



Determination of Quinolone Antibiotics in Bovine Liver Using Agilent SampliQ QuEChERS Kits by LC/MS/MS

Application Note

Food

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Abstract

This paper presents an analytical method which allows the determination of 11 quinolone antibiotic residue in bovine liver: pipemidic acid, ofloxacin, ciprofloxacin, danofloxacin, lomefloxacin, enrofloxacin, sarafloxacin, cinoxacin, oxolinic acid, nalidixic acid, and flumequine.

The procedure involves a rapid and efficient pretreatment by SampliQ QuEChERS kits. The homogenized liver sample was initially extracted in a buffered aqueous, 5% formic acid acetonitrile system. An extraction and partitioning step was performed after the addition of salts. Cleanup was done using dispersive solid phase extraction (dispersive-SPE). The final extracts allowed determination of all compounds in a single run using LC-ESI-MS-MS operating in positive ion multiple reaction monitoring (MRM) mode. Norfloxacin was selected as the internal standard. The accuracy of the method, expressed as recovery, was between 62 and 113%. The precision, expressed as RSD, was between 2.2 and 13.4%. The established limit of quantification (LOQ) was 5 ng/g and is significantly lower than the respective Maximum Residue Limit (MRL) for quinolones in food producing animals.



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Introduction

Quinolones are a family of synthetic broad-spectrum antibiotics. They prevent bacterial DNA from unwinding and duplicating. There is evidence that quinolones in food animals lead to the emergence of quinolone-resistant bacteria in animals. The resistant organisms are transmitted to humans via direct contact with the animal or through the consumption of contaminated food and water. Quinolone-resistant campylobacter is an example of animal-to-human transmission and has been observed in many European countries since the early 1990s [1]. Therefore, public health agencies in many countries such as the EU commission [2], the USA FDA administration [3], and the Chinese Ministry of Agriculture [4] have established maximum residue limits (MRLs) of veterinary drugs in food-producing animals. Given the different drugs in different food origins and in different countries, the MRLs of quinolones in food products of animal origin are usually at the level of 100 µg/kg or higher.

As animal food origins, such as muscle, liver, and eggs, are complicated matrices, it is critical to use an efficient sample pretreatment method for analyte extraction and concentration, and matrix cleanup. The established sample pretreatment methods used for determination of quinolones include traditional solvent extraction, solid phase extraction (SPE), or

a combination of both. Although they have been widely used, these traditional methods have inherent limitations.

Traditional methods are labor intensive, time consuming, require a large amount of solvent and waste disposal. In 2003, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method for pesticide residue analysis in fruit and vegetable matrices was introduced. [5] There are two validated QuEChERS methodologies: the AOAC and EN versions. Both are widely accepted and effective for the multiresidue analysis of pesticides in fruit, vegetables and other plant food matrices. The QuEChERS method contains significant advantages over traditional methods, including high recoveries for a wide range of pesticides, high sample throughput, minimal labor, time savings, limited solvent usage, and low waste. In addition, the method is manually accommodating which has made QuEChERS a very popular methodology for the analysis of pesticide residues in fruits and vegetables in recent years.

Although the current QuEChERS methodology has been designed for removing matrix interferences in food products of plant origin, such as polar organic acids, sugars, and lipids, it also has potential for other food matrices such as meat. Based upon the chemical properties of the compounds of interest and food matrices, some modifications of the original method might be necessary to obtain accurate and precise results. The purpose of this work is to extend the QuEChERS methodology to veterinary drug residues in food-producing animals. Agilent SampliQ QuEChERS EN buffered extraction kits (p/n 5982-5650) and dispersive-SPE 2 mL kits for drug residues in meat (p/n 5982-4921) were used for the analysis of 11 quinolone antibiotics in bovine liver: piperimide acid, ofloxacin, ciprofloxacin, danofloxacin, lomefloxacin, enrofloxacin, sarafloxacin, cinoxacin, oxolinic acid, nalidixic acid and flumequine (Figure 2). The method was validated in terms of recovery and reproducibility.

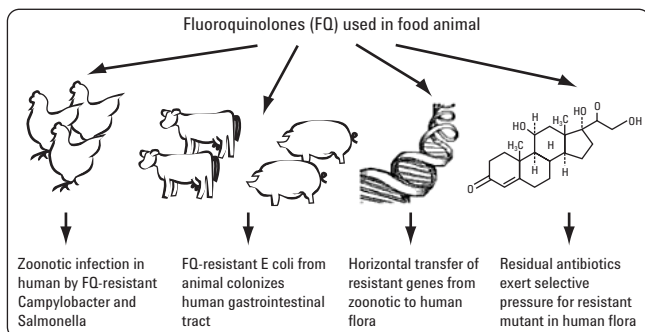


Figure 1. Animal to human transmission of resistant bacteria [1].

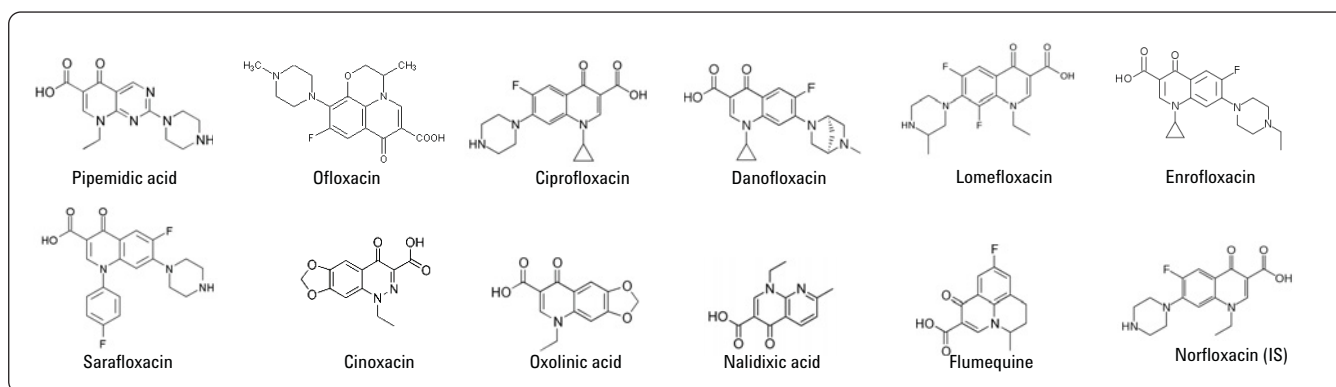


Figure 2. Chemical structures of the quinolone antibiotics investigated in this study.

Experimental

Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. Methanol (MeOH) was from Honeywell (Muskegon, MI, USA). Acetonitrile (ACN), dimethyl sulfoxide (DMSO) and glacial acetic acid (HAc) were from Sigma-Aldrich (St Louis, MO, USA). Ammonium acetate (NH₄OAc) was from Fisher Chemicals (Fair Lawn, NJ, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The quinolone standards and internal standard were purchased from Sigma-Aldrich (St Louis, MO, USA). Potassium phosphate, monobasic (KH₂PO₄), was from J.T. Baker (Phillipsburg, NJ, USA).

Solutions and Standards

1M ammonium acetate stock solution was made by dissolving 19.27 g NH₄OAc powder in 250 mL Milli-Q water. The solution was stored at 4 °C. A 5 mM ammonium acetate in water solution with pH 3 was made by adding 5 mL of 1M ammonium acetate stock solution into 1 L of Milli-Q water, then adjusting the pH to 3 with glacial acetic acid. A 1:1 MeOH/ACN solution was made by combining 500 mL of MeOH and ACN, then mixing well. A 5% formic acid solution in ACN was made fresh daily by adding 10 mL of formic acid to 190 mL of ACN, then mixing well. A 30 mM KH₂PO₄ buffer, pH 7.0, was made by dissolving 4.08 g KH₂PO₄ powder into 1 L Milli-Q water and adjusting the pH to 7.0 with 1 M KOH solution. A 1:1 ACN/H₂O with 0.1% FA was prepared by combining 50 mL of ACN and Milli-Q water, then adding 100 µL of formic acid. A 1:9 MeOH/H₂O solution with 0.1% FA was prepared by combining 10 mL of MeOH and 90 mL of Milli-Q water, then adding 100 µL of formic acid.

Standard and internal standard (IS) stock solutions (1.0 mg/mL for all, except 0.25 mg/mL for ciprofloxacin) were made in DMSO and stored at 4 °C. Due to the solubility of quinolones, it is essential to sonicate stock solutions to ensure they completely dissolve. Three combined QC spiking solutions of 0.2, 8 and 16 µg/mL were made fresh daily in 1:1 ACN/H₂O containing 0.1% FA. A 10 µg/mL standard spiking solution in 1:1 ACN/H₂O containing 0.1% FA was made for the preparation of calibration curves in the matrix blank extract. A 20 µg/mL IS spiking solution of norfloxacin was made in 1:1 ACN/H₂O containing 0.1% FA.

Equipment and Material

- Agilent 1200 Series HPLC with Diode Array Detector (Agilent Technologies Inc., CA, USA).
- Agilent 6410 Series triple quadrupole LC/MS system with Electrospray Ionization (Agilent Technologies Inc., CA, USA).
- Agilent SampliQ QuEChERS EN Extraction kits, p/n 5982-5650, and SampliQ QuEChERS dispersive-SPE kits for Drug Residues in Meat, 2 mL, p/n 5982-4921 (Agilent Technologies Inc., DE, USA).
- CentraCL3R Centrifuge (Thermo IEC, MA, USA)
- Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)
- 2010 Geno Grinder (Spex SamplePrep LLC, Metuchen, NJ, USA)
- Multi-tube Vortexer (Henry Troemner LLC, Thorofare, NJ, USA)

Instrument conditions

HPLC conditions

Column	Agilent ZORBAX Solvent Saver Eclipse Plus Phenyl-Hexyl 150 × 3.0 mm, 3.5 µm (p/n 959963-312)		
Flow rate	0.3 mL/min		
Column Temperature	30 °C		
Injection volume	10 µL		
Mobile Phase	A: 5 mM ammonium acetate, pH 3.0 in H ₂ O B: 1:1 MeOH/ACN		
Needle wash	1:1:1 ACN/ MeOH/ IPA/ H ₂ O with 0.2% FA.		
Gradient	Time	% B	Flow rate (mL/min)
	0	15	0.3
	0.2	15	0.3
	8.0	75	0.3
	9.0	100	0.3
	11.5	STOP	
Post run	4 min		
Total cycle time	~16 min.		

MS conditions

Polarity	positive
Gas Temp.	325 °C
Gas Flow	8 L/min
Nebulizer	50 Psi
Capillary	4000 V
Solvent cut	5 min

Other conditions relating to the analytes are listed in Table 1.

Table 1. Instrument Acquisition Data for the Analysis of 11 Quinolone Antibiotics by LC/MS/MS

Analyte	MRM channels (m/z)	Fragmentor (V)	CE (V)	RT (min)
Pipemidic acid	1) 304.1 → 286.1 2) 304.1 → 215.1	128	17 37	5.9
Ofloxacin	1) 362.2 → 318.1 2) 362.2 → 344.1	150	17 21	6.7
Ciprofloxacin	1) 332.1 → 314.1 2) 332.1 → 231.0	131	21 41	6.8
Danofloxacin	1) 358.2 → 340.2 2) 358.2 → 82.1	159	25 49	6.9
Lomefloxacin	1) 352.2 → 265.2 2) 352.2 → 334.1	144	21 21	7.0
Enrofloxacin	1) 360.2 → 342.2 2) 360.2 → 316.2	159	21 17	7.3
Sarafloxacin	1) 386.1 → 368.1 2) 386.1 → 348.2	144	21 33	7.9
Cinoxacin	1) 263.1 → 217.1 2) 263.1 → 189.0	103	21 29	8.8
Oxolinic acid	1) 262.1 → 216.0 2) 262.1 → 160.0	106	29 41	9.2
Nalidixic acid	1) 233.1 → 104.1 2) 233.1 → 159.1	94	45 33	10.3
Flumequine	1) 262.1 → 202.0 2) 262.1 → 126.0	106	33 50	10.8
Norfloxacin (IS)	320.1 → 302.1	134	17	6.6

1) Quantifier transition channel

2) Qualifier transition channel

Sample preparation

The sample preparation procedure includes sample homogenization, extraction/partitioning, and dispersive-SPE cleanup. As mentioned previously the QuEChERS methods were designed for pesticides analysis in fruit and vegetable matrices; therefore modifications were necessary to optimize the results for the determination of quinolones in bovine liver.

Bovine liver was purchased from a local grocery store. It was washed and chopped into small pieces. The chopped liver was homogenized thoroughly with a food grinder and stored at -20 °C. Two-gram (± 0.05 g) samples of homogenized liver were placed into 50 mL centrifuge tubes. The tubes were centrifuged for 30 s to move the sample from the inside tube wall to the bottom of the tube. Samples were then fortified with appropriate QC spiking solutions (50 μ L) when necessary, then 50 μ L of IS spiking solution (20 μ g/mL of norfloxacin). After vortexing the sample for 30 s, 8 mL of 30 mM KH_2PO_4 buffer, pH 7.0, were added. Tubes were then vortexed for 10 s to mix. A 10 mL volume of 5% FA in ACN was added to each tube. Tubes were capped and shaken by a 2010 Geno Grinder for 30 s. An Agilent SampliQ QuEChERS EN extraction salt

packet (p/n 5982-5650) was added to each tube. Sample tubes were capped tightly and shaken vigorously for 1 min by a 2010 Geno Grinder. Tubes were centrifuged at 4,000 rpm for 5 min at 4 °C.

A 1 mL aliquot of the upper ACN layer was transferred into an Agilent SampliQ QuEChERS dispersive-SPE 2 mL tube for Drug Residues in Meat (p/n 5982-4921). This 2 mL dispersive-SPE tube contained 25 mg of C18 and 150 mg of anhydrous MgSO_4 . The tubes were tightly capped and vortexed for 1 min. The 2 mL tubes were centrifuged with a microcentrifuge at 13,000 rpm for 3 min. An 800 μ L volume of extract was transferred into another tube and dried by N_2 flow at 40 °C. Samples were reconstituted into 800 μ L of 1:9 MeOH/ H_2O with 0.1% FA. After vortexing and sonicating for 10 min, the sample was filtered by a 0.22 μ m Cellulose Acetate Spin Filter (p/n 5185-5990). The clear filtered sample was transferred into an autosampler vial. The samples were capped and vortexed thoroughly in preparation for LC/MS/MS analysis. Figure 2 shows the flow chart of the entire extraction procedure for bovine liver sample.

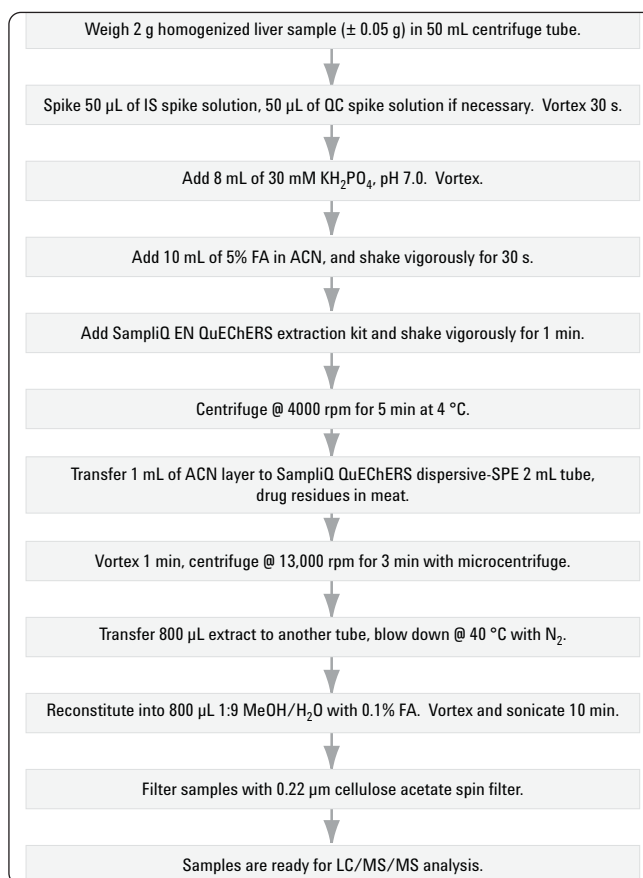


Figure 3. Flow chart of QuEChERS procedure for the determination of quinolones in bovine liver.

Results and Discussion

Feasibility Test

Quinolones are a group of relatively new antibacterials synthesized from 3-quinolone carboxylic acid. As shown in Figure 2, they all contain the carboxylic group, and are weakly acidic (pKa 4-6). Since this is the first time for quinolones determination by the QuEChERS method, the feasibility test was done by extracting 50 ng/mL of neat quinolone solution (prepared in water) with different SampliQ QuEChERS kits, including the SampliQ AOAC extraction kit, SampliQ EN extraction kit, and SampliQ Original extraction kit. In addition, bovine liver is a very different matrix than fruit and vegetables. Therefore, the cleanup was followed by the corresponding fatty dispersive-SPE kit (AOAC and EN fatty dispersive-SPE kit) because these fatty dispersive-SPE kits contain C18 which is critical for removing lipids from liver matrix.

However, the test results were initially very disappointing. All of the analytes had extremely low or nonexistent recoveries. The ACN extracts were tested at two points in the procedure to investigate where the analytes were being lost. The first test was made after the extraction step. The second test was made after both the extraction and the dispersive-SPE steps. Figure 3 shows the chromatogram comparison for the neat

ACN extracts after the extraction step using different extraction kits. The ACN extracts using the EN extraction kit (p/n 5982-5650) showed much higher responses than those using the AOAC extraction kit (p/n 5982-5755) and the original extraction kit (p/n 5982-5550). The buffer system in the extraction/partitioning step provided by the addition of salts plays a key role in the extraction efficiency. The pH when the acidic analytes exist in their neutral forms facilitates the extraction. Both the EN and AOAC extraction kits provide a buffer system of approximately pH 5.0 [6, 7], which is the point where most quinolones are neutral. Therefore, these extraction kits generate better extraction efficiency than the original nonbuffered extraction kit. However, it is unknown why the neat extract from the EN extraction buffer system produced higher responses than that from the AOAC extraction buffer system, especially for the early eluted analytes. From these results, the SampliQ EN buffered QuEChERS extraction kit was selected for future work.

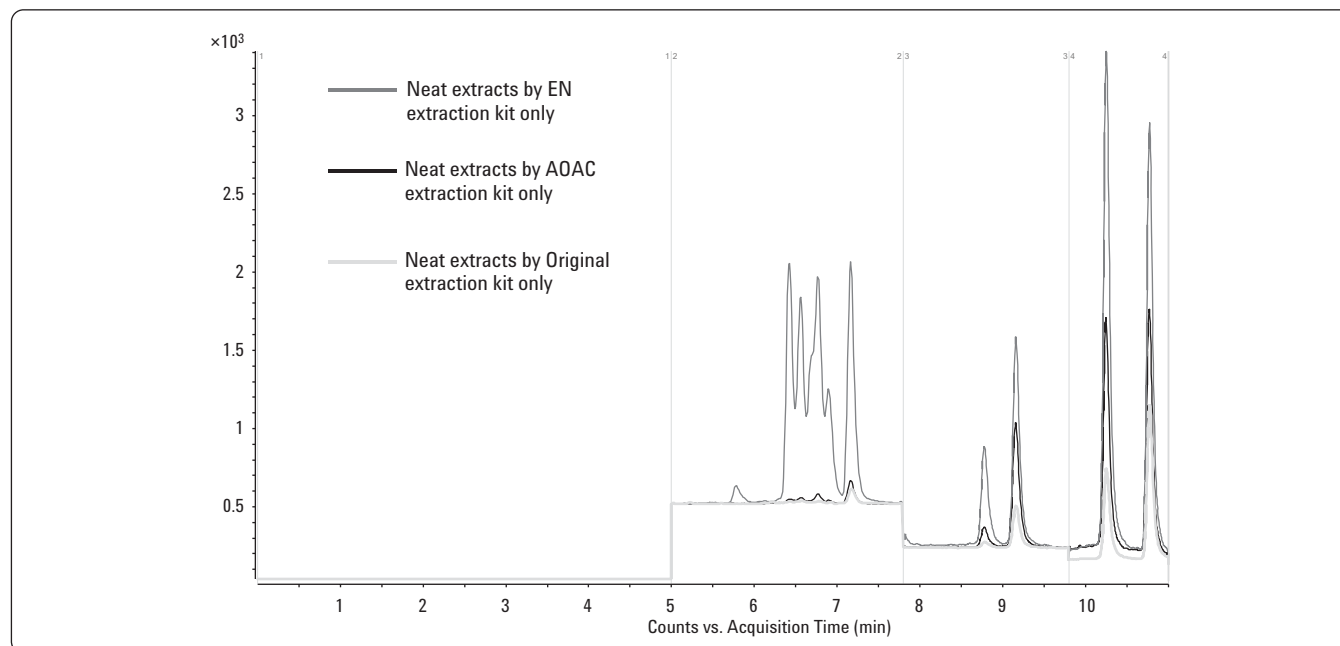


Figure 4. Feasibility test results 1: chromatogram comparison of the neat extracts (no dispersive-SPE) obtained by SampliQ QuEChERS EN extraction kit, AOAC extraction kit, and original extraction kit.

The addition of acid to acetonitrile during the extraction/partitioning step was also investigated. Acetonitrile only, used in the original EN method, and acidified acetonitrile with 5% formic acid were evaluated for their efficiency. As demonstrated in Figure 5 by comparing the results from columns A and D, better analyte recoveries were achieved (10-30% higher) with the acidified acetonitrile. The addition of formic acid into solvent extraction inhibits the acid dissociation for quinolones. Therefore, their protonated neutral form can be extracted easily into the solvent phase [8]. Furthermore, the addition of acid into acetonitrile greatly decreased the negative impact caused by PSA in the dispersive-SPE step (Figure 5, columns C and E). The formic acid in ACN extract interacts with PSA in the dispersive-SPE step, greatly decreasing the binding of PSA with the target quinolones. From these results, 5% (vol/vol) formic acid in acetonitrile was chosen as an extraction solvent for further study.

Although the EN extraction kit generated better recovery, the cleanup using the fatty dispersive-SPE kit in step two significantly lowered extraction efficiency (Figure 5). The selected fatty dispersive-SPE kit contains PSA (primary secondary amine), C18, and $MgSO_4$; however the loss of quinolones was mostly due to the PSA. In the QuEChERS method, PSA is used in all dispersive-SPE kits, because it acts as a weak anion exchanger. It strongly interacts with acidic interferences from fruits and vegetables such as polar organic acids, sugars, and fatty acids. However, it can also strongly interact with the tar-

get analytes, the quinolones, leading to the loss of analytes. When acetonitrile was used in the extraction step, PSA from the dispersive-SPE kit caused almost total loss of all of analytes (Figure 5, columns D and E). When acidified acetonitrile was used in the extraction step, the existence of PSA in the dispersive-SPE kit still caused a 10-40% loss of analytes (Figure 5, columns A and C). Because of these results, a brand new SampliQ dispersive-SPE kit for Drug Residues in Meat (p/n 5982-4921) was used for this study. This new SampliQ dispersive-SPE kit contains 25 mg C18 and 150 mg $MgSO_4$ per mL of ACN extract. The new dispersive-SPE kit's effect on the analytes recovery is negligible (Figure 5, columns A and B).

According to the above feasibility test results, a QuEChERS method was developed and applied for the subsequent study in the liver matrix. This method uses the SampliQ EN buffered extraction kit and 5% FA in ACN for the extraction/partitioning step as well as the new SampliQ dispersive-SPE kit for drug residues in meat for the following cleanup.

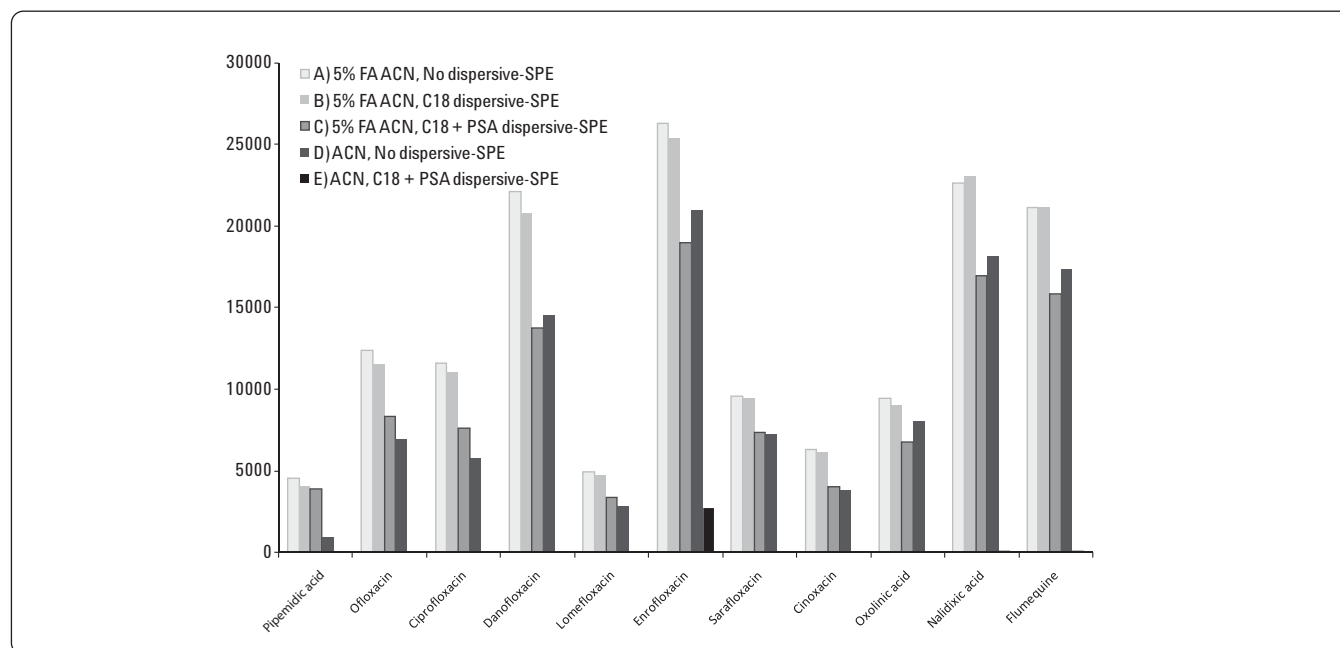


Figure 5. Feasibility test 2. Analytes peak area comparison for the neat extract processed by different procedures. Comparisons include pure ACN and acidified ACN, with and without PSA dispersive-SPE.

Method Optimization in the Liver Matrix

The QuEChERS method established from the results of the feasibility test was applied to the determination of quinolones in bovine liver.

The homogenized liver sample was very thick and could not be used directly for the extraction. Therefore, it was necessary to dilute the liver sample with an aqueous buffer (30 mM KH_2PO_4 in water, pH 7.0) before the extraction. Different sample/buffer ratios including 1:4, 3:7, 1:1, were investigated by adding 8 mL, 7 mL and 5 mL of buffer to 2 g, 3 g, and 5 g of homogeneous liver sample. After dilution, 10 mL of 5% FA in ACN was added. Visually, the more sample used, the more foam was generated during the extraction/partitioning step resulting in a darker red ACN extract. Although more sample should lead to a lower detection limit, it simultaneously introduced more matrix interferences and higher matrix effect. Since the addition of 5% FA ACN to the liver sample is also a protein precipitation procedure, a sample/ACN ratio of 1:4 to 1:5 usually provides the best precipitation effect and sufficient cleanup for proteins. Therefore, a sample/buffer ratio of 1:4 (2 g of liver sample and 8 mL of buffer) was employed.

After the extraction/partitioning step, the sample was centrifuged at 4,000 rpm and 4 °C for 5 min. The low temperature helped to remove lipids from the ACN extracts. After centrifuging, a thin layer of lipids might show up on the surface of the ACN layer. Additional lipids will be removed by C18 in the dispersive-SPE step. A 1 mL amount of ACN extract was transferred into a 2 mL dispersive-SPE tube containing 25 mg C18 and 150 mg MgSO_4 for cleanup. An 800 μL amount of upper solvent was transferred into another tube by vortexing and centrifuging. This was the final extract after the QuEChERS extraction and cleanup. It appeared light brown to red in color and was transparent. In order to get sufficient sensitivity and integrity of peak shape, the sample was dried under N_2 flow and reconstituted into 800 μL 1:9 MeOH/ H_2O with 0.1% FA. The reconstituted sample was cloudy and filtration was necessary, which was done by a 0.22 μm cellulose acetate spin filter. The sample became colorless and clear after filtering, and was ready for LC/MS/MS injection.

Figure 6 shows the MRM chromatograms of liver control blank and 5 ng/g fortified liver extract (LOQ). The liver control blank chromatogram indicated that it was free from any interference to the target analytes. The 5 ng/g fortified liver extract chromatogram demonstrated that the 5 ng/g limits of quantitation (LOQ) for all of analytes were well established with a signal-to-noise ratio (S/N) greater than 5.

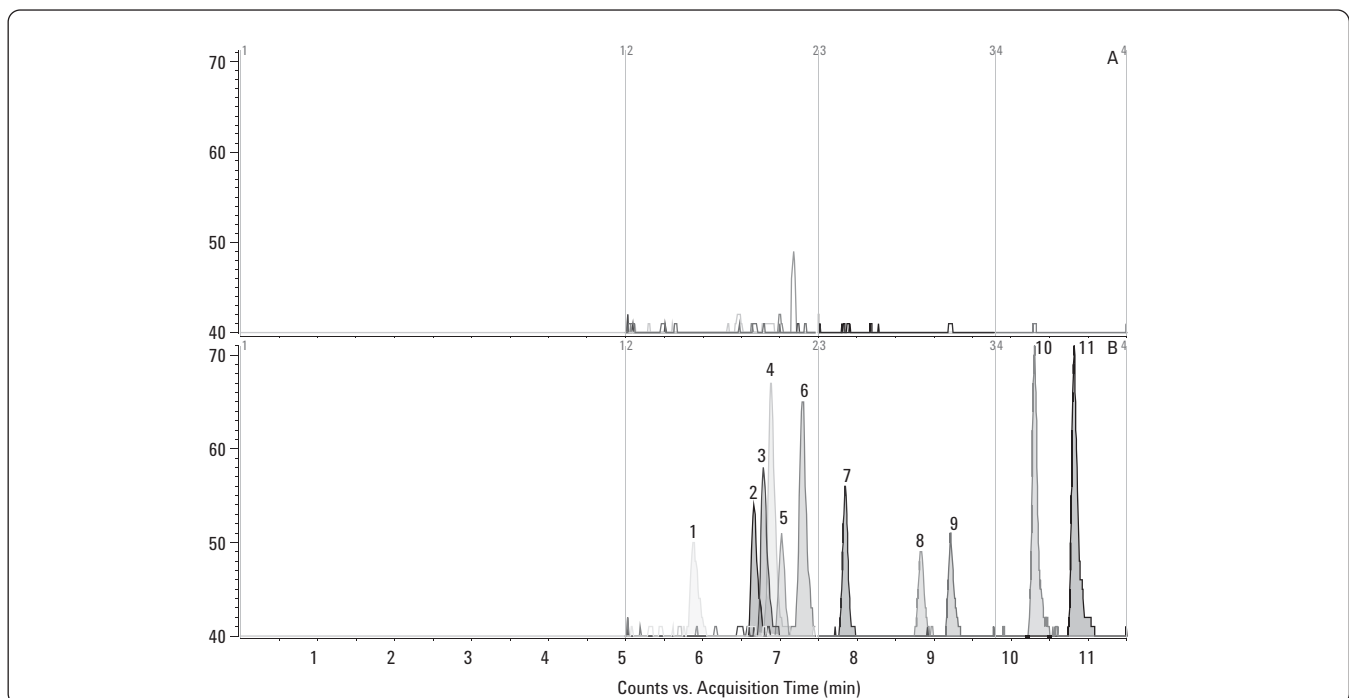


Figure 6. LC/MS/MS chromatograms of A) liver blank extract, and B) 5 ng/g fortified liver extract (LOQ). Peaks identification: 1. Pipemidic acid, 2. Ofloxacin, 3. Ciprofloxacin, 4. Danofloxacin, 5. Lomefloxacin, 6. Enrofloxacin, 7. Sarafloxacin, 8. Cinoxacin, 9. Oxolinoc acid, 10. Nalidixic acid, 11. Flumequine.

Linearity and limit of quantification (LOQ)

The linear calibration range for all of the quinolone antibiotics was 5 – 400 ng/g and matrix blanks were prepared for evaluation. Calibration curves spiked in matrix blanks were made at levels of 5, 10, 50, 100, 200, 300, and 400 ng/g for each analyte. The norfloxacin was used as an internal standard at 200 ng/g. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte / peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). The 5 ng/g limit of quantification LOQ (5 ppb) established for all of the quinolones is far below the MRLs for residues of these antibiotics in animal food products. Table 2 shows the regression equation and correlation coefficient (R^2). Linear regression fit was used with $1/x^2$ weight. Results indicated excellent linearity for all of the analytes calibration curves over a broad quantification range.

Table 2. Linearity of Quinolone Antibiotics in Bovine Liver.

Analytes	Regression equation	R^2
Pipemidic acid	$Y = 0.2081X - 0.00002$	0.9966
Ofloxacin	$Y = 0.2221X + 0.00001$	0.9964
Ciprofloxacin	$Y = 0.2971X - 0.00005$	0.9975
Danofloxacin	$Y = 0.6861X - 0.0039$	0.9957
Lomefloxacin	$Y = 0.1702X - 0.00003$	0.9958
Enrofloxacin	$Y = 0.6530X - 0.0020$	0.9962
Sarafloxacin	$Y = 0.2132X - 0.0004$	0.9937
Cinoxacin	$Y = 0.0933X - 0.0004$	0.9959
Oxolinic acid	$Y = 0.1043X + 0.0003$	0.9939
Nalidixic acid	$Y = 0.3223X + 0.0005$	0.9974
Flumequine	$Y = 0.3232X + 0.0003$	0.9966

Recovery and Reproducibility

The recovery and reproducibility were evaluated by fortifying quinolone standards in homogenized liver sample at levels of 5, 200 and 400 ng/g. These QC samples were quantified against the matrix spiked calibration curve. The analysis was performed in replicates of six at each level. The recovery and reproducibility (shown as RSD) data are shown in Table 3. It can be seen from the results that all of quinolones except pipemidic acid gave excellent recoveries (average of 95.9%) and precision (average of 6.6% RSD). Pipemidic acid gave lower recovery (average of 66.7%) but great precision (average of 5.7% RSD). Additionally, it still meets the 5 ng/g LOQ requirement. Therefore, the results are acceptable.

Table 3. Recovery and Repeatability of Pesticides in Fortified Liver with 2 mL Dispersive-SPE Tube (p/n 5982-4921)

Analytes	5 ng/g fortified QC		200 ng/g fortified QC		400 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Pipemidic acid	71.6	8.1	62.0	6.8	66.4	2.2
Ofloxacin	72.9	9.7	101.0	7.7	102.4	5.7
Ciprofloxacin	108.2	8.3	101.4	4.2	98.9	2.3
Danofloxacin	88.2	7.9	109.3	7.8	114.0	6.1
Lomefloxacin	82.6	13.4	96.8	8.5	97.8	5.3
Enrofloxacin	88.6	7.5	109.5	8.3	113.1	5.8
Sarafloxacin	99.6	9.0	97.7	8.4	97.0	4.6
Cinoxacin	92.3	9.3	95.1	7.9	93.5	2.6
Oxolinic acid	95.1	9.8	92.7	4.3	87.6	2.9
Nalidixic acid	92.7	6.0	90.2	5.3	87.7	3.5
Flumequine	91.6	6.6	93.3	5.3	89.9	2.9

Conclusions

The Agilent SampliQ Buffered Extraction EN kit and the SampliQ dispersive-SPE kit for Drug Residues in Meat provide a simple, fast and effective method for the purification of quinolone antibiotics in bovine liver. Compared to the other sample pretreatment methods, such as LLE and SPE, the QuEChERS method is easier to handle, faster, labor-saving, and cheaper. The recovery and reproducibility, based on matrix spiked standards, were acceptable for multiresidue quinolone determination in bovine liver. The impurities and matrix effects from liver were minimal and did not interfere with the quantification of any target compound. The LOQs of the quinolones were much lower than their regulated MRLs in animal food products. On the whole, the QuEChERS procedures presented here appear to be a promising reference method for the quantitative analysis of quinolones in food products of animal origin. This method also has the potential to extend the applications of SampliQ QuEChERS extraction and dispersive-SPE kits to the quantitative analysis in other bio-matrices, such as animal food products and bio-fluids, rather than just plant matrices.

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