# WP-CASE 2
# Validation on analytical method for NSAID analysis in water samples

## Interlaboratories Exercises on NSAIDs


Consejo Superior de Investigaciones Científicas, Barcelona, Spain

Jožef Stefan Institute, Ljubljana, Slovenia

- WP-CASE 2

  Validation on analytical method for NSAID analysis in water samples

  2 Main Activities

  - 1st Interlaboratory Exercise

  Consejo Superior de Investigaciones Científicas, Barcelona, Spain

  - 2nd Interlaboratory Exercise

  Jožef Stefan" Institute, Ljubljana, Slovenia

  Consejo Superior de Investigaciones Científicas, Barcelona, Spain

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

The main objectives:

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

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

## Participant Laboratories:

1. CNRS, LPTC - Université Bordeaux 1,Talence – France
2. Eawag, Environmental Chemistry, Duebendorf, Switzerland,
3. Environmental Institute, Kos, Slovak Republic
4. EPF Lausanne, Switzerland
5. Europa Fachhochschule Fresenius, University of Applied Science, Idstein, Germany
6. Federal Institute of Hydrology (BFG), Kablenz, Germany
7. IIQAB-CSIC, Barcelona, Spain
8. Institute for Environment and Sustainability, JRC, Ispra, Italy
9. Jožef Stefan Institute, Ljubljana, Slovenia
10. Mario Negri Institute for Pharmacological Research, Milan, Italy
11. Pesticide Residues Laboratory, General Chemical State Laboratory, Athens, Greece
12. Umweltbundesamt GmbH, Wien, Austria.
13. Universidade de Santiago de Compostela, Spain
14. Universita "La Sapienza" di Roma, Italy

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

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

A total number of **162 samples** were distributed to 17 laboratories (19 participations) distributed along 11 European Countries (Austria, France, Germany, Greece, Italy, Norway, Slovak Republic, Slovenia, Spain, Switzerland, and UK) that initially took part on the exercise. A final number of **14 participations** concluded the ring exercise.

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

## 2 method were recommended

- <u>LC-MS Analytical Protocol</u>
- *Extraction and Pre-treatment*
- Neutral pH
- Extraction Volumes: 500 mL of MilliQ water and river samples
- 200 mL of wastewater effluent
- SPE using Oasis HLB (60 mg, 3mL) polymeric cartridges.
- Elution of cartridges with methanol
- Reconstitution of extracts: 1mL of methanol-water (25:75, v/v).
- LC-ESI-tandem MS analysis
- Analysis of extracts: LC-ESI-tandem MS
- Chromatographic separation: RP-18 column.
- Analysis under NI mode, using as eluent A methanol and water as eluent B.
- 2 transitions, one for identification and one for quantification

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

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

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

## Results

- A total number of 126 samples were analyzed along the ring exercise and 486 results were collected corresponding to 14 participations in 13 laboratories.
- Total No. Results = 486
- Total No. Outliers = 23 (4.7%)
- No. of participations using Liquid Chromatography = 7 (50%)
- No. of participations using Gas Chromatography = 7 (50%)
- No. of outliers using Liquid Chromatography = 8 (3.3% of results)
- No. of outliers using Gas Chromatography = 15 (6.1 % of results)
- For each series of samples (batch 1, batch2, and batch3) the initial mean value (Xi), the initial standard deviation (σi), the upper warning limit (UWL), and the lower warning limit (LWL) values were calculated.
- The limits were calculated as: UWL = (Xi+2 σi), and LWL = (Xi-2 σi)

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

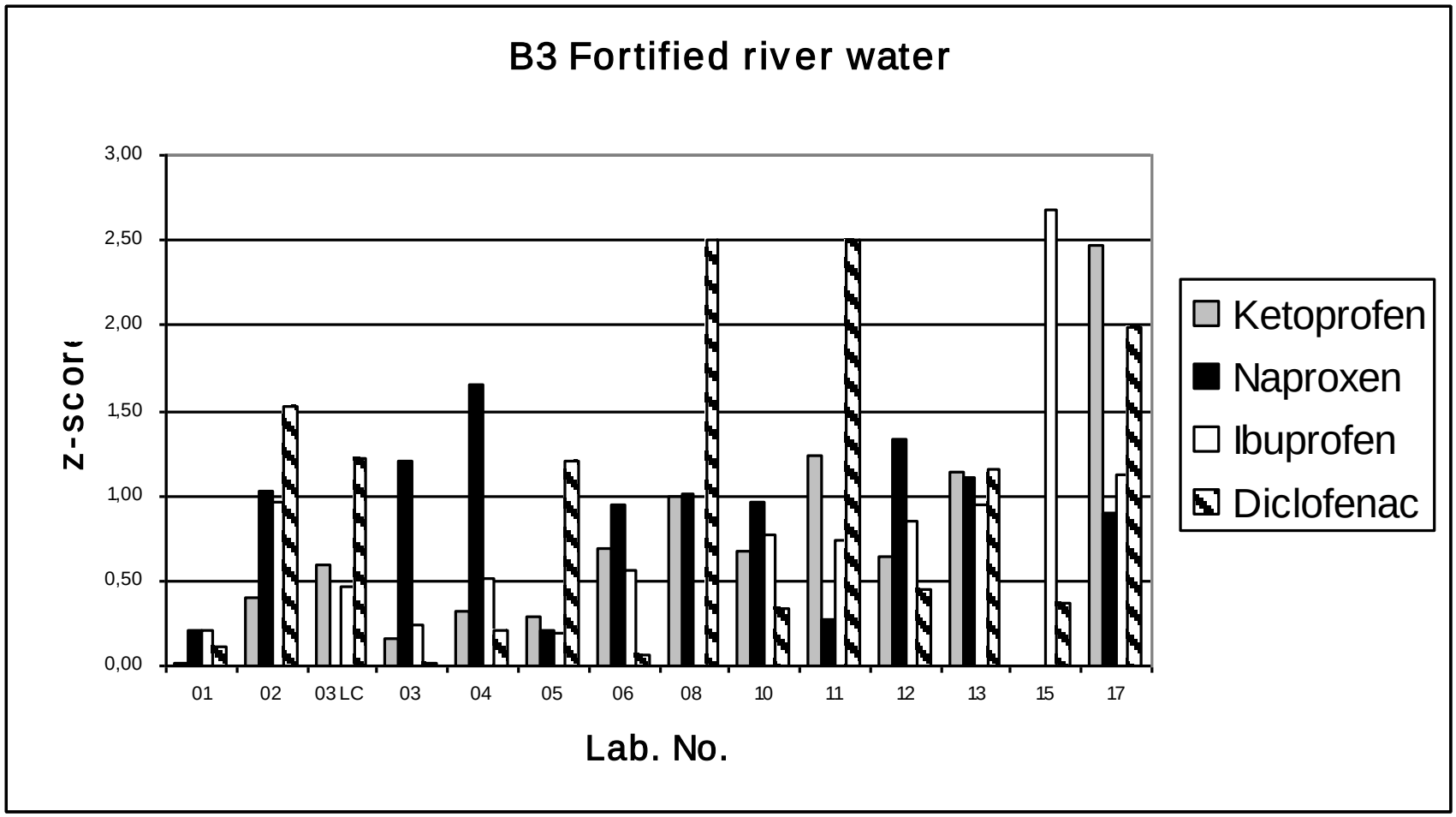
As **acceptance criteria** for each result was used the *Z-score* function according to the

"Laboratory Accreditation & Audit Protocol: Food Inspection Directorate":

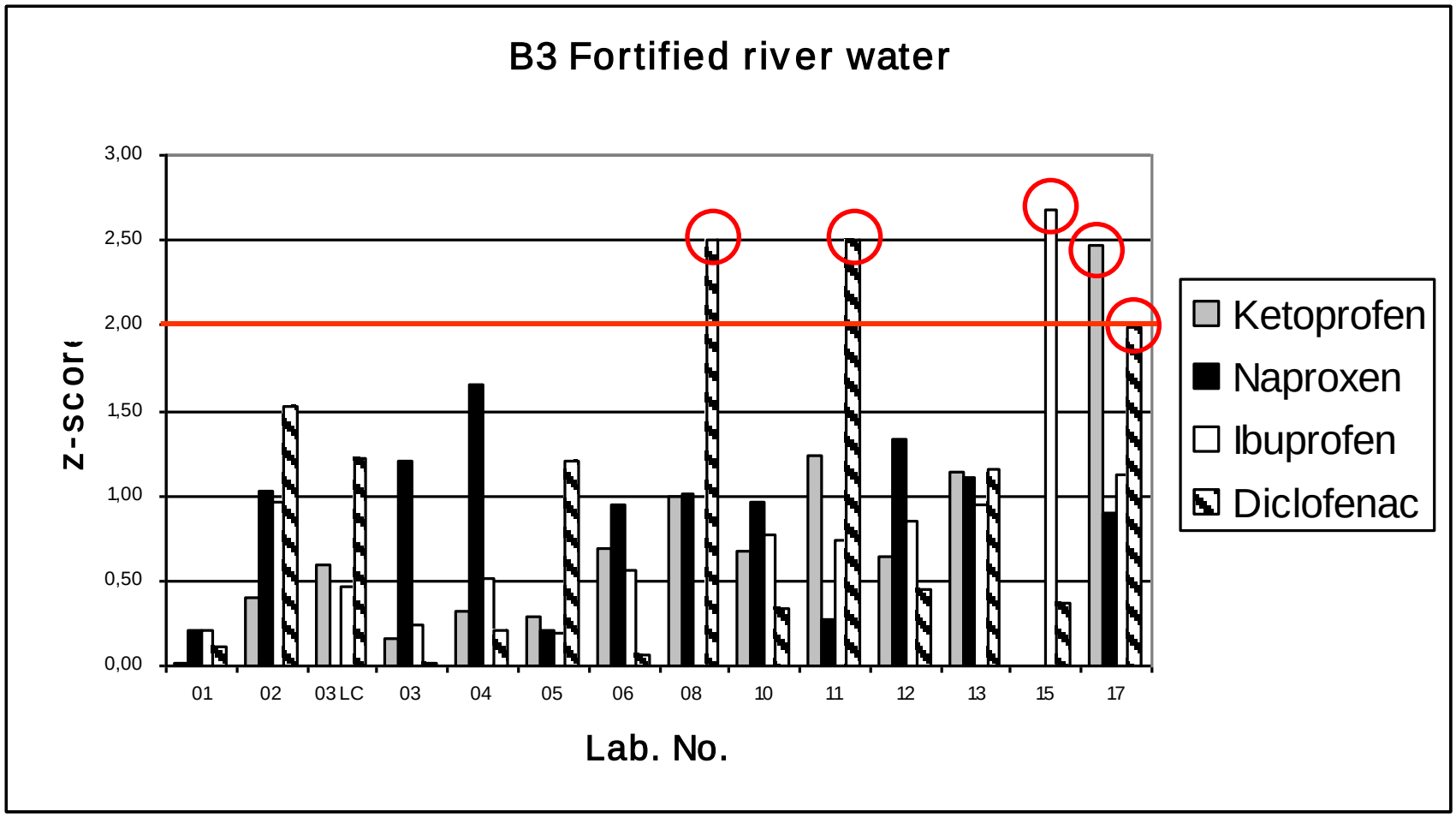
$$Z = (Xlab - Xi) / \sigma i$$

- Where Xlab is a result, Xi is the initial mean value and the σi the initial standard deviation.
- The results whose ***Z*-value was over 3** was directly **excluded** and when the ***Z-score* value was between 2 and** 3 was applied the **Dixon test with a 5%** of significance level.

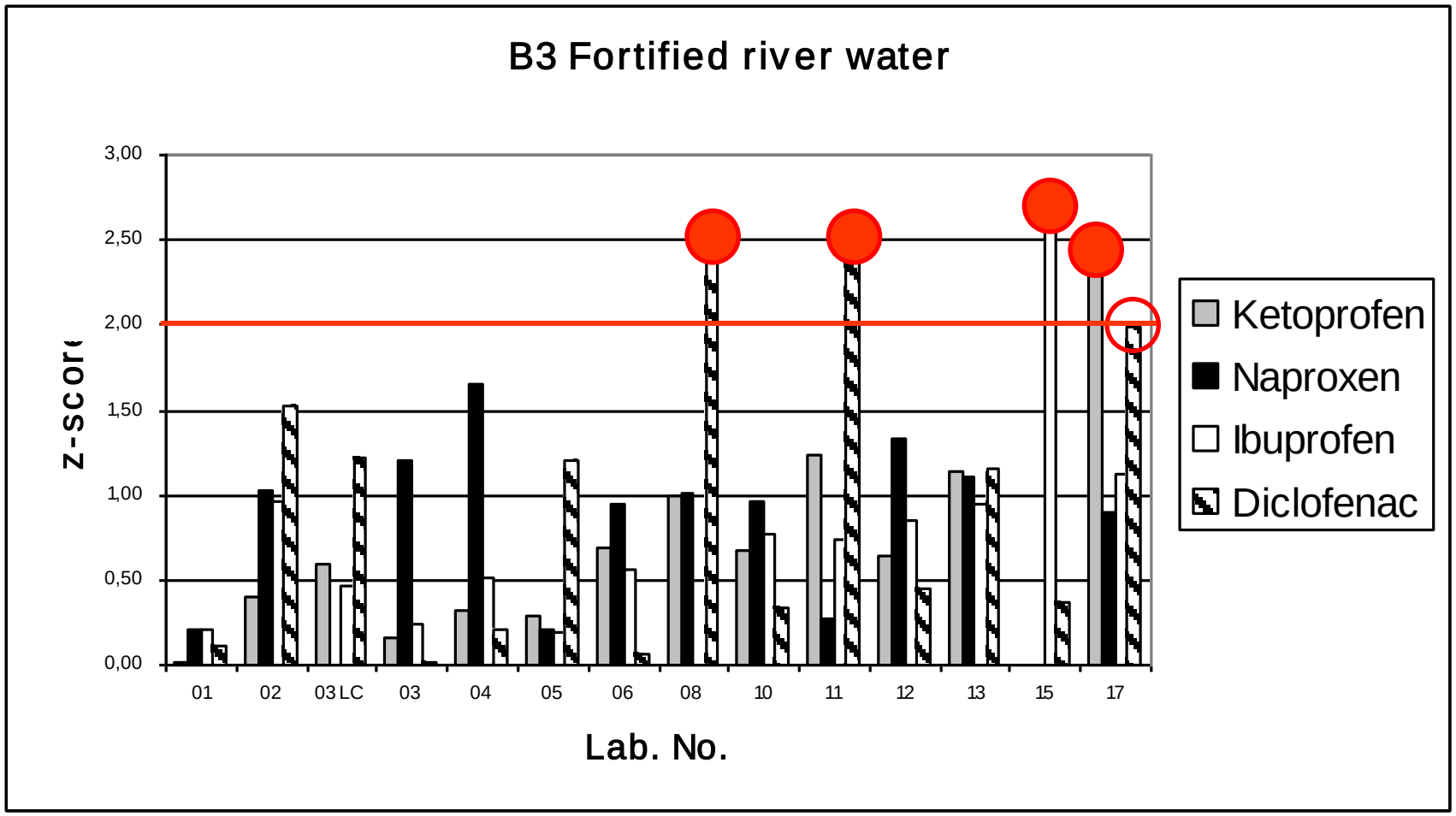
# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER



# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER



# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER



# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

**Reproducibility and repeatability:**

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

$$R = \frac{\sqrt{rlab}}{N}$$

*Where*

$rlab = \sqrt{(2 \cdot 2^{1/2})} \cdot lab$

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

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

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

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

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

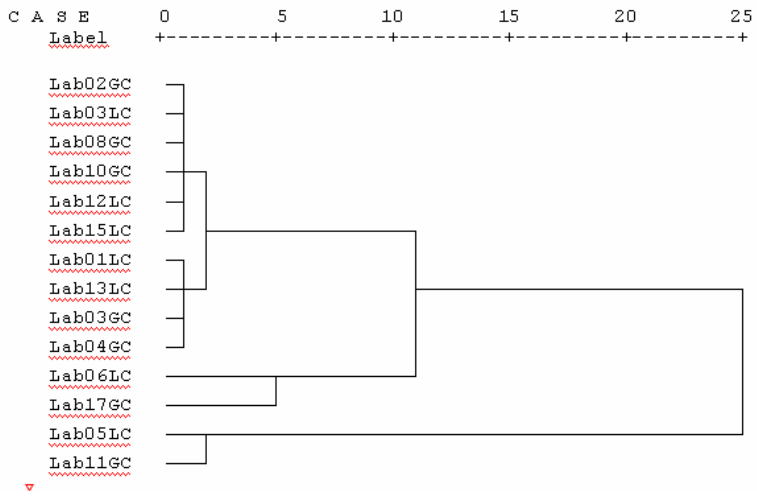
# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

## Hierarchical cluster analysis

**RIVER WATER**

Dendrogram using Single Linkage

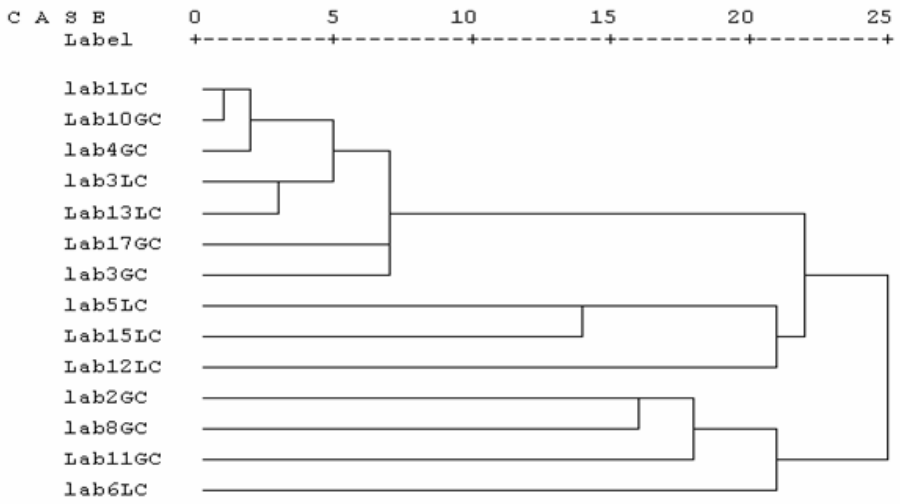
Rescaled Distance Cluster Combine




**Wastewater**

Dendrogram using Single Linkage

Rescaled Distance Cluster Combine



# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

- The final number of participants was 13 (**77%**).
- The final number of results collected was 486 and 23 values were **outliers (4,7%),** and were discarded.
- outlier values by **liquid chromatography** was **8 (3,3% of results)**
- Outlier values **gas chromatography** was superior **15 (6,1 %),** as expected because of the necessary additional step (derivatization)
- The sample with higher number of outlier was the fortified **MilliQ water**, because a low level of concentration, (nature of the matrix).
- The second sample with more outlier values was **wastewater** due to the complexity of the matrix. Also for this sample were obtained the higher levels of variability.

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

- A general good agreement was obtained between the concentrations of fortification and the mean values recorded by the participants. However, the precision of individual participants was low along the exercise, and that means a necessary protocol of sample treatment including (manipulations, how to defreeze the samples and during how long, etc…) in order to minimize sources of variation in the second ring

- About reproducibility of values recorded for the analyzed compound in the different types of samples was as well low, but that was expected due the high number of small different methods involved in the present edition.

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

- The hierarchical cluster analysis concluded that no relation can be found between the results and if the samples were analyzed following a GC or a LC based method.

- No relation was observed between the results and the temperature at reception, but it is an important source of variation

- The number of outliers was linked to the number of steps and the complexity of the method.

- Repeatability (r) and reproducibility (R) values were in agreement with data from other interlaboratories on chromatographic methods using different protocols

# FIRST INTERLABORATORY STUDY ON NSAID RESIDUE ANALYSIS IN WATER

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## First interlaboratory exercise on non-steroidal anti-inflammatory drugs analysis in environmental samples

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# 2nd Interlaboratory Exercise on NSAIDs

## PARTICIPANTS (12/13)

1. Environmental Institute, Kos, Slovak Republic (Peter Oswald)
2. General State Chemical Laboratory, Greece (Pigi Kormali)
3. Institute for Environment and Sustainability, JRC, Ispra, Italy (Robert Loos)
4. “Jožef Stefan” Institute, Ljubljana, Slovenia (Ester Heath, Tina Kosjek)
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9. Norwegian Institute for Water Research (NIVA), Oslo, Norway (Katherine Langford)
10. Umweltbundesamt GmbH, Abt. Umweltwirksame Stoffe und Metaboliten, Wien, Austria (Oliver Gans)
11. University of A. Coruña, Spain (José Benito Quintana)
12. Universita "La Sapienza" di Roma, Italy (Lucia Mainero Rocca, Federico Pastori)

# 2nd INTERLABORATORY STUDY

## The main objectives

- To carry out the second validation level of chromatographic methods of analysis of Ketoprofen, Naproxen, Ibuprofen and Diclofenac (preset protocols, dry ice)
- To evaluate the main sources of variation
- To evaluate the possible significant differences between methods based on liquid chromatography and gas chromatography
- To assess the influence of sample matrix on the different chromatographic approaches
- To assess the effect of filtration, taking into account different matrices, concentrations and filter material
- To evaluate preset analytical protocols, find possible drawbacks and try to improve the methods

# 2nd INTERLABORATORY STUDY

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

| SAMPLE CODES | | |
|---|---|---|
| **A1**<br>Natural wastewater | **B1**<br>Natural river water | **C1**<br>Spiked deionised water |
| **A2**<br>Fortified wastewater | **B2**<br>Fortified river water | **C2**<br>Spiked deionised water |
| **A3**<br>Fortified wastewater | **B3**<br>Fortified river water | **C3**<br>Spiked deionised water |

# LC-MS Analytical Protocol

- Neutral pH
- Internal standard d3 ibuprofen
- Extraction Volumes
  - 400 mL of MilliQ water and river samples
  - 200 mL of wastewater effluent
  - 1L of each sample
- Additional filtration (2 out of 3 samples)
- SPE using Oasis HLB (60 mg, 3mL) polymeric cartridges
- Cartridge elution: 8ml methanol
- Extract reconstitution: 1mL 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 5mM $NH_4$ acetate
  - Mobile phase B: water with 5mM $NH_4$ acetate
- 2 transitions when possible (one for identification and one for quantification)

# GC-MS Analytical Protocol

- Samples sent on dry ice
- Additional filtration (2 out of 3 samples)
- Volumes of extraction:
  - wastewater: 200 mL
  - surface and MiliQ water: 400 mL
  - 1L of each sample
- No acidification
- Int. std.: d3 ibuprofen
- SPE: Oasis HLB/60 mg
- Elution: EtAc 2 ml
- 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)

# RESULTS

- 12 out of 13 sets of samples in 11 laboratories from 8 European Countries (Austria, France, Greece, Italy, Norway, Slovenia, Spain, Switzerland)
- 12 participations concluded the ring exercise:
  7 LC and 5 GC
- total number of analysed samples: 108
- 773 results submitted (incl. paralell and ⊠LOD)

| Column1 | Column2 | comments |
|---|---|---|
| total number of samples distributed | 117 | |
| total number of samples analysed | 108 | |
| number of results collected* | 773 | * including <LODs, paralels |
| number of data processed | 428 | |
| number of participants | 12 | |
| number of laboratories | 11 | |
| GC labs | 5 | 42% |
| LC labs | 7 | 58% |
| Total No. of outliers | 15 | |
| No. of outliers in LC labs | 12 | 80% |
| No. of outliers in GC labs | 3 | 20% |

## Determination of outliers

- Acceptance criterion: z-score

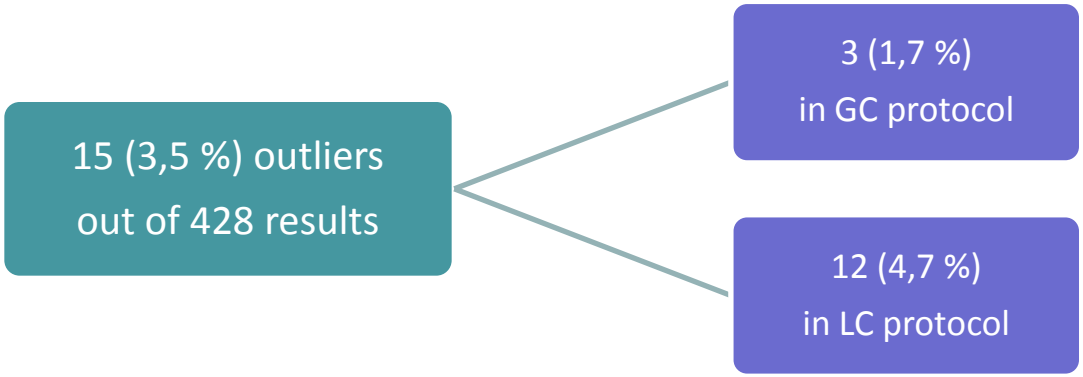
  |z| < 2 → accepted

  2 < |z| < 3 → questionable

  |z| > 3 → outlier

$$z = \frac{x_{lab} - x_0}{?_0}$$

- 2 < |z| < 3 → suspect outliers → Dixon test (T – tau value); α = 5 %

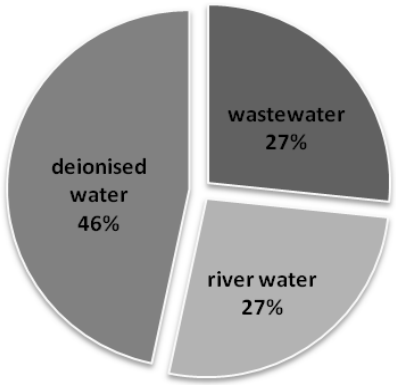


# 2nd INTERLABORATORY STUDY

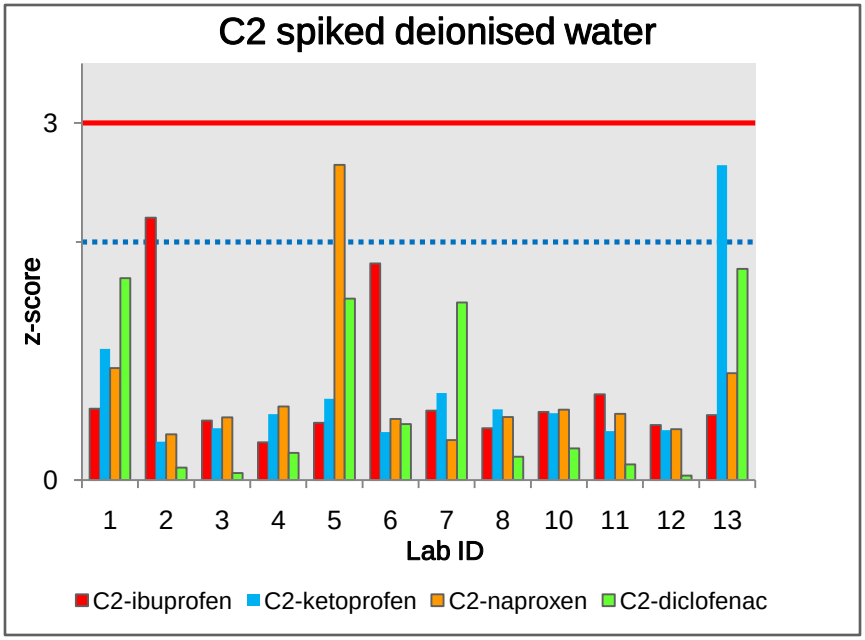
## Results

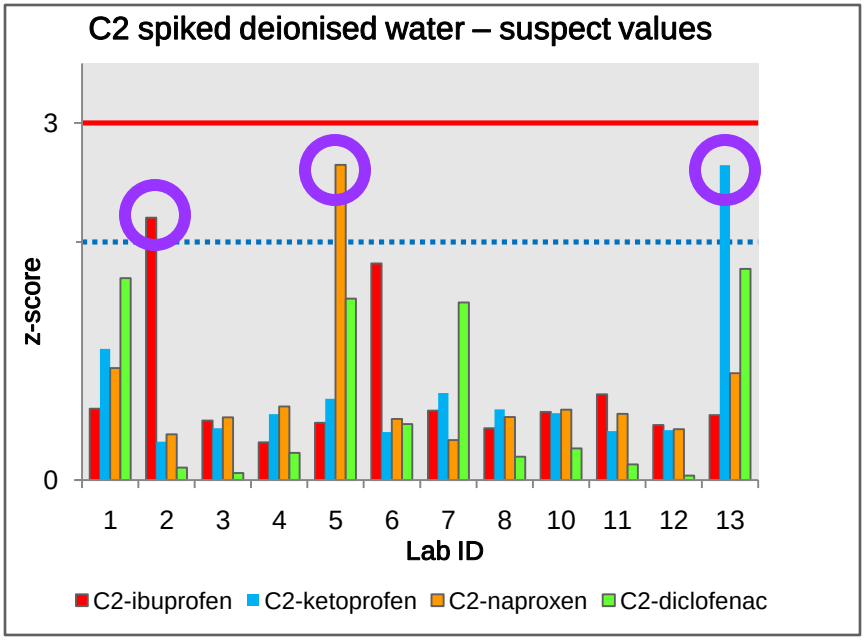
### Determinatin of outliers (cont'd)

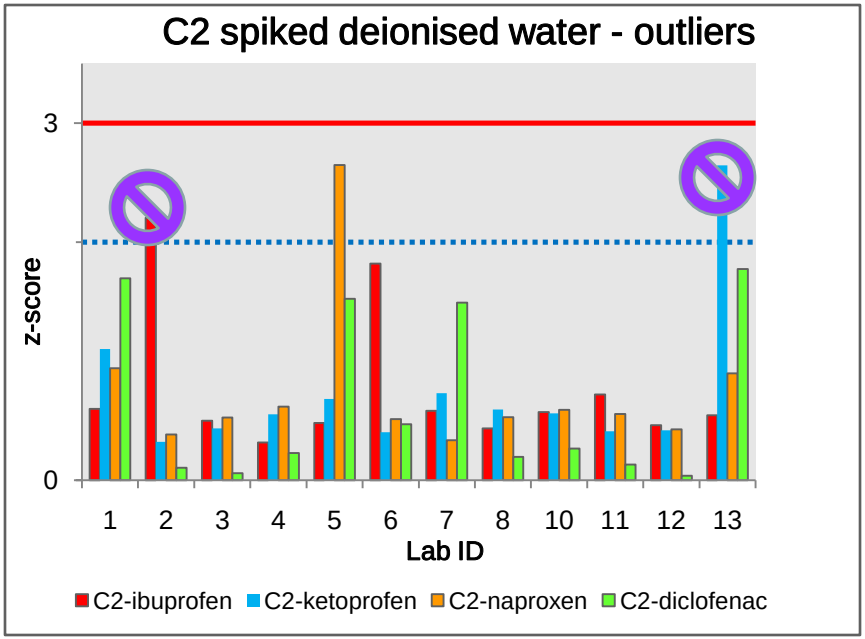
- Most outliers were found in DIW (lowest variance)




| 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** | |


C2 spiked deionised water
3
0
z-score
1 2 3 4 5 6 7 8 10 11 12 13
Lab ID
C2-ibuprofen C2-ketoprofen C2-naproxen C2-diclofenac


C2 spiked deionised water – suspect values
3
0
z-score
1 2 3 4 5 6 7 8 10 11 12 13
Lab ID
C2-ibuprofen C2-ketoprofen C2-naproxen C2-diclofenac


C2 spiked deionised water - outliers
3
0
z-score
1 2 3 4 5 6 7 8 10 11 12 13
Lab ID
C2-ibuprofen C2-ketoprofen C2-naproxen C2-diclofenac

# Analytical issues

## Occurrence of NSAIDs in RW and WW

### RIVER WATER

- **Ibuprofen**: **7,5 µg/L** !!
- **naproxen**: 1,8 µg/L
- **diclofenac**: 2,0 µg/L
- **ketoprofen**: 0,3 µg/L

### WASTEWATER

- **Ibuprofen**: 1,2 µg/L
- **Ketoprofen**: 0,3 µg/L
- **Naproxen**: 0,5 µg/L
- **Diclofenac:** 0,5 µg/L

## Statistical evaluation / analytical issues

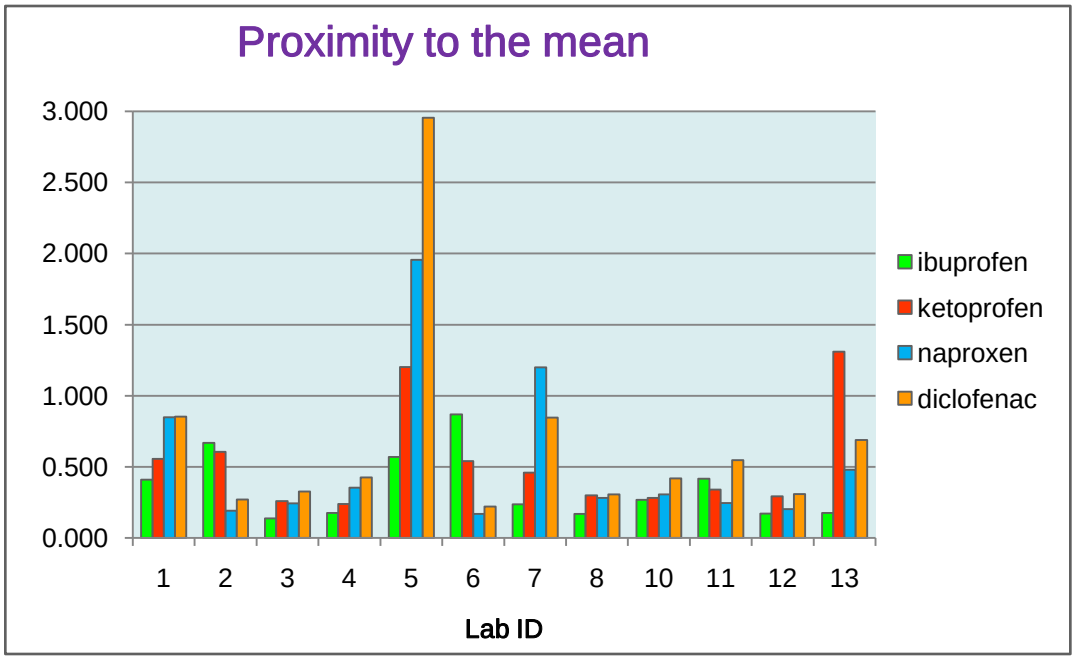
- Outlier exclusion: z-score (Dixon test)
- Mean, standard deviation, standard error of mean, median, minimum and maximum value
- Repetability, reproducibility
- Proximity to the mean
- Sample preparation: homogeneity of mixing ($x^2$-test)
- Stability in cartridges
- Effect of filtration / matrix (F-test, t-test, ANOVA)
- Effect of the filter material (F-test)
- Distribution of the results (Lilliefor's test for normality)
- Analytical issues: pH prior to extraction
- Sample preparation: standard mixture

# 2nd INTERLABORATORY STUDY

## Results

### Laboratory performance

- Proximity to the mean is a general measure of a laboratory capability to determine a specific analyte
- Influence of matrix and concentration excluded
- Calculated as for each compound as an average value of relative biases




$$prox = \frac{1}{n} \sum \frac{|x_i - \bar{x}|}{\bar{x}}$$

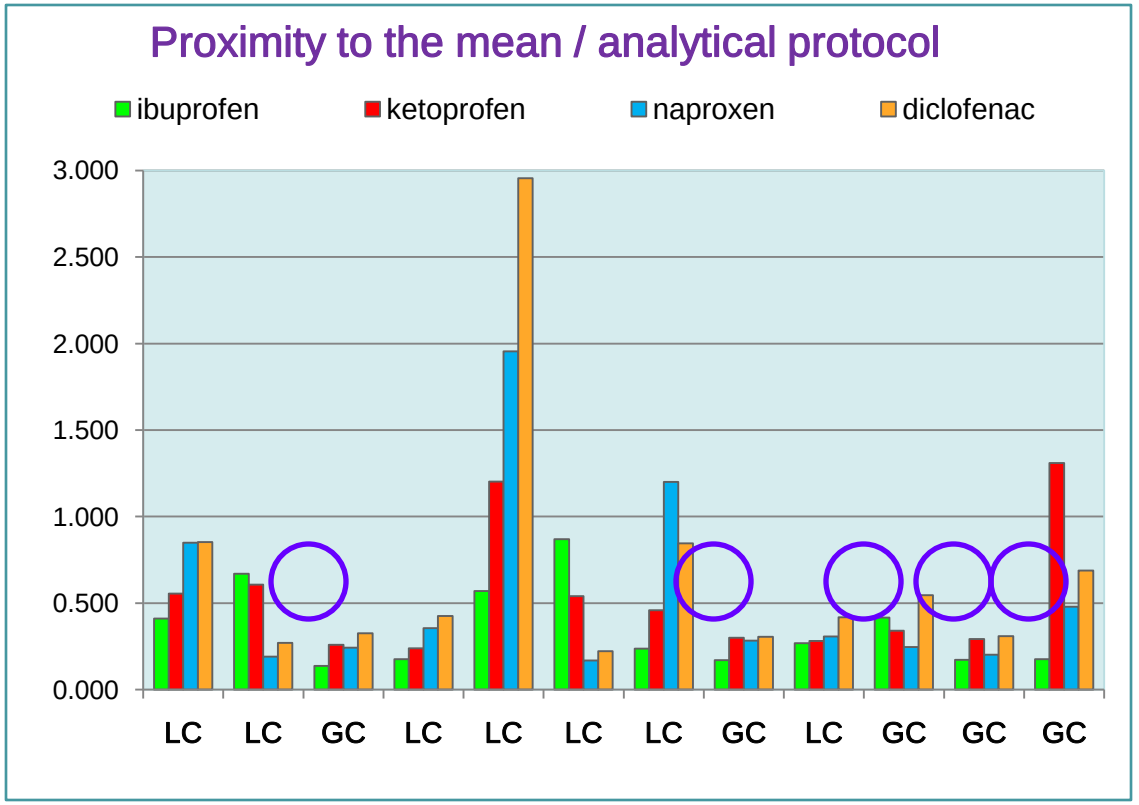
# 2nd INTERLABORATORY STUDY

## Results

### Proximity to the mean – according to the analytical protocol

- The deviations from the mean did not depend on the analytical protocol used

- * The mean is average; not neccessarily true mean value (eventhough it's close according to ISO/DIS 13528)



## Analytical issues

### Homogeneity of samples

- to assure and confirm the quality of sample preparation
- measured on spiked samples: A2 & A3, B2 & B3, C2 & C3
- Five samples taken from different layers in polyethylene bucket; two parallels analysed per each sample

$x^2$ test: $x^2 = \sum \frac{(O_i - E_i)^2}{E_i}$ *$O_i$: observed value; $E_i$: average*

- *H0* hypothesis: the determined concentrations are only affected by random error  homogeneity of mixing
-

$X^2_{exp.} < X^2_{crit}$ at Df = 4; α = 5%  homogeneity

- Confirmed for *A2 & A3, C2 & C3, B2 & B3* samples

## Analytical issues

## Experimental design / homogeneity

| sample | matrix | Fortification level (ng/L) |
|---|---|---|
| A1 | wastewater | - |
| A2 | fortified wastewater | A2 = A3 |
| A3 | fortified wastewater | A2 = A3 |
| B1 | river water | - |
| B2 | fortified river water | B2 = B3 |
| B3 | fortified river water | B2 = B3 |
| C1 | spiked deionised water | ibuprofen: C1 = C2 = C3 |
| C2 | spiked deionised water | C2 = C3 |
| C3 | spiked deionised water | C2 = C3 |

## Analytical issues

### Stability between SPE and analysis

- Stability in different matrices was assessed during 1st Interlaboratory exercise
- 2nd Interlaboratory exercise: request to extract immediately after the sample receipt  no need to confirm the stability in matrices; instead, assessment of stability between SPE and analysis
- Uneluted cartridges for stability tests were kept in a fridge since extracted (June 2007)
- 6 - 10 samples per a batch (A, B, C)
- Elution and analysis: June 2007, September 2007, May 2008

## Analytical issues

# Effect of filtration: experimental design

| sample | matrix | Fortification level (ng/L) | filtration |
|---|---|---|---|
| A1 | wastewater | - | YES |
| A2 | fortified wastewater | A2 = A3 | YES |
| A3 | fortified wastewater | A2 = A3 | NO |
| B1 | river water | - | YES |
| B2 | fortified river water | B2 = B3 | YES |
| B3 | fortified river water | B2 = B3 | NO |
| C1 | spiked deionised water | ibuprofen: C1 = C2 = C3 | YES |
| C2 | spiked deionised water | C2 = C3 | YES |
| C3 | spiked deionised water | C2 = C3 | NO |

## Analytical issues

## Effect of filtration

- filtration / NO filtration
- effect of matrices

- Samples numbered with “2” and “3” are parallels;
  2: filtration performed prior to SPE
  3: NO filtration

### Three statistical tests for determination of filtration effect:

1.) F-test for comparison of “2” and “3” variances within each batch (A, B, C)

$$F_{exp} = \frac{s^2_{\text{sample No.2}}}{s^2_{\text{sample No.3}}}$$

H0 hypothesis: samples were drawn from the same group (assuming normal distribution)  filtration had no effect; Df (degree of freadom) = 10 - 12; α = 5%

## Analytical issues

## Effect of filtration (cont'd)

**2.) t-test:** Paired two sample test for comparison of "2" and "3" means within each laboratory

$$D_{i(2)} = (x_{i(2)} - \overline{x_{(2)}})$$
$$D_{i(3)} = (x_{i(3)} - \overline{x_{(3)}})$$

$$t = (\overline{x_{i(2)}} - \overline{x_{i(3)}}) \sqrt{\frac{n(n-1)}{\sum (D_{i(2)} - D_{i(3)})^2}}$$

where $D_{i(2)}$ is laboratory bias in series "2" and $D_{i(3)}$ is laboratory bias in series "3"
Df (degree of freedom) = n-1; α = 5%.

- **Results:**
  - F-test: filtration had no effect except for naproxen in deionised water
  - t-test: in all cases filtration showed no effect on the analyses

## Analytical issues

# Effect of filtration (cont'd): ibuprofen in series C

- filtration / NO filtration
- effect of matrices

- Samples numbered with "2" and "3" were not additionally spiked with ibuprofen;
  1 and 2: filtration performed prior to SPE
  3: NO filtration

### 3.) One-way Analysis of Varianes (ANOVA) for comparison of "1", "2" and "3" variances within each batch (A, B, C)

Anova: Single Factor

SUMMARY

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

- H0 hypothesis: samples were drawn from the same group (assuming normal distribution)
  - filtration had no effect

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 | | | | |

- **Result:** filtration had no effect on determination of ibuprofen in batch C

## Analytical issues

### Effect of filtration:

- filter material

- Different types of filter material were used: glass fibre, nitrocellulose membrane, nylon membrane, cellulose acetate
- Smallest pore size: 0,45 µm
- Filtered samples (numbered “1” and “2”) were devided into two groups within each series in batches A and B :
  - glass fibre filters (G1: 7 laboratories)
  - membrane filters (G2: 5 laboratories)
- F-test for comparison of “G1” and “G2” variances within each batch for each NSAID

$$F_{\exp} = \frac{s^2_{G1}}{s^2_{G2}}$$

- H0 hypothesis: samples were drawn from the same group (assuming normal distribution)
  - filter material had no effect

# Conclusions:

- The final number of participants was 12
- The final number of results collected was 773
- The final number of 428 values was pooled out for further data analysis
- Between them 15 (3,5 %) were outliers
- Outlier values by liquid chromatography was 12 (4,7% of LC results)
- Outlier values gas chromatography was 3 (1,7 % of GC results)
- Oposite distribution of outliers than 1st round
- Tip: number of outliers would significantly decrease (up to 47%) by improving naproxen det. (Lab 7) and ketoprofen det. (Lab 13)

  ≠

  number of outliers cannot be used as a measure for assessment of method capability, better: parameter decribing a lab performance
- The sample matrix yielding the highest number of outliers: deionised water
  - as 1st Interlab
  - 47%
  - possible reason: lower level of concentration (greatest RSD)
- The stability of samples: measured in June, Sept. (2007) and May 2008
  - no significant difference June and Sept, 2007
- A general good agreement was obtained between the concentrations of fortification and the mean values recorded by the participants (not exact V)

# 2nd INTERLABORATORY STUDY

- 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 final determination of NSAIDs in each of the relevant matrices was studied by comparison of filtered and unfiltered parallel samples. Except for naproxen in deionised water, the filtration did not reveal a statistically significant effect on the results. Also, the filter material did not reveal an influence on determination of NSAIDs in all relevant matrices.

- For the results statistically incorporated into the same original group (with respect to the pre-filtration of matrices) the repeatability and reproducibility were calculated resulting in dispersion (R) ranging from 21 (IP in C1 & C2 & C3) to 904 (DF in river water).

# 2nd INTERLABORATORY STUDY

- 1st NORMAN Interlaboratory Exercise focusing on the stability of compounds during sample storage under freezing conditions
- 2nd round avoided the weaknesses recognized in the 1st round

  samples shipped on dry ice and 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.

- 1st and 2nd Interlaboratory Exercise Conclusions:
  - shipping samples on dry ice, as well as predetermined laboratory protocol contributed towards reduced number of outliers and improved the laboratory performance.
  - pre-filtration test: results 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.

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*CSIC and JSI team*

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