



## **Surveillance of chloramphenicol residues in milk, eggs and chicken meat by LCMSMS**

**Dr. G. SARATH CHANDRA, Ph.D**

**Professor and Head**

**Pharmacovigilance Laboratory for Animal Feed and Food Safety**

**Directorate of Centre for Animal Health Studies**

**Tamil Nadu Veterinary and Animal Sciences University**

**Chennai – 600 051, INDIA**





**Residues are the metabolites of veterinary drugs, and their associated parent compounds, that remain in the animal or its produce (eggs, milk and honey) after treatment.**

**Their behaviour depends on the nature of the drug and its metabolites and on the pharmacokinetics of the drug in the animal concerned.**

**Those that are metabolised and excreted rapidly also rapidly deplete in the animal.**

**Those that are slowly metabolised may also deplete rapidly if their excretion is not dependent on metabolism.**

**Others may be subject to slow excretion, especially those that bind to macromolecules and are thus not available for metabolism and/or excretion.**



**Humans health and veterinary drugs:**  
**Humans health and veterinary drugs Introduction** A variety of animal species, including cattle, sheep, goats, pigs, horses, fish, birds and bees, are kept for the purpose of providing food for the human population. In order to maintain their wellbeing, it is sometimes necessary to treat these animals with pharmaceutical products and such treatments can result in residues of the active ingredients, or their metabolites, entering the human food chain. The European Union, for example, in 1999 banned all antibiotics used on humans from being used on animals as growth promotants (AGPs), and similarly the EU has banned the use of hormonal growth promotants (HGPs) on cattle. The USA, Canada and Australia allow such hormone use (subject to maximum residue levels)



**The growing food safety concerns call for intensive surveillance of chloramphenicol in food products.**

**The objective of the study was to assess whether milk, eggs and chicken meat produced by the livestock farmers in TamilNadu state of India were contaminated with chloramphenicol residues**



**Chloramphenicol has been banned for use in all food-producing animals by the European Union (EU), and Most of the developed countries.. The EU recently set a minimum required performance limit (mrpl) for chloramphenicol determination at 0.3 µg/kg (ppb) in all foods of animal origin**



**Liquid chromatography/mass spectrometry (LC/MSMS) method was employed for the determination of chloramphenicol (CAP) residues in milk, eggs, chicken muscle and liver, and kidney**





## MATERIALS AND METHODS

✓ 120 samples –milk, eggs, chicken meat, milk powder and egg powder were analysed during 2011 to 2012.

**CAP was extracted from the samples with acetonitrile and defatted with hexane.**

**The acetonitrile extracts were then evaporated, and residues reconstituted in 10mM ammonium acetate--acetonitrile mobile phase and injected into the LC system, and detection was by a triple quadrupole mass spectrometer operated in selected reaction monitoring (SRM) mode**





AP\_160209

View Apps Options Help

### Instrument Setup - Define Instrument Methods

Startup Method

Instrument Method

Shutdown Method

Accela AS Method

Sample Preparation

Reservoir Content

Timed Events

Injection volume (ul): 10.0

Needle height from bottom (mm): 2.0

Syringe speed (ul/s): 8.0

Flush volume (ul): 2000

Flush/Wash source: bottle

Wash volume (ul): 2000

Flush speed (ul/s): 100.00

Post-injection valve switch time (min): 0.0

Loop loading speed (ul/s): 8.00

#### Injection Mode

- Partial loop
- Full loop
- No waste

#### Tray Temperature Control

Enable tray temperature control

Temperature (°C): 10.0

#### Column Oven Control

Enable column oven control

Temperature (°C): 30.0

Help





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Accela Pump View Apps Options Help

**Instrument Setup - Define Instrument Methods**

Startup Method  **Instrument Method**  Shutdown Method

Pump General **Gradient Program**

Pump 1

	Time	A%	B%	C%	D%	$\mu\text{l}/\text{min}$	P2
0	0.00	95.0	5.0	0.0	0.0	500.0	
1	0.60	95.0	5.0	0.0	0.0	500.0	
2	2.30	0.0	100.0	0.0	0.0	500.0	
3	2.35	0.0	100.0	0.0	0.0	500.0	
4	3.00	95.0	5.0	0.0	0.0	500.0	
5	4.00	95.0	5.0	0.0	0.0	500.0	
6		100.0	0.0	0.0	0.0	500.0	

Solvent colors:  
A B  
C D

Type of view: Solvent Gradient

press F1

2/16/2009 2:03 PM

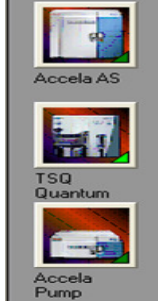


LCquan - CAP\_160209

File Quantum View Apps Options Help

Instruments

- Acquisition
- Explore
- Quantitate



Instrument Setup - Define Instrument Methods

- Startup Method
- Instrument Method
- Shutdown Method

Scan Editor Syringe Pump Divert Valve Method Summary

Calibration Correction Method

Run Settings  
MS Acquire Time (min): 4.00 Segments: 1 Current Segment: 1

Segment 1  
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6 3.8 4.0 Retention Time (min)

Segment 1 Settings  
Segment Time (min): 4.00 Tune Method: C:\Xcalibur\methods\CAP\_TUNE\_140209.TSQTune  
Scan Events: 1 Chrom Filter Peak Width (s):  15.0 Collision Gas Pressure (mTorr):  1.5  
Current Scan Event: 1 Scan Event 1

Scan Event 1  
Scan Type: SRM  
Same value for all SRMs  
Scan Width (m/z):  0.010  
Scan Time (s):  0.100  
Coll. Energy (V):  10  
Peak Width  
Q1 (FWHM): 0.70  
Use Tuned Tube Lens Value:

	Parent Mass	Product Mass	Collision E	T Lens
1	321.000	152.000	21	104
*	321.000	152.000	21	104

Polarity:  
 Positive  Negative

Data Type:  
 Centroid  Profile

Skimmer Offset:  
Skimmer Offset (V):  7

Micro Scans: 1

Copy ScanEvent Paste ScanEvent

Help Tune

For Help, press F1

2/16/2009 2:04 PM



Agilent - CAP... 145000

File View Apps Options Help

Tools

### Create Method - Identify components and define calibration settings

Identification | Calibration

#### Chromatogram Definition

Detection:  Smoothing:

Trace: TIC

Mass Intensity:

Filter:

Retention Time: Expected (min):  Window (sec):

Use as RT List View Width (min):

Adjust Using:

#### Generic Peak Integration

Peak Detection Algorithm: Generic

S/N Threshold:

Valley Detection Enabled

Expanded Width (sec):

Combine Peak Widths

Peak Int. (n):

Scaling Factor:

#### Peak Identification

Highest Peak  Maximal RT

Min. Peak Height (n):

Min. Peak Width (n):

Relative Concentration

	Min.	Max. (n)	Min. (n)
1			

Window:

Relative  Absolute

Quality Concentration:

#### Component

Component
1

---

#### Chromatogram

Sample: Cap... TIC - RT: 1.84 - 2.58 nL: 1.00000  
T: 1.84 2.00 2.10 2.20 2.30 2.40 2.50

#### Spectrum

Sample: Cap... RT: 2.24 nL: 1.00000  
T: 1.84 2.00 2.10 2.20 2.30 2.40 2.50

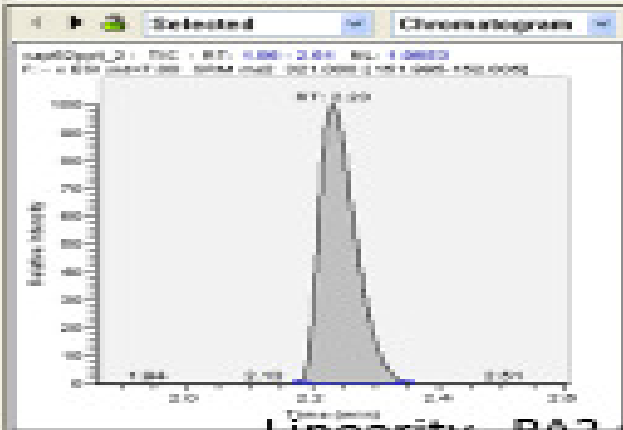
Agilent - CAP... 145000 2/14/2009 2:04 PM

Taskbar: File Explorer, CAP... 145000, Windows Explorer, Report Application..., Report Application..., Agilent - CAP... 145000, Microsoft PowerPoint, Outlook

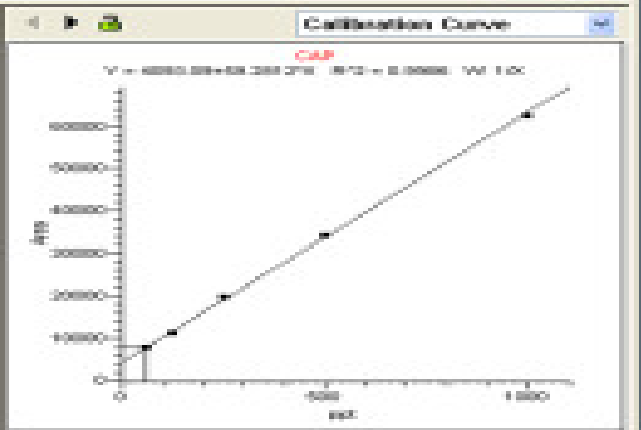


File Name	Sample Type	Response	Response Ratio	Specific ID	Calculated Conc	Level	Units	RT	Sample ID	Excitable	Int. Pct	% CV	% Bias	% Error
cap100ppm_2	Standard	11981	11981.000	62.000	62.000	Level_1	ppm	2.23	cap100ppm_2	<input type="checkbox"/>	A-2	0.00	0.00	0.00
cap120ppm_2	Standard	11981	11981.000	1.20.000	1.17.000	Level_2	ppm	2.23	cap120ppm_2	<input type="checkbox"/>	A-3	0.00	-0.16	0.00
cap200ppm_2	Standard	10493	10493.000	200.000	200.000	Level_3	ppm	2.23	cap200ppm_2	<input type="checkbox"/>	A-4	0.00	0.00	0.00
cap500ppm_2	Standard	34302	34302.000	500.000	500.000	Level_4	ppm	2.23	cap500ppm_2	<input type="checkbox"/>	A-5	0.00	1.00	0.00
cap1000ppm_2	Standard	62430	62430.000	1000.000	993.000	Level_5	ppm	2.23	cap1000ppm_2	<input type="checkbox"/>	A-6	0.00	-1.01	0.00

Units



No STD Peak for this Component



Linearity, R<sup>2</sup> = 0.9986



Quantum Tune Master - ESI- Instrument Method Development Workspace - POLYTYROSINE 010908 Neg\_Calibration.TSQCalib



Type: **Full Scan** SIM SRM

Time value for all SRM(s):

Scan Width:  0.010

Scan Time:  0.100

Collision Energy:  21

Q1 Peak Width: 0.70

Tuned Tube Lens Value:

	Parent Mass	Product Mass
1	321.000	152.000

Skimmer Offset:  15.0

Data Processing:  Average Chrom. Filter

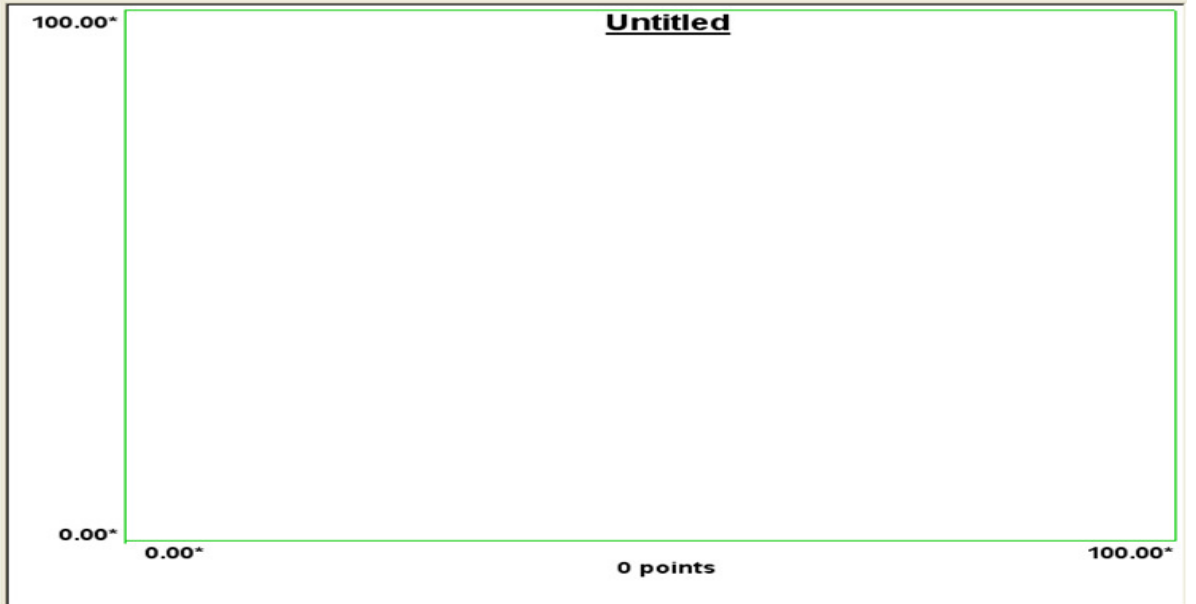
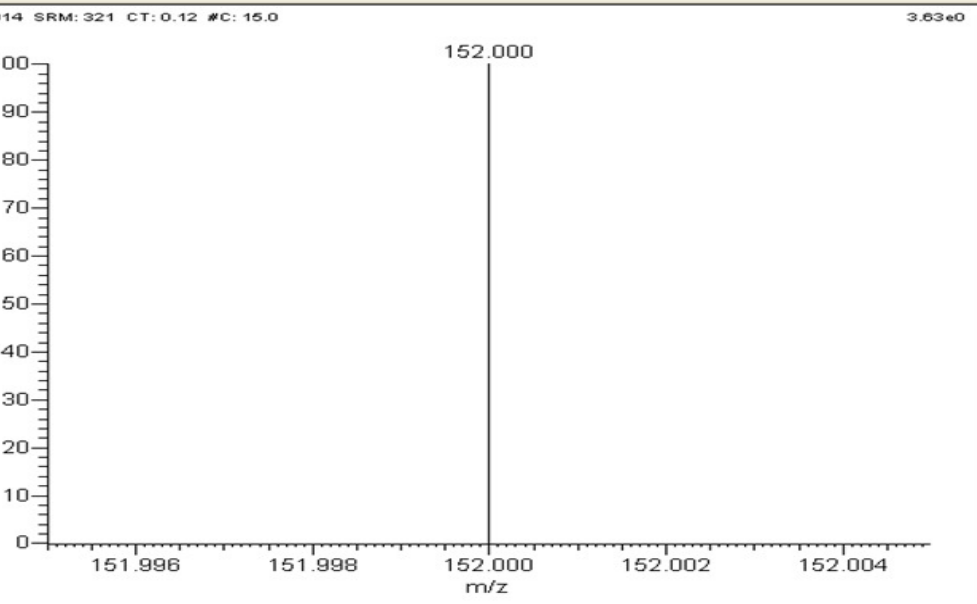
Q2 CID Gas:  1.5

Micro Scans:  1

Apply Abort

Device	Value	Readback
<input type="checkbox"/> <input checked="" type="checkbox"/> Spray Voltage	3000	3016
<input type="checkbox"/> <input checked="" type="checkbox"/> Sheath Gas Pressure	45	45
<input type="checkbox"/> <input checked="" type="checkbox"/> Ion Sweep Gas Pressure	0.0	0.0
<input type="checkbox"/> <input checked="" type="checkbox"/> Aux Gas Pressure	10	10
<input type="checkbox"/> <input checked="" type="checkbox"/> Capillary Temperature	325	326
<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Tube Lens Offset	SRM Table	-105
<input type="checkbox"/> <input checked="" type="checkbox"/> Skimmer Offset	7	7
<input type="checkbox"/> <input checked="" type="checkbox"/> Collision Pressure	1.5	1.5
<input type="checkbox"/> <input checked="" type="checkbox"/> Collision Energy	SRM Table	28 - 7

Tube Lens Offset:





The method studied was sensitive enough to detect and quantify 0.050 ug/kg (ppb) chloramphenicol for screening purposes, much lower than the Minimum Required Performance Limit (MRPL) of 0.3  $\mu\text{g}/\text{kg}$  imposed by European Commission's regulation. The study revealed that most of the samples were in compliance with MRL and growing awareness amongst farmers to avoid banned antibiotic CAP.

# Remembering Gandhi



## Thank You

Be The  
Change  
You Wish To See In  
The World

**Slide 15**

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**Sc1**

Sarath chandra, 03-08-2004