JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

ESTIMATION OF ANTIBIOTIC RESIDUEs IN AQUACULTURE PRODUCTS (FISH AND FISHERY PRODUCTS) AS PER 2002/657/EC GUIDANCE

K.VANITHA[1], T.GEETHARATHAN*[1], M.HEMALATHA[2]

Department of Biotechnology, Sri Venkateswara University, Tirupati, A.P.

Abstract:

In recent years, antibiotics have been used widely in intensive shrimp culture and this may lead to their contamination of the environment. Surveys on residues of Nitro furan metabolites [AOZ, AMOZ, AHD, SEM], Chloramphenicol in water and mud in shrimp ponds in mangrove areas were conducted in then or this well as in south of Viet Namin July and August,2002.The results show that these antibiotics are found in all samples in both shrimp ponds and surrounding canals. The highest concentrations ofNitrofuranmetabolites[AOZ,AMOZ,AHD,SEM],Chloramphenicolare1.04,2.39,6.06,and2.50ppm in waters amples;and734.61,820.49,2615.96,426.31ppm(based on wet mud weight), respectively. The comparison of antibiotics residues between study sites and types of shrimp ponds will be discussed in this paper. The results also suggest that antibiotics residues may cause harmful effect on ecosystems in the study sites. An analytical method based on LCMS was developed and optimized in order to determine the most useful antibiotics (Nitrofuranmetabolites [AOZ, AMOZ, AHD, SEM], Chloramphenicol) used in aquaculture. A simple extraction procedure, without any clean-up step, was evaluated in order to obtain maximum analyte recovery from shrimp samples (Machobrachiumrosenbergi, Machobrachiummalcolmsoni, Panaeusmonodo, Fenneropanaeusindicus, Litopanaeus Vannamei, and Litopanaeus).

Keywords: Aquaculture, Antibiotics, Shrimps, Nitro furan metabolites**,** LCMS.

Introduction:

Veterinary drug residue analysis of meat and seafood products is an important part of national regulatory agency food safety programs to ensure that consumers are not exposed to potentially dangerous substances. Toussaint et al. (2005) established a method for determination of quinolone antibiotic residues in pig kidney using liquid chromatography-tandem mass spectrometry. Hammel et al. (2008) developed a multi-screening approach for monitoring potential chemical contaminants in honey by liquid chromatography –electrospray ionization tandem mass spectrometry. Draisci et al. (2001) created a new sensitive electrochemical enzyme-linked immune sorbent assay (ELISA) for detection of two macrolides (erythromycin and tylosin) in bovine muscle. Okermanetal. (2003) analysed antibiotic residue of bovine and porcine kidney tissue by a solid-phase fluorescence immunoassay. However, pharmaceuticals identified as emerging contaminants are used in large quantities in human and veterinary medicine for treatment of different diseases. Among pharmaceuticals, antibiotics in the aquatic environment are a big challenge as prolonged exposure to low does may promote antibiotic resistance. The accelerated growth of aquaculture has resulted in a series of harmful effects to human health. The widespread and unrestricted use of antibiotics in this industry, to prevent bacterial infections, has leaded some excess amount of unutilized antibiotics to remain in the environment. The use of antibiotics and drugs in fish farms has prompted an investigation into the elimination of these residues from fish muscle. Won et al. (2011) analyzed both domestic and imported marine products in Korea for 14 sulphonamide antimicrobials by UPLC-MS / MS and found out that the samples include flat fish, jacopever, sea bream, common eel, blue crab, shrimp and abalone.

Swapna et al. (2012) detected the antibiotic residues of farmed shrimps from the southern states of India. The results were showing that streptomycin, tetracycline and β - lactam could not be detected in any of the samples and sulfonamides and erythromycin were detected in farmed shrimps at a level <100 ppb. Such findings are not limited to abroad; there are many studies on detecting antibiotics in China too. The case in China is much more sensitive in compare to other regions as China is not just a big consumer but a large producer of antibiotics too. With the decline of fishery resources, aquaculture has gradually become a major food industry to partially solve the "food shortage" and to provide food safety. In the past three decades, aquaculture industry has developed rapidly, and the output value of aquatic products has increased from1.30% in 1952 to 11.19% in 2003. The aquatic productions ranks as first in the world's aquaculture production and has reached 35.97 million tons in 2006. These statistics are based on68%of total fisheries production and70%of the world’s total aquaculture production.

Antibiotics are widely used in aquaculture. The positive effects of antibiotics are mainly reflected in the prevention and control of aquatic animal diseases, promoting growth, saving nutrition and other aspects. Especially that it can efficiently control the occurrence of many aquatic diseases and promote the development of aquaculture. This paper mainly introduces the rapid detection of antibiotics in aquatic products in China. However, one of the major threats to the aquaculture industry worldwide is bacterial infection. More than USD 6 billion per annum is lost from the aquaculture industry due to disease(Stentifordetal.2017).Both extensive and intensive aquaculture farming have greatly enhanced the transmission opportunities for waterborne pathogens that can spread at faster rates compared with terrestrial

systems (McCallum, Harvell, and Dobson 2003). For example, Vibrioparahaemolyticus, the causative agent of acute hepato pancreatic necrosis disease (AHPND) and formerly known as early mortality syndrome (EMS) causes devastating losses that reached billions of dollars annually since its first outbreak in Southern Chinain2009 (Lightneret al.2012a, 2012b; Tran et al. 2013). This disease is rapidly spreading and has affected several countries in Southeast Asia consecutively, e.g., Vietnam in the year 2010, Malaysia (2011), Thailand (2012), Philippines (2013), and has even spread to the Americas e.g., Mexicoin2013 (Tranetal. 2013; Nunanet al. 2014; DeLaPeña et al. 2015).

In order to treat and prevent bacterial diseases in aquaculture, antibiotics are commonly used as therapeutic and/or prophylactic agents. Tetracycline's, sulfonamides, oxolinic acid and erythromycin are commonly used antibiotics in aquaculture farming (ASEAN, 2013). These antibiotics are permitted for use in food producing animal based on the recommended Maximum Residue Level (MRL) set by joint Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO), Codex Alimentarius Commission, and European Union legislation (FAO and WHO 2020). However, MRLs differ between geographic regions depending on the antibiotic usage profiles and local food safety regulatory agencies. Moreover, most Southeast Asian countries lack legislation, regulatory surveillance and monitoring systems on the use of antibiotics(Chuah et al. 2016;FAO2016).Antibiotic contamination continues to be found in the environment and aquaculture products(LeandMunekage2004;Lin,Yu,andLin2008;Oliveiraetal. 2014; Xionget al.2015; Lai etal. 2018).Although Malaysia has banned the use of nitro furans and chloramphenicol in aquaculture farming, the United States Food and Drug Administration (FDA) continues to detect both these residues in seafood from Malaysia, in which 44cases were reported between 2009 and 2018 (Food and Drug Administration2018).

Aquaculture waste has been identified as one of the main contributors of antibiotic pollution in the environment (DeJesus Gaffneyet al. 2016) as the infrastructure for proper aquaculture waste management is critically lacking. Furthermore, many countries, particularly those in the developing countries have yet to develop standards on concentrations of antibiotics discharge from wastewater effluents (Sasikaladevi, KiruthikaEswari, and Nambi 2020). Therefore antibiotics, antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) from aquaculture are released directly to the environment. These chemical and biological pollutants can impact public health and marine ecosystems (World Health Organization (WHO) 2018).

However, quantitative data on the residual levels of antibiotic and antimicrobial resistance in water samples from aquaculture remain scarce (Managaki et al. 2007; Suzuki and Hoa 2012; Shimizu et al. 2013; Yan et al. 2018). Current levels of antibiotic use in aquaculture worldwide are difficult to determine as different countries have different distribution and standards to assess the pollution levels (Romero, Feijoó, and Navarrete 2012). With limited information on the level of contamination of antibiotics in Malaysian aquaculture farms, the potential risk of residual antibiotic toward the ecosystem remains unclear. Hence, the aim of this study is to examine the distribution and composition of antibiotics in aquaculture farms, and their ecological risk, as well as determine the prevalence of ARGs in bacteria from aquaculture farms.

Sulfonamides [12]
Sulfacetamide
Sulfadiazine
Sulfathiazole
Sulfapyridine
Sulfamerazine
Sulfamethazine
Sulfamethoxypyridazine
Sulfchloropyridazine
Sulfamethoxazole
Sulfisoxazole
Sulfadimethoxine
Sulfachinoxalin

Quinolones [10]
Pipemidic acid
Enoxacin sesquihydrate
Ofloxacin
Norfloxacin
Ciprofloxacin hydrochloride
Lomefloxacin
Danofloxain
Enrofloxacin
Sarafloxacin hydrochloride
Cinoxacin

Hormones [17]
19-nor-4-androstene-3,17-dione
1-Dehydrotestosterone Sulfate
Danazol
Fluoxymesterone
Testosterone
17-alpha-methyltestosterone
Methadrostenolone
Nandrolone
19-nor-4-androstene-3,17-dione
Trenbolone
Megestrol-17-acetate
Medroxyprogesterone
Medroxyprogesterone-17-acetate
Norgestrel
Chloromadinone 17-acetate
Norethindrone
Progesterone

Macrolides [5]
Spiramycin
leucomycin hydrate
Erythromycin
Tilmicosin
Acetylisovaleryltylosin Tartrate

Chloramphenicols [3]
Thiamphenicol
Florfenicol
Chloramphenicol

Nitro imidazoles [5]
Ronidazole
2-methyl-5-nitroimidazole
Metronidazole
4-Nitroimidazole
Ipronidazole

Nitrofuran metabolites [4]
Furazolidone
Furaltadone
Nitrofurantion
Furacilinum

Tetracyclines [5]
Tetracycline hydrochloride
Oxytetracycline
Demeclocycline hydrochloride
Chlorotetrachclie hydrochloride
Doxycycline

Lincosamides [2]
Lincocin Hydrochloride
Clindamycin

Other drug [5]
Trimethoprin
Malachite green oxalate
Leucomalachite green
Basic violet 3
Leucocrystal violet

3.0. PROCEDURE

3.1 Requirements

3.1.1 Glassware

- Volumetric flasks - 10 mL and 25 mL
- Glass tray

3.1.2 Consumables

- Centrifuge tubes - 50 mL
- Ria vials

3.1.3 Chemicals

- Ethyl acetate
- Methanol
- Type -1 water
- 2- Nitrobenzaldehyde
- Sodium dihydrogen phosphate monohydrate

3.1.4 Equipment

- Analytical balance
- Micro Balance
- Micropipettes 20-200µL, 100-1000µL
- Centrifuge
- Cyclomixer
- Nitrogen Evaporator
- Vortexes
- Shaking incubator
- LC-MS/MS

4.0 Types of shrimps:

Major Farm-Raised Species	
Old Name	**New Name**
Penaeus vannamei	*Litopenaeus vannamei*
Penaeus stylirostris	*Litopenaeus stylirostris*
Penaeus chinensis	*Fenneropenaeus chinensis*
Penaeus indicus	*Fenneropenaeus indicus*
Penaeus japonicus	*Marsupenaeus japonicus*
Minor Farm-Raised Species	
Old Name	**New Name**
Penaeus schmitti	*Litopenaeus schmitti*
Penaeus setiferus	*Litopenaeus setiferus*
Penaeus occidentalis	*Litopenaeus occidentalis*
Penaeus brasiliensis	*Farfantepenaeus brasiliensis*
Penaeus aztecus	*Farfantepenaeus aztecus*
Penaeus californiensis	*Farfantepenaeus californiensis*
Penaeus duorarum	*Farfantepenaeus duorarum*
Penaeus notialis	*Farfantepenaeus notialis*
Penaeus subtilis	*Farfantepenaeus subtilis*
Penaeus paulensis	*Farfantepenaeus paulensis*
Penaeus merguiensis	*Fenneropenaeus merguiensis*
Penaeus penicillatus	*Fenneropenaeus penicillatus*
No Name Change	
Penaeus monodon, esculentus and semisulcatus	

Machobrachium rosenbergi

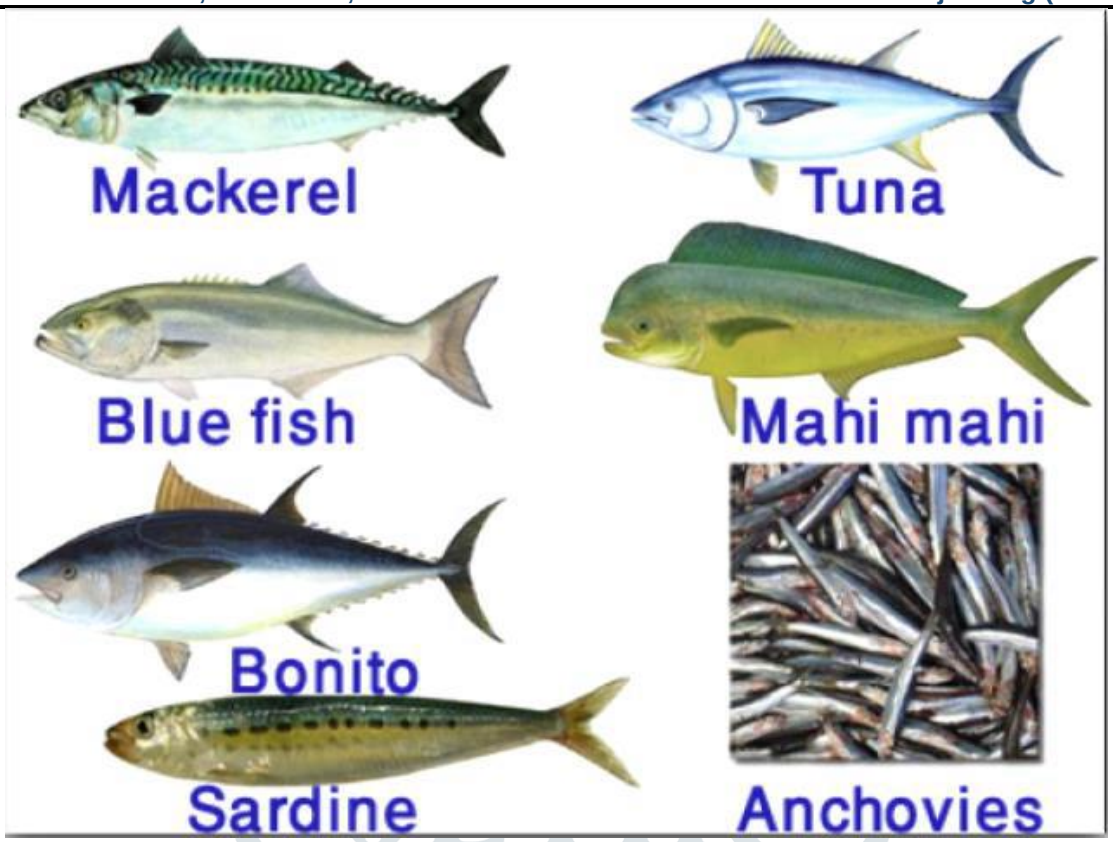
Machobrachium malcolmsoni

Panaeus monodon

Fenneropanaeus indicus

Litopanaeus Vannamei

Litopanaeus stylirostris



Histamine forming fish

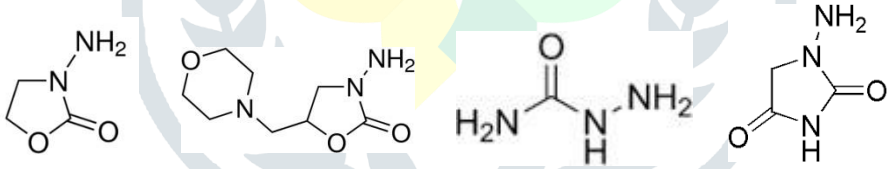
Shrimp Homogenization:-

Procedure for sample peeling, Blending and Homogenization of shrimp;

1. **Thawing process:-** Take out the sample portion marked s 'for analysis' from freezer and place it at room temperature which is less than 25ºC and place the sample pouch in a glass tray filled with water until they feel limber [soft/supple].care shall be taken to avoid sample contact with water used for thawing.
2. If shrimp samples are received with head and tail, remove the head, shell and tail of the thawed shrimp sample
3. Place the peeled shrimp sample in the blender
4. Blend the shrimp sample with pulsed action until contents are uniform
5. Label the pouch /container with the registration number provided
6. Transfer the homogenized portion into the labeled pouch/container
7. Store the homogenized sample in freezer at a temperature of not more than -18ºC until analysis

Nitro furan metabolites:

Structure of Nitro furans metabolites



Name of the compound	Chemical formula	Molecular weight
AOZ	C3H6N2O2	102.1
AMOZ	C8H15N3O3	201.22
AHD	C3H5N3O2	151.55
SEM	CH5N3O	111.53
AOZ-D4	C3H2N2O2D4	106.1
AMOZ-d5	C8H10N3O3D5	206.25
AHD-13C3	C3H5N3O2	118.07
SEM-13C15N2.HCl	CH6ClNN2O	114.51

MATERIALS METHODS AND RESULTS:

AOZ, AMOZ, AHD, SEM, Standard of highest purity with traceability to National / International standards in suitable solvents as per the COA/ MSDS.

Reagents Preparation

Preparation of diluent:

Use Methanol as diluent.

Preparation of Reagent Blank:

Prepare reagent blank as per sample preparation without sample and standard.

Preparation of Sample Blank:

Prepare sample blank as per sample preparation without analyte.

Preparation of Mobile phase blank:

Use mobile phase as mobile phase blank

Preparation of Mobile Phase:

Pump - A: 0.05% Acetic acid in type-1 water

Pump – B: 0.05% Acetic acid in Methanol

Preparation of 0.05% Acetic Acid in Water: Take 0.5mL concentrated acetic acid in 1000mL of volumetric flask and make up with type 1 water, mix well and sonicate.

Preparation of 0.05% Acetic Acid in Methanol: Take 0.5mL concentrated acetic acid in1000mL of volumetric flask and make up with Methanol.

Preparation of 0.125M HCl: Take 10.4 ml of HCL in 1000 mL volumetric flask and finally make with type -1water, mix well and sonicate.

Preparation of 50mM 2-Nitro Benzaldehyde: Weigh approximately 0.075g of 2 Nitro Benzaldehyde in 10mL Methanol and sonicate

Preparation of 0.2M Phosphate buffer: Take 17.42g of di potassium Hydrogen Phosphate in 1000mL type 1 water.

Preparation of 0.8M Sodium Hydroxide: Take Approximately 3.20g of sodium hydroxide in 100 mL.

Preparation of Standard solutions:

Preparation of Standard Stock Solutions

Weigh and transfer equivalent amount of 10mg of standard into 10mL volumetric flask and dissolve in 2 mL Methanol and make up the volume upto the mark with the same. Calculate the Concentration of stock by considering its purity. Label the stock solution (approximately 1000 mg/ L) and store the solution at -20 ± 4 °C.

Preparation of internal standard solution

Weigh and transfer about 10 mg of AOZ D4, AMOZ D5, AHD C133, SEM C13N152, standard into 10 mL volumetric flask and dissolve in Methanol. Make up the volume with the same. Calculate the concentration of resulting solutions by considering the purity of the standards. Label the solution and store in cold room at -20 ± 4 °C.

Preparation of Standard Solution (working standard solution)

Transfer each 0.100 mL of the stock solution into 10 mL volumetric flask containing 4 mL of Methanol and make up the volume up to the mark with the same. This standard solution concentration gives

10 mg/L. Label the solution and store at -20 ± 4 °C. Further dilute standard solution with Methanol. Shelf life of working standard is 6 months.

CC alpha standard solution preparation:

Table-1

Stock conc. (mg/ L)	Volume of Stock each(mL)	Volume of diluent (mL)	Final volume (mL)	Final conc. (mg /L)
1000 (AOZ)	1.000	9.000	10.00	10.00
1000 (SEM)	1.000	9.000	10.00	10.00
1000 (AMOZ	1.000	9.000	10.00	10.00
1000 (AHD)	1.000	9.000	10.00	10.00
10 (AOZ)	0.100	9.900	10.00	0.100
10 (SEM)	0.100	9.900	10.00	0.100
10 (AMOZ)	0.100	9.900	10.00	0.100
10 (AHD)	0.100	9.900	10.00	0.100
0.1 (AOZ)	0.200	0.800	1.00	0.020
0.1 (SEM)	0.200	0.800	1.00	0.020
0.1 (AMOZ)	0.200	0.800	1.00	0.020
0.1 (AHD)	0.200	0.800	1.00	0.020

Conc. of analyte (µg/L)	Volume of stock(mL)	Volume of Diluent(mL)	Final Volume (mL)	Final Standard Conc. (µg/ L)
1000.000	0.100	0.900	1.000	100.00
100.000	0.100	0.900	1.000	10.00
10.000	0.100	0.900	1.000	1.00

Conc. of analyte (µg/L)	Volume of stock(mL)	Volume of Diluent(mL)	Final Volume (mL)	Final Standard Conc. (µg/ L)
500.00	0.080	0.920	1.000	40.00
500.00	0.060	0.940	1.000	30.00
100.000	0.200	0.800	1.000	20.00
100.00	0.150	0.850	1.000	15.00
100.00	0.100	0.900	1.000	10.00
100.00	0.050	0.950	1.000	5.00
AOZ(C1-20)	0.075	0.925	1.000	1.50
AHD, AMOZ,SEM (C2,C3,C4-20)	0.050	0.950	1.000	1.00
AMOZ,AOZ(20)	0.055	0.945	1.000	1.10
SEM (20)	0.070	0.930	1.000	1.40
AHD(20)	0.060	0.940	1.000	1.20

Internal Standard Preparation (Mix):

Preparation of matrix calibration curve (CC) standards

Prepare following concentrations ranging from 0 to 4.00 µg /kg in matrix using final concentrations from Table-9 and label them as CC0 to CC7.

Concentration of the standard solution (µg/ L)	Weight of matrix(g)	Volume of Stock(mL)	Volume of IS mix added from 40µg/ L (mL)	Final conc (µg/ kg)	Label
40	2.000	0.200	0.100	4.0	CC-7
30	2.000	0.200	0.100	3.0	CC-6
20	2.000	0.200	0.100	2.0	CC-5
15	2.000	0.200	0.100	1.5	CC-4
10	2.000	0.200	0.100	1.0	CC-3
5	2.000	0.200	0.100	0.5	CC-2
*AOZ1.5	2.000	0.200	0.100	0.15	AOZ0.15
*AMOZ1.0	2.000	0.200	0.100	0.10	AMOZ0.10
*AHD1.0	2.000	0.200	0.100	0.10	AHD0.10
*SEM1.0	2.000	0.200	0.100	0.10	SEM0.10
#AMOZ,AOZ1.1	2.000	0.200	0.100	0.11	AMOZ,AOZ0.11
# SEM 1.4	2.000	0.200	0.100	0.14	SEM 0.14
#AHD 1.2	2.000	0.200	0.100	0.12	AHD0.12

Sample Storage and Procedure of sample extraction:

On receipt, the sample should be stored below -18 ^{0}C

Procedure of Sample Extraction:

• Weigh homogenized sample (Shrimp) 2.00 g ± 0.1g into a 50 mL centrifuge tube.

• Add 10mL of 0.125M HCl

• Add 300 µL of 50mM 2-Nitro benzaldehyde.

• Shake vigorously for few seconds

• Keep in shaking incubator for 16 hours at 37 ºC with 80 RPM.

• Wait for few minutes to reach to room temperature

• Transfer the sample solution to a 50mL centrifuge tube

• Add 15.0 mL of 0.2M Phosphate buffer and adjust the pH to 7.0 ±0.2 with 0.125M HCl, 0.8M NaOH

• Add 10 mL of Ethyl acetate and vortex for 10 minutes

• Centrifuge the sample at 4000 rpm at 20°C for 10 min

• Collect 6 mL of the supernatant layer into aria vial

• Keep for drying in Xcel Vap evaporator up to dry at 45°C under Nitrogen

• Finally reconstitute with 0.600 mL of water

• Clean-up with Iso-octane up to clear solution.

• Then transfer to auto sampler vial for loading in LC-MS/MS instrument.

Instrument Conditions of LC-MS/MS

LC-MS/MS	LCwith triplequadrapolemass spectrometer
IonSource	ESI
Polarity	PositiveIonmode
Columnoven temp	40 °C
Mobile PhaseA	0.05%acetic acidinwater
Mobile PhaseB	0.05%acetic acidinmethanol
AutosamplerTemperature	15 ºC
Column	Shim-PAC-XR-ODSIII,2mmIDx150mm
TotalRun Time	15 minutes
FlowRate	0.300 mL/ min
HeatBlockTemperature	450 ºC
DLTemperature	280 ºC
Dryinggasflow	15 L /mL

Gradient Program:

Time (min)	Channel	B %
0.10	Pump-B	20
2.00	Pump– B	20
3.00	Pump– B	30
10.00	Pump– B	50
10.50	Pump– B	100
13.00	Pump– B	100
13.10	Pump-B	20
15.00	Stop	-

MRM Conditions

COMPOUND NAME	PRECURSOR ION	PRODUCT ION	Q1*	CE*	Q3*
AOZ	236.05	134.10	-26	-13	-22
		104.10	-26	-20	-16
AOZ-D4	257.0	240.15	-13	-9.0	-24
AMOZ	334.85	291.15	-24	-13	-18
		128.15	-24	-22	-21
AMOZ-D5	340.25	296.25	-17	-12	-30
AHD	248.90	134.00	-26	-12	-23
		104.00	-17	-21	-17
AHD-C13	251.90	134.05	-27	-11	-23
SEM	209.10	166.10	-10	-11	-26
		192.05	-10	-12	-19
SEM-C13	212.05	168.10	-24	8	-10

Spike recovery

% Recovery, = (S-U) X 100/ CSA

S= Measured concentration of analyte in the matrix spike sample result

U = Measured concentration of an analyte in the unspiked sample

CSA = Spiking level

Acceptance Criteria:

- Relative Retention Time of analyte shall correspond to that the calibration solution at a tolerance of + 2.5%
- The signal to noise ratio for each diagnostic ion shall be > 3:1
- The plot of peak area ratio versus concentration should be linear with a correlation coefficient (r) > 0.990.
- A minimum of 4 identification points shall be required. In this 1 precursor ion and 2 daughter ions shall be monitored.
- Obtained recovery shall be within the limits of control chart.
- Ion Ratios.

The relative ion intensities (area ratio of qualifier and quantifier) shall be within the below mentioned Criteria.

Relative intensity (%of base peak)	**LC-MS (relative)**
>50 %	±20 %
>20 %to 50 %	±25 %
>10 %to 20 %	±30 %
<10 %	±50 %

Final antibiotic residue levels will be estimated in µg/ kg

Batch Sequence for LC-MSMS:

Name of the Parameter	**Number of Injections**
Reagent Blank	1
Matrix Blank	1
Matrix Calibration Curve	7
Matrix Blank	1
Sample	1
MatrixBlank	1
Spike/QC	1

Spike / QC sample shall be kept after every 10 samples in a sequence there is no need of considering Blank injections if injected before or after sample injections.

Data Processing

Concentration of the analytes shall be generated through software auto-integration. Calculate the concentration of the unknown from the equation given below using regression analysis of spiked matrix calibration standard with the reciprocate of the analyte concentration as weighing factor is 1/C2.

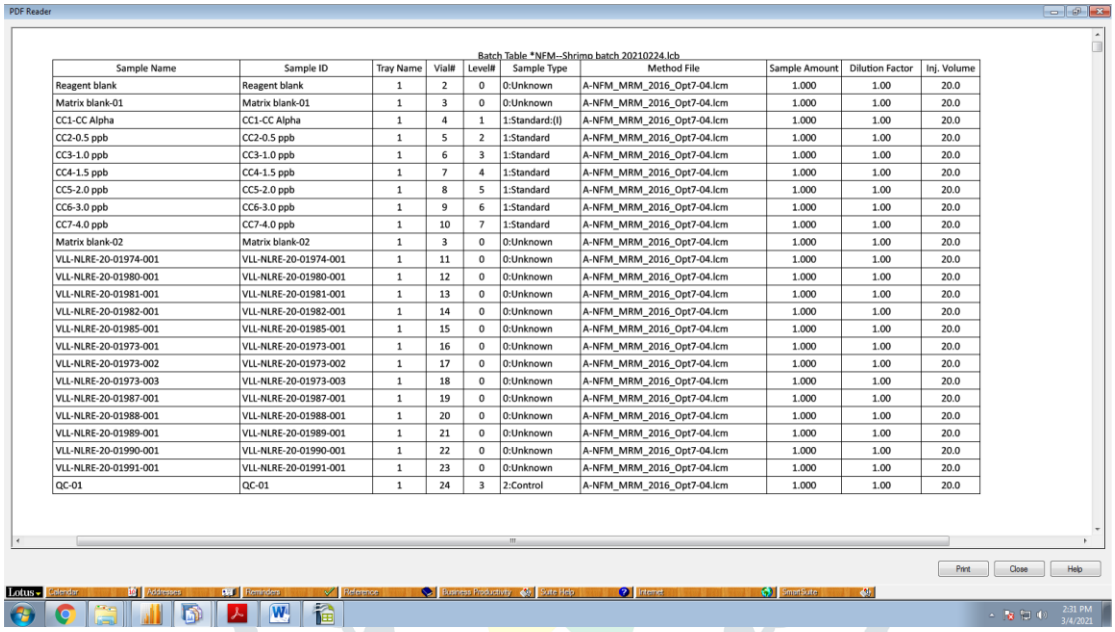
$y = mx + c$

Where, y = analyte area,

x = concentration of analyte

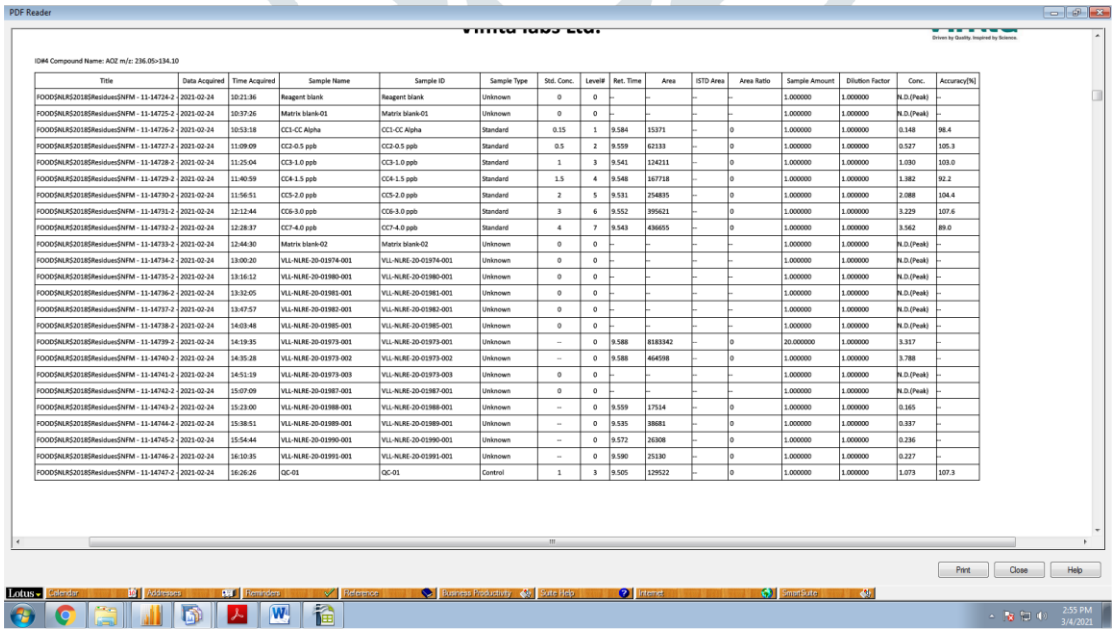
m = slope of the calibration curve,

c = y-axis intercept value



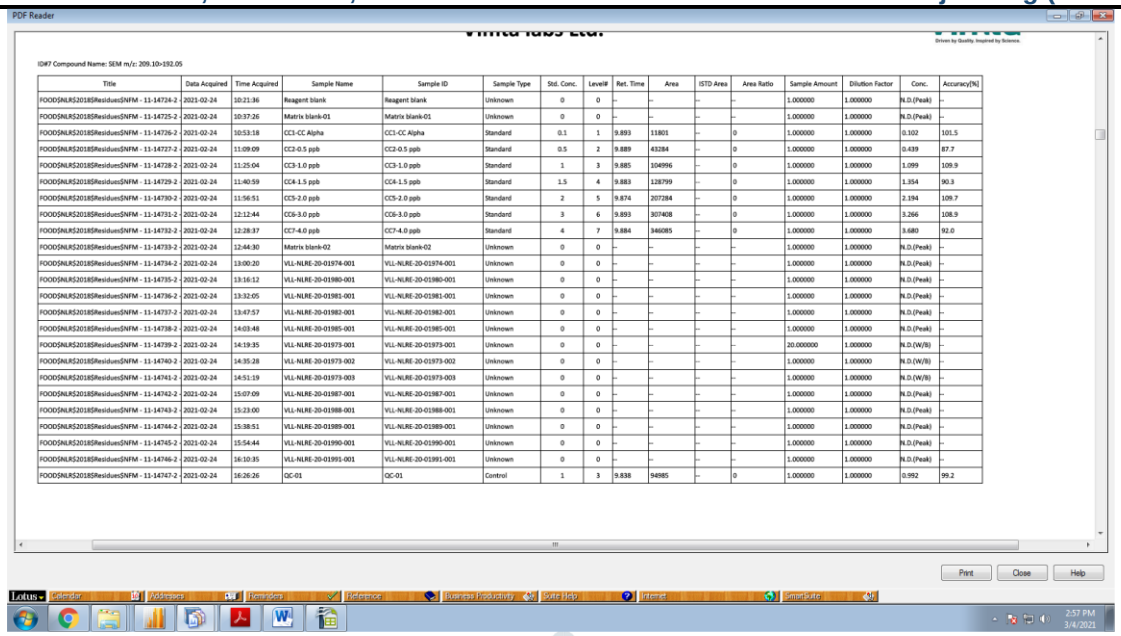
Batch Table *NFM--Shrimp batch 20210224.lcb

Sample Name	Sample ID	Tray Name	Vial#	Level#	Sample Type	Method File	Sample Amount	Dilution Factor	Inj. Volume
Reagent blank	Reagent blank	1	2	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
Matrix blank-01	Matrix blank-01	1	3	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
CC1-CC Alpha	CC1-CC Alpha	1	4	1	1:Standard:(I)	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
CC2-0.5 ppb	CC2-0.5 ppb	1	5	2	1:Standard	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
CC3-1.0 ppb	CC3-1.0 ppb	1	6	3	1:Standard	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
CC4-1.5 ppb	CC4-1.5 ppb	1	7	4	1:Standard	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
CC5-2.0 ppb	CC5-2.0 ppb	1	8	5	1:Standard	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
CC6-3.0 ppb	CC6-3.0 ppb	1	9	6	1:Standard	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
CC7-4.0 ppb	CC7-4.0 ppb	1	10	7	1:Standard	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
Matrix blank-02	Matrix blank-02	1	3	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01974-001	VLL-NLRE-20-01974-001	1	11	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01980-001	VLL-NLRE-20-01980-001	1	12	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01981-001	VLL-NLRE-20-01981-001	1	13	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01982-001	VLL-NLRE-20-01982-001	1	14	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01985-001	VLL-NLRE-20-01985-001	1	15	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01973-001	VLL-NLRE-20-01973-001	1	16	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01973-002	VLL-NLRE-20-01973-002	1	17	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01973-003	VLL-NLRE-20-01973-003	1	18	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01987-001	VLL-NLRE-20-01987-001	1	19	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01988-001	VLL-NLRE-20-01988-001	1	20	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01989-001	VLL-NLRE-20-01989-001	1	21	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01990-001	VLL-NLRE-20-01990-001	1	22	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
VLL-NLRE-20-01991-001	VLL-NLRE-20-01991-001	1	23	0	0:Unknown	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0
QC-01	QC-01	1	24	3	2:Control	A-NFM_MRM_2016_Opt7-04.lcm	1.000	1.00	20.0



ID#4 Compound Name: AOZ m/z: 236.05>134.10

Title	Data Acquired	Time Acquired	Sample Name	Sample ID	Sample Type	Std. Conc.	Level#	Ret. Time	Area	ISTD Area	Area Ratio	Sample Amount	Dilution Factor	Conc.	Accuracy[%]
FOOD$NLRS2018$ResiduesSNFM - 11-14724-2	2021-02-24	10:21:36	Reagent blank	Reagent blank	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14725-2	2021-02-24	10:37:26	Matrix blank-01	Matrix blank-01	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14726-2	2021-02-24	10:53:18	CC1-CC Alpha	CC1-CC Alpha	Standard	0.15	1	9.584	15371	--	0	1.000000	1.000000	0.148	98.4
FOOD$NLRS2018$ResiduesSNFM - 11-14727-2	2021-02-24	11:09:09	CC2-0.5 ppb	CC2-0.5 ppb	Standard	0.5	2	9.559	62133	--	0	1.000000	1.000000	0.527	105.3
FOOD$NLRS2018$ResiduesSNFM - 11-14728-2	2021-02-24	11:25:04	CC3-1.0 ppb	CC3-1.0 ppb	Standard	1	3	9.541	124211	--	0	1.000000	1.000000	1.030	103.0
FOOD$NLRS2018$ResiduesSNFM - 11-14729-2	2021-02-24	11:40:59	CC4-1.5 ppb	CC4-1.5 ppb	Standard	1.5	4	9.548	167718	--	0	1.000000	1.000000	1.382	92.2
FOOD$NLRS2018$ResiduesSNFM - 11-14730-2	2021-02-24	11:56:51	CC5-2.0 ppb	CC5-2.0 ppb	Standard	2	5	9.531	254835	--	0	1.000000	1.000000	2.088	104.4
FOOD$NLRS2018$ResiduesSNFM - 11-14731-2	2021-02-24	12:12:44	CC6-3.0 ppb	CC6-3.0 ppb	Standard	3	6	9.552	395621	--	0	1.000000	1.000000	3.229	107.6
FOOD$NLRS2018$ResiduesSNFM - 11-14732-2	2021-02-24	12:28:37	CC7-4.0 ppb	CC7-4.0 ppb	Standard	4	7	9.543	436655	--	0	1.000000	1.000000	3.562	89.0
FOOD$NLRS2018$ResiduesSNFM - 11-14733-2	2021-02-24	12:44:30	Matrix blank-02	Matrix blank-02	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14734-2	2021-02-24	13:00:20	VLL-NLRE-20-01974-001	VLL-NLRE-20-01974-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14735-2	2021-02-24	13:16:12	VLL-NLRE-20-01980-001	VLL-NLRE-20-01980-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14736-2	2021-02-24	13:32:05	VLL-NLRE-20-01981-001	VLL-NLRE-20-01981-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14737-2	2021-02-24	13:47:57	VLL-NLRE-20-01982-001	VLL-NLRE-20-01982-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14738-2	2021-02-24	14:03:48	VLL-NLRE-20-01985-001	VLL-NLRE-20-01985-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14739-2	2021-02-24	14:19:35	VLL-NLRE-20-01973-001	VLL-NLRE-20-01973-001	Unknown	--	0	9.588	8183342	--	0	20.000000	1.000000	3.317	--
FOOD$NLRS2018$ResiduesSNFM - 11-14740-2	2021-02-24	14:35:28	VLL-NLRE-20-01973-002	VLL-NLRE-20-01973-002	Unknown	--	0	9.588	464598	--	0	1.000000	1.000000	3.788	--
FOOD$NLRS2018$ResiduesSNFM - 11-14741-2	2021-02-24	14:51:19	VLL-NLRE-20-01973-003	VLL-NLRE-20-01973-003	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14742-2	2021-02-24	15:07:09	VLL-NLRE-20-01987-001	VLL-NLRE-20-01987-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14743-2	2021-02-24	15:23:00	VLL-NLRE-20-01988-001	VLL-NLRE-20-01988-001	Unknown	--	0	9.559	17514	--	0	1.000000	1.000000	0.165	--
FOOD$NLRS2018$ResiduesSNFM - 11-14744-2	2021-02-24	15:38:51	VLL-NLRE-20-01989-001	VLL-NLRE-20-01989-001	Unknown	--	0	9.535	38681	--	0	1.000000	1.000000	0.337	--
FOOD$NLRS2018$ResiduesSNFM - 11-14745-2	2021-02-24	15:54:44	VLL-NLRE-20-01990-001	VLL-NLRE-20-01990-001	Unknown	--	0	9.572	26308	--	0	1.000000	1.000000	0.236	--
FOOD$NLRS2018$ResiduesSNFM - 11-14746-2	2021-02-24	16:10:35	VLL-NLRE-20-01991-001	VLL-NLRE-20-01991-001	Unknown	--	0	9.590	25130	--	0	1.000000	1.000000	0.227	--
FOOD$NLRS2018$ResiduesSNFM - 11-14747-2	2021-02-24	16:26:26	QC-01	QC-01	Control	1	3	9.505	129522	--	0	1.000000	1.000000	1.073	107.3

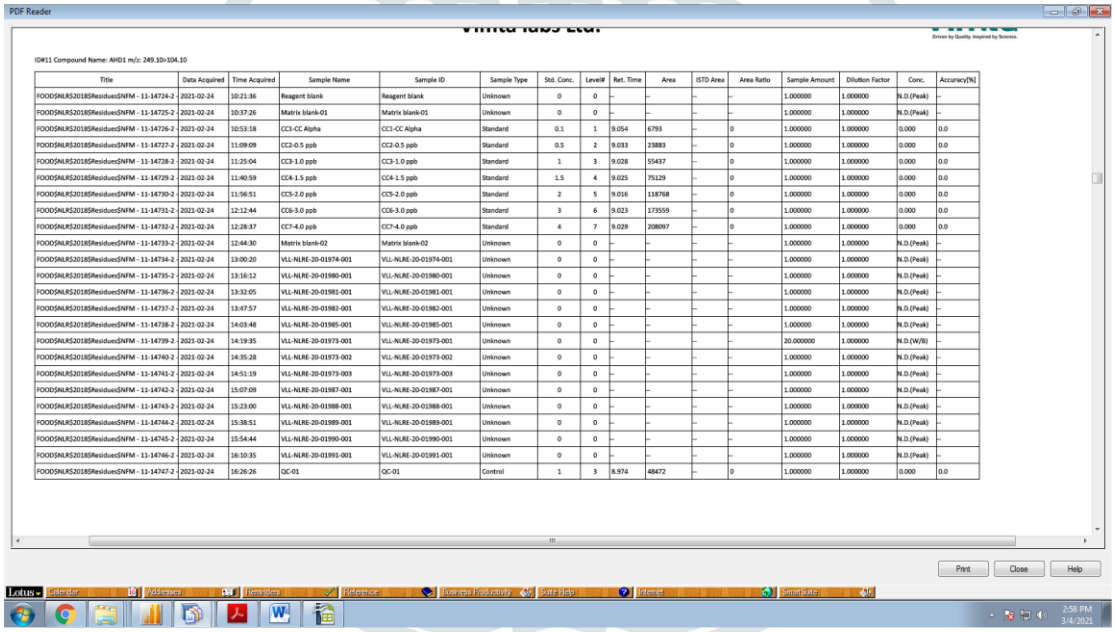


PDF Reader

ID#7 Compound Name: SEM m/z: 209.10>192.05

Title	Data Acquired	Time Acquired	Sample Name	Sample ID	Sample Type	Std. Conc.	Level#	Ret. Time	Area	ISTD Area	Area Ratio	Sample Amount	Dilution Factor	Conc.	Accuracy[%]
FOOD$NLRS2018$ResiduesSNFM - 11-14724-2	2021-02-24	10:21:36	Reagent blank	Reagent blank	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14725-2	2021-02-24	10:37:26	Matrix blank-01	Matrix blank-01	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14726-2	2021-02-24	10:53:18	CC1-CC Alpha	CC1-CC Alpha	Standard	0.1	1	9.893	11801	--	0	1.000000	1.000000	0.102	101.5
FOOD$NLRS2018$ResiduesSNFM - 11-14727-2	2021-02-24	11:09:09	CC2-0.5 ppb	CC2-0.5 ppb	Standard	0.5	2	9.889	43284	--	0	1.000000	1.000000	0.439	87.7
FOOD$NLRS2018$ResiduesSNFM - 11-14728-2	2021-02-24	11:25:04	CC3-1.0 ppb	CC3-1.0 ppb	Standard	1	3	9.885	104996	--	0	1.000000	1.000000	1.099	109.9
FOOD$NLRS2018$ResiduesSNFM - 11-14729-2	2021-02-24	11:40:59	CC4-1.5 ppb	CC4-1.5 ppb	Standard	1.5	4	9.883	128799	--	0	1.000000	1.000000	1.354	90.3
FOOD$NLRS2018$ResiduesSNFM - 11-14730-2	2021-02-24	11:56:51	CC5-2.0 ppb	CC5-2.0 ppb	Standard	2	5	9.874	207284	--	0	1.000000	1.000000	2.194	109.7
FOOD$NLRS2018$ResiduesSNFM - 11-14731-2	2021-02-24	12:12:44	CC6-3.0 ppb	CC6-3.0 ppb	Standard	3	6	9.893	307408	--	0	1.000000	1.000000	3.266	108.9
FOOD$NLRS2018$ResiduesSNFM - 11-14732-2	2021-02-24	12:28:37	CC7-4.0 ppb	CC7-4.0 ppb	Standard	4	7	9.884	346085	--	0	1.000000	1.000000	3.680	92.0
FOOD$NLRS2018$ResiduesSNFM - 11-14733-2	2021-02-24	12:44:30	Matrix blank-02	Matrix blank-02	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14734-2	2021-02-24	13:00:20	VLL-NLRE-20-01974-001	VLL-NLRE-20-01974-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14735-2	2021-02-24	13:16:12	VLL-NLRE-20-01980-001	VLL-NLRE-20-01980-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14736-2	2021-02-24	13:32:05	VLL-NLRE-20-01981-001	VLL-NLRE-20-01981-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14737-2	2021-02-24	13:47:57	VLL-NLRE-20-01982-001	VLL-NLRE-20-01982-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14738-2	2021-02-24	14:03:48	VLL-NLRE-20-01985-001	VLL-NLRE-20-01985-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14739-2	2021-02-24	14:19:35	VLL-NLRE-20-01973-001	VLL-NLRE-20-01973-001	Unknown	0	0	--	--	--	--	20.000000	1.000000	N.D.(W/B)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14740-2	2021-02-24	14:35:28	VLL-NLRE-20-01973-002	VLL-NLRE-20-01973-002	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(W/B)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14741-2	2021-02-24	14:51:19	VLL-NLRE-20-01973-003	VLL-NLRE-20-01973-003	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(W/B)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14742-2	2021-02-24	15:07:09	VLL-NLRE-20-01987-001	VLL-NLRE-20-01987-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14743-2	2021-02-24	15:23:00	VLL-NLRE-20-01988-001	VLL-NLRE-20-01988-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14744-2	2021-02-24	15:38:51	VLL-NLRE-20-01989-001	VLL-NLRE-20-01989-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14745-2	2021-02-24	15:54:44	VLL-NLRE-20-01990-001	VLL-NLRE-20-01990-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14746-2	2021-02-24	16:10:35	VLL-NLRE-20-01991-001	VLL-NLRE-20-01991-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14747-2	2021-02-24	16:26:26	QC-01	QC-01	Control	1	3	9.838	94985	--	0	1.000000	1.000000	0.992	99.2

Print Close Help

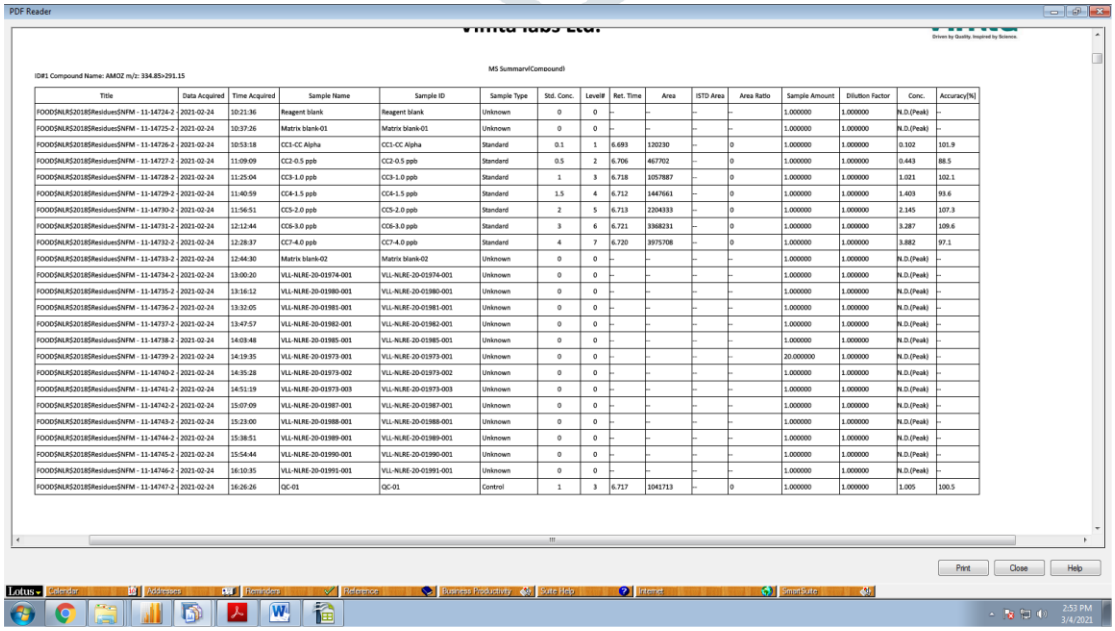


PDF Reader

ID#11 Compound Name: AHD1 m/z: 249.10>104.10

Title	Data Acquired	Time Acquired	Sample Name	Sample ID	Sample Type	Std. Conc.	Level#	Ret. Time	Area	ISTD Area	Area Ratio	Sample Amount	Dilution Factor	Conc.	Accuracy[%]
FOOD$NLRS2018$ResiduesSNFM - 11-14724-2	2021-02-24	10:21:36	Reagent blank	Reagent blank	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14725-2	2021-02-24	10:37:26	Matrix blank-01	Matrix blank-01	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14726-2	2021-02-24	10:53:18	CC1-CC Alpha	CC1-CC Alpha	Standard	0.1	1	9.054	6793	--	0	1.000000	1.000000	0.000	0.0
FOOD$NLRS2018$ResiduesSNFM - 11-14727-2	2021-02-24	11:09:09	CC2-0.5 ppb	CC2-0.5 ppb	Standard	0.5	2	9.033	23883	--	0	1.000000	1.000000	0.000	0.0
FOOD$NLRS2018$ResiduesSNFM - 11-14728-2	2021-02-24	11:25:04	CC3-1.0 ppb	CC3-1.0 ppb	Standard	1	3	9.028	55437	--	0	1.000000	1.000000	0.000	0.0
FOOD$NLRS2018$ResiduesSNFM - 11-14729-2	2021-02-24	11:40:59	CC4-1.5 ppb	CC4-1.5 ppb	Standard	1.5	4	9.025	75129	--	0	1.000000	1.000000	0.000	0.0
FOOD$NLRS2018$ResiduesSNFM - 11-14730-2	2021-02-24	11:56:51	CC5-2.0 ppb	CC5-2.0 ppb	Standard	2	5	9.016	118768	--	0	1.000000	1.000000	0.000	0.0
FOOD$NLRS2018$ResiduesSNFM - 11-14731-2	2021-02-24	12:12:44	CC6-3.0 ppb	CC6-3.0 ppb	Standard	3	6	9.023	173559	--	0	1.000000	1.000000	0.000	0.0
FOOD$NLRS2018$ResiduesSNFM - 11-14732-2	2021-02-24	12:28:37	CC7-4.0 ppb	CC7-4.0 ppb	Standard	4	7	9.029	208097	--	0	1.000000	1.000000	0.000	0.0
FOOD$NLRS2018$ResiduesSNFM - 11-14733-2	2021-02-24	12:44:30	Matrix blank-02	Matrix blank-02	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14734-2	2021-02-24	13:00:20	VLL-NLRE-20-01974-001	VLL-NLRE-20-01974-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14735-2	2021-02-24	13:16:12	VLL-NLRE-20-01980-001	VLL-NLRE-20-01980-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14736-2	2021-02-24	13:32:05	VLL-NLRE-20-01981-001	VLL-NLRE-20-01981-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14737-2	2021-02-24	13:47:57	VLL-NLRE-20-01982-001	VLL-NLRE-20-01982-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14738-2	2021-02-24	14:03:48	VLL-NLRE-20-01985-001	VLL-NLRE-20-01985-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14739-2	2021-02-24	14:19:35	VLL-NLRE-20-01973-001	VLL-NLRE-20-01973-001	Unknown	0	0	--	--	--	--	20.000000	1.000000	N.D.(W/B)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14740-2	2021-02-24	14:35:28	VLL-NLRE-20-01973-002	VLL-NLRE-20-01973-002	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14741-2	2021-02-24	14:51:19	VLL-NLRE-20-01973-003	VLL-NLRE-20-01973-003	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14742-2	2021-02-24	15:07:09	VLL-NLRE-20-01987-001	VLL-NLRE-20-01987-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14743-2	2021-02-24	15:23:00	VLL-NLRE-20-01988-001	VLL-NLRE-20-01988-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14744-2	2021-02-24	15:38:51	VLL-NLRE-20-01989-001	VLL-NLRE-20-01989-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14745-2	2021-02-24	15:54:44	VLL-NLRE-20-01990-001	VLL-NLRE-20-01990-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14746-2	2021-02-24	16:10:35	VLL-NLRE-20-01991-001	VLL-NLRE-20-01991-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14747-2	2021-02-24	16:26:26	QC-01	QC-01	Control	1	3	8.974	48472	--	0	1.000000	1.000000	0.000	0.0

Print Close Help



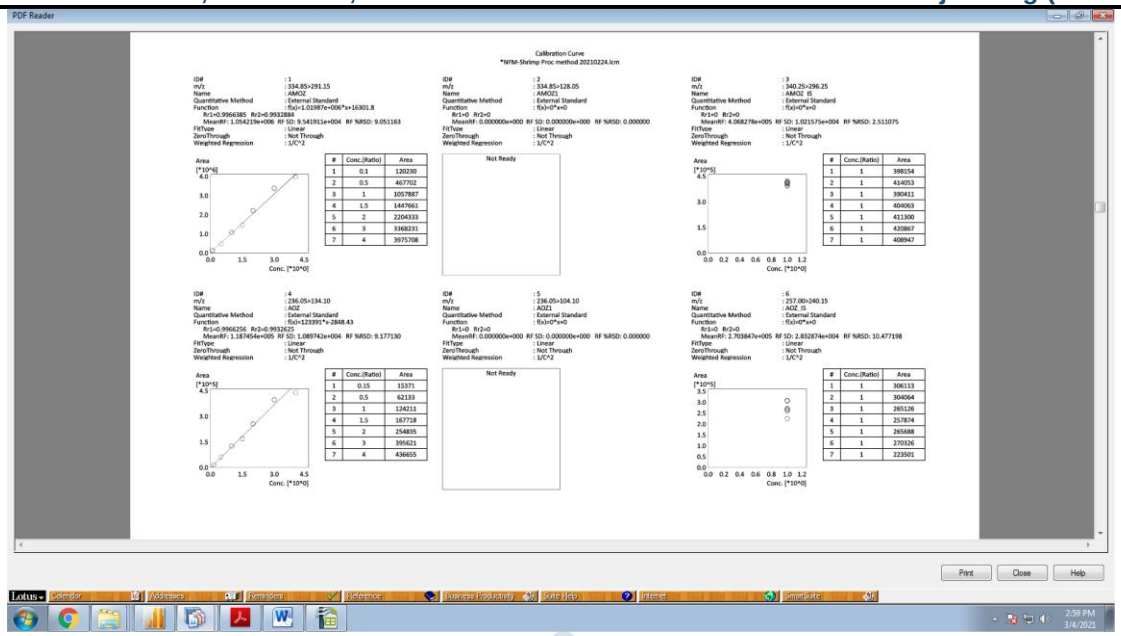
PDF Reader

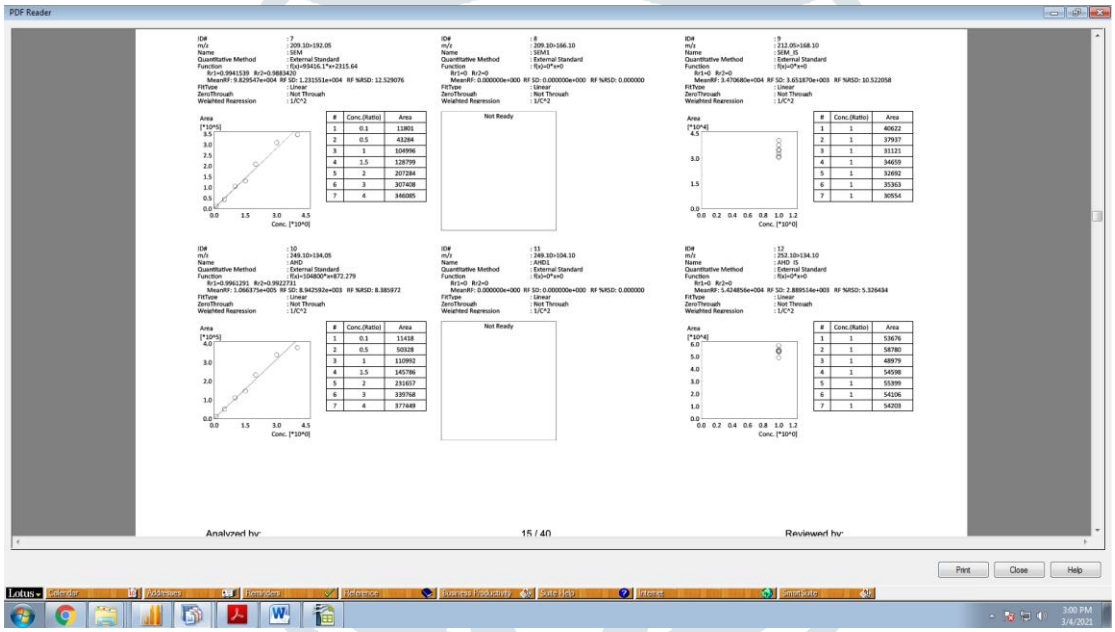
MS Summary\Compound\

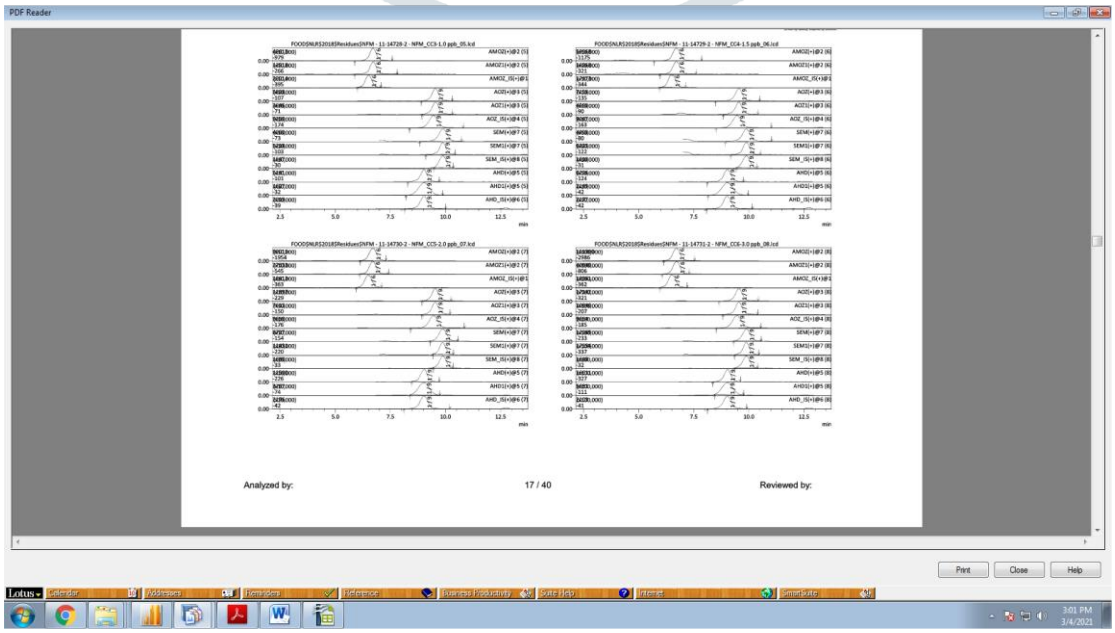
ID#1 Compound Name: AMOZ m/z: 334.85>291.15

Title	Data Acquired	Time Acquired	Sample Name	Sample ID	Sample Type	Std. Conc.	Level#	Ret. Time	Area	ISTD Area	Area Ratio	Sample Amount	Dilution Factor	Conc.	Accuracy[%]
FOOD$NLRS2018$ResiduesSNFM - 11-14724-2	2021-02-24	10:21:36	Reagent blank	Reagent blank	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14725-2	2021-02-24	10:37:26	Matrix blank-01	Matrix blank-01	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14726-2	2021-02-24	10:53:18	CC1-CC Alpha	CC1-CC Alpha	Standard	0.1	1	6.693	120230	--	0	1.000000	1.000000	0.102	101.9
FOOD$NLRS2018$ResiduesSNFM - 11-14727-2	2021-02-24	11:09:09	CC2-0.5 ppb	CC2-0.5 ppb	Standard	0.5	2	6.706	467702	--	0	1.000000	1.000000	0.443	88.5
FOOD$NLRS2018$ResiduesSNFM - 11-14728-2	2021-02-24	11:25:04	CC3-1.0 ppb	CC3-1.0 ppb	Standard	1	3	6.718	1057887	--	0	1.000000	1.000000	1.021	102.1
FOOD$NLRS2018$ResiduesSNFM - 11-14729-2	2021-02-24	11:40:59	CC4-1.5 ppb	CC4-1.5 ppb	Standard	1.5	4	6.712	1447661	--	0	1.000000	1.000000	1.403	93.6
FOOD$NLRS2018$ResiduesSNFM - 11-14730-2	2021-02-24	11:56:51	CC5-2.0 ppb	CC5-2.0 ppb	Standard	2	5	6.713	2204333	--	0	1.000000	1.000000	2.145	107.3
FOOD$NLRS2018$ResiduesSNFM - 11-14731-2	2021-02-24	12:12:44	CC6-3.0 ppb	CC6-3.0 ppb	Standard	3	6	6.721	3368231	--	0	1.000000	1.000000	3.287	109.6
FOOD$NLRS2018$ResiduesSNFM - 11-14732-2	2021-02-24	12:28:37	CC7-4.0 ppb	CC7-4.0 ppb	Standard	4	7	6.720	3975708	--	0	1.000000	1.000000	3.882	97.1
FOOD$NLRS2018$ResiduesSNFM - 11-14733-2	2021-02-24	12:44:30	Matrix blank-02	Matrix blank-02	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14734-2	2021-02-24	13:00:20	VLL-NLRE-20-01974-001	VLL-NLRE-20-01974-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14735-2	2021-02-24	13:16:12	VLL-NLRE-20-01980-001	VLL-NLRE-20-01980-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14736-2	2021-02-24	13:32:05	VLL-NLRE-20-01981-001	VLL-NLRE-20-01981-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14737-2	2021-02-24	13:47:57	VLL-NLRE-20-01982-001	VLL-NLRE-20-01982-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14738-2	2021-02-24	14:03:48	VLL-NLRE-20-01985-001	VLL-NLRE-20-01985-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14739-2	2021-02-24	14:19:35	VLL-NLRE-20-01973-001	VLL-NLRE-20-01973-001	Unknown	0	0	--	--	--	--	20.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14740-2	2021-02-24	14:35:28	VLL-NLRE-20-01973-002	VLL-NLRE-20-01973-002	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14741-2	2021-02-24	14:51:19	VLL-NLRE-20-01973-003	VLL-NLRE-20-01973-003	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14742-2	2021-02-24	15:07:09	VLL-NLRE-20-01987-001	VLL-NLRE-20-01987-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14743-2	2021-02-24	15:23:00	VLL-NLRE-20-01988-001	VLL-NLRE-20-01988-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14744-2	2021-02-24	15:38:51	VLL-NLRE-20-01989-001	VLL-NLRE-20-01989-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14745-2	2021-02-24	15:54:44	VLL-NLRE-20-01990-001	VLL-NLRE-20-01990-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14746-2	2021-02-24	16:10:35	VLL-NLRE-20-01991-001	VLL-NLRE-20-01991-001	Unknown	0	0	--	--	--	--	1.000000	1.000000	N.D.(Peak)	--
FOOD$NLRS2018$ResiduesSNFM - 11-14747-2	2021-02-24	16:26:26	QC-01	QC-01	Control	1	3	6.717	1041713	--	0	1.000000	1.000000	1.005	100.5

Print Close Help


PDF Reader
Calibration Curve
*NFM-Shrimp Proc method 20210224.lcm
Not Ready
Print
Close
Help


PDF Reader
Not Ready
Analyzed by:
15 / 40
Reviewed by:
Print
Close
Help


PDF Reader
Analyzed by:
17 / 40
Reviewed by:
Print
Close
Help

DISCUSSION:

A quick and sensitive analytical method was developed, optimized, and validated for the simultaneous determination of four NF metabolites in fish samples by HPLC. When the sample was acidified and derivatized using ultrasound-assisted technology, under acidic conditions at 60 °C, acid hydrolysis and derivatization reactions proceeded quickly and completed within 2 h. During the pre-treatment process, a one-pot method was used for acid hydrolysis and derivatization, which simplifies the pre-treatment steps, and constant-temperature ultrasound assisted derivatization is used, which shortens the derivatization time. For the determination of NF metabolites in fish, the HPLC detection method is cheaper and simpler than the LC-MS method, so this analysis method can be better popularized and used in developing countries and regions.

REFERENCES:

1. Toussaint B, Chedin M, Bordin G, Rodriguez AR.J Chromatogr A. 2005 Sep 23; 1088(1-2):32-9. doi: 10.1016/j.chroma.2005.02.057.PMID: 16130730.
2. Yves-Alexis Hammel , Rayane Mohamed, Eric Gremaud, Marie-Helene LeBreton, Philippe A Guy PMID: 18054364 DOI: 10.1016/j.chroma.2007.10.112 .
3. Draisci R, Palleschi L, Ferretti E, Achene L, Cecilia A. J Chromatogr A. 2001 Aug 10; 926(1):97-104. doi: 10.1016/s0021-9673(01)00838-x. PMID: 11554423
4. LieveOkerman , Katia De Wasch, Jan Van Hoof, Walter SmedtsPMID: **12723911**
5. So Young Won, Chang Hee Lee, HyeSook Chang, Dong Sul Kim , July 2011, Food Control 22(7):1101-1107, DOI: 10.1016/j.foodcont.2011.01.005.
6. Lundgren, R., D. J. Staples, S. J. Funge-Smith, and J. Clausen. 2006. “Status and Potential of Fisheries and Aquaculture in Asia and the Pacific.” FAO Regional Office for Asia and the Pacific.” RAP PUBLICATION 2006/22. Retrieved from:http://www.fao.org/tempref/docrep/fao/009/ag110e/ag110e00.pdf.
7. Stentiford, G. D., K. Sritunyalucksana, T. W. Flegel, B. A. Williams, B. Withyachumnarnkul, O. Itsathitphaisarn, and D. Bass. 2017. “New Paradigms to Help Solve the Global Aquaculture Disease Crisis.” *PLoS Pathogens*13 (2): e1006160. doi:https://doi.org/10.1371/journal.ppat.1006160.
8. McCallum, H., Harvell, D. &Dobson, A. (2003) Rates of spread of marine pathogens. Ecology Letters, 6, 1062–1067.
9. Oliveira, R. A., R. McDonough, S. Matser, A. Khatikarn, J. Satapornvanit, K. Nogueira, A. J. A. Soares, A. M. V. M. Domingues, I., and P. J. Van Den Vrink. 2014. “Use, Fate and Ecological Risks of Antibiotics Applied in Tilapia Cage Farming in Thailand.” Environmental Pollution192: 8–16. Doi:https://doi.org/10.1016/j.envpol.2014.04.002. [Crossref], [GoogleScholar].
10. Xiong, W., Y. Sun, T. Zhang, X. Ding, Y. Li, M. Wang, and Z. Zeng. 2015. “Antibiotics, Antibiotic Resistance Genes, and Bacterial Community Composition in Fresh Water Aquaculture Environment in China.” *Microbial Ecology*70 (2): 425–432. Doi:https://doi.org/10.1007/s00248-015-0583-x. [Crossref][PubMed][Web of Science ®], [Google Scholar].
11. Lai, W. W. P., Y. C. Lin, Y. H. Wang, Y. L. Guo, and A. Y. C. Lin. 2018. “Occurrence of Emerging Contaminants in Aquaculture Waters: Cross-Contamination between Aquaculture Systems and

Surrounding Waters." *Water, Air, and Soil Pollution*229 (8): 249. doi:https://doi.org/10.1007/s11270-018-3901-3. [Crossref][Web of Science ®], [Google Scholar]

12. De Jesus Gaffney, V., V. V. Cardoso, M. J. Benoliel, and C. M. Almeida. 2016. "Chlorination and Oxidation of Sulfonamides by Free Chlorine: Identification and Behaviour of Reaction Products by UPLC-MS/MS." *Journal of Environmental Management*166:466–477.doi:https://doi.org/10.1016/j.jenvman.2015.10.048. [Crossref][Web of Science ®], [Google Scholar].

13. Sasikaladevi, R., V. KiruthikaEswari, and I. M. Nambi. 2020. "Antibiotic Resistance and Sanitation in India: Current Situation and Future Perspectives." Antibiotic Resistance in the Environment: A Worldwide Overview 91: 217–244. Doi: https://doi.org/10.1007/698_2020_608. [Crossref], [Google Scholar].

14. Suzuki, S., and P. T. P. Hoa. 2012. "Distribution of Quinolones, Sulfonamides, Tetracyclines in Aquatic Environment and Antibiotic Resistance in Indochina." *Frontiers in Microbiology*3: 67. doi:https://doi.org/10.3389/fmicb.2012.00067. [Crossref][PubMed][Web of Science ®], [Google Scholar].

15. Romero, J., C. G. Feijoó, and P. Navarrete. 2012. "Antibiotics in Aquaculture–use, Abuse and Alternatives." Health and Environment in Aquaculture 159. doi:https://doi.org/10.5772/28157. [Crossref], [Google Scholar].

16. Shimizu, A., H. Takada, T. Koike, A. Takeshita, M. Saha, N. Nakada, A. Murata, et al. 2013. "Ubiquitous Occurrence of Sulfonamides in Tropical Asian Waters." Science of the Total Environment452: 108–115. doi:https://doi.org/10.1016/j.scitotenv.2013.02.027. [Crossref][PubMed][Web of Science ®], [Google Scholar]

17. Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities, Document No. 2002/657/EC.

18. Determination of Chloramphenicol in Fish and Fishery products by Electrospray Triple Quadrapole LC-MS/MS, FDA Lib No.4306

19. Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities, Document No. 2002/657/EC.

20. National Residue Control Plan for Aqua Culture Products Year 2017.

21. Determination of Nitrofuran Metabolites in Shrimp ,USFDA Method April 1,2004

22. Nitrofuran Metabolites by LCMSMS, USDA method CLG-NFUR 3.00.

23. Food safety manual application by Shimadzu ISO 11843