

Increasing Productivity in FAMES Analysis through Increased Selectivity

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Key Words

Chromleon Chromatography Data System, AutoSRM, Blood Spot, Fatty Acid Methyl Esters (FAMES), GC-MS/MS, Quantification

Goals

To demonstrate that a single quadrupole method for FAMES analysis provides robust results and that the transition to a triple quadrupole method using automated SRM development tools is a simple process. To illustrate improved productivity through more reliable data processing using triple quadrupole methods on the same MS platform.

Introduction

Fatty acids (FAs) are carboxylic acids with long, unbranched aliphatic chains, which can either be saturated or unsaturated. FAs differ in their chain length, as well as the number and location of the double bonds in the acyl chains. These subtle differences in structure determine the benefit or detriment of FAs to a biological system. For example, a primary function of FAs is supplying chemical energy to muscles in the form of adenosine triphosphate (ATP).¹ They also serve as signaling molecules and are linked to the prevention and development of many diseases, such as diabetes and coronary heart disease. Previous studies have shown that many n-3 FAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential to the normal development of young children, in contrast to trans-FAs which have been linked to increased risk of developing coronary heart disease.² Therefore, there is a need for reliable and precise methods to determine fatty acids profile and concentrations as part of nutritional, epidemiological, and clinical studies.

Until recently, most analytical methods for determining fatty acid methyl esters have used gas chromatography coupled to flame ionization detection (GC-FID). While this approach is still widely used, a powerful alternative is gas chromatography coupled to mass spectrometry, which provides structural information and improved selectivity and sensitivity. In this work, the performance of the Thermo Scientific™ TSQ™ Duo system is assessed for the



quantitative analysis of 11 FAMES in human blood spot extracts. The TSQ Duo system offers analysts a unique choice by allowing the use of single quadrupole methods and enabling the automatic transition, when appropriate, to triple quadrupole on the same system. The transition becomes a simple process when it is made on a familiar system and when using walkthrough method development tools and fully automatic tuning. Triple quadrupole methods may be required to meet future demands, such as lower detection limits, more specificity, and improved laboratory efficacy. This application note illustrates the stepwise process of upgrading methods from single quadrupole to triple quadrupole using integrated software.

Experimental Conditions

Sample Preparation

Samples consisted of human blood spots set on filter paper and were stored at 4 °C prior to extraction. The extraction procedure was as follows: 1 mL of 0.5 M HCl in methanol + 100 µL of internal standard was added to the blood spots, mixed and incubated at 70 °C for 1 hour. After cooling, 1 mL of deionized water and 1 mL of saturated potassium chloride were added and thoroughly mixed. To this solution, 2 mL of hexane was added, mixed, and centrifuged for 5 minutes. The samples were then frozen in liquid nitrogen and the hexane layer was transferred into a clean vial and dried under nitrogen at 40 °C for 20 minutes. The sample was reconstituted in 50 µL hexane. A standard calibration series ranging from 0.2–1200 µg/mL of FAMES was prepared in hexane.

Method Setup

FAMES were analyzed in standards and blood spot extracts using a TSQ Duo triple quadrupole GC-MS/MS instrument coupled with a Thermo Scientific™ TRACE™ 1310 GC. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and compound separation was achieved on a TRACE TR-FAME 30 m × 0.22 mm I.D. × 0.25 µm film capillary column (P/N: 260M141P). Additional instrument parameters used to acquire data are displayed in Tables 1 and 2.

The Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software was used as the data system for sample analysis and quantitative data processing. The unique functionality of the Chromeleon CDS software makes routine analysis fast and simple. All analytical information, with retention times and SRM transitions, is stored in the Chromeleon CDS method database. This information is also used for the quantitation processing method comprising all compounds, their transitions, and retention times. This information storage strategy avoids potential human error in writing or copying from one file to another and simplifies the complete method setup.

Table 1. GC and injector conditions.

TRACE 1310 GC Parameters	
Injection Volume (µL):	1
Liner:	Split quartz wool (P/N: 453A1295)
Inlet (°C):	260
Inlet Module and Mode:	Split
Split Flow (mL/min):	80
Split Ratio:	100
Carrier Gas, (mL/min):	Helium, 1.8
Oven Temperature Program:	Temperature 1 (°C): 150
	Hold Time (min): 1
	Temperature 2 (°C): 250
	Rate (°C/min): 10
	Hold Time (min): 3

Table 2. TSQ Duo Mass Spectrometer parameters.

TSQ Duo Mass Spectrometer Parameters	
Transfer Line (°C):	260
Ionization Type:	EI
Ion Source (°C):	320
Electron Energy (eV):	70
Emission Current (µA):	50
Acquisition Modes:	Full Scan/SIM/SRM
Q2 Gas Pressure(argon) (psi):	60
Q1 Peak Width (Da):	0.7
Q3 Peak Width (Da):	0.7

Table 3. SRM transitions and collision energies used for FAMES GC-MS/MS analysis.

Compound Name	Compound Abbreviation	Retention Time [min]	Precursor Ion [Da]	Product Ion [Da]	Collision Energy [V]
Palmitic acid	PALMA	3.6	74	43	10
			87	55	10
Stearic acid	STEARA	4.75	74	43	10
			87	55	10
Oleic acid	OLEICA	4.96	97.1	55	10
			97.1	69	5
Linoleic acid	LINOLA	5.35	95	67	10
			81	79	10
Gamma linolenic acid	GLA	5.6	79.1	51	20
			79.1	77	10
Alpha linolenic acid	ALA	5.81	87	55	10
			74	43	10
Dihomo- γ -linolenic acid	DHGLA	6.8	87	55	20
			74	43	10
Arachidonic acid	ARA	6.96	87	55	10
			74	43	10
Eicosapentaenoic acid	EPA	7.43	91	65	20
			79.1	51	15
Docosapentaenoic acid	DPA	8.6	79.1	51	20
			79	77	15
Docosahexaenoic acid	DHA	8.75	79	51	20
			91	65	15
17:0 (Internal standard)	17:0	4.15	74	43	10
			87	55	10
23:0 (Internal standard)	23:0	7.63	87	55	10
			74	43	10

Quantification ion in bold

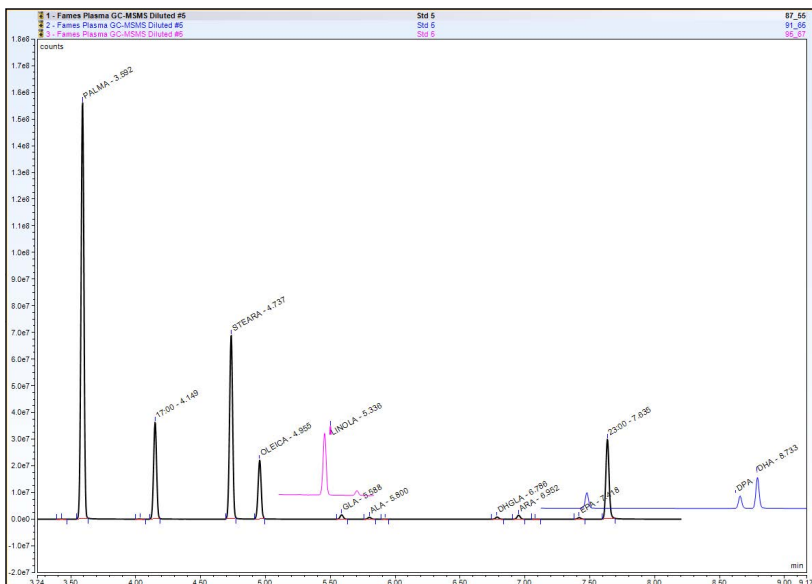


Figure 1. Chromatogram of 11 FAMES and two internal standards in standard 5 at concentrations ranging from 80–800 $\mu\text{g}/\text{mL}$.

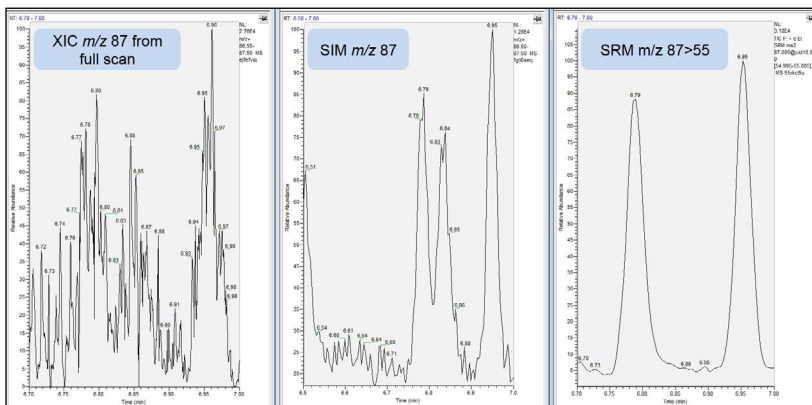


Figure 2. Resolution from blood matrix using full scan XIC m/z 87, SIM, and SRM for DHGLA (6.79 DHGLA) and ARA (6.95 min). The peaks are better resolved with an improved signal to noise in the SRM chromatogram. Data from the analysis of the blood QC sample.

Results and Discussion

An initial full scan analysis (m/z 50–500) was run in order to identify individual FAMES using a spectral library and to determine their retention times. The separation of the 11 FAMES was achieved in a relatively fast run time of 14 minutes (Figure 1) compared to typical analysis times of 30–40 minutes, significantly improving sample throughput. Once the GC method was optimized, the standards, quality controls and samples were analyzed using two MS methods — first with single quadrupole MS and then with triple quadrupole MS.

Traditionally, the most complex aspect of adopting triple quadrupole technology is the creation of MS/MS methods, particularly if the method contains a significant number of compounds. To solve this problem, integrated AutoSRM software was used to quickly develop SRM methods for each of the FAMES. This integrated tool takes the user from an existing single quadrupole method to a fully developed, optimized SRM method on the TSQ Duo GC-MS/MS system. The precursor ion, product ion, and collision energy are optimized in a logical, quick, and easy-to-follow process. The AutoSRM procedure is described in the following section. For SRM data acquisition, two SRM transitions per compound were selected and data acquired using timed-SRM with a minimum of 12 points/chromatographic peak. Timed-SRM uses a completely different analytical strategy than the “classical” segmented setup, allowing data acquisition for a target compound in a short retention window around the known compound retention time, and not in a wide retention time segment. As a result, dwelling on the target compounds is very effective, ultimately improving the sensitivity and lowering the method detection limit. Triple quadrupole MS enables higher compound selectivity, which is a particular advantage in overcoming matrix interferences and simplifying data processing.

For the SIM analysis (single quadrupole mode) in this study, the most intense fragments were used as diagnostic ions. FAMES can be classified according to their diagnostic ion; m/z 74 and 87 for saturated FAMES; m/z 55, 69, and 74 for monounsaturated; m/z 55 and 67 for polyunsaturated. These fragments are relatively low in mass and are common to many FAMES. Therefore, additional specificity of individual FAMES was achieved by retention time. When MS/MS was used, the same issues around specificity between FAMES existed. However, the improvement in resolution from background interferences when using SRM is evident. Figure 2 shows three chromatograms for DHGLA and ARA in a blood QC sample using full scan, SIM, and SRM acquisition methods. The peaks are poorly defined in the full scan and SIM data, but clearly resolved when using SRM. Using SRM has the benefit of improved signal to noise and results in cleaner chromatograms, ultimately allowing for faster data processing as little or no user intervention is required.

Automated SRM Method Development

The transition from GC-MS to GC-MS/MS for the analysis of FAMES was simplified using AutoSRM. For every compound, one precursor ion, but preferably two or more precursor ions, needs to be selected. Subsequently, the optimum collision energy and product ions for detection need to be defined for each precursor ion. In addition to all the necessary injections and data gathering, the primary challenge lies in handling the data and carefully monitoring which precursor yields the best product ion, at which collision energy, at a certain retention time; and determining this information for dozens of compounds at the same time.

The automated SRM feature of the Chromeleon CDS software was designed to perform these tasks automatically using the following workflow:

1. A full scan experiment identifies the target compounds.
2. The most intensive or selective ions are selected and put into a working list.
3. A product scan experiment is run in the next injection using the working list.
4. The method and the sequence for the product scan experiments is prepared automatically.
5. The product scan spectra are clearly shown by compound and by precursor ion.
6. The most intensive or selective product ions are selected and put into the working list.
7. Collision energy is optimized for sensitivity.
8. The method and the sequence for the optimal collision energies is set up automatically.
9. The completed working list is stored as a compound database .csv file. This file can be imported or attached to the instrument method and is also used as the quantifying processing method.

The sequence of the AutoSRM steps is illustrated in Figures 3–5. The Chromeleon CDS software can perform library searches for compound identification. Optionally, Chromeleon CDS software can also import a list of compounds with retention times, etc. All of the data can be reviewed and edited manually.

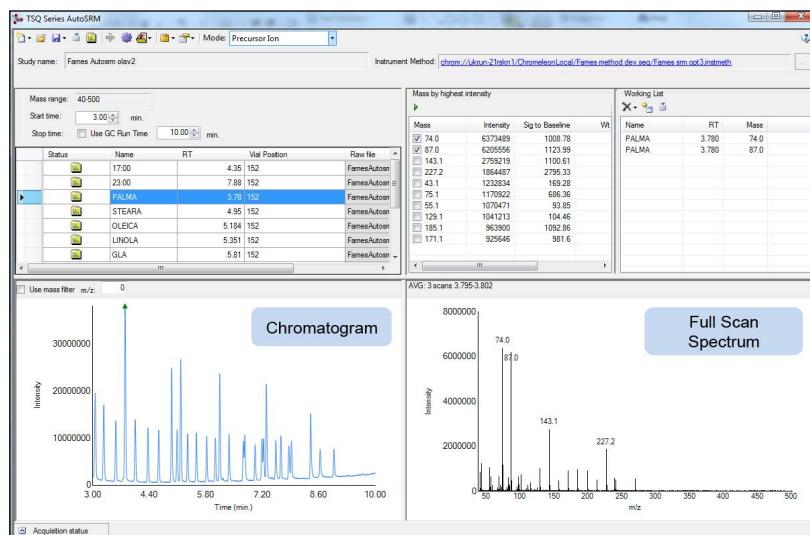


Figure 3. AutoSRM Step 1: Precursor identification. The upper left box shows the list of compounds and retention times. The upper middle box shows a list of the ions and their intensities. The upper right box shows the ions selected for Step 2: Product ion identification.

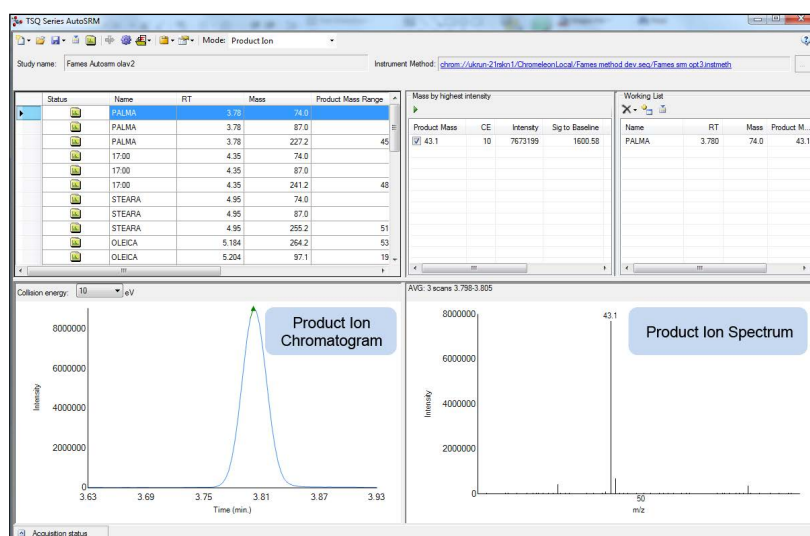


Figure 4. AutoSRM Step 2: Product ion identification. The upper left box shows the list of compounds, retention times, and precursor ions. The upper middle box now shows the product ions and intensities. The upper right box shows the SRM selected for Step 3: SRM optimization.

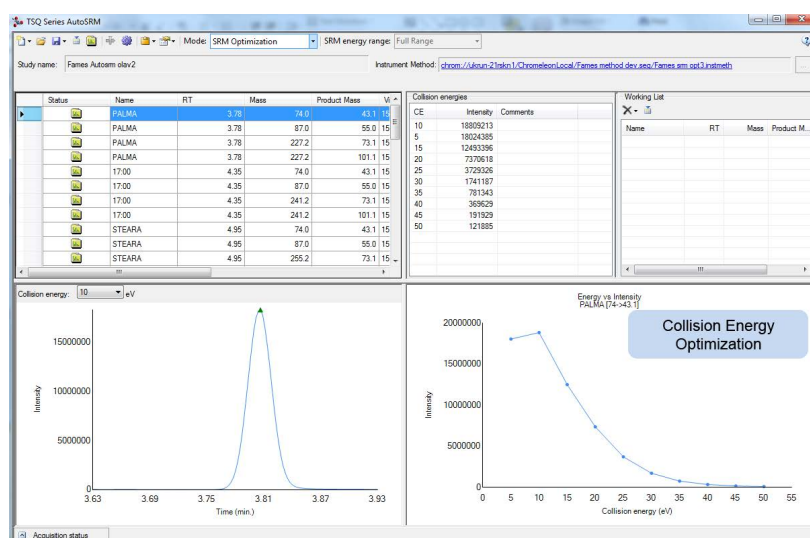


Figure 5. AutoSRM Step 3: SRM optimization. The upper left box shows the list of compounds, retention times, and SRMs. The upper middle box now shows the SRM intensities at different collision energies.

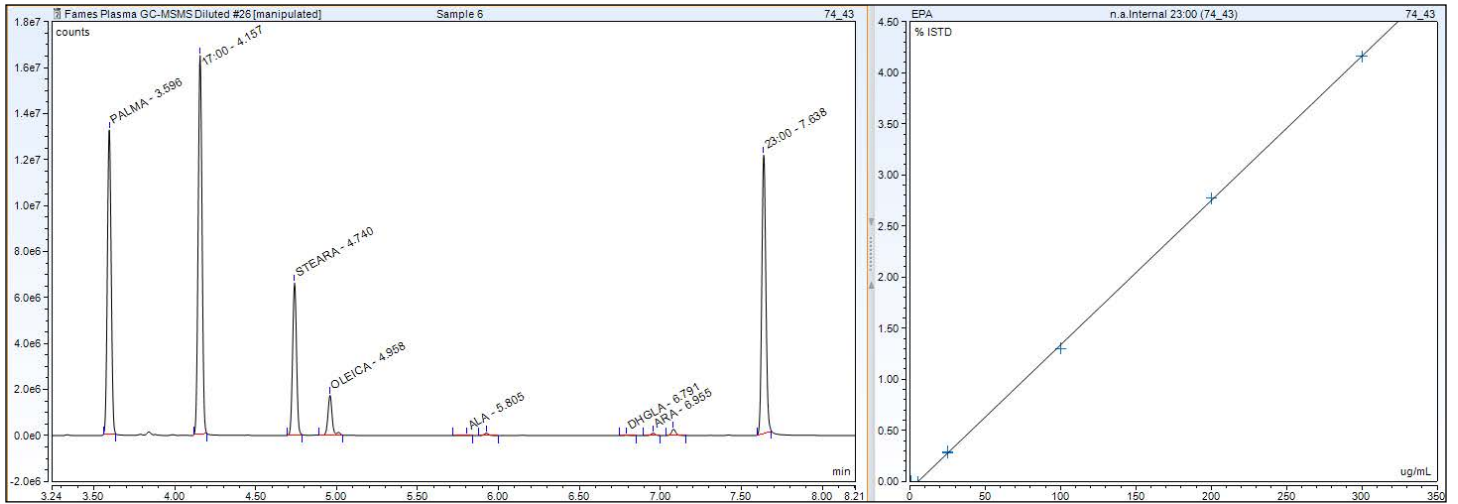


Figure 6. Chromatogram of SRM 74>43 in blood sample 6 and calibration graph for EPA.

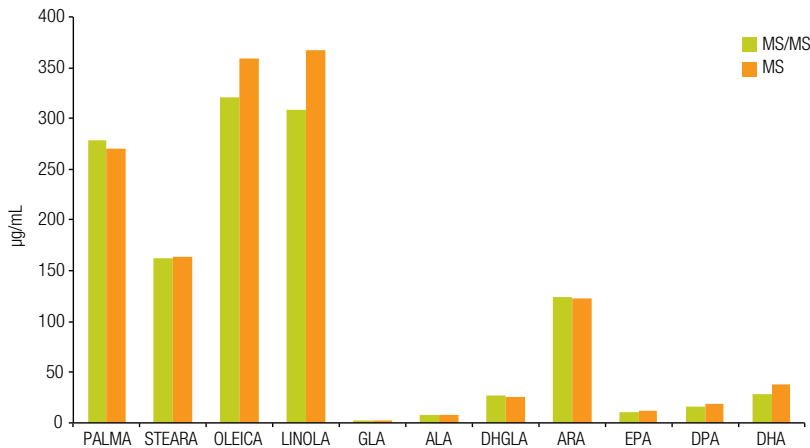


Figure 7. Comparison of FAME concentrations in blood sample 2 between GC-MS/MS and GC-MS.

Quantitative Results

The TSQ Duo provided excellent quantitative results in both single and triple quadrupole acquisition modes. The internally standardized calibration curves for both the GC-MS and the GC-MS/MS data show good linearity with $R^2 > 0.99$ over 0.2–1200 µg/mL. Figure 6 shows a typical calibration curve for EPA and the chromatogram of SRM 74>43 in blood sample 6. The concentration of FAMES in blood spot samples can vary significantly. Therefore, the analytical method needs to be capable of accurately measuring both low and high concentrations. The 100:1 split injection used in this method provides enough scope for further sensitivity when required. A summary of the duplicate injection results for the six blood samples run by GC-MS and GC-MS/MS is shown in Table 4. The duplicate injection values show excellent repeatability for both methods demonstrating that the TSQ Duo offers a robust and accurate system no matter which mode is used. As a further example, Figure 7 shows a comparison of the blood sample 2 results. Although the single quadrupole method provided good results, data processing in triple quadrupole mode is more efficient by virtue of the improved resolution of target chromatographic peaks from background noise. This additional resolution provides improved confidence in identification, increases sample throughput, and ultimately reduces the per-sample cost of analysis.

Table 4: Concentration of FAMES in the samples using GC-MS and GC-MS/MS. Results from duplicate injections.

	PALMA µg/mL	STEARA µg/mL	OLEICA µg/mL	LINOLA µg/mL	GLA µg/mL	ALA µg/mL	DHGLA µg/mL	ARA µg/mL	EPA µg/mL	DPA µg/mL	DHA µg/mL
Blood control (QC) MS/MS	218.3	113.2	184.3	67.7	< LOQ	1.7	5.0	15.6	2.7	1.0	2.8
Blood control (QC) MS	211.9	111.6	209.6	97.9	< LOQ	0.4	8.2	14.7	3.0	5.7	12.2
FAME Mix MS/MS	99.9	62.3	161.7	119.6	5.1	10.9	13.0	64.5	29.7	24.3	50.1
FAME Mix MS	95.0	62.3	120.1	127.3	4.8	9.9	9.0	61.7	28.3	26.1	54.3
Sample 1 MS/MS	214.8	118.1	231.4	209.7	< LOQ	4.4	11.3	78.7	10.6	10.0	33.2
Sample 1 MS/MS	215.3	120.5	266.2	219.8	< LOQ	4.8	11.7	76.2	11.6	10.6	34.3
Sample 1 MS	206.9	118.0	273.4	238.5	< LOQ	4.0	7.8	74.8	10.1	12.2	34.9
Sample 1 MS	205.5	119.7	275.1	244.4	< LOQ	4.2	7.7	75.6	11.1	13.8	39.8
Sample 2 MS/MS	278.5	162.8	320.9	308.8	2.1	8.1	27.0	123.3	10.4	15.8	29.0
Sample 2 MS/MS	281.4	162.9	324.3	314.7	1.8	8.5	27.5	124.7	10.8	15.9	29.7
Sample 2 MS	270.8	162.9	359.2	367.8	2.7	7.8	25.8	122.2	11.6	19.4	37.8
Sample 2 MS	270.1	160.3	357.4	369.5	2.6	7.7	27.4	123.3	11.3	19.6	38.2
Sample 3 MS/MS	190.6	123.7	213.7	228.9	1.9	5.9	15.0	128.4	14.1	12.1	43.2
Sample 3 MS/MS	193.2	127.5	258.0	235.3	2.5	6.0	15.4	125.7	13.3	13.0	44.2
Sample 3 MS	185.6	125.0	240.8	268.9	2.1	5.1	19.4	127.5	13.7	16.3	48.2
Sample 3 MS	185.5	123.9	237.8	266.6	1.3	5.7	14.6	126.1	14.2	16.7	48.5
Sample 4 MS/MS	86.0	47.5	156.8	87.1	< LOQ	4.4	1.4	14.5	15.7	3.1	8.1
Sample 4 MS/MS	86.6	47.7	154.0	89.2	< LOQ	4.7	1.6	14.5	14.4	3.4	8.5
Sample 4 MS	81.4	48.3	124.3	107.3	< LOQ	3.7	2.0	13.0	13.7	7.2	20.4
Sample 4 MS	82.7	48.0	124.3	103.9	< LOQ	4.4	1.0	13.5	12.9	7.3	20.3
Sample 5 MS/MS	220.0	122.5	248.4	194.6	1.1	7.7	12.5	56.9	12.5	6.6	17.0
Sample 5 MS/MS	222.6	121.3	166.2	197.7	1.0	7.5	14.7	57.4	12.4	7.0	15.9
Sample 5 MS	219.0	121.6	200.6	218.9	0.5	7.8	9.7	56.9	12.9	11.4	24.6
Sample 5 MS	218.4	121.2	199.5	217.6	0.8	7.0	7.9	58.0	12.1	11.4	25.4
Sample 6 MS/MS	142.9	80.8	165.2	132.1	< LOQ	2.8	6.3	49.9	17.2	5.8	11.4
Sample 6 MS/MS	148.0	83.1	175.5	144.6	< LOQ	2.8	6.4	48.6	14.3	6.2	11.5
Sample 6 MS	134.2	77.8	135.6	139.9	0.5	2.7	3.8	49.9	14.6	10.3	23.5
Sample 6 MS	130.5	76.2	131.5	140.8	0.5	2.7	3.9	48.8	15.0	10.6	23.0

Conclusion

The results of this evaluation demonstrate that the TRACE TR-FAME column and the TSQ Duo GC-MS/MS system, in combination with the Chromeleon CDS software, make for an extremely effective tool for the routine analysis of FAMES in blood spot samples using either GC-MS or GC-MS/MS.

- The TSQ Duo is a robust and accurate GC-MS system for the analysis of FAMES. The ability to upgrade a traditional single quadrupole method, such as FAMES analysis, to triple quadrupole has been demonstrated. Moving to a triple quadrupole method when the time is right is a simple process for any laboratory.
- The TSQ Duo and AutoSRM enable fast and reliable upgrading from GC-MS to GC-MS/MS on a single MS platform. The additional selectivity of GC-MS/MS provides improved resolution from matrix interferences.
- Increased productivity can be achieved using the selectivity that triple quadrupole brings by speeding up data processing and review. Ultimately this increased speed improves sample analysis turnaround times for this competitive application. Fast analysis was achieved in less than 15 minutes for all 11 FAMES with each showing good reproducibility, linearity, and sensitivity. The results of the analysis of six blood spot samples show excellent agreement using either single or triple quadrupole MS.
- Chromeleon CDS software offers excellent processing options for data reviewing and reporting.

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