

Food Safety: A New Standard in Nitrofurans Analysis

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Partnership between EU and China

Collaborative work on nitrofuran analysis between Teagasc Food Research Centre in Ireland and China Agricultural University (CAU).

- Teagasc: development of a confirmatory method.
- CAU: development of screening methods.
- Methods exchanged between both laboratories.
- Aim to harmonise food trade between EU countries and China.



What are nitrofurans?

Nitrofurans are a class of synthetic, broad spectrum antibiotics that were previously licensed for use in veterinary medicine.

- Administered in their parent form and are rapidly metabolized *in vivo* to form highly stable protein-bound metabolites.
- Metabolites persist for long periods of time and are used as marker residues for nitrofuran analysis.
- Pose a threat to consumer safety.
 - Genotoxic, carcinogenic and mutagenic

Mutagenicity studies of a carcinogenic nitrofuran and some analogues

R Jung, JY Le, F Wengenmayer, E Wolf, M Kramer

Carcinogenicity of 5-Nitrofurans and Related Compounds With Amino-Heterocyclic Substituents

Samuel M. Cohen, E. Ertürk, A. M. Von Esch, A. J. Crovetti, George T. Bryan

Genotoxic action of nitrofuran derivative drugs

G. N. Zolotareva, L. P. Akin'shina & L. U. Radchenko



Definition of problem

Nitrofurans are completely banned from used in food producing animals due to concerns regarding their undesirable toxicological properties.

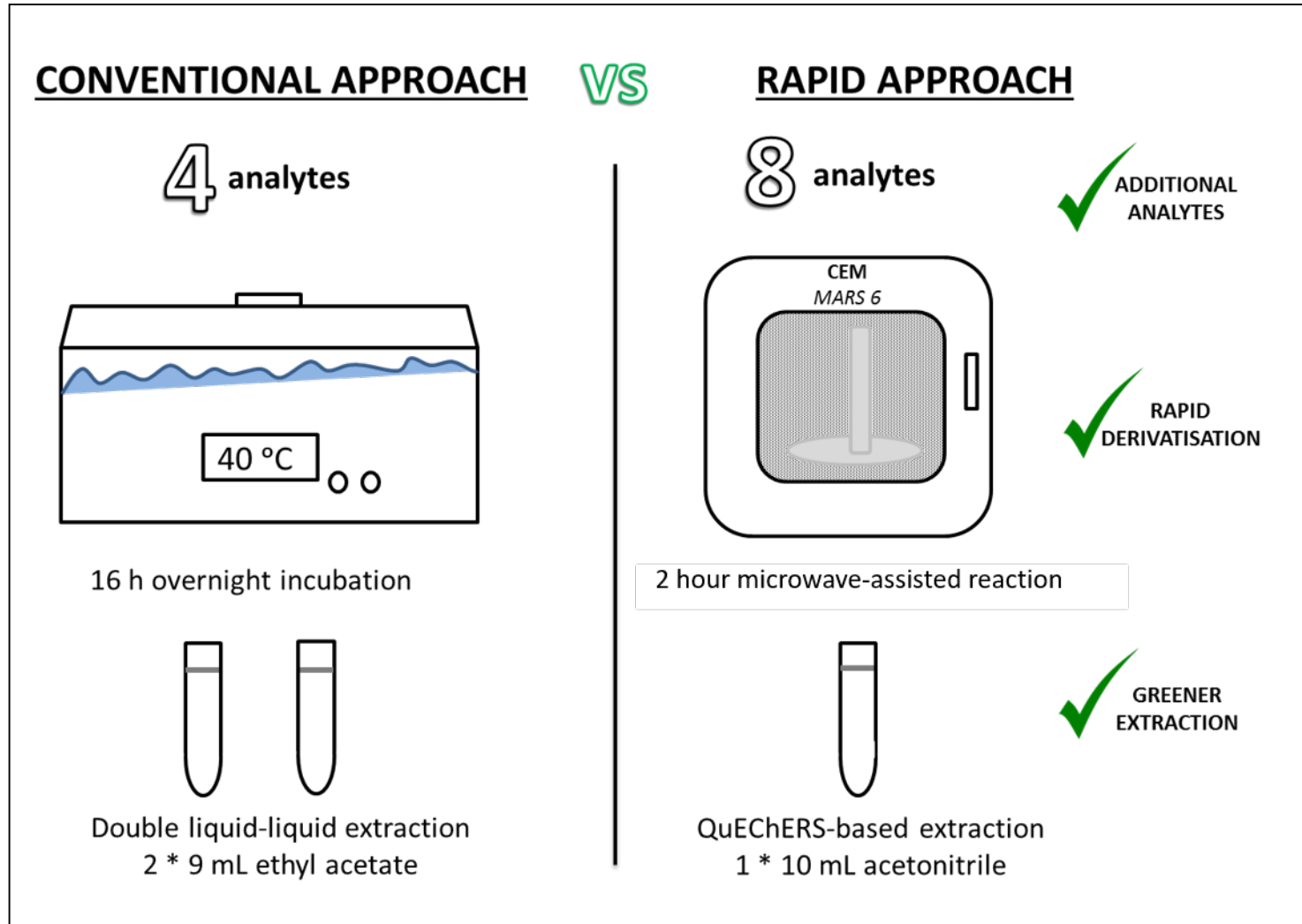
- To ensure food safety and consumer protections, strict legislation exists to monitor the levels of the marker residues in food.
- Recently, the EU Reference Point for Action (RPA) has been reduced from $1.0 \mu\text{g kg}^{-1}$ to $0.5 \mu\text{g kg}^{-1}$.

Nitrofurans and their metabolites	0,5 (1)	0,5 $\mu\text{g/kg}$ for each of the metabolites of furazolidone (AOZ or 3-amino-2-oxazolidinone), furaltadone (AMOZ or 3-amino-5-methylmorpholino-2-oxazolidinone), nitrofurantoin (AHD or 1-aminohydantoin), nitrofurazone (SEM or semicarbazide) and nifursol (DNSH or 3,5-dinitrosalicylic acid hydrazide)
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Commission Regulation (EU) No. 2019/1871 of 7 November 2019 on reference points for action for non-allowed pharmacologically active substances present in food of animal origin

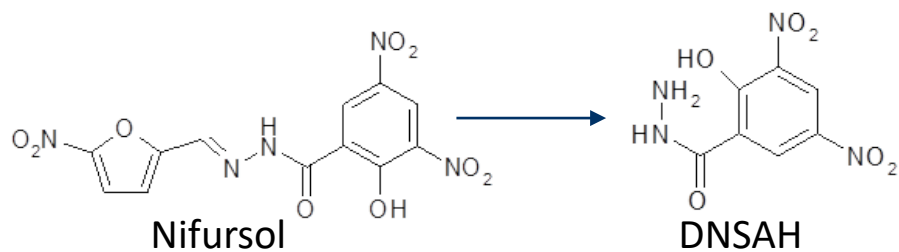


Pathway to solution

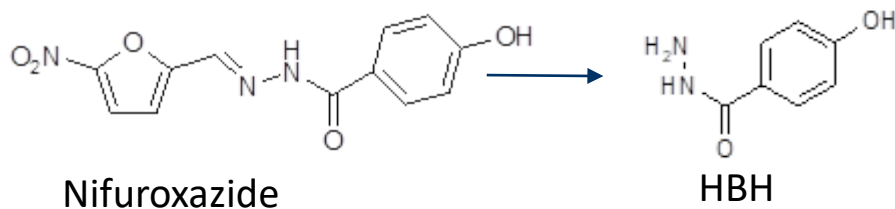


Identification of new marker metabolites

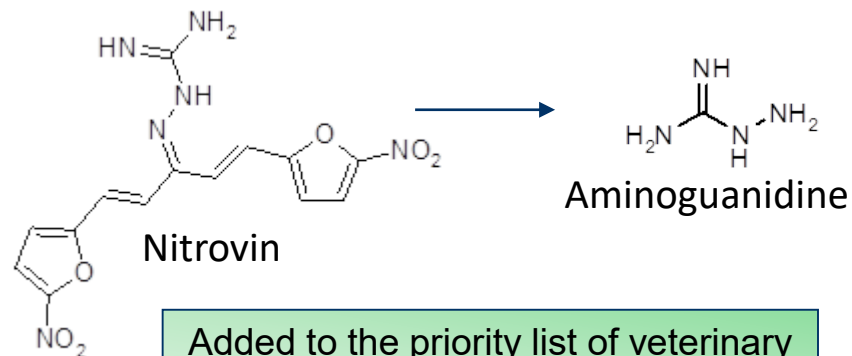
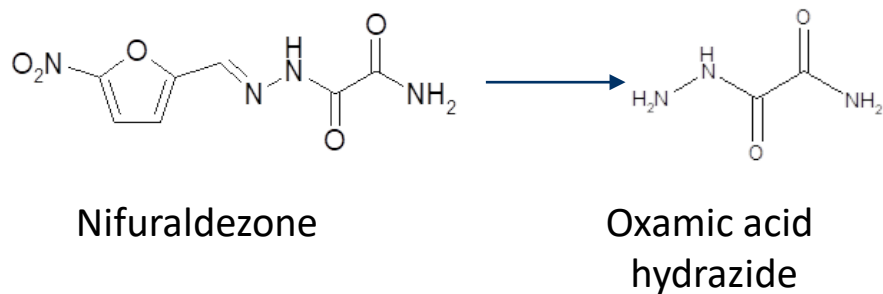
Four additional nitrofuran drugs and their respective marker residues were identified for inclusion in the method.



Added to the monitoring list in the EU



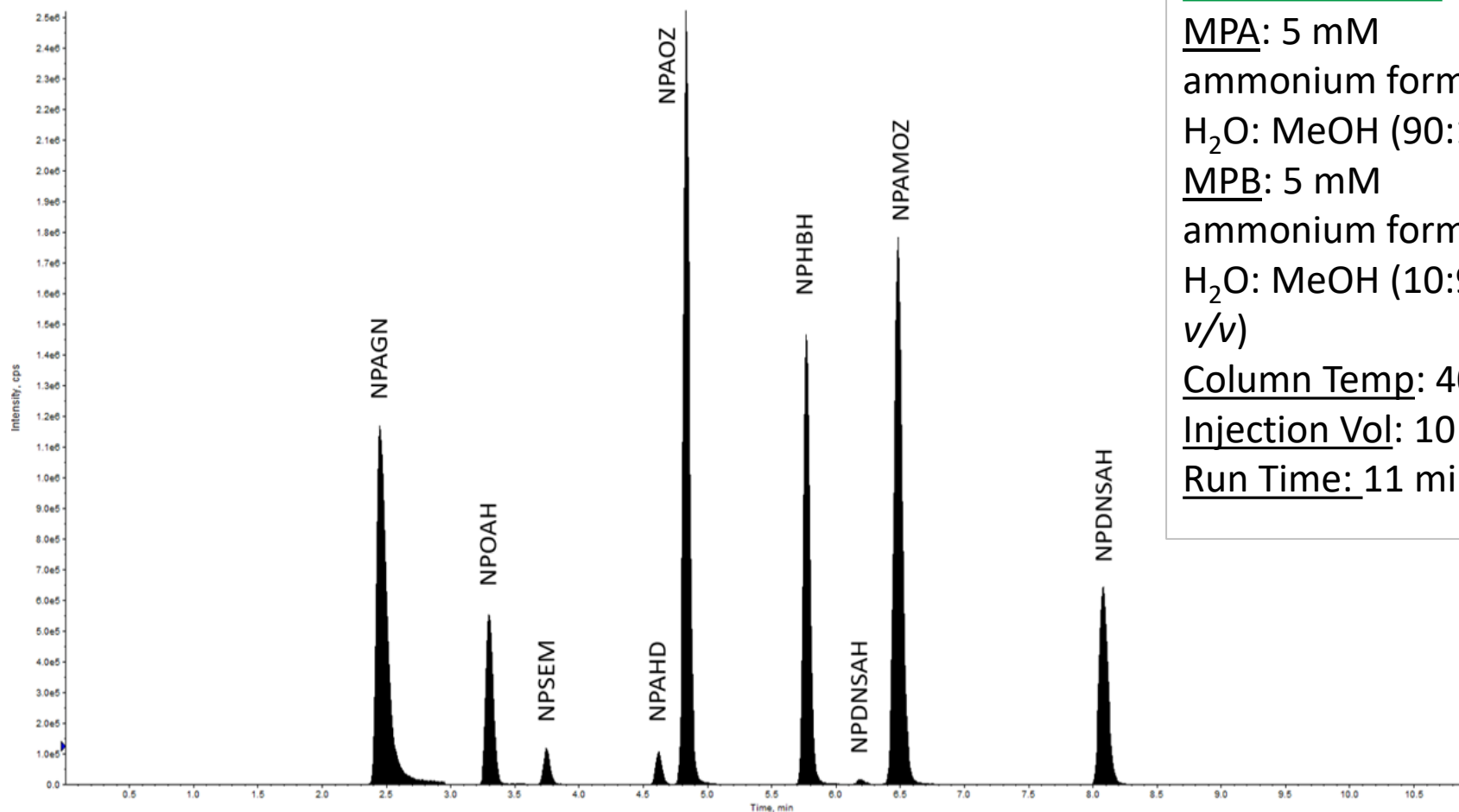
Identified as a NF drug that can result in bound residues in animal tissues



Added to the priority list of veterinary drugs in China



LC Method Development



LC Conditions

MPA: 5 mM
ammonium formate in
H₂O: MeOH (90:10, v/v)

MPB: 5 mM
ammonium formate in
H₂O: MeOH (10:90,
v/v)

Column Temp: 40 °C

Injection Vol: 10 µL

Run Time: 11 min

Chromatogram of a muscle sample spiked at 0.5 µg kg⁻¹ for the quantifier transitions

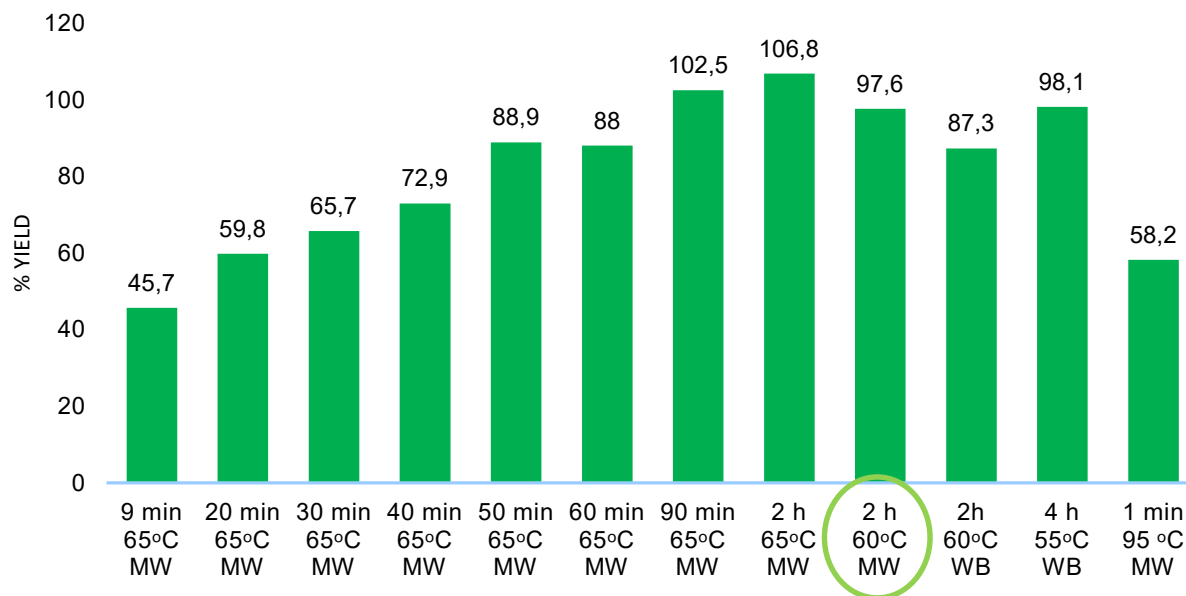


Microwave-assisted reaction

- Developed an alternative approach to the lengthy overnight waterbath incubation.
- Various conditions were assessed, and their impact on analyte stability was evaluated.

**4 min ramp to 60 °C,
with a 2 h hold time**

Comparison of derivatisation conditions for AOZ incurred material



Comparison of the performance of various derivatisation conditions. % yield shown is determined by calculating the mean AOZ concentration ($n = 3$) measured with each set of conditions and expressing each value as a percentage of the AOZ concentration measured using the traditional overnight incubation at 37 °C. Time shown = hold time; MW = microwave reaction; WB = heated waterbath.



Method Validation

The new method has been fully validated in accordance with the new guidelines set out in 2021/808 legislation.

- Met all the performance criteria for the following:
 - Identification
 - Selectivity
 - Linearity
 - Matrix effects
 - Trueness
 - Within-lab repeatability (WLr)
 - Within-lab reproducibility (WLR)
 - Decision limits (CC α)
- Multi-species validation for bovine, ovine, avian and porcine muscle tissues.
- Awarded accreditation by the Irish National Accreditation Board (INAB) in conformity with the ISO/IEC 17025:2017 standards.



New marker residues confirmed

The metabolites of nitrovin and nifuraldezone had previously been predicted through the assessment of their chemical structures, but they had not been verified.




- Two animal studies were conducted to verify the formation of the respective metabolites *in vivo*.
- Analysis of incurred **porcine tissues** confirmed that **aminoguanidine** is a suitable marker residue for NTV detection.
- Analysis of incurred **avian tissues** confirmed that **oxamic acid hydrazide** is a suitable marker residue for NDZ detection.



Long-term impact: Harmonisation of trade

(1) A new faster, more sensitive and more comprehensive confirmatory method has been developed for nitrofurans analysis.

(2) New marker residues have been confirmed for the first time *in vivo* for the detection of nifuraldezone and nitrovin, which can be used in future legislation.

- The technology can be used by laboratories in the EU and China. 
- Ensures the safety of products traded between the two regions. 
- Ensures supply lines for importers and retailers. 



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