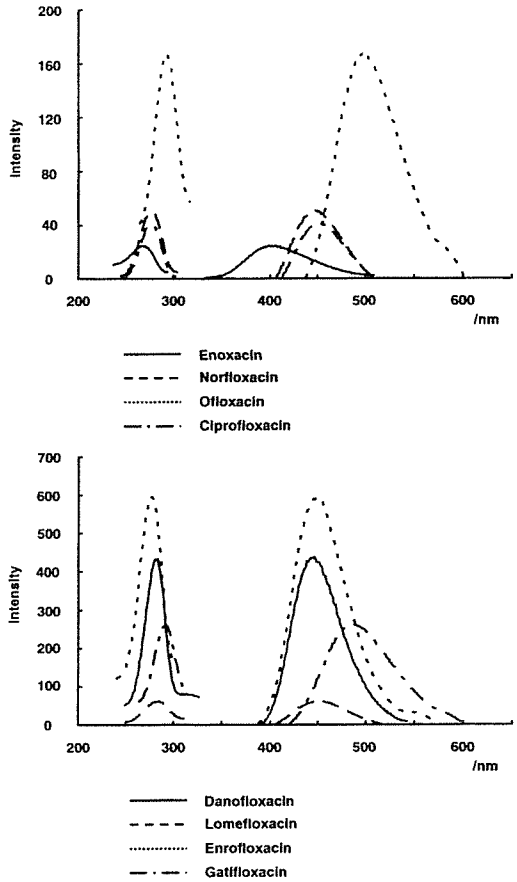

Fig. 2 Optimal excitation and emission wavelengths for detection of fluoroquinolones

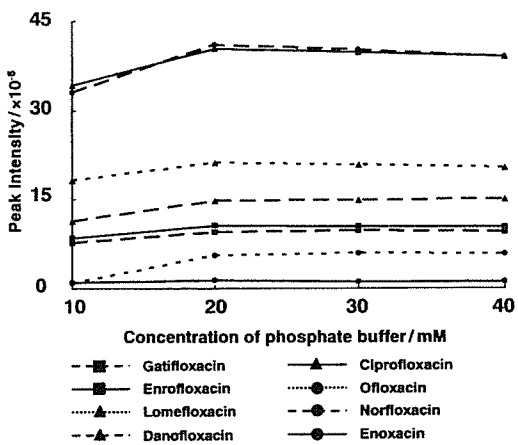

Fig. 3 Optimal concentration of formate buffer in the mobile phase

キノロン系抗菌剤はpH 7以上で蛍光を示さず，中性から酸性条件化で一定の蛍光強度が得られたことから，キノロン系抗菌剤の測定には液性をpH 3.0に調整した．同様に，最大の強度を示した，塩濃度20 mMを最適条件とした(Fig. 3)．

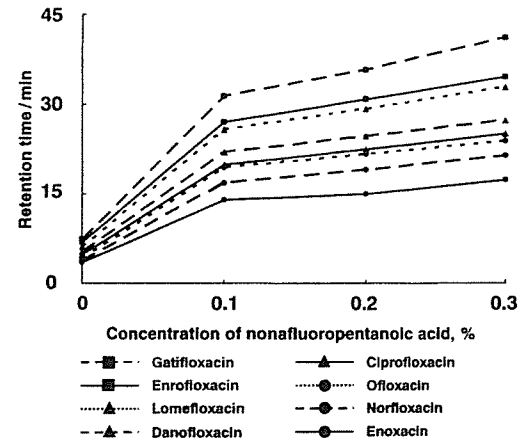

Fig. 4 Optimal concentration of nonafluoropentanoic acid and

Table 2 Recoveries of fluoroquinolones in meat sample using MCX cartridge

Analytical compound	Amount spiked/ ng g^{-1}	Recovery, % (mean ± S.D., n = 6)
Enoxacin	1000	105.7 ± 5.7
Norfloxacin	50	105.6 ± 4.3
Ofloxacin	500	106.3 ± 4.0
Ciprofloxacin	50	107.7 ± 6.1
Danofloxacin	50	109.2 ± 4.0
Lomefloxacin	125	105.0 ± 4.9
Enrofloxacin	50	107.8 ± 4.2
Gatifloxacin	500	104.2 ± 4.5

3・3　キノロン系抗菌剤の分離検討

キノロン系抗菌剤標準品を用い，HPLC/FLにおける分離条件をリン酸塩緩衝液で検討したところ，すべてのキノロン系抗菌剤の相互分離が不十分であった．そこで，NFPAをイオンペアー試薬として添加し，分離条件を検討したところ，NFPAが0.2%で良好な相互分離が達成された(Fig. 4)．これらの結果より，イオンペアー試薬を用いたことで，構造が類似している8種類のキノロン系抗菌剤を一斉分析することが可能となった．

3・4　試料の前処理方法

試料に含まれる他の共存物質の影響を取り除くため，Oasis HLB® 及びOasis MCX® を使用した前処理方法を検討した．Oasis HLB® を使用した場合，平均回収率が50%と低く，HPLC/FLにおいても共存物質の影響を完全に除去することができなかった．しかし，陽イオン交換系カートリッジであるOasis MCX® を用いた場合，標準品添加における平均回収率は106%と良好な結果を得ることができた(Table 2)．

Table 3 Validation of the HPLC/FL method

	Enoxacin	Norfloxacin	Ofloxacin	Ciprofloxacin	Danofloxacin	Lomefloxacin	Enrofloxacin	Gatifloxacin
Limit of detection	20	2	20	2	1	5	2	20
Limit of quantification	200	10	100	10	5	25	10	100
Resolution	1.38	1.39	1.08	1.49	1.90	1.71	4.97	
Correlation coefficient	0.998 (200～20000)	0.999 (10～1000)	0.999 (100～10000)	0.999 (10～1000)	0.999 (5～500)	0.999 (25～2500)	0.999 (10～1000)	0.999 (100～10000)

(ng g^{-1})

Table 4 Intraday and interday precision of quantifying fluoroquinolones using HPLC/FL

Intraday

Analytical compound	Actual concentration/ ng g^{-1}	Detected concentration/ng g^{-1} (mean ± S.D., n = 5)	Precision (R.S.D.), %
Enoxacin	200	205 ± 13.1	6.6
	20000	20043 ± 1364	6.8
Norfloxacin	10	11.0 ± 1.1	10.9
	1000	1053 ± 116	11.6
Ofloxacin	100	88.9 ± 8.2	8.2
	10000	10364 ± 714	7.1
Ciprofloxacin	10	10.4 ± 1.1	10.7
	1000	1033 ± 99.4	9.9
Danofloxacin	10	9.7 ± 1.2	12.3
	1000	1026 ± 19.5	2.0
Lomefloxacin	25	23.7 ± 1.7	6.8
	2500	2558 ± 196	7.9
Enrofloxacin	10	9.8 ± 1.2	12.0
	100	1007 ± 69.0	6.9
Gatifloxacin	100	82.6 ± 8.1	8.1
	10000	10574 ± 833	8.3

Interday

Analytical compound	Actual concentration/ ng g^{-1}	Detected concentration/ng g^{-1} (mean ± S.D., n = 5)	Precision (R.S.D.), %
Enoxacin	200	209 ± 1.6	0.8
	20000	18931 ± 919	4.6
Norfloxacin	10	11.2 ± 1.1	10.9
	1000	984 ± 61	6.1
Ofloxacin	100	97.4 ± 11.9	11.9
	10000	9636 ± 599	6.0
Ciprofloxacin	10	9.1 ± 1.3	13.0
	1000	972 ± 60.9	6.1
Danofloxacin	10	9.5 ± 1.2	12.2
	1000	1019 ± 13.5	1.3
Lomefloxacin	25	25.4 ± 3.2	12.9
	2500	2367 ± 209	8.4
Enrofloxacin	10	12.3 ± 1.2	12.2
	100	938 ± 68.3	6.8
Gatifloxacin	100	85.2 ± 6.2	6.2
	10000	9980 ± 953	9.5

3・5 HPLC/FL の分析法バリデーション

本分析法の，食肉中における検出限界，定量限界及び分離度を求めた[8]．検出限界は 1～20 ng/g，定量限界は 5～200 ng/g であり，各キノロン系抗菌剤の相互分離が達成された（Table 3）．本分析法の日内及び日間変動を求めたところ，定量限界付近にてバラツキと回収率の低下が見られたものの，13% 以下と良好な結果が得られた（Table 4）．これらの結果より，本分析法は食肉中キノロン系抗菌

Table 5　Concentration of fluoroquinolones in meat samples by ELISA and HPLC/FL

	ELISA	HPLC/FL							
	Enrofloxacin equivalent	Enoxacin	Norfloxacin	Ofloxacin	Ciprofloxacin	Danofloxacin	Lomefloxacin	Enrofloxacin	Gatifloxacin
Round	(<8 ng g^{-1})	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Liver-1	(<8 ng g^{-1})	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Liver-2	(<8 ng g^{-1})	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Breast meat	(<8 ng g^{-1})	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sirloin	(<8 ng g^{-1})	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sasami	(<8 ng g^{-1})	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Breast meat + Norfloxacin (50 ng g^{-1})	44.2	N.D.	53.5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Breast meat + Ciprofloxacin (50 ng g^{-1})	50.2	N.D.	N.D.	N.D.	59.0	N.D.	N.D.	N.D.	N.D.
Breast meat + Danofloxacin (50 ng g^{-1})	43.4	N.D.	N.D.	N.D.	N.D.	49.5	N.D.	N.D.	N.D.
Breast meat + Enrofloxacin (50 ng g^{-1})	53.6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	58.4	N.D.

(ng g^{-1}), N.D.: Not detection

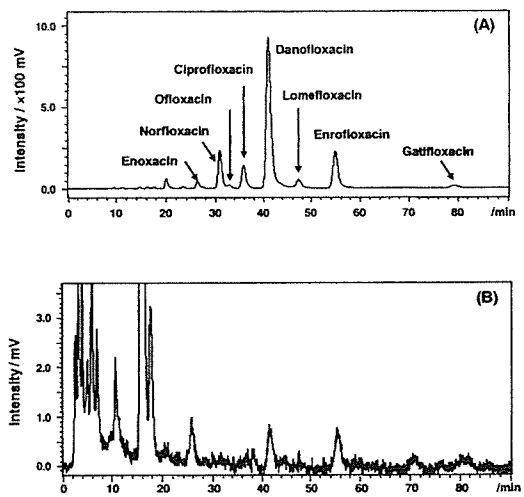

Fig. 5　Chromatograms of (A) fluoroquinolone standard and (B) meat sample

The mobile phase was 20 mM phosphate buffer (pH = 3.0)/acetonitrile (83 : 17, v/v) containing 0.2% nonafluoropentanoic acid.　Flow rate was 0.2 ml/min.

剤の分析に適用可能であると考えられる．

3・6　実試料の測定結果

キノロン系抗菌剤標準品及び実試料のクロマトグラムをFig. 5に示す．キノロン系抗菌剤は他の共存物質の影響を受けることなく良好に分離された．本分析法を用いて，食肉中キノロン系抗菌剤を測定した．その結果，HPLC/FLにおいて，すべての食肉からは検出限界未満であった．また，同一の試料をELISAで測定したところ，同様に定量限界値（8 ng/g）未満となった．更に，食肉に既知の濃度のキノロン系抗菌剤を添加し，HPLC/FL及びELISAで測定したところ，両者の間に有意な相関性が得られた(Table 5)．

4　結　　言

ELISA法は簡便な方法で，多検体を測定できる利点を有している．しかしながら，交差反応性などの問題から，定性能力に欠け，はん用性に乏しかった．そこで，キノロン系抗菌剤を対象薬剤として，HPLC/FLとELISA法を用いて食肉中の残留分析法を検討し，実試料へと適用したところ，両者の値に相関性が認められた．

HPLC/FLにおける前処理等煩雑な操作及び約120分の分析所要時間を考慮すると，ELISAキットは1次スクリーニング法としての有用性を示唆することができ，今後，更なる研究を進めていく必要があると思われる．

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Analysis of Fluoroquinolones in Meat Samples by Enzyme-Linked Immunosorbent Assay and HPLC

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Fluoroquinolones (FQs) are an important group of synthetic antibacterial, which are widely used to treat human and veterinary diseases. In recent years, FQs residues were detected in several samples. An enzyme-linked immunosorbent assay (ELISA) is useful to determine many samples simultaneously. However, there is a problem of not having qualification because of cross-reactivity. In the present study, we developed an analytical method using HPLC/FL for a comparative study with ELISA. Moreover, an HPLC/FL system was applied to the determination of FQs residues in meat samples. The LOD and LOQ of HPLC/FL were 2 ng/g and 10 ng/g for enrofloxacin, respectively. The extraction recovery of enrofloxacin spiked concentration of 50 ng/g was 107.8%. The HPLC method and an enzyme-liked immunosorbent assay for the analysis of fluoroquinolones in meat samples were compared. FQs were detectable in meat samples added FQs standard by HPLC/FL and ELISA. Moreover, there was a correlation between HPLC/FL and ELISA. In conclusion, ELISA may be useful to rapid monitoring of residues for FQs instead of HPLC/FL, which requires analytical times of 120 min in meat samples.

Keywords : fluoroquinolones; enzyme-linked immunosorbent assay; HPLC/FL.