# DETERMINATION OF THE METABOLITES OF NITROFURAN ANTIBIOTICS IN A RANGE OF ANIMAL TISSUES AND ASSOCIATED PRODUCTS BY LIQUID CHROMATOGRAPHY-TANDEM QUADRUPOLE MASS SPECTROMETRY

Simon Hird[1], Richard Ginn[2] and Matthew Sharman[2]
[1] Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, Cheshire, SK94AX, UK
[2] Fera Science Ltd, Sand Hutton, York, YO41 1LZ, UK

## Waters
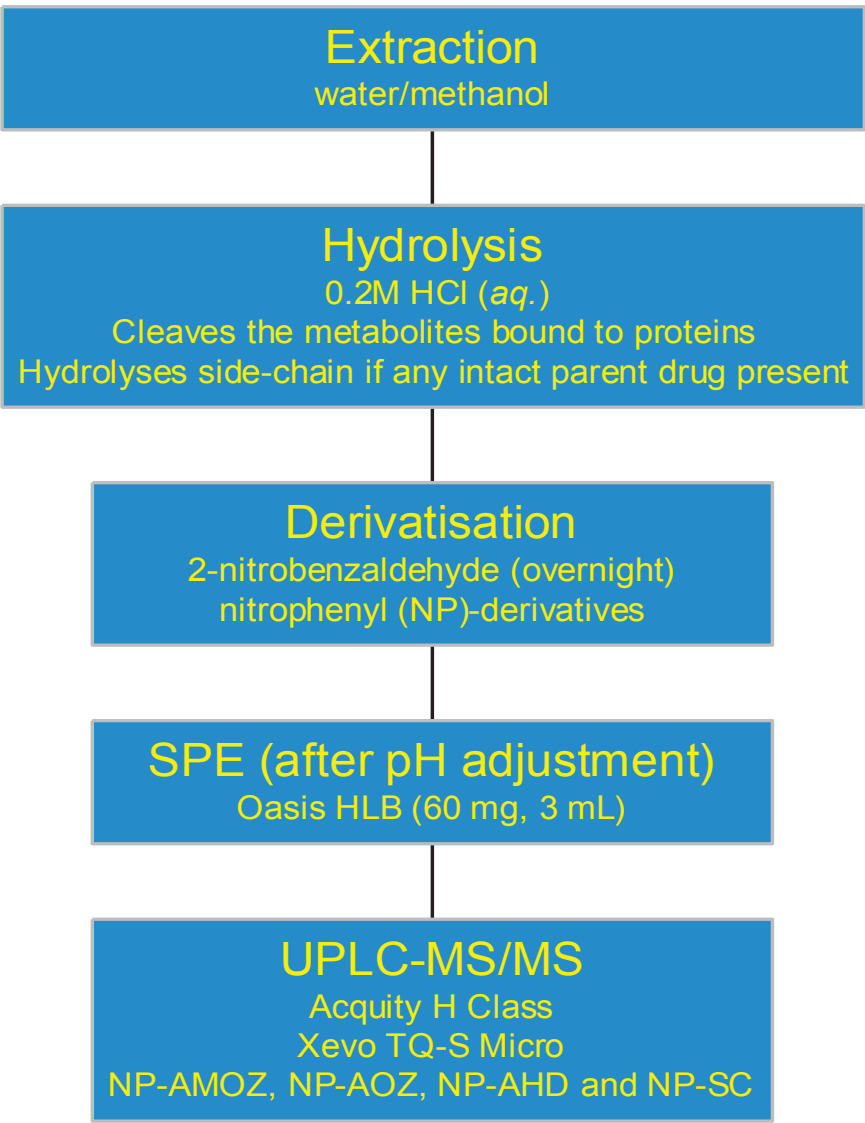### THE SCIENCE OF WHAT'S POSSIBLE.®

## INTRODUCTION

Nitrofurans (NFs) are a group of broad spectrum antibiotics. Due to health concerns, nitrofurans are now prohibited for use in food-producing animals in most jurisdictions. They are still authorized for human medicine and for the treatment of non-food animals. They are widely manufactured, sold and hence available for misuse [1]. There have been frequent findings of residues in honey, poultry and aquaculture products imported to EU countries, which has led to product recalls, border rejections and de-listed suppliers. Violations have resulted in implementation of emergency measures requiring mandatory pre-export testing (PET), widespread voluntary pre-harvest tests (PHT) and an increase both in analysis of imports at border control within the EU and in the frequency of FVO visits.

The EU MRPL in poultry meat and aquaculture is 1 µg/kg for each of the four nitrofurans, measured as their respective tissue-bound metabolites, [2]. MRPLs are 'the minimum content of an analyte in a sample, which at least has to be detected and confirmed'. They are the reference point for action ('Action levels') when evaluating food consignments. Laboratories must demonstrate that their calculated Detection Capability (CC β) and Decision Limit (CC α) values are at or below the MRPL. Suppliers and importers can set even lower limits for PET based upon trading decisions to provide better warranties to their customers and gain commercial advantage.

Here we demonstrate rugged, robust and reliable performance for the determination (screening and confirmation) of nitrofurans in a range of sample matrices using the Xevo TQ-S micro, which will allow confident screening and confirmation to concentrations well below the MRPL value.

## METHODS

Previous studies have demonstrated that parent nitrofurans deplete rapidly in animals and that they are extensively metabolized to tissue-bound metabolites [1]. Methods have been described for various animal tissues e.g. kidney for official control, muscle for consumer risk, honey, shrimp, eggs and milk. Parent nitrofurans are only sought in medicated feeds used for animal production and aquaculture. Commonly sought parent nitrofurans and associated metabolites include: furazolidone as 3-amino-2-oxazolidinone (AOZ), nitrofurazone as semicarbazide (SCA), furaltadone as 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ) and nitrofurantoin as 1-aminohydantoin (AHD). The method described here is based upon that originally developed as part of the "FoodBRAND" project [3], and although newer technology has enabled improvements in performance, the principles behind procedure have changed little.

### Extraction
water/methanol

### Hydrolysis
0.2M HCl (aq.)
Cleaves the metabolites bound to proteins
Hydrolyses side-chain if any intact parent drug present

### Derivatisation
2-nitrobenzaldehyde (overnight)
nitrophenyl (NP)-derivatives

### SPE (after pH adjustment)
Oasis HLB (60 mg, 3 mL)

### UPLC-MS/MS
Acquity H Class
Xevo TQ-S Micro
NP-AMOZ, NP-AOZ, NP-AHD and NP-SC

## RESULTS



Figure 1 Chromatograms showing the detection of different NFs extracted from a prawn sample spiked at screening target concentration (STC) of @ 0.5 µg/kg

### Screening results

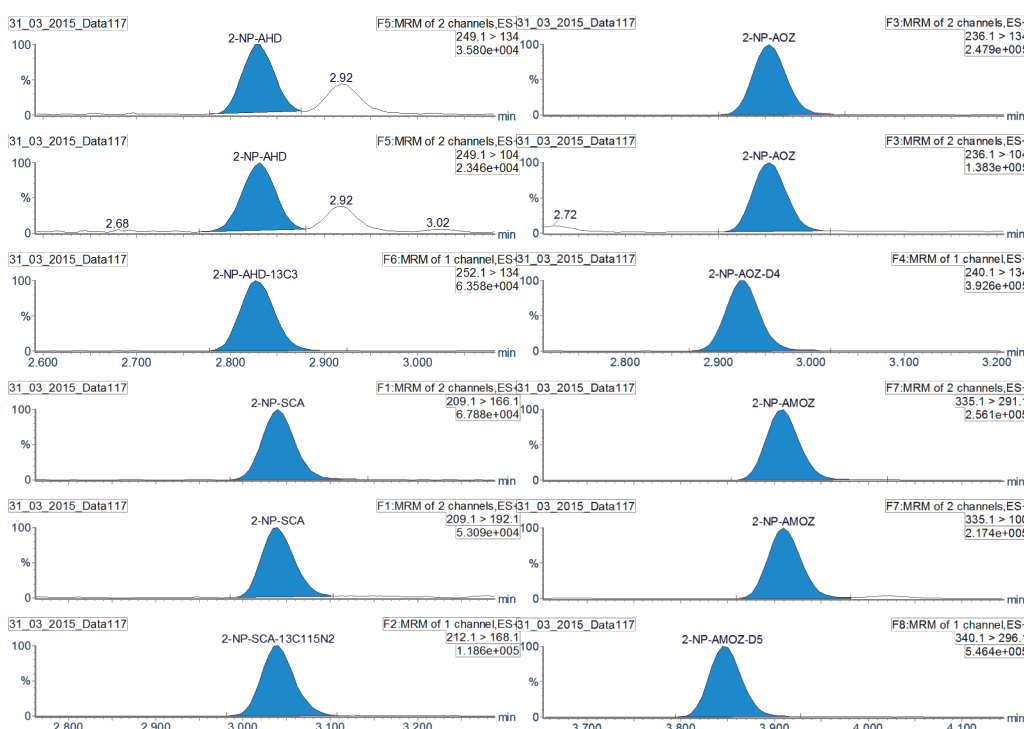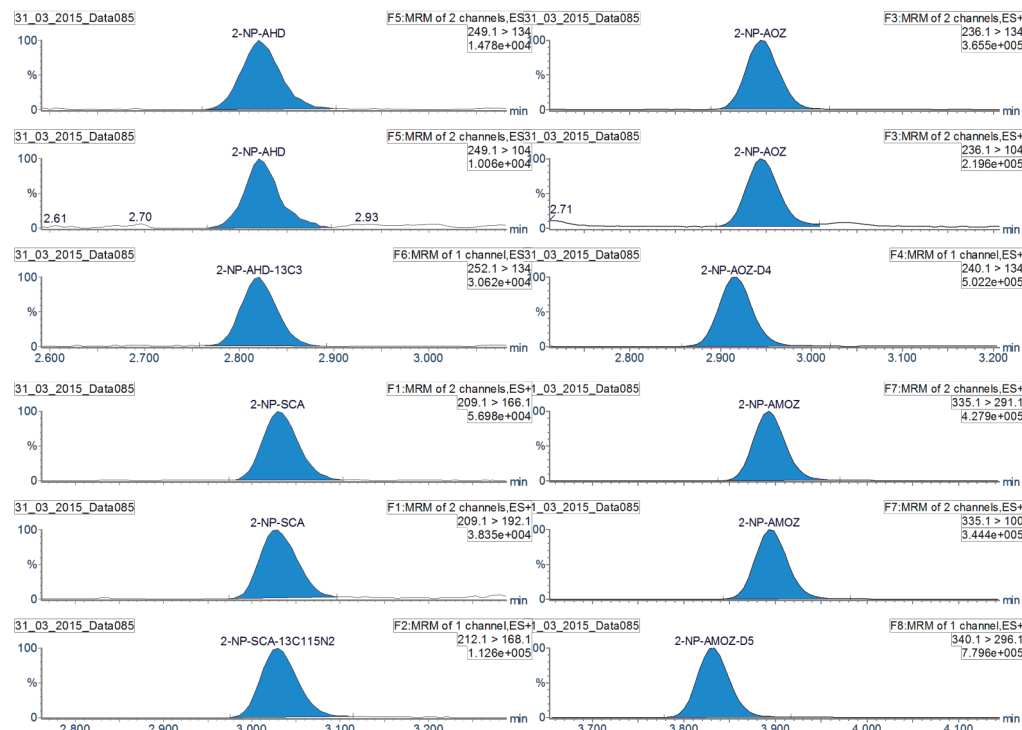- Excellent sensitivity and selectivity demonstrate at the STC in various matrices



Figure 3 Chromatograms showing the detection of different NFs extracted from a honey sample spiked at screening target concentration (STC) of @ 0.5 µg/kg

### Confirmation results

- 10 portions of a FAPAS honey test material were prepared and analyzed in duplicate
- Measured 1.41 µg/kg AMOZ cf. 1.50 µg/kg added
- Precision (RSD) for calculated AMOZ concentration in replicates of incurred sample was 2.9 %
- The range of ion ratios (0.81 to 0.84) was well within tolerance of ±20 % (0.66 to 0.99)
- Precision (RSD) for AMOZ retention time in replicates of incurred sample was 0.1 %, well within tolerance of ±2.5%
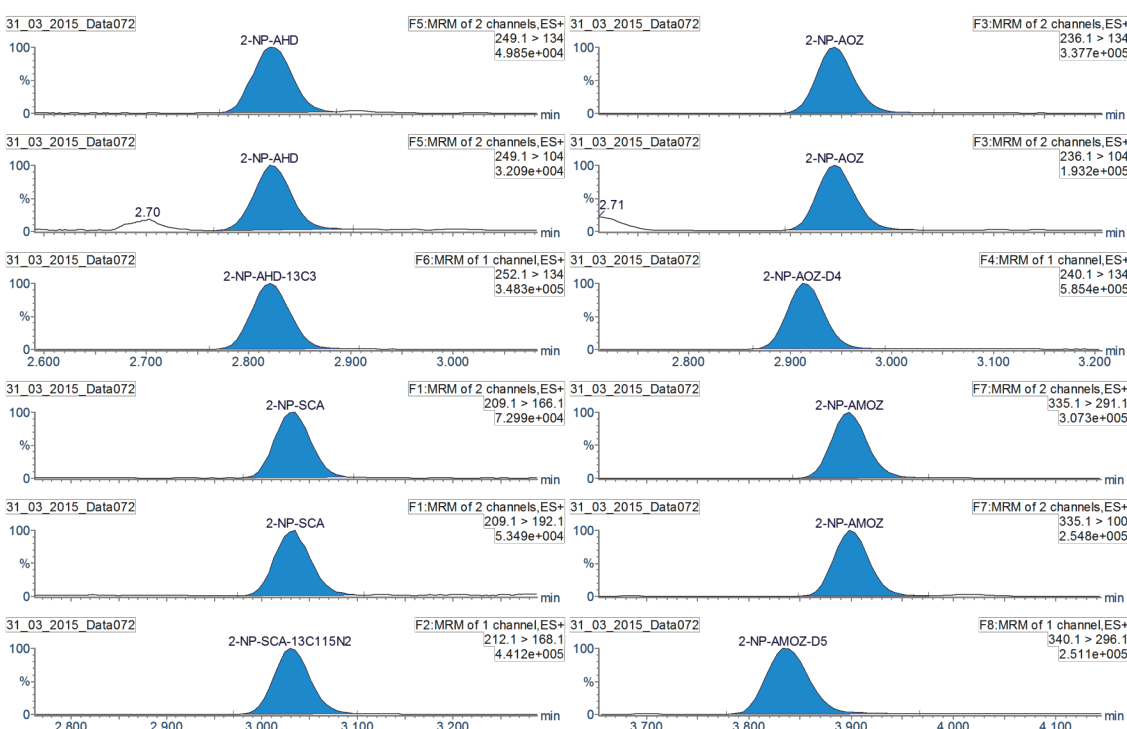


Figure 2 Chromatograms showing the detection of different NFs extracted from a poultry muscle sample spiked at screening target concentration (STC) of @ 0.5 µg/kg

Compound name: 2-NP-AHD
Correlation coefficient: r = 0.998009, r^2 = 0.996022
Calibration curve: 1.17322 * x + -0.0537145
Response type: Internal Std ( Ref 2 ), Area * ( IS Conc. / IS Area )
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None
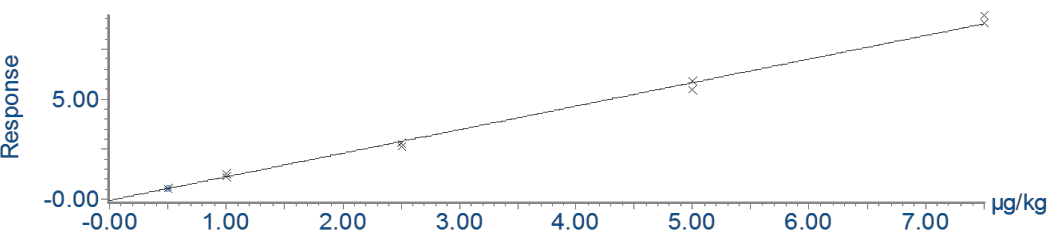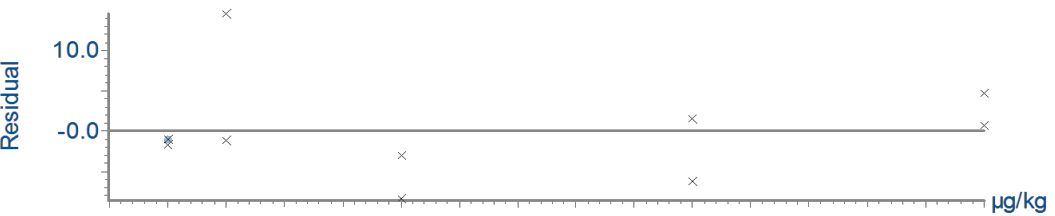
Compound name: 2-NP-AMOZ
Correlation coefficient: r = 0.999901, r^2 = 0.999802
Calibration curve: 1.10779 * x + -0.0094808
Response type: Internal Std ( Ref 4 ), Area * ( IS Conc. / IS Area )
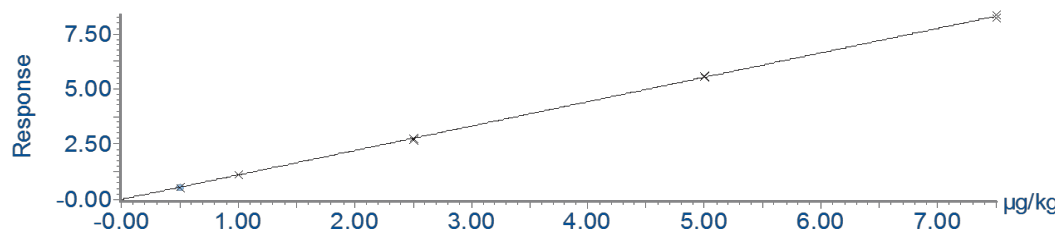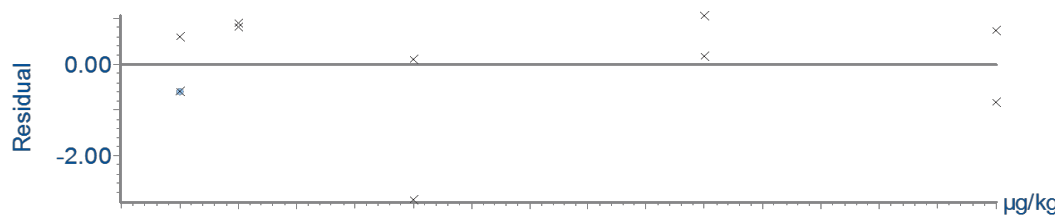Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Figure 4 Typical calibration graphs for the quantification of NFs in a honey proficiency test sample

## CONCLUSIONS

The ACQUITY H-Class with the Xevo TQ-S micro provides sufficient sensitivity for detection, identification and quantification of nitrofurans in a range of products.

The method is suitable for both official control purposes but also to meet the demands of pre-export testing, which may demand lower limits of quantification.

### References

1. Vass M et al. (2008). Nitrofuran antibiotics: a review on the application, prohibition and residual analysis. Veterinarni Medicina 53(9):469–500
2. CD. (2003). Commission decision 2003/181/EC of 13 March 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin
3. Cooper, K et al. (2005). Depletion of four nitrofuran antibiotics and their tissue-bound metabolites in porcine tissues and determination using LC-MS/MS and HPLC-UV . Food Additives & Contaminants: Part A 25: 406–414

### LC System:
**ACQUITY UPLC H Class with FTN autosampler**
Runtime: 11 min (to separate AHD from interference)
Column: ACQUITY UPLC BEH $C_{18}$ 1.7 µm, 2.1 × 100 mm
Mobile phase A: 0.5 mM ammonium formate (aq.)
Mobile phase B: Methanol
Flow rate: 0.45 ml.min$^{-1}$
Injection volume: 5 µl
Gradient:

| Time (min) | %A | %B |
|---|---|---|
| Initial | 80 | 20 |
| 0.2 | 80 | 20 |
| 7.0 | 25 | 75 |
| 7.25 | 0 | 100 |
| 8.25 | 0 | 100 |
| 8.26 | 80 | 20 |

### MS system:
**Xevo TQ-S micro**

| | | | |
|---|---|---|---|
| Ionisation mode: ESI | | Polarity: | +ve |
| Capillary voltage: 0.5kV | | Source temperature: | 150°C |
| Desolvation temperature: | 650°C | | |
| Desolvation gas: 1000 l.hr$^{-1}$ | | Cone gas flow: | 150 l.hr$^{-1}$ |

| Compound | RT (min) | MRM | Cone | CE |
|---|---|---|---|---|
| NP-AHD | 2.82 | 249.1>134.0 | 50 | 10 |
| | | 249.1>104.0 | 50 | 20 |
| NP-AHD-$^{13}C_3$ | 2.82 | 252.1>134.0 | 50 | 10 |
| NP-AOZ | 2.94 | 236.1>134.0 | 50 | 10 |
| | | 236.1>104.0 | 50 | 20 |
| NP-AOZ-d4 | 2.91 | 240.1>134.0 | 50 | 10 |
| NP-SCA | 3.03 | 209.1>166.1 | 30 | 8 |
| | | 209.1>192.1 | 30 | 10 |
| NP-SCA-$^{13}C^{15}N_2$ | 3.03 | 212.1>168.1 | 30 | 8 |
| NP-AMOZ | 3.90 | 335.1>291.1 | 35 | 10 |
| | | 335.1>100.0 | 35 | 30 |
| NP-AMOZ-d5 | 3.83 | 340.1>296.1 | 35 | 10 |

## TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS