
Determination of Antibiotics in animal feeds by Ion Chromatography



Jay Gandhi, *PhD*

Metrohm USA, Riverview FL

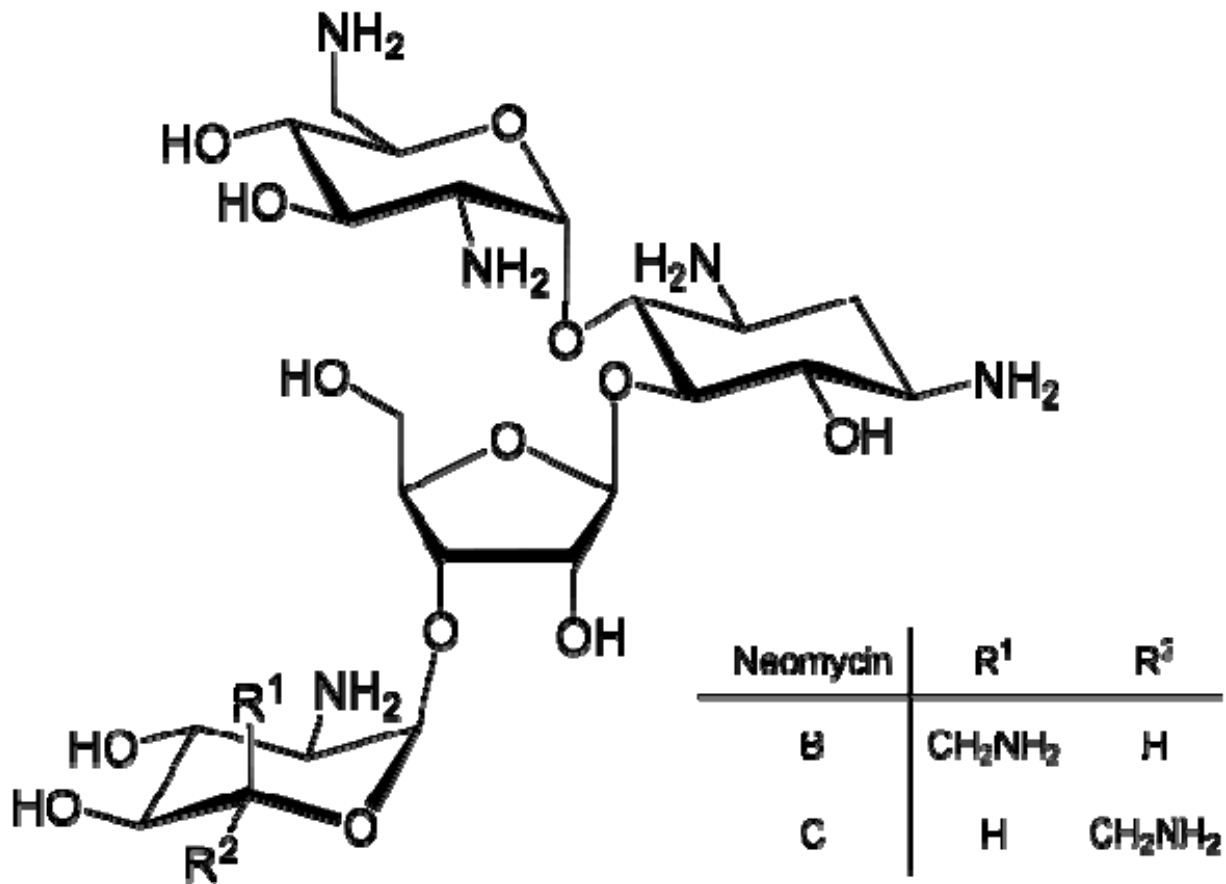
Outline

- **Data presented in June 2009**
 - *Chromatograms and Data*
- **Neomycin + Tetracycline + Tylosin**
 - *Statement: Chromatography Method should be able to Analyze Neomycin B and Neomycin C Sulfate as two separate peaks*
- **Ion Chromatography Work in progress between June and December 2009**
 - *Simple sample preparation technique*
 - *Chromatograms and Data*
- **Summary**



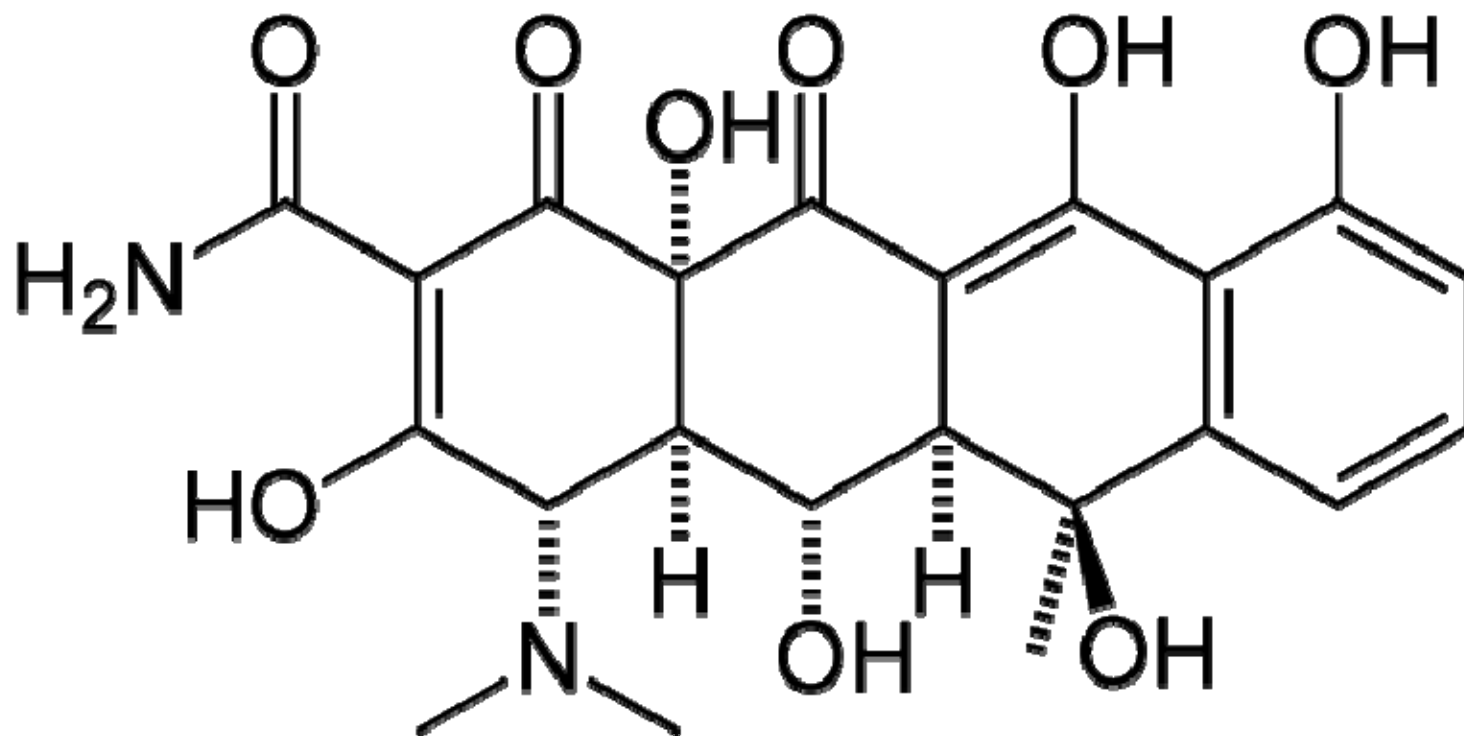
What is Neomycin?

Neomycin – Chemical Structure and Chemistry



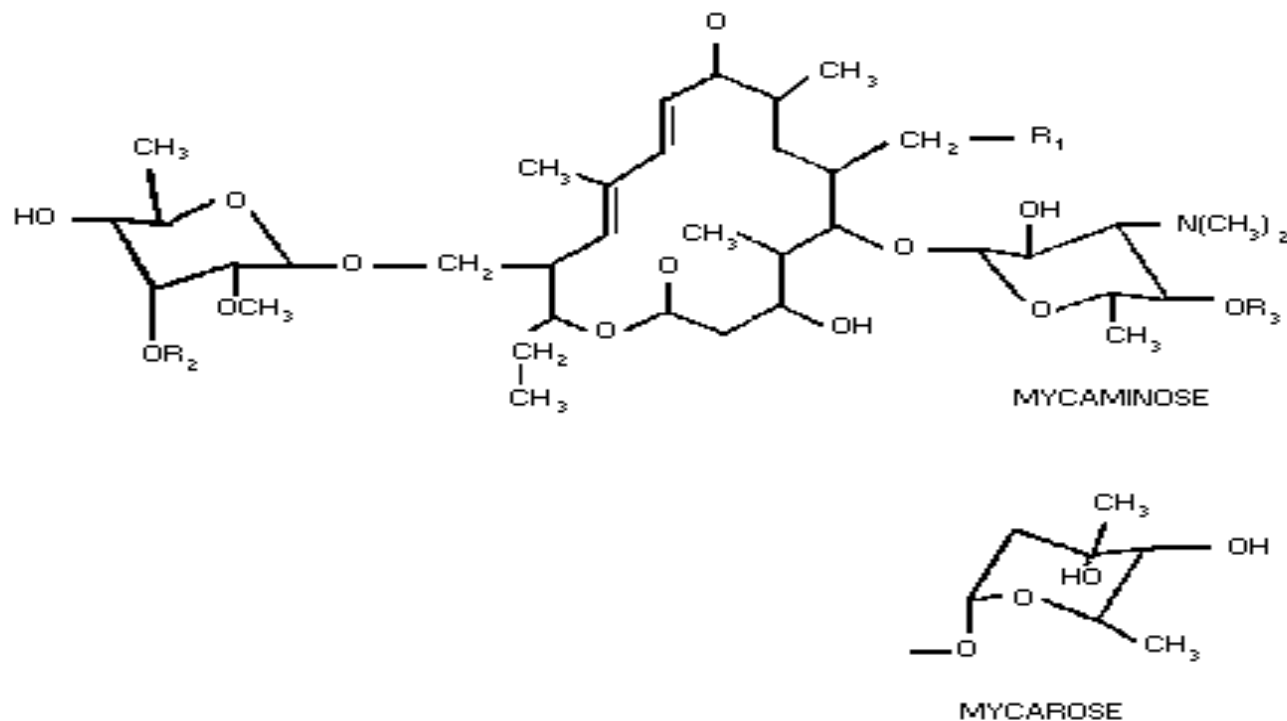
Aminoglycoside antibiotic obtained from *Streptomyces fradiae*

Terramycin[®] – Tetracycline Antibiotic



Tylosin

Figure I. Structures for tylosin factors and dihydrodesmycosin



COMPOUND	R_1	R_2	R_3
TYLOSIN (A)	-CHO	-CH ₃	MYCAROSE
DESMYCOSIN (B)	-CHO	-CH ₃	-H
MACROSIN (C)	-CHO	-H	MYCAROSE
RELOMYCIN (D)	-CH ₂ OH	-CH ₃	MYCAROSE
DIHYDRODESMYCOSIN	-CH ₂ OH	-CH ₃	-H

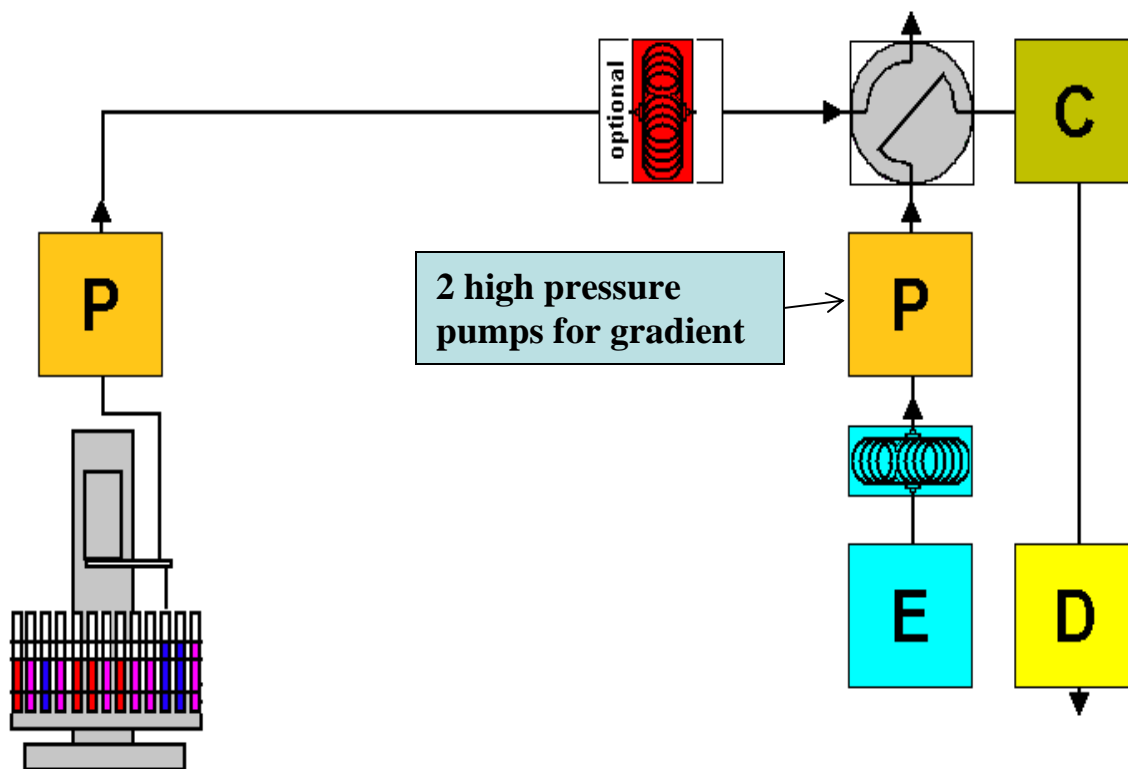


Simple Sample Preparation Technique

Sample Preparation

- Sample (1 gram)
- Add Water/Methanol – 75:25 (25mls)
- Shake well for 5 minutes
- Centrifuge
- Filter and analyze
- Dilute as required

Instrument Set-up

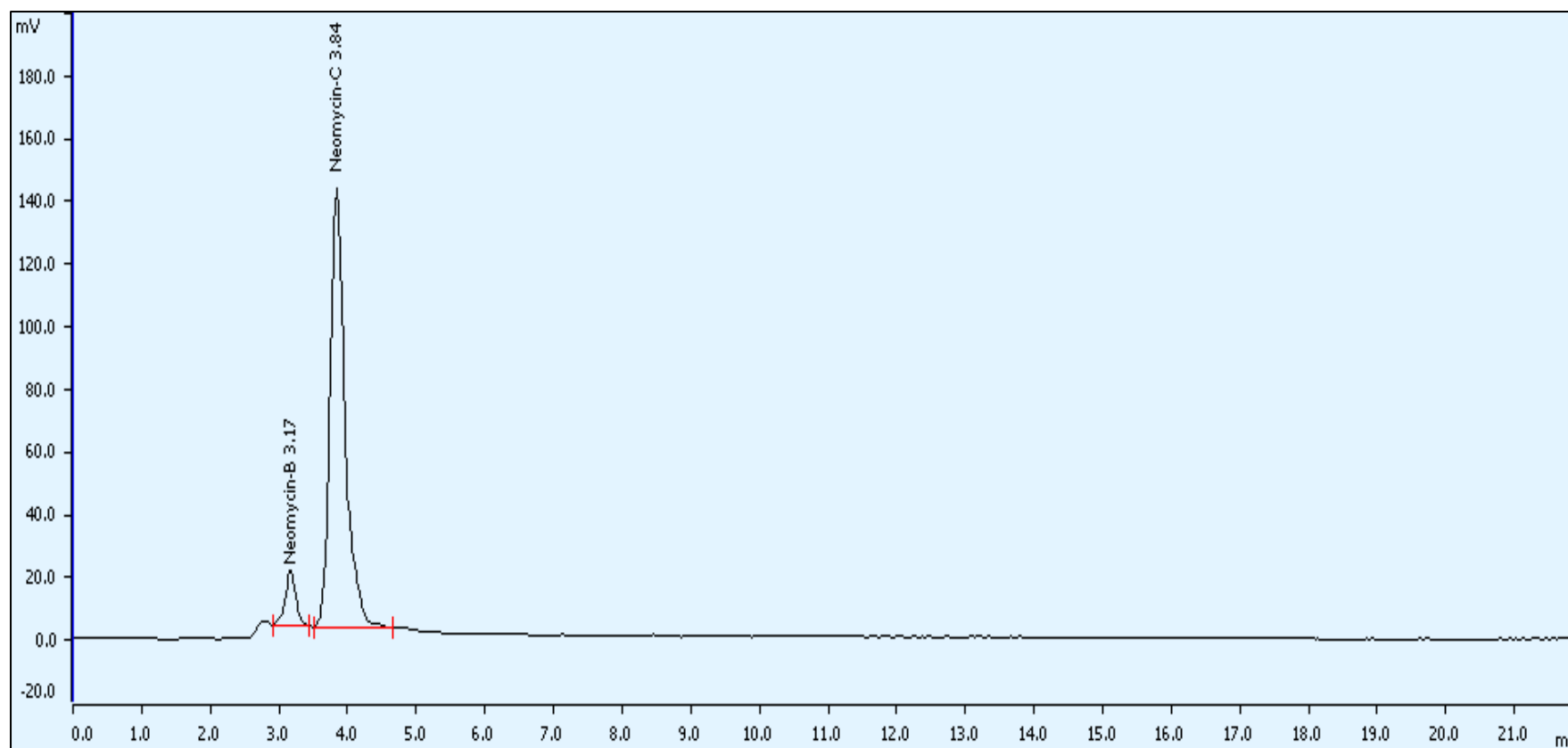




Experimental Conditions

- Metrohm dual piston high pressure metal-free Pump
- Metrohm Model 871 Electro-chemical Detector (with gold electrode and solid state reference electrode)
- Metrohm-Spark Triathlon Auto Sampler with PCT
- Metrosep Carb1- 250 analytical column + Carb1 guard column
- Eluent A : 150mM Sodium Hydroxide
- Eluent B : UHP Di Water
- Flow Rate: 1.5mls per minute
- Injection Loop Size: 10uL

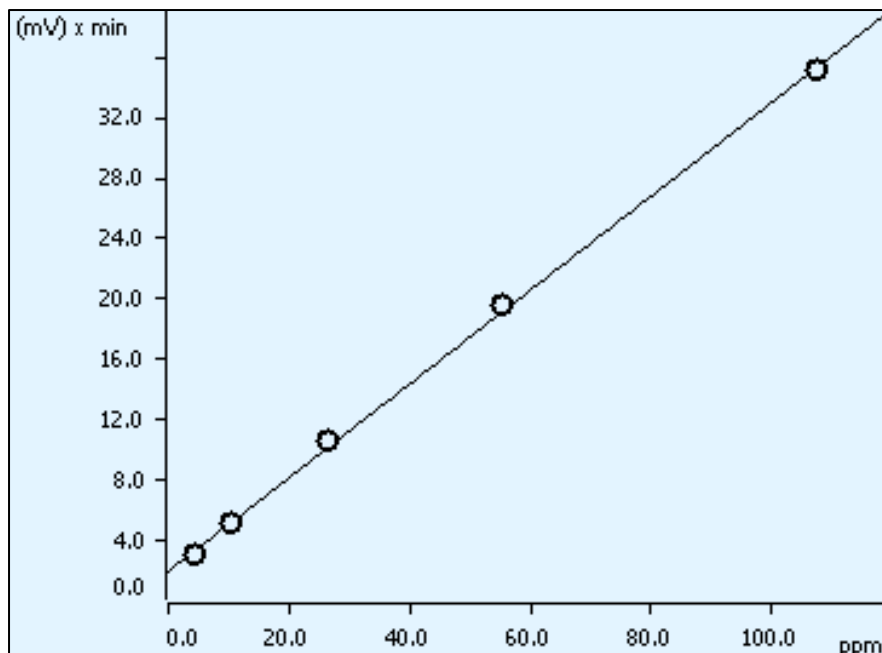
Calibration Standard



Neomycin B @15mg/Kg and C @ 85 mg/Kg

Calibration Curves – Linear regression

Neomycin C



Function: $A = 1.95901 + 0.0155156 \times Q$

Relative standard deviation 2.965%

Correlation coefficient 0.999587

Curve type Linear

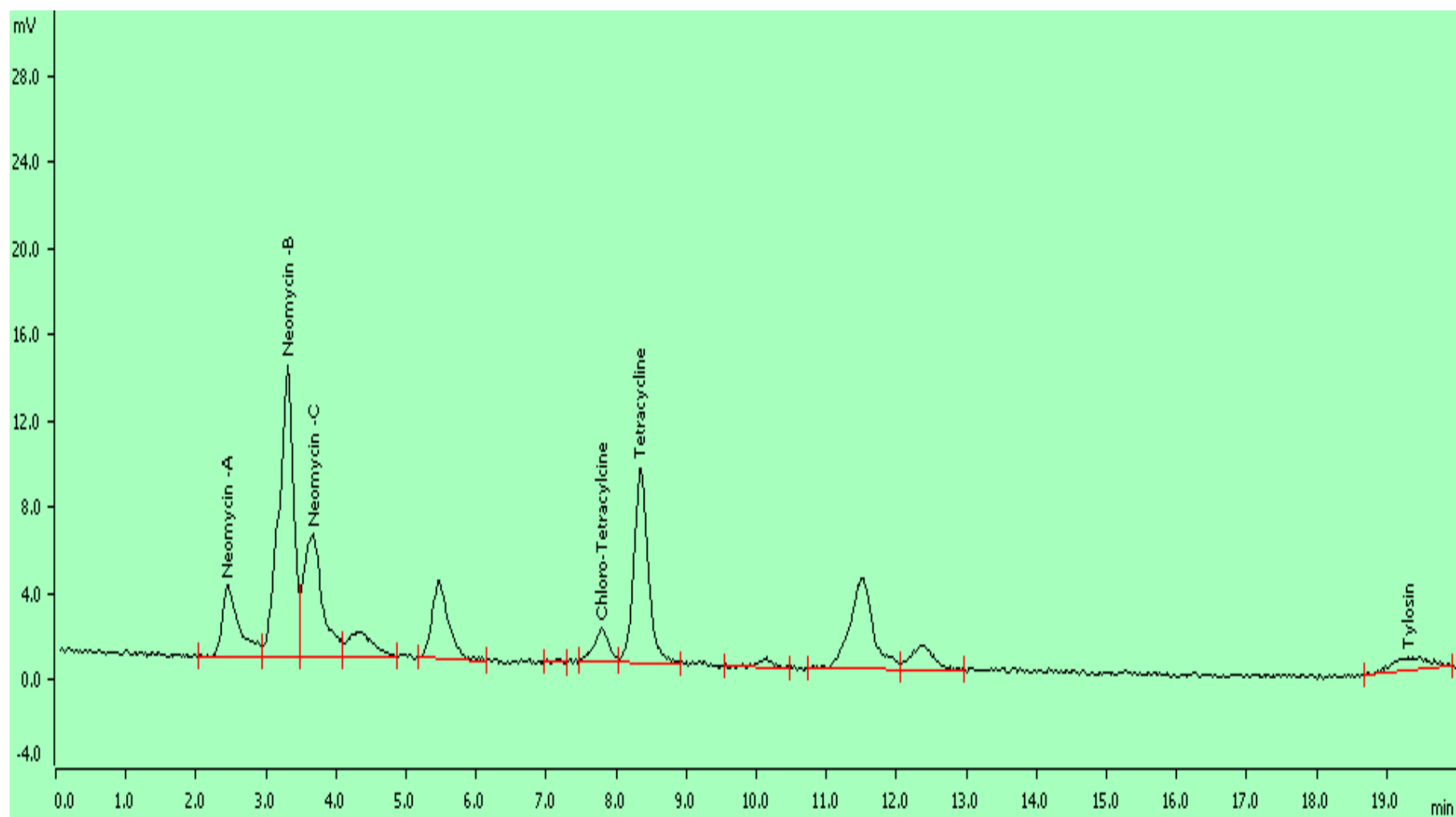
Weighting 1

Calibration range = 5ug/Kg to 100ug/Kg

Results (Data)

Sample Description	Total Neomycin*, mg/Kg		% Recovery
	Expected Value	Measured Value	
Duralife Regular	200	193.4	96.70%
Poultry Pellets	200	189.6	94.80%
Poultry Mash	20000	19587	97.94%
Non-Medicated Poultry Mash	0	0	
Unimilk Non-medicated Milk replacer	0	0	
Neo - Terramycin 50/50	100000	96733	96.73%
Advanced Calf Medi	1600	1690	105.63%
Ulti-Lact 369	1280	1246	97.34%

* This results are for Neomycin C only as Neomycin B standard was not available at the time of analysis



Summary

- **Ion Chromatography method is simple and applicable for this project**
 - *Extraction of the samples is simple due to water/methanol solubility of the antibiotics*
- **This method is adaptable to other antibiotics like**
 - *Tetracycline*
 - *Tylosin*
- **Further study is in process for optimizing chromatographic separation**

Thank you for listening.....

