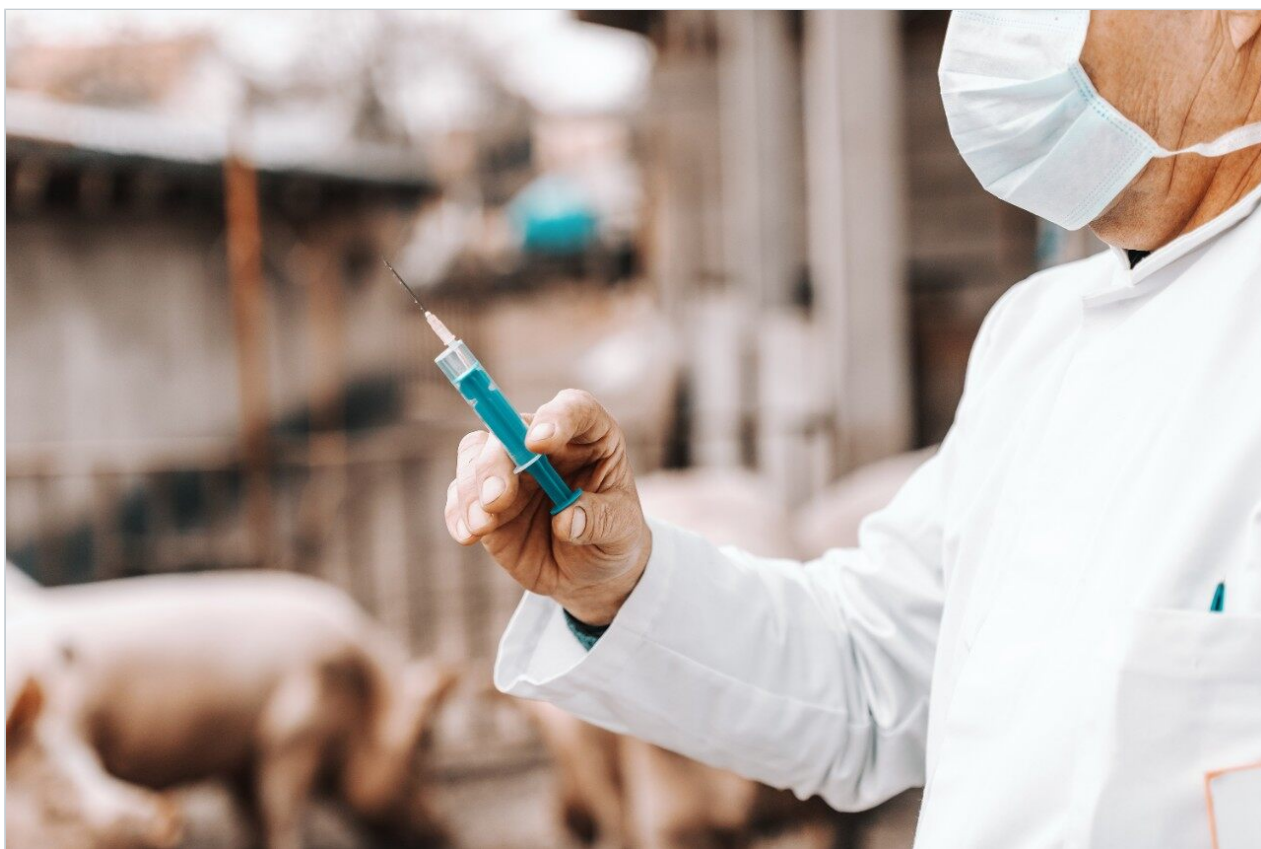


Note d'application

Determination of Nitrofurant Veterinary Drug Residues Using Waters Micromass Quattro Premier: Tandem Mass Spectrometer

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Abstract

This application note describes the performance of the Quattro Premier Mass Spectrometer for the analysis of bound nitrofurán metabolites in chicken meat.

Introduction

The four nitrofurán antibiotics furazolidone, furaltadone, nitrofurazone and nitrofurantoin are banned from use in the medication of animals destined for human consumption.¹ Once administered to livestock, the drugs are rapidly metabolized and, after a few hours, cannot be detected in edible tissues. Certain metabolites of these drugs are more persistent and their presence can be used as a marker of illegal use. Proportions of these metabolites exist as protein adducts and the established method of analysis contains an acid hydrolysis step prior to extraction. A less complex sample matrix may be obtained if the homogenized sample tissue is washed with organic solvents prior to the hydrolysis step. Such a method is used to target only the bound residues since any free metabolite will be removed during the washing stages.² This has the advantage of reducing sample matrix effects and minimizing the amount of routine instrument maintenance required.

Previous Waters application notes have described the analysis of nitrofurán metabolites using the Waters Micromass Quattro Ultima Platinum Mass Spectrometer.³ The Quattro Premier is a tandem quadrupole mass spectrometer that incorporates novel travelling wave (T-Wave) ion transfer and collision cell optics, together with improved detector technology in order to deliver unsurpassed sensitivity and method flexibility.⁴ This note describes the performance of the Quattro Premier Mass Spectrometer for the analysis of bound nitrofurán metabolites in chicken meat.

Experimental

Method

Extraction

- 2 g Chicken meat is homogenized
- Washed with methanol, ethanol, diethyl ether
- Calibration, recovery and internal standards are spiked
- Metabolites are hydrolyzed from protein at low pH and derivatized with 2-nitrobenzaldehyde
- Extracted with ethyl acetate
- Reconstituted in 500 μ L (1:4) methanol/water
- 50 μ L injected on column

Analysis

Analyses were carried out using an Alliance 2795 HPLC System with a Quattro Premier Mass Spectrometer. Mobile phase A was water/methanol (4:1) with 0.5 mM ammonium acetate; mobile phase B was water/methanol (1:9) with 0.5 mM ammonium acetate. The LC column was a Symmetry C₈, 2.1 \times 100 mm, 3.5 μ m with a 2.1 \times 10 mm pre-column of the same stationary phase. The injection volume was 50 μ L and mobile phase flow rate was 0.3 mL/min. The eluent was directed into the electrospray source of the mass spectrometer, which was operated in positive ion, multiple reaction monitoring mode. Two MRM transitions are followed for each analyte. The HPLC gradient program is shown in Table 1.

Time	0.0	0.1	4.9	5.0	7.0	7.1	End
% B	20	35	35	100	100	20	Re-equilibration

Table 1. HPLC gradient program.

Five calibration standards were prepared at 0.05, 0.1, 0.5, 1.0 and 2.0 ppb together with five recovery samples at 0.1 ppb and five at 1.0 ppb.

Results and Discussion

The chromatographic separation of analytes, in a matrix matched calibration standard, is shown in Figure 1. Both MRM transitions may be seen for each analyte.

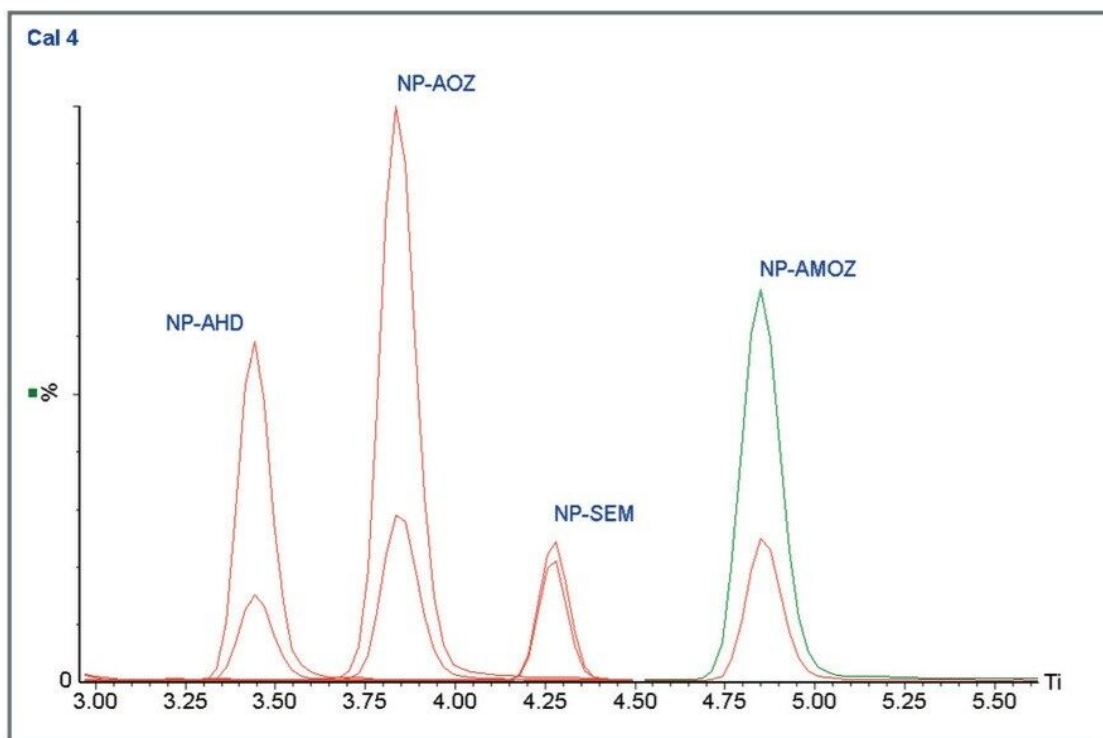


Figure 1. Chromatographic separation of the analytes in a 2 ppb matrix matched calibration standard.

Calibration graphs for NP-SEM, NP-AHD, NP-AMOZ and NP-AOZ are shown in Figures 2 to 5.

Compound name: NPSEM
Correlation coefficient: $r = 0.999270$, $r^2 = 0.998541$
Calibration curve: $0.176093 * x + 0.00154883$
Response type: Internal Std (Ref 5), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: $1/x$, Axis trans: None

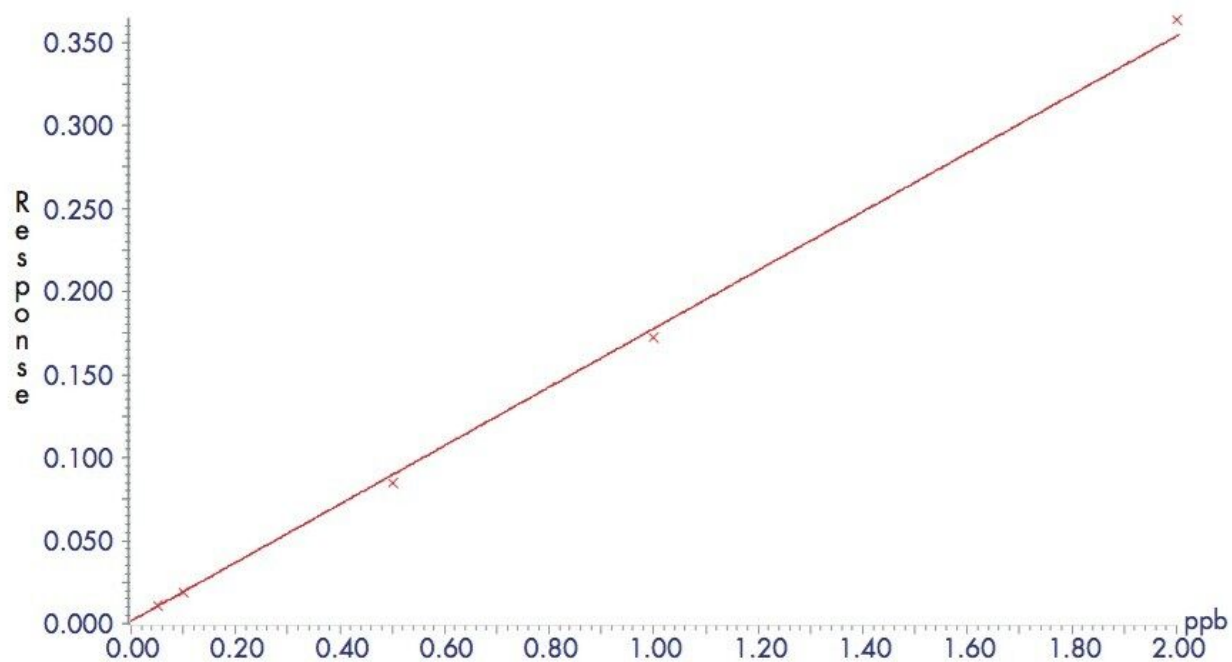


Figure 2. Calibration graph for NPSEM.

Compound name: NPAHD

Correlation coefficient: $r = 0.999018$, $r^2 = 0.998037$

Calibration curve: $0.617368 * x + 0.00522529$

Response type: Internal Std (Ref 5), Area * (IS Conc. / IS Area)

Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

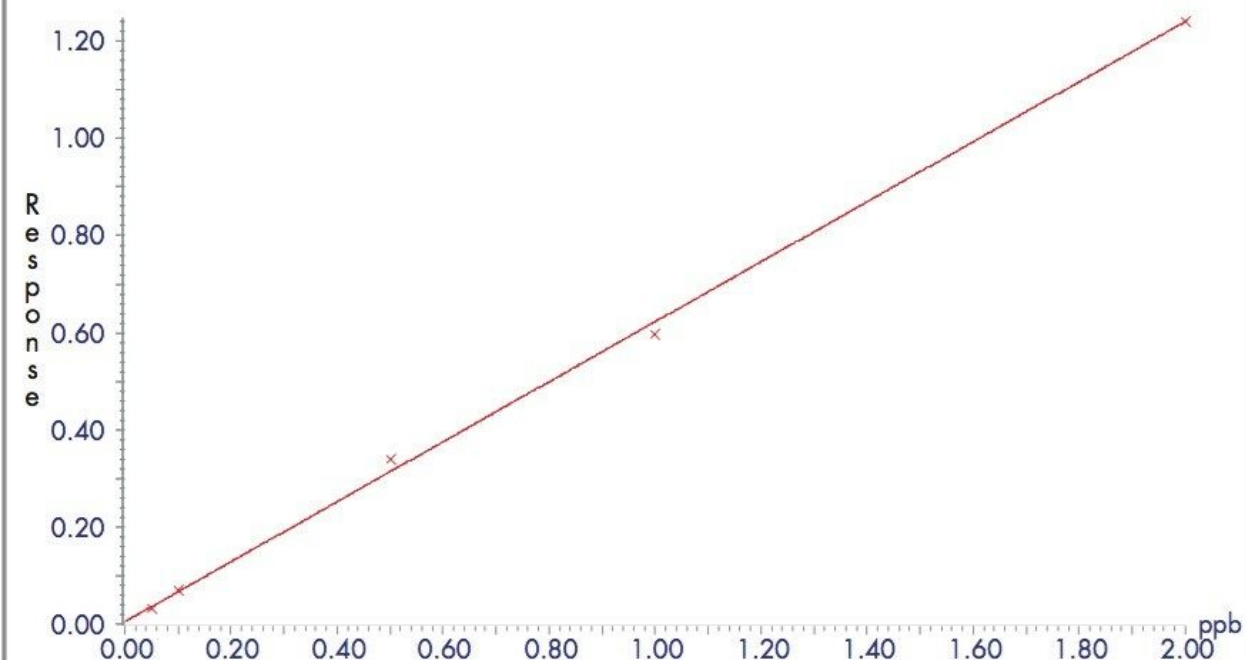


Figure 3. Calibration graph for NPAHD.

Compound name: NPAMTZ

Correlation coefficient: $r = 0.999772$, $r^2 = 0.999543$

Calibration curve: $1.24893 * x + -0.00511808$

Response type: Internal Std (Ref 6), Area * (IS Conc. / IS Area)

Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

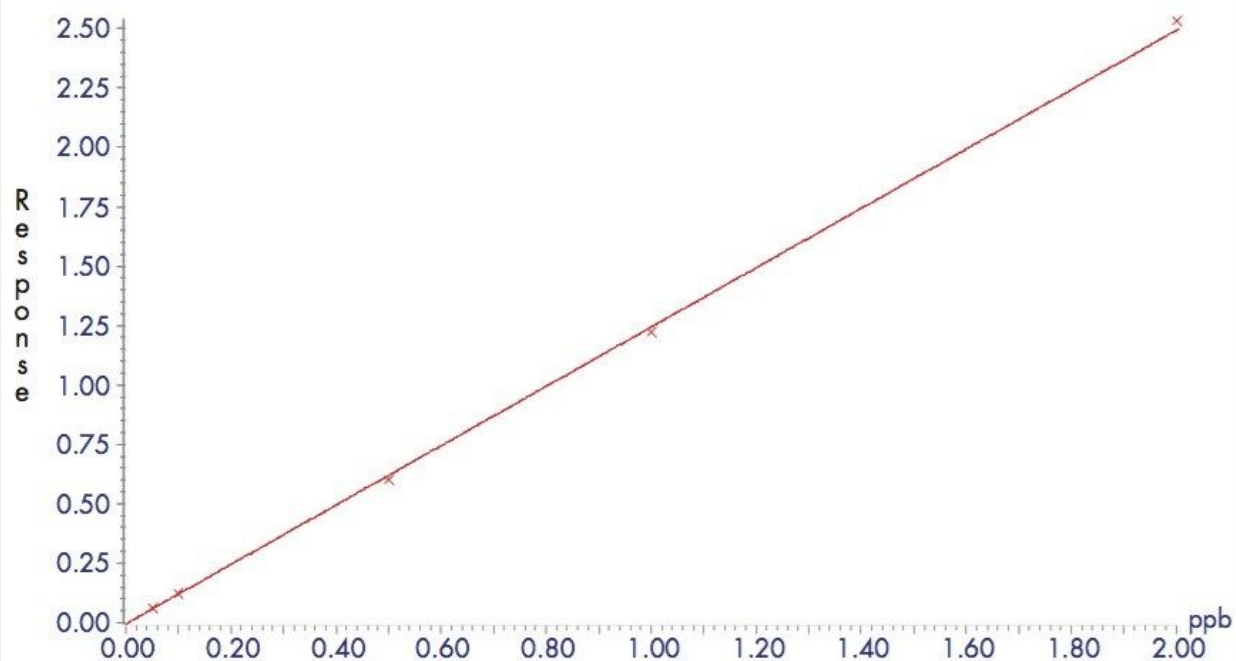


Figure 4. Calibration graph for NPAMTZ.

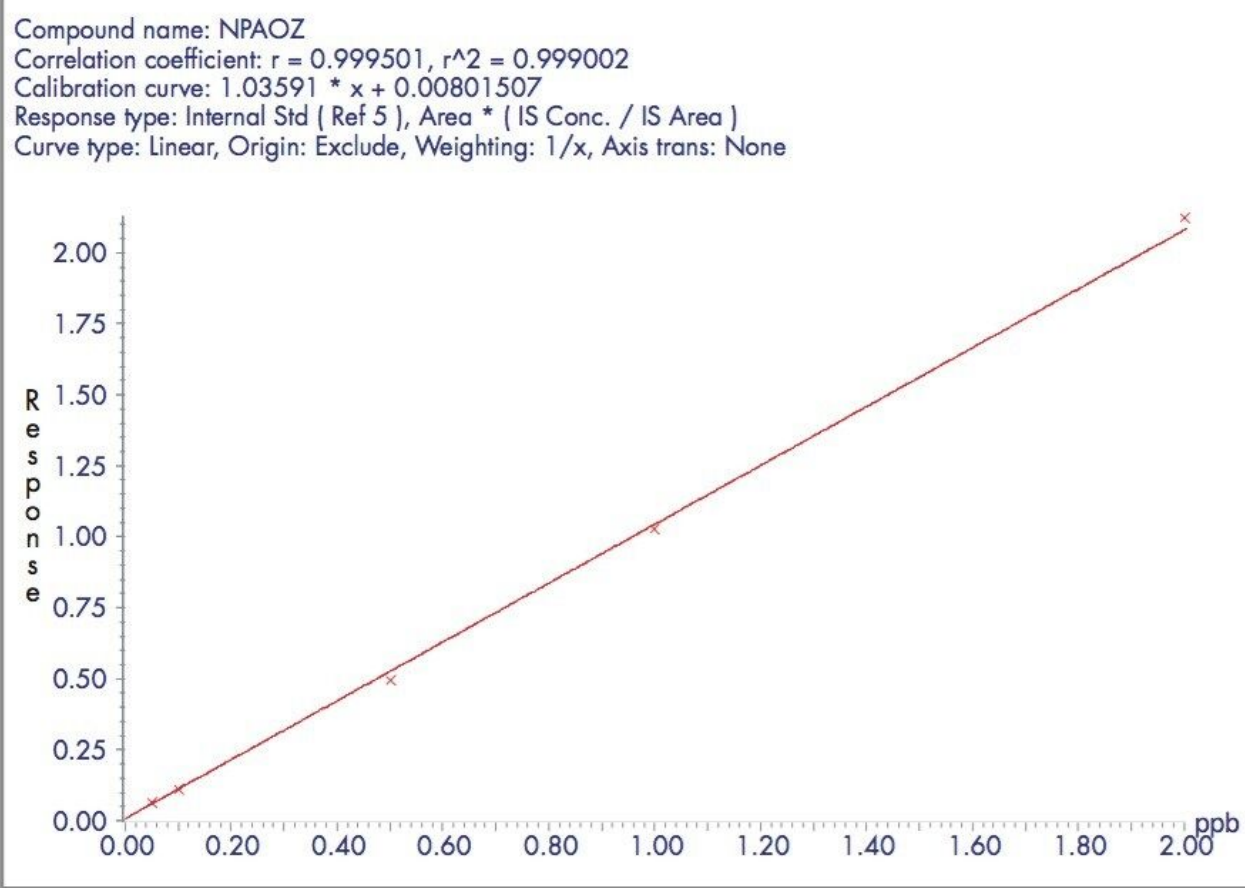


Figure 5. Calibration graph for NPAOZ.

Figure 6 shows the response factor for NP-AOZ over the course of an analytical batch. This is an indication of method repeatability.

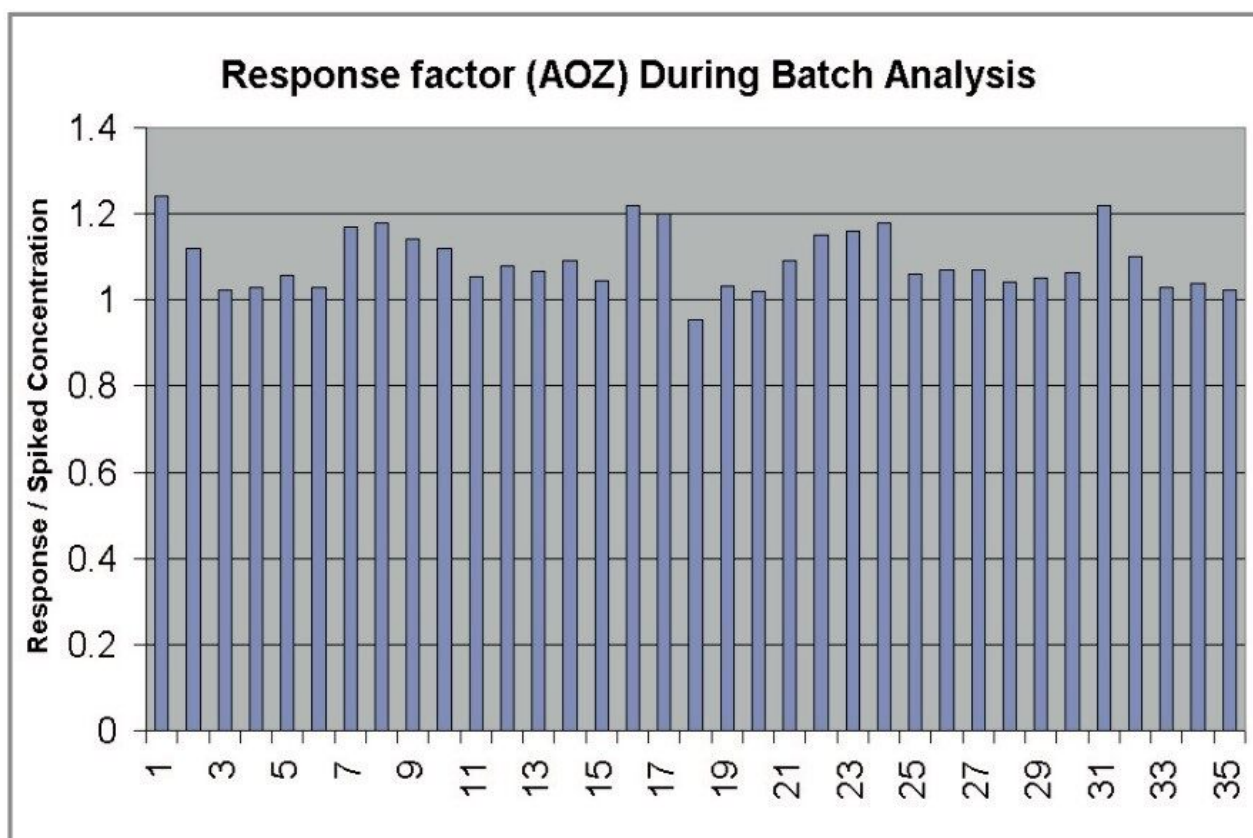


Figure 6. Response factor for NPAOZ during a batch analysis.

The method accuracy and precision can be seen in Table 2. The upper rows show mean observed concentration in the 0.1 ppb recovery samples together with %CVs. The lower rows show the same information for the 1.0 ppb recovery samples.

	AOZ	SEM	AMAZ	AHD
Mean	0.1	0.094	0.093	0.093
% CV	9.1	12.2	5.0	12.7
Mean	1.0	0.98	0.95	0.98
% CV	2.2	6.4	1.4	7.5

Table 2. Accuracy and precision for 0.1 and 1.0 ppb recovery samples.

Estimated instrument LODs for chicken matrix are shown in Table 3. These values were estimated from the response of the lowest matrix matched calibration standards using the 3:1 signal to noise (S/N) definition.

Estimated LOD (S:N = 3:1)

Using 0.05 ppb MM standard

	Daughter	S/N	LoD/ppb
AOZ	134	11	0.01
	104	8	0.02
SEM	192	7	0.02
	166	10	0.02
AMTZ	291	18	0.01
	262	11	0.01
AHD	134	12	0.01
	104	4	0.04

Table 3. Estimated instrument LODs in chicken matrix.

Conclusion

The established method for the analysis of bound nitrofuran metabolites in animal tissues has been successfully applied to the Waters Micromass Quattro Premier Mass Spectrometer. A novel LC method provides baseline separation of the derivatized metabolites and the instrumentation is sensitive enough to detect very low levels of these compounds in chicken meat.

References

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