



FLUOROQUINOLONE RESIDUES IN FOOD: OCCURRENCE AND ANALYTICAL METHODS - A MINI REVIEW FROM 2000 TO 2022

(Sisa Fluorokuinolon di dalam Makanan: Kemunculan dan Kaedah Analisis – Satu Ulasan Mini Tempoh 2000 hingga 2022)

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Abstract

Fluoroquinolones are used to treat and prevent serious bacterial infections. They are widely applied in the husbandry and veterinary areas as well as in the human medication scope. There is an emerging concern about the residues of fluoroquinolones in the environment. Once in the environment, especially in the water bodies, they will be taken up and accumulated in our livestock, which subsequently enters our body through our food resources. Therefore, the residues of fluoroquinolones in animal-based food are required to be monitored and assessed. The development of existing methods is crucial to provide accurate data on the fluoroquinolone residues in different food samples. This review presents an overview of the proposed methods from 2000 to 2022 for the analysis of fluoroquinolones in food samples. The extraction techniques were mostly combined with liquid chromatography and fluorescent detection. These methods allow for low detection limits and high recovery values. The ideal detection limit is less than $10 \mu\text{g L}^{-1}$. The main drawback of the screening test is low sensitivity and it may provide false positive results. A quantitative analytical method is important in giving the most accurate result from the determination of fluoroquinolones residues in different food matrices. One of the important factors to allow a low detection limit is the extraction method used. The solid phase extraction methods are the most widely used method for sample preparation. In addition, the physical and chemical properties, classification, toxicology, side effect of fluoroquinolones and occurrence of these residues in food samples were discussed in this paper.

Keywords: antibiotics, fluoroquinolones, food, extraction

Abstrak

Fluorokuinolon digunakan untuk merawat dan mencegah jangkitan bakteria yang serius. Ia digunakan secara meluas dalam bidang penternakan dan veterinar serta dalam skop perubatan manusia. Terdapat kebimbangan yang timbul tentang sisa fluorokuinolon di alam sekitar. Apabila berada di alam sekitar, terutamanya di dalam air, ia akan diambil dan terkumpul dalam ternakan kita, dan seterusnya memasuki badan kita melalui sumber makanan kita. Oleh itu, sisa fluorokuinolon dalam makanan berasaskan haiwan perlu dipantau dan dinilai. Pembangunan kaedah sedia ada adalah penting untuk menyediakan data yang tepat tentang sisa fluorokuinolon dalam sampel makanan yang berbeza. Kajian ini membentangkan gambaran keseluruhan kaedah yang dicadangkan terutamanya dari 2000 hingga 2022 untuk analisis fluorokuinolon dalam sampel makanan. Kaedah pengekstrakan yang paling

banyak bergabung dengan kromatografi cecair dan pengesanan pendarfluor. Kaedah ini membenarkan had pengesanan rendah dan nilai pemulihan yang tinggi. Had pengesanan yang ideal adalah kurang daripada $10 \mu\text{g L}^{-1}$. Kelemahan utama ujian saringan ialah kepekaan yang rendah dan ia mungkin memberikan keputusan positif palsu. Kaedah analisis kuantitatif adalah penting dalam memberikan hasil yang paling tepat daripada penentuan sisa fluorokuinolon dalam matriks makanan yang berbeza. Salah satu faktor penting untuk membenarkan had pengesanan yang rendah ialah kaedah pengekstrakan yang digunakan. Kaedah pengekstrakan fasa pepejal ialah kaedah yang paling banyak digunakan untuk penyediaan sampel. Di samping itu, sifat fizikal dan kimia, klasifikasi, toksikologi, kesan sampingan fluorokuinolon dan kejadian sisa ini dalam sampel makanan turut dibincangkan dalam kertas ini.

Kata kunci: antibiotik, fluorokuinolon, makanan, pengekstrakan

Introduction

Antibiotics have been used since old times. The first modern antibiotic, namely penicillin was discovered by Alexander Fleming in the 20th century. Penicillin has been widely applied in wartime mainly because no significant medicine is available to treat infections such as pneumonia, gonorrhoea or rheumatic fever [1]. The introduction of antibiotics has been recognised as one of the greatest inventions due to its effectiveness in treating various diseases in the form of microbial. Since penicillin was recognised in World War II, it has been manufactured in massive-scale production, making it a life-saving substance from the limited supply into a widely available medicine.

Antibiotics are antimicrobial substances that fight against bacteria. It is one of the most efficacious medicines that fight against bacteria and infection. However, virus-form diseases such as flu cannot be treated by antibiotics. Generally, the immune system in our bodies produces natural killer cells to function as bacterial killers. However, when the rapid propagation of bacteria occurs in our bodies, our immune system fails to cope with the infection. Therefore, antibiotics are needed to assist in curing infections. Antibiotics can be classified into several groups based on the type of action, sources to make antibiotics, the spectrum of activity, chemical structure and function of the antibiotics [2]. In the early stage, antibiotics are introduced from the natural plant extract while in the latter day, it has been produced chemically either in the form of fully synthetic or semi-synthetic. To date, the application of antibiotics has been widespread and extended to prevent and treat bacterial infection in the veterinary, aquaculture and domestic fields. In the United States, antibiotics are employed in livestock to speed up growth and increase

the size of animals bred for food [3]. In aquaculture enterprises, antibiotics are frequently applied to those aquafarming organisms with impairing immune systems. Some household cleaning products are fortified with synthetic antibacterial, antiseptic and antimicrobial substances especially triclosan against bacteria on the household surface [4].

The issue of antibacterial resistance detected in humans has become the biggest concern nowadays with the widespread application and improper use of antibiotics. In consequence, the bacteria may mutate and emerge into the immortal stage, making resistant bacteria survive antibiotic treatment. This will then threaten our lives, causing antibiotics to be ineffective in treating diseases. Fluoroquinolones are one of the frequently applied antibacterial in food animals. Fluoroquinolones are used to treat enteric infections and respiratory diseases. This antibiotic possesses low toxicity and it can fight a broad spectrum of pathogenic bacteria towards farmed animals [5]. Therefore, the food animals sold may retain the trace residue of fluoroquinolones and the transmission from food to humans may be biomagnified.

Physical and chemical properties of fluoroquinolones

Fluoroquinolones are a group of broad-spectrum antimicrobial drugs from the class of quinolones with the attachment of the fluorine group in the ring. It is active against Gram-negative and Gram-positive bacteria [6]. As shown in Figure 1, the position of R_1 increases the potency of the molecules where the alkyl substituent is usually attached to it. R_2 and X improve the activities of both Gram-negative and Gram-positive. F refers to the fluorine atom that increases the Gram-

negative activity and the position of R_3 increases only the Gram-positive activity [7]. Different classes of fluoroquinolones have different substituent rings attached to them and thus affect the physical and chemical properties in terms of solubility in different parts of the membrane in the body. Fluoroquinolones are effective in the inhibition of bacterial chromosome

replication and long-established in treating respiratory diseases and bacterial infections in food animals [8]. Fluoroquinolones are synthetic antibacterial agents which are not derived from living organisms [9]. Recently, there is a report on quinolone resistance due to bacteria mutation as a result of improper overuse of this antibiotic [10].

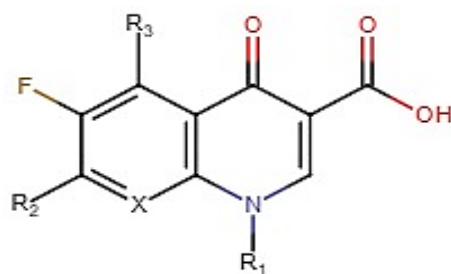


Figure 1. Molecular structure of fluoroquinolones. Ciprofloxacin denotes as $X=C-H$, R_1 =cyclo propyl, R_2 =piperazinyl, and $R_3=H$. Gatifloxacin denotes as $X=C-OMe$, R_1 =cyclo propyl, R_2 =piperazinyl, and $R_3=H$. Ofloxacin denote as X and R_1 =benzoxazine, R_2 =piperazinyl, and $R_3=H$

Generally, fluoroquinolones can be absorbed well in gastrointestinal and endocrine organs, as well as penetrate through the bones and cerebrospinal fluid. The partitioning coefficient ($\log K_{ow}$) and bioconcentration factor (BCF) values of fluoroquinolone are considered low [11]. Fluoroquinolones are slightly water soluble over a pH range from 6 to 8 which is known as amphoteric character, acting as base and acid. They can be absorbed via oral administration [12] as well as injection. For instance, ciprofloxacin is a light-yellow crystalline substance with a melting point of 255-257°C and the water solubility is 86 mg L⁻¹ at 25°C [13]. Flumequine is a white powder which is insoluble in water but soluble in organic solvents [14]. Thus, the flumequine is absorbed in the lipid membrane and it possesses strong stability. The melting point is 253-255°C.

Fluoroquinolone antibiotics

Fluoroquinolones are used to treat serious bacterial infections, especially infections in the skin, bone, joint,

urinary tract and respiratory tract. It is also widely applied in husbandry and veterinary fields to prevent the action. Fluoroquinolone is a strong antibiotic where it can easily penetrate the body cells and destruct the DNA in the cell. However, it cannot treat serious contagious infections such as colds and flu [15].

Classification of fluoroquinolones

More and more fluoroquinolones are being invented and it is hard to prescribe antibiotics. The most common synthetic fluoroquinolone antimicrobial is ciprofloxacin which is the cheapest and most frequently employed in treating animals [16]. The position of fluorine atoms substituted in each molecule seems to enhance the phototoxicity and their origin modification [17]. This enhancement provides more effective microbial activities and broadens the spectrum of activities. However, this modification also increases bacteria resistance. Table 1 shows the classification of fluoroquinolones according to their antimicrobial spectrum.

Table 1. The classification of fluoroquinolones [18]

Classification	Examples of Agents	Antimicrobial Spectrum	Indication
First generation	Nalidixic acid, Cinoxacin	Gram-negative organisms except for <i>Pseudomonas</i> species.	Uncomplicated urinary tract infections.
Second generation	Norfloxacin, Lomefloxacin, Enoxacin, Ofloxacin, Ciprofloxacin	All gram-negative organisms but some gram-positive organisms.	Uncomplicated and complicated urinary tract infections, pyelonephritis, sexually transmitted diseases, prostatitis, and skin infection.
Third generation	Lexofloxacin, Sparfloxacin, Gatifloxacin, Moxifloxacin	Expanded spectrum in gram-positive coverage, atypical pathogens.	Acute exacerbations of chronic bronchitis, and pneumonia.
Fourth generation	Trovafoxacin	Broader coverage than the third generation.	Abdominal infections, nosocomial, pneumonia, pelvic infections.

Transmission route of fluoroquinolones into human

Fluoroquinolone's main administration is via oral but some fluoroquinolones are taken intravenously [19]. The fluoroquinolones are manufactured in tablet or capsule forms. The fluoroquinolones enhance their function by inhibiting the bacteria from building the cell wall and altering the ability to the reproduction of the bacteria. When fluoroquinolones are consumed, they penetrate the membrane and are stored in the gastrointestinal tract, which is then absorbed as nutrients. After a while, they circulate in the blood and reach the target bacteria [20].

Fluoroquinolones can be transferred through the maternal plasma into breast milk. The rate of transmission depends on the maternal factor, physiochemical properties and infant factor [21]. The composition of particular breast milk and the pH affect the number of antibiotics transferred. However, fluoroquinolones are most likely to concentrate in the plasma compared to breast milk because fluoroquinolones are weak acidic drugs [22]. Hence, the

antibiotic has a lower chance to transmit to the infant. Indeed, fluoroquinolones are not recommended for breastfeeding mothers although ciprofloxacin is proven with no harm to both the breastfeeding mother and the infant [23-24].

Some of the transmission of fluoroquinolones is from food-producing animals such as livestock and poultry [25]. The by-products especially meat, dairy products and eggs may contain the residue of fluoroquinolones and thus food chain is also one of the transmission sources.

Transmission route of fluoroquinolones into animal

The fluoroquinolones used in livestock, poultry and veterinary are not only for the treatment of the illness of animals but also for illness prevention. This practice has been a part of the modern food-animal production industries. The utilization of the antibiotic as a therapy for preventing bacteria and promoting growth [26]. In general, antibiotics are added to feed for growth promotion [27]. The dosage applied for growth promo-

tion might be low, but it is a long-term application. Their accumulation of the antimicrobial is then causing drug resistance in the tissues of these animals. Fluoroquinolones are prescribed to treat gastrointestinal infections and microbiota modification in the intestine, as well as enhance the immune system [28]. The fluoroquinolones are given in form of oral, by injection, by drinking water, intra-mammary and mostly by feeding [25]. For example, enrofloxacin is injected into cattle for the treatment of bovine respiratory disease

[29].

Effects of fluoroquinolones towards living organisms

Fluoroquinolone residues have brought a lot of negative effects on humans and animals. The maximum residue limits of some fluoroquinolones from liver, muscle and kidney tissues, such as enrofloxacin, and ciprofloxacin have been established as no more than 200, 100, and 300 $\mu\text{g kg}^{-1}$, respectively by European Union [30], as summarized in Table 2.

Table 2. Maximum residue limits of fluoroquinolones by the European Union [30]

Fluoroquinolone Active Substances	Marker Residue	Animal Species	MRL ($\mu\text{g/kg}$)	Target Tissues
Enrofloxacin	Sum of enrofloxacin and ciprofloxacin	Bovine, ovine, caprine	100	Muscle
			100	Fat
			300	Liver
			200	Kidney
		Porcine, rabbit	100	Milk
			100	Muscle
			100	Fat
			200	Liver
		Poultry	300	Kidney
			100	Muscle
			100	Skin and fat
			200	Liver
		All other food producing species	300	Kidney
			100	Muscle
			100	Fat
			200	Liver
			200	Kidney

Side effects on human

The most general side effects of fluoroquinolones are diarrhoea, vomiting, nausea and dyspepsia which belong to indigestion problems. One of the serious adverse side effects is the central nervous system (CNS) effect which will lead to headaches, dizziness, convulsion and confusion. Dairy products which consist of fluoroquinolones, especially the ciprofloxacin will cause accumulation in the cartilage of bones and arthropathy which then lead to joint's disease, as well as foetal impairment [31]. According to the United States Food and Drug Administration (USFDA) (2018), fluoroquinolones increase the risk of mental health

including disturbances in attention, nervousness, disorientation, as well as memory impairment [32]. Some people get serious blood sugar disturbances such as hypoglycaemia, which is suffering from low blood sugar.

Side effects on animals

Fluoroquinolones are evenly applied in veterinary medicine, cattle management and aquaculture. The application of fluoroquinolones has resulted in diffuse retinal degeneration which then leads to blindness in animals [33]. However, it depends on the accumulation of drugs and the duration of the treatment. Some of the

side effects that occur in animals from the drug administration are similar to humans.

Antibiotics resistance of fluoroquinolones

The residues are risky to public health as the stimulation of bacterial resistance is getting worse and the bacteria may be mutated in the tissues [34]. Fluoroquinolones are broad-spectrum antibiotics which accelerate the spread and development of the multi-drug resistant strain. Overuse of antibiotics leads to the change and elimination of their effectiveness which then causes difficulty in curing the serious infection. For instance, medical advance treatments like organ transplantation, cancer chemotherapy and some major surgery need antibiotics for the prevention of infection [35]. The bacterial resistance may jeopardise these treatments. According to Institute for Medical Research (IMR), gram-negative bacilli such as *E. coli* have shown resistance to one of the fluoroquinolones, ciprofloxacin. The rise of fluoroquinolone resistance infections in Malaysia is mainly due to medical control practices and animal husbandry [36].

Restriction of fluoroquinolones in food products

To address the bacterial resistance issues, the Food and Drug Administration (FDA) has warned about the effect of overusing fluoroquinolones, that these antibiotics are only applied when there are no alternative treatment options [32]. Several restrictions have been applied to the use of fluoroquinolones by the FDA. Some of the classes of the fluoroquinolones such as Marbofloxacin which is generally applied in veterinary medicine and cattle management have been banned due to their high absorptivity and penetration into the tissue [33]. According to European Medicines Agency (EMA's) Pharmacovigilance Risk Assessment Committee (PRAC), fluoroquinolones are not recommended in treating mild infection and lower urinary infections [37]. According to the European Union (EU), fluoroquinolones as growth promoters have been banned in food processing animals because they are considered a non-essential antibiotic [36].

Poultry is the most important food industry in Malaysia as poultry including beef, chicken and pork are the main protein sources for Malaysian [38]. According to Lai

(2020), 49.4 kg of poultry meat is consumed per person in 2020. The consumption of poultry has increased by 40% from 2006 to 2019 and it is estimated to exceed 50 kg in 2025 [39]. In poultry farming, fluoroquinolones are often used for therapeutic. The most common fluoroquinolone used in poultry production is enrofloxacin (ENRO). The application of other groups of fluoroquinolones such as flumequine (FLU), danofloxacin (DANO), difloxacin (DIFLO), oxolinic acid (OXO) and sarafloxacin (SARA) has been licensed in the European Union (EU) and these medicines are only allowed for veterinary medicinal treatment but not for growth promoter [40]. The use of ENRO for the treatment of bacterial infections in poultry has been banned in 2005 according to USFDA. This was due to the presence of *Campylobacter* resistance species in chickens cured with this antimicrobial. Fluoroquinolones no longer inhibit the growth of *Campylobacter* in poultry as the fluoroquinolones-resistance *Campylobacters* have been detected in poultry faeces [41]. This indicated that the antibiotics have accumulated in the animal body and contaminated the agricultural production indirectly as the excrement may be the organic fertilizers to the fruits and vegetables [42]. It caused antibiotic pollution in the soil and affected the nutrition intake of the crops. Apart from being released to the land, the antibiotics may be accumulated and they may either leach into groundwater or be transported to the surface waters [43]. Since poultry has been improved from a small food production unit to a large-scale supply chain over these years, the spread of antimicrobials is increasing dramatically. The use of antimicrobials in food-producing animals has to be controlled and restricted under Maximum Residue Limits (MRLs).

As mentioned earlier, the antibiotics that are not fully absorbed in the animals will be excreted in faeces and this will contaminate the soil system [44]. Soil manure which consists of animal faeces, compost and green manure is great in providing fertility to the soil. It contains the main nutrients required for vegetable growth. However, the repeated fertilisation in the agriculture farming system with the soil manure has led to the accumulation of antibiotics in the soil, and in turn,

to food such as vegetables and fruits [45]. There is lacking research on the accumulation of antibiotics in ripened plants, although the antibiotics uptakes are mainly via leaves and roots [46]. The presence of antibiotics has affected plant growth and the seed germination process indirectly. Although the detection of fluoroquinolones was only 0.2 mg kg^{-1} in soil manure, the biotransformation of antibiotics from the soil to the plants could occur, leading to the promotion of antibiotic resistance to humans, animals and the environment [47]. Although the residues of antibiotics in soil (8.4%) are more than in crops (5.6%), the accumulation can be increased with large-scale production of crops and the rise of crop consumption by humans [48].

Some of the antibiotic contamination in the environment is caused by antibiotic manufacture. According to Fick et al. the concentration of quinolones was found 14 mg L^{-1} in the wastewater effluent. It may be caused by the illegal dumping of antibiotic residues and this affected the surrounding environment including the groundwater and the soil [49]. Thus, the runoff of surface water or leaching of groundwater will affect the crop indirectly, affecting the consumers' health. Moreover, the transport of the residues in the waterway may influence the aquatic organisms [50].

Antibiotic residues can be detected in other food, especially honey and baby food. Honey is a natural by-product that is mainly used for nutritional and medicinal purposes. It contains hydrogen peroxide which was used to be an antibacterial treatment in old times. The unusual antibiotic residue found in honey was mainly caused by the environment and inappropriate beekeeping practices [51]. The sugar syrup solution which is mixed with the antibiotics is introduced into bee hives and consumed by bees [52]. This leads to antibiotic contamination in honey. Research revealed that the honey consisted of antibiotics residues namely oxytetracycline and chloramphenicol that exceeded regulatory values [53]. Although the European Community regulations did not set up the MRL for antibiotics in honey, the use of antibiotics in bee farming has been banned by some countries [54].

Some the fluoroquinolones such as ciprofloxacin, levofloxacin and moxifloxacin are used to treat infection of the urinary tract, pneumonia and mycobacterium tuberculosis in paediatrics. However, fluoroquinolones are banned for paediatrics in Korea because the study showed that fluoroquinolones have lost their function gradually in treating multidrug-resistant infections [55]. Some of the meat containing baby food such as beef and chicken might contain antibiotics residue too. There are lacking regulations and legislation concerning the tolerance value of fluoroquinolones in processed food [56]. Apart from meat and vegetables, the egg is an important protein source for children. When the animals are injected with veterinary drugs, the residues remain in various tissues in the body, carrying the residues to the eggs laid directly. Moreover, antibiotics are also used in farmed fish to treat diseases by treating the water or aquaculture. This results in the spread of the residues among humans and the environment [57].

Sample preparation for the extraction of fluoroquinolones in food

Several studies are reporting the detection of fluoroquinolones in animal-based food such as milk, honey, chicken, egg, beef and yoghurt. Therefore, it is important to determine the antibiotic residues in food to guarantee food safety and ensure the consumer health policy.

Screening assay testing

Screening assay testing is commonly applied as a semi-quantitative method to identify the presence of the analytes in the sample. Screening is one of the easiest, most rapid and low-cost assay methods that provides high reproducibility and reliable results.

The presence of ciprofloxacin can be detected by β -gal colorimetric assay. This assay is commonly used in inspecting cultured cells and recognising the recombinant fusion genes. It provides a linear correlation between the bacteria inside the fluoroquinolones and β -gal activity. Ortho-nitrophenyl- β -galactoside (ONPG) increases the sensitivity of β -gal activity detection in ciprofloxacin [58]. The interaction between bacteria strains with antimicrobial detection in milk has proven its specificity without giving false

positive results. One of the greatest benefits of using the screening method is less amount of additional reagent is required. The screening method usually examines the optical density (OD) and the growth curve of the bacterial strain [59]. Although screening testing is simple and able to give a rapid response, it only detects the presence of fluoroquinolones without sufficient sensitivity [60].

Chromatographic techniques

From 2000 to 2022, liquid chromatography is still the most frequently employed quantitation instrument for the determination of fluoroquinolones. It is always coupled to sample preparation such as solid phase extraction, micro-solid phase extraction, and dispersive liquid-liquid microextraction to eliminate matrix effects and improve sensitivity.

Solid phase extraction

Solid phase extraction (SPE) is a sample extraction technique that concentrates and purifies analytes from complex sample matrix by sorption onto sorbents loaded in a cartridge device. The SPE isolates a species from a sample or cleans up a sample before analysis using an instrument. The general steps in performing SPE are washing, conditioning, loading and eluting. Compared to liquid-liquid extraction which separates analytes from the sample via partitioning between two immiscible solvents, SPE does not require large amounts of organic solvents. SPE provides a more complete extraction of the analytes and a more efficient separation of the interferences from analytes because there is a variety of sorbents and elution solvents that differ in polarity.

SPE of fluoroquinolones is an effective way for both extraction and clean-up especially after the samples undergo deproteination with an acid such as trichloroacetic acid (TCA) and trifluoroacetic acid (TFA) [61]. The use of SPE offers clean extract to reduce instrument maintenance. According to Herrera-Herrera (2009), the use of SPE with pre-treatment using acids (TCA, TFA) resulted in high recovery that was more than 75% and ultra-trace LOD that was in the range of 0.5-6.8 $\mu\text{g L}^{-1}$ [61].

The fluoroquinolones namely norfloxacin, ofloxacin, ciprofloxacin, pefloxacin, lomefloxacin, danofloxacin, enrofloxacin, sarafloxacin, difloxacin, oxolinic acid, and flumequine were separated with a reversed-phase HPLC coupled with fluorescence detection. The clean-up of the milk samples was succeeded by SPE with LODs ranging from 1-9 $\mu\text{g L}^{-1}$ except for enrofloxacin which was 23 $\mu\text{g L}^{-1}$. The residues were not exceeding MRL established by Professional Regulation Commission (PRC) and European Union Legislation.

The combination of molecularly imprinted polymers (MIPs) with the SPE cartridge demonstrated high recovery of the ciprofloxacin, enrofloxacin, norfloxacin, lomefloxacin and sarafloxacin from milk samples that were more than 96%. The LODs were ranging 10 and 20 ng mL^{-1} , indicating that MIP-based SPE offered superior purification and sensitivity to the normal SPE [62]. MIP is a synthetic polymer for targeted molecules or selectively given analyte or a group of compounds [63]. MIP is capable to withstand various conditions such as extreme sample pH values, high temperature, and high pressure and it can be reused multiple times [64]. MIP allows better sample clean-up and enhances the detection sensitivity of the analytes. There were several studies reveal that MIP-based SPE was successful as a sample clean-up tool for the isolation of tetracycline antibiotics in lobster, duck, honey and egg. The recovery was above 95% and the LODs were in the range of 0.1-0.3 $\mu\text{g kg}^{-1}$ [65]. The determination of tetracycline in milk and honey with the molecularly imprinted monolith SPE achieved the recoveries of 73.3% - 90.6% from the milk samples and 62.6-82.3% from the honey samples [66]. The MIP monolithic column was polymerised directly and saved lots of procedures like grinding and sieving. However, some studies were reporting the limitation of using MIP such as template leaching problems, low loading capacity, high consumption of organic solvents in conditioning, low affinity binding and the application being limited to polar compounds [42, 56].

The development of magnetic graphene dispersive SPE provides higher stability, larger surface area for adsorption and resistance to acid and alkali. Most of the studies have proven that magnetic graphene is efficient

in extracting pesticides, oxytetracycline in water samples and sulfonamides in milk samples [67-69]. The determination of fluoroquinolones in bovine milk, chicken muscle and egg using magnetic graphene dispersive SPE shows that the recoveries, LOQs and LODs ranged 82.4-108.5%, 0.2-0.8 ng g⁻¹, and 0.05-0.3 ng g⁻¹ respectively. The development of ionic liquid-modified Fe₃O₄ as the adsorbent in magnetic SPE for the determination of fluoroquinolones in milk and pork offered good sensitivity with LODs ranged 0.33-0.78 ng mL⁻¹ and LOQs ranged 1.09-2.60 ng mL⁻¹, where the analytes responses were enriched for 29.1-43.9 folds, succeeding the ultra-trace detection of fluoroquinolones in complicated food samples. The recovery of fluoroquinolones from milk and pork samples gained insignificant matrices ranging from 85.4-105.9% and 85.2-103.7%, respectively [70].

The detection of five fluoroquinolones namely norfloxacin, ciprofloxacin, enrofloxacin, danofloxacin and sarafloxacin in fish using ultra-performance liquid chromatography (UPLC) with a fluorescence detector (FD) demonstrated fast analysis and high-resolution selectivity. The fish samples underwent microwave-assisted extraction and clean-up with SPE. The combination of both techniques resulted in a clean extract with recoveries ranged 90-107%. The microwave-assisted extraction utilized a shorter extraction time and minimal solvent. Nevertheless, the sample required further SPE clean-up to eliminate the matrix effect from fish [71].

The development of another sample preparation technique known as QuEChERS is quick, easy, cheap, effective, rugged and safe. This method is rapid and easy to use to separate the sample matrix. Generally, this method is widely applied in the clean-up of food samples with high water content such as fruits and vegetables, especially for the determination of pesticide residues. However, this method is lacking attention to determining the residues of the antibiotics in the food samples. According to Aguilera-Luiz et al. the clean-up step is straightforward with QuEChERS by the addition of EDTA in the extractant solution. It acts as a buffer to QuEChERS and increases the recoveries of the analytes. The recovery study was performed using a spiked milk

sample and acceptable recoveries were attained in the range of 70.6-111.3%. However, the intra-day repeatability was recorded as 20% which was considered high as compared to inter-day repeatability [72]. The coupling of QuEChERS clean-up and the UPLC-MS/MS was applied for the determination of veterinary drugs in fish and shrimp samples. Enrofloxacin which is not permitted for use was found in freshwater and marine seafood as high as 7% [73]. In conclusion, the QuEChERS technique is simple, eco-friendly, low cost and fast. There is no filtration and condensation step as well as lesser solvent is consumed. It can be applied to a wide range of complex matrices especially those with high moisture content. Nevertheless, this method could be further modified and improved for better precision.

Apart from clean-up, sample pre-treatment is also required attention to remove interferences with larger particle sizes. For instance, the combination of methanol and trichloroacetic acid (TCA) can easily clear unwanted precipitation and reduce the interaction of lipids and proteins [74]. The combination of methanol with base has shown ineffective sensitivity. Ultra-filtration is needed to increase the detectability of fluoroquinolones without any other protein and lipids component [75].

Liquid-liquid extraction

Liquid-liquid extraction (LLE) is an extraction method that separates compounds according to their solubility in two different immiscible solvents. It is commonly used in the petroleum industry because of its versatility and effectiveness in reducing complex samples [76]. However, there is lacking studies on the extraction of fluoroquinolones in food samples by LLE probably due to the efficiency in extracting organic compounds from complicated matrices.

18 types of veterinary drugs including tylosin, oxolinic acid, flumequine and sulphadimidine were extracted from baby food by LLE. Acidified acetonitrile was added as sample pre-treatment steps to denature the precipitation of proteins and enzymes in the sample. The interferences were reduced before the UPLC tandem mass spectrometer analysis. The results revealed that

this sample pre-treatment step successfully offered a cleaner extract and recovered 120% of one of the fluoroquinolones, flumequine from the samples [77]. Although LLE provides high recovery, the drawbacks of this method include high solvent consumption and waste generation as compared to SPE. It also results in poorer selectivity, emulsion formation and high labour costs [78]. Hence, the SPE is far better than LLE. SPE can extract diverse organic analytes from different samples with superior selectivity compared to LLE [79].

Micro-solid phase extraction

Micro-solid phase extraction (μ -SPE) is developed for rapid sample preparation procedures with minimal solvent. It is known as a miniature SPE. According to Vakh (2018), the fluoroquinolones were extracted by automated magnetic dispersive μ -SPE where the sample solution was injected into a fluidized reactor followed by online separation based on fluidized beds using magnetic nanoparticles, Zr-Fe-C that acted as adsorbents. The LODs and LOQs were in the range of 1.5 to 3.0 $\mu\text{g L}^{-1}$ and 5 to 10 $\mu\text{g L}^{-1}$. High recovery was obtained for all of the analytes [80]. A core-shell magnetic porous organic polymer has been utilized to extract fluoroquinolone antibiotics in honey samples. The technique was integrated with HPLC-FD and achieved superior sensitivity with LODs in the range of 1.5 to 5.4 ng L^{-1} and enrichment of 84.4-91.7 for ofloxacin, ciprofloxacin and lomefloxacin, and enrofloxacin [81]. The magnetic nanoparticles offered a large ratio of surface area to sample solution volume, a safer technique with low toxic levels and cost-effectiveness [80]. Although μ -SPE applies minute amounts of solvent and indirectly enhances the sensitivity, the major drawbacks are the relative recovery is slightly lower and the insignificant reproducibility problem [42]. Apart from these, μ -SPE is also lacking commercially available stationary phases [82].

Dispersive liquid-liquid microextraction

Dispersive liquid-liquid microextraction (DLLME) was introduced by Assadi et al. to isolate polyaromatic hydrocarbons (PAHs) from water samples [83]. The main advantage of DLLME is its simple, rapid and inexpensive operation [84]. The criteria for an extraction

solvent in DLLME is it must have low solubility in the aqueous phase, whereas the dispersive solvent must be miscible with both the extraction solvent and aqueous sample [85]. The DLLME only involves two steps, where both the extraction solvent and disperser solvent are injected rapidly into the sample solution with a syringe. The formation of a cloudy solution containing fine droplets of extraction will be fully dispersed and removed by centrifugation to separate the solutions based on their densities [86].

Yu demonstrated DLLME by employing the deep eutectic solvent (DES) as the extraction solvent. The technique provided fast kinetics to enhance mass transfer because of the large surface area with fine droplets [87]. This method produced good reproducibility which was less than 7.6%. The enrichment factor was in the range of 531-838 folds. DES used in this method is non-toxic and environmentally friendly. Except for milk samples, the DLLME offered good linearity ranged 0.02 to 3.2 $\mu\text{g mL}^{-1}$ and 0.03 to 4.8 $\mu\text{g mL}^{-1}$ with LOD less than 0.01 $\mu\text{g mL}^{-1}$ in determining fluoroquinolones in the honey and water samples [87].

The development of DLLME with the use of switchable polarity dispersive solvent (DLLME-SPDS) has overcome the drawback of conventional DLLME. The centrifugation step was eliminated to simplify the procedure. The determination of ofloxacin in chicken meat samples has applied acrylic acid as the solvent and sodium hydroxide as the alkaline agent in this method. The recoveries obtained were satisfactory in the range of 90.4-102.1% for the spiked chicken meat samples [88]. Despite the various advantages, DLLME is lacking selectivity for complicated matrices [42].

Accelerated solvent extraction

Accelerated solvent extraction (ASE) works at elevated temperatures and high pressure to keep the solvent in a liquid state. The high pressure and increased temperature increase the extraction yield and shorten the extraction time [89]. This method had been applied for the extraction of 15 types of fluoroquinolones in swine, bovine and chicken. The ASE extract required further clean-up by SPE before HPLC-UV analysis. The

recoveries obtained ranged from 70.7-111.1%. The ASE coupled with SPE provided high recoveries and the ASE system was fully automated to ease the operation. The combination of these techniques can separate efficiently, characterise and quantify multiple residues in the food samples [90]. The ASE combined with magnetic SPE was also applied for the extraction of 10 fluoroquinolones in aquatic products. The recoveries obtained were satisfactory in the range of 81.6-105.8% [70]. Overall, the ASE increases the extraction yield, enhances the quality of the extracts, and it is rapid and fully automated. The main drawbacks are the preparation of the extraction cell is time-consuming and a clean-up process is needed. This extraction method is considered limited due to the high operation cost. The high pressure has to be maintained and the temperature is kept elevated along the process. Table 3 summarizes the previously reported techniques for the determination of fluoroquinolones in food.

Conclusion

The lack of analysis of fluoroquinolones in our food samples has to be a concern to maintain a healthy life. The development of a more efficient and effective quantitative analysis is important to obtain reliable

residue data. Even though some of the techniques allow for low detection limits and high recovery values, many come with challenging extraction methods to gain better results for ultra-trace levels in food analysis. Most of the studies applied SPE as sample preparation. However, the modified SPE such as magnetic SPE and MIP-SPE are proven to be better than the conventional cartridge-based SPE especially in sensitivity attained. μ -SPE provides a greener technique but the extraction capacity is limited, leading to lower recovery and selectivity as compared to SPE. SPE can extract diverse analytes from different samples and this approach is simple compared to the other techniques. However, it is time-consuming and labour-intensive. Thus, more studies are needed regarding the advanced modification of SPE in sample preparation of food samples as SPE is the most widely used method from 2000 till 2022. In view of that, synthesis of selective adsorbent and liquid extractant for the recognition and extraction of fluoroquinolones is the way forward to enhance the clean-up procedure to offer clean extracts and ultra-trace detection from food samples. On top of that, consolidating automation into sample preparation could further simplify the routine analysis and increase the sample throughput to overcome the shortcoming of microextraction techniques.

Table 3. Summary of previously reported techniques for the determination of fluoroquinolones in food

Food	Analytes	Extraction	Recovery (%)	LOD and LOQ ($\mu\text{g L}^{-1}$)	Instrumentation	References
Milk	Enrofloxacin, Ciprofloxacin, Sarafloxacin, Difloxacin	Acidified ethanol, isolation & retention on cation exchange solid phase extraction, elution with basic ethanol	70-90	0.3-1.2 5.0	HPLC-FD	[91]
Bovine Milk	Fleroxacin, Ciprofloxacin, Lomefloxacin, Danofloxacin, Enrofloxacin, Sarafloxacin, Difloxacin	Deproteinization with TCA, solid phase extraction	73-113	0.5-6.8 1.7-22.2	HPLC-FD	[61]
Milk	Norfloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin,	Solid phase extraction	69-88	1-9 except for enrofloxacin which is 23	HPLC-FD	[92]

	Lomefloxacin, Danofloxacin, Enrofloxacin, Sarafloxacin, Difloxacin, Oxolinic acid, Flumequine					
Milk	Ciprofloxacin, Norfloxacin, Enrofloxacin, Lomefloxacin	MIP-SPE	96	10-20 20-50	HPLC	[62]
Baby Food	Fleroxacin, Norfloxacin, Ofloxacin	Automated magnetic dispersive (SPME)	86-122	1.5-3 5-10	HPLC-FD	[80]
Honey, Milk, Water.	Enrofloxacin, Ciprofloxacin, Lomefloxacin, Gatifloxacin, Sparfloxacin	SO-DLLME-DES- BE	90-110 95-104 87-114	<10 <30	MECC	[87]
Baby Food	18 types of veterinary drugs	LLE with acidified acetonitrile	120	1.0 3.3	UPLC-MS/MS	[77]
Milk	18 types of veterinary drugs	QuEChERS, addition of EDTA as buffer	70.6-111.3	1-4 3-10	UPLC-MS/MS	[72]
Lobster, Duck, Honey, Egg	Tetracycline	MIP-SPE		0.1-0.3	HPLC-MS/MS	[65]
Milk, Honey	Tetracycline	MIP-SPE	73.3-90.6; and 62.6-82.3	10.2-18.8	HPLC-DAD	[66]
Chicken Meat	Ofloxacin	DLLME with switchable polarity dispersive solvent. (DLLME-SPDS)	90.4-92.6 for 5µg/kg 98.7-102.1 for 50 µg/kg	0.071-0.214	HPLC-FD	[88]
Fish	Norfloxacin, Ciprofloxacin, Enrofloxacin, Danofloxacin, Sarafloxacin	Microwave-assisted extraction-SPE	90-107	0.1-1.2 0.2-20.2	UPLC-FD	[71]
Goat Milk	Enrofloxacin, Ciprofloxacin	SPE	84-88	15 20	HPLC-DAD	[93]
Milk, Pork	Norfloxacin, Ciprofloxacin, Lomefloxacin, Enrofloxacin	Magnetic solid phase extraction, Fe ₃ O ₄ as sorbent	85.4-105.9 85.2-103.7	0.33-0.78 1.09-2.60	HPLC-DAD	[94]

Water, Meat	Ofloxacin, Norfloxacin, Ciprofloxacin, Enrofloxacin, Difloxacin, Sarafloxacin, Pefloxacin, Danofloxacin	Magnetic solid phase extraction, Fe ₃ O ₄ as sorbent	82.5-112.5 75.3-104.7	0.0007-0.006 0.0025-0.02	HPLC-MS	[95]
Milk	Fleroxacin, Enoxacin, Pefloxacin, Norfloxacin, Ciprofloxacin, Lomefloxacin, Enrofloxacin, Gatifloxacin, Sparfloxacin, Levofloxacin	MIP-SPE	84.1-104.7	1.34 7.35	HPLC-DAD	[96]
Fish, Shrimp	Flumequine, Ciprofloxacin, Enrofloxacin, Ofloxacin	QuEChERS	72-114	0.01-0.3	UPLC-MS/MS	[73]
Swine, Bovine, Chicken	15 types of fluroquinolones	Accelerated solvent extraction, clean up with solid phase extraction	70.7-111.1	3 10	HPLC-UV	[90]
Aquatic Products	Sarafloxacin, Ofloxacin, Enrofloxacin, Danofloxacin, Lomefloxacin, Pefloxacin, Ciprofloxacin, Enoxacin, Norfloxacin, Difloxacin	Accelerated solvent extraction and magnetic solid- phase extraction.	81.6-105.8	0.02-0.29	HPLC-MS	[70]

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