

SECOND INTERLABORATORY EXERCISE ON DETERMINATION OF NSAIDs IN WATER SAMPLES

Report

Ester Heath and Tina Kosjek
“Jožef Stefan” Institute, Slovenia

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

| | | |
|------|---|----|
| I. | Participant Laboratories | 3 |
| II. | General information | 4 |
| 1. | <i>Sample preparation and transport</i> | 4 |
| 2. | <i>Homogeneity of samples</i> | 5 |
| 3. | <i>Stability of samples</i> | 5 |
| 4. | <i>Analytical protocols for NORMAN participants and NORMAN Validation</i> | 6 |
| 5. | <i>Data collection</i> | 7 |
| III. | Results | 8 |
| 6. | <i>Determination of outliers</i> | 8 |
| 7. | <i>Summary of the results</i> | 19 |
| 8. | <i>Laboratory performance</i> | 24 |
| a) | <i>Deviations from the mean (classical approach)</i> | 24 |
| b) | <i>Deviations from the median (robust approach)</i> | 36 |
| c) | <i>Laboratory performance according to ISO/DIS 13528</i> | 48 |
| d) | <i>Proximity to the mean</i> | 51 |
| e) | <i>Proximity to the median</i> | 52 |
| 9. | <i>Effect of filtration</i> | 52 |
| a) | <i>Effect of filtration with respect to the matrices</i> | 52 |
| b) | <i>Effect of the filter material</i> | 56 |
| 10. | <i>Repeatability and reproducibility</i> | 57 |
| IV. | Conclusions | 59 |
| V. | References | 61 |

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 Toxicologie-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 χ^2 -test, proposing the H0 hypothesis that the homogeneity of mixing is achieved, when samples are only affected by random error. χ^2 -test was performed by Equation 1,

$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i} \quad \text{Equation 1}$$

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 $\chi_{\text{exp}}^2 < \chi_{\text{crit}}^2$ at five degrees of freedom and $\alpha=5\%$.

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
 - o 400 mL of deionised water and river samples
 - o 200 mL of wastewater effluent
 - o 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
 - o Mobile phase A: methanol with 5 mM NH₄ acetate
 - o Mobile phase B: water with 5 mM NH₄ 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
 - o 400 mL of deionised water and river samples
 - o 200 mL of wastewater effluent
 - o 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
 - o IB:263
 - o NP:287
 - o KT:311
 - o DF:352 and 354
- GC column: HP-5MS, 30m, 0.25mm, 0,25µm
- GC oven: 65° (2min), rate 30°/min to 180°, rate 5°/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 than the critical value at 5 % significance level for a given number of observations, the H_0 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.

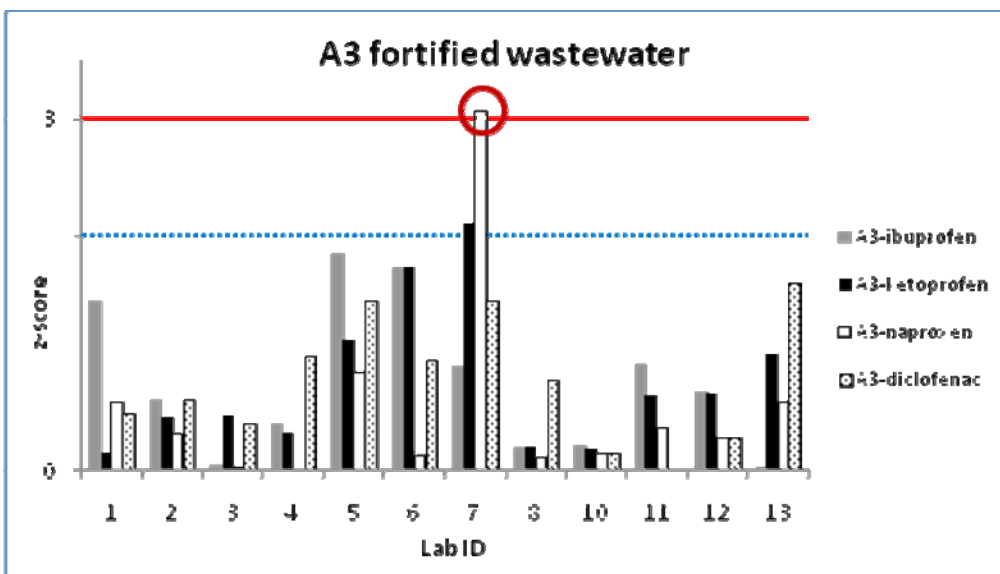
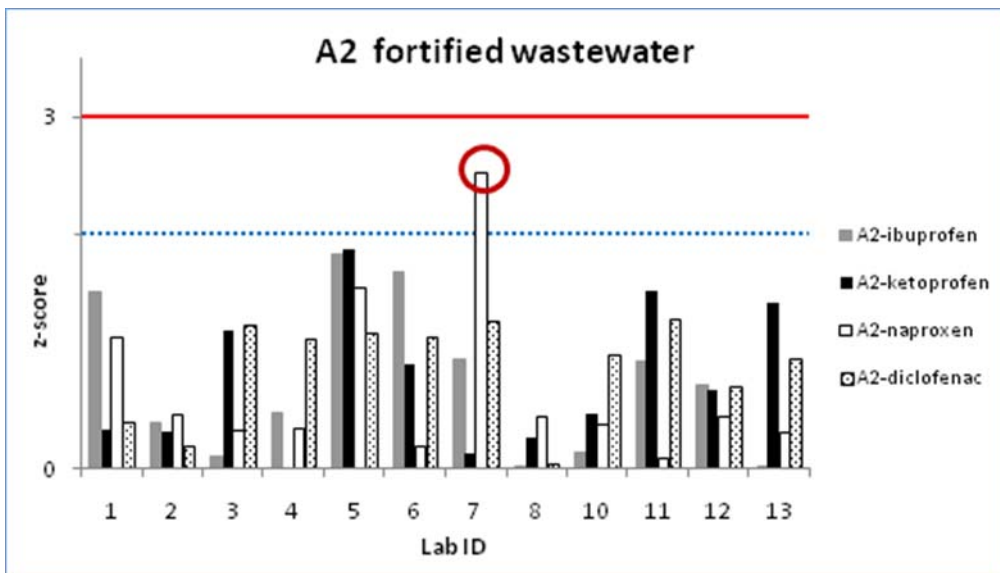
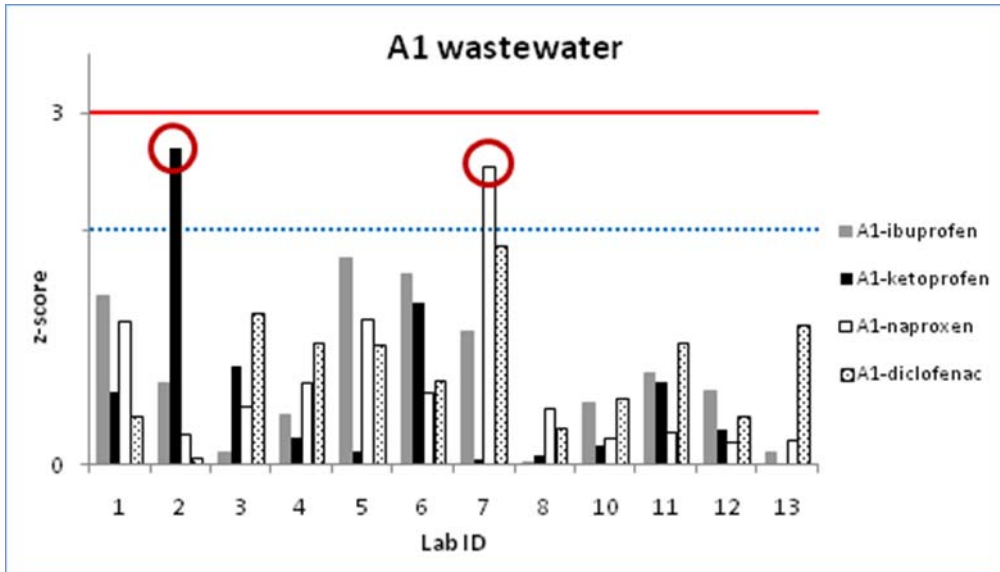


Figure 1 (1/3)

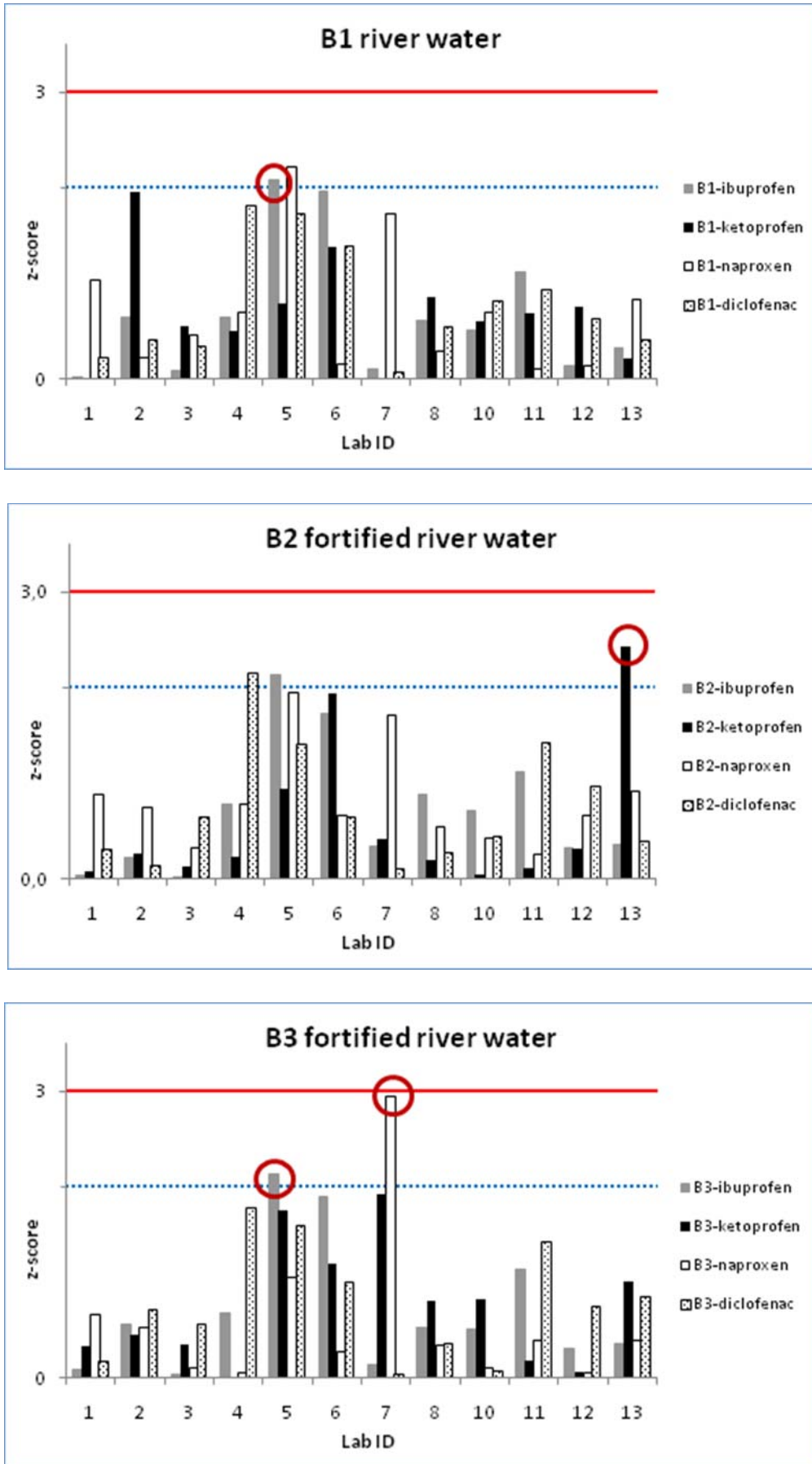


Figure 1 (2/3)

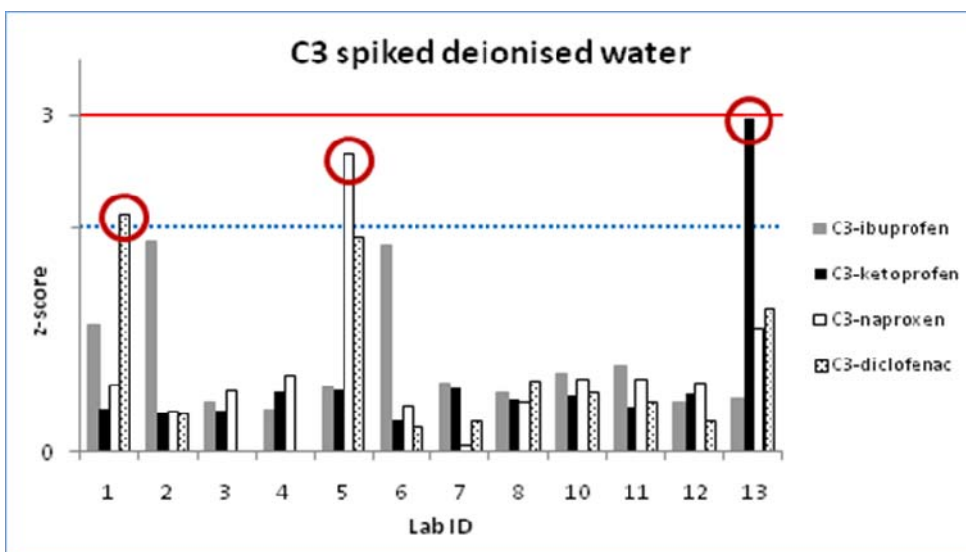
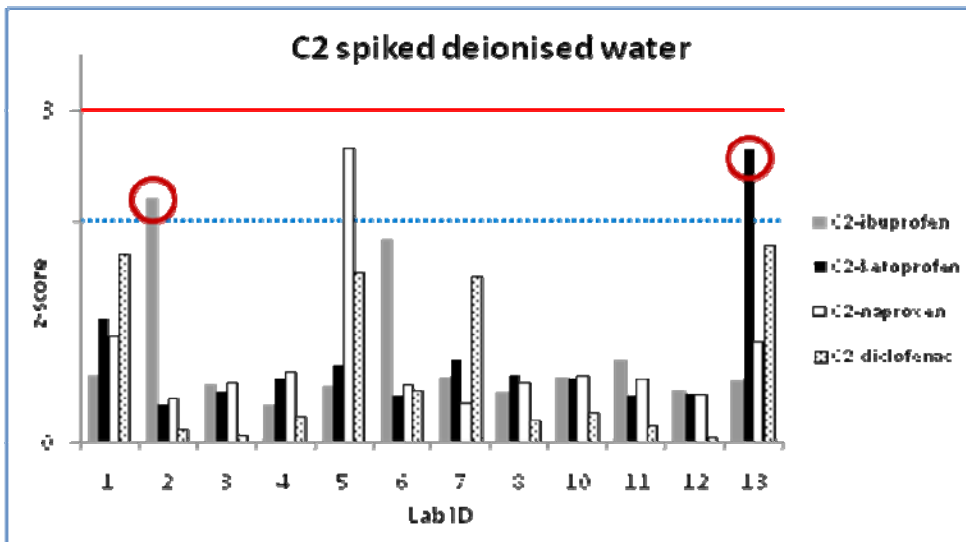
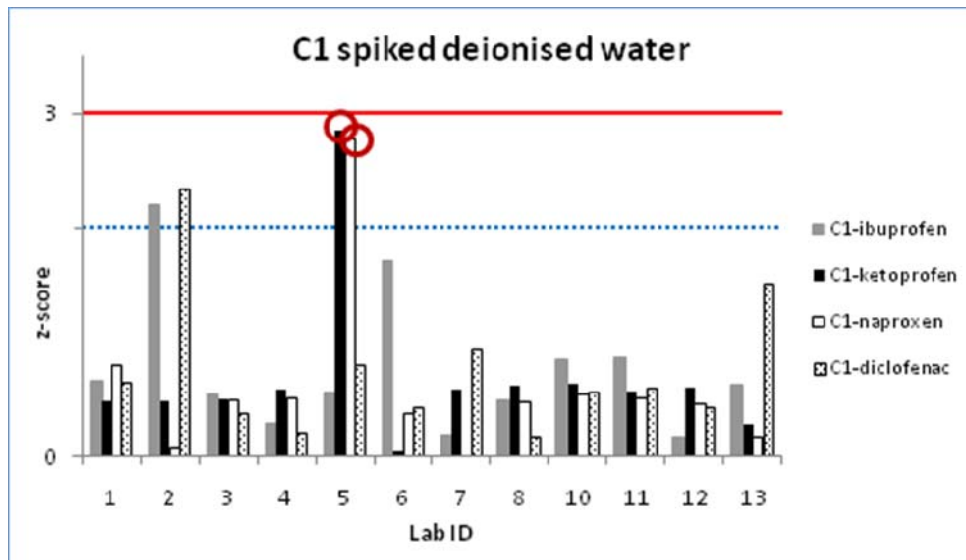


Figure 1 (3/3)

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 from 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 | |

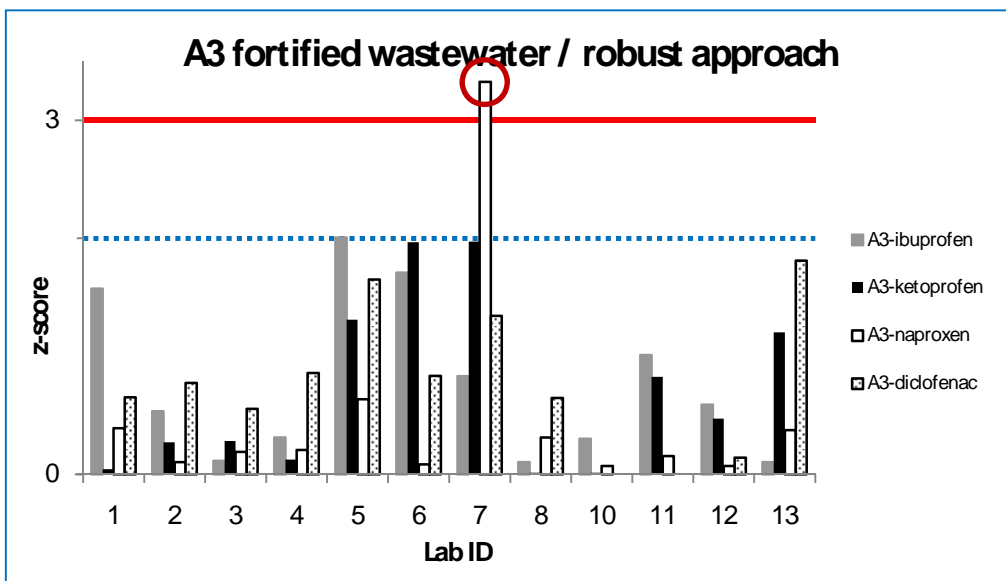
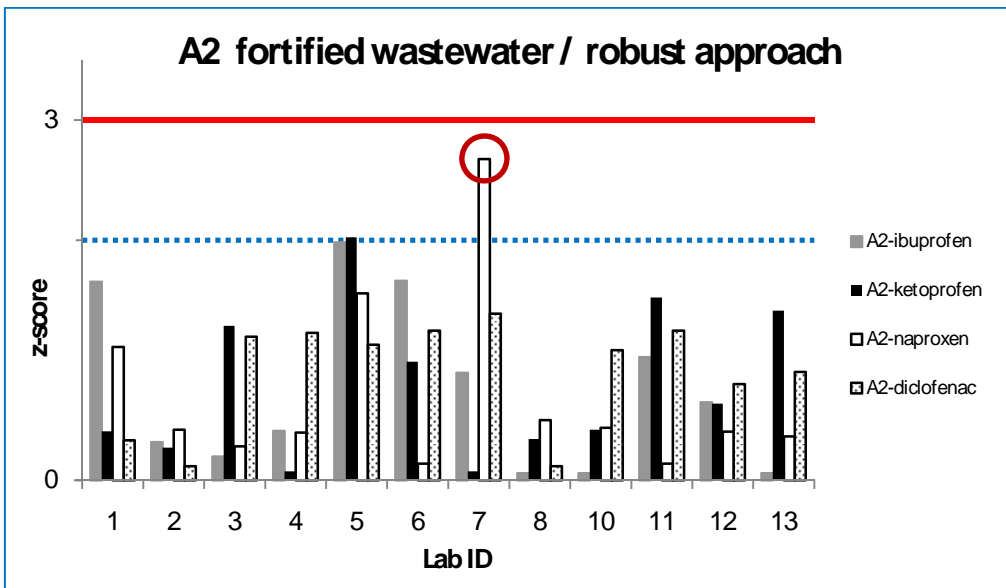
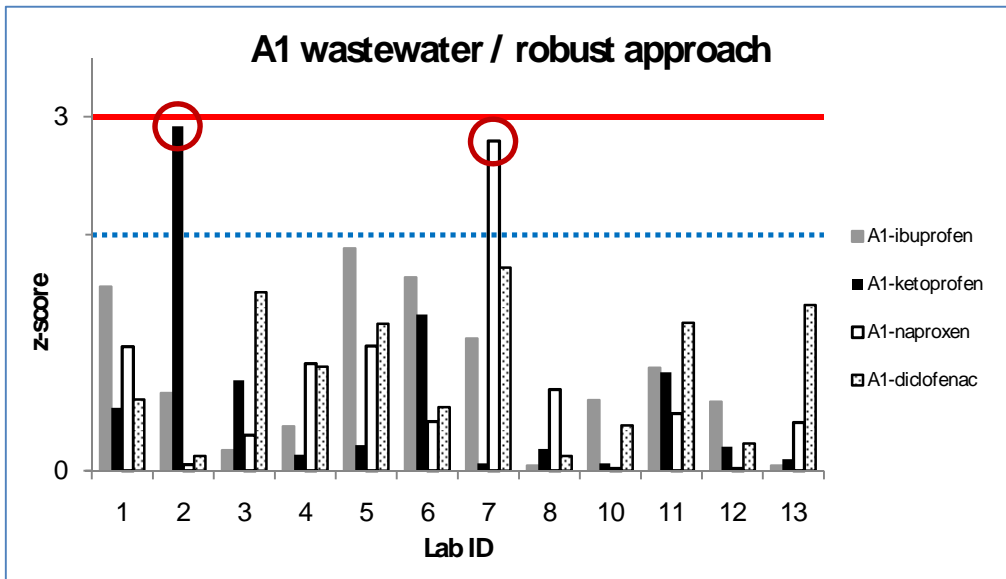


Figure 2 (1/3)

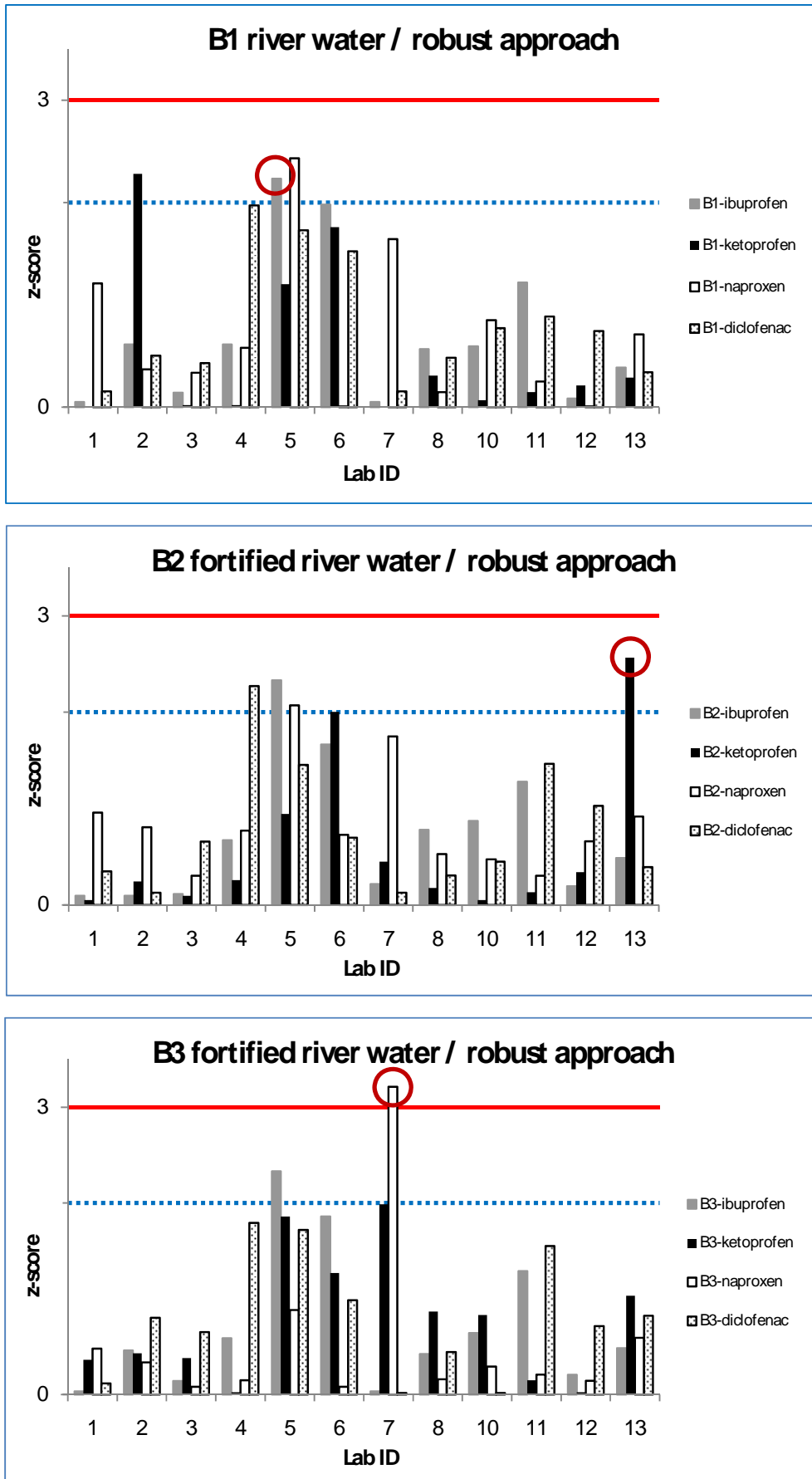


Figure 2 (2/3)

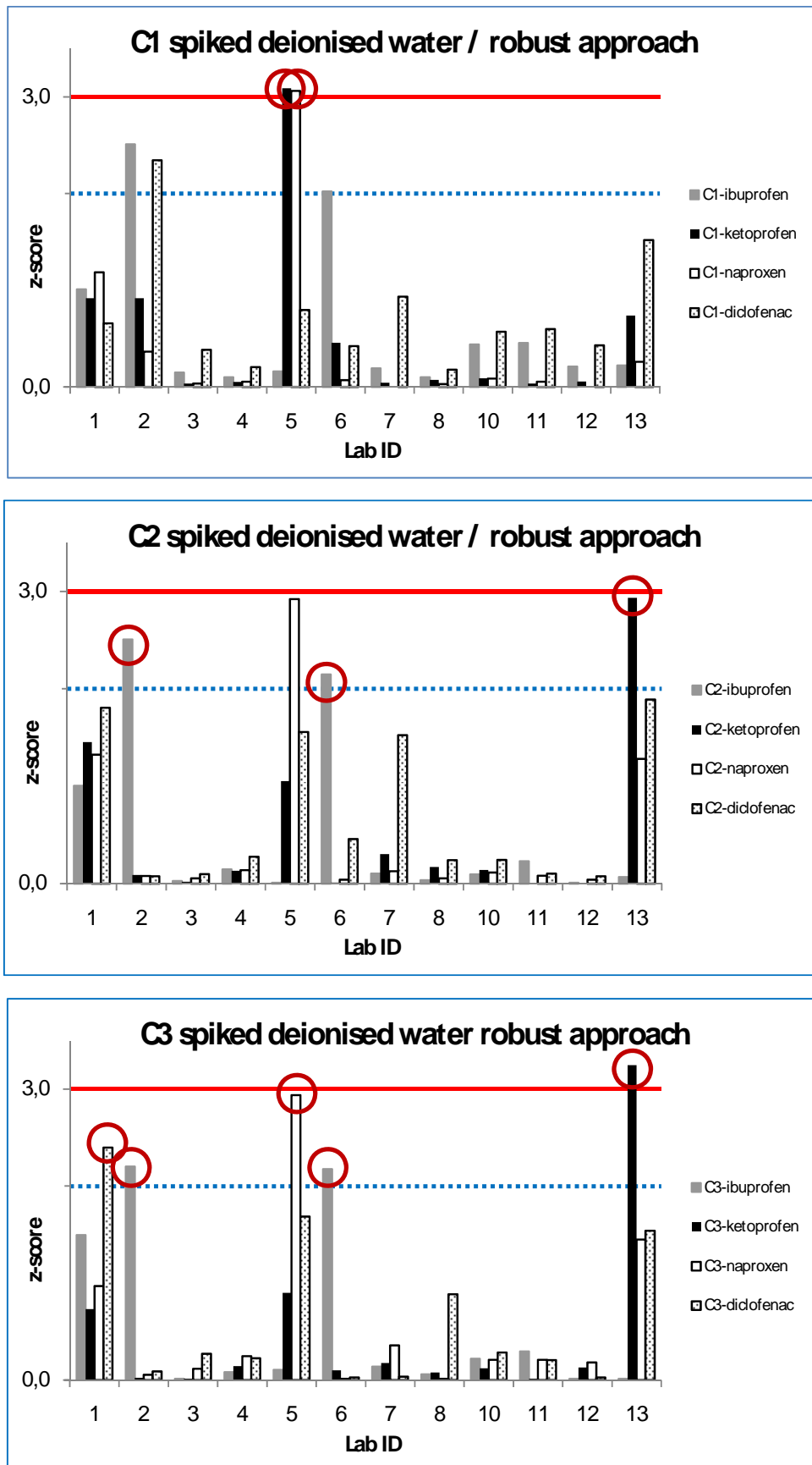


Figure 2 (3/3)

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.

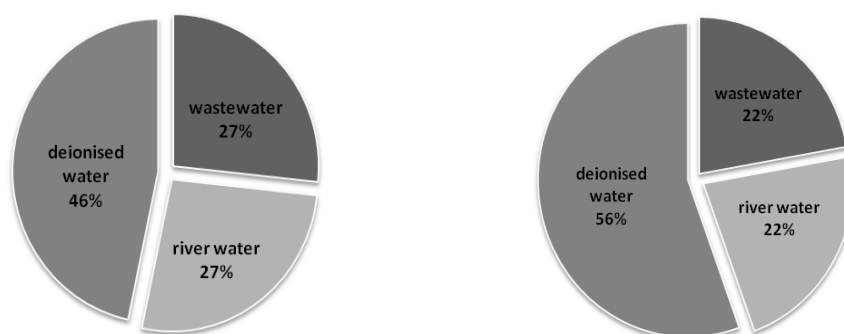


Figure 3: Pie-chart showing the percentage of outliers in relation to the sample matrices: classical approach (left) and robust approach (right)

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).

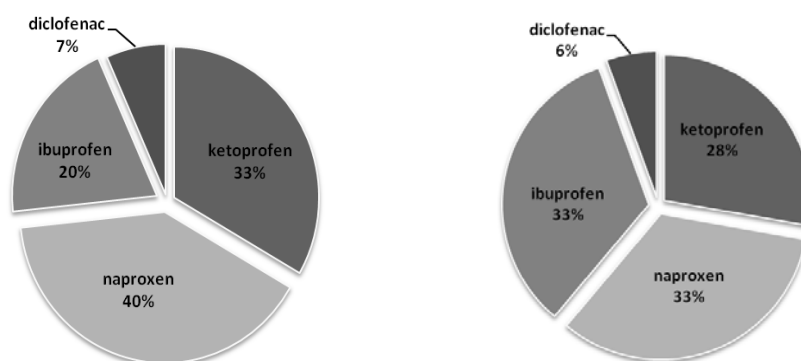


Figure 4: Pie-chart showing the percentage of outliers per analyte: classical approach (left) and robust approach (right)

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.

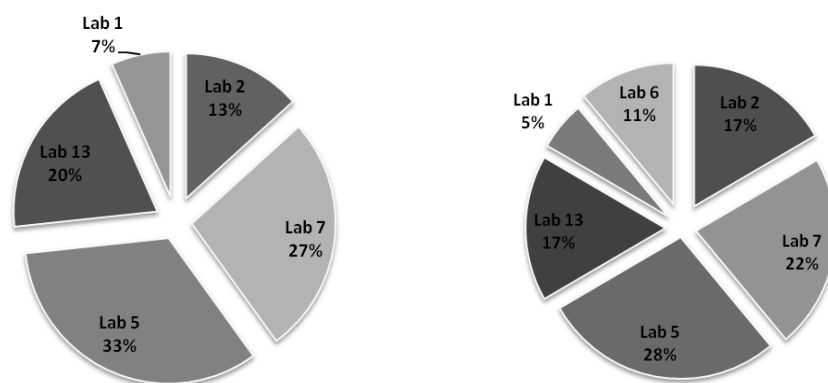


Figure 5: A pie-chart illustrating the percentage of outliers per participant: classical approach, left and robust approach, right

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.

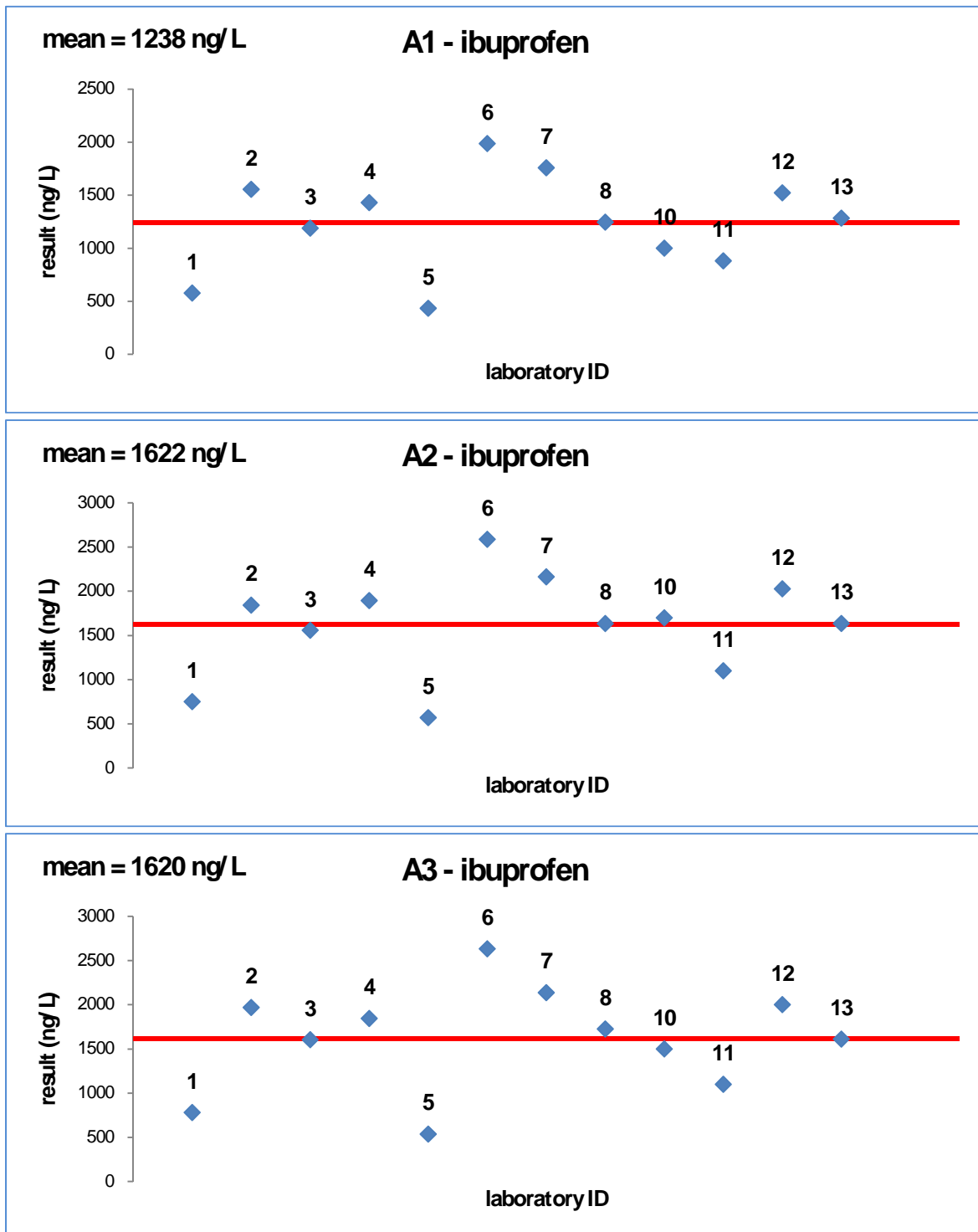


Figure 6 (1/12)

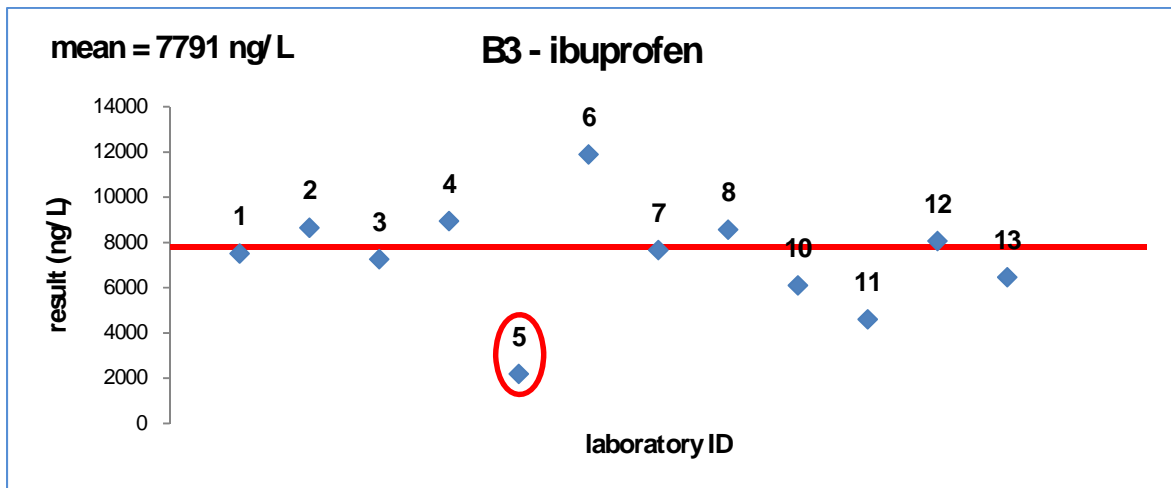
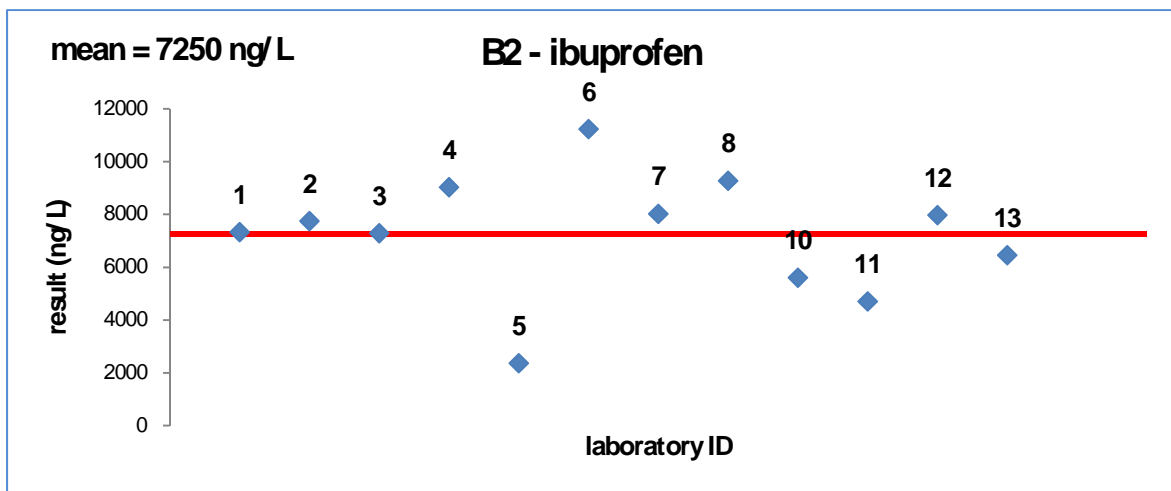
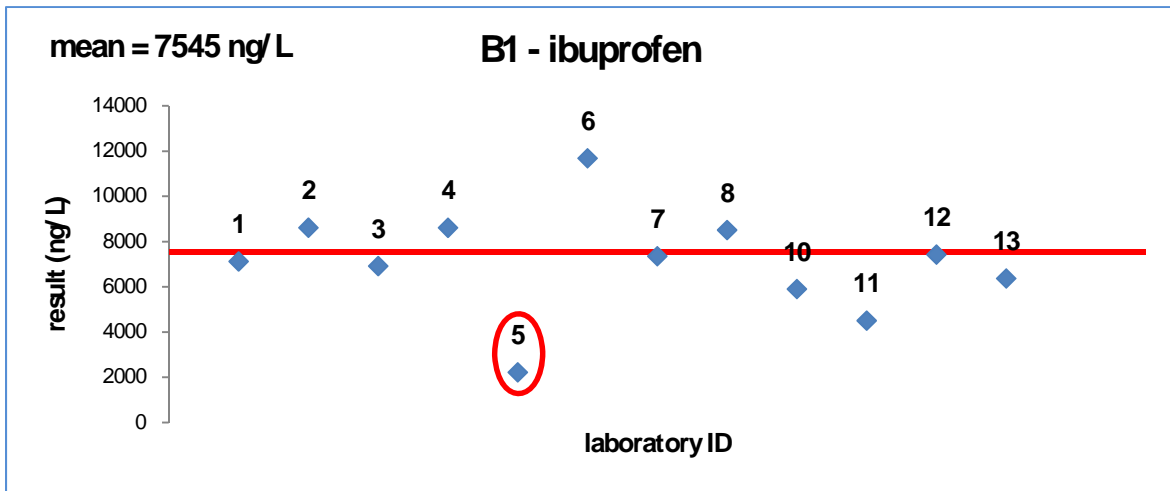


Figure 6 (2/12)

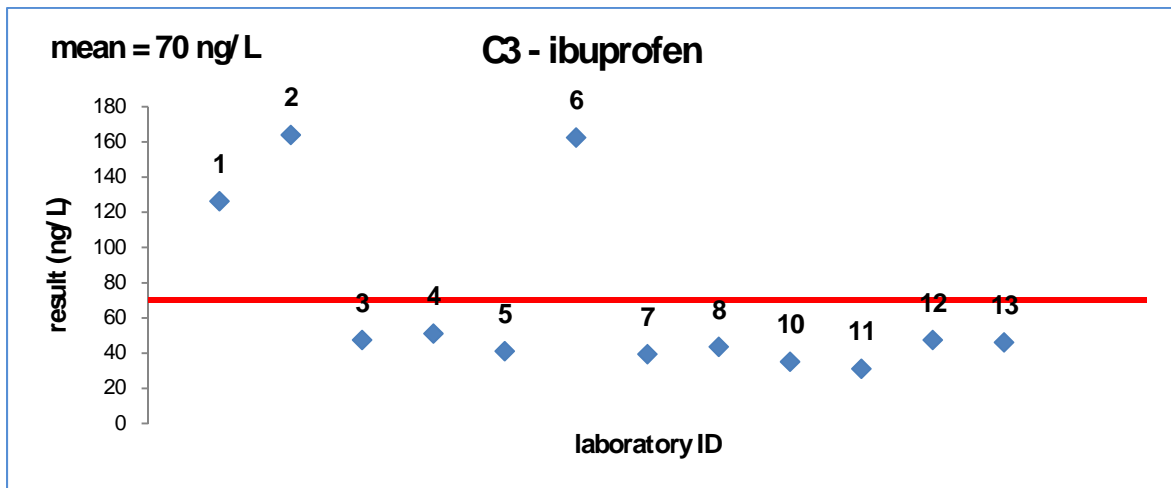
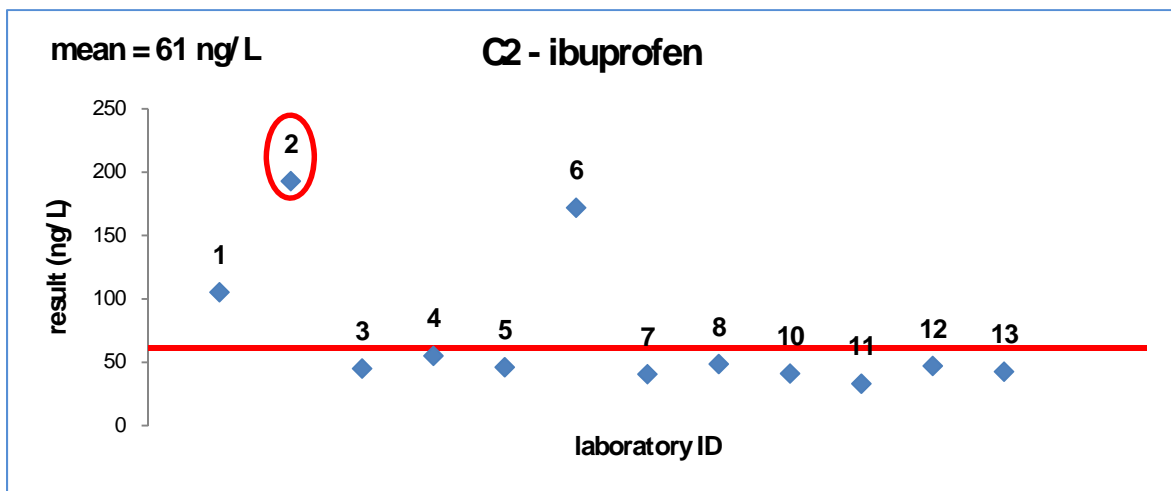
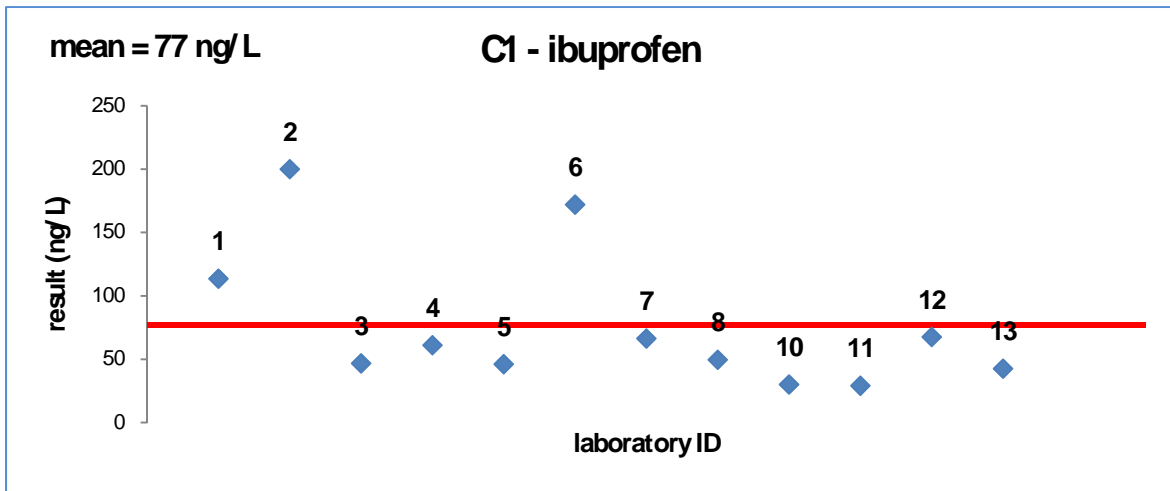


Figure 6 (3/12)

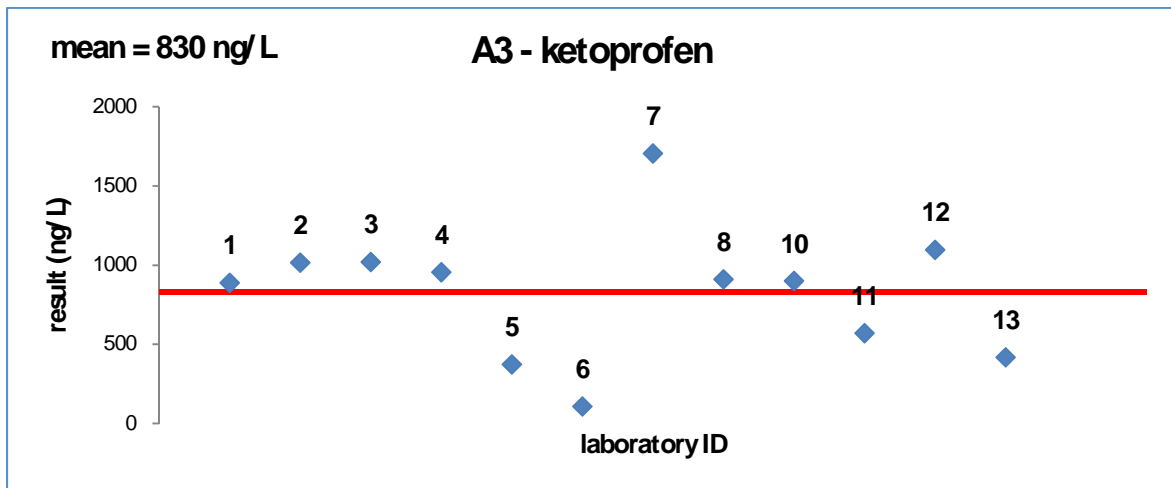
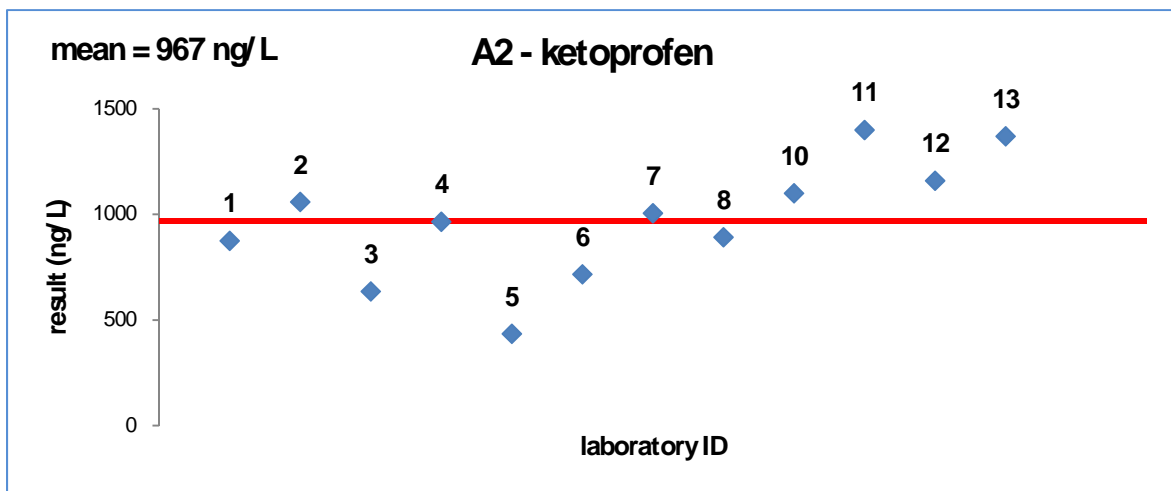
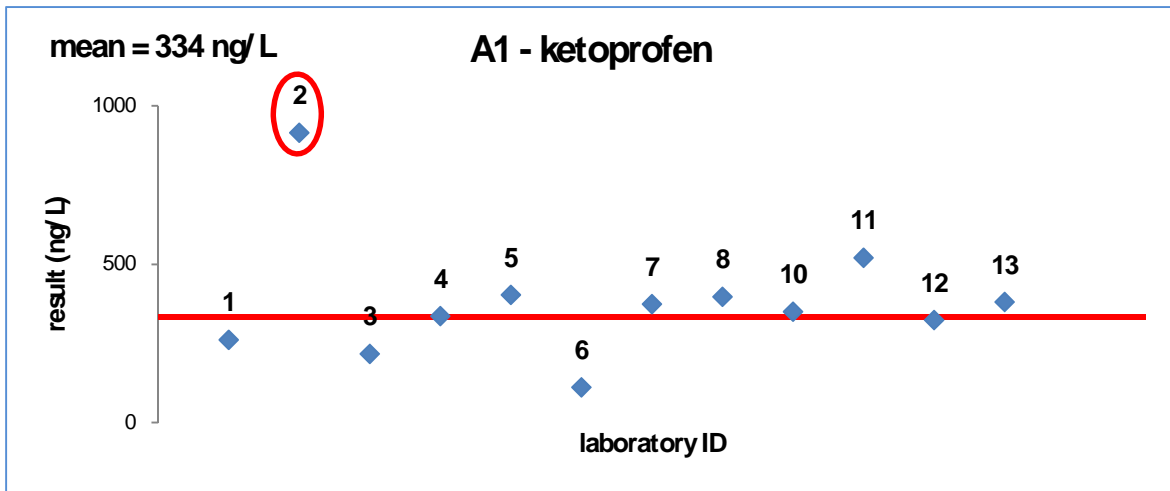


Figure 6 (4/12)

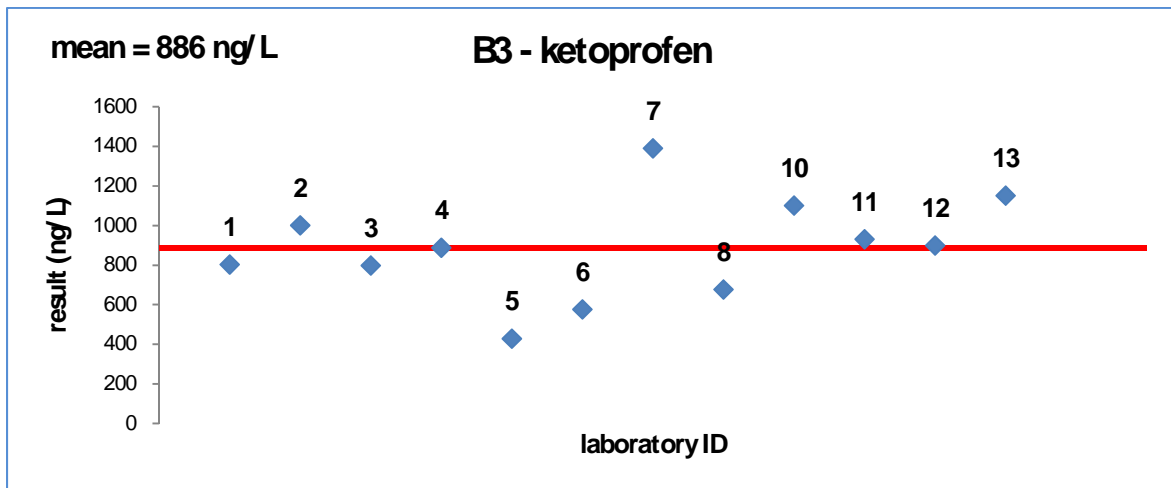
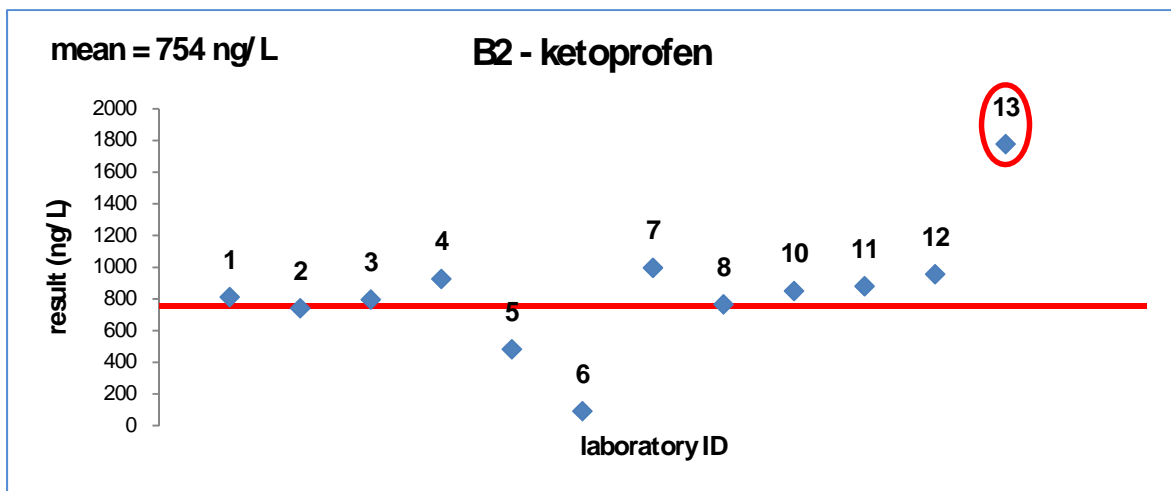
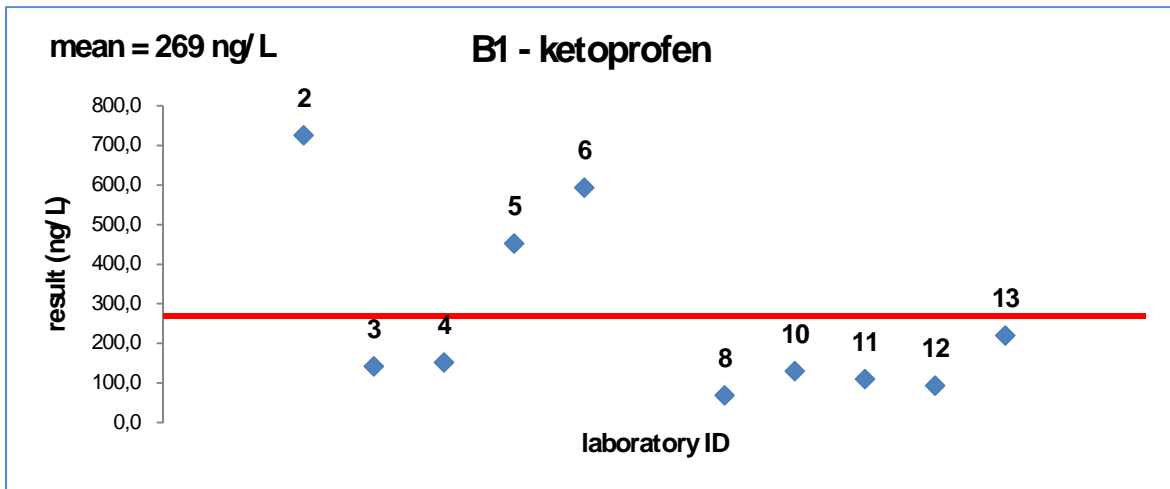


Figure 6 (5/12)

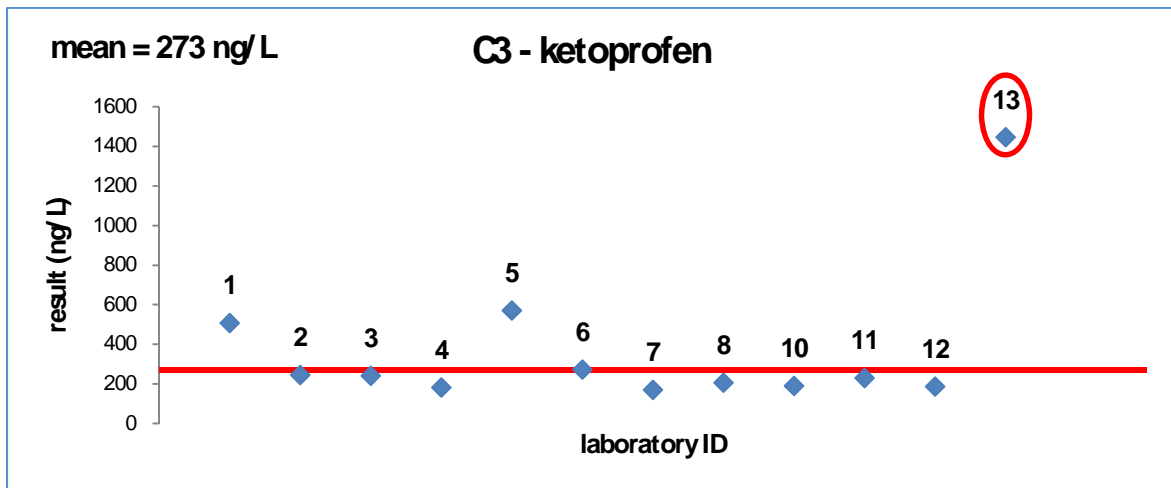
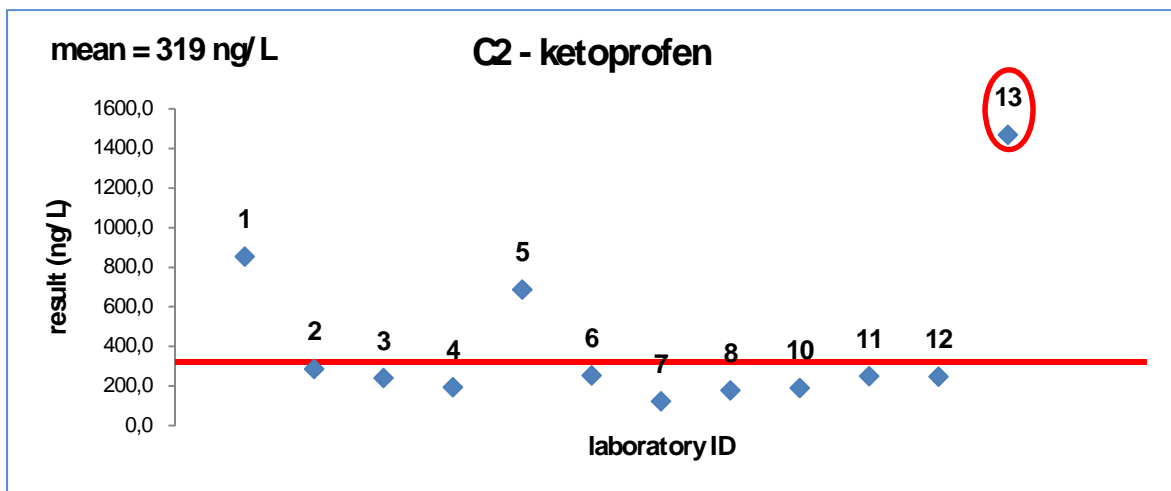
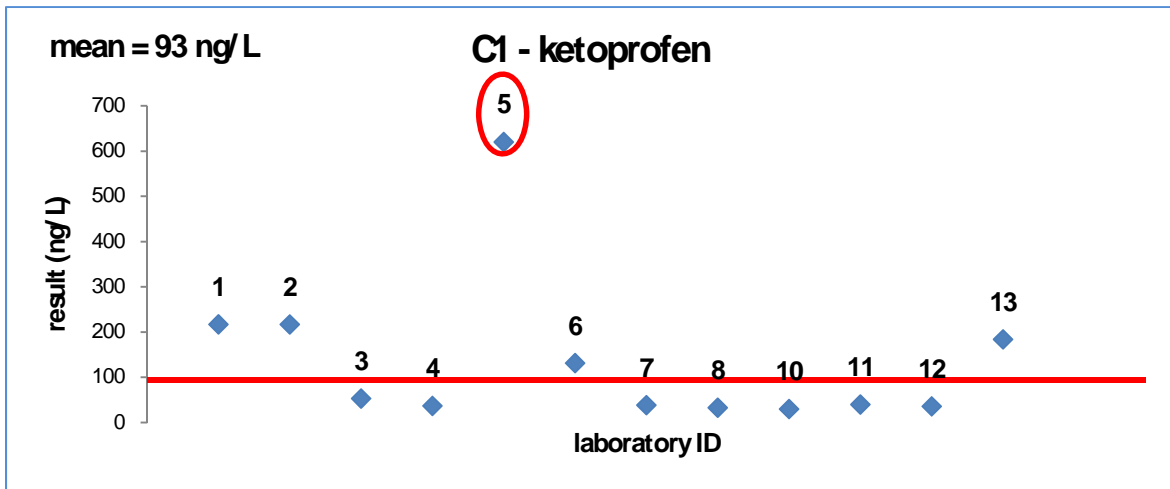


Figure 6 (6/12)

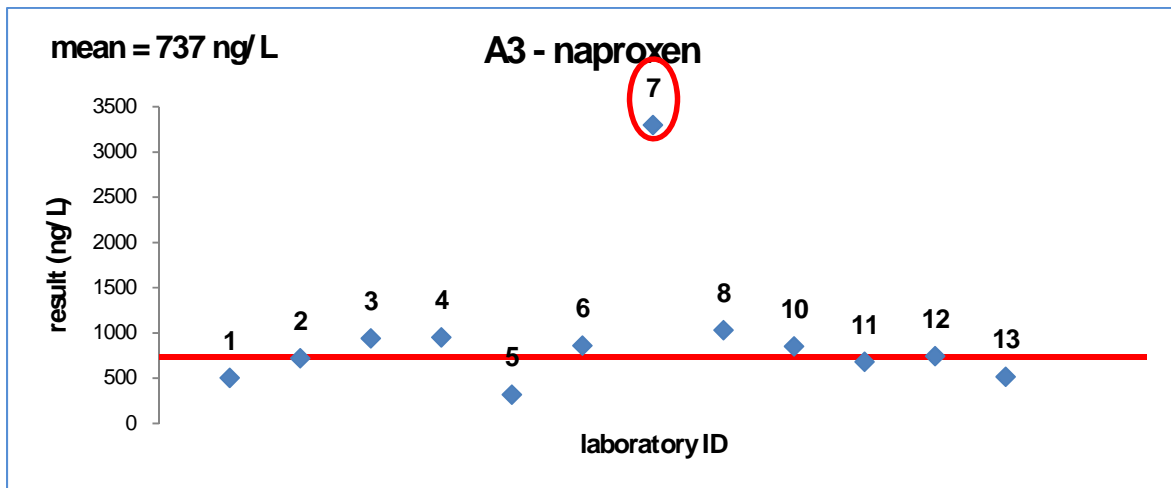
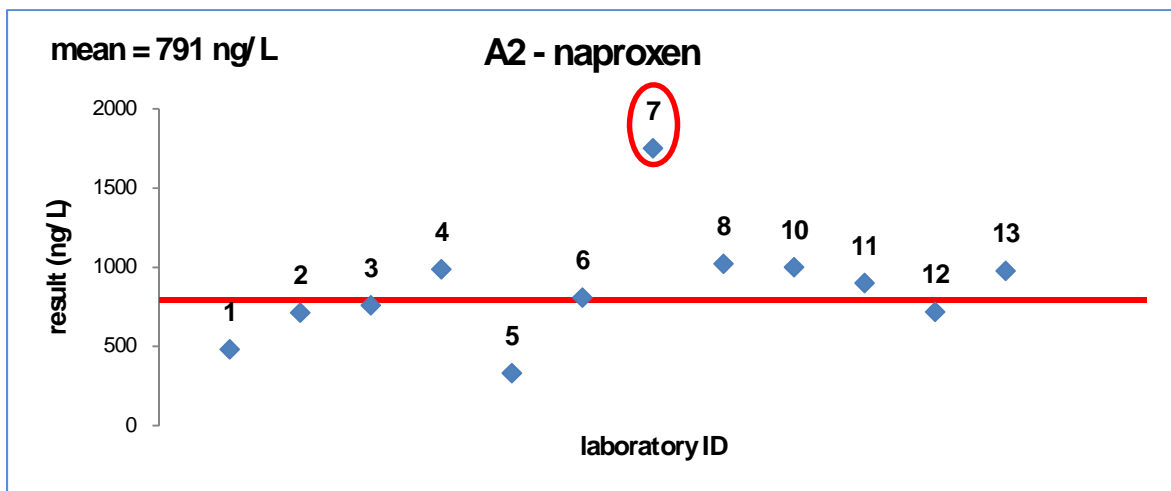
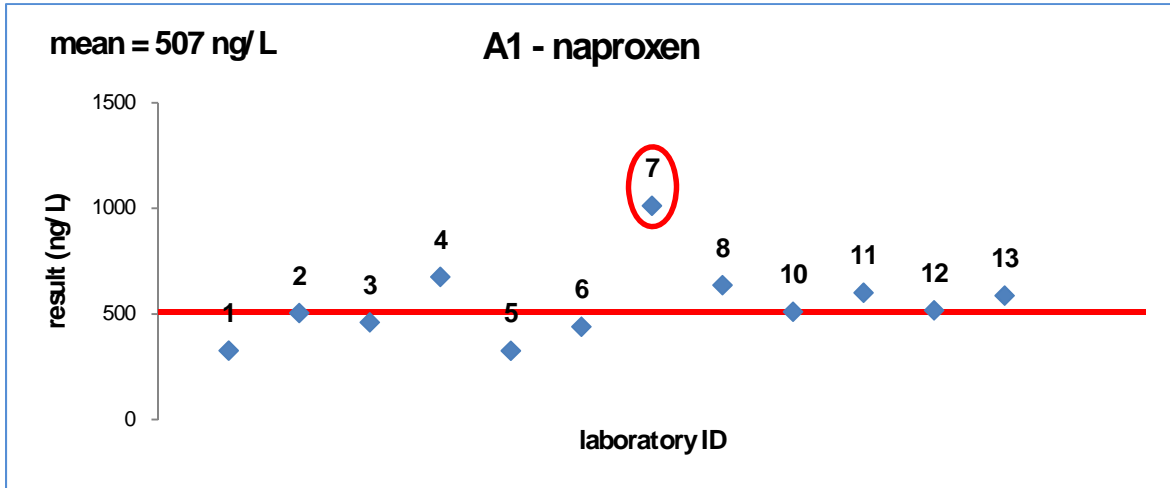


Figure 6 (7/12)

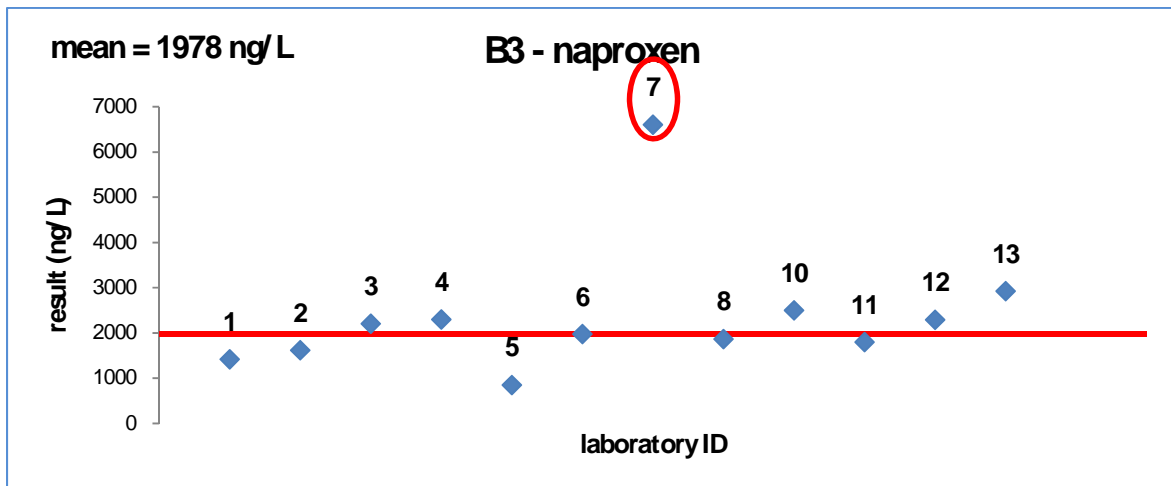
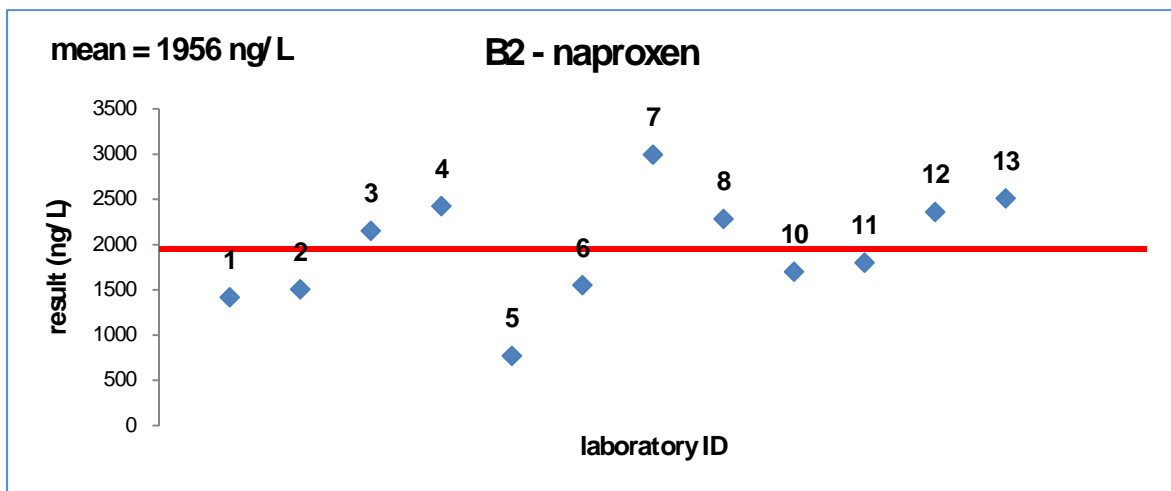
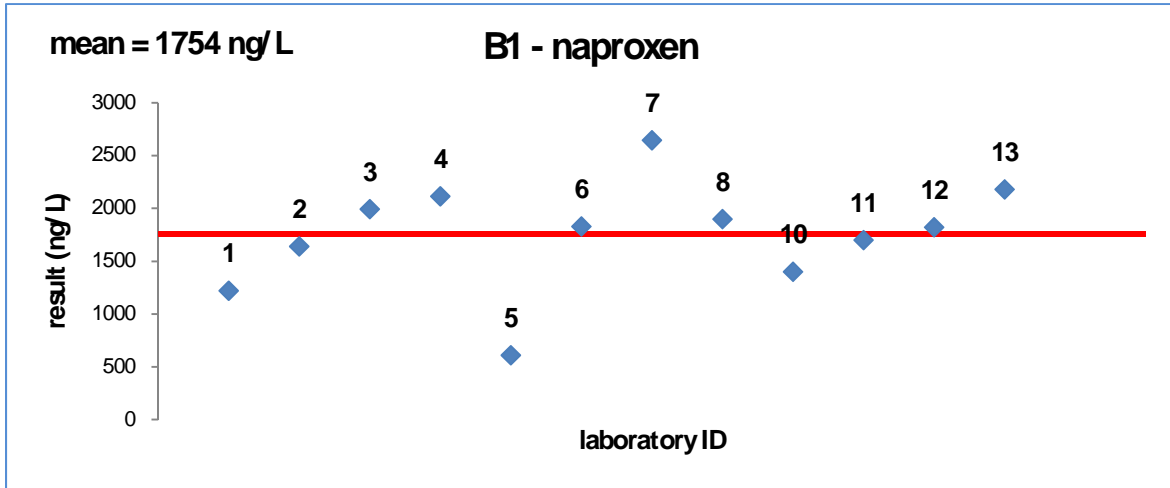


Figure 6 (8/12)

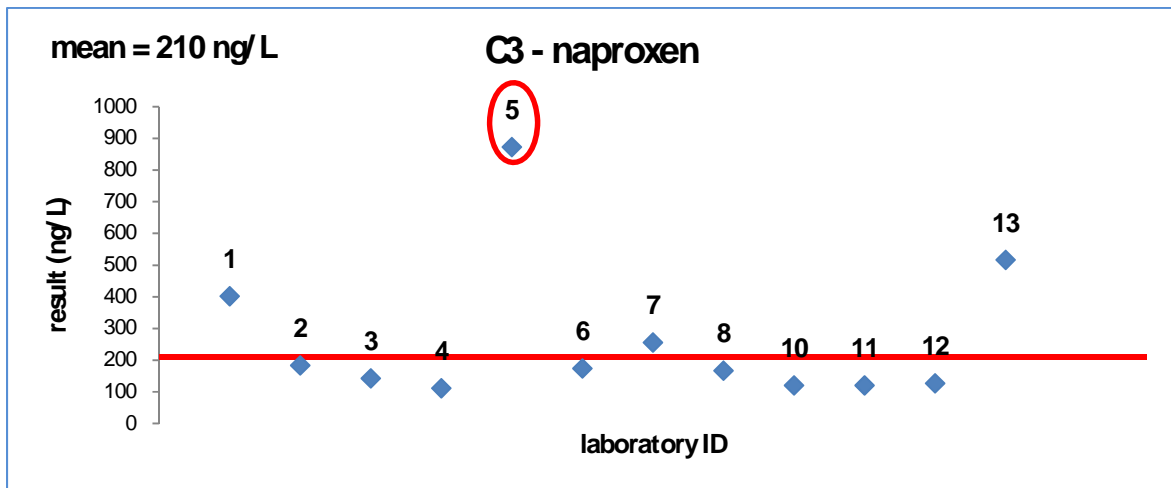
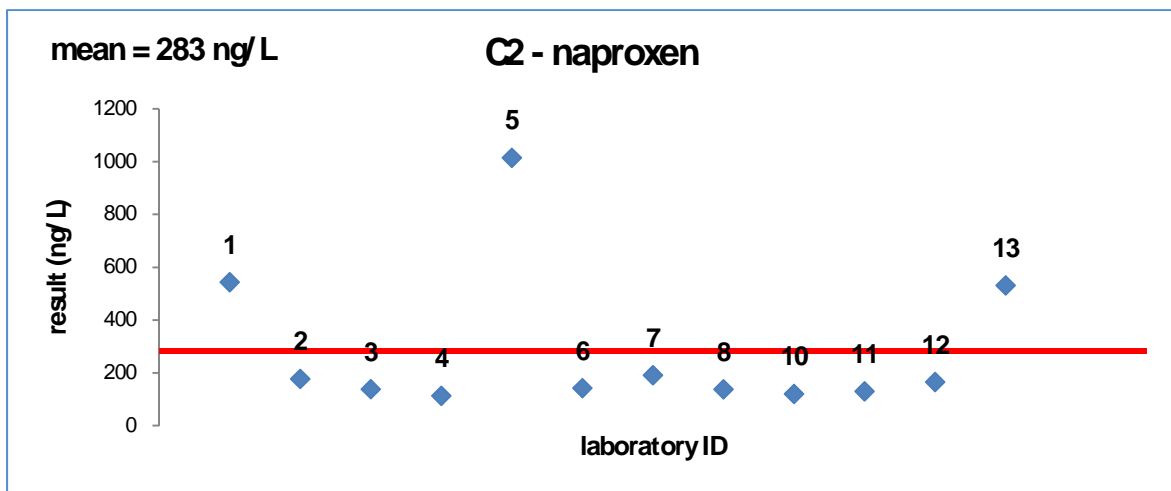
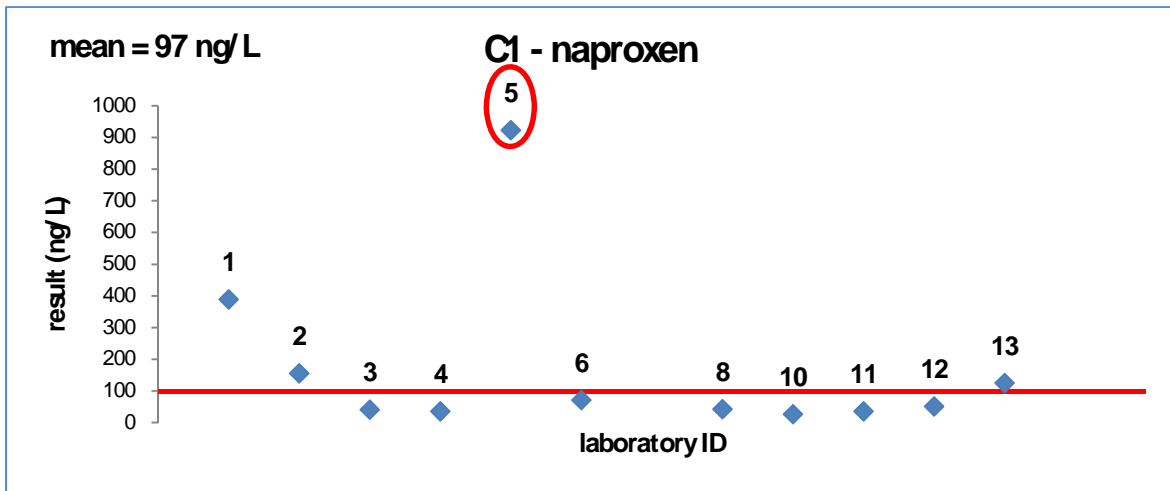


Figure 6 (9/12)

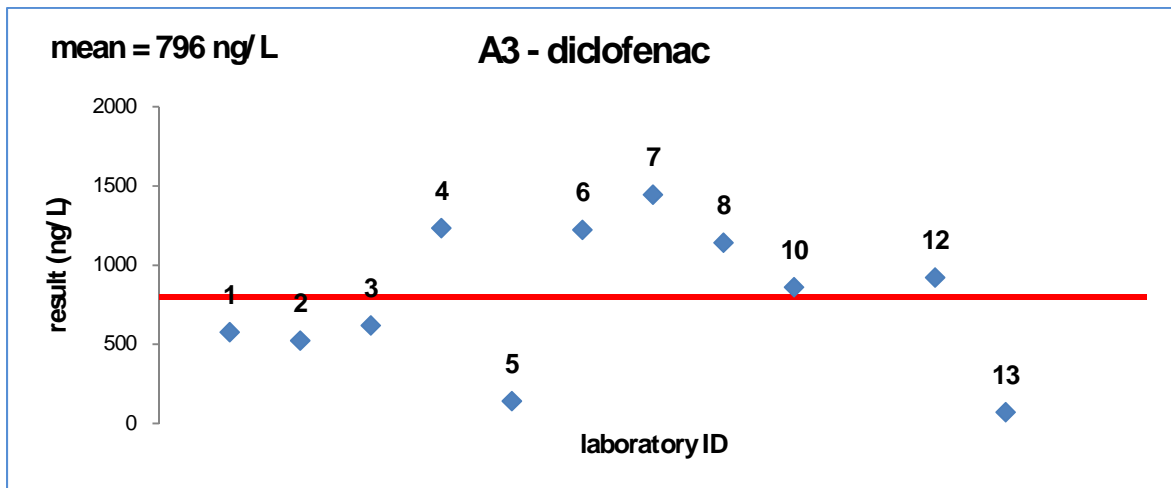
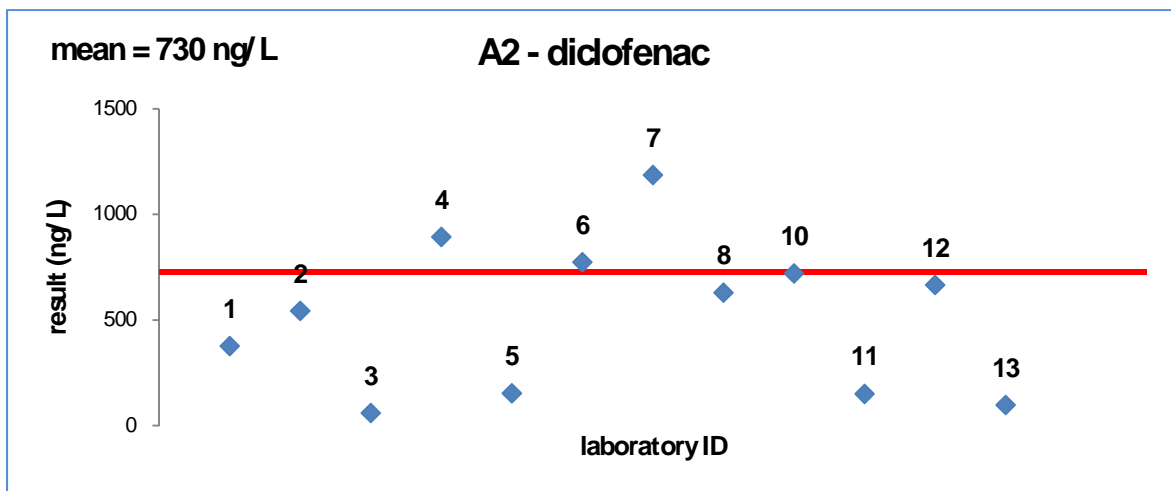
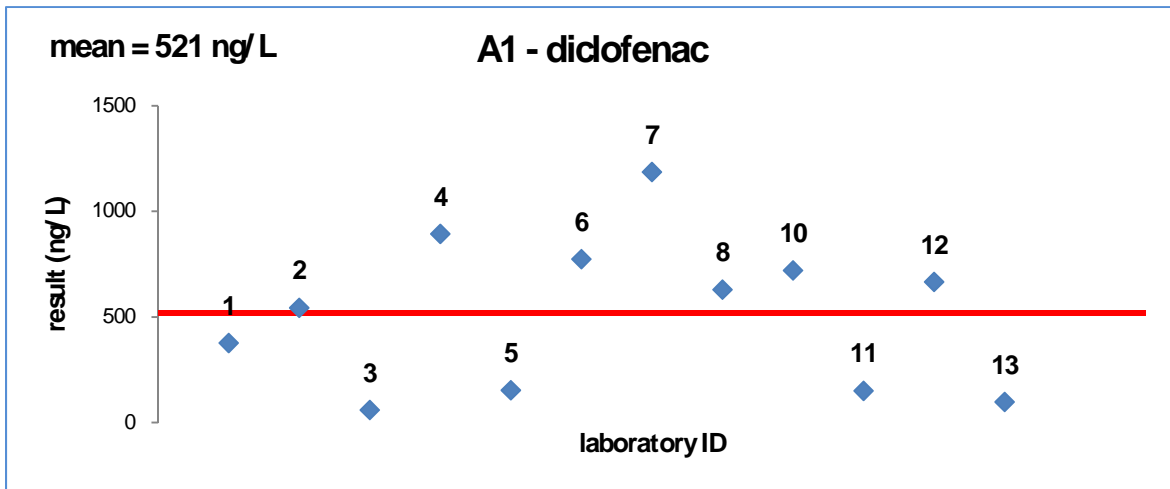


Figure 6 (10/12)

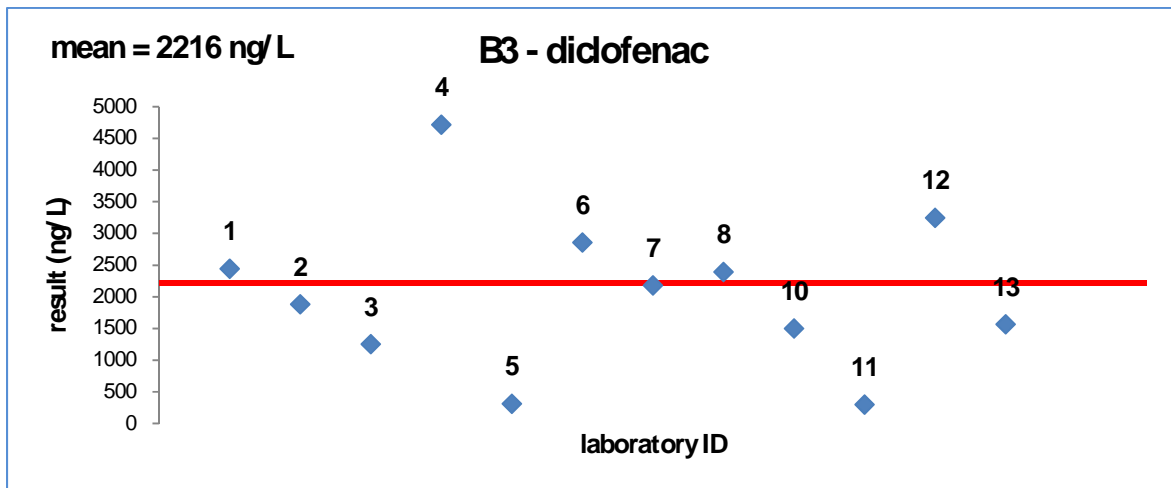
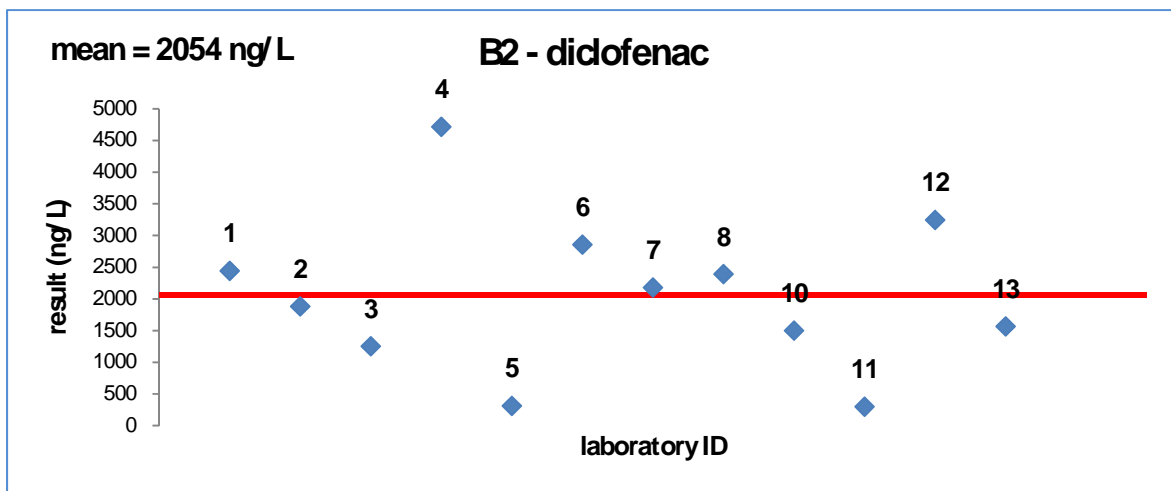
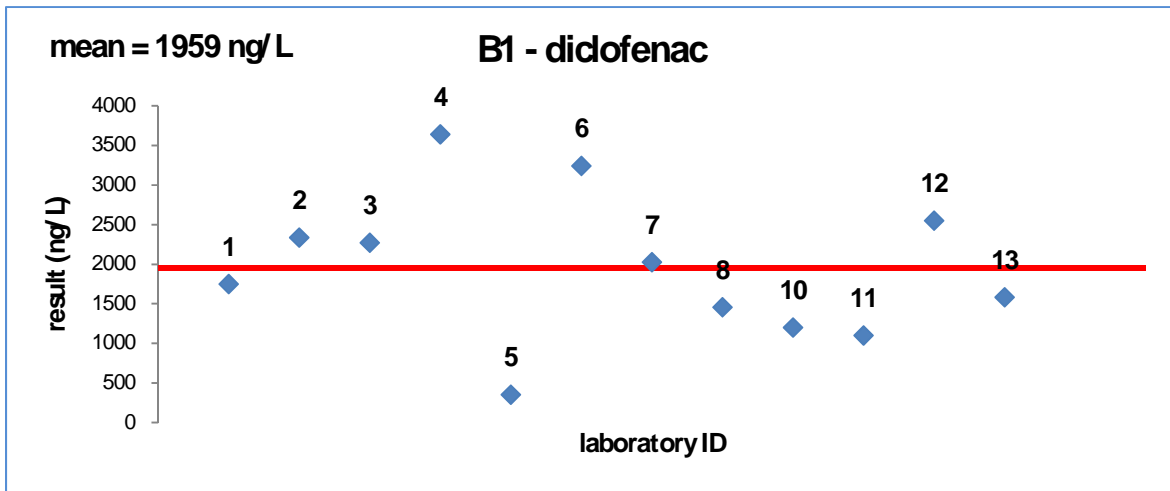


Figure 6 (11/12)

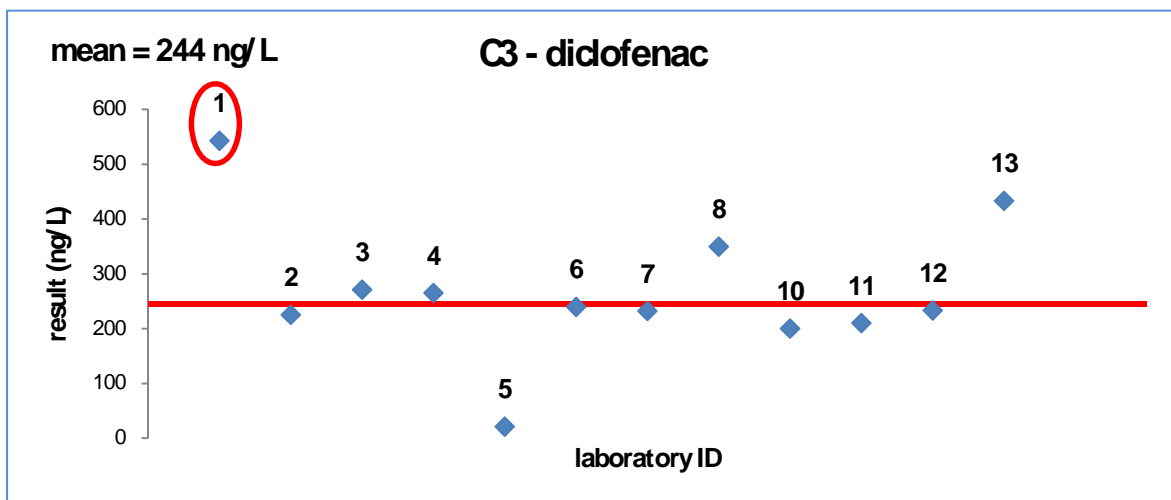
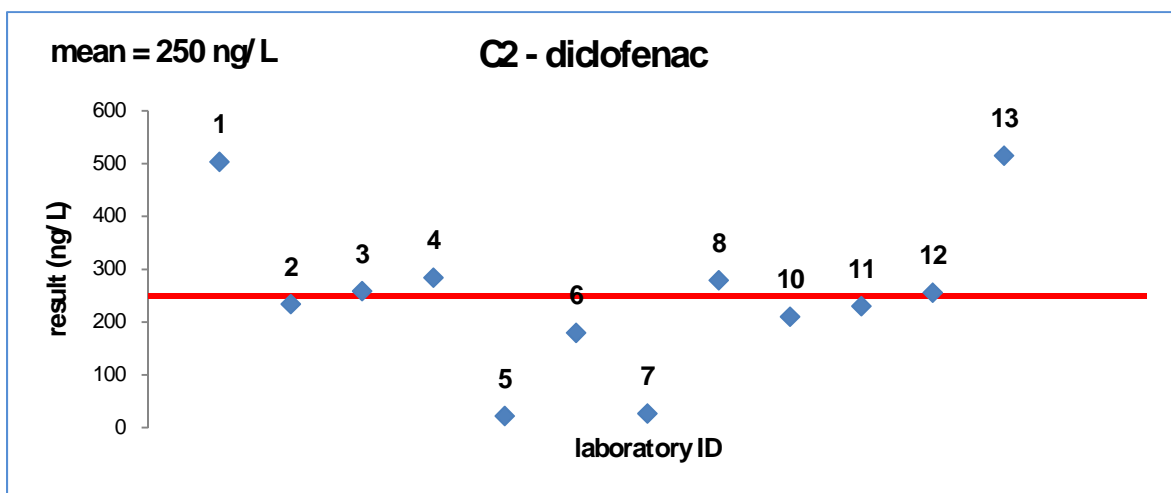
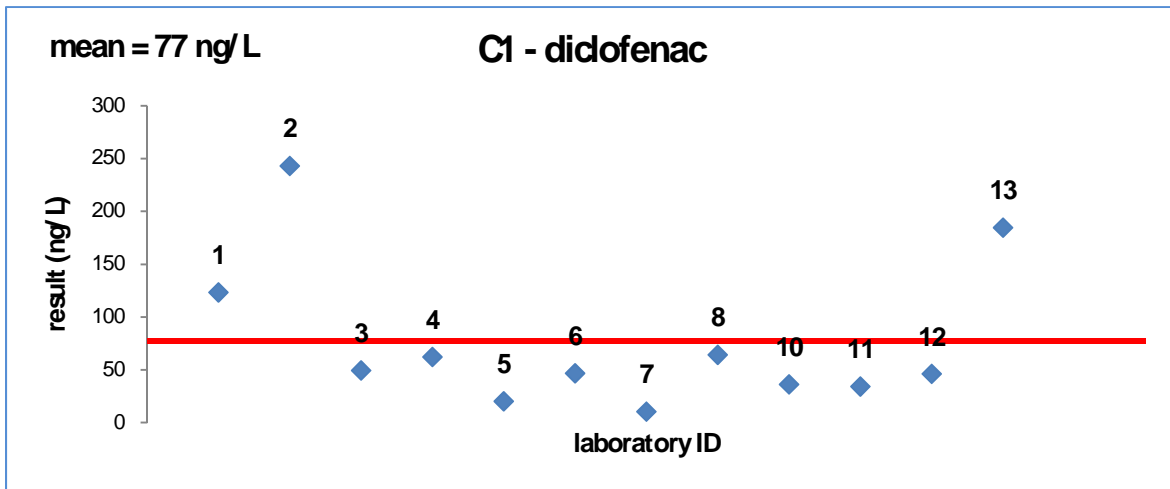


Figure 6 (12/12)

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.

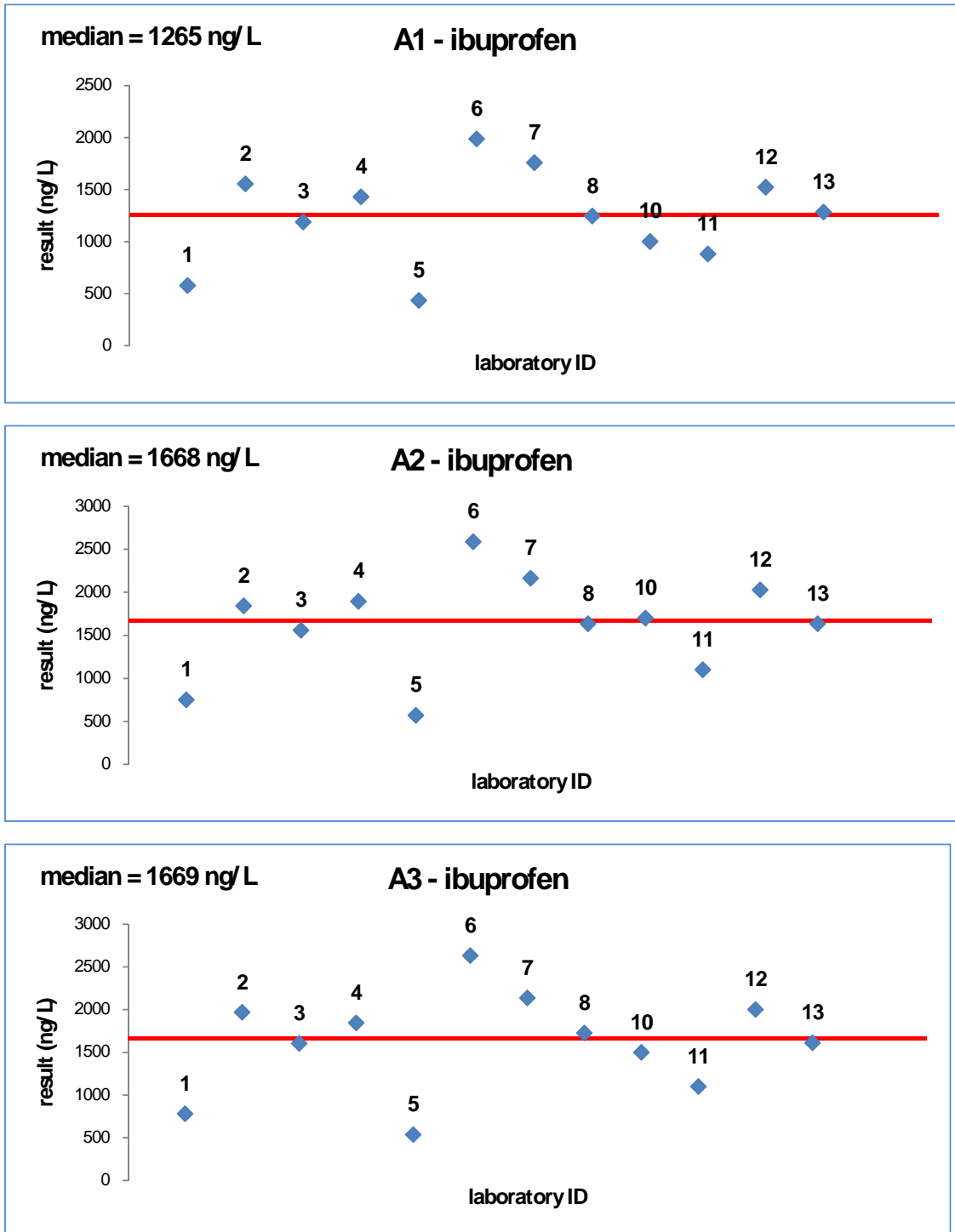


Figure 7 (1/12)

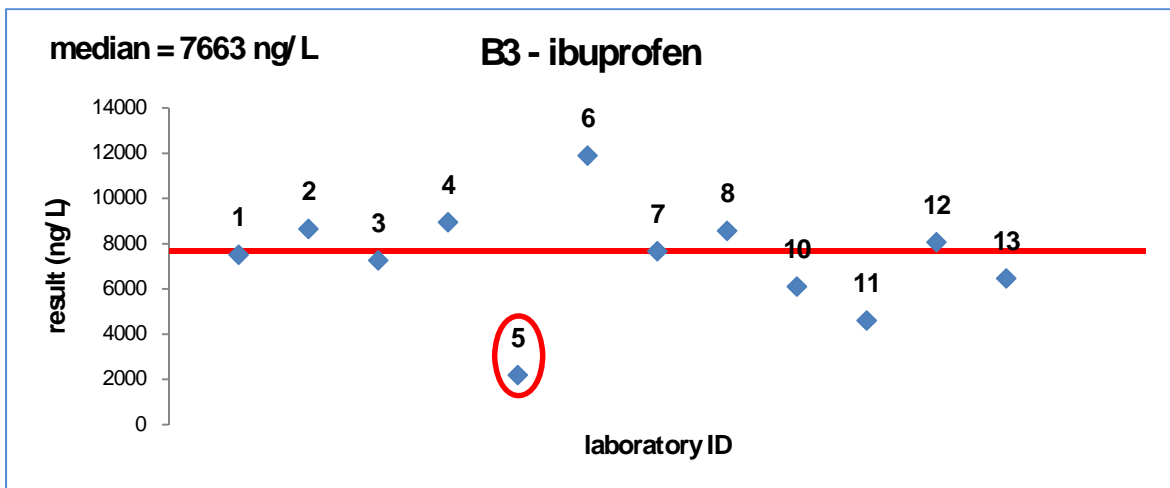
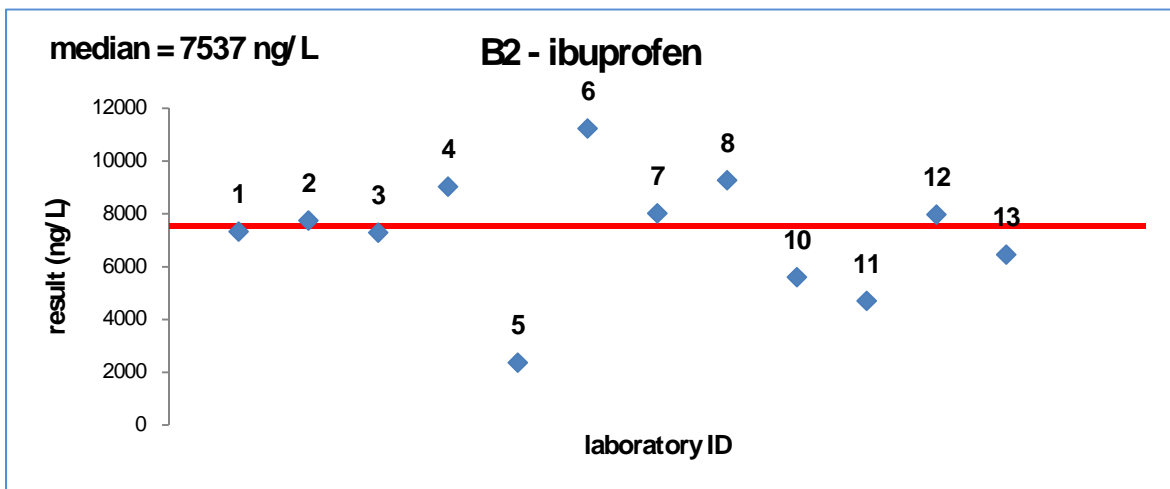
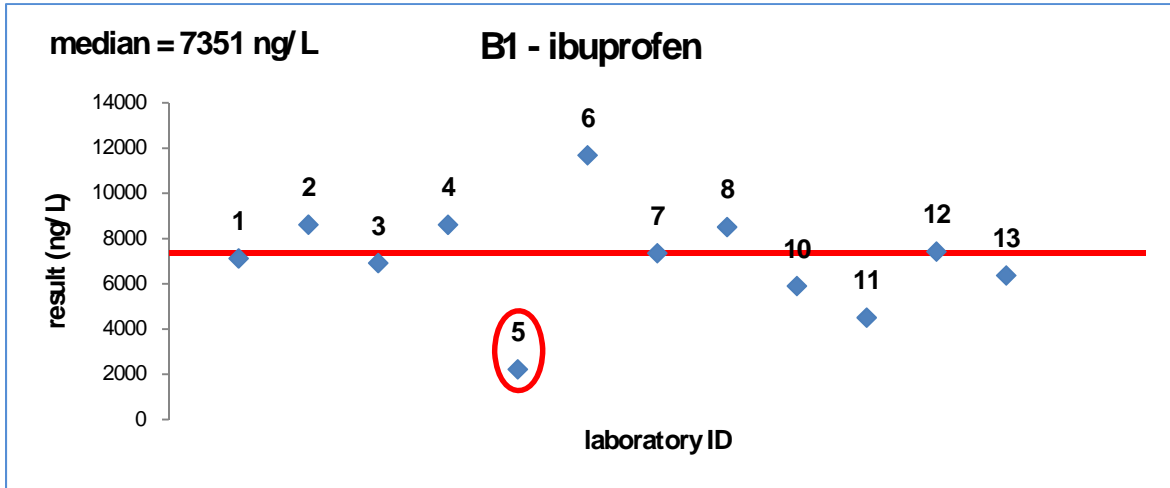


Figure 7 (2/12)

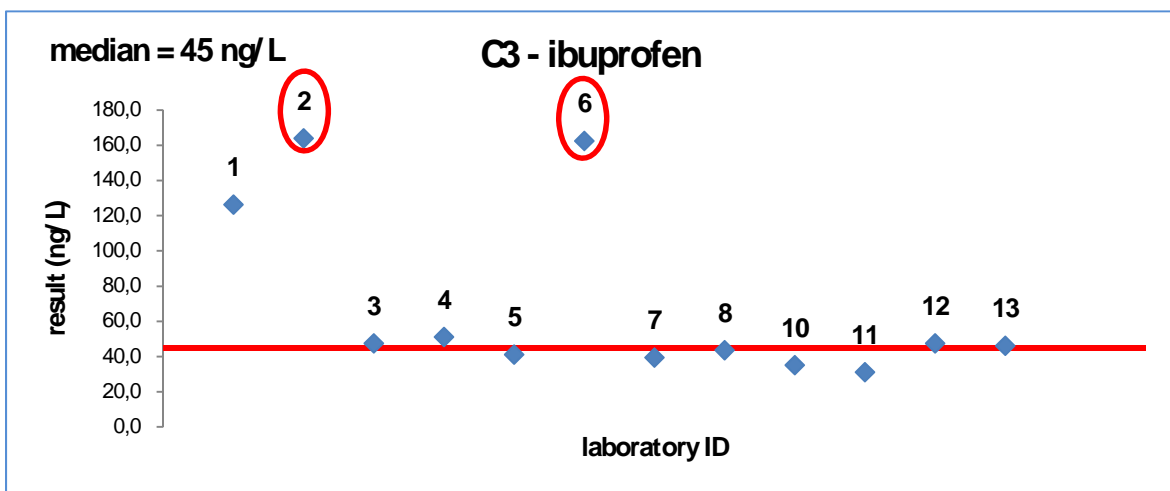
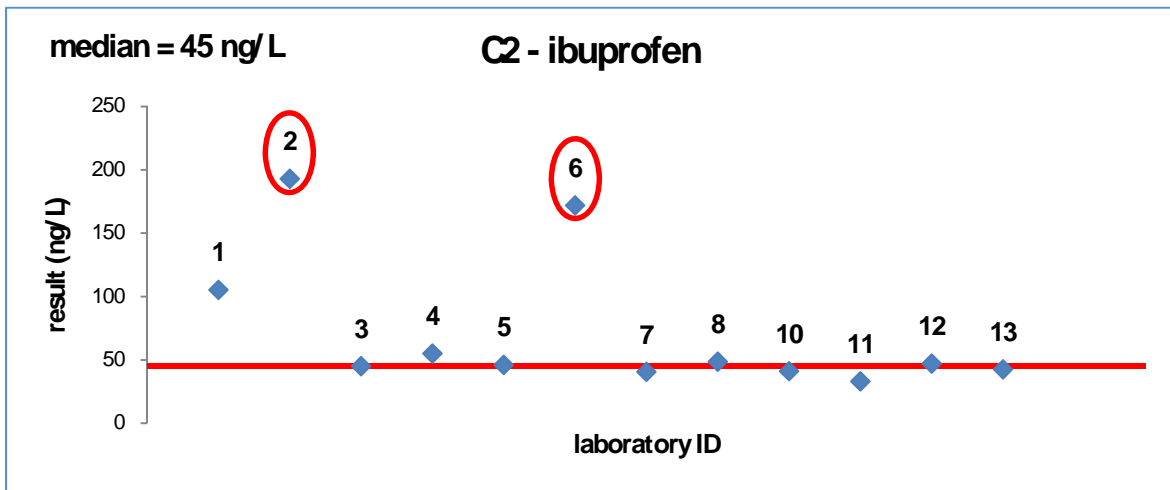
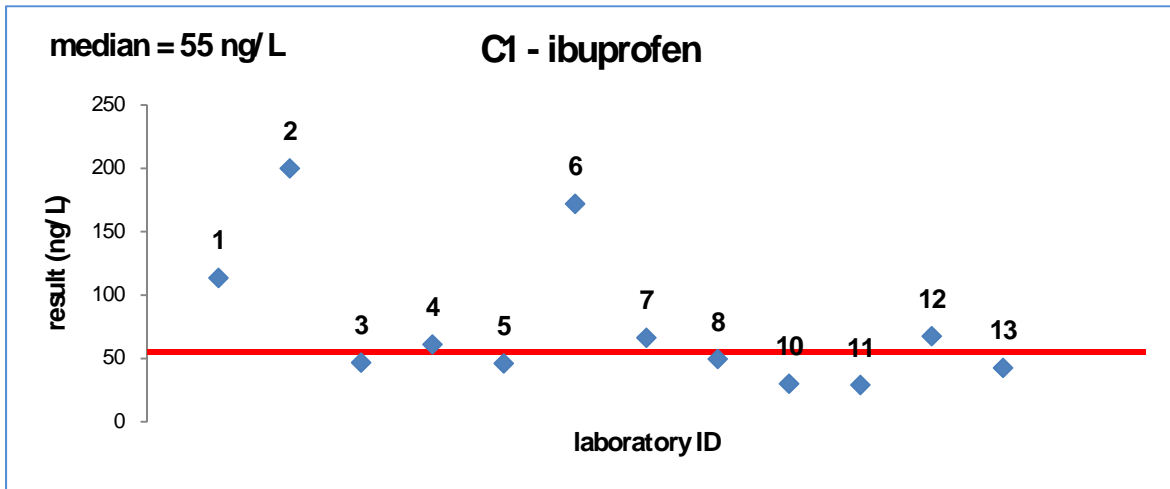


Figure 7 (3/12)

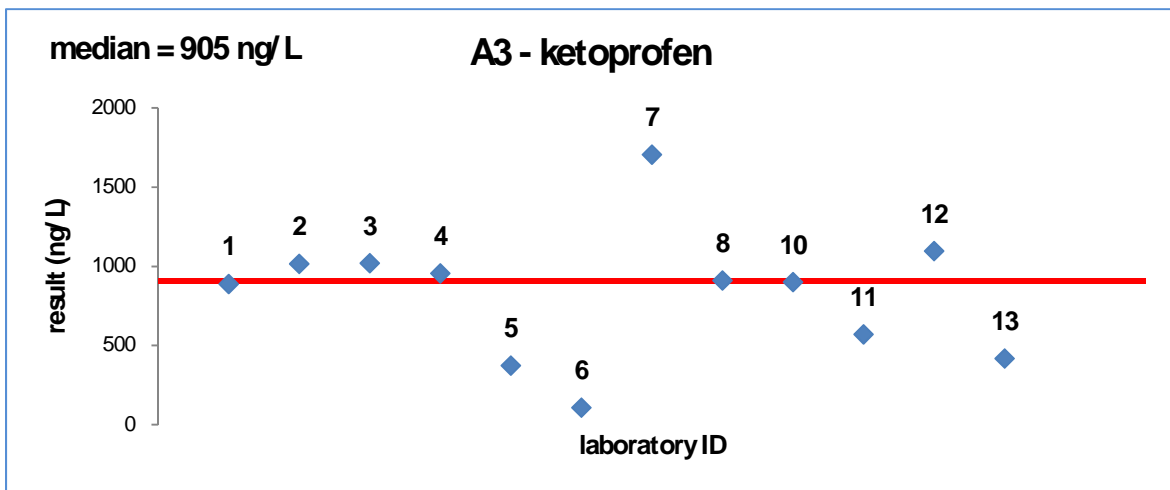
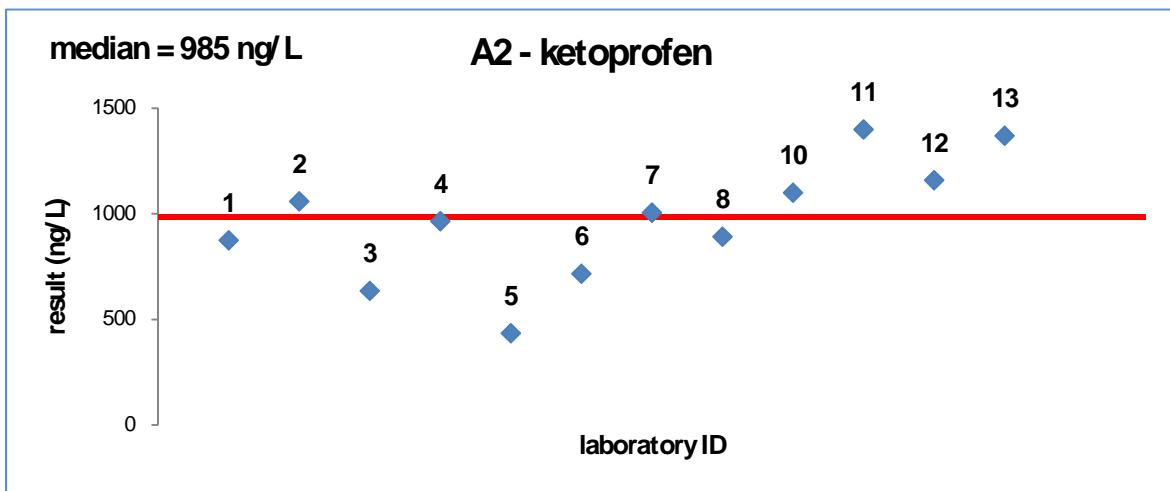
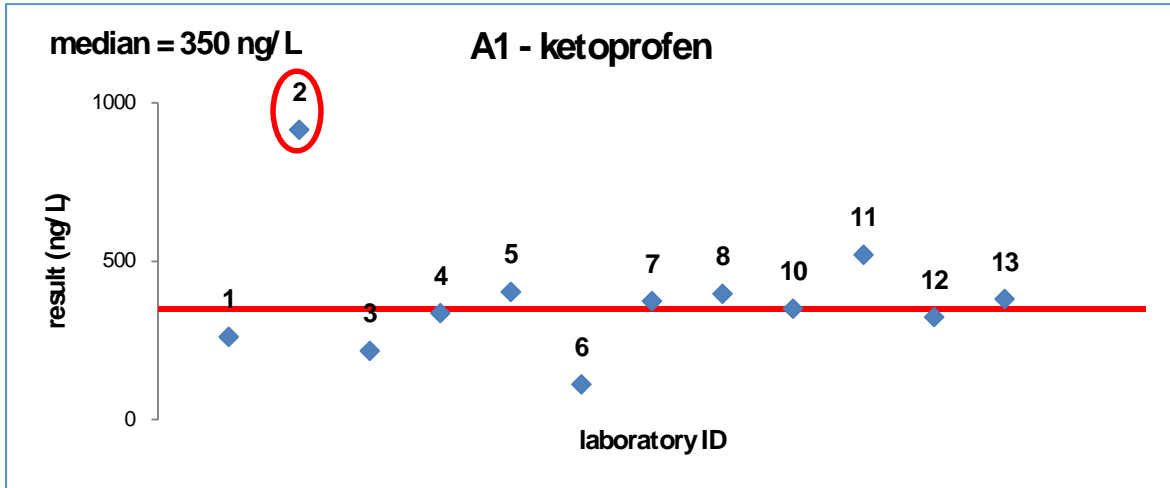


Figure 7 (4/12)

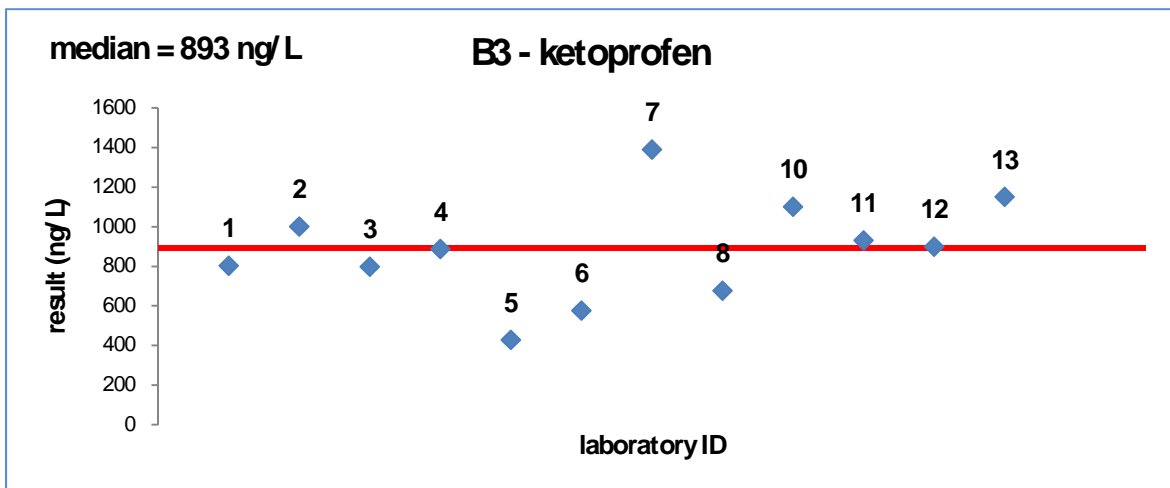
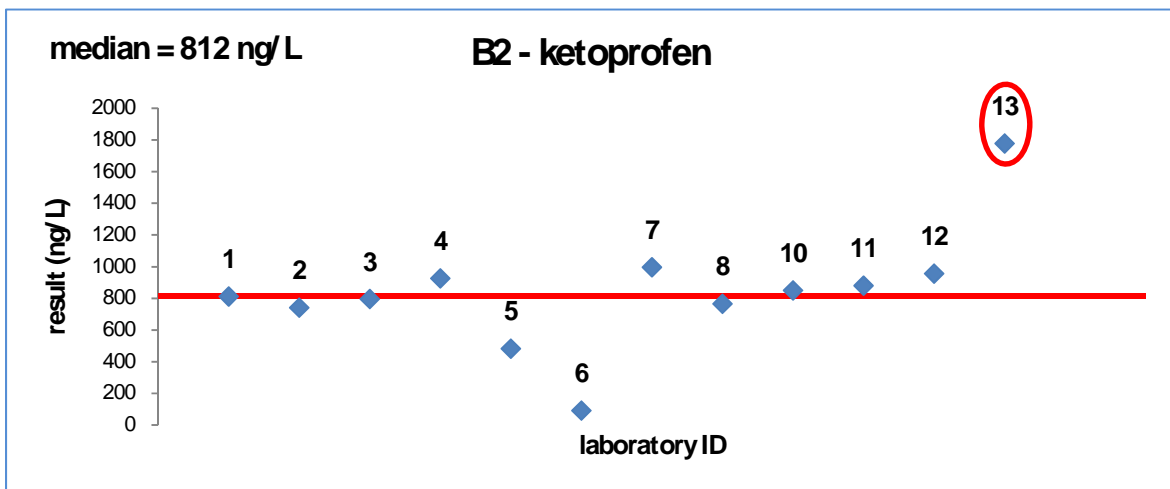
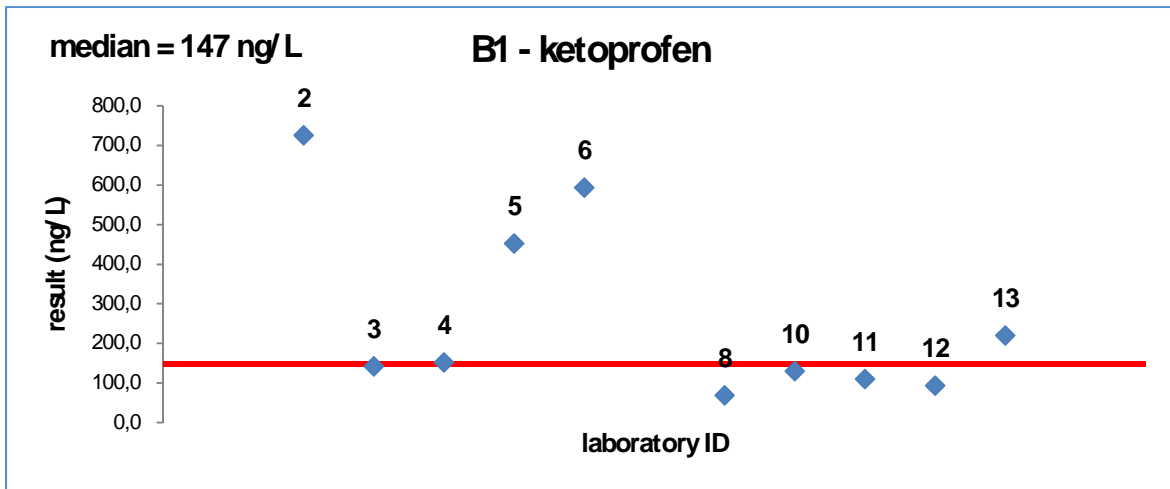


Figure 7 (5/12)

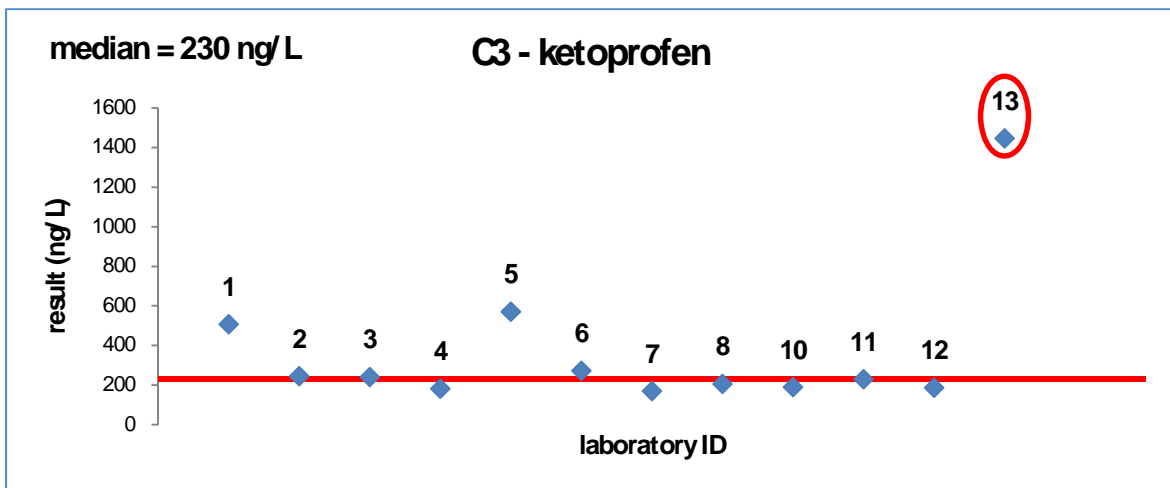
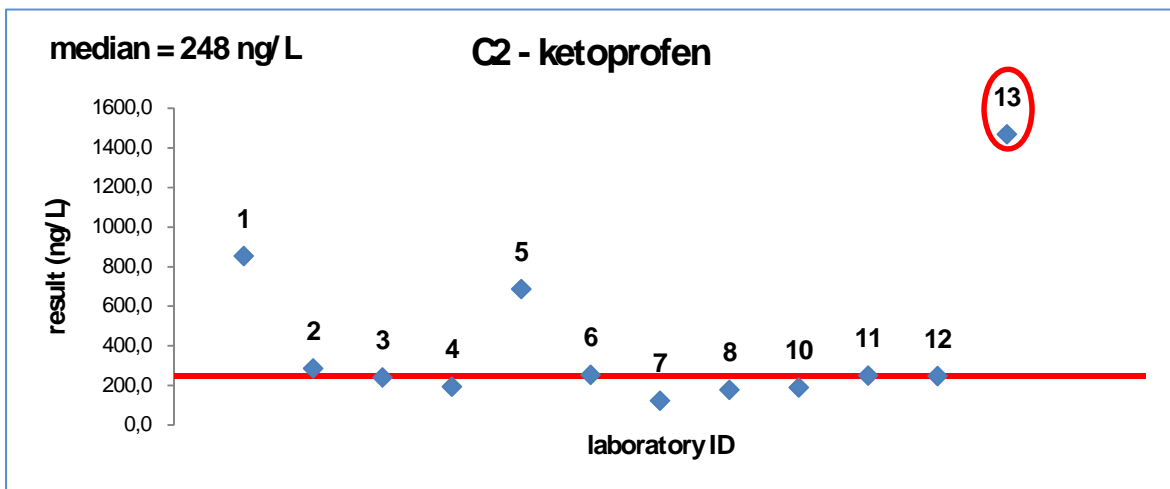
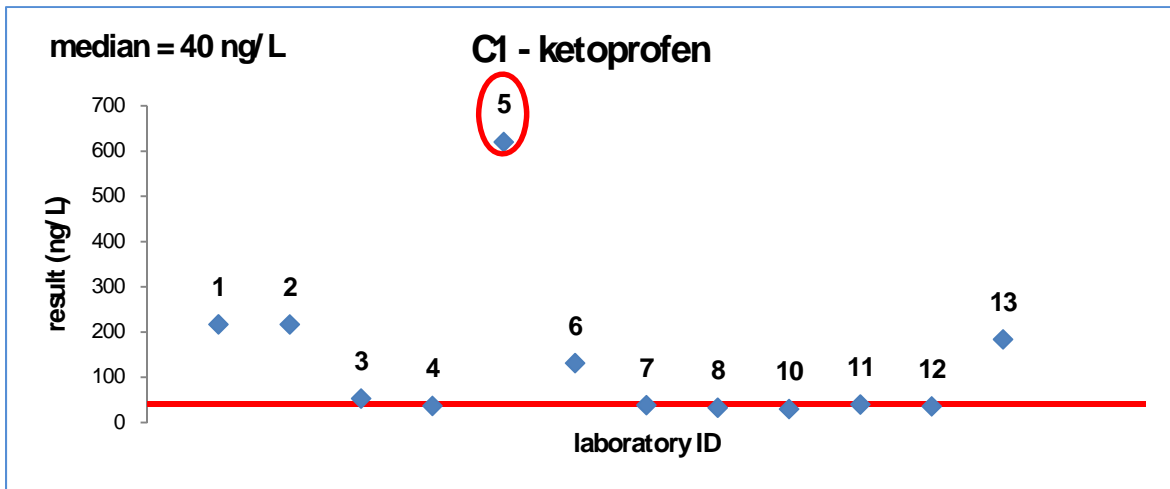


Figure 7 (6/12)

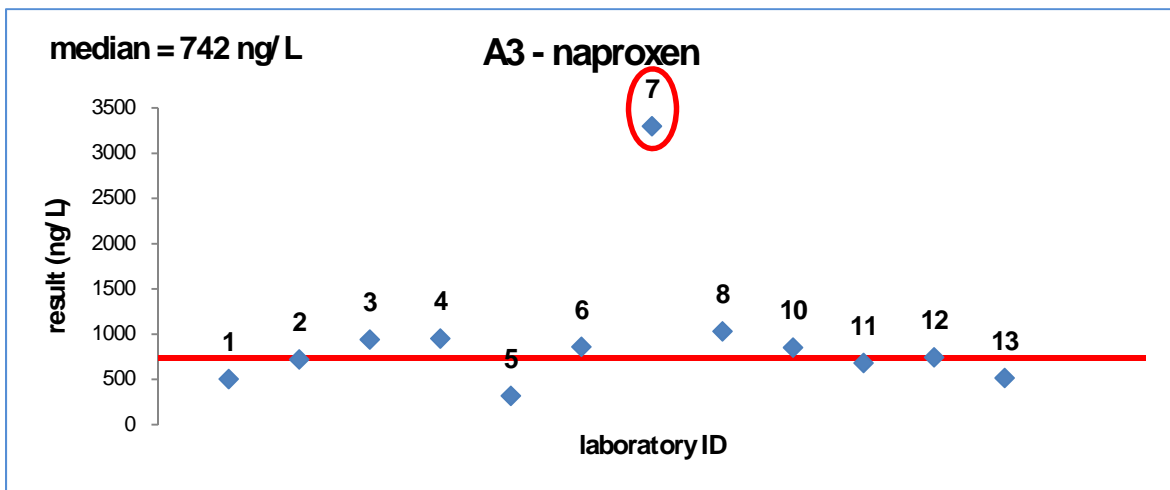
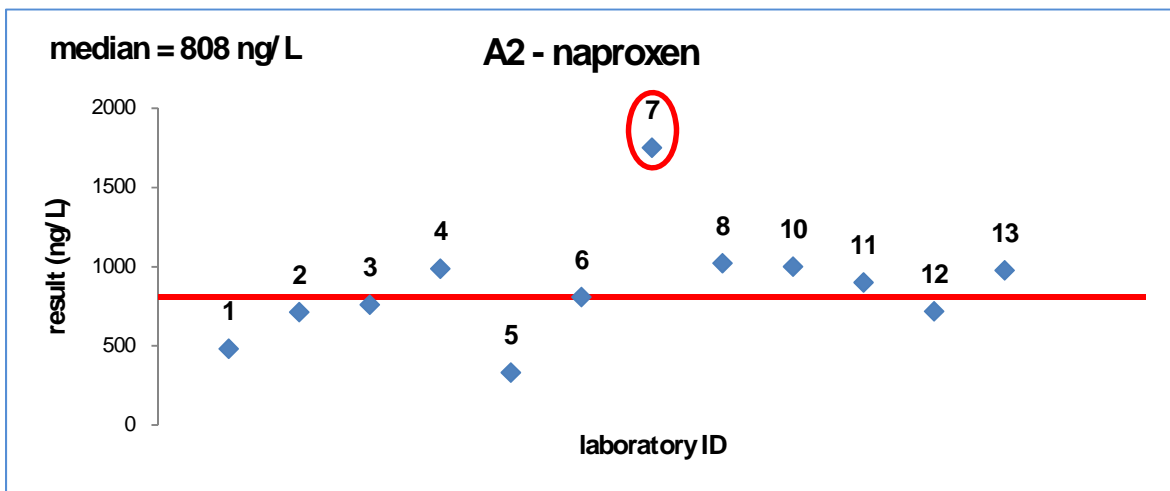
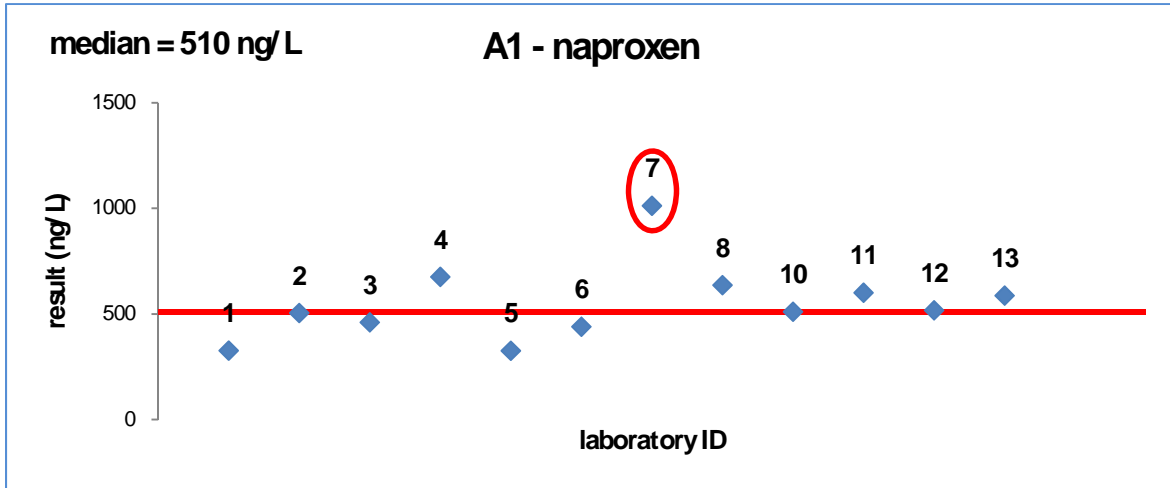


Figure 7 (7/12)

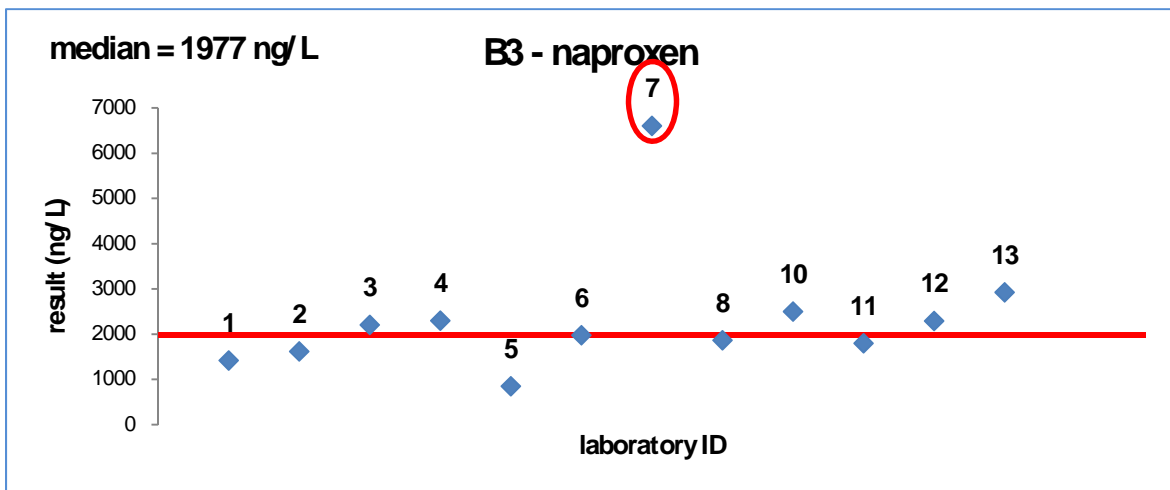
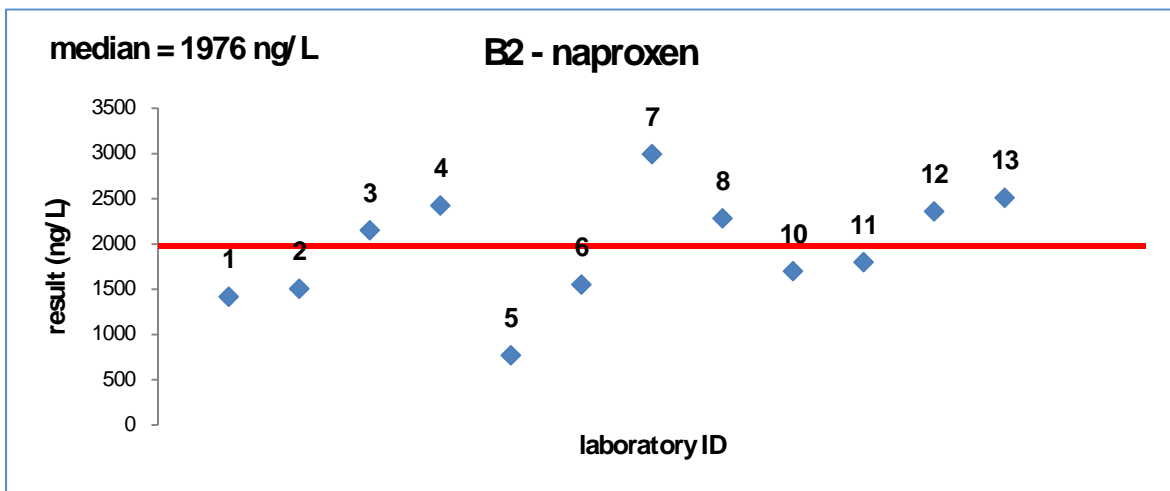
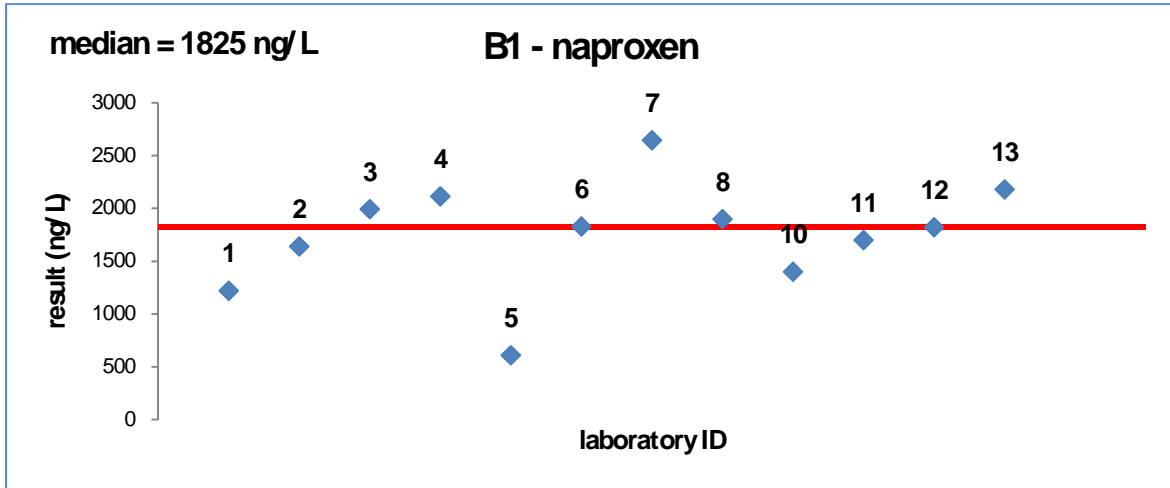


Figure 7 (8/12)

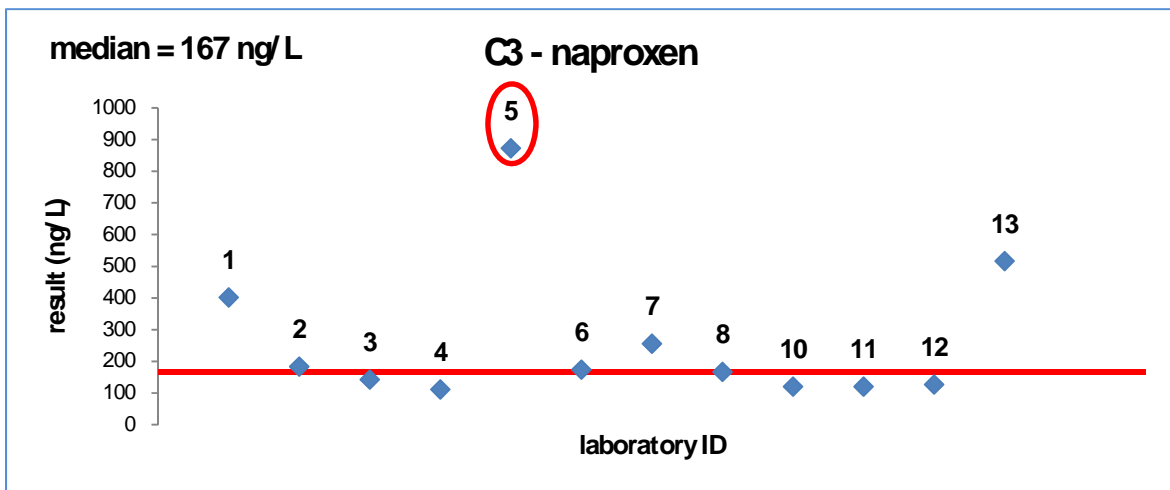
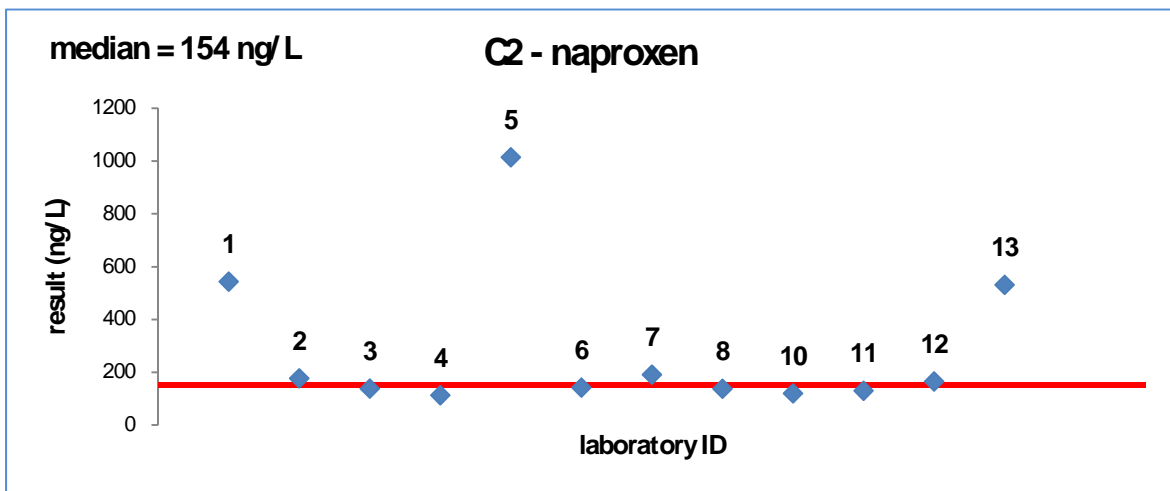
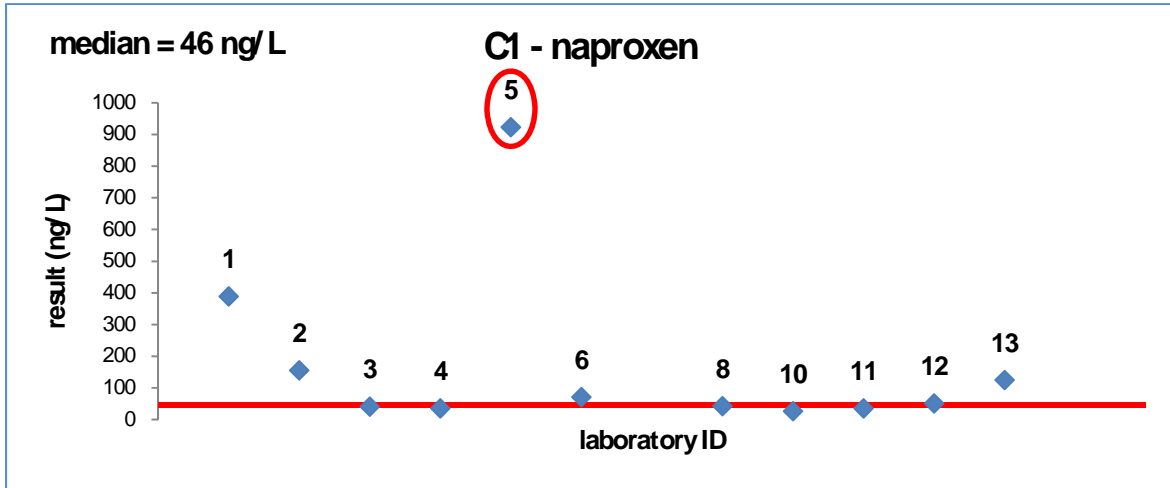


Figure 7 (9/12)

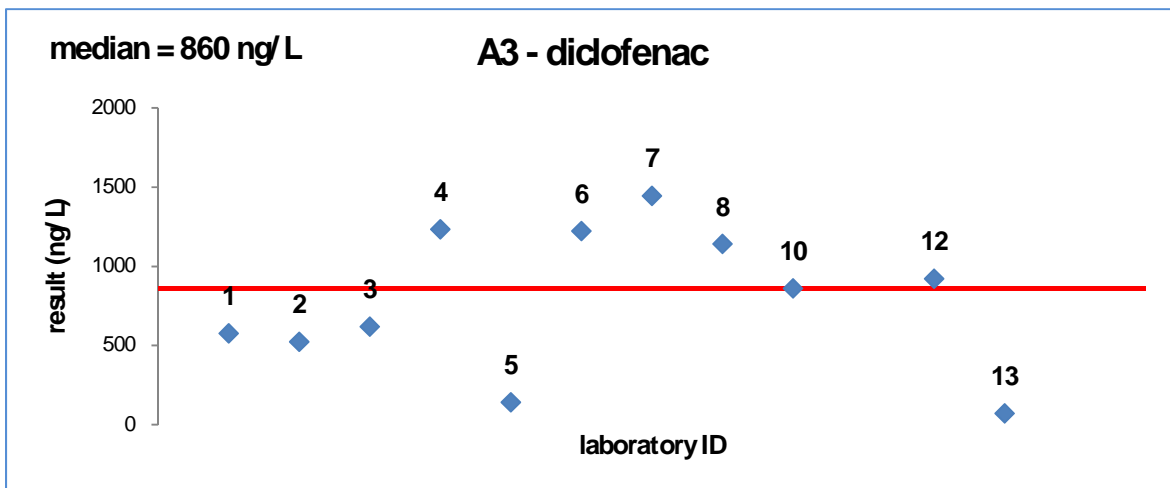
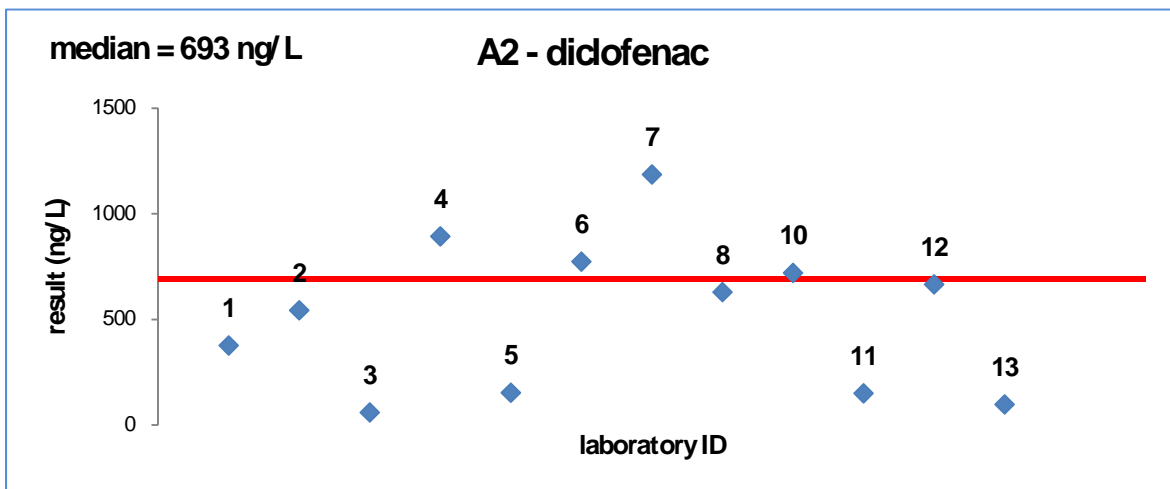
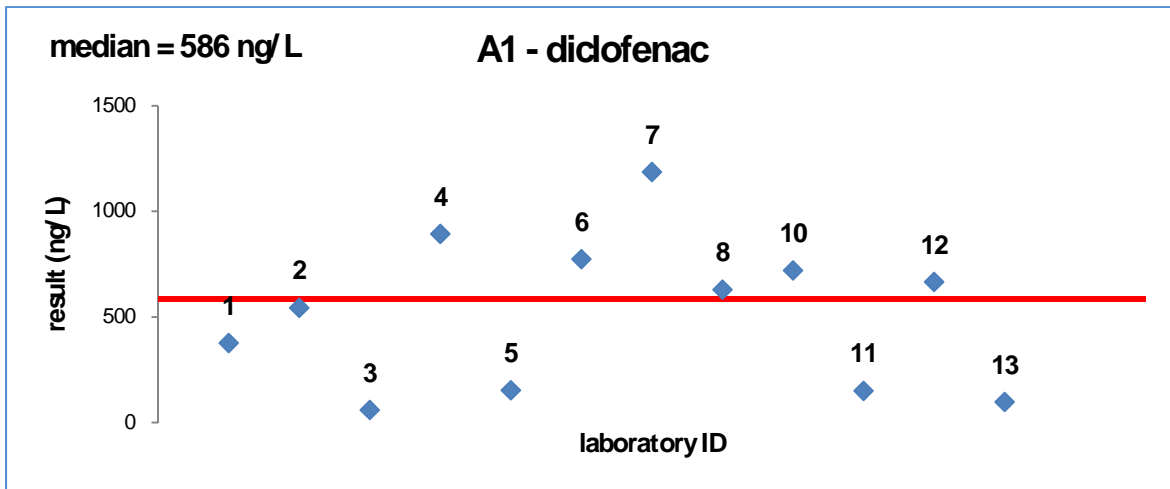


Figure 7 (10/12)

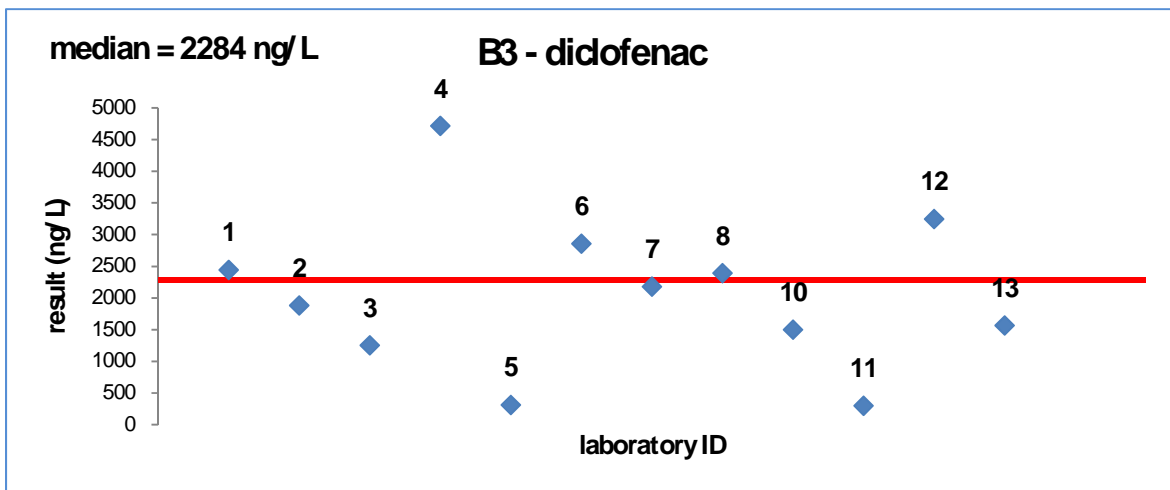
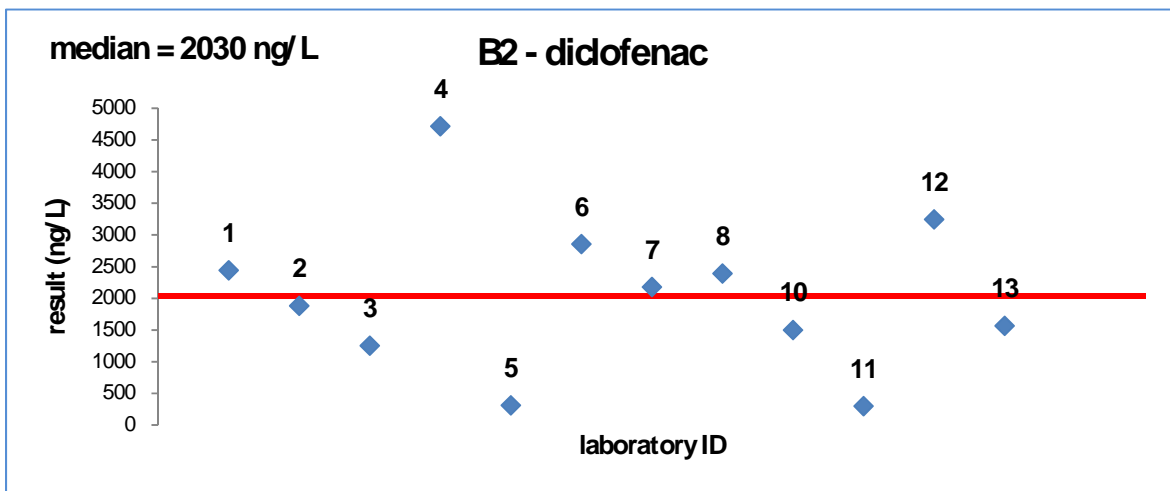
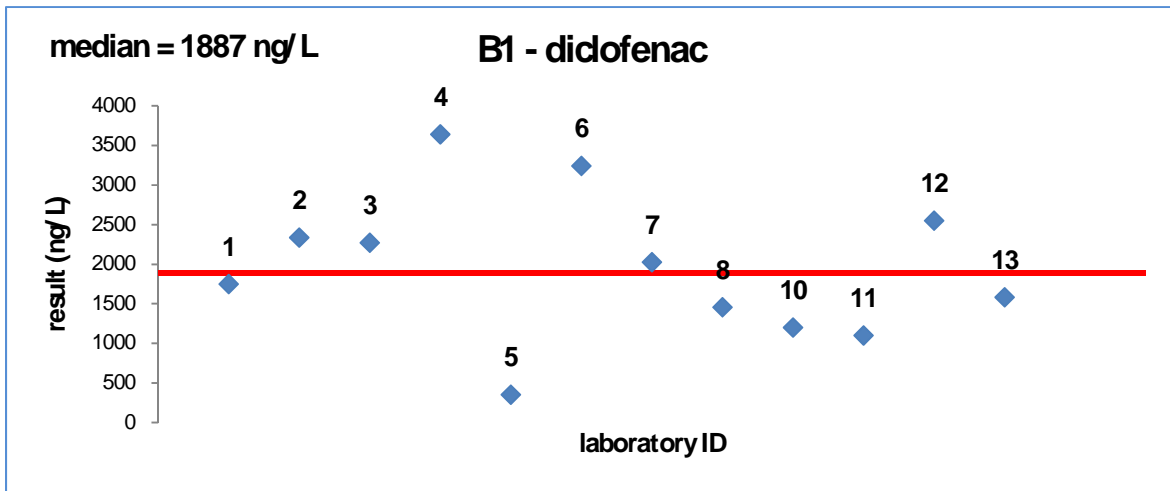


Figure 7 (11/12)

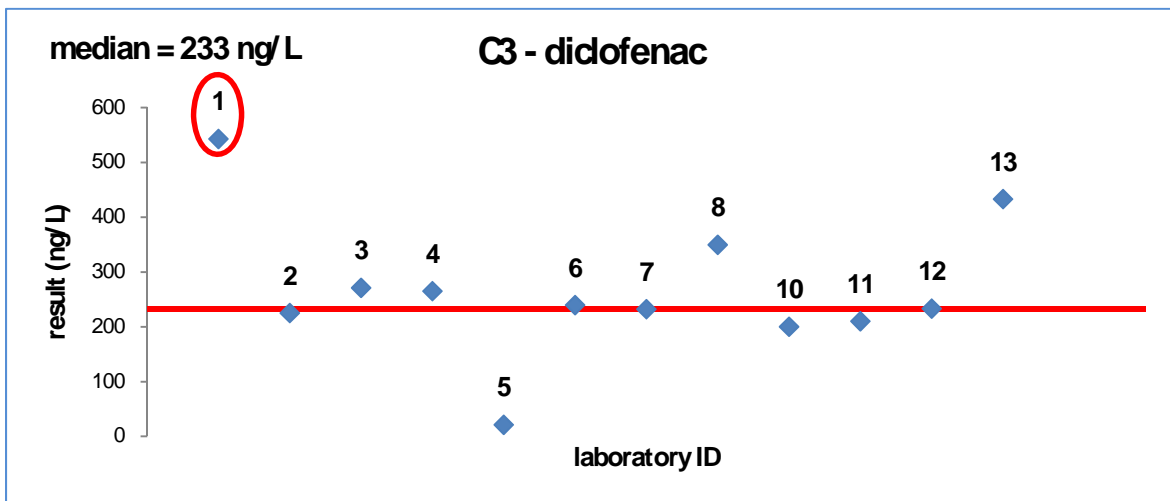
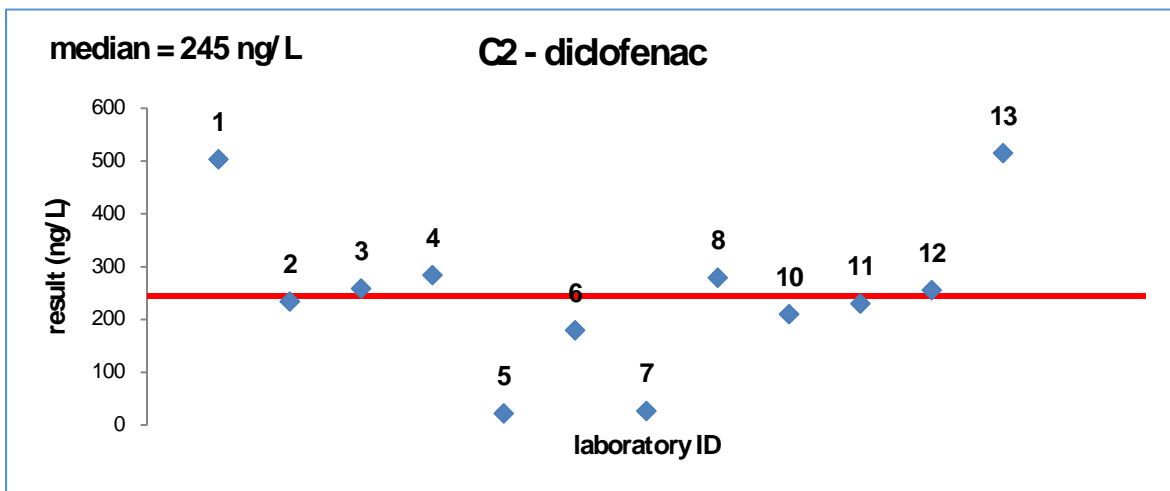
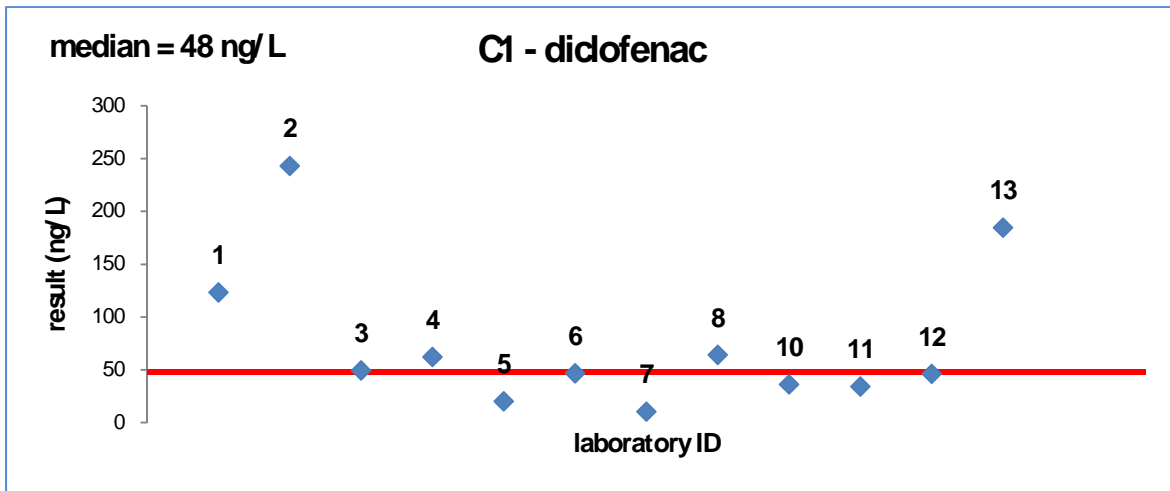


Figure 7 (12/12)

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 (\bar{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). $\pm 2\sigma$ biases are coloured light grey. The estimates for $\pm 3\sigma$ 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 | > $\pm 2\sigma$ | > $\pm 3\sigma$ |
|---------------------|--------|------|------|------|-------|------|------|------|-------|-------|------|-------|--------------|-----------------|-----------------|
| | 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 | > $\pm 2\sigma$ | > $\pm 3\sigma$ |
|----------------------|--------|-----|------|------|------|------|------|------|------|------|------|------|-------------|-----------------|-----------------|
| | 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,

$$prox. = \frac{1}{n} \times \sum \frac{|x_i - \bar{x}|}{\bar{x}} \quad \text{Equation 3}$$

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)

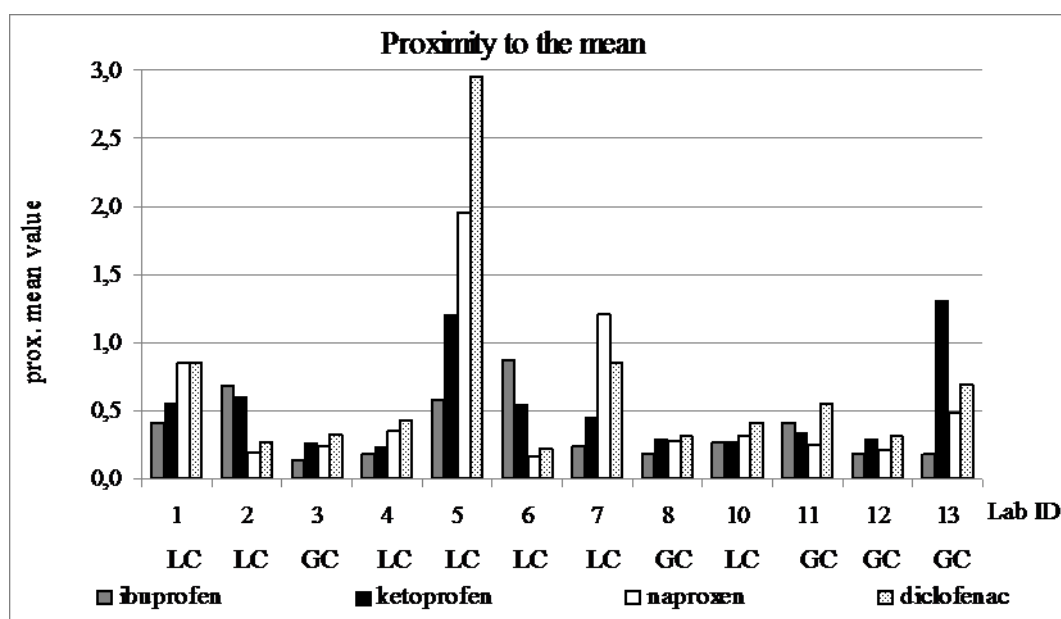


Figure 8: Bar-chart showing the “proximity to the mean” values

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).

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

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

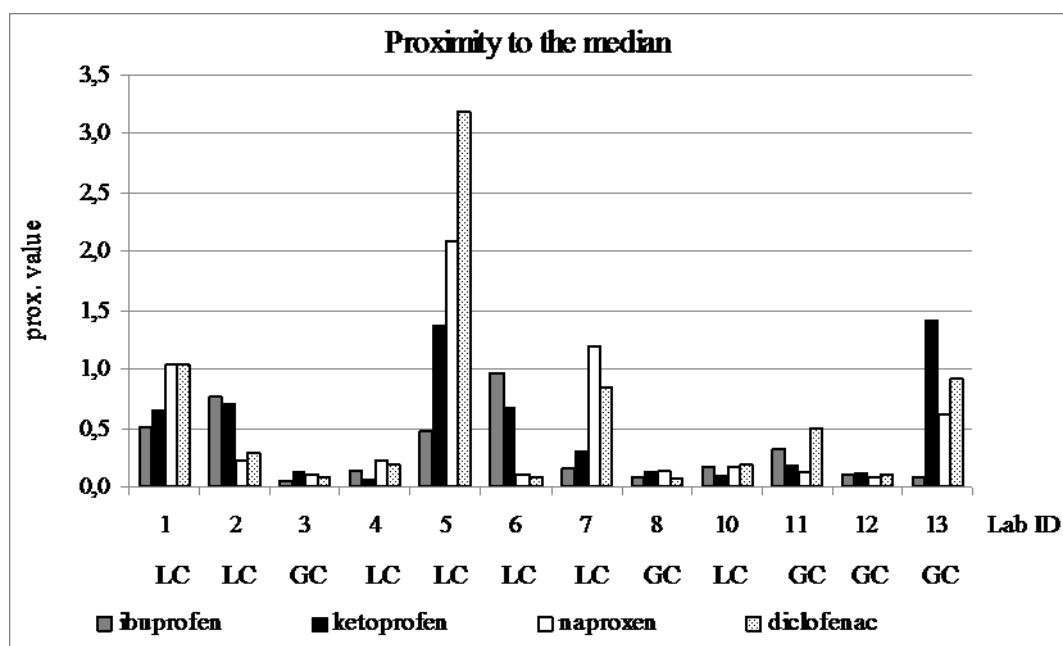


Figure 9: Bar-chart illustrating the proximity to the median

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].

$$F_{\text{exp.}} = \frac{\sigma_{\text{sampleNo.2}}^2}{\sigma_{\text{sampleNo.3}}^2} \quad \text{Equation 5}$$

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.)

| <i>IBUPROFEN</i> | <i>IP-A2</i> | <i>IP-A3</i> | <i>IP-B2</i> | <i>IP-B3</i> |
|---------------------|--------------|--------------|--------------|--------------|
| 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.)

| <i>KETOPROFEN</i> | <i>KP-A2</i> | <i>KP-A3</i> | <i>KP-B2</i> | <i>KP-B3</i> | <i>KP-C2</i> | <i>KP-C3</i> |
|---------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 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.\

| <i>NAPROXEN</i> | <i>NP-A2</i> | <i>NP-A3</i> | <i>NP-B2</i> | <i>NP-B3</i> | <i>NP-C2</i> | <i>NP-C3</i> |
|---------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 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.\

| <i>DICLOFENAC</i> | <i>DF-A2</i> | <i>DF-A3</i> | <i>DF-B2</i> | <i>DF-B3</i> | <i>DF-C2</i> | <i>DF-C3</i> |
|---------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 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.\

| <i>IBUPROFEN</i> | <i>IP-A2</i> | <i>IP-A3</i> | <i>IP-B2</i> | <i>IP-B3</i> |
|------------------------------|--------------|--------------|--------------|--------------|
| 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.\

| <i>KETOPROFEN</i> | <i>KP-A2</i> | <i>KP-A3</i> | <i>KP-B2</i> | <i>KP-B3</i> | <i>KP-C2</i> | <i>KP-C3</i> |
|------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| 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.\

| <i>NAPROXEN</i> | <i>NP-A2</i> | <i>NP-A3</i> | <i>NP-B2</i> | <i>NP-B3</i> | <i>NP-C2</i> | <i>NP-C3</i> |
|------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| 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.\

| <i>DICLOFENAC</i> | <i>DF-A2</i> | <i>DF-A3</i> | <i>DF-B2</i> | <i>DF-B3</i> | <i>DF-C2</i> | <i>DF-C3</i> |
|------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| 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

| <i>Groups</i> | <i>No. observations</i> | <i>Sum</i> | <i>Average</i> | <i>Variance</i> |
|---------------|-------------------------|------------|----------------|-----------------|
| C1-IP | 12 | 924 | 77 | 3117 |
| C2-IP | 11 | 676 | 61 | 1709 |
| C3-IP | 12 | 834 | 70 | 2523 |

ANOVA

| <i>Source of Variation</i> | <i>Sum of Squares</i> | <i>Degrees of freedom</i> | <i>Mean Square</i> | <i>F</i> | <i>P-value</i> | <i>F crit</i> |
|----------------------------|-----------------------|---------------------------|--------------------|----------|----------------|---------------|
| 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).

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

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 performed 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 excused 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.A

| 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), Statistical 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-wayANOVAindependentgroups.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 Exercise, 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.