#A06

Analysis of Beer by Comprehensive 2D-LC with the Agilent 1290 Infinity 2D-LC system

Application Note

Food Testing & Agriculture

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Abstract

This Application Note demonstrates the use of two-dimensional liquid chromatography for the separation of complex compounds mixtures inherent in commercially available beer samples. Comprehensive two-dimensional liquid chromatography is a versatile tool for the separation of samples of highest complexity with the possibility to combine a large number of chromatographic separation mechanisms. The separation of beer constituents with three different separation mechanisms is demonstrated in this Application Note.

Introduction

Two-dimensional liquid chromatography is the method of choice for the separation of mixtures of highest complexity because the peak capacity of the first and second dimension columns combines idealistically in a direct manner to the complete system peak capacity[1]. This approach works if the separation mechanisms are truly orthogonal, for example, size exclusion chromatography versus reversed phase chromatography or ion exchange chromatography versus reversed phase chromatography. The combination of separation mechanisms with similar selectivities such as reversed phase versus reversed phase needs careful optimization of the chromatographic conditions to achieve optimum peaks capacity[2,3].

To get a separation of largely different compound classes inherent in one sample type, analyze them using 2D-LC with different selectivity combinations. Typical examples can be found in the analysis of beverages like fruit juices, wine, and beer. These beverages typically contain polyphenolic compounds which can be resolved by a combination of different reversed phase selectivities[4,5,6]. Beer contains not only the polyphenolic compounds from hops but other large molecules such as carbohydrates. These compounds can be analyzed through the combination of SEC and IEX with RP chromatography[7,8].

Experimental

Instruments

Agilent 1290 Infinity 2D-LC solution

- Agilent 1260 Quaternary Pump G1311B (for 1st dimension)
- Agilent 1290 Infinity Binary pump G4220A (for 2nd dimension)
- Agilent 1290 Infinity Autosampler G4226A
- Agilent 1290 Infinity Thermostatted Column Compartment G1316C with 2-position/4-port duo valve (G4236A) for 2D-LC
- Agilent 1290 Infinity DAD G4212A (diode array detector) with 10-mm standard MaxLight flow cell

Software

Open Lab CDS ChemStation Edition C01.04. LCxLC Software for 2D-LC data analysis from GC Image LLC, Lincoln, NE, USA

Chromatographic Methods

Size exclusion X Reverse Phase

First dimension

Column:	TOSOH TSKgel SuperOligoPW 6.0 × 150 mm
Mobile phase:	A: Water/MeOH = 8/2, 50 mM ammonium acetate, B: MeOH
Flow rate:	0.1 mL/min
Gradient:	0 minutes – 0% B, 60 minutes – 0% B, 65 minutes – 20% B, 70 minutes – 20% B

Second dimension

Column:	Agilent ZORBAX RRHT SB-Aq 3.0 × 50 mm, 1.8 µm (p/n 827975-314)
Mobile phase:	A: Water, B: ACN
Flow rate:	2.0 mL/min
Gradient:	0 minutes – 0% B, 0.5 minutes – 20% B, 0.51 minutes – 0% B, 0.65 minutes – 0% B
Modulation time:	0.65 minutes
Total run time:	70 minutes
Temperature:	40 °C (1st column) 50 °C (2nd column)
Loop size:	40 µL
Injection volume:	5 µL
Detection:	210 nm/4 nm, reference off 254 nm/4 nm, reference off

Ion exchange X Reverse phase

First dimension

Column:	Agilent ZORBAX 300SCX 2.1 × 150 mm, 5 µm (p/n 883700-714)
Mobile phase:	10 mM ammnonium acetate (isocratic)
Flow rate:	0.08 mL/min

Second dimension

Column:	Agilent ZORBAX RRHT SB-Aq 3.0 × 50 mm, 1.8 µm (p/n 827975-314)
Mobile phase:	A: Water, B: ACN.
Flow rate:	2.0 mL/min
Gradient:	0 minutes – 0% B, 0.5 minutes – 20% B, 0.51 minutes – 0% B, 0.65 minutes – 0% B.
Modulation time:	0.65 minutes
Total run time:	25 minutes
Temperature:	40 °C (1st column) 50 °C (2nd column)
Loop size:	40 µL
Injection volume:	5 µL
Detection:	210 nm/4 nm, reference off

254 nm/4 nm, reference off

Reverse phase X Reverse phase

First dimension

Column:	Agilent ZORBAX Poroshell 120 SB-C18 2.1 × 150 mm, 2.7 µm (p/n 683775-902)
Mobile phase:	A: Water, B: ACN
Flow rate:	0.1 mL/min
Gradient:	0 minutes – 0% B, 20 minutes – 50% B, 30 minutes – 50% B,

Second dimension

Column:	Agilent ZORBAX RRHD SB-Phenyl 3.0 × 50 mm, 1.8 µm (p/n 857700-312)
Mobile phase:	A: Water, B: ACN
Flow rate:	2.5 mL/min
Gradient:	0 minutes – 0% B, 0.5 minutes – 60% B, 0.51 minutes – 0% B, 0.65 minutes – 0% B
Modulation time:	0.65 minutes
Total run time:	30 minutes
Temperature:	40 °C (1st column), 50 °C (2nd column)
Injection volume:	5 µL
Detection:	210 nm/4 nm, reference off, 254 nm/4 nm, reference off

Sample preparation

Commercially available beer was ultrasonically shaken to expel carbon dioxide, then filtrated by a syringe filter, and directly used for injection.

Results and discussion

Beer is a special example due to its variability in compound composition. To examine the content it is necessary to apply different separation techniques even in a 2D-LC set up, to get the most analytical information out of the sample.

In the following examples, two different brands of beer were analyzed by combinations of different chromatographic selectivity mechanisms.

Figure 1 shows a separation by size exclusion chromatography (under the given chromatographic conditions size exclusion is the favored separation mechanism of this column) in the first and reversed phase is the second dimension for the separation of the more complex and large molecules like glycosidic and protein like compounds. To compare all analytical runs, the modulation time in the second dimension was keep constant at 0.65 minutes. Both samples show compounds which are retained on SEX phases and then separated on RP phases. The very polar compounds seem to be the same in both samples. The difference is seen in the middle of the plot where the compounds differ by their retention from the first dimension.

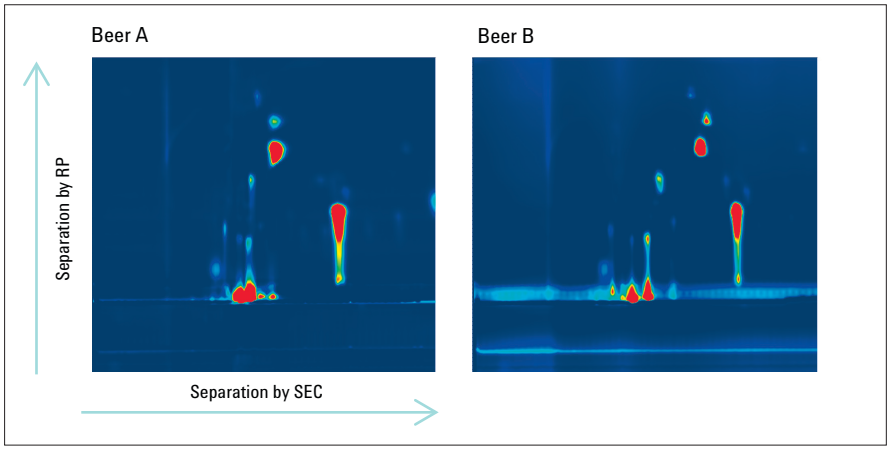

Figure 1
2D-LC plot by 1st dimension – SEC and 2nd dimension – Reversed phase separation.

In the second separation the compounds were separated by ion exchange chromatography in the first dimension and reversed phase in the second dimension (Figure 2). In this case, ionic compounds were retained on the first dimension and then separated by polarity on the second dimension. The comparison of both brands of beer shows, that Beer A has ionic compounds which elute early from the second dimension. This set of compounds is not present in Beer B. However, there are some stronger ionic compounds with higher hydrophobicity in Beer B, which elute later in the first and second dimension.

The nonionic compounds were separated by a combination of two reversed phase separations in the first and second dimension (Figure 3). The 2D-LC plot shows good separation in the first part of the 2D plot but later both separation mechanisms seem to be comparable. Hence, in the first left part of the plot some differences in compounds and their relative concentrations are seen.

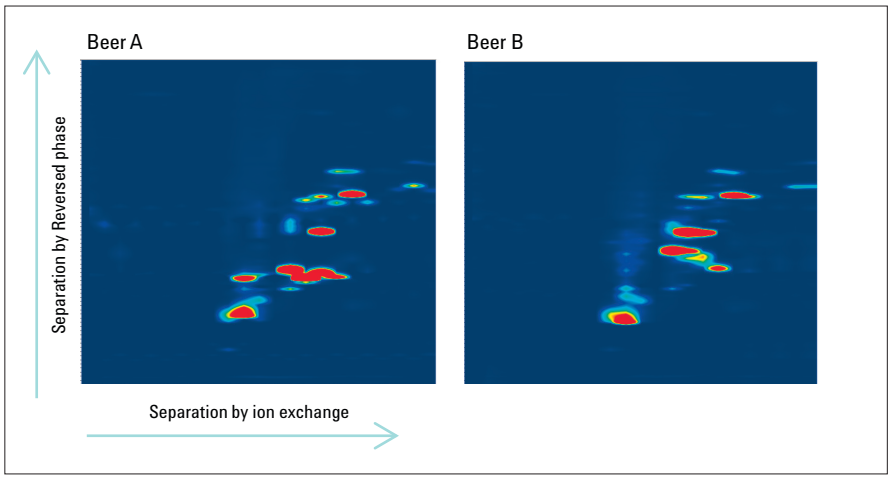

Figure 2
2D-LC plot by 1st dimension – ion exchange and 2nd dimension – Reversed phase separation.

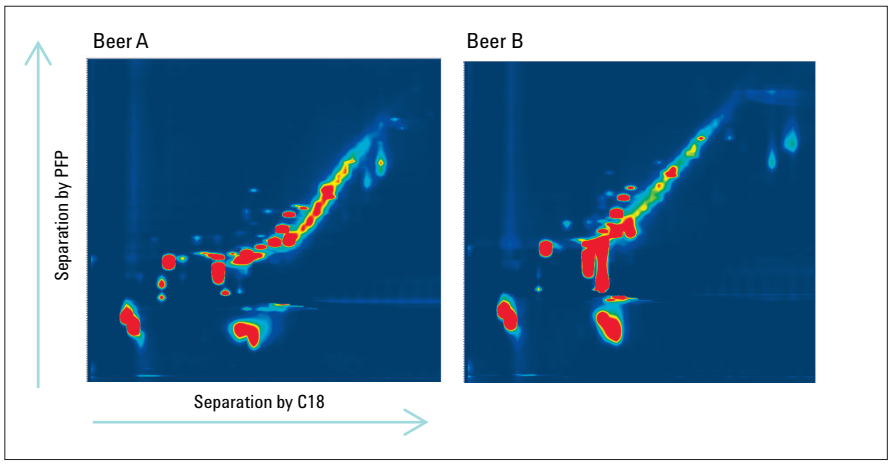

Figure 3
2D-LC plot by 1st dimension – Reverse phase C18) and 2nd dimension – Reversed phase PFP) separation.

Conclusion

The Agilent 1290 Infinity 2D-LC System is able to separate mixtures of highest complexity by the combination of columns of different selectivity such as SEC, IEX and RP in the first and second dimension. This gives the maximum information out of mixtures of highest complexity.

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