COMPREHENSIVE LC X LC ANALYSIS OF FLAVORING INGREDIENTS IN DISTILLED SPIRITS

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Introduction

Composition of flavoring ingredients in distilled spirits is largely dependent on make and storage of the product after distillation. The so-called "aging" of alcoholic beverages takes place in charred wooden, mostly oak barrels. During this process of maturation, wood components are extracted into the distillate, which has a major effect on their final aroma.
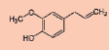
The complex composition and a distinct change over the period of storage in the cask provide a unique profile for any distilled spirit after "aging".

Due to anaerobic decomposition of the lignin underneath the wood charcoal layer in the cask, aromatic aldehydes, such as vanillin or p-anisaldehyde are formed. Colour and (poly-)phenol content of the distillate are a result of extractable wood components. Additional flavoring ingredients are formed by oxidative, interactive chemical reactions of the various constituents.

Table 1: Examples of flavoring ingredients in distilled spirits

Component	Flavor	Color
p-Anisaldehyde	Woodruff	Yellowish
Caffeic acid	Coffee	Brownish yellow
Vinylguaiacol	Tangy, spicy	Colorless
Cinnamic aldehyde	Balmy, cinnamon	Yellowish
Vanillin	Sweet, vanilla-like	Colorless
Eugenol	Clove	Colorless
Ionone	Violet, raspberry	Yellowish

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A comprehensive two-dimensional LC method for the simultaneous determination of polyphenols, and a large number of different flavouring substances has been developed. This online 2D approach offers enhanced resolving power compared to a one dimensional run by employing two successive separations, with different selectivities in a single analysis. This LC x LC analysis also allows for 2D pattern profiling for quick and easy quality control and identification of counterfeit products.
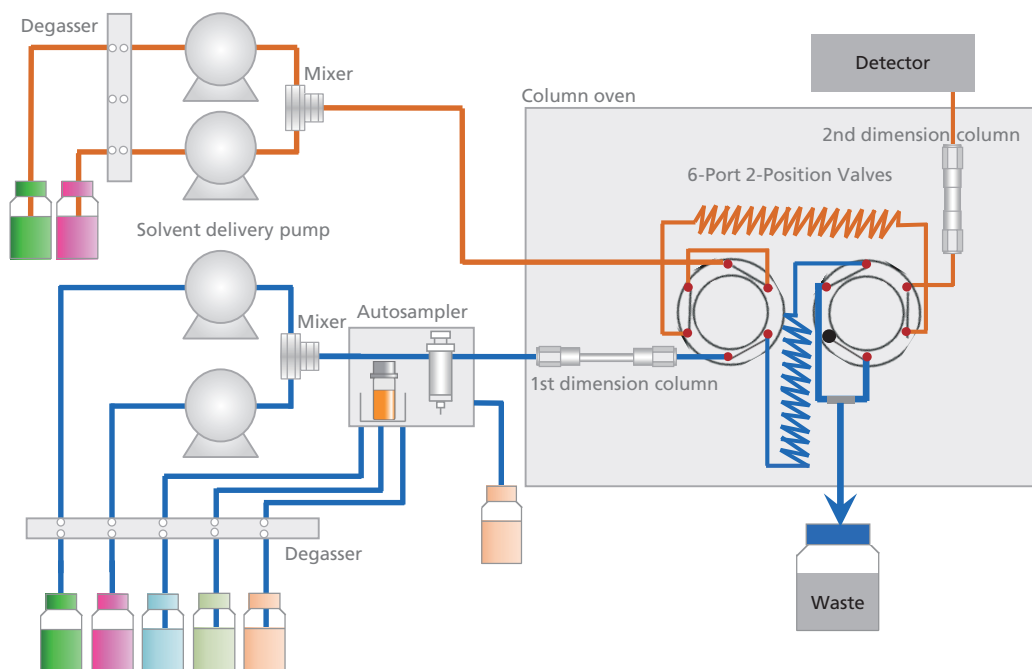

Figure 1: System configuration of Nexera-e two-dimensional LC system

Instrument and Software

A comprehensive separation is achieved on two distinct columns connected in series with a transfer system of two sample loops on 6-port 2-position valves located between them (figure 1). This interface cuts and then releases continuous fractions of the primary column effluent onto a fast separation column in the second dimension. This way, through continuous 'heart-cutting', the entire sample is subjected to two different separations. An ultra-fast UHPLC separation in the second dimension ensures that the bands injected onto the secondary column elute before the next fraction is injected.
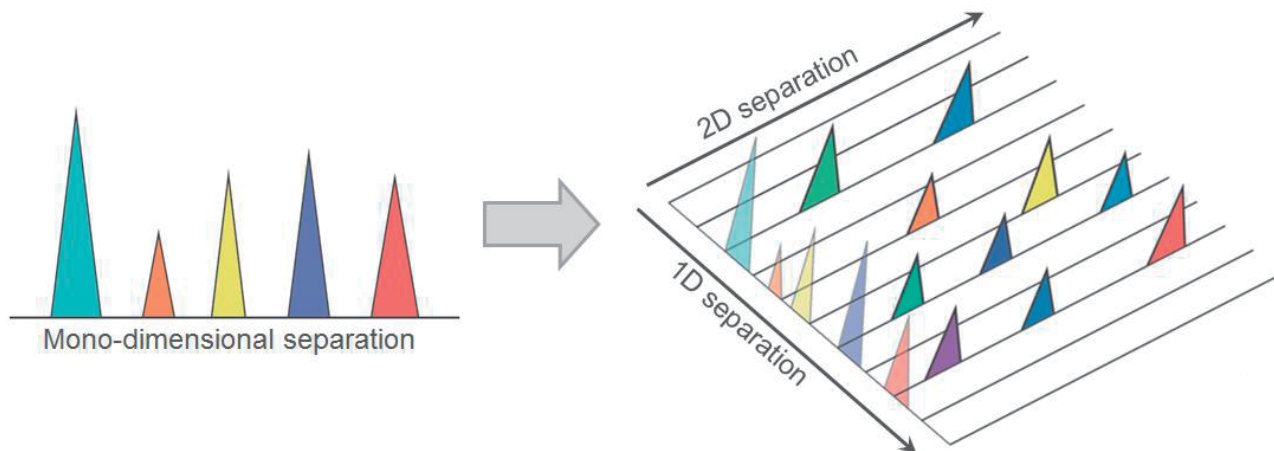
Figure 2: Schematic demonstration of a two-dimensional LC x LC separation

Figure 2 shows the schematic demonstration of a two-dimensional LC x LC separation. The wide compound peaks from the slow first dimension method undergo a fast second separation, that allows for co-eluting peaks to be resolved by the different selectivity of the 2nd dimension column. In a two dimensional approach the chances for full separation even of complex samples are multiplied as peak capacity is increased and two different separation mechanisms are exploited. However, not only the instrument set-up is crucial, but also the software for visualization of the results. Even though the system is running two separate methods, data is recorded by only one detector. The ChromSquare software produces a 2D contour plot (see figure 3) based on the recorded data and the modulation time in the method – the 2nd dimension analysis time.
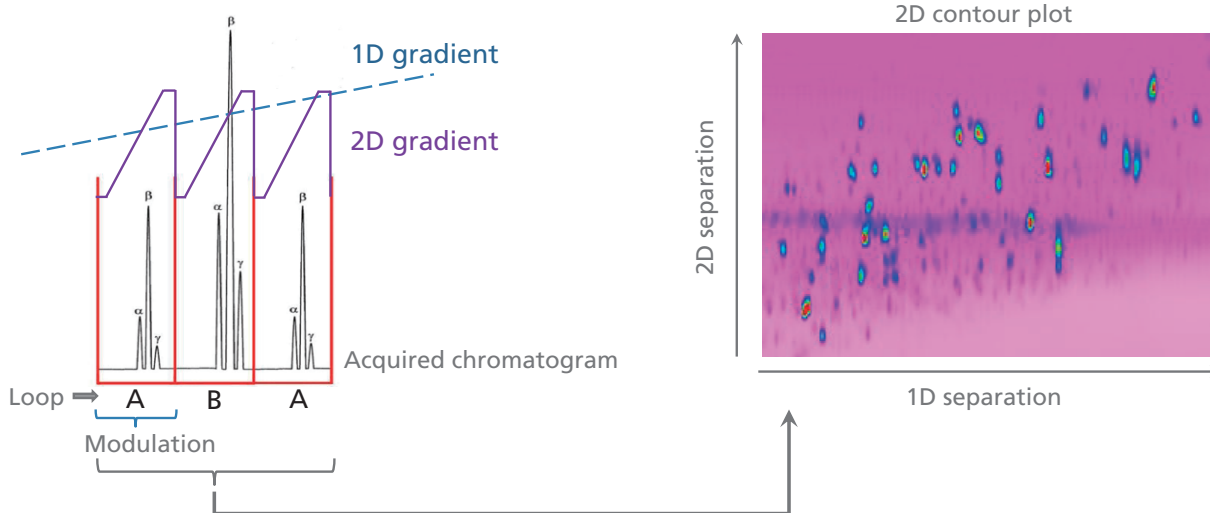

Figure 3: Nexera-e System Operation Overview

Separation Method

In order to benefit from the two-dimensional method set-up, the two separations must offer different selectivty. As there are lipophilic flavonoids, as well as very polar analytes among the compounds of interest, a RPxRP system was chosen with a purely lipophilic ODS (ACE 3 Super C18, 150 x 1.0 mm) and a polar embedded RP Cyano phase (ACE Excel 2 CN-ES; 100 x 2.1 mm). To further increase the differences between the two methods, ammonium formate buffer, pH 2.8 and acetonitrile were used as mobile phase in the first dimension the same buffer and methanol in the second separation method. Oven

temperature was set to 45 °C. The LCxLC assist software created the two-dimensional time program for a very slow 1D gradient of 8 to 43 %B in 60 min at a flow rate of 0.02 ml/min and an ultra-fast 2D gradient of just 1.6 min at a flow rate of 1.2 ml/min with increasing start %B from 30 to 40 over the time of the 1D gradient to match the increasing content of organic solvent from the first dimension effluent.

Detection was carried out with a PDA detectot to evaluate the most sensitive wavelength for all compounds of interest during data analysis.
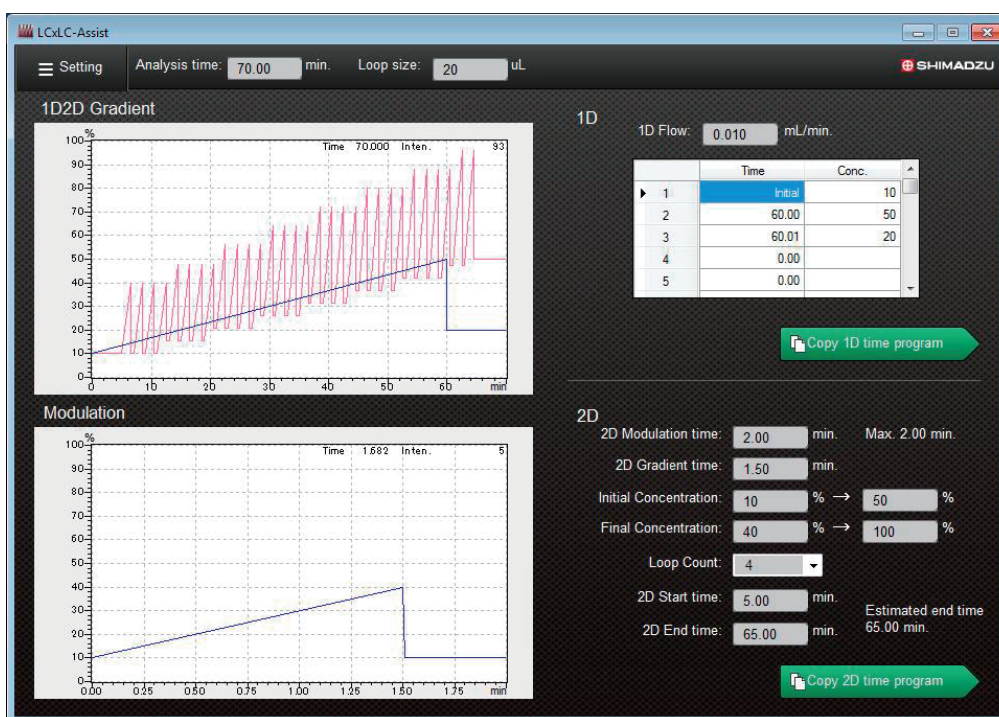

Figure 4: Creation of 2D time program in LCxLC assist software

Results

After separate optimisation of the two gradient methods, over 20 compounds of interest with large differences in structure and physicochemical properties could be separated and quantified in one two-dimensional LCxLC separation. The contour plot of the analysis of a mixed standard solution is displayed in figure 5. In the

chromatogram below the 2D plot the peaks separated in one chosen modulation time can be identified. Integration contours and peaks in the chromatogram are colour coded for clarification. UV spectra of each blob are also available.
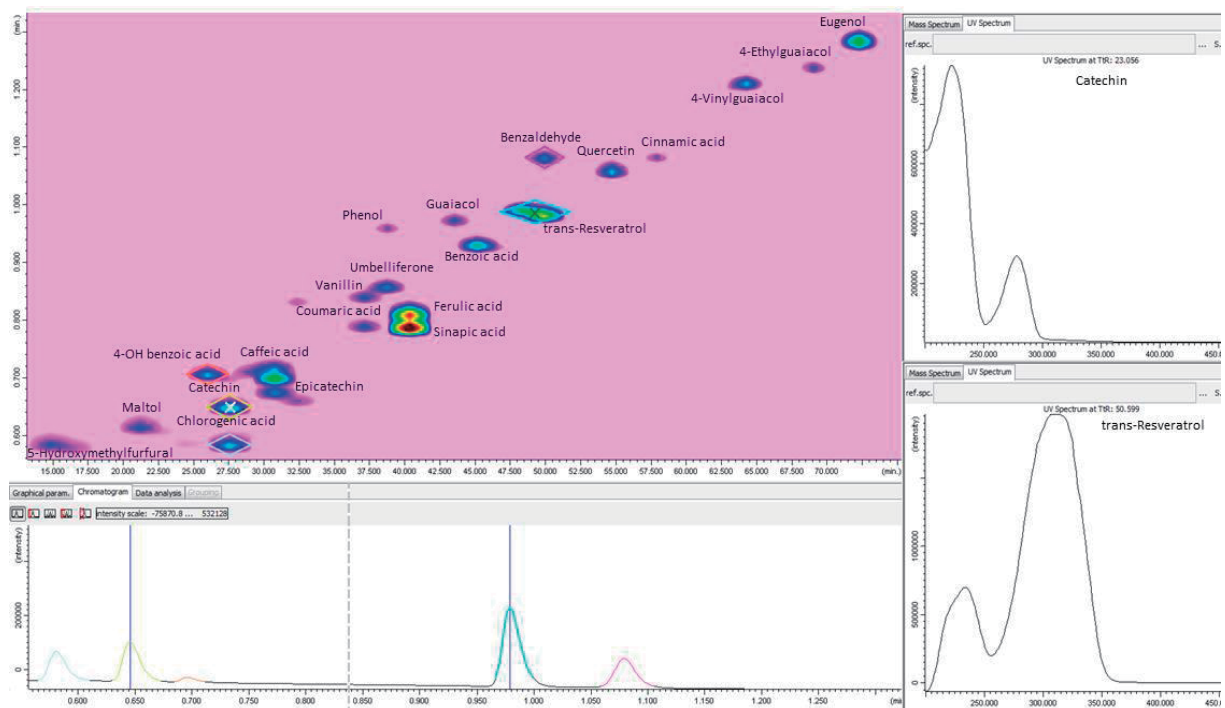
Figure 5: Contour plot of the 2D separation of polyphenols, and different flavouring substances – mixed standard sample

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