

## Analyses of Various Chemical Residues in a Food Matrix Using LC/MS/MS

Presented by  
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FPRW – 2006  
Orlando, FL



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## Decision 2002/657/EC

Substance	Matrix	MRPL
Chloramphenicol	Meat	0.3 µg/kg
	Eggs	
	Milk	
	Urine	
	Aquaculture products	
Medroxyprogesterone acetate	Pig kidney fat	1 µg/kg

MRPL = minimum required performance limit

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## The Identification Point System

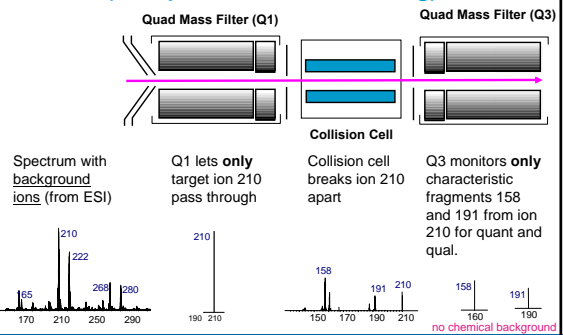
MS – Technique	IP / ion
Low resolution mass spectrometry (LR-MS)	1.0
LR-MS <sup>n</sup> precursor ion	1.0
LR-MS <sup>n</sup> transition products	1.5
High resolution mass spectrometry (HR-MS)	2.0
HR-MS <sup>n</sup> precursor ion	2.0
HR-MS <sup>n</sup> transition products	2.5

4 ions are needed when using GC/MS or LC/MS but only 2 transitions when applying LC-MS/MS

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## LC/MS QQQ: Excellent Solution for Sensitivity MRM (Multiple Reaction Monitoring)



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## Tolerances of Relative Intensities

Rel. intensity (% of base peak)	GC-MS/EI	GC-MS/CI, GC-MS/MS <sup>n</sup> LC-MS, LC-MS-MS <sup>n</sup>
> 50 %	± 10 %	± 20 %
> 20 % - 50 %	± 15 %	± 25 %
> 10 % - 20 %	± 20 %	± 30 %
≤ 10 %	± 50 %	± 50 %

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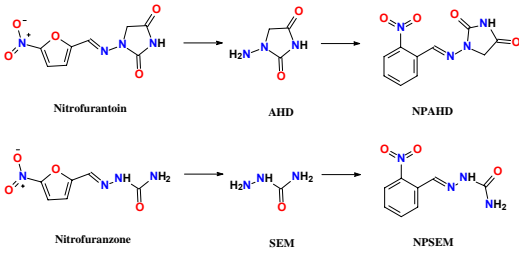
## Nitrofurans - Background

- ✚ The four drugs furazolidone, furaltadone, nitrofurazone and nitrofurantoin are veterinary drugs that belong to family of nitrofuran antibiotics
- ✚ Widely used for the treatment of gastrointestinal infections in cattle, pigs and poultry
- ✚ The European Union (EU) banned the use of nitrofuran antibiotics in food-producing animals by listing them in Annex IV of Council Regulation 2377/90
- ✚ The EU set a Minimum Required Performance Limit (MRPL) of 1 µg/kg (1 ppb) that laboratories should at least detect and confirm

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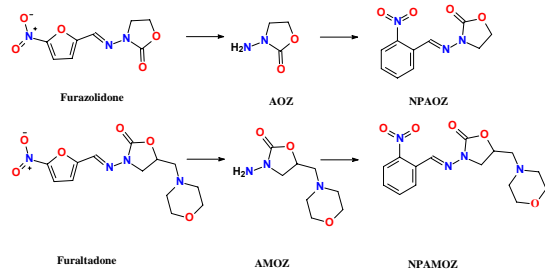
## Structures of Nitrofurans and Metabolites



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## Structures of Nitrofurans and Metabolites



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## Sample Preparation of Nitrofuran Metabolites

- Homogenization of blank control tissue (Tilapia) with addition of acid (HCl) to free tissue-bound residues
- L-L extraction by centrifugation
- Aliquot and evaporate the organic layer
- Reconstitute in 100  $\mu$ L LC mobile phase with addition of control standard spikes (derivatized metabolites)

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## HPLC Parameters

- ◆ HPLC system Agilent 1100 Series
- ◆ Column  $C_{18}$ , 2.1 x 150 mm, 5  $\mu$ m
- ◆ Injection volume 50  $\mu$ L
- ◆ Flow rate 0.3 mL/min
- ◆ Temp 40 C
- ◆ Mobile phase A: 0.1% Formic acid; B: Acetonitrile
- ◆ Gradient



Time	A	B
0	90	10
10	50	50
12	40	60
12.01	5	95
17	5	95
17.01	90	10
22	90	10

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## MS Parameters

- ◆ MS system Agilent 6410 LC/MS/MS
- ◆ Ion source ESI
- ◆ Polarity Positive
- ◆ Nebulizer gas Nitrogen
- ◆ Ion spray voltage 4000 V
- ◆ Source temp 350 C
- ◆ Resolution Q1 (unit); Q3 (unit)
- ◆ Scan mode Multiple Reaction Monitoring (MRM)
- ◆ Conditions of MRM



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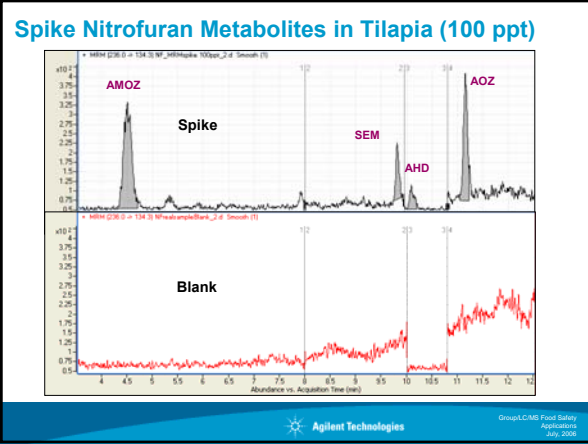
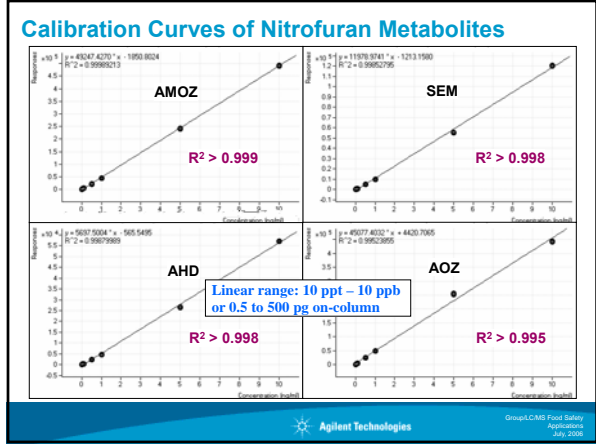
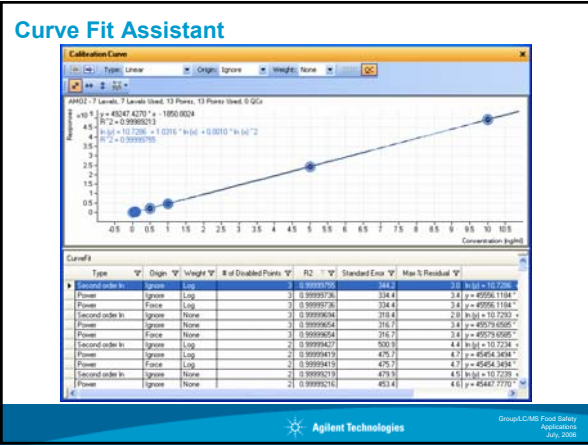
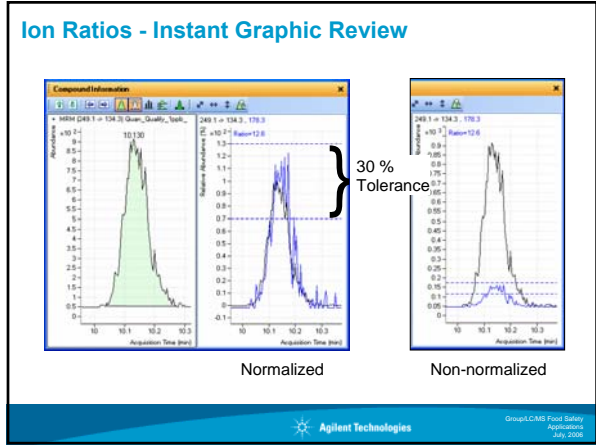
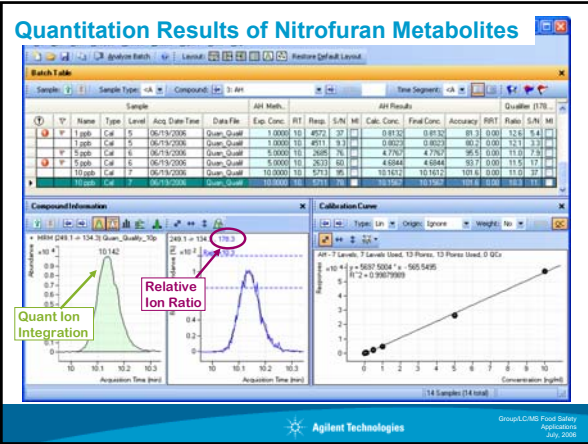
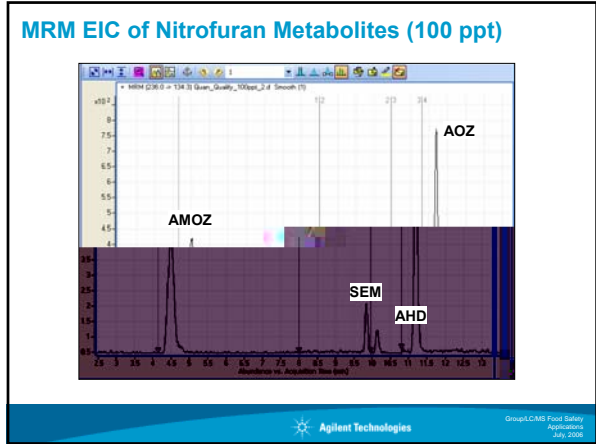
## MRM Conditions of Nitrofuran Metabolites



Time	Compound	Precursor	Product	Dwell (ms)	Fragmentor (V)	Collision Energy (V)
0.0	AMOZ	335.1	291.4	150	90	5
		335.1	262.4			
8.0	SEM	209.1	166.3			
		209.1	192.3			
9.9	AHD	249.1	134.3			
		249.1	178.3			
10.8	AOZ	236.0	134.3			
		236.0	104.3			

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## The Reproducibility of Nitrofuran Metabolites

Compounds	Concentration (ng/mL)	RSD <sup>1</sup> (%)
AMOZ (m/z 335.1 > 291.4)	0.1	1.05
SEM (m/z 209.1 > 166.3)	0.1	2.01
AHD (m/z 249.1 > 134.3)	0.1	8.07
AOZ (m/z 236.0 > 134.3)	0.1	1.58

<sup>1</sup> From 8 replicate injections.

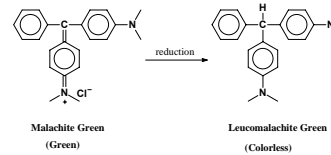
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## Malachite Green (MG)

Among other veterinary drugs, the triphenyl-methane dye malachite green (MG) is a popular substance, and prevents fungal and parasitic infections in aquaculture, including saprolegniasis, white spot disease, and ciliates.

MG is prevalently reduced into leuco-malachite green (LMG) and deposited in fatty tissue of the fish.



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## Analytical approaches for determination of residues of MG

HPLC equipped with a post-column unit for oxidation of LMG and with either an absorbance or MS detector for detection in Trout (eggs, fry and muscle), catfish (muscle and plasma) and in Trout muscle. The reactors (5 cm×4 mm) used in these studies were filled with 25% PbO<sub>2</sub>.

Other method LC-APCI-MS in Catfish and Trout without the use of a reactor for the conversion of LMG. This study aimed at the development and validation of a procedure for sample processing and of an HPLC analysis method for determination of residues of MG.

The use of LC-ESI-MS-MS analysis was explored for confirmation of detected residues of this drug in such matrices.

Sample preparation is fundamentally similar to nitrofurans except no derivatization required. Again, control spikes added post-extraction.

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## HPLC Parameters

- HPLC system: Agilent 1100 Series
- Column: C<sub>18</sub>, 2.1 × 150 mm, 5 μm
- Injection volume: 10 μL
- Flow rate: 0.3 mL/min
- Mobile phase: A: 10mM NH<sub>4</sub>Ac (pH=4.5); B: Acetonitrile
- Gradient



Time	A	B
0	70	30
1	50	50
2	5	95
8	5	95
8.01	70	30
13	70	30

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## MS Parameters

- MS system: Agilent 6410 LC/MS/MS
- Ion source: ESI
- Polarity: Positive
- Nebulizer gas: Nitrogen
- Ion spray voltage: 4000 V
- Source temp: 350 C
- Resolution: Q1 (unit); Q3 (unit)
- Scan mode: Multiple Reaction Monitoring (MRM)
- Conditions of MRM



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## MRM Conditions of MG and LMG

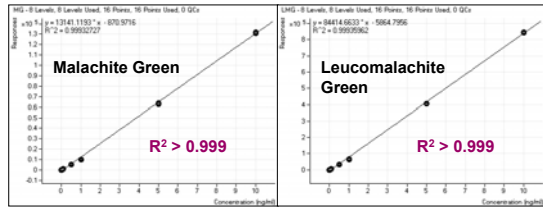


Time	Compound	Precursor	Product	Dwell (ms)	Fragmentor (V)	Collision Energy (V)
0.0	Malachite Green	329.3	313.3	40	100	40
		329.3	208.2	40	100	40
7.0	Leucomalachite Green	331.3	316.3	40	100	30
		331.3	239.2	40	100	30

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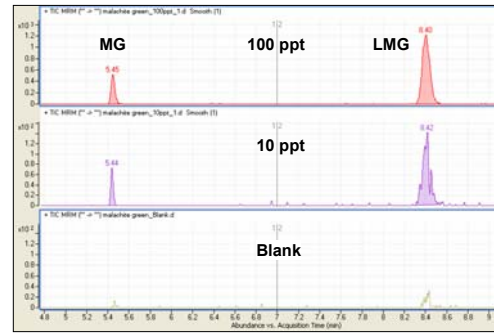
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## Calibration Curves



Linear range: 10 ppt – 10 ppb  
 or 0.1 to 100 pg on-column

## MRM EIC of MG and LMG (100 ppt) in Tilapia



## The Reproducibility of MG and LMG in Tilapia

Compounds	Concentration (ng/mL)	RSD <sup>1</sup> (%)
Malachite Green ( <i>m/z</i> 329.3 > 313.3)	0.1	3.52
Leucomalachite Green ( <i>m/z</i> 331.3 > 239.2)	0.1	2.25

<sup>1</sup> From 8 replicate injections.

## Conclusions

- ◆ LCMS, in general, eliminates the need for derivatization typically required for GCMS. Nitrofurans are an exception.
- ◆ Triple quadrupole mass spectrometer has more than enough sensitivity and reproducibility for quantitating below MRPLs.
- ◆ Linearity over two orders of magnitude without internal standards.
- ◆ Sample extraction / cleanup still an important part for minimizing matrix effects and reducing maintenance.