Supporting Information

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An HPLC/CAD method for determination of fatty acids in metered dose inhalation products: a candidate leachable test method

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S1: The Interaction Between the Packaging System and The Dosage Form

Table S1: The interaction between the packaging system and the dosage form

Degree of concern associated with the route of administration	**Likelihood of packaging component-dosage form interaction**		
	High	Medium	Low
Highest	Inhalation Aerosols and Sprays	Injections and Injectable Suspensions; Inhalation Solutions	Sterile Powders and Powders for Injection; Inhalation Powders
High	High Transdermal Ointments and Patches	Ophthalmic Solutions and Suspensions; Nasal Aerosols and Sprays	-
Low	Low Topical Solutions and Suspensions; Topical and Lingual Aerosols; Oral Solutions and Suspensions	-	Oral Tablets and Oral (Hard and Soft Gelatin) Capsules; Topical Powders; Oral Powders

S2: Preparation of Mobile Phase

In order to determine the appropriate mobile phase to be used in the method, 1000.0 mL of purified water was taken for mobile phase A and 1000.0 mL of acetonitrile was taken for mobile phase B, and 0.1-1.0 mL (0.1 mL increments) of formic acid solution was added. As a result of the evaluation, 0.5 mL formic acid was found suitable for both phases. The applied gradient program is summarized in the Table 2.

Mobile Phase A: 0.5 mL of formic acid into the 1000.0 mL of pure water.

Mobile Phase B: 0.5 mL of formic acid into the 1000.0 mL of acetonitrile.

Table S2: Gradient program

Time (minute)	Mobile phase A (%)	Mobile phase B (%)
0.0	37.0	63.0
28.0	15.0	85.0
40.0	15.0	85.0
40.01	37.0	63.0
45.0	37.0	63.0

S3 : Calculation

The following equations were used to calculate amount of sample.

$$\frac{Asmp}{Astd} \times \frac{Wstd}{100.0} \times \frac{2.5}{100.0} \times \frac{P}{100} \times \frac{10.0}{Wsmp} \times \frac{1}{L} \times 100 \times \mathrm{CF} = mcg/canister$$

Asmp : Impurity peak area obtained from the sample solution
Astd : Stearic acid peak area obtained from standard solution
Wstd : Standard weight (mg)
P : Standard potens (%)
L : Label value (µg/ canister)
CF : Correction factor

S4 : Validation Parameters

S4.1. Specificity

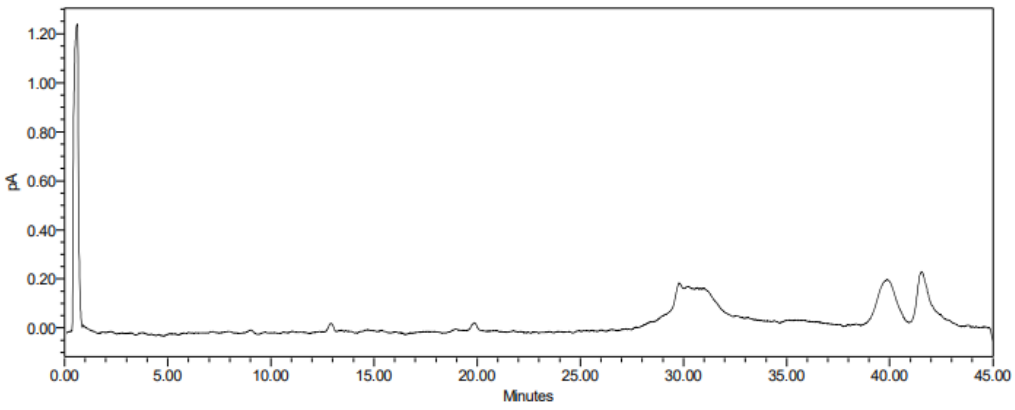

Figure S1:. Blank chromatograph

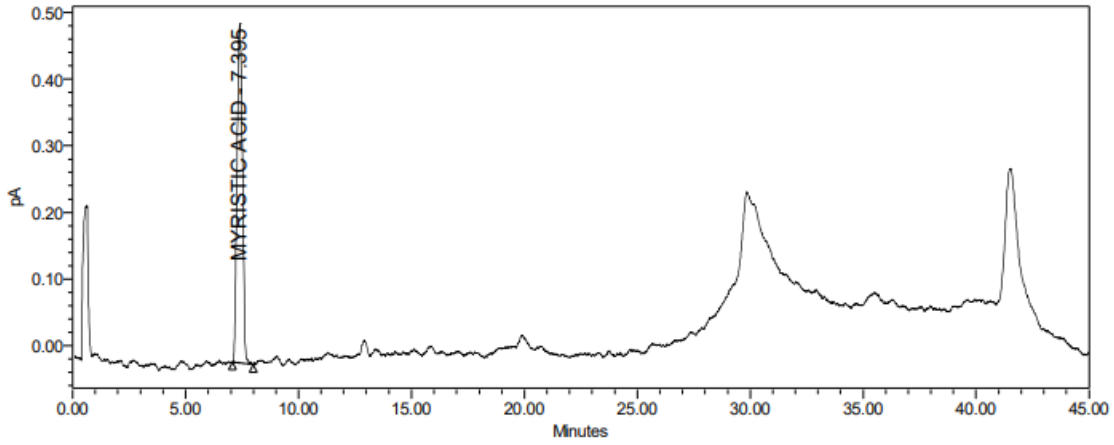

Figure S2: Chromatograph of myristic acid

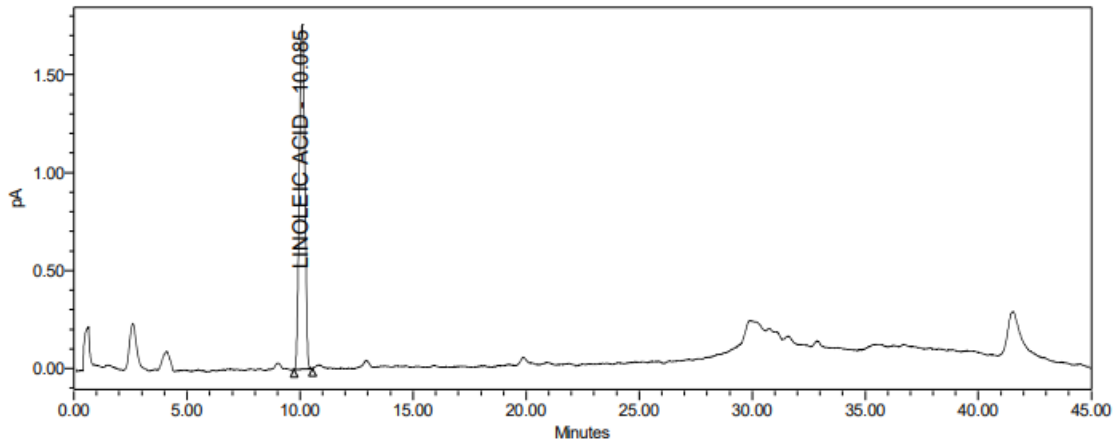

Figure S3: Chromatograph of linoleic acid

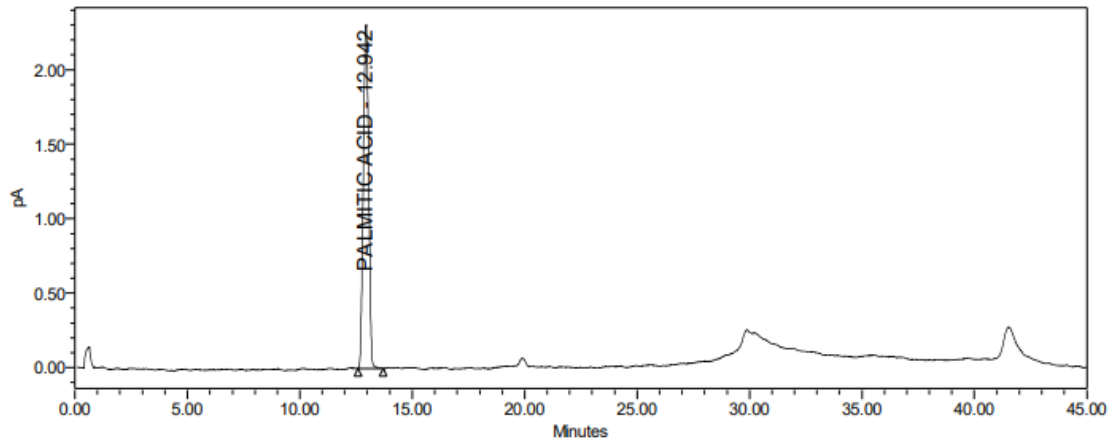

Figure S4: Chromatograph of palmitic acid

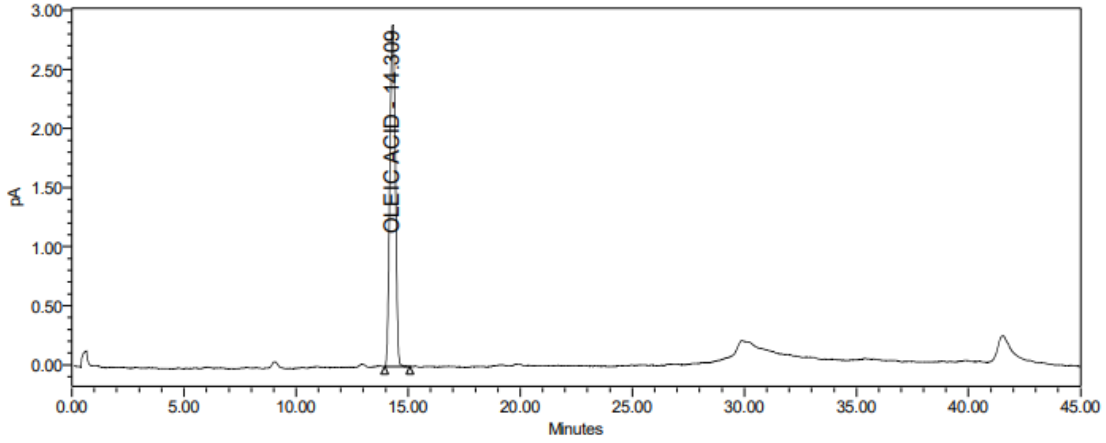

Figure S5: Chromatograph of oleic acid

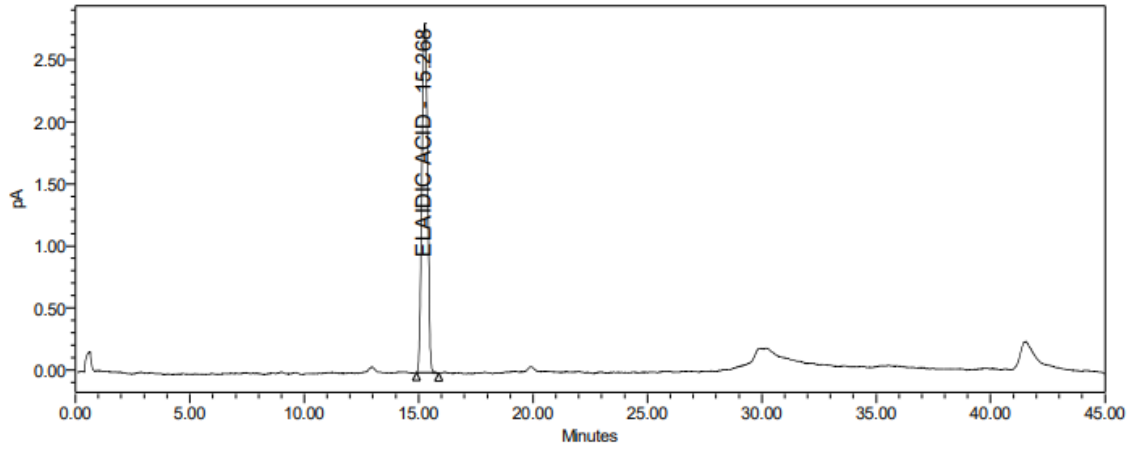

Figure S6: Chromatograph of elaidic acid

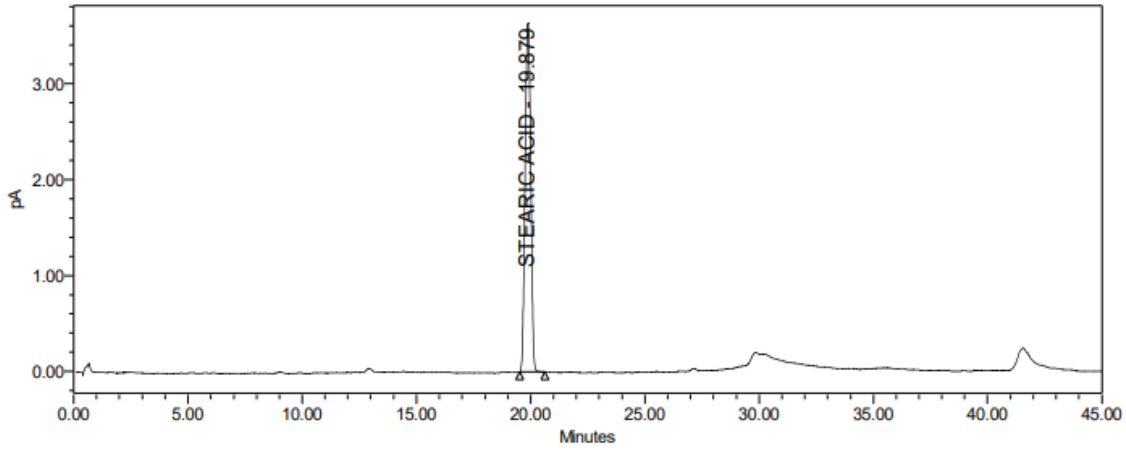

Figure S7: Chromatograph of stearic acid

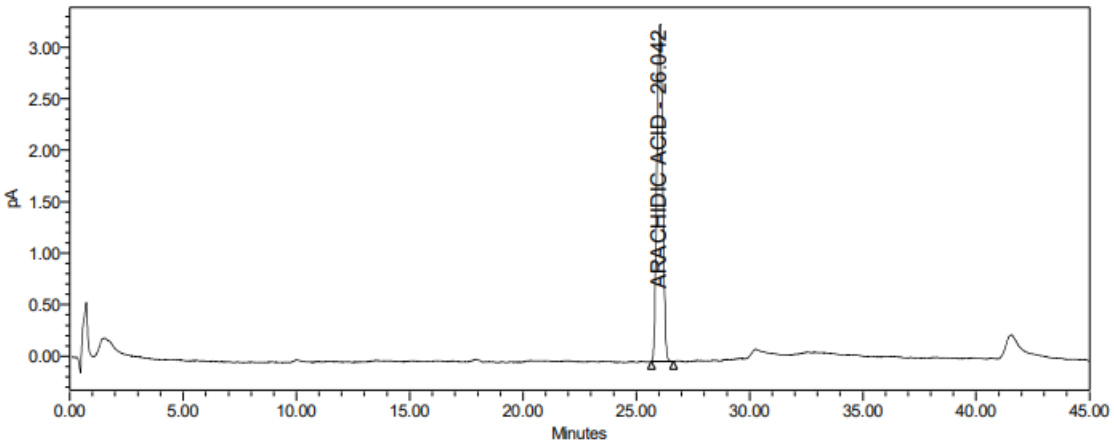

Figure S8: Chromatograph of arachidic acid

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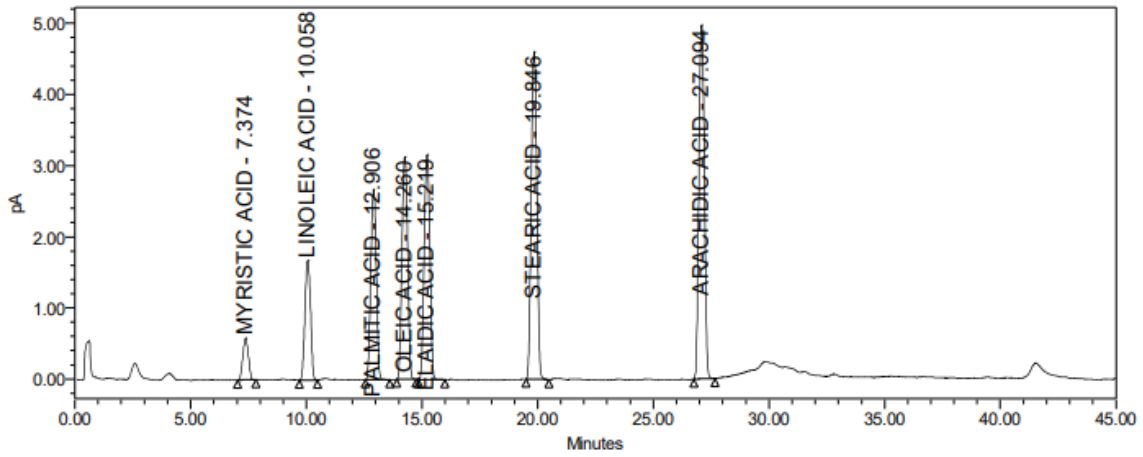

Figure S9: Chromatograph of standard mixture

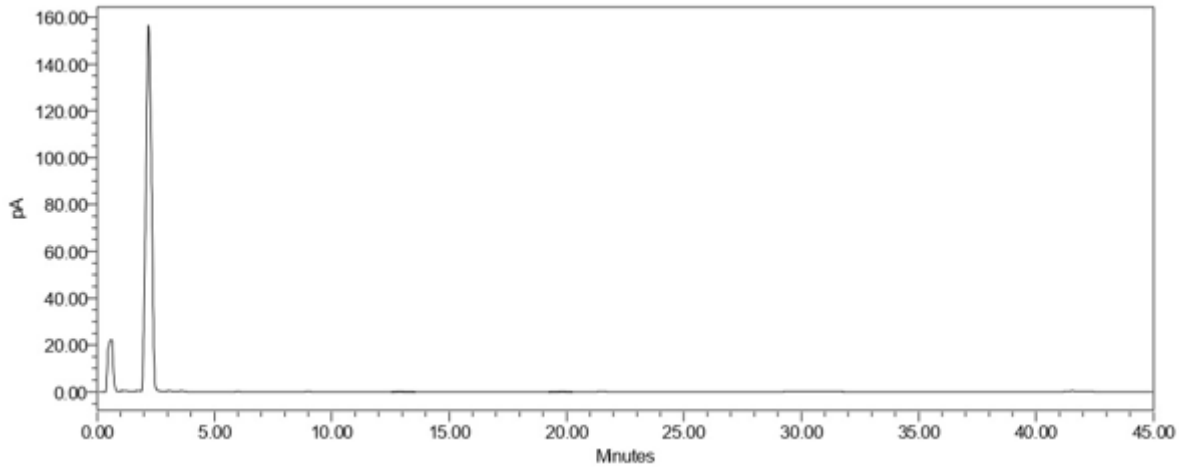

Figure S10: Chromatograph of unspike sample

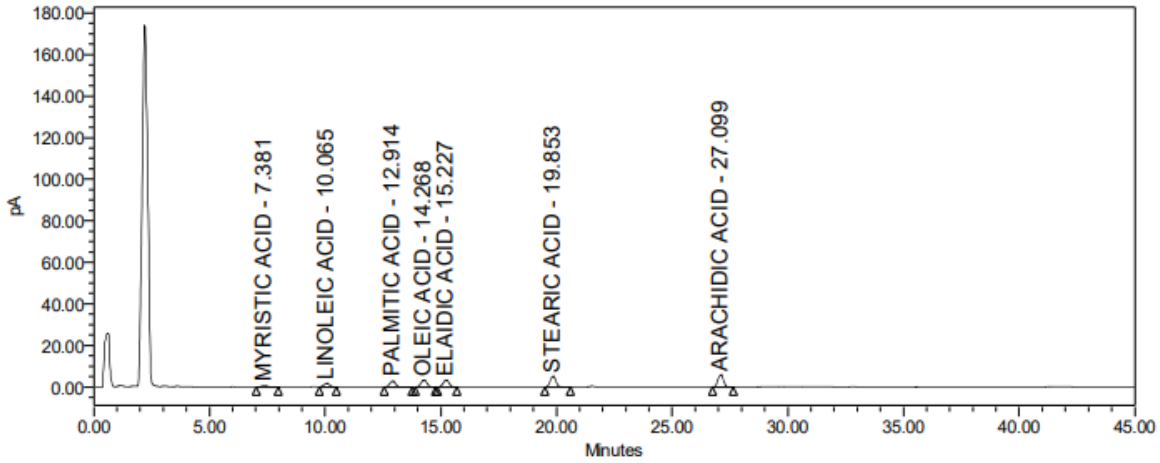

Figure S11: Chromatograph of spike sample

S4.2. AccuracyRresults

Table S3 : Accuracy results for myristic acid

Level (%)	Experimental Value (µg/mL)	Theoretical Value (µg/mL)	Recovery (%)	Average (%)	SD	RSD (%)
	0.000331	0.000328	99.1			
LQ	0.000327	0.000331	101.2	99.8	1.2	1.2
	0.000332	0.000329	99.1			
	0.007516	0.007536	100.3			
100	0.007430	0.007551	101.6	100.3	1.3	1.3
	0.007556	0.007481	99.0			
	0.011274	0.011299	100.2			
150	0.011145	0.011569	103.8	101.1	2.4	2.4
	0.011334	0.011251	99.3			
		Average	100.4			
		SD	1.6			
		% RSD	1.6			
		95% CI	1.2			

Table S4 : Accuracy results for linoleic acid

Level (%)	Experimental Value (µg/mL)	Theoretical Value (µg/mL)	Recovery (%)	Average (%)	SD	RSD (%)
	0.000343	0.000352	102.6			
LQ	0.000343	0.000359	104.7	103.5	1.1	1.1
	0.000344	0.000355	103.2			
	0.007453	0.007515	100.8			
100	0.007454	0.007610	102.1	101.4	0.7	0.7
	0.007470	0.007574	101.4			
	0.011179	0.011054	98.9			
150	0.011179	0.011021	98.6	100.3	2.6	2.6
	0.011179	0.011544	103.3			
		Average	101.7			
		SD	2.0			
		% RSD	2.0			
		95% CI	1.5			

Table S5 : Accuracy results for palmitic acid

Level (%)	Experimental Value (µg/mL)	Theoretical Value (µg/mL)	Recovery (%)	Average (%)	SD	RSD (%)
	0.000121	0.000120	99.2			
LQ	0.000121	0.000120	99.2	99.5	0.5	0.5
	0.000120	0.000120	100.0			
	0.007536	0.007596	100.8			
100	0.007536	0.007499	99.5	100.0	0.7	0.7
	0.007536	0.007515	99.7			
	0.011304	0.011256	99.6			
150	0.011304	0.011245	99.5	100.7	2.1	2.1
	0.011304	0.011652	103.1			
		Average	100.1			
		SD	1.2			
		% RSD	1.2			
		%95	0.9			
		95% CI				

Table S6 : Accuracy results for oleic acid

Level (%)	Experimental Value (µg/mL)	Theoretical Value (µg/mL)	Recovery (%)	Average (%)	SD	RSD (%)
	0.000751	0.000781	104.0			
LQ	0.000753	0.000784	104.1	104.4	0.6	0.6
	0.000745	0.000783	105.1			
	0.007512	0.007572	100.8			
100	0.007525	0.007612	101.2	101.3	0.5	0.5
	0.007451	0.007588	101.8			
	0.011269	0.011263	99.9			
150	0.011269	0.011314	100.4	100.3	0.4	0.4
	0.011269	0.011333	100.6			
		Average	102.0			
		SD	1.9			
		% RSD	1.9			
		95% CI	1.5			

Table S7 : Accuracy results for elaidic acid

Level (%)	Experimental Value (µg/mL)	Theoretical Value (µg/mL)	Recovery (%)	Average (%)	SD	RSD (%)
	0.000733	0.000741	101.1			
LQ	0.000731	0.000749	102.5	101.7	0.7	0.7
	0.000744	0.000755	101.5			
	0.007330	0.007415	101.2			
100	0.007330	0.007503	102.4	102.3	1.0	1.0
	0.007330	0.007562	103.2			
	0.010995	0.011263	102.4			
150	0.010995	0.011412	103.8	104.0	1.7	1.6
	0.010995	0.011632	105.8			
		Average	102.7			
		SD	1.5			
		% RSD	1.5			
		95% CI	1.2			

Table S8 : Accuracy results for arachidic acid

Level (%)	Experimental Value (µg/mL)	Theoretical Value (µg/mL)	Recovery (%)	Average (%)	SD	RSD (%)
	0.000735	0.000751	102.2			
LQ	0.000737	0.000766	103.9	103.1	0.9	0.9
	0.000737	0.000761	103.3			
	0.007354	0.007415	100.8			
100	0.007354	0.007462	101.5	101.5	0.7	0.7
	0.007354	0.007510	102.1			
	0.011031	0.011290	102.3			
150	0.011031	0.011312	102.5	102.5	0.2	0.2
	0.011031	0.011333	102.7			
		Average	102.4			
		SD	0.9			
		% RSD	0.9			
		95% CI	0.7			

S4.3. Repeatability Results

Table S9 : Intermediate repeatability results

	C14:0	C18:2$^{\Delta 9, 12}$	C16:0	C18:1 n9	C18:1 n9t	C18:0	C20:0
1	81.917	75.868	79.464	76.809	75.402	79.371	76.962
2	75.734	75.340	81.171	76.427	74.273	78.454	79.416
3	75.457	73.254	75.447	71.807	70.349	75.690	72.608
4	75.933	71.712	74.810	71.756	70.463	74.319	73.547
5	80.113	76.023	80.483	76.858	74.994	79.705	75.065
6	80.226	75.812	80.829	77.022	75.391	79.092	74.580
Average	78.230	74.668	78.701	75.113	73.5	77.8	75.4
SD	2.840	1.776	2.833	2.588	2.415	2.225	2.473
% RSD	3.6	2.4	3.6	3.4	3.3	2.9	3.3
95% CI	2.981	1.864	2.974	2.716	2.535	2.335	2.596
Absolute Difference	9.3	4.5	2.1	4.4	3.8	2.8	3.1

S4.4. Linearity Results

Table S10 : Linearity results for myristic acid

Standard No	Level	Cstandard µg/mL	Astandard	Average Astandard (Y1)	Calculated Area (Y2)	Difference Area (Y1-Y2)	Residual of Squares $(Y1-Y2)^2$
1	LOQ	0.0003304	675633 681414 648611 679544 665122 697369	674616	1073368	39752	159003157504
2	50%	0.0037610	5567639 5759085 5824541	5717088	5404766	312322	97545031684
3	80%	0.0060176	8445116 8842597 8463622	8583778	8253898	329880	108820814400
4	100%	0.0075220	10343010 10787787 10617506 10261196 10267666 10449998	10454527	10153319	301208	90726259264
6	120%	0.0090264	11571383 11636340 11818203	11675309	12052741	377432	142454914624
7	150%	0.0112830	14900219 14935456 14989248 14729228 14330646 14523086	14734647	14901879	167226	27964535076

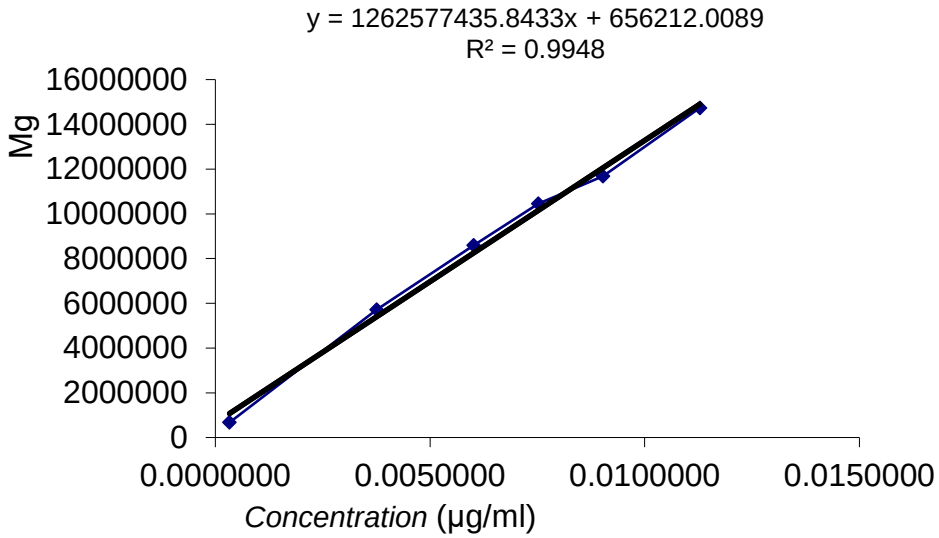

Figure S12: Linearity graphic for myristic acid

Table S11 : Linearity results for linoleic acid

Standard No	Level	Cstandard µg/mL	Astandard	Average Astandard (Y1)	Calculated Area (Y2)	Difference Area (Y1-Y2)	Residual of Squares $(Y1-Y2)^2$
1	LOQ	0.0003560	1186184 1281414 872203 1167083 980198 1162788	1108312	2195435	1087123	1181836417129
2	50%	0.0038612	11799368 11842762 11775415	11805848	11169489	636359	404952776881
3	80%	0.0061779	17748560 17839174 17878163	17821966	17100732	721234	520178482756
4	100%	0.0077224	22488110 21832437 21878132 22074044 22015078 21701802	21998267	21054979	943288	889792250944
6	120%	0.0092669	24801874 24883211 25019591	24901559	25009229	107667	11592182889
7	150%	0.0115836	29301794 29933192 29996724 29886760 29859459 30028339	29834378	30910469	1106091	1223437300281

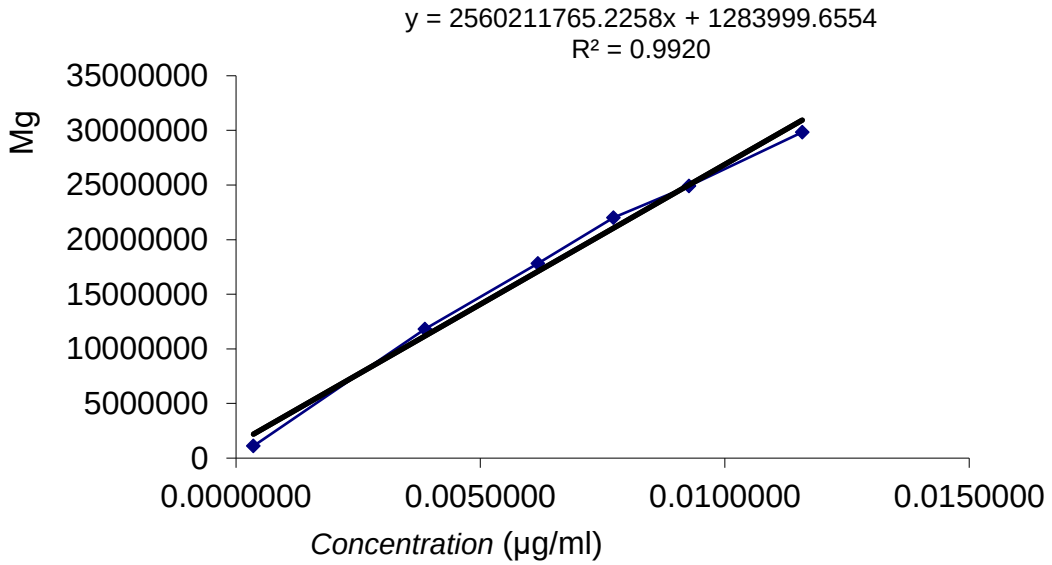

Figure S13: Linearity graphic for linoleic acid

Table S12 : Linearity results for palmitic acid

Standard No	Level	Cstandard µg/mL	Astandard	Average Astandard (Y1)	Calculated Area (Y2)	Difference Area (Y1-Y2)	Residual of Squares $(Y1-Y2)^2$
			1628545				
			1363729				
1	LOQ	0.0001203	1548971	1542605	1417581	125024	15631000576
			1493821				
			1588301				
			1632265				
			26957404				
2	50%	0.0037550	26389675	26481217	26209179	272038	74004673444
			26096571				
			41086411				
3	80%	0.0060080	41616511	41472691	41576464	103773	10768835529
			41715152				
			52184335				
			52898343				
4	100%	0.0075100	53427524	53048934	51821320	1227614	1507036132996
			53796877				
			53674324				
			52312201				
			58193103				
6	120%	0.0090120	59376830	58737054	62066177	3329123	11083059949129
			58641229				
			78177132				
			79400563				
7	150%	0.0112650	79895328	79241682	77433462	1808220	3269659568400
			79759205				
			79104755				
			79113109				

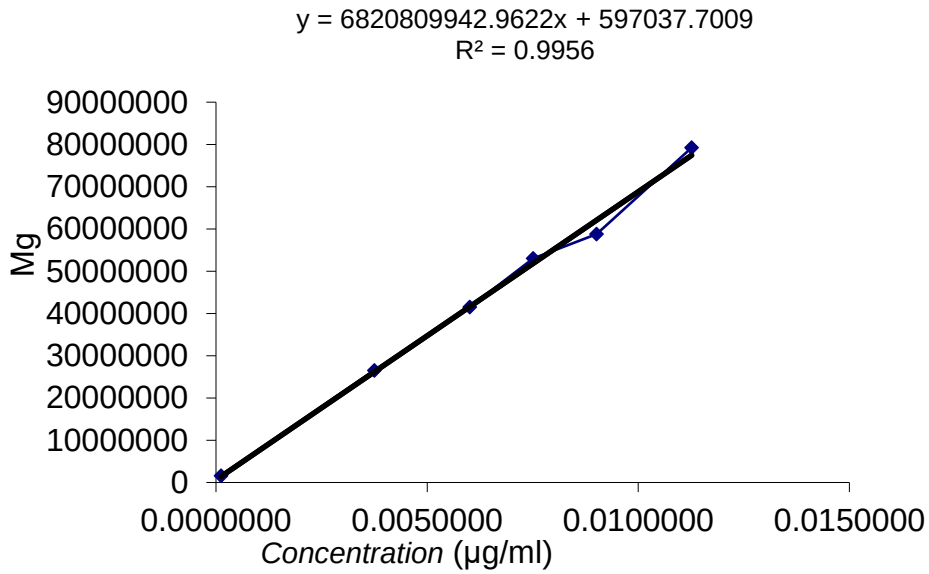

Figure S14: Linearity graphic for palmitic acid

Table S13 : Linearity results for oleic acid

Standard No	Level	Cstandard µg/mL	Astandard	Average Astandard (Y1)	Calculated Area (Y2)	Difference Area (Y1-Y2)	Residual of Squares $(Y1-Y2)^2$
1	LOQ	0.0007840	1133048 1363729 1440269 1319854 1408024 1274458	1323230	2214495	891265	794353300225
2	50%	0.0039700	25006845 24838947 24047454	24631082	24866015	234933	55193514489
3	80%	0.0063520	42227126 42008635 42928075	42387945	41801332	586613	344114811769
4	100%	0.0079400	55935785 53091501 56386072 53616290 56351012 54188089	54928125	53091544	1836581	3373029769561
6	120%	0.0095280	64475515 64858922 65359895	64898111	64381756	516355	266622486025
7	150%	0.0119100	79953299 79715396 78431241 81072685 78466433 79383279	79503722	81317073	1813351	3288241849201

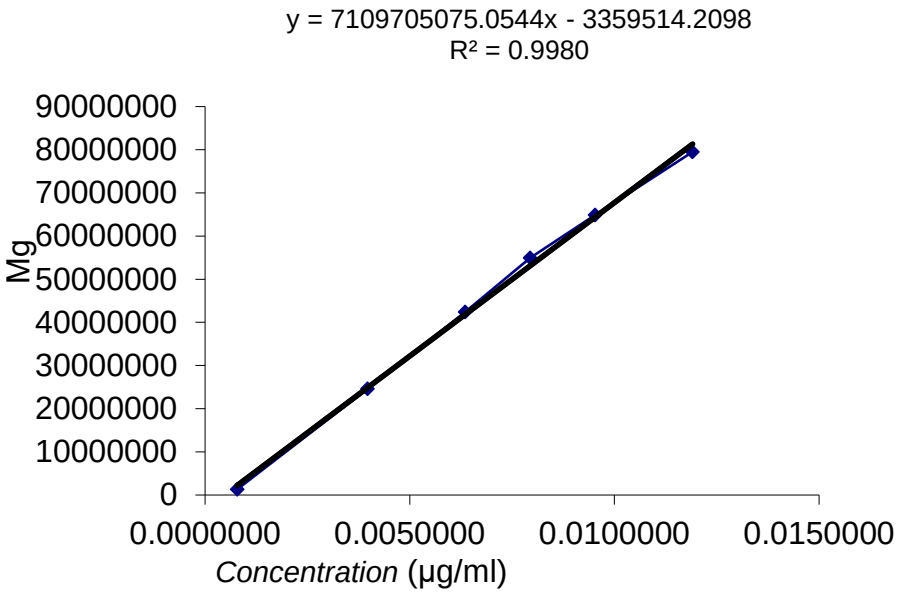

Figure S15: Linearity graphic for oleic acid

Table S14 : Linearity results for elaidic acid

Standard No	Level	Cstandard µg/mL	Astandard	Average Astandard (Y1)	Calculated Area (Y2)	Difference Area (Y1-Y2)	Residual of Squares $(Y1-Y2)^2$
1	LOQ	0.0007494	1044437 1168025 1062827 922207 1169963 1197526	1094164	3850073	2755909	7595034416281
2	50%	0.0035644	28072977 28524726 27196923	27931542	25950552	1980990	3924321380100
3	80%	0.0057030	44492581 45355580 44776577	44874913	42740635	2134278	4555142581284
4	100%	0.0071288	55533229 55936389 55410339 55875528 56304105 55559594	55769864	53934547	1835317	3368388490489
6	120%	0.0085546	63120392 63276102 63503776	63300090	65128459	1828369	3342933200161
7	150%	0.0106932	78009028 79532049 81000564 80510277 81392095 82869394	80552235	81918542	1366307	1866794818249

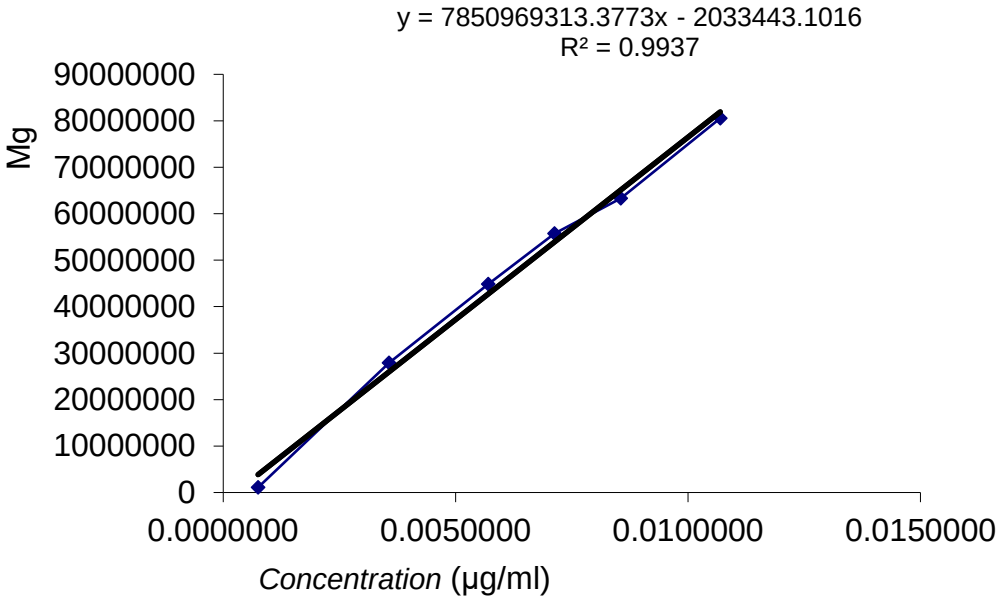

Figure S16: Linearity graphic for elaidic acid

Table S15 : Linearity results for stearic acid

Standard No	Level	Cstandard µg/mL	Astandard	Average Astandard (Y1)	Calculated Area (Y2)	Difference Area (Y1-Y2)	Residual of Squares $(Y1-Y2)^2$
1	LOQ	0.0008259	1029871 1051463 1038164 916825 1115018 1028973	1030052	5503868	4473816	20015029601856
2	50%	0.0037410	39485598 39108731 38614644	39069658	35953681	3115977	9709312664529
3	80%	0.0059856	61949769 61770633 61730857	61817086	59399755	2417331	5843489162561
4	100%	0.0074820	78702228 78632326 78152813 78816638 78572844 78672821	78591612	75030471	3561141	12681725221881
6	120%	0.0089784	89581471 89173427 89510686	89421861	90661187	1236326	1535928934276
7	150%	0.0112230	110126356 109570086 110393434 110480788 111545288 112239772	110725954	114107261	3381307	11433237028249

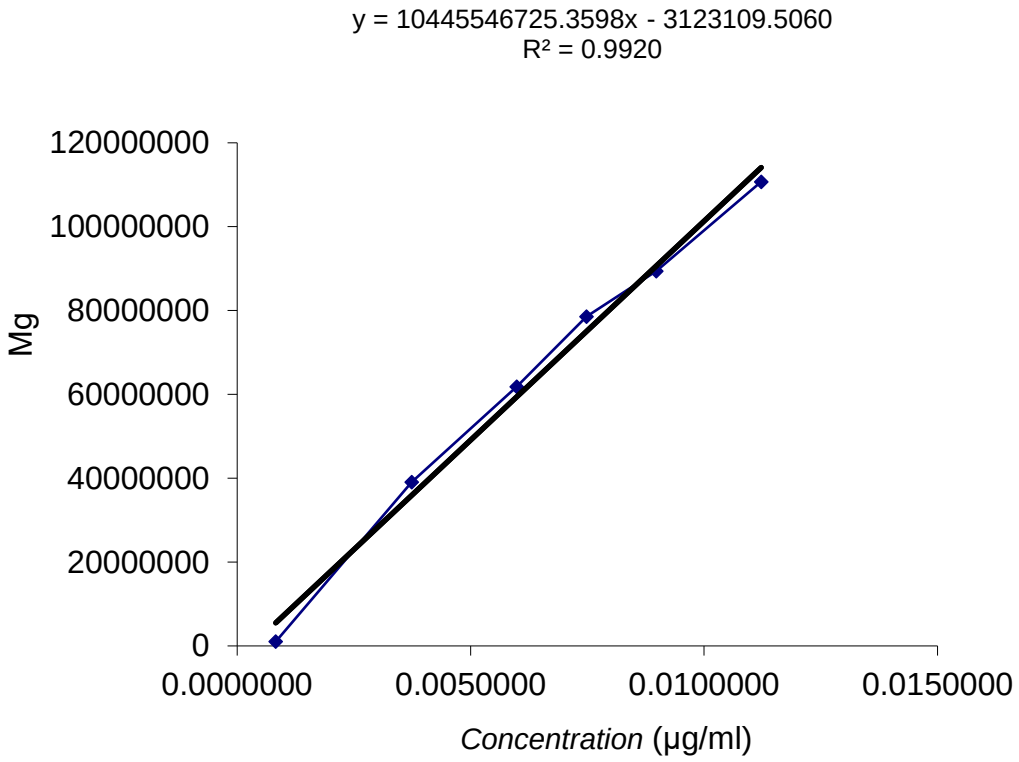

Figure S17: Linearity graphic for stearic acid

Table S16 : Linearity results for arachidic acid

Standard No	Level	Cstandard µg/mL	Astandard	Average Astandard (Y1)	Calculated Area (Y2)	Difference Area (Y1-Y2)	Residual of Squares $(Y1-Y2)^2$
1	LOQ	0.0007492	995044 1131110 1251520 1051459 1135631 1161781	1121091	2788961	1667870	2781790336900
2	50%	0.0036927	37613617 37637812 37350018	37533816	34465564	3068252	9414170335504
3	80%	0.0059082	58083826 57310204 57459210	57617747	57537265	80483	6477513289
4	100%	0.0073853	68892914 68160596 68610434 68288436 68125078 68018969	68349405	72919440	4570035	20885219901225
6	120%	0.0088624	89983567 89860895 90998548	90281003	88301615	1979388	3917976854544
7	150%	0.0110780	111418944 111379438 112289960 111043858 112072036 112842672	111841151	111374357	466794	217896638436

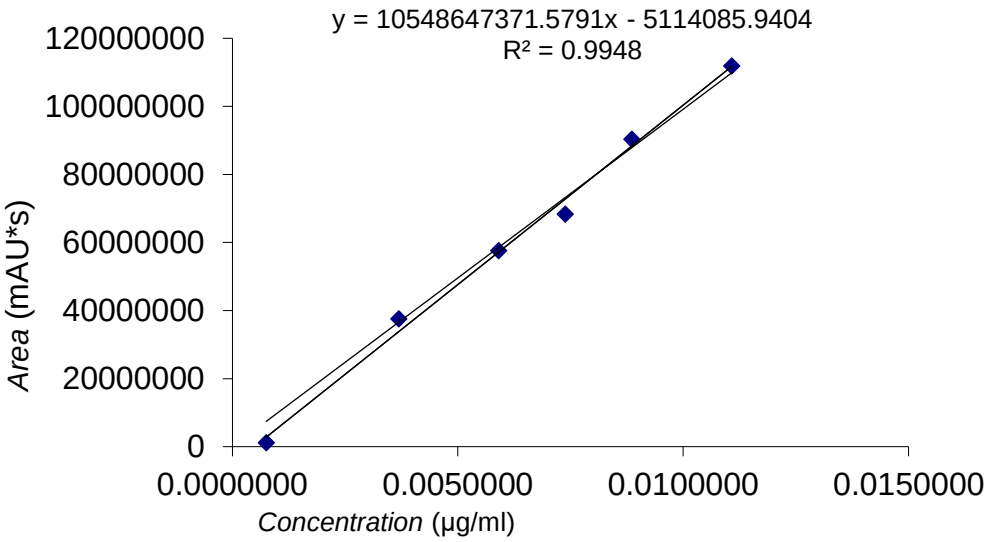

Figure S18: Linearity graphic for arachidic acid