ApplicationNOTE

Waters

DETERMINATION OF NITROFURAN VETERINARY DRUG RESIDUES USING WATERS MICROMASS QUATTRO PREMIER: TANDEM MASS SPECTROMETER

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Introduction

The four nitrofuran 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 nitrofuran 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 nitrofuran metabolites in chicken meat.

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 µL (1:4) methanol/water
- 50 µL 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® C_8 2.1 × 100 mm 3.5 µ with a 2.1 × 10 mm pre-column of the same stationary phase. The injection volume was 50 µL 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

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.

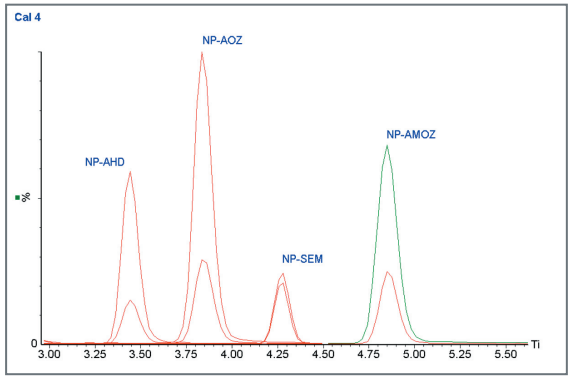

Figure 1. Chromatographic separation of the analytes in a 2 ppb matrix matched calibration standard.

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

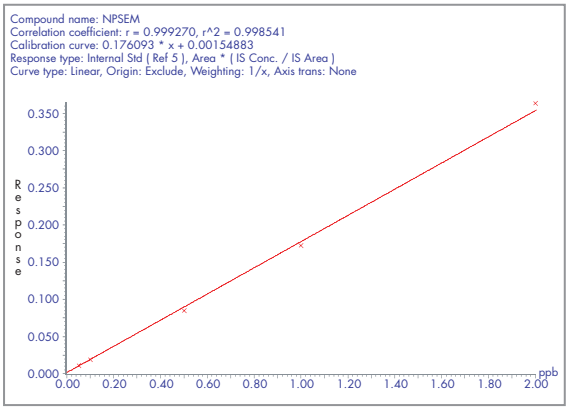

Figure 2. Calibration graph for NPSEM.

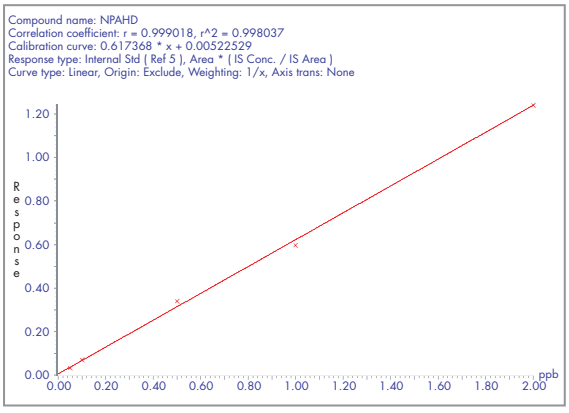

Figure 3. Calibration graph for NPAHD.

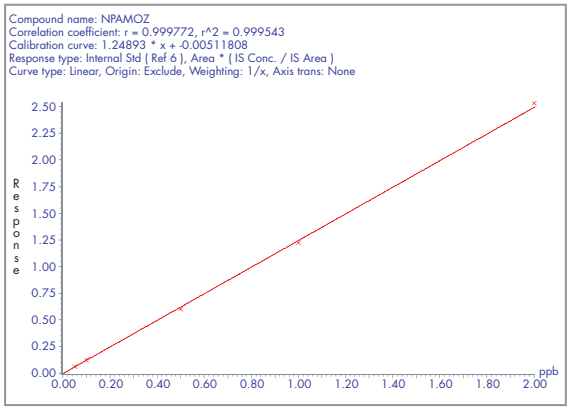

Figure 4. Calibration graph for NPAMOZ.

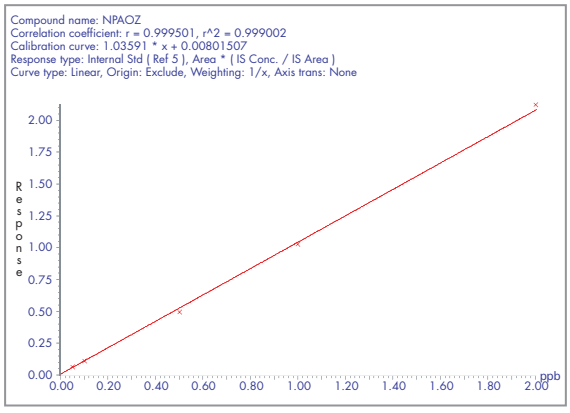

Figure 5. Calibration graph for NPAOZ.

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

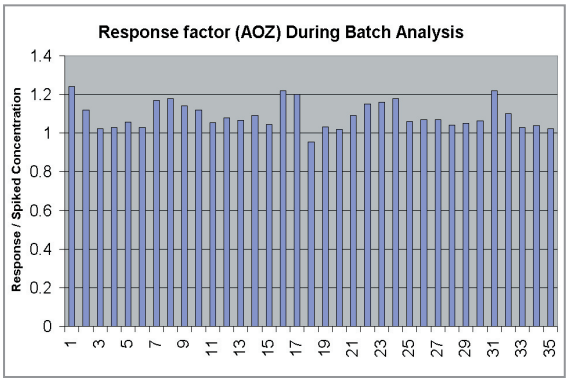

Figure 6. Response factor for NPAOZ during a batch analysis.

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	AMOZ	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
AMOZ	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.

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References

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2. Isolation of bound residues of nitrofuran drugs from tissue by solid-phase extraction with determination by liquid chromatography with UV mass spectrometric detection, Conneely A., A. Nugent, M.O. Keeffe, P.P.J. Mulder, J.A. van Rhijn, L. Kovacsics, A. Fodor, R.J. McCracken, D.G. Kennedy, Analytica Chimica Acta, 483, (2003), pp 91-98
3. A Method for the Determination of Nitrofuran Veterinary Drug Residues by LC/MS/MS, Waters Application Note 720000705EN
4. Travelling Wave Ion Propulsion in Collision Cells, Kevin Giles , Steven D Pringle; Kenneth R Worthington; Robert H Bateman, Proceedings of the 51st ASMS Conference, Montreal, Canada, 8th–12th June 2003.

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