Fast Analysis of natural and artificial vanilla flavorings

Computer assisted development of a robust, fast and sensitive UHPLC method

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1. Introduction

Vanilla is one of the most important flavors worldwide and is widely used in foods, beverages and perfumes [1]. Natural vanilla extract contains up to several hundred substances with vanillin, vanillic acid, 4-hydroxybenzoic acid and 4-hydroxybenzaldehyd as the major components [2]. Due to the continuously increasing demand and the resulting high costs of natural vanilla extracts, artificial flavorings are often used instead. Vanillin can be obtained through various methods like chemical synthesis and biotransformation [3, 4]. These artificial flavors can contain synthetic vanillin, ethyl vanillin, eugenol, guaiacol, vanillin mandelic acid and others.

As authenticity criteria for vanilla, the ratios of the major components vanillin, 4-hydroxybenzaldehyde, vanillic acid and 4-hydroxybenzoic acid are frequently used. In order to monitor the composition and therefore quality of vanilla flavors contained in food, an analytical method needs to enable individual quantification of any of these ingredients as well as possible ingredients like the precursors from the synthesis of vanillin.

This poster shows the development and optimization of a rapid UHPLC screening test for the separation and quantification of natural and artificial vanilla flavoring substances as well as some precursors for the quality control of vanilla products using an automated method scouting / method optimization workflow.

2. Method Development

For UHPLC method scouting, a Shimadzu Nexera X2 Method Scouting System (Figure 1) was used, consisting of two quaternary solvent pumps, autosampler and column oven including a six column switching valve. The system was also equipped with a high resolution photo diode array detector.

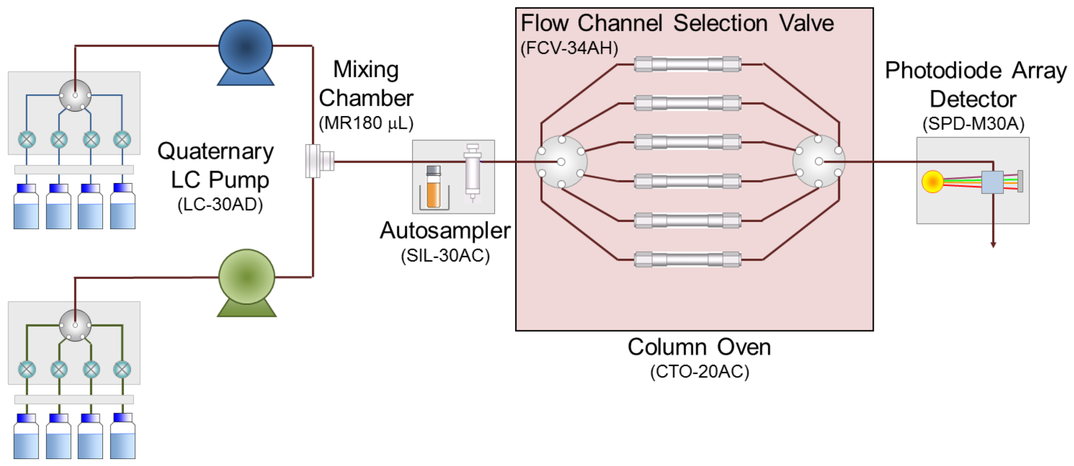

Figure 1: Schematic diagram of the Nexera X2 Method Scouting System

Method scouting for eleven different compounds was performed in an overnight sequence using 7.5 min gradient runs at 40° C with a flow rate of 0.5 ml/min. Twelve combinations of stationary and mobile phases were selected (six different columns, two different organic solvents). Methods and sequence were created using the dedicated Method Scouting Solution Software (Figure 2).

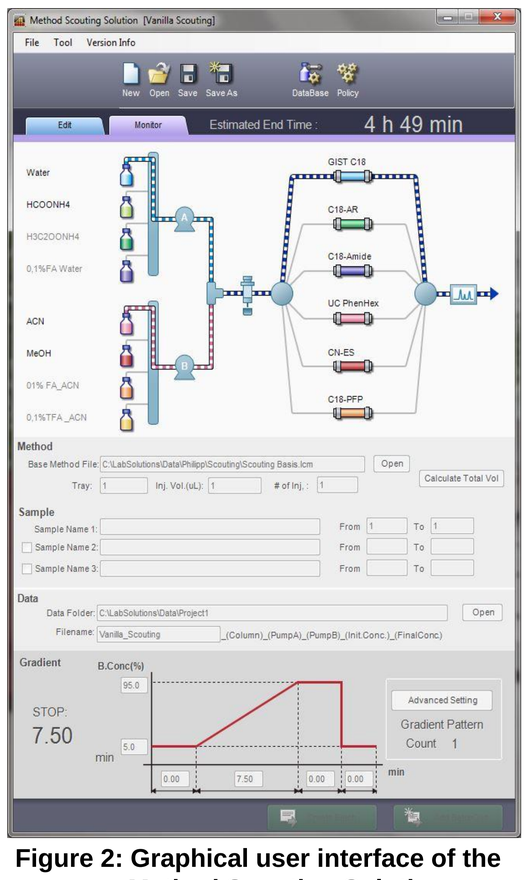

Figure 2: Graphical user interface of the Method Scouting Solution

Table 1: Columns used for method scouting

Shim-Pack GIST 3 µm C18, 100 x 2,1 mm
ACE Excel 3 C18-AR, 100 x 2.1 mm
ACE Excel 3 C18-Amide, 100 x 2.1 mm
ACE UltraCore 2.5 PhenHex, 100 x 2.1 mm
ACE Excel 3 CN-ES, 100 x 2.1 mm
ACE Excel 3 C18-PFP, 100 x 2.1 mm

Table 2: Scouting base method

Mobile Phase:	A1: 10 mM NH_4HCO_2 pH 2.8 in H_2O B1: Acetonitrile B2:Methanol
Gradient:	5 – 95 %B in 7.5 min
Flow rate:	0.5 ml/min
Temp.:	40 °C

3. Results

The ACE UltraCore 2.5 SuperPhenylHexyl column showed the most promising results with all analytes of interest at least partly separated using a mobile phase consisting of A: 10 mM ammonium formate in H_2O (pH 2.8) and B: acetonitrile. These conditions were used to create a two-dimensional DryLab© model (Molnár Institute) using 2 min and 6 min gradient runs at 25° C and 50° C as input data. These experiments resulted in a color-coded resolution map for simple identification of the optimum separation conditions (Figure 3). The figure shows the calculated resolution (color-coded) for the different simulated combinations of gradient time (x-axis) and oven temperature (y-axis).

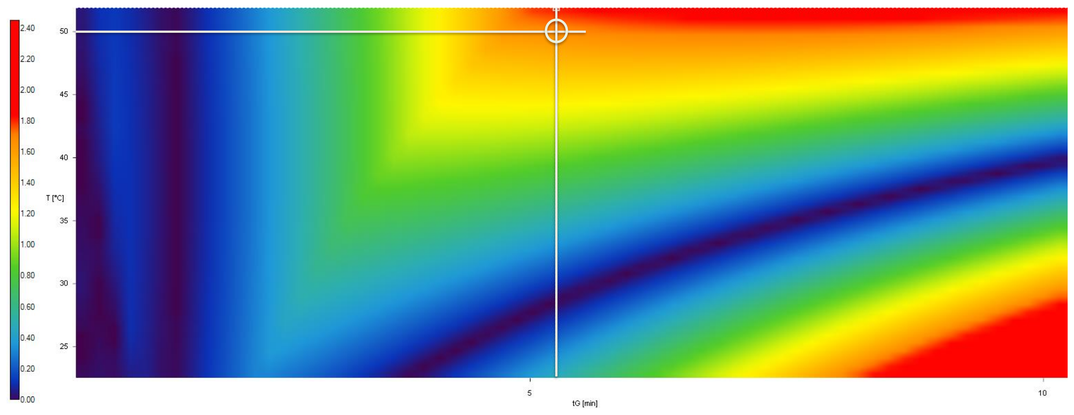

Figure 3: Color-coded DryLab resolution map for UHPLC method development

The software predicted an optimum separation with a minimum resolution of the critical peak pair of 2.1 in a gradient run using the conditions shown in table 3. A comparison of the predicted chromatogram with an actual sample run is displayed in Figure 4.

Table 3: Resulting UHPLC method

Column:	ACE UltraCore 2.5 SuperPhenylHexyl, 100 x 2.1 mm
Mobile Phase:	A: 10 mM NH_4HCO_2, pH 2.8 in H_2O; B: Acetonitrile
Gradient:	5 – 70 % B in 5.5 min
Flow rate:	0.5 ml/min
Temperature:	50 °C

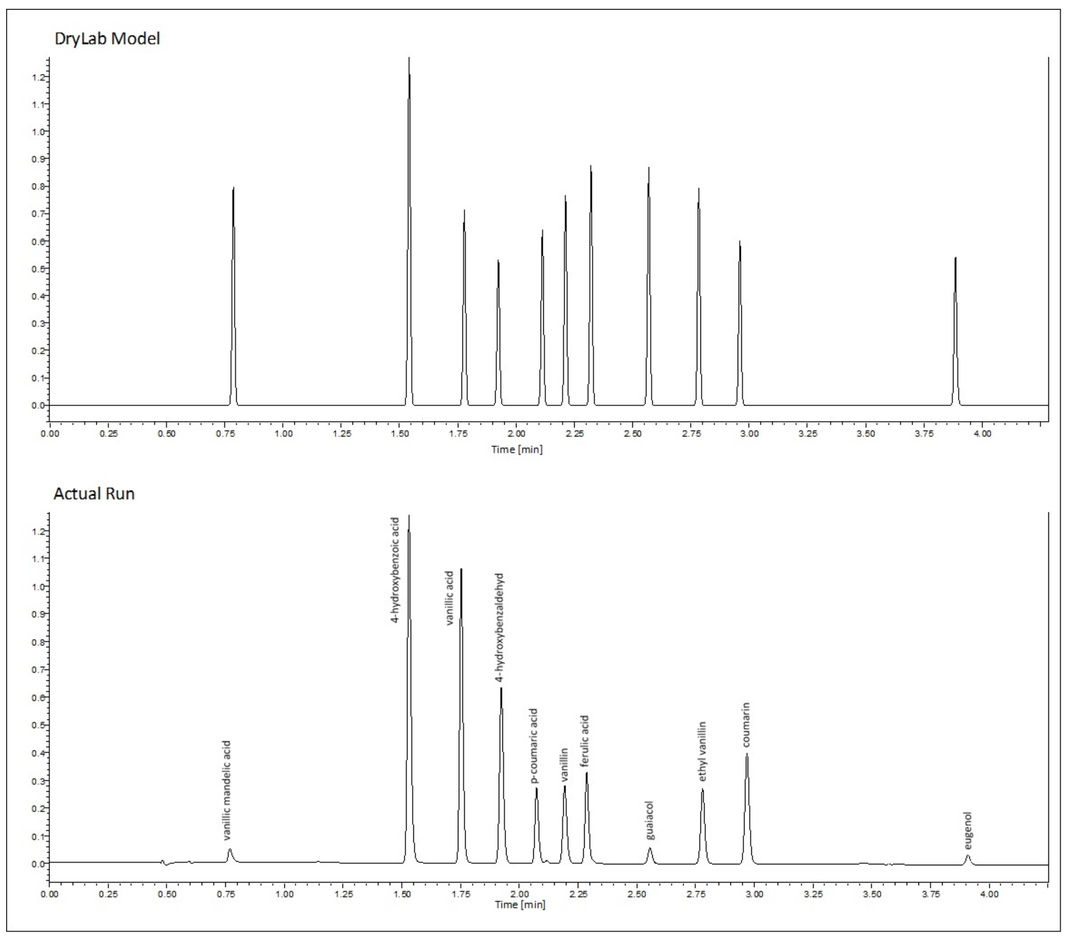

Figure 4: Comparison of DryLab predicted and actual chromatogram of the UHPLC analysis of natural and artificial vanilla flavorings

4. Conclusion

- A robust, fast and sensitive UHPLC method for the simultaneous separation of natural and artificial vanilla flavoring substances as well as some precursors has been developed.
- The automated workflow (figure 5) starting with a method scouting experiment and further optimization using a computer simulation software package saved time and offered visualization of the design space in a resolution map to establish the most robust separation method.

Figure 5: Computer assisted method development workflow

[1] A.S. Ranadive; in Charalambous G. 34 (1994) [2] J. Adeji, T.G. Hartmann, C.O. Ho; in Perf. Flav. 18 (1993) [3] J.R. Desmurs et al.; in Perf. Flav. 29 (2004) [4] R. Berger; in Flavours and Fragrances 1 (2007)