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:

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Checked and approved by:

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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.

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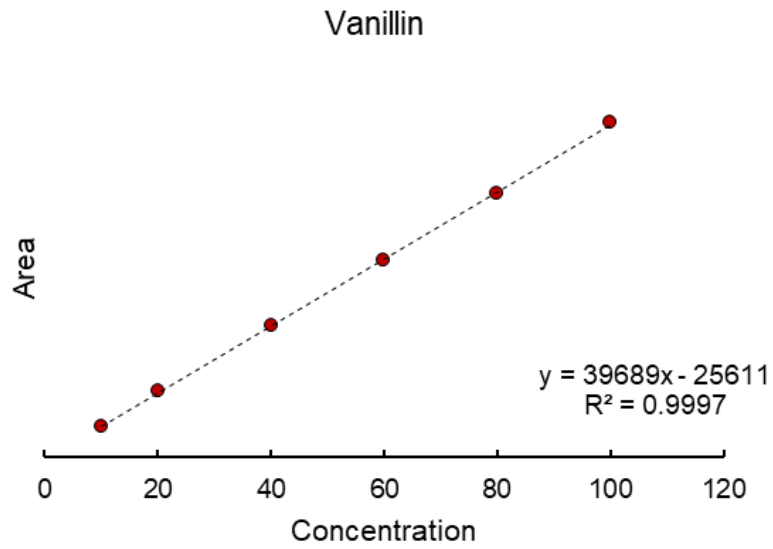
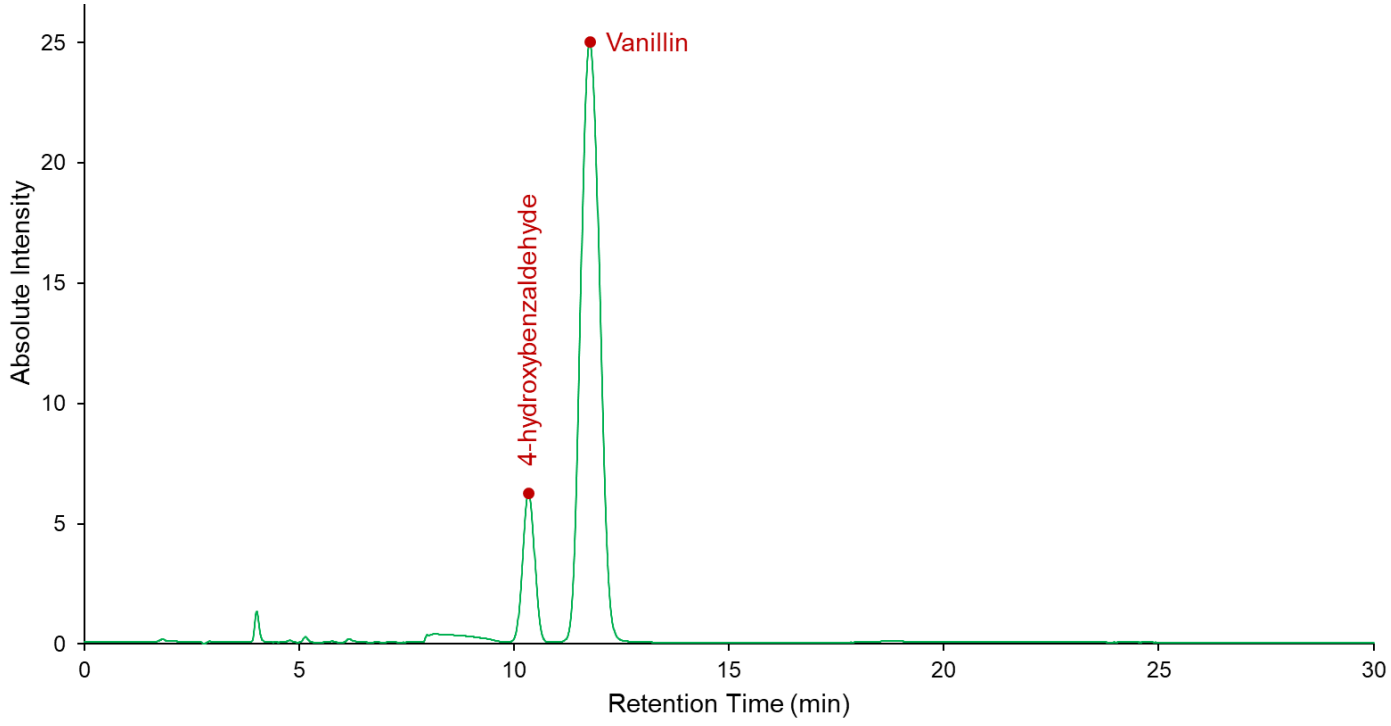
Results – Vanillin calibration data

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)*
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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

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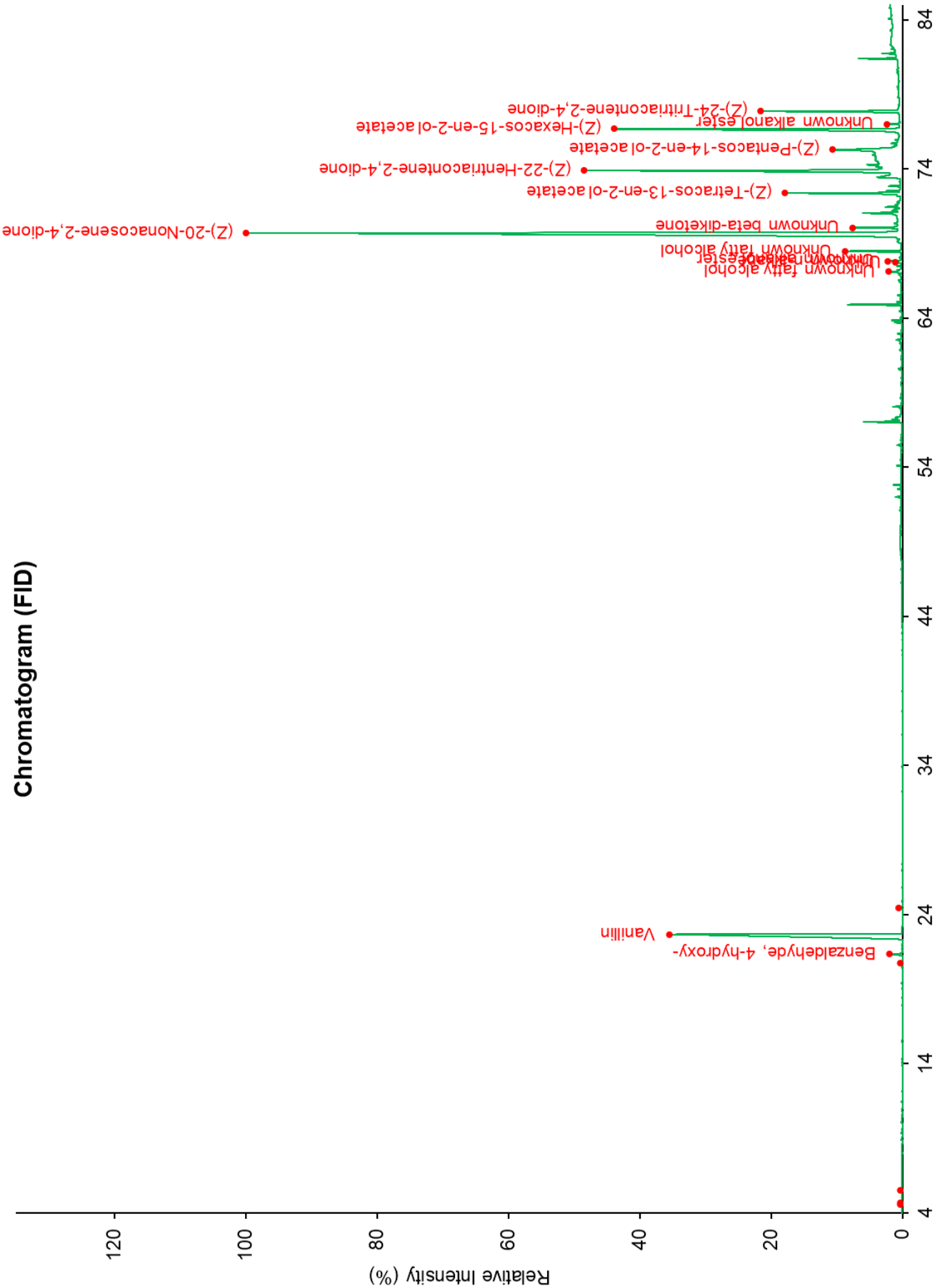
Results – Example chromatogram (GC-FID)



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