Journal of Chromatography B, 893–894 (2012) 187–192

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

# Analysis of fatty acid composition in insulin secreting cells by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry

Amy L. Payeur[a], Matthew A. Lorenz[a], Robert T. Kennedy[a,b,*]

[a] Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA
[b] Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109, USA

ARTICLE INFO

*Article history:*
Received 3 June 2011
Accepted 1 March 2012
Available online 7 March 2012

*Keywords:*
Lipids
GC × GC
Insulin

ABSTRACT

A comprehensive two-dimensional gas chromatography (GC × GC) time-of-flight mass spectrometry method was developed for determination of fatty acids (irrespective of origin, i.e., both free fatty acids and fatty acids bound in sources such as triglycerides) in cultured mammalian cells. The method was applied to INS-1 cells, an insulin-secreting cell line commonly used as a model in diabetes studies. In the method, lipids were extracted and transformed to fatty acid methyl esters for analysis. GC × GC analysis revealed the presence of 30 identifiable fatty acids in the extract. This result doubles the number of fatty acids previously identified in these cells. The method yielded linear calibrations and an average relative standard deviation of 8.4% for replicate injections of samples and 12.4% for replicate analysis of different samples. The method was used to demonstrate changes in fatty acid content as a function of glucose concentration on the cells. These results demonstrate the utility of this method for analysis of fatty acids in mammalian cell cultures.



## 1. Introduction

Fatty acids play an important role in nutrition, health, and biochemistry [1–6]. Fatty acid determinations are complicated by their variety of chemical forms. They occur with a wide range of carbon chain lengths, with different degrees of saturation, and may be free or bound as esters in lipids such as triacylglycerols and glycerophospholipids. Fatty acids are commonly analyzed via gas chromatography (GC) after transformation into methyl esters. Other methods such as chemo-thermolysis GC [7] and high performance liquid chromatography (HPLC) have also been applied [8]. Comprehensive two-dimensional gas chromatography (GC × GC) is also gaining popularity [9,10].

The use of GC × GC instead of traditional GC to determine fatty acid methyl esters (FAMEs) enhances overall resolution which is important because these samples often contain compounds with similar structures that are difficult to separate in a one-dimensional analysis [3,11]. In GC × GC, FAMEs with the same carbon number elute as clusters with predictable patterns. As carbon number increases, first dimension retention time increases so that a homologous series appears along an arc in a traditional contour plot. Esters with the same number of double bonds appear on arcs offset from that for saturated fatty acids. The double bond location, or ω number, also results in predictable shifts in retention times. The inherent structure of these chromatograms aids in identification of FAMEs even when a pure standard is not available. Improved resolution and detectability, compared to one-dimensional GC, also make it possible to detect and identify trace fatty acids [9,11].

GC × GC has previously been used to analyze fatty acids in biodiesel [12,13], human plasma [3], algae [14], bacteria [15–18] and edible fats and oils [9–11,19]. Despite this extensive list of applications, little work has focused on analysis of fatty acids in cell culture lines by GC × GC. Such analysis is likely to be of value in understanding the role of fatty acids in signaling and disease pathophysiology. In this work we demonstrate the applicability of GC × GC for determination of total fatty acid content in the INS-1 cell culture line.

INS-1 cells are a β-cell line used as a model for insulin secretion and diabetes studies. In β-cells, fatty acids may serve as signaling molecules or energy sources [6,20,21]. Determination of fatty acid content may help elucidate the roles of fatty acids in these cells and their link to insulin secretion and diabetes. Previous methods for determining fatty acids in INS-1 cells include HPLC [8] and GC [22]. In this work we use GC × GC for fatty acid determinations in INS-1 cells to demonstrate

* Corresponding author at: Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA. Tel.: +1 734 615 4363; fax: +1 734 647 4865.
*E-mail address:* rtkenn@umich.edu (R.T. Kennedy).

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2012.03.003

that the method has advantages over previously used methods.

## 2. Experimental

### 2.1. Chemicals

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO) unless otherwise noted. Rosewell Park Memorial Institute (RPMI) media, fetal bovine serum (FBS), HEPES, and penicillin-streptomycin were purchased from Invitrogen Corp (Carlsbad, CA). Cells lifters and 10 cm polystyrene non-pyrogenic culture dishes were purchased from Corning (Lowell, MA). 15 mL polypropene sterile centrifuge tubes, HPLC grade methanol, ACS certified sulfuric acid and potassium hydroxide were obtained from Fisher Scientific (Fairfield, NJ). Hexane was from Acros Organics (Morris Plain, NJ) and the 37 component FAME mix was from Supelco (Bellefonte, PA).

### 2.2. Samples

INS-1 cells were cultured on 10 cm plates in RPMI-1640 (+L-glutamine) supplemented with 10% FBS, 1 mM pyruvate, 10 mM HEPES, 50 μM 2-β-mercaptoethanol, and 1 unit penicillin–streptomycin. INS-1 cells were grown to confluence ($\sim 4 \times 10^7$ cells) in 10 cm polystyrene dishes with RPMI culture media. All cells used in a particular experiment were seeded at the same time taking care to minimize variability by using precise volumes of reagents and seed cells.

Krebs-Ringer-HEPES buffer (KRHB) was prepared to contain 20 mM HEPES, 118 mM NaCl, 5.4 mM KCl, 2.4 mM CaCl, 1.2 mM $MgSO_4$, and 1.2 mM $KH_2PO_4$ and adjusted to pH 7.4 with HCl. Cells were washed once with 10 mL of KRHB prior to treatment in 10 mL of KRHB containing 0, 0.5, 10 or 20 mM glucose for 1 h at 37 °C. Each concentration was prepared in triplicate. After treatment, cells were washed once with 10 mL milli-Q water and snap frozen with liquid nitrogen. Plates were stored at −80 °C until extraction.

### 2.3. Fatty acid methyl ester synthesis

Lipid material was converted to fatty acid methyl esters from the total lipid content as described before [23]. Briefly, 5.3 mL methanol was added to the cell plate which was then scraped and transferred to a 15 mL centrifuge tube. 700 μL of 10 M potassium hydroxide was added followed by incubation in a 55 °C water bath for 1.5 h with vigorous shaking every 20 min. The samples were cooled with a water bath followed by the addition of 580 μL of 12 M sulfuric acid and an additional 1.5 h of incubation at 55 °C with shaking every 20 min. The tubes were cooled again to below room temperature with a cool water bath and 3 mL of hexane was added to each. The samples were vortexed for 5 min followed by centrifugation at 1600 *g* for 5 min. The hexane layer was transferred to a capped 15 mL vial and stored at −20 °C until analysis.

### 2.4. Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry

GC × GC analysis was performed on a Leco Pegasus III with 4D upgrade (St. Joseph, MI). The primary column was a 30 m Rxi®-1ms (0.25 mm i.d., 0.18 μm film of Crossbond® 100% dimethylpolysiloxane) and the secondary column was a 2 m Rxi®-17Sil MS (0.1 mm i.d., 0.1 μm film of Crossbond® silarylene phase) both from Restek Corporation (Bellefonte, PA). A 1 μL injection was made with an Agilent 7683 automatic liquid sampler (Palo Alto, CA) in splitless mode and four replicates were completed for each sample. For temperature programming, the primary oven was maintained at 40 °C for 2 min and then increased at a rate of 30 °C/min to 160 °C, the rate was then slowed to 2 °C/min until 260 °C was reached and maintained for 0.5 min. The secondary oven and the thermal modulator were offset from the primary oven by 5 °C and 30 °C respectively. A modulation period, or second dimension injection frequency, of 7 s was used. A flow rate of 2 mL/min ultra high purity helium with an inlet and mass spectral transfer line temperature of 250 °C were maintained. A mass range of *m/z* 45–650 was collected at a rate of 200 spectra/s after a 400 s solvent delay. The ion source was maintained at 200 °C.

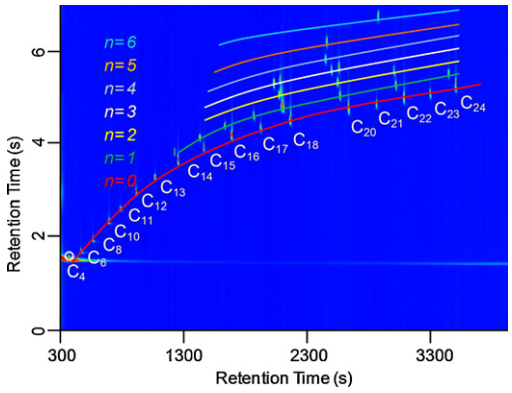


**Fig. 1.** GC × GC chromatogram of neat 37 component FAMEs mix where *n* is the number of double bonds.

Supelco 37 component FAME mix was analyzed at varying concentrations, in triplicate, under identical conditions to the cell extracts for validation of the method. Additionally, the mix was analyzed neat with the following chromatographic differences. A 150:1 split ratio was used in addition to a 1 mL/min flow rate. The final temperature was 270 °C and was maintained for 5 min. The mass spectral solvent delay was 300 s.

### 2.5. Data analysis

Leco ChromaTOF version 4.22 was used for instrument control and data processing. The National Institutes of Standards and Technology (NIST) mass spectral library (version 2.0) was used to aid in peak identification. Statistical significance was determined in GraphPad Prism version 3.03 (La Jolla, CA) using a one way ANOVA analysis and a Newman–Keuls post hoc test.

## 3. Results and discussion

### 3.1. Analysis of 37 component FAME standard

A standard mixture of 37 FAMEs was analyzed to ensure that the column set and conditions used would provide adequate resolution. As shown in Fig. 1, good resolution and ordered retention

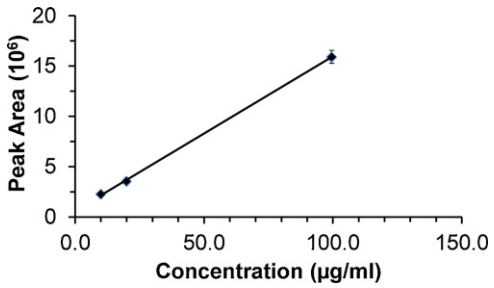


**Fig. 2.** Calibration curve for myristic acid. Samples were analyzed in triplicate and error bar is ±1 SEM.

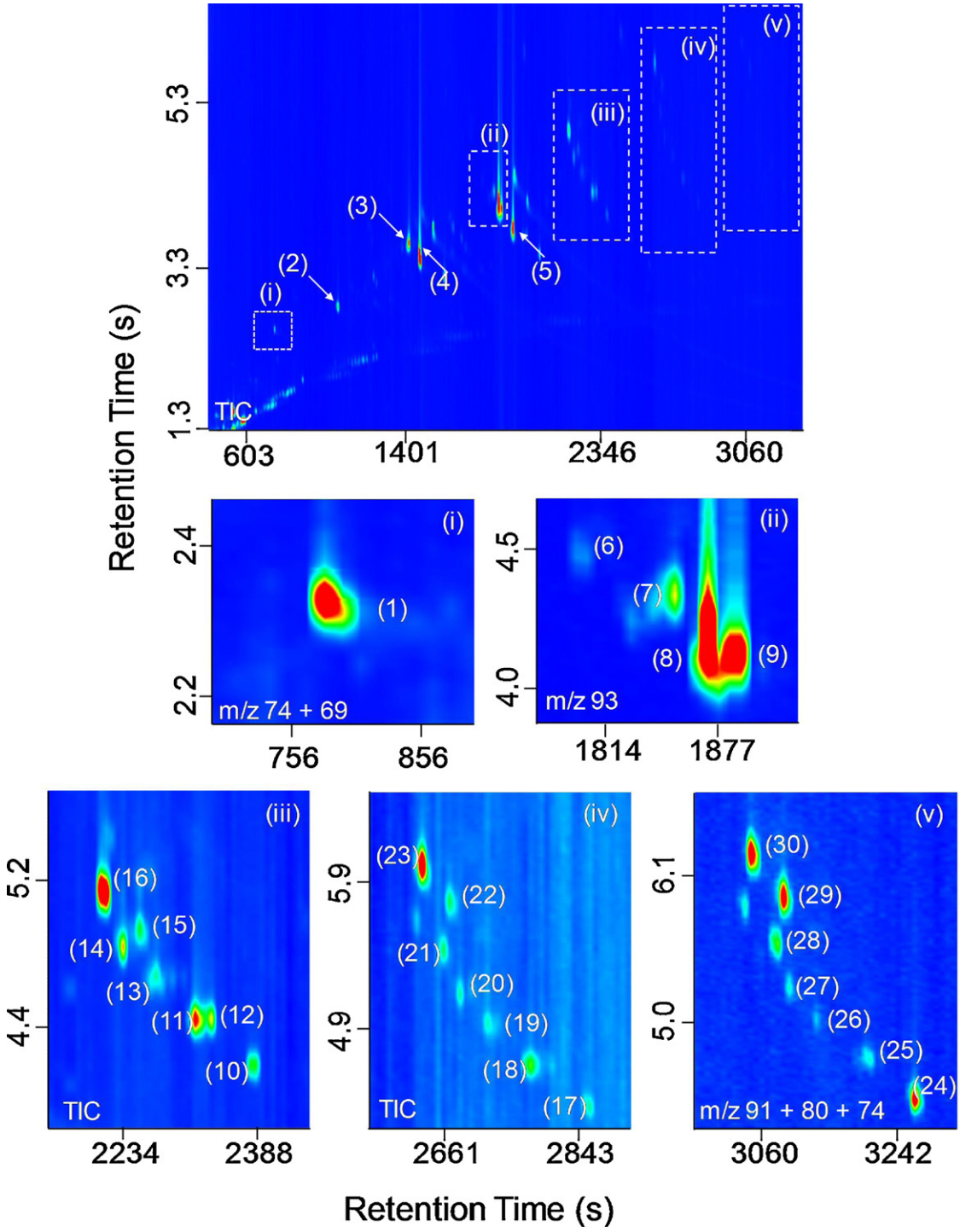


**Fig. 3.** GC × GC total ion chromatogram (TIC) of a representative INS-1 cell extract (top). Peaks identified include lauric acid (1), myristic acid (2), palmitoleic acid (3), palmitic acid (4), stearic acid (5), linolenic acid (6), linoleic acid (7), oleic acid (8), elaidic acid (9), arachidic acid (10), cis-11-eicosenoic acid (11), cis-13-eicosenoic acid (12), eicosadienoic acid (13), cis-11,14,17-eicosatrienoic acid (14), cis-8,11,14-eicosatrienoic acid (15), arachidonic acid (16), behenic acid (17), erucic acid (18), brassic acid (19), eicosatetraenoic acid (20), eicosapentaenoic acid (21), clupanodonic acid (22), docosahexaenoic acid (23), lignoceric acid (24), nervonic acid (25), tetracosadienoic acid (26), tetracosatrienoic acid (27), tetracosatetraenoic acid (28), tetracosapentaenoic acid (29), tetracosahexaenoic acid (30). Boxes (i)–(v) are zoomed in portions of the corresponding broken boxes in the top chromatogram at either TIC, *m/z* 74 + 69, *m/z* 93, or *m/z* 91 + 80 + 74, as indicated.

is achieved with these conditions. Clustered elution of FAMEs with the same carbon number but varying degree of saturation is readily observed in the $C_{18}$ and $C_{20}$ range of the standard, for example. Elution of FAMEs with the same degree of saturation along a horizontal axis is highlighted by the colored lines. FAMEs with the same degree of saturation but different ω numbers can be seen in Fig. 1 where both α-linolenic acid (C18:3 ω3) and γ-linolenic acid (C18:3 ω6) as well as cis-11,14,17-eicosatrienoic acid (C20:3 ω3) and cis-8,11,14-eicosatrienoic acid (C20:3 ω6) are fully resolved.

Method linearity was determined by the creating calibration curves for 22 FAMES. Linear correlation coefficients of 0.99 or greater were achieved for 17 of the 22 FAMES in the concentration

**Table 1**
Determination of fatty acids in INS-1 cells incubated for 60 min at different glucose concentrations. Average peak area and ±1 SEM at 0 mM, 0.5 mM, 10 mM and 20 mM glucose.

| FAME | Peak area 0 mM glucose | Peak area 0.5 mM glucose | Peak area 10 mM glucose | Peak area 20 mM glucose |
|---|---|---|---|---|
| C12:0 | 1.1E ± 0.04 | 0.99 ± 0.04 | 0.99 ± 0.04 | 0.79 ± 0.03[a,b,c] |
| C14:0 | 20 ± 0.8 | 20 ± 0.5 | 22 ± 0.5 | 17 ± 0.7[a,b,c] |
| C16:0 | 220 ± 1 | 240 ± 5 | 250 ± 6 | 210 ± 11[c] |
| C16:1 | 7.8 ± 0.2 | 4.6 ± 1.0[a] | 5.7 ± 0.7[a] | 6.6 ± 0.3 |
| C18:0 | 95 ± 4 | 110 ± 3[a] | 110 ± 2[a] | 88 ± 4[b,c] |
| C18:1 (ω9, cis) | 20 ± 0.6 | 20 ± 0.4 | 20 ± 0.04 | 18 ± 0.6[a,c] |
| C18:1 (ω9, trans) | 8.0 ± 0.6 | 6.5 ± 1 | 9.6 ± 0.4[a] | 7.4 ± 0.5[c] |
| C18:2 | 3.4 ± 0.1 | 3.3 ± 0.1 | 2.5 ± 0.4 | 2.6 ± 0.1 |
| C18:3 | 0.16 ± 0.004 | 0.15 ± 0.006 | 0.17 ± 0.003 | 0.13 ± 0.006[a,b,c] |
| C20:0 | 2.6 ± 0.06 | 2.8 ± 0.01 | 3.0 ± 0.07[c] | 2.3 ± 0.01[a,b,c] |
| C20:1 (cis-11) | 1.7 ± 0.07 | 1.8 ± 0.1 | 1.9 ± 0.04 | 1.5 ± 0.08[b,c] |
| C20:1 (cis-13) | 1.1 ± 0.03 | 1.1 ± 0.03 | 1.2 ± 0.02 | 0.93 ± 0.05[a,b,c] |
| C20:2 | 0.9 ± 0.03 | 0.93 ± 0.04 | 0.97 ± 0.03 | 0.76 ± 0.03[a,b,c] |
| C20:3 (ω3) | 1.7 ± 0.06 | 1.7 ± 0.04 | 1.7 ± 0.05 | 1.3 ± 0.05[a,b,c] |
| C20:3 (ω6) | 1.2 ± 0.02 | 1.2 ± 0.05 | 1.3 ± 0.03 | 1.1 ± 0.05[a,b,c] |
| C20:4 | 7.4 ± 0.2 | 7.1 ± 0.2 | 7.5 ± 0.2 | 6.0 ± 0.2[a,b,c] |
| C22:0 | 0.80 ± 0.02 | 0.97 ± 0.05[a] | 1.0 ± 0.04[a] | 0.775 ± 0.04[b,c] |
| C22:1 | 0.30 ± 0.07 | 0.24 ± 0.05 | 0.43 ± 0.02[a,b] | 0.22 ± 0.02[c] |
| C22:2 | 0.35 ± 0.02 | 0.38 ± 0.04 | 0.35 ± 0.03 | 0.32 ± 0.01 |
| C22:3 | 0.31 ± 0.05 | 0.32 ± 0.09 | 0.33 ± 0.1 | 0.25 ± 0.01 |
| C22:4 | 0.50 ± 0.01 | 0.48 ± 0.02 | 0.51 ± 0.02 | 0.40 ± 0.02[a,b,c] |
| C22:5 | 1.1 ± 0.02 | 1.1 ± 0.04 | 1.1 ± 0.03 | 0.91 ± 0.03[a,b,c] |
| C22:6 | 5.4 ± 0.1 | 5.3 ± 0.2 | 5.4 ± 0.3 | 4.5 ± 0.2[a,b,c] |
| C24:0 | 0.40 ± 0.01 | 0.47 ± 0.02 | 0.47 ± 0.03 | 0.35 ± 0.02[b,c] |
| C24:1 | 0.091 ± 0.002 | 0.11 ± 0.003[a] | 0.11 ± 0.004[a] | 0.097 ± 0.004 |
| C24:2 | 0.073 ± 0.005 | 0.088 ± 0.005 | 0.082 ± 0.004 | 0.063 ± 0.004[b,c] |
| C24:3 | 0.048 ± 0.003 | 0.051 ± 0.002 | 0.47 ± 0.002 | 0.040 ± 0.003 |
| C24:4 | 0.13 ± 0.003 | 0.14 ± 0.003 | 0.14 ± 0.003 | 0.10 ± 0.008[a,b,c] |
| C24:5 | 0.70 ± 0.01 | 0.69 ± 0.01 | 0.70 ± 0.02 | 0.59 ± 0.03[a,b,c] |
| C24:6 | 0.77 ± 0.03 | 0.79 ± 0.02 | 0.81 ± 0.02 | 0.63 ± 0.03[a,b,c] |

[a] Significantly different from 0 mM glucose.
[b] Significantly different from 0.5 mM glucose.
[c] Significantly different from 10 mM glucose.
Significance was determined with one way ANOVA test and Newman–Keuls post hoc test. Three plates per concentration, four replicates per plate.

range of 5–150 μg/mL. A representative curve for myristic acid is shown in Fig. 2. The average RSD for replicate injections of the same standard was 8.4% with a range of 3.1–23.2%. These results validate the column set and chromatographic conditions for FAMEs used in this work.

### 3.2. Analysis of INS-1 cell extracts

Extracts of INS-1 cells incubated in 10 mM glucose were analyzed via GC × GC after transformation of the total lipid content to fatty acid methyl esters. A representative chromatogram is shown in Fig. 3. Peaks for myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1) and stearic acid (C18:0) dominate the chromatogram; however, signals for other analytes are made apparent by expanding the scale or plotting select mass channels (Fig. 3i–v).

In these samples, 30 FAMEs were identified with 12–24 carbons and 0–6 double bonds. 15 fatty acids, of higher carbon number and number of double bonds, not previously identified by GC analysis of lipids in INS-1 cells [22] are detected and identified. Of the 30 FAMEs identified, 11 were not available as standards. Identification of these 11 analytes was performed by utilizing the chromatogram structure, NIST 02 mass spectral database matches and manual inspection of the mass spectra. The new lipids identified in the INS-1 cells are: lauric, linolenic, eicosadienoic, eicosatrienoic, erucic, brassic, eicosatetraenoic, eicosapentaenoic, clupanodonic, nervonic, tetracosenoic, tetracosatrienoic, tetracosatetraenoic, tetracosapentaenoic, and tetracosahexaenoic acid.

Replicate analyses of the samples (4 different plates of cells) yielded an average RSD of 12% with a range of 4.6–36% for all analytes except for the following fatty acids: C16:1 (52 and 44% at 0.5 and 10 mM glucose respectively), C18:1 ω9, trans (42% at 0.5 mM glucose), C18:2 (57% 10 mM glucose) and C22:1 (66 and 53% 0 and 0.5 mM glucose respectively). The reason for this variability is not well understood; however, the results suggest that the method can be used for semi-quantitative analysis for most analytes.

### 3.3. Glucose stimulated changes in fatty acids

Glucose evokes a number of metabolic changes in β-cells that are linked to cell function such as insulin secretion and adaptation (such as cell growth). In view of the important role of glucose for β-cell function, we evaluated the acute effect of incubation in different concentrations of glucose (0–20 mM) on fatty acid content of INS-1 cells (Table 1). Several fatty acids showed increases with glucose until the highest concentration where a decrease was observed (Fig. 4). Another group of fatty acids that included lauric, myristic, linolenic, cis-13-eicosenoic, eicosadienoic, cis-11,14,17-eicosatrinoic, cis-8,11,14-eicosatrienoic, arachidonic acids experienced no significant change in peak area at 0, 0.5, and 10 mM glucose but showed a significant decrease at 20 mM glucose (see Table 1 and Fig. 4). The decreases that occur at high glucose may reflect secretion of fatty acids stimulated by glucose. The significance of these changes to cell physiology cannot be discerned without further experimentation; however, these changes do illustrate the potential of the method to provide detailed analysis of the fatty acid profile.

We can compare our results to a previous study that measured 11 fatty acids (total fatty acid content) by HPLC in islets of Langerhans in the presence of 0, 5.6, 8.3 and 16 mM glucose [8]. In most cases we observer similar trends; however, at high glucose, we observe a decrease in arachidonic and docosahexaenoic acids that was not previously observed in islets [8]. Additionally, an increase

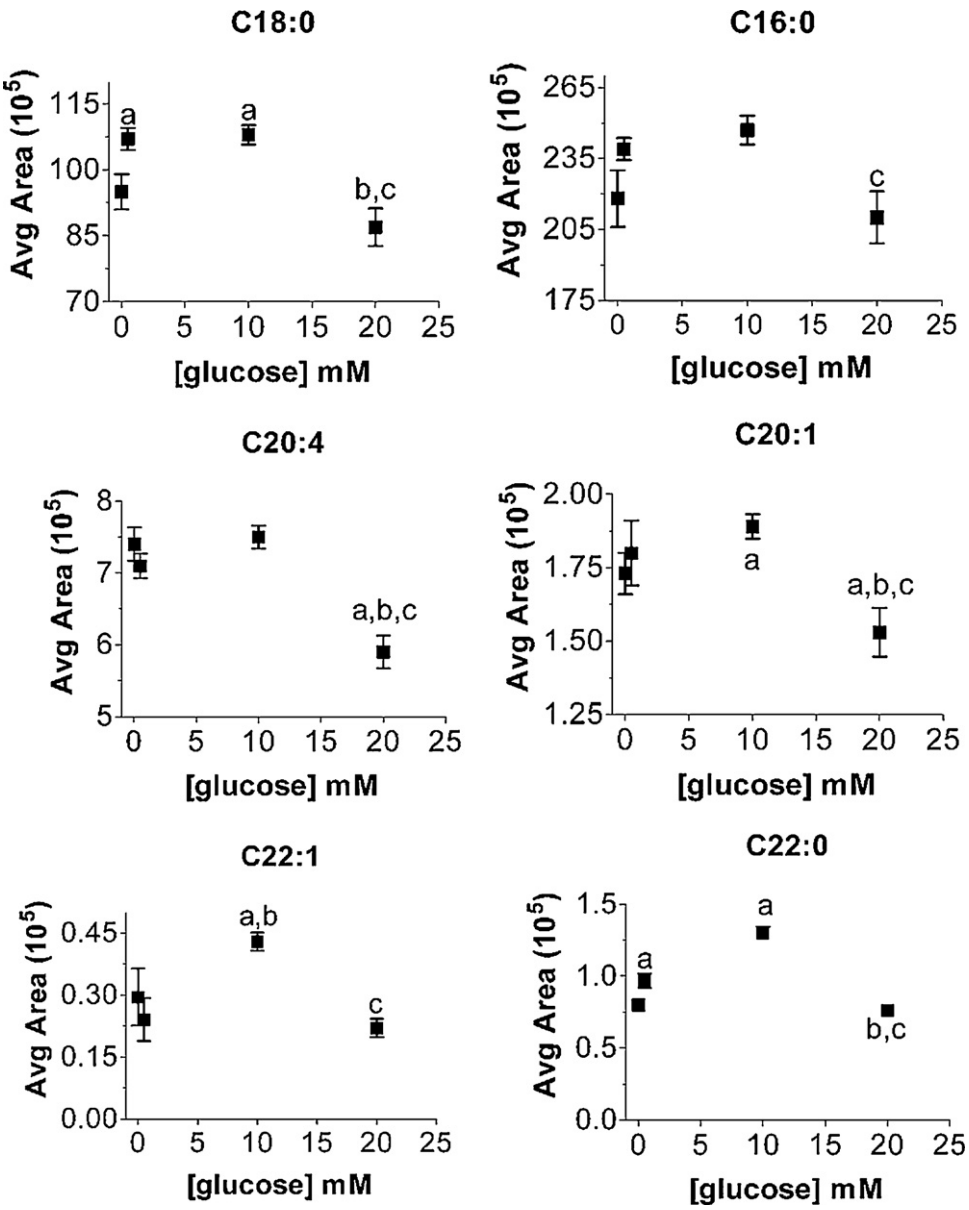


**Fig. 4.** Average area of palmitic acid (C16:0), stearic acid (C18:0), eicosenoic acid (C20:1), arachidonic acid (C20:4), behenic acid (C22:0) and erucic acid (C22:1) at 0 mM, 0.5 mM, 10 mM, and 20 mM glucose. Error bars are ± 1 SEM; a = significantly different from 0 mM glucose, b = significantly different from 0.5 mM glucose, c = significantly different from 10 mM glucose. Significance was determined with one way ANOVA test and Newman–Keuls post hoc test.

in lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and palmitoleic acid (C16:1) concentrations when compared to the absence of glucose were reported in the previous work [8] but not here. The changes observed previously in lauric acid and myristic acid are only seen at 16 mM glucose, thus, in view of the inverted U-shaped curve observed in our work, it is possible that these changes are missed in our experiment. The difference in palmitic and palmitoleic acid results may be explained by the high variability observed for these analytes. Another important point is that although islets and INS-1 cells are metabolically similar; they also have substantial differences that could easily affect the lipid profile and content.

## Acknowledgments

This work was supported by NIH R37-DK046960. Leco Corporation provided use of the Pegasus GC × GC and Restek Corporation provided GC columns used in this work.

## References

[1] A.P. Simopoulos, Am. J. Clin. Nutr. 70 (1999) 560S.
[2] T. Seppänen-Laakso, I. Laakso, R. Hiltunen, Anal. Chim. Acta 465 (2002) 39.
[3] P. Quinto Tranchida, R. Costa, P. Donato, D. Sciarrone, C. Ragonese, P. Dugo, G. Dugo, L. Mondello, J. Sep. Sci. 31 (2008) 3347.
[4] H. Rupp, T.P. Rupp, P. Alter, B. Maisch, Lancet 9661 (2009) 378.
[5] M. Rokling-Andersen, A. Rustan, A. Wensaas, O. Kaalhus, H. Wergedahl, T. Rost, Br. J. Nutr. 102 (2009) 995.
[6] M.P. Wymann, R. Schneiter, Nat. Rev. Mol. Cell Biol. 9 (2008) 162.
[7] D. Fabbri, V. Baravelli, G. Chiavari, S. Prati, J. Chromatogr. A 1100 (2005) 218.
[8] E.F. Martins, C.K. Miyasaka, P. Newsholme, R. Curi, A.R. Carpinelli, Diab. Metab. 30 (2004) 21.
[9] M. Adahchour, J. Beens, U.A.T. Brinkman, J. Chromatogr. A 1186 (2008) 67.
[10] S.-T. Chin, Y.B. Che Man, C.P. Tan, D.M. Hashim, J. Am. Oil Chem. Soc. 86 (2009) 949.
[11] J. Dalluge, J. Beens, U.A.T. Brinkman, J. Chromatogr. A 1000 (2003) 69.
[12] W. Tiyapongpattana, P. Wilairat, P.J. Marriott, J. Sep. Sci. 31 (2008) 2640.
[13] F. Adam, F. Bertoncini, V. Coupard, N. Charon, D. Thiebaut, D. Espinat, M. Hennion, J. Chromatogr. A 1186 (2008) 236.
[14] L. Akoto, F. Stellaard, H. Irth, R. Vreuls, R. Pel, J. Chromatogr. A 1186 (2008) 254.

[15] L. Mondello, P.Q. Tranchida, G. Purcaro, C. Fanali, P. Dugo, E. La Camera, C. Bisignano, in: J. Banoub (Ed.), Detection of Biological Agents for the Prevention of Bioterrorism, Springer, Dordrecht, 2011, 231 pp.
[16] Q. Gu, F. David, F. Lynen, K. Rumpel, G.W. Xu, P. De Vos, P. Sandra, J. Chromatogr. A 1217 (2010) 4448.
[17] J.Y. Gardner, D.E. Brillhart, M.M. Benjamin, L.G. Dixon, L.M. Mitchell, J.M.D. Dimandja, J. Sep. Sci. 34 (2011) 176.
[18] G. Purcaro, P.Q. Tranchida, P. Dugo, E. La Camera, G. Bisignano, L. Conte, L. Mondello, J. Sep. Sci. 33 (2010) 2334.
[19] L. Mondello, A. Casilli, P.Q. Tranchida, P. Dugo, G. Dugo, J. Chromatogr. A 1019 (2003) 187.
[20] G.C. Yaney, B.E. Corkey, Diabetologia 46 (2003) 1297.
[21] C.J. Nolan, M.S.R. Madiraju, V. Delghingaro-Augusto, M.-L. Peyot, M. Prentki, Diabetes 55 (2006) S16.
[22] M.J. MacDonald, A. Dobrzyn, J. Ntambi, S.W. Stoker, Arch. Biochem. Biophys. 470 (2008) 153.
[23] J.V. O'Fallon, J.R. Busboom, M.L. Nelson, C.T. Gaskins, J. Anim. Sci. 85 (2007) 279.