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## Detection of Bacterial Lipopolysaccharides of Escherichia coli by Direct Analysis in Real Time/Time-of-Flight Mass Spectrometry

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**Detection of Bacterial Lipopolysaccharides of *Escherichia coli* by Direct Analysis in Real Time/Time-of-Flight Mass Spectrometry**

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## ABSTRACT

Lipopolysaccharides (LPS) are a major component of the outer membrane in Gram-negative bacteria species and are responsible for toxicity caused by organisms such as *Escherichia coli* (*E. coli*). Conventional methods for characterizing LPS utilize mass spectrometry, particularly matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) and liquid chromatography mass spectrometry (LC-MS). Although these are successful in analyzing intact LPS molecules, they can be time-consuming. Other mass spectrometry techniques, such as direct analysis in real time/time-of-flight mass spectrometry (DART-TOF-MS) could also be helpful in the rapid analysis of LPS molecules without sample extraction procedures. This research focuses on the application of a DART-TOF-MS method to rapidly identify LPS molecules from *E. coli* as well as an analysis of LPS extracts generated using a commercial LPS extraction kit by gas chromatography-flame ionization detection (GC-FID). It was determined that DART-TOF-MS allows for the direct detection of LPS-specific fatty acids from solutions containing intact *E. coli* cells. Finally, the GC-FID based evaluation of the commercial LPS extraction kit showed that resulting extracts were enriched in LPS-specific fatty acids compared to intracellular/membrane fatty acids.

Keywords: *E. coli*, Lipopolysaccharide, LPS, Direct Analysis in Real Time/Time-of-Flight Mass Spectrometry, Mass Spectrometry, MS, DART-TOF-MS, GC-FID, LPS Extraction

## INTRODUCTION

One of the earliest and simplest classifications of bacteria is into Gram-positive and Gram-negative groups. Hans Christian Gram first developed the Gram Stain test to discern Gram-positive bacteria with a blue stain and Gram-negative with a red stain.<sup>1</sup> These two types of bacteria differ in their cell wall composition. Gram-positive bacteria have only one membrane with a thick layer of peptidoglycan, which provides bacteria protection and defines bacterial shape, and cause illnesses that respond positively to antibiotics due to the lack of endotoxin within this bacterial type.<sup>1</sup>

Gram-negative bacteria have two phospholipid bilayer membranes. The inner membrane is used to protect the intracellular components of the bacteria, where the outer membrane acts as a protective unit that prevents immune response cells from ingesting the bacteria. Gram-negative bacteria have a thin layer of peptidoglycan within the periplasmic space, which helps the cell maintain its shape and rigidity. Most importantly, Gram-negative bacteria produce lipopolysaccharide (LPS), which is the main constituent to the surface of the outer membrane. The LPS is responsible for toxic effects and the antibiotic resistance that are observed within some bacteria.<sup>2,3</sup> The LPS of Gram-negative bacteria are known to contribute to many types of foodborne illnesses (*E.coli*), sexually transmitted diseases (*N. gonorrhoeae*), and respiratory illnesses (*Y.pestis*).<sup>1,2</sup>

A general schematic of the structure of an LPS molecule can be seen in Figure 1. LPS molecules are known to vary in molecular weights from between 10-40 kDa. This molecular variation is caused not only by species of bacteria, but by variation between the LPS structure between different strains of the same bacteria. This molecule consists primarily of lipid and

carbohydrate regions and can be further divided into three regions: the o-antigen repeats, the core polysaccharide and the Lipid A.<sup>4</sup>

The o-antigen unit is present on the outermost part of the LPS and is hydrophilic in nature.<sup>5</sup> The o-antigen unit consists of one to eight glycosyl residues and the composition can have wide variety diversity among bacteria and their strains. These residues can repeat between up to 40 times depending on the bacteria species. This region is often targeted by the host antibody response as the major antigen and plays a major role in the suppression of the host's immune system.<sup>4,5</sup>

The core polysaccharide region is also located on the exterior of the cell and consists of the outer and inner cores. The outer core consists of a common hexose sugar such as glucose, galactose or N-acetyl glucosamine, while the inner core consists of other sugars such as 3-Deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) and heptose. The Kdo sugar is bound to the carbohydrate backbone of Lipid A by an  $\alpha$ -linkage bond and is seen in all LPS molecules, except those of *Acinetobacter* and *Burkholderia cepacia*.<sup>6</sup> The structure of the core polysaccharide is much less variable among species, which indicates that is needed for the structural integrity of the lipopolysaccharide; within *E. coli* only five different core structures have been identified.<sup>4</sup>

The lipid A region is the hydrophobic region of the molecule and is embedded within the outer membrane.<sup>5</sup> The Lipid A chain consists of a  $\beta$ -D-GlcN-(1-6)- $\alpha$ -D-GlcN disaccharide with two phosphoryl groups that can be further substituted.<sup>8</sup> These sugars and phosphoryl group structures are attached to a maximum of four acyl chains through an ester or amide linkage. These acyl chains are often substituted with fatty acids. Like the o-antigen repeats unit, the Lipid A portion can vary widely between species and strains of bacteria. The fatty acid chains within the hydrophobic tail can also vary between species in abundance, length, and degree of

saturation. It is thought that the fatty acid chains may help to regulate membrane fluidity in the bacteria.<sup>6</sup>

The core region represents one of the critical components that are used for the development and maintenance of the bacterial cell. However, the o-antigen repeats or the lipid A can vary without survival implications. While the changes to the o-antigen or lipid A chain can have major effects on configuration, composition or change of charged groups, there is not a major impact on its life cycle. Variation between these units instead may affect the virulence and toxicity of the bacteria.<sup>5,8</sup> The o-antigen and Lipid A regions of the LPS act as the main source of virulence for Gram-negative bacteria within human hosts.

The direct mechanism of action for LPS molecules varies between organisms, but within *E. coli*, it can follow a general scheme. The o-antigen repeats are nontoxic, but will suppress the immunogenic action against the host cell. The o-antigen repeats will help the LPS to adhere to the host cells. Once adhered, the o-antigen acts a protection barrier for the rest of the molecule and will allow the molecule for its own self destruction in order to help facilitate the release of Lipid A.<sup>7,9</sup> Once released, the Lipid A will bind to lipopolysaccharide-binding protein, before interaction with the Toll-like receptor 4 (TLR4).<sup>9</sup> The TLR4 receptor is a pattern recognition receptor that is used to recognize foreign microbes in the body. This interaction will activate the body's innate immune system, which is just the main response to microbial infections. This immune defense results in the activation of several signaling cascades that produce cytokines in order to mediate bacterial clearance. Lipid A is responsible for the toxic physiological reactions seen in illnesses like food poisoning. These symptoms include fever, diarrhea, dehydration, and, in severe cases, can cause sepsis.<sup>9</sup>



The rapid detection of LPS molecules would be advantageous due to its potent bioactivity. LPS is active within human hosts at quantities as low as 2ng/kg of body weight.<sup>10</sup> Further LPS has shown evidence of having both *in vivo* and *in vitro* actions.<sup>5</sup> LPS analysis can be useful when analyzing possible bioterrorism cases, especially vulnerable water systems. Analyzing for these pathogens by targeting the biomarkers expressed by the LPS on these bacteria can help to determine if these biological agents have been used.<sup>11</sup>

Rapid characterization would also be useful since most existing analytical methods are often time-consuming and laborious. Previously published studies typically employ mass spectrometry (MS)-based techniques, but before any analytical methods can be applied, the LPS molecule must first be extracted from the outer membrane, which can take 7-10 hours to complete.<sup>13</sup> MS on extracted LPS molecules can be performed by top-down or bottom-up proteomic techniques. In top-down proteomics, intact molecular ions are introduced to a mass analyzer and then fragmented in the gas-phase. In bottom-up proteomics, analytes are entered into the mass spectrometer after they are subjected to enzymatic cleavage assays.<sup>14</sup> In order to detect LPS, top-down proteomic strategies are employed. Some laboratories utilize liquid chromatography-mass spectrometry (LC-MS), which is a sensitive method, but the analytical scheme can be difficult to optimize.<sup>15</sup> Other labs implement matrix-assisted laser desorption ionization (MALDI-MS) in the analysis of LPS and rough LPS, which just refers to lipopolysaccharides that lack the o-antigen regions. MALDI-MS has been applied to general structure analysis but also to Lipid A fatty acid analysis and rough LPS, or LPS that lack the o-antigen repeats, analyses.<sup>16-19</sup> MALDI-MS uses soft ionization techniques that allow the analytes to enter the gas phase without being decomposed, which is especially helpful for large molecules, like LPS, that can be destroyed when traditional ionization techniques are

employed.<sup>19, 20</sup> While MALDI-MS has many advantages, there are a few notable disadvantages. The main limitation in MALDI-MS is the limited sensitivity without prior culturing or differentiation of related microorganisms, such as *E. coli* or *Shigella*.<sup>21</sup> Also, optimizing matrix compounds to prepare samples can be time-consuming, and analysts must be aware of the limitations, such as solubility, of their analytes within a chosen matrix.<sup>19</sup> Additionally, other techniques are needed to differentiate between nonvirulent and virulent bacterial strains. For example, *E. coli* lives in the intestines of most mammals, but only a few strains of *E. coli* are virulent.<sup>22</sup> Another emerging MS technique, direct analysis in real time/time-of-flight mass spectrometry (DART-TOF-MS), may prove to be useful in the rapid characterizations of LPS molecules.

DART-TOF-MS is also a soft ionization technique, but unlike MALDI-MS, DART-TOF-MS analyzes low molecular weight fragments between  $m/z$  50-1200. The TOF-MS allows for high mass accuracy and resolution. The analysis time itself is quick and takes seconds from the time the sample is introduced to the ionization stream. This allows for rapid screening to be completed, prior to other confirmatory MS techniques being performed. Further, DART-TOF-MS requires little to no sample preparation, can be done on solid, liquid or gas samples, and is done at ambient temperatures.<sup>23,24</sup>

DART-TOF-MS differs from other traditional MS techniques due to the ionization process. The ionization gas, normally helium, enters the ion source, and electric potential is applied to the flow, which results in the formation of plasma which contains electrons and other ions, which are later recombined in order to form stable excited molecules. The gas may pass through a heater, which allows for desolvation of the analyte molecule. The ionizing gas is then passed through a gas electrode, which can be set to a positive or negative potential. The analyte

is placed into the gas stream and ionized.<sup>24</sup> This ionization occurs at ambient pressure and once ionized the analyte will enter the mass spectrometer, which is performed under normal high vacuum conditions. Once ions enter the mass analyzer, they are pushed through two orifices that are staggered both in position and voltage in order to stop contamination from the atmosphere from entering the vacuum system.<sup>23,24</sup>

DART-TOF-MS can be run in positive mode, which primarily produces  $[M+H]^+$  fragments, or in negative mode, which produces  $[M-H]^-$  fragments, though adducts of oxygen, chlorine, and ammonium can be produced in both positive and negative modes.<sup>26</sup> The ionization method for both modes is not well understood, but some hypotheses have been made as to how ions are formed. For positive mode, it is believed that once the ionizing gas passes through the final electrode, it initiates direct excitation of the analyte, while other molecules rely on Penning ionization of nitrogen or water in the atmosphere. Penning ionization is a form of chemi-ionization that involves reactions between neutral atoms and the ionization gas in order to form an excited species that is then used in a secondary collision of the analyte to generate excitation. This mode primarily results in protonated species but adduct formation can occur.<sup>23</sup> For negative mode, the gas electrode has a negative potential and is thought to use Penning ionization in order to excite the oxygen in order to promote radical formation. The efficiency for ionization in negative mode is primarily determined by the gas used. Negative mode primarily results in deprotonated species and adduct formation.<sup>23</sup>

For analysis of LPS, DART-TOF-MS methods have been developed that target the fatty acid methyl esters (FAME) composition of bacteria in order to identify pathogens.<sup>25</sup> FAME analysis has been validated for the use of rapid identification of bacteria by gas chromatography (GC). These methods are time-consuming for the analyst, though this has been alleviated by the

2007 introduction of the MIDI Instant Fame Extraction kit, which reduces the need for heated incubation.<sup>26,27</sup> The methylation enhances the volatility of FAMES needed for GC analysis, as fatty acids are not naturally volatile.<sup>27</sup> Using DART-TOF-MS for rapid direct analysis of fatty acids in untreated bacteria would be advantageous since the FAME extraction step would not be necessary.

DART-TOF-MS has been previously used in the analysis of LPS.<sup>28</sup> In this study, DART-TOF-MS was used in order to rapidly screen LPS molecules that could be found on viscosurgical device materials and found that DART-TOF-MS allowed for a direct, rapid technique for the detection of endotoxins in different matrices. Further, DART-TOF-MS produced low mass characteristic ions from the overall complex endotoxin.<sup>28</sup>

The goal of this research was to develop a DART-TOF-MS protocol for rapid detection and screening of LPS molecules from *E. coli*. This has important implications for rapid analysis of biothreat agents. A secondary part of this research focused on the evaluation of a rapid extraction technique by GC-FID in order to determine the fatty acid composition of the extracts and compare these compositions to those found in whole cell *E. coli*.

## **RESEARCH MATERIALS AND METHODS**

### *E.coli Preparation*

*Escherichia coli* O157 (ATCC strain 43888, Manassas, VA, USA) was inoculated on Trypticase Soy Agar (TSA, Benton Dickinson, Franklin Lakes, NJ, USA) and incubated at 37 °C for 18-24 hours. Cultures were harvested and resuspended in two milliliters of ultrapure water

(18MΩ, 0.2μm filtered). After resuspension, 500 μL of the bacteria suspension was aliquoted into microcentrifuge tubes. Whole cell *E. coli* samples had no further preparation prior to DART analysis.

For lysed cell samples, direct lysis was performed by boiling the cellular material at 100 °C for 10 minutes. For the fatty acid methyl ester (FAME), the Instant FAME™ Extraction Kit (MIDI Inc., Newark, DE, USA) was utilized. Whole cell samples were first treated with 250 μL of Reagent 1 (5% KOH, 95% methanol). After vortexing for 10 seconds, 250 μL of Reagent 2 (analytical grade hexane) was added, followed by 3 seconds of vortexing. Afterward, 250 uL of Reagent 3 (dilute HCl with red dye) was added, and the clear layer was removed and placed in a microcentrifuge tube for later DART-TOF-MS analysis.

Lipopolysaccharides from *E. coli* O127: B8 were purchased from Sigma-Aldrich (St. Louis, MO, USA). The lyophilized powder was analyzed directly by DART-TOF-MS.

#### *Extraction of E.coli LPS commercial LPS Extraction Kit*

A commercial LPS Extraction Kit (iNtRON Biotechnology, Seongnam, Gyeonggi, South Korea) was used to extract LPS from *E. coli* following the manufacturer's protocol. The entire TSA plate of cellular material was harvested for extraction, resuspended in 1X Phosphate Buffered Saline (PBS), and centrifuged at 13,000 rpm for 30 seconds. After centrifugation, the supernatant was removed. One milliliter of Lysis Buffer was added to the cell pellet and the sample was vortexed until the visible biomass was resuspended. Next, 200 μL of chloroform (Alfa Aesar, Ward Hill, MA, USA) was added followed by vortexing and incubation at room temperature for five minutes.

After incubation, the samples were centrifuged at 13,000 rpm for 10 minutes at 4 °C. The supernatant (~400 µL) was transferred to a clean microcentrifuge tube. Next, 800 µL of Purification Buffer was added to the supernatant and the samples were mixed using a vortexer. The samples were then incubated at -20 °C for 10 minutes.

After incubation, the samples were centrifuged for 15 minutes at 4 °C at 13,000 rpm. The supernatant liquid was removed, leaving the pellet behind. The pellet was washed with 1 mL of 70% EtOH and left to dry in the hood for 15-30 minutes until dry. After the pellet was dried, 70 µL of 10 mM Tris-HCl at pH 8.0, was added to the LPS pellet and it was sonicated for 10 minutes. The LPS pellet was stored at 4 °C for later analysis.

#### *DART-TOF-MS Parameters*

A JEOL JMS-I1100LC AccuTOF™ mass spectrometer (JEOL, Peabody, MA, USA) coupled with an IonSense DART controller ion source (Saugus, MA, USA) was used in both negative and positive mode for all experimentation. Ultra-high purity (99.999%) helium (Airgas, Radnor, PA, USA) was used as the ionizing gas, while compressed nitrogen (Praxair, Danbury, CT, USA) was used in standby mode.

Positive mode DART-TOF-MS settings were as follows: heater temperature: 350 °C; desolvating chamber: 250 °C; grid voltage: 100 V; ring lense voltage: 5 V; orifice 1 voltage: 20V; orifice 2 voltage: 5 V; focus voltage: -120 V; reflectron voltage: 900 V; detector voltage: 2000 V.

Negative mode DART-TOF-MS settings were as follows: heater temperature: 350 °C; desolvating chamber: 250 °C; grid voltage: 150 V; ring lense voltage: -5V; orifice 1 voltage: -20

V; orifice 2 voltage: -5 V; focus voltage: 120 V; reflectron voltage: -900 V; detector voltage: 2000 V.

For each sample, a melting point capillary tube was dipped into the sample and placed into the helium stream five times in order to obtain DART-TOF-MS spectra. Polyethylene glycol, PEG 600, (Sigma-Aldrich, St. Louis, MO, USA) was used as a calibrator. The PEG calibrator analyzed in positive mode was prepared by adding 50  $\mu$ L of PEG 600 to 5 mL of Methanol. The PEG calibrator analyzed in negative mode was prepared by adding 50  $\mu$ L of PEG 600 in 10 mL of 1:1 Methanol: Dichloromethane. All DART-TOF-MS spectra were acquired in both positive and negative modes, over a mass range of 50-1000  $m/z$  with the Mass Center Main software. Data reduction and analysis were done using the TSSPro 3.0 software and the resulting spectral data was an average mass spectrum of one peak in the total ion chromatogram produced by the software.

#### *FAME Extract Preparation and GC-FID Parameters*

As described above the Instant FAME™ Extraction Kit was used to isolate FAMES from whole cell *E. coli* and the LPS extracts obtained from the rapid extraction procedure.

An Agilent 7890A GC system equipped with a 25m x .200mm x .33  $\mu$ m HP-ULTRA 2 column (Agilent Technologies, Santa Clara, CA, USA) was used for the analysis. The injection parameters were as follows: injection volume: 2  $\mu$ L, mode: split, split ratio: 20:1, inlet temperature: 250 °C. The oven program had an initial temperature of 172.3 °C, after the sample was introduced the first ramp was 28 °C/min until a temperature of 290.3 °C was reached; the hold time was 0 min. The second ramp was 60 °C/min to 310 °C and the hold time for this was

1.25 min. The flow rate was 1.352 mL/min. The FID parameters were as follows: FID temperature: 310 °C, air flow: 350 mL/min, hydrogen flow: 30mL/min, nitrogen (makeup) flow: 30 mL/min. All gas was supplied by Praxair and ultra-zero purity air, ultra-high purity nitrogen and compressed hydrogen was used. The total run time was 5.793 min. The FAME profiling was performed using the MIDI Microbial Identification Sherlock software. The MIDI calibration standard (MIDI Inc., Newark, DE, USA) was used prior to each run for identification and quantitation of the fatty acid peaks.

## RESEARCH RESULTS AND DISCUSSION

### *Detection of E. coli O127:B8 LPS standard*

*E. coli* LPS standard was analyzed using both positive and negative mode in DART-TOF-MS. Analyzing in both positive and negative mode allows for structural determination, especially in complex samples, like LPS.<sup>29</sup> The spectra obtained in positive mode can be found in Figure 2, while the spectra obtained in negative mode spectra can be found in Figure 3.

In positive mode, the major peak at mass to charge ratio of ( $m/z$ ) 89.10 is consistent with alanine. Alanine has a molecular weight of 89.09 Da and while this does not follow the normal  $[M+H]^+$  ionization expected in DART-TOF-MS, the presence of some  $M^+$  peaks are anticipated as a result of the rapid ionization.<sup>21</sup> Further, amino acids are expected to be present as the LPS core is composed of both carbohydrate and non-carbohydrate regions; the non-carbohydrate regions mainly consist of phosphate and amino acids.<sup>3,4</sup> The  $m/z$  of 122.08 in the positive mode spectra is consistent with cysteine as highlighted in Table 1. Cysteine has a molecular weight of



121.16 Da and this ion is the expected result of  $[M+H]^+$  ionization. Cysteine residues are an amino acid component of the LPS assembly proteins found in lysis cytoplasm and its detection is expected in the analysis. LPS assembly proteins are used to build and then transport LPS from the inner membrane to the outer membrane.<sup>30</sup> Further, the presence of amino acids, like cysteine, can be indicative of intracellular protein impurities from the LPS extraction. LPS standards are not chemically synthesized, rather they are extracted from whole cell *E. coli* and purified. The LPS standard used in this study was extracted and purified via a phenol extraction. Most likely the amino acids seen in the spectra could be from the intracellular protein residuals that remained in the purified sample after extraction.

In previous studies using DART-TOF-MS to analyze *E.coli*, LPS major peaks were observed for monosaccharides, such as galactose, or mannose, which are common within LPS in the o-antigen regions in negative mode.<sup>5,28</sup> They also report the  $[M+H]^+$  and  $[M-H]^-$  of a disaccharide in positive mode.<sup>28</sup> In this study, the  $[M-H]^-$  peak for the monosaccharides is located at  $m/z$  179.28 and has an intensity less than 100. Further, in both negative and positive mode, neither of the peaks corresponding to a disaccharide were detected. It should also be noted that Li *et al.* did not detect any of the fatty acids that were detected in the present study.<sup>28</sup> Parameters such as the amount of voltage applied and the heater temperature used by Li *et al.* differed from this study, which could have resulted in the observed differences. The presence of monosaccharides could be useful in the detection of carbohydrate regions of LPS.

The  $m/z$  136.06 in positive mode and its corresponding  $m/z$  134.05 in negative mode, indicates that a species with a molecular weight of 135 Da is present. While this peak could be a  $C_{10}H_{15}$  group (135.23 Da) as a result of fragmentation of the fatty acids as seen in other MS methods, the fragmentation of the fatty acids has not been observed in previous DART-TOF-MS

studies.<sup>29</sup> These peaks may be caused by compounds present in the ambient background which was observed intermittently throughout this study.

Previous research has determined the limit of detection of fatty acids for the DART-TOF-MS which is approximately  $1 \times 10^{-5}$   $\mu\text{g}$ , which is equal to about 100 bacterial cells worth of fatty acid.<sup>31</sup> Fatty acids were detected in both positive and negative mode. Due to the loss of the hydrogen on the carboxylic acid, deprotonated anions commonly seen in fatty acids are produced.<sup>29</sup> Fatty acid chains are present in the Lipid A portion of the molecule as seen in Figure 1. All of the  $m/z$  produced in negative mode are shown in Table 1. Dodecanoic Acid (12:0, 200.32 Da) has a protonated peak at  $m/z$  201.18 and deprotonated peak at  $m/z$  199.17. Dodecanoic acid has been determined to be a common fatty acid constituent in *E. coli* LPS by Varbanets *et al.*, in their study on the characterization of *E. coli* 126 LPS. As a result, the presence of dodecanoic acid in a sample can help validate the presence of LPS.<sup>32</sup>

Another fatty acid present in *E. coli* LPS, is tetradecanoic acid (14:0, 228.37 Da).<sup>32</sup> Peaks at  $m/z$  at 227.19 and  $m/z$  227.20 were present in both positive and negative mode and are consistent with tetradecanoic acid. While the  $[\text{M}-\text{H}]^-$  follows the expected ionization producing the peak at 227.20, the presence of an  $[\text{M}-\text{H}]^+$  peak (227.19) in positive mode may be unusual. It was possible that a proton was lost from the carboxylic acid of the fatty acid prior to introduction to the ionization stream, resulting in the  $[\text{M}-\text{H}]$  peak, which was detected in positive mode. In positive mode, the  $m/z$  of 227.19 was the most abundant fatty acid, while in negative mode  $m/z$  of 227.20 was the second most abundant. The 14:0 fatty acid dimer (456.74 Da) was detected in both positive and negative mode at  $m/z$  455.41 and 453.39, respectively. Dimerization commonly occurs in DART-MS and is possibly due to the high concentration of fatty acids within the lyophilized powder. Dimers are most likely the result of noncovalent dimerization, which results

from the acidic hydrogen on carboxylic acid being deprotonated and then reforming with another carboxylic acid of a second fatty acid.<sup>23</sup> Further studies with the DART-TOF-MS, changing the ionization voltage is required to further explore this.

3-Hydroxy-tetradecanoic acid (3OH-14:0, 244.37 Da) was the most abundant fatty acid found within the LPS of *E. coli*.<sup>32</sup> A peak at  $m/z$  of 244.22 was present in positive mode. Again, it was not uncommon that some  $M^+$  species were present as a result of the ionization efficiency.<sup>21</sup> The corresponding  $[M-H]^-$  was at  $m/z$  of 243.19. Consistent with previous studies, the  $m/z$  of 243.19 was the most abundant fatty acid, which indicates that this ion could be used in the differentiation of *E. coli* LPS from other samples.

The last fatty acid was detected at a molecular weight of approximately 261.39 Da. The  $m/z$  in positive mode for this species was at 262.24, while the negative mode  $m/z$  was at 260.20. This was consistent with 3OH-14:0, but as a result of an ammonium ( $NH_4^+$ ) and ammonia ( $NH_3$ ) adduct in positive and negative mode, respectively. Since  $NH_4^+$  is a common adduct in DART-TOF-MS, an adduct being present was not unreasonable.<sup>21</sup>  $NH_4^+$  has a molecular weight of 18.04 Da and was most likely an ionized component of sweat; ammonia is known to be present due to the body's excretion of nitrogen via ammonia.<sup>34</sup> Both ammonia and ammonium have been commonly identified in both positive and negative modes in DART-MS.<sup>35,36</sup> The  $m/z$  of 262.24 may have been a result of an  $[M^+H+NH_4]^+$  adduct; the expected molecular weight, after subtraction of the  $NH_4^+$ , would be 244 Da, the same as 3OH-14:0 and at the same  $m/z$  where the 3OH-14:0 peak occurs in the positive mode spectra. The negative mode  $m/z$  of 260.20 was a result of an  $[M-H+NH_3]^-$  adduct; this expected molecular weight would be 243 Da; which was the same as the  $m/z$  for 3OH-14:0 detected in the negative mode spectra. Another candidate for this peak could be hexadecanoic acid (16:0, 256.42 Da), as this is a known component in *E. coli*

LPS determined in previous studies (Varbanets).<sup>32</sup> This ion was unlikely produced through the expected  $[M+H]^+$  ionization results as there are 5 mass units between 256 and 261.<sup>33</sup> Further analysis is required in order to determine if the  $m/z$  at 261.20 is a result of an adduct or another fatty acid component.

#### *Analysis of Whole Cell E. coli, Lysed E. coli, and FAME extracts*

In order to characterize diversity and abundance of compounds detected in different types of cell preparations of *E. coli*, we also analyzed untreated suspensions of *E. coli*, lysed *E. coli* and FAME methylated extracts in both positive and negative modes. The untreated *E. coli* suspensions (i.e., ‘whole cell’), lysed cell and the FAME extracts showed distinct spectra from the LPS standard. Figures 4 and 5 show the whole cell *E. coli* spectra in positive and negative mode. Figures 6 and 7 are the lysed *E. coli* spectra and Figures 8 and 9 are the FAME extracts spectra in positive and negative mode, respectively. Data from whole cell suspensions of *E. coli* suggest that only fatty acids from the outer membrane surface primarily being detected, indicating that the integrity of the inner cell membrane is not compromised after introduction to the DART-MS. Profiles generated from *E. coli* lysate indicate both surface and intracellular fatty acids consistent with the extraction procedure.

Since FAMEs have been previously validated as biochemical markers for classification and differentiation of bacteria, FAME extracts of *E. coli* were also analyzed by GC-FID. These analyses compared the GC-FID fatty acid profile results with profiles generated with DART-TOF-MS. The differences of the FAME spectra were compared to the LPS standard and *E. coli* suspensions spectra were expected. Since the Instant FAME™ Extraction Kit focuses on the

direct extraction and methylation of the fatty acids over other cellular components, it was predicted that the FAME spectra produced primarily fatty acids. The method was chosen because it requires about minimal material (~1mg biomass) and approximately five minutes of preparation time.

#### *Identification of Components of Untreated E. coli by DART-TOF-MS*

As seen in Figure 4, there are only three significant peaks at 118.06, 132.10 and 166.08  $m/z$  in the positive mode profiles of whole cell suspensions of *E. coli*. Corresponding peaks at 116.08, 130.11, and 164.08  $m/z$  were observed in negative mode as well (Figure 5; Table 2). Also present in negative mode, were peaks at 227.20 and 243.19  $m/z$ .

The  $m/z$  of 118.06 (positive mode) and the  $m/z$  of 116.08 (negative mode) were consistent with valine, which has a molecular weight of 117.15 Da. These peaks are the most abundant non fatty acid in both positive and negative mode. The  $m/z$  of 132.10 in positive mode and  $m/z$  of 130.11 in negative mode were consistent with leucine or isoleucine. Finally, the  $m/z$  of 166.08 (positive mode) and the  $m/z$  164.08 (negative mode) correspond to phenylalanine. Leucine/ isoleucine and phenylalanine have molecular weights of 131.17 Da and 165.19 Da, respectively. Another possibility for the differences in the amino acid composition between the whole cell *E. coli* and the LPS standard was that these free amino acids were present in the TSA growth medium and adhered to the cell surface during culturing, as has been observed in other studies of gram-negative organisms.<sup>38</sup>

The negative mode spectra (Figure 5) had two fatty acids, 14:0 and 3OH-14:0, present. As discussed earlier, the presence of fatty acids in negative mode ionization was expected since

the negative ionization was more conducive to the fatty acids.<sup>29</sup> The  $m/z$  of 227.20 is consistent with 14:0 fatty acid, while the  $m/z$  of 243.19 indicates the 3OH-14:0 fatty acid. Both followed the expected  $[M-H]^-$  ionization. Additionally, it was expected that these fatty acids would be present as it has been shown that the 14:0 and 3OH-14:0 acids are components of *E. coli*. The 14:0 was the most abundant fatty acid detected in negative mode. Since the 14:0 and 3OH-14:0 are common fatty acids found in the Lipid A portion of LPS, these two fatty acids indicated that LPS was present in the whole cell *E. coli*. Their presence also indicated that the DART-TOF-MS was predominantly ionizing and detecting constituents on the surface of the *E. coli* cell.

The hexadecanoic acid (16:0) was not detected, even though it is a major fatty acid component in whole cell *E. coli*<sup>39</sup> Its absence here may reflect slight variation in the culturing conditions between studies or that the 16:0 fatty acid was present but not above the limit of detection in the DART-TOF-MS in this particular sample.

#### *Identification of Components of Heat-lysed E. coli by DART-TOF-MS*

The spectra of lysed *E. coli* for positive and negative mode can be found in Figures 6 and 7, while Table 3 summarizes the  $m/z$  and proposed ID of these peaks. The positive mode spectra have the same amino acid components that were present in the whole cell sample: valine, leucine/ isoleucine, and phenylalanine at  $m/z$  of 118.06, 132.10, and 166.08 respectively. The amino acids also had corresponding peaks in negative mode at  $m/z$  of 116.08, 130.11 and 164.08, similar to the whole cell *E. coli* profile. Again, valine was the most abundant of the non-fatty acid components. It is possible that these amino acids were from proteins on the cell, but could also been due to free amino acids within the growth media.<sup>38</sup>

Different fatty acids were present in the lysed cell than in the whole cell; though this indicated that the lysis was successful and the intracellular fatty acids of *E. coli* could be detected. These differences in fatty acid composition could be used to indicate purity of an LPS extract as pure LPS would be expected to have 12:0, 14:0 3OH-14:0 and 16:0 fatty acid components. The presence of other fatty acids could indicate partial or incomplete extraction.<sup>32</sup>

All of the fatty acids were detected in negative mode. As shown in Table 4, there were six fatty acids present that could have been determined. The  $m/z$  of 199.18 was 12:0 fatty acid. The  $m/z$  of 227.20 was 14:0 fatty acid and was the second most abundant fatty acid. The fatty acid present in the lysate spectra was at  $m/z$  of 243.19 and was consistent with the 3OH-14:0 fatty acid.

The 16:0 fatty acid has a molecular weight of 256.42 Da and its  $[M-H]^-$  peak was present at  $m/z$  of 255.23. 16:0 was the most abundant fatty acid in the lysed sample. The peak at  $m/z$  of 267.23 was consistent with *cis*-9,10-methylenehexadecanoic acid (17cyclo, 268.40 Da). The  $m/z$  of 281.25 was consistent with *cis*-11-octadecenoic acid (18:1 $\Delta$ 11*cis*, 282.50 Da). All of these listed fatty acids were found in previously published literature to be the dominant fatty acids in *E. coli*. Both 17:0 cyclo and 18:1 $\Delta$ 11*cis* are significant fatty acids within the plasma membrane of *E. coli*.<sup>40,41</sup>

The presence of intracellular fatty acids along with the expected surface fatty acids, show that the lysis was successful and that *E. coli* lysates could be differentiated from intact *E. coli* and *E. coli* LPS using DART-TOF-MS.

## *Identification of Components of E. coli FAME Extracts by DART-TOF-MS and GC-FID*

Whole cell *E. coli* fatty acids were extracted via FAME methylation and then analyzed by DART-TOF-MS and as a confirmatory analysis, whole cell *E. coli* FAME extracts were also analyzed by GC-FID. The positive mode spectra can be found in Figure 8 and the negative mode spectra is in Figure 9. Table 4, which is the DART-TOF-MS data for the FAME extracts, contains the expected molecular weight of the FAME product of the fatty acid, rather than the molecular weight of the original fatty acid. The FAME molecular weight represents the original molar mass plus the addition of a methyl group.

The  $m/z$  of 118.06 was only present in positive mode and was consistent with valine. Though valine was present in the other samples, the expected results of the FAME extraction were primarily the FAMEs of the fatty acids.

The remaining identified peaks were FAMEs. The  $m/z$  of 241.21 in positive mode was consistent with the methyl ester of (9Z)-9-tetradecanoic acid (14:1), which has a molecular weight of 240.41 Da. This FAME was identified in previous studies at low intensity, similar to the findings presented herein; suggesting that 14:1 is not a significant fatty acid.<sup>25</sup> The next identified  $m/z$  is of 257.24 and 255.08 detected in positive and negative mode, respectively were the methyl ester of pentadecanoic acid (15:0), which has a molecular weight of 256.43. It was also the most abundant FAME in the negative spectra. The 15:0 FAME was detected in Pierce *et al.*, but the existence of odd chain unsaturated fatty acids in *E. coli* is not typical.<sup>25</sup> This may have been the result of possible contamination from the strains of *Bacillus* that was maintained in the laboratory. *Bacillus* unlike *E. coli* has 15:0 iso as a major component of the membrane fatty acid profile,<sup>27</sup> *Bacillus* is also known to have heptadecanoic acid (17:0), which would also have



a FAME molecular weight of 283.46. However, these structural isomers are not able to be differentiated using peak position. For *Bacillus* organisms, 12:0, 13:0 and 16:0 peaks would also be expected.<sup>26</sup> While the 12:0 and 13:0 were not present in either mode, 16:0 was present within positive mode. Since these are common fatty acids between the two, it is difficult to conclusively determine if this *E. coli* sample was contaminated with *Bacillus* without further analysis by GC-FID.

The next FAMES identified were the methyl esters of (9Z)-9-hexadecenoic acid (16:1, 268.46) at  $m/z$  269.24 and 16:0 (270.55 Da) at  $m/z$  of 271.26 in positive mode. Pierce *et al.* described detection of both the unsaturated (16:1) and saturated (16:0) FAMES in their study.<sup>25</sup> The last three FAMES detected were also identified in Pierce *et al.* and are known intracellular fatty acids of *E. coli*.<sup>25,42</sup> The next FAME was determined to be 18:1 $\Delta$ 11*cis* (methyl ester molecular weight: 296.51 Da) at  $m/z$  of 297.27 in positive mode and had the second most abundance. The final detected FAME was at  $m/z$  of 311.29 in positive mode and was consistent with *cis*-11,12-methyleneoctadecanoic acid (19cyclo), whose FAME has a molecular weight of 310.54 Da.

GC-FID analysis of whole cell *E. coli* after FAME extraction was also completed in order to compare and confirm results of the DART-TOF-MS analysis. The chromatogram for GC-FID can be seen in Figure 10. Table 5 represents the average fatty acid composition as determined by whole cell FAME extracts and the MIDI software. The most abundant fatty acid was the 16:0 at 33.3% relative intensity, which is consistent with literature on the fatty acid composition of *E. coli*.<sup>39</sup> The next most abundant were 18:1 $\Delta$ 11*cis* at 23.0%, 17cyclo at 17.7% and 16:1 at 15.0%; according to the findings in Li *et al.* the 18:1 $\Delta$ 11*cis* was found to have 6.6% composition and the 16:1 was found to have 5.9% composition.<sup>39</sup> The variation in fatty acid composition may be due

to the differences between the strains of *E. coli* organisms used in the Li et al study compared to this one since there can be wide variation between strains.<sup>27,39</sup> The 17:0 cyclo abundance was found to match previously reported values of 17.2%.<sup>30</sup> The 15:0 fatty acid was not detected in any *E. coli* sample that was analyzed by GC-FID. This further supported the DART profile containing the 15:0 was the result of non-*E.coli* material. The presence of these fatty acids does not reflect the abundances of the FAMES found in DART-TOF-MS; but the presence of common compounds detected in the GC-FID and the DART-TOF-MS was strong evidence that the FAMES detected by DART-TOF-MS were the major fatty acids within *E. coli* and differences in these fatty acids could be used to assess LPS of *E. coli*.

#### *Evaluation of the Extracts from the Commercial LPS Extraction Kit by FAME extraction and GC-FID*

The iNtRON Biotechnology LPS extraction kit is advertised as being able to isolate and purify LPS from Gram negative bacteria in approximately 60 minutes. This rapid extraction is based on the phenol-water extraction that is employed in conventional protocols for LPS extraction. However, conventional methods using phenol-water extraction typically takes several hours to complete.<sup>13,42</sup> In this extraction, the lysis buffer was used to lyse the cellular material and form separate the components into organic and water-soluble layers.<sup>42</sup> After incubation and centrifugation, a white precipitate consisting of DNA and cellular debris separates the organic layer from the aqueous layer. The aqueous layer was collected and Purification Buffer was added. This Purification Buffer is a high-level salt solution and allows for purification of LPS

from the RNA and nucleic acid components. Further, the ethanol wash and drying period were used to dilute and subsequently remove any salts from the purification step.<sup>42</sup>

After extraction, the pellet containing LPS underwent subsequent methylation from the FAME extraction. The goal of this study was to determine the GC-FID profile of extracts from this extraction and determine extraction purity based off of the expected fatty acids present in *E. coli* LPS.

The GC-FID chromatogram is shown in Figure 11. Table 6 is the average percent composition of the fatty acids found in the LPS extracts. The expected LPS fatty acids were 12:0, 14:0, 3OH-14:0 and 16:0. All of these were present in varying compositions. The 12:0 was found to have a 32.9% relative abundance, which has drastically increased from the 2.18% found in the whole cell FAME analysis. The 14:0 had 9.89% composition, which again has increased from the 2.65% found in whole cell analysis. The 3OH-14:0 also increased from 1.06% to 4.90%. The 16:0 abundance decreased from 33.3% to 29.8%, but this could be due to the variation in growth conditions of the *E. coli* prior to LPS extraction. While this indicates the presence of LPS within these extracts, 17cyclo, 18:1 $\Delta$ 11*cis* and 19cyclo were also detected. The fatty acids are primarily intracellular and were not expected to be on the cell surface, with the LPS molecule. All of the intracellular fatty acid compositions decrease in abundance from that of the whole cell *E. coli*, but their presence within the extract may indicate that there was also some intracellular material present. Since 18:1 $\Delta$ 11*cis*, 17cyclo, and 19:0 cyclo were components of this profile, it was determined that this extract was not pure, but rather enriched with LPS. This was a result of the abundance of 12:0, 14:0, 3OH-14:0 and 16:0 within these extracts. An interesting future project would be repeating the rapid extraction on the LPS pellet to determine if purity can be increased.

## RESEARCH CONCLUSIONS

This research demonstrated that DART-TOF-MS can be used to detect LPS specific fatty acids, such as 12:0, 14:0 and 3OH-14:0 and were able to differentiate the LPS from whole cell *E. coli*, lysed *E. coli*, and the *E. coli* FAME extracts. It was also determined that analyzing whole cell *E. coli* by DART-TOF-MS shows compounds consistent with cell surface components without significant evidence for components from the inner plasma membrane. This work also showed that DART-TOF-MS could be a viable analytical tool for the rapid analysis of bacterial LPS that does not require the cumbersome extraction that current methods use. Whole cell samples were differentiated from LPS samples by the abundance of the 14:0 and 3OH-14:0 fatty acids present within the sample. The whole cell *E. coli* showed less abundance for these fatty acids, whereas these were the major fatty acids within the LPS standard.

This research has also demonstrated that there is not a similarity of amino acids present in between the LPS standard and the other *E. coli* samples. The LPS standard has alanine and cysteine present, and while they may have been artifacts from the standard purification, the other *E. coli* samples do not show the same amino acid constituents. Whole cell *E. coli* and the lysed samples had valine, leucine/ isoleucine and phenylalanine present as detected in DART-TOF-MS in both positive and negative ion modes. The FAME extraction only had valine present in a lower abundance in only positive mode. The abundances of these amino acids could be due to free amino acids within the growth medium adhering to the cell, but overall the LPS standard was distinguished between the whole cell, lysed and FAME extracts of *E. coli*.

Finally, a preliminary evaluation of a commercial LPS rapid extraction kit showed complex profiles containing components from both the LPS and the plasma membrane.

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## APPENDICES

### A. LIST OF ABBREVIATIONS

LPS- Lipopolysaccharide

*E. Coli- Escherichia coli*

MS- Mass Spectrometry

MALDI-MS- Matrix Assisted Laser Desorption Ionization- Mass Spectrometry

DART-TOF-MS- Direct Analysis in Real Time/Time-of-Flight Mass Spectrometry

GC-FID- Gas Chromatography- Flame Ionization Detection

Kdo- 3-Deoxy-D-manno-oct-2-ulosonic acid

TLR4- Toll-like Receptor 4

FAME- Fatty Acid Methyl Esters

GC- Gas chromatography

TSA- Trypticase Soy Agar

PBS- Phosphate Buffered Saline

EtOH- Ethanol

SDS- Sodium Dodecyl Sulfate

PEG- Polyethylene Glycol

*m/z*- Mass to Charge

LC-MS- Liquid Chromatography- Mass Spectrometry

12:0- Dodecanoic Acid

14:0- Tetradecanoic Acid

3OH-14:0- 3-Hydroxy-Tetradecanoic Acid

16:0- Hexadecanoic Acid

17cyclo- *cis*-9,10-Methylenehexadecanoic Acid

18:1 $\Delta$ 11*cis*- *cis*-11-Octadecenoic Acid

14:1- (9Z)-9-Tetradecanoic Acid

15:0- Pentadecanoic Acid

16:1- (9Z)-9-Hexadecenoic Acid

19cylco- *cis*-11,12-Methyleneoctadecanoic Acid

18:0- Octadecanoic Acid

## B. FIGURES AND TABLES

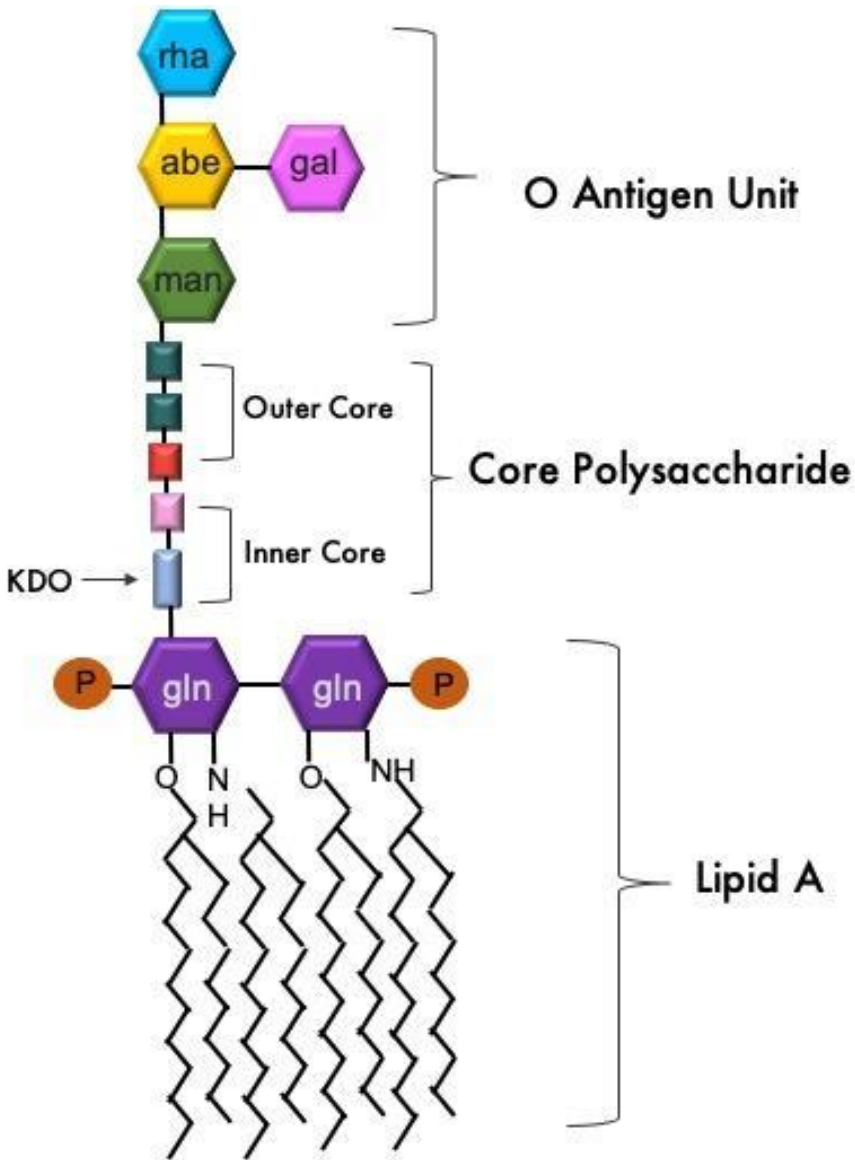


Figure 1. General Structure of Lipopolysaccharide (LPS) molecule.

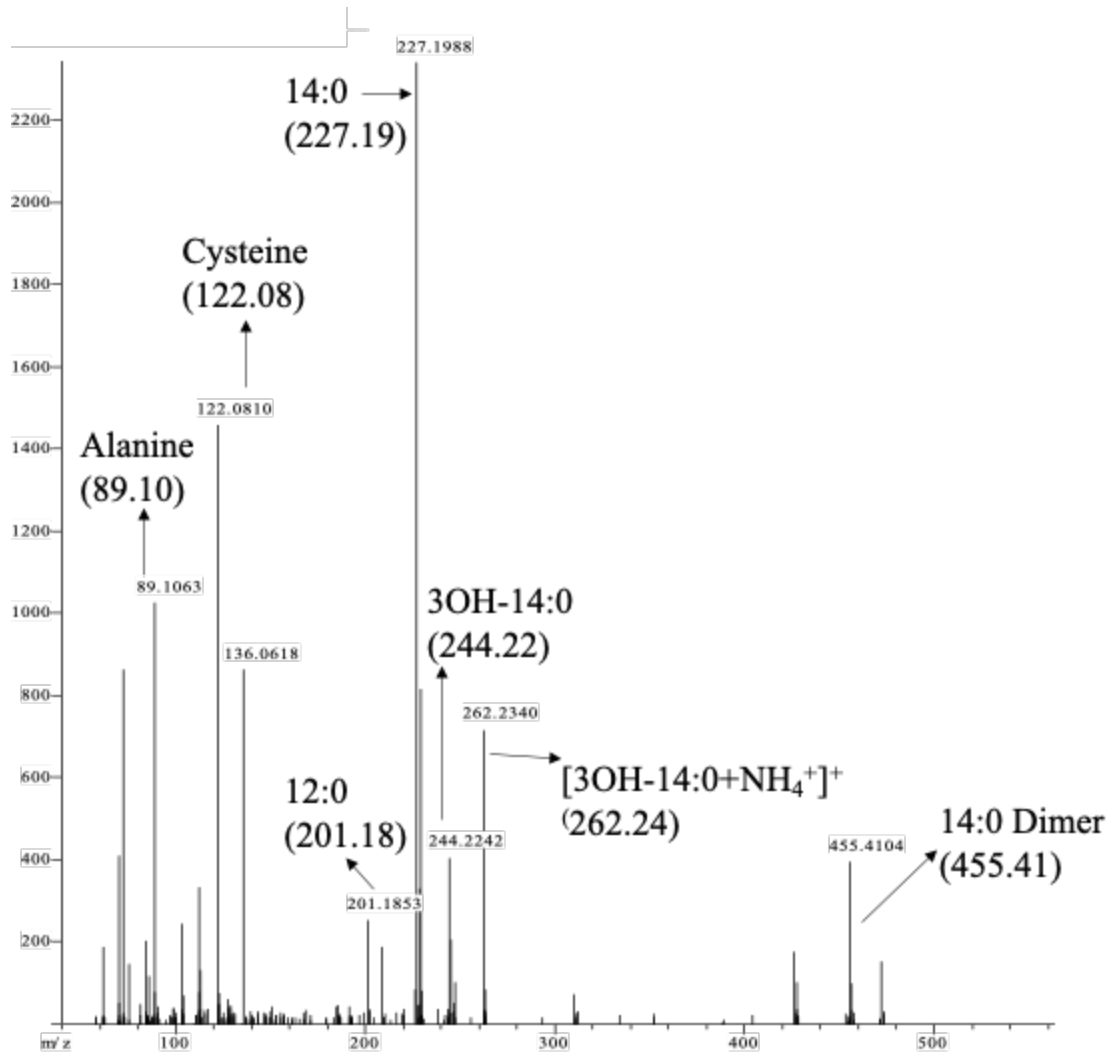


Figure 2. Positive ion mass spectrum of *E. coli* Lipopolysaccharides acquired by DART-TOF-MS spectra

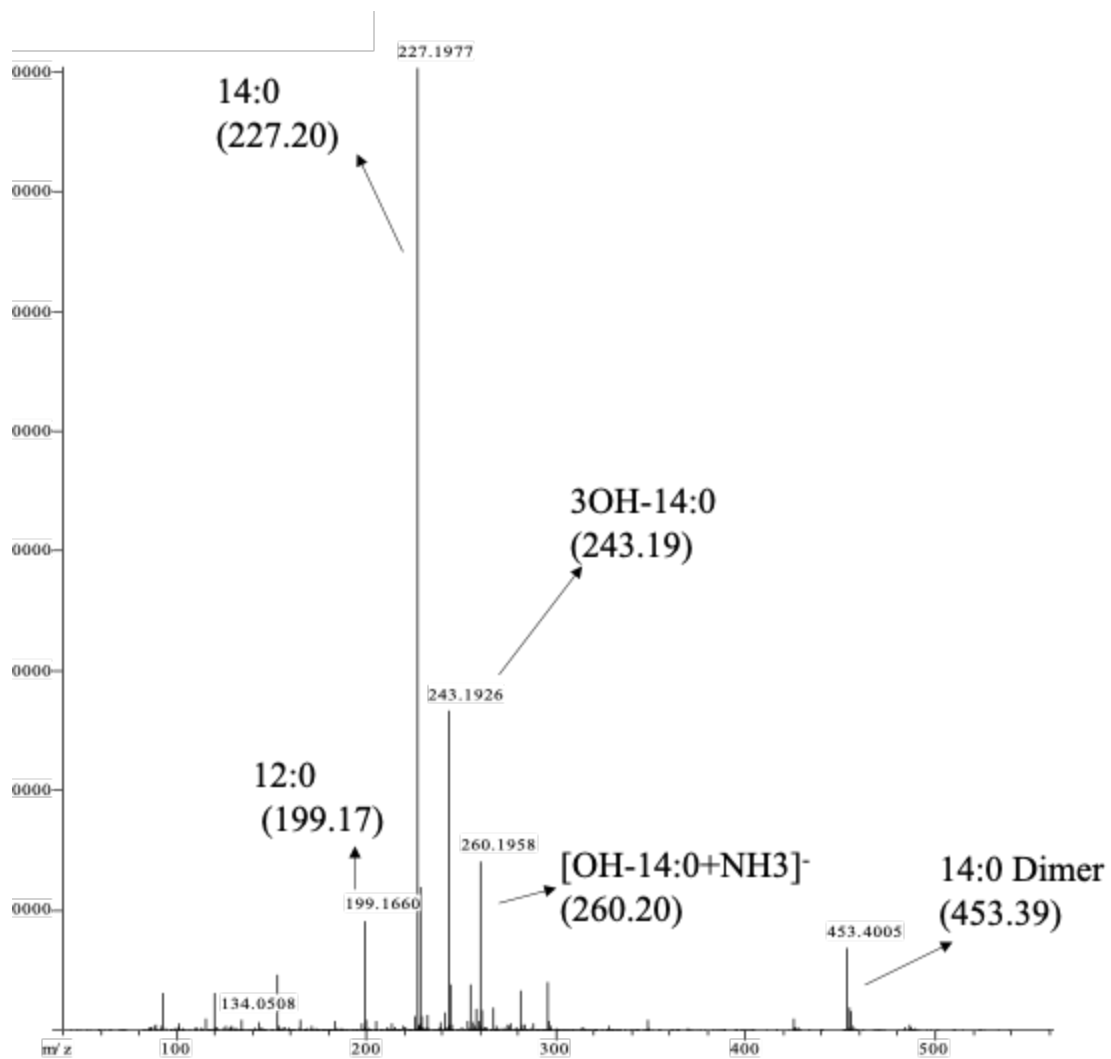


Figure 3. Negative ion mass spectrum of *E. coli* Lipopolysaccharides acquired by DART-TOF-MS spectra.

Table 1.

<b>DART-TOF-MS <i>m/z</i> of <i>E. coli</i> O127:B8 LPS Standard</b>			
<i>m/z</i>	Ionization Mode	Proposed Identity	Proposed Identity Molecular Weight (Da)
89.10	POS	Alanine	89.09
122.08	POS	Cysteine	121.16
136.06	POS	C <sub>10</sub> H <sub>15</sub>	135.23
		Background	~135.00*
201.18	POS	12:0	200.32
227.19	POS	14:0	228.37
244.22	POS	3OH-14:0	244.37
262.24	POS	[3OH-14:0 + NH <sub>4</sub> ] <sup>+</sup>	262.41
455.41	POS	14:0 Dimer	454.76
134.05	NEG	C <sub>10</sub> H <sub>15</sub>	135.23
		Background	~135.00*
199.17	NEG	12:0	200.32
227.20	NEG	14:0	228.38
243.19	NEG	3OH-14:0	244.37
260.20	NEG	[3OH-14:0 + NH <sub>3</sub> ] <sup>-</sup>	262.39
453.39	NEG	14:0 Dimer	454.76
Abbreviations: POS, positive; NEG, negative; 12:0, dodecanoic acid; 14:0, tetradecanoic acid; 3-OH-14:0, 3-Hydroxy-tetradecanoic acid			
*The exact mass of this background component is unknown			

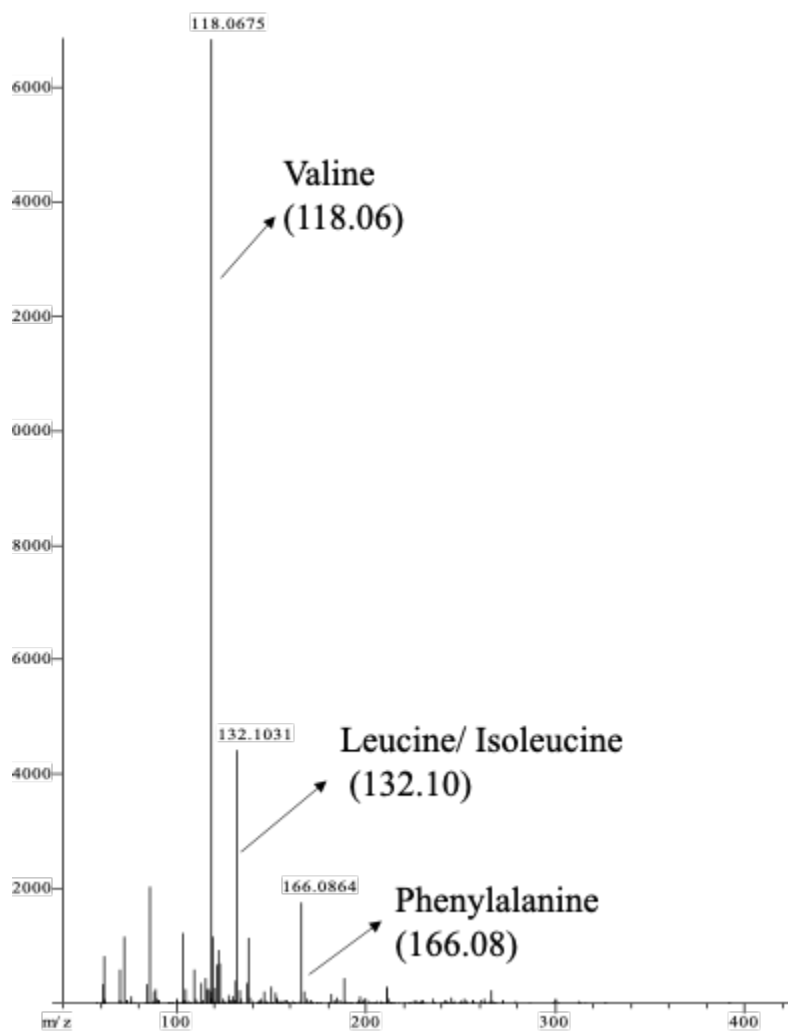


Figure 4. Positive ion mass spectrum of whole cell *E. coli* 43888 acquired by DART-TOF-MS.

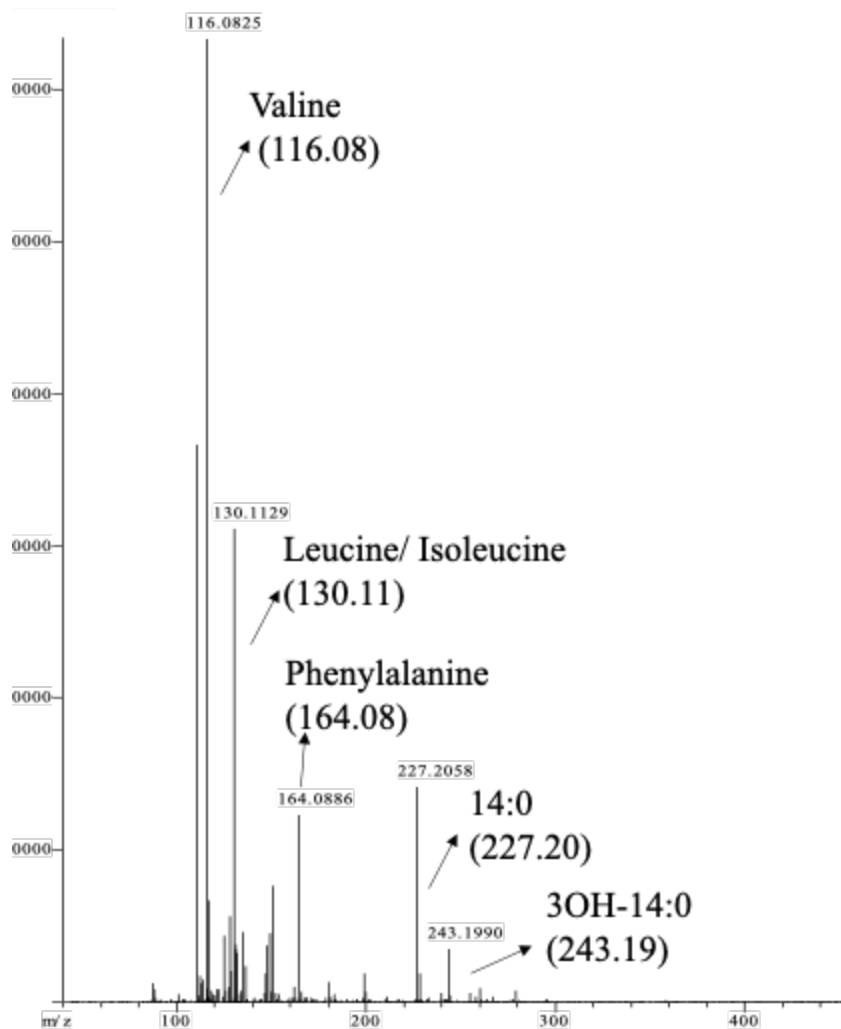


Figure 5. Negative ion mass spectrum of whole cell *E. coli* 43888 acquired by DART-TOF-MS.

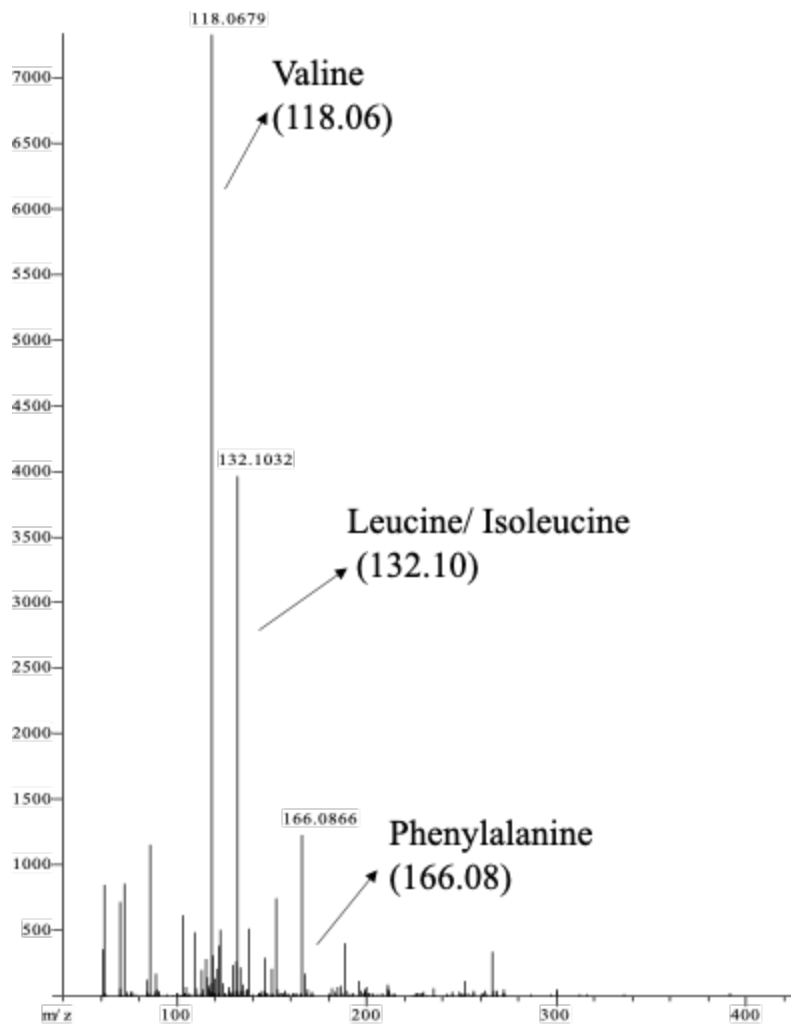


Figure 6. Positive ion mass spectrum of *E. coli* 43888 after direct lysis acquired by DART-TOF-MS.



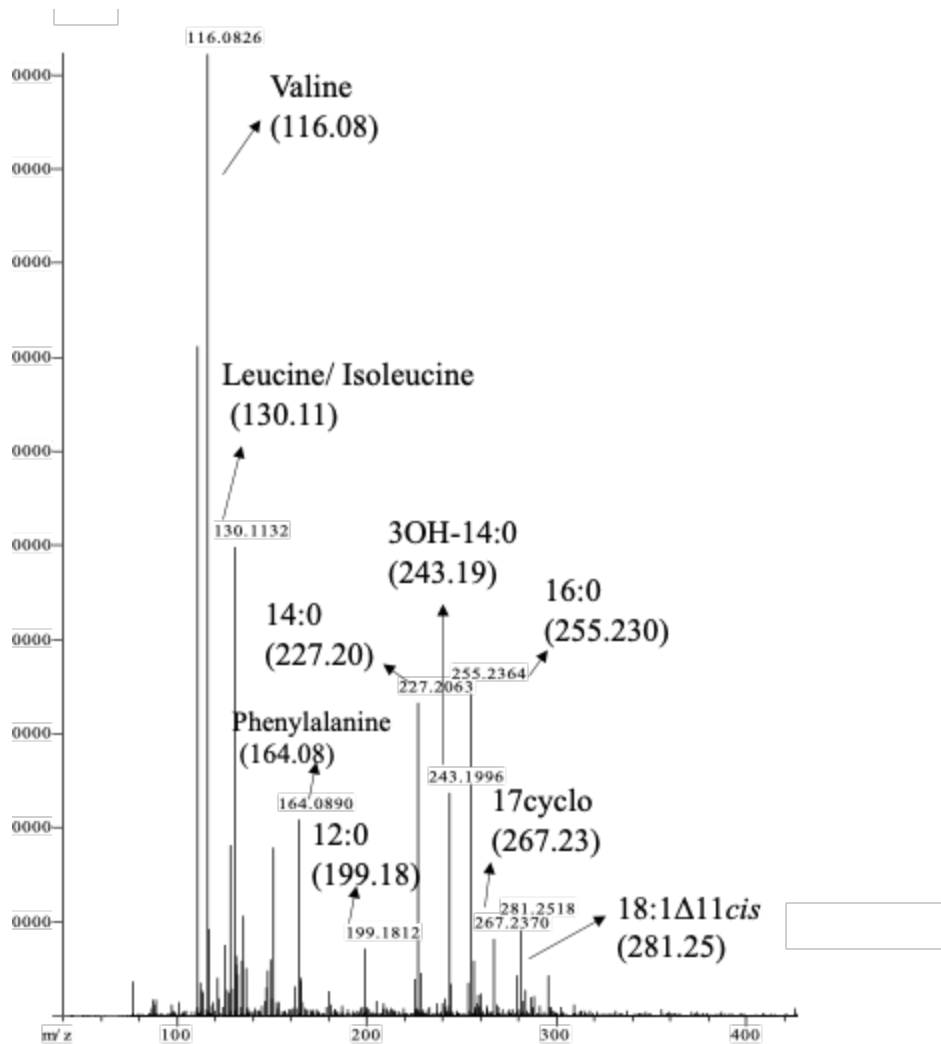


Figure 7. Negative ion mass spectrum of *E. coli* 43888 after direct lysis acquired by DART-TOF-MS.

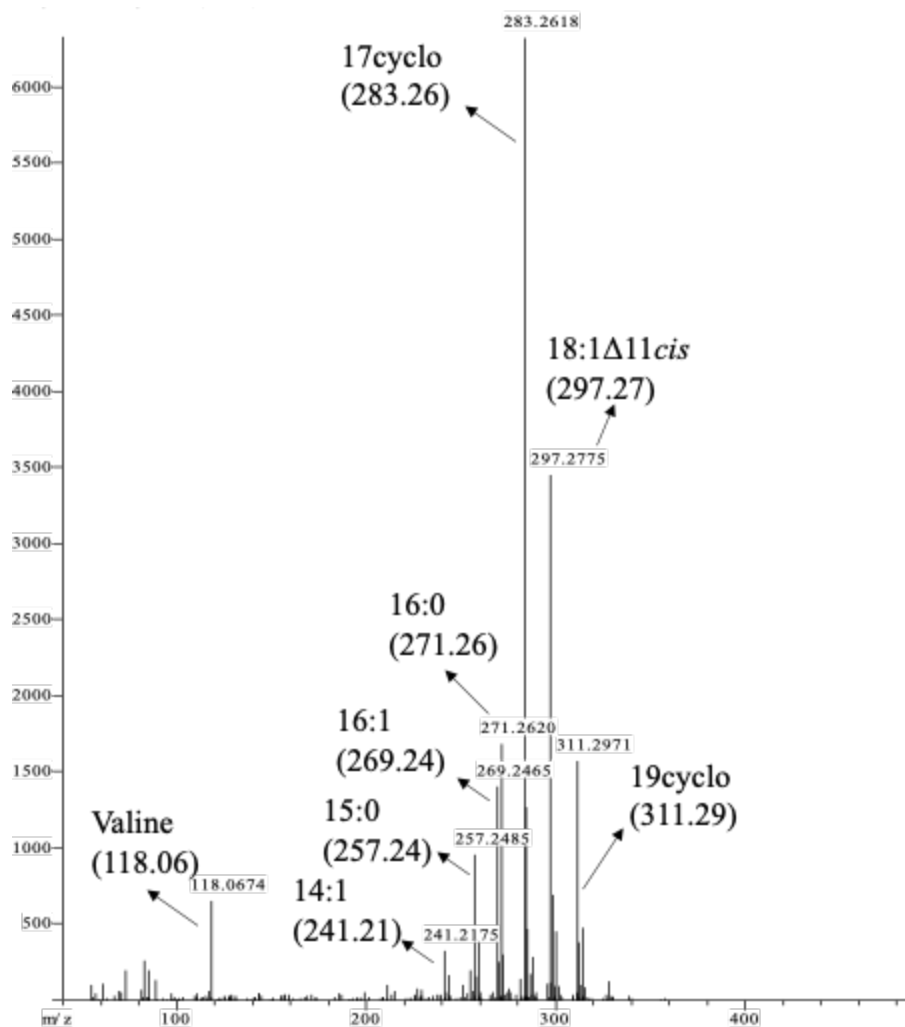


Figure 8. Positive ion mass spectrum of Fatty Acid Methyl Esters (FAMES) of *E. coli* 43888 acquired by DART-TOF-MS.

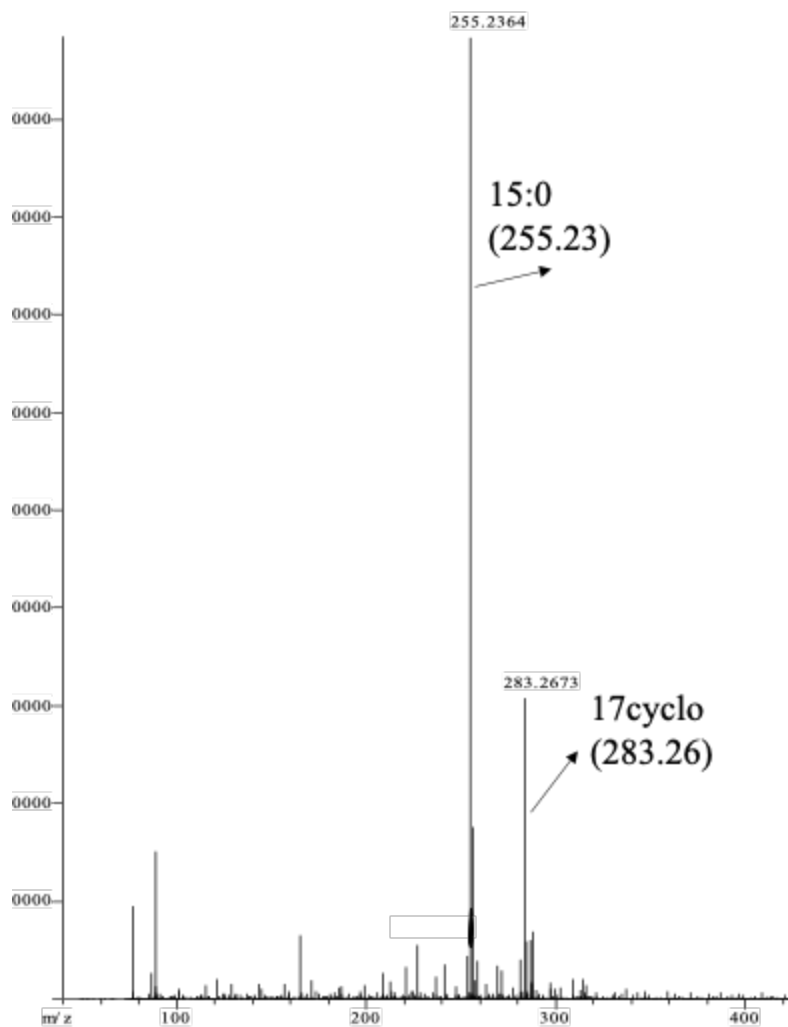


Figure 9. Negative ion mass spectrum of Fatty Acid Methyl Esters (FAMES) of *E. coli* 43888 acquired by DART-TOF-MS.

Table 2.

<b>DART-TOF-MS <i>m/z</i> of whole cell <i>E. coli</i> 43888</b>			
<i>m/z</i>	Ionization Mode	Proposed Identity	Proposed Identity Molecular Weight (Da)
118.06	POS	Valine	117.15
132.10	POS	Leucine/ Isoleucine	131.17
166.08	POS	Phenylalanine	165.19
116.08	NEG	Valine	117.15
130.11	NEG	Leucine/ Isoleucine	131.17
164.08	NEG	Phenylalanine	165.19
227.20	NEG	14:0	228.37
243.19	NEG	3OH-14:0	244.37
Abbreviations: POS, positive; NEG, negative; 14:0, tetradecanoic acid; 3OH-14:0, 3-hydroxy-tetradecanoic acid			

Table 3.

<b>DART-TOF-MS m/z of <i>E. coli</i> 43888 after Direct Lysis</b>			
<i>m/z</i>	Ionization Mode	Proposed Identity	Proposed Identity Molecular Weight (Da)
118.06	POS	Valine	117.15
132.10	POS	Leucine/ Isoleucine	131.17
166.08	POS	Phenylalanine	165.19
116.08	NEG	Valine	117.15
130.11	NEG	Leucine/ Isoleucine	131.17
164.08	NEG	Phenylalanine	165.19
199.18	NEG	12:0	200.32
227.20	NEG	14:0	228.37
243.19	NEG	3OH-14:0	244.37
255.23	NEG	16:0	256.42
267.23	NEG	17cyclo	268.4
281.25	NEG	18:1 $\Delta$ 11 <i>cis</i>	282.5

Abbreviations: POS, positive; NEG, negative; 12:0, dodecanoic acid; 14:0, tetradecanoic acid; 3OH-14:0, 3-hydroxy-tetradecanoic acid; 17 cyclo, *cis*-9,10-methylenehexadecanoic acid; 18:1 $\Delta$ 11*cis*, (11*Z*)-11-octadecenoic acid

Table 4.

<b>DART-TOF-MS <i>m/z</i> of FAMES of <i>E. coli</i> 43888</b>			
<i>m/z</i>	Ionization Mode	Proposed Identity	Proposed Identity Molecular Weight (Da)*
118.06	POS	Valine	117.15
241.21	POS	14:1	240.41
257.24	POS	15:0	256.43
269.24	POS	16:1	268.46
271.26	POS	16:0	270.55
283.26	POS	17cyclo	283.46
297.27	POS	18:1 $\Delta$ 11 <i>cis</i>	296.51
311.29	POS	19cyclo	310.54
255.08	NEG	15:0	256.43
283.00	NEG	17cyclo	283.46
Abbreviations: POS, positive; NEG, negative; 14:1, (9Z)-9-tetradecenoic acid; 15:0, pentadecanoic acid; 16:1, (9Z)-9-hexadecenoic acid; 16:0, hexadecanoic acid; 17 cyclo, <i>cis</i> -9-,10-methylenehexadecanoic acid; 18:1 $\Delta$ 11 <i>cis</i> , (11Z)-11-octadecenoic acid; 19:0cyclo, <i>cis</i> -11,12-Methylene-Octadecanoic Acid			
*Molecular weights are reported as FAME product molecular weights, not the original fatty acid			

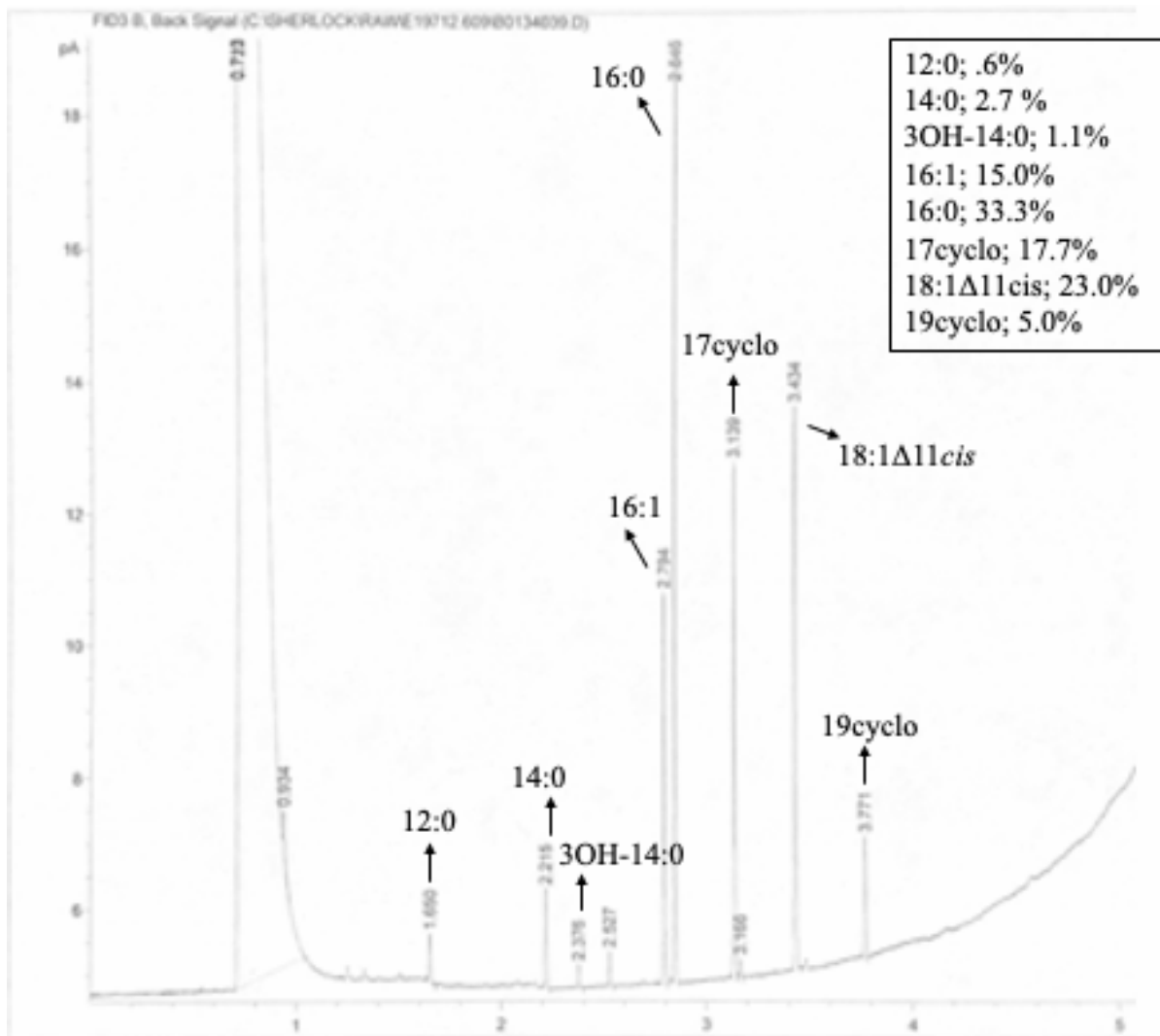


Figure 10. GC chromatogram of Fatty Acid Methyl Esters (FAMES) of *E. coli* 43888 acquired by GC-FID after Instant FAME™ Extraction.

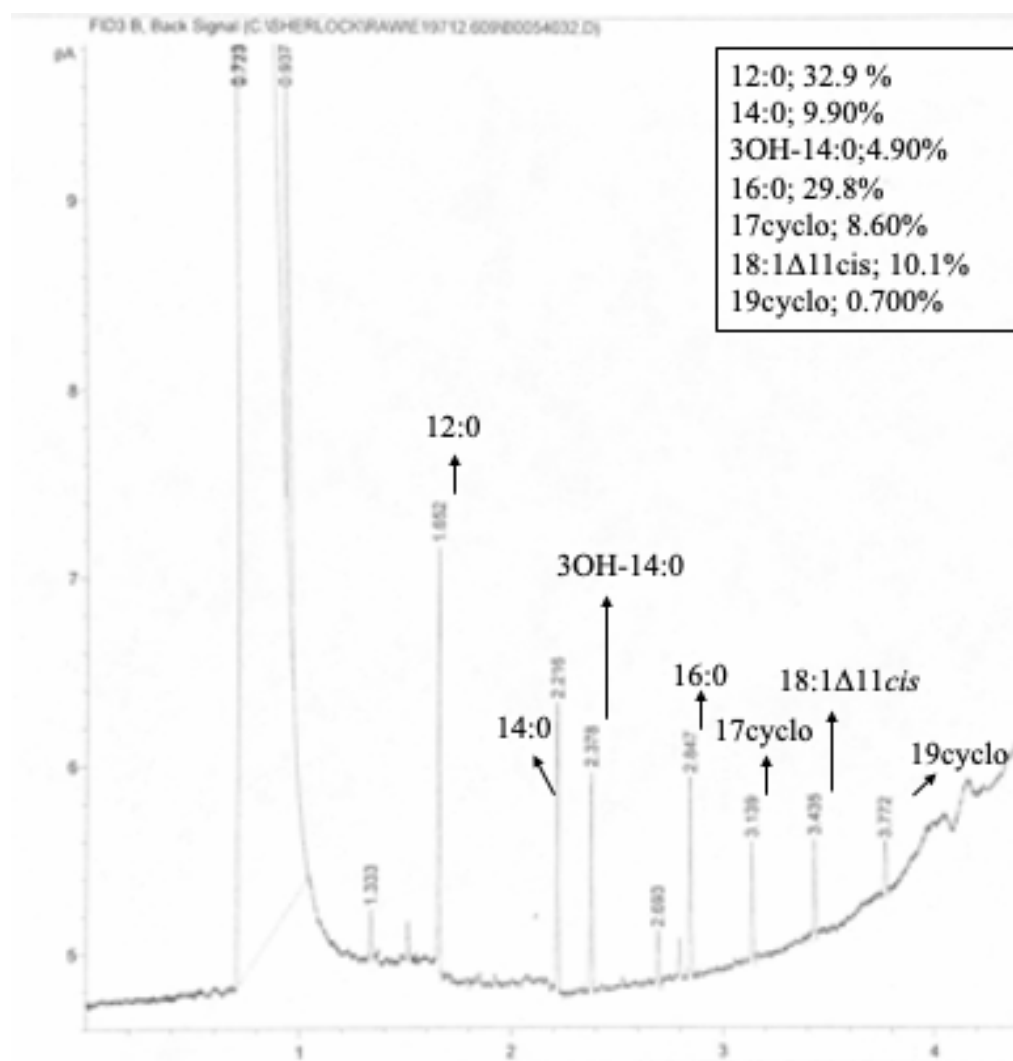


Figure 11. GC chromatogram of Fatty Acid Methyl Esters (FAMES) of *E. coli* 43888 LPS acquired by GC-FID after the iNtRON Biotechnology LPS extraction kit was used.



Table 5.

Average Percent Composition of Fatty Acids from <i>E. coli</i> 43888	
Fatty Acid	Percent Composition (%)
12:0	2.18
14:0	2.65
3OH-14:0	1.06
16:1	15.0
16:0	33.3
17cyclo	17.7
18:1 $\Delta$ 11 <i>cis</i>	23.0
19:0cyclo	4.99
Abbreviations: 12:0, dodecanoic acid; 14:0, tetradecanoic acid; 3OH-14:0, 3-hydroxy-tetradecanoic acid; 16:1, (9Z)-9-hexadecenoic acid; 16:0, hexadecanoic acid; 17 cyclo, <i>cis</i> -9,10-methylenehexadecanoic acid; 18:1 $\Delta$ 11 <i>cis</i> , (11Z)-11-octadecenoic acid; 19:0cyclo, <i>cis</i> -11,12-Methylene-Octadecanoic Acid	

Table 6.

Average Percent Composition of Fatty Acids from <i>E. coli</i> 43888 LPS Rapid Extract Protocol	
Fatty Acid	Percent Composition (%)
12:0	32.9
14:0	9.89
3OH-14:0	4.90
16:0	29.8
17cyclo	8.60
18:1 $\Delta$ 11 <i>cis</i>	10.1
19:0cyclo	0.730
Abbreviations: 12:0, dodecanoic acid; 14:0, tetradecanoic acid; 3OH-14:0, 3-hydroxy-tetradecanoic acid; 16:0, hexadecanoic acid; 17 cyclo, <i>cis</i> -9,10-methylenehexadecanoic acid; 18:1 $\Delta$ 11 <i>cis</i> , (11 <i>Z</i> )-11-octadecenoic acid; 19:0cyclo, <i>cis</i> -11,12-Methylene-Octadecanoic Acid	

## VITAE

Dakotah M. Biller attended Shippensburg University of Pennsylvania, where she received a Bachelor of Science in Chemistry in 2018. During her time there, she researched with Dr. Thomas Frielle on the development of a method to extract and analyze synthetic cannabinoids out of blood, which was presented at the National American Chemical Society (ACS) conference in March of 2018. Miss Biller completed an internship with the Cumberland County District Attorney's Office in the forensic laboratory. Along with her educational pursuits, Miss Biller was also the president of the Chemistry Club, the representative for the Department of Chemistry on the College of Arts and Sciences Dean's Advisory Board, and the 2018 recipient of the Class of 1920 - Chemistry Award. In the Master's program at Virginia Commonwealth University, Miss Biller pursued a degree in Forensic Chemistry, specializing in drugs and toxicology. In addition to her coursework and research, Miss Biller was also a Laboratory Teaching Assistant for the Chemistry Department and a mentor in the Virginia Commonwealth University Graduate School Mentorship Program.