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Inter-laboratory exercise on antibiotic drugs analysis in aqueous samples

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ABSTRACT

An inter-laboratory exercise was organized under the PHARMAS EU project, by the Advanced School of Public Health (EHESP), in order to evaluate the performances of analytical methods for the measurement of antibiotics in waters (surface and tap). This is the first time such an exercise on antibiotics has been organized in Europe, using different kinds of analytical methods and devices. In this exercise thirteen laboratories from five countries (Canada, France, Italy, the Netherlands and Portugal) participated, and a total number of 78 samples were distributed.

During the exercise, 2 testing samples (3 bottles of each) prepared from tap water and river water, respectively, spiked with antibiotics, were sent to participants and analyzed over a period of one month.

A final number of 77 (98.7%) testing samples were considered. Depending on substances studied by each participant, 305 values in duplicate were collected, with the results for each sample being expressed as the target concentration.

A statistical study was initiated using 611 results. The mean value, standard deviation, coefficient of variation, standard uncertainty of the mean, median, the minimum and maximum values of each series as well as the 95% confidence interval were obtained from each participant laboratory.

In this exercise, 36 results (6% of accounted values) were outliers according to the distribution over the median (box plot). The outlier results were excluded.

In order to establish the stability of testing samples in the course of the exercise, differences between variances obtained for every type of sample at different intervals were evaluated. The results showed no representative variations and it can be considered that all samples were stable during the exercise.

The goals of this inter-laboratory study were to assess results variability when analysis is conducted by different laboratories, to evaluate the influence of different matrix samples, and to determine the rate at which participating laboratories successfully completed the tests initiated.

1. Introduction

'Pharmaceuticals in the Environment' is no longer a new issue but recently it has become a priority concern, particularly for politicians and the general public. Indeed, the occurrence of active pharmaceutical ingredients in water bodies is an undesired side effect of their normal use. In order for the substances to develop their intended effects in the human body, sufficient intact molecules must reach the diseased cell before they are broken down into a multitude of metabolites by the body's biochemical processes. In order to achieve this goal, pharmaceuticals are optimized for stability. This has two consequences: on the one hand, the active ingredients are not metabolized completely in the human body but excreted primarily via urine and thus reach domestic wastewater [1]; on the other hand, the desired stability of the molecules hinders their biological degradation in conventional sewage treatment plants [2–4].

Many of the active ingredients studied so far are only partially removed, whilst others are not removed at all. On reaching surface water (rivers and lakes), stable molecules can then make their way into drinking water and finally – via groundwater – back to humans [5–9].

According to the published literature, it appears that most pharmaceuticals do not pose a threat to the environment, although a small number do, and that none appear likely to pose

Abbreviations: ERY, erythromycin; CIP, ciprofloxacin; OFL, ofloxacin; SMX, sulfamethoxazole; TMP, trimethoprim; LOQ, limit of quantification; RRLC/MS/MS, rapid resolution liquid chromatography/mass spectrometry in tandem; s_s, between-sample standard deviation; s_w, the standard deviation of the concentration; $\bar{x}$, the mean value; σ, the population standard deviation; *CV*, coefficient of variation; *u*, standard uncertainty of the mean; *M*, median value; Min, minimum value of a series; Max, maximum value of a series; *D*, bias

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a significant threat to human health (via environmental exposure) [10–13]. But this apparent certainty that no human health risk is posed by the presence of human pharmaceuticals in the environment is not supported by more in-depth thinking. The real situation is that, currently, there are many more uncertainties than certainties, which leaves the public and the press still unconvinced that drinking water containing a tiny quantity of a pharmaceutical is completely harmless.

In this context, the Pharmas project (N° 265346, www.pharmas-eu.org) has been funded under the 7th European framework programme to significantly advance the science, so that risks to the environment and public health can be more accurately defined. More precisely, Pharmas develops a general procedure taking into account scenarios of exposure to human pharmaceuticals, their effect and impact on organisms, in order to define the range of possible risks for these populations. In addition, social and policy considerations are considered in order to, respectively, inform the public and to support appropriate changes in regulation. The project focuses on the groups of pharmaceuticals considered most likely to be a threat to the environment and/or human health, in particular antibiotics, the presence of which has been reported extensively and that are among the most investigated class of pharmaceuticals in the environment including drinking water [11–16].

Within the framework of the project, the implementation of efficient risk assessment procedures requires preliminary production of reliable data in particular concerning the exposure data. Thus an intercalibration study was performed in order to assess and understand the uncertainty of the data produced by laboratories.

The exercise was conducted among thirteen laboratories covering different EU countries (+Canada) using their own analytical protocols. To our knowledge, such an exercise for antibiotics is the first one performed in these conditions. Other interlaboratory exercises have been performed for other pharmaceutical products (anti-inflammatory in the frame of Norman network for example, individual antibiotics), but based on same analytical procedures [17–20].

In this study, 2 samples were analyzed over a period of one month. The reference samples (homemade and calibrated solutions) correspond to 3 glass bottles of tap water for the first and 3 glass bottles of river water (filtered sample from La Vilaine river in Brittany, France) for the second. 5 antibiotics were selected for the exercise: erythromicyn (ERY), ciprofloxacin (CIP), ofloxacin (OFL), sulfamethoxazole (SMX) and trimethoprim (TMP). This choice was dictated taking into account the performances of the participant laboratories (via a questionnaire) and the organising laboratory's ability to produce reference samples.

The principal aims of the inter-laboratory study were to evaluate the variability of results between different laboratories and to evaluate the rate at which participating laboratories successfully completed the exercise.

2. Participant profiles

13 laboratories participated in the exercise. 5 countries were represented (Canada, France, Italy, The Netherlands, and Portugal). One laboratory used two analytical methods. 14 labs were then considered. For reasons of anonymity, they have been randomly numbered from lab1 to lab14.

Participants were offered a questionnaire aimed at assessing their profiles and analytical performances in terms of analysis of the five antibiotics (Tables 1 and 2).

Table 2 has considered the limit of quantification of each laboratory. *n* is the number of laboratories analysing the corresponding substances. Min and Max correspond to the lower and higher value of LOQ among the laboratories. Median and mean have been calculated, for each substance, from the n value of LOQ declared by the laboratories.

Table 1
ILE participant profiles.

Questions	Answer
ILE participation	Yes: 54% (7/13)
Experience in analysis of ATB in water	<2 years: 38.5% (5/13) >6 years: 23% (3/13) 3–5 years: 38.5% (5/13)
Analysis frequency of ATB in participants laboratories	46% (6/13) several times/week 46% (6/13) at least once every 6 months 8% (1/13) not frequent

Table 2
Analytical performances of participating laboratories.

	n	LOD distribution (ng/L)			
		Min	Max	Mean	Median
Erythromycin (ERY)	10	1	500	80.3	10
Trimethoprim (TMP)	10	1	50	16.6	5.5
Sulfamethoxazole (SMX)	9	1	50	13	10
Ciprofloxacin (CIP)	8	1	100	32.13	20.5
Ofloxacin (OFL)	7	1	20	12.2	11

3. Experimental

3.1. Chemicals and solutions

Acetonitrile (HPLC grade, JT Baker, distributed by Atlantic labo ICS, Bruges, France), methanol (HPLC grade, JT Baker, distributed by Atlantic labo ICS, Bruges, France), formic acid (JT Baker, distributed by Atlantic labo ICS, Bruges, France), NH_4OH 25% (Carlo Erba, Val de Reuil, France), sodium nitrite 99% (ACS Reag. PhEur, Merck) and ultrapure water were used for the preparation of standard solutions.

Antibiotics are commercialized in powder form with purity between 97 and 99.9%. Trimethoprim standard was purchased from VWR (Fontenay sous Bois, France; certified quality, from Dr. Ehrenstorfer GmbH, Bgm.-Schlosser-Str. 6 A, 86199 Augsburg, Germany). Standards of other antibiotics were purchased from Sigma Aldrich (St Quentin Fallavier, France). Life durations are not certified by the provider but 3 years of preservation are guaranteed by a certificate.

Stock solutions of individual compounds were prepared in HPLC grade methanol. CIP and OFL solutions were prepared as 0.5 g/L; the others at 1 g/L. CIP solution required the addition of NaOH 1M to increase solubility of CIP in methanol.

Individual solutions (DS) were prepared at 0.5 mg/L in HPLC grade acetonitrile by two successive dilutions of the stock solutions and were preserved at −20 °C.

Reference samples were characterized (using stability and homogeneity tests) by RRLC/MS/MS (rapid resolution liquid chromatography/mass spectrometry in tandem) using isotopic internal standards. Internal standard solutions were individually prepared from: Ofloxacin-d3, Trimethoprim-13C, Ciprofloxacin-13C-15N, Sulfamethoxazole-13C, Erythromycin-13C purchased from Sigma Aldrich (St Quentin Fallavier, France).

Stock solutions of OFL-d3 and SMX-13C were prepared in HPLC grade methanol (at 1 g/L) followed by dilution in acetonitrile up to a solution at 10 mg/L. Stock solutions of ERY-13C, TMP-13C, CIP-13C-15C were prepared directly in acetonitrile (at 12 mg/L). Finally, a mixture standard solution of all isotopic reference compounds was prepared at 0.5 mg/L in acetonitrile and preserved at −20 °C.

3.2. Reference sample preparation

Two reference samples (sample A and sample B) were prepared corresponding to treated (distributed) water and filtrated surface water, respectively. Both samples were spiked with the 5 antibiotics in which we are interested. Physico-chemical properties and content of organic carbon and free residual chlorine for samples A and B are listed in Table 3. The measurement of all ATB before spiking shows concentration below the limit of detection.

In order to minimize sources of variation, samples A and B were collected, homogenized and prepared at EHESP-LERES, Rennes, France. River water (70 L) was collected and transported to the laboratory where it was filtered through 0.7 μm glass fibre filters. Treated water (70 L) was directly collected from the laboratory tap and free chlorine was removed with sodium nitrite. Afterwards, waters were spiked, homogenized, and sub-sampled for homogeneity and stability testing.

Triplicates of samples A and B were prepared for each laboratory by transferring each sample into 3 × 1 L amber glass bottles.

3.3. Homogeneity of samples

Homogeneity of the reference solution was tested according to ISO 13528 guidelines. Treated (sample A) and surface water (sample B), were sub-sampled after spiking and homogenization. A total of ten subsamples per sample were taken from different layers in the container. Two parallels (duplicate) were analyzed per each sample, in total 20 samples were analyzed per each sample. The homogeneity was statistically evaluated by using the comparison of the between-sample standard deviation to the total standard deviation. According to ISO 13528, the ratio of between-samples standard deviation to total standard deviation must be below 0.3.

Between-sample standard deviation has been calculated as follows:

$$s_s = \sqrt{s_x^2 - \left(\frac{s_w^2}{2}\right)}$$

where

- $s_x = \sqrt{\frac{\sum (x_i - \bar{x})^2}{(n-1)}}$ is the standard deviation of the mean concentration of n samples.
- $s_w = \sqrt{\frac{\sum w_i^2}{2n}}$ is the standard deviation of the concentration of n samples. with
- n the number of samples,
- $x_{i,k}$ are the data (i represents the sample and k the parallel, $k=2$),
- $x_{i,} = (x_{i,1} + x_{i,2})/2$ is the mean concentration of the samples,
- $w_i = |x_{i,1} - x_{i,2}|$ is the range of duplicate concentration,
- $x^G = \sum x_i / n$ is the general mean.

Table 4 shows the calculation of the between-sample standard deviation for each antibiotic in both samples.

The results of the controls showed that the samples for that homogeneity target are not notably different. It can be concluded that reference samples A and B may be considered homogeneous; this means that all the manufactured samples may be considered homogeneous for a given proficiency test.

Ciprofloxacin in sample A was found problematic to detect in some of the laboratories involved in its measurement, probably due to low concentration and the effect of the matrix. Consequently, the number of values obtained was too low to conduct a statistical analysis.

3.4. Stability of reference samples

Verification of stability was assessed using the same protocol as for verification of homogeneity. Temperature preservation and storage time were assessed for evaluation of the stability of the reference samples.

Three sub-samples of each type of water were randomly selected during the reference sample preparation. They were preserved at room temperature (20 °C ± 2) and +4 °C during two, seven and ten days (total of 18 samples).

Each antibiotic was analysed in each sub-sample, in duplicate. The average concentration ($\bar{y}$) was compared with the average concentration of all sub-samples used for the stability and homogeneity tests ($\bar{x}$). The total standard deviation has been calculated from the results of the exercise for each antibiotic (Tables 5 and 6).

The stability of the reference sample is considered acceptable if $|\bar{x} - \bar{y}| \leq 0.3\hat{\sigma}$. Results showed that the reference samples can be considered as stable, both at room temperature and at +4 °C.

3.5. Sample distribution

Samples were shipped by express service on dry-ice to the participant laboratories on October, 5th, 2011. A total of 78 bottles were sent to 13 participants (3 bottles each, of each sample). The samples arrived at participant laboratories in 24 to 72 h, in frozen state.

Table 3
Characteristics of the matrices used to prepared samples A and B.

Parameters	Distributed water (LERES tap)	Raw water after filtration (0.7 μ)
pH at 20 °C	7.85	7.95
Conductivity at 25 °C (μS/cm)	697	516
Total organic carbon (mg/L)	1.3	7.3
Dissolved organic carbon (mg/L)	1.3	7.0
Total chlorine (mg/L)	0.2	< 0.1
Free chlorine (mg/L)	< 0.1	< 0.1

Table 4
Ratios between S_s (between-samples standard deviation) and σ (standard deviation of the round calculated from the results of the laboratories) for all the homogeneity controls.

Target	Sample A			Sample B		
	s_s	σ	s_s/σ	s_s	σ	s_s/σ
ERY	7.1	43.1	0.16	11.7	252.9	0.04
OFL	2.7	21.6	0.13	9.6	83.1	0.12
CIP	ND			11.0	62.4	0.18
SMX	2.6	6.8	0.38	2.8	26.7	0.1
TMP	4.5	12.8	0.35	0.8	33.0	0.02

3.6. Analytical methods

No standard recommendation was sent to the participants, either for the sample treatment (extraction, preconcentration) or for the analytical method. Participants were required to extract and analyze samples within a maximum of 2 and 10 day after receipt, respectively.

In general, antibiotics were extracted from surface and treated water samples by solid phase extraction and analyzed by liquid chromatography with mass spectrometry detection.

Table 7 lists the participant laboratories as well as the main characteristics (where provided) of the analytical methods used.

Table 5
Concentration values (ng/L) for verification of stability in treated (distributed) water.

SAMPLE A: treated water					
		OFL	TMP	SMX	ERY
$\bar{x}$		28.63	43.76	26.81	43.03
σ		21.6	12.8	5.7	43.1
$\bar{y}$	Room T° Day 2	33.81	40.67	24.29	44.74
	Room T° Day 7	28.43	43.04	24.45	38.04
	Room T° Day 10	30.43	42.36	23.87	41.81
	4 °C Day 2	25.86	42.42	25.47	46.97
	4 °C Day 7	26.13	43.23	25.19	41.92
	4 °C Day 10	20.33	41.8	24.45	41.79

Table 6
Concentration values (ng/L) for verification of stability in surface water.

SAMPLE B: surface water						
		OFL	TMP	CIP	SMX	ERY
$\bar{x}$		208.96	121.06	179.35	116.21	164.85
σ		83.1	33	62.4	26.7	252.9
	Room T° Day 2	234.2	106	211.44	104.48	162.09
	Room T° Day 7	200.81	120.63	156.5	112.07	171.81
	Room T° Day 10	207.27	117.77	168.3	104.59	162.02
	4 °C Day 2	234.65	117.94	215.3	112.41	169.14
	4 °C Day 7	205.91	123.7	169.1	119.72	184.86
	4 °C Day 10	206.37	120.48	168.24	118.14	178.04

4. Evaluation procedure

Statistical evaluation was executed according to ISO 13528 "Statistical methods for use in proficiency testing by inter-laboratory comparisons".

For each series, the mean value ($\bar{x}$), the standard deviation (σ), coefficient of variation (CV), standard uncertainty of the mean (u), median (M), the minimum (Min) and maximum (Max) values of each series, as well as the 95% confidence interval over the mean have been calculated (by using StatSoft (2011). STATISTICA (data analyses software), version 10. www.statsoft.fr) and reported after exclusion of outlier values.

In this study, uncertainty of the measurement is represented by the standard uncertainty of the mean (u) because of the lack of information of the other source of uncertainty in each laboratory. The uncertainty is then obtained by the ratio: $u = (\sigma/\sqrt{n})$ with n the number of measurement and σ the standard deviation. The uncertainty of the measurement for each substance in all individual laboratories has been also assessed.

Outlier values were identified with the interquartile range test (test representing the distribution of the data toward the median) by using the graphical representation box plot. This standardized representation is based on five numbers: minimum, first quartile, median, third quartile, and maximum. In the simplest box plot the central rectangle spans the first quartile to the third quartile. A segment inside the rectangle shows the

Table 7
Analytical methods used by the different participants.

Participant	Pre-treatment	Extraction cartridges	Extraction solvent(s)	Internal standard	Separation/ detection	Chromatographic column
1	Before extraction 2.5 mL EDTA solution of 2.5 g/L added (pH8), and pH changed to 3.2 with 100 uL formic acid	SPE Oasis HLB	n.c	Ciprofloxacin 13C3 Sulfamethoxazole 13C6	UPLC-MS–MS	Acquity BEH 50 × 2.1 mm; 1.7 um
2	None	SPE Oasis HLB	Methanol/Ethyl acetate (50/50)	13 C compound	UPLC-MS/MS	Hypersil Gold 1.9 μm-2.1∗100 mm
3	None	SPE reverse phase	n.c	None	LC-MS/MS	Acquity HSS-T3
4	None	SPE ENVI CHROM-P 250 mg	Methanol	Carbamazepine D10	HPLC-MS/MS	XBridge C18 3.5 um, 2.1∗150 mm Column
5	Acidification pH2	SPE JTBaker H_2O philic	n.c	None	HPLC-MS–MS	Varian Pursuit UPS (2.1 mm × 50 mm × 2.4 um)
6	n.c	n.c	n.c	n.c	HPLC-MS/MS	Zorbax eclipse plus C18
7	n.c	SPE Oasis HLB	Acetone/methanol/ acetonitrile	Yes	HPLC-MS/MS	Acquity BEH C18, 2.1∗150 mm, 1.7 μm
8	n.c	Polymeric reversed phase	n.c	Yes	HPLC-MS/MS	C18
9	n.c	SPE Oasis HLB (pH 7)	Methanol	Yes	HPLC-MS/MS	Agilent ZORBAX eclipse plus C18 (3,5 um 2,1∗100 mm)
10	n.c	SPE Oasis HLB	Acetonitrile	Carbamazepine D		Polaris
11	Cooling+dark	SPE Polymer	n.c	Yes	HPLC-MS	C18
12	Cooling+dark	SPE polymer	n.c	Yes	HPLC-MS/MS	C18
13	pH 6.95 ± 0.2	SPE water HLB	n.c	13C3-analog	HPLC-MS/MS	Thermo Betasil, 2.1 × 100 m
14	pH 7	SPE Oasis HLB	Methanol	13C compound	RRLC-MS/MS	Zorbax Eclipse Plus C18 (Agilent) 2.1∗100 mm, 1.8 μm

n.c: not communicated; HPLC: high performance liquid chromatography; UPLC: ultra performance liquid chromatography; RRLC: rapid resolution liquid chromatography; MS: mass spectroscopy; SPE: solid phase extraction.

median, and "whiskers" above and below the box show the locations of the minimum and maximum values.

Outlier values are identified if:

- value < 1st Quartile-1,5∗InterQuartile Range and
- Value > 3rd Quartile+1,5∗InterQuartile Range

The ***interlaboratory variability*** for each substance was estimated by calculation of the coefficient of variation (*CV*). It is expressed, for each substance, as a % and represents the ratio of the standard deviation (σ) to the mean of all recorded values ($\bar{x}$)

$$CV = 100 * \frac{\sigma}{\bar{x}}$$

Table 8
Reference value for each antibiotic in the two matrices[a].

	Sample A: treated water				Sample B: surface water				
	ERY	OFL	SMX	TMP	ERY	CIP	OFL	SMX	TMP
Spiking level (ng/L)	55	40	30	50	200	180	200	100	150

[a] Concentration of ATB in the two matrices was < LOD before spiking.

Performances of the participants were assessed by the estimation of the intralaboratory variability (repeatability) and the bias.

For each laboratory, ***individual repeatability*** (CV_{lab} in %) was calculated, by the comparison of the mean ($\bar{x}_{lab}$) of the recorded values (3 to 6 replicates) for a substance with the standard deviation (σ_{lab}) according to the relation:

$$CV = 100 * \frac{\sigma_{lab}}{\bar{x}_{lab}}$$

Laboratory biases (*D*) were estimated for each set of results (or average of results) reported by each participant. *D* corresponds to the difference between the value (or average value) of laboratory (x_i) with the reference value (*X*):

$$D = x_i - X$$

According to ISO 13528, the biases were classified into three categories: $D \geq 3.0\sigma$ indicating an "action signal", $2.0\sigma \leq D < 3.0\sigma$ considered as "warming signal" and $-2.0\sigma \leq D < 2.0\sigma$ indicating "acceptable value". The outlier results were excluded from the calculation of *D*.

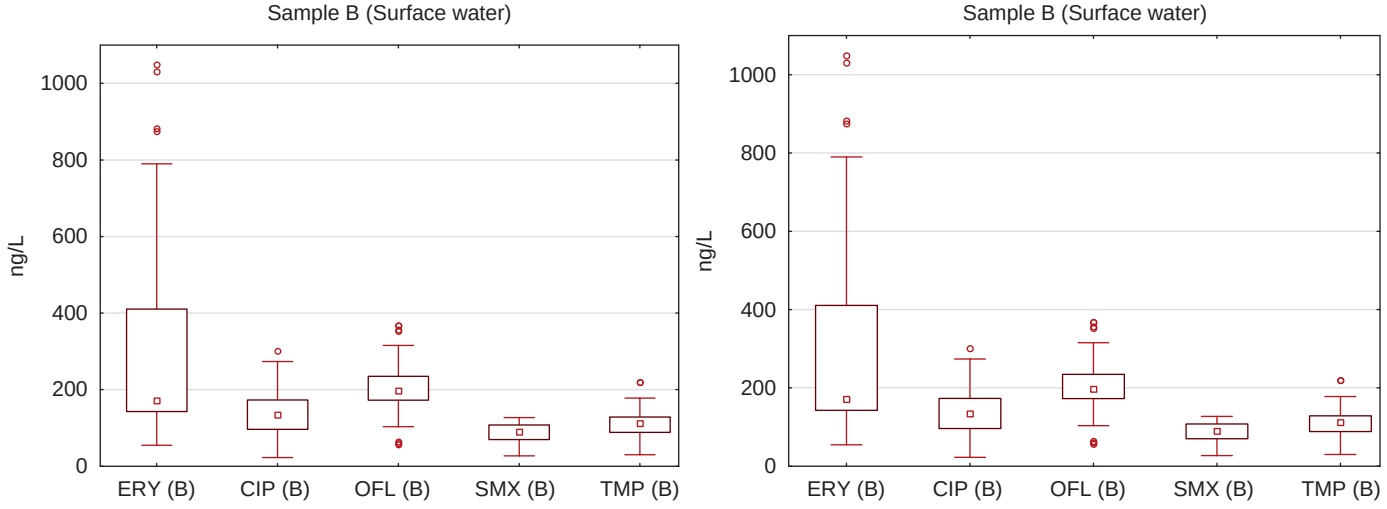

Fig. 1. Box plot diagram for the distribution of the value for treated water and surface water (circle represent outliers).

Table 9
Statistical values corrected after outlier exclusion for each compound in the different types of water: mean (*x*), standard deviation (σ), coefficient of variation (*CV*), standard error of mean (σ_M), median (*M*), minimum value (Min), maximum value (Max).

Substance *Matrix*	**Spiking level (ng/L)**	**No. of accepted results**	***x* (ng/L)**	**σ (ng/L)**	***CV***	***u* (ng/L)**	***M* (ng/L)**	**Min (ng/L)**	**Max (ng/L)**	**95% confidence interval**		**No. of outliers**
										From	**To**	
Cyprofloxacin												
Surface Water (B)	180	54	135.0	59.0	0.44	8.02	134.4	22.6	273.6	118.9	151.1	1
Erythromycin												
Treated water (A)	55	56	53.6	26.7	0.50	3.56	44.8	13.7	136.9	46.4	60.7	4
Surface water (B)	200	54	247.9	181.2	0.73	24.7	165.5	54.7	790.0	198.4	297.4	4
Ofloxacin												
Treated water (A)	40	54	45.2	21.6	0.48	2.9	40.4	6.3	93.1	39.3	51.1	0
Surface Water (B)	200	45	198.6	48.7	0.25	7.3	196.6	103.4	315.5	184.0	213.2	9
Sulfamethoxazole												
Treated water (A)	30	70	20.0	6.4	0.32	0.76	19.83	5.6	30.5	18.5	21.5	6
Surface water (B)	100	76	87.1	26.7	0.27	3.07	89.3	26.9	127.1	81.0	93.2	0
Trimetoprim												
Treated water (A)	50	74	37.3	9.3	0.25	1.08	38.6	18.9	64	37.2	39.5	10
Surface water (B)	150	80	105.8	32.9	0.31	3.67	110.7	30	178	98.5	113.1	2
Total		**563**										**36**

5. Results

A total number of 13 participants took part in this study, using methods detailed in Table 7. One participant used two analytical methods. We have considered 14 series of data. Each participant received 3 bottles of sample A (spiked treated water) and 3 bottles of sample *B* (spiked surface water). Statistical analysis was performed by considering the spiking value as the reference value of the antibiotic concerned (Table 8).

5.1. Accepted values and outliers

The different antibiotics were not measured by all laboratories: ERY, CIP, OFL, SMX and TMP gave 11, 9, 9, 13 and 14 series of data, respectively. A total number of 611 results were collected.

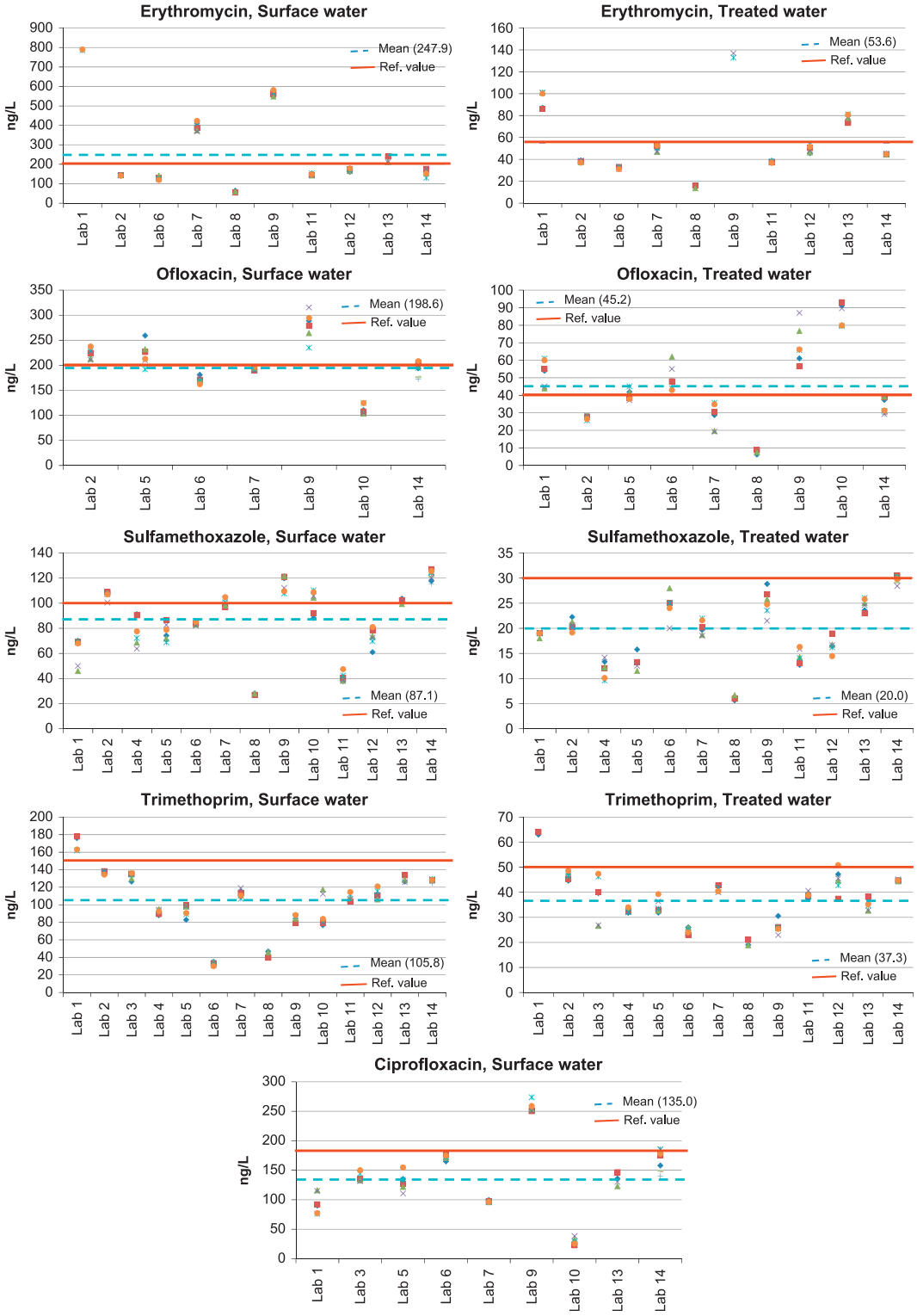

Fig. 2. Results obtained for each participant for ERY, OFL, SMX, TMP and CIP expressed in ng/L in the different samples, and mean values of results.

A first selection was operated by considering the LOQ of each laboratory compared to the spiking values and data from laboratories with LOQ > spiking level was excluded. 1 series of ERY in sample A and 1 series of ERY in sample B were excluded.

In a second time, the normality of each series has been tested by using the Kolmogorov–Smirnov test. Excepted for ERY (in both A), a normal distribution was observed. Consequently, and in order to be homogeneous, the interquartile range test (non parametric) has been used to exclude outlier values.

Fig. 1 shows the distribution of the values for each series and highlight the outliers: in sample A, 4, 6 and 10 outliers for ERY (60 values), SMX (76 values) and TMP (84 values) series, respectively and in sample B, 4, 1, 9 and 2 outliers for ERY (58 values), CIP (55 values), OFL (54 values) and TMP (82 values) series, respectively.

Finally, the calculation gave 36 outliers (6% of the total number of results, see Table 9). The outliers were not distributed evenly across all labs, but only in 4 of them.

According to the difference between the analytical protocols used and the number of produced value (600), the number of outliers obtained in this exercise can be considered low. The highest number of outliers was determined for treated water, probably due to the lower concentration levels and the limitation in analytical performances. Such results have been also shown in the two inter-laboratory exercises on anti-inflammatory drugs [9,17].

The outlier values were excluded for the final data treatment and the statistical parameters (mean values, standard deviation, variance, coefficient of variation) were calculated. Table 9 shows the corrected statistical values (after outlier exclusion) obtained for each compound in the different samples.

5.2. Results by molecules and matrices

Results from the different laboratories, mean between laboratories and reference values of each antibiotics in the two matrices are plotted in Fig. 2.

Except for ERY in sample A and OFL in sample B, the mean of all values is below than the reference value for each substance.

The deviation between the two values (calculated by the ratio between the observed mean concentration and the reference one) is important for SMX in surface water (33%) and TMP in treated water (29%). It corresponds to 2.6%, 13% 25%, 25.4% in surface water for ERY, OFL, TMP, CIP, respectively and to 23.5%, 0.7%, 13% in treated water for ERY, OFL, SMX.

This difference can be considered as representative in the case of SMX in treated water and TMP in surface water because the value is higher than the standard deviation (Table 9).

5.3. Interlaboratory variability

A comparison of the *CV* values (Fig. 3) in both matrices (surface and treated water) for all laboratories resulted in a slight difference. The highest *CV* was observed for erythromycin in both matrices. It is particularly high in surface water (highest concentration). In addition the highest uncertainty was found in the determination of ERY in surface water (Table 9).

The lowest *CV* was observed for sulfamethoxazole and trimethoprim and is very closed in the two matrices in spite of a difference in the spiked concentration. It corresponds also to the lowest uncertainty values.

According to the heterogeneity of the methods employed, we can consider the *CV* relatively low for the targets except for erythromycin.

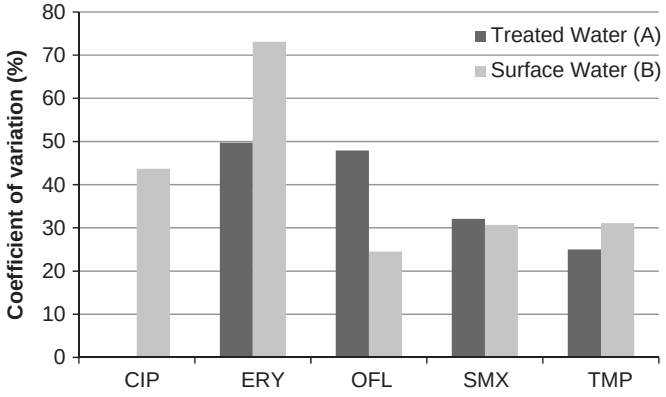

Fig. 3. Coefficient of variation for all laboratories.

5.4. Laboratory performances

Intralaboratory repeatability has been evaluated for each substance in the two matrices and for each laboratory by the calculation of the internal coefficient of variation (Fig. 4).

Good intralaboratory repeatability has been observed. *CV* varies from 0.5 to 25.5% (median 6.8%) and to 0.2 to 19.8% (median 5.0%) for sample A and B, respectively. This result illustrates the high potential of the different laboratories in producing representative data. This was confirmed by the calculation of the uncertainties of the measurement: range from 0.17 to 10.4% (median 3%) and from 0.14 to 8.1% (median 2.19%) for the individual lab in the analyses of the substances in sample A and B, respectively.

On the other hand, the difference between the values produced by each laboratory and the reference value was estimated by calculation of the bias.

By considering all values, the ISO 13528 classification of the laboratory biases resulted in 85% of acceptable values (falling outside the range $-2.0\sigma < D < 2.0\sigma$), 11% of values to be monitored and 4% of unacceptable values ($-3.0\sigma < D < 3.0\sigma$), as reported in Table 10.

Of the 14 participating laboratories, 5 showed excellent performance (all using internal standards), never reaching the range outside $-2.0\sigma < D < 2.0\sigma$, and only 2 laboratories having the warning signals.

Half of the signals (warming and action) come only from 2 laboratories, the analysis of antibiotic of which is very recent (below than two years) and with a frequency of analysis relatively low (at least once every 6 month).

Considering the ILE conditions (no analytical procedure recommendation) and the profile of the participant laboratories, the exercise can be considered as very positive. Indeed, around 40% of the laboratories have only 2 years of experience in the analysis of ATB and less than half of them analyses ATB routinely. Moreover, they show very interesting internal performances and an acceptable variability among them. However, some improvements are required for some of them to be very effective in the measurement of ATB. A second exercise, by using the same analytical protocol would be useful for a better comparison between the participants.

6. Conclusion

Pharmas Project is an FP7 European project dealing with human and ecological risk assessment for a selection of antibiotics and anticancer drugs. To perform it, accurate exposure data are necessary. Within the framework of the

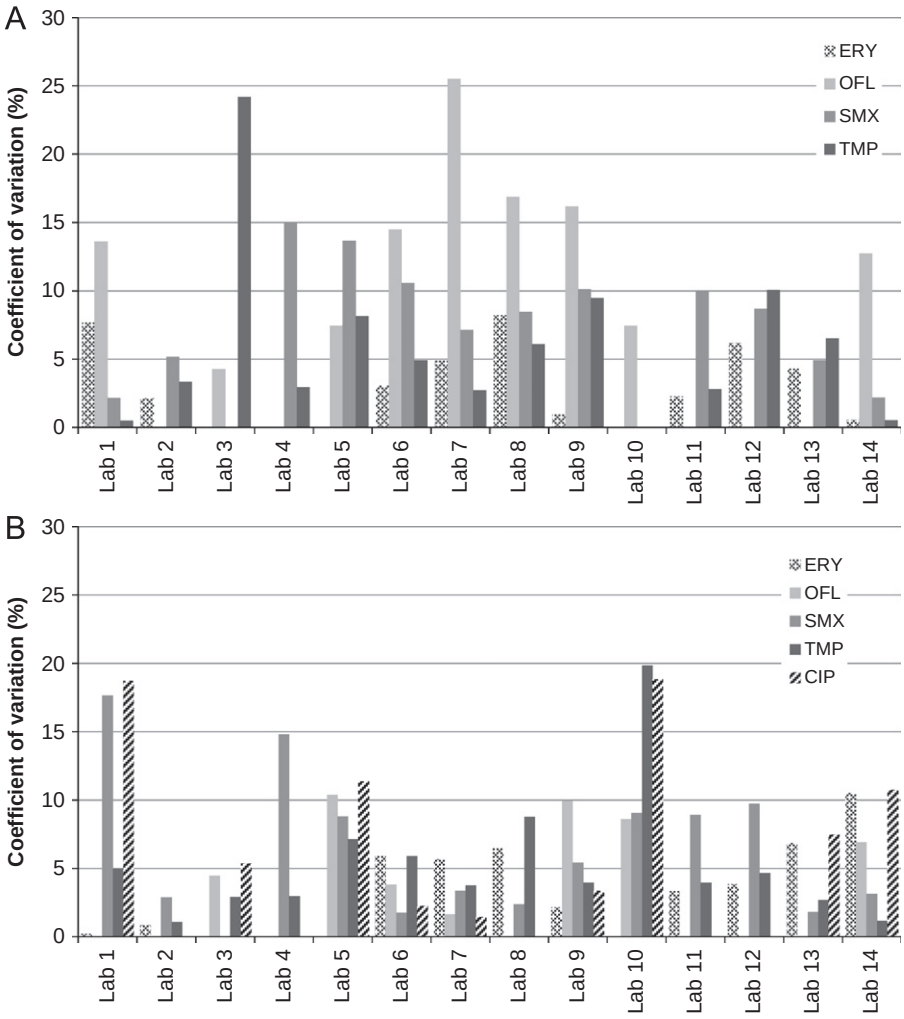

Fig. 4. Intra-laboratory repeatability in samples A (tap water) and B (surface water).

Table 10
Laboratory biases for each value reported by participant laboratories in the two matrices (surface (SW) and treated (TW) water). 0, 1 and 2 correspond to acceptable values, warning signal and action signal, respectively (grey boxes correspond to non produced data).

	ERY		CIP	OFL		TMP		SMX	
	TW	SW	SW	TW	SW	TW	SW	TW	SW
Lab 1	0	2	0	0		0	0	0	0
Lab 2	0	0				0	0	0	0
Lab 3			0	0	0	0	0		
Lab 4						0	0	1	0
Lab 5		0	0	0	0	0	0	1	0
Lab 6	0	0	0	0	0	1	0	0	0
Lab 7	0	0	0	0	0	0	0	0	0
Lab 8	0	0	0	0		2	2	2	1
Lab 9	1	1	0	0	0	1	0	0	0
Lab 10			1	1	0		0		0
Lab 11	0	0				0	0	1	1
Lab 12	0	0				0	0	1	0
Lab 13	0	0	0	0		0	0	0	0
Lab 14	0	0	0	0	0	0	0	0	0

project an inter-laboratory exercise was proposed to evaluate performance in the analysis of antibiotics in resource and tap (treated) water.

Thirteen participants from five different countries participated in the exercise. 77 reference samples were analyzed to determine concentration of selected antibiotics and 611 results (including parallels, excluding $<$LOQ) were collected for data evaluation. The final number of 563 values was pooled out for further data analysis, where 36 of them (6%) were determined to be outliers, according to the interquartile range test (box plot).

The sample matrix yielding the highest number of outliers was treated water (55%).

Finally, according to the scheme of the exercise (each laboratory implementing its own analyses), most of the participant show results in close agreement with the expected ones and the variability between them can be considered acceptable. However, it is difficult, from this study, to identify the limiting factors in the analysis of ATB and a second exercise, based on the same analytical protocol, would be necessary.

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