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Systematic Analysis of Fatty Acids in Human Cells with a Multiplexed Isobaric Tag (TMT)-Based Method

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Abstract

Fatty acids (FAs) are essential components in cells and are involved in many cellular activities. Abnormal FA metabolism has been reported to be related to human diseases such as cancer and cardiovascular diseases. Identification and quantification of FAs provide insights into their functions in biological systems, but it is very challenging to analyze them due to their structures and properties. In this work, we developed a novel method by integrating FAs tagged with stable isotope labeled aminoxy tandem mass tags (aminoxyTMTs) and mass spectrometric analysis in the positive mode. On the basis of their structures, the aminoxyTMT reagents reacted with the carboxylic acid group of the FAs, resulting in an amine group with high proton affinity covalently attached to the analytes. This enabled the analysis of FAs under the positive electrospray ionization–mass spectrometry (ESI–MS) mode, which is normally more popular and sensitive compared to the negative mode. More importantly, the multiplexed TMT tags allowed us to quantify FAs from several samples simultaneously, which increased the experimental throughput and quantification accuracy. FAs extracted from three types of breast cells, i.e., MCF 10A (normal), MCF7 (minimally invasive) and MDA-MB-231 (highly invasive) cells, were labeled with the six-plexed aminoxyTMTs and quantified by LC–MS/MS. The results demonstrated that the abundances of some FAs, such as C22:5 and C20:3, were markedly increased in MCF7 and MDA-MB-231 cancer cells compared to normal MCF 10A cells. For the first time, aminoxyTMT reagents were exploited to label FAs for their identification and quantification in complex biological samples in the positive MS mode. The current method enabled us to confidently identify FAs and to accurately quantify them from several samples simultaneously. Because this method does not have sample restrictions, it can be extensively applied for biological and biomedical research.

Graphical abstract

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Notes

The authors declare no competing financial interest.

The raw files are publically accessible at <http://www.peptideatlas.org/PASS/PASS01162>.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jproteome.7b00896.

Tandem mass spectrum of derivatized C16:0 and the corresponding structures of the fragments (Figure S1); calibration curve of the C16:0 derivative over a concentration range from 22 to 2200 ng/mL (Figure S2) (PDF)

Keywords

fatty acids; LC-MS/MS; identification and quantification; aminoxyTMT; cancer cells

INTRODUCTION

Fatty acids (FAs) are critically important in biological systems and essential for cell survival.^{1,2} They are highly diverse because their carbon chain can vary dramatically, and they can be bound to different functional groups to form glycolipids, phospholipids, and triglycerides. FAs are essential components for membranes, including the plasma membrane in mammalian cells, and they also play an important role in metabolic processes.³⁻⁵ Aberrant FAs have been reported to be related to human diseases, and previous studies indicated that the development of cancers was closely correlated with changes in the composition and abundance of FAs.⁶⁻⁸ Comprehensive identification and quantification of FAs aid in a better understanding of their functions and cellular activities. Considering the importance of FAs in human diseases, investigation of FAs provides insight into the molecular mechanisms of diseases and leads to the identification of FAs as biomarkers for disease detection.⁹⁻¹⁵

Modern mass spectrometry is extremely powerful, allowing the analysis of biological molecules,¹⁶⁻²³ including lipids.²⁴⁻³³ Gas chromatography/mass spectrometry (GC/MS) with electron ionization (EI) is commonly employed to analyze FAs, in which it is necessary to perform silylation or methyl ester derivatization before ionization.^{34,35} Recently, liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) has emerged as a highly promising technique to study FAs.^{24,36-38} Because of the common structure of the carboxylic acid group in FAs, the negative mode in the electrospray ionization source is normally used to ionize them, and negatively charged FAs are analyzed in negative mode MS. However, the drawback is that the negative mode for MS detection is relatively less sensitive than the positive mode. In addition, the positive mode is typically much more popular compared to the negative mode for MS.^{39,40} In order to improve their detection sensitivity, FAs with a carboxyl acid group were chemically derivatized; then, a functional tag carrying a positive charge under acidic conditions was attached. Chemical derivatization-based methods, including the generation of a tag containing an amine group with high proton affinity, have been reported in the literature and have allowed FAs to be detected under the positive mode of MS with improved sensitivity.⁴⁰⁻⁴⁶

When an isotopic tag is employed in the chemical derivatization, it is possible to achieve relative quantification of FAs with MS. Isotopic labeling reagents containing particular isotopic tags can produce “heavy” and “light” versions of derivatized FAs, which results in a

certain mass difference. Therefore, the same FA from different samples can be quantified by MS.^{40,41,47} However, this method is usually limited to quantifying two samples because of the lack of corresponding isotopic reagents and the complexity of the mass shift from the isotopic tags in the full mass spectra. In recent years, isobaric tag-based methods have been widely used in the proteomics field to quantify proteins in a tandem mass spectrum, such as isobaric tag for relative and absolute quantitation (iTRAQ) and tandem mass tags (TMT).⁴⁸⁻⁵⁴ On the basis of the reporter ion intensities in tandem MS, simultaneous and accurate quantification of multiple samples in a single run can be achieved. Even though these multiplex methods have become very popular for protein analysis, they have not yet been reported for lipid analysis.

AminoxyTMT reagents are composed of an aminoxy group and an isotopic reporter group, which are connected by an isotopic balance group to normalize the total mass of the tag.^{55,56} The commercially available aminoxyTMTs can be used to quantify up to six different samples simultaneously based on the reporter ion intensities in tandem MS. These reagents were successfully used for glycan analysis.⁵⁵⁻⁵⁷ Furthermore, aminoxyTMTs contain heavy C and N isotopes, instead of H isotopes, which guarantees that the same FA from different samples labeled with different isobaric tags will have the same retention time during separation with liquid chromatography. Moreover, other physicochemical properties also remain the same, especially the ionization efficiency during MS analysis.

Here, we developed a novel method by employing aminoxyTMT reagents to label FAs for their identification in the positive MS mode and simultaneous quantification in multiple samples. First, we used a FA compound (C16:0) as a model to test the method. The results clearly demonstrated that this FA can be coupled with the aminoxyTMT reagent and then analyzed under the positive ESI-MS mode with high sensitivity. After FAs extracted from MCF7 cells were derivatized with aminoxyTMT reagents, global identification of FAs was achieved through a combination of the highly accurate mass-to-charge ratios in the full mass spectra and the reporter ions in the tandem mass spectra. The practical utility of the developed method was demonstrated through the identification and quantification of FAs in benign MCF 10A cells as well as minimally invasive MCF7 and highly invasive malignant MDA-MB-231 breast cancer cells. The results revealed that there were dramatic changes for some FAs between normal and cancerous cells. Because this method does not have sample restrictions, it can be extensively applied for MS-based FA analysis in complex biological samples.

EXPERIMENTAL SECTION

Materials and Reagents

FAs, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM), 4-methylmorpholine (NMM), chloroform (CHCl₃), Dulbecco's modified Eagle's medium (DMEM), and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. AminoxyTMT labeling reagents were purchased from Thermo.

Cell Culture

All cells were cultured in a humidified incubator at 37 °C and 5.0% CO₂. Both MCF7 and MDA-MB-231 cells (from American Type Culture Collection (ATCC)) were grown in DMEM containing 10% fetal bovine serum (FBS) (Thermo). MCF 10A cells were cultured in defined mammary epithelial growth media (CC-3150, MEGM Bullet Kit, Lonza Inc.) supplemented with bovine pituitary extract, epidermal growth factor, and 100 ng/mL cholera toxin (Sigma-Aldrich). Cells were harvested when the confluence reached about 70%.

Lipid Extraction

Lipids were extracted from cells according to a previous report in the literature with slight modifications.⁴² Briefly, cells were scrapped, pelleted by centrifugation at 300g for 3 min, and washed twice with PBS. The cell pellet was mixed with 1 mL of 2-propanol/dichloromethane (11/7 (v/v)). The resulting mixture was rotated at 4 °C for 60 min. After centrifugation, the organic portion was separated and dried. Free FAs were subsequently released from lipids under basic hydrolysis conditions. The lipid extract was dissolved in 850 μ L of methanol/dichloromethane (8/1 (v/v)), and then 150 μ L of 40% aq. potassium hydroxide was added. The mixture was incubated at 60 °C for 30 min followed by the addition of 700 μ L of 75 mM Na₂HPO₄ and acidification to pH < 2 with hydrochloric acid. The FAs were extracted with chloroform/hexane (1/4 (v/v)) four times (4 $\times$ 1 mL). The organic portions were combined, dried, and subjected to derivatization.

FAs Labeling with AminoxyTMT Reagents

Palmitic acid (C16:0, 40 nmol) or FAs extracted from MCF 10A, MCF7, or MDA-MB-231 cells were dissolved in CHCl₃ and mixed with NMM (44 nmol). DMTMM (44 nmol) was dissolved in acetonitrile (ACN) and added to the mixture. The reaction was incubated at room temperature for 3 h. The aminoxyTMT reagent (44 nmol) in ACN was added to the solution, which was then incubated at room temperature for another 4 h. The reaction was quenched by the addition of 40 μ L of water and incubation for 30 min. For quantification experiments, the cell number was normalized on the basis of cell counting using a hemocytometer. Cells were first detached with trypsin/EDTA and counted under a microscope after trypan blue staining. The final cell number was kept the same for each sample. For each type of cell, duplicate experiments were performed. FA derivatives from all six samples were then mixed for LC-MS analysis.

Because the aminoxyTMT reagents and the labeled FAs have dramatically different hydrophobicities, the TMT reagents elute much earlier than the labeled FAs during separation using reversed-phase liquid chromatography. Therefore, they do not interfere with the separation and MS analysis of the labeled FAs.

LC-MS/MS Analysis

Dried FA derivatives were dissolved in ethanol, and then the equivalent volume of solvent containing 5% ACN and 4% FA was added. The dissolved sample (2 μ L) was loaded onto a microcapillary column packed with C18 beads (Magic C18AQ, 3 μ m, 200 Å, 75 μ m $\times$ 15 cm, Michrom Bioresources) by a Dionex WPS-3000TPLRS autosampler (UltiMate 3000 Thermostated Rapid Separation Pulled Loop Wellplate Sampler). FA derivatives were

separated by reverse-phase liquid chromatography using an UltiMate 3000 binary pump with a 40 min gradient of 15–60% ACN (with 0.125% FA). FAs were detected with a data-dependent Top15 method in a hybrid dual-cell quadrupole linear ion trap-Orbitrap mass spectrometer (LTQ Orbitrap Elite, Thermo Scientific, with Xcalibur 3.0.63 software). For each cycle, one full MS scan (resolution: 60 000) in the Orbitrap at an automatic gain control (AGC) target of 10^6 was followed by up to 15 MS/MS scans for the most intense ions. The selected ions with 1.2 m/z isolation width were activated and fragmented with high-energy collision dissociation (HCD) at 40% normalized collision energy. The resulting fragments were detected in the Orbitrap cell. FA identification was performed manually based on their observed and theoretical masses within ± 3 ppm. The FA database was constructed from LIPID MAPS.⁵⁸

RESULTS AND DISCUSSION

Principle of Tagging FAs with AminoxyTMT Reagents

Typically, FAs contain a long carbon chain and a carboxylic acid group, and for MS analysis, the negative mode is normally employed to analyze deprotonated FAs. However, for MS, the positive mode is much more popular and sensitive. To analyze FAs in the positive mode, several chemical derivatization methods have been reported, such as introducing a pyridinium or dimethylaminoethyl functional group.^{39,42} Although those methods improve the ionization efficiency and detection sensitivity of FA analysis, they still have some limitations. For instance, these derivatization reagents are not commercially available and they are usually synthesized through several steps, which is laborious and time-consuming. Furthermore, relative quantification using those methods is dependent on differential isotopic labeling, and usually only two samples may be quantified at a time.

To circumvent the limitations discussed above, we developed a novel method based on labeling FAs with commercially available aminoxyTMT reagents, followed by MS analysis in the positive mode. The current method is simple and easy to operate. Since its inception, TMT-based multiplexed proteomics has been extensively used to globally quantify proteins.^{48,59} On the basis of the reporter ion intensities, multiple samples can be quantified simultaneously, which dramatically increases the experimental throughput and quantification accuracy. AminoxyTMT was initially designed for glycan labeling, and its quantification is similar in principle to that of common TMT.⁵⁵ However, these reagents contain an aminoxy group, which can be used to tag an aldehyde or carboxylic acid group.

Benefiting from the common feature of a carboxylic acid group in FAs, we tagged them with aminoxyTMT reagents, resulting in derivatized FAs containing a group with high proton affinity. The derivatization procedure was carried out via a one-pot reaction involving two steps (Scheme 1). FAs were first activated by DMTMM and were then covalently attached to the TMT containing a tertiary amine group through an amide bond. Finally, the tagged FAs were readily protonated under acidic conditions and analyzed using positive mode MS with high sensitivity.

Testing the Feasibility of the Labeling Method Using a FA Standard

First, we used a standard FA (C16:0) to test the feasibility of the current method. This FA reacted with the aminoxyTMT¹²⁶ reagent (the reporter ion with an m/z of 126 Da in the tandem mass spectrum). To improve its solubility, the derivatized FA was dissolved in ethanol and then added to an equivalent volume of 5% ACN and 4% formic acid solvent. The C16:0 derivative gave an intense protonated $[M + H]^+$ signal with an m/z of 553.4679 in the full mass spectrum with a mass accuracy of 2.3 ppm (Figure 1). The total ion chromatograph and extracted ion chromatograph (553.4679 ± 3 ppm) showed excellent separation (Figure 1A,B). After the protonated ion was fragmented with high-energy collision dissociation (HCD), the tandem mass spectrum was recorded in the Orbitrap cell. The singly charged peak at m/z of 126.1278 was the reporter ion generated from the tandem mass tag (Figure 1D). The peaks located at m/z of 225.1601 and 300.2286 were also generated from the tag (the corresponding structures are shown in Figure S1).

The labeling with aminoxyTMT enabled us to analyze FAs in the positive ESI-MS mode because the tag has a tertiary amine, which carries a positive charge under acidic conditions. Furthermore, it allowed us to achieve the quantification of FAs in six samples simultaneously, which increases the throughput and quantification accuracy. After HCD fragmentation, the tag containing a tertiary amine with high proton affinity carries a positive charge, but the FA component does not bear a protonation site. Therefore, it is difficult to observe the fragments from the FA backbone in tandem MS. It is well-known that it is generally very challenging to determine a FA structure using tandem MS, but the aim of the current method is not to overcome this difficulty. Here, the most important purpose is to analyze FAs in the popular and sensitive positive ESI-MS mode and to achieve accurate and high-throughput quantification of FAs. By using this FA, we also tested the detection limit and dynamic range of the current method. The limit of detection (LOD) was in the low femtomole range (~ 40 fmol), which indicated that this new method was highly sensitive and was comparable to that of a previously reported chemical derivation method.⁴² The calibration curve of the C16:0 derivative was linear ($R^2 > 0.99$) in the concentration range from 22 to 2200 ng/mL (Figure S2). On the basis of the results from the standard FA (C16:0), the current method is clearly effective for FA analysis.

Profiling of FAs in MCF7 Cells

The methods combining chemical isotope labeling (CIL) with high performance LC-MS have been reported to be useful to analyze metabolites with a specific functional group.⁶⁰⁻⁶² Labeling reagents can be coupled with a functional group among metabolites of the same type, such as amine, carboxyl, hydroxyl, or carbonyl, which enhances the separation efficiency and ionization in positive mode MS because of changes in the chemical and physical properties of the metabolites. For example, dansylhydrazine (DnsHz) was employed to label carbonyl-containing metabolites. On the basis of the peak pair of differential ¹²C- and ¹³C-DnsHz labeling, profiling of carbonyl metabolites was achieved.⁶³

Here, we employed the current method to profile FAs in human cells. FAs were extracted from MCF7 cells as described in the Experimental Section. After labeling the extracted FAs with aminoxyTMT reagents, they were injected into LC-MS/MS for analysis in the positive

mode. The top15 method was used, and dynamic exclusion was set up to avoid the fragmentation of the same ions again. After fragmentation, every ion was put in the exclusion list for 90 s. The identification of FAs can be achieved through full MS with high resolution and mass accuracy, and this can be further verified using the characteristic reporter ions in tandem MS produced by the tags from the aminoxyTMT reagents.

FAs were identified by manually comparing the observed m/z with the theoretical m/z from the FA database.⁵⁸ To improve the confidence of the identification, the mass accuracy of $[M + H]^+$ was restricted to be less than 3 ppm. In biologically duplicate experiments, 26 and 28 fatty acids were identified, respectively. Most of the FAs (24) were identified in both experiments (Figure 2), which means that the current method is highly reproducible considering the complexity of the biological sample. Differences among the samples, the dynamic nature of FAs, or the sample preparation process (sample loss) could lead to the variation between the biologically duplicate experiments. The identified FAs are listed in Table 1. One example is C16:0, which is the precursor to unsaturated fatty acids and is used for energy storage.⁶⁴ It was identified with a mass accuracy of 0.64 ppm, and its reporter ion from the TMT tag was found at 126.1279. Another example is C18:1, an unsaturated fatty acid, which was identified with a mass accuracy of 0.95 ppm, and its reporter ion appeared at 126.1272. This FA is an important component of the cell membrane and is a precursor to many other lipids, such as phospholipids and glycolipids.⁶⁴

Quantification of FAs in Different Types of Breast Cells

Lipid metabolism, particularly FA oxidation, is related to cancer cell metastasis and adaptation of the microenvironment changes due to hypoxia and acidosis.^{65,66} Cancer cell proliferation, however, relies on building blocks like FAs and their derivatives.⁶⁷ Previous studies demonstrated that the type and abundance of FAs changed dramatically from normal to cancer cells due to altered cellular metabolism.^{68,69} For example, unsaturated FAs in normal RWPE1 and cancerous PC3 prostate cells were studied, and the abundances of FAs 18:1 and 16:1 in PC3 cells were upregulated compared to those in RWPE1 cells.⁷⁰ Therefore, the quantification of FAs in cancer cells may result in the discovery of effective disease biomarkers and provide insight into the mechanisms of the disease.

Here, we quantified six samples simultaneously, benefiting from the high throughput of this newly developed method. As an example, we quantified FAs in benign breast cells (MCF 10A) as well as minimally invasive (MCF7) and highly invasive (MDA-MB-231) breast cancer cells. Instead of using six different samples, we performed duplicate experiments for these three types of breast cells.

To further assess the quantification accuracy of the current method, we extracted FAs from MCF7 cells and labeled them with the aminoxyTMT¹²⁶ and aminoxyTMT¹³¹ reagents, respectively. Then, different ratios of FAs from the two samples with the 126 or 131 tag were mixed for LC-MS analysis. For example, the ratios based on the report ion intensities from the MS/MS spectra of aminoxyTMT-labeled C22:5 were in excellent agreement with the mixed ratios. The plot between the expected ratios and observed values showed high linearity ($R^2 > 0.99$) in the range from 1:1 to 1:50 (Figure 3). Thus, the FA quantification results based on the current method are reliable.

Next, we identified and quantified FAs in MCF 10A, MCF7, and MDA-MB-231 cells, and the experimental procedure is shown in Figure 4. We tried to keep the number of cells the same for each sample. FAs were extracted from the three different cell lines (see the detailed procedure in the Experimental Section) and divided equally into two samples for each type of cell. After labeling with the aminoxyTMT reagents, six samples were mixed and analyzed by LC–MS/MS. Both full and tandem mass spectra were recorded in the Orbitrap cell with high resolution and high mass accuracy.

Quantified FAs with their ratios from (1) MCF7 versus MCF 10A and (2) MDA-MB-231 versus MCF 10A are listed in Table 2. For instance, C16:0, which was identified with a precursor mass accuracy of -0.13 ppm, was not upregulated in MCF7 cells compared to that in MCF 10A cells, but its abundance in MDA-MB-231 cells increased about 2-fold, as shown by the reporter ion intensities in Figure 5A. Long-chain FAs, including C16:0, were produced by successive condensation reactions from malonyl-CoA and acetyl-CoA substrates catalyzed by an enzyme encoded by the FASN gene. Increased levels of FASN have been observed in many cancer cells, and this was shown to lead to the upregulation of long-chain FAs including C16:0.^{7,69,71}

Previously, C18:1 and C16:1 were found to be increased by 6.0- and 3.7-fold in cancerous human prostate cells (PC3 cells) compared to normal (RWPE1) cells, respectively.⁷⁰ In another report, FAs were compared between human breast cancer tissue and normal breast tissue, and the cancer tissue had higher levels of monounsaturated FAs, including C18:1.⁷² In the current work, compared to MCF 10A cells (normal cells), C16:1 was upregulated by 2.8-fold in MCF7 cells and C18:1 was increased by about 1.6-fold in MDA-MB-231 cells.

Interestingly, a markedly increased quantity of C22:5 was observed in both minimally and highly invasive cancer cells (MCF7 and MDA-MB-231) (Figure 5B). Previously, the relationship between the abundance of C20:5 or C22:6 and proliferation of cancer cells was extensively studied.^{73,74} However, little attention has been paid to C22:5.⁷⁵⁻⁷⁷ Here, C22:5 was identified with a mass accuracy of 0.88 ppm, and the quantification results demonstrated that it increased by 8.0- and 18.4-fold in MCF7 and MDA-MB-231 cancer cells compared to normal MCF 10A cells, respectively. Meanwhile, C20:3 and C22:4 were also dramatically upregulated in both MCF7 and MDA-MB-231 cancer cells. These quantification results can provide valuable information regarding the changes in FA metabolism in cancer cells at different stages.

CONCLUSIONS

FAs are highly diverse and critically important in biological systems. Because of their structures and properties, it is very challenging to analyze them. Here, a novel method was developed to identify and quantify FAs with high sensitivity and high throughput by LC–MS/MS in the positive mode. For the first time, we employed commercially available aminoxyTMT reagents to label FAs, followed by MS analysis. There are several advantages of the current method. First, it enabled the detection of FAs under the positive ESI–MS mode, which is more popular and normally has a higher sensitivity than the negative mode. Second, more importantly, the reporter ion intensities allowed FAs to be quantified

accurately. Furthermore, because the commercially available aminoxyTMT reagents have six channels, FA quantification was achieved in six different samples simultaneously, which increases the throughput and quantification accuracy. Because the current method is robust and does not have any sample restrictions, it can be extensively applied for FA studies in biological and biomedical fields, which will lead to a better understanding of the functions of FAs and their mechanisms in disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

LC-MS analysis results using C16:0 as a model FA to test the feasibility of the current method. (A) Total ion chromatogram (TIC); (B) extracted ion chromatogram (EIC) of derivatized C16:0 within 3 ppm; (C) full mass spectrum of derivatized C16:0; (D) tandem mass spectrum (MS2) of derivatized C16:0.

Figure 2.
Overlap of FAs (26 vs 28) identified from MCF7 cells in biologically duplicate experiments.

Figure 3.
Comparison of the expected (mixed) ratios and measured ratios of C22:5 after labeling with aminoxyTMT reagents. The results show excellent agreement.

Figure 4.
Experimental procedure for the quantification of FAs in MCF 10A, MCF7, and MDA-MB-231 cells.

Figure 5. Quantification of C16:0 (A) and C22:5 (B) based on the reporter ion intensities in three different types of breast cells (MCF 10A (channels 126 and 127), MCF7 (128 and 129), and MDA-MB-231 (130 and 131)).

Scheme 1.
Fatty Acid Derivatization Reactions Using AminoxyTMT Reagents

Table 1

FAs Identified from MCF7 Cells in Biologically Duplicate Experiments

fatty acid	formula	theoretical m/z	observed m/z	mass accuracy (ppm)	reporter ion	identified in exp #1	identified in exp #2
C6:0	C ₆ H ₁₂ O ₂	418.3232	418.3224	2.07	126.1271	Y	Y
C8:0	C ₈ H ₁₆ O ₂	446.3545	446.3543	0.48	126.1274	Y	Y
C10:0	C ₁₀ H ₂₀ O ₂	474.3858	474.3854	0.81	126.1273	Y	Y
C12:0	C ₁₂ H ₂₄ O ₂	502.4171	502.4167	0.87	126.1276	Y	Y
C14:1	C ₁₄ H ₂₆ O ₂	528.4328	528.4321	1.43	126.1281	Y	Y
C14:0	C ₁₄ H ₂₈ O ₂	530.4484	530.4479	0.91	126.1272	Y	Y
C16:2	C ₁₆ H ₂₈ O ₂	554.4484	554.4482	0.44	126.1274	N	Y
C16:1	C ₁₆ H ₃₀ O ₂	556.4641	556.4635	1.12	126.1277	Y	Y
C16:0	C ₁₆ H ₃₂ O ₂	558.4797	558.4794	0.64	126.1279	Y	Y
C18:3	C ₁₈ H ₃₀ O ₂	580.4641	580.4634	1.18	126.1276	N	Y
C18:2	C ₁₈ H ₃₂ O ₂	582.4797	582.4794	0.61	126.1281	Y	Y
C18:1	C ₁₈ H ₃₄ O ₂	584.4954	584.4949	0.95	126.1272	Y	Y
C18:0	C ₁₈ H ₃₆ O ₂	586.5110	586.5107	0.49	126.1269	Y	Y
C20:6	C ₂₀ H ₂₈ O ₂	602.4484	602.4481	0.51	126.1276	Y	Y
C20:5	C ₂₀ H ₃₀ O ₂	604.4641	604.4638	0.52	126.1281	N	Y
C20:4	C ₂₀ H ₃₂ O ₂	606.4797	606.4793	0.68	126.1272	Y	Y
C20:3	C ₂₀ H ₃₄ O ₂	608.4954	608.4953	0.21	126.1272	Y	Y
C20:2	C ₂₀ H ₃₆ O ₂	610.5110	610.5103	1.17	126.1274	Y	Y
C20:1	C ₂₀ H ₃₈ O ₂	612.5267	612.5266	0.29	126.1277	Y	Y
C20:0	C ₂₀ H ₄₀ O ₂	614.5423	614.5425	-0.35	126.1279	Y	Y
C22:6	C ₂₂ H ₃₂ O ₂	630.4797	630.4793	0.66	126.1279	Y	N
C22:5	C ₂₂ H ₃₄ O ₂	632.4954	632.4952	0.39	126.1271	Y	Y
C22:4	C ₂₂ H ₃₆ O ₂	634.5110	634.5106	0.73	126.1276	Y	Y
C22:2	C ₂₂ H ₄₀ O ₂	638.5423	638.5422	0.23	126.1271	Y	Y
C22:1	C ₂₂ H ₄₂ O ₂	640.5580	640.5579	0.26	126.1274	Y	N
C22:0	C ₂₂ H ₄₄ O ₂	642.5736	642.5740	-0.54	126.1273	Y	Y

fatty acid	formula	theoretical m/z	observed m/z	mass accuracy (ppm)	reporter ion	identified in exp #1	identified in exp #2
C24:5	C ₂₄ H ₃₈ O ₂	660.5267	660.5264	0.54	126.1276	Y	Y
C24:4	C ₂₄ H ₄₀ O ₂	662.5423	662.5426	-0.42	126.1281	N	Y
C24:1	C ₂₄ H ₄₆ O ₂	668.5893	668.5895	-0.23	126.1272	Y	Y
C24:0	C ₂₄ H ₄₈ O ₂	670.6049	670.6050	-0.08	126.1279	Y	Y

Table 2

Quantification Results of FAs from MCF 10A, MCF7, and MDA-MB-231 Cells

Fatty acid	formula	theoretical m/z	observed m/z	mass accuracy (ppm)	MCF7/MCF 10A	MDA-MB-231/MCF 10A
C4:1	C ₄ H ₆ O ₂	388.2763	388.2760	0.91	0.77	1.42
C6:0	C ₆ H ₁₂ O ₂	418.3232	418.3226	1.42	1.08	1.80
C8:0	C ₈ H ₁₆ O ₂	446.3545	446.3541	0.95	0.98	1.64
C12:1	C ₁₂ H ₂₂ O ₂	500.4015	500.4008	1.41	0.75	0.97
C12:0	C ₁₂ H ₂₄ O ₂	502.4171	502.4168	0.75	0.94	1.67
C14:1	C ₁₄ H ₂₆ O ₂	528.4328	528.4325	0.62	2.71	0.98
C14:0	C ₁₄ H ₂₈ O ₂	530.4484	530.4478	1.27	1.28	1.83
C16:1	C ₁₆ H ₃₀ O ₂	556.4641	556.4635	1.23	2.76	0.56
C16:0	C ₁₆ H ₃₂ O ₂	558.4797	558.4798	-0.13	1.18	1.99
C18:3	C ₁₈ H ₃₀ O ₂	580.4641	580.4638	0.65	1.52	2.05
C18:1	C ₁₈ H ₃₄ O ₂	584.4954	584.4942	2.10	1.12	1.57
C18:0	C ₁₈ H ₃₆ O ₂	586.5110	586.5119	-1.49	1.16	2.10
C20:5	C ₂₀ H ₃₀ O ₂	604.4641	604.4636	0.93	0.97	1.65
C20:3	C ₂₀ H ₃₄ O ₂	608.4954	608.4949	0.91	7.92	27.96
C20:1	C ₂₀ H ₃₈ O ₂	612.5267	612.5269	-0.22	0.86	5.00
C20:0	C ₂₀ H ₄₀ O ₂	614.5423	614.5429	-0.85	0.77	1.51
C22:5	C ₂₂ H ₃₄ O ₂	632.4954	632.4949	0.88	7.95	18.35
C22:4	C ₂₂ H ₃₆ O ₂	634.5110	634.5110	0.06	13.21	65.39
C22:1	C ₂₂ H ₄₂ O ₂	640.5580	640.5582	-0.23	1.55	2.11
C22:0	C ₂₂ H ₄₄ O ₂	642.5736	642.5742	-0.83	0.77	1.51