

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.

Improving Analytical Efficiencies in Single-Analyte Methods for Veterinary Drug Residues in Foods of Animal Origin

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Centre for Veterinary Drug Residues (CVDR)

Saskatoon Laboratory



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Outline

Introduction

- CFIA – Who are we?
- Regulatory Context

Chromatography of Single-class Methods

- Tranquilizers (LC-MS)
- Fluoroquinolones (LC-Fluorescence)

CFIA Vision and Mission

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

Mission:

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

Acts and Regulations

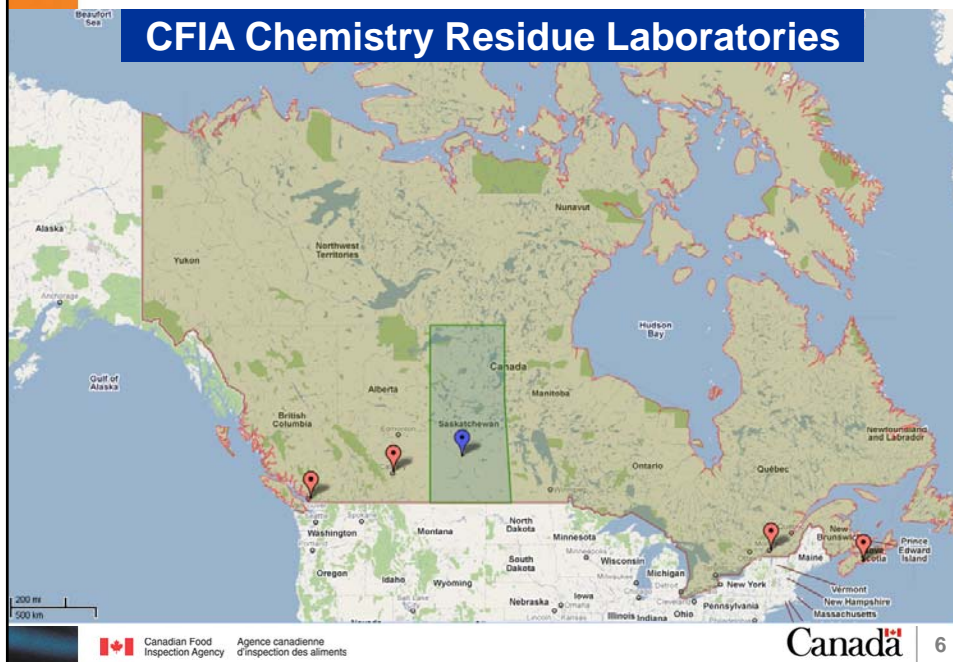
- Canada Agricultural Products Act
- Meat Inspection Act
- Food and Drug Act:

4. (1) No person shall sell an article of food that... (d) is **adulterated**

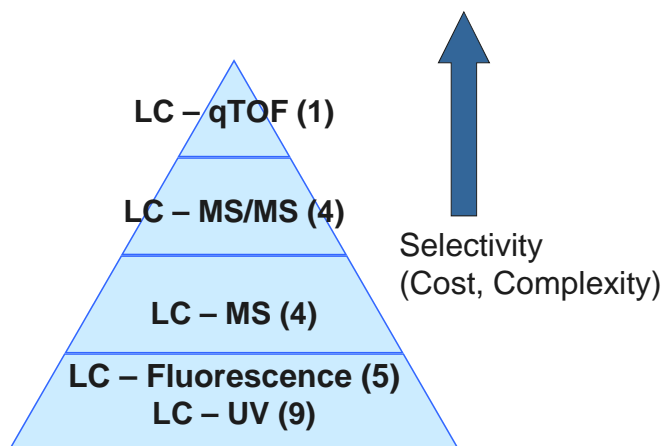
CFIA Activities

- ❑ registration/ and inspection of slaughter and processing establishments
- ❑ inspection and grading
- ❑ policy and program development
- ❑ food advertising compliance
- ❑ retail inspection
- ❑ **residue testing**

CFIA Chemistry Residue Laboratories

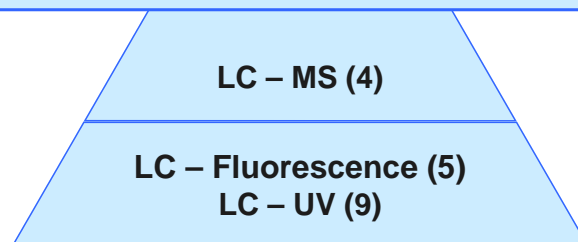


Saskatoon Lab Instrumentation



Why use less selective detectors?

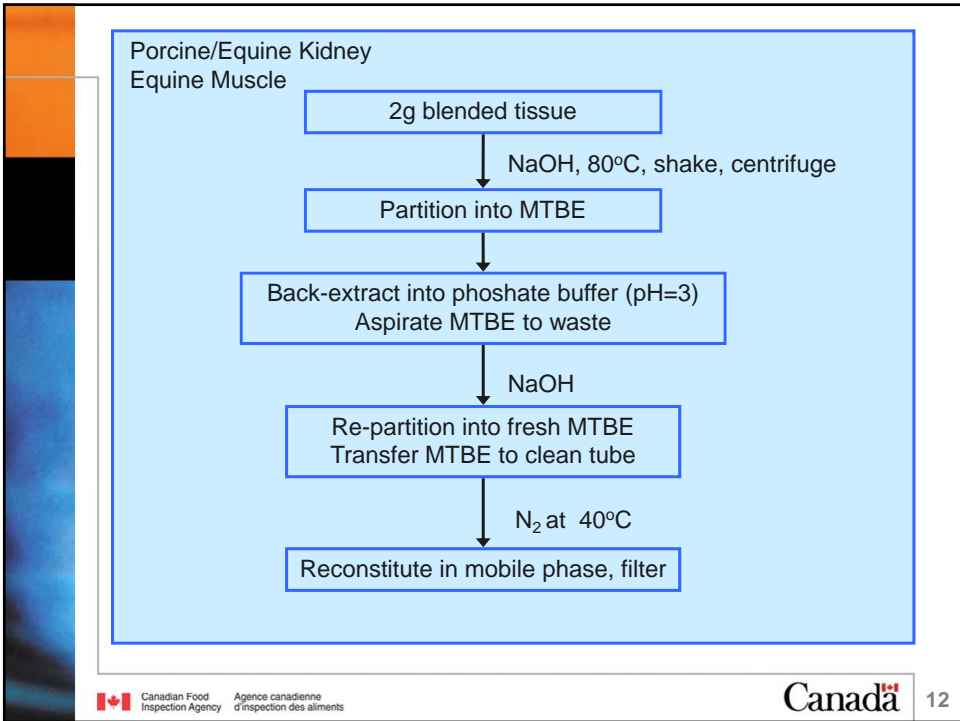
- Instrument availability
- Method portability
- Ease of use / analyst comfort level



Tranquilizers

Tranquilizers Method – Target Analytes

Analyte	Abbrev	[M+H ⁺]
Xylazine	XYL	221
Azaperol	AZAL	330
Carazolol	CARA	299
Azaperone	AZPN	328
Haloperidol	HAL	376
Acepromazine	ACE	327
Propionylpromazine	PPRMZ	341
Chlorpromazine	CHLPRM	319



Waters Nova-Pak C₁₈ 2.1 x 150mm, 4µm

A = 0.01M NH₄HCO_{2(aq)}
B = Acetonitrile
C = Methanol
Flow Rate = 0.2mL/min.
Temperature = 35°C
Injection Volume = 20µL

Standard Column

Time (min.)	%A	%B	%C
0.0	70	15	15
4.0	70	15	15
9.0	40	15	45
25.0	35	15	50
28.0	20	30	50
30.0	70	15	15

Waters Atlantis dC₁₈ 2.1 x 10mm, 3µm

A = 0.01M NH₄HCO_{2(aq)}
B = Acetonitrile
Flow Rate = 0.45mL/min.
Temperature = 45°C
Injection Volume = 5µL

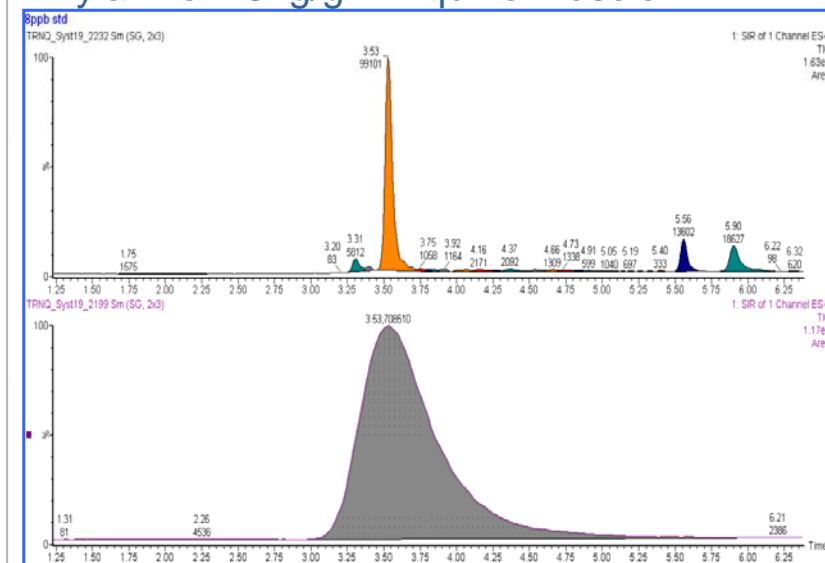
"Mini" Column

Time (min.)	%A	%B
0.0	99	1
1.0	99	1
2.5	65	35
5.5	1	99
8.5	1	99
8.6	99	1
12.0	99	1

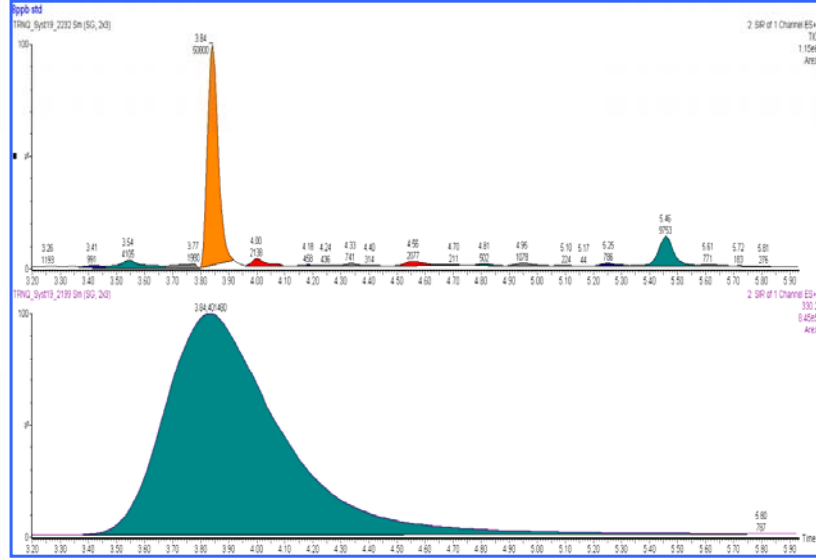
Comparison of Retention Times and Peak Widths

Analyte	1cm Column		15cm Column	
	t_R (min.)	$W_{1/2}$ (min.)	t_R (min.)	$W_{1/2}$ (min.)
XYL	3.53	0.06	7.40	0.52
AZAL	3.84	0.04	10.13	0.58
CARA	3.93	0.03	11.18	0.30
AZPN	3.99	0.04	12.79	0.36
HAL	4.32	0.04	16.19	0.19
ACE	4.34	0.04	18.06	0.25
PPRMZ	4.54	0.04	22.05	0.36
CHLPRM	4.61	0.04	23.88	

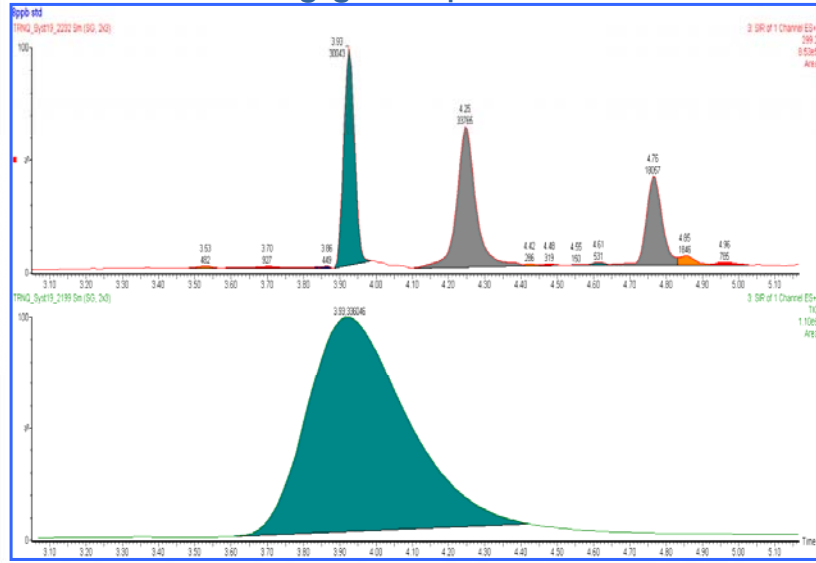
Xylazine – 8ng/g in Equine Muscle



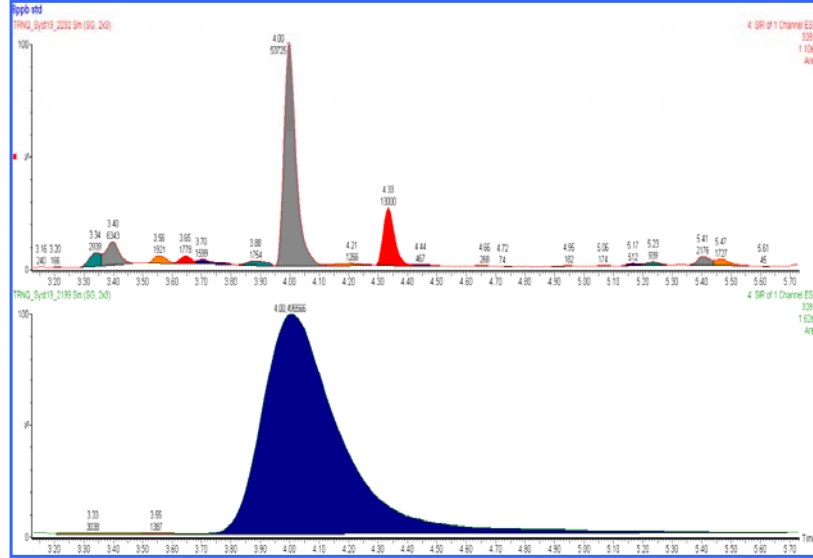
Azaperol – 8ng/g in Equine Muscle



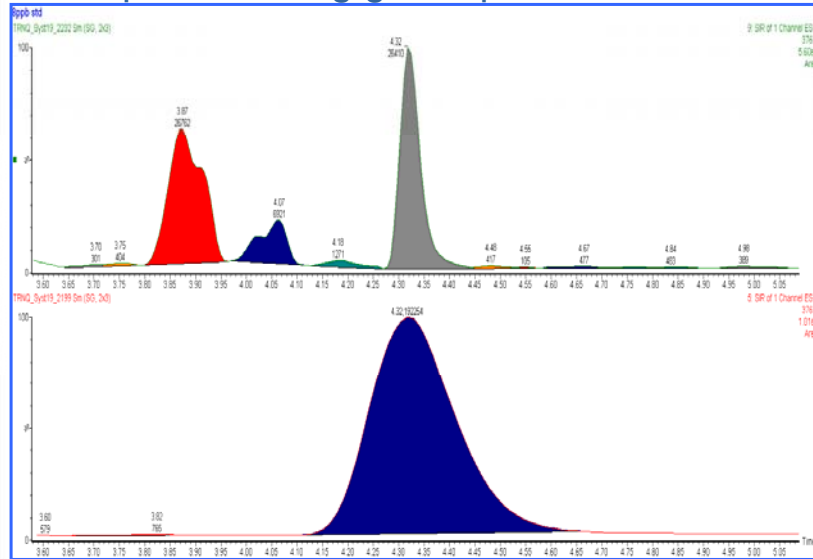
Carazolol – 8ng/g in Equine Muscle



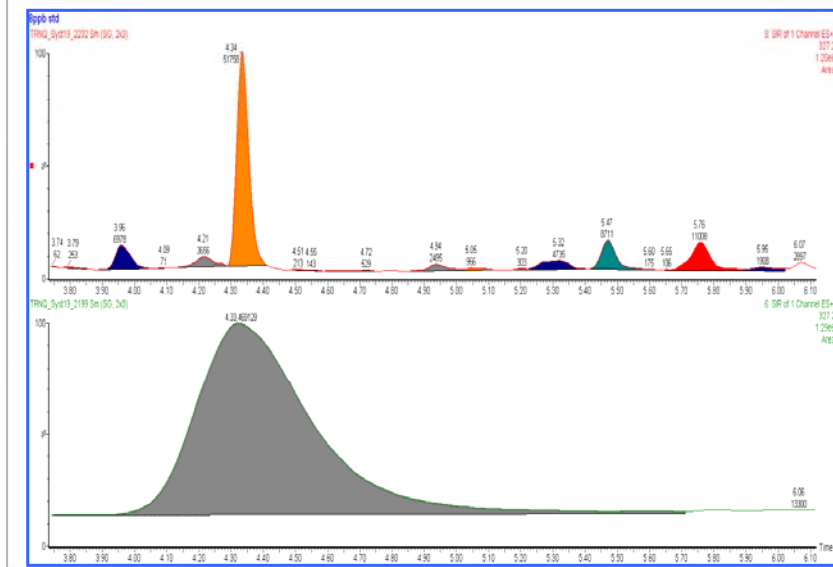
Azaperone – 8ng/g in Equine Muscle



Haloperidol – 8ng/g in Equine Muscle



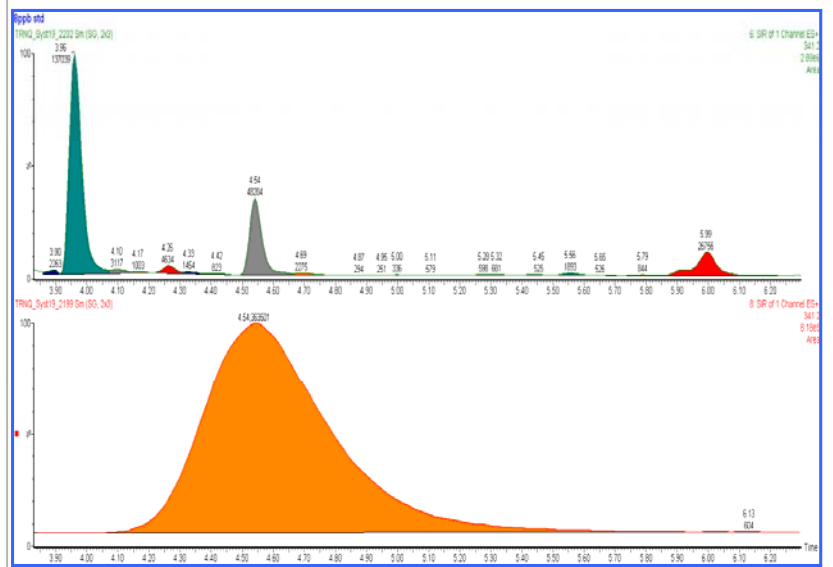
Acepromazine – 8ng/g in Equine Muscle



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Canada 21

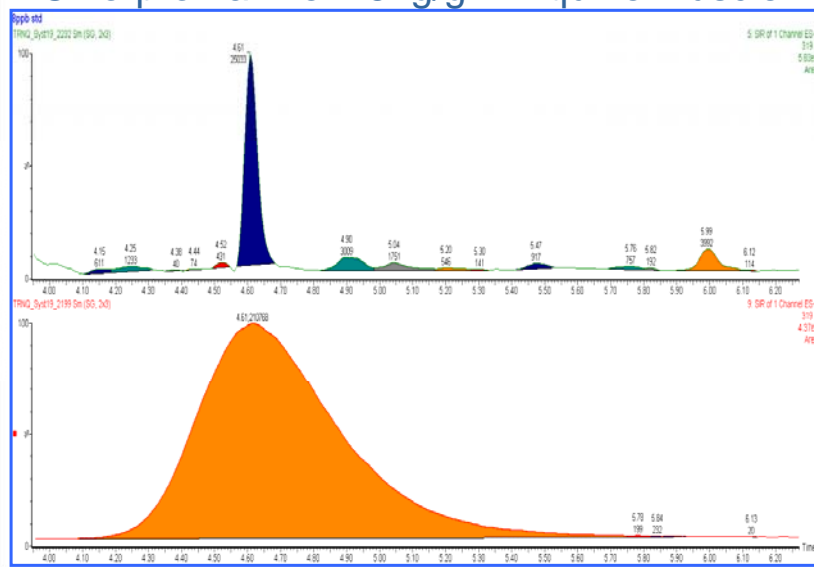
Propionylpromazine – 8ng/g in Equine Muscle



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Canada 22

Chlorpromazine – 8ng/g in Equine Muscle



Retention Time Reproducibility

	XYL	AZAL	CARA	AZPN
Avg	3.53	3.84	3.93	3.99
%RSD	0.23%	0.24%	0.20%	0.32%
	HAL	ACE	PRPMZ	CHLPRM
Avg	4.32	4.34	4.54	4.61
%RSD	0.30%	0.30%	0.33%	0.31%

Duration: October 2009 – March 2010

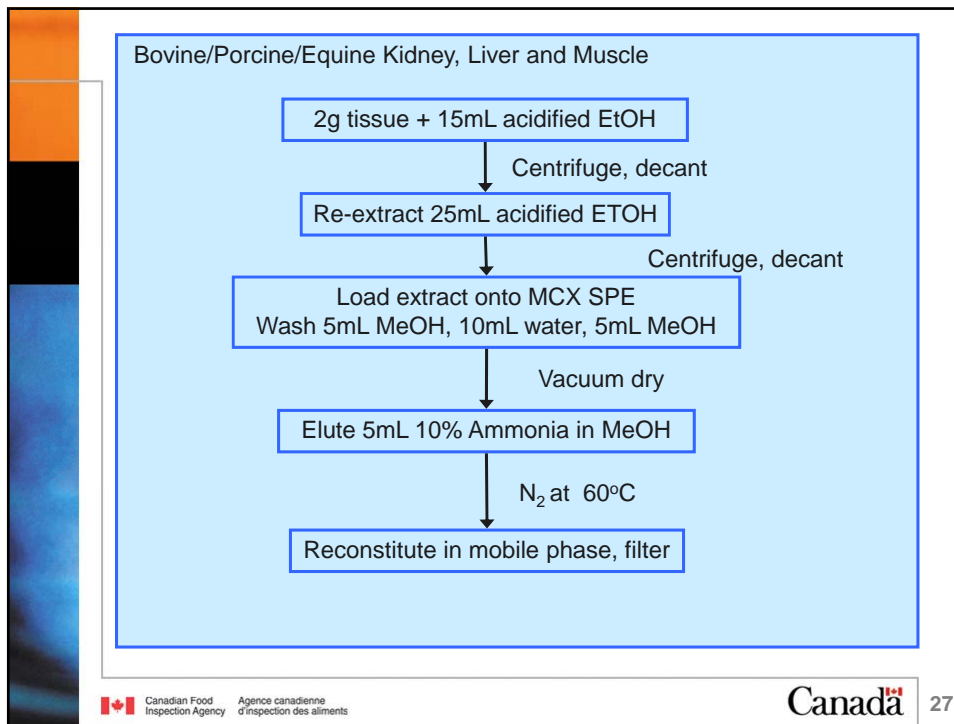
Number of injections: 207

2ng/g spikes as measure of trueness at the method reporting limit

	XYL	AZAL	CARA	AZPN
Avg	104.0%	102.3%	100.7%	99.2%
%RSD	15.3%	9.2%	9.5%	12.3%
	HAL	ACE	PRPMZ	CHLPRM
Avg	99.0%	98.8%	101.5%	97.6%
%RSD	10.1%	11.8%	9.5%	9.3%

Duration: October 2009 – March 2010
n=7

Fluoroquinolones



Inertsil ODS-3, 4.6 x 150 mm, 5 μm

A = 0.05 M sodium phosphate + 0.0025 M 1-heptanesulfonic acid pH = 2.4
 B = Acetonitrile

Flow Rate = 1.0 mL/min.
 Temperature = 28 °C.
 Injection Volume = 25 μL.

Time (min.)	%A	%B
0.0	78	22
17.0	78	22

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Canada 28

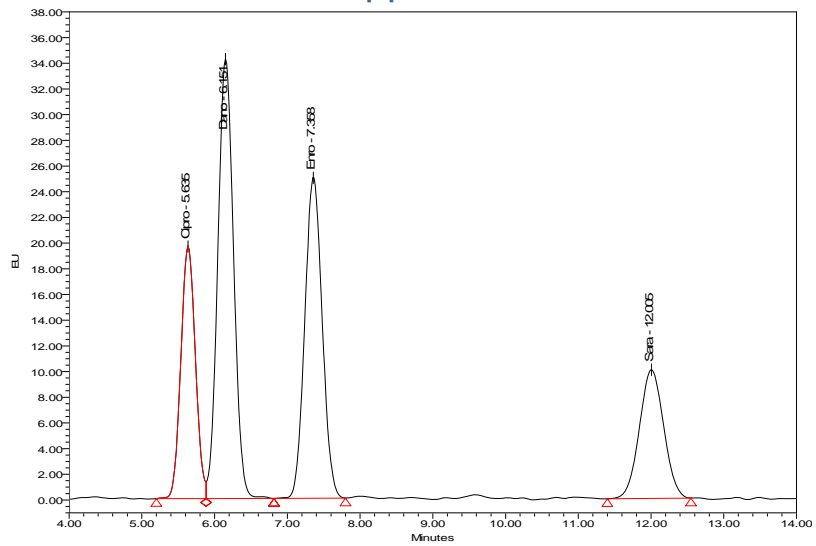
Halo C₁₈, 2.1 x 30 mm, 2.7 µm.

A = 0.1 % Formic acid(aq)
 B = Acetonitrile
 Flow Rate = 1.0 mL/min.
 Temperature = 50 °C
 Injection Volume = 10 µL

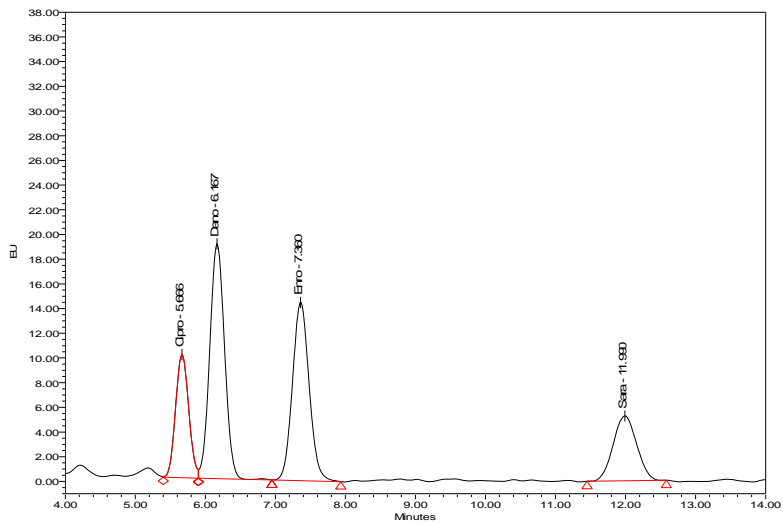
No Ion Pairing Reagent

Time (min.)	%A	%B
0.0	99	1
0.1	99	1
5.0	94.	6
5.1	10	90
6.6	10	90
6.7	99	1
11	99	1

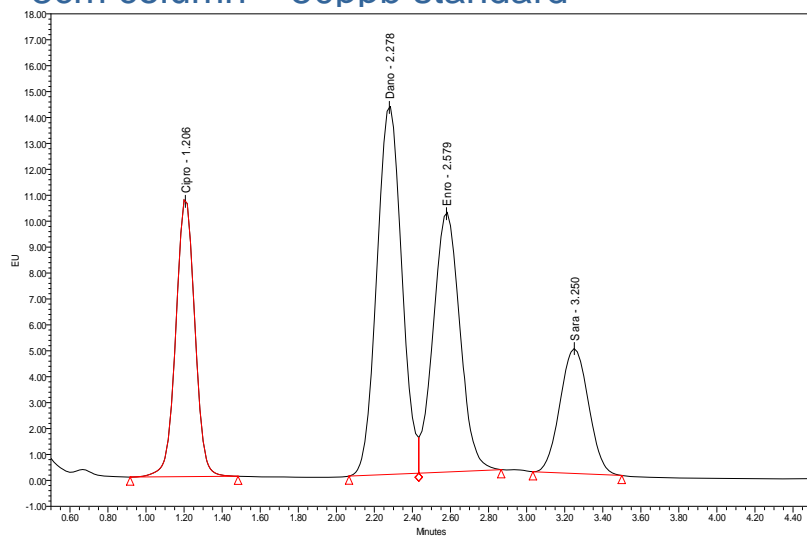
15cm Column – 50ppb standard



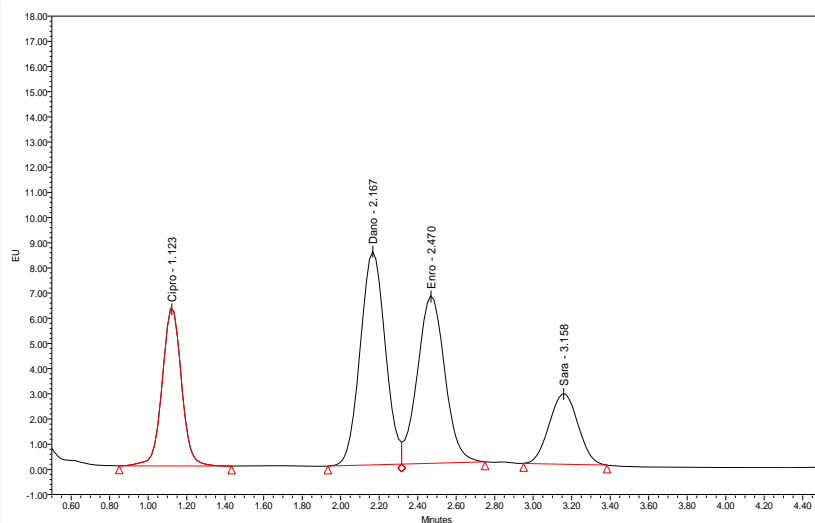
15cm Column – 30ppb Bovine Kidney Spike



3cm column – 50ppb standard



3cm column – 30ppb Bovine Kidney Spike



Conclusions

Using existing methods and equipment

- Shorter run-times
- Higher throughput
- Lower injection volumes
- Improved peak shapes

Acknowledgements

- Carol Barriault
- Ryan Denkert
- Collin O'Byrne
- Becky Schurman
- Kim Souster
- Kathleen Verity

Canada

Saskatchewan: Famous for...



SASKATOON INTERNATIONAL WORKSHOP VALIDATION AND REGULATORY ANALYSIS

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