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Analysis of Panax ginseng extracts by comprehensive Two-Dimensional Ultra High Performance Liquid Chromatography coupled with IT-TOF

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Introduction

The comprehensive two-dimensional Liquid Chromatography (2D-LC) is a powerful tool for the analysis of complex samples including pharmaceutical, biological, and natural products. Recently, UHPLC (Ultra High Performance Liquid Chromatograph) has been successfully employed to remarkably decrease analysis time in the second dimension of comprehensive 2D-UHPLC system, and it is becoming popular to speed up 2D-LC separations. In this study, we employed 2D-UHPLC for the analysis of Panax ginseng components, a popular Chinese herbal medicine. A combination of RPLC (low pH) x RPLC (high pH) was selected and the chromatographic behavior of five major ginsenosides (ginsenoside Rg1, Re, Rb1, Rc, and Rd) and other minor compounds was monitored. All experiments have been run on a 2D-UHPLC system (Shimadzu Prominence), equipped with four high pressure pumps, and interfaced through ESI to an hybrid mass spectrometer (Shimadzu LCMS-IT-TOF), which possesses both MSn ability of an ion trap and the excellent resolution and mass accuracy of time-of-flight.

Apparatus & Conditions

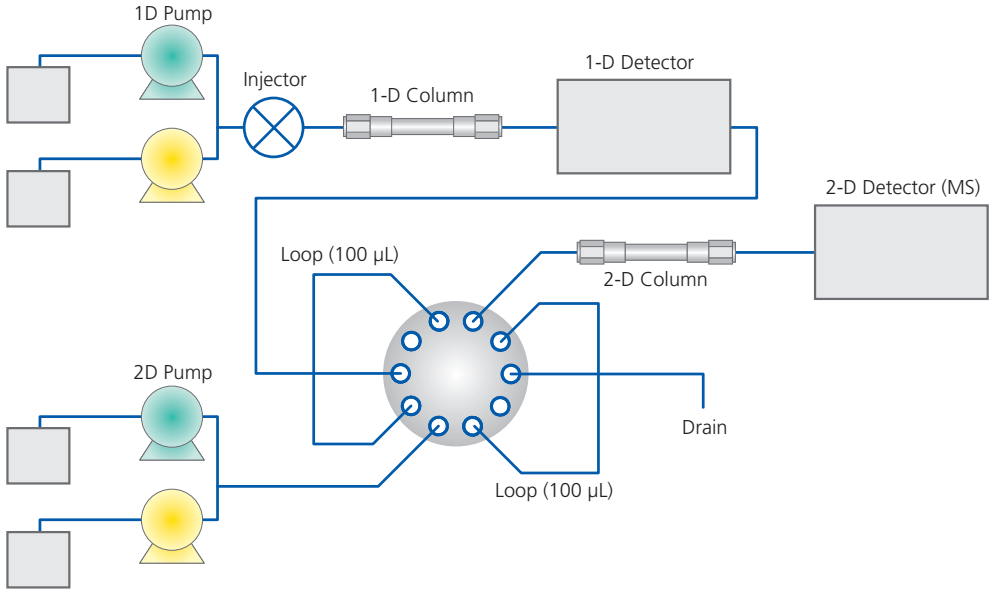

Fig. 1 Schematic of comprehensive 2-D UHPLC system using a 10-port switching valve between two dimensions.

Table 1 Analytical Condition

LC Condition ;	
Column (1D)	: Develosil ODS (0.5 mm I.D.×100 mm L, 3 mm)
Column (2D)	: ZORBAX Extend-C18 (2.0 mm I.D.×50 mm L., 1.8 mm)
Mobile phase (1D)	: Buffer (pH 2), Acetonitrile
Mobile phase (2D)	: Buffer (pH 9), Acetonitrile
Injection volume	: 2 mL
Column temp	: 50°C
2nd sample loop	: 100 mL
Flow rate (1D)	: 0.05 mL/min
Flow rate (2D)	: 0.6 mL/min
Modulation time	: 2 min

MS Condition ;	
Ionization mode	: ESI positive or negative (Auto MS/MS functionality)
Applied voltage	: +4.5 kV or -3.5kV
Nebulizer gas flow	: 1.5 L/min
Drying gas	: 0.1 MPa
BH temperature	: 200°C
CDL temp	: 200°C
Scan range	: m/z 100-1500 or 500-1500
Software ;	
Instrument control	: LCsolution (Shimadzu)
Formula prediction	: Formula Predictor (Shimadzu)
Visualization	: ChromSquare ver.1.0 (Chromaleont)

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Results

Two kinds of Panax ginseng samples were analyzed by the Comprehensive 2D-UHPLC IT-TOF system. Two dimentional plots of the TIC are shown in Fig. 3. The visualized results gave differential display of the samples. Fourteen ginsenosides were identified by LCMS-IT-TOF. The peak identification results are shown in Table 2.

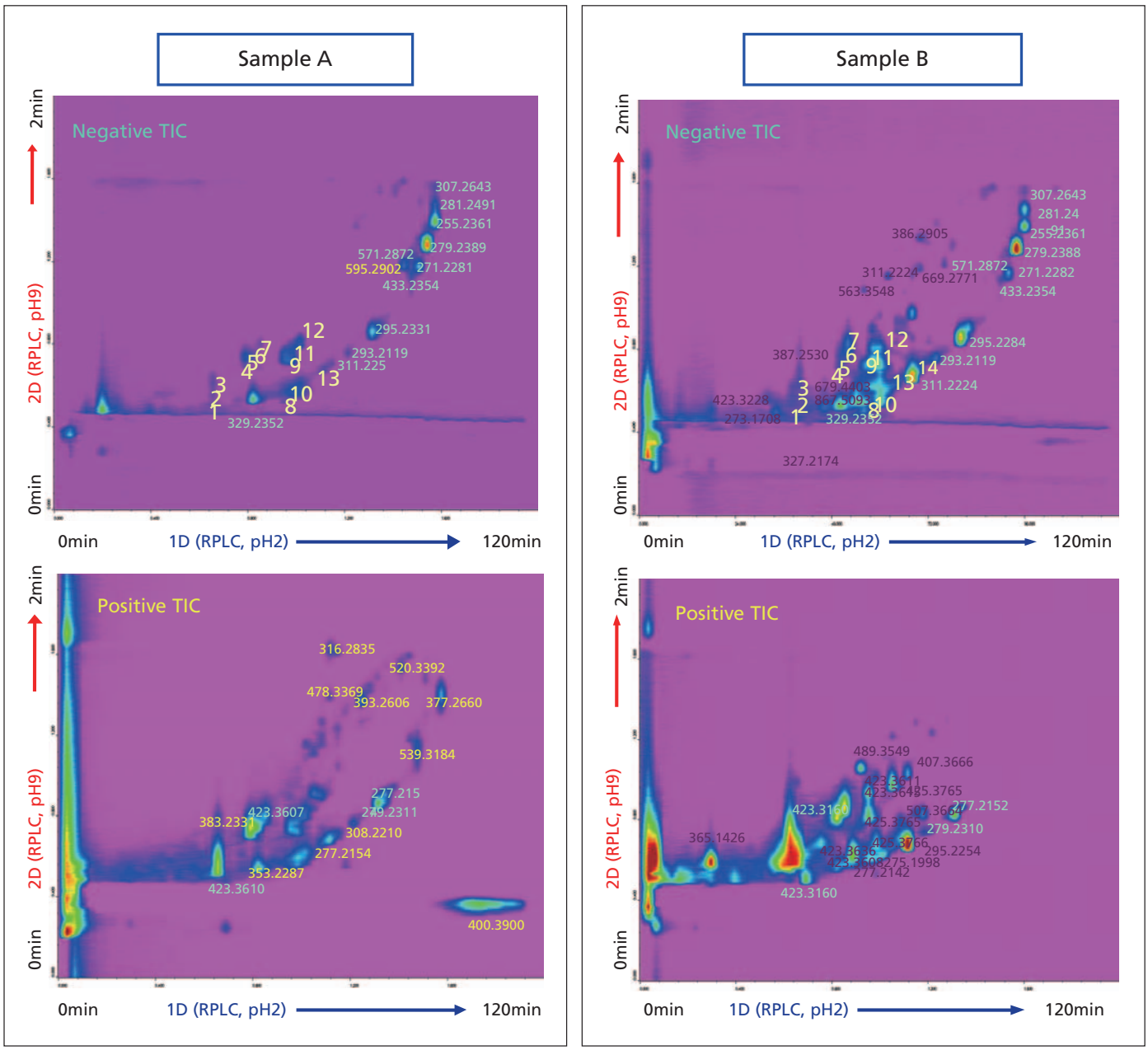

Fig.2 2-D plots of the TIC of comprehensive 2-D analysis of the Panax ginseng extract using RPLC(pH2) in the first dimension and RPLC(pH9) in the second dimension.
Upper Left : Sample A (Negative TIC), Upper Right : Sample B (Negative TIC),
Lower Left : Sample A (Positive TIC), Lower Right : Sample B (Positive TIC)

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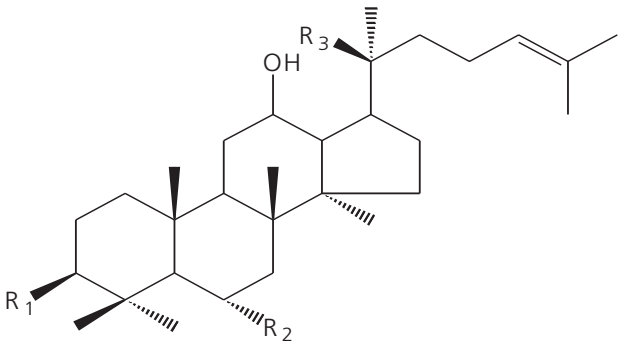
Table 2 Peak identification and mass accuracy for the analysis of ginsenosides

No.	Compound	Formula [M]	$[M-H]^-$ Calculated (monoisotopic)	$[M-H]^-$ Observed (monoisotopic)	Mass Accuracy (ppm)
1	notoginsenoside R1	C47 H80 O18	931.5252	931.5272	-2.2
2	ginsenoside Rg1	C42 H72 O14	799.4844	799.4849	-0.6
3	ginsenoside Re	C48 H82 O18	945.5397	945.5428	-3.3
4	ginsenoside Rf	C42 H72 O14	799.4817	799.4849	-4.0
5	notoginsenoside R2	C41 H70 O13	769.4755	769.4744	1.4
6	ginsenoside Rg2 or Rg3 or F2	C42 H72 O13	783.4899	783.4900	-0.1
7	ginsenoside F1 or Rh1	C36 H62 O9	637.4326	637.4321	0.8
8	ginsenoside Ro	C48 H76 O19	955.4893	955.4908	-1.6
9	ginsenoside Rb1	C54 H92 O23	1107.5913	1107.5957	-4.0
10	malonyl-ginsenoside Rb1	C57 H94 O26	1193.5902	1193.5961	-4.9
11	ginsenoside Rb2 or Rc	C53 H90 O22	1077.5830	1077.5851	-2.0
12	ginsenoside Rd	C48 H82 O18	945.5423	945.5428	-0.5
13	malonyl-ginsenoside Rd	C51 H84 O21	1031.5414	1031.5432	-1.7
14	ginsenoside Rh4	C36 H60 O8	619.4219	619.4215	0.7



Compound	R1	R2	R3
notoginsenoside R1	OH	O-Glc(2-1)Xyl	O-Glc
ginsenoside Rb1	O-Glc(2-1)Glc	H	O-Glc(6-1)Glc
ginsenoside Rb2	O-Glc(2-1)Glc	H	O-Glc(6-1)Arap
ginsenoside Rb3	O-Glc(2-1)Glc	H	O-Glc(6-1)Xyl
ginsenoside Rc	O-Glc(2-1)Glc	H	O-Glc(6-1)Araf
ginsenoside Rd	O-Glc(2-1)Glc	H	O-Glc
ginsenoside Re	OH	O-Glc(2-1)Rha	O-Glc
ginsenoside Rf	OH	O-Glc(2-1)Glc	OH
ginsenoside Rg1	OH	O-Glc	O-Glc
ginsenoside Rg2	OH	O-Glc(2-1)Rha	OH
ginsenoside Rg3	O-Glc(2-1)Glc	H	OH

Fig. 3 Structural formula of ginsenosides

Conclusions

1. The visualized results using the comprehensive 2-D UHPLC system and ChromSquare Software gave an easy and intuitive differential display of analysis two Panax ginseng samples. This method would be useful for not only Differential Display Analysis but also Principal Component Analysis.

2. The employment of the hybrid mass spectrometer (LCMS-IT-TOF) and Formula Predictor Software allowed the structural assignment of ginsenosides in Panax ginseng with similar formula below 5 ppm for analysis.

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