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Determination and characterization of quinolones in foodstuffs of animal origin by CE-UV, LC-UV, LC-FL, LC-MS AND LC-MS/MS

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Abstract In this work methods for the multiresidue determination of the series of quinolones include in the European regulation in food of animal origin are developed and validated in line with Commission Decision 2002/657/EC in terms of linearity, decision limit, capability detection, precision and stability. Multiresidue methods were established to allow the determination of quinolones covered by EU legislation in 2377/90/EC in muscle of chicken, turkey, pig and cow, plasma of cow and pig, liver of pig and milk of cow. First an extraction step was optimized and a SPE step was applied to clean-up and preconcentrate quinolones prior to their separation by CE or LC and determination by CE-UV, LC-UV, LC-Fl, LC-MS with different ion sources (ESI, ApCI) and different mass analyser (Q, ToF) and LC-ESI-QqQ tandem mass spectrometry. The limits of quantification obtained are always lower than Maximum Residue Limit (MRL) established by EU for quinolones in animal products and they can be applied to the control of quinolones in foodstuffs of animal origin. Finally the proposed methods were applied to determine quinolones in samples of turkey and pig muscle, pig plasma and milk of cow. Excellent quality parameters and reduced time of analysis were obtained when LC-ESI-MS/MS is used, although the others techniques presented too satisfactory results.

Keywords: Quinolones, animal tissues, LC-UV, CE-UV, LC-Fl, LC-MS, LC-MS/MS.

1. Introduction

Quinolones are a group of synthetic antibacterial compounds that operate by inhibiting bacterial DNA synthesis. They are used in the treatment of respiratory diseases and enteric bacterial infections in humans and in food-producing animals such as cattle, swine, turkey and chicken. Since 2000 there has been a significant progressive increase in the use of quinolones in animal production, which has inevitably caused residues in food. These residues are a source of concern due to the emergence of drug-resistant bacteria and they are also a potential health hazard for consumers. Therefore, the European Union has set Maximum Residue Limits (MRL) for quinolones in animal products such as muscle, liver and milk, with the aim of minimizing the risk to human health associated with their residue consumption. There are eight quinolones regulated by EU. The MRL established for these quinolones ranges between 30 and 800 μg kg^{-1}. These low values demand the development of analytical methods that are sensitive enough to monitor and determine these drugs in biological material.

There are numerous studies and reviews in the literature addressing quinolones determination in veterinary field [1-12]. Most papers focus on a specific matrix and generally analysing only two or three quinolones. The development of accurate and sensitive methods of determination and characterization of the series of quinolones regulated in the European normatives in foodstuff of animal origin could be of great interest to ensure safety and minimize the risks to human health.

In this study CE-UV, LC-UV, LC-Fl, LC-MS and LC-MS/MS multiresidue methods were developed and optimized to allow the determination of the

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series of quinolones covered by European Union legislation in muscle of chicken, turkey, pig and cow, plasma of cow and pig, liver of pig and milk of cow. First an extraction step was optimized and a SPE step was applied to clean-up and preconcentrate quinolones prior to their separation by CE or LC and determination by CE-UV, LC-UV, LC-Fl, LC-MS with different ion sources (ESI, ApCI) and different mass analysers (Q, ToF) and LC-ESI-QqQ tandem mass spectrometry. All methods were validated in line with Commission Decision 2002/657/EC in terms of linearity, decision limit, detection capability, detection limit, quantification limit, recovery, precision, selectivity and stability. Finally, the established methods were applied to determine quinolones in samples of product of animals medicated with quinolones. LOQ of the developed methods are always lower than MRL established in council regulation and then can be applied to determine quinolones residues in product of animal origin.

2. Experimental

Reagents

The quinolones were purchased from different pharmaceutical firms: norfloxacin, NOR (Liade, Boral Quimica, Barcelona, Spain); ciprofloxacin, CIP (Ipsen Pharma, Barcelona, Spain); sarafloxacin, SAR and difloxacin, DIF (Abbot, Madrid, Spain); enrofloxacin, ENR (Cenavisa, Reus, Spain); danofloxacin, DAN (Pfizer, Karlsruhe, Germany); marbofloxacin, MAR (Vetoquinol, Barcelona, Spain); oxolinic acid, OXO and flumequine, FLU (Sigma, St. Louis, MO, USA). Structures of quinolones are shown in figure 1.

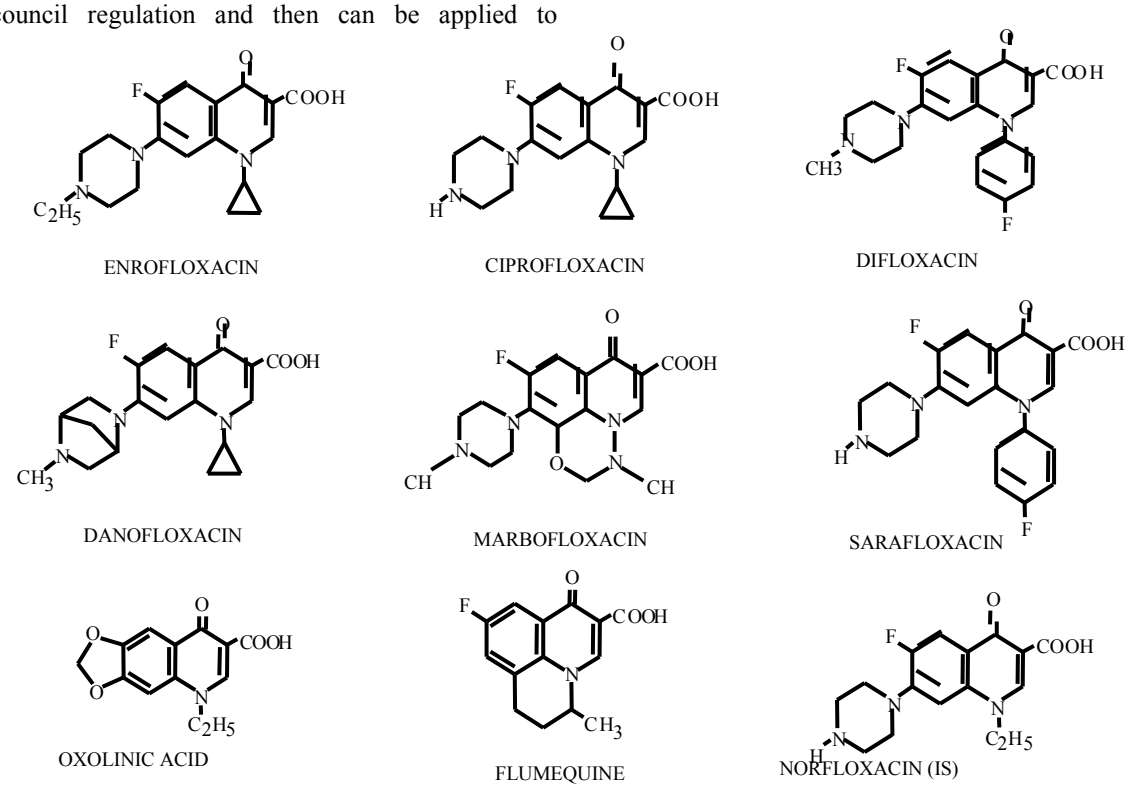


Fig. 1. Structures of the studied quinolones.

All reagents were of analytical grade. Merck (Darmstadt, Germany) supplied ammonium acetate, potassium hydrogenphosphate, formic, trifluoroacetic (TFA) and acetic acid, hexane, acetonitrile (MeCN) and methanol. Sigma-Aldrich had supplied *m*-phosphoric acid (HPO_3). Citric acid and potassium hydrogenphtalate were supplied by Fluka (Buchs, Switzeland). Ultrapure water generated by the Milli-Q system (Millipore, Billerica, USA) was used.

The solid-phase extraction (SPE) cartridges used in these studies were of hydrocarbon chain (Bond Elut C_{18} and Zorbax C_{18} encapped), polymeric sorbents (Isolute ENV+, Oasis HLB and Strata X) and mixed polymeric sorbent with amino groups (Oasis MAX) and sulfonated groups (SDB-RPS).

Instrumentation

Chromatographic separation was achieved on a 150 x 4.6 mm Zorbax Eclipse XDB-C8 column (Agilent Technologies, Waldbronn, Germany) using a pre-column Kromasil C8 20 x 4.5 mm (Aplicaciones Analíticas, Barcelona, Spain). A Crison 2002 potentiometer (± 0.1 mV) (Crison S.A., Barcelona, Spain) and an Orion 8102SC Ross combination pH electrode (Orion Research, Boston, MA, USA) was used to measure the pH of the mobile phase. The electrode was stored in water when not in use and soaked for 15-20 min in a MeCN-water mixture (14%) before pH measurements of the mobile phase [13].

A centrifuge Macrotronic Selecta (J.P. Selecta S.A., Abrera, Spain) was used to perform the extractions. A mini-centrifuge Mikro 20 (Hettich Zentrifugen, Germany) was used to centrifuge the final extract. SPE was carried out on a Supelco vacuum manifold for 12 cartridges (Bellefonte, PA, USA) connected to a Supelco vacuum tank.

CE-UV instrument

A CE Beckman P/ACE system 5500 (Beckman Instruments) equipped with a photodiode array detector was used, with an uncoated fused-silica CE capillary column of 57 cm (50 cm from the inlet to the detector) and 75 μm internal diameter (Polymicro Technologies).

LC-UV and LC-Fl instrument

The LC-UV and LC-Fl equipment consisted of an HP 1100 series with an autosampler injector. UV detection was performed by a diode array detector (DAD) at maximum wavelength of quinolones (250 nm for FLU, 300 nm for MAR and 280 nm for the other quinolones). Fluorescence detection was performed at their maximum excitation/emission wavelength (300/508 nm for MAR; 280/450 nm for CIP, DAN and ENR; 245/360 for FLU; and 270/450 for PIP). A Chemstation for LC 3D Rev. A 08.03(847) and A 07.01 software (Agilent Technologies) was used for data analysis.

LC-ToF-MS instrument

The LC system was an HP Agilent Technologies 1100 series with an autosampler, a pump (quaternary solvent manager) and an orthogonal acceleration MSD Sciex MassHunter time-of-flight mass spectrometer with an electrospray ionization (ESI) interface from Agilent Technologies. The software Analyst QS 1.1. (Applied Biosystems, Foster City, CA, USA) was used to treat the data.

LC-Q-MS and LC-QqQ-MS/MS instruments

The LC-Q-MS and LC-QqQ-MS/MS analyses were performed on an HP Agilent Technologies 1100 series liquid chromatography system equipped with an autosampler and a quaternary pump coupled to a PE Sciex API 3000 triple-quadrupole mass spectrometer with a turbo ion spray source. Both the system and the data treatment were controlled by Analyst 1.4.2. software (Applied Biosystems).

Procedures

The characterization and determination of quinolones in different biological samples requires the development of methodologies of extraction, clean-up, separation and detection sensitive enough to allow the determination of the analytes having concentration lower than the MRL set by the EU. For the first step, the extraction of quinolones from the different samples, organic solvents water immiscible and miscible, different buffers and pH and the use of microwaves were tested. The best results were obtained using CH_2Cl_2 and hydroorganic solutions of MeCN (25-30%) and water with 0.3-0.4% of HPO_3 [14-17]. The method using HPO_3 water/MeCN is shorter than CH_2Cl_2

method and is the method of choice. The extracting agent was optimized with experimental design [15].

In previous studied [14,18-19], different cartridges were examined for the clean up and preconcentration of analytes, using SPE from different matrices such as muscle of chicken, turkey, pig and cow, pig liver, pig and cow plasma and milk of cow. The best results were obtained using polymeric sorbents of the type of Isolute ENV+, Strata X and SDB-RPS [16,18-19].

After the treatment of the sample, separation LC and CE methodologies were optimized using Linear Solvation Energy Relationship methods (LSER) and the relationships of electrophoretic mobility of analytes versus pH values, respectively [20-21].

For detection and characterization of analytes the use of ultraviolet, fluorescence, mass spectrometric (MS) and tandem mass spectrometric (MS/MS) detectors was studied. In previous works [15,19], the ESI-MS and ESI-MS/MS conditions were first optimized by direct infusion of each compound individually following an experimental design [22].

Quality parameters

Validation of methods was performed according to the FDA guideline for bioanalytical assay procedure [23] and the EU guideline [24]. The quality parameters established were limit of detection and limit of quantification, decision limit and detection capability, linearity range, recovery, precision and selectivity.

The limit of detection (LOD), defined as the lowest concentration that the analytical process can reliably differentiate from background levels was estimated for a signal-to-noise ratio of 3 from the chromatograms of spiked samples at the lowest analyte concentration tested. Similarly, the limit of quantification (LOQ) was determined for a signal-to-noise ratio of 10. Spiked samples at six different concentration levels were prepared in duplicate in order to establish the LOD and LOQ in the different detection systems. The LOD and LOQ in LC-TOF-MS were obtained in extracted ion chromatogram (XIC) mode. In LC-Q-MS, the LOD and LOQ were obtained by defining it as the minimum detectable amount of analyte in selected ion monitoring (SIM) mode of the most abundant ion. On the other hand, while LC-QqQ-MS/MS was used, we considered in multiple reaction monitoring (MRM) mode of the transition with higher signal/noise values.

To calculate the decision limits (CCα, alpha error 5%) and detection capability (CCβ, beta error 5%), we have prepared 20 samples for all quinolones and analysed these samples by the corresponding calibration curves. For compounds with MRL established, CCα was determined as the concentration at the MRL level for each quinolone plus 1.64 times the standard deviation at the 100 μg kg^{-1} level. CCβ was calculated as the corresponding concentration at the decision limit plus 1.64 times the standard deviation of the within-laboratory reproducibility [15, 24].

Linearity was tested by assessing signal responses of target analytes from samples spiked in a concentration ranging from the LOQ for each analyte to 300 μg kg^{-1}. Spiked samples at eight different concentration levels were prepared in duplicate. Calibration curves were constructed using analyte/internal standard peak area ratio versus concentration of analyte/internal standard ratio.

Recovery experiments were performed by comparing the analytical results for extracted standard samples and internal standard added before the extraction procedure, with unextracted standards prepared at the same concentrations in blank extract representing 100% recovery. The prepared concentration ranges were in accordance with the concentration ranges established in the calibration line.

To assess intra-day precision, five blank samples spiked at three concentration levels (50, 100 and 200 μg kg^{-1}) were prepared and analysed. The procedure was repeated on three different days to determine inter-day-precision. Acceptance criteria for precision are that relative standard deviation (RSD) was lower than 15%. Spiked samples used to assess intra- and inter-day precision were prepared daily.

Different quinolones positive samples of turkey and pig muscle, pig liver, pig plasma and milk of cow, were purchased and treated with the same procedure optimized for spiked samples in order to evaluate the presence or absence of these antibiotics.

3. Results and Discussions

The traditional optimisation procedures consist in studying each factor separately. This kind of

optimisation if often time consuming, and attainment of true optimum conditions cannot be guaranteed, especially when factors are not really independent. Experimental designs reduce the number of experiments in comparison with the traditional methodology, while providing a much more accurate approach to the optimum information.

In previous works [15, 22], studies of liquid-liquid extraction of quinolones were reported using an experimental design, because of high influence of solvent in the extraction of quinolones from the matrix. Two main parameters to provide high efficiency in the extraction of these antibiotics are studied, the percentage of MeCN and *m*-HPO_3. In the optimisation step, the maximum recovery in percentage terms of each compound was chosen as the target function for the optimisation procedure. The experimental domain was defined taking into account preliminary experiments, and instrumental and operative limits. Each factor was evaluated at 5 levels at the following ranges: 10 - 30% for MeCN and 0.1 - 0.4% for *m*-HPO_3.

The surface response and its two-dimensional (2D) plots (Isoresponse curves) for the overall desirability function in the experimental domain studied are shown in Figure 2. The surface response (Figure 2a) shows that maximum desirability was close to 1 when we have worked at high values of the concentration of organic solvent and *m*-phosphoric acid. The solution chosen (25-30% MeCN and 0.3-0.4% of HPO_3) for the validation of the method is in the optimum region.

Also in a previous work [22], chemometrical approaches, based on the use of a second order polynomial central composite design to explore the considered variables affecting time-of-flight detection were studied by Statgraphics Plus 5.1 software. The studied variables with high influence on the intensity of the molecular ion of each quinolone by high-resolution time-of-flight mass spectrometry detection were: voltage of fragmentor, OCT RF V, and skimmer. Fragmentor voltage is the maxim factor that it is statistically significant in the quinolones detection. The optimized values of the considered variables are shown in Fig. 3 In this figure is also shown the fragmentation pathway of FLU established with a ToF analyses.

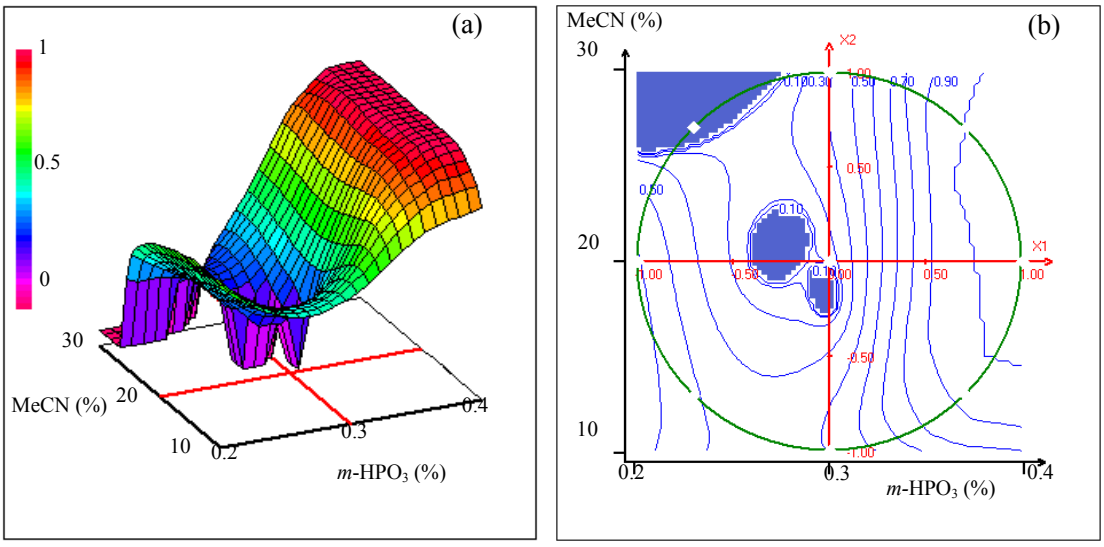


Fig. 2. (a) Response surface (3D) of global desirability as a function of MeCN and *m*-phosporic acid composition. (b) Curves of isoresponse (2D) in the experimental range.

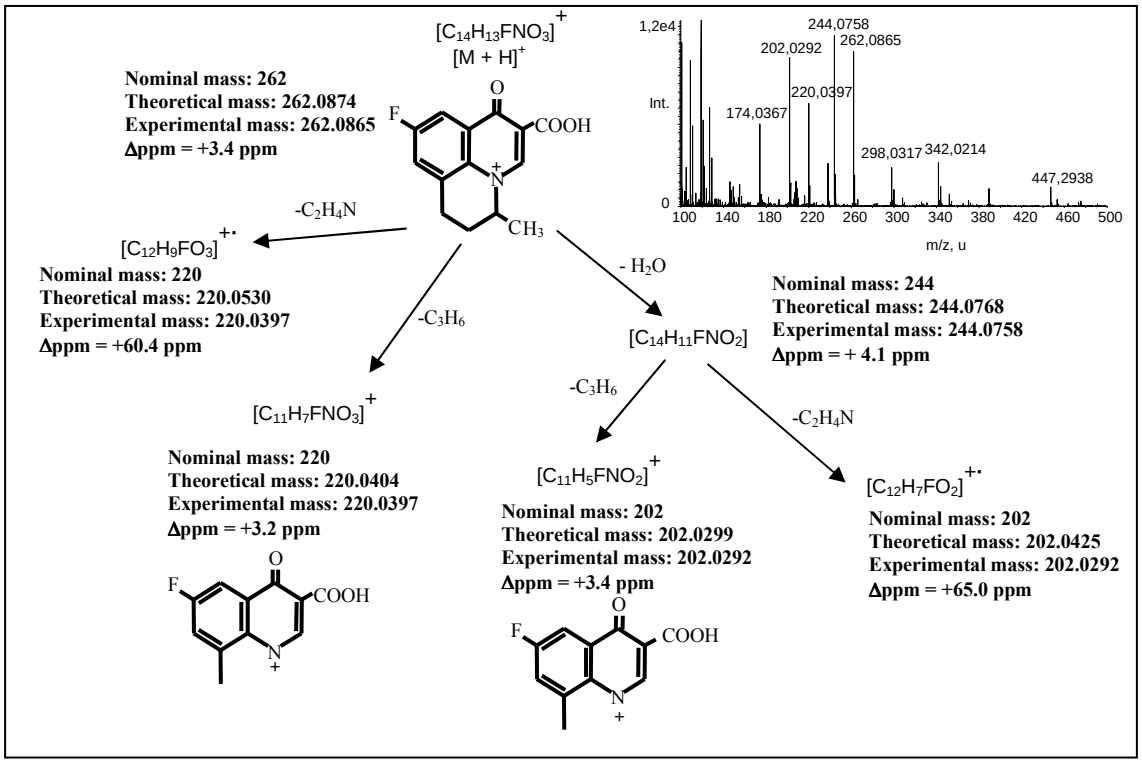


Fig 3. Fragmentation pathway of standard solution of FLU using a ToF analyser at fragmentator voltage 300 V, skimmer 60 V and OCT RF V 250 V. The used of ToF permits accurate identification of the obtained ions.

In our works, a lot of sorbents and different conditions, washing and elution steps in SPE were studied to improve the clean-up and pre-concentration of antibiotics from food and biological tissues [14]. The optimal sorbent for any given extraction problem is dependent on the properties of the target analyte and the sample/matrix composition. From previous studies we concluded that the best results in the extraction of quinolones from several tissues are obtained when polymeric sorbents are used. After extraction of quinolones with HPO_3/MeCN hydroorganic solutions, the best results were obtained with a polymeric hidroxylated sorbent (Isolute ENV+) used for the analysis of chicken, pig, turkey and cow muscle and pig liver. For pig and cow plasma and milk, the polymeric Strata X sorbent gives the best results. When CH_2Cl_2 is used for the extraction of quinolones, we use a polymeric cationic exchange (SDB-RPS) sorbent applied to the analysis of chicken tissues.

Electrophoretic and chromatographic results are shown in Fig. 4. Short time of analysis and small volumes of samples were needed using CE but the LOD are higher (Table 1). Using CE cleaner extracts obtained with CH_2Cl_2 extraction permits lower LOD but the method is longer (Table 1). Using LC the shorter method with HPO_3/MeCN is the method of choice.

For determination of quinolones by LC-MS, different ion sources (ESI and ApCI) and mass analyser (Q, ToF and QqQ) were studied. Figure 5 show, as an example, the optimized parameters used for LC-QqQ-MS/MS analysis. Figure 6 shows the MS/MS spectrum of DAN. When MS detection is used, Selected Ion Monitoring (SIM) mode and the molecular ions are selected.

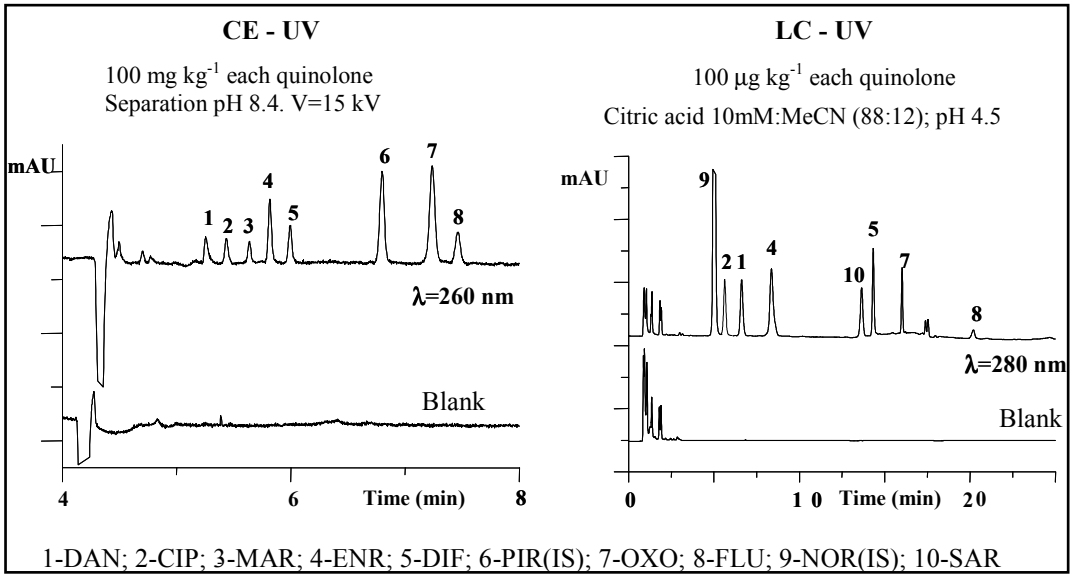


Fig. 4. Electropherogram and chromatogram of quinolones in chicken muscle

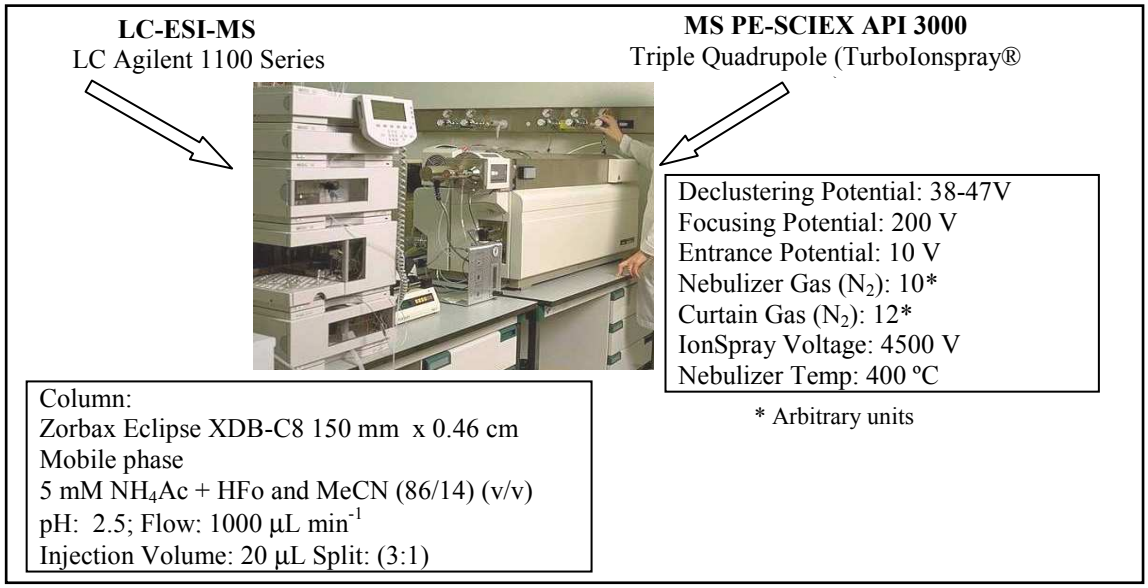


Fig. 5. Optimized parameters for quinolones separation and determination using LC-QqQ-MS/MS.

But if MS/MS detection is used, the analysis data were obtained in Multiple Reaction Monitoring (MRM) mode, using transitions between molecular ion and the more abundant fragmented ion for quantification and transition between molecular ion and the second more abundant ion for the identification transition. For DAN are 358 → 340 and 358 → 283 respectively. The reason because two different transitions were used remained in the possibility that the molecular ions and also one of the transitions can be the same for two analytes, as it is shown in Fig. 7 for OXO and FLU.

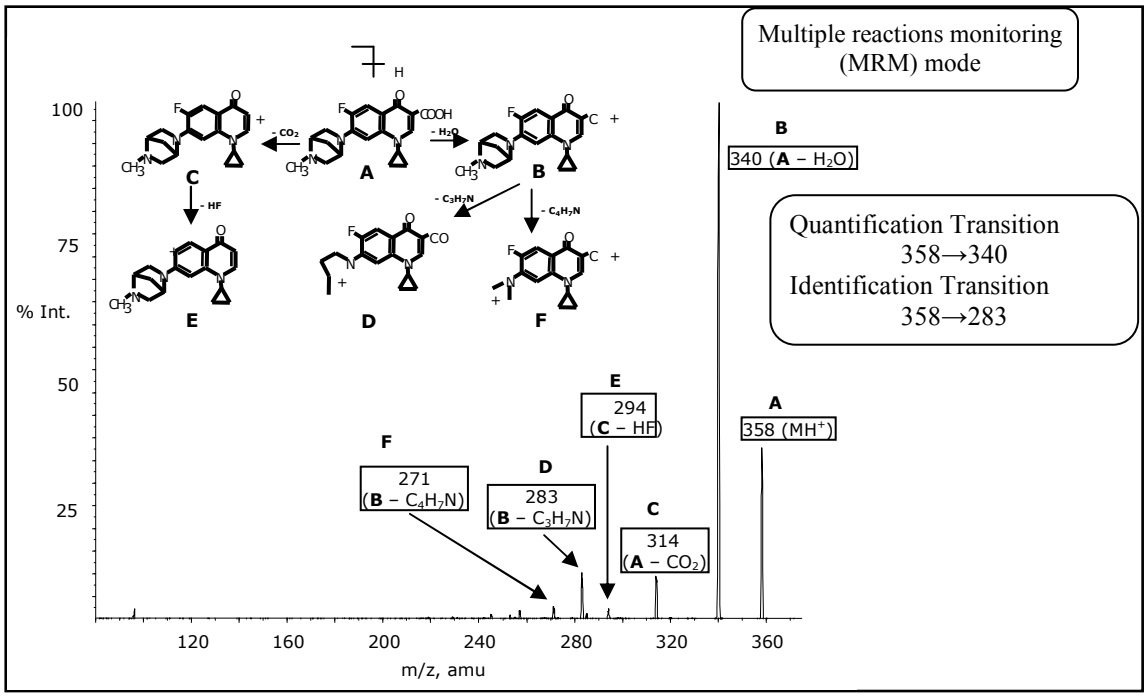


Fig 6. MS/MS spectrum of Danofloxacin

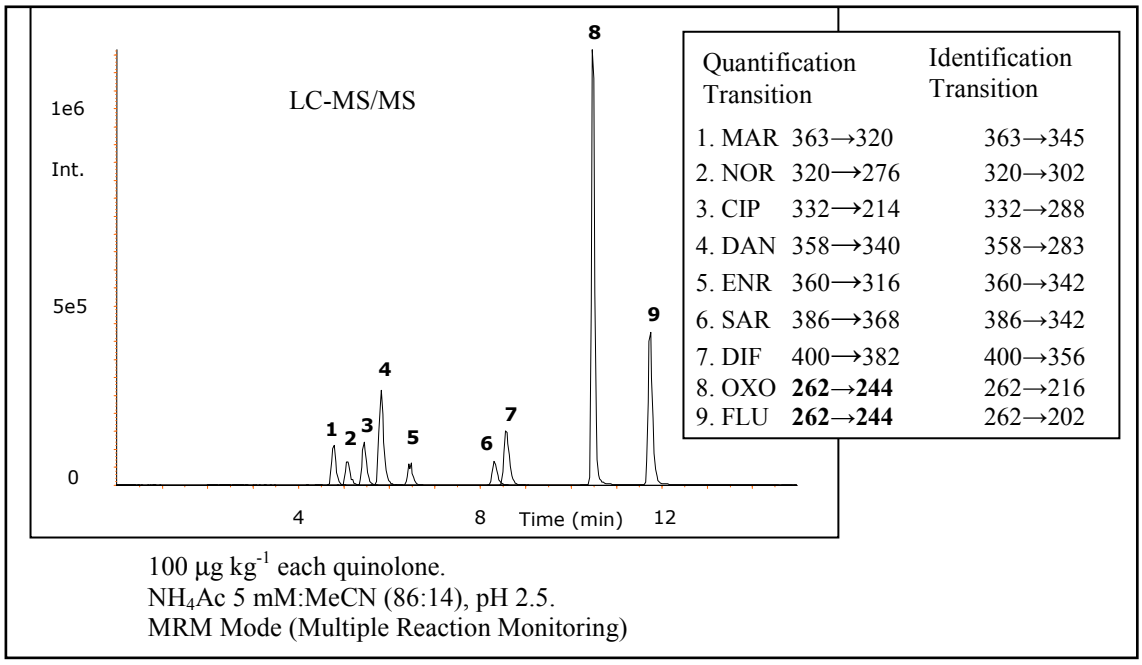


Fig. 7. Quinolones determination in pig muscle (Ext.:HPO_3 water/MeCN; SPE: Isolute ENV+).

Table 1. LOD (μg kg^{-1}). Chicken muscle (CH_2Cl_2; SDB-RPS). Pig plasma (HPO_3 water/MeCN, Strata X).

Quinolone	CE-UV		LC-UV		LC-MS (SIM)	
	Chicken muscle	Pig plasma	Chicken muscle	Pig plasma	Chicken muscle	Pig plasma
MAR	17	150	-	12	-	0.8
CIP	25	150	5	12	1	0.8
DAN	17	150	10	10	1	0.8
ENR	14	100	5	10	0.5	10
SAR	-	-	5	7	0.5	0.8
DIF	17	100	10	10	0.5	1.0
OXO	16	100	5	6	0.25	0.9
FLU	14	150	10	10	5	1.2

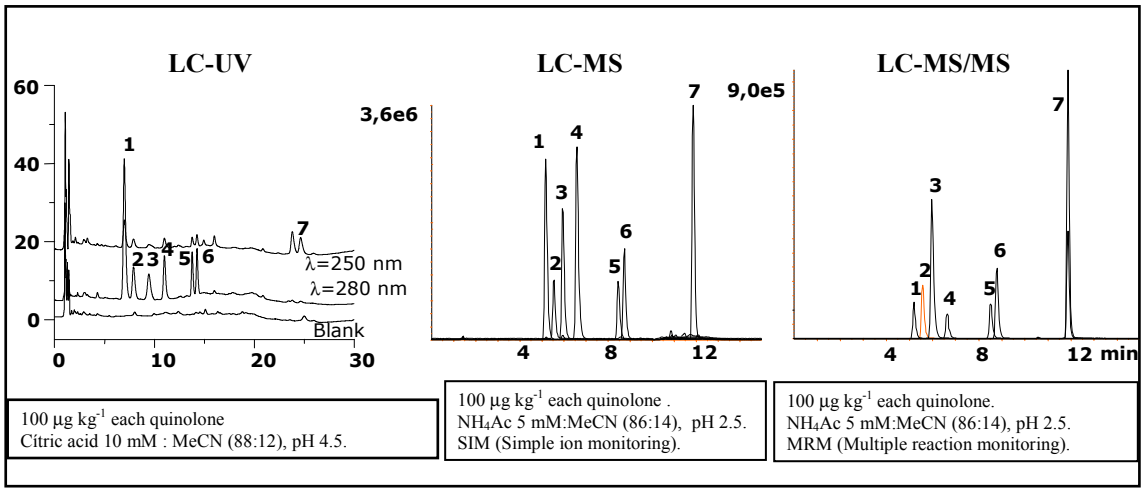


Fig. 8. Chromatograms of quinolones in turkey muscle samples. (HPO_3 water/MeCN; SPE: Isolute ENV+).

Figure 8 shows the chromatograms of the series of quinolones in turkey muscle samples using UV, MS and MS/MS detection. As it is shown, shorter time of analysis is achieved using MS detection, because excellent separations is not need, but lower linearity ranges is obtained using LC-UV.

After the different analysis methods were developed for the analysis of quinolones, the quality parameters have been assessed as summarized in Fig. 9. In Tables 2, 3 and 4, different quality parameters for the established methods are shown. The recoveries obtained exceeded 80% in most cases and the lower recoveries were obtained for pig liver samples. As it is shown in Table 3, LOQ are approximately 10 times lower by LC-Q-MS than by LC-UV and 5 times lower by LC-QqQ-MS/MS than LC-MS, while LOQ for LC-ToF-MS are in-between those for LC-Q-MS and LC-QqQ-MS/MS. Background noise makes LOD for LC-QqQ-MS/MS to be better than those for LC-Q-MS as can be seen in Fig.10. It is important to point out that the LOQs obtained are always lower than MRL established by EU. The sensitivities of the methods for the quinolones determination in different samples (chicken, cow, pig and turkey muscle, pig liver, cow and pig plasma and cow milk) are summarized in Fig. 11. The best results are obtained using LC-MS/MS, 100 times more sensitive than CE-UV and 50 times more sensitive than LC-UV.

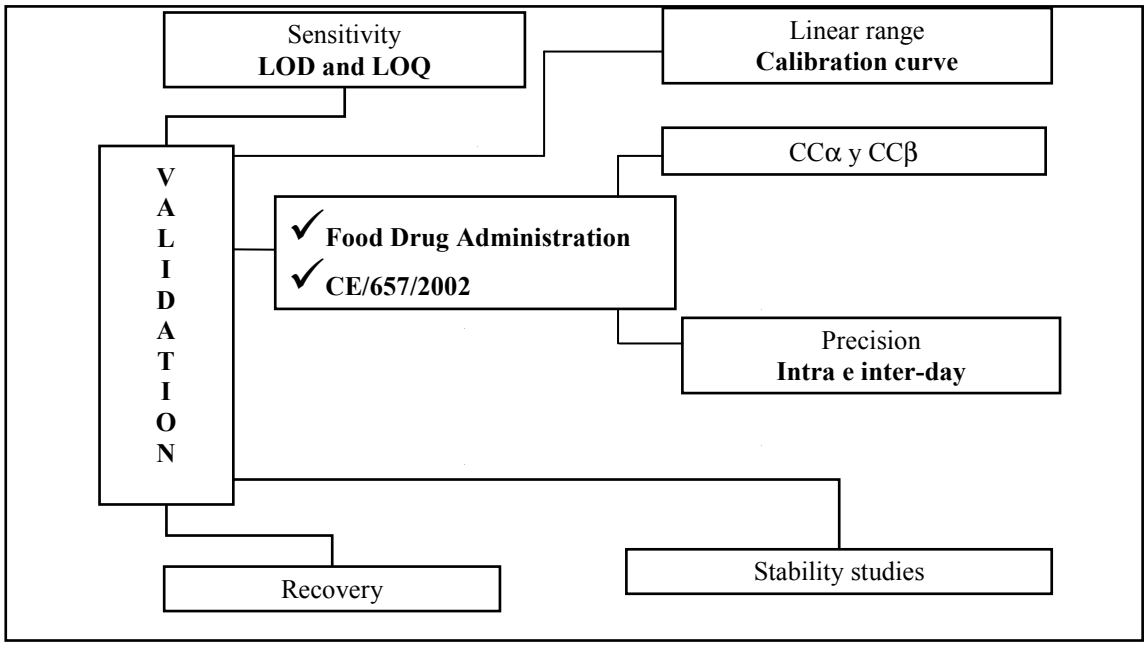


Fig 9. Quality parameters of the developed methods proposed for quinolones determination.

Table 2. Recoveries obtained using the developed methods for quinolones determination in animal materials

Sample		Technique	MAR	CIP	DAN	ENR	SAR	DIF	OXO	FLU
Pig	Muscle	LC-UV	93±2	87±2	87±1	92±3	82±3	81±2	99±4	95±6
		LC-MS	90±2	81±1	91±2	87±3	82±1	78±1	79±3	94±4
		LC-MS/MS	92±3	87±2	90±2	86±2	87±2	82±2	77±2	90±3
	Liver	LC-Tof-MS	82±2	70±1	66±2	76±5	63±2	74±6	80±2	79±4
		LC-MS	80±5	79±5	70±3	81±3	65±4	76±3	81±5	72±5
		LC-MS/MS	79±2	83±2	75±3	76±6	63±6	76±4	89±5	74±5
	Plasma	LC-UV	96±0.5	86±0.5	96±0.5	99±0.5	93±0.5	100±0.5	100±1	94±0.5
		CE-UV	93±2	93±3	83±2	86±2	-	97±4	100±3	99±2
Turkey	Muscle	LC-UV	-	70±7	77±5	79±2	72±3	74±3	-	87±6
		LC-MS	-	76±3	85±4	72±9	72±8	72±6	-	72±5
		LC-MS/MS	-	76±9	85±7	75±5	76±8	75±4	-	73±8
Cow	Muscle	LC-Fl	100±2	99±4	99±3	100±2	100±2	99±3	98±3	100±3
	Plasma	LC-UV	96±1	93±1	95±1	97±1	94±1	96±1	99±2	96±1
		LC-MS	91±4	90±2	91±2	91±3	94±2	98±4	93±3	91±2
		LC-MS/MS	99±2	94±1	95±1	95±3	90±2	90±2	96±4	93±1
	Milk	LC-UV	84±1	86±2	85±2	91±3	-	-	-	98±4
		LC-Fl	84±3	83±4	85±4	92±4	-	-	-	99±2
		LC-MS	91±2	80±2	82±3	89±6	-	-	-	100±3
		LC-MS/MS	84±3	87±2	84±3	91±5	-	-	-	100±3

Table 3. LOQ (μg kg^{-1}) for quinolones separation and determination by LC using different detectors in pig and turkey muscle and in pig liver.

Quinolone/ Technique/ Sample	**LC-UV**		**LC-Q-MS (SIM)**		**LC-ToF-MS (XIC)**		**Lc-QqQ-MS/MS (MRM)**		
	Pig	Turkey	Pig	Turkey	Pig			Pig	Turkey
	Muscle		Muscle		Liver			Muscle	
MAR	31	-	2	-	3	1.5	1	0.5	-
CIP	42	18	6	6	6	4	1	1	0.5
DAN	25	13	2	2	3	3	1	0.5	0.2
ENR	38	13	2	2	2	1.5	0.5	0.5	0.5
SAR	40	17	4	3	2	6	1	0.5	0.5
DIF	38	13	2	3	6	4	1	0.5	0.5
OXO	35	-	1	-	6	3	0.5	0.5	-
FLU	31	33	1	2	6	1.5	0.5	0.5	0.5

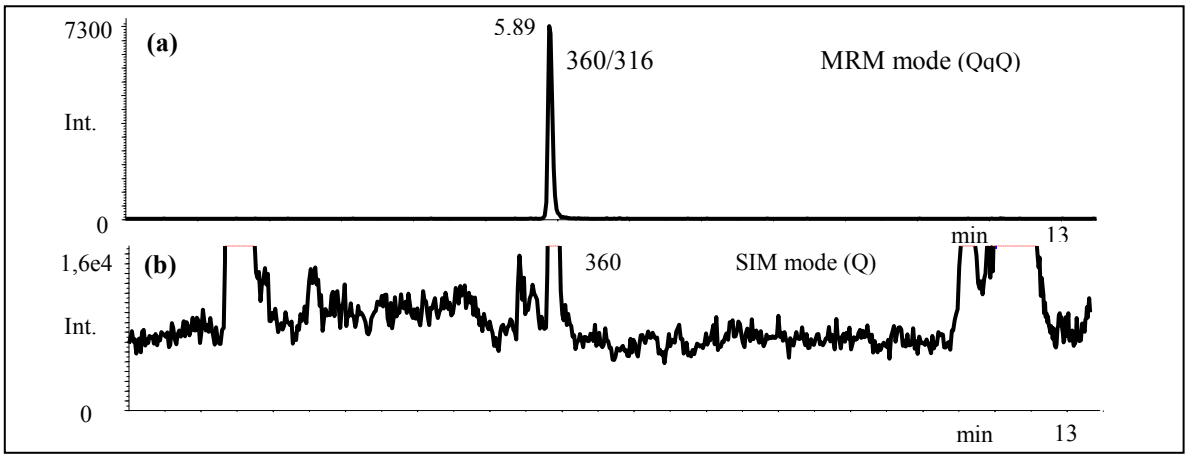


Fig. 10. Chromatogram of ENR, obtained for the analysis of milk raw sample at a concentration of 5 μg kg^{-1}, using (a) LC-QqQ-MS/MS and (b) LC-Q-MS.

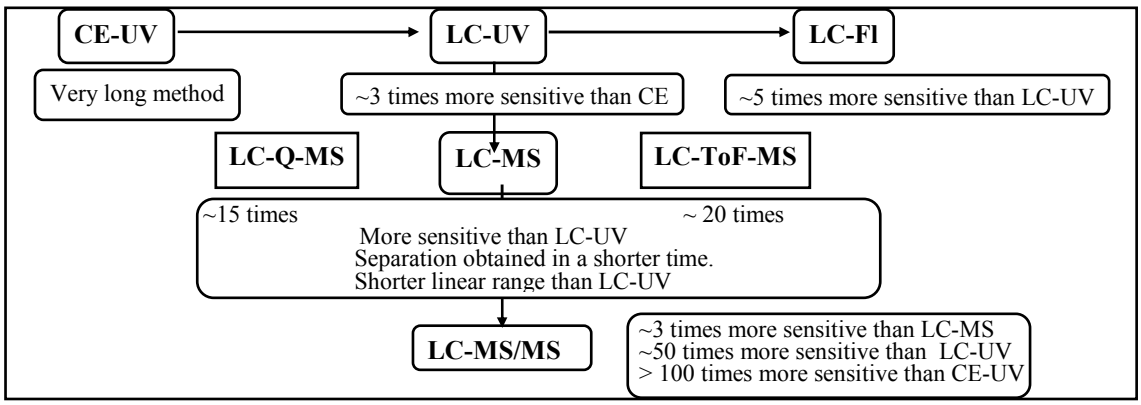
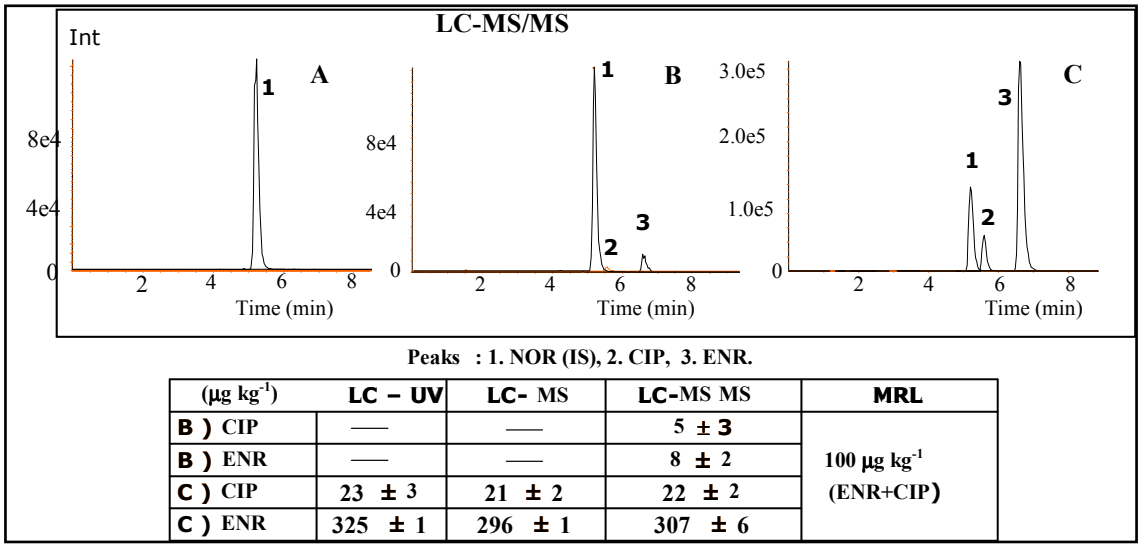


Fig. 11. Sensitivities of the methods for the determination of quinolones in different samples (chicken, cow, pig and turkey muscle, pig liver, cow and pig plasma and cow milk).



Peaks : 1. NOR (IS), 2. CIP, 3. ENR.

(μg kg^{-1})	LC – UV	LC- MS	LC-MS MS	MRL
B) CIP	——	——	5 ± 3	100 μg kg^{-1} (ENR+CIP)
B) ENR	——	——	8 ± 2	
C) CIP	23 ± 3	21 ± 2	22 ± 2	
C) ENR	325 ± 1	296 ± 1	307 ± 6	

Fig. 12. Determination of ENR and its metabolite, CIP, in turkey muscle samples, (HPO_3, Isolute ENV+) treated with: A.) 20 mg.L^{-1} Vitamine E + Fat (no signal was obtained); B.) ENR 10 mg L^{-1} in water for 5 consecutive days. The drug was taken away and 12 days later the animal was slaughtered (minima quantities were obtained and only using LC-MS/MS); C.) ENR 10 mg L^{-1} in water for 5 consecutive days and then the animal was slaughtered (positive samples)

The established methodologies were applied to the analysis of quinolones in positive samples. Figure 12 shows the results obtained with turkey muscle samples treated with ENR.
In case A, obviously no quinolones signal was obtained. In case B, little peaks can be seen in Fig. 12, only if LC-MS/MS is used and in case C positive data are obtained using UV, MS and MS/MS detection. Figure 13 shows the results concerning 6 samples of pig muscle from different markets of Barcelone (Spain) analysis.

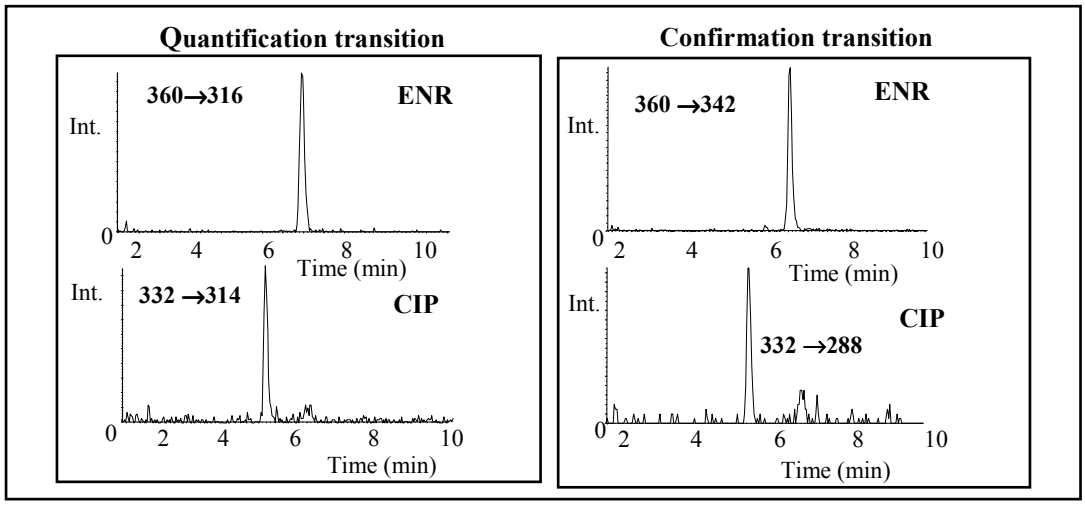


Fig. 13. Quinolones determination in pig muscle from Barcelone market (HPO_3, Isolute ENV+).

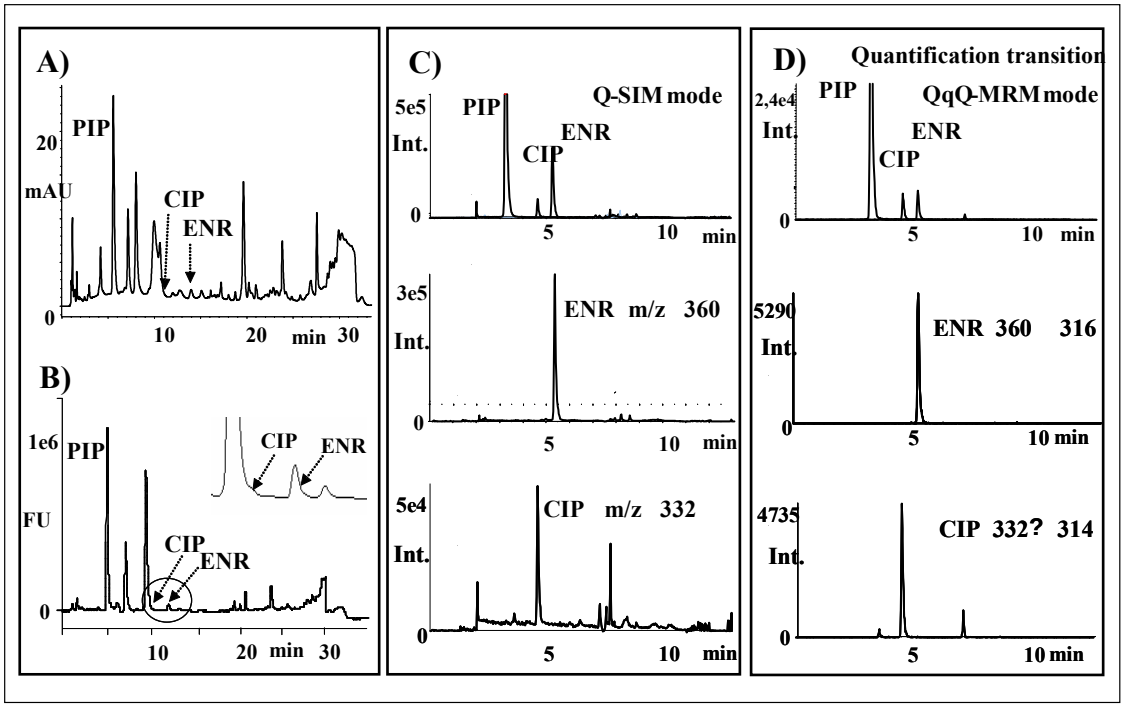


Fig. 14. Quinolones in raw milk positive samples. A) LC-UV (λ 208 nm), B) LC-Fl (λ_{exc} 280 nm and λ_{emi} 450nm), C) LC-MS, and D)LC-MS/MS. PIP (IS) 300 μg L^{-1}.

Only one of the analyzed samples presented residues of ENR and CIP demonstrating that the quantification of low concentrations of ENR (~ 0.7 μg kg^{-1}) and CIP (~0.5 μg kg^{-1}) was only possible using ESI-MS/MS detection.

Figure 14 shows graphically the significant different signals obtained using UV, Fl, MS and MS-MS detection in the analysis of ENR and CIP in positive samples of milk. The quinolones can not be analysed by LC-UV and LC-Fl but using LC-MS and LC-MS/MS the concentration of 6 μg kg^{-1} of ENR and CIP is obtained.

4. Conclusions

In this work multiresidue methods were developed and optimized to allow the analysis of quinolnes included in the European Union regulations in samples of chicken, turkey, pig and cow muscle, pig and cow plasma, pig liver and bovine milk. Different solvents to the extraction of quinolones compounds were studied. The best results were obtained using HPO3 water:MeCN hydroorganic mixtures.

A SPE step was established in order to clean-up and preconcentrate quinolones prior to their separation by CE or LC. Polymeric sorbents give the best results. Determination methods have been developed using CE-UV, LC-UV, LC-Fl, LC-ESI-Q-MS, LC-ESI-ToF-MS and LC-ESI-QqQ-MS/MS.

Excellent quality parameters and reduced time of analysis were obtained when LC-ESI-MS/MS is used, although the others techniques also presented satisfactory results. All methods were validated in line with commission decision 2002/657/EC.

The optimized methods were applied to determine quinolones on samples of products of animals medicated with quinolones. LOQ of the developed methods are always lower than MRL established in council regulation and then can be applied to determine quinolones residues on products of animal origin.

5. References

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Table 4. Quality parameters LOD, LOQ, calibration curves, recoveries curves, intra and inter-day precision, CCα and CCβ,.

	MAR	CIP	DAN	ENR	SAR	DIF	OXO	FLU
LC-ToF-MS								
LOD (μg kg^{-1})	0.5	1.5	1	0.5	2	1.5	1	0.5
LOQ (μg kg^{-1})	1.5	4	3	1.5	6	4	3	1.5
Calibration curve (*r*)[a]	*y* =2.11C -0.0109 (*r=0.998*)	*y* =0.914*C* - 0.0052 (*r=0.997*)	*y* =1.58C + 0.0009 (*r=0.995*)	*y* =2.40C +0.0333 (*r=0.994*)	*y* =0.541C -0.0126 (*r=0.995*)	*y* =1.37*C* -0.0073 (*r=0.984*)	*y* =1.48*C* -0.0212 (*r=0.998*)	*y* =1.67*C*+0.0011 (*r=0.994*)
Recovery curve[a,c]	*y*=0.820C-0.0004 (*r* = 0,994)	*y*=0.699C-0.0024 (*r* = 0,999)	*y*=0.6574C+0.009 (*r* = 0,993)	*y*=0.7638C-0.002 (*r* = 0,980)	*y*=0.6281C+0.004 (*r* = 0,992)	*y*=0.7377C-0.0109 (*r* = 0,970)	*y*=0.7955C+0.0053 (*r* = 0,995)	*y*=0.7872C+0.0002 (*r* = 0,987)
Precision, intra-day[b] (n=15), (%RSD)	3-6	3-4	1-6	2-5	4-6	4-7	6-9	6-9
Precision, inter-day[b] (n=45), (%RSD)	5-10	3-9	5-9	6-11	6-8	10-13	7-10	9-13
CCα (n=20)	164	209	213	220	811	818	165	515
CCβ (n=45)	175	218	222	229	822	828	179	535
LC-Q-MS								
LOD (μg kg^{-1})	1	2	1	0.5	0.5	2	2	2
LOQ (μg kg^{-1})	3	6	3	2	2	6	6	6
Calibration curve (*r*)[a]	*y* =2.96*C*+0.0607 (*r=0.990*)	*y* =1.08*C*+0.0144 (*r=0.998*)	*y* =2.60*C*+0.0390 (*r=0.995*)	*y* =4.89*C*+0.0697 (*r=0.990*)	*y* =1.14*C*+0.0010 (*r=0.996*)	*y* =2.83*C*+0.00314 (*r=0.994*)	*y* =1.54*C*+0.0219 (*r=0.988*)	*y* =2.89*C*+0.0124 (*r=0.992*)
Recovery curve[a,c]	*y*=0.7998C-0.0313 (*r* = 0,970)	*y*=0.7853C-.0001 (*r* = 0,983)	*y*=0.6997C+0.015 (*r* = 0,990)	*y*=0.8104C-0.0185 (*r* = 0,983)	*y*=0.6535C-0.0173 (*r* = 0,988)	*y*=0.7575C-0.0107 (*r* = 0,984)	*y*=0.8145C-0.0114 (*r* = 0,988)	*y*=0.7225C-0.0177 (*r* = 0,970)
Precision, intra-day[b] (n=15), (%RSD)	2-9	1-7	3-7	4-7	1-11	5-13	2-15	2-14
Precision, inter-day[b] (n=45), (%RSD)	5-6	3-5	5-6	6-8	7-8	10-11	8-13	5-15
CCα (n=20)	161	208	212	220	808	819	164	517
CCβ (n=45)	169	216	224	230	819	834	177	526
LC-QqQ-MS/MS								
LOD (μg kg^{-1})	< 0.5	< 0.25	< 0.25	< 0.25	< 0.5	< 0.25	< 0.1	< 0.25
LOQ (μg kg^{-1})	1	1	1	0.5	1	1	0.5	0.5
Calibration curve (*r*)[a]	*y* =2.04*C*+0.0021 (*r=0.998*)	*y* =4.63*C*+0.0633 (*r=0.983*)	*y* =12.1*C*+0.2010 (*r=0.993*)	*y* =3.57*C*+0.0752 (*r=0.993*)	*y* =3.34*C*+0.0142 (*r=0.997*)	*y* =8.27+0.131 (*r=0.981*)	*y* =7.64*C*+0.169 (*r=0.988*)	*y* =12.00*C*+0.21 (*r=0.990*)
Recovery curve[a,c]	*y*=0.7857C–0.0115 (*r* = 0.994)	*y*=0.8314C-0.001 (*r* = 0.996)	*y*=0.7521C-7e-06 (*r* = 0.988)	*y*=0.7604C+0.005 (*r* = 0.984)	*y*=0.6256C-0.003 (*r* = 0.966)	*y*=0.7609C-0.007 (*r* = 0.987)	*y*=0.8946C-0,011 (*r* = 0.990)	*y*=0.7373C-0.0056 (*r* = 0.970)
Precision, intra-day[b] (n=15), (%RSD)	2-10	4-15	3-15	4-6	3-8	5-11	7-10 (n=10)	6-14
Precision, inter-day[b] (n=45), (%RSD)	5-9	7-13	5-9	5-10	6-10	7-11	9-15 (n=30)	13-15
CCα (n=20)	161	216	215	215	817	816	163	515
CCβ (n=45)	169	230	224	223	833	827	178	536

[a] The interval range of calibration curve was LOQ-300 μg kg^{-1}, except OXO and FLU (ToF) LOQ-250 μg kg^{-1}; MAR, DAN, ENR, SAR, DIF, OXO and FLU (Q) LOQ-250μg kg^{-1} ; CIP, DAN, SAR and DIF (QqQ) LOQ-250 μg kg^{-1}; OXO (QqQ) LOQ-150 μg kg^{-1}; *y*= area quinolone/ internal standard ratio ; *C*= conc. quinolone/ internal standard ratio.

[b] The intra day and inter day data showed are the minimum and maximum values obtained in the analysis of the Samples, that were prepared at 50, 100 and 200 μg kg^{-1}.

[c] The % recovery is the slope recovery calibration line ∗ 100.