RESTEK HPLC

Applications note

Analysis of Vanillin and Ethyl Vanillin in Vanilla Flavors Using Ultra C8 Column

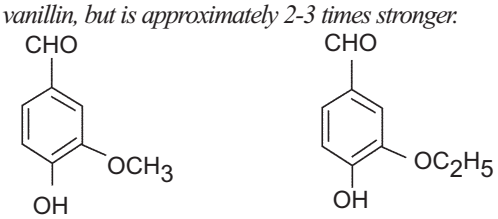
Introduction

Vanilla extracts and flavorings are used in a wide range of food products, including dairy products, beverages, baked goods, and confections. They also are used as a background note or flavor enhancer in products such as sauces, soups, and vegetables. In fact, vanilla is the only flavor with a US Federal Department of Agriculture (FDA) standard of identity (21 CFR 169), which states that vanilla extract must contain the extractive material from 13.35 oz. of vanilla beans per gallon and at least 35% alcohol by volume. If the alcohol content is less than 35%, the solution is "vanilla flavor." Imitation vanilla extract contains natural and artificial flavorings, including vanillin, and has alcohol as the solvent. Imitation vanilla flavors contain vanillin, ethyl vanillin, or other acceptable flavoring materials, with or without "real" vanilla.

The flavor profile of vanilla contains over 250 components, with vanillin present at levels between 0.5-2%. A curing process creates the characteristic odor and flavor by promoting an enzymatic reaction that transforms glucovanillin and other substances into vanillin and other aromatic compounds. In general, vanillin is the component of vanilla extract that is imitated in artificial flavorings. United States Pharmacopoeia (USP) defined vanillin can be synthesized from sources such as lignin, a byproduct of the paper industry. Ethyl vanillin also is synthesized for use in vanilla flavorings, and has a taste very similar to vanillin. Figure 1 shows the chemical structures for vanillin and ethyl vanillin. The aromatic rings on these compounds allow sensitive detection based on their UV absorbance at 254nm.

Figure 1

Ethyl vanillin is a synthetic molecule that has a similar taste to vanillin, but is approximately 2-3 times stronger.



Vanillin **Ethyl Vanillin**

Analysis

In the Association of Official Analytical Chemists (AOAC) Method 990.25, flavor compounds in vanilla extract and artificial vanilla flavor are analyzed using HPLC. The analytes are separated on a C8 column and quantitated by comparing their UV absorbance at 254nm to an external standard. The analysis is performed isocratically, with a mobile phase of acidified water:methanol (90:10) and a flow rate of 2.5mL/min. Even at this high flow rate, the run time is long (approximately 40 minutes) and there is significant broadening of the late-eluting ethyl vanillin peak.

An efficient separation can be performed using the Restek Ultra C8 reverse phase column (150 x 4.6mm, with 5µm particles) and a gradient pump program, such as acidified water:methanol (80:20 to 60:40). By using a gradient program, the run time can be reduced to 25 minutes at a flow rate of 1mL/min (Figures 2 and 3).

Figure 2 shows the analysis of a standard solution of vanillin and ethyl vanillin. Using these parameters, the k' values for vanillin and ethyl vanillin are 4.8 and 6.8, respectively. The resolution of these compounds is excellent under these run conditions. Figure 3 shows the injection of a solution of vanilla extract. In addition to vanillin, there are other aromatic compounds present in the extract. Vanillin is well resolved from these components, and the vanillin can be easily quantitated in this sample. Other flavor components present in vanilla extracts and flavorings also can be analyzed using this procedure.

Figure 2

The Ultra C8 column achieves excellent resolution of vanillin and ethyl vanillin.

Peak List: **Conc.:** (mg/mL)
1. vanillin 0.12
2. ethyl vanillin 0.04

Column: **Ultra C8**
Catalog #: 9103565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Sample:
Inj.: 10µL
Solvent: 40% ethanol

Conditions:
Mobile phase: A:1.2% acetic acid
B:methanol

Time (min.)	%B
0.0	20.0
5.0	20.0
15.0	40.0
20.0	40.0
25.0	20.0

Flow: 1.0mL/min.
Temp.: 28°C
Det.: UV @ 254nm

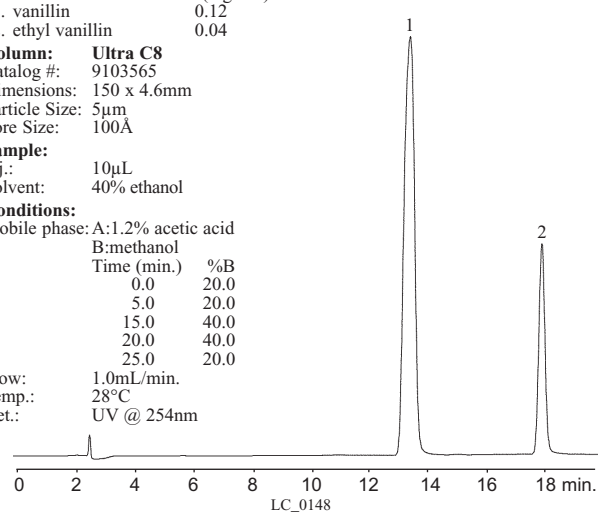


Figure 3

The analysis of a 5% solution of a vanilla extract allows easy quantitation of vanillin.

Peak List:
1.vanillin

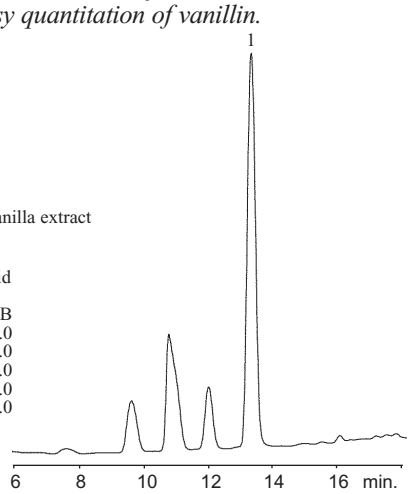
Column: Ultra C8
Catalog #: 9103565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Sample:
Inj.: 10µL
Conc.: 5% solution of vanilla extract
Solvent: 40% ethanol

Conditions:
Mobile phase: A:1.2% acetic acid
B:methanol

Time (min.)	%B
0.0	20.0
5.0	20.0
15.0	40.0
20.0	40.0
25.0	20.0

Flow: 1.0mL/min.
Temp.: 28°C
Det.: UV @ 254nm



HPLC

Summary

The analysis of vanillin and ethyl vanillin in vanilla flavors can be performed efficiently by liquid chromatography using an Ultra C8 column and UV detection at 254nm. The separation can be carried out using either an isocratic or a gradient elution program; however, in the gradient mode the run time is significantly reduced and less solvent is needed. In addition, much less peak broadening is observed with the gradient program, resulting in higher sensitivity for the later-eluting compounds.

References

1. AOAC Official Methods of Analysis (2000), 17th edition, method 990.25.
2. Brandt, Laura. "The Creation and Use of Vanilla," Food Product Design (1996), editorial archives.

Ultra C8, 3µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9103331	9103332	9103333	9103335
50mm	9103351	9103352	9103353	9103355
100mm	9103311	9103312	9103313	9103315

Ultra C8, 3µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9103332-700	9103333-700	9103335-700
50mm	—	9103352-700	9103353-700	9103355-700
100mm	—	9103312-700	9103313-700	9103315-700

Ultra C8, 5µm Columns

Length:	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9103531	9103532	9103533	9103535
50mm	9103551	9103552	9103553	9103555
100mm	9103511	9103512	9103513	9103515
150mm	9103561	9103562	9103563	9103565
200mm	9103521	9103522	9103523	9103525
250mm	9103571	9103572	9103573	9103575

Ultra C8, 5µm Columns with Trident™ Inlet Fitting

Length:		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	—	9103532-700	9103533-700	9103535-700
50mm	—	9103552-700	9103553-700	9103555-700
100mm	—	9103512-700	9103513-700	9103515-700
150mm	—	9103562-700	9103563-700	9103565-700
200mm	—	9103522-700	9103523-700	9103525-700
250mm	—	9103572-700	9103573-700	9103575-700