Development of Analytical Methods for the Determination of Flunixin and Phenylbutazone Drug Residues in Edible Bovine Tissues

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Scope of Presentation

- Need for the development of analytical methods for Nonsteroidal Anti-inflamatory Drugs (NSAIDs).
- Method development and their analytical parameters.
- Results of application of methods to Residue Monitoring.
- Impact of the developed technology.

Need for the Development of Analytical Methods for Flunixin & Phenylbutazone in Edible Tissues

- Flunixin and Phenylbutazone are not approved for use in food animals.
- NSAIDs exhibit anti-inflamatory, antipyretic and analgesic characteristics.
- NSAIDs approved for treating arthritis and associated muscular-skeletal disorders in dogs and horses.

Catagories of NSAIDs - Carboxylic Acid Derivatives

Salicylic	**Acetic**	**Propionic**	**Fenamic**
Aspirin	Diclofenac Na	Fenoprofen Ca	Meclofenamate Na
Diflusinal	Diclofenac K	Naproxen*	Meclofenamic* acid
	Indomethacin	Fenbufen	Flunixin meglumine*
	Tolmetin Na	Fluribiprofen	Flufenamic acid
		Benoxaprofen	Mefanamic acid
	Sulindac	Indoprofen	
	Alclofenac	Ibuprofen	
	Fenclofenac		

Catagories of NSAIDs - Enolic Acid Derivatives

Pyrazolons	**Oxicans**	**Nonacidic agents**
Oxyphenbutazone	Piroxicam	Tiaramide HCl
Phenylbutazone*	Isoxicam	Epirizole
Dipyrazone*	Sudoxicam	Flumizole
Apazone	Tenoxicam	Proquazone
Isopyrin		

Phenylbutazone Use in Veterinary medicine

- Useful anti-inflammatory analgesic for treating various forms of lameness in dogs and horses.
- Useful for treating acute laminitis in horses.
- Misuse…. to mask lameness during examination e.g. for sale, 3 to 4 days post administration signs of lameness re-appear.

Phenylbutazone Precautions & Contraindications

- Not approved for use in food-producing animals
- Must not be administered to animals afflicted with serious cardiac, renal, hepatic injury or hemacytological disorders.

  Injection of PBZ into the carotid artery will result in immediate excitement, prostration, and even death.

Flunixin meglumine use in Veterinary Medicine

- In horse FLX is approved for use to reduce inflammation and pain associated with musculo-skeletal disorders.

  - approved for alleviation of pain associated with spasms (colic) of the GI tract.

  - approved for IV use once daily for 2 – 5 days in the post operative care after surgical repair of the cornea.

Need to Develop Analytical Methods for NSAIDs

- Visits to some farms have indicated large supplies of these NSAIDs where there is no equine husbandry.
- Field Staff suspect some off-label use e.g. ‘to mask lameness of cows being shipped to slaughter’.
- To primarily protect the Canadian consumer and the producer’s market access while facilitating monitoring production methods.

Limitations of Field Test for Anti-microbials in Meat

- The Swab Test On Premises (STOP), is used in both Canadian and U.S. federally inspected abattoirs. It screens for anti-microbial contamination in meat but does not detect NSAIDs.
- Till now, there were no analytical methods for detecting these drugs in edible bovine tissues.

Flunixin Analytical Method

- Adjust pH of 5 g pre-homogenized tissue sample (add Diclofenac as IS) to 4.5 with potassium acetate (0.04M) and digest for 16 h at 37 °C with $-glucoronidase.
- Extract enzyme digest with acetonitrile.
- Wash the acetonitrile fraction with n-hexane to waste.
- Evaporate the acetonitrile fraction dry under nitrogen at 55 °C.

Flunixin Analytical Method cont.

- Reconstitute the residue in pH 7 phosphate buffer and load on bimodal solid phase extraction cartridge (Certify II, Varian).
- The loaded cartridge is cleaned with water, methanol, air dried, and rinsed with n-hexane.
- Elute flunixin and diclofenac (IS) with acidified hexane.
- Evaporate solvent under nitrogen at 55 °C; reconstitute residue in 1 mL mobile phase, filter and analyse by RP-HPLC on a 3.0 x 250 mm 5um Inertsil ODS (3) analytical column, with UV detection at 285 nm.

Flunixin Analytical Parameters

- Detection Limit 5 ppb in muscle and injection site tissue samples.
- Lower Limit of Quantitation 10 ppb
- Higher Limit of Quantitation 200 ppb
- Recovery from tissue > 85%
- Repeatability from tissue CV < 20%

Results of Flunixin Study

Fiscal Year	**# Samples Tested**	**# Flunixin +ve**
1995-1996 (IS)	80	1
1996-1997 (IS)	204	0
1997-1998 (IS)	101	1
1997-1998 (veal)	320	0

Phenylbutazone Analytical Method

- Take 2 g pre-homogenized bovine muscle tissue, add Internal Standard (diclofenac 100 uL of 2 ppm working solution).
- Extract tissue 3 x with extraction solution, centrifuge 2800 x g for 5 min. at 5 °C. Combine extracts and evaporate to minimum volume under nitrogen.
- Cleanup extract on conditioned Florisil (2 g) cartridge. Elute phenylbutazone and internal standard using elution solution.
- Evaporate to minimum volume under nitrogen at 55 °C; reconstitute in 300 uL mobile phase.

Phenylbutazone Analytical Method

- Filter samples and analyse using gradient conditions below on a Symmetry C_8 analytical column with detection at 270 nm.
- Gradient conditions:

Time	Solvent A	Solvent B	Solvent C
0	45	45	10
5	45	45	10
15	50	40	10
21	50	30	20
22	45	45	10
45	45	45	10

Phenylbutazone Analytical Parameters

- Detection Limit 2 ppb in muscle & injection site tissue samples.
- Lower Limit of Quantitation 10 ppb
- Upper Limit of Quantitation 200 ppb
- Recovery from tissue > 85%
- Repeatability from tissue CV < 10%
- Time to prepare 12 samples 4 hr.

Phenylbutazone Preliminary Results

Sample ID	Previous Analysis Data	PBZ ppb
A0025	TTC (100 ppb); OTC (110 ppb)	28
A0016	NAD	156
A0005	PENG(7 ppb)	16000
A0549	NAD	nd
A0039	OTC(50 ppb)	nd
A0038	PENG(6 ppb)	nd
A0023	NAD	nd
A0009	OTC(110 ppb)	nd

Impact of Method Development [1]

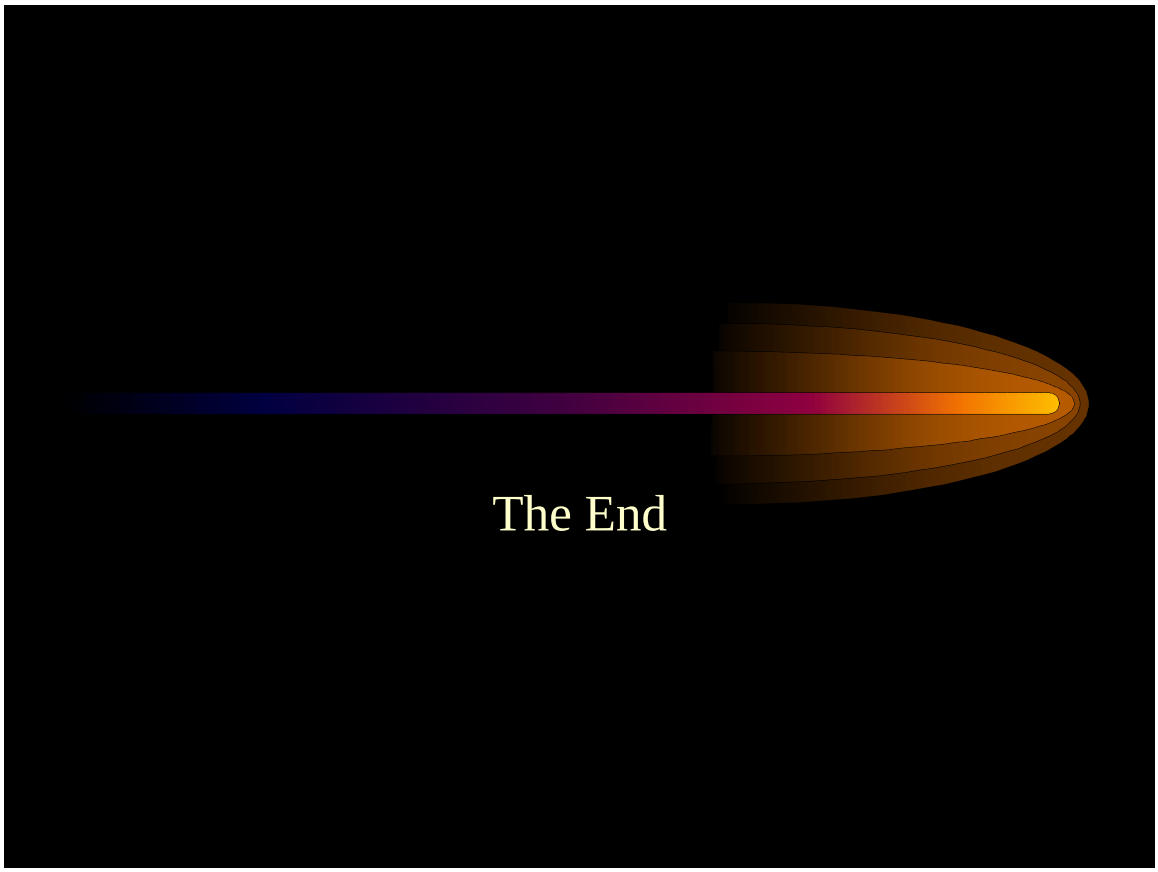
- Preliminary results suggest that;
  (1) both flunixin and phenylbutazone are being used by producers,
  (2) suspicions of off-label use of flunixin may be valid but not so for phenylbutazone,
  (3) using this technology we will soon be able to better define the incidence of phenylbutazone residues in food animals at slaughter.

Impact of Method Development [2]

- Using these methods CVDR will be able to reduce the number of incidences of Unidentified Microbial Inhibitors (UMI) from the STOP positive submissions originating from federally inspected abattoirs.
- These analytical methodologies have broadened CFIA's analytical capabilities.
- Reassures our global trade partners that CFIA has appropriate science based technologies to both monitor and regulate the proper use of veterinary drugs in food animals.

Conclusion

- The development of new analytical methods for the detection of veterinary drug residues in edible tissues is an essential contribution of CVDR to CFIA's function of ensuring that Canadian consumers are protected from undesirable drug residues in both domestic and imported meat products.
- They also enable the verification of residue status of meat to satisfy export requirements in order to maintain foreign market access for our producers.


The End

NSAID's Mechanism of Action

- The 1982 Nobel Prize for Physiology and Medicine was awarded to John Vane for his discovery in 1971 that aspirin blocks or inhibits the enzymatic conversion of arachidonic acid (AA) to prostoglandins (PGs); PGs are potent mediators of tissue inflammation in mammals.

NSAIDs Mechanism of Action

- NSAIDs [salicylates, indomethacin, phenylbutazone, naproxen, flunixin etc.] operate by disrupting the cellular release of PGs by interfering with their biosynthesis ie., the conversion of AAs to the endoperoxides PGG_2 and PGH_2. This being the most vulnerable target for pharmacological manipulation of the PG system and is very effective.