Sensitive monoclonal antibody-based immunoassays for kresoxim-methyl analysis in QuEChERS-based food extracts

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ABSTRACT

Kresoxim-methyl is nowadays widely used to combat a diversity of common diseases affecting high-value crops. In this article, we report the development and characterization of two novel immunoassays for the analysis of this pioneer strobilurin fungicide, and for the first time, a validation study with food samples was performed. A direct and an indirect competitive immunoassay based on a new anti-kresoxim-methyl monoclonal antibody were developed for sensitive and specific chemical analysis. Optimized assays showed limits of detection of 0.1 µg/L. Fruit and vegetable samples were extracted with acetonitrile by the QuEChERS procedure and analyzed by the developed immunoassays after a simple dilution in buffer, affording limits of quantification below US and European maximum residue limits. Immunochemical results of samples from kresoxim-methyl-sprayed strawberry fields demonstrated good statistical agreement with gas chromatography coupled to mass spectrometry as reference technique.

INTRODUCTION

Kresoxim-methyl was one of the two first strobilurin pesticides to be registered back in 1992 (1). This fungicide is particularly active against Ascomycetes, such as *Venturia inaequalis*, *Podosphaera leucotricha*, *Leveillula taurica*, and *Botrytis cinerea*, which are responsible of a large variety of plant diseases (2). Like other strobilurins, kresoxim-methyl inhibits mitochondrial respiration of fungi, which prevents infection and makes it highly active (3). Although it is considered a low-hazard chemical to mammals (oral LD_{50} > 2000 mg/kg in rats) and bees, kresoxim-methyl is very toxic to aquatic organisms including fish (LD_{50} = 150 µg/L), plankton, and algae (4–6). Nowadays, kresoxim-methyl is being extensively used worldwide, with total global sales reaching 400 M€ in 2010 (7). Concomitantly, its residues in food have also risen, though to concentrations mainly below the legal maximum residue limits (MRL) – in the European monitoring program for 2006, kresoxim-methyl was encountered only in strawberries, whereas just two years later its residues were also found in carrots, cucumbers, and pears; and in 2009, up to six different food commodities contained measurable levels of this chemical (www.efsa.europa.eu/en/topics/topic/pesticides.htm). Kresoxim-methyl displays low metabolism in plants, being the original compound the main residue, and therefore the only target chemical for residue monitoring. European and US MRLs range between 0.05 and 1 mg/kg and between 0.15 and 1.5 mg/kg, respectively, for most fruits and vegetables [www.ec.europa.eu/sanco_pesticides; www.mrldatabase.com].

Current health and ecological concerns about chemical residues in food and environment has compelled private and public organizations to reinforce pesticide monitoring programs. Consequently, the diversity of analytical applications has grown, and tools with alternative performing properties are demanded. Since it is highly difficult for a method to be simultaneously sensitive, accurate, high capacitive, rapid, cheap, portable, and user and environmental friendly,

60 analysts should choose the best strategy for each particular demand. Nowadays, chromatographic
61 methods are the most employed techniques for pesticide residue determination. In 1998, Cabras
62 et al. (8) described the first applied approach for kresoxim-methyl analysis using gas
63 chromatography (GC) with mass spectrometry (MS) detection. Since then, a diversity of methods
64 for this chemical has been published, mainly as multiresidue strategies (9–11). Besides,
65 immunochemical methods have become complementary analytical tools for chemical residue
66 analysis. During the last decade, novel antibody-based kits for pesticide determination have been
67 steadily introduced into the market with different applications, also for strobilurin fungicides
68 [www.abraxiskits.com]. Undoubtedly, the most extended immunoanalytical method for small
69 organic chemicals is the competitive enzyme-linked immunosorbent assay (cELISA).

70 During the last years, basic studies regarding the relationship between the structure of
71 kresoxim-methyl haptens and the activity of the generated antibodies have been published by our
72 group (12, 13). Now, we herein report for the first time the application of competitive
73 immunoassays to the analysis of kresoxim-methyl in foodstuffs. Following development and
74 characterization of monoclonal antibody-based direct and indirect cELISAs, samples of
75 strawberries, cucumbers, and tomatoes were fortified with kresoxim-methyl and extracted using
76 the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) methodology (14) in order to
77 evaluate immunoassay performance. Validation of the newly developed rapid methods was
78 carried out by comparison with GC–MS through Deming regression analysis and Bland–Altman
79 plots using samples from sprayed crops.

80 **MATERIALS AND METHODS**

81 **Reagents and instruments.** The employed monoclonal antibody (KM*o*#117) and the
82 homologous assay conjugates using horseradish peroxidase (HRP–KM*o*) and ovalbumin (OVA–
83 KM*o*) were generated in our lab, and their preparation was described elsewhere (13). The affinity

84 to kresoxim-methyl of monoclonal antibody (mAb) KM*o*#117 clearly improves that of a former
85 antibody that we produced some years ago, at the beginning of this project (12). Rabbit anti-
86 mouse immunoglobulin polyclonal antibody conjugated to peroxidase (RAM–HRP) was from Dako
87 (Glostrup, Denmark). *o*-Phenylenediamine was purchased from Sigma/Aldrich (Madrid, Spain). 96-
88 well Costar flat-bottom high-binding polystyrene ELISA plates were purchased from Corning
89 (Corning, NY, USA). ELISA absorbances were read with a PowerWave HT from BioTek Instruments
90 (Winooski, VT, USA). Microwells were washed with an ELx405 microplate washer also from BioTek
91 Instruments.

92 Pestanal-grade kresoxim-methyl (methyl (*E*)-methoxyimino[α-(*o*-tolyloxy)-*o*-tolyl]acetate, CAS
93 registry number 143390-89-0, Mw 313.35) was purchased from Fluka/Riedel-de-Haën (Seelze,
94 Germany). Other pesticide standards were also form Fluka/Riedel-de-Haën or from BASF
95 (Limburgerhof, Germany), Bayer CropScience (Frankfurt, Germany), Dr. Ehrenstorfer (Augsburg,
96 Germany), or Syngenta (Basel, Switzerland). Triphenylphosphate (TPP) was from Sigma/Aldrich
97 (Madrid, Spain), and primary–secondary amine (PSA) for dispersive solid phase extraction cleanup
98 was from Scharlab (Barcelona, Spain). Chromatographic determinations were carried out with a
99 6890N GC apparatus furnished with a 7683 Series autosampler, a HP-5MS (30 m × 0.25 mm × 0.25
100 µm) capillary column, and a quadrupole 5973N mass detector, all from Agilent Technologies (Santa
101 Clara, CA, USA).

102 **Immunoassays.** *General procedures.* Eight-point standard curves, including a blank, were
103 prepared in borosilicate glass tubes by 10-fold serial dilution in PBS starting from a 100 µg/L
104 solution also in PBS. Pesticide concentrated stock solutions (100 mg/L) in anhydrous
105 *N*,*N*-dimethylformamide – kept at −20 °C in amber glass vials – were used to prepare the first
106 standard point. Experimental values were fitted using the SigmaPlot software (Systat Software
107 Inc., Chicago, IL, USA) to a four-parameter logistic equation:

$$y = (A_{max} - A_{min})/[1+(x/C)^B]+A_{min}$$

were A_{max} is the absorbance that was reached in the absence of analyte, A_{min} is the background signal, C is the analyte concentration at the inflexion point of the sigmoidal curve, and B is the slope at the inflexion point. Curves were normalized and average values were calculated from independent experiments.

The concentration of kresoxim-methyl inducing a 50% inhibition (IC_{50}) of the reaction between antibody and hapten conjugate was taken as the key criteria for assay characterization. The limit of detection (LOD) was calculated as the analyte concentration causing a 10% inhibition (IC_{10}) of the immunochemical reaction. Cross-reactivity (CR) was estimated as a percentage value from the quotient between the IC_{50} value for kresoxim-methyl and the IC_{50} for the studied compound, both in molar concentration units.

Antigen-coated indirect competitive ELISA. Microplates were coated by overnight incubation with 100 µL per well of OVA–KM*o* conjugate solution in 50 mM carbonate buffer, pH 9.6. All incubation steps were performed at room temperature with sealed plates, and after each step, microwells were washed four times with washing solution (150 mM NaCl with 0.05% (v/v) Tween 20). The competitive step was carried out during 1 h with 50 µL per well of analyte solution in PBS plus 50 µL per well of a dilution of antibody KM*o*#117 in PBST (PBS containing 0.05% (v/v) Tween 20). Retained mAb was amplified by incubation during 1 h with 100 µL per well of RAM–HRP diluted 1/2000 in PBST. Finally, signal was generated with 100 µL per well of 0.012% (v/v) H_2O_2 in 62 mM phosphate and 25 mM citrate, pH 5.4 containing 2 g/L of *o*-phenylendiamine. Ten minutes later, enzymatic activity was stopped with 100 µL per well of 2.5 M H_2SO_4. Absorbance was immediately read at 492 nm using 650 nm as reference wavelength.

Antibody-coated direct competitive ELISA. Polystyrene plates were coated by overnight incubation with 100 µL per well of a solution of antibody KM*o*#117 in 50 mM carbonate buffer, pH

9.6. All incubation steps were carried out at room temperature, and after each incubation step, microwells were washed as described above. The competitive step was performed during 1 h with sequential addition of 50 µL per well of analyte solution in PBS and 50 µL per well of a solution of tracer HRP–KM*o* in PBST. Signal was generated and plates were read as before.

Solvent, detergent, and buffer studies. The influence of acetonitrile was evaluated using kresoxim-methyl standard curves in PBS with different solvent contents, whereas antibody or tracer solutions were prepared in PBST. Variation of A_{max} and IC_{50} values due to Tween 20 concentration was also assessed. Moreover, a central composite design was followed for buffer studies, consisting of a two-level full factorial design (α = 1.414) with two factors (pH and ionic strength) and three replicates that included 12 cube, 12 axial, and 15 center points; that is, 39 randomized assays under 9 different buffer conditions (**Table S1** in the Supplementary Material file). The corresponding 9 buffers were set up from a 40 mM citrate, 40 mM phosphate, and 40 mM Tris solution, as described elsewhere (15). Ionic strength and pH values of each buffer were adjusted using 2 M NaCl and 5 M HCl, respectively. All buffers contained 0.05% (v/v) Tween 20. Kresoxim-methyl was prepared in Milli-Q water, and antibody or tracer solutions were diluted in the studied buffers. A_{max} and IC_{50} values of the 39 resulting curves were fitted as functions of pH and ionic strength using the Minitab software (Minitab Inc., State College, PA, USA).

150 **Sample treatment and analysis.** Tomatoes, cucumbers, and strawberries were obtained from
151 local supermarkets and extracted using the QuEChERS method (14). Briefly, 20 g of homogenate
152 was mixed under vigorous stirring with 2 g of sodium acetate and 8 g of anhydrous magnesium
153 sulfate in 20 mL of acetonitrile containing 1% (v/v) acetic acid. The organic phase was separated by
154 centrifugation at 2200×g during 5 min, and a 10 mL aliquot was cleaned up by vortexing with PSA
155 (500 mg) in the presence of 1.5 g of anhydrous magnesium sulfate. After a second centrifugation
156 step, extracts were filtrated with a Teflon filter (0.2 µm) and stored at −20 °C. Extracts were
157 fortified with kresoxim-methyl, diluted 50-fold in PB (100 mM phosphate, pH 7.4), and analyzed by
158 the developed cELISAs using antibody or tracer solutions prepared in PBT (PB containing 0.05%
159 (v/v) Tween 20). A kresoxim-methyl standard curve in PB was run in each plate.

160 In order to better simulate real-world situations, greenhouse strawberry crops were sprayed
161 with a nebulizer using a commercial kresoxim-methyl formulation from BASF (Stroby), which was
162 prepared as recommended by the manufacturer (100 mg/L kresoxim-methyl in water containing
163 20% (v/v) alkyl polyglycol ether). Samples were collected at different days to obtain positive
164 samples covering a wide range of kresoxim-methyl concentrations, homogenized, and stored
165 frozen at −20 °C. Residues were extracted by the described QuEChERS procedure and analyzed by
166 the optimized cELISAs and GC–MS. Immunochemical determinations were performed as described
167 for spiked samples. For chromatographic analysis, one microliter of clean extract containing
168 500 µg/L of TPP as internal standard was injected in splitless mode at 280 °C, employing helium as
169 carrier with a steady flow of 1 mL/min. The temperature of the oven (110 °C) was held during 1
170 min; then, it was increased at a rate of 15 °C/min until 280 °C and kept constant at the final
171 temperature during 15 min. Electron impact ionization at 70 eV was used with the ion source at
172 225 °C. The employed quantification ions were *m/z* 116 and 131 for kresoxim-methyl and *m/z* 325
173 and 326 for TPP. Retention times were 11.0 and 14.0 min for kresoxim-methyl and TPP,

174 respectively. For method validation, Deming regression and Bland–Altman analysis were applied
175 using the SigmaPlot software (version 12.0).

176 **RESULTS AND DISCUSSION**

177 **Assay selectivity.** Two mAb-based immunoassays for kresoxim-methyl were studied. Both
178 assays employed mAb KM*o*#117 and the homologous conjugate but different cELISA formats (for
179 hapten structure, see **Figure S1** in the Supplementary Material file). Immunoassay selectivity with
180 structurally related compounds was assessed towards the main strobilurin fungicides
181 (trifloxystrobin, azoxystrobin, picoxystrobin, dimoxystrobin, metominostrobin, orysastrobin,
182 pyraclostrobin, and fluoxastrobin; see **Figure S2**). In both cELISA formats, antibody KM*o*#117 was
183 highly selective to kresoxim-methyl (CR values with other strobilurins were below 1%). On the
184 other hand, recognition towards chemicals potentially present in real samples was verified using
185 active principles that are commonly formulated together with kresoxim-methyl, such as boscalid,
186 fenpropimorph, epoxiconazole, propiconazole, tebuconazole, and pyrimethanil, and none of them
187 was noticeably recognized by mAb KM*o*#117.

188 **Tolerance to solvents and detergents.** QuEChERS methodology for pesticide extraction from
189 food matrices consists of a liquid phase extraction of homogenates with acetonitrile. Hence,
190 tolerance of the described cELISAs to that solvent was appraised. The A_{max} and IC_{50} values of
191 inhibition curves that had been run in the presence of different amounts of acetonitrile (from 0.5%
192 to 10%, v/v) were compared to those obtained in the absence of solvent (see **Figure 1**). Only a
193 slight influence over the assay signal was observed, and up to 2% acetonitrile was fairly well
194 tolerated by the studied immunoassays.

195 Immunochemical competitive reactions are usually performed in the presence of different
196 additives. Tween 20 is a common detergent for unspecific binding minimization; however, it has

been often shown to exert a negative effect over the analytical parameters of immunoassays for small organic analytes (16). The relative variation of A_{max} and IC_{50} values in the presence of different concentrations of Tween 20 (from 0% to 0.1%, v/v), taking 0.025% as a reference detergent concentration, is depicted in **Figure 1**. A similar behavior was observed with the two studied cELISAs, *i.e.*, Tween 20 increased the A_{max} of both assays, but it also increased the IC_{50} value.

Influence of pH and ionic strength. The analytical influence of physicochemical conditions such as pH and ionic strength was evaluated. Competitive assays were performed following a biparametric study with composite design in which the center point conditions (pH = 7.5 and *I* = 175 mM at 25 °C) were similar to those of PBS. A series of buffers was prepared with a mixture of citrate, phosphate, and Tris in order to cover a wide effective pH range. The ionic strength of each buffer was fixed with NaCl, and Tween 20 was added. **Figure 2** shows the overlaid contour plots for the responses (A_{max} and IC_{50} values) to pH and ionic strength changes of the two studied immunoassays; taking the results at center point conditions as the reference values. A constricted area for acceptable pH and ionic strength variations (the area stretching changes on A_{max} and IC_{50} values below ±20%; area in white color) was found for the indirect cELISA. With this assay, at pH 7.5, ionic strength values below 150 mM increased both A_{max} and IC_{50} values above tolerable levels (> 120%), whereas values over 200 mM decreased excessively the A_{max} (< 80%). On the contrary, the direct cELISA was very robust – A_{max} and IC_{50} changes stayed below ±20% – to alterations of either pH or ionic strength conditions (white area in lower graph of **Figure 2**).

Immunoassay validation. The assays were validated by investigating the LOD, LOQ, trueness, and repeatability. Final assay conditions of the developed cELISAs and the optimized standard inhibition curves can be found in **Table 1**. Optimum antibody concentrations were 100 and 300 µg/L for the indirect and direct format, respectively, whereas optimum assay conjugate concentrations were 100 µg/L for the former and 30 µg/L for the latter. Signals in the absence of analyte (A_{max}) were high (around 2.0), background signals (A_{min}) were low, and slopes were moderate (between −1.0 and −1.2). Under those conditions, the IC_{50} values for kresoxim-methyl were below 1 µg/L for both cELISAs, and the LODs were around 0.10 µg/L. Attempts to reduce these values by decreasing immunoreagent concentrations just resulted in lower A_{max} without a concomitant effect on assay sensitivity.

To evaluate the trueness and precision, the described immunoassays were applied to the analysis of kresoxim-methyl in diverse fortified foodstuffs. Nowadays, this pesticide is employed against a variety of plant diseases in cucumber, tomato, and strawberry crops. Extracts of those food samples were prepared following a QuEChERS procedure that was essentially based on the AOAC Official Method 2007.01 for pesticide extraction from food matrices (17). Homogenized foodstuffs were treated with acetonitrile in the presence of buffered saline and $MgSO_4$, and then a clean-up step using PSA was performed. Extracts from kresoxim-methyl-free samples (as judged by GC–MS) were fortified at four concentration levels and analyzed with the optimized immunoassays after a simple dilution in buffer. In general, quantitative recoveries (between 70% and 120%) and good precision values (relative standard deviation below 20%) were retrieved with each cELISA (**Table 2**), in accordance to the EU validation guidelines for pesticide residues analysis in food (18). For both immunoassays, the limit of quantification (LOQ) for the studied foodstuffs was set at 0.01 mg/kg, which is lower than the European MRLs for kresoxim-methyl in these food products (0.05, 0.5, and 1.0 mg/kg for cucumbers, tomatoes, and strawberries, respectively).

As further validation of the developed cELISAs, strawberry samples from crops that we had sprayed with a commercial formulation of kresoxim-methyl were employed as model and relevant commodity in order to evaluate the performance of our novel analytical methods under real-like conditions. Fruits were extracted following a QuEChERS procedure and then analyzed by the optimized immunoassays and by GC–MS (**Table S2**). Trueness of the developed cELISAs was also assessed by statistical method comparison using the Deming regression, which accounts for errors in observations on both methods. Orthogonal regression analysis showed that the results provided by both cELISAs were statistically comparable to those retrieved by the reference method; *i.e*. the 95% confidence intervals for the intercept and for the slope included 0 and 1, respectively (**Table 3**). The corresponding regression lines can be seen in **Figure S3**. Moreover, good correlation between the studied immunochemical technique and the reference chromatography approach was evidenced by the Bland–Altman plot (**Figure 3**), as the average values of both methods were randomly distributed around the average difference, and they were mostly inside the limits of agreement (average difference ± 1.96s), meaning that only random deviations occurred.

In summary, two mAb-based immunoassays to kresoxim-methyl – one indirect and one direct cELISA – have been characterized, optimized, and validated using food samples. These assays showed no CR with the most common strobilurin pesticides or a series of fungicides potentially present in relevant foodstuffs. A relative tolerance to acetonitrile contents was observed, and lower influence of pH and ionic strength changes over assay analytical parameters was found with the direct assay. Both immunoassays showed IC_{50} values below 1 µg/L under optimized conditions. The LOQs were fixed at 10 µg/kg from the analysis of fortified tomato, cucumber, and strawberry samples which had been extracted by the QuEChERS method. Overall, good recoveries and precision values were found. Method trueness was also demonstrated by comparison with a

reference chromatographic method through statistical analysis using Deming regression and Bland–Altman plot.

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Limited amounts of the described immunoreagents are available upon request.

ASSOCIATED CONTENT

Supporting Information

Studied buffer conditions, chemical structure of hapten KM*o*, chemical structures of strobilurins, raw data from the analysis of in-field treated samples, and Deming regression lines. This material is available free of charge via de Internet at http://pubs.acs.org.

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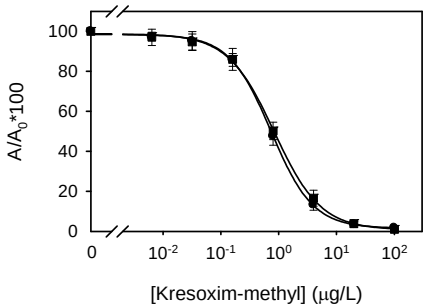
FIGURE LEGENDS

Figure 1. Influence of acetonitrile (upper graph) and Tween 20 (lower graph) contents over A_{max} and IC_{50} values of the studied immunoassays.

Figure 2. Overlaid contour plots for the A_{max} and IC_{50} dependence upon pH and ionic strength conditions of the studied immunoassays. White areas set the limits of acceptable pH and *I* conditions; those with A_{max} (red) and IC_{50} (green) variations between 80% (solid line) and 120% (dashed line), taking as a reference (100%) the average of the A_{max} and IC_{50} values of the center point conditions of the composite design.

Figure 3. Bland–Altman dispersion for comparison of results obtained by the developed cELISAs and by a reference chromatographic method. Samples were analyzed three times by the immunochemical methods and twice by GC–MS.

Table 1. Conditions and parameters of the optimized immunoassays.[a]



	Immunoassay	
Format	Indirect cELISA	Direct cELISA
mAb	KM*o*#117	KM*o*#117
	100 μg/L	300 μg/L
Conjugate	OVA–KM*o*	HRP–KM*o*
	100 μg/L	30 μg/L
A_{max}	2.390 ± 0.254	2.037 ± 0.208
A_{min}	0.039 ± 0.015	0.021 ± 0.009
Slope	−1.198 ± 0.059	−1.082 ± 0.056
IC_{50} (μg/L)	0.738 ± 0.032	0.838 ± 0.044
LOD (μg/L)	0.103	0.097
Buffer	PB + 0.025% Tween 20	PB + 0.025% Tween 20
Time (h)	2.5	1.5

[a] Values are the mean of 16 independent experiments.

Table 2. Recoveries and precision values obtained by analyzing replicate spiked samples (n=4).

Sample	Spiked [KM][a] (µg/kg)	Indirect			Direct		
		Found [KM] (µg/kg)	RSD (%)	Recovery (%)	Found [KM] (µg/kg)	RSD (%)	Recovery (%)
Tomato	10	9.1 ± 0.8	8.8	91.3 ± 7.7	11.6 ± 1.4	12.1	75.5 ± 21.9
	30	29.1 ± 3.8	13.1	97.0 ± 12.8	32.8 ± 4.8	14.6	95.8 ± 15.2
	100	103.2 ± 7.5	7.3	103.2 ± 7.5	110.8 ± 3.9	3.5	110.8 ± 3.9
	300	313.2 ± 28.8	9.2	104.4 ± 9.6	361.2 ± 9.3	2.6	120.5 ± 3.1
Cucumber	10	8.1 ± 0.9	11.1	81.3 ± 9.1	7.5 ± 1.1	14.7	75.4 ± 10.7
	30	30.4 ± 5.5	18.1	101.2 ± 18.2	29.5 ± 3.3	11.2	98.3 ± 10.9
	100	104.4 ± 6.4	6.1	104.4 ± 6.4	116.3 ± 4.0	3.4	116.3 ± 4.0
	300	306.0 ± 12.3	4.0	102.0 ± 4.1	336.0 ± 26.4	7.9	112.0 ± 8.8
Strawberry	10	9.7 ± 1.9	19.6	97.2 ± 18.7	11.5 ± 1.6	13.9	115.1 ± 15.8
	30	29.0 ± 5.3	18.3	96.7 ± 17.6	34.4 ± 5.6	16.3	114.7 ± 18.7
	100	100.0 ± 19.2	19.2	100.0 ± 19.2	120.3 ± 8.3	6.9	120.3 ± 8.3
	300	308.4 ± 60.9	19.7	102.8 ± 20.3	360.6 ± 12.0	3.3	120.7 ± 4.0

[a] KM: kresoxim-methyl

Table 3. Correlation between the developed immunoassays and GC–MS by Deming regression of in-field treated samples.

cELISA	Intercept	95% confidence interval for intercept	Slope	95% confidence interval for slope	r[a]	N[b]
Indirect	0.015 ± 0.027	[−0.045; 0.074]	1.03 ± 0.04	[0.94; 1.12]	0.9838	14
Direct	0.055 ± 0.026	[−0.003; 0.112]	0.99 ± 0.03	[0.91; 1.06]	0.9882	14

[a] Correlation coefficient. [b] Number of samples.

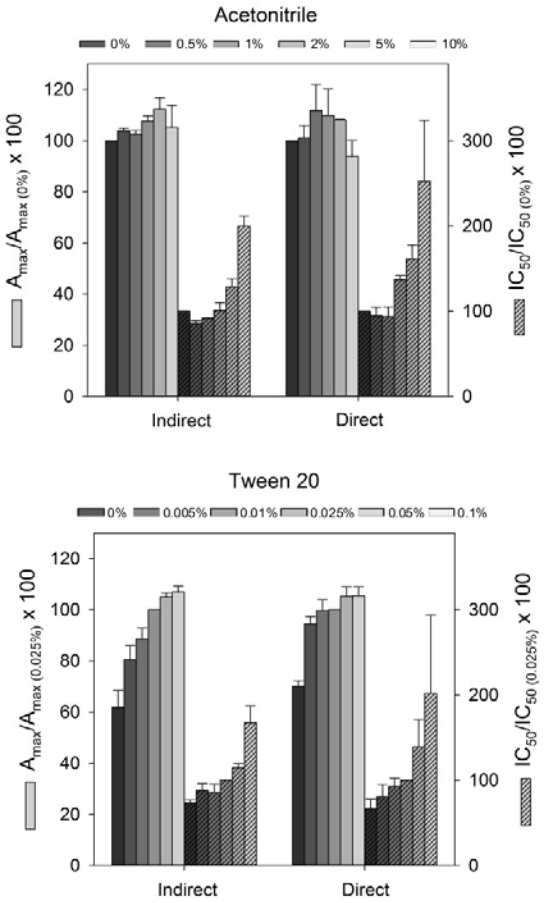

Acetonitrile
0%
0.5%
1%
2%
5%
10%
$A_{max}/A_{max\ (0\%)}$ x 100
$IC_{50}/IC_{50\ (0\%)}$ x 100
Indirect
Direct
Tween 20
0%
0.005%
0.01%
0.025%
0.05%
0.1%
$A_{max}/A_{max\ (0.025\%)}$ x 100
$IC_{50}/IC_{50\ (0.025\%)}$ x 100
Indirect
Direct

Figure 1

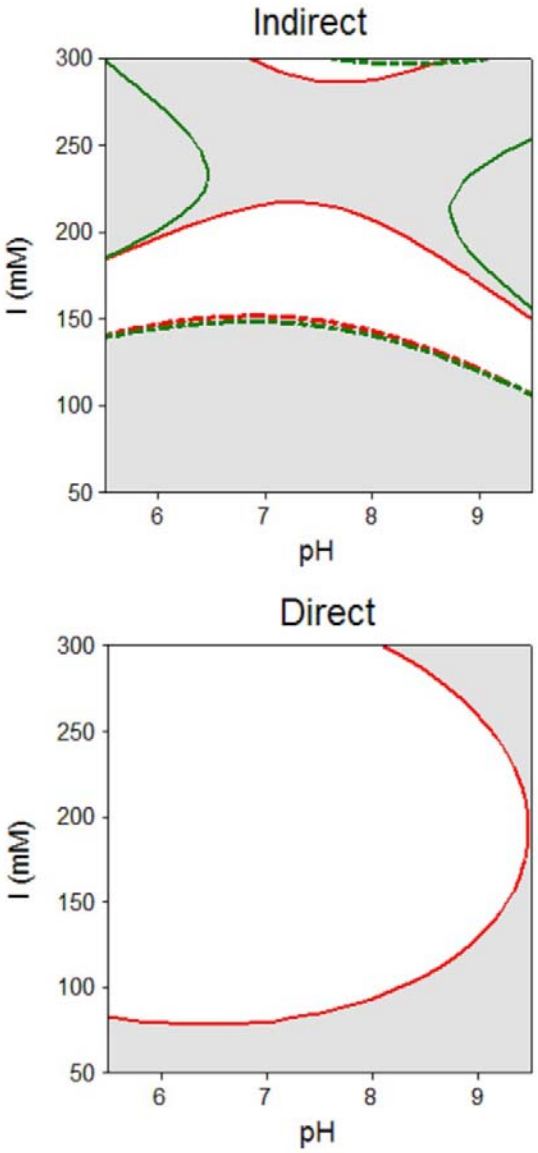

Indirect
300
250
200
150
100
50
I (mM)
6
7
8
9
pH
Direct
300
250
200
150
100
50
I (mM)
6
7
8
9
pH

Figure 2

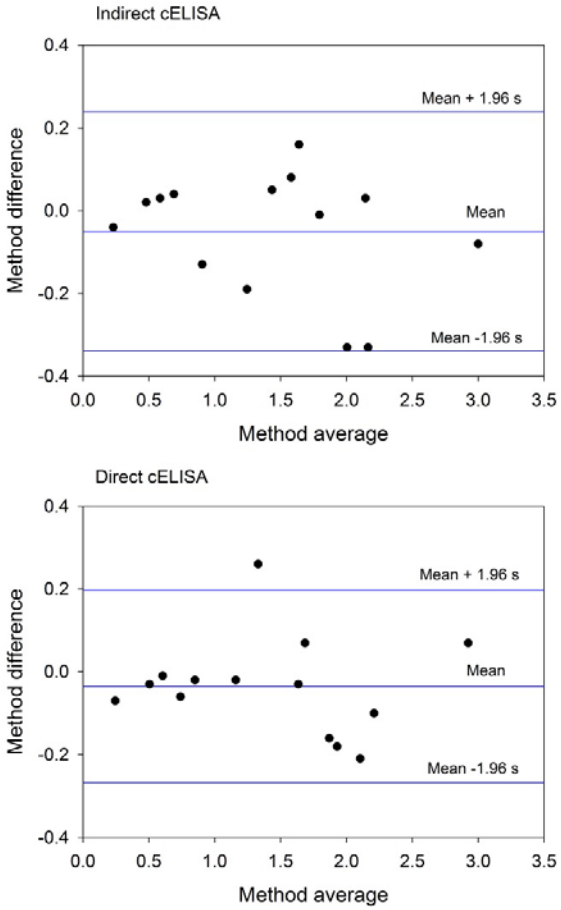
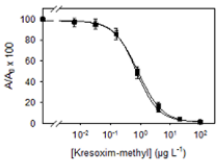
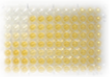
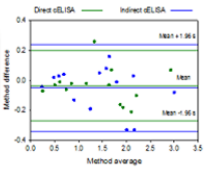

Indirect cELISA
Mean + 1.96 s
Mean
Mean -1.96 s
Method difference
Method average
Direct cELISA
Mean + 1.96 s
Mean
Mean -1.96 s
Method difference
Method average

Figure 3

TOC Graphic







	cELISA	
Format	Direct	Indirect
mAb	KMo#117	KMo#117
Conjugate	HRP–KMo	OVA–KMo
A_{max}	2.037	2.390
IC_{50} (µg L^{-1})	0.838	0.738
LOD (µg L^{-1})	0.097	0.103