APPLICATION NOTE

Liquid Chromatography

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Analysis of Vanillin, Ethyl Vanillin, and Coumarin in Vanilla Extract Products by UHPLC with PDA Detection

Introduction

Vanilla is one of the most popular flavoring ingredients in confections, food, and beverages. The high demand for natural vanilla far exceeds the supply from all sources. Due to the limited supply and high prices for natural vanilla, artificial vanilla flavorings are often used. Artificial vanilla flavorings usually contain synthetically produced vanillin and/or ethyl vanillin.

Also, some vanilla extract manufacturers have adulterated vanilla extracts with coumarin in order to increase the perceived vanilla flavor. Coumarin is a phytochemical found in many plant species, it has a sweet herbaceous odor and has been used in food, tobacco and cosmetics as a flavoring and fragrance enhancer.[1] However, coumarin has been shown to cause hepatotoxcity in animals and has been banned for use as a food additive in the U.S. since 1956.[2] It is toxic to the liver and kidneys and causes thinning of the blood.[3]

With the focus on possible vanilla extract adulteration, this application focuses on the HPLC separation and quantitation of vanillin, ethyl vanillin, and coumarin in three store-bought vanilla extracts. Method conditions and performance data, including linearity and repeatability, are presented.

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Experimental

Hardware/Software

For all chromatographic separations, a PerkinElmer Altus™ UPLC® System was used, including the Altus A-30 Solvent/Sample Module, integrated vacuum degasser, A-30m Column Module and A-30 PDA (photodiode array) detector. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 Chromatography Data Software (CDS) platform.

Method Parameters

The LC method parameters are shown in Table 1.

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade and filtered via 0.45-µm filters. The diluent was 60% H_2O: 40% ACN throughout.

The standards were obtained from Sigma Aldrich®, Inc (Allentown, PA) and consisted of vanillin, ethyl vanillin, and coumarin. Individual 1-mg/mL stock solutions of each analyte were prepared using the diluent. From these standards, a Standard Mix (StdMix) was prepared containing 50-ppm of both vanillin and ethyl vanillin, and 25-ppm of coumarin. The lower level standards were then made by serially diluting the StdMix with diluent.

The vanilla extract samples were purchased at a local store. They were labeled Vanilla1, Vanilla2, and Vanilla3. Each vanilla extract was prepared by a 1:50 dilution with diluent.

Table 1. LC Method Parameters.

Column:	PerkinElmer Brownlee™ Analytical C18 5 µm, 4.6 x 150-mm (Part# N9303513)
Mobile Phase:	Solvent A: Water Solvent B: Acetonitrile (ACN) Solvent program: isocratic, 60% A / 40% B
Analysis Time:	5 min.
Flow Rate:	1.0 mL/min. (4100 psi)
Oven Temp.:	35 °C
Detection:	Altus A-30 PDA; wavelength: 280 nm
Injection Volume:	10 µL
Sampling (Data) Rate:	20 pts./sec

Prior to injection, all calibrants and samples were filtered through 0.45-µm filters to remove small particles.

Results and Discussion

Figure 1 shows the chromatographic separation of the StdMix using the optimized conditions described above. The analysis time was under five minutes, with all three analytes well resolved.

Figure 2 shows the overlay of 15 replicate StdMix injections, demonstrating exceptional reproducibility. The retention time %RSD for coumarin was 0.09%.

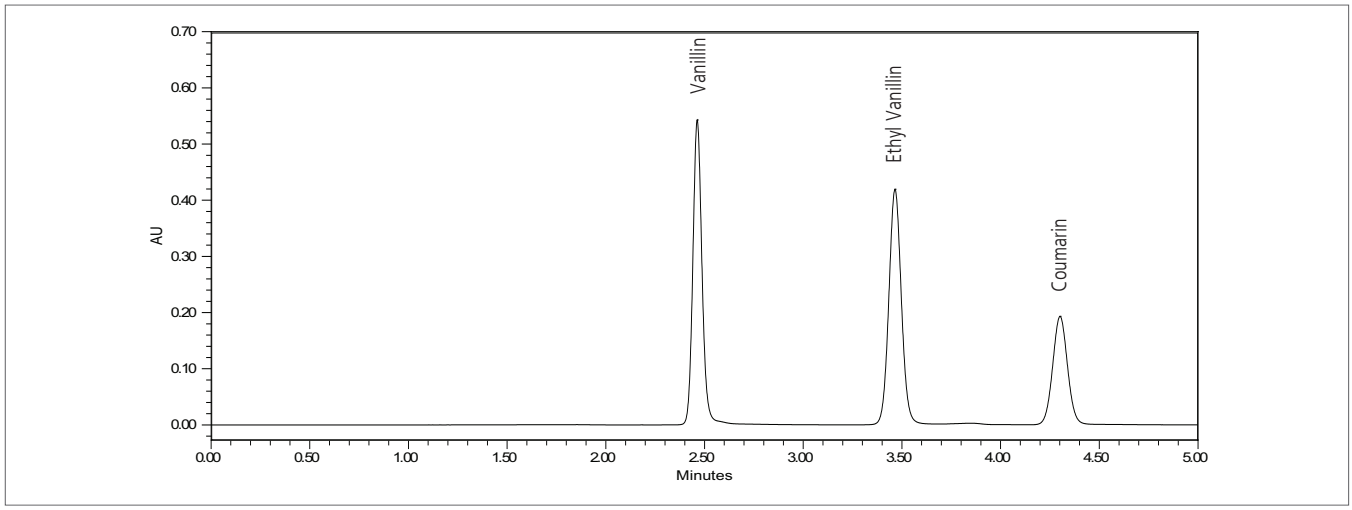

Figure 1. Chromatogram of the 1-mg/mL StdMix.

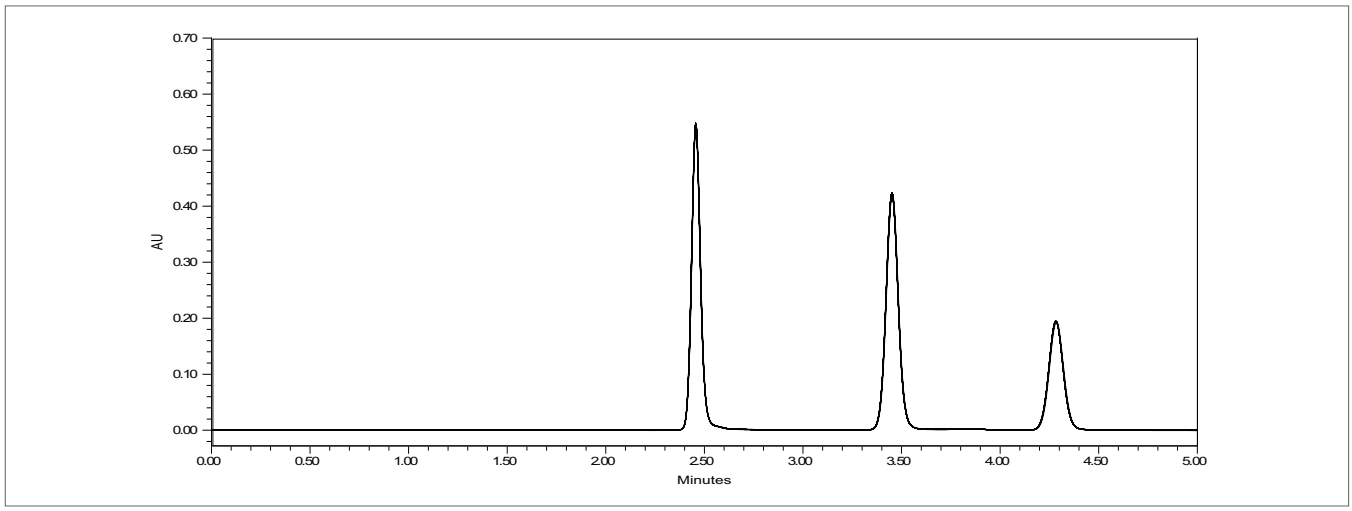

Figure 2. Overlay of 15 replicates of the 1-mg/mL StdMix.

Figure 3 shows the calibration results for vanillin and ethyl vanillin over a concentration range of 0.5 to 50 ppm and for coumarin over a concentration range of 0.25 to 25 ppm. All the standards followed a linear (1st order) fit and had R^2 coefficients > 0.999 (n = 3 at each level).

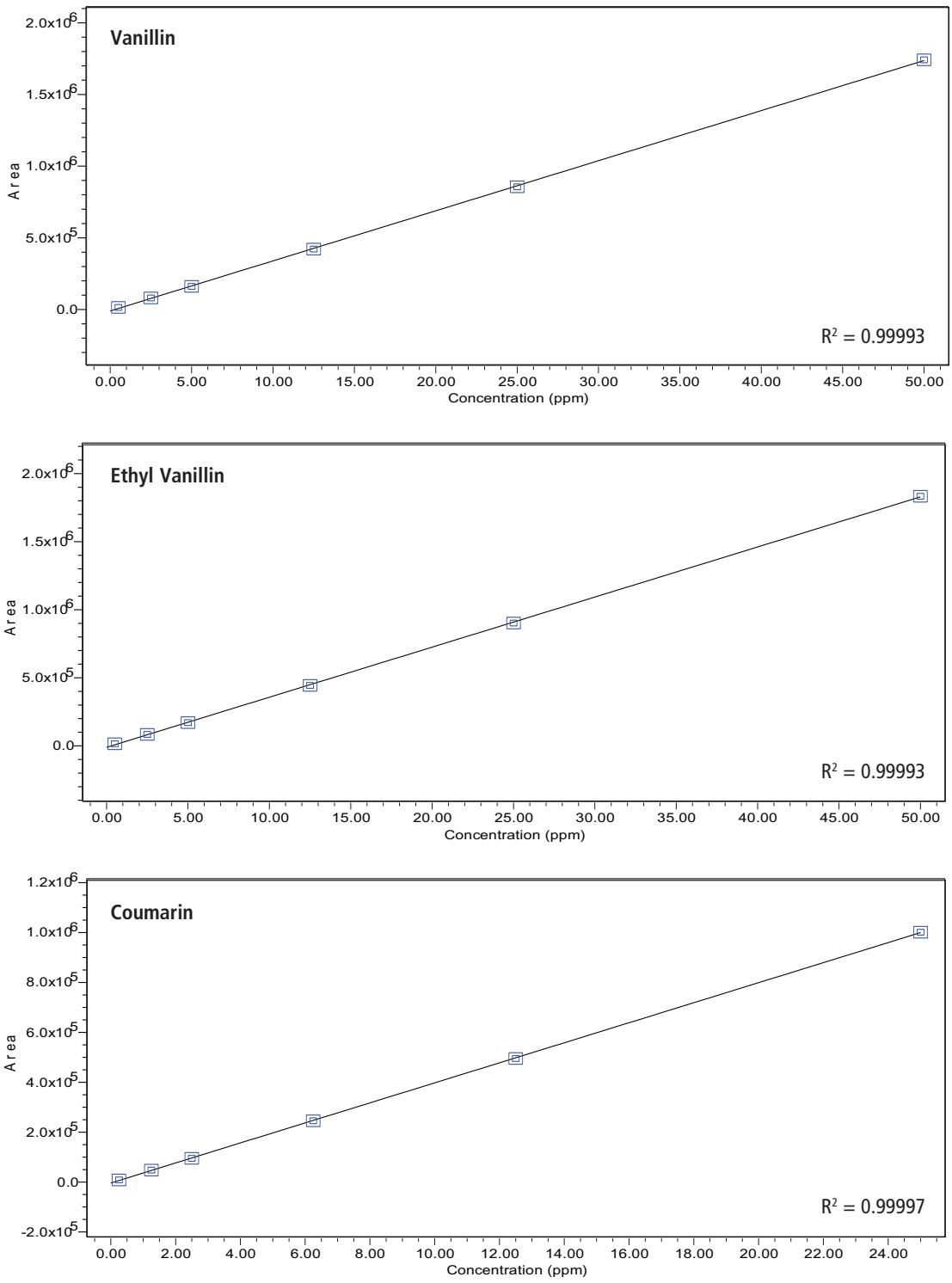

Figure 3. Results of 6-level calibration sets for vanillin, ethyl vanillin, and coumarin.

As listed in Table 2, LOD (limit of detection) and LOQ (limit of quantitation) levels were established for each analyte, based upon a s/n of > 3/1 for the LOD and >10/1 for the LOQ.

Table 2. LOD and LOQ values for the three analytes.

Analyte	LOD (ppb)	LOQ (ppb)
Vanillin	10.5	35.0
Ethyl Vanillin	14.8	45.1
Coumarin	9.7	32.4

Using the same chromatographic conditions, three vanilla extract samples were analyzed: two pure vanilla extracts and one imitation vanilla extract. The results compared to the StdMix are shown in Figure 4. As expected, it can be observed that all three vanilla extracts contain vanillin. Per chromatographic inserts, it can be observed that ethyl vanillin doesn't appear in any of the three samples. For Vanilla1 and Vanilla2, although a peak corresponding to coumarin was detected, it was below quantitable limits in both samples.

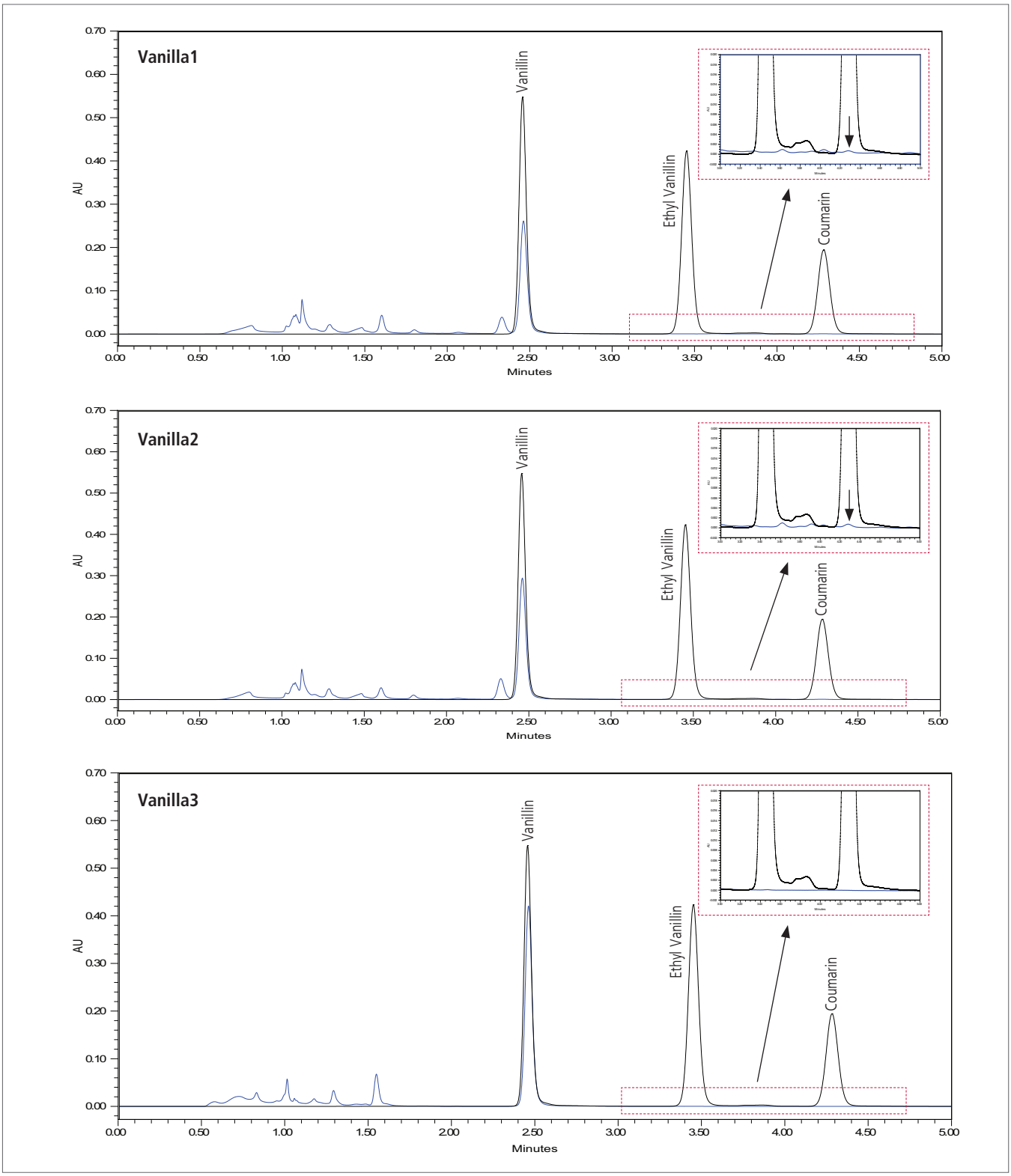

Figure 4. Overlaid chromatograms of Vanilla1, Vanilla2, Vanilla3 (blue) and 1-mg/mL StdMix (black).

Based on the standard calibration plots, the quantitative results for each vanilla extract sample are shown in Table 3. All three vanilla extracts contained appreciable amounts of vanillin and the imitation vanillia extract contained significantly more vanillin than the two pure vanilla extract samples.

Table 3. Quantitative Results.

Sample	Labeled Artifical	Vanillin (ppm)	Ethyl Vanillin (ppm)	Coumarin (ppm)
Vanilla1	No	1176	ND	NQ
Vanilla2	No	1305	ND	NQ
Vanilla3	Yes	1865	ND	ND

Conclusion

This work has demonstrated the effective chromatographic separation of vanillin, ethyl vanillin, and coumarin using a PerkinElmer Altus UPLC® System with PDA detection. The results exhibited very good retention time repeatability as well as excellent linearity over the tested concentration ranges.

From a food quality perspective, there is an ever growing emphasis on food monitoring, especially regarding adulteration. With this in mind, this work provides an effective chromatographic method for the analysis of vanillin, ethyl vanillin and coumarin in vanilla extracts.

References

1. C. Sproll, W. Ruge, C. Andlauer and R. Godelmann. *Food Chemistry.* 109, 462, 2008.
2. L. de Jager, G. Perfetti and G. Diachenko. *Journal of Chromatography A.* 1145, 83, 2007.
3. Scientific Committee on Food, SCF/CS/ADD/FLAV/61 final 29/9/99.

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