

Fast Determination of Vanillin and its Synthesis Precursor by HPLC

INTRODUCTION

Vanillin (Figure 1A) is the primary chemical component of the extract of vanilla bean. Natural vanilla extract is a mixture of several hundred compounds in addition to vanillin. Artificial vanilla flavoring is a solution of pure vanillin, usually of synthetic origin. Synthetic vanillin and ethyl vanillin (Figure 1B) are used as flavoring agents in foods, beverages, and pharmaceuticals. Ethyl vanillin is more expensive and has a stronger flavor. Compared to vanillin, ethyl vanillin has an ethoxy group (-O-CH₂CH₃) rather than a methoxy group (-O-CH₃). The largest single use of vanillin is for flavoring.

Vanillin was first synthesized from eugenol (found in oil of clove) and later synthesized from lignin-containing sulfite liquor, a byproduct of wood pulp processing in paper manufacture. While some vanillin is still made from lignin wastes, today most synthetic vanillin is synthesized in a two-step process from the petrochemical precursors: guaiacol (Figure 2A) and glyoxylic acid (Figure 2B).¹

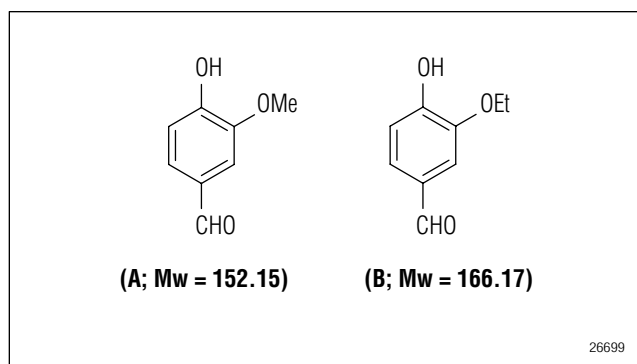


Figure 1. Structures of (A) vanillin and (B) ethyl vanillin.

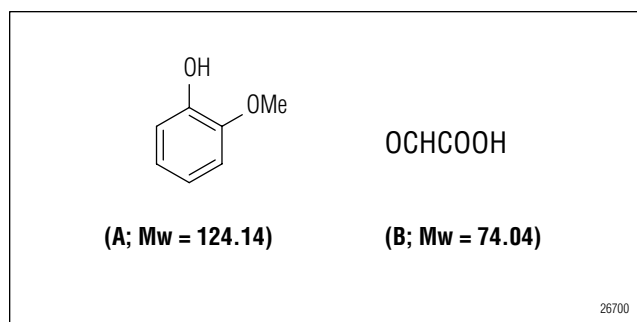


Figure 2. Structures of (A) guaiacol and (B) glyoxylic acid.

This application update describes a fast HPLC method to determine vanillin and its synthetic precursors for process monitoring and quality control of the two-step synthetic process. An Acclaim® PA2 column (3 mm × 75 mm format) is used for fast analysis. The total analysis time is 9 min. The PA2 column delivers good retention for the polar compounds in these samples, which are difficult to determine with a typical C18 column. This method can be used to analyze the solutions from the first (condensation) and second (oxidation) synthetic steps of both vanillin and ethyl vanillin synthesis. Most byproducts during the synthesis process can be separated and identified using MS with the same chromatographic conditions.

EQUIPMENT

UltiMate® 3000 HPLC
 DGP 3600M pump with SRD 3600 degasser
 WPS 3000 Micro TSL autosampler
 TCC 3000 thermostatted column compartment
 VWD-3400 UV-vis detector with 2.5 µL semi-micro cell
 MSQ™ Plus single quadrupole mass spectrometer with APCI source.
 Chromeleon® 6.80 SP5 Chromatography Data System with MS option

REAGENTS

Water, Milli-Q® water from Milli-Q Gradient A10
 Acetonitrile (CH₃CN), Fisher, HPLC grade
 Acetic acid, reagent grade, (AR, analytical pure grade, China)

PREPARATION OF SAMPLES

Reaction solutions (both condensation and oxidation) are diluted 1:1000, using 1% acetic acid. Prior to injection, filter the solution through a 0.45 µm filter (Millex®-HV).

CONDITIONS

CHROMATOGRAPHIC CONDITIONS

Column: Acclaim PA2, 3 µm, 3.0 × 75 mm (P/N 066277)
 Temperature: 30 °C
 Inj. Volume: 1 µL
 Mobile Phase: A: Water
 B: Acetonitrile (CH₃CN)
 C: 10% Acetic acid (HAc)
 UV Detection: Absorbance at 280 nm
 MS Detection:
 Ionization Mode: APCI
 Operating Mode: Negative Scan
 Probe Temperature: 550 °C
 Corona: 10 µA
 Mass Range: 65 ~ 300
 Scan Time: 0.5 sec
 Cone Voltage: 50 V
 Nebulizer Gas: Nitrogen at 45 psi

Gradient Table:

Time (min)	Flow Rate (mL/min)	Water (%)	CH ₃ CN (%)	10% HAc (%)	Curve
0.0	0.5	85	5	10	
1.0	0.5	85	5	10	5
5.0	0.5	40	50	10	5
6.0	0.5	40	50	10	5
6.1	0.5	85	5	10	5
9.0	0.5	85	5	10	5

RESULTS AND DISCUSSION

The reaction between guaiacol and glyoxylic acid will yield vanillyl mandelic acid (Figure 3A) as the precursor for the second step of vanillin synthesis. Two major byproducts (Figures 3B, 3C) also form in this step, along with the remaining raw material. The vanillyl mandelic acid and the two byproducts exhibit significant peak tailing and weak retention on a typical C18 column, making their separation difficult. Polar-embedded stationary phases are ideal for polar acidic analytes, like those determined here, because these phases can be used at low pH, where there is enhanced hydrophobic retention due to the suppression of analyte ionization. The PA2 column is an amide-embedded, reversed-phase column with enhanced hydrolytic stability in a wide pH range (pH 1.5–10), allowing it to be used with high concentrations of acetic acid to promote retention of acidic compounds. This makes the Acclaim PA 2 column an ideal choice for this application.

Figures 4B and 5B show chromatograms of the first reaction step of vanillin and ethyl vanillin respectively. Vanillyl mandelic acid and two possible byproducts (Figure 4B, peaks 4 and 6) have good resolution. Guaiacol and other impurities that have less polarity were separated in the same run.

The same conditions can be used to analyze the outcome of the second reaction step. Figures 4A and 5A show chromatograms for vanillin and ethyl vanillin respectively. This method can also be used to assess final vanillin product quality.

Other buffers were used during method development. A phosphate buffer with a pH in the range of 3 ~ 3.4 will yield similar results as 1% HAC. In fact, the phosphate buffer delivers slightly better peak shape and peak response, but it is not a MS compatible buffer and because pH adjustment with phosphoric acid is needed, it is not as easy to prepare as the 10% HAC. Phosphate buffers with pH values greater than 4.0 had poor retention for peaks 4 and 5. Lower concentrations of HAC were tried, but they were not as good as 1% because of poor retention and peak shapes for peaks 4 and 5.

Confirming Peak Identity Using MS Detection

Monitoring a synthetic process and the results of adjustments to that process, allows for optimization to achieve the highest yield of desired product(s). To better understand a synthetic process and to optimize chromatographic conditions, it is important to identify the desired products as well as reaction byproducts. Using MS detection after the UV-vis detector can assist in identifying synthetic byproducts and other impurities.

The main peaks in Figures 4A and 5A (peak 2) are vanillin and ethyl vanillin peak with molecular ions of $m/z = 151$ and 165 , respectively. The small peak (peak 7) in Figures 4B and 5B is also vanillin and ethyl vanillin which has the same retention times and molecular ions as peak 2 in Figures 4A and 5A. The other two small peaks in Figures 4A and 5A can be also identified using MS detection. Peak 1 in Figure 4 has a molecular ion at $m/z = 225$, which may be the structure in Figure 3E. Peak 3 has a molecular ion at $m/z = 179$, which may be the structure in Figure 3D.

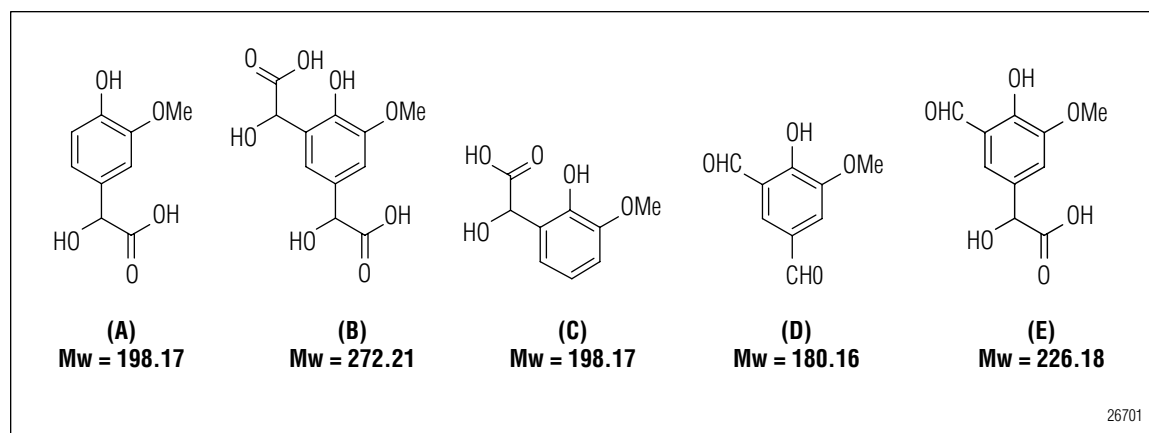


Figure 3. Structures of vanillyl mandelic acid (A) and byproducts.

The main peak (peak 5) in Figures 4B and 5B is vanillyl mandelic acid and ethyl vanillyl mandelic acid with molecular ions at $m/z = 197$ (Figure 3A) and 211 (ethyl), respectively. Peaks 4 and 6 in Figure 4B are byproducts of vanillyl mandelic acid with molecular ions of $m/z = 271$ and 197 respectively. These molecular ions are consistent with the structures shown in Figures 3B and 3C. In Figure 5B, molecular ions of $m/z = 285$, 211 are found for byproducts of ethyl vanillyl mandelic acid. Peak 8 is the remaining raw material guaiacol, with a $m/z = 123$ (Figure 4B). In Figure 5B, peak 8 is guaethol, having a $m/z = 137$.

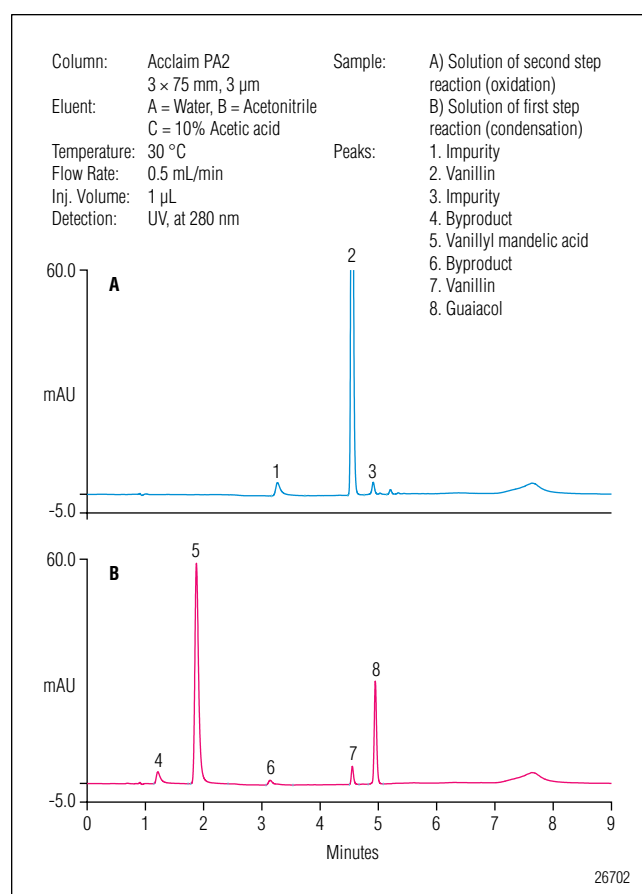


Figure 4. Chromatograms of solutions of the vanillin synthetic process A) solution of the second step of the synthetic process. Peak 2 is vanillin, and peaks 1 and 3 are impurities as described in the text, and B) solution of the first step of the synthetic process.

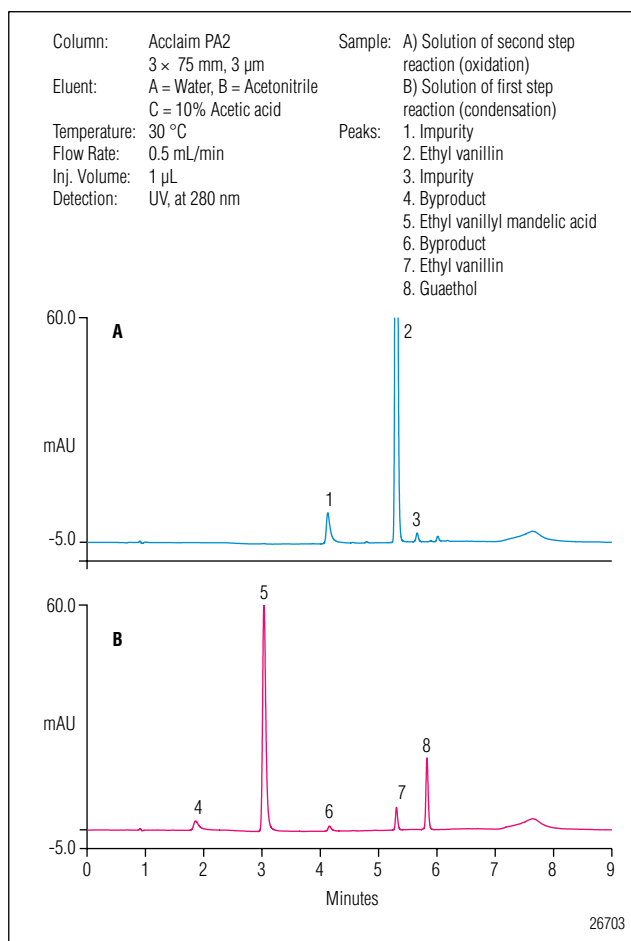


Figure 5. Chromatograms of solutions of ethyl vanillin synthesis process A) solution of the second step of the synthetic process. Peak 2 is ethyl vanillin, and peaks 1 and 3 are impurities, and B) solution of the first step of the synthetic process.

The peaks with lower polarity, such as guaiacol, were difficult to detect by MS detection with the low pH mobile phase. To achieve higher sensitivity for MS detection, a higher pH mobile phase could be used or the acetic acid could be removed from the mobile phase. These changes will cause some loss of resolution and peak tailing.

REFERENCES

1. Dignum, M.J.W.; Kerler, J.; Verpoorte, R. Vanilla Production: Technological, Chemical, and Biosynthetic Aspects, *Food Reviews International*, **2001**, 17 (2): 119–120.

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