VALIDATION OF AN ELISA KIT FOR THE DETECTION OF FLUOROQUINOLONES IN HONEY

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Introduction

Quinolones are antibiotics used in veterinary medicine in meat production, aquaculture and even apiculture. Their use, especially in Asia, has increased during the last few years and this use was confirmed by the detection of residues in honey from this area.

No legislation exists for quinolone residues in honey and no residues should be detected. To ascertain that honey raw material is free of fluoroquinolone residues, we have successfully tested and validated the ELISA fluoroquinolones kit from EuroProxima (previously Euro Diagnostica).

Sample preparation for honey

1. Weigh 0.5 g honey sample in a Falcon tube of 14 mL.
2. Add 4.5 mL dilution buffer / 8% MeOH.
3. Shake vigorously on a mechanical shaker for 30 min.
4. Centrifuge 10 min at 2000 G.
5. The clear solution is applied on the ELISA assay.

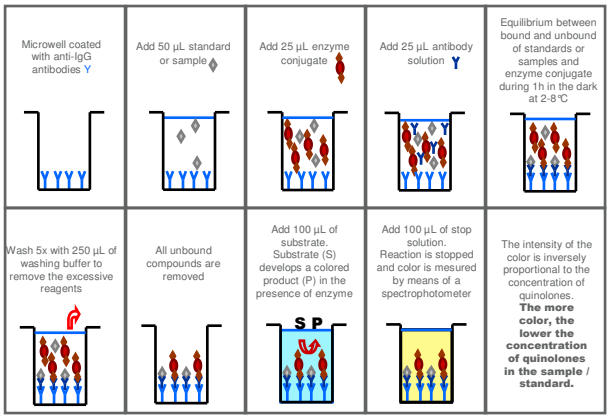
Kit

Euro-Proxima is the supplier of the described Elisa kit.
The detection limit is 3.13 µg/kg for norfloxacin.
The analysis time for one plate (96 tests) is ~2h.

Method validation

The validation was performed according to the EU Commission Decision 2002/657/EC.
The selectivity of the kit has been tested with each fluoroquinolone. The specificity has been evaluated with antibiotics of various families.
The ELISA KIT has been validated on 20 spiked samples at 0, 2.5, 5 and 10 µg/kg and on incurred samples.
A detection limit of 3.13 µg/kg for norfloxacin was obtained.

Reaction mechanism and test procedure



Results & discussion

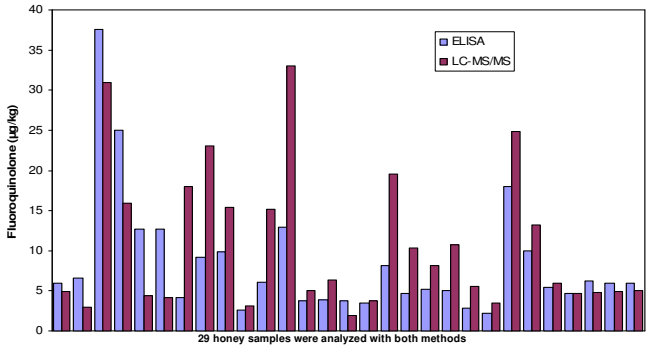
Performance indicators using table of ISO 16140, Afnor

RESPONSE	LC-MS/MS positive (reference methode positive) (R+)	LC-MS/MS negative (reference methode negative) (R-)	Total (N)
ELISA positive (alternative method positive) (A+)	+/+ positive agreement (PA) **30 (100%)**	-/+ positive deviation (PD) **4 (15%)**	**34**
ELISA negative (alternative method negative) (A-)	+/- negative deviation (ND) **0 (0%)**	-/- negative agreement (NA) **23 (85%)**	**23**
Total (N)	(N+) **30**	(N-) **27**	**57**

Matrices	False negative (%)	False positive (%)	Accuracy (%)	Specificity (%)	Sensitivity (%)
Incurred Honey	0	15	93	85	100

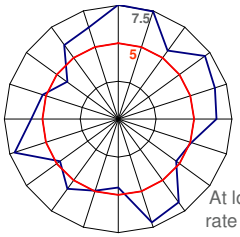
No false negative and less than 20% of false positive were obtained during the validation.

Compararative analyses Elisa & LC-MS/MS
Concentration of fluoroquinolones in honey in µg/kg



A good correlation was found with the two methods.The observed variations are due to the different cross-reactivity of the fluoroquinolones.

Recoveries at 5 µg/kg



20 different honeys were spiked with norfloxacin at a concentration of 5 µg/kg.

At low concentration a higher recovery rate was observed due to the matrix effect.

Selectivity & Specificity

Family	Antibiotics	Results (%)
Sulfonamides	Sulfathiazole	<1%
	Sulfamethazine	<1%
Macrolides	Tylosin	<1%
Tetracyclines	Oxytetracycline	<1%
Amphenicol	Chloramphenicol	<1%
Quinolones	Norfloxacin	100%
	Enrofloxacin	110%
	Ciprofloxacin	106%
	Ofloxacin	24%
	Danofloxacin	140%
	Marbofloxacin	20%
	Sarafloxacin	16%
	Difloxacin	<15%

Among the fluoroquinolone family, different cross-reactivity values have been obtained, from <15% for difloxacin to 140% for danofloxacin.
The test does not react with other antibiotic families; it is specific for quinolones.

Conclusions

The results obtained with the EuroProxima Elisa kit demonstrate that the kit is reliable and suitable for the determination of quinolone residues in honey achieving a good level of sensitivity of 3.13 µg/kg norfloxacin

The performance indicator table shows good results of accuracy, sensitivity and specificity.

The test does not detect a single quinolone but a broad range of quinolone antibiotics. The semi quantitative results depend on the cross reactivity and the recovery of each quinolone. Each positive result should be confirmed with a confirmatory method to identify and quantify the quinolone present.

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