Inter-laboratory test on drug residues in water intended for human consumption

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Abstract. In June 2009, the Laboratory in hydrology of Nancy (Ansès-LHN, Nancy) conducted an exploratory inter-laboratory proficiency tests (ILT) test in collaboration with the AQUAREF network to identify laboratory practices and estimate inter-laboratory uncertainty for the compounds under study. There were a total of 12 compounds belonging to six families (hormones, antibiotics, non-steroidal anti-inflammatory drugs, beta blockers, antipsychotics and others) including one compound used in veterinary medicine. It was the first ILT conducted in France on water intended for human consumption. Thirty-one laboratories participated, 4 of which were located abroad.

Keywords: Interlaboratory test; hydrology; uncertainty; drugs; human consumption

1 Introduction

In accordance with French regulations and the recommendations of the French Accreditation Committee (COFRAC) in French Standard NF EN 17025, laboratories are required to take part in inter-laboratory proficiency tests (ILPT). The goal of these ILPT is to verify the laboratories’ analytical proficiency for given parameters and methods. Exploratory ILT aim to provide a snapshot of the profession at a point in time \( t \) in preparation for a method’s standardisation.

In this context, Ansès’s Laboratory in hydrology of Nancy, in collaboration with the AQUAREF network, organised an inter-laboratory test on drug residues in June 2009. After consultation with the various participants, a list of 12 compounds was established. This list included various families of human compounds (hormones, antibiotics, non-steroidal anti-inflammatory drugs, beta blockers, antipsychotics, and others) and Tylosin, an antibiotic used specifically in veterinary medicine. Thirty-one laboratories participated in this test, 4 of which were located abroad. The goals of this test on drug residues in water are (the following):

- to compile a list of analytical laboratories that had started developing analysis techniques;
- to obtain information related to analytical performance and uncertainties;
- to stimulate inter-laboratory cooperation on extraction and detection-quantitation methods;
- to draw up legislative and regulatory texts.

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2 The samples

Five samples were studied: a standard solution in a 50-50 methanol-acetonitrile mixture (for gas or liquid chromatography injection) which was to be diluted 1:10 in the laboratories’ injection conditions, a spiked and a non-spiked natural mineral water, a raw water, and a treated water. The various formulations were used to test the various stages of analysis:

- the sample in the solvent was used to test the analytical system without taking the extraction phase into account;
- the mineral water was used to:
  - test contaminant control with the non-spiked solution,
  - verify the extraction protocol’s effectiveness in relation to theoretical spiking values on an eigenmatrix with little organic matter,
  - compare the data with those from the raw water;
- the treated water matrix was used to assess the impact of the sodium thiosulfate stabilisation protocol on values close to analytical systems performance.

The samples were prepared and sent on the same day. They were packaged in 1-L brown glass flasks and did not undergo stabilisation treatment with the exception of the treated water (350 mg sodium thiosulfate per litre). Extraction was to take place within 48 h. after the samples were prepared. This timetable was followed in 85% of cases.

Sample pre-treatment and analysis methods were not imposed in order to assess the robustness of the various extraction and analysis techniques on the four matrix types.
the following form: ‘limit of quantitation could be submitted, for example, in
peatability conditions (2 extractions). Results below the
3 The results
Statistical processing was performed in accordance with the procedure used by Ansès.
As a result, accuracy and precision information was obtained according to Mandel’s $h$ and $k$ criteria. Robust
tests were favoured when obtaining means and standard deviations so that all of the laboratories’ data would be
taken into account. No outliers were eliminated. The last step of statistical processing consisted in a study of in-
dividual performance in order to obtain results to calcu-
late Z-scores, so as to identify ‘compliant values’, ‘val-
es to be monitored’ and ‘non-compliant values’ for each
compound-matrix pair.
These results can be used to assess a laboratory’s per-
formance in relation to the profession, which can be il-
lustrated in the form of a histogram and a bell curve, as shown in Figure 1.
This figure shows the number of laboratories in each
class. If we take the example of class [0-1], the histogram
shows that 7 laboratories have a Z-score value (the labora-
tory’s value minus the mean value divided by the standard
deviation) between 0 and 1.
The tolerance levels obtained through the Z-score cal-
culation as described in Table 1 show that in the case of
ketoprofen, 95% of the laboratories that submitted a raw
water result had satisfactory performance.
Similar work was undertaken for the 12 compounds un-
der study (17-α-estradiol, 17-β-estradiol, ethinylestradiol,
erthyromycin, ofloxacin, tylosin, ibuprofen, diclofenac, ke-
toprofen, carbamazepine, atenolol, paracetamol) and the
5 samples.
The ILPT allowed each laboratory to compare its per-
formance to other laboratories and to determine labora-
tory potential for possible standardisation and regulation
in the current legislative corpus.
It is worthwhile to consider the extraction yields ob-
tained by the laboratories against the performance cri-
tera set out in the French Ministry of Health’s Circu-
60–120% tolerance levels on extraction yields of pesticides
and similar products. When these values are applied to
pharmaceutical products, Figure 2 shows 8 compounds
that have extraction yields between 60 and 120%.
This interpretation is based on Tukey diagrams of each
compound. Note that quartiles 1 and 3 fall between 60 and
120%. The methods that were used for two-thirds of the
compounds studied in this ILT complied with the Ministry
of Health’s Circular. We can also illustrate that based on
the same criteria, the methods used for 50% of the com-
ponds had yields greater than 80%.
The laboratories’ protocol descriptions were used to draw up a diagram of analytical phases on the basis of
4 influential factors: the sample’s pre-treatment upon re-
cipient, the extraction protocol, the storage method and the
detection-quantitation method. Figure 3 shows the labora-
tories’ main practices but does not take performance into
account since there were not enough responses for each
standard methodology to statistically process the results.
On the basis of the participants’ results (mean and
standard deviation), relative standard deviation (RSD)
were calculated for each compound and within each ma-
trix, in accordance with French standard NF EN ISO
16140 from 2003 [2]. The profession’s inter-laboratory un-
certainty was obtained by applying the formula of ex-
panded uncertainty. The results given in Table 2 illustrate
uncertainty ranging from 47% to 157%, where 6% of the
data have uncertainty under 50%, 15% of the data have
uncertainty ranging from 50% to 60%, and 35% of the
data have uncertainty greater than 100%. There are at
least two explanations:
− laboratories’ progress in relation to the method (devel-
opment, validation, undergoing accreditation, accred-
ited) can cause significant variations in results;
− since there are no guides on the assaying of pharmaeu-
catical products in water, laboratories adapt or develop

<table>
<thead>
<tr>
<th>$z$</th>
<th>Interpretation</th>
</tr>
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<tr>
<td>$</td>
<td>z</td>
</tr>
<tr>
<td>$0.0 &lt;</td>
<td>z</td>
</tr>
<tr>
<td>$2.0 &lt;</td>
<td>z</td>
</tr>
<tr>
<td>$</td>
<td>z</td>
</tr>
</tbody>
</table>

Table 1. Z-score interpretation table.
their own protocol, even if a comparable technique has been used for analysing these compounds. This lack of consensus leads to biased results.

4 Conclusion

To conclude, this first French inter-laboratory test in the field of drinking water has generated a better view of current performance levels and laboratory practices. The latter appear to be fairly consistent from one laboratory to another.

The high level of participation shows the extent to which public and private laboratories are interested in these new analytical developments. The study of the comparability of results is especially important given that water prevalence campaigns are increasing in number.

This test also highlighted sensitive points such as the number of compounds studied compared to the number of compounds used in France (12 compounds studied/3000 compounds used in France). Inter-laboratory uncertainties indicated a high level of inter-laboratory performance variability which demonstrates a need to harmonise analytical practices.
Table 2. Mean concentration and uncertainty by compound and by matrix.

<table>
<thead>
<tr>
<th>Family</th>
<th>Molecules</th>
<th>Standard solution</th>
<th>Spike sample</th>
<th>Tap water</th>
<th>Groundwater</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Uncertainty</td>
<td>Average</td>
<td>Uncertainty</td>
<td>Average</td>
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<tr>
<td></td>
<td>(μg/L)</td>
<td>(%)</td>
<td>(μg/L)</td>
<td>(%)</td>
<td>(μg/L)</td>
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<tr>
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<td>(μg/L)</td>
<td>(%)</td>
<td>(μg/L)</td>
<td>(%)</td>
<td>(μg/L)</td>
</tr>
<tr>
<td>Non-steroidal</td>
<td>Diclofenac</td>
<td>277</td>
<td>47</td>
<td>27.15</td>
<td>63</td>
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<tr>
<td></td>
<td>Ibuprofen</td>
<td>255</td>
<td>55</td>
<td>38.07</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>Ketoprofen</td>
<td>255</td>
<td>52</td>
<td>31.61</td>
<td>73</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Erythromycin</td>
<td>356</td>
<td>157</td>
<td>36.07</td>
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<tr>
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<td>Ofloxacin</td>
<td>201</td>
<td>92</td>
<td>33.49</td>
<td>102</td>
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<td>Tylosin</td>
<td>283</td>
<td>130</td>
<td>26.67</td>
<td>97</td>
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<tr>
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<td>Atenolol</td>
<td>239</td>
<td>101</td>
<td>29.09</td>
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<td>Paracetamol</td>
<td>251</td>
<td>48</td>
<td>31.94</td>
<td>70</td>
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<td>Beta-blockers</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17-α-estradiol</td>
<td>244</td>
<td>139</td>
<td>39.91</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>17-β-estradiol</td>
<td>277</td>
<td>100</td>
<td>36.22</td>
<td>108</td>
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<tr>
<td></td>
<td>Ethynilestradiol</td>
<td>234</td>
<td>121</td>
<td>30.91</td>
<td>90</td>
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<tr>
<td></td>
<td>Carbamazepin</td>
<td>225</td>
<td>103</td>
<td>31.26</td>
<td>77</td>
</tr>
</tbody>
</table>

This work will be followed by:

- inter-laboratory collaborative work in preparation for feedback and a standardised protocol that is scheduled to start in the second half of 2010;

Other inter-laboratory tests are planned, including a test on natural waters, which will be undertaken together with the AQUAREF network. Laboratories will be contacted in the second half of 2010 and the test will be conducted in the third quarter of 2011.

References