ALTRAFLORA
NATURAL EXTRACTS

Date: 09.02.2026

Certificate of Analysis – SFE Extract

Sample information

Sample name: Vanilla (fermented beans)

Sample type: SFE extract

Scientific name: *Vanilla planifolia*

Sample amount: 100–300 mg (a total of 15 samples were submitted for analysis)

Customer: -

Applied sample preparation and analysis procedures

Analysis date: 07.02.2026

Analyst: Dr. Deniz Can Köseoğlu

Sample preparation:

Dilution in ethyl acetate (GC) or 1:1 methanol : ethyl acetate (HPLC-DAD) at a concentration of 1–5 mg mL^{-1}

Analysis:

Identification of components via GC-MS

Determination of relative component amounts via GC-FID using peak area normalization

Quantitative analysis of vanillin via HPLC-DAD

Additional data processing:

-

Checked and approved by:

Altra Flora Doğal Bitki Ürünleri San. Tic. A.Ş.
Zafer Mahallesi 1038 Sokak Kapı No:16/A
Merkezaefendi - DENİZLİ
Telefon: 0 538 051 74 05
Gökpınar V.D. 065 083 2816

Professor Abdullah Akdoğan

This report may not be published online or in any other media without the written consent of AltraFlora Natural Extracts Inc. The results presented herein pertain only to the samples submitted for analysis.

Zafer Mahallesi, 1033/2 Sokak, No: 16/A, Merkezefendi/DENİZLİ || www.altraflora.com

Description of laboratory sample preparation and analysis protocols

Supercritical fluid extraction

Fermented vanilla beans were comminuted to achieve a particle size of < 1 mm using a rotating cutter at a blade speed of 28000 rpm. SFE and SWE were carried out using a SuperEx P-30 pilot-scale system with a working volume of 30 L at a maximum operating pressure and temperature of 500 bar and 100 °C, respectively. Dynamic extraction mode with a continuous flow-through of $scCO_2$ was employed to minimize solvent saturation, maximize mass transfer, and improve yield. Extraction was carried out at 400 bar, 40 °C, and 2700 g min^{-1} CO_2 flow until a **Sample-Material (S/M)** ratio of at least **65** was achieved. Samples were collected every 30–60 minutes for analysis throughout a total extraction time of 3 hours.

HPLC-DAD analysis of vanillin

A Shimadzu i-Series 2050-3D system was employed for HPLC analysis. The mobile phase consisted of methanol (A) and milli-Q water (B) eluted at 1 mL min^{-1} isocratically for 30 minutes. The column was thermostated at 30 °C. Vanillin was detected at 275 nm. Initial sample solutions were additionally diluted 20-fold prior to injection. A calibration standard series of vanillin ranging from 10–100 ppm was prepared in 1:1 methanol : ethyl acetate.

GC-FID and GC-MS analysis of extracts

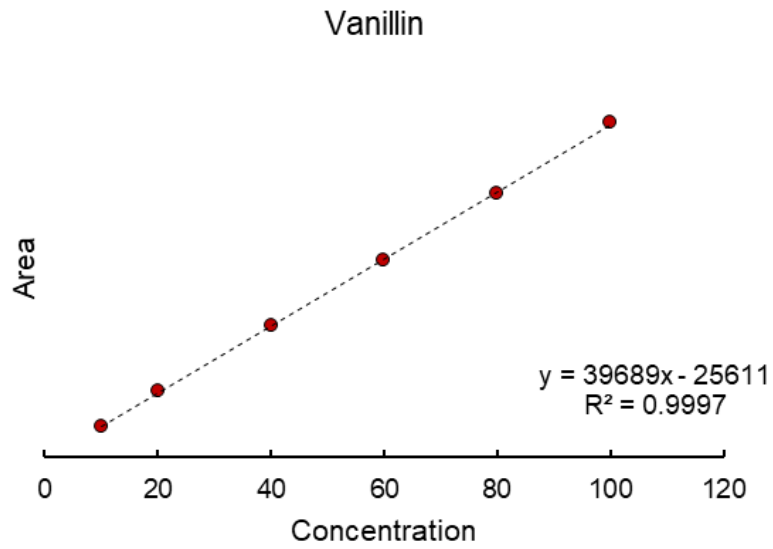
Prior to analysis, extracts were dissolved in ethyl acetate at a concentration of ca. 5 mg mL^{-1} and filtered through a 0.22 µm PVDF membrane filter. The following equipment and parameters were used to analyze the obtained extract solution: Shimadzu GC-2030 gas chromatograph (GC-FID) and a Shimadzu QP2020NX gas chromatograph – mass spectrometer (GC-MS), Restek Rxi-5SilMS non-polar column (30 m × 0.25 mm × 0.25 µm), hydrogen carrier gas at 1.2 mL min^{-1}, and flame ionization detector auxiliary gas flow rates of 32 mL min^{-1} (hydrogen), 30 mL min^{-1} (nitrogen makeup), and 350 mL min^{-1} (air). The injector temperature was 260 °C, while the FID and MS interface were maintained at 320 °C and 280 °C, respectively. The MS ion source was thermostated at 230 °C. The split ratio was 5. The injection volume was 1 µL. Temperature program used a linear increase from 60–300 °C at 3 °C min^{-1}, then 320 °C at 10 °C min^{-1}, which was finally held for 8 minutes. Relative abundances of major constituents were determined semi-quantitatively from their GC -FID peak areas.

Zafer Mahallesi, 1033/2 Sokak, No: 16/A, Merkezefendi/DENİZLİ || www.altraflora.com

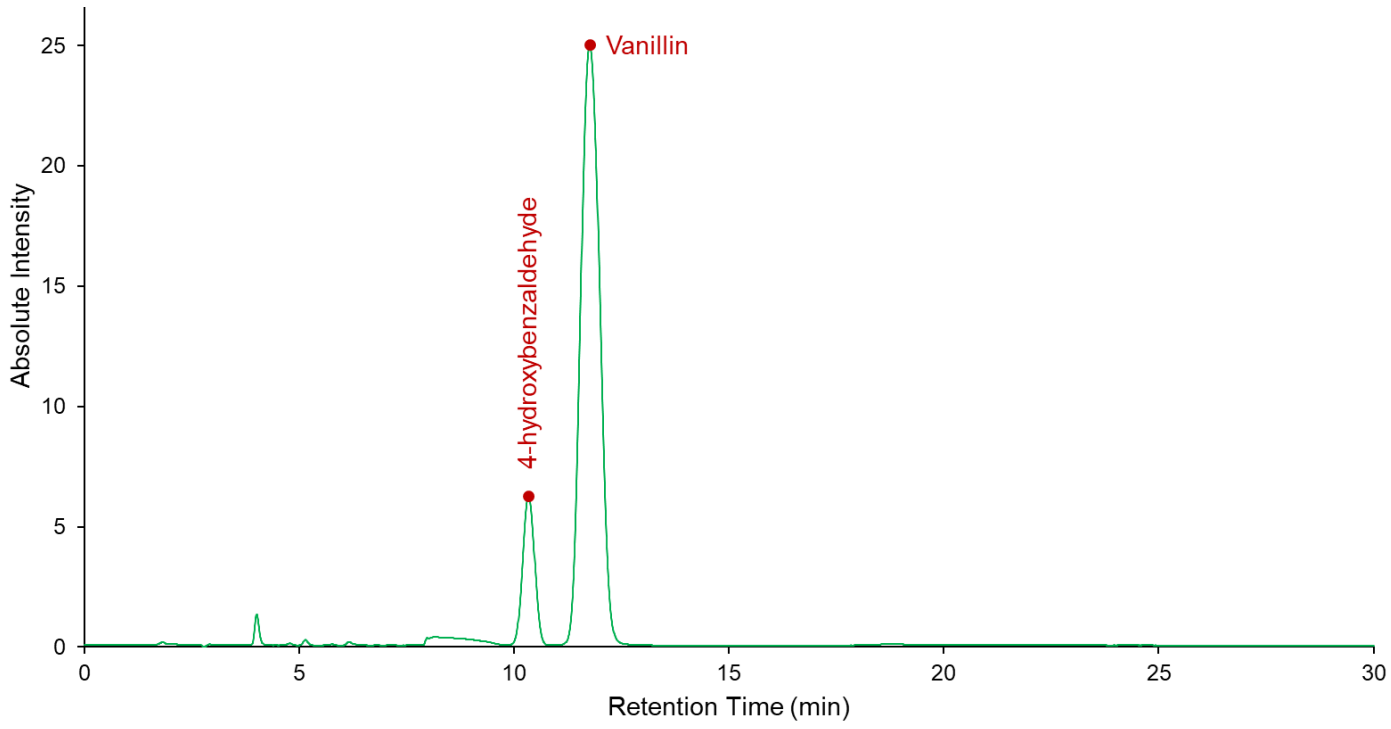
Results – Vanillin calibration data

Name	CAS	RT (min)*	Concentration (ppm)	Peak Area
Vanillin	121-33-5	13.949	10	368599
		13.316	20	788796
		12.959	40	1561951
		12.763	60	2333959
		12.628	80	3122589
		12.422	100	3974026

*Retention Time (min)



Results – Example chromatogram (HPLC-DAD)



SFE extract, fermented vanilla beans *(Vanilla planifolia)*
This report was prepared for -.

Zafer Mahallesi, 1033/2 Sokak, No: 16/A, Merkezefendi/DENİZLİ || www.altraflora.com

Results – Overall yield, vanillin concentration (% extract), and recovery (mg g^{-1} d.w.)

Process Parameters						Vanillin	
***P* (bar)**	***T* (°C)**	**Flow (g min^{-1})**	**Time (min)**	**S/M***	**Yield (% d.w.)**	***C* (% ex.)**	***R* (mg g^{-1} d.w.)**
400	40	2700	30	11	0.76	3.08	0.23
			60	22	6.58	8.80	5.79
			90	33	8.73	8.55	7.47
			120	45	9.64	8.56	8.26
			180	67	10.59	8.70	9.21

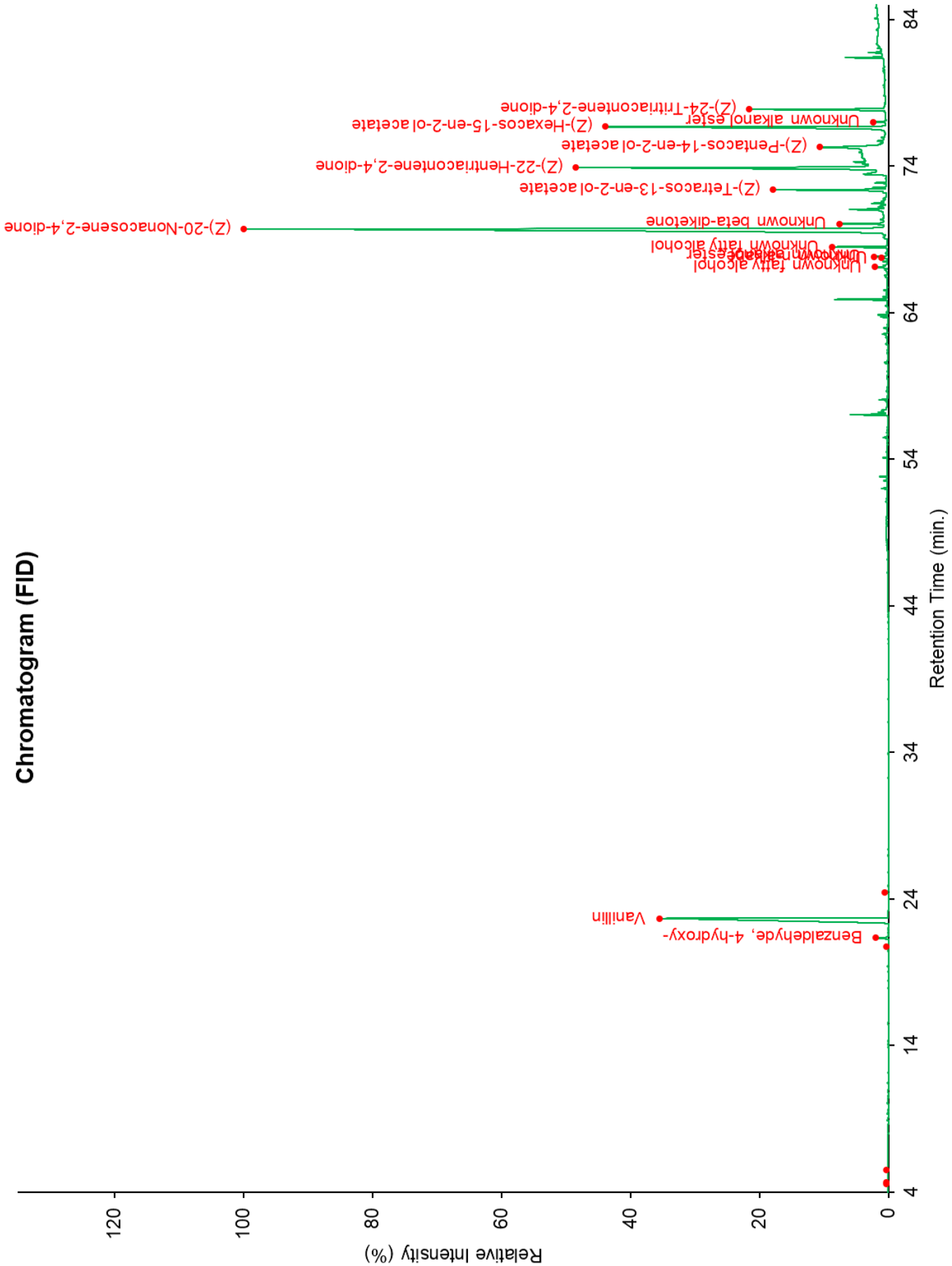
*Sample to Material Ratio

Results – Determination of the relative amounts (%) of key constituents using GC-FID

Name	Chemical Class	Peak area (%) based on extraction time (min)				
		30	**60**	**90**	**120**	**180**
4-Hydroxybenzaldehyde	Vanilla biomarkers	0.00	0.00	0.00	0.35	0.52
Vanillin	**Vanillin**	**4.71**	**10.29**	**9.65**	**12.26**	**13.47**
(Z)-20-Nonacosene-2,4-dione	β-diketone	32.46	32.01	28.20	30.23	29.28
(Z)-22-Hentriacontene-2,4-dione	β-diketone	10.92	9.99	9.11	10.01	9.72
(Z)-24-Tritriacontene-2,4-dione	β-diketone	3.72	3.21	3.13	3.42	3.35
(Z)-Tetracos-13-en-2-ol acetate	Alkanol ester	3.97	4.01	3.50	3.48	3.32
(Z)-Pentacos-14-en-2-ol acetate	Alkanol ester	3.08	3.16	3.07	3.77	3.74
(Z)-Hexacos-15-en-2-ol acetate	Alkanol ester	16.54	15.45	13.90	13.35	12.81
TOTAL DIKETONES:			47.10	45.21	40.44	43.66
TOTAL ALKANOL ESTERS:			23.59	22.62	20.47	20.60

Zafer Mahallesi, 1033/2 Sokak, No: 16/A, Merkezefendi/DENİZLİ || www.altraflora.com

Results – Example chromatogram (GC-FID)



SFE extract, fermented vanilla beans *(Vanilla planifolia)*
This report was prepared for -.