

Introduction

Endoparasites known as helminths may be divided into three categories: nematodes (roundworms); cestodes (tapeworms); and trematodes (flukes). Infections with parasitic helminths represent a significant economic and welfare burden to the global ruminant livestock industry. Their extensive use in food producing animals could result in the presence of residues in milk. For industry and consumer protection, it is critical to monitor the levels of these residues to ensure regulatory compliance.

Therefore, screening methods for their detection are beneficial in test settings. Biochip Array Technology (BAT) is a multi-analytical platform which allows the simultaneous detection of multiple analytes from a single undivided sample. The aim of this study was to apply BAT to the quantitative screening of anthelmintics in milk samples to comply with EU regulatory limits.

Methodology

Twelve competitive chemiluminescent immunoassays were employed. The immunoassays were applied to the semi-automated biochip analyser, Evidence Investigator (EV3602, Radox Food Diagnostics, Crumlin, UK). The instrument

includes dedicated software which automatically processes, reports and archives the sample data generated.

Milk samples were prepared by a single, simple solvent extraction.

Immunoassays			
Amino benzimidazoles	Clorsulon	Morantel/Oxantel/Pyrantel	Oxyclosanide
Avermectins	Closantel/Rafoxanide	Moxidectin	Thiabendazole
Benzimidazoles	Levamisole	Nitroxynil	Triclabendazole

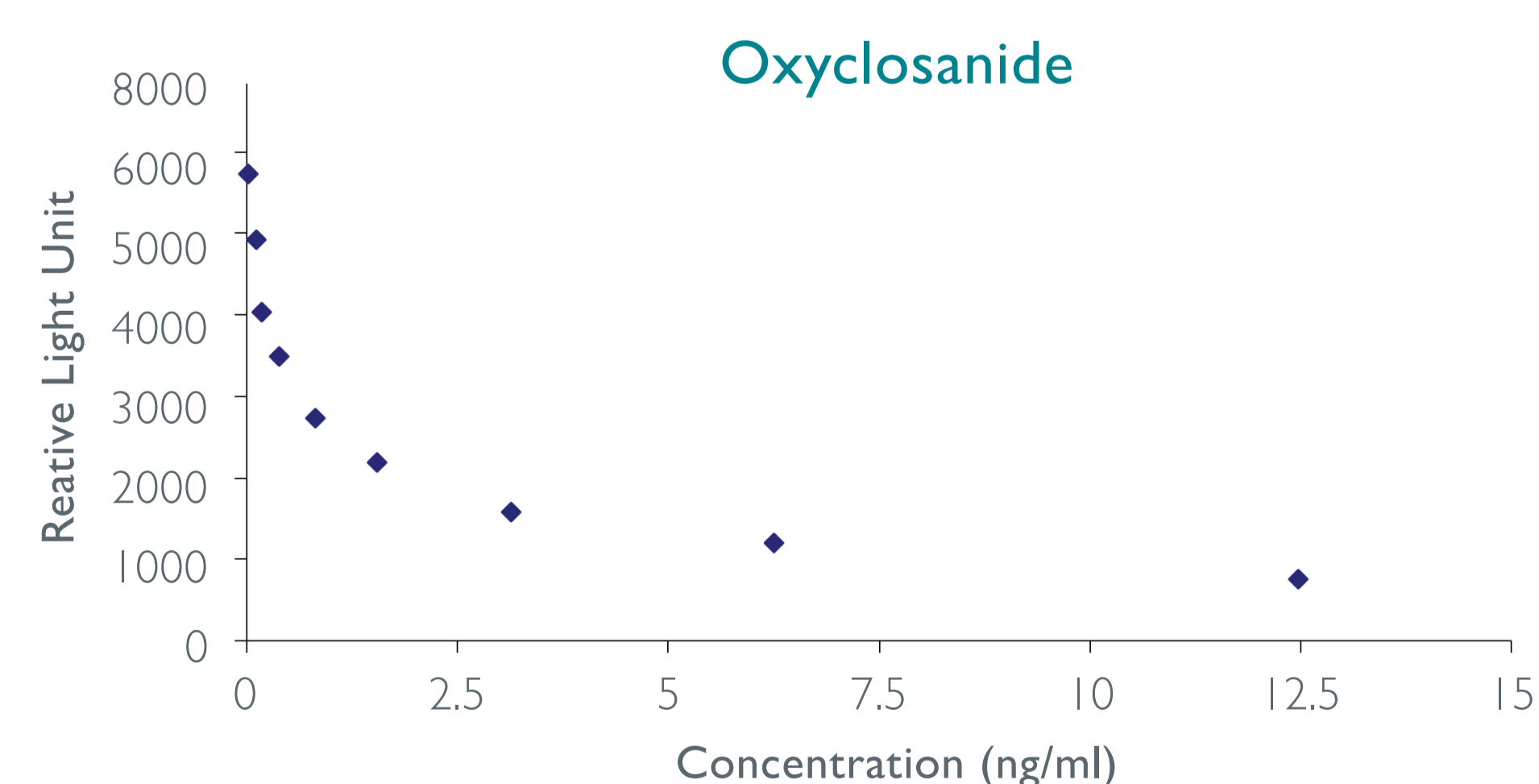
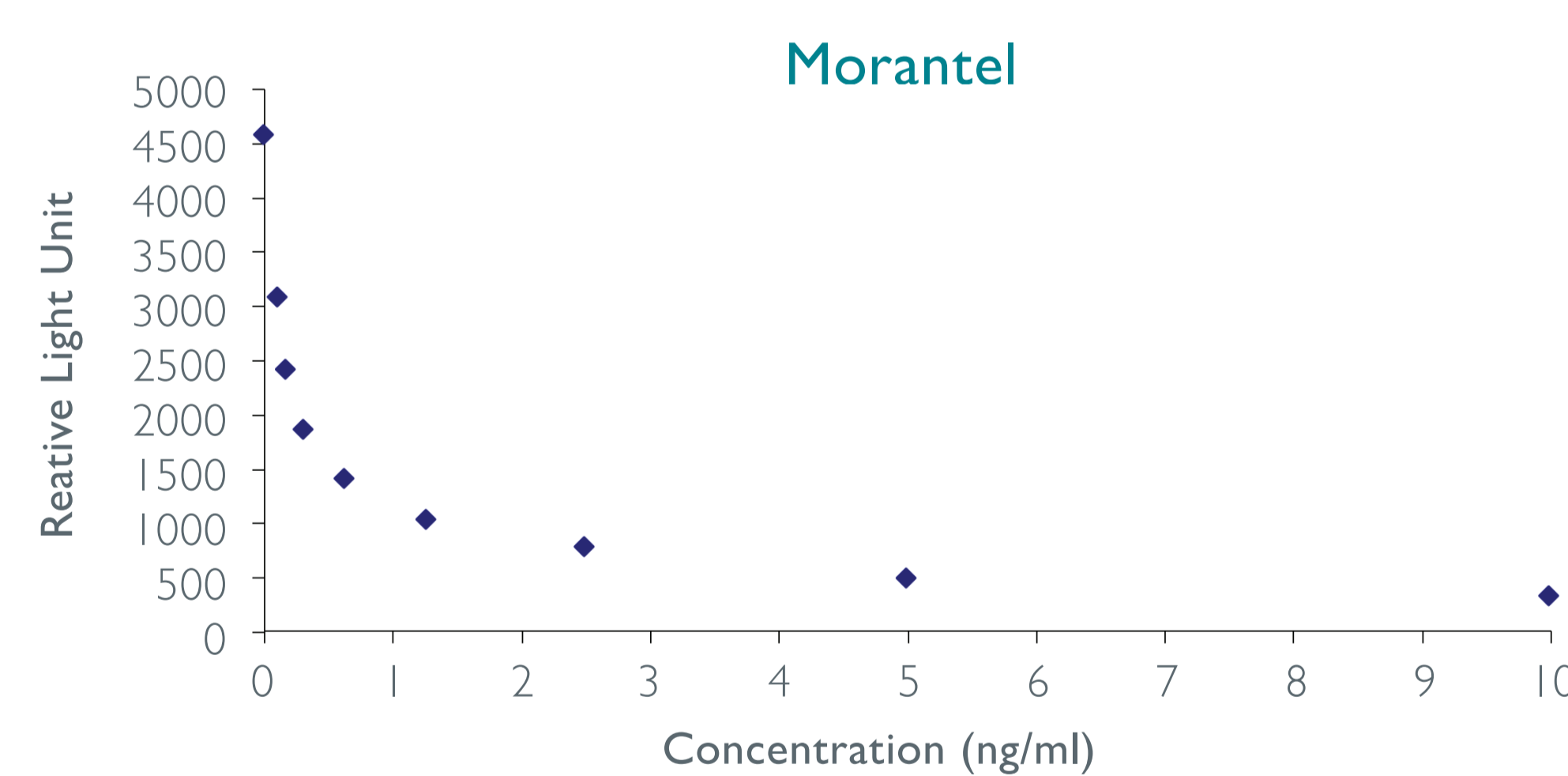
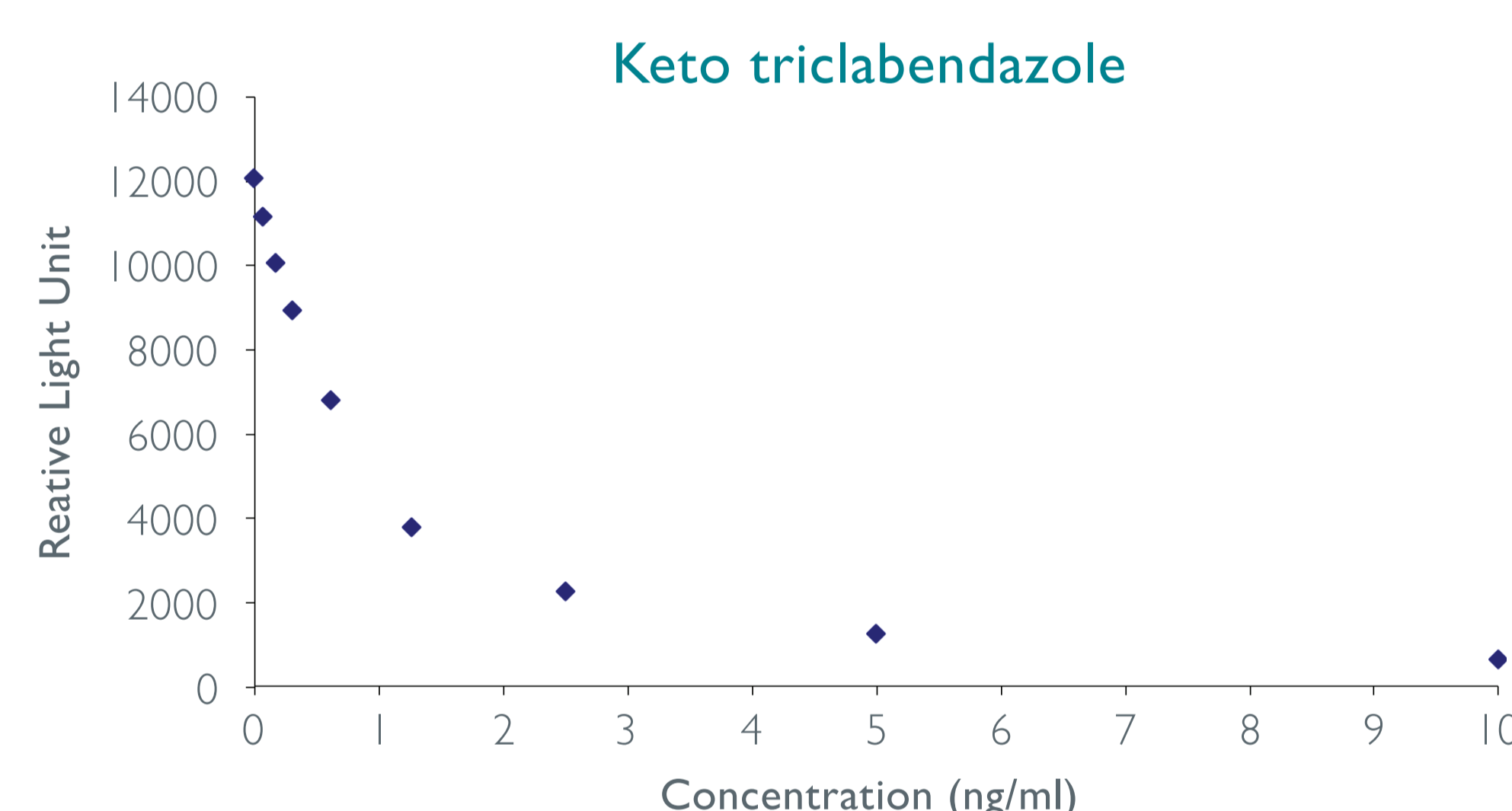
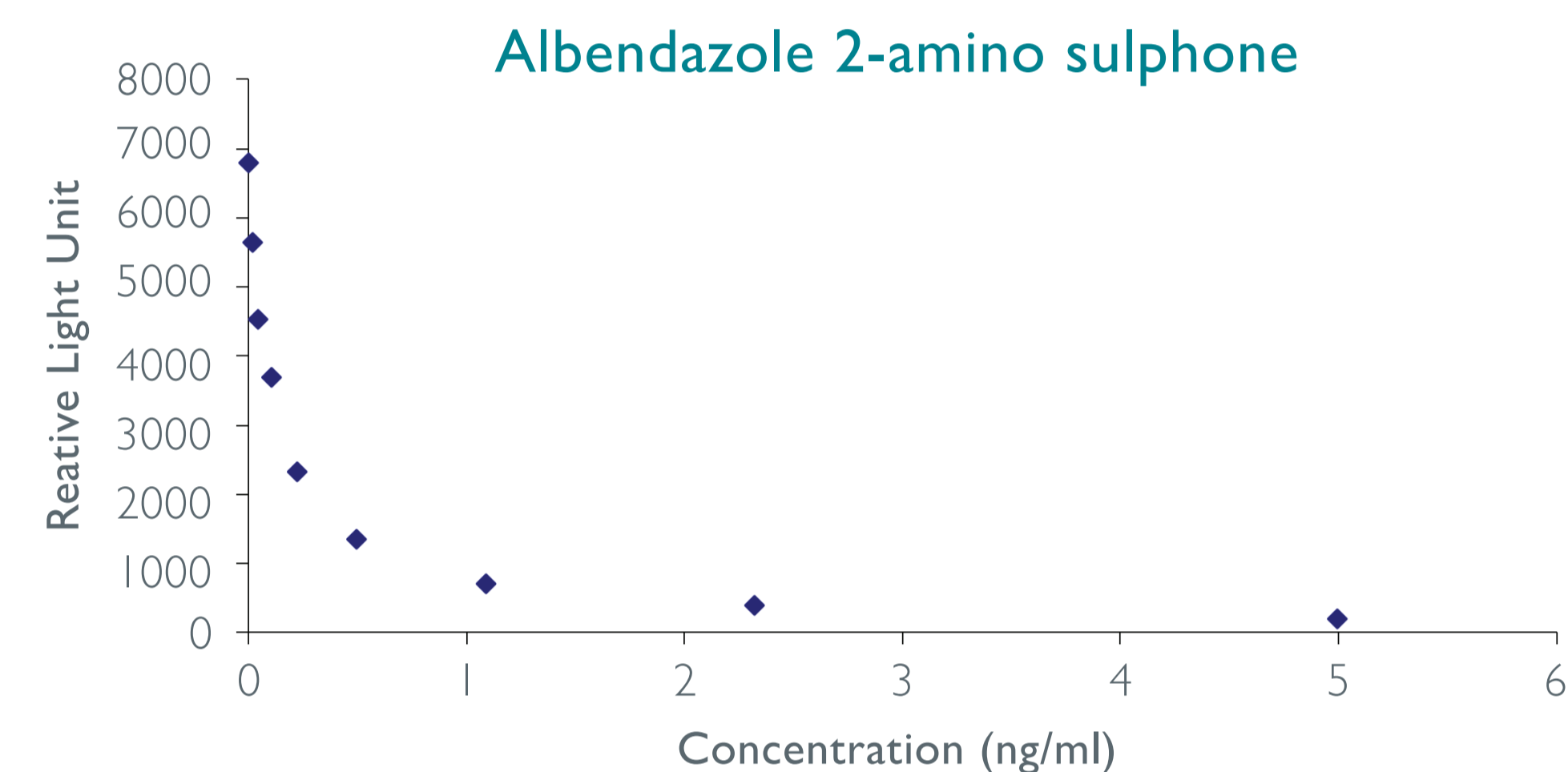
Results

Initial evaluation of the biochip based immunoassays is presented.

Sensitivity

Analyte	IC ₅₀ (ng/mL)	LOD (ng/mL)
Abamectin	1.36	0.42
Albendazole	0.32	1.00
Albendazole sulphoxide	0.32	1.01
Albendazole sulphone	0.18	0.56
Albendazole 2-amino sulphone	0.14	0.30
Amino-flubendazole	0.14	0.30
Amino-mebendazole	0.10	0.21
Cambendazole	0.32	0.06
Carbendazim	2.45	10.00
Closantel	27.61	<27.61
Clorsulon	3.02	<3.02
Doramectin	3.23	1.00
Emamectin Benzoate	0.96	0.30
Eprinomectin	1.27	0.39
Fenbendazole	3.12	10.00
Flubendazole	1.13	3.45
Hydroxy Flubendazole	18.67	50.00
Hydroxy Mebendazole	21.98	100.00
5-Hydroxythiabendazole	1.25	0.55
Ivermectin	2.43	0.75
Keto-triclabendazole	0.62	0.16
Levamisole	21.50	2.00
Mebendazole	1.84	5.56
Morantel	0.19	<0.19
Moxidectin	4.48	1.60
Nitroxynil	0.31	<0.31
Oxantel	0.26	<0.26
Oxfendazole (Fenbendazole sulphoxide)	0.81	2.50
Oxfendazole sulphone (Fenbendazole sulphone)	1.81	7.14
Oxibendazole	0.68	2.08
Oxyclosanide	0.70	<0.7
Parbendazole	0.67	3.33
Pyrantel	0.08	<0.08
Rafoxanide	33.09	<33.09
Thiabendazole	1.14	0.50
Triclabendazole	2.27	0.60
Triclabendazole Sulphoxide	1.97	0.52
Triclabendazole Sulphone	20.57	5.45

Typical calibration curves



Conclusion

The application of Biochip Array Technology to the detection of anthelmintic drugs shows that all marker residues for endoparasites in milk stipulated in Commission Regulation (EU) No 37/2010 of 22 December 2009 can be detected simultaneously.

This application greatly assists both routine monitoring or surveillance of milk compliance.

Reference

Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Off J Eur Commun L15.