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A microfluidic device for the automated derivatization of free fatty acids to fatty acids methyl esters

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Abstract

Free fatty acid (FFA) compositions are examined in feedstock for biodiesel production, as source-specific markers in soil, and because of their role in cellular signaling. However, sample preparation of FFAs for gas chromatography-mass spectrometry (GC-MS) analysis can be time and labor intensive. Therefore, to increase sample preparation throughput, a glass microfluidic device was developed to automate derivatization of FFAs to fatty acid methyl esters (FAMES). FFAs were delivered to one input of the device and methanolic-HCl was delivered to a second input. FAME products were produced as the reagents traversed a 29 μ L reaction channel held at 55 °C. A Design of Experiment protocol was used to determine the combination of derivatization time (T_{der}) and ratio of methanolic-HCl:FFA (R_{der}) that maximized the derivatization efficiencies of tridecanoic acid and stearic acid to their methyl ester forms. The combination of $T_{\text{der}} = 0.8$ min and $R_{\text{der}} = 4.9$ that produced optimal derivatization conditions for both FFAs within a 5 min total sample preparation time was determined. This combination of T_{der} and R_{der} was used to derivatize 12 FFAs with a range of derivatization efficiencies from 18% to 93% with efficiencies of 61% for tridecanoic acid and 84% for stearic acid. As compared to a conventional macroscale derivatization of FFA to FAME, the microfluidic device decreased the volume of methanolic-HCl and FFA by 20- and 1300-fold, respectively. The developed microfluidic device can be used for automated preparation of FAMES to analyze the FFA compositions of volume-limited samples.

Introduction

Free fatty acids (FFAs) are major components of triacylglycerols and are ubiquitous in biological and environmental samples. In many cases, analysis of the FFA composition, as well as the total amount of fatty acids, is required. As an example, FFA compositions of soil,¹ cellular secretions,² and in foodstuffs^{3,4} have been performed. To underscore the importance of determining FFA composition, the relative amounts of the FFAs in dietary milk fats has been proposed as a potential determinant of childhood obesity.⁵

Gas chromatography-mass spectrometry (GC-MS) is often used for both quantitative and qualitative analysis of FFA. Prior to GC-MS analysis, FFAs must be derivatized to a more volatile form, such as a fatty acid methyl ester (FAME), but the sample preparation procedure can be laborious and time-intensive. The derivatization process may require up to

24 hours of reaction time⁶, high temperature,^{7,8} and/or large volumes of hazardous reagents and solvents. One way to decrease reagent consumption and increase sample preparation throughput would be to automate the derivatization process. Automation of the FAME preparation procedure using a robotic system with acetyl chloride derivatization reagent has been performed, but the overall derivatization reaction required up to 1 hour at elevated temperatures with multiple liquid-liquid extraction steps⁹, making the system expensive and complicated.

Microfluidic devices, and more generally continuous-flow microreactors, are ideal for automation because they can integrate multiple sample preparation steps, while decreasing reagent and sample consumption.¹⁰ These devices have been used for organic synthesis^{11–13} and have increased product yield in a shorter amount of time than comparable macroscale reactions. Integrated systems also open the possibility for rapid on-line screening allowing highly functional devices.¹⁴ To date, there have been no reports in the development of a microfluidic device for derivatization of FFAs to FAMES prior to GC-MS analysis.

In this report, we describe a microfluidic chip that can be used to derivatize FFA with methanolic-HCl to produce the corresponding FAME. Tridecanoic acid and stearic acid were used as representative short and long chain FFA, respectively, to optimize reaction conditions. Through initial screening, derivatization time (T_{der}) and the relative ratio of methanolic-HCl to FFA (R_{der}) were two variables that could be easily varied on the device and alter the amount of FAME generated. Since the method development would be time-consuming and inefficient if a univariate approach was taken, a Design of Experiment (DOE) protocol was used to screen 13 combinations of T_{der} and R_{der} to maximize the responses, in this case, derivatization efficiencies of tridecanoic acid and stearic acid. After performing these experiments, equations were fit to these responses and used to predict responses at experimental conditions that were not performed. A numerical optimization was performed to determine the combination of T_{der} and R_{der} which would optimize the derivatization efficiency of all FFAs at one condition. The predicted response at the optimal combination was validated and used to derivatize a mixture of 12 FFAs with varying chain lengths and degrees of saturation to their corresponding FAMES.

Materials and Methods

Reagents and materials

Heptadecanoic acid, gamma-linolenic acid, oleic acid, eicosadienoic acid, and gondoic acid were from Nu-Chek Prep, Inc. (Elysian, MN, USA). All other FFAs, methanolic-HCl, and a 37-component FAME mixture were from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol and hexane were from EMD Chemicals (Gibbstown, NJ, USA). All PEEK tubings, microfluidic reservoirs, and coupling adapters were purchased from Upchurch Scientific (Oak Harbor, WA, USA). Capillary was from Polymicro Technologies (Phoenix, AZ, USA).

Fabrication of microfluidic devices

The design of the microfluidic derivatization device had two inlet channels (1 cm length each) that intersected and formed a reaction channel 20 cm in length (29 μ L volume). The devices were fabricated using conventional photolithography and wet etching with hydrofluoric acid as described¹⁵ with the following additional steps. After the exposed photoresist and chrome layer were removed, the glass piece was baked at 105 °C for 30 min to harden the unexposed photoresist. Channels were etched to 230 $\times$ 510 μ m (depth $\times$ width). Fluidic access holes for the channel inlets were drilled with a 0.012" (Industrial Power Tools, North Tonawanda, NY, USA) diamond drill bit, whereas the outlet access hole was made with a 0.04" size drill bit (Abrasive Technology, Inc., Lewis Center, OH, USA). The different sized access holes were to minimize dead volume at the inlets and to decrease backpressure at the outlet. After the microfluidic device was thermally bonded, microfluidic reservoirs for 360 μ m o.d. capillary (Upchurch Scientific) were attached to the device according to the manufacturer's instructions.

Experimental system

A Rheodyne 6-port HPLC injector (IDEX Health & Science, Rohnert Park, CA, USA) and two syringe pumps (KDScientific, Inc., Holliston, MA, USA) for methanolic-HCl and water (18 M Ω -cm water, Millipore, Bedford, MA, USA) were connected to the two inlets of the microfluidic device. A 7.4 μ L loop made with a 250 μ m i.d. capillary was used with the 6-port injector. The syringe pump pushing water was connected to the injector using 0.04" i.d. PEEK tubing. Connections from the 6-port injector to the device, and from the other syringe pump pushing methanolic-HCl to the device, were made with 100 μ m i.d. capillaries. The outlet reservoir of the device was connected to a 50 μ m i.d. $\times$ 14 cm length capillary. The temperature of the derivatization reaction channel was maintained at 55 °C by a Kapton flexible heater (Omega Engineering, Inc., Stamford, CT, USA) and a temperature controller (Omega Engineering, Inc., Stamford, CT, USA).

FFA derivatization

Off-chip preparation of internal standard—Methyl pentadecanoate solution was used as the IS and was made by reacting 6.0 mg of pentadecanoic acid in 2 mL of methanolic-HCl at 55 °C for 5 min. Then, 10 mL of hexane was added and mixed by a vortexer for 2 min. 9 mL of the hexane phase was then removed. A second extraction of the aqueous phase was performed by addition of 2 mL of hexane to the original methanolic-HCl solution and extracted as before. 1 mL of the hexane phase was removed and combined with the 9 mL from the first extraction and placed in a 50-mL volumetric flask. Hexane was added to obtain approximately 100 μ g mL⁻¹ IS solution.

On-chip derivatization

Volumetric flow rates and the sample collection and extraction time were calculated from the combination of T_{der} and R_{der} being tested. The reaction channel was 29 μ L and at a given T_{der} , the total volumetric flow rate was calculated. Using this total flow rate at a given R_{der} , the flow rates of both the methanolic-HCl and FFA were calculated. For each on-chip derivatization experiment, 5 μ L of IS and 5 mL of hexane were added to a glass vial and

stirred while held in an ice bath. The outlet capillary from the microfluidic device was placed into the hexane in this vial for a given time as described in the next paragraph.

Reaction conditions were optimized by using a mixture of $100\text{ }\mu\text{g mL}^{-1}$ of tridecanoic acid and stearic acid in methanol. This solution filled the $7.4\text{ }\mu\text{L}$ injection loop and water was used to push this solution through the device. The start time for collecting the derivatized FAMES was when the HPLC injector valve was actuated. The end of the collection time was twice the calculated time it took for the sample plug to flow through the sample loop, tubings, microfluidic chip, and outlet capillary at a given flow rate. After sample collection, the stirring in the collection vial was stopped and 1 min was allowed for phase separation. 4 mL of the hexane phase was transferred into another vial and evaporated using a stream of N_2 . After evaporation, the sample was reconstituted in $100\text{ }\mu\text{L}$ hexane for GC-MS analysis. Between each derivatization, the device was flushed with $500\text{ }\mu\text{L}$ of methanolic-HCl and methanol.

GC-MS analysis

FAME detection was performed with an HP 6890 gas chromatograph with 5973N mass selective detector (Agilent Technologies, Inc., Santa Clara, CA, USA). $1\text{ }\mu\text{L}$ splitless injection was performed onto the GC column (Restek Corp. RTX-5SIL MS column, $0.32\text{ mm ID} \times 30\text{ m length}$, $0.25\text{ mm film thickness}$, Bellefonte, PA, USA) with the injection port temperature held at $250\text{ }^\circ\text{C}$. The carrier phase was ultrahigh purity He at a flow rate of 1.0 mL min^{-1} . The chromatographic separation was $t = 0\text{ min}/135\text{ }^\circ\text{C}$; $t = 7.5\text{ min}/180\text{ }^\circ\text{C}$; $t = 22\text{ min}/180\text{ }^\circ\text{C}$. For GC-MS analysis of the 37-component FAME mixture and 12 FFAs mixture, the temperature program was $t = 0\text{ min}/135\text{ }^\circ\text{C}$; $t = 7.5\text{ min}/180\text{ }^\circ\text{C}$; $t = 22\text{ min}/180\text{ }^\circ\text{C}$; $t = 23\text{ min}/208\text{ }^\circ\text{C}$; $t = 34\text{ min}/230\text{ }^\circ\text{C}$; $t = 44\text{ min}/230\text{ }^\circ\text{C}$. The temperature of the MS inlet and quadrupoles were $150\text{ }^\circ\text{C}$ while the MS source was $230\text{ }^\circ\text{C}$. The m/z was scanned from 40–550 with a sampling rate of $1.47\text{ scan sec}^{-1}$. On-chip derivatized peaks were identified by comparing their retention times with the 37-component FAME mixture and matching their mass spectra against a NIST reference mass spectra library (v 2.0d). Total ion count was used for integration of FAME peak areas. Calibration curves were made by diluting the 37-component FAME mixture 500, 167, 50 and 33-fold with hexane. These curves were used for calculating derivatization efficiency of the on-chip reaction, expressed as a percent of the experimentally determined amount of FAME to the theoretical amount of FAME obtained if the derivatization efficiency was 100%.

Off-chip extraction efficiency

To test if the range of T_{der} and R_{der} used in the on-chip reactions affected off-chip extractions, four sets of samples at 1.2 min and 6.2 min extraction times at 0.4 and 5.0 R_{der} were generated with a minimum of four replicates. Each sample contained identical volumes of methanolic-HCl, water and methanol, as well as identical concentrations of $100\text{ }\mu\text{g mL}^{-1}$ methyl tridecanoate and methyl stearate mixture solution (prepared using the sample procedures as for IS solution), and IS. These volumes and concentrations were also identical to those samples derivatized in the microfluidic device. The samples were extracted and prepared for GC-MS analysis as described in GC-MS analysis section. A two-tailed t -test was used to compare the average FAME:IS peak areas.

Mixture of 12 FFAs

Individual stock solutions of 2 mg mL⁻¹ FFA in methanol were prepared for decanoic acid, lauric acid, tridecanoic acid, myristic acid, palmitoleic acid, palmitic acid, heptadecanoic acid, gamma-linolenic acid, oleic acid, stearic acid, eicosadienoic acid, and gondoic acid. A final mixture of the 12 FFAs was prepared with 300 µg mL⁻¹ decanoic acid and all other FFAs at 50 µg mL⁻¹.

Design of Experiment method

Design Expert® 7 software (StatEase, Minneapolis, MN, USA) was used to guide the selection of T_{der} and R_{der} combinations to be tested. All statistical tests were deemed significant when $p < 0.05$. All values are given as the average peak area $\pm$ one standard deviation with the number of replicates given in the text.

Results and discussion

To increase sample preparation throughput, a rapid method for converting FFAs to FAMES prior to GC-MS analysis is needed. The microfluidic system shown in Figure 1 was developed for the rapid and simple derivatization of FFA to their corresponding FAME. Briefly, a 6-port HPLC injector was used to introduce a plug of FFA to one arm of a microfluidic device where it was mixed with methanolic-HCl derivatization reagent as it traversed a heated serpentine reaction channel. The FAME products then exited the device and were collected in a vial off-chip and extracted with hexane prior to injection onto a GC-MS system. Tridecanoic acid and stearic acid were used as model FFAs to be derivatized to methyl tridecanoate and methyl stearate, respectively.

To demonstrate the possibility of rapid on-chip derivatization of FFA, both T_{der} and R_{der} were optimized using a DOE protocol with the goal of the highest derivatization efficiency within a 6 min total sample preparation time. To accomplish this goal, T_{der} was varied between 0.4 and 1.0 min, and R_{der} varied between 0.4 and 5.0. T_{der} was the time that the FFA had to react with methanolic-HCl in the heated reaction channel, while R_{der} was the ratio of the volumetric flow rates of methanolic-HCl to FFA. The use of a DOE protocol allowed determination of optimum T_{der} and R_{der} without the need to test a large experimental space. DOE protocols have been used to optimize reaction conditions, for example, to optimize the temperature, stoichiometry, and reaction temperature for removal of a protecting group from an amine in a continuous flow microreactor.¹⁶ In our optimization, 13 experiments with different combinations of T_{der} and R_{der} were performed with additional replicates added for assessment of precision, producing a total of 27 experiments over 3 days. Response surfaces were used to determine the condition where the derivatization efficiencies of tridecanoic acid and stearic acid were maximized.

Extraction variability

For each experiment, the combination of T_{der} and R_{der} being tested resulted in different extraction times and volume ratios of hexane:aqueous in the off-chip collection vial, which may have influenced the post-derivatization extractions of the FAMES. To test if the different conditions affected the off-chip extraction, four samples with combinations of the smallest

and largest sample collection time and R_{der} used in the on-chip experiments, were extracted off-chip. We hypothesized that if the amount of FAME detected in these samples were not significantly different, the various extraction times and volume ratios of hexane:aqueous would not influence the amount of FAME extracted for all other samples.

Extraction with R_{der} of 0.4 resulted in the same average FAME:IS peak area whether the extraction occurred for 1.2 min, 0.46 ± 0.09 for methyl tridecanoate and 0.54 ± 0.04 for methyl stearate ($n = 9$), or as long as 6.2 min, 0.50 ± 0.11 for methyl tridecanoate ($p = 0.38$) and 0.51 ± 0.05 ($n = 6$, $p = 0.19$). Similarly, when extracting with R_{der} of 5.0, extraction times of 1.2 ($n = 8$) and 6.2 min ($n = 8$) resulted in the same FAME:IS ratio of 0.47 ± 0.08 versus 0.54 ± 0.04 for methyl tridecanoate ($p = 0.14$) and 0.55 ± 0.04 versus 0.56 ± 0.02 for methyl stearate ($p = 0.46$), respectively. The average FAME:IS peak area of these samples showed no statistical difference ($p > 0.05$) for both methyl tridecanoate and methyl stearate at any extraction time or R_{der} . The lack of statistical significance in these off-chip experiments implied that any differences in the FAME levels detected by the GC-MS were due to varying amounts of FAME produced on-chip because of differences in the T_{der} and R_{der} , not to processes outside the device.

On-chip derivatization

After demonstrating that the off-chip extraction procedure was not influencing the amount of FAME detected by the GC-MS, 27 on-chip derivatization experiments were performed with 13 different T_{der} and R_{der} combinations. After every two samples, a blank injection of hexane was made into the GC-MS and indicated no detectable carryover between runs. Blank runs were also performed through the device and showed no detectable FFA or FAME. In each of the 27 experiments, the same mass of FFA was subjected to derivatization. In most cases, unreacted FFA was not detected in the GC-MS chromatograms, except when $T_{\text{der}} = 0.7$ min. To assess the inter-day precision, the percent relative standard deviation (%RSD) of the FAME:IS peak area ratio was calculated from replicates ($n = 7$) of the DOE centerpoint over 3 days. The DOE centerpoint was defined as the median values of the range of the two variables tested, $T_{\text{der}} = 0.7$ min and $R_{\text{der}} = 2.7$. A 13% inter-day precision was obtained for tridecanoic acid and 15% for stearic acid. This poor precision may be due to incomplete derivatization for tridecanoic acid and stearic acid at these reaction conditions (67% and 86% derivatization efficiency, respectively). The DOE model assumed the same inter-day precision throughout all combinations of T_{der} and R_{der} tested. Intra-day precision ($n = 3$) was assessed as the %RSD of FAME:IS peak area ratio at one condition in one day and was found to be 14% for both tridecanoic acid and stearic acid.

Analysis of design models

ANOVA analysis of model designs—The model used to fit the derivatization response from the 27 samples was the quadratic model with the general form of:

$$Y = \beta + A + B + (AB) + A^2 + B^2 + E \quad (1)$$

where Y was the FFA derivatization efficiency, β was the model coefficient, A was T_{der} , B was R_{der} , and E the error term, while the terms in parentheses were interactions between the variables, and squared terms were the quadratic effects of the variables.¹⁷ Peak areas of methyl tridecanoate, methyl pentadecanoate, and methyl stearate from each on-chip derivatization sample were used to calculate the response expressed as the FFA derivatization efficiency as described in GC-MS analysis section. Analysis of variance (ANOVA) was performed to aid in the selection of the lowest-order polynomial model that adequately described the derivatization process. To perform ANOVA, an F -value for each variable was tabulated. In our experiments, F -values were a test statistic for the null hypothesis, which stated that the variable had no effect on the response.^{17,18} A p -value was calculated for each of the variables by comparing calculated F -values to a one-tailed F distribution table. The p -value was the probability that the effect of the variable on the response was due to random error. Therefore, when $p < 0.05$, the effect was not due to random error and the variable had a significant effect on the response. A model reduction was done, which excluded the insignificant variables in equation 1 to the final reduced model form (equations 2 and 3), but still accounted for the variation of these terms in the E term.

The final reduced model for the derivatization to methyl tridecanoate was linear with the form:

$$\text{Tridecanoic acid derivatization efficiency} = \beta + A + B + E \quad (2)$$

ANOVA results (Table 1) for the variables in Equation 2 indicated that T_{der} was not significant ($p = 0.3086$), but was retained in the reduced model because T_{der} was one of the tested variables. The model term estimated the factor effects and with $p = 0.0116$, T_{der} and R_{der} had significant effects on the derivatization of tridecanoic acid.

A reduced quadratic model was fit to the derivatization efficiency of stearic acid as described by Equation 3:

$$\text{Stearic acid derivatization efficiency} = \beta + A + B + (AB) + B^2 + E \quad (3)$$

The ANOVA results for the variables in equation 3, as well as the excluded variables are shown in Table 1. Similar to the model for tridecanoic acid, the model term indicated these factors had significant effects ($p < 0.0001$) on the response.

Derivatization of tridecanoic acid

Equations 2 and 3 can be represented by multi-dimensional plots to visualize the derivatization efficiency response at a range of T_{der} and R_{der} values. The response surface plot for derivatization of tridecanoic acid to methyl tridecanoate is shown in Figure 2A. The shallow slope of the response over the range of T_{der} demonstrates that this variable had less of an effect on the derivatization than R_{der} , which had a steeper slope. Using equation 2, the predicted vs. actual derivatization efficiency was plotted and showed a quality of fit (R^2) of

0.6042 (Figure 2B). One possibility for the low R^2 was that the model assumed the variability at all combinations of T_{der} and R_{der} were equal to the intra-day error of 13% found from replicates of a single combination of these variables, as mentioned in the on-chip derivatization section. If the variability in some responses were larger than this intra-day error, R^2 would be reduced.¹⁷ FFAs are known to adsorb onto silica in a chain-length dependent manner¹⁹ which may be pertinent in our system. Regardless of the low R^2 , as stated in ANOVA analysis discussion, the overall model was statistically significant (Table 1).

Derivatization of tridecanoic acid had the lowest derivatization efficiency of 10% at the shortest T_{der} and lowest R_{der} , and the highest conversion of 83% at the longest T_{der} and highest R_{der} . While larger ranges of T_{der} and R_{der} could have been used in an attempt to obtain 100% derivatization efficiency, the ranges of T_{der} and R_{der} were limited intentionally to keep the total sample preparation time less than 6 min, which was the approximate time for macroscale sample preparation. There may be methods to increase tridecanoic acid derivatization efficiency without increasing total sample preparation, such as mixing the FFAs with the derivatization reagent in a faster manner, or by increasing the reaction temperature. Reproducibility of the tridecanoic acid derivatization varied from 13% – 28% in the range of T_{der} and R_{der} where no unreacted tridecanoic acid was detected.

Derivatization of stearic acid

Similar to tridecanoic acid, stearic acid derivatization efficiency (Figure 3A) was lowest at the shortest T_{der} and lowest R_{der} , and was the highest at the longest T_{der} and highest R_{der} .

The derivatization efficiency of stearic acid was 100% when $T_{\text{der}} = 1.0$ min and $R_{\text{der}} = 5.0$. Curvature in the response near the longest T_{der} and highest R_{der} indicated that the response did not increase further and the optimum result was contained within the model. The R^2 value, 0.9235, of the reduced quadratic model for stearic acid derivatization (Figure 3B) showed the predictive power of the model. Reproducibility of stearic acid derivatization ranged from 15% – 23%.

Numerical optimization

To determine the T_{der} and R_{der} that would generate the highest simultaneous derivatization of tridecanoic acid and stearic acid, a numerical optimization of the response surfaces was performed. Since unreacted tridecanoic acid was detected in most samples with $T_{\text{der}} = 0.7$ min at $2.7 R_{\text{der}}$, T_{der} was restricted to 0.8 min. With this constraint on T_{der} , R_{der} was set to be in the range of 4.9 – 4.95 to ensure that the total sample preparation time was less than 5 min. Tridecanoic acid derivatization efficiency was constrained to the range of 70% – 83%, the maximum experimentally obtained efficiency. Stearic acid derivatization efficiency was constrained to the range of 90% – 100%. Priority was given to tridecanoic acid derivatization being in the set range because derivatization of stearic acid was complete in this region. The solution meeting these criteria was $T_{\text{der}} = 0.8$ min and $R_{\text{der}} = 4.9$, which produced a 5 min sample collection time. On-chip derivatization of tridecanoic acid and stearic acid at these values of T_{der} and R_{der} was performed to validate the predicted solution. The derivatization efficiencies of $59 \pm 14\%$ and $94 \pm 4\%$ for tridecanoic acid and stearic acid, respectively,

were statistically similar to the predicted solutions of $72 \pm 11\%$ and $94 \pm 8\%$, respectively ($p > 0.05$)

Derivatization of 12 FFAs

The microfluidic device was developed to increase sample preparation throughput by automating the derivatization of FFA to FAME prior to GC-MS analysis. To demonstrate the potential application for on-chip derivatization of a variety of FFAs, a mixture of 12 FFAs was derivatized to the corresponding FAMES using the optimized combination of T_{der} and R_{der} (Figure 4). All FFAs derivatized to their FAME forms with derivatization efficiencies ranging from 18% to 93% (Table 2).

Decanoic acid had the lowest derivatization efficiency (18%) with a large percentage remaining as underivatized FFA, indicated by an asterisk in Figure 4. We believe the low derivatization efficiency of decanoic acid may be due to non-optimal reaction conditions for this FFA, as well as decanoic acid lost in pre-chip sample handling due to its high volatility. Derivatization efficiencies of tridecanoic acid and stearic acid correlated to the predicted solution described in the analysis of design models, indicating that derivatization efficiencies of the individual FFAs were not altered by inclusion in a more complex sample, making the applicability of this method for other samples feasible. One caveat of this approach was that FFAs longer than 20-carbons in chain length had limited solubility in methanol making their derivatization difficult with this procedure. However, in volume-limited samples, the method described here can be used for rapid derivatization of a number of short and medium chain length FFAs.

Conclusions

DOE was used to optimize derivatization efficiencies of tridecanoic acid and stearic acid to their methyl ester forms. When compared to the typical sample preparation procedure listed in the product information sheet provided with methanolic-HCl, the optimized microfluidic derivatization resulted in a 20-fold decrease in the required methanolic-HCl and 1300-fold decrease in the amount of FFA required, all while maintaining a 5 min total sample preparation (derivatization and extraction) time. Future work will aim to integrate extraction of FAMES with this derivatization module with coupling of the integrated microfluidic device to a GC-MS to realize continuous-flow preparation of FAMES for an increased sample preparation throughput method.

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Fig. 1.

A syringe pump used water to drive mixtures of FFA from the sample loop of a 6-port injector into one arm of a microfluidic device. The conversion of FFAs to FAMES occurred as the FFAs solution mixed with methanolic-HCl within the heated reaction channel. The flow rate ratio of the two syringe pumps (R_{der}) and the duration of time in the heated reaction channel (T_{der}) were varied using a DOE protocol to optimize the derivatization efficiency of two model FFAs.

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(A) Tridecanoic acid derivatization efficiencies are shown with respect to the two experimental variables, R_{der} and T_{der} . (B) Predicted vs. actual tridecanoic acid derivatization efficiency was linear with an R^2 of 0.6042.

Fig. 3.

(A) The response surface for stearic acid derivatization is shown with maximum response at $T_{der} = 1$ min and $R_{der} = 5.0$. (B) The plot of predicted vs. actual stearic acid derivatization efficiency had an R^2 of 0.9235.

Fig. 4.

A mixture of 12 FFAs was derivatized using the optimal derivatization condition of $T_{\text{der}} = 0.8$ min and $R_{\text{der}} = 4.9$ with the IS added. FAME peaks are (1) methyl decanoate, (2) methyl laurate, (3) methyl tridecanoate, (4) methyl myristate, (5) methyl palmitoleate, (6) methyl palmitate, (7) methyl heptadecanoate, (8) methyl gamma-linolenate, (9) methyl oleate, (10) methyl stearate, (11) methyl eicosadienoate, and (12) methyl gondoate. The asterisk (*) denotes underivatized decanoic acid.

Table 1.

ANOVA table for the response surface models

Source	Tridecanoic acid derivatization efficiency			Stearic acid derivatization efficiency		
	<i>df</i> ^a	<i>F</i> value	<i>p</i>	<i>df</i> ^a	<i>F</i> value	<i>p</i>
<i>A</i>	1	1.14	0.3086	1	19.06	0.0011
<i>B</i>	1	20.91	0.0008	1	96.00	<0.0001
<i>AB</i>	1	0.41	0.5372 ^b	1	5.25	0.0426
<i>A</i> ²	1	0.53	0.4809 ^b	1	0.011	0.9187 ^b
<i>B</i> ²	1	1.20	0.2963 ^b	1	10.45	0.0080
Model	5	5.10	0.0116	5	26.60	<0.0001
Error	11	1.70	0.3174	11	1.49	0.3664

^aDegrees of freedom for each source of variation^bNot significant and moved to the Error term in the final model

Table 2.

On-chip derivatization efficiencies of 12 FFAs

Peak number	FAME common name	Derivatization efficiency (%)
1	Methyl decanoate	18
2	Methyl laurate	69
3	Methyl tridecanoate	61
4	Methyl myristate	78
5	Methyl palmitoleate	76
6	Methyl palmitate	93
7	Methyl heptadecanoate	71
8	Methyl gamma-linolenate	60
9	Methyl oleate	33
10	Methyl stearate	84
11	Methyl eicosadienoate	50
12	Methyl gondoate	45