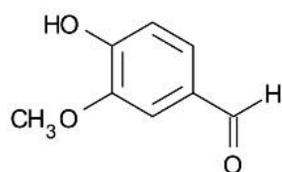


Introduction

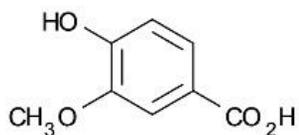
Vanilla is a natural flavouring derived from orchids of the genus *Vanilla* and is one of the most widely used flavouring ingredients in foods and beverages. The cultivation and processing of vanilla is very labour-intensive because the flowers are hand-pollinated and the pods are hand-picked. Vanilla extract is produced by splitting and soaking the vanilla pods in a mixture of water and ethanol. As a result of the production process, and of the relatively low production volumes world-wide, vanilla is considered the second most expensive spice in the world after saffron.

The high cost and low availability of natural vanilla extract means that much cheaper artificial vanilla extract is readily available. Vanillin is the main component of vanilla extract, but whereas natural vanilla extract contains many additional flavour compounds, artificial vanilla extract is often simply a solution of synthetic vanillin. Due to the high demand and limited supply of natural vanilla, confirmation of quality and authenticity and detection of adulterated sources is of high importance.

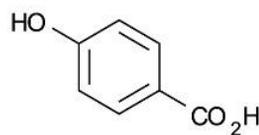
This application note describes a method for the rapid separation of vanillin and seven related compounds under fast UHPLC conditions, that could be utilised for the high throughput screening of vanilla extract authenticity. Small 1.7 μm particles enabled the flow rate to be increased without the efficiency loss associated with 3 or 5 μm particles, thereby speeding up the analysis without losing resolution. The increased back-pressure associated with higher flow rates were not a problem when using a UHPLC compatible instrument. Performing this method on a 50 x 3.0 mm ACE 1.7 μm C18-Amide column at a flow rate of 1.3 mL/min allowed for the full separation of all compounds in less than 1.5 minutes, while traditional HPLC analysis on a 150 x 4.6 mm column at 1.0 mL/min would have taken over 10 minutes.



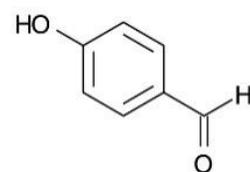
Vanillin



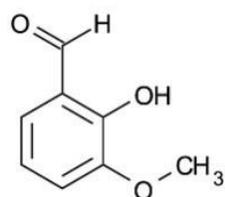
Vanillic acid



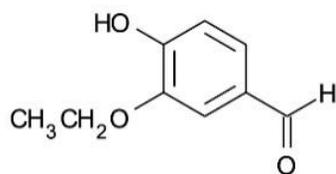
4-Hydroxybenzoic acid



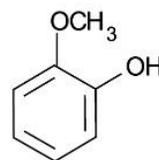
4-Hydroxybenzaldehyde



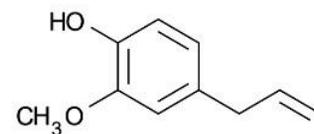
o-Vanillin



Ethyl vanillin



Guaiacol



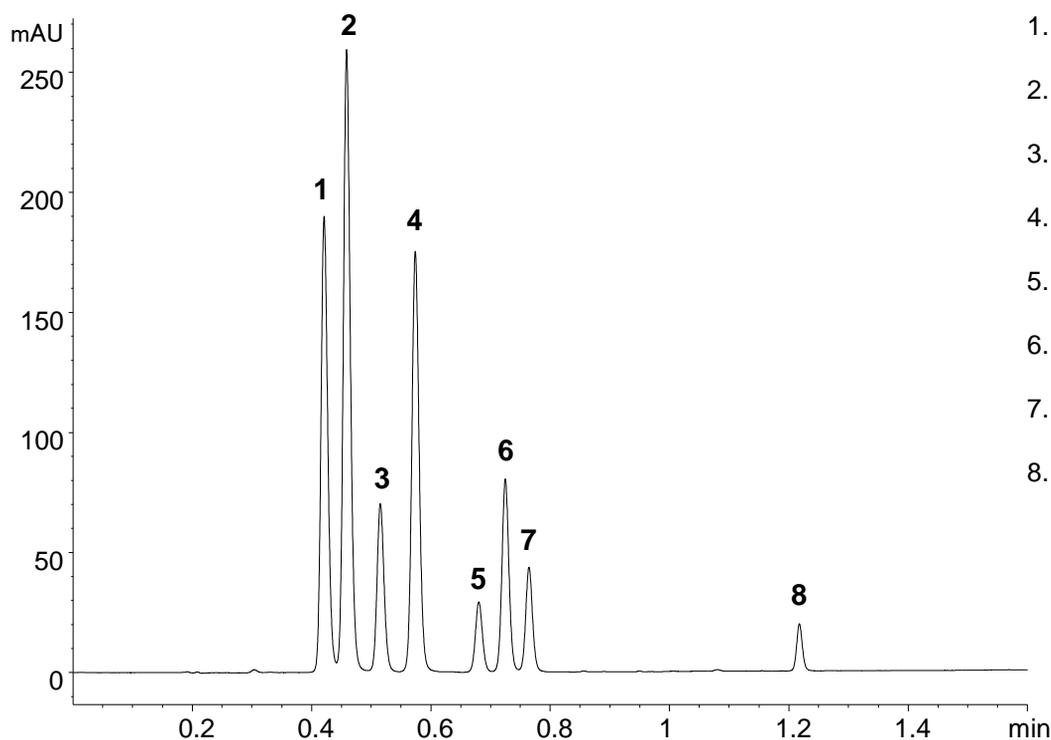
Eugenol

Method

Column: ACE Excel 1.7 C18-Amide
 Dimensions: 50 x 3.0 mm
 Part Number: EXL-1712-0503U
 Mobile Phase: A: 0.1% formic acid in H₂O
 B: 0.1% formic acid in MeCN

Time (mins)	%B
0.00	25
1.32	75
1.49	75
1.60	25

Flow Rate: 1.3 mL/min
 Injection: 1 µL
 Temperature: 45 °C
 Detection: UV, 260 nm



1. Vanillic acid
2. 4-Hydroxybenzoic acid
3. Vanillin
4. 4-Hydroxybenzaldehyde
5. Guaiacol
6. o-Vanillin
7. Ethyl vanillin
8. Eugenol

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