SUPPLEMENTARY DATA

Table 1 Visual stability evaluation of DHA nanoemulsions prepared using various emulsifiers

Emulsifier	Concentration (%)	T80 (%)	Poor dissolution of emulsifier	Visible floating of oil after ultrasonication	Phase separation after 24 h	Phase separation after 7 days
Casein	3	1	Nil	*	*	*
	5	1	Nil	Nil	**	*
	8	1	Nil	Nil	**	*
	10	1	Nil	Nil	**	*
	13	1	Nil	Nil	**	*
	15	1	Nil	Nil	**	*
	20	1	*	Nil	*	*
Chitosan	0.5	1	*	Nil	*	*
	1	1	Nil	Nil	**	**
	1.5	1	Nil	Nil	**	**
	2	1	Nil	Nil	**	*
	3	1	*	Nil	**	*
Pectin	2	1	Nil	*	*	*
	3	1	Nil	Nil	**	*
	3.5	1	Nil	Nil	**	**
	4	1	Nil	Nil	**	*
	5	1	Nil	Nil	**	*
	6	1	Nil	Nil	**	*
	6.5	1	*	Nil	*	*

* Phase separation/ unstable, **No phase separation/ stable

Table 2 Color analysis of kalakand fortified with DHA nanoemulsion

Number of days	Color parameters	KCON	KDHO	KCDN
	L*	65.56±0.12^{d}	67.08±0.29^{d}	68.70±0.05^{d}
Day 1	**a***	1.05±0.04^{d}	1.14±0.02^{d}	0.66±0.1^{c}
	b*	22.43±0.06^{d}	22.71±0.06^{d}	20.35±0.12^{d}
	L*	66.62±0.28^{c}	68.52±0.1^{c}	69.5±0.05^{c}
Day 4	**a***	1.17±0.02^{c}	1.29±0.03^{c}	0.71±0.03^{b}
	b*	22.52±0.06^{c}	23.90±0.21^{c}	21.62±0.02^{c}
	L*	67.52±0.01^{b}	69.5±0.21^{b}	71.66±0.28^{b}
Day 7	**a***	1.22±0.03^{b}	1.33±0.02^{b}	0.82±0.02^{a}
	b*	23.59±0.02^{b}	24.11±0.06^{b}	22.48±0.09^{b}
	L*	68.67±0.01^{a}	71.47±0.06^{a}	72.63±0.05^{a}
Day 10	**a***	1.31±0.02^{a}	1.4±0.02^{a}	1.12±0.02^{a}
	b*	24.63±0.06^{a}	22.56±0.12^{a}	22.68±0.05^{a}
	ΔE	3.32±0.2^{b}	4.41±0.1^{a}	3.95±0.1^{ab}

KCON- Kalakand control; KDHO- Kalakand with neat DHA oil; KCDN- Kalakand with DHA nanoemulsion.

Data are articulated as mean ± standard deviation (n = 3). In an individual row, each value tailed by a different letter is significantly different ($P \leq 0.05$) between samples as determined by Tukey's test

Step 1: Preparation of milk solids

Boiling of standardized milk

(Total fat 3 g, carbohydrates 4.7 g and protein 3.4 g)

↓

Addition of citric acid (0.02% w/v) to initiate coagulation

↓

Separation of whey by draining the coagulated mass through a muslin cloth

↓

Mashing of the obtained milk solids to a smooth texture

Step 2: Preparation of kalakand

Boiling 100 ml of milk separately in a vessel

↓

Addition of (1) milk solids prepared in step 1, (2) condensed milk (30 g) and table sugar (10 g) while maintaining temperature at 60-70 ℃

↓

Cooking the mixture until a thick consistency (only milk solids, no free water) was obtained

↓

Maintaining the temperature of mixture at 40-45 ℃ to ensure proper mixing of DHA during fortification

↓

Incorporation of both chitosan-stabilized DHA nanoemulsion (CHDN) and DHA oil (each providing 0.5g DHA) into separate lots under gentle stirring

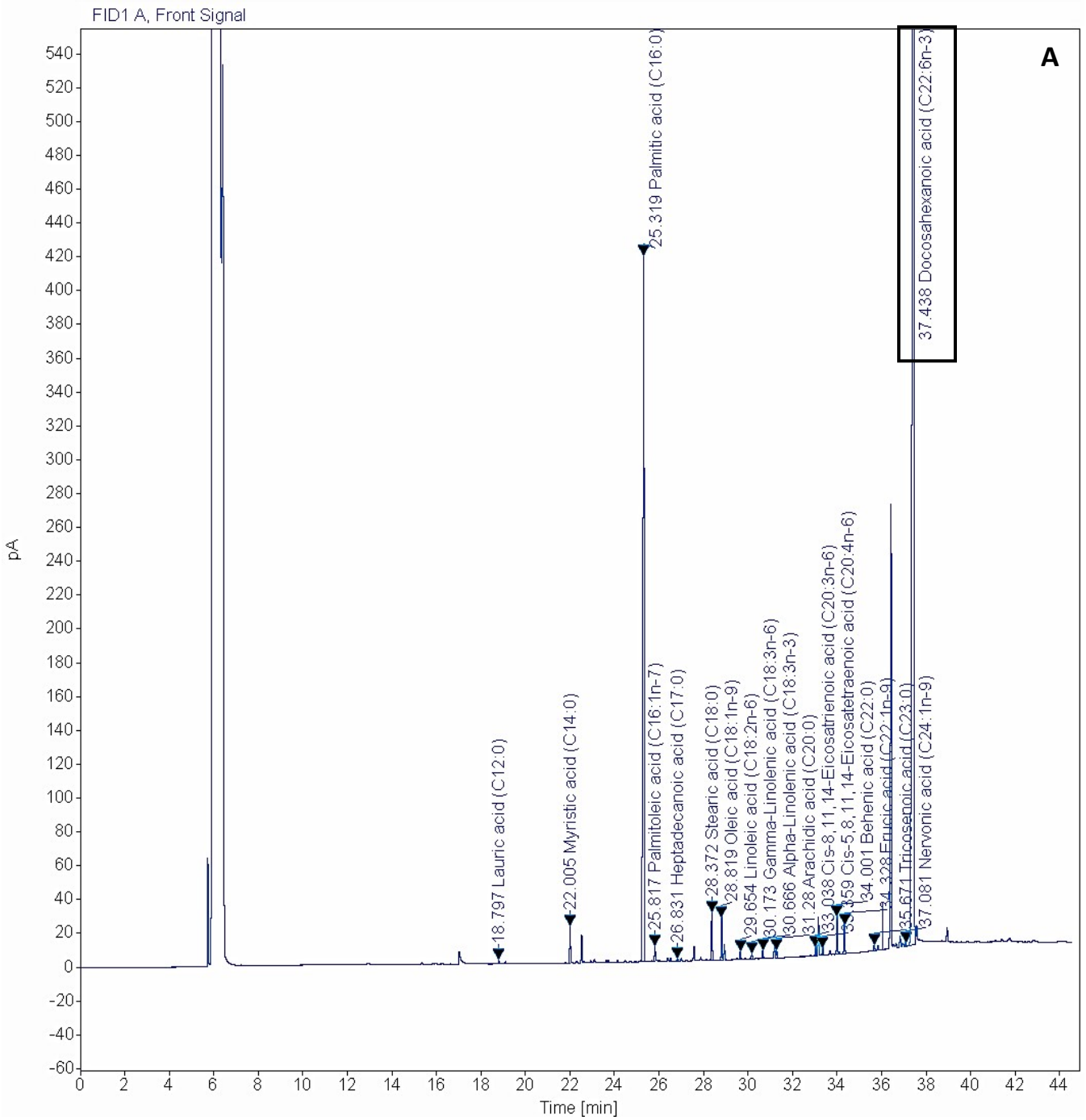
↓

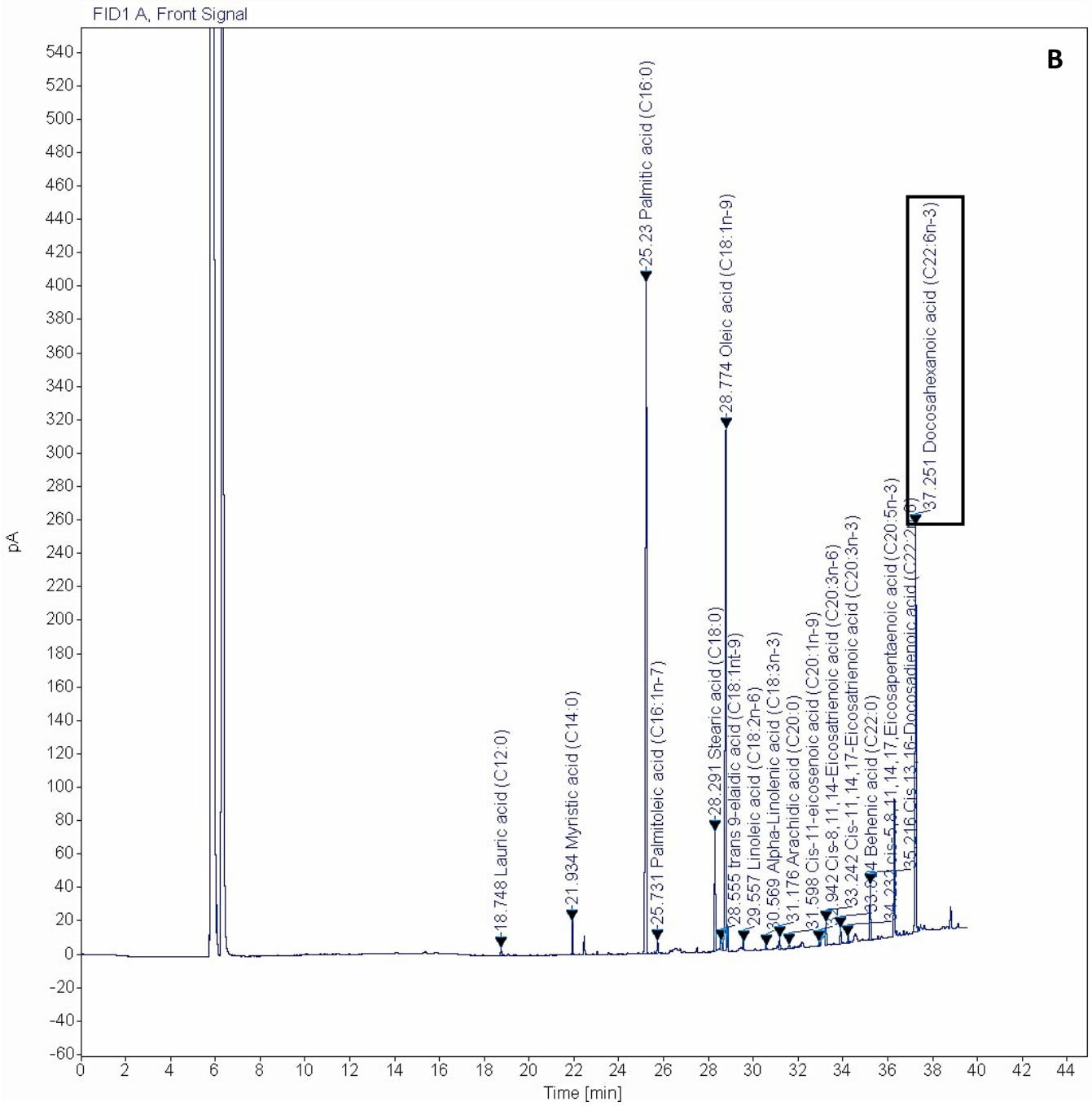
Transferring the DHA fortified kalakand to a clean, sterilized container

↓

Setting the product overnight in a refrigerator (4 ℃).

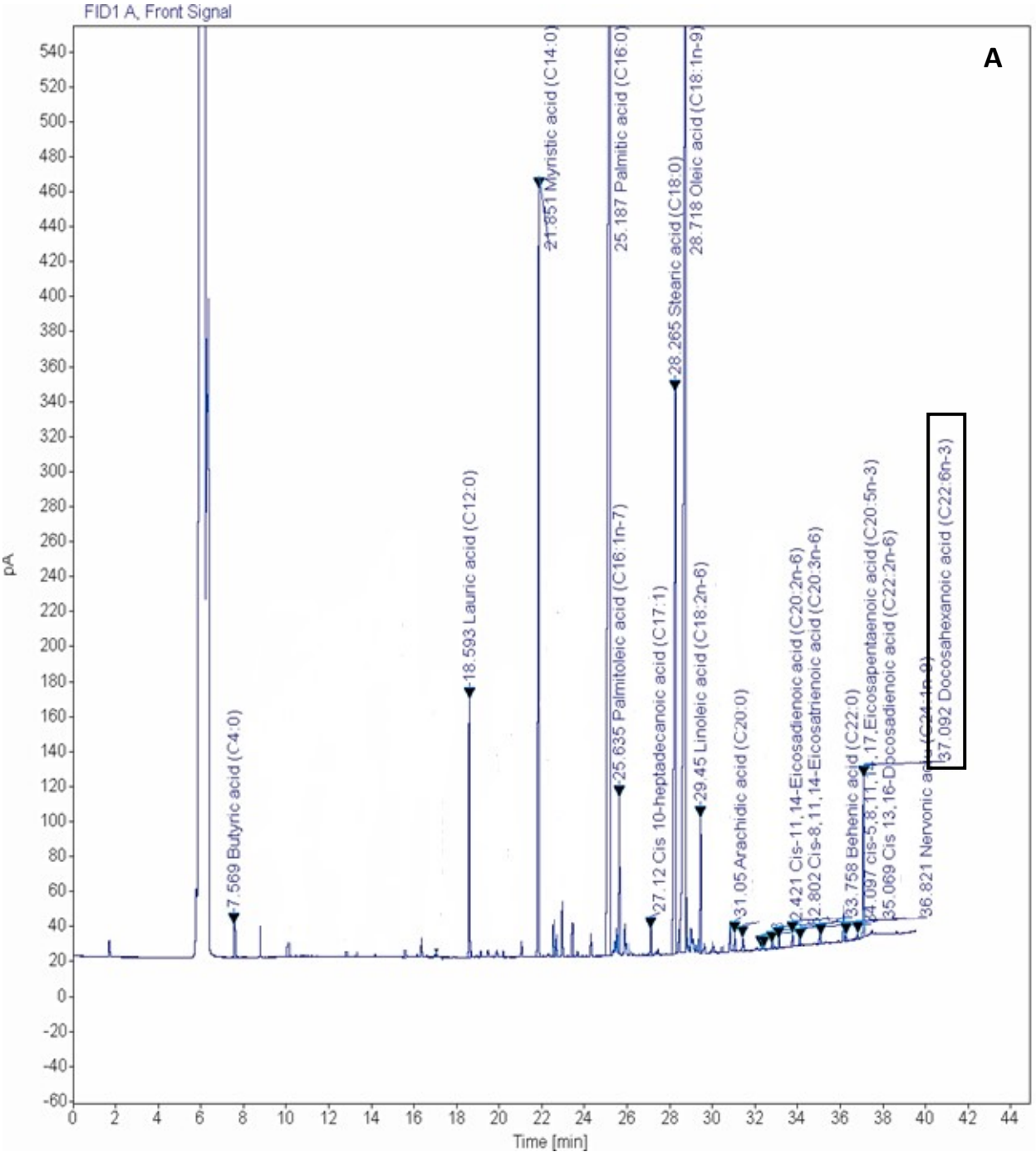
Figure 1- Flowchart of the preparation of DHA fortified kalakand


FID1 A, Front Signal
A
pA
Time [min]
18.797 Lauric acid (C12:0)
22.005 Myristic acid (C14:0)
25.319 Palmitic acid (C16:0)
25.817 Palmitoleic acid (C16:1n-7)
26.831 Heptadecanoic acid (C17:0)
28.372 Stearic acid (C18:0)
28.819 Oleic acid (C18:1n-9)
29.654 Linoleic acid (C18:2n-6)
30.173 Gamma-Linolenic acid (C18:3n-6)
30.666 Alpha-Linolenic acid (C18:3n-3)
31.28 Arachidic acid (C20:0)
33.038 Cis-8,11,14-Eicosatrienoic acid (C20:3n-6)
33.359 Cis-5,8,11,14-Eicosatetraenoic acid (C20:4n-6)
34.001 Behenic acid (C22:0)
34.328 Erucic acid (C22:1n-9)
35.671 Tricosenoic acid (C23:0)
37.081 Nervonic acid (C24:1n-9)
37.438 Docosahexanoic acid (C22:6n-3)


FID1 A, Front Signal
B
pA
Time [min]
18.748 Lauric acid (C12:0)
21.934 Myristic acid (C14:0)
25.23 Palmitic acid (C16:0)
25.731 Palmitoleic acid (C16:1n-7)
28.291 Stearic acid (C18:0)
28.774 Oleic acid (C18:1n-9)
28.555 trans 9-elaidic acid (C18:1nt-9)
29.557 Linoleic acid (C18:2n-6)
30.569 Alpha-Linolenic acid (C18:3n-3)
31.176 Arachidic acid (C20:0)
31.598 Cis-11-eicosenoic acid (C20:1n-9)
33.242 Cis-11,14,17-Eicosatrienoic acid (C20:3n-3)
34.233 cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n-3)
35.216 Cis-13,16-Docosadienoic acid (C22:2n-6)
37.251 Docosahexanoic acid (C22:6n-3)

C

Figure 2- GC-FID chromatograms of algal DHA oil **(A)** and chitosan stabilized DHA nanoemulsion at **(B)** day 0 and **(C)** day 28.


FID1 A, Front Signal
A
pA
Time [min]
7.569 Butyric acid (C4:0)
18.593 Lauric acid (C12:0)
21.851 Myristic acid (C14:0)
25.187 Palmitic acid (C16:0)
25.635 Palmitoleic acid (C16:1n-7)
27.12 Cis 10-heptadecanoic acid (C17:1)
28.265 Stearic acid (C18:0)
28.718 Oleic acid (C18:1n-9)
29.45 Linoleic acid (C18:2n-6)
31.05 Arachidic acid (C20:0)
32.421 Cis-11,14-Eicosadienoic acid (C20:2n-6)
32.802 Cis-8,11,14-Eicosatrienoic acid (C20:3n-6)
33.758 Behenic acid (C22:0)
34.097 cis-5,8,11,14,17,Eicosapentaenoic acid (C20:5n-3)
35.069 Cis 13,16-Docosadienoic acid (C22:2n-6)
36.821 Nervonic acid (C24:1n-9)
37.092 Docosahexanoic acid (C22:6n-3)

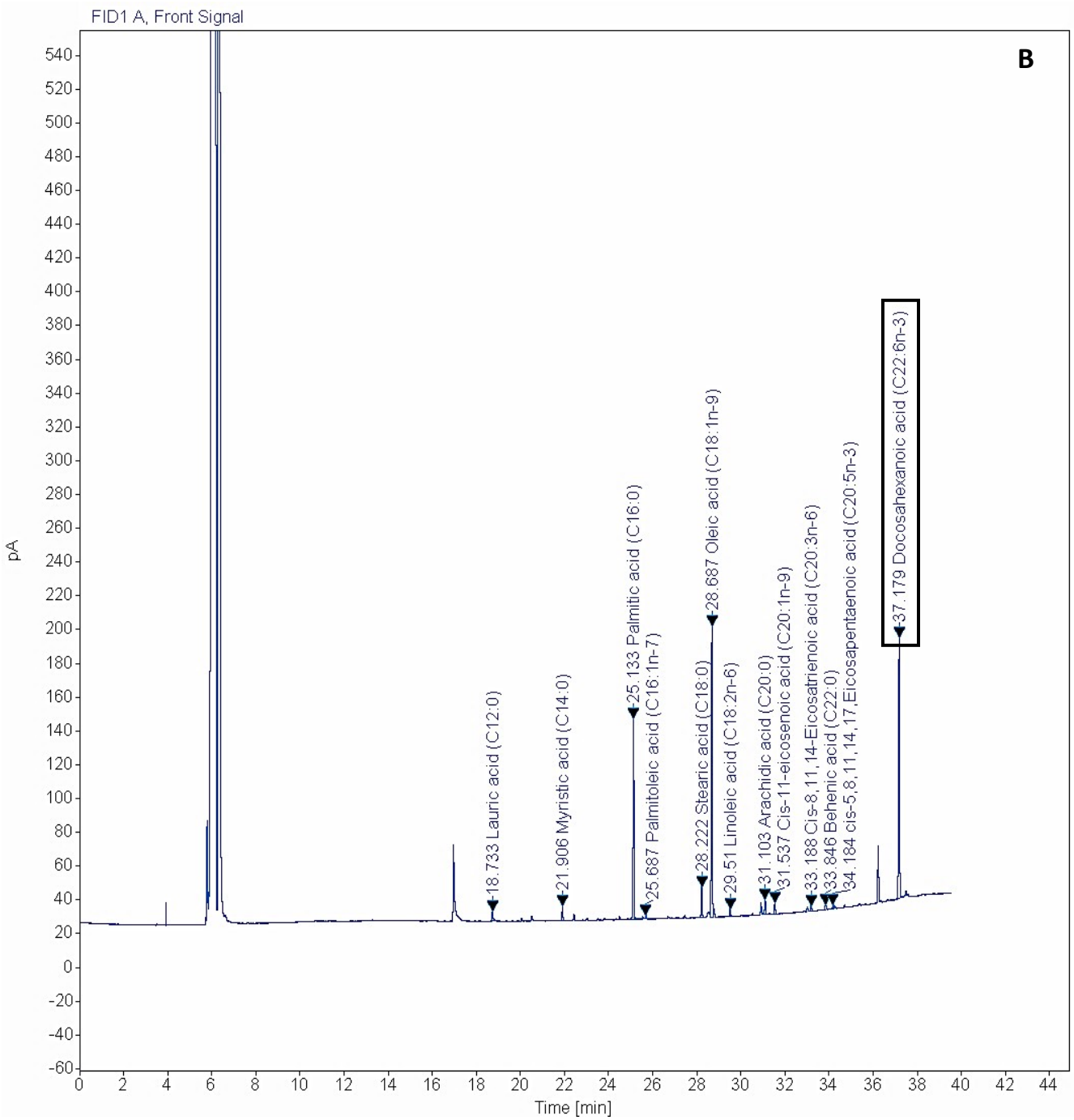


Figure 3- GC-FID chromatograms of DHA fortified kalakand at day 28. **(A)** DHA oil fortified kalakand and **(B)** chitosan DHA nanoemulsion fortified kalakand.