

APPLICATION OF AN ENZYME LINKED IMMUNOSORBENT ASSAY TO THE DETECTION OF ALL PREVALENT TWELVE ERGOT ALKALOIDS IN CEREALS AND CEREAL BASED ANIMAL FEED PROPOSED FOR EUROPEAN COMMISSION REGULATION

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FOOD DIAGNOSTICS

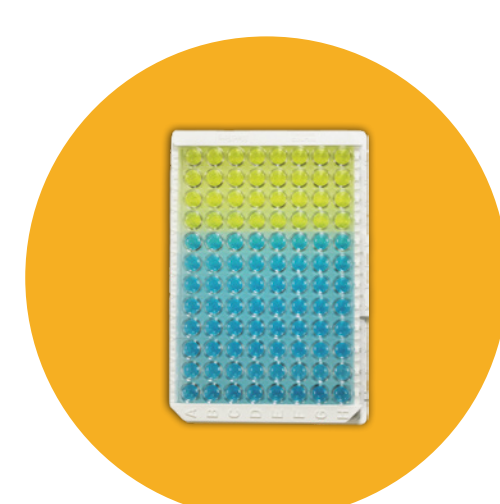
Introduction

The determination of ergot alkaloids in cereals and feed is important for food and feed safety as they are undesirable contaminants and can cause adverse health effects in humans and animals. The European Commission (EC) is considering implementation of maximum levels for a total of twelve main ergot alkaloids in cereal grains for human consumption. Recently, the draft of the proposed legislation with the proposed matrices was released. In this context, the availability of screening methods allowing the detection of ergot alkaloids from proposed cereal grains and their corresponding milling products is relevant.

Aiming to the detection of the twelve ergot alkaloids (ergotamine, ergocristine, ergocryptine, ergocornine, ergometrine, ergosine and their inine forms) in cereal grains, cereal milling product and cereal-based feed, as proposed by the EC; this study reports the analytical performance of an enzyme-linked immunosorbent assay (ELISA) for the detection of these ergot alkaloids in the proposed matrices.

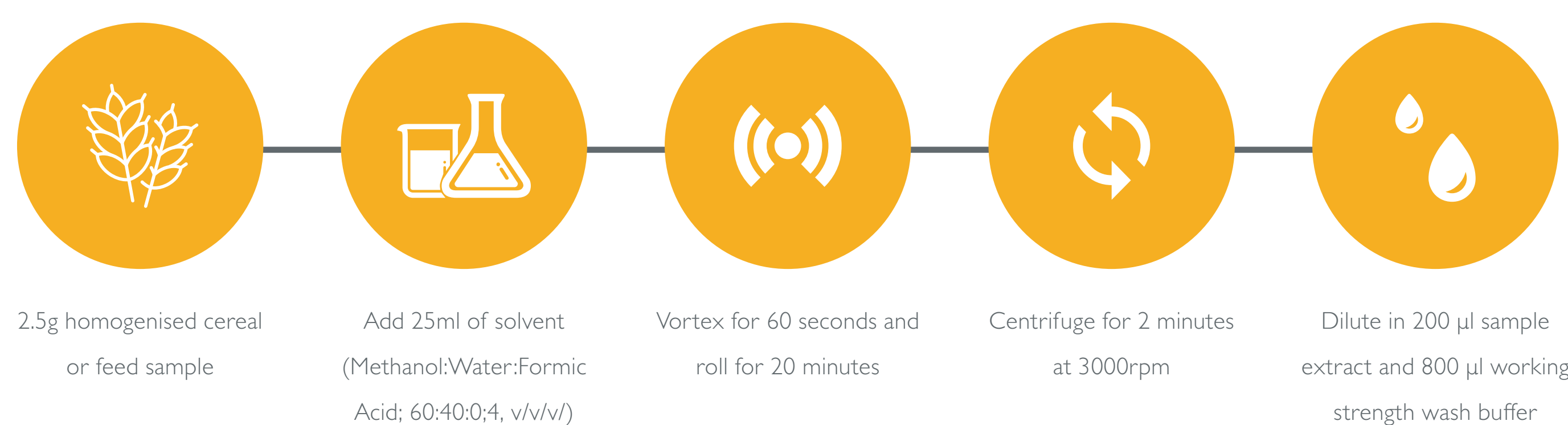
Methodology

The competitive ELISA was validated according to Commission Regulation (EU) 519/2014 for proposed in draft legislation matrices.



Detection of 12 prevalent ergot alkaloids

Cereal grains, cereal milling products and feed samples were extracted by a single generic solid/liquid extraction. Screening results were semi-quantitative.

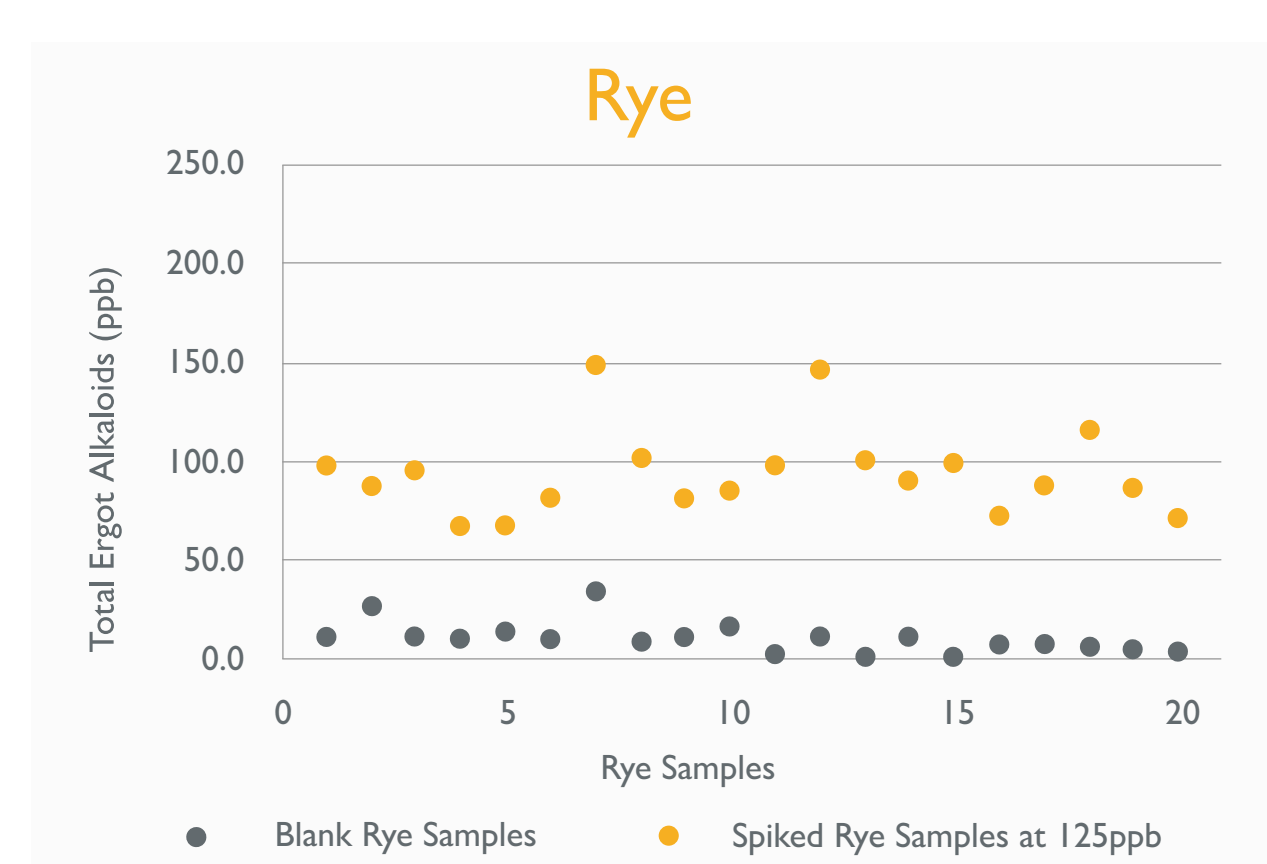
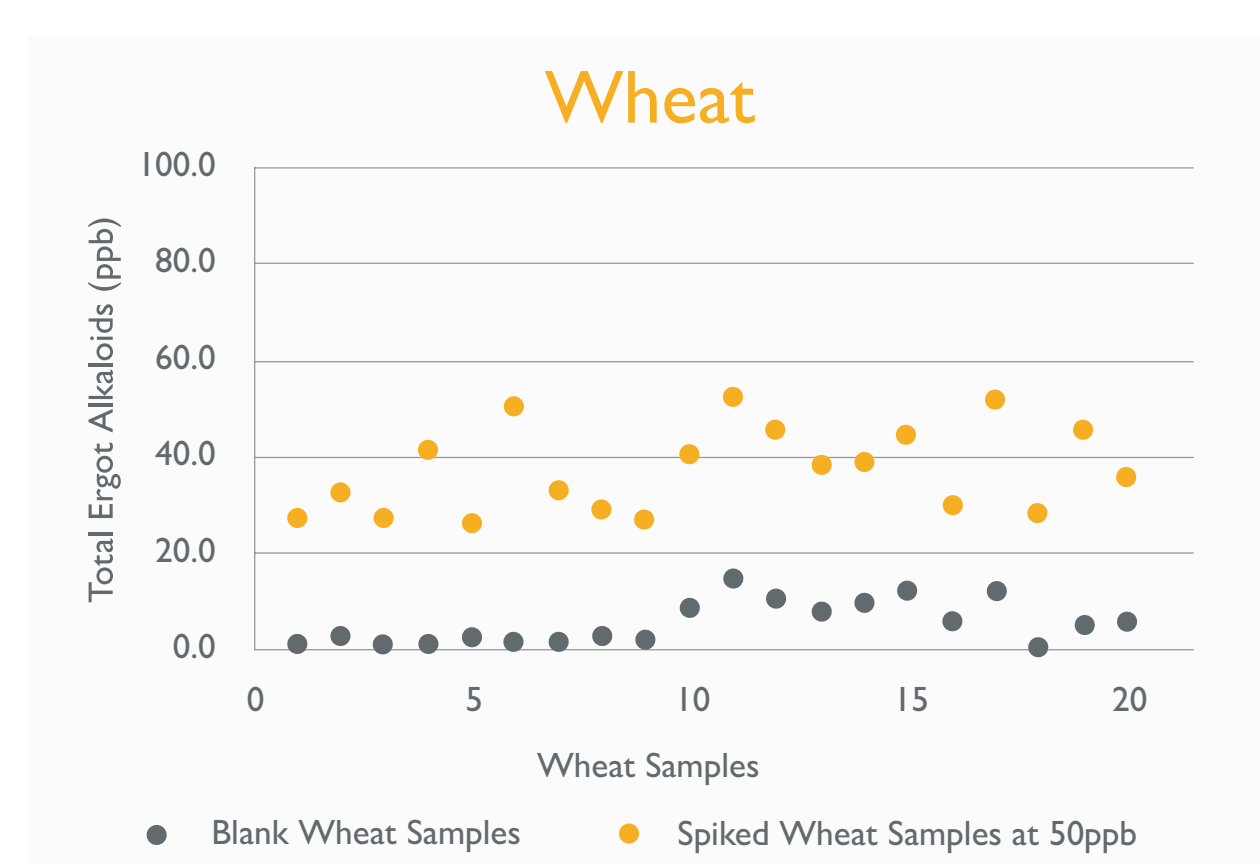


Results

Screening Target Concentration (STC)

Matrix	n	Sample dilution factor	Measuring range (ppb)
Feed	20	50	0-1100
Wheat	20	50	0-1100
Rye	20	50	0-1100

Matrix	STC (ppb)	Cut-off (ppb)	Detection capability validated at STC level	Fitness for purpose assessment: Rate of false suspected results (TDIST)
Feed	50.00	33.07	Passed	<0.1%
Wheat	50.00	21.67	Passed	<0.1%
Rye	125.00	56.62	Passed	<0.1%



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Recovery

Matrix	Spiked concentration (ppb)	Level	Recovery (%)	CV (%)	n
Feed	50.00	CC beta	102	10.3	12
	75.00	MRL*	94	13.6	16
	150.00	2 x MRL*	79	17.7	16
Wheat	50.00	CC beta	87	14.6	12
	75.00	MRL*	85	17.4	16
	150.00	2 x MRL*	83	11.3	16
Rye	125.00	CC beta	104	15.6	16
	250.00	MRL*	99	19.5	16
	500.00	2 x MRL*	96	6.9	8

* Still as a draft of legislation 19/004.193

Intra-assay Precision

Concentration, [ppb]	n	CV (%)
0.00	12	3.9
0.29	12	2.5
0.67	12	5.1
1.60	12	3.5
3.87	12	2.5
22.22	12	2.8

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Comparison with Confirmatory Methods

Sample Type	Ergot Alkaloids	Confirmatory 1	Confirmatory 2	Confirmatory 3	Correlation (%)
	ELISA (ppb)	LC-MS/MS (ppb)	LC (ppb)	LC (ppb)	
Barley	731	907	-	-	81
Buckwheat	132	134	-	-	98
Wheat	988	935	-	-	106
Rye	4290	3552	-	-	121
Bovine Feed	1105	1219	-	-	91
Porcine Feed	1745	2101	-	-	83
Broiler Feed	251	249	-	-	101
Rye Flour	4434	-	-	4190	106
Rye Flour	3158	-	3200	-	99
Rye Flour	2415	-	2000	-	121
Rye Flour	29	-	<36	-	Correct
Rye	10	<24	-	-	Correct
Rye	884	640	-	-	138
Barley	5	<24	-	-	Correct
Buckwheat	7	<24	-	-	Correct
Wheat	9	<24	-	-	Correct
Rye	5	<24	-	-	Correct
Bovine Feed	17	<24	-	-	Correct
Porcine Feed	19	<24	-	-	Correct
Broiler Feed	14	<24	-	-	Correct

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Proficiency Testing



Fapas® - Food Chemistry Proficiency Test Report 22158

Ergot Alkaloids in Rye Flour (March - April 2019)

Analyte	Assigned value, X _s µg/kg	Number of Scores, z ≤ 2	Total Number of Scores	% z ≤ 2
Ergocornine	18.4	23	26	88
Ergocorninine	7.11	21	21	100
Ergocristine	64.7	23	27	85
Ergocristinine	22.1	23	25	92
α-and-β-Ergocryptine (sum)	not issued	-	-	-
α-and-β-Ergocryptinine (sum)	10.0	22	23	96
Ergometrine	15.6	21	24	88
Ergometrinine	2.30	12	15	80
Ergosine	10.0	21	25	84
Ergosinine	3.35	17	18	94
Ergotamine	26.0	22	27	81
Ergotaminine	7.00	17	19	89

Total Ergot Alkaloids Content: 186.54 µg/kg

Randox Ergot Alkaloids ELISA Results

Laboratory Number	Result Submitted for Total Ergot Alkaloids (µg/kg)
015	176.4

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Conclusion

Data indicate applicability of the reported ELISA to the detection of twelve predominant ergot alkaloids in wheat, rye and cereal based feed. This application facilitates the testing process and allows the detection of the proposed ergot alkaloids in the proposed matrices considered by the EC.

Reference

<https://prodstoragehoeringspo.blob.core.windows.net/6e4b0ebb-a9f0-4f30-a350-06e12c851375/Ergot-alkaloids-stakeholder-consultation.pdf>