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QUANTIFICATION OF TETRAMETHYLENEDISULFOTETRAMINE (TETS) IN VARIOUS FOOD MATRICES BY SOLID-PHASE EXTRACTION LIQUID CHROMATOGRAPHY-ION TRAP MASS SPECTROMETRY

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RESEARCH AND TECHNOLOGY DIRECTORATE

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14. ABSTRACT: Tetramethylenedisulfotetramine (TETS), commonly known as tetramine, is a highly neurotoxic rodenticide (the LD ₅₀ , or lethal dose for 50% of test subjects, is 0.1 mg/kg). TETS has been used in hundreds of deliberate and accidental food poisoning events in China. In this report, we describe an extraction method for the quantitation of TETS as spiked into various food matrices, including fruit juices, egg, hot dog, chicken nuggets, turkey deli meat, and ground beef. Quantitation by liquid chromatography–ion trap mass spectrometry was based upon selected reaction monitoring of mass-to-charge ratio (<i>m/z</i>) 347 → 268 and consecutive reaction monitoring of <i>m/z</i> 347→268→175. An ~5 mg TETS concentration was spiked into each food sample. The linear range of quantitation for TETS was 0.5–20 µg/mL. Total recoveries (and percent relative standard deviations) for TETS in various food samples are reported.					
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PREFACE

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CONTENTS

1.	INTRODUCTION	1
2.	EXPERIMENTAL METHODS.....	2
2.1.	Reagents and Chemicals	2
2.2.	Instrumentation	2
2.3.	Sample Preparation and Extraction Procedure	3
2.3.1	Juice Sample Preparation.....	3
2.3.2	Egg Sample Preparation.....	3
2.3.3	Hot Dog, Chicken Nuggets, Turkey Deli Meat, and 80/20 Ground Beef Preparation	4
2.3.4	Extraction Procedures	4
3.	RESULTS AND DISCUSSION	5
3.1.	Apple and Orange Juices	5
3.2.	Egg Beaters Egg Whites; Whole Egg; and USDA Egg White, Salted Egg Yolk 2551, and P. Sugared Egg Yolk C24410.....	7
3.3.	Hot Dog, Chicken Nuggets, Turkey Deli Meat, and 80/20 Ground Beef.....	9
4.	CONCLUSION.....	11
	LITERATURE CITED	13
	ACRONYMS AND ABBREVIATIONS	15
	APPENDIX: SELECTED REACTION MONITORING (SRM) AND CONSECUTIVE REACTION MONITORING (CRM) CHROMATOGRAMS AND MASS SPECTRA OF TETRAMETHYLENEDISULFOTETRAMINE (TETS) EXTRACTED FROM VARIOUS FOOD MATRICES	17

FIGURES

1.	Chemical structure of TETS	2
2.	Schematic of RediSep Rf normal-phase silica column.....	4
3.	External calibration curve for TETS in acetonitrile and water for juice analyses	5
4.	Representative (a) SRM and (b) MS results for TETS extracted from apple juice matrix.....	6
5.	External calibration curve for TETS in acetonitrile and water for various egg sample analyses	7
6.	Representative (a) CRM and (b) MS results for TETS extracted from Egg Beaters egg whites matrix.....	8
7.	External calibration curve for TETS in acetonitrile and water for various hot dog, chicken nugget, turkey deli meat, and 80/20 ground beef sample analyses	9
8.	Representative (a) CRM and (b) MS for TETS extracted from 80/20 ground beef matrix.....	10

TABLES

1.	LC–IT-MS Parameters.....	3
2.	Percent Recovery of TETS Extracted from Juice Matrices	7
3.	Percent Recovery of TETS Extracted from Various Egg Matrices	9
4.	Percent Recovery of Extracted TETS from Hot Dog, Chicken Nugget, Turkey Deli Meat, and 80/20 Ground Beef Matrices	11

QUANTIFICATION OF TETRAMETHYLENEDISULFOTETRAMINE (TETS) IN VARIOUS FOOD MATRICES BY SOLID-PHASE EXTRACTION LIQUID CHROMATOGRAPHY–ION TRAP MASS SPECTROMETRY

1. INTRODUCTION

Tetramethylenedisulfotetramine (TETS), commonly known as tetramine, is a highly neurotoxic rodenticide (the LD₅₀, or lethal dose for 50% of test subjects, is 0.1 mg/kg).¹ TETS has been used in hundreds of deliberate and accidental food poisoning events in China. Banned rodenticides illicitly imported into the United States have been responsible for numerous intentional and unintentional human poisonings.^{2–4} TETS is slightly soluble in water (0.25 mg/mL),⁵ and it is dangerous when used to deliberately contaminate food or water. TETS is not absorbed through the skin; the most common route of exposure is ingestion of contaminated foods. Thus, the development of a reliable extraction and detection technique for TETS in different foods is essential: when accidental and intentional poisonings occur, responders must be able to identify the presence of potentially toxic substances in foods.

Most of the TETS detection in food and biological sample matrices has been achieved using gas chromatography coupled with mass spectrometry (GC–MS),^{6–10} and limited literature exists regarding the direct detection of TETS in food via liquid chromatography coupled with mass spectrometry (LC–MS).¹¹ Several reports have been published describing sample preparation and cleanup techniques that rely on solid-phase microextraction,^{9,12} however, the longer extraction time (>60 min) and analysis time (>10 min) make this technique inefficient for processing large numbers of samples.

This report documents the efforts of the Agent Chemistry Team from the Research and Technology Directorate of the U.S. Army Edgewood Chemical Biological Center (ECBC; Aberdeen Proving Ground, MD) in developing new extraction and analytical detection methodologies using liquid chromatography–ion trap mass spectrometry (LC–IT-MS). The objective of this task was to provide development and laboratory support for extraction of TETS (Figure 1) from various food samples. This included detection and quantitative and qualitative analyses of complex matrices, such as foods with high salt and fat contents. In support of this objective, we examined 11 food samples using individual agents, including apple and orange juices; Egg Beaters processed egg whites; U.S. Department of Agriculture (USDA) egg white, salted egg yolk 2551, and p. sugared egg yolk C24410; whole egg; chicken nuggets; hot dog; precooked, 99% fat free turkey deli meat; and 80/20 hamburger meat (80% lean and 20% fat). The choice of food types arose from collaboration and conversations with USDA personnel and represent items that are commonly associated with school lunch programs. Foods were cleaned using commercially available normal-phase separation columns.

The use of LC–IT-MS, or any comparable high-resolution tandem mass spectrometry, has become common. From an affordability standpoint, this technology is currently within reach for most laboratories. For this work, extracted TETS was analyzed using LC–IT-MS, and percent recoveries were calculated from external calibration curves.

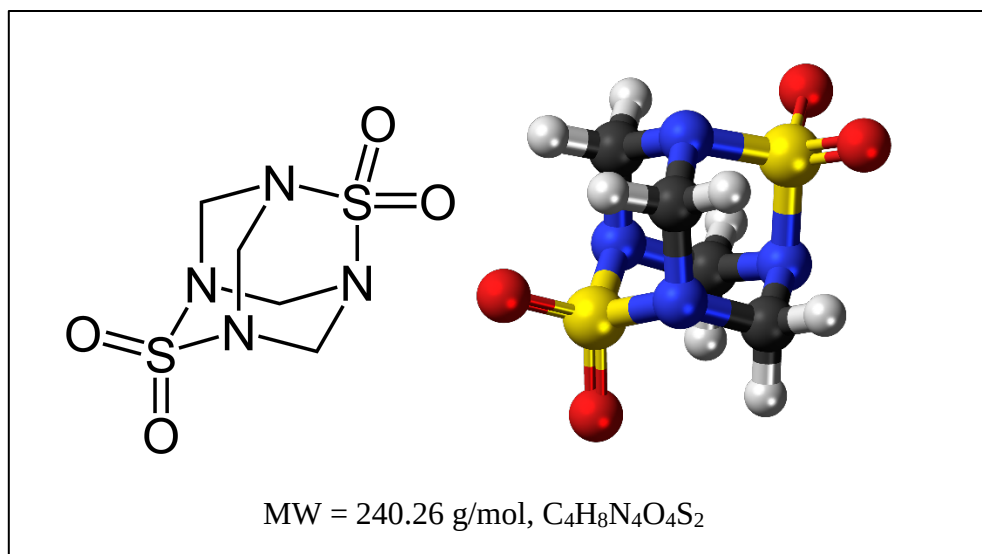


Figure 1. Chemical structure of TETS.

2. EXPERIMENTAL METHODS

2.1 Reagents and Chemicals

The TETS (>98% purity) was provided by ECBC. All reagents and solvents were LC–MS grade. Acetonitrile, water, and triethylamine (TEA) were purchased from Sigma-Aldrich (St. Louis, MO). A RediSep Rf normal-phase silica gel column (5 g) was obtained from Teledyne Isco (Lincoln, NE). Juicy Juice apple juice (Harvest Hill Beverage Company; Stamford, CT), Minute Maid orange juice (Coca Cola Company; Atlanta, GA), Egg Beaters processed egg whites (ConAgra Foods; Chicago, IL), whole egg, Smart Option chicken nuggets (Food Lion; Salisbury, NC), Esskay Oriole hot dog (Smithfield Foods; Smithfield, VA), Buddig original thin turkey deli meat (Carl Buddig and Company; Homewood, IL), and 80/20 ground beef food samples were purchased from a local grocery store (Food Lion; Edgewood, MD). Four egg samples were provided from USDA: egg white, salted egg yolk 2551, and p. sugared egg yolk C24410.

2.2 Instrumentation

All samples were characterized using an LTQ ion trap mass spectrometer (Thermo Fisher Scientific; Waltham, MA) equipped with an electrospray ionization (ESI) interface. The operating parameters are tabulated in Table 1.

Table 1. LC–IT-MS Parameters

Column: Phenomenex Gemini-NX 3 μ m, C18 50 $\times$ 2 mm				
Mobile phase A: water				
Mobile phase B: acetonitrile				
Gradient	Time (min)	A (%)	B (%)	Flow Rate (μ L/min)
	0	75	25	350
	1	75	25	
	3	5	95	
	4.5	5	95	
	4.6	75	25	
	7	75	25	
Injection volume: 5 μ L				
Sheath gas flow rate: 50 L/min				
Auxiliary gas flow rate: 20 L/min				
Sweep gas flow rate: 10 L/min				
Ion spray voltage: 3.5 kV				
Capillary temperature: 300 $^{\circ}$ C				
Capillary voltage: -50 V				
Tube lens voltage: -86 V				
Ionization mode: ESI				

2.3 Sample Preparation and Extraction Procedure

2.3.1 Juice Sample Preparation

Juicy Juice apple juice and Minute Maid orange juice were purchased from a local grocery store. Two milliliters of each sample was spiked with ~5 mg of TETS and diluted with 20 mL of acetonitrile. The food sample was then passed through a RediSep Rf column, and the eluents were collected.

2.3.2 Egg Sample Preparation

Original Egg Beaters egg whites and whole eggs were purchased from a local grocery store. USDA egg white, salted egg yolk 2551, and p. sugared egg yolk C24410 were provided by USDA. Approximately 5 g of each food sample was spiked with the desired TETS quantity and diluted with 10 mL of acetonitrile. The mixture was centrifuged for 15 min at 6000 rpm, and the supernatant was decanted. A second 10 mL portion of acetonitrile was added, and the mixture was vortexed or sonicated for 1 min and again centrifuged for 15 min at 6000 rpm. The supernatant was removed, combined, and passed through a RediSep Rf column. The eluents were collected for analysis.

2.3.3 Hot Dog, Chicken Nuggets, Turkey Deli Meat, and 80/20 Ground Beef Preparation

Esskay hot dogs, Smart Option chicken nuggets, Buddig turkey deli meat, and 80/20 ground beef were purchased from a local grocery store. Approximately 5 g of each food sample was spiked with TETS and diluted with 10 mL of acetonitrile. The whole sample was homogenized using a Kinematica Polytron homogenizer (Kinematica AG; Luzern, Switzerland) at 20,000 rpm for 1–2 min. The mixture was then centrifuged for 15 min at 6000 rpm, and the supernatant was removed. A second 10 mL portion of acetonitrile was added, and the sample was vortexed or sonicated for 1 min and centrifuged for 15 min at 6000 rpm, and the supernatant was removed. The combined supernatant was passed through a RediSep Rf column, and the eluents were collected for analysis.

2.3.4 Extraction Procedures

A packed RediSep Rf normal-phase silica gel column (shown in Figure 2) was used in this study to separate TETS from the food samples. First, the column was eluted with 25 mL of 0.1% TEA/acetonitrile, using in-house air to pass the solution through the column, and the 0.1% TEA/acetonitrile solution was collected for later use. Second, the supernatant was passed through the column, and the sample was collected. Third, 1 mL of 0.1% TEA/acetonitrile solution was added to the column and pushed slightly into the silica gel until 1 mL of the solution just cleared the top of the silica gel. This step was repeated four times. Finally, the remaining 0.1% TEA/acetonitrile solution was added to the column and passed through the bed. A small aliquot of the extracted solution was filtered through a 0.45 μm poly(tetrafluoroethylene) membrane filter. The final solution was diluted with mobile phase and analyzed using LC–MS.



Figure 2. Schematic of RediSep Rf normal-phase silica column.

3. RESULTS AND DISCUSSION

3.1 Apple and Orange Juices

The quantitation was evaluated by linear regression with an external calibration curve (Figure 3) that ranged from 0.10 to 20 $\mu\text{g/mL}$ with $n \geq 9$ measurements per standard. A standard curve was prepared at the beginning of each sequence run. In the presence of heat (i.e., mass spectrometer collision cell), TETS forms the TETS dimer,¹¹ which in turn becomes protonated before losing methylene to form an ion of mass-to-charge ratio (m/z) 347. The possible product ions were m/z 268, 227, 175, 148, and 134. TETS was analyzed using selected reaction monitoring (SRM) of the m/z 347 $\rightarrow$ 268 transition. A representative SRM for TETS in an apple juice sample is shown in Figure 4a. The mass spectrum, shown in Figure 4b, exhibits mass ions at m/z 268.09 due to $[\text{M}-\text{SO}_2\text{NH}]^-$ for TETS. The percent recovery was calculated based on an external calibration curve for TETS, and the results revealed a $>95\%$ recovery of TETS from apple juice and orange juice (Table 2).

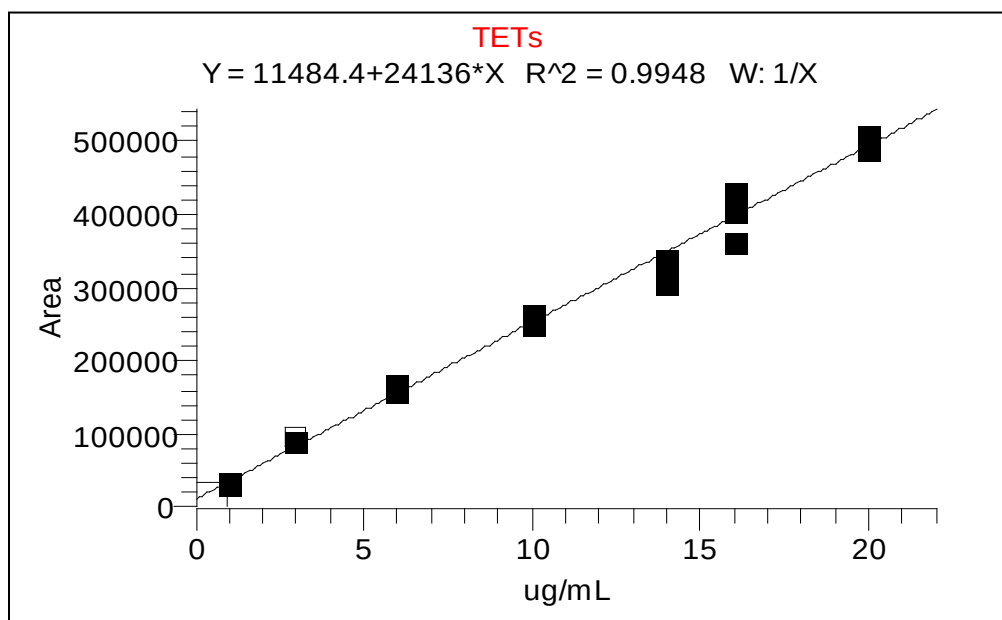


Figure 3. External calibration curve for TETS in acetonitrile and water for juice analyses.

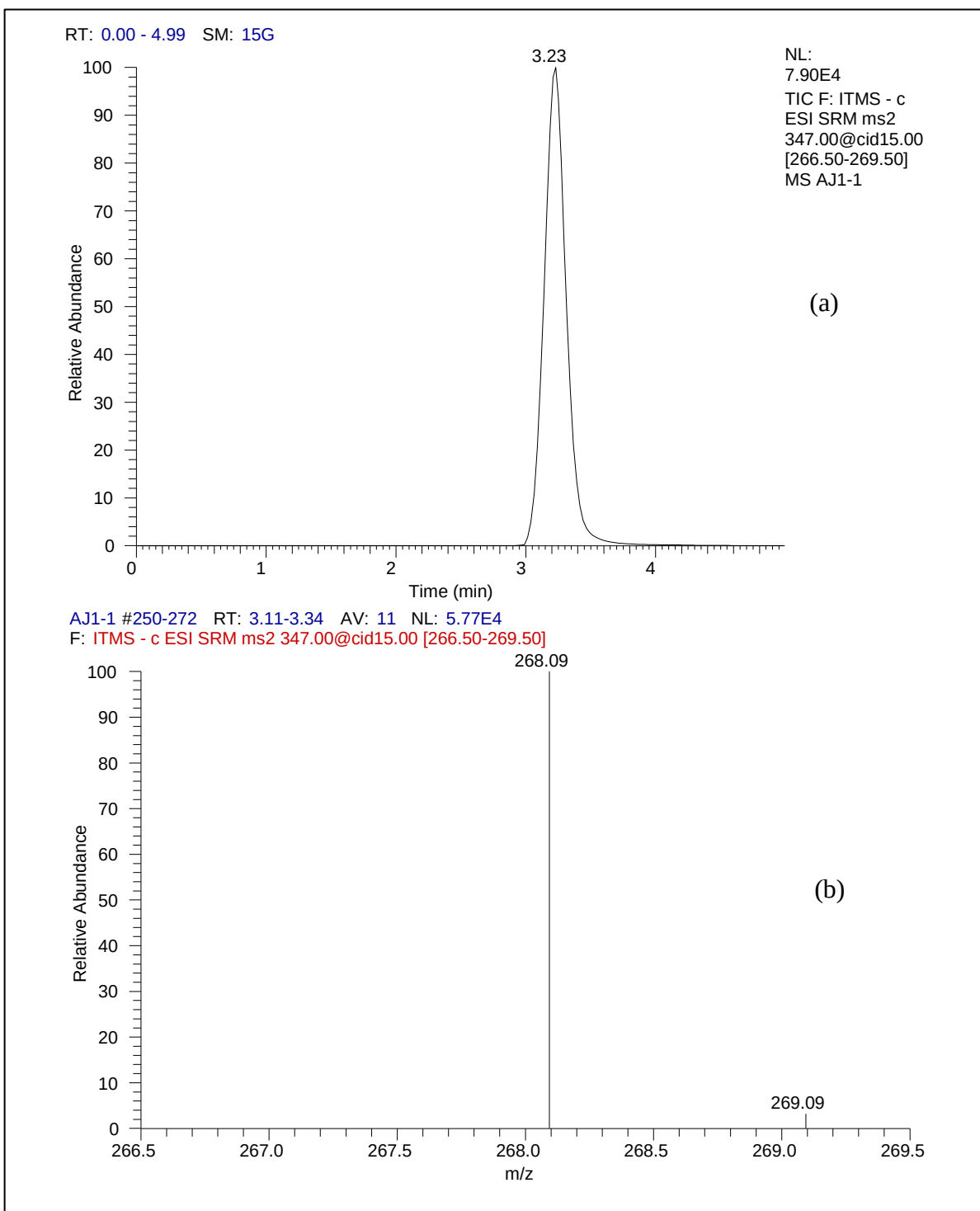


Figure 4. Representative (a) SRM and (b) MS results for TETS extracted from apple juice matrix.

Table 2. Percent Recovery of TETS Extracted from Juice Matrices

Sample	pH	Recovery (%)
Apple juice	3.5	100.7 ± 2.3
Orange juice	4.0	96.9 ± 2.0

3.2 Egg Beaters Egg Whites; Whole Egg; and USDA Egg White, Salted Egg Yolk 2551, and P. Sugared Egg Yolk C24410

The quantitation was evaluated by linear regression with an external calibration curve (Figure 5) that ranged from 0.10 to 20 µg/mL with $n \geq 9$ measurements per standard. A standard curve was prepared at the beginning of each sequence run. TETS was analyzed using consecutive reaction monitoring (CRM), and the m/z 347 → 268 → 175 transitions were monitored. A representative CRM for TETS in an Egg Beaters sample is shown in Figure 6a. The mass spectrum, shown in Figure 6b, exhibits mass ions at m/z 175.05 due to $[M-CH_4N_2O_4S_2]^-$ for TETS. The percent recovery was calculated based on an external calibration curve for TETS, and the results showed >88 % recovery of TETS from Egg Beaters egg whites, whole egg, and USDA egg white. However, the percent recoveries of TETS from USDA salted egg yolk 2551 and p. sugared egg yolk C24410 were <90% (Table 3). Two of the USDA egg yolk samples were very thick and sticky. A procedure that includes multiple centrifuge and extraction steps could improve the percent recovery of TETS from the salted and p. sugared egg yolk samples.

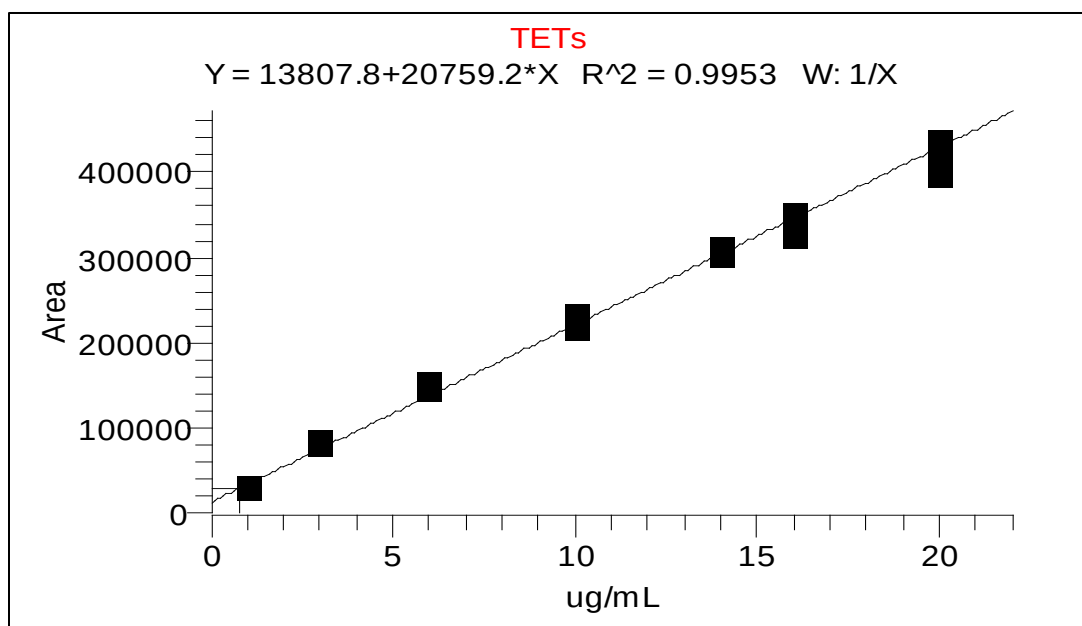


Figure 5. External calibration curve for TETS in acetonitrile and water for various egg sample analyses.

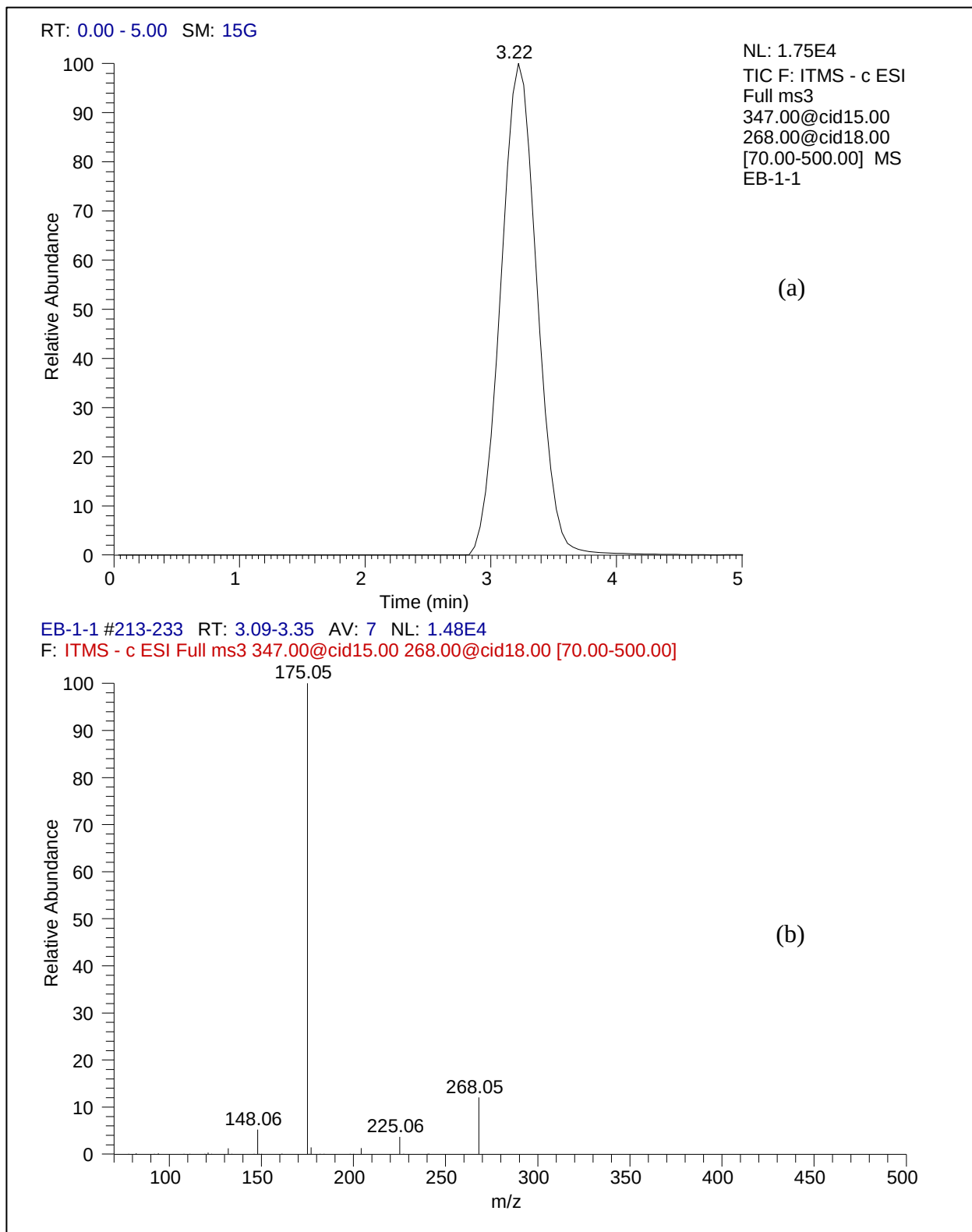


Figure 6. Representative (a) CRM and (b) MS results for TETS extracted from Egg Beaters egg whites matrix.

Table 3. Percent Recovery of TETS Extracted from Various Egg Matrices

Sample	pH	Recovery (%)
Whole egg	7	98.7 ± 1.4
USDA egg white	7	96.1 ± 2.7
Egg Beaters egg white	7	91.5 ± 1.6
USDA p. sugared egg yolk C24410	7	88.9 ± 3.2
USDA salted egg yolk 2551	7	79.1 ± 8.4

3.3 Hot Dog, Chicken Nuggets, Turkey Deli Meat, and 80/20 Ground Beef

The quantitation was evaluated by linear regression with an external calibration curve (Figure 7) that ranged from 0.10 to 20 µg/mL with $n \geq 9$ measurements per standard. A standard curve was prepared at the beginning of each sequence run. TETS was analyzed using CRM, and the m/z 347 → 268 → 175 transitions were monitored. A representative CRM for TETS in an 80/20 ground beef sample is shown in Figure 8a. The mass spectrum, shown in Figure 8b, exhibits mass ions at m/z 175.05 due to $[M-CH_4N_2O_4S_2]^-$ for TETS. Percent recovery was calculated based on an external calibration curve for TETS, and results showed that >88% recovery of TETS was realized from hot dog, chicken nuggets, turkey deli meat, and 80/20 ground beef (Table 4).

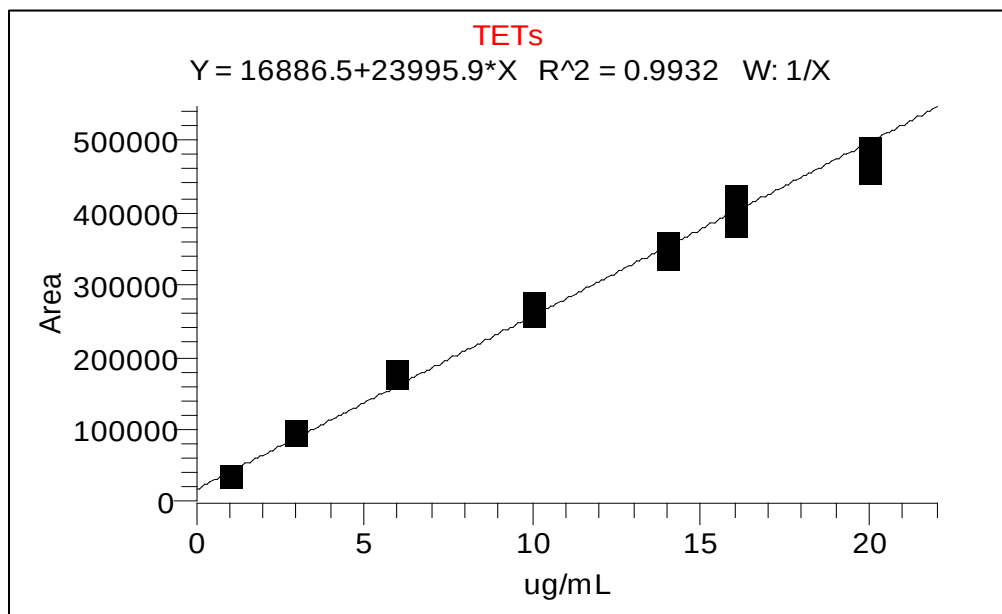


Figure 7. External calibration curve for TETS in acetonitrile and water for various hot dog, chicken nugget, turkey deli meat, and 80/20 ground beef sample analyses.

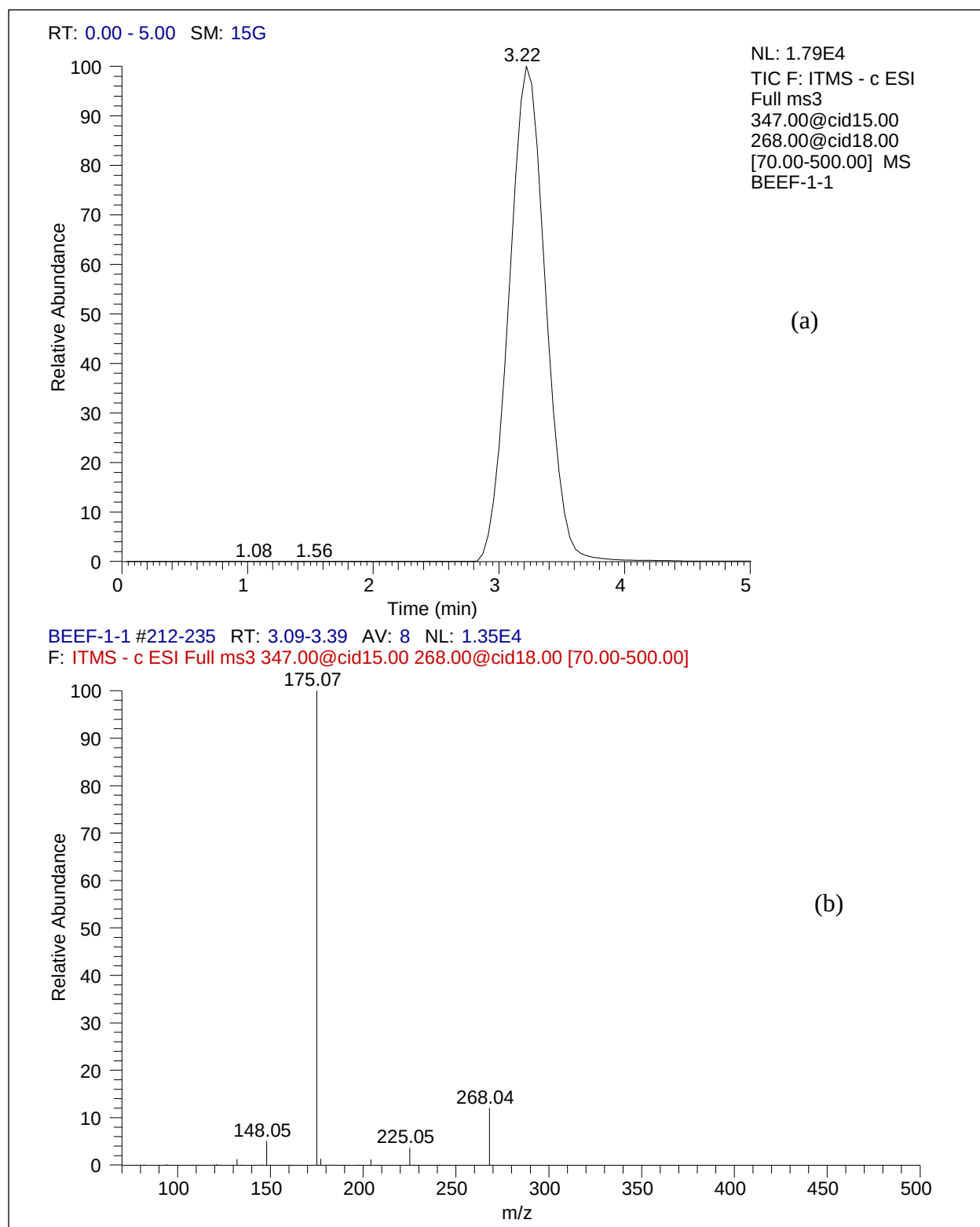


Figure 8. Representative (a) CRM and (b) MS for TETS extracted from 80/20 ground beef matrix.

Table 4. Percent Recovery of Extracted TETS from Hot Dog, Chicken Nugget, Turkey Deli Meat, and 80/20 Ground Beef Matrices

Sample	pH	Recovery (%)
Hot dog	7	93.5 $\pm$ 1.6
Chicken nuggets	7	87.4 $\pm$ 1.3
Turkey deli meat	7	90.1 $\pm$ 1.2
80/20 ground beef	7	88.8 $\pm$ 3.3

4. CONCLUSION

The techniques developed for extracting TETS from various food matrices were accomplished, and recoveries >95% were obtained from most of the liquid and semi-liquid food matrices. The recoveries were >88% from all of the solid food matrices, including both of the USDA egg yolk samples (C24410 and 2551). We should be able to optimize the cleanup and extraction procedures to achieve greater TETS recovery from the high-fat, -salt, and -sugar food matrices. At present, we are working on the optimization process.

The extraction method was easy to use, and from it, we could determine the amounts of TETS in various complex food matrices. TETS is a relatively persistent environmental contaminant due to its high stability in water. This extraction method is applicable to a wide variety of complex foods, yet it requires minimal sample handling. The method is simple and inexpensive; thus, food samples could be extracted in the field by individuals who have minimal training. This would be ideal for quickly identifying potentially toxic food products anywhere in the United States.

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ACRONYMS AND ABBREVIATIONS

CRM	consecutive reaction monitoring
ESI	electrospray ionization
GC	gas chromatography
IT-MS	ion trap mass spectrometry
LC	liquid chromatography
LD ₅₀	lethal dose for 50% of test subjects
MS	mass spectrometry
<i>m/z</i>	mass-to-charge ratio
SRM	selected reaction monitoring
TEA	trimethylamine
TETS	tetramethylenedisulfotetramine
USDA	U.S. Department of Agriculture

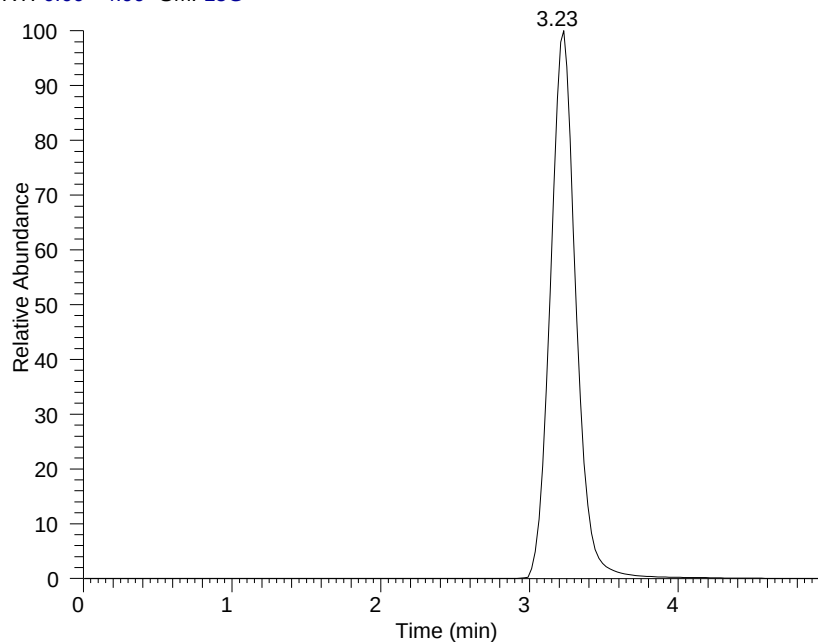
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APPENDIX

SELECTED REACTION MONITORING (SRM) AND CONSECUTIVE REACTION MONITORING (CRM) CHROMATOGRAMS AND MASS SPECTRA OF TETRAMETHYLENEDISULFOTETRAMINE (TETS) EXTRACTED FROM VARIOUS FOOD MATRICES

A.1. TETS and Apple Juice

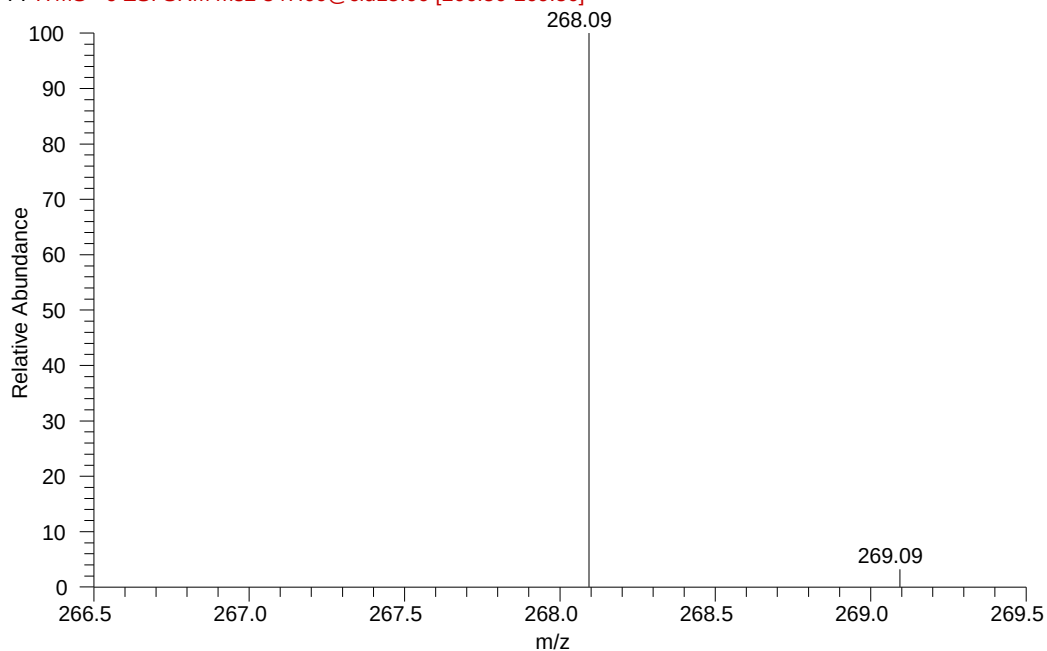
RT: 0.00 - 4.99 SM: 15G



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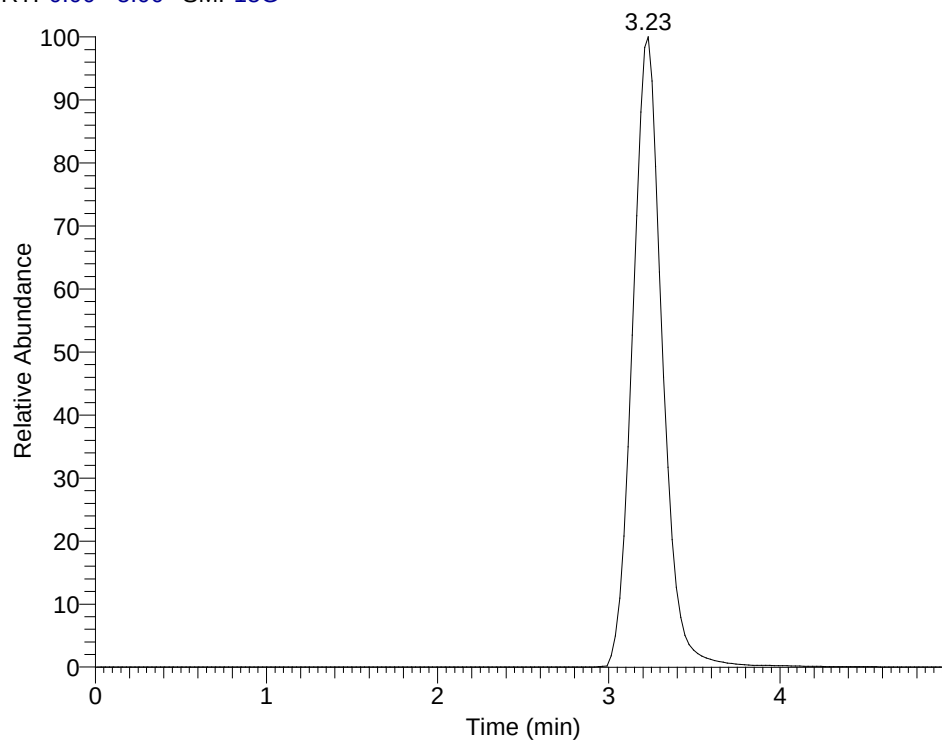
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A.2. TETS and Orange Juice

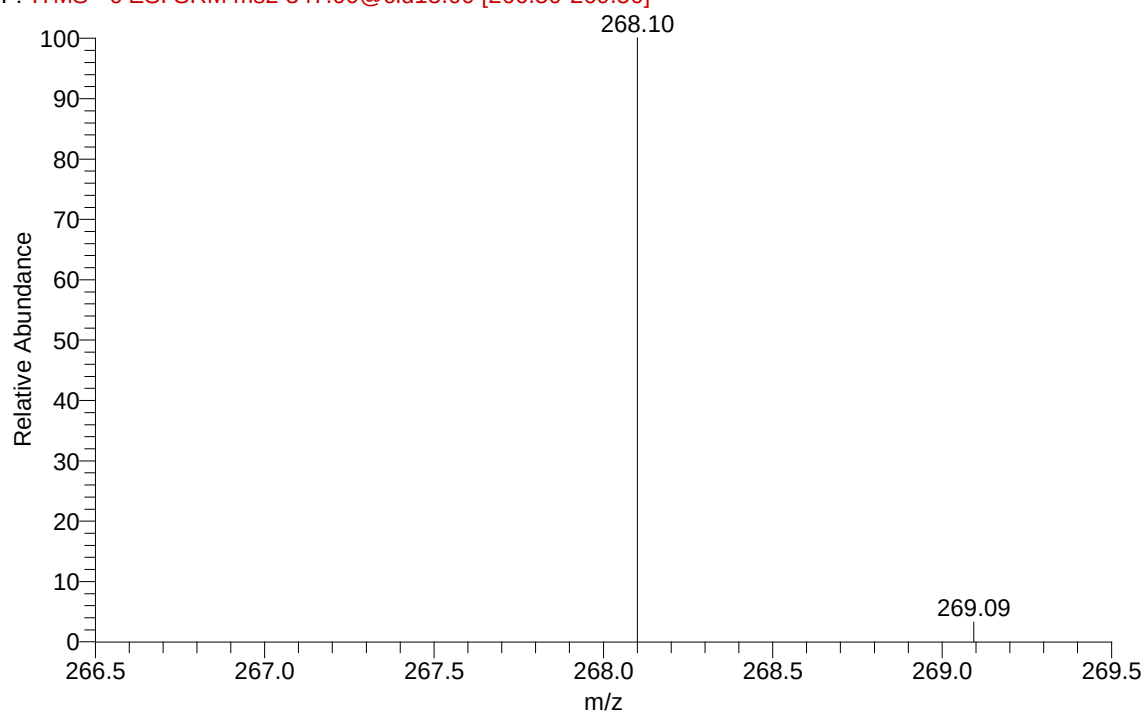
RT: 0.00 - 5.00 SM: 15G



NL:
6.73E4
TIC F: ITMS - c
ESI SRM ms2
347.00@cid15.00
[266.50-269.50]
MS OJ1-1

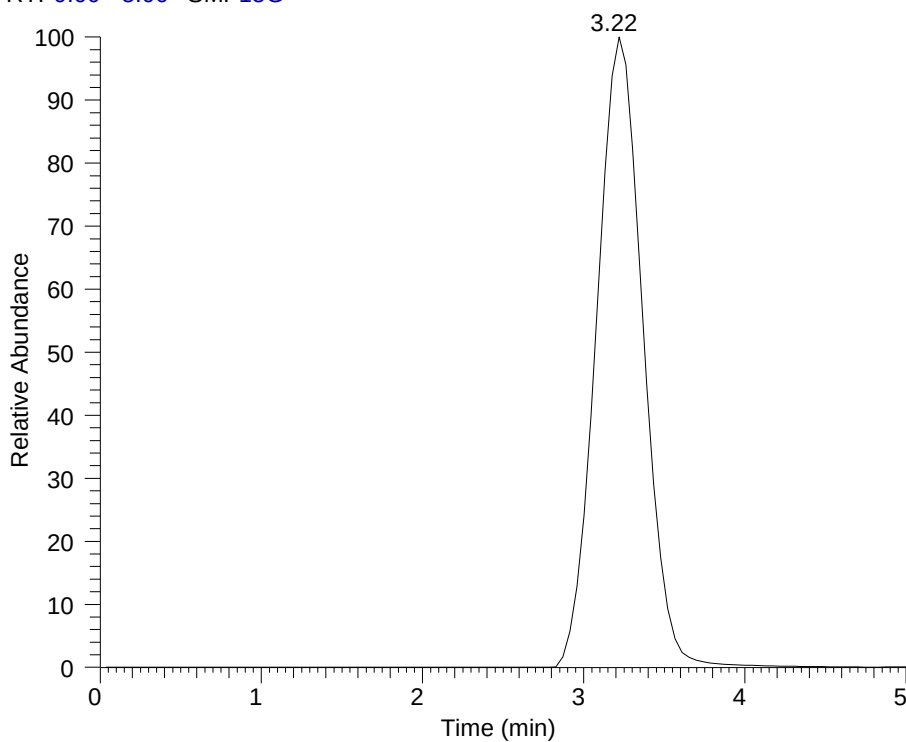
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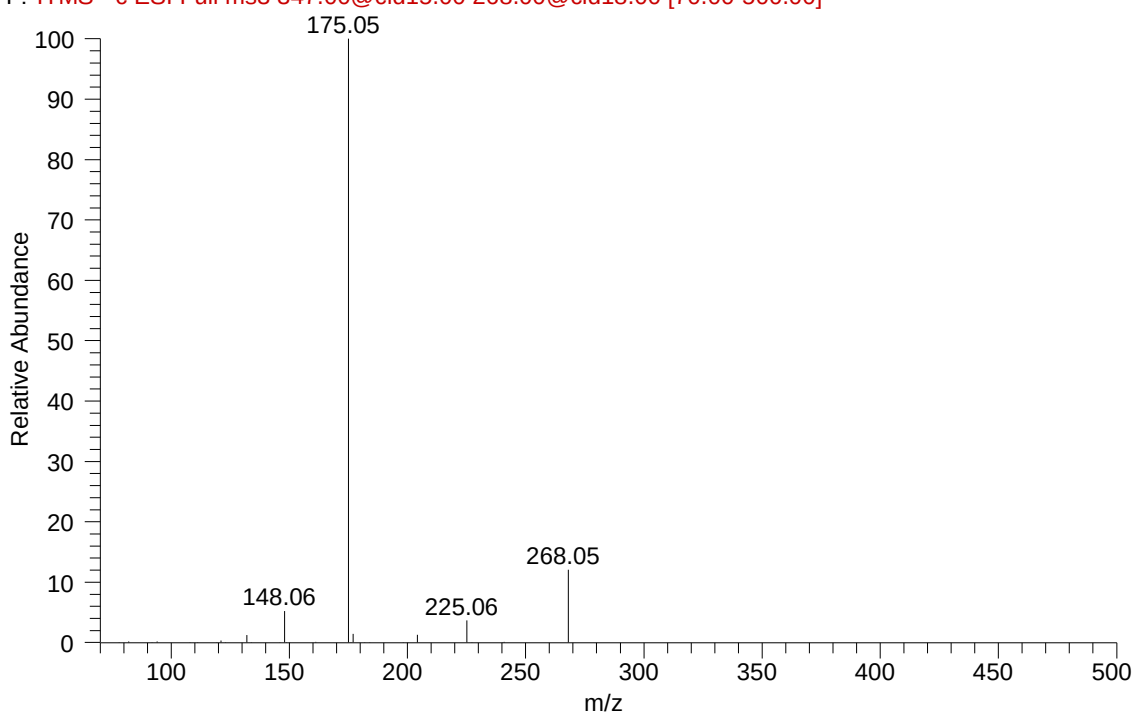
A.3. TETS and Egg Beaters Egg Whites

RT: 0.00 - 5.00 SM: 15G



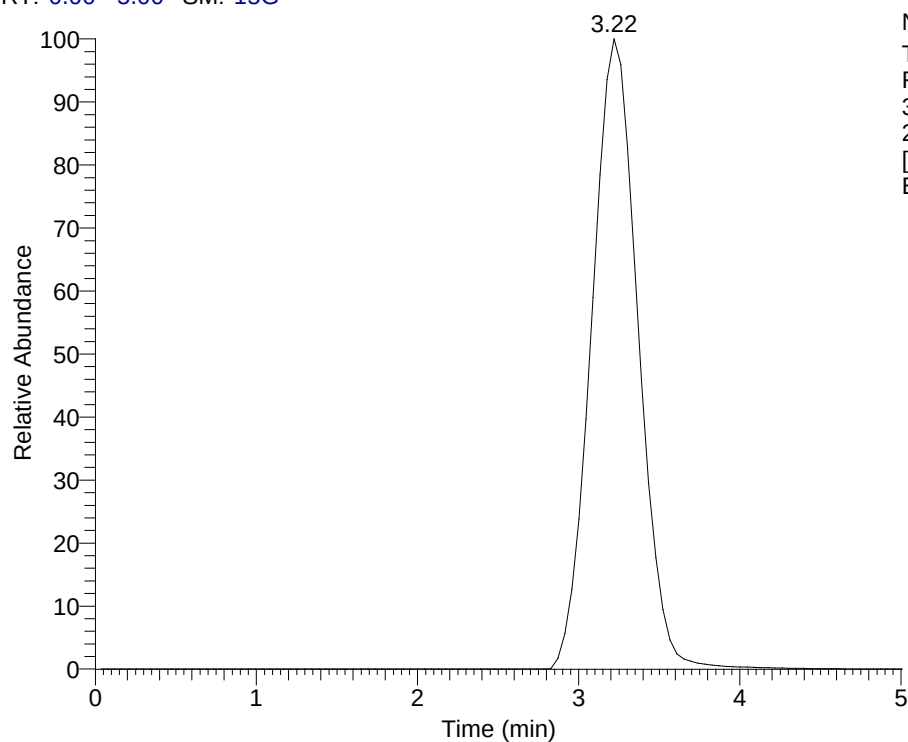
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Full ms3
347.00@cid15.00
268.00@cid18.00
[70.00-500.00] MS
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EB-1-1 #213-233 RT: 3.09-3.35 AV: 7 NL: 1.48E4
F: ITMS - c ESI Full ms3 347.00@cid15.00 268.00@cid18.00 [70.00-500.00]



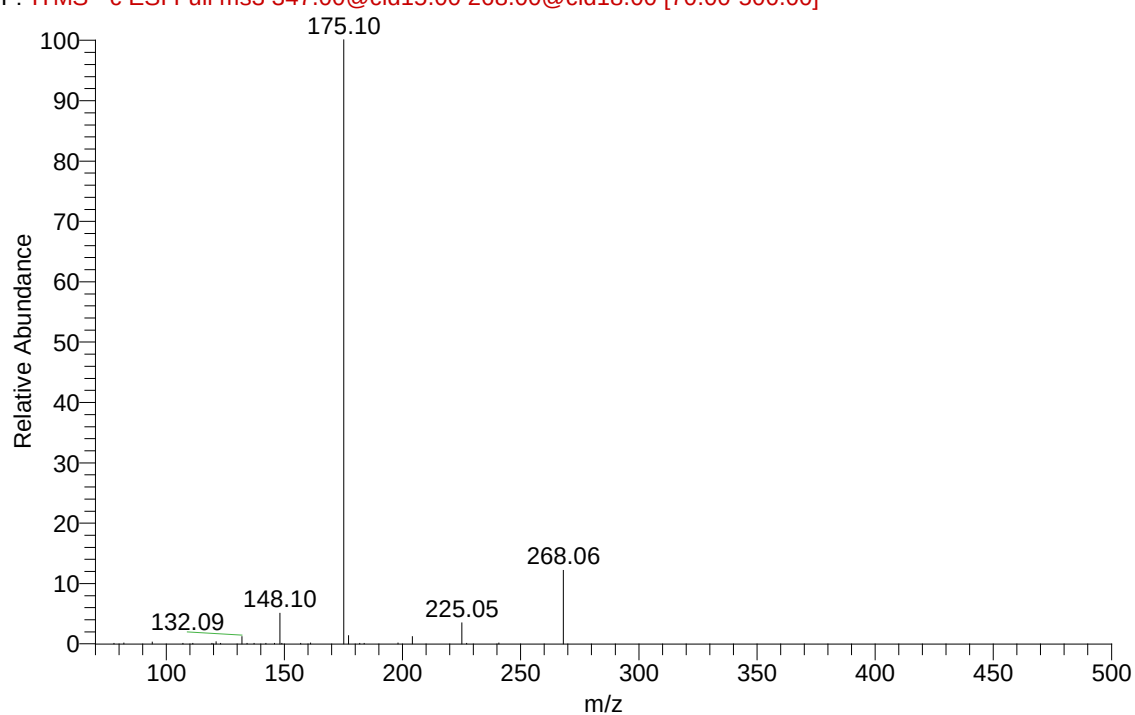
A.4. TETS and USDA Egg White

RT: 0.00 - 5.00 SM: 15G



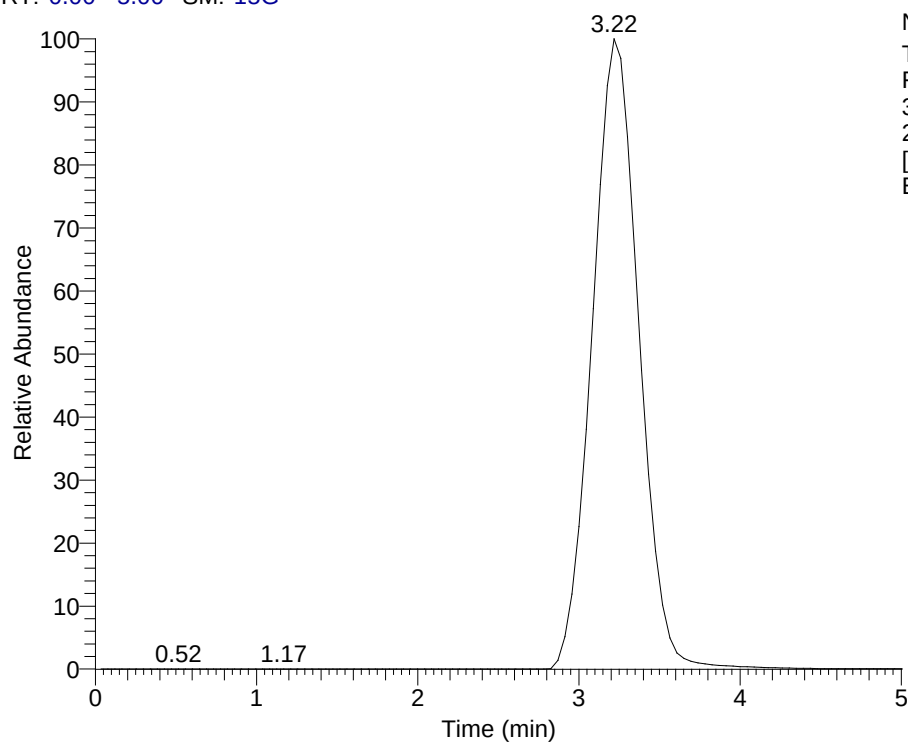
NL: 1.77E4
TIC F: ITMS - c ESI
Full ms3
347.00@cid15.00
268.00@cid18.00
[70.00-500.00] MS
EW-1-1

EW-1-1 #209-236 RT: 3.05-3.39 AV: 9 NL: 1.18E4
F: ITMS - c ESI Full ms3 347.00@cid15.00 268.00@cid18.00 [70.00-500.00]



A.5. TETS and USDA Egg Yolk 2551

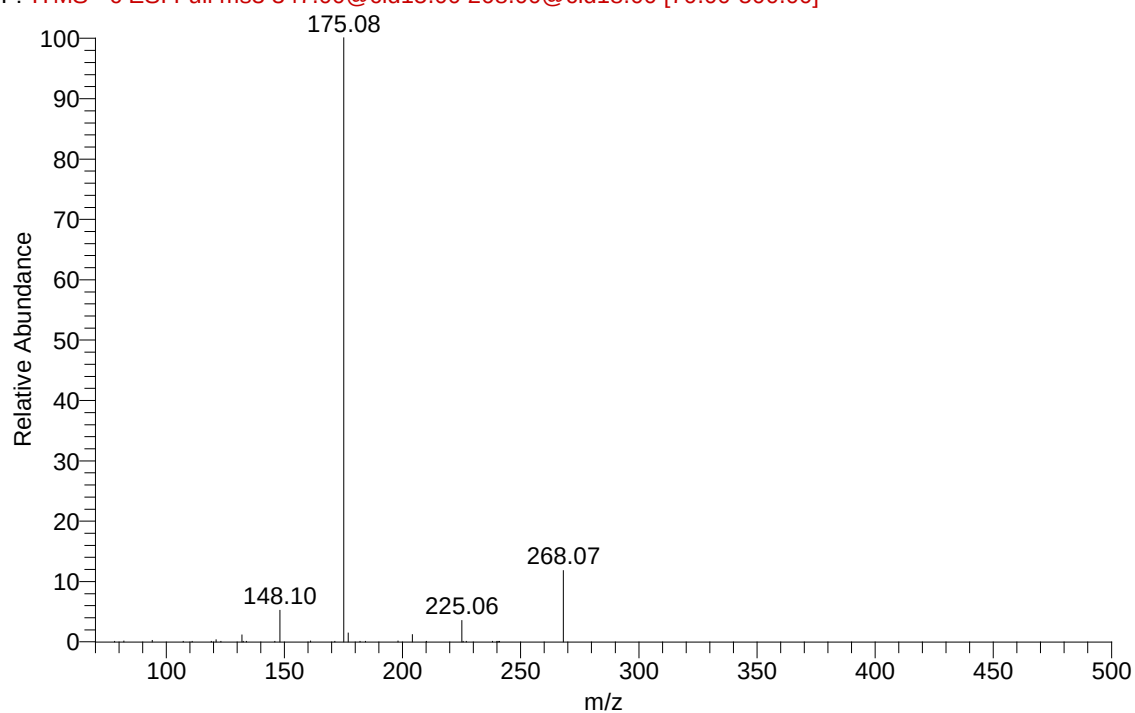
RT: 0.00 - 5.00 SM: 15G



NL: 1.85E4
TIC F: ITMS - c ESI
Full ms3
347.00@cid15.00
268.00@cid18.00
[70.00-500.00] MS
EY-1-1

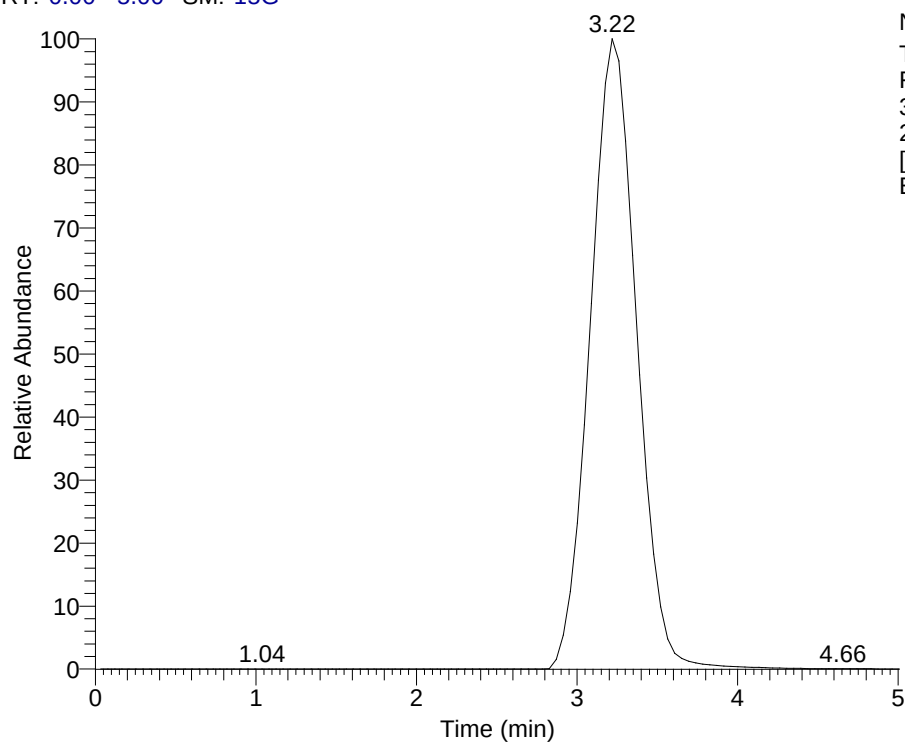
EY-1-1 #212-235 RT: 3.09-3.39 AV: 8 NL: 1.39E4

F: ITMS - c ESI Full ms3 347.00@cid15.00 268.00@cid18.00 [70.00-500.00]



A.6. TETS and USDA Egg Yolk C24410

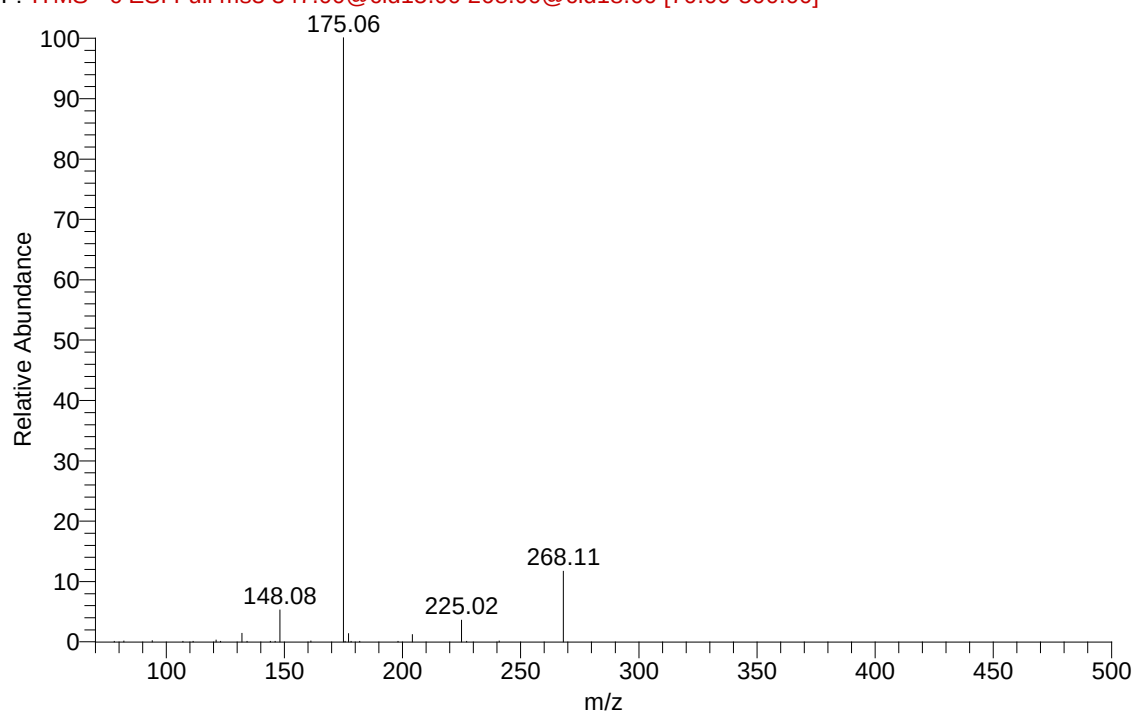
RT: 0.00 - 5.00 SM: 15G



NL: 1.83E4
TIC F: ITMS - c ESI
Full ms3
347.00@cid15.00
268.00@cid18.00
[70.00-500.00] MS
EYC-1-1

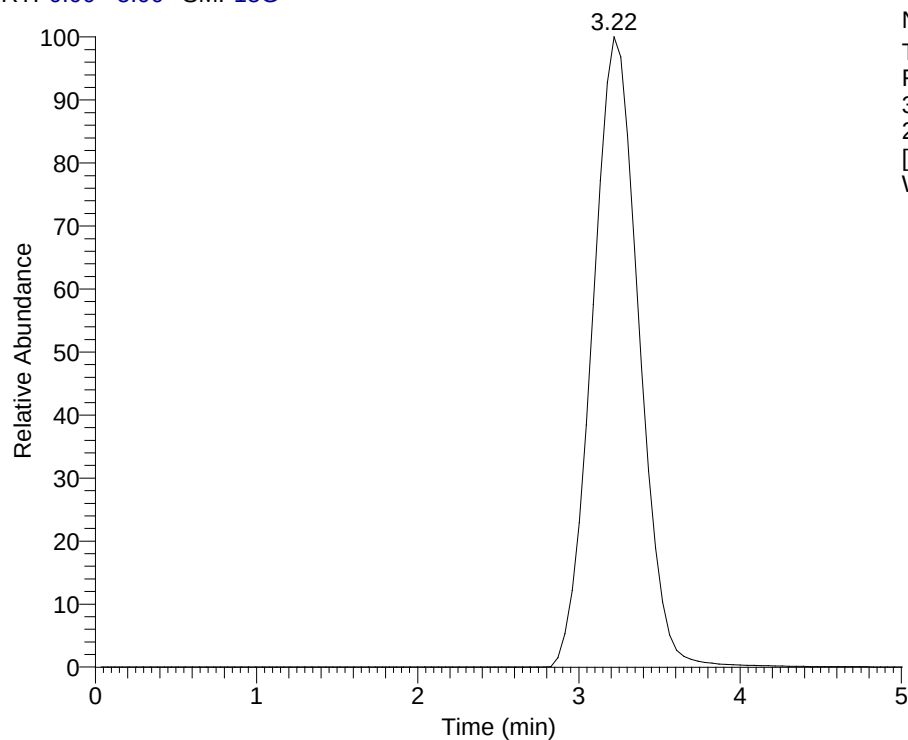
EYC-1-1 #214-231 RT: 3.13-3.35 AV: 6 NL: 1.81E4

F: ITMS - c ESI Full ms3 347.00@cid15.00 268.00@cid18.00 [70.00-500.00]



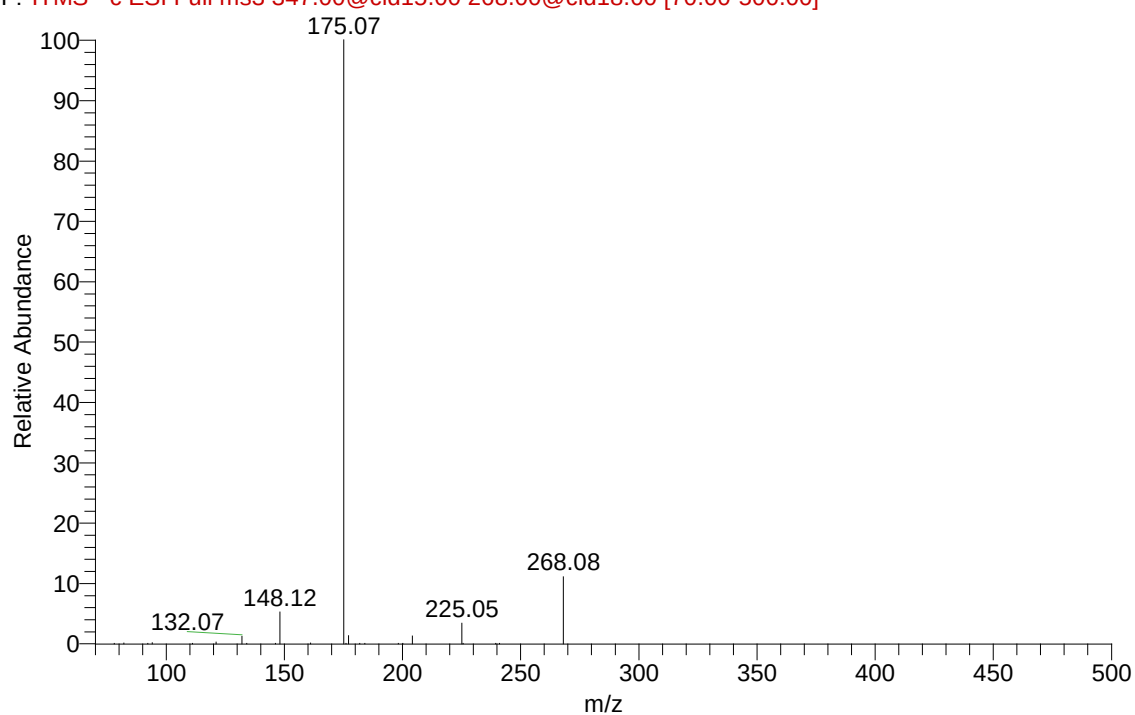
A.7. TETS and Whole Egg

RT: 0.00 - 5.00 SM: 15G



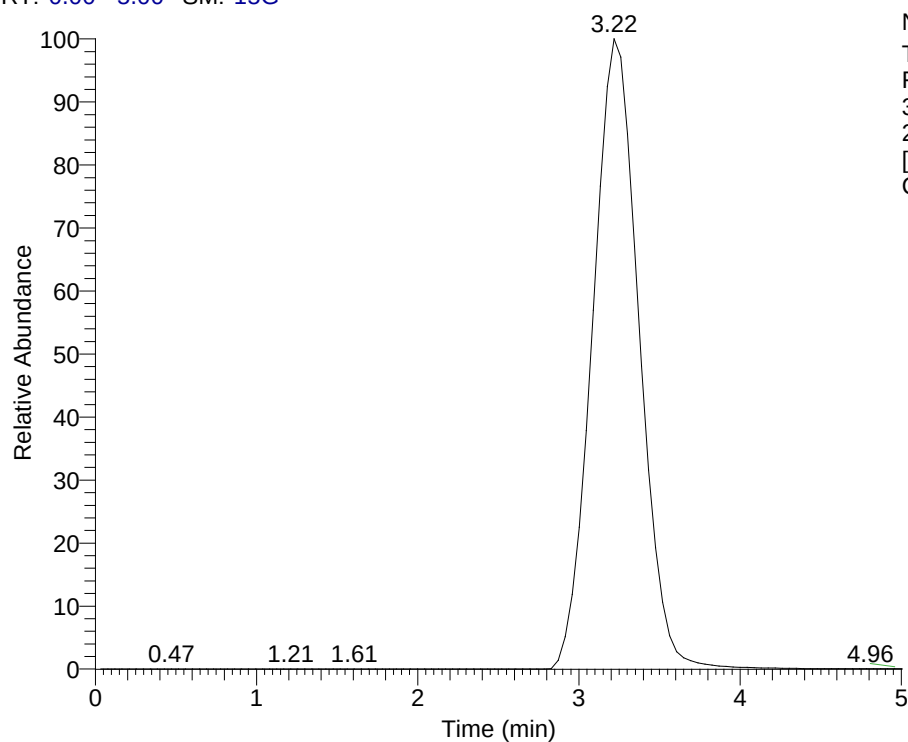
NL: 1.76E4
TIC F: ITMS - c ESI
Full ms3
347.00@cid15.00
268.00@cid18.00
[70.00-500.00] MS
WE-1-1

WE-1-1 #210-236 RT: 3.05-3.39 AV: 9 NL: 1.19E4
F: ITMS - c ESI Full ms3 347.00@cid15.00 268.00@cid18.00 [70.00-500.00]



A.8. TETS and Chicken Nuggets

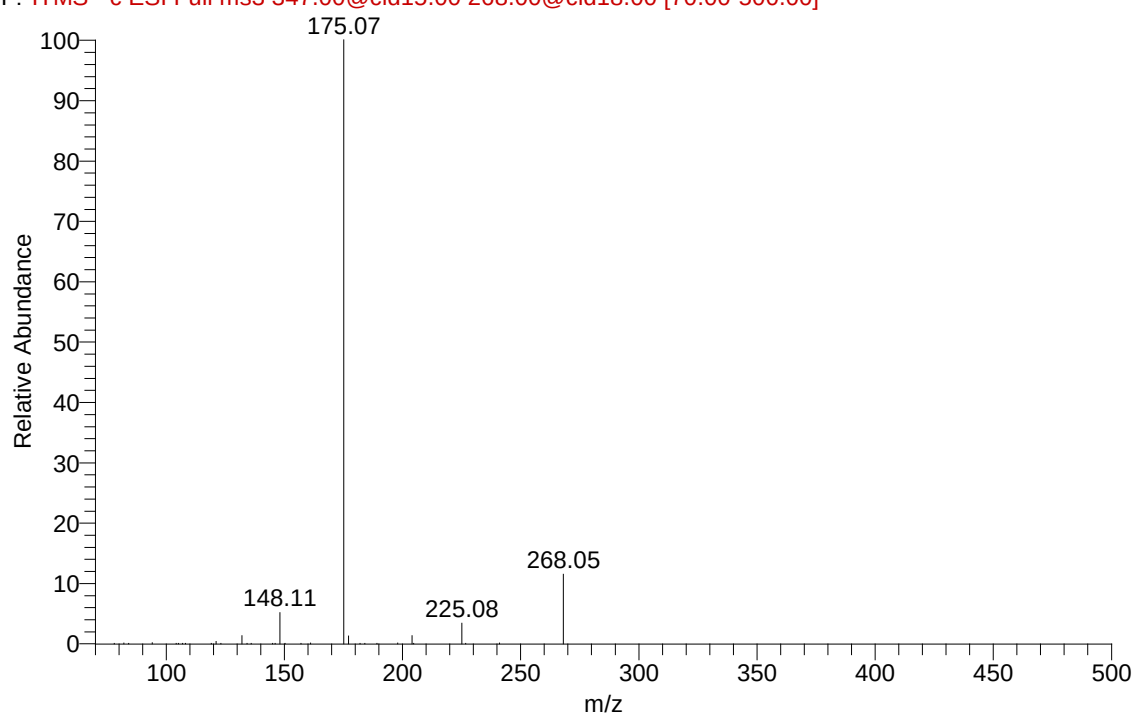
RT: 0.00 - 5.00 SM: 15G



NL: 2.01E4
TIC F: ITMS - c ESI
Full ms3
347.00@cid15.00
268.00@cid18.00
[70.00-500.00] MS
CN-1-1

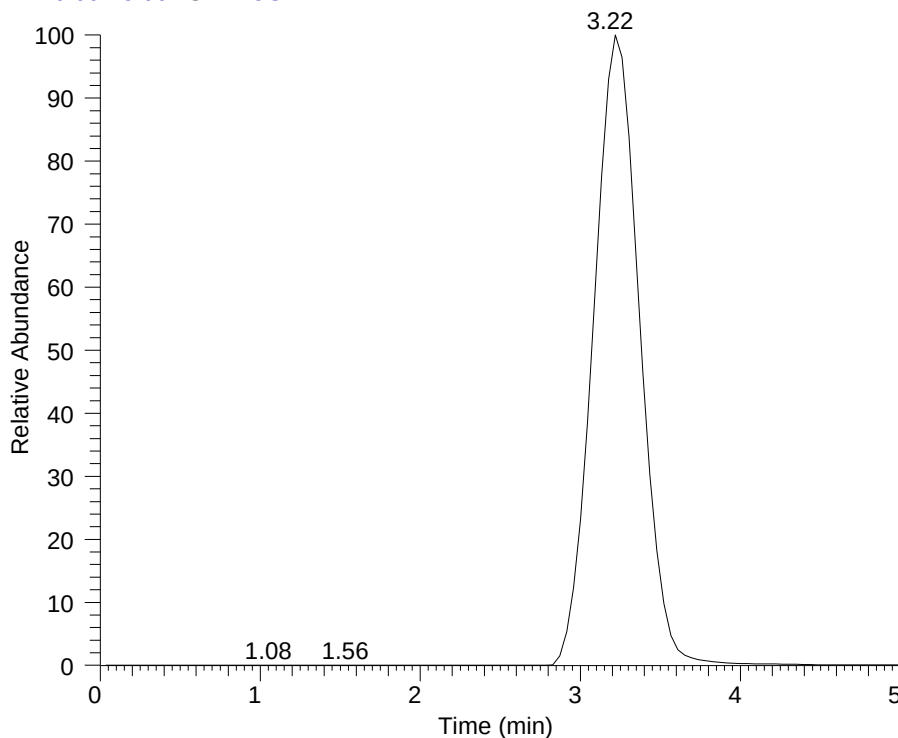
CN-1-1 #207-240 RT: 3.00-3.48 AV: 12 NL: 1.03E4

F: ITMS - c ESI Full ms3 347.00@cid15.00 268.00@cid18.00 [70.00-500.00]



A.9. TETS and 80/20 Ground Beef

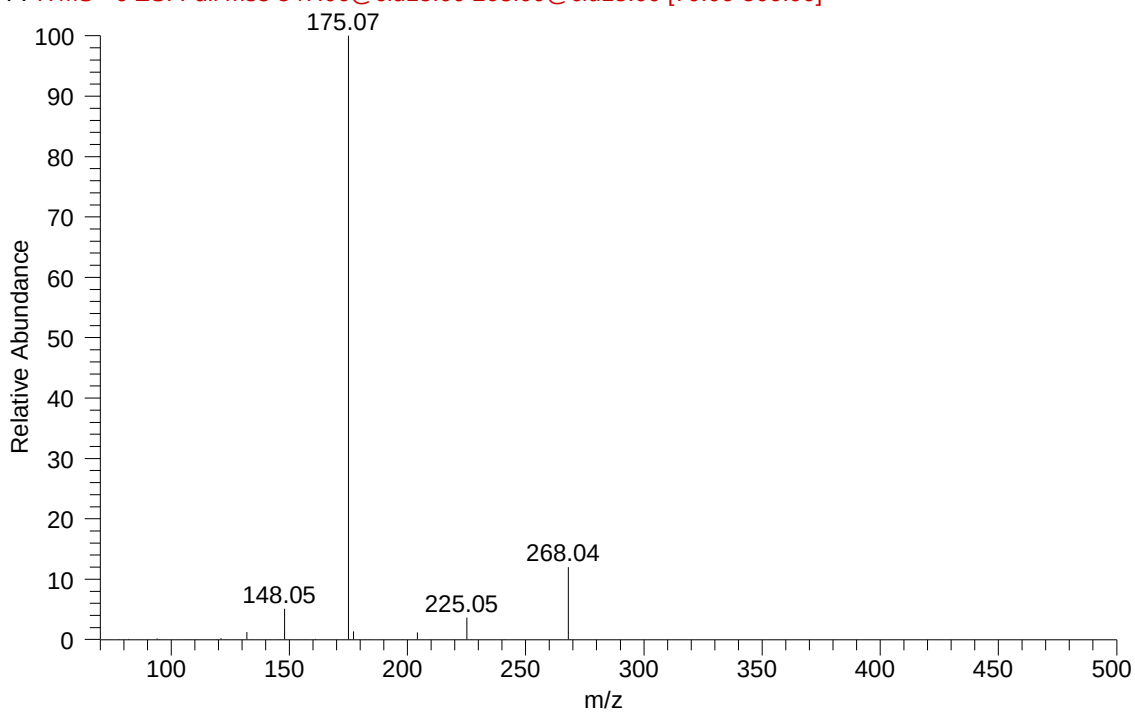
RT: 0.00 - 5.00 SM: 15G



NL: 1.79E4
TIC F: ITMS - c ESI
Full ms3
347.00@cid15.00
268.00@cid18.00
[70.00-500.00] MS
BEEF-1-1

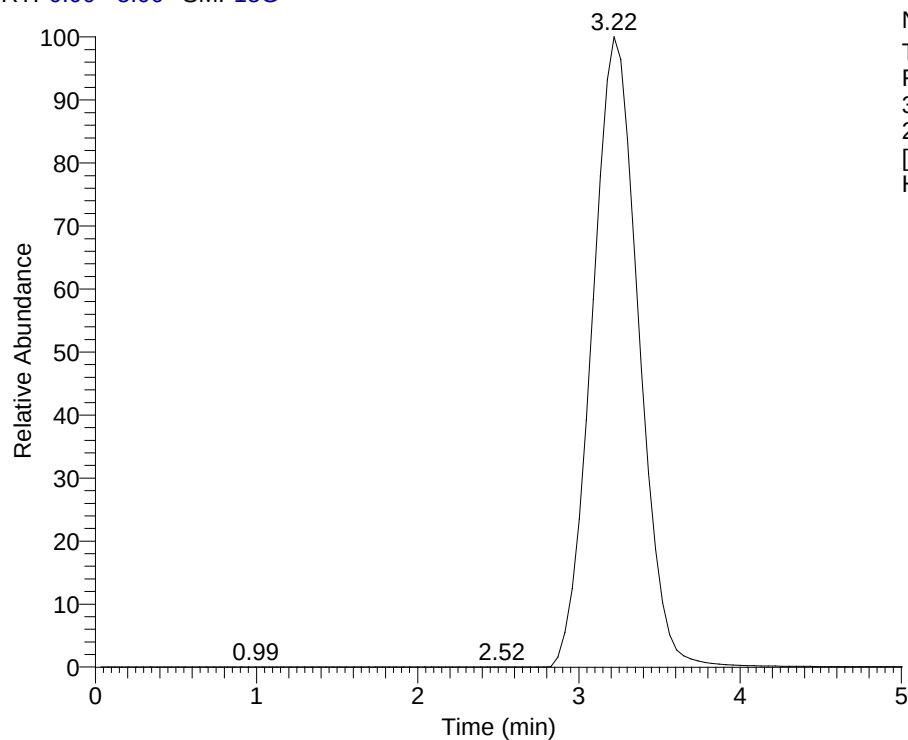
BEEF-1-1 #212-235 RT: 3.09-3.39 AV: 8 NL: 1.35E4

F: ITMS - c ESI Full ms3 347.00@cid15.00 268.00@cid18.00 [70.00-500.00]



A.10. TETS and Hot Dog

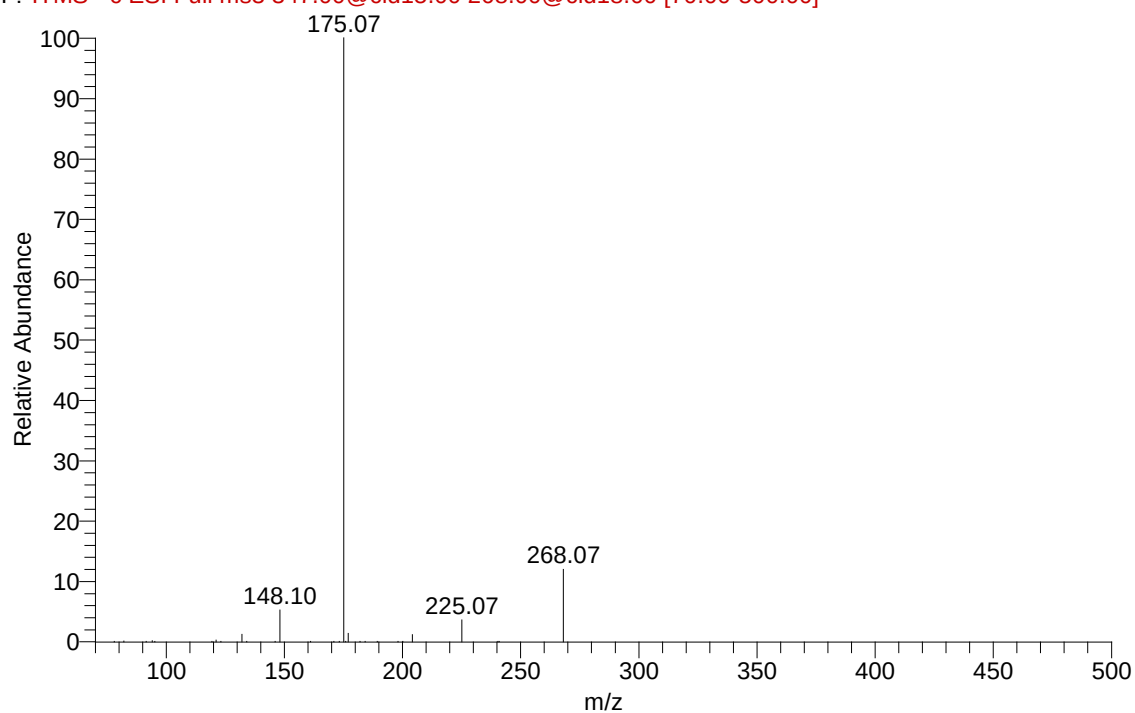
RT: 0.00 - 5.00 SM: 15G



NL: 2.12E4
TIC F: ITMS - c ESI
Full ms3
347.00@cid15.00
268.00@cid18.00
[70.00-500.00] MS
HD-1-1

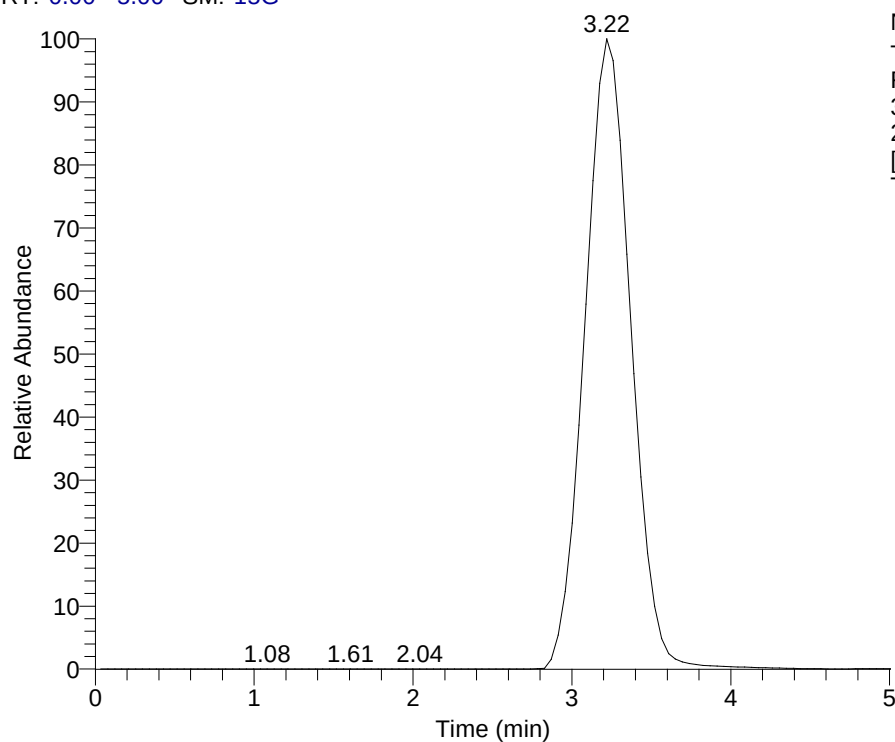
HD-1-1 #212-235 RT: 3.09-3.39 AV: 8 NL: 1.60E4

F: ITMS - c ESI Full ms3 347.00@cid15.00 268.00@cid18.00 [70.00-500.00]



A.11. TETS and Turkey Deli Meat

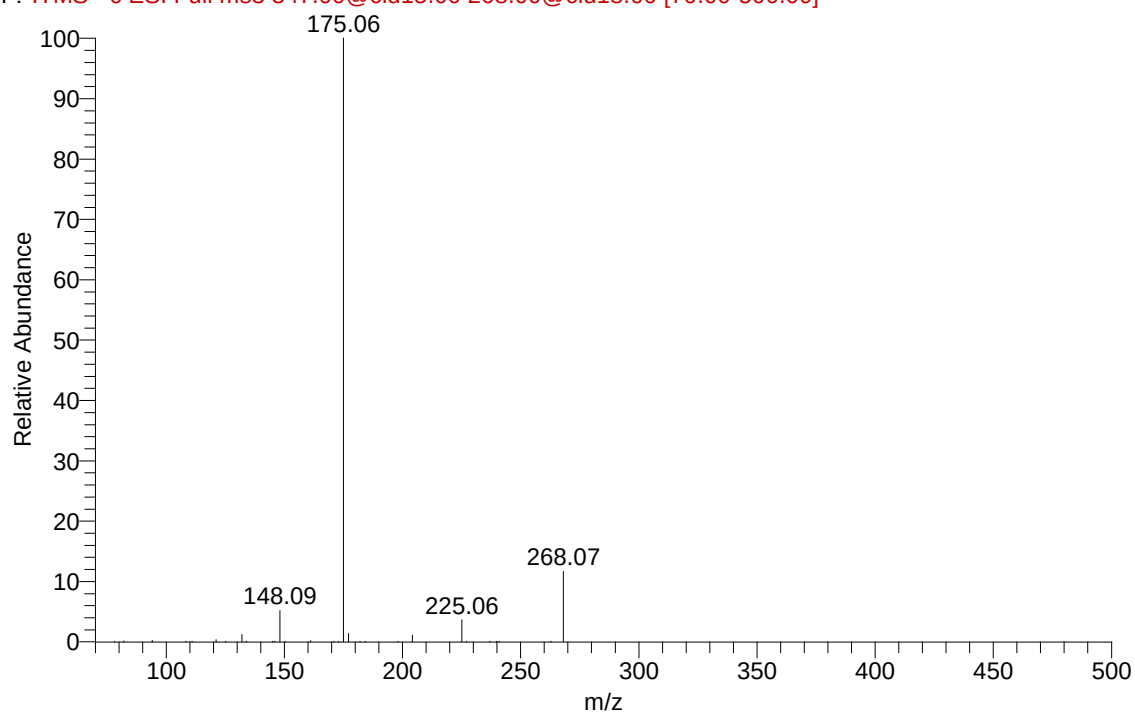
RT: 0.00 - 5.00 SM: 15G



NL: 1.70E4
TIC F: ITMS - c ESI
Full ms3
347.00@cid15.00
268.00@cid18.00
[70.00-500.00] MS
TURKEY-1-1

TURKEY-1-1 #212-235 RT: 3.09-3.39 AV: 8 NL: 1.29E4

F: ITMS - c ESI Full ms3 347.00@cid15.00 268.00@cid18.00 [70.00-500.00]



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