Rapid Screening Detection of DNSH (Nifursol) Residues: A Cost-Effective Solution for Ensuring EU Compliance

Biorex Food Diagnostics

Overview

Purpose: Detect illegal use of Nifursol in food through the metabolite DNSH, to allign with EU safety standards.

Methods: Sample extraction via acid hyrolysis; derivatisation, and incubation (30 minutes), with the complete test protocol taking 45 minutes.

Results: With high specificity, low cross-reactivity, and efficient sample preparation, the kit ensures accurate results and relieable preformance, meeting EU regulations.

Introduction

Nifursol belongs to the broad-spectrum class of antibiotics known as Nitrofurans, which are prohibited in the EU for use in food-producing animals due to their potential carcinogenic effects. Nifursol metabolises rapidly when ingested, making it difficult to detect directly. However, its protein-bound metabolite, DNSH, is more easily detectable, serving as a key marker.

EU Commission Regulation (EU) 2019/1871, as amended by Commission Regulation (EU) 2023/411, sets a Reference Point for Action (RPA) of 0.5 µg/kg (0.5 ppb) for Nifursol and its metabolite DNSH. Therefore, reliable detection methods with good sensitivity are essential to monitor the illegal use of Nifursol in the food industry. The Biorex Food Diagnostics DNSH (Nifursol) ELISA kit, designed to detect residues in prawns and shrimp, meets these requirements and is outlined in this study.

Method

A highly sensitive ELISA kit was developed to detect the Nifursol metabolite DNSH in prawn and shrimp samples, with a test protocol completed in 45 minutes. Metabolites are extracted and quantified using acid hydrolysis, followed by a 30-minute derivatisation incubation and solvent extraction from a 1g sample. The steps are outlined in the diagram below.

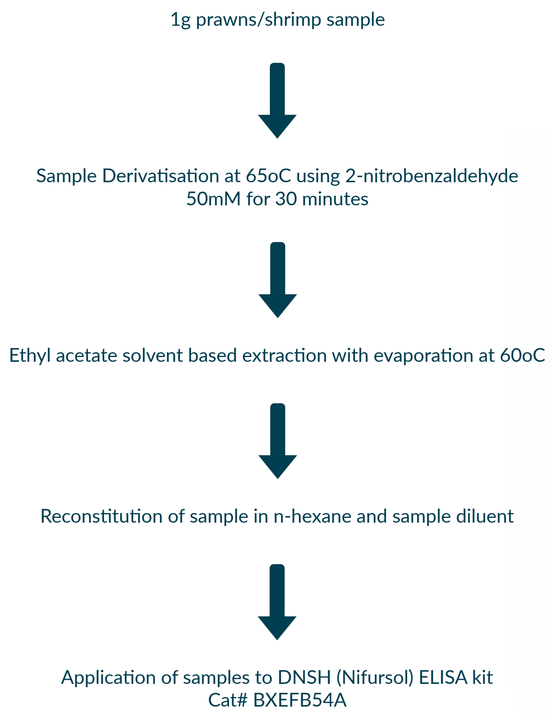

Figure 1: Prawns/Shrimp Sample Preparation Method

The assay kit includes 2-nitrobenzaldehyde (50nM) and sample diluent for preparation, along with ready-to-use reagents such as standards, spiking solution, and primary and secondary antibodies.

Results

Calibration Range

Concentration (ppb)	Absorbance (nm)	Binding (%)
0	2.182	100
0.05	1.825	85
0.125	1.565	72
0.25	1.322	61
0.5	0.893	41
4	0.368	17

Table 1: Typical results obtained for a calibration curve using the DNSH kit, ranging from 0.05ppb to 4.00ppb.

Reactant/ Cross reactant	% Cross Reactivity
2-NP-DNSH	100%
Nifursol	117%
1-aminohydantoin (AHD)	<0.1%
3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ)	<0.1%
3-amino-2-oxazolidinone (AOZ)	<0.1%
Semicarbazide (SEM)	<0.1%

Table 2: Cross-reactants of the DNSH kit.

Results demonstrate a high cross-reactivity between DNSH and Nifursol, with very low cross-reactivity observed for the other four Nitrofurans—AHD, AMOZ, AOZ, and SEM—ensuring excellent specificity for the target analytes.

Intra-precision

	Level 1 (QCL) Expected Concentration_0.125		Level 1 (QCL) Expected Concentration_0.125	
Replicate	Absorbance (nm)	Concentration (ppb)	Absorbance (nm)	Concentration (ppb)
1	1.598	0.125	0.988	0.432
2	1.579	0.132	0.940	0.472
3	1.510	0.157	0.926	0.485
4	1.587	0.129	0.944	0.468
5	1.563	0.137	0.934	0.477
6	1.560	0.139	0.996	0.425
7	1.353	0.148	0.912	0.498
8	1.579	0.132	0.978	0.439
9	1.525	0.151	0.957	0.457
10	1.561	0.138	0.982	0.437
11	1.500	0.161	0.945	0.467
12	1.514	0.155	0.944	0.469
13	1.541	0.146	0.903	0.507
14	1.500	0.161	0.964	0.458
15	1.548	0.143	0.999	0.422
16	1.533	0.149	1.004	0.419
17	1.574	0.134	0.934	0.477
18	1.552	0.142	0.998	0.423
19	1.502	0.160	0.905	0.505
20	1.517	0.154	0.945	0.468
	MEAN SD %CV	0.145 0.011 7.8	MEAN SD %CV	0.460 0.027 6.0

Table 3: Intra-assay precision for expected DNSH concentrations of 0.125 ppb and 0.5 ppb.

Two control levels were examined by running 20 replicates of each concentration in a single assay run of the DNSH (Nifursol) ELISA kit. The %CV for both concentrations was below 10%, indicating excellent intra-assay precision.

Replicate Number	Control (Exp. 0.25ppb)
	Concentration (ppb)
1	0.27
2	0.26
3	0.26
4	0.23
5	0.29
6	0.23
7	0.26
8	0.26
9	0.24
10	0.28
11	0.23
12	0.26
13	0.27
14	0.26
15	0.27
16	0.24
17	0.25
18	0.27
19	0.23
20	0.24
Mean	0.26
Standard Deviation	0.018
CV (%)	7.02

Table 4: Inter-assay precision observed for DNSH at a concentration of 0.25 ppb.

Inter-assay precision achieved was good, with a %CV of less than 10% for the 20 independent runs at this control level. This demonstrates a high degree of reproducibility expected with this assay kit.

Limit of Detection

Replicate Number	Prawns / shrimp DNSH Concentration (ppb)
1	0.13
2	0.13
3	0.14
4	0.12
5	0.06
6	0.07
7	0.03
8	0.09
9	0.06
10	0.12
11	0.16
12	0.12
13	0.06
14	0.02
15	0.11
16	0.08
17	0.03
18	0.05
19	0.07
20	0.17
Mean Conc.	0.091
Standard Deviation	0.044
Limit of Detection	0.22

Table 5: Limit of Detection (LOD) for DNSH in Prawn/Shrimp Samples.

The LOD was determined by running 20 known negative samples using the DNSH (Nifursol) kit. The mean concentration and standard deviation from these results were calculated, yielding an LOD of 0.22 ppb.

Recovery

	Prawns/ Shrimp Samples	
	Concentration(ppb) N=5	% Recovery
0.25ppb	0.28	112%
0.5ppb	0.42	84%
1ppb	0.82	82%

Table 6: Recovery of Prawn/Shrimp Matrix Using the DNSH (Nifursol) Kit at Concentrations of 0.25 ppb, 0.5 ppb, and 1 ppb.

A recovery run of five samples at three separate concentrations was assayed using the DNSH (Nifursol) kit. The mean percentage for all three concentrations fell within the range of 70-130%, demonstrating good accuracy in the detection of the target analyte.

CC-β Testing(Detection Capability)

	Negative Samples		Spiked Samples	
Replicate	Prawn Sample ID	Concentration (ppb)	Prawn Sample ID	Concentration (ppb)
1	1	0.05	1	0.20
2	2	0.04	2	0.16
3	3	0.09	3	0.23
4	4	0.06	4	0.17
5	5	0.001	5	0.16
6	6	0.09	6	0.18
7	7	0.10	7	0.17
8	8	0.04	8	0.16
9	9	0.09	9	0.21
10	10	0.08	10	0.20
11	11	0.09	11	0.25
12	12	0.00	12	0.33
13	13	0.03	13	0.16
14	14	0.04	14	0.26
15	15	0.10	15	0.27
16	16	0.00	16	0.28
17	17	0.06	17	0.23
18	18	0.06	18	0.31
19	19	0.01	19	0.21
20	20	0.05	20	0.23

Highest Concentration Negative Sample (ppb) 0.10	Overlap Evident	No
Lowest Concentration Spiked Sample (ppb) 0.16		

Table 7: Detection capability of the DNSH (Nifursol) ELISA kit using prawn/shrimp samples.

To assess the CCβ of this kit, 20 negative samples were tested alongside the same samples spiked at 0.25 ppb. No overlap between the negative and spiked samples was observed. The kit's detection capability (CCβ) of 0.25 ppb in prawn/shrimp complies with the EU directive outlined in Commission Regulation (EU) 2019/1871, as amended by Commission Regulation (EU) 2023/411, which sets a reference point of action (RPA) of 0.5 µg/kg for Nitrofurans and their metabolites. This kit, therefore, achieves a detection threshold that is half the required RPA.

Conclusion

The DNSH (Nifursol) ELISA kit developed demonstrates high cross-reactivity with Nifursol and exhibits excellent recovery and detection capability using prawn/shrimp samples. The kit offers good sensitivity and a broad standard range, allowing for effective sample detection across a wide measuring range. Compared to commercially available options, this kit has several advantages, including ready-to-use reagents, a shorter 30-minute sample incubation period, and a lower detection limit. The Biorex Food Diagnostics DNSH (Nifursol) ELISA kit is a reliable and cost-effective option for the detection of DNSH (Nifursol) in prawn/shrimp samples, aligning with the EU Directive 2023/411 for Nitrofurans and their metabolites.

Contact Us

biorexfooddiagnostics.com

sales@biorexfooddiagnostics.com

Nicole Haveron, Mark Squance, Nicola Kane*, Morteza Afrasiabi; Biorex Food Diagnostics | Corresponding Author: nicola@biorexfooddiagnostics.com