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VALIDATION OF A HIGH SENSITIVITY ELISA KIT FOR A BROAD RANGE SULFONAMIDES DETECTION IN FOOD AND FEED

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Introduction

For a first screening in food and feed, the enzyme-linked immunosorbent assay (ELISA) technique is widely used. However in ELISA screening of veterinary drugs one of the most frequent drawbacks is the limited antibody cross-reactivity and the consequent difficulty to detect a broad range of compounds belonging to a certain drug group. Recently an immunoenzymatic assay was developed by TECNA S.r.l. (Trieste, Italy), *I'Screen* SULFA, reporting suitable cross-reactivities towards the mostly employed sulfonamides. This work describes the validation of this test in some matrices (muscle, feed and honey) collected within the official monitoring plans.

Experimental

In ELISA experiments, the manufacturer protocol was applied for sample preparation of muscles; whereas new protocols were introduced for honey and feed. The considered sulfonamides were: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline and sulfathiazole. The validation approach has followed the criteria of Commission Decision 2002/657/EC [1, 2]. The relative cross-reactivities were established in matrix by simultaneous spiking experiments with all sulfonamides. Therefore for each matrix twenty representative blank samples were carried out and, at the same time, these blank samples were fortified at suitable concentration levels with sulfamethazine (representative analyte).

Results

The estimated detection capabilities (CCβ) were 10, 5 and 1000 µg/kg in muscle, honey and feed, respectively (Table 1). Since these values were established with the lowest cross-reactant sulfonamide within those included in the scope of the method, all the others were detectable at lower concentrations.The decision limit (CCα) represents the cut-off (discriminant value) at which a sample is evaluated as compliant (negative) or suspect and it was estimated from the data of the twenty blank samples [3]. Considering these CCα values, in routine analyses some samples of muscle, honey and feed were identified as being suspect by *I'Screen* SULFA; therefore they were re-analysed with a sensitive confirmatory technique i.e. liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Traces of sulfonamides were detected in all cases (Table 2). As an example, the LC-MS/MS chromatogram of the suspect multifloral honey is shown (Figure 1b). The presence of sulfathiazole was unequivocally confirmed. As comparison in the same Figure 1 a honey sample judged compliant when analysed with ELISA test is reported in the chromatogram (a).

Table 1 – Main validation parameters of *I'Screen* SULFA ELISA test

Matrix	Decision limit (CCα) B/B0 (%)	Decision limit (CCα) µg/kg[a]	Detection capability (CCβ) (µg/kg of sulfamethazine)
Muscle	66.6	3.16	10
Honey	63.7	2.02	5
Feed	94.9	24.8	1000

[a]As sulfamethazine equivalents

Table 2 – Comparison screening *vs* confirmatory method for some suspect samples

	Screening method (ELISA)			Confirmatory method (LC-MS/MS)
Matrix/species	Result B/B0 (%)	Result µg/kg[a]	Interpretation	
Muscle/swine	41.6	10.4	Suspect	1.7 µg/kg sulfadiazine
Muscle/swine	41.9	8.94	Suspect	6.0 µg/kg sulfadimethoxine
Muscle/swine	60.9	3.38	Suspect	1.0 µg/kg sulfadimethoxine
Muscle/bovine	33.2	28.2	Suspect	27 µg/kg sulfadiazine
Muscle/trout	23.7	29.4	Suspect	41 µg/kg sulfadiazine
Multifloral honey	54.4	4.14	Suspect	1.3 µg/kg sulfathiazole
Feed/poultry	54.6	532	Suspect	1036 µg/kg sulfadiazine

[a]As sulfamethazine equivalents

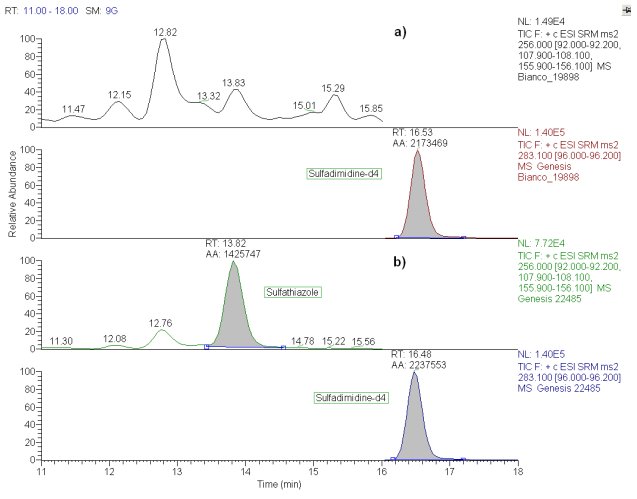

Figure 1 - LC-MS/MS chromatograms: a) compliant honey sample; b) multifloral honey sample containing 1.3 µg/kg of sulfathiazole

Conclusions

The results demonstrated that ELISA screening by *I'Screen* SULFA is fit to purpose permitting rapid and reliable analysis in muscle, honey and feed. Futhermore it reaches sensitivity of the LC-MS/MS confirmation method. Further experiments are in progress to validate the assay also in other matrices.

References

[1] Commission Decision 2002/657/EC, *Off. J. Eur. Comm.,* L221 (2002) 8-36.
[2] Community Reference Laboratories (CRL) Guidelines for the Validation of Screening Methods for Residues of Veterinary Medicines, 20/01/2010.
[3] R. Galarini, R. Buratti, L. Fioroni, L. Contiero, F. Lega, *Anal. Chim. Acta,* 700 (2011) 2-10.

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