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Journal of Chromatography A, 1088 (2005) 40–48
JOURNAL OF CHROMATOGRAPHY A
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Determination of (fluoro)quinolone antibiotic residues in pig kidney using liquid chromatography-tandem mass spectrometry Part II: Intercomparison exercise

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Available online 24 February 2005

Abstract

A recently in-house validated method for the liquid chromatography-tandem mass spectrometry (LC-MS/MS) determination of eleven (fluoro)quinolone antibiotics (FQs) in pig kidney has been fully validated through an intercomparison exercise. This ring trial involved eight European laboratories and was based on the Commission Decision 2002/657/CE for validation of method and on the IUPAC protocol for method–performances studies. The laboratories data were submitted to a one-way analysis of variance. Satisfactory results were obtained for each FQ with regards to within- and between-laboratory reproducibility and accuracy. The method was validated for the simultaneous qualitative and quantitative determination of the eleven FQs in pig kidney around their maximum residue limit (MRL) as defined in the European Council Regulation 2377/90/EEC.

Keywords: (Fluoro)quinolone; Pig kidney; Liquid chromatography-tandem mass spectrometry; Intercomparison exercise; Ring trial

1. Introduction

The (fluoro)quinolones (FQs) represent a recent and highly potent group of antibiotics used in human and in veterinary medicine. The widespread use of FQs and other antibiotics in agriculture has resulted in the potential presence of these compound residues in foodstuffs from animal origin and, in parallel, to an upsetting increase of resistant human pathogens. In the frame of its policy on consumer health protection, the European Union (EU) established maximum residue limits (MRLs) for various classes of antibiotics among which (fluoro)quinolones, in different animal tissues [1]. State laboratories of the EU have to monitor the residues possibly present in samples coming from the slaughterhouses [2]. The great chemical variety of FQs and the possibility of trace level residues made it necessary to develop sensitive multi-residue screening methods.As described in a previous work [3], a multi-residue method for the determination of FQs, based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and validated in-house. The targeted FQs include seven amphoteric [norfloxacin (No), ofloxacin (Of), enoxacin (En), enrofloxacin (Er), ciprofloxacin (Cp), marbofloxacin (Ma) and danofloxacin (Da)] and four acidic [cinoxacin (Cn), flumequine (Fl), oxolinic acid (Ox) and nalidixic acid (Na)] compounds, which constitute a challenge, as usually amphoteric and acidic FQs can hardly be analysed together [4–8]. In this procedure a single sample preparation procedure is followed by the separation, the identification and the quantification of all eleven FQs in pig kidney in a single analysis by LC-MS/MS. In order to propose a candidate reference method to the EU Member States, this method was validated through an intercomparison exercise involving eight different laboratories. Each laboratory was provided with 10 unknown spiked pig kidney samples containing between 1 and 8 FQs which had to be identified and quantified. The intercomparison trial was organised by the Institute for Reference Materials and

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doi:10.1016/j.chroma.2005.02.016

Measurements of the Joint Research Centre of the European Commission (EC-JRC-IRMM). It was set up according to the Commission Decision 2002/657/CE [9] and to the IUPAC protocol for method–performances studies [10]. The data were submitted to a one-way analysis of variance (ANOVA). The results were evaluated for each FQ respectively in terms of within- and between-laboratory reproducibility and accuracy. The validation results which were discussed and agreed by all participants are presented in this paper.

2. Experimental

The detailed experimental conditions are given in a previous publication [3].

2.1. Reagents

The FQ standards were obtained from Sigma–Aldrich (St. Louis, MO, USA) except danofloxacine mesylate which was provided by Pfizer (Groton, CT, USA) and marbofloxacine by Vetoquinol (Lure, France). All reagents and water used were of analytical purity and suitable for HPLC.

2.2. Samples

Fifty pig kidney samples, reacting negatively to an inhibition test for detection of quinolones in meat [11], were provided by the University of Ghent (Faculty of Veterinary Medicine, Ghent, Belgium). All pig kidneys were pooled and minced and samples were tested for FQ contamination by LC-MS/MS at IRMM.

Each sample submitted to the intercomparison exercise consisted of a minced fresh blank pig kidney spiked with FQ standards and was frozen at −20 °C. Two hundred and sixty vials of ±1 g of FQ standards and internal standards, 40 vials of ±50 g of blank pig kidney and 1200 vials ±1 g of spiked pig kidney samples were prepared at IRMM. Each gram of spiked pig kidney sample was spiked independently.

2.3. Standard solutions preparation

Standard stock solutions were prepared for each FQ and for each internal standard in methanol [3]. Intermediate solutions were obtained by dilution with diluted formic acid (pH 2.5). Final solutions in diluted formic acid (pH 2.5) were prepared at concentrations in the range MRL/4 to MRL × 2 except for flumequine that was prepared at a concentration above MRL × 10.

A single internal standard solution was also prepared for spiking of the unknown pig kidney samples before analysis. This solution was prepared in diluted formic acid (pH 2.5) at 300 ng ml^{-1} (around MRL × 2).

2.4. Instrumentation and methods

The sample preparation consisted of a liquid extraction followed by a solid-phase extraction (SPE) for the clean-up of the samples [3]. The SPE was performed using SDB-RPS disposable extraction disk cartridges (10 mm, 6 ml) from 3 M Empore (St. Paul, MN, USA) containing mixed C_8 and cation-exchange phases. The extracted FQs were separated by liquid chromatography using a Symmetry Shield RP-8 (150 mm × 3.9 mm; 5 μm particule diameter) reversed-phase analytical column from Waters. A guard column Symmetry Shield RP-8 was placed in front of the analytical column.

The LC separation of the FQs was achieved using a gradient elution. The initial mobile phase consisted of 90% A and 10% B, where A was diluted formic acid (pH 2.5) and B was acetonitrile containing 0.14% (v/v) of formic acid. From 0 to 10 min, the percentage of B increased from 10 to 62%. At 10.5 min, B percentage was set at 100% and was stable for 2 min. Finally at 13 min, B percentage was set at 10% and was stable for 2 min for reconditioning of the analytical column.

The flow-rate of the mobile phase was 1.0 ml min^{-1}. A T-piece splitter (4:1) was used between the LC column and the MS detector in order to introduce 200 μl min^{-1} efluent into the ion source of the mass spectrometer. The column temperature was 25 °C. Aliquots of 50 μl of the extracts were injected in the LC-MS/MS system.

The separated FQs were detected by (ESI +)MS/MS using a triple quadrupole mass spectrometer. The (ESI)MS/MS conditions were the followings: + 3.2 kV capillary voltage, source block and desolvation temperatures at 130 and 400 °C, respectively. Desolvation and nebuliser gas (N_2) flows were 650 and 80 l h^{-1}, respectively. Argon pressure in the collision cell was 2.5×10^{-3} mbar. The cone voltage and collision energy for MRM acquisitions are presented in Table 1. The dwell time was 100 ms/transition. Two MS transitions were followed for the FQ identification among which one was used for quantification (in bold in Table 1).

2.5. Results calculation

The results were calculated as a ratio of the response of the respective FQ and the internal standard. A linear calibration curve was established using lomefloxacine as an internal standard for the quantitation of the amphoteric FQs while cinchophen was used for the quantitation of the acidic FQs. A linear calibration curve between the ratio of the response and the concentration of the target FQ was established by linear regression. The curve was not forced through zero. A weighing factor equal to $1/X$ was applied. The concentration of the FQs in the solution was determined using the calibration curve.

3. Participating laboratories

The following eight laboratories took part in the exercise:

Table 1
MRM conditions for the detection of the (fluoro)quinolones in pig kidney using MS/MS

FQ	Parent ion (*m*/*z*)	Cone voltage (V)	Coll. En. (eV)	Fragment ion (*m*/*z*)
Amphoteric FQs				
Norfloxacine	320.14	35	15	**276**.**15**
			25	233.11
Ofloxacine	362.15	25	25	**261**.**10**
			20	318.17
Enoxacine	321.14	35	30	**206**.**07**
			20	257.14
Marbofloxacine	363.15	30	20	345.20
			15	**320**.**10**
Enrofloxacine	360.17	35	20	**316**.**20**
			30	245.20
Ciprofloxacine	332.14	30	15	**288**.**13**
			25	245.20
Danofloxacine	358.16	35	25	**96**.**10**
			20	314.20
Acidic FQs				
Cinoxacine	263.10	35	20	**217**.**10**
			15	245.20
Flumequine	262.20	35	35	**202**.**10**
			20	244.20
Oxolinic acid	262.14	40	30	**216**.**10**
			20	244.10
Nalidixic acid	233.17	40	15	**215**.**15**
			25	187.10
Internal standards				
Lomefloxacine	352.20	35	25	**265**.**18**
			25	308.26
Cincophen	250.04	45	35	**128**.**02**
			30	222.17

Fragment ions in bold were used for quantification.

(1) Federal Office of Consumer Protection and Food Safety (BVL) (CRL/NRL for Veterinary Drug Residues), Berlin, Germany.
(2) National Veterinary Institute, Uppsala, Sweden.
(3) Advanced Technology Corporation (ATC), Liège (Sart-Tilman), Belgium.
(4) Centre d'Economie Rurale (CER), Division Hormonologie Animale, Marloie, Belgium.
(5) Agence Française de Sécurité Sanitaire des Aliments (AFSSA) (CRL/NRL for Antimicrobial Residues in Food), LERMVD, Fougères, France.
(6) Centre d'Analyse des Résidus en Traces (CART), Liège (Sart-Tilman), Belgium.
(7) Institut de Santé Publique (ISP) – (WIV), Brussels, Belgium.
(8) Universitat Barcelona, Química Analítica, Barcelona, Spain.

IRMM also participated to the intercomparison exercise but its results were not included in the statistics to prevent any bias compared to the external users of the method.

From the beginning of the study each laboratory was assigned a random and unique code (01–18).

Before launching the actual ring trial, the experimental protocol proposed by IRMM was extensively discussed during a preliminary meeting involving participating laboratories and IRMM.

4. Design of the exercise

In a first step, blanks and standards were sent to the participating laboratories for preliminary training on the extraction procedure. The spiked samples to be analysed were sent to the laboratories in a second step.

Each laboratory was provided with 10 sets of samples. Each set of sample was assigned a unique code (001–240) and contained 5 replicates (±1 g) so that each laboratory received 50 samples in total. The sets of samples sent to the different laboratories were strictly similar and were identified with different codes.

Among the 10 sets of samples, 5 sets corresponded to 5 different materials (A, B, C, D and E) in order that each laboratory analysed each material in blind replicates. The 5 different materials consisted of pig kidney spiked with 5 different mixtures made of 1–8 FQs. Each of the 11 targeted FQs was present in 2 different mixtures, at two different concentrations between MRL/4 and 2 MRL, once below MRL and once above MRL. The five different sets of samples are presented in Table 2.

Table 2
Five different materials provided to the laboratories

Material	Compounds added		Nominal concentration ($\mu g\,kg^{-1}$)
A	Amphoteric FQs	Norfloxacin	70
		Enrofloxacin	252
		Danofloxacin	261
		Ciprofloxacin	99
		Ofloxacin	155
		Marbofloxacin	101
	Acidic FQs	Oxolinic acid	119
		Flumequine	6988
B	Amphoteric FQs	Danofloxacin	97
		Ciprofloxacin	168
		Ofloxacin	259
	Acidic FQs	Oxolinic acid	218
		Nalidixic acid	224
		Cinoxacin	130
C	Amphoteric FQs	Norfloxacin	160
		Enoxacin	50
	Acidic FQs	Nalidixic acid	61
		Flumequine	16971
D	Amphoteric FQs	Enoxacin	70
		Marbofloxacin	202
	Acidic FQ	Cinoxacin	261
E	Amphoteric FQ	Enrofloxacin	141

Table 3
Experimental work: sequence of analysis in LC-MS/MS

Sample list		FQs concentration	IS concentration	Number of injections
Day 1				
Mobile phase		0	0	1
Blank pig kidney	No. 1	0	0	2
	No. 2	0	0	2
Calibration no. 1 with spiked pig kidney samples		0	2 MRL	2
		0	2 MRL	2
		MRL/4	2 MRL	2
		MRL/4	2 MRL	2
		MRL/2	2 MRL	2
		MRL/2	2 MRL	2
		MRL	2 MRL	2
		MRL	2 MRL	2
		MRL × 1.5	2 MRL	2
		MRL × 1.5	2 MRL	2
		MRL × 2	2 MRL	2
		MRL × 2	2 MRL	2
Sample no. 1	1	To be determined	2 MRL	3
	2	To be determined	2 MRL	3
Sample no. 2	1	To be determined	2 MRL	3
	2	To be determined	2 MRL	3
Sample no. 3	1	To be determined	2 MRL	3
	2	To be determined	2 MRL	3
Sample no. 4	1	To be determined	2 MRL	3
	2	To be determined	2 MRL	3
Sample no. 5	1	To be determined	2 MRL	3
	2	To be determined	2 MRL	3
Quality control	No. 1	MRL	2 MRL	3
	No. 2	MRL	2 MRL	3
Day 2				
Mobile phase		0	0	1
Blank pig kidney	No. 1	0	0	2
	No. 2	0	0	2
Calibration no. 2 with spiked pig kidney samples		0	2 MRL	2
		0	2 MRL	2
		MRL/4	2 MRL	2
		MRL/4	2 MRL	2
		MRL/2	2 MRL	2
		MRL/2	2 MRL	2
		MRL	2 MRL	2
		MRL	2 MRL	2
		MRL1.5	2 MRL	2
		MRL × 1.5	2 MRL	2
		MRL × 2	2 MRL	2
		MRL × 2	2 MRL	2
Sample no. 6	1	To be determined	2 MRL	3
	2	To be determined	2 MRL	3
Sample no. 7	1	To be determined	2 MRL	3
	2	To be determined	2 MRL	3
Sample no. 8	1	To be determined	2 MRL	3
	2	To be determined	2 MRL	3
Sample no. 9	1	To be determined	2 MRL	3
	2	To be determined	2 MRL	3
Sample no. 10	1	To be determined	2 MRL	3
	2	To be determined	2 MRL	3
Quality control	No. 3	MRL	2 MRL	3
	No. 4	MRL	2 MRL	3

For materials A and C the laboratories were asked to dilute ten-fold the final extract of each sample, in order to deal with the high content of flumequine, and to provide results for the diluted and non-diluted flumequine.

A detailed protocol was provided to each laboratory. As shown in Table 3, the experimental work to be carried out by the laboratories was planned over 2 days.

An Excel spreadsheet developed in IRMM was sent to all participants for reporting their results. Results were expressed as $\mu g\,kg^{-1}$ of wet tissue.

Any deviation to the given protocol had to be reported. The analysis of data was carried out and a final evaluation meeting involving all participants was held at IRMM. During this meeting, possible experimental/technical justifications to explain discrepancies were discussed. When an explanation was found, the values were withdrawn to lead to the final set of results for the FQs. The trend for bias between blind replicates was also investigated.

5. Results and discussion

5.1. Follow-up of the protocol

It appeared that all laboratories followed the experimental conditions specified in the protocol, with otherwise only minor changes. Calibration curves were achieved in the range 37.5–300 $\mu g\,kg^{-1}$, which corresponds to the range MRL/4 to 2 MRL for most FQs, except danofloxacin (50–400 $\mu g\,kg^{-1}$) and flumequine (375–3000 $\mu g\,kg^{-1}$).

5.2. Qualitative determination

First, it has to be noted that no traces of FQs were found in the blank pig kidney samples. Then, all laboratories performed satisfactorily the identification of the FQs in material A to E and blind replicates A′ to E′, with the exception of laboratory 03 which did not found enoxacin in material C at a concentration of 50 $\mu g\,g^{-1}$ but could identify enoxacin in material D at a concentration of 70 $\mu g\,kg^{-1}$.

Traces of flumequine were found in material B (three laboratories), in material D (three laboratories) and in material E (two laboratories). Traces of oxolinic acid were found in material C (one laboratory).

The traces of flumequine found in materials B and D might be due to a cross-contamination from materials A and C, related to the high sample content of flumequine compared to the other FQs: the maximum flumequine concentration in the samples was 16971 $\mu g\,kg^{-1}$, compared to a maximum of 261 $\mu g\,kg^{-1}$ for the other FQs.

5.3. Quantitative results

The limits of detection (LOD) and quantification (LOQ) were established by the laboratories as the (fluoro)quinolone concentration corresponding to a signal to noise ratio of 3 and 10 for LOD and LOQ, respectively. The results are given in Table 4. L04 and L14 reported LOQs corresponding to the lower point of the calibration curve and are indicated in italic as these results are not comparable to the others.

The concentration of each FQ was determined in the ten sets of samples by every participant.

The analysis of data was carried out at IRMM in terms of:

- Difference between nominal and experimental values obtained in each laboratory taken individually.
- One-way ANOVA with subsequent application of *F*-test to control between and intra-bottle variability, hence:
  - to compare and estimate the between- and within-laboratory components of the overall variance of all individual results, allowing the computation of the repeatability and reproducibility values;
  - to determine the accuracy of the method for each material.

5.3.1. Difference between nominal and experimental values in each laboratory

A schematic overview of all calculated experimental concentrations compared to the nominal value ±20% is given in

Table 4
Limits of detection (LOD) and quantification (LOQ) obtained in each laboratory and expressed in $\mu g\,kg^{-1}$

Compound	Laboratory															
	01		02		03		04		05		08		14		15	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
Norfloxacin	6.87	9.56	5.00	10.00	12.78	16.76	10.00	*37.50*	2.50	NR	10.00	15.00	10.00	*37.50*	NR	38.59
Ofloxacin	2.17	2.98	5.00	10.00	10.63	13.94	10.00	*37.50*	3.00	NR	10.00	15.00	5.00	*37.50*	NR	42.19
Cinoxacin	5.40	5.82	5.00	10.00	27.50	36.05	10.00	*37.50*	2.00	NR	10.00	15.00	5.00	*37.50*	NR	39.00
Enrofloxacin	5.55	5.87	5.00	10.00	19.03	24.96	10.00	*37.50*	5.00	NR	10.00	15.00	5.00	*37.50*	NR	39.60
Flumequine	51.20	52.66	NR	NR	288.00	378.00	10.00	*375.00*	2.00	NR	100.00	150.00	5.00	*37.50*	NR	376.70
Enoxacin	7.83	12.83	5.00	10.00	22.20	29.11	10.00	*37.50*	10.00	NR	10.00	15.00	10.00	*37.50*	NR	46.16
Ciprofloxacin	2.21	3.59	5.00	10.00	17.39	22.80	10.00	*37.50*	3.50	NR	10.00	15.00	10.00	*37.50*	NR	39.19
Marbofloxacin	3.86	5.47	5.00	10.00	11.39	14.94	10.00	*37.50*	6.00	NR	10.00	15.00	10.00	*37.50*	NR	39.30
Danofloxacin	4.70	6.69	5.00	10.00	27.38	35.92	10.00	*37.50*	10.00	NR	15.00	20.00	10.00	*50.00*	NR	70.54
Oxolinic acid	2.34	2.80	5.00	10.00	35.06	45.99	10.00	*37.50*	1.00	NR	10.00	15.00	5.00	*37.50*	NR	40.09
Nalidixic acid	5.74	6.24	5.00	10.00	23.83	31.26	10.00	*37.50*	1.00	NR	10.00	15.00	5.00	*37.50*	NR	38.10

NR = not reported, LOQ value in italic = lower point of the calibration curve.

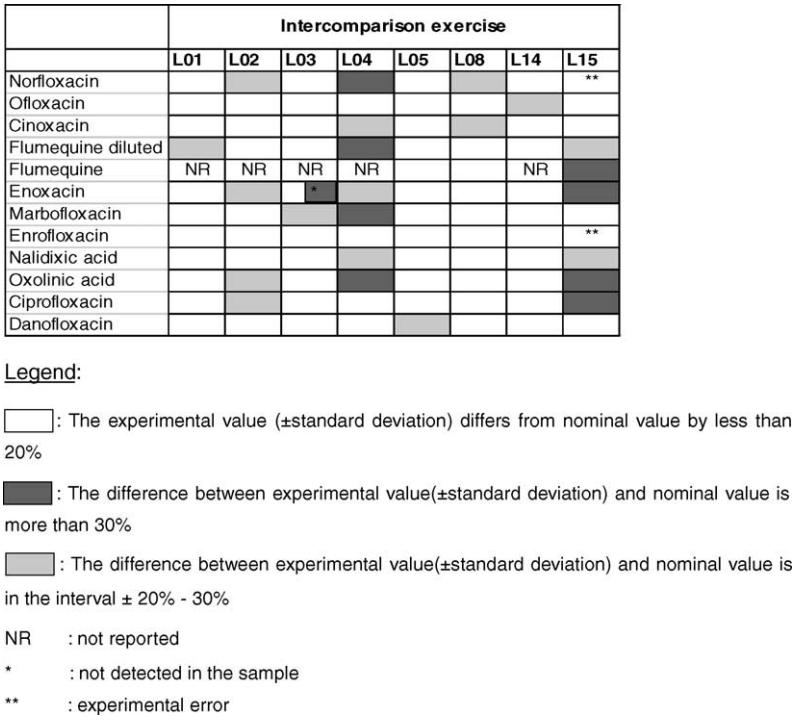

Fig. 1. Schematic overview of the difference between nominal and experimental values obtained in each laboratory (average of blind replicates).

Fig. 1. A white shade is given when the experimental value (±standard deviation) differs from the known nominal value (provided by IRMM) by less than 20%. A dark grey shade is given when the difference between the two values is superior to 30%. Finally, a light grey shade is given when the difference between the two values lies in the interval ±20–30%.

Laboratory 04 and laboratory 15 provided in some cases high values, probably due to calibration problems. However, considering ANOVA, the overall accuracy result was satisfactory. Concerning flumequine, no systematic bias could be shown from the different laboratory results. The quantification of flumequine, diluted and non-diluted, seemed to be identically performant. Moreover, the quantification of flumequine in non-diluted samples, achieved by extrapolation of the calibration curve, showed, when reported, that the method is rather robust. And, taking into account all participants, the high content of flumequine in the samples had no negative influence on the quantification of the other FQs simultaneously present in the samples.

5.3.2. Analysis of variance (ANOVA)

5.3.2.1. Between- and within-laboratory values. The *between-laboratory reproducibility* has been evaluated using the Horrat ratio described as follows [12]:

$$\text{Horrat ratio} = \frac{\text{RSD}_\text{R}}{\text{RSD}_\text{Horwitz}}$$

where RSD_R is the relative standard deviation of reproducibility obtained by ANOVA, and $\text{RSD}_\text{Horwitz}$ is calculated using the Horwitz equation [9]:

$$\text{RSD}_\text{Horwitz} = 2^{(1-0.5\ \log\ C)}$$

where C is the mass expressed in power of 10 (i.e. $1\ \text{mg g}^{-1} = 10^{-3}$).

The acceptance limit is: Horrat ratio $\leq$1.5–2.0.

The results obtained for each FQ in each material are presented in Fig. 2. Detailed data are given in Table 5. The between-laboratory reproducibility was very good (Horrat ratio < 1.5) for all FQs except flumequine, which shows Horrat ratio values of 2.0 and 2.3 in materials A and C, respectively. This might be due to the additional dilution required for flumequine determination in order to obtain data within the calibration range.

The *within-laboratory reproducibility* has been evaluated using RSD_r obtained by ANOVA [9].

The acceptance limit is: $0.75 \times \text{RSD}_\text{Horwitz}$.

The results obtained for each FQ in each material are presented in Fig. 3. Detailed data are given in Table 5. Good results, with RSD_r lower than 15%, were obtained for each FQ in the different materials. In particular, flumequine results were also satisfactory with $\text{RSD}_\text{r} \leq$ the acceptance limit and lower than 8%.

5.3.2.2. Accuracy of the method. The accuracy of the method, considering all laboratories together, has been evaluated for each FQ in each material. The uncertainty on the accuracy has been calculated using ANOVA.

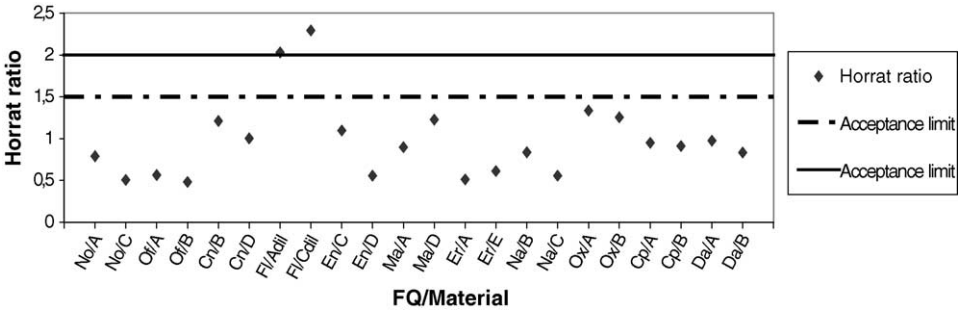

Fig. 2. Between-laboratory reproducibility.

Table 5
Between- and within-laboratory reproducibility and accuracy results – detailed data given per FQ and per material

Material	Between-laboratory			Within-laboratory		Accuracy		
	RSD_R	Horwitz RSD_R	Horrat ratio	RSD_r	Acceptance limit	Average (%)	Uncertainty	
No/A	18	23	0.8	4	17	119	103	136
No/C	11	21	0.5	4	16	109	100	118
Of/A	12	21	0.6	8	16	104	95	112
Of/B	9	20	0.5	6	15	102	96	109
Cn/B	26	22	1.2	8	16	100	81	118
Cn/D	20	20	1.0	7	15	93	80	106
Fl/Adil	24	12	2.0	6	9	96	78	113
Fl/Cdil	24	11	2.3	8	8	93	76	110
En/C	27	25	1.1	12	18	115	92	139
En/D	13	24	0.6	5	18	111	101	121
Ma/A	20	23	0.9	10	17	94	80	107
Ma/D	25	20	1.2	4	15	107	88	125
Er/A	10	20	0.5	9	15	104	96	112
Er/E	13	21	0.6	8	16	100	91	110
Na/B	17	20	0.8	9	15	91	80	102
Na/C	14	24	0.6	5	18	100	91	110
Ox/A	30	22	1.3	8	17	96	76	116
Ox/B	26	21	1.3	7	15	88	72	104
Cp/A	21	22	0.9	6	17	112	95	129
Cp/B	19	21	0.9	6	16	99	86	113
Da/A	19	20	1.0	14	15	100	85	114
Da/B	19	23	0.8	14	17	96	83	109

The results are presented in Fig. 4 and in Table 5. Satisfactory accuracy results were obtained in 20 cases out of 22. The two unsatisfactory results were those for norfloxacin in material A and for enoxacin in material D. In these cases, the uncertainty interval did not include the 100% value (lower limit = 103, 101%, respectively). However, the accuracy results obtained in each individual laboratory show that six laboratories out of eight obtained satisfactory results. Moreover, the results obtained for these FQs in a different material were satisfactory.

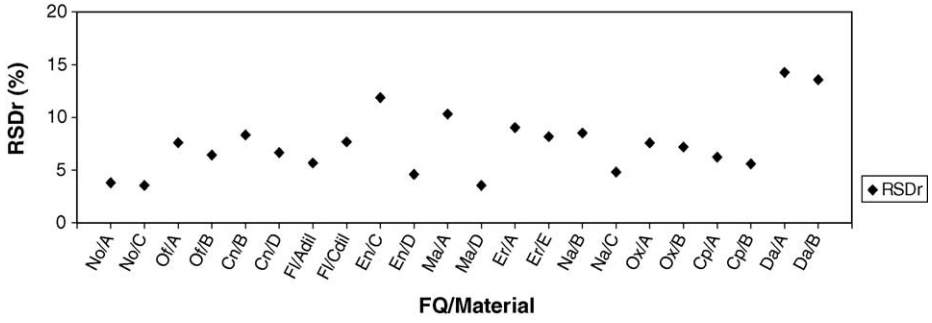

Fig. 3. Within-laboratory reproducibility.

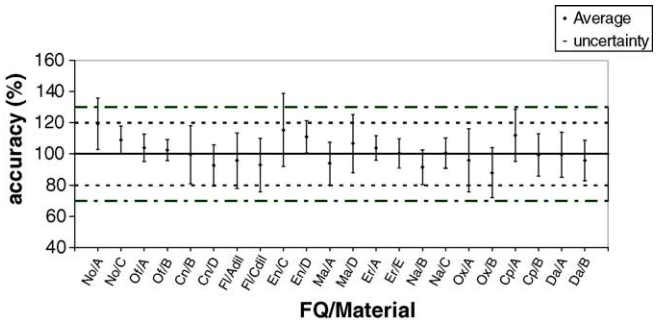

Fig. 4. Accuracy.

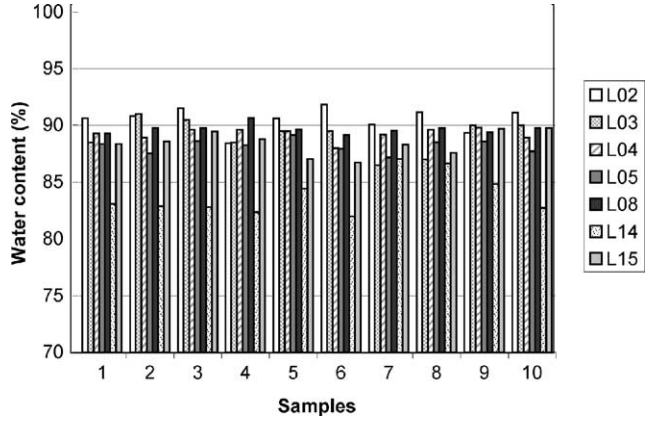

Fig. 5. Water content determination in spiked pig kidney samples.

5.4. Water content determination (or moisture determination)

The water content was determined on each spiked pig kidney sample using the oven procedure. The oven temperature was around 105 °C and the drying time was between 3 and 8 h (48 h for laboratory 08).

The results, summarised in Fig. 5, show an average water content of 88.4%, which, taking into account the spiking of the samples with 1 ml of standard solution, is coherent with values given in the literature for the unspiked sample (76% water in fresh pig kidney) [13]. L01 performed the water content determination of blank pig kidney samples (78.9%).

In order to avoid the uncertainty contribution related to the drying procedure, these results were not taken into account for the determination of the (fluoro)quinolone content of the pig kidney samples, expressed in $\mu g\ kg^{-1}$ of wet tissue. However they gave an indication about the homogeneity of the spiking of the samples and about the stability of the samples during dispatch.

6. Conclusions

The intercomparison committee composed of all participating laboratories and IRMM concluded that:

(1) This method, after the present validation, is suitable without any restriction in terms of repeatability, reproducibility, precision and accuracy for the identification and quantification of the eleven following FQs in spiked pig kidney samples, in a single run: norfloxacin, ofloxacin, enoxacin, enrofloxacin, ciprofloxacin, marbofloxacin and danofloxacin as amphoteric FQs; cinoxacin, flumequine, oxolinic acid and nalidixic acid as acidic FQs.
(2) No significant accuracy difference was observed between blind replicates, indicating a good performance of the participating laboratories.
(3) No significant accuracy neither within-laboratory nor between-laboratory reproducibility difference was obtained for any FQ comparing two different materials. This reflects the suitability of the method for the determination of FQ mixtures with different FQ composition and FQ concentration.
(4) No qualitative neither quantitative bias was observed when analysing simultaneously acidic and amphoteric FQs.
(5) According to participants' comments, it seems that this method could also probably be applied with minor changes to other biological matrices such as pig or calf muscle.

Acknowledgments

The authors acknowledge the University of Ghent, Faculty of Veterinary Medicine (Ghent, Belgium), for the gift of pig kidney samples. The authors are also grateful to Christoph von Holst and Piotr Robouch from IRMM for their support to the preparation of the validation experimental design and the statistical treatment of the data.

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