DEVELOPMENT OF A RAPID HONEY SCREENING IMMUNOASSAY FOR RESIDUES OF NITROFURANS AOZ, AMOZ, AHD, SEM

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BACKROUND

Nitrofurans are a class of broad spectrum antibiotics which have potentially carcinogenic effects following human consumption. As a result, their use in food-producing animals has been banned in many jurisdictions including the European Union and USA. Reliable methods are therefore required to monitor their illegal use in food produce to ensure food safety.

Monitoring of the illegal use of Nitrofurans complicated by the short *in vivo* half life of the parent compounds. However, their tissue-bound metabolites – AMOZ, AOZ, AHD and SEM – are stable for several weeks after use of the parent compound and are therefore more reliable markers of the illegal use of Nitrofurans. This study reports the use of ELISA to quantify Nitrofuran metabolites in honey following their release from the sample matrix.

Parent Compound	Metabolite
Furazolidone	AOZ
Furaltadone	AMOZ
Nitrofurantoin	AHD
Nitrofurazone	SEM

METHODS

Honey samples were prepared following a single sample preparation protocol for all four Nitrofuran metabolites, as illustrated in the diagram below.

Single Sample Preparation method

1g Honey

Sample Derivatisation at 60˚C under using 2-NBA (supplied in kit) for only 90 minutes – 30 minutes shorter than is currently available on the market

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Solvent-based extraction using ethyl acetate which is evaporated at 50˚C

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Reconsitution of sample in hexane and buffer (supplied in kit

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Application of samples to ELISA test kit

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AMOZ ELISA Cat# BXEFB42A | **AOZ ELISA Cat # BXEFB41A** | **AHD ELISA Cat# BXEFB43A** | **SEM ELISA Cat# BXEFB44A**

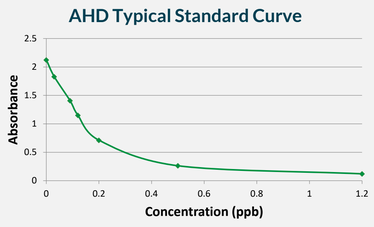
Many of the reagents required for the sample preparation, including 2-NBA and spiking material, are provided in the assay kit. All reagents, including standards and antibodies required for the ELISA are also provided in ready-to-use form as part of the assay kit.

RESULTS

Calibration Range

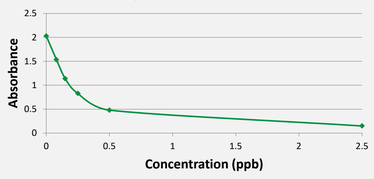
AOZ: 0.05 – 1.5 ppb
AMOZ: 0.08 – 2.5 ppb
AHD: 0.03 – 1.2 ppb
SEM: 0.05 – 5.0 ppb

AHD Typical Standard Curve



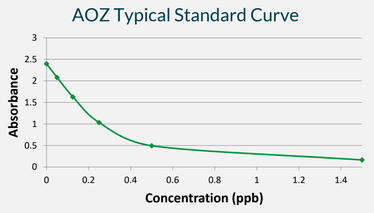
Cross Reactivity	
Metabolite	% Cross Reactivity
AHD	100
AOZ	0.01
SEM	<0.01
AMOZ	<0.003

AMOZ Typical Standard Curve



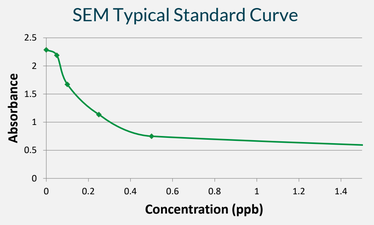
Cross Reactivity	
Metabolite	% Cross Reactivity
AMOZ	100
AHD	0.02
SEM	0.02
AOZ	<0.01

AOZ Typical Standard Curve



Cross Reactivity	
Metabolite	% Cross Reactivity
AOZ	100
AMOZ	0.11
AHD	0.07
SEM	0.03

SEM Typical Standard Curve



Cross Reactivity	
Metabolite	% Cross Reactivity
SEM	100
AMOZ	<0.04
AOZ	<0.04
AHD	<0.04

Figure 1: *Typical curves obtained using each Nitrofuran ELISA kit, with cross reactivities with other Nitrofuran metabolites highlighted in corresponding tables.*
The results highlight that cross-reactivities with other Nitrofuran metabolites are extremely low, enabling honey samples prepared in one run to be applied to all four Nitrofuran ELISA kits.

AHD Intra-Assay Precision

Expected Concentration: 0.09ppb				Expected Concentration: 0.2ppb		
	ABSORBANCE (nm)	CONCENTRATION (ppb)			ABSORBANCE (nm)	CONCENTRATION (ppb)
MEAN	1.043	0.100		MEAN	0.529	0.210
SD	0.04	0.01		SD	0.02	0.01
%CV	4.2	6.7		%CV	4.3	3.5

AMOZ Intra-Assay Precision

Expected Concentration: 0.08ppb				Expected Concentration: 0.25ppb		
	ABSORBANCE (nm)	CONCENTRATION (ppb)			ABSORBANCE (nm)	CONCENTRATION (ppb)
MEAN	1.778	0.100		MEAN	0.938	0.340
SD	0.09	0.01		SD	0.04	0.02
%CV	5.0	15.1		%CV	3.9	5.4

AOZ Intra-Assay Precision

Expected Concentration: 0.125ppb				Expected Concentration: 0.5ppb		
	ABSORBANCE (nm)	CONCENTRATION (ppb)			ABSORBANCE (nm)	CONCENTRATION (ppb)
MEAN	1.883	0.122		MEAN	0.455	0.714
SD	0.09	0.01		SD	0.03	0.04
%CV	4.6	9.0		%CV	6.7	5.6

SEM Intra-Assay Precision

Expected Concentration of 0.05ppb				Expected Concentration: 0.5ppb		
	ABSORBANCE (nm)	CONCENTRATION (ppb)			ABSORBANCE (nm)	CONCENTRATION (ppb)
MEAN	1.887	0.081		MEAN	0.422	0.800
SD	0.06	0.01		SD	0.04	0.12
%CV	3.4	10.7		%CV	9.5	14.6

Figure 2: *Intra-assay precision for each of the four Nitrofurans*
The results demonstrate good intra-assay precision for all four Nitrofuran ELISAs, with CV for absorbance all much less than 10%

RESULTS

AHD Inter-Assay Precision

Expected Concentration: 0.2 ppb	
MEAN	0.200
SD	0.02
%CV	9.6

AMOZ Inter-Assay Precision

Expected Concentration: 0.1 ppb	
MEAN	0.090
SD	0.01
%CV	10.0

AOZ Inter-Assay Precision

Expected Concentration: 0.25 ppb	
MEAN	0.250
SD	0.02
%CV	9.9

SEM Inter-Assay Precision

Expected Concentration: 0.25 ppb	
MEAN	0.270
SD	0.00
%CV	9.3

Figure 3: *Inter-assay precision for each of the four Nitrofurans*
Inter-assay precision was examined by running one control level across up to twenty independent test runs. Inter-assay precision is good across all four Nitrofuran kits, with %CV ≤ 10%.

CONCLUSIONS

Biorex Food Diagnostics have developed four ELISA kits for the detection of four individual Nitrofuran Metabolites. Each of the kits exhibits very low cross-reactivity with the other Nitrofuran metabolites, enabling one sample preparation to be used across all four kits.

Each of the Nitrofuran ELISA kits have a number of advantages over other commercially-available kits:

- **Short assay time**: 45 minutes
- **Shorter sample derivatisation time** of 90 minutes as opposed to 120 minutes
- **One sample preparation** can be used across all four Nitrofuran kits
- **Reduced solvent volumes**. This offers a number of benefits including:
  - Lower reagent costs
  - Faster sample evaporation
  - Preparation can be completed in one 15ml tube
- Spiking material is provided in the assay kit in a **ready-to-use** format
- Conjugate is supplied **ready-to-use**, removing the need for dilution prior to use
- The **derivatisation reagent** 2-nitrobenzaldehyde is supplied as a **ready-to-use** liquid