Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Canadian Food Inspection Agency




Our vision:
To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Multi-Residue Determination of Organic Arsenical Drugs in Feeds by LC-MS/MS

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AAFCO Laboratory Methods and Services Committee meeting

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Canada

Background

- Animal Feed Division of CFIA identified a high priority need for the determination of three organic arsenicals (arsanilic acid, roxarsone and nitarsone) at residue levels in animal feed
- These are withdrawal drugs and are priority food contaminants
- Current test methods are at guarantee levels greater than 10% minimum use rate
- Therefore, current methods not well suited for residue or traceback testing
- Requested feed residue LOQ of 1 mg/kg for all three organic arsenicals

Background

- UHPLC-PDA Challenges
  - Extract were very dirty
    - Tried sample clean-up using Oasis MAX SPE
      - Still very dirty
- HPLC Challenges
  - Compounds elute too easily
  - Analytical column must : retain and separate compounds, and give good peak shape
  - Analytical column : Phenomenex Onyx Monolithic C18 100 X 3.0mm

Background

- LC/MS/MS method (positive mode)
  - Column: Phenomenex Onyx Monolithic C18 100 X 3.0mm
  - Linearity problems with Internal Standard (IS)
  - Internal standard – 4-hydroxyphenylarsonic acid
  - Peak area of the internal standard increased with increasing analyte concentration
- Cause
  - 4-hydroxyphenyl arsanic acid co-elute with Arsanilic acid and have similar m/z

New method - summary

- *Liquid chromatography combined with atomic and molecular mass spectrometry for speciation of arsenic in chicken liver.* Peng et. al., Journal of Chromatography A, 1370 (2014) 40-49
- Analytes: 3-nitro-4-hydroxyphenylarsonic acid (Roxarsone), *p*-arsanilic acid, 4-nitrophenylarsonic acid (Nitarsone)
- Internal standard: 4-hydroxyphenylarsonic
- Extraction: 2% K_2HPO_4 in MeOH/H_2O (10+90)
- Column: Hamilton PRP-X110S anion exchange column (7 µm x 100 mm x 2.1 mm)
- Mobile phase: 80mM Ammonium Bicarbonate in MeOH/H_2O (10+90), pH 10.0
- Detection: API 5000 LC/MS/MS, negative ionization

Method Development - Infusion

LC-MS/MS transitions

- "a" used for quantitation and "b" used for confirmation

Name	Q1 (m/z)	Q3 (M/z)
Arsanilic acid a	215.90	106.90
Arsanilic acid b	215.90	122.80
Roxarsone a	262.00	106.90
Roxarsone b	262.00	122.80
Nitarsone a	246.00	106.90
Nitarsone b	246.00	122.80
4- hydroxyphenyl arsonic acid (IS)	216.90	106.90

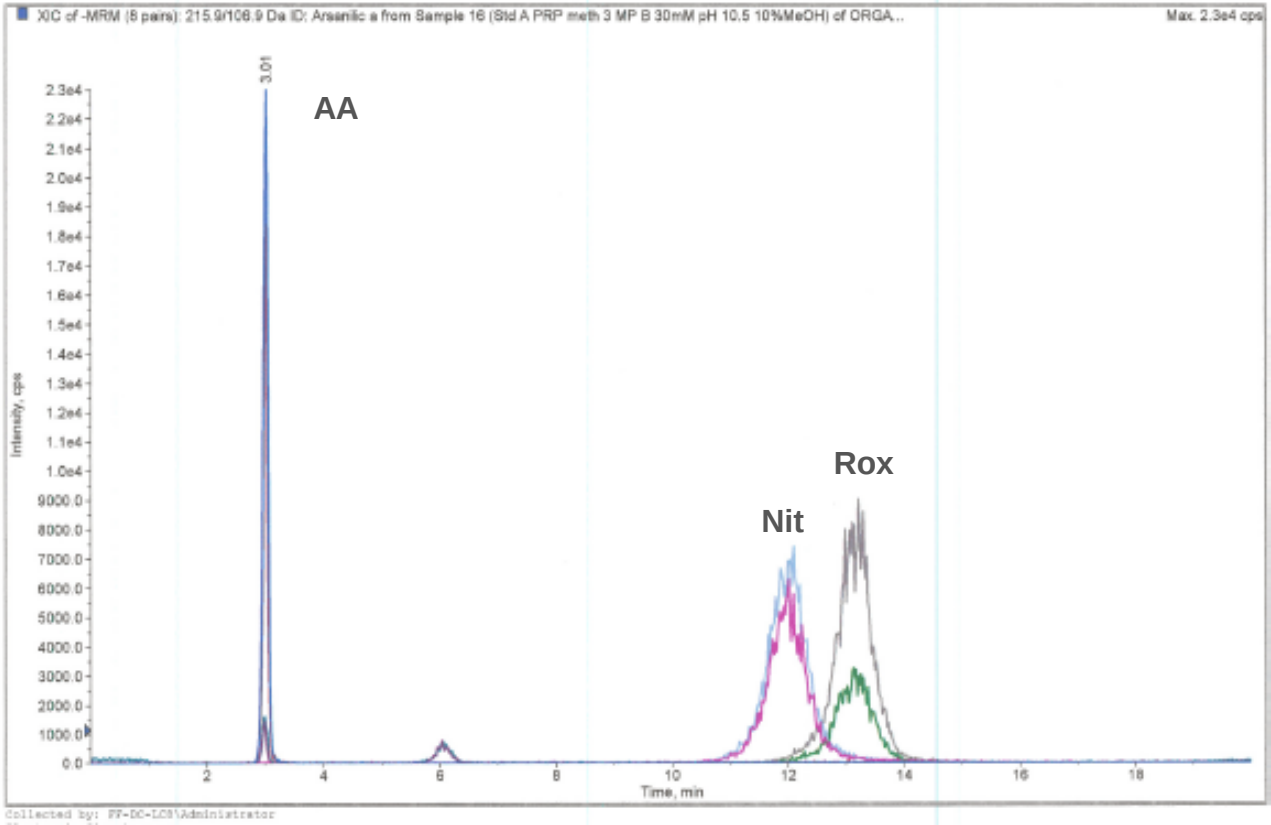
Method Development

Optimization of MS/MS Parameters

CUR	30.00
GS 1	60.00
GS 2	70.00
Ihe	ON
CAD	9.00
IS	-3000
TEM	750.00
DP	-65.00
EP	-10.00
Polarity	negative

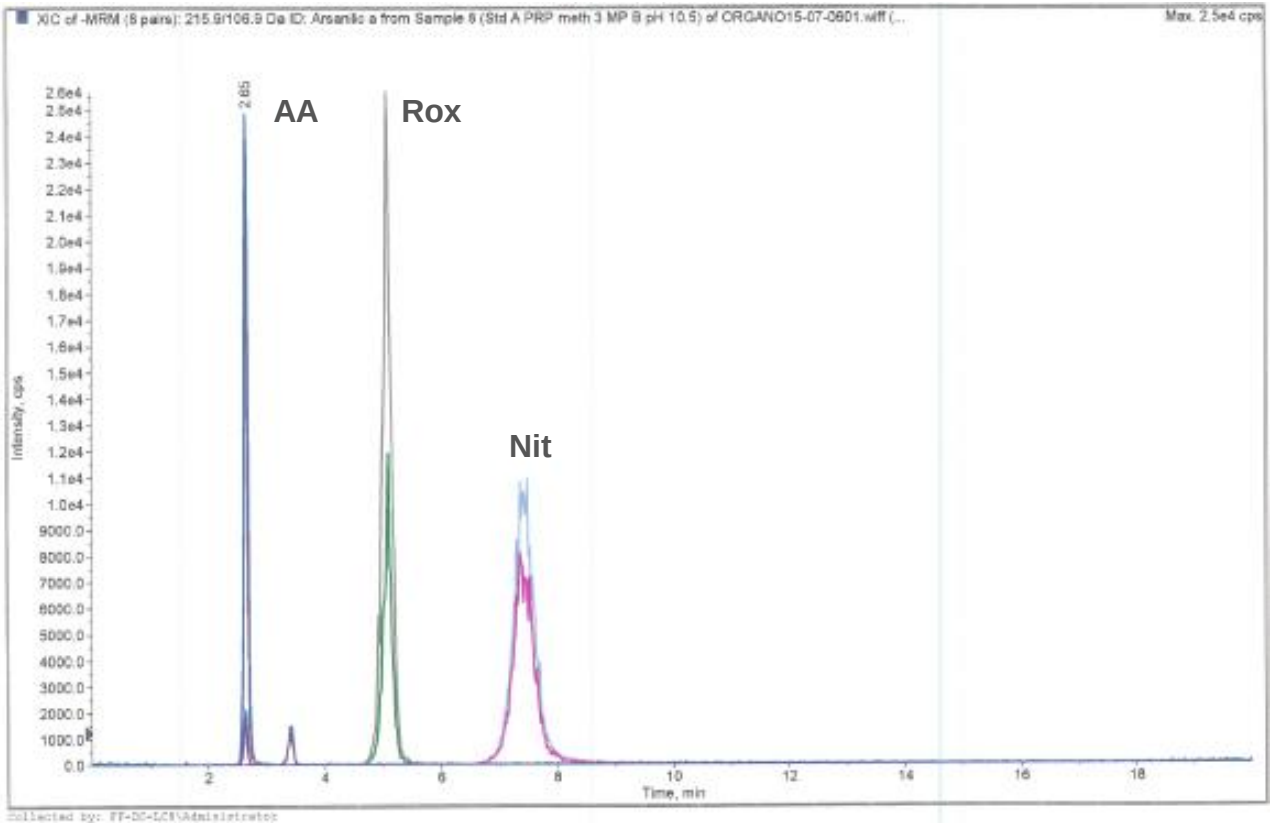
Method Development – Mobile phase

- Mobile Phase A – 30 mM Ammonium bicarbonate



Method Development

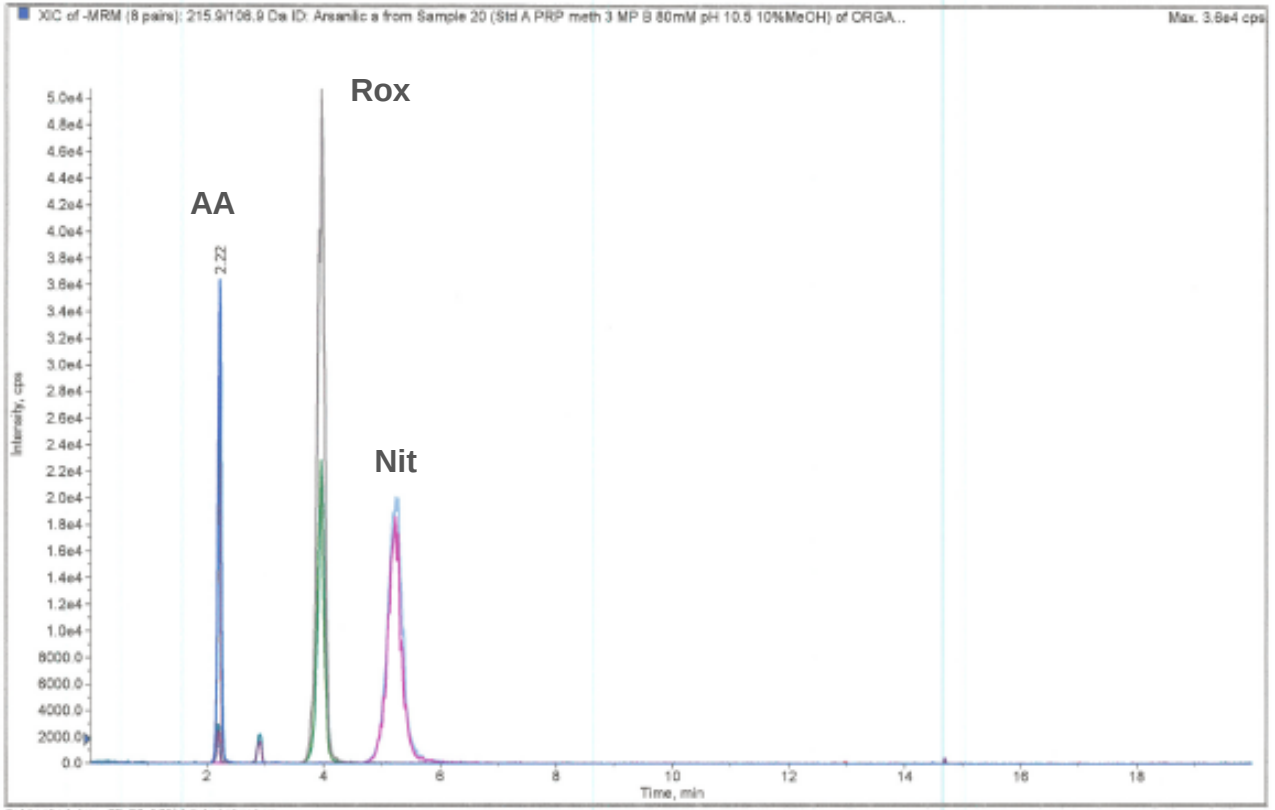
- Mobile Phase A – 60 mM Ammonium bicarbonate



Method Development

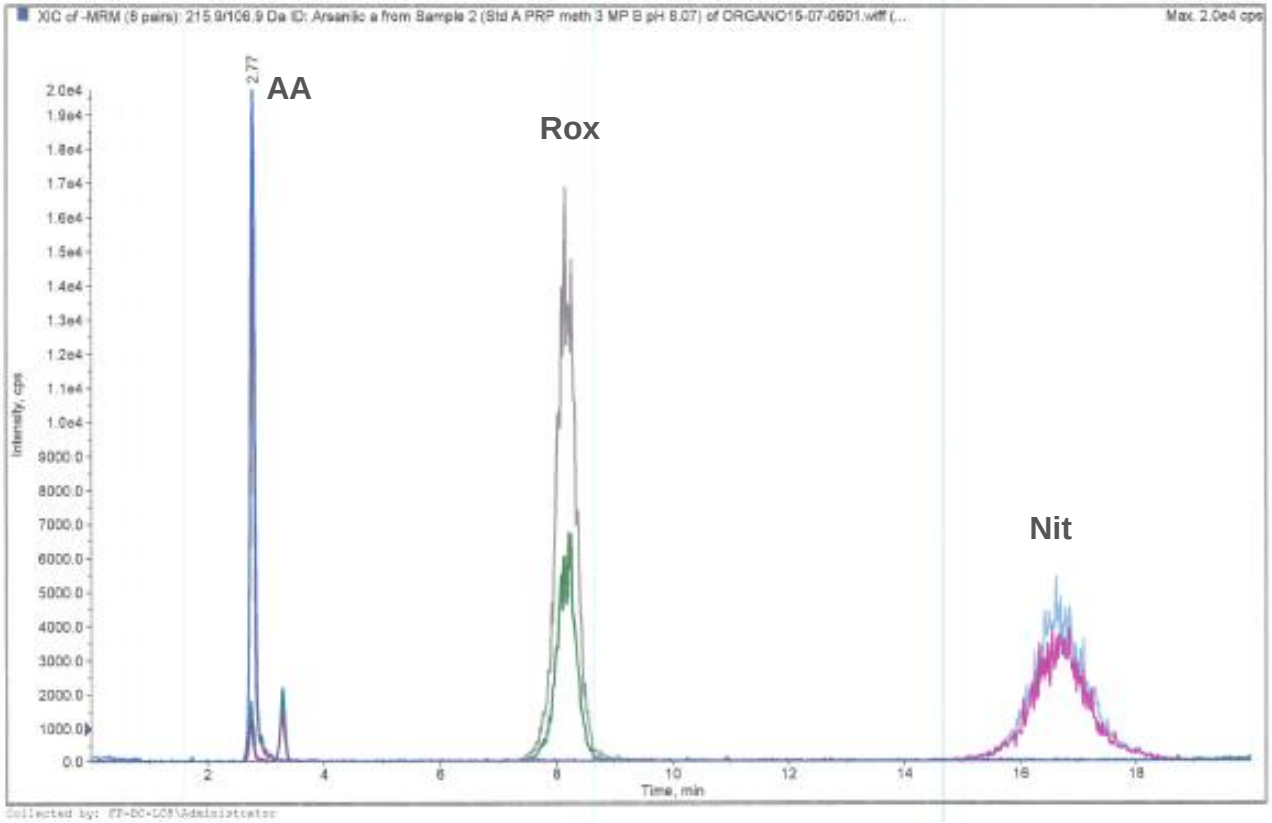
Mobile Phase A – 80 mM Ammonium bicarbonate

Shorter RTs and sharper peaks!



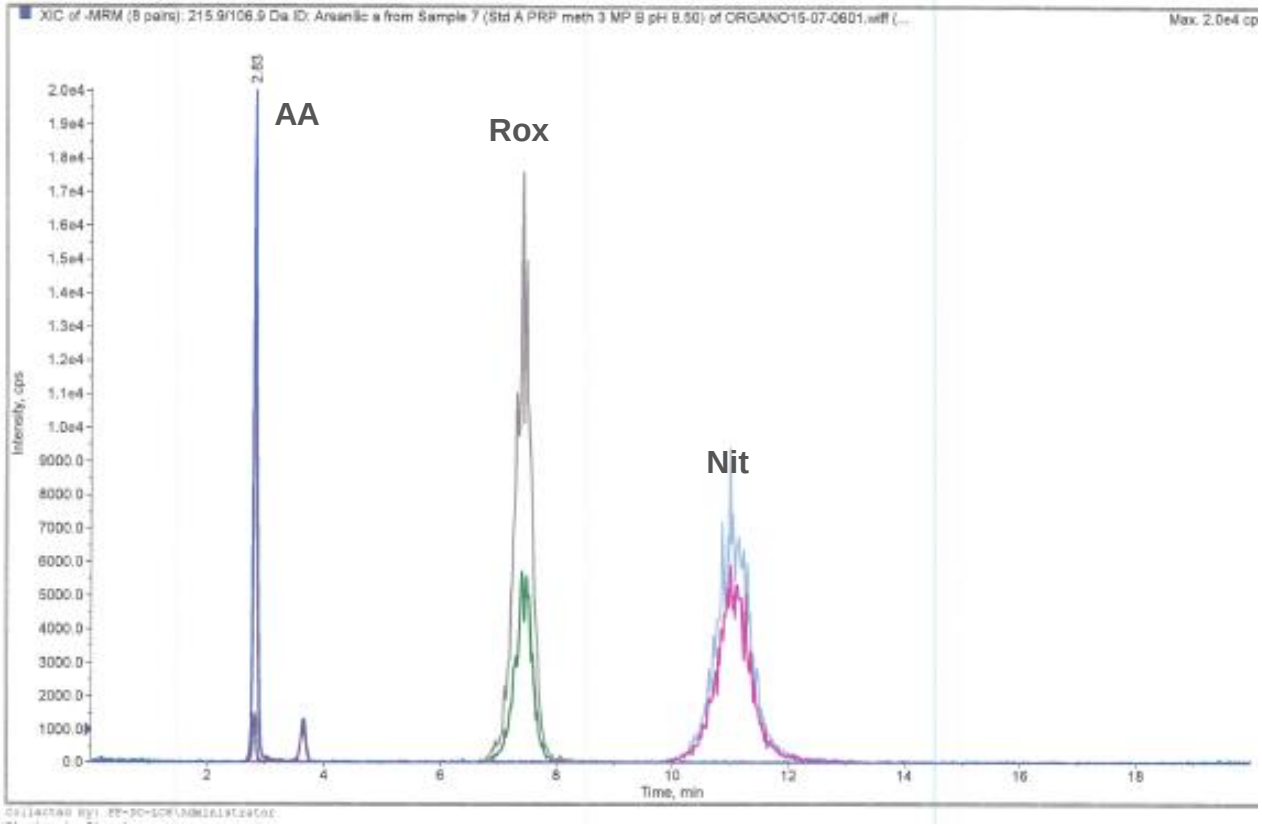
Method Development

Mobile Phase A – pH 8.07 (60 mM Ammonium Bicarbonate)



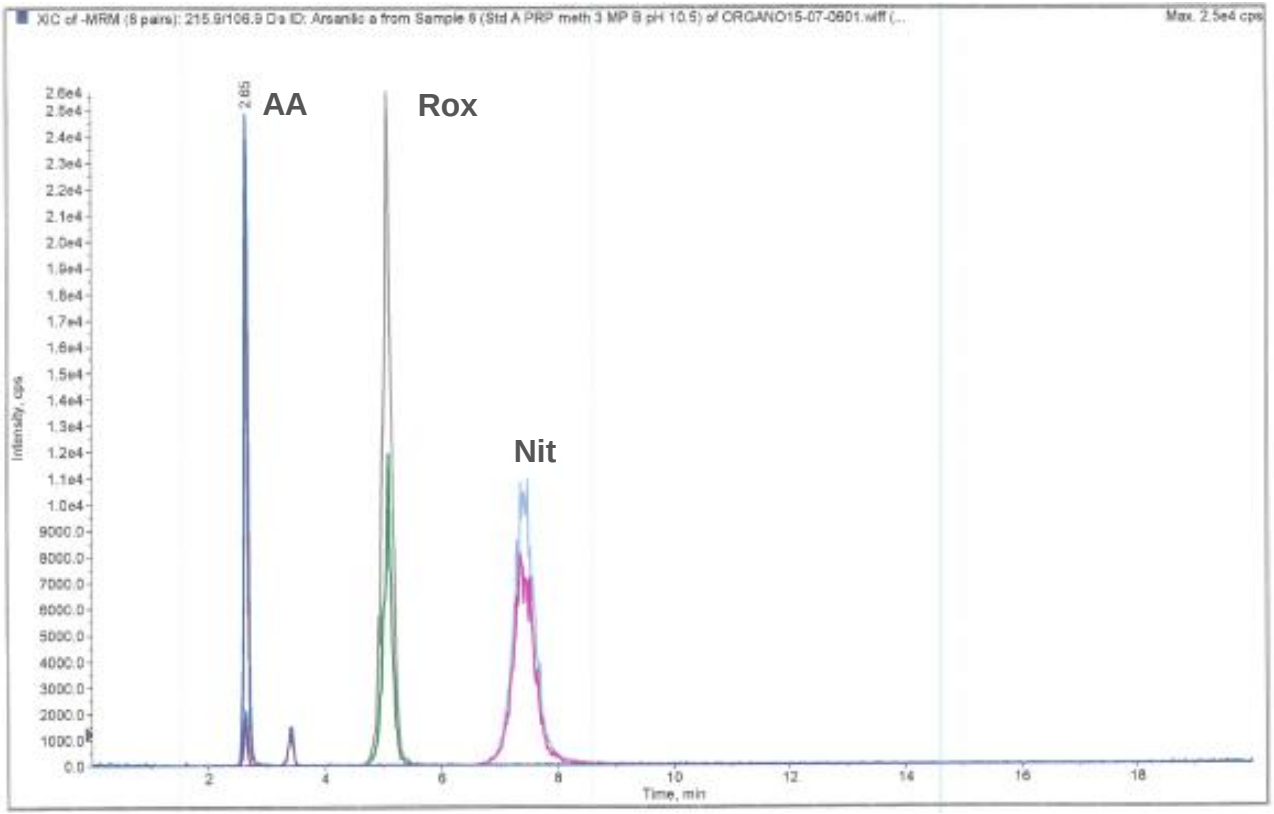
Method Development

Mobile Phase A – pH 9.50 (60 mM Ammonium Bicarbonate)



Method Development

Mobile Phase A – pH 10.50 (60 mM Ammonium Bicarbonate)



Method Development

Mobile Phase A = 80 mM Ammonium Bicarbonate in MeOH/H_2O (10+90), pH adjusted to 10.0

Mobile Phase B = MeOH/H_2O (10+90), column wash solution

Column: Hamilton PRP-X110S anion exchange column (7µm x 100mm x 2.1mm)

Injection volume = 25 µL

Flow rate = 1000 µL/min

Run time = 4 min

Method Development

- Extraction solution:

  #1 – MeOH/1% acetic acid (95+5): poor extraction efficiency for “real” sample (vs. spiked sample).

  #2 – 2% K_2HPO_4 (aqueous)

  #3 – 2% K_2HPO_4 in MeOH/H_2O (10+90). MeOH improves extraction efficiency for arsanilic acid.

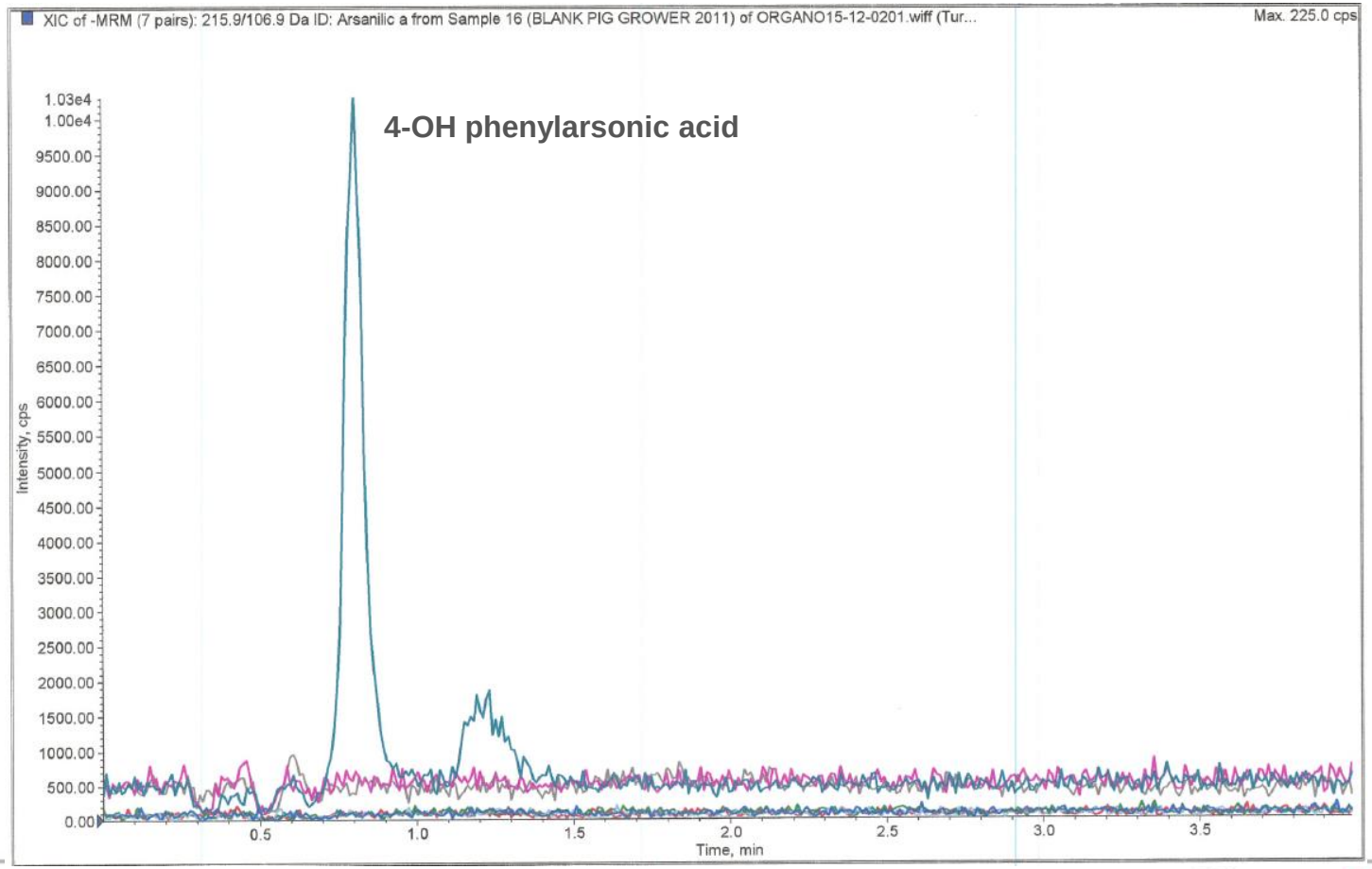
- Shaking time (tried 15 min, 30 min, 1 hr, 3 hrs, overnight)

Method Development

- Extraction
  - 20g sample
  - 100mL of % K_2HPO_4 in MeOH/H_2O (10+90)
  - Shake 30 min
  - Centrifuge ~ 30 mL of extract for 10 min
  - Filter supernatant through autovial syringeless filters
  - Dilute 50uL of filtered solution with 5 mL MeOH/H_2O (10+90)

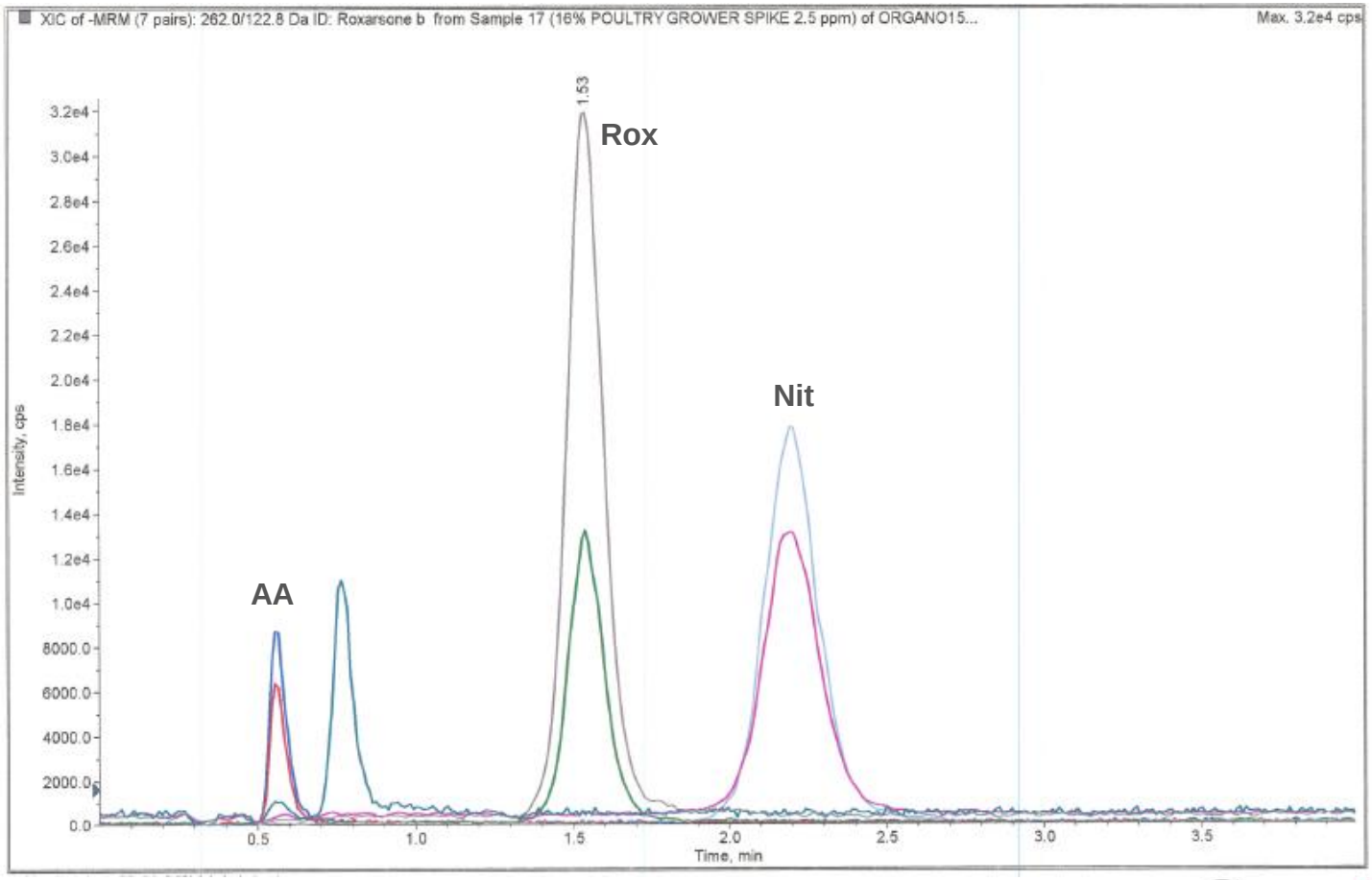
Method Development

Blank Poultry grower (with IS)



Method Development

Spiked Poultry grower at 2.5 ppm



Method Validation

Performance criteria evaluated

- Ruggedness
- Matrix effects
- LOD/LOQ
- Analytical Range
- Linearity
- Analyte stability
- Accuracy
- Repeatability
- Measurement uncertainty

Method Validation – matrix effects

- Even though we used an internal standard, matrix effects were observed. Matrix fortified standards used to compensate for the matrix effects.

Method Validation - matrices

- Pig grower
- Lamb grower
- Horse premix
- Dairy supplement
- Poultry grower
- Duck
- Lactating swine
- Beef ration
- Turkey finisher
- Dairy premix
- DDG
- Horse ration

Method Validation – LOD and LOQ

- LOD and LOQ was evaluated for each transition in different types of feed and premixes
- 12 blank matrices spiked at 0.5 ppm

Transition name	LOD (ppm)	LOQ (ppm)
Arsanilic acid a	0.13	0.37
Arsanilic acid b	0.12	0.33
Roxarsone a	0.10	0.27
Roxarsone b	0.09	0.25
Nitarsone a	0.10	0.29
Nitarsone b	0.12	0.32

Method Validation – Linearity and Analytical Range

- 5 mixed working standards with concentration range from 0.5 to 50 ng/mL
- Sample dilution is 1 g/500 mL = Range of 0.25 ppm to 25 ppm
- Coefficient of correlation ≥ 0.999 for all analytes

Method Validation – Linearity and Analytical Range

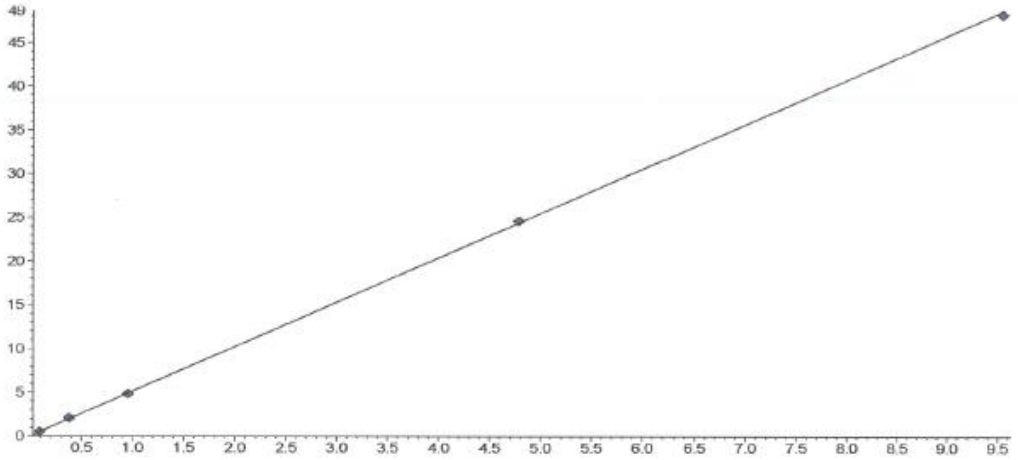
Analyte Name: Nitarsone a
Internal Standard: 4-HPA acid a

Data File	ORGANO15-12-2201.wiff	**Result Table**	ORGANOARS15-12-2201.rdb
Acquisition Date	12/22/2015 3:20:47 PM	**Algorithm Used**	IntelliQuan
Acquisition Method	Organoarsenicals.dam	**Instrument Name**	API 5000
Project	Organo\2015		

Regression Equation: y = 5.08 x + 0.0467 (r = 0.9999)

Expected Concentration	Number of Values	Mean Calculated Concentration	% Accuracy	Std. Deviation	%CV
0.515	1	0.49	95.4	N/A	N/A
2.06	1	2.18	105.7	N/A	N/A
5.15	1	5.06	98.3	N/A	N/A
25.80	1	26.08	101.1	N/A	N/A
51.50	1	51.17	99.4	N/A	N/A

ORGANOARS15-12-2201.rdb (Nitarsone a): "Linear" Regression ("1 / x" weighting):
y = 5.08 x + 0.0467 (r = 0.9999)



Method Validation – Accuracy and Repeatability

Samples spiked at 0.5 ppm, 2.5 ppm and 20 ppm.

Arsanilic Acid a

Matrix	Recovery mean (%)	CV (%)
16% Poultry grower	95.1	4.7
Duck	92.5	6.6
14% Horse Ration	90.1	4.2
DDG	84.2	7.3
Pig grower	98.1	6.6
Lamb grower	82.1	7.3
Horse premix	75.4	6.3
Dairy supplement	98.8	4.5

Method Validation – Accuracy and Repeatability

Roxarsone a

Matrix	Recovery mean (%)	CV (%)
16% Poultry grower	99.3	3.8
Duck	94.3	2.6
14% Horse Ration	95.0	3.7
DDG	108.5	5.4
Pig grower	103.0	4.7
Lamb grower	107.5	9.9
Horse premix	93.6	4.1
Dairy supplement	103.0	4.1

Method Validation – Accuracy and Repeatability

Nitarsone a

Matrix	Recovery mean (%)	CV (%)
16% Poultry grower	98.5	4.1
Duck	93.8	2.2
14% Horse Ration	93.9	5.4
DDG	116.4	4.9
Pig grower	108.7	5.8
Lamb grower	107.2	9.0
Horse premix	98.4	3.3
Dairy supplement	103.1	4.0

Method Validation

- All analytes have recovery means between 75 – 120%
- Coefficients of variation all < 10%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