I'screen SULFA

Product code:	HU50003
Format:	96 determinations
Detection limits	Muscle, Egg (METHOD I) and honey (METHOD III, high sensitivity): 0.5ppb Milk (METHOD I): 0.25 ppb Muscle, Egg, Milk, Honey (METHOD II; solvent free): 5 ppb Feed: 66.6 ppb
Assay time:	75 minutes
Sample preparation:	Muscle, egg, milk (METHOD I, high sensitivity): Homogenization (skimming for milk), solvent extraction, centrifugation, evaporation. Muscle, egg, honey (METHOD II, solvent free): Homogenization (acid hydrolysis for honey), buffer extraction, centrifugation, filtration. Milk (METHOD II): centrifugation and dilution. Honey (METHOD III): Acidic hydrolysis, purification on SPE column, elution, evaporation. Feed: grinding, solvent extraction, dilution.

Calibration curve

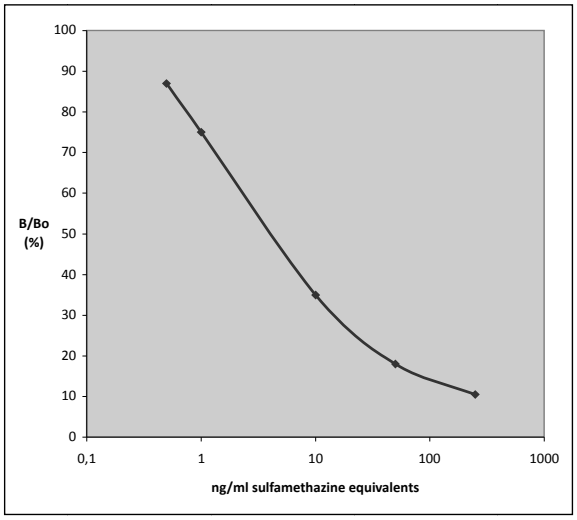

Figure 1 Calibration curve example.

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Cross-reactivity

I'Screen SULFA is a broad-spectrum enzyme immunoassay; the antibody shown the capability to detect at least fifteen main sulfonamides. Buffer cross-reactivities are minuted in the table 1.

Analyte	Cross-reactivity (%)
Sulfamerazine	>100
Sulfamonomethoxine	>100
Sulfadiazine	>100
Sulfachloropyridazine	>100
Sulfamethoxydiazine (sin. Sulfamethoxine, Sulfameter)	>100
Sulfamethoxypyridazine (sin. Sulfapyridazine)	>100
Sulfadimethoxine	>100
Sulfaquinoxaline	>100
Sulfathiazole	>100
Sulfamethizole	>100
Sulfamethazine	**100**
Sulfamethoxazole	90
Sulfisoxazole	50
Sulfapyridine	36
Sulfadoxine	24
Sulfaphenazole	< 2
Sulfabenzamide	< 2
Sulfaguanidine	< 0.1
Sulfanilamide	< 0.1

Table 1. Buffer cross-reactivities

Matrix validation

Solvent extraction (HIGH SENSITIVITY PROCEDURE)

A high sensitive ethyl–acetate extractive procedure was developed and validated. 20 blank bovine muscle samples were analysed *in-house* as blanks to set the Limit Of Decision (LOD); the same 20 blanks were spiked with 10 ppb of sulfamethazine (**SMZ**) to check the sensitivity and set the CCβ. Results are shown in Table 2 and Figure 2.

A collaborative study was carried out with "IZS" Istituto Zooprofilattico Sperimentale Umbria e Marche (Perugia, I-Italy) an Italian official routine laboratory upon swine muscle samples. Different blanks were analysed and then spiked at 10 ppb with **SMZ**, sulfaquinoxaline (SQX) and sulfamethoxazole (SMO) by IZS and by Tecna's team. Results are shown in Table 3 and Figure 2.

Materials	B/B0 (%)	Comments
Blank bovine samples	103±6%, *n*=20	LOD was set at 92% B/Bo, thus obtaining 95% specificity
10 ppb **SMZ** spiked samples	46±7%, *n* = 20	CCβ was set at 10 ppb of sulfamethazine with 100% sensitivity and a mean difference to blanks of 57%.

Table 2. Bovine muscle: results obtained in-house analysing 20 blanks and spiking the same samples at 10 ppb of SMZ.

Materials	B/B0 (%)	Comments
Blank swine samples	93±12%, *n* = 48	LOD was set at 70% B/Bo, thus obtaining 98% specificity
10 ppb **SMZ** spiked samples	48±7%, *n* = 30	CCβ was set at 10 ppb of **SMZ** with 100% sensitivity and a mean difference to blanks of 45%.
10 ppb SQX spiked samples	50±4%, *n* = 10	CCβ was set at 10 ppb of SQX with 100% sensitivity and a mean difference to blanks of 43%.
10 ppb SMO spiked samples	60±6%, *n* = 19	One sample up to 19 turned to be less inhibited than the CCα, the sensitivity was therefore 94%.

Table 3. Swine muscle: results of in-house validation and the test performed by the IZS Umbria e Marche. An overall amount of 48 blank samples were analysed, then a number of samples were spiked at 10 ppb of SMZ, SQX and SMO.

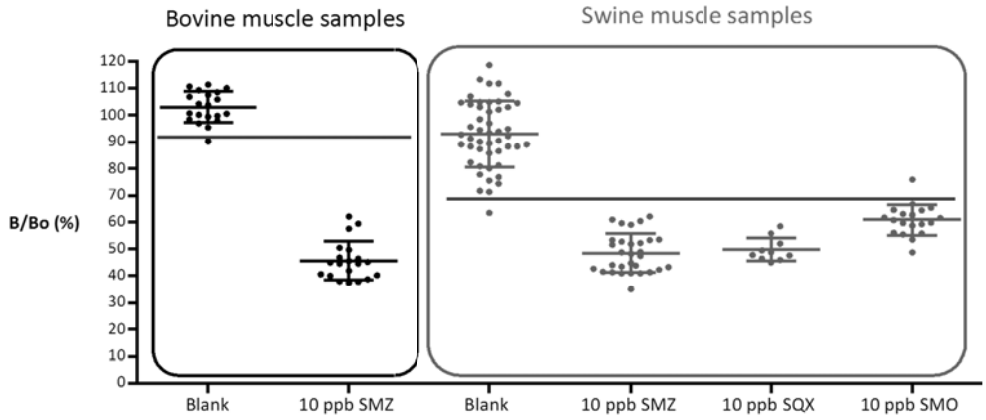

*Figure 2. Bovine and swine muscle tissue: comparison between blank and spiked samples. In the left box, bovine samples results. In the right box, swine muscle samples results. Blank swine samples were spiked with 10 ppb of **SMZ**, SQX and SMO. Each dot represents one single result, bars are mean B/Bo values±SD. The lines represent the B/B0 value of the LOD.*

As a result, considering all the data belonging to the bovine and swine muscle validation, the mean B/B0 value of blanks turned to be 96±11%. The LOD was set at 72% relative signal, corresponding to a CCα 1.5 ppb of **sulfamethazine** equivalents. The CCβ was defined at 10 ppb of **sulfamethazine** and sulfaquinoxaline and no false compliant results were obtained.

While performing the validation study, some "blank" swine muscle routine samples (< 10 ppb of sulfonamides, by HPLC-DAD) were "suspect not compliant" by the *I'Screen* SULFA kit. Once analysed by LC-MS, samples turned to be actually contaminated with low levels of sulfonamides. This case demonstrated that this ELISA kit has an higher sensitivity than the HPLC analysis.

Buffer extraction

20 blank bovine and 20 swine muscle samples were analysed within three different *in-house* sessions to validate an easier, solvent-free procedure. The main relative signal value were 66±6% and 68±8% respectively (*n* = 60). The LOD was set at 54% of B/B0 to obtain 98% specificity.

The same blanks were then spiked with different concentrations of **SMZ** and analysed. 98% sensitivity was found by spiking 50 ppb. The CCβ was therefore 50 ppb of **SMZ**. The mean B/B0 of bovine and swine spiked samples was 46±6% and 44±5%, with a main difference to blanks of 20 and 24 B/B0 points, respectively.

Hydrolysis and SPE preparation

20 blank honey samples of different type and origin were analysed to establish the specificity of the assay. Data were collected by the IZS Umbria e Marche. The mean relative binding signal was 83±8%. To obtain 90% specificity, the LOD was set at 70% B/Bo, corresponding to a CCα of 1 ppb of **SMZ** equivalents.

The same samples were spiked with 5 ppb of **SMZ** and analysed. The mean B/B0 was 45±7%, no determinations had a relative binding signal above 70% and the mean difference to blanks' B/Bo was 38%. The sensitivity of the assay was therefore 100% at 5 ppb. The average measured value of spiked samples was 5.11±1.35 ppb. Results are shown in Figure 3

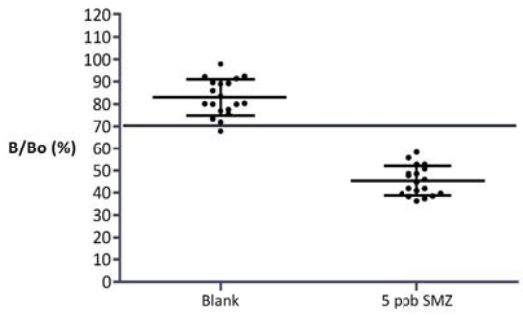

Figure 3. Comparison between blank and CCβ spiked honey samples (n=20). Each dot represents one sample, bars are mean B/B0 values±SD. The line represents the B/B0 value of the LOD.

Buffer extraction

Another possibility is to dilute the honey samples within an easy, solvent-free procedure. 20 blank between blank and **SMZ**, STZ and SMR spiked honey samples (n = 60). Each dot represents one single result, bars honey samples of different origins and types were analysed within an *in-house* validation of three replicates (*n* = 60 determinations). The mean B/B0 was 70±4%.

To obtain 95% specificity, the LOD was set at 62% B/B0. The same honey samples were spiked with 25 ppb of (**SMZ**), 8 ppb of STZ, 2 ppb of sulfamerazine (SMR). One determination up to 60 sulfathiazole spiked samples turned to be less inhibited than the LOD; the sensitivity was hence 98% for STZ and 100% for **SMZ** and SMR. Results shown in Figure 4.

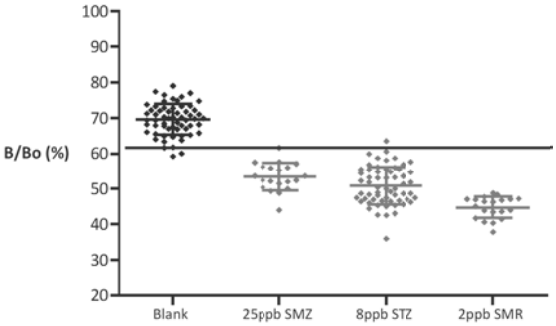

Figure 4. Comparison between blank and CCβ spiked honey samples (n=20). Each dot represent one sample, bars are the mean B/B0 ± SD. The line represents the LOD at 62% B/B0.

MILK

Solvent extraction

20 blank milk samples were analysed to establish the specificity of the assay. Data were collected by the IZS Umbria e Marche.

The mean B/Bo value was 94±10%, to obtain 95% specificity the LOD was set at 77% relative signal. The same samples were spiked with 10 ppb of **SMZ**. The mean relative binding value was 38±10%, with no determinations less inhibited than 77% and a mean difference to blanks of 56% in terms of B/Bo. The CCβ was hence set at 10 ppb of **SMZ**, with 100% sensitivity. Results shown in Fig. 5.

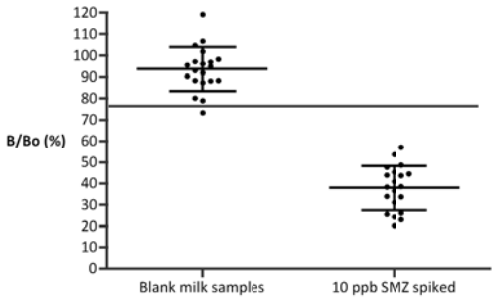

Figure 5. Comparison between blank and CCβ spiked milk samples (n = 20). Each dot represents one single result, bars are mean B/B0 values±SD. The red line represents the B/B0 value of the LOD.

Buffer dilution

An easier, solvent-free procedure was validated by the IZS Umbria e Marche.

20 blank samples were analysed to establish the specificity. The mean B/Bo value was 78±5%, to obtain 90% specificity the LOD was set at 68% relative signal, corresponding to a CCα of 14 ppb of **SMZ** equivalents. The same samples were spiked with 25 ppb of **SMZ**. The mean B/Bo was 47±9%, with no determinations less inhibited than 68% (100% sensitivity) and a mean difference of 31% to blanks.

An *in-house* validation was carried out by spiking 20 other blank milk samples with 5 ppb of STZ. The mean B/Bo was 26±3%, with no false compliant results (100% sensitivity) and a difference to blanks of 52%. Results shown in Fig. 6.

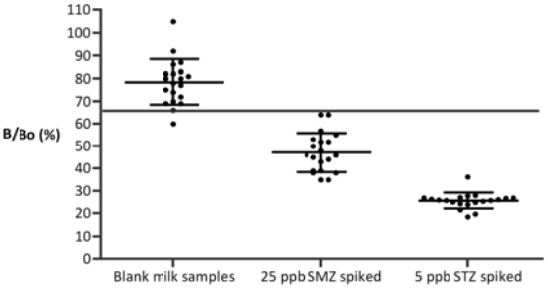

*Figure 6. Comparison between blank and 25 ppb **SMZ** – 5 ppb STZ spiked milk samples (n = 20). Each dot represents one single result, bars are mean B/B0 values±SD. The red line represents the B/B0 value of the LOD.*

EGGS

Solvent extraction

20 blank eggs were analysed by the IZS Umbria e Marche.

As a result, the mean B/B0 was 100±4%. To achieve 100% specificity, a LOD could be set at 93%. The same eggs were spiked with 10 ppb of **SMZ**, finding a mean relative binding of 36±7%, no false compliant results (100% sensitivity) and a mean difference to blanks' B/B0 of 64%. Results shown in Figure 7.

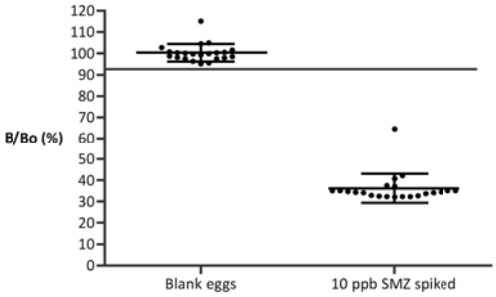

Figure 7. Comparison between blank and CCβ spiked eggs (n = 20). Each dot represents one single result, bars are mean B/B0 values ± SD. The red line represents the B/B0 value of the LOD.

Buffer extraction

An alternative, easier sample preparation was *in-house* validated on 60 eggs of different type, origin and farming procedure. Blanks had a mean relative signal of 93±8%. To achieve 100% specificity, the LOD was set at 77%. The same eggs were spiked with 15 ppb of **SMZ**, finding a mean relative binding of 64±7%, one false compliant result up to 60 determinations (99% sensitivity) and a mean difference to blanks' B/B0 of 29%.

FEED

Solvent extraction

The validation on 22 blank feed samples was carried out by the IZS Umbria e Marche.

Blanks had a relative signal of 96±7%. 90% specificity was achieved at 89% B/B0, corresponding to a concentration of **SMZ** lower than 66 ppb.

The same blank samples were then spiked at 1 ppm of **SMZ** and analysed by *I'Screen* SULFA. The mean B/B0 was 58±8%, with 100% sensitivity and a mean difference to blanks of 38%. Results shown in Figure 8.

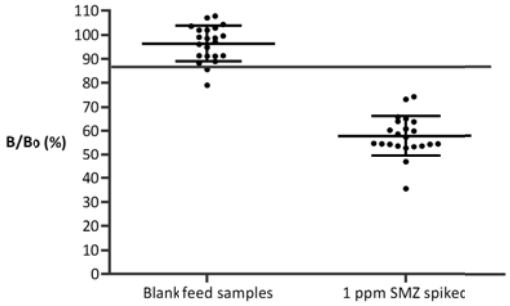

Figure 8. Comparison between blank and CCβ spiked feed samples (n = 22). Each dot represents one single result, bars are mean B/B0 values±SD. The red line represents the B/B0 value of the LOD.

References

R. Galarini, R. Buratti, B. Bertini, L. Persic. Validation of a high sensitivity ELISA kit for a broad range sulfonamides detection in food and feed. Poster presentation at RAFA. November 1st-4th, 2011. Prague, Czech Republic.

R. Galarini, F. Diana, S. Moretti, B. Puppini, G. Saluti, L. Persic. Development and validation of a new qualitative ELISA screening for multiresidue detection of sulfonamides in food and feed. Food control. 35. 2014. 300 – 310