LC/MS Applications for Drug Residues in Foods

Solutions Guide

General Description

Agilent Technologies has developed some key liquid chromatography/mass spectrometry (LC/MS) applications for the analysis of drug residues in foods.

This guide provides an overview of the analysis of these residues using LC/MS, including single quadrupole (SQ), triple quadrupole (QQQ), time-of-flight (TOF), and hybrid quadrupole time-of-flight (Q-TOF). The guide will be useful as a starting point for application development and as a way to easily order the LC/MS system. Application examples include detailed chromatographic and mass spectrometer conditions, MS performance data, and representative chromatograms. An appendix provides some general sample preparation procedures.

Agilent Technologies

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Introduction

Major Classes of Veterinary Drugs

Veterinary drugs are used in animal husbandry to cure diseases or prevent sickness. They can also promote growth, enhance feed efficiency, and keep animals calm during transport. However, all drugs that are administered to food-producing animals may lead to residues in the edible tissues, milk, or eggs. The presence of these residues may pose potential health risks, including allergic reactions, direct toxic effects, and a change in the resistance of bacteria exposed to antibiotics.

During the last several decades, many veterinary drugs were used around the world and some can still be detected in the food chain. The list below shows the major classes of veterinary drugs.

1. Antimicrobial drugs
    - Aminoglycosides and aminocyclitols
    - Amphenicols
    - β-Lactams (penicillins and cephalosporins)
    - Fluoroquinolones
    - Macrolides and lincosamides
    - Nitrofurans
    - Sulfonamides
    - Tetracyclines
2. Anthelmintic drugs
    - Benzimidazoles
    - Imidazothiazoles
    - Organophosphates
    - Tetrahydropyrimidines
    - Salicylanilides
    - Substituted phenols
    - Macrocyclic lactones
    - Piperazine derivatives
3. Antiprotozoal (anticoccidial) drugs
    - Benzamides
    - Carbanilides
    - Nitroimidazoles
    - Polyether ionophores
    - Quinolone derivatives
    - Triazines
4. Antimicrobial growth promoters
    - Organic arsenicals
    - Peptide antibiotics
    - Quinoxaline-1,4-dioxides
5. Hormonal-type growth promoters
    - Endogenous sex steroids
    - Synthetic steroidal compounds
    - Synthetic nonsteroidal compounds
    - Polypeptide hormones
6. Other drugs
    - Antifungal drugs
    - β-Adrenergic agonists
    - Corticosteroids
    - Diuretic drugs
    - Dye drugs
    - Nonsteroidal anti-inflammatory drugs (NSAIDs)
    - Sedatives and β-blockers
    - Thyreostatic drugs

Many countries, such as the United States and those in the European Union, have set maximum residue limits (MRLs) for different foods. Japan also has set up MRLs for compounds included in the Japanese Positive List. Recently, China has defined some new national standards to monitor banned antibiotics in foods.

Analytical Challenges in Drug Residue Analysis

There are two main challenges analysts face when testing for drug residues in food matrices:

1. Analyte Diversity
   Because the list of possible residues that must be analyzed is so long, there are significant differences in their characteristics, which impact the chromatographic separation and detection.
   - Physical-chemical properties (for example, polarity and pKa)
   - Stability

2 Matrix Diversity
   Veterinary drugs are more difficult to analyze than pesticides using a single method, and tissues are more difficult matrices than fruits and vegetables. The matrix may be muscle, kidney, liver, milk, eggs, urine, blood, etc.
   - Matrix composition (for example, lipids, proteins, carbohydrates, water, and salts)
   - Analyte-matrix interactions
   - Matrix coextractives

The growing number of veterinary drug residues to be tested in food laboratories is hastening the search for high-throughput techniques that are more effective in tackling large numbers of samples in a limited time. Several liquid chromatography/mass spectrometry (LC/MS) methods have been developed for the analysis of multi-class veterinary drug residues in different food matrices such as eggs, shrimp, and animal muscle. Because sample extraction and cleanup are the rate-determining steps in drug analyses, the combination of solid-phase extraction (SPE) with chromatography coupled with mass spectrometry or other spectroscopic techniques is widely used in modern food analysis.

Overview of LC/MS Technologies – Which Technique to Use

Sample preparation	Instrument options	Screening	Confirmation	Quantitation
SPE or QuEChERs • Extraction • Cleanup	LC/QQQ Target, trace analysis in the "dirty" or complex matrix 	– Highly selective, sensitive detection of a large number of compounds in a single injection – Low detection limits in complex matrices – Novel Agilent Jet Stream technology improves sensitivity	– MRM provides positive confirmation and low detection limits to meet all regulatory requirements – One-run screening and confirmation	– Routine, high-accuracy MS/MS quantification – Batch-at-Glance for fast view of quantification results
	LC/Q-TOF or LC/TOF Nontarget, unknown compounds analysis 	– High resolution reduces noise and matrix interference – Accurate mass provides added compound selectivity and unlimited screening using exact mass databases – Novel Agilent Jet Stream technology improves sensitivity	– Automated, accurate mass searches against public and private databases – Molecular Formula Generator feature reduces data interpretation time	– Very narrow mass window for extracted ion – Chromatograms provide precise MS and MS/MS quantification – High dynamic range to quantitate a wide range of concentrations
	LC/SQ Screening and quantification in the clean matrix 	– Selective detection of a large number of compounds in a single injection – Low detection limits in simple matrices – Complementary structure information with DAD and FLD		– High dynamic range to quantitate a wide range of concentrations

1
Determination, confirmation, and quantitation of trace ß-lactam antibiotics in milk using the Agilent 6410 LC/MS/MS

Abstract

The ß-lactam antibiotics are widely used in veterinary medicine for the treatment and prevention of disease. This use can result in the presence of residues in milk and edible tissues which can lead to problems in the fermentation processes and to health problems for individuals who are hypersensitive to ß-lactams.

Sample Preparation Procedure

1. **Pipet** 2 mL milk and mix with 4 mL acetonitrile.
2. **Vortex** to complete protein precipitation.
3. **Centrifuge** at 500 rpm for 10 min and filter (0.45 µm).
4. **Inject** 20 µL.

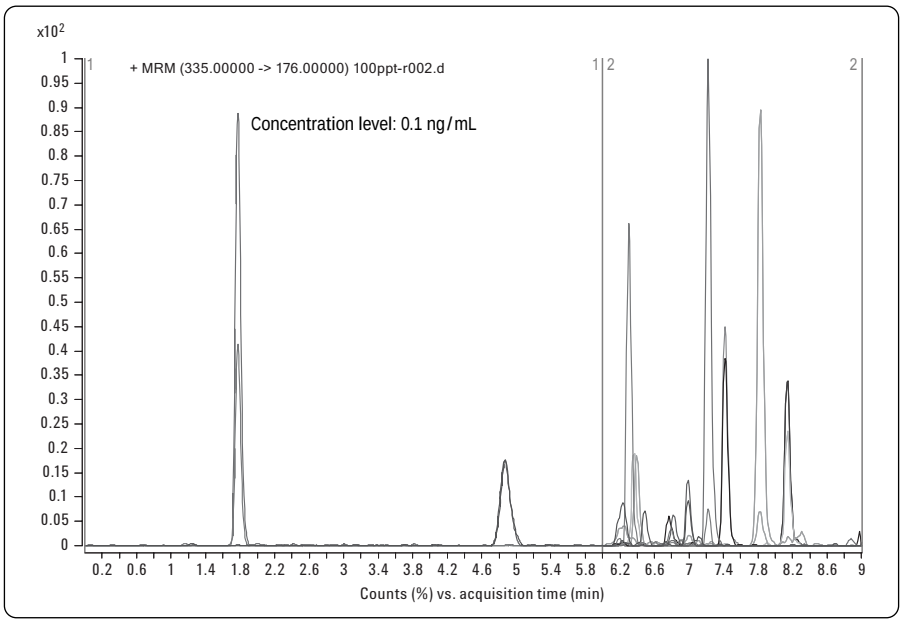
Instrument Settings

LC Conditions	
Column	Agilent ZORBAX SB-C18, 2.1 × 150 mm, 3.5 µm
Mobile phase	A: water/0.3% acetic acid B: acetonitrile/0.3% acetic acid
Flow rate	0.3 mL/min
Gradient	0–2 min/A 90% 2.01–8 min/A 35% 8.01–9/A 5%
Column compartment temperature	30 °C
Stop time	9 min
Post time	6 min
Injection volume	10 µL
MSD Conditions	
Instrument	Agilent 6410 triple quadrupole LC/MS system
Source	ESI +

MRM Settings

TS	Compound	Precursor	Product	Dwell (ms)	Frag (V)	CE (V)
0	Amoxicillin	366	114	100	110	15
			208	100	110	5
	Ampicillin	350	160	100	100	5
			192	100	110	10
6	Dicloxacillin	470	311	70	110	10
			160	70	110	10
	Nafcillin	415	256	70	110	15
				70	110	10
	Oxacillin	402	243	70	110	10
			160	70	110	10
	Penicillin V	351	192	70	100	5
			160	70	100	10
	Penicillin G	335	176	70	100	10
			160	70	100	5

Results



LC/MS/MS Method Performance

Compounds	S/N*	R^2
Amoxicillin 366-114	224	0.992
Ampicillin 350-160	61.6	0.984
Dicloxacillin 470-160	48.5	0.981
Nafcillin 415-199	52.6	0.998
Oxacillin 402-160	70.9	0.993
Penicillin V 351-160	225.9	0.998
Penicillin G 335-160	33.2	0.981

* These results were obtained in the real milk matrix spiked at 0.1 ppb each.

Ordering Information

Description	Part number
Agilent LC/MS/MS	G6410A or G6460AA
Agilent ZORBAX SB-C18, 2.1 × 150 mm, 3.5 µm	830990-902
13mm, 45 µm PTFE syringe filter	5185-5836

References

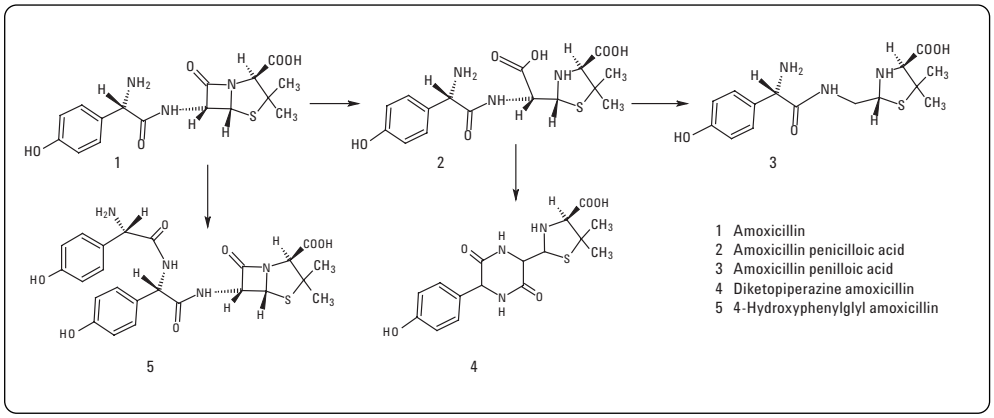
Agilent Technologies publication 5990-3722EN, “Determination, Confirmation, and Quantification of Trace β-Lactam Antibiotics in Milk by 6410 LC/MS/MS”

Agilent Technologies publication 5990-3364EN, “Determination of Penicillins in Meat by HPLC/UV and HPLC/MS/MS”

2
Structure elucidation of degradation products of amoxicillin by LC/TOF

Abstract

The confirmation of degradation products from a final dosage of the antibiotic drug amoxicillin obtained under stress conditions is demonstrated by accurate mass measurement and molecular formula confirmation for the molecular ions and their in-source CID fragments by LC/ESI TOF.



Sample Preparation Procedure

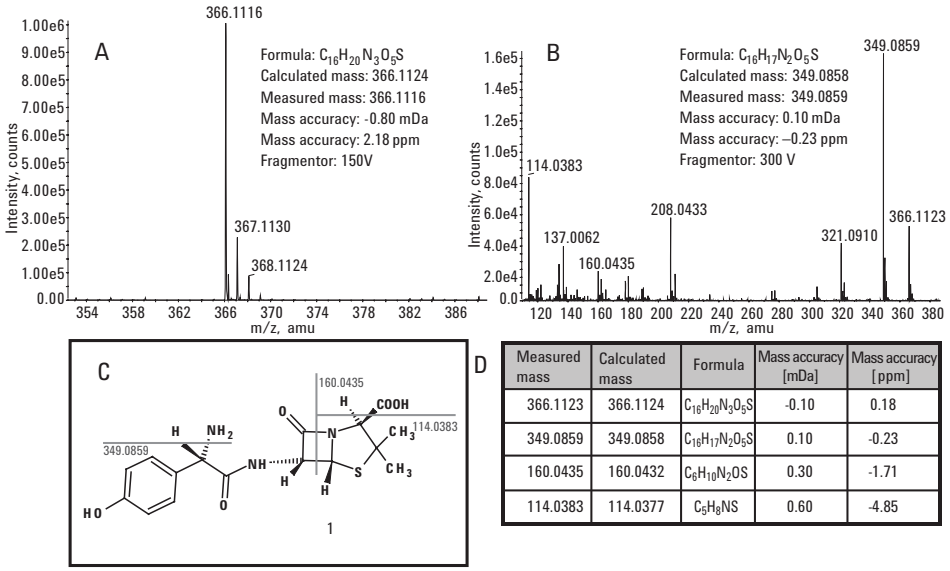
The antibiotic amoxicillin was stressed under acidic conditions.

1. **Add** approximately 1 mL of amoxicillin solution (25 mg/mL in DMSO) to 1 mL 0.1 M HCl solution.
2. **Stir** the sample for 1 hour at room temperature (RT = 25 ºC).
3. **Dilute** 1:10 with 20 mL of DMSO.

Instrument Settings

LC Conditions	
Agilent 1100 Series capillary pump	
Solvent A	Water, 10 mM ammonium formate, pH 4.1
Solvent B	ACN
Column flow	8 µL/min
Primary flow	500 – 800 µL/min
Gradient	0 min 0% B
	1 min 0% B
	13 min 25% B
	23 min 25% B
Stop time	23 min
Post time	15 min
MSD Conditions	
Source	ESI(+)
Drying gas	7.0 L/min @ 300 °C
Nebulizer	15 psi
Fragmentor	150 V or 300 V for CID

Results



Measured mass	Calculated mass	Formula	Mass accuracy [mDa]	Mass accuracy [ppm]
366.1123	366.1124	$C_{16}H_{20}N_3O_5S$	-0.10	0.18
349.0859	349.0858	$C_{16}H_{17}N_2O_5S$	0.10	-0.23
160.0435	160.0432	$C_6H_{10}N_2OS$	0.30	-1.71
114.0383	114.0377	C_5H_8NS	0.60	-4.85

Note: Amoxicillin penicilloic acid (2), ($C_{16}H_{21}N_3O_6S$), $[M+H]^+$ = 384.1229 *m/z*

A) Accurate mass measurement of the molecular ion of amoxicillin penicilloic acid

B) Accurate mass measurement of the CID fragment ions of amoxicillin penicilloic acid

C) Structural CID fragmentation of amoxicillin penicilloic acid

D) Mass accuracy of all CID fragments of amoxicillin penicilloic acid

Ordering Information

Description	Part number
Agilent LC/MS TOF	G6224AA or A6230AA

References

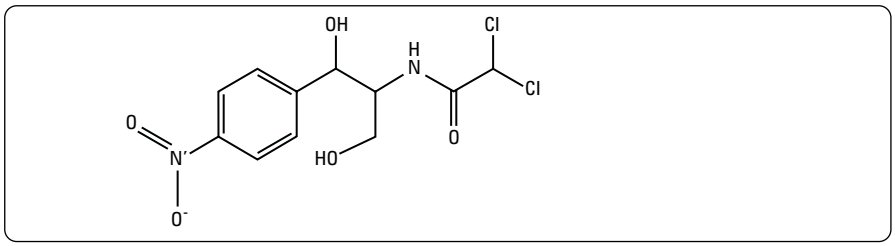
Agilent Technologies publication 5989-2348EN, “Structure Elucidation of Degradation Products of the Antibiotic Drug Amoxicillin Part 2: Identification and Confirmation by Accurate Mass Measurement With ESI TOF of the Compound Ions and the Fragments After CID”

Agilent Technologies publication 5989-3561EN, “Statistic Evaluation of Mass Accuracy Measurements by ESI-TOF with a Sample of Degradation Products from the Antibiotic Drug Amoxicllin”

3
Chloramphenicol in honey, shrimp, and chicken using the Agilent 6410 LC/MS/MS

Abstract

A method for chloramphenicol in honey, shrimp, and chicken was developed using the Agilent 6410A triple quadrupole (QQQ) LC/MS System. The sensitivity obtained exceeds the minimum required performance level (MRPL) established by the European Union regulation for food monitoring programs. Using a deuterated internal standard and one simple solid phase extraction (SPE) procedure can provide a limit of detection of 10 ppt in the sample matrix.



Sample Preparation Procedure

1. **Internal Standard**

   Prepare standard solutions: 1, 0.2, 0.1, 0.02, and 0.01 ppb, with IS (CAP-d5) at 0.25 ppb level.

   Honey

   1. **Dilute** 1 g of sample to 5 mL with water. Add 25 µL of 10 ppb IS.
   2. **Load** the solution onto the SPE cartridge and allow to stand for 5 min.
   3. **Elute** the sample with 10 mL ethyl acetate.
   4. **Collect** the eluate and evaporate the solvent under a nitrogen stream at 40 °C.
   5. **Dissolve** the residue in 1 mL of methanol and put in an ultrasonic bath for 1 minute.
   6. **Filter** the solution for injection.

Instrument Settings

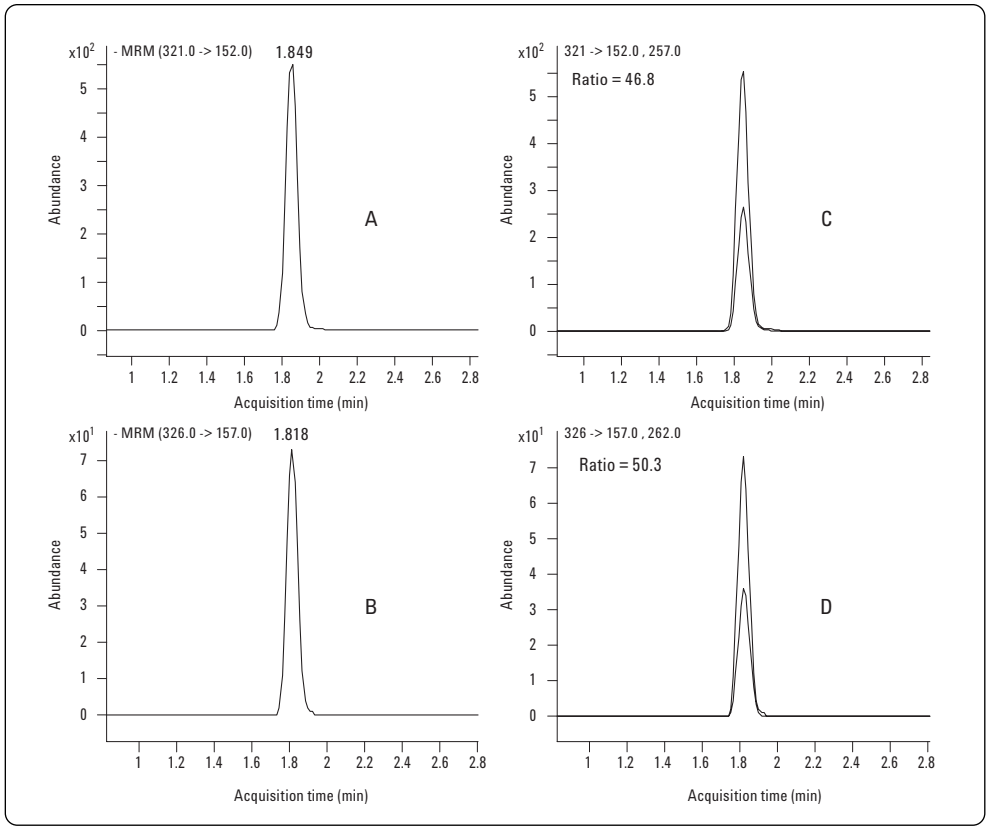
LC Conditions

Column	Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8 µm (p/n 827700-902)
Flow rate	0.4 mL/min
Mobile phase	A: water; B: methanol
Gradient	0–5 min, 30~70% B 5–6 min, 70~100% B 8 min, 100% B
Post time	4 min
Temperature	45 °C
Injection	5 µL

MSD Conditions

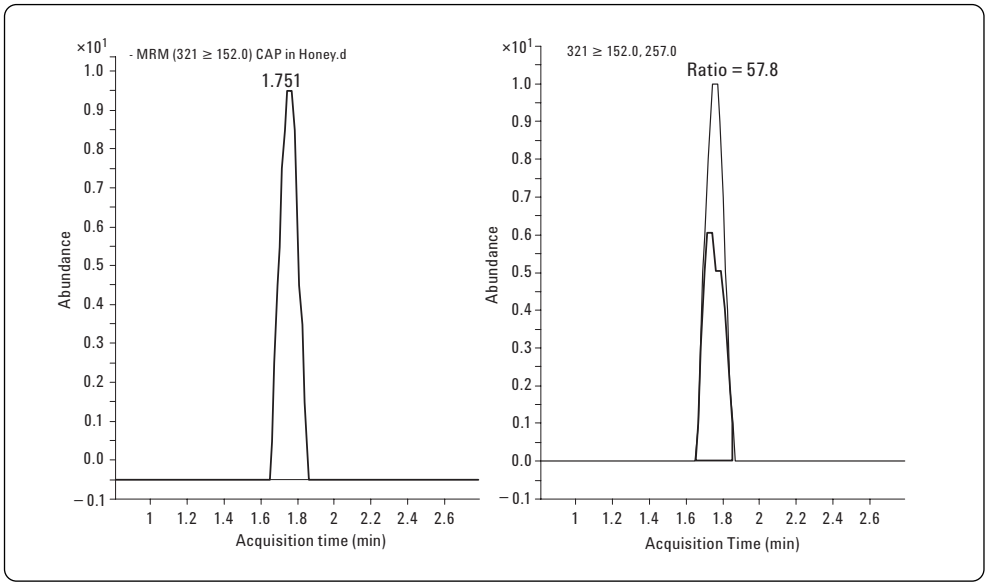
Source	ESI
Ion polarity	Negative
Drying gas	10 L/min @ 350 °C
Nebulizer	45 psi
V_{cap}	3500 V
Fragmentor	100 V
Collision energy	10 V for *m/z* 257 (qualifier ion) 15 V for *m/z* 152 (quantitation ion)

Results



Sensitivity

Standard in water and 10 ppt/10 µL injection



Ordering Information

Description	Part number
Agilent LC/MS/MS	G6410B or G6460AA
Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8 µm	827700-902
Agilent AccuBond SPE ENV PS DVB Cartridges	188-3060

Reference

Agilent Technologies publication 5989-5975EN, “Detection, Confirmation, and Quantification of Chloramphenicol in Honey, Shrimp, and Chicken Using the Agilent 6410 LC/MS Triple Quadrupole”

4
Using LC/MS/MS 6410 for Analysis of Chloramphenicol, Thiamphenicol, and Florfenicol in Fish Samples

Abstract

Chloramphenicol (CAP), thiamphenicol (TAP), and florfenicol (FF) are detected in a single run with very high sensitivity and selectivity using the Agilent 6410A triple quadrupole LC/MS system in negative ion mode .

Sample Preparation

See Chloramphenicol Sample Preparation on page 11.

Instrument Settings

LC Conditions

Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm
Mobile phase	A: water; B: acetonitrile
Flow rate	0.4 mL/min
Gradient	0–2min/B 10–90% 2–3min/B 90% 3.01/B 10%
Stop time	6 min
Column compartment temperature	45 °C
Injection volume	5 µL

MSD Condition

6410A ESI (–)

MRM Setting

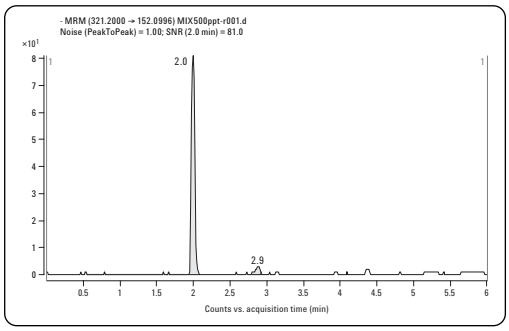
Name	Precursor ion	Product ion	Frag (V)	CE (V)	Dwell (ms)
TAP	354.1	185.1*	120	20	60
	354.1	289.9	120	10	60
	354.1	227.1	120	10	60
FF	356	185.1*	120	20	60
	356	335.8	120	5	60
	356	219.1	120	5	60
CAP	321.2	152.1*	120	10	60
12	321.2	176.2	120	10	60
	321.2	257.1	120	5	60
D5-CAP (ISTD)	326.2	157.2	130	15	60
	326.2	262.2	130	5	60

Results

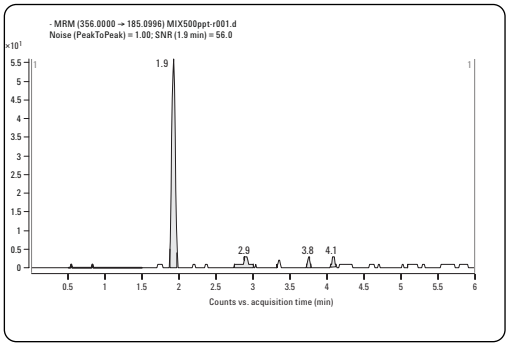
Name	**Linearity** (0.5–20 ppb)
TAP	0.994
FF	0.992
CAP	0.994

Sensitivity

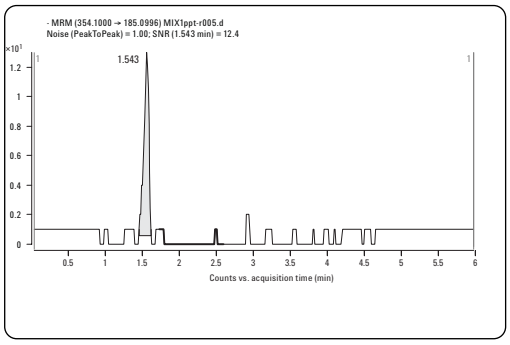
1. CAP: 0.5 ppb S/N = 81



2. FF: 0.5 ppb S/N = 56



3. TAP: 1 ppb, S/N = 12



Ordering Information

Description	Part number
Agilent LC/MS/MS	G6410A or G6460AA
Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm	959741-902
Agilent AccuBond SPE ENV PS DVB Cartridges	188-3060

Reference

Agilent Technologies publication 5990-3734EN, “Using LC/MS/MS 6410 for Analysis of Chloramphenicol, Thiamphenicol, and Florfenicol in Fish Samples”

5
Chloramphenicol in fish meat by LC / APPI / MS

Abstract

This example shows the determination of chloramphenicol in fish meat by liquid chromatography / atmospheric pressure photoionization / mass spectrometry (LC / APPI / MS).

Sample Preparation

1. **Weigh** 5 g fish meat and 5 g anhydrous sodium sulfate.
2. **Add** 10 mL ethyl acetate.
3. **Homogenize** the sample for 20 seconds.
4. **Centrifuge** the sample for 5 min at 6000 rpm.
5. **Remove** supernatant and repeat steps 2 to 4.
6. **Combine** the two ethyl acetate extracts and take to dryness with a rotovap at 40 °C.
7. **Reconstitute** with 1 mL acetonitrile, add 1 mL hexane, shake and discard the hexane layer.
8. **Add** another 1 mL hexane, shake, and discard the hexane layer.
9. **Take** the acetonitrile to dryness and reconstitute with 10 % acetonitrile in 10 mM aqueous ammonium acetate.
10. **Filter** into autosampler vial.

Instrument Settings

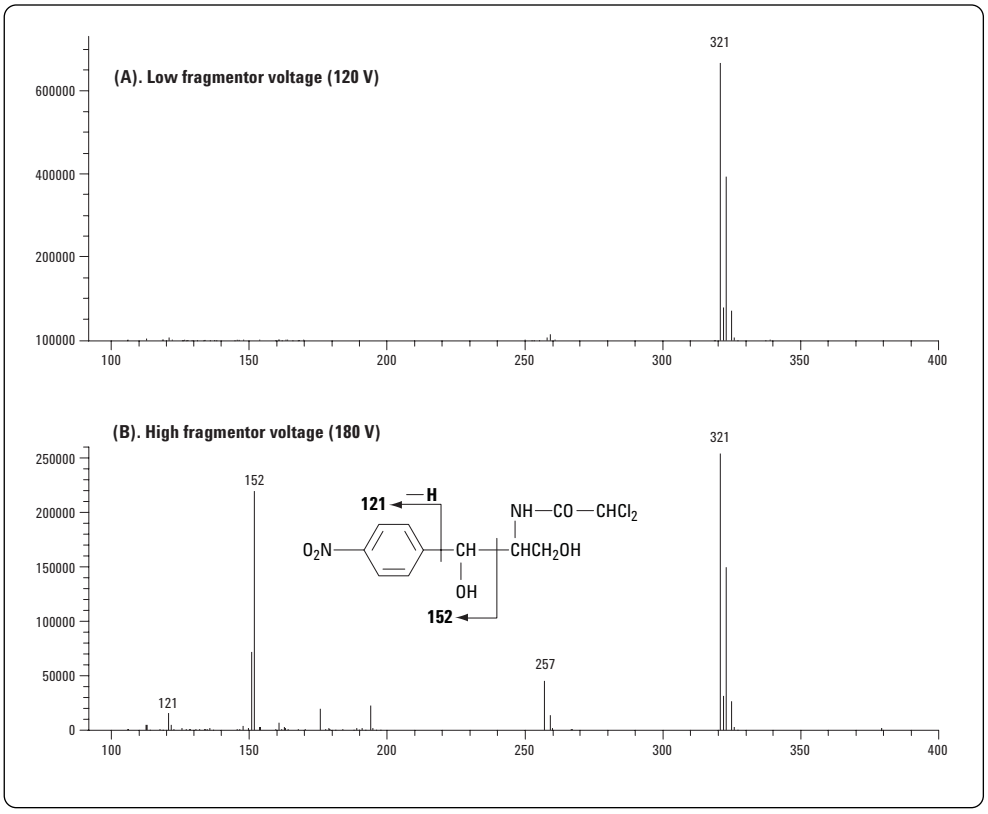
LC Conditions

Column	Agilent ZORBAX Eclipse XDB C18, 3 × 150 mm, 5 µm
Solvent A:	Water with 10 mM ammonium acetate
Solvent B:	Methanol
Dopant:	Acetone at 0.05 mL / min
Gradient:	90 / 10 A / B 15 min to 70 / 30 A / B
Column temperature:	40 °C
Sample volume:	20 µL
Flow rate:	0.5 mL / min

MSD Conditions

Ionization:	APPI (Negative)
Scan range:	100–400 *m/z* for optimization
SIM ion:	321 *m/z*; $(M\text{-}H)^-$
Drying gas:	Nitrogen, 7 L / min @ 350 °C
Nebulizer gas:	Nitrogen, 50 psi
Fragmentor:	120 V
Capillary:	3500 V
Vaporizer temperature:	350 °C

Results



Recovery of Chloramphenicol for Spiked Fish Meat

Spiking levels (ng/g)	Recovery [±RSD (%)] Young yellowtail	Flatfish
0.1	89.3 ± 5.1	87.4 ± 6.1
0.5	102.5 ± 4.9	94.8 ± 6.7
2.0	96.1 ± 4.3	91.8 ± 4.9

Ordering Information

Description	Part number
Agilent LC/MSD	G6120AA, G6130AA, or G6140AA
APPI source	G1971B
Agilent ZORBAX Eclipse Plus C18, 3 × 150 mm, 5 µm	993967-302

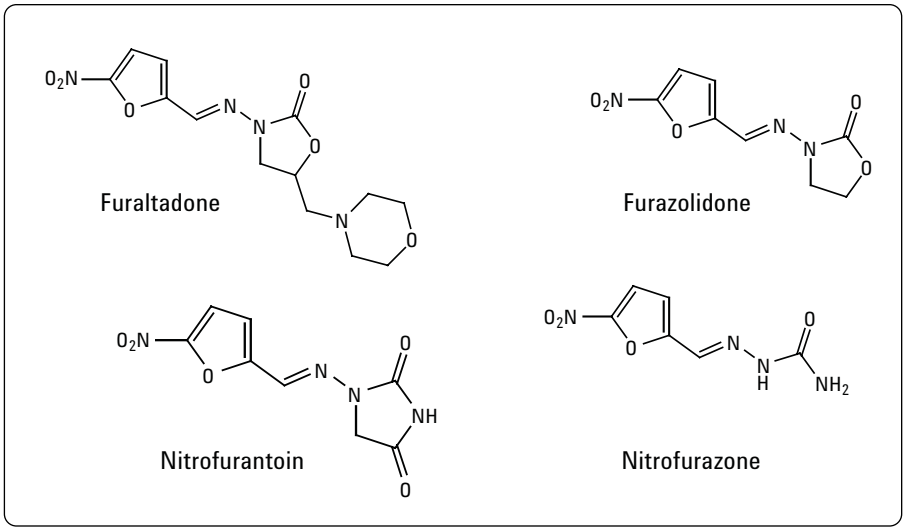
Reference

Agilent Technologies publication 5988-8999EN, “Determination of Chloramphenicol in Fish Meat by Liquid Chromatograph-Atmospheric Pressure Photo Ionization-Mass Spectrometry (LC-APPI-MS)”

6
Determination of the metabolites of nitrofurans in chicken tissue using the Agilent 6410A LC/ESI/MS

Abstract

The metabolites of nitrofuran antibacterial drugs in chicken tissue are measured by liquid chromatrograph/electrospray ionization/mass spectrometry (LC/ESI/MS). (Shown below are the nitrofuran drugs, not their metabolites.)



Sample Preparation

1. **Prepare** four metabolite solutions in water (12.5, 25.0, 37.5, and 50 µL at 100 ng/mL).
2. **Transfer** the samples to separate 40-mL glass vials with screw-top caps.
3. **Add** 10 mL of 125 mM HCl in water, and 200 µL of 50 mM 2-NBA in DMSO to each vial cap.
4. **Maintain** the reaction mixtures in a water bath at 37 °C for 16 hours.
5. **Cool** the solutions to room temperature.
6. **Adjust** the pH 7.4 by adding 0.1 M aqueous $KHPO_4$ and 0.8 M aqueous NaOH.
7. **Extract** the samples with ethyl acetate and dry with a nitrogen stream.
8. **Reconstitute** the samples in 1.0 mL initial mobile phase and inject.

Instrument Settings

LC Conditions	
LC	Agilent 1100 Series LC
Column	Inertsil ODS3, 2.1 × 150 mm, 5 µm (GL Science, Tokyo, Japan)
Solvent A	Acetonitrile
Solvent B	Aqueous 0.5% formic acid
Gradient	20/80A/B to 70/30 A/B in 20 min
Column temperature	20 °C
Sample volume	30 µL
Flow rate	200 µL/min

MSD Settings	
MS	Agilent 1100 MSD, SL
Ionization	ESI (positive)
Scan range	100–500 *m/z* for optimization
SIM ion	Base peak for quantitation
Drying gas	Nitrogen, 10 L/min @ 350 °C
Nebulizer gas	Nitrogen, 50 psi
Fragmentor	120 or 140 V
V_{cap}	2000 V

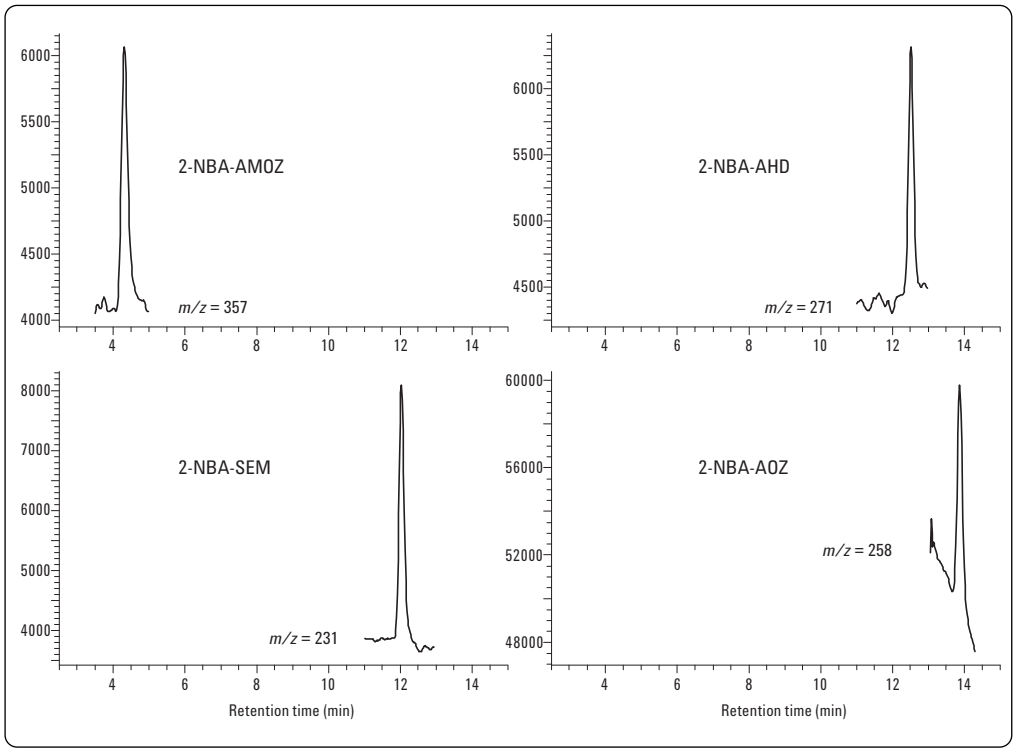
Results

Linearity, LOD, and Instrument Precision of Metabolites in Aqueous Solutions

Metabolites	R^2	LOD (ng/mL)	RSD 1 day	RSD 3 day
AMOZ	0.9999	0.04	5.0	7.3
SEM	0.9998	0.02	4.7	8.1
AHD	0.9989	0.06	4.9	7.9
AOZ	0.9997	0.06	3.1	8.2

Results

SIM chromatograms of aqueous 2-NBA nitrofuran metabolites solution at 0.25 ng/mL



Ordering Information

Description	Part number
Agilent LC/MSD	G6110AA, G6120AA, G6130AA, or G6140AA

Reference

Agilent Technologies publication 5988-8903EN, “Determination of the Metabolites of Nitrofuran Antibacterial Drugs in Chicken Tissue by Liquid Chromatograph/Electrospray Ionization/Mass Spectrometry (LC/ESI/MS)”

7
Analysis of nitrofuran metabolites in tilapia by LC/MS/MS

Abstract

The metabolites of nitrofuran antibiotics banned in meat and meat products are analyzed by LC/MS/MS with the new Agilent 6410 triple quadrupole. The method is shown to be highly sensitive, to 0.01 ppb (10 ppt), for each of the four analytes. Calibration from 0.1 ppb to 10 ppb is presented with all criteria for confirmation as set by the European Union decisions for analytical method performance.

Instrument Settings

LC Conditions

Column	Agilent ZORBAX C18, 2.1 × 150 mm, 3 µm
Column temperature	40 °C
Mobile phase	A: 0.1% formic acid in water B: acetonitrile
Gradient	22% B at 0 min 99% B at 6 min 99% B at 9 min
Flow rate	0.3 mL/min
Injection volume	50 µL

MSD Conditions

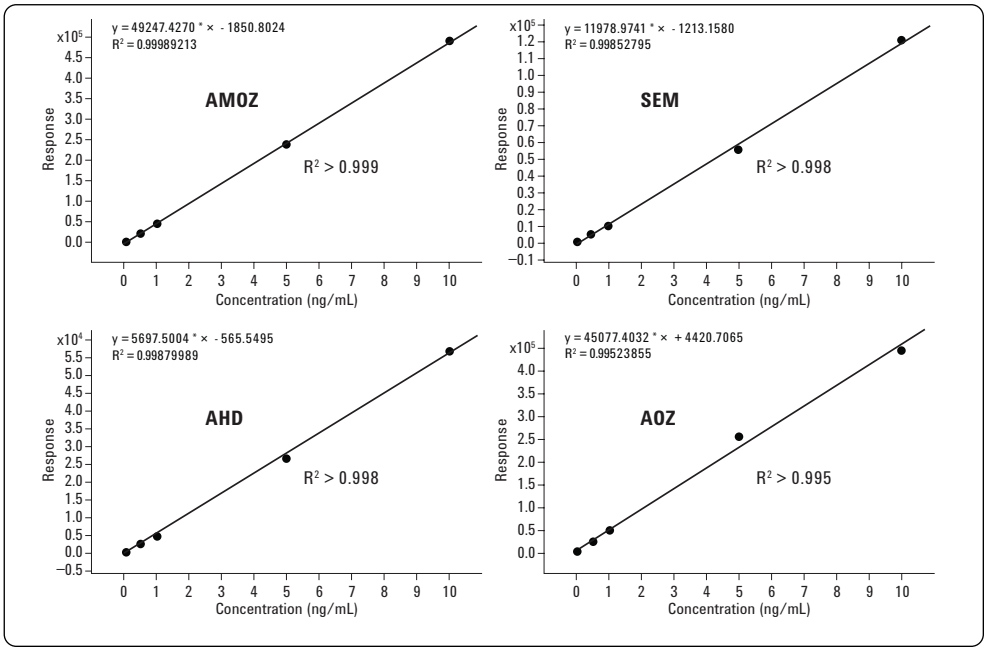
Ionization mode	Positive ESI
Drying gas	10 L/min @ 350 °C
Nebulizer	35 psig
V_{cap}	5000 V

MRM Method Setup

Name	Transitions	Dwell time (ms)	Frag (V)	CE
AMOZ	335.1→291.4	60	100	
	335.1→262.4	60	100	5
SC	209.1→192.3	60	100	5
	209.1→166.3	60	100	5
AH	249.1→134.2	60	100	5
	249.1→104.2	60	100	5
AOZ	236.0→134.1	60	100	5
	236.0→104.1	60	100	5

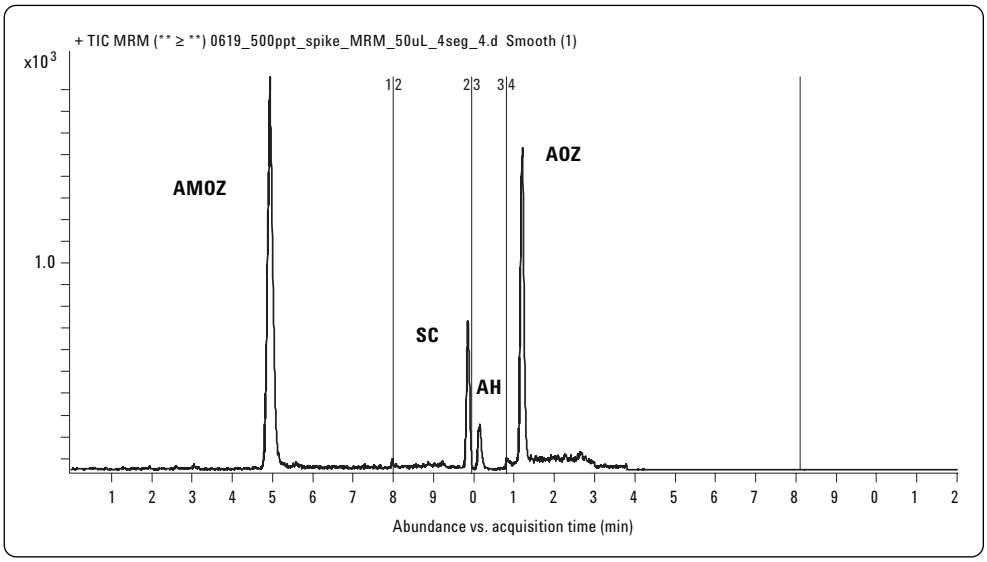
Results

Calibration curves of nitrofuran metabolites show a linear range from 10 ppt to 10 ppb.



Sensitivity

Spiked tilapia sample extract at 500 ppt each



Ordering Information

Description	Part number
Agilent LC/MS/MS	G6410BA or G6460AA

Reference

Agilent Technologies publication 5989-5808EN, “Analysis of Nitrofuran Metabolites in Tilapia Using Agilent 6410 Triple Quadrupole”

8
Detection of sulfonamides in pork by LC/MSD with the APCI source

Abstract

A simple LC/MS method is described for the detection of sulfonamides in pork using the Agilent single-quadrupole LC/MSD with an APCI source.

1. **Weigh** 3-g samples of pork muscle directly into 50-mL polypropylene centrifuge tubes.
2. **Homogenize** 3 minutes with 10 mL of acidified methanol.
3. **Centrifuge** 10 minutes.
4. **Extract** 10 mL of acidified methanol, filter and inject.

Instrument Settings

Sample Preparation

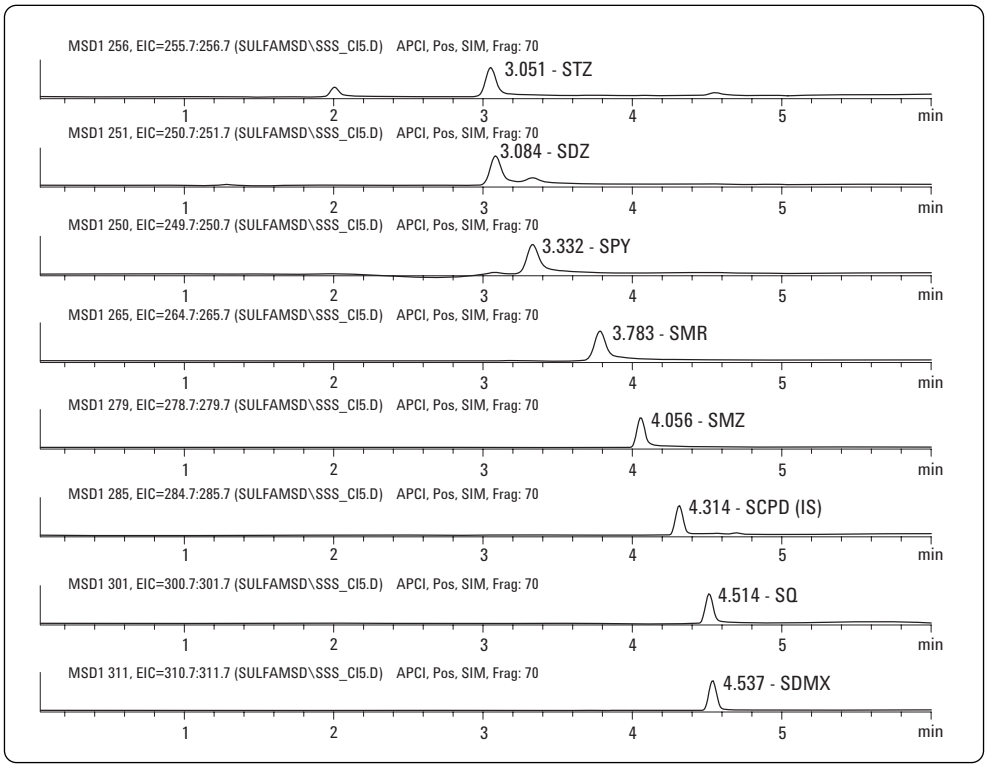
LC Conditions

Column	Agilent ZORBAX Eclipse XDB-C8, 4.6 × 150 mm, 5 µm (p/n 993967-906)
Solvent A	0.1% formic acid in water
Solvent B	0.1% formic acid in acetonitrile
Gradient	0 min 20% B 1 min 20% B 3 min 90% B 6.5 min 90% B Post time = 1.5 min
Flow rate	1.0 mL/min
Injection volume	50 µL
Column temperature	30 °C

MSD Conditions

Source	APCI (positive ion mode)
Ion dwell time	8 ions at 63 ms each
Fragmentor	70 V
Drying gas	6.0 L/min @ 350 °C
Nebulizer pressure	60 psi
Vaporizer temperature	400 °C
Capillary voltage	3000 V
Corona current	4 µA

Results



	Amount recovered (ng)							
Description	**STZ**	**SDZ**	**SPY**	**SMR**	**SMZ**	**SCPD(IS)**	**SQ**	**SDMX**
Pork spike 1	161	157	132	273	149	2,000	139	126
Pork spike 2	154	156	132	293	157	2,000	153	131
Pork spike 3	149	158	124	267	155	2,000	132	113
Pork spike 4	145	152	122	279	144	2,000	119	111
Pork spike 5	151	162	127	294	149	2,000	127	121
Pork spike 6	136	147	127	274	136	2,000	116	108
Pork spike 7	148	161	128	275	155	2,000	124	116
Amount spiked (ng)	150	150	150	300	150	2,000	150	150
Mean	149	156	127	279	149	2,000	130	118
SD (Precision)	8	5	4	10	7	–	13	8
MDL (SD × t-stat) ng	24	17	11	33	23	–	40	26
LOQ (SD × 10) ng	76	53	36	104	73	–	128	82
RSD (SD × 100/Mean)	5	3	3	4	5	–	10	7
Accuracy (%)	99	104	85	93	100	100	87	79
Linearity (R^2)	0.9994	0.9994	0.9997	0.9979	0.9998	1.0000	0.9989	0.9989
t-stat (N = 7)	3.14	3.14	3.14	3.14	3.14	3.14	3.14	3.14

Ordering Information

Description	Part number
Agilent LC/MSD	G6110AA, G6120AA, G6130AA, or G6140AA
Agilent ZORBAX Eclipse XDB-C8, 4.6 × 150 mm, 5 µm	993967-906

Reference

Agilent Technologies publication 5989-0182EN, “A Validated Atmospheric Pressure Chemical Ionization Method for Analyzing Sulfonamides in Pork Muscle”

9
Screening, determination, and quantitation of sulfonamides in meat using the Agilent 6410A LC/MS/MS

Abstract

An Agilent 6410A LC/MS/MS is used to detect, monitor, and quantitate trace sulfonamides in pork with high sensitivity results.

Sample Preparation

1. **Weigh** 3-g samples of pork muscle directly into 50-mL polypropylene centrifuge tubes.
2. **Homogenize** 3 minutes with 10 mL acidified methanol.
3. **Centrifuge** 10 minutes.
4. **Extract** 10 mL acidified methanol, filter and inject.

Instrument Settings

LC Conditions

LC	Agilent 1200 Series LC
Column	Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8 µm
Gradient	A: 0.1% TFA, B: Acetonitrile 0 min – 5% B; 6 min – 23% B; 9 min – 23% B; 9.01 min – 90% B
Stop time	10 min
Column temperature	30 °C
Injection volume	1 µL
Flow rate	0.3 mL/min

MSD Conditions

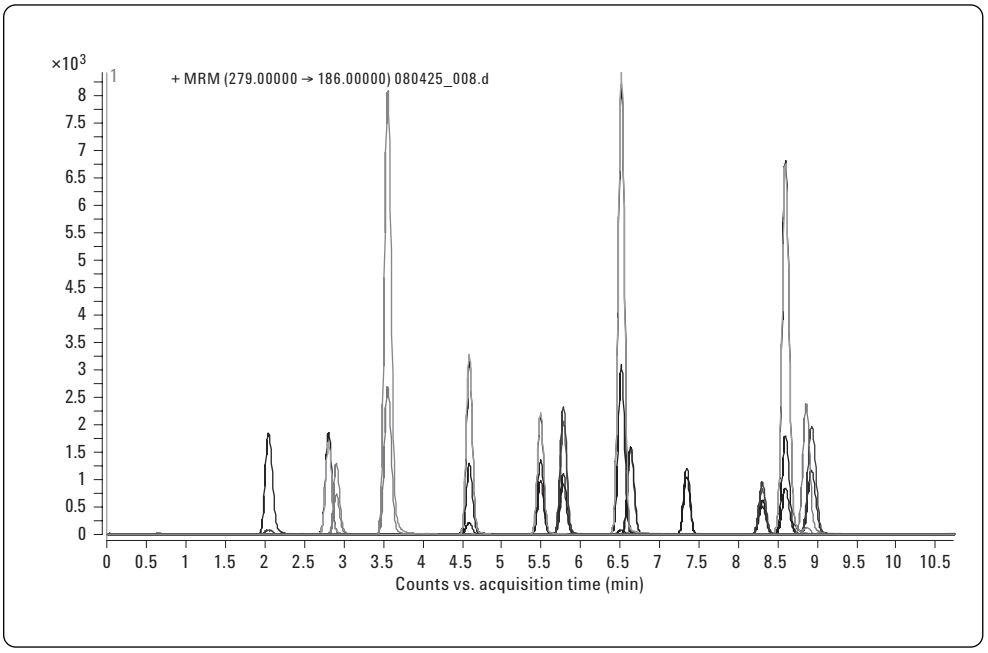
Ionization	ESI (Positive)
Drying gas	7 L/min @ 350 °C
Nebulizer gas	30 psi

MRM Settings

Compound	MRM	Frag	CE (V)
Sulfachlororyridazine (SCP)	285–156 285–108	100	15 20
Sulfadiazine (SD)	251–156 251–185	120	10 10
Sulfamethazine (SDM)	311–156 311–218	140	15 15
Sulfamethoxypyridazine (SMP)	281–156 281–215	120	10 15
Sulfamerazine (SM1)	265–156 265–172	120	15 15
Sulfamethazin (SM2)	279–156 279–204	140	15 15
Sulfafurazole (MSZ)	254–156 254–147	120	15 20
Sulfamonomethoxine (SMM)	281–156 281–126	120	10 20
Sulfathiazole (ST)	256–156 256–107	120	15 15
Sulfaquinoxaline (SQX)	301–156 301–208	140	15 15
Sulfadoxine (SDM)	311–156 311–108	140	15 20
Sulfaphenazole (SPP)	315–156 315–160	140	20 20
Sulfachlorpyridazine	285–156 285–131	100	15 20
Sulfafurazole (SIZ)	268–156 268–113	120	5 10

Results

The resulting chromatogram illustrates good separation and response.



Ordering Information

Description	Part number
Agilent LC/MS/MS	G6410BA or G6460AA

Reference

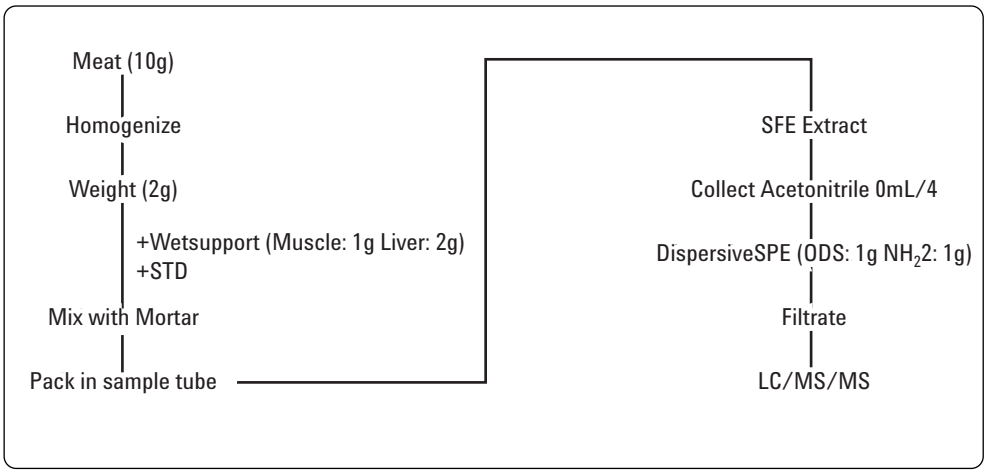
Agilent Technologies publication 5990-3761EN, “Detection, Monitoring, and Quantitation of Trace Sulfonamides in Pork Muscle Using the Agilent 6410A LC/MS/MS”

10
Rapid multi-residue screening for fluoroquinolones in beef using the Agilent 6410A LC/MS/MS

Abstract

This example highlights a rapid multi-residue screening method for veterinary drugs in meat by supercritical fluid extraction combined with liquid chromatography/tandem mass spectrometry (LC/MS/MS).

Sample Preparation



Instrument Settings

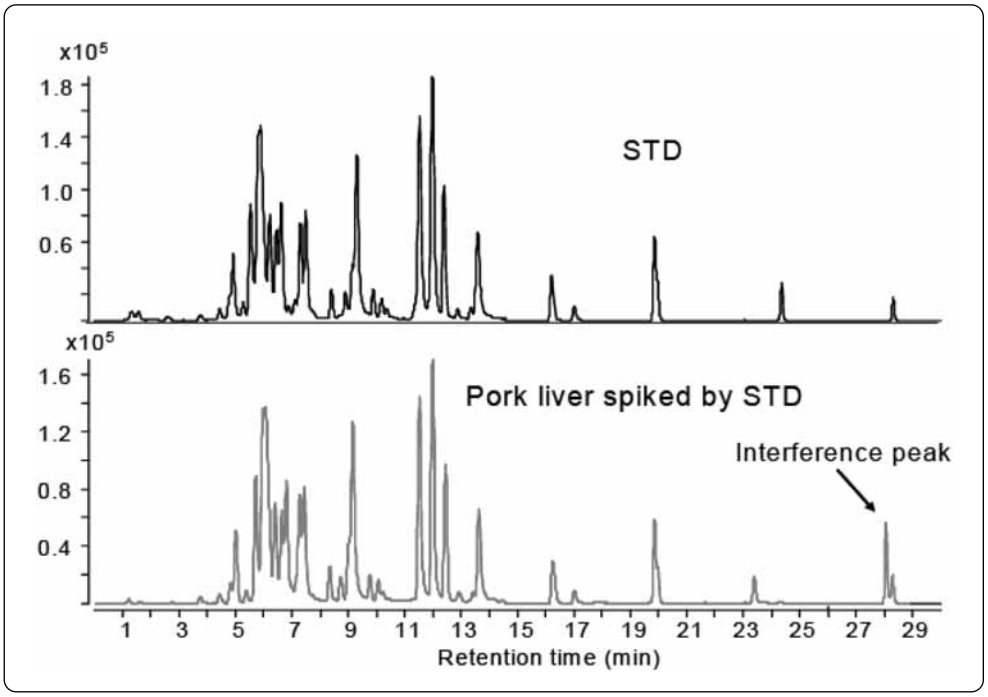
LC Conditions

Column	Agilent ZORBAX Extend-C18, 2.1 mm × 100 mm, 1.8 µm
Oven temperature	40 °C
Mobile temperature	A: Acetonitrile, B: 0.1% formic acid/10 mM $HCOONH_4$ 10% A – (30 min) – 100% A
Flow rate	0.2 mL/min
Injection	1 µL

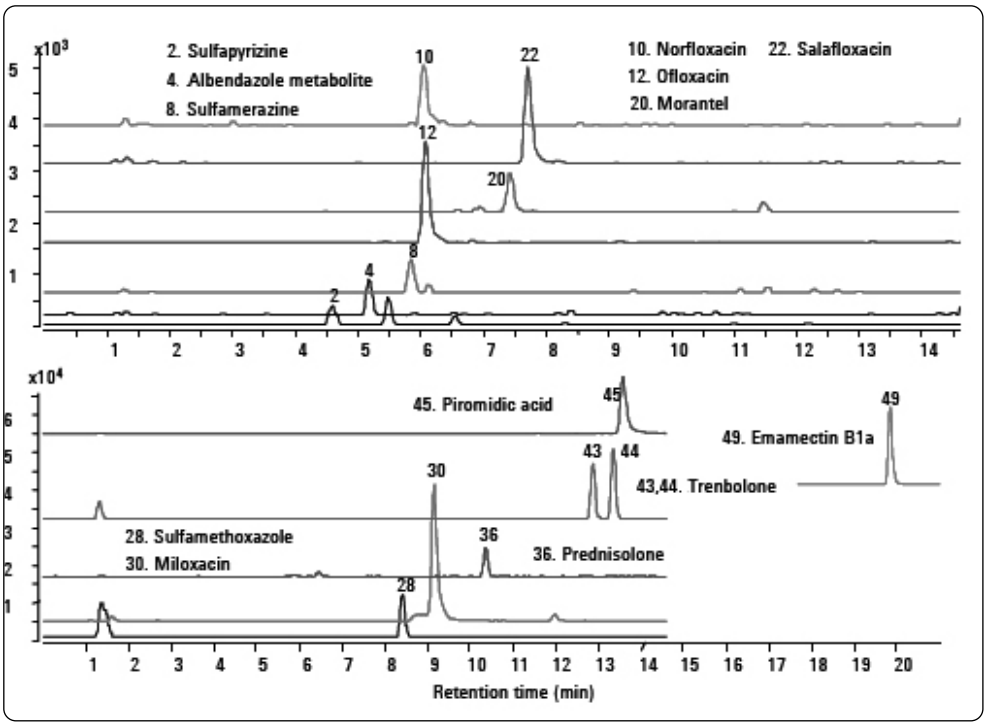
MSD Conditions

Ionization	ESI (+)(–)
Nebulizer gas	345 kPa
Drying gas	10L/min @ 350 °C
Fragmentor	100 V

Results



MRM chromatogram of veterinary drugs in pork liver at 100 ppb



Ordering Information

Description	Part number
Agilent LC/MS/MS	G6410BA or G6460AA
Agilent ZORBAX Extend C18, 2.1 × 100 mm, 1.8 µm	

Reference

Agilent Technologies poster ASMS07-25, “Rapid Multiresidue Screening for the Veterinary Drugs in Meat by Supercritical Fluid Extract Combined With Liquid Chromatography-Tandem Mass Spectrometry”

11
Determination of malachite green and leucomalachite green in food by Agilent 6410A Series LC/MS/MS

Abstract

This example uses a method to rapidly and precisely determine residue levels of malachite green and leucomalachite green in fish with the new Agilent 6410 LC/MS triple quadrupole system. Using positive mode electrospray ionization (ESI+) and multiple reaction monitoring (MRM), qualification and quantification were accomplished without the traditional tedious PbO_2 oxidation process.

Sample Preparation

1. **Add** to 5 g tilapia tissue 1 mL (0.25 mg/mL) hydroxylamine, 2 mL 1 M toluene sulfonic acid, 2 mL of 0.1 M ammonium acetate buffer (pH 4.5), and 40 mL acetonitrile.

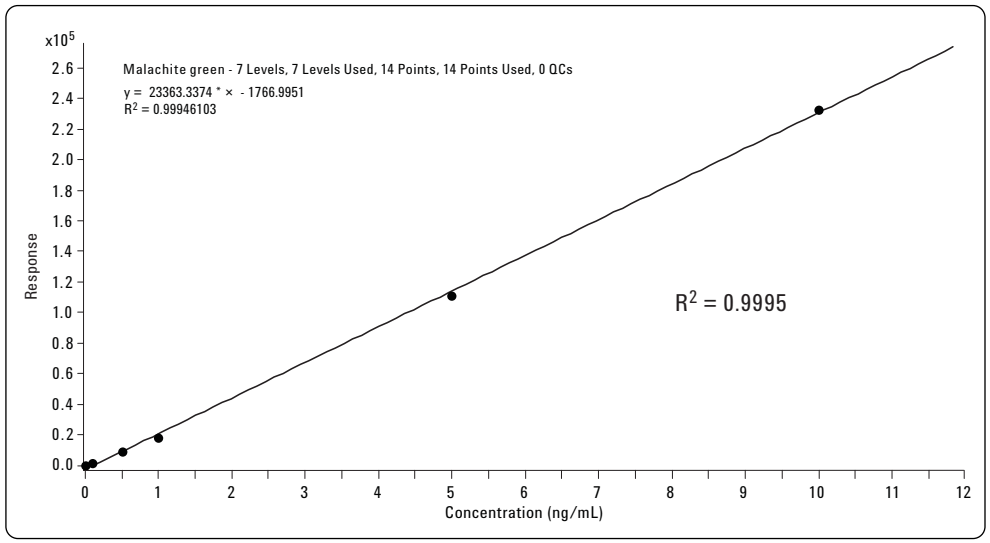
2. **Extract** using acetonitrile and methylene chloride.

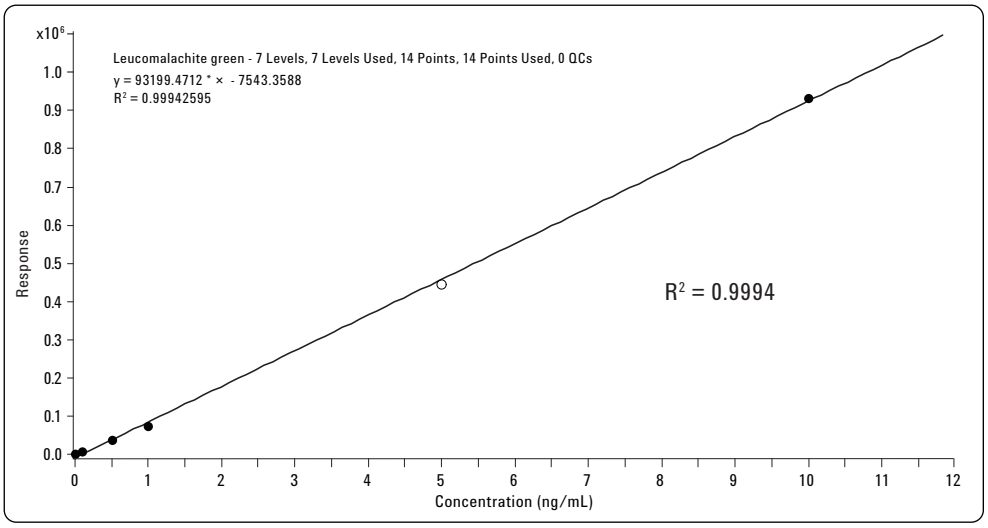
Instrument Settings

LC Conditions	
Column	Agilent ZORBAX C18, 2.1 × 150 mm, 5 µm
Column temperature	40 °C
Mobile temperature	A: 10 mmol/L ammonium acetate (adjust to pH 4.5 with acetic acid) B: acetonitrile
Flow rate	0.3 mL/min
Gradient %B	0 min, 30 1 min, 50 2 min 95 8 min 95 8.01 min, 30
Injection	10 µL
MS Conditions	
Ionization	ESI (+)
Capillary	4000 V
Nebulizer pressure	35 psi
Drying gas	11 L/min @ 350 °C
Skimmer	15 V

Results

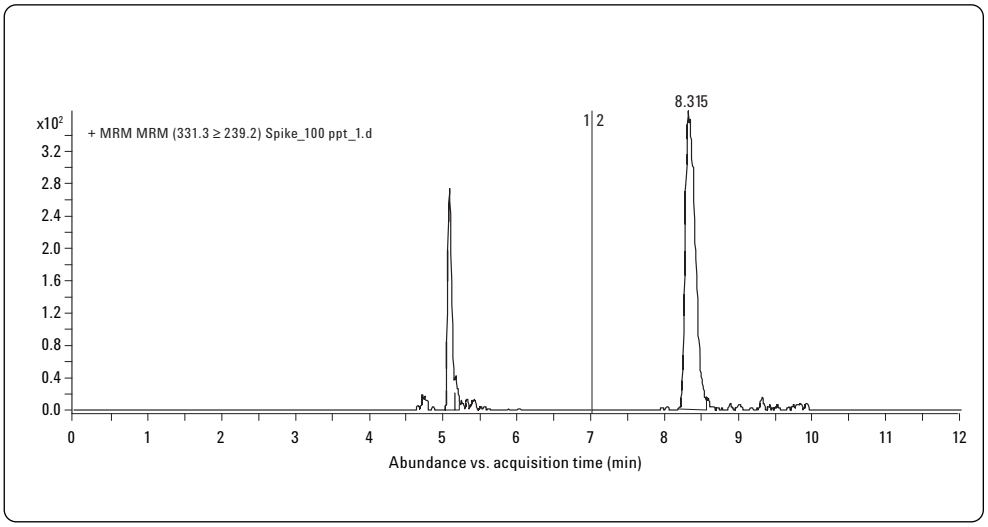
Linearity: 10 ppt – 10 ppb





Sensitivity

100 ppt level



Ordering Information

Description	Part number
Agilent LC/MS/MS	G6410BA or G6460AA

Reference

Agilent Technologies publication 5989-5807EN, “Determine Malachite Green and Leucomalachite Green in Food by LC/MS/MS”

12
Rapid confirmation and multi-residue quantitation of β_2-agonist in animal food using the Agilent 6410A LC/MS/MS

Abstract

β_2-agonist acts as a growth promoter and also influences lypolysis, transferring fat to muscle. In this analysis, liver and urine were selected as suitable matrices. Molecular imprinted polymers were used for the isolation of the β_2-agonists from the matrices and an Agilent 6410A LC/MS/MS was used for quantitation and confirmation. Eleven β_2-agonists are included in this example. The aim of the study was to develop a method suitable for both screening and confirmation of the β_2-agonists at the proposed minimum required performance level (MRPL) or lower.

Sample Preparation

1. **Transfer** 5 mL of urine or 5 g of ground animal liver tissue sample into a centrifuge tube.
2. **Add** 10 mL of 0.1 M HCl solution to the tube.
3. **Shake** vigorously for 5 min, stir for 3 min, ultrasound for 10 min and centrifuge for 5 min at 12000r/min.
4. **Decant** and repeat the above two steps twice, combining all the supernatant.
5. **Rinse** a C18 SPE tube with 6 mL of methanol and then 6 mL de-ionized water.
6. **Load** the extract onto the C18 tube and rinse with 5 mL de-ionized water.
7. **Wash** the tube with 0.8 mL of methanol and 0.8 mL of de-ionized water, and collect the effluent.
8. **Dry** the effluent under a stream of nitrogen and re-constitute in initial mobile phase.
9. **Pass** the sample through a 0.45-µm membrane filter and into an autosampler vial.

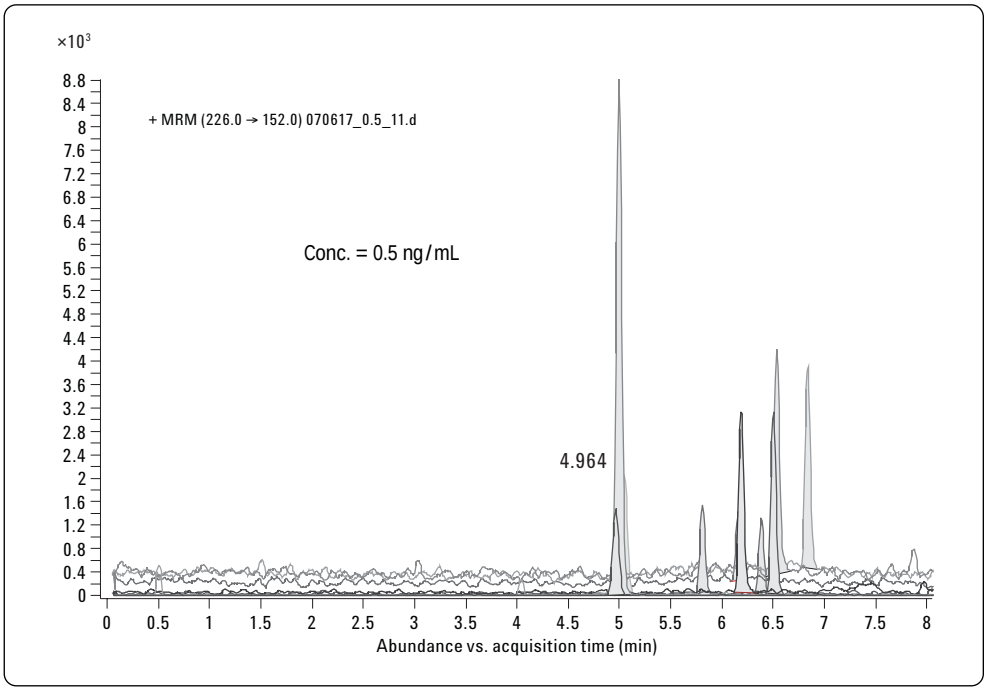
Instrument Settings

LC Conditions	
Column	Agilent ZORBAX SB-C18, 2.1 × 100 mm, 1.8 µm
Column temperature	40 °C
Mobile temperature	A: 10 mmol/L ammonium formate plus 0.1% formic acid; B: acetonitrile
Flow rate	0.3 mL/min
Gradient	0–5 min, B% 1% to 90% 5–8 min, B% 90% 8.1–14 min B% 1% at 0.5 mL/min
Injection	5 µL
MS Conditions	
Ionization	ESI (+)
Capillary	4000 V
Nebulizer pressure	35 psi
Drying gas	11 L/min @ 350 °C

MRM Settings

Time	Compound	Precursor	Product	Dwell (ms)	Fragmentor (V)	Collision energy (V)
4.95	Terbutaline	226	152	10	100	15
			170	10	100	30
4.98	Zilpaterol	262	244	10	100	10
			185	10	100	25
4.98	Salbutemol	240	222	10	100	5
			148	10	100	15
5.04	Cimaterol	220	202	10	80	5
			160	10	80	15
5.80	Ractopamine	302	284	10	100	10
6.15	Tulobuterol	228	119	10	100	30
			172	10	100	10
6.18	Clenbuterol	277	203	10	100	10
			259	10	100	5
6.37	Bromobuterol	367	349	10	100	10
			293	10	100	15
6.49	Clenpenterol	291	203	10	100	15
			273	10	100	5
6.52	Mabuterol	311	237	10	100	15
			293	10	100	10
6.83	Mapenterol	325	237	10	100	15
			217	10	100	25

Results



Compound	S/N (p-p)
Terbutaline	374.6
Zilpaterol	39.0
Sabutemol	1759.8
Cimaterol	48.8
Ractopamine	1539.0
Tulobuterol	7.1
Clenbuterol	30.2
Bromobuterol	53.8
Clenpenterol	43.0
Mabuterol	15.8
Mapenterol	58.5

Ordering Information

Description	Part number
Agilent LC/MS/MS	G6410BA or G6460AA
C18 ODS – 6 mL tubes, 500 mg SampliQ	5982-1165

Reference

Inspection and Quarantine Science, Vol. 18 No. 5, pp 39–41

13
Determination of multi-residues of tetracyclines and their metabolites in milk by high-performance liquid chromatography (HPLC) and an Agilent 6410A LC/MS/MS

Abstract

Tetracyclines are probably the most frequently used antibiotics in animal husbandry. In this paper, a high-performance liquid chromatograph/tandem mass spectrometric (HPLC /MS/MS) method is developed for the simultaneous determination of 10 antibiotic residues (minocycline, 4-epioxytetracycline, 4-epitetracycline, tetracycline, 4-epichlortetracycline, demeclocycline, chlortetracycline, methacycline, doxycycline, and oxytetracycline) in milk and animal tissue.

Sample Preparation

Extraction

1. **Weigh** a 5-g milk sample (accurate to 0.01 g) into a 50-mL colorimetric tube.
2. **Dissolve** with 0.1 mol/L Na2EDTA-McIlvaine buffer solution, and adjust the volume to 50 mL.
3. **Vortex** mix for 1 min.
4. **Ultrasonically** extract in an ice water bath for 10 min, then transfer to a 50-mL polypropylene centrifugal tube.
5. **Cool** down to 0~4 °C.
6. **Centrifuge** at a rotate speed of 5000 r/min for 10 min (below 15 °C) and filter with fast filter paper.

Purification

1. **Draw** 10 mL extract (equivalent to 1 g sample) and put it through the Agilent SampliQ cartridge at a speed of 1 drop/s.
2. **Clean** the cartridge with 5 mL water and 5 mL methanol and water after the sample effuses completely.
3. **Discard** the effluent fluid.
4. **Drain** the cartridge for 5 min. under a negative pressure below 2.0 kPa.
5. **Elute** with 10 mL of 10 mmol oxalic acid in methanol.
6. **Collect** the eluent and dry under nitrogen below 40 °C.
7. **Dissolve** the residue with mobile phase to 1.0 mL (LC/MS/MS), filter with 0.45 µm filter membrane, and inject.

Instrument Settings

LC Conditions

Column	Agilent ZORBAX RX-C8, 2.1 × 150 mm, 5 µm (p/n 883700-906)
Flow rate	0.3 mL/min
Mobile phase	A: water/0.1% formic acid B: methanol
Gradient	0–10 min, B from 5% to 30% 10–12 min, B from 30% to 40% 12.5–18 min, B 65% 18.5–25 min, B 95% 25.5 min, B 5.0%
Total run	28 min
Post time	5 min
Temperature	30 °C
Injection	5 µL

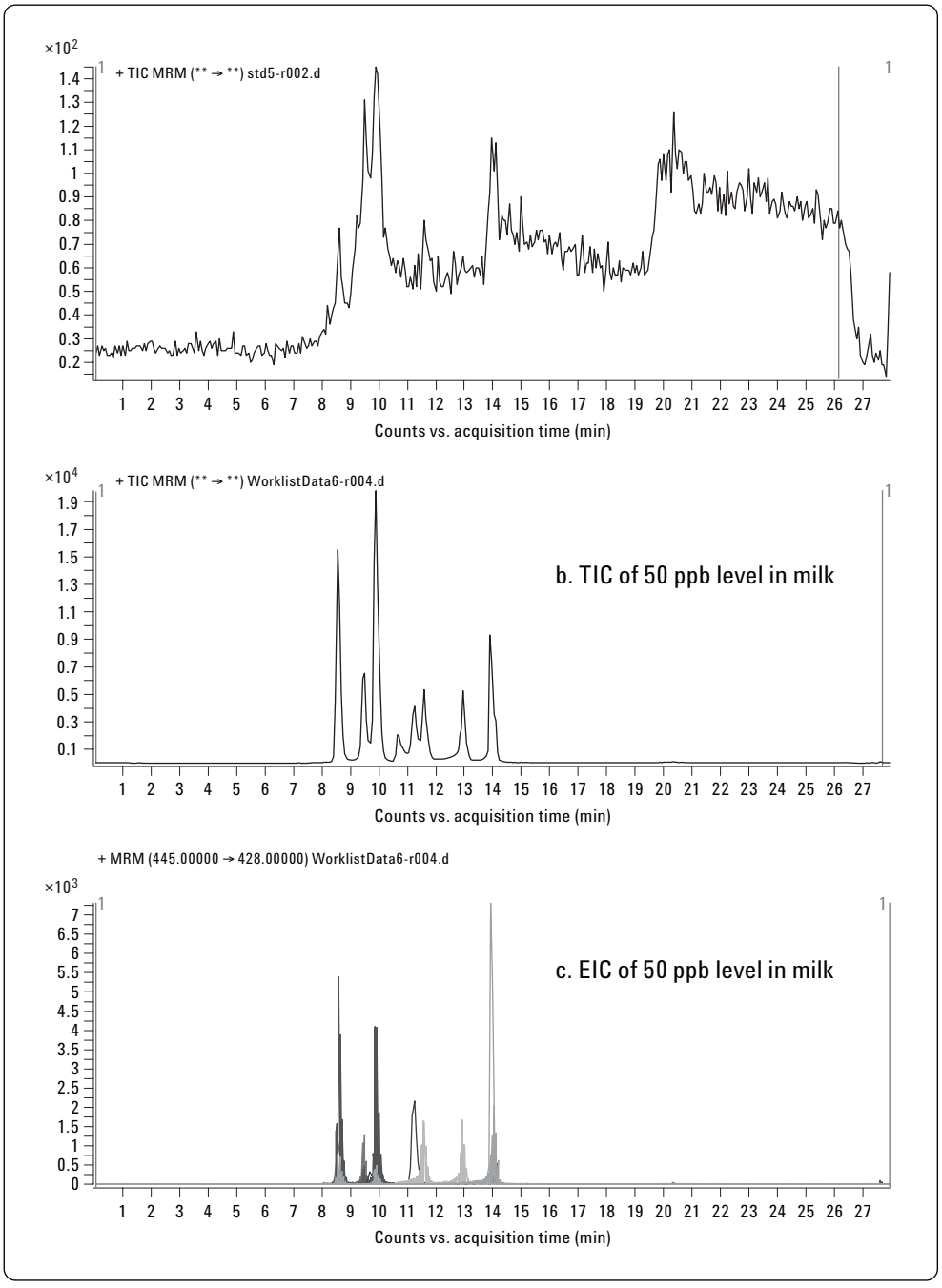
MS Source Settings

Source	ESI
Ion polarity	Positive
Drying gas	10 L/min @ 350 °C
Nebulizer	45 psi
V_{cap}	4000 V

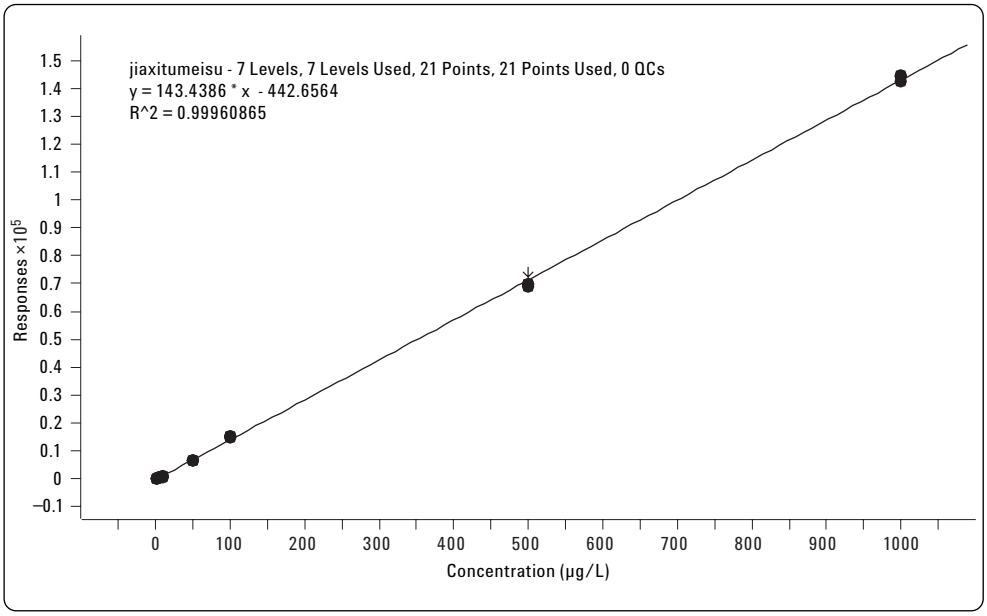
MRM Settings

Name	Fragment	Precursor ion	Product ion	CE	Rt. (min)
Minocycline	120	458	352 441	35 20	8.58
4-Epitetracycline	120	445	410 427	20 10	8.60
4-Epioxytetracycline	120	461	426 444	20 15	9.47
Tetracycline	120	445	410 427	20 15	9.90
Oxytetracycline	120	461	426 443	20 10	9.95
Demethylclocycline	120	465	430 448	25 15	11.25
4-Epichlortetracycline	120	479	444 462	22 15	11.59
Chlortetracycline	120	479	444 462	22 15	12.95
Methacycline	120	443	381 426	25 15	13.98
Doxycycline	120	445	154 428	30 15	14.08

Results


×10^2
+ TIC MRM (** → **) std5-r002.d
Counts vs. acquisition time (min)
×10^4
+ TIC MRM (** → **) WorklistData6-r004.d
b. TIC of 50 ppb level in milk
Counts vs. acquisition time (min)
+ MRM (445.00000 → 428.00000) WorklistData6-r004.d
×10^3
c. EIC of 50 ppb level in milk
Counts vs. acquisition time (min)

Name	Standards in solvent* R^2	LOQ (S/N = 20) pg on column	LOD (S/N = 3) pg on column	Standards in Milk matrix* R^2	LOD (S/N = 3) pg on column
Minocycline	0.999	41.5	6.2	0.990	16.3
4-Epitetracycline	0.991	10.8	1.6	0.994	8.7
4-Epioxytetracycline	0.996	14.7	2.2	0.996	12.8
Tetracycline	0.998	9.4	1.4	0.994	10.2
Oxytetracycline	0.996	10.7	1.6	0.991	8.6
Demethylclocycline	0.999	22.8	3.4	0.993	8.1
4-Epichlortetracycline	0.986	38.2	5.7	0.987	11.9
Chlortetracycline	0.986	8.1	1.2	0.994	7.6
Methacycline	0.999	20.8	3.1	0.994	12.3
Doxycycline	0.999	32.2	4.8	0.995	11.2



Ordering Information

Description	Part number
Agilent LC/MS/MS	G6410BA or G6460AA
Agilent ZORBAX RX-C8, 2.1 x 150 mm, 5 µm	883700-906
SampliQ OPT SPE Cartridge 3mL 60 mg	5982-3036

Reference

Agilent Technologies publication 5990-3816EN, “Determination of Multi-Residue Tetracyclines and their Metabolites in Milk by High Performance Liquid Chromatography- Tandem Mass Spectrometry”

Appendix

General Sample Preparation Procedure for Reference

- Nitrofuran and its metabolites

    1. Weigh a 2-g tissue sample and add 5 mL of 0.2 N HCl and 50 µL of 50 mM 2-NBA.
    2. Vortex the sample for 1 min.
    3. Place in a water bath and shake for 16 hr (Note: lightproof).
    4. Add 0.3 mL of 0.3 M Na_3PO_4 and stir.
    5. Adjust pH to 7.4 (± 0.2) using 1M NaOH.
    6. Extract using 5 mL of acetate.
    7. Vortex 10 min, then centrifuge 3 min at 4000 rpm.
    8. Repeat Steps 6 and 7.
    9. Combine acetate extracts and dry using nitrogen at 40 °C. Reconstitute in 1 mL initial mobile phase.
    10. Filter 1 mL of the mobile phase for injection.

- Chloramphenicol

    1. Weigh a 10-g sample and extract twice with 50 mL ethyl acetate.
    2. Combine the two supernatants and concentrate at 40 °C to almost dry.
    3. Add 2 mL of methanol and 50 mL of 4% NaCl to dissolve the residue.
    4. Extract twice with 20 mL n-hexane. Discard the hexane.
    5. Extract twice with 20 mL acetate, collecting the ethyl acetate.
    6. Combine two ethyl acetate extracts, concentrated at 40 °C to almost dry.
    7. Dissolve using 5 mL of water.
    8. Activate Agilent ZORBAX C18 SPE cartridge using 5 mL of methanol, 5 mL of chloroform, 5 mL of methanol, and 10 mL of water.
    9. Load the 5 mL of sample onto the SPE cartridge.
    10. Using 5 mL acetonitrile/H_2O(5:95, V/V) and 3 mL acetonitrile, elute the analyte from the SPE cartridge.
    11. Dry the eluant using nitrogen at 40 °C.
    12. Dissolve the dried extract in 1-mL mobile phase and filter for injection.

- Glucocorticoid

    1. Weigh a 3-g sample.
    2. Add 15 mL of acetate.
    3. Vortex 15 min and centrifuge for 5 min at 16,000 rpm.
    4. Repeat and combine two acetate layers.
    5. Evaporate until almost dry at 40 °C.
    6. Activate a Silica SPE column using 6 mL n-hexane.
    7. Load samples.
    8. Wash with 6 mL of n-hexane.
    9. Elute with 6 mL of n-hexane/acetone (6:4).
    10. Using N_2 to dry at 40 °C, dissolve in 1 mL and filter for injection.

www.agilent.com/chem

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