Determination of Tylosin Residues in Large Animal Feeds by LC-MS/MS

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AAFCO
Association of American Feed Control Officials

OVERVIEW

- Method Developments
  - Initial Developments
  - Laboratory Methods
- Where do we go next?

Method Development and Modifications

- Method development under way:
  - Tylosin:
    - Developing and validating method for tylosin in animal feeds, and premixes, both at medicated levels and for residues.
    - Using Gramse method from Wisconsin Department of Agriculture as a starting point.
    - SCS will be evaluating SPE cleanups, as well as a variety of different extraction techniques.
    - Instrumental work will be done on the LC-DAD and LC-MS/MS.

Tylosin

LC-MS/MS Residue Method Development

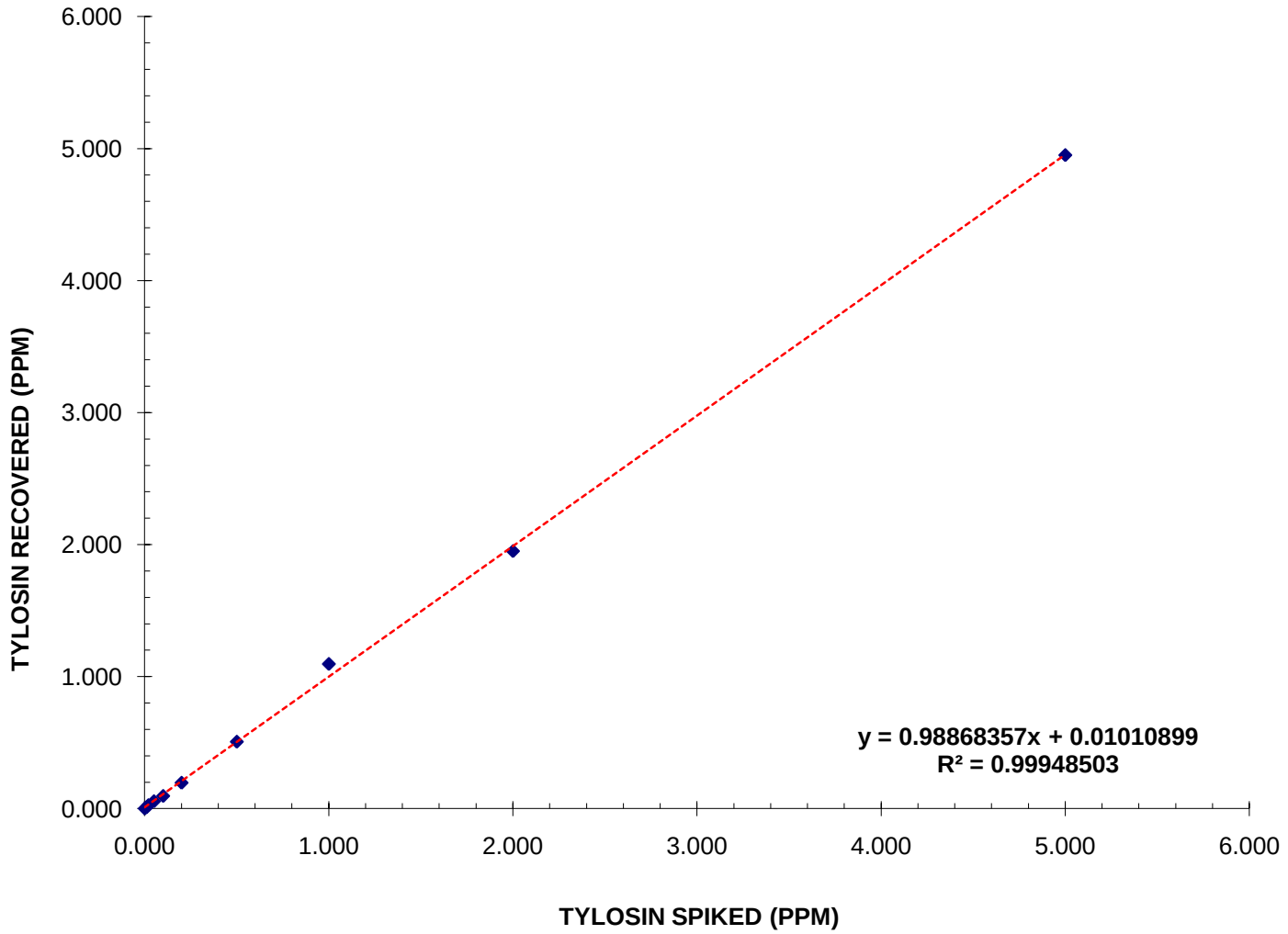
- Developmental Strategies
  - Medicated vs. residue levels
    - Do we need different extraction, clean-up and detection methods?
    - AAFCO method needs requirements
      - Accuracy
      - Applicability
      - DL, QL
      - Precision – repeatablity and reproducibility
      - Selectivity and performance

Tylosin

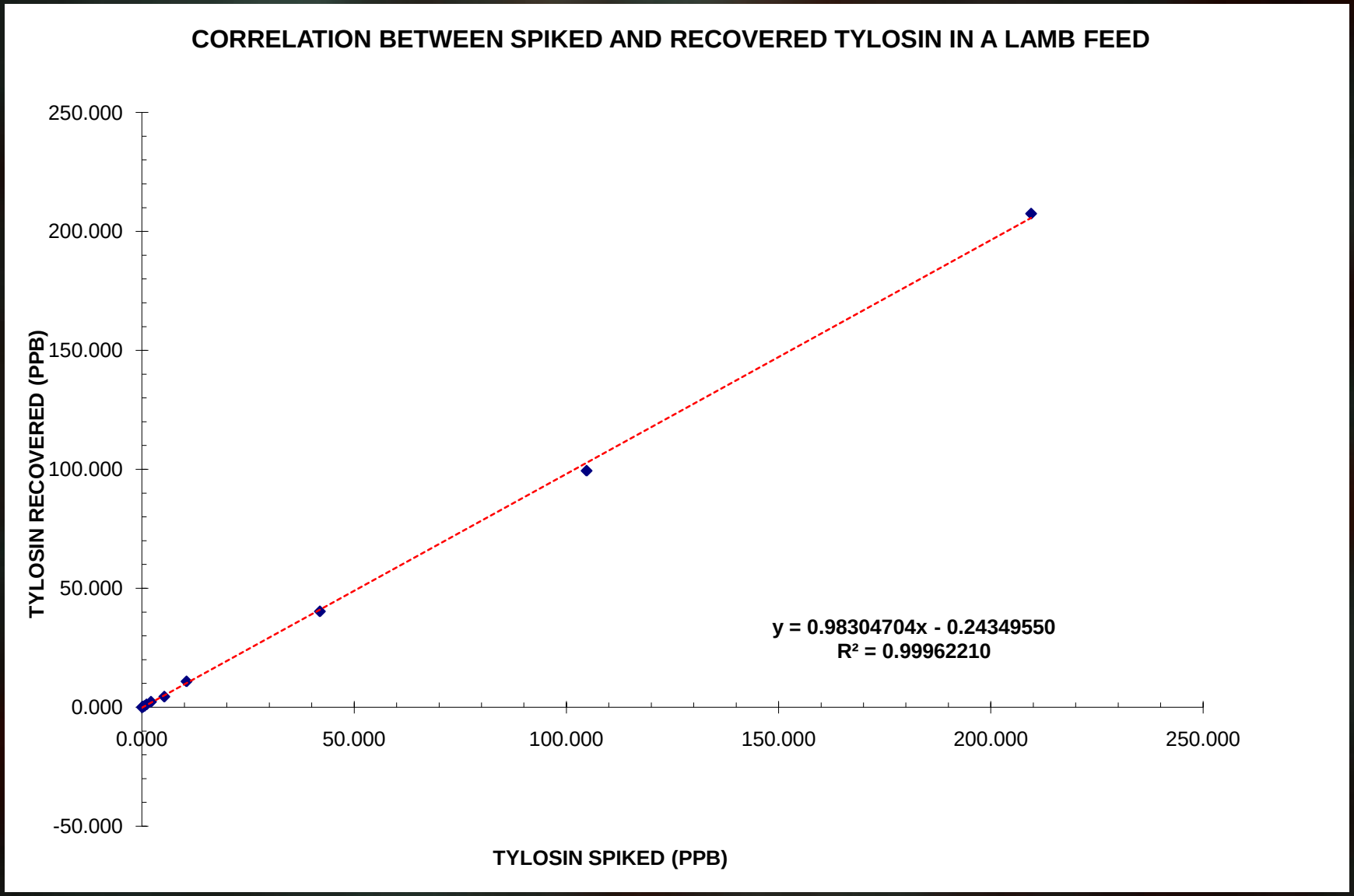
LC Method Development

- Extraction and clean up methods
  - Extraction solvent – currently using methanol
    - QuEChER'S modifications
    - Other solvents, ACN, or combinations
    - Evaporation step without clean up
    - Evaporation step with clean up
    - ASE technique
  - Cleanup
    - QuEChER'S modifications
    - Evaluate the use of different SPE packings
      - HLB, DSC-18, DSC-CN, Envi-18, PSA, GCB or combinations of these

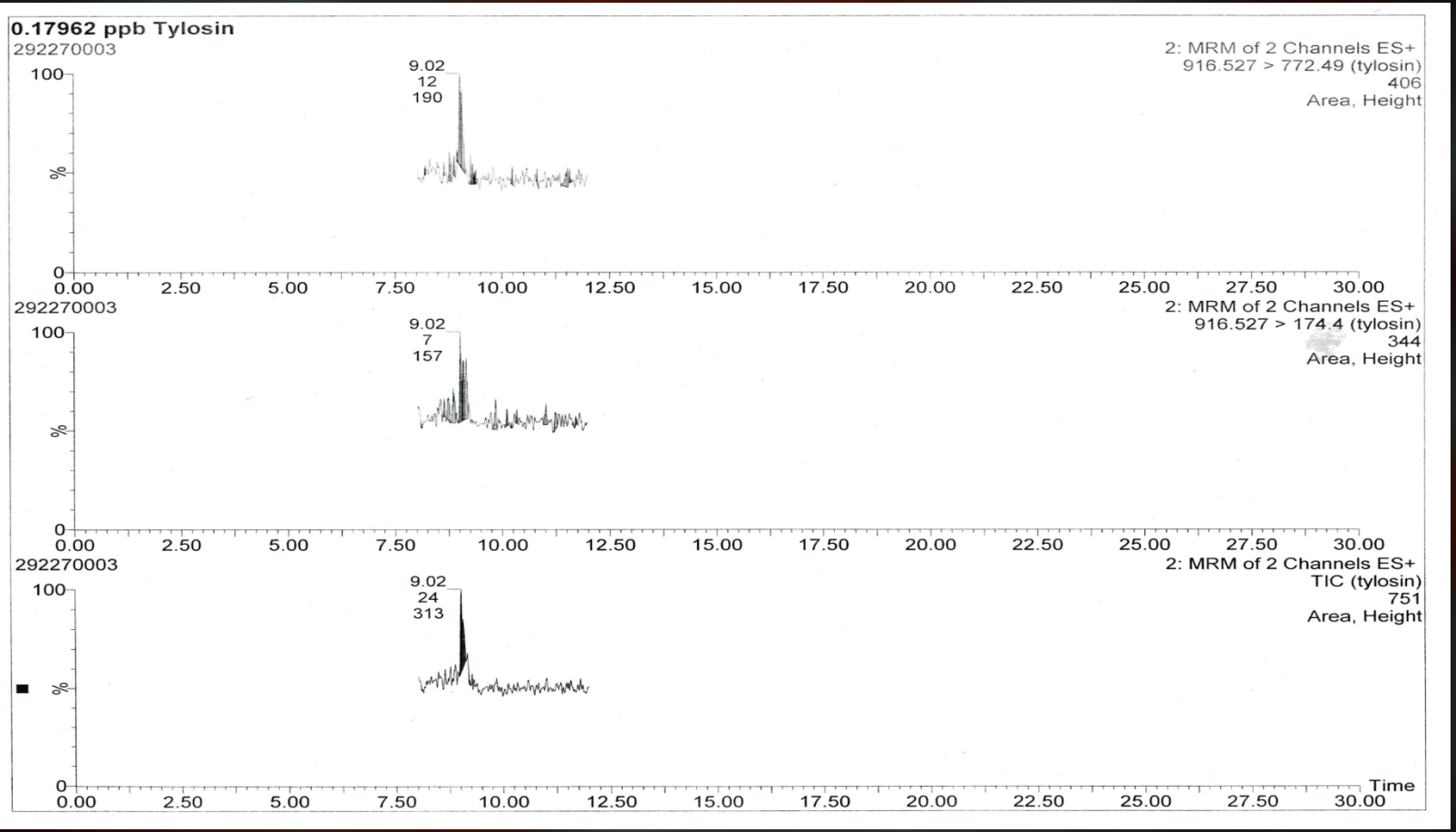
Tylosin


CORRELATION BETWEEN SPIKED AND RECOVERED TYLOSIN IN A HORSE FEED
TYLOSIN RECOVERED (PPM)
6.000
5.000
4.000
3.000
2.000
1.000
0.000
0.000
1.000
2.000
3.000
4.000
5.000
6.000
TYLOSIN SPIKED (PPM)
y = 0.98868357x + 0.01010899
R² = 0.99948503

Tylosin

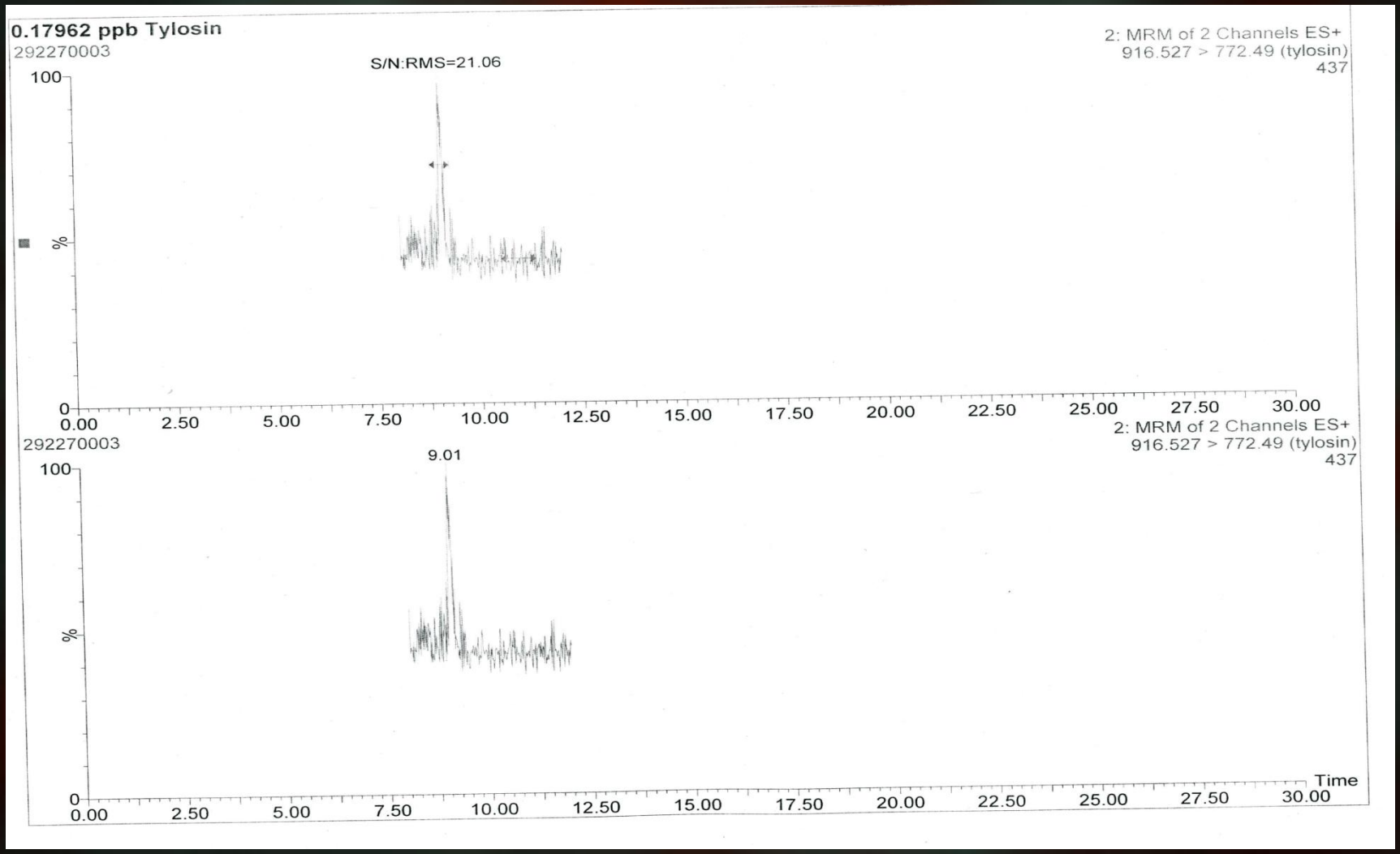

CORRELATION BETWEEN SPIKED AND RECOVERED TYLOSIN IN A LAMB FEED
250.000
200.000
150.000
100.000
50.000
0.000
-50.000
TYLOSIN RECOVERED (PPB)
0.000
50.000
100.000
150.000
200.000
250.000
TYLOSIN SPIKED (PPB)
y = 0.98304704x - 0.24349550
R² = 0.99962210

Tylosin

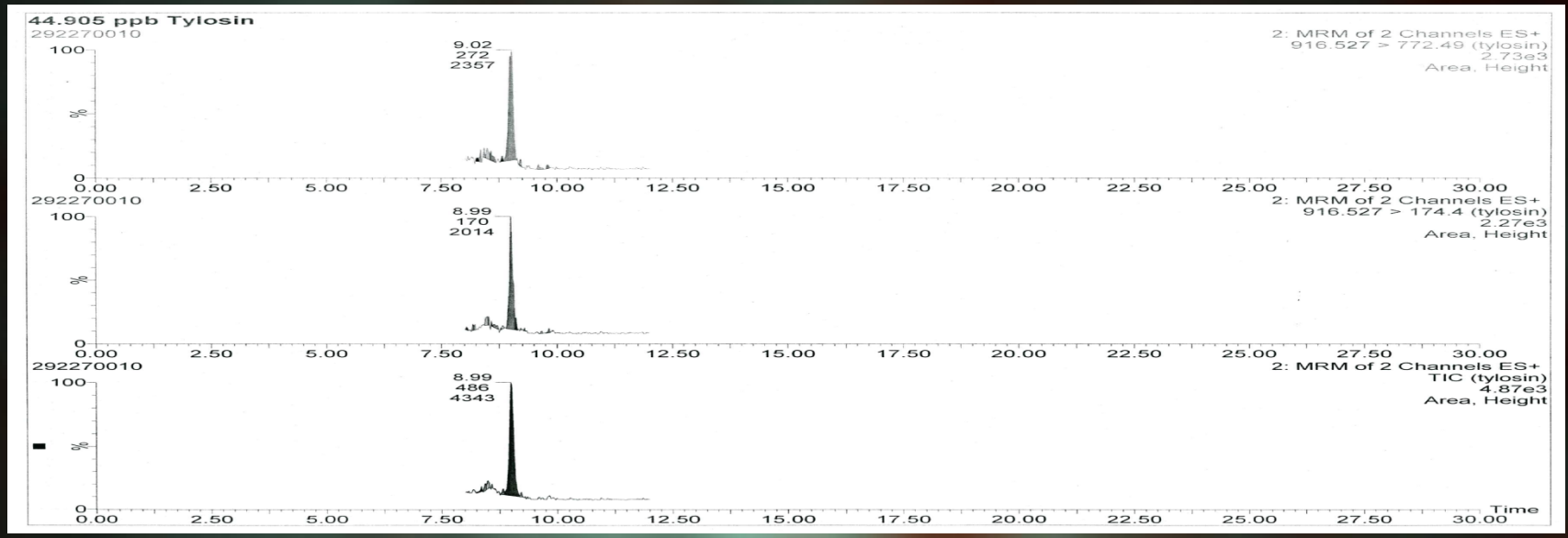


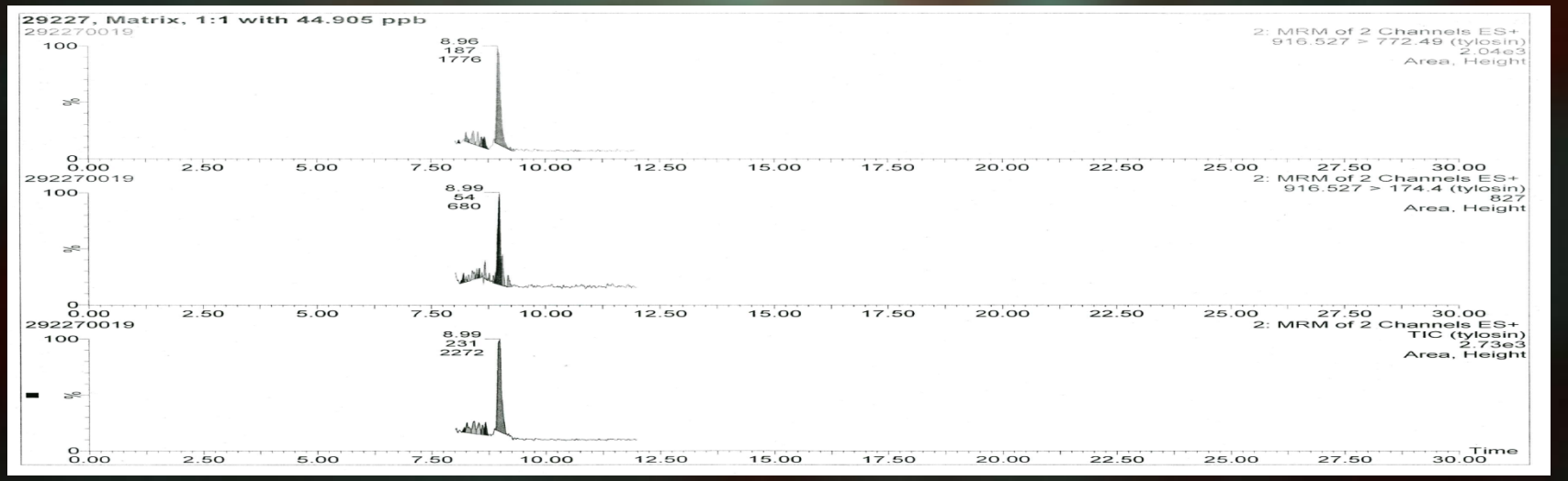
0.18 ppb Tylosin

Tylosin

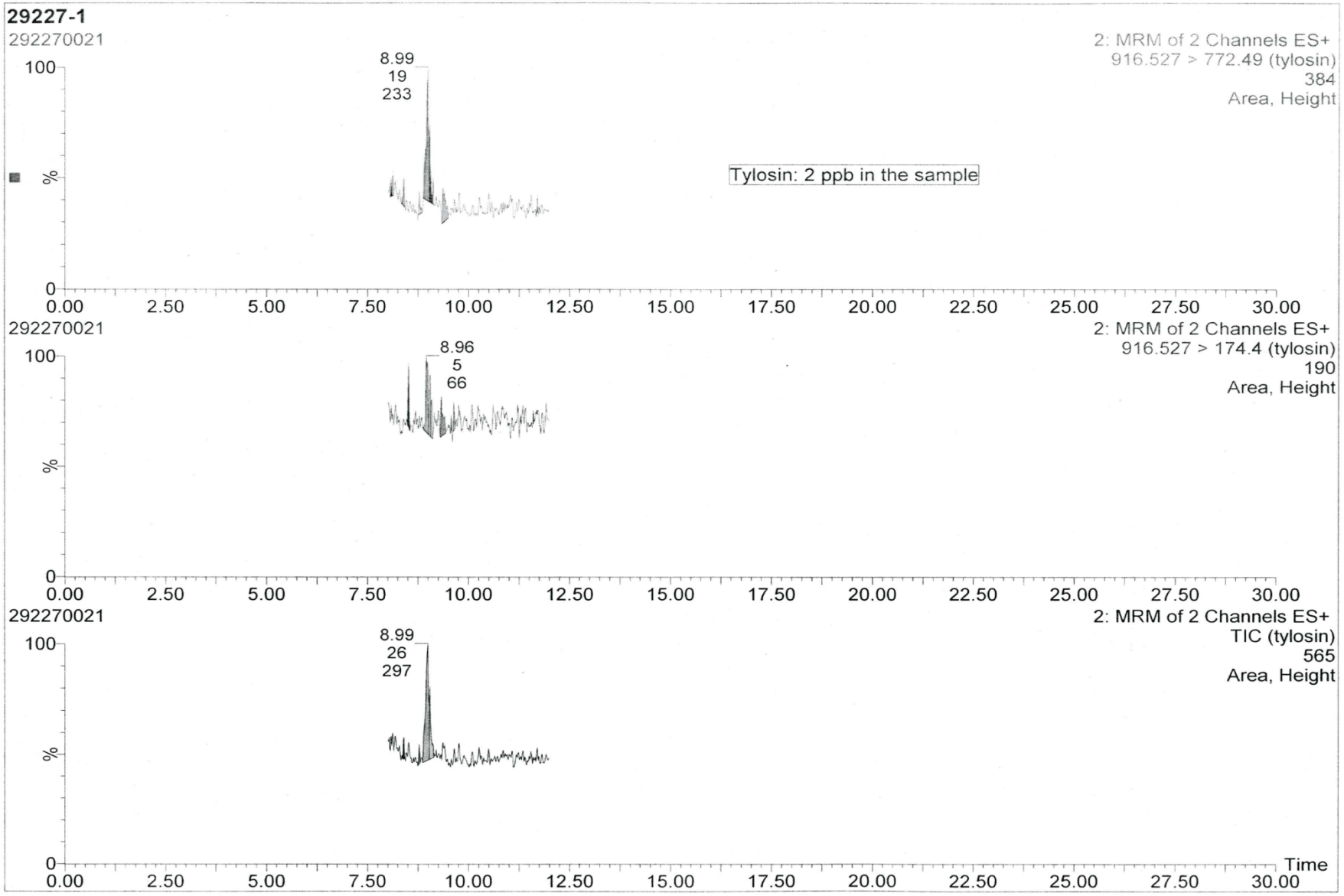

0.17962 ppb Tylosin
292270003
S/N:RMS=21.06
2: MRM of 2 Channels ES+
916.527 > 772.49 (tylosin)
437
100
%
0
0.00
2.50
5.00
7.50
10.00
12.50
15.00
17.50
20.00
22.50
25.00
27.50
30.00
292270003
9.01
2: MRM of 2 Channels ES+
916.527 > 772.49 (tylosin)
437
100
%
0
Time
0.00
2.50
5.00
7.50
10.00
12.50
15.00
17.50
20.00
22.50
25.00
27.50
30.00

Tylosin

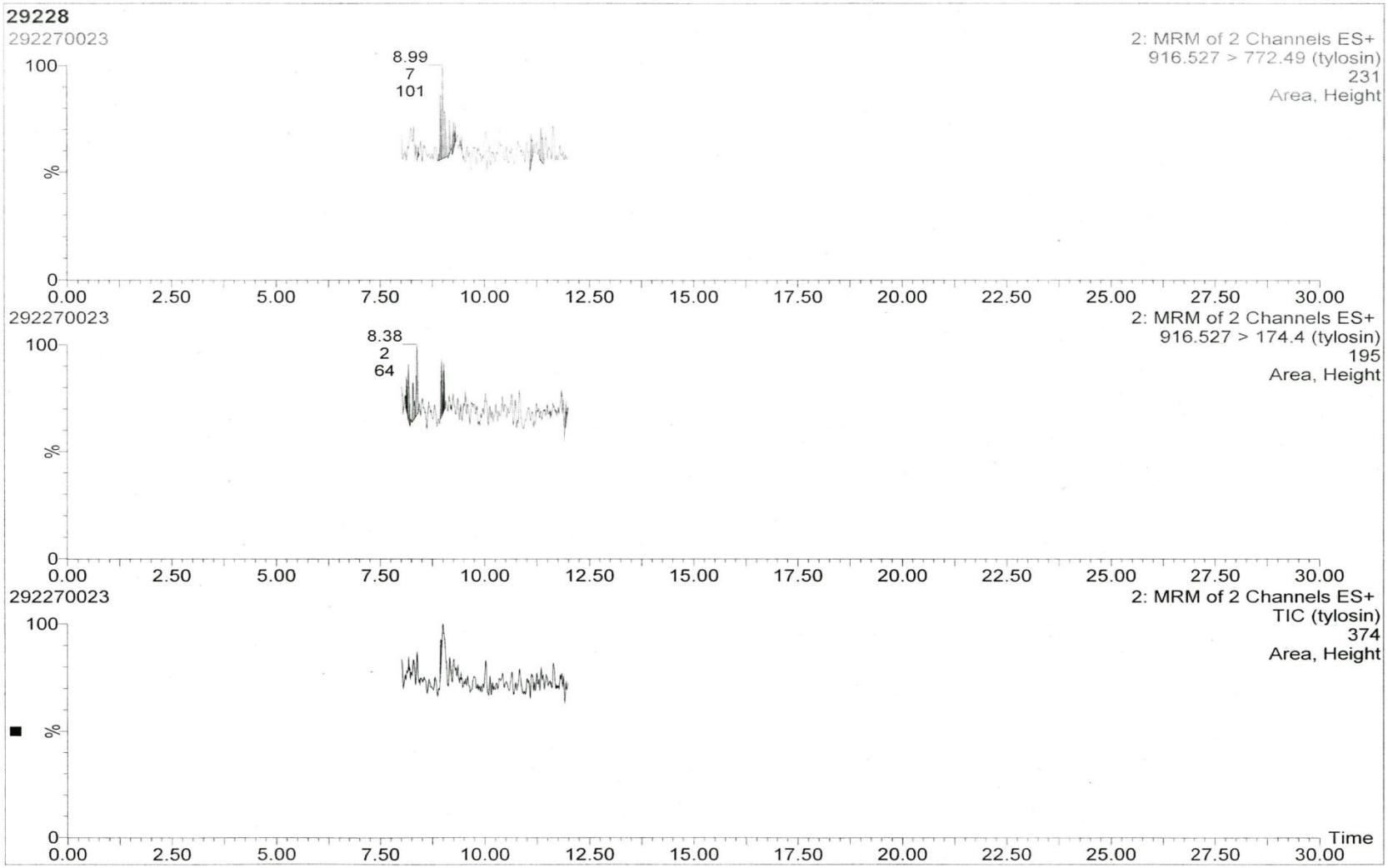

44.905 ppb Tylosin
292270010
9.02
272
2357
2: MRM of 2 Channels ES+
916.527 > 772.49 (tylosin)
2.73e3
Area, Height
292270010
8.99
170
2014
2: MRM of 2 Channels ES+
916.527 > 174.4 (tylosin)
2.27e3
Area, Height
292270010
8.99
486
4343
2: MRM of 2 Channels ES+
TIC (tylosin)
4.87e3
Area, Height
100
%
0
0.00
2.50
5.00
7.50
10.00
12.50
15.00
17.50
20.00
22.50
25.00
27.50
30.00
Time


29227, Matrix, 1:1 with 44.905 ppb
292270019
8.96
187
1776
2: MRM of 2 Channels ES+
916.527 > 772.49 (tylosin)
2.04e3
Area, Height
292270019
8.99
54
680
2: MRM of 2 Channels ES+
916.527 > 174.4 (tylosin)
827
Area, Height
292270019
8.99
231
2272
2: MRM of 2 Channels ES+
TIC (tylosin)
2.73e3
Area, Height
100
%
0
0.00
2.50
5.00
7.50
10.00
12.50
15.00
17.50
20.00
22.50
25.00
27.50
30.00
Time

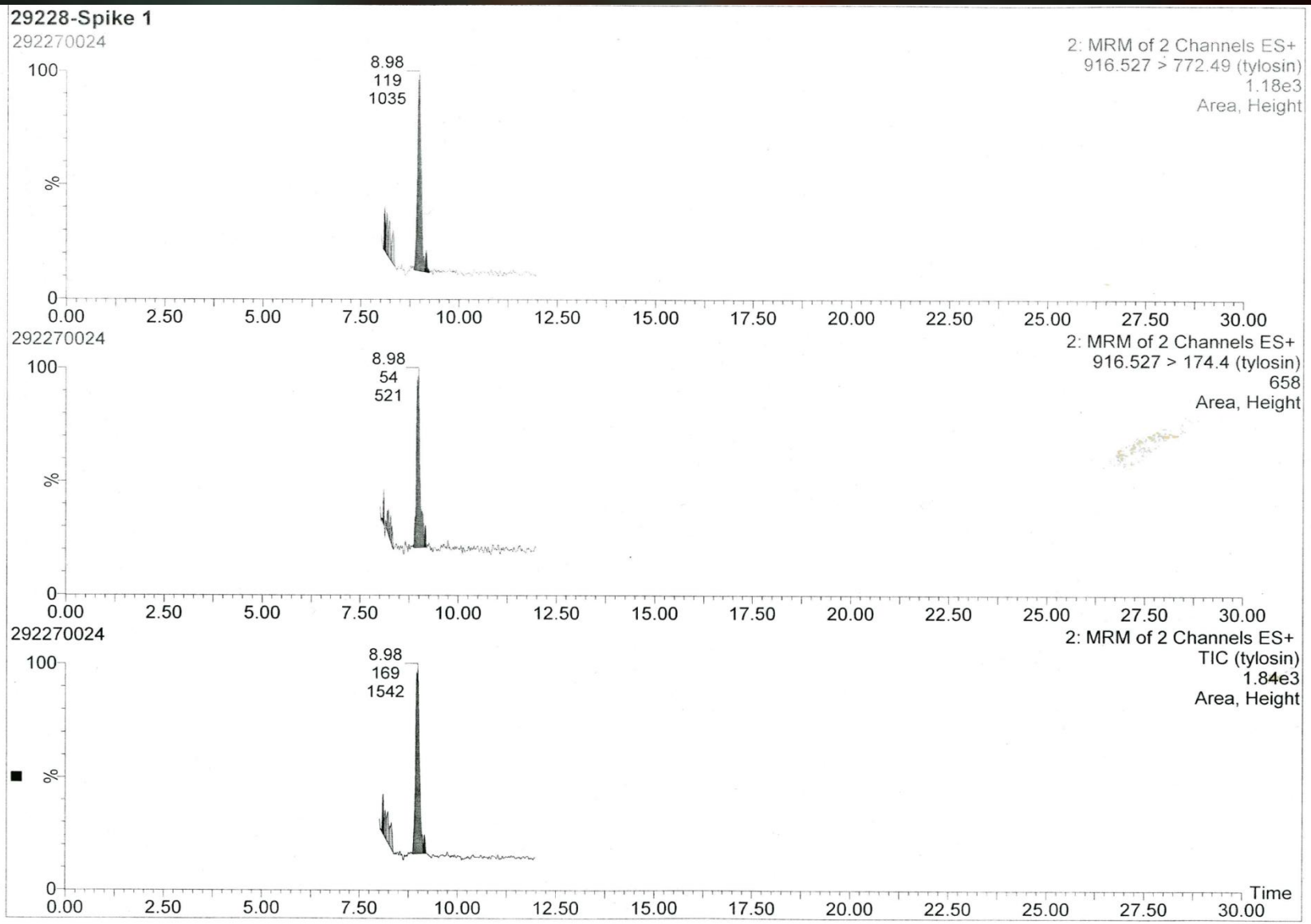
Tylosin


29227-1
292270021
2: MRM of 2 Channels ES+
916.527 > 772.49 (tylosin)
384
Area, Height
8.99
19
233
Tylosin: 2 ppb in the sample
100
%
0
0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00
292270021
2: MRM of 2 Channels ES+
916.527 > 174.4 (tylosin)
190
Area, Height
8.96
5
66
100
%
0
0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00
292270021
2: MRM of 2 Channels ES+
TIC (tylosin)
565
Area, Height
8.99
26
297
100
%
0
Time
0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00

Tylosin


29228
292270023
2: MRM of 2 Channels ES+
916.527 > 772.49 (tylosin)
231
Area, Height
8.99
7
101
100
%
0
0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00
292270023
2: MRM of 2 Channels ES+
916.527 > 174.4 (tylosin)
195
Area, Height
8.38
2
64
100
%
0
0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00
292270023
2: MRM of 2 Channels ES+
TIC (tylosin)
374
Area, Height
100
%
0
Time
0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00

Tylosin


29228-Spike 1
292270024
2: MRM of 2 Channels ES+
916.527 > 772.49 (tylosin)
1.18e3
Area, Height
8.98
119
1035
100
%
0
0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00
292270024
2: MRM of 2 Channels ES+
916.527 > 174.4 (tylosin)
658
Area, Height
8.98
54
521
100
%
0
0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00
292270024
2: MRM of 2 Channels ES+
TIC (tylosin)
1.84e3
Area, Height
8.98
169
1542
100
%
0
0.00 2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00
Time

Tylosin

Clean Chicken Feed

Spike No.	Tylosin (ppm)	Grand Mean	With-in Day RSD	Between Day RSD	Total RSD	HorRAT	F-Value (2.773)
Spike 1	0.030	0.0311	15.623	6.645	16.98	2.52	0.181
Spike 2	0.100	0.103	7.078	1.537	7.24	1.29	1.188
Spike 3	0.300	0.307	1.827	1.762	2.54	0.53	4.720
Spike 4	1.000	1.031	6.271	0.897	6.33	1.59	1.082

Tylosin

Clean Horse Feed

Spike No.	Tylosin (ppm)	Grand Mean	With-in Day RSD	Between Day RSD	Total RSD	HorRAT	F-Value (2.773)
Spike 1	0.030	0.0305	7.290	5.645	8.29	1.14	0.301
Spike 2	0.100	0.108	5.290	1.645	4.29	0.71	0.305
Spike 3	0.300	0.310	2.105	1.562	2.05	0.97	3.250
Spike 4	1.000	0.997	3.215	2.850	5.215	1.62	2.750

Tylosin

Clean Swine Feed

Spike No.	Tylosin (ppm)	Grand Mean	With-in Day RSD	Between Day RSD	Total RSD	HorRAT	F-Value (2.773)
Spike 1	0.030	0.028	7.298	5.456	10.29	1.41	0.405
Spike 2	0.100	0.098	4.238	2.057	4.50	1.06	2.085
Spike 3	0.300	0.295	2.052	2.012	3.85	1.88	2.750
Spike 4	1.000	1.100	3.850	4.258	7.50	1.95	2.158

Tylosin

Where do we go from here?

- Further residue work
  - Determine robustnness of method by varying LC conditions, extraction conditions, and clean-up conditions.
  - Have second analyst conduct SLV
- Medicated feeds/premixes
  - Gather more samples, i.e., ones with other drugs, especially
  - Determine robustness of method by varying LC conditions, extraction conditions, and clean-up conditions
  - Have second analyst conduct SLV

Tylosin

Got Questions?

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