Destruction of Antibiotic Residues in the Rendering Process

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Objective

Method development

Experimental Design

Results

Thoughts and Questions

Antibiotics in chicken meat

Muaz et al. (2018): 8 to 90% positive chicken samples, including fresh whole chicken, frozen chicken meat, and chicken liver GLOBALLY

- less than 1 µg/kg of chloramphenicol and sulfonamides
- 1 mg/kg of tetracycline
- 106º C for 24 to 100 min – reducing 50% tetracyclines

No literature data available in the U.S.

Muaz, K., Riaz, M., Akhtar, S., Park, S., & Ismail, A. (2018). Antibiotic residues in chicken meat: global prevalence, threats, and decontamination strategies: a review. Journal of food protection, 81(4), 619-627.

FDA Directive

21 CFR Part 558

- Requesting voluntary withdrawal of antibiotics from feeds
- Eliminating FDA approval of antibiotic use as growth promoters
- 2 categories for new drug approval
  - Cat II with no residue or zero tolerance

NATIONAL ARCHIVES

Code of Federal Regulations

A point in time eCFR system

Title 21

Displaying title 21, up to date as of 10/15/2021. Title 21 was last amended 10/08/2021. view historical versions

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PART 558 - NEW ANIMAL DRUGS FOR USE IN ANIMAL FEEDS

Authority: 21 U.S.C. 354, 360b, 360ccc, 360ccc-1, 371.

Source: 40 FR 13959, Mar. 27, 1975, unless otherwise noted.

Subpart A - General Provisions

§ 558.3 Definitions and general considerations applicable to this part.

(a) Regulations in this part provide for approved uses of drugs and combinations of drugs in animal feeds. Approved combinations of such drugs are specifically identified or incorporated by cross-reference. Unless specifically provided for by the regulations, a combination of two or more drugs is not approved.

(b) The following definitions apply to terms used in this part:

(1) New animal drugs approved for use in animal feed are placed in two categories as follows:

(i) Category I - These drugs require no withdrawal period at the lowest use level in each major species for which they are approved or are approved for use only in minor species.

Food and Drug Administration (FDA). (2015). Veterinary feed directive. Federal Code of Regulations, 80 (106, 31708-31735)

Objective

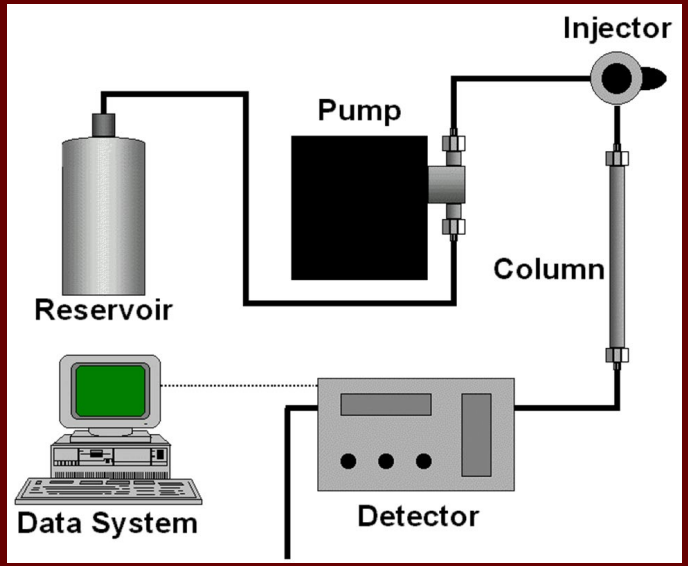
To measure the destruction of antibiotics in spiked samples by the rendering process

Classes of antibiotics	Antibiotics of interest	Notes	LOD	LOQ
Aminoglycosides	Gentamicin		15	50
Beta-Agonists	Ractopamine		1	3
	Zilpaterol	*if we can get access	6	20
Cephalosporins	Ceftiofur		9	30
Ionophore	Narasin		5	10
	Nicarbizine (DNC - dinitrocarbanilide)		5	10
Lincosamides	Lincomycin		6	20
Macrolides	Tylosin		6	20
Penicillins	Penicillin G		9	30
Polypeptides	Bacitracin		15	50
Quinolones	Ciprofloxacin		6	20
	Enrofloxacin	*commonly a false positive	6	20
Sulfonamides	Sulfadiazine		6	20
	Sulfaquinoxaline		6	20
	Sulfadimethoxine		6	20
Streptogramins	Virginiamycin		6	20
Tetracyclines	Tetracycline		9	30

Class of antibiotics

Classes of antibiotics	Antibiotics of interest	Standard compounds	Sol in water, mg/mL
Aminoglycosides	Gentamicin	GENTAMICIN SULFATE SALT	50
Beta-Agonists	Ractopamine	RACTOPAMINE HYDROCHLORIDE	52
	Zilpaterol	N/A	
Cephalosporins	Ceftiofur	CEFTIOFUR SODIUM	Insoluble
Ionophore	Narasin	NARASIN IN DMSO	0.00241
	Nicarbazin (DNC - dinitrocarbanilide)	NICARBAZIN	
Lincosamides	Lincomycin	LINCOMYCIN HYDROCHLORIDE	29.3
Macrolides	Tylosin	TYLOSIN in 0.9% NACL	50
Penicillins	Penicillin G	PENICILLIN G SODIUM SALT	0.2
Polypeptides	Bacitracin	BACITRACIN FROM BACILLUS LICHENIFORMIS	50
Quinolones	Ciprofloxacin	CIPROFLOXACIN	36
	Enrofloxacin	ENROFLOXACIN	0.145
Sulfonamides	Sulfadiazine	SULFADIAZINE	0.75
	Sulfaquinoxaline	SULFAQUINOXALINE SODIUM SALT	0.72
	Sulfadimethoxine	SULFADIMETHOXINE	0.34
Streptogramins	Virginiamycin	VIRGINIAMYCIN S1	Insoluble
Tetracyclines	Tetracycline	TETRACYCLINE HYDROCHLORIDE	500

LCMS Method: Method development & validation



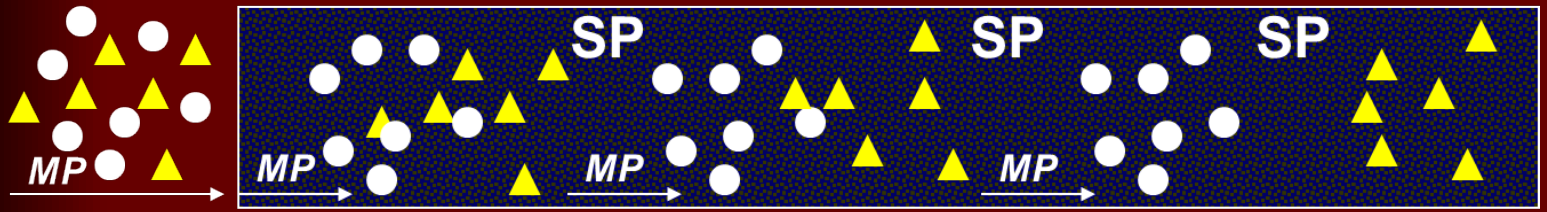
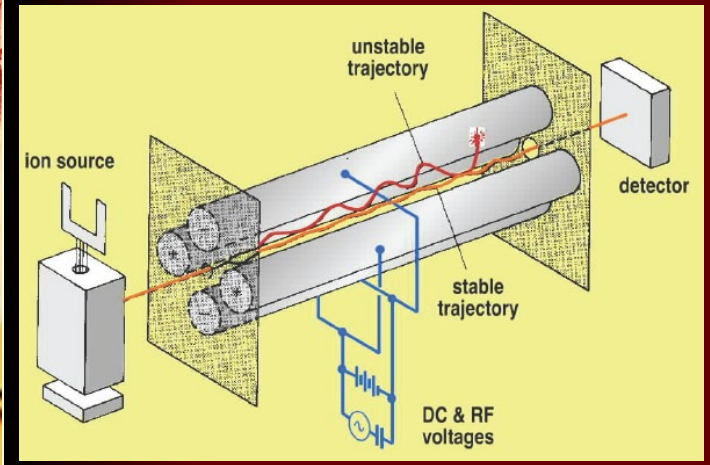
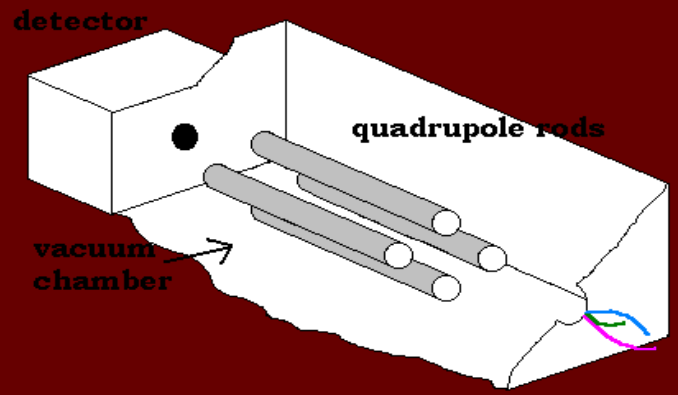


Figure 1: LC Principles

LCMS Method: Method development & validation





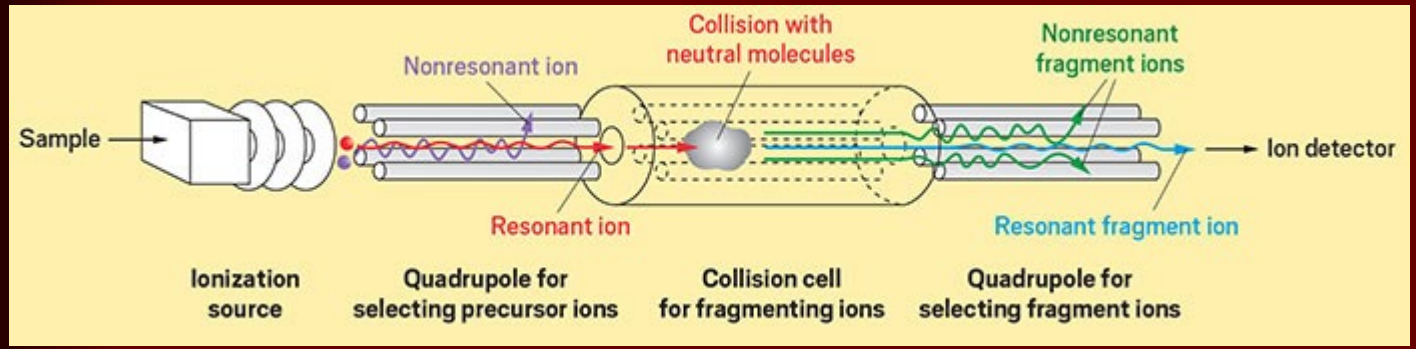
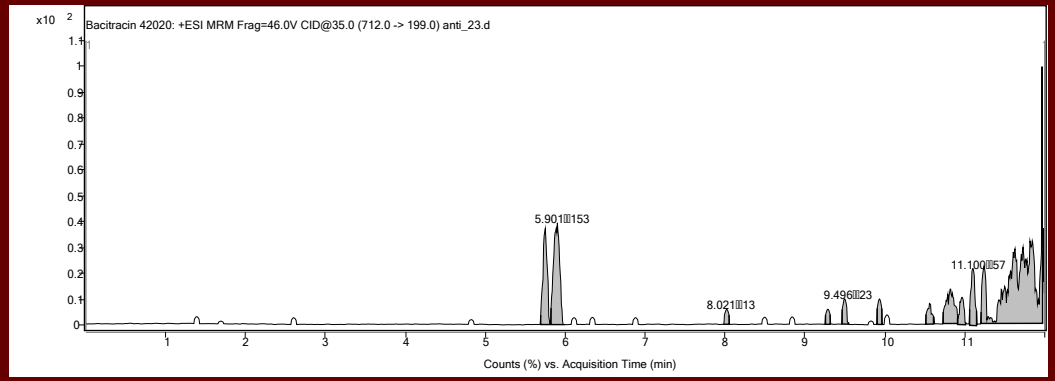


Figure 2: Quad MS Principles

LCMS Method: Method development & validation



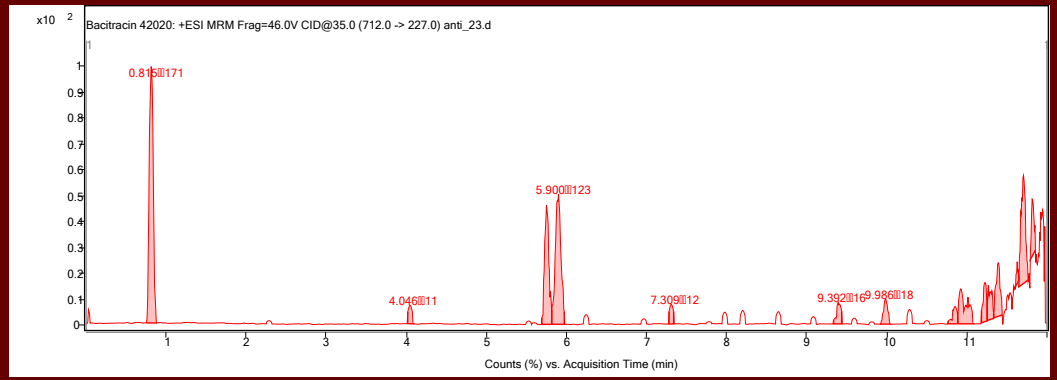
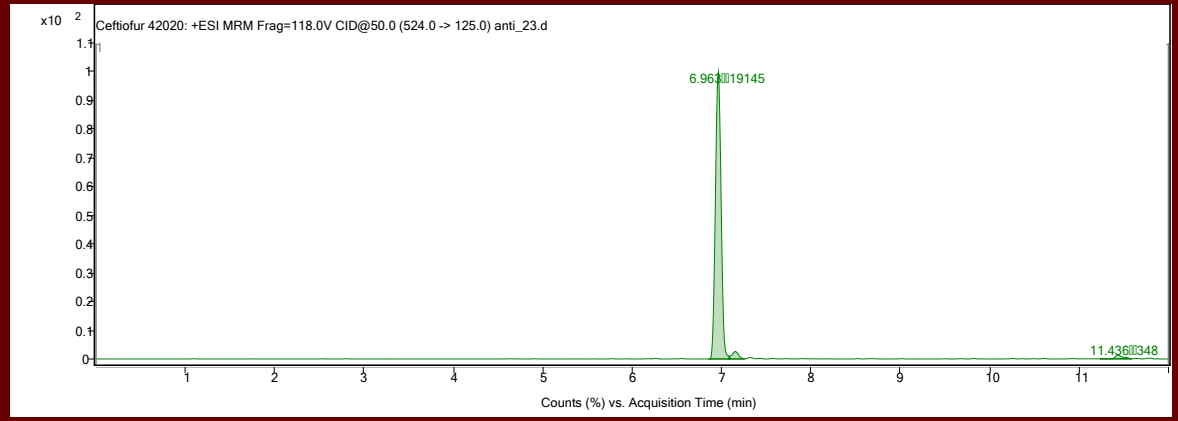


Figure 3: Bacitracin MRM fragmentation

LCMS Method: Method development & validation



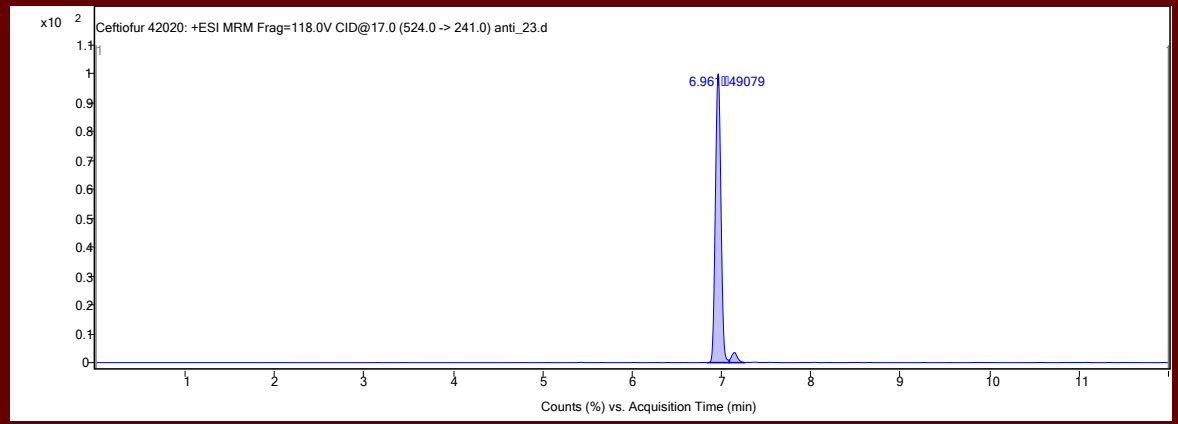


Figure 4: Ceftiofur MRM fragmentation

LCMS Method: Method development & validation

Acquisition Method Report

Agilent Technologies

Scan Segments

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
Tylosin	No	916.51	Unit/Enh (6490)	174	Unit/Enh (6490)					
Tylosin	No	916.51	Unit/Enh (6490)	101	Unit/Enh (6490)					
Virginiamycin S1	No	824	Unit/Enh (6490)	205	Unit/Enh (6490)					
Virginiamycin S1	No	824	Unit/Enh (6490)	177.2	Unit/Enh (6490)					
Narasin	No	787.4	Unit/Enh (6490)	531.2	Unit/Enh (6490)					
Narasin	No	787.4	Unit/Enh (6490)	431.2	Unit/Enh (6490)					
Bacitracin	No	712	Unit/Enh (6490)	227	Unit/Enh (6490)					
Bacitracin	No	712	Unit/Enh (6490)	199	Unit/Enh (6490)					
Ceftiofur	No	524	Unit/Enh (6490)	241	Unit/Enh (6490)					
Ceftiofur	No	524	Unit/Enh (6490)	125	Unit/Enh (6490)					
Gentamicin	No	478.3	Unit/Enh (6490)	322.2	Unit/Enh (6490)					
Gentamicin	No	468.2	Unit/Enh (6490)	163.1	Unit/Enh (6490)					
Tetracycline	No	445.2	Unit/Enh (6490)	410.1	Unit/Enh (6490)					
Tetracycline	No	445.2	Unit/Enh (6490)	154	Unit/Enh (6490)					
Nicarbazin	No	427.1	Unit/Enh (6490)	410.1	Unit/Enh (6490)					
Nicarbazin	No	427.1	Unit/Enh (6490)	154	Unit/Enh (6490)					
Lincomycin	No	407.5	Unit/Enh (6490)	359.2	Unit/Enh (6490)					
Lincomycin	No	407.5	Unit/Enh (6490)	126.1	Unit/Enh (6490)					
Enrofloxacin	No	360.2	Unit/Enh (6490)	342.1	Unit/Enh (6490)					
Enrofloxacin	No	360.2	Unit/Enh (6490)	286	Unit/Enh (6490)					
Penicillin G	No	335.1	Unit/Enh (6490)	128	Unit/Enh (6490)					
Penicillin G	No	335.1	Unit/Enh (6490)	91	Unit/Enh (6490)					
Ciprofloxacin	No	332.1	Unit/Enh (6490)	314.1	Unit/Enh (6490)					
Ciprofloxacin	No	332.1	Unit/Enh (6490)	231	Unit/Enh (6490)					
Sulfadimethoxine	No	311.1	Unit/Enh (6490)	156	Unit/Enh (6490)					
Sulfadimethoxine	No	311.1	Unit/Enh (6490)	92	Unit/Enh (6490)					
Ractopamine	No	302.2	Unit/Enh (6490)	164.1	Unit/Enh (6490)					
Ractopamine	No	302.2	Unit/Enh (6490)	107	Unit/Enh (6490)					
Sulfaquinoxaline	No	301.1	Unit/Enh (6490)	156	Unit/Enh (6490)					
Sulfaquinoxaline	No	301.1	Unit/Enh (6490)	92.1	Unit/Enh (6490)					
Zilpaterol	No	262	Unit/Enh (6490)	244	Unit/Enh (6490)					
Zilpaterol	No	262	Unit/Enh (6490)	185	Unit/Enh (6490)					
Sulfadiazine	No	251.1	Unit/Enh (6490)	156	Unit/Enh (6490)					
Sulfadiazine	No	251.1	Unit/Enh (6490)	108.1	Unit/Enh (6490)					
Sulfadiazine	No	251.1	Unit/Enh (6490)	92	Unit/Enh (6490)					
						10	94	33	4	Positive

Scan Parameters

Data Stg	Threshold
Centroid	0

MS QQQ Mass Spectrometer

Ion Source	AJS ESI	Tune File	atunes.tune.xml
Stop Mode	No Limit/As Pump	Stop Time (min)	1.0
Time Filter	On	Time Filter Width (min)	0.07

Time Segments

30	46	35	4	Positive
10	118	17	4	Positive
10	118	50	4	Positive
10	70	21	4	Positive
10	40	31	4	Positive
10	109	21	4	Positive
10	109	29	4	Positive
30	153	17	4	Positive
30	153	29	4	Positive
10	133	21	4	Positive
10	133	29	4	Positive
10	118	21	4	Positive
10	118	37	4	Positive
30	133	31	4	Positive
30	133	50	4	Positive
10	109	25	4	Positive
10	109	45	4	Positive
10	118	21	4	Positive
10	118	37	4	Positive
10	104	17	4	Positive
10	104	45	4	Positive
10	104	17	4	Positive
10	104	37	4	Positive
10	46	20	4	Positive
10	46	35	4	Positive
10	94	17	4	Positive
10	94	20	4	Positive
10	94	33	4	Positive

LCMS Method: Method development & validation

LOQ: 0.078 ppb

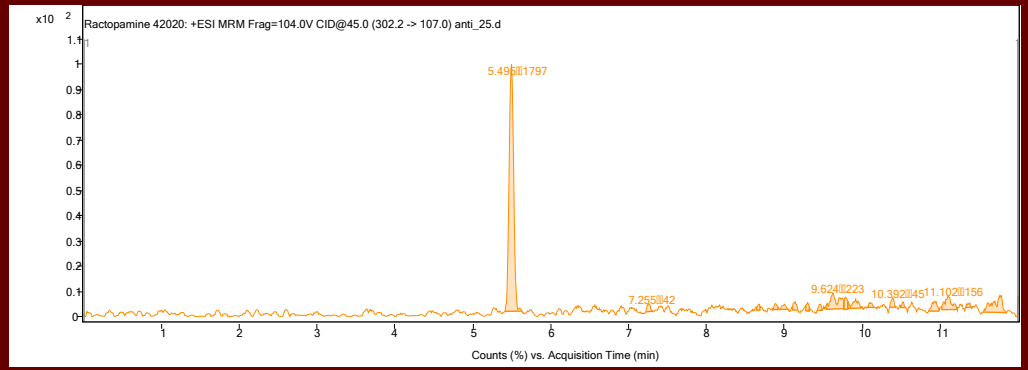
Calibration range: 0.078 to 200 ppb

LCMS Method: Method development & validation

Ractopamine

LOQ below 0.08 ppb

The MRLs are 10 ppb in pork and beef meat, 40 ppb in livers and 90 ppb in kidneys.



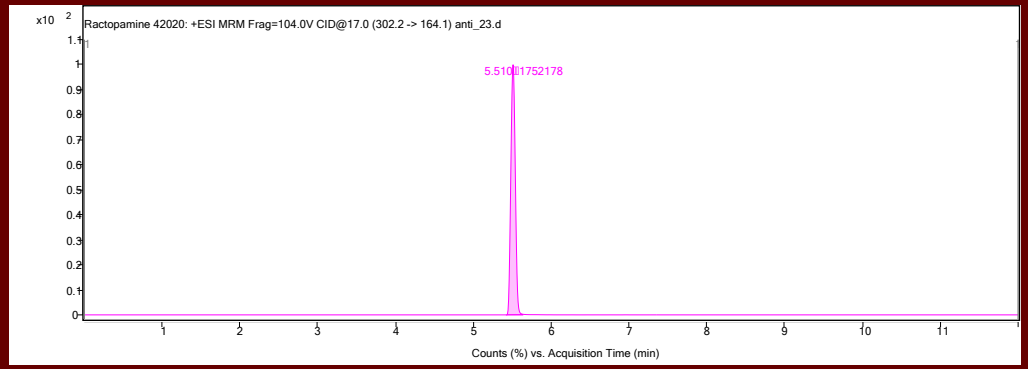


Figure 5: Ractopamine MRM fragmentation

Experimental Design

Materials: chicken meals, chicken meat, chicken fat

Matrix weight: 1 g

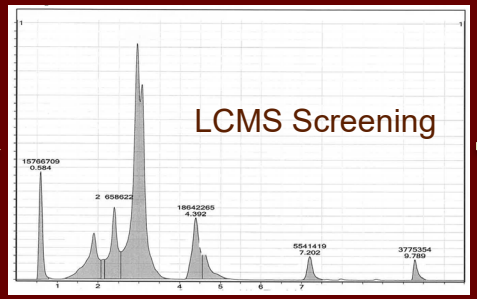
Added fat: 0.5 g (33%)

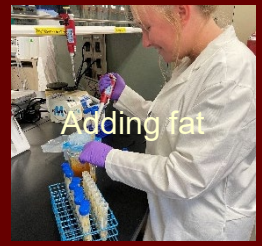
Spiking levels: 20 or 200 ppb using authentic standard mix

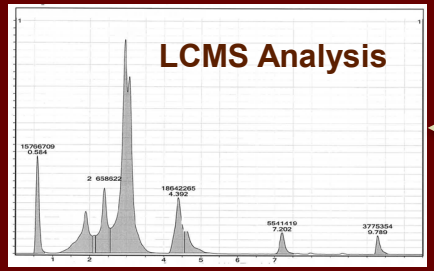
Cooking temperature: 95 or 105°C

Replication: minimum 5 per experiment

Experimental Design







Chicken meal

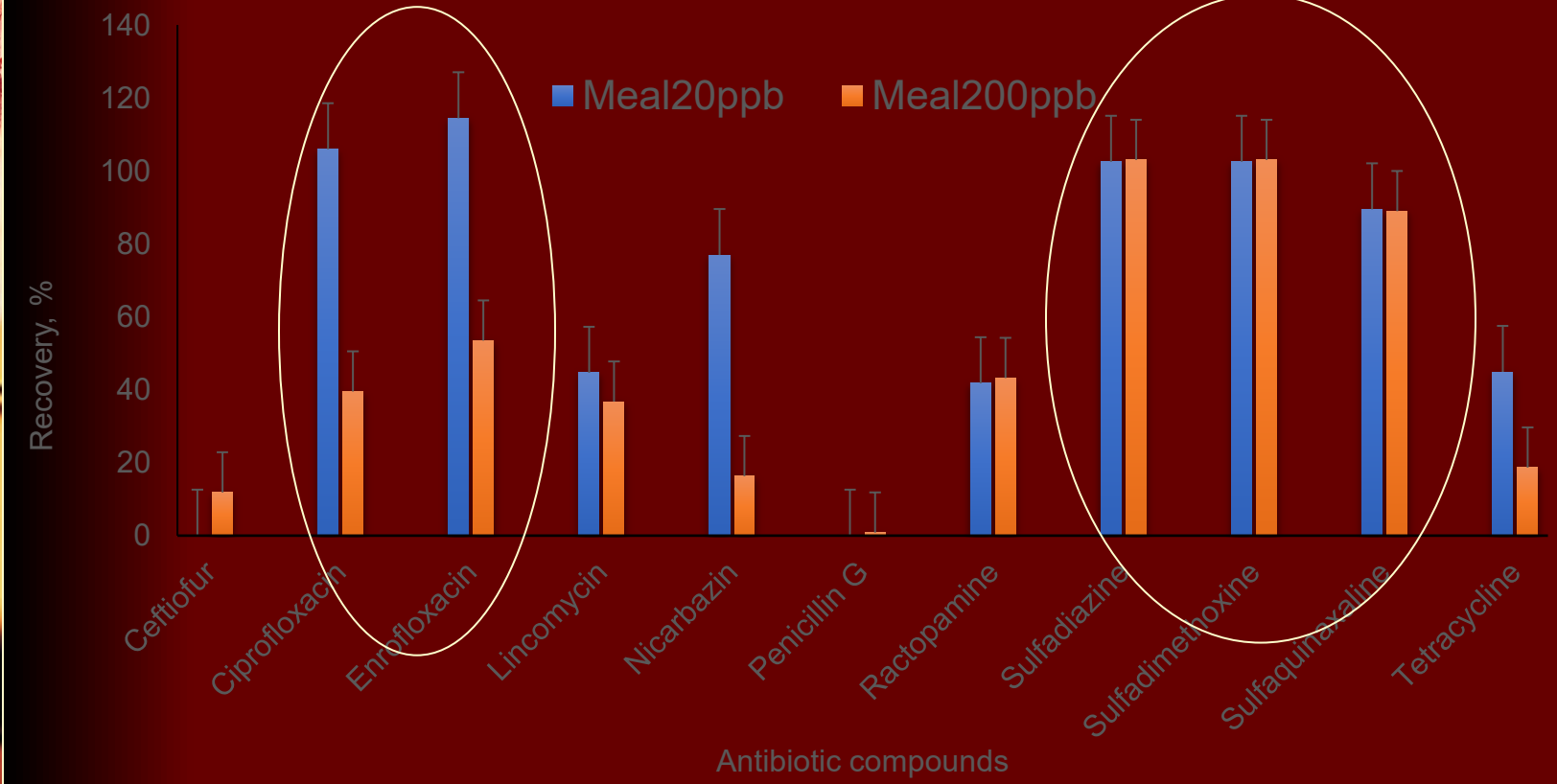


Figure 6: Recovery of antibiotics in heated chicken meal

Chicken meat only

1 g ground chicken breast meat
1 mL water
95°C in water bath
30 min

Chicken meat only, 20- and 200-ppb spiking

Antibiotics	Spiked level	Recovery, %	Loss, %
Bacitracin	200	Not Detected	100%
Bacitracin	20	Not Detected	100%
Ceftiofur	200	Not Detected	100%
Ceftiofur	20	Not Detected	100%
Ciprofloxacin	200	88.70	11.30
Ciprofloxacin	20	86.60	13.40
Enrofloxacin	200	112.90	N/A
Enrofloxacin	20	117.10	N/A
Gentamicin	200	Not reported	N/A
Gentamicin	20	Not reported	N/A
Lincomycin	200	110.80	N/A
Lincomycin	20	106.40	N/A
Narasin	200	17.90	82.10
Narasin	20	10.09	89.91
Nicarbizine	200	51.76	48.24
Nicarbizine	20	67.6	32.40
Penicillin G	200	Not reported	N/A
Penicillin G	20	Not reported	N/A
Ractopamine	200	80.3	19.70
Ractopamine	20	66.9	33.10
Sulfadiazine	200	84.7	15.30
Sulfadiazine	20	83.7	16.30
Sulfadimethoxine	200	111.5	N/A
Sulfadimethoxine	20	122.9	N/A
Sulfaquinaxaline	200	131.5	N/A
Sulfaquinaxaline	20	144.4	N/A
Tetracycline	200	56.61	43.39
Tetracycline	20	52.6	47.40
Tylosin	200	27.68	72.32
Tylosin	20	22.61	77.39
Virginiamycin	200	58.6	41.40
Virginiamycin	20	56.28	43.72

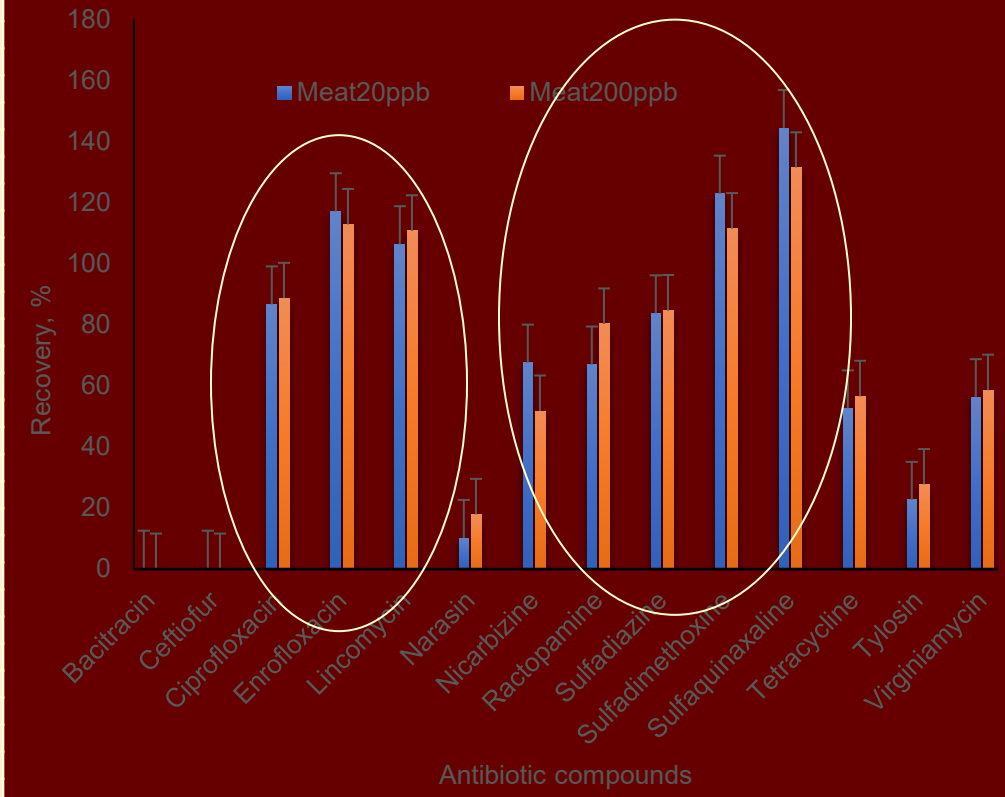


Figure 6: Recovery of antibiotics in heated chicken meat

Chicken meat + fat, 20-ppb spiking

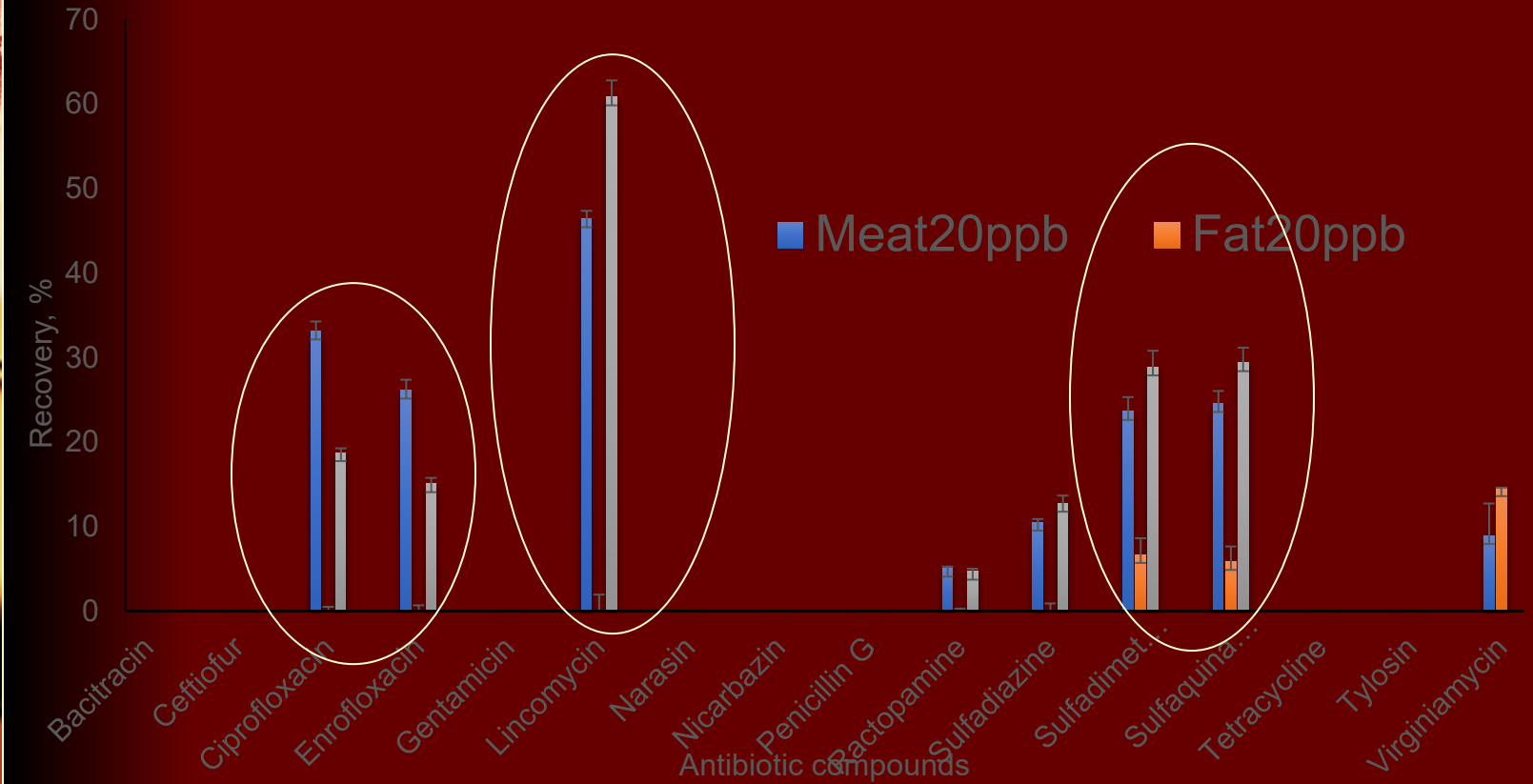


Figure 7: Recovery of antibiotics in heated chicken meat with added fat

Chicken meat + fat, 200-ppb spiking

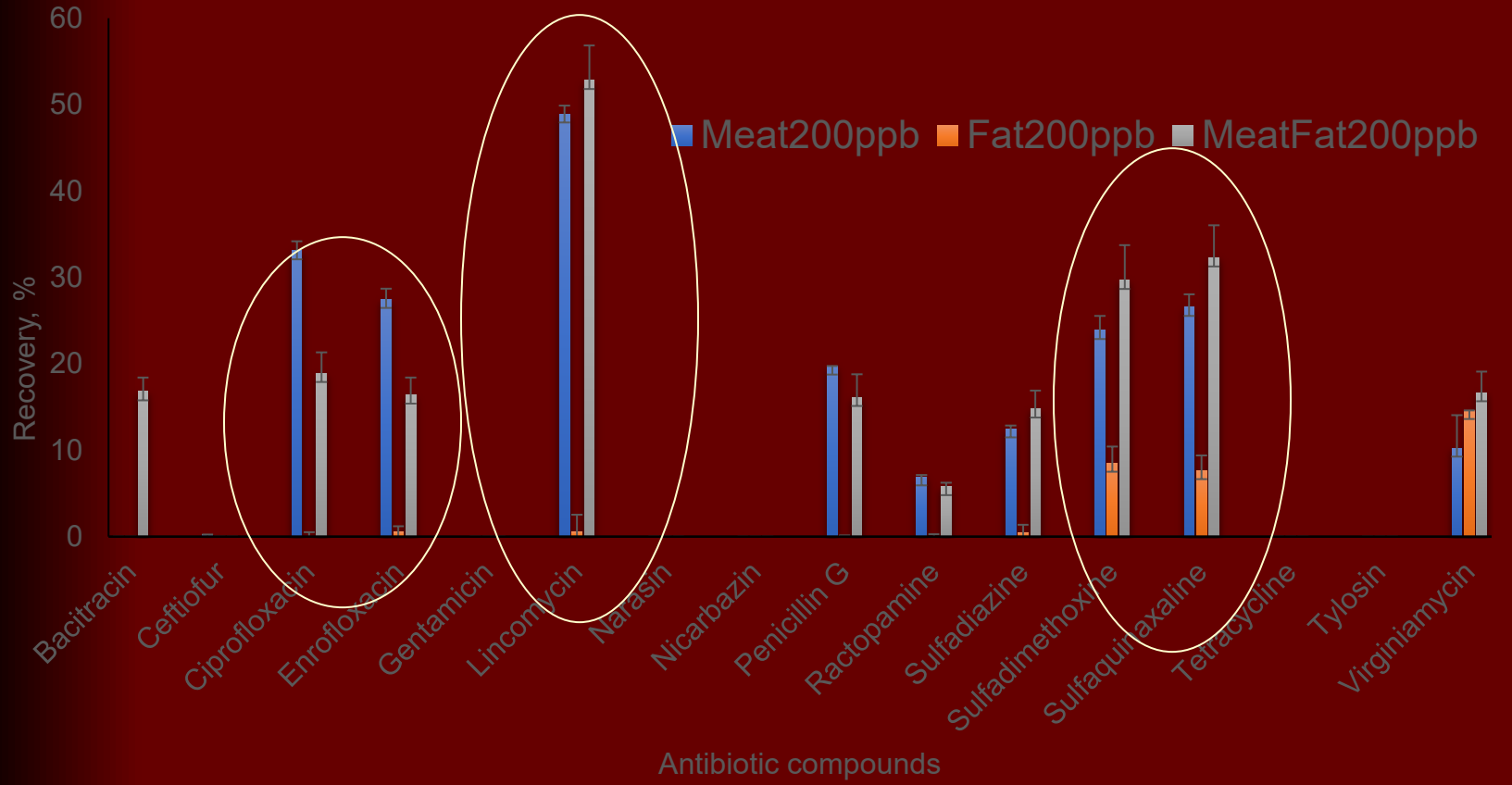


Figure 8: Recovery of antibiotics in heated chicken meat with added fat

Initial Thoughts

No residue detected in samples

Greater temperatures increase destruction

- Higher fat content increases destruction

Antibiotics compounds are not the same

- Greater water-solubility = greater destruction
- Low water-solubility = more migration into fat fraction

Major outcomes

Analytical methodology for antibiotics in rendered products

- LCMS method
- Extraction, cleanup
- LOD, LOQ – instrumental capability

Initial understanding of how rendering impacts antibiotics residues

- Recovery %
- Temperature
- Fat content
- Chemical nature of antibiotics compounds

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