

Biosensor immunoassay for (fluoro)quinolone antibiotics in fish, egg, and poultry meat

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I. Introduction

Due to the broad spectrum of activity for various animal infections, different quinolones and fluoroquinolones have been approved around the world. Few screening methods for the analysis of quinolone residues have been proposed; moreover, there is a lack of multiresidue procedures. The present study utilises the BIAcore® surface plasmon resonance instrument for the development of an inhibition assay for the determination of thirteen (fluoro)quinolones used as veterinary drugs in food-producing animals. Techniques used in method development and validation as required by 2002/657/EC are presented.

II. Sample Preparation

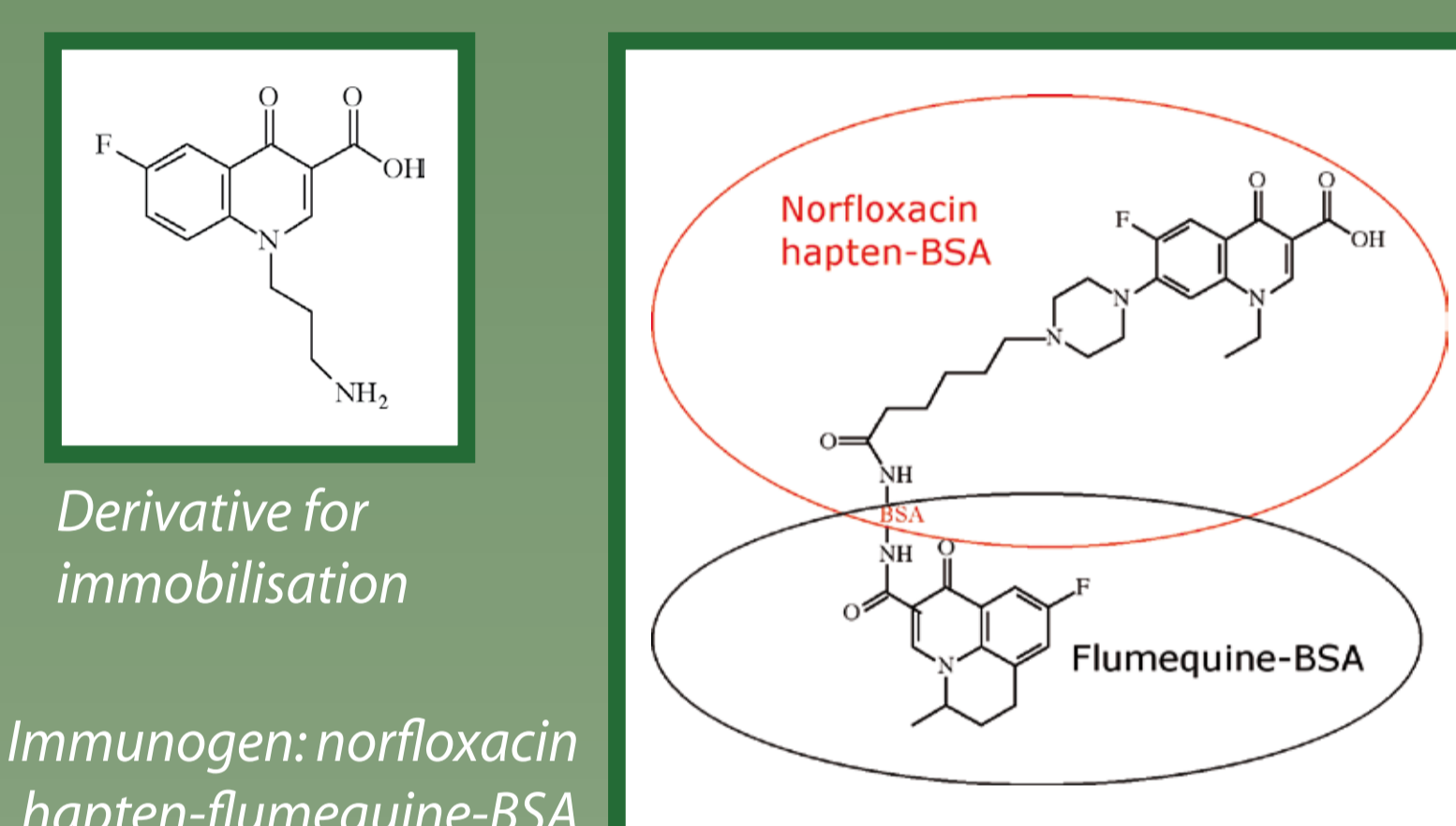
Egg and poultry meat: 2g homogenized muscle or whole egg were extracted with 8 mL acetonitrile. Each tube was immediately vortexed before vigorous head-over-head shaking for 10 minutes. After centrifugation, all of the supernatant was removed and evaporated to dryness. Residue was reconstituted in 1 mL Phosphate Buffered Saline (PBS) pH 7.4 and mixed. Then 1 mL hexane was added and the tubes were vortexed. The sample was centrifuged and the hexane layer and any traces of emulsion at the interface were removed. The washing step was repeated once again and the reconstituted sample was quickly vortexed and centrifuged just before application to the 96-well plate.

Fish: As above except only a 2 mL aliquot of the supernatant was transferred into new tube.

III. Biosensor Analysis

The CM5 sensor chip was immobilized with an amine derivative of the quinolone using an EDC/NHS reaction. A bi-active polyclonal antibody was produced and used as binding protein thanks to a new immunogen combining features of two preparations (flumequine-BSA and norfloxacin hapten-BSA).

The samples and calibration standards in blank extracts were mixed with the binding protein using a ratio antibody:extract equal to 25/75. Each sample was injected at a flow rate of 25 µl/min for 210 seconds. Regeneration of the sensor surface was achieved by injection of 5 µl of 10 mM NaOH (10 µl/min).



IV. Results

This assay has the optimal range between 0.1 µg kg⁻¹ and 100 µg kg⁻¹ norfloxacin in fish matrix, between 0.1 µg kg⁻¹ and 10 µg kg⁻¹ norfloxacin in egg and poultry meat matrixes (figure 1). Based on this standard curve, the assay generated a midpoint curve of 3.1 µg kg⁻¹, 1.5 µg kg⁻¹ and 1 µg kg⁻¹ for fish, egg and chicken muscle, respectively. No significant loss of the surface activity could be observed after more than 200 cycles.

The cross-reactivity was determined for 13 related compounds. As demonstrated in table 1, the SPR-assay can detect 13 of the most widely used (fluoro)quinolones at levels below their established maximum residue limits (MRLs).

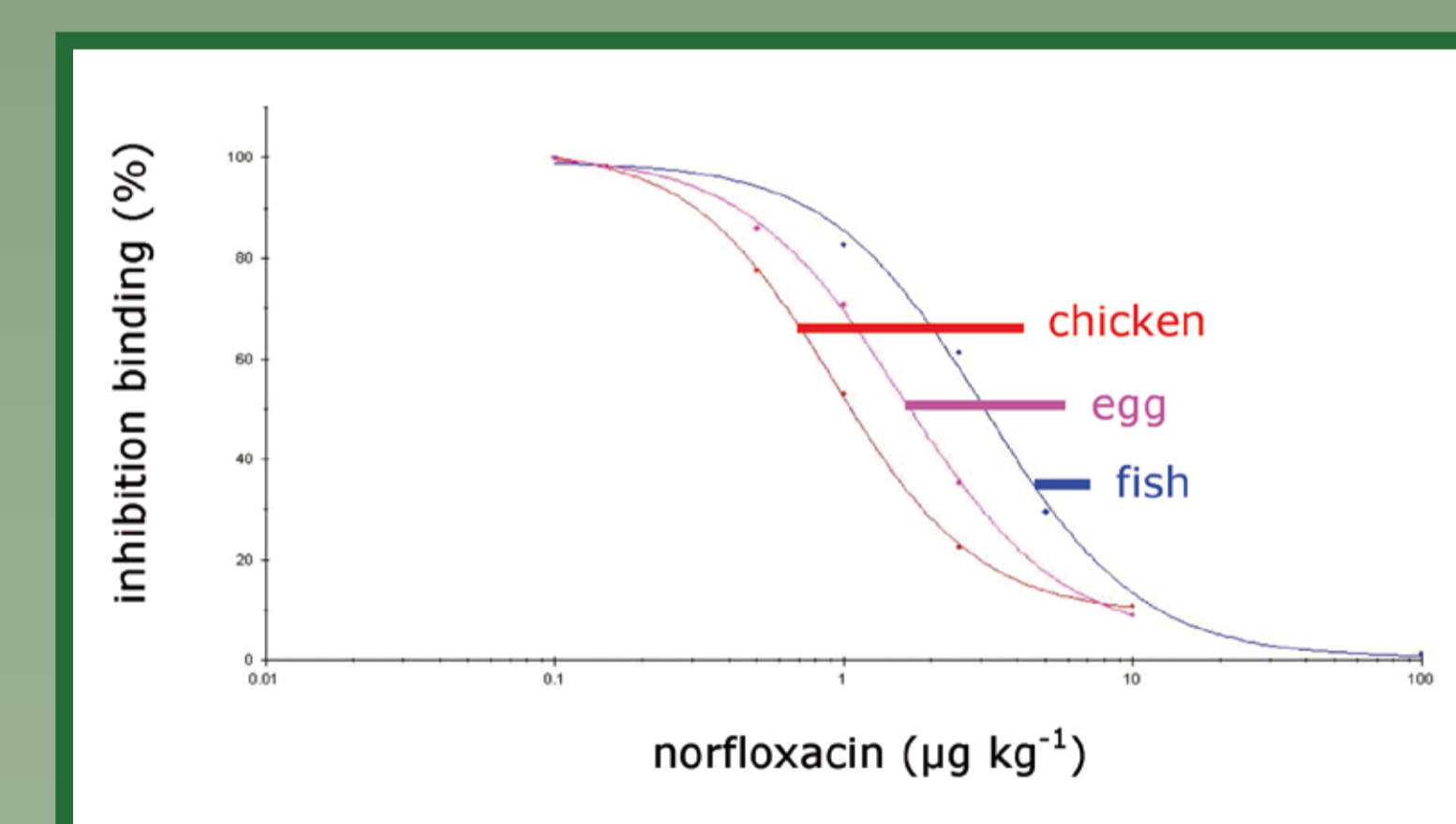


Figure 1: four parameter fit of norfloxacin calibration curve in the presence of extracts

	fish		egg**		chicken muscle	
	IC ₅₀ (µg kg ⁻¹)	CR (%)	IC ₅₀ (µg kg ⁻¹)	CR (%)	IC ₅₀ (µg kg ⁻¹)	CR (%)
norfloxacin	2.4	100	0.9	100	0.6	100
ciprofloxacin	2.3 (50 [*])	106	1.1	82	0.7 (50 [*])	82
danofoxacin	3.4 (100)	71	0.7	124	1.1 (200)	50
difloxacin	4.3 (300)	57	0.8	106	1.2 (300)	45
enoxacin	5.7	43	1.6	56	2.1	26
enrofloxacin	1.5 (50 [*])	163	0.5	183	0.5 (50 [*])	118
flumequine	106.1	2	45.6	2	74.9	1
lomefloxacin	5.1	48	2.0	45	1.3	43
marbofloxacin	3.6	67	0.7	122	0.7	80
ofloxacin	2.7	91	0.8	117	0.9	65
oxolinic acid	22.6 (100)	11	6.1	14	11.7 (100)	5
pefloxacin	2.0	125	0.5	180	0.5	116
sarafloxacin	6.0 (30)	41	1.9	47	1.9	30

* sum of ciprofloxacin and enrofloxacin = 100 µg kg⁻¹
** not for use in animals from which eggs are produced for human consumption

Table 1: IC₅₀ values and cross-reactivities (CR) between (fluoro)quinolones in the matrixes. The corresponding MRL value is indicated between ()

This qualitative screening test was validated according to the European Decision 2002/657/EC (table 2). Decision limit was defined as the concentration corresponding to the average signal from blank samples plus three standard deviations. The detection capabilities (CCbeta) for norfloxacin residue were also determined in each matrix. Repeatability study was done using spiked samples with norfloxacin at their respective CCbeta values and the coefficient of variation (CV) was calculated.

	fish	egg	chicken muscle
decision limit (µg kg ⁻¹)	0.30	0.29	0.13
detection capability for norfloxacin (µg kg ⁻¹)	< 1.5	< 1	< 0.5
Intra-day variability (n=6)	CV = 5.8 %	CV = 4.4 %	CV = 3.6 %
Inter-day variability (n=3)	CV = 5.7 %	CV = 5.4 %	CV = 1.9 %

Table 2: validation data done with the optimised assay

V. Conclusions

We developed and validated a qualitative analysis of main fluoroquinolones plus oxolinic acid in three matrixes using a simple and rapid immunobiosensor method. Moreover, the concept of the bi-active antibody was experimentally used and its efficiency was proved for the first time.

VI. Acknowledgement

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