



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.

## ***Development and Validation of a Method for the Determination of Ractopamine at Residue Levels***

***Robert Lemieux and Aaron Price***

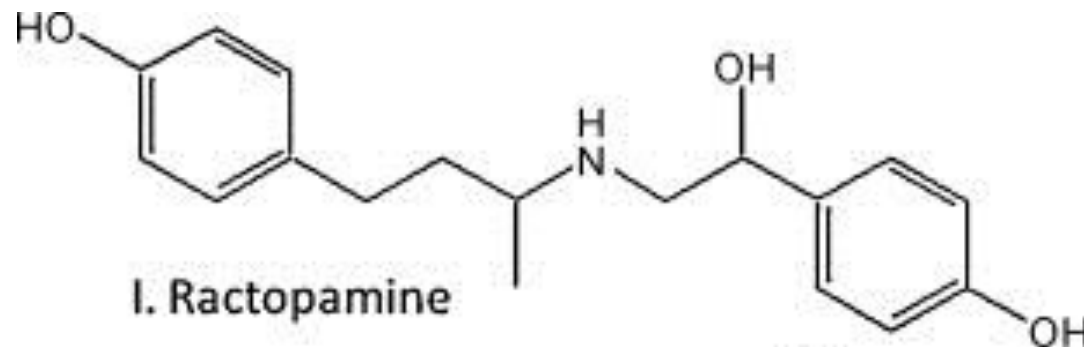
***AAFCO Laboratory Methods and Service Committee meeting***

***2015-01-14***

Canada

# Background

- Ractopamine is a  $\beta$ -agonist used in Canada for improved weight gain, feed efficiency and leanness (swine, cattle and turkey).
- Added to feed at the following levels:
  - 5-10 ppm (finishing barrows and gilts)
  - 10-30 (finishing beef cattle)
  - 5-9 (finishing heavy tom and hen turkeys)



# Background

- EU, Russia, mainland China, and Taiwan have banned ractopamine - Leads to export and trade issues
- Ractopamine free meat export program in Canada:
  - Codex MRL's for ractopamine have been developed (muscle cuts of beef and pork)
- Ractopamine is a prohibited substance in Canada for race horses:
  - Canadian Pari-Mutuel Agency (CPMA) tests horse urine for banned substances, including ractopamine

# Method Needs

- CFIA Animal Feed Division Needs:
  - Ractopamine test method that supports residue and traceback sampling programs
  - Respond to EU audit findings
  - Feed method in place to support CFIA ractopamine free meat export certification program
- Target LOQ in feed: LOQ of 0.05 mg/kg
- Current lab capability: LOQ of 1.0 mg/kg
- Ideally a simple (dilute and shoot) procedure that could be combined with other analytes for a multi-analyte method.

# Method Development

## Reference methods

- Internal CFIA method LCMSMS2R: *Determination of Tylosin, Lincomycin, Virginiamycin, Erythromycin and Novobiocin at Low Levels in Animal Feed by ESI LC-MS/MS.*
- *C. Juan et al, 2010. Development and Validation of a Liquid Chromatography Tandem Mass Spectrometry Method for the Analysis of  $\beta$ -agonists in Animal Feed and Drinking Water, Journal of Chromatography A (1217) 6061-6068.*

## Instrument

- LC: Agilent 1200 Series  
MS: Applied Biosystems API5000

# Method Development

- Extraction: 20 g in 100 mL of methanol: 5 mM ammonium acetate (90:10), shake for 1 hour.
- Ractopamine-d6 internal standard added to each sample prior to shaking (0.05 mL of 100 ng/mL).
- Filter then combine 25  $\mu$ L with 5 mL extraction solvent (no sample clean-up)
- Equivalent to dilution ratio of 1g/1000 mL
  - Reduces matrix effects, but sacrifices sensitivity



# Method Development

## LC Conditions

Mobile phase A:

10mM Ammonium Acetate

Mobile Phase B: MeOH

Flow Rate: 0.5 mL/min

Time	%A	%B
0	90	10
1.0	90	10
5.0	0	100
8	0	100
8.5	90	10
12.0	90	10

Column: Phenomenex Synergi 2.5  $\mu$ m Fusion-RP  
(polar C18 phase) with guard column

Column oven temp: 50°C





# Method Development

## MS/MS Conditions

Positive polarity used for ionization mode

### Quantitation Ion Masses

Name	Parent Q <sub>1</sub>	Product Q <sub>1</sub>
RAC1	302.2	164.2
RAC-d <sub>6</sub>	308.18	166.18

### Confirmation Ion Masses

Name	Parent Q <sub>1</sub>	Product Q <sub>1</sub>
RAC2	302.2	121.18

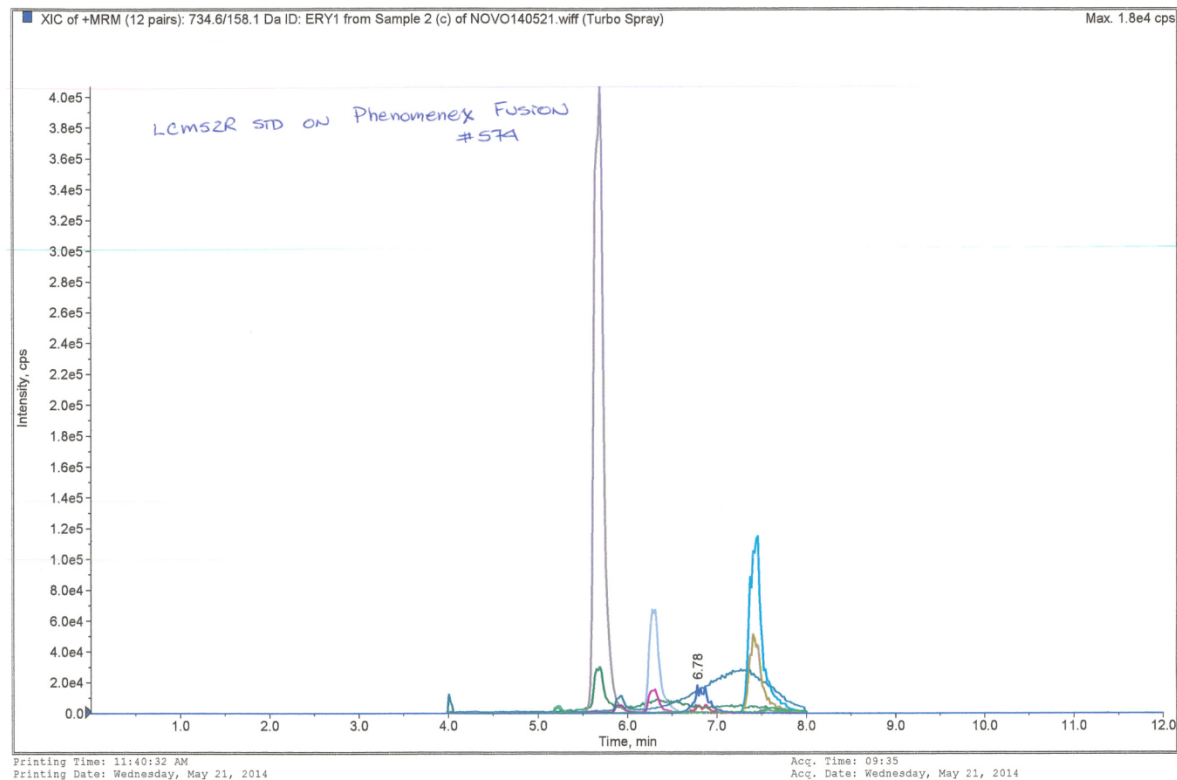




# Method Development

## Challenges

- Poor peaks when RAC added to another method
- Poor peaks when other drugs added to RAC method



# Method Development

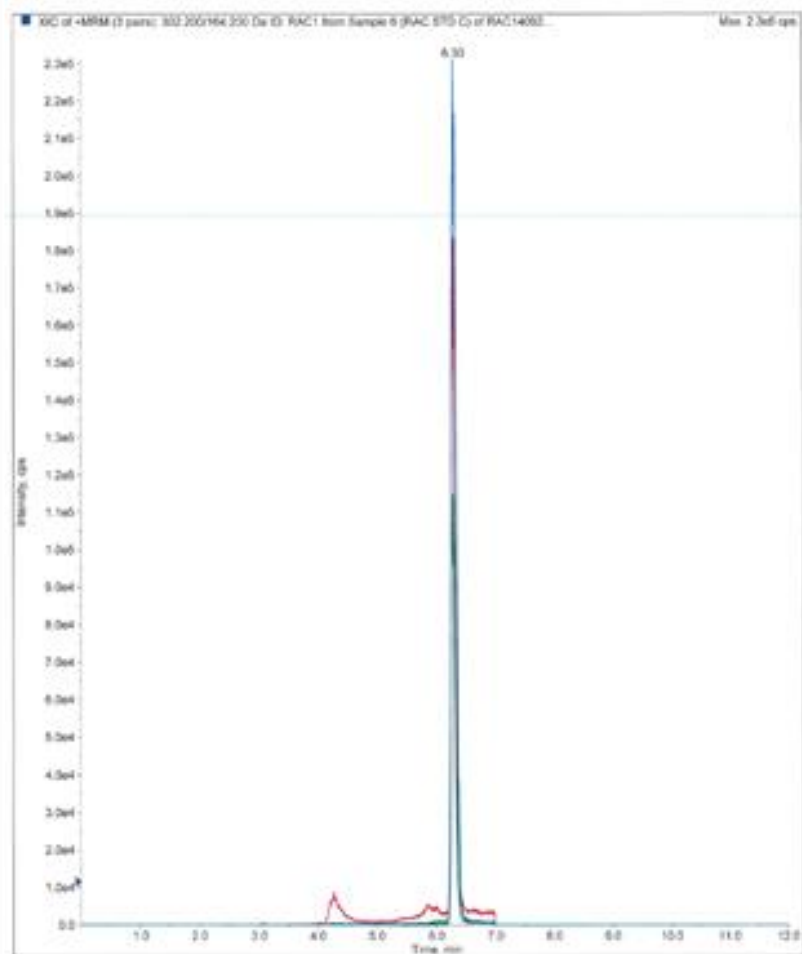
## Challenges

- Column degradation:
  - Wash not sufficient, changed to 50/50 MeOH/water
  - Phenomenex Synergi column changed to Agilent Zorbax Phenyl-SB
- Peak width for working std C

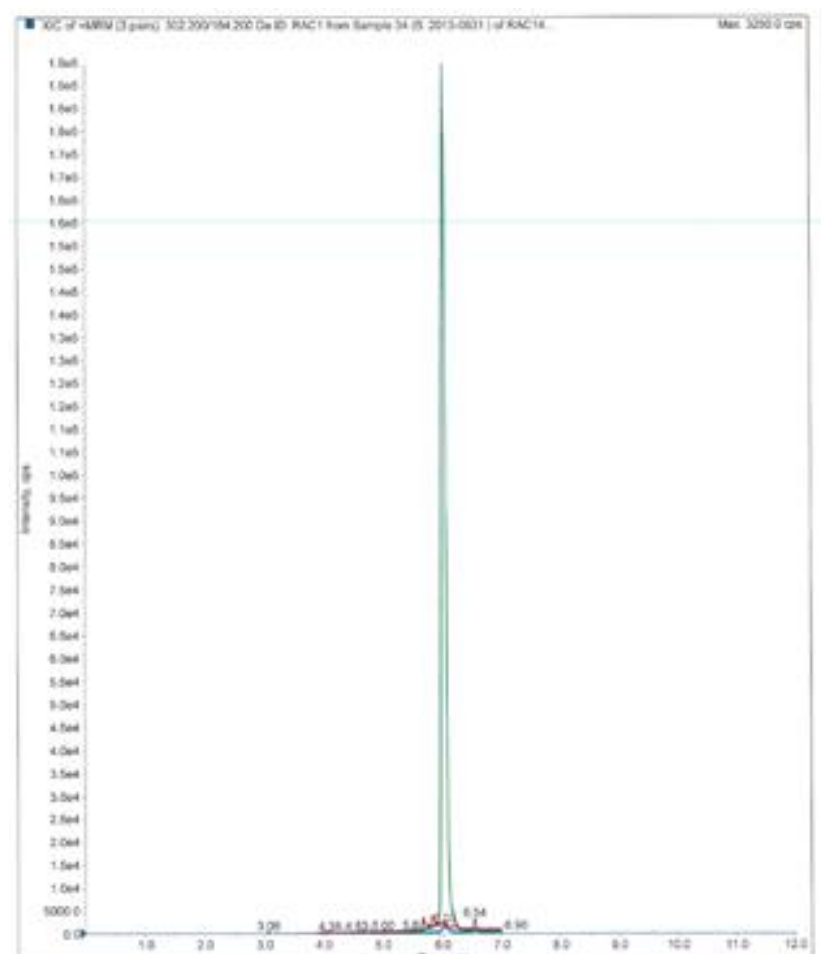
Date	Peak width
17 July 2014	0.473
8 Aug. 2014	0.594
8 Sep. 2014	0.573
25 Sep. 2014	0.478

# Method Development

Working standard C (eq. 2 mg/kg)



Blank lamb feed sample



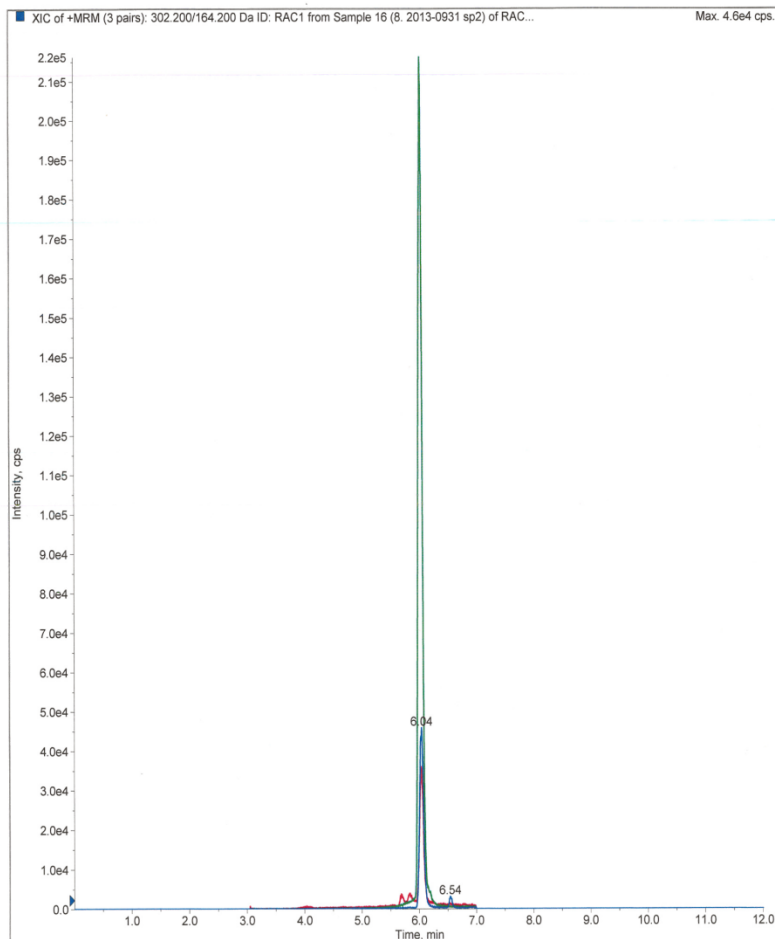
Canadian Food  
Inspection Agency

Agence canadienne  
d'inspection des aliments

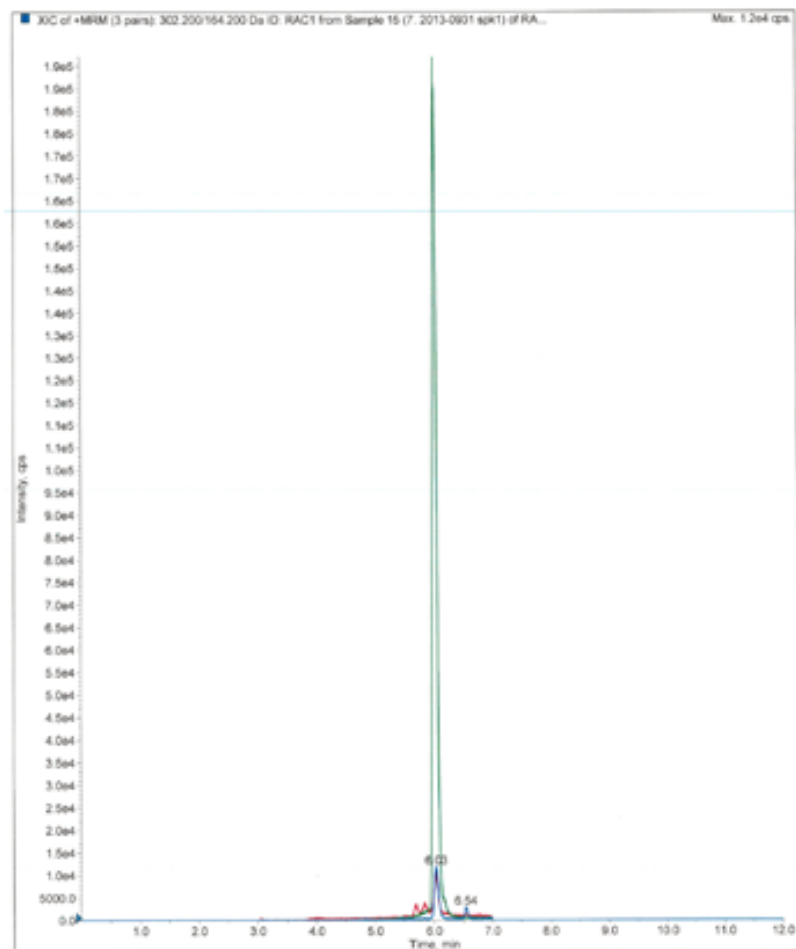
Canada

# Method Development

Lamb feed spiked at ~0.20 mg/kg



Lamb feed spiked at ~0.05 mg/kg



Canadian Food  
Inspection Agency

Agence canadienne  
d'inspection des aliments

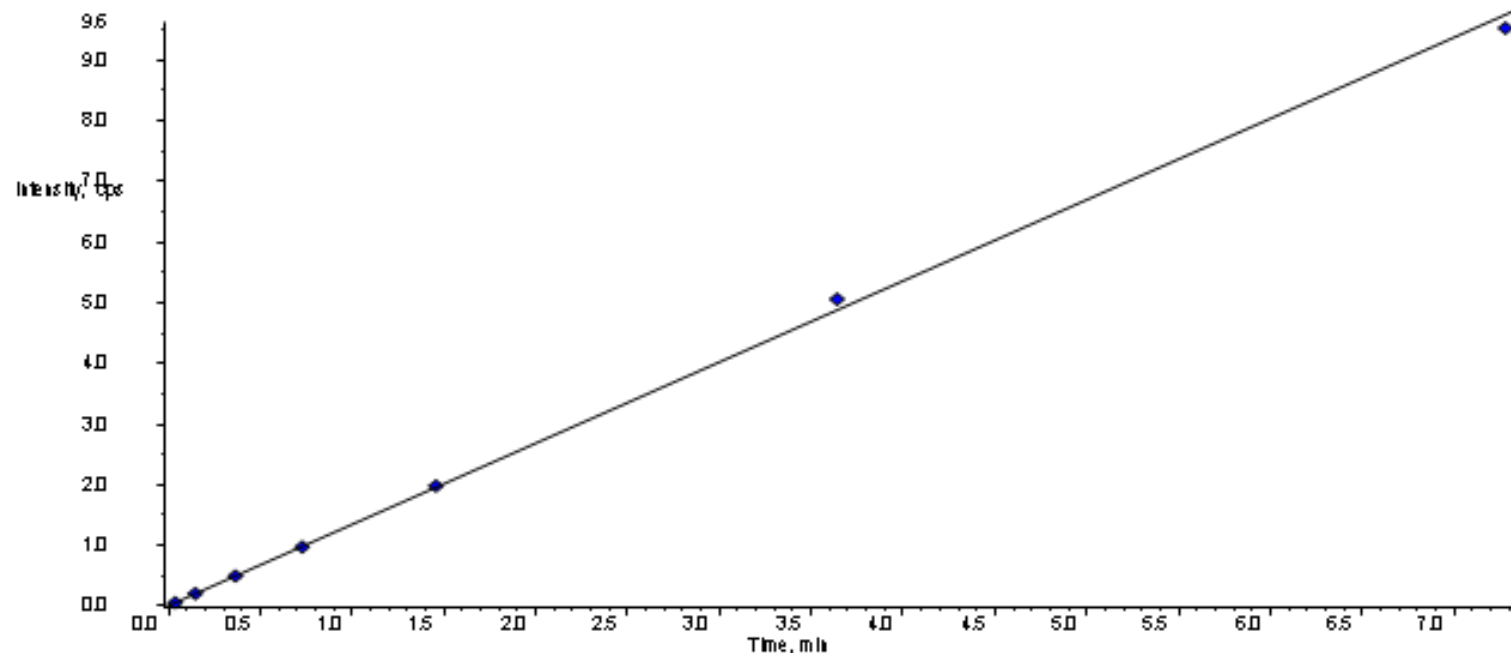
Canada

# Method Development

- Good linear correlation for standards:  $r = 0.9996$

RAC140923we.mdb (RAC1): "Linear" Regression (1/x<sup>2</sup> weighting):  
 $y = 1.34 x + 0.00874$  ( $r = 0.9996$ )

Max. 5570.0 cps.



# Validation

Complete Feeds: Horse feed, chicken starter, sow feed, poultry grower, layer feed, barley, lamb starter, milk replacer

Ingredients: Various mineral supplements and premixes

LOQ:  $10 \times \text{Std Dev}$  of 8 replicate spikes at 0.05 mg/kg

Recovery:

4X4 for each matrix: four spikes at four level of spiking (0.05, 0.2, 1.0, and 5.0 mg/kg) analysed on four different days



# Validation

LOQ study: spiked at 0.047 mg/kg

Complete Feed	Value (mg/kg)	Std Dev.	LOD/LOQ (mg/kg)	Feed Ingredient	Value (mg/kg)	Std Dev.	LOD/LOQ (mg/kg)
Chicken Starter	0.0538	0.0008	0.003/ 0.008	Dairy Premix	0.0481	0.0016	0.006/ 0.016
Horse Feed	0.0563			Dairy Supplement	0.0510		
Lamb Starter	0.0554			Calf Premix	0.0482		
Barley	0.0546			Beef Supplement	0.0504		
Sow Feed	0.0551			Calf Supplement	0.0486		
Poultry Grower	0.0544			Horse Premix	0.0497		
Milk Replacer	0.0554			Turkey Premix	0.0463		
Layer Feed	0.0558			Mineral	0.0480		





# Validation

## Results: Accuracy and Precision

	0.05 mg/kg		0.20 mg/kg		1.0 mg/kg		5.0 mg/kg	
Matrix	Recovery (%)	CV (%)	Recovery (%)	CV (%)	Recovery (%)	CV (%)	Recovery (%)	CV (%)
Horse Feed	109	9.6	94.3	5.3	96.2	5.1	98.7	1.7
Chicken Starter	106	10.9	91.0	5.6	93.5	9.0	93.5	3.0
Sow Feed	103	9.9	90.9	2.5	94.1	3.9	94.9	2.0
Layer Feed	100	14.3	92.5	1.7	98.3	3.2	97.5	0.70
Lamb Feed	102	14.3	90.3	5.2	95.1	2.8	95.1	1.0
Milk Replacer	94.6	7.1	89.7	4.5	93.0	5.1	96.1	1.5
Mineral Supplement	98.6	9.5	89.6	3.3	94.2	4.3	96.5	3.1
Premix	105	7.2	93.3	8.6	84.7	7.0	87.4	7.5
<b>Overall</b>	<b>102</b>	<b>10.3</b>	<b>91.5</b>	<b>4.8</b>	<b>94.3</b>	<b>5.3</b>	<b>95.0</b>	<b>4.4</b>



# Validation

## Performance Summary:

- Range: 0.02 – 10.0 mg/kg
- LOQ:  $\leq 0.02$  mg/kg for all matrices (0.008 – 0.016)
- Precision:  $\leq 14.3\%$
- Accuracy: 81.0-124%
- Confirmation: Second product ion used to confirm
- Solution Stability: Stock 3 months, Stds 1 month

# Conclusions

- LOQ need met; 0.05 mg/kg required and 0.02 mg/kg obtained
- Straight forward “dilute and shoot” method achieved
- Method not incorporated with other vet drugs to make a multi-analyte method (yet?)
- Lower detection limits could be obtained with a clean-up/concentration step
  - Reference used a cation exchange SPE for clean-up, then drying and reconstituting followed by 2 liquid-liquid extractions, and finally another drying and reconstituting step. Achieves LOQ around 1 µg/kg.

# What's next

- More matrices to validate (e.g. cattle feed)
- Look further into combining with any other analytes?
- Run method using in-house generated control samples to generate a control chart
  - 2 g RAC medicated feed added to 18 g blank feed for each sample instead of one large mixed feed

Canada