Comprehensive 2D-LC Analysis of Chinese Herbal Medicine

The Agilent 1290 Infinity 2D-LC Solution

Suitable for Agilent
1290 Infinity III LC

Application Note

Small Molecule Pharmaceuticals & Generics

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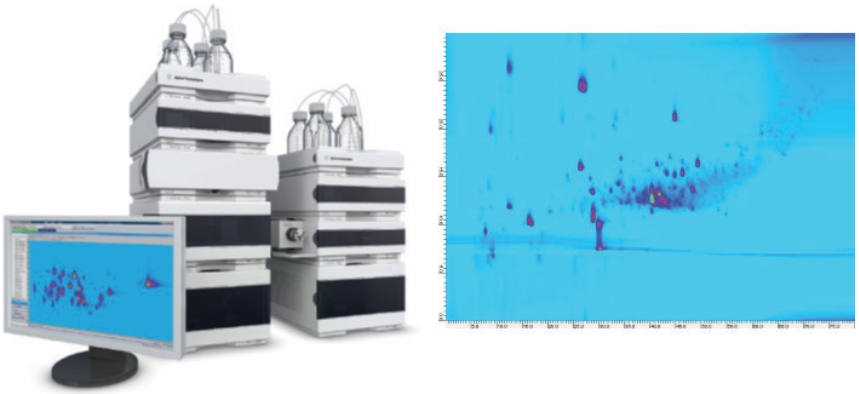
Abstract

Chinese herbal medicine (CHM), one aspect of traditional Chinese medicine (TCM), uses single plants or preparations of several plants. Their effectiveness depends on the synergistic effects of multiple components in the plants. The plants used in CHM present extremely complex samples. Therefore, comprehensive two-dimensional liquid chromatography (comprehensive 2D-LC) is the method of choice for their analysis. This Application Note shows the development of a comprehensive 2D-LC method for the analysis of a decoction from mulberry twigs (mori ramulus; Sang Zhi). The method was then applied to the analysis of decoctions from four different plants used in CHM. The method provided good separation of the components of all four plants, without any method adjustments. The very complex nature of the four plants is revealed by their comprehensive 2D-LC analyses.



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Introduction

Traditional Chinese medicine (TCM) was developed in China, and is based on human experience over the past 3,000 years. It has become increasingly popular in Western countries during the last decade. TCM includes various treatments, such as Chinese herbal medicine (CHM), acupuncture, and moxibustion, amongst others[1-3]. In CHM, single plants or preparations of several plants are mainly used as decoctions prepared by extraction with boiling water[2-4].

The pharmaceutical efficacy of CHM is thought not to result from the presence of a single bioactive component, but to depend on the synergistic effects of multiple components of the plants[1-3]. The plants used in CHM present extremely complex samples that can consist of hundreds of components with different physicochemical properties, and in different amounts[3,4]. For a comprehensive analysis of these very complex samples, one-dimensional liquid chromatography (1D-LC) does not provide enough resolving power. Therefore, comprehensive two-dimensional liquid chromatography (comprehensive 2D-LC) is the method of choice for the analysis of CHM.

This Application Note shows the development of a comprehensive 2D-LC method for the analysis of a decoction from mulberry twigs (mori ramulus; Sang Zhi). A suitable combination of reversed-phase columns and eluents for the first and second dimension separation, providing maximum coverage of the two-dimensional separation space, was identified. To enlarge the accessible two-dimensional separation space, a complex gradient was designed for the second-dimension separation[5]. This comprehensive 2D-LC method was then applied to the analysis of decoctions from four different plants used in CHM.

Experimental

The Agilent 1290 Infinity 2D-LC Solution comprised the following modules:

- Two Agilent 1290 Infinity Binary Pumps (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A) with 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Valve Drive (G1170A) with 2-position/4-port-duo valve (2D-LC Valve Head, 1,200 bar, p/n 5067-4214) equipped with two 60-µL loops
- Agilent 1290 Infinity Diode Array Detector (G4212A) with Max-Light Cartridge Cell, 60-mm (G4212-60007)

Software

- Agilent OpenLAB CDS ChemStation Edition Rev. C.01.05 [38] with 1290 Infinity 2D-LC Acquisition Software, Product Version A.01.01 [26].
- GC Image LCxLC-HRMS Edition Software for 2D-LC data analysis from GC Image, LLC, Lincoln, NE, USA.

Columns

First dimension

Agilent ZORBAX Narrow Bore RR SB-Aq, 2.1 × 100 mm, 3.5 µm (p/n 861753-914)

Second dimension

Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl, 3.0 × 50 mm, 1.8 µm (p/n 959757-312)

Agilent ZORBAX RRHD SB-CN, 3.0 × 50 mm, 1.8 µm (p/n 857700-305)

Agilent ZORBAX RRHT Bonus-RP, 3.0 × 50 mm, 1.8 µm (p/n 827668-301)

Solvents

All solvents were LC grade. Acetonitrile and methanol were purchased from Merck, Darmstadt, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). Formic acid was from Agilent (p/n G2453-85060).

Samples

The plants used in Chinese herbal medicine were kindly provided by Patrick Kwik from Congress Pharmacy in Karlsruhe, Germany (Table 1).

Table 1. Chinese herbal medicine plants analyzed using comprehensive 2D-LC.

Latin name	CHM name, English	CHM name, Chinese
Morus alba L.	Mori ramulus	Sang Zhi
Lycium barbarum L.	Lycii fructus	Gou Qi Zi
Angelica sinensis (Oliv.) Diels	Angelicae sinensis radix	Dang Gui
Atractylodes macrocephala Koidz	Atractylodis macrocephalae rhizoma	Bai Zhu

Samples were prepared as decoctions, in the same manner they are prepared for pharmaceutical use. Approximately 2.5 g of each sample (whole berries for lycii fructus, cut pieces for the others) was weighed, and 25 mL of water was added. The samples were allowed to soak in the cold water for 30 minutes, and then boiled for 20 minutes. After cooling, aliquots of the decoctions were centrifuged at 10,000 rpm for 15 minutes. Aliquots of the supernatant phases were filtered using a 1-mL plastic syringe with Agilent Captiva Premium Syringe filters, regenerated cellulose, 15-mm, 0.45 µm (p/n 5190-5109) before injection into the HPLC system.

Comprehensive 2D-LC method

First-dimension pump	
Solvent A	Water + 0.1 % formic acid
Solvent B	Methanol + 0.1 % formic acid
Flow rate	0.05 mL/min
Gradient	Time (min) % B 0 0 10 0 70 95 80 95
Stop time	80 minutes
Post time	30 minutes
Second-dimension pump	
Solvent A	Water + 0.1 % formic acid
Solvent B	Acetonitrile + 0.1 % formic acid
Column testing method	
Flow rate	3.0 mL/min (for Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl and Agilent ZORBAX RRHD SB-CN) 2.5 mL/min (for Agilent ZORBAX RRHT Bonus-RP)
Gradient	Time (min) % B 0 5 0.35 95 0.36 5 0.50 5
Final method	
Second-dimension column	Agilent ZORBAX RRHT Bonus-RP
Flow rate	2.5 mL/min
Gradient	Time (min) % B 0 2 0.35 50 0.36 2 0.50 2
Gradient modulation	0.00 minutes – 2 % B to 30 minutes – 2 % B to 70 minutes – 40 % B 0.35 minutes – 50 % B to 30 minutes – 50 % B to 70 minutes – 95 % B 0.36 minutes – 2 % B to 30 minutes – 2 % B to 70 minutes – 40 % B 0.50 minutes – 2 % B to 30 minutes – 2 % B to 70 minutes – 40 % B
Thermostatted column compartment	
First-dimension column on the right side at 25 °C, second-dimension column on the left side at 60 °C.	
2-position/4-port-duo valve	
The 2-position/4-port-duo valve was switched automatically after each second-dimension modulation cycle of 30 seconds. The loops were used in a “cocurrent” manner (filling and elution of the loops in the same flow direction).	
Autosampler	
Injection volume	10 µL
Sample temperature	6 °C
Needle wash	6 seconds in methanol
Diode array detector	
Wavelength	254 nm/4 nm; ref, 380 nm/40 nm 280 nm/4 nm; ref, 380 nm/40 nm
Data rate	80 Hz

Results and Discussion

For comprehensive 2D-LC analysis, samples of the plants were prepared as decoctions. It can be expected that decoctions of plants used in CHM contain a range of very polar compounds. For this reason, a ZORBAX SB-Aq column was chosen for the first-dimension separation as it retains hydrophilic compounds, and can be run under 100 % aqueous conditions. Figure 1 shows the 1D-LC analysis of a decoction of mori ramulus on the ZORBAX SB-Aq column using gradients from 100 % water to 95 % acetonitrile, and from 100 % water to 95 % methanol (each containing 0.1 % formic acid). It can be seen that the use of methanol provided a better separation of the compounds contained in the decoction from mori ramulus. Therefore, a ZORBAX SB-Aq column in combination with a gradient of water and methanol was chosen for the first-dimension separation.

A suitable column for the second-dimension separation should provide maximum coverage of the two-dimensional separation space in combination with the ZORBAX SB-Aq column used in the first dimension. To identify a suitable second-dimension column, ZORBAX Eclipse Plus Phenyl-Hexyl, ZORBAX SB-CN, and ZORBAX Bonus-RP columns were tested in a comprehensive 2D-LC analysis of the decoction from mori ramulus. To enhance selectivity differences between the first-dimension separation (ZORBAX SB-Aq column used with methanol) and the second-dimension separation, the different columns in the second dimension were used with acetonitrile. Using acetonitrile in the second dimension was also advantageous because of the lower backpressure generated compared to methanol. Hence, higher flow rates could be employed in the second dimension. During column testing, the different second-dimension columns were all run with repeating gradients from 5 to 95 % acetonitrile (containing 0.1 % formic acid). Figure 2 shows the resulting 2D-LC separations of the compounds contained in the decoction from mori ramulus.

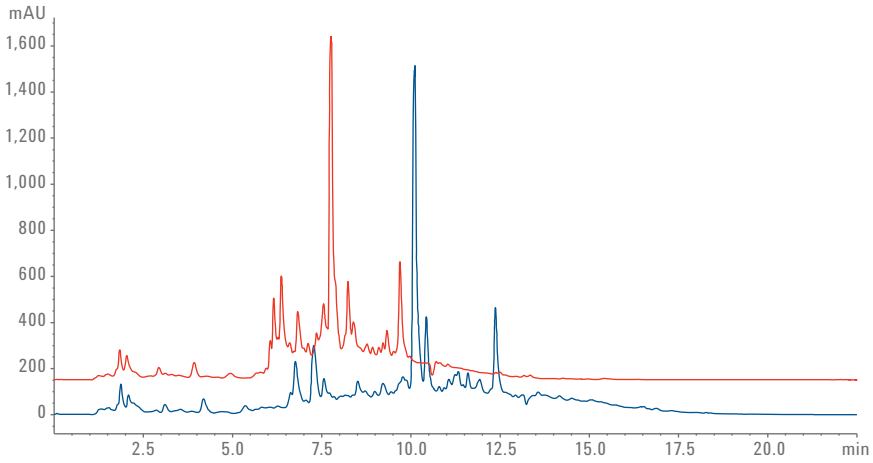

Figure 1. 1D-LC analysis of a decoction from mori ramulus on an Agilent ZORBAX Narrow Bore RR SB-Aq column (2.1 × 100 mm, 3.5 µm); red, gradient from 0 to 95 % acetonitrile; blue, gradient from 0 to 95 % methanol; flow rate, 0.2 mL/min; detection, 280 nm.

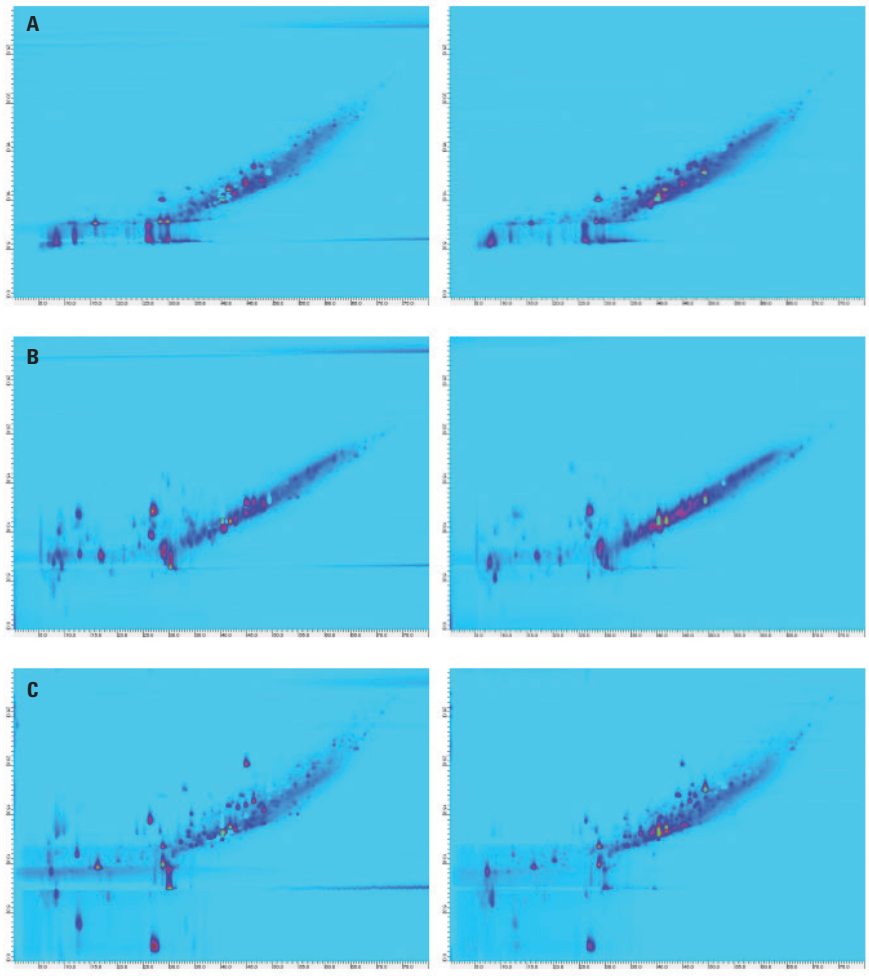

Figure 2. Comprehensive 2D-LC analysis of a decoction from mori ramulus; (A) second-dimension column, Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl (3.0 × 50 mm, 1.8 µm); (B) second-dimension column, Agilent ZORBAX RRHD SB-CN (3.0 × 50 mm, 1.8 µm); (C) second-dimension column, Agilent ZORBAX RRHT Bonus-RP (3.0 × 50 mm, 1.8 µm); left, detection at 254 nm; right, detection at 280 nm.

Using the ZORBAX Eclipse Plus Phenyl-Hexyl column in the second dimension (Figure 2A), the most polar compounds eluting early from the first-dimension column were not sufficiently retained in the second dimension. The use of a ZORBAX SB-CN column in the second dimension (Figure 2B) provided retention of compounds eluting early from the first-dimension column. However, for compounds eluting later from the first-dimension column, there was almost no difference in the retention behavior on the ZORBAX SB-Aq and ZORBAX SB-CN columns. The ZORBAX Bonus-RP column in the second dimension (Figure 2C) provided strong retention of compounds eluting early from the first-dimension column (several compounds show wrap-around). Additionally, differences in the retention behavior on the ZORBAX SB-Aq and ZORBAX Bonus-RP columns were observed for compounds eluting later from the first-dimension column. Therefore, the combination of a ZORBAX SB-Aq column with methanol in the first dimension and a ZORBAX Bonus-RP column with acetonitrile in the second dimension was chosen for the analysis of the decoction from mori ramulus.

To enlarge the accessible two-dimensional separation space, complex gradients may be designed for the second-dimension separation. Interestingly, a shallower second-dimension gradient, up to only 50 % acetonitrile, led to compounds eluting early from the first-dimension column eluting within one modulation cycle from the second-dimension column (avoiding wrap-around). Figure 3 shows the complex gradient designed for the second-dimension separation of components of the decoction from mori ramulus. Figure 4 compares the comprehensive 2D-LC separation of components of the decoction from mori ramulus obtained using the complex second-dimension gradient (Figure 4B) to the separation obtained using repeating second-dimension gradients of 5 to 95 % acetonitrile (Figure 4A). A more complete coverage of the two-dimensional separation space was observed using the complex gradient, and wrap-around was avoided.

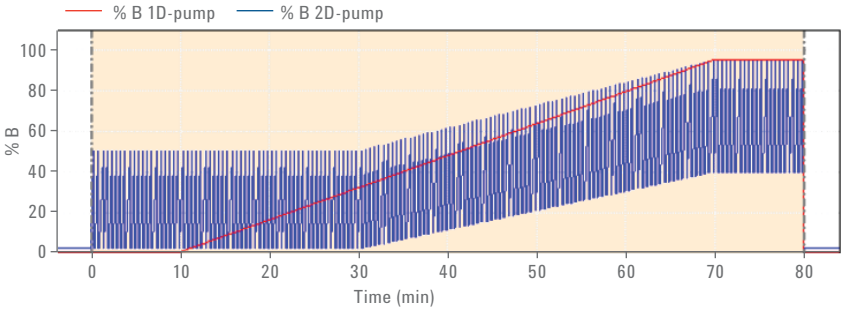

Figure 3. A complex gradient designed for the second-dimension separation of components of a decoction from mori ramulus.

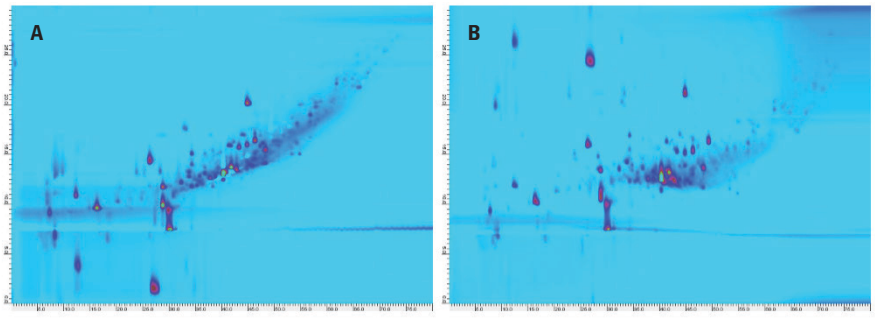

Figure 4. Comprehensive 2D-LC analysis of a decoction from mori ramulus; (A) using repeating second-dimension gradients from 5 to 95 % acetonitrile; (B) using the complex second-dimension gradient shown in Figure 3; detection at 254 nm.

The comprehensive 2D-LC method developed using a decoction from mori ramulus was then applied to the analysis of decoctions from the plants shown in Table 1. The resulting separations are shown in Figure 5. It can be seen that the comprehensive 2D-LC method provided good separation of the compounds contained in the decoctions from all four plants, without any method adjustments. The decoctions from the plants all present very complex samples not amenable to comprehensive analysis using 1D-LC. Furthermore, differences in the elution pattern (fingerprint) of components of the decoctions from the individual plants can be observed.

Conclusions

This Application Note demonstrates the comprehensive 2D-LC analysis of plants used in Chinese herbal medicine with the Agilent 1290 Infinity 2D-LC Solution. The development of a comprehensive 2D-LC method for the analysis of a decoction from mulberry twigs (mori ramulus; Sang Zhi) is shown. The developed method was applied to the analysis of decoctions from four different plants used in CHM. Good separation of the components of all four plants was achieved without any method adjustments. The very complex nature of the four analyzed plants used in CHM was revealed by their comprehensive 2D-LC analyses.

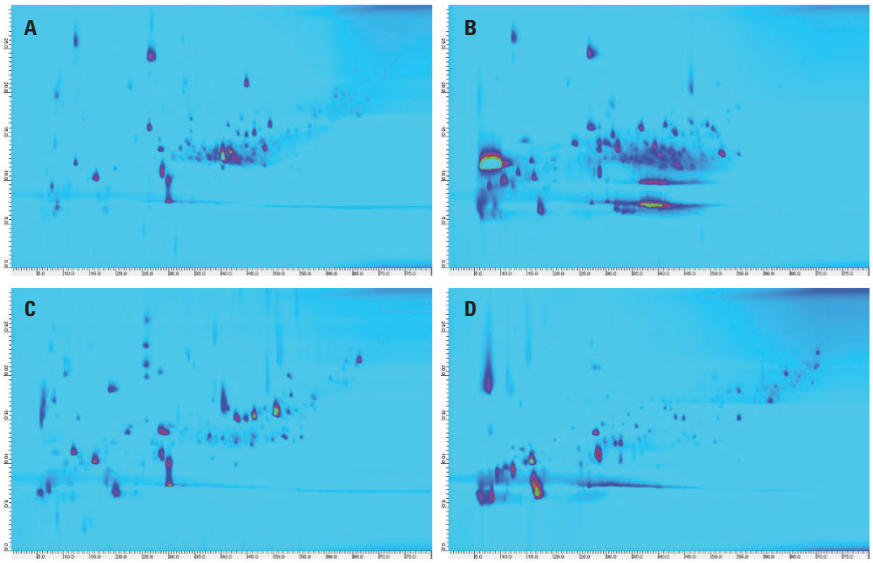

Figure 5. Comprehensive 2D-LC analysis of decoctions from plants used in Chinese herbal medicine; (A) mori ramulus; (B) lycii fructus; (C) angelicae sinensis radix; (D) atractylodis macrocephalae rhizoma; detection at 254 nm.

References

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