ALTRAFLORA
NATURAL EXTRACTS

Date:
13.04.2026

Application Note — Parsley Seed Supercritical CO_2 Extract

Sample information

Sample name: Parsley_SC-1000_STAGE-1+2_SFE_06-04-2026 (combined extract; 06.04.2026 batch)

Sample type: Single combined $scCO_2$ extract of dried, milled parsley seed, produced by a two-stage isothermal extraction at 40 °C in pure CO_2 (no co-solvent). STAGE 1 (100 bar) and STAGE 2 (400 bar) extracts were pooled to give the TOTAL extract reported throughout this note.

Scientific name: *Petroselinum crispum* (Mill.) Fuss

Number of samples and mass: 1 combined extract from one run; raw-material loading **517.88 g** (moisture 7.60%, dry basis 478.52 g); FAME aliquot 24.9 mg of STAGE 2 extract.

Number of analyses: n = 1 per stage (GC-MS qualitative + GC-FID quantitative on the STAGE 1 and STAGE 2 extracts separately); FAME quantification, TPC and DPPH on the STAGE 2 extract (62.98 % of combined extract mass).

Sample preparation and analytical procedures applied

Analysis date: 07–13 April 2026

Analyst: Dr. Deniz Can Köseoğlu

Sample preparation (GC-MS / GC-FID scan): Extract aliquots (~10 mg in 1 mL *n*-hexane) injected directly without derivatisation.

Sample preparation (FAME): Base-catalysed transesterification (KOH / methanol); the *n*-hexane phase was recovered and injected directly into GC-FID.

GC method: Shimadzu LabSolutions GC-MS/FID dual-detector system, HP-5 capillary column; carrier gas helium, split injection. Identification by NIST mass-spectral library matching on the MS channel; quantification by peak-area normalisation on the FID channel. Method file: *Oak_LONGSCAN_90min.gcm* (STAGE 1) / *Oak_LONGSCAN_MEDSPLIT_90min.gcm* (STAGE 2). The five-FAME quantification used an external five-point calibration against authentic methyl-ester standards (palmitate, linoleate, linolenate, petroselinate-*cis*-6, stearate; R^2 ≥ 0.9969 for all five).

TPC method: Folin–Ciocalteu protocol against a gallic-acid calibration curve (R^2 = 0.99964), absorbance at 765 nm. Results reported as mg gallic-acid equivalents (GAE) per g of dry extract.

DPPH method: DPPH radical-scavenging activity (RSA, %) measured at seven concentrations (10–120 ppm). IC_{50} obtained by linear interpolation of RSA versus concentration at the 50 % scavenging level. Lower IC_{50} = stronger antioxidant.

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Extraction conditions and yields

Dried, milled parsley seed (loading **517.88 g**; raw-material moisture **7.60 %**; dry-feed basis **478.52 g**) was extracted on the AltraFlora SC-1000 supercritical CO_2 system in dynamic mode under two consecutive pressure stages at the same temperature (40 °C) in pure CO_2 with no co-solvent:

- **STAGE 1** — 100 bar / 40 °C / pure CO_2 (light-volatile fraction)
- **STAGE 2** — 400 bar / 40 °C / pure CO_2 (heavy-lipid fraction)

STAGE 1 and STAGE 2 extracts were collected separately, weighed, and pooled to produce the COMBINED extract for which all numbers in this note are reported. The yield breakdown is as follows:

Stage	Extract mass (g)	Yield (% as-received)	Yield (% dry feed)	Mass fraction of COMBINED (%)
STAGE 1 (100 bar / 40 °C)	19.09	3.69	3.99	37.02
STAGE 2 (400 bar / 40 °C)	32.48	6.27	6.79	62.98
COMBINED	**51.57**	**9.96**	**10.78**	**100.00**

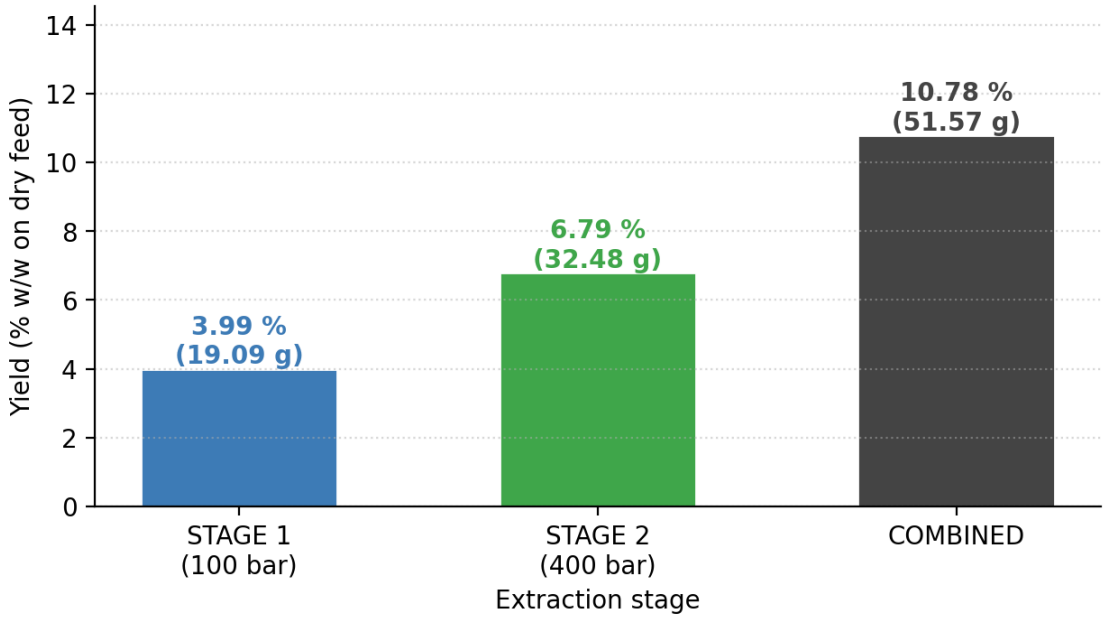


Figure 1. Extraction yield by stage and combined (% on dry-feed basis; extract mass in parentheses).

Composition — GC-MS / GC-FID quantification

GC-MS identifications (NIST library matching, qualitative ID) were paired with co-injected GC-FID peaks via the AltraFlora GC-MS/FID peak-matching procedure, which builds a PCHIP-interpolated RT mapping anchored on shared landmarks. Combined FID Area % values use mass-weighted averaging (w_1 = 0.3702, w_2 = 0.6298). Each compound's row gives the Stage 1 and Stage 2 FID Area % and the resulting mass-weighted Area % for the COMBINED extract.

#	Compound	CAS	R_T MS (min)	R_T FID (min)	FID Area Percentage (%)		
					STAGE 1	STAGE 2	COMBINED
1	α-Pinene	80-56-8	3.89	5.49	0.200	0.639	0.477
2	β-Pinene	127-91-3	4.80	6.66	0.220	0.601	0.460
3	**D-Limonene**	**5989-27-5**	**6.11**	**8.21**	**4.058**	**8.282**	**6.719**
4	Linalool	78-70-6	8.33	10.66	0.114	0.326	0.248
5	**(Z)-Dihydrocarvone**	**3792-53-8**	**11.83**	**14.51**	**3.625**	**3.915**	**3.808**
6	**(E)-Dihydrocarvone**	**5948-04-9**	**12.11**	**14.77**	**8.948**	**9.523**	**9.310**
7	neo-Dihydrocarveol	18675-34-8	12.55	15.32	0.465	0.319	0.373
8	8-p-Menthen-2-ol	619-01-2	13.07	15.87	0.501	0.326	0.391
9	**Carvone**	**99-49-0**	**13.65**	**16.38**	**20.173**	**19.751**	**19.907**

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#	Compound	CAS	R_T MS (min)	R_T FID (min)	FID Area Percentage (%)		
					STAGE 1	STAGE 2	COMBINED
10	8-p-Menthadien-1,2-diol	1946-00-5	17.50	20.51	0.103	0.00	0.038
11	**Myristicin**	**607-91-0**	**24.73**	**27.44**	**1.476**	**2.359**	**2.032**
12	Elemicin	487-11-6	26.20	28.54	0.438	0.508	0.482
13	**1-Allyl-2,3,4,5-tetramethoxybenzene**	**15361-99-6**	**27.74**	**29.99**	**1.884**	**3.076**	**2.635**
14	**Dillapiole**	**23731-65-9**	**28.64**	**31.16**	**40.444**	**29.875**	**33.787**
15	**Apiol**	**523-80-8**	**30.71**	**33.14**	**2.153**	**3.516**	**3.012**
16	Palmitic acid	57-10-3	40.22	50.43	0.344	0.169	0.234
17	Linoleic acid	60-33-3	45.35	51.31	0.00	0.162	0.102
18	Petroselinic acid (*cis*-6)	593-39-5	45.66	51.87	0.00	0.681	0.429
19	Oleic acid (*cis*-9)	112-80-1	46.38	52.57	0.00	0.133	0.084
20	Unknown *n*-alkane	—	60.32	64.69	0.358	0.455	0.419
21	Unknown *n*-alkane	—	64.89	69.45	0.705	0.765	0.743
22	Unknown *n*-alkane	—	69.17	71.40	0.00	0.908	0.572
23	Stigmasterol	83-48-7	71.86	75.34	0.00	0.669	0.421
	Σ identified	**—**	**—**	**—**	**86.21**	**86.96**	**86.68**

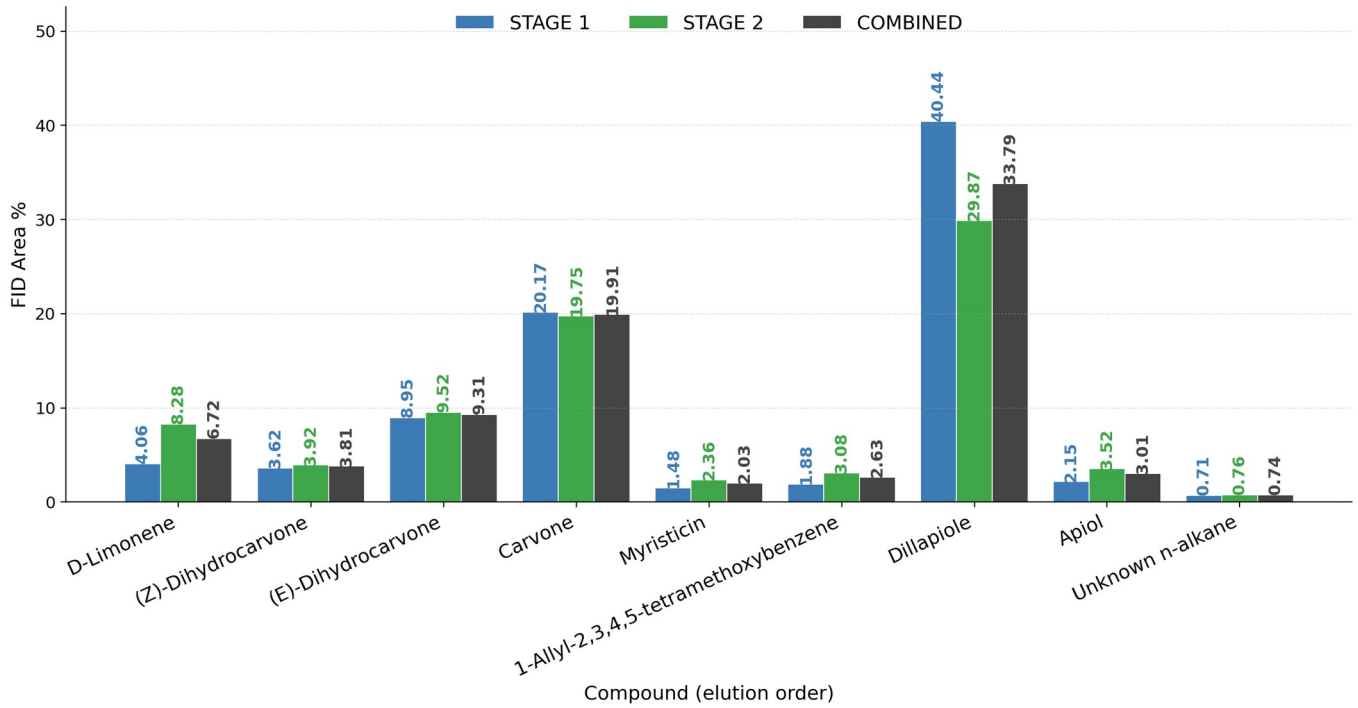


Figure 2. Major identified GC-MS/FID constituents by stage and combined — top 9 by combined FID Area %, in elution order.

Composition — Fatty-acid (FAME) quantification

FAMEs were quantified by GC-FID against an externally-calibrated five-FAME standard curve (one curve per analyte; R^2 ≥ 0.9969 for all five). Quantification was performed on the STAGE 2 derivatised aliquot, which represents 62.98 % of the combined extract mass; the STAGE 1 contribution to combined-extract FA content is small (SCAN FID Area % for free palmitic acid in STAGE 1 = 0.34 %; all other FAs below LOD on the SCAN) and is incorporated as a STAGE-2-mass-fraction scaling. Concentrations are reported as % w/w of dry extract; recovery is reported as mg analyte per gram of dry raw material in the combined extract.

Fatty acid (FAME)	R_T FID (min)	Stage 2 (% *w/w*)	Combined (% *w/w*)	Recovery (mg g^{-1} RM)
C16:0 — Methyl Palmitate	22.98	1.973	1.243	1.339

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Fatty acid (FAME)	R_T FID (min)	Stage 2 (% w/w)	Combined (% w/w)	Recovery (mg g^{-1} RM)
C18:2 ω6 — Methyl Linoleate	27.61	6.281	3.956	4.263
C18:3 ω3 — Methyl Linolenate (α-)	27.75	1.062	0.669	0.721
C18:1 ω12 — Methyl Petroselinate (cis-6)	27.92	42.746	26.922	29.014
C18:0 — Methyl Stearate	28.65	0.759	0.478	0.515
TOTAL FAME	**—**	**52.821**	**33.268**	**35.853**

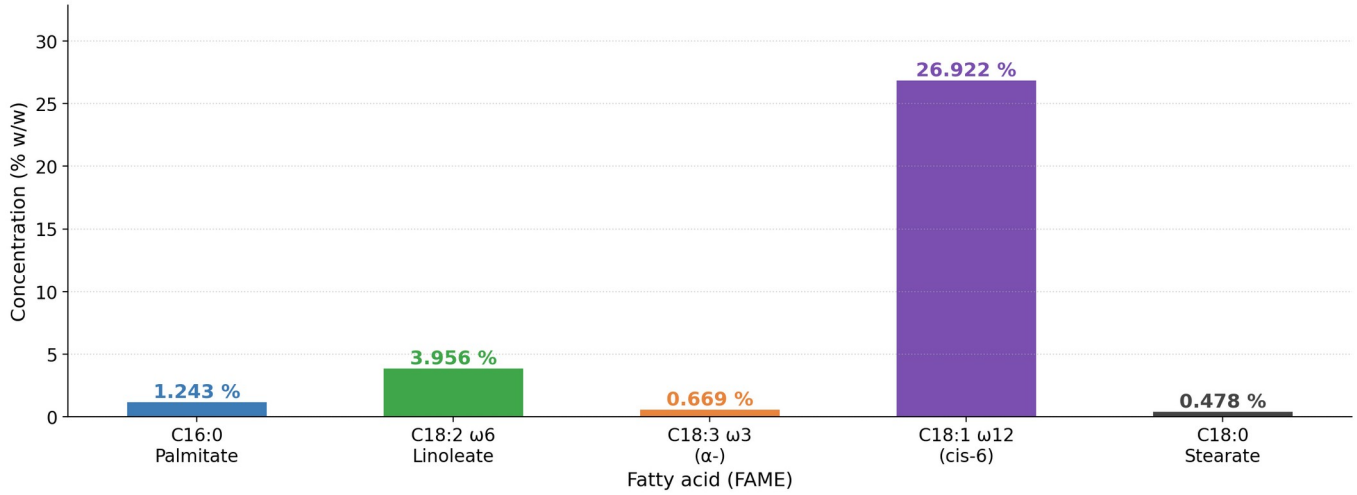


Figure 3. Fatty acid composition of the combined extract (% w/w as methyl ester).

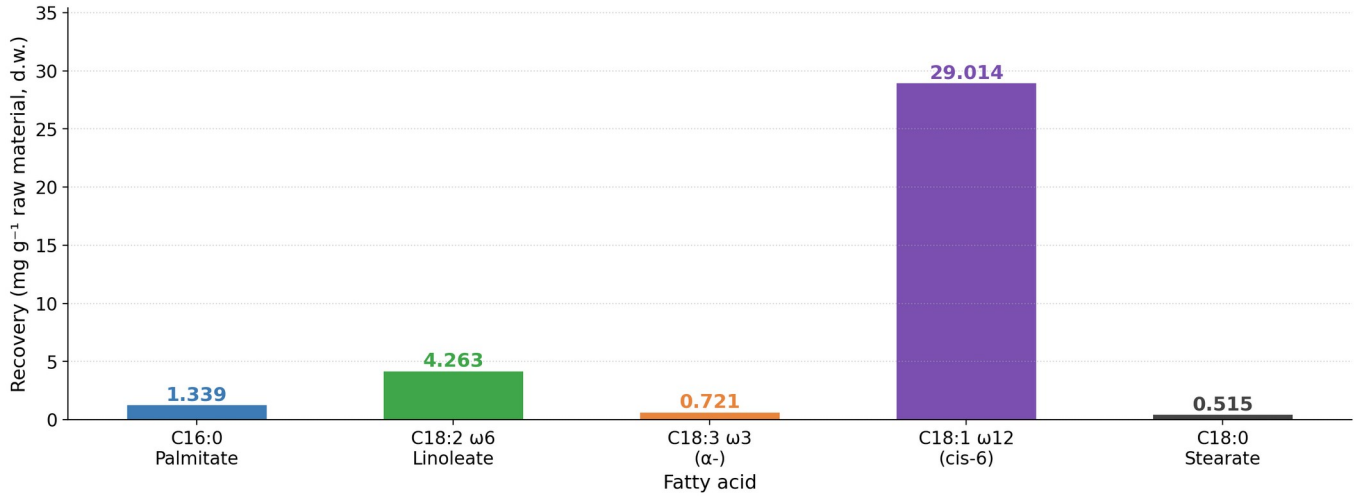


Figure 4. Fatty acid recovery from parsley seed — mg analyte per g dry raw material (combined extract).

Results — TPC and DPPH antioxidant activity

Total phenolic content (TPC) in the STAGE 2 extract is **32.35 mg GAE g^{-1}** (Folin–Ciocalteu, gallic-acid equivalents); applying the STAGE 2 mass fraction (0.6298) gives an estimated **20.38 mg GAE g^{-1}** for the COMBINED extract (the STAGE 1 fraction was not analysed separately for TPC and is conservatively assumed negligible at 100 bar). Recovery into the combined extract is **2.196 mg GAE g^{-1}** of dry raw material. DPPH radical scavenging IC_{50} is **1700 ppm**. Both values are characteristic of a lipid-dominant $scCO_2$ extract: pure-CO_2 extraction (no co-solvent) leaves polar phenolics in the raffinate, and the residual antioxidant activity comes mostly from minor terpenoid and lipid-soluble constituents carried with the seed-oil fraction.

Annotated GC-FID chromatograms

The chromatograms below show the labelled GC-FID traces for STAGE 1 and STAGE 2 of the parsley seed extraction. Each major identified peak is labelled directly with its compound name (peaks below 1 % FID Area in a given stage are

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left unlabelled but remain listed in the table above). Note the compositional shift between stages: the 100-bar STAGE 1 fraction is already lipid-bearing for parsley (dillapiole 40.4 %, carvones 20.2 + 12.6 % FID Area %, and minor petroselinic and palmitic acids), while the 400-bar STAGE 2 extract — the bulk of the combined product mass — dilutes dillapiole to 29.9 % and brings in the full lipid fraction (palmitic, linoleic, petroselinic, oleic; plus stigmasterol at 0.67 %).

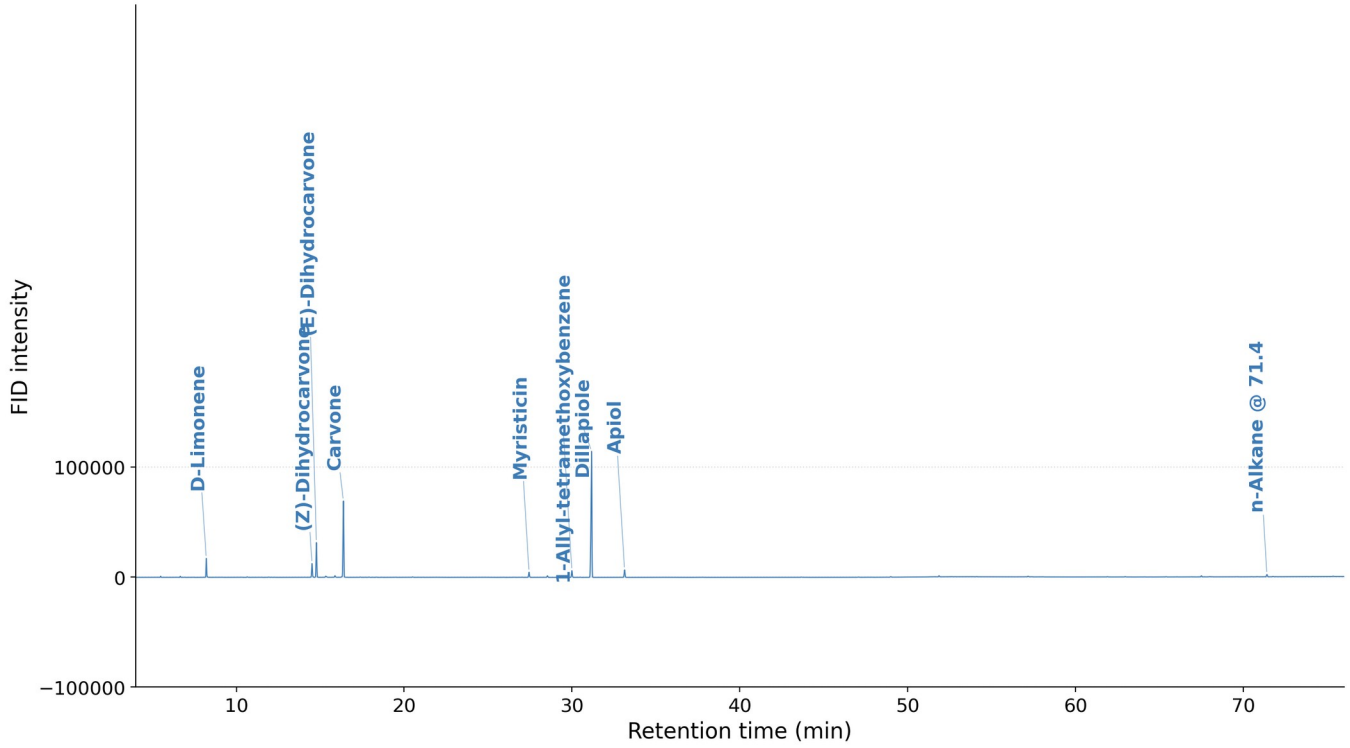


Figure 6. STAGE 1 GC-FID chromatogram of the parsley seed scCO₂ extract (100 bar / 40 °C / pure CO₂; 19.09 g; Σ identified = 86.21 %). Peaks are labelled with the compound name; identifications and FID Area % are tabulated above.

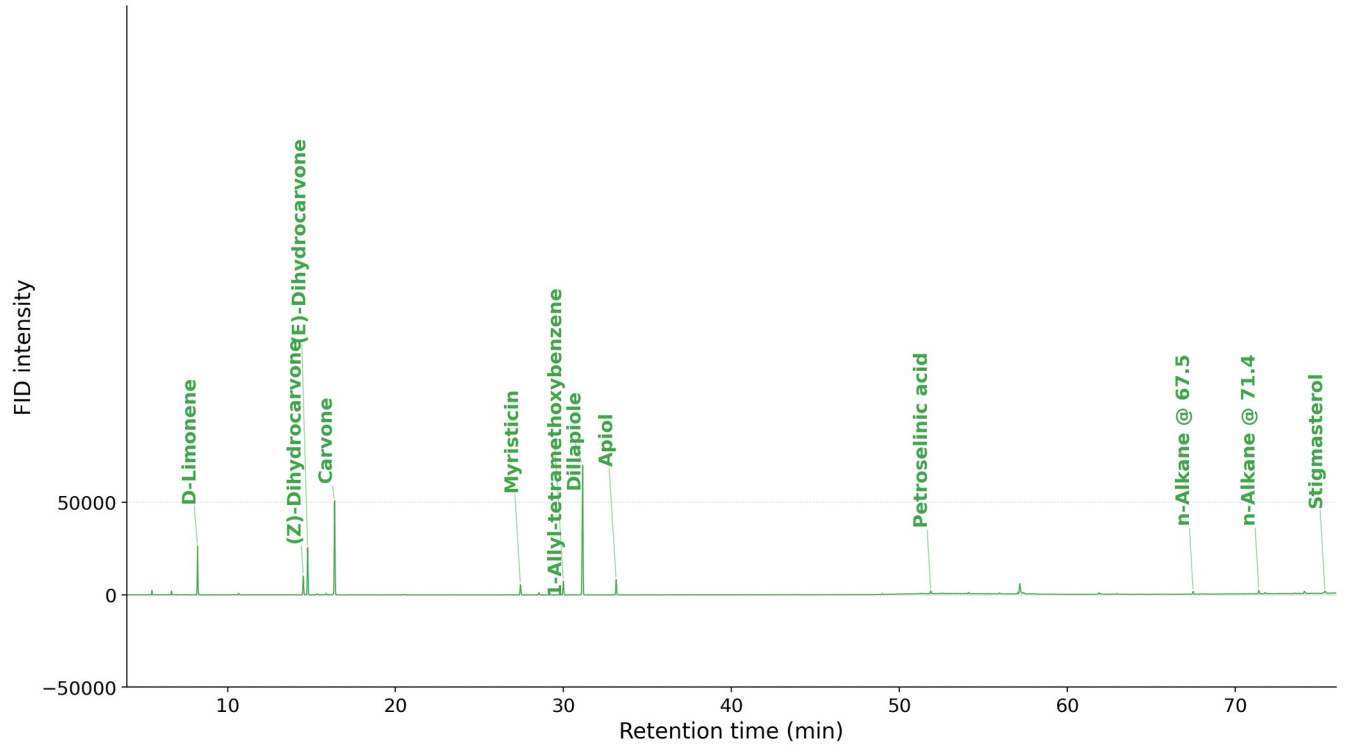


Figure 7. STAGE 2 GC-FID chromatogram of the parsley seed scCO₂ extract (400 bar / 40 °C / pure CO₂; 32.48 g; Σ identified = 86.96 %). Peaks are labelled with the compound name; identifications and FID Area % are tabulated above.

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The chromatogram below shows the GC-FID trace of the FAME-derivatised STAGE 2 extract, with the five quantified fatty-acid methyl esters labelled directly on the peaks. Petroselinate (C18:1 ω12, cis-6) is the dominant FAME (~42 % w/w of the dry extract) and elutes between linoleate and the trace stearate peak; the linoleate and linolenate peaks are well-resolved at 27.61 and 27.75 min respectively. Cumulative identified peak area Σ = 82.23 % in the FAME trace (the remainder are minor unresolved derivatisation by-products and an unidentified C16-region pair at 22.98 min with very small area).

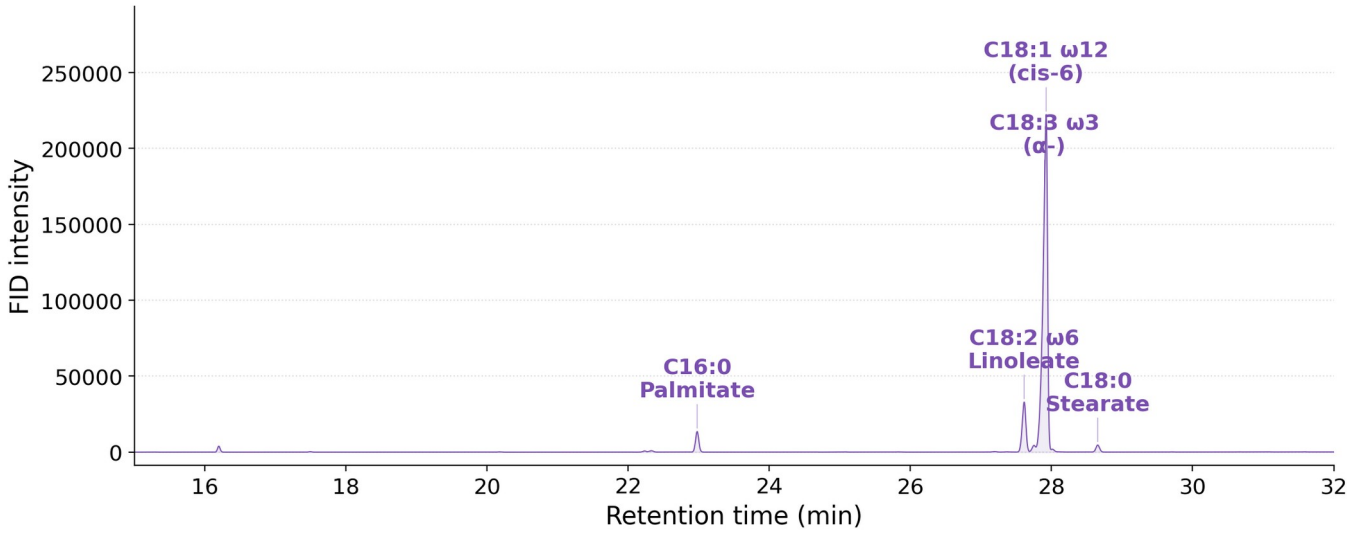


Figure 8. GC-FID chromatogram of the FAME-derivatised STAGE 2 extract. The five peaks identified against authentic FAME-standard retention times are labelled by symbol.

Discussion and application notes

The two-stage $scCO_2$ extraction of parsley seed produces a **combined yield of 51.57 g** (= 10.78 % on dry-feed basis), of which STAGE 2 contributes 62.98 % of the mass. GC-MS/FID profiling identifies **23 distinct constituents** covering ~86.7 % of the combined-extract FID area, dominated by two compositional families: (i) **phenylpropene oxygenates** — dillapiole (33.79 % FID Area in combined), apiol (3.01 %), 1-allyl-2,3,4,5-tetramethoxybenzene (2.63 %), myristicin (2.03 %), and elemicin (0.48 %); and (ii) **monoterpenoid carvones** — carvone (19.91 %), (E)-dihydrocarvone (9.31 %), (Z)-dihydrocarvone (3.81 %). D-Limonene (6.72 %) completes the volatile signature. The lipid fraction — dominated by petroselinic acid (C18:1 ω12, cis-6, the Apiaceae-diagnostic fatty acid) at **26.92 % w/w** of the combined extract — is largely subsumed in the unidentified 13 % of FID area in the SCAN runs (free fatty acids elute as broad late peaks with poor FID response factors); the FAME derivatisation cleanly resolves them and shows that total fatty-acid content of the combined extract is **33.27 % w/w**.

Application notes. The combined extract is a multifunctional ingredient combining (a) a fatty-oil base rich in *cis*-6 petroselinic acid — a monounsaturated C18 acid with reported skin-barrier benefits and emolliency — with (b) the characteristic parsley aromatic-phenylpropene signature (dillapiole + apiol + myristicin) and a monoterpene carvone top-note. Recommended applications include natural-flavour concentrate for food and beverage formulations, cosmetic / personal-care ingredient targeting petroselinate-mediated skin functionality, and nutraceutical or traditional-medicine preparations where the apiol/dillapiole carminative and diuretic activities are desired. The very weak DPPH activity (IC_{50} ≈ 1700 ppm) indicates the extract should not be relied on as a primary antioxidant; this is expected for a pure-CO_2 lipid-rich extract.

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