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Review

Analysis of quinolone residues in edible animal products

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Abstract

A comprehensive review on the analysis of quinolone antibacterials is presented. The review covers most of the methods described for the determination of quinolone residues in edible animal products. Sample handling, chromatographic conditions and detection methods have been discussed. A summary of the most relevant information about the analytical procedures has been included.

Keywords: Reviews; Food analysis; Quinolones

Contents

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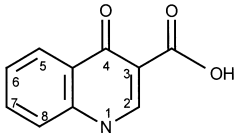
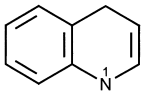
1. Introduction

Quinolones are antibacterial agents widely used in the treatment of infections in both humans [1] and animals [2]. Their primary target is the bacterial enzyme DNA gyrase or topoisomerase II, which renders the DNA molecule compact and biologically active [1].

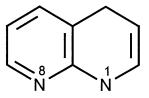
Their basic structure is shown in Fig. 1. They are nitrogen-containing, eight-membered heterocyclic aromatic compounds with a ketone group at position 4 and a carboxylic group at position 3. The main nucleus usually contains one nitrogen atom (quinolines), but analogues have additional nitrogens at position 2 (cinolines), position 8 (naphthyridines) or positions 6 and 8 (pyridopyrimidines). Since the discovery of nalidixic acid (the prototype antibacterial quinolone) in 1962, several structural modifications have enhanced their biological and pharmacological activities. These modifications include the introduction of alkyl or aryl groups at position 1 and fluoro and piperazinyl substitutions at positions 6 and 7, respectively. The fluoro group at position 6, which originates fluoroquinolones, widens the spectrum of activity against both gram-negative and gram-positive pathogens. The introduction of a piperazinyl group at position 7 improves activity against *Pseudomonas aeruginosa* [3]. The structure of the quinolones described in this review is shown in Fig. 2.



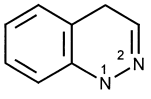
Basic structure of the quinolones



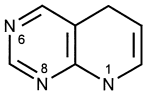
Quinoline



Naphthyridine



Cinoline



Pyridopyrimidine

Fig. 1. Basic structure of the quinolone antibacterials.

The carboxylic group at position 3 makes these compounds acidic. In addition, the 7-piperazinylquinolones include additional amine groups, which are basic. Therefore, in aqueous solution, the 7-piperazinylquinolones show three different species, which are cationic, zwitterionic and anionic, while the other quinolones can only be neutral or anionic. The existing equilibria for these two types of quinolones in the physiological pH range are shown in Fig. 3. In what follows, those with only one pK_a value in the physiological region of pH are referred to as acidic quinolones (AQ) and those with two pK_a values (due to the presence of a piperazinyl ring) are called piperazinyl quinolones (PQ). The reported values of pK_a for AQ range from 6.0 to 6.9, whereas in the case of PQ, the proposed values fall in the ranges 5.5–6.6 and 7.2–8.9 for pK_1 and pK_2, respectively.

Quinolones are widely used in veterinary medicine, and they are subjected to regulation. The European Union (EU) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have established maximum residue limits (MRL) for several quinolones, which are shown in Table 1 [4]. Since the beginning of year 2000, an MRL is required for every new substance that might be commercialised in the EU for veterinary use.

To our knowledge, only two reviews on the analysis of quinolones have been published to date. The first deals with the determination of quinolones in biological fluids, mainly for pharmacokinetic studies in human medicine [5]. The other concentrates on determination techniques but the sample treatment is hardly described [6]. Other reviews on the analysis of antibiotics can be found [7–11], however, since they focused on antibiotics in general, there is little information on quinolones. The present review focuses on the analysis of quinolones in edible animal products. The papers reviewed describe methodology for: (i) the control of quinolones at residue level, (ii) pharmacokinetic studies, and (iii) the study of the environmental impact of the quinolones used in fish farming.

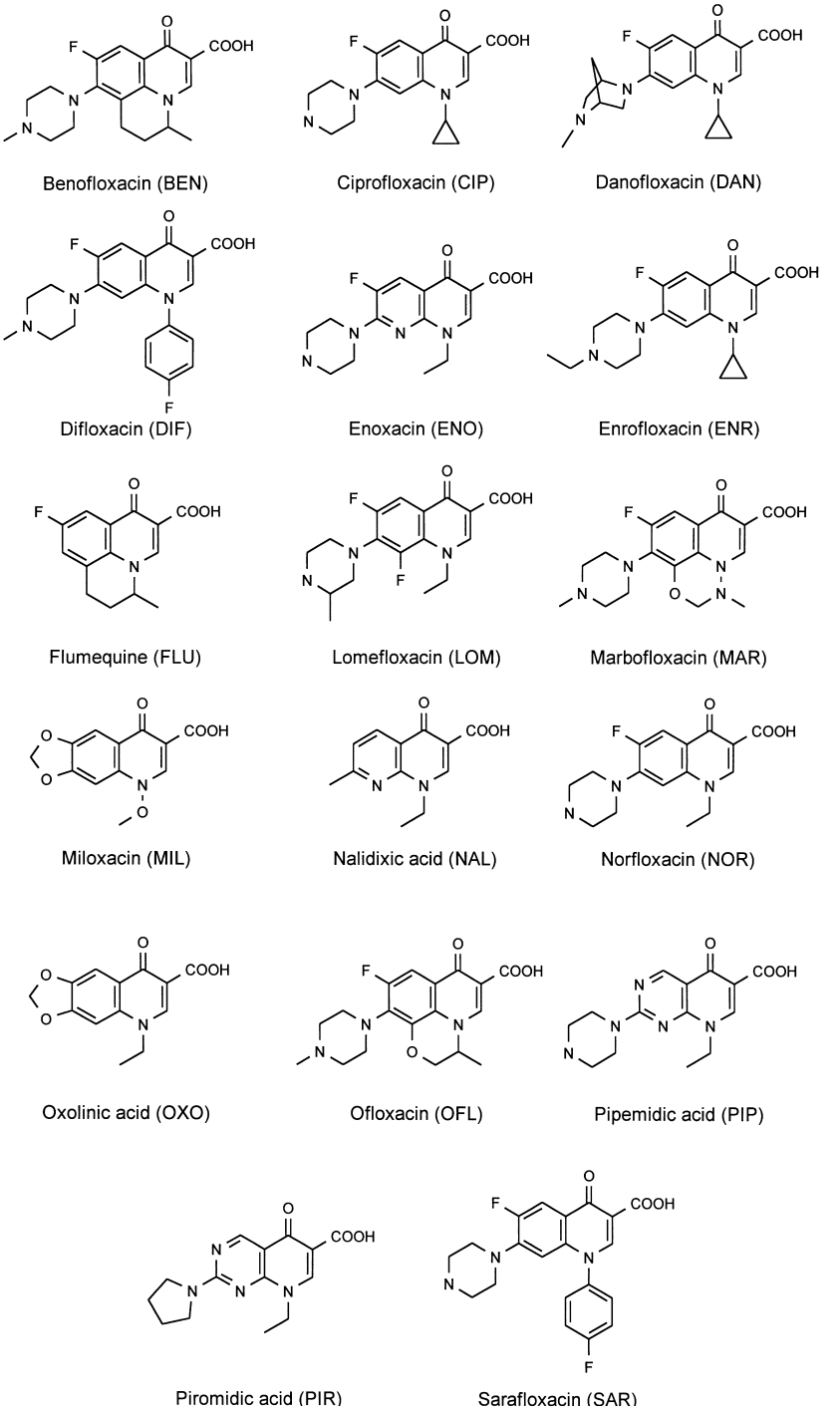


Fig. 2. Structure of the quinolones considered in this review.

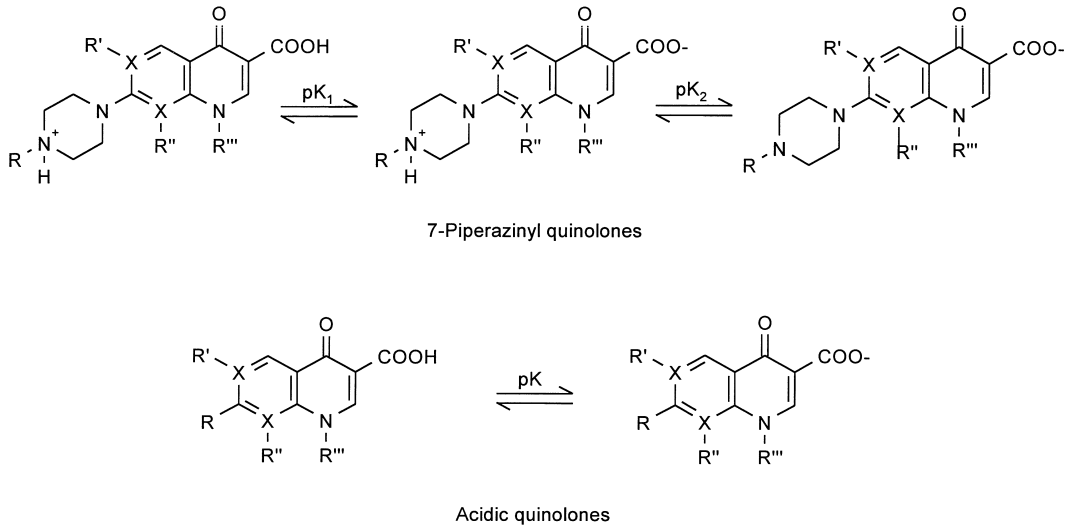


Fig. 3. Acid–base equilibria for the quinolones.

The papers considered were written in English, French, Italian or German. Articles written in other languages were included if the information given in the abstract is sufficient. These abstracts are marked in the table with an asterisk. The secondary sources consulted cover a wide field of applications: Food Science and Technology Abstracts, Science Citation Index and Chemical Abstracts.

Bibliography has been reviewed from 1969 to 2000, but since quinolones have been applied to veterinary medicine only recently, only 10 papers have been found in the period 1969–1988. Therefore, most of the papers belong to the period 1989–2000.

1.1. Samples and analytes

Most of the papers reviewed refer to the analysis of fish. Although most MRLs are established in poultry, pigs and cattle, less than half of the papers deal with the analysis of tissues of these animals. Aquatic wild fauna (mainly molluscs) and sheep have also been analysed. The most often analysed tissue is muscle (82%), followed by liver, kidney, skin and fat. Milk and eggs are also often analysed and one paper deals with honey. Since OXO is also used as fungicide, one method for its monitoring in crops has also been reported. With respect to analytes, most papers deal with OXO, ENR and FLU. Many analytical procedures are also available for CIP, NAL, SAR and PIR. As expected, the AQ have been studied extensively since they belong to the first generation of quinolones. Because of the broad activity spectrum of ENR (which belongs to the second generation of quinolones), many methodologies have also been developed for the analysis of this compound and its metabolite CIP. Other PQ such as DAN, DIF, NOR, OFL, BEN, MAR, and PIP have occasionally been determined in food.

The methods described usually allow the determination of one or at the most two quinolones (68% of the papers) but papers dealing with three or more quinolones are also proposed. In some cases simultaneous determination of quinolones and other antibacterial agents, such as sulphonamides and/or nitrofurans, has been reported [12–18].

2. Analytical methods

Quinolone residue analysis involves extraction with an appropriate solvent followed by one or more clean-up processes and determination by liquid chromatography (LC), or in a few cases by gas chromatography (GC), high-performance thin-layer chromatography (HPTLC) or even a non-chromatographic

Table 1
MRL values established by the EU and the JECFA for quinolones of veterinary use

Marker residue	Animal species	Target tissues	MRL (EU) ($\mu g\ kg^{-1}$)	MRLs (JECFA) ($\mu g\ kg^{-1}$)
DAN	Bovine, chicken	Muscle	200	200
		Fat	100	100
		Liver, kidney	400	400
		Milk	30	–
	Porcine	Muscle	100	100
		Skin+fat	50	–
		Fat	–	100
		Liver	200	50
		Kidney	200	200
DIF	Bovine, porcine	Muscle	400	–
		Fat, skin+fat	100	–
		Liver	1400	–
		Kidney	800	–
	Chicken, turkey	Muscle	300	–
		Skin+fat	400	–
		Liver	1900	–
		Kidney	600	–
ENR+CIP	Bovine, ovine	Muscle, fat	100	–
		Liver	300	–
		Kidney	200	–
		Milk	100	–
	Porcine, poultry, rabbit	Muscle, skin+fat, fat	100	–
		Liver	200	–
		Kidney	300	
FLU	Bovine, ovine, porcine	Muscle	200	500
		Fat, skin+fat	300	1000
		Liver	500	1000
		Kidney	1500	3000
		Milk	50	–
	Chicken, turkey	Muscle	400	500
		Skin+fat	250	–
		Fat	–	1000
		Liver	800	1000
		Kidney	1000	3000
	Salmonidae	Muscle+skin in natural proportions	600	500
MAR	Bovine, porcine	Muscle, liver, kidney	150	–
		Fat	50	–
		Milk	75	–
OXO	Bovine, porcine, chicken	Muscle	100	–
		Fat, skin+fat	50	–
		Liver, kidney	150	–
		Eggs	50	–
	Fin fish	Muscle+skin in natural proportions	300	–
SAR	Chicken	Muscle	–	10
		Skin+fat	10	–
		Fat	–	20
		Liver	100	80
		Kidney	–	80
	Turkey	Muscle	–	10
		Fat	–	20
		Liver, kidney	–	80
	Salmonidae	Muscle+skin in natural proportions	30	–

Table 2
Extraction with water immiscible organic solvents

Sample	Analyte	Sample treatment	Determination technique	Recovery (%)	Comments	Ref.
Fish	NAL, OXO, PIR	(1) Dry Na_2SO_4. Ex EtAc-hex (3:1) (2) SPE (amino, mph)	C_{18} LC–UV ACN–MeOH–H_2O (oxalic ac)	NAL: 81–95 OXO: 75–92 PIR: 74–93	LOD: 50 ng g^{-1} 2C+2Ex+SPE	[26]
Pig: kidney	FLU, FLUOH	(1) Dry Na_2SO_4. Ex EtAc (2) Ev. Add mph	C_{18} LC–Fl ACN–H_2O (oxalic bf), gradient	FLU: 90 FLUOH: 91	LOD: 15–24 ng g^{-1} 2C+Ev+2Ex+So	[24]
Fish: muscle	FLU, OXO	(1) Dry Na_2SO_4. Ex EtAc (2) Ev. Add oxalic bf pH 3. W hex	C_8 LC–Fl ACN–H_2O (oxalic bf)	FLU: 51–70 OXO: 70–89	LOD: 20–50 pg 3C+Ev+3Ex	[22]
Fish: muscle	OXO	As Ref. [22]	C_{18} LC–Fl ACN–MeOH–H_2O (oxalic ac)	71–83	LOD: 2 ng g^{-1} 3C+Ev+3Ex	[19] [20]
Eggs	FLU	(1) Dry Na_2SO_4. Ex EtAc (2) Ev. Add aq. oxalic ac. W petroleum ether	C_8 LC–Fl ACN–MeOH–H_2O (oxalic bf, TEA)	75	LOD: 5 ng g^{-1} 3C+Ev+3Ex	[23]
Fish: kidney liver, muscle	FLU, FLUOH	(1) Dry Na_2SO_4. Ex EtAc (2) (a) Add hex. Dry Na_2SO_4. SPE (amino, mph) (b) Ev. Add hex. Ex aq. NaOH	C_8 LC–Fl ACN–H_2O (oxalic bf)	(a) 70–91 (b) 65–87	LOD: 0.2 ng (a) 2C+2Ex+F+SPE (b) 4C+Ev+3Ex	[21]
Shrimps	OXO	(1) Ex EtAc (2) rEv. Add phosphate bf pH 8. Remove fat layer	C_{18} LC–UV ACN–H_2O (H_3PO_4)	92–103	LOD: 3.5 ng g^{-1} 3C+2Ex+rEv	[25]
Fish: muscle	NAL, OXO, PIR	(1) H phosphate bf pH 6. Ex EtAc (2) Ex aq. $NaHCO_3$. Add aq. HCl to pH 6. Ex EtAc. Dry Na_2SO_4. rEv. Add MeOH (3) $NaBH_4$	DB-5 GC–MS	NAL: 76–116 OXO: 75–96 PIR: 63–96	LOD: 3 ng g^{-1} 2C+Ev+13Ex+F+H+rEv+SPE	[27]
Fish: kidney muscle, liver	NAL	(1) H phosphate bf pH 6. Ex EtAc (2) Ev. Add ACN-hex. Ex aq. NaCl. Ex $CHCl_3$. Ev. Add $CHCl_3$. Ex borate bf pH 10	DEAE LC–UV ACN–H_2O (phosphate bf)	74–92	LOD: 50–100 ng g^{-1} 2C+2Ev+4Ex+H	[31]
Sheep: liver muscle, fat kidney	FLU, FLUOH	(1) H phosphate bf pH 7.8. Ex EtAc (2) Ev. Add phosphate bf pH 7.8. W hex	C_{18} LC–Fl (UV) ACN–DMF–H_2O (H_3PO_4)	FLU: 80 FLUOH: 65	LOD: UV: 100 ng g^{-1} Fl: 0.5–2.8 ng g^{-1} 2C+Ev+2Ex+H+So	[29]
Fish: muscle	OXO	(1) H phosphate bf pH 6. Ex EtAc (2) Ex aq. $NaHCO_3$. Add aq. HCl to pH 1–2 and NaCl. Ex EtAc. W phosphate bf pH 6. Dry Na_2SO_4. rEv. Add MeOH (3) $NaBH_4$	DB-5 GC–MS	65–108	LOD: 1 ng g^{-1} 2C+11Ex+2F+H+3rEv+SPE	[28]
Chicken: liver, muscle	NAL	(1) H phosphate bf pH 6. Ex EtAc (2) Ev. SPE (Al_2O_3, borate bf pH 10). W Et_2O. Add aq. H_2SO_4. Ex $CHCl_3$. Dry. Ev. Add aq. H_2SO_4	Fl	71–149	LOD: nr C+4Ex+2Ev+H+SPE	[32] [33]
Fish	NAL	(1) H phosphate bf. Ex EtAc (2) Ev. Add aq. NaCl. Ex $CHCl_3$. Ev. Add borate bf pH 10	SAX LC–UV H_2O (Na_2SO_4, borate bf)	78–122	LOD: 50 ng ml^{-1} C+2Ex+2Ev+H	[35]*

Table 2. Continued

Sample	Analyte	Sample treatment	Determination technique	Recovery (%)	Comments	Ref.
Fish	OXO, PIR	(1) H phosphate bf pH 6.0. Ex EtAc (2) Ev. Add ACN. Add aq. NaCl. W hex. Ex $CHCl_3$. Ex borate bf pH 10	C_{18} LC–UV MeOH–H_2O (phosphate bf, TMABr)	nr	LOD: nr 4Ex+Ev+H	[30]*
Fish: muscle, skin	OXO	(1) H H_2O. Add HCl. Ex EtAc (2) rEv. Add mph and aq. NaOH. W $CHCl_3$. Add aq. HCl. Ex $CHCl_3$. rEv. Add mph	C_{18} LC–UV MeOH–H_2O (Na_2HPO_4, CTAB)	80	LOD: <10 ng g^{-1} 2C+3Ex+F+H+2rEv	[34]
Eggs	OXO	(1) H aq. HCl. Ex EtAc (2) Ev. (a) Albumen: Add mph (b) Yolk: Add $CHCl_3$. Ex aq. NaOH. Add aq. HCl. Ex $CHCl_3$. Ev. Add mph	C_{18} LC–Fl ACN–H_2O (HAc)	Albumen: 75–85 Yolk: 60–70	LOQ: 5 ng g^{-1} Albumen: C+Ev+Ex+H Yolk: 3C+2Ev+3Ex+H	[36]
Chicken: fat, liver, kidney, skin, muscle	CIP, ENR	(1) H phosphate bf pH 7.4. Ex DCM (2) Ev. Add mph	C_{18} LC–UV MeOH–H_2O (H_3PO_4, TBAI)	>70	LOD: 3 ng ml^{-1} 3C+Ev+3Ex+H	[37]
Fish: skin, muscle, fat, kidney, liver	FLU	As Ref. [37]	C_{18} LC–Fl ACN–DMF–H_2O (H_3PO_4, TMACl)	69–88	LOD: 10 ng g^{-1} C+Ev+Ex+H	[38]
Chicken: muscle	ENR	(1) Add phosphate bf pH 7.2. Ex DCM (2) Ev. Add mph	C_{18} LC–Fl ACN–H_2O (phosphate bf, TEA)	71–100	LOD: 0.5 ng g^{-1} 2C+Ev+2Ex+F	[39]
Chicken, fish muscle	CIP, ENR	(1) Add diethylmalonic bf pH 7.4. Ex DCM (2) Add aq. NaCl and aq. HNO_3. rEv org. W hex	Fl (Tb^{3+}, SLS, HAc bf)	CIP: 47–62 ENR: 64–67	LOD: 3–4 ng g^{-1} 2C+3Ex+rEv	[41] [42]
Chicken, fish pig: muscle	CIP, DAN, DIF, ENR, MAR, NOR, SAR	(1) Add diethylmalonic bf pH 7. Ex DCM (2) Ex aq. NaOH. Add aq. oxalic ac	C_8 LC–Fl ACN–H_2O (oxalic bf), gradient	30–92[a]	LOD: nr 3C+3Ex	[40]
Chicken, turkey: liver, muscle, skin	ENR	(1) Soxhlet DCM–MeOH (9:1) (2) Ev. Add hex. Ex phosphate bf pH 2. W hex. W DCM. Add aq. NaOH to pH 12. W DCM. Add aq. H_3PO_4 to pH 7. Ex DCM. Add diethylenglycol–DCM. rEv (a) Fl: Add phosphate bf pH 3.5 (b) LC–Fl: Add mph	Fl, C_{18} LC–Fl (UV) ACN–H_2O (phosphate bf, TEA)	82–93	LOD: 20 ng g^{-1} 3C+9Ex+Ev+2F+rEv+So	[44]
Fish: muscle	FLU	(1) Ex $CHCl_3$	HPTLC–Fl THF–DCM–acetone–HAc	nr	LOD: 10 000 ng ml^{-1} C+Ex	[45]
Chicken, fish, pig	FLU, OXO	(1) Ex DCM (2) Ex aq. NaOH	C_8 LC–Fl ACN–H_2O (oxalic ac)	FLU: 77–91 OXO: 83–95	LOD: 0.2 ng ml^{-1} 3C+3Ex	[46]
Fish: liver, muscle	FLU	(1) H H_2O. Add aq. $HClO_4$ and aq. HCl. Ex $CHCl_3$ (2) rEv. Add $CHCl_3$. Ex aq. NaOH. Add aq. H_2SO_4	Fl	nr	LOD: nr 2C+2Ex+F+H+rEv	[43]

Acronyms: ac, acid; aq, aqueous; bf, buffer; C, centrifugation; CTAB, cetyltrimethylammonium bromide; DMF, dimethylformamide; Ev, evaporation; Ex, extraction; F, filtration; Fl, fluorescence; FLUOH, 7-hydroxyflumequine; H, homogenisation; hex, hexane; mph, mobile phase; nr, not reported; org, organic phase; rEv, rotaevaporation; SLS, sodium laurylsulfate; So, sonication; TBA, tetrabutylammonium; TMA, tetramethylammonium; W, wash.

[a] Recovery range covers all the analytes and samples.

* Abstract.

method. Tables 2–5 summarise the most relevant information about the analytical methods for animal tissues, grouped according to the extraction solvent. Further, methods developed for milk samples are grouped in Table 6.

The column labelled *Sample treatment* includes all the steps carried out before the determination, divided into two parts: (1) extraction and (2) clean-up. In the case of determination by GC, an additional derivatization step (3) is considered. *Extraction* includes all the steps performed while the matrix is in contact with the extracting agent. The heading *Determination technique* specifies the separation and detection techniques, columns in GC, columns and mobile phases in LC and mobile phase in HPTLC.

The column labelled *Recovery* includes data from spiked materials. When only one material was analysed at one spiking level, the recovery for each quinolone is shown as a single value. When either several materials or several spiking levels were used, the data are usually expressed as a range.

The last column *Comments* includes information about limits of detection and an equation that summarises the steps of the sample treatment in order to reflect the complexity of the method.

In order to make reading easy, no references have been included in the discussion unless they were necessary to explain some facts.

2.1. Extraction methods

Quinolones are soluble in polar organic solvents, but not in non-polar ones, such as hexane or toluene. They are also soluble in hydro-organic or aqueous acidic and basic media. Thus, the extraction of quinolones from biological matrices has been tackled by several approaches. Systems include: (i) lixiviation with organic solvents of medium to high polarity, such as ethyl acetate (EtAc), acetone, acetonitrile (ACN), ethanol (EtOH) or methanol (MeOH); (ii) partition between a sample homogenate in aqueous buffer solution and a not-miscible organic solvent, such as EtAc, chloroform or dichloromethane (DCM); (iii) extraction with acidic or basic hydro-organic mixtures, or even buffered aqueous solutions.

In this review, extraction methods are described according to the categories outlined in Tables 2–5. Tables 2 and 3 refer to the extraction of the neutral molecular form of analytes with organic solvents, which have been classified according to their miscibility with water. Thus, Table 2 groups methods based on water-immiscible solvents, irrespective of whether they are used alone or in combination with a liquid–liquid partition, and extraction methods with water miscible organic solvents are listed in Table 3. Table 4 corresponds to extraction with acidic polar solvents and Table 5 corresponds to extraction of anionic species using basic media.

Fig. 4 shows the percentage distribution of the analytical methodologies according to the solvent used in the extraction step.

2.1.1. Water-immiscible organic solvents

Extraction is usually performed with EtAc, but chloroform or DCM have also been reported. Although EtAc has been applied to various matrices, all the methods refer to analysis of AQ, which are more hydrophobic than PQ. Quinolones are extracted either from chemically dried samples or samples homogenised in an aqueous buffer. In the former, anhydrous sodium sulfate is used to dry samples. Extraction is usually performed by blending or shaking the sample with EtAc. In most cases, two equilibration steps using a total volume of 20–50 ml EtAc were performed. The reported recoveries for NAL, OXO and PIR are satisfactory, and although lower global recoveries were obtained for FLU, this seems to be due to the clean-up step rather than to low efficiency in the lixiviation.

Extraction from wet samples involves previous homogenisation with an aqueous solution, which usually consists of phosphate buffer solutions at pH 6. Although at this pH FLU is an anionic species in aqueous solution, its great hydrophobicity nevertheless allows for good recoveries. The volumes of organic solvent used in these methods range from a few milliliters to some hundreds.

Conditions for extraction of quinolones with chloroform and DCM vary widely, and include partition between samples homogenised in an aqueous buffered solution and DCM or chloroform, Soxhlet extraction with DCM–MeOH or lixiviation with chloroform or DCM. None of the papers dealing with chloroform reports recovery data. Soxhlet extraction with DCM gives high recoveries, but in-

Table 3
Extraction with water miscible organic solvents

Sample	Analyte	Sample treatment	Determination technique	Recovery (%)	Comments	Ref.
Fish	FLU, NAL, OXO, PIR	(1) Ex acetone (2) Add propanol. rEv. Add acetone–H_2O (NaCl). W hex. Ex $CHCl_3$. Ex aq. NaOH. W $CHCl_3$. Add aq. H_3PO_4. Ex $CHCl_3$. rEv. Add mph	Ph(C_{18}) LC–ESI-MS (MS–MS) ACN–H_2O (formic bf)	56–95[a]	LOD: 1 ng g^{-1} 5C+11Ex+5F+2rEv+So	[53]
Shrimps, fish: muscle	FLU, NAL, OXO, PIR	As Ref. [53]	PLRP–S LC–Fl, PLRP–S LC–UV ACN–THF–H_2O (H_3PO_4)	FLU: 85–90 OXO: 77–91 NAL: 80–103 PIR: 75–82	LOQ: 5 ng g^{-1} 5C+11Ex+5F+2rEv+So	[50] [49]
Fish: muscle	FLU, NAL, OXO, PIR	As Ref. [53]	NS1 LC–APCI-MS (MS–MS) ACN–THF–H_2O (HAc), gradient	nr	LOD: MS: 0.8–1.7 ng g^{-1} MS/MS: 0.08–0.16 ng g^{-1} 5C+11Ex+5F+2rEv+So	[51]
Shrimps, fish: muscle	FLU, NAL, OXO, PIR	(1) (2) As Ref. [53] (3) $NaBH_4$	DB-5-GC–MS	FLU: 79–108 OXO: 87–94 NAL: 72–93 PIR: 65–96	LOD: 10 ng g^{-1} 5C+2Ev+16Ex+5F+2rEv	[49] [48]
Fish: muscle	NAL, OXO, PIR	(1) Ex acetone (2) Add propanol. rEv. Add acetone–H_2O (NaCl). W hex. Ex $CHCl_3$. Dry Na_2SO_4. rEv. Add ACN. SPE (Al_2O_3, NaOH). W $CHCl_3$. Add H_3PO_4. Ex $CHCl_3$. Dry Na_2SO_4. rEv. Add THF–H_2O	C_{18} LC–UV ACN–THF–H_2O (H_3PO_4)	NAL: 83–93 OXO: 74–95 PIR: 72–84	LOD: 20–80 ng g^{-1} 10Ex+2F+3rEv+SPE	[12]
Chicken: liver	FLU, OXO, SAR	(1) Ex acetone (2) Add acetone–H_2O (NaCl). W hex. Ex $CHCl_3$. Ex phosphate bf pH 9. W $CHCl_3$. Di. OLPC (PLRP–S or C_{18}, mph)	Amide LC–Fl ACN–H_2O (phosphate bf, TEA) gradient	FLU: 88 OXO: 97 SAR: 95	LOD: 0.2 ng ml^{-1} 4C+Di+6Ex+OLPC	[47]
Chicken: liver	FLU, OXO	As Ref. [47]	PLRP–S LC–Fl ACN–THF–H_2O (phosphate bf)	FLU: 94–96 OXO: 98–115	LOD: 2.5–5 ng g^{-1}, 4C+Di+6Ex+OLPC	[52]
Pig	NAL, OXO, PIR	(1) Dry Na_2SO_4. Ex ACN (2) W hex. Ev. Add ACN. W hex	C_{18} LC–ESI-MS ACN–H_2O (HAc bf), gradient	NAL: 82 OXO: 85 PIR: 90	LOD: 50–100 ng g^{-1} 3C+Ev+4Ex	[18]
Chicken: liver, eggs, muscle	FLU, NAL, OXO	(1) Ex ACN. Dry Na_2SO_4 (2) Ev. Add phosphate bf pH 11. SPE (AGMP-1, MeOH–HAc). Ev. Add aq. oxalic ac	C_8 LC–Fl ACN–MeOH–H_2O (oxalic ac)	FLU: 48–70 NAL: 44–64 OXO: 42–63	LOQ: 10 ng g^{-1} C+2Ev+Ex+SPE	[58]
Eggs	CIP, ENR	(1) Ex ACN (2) rEv. Add mph	C_{18} LC–UV ACN–H_2O (TEA)	CIP: 36–50 ENR: 49–85	LOD: ENR: 19 ng g^{-1}, CIP: 156 ng g^{-1} 2C+Ex+rEv	[56]
Cattle: liver, muscle	FLU	(1) Ex ACN (2) W hex. Add NaCl. Ex DCM and EtAc. rEv. Add Me–OH–H_2O. W hex. rEv. Ex EtAc. Ev org. Add ACN–MeOH–H_2O. SPE (SiO_2)	C_{18} LC–UV ACN–H_2O (H_3PO_4)	41	LOD: 15 ng g^{-1} 2C+Ev+8Ex+2F(2C) +2rEv+SPE	[55]
Eggs	SAR	(1) Ex ACN. Add aq. NaCl. Ex ACN (2) W hex. Add EtOH. Di. OLPC (C_{18}, mph)	Amide LC–Fl ACN–H_2O (H_3PO_4, TEA), gradient	87–102	LOD: 0.2 ng g^{-1} 3C+3Ex+Di+OLPC	[57]
Shrimps, fish: muscle	MIL, OXO	(1) Ex ACN–THF (19:1)	Hisep LC–UV ACN–H_2O (citric bf, phosphate bf, TBABr)	MIL: 72–88 OXO: 80–98	LOD: 20–100 ng g^{-1} C+Ex	[14] [16]
Sheep: lung, brain, skin	DAN	(1) C. Ex ACN–H_2O (3:2) (2) Add H_2O	C_{18} LC–Fl ACN–H_2O (phosphate bf)	77–86	LOD: 40 ng g^{-1} 3C+Ex	[54]
Fish: muscle	SAR	(1) Ex ACN–H_2O (1:1) (2) W hex. rEv. Add ACN–MeOH–H_2O (CF_3COOH). UF	C_{18} LC–Fl (LSC) ACN–MeOH–H_2O (CF_3COOH), gradient	LC–Fl: 85–104 LC–LSC: 80–85	LOD: 1.4 ng g^{-1} 5C+7Ex+F+rEv+UF	[59]
Fish: muscle	NAL, OXO, PIR	(1) Ex MeOH (2) Add succinic bf pH 4 and NaCl. Ex EtAc. W H_2O. Ex borate bf pH 10. Add aq. HCl. Ex EtAc. W H_2O. Dry Na_2SO_4. Ev. Add mph	C_8 LC–UV ACN–MeOH–H_2O (citric ac, TBABr)	NAL: 84 OXO: 93 PIR: 83	LOD: 1 ng 2C+Ev+10Ex	[60]
Fish: muscle	OXO	MSPD (C_{18}, ACN–MeOH). Ev. Add MeOH–HAc	C_{18} LC–UV MeOH–H_2O (HAc), gradient	63–100	LOD: 50 ng g^{-1} Ev+MSPD	[61]

Acronyms: ac, acid; aq, aqueous; bf, buffer; C, centrifugation; Di, dialysis; Ev, evaporation; Ex, extraction; F, filtration; Fl, fluorescence; hex, hexane; LSC, liquid scintillation counting; mph, mobile phase; MSPD, matrix solid-phase dispersion; nr, not reported; OLPC, on-line pre-concentration; org, organic phase; Ph, phenyl; rEv, rotaevaporation; So, sonication; TBA, tetrabutylammonium; UF, ultrafiltration; W, wash.

[a] Recovery range covers all the analytes and samples.

Table 4
Extraction with acidic solutions

Sample	Analyte	Sample treatment	Determination technique	Recovery (%)	Comments	Ref.
Poultry, pig, cattle, eggs, honey	CIP, DAN, ENO, ENR, LOM, MAR, NOR, OFL, SAR	(1) Ex ACN (HAc). Dry Na_2SO_4 (2) Add HAc. SPE (SCX, MeOH–NH_3). Ev. Add mph	C_8 LC–Fl (UV for MAR) ACN–H_2O (phosphate bf, TEA)	42–105[a]	LOQ: CIP, DAN, ENR, MAR, NOR, 5–10 ng g^{-1}, ENO, LOM, OFL: 50 ng g^{-1} C+Ev+Ex+F+SPE	[58]
Chicken: muscle	CIP, ENR	(1) Ex ACN (HAc) (2) Ev. Add phosphate bf pH 7.4. W hex SPE (C_{18}, MeOH–NH_3). Add mph	C_{18} LC–Fl ACN–H_2O (phosphate bf, pentanesulphonate)	92–111	LOD: 1 ng g^{-1} C+Ev+Ex+So+SPE	[84]
Pig, beef: muscle, fat	CIP, ENR	(1) Ex EtOH (HAc) (2) SPE (SCX, MeOH–NH_3). Add ACN Ev. Add mph	C_8 LC–Fl ACN–H_2O (phosphate bf,TEA)	CIP: 53–81 ENR: 67–90	LOD: <1 ng g^{-1} 4C+Ev+2Ex+3So+SPE	[78] [79]
Chicken, fish, pig, beef	CIP, ENR	(1) Ex EtOH (HAc) (2) Add TEA. Ev. Add phosphate bf pH 7.5 W hex. SPE (C_{18}, ACN–TEA). Ev. Add phosphate bf pH 3.5	C_{18} LC–Fl ACN–H_2O (phosphate bf, TEA), gradient	CIP: 48–53 ENR:73–77	LOD: 2 ng g^{-1} 2C+2Ev+5Ex+SPE	[81]
Poultry: eggs, muscle	CIP, ENR	(1) Ex EtOH (HAc) (2) rEv. Add EtOH and mph	C_{18} LC–Fl ACN–H_2O (phosphate bf, TEA)	CIP: 90–98 ENR: 91–99	LOQ: 7 ng g^{-1} 3C+2Ex+rEv	[82]
Fish: muscle	CIP, DIF, ENR, SAR	(1) Ex EtOH (HAc) (2) Add aq. HAc. Fr. SPE (SCX, MeOH–NH_3). Ev. Add mph	Ph LC–ESI-MS ACN–H_2O (formic ac)	CIP: 35 DIF, ENR, SAR: 60	LOD: nr 3C+2Ex+Ev+Fr+SPE	[80]
Fish: liver, muscle	CIP, ENR	(1) Ex EtOH (HAc). In (2) rEv. Add mph	C_{18} LC–Fl ACN–H_2O (phosphate bf, TEA)	CIP: 65 ENR: 80	LOD: nr 2C+2Ex+2H+2In+rEv	[83]
Fish: muscle	FLU	(1) Ex MeOH (HAc) (2) rEv. Add aq. HAc. SPE (C_{18}, MeOH–NH_3). rEv. Add mph (or MeOH for MS)	C_{18} LC–Fl (ESI-MS) ACN–H_2O (HAc)	87–94	LOD: 3 ng g^{-1} 2C+2Ex+2rEv+SPE	[87]
Fish: kidney, liver, skin, muscle	FLU	As Ref. [87]	C_{18} LC–LSC (ESI-MS, ESI-MS–MS) ACN-H_2O (HAc), gradient	>90	LOD: nr 2C+Ex+2rEv+SPE	[88]
Pig: kidney	FLU	(1) Ex MeOH (HAc) (2) W hex. Ev. Add mph	C_8 LC–Fl ACN–H_2O (phosphate bf, TEA), gradient	32–41	LOD: 90 ng g^{-1} C+Ev+2Ex	[85]
Mussel: shell	OXO	(1) Ex MeOH (oxalic ac) (2) rEv. Add H_2O	C_{18} LC–UV ACN–H_2O (phosphate bf)	73	LOD: 12 ng g^{-1} 3C+2Ex+F+rEv	[15]
Fish: kidney, liver, skin, muscle	OXO	(1) Ex MeOH (HCl). In (55 °C) (2) Add aq. sodium citrate	C_8 LC–Fl MeOH–H_2O (citrate bf)	94	LOD: <0.31 ng 2C+Ex+In+So	[86]
Crops	OXO	(1) Ex MeOH–H_2O (HCl) (2) Add aq. NaCl. Ex DCM. rEv. Add MeOH–H_2O (KOH, NaCl). W DCM. Add aq. HCl. Ex DCM. rEv. Add DCM. SPE (SiO_2, DCM). rEv. Add MeOH–H_2O (KOH)	C_{18} LC–Fl ACN–THF–H_2O (citrate bf, TOA)	78–95	LOD: 0.5 ng 7Ex+4F+3rEv+SPE	[65]
Chicken, beef; liver, muscle, fat, kidney	DAN, NDAN	(1) Ex MeOH–H_2O (1:1) ($HClO_4$, H_3PO_4). In (50 °C)	C_8 LC–Fl ACN–H_2O (phosphate bf)	DAN: 93–111 NDAN: 92–112	LOQ: 10 ng g^{-1} C+Ex+In	[62]
Chicken: liver, kidney, muscle, skin	DAN, NDAN	As Ref. [62]	C_{18} LC–Fl ACN–H_2O (phosphate bf, TBAOH, Waters D-4 reagent)	DAN: 89 NDAN: 94	LOQ: 10 ng g^{-1} C+Ex+In	[68]
Chicken, cattle: liver	DAN	(1) Ex MeOH–H_2O (1:1) ($HClO_4$, H_3PO_4). In (55 °C) (2) Add NH_3 to pH 8.5. Ex DCM. rEv. Add mph	C_{18} LC–ISP–MS–MS ACN–H_2O (CF_3COOH)	nr	LOD: nr 2C+2Ex+In+rEv+So	[66]
Fish: muscle	ENR, FLU, NAL, OXO	(1) Ex MeOH–H_2O (2:3) (HPO_3) (2) rEv. SPE (C_{18}, MeOH). Ev. Add mph	PLRP–S LC–Fl (UV) ENR: ACN–H_2O (H_3PO_4) FLU, NAL, OXO: ACN–THF–H_2O (H_3PO_4)	ENR: 64–70 FLU: 60–92 NAL: 60–73 OXO: 65–88	LOD: ENR, OXO: 5 ng ml^{-1}, NAL: 20 ng ml^{-1}, FLU: 40 ng ml^{-1} C+Ev+Ex+F+rEv+SPE	[63]

Table 4. Continued

Sample	Analyte	Sample treatment	Determination technique	Recovery (%)	Comments	Ref.
Fish: muscle	NAL, OXO, PIR	(1) Ex MeOH–H_2O (2:3) (HPO_3). F Hyflo Super-Cel (2) rEv. SPE (C_{18}, MeOH). rEv. Add mph	C_{18} LC–UV ACN–H_2O (oxalic ac)	NAL: 86–88 OXO: 87–90 PRI: 85–87	LOD: NAL, PIR: 50 ng g^{-1}, OXO: 20 ng g^{-1} Ex+F+2rEv+SPE	[13]
Fish: muscle	NAL, OXO, PIR	As Ref. [13]	C_{18} LC–TSP–MS ACN–H_2O (NH_4 Ac bf)	nr	LOD: OXO, PIR: 10 ng g^{-1}, NAL: 1 ng g^{-1} Ex+F+2rEv+SPE	[69]
Fish: muscle	NAL, OXO, PIR	As Ref. [13]	C_{18} LC–Fl (UV for PIR) ACN–H_2O (NaH_2PO_4)	NAL: 82–85 OXO: 84–89 PIR: 81–85	LOD: 10 ng g^{-1} Ex+F+2rEv+SPE	[67]
Fish: muscle	MIL	As Ref. [13]	C_{18} LC–Fl (UV) ACN–H_2O (NaH_2PO_4)	85–88	LOD: 10 ng g^{-1} Ex+F+2rEv+SPE	[71]
Chicken: liver, muscle	BEN, DAN, ENR, OFL	(1) Ex ACN–H_2O (3:7) (HPO_3). F Hyflo Super-Cel (2) rEv. SPE (C_{18}, MeOH). rEv. Add mph	C_{18} LC–Fl ACN–H_2O (heptanesulfonic ac, phosphate bf)	BEN: 84–85 DAN: 81–83 ENR: 82–85 OFL: 85–90	LOD: 10–20 ng g^{-1} Ex+F+2rEv+SPE	[72]
Meat, fish	BEN, DAN, ENR, FLU, NAL, OFL, OXO, PIR	(1) Ex ACN–H_2O (3:7) (HPO_3) (2) SPE (C_{18})	C_{18} LC–Fl (UV) ACN–H_2O (phosphate bf, SLS)	81–92	LOD: 10 ng g^{-1}	[73]*
Chicken, fish, beef: muscle, liver	ENR	As Ref. [73]	C_{18} LC–Fl (UV) ACN–H_2O (NaH_2PO_4)	80–85	LOD: 10 ng g^{-1}	[77]*
Chicken, fish, pig	FLU, MIL, NAL, OXO, PIR	As Ref. [73]	C_{18} LC–Fl (UV) ACN–H_2O (NaH_2PO_4)	78–87	LOD: 10 ng g^{-1}	[75]*
Chicken: muscle	BEN, ENR, OFL	(1) Ex ACN–H_2O (1:4) (EDTA, McIlvaine bf) (2) SPE (C_{18}, MeOH). Ev. Add mph	C_{18} LC–UV ACN–H_2O (McIlvaine bf, TBABr)	73–91	LOD: 20 ng g^{-1} Ex+Ev+SPE	[76]*
Crab, mussel, fish: liver, muscle	OXO	(1) Ex MeOH–H_2O (9:11) (McIlvane bf pH 3.6) (2) rEv. Add McIlvane bf pH 3.6. Ex DCM. W McIlvane bf. Ev. Add aq. NaOH	C_8 LC–Fl ACN–MeOH–THF–H_2O (oxalic bf), gradient	85–93	LOD: 3–10 ng g^{-1} 3C+Ev+4Ex+2F+rEv	[70]
Fish: muscle	FLU, OXO	(1) Ex MeOH–H_2O (9:11) (McIlvane bf pH 3.6) (2) rEv. Add McIlvane bf pH 3.6. Ex DCM. W McIlvane bf. Ev. Add HCO_3^- bf pH 9.0	C_{18} LC–Fl ACN–MeOH–THF–H_2O (citric bf)	FLU: 84–87 OXO: 90–93	LOD: 5 ng g^{-1} 3C+Ev+4Ex+rEv	[64]
Pig, beef: muscle, liver, kidney	CIP, DIF, ENR, SAR	(1) Ex ACN–H_2O (3:7) (TCA) (2) Add H_2O. SPE (PSDVB, mph). Ev. Add ACN	C_8 LC–Fl (DAD) ACN–H_2O (heptanesulfonic ac, H_3PO_4)	CIP: 78–91 DIF: 79–90 ENR: 79–93 SAR: 79–92	LOD: 5–10 ng g^{-1} C+Ev+Ex+F+SPE	[74]
Fish: muscle, liver	FLU, OXO	(1) Ex MeOH–TCA (1:4) (2) Add aq. NaOH. Add MeOH–H_2O (H_3PO_4)	PLRP–S LC–Fl ACN–THF–H_2O (H_3PO_4)	FLU: 51–64 OXO: 56–71	LOQ: 30–35 ng g^{-1} C+Ex+F	[89]
Eggs, beef: kidney, muscle	CIP, DIF, ENR, MAR, NOR	(1) Ex aq. HCl (2) SPE (C_{18}, MeOH–phosphate bf). Ev	C_{18} LC–DAD ACN–H_2O (phosphate bf)	64–99[a]	LOD: 1–4 ng C+Ev+Ex+So+SPE	[90]

Acronyms: ac, acid; aq, aqueous; bf, buffer; C, centrifugation; Ev, evaporation; Ex, extraction; F, filtration; Fl, fluorescence; Fr, freeze; H, homogenisation; hex, hexane; In, incubation; mph, mobile phase; NDAN, *N*-desmethyl-danofloxacin; nr, not reported; Ph, phenyl; rEv, rotaevaporation; SLS, sodium laurylsulfate; So, sonication; TBA, tetrabutylammonium; TOA, tri-*n*-octylamine; W, wash.

[a] Recovery range covers all the analytes and samples.

***** Abstract.

Table 5
Extraction with basic or neutral buffered solutions

Sample	Analyte	Sample treatment	Determination technique	Recovery (%)	Comments	Ref.
Chicken: liver, eggs	CIP, DAN, DCIP, ENR, NOR, SAR	(1) Ex ACN (NH_3) (2) Add aq. NaCl. W Et_2O-hex. Ev. Add phosphate bf pH 9. Di. OLPC (C_{18}, phosphate bf pH 5)	C_{18} LC–Fl ACN–H_2O (phosphate bf, TEA), gradient	66–110[a]	LOD: CIP, ENR, NOR,SAR: 1–3 ng g^{-1}; DAN, DCIP: 0.3 ng g^{-1} 3C+Di+Ev+3Ex+OLPC+So	[97]
Chicken: liver	FLU, OXO, SAR	(1) Ex ACN (NH_3) (2) Add aq. NaCl. W hex-Et_2O. Di. OLPC (C_{18}, mph)	Amide LC–Fl ACN–H_2O (phosphate bf, TEA), gradient	FLU: 78–106 OXO: 85–115 SAR: 83–114	LOD: 0.2 ng ml^{-1} 4C+Di+3Ex+OLPC	[98]
Beef: muscle	ENR	(1) Ex ACN (NH_3) (2) Add aq. NaCl. W Et_2O-hex. Add aq. H_3PO_4	PLRP–S LC–Fl ACN–MeOH–H_2O (H_3PO_4, heptanesulfonate)	89–93	LOD: 5 ng g^{-1} 3C+2Ex+F	[92]
Fish: liver, muscle	ENR, SAR	As Ref. [92]	PLRP-S LC–Fl ACN–MeOH–H_2O (H_3PO_4)	nr	LOQ: 5–10 ng g^{-1} 2C+2Ex+F	[91]
Fish: liver, muscle	FLU, OXO	(1) Ex ACN (NH_3) (2) Add aq. NaCl. Add H_3PO_4. Ex $CHCl_3$. Ev. Add mph	PLRP–S LC–Fl ACN–THF–H_2O (H_3PO_4)	FLU: 88–94 OXO: 101–104	LOD: 5–10 ng g^{-1} 3C+Ev+2Ex+F	[96]
Fish: liver, muscle	FLU, OXO	(1) Ex acetone–H_2O (NaOH) (2) Add aq. H_3PO_4. Ex $CHCl_3$. rEv Muscle (a) Add $CHCl_3$. Ex aq. NaOH. Add H_3PO_4. Ex $CHCl_3$. Ev. Add mph (b) Add hex. Ex aq. NaOH. W hex. Add MeOH–H_2O (H_3PO_4). SPE (C_2, HAc–MeOH). Ev. Add mph Liver Add hex. SPE (CN, MeOH–$CHCl_3$). Ev. Add $CHCl_3$ Ex. aq. NaOH. Add H_3PO_4. Ex $CHCl_3$. Ev. Add mph	PLRP–S LC–Fl ACN–THF–H_2O (H_3PO_4)	Muscle a) FLU: 89–92 OXO: 97 Muscle b) FLU: 77 OXO: 78 Liver FLU: 88–91 OXO: 90–98	LOD: 0.5–2 ng g^{-1} Muscle a) 3C+Ev+8Ex+rEv Muscle b) 3C+Ev+7Ex+rEv+SPE Liver 3C+2Ev+8Ex+rEv+SPE	[102]
Fish: liver, muscle	CIP, ENR	(1) Ex acetone–H_2O (NaOH) (2) W hex. rEv org. Add aq. H_3PO_4. W hex. Add NaCl. W hex. SPE (C_{18}, MeOH–H_2O (H_3PO_4))	PLRP–S LC–Fl (UV) ACN–H_2O (H_3PO_4)	ENR: 96–98	LOD: 1 ng g^{-1} 3C+7Ex+F+rEv+SPE	[101]
Pig: muscle	CIP, DAN, ENR, FLU, NAL, NOR, OXO	(1) Ex ACN–H_2O (10:1) (NaOH) (2) Ev. Add phosphate bf pH 7.4. W hex. SPE (C_8, MeOH–NH_3). Ev. Add MeOH	HPTLC–Fl MeOH–NH_3	nr	LOD: 5–15 ng g^{-1} 2C+2Ev+2Ex+So+SPE	[99]
Shrimps	SAR	(1) H H_2O. Ex ACN–H_2O (4:1) (NaOH) (2) Add aq. H_3PO_4. Add mph	C_{18} LC–UV ACN–H_2O (acetohydroxamic ac, SLS, phosphate bf)	75	LOD: 50 ng g^{-1} C+Ex+H	[95]
Fish: muscle	SAR	(1) H aq. NaOH. Ex ACN (H_3PO_4) (2) Add H_3PO_4. SPE (C_{18}, ACN–NH_4Ac bf pH 3) (a) UV: Add NH_4Ac bf pH 3 (b) MS–MS: rEv. Add mph	C_{18} LC–UV (ISP–MS–MS) UV: ACN–H_2O (citric bf) MS–MS: ACN–H_2O (NH_4Ac bf)	91–99	LOD: 64 ng ml^{-1} UV: 2C+Ex+H+SPE MS–MS: 2C+Ex+H+rEv+SPE	[100]
Chicken: liver	CIP, DIF, ENR, SAR	(1) H aq. NaOH. Add aq. H_3PO_4. Ex MeOH–H_2O (1:9) (PBS) (2) OLPC (IAC, HAc bf)	Ph LC–Fl ACN–H_2O (HAc bf)	CIP: 88–91 ENR: 91–94 SAR: 86–89	LOD: 3–9 ng g^{-1} C+H+Ex+OLPC	[103]

Table 5. Continued

Sample	Analyte	Sample treatment	Determination technique	Recovery (%)	Comments	Ref.
Chicken: muscle	CIP, DAN, DIF, ENR, FLU, MAR, NAL, OXO, SAR	(1) H Tris bf pH 9.1. Ex ACN (2) Ev. Add Tris bf pH 9.1. W hex	PLRP–S LC–Fl ACN–THF–H_2O (H_3PO_4)	59–77[a]	LOD: CIP, DIF, ENR, FLU, SAR: 0.5–3 ng g^{-1}, DAN, NAL, OXO: 7.5–12 ng g^{-1}, MAR: 35 ng g^{-1} 2C+Ev+Ex+H+So	[93]
Fish: muscle	FLU, OXO	(1) H phosphate bf pH 8. Ex ACN–H_2O (16:5) (KCl, KOH, NaOH) (2) Dry Na_2SO_4. Ev. Add ACN–H_2O (KCl, KOH)	HPTLC–Fl (UV) Toluene–EtAc–formic ac	nr	LOD: UV 8–9 ng, Fl: 0.2 ng C+Ev+Ex+H+So	[94]
Chicken, fish, pig, beef	OXO	(1) H MeOH–H_2O (3:1) (Tris bf pH 8). In (55 °C) (2) Fish: Add HAc. Ev. Add aq. H_3PO_4. SPE (C_{18}, ACN–TEA). Ev. Add aq. H_3PO_4	C_{18} LC–Fl ACN–THF–H_2O (H_3PO_4), gradient	57–60	LOD: 1–2 ng g^{-1} C+H+In+So Fish: C+2Ev+H+In+So+SPE	[81]
Fish: muscle	PIR	(1) Ex aq. NaOH (2) Add aq. $ClCH_2COOH$ to pH 3. Ex $CHCl_3$. rEv. Add mph	C_{18} LC–Fl ACN–H_2O (oxalic ac) Post-column: NaOH	77–81	LOD: 5.9 ng g^{-1} 3C+4Ex+rEv	[104]
Fish: muscle	CIN, NAL, NALOH, OXO	(1) Ex aq. NaOH (2) Add aq. $ClCH_2COOH$ to pH 3. Ex $CHCl_3$. Dry Na_2SO_4. rEv. Add mph	C_{18} LC–Fl ACN–H_2O (oxalic ac)	CIN: 74–93 NAL: 70–79 NALOH: 61–97 OXO: 69–99	LOD: nr 2C+4Ex+rEv+So	[113]
Chicken: muscle, eggs	CIP, ENR, FLU, OXO	(1) (a) Eggs: Ex aq. NaOH–PBS (b) Chicken muscle: Ex PBS (2) Di. OLPC (XAD-4)	C_{18} LC–Fl ACN–H_2O (phosphate bf), gradient	75–85	LOD: 2.5–3.5 ng g^{-1} Eggs: Di+Ex+OLPC Chicken: C+Di+Ex+OLPC	[112]
Pig: muscle	CIP, DAN, DIF, ENR, MAR, SAR	(1) Ex phosphate bf pH 7.4 (2) SPE (C_{18}, ACN (CF_3COOH)). Ev. Add ACN–H_2O	C_{18} LC–APCI-MS ACN–H_2O (NH_4Ac bf, formic bf), gradient	CIP 105 ENR 98	LOD: 2 ng g^{-1} C+Ev+Ex+SPE	[110]
Chicken: muscle	CIP, DAN, DIF, ENR, FLU, MAR, NAL, NOR, OXO, SAR	As Ref. [110]	C_{18} LC–APCI-MS–MS ACN–H_2O (formic bf), gradient	80–100	LOD: 1 ng g^{-1} C+Ev+Ex+F+SPE	[111]
Oyster	OXO	(1) Ex phosphate bf pH 7 (2) SPE (C_{18}, MeOH–H_3PO_4). Ev. Add mph	C_{18} LC–UV ACN–H_2O (phosphate bf)	86–91	LOD: 10 ng g^{-1} 4C+Ev+3Ex+SPE	[109]
Fish: liver, muscle	OXO	(1) Ex phosphate bf pH 7 (or pH 4.5 for liver) (2) SPE (C_{18}, MeOH–H_3PO_4). rEv	ISRP LC–UV ACN–H_2O (phosphate bf)	84–88	LOD: 10 ng g^{-1} 4C+3Ex+3F+rEv+3So+SPE	[108]
Fish: liver	FLU, OXO	(1) Add hex. Ex phosphate bf pH 10 (2) Di. OLPC (PSDVB, mph)	PLRP–S LC–Fl ACN–THF–H_2O (H_3PO_4)	FLU: 83 OXO: 84	LOD: 4–7 ng g^{-1} C+Di+Ex+So+OLPC	[106]
Fish: liver, muscle, skin	FLU	(1) Add hex. Ex phosphate bf pH 10 (2) Add H_3PO_4. Ex DCM. W McIlvaine bf pH 3.6 Dry Na_2SO_4. rEv. Add aq. NaOH	C_{18} LC–Fl ACN–THF–H_2O (phosphate bf)	FLU: 73–86 OXO: 76–81	LOD: 2–5 ng g^{-1} 2C+3Ex+rEv+So	[105]
Fish: muscle	FLU, OXO	(1) Add hex. Ex phosphate bf pH 9 (2) (a) OLPC (PSDVB, mph) (b) Di. OLPC (PSDVB, mph)	PLRP–S LC–Fl (UV) ACN–THF–H_2O (H_3PO_4)	(a) 85 (b) nr	LOD: 2–3 ng g^{-1} (a) 2C+Ex+OLPC+So (b) 2C+Di+Ex+OLPC+So	[107]

Acronyms: ac, acid; aq, aqueous; bf, buffer; C, centrifugation; DCIP, des-ethylene-ciprofloxacin; Di, dialysis; Ev, evaporation; Ex, extraction; F, filtration; Fl, fluorescence; H, homogenisation; hex, hexane; In, incubation; ISRP, internal surface reversed-phase; mph, mobile phase; NALOH, 7-hydroxymethyl-nalidixic acid; nr, not reported; OLPC, on-line pre-concentration; org, organic phase; PBS, phosphate buffer saline; Ph, phenyl; rEv, rotaevaporation; SLS, sodium laurylsulfate; So, sonication; Tris, tris-hydroxymethylaminomethane; W, wash.

[a] Recovery range covers all the analytes and samples.

Table 6
Milk samples

Analyte	Sample treatment	Determination technique	Recovery (%)	Comments	Ref.
CIP, DIF, ENR, SAR	Add Na_2SO_4. Ex EtOH (HAc). Add aq. HAc to supernatant. Fr. SPE (SCX, MeOH–NH_3). Ev. Add HAc	Ph LC–Fl ACN–H_2O (HAc)	CIP: 80 DIF: 85 ENR: 90 SAR: 74	LOD: 0.3–1.2 ng ml^{-1} 3C+Ev+2Ex+Fr+So+SPE	[114]
CIP, ENR, SAR	Add ACN–TCA–H_2O (3:2:5). Add HAc bf to supernatant. SPE (C_{18}, mph). Ev ACN. Add mph	C_8 LC–Fl ACN–H_2O (oxalic bf, TEA), gradient	CIP: 68–86 ENR: 72–78 SAR: 76	LOD: 1–1.5 ng g^{-1} C+Ev+F+H+SPE	[115]
ENR	Add ACN–H_2O (5:1) (NaOH). W Et_2O-hex. Add MeOH–H_2O (H_3PO_4)	PLRP–S LC–Fl ACN–MeOH–H_2O (H_3PO_4, heptanesulfonate)	86–87	LOD: 3 ng ml^{-1} 2C+Ex+H	[92]
CIP, ENO, ENR, LOM, NOR, PIP, SAR	Add ACN–H_2O (5:1) (NaOH). W Et_2O-hex. Add MeOH–H_2O (H_3PO_4). rEv. Add mph	Ph LC–ESI-MS (MS–MS, quasi MS–MS–MS) ACN–H_2O (formic bf), gradient	65–86[a]	LOD: 0.2–2 ng ml^{-1} 2C+Ex+H+rEv	[53]
CIP, ENR	Add ACN–H_2O (1:1) (NaOH). UF	Ph LC–DAD ACN–MeOH–H_2O (phosphate bf, TEA, SLS)	CIP: 92–105 ENR: 92–107	LOD: 5 ng ml^{-1} H+UF	[116]
CIP, DIF, ENR, SAR	C. Fr. Remove fat layer. OLPC (IAC, HAc)	Ph LC–Fl ACN–H_2O (HAc)	CIP: 83–86 DIF: 77–88 ENR: 85–91 SAR: 72–79	LOD: 0.25–0.83 ng g^{-1} C+Fr+OLPC	[117]
NAL	Add aq. $ZnSO_4$–$K_4[Fe(CN)_6]$	Phosphorescence	97–102	LOD: 20 ng ml^{-1} F	[118]
ENR	Add aq. oxalic acid	ELISA	nr	LOD: 1.56 ng g^{-1}	[119]
ENR	–	BIA	–	LOD: 2.4 ng g^{-1}	[17]
CIP, ENR	–	BIA	–	LOD: 2 ng g^{-1}	[120]
CIP, ENR	–	BIA	–	LOD: 1.5 ng g^{-1}	[121]
ENR	nr	ELISA Immunochromatography	–	ELISA: 2–50 ng g^{-1} Immunochromatography: 10–300 ng g^{-1}	[122]

Acronyms: ac, acid; aq, aqueous; BIA, biospecific interaction analysis; bf, buffer; C, centrifugation; Ev, evaporation; Ex, extraction; F, filtration; Fl, fluorescence; Fr, freeze; H, homogenisation; hex, hexane; mph, mobile phase; nr, not reported; OLPC, on-line preconcentration; Ph, phenyl; rEv, rotaevaporation; SLS, sodium laurylsulfate; So, sonication; UF, ultrafiltration; W, wash.

[a] Recovery range covers all the analytes and samples.

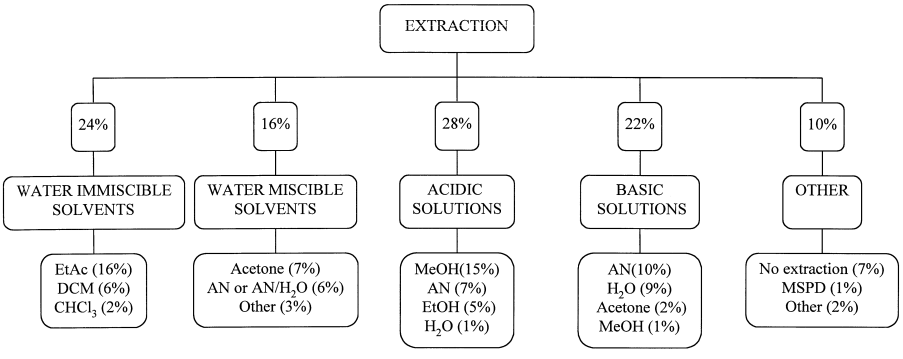


Fig. 4. Extraction step. Schematic summary of the solvent used.

volves complex and time-consuming treatments and large organic solvent volumes. In contrast, extraction methods using pH 7.4 buffer and DCM are simpler and faster and, although they lead to lower recoveries, they seem suitable for routine analysis.

2.1.2. Water-miscible organic solvents

Acetone, ACN, MeOH and ACN–tetrahydrofuran (THF) mixtures are the solvents proposed. As in the case of EtAc, extraction methods using acetone have been applied to AQ, and ACN has also been used in the analysis of SAR, DAN and ENR and CIP. However, the efficiency with which ENR and CIP are extracted with ACN is lower than that achieved when acetic acid (HAc) is added to the organic solvent. Moreover, for the extraction of AQ, methods based on ACN led to lower recoveries than acetone.

The volumes of organic solvent used are often higher than 50 ml (consumed in one or more extraction steps) and in a few cases, around 1 ml. In most cases, extraction is performed by mechanical shaking, although manual shaking has also been used.

2.1.3. Acidic solutions

The most frequent extracting agents are MeOH–water and ACN–water mixtures containing HCl, trichloroacetic acid (TCA), HPO_3, $HClO_4$–H_3PO_4 mixtures or McIlvaine buffer at pH 3.6–4.0. EtOH or ACN acidified with HAc, and MeOH containing acetic, oxalic or hydrochloric acids have also been proposed. Although there is no chemical justification, methods using acidified MeOH or MeOH–water mixtures are used almost exclusively for the extraction of AQ, whereas EtOH is used mainly in connection with PQ. This may be due to the fact that a method based on EtOH–HAc has been accepted by the EU as a routine method for ENR and CIP [79].

In most cases, extraction is carried out by manual shaking. In a few cases, sonication or mechanical shaking are applied. Incubation at about 50 °C is sometimes used. In about half the methods two extractions are carried out, while in the rest only one equilibration step is performed. Volumes of extractant vary from 5 to 100 ml.

2.1.4. Basic or neutral buffered solutions

Extraction is usually carried out with hydro-organic mixtures containing ACN and NaOH or NH_3. Basic solutions containing acetone or MeOH are also sometimes used. In the remaining papers, extraction is performed with aqueous solutions, usually phosphate buffer or NaOH. The most basic solutions are used almost exclusively for AQ, whereas PQ are preferably extracted at pH close to 7. In some cases, a defatting process with hexane or Et_2O/hexane mixtures is simultaneously conducted with the extraction step.

Extraction equilibrium is usually reached by manual shaking, but sonication is also reported. The amounts of sample processed range from 0.5 to 10 g, and the volumes of the extracting solutions vary from 1 to 50 ml.

2.2. Clean-up

Because of the complex nature of the sample matrix, a clean-up step is required prior to the chromatographic determination. Most of the treatments described consist of liquid–liquid extraction (LLE) and/or solid-phase extraction (SPE). However, clean-up procedures vary widely and do not necessarily depend on either the sample matrix or the solvent used in the previous extraction step. Whereas some authors report extensive clean-up treatment, which includes several LLE steps or combinations of LLE with SPE, dialysis or ultrafiltration (UF), about 21% of the methods reviewed involve little or no clean-up, even when tissue samples are analysed.

LLE is the preferred clean-up technique after extraction of quinolones with pure organic solvents. In some cases, extracts from sample matrix were simply defatted with a non-polar solvent. This approach is often applied after extraction of AQ with EtAc or DCM and therefore evaporation to dryness followed by the addition of an aqueous buffered phase is necessary before the defatting step. PQ-containing samples are seldom defatted with hexane–ether mixtures.

About 26% of the procedures include clean-up by LLE in which the analytes are transferred from one phase to another by controlling the pH of the aqueous phase. Chloroform is the solvent preferred

for liquid–liquid partitioning, but DCM or EtAc are also used. NaCl is sometimes added to increase the efficiency of the extraction of quinolones into the organic phase. Partitioning into these solvents is often combined with defatting with hexane or ether and in a few cases with an additional SPE using alumina, silica or ciano-cartridges. These procedures are used before LC separation and also in methods based on GC, which also include an additional clean-up step after derivatization.

Clean-up procedures based on SPE are mainly applied after lixiviation of quinolones with polar solvents. In some cases, defatting with hexane is performed prior to SPE. Reversed phase cartridges, mainly C_{18} and in some cases C_8, C_2 or polystyrene–divinylbenzene (PSDVB), are commonly used and have been applied to both AQ and PQ. Since these non-polar sorbents retain the uncharged quinolones when dissolved in a polar solvent, sample is added to the cartridge from water or hydroorganic solutions at acidic or neutral pH. Cartridges are usually washed with water, aqueous acidic solutions, ACN–water or MeOH–water mixtures with a low content of organic solvent or even hexane. Acidic or basic MeOH–water mixtures (both containing more than 75% MeOH) or pure MeOH are the most common eluent. Acidic and basic solutions of ACN have also been reported but in few cases. Matrix-solid-phase dispersion on a C_{18} phase, which combines extraction and clean-up in one step has also been proposed for the analysis of OXO in fish muscle.

Alternatively, cation-exchange cartridges are used when analysing PQ, which may be found as cationic species. In this case, the cartridge is washed with pure organic solvents and analytes were eluted with organic-rich MeOH–NH_3 mixtures. The use of anionic exchange cartridges has been reported in only one method for OXO, NAL and FLU. Recently, immunoaffinity columns (IAC) coupled on-line with the LC system have been used for clean-up in the analysis of several PQ.

In contrast, SPE was reported in a few cases after lixiviation of analytes with solvents of medium polarity. Polar cartridges, such as amino, are used and elution has been achieved using hydroorganic oxalic acid buffers.

A commercially available automated sequential trace enrichment of dialysate (ASTED) system, which combines on-line dialysis with concentration on a polymeric pre-column and LC, has also been used for clean-up of aqueous extracts from several samples. However, ASTED is only a part of the overall clean-up treatment, which also includes several partitioning steps, which are carried out off-line prior to loading onto the ASTED system.

A schematic summary of the clean-up strategies is shown in Fig. 5.

2.3. Determination techniques

Most methods (84%) are based on a LC separation, whereas GC methods account for only 4% of the determination techniques reviewed. Few methods are based on HPTLC. Although capillary electrophoresis has proved to be effective for the separation of quinolones, no application to residue analysis has been found.

Some analytical techniques, such as luminescence

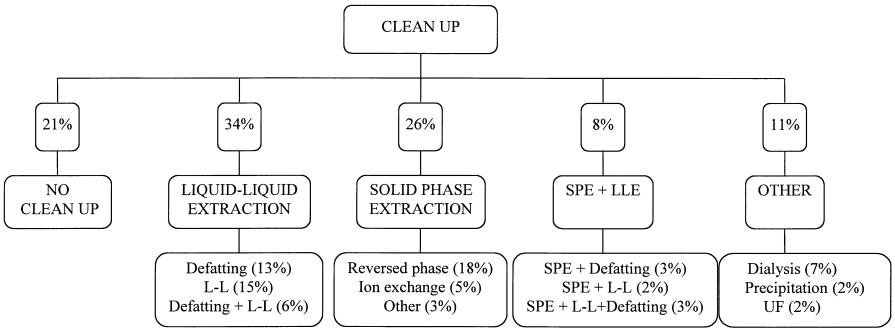


Fig. 5. Clean-up step. Schematic summary for the techniques used.

or immunochemical methods, are used without previous chromatographic separation.

2.3.1. Liquid chromatography

Separation is usually performed with silica-based reversed-phased columns, mainly C_{18} or C_8 but in some cases phenyl or amides. Because of the residual silanol groups and metal impurities in column-packing materials, conventional reversed-phase columns lead to severely tailing peaks. Therefore, most methods used endcapped columns or even high purity silica columns, such as Inertsil, Kromasil, Puresil, Versapack, Wakosil, L-column, LUNA or Zorbax RX which are free of trace metals responsible for the strengthening of the acidic properties of silanol groups.

Separation using PSDVB polymeric analytical columns is another approach to avoid peak tailing. However, these columns are less efficient than silica-based ones. They are applied mainly to the analysis of FLU and OXO but in some cases to that of ENR, CIP and SAR. Since the piperazinyl group is positioned perpendicularly to the quinolone ring and the phenyl groups in the stationary phase face each other, there is steric hindrance between the stationary phase and piperazinyl group and therefore PQ are hardly retained in these columns [123]. Ion exchange chromatography is also used in the determination of NAL.

Mobile phases consist mainly of ACN–water mixtures, but ternary mixtures of ACN–MeOH–water, ACN–THF–water or ACN–dimethylformamide–water are also used. ACN–MeOH–THF–water mixtures are also reported. Few papers describe mobile phases that do not contain ACN and these consist of MeOH–water mixtures, and MeOH percentage is always over 30%.

ACN–water and ACN–MeOH–water mixtures are used in about 60 and 12% of the reviewed LC methods, respectively. They are usually applied in the isocratic mode for both AQ and PQ. ACN content ranges from 10 to 20% for PQ and from 25 and 50% for AQ. Gradient elution is also used for the analysis of a single quinolone and for mixtures of several quinolones with large differences in polarity. In the former, the increase in the proportion of organic solvent seems to be used only as a cleaning step between samples.

Mobile phases containing ACN and THF are applied almost exclusively to the determination of AQ, especially when a polymeric column is used. THF content is about 15% and ACN about 20%. Some of them also contain MeOH.

Several tail-reducing agents are added to these mobile phases to improve peak shape. The pH was kept in the range 2–4 in order to reduce silanol ionisation and minimise its interaction with quinolones, which are present as cationic species. Although phosphate buffer solutions are usually added to adjust the pH, other solutions, such as citric or oxalic acid are also used. Other selected additives include anionic species, such as sodium alkylsulfates or alkylsulfonates, which form ion pairs with the protonated analytes, and triethylamine (TEA) or quaternary ammonium salts, which compete with the analytes for the active residual silanol groups. Generally, no additive is used with mobile phases containing THF, since THF itself reduces peak tailing [64].

Since mass spectrometry (MS) is incompatible with most of the mobile phases, volatile additives must be used when LC is coupled to MS. Thus, ammonium acetate or formic, acetic and trifluoroacetic acid are added to ACN–water mixtures.

Separation is mostly carried out at room temperature, although it is sometimes done at 30–50 °C. This may reduce mobile phase viscosity and decrease backpressure. In some cases, separation is achieved at 14 °C, although no justification is given.

Several spectroscopic techniques, such as ultraviolet–visible (UV) absorption, fluorescence or MS are used for detection in LC. Earlier methods used UV almost exclusively, but more recent systems use fluorimetric detection, which is more sensitive and selective. Quinolones show two absorption bands: a broad one (300–350 nm), which is the same for all quinolones, and a second centred at 245–290 nm, which is specific to each quinolone. The latter band shows higher absorptivity than the former and is therefore usually selected. However, in some cases, detection is carried out at 325–330 nm, which reduces interference and baseline noise.

About 62% of the LC determinations use fluorimetric detection. The emission spectrum consists of a wide band centred at 350–400 nm for AQ and 440–500 nm for PQ. Unlike UV absorption,

fluorescence depends strongly on the pH of the medium. Thus, the anionic species do not generally show native fluorescence, whereas the highest fluorescence is obtained at low pH (from 2.5 to 4.5). At these pH values, neutral and cationic species prevail for AQ and PQ, respectively. Since separation is usually performed at pH from 2 to 4, the pH of the mobile phase is optimal for fluorimetric detection. For AQ, excitation and emission wavelengths are set around 325 and 360 nm, respectively, although in some cases excitation is made at the wavelength of the first band of the spectrum. For the PQ, they are set at 275–280 nm and 440–450 nm, respectively. Because of its poor native fluorescence, PIR and MAR have almost exclusively been determined with UV detection.

Wavelength programming, which allows the detection of each analyte at its own wavelength, is a good alternative for the determination of analytes with large differences in their spectroscopic characteristics. It has been applied to the UV detection of OXO, NAL and PIR mixtures. Programming wavelength is also applied to the fluorimetric detection of PQ or mixtures of AQ and PQ in several matrices. Determination of fluorescent and non-fluorescent quinolones in one chromatographic run is achieved using UV and fluorescence detectors in tandem.

Diode array detectors (DAD), which provide spectral information for confirmatory purposes have been used for the detection of PQ. The analytes are monitored in wavelength ranges covering the two characteristic absorption bands of quinolones. Fast-scanning fluorescence detectors, which provide for excitation or emission spectra along the chromatogram, might be a suitable alternative to DAD but we have found no reference to its use for quinolones.

Since it is highly specific, MS is normally used for confirmatory analysis. Fragmentation pathways involving distinct functional groups are preferred because they provide higher selectivity [100]. Several interfaces have been used for LC–MS (or LC–MS/MS), always in the positive mode. Thermospray was initially used to analyse AQ in fish but, since it is a fairly gentle ionisation technique, only peaks corresponding to $[MH]^+$ are obtained. No further references have been found.

Atmospherical pressure chemical ionisation (APCI) has been used for both LC–MS and LC–MS/MS with in source collision-induced dissociation (CID). For PQ, only the peaks corresponding to $[MH]^+$ and $[MH–H_2O]^+$ and their isotopes were observed at low CID voltage. For AQ, the mass spectrum obtained at low cone voltage consists of $[MH]^+$, $[M+Na]^+$ and $[MH–CO_2]^+$, and at higher cone voltages, a peak corresponding to $[MH–H_2O]^+$ also appears. APCI-MS/MS was used to determine product ions from the $[MH]^+$ peak following CID in the gas cell. Loss of H_2O was the first transition observed followed by loss of $CH_2{=}CH_2$. However, the second transition depended highly on the analyte and no conclusion can be drawn.

Ionspray (ISP) interface has been applied to the LC–MS/MS determination of DAN and SAR. Two approaches have been proposed to reduce the amount of eluent in the interface: the use of microbore columns and the use of a splitter. In the CID daughter spectrum, both SAR and DAN show peaks corresponding to $[MH]^+$, $[MH–H_2O]^+$ and another peak corresponding to losses in the piperazinyl substituent. In both cases, the predominant fragment is loss of water.

Several studies describe the use of electrospray ionisation (ESI) interface. Volmer et al. studied the separation and fragmentation of 15 quinolones (10 PQ and five AQ). As reported with other ionisation techniques in LC–MS, mainly $[MH]^+$ is obtained at low voltage, but $[MH–H_2O]^+$ and $[MH–CO_2]^+$ are obtained at higher voltage. Similar sensitivities were obtained for all the PQ, whereas lower responses were obtained for the AQ. The use of LC–MS/MS led to two major fragmentation pathways after CID of the $[MH]^+$ for PQ: (a) loss of water followed by loss of the N1-substituent (ethyl or cyclopropyl); (b) loss of CO_2 followed by fragmentation of the piperazinyl ring (C_2H_5N, C_3H_7N, C_4H_9N). AQ show less fragmentation because they do not possess any substituent at C7. Moreover, they do not even show the fragment $[MH–CO_2]^+$. LC-quasi-MS/MS/MS was performed to obtain an additional degree of specificity. Here, in-source CID was used as the first quasi-MS/MS stage to generate first-generation fragments from the $[MH]^+$ ion. In the second MS/MS step, the ions of interest were isolated and subjected to CID in the quadrupole to yield second-generation product ions. Since fragmentation of $[MH–CO_2]^+$ provides mass spectra that are virtually identical to those of MS/MS, $[MH–H_2O]^+$ was chosen as an intermediate ion in order to improve selectivity.

Similar fragmentations were observed by Turnipseed et al. in the analysis of ENR, CIP, SAR and DIF in catfish muscle. On the other hand, some authors do not report mass losses but the formation of $[M+Na]^+$ adducts for some AQ even after in-source CID [18]. The authors explain this behaviour in terms of the relatively weak basicity of these compounds [18].

Signal monitoring in LC–MS is usually performed in the selective ion monitoring (SIM) mode. In some cases, only $[MH]^+$ was monitored, whereas other authors report the use of three or four diagnostic ions. In the case of MS/MS, both constant neutral loss (CNL) and multiple reaction monitoring (MRM) were used. Whereas a CNL of 18 Da was proposed for some authors as suitable for all the quinolones, others found it unspecific and proposed a mass loss of 87 Da, which is common to all the non-*N*-substituted PQ. Although MRM is about 10-fold more sensitive than SIM [51], the latter is preferred because it shows better precision [53].

2.3.2. Gas chromatography

Since quinolones are quite polar compounds, volatile derivatives must be obtained prior to GC analysis, and therefore few papers describe GC methods for these compounds. All the GC methods proposed have been applied to the analysis of AQ and reduction with $NaBH_4$ is the derivatization method of choice, since esterification lead to too polar compounds [28].

All authors agreed on the use of a DB-5 column for separation and a temperature gradient from 100 to 270 °C. In all cases, detection was carried out by MS in the positive ion mode and signal monitoring was performed in the SIM mode. GC–MS was used as a confirmatory tool after determination by LC-Fl, as well as for quantification of analytes.

2.3.3. Thin layer chromatography

Only three studies describe methods based on this technique and all use silica gel 60 HPTLC plates. Whereas two of the reported methods are devoted to identification, the other allows quantitative analysis of FLU and OXO. Medium to low polarity organic solvents containing formic or acetic acid have been proposed as mobile phases for AQ, and MeOH–NH_3 for the simultaneous determination of AQ and PQ. At basic pH, both AQ and PQ prevail as anionic species, which strongly interact with silica and therefore a mobile phase with great eluotropic strength is needed. In contrast, at low pH, AQ prevail as neutral molecules, which are less polar than anionic species and can be eluted with a low-polarity mobile phase. Several detection systems have been used, such as UV-absorption, native fluorescence, indirect fluorescence and terbium sensitised luminescence.

2.3.4. Non-chromatographic methods

Few non-chromatographic methods for quinolone residue are available and they are based on immunoassay and luminescence techniques. Although direct measurement of native fluorescence usually lacks the required selectivity for complex mixtures, it has been applied to the determination of individual quinolones, such as FLU, NAL and ENR. A method based on terbium-sensitised luminescence has been established for the determination of the sum of ENR and its metabolite CIP. Recently, the application of a sensor based on room temperature phosphorescence has been reported for the determination of NAL in milk.

Immunochemical methods based on ELISA and on optical immunosensors have also been applied for the determination of ENR and/or CIP in several matrices. These methods have shown great potential as a screening tool, although most only allow semi-quantitative analysis of the sum of ENR and CIP.

2.4. Method validation

The degree of validation of the methods reviewed varies widely, whereas some information on accuracy, precision and detection limits is usually included, only three of these methods have been validated through interlaboratory studies. In the first, which determines NAL in chicken tissues [32], the seven participating laboratories analysed 10 samples each. This procedure has been an official method of the AOAC (970.84) since 1974. In the second case, five laboratories, analysing six samples each, validated a method for OXO in salmon muscle [20]. The third method is a peer-verified method of the AOAC for FLU, NAL, OXO and PIR in catfish [49]. In this case, 16 samples of incurred or spiked fish were analysed by the submitting laboratory and the peer laboratory.

We must also mention a method for CIP and ENR

determination in pig muscle, bacon and bovine muscle, which is included in a book about residue analysis published by the Commission of the EU [79]. The methods in this book are routinely used in the member states and meet the criteria for routine methods given in the Commission Decision 93/256/EEC. However, currently they are not classified as a Community Reference Method.

2.4.1. Recovery

Recovery studies are essential to evaluate the accuracy of analytical methodologies. The best approach for determining recoveries is the analysis of a reference material for the matrices and analytes concerned. Although a reference material for OXO and FLU in salmon muscle is now being prepared, to our knowledge, no reference material for quinolones in edible animal tissues is currently available. Therefore, recoveries are estimated by means of surrogates that are assumed to match the behaviour of the native analyte in the extraction procedure. However, there is a risk that spiked samples lead to an overestimation of the extraction efficiency.

Although 90% of the papers reviewed reported the use of spiked samples, in most of them, information about spiking procedures and spiking levels is either too brief or non-existent. Whereas the spiking levels were usually given, only one-third described the spiking process. The papers that do not report the use of spikes include pharmacokinetic studies and those that develop methodology focused on the measurement step, mainly by LC–MS, without dealing with sample treatment.

Spiking is usually performed in each portion of the sample to be analysed and only in a few cases is an aliquot of a sample previously spiked analysed. The quinolones are usually dissolved in the solvent of the working standard solutions (mainly aqueous solutions or ACN, EtOH or MeOH hydroorganic mixtures at several pH values). However, in some cases, the solvent used in the spiking process was the same or almost the same as the extractant or even spikes are simply added to the sample in contact with the extracting solvent. The latter approaches are inadvisable, since the spike may not interact with the matrix.

Typical spiking volumes range from 10 to 500 μl. It must be taken into account that if the spiking solvent is not eliminated, its presence could modify the subsequent extraction. However, if a low volume (compared with that of the extractant) is used, no significant effect would be expected.

Few papers reported information about the equilibration step, which is carried out by leaving the sample to stand for a time ranging from several minutes to overnight. In the latter case, agitation of the sample in contact with the spike for 30 min at room temperature is usually performed prior to leaving the sample overnight at 4 °C. Some papers do not report any equilibration step and it is assumed to be zero because spiking is carried out over the sample which is in contact with the extracting solvent.

Some studies about the effect of storage of spiked samples on recoveries are reported. OXO has been found to be stable in spiked crops and oyster stored at −20 °C over several months [65,109]. Similar results have also been reported for FLU in sheep [29] and fish [107] tissues even after 24 h at room temperature [107]. OXO and FLU are stable in salmon muscle stored at −20 °C at least for a week [46]. However, when it is stored at 4 °C, recovery decreases after only 24 h [46].

The spiking levels range from 1 ng g^{-1} to 50 μg g^{-1}, although in about one-half of the cases spiking levels are below 200 ng g^{-1}. In a few cases, they are over 500 ng g^{-1}, which is inappropriate for residue analysis. The most advisable option of spiking at two or more levels has been often reported and no differences in extraction recoveries were observed at different levels.

Because of the great variety of extraction and clean-up procedures, it is not easy to draw conclusions about recoveries. In general, recoveries are over 60% for most of the analytes and matrices. However, in some PQ multiresidue procedures they fall below 40%.

2.4.2. Detection limits

Almost all the published methods report either the limit of detection (LOD) or the limit of quantification (LOQ). However, these limits are not calculated in the same way and often authors do not report how LOD was determined. According to the EU Commission Decision 93/256/EEC, LOD must be calculated as the concentration corresponding to three times the

peak-to-peak noise. This approach is followed by many authors but more conservative options such as using four or five times the standard deviation of the blanks are also used.

Some authors report LOQ (referred as the limit of determination in the aforementioned EU Decision) instead of LOD. It usually corresponds to the lower limit of the dynamic range.

For most of the proposed methods, LODs are in the low ng g^{-1} range (10 ng g^{-1} or lower), which are suitable for residue analysis. In some LC–UV methods, the LODs are around 100 ng g^{-1}, whereas the highest are obtained using HPTLC (in the μg g^{-1} range).

2.4.3. Calibration

Although calibration is a fundamental step in any analytical method, in some of the methods reviewed, information about the calibration technique is not included or not available. These methods are reported mainly in papers dealing with pharmacokinetic studies or abstracts and they probably use external standards.

Calibration is usually carried out by means of external standards, which is the most common approach in LC-based methodologies. Standards usually consist of solutions of pure analytes in the mobile phase or in an appropriate solvent, which are injected into the chromatographic system. Since in most cases quinolone-free matrices are available, some authors use matrix matched standards (MMS). The solutions coming from standards are similar to those coming from samples and, therefore any influence of matrix on the response would be the same for both. Moreover, if the analyte added to the quinolone-free matrices (standards) behaves in the same way as the incurred, this approach allows for the correction of any loss of analyte.

Internal standards (IS) are also used for calibration in some of the methods reviewed. The IS or surrogate, which is always another quinolone, is added to the samples at the beginning of the sample treatment in order to compensate for any uncontrolled effect. However, because sample treatments are in general quite complicated, in a half of methods that use an IS as a surrogate, MMS is also applied. No references to the use of the standard additions method were found.

3. Conclusions

Many papers have been devoted to the analysis of quinolone residues in animal products over the last 10 years. Although most methods have been designed for the analysis of individual quinolones or for only two or three compounds, an increase in the number of multiresidue methods, which offer great advantages for monitoring purposes, has recently been observed.

Since quinolones are polar compounds and most of them are fluorescent, reversed-phase liquid chromatography with fluorimetric detection is the determination technique mainly used for routine residue analysis. However, several methods dealing with LC–MS have recently been reported for confirmatory analysis.

In contrast, sample treatment varies greatly among the methods proposed, often irrespective of the sample matrix and the analyte. Moreover few authors have reported optimisation of both the extraction and the clean-up steps and, in most of the reviewed papers, there is a lack of information concerning the criteria used for the selection of the experimental conditions.

Many of the methods used to monitor these residues are time consuming and solvent intensive. Therefore, analytical methods that can increase sample throughput and reduce environmental impact are required. Examples of such preparation techniques could be automated on-line SPE, solid-phase microextraction, on-line dialysis and immunoaffinity extraction.

There are also few data about method validation. The lack of reference materials with certified values for quinolones is an added difficulty for method validation, and thus the preparation of this material should be a priority.

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