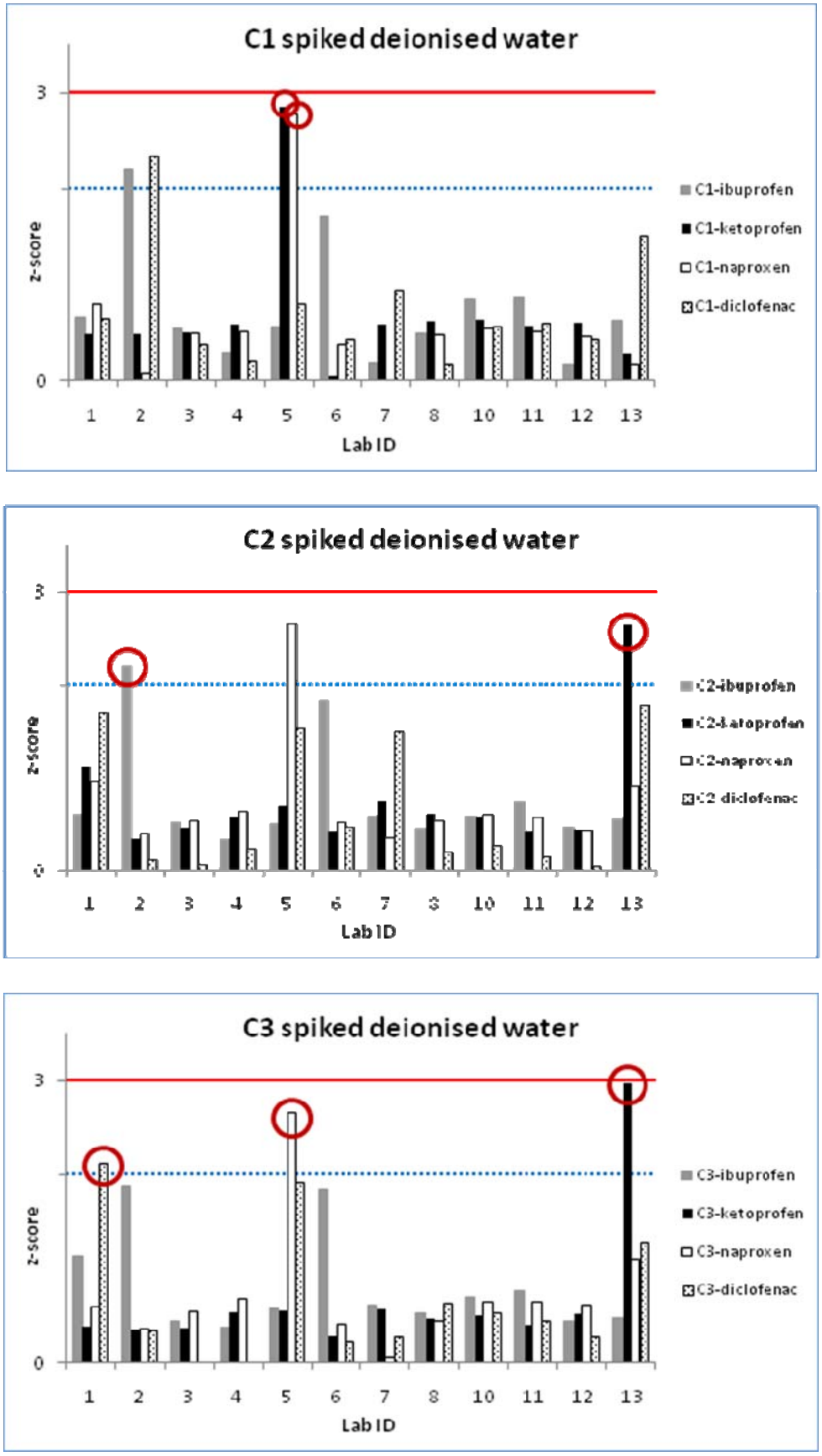


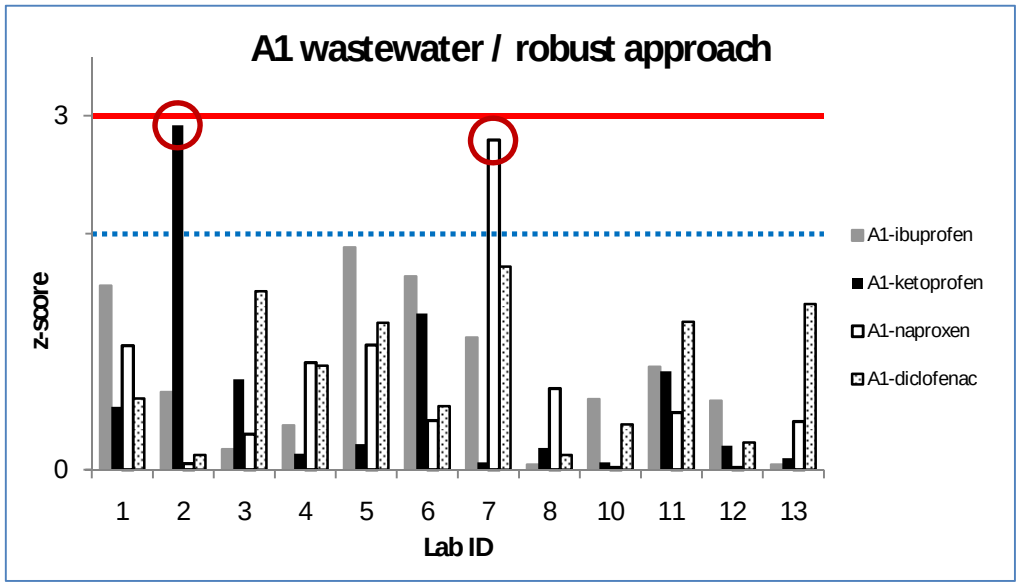
Figure 1 (3/3)

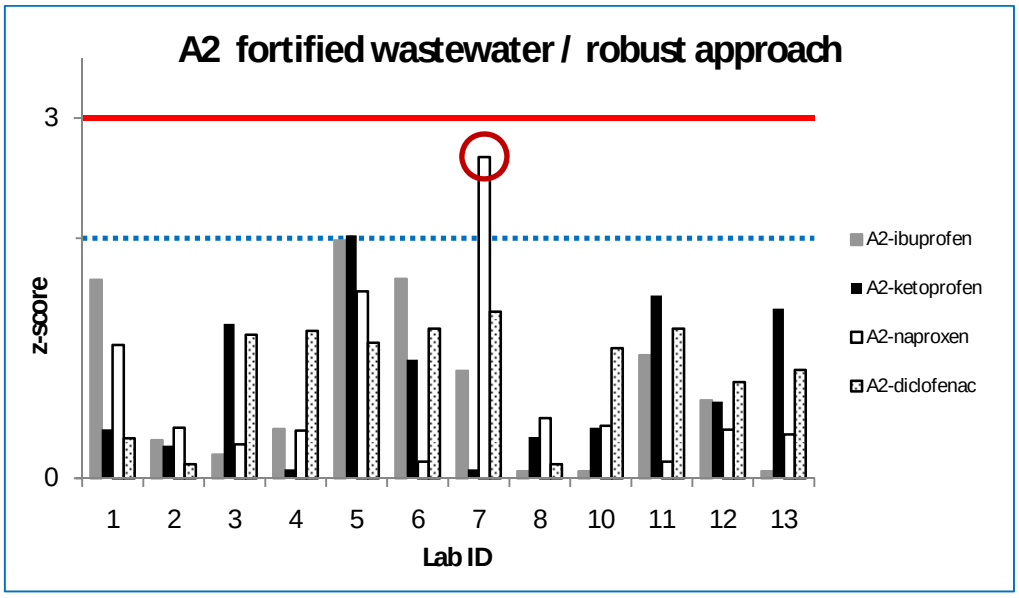
Figure 1: z-score values for each of the participant laboratories (Lab ID 1-13). The outliers are marked with red circles.

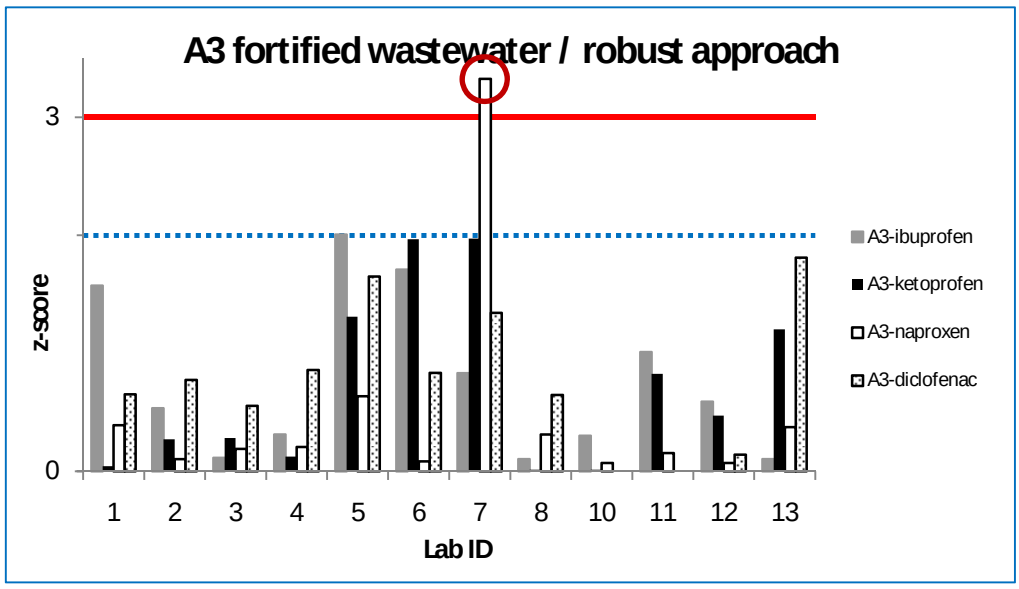
In addition to the classical approach to determine the outliers, the process was repeated using the robust approach, i.e. by using median as a middle value form which the deviations (robust z-score) were assessed. Table 4 and Figure 2 illustrate the determination of outliers using the robust approach, which in general gave similar results as the classical approach. However, in C2 the use of the robust approach resulted in one and in C3 samples in two additional values assessed as outliers.

Table 4: Outliers by robust approach

sample-compound	**τ**	**sample size**	**outlier**	**Lab ID**
A1-ketoprofen	0,733	12	**YES**	2
A1-naproxen	0,548	12	**YES**	7
A2-naproxen	0,592	12	**YES**	7
A3-naproxen		12	**YES (z = 3.3)**	7
Total outliers (A)			**4**	
sample-compound	**τ**	**sample size**	**outlier**	**Lab ID**
B1-ibuprofen	0,576	12	**YES**	5
B1-ketoprofen	0,209	10	**NO**	
B1-naproxen	0,503	12	**NO**	
B2-diclofenac	0,422	12	**NO**	
B2-ibuprofen	0,469	12	**NO**	
B2-naproxen	0,444	12	**NO**	
B2-ketoprofen	0,634	12	**YES**	13
B3-ibuprofen	0,579	12	**YES**	5
B3-naproxen		12	**YES (z = 3.2)**	7
Total outliers (B)			**4**	
sample-compound	**τ**	**sample size**	**outlier**	**Lab ID**
C1-ibuprofen	0,509	12	**NO**	
C1-diclofenac	0,537	12	**NO**	
C1-ketoprofen		12	**YES (z = 3.1)**	5
C1-naproxen		11	**YES (z = 3.1)**	5
C2-ibuprofen	0,905	11	**YES**	2
C2-ibuprofen	0,890	11	**YES**	6
C2-naproxen	0,540	12	**NO**	
C2-ketoprofen	0,606	12	**YES**	13
C3-ibuprofen	0,876	11	**YES**	2
C3-ibuprofen	0,875	11	**YES**	6
C3-diclofenac	0,564	12	**YES**	1
C3-naproxen	0,626	12	**YES**	5
C3-ketoprofen		12	**YES (z = 3.2)**	13
Total outliers (C)			**10**	


A1 wastewater / robust approach
3
0
z-score
1 2 3 4 5 6 7 8 10 11 12 13
Lab ID
A1-ibuprofen
A1-ketoprofen
A1-naproxen
A1-diclofenac


A2 fortified wastewater / robust approach
3
0
z-score
1 2 3 4 5 6 7 8 10 11 12 13
Lab ID
A2-ibuprofen
A2-ketoprofen
A2-naproxen
A2-diclofenac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3
0
z-score
1 2 3 4 5 6 7 8 10 11 12 13
Lab ID
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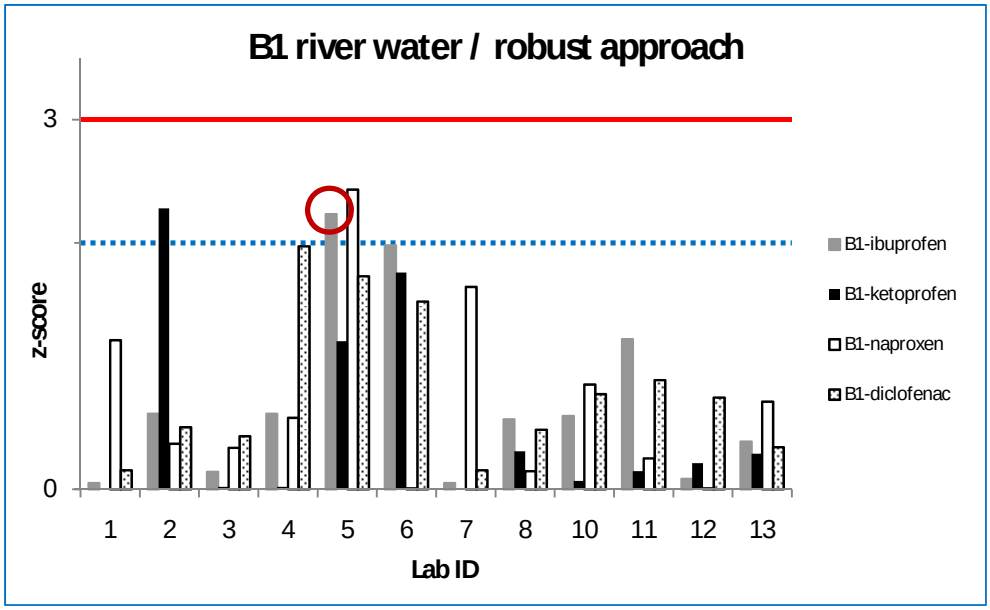
A3-ketoprofen
A3-naproxen
A3-diclofenac

Figure 2 (1/3)


B1 river water / robust approach
3
0
z-score
1
2
3
4
5
6
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8
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11
12
13
Lab ID
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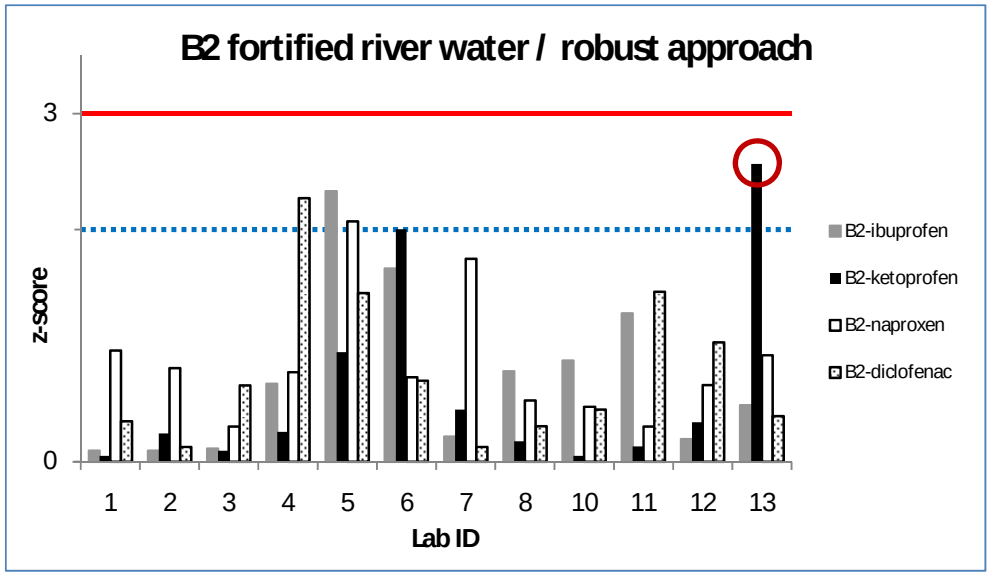
B1-ketoprofen
B1-naproxen
B1-diclofenac


B2 fortified river water / robust approach
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0
z-score
1
2
3
4
5
6
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12
13
Lab ID
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B2-ketoprofen
B2-naproxen
B2-diclofenac

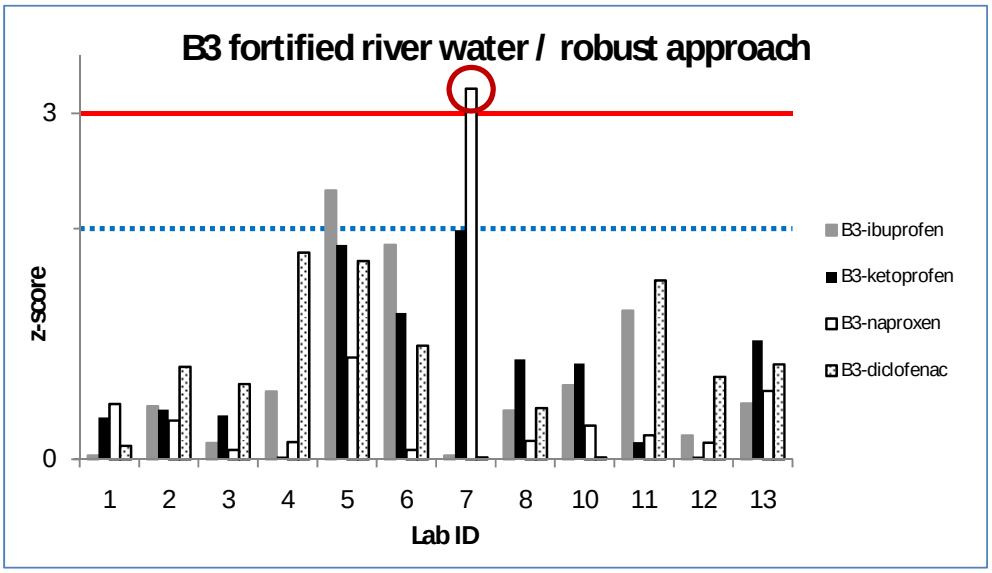
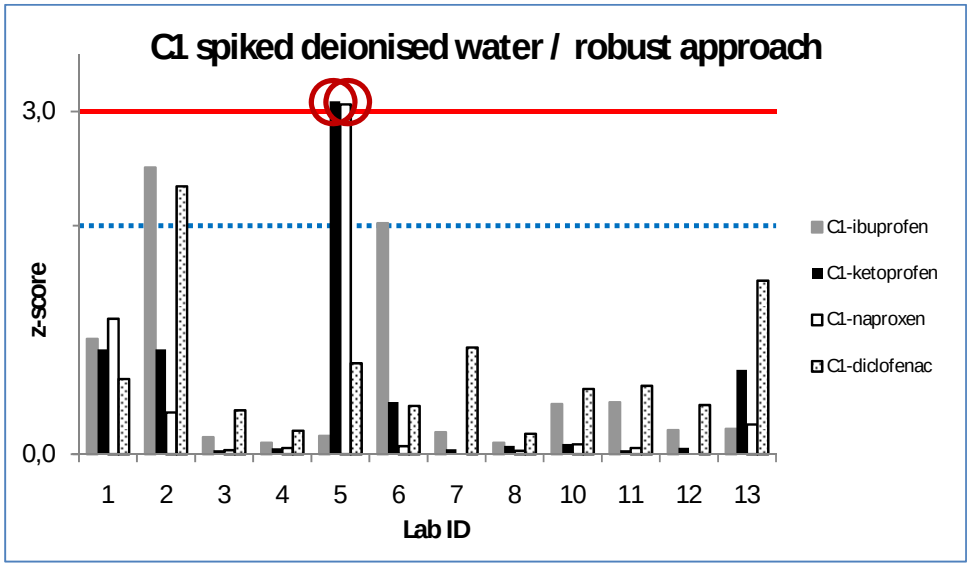
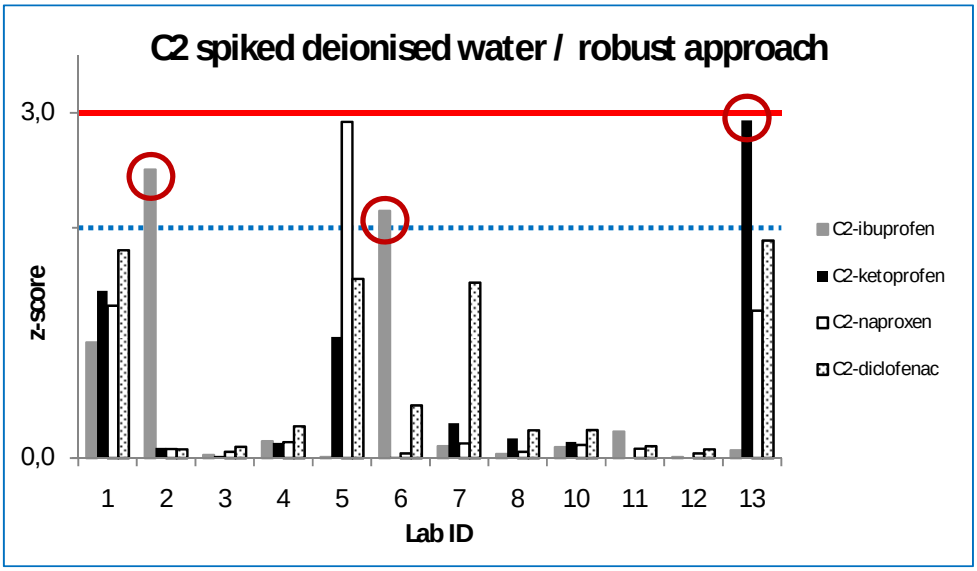

B3 fortified river water / robust approach
3
0
z-score
1
2
3
4
5
6
7
8
10
11
12
13
Lab ID
B3-ibuprofen
B3-ketoprofen
B3-naproxen
B3-diclofenac

Figure 2 (2/3)





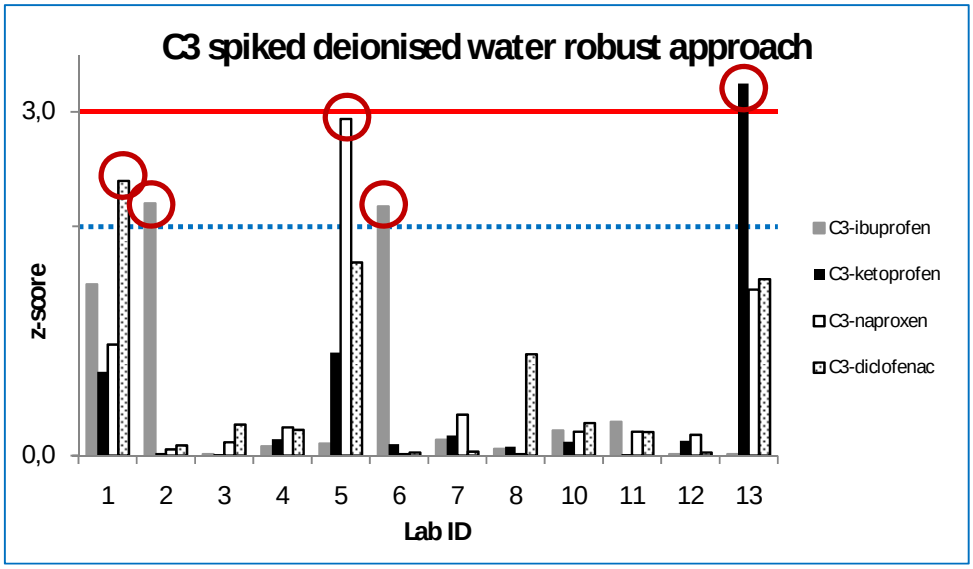


Figure 2 (3/3)
Figure 2: z-score values of each participant laboratory, determined according to the robust approach. The outliers are marked with red circles

The number of outliers in relation to the sample matrix shows that the highest number of outliers (46 %) was found in deionised water (Figure 3), which is in agreement with the results of the 1st Interlaboratory Exercise on NSAIDs analysis. However, according to the robust approach three more outlier values were determined in deionised water (56 % of the total number of outliers), while the number of outliers in the wastewater and river water was the same as in the classical approach.

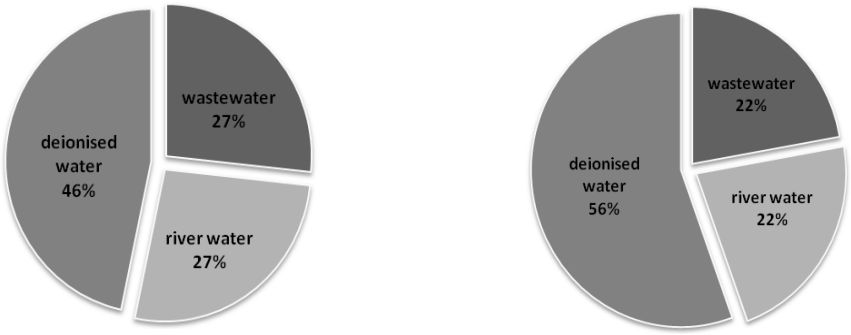


Figure 3: Pie-chart showing the percentage of outliers in relation to the sample matrices: classical approach (left) and robust approach (right)

Regarding the analyte, most of the outliers were found for naproxen (40% and 33%), while only one outlier value was determined for diclofenac (Figure 4).

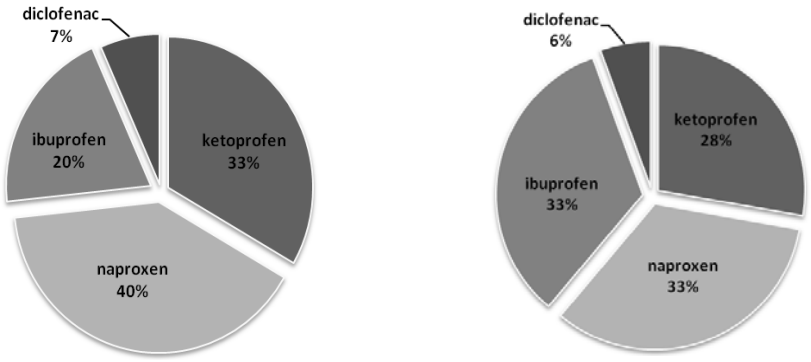


Figure 4: Pie-chart showing the percentage of outliers per analyte: classical approach (left) and robust approach (right)

As illustrated in Figure 5, the outliers were obtained for only five (classical approach) and six (robust approach) of twelve participants, which suggests a good quality of sample preparation, but insufficient method performance in some laboratories. Thus, according to the Table 3 and 4, the number of the outliers would significantly decrease (up to 44 - 47 %) merely by improving the determination of naproxen in the Lab 7 and ketoprofen in the Lab 13.

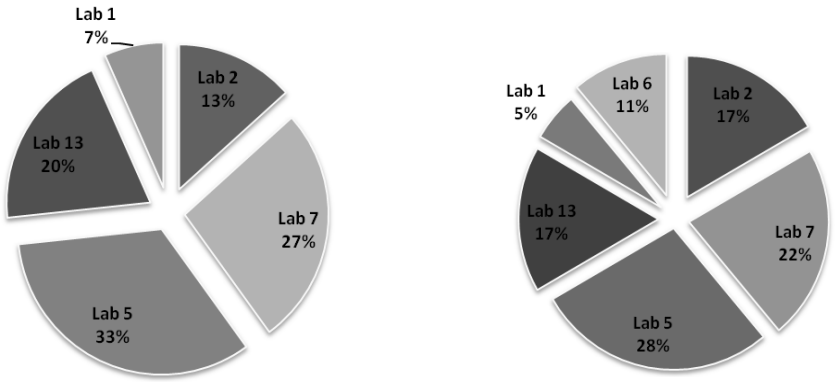


Figure 5: A pie-chart illustrating the percentage of outliers per participant: classical approach, left and robust approach, right

According to the method of analysis 5 GC and 7 LC laboratories participated the NORMAN 2nd Interlaboratory exercise, where the LC laboratories gave 80 % (83 % using robust approach) of the total number of outliers. From the total number of 15 outlier values, GC methods yielded 3 (1.7 % of the GC results) and LC 12 (4.7 % of the LC results) outliers. Accordingly, using the robust approach, the LC methods yielded 15 (6,0 % of total LC results) outliers, while the number of GC results didn't differ from the classical approach (1.7 %).

7. *Summary of the results*

After the outlier exclusion the mean, standard deviation, median, minimum and maximum value were calculated for each NSAID in each of the 9 samples. The results are summarized in Table 5. Samples marked with A1 were natural wastewater samples, while A2 and A3 were parallels fortified together in the polyethylene bucket. Similarly, samples marked with "B" were river water samples, where again the latter two (B2 and B3) were obtained by fortification of authentic river water (B1). Finally, C1 was deionised water spiked with each of the NSAIDs, while C2 and C3 were parallels additionally spiked with ketoprofen, naproxen and diclofenac. The levels of NSAID additions (Table 5) are only approximate values, as during the sample preparation the total volumes of matrices to be spiked were not determined accurately. In general, the median values are better approximates to the spiked NSAID concentrations than the mean values.

Table 5: Summary of the corrected results after the outlier exclusion

IBUPROFEN (ng/L)											
Sample	**Matrix**	**Approx. fortif. level (ng/L)**	**Filtration**	**No. accepted results**	**Mean**	**Standard deviation**	**Standard error of mean**	**Median**	**Minimum value**	**Maximum value**	**No. of outliers**
A1	wastewater	-	YES	12	1238	460	133	1265	433	1987	0
A2	fortified wastewater	416	YES	12	1622	577	167	1668	570	2588	0
A3	fortified wastewater	416	NO	12	1620	586	169	1669	537	2633	0
B1	river water	-	YES	11	7545	1853	559	7351	4500	11684	1
B2	fortified river water	416	YES	12	7250	2302	665	7537	2358	11235	0
B3	fortified river water	416	NO	11	7791	1864	332	7663	4600	11891	1
C1	spiked deionised water	50	YES	12	77	56	16	55	29	200	0
C2	spiked deionised water	50	YES	11	61	41	12	46	33	172	1
C3	spiked deionised water	50	NO	12	70	50	14	47	31	571	0

Table 5 (1/4)

KETOPROFEN (ng/L)											
Sample	**Matrix**	**Approx. fortif. level (ng/L)**	**Filtration**	**No. accepted results**	**Mean**	**Standard deviation**	**Standard error of mean**	**Median**	**Minimum value**	**Maximum value**	**No. of outliers**
A1	wastewater	-	YES	11	334	108	33	350	111	520	1
A2	fortified wastewater	790	YES	12	967	284	82	985	434	1400	0
A3	fortified wastewater	790	NO	12	830	416	120	905	107	1705	0
B1	river water	-	YES	10	269	234	74	147	69	725	0
B2	fortified river water	790	YES	11	754	259	78	812	91	997	1
B3	fortified river water	790	NO	12	886	261	75	893	428	1389	0
C1	spiked deionised water	47	YES	11	93	79	24	40	30	217	1
C2	spiked deionised water	205	YES	11	319	231	70	248	123	854	1
C3	spiked deionised water	205	NO	11	273	136	41	230	170	571	1

Table 5 (2/4)

NAPROXEN (ng/L)											
Sample	**Matrix**	**Approx. fortif. level (ng/L)**	**Filtration**	**No. accepted results**	**Mean**	**Standard deviation**	**Standard error of mean**	**Median**	**Minimum value**	**Maximum value**	**No. of outliers**
A1	wastewater	-	YES	11	507	115	35	510	325	675	1
A2	fortified wastewater	412	YES	11	791	224	67	808	332	1022	1
A3	fortified wastewater	412	NO	11	737	220	66	742	317	1030	1
B1	river water	-	YES	12	1754	516	149	1825	609	2646	0
B2	fortified river water	412	YES	12	1956	608	175	1976	771	2993	0
B3	fortified river water	412	NO	11	1978	563	170	1977	852	2925	1
C1	spiked deionised water	45	YES	10	97	111	35	46	26	388	1
C2	spiked deionised water	120	YES	12	283	276	80	154	113	1014	0
C3	spiked deionised water	120	NO	11	210	132	40	167	111	516	1

Table 5 (3/4)

DICLOFENAC (ng/L)											
Sample	**Matrix**	**Approx. fortif. level (ng/L)**	**Filtration**	**No. accepted results**	**Mean**	**Standard deviation**	**Standard error of mean**	**Median**	**Minimum value**	**Maximum value**	**No. of outliers**
A1	wastewater	-	YES	12	521	357	103	586	59	1186	0
A2	fortified wastewater	523	YES	12	730	487	141	693	110	1341	0
A3	fortified wastewater	523	NO	11	796	452	136	860	71	1444	0
B1	river water	-	YES	12	1959	924	267	1887	352	3640	0
B2	fortified river water	523	YES	12	2054	1234	356	2030	300	4715	0
B3	fortified river water	523	NO	12	2216	1152	332	2284	386	4262	0
C1	spiked deionised water	63	YES	12	77	71	21	48	10,2	243	0
C2	spiked deionised water	220	YES	12	250	149	43	245	22	515	0
C3	spiked deionised water	220	NO	11	244	101	30	233	21	433	1

Table 5 (4/4)

8. *Laboratory performance*

a) Deviations from the mean (classical approach)

The deviations of laboratories from the sample corrected mean (stated in Table 5) for each analyte are illustrated in the following graphs (Figure 6/1-12), where the outliers are circled. The outliers were excluded from the mean value calculation.

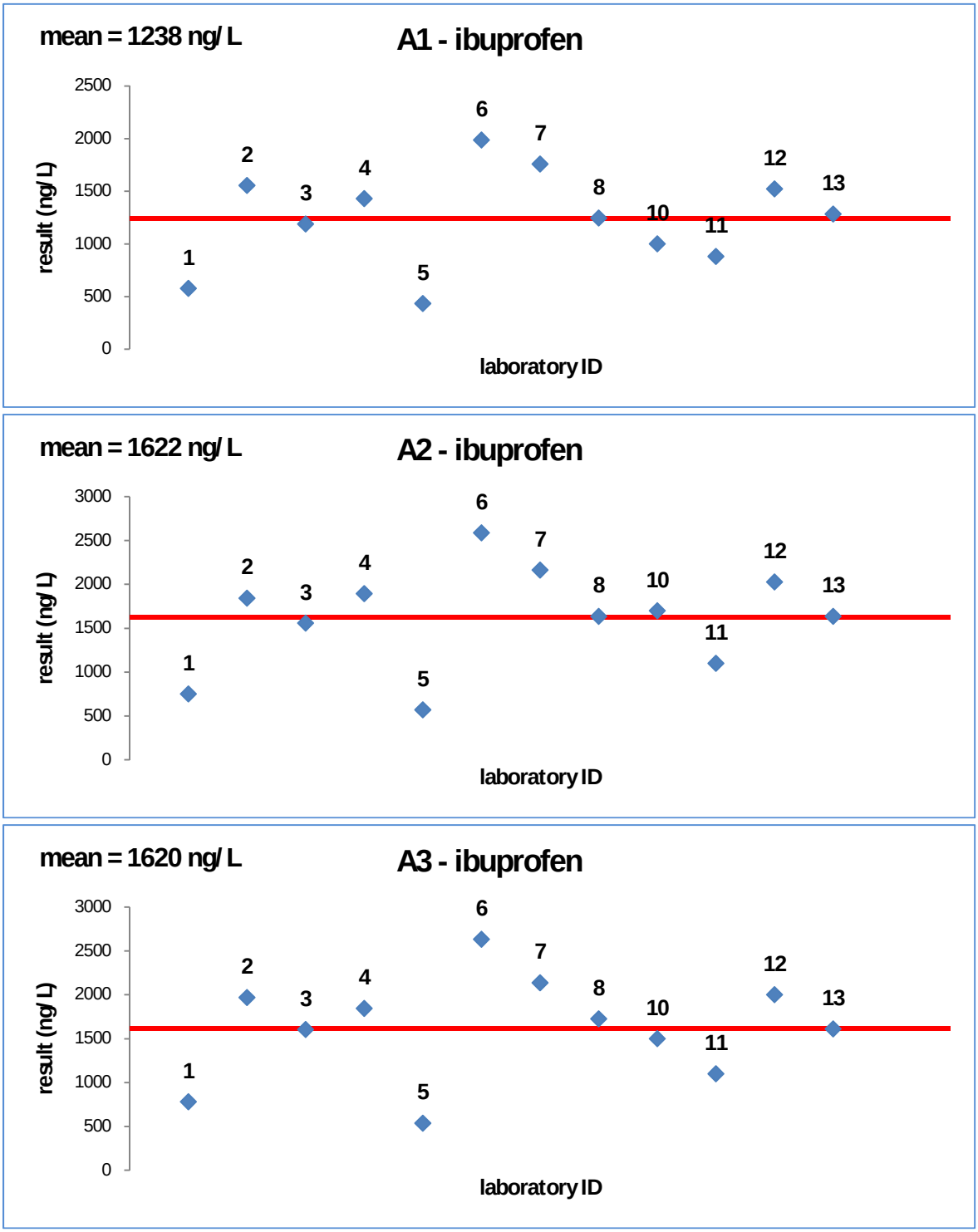
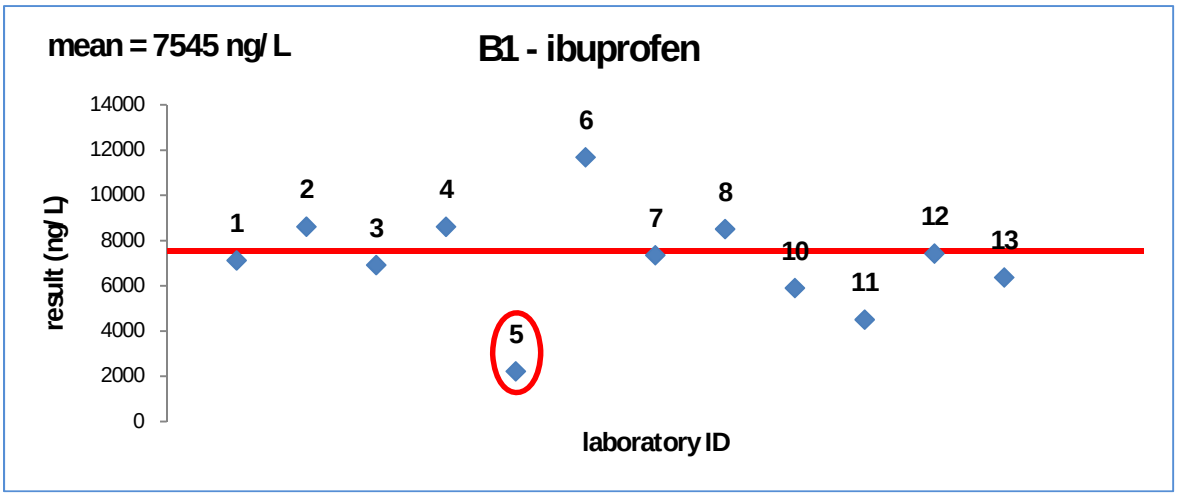
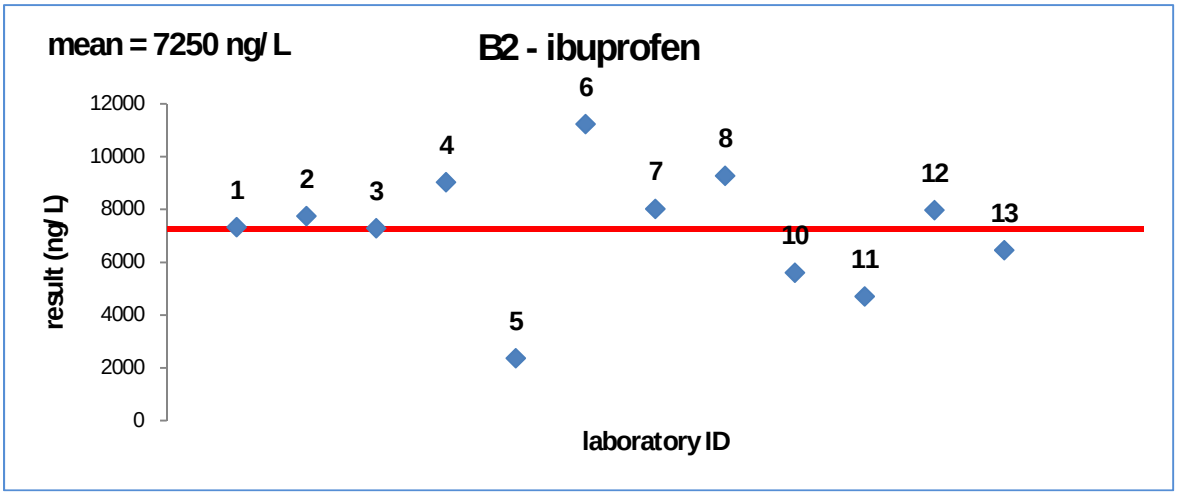


Figure 6 (1/12)


mean = 7545 ng/ L
B1 - ibuprofen
result (ng/ L)
14000
12000
10000
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


mean = 7250 ng/ L
B2 - ibuprofen
result (ng/ L)
12000
10000
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

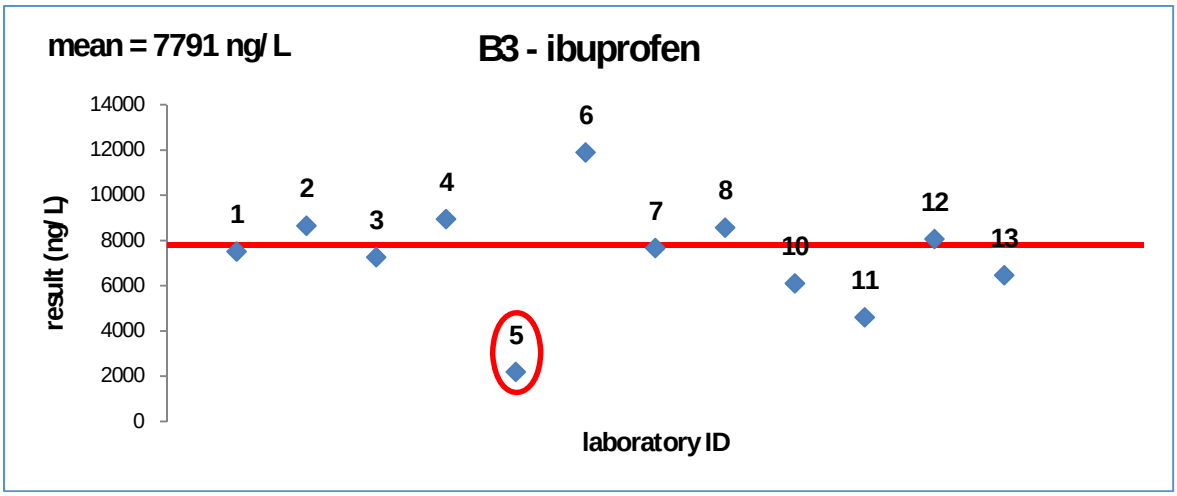
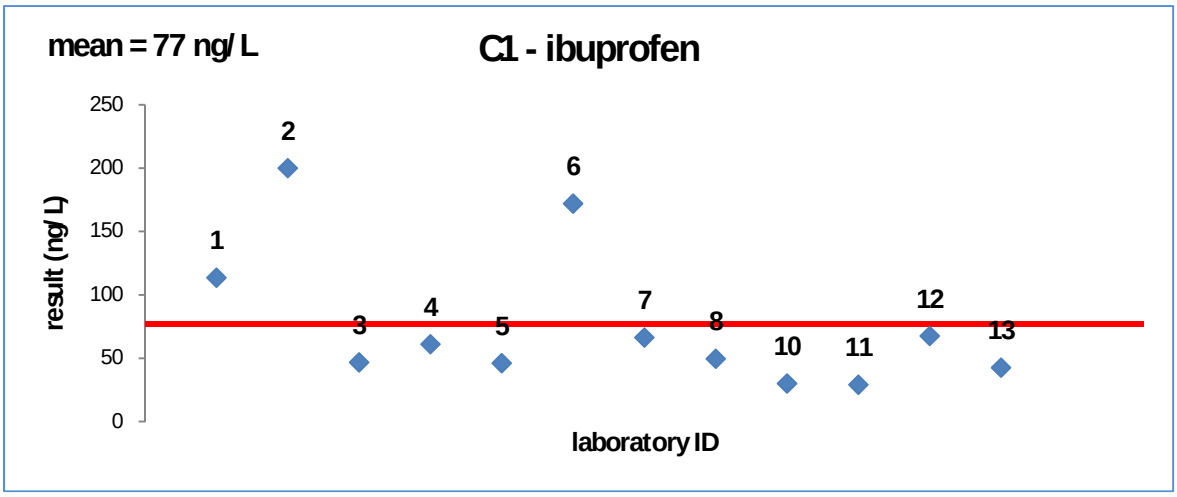
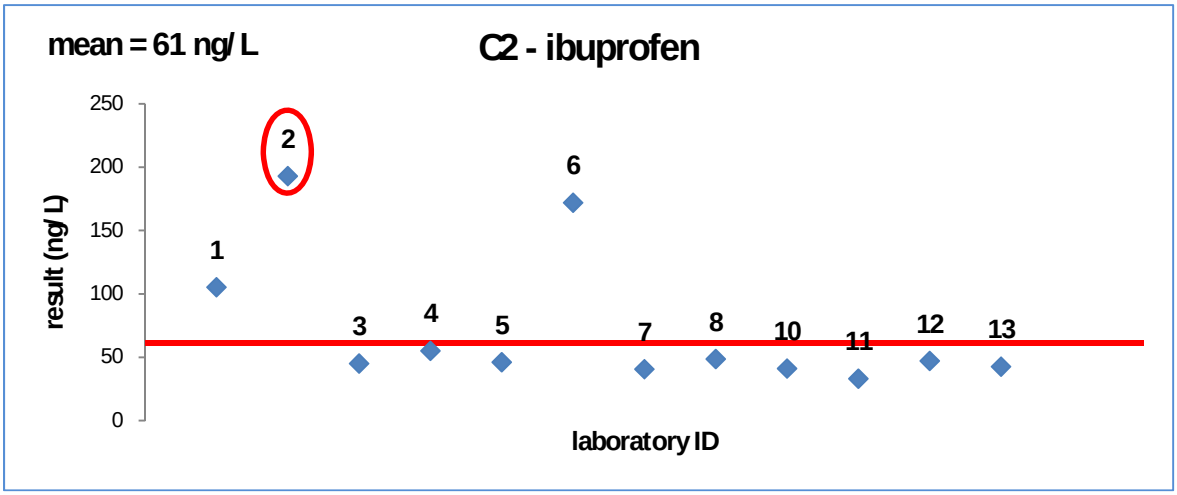

mean = 7791 ng/ L
B3 - ibuprofen
result (ng/ L)
14000
12000
10000
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 6 (2/12)


mean = 77 ng/ L
C1 - ibuprofen
result (ng/ L)
250
200
150
100
50
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


mean = 61 ng/ L
C2 - ibuprofen
result (ng/ L)
250
200
150
100
50
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

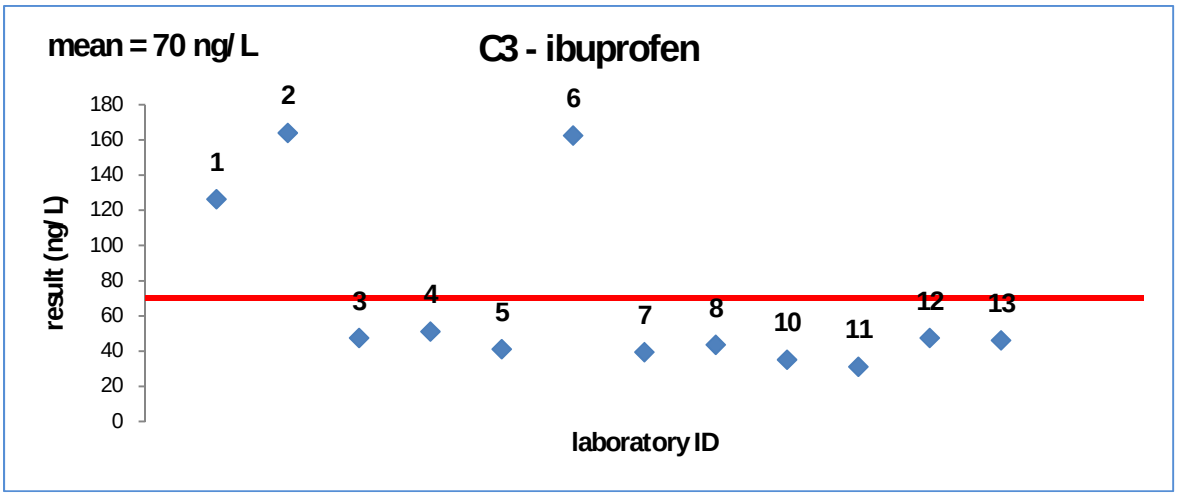
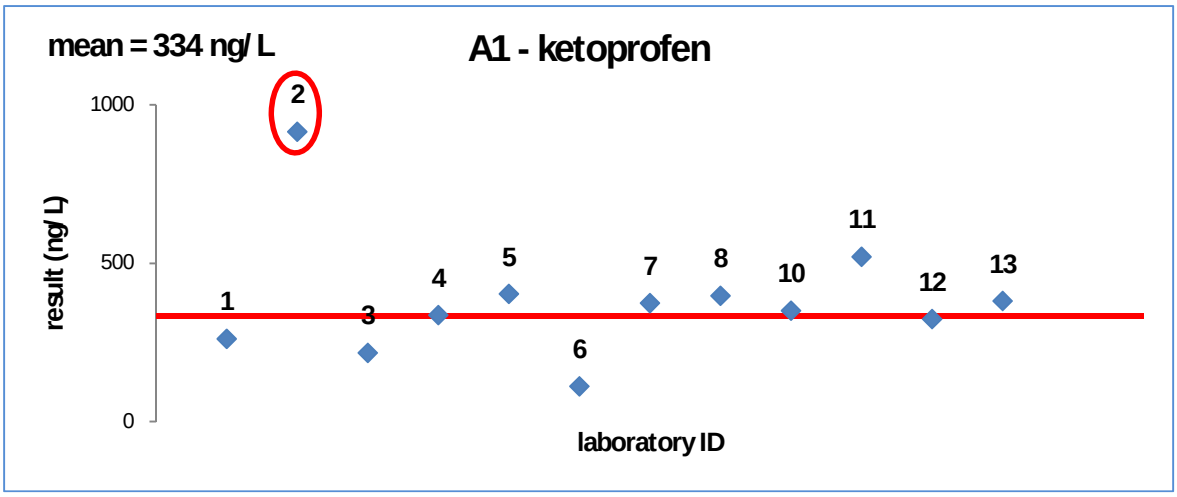
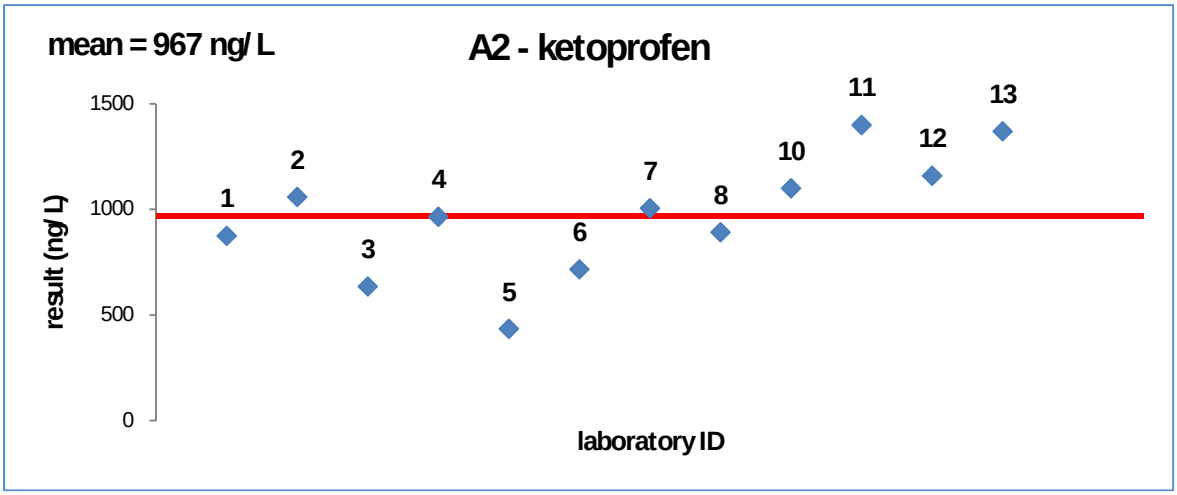

mean = 70 ng/ L
C3 - ibuprofen
result (ng/ L)
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 6 (3/12)


mean = 334 ng/ L
A1 - ketoprofen
result (ng/ L)
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


mean = 967 ng/ L
A2 - ketoprofen
result (ng/ L)
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

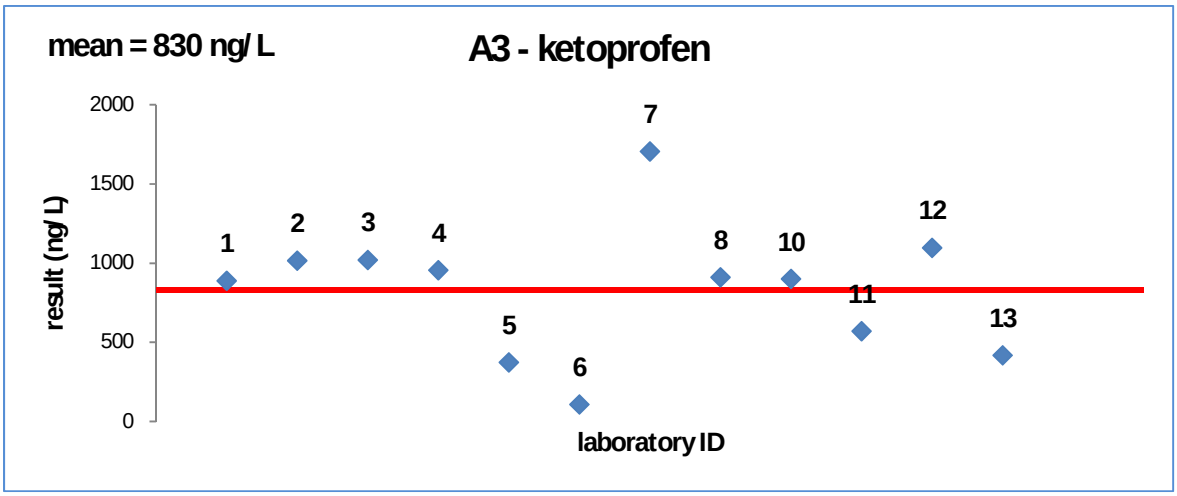
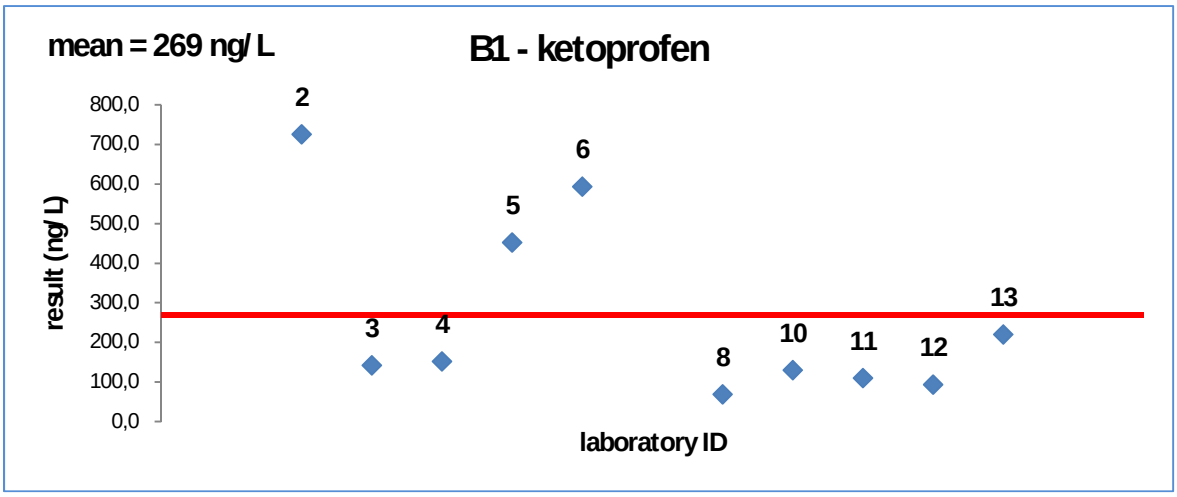
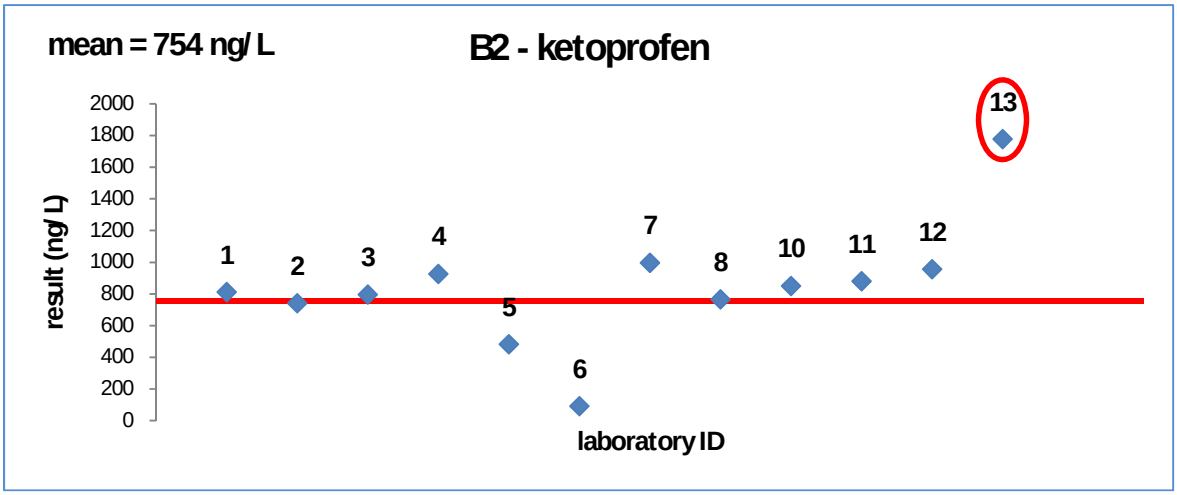

mean = 830 ng/ L
A3 - ketoprofen
result (ng/ L)
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 6 (4/12)


mean = 269 ng/ L
B1 - ketoprofen
result (ng/ L)
800,0
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
2
6
5
3
4
8
10
11
12
13
laboratory ID


mean = 754 ng/ L
B2 - ketoprofen
result (ng/ L)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
13
1
2
3
4
7
8
10
11
12
5
6
laboratory ID

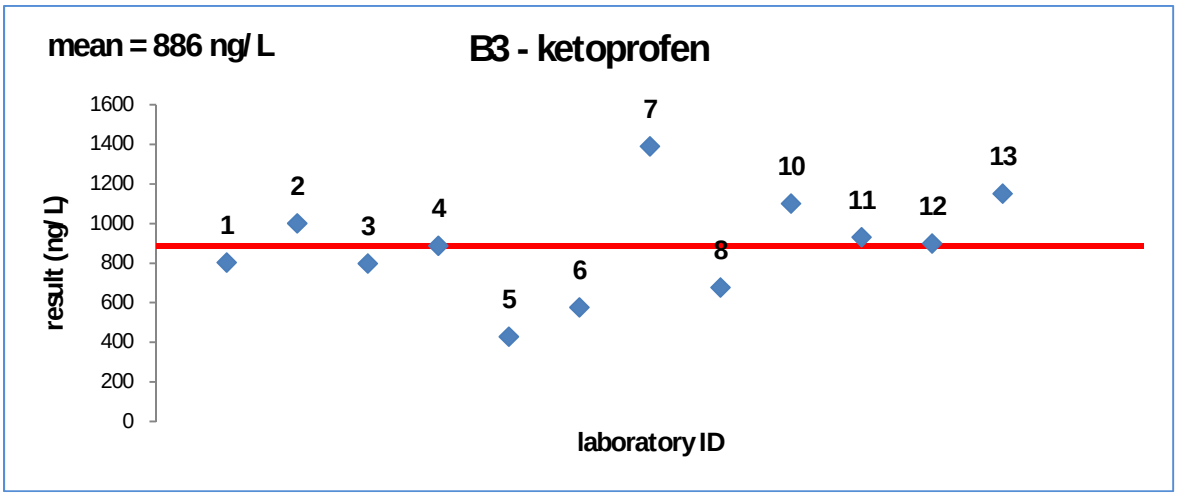
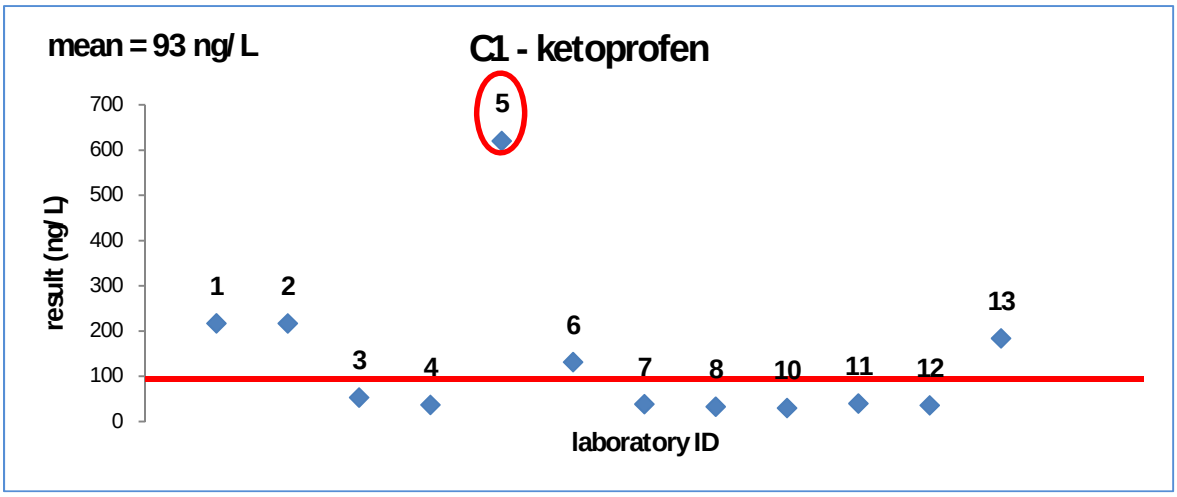
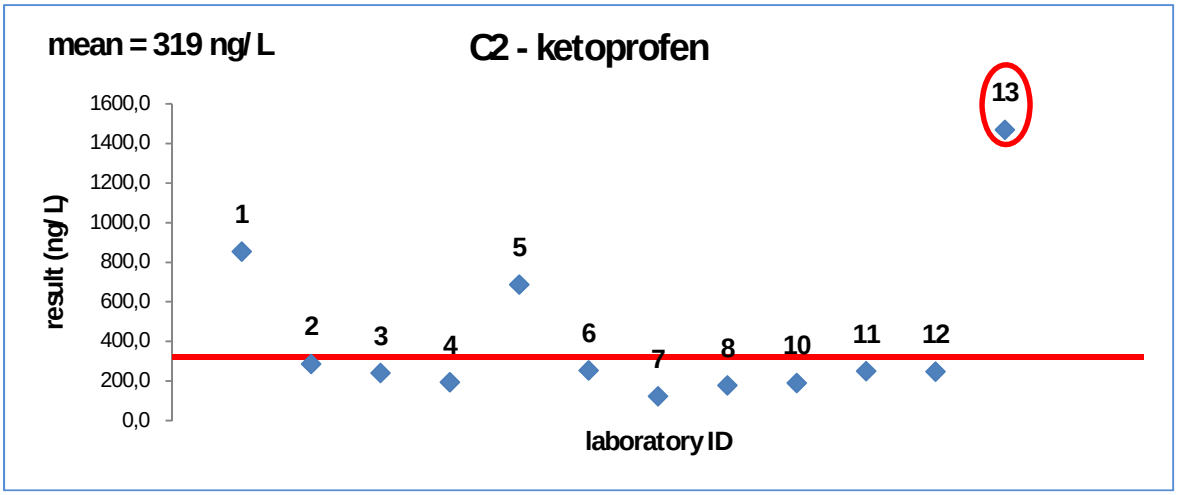

mean = 886 ng/ L
B3 - ketoprofen
result (ng/ L)
1600
1400
1200
1000
800
600
400
200
0
7
10
13
2
11
1
4
12
3
8
6
5
laboratory ID

Figure 6 (5/12)


mean = 93 ng/ L
C1 - ketoprofen
result (ng/ L)
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


mean = 319 ng/ L
C2 - ketoprofen
result (ng/ L)
1600,0
1400,0
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

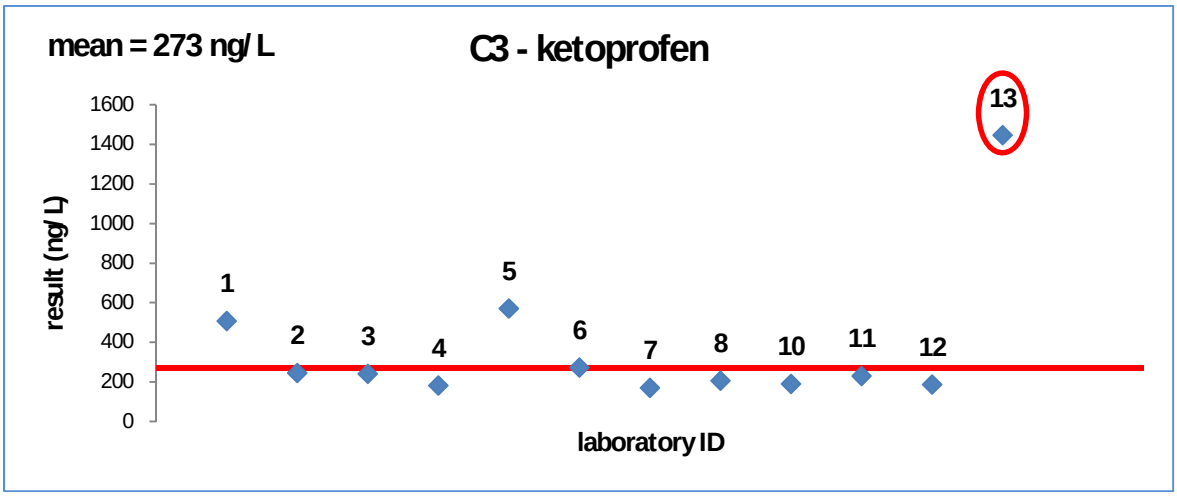
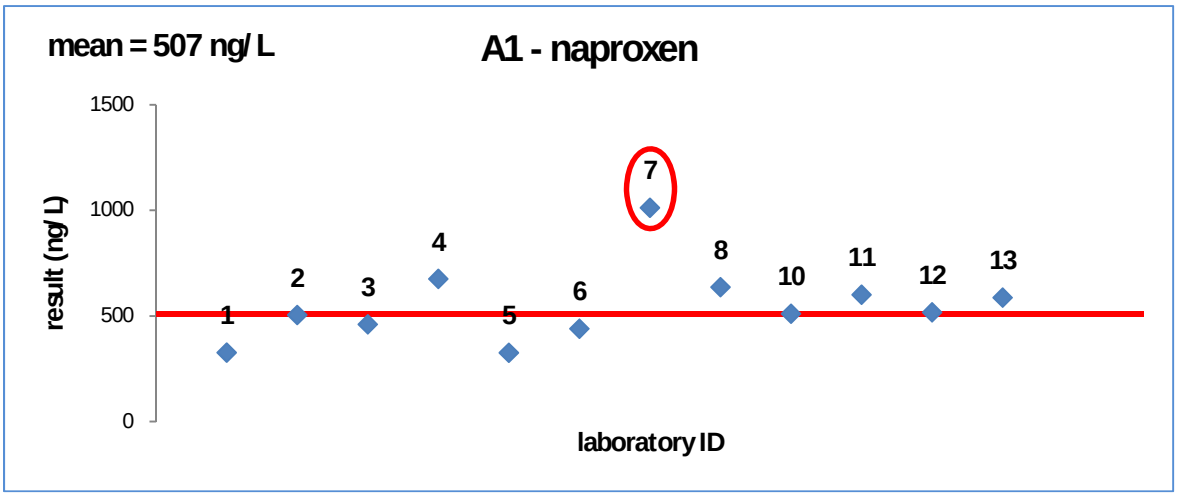
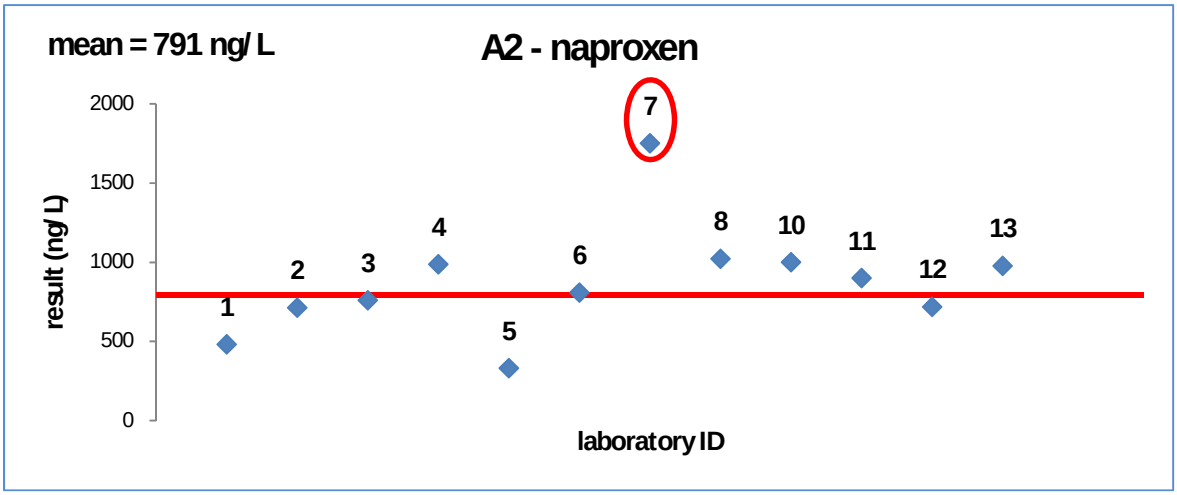

mean = 273 ng/ L
C3 - ketoprofen
result (ng/ L)
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 6 (6/12)


mean = 507 ng/ L
A1 - naproxen
result (ng/ L)
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


mean = 791 ng/ L
A2 - naproxen
result (ng/ L)
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

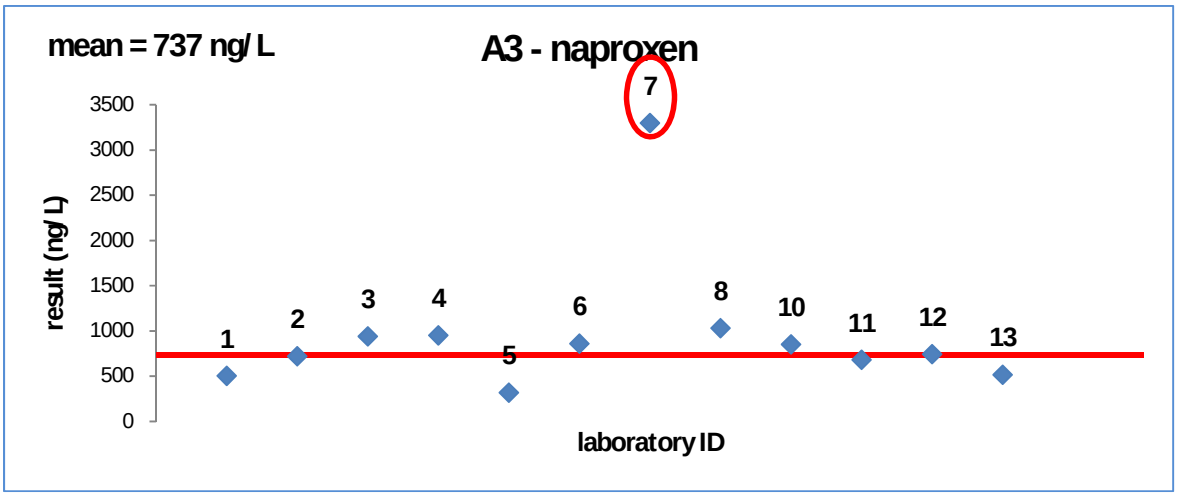
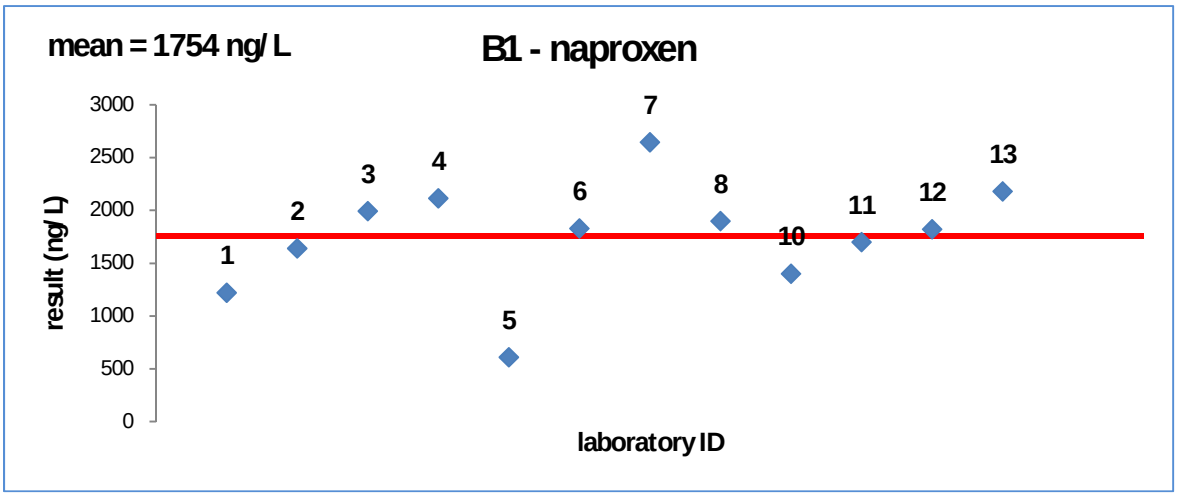
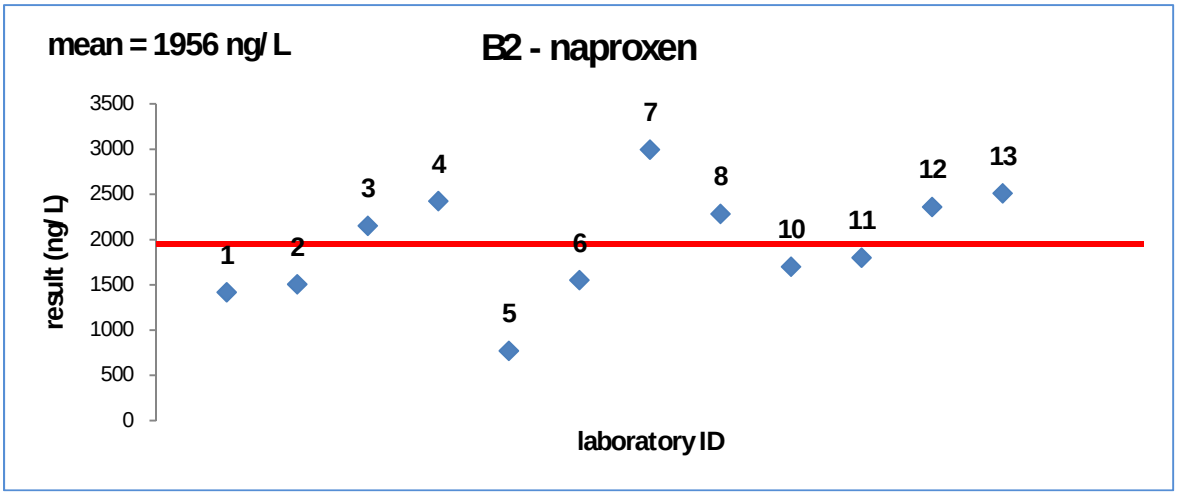

mean = 737 ng/ L
A3 - naproxen
result (ng/ L)
3500
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 6 (7/12)


mean = 1754 ng/ L
B1 - naproxen
result (ng/ L)
laboratory ID


mean = 1956 ng/ L
B2 - naproxen
result (ng/ L)
laboratory ID

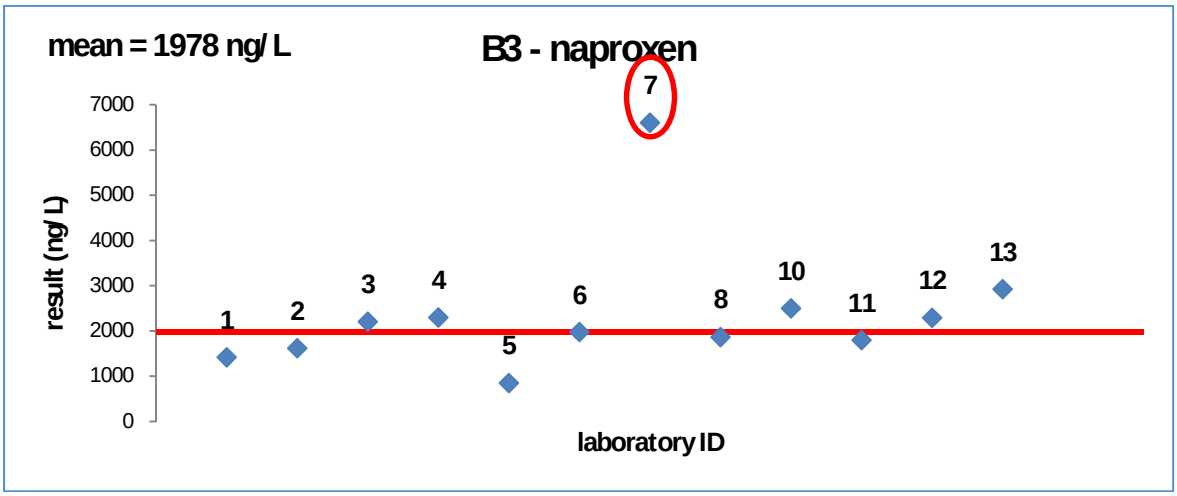
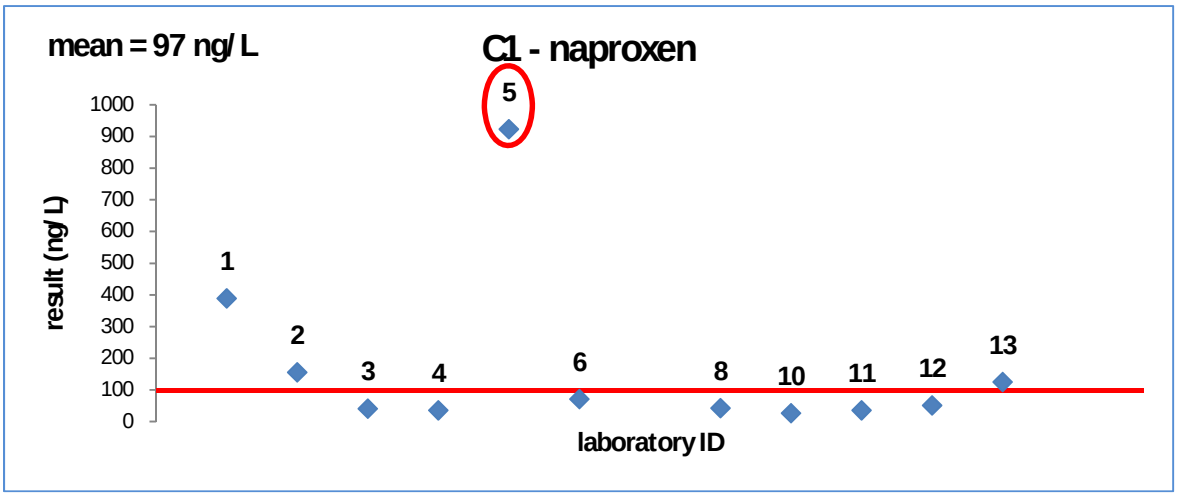
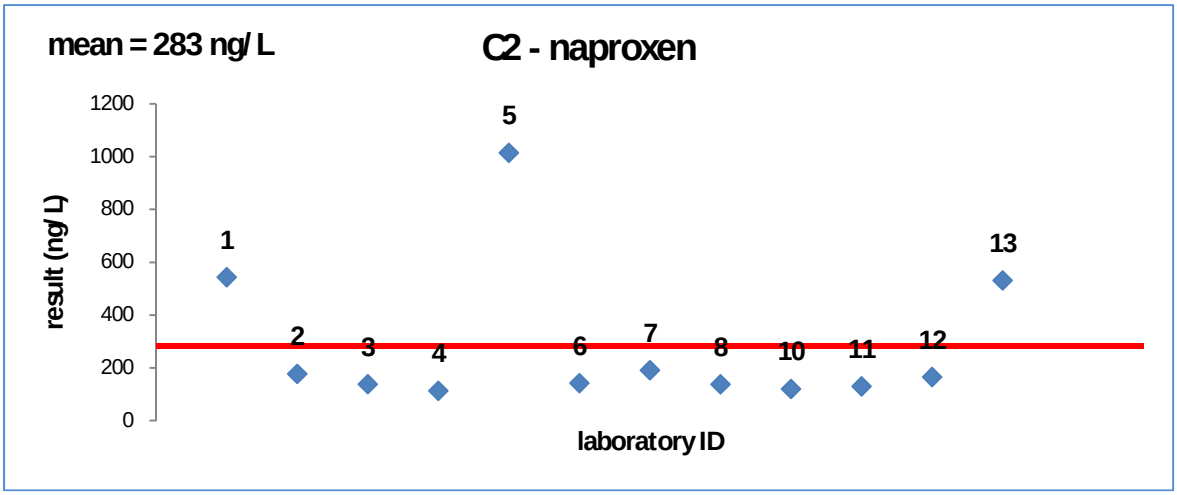

mean = 1978 ng/ L
B3 - naproxen
result (ng/ L)
laboratory ID

Figure 6 (8/12)


mean = 97 ng/ L
C1 - naproxen
result (ng/ L)
laboratory ID


mean = 283 ng/ L
C2 - naproxen
result (ng/ L)
laboratory ID

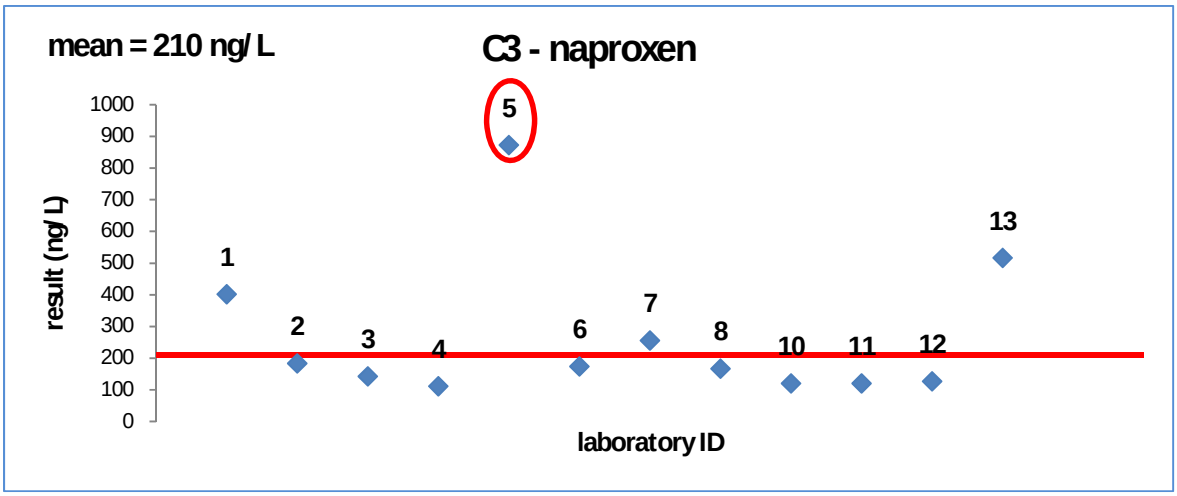
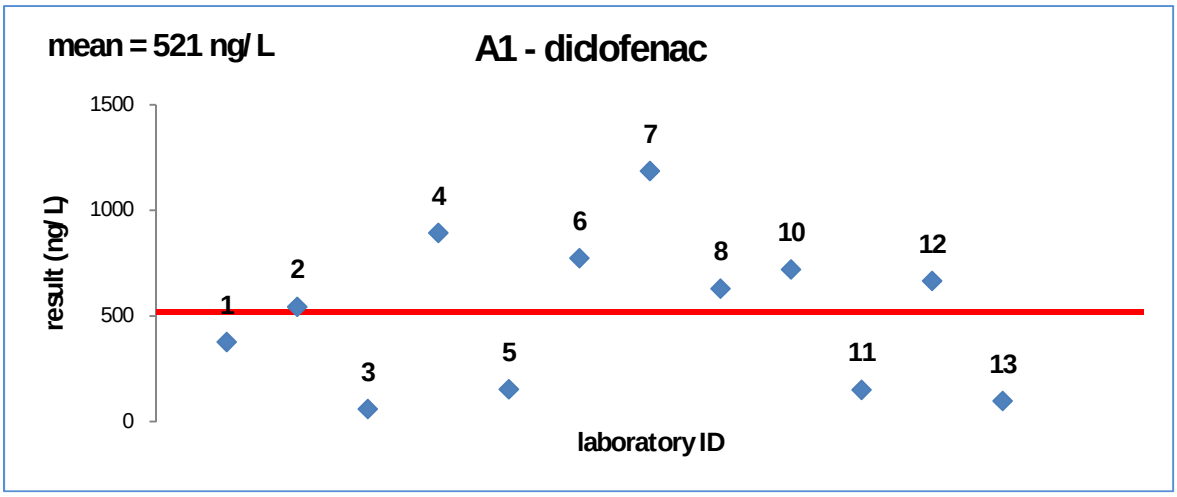
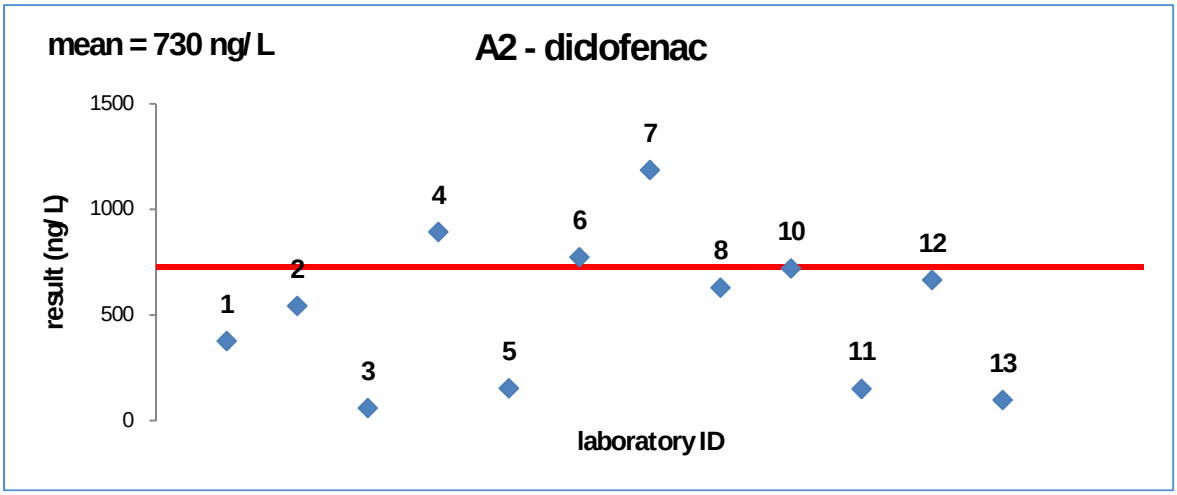

mean = 210 ng/ L
C3 - naproxen
result (ng/ L)
laboratory ID

Figure 6 (9/12)





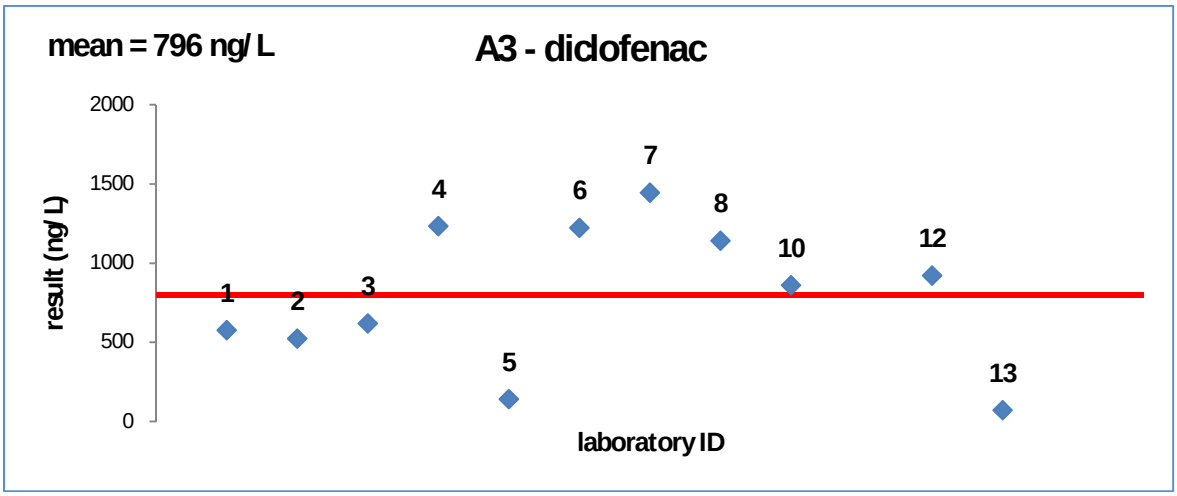
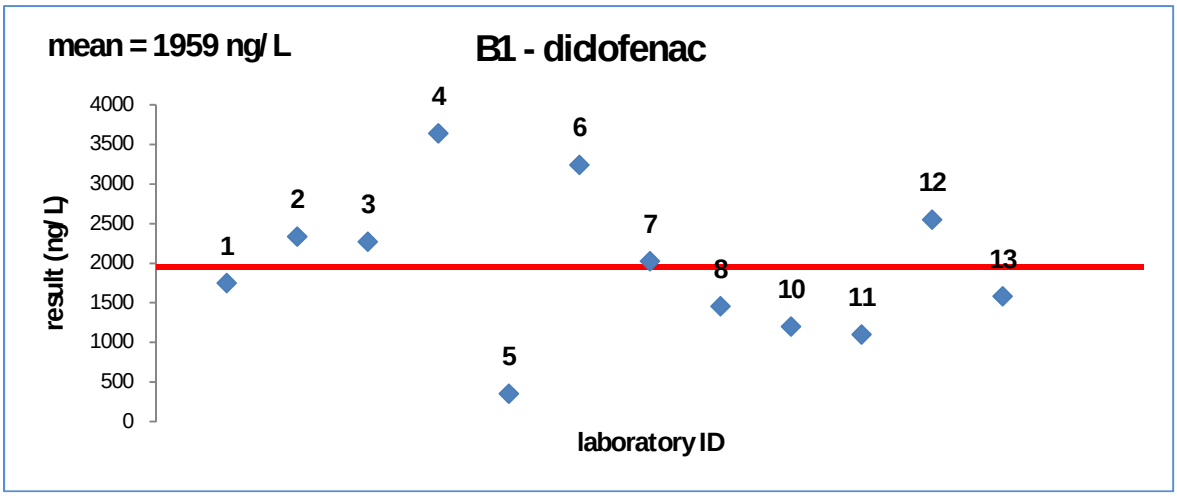
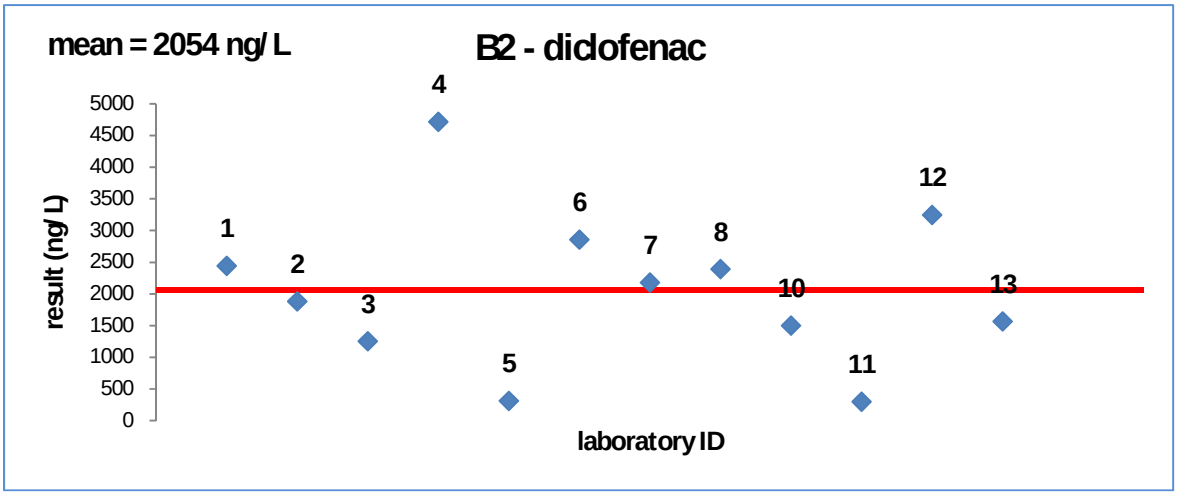


Figure 6 (10/12)





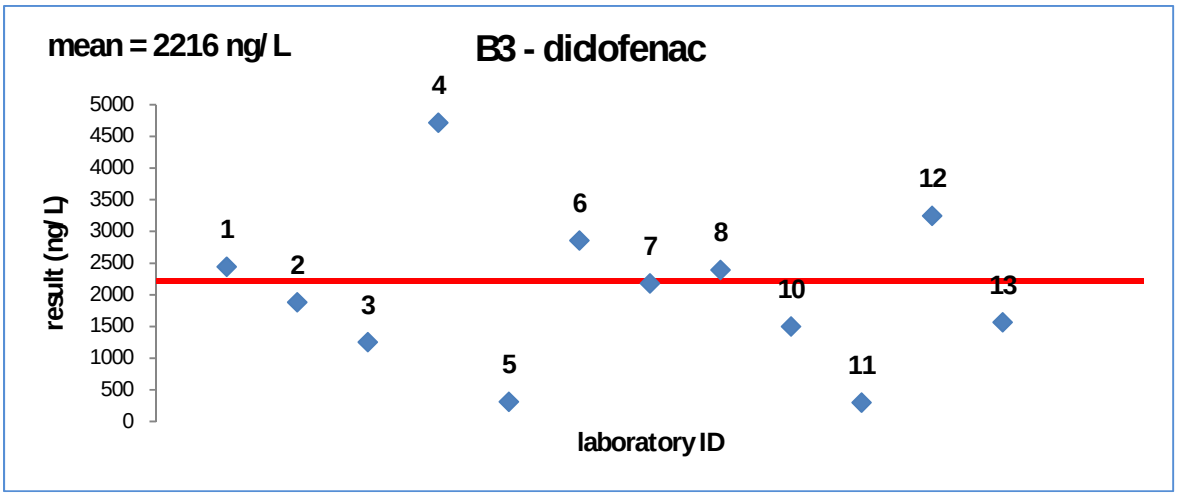
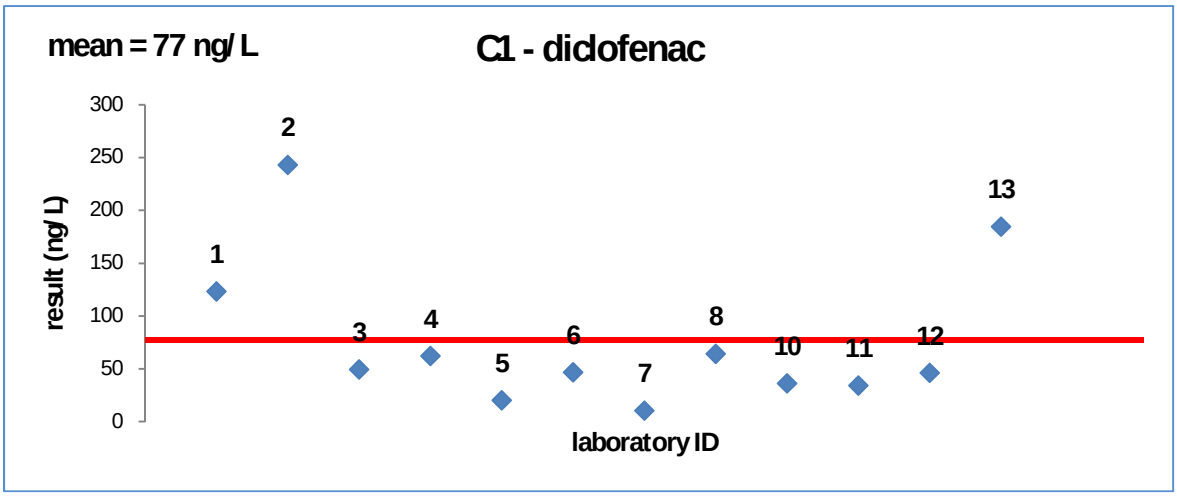
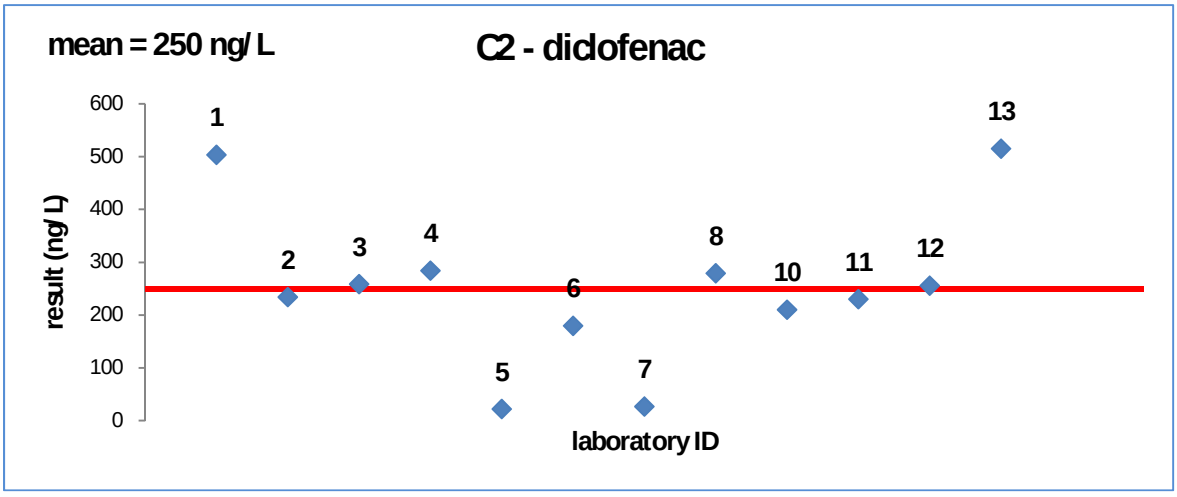


Figure 6 (11/12)





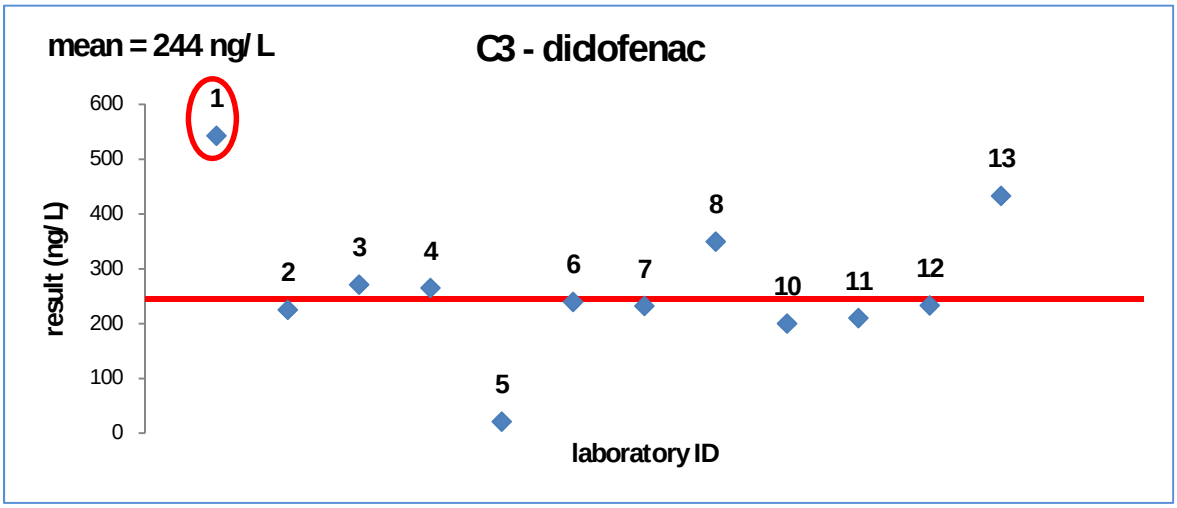
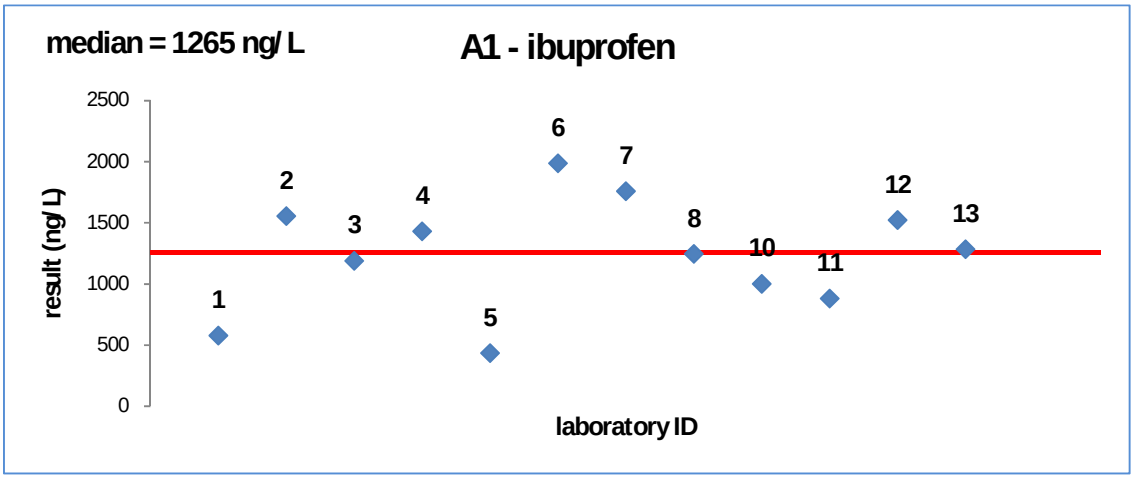


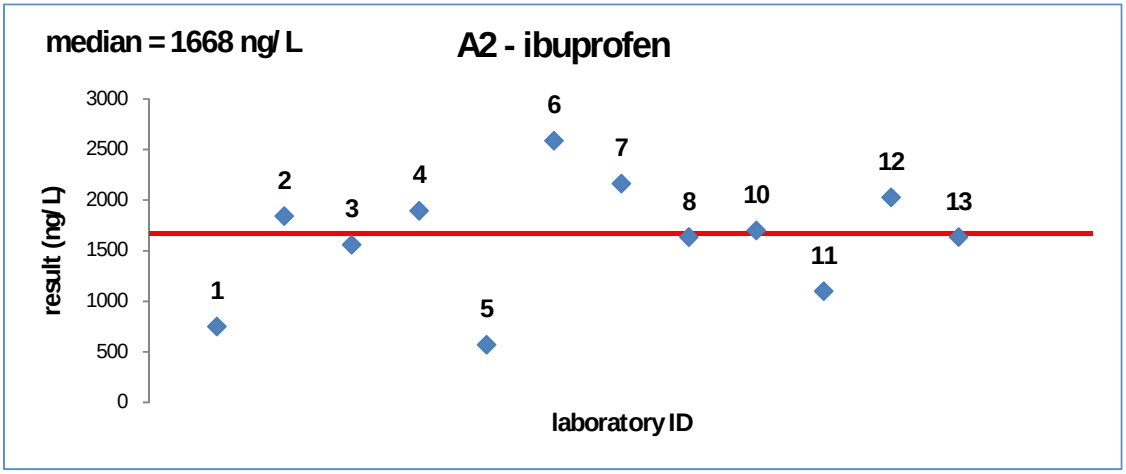
Figure 6 (12/12)

Figure 6: Laboratory performance: graphs showing the corrected sample mean (after the exclusion of the outliers), drawn by the red line and deviation of each laboratory (blue dots, numbered by laboratory ID). The outlier values are labelled with red circles and are not taken into account for the mean value calculation.

b) Deviations from the median (robust approach)

Figure 7 presents the laboratory performance presented as a deviation of each laboratory from the corrected (excluded outliers) sample mean. In addition, the robust approach was also used, illustrating the deviations from the corrected median. The results are presented in Figure 8.





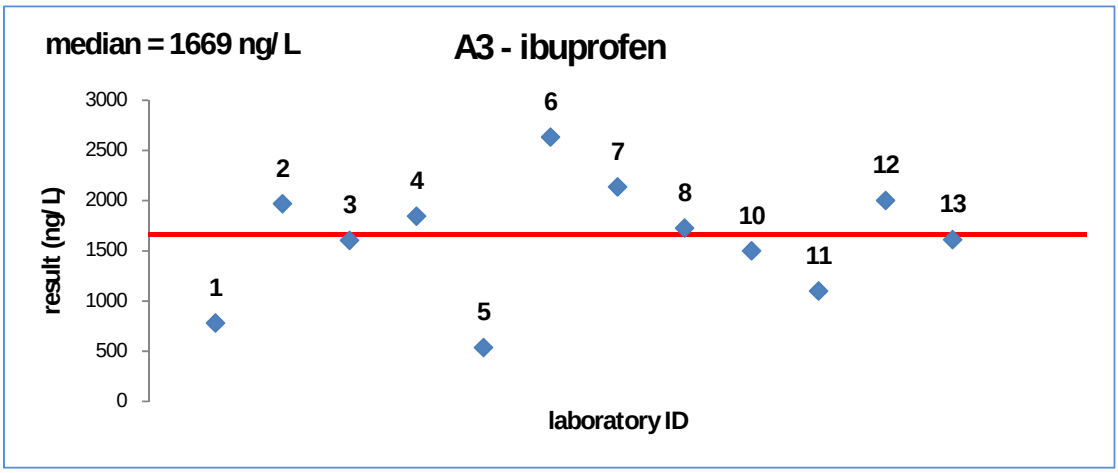
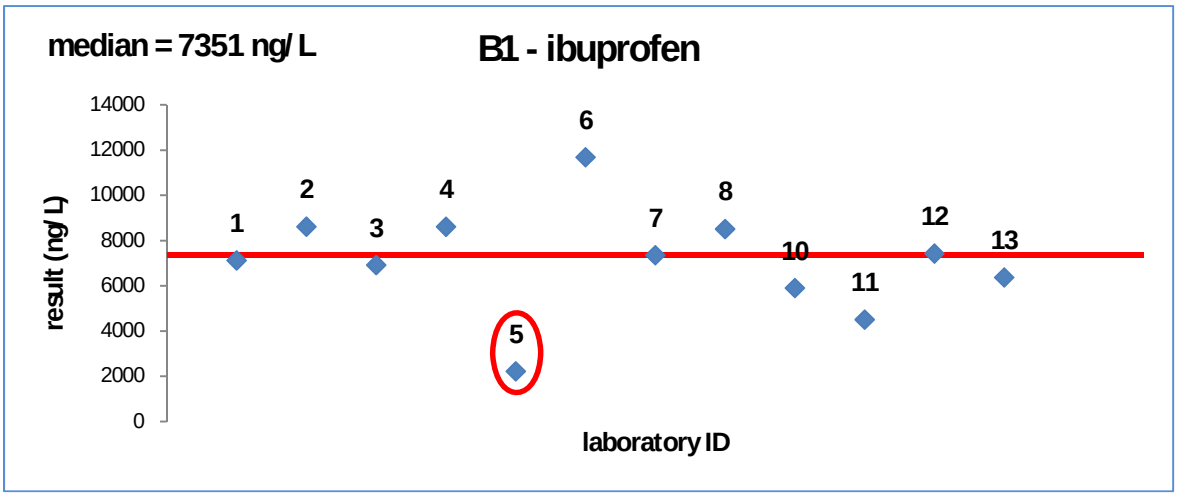
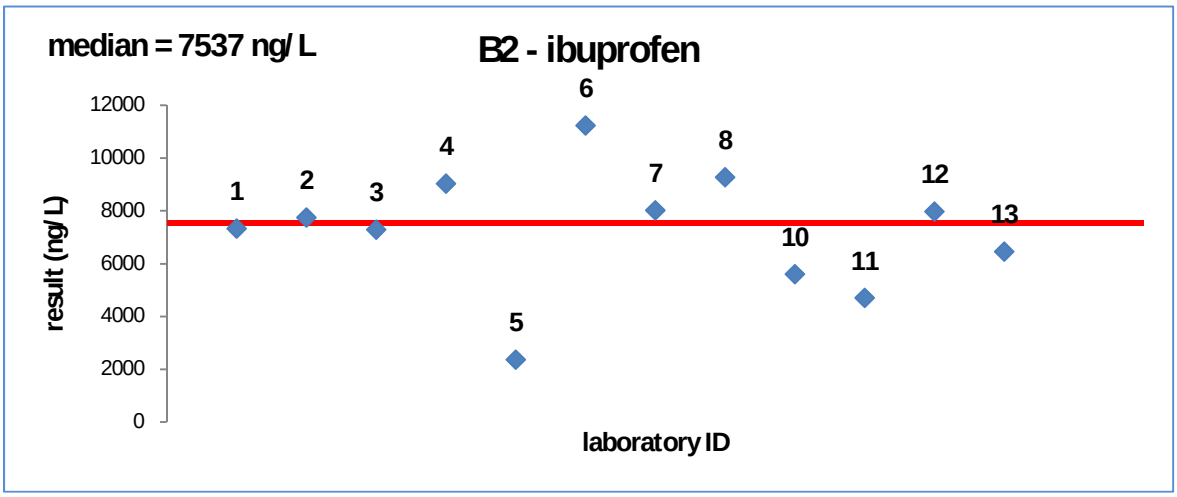


Figure 7 (1/12)


median = 7351 ng/ L
B1 - ibuprofen
result (ng/ L)
14000
12000
10000
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


median = 7537 ng/ L
B2 - ibuprofen
result (ng/ L)
12000
10000
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

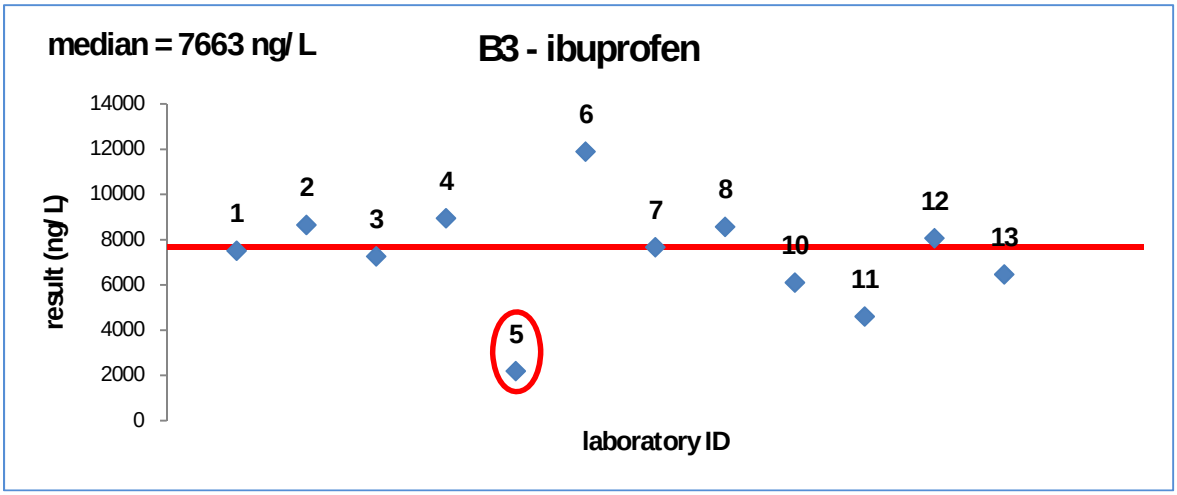
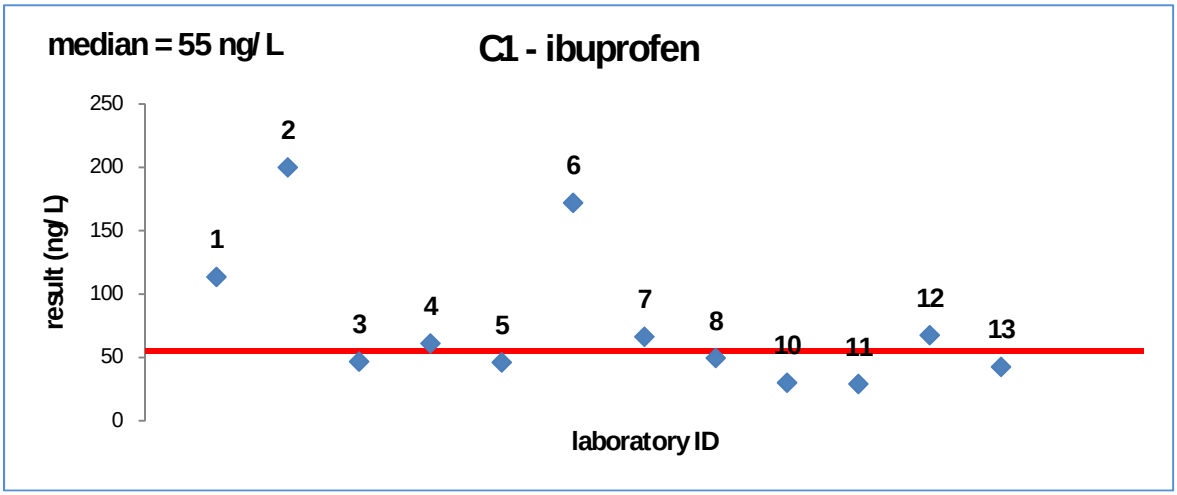
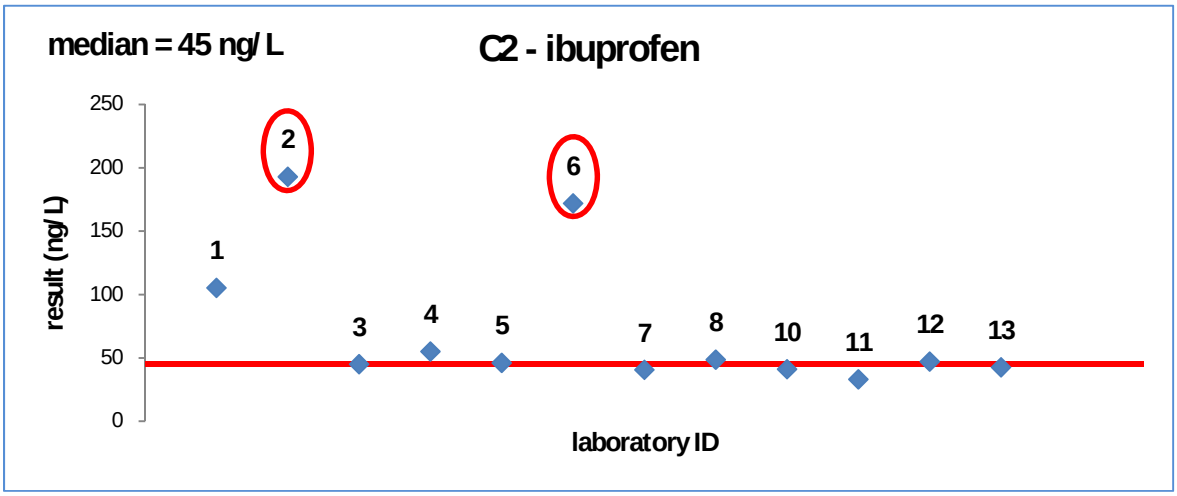

median = 7663 ng/ L
B3 - ibuprofen
result (ng/ L)
14000
12000
10000
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 7 (2/12)


median = 55 ng/ L
C1 - ibuprofen
result (ng/ L)
250
200
150
100
50
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


median = 45 ng/ L
C2 - ibuprofen
result (ng/ L)
250
200
150
100
50
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

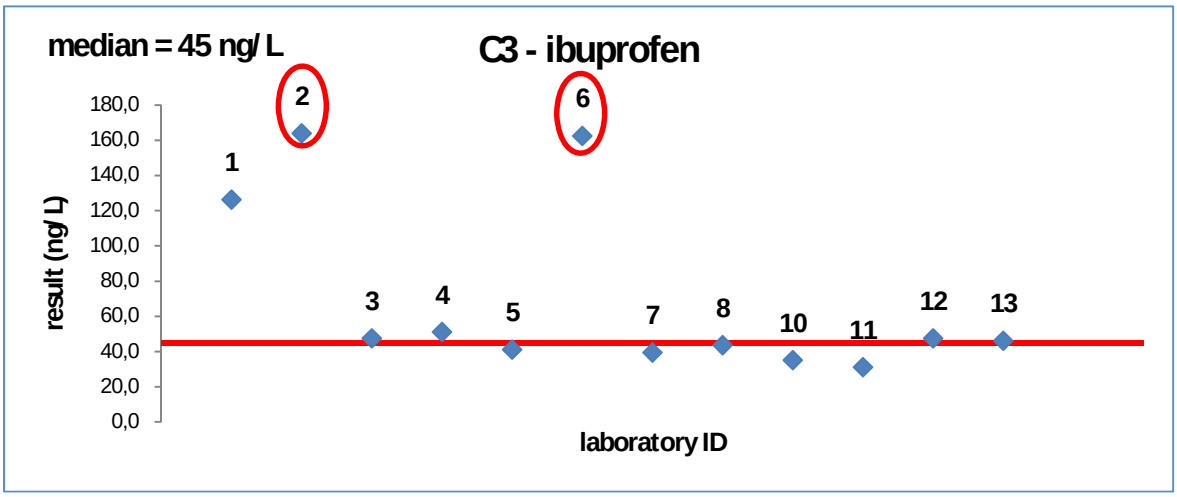
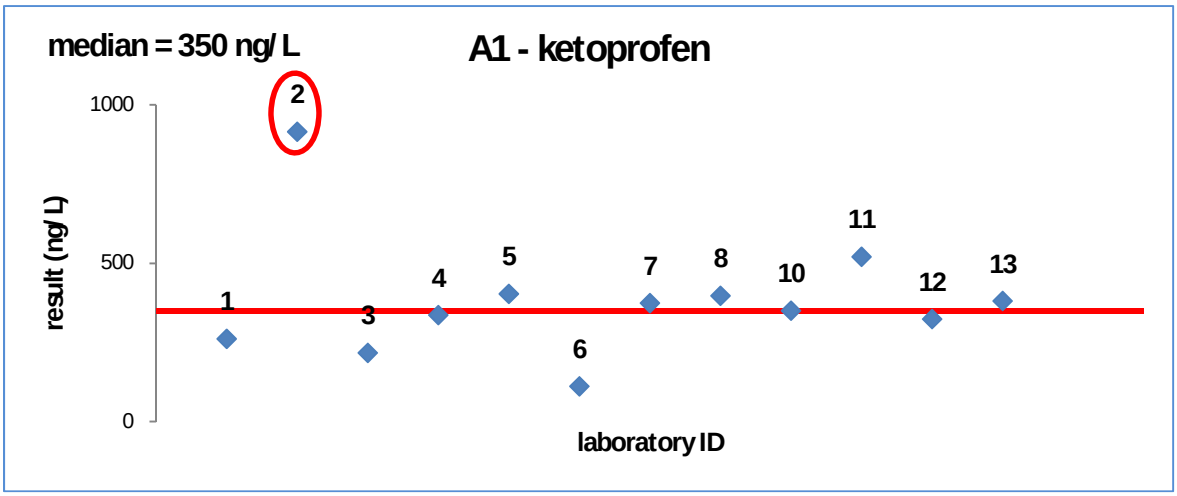
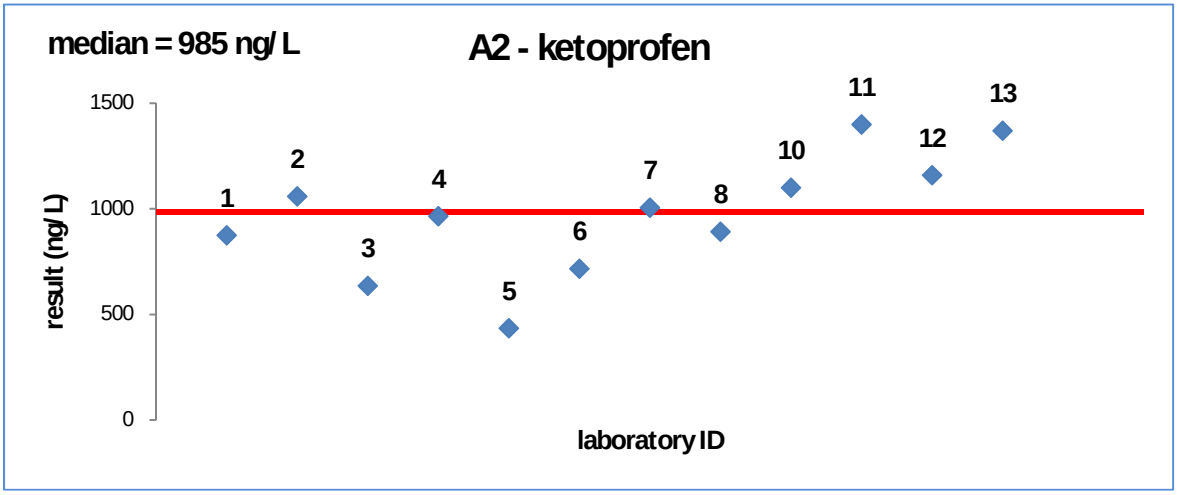

median = 45 ng/ L
C3 - ibuprofen
result (ng/ L)
180,0
160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 7 (3/12)


median = 350 ng/ L
A1 - ketoprofen
result (ng/ L)
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


median = 985 ng/ L
A2 - ketoprofen
result (ng/ L)
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

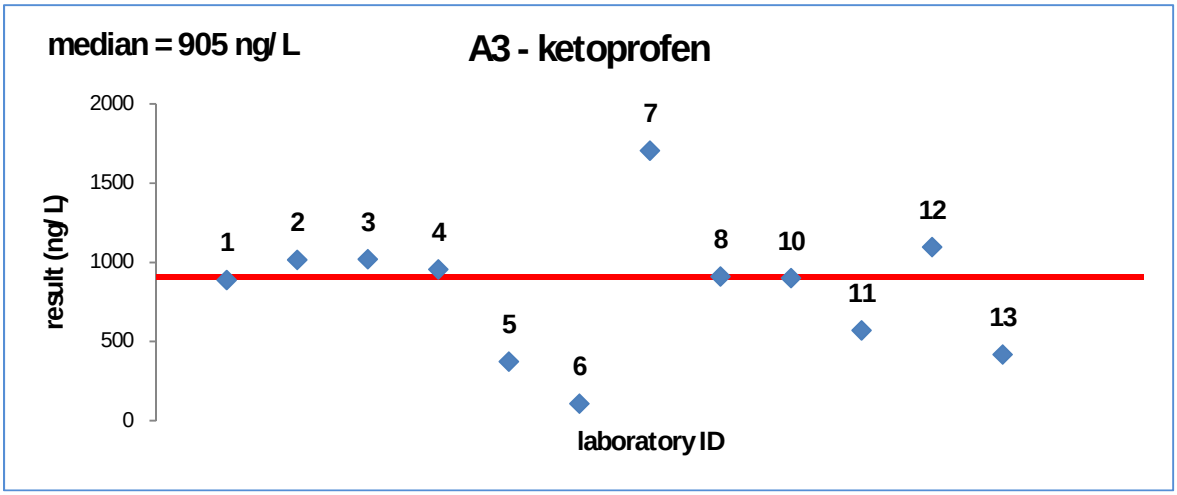
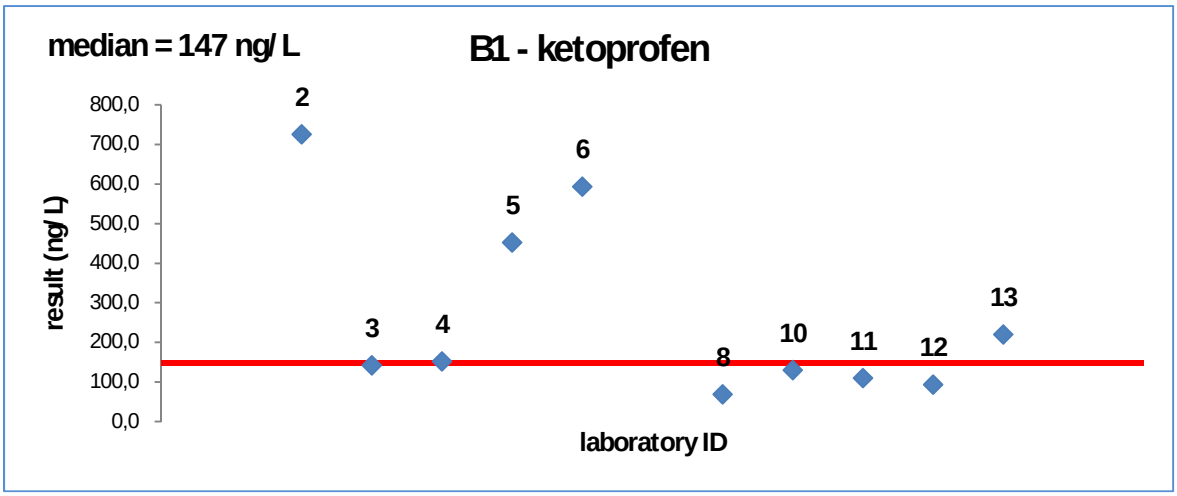
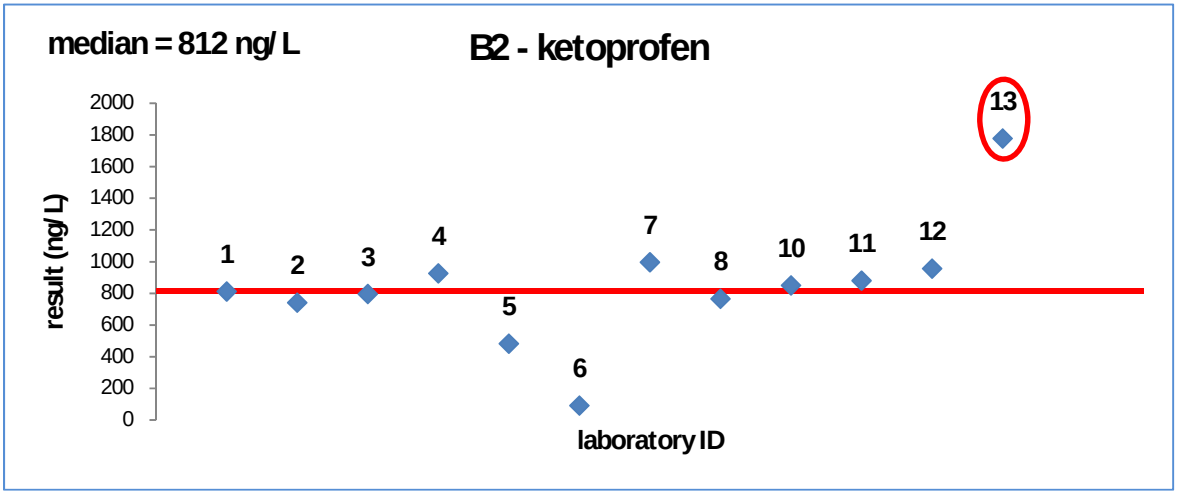

median = 905 ng/ L
A3 - ketoprofen
result (ng/ L)
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 7 (4/12)


median = 147 ng/ L
B1 - ketoprofen
result (ng/ L)
800,0
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
2
6
5
3
4
8
10
11
12
13
laboratory ID


median = 812 ng/ L
B2 - ketoprofen
result (ng/ L)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
13
1
2
3
4
7
8
10
11
12
5
6
laboratory ID

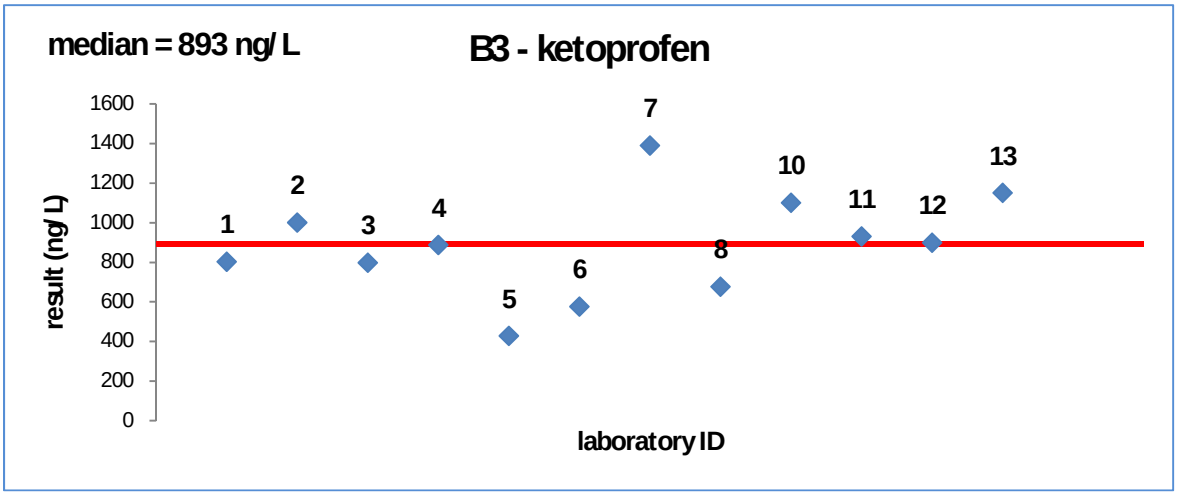
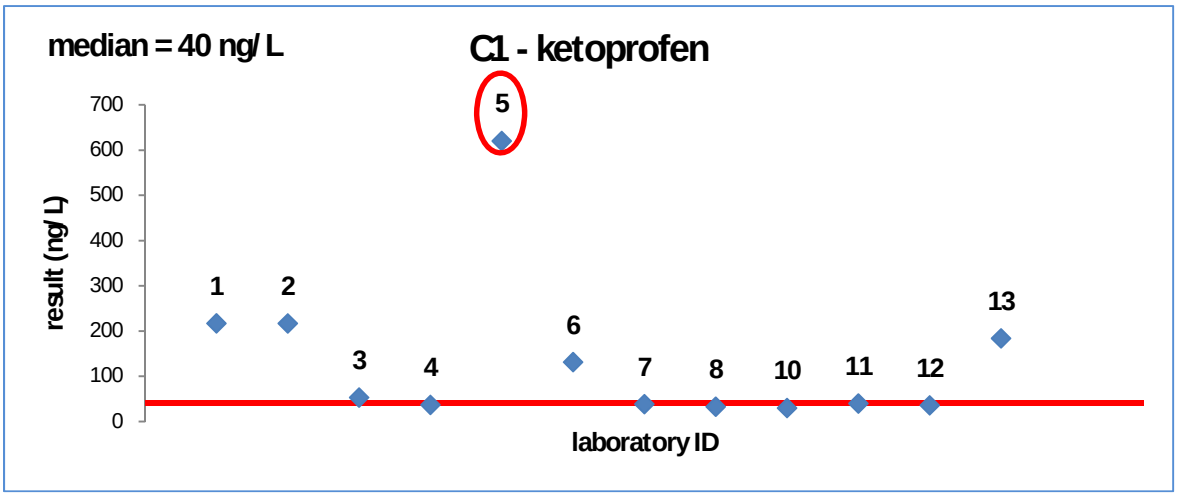
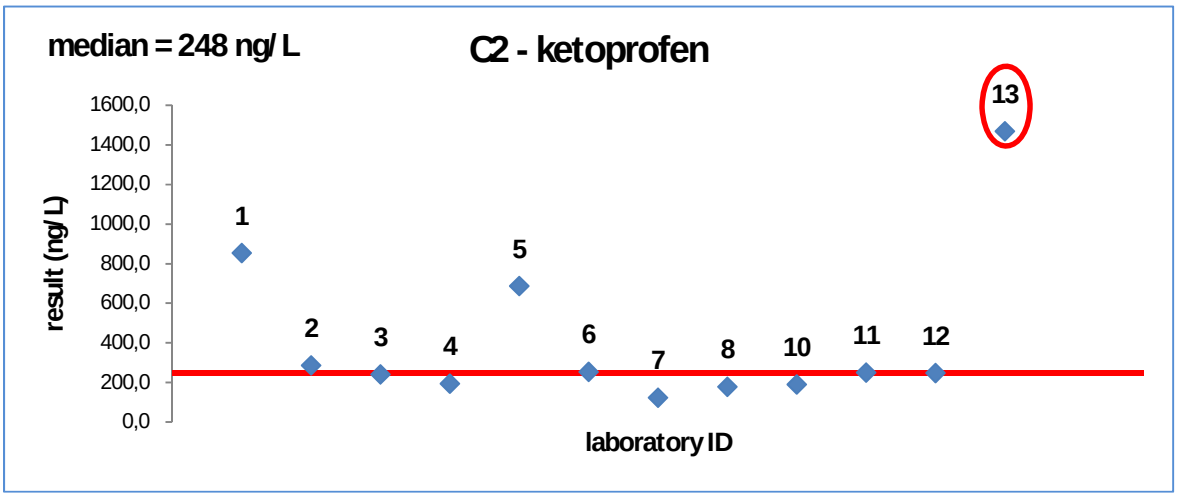

median = 893 ng/ L
B3 - ketoprofen
result (ng/ L)
1600
1400
1200
1000
800
600
400
200
0
7
10
13
2
11
1
4
12
3
8
6
5
laboratory ID

Figure 7 (5/12)


median = 40 ng/ L
C1 - ketoprofen
result (ng/ L)
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


median = 248 ng/ L
C2 - ketoprofen
result (ng/ L)
1600,0
1400,0
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

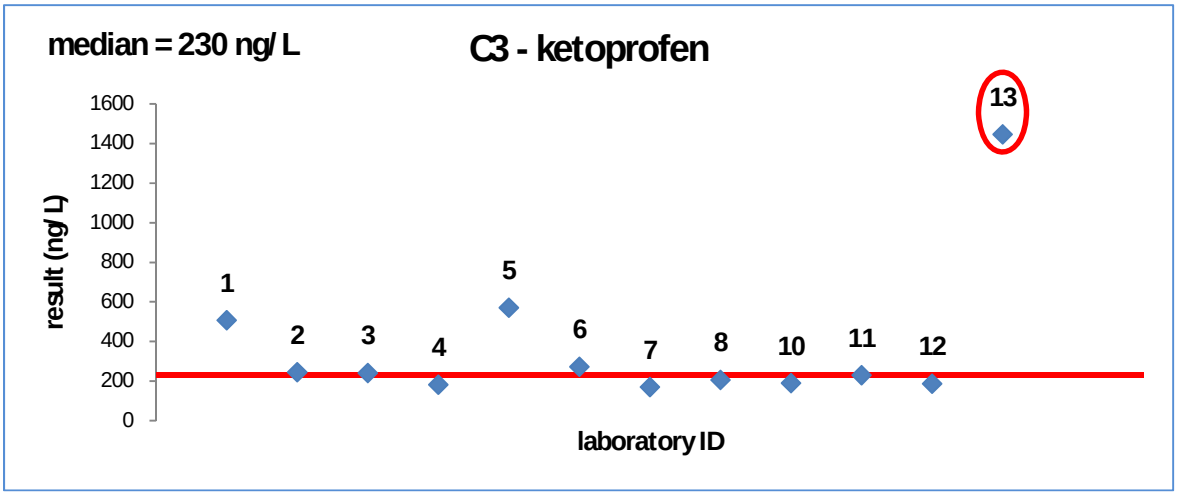
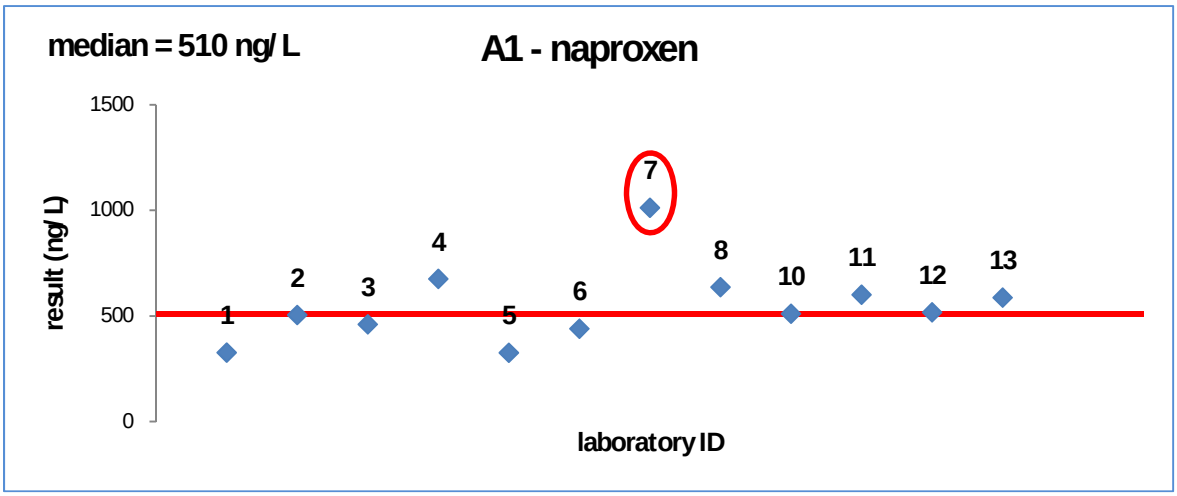
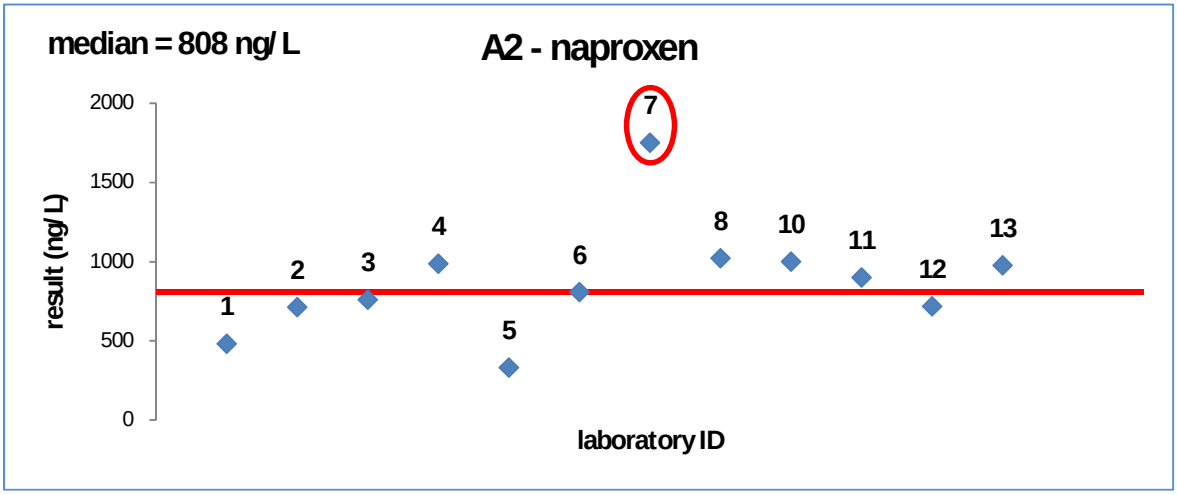

median = 230 ng/ L
C3 - ketoprofen
result (ng/ L)
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 7 (6/12)


median = 510 ng/ L
A1 - naproxen
result (ng/ L)
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


median = 808 ng/ L
A2 - naproxen
result (ng/ L)
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

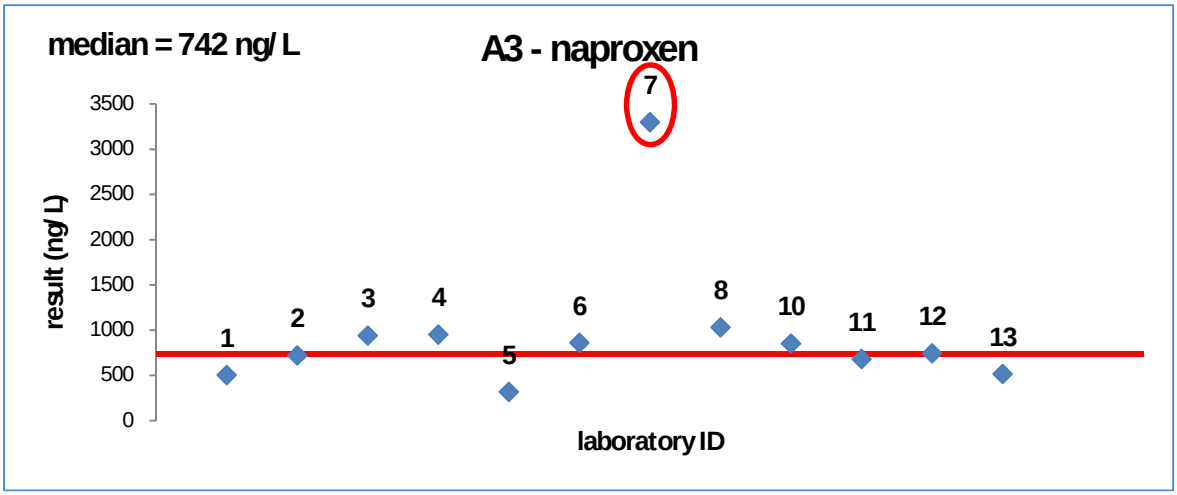
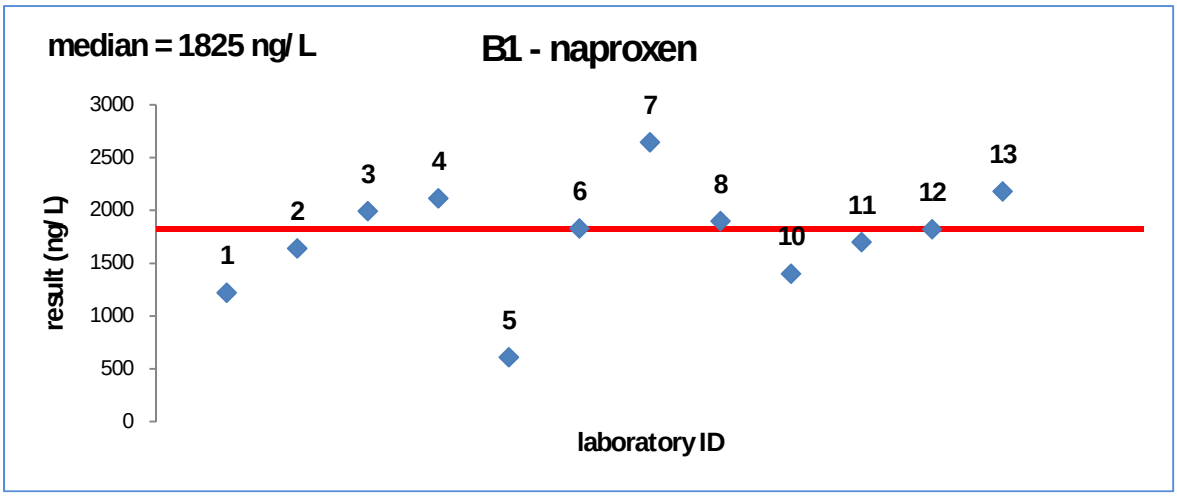
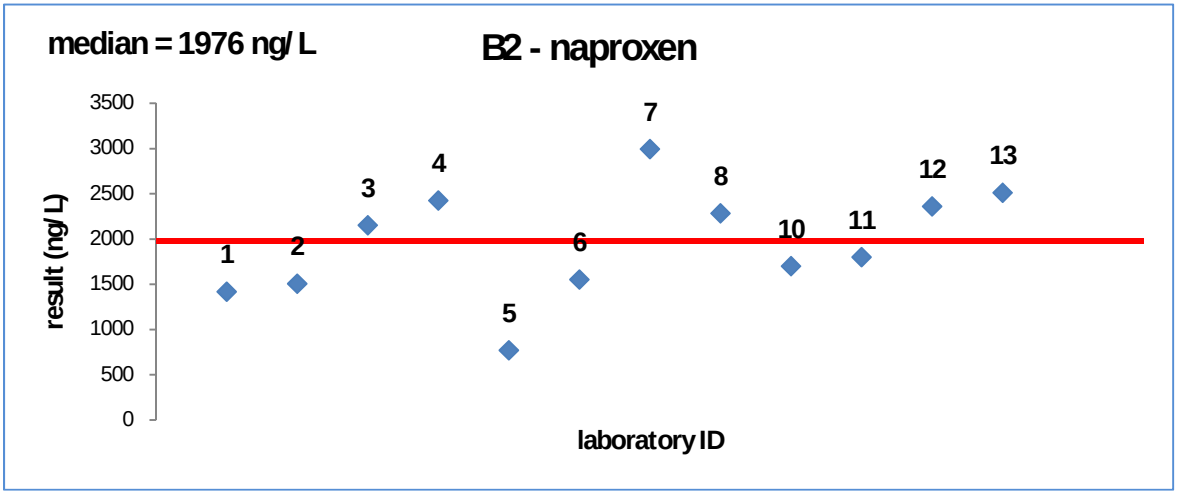

median = 742 ng/ L
A3 - naproxen
result (ng/ L)
3500
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 7 (7/12)


median = 1825 ng/ L
B1 - naproxen
result (ng/ L)
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID


median = 1976 ng/ L
B2 - naproxen
result (ng/ L)
3500
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

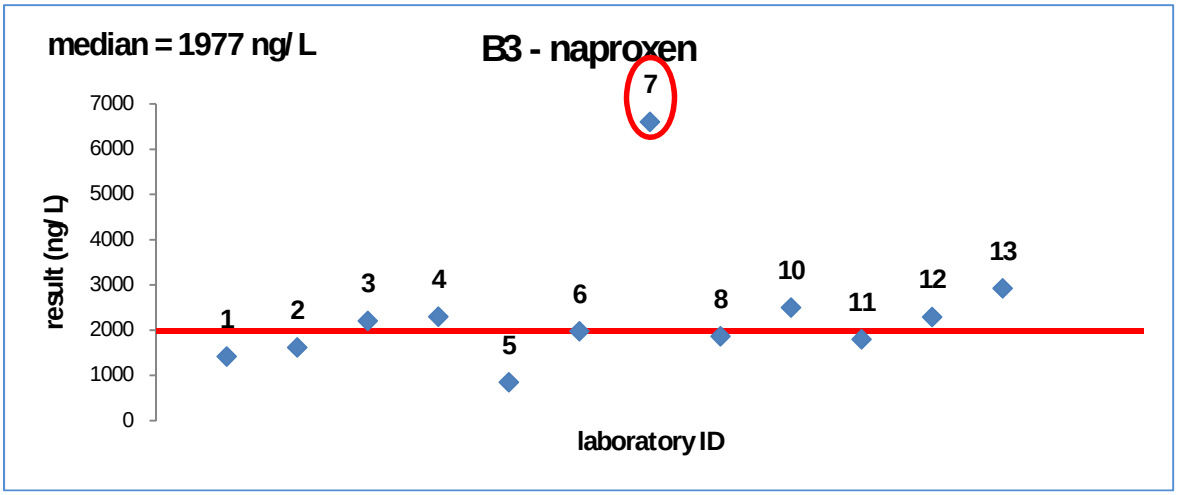
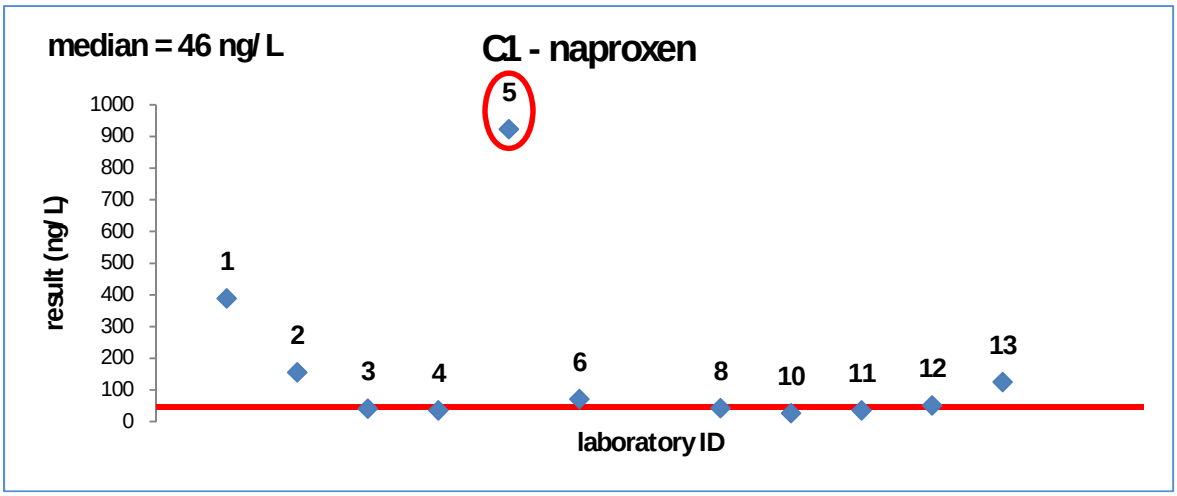
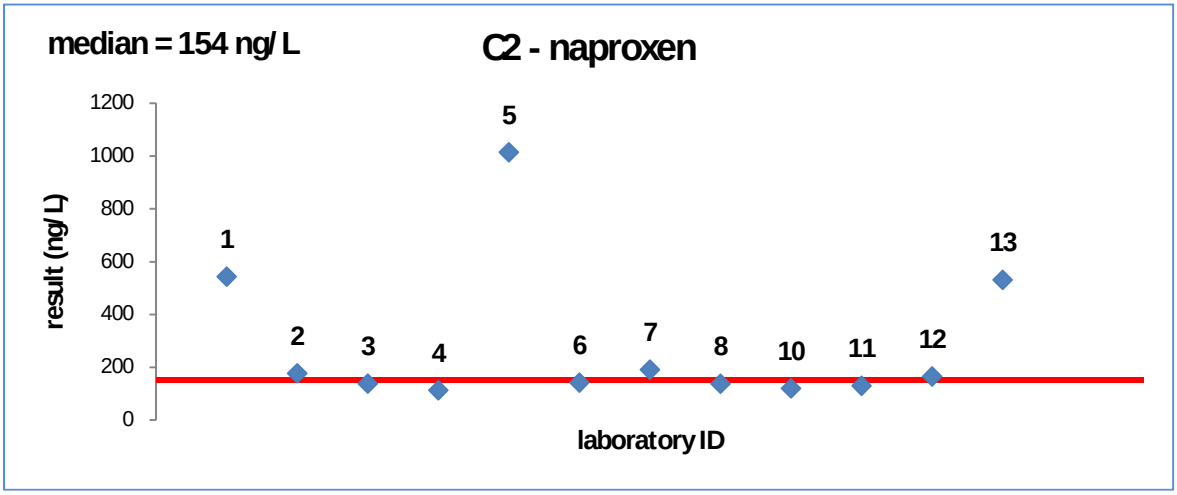

median = 1977 ng/ L
B3 - naproxen
result (ng/ L)
7000
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
6
7
8
10
11
12
13
laboratory ID

Figure 7 (8/12)


median = 46 ng/ L
C1 - naproxen
result (ng/ L)
laboratory ID


median = 154 ng/ L
C2 - naproxen
result (ng/ L)
laboratory ID

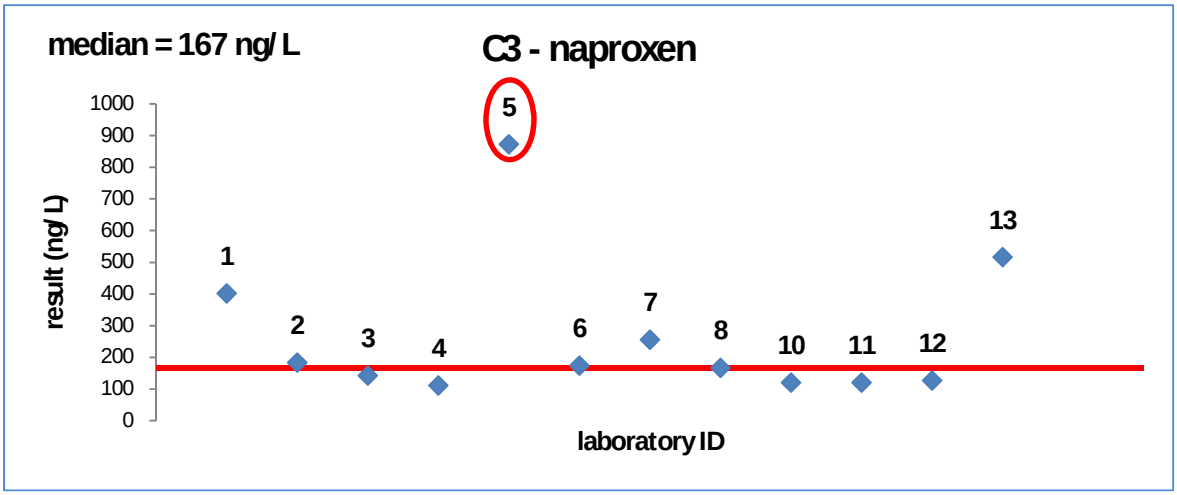
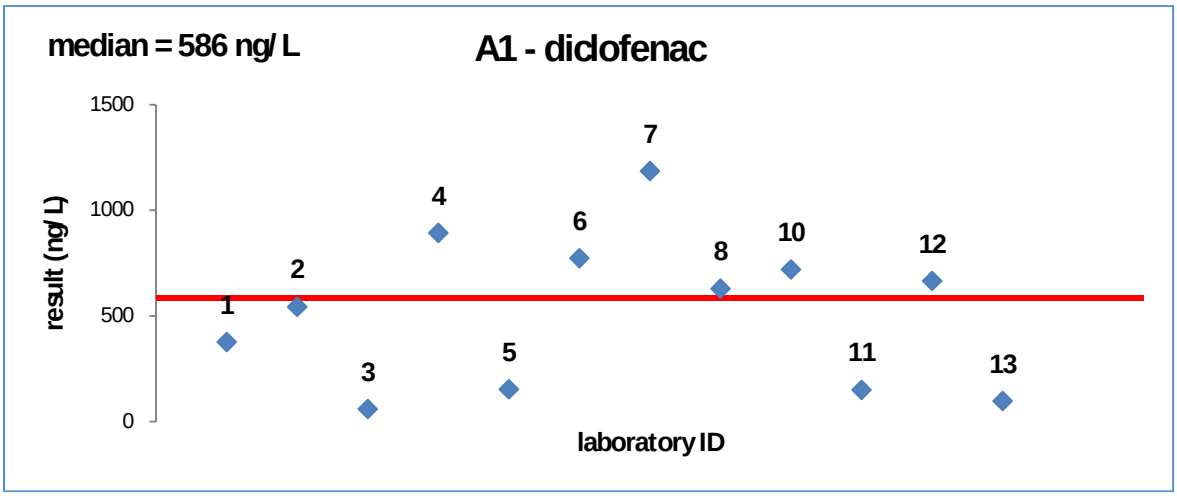
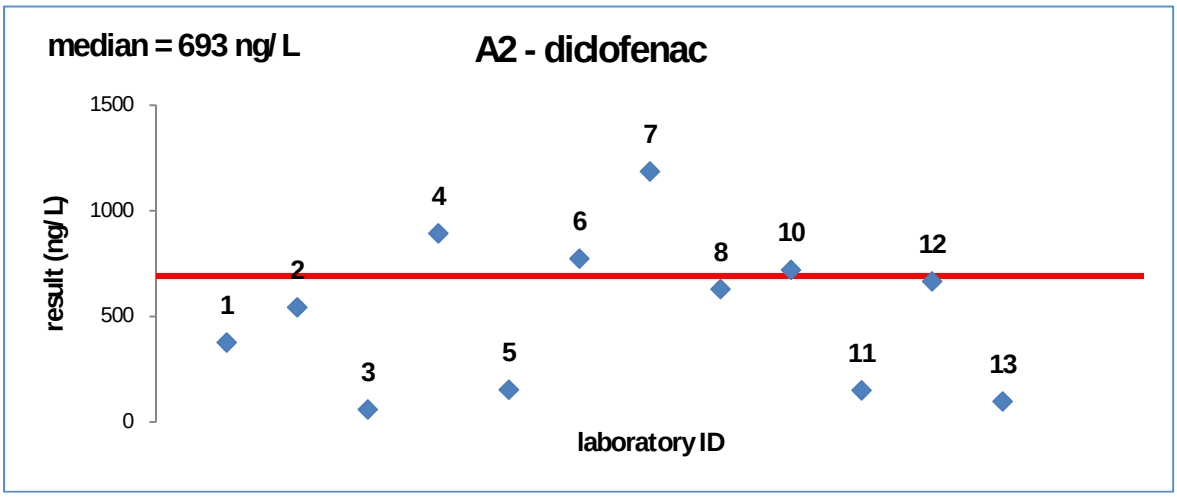

median = 167 ng/ L
C3 - naproxen
result (ng/ L)
laboratory ID

Figure 7 (9/12)





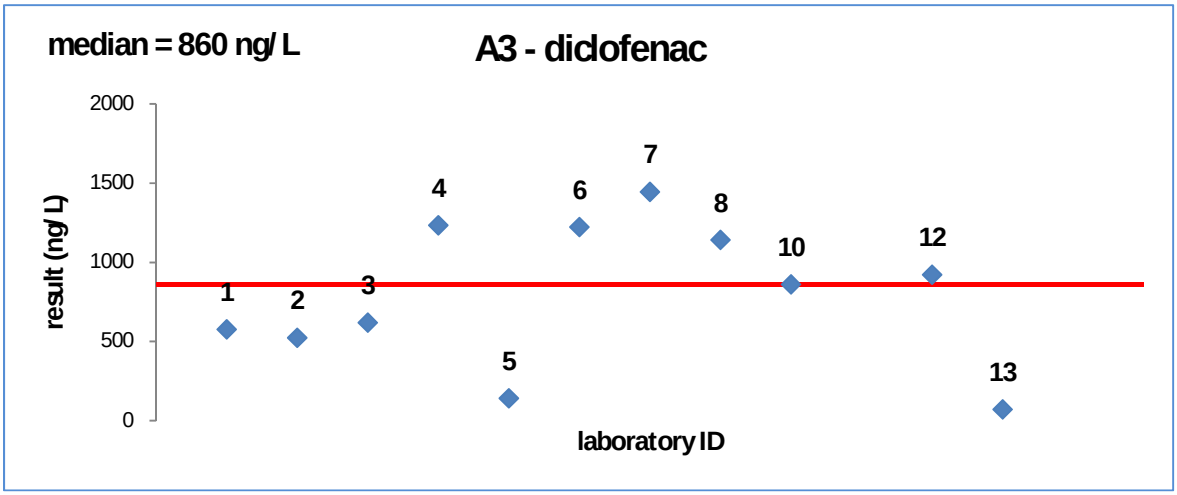
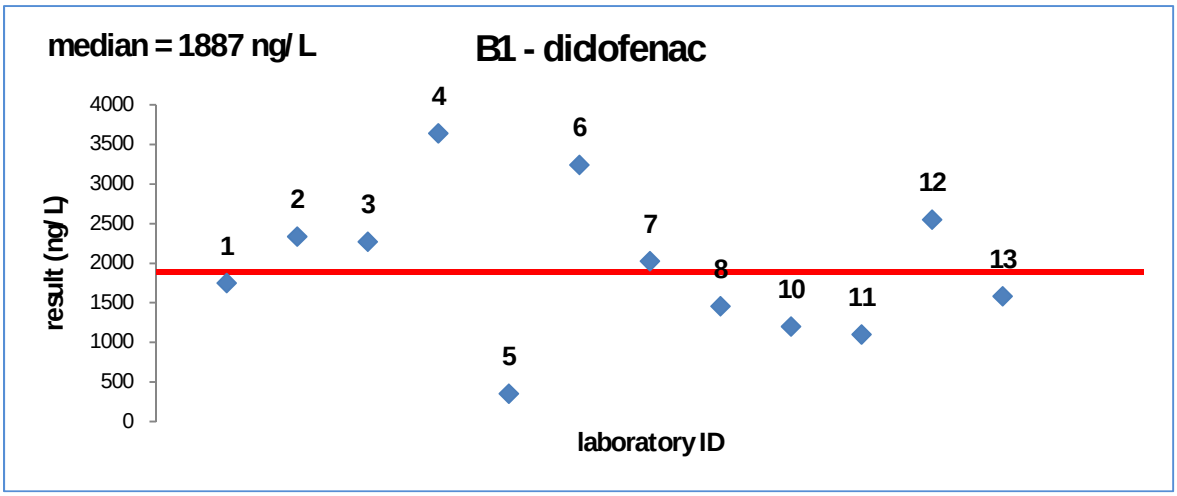
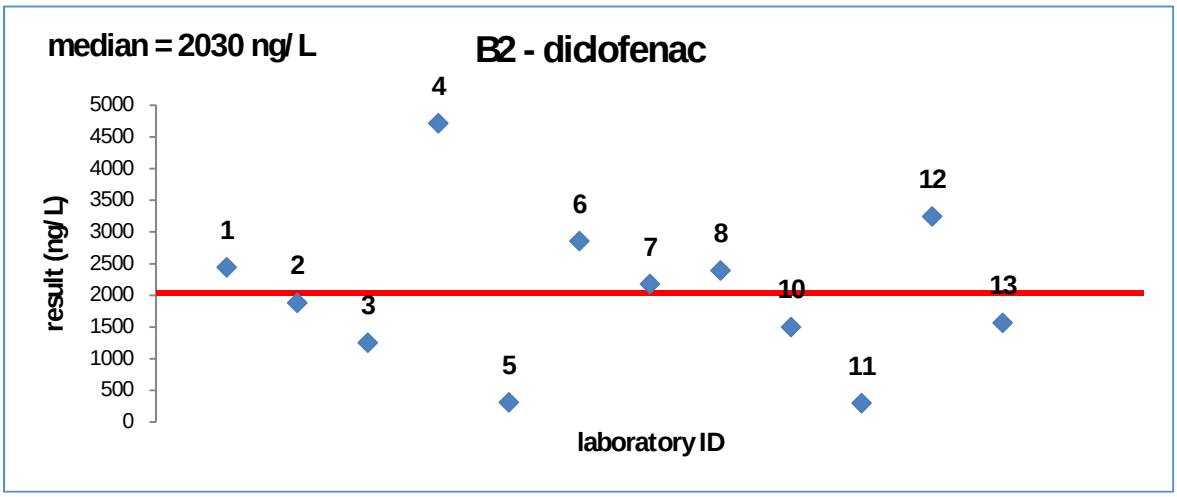


Figure 7 (10/12)





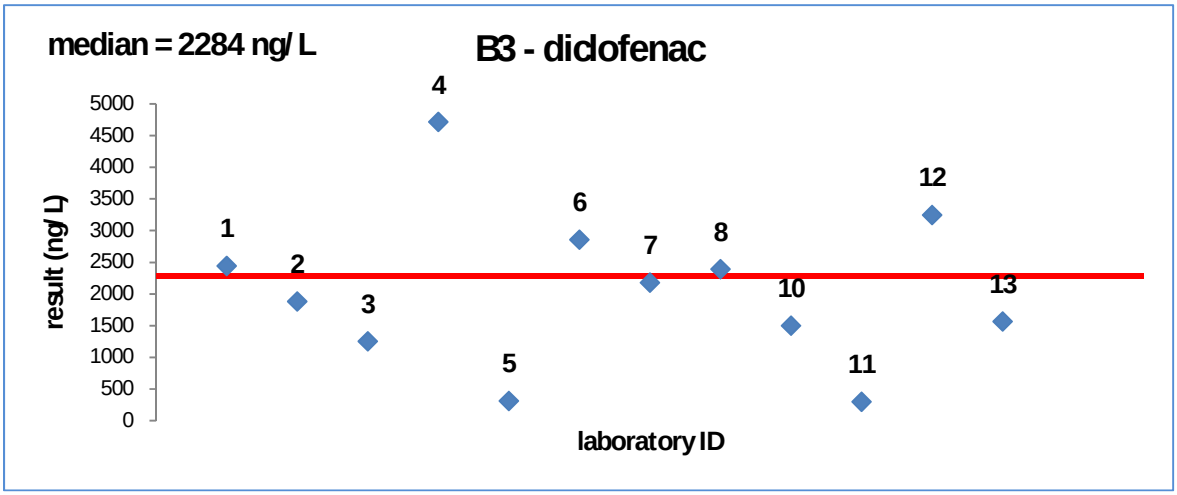


Figure 7 (11/12)

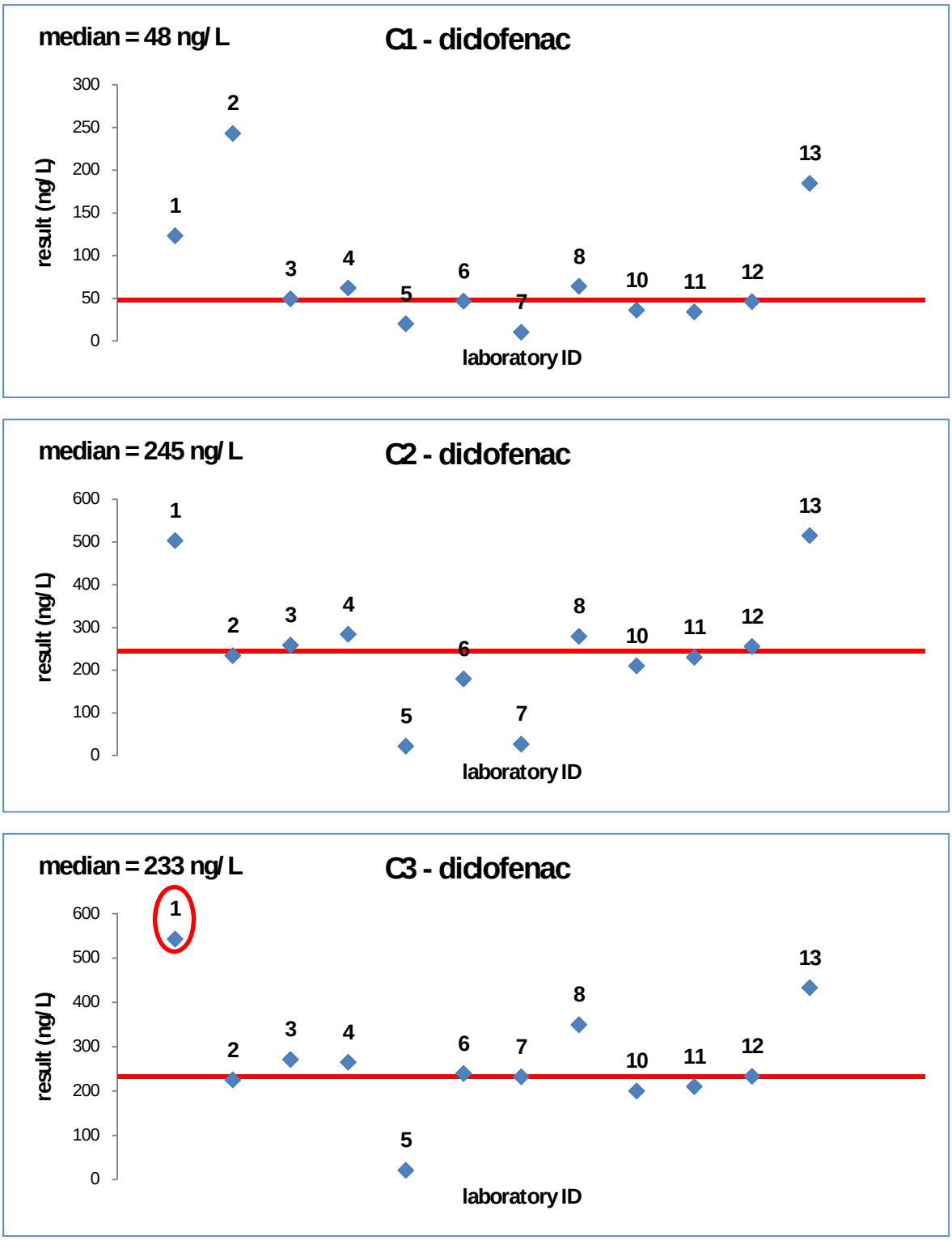


Figure 7 (12/12)

Figure 7: The laboratory performance using the robust approach. The outliers are labelled by red circles and are not taken into account for the median value calculation.

c) Laboratory performance according to ISO/DIS 13528 [4]

Laboratory biases (D) were estimated for each result (or average of results) reported by a participant. When a participant reports a result that gives rise to a laboratory bias outside the range $-3.0\ \sigma < D < 3.0\ \sigma$, then such result shall be considered to give an "action signal" [4]. Likewise, laboratory bias outside $-2.0\ \sigma < D < 2.0\ \sigma$ (light grey fields in Table 6) shall be considered to give a "warning signal". The outlier results are marked with circles (Table 6) and were previously excluded from the calculation of the assigned values. Table 6 shows no "action signals" and maximum one "warning signal" per a series of results (series = one analyte in one sample / all participating laboratories; represented by one line in Table 6) considered for the assigned value calculation. According to International standard ISO/DIS 13528 [4] the complete absence of "action signals" and less than two "warning signals" in a single run indicate that the mean (x_{AV}) and standard deviation (σ), with the underlying normal distribution, are good approximates for the true mean and standard deviation values.

Table 6: Laboratory biases for each result (or average of results). ± 2 σ biases are coloured light grey. The estimates for ± 3 σ biases are also shown in the right column of the table. The outlier values are marked with circles and were excluded in calculation of the assigned values.

bias (D): ibuprofen	Lab ID	1	2	3	4	5	6	7	8	10	11	12	13	> ± 2σ	> ± 3σ
	A1	-662	317	-50	192	-805	748	520	7	-238	-358	284	45	> ± 919	> ± 1379
	A2	-871	221	-63	273	-1052	966	541	13	78	-522	405	13	> ± 1154	> ± 1731
	A3	-839	349	-16	225	-1083	1012	516	106	-120	-520	381	-9	> ± 1173	> ± 1759
	B1	-425	1067	-629	1065	**-5330**	**4139**	-194	965	-1645	-3045	-121	-1178	> ± 3706	> ± 5559
	B2	77	497	37	1780	**-4892**	3985	770	2022	-1650	-2550	722	-798	> ± 4604	> ± 6906
	B3	-283	858	-533	1154	**-5602**	**4099**	-129	774	-1691	-3191	271	-1329	> ± 3727	> ± 5591
	C1	37	**123**	-30	-16	-31	95	-11	-27	-47	-48	-9	-34	> ± 112	> ± 167
	C2	44	**132**	-16	-6	-15	**111**	-21	-13	-20	-28	-14	-19	> ± 83	> ± 124
	C3	57	94	-22	-19	-29	93	-30	-26	-35	-39	-22	-24	> ± 100	> ± 151

bias (D):ketoprofen	Lab ID	1	2	3	4	5	6	7	8	10	11	12	13	> ± 2σ	> ± 3σ
	A1	-73	**581**	-117	2	69	**-223**	40	63	16	186	-10	47	> ± 216	> ± 323
	A2	-93	92	-333	-2	-533	-251	38	-76	133	433	192	403	> ± 568	> ± 852
	A3	58	185	190	125	-457	-722	**875**	80	70	-260	266	-412	> ± 832	> ± 1248
	B1		**456**	-127	-117	183	324		-200	-139	-159	-175	-49	> ± 469	> ± 703
	B2	57	-13	42	172	-272	**-663**	242	11	96	126	202	**1024**	> ± 519	> ± 778
	B3	-84	114	-89	1	-458	-310	503	-210	214	44	12	264	> ± 522	> ± 782
	C1	125	124	-39	-56	**527**	39	-54	-60	-63	-53	-57	91	> ± 157	> ± 236
	C2	**535**	-33	-78	-125	368	-65	-195	-141	-129	-69	-71	**1150**	> ± 462	> ± 693
	C3	235	-28	-32	-91	**298**	0	-103	-67	-83	-43	-86	**1174**	> ± 272	> ± 408

Table 6(1/2)

bias (D):naproxen	Lab ID	1	2	3	4	5	6	7	8	10	11	12	13	> ± 2σ	> ± 3σ
	A1	-181	-3	-47	168	-182	-68	**505**	129	3	93	10	79	> ± 230	> ± 346
	A2	-309	-78	-31	196	**-459**	17	**959**	231	209	109	-73	186	> ± 447	> ± 671
	A3	-234	-18	202	213	-420	122	**2559**	293	113	-57	6	-222	> ± 440	> ± 660
	B1	-534	-114	239	361	**-1145**	75	891	145	-354	-54	67	426	> ± 1033	> ± 1549
	B2	-538	-450	196	469	-1185	-404	1037	327	-256	-156	404	555	> ± 1215	> ± 1823
	B3	-558	-358	228	322	-1126	-1	**4621**	-112	522	-178	314	947	> ± 1127	> ± 1690
	C1	**292**	58	-57	-62	**826**	-26		-55	-71	-62	-46	28	> ± 222	> ± 333
	C2	260	-106	-146	-170	**731**	-141	-93	-146	-163	-153	-118	248	> ± 552	> ± 828
	C3	191	-27	-68	-99	**662**	-37	45	-44	-90	-90	-84	**306**	> ± 264	> ± 396

bias (D):diclofenac	Lab ID	1	2	3	4	5	6	7	8	10	11	12	13	> ± 2σ	> ± 3σ
	A1	-144	22	-461	372	-368	253	665	108	199	-371	145	-423	> ± 714	> ± 1071
	A2	-192	-92	-596	537	-565	546	612	18	470	-620	338	-459	> ± 974	> ± 1461
	A3	-219	-273	-177	437	-655	427	649	345	64		126	-725	> ± 903	> ± 1355
	B1	-209	377	312	1681	-1607	1282	67	-503	-759	-859	591	-376	> ± 1848	> ± 2773
	B2	388	-172	-801	**2661**	-1742	803	125	338	-554	-1754	1192	-488	> ± 2468	> ± 3702
	B3	194	-817	-653	2046	-1830	1156	51	-421	84	-1646	856	976	> ± 2303	> ± 3455
	C1	47	**166**	-27	-15	-57	-30	-66	-13	-41	-43	-31	108	> ± 142	> ± 213
	C2	253	-16	9	34	-228	-70	-223	29	-40	-20	6	265	> ± 299	> ± 448
	C3	**299**	-19	27	21	**-223**	-4	-12	106	-44	-34	-10	189	> ± 201	> ± 302

Table 6(2/2)

d) Proximity to the mean

"Proximity to the mean" is a general measure of a laboratory capability to determine a specific analyte. In the calculation the influence on matrix and concentration are excluded, instead only the relative biases in determination of each compound are taken into account. The proximity to the mean was calculated as shown in the following equation,

$$prox. = \frac{1}{n} \times \sum \frac{\left| x_i - \bar{x} \right|}{\bar{x}} \qquad \textit{Equation 3}$$

where x_i is an observed value and $\bar{x}$ is the interlaboratory mean. The proximity to the mean values were plotted for each analyte in each laboratory as shown in (Figure 8)

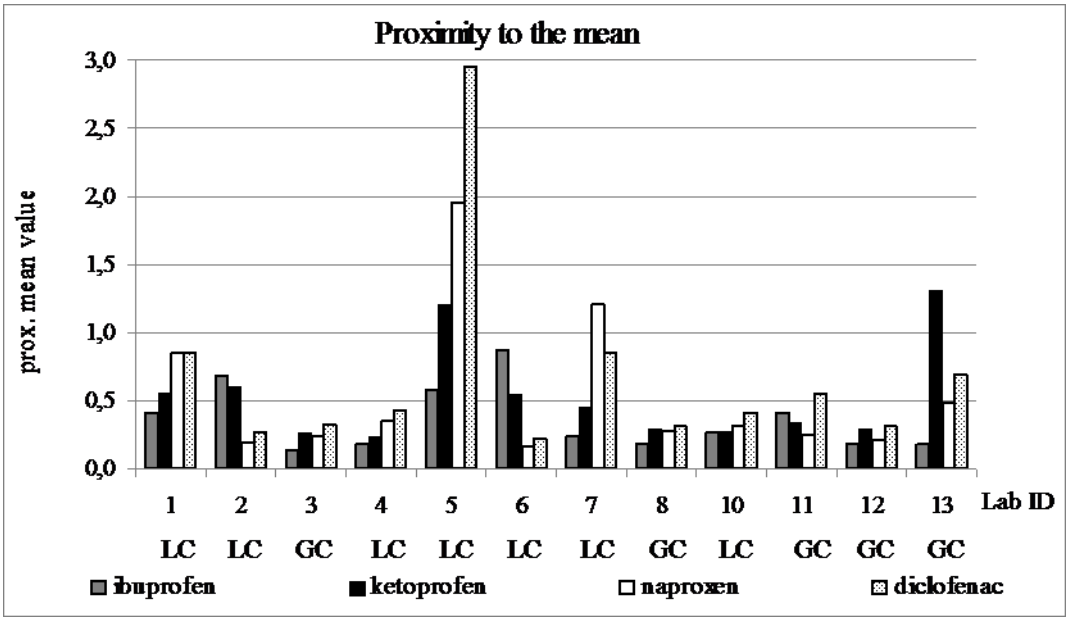


Figure 8: Bar-chart showing the "proximity to the mean" values

In addition, the x-axes illustrates the analytical protocol used, which, in contrast with the results of the 1st Interlaboratory exercise, shows a relatively good performance of GC laboratories. This leads to the conclusion that the deviations from the mean value did not depend on the analytical protocol used.

e) Proximity to the median

According to the robust approach, also the proximity to the median was calculated, where in its calculation the mean value was replaced by the corrected median (Equation 4).

$$prox.(MED) = \frac{1}{n} \times \sum \frac{|x_i - MED|}{MED} \qquad \textit{Equation 4}$$

Figure 10 shows the "proximity to the median" performance of the participating laboratories and the analytical protocol used.

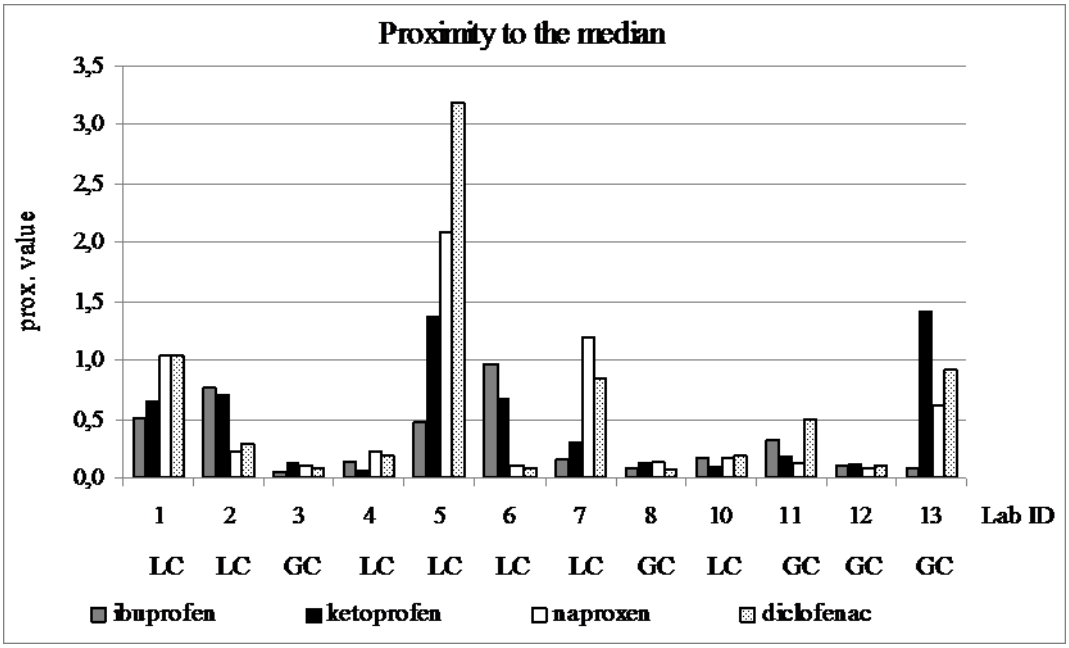


Figure 9: Bar-chart illustrating the proximity to the median

9. Effect of filtration

a) Effect of filtration with respect to the matrices

In order to evaluate the effect of filtration on determination of NSAIDs in different matrices, the samples numbered with "2" and "3" in each series (A, B, C) were prepared in parallel. Participants were asked to filter the samples "2", while samples "3" were extracted without

the pre-filtration. To compare the variances of each NSAID in filtered and unfiltered matrix three statistical tests were used. First, to assess the effect of filtration in different matrices to the final determination F-test at 5 % significance level (Equation 5) was used for comparison of the variances within each batch[5].

$$F_{\exp.} = \frac{\sigma^2_{sampleNo.2}}{\sigma^2_{sampleNo.3}} \qquad \textit{Equation 5}$$

By accepting the H0 hypothesis it was proved that the samples "2" and "3" were drawn from the same group with underlying normal distribution, meaning that the filtration had no effect on the sample mean. As presented in Table 7, the F-tests did not show a significant difference between the filtered and unfiltered parallels, except in the case of naproxen in deionised water.

Table 7: Results of the F-test for ibuprofen in wastewater and river water (A) and ketoprofen (B), naproxen (C) and diclofenac (D) in wastewater, river water and deionised water

A.

IBUPROFEN	***IP-A2***	***IP-A3***	***IP-B2***	***IP-B3***
Mean	1622	1620	7250	7791
Variance	333039	343906	5299889	3473365
Observations	12	12	12	11
Degrees of freedom	11	11	11	10
F	0,9684		1,5259	
P(F<=f) one-tail	0,4792		0,2568	
F Critical one-tail	0,3549		2,9430	
H0 accepted?	**YES**		**YES**	

B.

KETOPROFEN	***KP-A2***	***KP-A3***	***KP-B2***	***KP-B3***	***KP-C2***	***KP-C3***
Mean	967	830	754	886	319	273
Variance	80571	173192	67330	68002	53346	18515
Observations	12	12	11	12	11	11
Degrees of freedom	11	11	10	11	10	10
F	0,4652		0,9901		2,8812	
P(F<=f) one-tail	0,1101		0,4977		0,0551	
F Critical one-tail	0,3549		0,3398		2,9782	
H0 accepted?	**YES**		**YES**		**YES**	

C.\

NAPROXEN	*NP-A2*	*NP-A3*	*NP-B2*	*NP-B3*	*NP-C2*	*NP-C3*
Mean	791	737	1956	1978	283	210
Variance	50037	48359	369326	317426	76193	17386
Observations	11	11	12	11	12	11
Degrees of freedom	10	10	11	10	11	10
F	1,0347		1,1635		4,3824	
P(F<=f) one-tail	0,4790		0,4094		0,0136	
F Critical one-tail	2,9782		2,9430		2,9430	
H0 accepted?	**YES**		**YES**		**NO**	

D.\

DICLOFENAC	*DF-A2*	*DF-A3*	*DF-B2*	*DF-B3*	*DF-C2*	*DF-C3*
Mean	730	796	2054	2216	250	244
Variance	237328	204049	1522998	1325984	22320	10148
Observations	12	11	12	12	12	11
Degrees of freedom	11	10	11	11	11	10
F	1,1631		1,1486		2,1995	
P(F<=f) one-tail	0,4097		0,4112		0,1125	
F Critical one-tail	2,9430		2,8179		2,9430	
H0 accepted?	**YES**		**YES**		**YES**	

To confirm the results of *"F-test"* paired *"t-test"* for comparison of means "2" and "3" within each laboratory was applied. The results are presented in Table 8.

Table 8: Results of the t-test for ibuprofen in wastewater and river water (A) and ketoprofen (B), naproxen (C) and diclofenac (D) in wastewater, river water and deionised water

A.\

IBUPROFEN	*IP-A2*	*IP-A3*	*IP-B2*	*IP-B3*
Mean	1622	1620	7695	7791
Variance	333039	343906	3219075	3473365
Observations	12	12	11	11
Pearson Correlation	0,9901		0,9697	
Hypothesized Mean Difference	0		0	
Df	11		10	
t Stat	0,0827		-0,7023	
P(T<=t) one-tail	0,4678		0,2493	
t Critical one-tail	1,7959		1,8125	
P(T<=t) two-tail	0,9356		0,4985	

B.\

KETOPROFEN	***KP-A2***	***KP-A3***	***KP-B2***	***KP-B3***	***KP-C2***	***KP-C3***
Mean	967	830	754	862	319	273
Variance	80571	173192	67330	67212	53346	18515
Observations	12	12	11	11	11	11
Pearson Correlation	0,1407		0,6964		0,9527	
Hypothesized Mean Difference	0		0		0	
Df	11		10		10	
t Stat	1,0170		-1,7740		1,3823	
P(T<=t) one-tail	0,1655		0,0532		0,0985	
t Critical one-tail	1,7959		1,8125		1,8125	
P(T<=t) two-tail	0,3310		0,1065		0,1970	
t Critical two-tail	2,2010		2,2281		2,2281	

C.\

NAPROXEN	***NP-A2***	***NP-A3***	***NP-B2***	***NP-B3***	***NP-C2***	***NP-C3***
Mean	791	737	1862	1978	217	210
Variance	50037	48359	288864	317426	25594	17386
Observations	11	11	11	11	11	11
Pearson Correlation	0,7061		0,8261		0,9535	
Hypothesized Mean Difference	0		0		0	
Df	10		10		10	
t Stat	1,0561		-1,1851		0,4163	
P(T<=t) one-tail	0,1579		0,1317		0,3430	
t Critical one-tail	1,8125		1,8125		1,8125	
P(T<=t) two-tail	0,3158		0,2634		0,6860	
t Critical two-tail	2,2281		2,2281		2,2281	

D.\

DICLOFENAC	***DF-A2***	***DF-A3***	***DF-B2***	***DF-B3***	***DF-C2***	***DF-C3***
Mean	786	796	2054	2216	227	244
Variance	219185	204049	1522998	1325984	17544	10148
Observations	11	11	12	12	11	11
Pearson Correlation	0,8599		0,8667		0,8281	
Hypothesized Mean Difference	0		0		0	
df	10		11		10	
t Stat	-0,1317		-0,9037		-0,7448	
P(T<=t) one-tail	0,4489		0,1928		0,2368	
t Critical one-tail	1,8125		1,7959		1,8125	
P(T<=t) two-tail	0,8978		0,3855		0,4735	
t Critical two-tail	2,2281		2,2010		2,2281	

The results of t-test are in general agreement with the results of F-test, showing that the prefiltration did not lead to changes in concentration in the samples "2" compared to the samples "3".

Ibuprofen in C1 was not additionally spiked to produce C2 and C3 samples, hence it was possible to compare the variances of all three samples in series C by One-way Analysis of Variance (ANOVA) [6]. The results are summarised in Table 9, again showing that the filtration did not have any effect on determination of ibuprofen in deionised water.

Table 9: The results of the ANOVA for ibuprofen in spiked deionised water samples C1, C2 and C3

Groups	*No. observations*	*Sum*	*Average*	*Variance*
C1-IP	12	924	77	3117
C2-IP	11	676	61	1709
C3-IP	12	834	70	2523

ANOVA

Source of Variation	*Sum of Squares*	*Degrees of freedom*	*Mean Square*	*F*	*P-value*	*F crit*
Between Groups	1391	2	696	0,2813	0,7567	3,2945
Within Groups	79133	32	2473			
Total	80524	34				

b) Effect of the filter material

As the filter material was not specified in the analytical protocols at least four different types of materials were used in different laboratories: glass fibre, nitrocellulose membrane, nylon membrane, cellulose acetate, membrane (not specified). Between twelve participating laboratories, 7 of them used glass microfibre filters (Group 1 of laboratories, G1), while 5 (Group 2 of laboratories, G2) used membrane filters. In order to test the influence of the filter material, F- test (Equation 6) was applied to compare the variances of G1 with G2 for each NSAID in all filtered samples (A1, A2, B2, B2, C1 and C2).

$$F_{\exp.} = \frac{\sigma_{G1}^2}{\sigma_{G2}^2} \qquad \textit{Equation 6}$$

The H0 hypothesis at 95 % confidence level was not rejected in none of the cases, meaning that the filter material did not influence the final determination of NSAIDs.

10. Repeatability and reproducibility

In all cases, where the effect of filtration was shown insignificant (i.e. IP, KP, NP and DF in A2 & A3, B2 & B3; IP in C1 & C2 & C3, KP and DF in C2 & C3) the repeatability and reproducibility were calculated.

Repeatability is a measure of closeness of agreement between independent results obtained with the same method on identical test material, under the same conditions (same operator, same apparatus, same laboratory and after short intervals of time). The measure of repeatability is the standard deviation qualified with the term: 'repeatability' as repeatability standard deviation, σ_r [7,8,9,10]. Repeatability standard deviation is a standard deviation obtained under repeatability conditions and was calculated for each compound form "2" and "3" samples and for ibuprofen in "C1", "C2" and "C3" samples (Table 10).

Reproducibility (R) is a precision under conditions, where test results are obtained with the same method on identical samples, but the analyses are preformed in different laboratories with different operators and using different equipment [7]. Reproducibility standard deviation is the standard deviation under reproducibility conditions (σ_R).The reproducibility standard deviation is given in the last lines of Table 10A.\, 10B.\ and 10C.\. The outlier values were excuded from the reproducibility calculation, while repeatability values are shown in coloured fields.

Table 10: Repeatability (r_{lab}) and reproducibility (R) of the results. The parameters were calculated for each NSAID in all tested matrices. Table 8/A: wastewater; 8/B: river water; 8/C: deionised water. The grey coloured fields illustrate the outlier repeatability results, which were excluded from the calculation of reproducibility

A.

A2 & A3 (σ_r)	ibuprofen	ketoprofen	naproxen	diclofenac
Lab 1	21	9	15	28
Lab 2	89	31	4	81
Lab 3	32	272	126	343
Lab 4	35	7	26	24
Lab 5	23	43	11	17
Lab 6	31	430	36	38
Lab 7	19	494	1093	73
Lab 8	64	13	6	278
Lab 10	141	141	106	240
Lab 11	0	587	156	
Lab 12	19	45	17	103
Lab 13	17	674	327	141
σ_R	**580**	**268**	**205**	**466**

B.

B2 & B3 (σ_r)	ibuprofen	ketoprofen	naproxen	diclofenac
Lab 1	128	7	2	23
Lab 2	638	183	81	342
Lab 3	20	1	38	219
Lab 4	60	28	88	320
Lab 5	120	38	57	52
Lab 6	464	343	301	365
Lab 7	253	277	2549	62
Lab 8	500	63	295	422
Lab 10	354	177	566	566
Lab 11	71	35	0	191
Lab 12	63	41	48	123
Lab 13	7	444	293	1150
σ_R	**2326**	**248**	**590**	**1152**

C.\

C2 & C3 (σ_r)	ibuprofen (C1&C2&C3)	ketoprofen	naproxen	diclofenac
Lab 1	11	245	100	28
Lab 2	19	29	4	6
Lab 3	1	0	3	9
Lab 4	5	8	1	13
Lab 5	3	82	100	1
Lab 6	5	13	22	42
Lab 7	15	33	46	145
Lab 8	3	20	21	50
Lab 10	6	0	0	7
Lab 11	2	14	7	14
Lab 12	12	43	27	16
Lab 13	2	16	11	58
σ_R	**42**	**181**	**269**	**132**

IV. Conclusions

Twelve participants from eleven different European research institutes and universities took part in NORMAN 2nd Interlaboratory exercise. 108 samples were analysed to determine concentration of selected NSAIDs and 773 results (including < LOD values and parallels) were collected for the data evaluation. The final number of 428 values was pooled out for further data analysis, where 15 of them (3.5 %) were determined as outliers according to classical approach and 18 (4.2 %) according to robust approach. Among 5 GC and 7 LC laboratories, which participated in this Interlaboratory exercise, GC methods yielded 3 (1.7 % of the GC results) and LC 12 (4.7 % of the LC results) outliers. The distribution of the outliers between the GC and LC protocols is contrary to the results of the 1st round of the NORMAN Interlaboratory exercise. However, as the outliers were distributed among only 5 participants this suggests that the performance of a single laboratory has a large impact on the final number of the outliers. Accordingly, the number of the outliers would significantly decrease (up to 47%) merely by improving the determination of naproxen in the Lab 7 and ketoprofen

in the Lab 13. In this view, the number of the outliers cannot be used as a measure for assessment of method capability, but rather as a parameter describing a laboratory performance. The sample matrix yielding the highest number of outliers was, as well as in the 1st Interlaboratory exercise, deionised water (47 %). In addition to the classical approach the evaluation of the outliers was also performed using the less common robust approach, which is based on deviation of results from the median and not mean value. The results for the batches A and B (waste and river water) were identical for both approaches, while the latter yielded three more outliers for the batch C.

The estimation of the laboratory biases (D) showed no results outside the range $-3.0\ \sigma < D < 3.0\ \sigma$ ("action signals"), while only 19 were "warning signals", falling outside the range $-2.0\ \sigma < D < 2.0\ \sigma$. As none of the series of results included more than 1 "warning signal", we can conclude that the estimated sample mean and standard deviation were good approximates to the true values. Between the 12 participating laboratories 5 laboratories showed an excellent performance, never reaching the range outside $-2.0\ \sigma < D < 2.0\ \sigma$.

The effect of filtration on the final determination of NSAIDs in each of the relevant matrices was studied by three statistical tests: F-test, paired t-test and ANOVA. The first was used for comparison of variances between the filtered and unfiltered parallel samples, while the paired t-test compared the effect of filtration within each laboratory. ANOVA was used for comparison of three parallel determinations of ibuprofen in deionised water. The tests were in general agreement, showing that the filtration did not reveal a statistically significant effect on the results. Also, the effect of the filter material was studied, where glass microfibre filters were compared by membrane filters, showing the filter material did not influence the determination of NSAIDs.

For the results statistically incorporated into the same original group (with respect to the pre-filtration of matrices) the repeatability and reproducibility were calculated and were presented as standard deviation repeatability and standard deviation reproducibility. To determine the repeatability, "2" and "3" samples were considered (C1, C2 and C3 for ibuprofen), while reproducibility was determined as an interlaboratory measure for a series of measurements.

While the 1st NORMAN Interlaboratory Exercise was a test round focusing on the stability of compounds during sample storage under freezing conditions, the 2nd round avoided the weaknesses recognized in the 1st round. Thus, in contrast to the 1st round, the samples were shipped on dry ice and were extracted as soon as possible after their arrival to the participant laboratories. In addition, for the sample preparation and analysis two laboratory protocols

(GC and LC), specified in details, were given. On the basis of 1st and 2nd Interlaboratory Exercise we can conclude that shipping samples on dry ice, as well as predetermined laboratory protocol contributed towards reduced number of outliers and improved the laboratory performance. Another aim of the 2nd round was to test, whether the pre-filtration affected the determination of the analytes in the tested matrices. The results of the test implied that the filtration itself as well as filter material, did not affect the analysis of selected NSAIDs in none of the three tested matrices.

V. References

[1] M. Thompson and R. Wood, The International Harmonized Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories (Technical Report), Pure & App. Chem. 65/9 (1993) 2123.

[2] S.P. Verma and A. Quiroz-Ruiz, Critical values for six Dixon tests for outliers in normal samples up to sizes 100, and applications in science and engineering, Revista Mexicana de Ciencias Geológicas 23/2 (2006) 133.

[3] http://www.cee.vt.edu/ewr/environmental/teach/smprimer/outlier/outlier.html

[4] International Organization for Standardization (2002), Satistical methods for use in proficiency testing by interlaboratory comparisons, Draft International Standard ISO/DIS 13528

[5] M. Pečanac, V. Hudnik, Merilna negotovost pri kemijskih meritvah (Measure uncertainty at chemical analyses), Workshop, ZTI, Ljubljana, March 12th-13th 2008.

[6] 1-way ANOVA independent groups (Copyright 1998, 2000 Tom Malloy); http://www.psych.utah.edu/stat/introstats/web-text/1-ANOVA_indep/1-wayANOVAindepenpendentgroups.pdf

[7] Definition in the IUPAC Gold Book (1994) 66, 598

[8] International Organization for Standardization (2006), Statistics -- Vocabulary and symbols -- Part 1: General statistical terms and terms used in probability, ISO 3534-1

[9] M. Farré, First NSAIDs Analysis Interlaboratory Excercise, Network of reference laboratories for monitoring of emerging pollutants: NORMAN No. 018486

[10] University of Ljubljana, Faculty of Chemistry and Chemical Technology and SENARC, Workshop on Validation, 12th International Symposium on Separation Sciences, Lipica, Slovenia, September 27-29, 2006.