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Inter-Laboratory Study for Quinolones in Poultry Muscle

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APPENDIX

SUMMARY

The inter-laboratory study for quinolones in poultry muscle was performed in accordance with ISO/IEC Guide 43-1 and 43-2 and ILAC-G13.

For this inter-laboratory study, three test materials were prepared:
- A blank material (A);
- A material containing ciprofloxacin and enrofloxacin, the sum of both being just below the MRL, danofloxacin and difloxacin both at levels of about the MRL (B);
- A material containing ciprofloxacin and enrofloxacin, the sum of both being just above the MRL, danofloxacin and difloxacin at levels of approximately 0.5*MRL (C);

Homogeneity and satisfactory stability of the materials was demonstrated.

Thirty four laboratories were invited to participate in the inter-laboratory study for quinolones in poultry muscle of which seventeen laboratories, i.e. 50%, subscribed. Each laboratory received six randomly coded samples including one sample of material A, three samples of material B and two samples of material C. The laboratories were asked to analyze the samples in duplicate.

Fifteen laboratories managed to submit results that could be included in the evaluation. The majority of those laboratories applied a validated and accredited method for the analysis of quinolones in poultry muscle.
Three laboratories used a method that did not include all the quinolones that are registered for medication in poultry in the EU.

The laboratories applied different methodologies. Four different sample clean-up procedures can be distinguished:
- Solid Phase Extraction (SPE): using reversed phase (C_{18} or OASIS® HLB) or reversed phase combined with cation exchange interaction (SDB-RPS);
- Filtration (0.45 µm) without any further purification;
- Ultrafiltration (30 kD) without any further purification;
- Partial evaporation of the solvent followed by dilution without further purification.

Two detection techniques were applied for the quantitative and confirmatory analysis of quinolones in poultry muscle: LC–MS/MS and LC-(UV)-FLU.
In accordance with the definition of the MRL, all laboratories considered the sum of enrofloxacin and ciprofloxacin in classifying the results as either compliant or non-compliant.

Most participating laboratories determined values for CCα and CCß and, hence, the majority already complies with the requirements of Commission Decision 2002/657/EC regarding CCα and CCß that apply for registered veterinary drugs as from the 1st of August 2007.
Some laboratories reported values for CCα and CCß below the MRL. This is not in compliance with the definition of CCα and CCß for compounds for which a permitted limit is established. For some laboratories the reported values for CCα and CCß are not in agreement with the reproducibility of the analysis calculated from the reported results in this inter-laboratory study. In both cases, reconsideration of the value of CCα and CCß could be necessary.

No false positive or false negative results occurred in this inter-laboratory study.
For all compounds and materials a considerable variation of the reported results is observed. In some cases the lowest and the highest value reported differ by a factor 40. In this inter-laboratory study a considerable number of results is classified as questionable or unsatisfactory. Those results could not be explained based on the applied detection technique. However, it is observed that filtration (0,45 μm) as sample preparation technique, without any further purification is not suitable for the analysis of quinolones in poultry muscle.
For each laboratory, the performance with respect to accuracy, reproducibility, false positive and false negative results was expressed in a laboratory performance score. Only 60% of the laboratories obtained the maximum score.

Based on the results, it is concluded that extra effort in the optimization of analytical methods for the analysis of quinolones in poultry muscle is urgently required:

- Danofloxacin, difloxacin and sarafloxacin should be included in the methods of analysis of quinolones by all laboratories, because those compounds are registered for medication in poultry in the EU;
- Reconsideration of numerical values determined for CCα and CCß may be necessary in some cases;
- An effort should be made regarding the quantitative analysis of all quinolones in poultry muscle with respect to the accuracy and the reproducibility.

1 INTRODUCTION

1.1 Inter-laboratory testing

Inter-laboratory testing is conducted to provide laboratories with a powerful tool to evaluate and demonstrate the reliability of the data that is produced. Next to validation and accreditation, inter-laboratory testing is an important requirement of the EU Additional Measures Directive 93/99/EEC [1] and is increasingly important in the new ISO 17025:2005 [2].
The aim of this inter-laboratory study was to give laboratories the possibility to evaluate or demonstrate their competence for the analysis of quinolones in poultry muscle. This study also provided an evaluation of the methods applied for quantitative and confirmatory analysis of quinolones. Since this study focuses exclusively on the analytical capability of laboratories rather than the correctness of the regulatory decision taken on the basis of the analytical results, throughout the report the terms "compliant" and "non-compliant" are avoided. Instead the terms "positive" and "negative" are used to indicate respectively the presence or absence of the analyte of interest.
The inter-laboratory study was carried out in accordance with guidelines ISO/IEC 43-1 [3], ISO/IEC 43-2 [4] and ILAC-G13 [5].

1.2 Quinolones

The discovery of the synthetic antibacterial agent nalidixic acid in 1962 marks the beginning of decades of quinolone development for human and veterinary use [6, 7, 8]. Nalidixic acid was discovered as a by-product of the production of the anti-malaria drug chloroquine. Nalidixic acid was found to be a rapid bactericidal agent by inhibition of the bacterial DNA gyrase synthesis [9]. Nalidixic acid is active against the majority of Gram-negative bacteria. Unfortunately it is not active against *Pseudomonas aeruginosa (*responsible for causing numerous infections), Gram-positive organisms and anaerobes. In addition, the clinical use of nalidixic acid is limited, because administration results in low drug concentrations in serum and tissues. Furthermore, resistance to nalidixic acid developed rapidly in numerous organisms. Derivatisation products of nalidixic acid, like oxolinic acid represented only marginal improvements over nalidixic acid.

In 1976, the development of flumequine, the first fluoroquinolone, offered significant improvement. This monofluoroquinolone indicated that the addition of a fluor atom in the molecule improved Gram-positive activity. In 1978 norfloxacin, a monofluorinated quinolone with a piperazinyl side-chain was developed. This fluoroquinolone has a longer half-time, less protein binding and improved Gram-negative activity compared to the earlier developed compounds. Still the pharmacokinetic profile and activity were not adequate for systemic use [10].
Very successful and widely used compounds of the fluoroquinolone group are ciprofloxacin, developed in 1981 and its counterpart in veterinary use enrofloxacin [11]. These compounds are active against a broad spectrum of Gram-positive as well as Gram-negative species, including *Pseudomonas aeruginosa*. Following oral administration, the drug is well distributed through the body with high concentrations in most tissues.

Gram-positive staphylococci became a major problem with increasing resistance to antibiotic compounds like ß-lactams and macrolides. Also for quinolones resistance in human pathogens was

demonstrated [12]. Therefore, the search for new fluoroquinolones continued, aiming for improved activity for ß-lactam, macrolide and quinolone resistant strains, and activity against Gram-positive staphylococci and anaerobes. This resulted in the development of fourth-generation quinolones.

1.3 Quinolones in animal health

The most notable fluoroquinolones used in veterinary medicine worldwide include ciprofloxacin, danofloxacin, enrofloxacin, marbofloxacin, norfloxacin and sarafloxacin [9]. Data gathered by The World Health Organization indicate that the use of quinolones differs greatly as regards animal species and geographical spread.
For instance, in the EU licensed quinolones for use in poultry are enrofloxacin, difloxacin, danofloxacin, oxolinic acid and sarafloxacin [13]. In Asia also the use of ciprofloxacin, flumequine, oxolinic acid and norfloxacin is licensed [14].
Quinolones have a very broad clinical application in livestock, poultry, fish and domestic animals in the treatment and prevention of respiratory, enteric and urinary tract infections [14].

Quinolone resistance has multiple mechanisms and significant clinical impact. Mutations may occur rapidly during fluoroquinolone therapy and may be the most significant factor limiting the use of these antimicrobials [15]. The toxicity of quinolones is mild at therapeutic doses and generally consists of gastrointestinal disturbances such as nausea and diarrhoea. At higher doses the central nervous system is affected resulting in dizziness, depression or insomnia [9].

The distribution and metabolism of enrofloxacin was studied in rats [15]. After oral administration enrofloxacin was well absorbed. The substance was widely distributed to all tissues with the highest concentration in liver and kidney. Elimination was rapid via both urine and faeces. Ciprofloxacin was indicated as the major metabolite of enrofloxacin [15]. The occurrence of metabolism of other quinolone compounds was not demonstrated.

1.4 Quinolones in poultry muscle

According to EU regulations, all substances for veterinary use need to be included in Annexes I, II or III of Council Regulation (ECC) No 2377/90 [13]. Quinolones are included in Annex I: pharmacologically active veterinary products for which a Maximum Residue Limit (MRL) is established. Because ciprofloxacin is the major metabolite of enrofloxacin, the MRL for enrofloxacin is established as the sum of enrofloxacin and ciprofloxacin.

This inter-laboratory study focuses on enrofloxacin (and its metabolite ciprofloxacin), danofloxacin and difloxacin (third generation fluoroquinolones) in poultry muscle. For these fluoroquinolones medication of poultry is described [12, 14]. The MRL for these compounds in poultry muscle is presented in Table 1. The structures of these fluoroquinolones are presented in Figure 1.

Table 1. MRL in poultry muscle of fluoroquinolones included in the inter-laboratory study [13]

Compound	Marker residue	MRL (µg/kg)
Enrofloxacin	Sum of enrofloxacin and ciprofloxacin	100
Danofloxacin	Danofloxacin	200
Difloxacin	Difloxacin	300

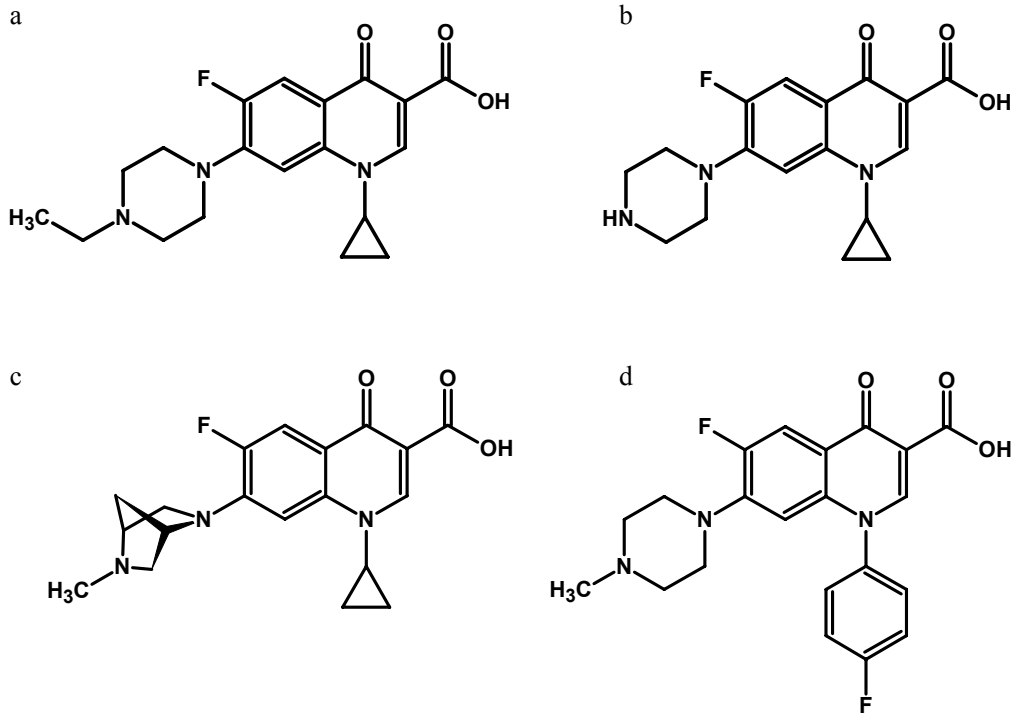

Figure 1. Molecular structure of (a) enrofloxacin, (b) ciprofloxacin, (c) danofloxacin and (d) difloxacin.

2 TEST MATERIALS

2.1 Sample preparation

Three test materials were prepared containing different amounts of ciprofloxacin (CIP), enrofloxacin (ENR), danofloxacin (DAN) and difloxacin (DIF) by adding methanolic solutions of these compounds to blank poultry muscle. The materials were homogenised under cryogenic conditions according to standard operating procedures. The materials presented in Table 2 were obtained.

Table 2.Target amount of quinolones in the inter-laboratory study test materials

Material code	Target amount of ENR+CIP*	Target amount of DAN	Target amount of DIF	Amount of material (g)
A	Blank	Blank	Blank	1000
B	Just below MRL	ca. MRL	ca. MRL	3000
C	Just above MRL	ca. 0.5*MRL	ca. 0.5* MRL	2200

* Contains both ENR and CIP (approximately 2:1).

2.2 Sample identification

Materials A, B and C were stored in poly propylene containers containing at least 25 gram of muscle, yielding a total of 30 containers of material A, 90 containers of material B and 60 containers of material C. For homogeneity and stability testing, 22 randomly chosen containers of material B and C were used. The other samples were randomly coded with a code from QUIN/2006/001 through QUIN/2005/180. Thirty sample sets consisting of one sample of material A, three samples of material B and two samples of material C were randomly prepared as presented in Appendix I.

2.3 Homogeneity study

Ten containers of materials B and C were each analyzed in duplicate for ENR, CIP, DAN and DIF to determine the homogeneity of the materials. The homogeneity study was carried out according to The International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [16] and ISO/DIS 13528 [17], taking into account the insights discussed by Fearn *et al*. [18] and Thompson [19].
The results of the homogeneity study and their statistical evaluation is presented in Appendices II and III for materials B and C respectively. All materials were demonstrated to be sufficiently homogenous for use in the inter-laboratory study.
Simultaneous with the homogeneity study, two samples of material A were analyzed. These analyses demonstrated that material A was free of residues of ciprofloxacin, enrofloxacin, danofloxacin, difloxacin, flumequine, marbofloxacin, nalidixic acid, norfloxacin, oxolinic acid and sarafloxacin (< 5 µg/kg). It was concluded that material A is suited for use as a blank material in the inter-laboratory study.

2.4 Sample distribution

Each of the participating laboratories received a randomly assigned laboratory code (lab1 through lab17). The sample sets with the corresponding number, consisting of six coded samples, were sent to the participating laboratory at the 16th of January. The sample sets were packed in an insulating box, containing dry ice and were dispatched to the participants immediately by courier. Due to Customs regulations the samples did not arrive at one of the laboratories (lab8). This laboratory was therefore not able to participate. Receipt of the samples in good condition (frozen) was confirmed by all other laboratories.
The samples were accompanied by a letter describing the requested analyses, an acknowledgement of receipt form and a results form. Furthermore, a reference standard of DAN, including a certificate of analysis, was included in the packages. The participants were asked to use this reference standard.
The laboratories were advised to store the samples at < -20 °C until analysis. A duplicate analysis of each sample was requested, resulting in two results for material A, six results for material B and four results for material C. The deadline for sending in results was 3rd of March, allowing the participants at least six weeks for analysis.

2.5 Stability

From the homogeneity data, the amount of quinolone residues in the materials, just after preparation, is calculated from the average of the 10 duplicate results.
The samples for the stability study were stored at -20 °C, corresponding to the advised storage conditions. On the 28th of January and the 17th of February, respectively 43 and 63 days after the initial analysis, three containers of material B and C were analyzed in duplicate. On the 15th of March, 88 days after the initial analysis and after the deadline of the inter-laboratory study, again three containers of material B and C were analyzed. For the three points in time, the average of the results was calculated. The results of the initial analysis were compared to the results of the analyses after the deadline of the study, using a Students *t*-test [20]. The hypothesis for this test is:

$$H_0 : \bar{x}_0 = \bar{x}_d$$

where: $\bar{x}_0$ = the average of the initial analyses;
$\bar{x}_d$ = the average of the analyses at time=d.

The standard deviation of both analyses are considered the same, because the same analytical procedure is applied to obtain the results. Therefore the value *t* is calculated by:

$$t = \frac{\bar{x}_0 - \bar{x}_d}{s\sqrt{\frac{1}{n_0} + \frac{1}{n_d}}}$$

where: $\bar{x}_0$ = the average amount calculated for the initial analyses;
$\bar{x}_d$ = the average amount calculated for the analyses at time=d;
n_0 = number of results of the initial analyses;

n_d = number of results of the analyses at time=d;

$$s = \sqrt{\frac{(n_0 - 1)s_0^2 + (n_d - 1)s_d^2}{(n_0 + n_d - 2)}}$$

where: s = pooled standard deviation;
n_0 = number of results of the initial analyses;
n_d = number of results of the analyses at time=d;
s_0 = standard deviation of the initial analyses calculated from the CV% resulting from the validation procedure;
s_d = standard deviation of the analyses at time=d calculated from the CV% resulting from the validation procedure.

The calculated value t is compared to a critical value (t_{crit}) derived from a Students-t table with t having $n_0 + n_d - 2$ degrees of freedom [20]. If $t < t_{crit}$ it is demonstrated that no significant difference between the average amount of the analyses at time=d and the initial analyses at time=0 is found. In this case the material is considered stable.

The results and statistical evaluation of the stability test are presented in Appendix IV. It was demonstrated that no significant loss of CIP, ENR and DIF occurred during the timescale of the inter-laboratory study at the chosen storage conditions. For material C, a significant loss of DAN is found. It was observed that a different reference standard of DAN was used for the analysis at t=0 compared to the analyses at the other points in time, which could influence the quantitative result. Furthermore, based on the results at t=43, 63 and 88 days, it is concluded that no significant loss of DAN is found from day 43 through day 88. If DAN would be unstable under the chosen conditions, an ongoing decrease of the amount of DAN is expected. Based on this and the results of the inter-laboratory study, it is concluded that the stability of DAN is satisfactory.

3 APPLIED METHODOLOGIES

The participating laboratories applied different sample preparation procedures and detection techniques for the analysis of quinolones in poultry muscle. A schematic overview of the methods applied is presented in Appendix V.

The participants extracted the samples in different ways. The majority used an aqueous extraction medium like water, trichloro acetic acid (5%) or a phosphate buffer at neutral pH. Other extraction media used are acetonitrile at neutral and low pH, acidic ethanol, acidic glycine and a mixture of acetonitrile and methanol at low pH.

Four sample clean-up procedures can be distinguished:

- Nine participants reported that they applied a Solid Phase Extraction (SPE) procedure. Some participants applied reversed phase materials, using C_{18} or OASIS® HLB. In those cases the quinolones were eluted using methanol or acetonitrile, both at neutral or low pH. Other participants combined reversed phase with cation exchange interaction using SDB-RPS. These participants eluted the quinolones using acetonitrile or methanol, both at high pH.
- Two participants (lab14 and 16) only filtered their extracts using a 0.45 µm filter without any further purification.
- Two participants (lab1 and 4) applied ultrafiltration (30 kD) as a sample clean-up technique.
- One participant (lab10) concentrated an aliquot of the raw extract by partial evaporation. Afterwards the extract was diluted using a mixture of ethanol, 25 mM phosphoric acid, acetic acid, triethylamine and acetonitrile. This participant did not apply any further clean-up.

Two detection techniques are applied for the analysis of quinolones in poultry muscle. Eight of the participants used LC–MS/MS. The other seven participants applied LC–Fluorescence, one participant (lab2) in combination with UV detection. According to Commission Decision 2002/657/EC [21], MS/MS as well as Fluorescence detection are suited for confirmatory analysis of group B substances.

Of the participants that used LC-MS/MS as a detection technique, six used one or more internal standards for the quantification of the quinolones. The internal standards used are:

- Cincophen (2-Phenyl-4-quinolinecarboxylic acid) from Sigma-Aldrich, St. Louis, MO (USA), 196479.
- Lomefloxacin from Sigma-Aldrich, L2906.
- Quinine from Sigma-Aldrich, 145904.
- d_8-Ciprofloxacin from Chemical Synthesis Services, County Armagh, Northern Ireland, UK, QC 1077.
- d_5-Enrofloxacin from Chemical Synthesis Services, QC 1138.
- d_5-Oxolinic acid from Chemical Synthesis Services, QC 0148.
- d_5-Norfloxacin.from Witega Laboratorien, Berlin-Adlershof, GE, OP010.

The laboratories that did not analyze for one or more of the quinolones mentioned in the invitation letter are presented in Table 3. It is noted that danofloxacin, difloxacin and sarafloxacin are not included by all laboratories. These compounds however, are registered for medication in poultry within the EU and a

MRL for poultry tissues is established. Therefore, these compounds should be included in a method of analysis used in the framework of regulatory control of residues in poultry muscle.

Table 3. Overview of laboratories that did not include all quinolones in the analysis.

Compound	Not included by (lab code)
Ciprofloxacin*	
Enrofloxacin*	
Danofloxacin*	5
Difloxacin*	3, 5, 7
Flumequine	
Marbofloxacin	2, 3, 5, 17
Nalidixic acid	5, 9, 17
Norfloxacin	5, 17
Oxolinic acid*	
Sarafloxacin*	3, 5, 7

* Compound registered for medication in poultry in the EU.

All laboratories comply with Council Regulation (ECC) No 2377/90 [13] regarding the definition of the MRL of enrofloxacin: all the participants considered the sum of enrofloxacin and ciprofloxacin in the classification of compliant or non-compliant results.

An overview of the method performance characteristics of the participating laboratories is presented in appendix VI. All values are presented as reported by the laboratories without any adjustments. Thirteen of the 15 participating laboratories (i.e. 87%) applied a validated method. Twelve of the participating laboratories (i.e. 80%) laboratories reported to have their method accredited for the analysis of quinolones in poultry muscle.
Amongst the participating laboratories, four did not report values for CCα. Hence, not all participating laboratories are yet ready to report their results as required by Commission Decision 2002/657/EC [21] per 1st of August 2007. It is noted that some laboratories (lab5, 7 and 17) report values for CCα and/or CCß below the MRL for all quinolones. For DAN laboratory 1 reported a CCα and CCß below the MRL. This is not in compliance with Commission Decision 2002/657/EC [21] considering the definition of CCα and CCß for compounds with an MRL.

4 STATISTICAL EVALUATION

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [16], elaborated by ISO, IUPAC and AOAC and ISO/DIS 13528 [17] in combination with the insights published by the Analytical Methods Committee [22, 23] regarding robust statistics.

4.1 Calculation of the assigned value

The assigned value (X) was determined using robust statistics [22, 23, 24]. The advantage of robust statistics is that all values are taken into account: outlying observations are retained, but given less weight. Furthermore, it is not expected to receive normally distributed data in an inter-laboratory proficiency test. When using robust statistics, the data does not have to be normally distributed in contrast to conventional outlier elimination methods.
The robust mean of the reported results of all participants was used as the assigned value.

4.2 Calculation of the uncertainty of the assigned value

The uncertainty of the assigned value is calculated to determine the influence of this uncertainty on the evaluation. A high uncertainty of the assigned value will lead to a high uncertainty of the calculated participants z_a-scores. If the uncertainty of the assigned value and thus the uncertainty of the z_a-score is high, the evaluation could indicate unsatisfactory method performance without any cause within the laboratory.
In other words, is it legitimate to draw any conclusion regarding the performance of the participating laboratories from the calculated assigned value and z_a-scores?
The uncertainty of the assigned value (the robust mean) is calculated from the estimate of the standard deviation of the assigned value and the number of values used for the calculation of the assigned value:

$$u = \frac{\hat{\sigma}}{\sqrt{n}}$$

where: u = uncertainty of the assigned value;
n = number of values used to calculate the assigned value;
$\hat{\sigma}$ = The estimate of the standard deviation of the assigned value resulting from robust statistics.

According to ISO/DIS 13528 [17] the uncertainty of the assigned value (u) is negligible and therefore does not have to be included in the statistical evaluation if:

$$u \leq 0.3\sigma_p$$

where: u = The uncertainty of the assigned value;
σ_p = target standard deviation (§ 4.3).

In case the uncertainty of the assigned value does not comply with this criterion, the uncertainty of the assigned value should be taken into account when evaluating the performance of the participants regarding the accuracy (§ 4.4).

4.3 Calculation of the target standard deviation

According to Commission Decision 2002/657/EC [21], the inter-laboratory coefficient of variation for the repeated analysis of a reference or fortified material, under reproducibility conditions, shall not exceed the level calculated by the Horwitz equation.
The Horwitz equation, $\sigma_H = 0.02c^{0.8495}$ presents a useful and widespread applied relation between the expected standard deviation under reproducibility conditions, σ_H and the concentration, c. It expresses inter-laboratory precision expected in inter-laboratory trials. Therefore, this relation is suitable for calculating the target standard deviation, σ_p in inter-laboratory trials.

Thompson [11] demonstrated that the Horwitz equation is not applicable to the lower concentration range (<120 µg/kg) as well as to the higher concentration range (>138 g/kg). Therefore a complementary model is suggested:

For analyte concentrations <120 µg/kg:
$\sigma_H = 0.22c$

For analyte concentrations >138 g/kg:
$\sigma_H = 0.01c^{0.5}$

where: σ_H = expected standard deviation in inter-laboratory trials;
c = concentration of the analyte.

The target standard deviation, σ_p, was determined using the equation for analyte concentrations <120 µg/kg for CIP, ENR and DAN (in material C) and the Horwitz equation for DAN (in material B) and DIF, with c = the assigned value (X) and $\sigma_H = \sigma_p$.

4.4 Performance characteristics with regard to the accuracy

For illustrating the performance of the participating laboratories with regard to the accuracy a z_a-score is calculated. For the evaluation of the performance of the laboratories, the Guidelines of ISO/IEC Guide 43-1 [3] and ISO/DIS 13528 [17] are applied. According to these guidelines z_a-scores are classified as presented in Table 4.

Table 4: Classification of z-scores

$\|z\| \leq 2$	satisfactory
$2 < \|z\| < 3$	questionable
$\|z\| \geq 3$	unsatisfactory

When the calculated uncertainty of the assigned value complies with the criterion mentioned in § 4.2, the uncertainty is negligible. In this case the accuracy *z*-score is calculated from:

$$z_a = \frac{\bar{x} - X}{\sigma_p}$$

where: z_a = accuracy *z*-score;
$\bar{x}$ = the average result of the laboratory;
X = assigned value;
σ_p = target standard deviation.

However, if the uncertainty of the assigned value does not comply with the criterion mentioned in § 4.2, it could influence the evaluation of the laboratories. Therefore this uncertainty is taken into account by calculating the accuracy z-score [13]:

$$z_a' = \frac{\bar{x} - X}{\sqrt{\sigma_p^2 + u^2}}$$

where: z_a' = accuracy *z*-score taking into account the uncertainty of the assigned value;
$\bar{x}$ = mean result of the laboratory;
X = assigned value;
σ_p = target standard deviation;
u = uncertainty of the assigned value.

4.5 Performance characteristics with regard to reproducibility

In addition to the evaluation of the accuracy, it is useful to inform the participants about the reproducibility of the results. In the design of this inter-laboratory study, three blind samples of material B and two blind samples of material C were submitted to the participants. Therefore, every laboratory reported multiple results for each material. From the results of the blind samples of material B and C the repeatability (s_r) and an estimate of the within-lab-reproducibility (s_{R_L}) were calculated [24].

The repeatability standard deviation is calculated from:

$$s_r = \sqrt{\frac{\sum d_i^{\,2}}{2p}}$$

where: s_r = repeatability standard deviation;
d_i = difference between the individual values for a pair;
p = number of pairs.

An estimate of the within-lab-reproducibility standard deviation is calculated from:

$$s_{R_L} = \sqrt{(s_L^2 + s_r^2)}$$

where: s_{R_L} = estimate of the within-lab-reproducibility standard deviation;
s_r = repeatability standard deviation;

$$s_L = \sqrt{\frac{p\Sigma\left(\bar{x}_p\right)^2 - \left(\Sigma\bar{x}_p\right)^2}{p(p-1)}} - \frac{s_r^2}{2}$$

where: s_L = between sample variance (if s_L <0 this value is assumed to be zero)
p = number of pairs;
$\bar{x}_p$ = average result of the duplicates;
s_r = repeatability standard deviation.

Because the samples are not analyzed under true within-lab reproducibility conditions, the estimate of the within-lab reproducibility standard deviation (s_{R_L}) will always be lower than the true within-lab reproducibility standard deviation.
To inform a laboratory of its performance for reproducibility, the Horwitz-ratio (*HORRAT*) is a suitable value [25]. In this report, the HORRAT is calculated from the estimate of the within-lab reproducibility, because it is not possible to calculate a reproducibility standard deviation from the laboratory data. The reproducibility standard deviation (s_R) includes inter-laboratory variation and must therefore always be higher than the estimate of the within-lab reproducibility (s_{R_L}).
Because the *HORRAT* value is calculated from s_{R_L} instead of s_R, this value is not for evaluation purposes but for information only.

The *HORRAT* is calculated from:

$$HORRAT = \frac{s_{R_L}}{\sigma_p}$$

where: *HORRAT* = Horwitz ratio;
s_{R_L} = estimate of the within-lab reproducibility standard deviation;
σ_p = target standard deviation (§ 4.3).

In this formula, a *HORRAT* value equal to 1.0 indicates that the estimate of the within-lab reproducibility is equal to the predicted maximum reproducibility standard deviation resulting from the Horwitz equation. However, the latter refers to reproducibility between laboratories and, hence, would normally be higher than the within-lab reproducibility. Therefore it is within reason that the HORRAT value calculated from the estimate of the within-lab reproducibility, as done in this report, should be substantially below 1.0.
Nonetheless in this report, a *HORRAT* value is not regarded as a questionable result unless it exceeds 1.0.

Furthermore, from the calculated inter-laboratory standard deviation (s_{R_L}) the expected decision limit ($CC\alpha_e$) is calculated:

$$CC\alpha_e = MRL + \frac{1.64 \times s_{R_L} \times MRL}{\bar{x}}$$

where: $CC\alpha_e$ = expected decision limit based on the inter-laboratory study results;
MRL = maximum residue limit (µg/kg);
s_{R_L} = estimate of the within-lab reproducibility standard deviation;
$\bar{x}$ = the average result of the laboratory (µg/kg).

For this calculation, it is assumed that the relative within-lab reproducibility standard deviation at the MRL is equal to the relative within-lab reproducibility standard deviation at $\bar{x}$. The $CC\alpha_e$, calculated based on the inter-laboratory study results is compared to the reported CCα.

4.6 Calculation of laboratory performance scores

In the evaluation of this proficiency test, a score is calculated to demonstrate the performance of the participating laboratories. This score accounts for the accuracy and reproducibility of the results, and the occurrence of false positive and false negative results. For each satisfactory result for the accuracy ($|z_a$-score$|\leq 2.0$) and for each satisfactory result for the reproducibility ($HORRAT \leq 1.0$), 1 point is earned. However, for each compound detected in material A and each compound other than CIP, ENR, DAN and DIF in material B and C (false positive results), two points are subtracted. Furthermore, if CIP, ENR, DAN or DIF was not detected in samples originating from material B or C, whilst these compounds were included in the method (false negative results), two points are subtracted from the score.
The laboratory performance score is calculated by comparing the points attained with the maximum score and is expressed as a percentage.

5 RESULTS AND DISCUSSION

Seventeen out of 34 (i.e. 50%) invited laboratories subscribed for the participation in the inter-laboratory study for quinolones in poultry muscle. Due to Customs regulations, it was not possible to ship the samples to one of the laboratories (lab8). Therefore, this laboratory was not able to participate. Fourteen laboratories (i.e. 82%) managed to submit valid results before the deadline of the 3rd of March. Laboratory 17 reported their results on the 6th of March. This however, is still within the time frame of the stability study. Therefore the results of this laboratory were included in the evaluation.

All laboratories analyzed the samples in duplicate. The number of laboratories included in the statistical evaluation is 15 for ciprofloxacin and enrofloxacin, 14 for danofloxacin and 12 for difloxacin. All results are presented as reported by the laboratories, without any correction or adjustments. For compounds other than ciprofloxacin, enrofloxacin, danofloxacin and difloxacin, only amounts above 5 µg/kg are taken into account.

None of the laboratories detected any quinolones in the blank material (material A). No false positive results occurred.

5.1 Evaluation of the results of ciprofloxacin

All laboratories that sent in results included CIP in their analysis. Therefore the evaluation of CIP is based on the results of 15 laboratories. Those results as well as the evaluation of CIP results are presented in Appendix VII for material B and VIII for material C.

All laboratories detected CIP in the samples originating from material B and C. No false negative results occurred.

Laboratory 11 reported very low amounts of CIP for one of their samples (QUIN/2006/041) originating from material B. This duplicate result was identified as an outlier and was therefore not included in the calculation of the average result for this laboratory. It was however included in the evaluation of reproducibility.

For material B the lowest value reported is 5 µg/kg and the highest value is 73 µg/kg. The assigned value of CIP in material B is 33.2 µg/kg with an uncertainty of 3.1 µg/kg. The uncertainty of the assigned value of CIP in material B exceeds $0.3\sigma_p$ (§4.2). Therefore, for this material, the uncertainty of the assigned value is taken into account in the evaluation of the laboratories. The z_a'-scores and *HORRAT* values for CIP obtained by each laboratory were calculated. The results are presented in Appendix VII. Graphical representations of the z_a'-scores and *HORRAT* values are included.

For material C the lowest value reported is 9 µg/kg and the highest value reported is 84.2 µg/kg. The assigned value of CIP in material C is 38.1 µg/kg with an uncertainty of 3.2 µg/kg. The uncertainty of the assigned value of CIP in material C exceeds $0.3\sigma_p$ (§4.2). Therefore, for this material, the uncertainty of the assigned value is taken into account with regard to the evaluation of the laboratories. The z_a'-scores and *HORRAT* values for CIP obtained by each laboratory were calculated. The results are

presented in Appendix VIII. Graphical representations of the z_a'-scores and *HORRAT* values are included.

With respect to the accuracy, for both materials the results of two laboratories were questionable and the results of two other laboratories were unsatisfactory. Differences in accuracy or reproducibility could not be attributed to differences in the applied detection technique. Also no effect of the use of an internal standard in case of mass spectrometric detection was demonstrated. However, it is observed that both laboratories that applied filtration as the only sample preparation technique, without any further purification, obtained unsatisfactory results for both materials.
The number of satisfactory results for accuracy for both materials is presented in Table 5.

The calculation of the *HORRAT* value results in a value above 1.0 for laboratory 11 for material B. This indicates questionable performance with respect to repeatability. This was caused by the low amount of CIP found in one of the samples. The number of satisfactory results for reproducibility for both materials is presented in Table 5.

Table 5. Number and percentage of satisfactory results for accuracy and reproducibility for ciprofloxacin

Material	No. of satisfactory results for accuracy	% of satisfactory results for accuracy	No. of satisfactory results for reproducibility	% of satisfactory results for reproducibility
B	11 of 15	73%	14 of 15	93%
C	11 of 15	73%	15 of 15	100%

The CCα reported by the laboratories are compared to the reproducibility calculated from the results in this inter-laboratory study (§4.5). For laboratories 1 and 4 the reproducibility of the results of CIP in material C are higher than is suggested by the reported CCα for this analyte.

5.2 Evaluation of the results of enrofloxacin

All laboratories that sent in results included ENR in their analysis. Therefore the evaluation of ENR is based on the results of 15 laboratories. The reported results and the results of the evaluation of ENR are presented in Appendix IX for material B and X for material C.

All laboratories detected ENR in the samples originating from material B and C. No false negative results occurred.

Laboratory 11 reported a very low amount of ENR for one of the duplicates of one sample (QUIN/2006/041) originating from material B. This result was identified as an outlier and was therefore not included in the calculation of the average result for ENR for this laboratory. It was however included in the evaluation of reproducibility.

For material B the lowest value reported is 4.8 µg/kg and the highest value reported is 206 µg/kg. The assigned value of ENR in material B is 68.1 µg/kg with an uncertainty of 5.7 µg/kg. The uncertainty of the assigned value of ENR in material B exceeds $0.3\sigma_p$ (§4.2). Therefore, for this material, the uncertainty of the assigned value is taken into account with regard to the evaluation of the laboratories.

The z_a'-scores and *HORRAT* values for ENR obtained by each laboratory were calculated. The results are presented in Appendix IX. Graphical representations of the z_a'-scores and *HORRAT* values are included.

For material C the lowest value reported is 22 µg/kg and the highest value reported is 281 µg/kg. The assigned value of ENR in material C is 81.9 µg/kg with an uncertainty of 5.6 µg/kg. The uncertainty of the assigned value of ENR in material C exceeds $0.3\sigma_p$ (§4.2). Therefore, for this material, the uncertainty of the assigned value is taken into account in the evaluation of the laboratories. The z_a'-scores and *HORRAT* values for ENR obtained by each laboratory were calculated. The results are presented in Appendix X. Graphical representations of the z_a'-scores and *HORRAT* values are included.

With respect to the accuracy, for material B, the result of one laboratory was questionable and the results of two laboratories were unsatisfactory. For material C, two laboratories obtained questionable results and two laboratories obtained unsatisfactory results. Differences in accuracy or reproducibility could not be attributed to differences in the applied detection technique. Also no effect of the use of an internal standard in case of mass spectrometric detection was demonstrated. However, it is observed that both laboratories that applied filtration as the only sample preparation technique, without any further purification, obtained questionable or unsatisfactory results for one or both materials. The number of satisfactory results for accuracy for both materials is presented in Table 6.

The calculation of the *HORRAT* value results in a value above 1.0 for laboratory 11, 14 and 16 for material B. For material C, only laboratory 16 obtained a HORRAT value above 1.0. This indicates questionable performance of the applied method with regard to repeatability. The number of satisfactory results for reproducibility for both materials is presented in Table 6.

Table 6. Number and percentage of satisfactory results for accuracy and reproducibility for enrofloxacin

Material	No. of satisfactory results for accuracy	% of satisfactory results for accuracy	No. of satisfactory results for reproducibility	% of satisfactory results for reproducibility
B	12 of 15	80%	12 of 15	80%
C	11 of 15	73%	14 of 15	93%

The CCα reported by the laboratories are compared to the reproducibility calculated from the results in this inter-laboratory study (§4.5). For laboratories 14 and 16 the reproducibility of the results of ENR in material B is higher than is suggested by the reported CCα for this analyte. For laboratories 1 and 16 the reproducibility of the results of ENR in material C is higher than is suggested by the reported CCα for this analyte.

5.3 Evaluation of the results of the sum of ciprofloxacin and enrofloxacin

Because the MRL for ENR is defined as the sum of CIP and ENR, also an evaluation of the sum of CIP and ENR was carried out. All of the laboratories that sent in results included CIP and ENR in their analysis. Therefore the evaluation for CIP+ENR is based on the results of 15 laboratories. The reported results and the results of the evaluation of CIP+ENR are presented in Appendix XI for material B and XII for material C.

Because laboratory 11 reported a very low amount of CIP for one of the sample (QUIN/2006/041) originating from material B, this result was not included in the evaluation of the accuracy of CIP+ENR. It was however included in the evaluation regarding the reproducibility.

For material B the lowest value reported is 30 µg/kg and the highest value reported is 278 µg/kg. The assigned value of CIP+ENR in material B is 101.4 µg/kg with an uncertainty of 7.4 µg/kg. The uncertainty of the assigned value of ENR in material C exceeds $0.3\sigma_p$ (§4.2). Therefore, for this material, the uncertainty of the assigned value is taken into account in the evaluation of the laboratories. The z_a'-scores and *HORRAT* values for CIP+ENR obtained by each laboratory were calculated. The results are presented in Appendix XI. Graphical representations of the z_a'-scores and *HORRAT* values are included.

For material C the lowest value reported is 34 µg/kg and the highest value reported is 356 µg/kg. The assigned value of CIP+ENR in material C is 122 µg/kg with an uncertainty of 7.3 µg/kg. The uncertainty of the assigned value of CIP+ENR in material C is below $0.3\sigma_p$ (§4.2). Therefore, the uncertainty of the assigned value is considered to be negligible. The z_a-scores and *HORRAT* values for CIP+ENR obtained by each laboratory were calculated. The results are presented in Appendix XII. Graphical representations of the z_a-scores and *HORRAT* values are included.

With respect to the accuracy for material B, the result of one laboratory was questionable and the results of two other laboratories were unsatisfactory. For material C, two laboratories obtained unsatisfactory results. Differences in accuracy or reproducibility could not be attributed to differences in the applied detection technique. Also no effect of the use of an internal standard in case of mass spectrometric detection was demonstrated.
The number of satisfactory results regarding the accuracy for both materials is presented in table 7.

The calculation of the *HORRAT* value results in a value above 1.0 for laboratory 11 and 16 for material B. For material C, only laboratory 16 obtained a HORRAT value above 1.0. This indicates questionable performance of the applied method with regard to repeatability. The number of satisfactory results for reproducibility for both materials is presented in Table 7.

Table 7. Number and percentage of satisfactory results for accuracy and reproducibility for ciprofloxacin + enrofloxacin

Material	No. of satisfactory results for accuracy	% of satisfactory results for accuracy	No. of satisfactory results for reproducibility	% of satisfactory results for reproducibility
B	12 of 15	80%	13 of 15	87%
C	13 of 15	87%	14 of 15	93%

5.4 Evaluation of the results of danofloxacin

Fourteen laboratories included DAN in their analysis. Therefore the evaluation of DAN is based on the results of 14 laboratories. Four laboratories indicated that they did not use the reference standard DAN that was supplied with the samples. The reported results and the results of the evaluation of DAN are presented in Appendix XIII for material B and XIV for material C.
All laboratories that included DAN in their analysis detected DAN in the samples originating from material B and C. No false negative results occurred.

Laboratory 11 reported very low amounts of DAN for both duplicates of one sample (QUIN/2006/041) originating from material B. These results were indicated as outliers and were therefore not included in the calculation of the average result for DAN for this laboratory. They were however included in the evaluation of the reproducibility.

For material B the lowest value reported is 18 µg/kg and the highest value reported is 540 µg/kg. The assigned value of DAN in material B is 192 µg/kg with an uncertainty of 11 µg/kg. The uncertainty of the assigned value of DAN in material B is below $0.3\sigma_p$ (§4.2). Therefore, the uncertainty of the assigned value is considered to be negligible. The z_a-scores and *HORRAT* values for ENR obtained by each laboratory were calculated. The results are presented in Appendix XIII. Graphical representations of the z_a-scores and *HORRAT* values are included.

For material C the lowest value reported is 8 µg/kg and the highest value reported is 295 µg/kg. The assigned value of DAN in material C is 118 µg/kg with an uncertainty of 9.3 µg/kg. The uncertainty of the assigned value of DAN in material C exceeds $0.3\sigma_p$ (§4.2). Therefore, for this material, the uncertainty of the assigned value is taken into account in the evaluation of the laboratories. The z_a'-scores and *HORRAT* values for DAN obtained by each laboratory were calculated. The results are presented in Appendix XIV. Graphical representations of the z_a'-scores and *HORRAT* values are included.

With respect to the accuracy, for both materials, the results of three laboratories were unsatisfactory. Differences in accuracy or reproducibility could not be attributed to differences in the applied detection technique. Also no effect of the use of an internal standard in case of mass spectrometric detection was demonstrated.
Surprisingly, no relation was found between the results and laboratories that did or did not use the supplied reference standard for DAN. It is observed that both laboratories that applied filtration as the only sample preparation technique, without any further purification, obtained unsatisfactory results for both materials.
The number of satisfactory results regarding the accuracy for both materials is presented in table 8.

The calculation of the *HORRAT* value results in a value above 1.0 for laboratory 16, 14 and 11 for material B. For material C, only laboratory 16 obtained a HORRAT value above 1.0. This indicates questionable performance of the applied method with regard to repeatability. The number of satisfactory results for reproducibility for both materials is presented in Table 8.

Table 8. Number and percentage of satisfactory results for accuracy and reproducibility for danofloxacin

Material	No. of satisfactory results for accuracy	% of satisfactory results for accuracy	No. of satisfactory results for reproducibility	% of satisfactory results for reproducibility
B	11 of 14	79%	11 of 14	79%
C	11 of 14	79%	13 of 14	93%

The CCα reported by the laboratories are compared to the reproducibility calculated from the results in this inter-laboratory study (§4.5). For laboratory 14 the reproducibility of the results of DAN in both materials is higher than is suggested by the reported CCα for this analyte. For laboratory 16 the

reproducibility of the result of DAN in material C is higher than is suggested by the reported CCα for this analyte.

5.5 Evaluation of the results of difloxacin

Twelve laboratories included DIF in their analysis. Therefore the evaluation of DIF is based on the results of 12 laboratories. The reported results and the results of the evaluation of DIF are presented in Appendix XV for material B and XVI for material C.

All laboratories that included DIF in their analysis detected DIF in the samples originating from material B and C. No false negative results occurred.

For material B the lowest value reported is 81 µg/kg and the highest value reported is 612 µg/kg. The assigned value of DIF in material B is 299 µg/kg with an uncertainty of 22 µg/kg. The uncertainty of the assigned value of DIF in material B is exceeds $0.3\sigma_p$ (§4.2). Therefore, for this material, the uncertainty of the assigned value is taken into account in the evaluation of the laboratories. The z_a'-scores and *HORRAT* values for DIF obtained by each laboratory were calculated. The results are presented in Appendix XV. Graphical representations of the z_a'-scores and *HORRAT* values are included.

For material C the lowest value reported is 50 µg/kg and the highest value reported is 362 µg/kg. The assigned value of DIF in material C is 188 µg/kg with an uncertainty of 20 µg/kg. The uncertainty of the assigned value of DIF in material C exceeds $0.3\sigma_p$ (§4.2). Therefore, for this material, the uncertainty of the assigned value is taken into account in the evaluation of the laboratories. The z_a'-scores and *HORRAT* values for DIF obtained by each laboratory were calculated. The results are presented in Appendix XVI. Graphical representations of the z_a'-scores and *HORRAT* values are included.

With respect to the accuracy, for both materials, the results of three laboratories were unsatisfactory. Differences in accuracy could not be attributed to the applied detection technique. Also no effect of the use of an internal standard in case of mass spectrometric detection was observed. The number of satisfactory results for accuracy for both materials is presented in Table 9.

The calculation of the *HORRAT* value results in a value above 1.0 for laboratory 16 and 14 for material B. This indicates questionable performance of the applied method for repeatability. For material C no HORRAT values above 1.0 were obtained. The number of satisfactory results for reproducibility for both materials is presented in Table 9.

Table 9. Number and percentage of satisfactory results for accuracy and reproducibility for difloxacin

Material	No. of satisfactory results for accuracy	% of satisfactory results for accuracy	No. of satisfactory results for reproducibility	% of satisfactory results for reproducibility
B	9 of 12	75%	10 of 12	83%
C	9 of 12	75%	12 of 12	100%

The CCα reported by the laboratories are compared to the reproducibility calculated from the results in this inter-laboratory study (§4.5). For laboratories 12, 14 and 15 the reproducibility of the results of DIF in material B is higher than is suggested by the reported CCα for this analyte. For laboratories 4, 12 and

14 the reproducibility of the results of DIF in material C is higher than is suggested by the reported CCα for this analyte.

5.6 Laboratory scores

The performance of each participating laboratory is expressed in a laboratory performance score (§4.6). The maximum attainable score is 100%. The laboratory performance score and the maximum attainable score per lab are presented in Appendix XVII.
From the 15 laboratories 9 (i.e. 60%) showed optimal performance for the analysis of quinolones in poultry muscle with respect to the accuracy, repeatability and the occurrence of false positive and false negative results.

6 CONCLUSION

Thirty four laboratories were invited to participate in the inter-laboratory study for quinolones in poultry muscle, of which seventeen laboratories subscribed.
Fourteen laboratories reported their results within the given time scale. One laboratory reported their results with a delay of three days. All reported results were included in the report without any modifications.

Three laboratories did not include all the quinolones that are registered for medication in poultry in the EU in their method. In these cases false negative results may occur. All laboratories comply with Council Regulation (ECC) No 2377/90 [13] the definition of the MRL of enrofloxacin: all participants considered the sum of enrofloxacin and ciprofloxacin in the characterisation of compliant or non-compliant results.

The majority of participants applied an accredited method for the analysis of quinolones in poultry muscle. Three laboratories did not report a value for CCα and CCß. Apparently some laboratories are not yet ready to comply with the requirements of Commission Decision 2002/657/EC [21] for registered veterinary drugs as per 1st of August 2007.
Some laboratories reported values for CCα and CCß below the MRL. This is not in accordance with Commission Decision 2002/657/EC [21] considering the definition of CCα and CCß for compounds with an MRL.
For some laboratories the values for CCα and CCß are not in agreement with the reproducibility of the analysis calculated from the reported results in this inter-laboratory study.

The results of material B and C are summarized in Table 10 and Table 11 respectively. No false negative or false positive results occurred.

Table 10. Summary of the results for material B

Compound	Assigned value (X) (µg/kg)	Uncertainty of X (µg/kg)	No. of labs that reported results	No. of satisfactory results	
				Accuracy	Reproducibility
CIP	33.2	3.1[1)]	15	11	14
ENR	68.1	5.7[1)]	15	12	12
CIP+ENR	101.4	7.4[1)]	15	12	13
DAN	192	11	14	11	11
DIF	299	22[1)]	12	9	10

[1)] The uncertainty of the assigned value exceeds $0.3\sigma_p$: the uncertainty of the assigned value is taken into account in the evaluation of the laboratories.

Table 11. Summary of the results for material C

Compound	Assigned value (X) (µg/kg)	Uncertainty of X (µg/kg)	No. of labs that reported results	No. of satisfactory results	
				Accuracy	Reproducibility
CIP	38.1	3.2[1)]	15	11	15
ENR	81.9	5.6[1)]	15	11	14
CIP+ENR	122	7.3	15	13	14
DAN	118	9.3[1)]	14	11	13
DIF	188	20[1)]	12	9	12

[1)] The uncertainty of the assigned value exceeds $0.3\sigma_p$: the uncertainty of the assigned value is taken into account in the evaluation of the laboratories.

For all compounds and materials a considerable variation for the reported amount is observed. This results in a substantial number of results that are characterised as questionable or unsatisfactory. The occurrence of questionable or unsatisfactory results could not be explained by the applied detection technique. However, it is observed that filtration as a sample preparation technique, without any further purification, is apparently not suitable for the analysis of quinolones in poultry muscle.
The performance with respect to accuracy, reproducibility, false negative and false positive results was expressed in a laboratory performance score for each laboratory. Only 60% of the laboratories obtained the maximum score.

Based on the results, it is concluded that extra effort is needed for the optimization of methods of analysis for quinolones in poultry muscle:

- Danofloxacin, difloxacin and sarafloxacin should be included in the method of analysis for quinolones, because those compounds are registered for medication in poultry in the EU;
- Reconsideration of numerical values determined for CCα and CCß may be necessary in some cases;
- An effort should be made to improve the quantitative analysis of all quinolones in poultry muscle with respect to the accuracy and reproducibility.

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APPENDIX I: Codification of the samples

Sample set	Material A	Material B	Material C
1	QUIN/2006/092	QUIN/2006/091	QUIN/2006/128
		QUIN/2006/124	QUIN/2006/116
		QUIN/2006/126	
2	QUIN/2006/130	QUIN/2006/019	QUIN/2006/177
		QUIN/2006/131	QUIN/2006/086
		QUIN/2006/141	
3	QUIN/2006/155	QUIN/2006/107	QUIN/2006/043
		QUIN/2006/001	QUIN/2006/178
		QUIN/2006/080	
4	QUIN/2006/170	QUIN/2006/166	QUIN/2006/084
		QUIN/2006/017	QUIN/2006/040
		QUIN/2006/061	
5	QUIN/2006/180	QUIN/2006/109	QUIN/2006/068
		QUIN/2006/133	QUIN/2006/162
		QUIN/2006/145	
6	QUIN/2006/042	QUIN/2006/137	QUIN/2006/082
		QUIN/2006/165	QUIN/2006/138
		QUIN/2006/159	
7	QUIN/2006/123	QUIN/2006/106	QUIN/2006/078
		QUIN/2006/073	QUIN/2006/049
		QUIN/2006/066	
8	QUIN/2006/100	QUIN/2006/102	QUIN/2006/054
		QUIN/2006/115	QUIN/2006/016
		QUIN/2006/139	
9	QUIN/2006/024	QUIN/2006/006	QUIN/2006/056
		QUIN/2006/004	QUIN/2006/158
		QUIN/2006/020	
10	QUIN/2006/029	QUIN/2006/048	QUIN/2006/090
		QUIN/2006/147	QUIN/2006/026
		QUIN/2006/119	
11	QUIN/2006/025	QUIN/2006/173	QUIN/2006/052
		QUIN/2006/012	QUIN/2006/003
		QUIN/2006/041	
12	QUIN/2006/171	QUIN/2006/063	QUIN/2006/150
		QUIN/2006/135	QUIN/2006/021
		QUIN/2006/028	
13	QUIN/2006/104	QUIN/2006/093	QUIN/2006/105
		QUIN/2006/065	QUIN/2006/179
		QUIN/2006/053	
14	QUIN/2006/089	QUIN/2006/037	QUIN/2006/161
		QUIN/2006/044	QUIN/2006/058
		QUIN/2006/153	
15	QUIN/2006/045	QUIN/2006/014	QUIN/2006/035
		QUIN/2006/117	QUIN/2006/114
		QUIN/2006/148	

APPENDIX I: Codification of the samples (continued)

Set No.	Material A	Material B	Material C
16	QUIN/2006/144	QUIN/2006/074	QUIN/2006/169
		QUIN/2006/087	QUIN/2006/088
		QUIN/2006/095	
17	QUIN/2006/121	QUIN/2006/136	QUIN/2006/015
		QUIN/2006/060	QUIN/2006/160
		QUIN/2006/167	
18	QUIN/2006/046	QUIN/2006/146	QUIN/2006/098
		QUIN/2006/094	QUIN/2006/022
		QUIN/2006/113	
19	QUIN/2006/027	QUIN/2006/122	QUIN/2006/143
		QUIN/2006/149	QUIN/2006/111
		QUIN/2006/013	
20	QUIN/2006/156	QUIN/2006/081	QUIN/2006/067
		QUIN/2006/059	QUIN/2006/072
		QUIN/2006/009	
21	QUIN/2006/099	QUIN/2006/151	QUIN/2006/085
		QUIN/2006/154	QUIN/2006/127
		QUIN/2006/071	
22	QUIN/2006/079	QUIN/2006/175	QUIN/2006/011
		QUIN/2006/033	QUIN/2006/038
		QUIN/2006/018	
23	QUIN/2006/051	QUIN/2006/076	QUIN/2006/164
		QUIN/2006/168	QUIN/2006/031
		QUIN/2006/101	
24	QUIN/2006/030	QUIN/2006/172	QUIN/2006/132
		QUIN/2006/077	QUIN/2006/050
		QUIN/2006/120	
25	QUIN/2006/007	QUIN/2006/047	QUIN/2006/112
		QUIN/2006/174	QUIN/2006/176
		QUIN/2006/008	
26	QUIN/2006/002	QUIN/2006/097	QUIN/2006/140
		QUIN/2006/062	QUIN/2006/110
		QUIN/2006/152	
27	QUIN/2006/064	QUIN/2006/157	QUIN/2006/032
		QUIN/2006/057	QUIN/2006/108
		QUIN/2006/023	
28	QUIN/2006/118	QUIN/2006/103	QUIN/2006/128
		QUIN/2006/134	QUIN/2006/129
		QUIN/2006/083	
29	QUIN/2006/039	QUIN/2006/142	QUIN/2006/070
		QUIN/2006/069	QUIN/2006/036
		QUIN/2006/163	
30	QUIN/2006/096	QUIN/2006/075	QUIN/2006/035
		QUIN/2006/010	QUIN/2006/005
		QUIN/2006/055	

APPENDIX IIa: Statistical evaluation of homogeneity data of material B for ciprofloxacin

	Ciprofloxacin (µg/kg)	
Sample No.	Replicate 1	Replicate 2
1	30.8	24.8
2	25.4	29.5
3	32.7	21.9
4	24.4	25.0
5	29.3	28.9
6	26.9	32.3
7	33.7	34.0
8*	30.5	98.4
9	29.0	32.0
10	31.0	25.1
Grand mean	28.7	
Cochran's test		
C	0.477	
C_{crit}	0.638	
$C < C_{crit}$?	NO OUTLIERS	
Target sd (σ_p)	Horwitz: 6.31	
s_{an}^2	13.4	
s_{sam}^2	0	
σ_{all}^2	3.6	
critical	20.3	
s_{sam}^2 < critical?	ACCEPTED	

* One of the duplicate results is very high due to a low signal of the internal standard. This duplicate was indicated as an outlier by the Cochran's test. The presented results of the Cochran's test were calculated after elimination of this outlying duplicate.

No flumequine, marbofloxacin, nalidixic acid, norfloxacin, oxolinic acid or sarafloxacin was detected in the samples (< 5 µg/kg).

s_{an}^2 = estimate of analytical variance
s_{sam}^2 = estimate of sampling variance
σ_{all}^2 = allowable sampling variance

APPENDIX IIb: Statistical evaluation of homogeneity data of material B for enrofloxacin

	Enrofloxacin (µg/kg)	
Sample No.	Replicate 1	Replicate 2
1	65.0	56.2
2	54.6	57.6
3	59.6	51.2
4	52.1	54.3
5	57.1	58.1
6	58.5	58.3
7	66.1	64.4
8*	60.5	198.8
9	59.9	59.4
10	62.3	56.7
Grand mean	58.4	
Cochran's test		
C	0.395	
C_{crit}	0.638	
$C < C_{crit}$?	NO OUTLIERS	
Target sd (σ_p)	Horwitz: 12.9	
s_{an}^2	10.9	
s_{sam}^2	6.7	
σ_{all}^2	14.9	
critical	39.0	
s_{sam}^2 < critical?	ACCEPTED	

* One of the duplicate results is very high due to a low signal of the internal standard. This duplicate was indicated as an outlier by the Cochran's test. The presented results of the Cochran's test were calculated after elimination of this outlying duplicate.

No flumequine, marbofloxacin, nalidixic acid, norfloxacin, oxolinic acid or sarafloxacin was detected in the samples (< 5 µg/kg).

s_{an}^2 = estimate of analytical variance
s_{sam}^2 = estimate of sampling variance
σ_{all}^2 = allowable sampling variance

APPENDIX IIc: Statistical evaluation of homogeneity data of material B for danofloxacin

	Danofloxacin (µg/kg)	
Sample No.	Replicate 1	Replicate 2
1	223	187
2	165	167
3	197	174
4	162	155
5	178	203
6	196	194
7	226	231
8*	227	678
9	225	204
10	205	208
Grand mean	195	
Cochran's test		
C	0.449	
C_{crit}	0.638	
$C < C_{crit}$?	NO OUTLIERS	
Target sd (σ_p)	Horwitz: 39.8	
s_{an}^2	167	
s_{sam}^2	415	
σ_{all}^2	165	
critical	478	
s_{sam}^2 < critical?	ACCEPTED	

* One of the duplicate results is very high due to a low signal of the internal standard. This duplicate was indicated as an outlier by the Cochran's test. The presented results of the Cochran's test were calculated after elimination of this outlying duplicate.

No flumequine, marbofloxacin, nalidixic acid, norfloxacin, oxolinic acid or sarafloxacin was detected in the samples (< 5 µg/kg).

s_{an}^2 = estimate of analytical variance
s_{sam}^2 = estimate of sampling variance
σ_{all}^2 = allowable sampling variance

APPENDIX IId: Statistical evaluation of homogeneity data of material B for difloxacin

	Difloxacin (µg/kg)	
Sample No.	Replicate 1	Replicate 2
1	300	229
2	207	203
3	253	210
4	196	193
5	206	252
6	224	258
7	275	269
8*	272	815
9	256	205
10	273	214
Grand mean	234	
Cochran's test		
C	0.314	
C_{crit}	0.638	
$C < C_{crit}$?	NO OUTLIERS	
Target sd (σ_p)	Horwitz: 46.7	
s_{an}^2	899	
s_{sam}^2	167	
σ_{all}^2	196	
critical	1277	
s_{sam}^2 < critical?	ACCEPTED	

* One of the duplicate results is very high due to a low signal of the internal standard. This duplicate was indicated as an outlier by the Cochran's test. The presented results of the Cochran's test were calculated after elimination of this outlying duplicate.

No flumequine, marbofloxacin, nalidixic acid, norfloxacin, oxolinic acid or sarafloxacin was detected in the samples (< 5 µg/kg).

s_{an}^2 = estimate of analytical variance
s_{sam}^2 = estimate of sampling variance
σ_{all}^2 = allowable sampling variance

APPENDIX IIIa: Statistical evaluation of homogeneity data of material C for ciprofloxacin

	Ciprofloxacin (µg/kg)	
Sample No.	Replicate 1	Replicate 2
1	36.3	36.3
2	36.4	36.3
3	39.9	37.6
4	36.8	37.8
5	32.3	32.5
6	31.8	32.8
7	26.7	30.3
8	29.8	35.3
9	32.5	30.1
10	34.9	35.3
Grand mean	34.1	
Cochran's test		
C	0.527	
C_{crit}	0.602	
$C < C_{crit}$?	NO OUTLIERS	
Target sd (σ_p)	Horwitz: 7.5	
s_{an}^2	2.8	
s_{sam}^2	8.7	
σ_{all}^2	5.1	
critical	12.3	
s_{sam}^2 < critical?	ACCEPTED	

No flumequine, marbofloxacin, nalidixic acid, norfloxacin, oxolinic acid or sarafloxacin was detected in the samples (< 5 µg/kg).

s_{an}^2 = estimate of analytical variance
s_{sam}^2 = estimate of sampling variance
σ_{all}^2 = allowable sampling variance

APPENDIX IIIb: Statistical evaluation of homogeneity data of material C for enrofloxacin

	Enrofloxacin (µg/kg)	
Sample No.	Replicate 1	Replicate 2
1	80.0	73.7
2	75.0	78.4
3	79.6	71.4
4	76.1	73.6
5	71.9	75.2
6	66.1	83.7
7	73.7	69.7
8	69.5	73.6
9	76.9	69.0
10	73.2	70.1
Grand mean	74.0	
Cochran's test		
C	0.562	
C_{crit}	0.602	
$C < C_{crit}$?	NO OUTLIERS	
Target sd (σ_p)	Horwitz: 16.3	
s_{an}^2	27.3	
s_{sam}^2	0	
σ_{all}^2	23.9	
critical	72.4	
s_{sam}^2 < critical?	ACCEPTED	

No flumequine, marbofloxacin, nalidixic acid, norfloxacin, oxolinic acid or sarafloxacin was detected in the samples (< 5 µg/kg).

s_{an}^2 = estimate of analytical variance
s_{sam}^2 = estimate of sampling variance
σ_{all}^2 = allowable sampling variance

APPENDIX IIIc: Statistical evaluation of homogeneity data of material C for danofloxacin

	Danofloxacin (µg/kg)	
Sample No.	Replicate 1	Replicate 2
1	130	127
2	132	147
3	155	123
4	134	122
5	119	130
6	143	146
7	121	116
8	123	140
9	109	105
10	133	119
Grand mean	129	
Cochran's test		
C	0.510	
C_{crit}	0.602	
$C < C_{crit}$?	NO OUTLIERS	
Target sd (σ_p)	Horwitz: 28.0	
s_{an}^2	103	
s_{sam}^2	80	
σ_{all}^2	72	
critical	240	
s_{sam}^2 < critical?	ACCEPTED	

No flumequine, marbofloxacin, nalidixic acid, norfloxacin, oxolinic acid or sarafloxacin was detected in the samples (< 5 µg/kg).

s_{an}^2 = estimate of analytical variance
s_{sam}^2 = estimate of sampling variance
σ_{all}^2 = allowable sampling variance

APPENDIX IIId: Statistical evaluation of homogeneity data of material C for difloxacin

	Difloxacin (µg/kg)	
Sample No.	Replicate 1	Replicate 2
1	133	142
2	143	152
3	126	147
4	156	141
5	136	152
6	127	124
7	135	135
8	115	148
9	143	117
10	150	117
Grand mean	137	
Cochran's test		
C	0.279	
C_{crit}	0.602	
$C < C_{crit}$?	NO OUTLIERS	
Target sd (σ_p)	Horwitz: 29.6	
s_{an}^2	194	
s_{sam}^2	0	
σ_{all}^2	79	
critical	343	
s_{sam}^2 < critical?	ACCEPTED	

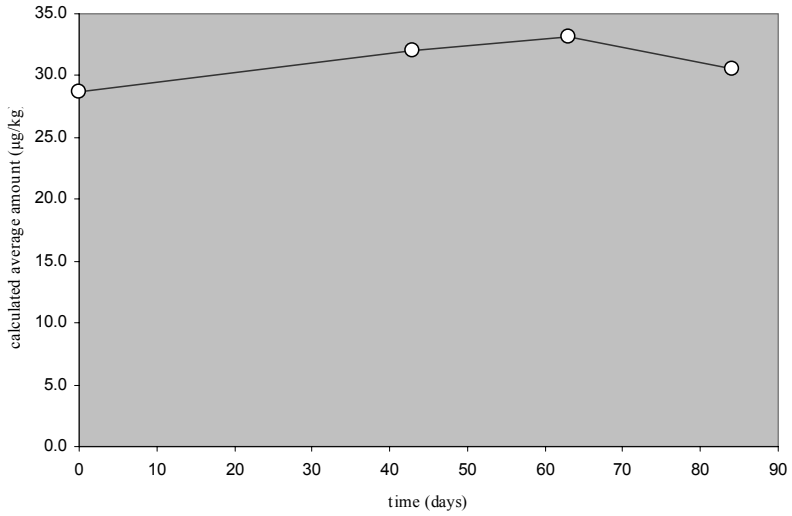
No flumequine, marbofloxacin, nalidixic acid, norfloxacin, oxolinic acid or sarafloxacin was detected in the samples (< 5 µg/kg).

s_{an}^2 = estimate of analytical variance
s_{sam}^2 = estimate of sampling variance
σ_{all}^2 = allowable sampling variance

APPENDIX IVa: Statistical evaluation of stability data of ciprofloxacin

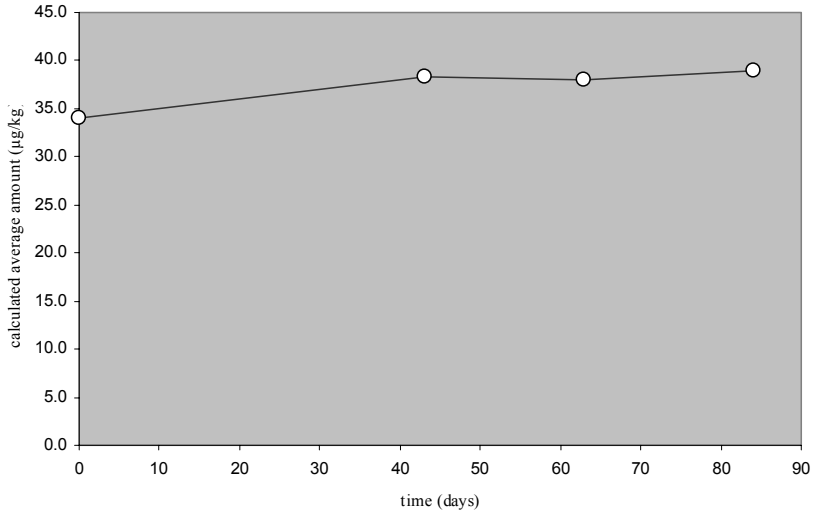
Statistical evaluation for ciprofloxacin in material B

Date of analysis	Time (days)	Average amount (µg/kg)	n	Pooled st. dev (µg/kg)	t	t_{crit}	$t < t_{crit}$
12-16-2005	0	28.7	18				
03-15-2006	88	30.5	6	5.1	0.75	2.08	ACCEPTED



Statistical evaluation for ciprofloxacin in material C

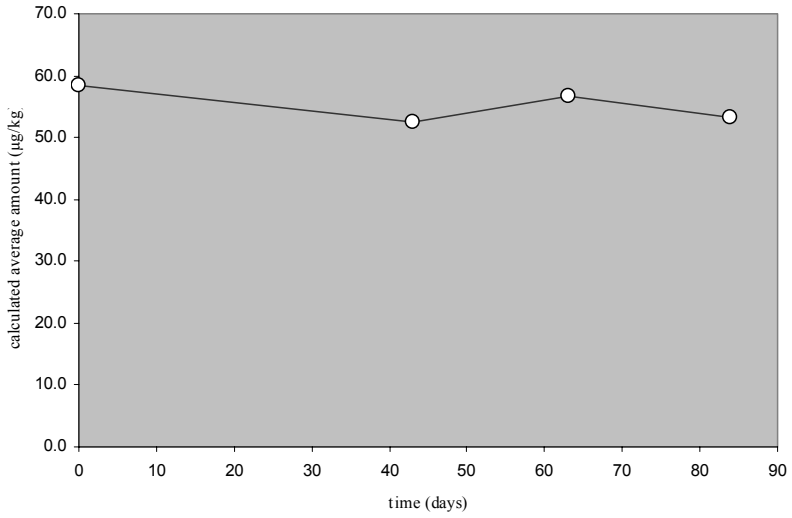
Date of analysis	Time (days)	Average amount (µg/kg)	n	Pooled st. dev (µg/kg)	t	t_{crit}	$t < t_{crit}$
12-16-2005	0	34.1	20				
03-15-2006	88	38.9	6	6.1	1.69	2.06	ACCEPTED



APPENDIX IVb: Statistical evaluation of stability data of enrofloxacin

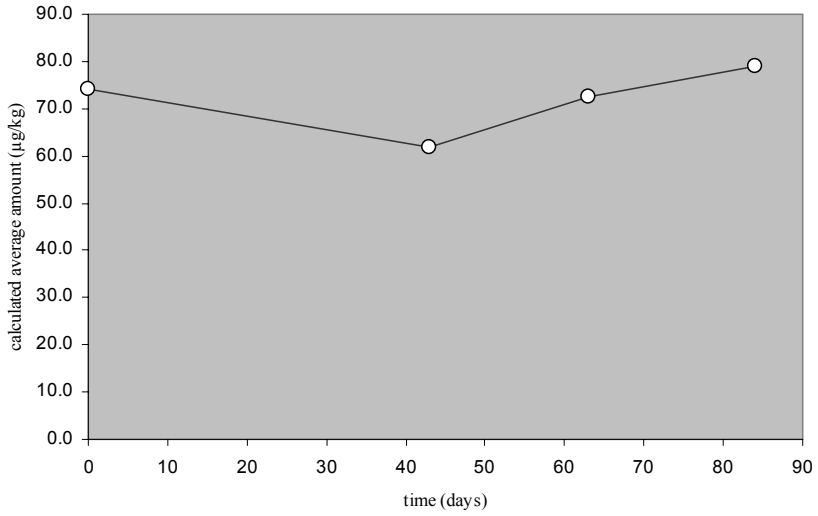
Statistical evaluation for enrofloxacin in material B

Date of analysis	Time (days)	Average amount (µg/kg)	n	Pooled st. dev (µg/kg)	t	t_{crit}	$t < t_{crit}$
12-16-2005	0	58.4	18				
03-15-2006	88	53.3	6	6.4	1.69	2.08	ACCEPTED



Statistical evaluation for enrofloxacin in material C

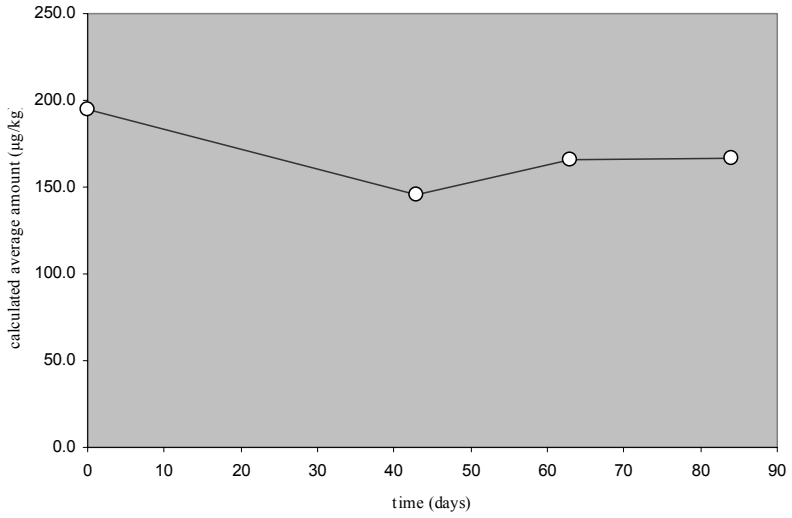
Date of analysis	Time (days)	Average amount (µg/kg)	n	Pooled st. dev (µg/kg)	t	t_{crit}	$t < t_{crit}$
12-16-2005	0	74.0	20				
03-15-2006	88	78.9	6	6.8	1.54	2.06	ACCEPTED



APPENDIX IVc: Statistical evaluation of stability data of danofloxacin

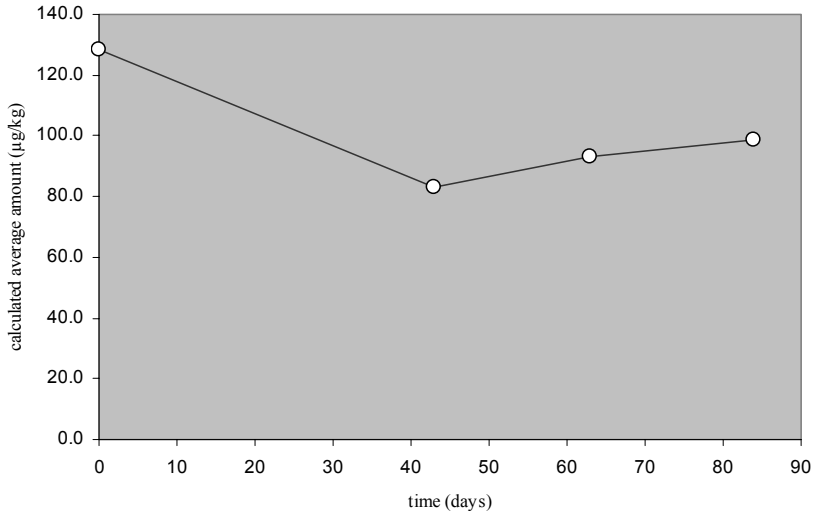
Statistical evaluation for danofloxacin in material B

Date of analysis	Time (days)	Average amount (µg/kg)	n	Pooled st. dev (µg/kg)	t	t_{crit}	$t < t_{crit}$
12-16-2005	0	195	18				
03-15-2006	88	167	6	30.7	1.90	2.08	ACCEPTED



Statistical evaluation for danofloxacin in material C

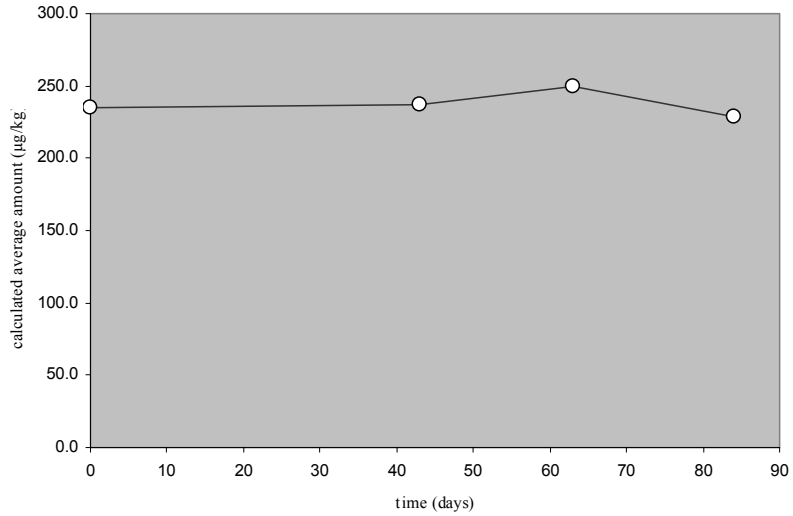
Date of analysis	Time (days)	Average amount (µg/kg)	n	Pooled st. dev (µg/kg)	t	t_{crit}	$t < t_{crit}$
12-16-2005	0	129	20				
03-15-2006	88	99	6	17.1	3.76	2.06	NOT ACCEPTED



APPENDIX IVd: Statistical evaluation of stability data of difloxacin

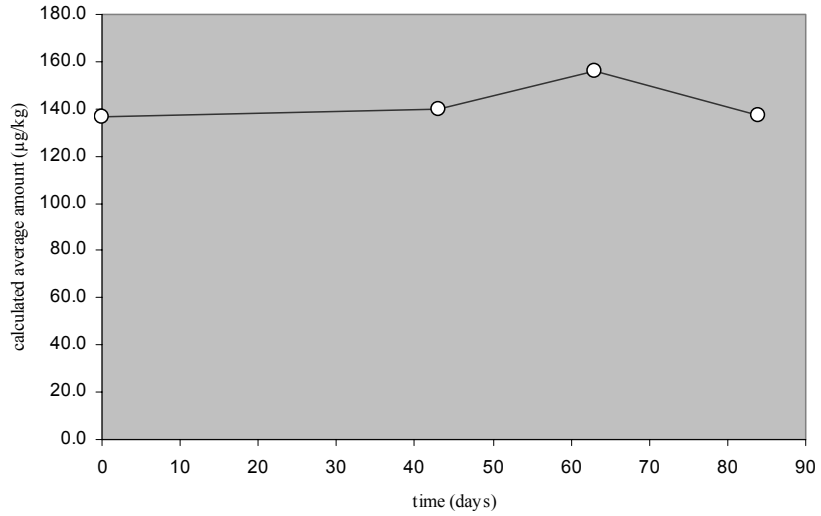
Statistical evaluation for difloxacin in material B

Date of analysis	Time (days)	Average amount (µg/kg)	n	Pooled st. dev (µg/kg)	t	t_{crit}	$t < t_{crit}$
12-16-2005	0	234	18				
03-15-2006	88	229	6	14.7	0.79	2.08	ACCEPTED



Statistical evaluation for difloxacin in material C

Date of analysis	Time (days)	Average amount (µg/kg)	n	Pooled st. dev (µg/kg)	t	t_{crit}	$t < t_{crit}$
12-16-2005	0	137	20				
03-15-2006	88	138	6	12.8	0.09	2.06	ACCEPTED



APPENDIX V: Overview of the applied methods

Lab code	Extraction	Sample purification	Internal standard	Detection method	Quinolones not analysed for	2002/657/EC ?
Lab1	PBS-EDTA buffer / ACN	Dilution, ultrafiltration (30kD)	-	LC-MS/MS		Yes
Lab2	Phosphoric acid, ACN, MeOH	Partial evaporation, SPE (Oasis® HLB), etulion MeOH, evaporation, reconstitution	-	LC-FLU-UV	marbofloxacin	Yes
Lab3	Phosphate buffer (pH=7.4)	SPE (C_{18}), elution TFA in ACN, evaporation, reconstitution	-	LC-FLU	difloxacin, marbofloxacin, sarafloxacin	NM
Lab4	Water	Filtration 0.45 µm, ultrafiltration (30 kD)	cincophen, lomefloxacin	LC-MS/MS	-	Yes
Lab5			-	LC-FLU	danofloxacin, difloxacin, marbofloxacin, nalidixic acid, norfloxacin, sarafloxacin	
Lab7	Acetonitrile	SPE (SDB-RPS)	cincophen, lomefloxacin	LC-MS/MS	difloxacin, sarafloxacin	Yes
Lab9	Phosphate buffer (pH=6.5)	SPE (C_{18}), elution acetic MeOH, evaporation, reconstitution	-	LC-FLU	nalidixic acid	Yes
Lab10	Acetic acid in ethanol	Evaporation of solvent, reconstitution, centrifugation	-	LC-FLU	-	Yes
Lab11	Acedic ACN	SPE (C_{18}), elution ACN, evaporation, reconstitution	-	LC-MS/MS	-	
Lab12	Glycine / HCl	SPE (OASIS® HLB), etution MeOH evaporation, reconstitution	d_8-ciprofloxacin d_5-enrofloxacin	LC-MS/MS		Yes
Lab13	ACN	Evaporation, reconstitution, SPE (SDB-RPS), elution ACN/NH_4OH, evaporation, reconstitution, filtration 0.45 µm	d_5-norfloxacin	LC-MS/MS	-	Yes
Lab14	Trichloroacetic acid (5%)	Filtration 0.45 µm	-	LC-FLU	-	Yes
Lab15	Water	SPE (C_{18}), elution: 1% TFA in ACN evaporation, reconstitution	quinine / sarafloxacin[1)]	LC-MS/MS	-	Yes
Lab16	Trichloricacetic acid (5%)	Filtration 0.45 µm	d_5-norfloxacin	LC-MS/MS	-	Yes
Lab17	Phosphate buffer (pH=7.4)	SPE (DSC-C_{18}), elution MeOH/NH_4OH, evaporation, reconstitution	-	LC-FLU	marbofloxacin, nalidixic acid, norfloxacin	Yes

NM = not mentioned

[1)] The screening analysis showed that no sarafloxacin was in the sample. Because of structure equivalency, sarafloxacin was used as the internal standard for the CIP, ENR, DAN and DIF.

APPENDIX VI: Overview of method characteristics as reported by the participants

		CIP		ENR		DAN		DIF	
Lab code	Validation / accreditation	CCα (µg/kg)	CCß (µg/kg)	CCα (µg/kg)	CCß (µg/kg)	CCα (µg/kg)	CCß (µg/kg)	CCα (µg/kg)	CCß (µg/kg)
Lab1	Yes (not for DIF, SAR, MAR) / No	112	125	110	120	120	139		
Lab3	Yes /Yes								
Lab4	Yes / No	117	141	115	141	252	310	333	383
Lab5	Yes / Yes	1	2	1	2				
Lab6									
Lab7	Yes / Yes		<0.25		<0.25		<50		
Lab10	Yes / Yes	115	130	115	130	230	260	345	390
Lab12	Yes / Yes	113	123	116	134	233	260	334	351
Lab13	Yes / Yes	111	136	114	142	228	288	362	503
Lab14	Yes / Yes			119	140	223	245	327	350
Lab15	Yes / Yes	119.1	137.7	117.7	136.3	262.2	316.5	458.1	516.9
Lab16	In progress / No	115	130	115	130	234	269	352	404
Lab17	Yes / Yes	59.4	69.1	65.0	79.3				

		CIP		ENR		DAN		DIF	
Lab code	Validation according to 2002/657/ accreditation	LoD (µg/kg)	LoQ (µg/kg)	LoD (µg/kg)	LoQ (µg/kg)	LoD (µg/kg)	LoQ (µg/kg)	LoD (µg/kg)	LoQ (µg/kg)
Lab2	Yes (validation not according to 2002/657/EC / Yes	10	20	10	20	5	10	10	20
Lab3	Yes / Yes	0.25	0.40	0.51	0.83	0.25	0.40		
Lab4	Yes / No	5	10	5	10	5	10	5	10
Lab6									
Lab7	Yes / Yes	1.91	6.38	0.87	2.98	0.87	2.90		
Lab9	Yes/ CIP+ENR	2	3	1	2				
Lab10	Yes / Yes		6		6		2		10
Lab11	No / Yes	0.02	0.05	0.02	0.05	0.02	0.05	0.02	0.05
Lab14	Yes / Yes			5	15	7	20	6	25

APPENDIX VII: The result for the analysis of ciprofloxacin in poultry muscle (material B)

Ciprofloxacin											
Assigned value: 33.2 µg/kg											
Uncertainty of assigned value: 3.1 µg/kg											
Target standard deviation (Horwitz, Thompson): 7.3 µg/kg											
Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Average	s_r	s_{R_L}	z_a'-score	*HORRAT*
Lab1	12	11	13	11	13	13	12.2	0.91	1.00	**-2.65**	0.14
Lab2	38.3	40.8	36.8	38.2	39.2	37.2	38.4	1.43	1.45	0.66	0.20
Lab3	19.7	18.3	19.4	19.3	20.3	18.0	19.2	1.10	1.10	-1.76	0.15
Lab4	35	35	32	32	30	30	32.3	0.00	2.52	-0.10	0.34
Lab5	24.9	27.4	26.7	30.2	25.8	28.8	27.3	2.14	2.14	-0.74	0.29
Lab7	26	26	29	24	26	27	26.3	2.08	2.08	-0.86	0.29
Lab9	34.5	34.2	35.1	34.5	31.0	32.4	33.6	0.63	1.73	0.06	0.24
Lab10	34.1	32.9	31.4	32.6	32.0	31.1	32.4	0.78	1.16	-0.10	0.16
Lab11	47.32	46.29	3.40*	9.20*	50.49	49.27	48.3	2.46	24.38	1.91	**3.32**
Lab12	41.3	42.1	45.6	39.5	42.2	42.9	42.3	2.53	2.53	1.15	0.35
Lab13	52.14	54.44	55.71	55.24	59.76	59.01	56.1	1.01	3.17	**2.89**	0.43
Lab14	7	8	9	8	6	5	7.2	0.71	1.61	**-3.28**	0.22
Lab15	34.1	38.0	38.0	42.7	40.4	42.7	39.3	2.66	3.45	0.78	0.47
Lab16	69.1	68.6	64.7	73.0	61.0	72.1	68.1	5.66	5.66	**4.40**	0.78
Lab17	31.8	29.8	33.1	28.9	27.0	26.9	29.6	1.90	2.65	-0.45	0.36

Bold values indicate a questionable or unsatisfactory performance ($|z_a$'-score$|>2$ or *HORRAT*>1)

* This value was indicated as an outlier and was therefore not included in the statistical evaluation regarding the accuracy.

Figure a: Graphical representation of the reported results

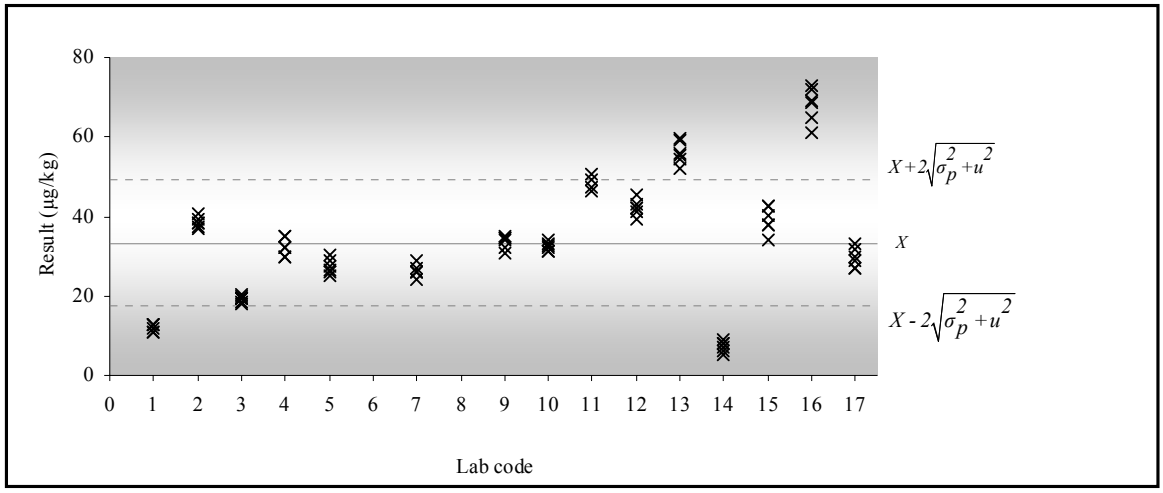


Figure b: Graphical representation of z_a'-score

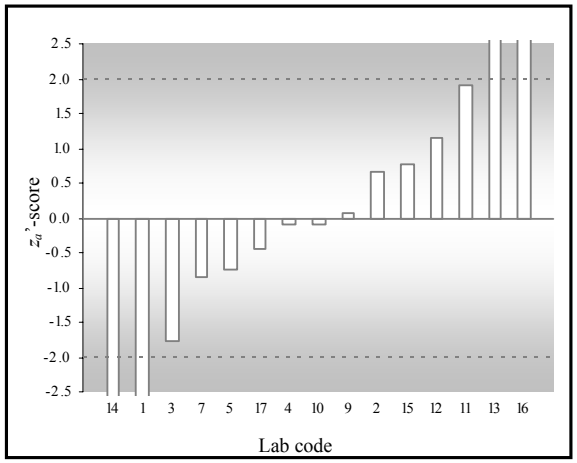
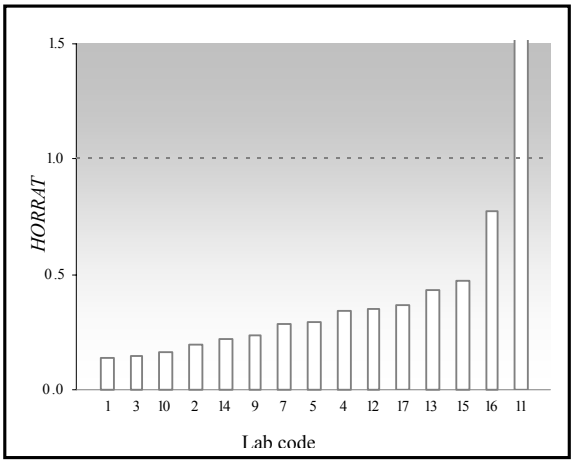


Figure c: Graphical representation of *HORRAT*



APPENDIX VIII: The result for the analysis of ciprofloxacin in poultry muscle (material C)

Ciprofloxacin									
Assigned value: 38.1 µg/kg									
Uncertainty of assigned value: 12.6 µg/kg									
Target standard deviation (Horwitz, Thompson): 3.2 µg/kg									
Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average	s_r	s_{R_L}	z_a'-score	*HORRAT*
Lab1	12	12	14	14	13.0	0.00	1.41	**-2.79**	0.17
Lab2	35.6	42.1	43.2	42.3	40.8	2.68	3.35	0.30	0.40
Lab3	18.6	20.1	26.8	25.1	22.7	0.93	4.71	-1.72	0.56
Lab4	36	31	27	40	33.5	5.69	5.69	-0.51	0.68
Lab5	25.1	31.8	27.2	31.4	28.9	3.23	3.23	-1.02	0.39
Lab7	26	30	33	33	30.5	1.63	3.72	-0.84	0.44
Lab9	42.9	39.1	37.5	40.9	40.1	2.08	2.08	0.23	0.25
Lab10	36.1	42.0	37.8	40.0	39.0	2.57	2.57	0.10	0.31
Lab11	61.61	53.06	54.08	62.22	57.7	4.82	4.82	**2.19**	0.58
Lab12	47.2	46.3	42.4	49.4	46.3	2.88	2.88	0.92	0.34
Lab13	61.11	64.09	55.81	60.32	60.3	2.21	3.57	**2.48**	0.43
Lab14	9	10	9	9	9.3	0.41	0.46	**-3.21**	0.05
Lab15	44.9	46.0	41.5	49.1	45.4	3.14	3.14	0.81	0.37
Lab16	84.2	69.9	62.8	74.8	72.9	7.62	7.94	**3.88**	0.95
Lab17	34.5	37.4	28.6	30.2	32.7	1.35	4.73	-0.60	0.56

Bold values indicate a questionable or unsatisfactory performance ($|z_a$'-score$|>2$)

APPENDIX VIII: The result for the analysis of ciprofloxacin in poultry muscle (material C) (continued)

Figure a: Graphical representation of the reported results

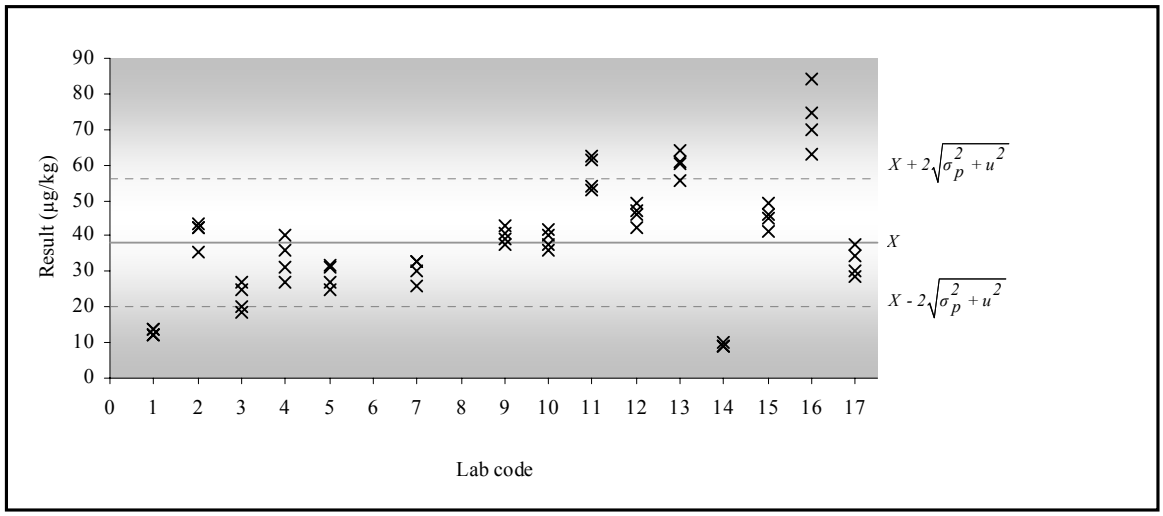


Figure b: Graphical representation of z_a'-score

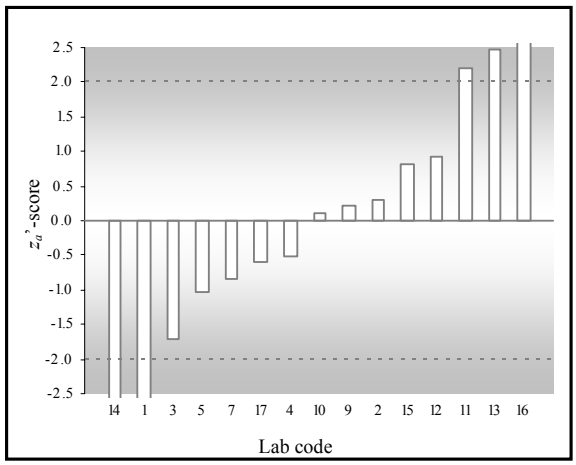
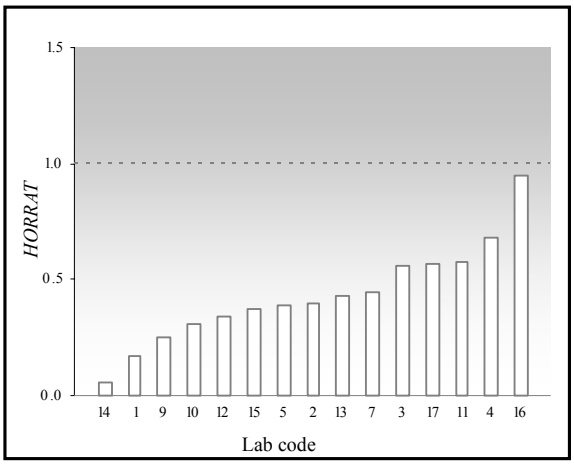


Figure c: Graphical representation of *HORRAT*



APPENDIX IX: The result for the analysis of enrofloxacin in poultry muscle (material B)

Enrofloxacin											
Assigned value: 68.1 µg/kg											
Uncertainty of assigned value: 5.7 µg/kg											
Target standard deviation (Horwitz, Thompson): 15.0 µg/kg											
Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Average	s_r	s_{R_L}	z_a'-score	*HORRAT*
Lab1	20	19	20	19	19	20	19.5	0.71	0.71	**-3.05**	0.05
Lab2	89.1	91.6	73.1	80.5	77.6	76.4	81.4	3.23	8.09	0.90	0.55
Lab3	72.1	72.5	71.0	67.0	63.1	61.5	67.9	1.77	5.25	0.03	0.35
Lab4	54	59	53	53	51	51	53.5	2.04	3.14	-0.88	0.21
Lab5	37.9	41.8	41.5	47.0	39.1	43.7	41.8	3.33	3.33	-1.63	0.22
Lab7	28	24	30	17	28	29	26.0	5.57	5.57	**-2.64**	0.38
Lab9	63.2	66.0	65.8	66.9	65.9	68.6	66.1	1.65	1.78	-0.08	0.12
Lab10	78.2	73.6	78.1	73.1	73.4	72.0	74.7	2.83	2.83	0.47	0.19
Lab11	86.50	96.50	4.76*	90.91	94.03	88.27	91.2	4.71	25.33	1.44	**1.71**
Lab12	59.1	57.4	64.5	63.3	60.5	59.0	60.6	1.05	3.02	-0.43	0.20
Lab13	72.69	70.57	71.40	73.21	74.77	71.62	72.4	1.72	1.72	0.32	0.12
Lab14	97	110	109	102	79	70	94.5	7.06	18.05	1.73	**1.22**
Lab15	72.7	78.6	75.9	90.3	74.8	87.6	80.0	8.23	8.23	0.81	0.56
Lab16	164	131	153	135	180	206	161.5	18.66	30.35	**6.01**	**2.05**
Lab17	65.4	66.7	65.7	63.2	58.9	61.2	63.5	0.71	3.28	-0.24	0.22

Bold values indicate a questionable or unsatisfactory performance ($|z_a$'-score$|>2$ or *HORRAT*>1)

* This value was indicated as an outlier and was therefore not included in the statistical evaluation regarding the accuracy.

APPENDIX IX: The result for the analysis of enrofloxacin in poultry muscle (material B) (continued)

Figure a: Graphical representation of the reported results

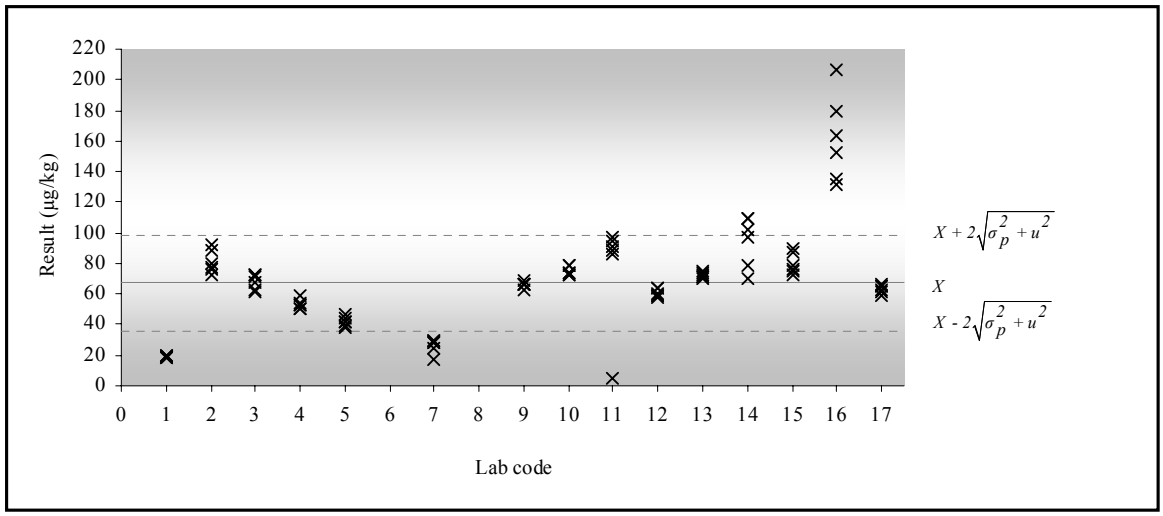


Figure b: Graphical representation of z_a'-score

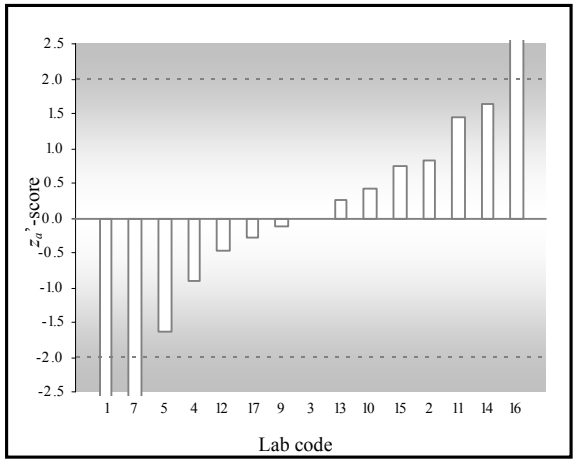
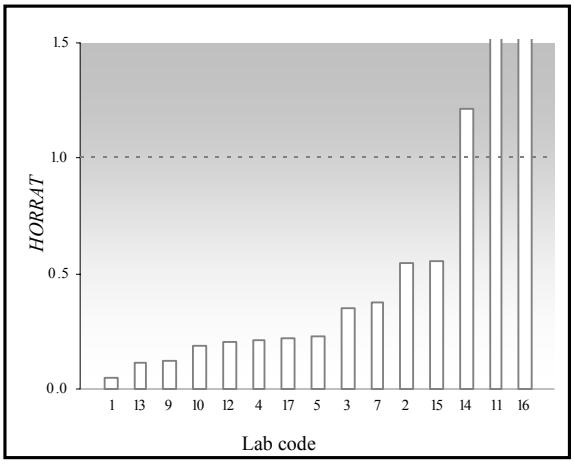


Figure c: Graphical representation of *HORRAT*



APPENDIX X: The result for the analysis of enrofloxacin in poultry muscle (material C)

Enrofloxacin									
Assigned value: 81.9 µg/kg									
Uncertainty of assigned value: 5.6 µg/kg									
Target standard deviation (Horwitz, Thompson): 18.1 µg/kg									
Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average	s_r	s_{R_L}	z_a'-score	*HORRAT*
Lab1	23	22	27	27	24.8	0.50	3.20	**-3.03**	0.18
Lab2	80.4	88.4	88.4	87.2	86.1	3.30	3.35	0.22	0.19
Lab3	65.2	70.0	80.6	83.4	74.8	2.27	10.31	-0.38	0.57
Lab4	71	77	69	73	72.5	2.94	2.97	-0.50	0.16
Lab5	39.6	50.1	47.1	54.6	47.9	5.27	5.65	-1.81	0.31
Lab7	37	41	49	38	41.3	4.78	4.78	**-2.16**	0.27
Lab9	83	80	78	80	80.1	1.71	2.05	-0.10	0.11
Lab10	90.3	90.9	93.2	88.7	90.8	1.85	1.85	0.47	0.10
Lab11	109.75	108.79	111.25	113.88	110.9	1.14	2.47	1.54	0.14
Lab12	74.1	73.5	82.7	82.2	78.1	0.32	6.12	-0.20	0.34
Lab13	85.24	93.74	83.02	88.45	87.6	4.12	4.12	0.30	0.23
Lab14	114	128	140	128	127.5	7.53	10.62	**2.41**	0.59
Lab15	98.2	112.2	101.2	106.9	104.6	6.17	6.17	1.20	0.34
Lab16	199	196	274	281	237.5	3.11	56.61	**8.24**	**3.14**
Lab17	79.9	83.4	60.4	70.9	73.7	4.52	11.76	-0.44	0.65

Bold values indicate a questionable or unsatisfactory performance ($|z_a$'-score$|>2$ or *HORRAT*>1)

APPENDIX X: The result for the analysis of enrofloxacin in poultry muscle (material C) (continued)

Figure a: Graphical representation of the reported results

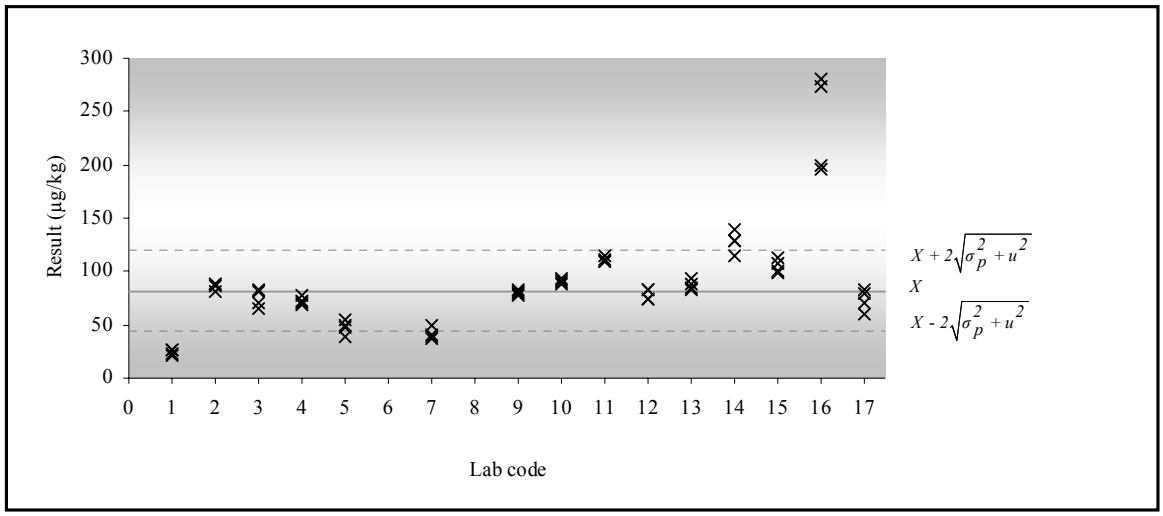


Figure b: Graphical representation of z_a'-score

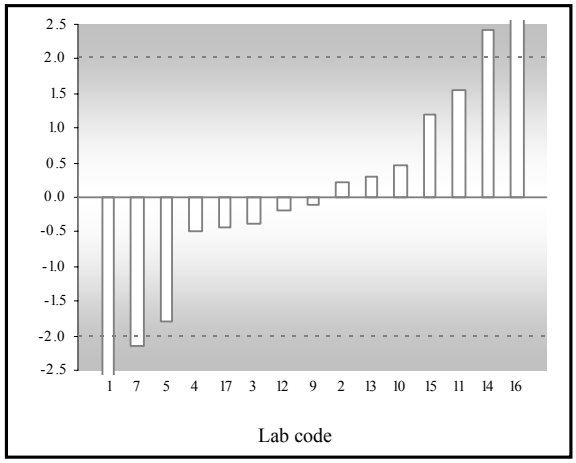
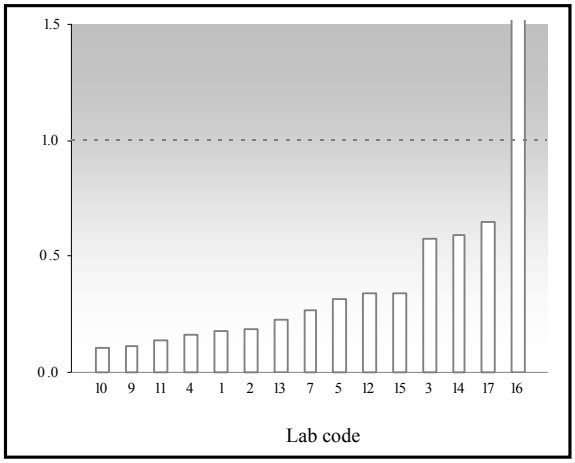


Figure c: Graphical representation of *HORRAT*



APPENDIX XI: The result for the analysis of the sum of ciprofloxacin and enrofloxacin in poultry muscle (material B)

Ciprofloxacin + enrofloxacin											
Assigned value: 101.4 µg/kg											
Uncertainty of assigned value: 7.4 µg/kg											
Target standard deviation (Horwitz, Thompson): 22.3 µg/kg											
Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Average	s_r	s_{R_L}	z_a'-score	*HORRAT*
Lab1	32	30	33	30	32	33	31.7	1.53	1.53	**-2.97**	0.07
Lab2	127.4	132.4	109.9	118.7	116.8	113.6	119.8	4.33	9.28	0.78	0.42
Lab3	91.8	90.8	90.4	86.3	83.4	79.5	87.0	2.35	5.32	-0.61	0.24
Lab4	89	94	85	85	81	81	85.8	2.04	5.49	-0.66	0.25
Lab5	62.8	69.2	68.2	77.2	64.9	72.5	69.1	5.47	5.47	-1.37	0.25
Lab7	54	50	59	41	54	56	52.3	7.57	7.57	**-2.09**	0.34
Lab9	97.7	100.2	100.9	101.4	96.9	101.0	99.7	1.97	1.97	-0.07	0.09
Lab10	112.3	106.5	109.5	105.7	105.4	103.1	107.1	2.98	3.36	0.24	0.15
Lab11	133.8	142.8	8.16*	100.08*	144.5	137.5	139.7	37.81	56.18	1.63	**1.71**
Lab12	100.4	99.5	110.1	102.8	102.7	101.9	102.9	3.02	3.92	0.06	0.18
Lab13	124.8	125.0	127.1	128.5	134.5	130.6	128.4	1.69	4.05	1.15	0.18
Lab14	104	118	118	110	85	75	101.7	7.75	19.60	0.01	0.88
Lab15	106.8	116.6	113.9	133.0	115.2	130.3	119.3	10.71	10.71	0.76	0.48
Lab16	233.1	199.6	217.7	208.0	241.0	278.1	229.6	20.79	29.88	**5.46**	**1.35**
Lab17	97.2	96.5	98.8	92.1	85.9	88.1	93.1	2.89	5.71	-0.35	0.26

Bold values indicate a questionable or unsatisfactory performance ($|z_a$'-score$|>2$ or *HORRAT*>1)

* This value was indicated as an outlier and was therefore not included in the statistical evaluation regarding the accuracy.

APPENDIX XI: The result for the analysis of the sum of ciprofloxacin and enrofloxacin in poultry muscle (material B) (continued)

Figure a: Graphical representation of the reported results

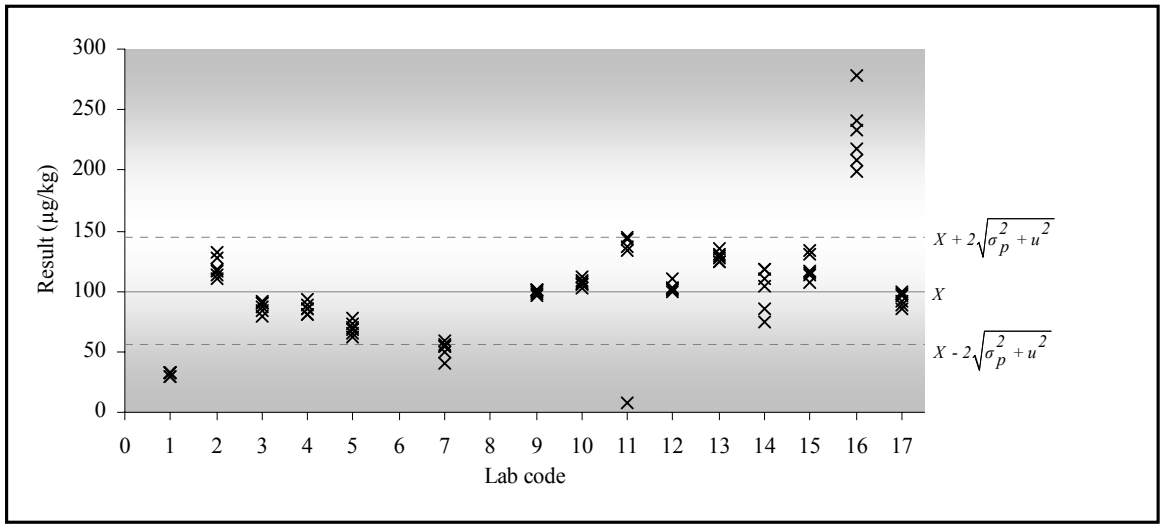


Figure b: Graphical representation of z_a'-score

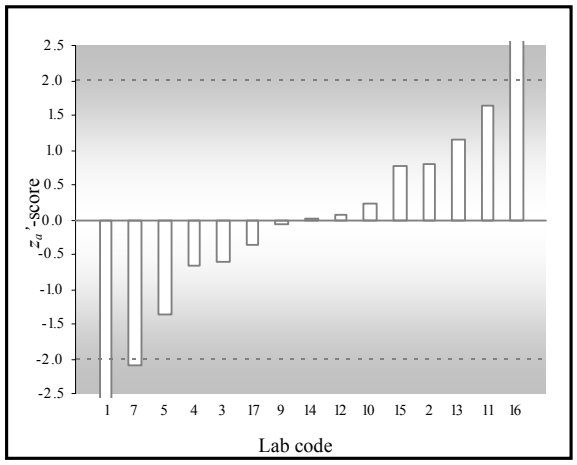
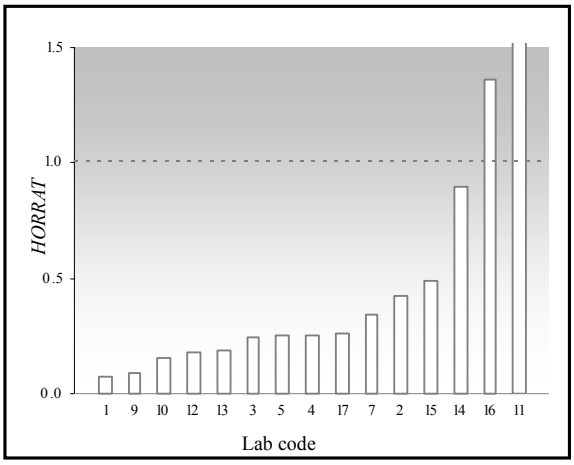


Figure c: Graphical representation of *HORRAT*



APPENDIX XII: The result for the analysis of the sum of ciprofloxacin and enrofloxacin in poultry muscle (material C)

Ciprofloxacin + enrofloxacin									
Assigned value: 122 µg/kg									
Uncertainty of assigned value: 7.3 µg/kg									
Target standard deviation (Horwitz, Thompson): 26.8 µg/kg									
Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average	s_r	s_{R_L}	z_a-score	*HORRAT*
Lab1	35	34	41	41	37.8	0.50	4.61	**-3.15**	0.17
Lab2	116.0	130.5	131.6	129.5	126.9	5.98	6.67	0.17	0.25
Lab3	83.8	90.1	107.4	108.5	97.5	2.61	14.96	-0.92	0.56
Lab4	107	108	96	113	106.0	6.95	6.95	-0.61	0.26
Lab5	64.7	81.9	74.3	86.0	76.7	8.49	8.49	-1.70	0.32
Lab7	63	71	82	71	71.8	5.55	7.78	-1.88	0.29
Lab9	125.8	118.8	115.1	121.2	120.2	3.79	3.97	-0.08	0.15
Lab10	126.4	132.9	131.0	128.7	129.8	2.81	2.81	0.28	0.10
Lab11	171.4	161.9	165.3	176.1	168.7	5.87	5.87	1.73	0.22
Lab12	121.3	119.8	125.1	131.6	124.5	2.72	5.84	0.08	0.22
Lab13	146.4	157.8	138.8	148.8	147.9	6.20	7.32	0.96	0.27
Lab14	123	138	149	137	136.8	7.84	10.43	0.54	0.39
Lab15	143.1	158.2	142.7	156.0	150.0	8.21	8.21	1.03	0.31
Lab16	283.2	265.9	336.8	355.8	310.4	10.49	51.27	**7.01**	**1.91**
Lab17	114.4	120.8	89.0	101.1	106.3	5.59	16.43	-0.59	0.61

Bold values indicate a questionable or unsatisfactory performance ($|z_a$-score$|>2$ or *HORRAT*>1)

APPENDIX XII: The result for the analysis of the sum of ciprofloxacin and enrofloxacin in poultry muscle (material C) (continued)

Figure a: Graphical representation of the reported results

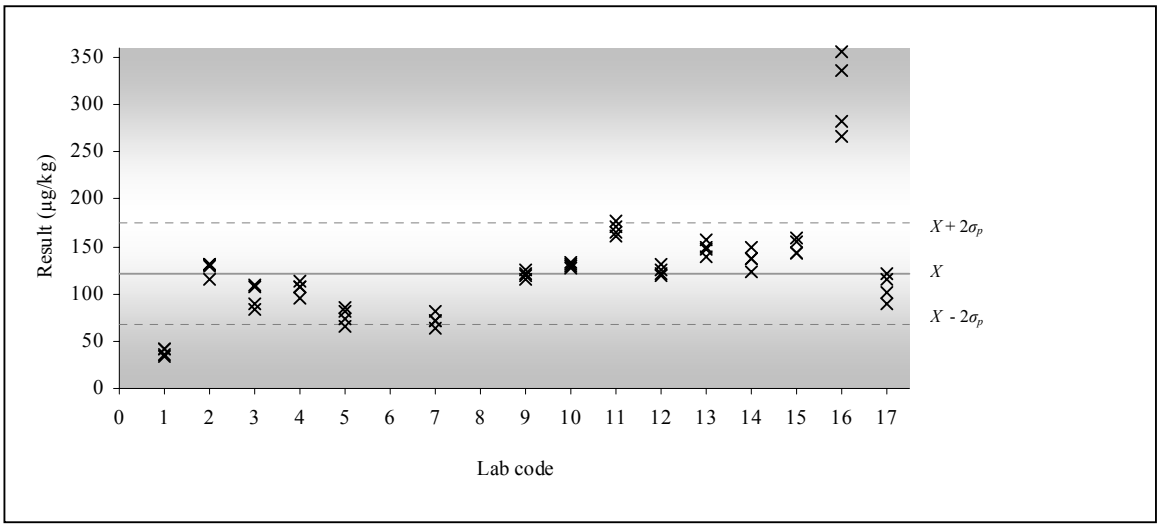


Figure b: Graphical representation of z_a-score

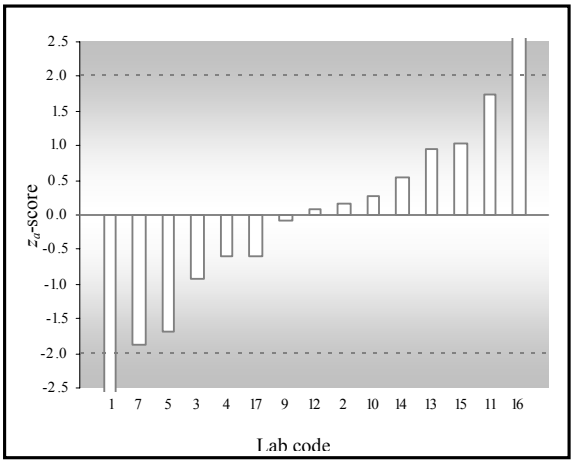
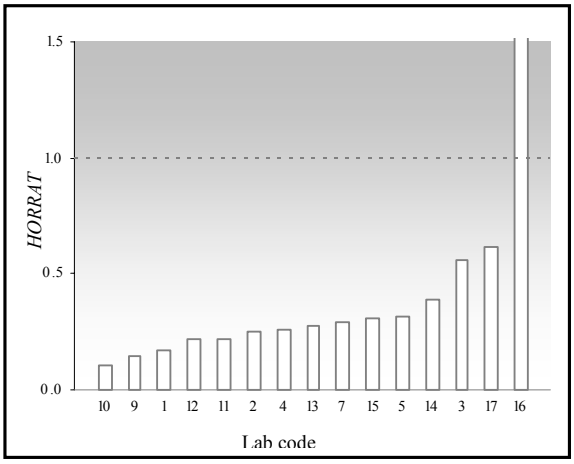


Figure c: Graphical representation of *HORRAT*



APPENDIX XIII: The result for the analysis of danofloxacin in poultry muscle (material B)

Danofloxacin											
Assigned value: 192 µg/kg											
Uncertainty of assigned value: 11 µg/kg											
Target standard deviation (Horwitz, Thompson): 39.3 µg/kg											
Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Average	s_r	s_{R_L}	z_a-score	*HORRAT*
Lab1	19	18	22	18	21	21	19.8	1.68	1.73	**-4.37**	0.05
Lab2	192.6	198.8	183.3	201.2	196.5	187.4	193.3	8.58	8.58	0.04	0.23
Lab3	137.7	148.8	170.8	159.7	139.9	145.9	150.5	6.86	13.69	-1.05	0.36
Lab4	161	173	176	159	163	160	165.3	8.58	8.58	-0.67	0.23
Lab7	158	154	154	122	148	148	147.3	13.17	13.17	-1.13	0.35
Lab9	192.0	192.0	194.0	190.0	191.0	200.0	193.2	4.02	4.02	0.04	0.11
Lab10	207.2	229.5	203.3	230.8	203.5	231.9	217.7	18.53	18.53	0.67	0.49
Lab11	214.65	225.45	0.81*	12.90*	231.59	235.60	226.8	6.82	127.27	0.90	**3.34**
Lab12	200.0	197.8	195.2	199.5	209.6	199.7	200.3	4.50	4.99	0.22	0.13
Lab13	173.64	185.39	164.40	173.12	188.98	176.50	177.0	7.85	9.19	-0.37	0.24
Lab14	365	396	408	370	280	239	343.0	26.10	74.75	**3.85**	**1.96**
Lab15	168.2	157.3	168.5	181.6	168.1	175.1	169.8	7.52	8.28	-0.55	0.22
Lab16	460.0	377.0	459.0	417.0	385.0	540.0	439.7	73.80	73.80	**6.31**	**1.94**
Lab17	204.8	229.1	219.9	203.9	183.7	187.4	204.8	11.97	18.87	0.34	0.50

Bold values indicate a questionable or unsatisfactory performance ($|z_a$-score$|>2$ or *HORRAT*>1)

* This value was indicated as outliers and was therefore not included in the statistical evaluation regarding the accuracy.

APPENDIX XIII: The result for the analysis of danofloxacin in poultry muscle (material B) (continued)

Figure a: Graphical representation of the reported results

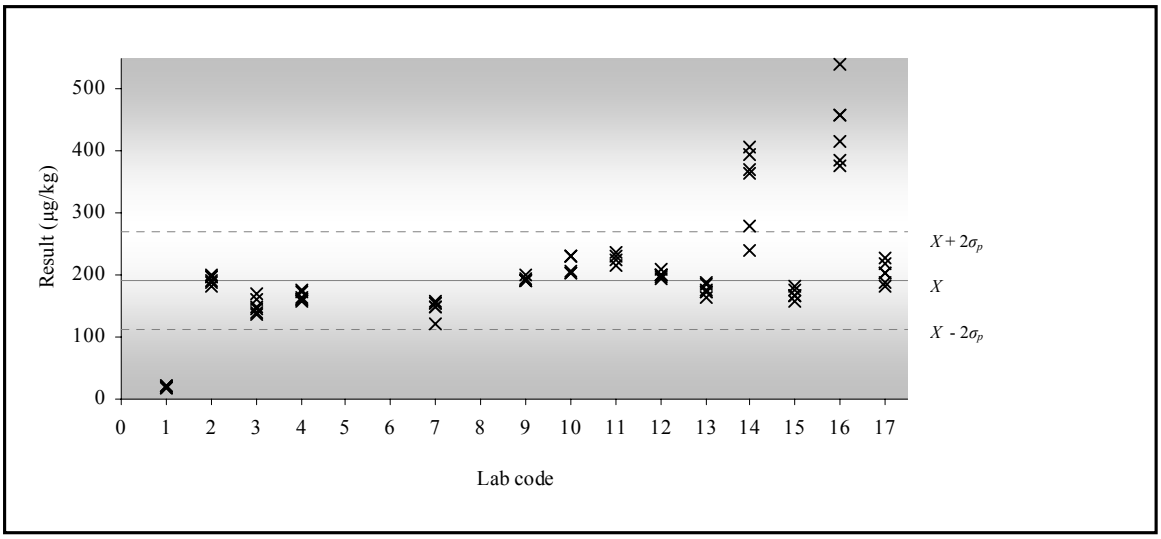


Figure b: Graphical representation of z_a-score

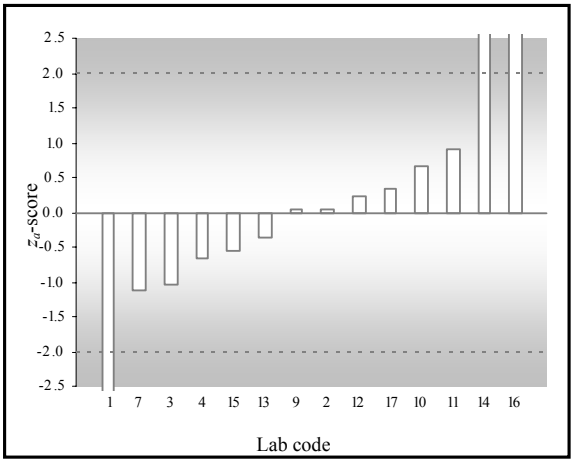
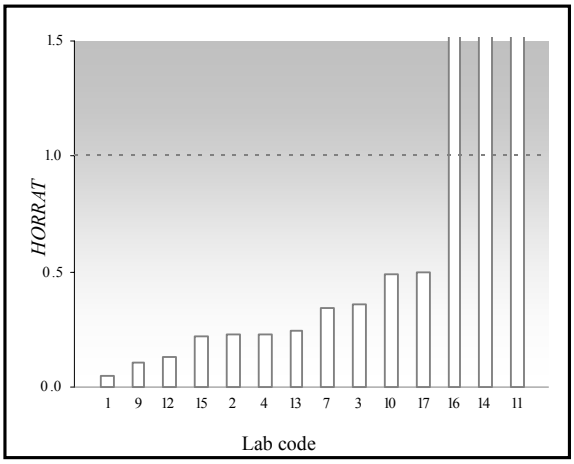


Figure c: Graphical representation of *HORRAT*



APPENDIX XIV: The result for the analysis of danofloxacin in poultry muscle (material C)

Danofloxacin									
Assigned value: 118 µg/kg									
Uncertainty of assigned value: 9.3 µg/kg									
Target standard deviation (Horwitz, Thompson): 26.0 µg/kg									
Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average	s_r	s_{R_L}	z_a'-score	*HORRAT*
Lab1	8	8	11	12	9.8	0.50	2.50	**-3.92**	0.10
Lab2	88.7	115.3	120.5	120.3	111.2	10.86	15.11	-0.25	0.58
Lab3	80.7	87.5	97.1	101.0	91.6	3.20	10.81	-0.96	0.42
Lab4	107	110	84	95	99.0	4.65	13.83	-0.69	0.53
Lab7	88	84	110	110	98.0	1.63	17.01	-0.72	0.66
Lab9	123.0	117.0	113.0	122.0	118.8	4.42	4.42	0.03	0.17
Lab10	123.4	145.3	128.0	145.0	135.4	11.32	11.32	0.63	0.44
Lab11	114.17	149.94	147.93	149.65	140.4	14.62	15.71	0.81	0.61
Lab12	129.6	126.8	130.2	132.4	129.8	1.45	2.42	0.43	0.09
Lab13	112.88	120.31	112.74	114.87	115.2	3.16	3.16	-0.10	0.12
Lab14	191	216	247	216	217.5	16.26	22.89	**3.61**	0.88
Lab15	94.5	106.4	88.2	102.8	98.0	7.69	7.69	-0.73	0.30
Lab16	276.0	231.0	284.0	295.0	271.5	18.91	28.75	**5.57**	**1.11**
Lab17	124.6	132.6	88.3	111.1	114.2	9.86	21.59	-0.14	0.83

Bold values indicate a questionable or unsatisfactory performance ($|z_a$'-score$|>2$ or *HORRAT*>1)

APPENDIX XIV: The result for the analysis of danofloxacin in poultry muscle (material C) (continued)

Figure a: Graphical representation of the reported results

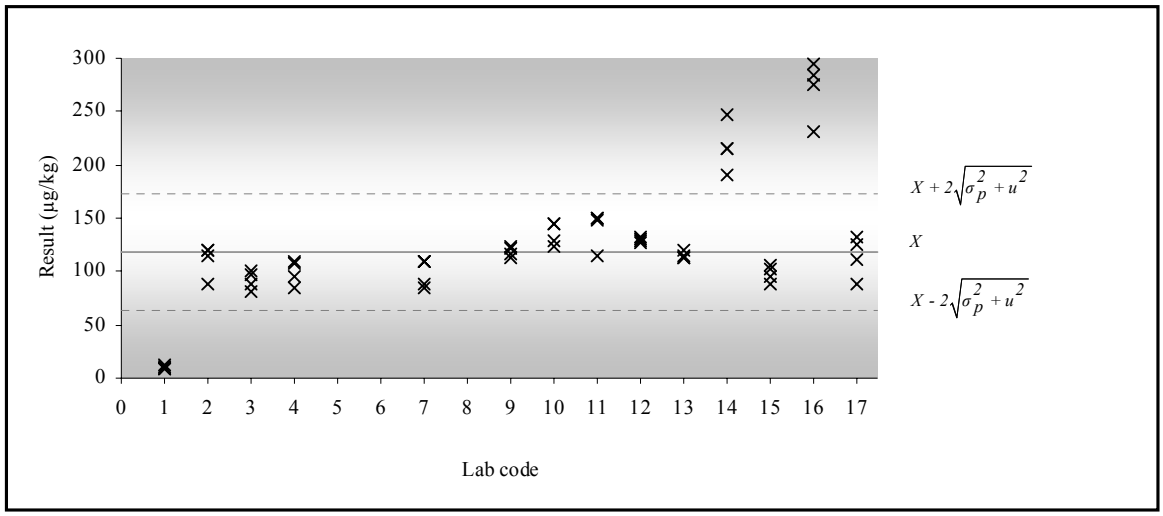


Figure b: Graphical representation of z_a'-score

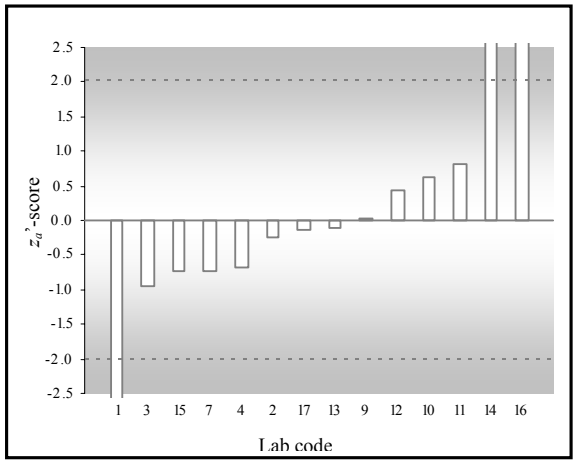
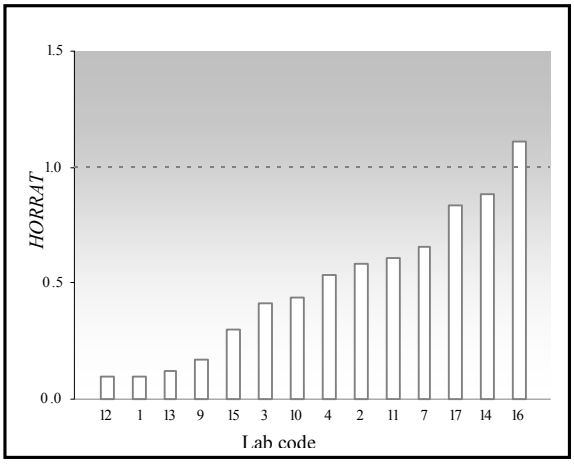


Figure c: Graphical representation of *HORRAT*



APPENDIX XV: The result for the analysis of difloxacin in poultry muscle (material B)

Difloxacin											
Assigned value: 299 µg/kg											
Uncertainty of assigned value: 22 µg/kg											
Target standard deviation (Horwitz, Thompson): 57.4 µg/kg											
Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Average	s_r	s_{R_L}	z_a'-score	*HORRAT*
Lab1	81	81	84	88	91	87	85.3	2.31	4.36	**-3.49**	0.08
Lab2	293.5	292.6	280.7	281.6	283.6	284.3	286.1	0.59	6.24	-0.21	0.11
Lab4	243	252	237	240	243	220	239.2	10.16	10.77	-0.98	0.19
Lab9	274	294	297	296	294	306	293.5	9.53	10.78	-0.09	0.19
Lab10	297.3	280.6	278.7	284.2	281.0	286.2	284.7	7.49	7.49	-0.24	0.13
Lab11	561.31	544.10	512.56	495.18	515.77	506.88	522.6	10.62	27.36	**3.64**	0.48
Lab12	322.5	282.6	409.9	373.7	407.1	376.2	362.0	25.36	54.52	1.02	0.95
Lab13	245.94	235.37	224.16	246.28	255.98	246.84	242.4	10.68	11.18	-0.93	0.19
Lab14	356	426	405	388	296	265	356.0	32.02	69.25	0.93	**1.21**
Lab15	277.8	241.3	276.4	344.0	317.6	343.4	300.1	33.08	43.39	0.01	0.76
Lab16	489	470	612	519	486	456	505.3	40.64	59.66	**3.36**	**1.04**
Lab17	256.5	263.1	246.3	249.8	231.0	235.5	247.0	3.56	13.54	-0.85	0.24

Bold values indicate a questionable or unsatisfactory performance ($|z_a$'-score$|>2$ or *HORRAT*>1)

APPENDIX XV: The result for the analysis of difloxacin in poultry muscle (material B) (continued)

Figure a: Graphical representation of the reported results

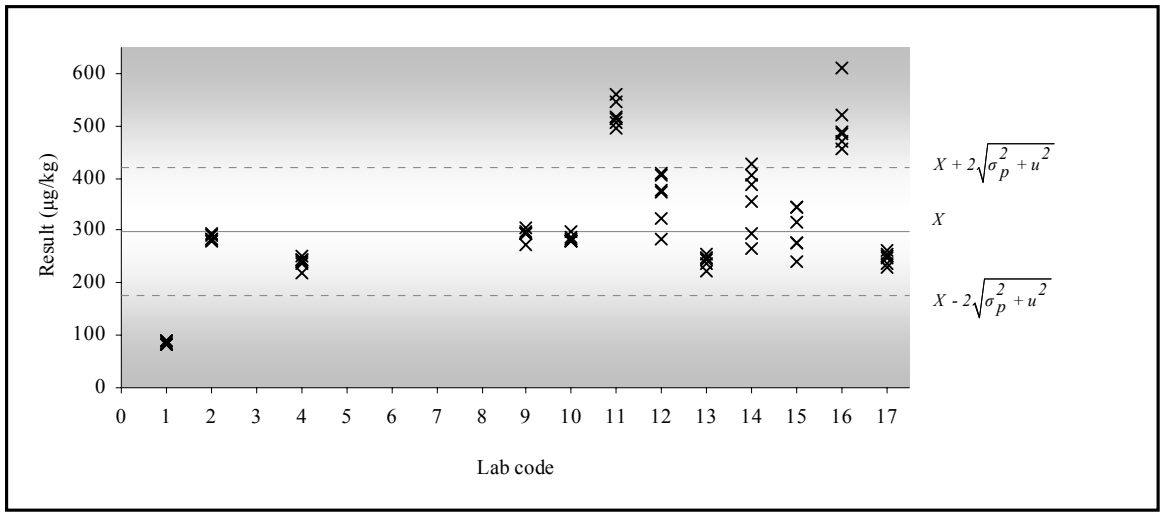


Figure b: Graphical representation of z_a'-score

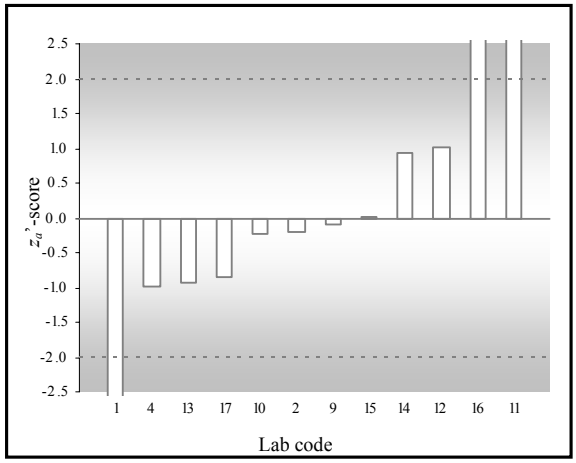
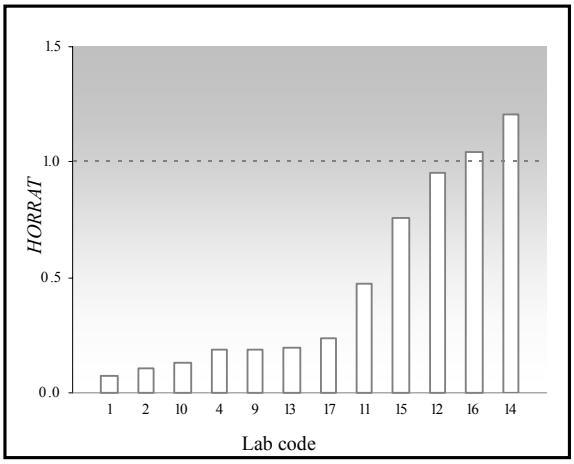


Figure c: Graphical representation of *HORRAT*



APPENDIX XVI: The result for the analysis of difloxacin in poultry muscle (material C)

Difloxacin									
Assigned value: 188 µg/kg									
Uncertainty of assigned value: 20 µg/kg									
Target standard deviation (Horwitz, Thompson): 38.6 µg/kg									
Code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average	s_r	s_{R_L}	z_a'-score	*HORRAT*
Lab1	51	50	57	62	55.0	2.55	6.61	**-3.05**	0.17
Lab2	167.1	169.8	173.1	173.2	170.8	1.10	3.41	-0.39	0.09
Lab4	147	139	114	152	138.0	15.85	15.85	-1.14	0.41
Lab9	186	177	170	181	178.5	5.80	5.90	-0.21	0.15
Lab10	185.6	181.2	184.1	179.2	182.5	2.69	2.69	-0.12	0.07
Lab11	318.47	353.29	346.83	362.46	345.3	15.58	17.25	**3.63**	0.45
Lab12	203.7	166.5	231.0	240.1	210.3	15.63	37.35	0.52	0.97
Lab13	156.54	172.08	147.17	157.94	158.4	7.72	9.94	-0.67	0.26
Lab14	208	249	278	247	245.5	20.98	28.25	1.33	0.73
Lab15	173.1	204.1	153.8	185.9	179.2	18.22	18.49	-0.19	0.48
Lab16	331	324	287	278	305.0	4.65	31.99	**2.70**	0.83
Lab17	154.8	161.1	119.1	133.7	142.2	6.49	22.78	-1.05	0.59

Bold values indicate a questionable or unsatisfactory performance ($|z_a$'-score$|>2$)

APPENDIX XVI: The result for the analysis of difloxacin in poultry muscle (material C) (continued)

Figure a: Graphical representation of the reported results

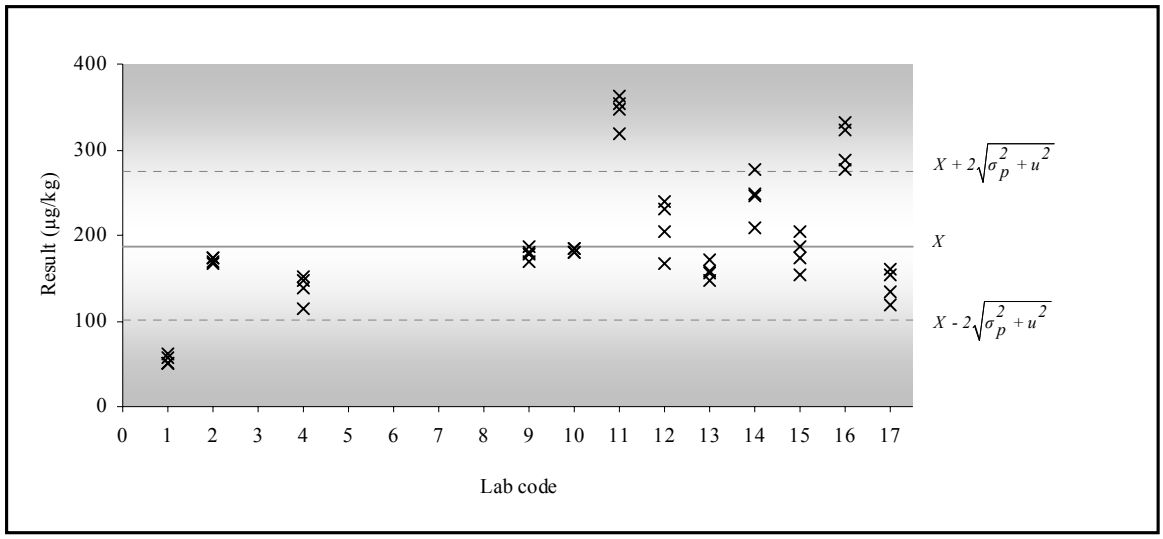


Figure b: Graphical representation of z_a'-score

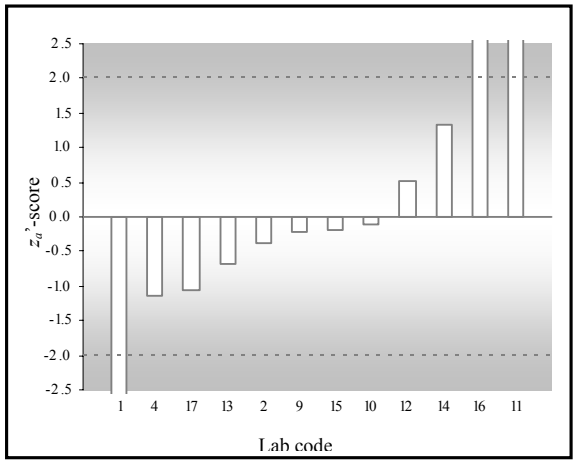
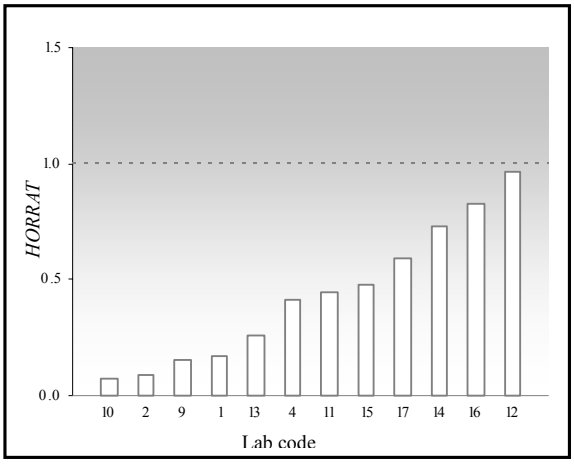


Figure c: Graphical representation of *HORRAT*



APPENDIX XVII: The calculation of the laboratory performance score

			Ciprofloxacin accuracy / reproducibility		Enrofloxacin accuracy / reproducibility		Danofloxacin accuracy / reproducibility		Danofloxacin accuracy / reproducibility				
Code	False pos.	False neg.	Material B	Material C	Material B	Material C	Material B	Material C	Material B	Material C	Accuracy score (%)	Reproducibility score (%)	Laboratory performance score (%)
Lab1	-	-	0 / 1	0 / 1	0 / 1	0 / 1	0 / 1	0 / 1	0 / 1	0 / 1	0	100	50
Lab2	-	-	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	100	100	**100**
Lab3	-	-	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	-	-	100	100	**100**
Lab4	-	-	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	100	100	**100**
Lab5	-	-	1 / 1	1 / 1	1 / 1	1 / 1	-	-	-	-	100	100	**100**
Lab7	-	-	1 / 1	1 / 1	0 / 1	0 / 1	1 / 1	1 / 1	-	-	67	100	83
Lab9	-	-	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	100	100	**100**
Lab10	-	-	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	100	100	**100**
Lab11	-	-	1 / 0	0 / 1	1 / 0	1 / 1	1 / 0	1 / 1	0 / 1	0 / 1	63	63	63
Lab12	-	-	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	100	100	**100**
Lab13	-	-	0 / 1	0 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	75	100	88
Lab14	-	-	0 / 1	0 / 1	1 / 0	0 / 1	0 / 0	0 / 1	1 / 0	1 / 1	38	62	50
Lab15	-	-	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	100	100	**100**
Lab16	-	-	0 / 1	0 / 1	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 1	0	38	19
Lab17	-	-	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1	100	100	**100**