
SUPPLEMENTARY INFORMATION TO SUPPORT PAPER: "HIGHLIGHTING EXTRACTION AND DERIVATIZATION METHOD COMPARISONS FOR OPTIMAL SAMPLE PREPARATION OF
NANNOCHLOROPSIS SP. ALGAL OILS PRIOR TO FAME DETERMINATION"

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1. PEAK AREA AND PEAK NUMBER RESULTS FOR OPTIMISATION OF METHODS

Table 1: Data obtained for optimisation of parameters for conventional extraction method

Parameter	Setting	Peak area (1)	Peak area (2)	Peak area (3)	Peak number (1)	Peak number (2)	Peak number (3)	Average peak area	Standard deviation peak area	RSD % peak area	Average peak number	Standard deviation peak number	RSD % peak number
Extracting solvent	Acetic acid	2.8E+08	2.8E+08	2.8E+08	1.2E+01	1.2E+01	1.2E+01	2.8E+08	1.8E+06	6.5E-03	1.2E+01	0.0E+00	0.0E+00
	Acetone	7.6E+08	7.6E+08	7.6E+08	1.4E+01	1.4E+01	1.4E+01	7.6E+08	1.8E+05	2.4E-04	1.4E+01	0.0E+00	0.0E+00
	Ethanol	6.8E+08	6.9E+08	6.8E+08	1.3E+01	1.3E+01	1.3E+01	6.8E+08	4.9E+06	7.2E-03	1.3E+01	0.0E+00	0.0E+00
	IPA	5.5E+08	5.5E+08	5.4E+08	1.3E+01	1.3E+01	1.3E+01	5.4E+08	1.2E+06	2.2E-03	1.3E+01	0.0E+00	0.0E+00
	Methanol	3.2E+07	3.2E+07	3.2E+07	1.3E+01	1.3E+01	1.3E+01	3.2E+07	4.7E+03	1.5E-04	1.3E+01	0.0E+00	0.0E+00
Fractionation solvent	Chloroform	1.7E+07	1.7E+07	1.7E+07	1.0E+01	1.0E+01	1.0E+01	1.7E+07	1.2E+04	6.8E-04	1.0E+01	0.0E+00	0.0E+00
	Cyclohexane	1.5E+07	1.9E+07	1.4E+07	7.0E+00	8.0E+00	7.0E+00	1.6E+07	2.6E+06	1.6E-01	7.3E+00	5.8E-01	7.9E-02
	Hexane	1.1E+09	1.1E+09	1.1E+09	1.4E+01	1.4E+01	1.4E+01	1.1E+09	4.3E+06	4.0E-03	1.4E+01	0.0E+00	0.0E+00
	Toluene	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00

Table 2: Data obtained for optimisation of parameters for conventional derivatisation method

Parameter	Setting	Peak area (1)	Peak area (2)	Peak area (3)	Peak number (1)	Peak number (2)	Peak number (3)	Average peak area	Standard deviation peak area	RSD % peak area	Average peak number	Standard deviation peak number	RSD % peak number
Hydrolysis reagent	H ₂ SO ₄	3.3E+08	3.1E+08	3.1E+08	1.2E+01	1.2E+01	1.2E+01	3.2E+08	1.5E+07	4.6E-02	1.2E+01	0.0E+00	0.0E+00
	HCl	1.5E+09	1.5E+09	1.5E+09	1.0E+01	1.0E+01	1.0E+01	1.5E+09	2.1E+05	1.4E-04	1.0E+01	0.0E+00	0.0E+00
	KOH	1.1E+08	1.1E+08	1.1E+08	1.4E+01	1.4E+01	1.4E+01	1.1E+08	1.5E+05	1.4E-03	1.4E+01	0.0E+00	0.0E+00
	Na ₂ CO ₃	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	#DIV/0!	0.0E+00	0.0E+00	#DIV/0!
	NaOH	1.7E+09	1.7E+09	1.7E+09	1.4E+01	1.4E+01	1.4E+01	1.7E+09	5.3E+06	3.2E-03	1.4E+01	0.0E+00	0.0E+00
Hydrolysis reagent volume (NaOH)	0.5 mL	5.5E+08	5.5E+08	5.6E+08	1.4E+01	1.4E+01	1.4E+01	5.5E+08	3.9E+05	7.1E-04	1.4E+01	0.0E+00	0.0E+00
	1 mL	5.0E+08	5.0E+08	5.0E+08	1.4E+01	1.4E+01	1.4E+01	5.0E+08	7.4E+05	1.5E-03	1.4E+01	0.0E+00	0.0E+00
	1.5 mL	1.4E+08	1.4E+08	1.5E+08	1.4E+01	1.4E+01	1.4E+01	1.4E+08	1.2E+06	8.0E-03	1.4E+01	0.0E+00	0.0E+00
	2 mL	1.2E+08	1.2E+08	1.2E+08	1.4E+01	1.4E+01	1.4E+01	1.2E+08	3.4E+06	2.8E-02	1.4E+01	0.0E+00	0.0E+00
Transesterification volume	1 mL	1.0E+09	1.0E+09	1.0E+09	1.3E+01	1.3E+01	1.3E+01	1.0E+09	4.3E+04	4.1E-05	1.3E+01	0.0E+00	0.0E+00
	2 mL	1.6E+09	1.6E+09	1.6E+09	1.4E+01	1.4E+01	1.4E+01	1.6E+09	2.5E+07	1.5E-02	1.4E+01	0.0E+00	0.0E+00
	3 mL	3.3E+08	0.0E+00	3.0E+08	9.0E+00	0.0E+00	9.0E+00	2.1E+08	1.8E+08	8.7E-01	6.0E+00	5.2E+00	8.7E-01
Hydrolysis reaction time	15 min	8.1E+07	8.1E+07	8.2E+07	1.2E+01	1.2E+01	1.2E+01	8.1E+07	1.0E+05	1.2E-03	1.2E+01	0.0E+00	0.0E+00
	30 min	2.3E+08	2.1E+08	2.1E+08	1.4E+01	1.4E+01	1.4E+01	2.1E+08	1.1E+07	5.2E-02	1.4E+01	0.0E+00	0.0E+00
	45 min	2.3E+08	2.3E+08	2.3E+08	1.4E+01	1.4E+01	1.4E+01	2.3E+08	2.0E+06	8.5E-03	1.4E+01	0.0E+00	0.0E+00
	60 min	1.8E+08	1.8E+08	1.8E+08	1.4E+01	1.4E+01	1.4E+01	1.8E+08	1.0E+06	5.6E-03	1.4E+01	0.0E+00	0.0E+00
	75 min	1.6E+08	1.6E+08	1.6E+08	1.4E+01	1.4E+01	1.4E+01	1.6E+08	1.5E+05	9.4E-04	1.4E+01	0.0E+00	0.0E+00
	90 min	5.7E+07	5.6E+07	5.9E+07	1.2E+01	1.2E+01	1.2E+01	5.8E+07	1.6E+06	2.8E-02	1.2E+01	0.0E+00	0.0E+00
Transesterification reaction time	10 min	2.9E+08	2.9E+08	2.9E+08	1.4E+01	1.4E+01	1.4E+01	2.9E+08	2.4E+05	8.2E-04	1.4E+01	0.0E+00	0.0E+00
	20 min	2.1E+08	2.1E+08	2.1E+08	1.4E+01	1.4E+01	1.4E+01	2.1E+08	1.9E+05	8.6E-04	1.4E+01	0.0E+00	0.0E+00
	30 min	2.4E+08	2.4E+08	2.4E+08	1.4E+01	1.4E+01	1.4E+01	2.4E+08	5.2E+05	2.1E-03	1.4E+01	0.0E+00	0.0E+00

40 min	1.8E+08	1.8E+08	1.8E+08	1.4E+01	1.4E+01	1.4E+01	1.8E+08	5.2E+05	2.9E-03	1.4E+01	0.0E+00	0.0E+00
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Table 3: Data obtained for optimisation of parameters for MAED method

Parameter	Setting	Peak area (1)	Peak area (2)	Peak area (3)	Peak number (1)	Peak number (2)	Peak number (3)	Average peak area	Standard deviation peak area	RSD % peak area	Average peak number	Standard deviation peak number	RSD % peak number
Extraction solvent	Acetone	1.8E+08	1.8E+08	1.8E+08	1.4E+01	1.4E+01	1.4E+01	1.8E+08	6.1E+05	3.4E-03	1.4E+01	0.0E+00	0.0E+00
	Methanol	1.5E+08	1.5E+08	1.5E+08	1.4E+01	1.4E+01	1.4E+01	1.5E+08	5.8E+05	3.9E-03	1.4E+01	0.0E+00	0.0E+00
	Acetic acid	3.8E+08	3.8E+08	3.8E+08	1.4E+01	1.4E+01	1.4E+01	3.8E+08	1.3E+06	3.6E-03	1.4E+01	0.0E+00	0.0E+00
	Ethanol	1.8E+07	1.9E+07	1.8E+07	6.0E+00	6.0E+00	6.0E+00	1.8E+07	8.0E+04	4.3E-03	6.0E+00	0.0E+00	0.0E+00
Fractionation solvent	Chlorform	2.6E+08	2.6E+08	2.6E+08	1.2E+01	1.2E+01	1.2E+01	2.6E+08	4.8E+05	1.8E-03	1.2E+01	0.0E+00	0.0E+00
	Cyclohexane	1.5E+07	1.5E+07	1.5E+07	7.0E+00	7.0E+00	7.0E+00	1.5E+07	1.3E+04	8.6E-04	7.0E+00	0.0E+00	0.0E+00
	Hexane	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
	Toluene	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Reaction time	10 min	2.4E+07	2.4E+07	2.4E+07	1.0E+01	1.0E+01	1.0E+01	2.4E+07	5.5E+04	2.3E-03	1.0E+01	0.0E+00	0.0E+00
	15 min	3.1E+07	3.1E+07	3.1E+07	1.4E+01	1.4E+01	1.4E+01	3.1E+07	3.4E+04	1.1E-03	1.4E+01	0.0E+00	0.0E+00
	20 min	2.1E+07	2.1E+07	2.0E+07	1.0E+01	1.0E+01	1.0E+01	2.0E+07	7.3E+04	3.6E-03	1.0E+01	0.0E+00	0.0E+00
Volume of NaCl	10 µL	1.7E+07	1.7E+07	1.7E+07	1.3E+01	1.2E+01	1.2E+01	1.2E+01	1.4E+04	8.5E-04	1.2E+01	5.8E-01	4.7E-02
	20 µL	2.1E+07	2.1E+07	2.1E+07	1.4E+01	1.4E+01	1.4E+01	2.1E+07	4.4E+04	2.1E-03	1.4E+01	0.0E+00	0.0E+00
	30 µL	1.6E+07	1.6E+07	1.6E+07	1.2E+01	1.2E+01	1.2E+01	1.6E+07	1.9E+03	1.1E-04	1.2E+01	0.0E+00	0.0E+00

2. FATTY ACID CONTENT OBTAINED BY THE THREE DIFFERENT SAMPLE PREPARATION METHODS

Table 4: Comparison of methods with FA content, RSD and standard deviation

	Direct synthesis (n = 3)			Conventional method (n = 3)			MAED method (n = 3)		
FA	FA content (%)	SD	RSD	FA content (%)	SD	RSD	FA content (%)	SD	RSD
C _{8:0}	0	0.1	16.6	0	0.5	8.4	0.7	1	10.6
C _{10:0}	0	0	6.2	0.1	0.1	9.5	0	0.3	6.3
C _{12:0}	0.1	0	10.3	0.2	0	12.5	0.2	0	11.8
C _{13:0}	1.2	0.1	11.3	2.1	0.1	4.3	2.1	0	8.2
C _{14:0}	0.1	1.4	15.4	0.4	0.1	15.9	0.2	0.1	10
C _{16:0}	8.4	2	7.4	9.8	2.5	13.9	9.2	0.8	7.5
C _{16:1ω7t}	9.8	0	8.2	14.8	2	11.1	10.2	1	8.1
C _{16:1 ω 7}	1.5	1.2	13.9	2	0.3	15	1.3	1.3	4.6
C _{18:0}	0.5	0	7	0.3	0.1	7.1	0.8	0.1	13.4
C _{18:1 ω 9}	2.5	0.2	9.1	2.2	0.5	8.2	1.3	0.1	6.7
C _{18:1 ω 7}	1.2	0.1	19.2	0.8	0.1	11.1	0.9	0.6	8.5
C _{18:2 ω 6}	2.7	0.3	12.8	2.7	0.3	12.4	4.8	0.3	10
C _{20:4 ω 6}	1.2	0	7.6	1.1	1.2	12.5	1.4	0.1	6.7
C _{20:5 ω 3}	5.1	0.2	4.8	6.2	1	15.2	18.7	1.3	7.2

Σ TFA	34.1	0.4	10.7	42.6	0.6	11.2	51.8	0.5	8.5
Σ SFA	10.3	1.1	10.6	12.8	1.3	10.2	13.2	1.3	9.7
Σ MUFA	5.6	0.7	12.3	5	0.6	11.4	3.5	0.2	6.6
Σ PUFA	18.7	1.6	8.4	24.8	3.2	12.8	35	2.8	8
Σ TUFA	23.9	2.6	10.8	29.7	3.6	12.2	38.5	2.9	7.4

3. ANALYSIS OF VARIATION (ANOVA) TESTS

3.1 CONVENTIONAL EXTRACTION

Table 5: ANOVA results obtained for the investigation of significance for extracting solvents in conventional extraction based on peak area

SUMMARY						
Groups	Count	Sum	Average	Variance		
Acetic acid	3	830792280	2.77E+08	3.23664E+12		
Acetone	3	2288011320	7.63E+08	32902280028		
Ethanol	3	2051088159	6.84E+08	2.40819E+13		
IPA	3	1633614990	5.45E+08	1.37756E+12		
Methanol	3	95679499	3.19E+07	21953622.33		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.09676E+18	4	2.74189E+17	47719.88526	2.37E-21	3.47804969
Within Groups	5.74581E+13	10	5.74581E+12			
Total	1.09681E+18	14				

Table 6: ANOVA results obtained for the investigation of significance for fractionation solvents in conventional extraction based on peak area

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>

Chloroform	3	52195696	17398565.33	139953082.3		
Cyclohexane	3	47796004	15932001.33	6.70245E+12		
Hexane	3	3234125539	1078041846	1.88298E+13		
Toluene	3	0	0	0		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	2.56183E+18	3	8.53944E+17	133782.0997	3.88459E-19	4.06618055
Within Groups	5.10647E+13	8	7.29E+12			
Total	2.56188E+18	11				

Table 7: ANOVA results obtained for the investigation of significance for extraction solvents in conventional extraction based on peak number

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Acetic acid	3	36	12	0
Acetone	3	42	14	0
Ethanol	3	39	13	0
IPA	3	39	13	0
Methanol	3	39	13	0
ANOVA				

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	6	4	1.5	65535	0	3.47804969
Within Groups	0	10	0			
Total	6	14				

Table 8: ANOVA results obtained for the investigation of significance for fractionation solvents in conventional extraction based on peak number

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Chloroform	3	30	10	0		
Cyclohexane	3	22	7.33333333	0.33333333		
Hexane	3	42	14	0		
Toluene	3	0	0	0		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	313	3	104.333333	1252	5.01756E-11	4.06618055
Within Groups	0.66666667	8	0.08333333			
Total	313.666667	11				

3.2 CONVENTIONAL DERIVATISATION

Table 9: ANOVA results obtained for the investigation of significance for hydrolysis reagent used in conventional derivatisation based on peak area

SUMMARY						
Groups	Count	Sum	Average	Variance		
Sulfuric acid	3	950927004	316975668	2.13893E+14		
Hydrochloric acid	3	4551980619	1517326873	42173766319		
Potassium hydroxide	3	338234161	112744720.3	23688352549		
Calcium carbonate	3	0	0	0		
Sodium hydroxide	3	5083130163	1694376721	2.85716E+13		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7.90315E+18	4	1.97579E+18	40732.84022	5.22363E-21	3.47804969
Within Groups	4.8506E+14	10	4.8506E+13			
Total	7.90364E+18	14				

Table 10: ANOVA results obtained for the investigation of significance for hydrolysis reagent volume for conventional derivatisation based on peak area

SUMMARY						
Groups	Count	Sum	Average	Variance		
0.5 mL	3	1664943629	554981209.7	1.53404E+11		
1 mL	3	1510387844	503462614.7	5.5339E+11		
1.5 mL	3	432080903	144026967.7	1.34028E+12		
2 mL	3	363759252	121253084	1.17526E+13		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4.76591E+17	3	1.58864E+17	46048.66252	2.76692E-17	4.06618055
Within Groups	2.75993E+13	8	3.44991E+12			
Total	4.76618E+17	11				

Table 11: ANOVA results obtained for the investigation of significance for transesterification reagent (BF_3) volume for conventional derivatisation based on peak area

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
1 mL	3	3144876149	1048292050	1881818577
2 mL	3	4896057068	1632019023	6.07156E+14
3 mL	3	626467216	208822405.3	3.30253E+16

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	3.07094E+18	2	1.53547E+18	136.9632042	9.84741E-06	5.14325285
Within Groups	6.72648E+16	6	1.12108E+16			
Total	3.1382E+18	8				

Table 12: ANOVA results obtained for the investigation of significance for hydrolysis reaction time for conventional derivatisation based on peak area

SUMMARY						
Groups	Count	Sum	Average	Variance		
15 min	3	244466809	81488936.33	10173007161		
30 min	3	638162161	212720720.3	1.21325E+14		
45 min	3	697329746	232443248.7	3.93619E+12		
60 min	3	544983737	181661245.7	1.04586E+12		
75 min	3	492275393	164091797.7	23635371240		
90 min	3	172844819	57614939.67	2.61868E+12		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit

Between Groups	7.5031E+16	5	1.50062E+16	698.1836251	2.35949E-14	3.10587524
Within Groups	2.57919E+14	12	2.14932E+13			
Total	7.5289E+16	17				

Table 13: ANOVA results obtained for the investigation of significance for transesterification reaction time for conventional derivatisation based on peak area

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
10 min	3	861085416	287028472	55549098549		
20 min	3	643646082	214548694	34389872131		
30 min	3	724005070	241335023.3	2.67063E+11		
40 min	3	542425866	180808622	2.68083E+11		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	1.81074E+16	3	6.0358E+15	38623.8809	5.5901E-17	4.06618055
Within Groups	1.25017E+12	8	1.56271E+11			
Total	1.81087E+16	11				

Table 14: ANOVA results obtained for the investigation of significance for hydrolysis reagent used for conventional derivatisation based on peak number

SUMMARY						
Groups	Count	Sum	Average	Variance		
Sulfuric acid	3	36	12	0		
Hydrochloric acid	3	30	10	0		
Potassium hydroxide	3	42	14	0		
Calcium carbonate	3	0	0	0		
Sodium hydroxide	3	42	14	0		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	408	4	102	65535	0	3.47804969
Within Groups	0	10	0			
Total	408	14				

Table 15: ANOVA results obtained for the investigation of significance for hydrolysis reagent volume used for conventional derivatisation based on peak number

SUMMARY						
Groups	Count	Sum	Average	Variance		
0.5 mL	3	42	14	0		
1 mL	3	42	14	0		
1.5 mL	3	42	14	0		
2 mL	3	42	14	0		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0	3	0	65535	0	4.06618055
Within Groups	0	8	0			
Total	0	11				

Table 16: ANOVA results obtained for the investigation of significance for transesterification volume used for conventional derivatisation based on peak number

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
1 mL	3	39	13	0
2 mL	3	42	14	0

3 mL	3	18	6	27		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	114	2	57	6.33333333	0.03320882	5.14325285
Within Groups	54	6	9			
Total	168	8				

Table 17: ANOVA results obtained for the investigation of significance for hydrolysis reaction time used for conventional derivatisation based on peak number

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
15 min	3	36	12	0
30 min	3	42	14	0
45 min	3	42	14	0
60 min	3	42	14	0
75 min	3	42	14	0
90 min	3	36	12	0
ANOVA				

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	16	5	3.2	65535	0	3.10587524
Within Groups	0	12	0			
Total	16	17				

Table 18: ANOVA results obtained for the investigation of significance for transesterification reaction time used for conventional derivatisation based on peak number

SUMMARY						
Groups	Count	Sum	Average	Variance		
10 min	3	42	14	0		
20 min	3	42	14	0		
30 min	3	42	14	0		
40 min	3	42	14	0		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0	3	0	65535	0	4.06618055
Within Groups	0	8	0			

Total	0	11
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3.3 MAED

Table 19: ANOVA results obtained for the investigation of significance for fractionation solvent used for MAED based on peak area

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Chloroform	3	791746012	263915337	2.3013E+11		
Cyclohexane	3	46376082	15458694	175419391		
Hexane	3	0	0	0		
Toluene	3	0	0	0		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	1.5113E+17	3	5.0378E+16	874964.902	2.1233E-22	4.06618055
Within Groups	4.6062E+11	8	5.7577E+10			
Total	1.5113E+17	11				

Table 20: ANOVA results obtained for the investigation of significance for extraction solvent used for MAED based on peak area

SUMMARY						
Groups	Count	Sum	Average	Variance		
Acetone	3	532994423	177664808	3.7376E+11		
Methanol	3	448589263	149529754	3.3542E+11		
Acetic acid	3	1135321787	378440596	1.8071E+12		
Ethanol	3	55424424	18474808	6372977088		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.992E+17	3	6.6399E+16	105285.992	1.0126E-18	4.06618055
Within Groups	5.0452E+12	8	6.3065E+11			
Total	1.992E+17	11				

Table 21: ANOVA results obtained for the investigation of significance for reaction time used for MAED based on peak area ($q_{crit} = 4.34$)

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
10 min	3	73400056	24466685.3	3063799070
15 min	3	92877801	30959267	1145419201
20 min	3	61460557	20486852.3	5321465400

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	1.6766E+14	2	8.3832E+13	26388.0581	1.4689E-12	5.14325285
Within Groups	1.9061E+10	6	3176894557			
Total	1.6768E+14	8				

Table 22: ANOVA results obtained for the investigation of significance for NaCl volume used for MAED based on peak area

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
10 µL	3	50183122	16727707.3	204438136
20 µL	3	61657204	20552401.3	1922962302
30 µL	3	48957840	16319280	3435031

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	3.2714E+13	2	1.6357E+13	23029.2862	2.2098E-12	5.14325285
Within Groups	4261670939	6	710278490			

Total	3.2719E+13	8
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Table 23: ANOVA results obtained for the investigation of significance for fractionation solvent used for MAED based on peak number

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Chloroform	3	36	12	0		
Cyclohexane	3	21	7	0		
Hexane	3	0	0	0		
Toluene	3	0	0	0		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	308.25	3	102.75	65535	0	4.06618055
Within Groups	0	8	0			
Total	308.25	11				

Table 24: ANOVA results obtained for the investigation of significance for extraction solvent used for MAED based on peak number

SUMMARY						
Groups	Count	Sum	Average	Variance		
Acetone	3	42	14	0		
Methanol	3	42	14	0		
Acetic acid	3	42	14	0		
Ethanol	3	18	6	0		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	144	3	48	65535	0	4.06618055
Within Groups	0	8	0			
Total	144	11				

Table 25: ANOVA results obtained for the investigation of significance for reaction time used for MAED based on peak number

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
10 min	3	30	10	0
15 min	3	42	14	0
20 min	3	30	10	0

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	32	2	16	65535	0	5.14325285
Within Groups	0	6	0			
Total	32	8				

Table 26: ANOVA results obtained for the investigation of significance for volume of NaCl used for MAED based on peak number

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
10 µL	3	37	12.3333333	0.33333333
20 µL	3	42	14	0
30 µL	3	36	12	0

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	6.88888889	2	3.44444444	31	0.00068695	5.14325285
Within Groups	0.66666667	6	0.11111111			

Total	7.5555556	8
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3.4 FATTY ACID CHARACTERISATION

Table 27: ANOVA results obtained for the investigation of significance for C_{8:0} recovery by different methods of sample preparation

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Direct	3	82183474.72	27394491.57	5.83739E+12		
Conventional	3	281617222	93872407.33	1.03489E+13		
MAED	3	113140129.6	37713376.54	5.28976E+12		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	7.67963E+15	2	3.83981E+15	536.3857195	1.72054E-07	5.14325285
Within Groups	4.29521E+13	6	7.15868E+12			
Total	7.72258E+15	8				

Table 28: ANOVA results obtained for the investigation of significance for C_{10:0} recovery by different methods of sample preparation

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Direct	3	22012990	7337663.333	46351336502
Conventional	3	57744375	19248125	1.09296E+12
MAED	3	43104694.67	14368231.56	47219844275
ANOVA				

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	2.15101E+14	2	1.07551E+14	271.928321	1.29929E-06	5.14325285
Within Groups	2.37307E+12	6	3.95511E+11			
Total	2.17474E+14	8				

Table 29: ANOVA results obtained for the investigation of significance for C_{12:0} recovery by different methods of sample preparation

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Direct	3	27927835.21	9309278.405	1.93468E+11		
Conventional	3	5714001	1904667	10890352837		
MAED	3	1622032.988	540677.6628	271799061.2		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	1.33577E+14	2	6.67885E+13	979.1610283	2.84981E-08	5.14325285
Within Groups	4.0926E+11	6	68209973679			
Total	1.33986E+14	8				

Table 30: ANOVA results obtained for the investigation of significance for C_{13:0} recovery by different methods of sample preparation

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Direct	3	94605761.36	31535253.79	2.82531E+12		
Conventional	3	88252097	29417365.67	1.94886E+11		
MAED	3	1456111.423	485370.4743	15800490.33		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	1.80564E+15	2	9.02821E+14	896.7784993	3.70644E-08	5.14325285
Within Groups	6.04042E+12	6	1.00674E+12			
Total	1.81168E+15	8				

Table 31: ANOVA results obtained for the investigation of significance for C_{14:0} recovery by different methods of sample preparation

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Direct	3	675084396.3	225028132.1	2.67424E+14		
Conventional	3	27679138	9226379.333	4.26577E+11		
MAED	3	8674540.467	2891513.489	2024589021		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>

Between Groups	9.59552E+16	2	4.79776E+16	537.3584873	1.71127E-07	5.14325285
Within Groups	5.35705E+14	6	8.92842E+13			
Total	9.64909E+16	8				

Table 32: ANOVA results obtained for the investigation of significance for C_{16:0} recovery by different methods of sample preparation

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Direct	3	2580512660	860170886.5	1.34276E+15		
Conventional	3	674771181	224923727	1.89665E+14		
MAED	3	85614306.86	28538102.29	90810396753		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	1.13372E+18	2	5.6686E+17	1109.666602	1.96006E-08	5.14325285
Within Groups	3.06503E+15	6	5.10838E+14			
Total	1.13678E+18	8				

Table 33: ANOVA results obtained for the investigation of significance for C_{16:1w7t} recovery by different methods of sample preparation

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
Direct	3	45915175.46	15305058.49	4.23636E+11

Conventional	3	552829205	184276401.7	7.74664E+13		
MAED	3	88601661.18	29533887.06	1.21445E+11		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	5.2699E+16	2	2.63495E+16	1013.293073	2.57221E-08	5.14325285
Within Groups	1.56023E+14	6	2.60038E+13			
Total	5.2855E+16	8				

Table 34: ANOVA results obtained for the investigation of significance for C_{16:1w7} recovery by different methods of sample preparation

SUMMARY						
Groups	Count	Sum	Average	Variance		
Direct	3	749997494.6	249999164.9	2.561E+14		
Conventional	3	71233902	23744634	2.48724E+12		
MAED	3	221970253.1	73990084.35	3.26433E+12		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8.46949E+16	2	4.23475E+16	485.1695311	2.32088E-07	5.14325285
Within Groups	5.23703E+14	6	8.72838E+13			
Total	8.52186E+16	8				

Table 35: ANOVA results obtained for the investigation of significance for C_{18:0} recovery by different methods of sample preparation

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Direct	3	51564870.43	17188290.14	3.7218E+11		
Conventional	3	57707672	19235890.67	2.93185E+11		
MAED	3	5072703.244	1690901.081	3802394398		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	5.52188E+14	2	2.76094E+14	1237.779871	1.41345E-08	5.14325285
Within Groups	1.33834E+12	6	2.23056E+11			
Total	5.53527E+14	8				

Table 36: ANOVA results obtained for the investigation of significance for C_{18:1w9} recovery by different methods of sample preparation

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Direct	3	280684167.4	93561389.13	2.4395E+13		
Conventional	3	253468856	84489618.67	1.56868E+13		
MAED	3	12446068.59	4148689.53	74021142.62		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>

Between Groups	1.45316E+16	2	7.2658E+15	543.8210102	1.65131E-07	5.14325285
Within Groups	8.01638E+13	6	1.33606E+13			
Total	1.46118E+16	8				

Table 37: ANOVA results obtained for the investigation of significance for C_{18:1w7} recovery by different methods of sample preparation

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Direct	3	54277569.74	18092523.25	3.58127E+12		
Conventional	3	32003356	10667785.33	2.57493E+11		
MAED	3	64950924.11	21650308.04	65505069546		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	1.884E+14	2	9.42002E+13	72.38241478	6.30309E-05	5.14325285
Within Groups	7.80854E+12	6	1.30142E+12			
Total	1.96209E+14	8				

Table 38: ANOVA results obtained for the investigation of significance for C_{18:2w6} recovery by different methods of sample preparation

SUMMARY						
Groups	Count	Sum	Average	Variance		
Direct	3	200508040.4	66836013.45	1.5178E+13		
Conventional	3	82664322	27554774	3.71746E+12		
MAED	3	20496715.05	6832238.349	20770256372		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5.57289E+15	2	2.78645E+15	441.9134338	3.06575E-07	5.14325285
Within Groups	3.78325E+13	6	6.30541E+12			
Total	5.61072E+15	8				

Table 39: ANOVA results obtained for the investigation of significance for C_{20:4w6} recovery by different methods of sample preparation

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Direct	3	45276121	15092040.33	3.03718E+11		
Conventional	3	630340352	210113450.7	1.29617E+14		
MAED	3	19700730.56	6566910.187	1.30052E+11		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>

Between Groups	7.95372E+16	2	3.97686E+16	917.3786784	3.46309E-08	5.14325285
Within Groups	2.60102E+14	6	4.33503E+13			
Total	7.97973E+16	8				

Table 40: ANOVA results obtained for the investigation of significance for C_{20:5w3} recovery by different methods of sample preparation

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Direct	3	906168115	302056038.3	2.81127E+13		
Conventional	3	451718758	150572919.3	1.04758E+14		
MAED	3	292718833.6	97572944.53	1.06123E+11		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	6.75695E+16	2	3.37847E+16	762.1969696	6.02621E-08	5.14325285
Within Groups	2.65953E+14	6	4.43255E+13			
Total	6.78354E+16	8				

Table 41: ANOVA results obtained for the investigation of significance for TFA recovered from different methods of analysis based on peak area

SUMMARY						
Groups	Count	Sum	Average	Variance		
MAED	3	979569705	326523235	1.7542E+13		
Direct	3	5816718671	1938906224	8.7852E+14		
Conventional	3	3267744437	1089248146	1.9859E+15		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3.9034E+18	2	1.9517E+18	2031.62776	3.2056E-09	5.14325285
Within Groups	5.764E+15	6	9.6067E+14			
Total	3.9092E+18	8				

Table 42: ANOVA results obtained for the investigation of significance for SFA recovered from different methods of analysis based on peak area

SUMMARY				
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>
MAED	3	258684519	86228173.1	3.4878E+12
Direct	3	3533891988	1177963996	5.7807E+14
Conventional	3	1193485686	397828562	3.0199E+14

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	1.8976E+18	2	9.488E+17	3221.55189	8.053E-10	5.14325285
Within Groups	1.7671E+15	6	2.9452E+14			
Total	1.8994E+18	8				

Table 43: ANOVA results obtained for the investigation of significance for MUFA recovered from different methods of analysis based on peak area

SUMMARY						
Groups	Count	Sum	Average	Variance		
MAED	3	299367246	99789081.9	2.5069E+12		
Direct	3	1084959232	361653077	2.0905E+14		
Conventional	3	356706114	118902038	1.4373E+13		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.2787E+17	2	6.3933E+16	848.914904	4.3669E-08	5.14325285

Within Groups	4.5187E+14	6	7.5312E+13
Total	1.2832E+17	8	

Table 44: ANOVA results obtained for the investigation of significance for PUFA recovered from different methods of analysis based on peak area

SUMMARY						
Groups	Count	Sum	Average	Variance		
MAED	3	421517940	140505980	9.6041E+11		
Direct	3	1197867452	399289151	6.4721E+13		
Conventional	3	1717552637	572517546	7.5105E+14		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.8361E+17	2	1.4181E+17	520.876261	1.8779E-07	5.14325285
Within Groups	1.6335E+15	6	2.7224E+14			
Total	2.8524E+17	8				

Table 45: ANOVA results obtained for the investigation of significance for TUFA recovered from different methods of analysis based on peak area

SUMMARY						
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Groups	Count	Sum	Average	Variance		
MAED	3	720885186	240295062	5.4222E+12		
Direct	3	2282826684	760942228	3.6644E+14		
Conventional	3	2074258751	691419584	7.7017E+14		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4.7942E+17	2	2.3971E+17	629.694931	1.0661E-07	5.14325285
Within Groups	2.2841E+15	6	3.8068E+14			
Total	4.817E+17	8				

4. TUKEY HSD ANALYSIS

4.1 CONVENTIONAL EXTRACTION

Table 46: Tukey HSD analysis for extracting solvents for conventional extraction based on peak area ($q_{crit} = 4.66$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for ($\mu_i - \mu_j$)		Significant at 0.05
			Positive	Negative	
Acetic acid - Acetone	-4.9E+08	2.0E+06	-4.8E+08	-4.9E+08	Yes
Acetic acid - Ethanol	-4.1E+08	2.0E+06	-4.0E+08	-4.2E+08	Yes
Acetic acid - IPA	-2.7E+08	2.0E+06	-2.6E+08	-2.8E+08	Yes
Acetic acid - Methanol	2.5E+08	2.0E+06	2.5E+08	2.4E+08	Yes
Acetone - Ethanol	7.9E+07	2.0E+06	8.8E+07	7.0E+07	Yes
Acetone - IPA	2.2E+08	2.0E+06	2.3E+08	2.1E+08	Yes
Acetone - Methanol	7.3E+08	2.0E+06	7.4E+08	7.2E+08	Yes
Ethanol - IPA	1.4E+08	2.0E+06	1.5E+08	1.3E+08	Yes
Ethanol - Methanol	6.5E+08	2.0E+06	6.6E+08	6.4E+08	Yes

IPA - Methanol	5.1E+08	2.0E+06	5.2E+08	5.0E+08	Yes
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Table 47: Tukey HSD analysis for fractionation solvents for conventional extraction based on peak area ($q_{crit} = 4.54$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for ($\mu_i - \mu_j$)		Significant at 0.05
			Positive	Negative	
Chloroform - cyclohexane	1.5E+06	2.2E+06	1.1E+07	-8.5E+06	
Chloroform - hexane	-1.1E+09	2.2E+06	-1.1E+09	-1.1E+09	Yes
Chloroform - toluene	1.7E+07	2.2E+06	2.7E+07	7.4E+06	Yes
Cyclohexane - hexane	-1.1E+09	2.2E+06	-1.1E+09	-1.1E+09	Yes
Cyclohexane - toluene	1.6E+07	2.2E+06	2.6E+07	5.9E+06	Yes
Hexane - toluene	1.1E+09	2.2E+06	1.1E+09	1.1E+09	Yes

Table 48: Tukey HSD analysis for extraction solvents for conventional extraction based on peak number ($q_{crit} = 4.66$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for ($\mu_i - \mu_j$)		Significant at 0.05
			Positive	Negative	
Acetic acid - Acetone	-2	0	-2	-2	Yes
Acetic acid - Ethanol	-1	0	-1	-1	Yes
Acetic acid - IPA	-1	0	-1	-1	Yes
Acetic acid - Methanol	-1	0	-1	-1	Yes
Acetone - Ethanol	1	0	1	1	Yes
Acetone - IPA	1	0	1	1	Yes
Acetone - Methanol	1	0	1	1	Yes
Ethanol - IPA	0	0	0	0	
Ethanol - Methanol	0	0	0	0	
IPA - Methanol	0	0	0	0	

Table 49: Tukey HSD analysis for fractionation solvents for conventional extraction based on peak number ($q_{crit} = 4.54$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for ($\mu_i - \mu_j$)		Significant at 0.05
			Positive	Negative	
Chloroform - cyclohexane	2.7	0.2	3.7	1.6	Yes
Chloroform - hexane	-4.0	0.2	-2.9	-5.1	Yes
Chloroform - toluene	10.0	0.2	11.1	8.9	Yes
Cyclohexane - hexane	-6.7	0.2	-5.6	-7.7	Yes
Cyclohexane - toluene	7.3	0.2	8.4	6.3	Yes
Hexane - toluene	14.0	0.2	15.1	12.9	Yes

4.2 CONVENTIONAL DERIVATISATION

Table 50: Tukey HSD analysis for hydrolysis reagent for conventional derivatisation based on peak area ($q_{crit} = 4.66$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
Sulfuric acid - hydrochloric acid	-1.2E+09	5.7E+06	-1.2E+09	-1.2E+09	Yes
Sulfuric acid - potassium hydroxide	2.2E+08	5.7E+06	2.5E+08	1.9E+08	Yes
Sulfuric acid - calcium carbonate	3.3E+08	5.7E+06	3.6E+08	3.1E+08	Yes
Sulfuric acid - sodium hydroxide	-1.4E+09	5.7E+06	-1.3E+09	-1.4E+09	Yes
Hydrochloric acid - potassium hydroxide	1.4E+09	5.7E+06	1.4E+09	1.4E+09	Yes
Hydrochloric acid - calcium carbonate	1.5E+09	5.7E+06	1.5E+09	1.5E+09	Yes
Hydrochloric acid - sodium hydroxide	-1.8E+08	5.7E+06	-1.5E+08	-2.1E+08	Yes
Potassium hydroxide - calcium carbonate	1.1E+08	5.7E+06	1.4E+08	8.6E+07	Yes
Potassium hydroxide - sodium hydroxide	-1.6E+09	5.7E+06	-1.6E+09	-1.6E+09	Yes
Calcium carbonate - sodium hydroxide	-1.7E+09	5.7E+06	-1.7E+09	-1.7E+09	Yes

Table 51: Tukey HSD analysis for hydrolysis reagent volume for conventional derivatisation based on peak area ($q_{crit} = 4.54$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
0.5 - 1 mL	5.2E+07	1.5E+06	5.8E+07	4.5E+07	Yes
0.5 - 1.5 mL	4.1E+08	1.5E+06	4.2E+08	4.0E+08	Yes
0.5 - 2 mL	4.3E+08	1.5E+06	4.4E+08	4.3E+08	Yes
1 - 1.5 mL	3.6E+08	1.5E+06	3.7E+08	3.5E+08	Yes
1 - 2 mL	3.8E+08	1.5E+06	3.9E+08	3.8E+08	Yes
1.5 - 2 mL	2.3E+07	1.5E+06	3.0E+07	1.6E+07	Yes

Table 52: Tukey HSD analysis for transesterification volume for conventional derivatisation based on peak area ($q_{crit} = 4.34$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
1-2 mL	-5.8E+08	8.6E+07	-2.1E+08	-9.6E+08	Yes
1-3 mL	8.4E+08	8.6E+07	1.2E+09	4.6E+08	Yes
2-3 mL	1.4E+09	8.6E+07	1.8E+09	1.0E+09	Yes

Table 53: Tukey HSD analysis for hydrolysis reaction time for conventional derivatisation based on peak area ($q_{crit} = 4.75$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
15-30 min	-1.3E+08	3.8E+06	-1.1E+08	-1.5E+08	Yes
15-45 min	-1.5E+08	3.8E+06	-1.3E+08	-1.7E+08	Yes
15-60 min	-1.0E+08	3.8E+06	-8.2E+07	-1.2E+08	Yes
15-75 min	-8.3E+07	3.8E+06	-6.5E+07	-1.0E+08	Yes
15-90 min	2.4E+07	3.8E+06	4.2E+07	5.9E+06	Yes
30-45 min	-2.0E+07	3.8E+06	-1.7E+06	-3.8E+07	Yes
30-60 min	3.1E+07	3.8E+06	4.9E+07	1.3E+07	Yes
30-75 min	4.9E+07	3.8E+06	6.7E+07	3.1E+07	Yes
30-90 min	1.6E+08	3.8E+06	1.7E+08	1.4E+08	Yes
45-60 min	5.1E+07	3.8E+06	6.9E+07	3.3E+07	Yes
45-75 min	6.8E+07	3.8E+06	8.6E+07	5.0E+07	Yes
45-90 min	1.7E+08	3.8E+06	1.9E+08	1.6E+08	Yes
60-75 min	1.8E+07	3.8E+06	3.6E+07	-4.1E+05	
60-90 min	1.2E+08	3.8E+06	1.4E+08	1.1E+08	Yes
75-90 min	1.1E+08	3.8E+06	1.2E+08	8.8E+07	Yes

Table 54: Tukey HSD analysis for transesterification reaction time for conventional derivatisation based on peak area ($q_{crit} = 4.54$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
10-20 min	7.2E+07	3.2E+05	7.4E+07	7.1E+07	Yes
10-30 min	4.6E+07	3.2E+05	4.7E+07	4.4E+07	Yes
10-40 min	1.1E+08	3.2E+05	1.1E+08	1.0E+08	Yes
20-30 min	-2.7E+07	3.2E+05	-2.5E+07	-2.8E+07	Yes
30-40 min	3.4E+07	3.2E+05	3.5E+07	3.2E+07	Yes
30-40 min	6.1E+07	3.2E+05	6.2E+07	5.9E+07	Yes

Table 55: Tukey HSD analysis for hydrolysis reagent for conventional derivatisation based on peak number ($q_{crit} = 4.66$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
Sulfuric acid - hydrochloric acid	2	0	2	2	Yes
Sulfuric acid - potassium hydroxide	-2	0	-2	-2	Yes
Sulfuric acid - calcium carbonate	12	0	12	12	Yes
Sulfuric acid - sodium hydroxide	-2	0	-2	-2	Yes
Hydrochloric acid - potassium hydroxide	-4	0	-4	-4	Yes

Hydrochloric acid - calcium carbonate	10	0	10	10	Yes
Hydrochloric acid - sodium hydroxide	-4	0	-4	-4	Yes
Potassium hydroxide - calcium carbonate	14	0	14	14	Yes
Potassium hydroxide - sodium hydroxide	0	0	0	0	Yes
Calcium carbonate - sodium hydroxide	-14	0	-14	-14	Yes

Table 56: Tukey HSD analysis for hydrolysis reagent volume for conventional derivatisation based on peak number ($q_{crit} = 4.54$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
0.5 - 1 mL	0	0	0	0	
0.5 - 1.5 mL	0	0	0	0	
0.5 - 2 mL	0	0	0	0	
1 - 1.5 mL	0	0	0	0	
1 - 2 mL	0	0	0	0	
1.5 - 2 mL	0	0	0	0	

Table 57: Tukey HSD analysis for transesterification volume for conventional derivatisation based on peak number ($q_{crit} = 4.34$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for ($\mu_i - \mu_j$)		Significant at 0.05
			Positive	Negative	
1-2 mL	-1.0	2.4	9.6	-11.6	
1-3 mL	7.0	2.4	17.6	-3.6	
2-3 mL	8.0	2.4	18.6	-2.6	

Table 58: Tukey HSD analysis for hydrolysis reaction time for conventional derivatisation based on peak number ($q_{crit} = 4.75$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for ($\mu_i - \mu_j$)		Significant at 0.05
			Positive	Negative	
15-30 min	-2	0	-2	-2	Yes
15-45 min	-2	0	-2	-2	Yes
15-60 min	-2	0	-2	-2	Yes
15-75 min	-2	0	-2	-2	Yes
15-90 min	0	0	0	0	
30-45 min	0	0	0	0	
30-60 min	0	0	0	0	
30-75 min	0	0	0	0	
30-90 min	2	0	2	2	Yes
45-60 min	0	0	0	0	
45-75 min	0	0	0	0	
45-90 min	2	0	2	2	Yes
60-75 min	0	0	0	0	

60-90 min	2	0	2	2	Yes
75-90 min	2	0	2	2	Yes

Table 59: Tukey HSD analysis for transesterification reaction time for conventional derivatisation based on peak number ($q_{crit} = 4.54$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
10-20 min	0	0	0	0	
10-30 min	0	0	0	0	
10-40 min	0	0	0	0	
20-30 min	0	0	0	0	
30-40 min	0	0	0	0	
30-40 min	0	0	0	0	

4.3 MAED

Table 60: Tukey HSD analysis for fractionation solvent for MAED based on peak area ($q_{crit} = 4.54$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for ($\mu_i - \mu_j$)		Significant at 0.05
			Positive	Negative	
Chloroform - cyclohexane	2.5E+08	2.0E+05	2.5E+08	2.5E+08	Yes
Chloroform - hexane	2.6E+08	2.0E+05	2.6E+08	2.6E+08	Yes
Chloroform - toluene	2.6E+08	2.0E+05	2.6E+08	2.6E+08	Yes
Cyclohexane - hexane	1.5E+07	2.0E+05	1.6E+07	1.5E+07	Yes
Cyclohexane - toluene	1.5E+07	2.0E+05	1.6E+07	1.5E+07	Yes
Hexane - toluene	0.0E+00	2.0E+05	8.9E+05	-8.9E+05	

Table 61: Tukey HSD analysis for extraction solvent for MAED based on peak area ($q_{crit} = 4.54$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for ($\mu_i - \mu_j$)		Significant at 0.05
			Positive	Negative	
Acetone - methanol	2.8E+07	6.5E+05	3.1E+07	2.5E+07	Yes
Acetone - acetic acid	-2.0E+08	6.5E+05	-2.0E+08	-2.0E+08	Yes
Acetone - ethanol	1.6E+08	6.5E+05	1.6E+08	1.6E+08	Yes
Methanol - acetic acid	-2.3E+08	6.5E+05	-2.3E+08	-2.3E+08	Yes
Methanol - ethanol	1.3E+08	6.5E+05	1.3E+08	1.3E+08	Yes
Acetic acid - ethanol	3.6E+08	6.5E+05	3.6E+08	3.6E+08	Yes

Table 62: Tukey HSD analysis for reaction time for MAED based on peak area ($q = 4.34$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for ($\mu_i - \mu_j$)		Significant at 0.05
			Positive	Negative	
10-15 min	-6.5E+06	4.6E+04	-6.3E+06	-6.7E+06	Yes
10-20 min	4.0E+06	4.6E+04	4.2E+06	3.8E+06	Yes
15-20 min	1.0E+07	4.6E+04	1.1E+07	1.0E+07	Yes

Table 63: Tukey HSD analysis for NaCl volume for MAED based on peak area ($q_{crit} = 4.34$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
10-20 μL	-2.1E+07	2.2E+04	-2.0E+07	-2.1E+07	Yes
10-30 μL	-1.6E+07	2.2E+04	-1.6E+07	-1.6E+07	Yes
20-30 μL	4.2E+06	2.2E+04	4.3E+06	4.1E+06	Yes

Table 64: Tukey HSD analysis for fractionation solvent for MAED based on peak number ($q_{crit} = 4.54$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
Chloroform - cyclohexane	5	0	5	5	Yes
Chloroform - hexane	12	0	12	12	Yes
Chloroform - toluene	12	0	12	12	Yes
Cyclohexane - hexane	7	0	7	7	Yes
Cyclohexane - toluene	7	0	7	7	Yes
Hexane - toluene	0	0	0	0	

Table 65: Tukey HSD analysis for extraction solvent for MAED based on peak number ($q_{crit} = 4.54$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
Acetone - methanol	0	0	0	0	
Acetone - acetic acid	0	0	0	0	
Acetone - ethanol	8	0	8	8	Yes
Methanol - acetic acid	0	0	0	0	
Methanol - ethanol	8	0	8	8	Yes
Acetic acid - ethanol	8	0	8	8	Yes

Table 66: Tukey HSD analysis for reaction time for MAED based on peak number ($q_{crit} = 4.34$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
10-15 min	-4	0	-4	-4	Yes
10-20 min	0	0	0	0	
15-20 min	4	0	4	4	Yes

Table 67: Tukey HSD analysis for volume of NaCl for MAED based on peak number ($q_{crit} = 4.34$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
10-20 μL	-1.7	0.3	-0.5	-5.7	Yes
10-30 μL	0.3	0.3	1.5	-3.7	
20-30 μL	2.0	0.3	3.2	-2.1	

4.4 FATTY ACID CHARACTERISATION

Table 68: Tukey HSD analysis for C_{8:0} recovery using different sample preparation methods ($q_{crit} = 4.34$)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	1.0E+07	3089483.632	2.4E+07	-3.1E+06	
MAED - conventional	-5.6E+07	3089483.632	-4.3E+07	-7.0E+07	Yes
Direct - conventional	-6.6E+07	3089483.632	-5.3E+07	-8.0E+07	Yes

Table 69: Tukey HSD analysis for C_{10:0} recovery using different sample preparation methods ($q_{crit} = 4.34$)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	7.0E+06	726187.4089	1.0E+07	3.9E+06	Yes
MAED - conventional	-4.9E+06	726187.4089	-1.7E+06	-8.0E+06	Yes
Direct - conventional	-1.2E+07	726187.4089	-8.8E+06	-1.5E+07	Yes

Table 70: Tukey HSD analysis for C_{12:0} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	-8.8E+06	301573.5923	-7.5E+06	-1.0E+07	Yes
MAED - conventional	-1.4E+06	301573.5923	-5.5E+04	-2.7E+06	Yes
Direct - conventional	7.4E+06	301573.5923	8.7E+06	6.1E+06	Yes

Table 71: Tukey HSD analysis for C_{13:0} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	-3.1E+07	1158583.893	-2.6E+07	-3.6E+07	Yes
MAED - conventional	-2.9E+07	1158583.893	-2.4E+07	-3.4E+07	Yes
Direct - conventional	2.1E+06	1158583.893	7.1E+06	-2.9E+06	

Table 72: Tukey HSD analysis for C_{14:0} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	-2.2E+08	10910799.66	-1.7E+08	-2.7E+08	Yes
MAED - conventional	-6.3E+06	10910799.66	4.1E+07	-5.4E+07	

Direct - conventional	2.2E+08	10910799.66	2.6E+08	1.7E+08	Yes
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Table 73: Tukey HSD analysis for C_{16:0} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	-8.3E+08	26098220.34	-7.2E+08	-9.4E+08	Yes
MAED - conventional	-2.1E+08	26098220.34	-9.8E+07	-3.2E+08	Yes
Direct - conventional	5.8E+08	26098220.34	7.0E+08	4.7E+08	Yes

Table 74: Tukey HSD analysis for C_{16:1w7t} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	1.4E+07	5888275.261	4.0E+07	-1.1E+07	
MAED - conventional	-1.5E+08	5888275.261	-1.3E+08	-1.8E+08	Yes
Direct - conventional	-1.8E+08	5888275.261	-1.5E+08	-2.0E+08	Yes

Table 75: Tukey HSD analysis for C_{16:1w7} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	-1.8E+08	10787884.24	-1.3E+08	-2.2E+08	Yes

MAED - conventional	5.0E+07	10787884.24	9.7E+07	3.4E+06	Yes
Direct - conventional	2.3E+08	10787884.24	2.7E+08	1.8E+08	Yes

Table 76: Tukey HSD analysis for C_{18:0} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	-1.5E+07	545351.2341	-1.3E+07	-1.8E+07	Yes
MAED - conventional	-1.8E+07	545351.2341	-1.5E+07	-2.0E+07	Yes
Direct - conventional	2.0E+06	545351.2341	4.4E+06	-3.2E+05	

Table 77: Tukey HSD analysis for C_{18:1w9} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	-8.9E+07	4220685.464	-7.1E+07	-1.1E+08	Yes
MAED - conventional	-8.0E+07	4220685.464	-6.2E+07	-9.9E+07	Yes
Direct - conventional	-9.1E+06	4220685.464	9.2E+06	-2.7E+07	

Table 78: Tukey HSD analysis for C_{18:1w7} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
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MAED - direct	3.6E+06	1317281.865	9.3E+06	4.9E+06	Yes
MAED - conventional	1.1E+07	1317281.865	1.7E+07	1.2E+07	Yes
Direct - conventional	-7.4E+06	1317281.865	-1.7E+06	-6.1E+06	Yes

Table 79: Tukey HSD analysis for C_{18:2w6} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	-6.0E+07	2899519.894	-4.7E+07	-7.3E+07	Yes
MAED - conventional	-2.1E+07	2899519.894	-8.1E+06	-3.3E+07	Yes
Direct - conventional	-3.9E+07	2899519.894	-2.7E+07	-5.2E+07	Yes

Table 80: Tukey HSD analysis for C_{20:4w6} recovery using different sample preparation methods (qcrit = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	-8.5E+06	7602654.592	2.4E+07	-4.2E+07	
MAED - conventional	-2.0E+08	7602654.592	-1.7E+08	-2.4E+08	Yes
Direct - conventional	2.0E+08	7602654.592	2.3E+08	1.6E+08	Yes

Table 81: Tukey HSD analysis for C_{20:5w3} recovery using different sample preparation methods (q_{crit} = 4.34)

	$\bar{x}_i - \bar{x}_j$	Standardized error	95% Confidence interval for $\mu_i - \mu_j$		Significant at 0.05?
MAED - direct	-2.0E+08	7687692.655	-1.7E+08	-2.4E+08	Yes
MAED - conventional	-5.3E+07	7687692.655	-2.0E+07	-8.6E+07	Yes
Direct - conventional	-1.5E+08	7687692.655	-1.2E+08	-1.8E+08	Yes

Table 82: Tukey HSD analysis for TFA extraction in different sample preparation methods based on peak area (q_{crit} = 4.34)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
MAED - direct	-1.6E+09	2.5E+07	-1.5E+09	-1.7E+09	Yes
MAED - conventional	-7.6E+08	2.5E+07	-6.5E+08	-8.7E+08	Yes
Direct - conventional	8.5E+08	2.5E+07	9.6E+08	7.4E+08	Yes

Table 83: Tukey HSD analysis for SFA extraction in different sample preparation methods based on peak area ($q_{crit} = 4.34$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
MAED - direct	-1.1E+09	1.4E+07	-1.0E+09	-1.2E+09	Yes
MAED - conventional	-3.1E+08	1.4E+07	-2.5E+08	-3.7E+08	Yes
Direct - conventional	7.8E+08	1.4E+07	8.4E+08	7.2E+08	Yes

Table 84: Tukey HSD analysis for MUFA extraction in different sample preparation methods based on peak area ($q_{crit} = 4.34$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
MAED - direct	-2.6E+08	1.4E+07	-2.0E+08	-3.2E+08	Yes
MAED - conventional	-1.9E+07	1.4E+07	4.2E+07	-8.0E+07	
Direct - conventional	2.4E+08	1.4E+07	3.0E+08	1.8E+08	Yes

Table 85: Tukey HSD analysis for PUFA extraction in different sample preparation methods based on peak area ($q_{crit} = 4.34$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
MAED - direct	-2.6E+08	1.3E+07	-2.0E+08	-3.2E+08	Yes
MAED - conventional	-4.3E+08	1.3E+07	-3.7E+08	-4.9E+08	Yes
Direct - conventional	-1.7E+08	1.3E+07	-1.1E+08	-2.3E+08	Yes

Table 86: Tukey HSD analysis for TUFA extraction in different sample preparation methods based on peak area ($q_{crit} = 4.34$)

Comparison	$\bar{x}_i - \bar{x}_j$	Standardized error	95% confidence interval for $(\mu_i - \mu_j)$		Significant at 0.05
			Positive	Negative	
MAED - direct	-5.2E+08	1.6E+07	-4.5E+08	-5.9E+08	Yes
MAED - conventional	-4.5E+08	1.6E+07	-3.8E+08	-5.2E+08	Yes
Direct - conventional	7.0E+07	1.6E+07	1.4E+08	3.8E+05	Yes