CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

Antibody production for alkaloids

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Introduction

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms with many having heterocyclic rings as a part of their structure. They are produced as secondary metabolites by bacteria, fungi, algae, animals but mainly by plants. There are over 3000 compounds known such as caffeine, nicotine, cocaine and these may be classified due to their biosynthetic origin, pharmacological activity, taxonomic source or chemical structure. The various pharmacological and toxicological activities have always captivated human interest, and for centuries selected plant products have been used as poisons, euphoriants, psychedelics, stimulants or medicines. Due to their profound biological effects the European Food Safety Authority (EFSA) are reviewing plant alkaloids as emerging toxins on behalf of their potential as food and feed contaminants. Some ELISA methods exist for the detection of definite alkaloids but most detection methods to date are based on GC or LC/MS which offer multi-screening possibilities. However, these techniques are laborious and expensive and hence multiplex rapid methods of analysis are still required.

Aim

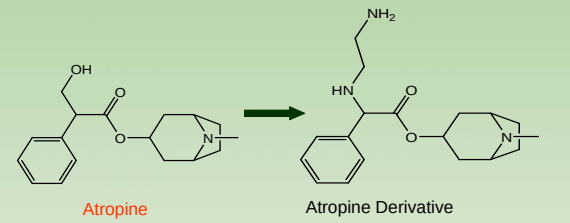
The aim of the CONffIDENCE project is to study three major groups of alkaloids: tropane alkaloids (TA); pyrrolizidine alkaloids (PA) and ergot alkaloids (EA). For all three groups EFSA scientific opinions have been prepared (EA in feed), are requested (EA in food) or are in preparation (TA and PA in feed). A major objective of the project was to produce antibodies for the most abundant alkaloids in each group for the development of multiplex dipstick assays.

Alkaloid		Source	Effect
Tropane	Atropine	Atropa belladonna	Anticholinergic drug
	Scopolamine	Solanaceae family	Anticholinergic drug
Pyrrolizidine	Jacobine	Senecio jacobae	Seneciosis
	Lycopsamine	Heliotropium keralense	Liver toxicity
Ergot	Ergotamine	Claviceps purpurea	Ergotism
	Ergocristine	Claviceps purpurea	Ergotism

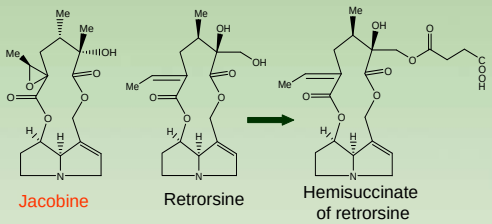
Methods

Six alkaloids were sourced commercially or obtained from RIVM and chemical derivatives were produced.

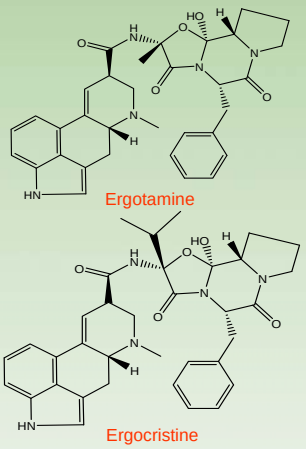
Tropane alkaloids (TA)



Pyrrolizidine alkaloids (PA)



Ergot alkaloids (EA)



Each of the six alkaloids (or derivatives there of) were chemically conjugated to carrier proteins using carbodiimide, acid anhydride, DSC, carbonyldimidazole or formaldehyde reactions

Results

The assessment and characterisation of antibody titre was performed by ELISA using both the HRP format and the OVA as a coating antigen.

Alkaloid	Protein	No. of rabbits immunised	No. of rabbits with antibody titre	Best IC_{50} ng/ml
Atropine Derivative	BTG*	2 3	2 3	1.9 0.1
	BSA*	2 3	2 3	2.5 0.2
Scopolamine	BTG	5	2	TBD
	BSA	5	3	TBD
Jacobine (Retrorsine Derivative)	BSA	5	2	3.4
	BTG	5	5	0.5

Alkaloid	Protein	No. of rabbits immunised	No. of rabbits with antibody titre	Best IC_{50} ng/ml
Lycopsamine (Retronecine Derivative)	BSA	5	2	21.4
	BTG	5	5	110
Ergot derivative	Jeffamine-BTG	3	3**	TBD
	Jeffamine-BSA	3	3**	TBD
	PEG-BSA	3	3**	TBD
	PEG-BTG	3	3**	TBD
Ergotamine	BTG	5	***	TBD
	BSA	5	***	TBD
Ergocristine	BTG	5	***	TBD
	BSA	5	***	TBD

TBD – To be determined * Two different immunisation protocols
** Testing method with coating antigen coupled to OVA *** First bleedings

Conclusion

This work provides on overview of the on-going antibody production for the tropane, pyrrolizidine and ergot alkaloids. Sensitive antibodies have successfully been produced for atropine, jacobine and lycopsamine. Antibody titres have been established for scopolamine and the ergot alkaloids with the IC_{50}s still to be determined. The antibodies will now be utilised in multiplex dipstick assays.

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