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R08. Characterization of Key Metabolites in Serum of Fuzheng Huayu Phase II Clinical Trial

Yan-Hong Wang
University of Mississippi, wangyh@olemiss.edu

ZaChara Catchings
University of Mississippi

Bharathi Avula
University of Mississippi

Mei Wang
University of Mississippi

Huashi Bian
Shanghai Sundise Traditional Chinese Medicine Co., (People's Republic of China)

See next page for additional authors

Authors
Yan-Hong Wang, ZaChara Catchings, Bharathi Avula, Mei Wang, Huashi Bian, and Ikhlas A. Khan

Characterization of Key Metabolites in Serum of Fuzheng Huayu (FZHY) Phase II Clinical Trial

Yan-Hong Wang[1], ZaChara Catchings[1], Bharathi Avula[1], Mei Wang[1], Huashi Bian[2], Ikhlas A. Khan[1,3]

[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, University, MS 38677, USA; [2]Shanghai Sundise Traditional Chinese Medicine Co., Ltd. F33 Zhaofeng Plaza, Changning District, Shanghai, P.R. China; [3]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA,

Goal of the Study

Qualitative analysis of thirty (30) marker components from fifteen (15) serum samples collected from FZHY Phase II clinical trial.

Serial No.	Marker Components
1	Danshensu
2	Tanshinone IIA
3	Salvianolic Acid B
4	Protocatechuic Aldehyde
5	Protocatechuic acid
6	Rosmarinic acid
7	Cryptotanshinone
8	Adenosine
9	Guanosine
10	Uridine
11	Ergosterol
12	Mannitol
13	Massoia Lactone
14	Amygdalin
15	Prunasin
16	Schisandrin A,
17	Schisandrin B
18	Schisandrin C
19	Schisandrol A
20	Schisandrol B
21	Schisantherin A
22	Ombuoside
23	Ombuin
24	Rutin
25	Quercetin
26	Kaempferol
27	Naringenin
28	Isoquercitrin
29	Gypenoside XLIX
30	Tanshinone I

Introduction

- Liver fibrosis is a reversible wound-healing response to cellular injury with the characteristics of the excessive accumulation of extracellular matrix (ECM) proteins including collagen that occurs in most types of chronic liver diseases.
- Fuzheng Huayu (FZHY) is a China Food and Drug Administration (CFDA) approved Traditional Chinese Medicines (TCM) product for the treatment of liver fibrosis in 2002. Its phase II clinical trial in the US had completed in 2013.
- FZHY is composed of six Chinese medicinal herbs and effective for treatment of liver fibrosis causing by chronic hepatitis B.
- The pharmacokinetics features of FZHY and bio-marker(s) to affect the pathological state of liver fibrosis are still not clear or well addressed.
- Systemically analyze the samples from phase II clinical trial will help to understand the absorption of FZHY in human body and to identify the key metabolites of FZHY.

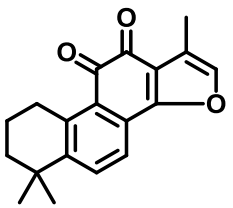
Formula of FZHY

Table 1
The formula of FZHY (one dose)[a]

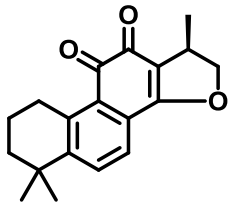
Chinese name	Plant sources	Medicinal parts	Amount in preparation (g)
Danshen	*Salvia Miltiorrhizae* Bge. (Labiatae)	Radix	8
Chongcao	Artifical fermentation cordyceps	Mycelia	4
Taoren	*Prunus persica* (L.) Batsch(Rosaceae)	Fruit	2
Jiaogulan	*Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae)	Whole herb	6
Songhuafen	*Pinus massoniana* Lamb.(Pinaceae)	Pollen	2
Wuweizi	*Schisandrae Chinensis* (Turcz.) Baill.(Magnoliaceae)	Fruit	2

T. Yang, S. Liu, et al., *J. Ethnopharm.* 2015, 166: 305-312

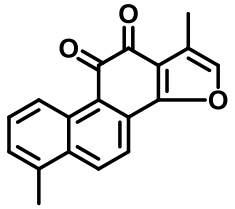
Major Marker Compounds in Danshen (*Salvia miltiorrhizea* Bge)



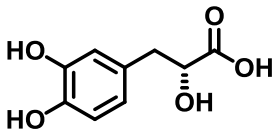
Tanshinone IIA
(CAS 568-72-9)
Chemical Formula: $C_{19}H_{18}O_3$
Exact Mass: 294.1256



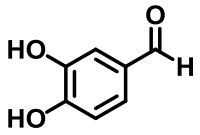
Cryptotanshinone
(CAS 35825-57-1)
Chemical Formula: $C_{19}H_{20}O_3$
Exact Mass: 296.1412



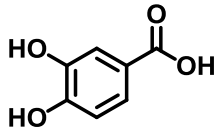
Tanshinone I
(CAS 568-73-0)
Chemical Formula: $C_{18}H_{12}O_3$
Exact Mass: 276.0786



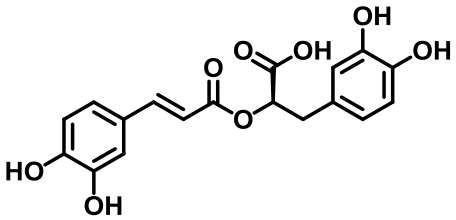
Danshensu
Chemical Formula: $C_9H_{10}O_5$
Exact Mass: 198.0528



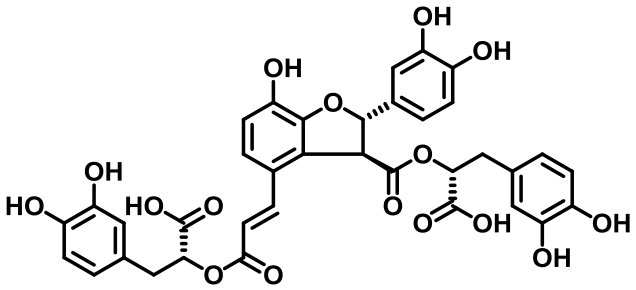
Protocatechuic Aldehyde
(CAS 139-85-5)
Chemical Formula: $C_7H_6O_3$
Exact Mass: 138.0317



Protocatechuic acid
(CAS 99-50-3)
Chemical Formula: $C_7H_6O_4$
Exact Mass: 154.0266

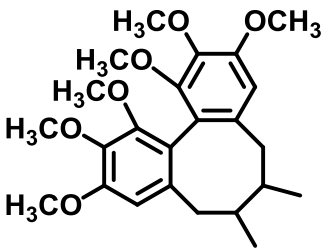


Rosmarinic acid
(CAS 20283-92-5)
Chemical Formula: $C_{18}H_{16}O_8$
Exact Mass: 360.0845



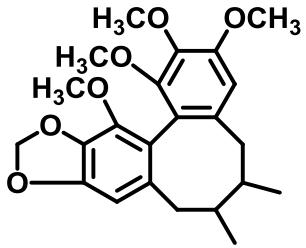
Salvianolic Acid B
(CAS 121521-90-2)
Chemical Formula: $C_{36}H_{30}O_{16}$
Exact Mass: 718.1534

Major Marker Compounds in Wuweizi (*Schisandrae chinensis*)



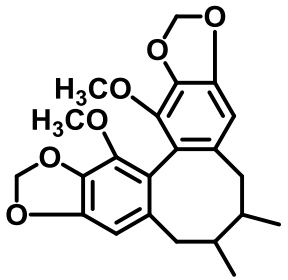
Schisandrin A
(CAS 61281-38-7)

Chemical Formula: $C_{24}H_{32}O_6$
Exact Mass: 416.2199



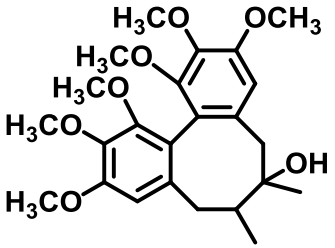
Schisandrin B
(CAS 61281-37-6)

Chemical Formula: $C_{23}H_{28}O_6$
Exact Mass: 400.1886



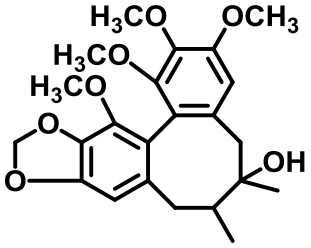
Schisandrin C
(CAS 61301-33-5)

Chemical Formula: $C_{22}H_{24}O_6$
Exact Mass: 384.1573



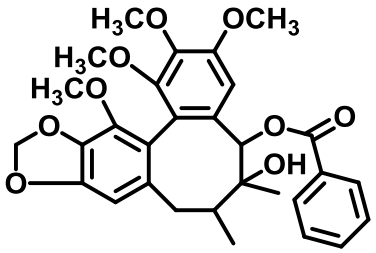
Schizandrin (Schisandrol A)
(CAS 7432-28-2)

Chemical Formula: $C_{24}H_{32}O_7$
Exact Mass: 432.2148



Schisandrol B
(CAS 58546-54-6)

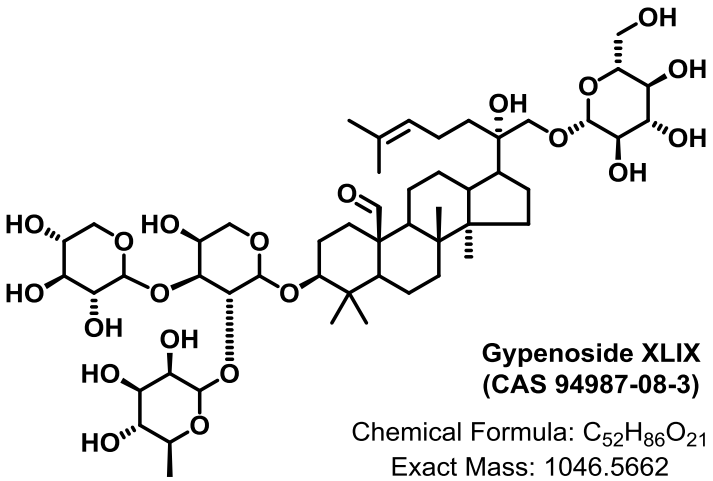
Chemical Formula: $C_{23}H_{28}O_7$
Exact Mass: 416.1835



Schisantherin A
(CAS 58546-56-8)

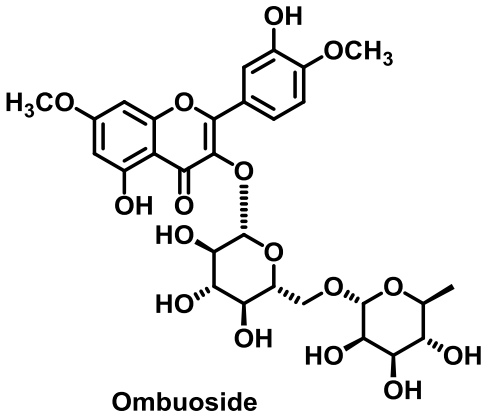
Chemical Formula: $C_{30}H_{32}O_9$
Exact Mass: 536.2046

Major Marker Compounds in Jiaogulan (*Gynostemma pentaphyllum*)



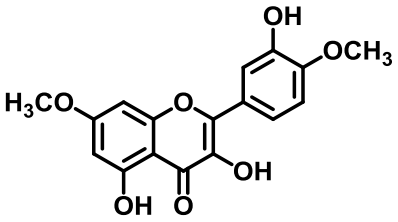
Gypenoside XLIX
(CAS 94987-08-3)

Chemical Formula: $C_{52}H_{86}O_{21}$
Exact Mass: 1046.5662



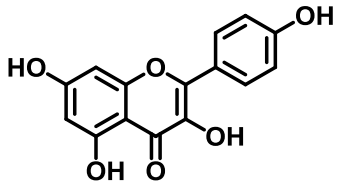
Ombuoside
(CAS 20188-85-6)

Chemical Formula: $C_{29}H_{34}O_{16}$
Exact Mass: 638.1847



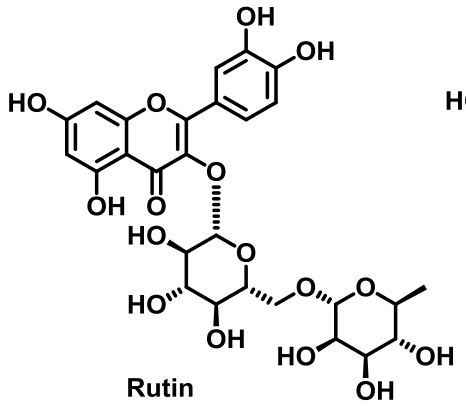
Ombuin
(CAS 529-40-8)

Chemical Formula: $C_{17}H_{14}O_7$
Exact Mass: 330.0740



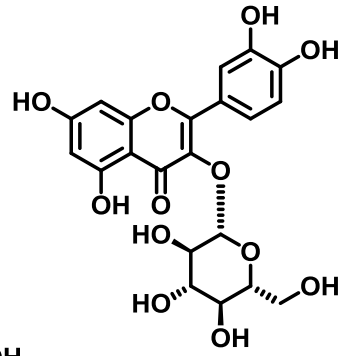
Kaempferol
(CAS 520-18-3)

Chemical Formula: $C_{15}H_{10}O_6$
Exact Mass: 286.0477



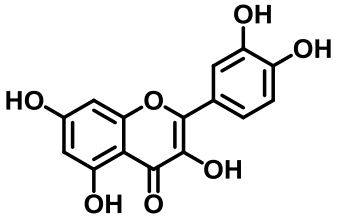
Rutin
(CAS 207671-50-9)

Chemical Formula: $C_{27}H_{30}O_{16}$
Exact Mass: 610.1534



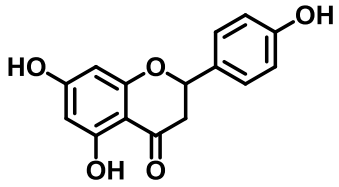
Isoquercitrin
(CAS 482-35-9)

Chemical Formula: $C_{21}H_{20}O_{12}$
Exact Mass: 464.0955



Quercetin
(CAS 849061-97-8)

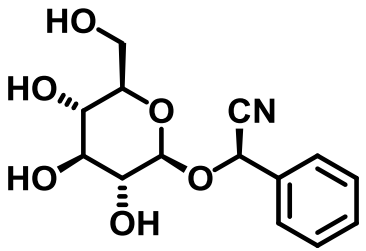
Chemical Formula: $C_{15}H_{10}O_7$
Exact Mass: 302.0427



Naringenin
(CAS 67604-48-2)

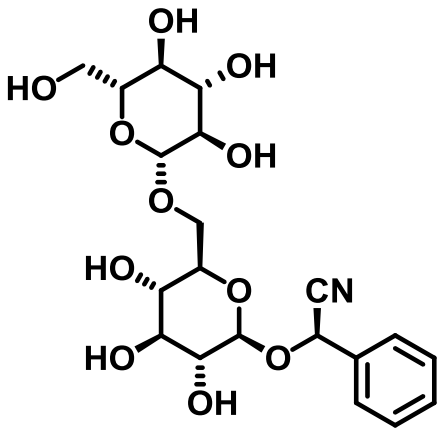
Chemical Formula: $C_{15}H_{12}O_5$
Exact Mass: 272.0685

Major Marker Compounds in Taoren (*Prunus persica* L.)



Prunasin
(CAS 99-18-3)

Chemical Formula: $C_{14}H_{17}NO_6$
Exact Mass: 295.1056



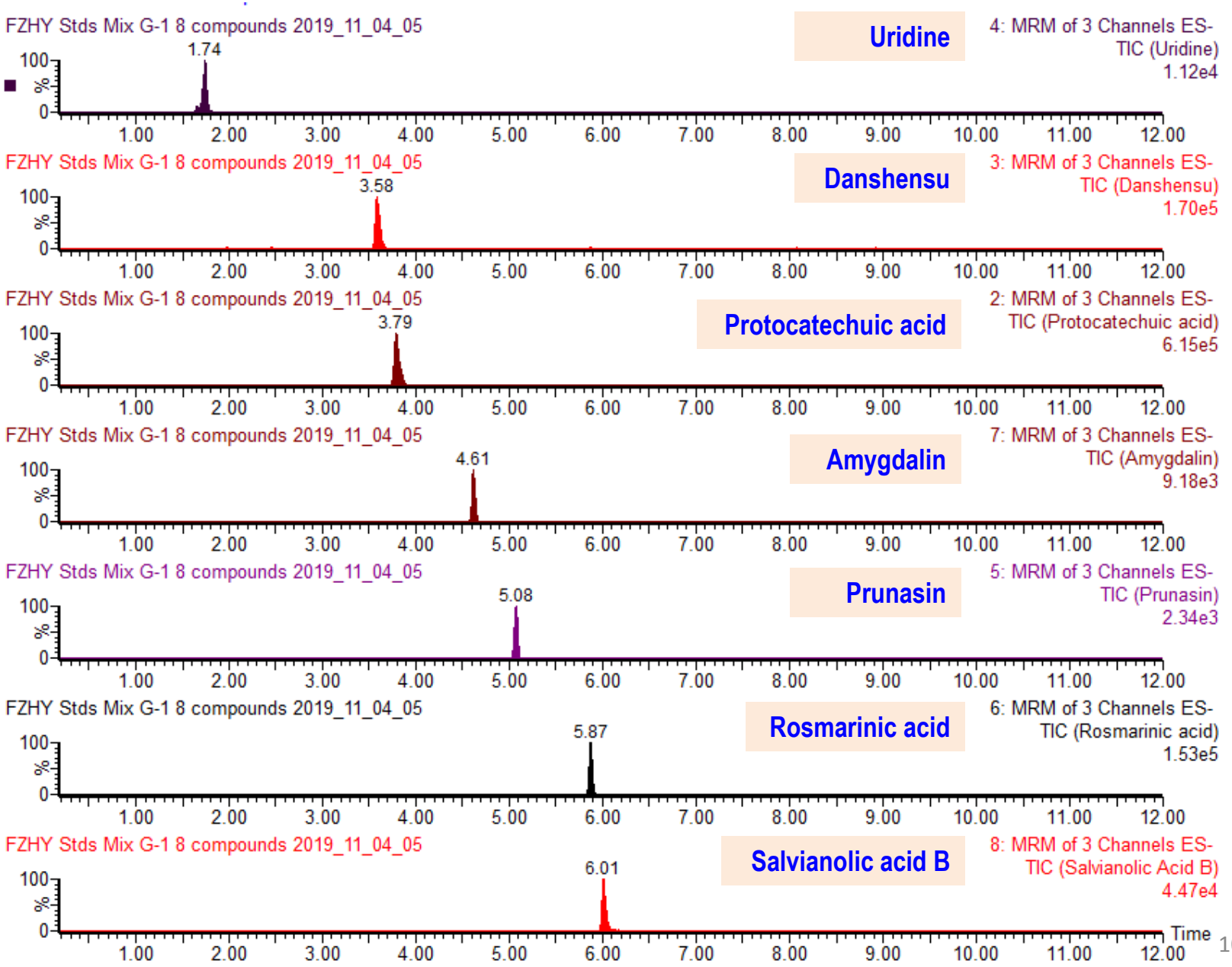
Amygdalin
(CAS 29883-15-6)

Chemical Formula: $C_{20}H_{27}NO_{11}$
Exact Mass: 457.1584

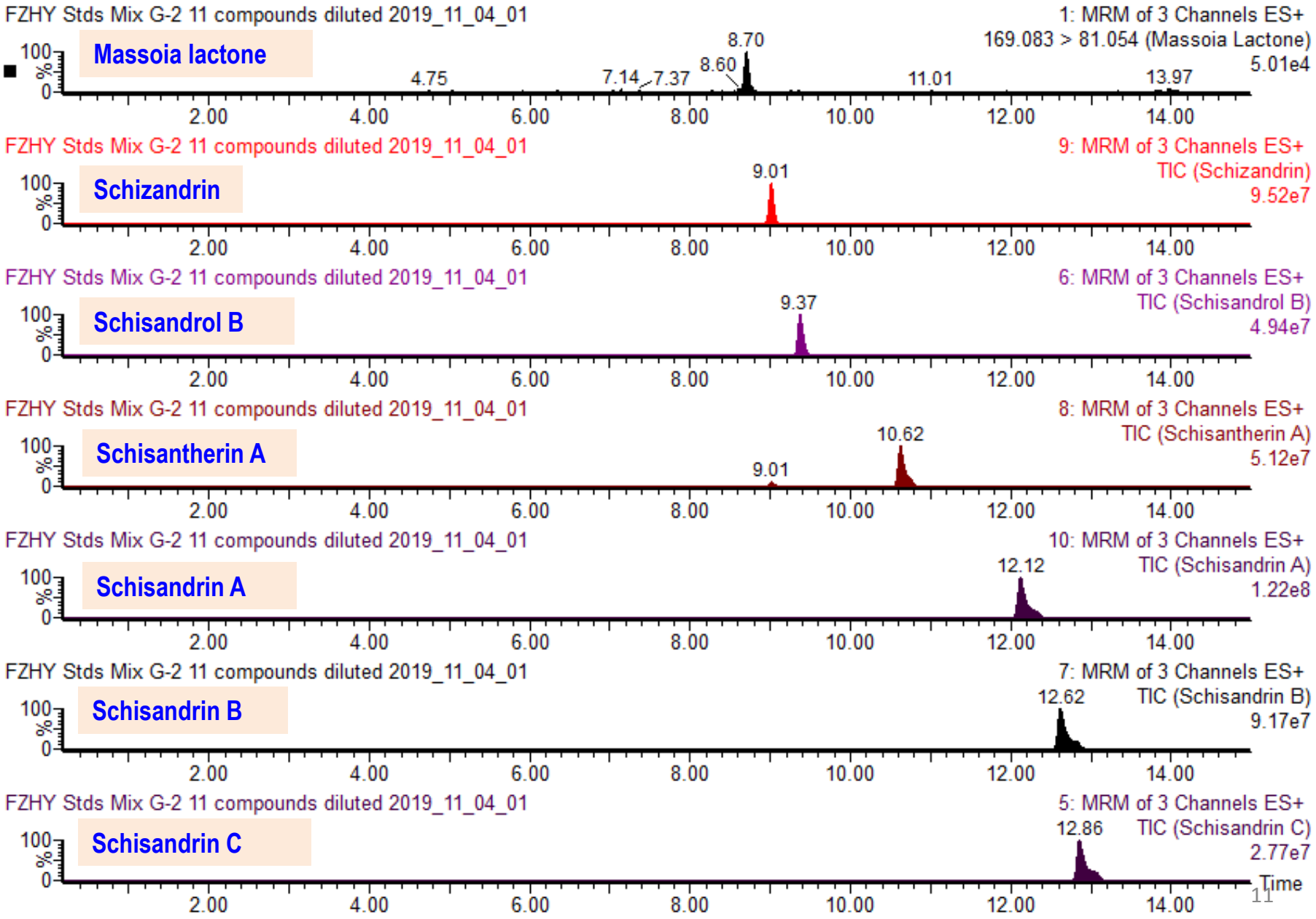
LC-MS/MS Method

UHPLC-UV System	
UPLC	**Waters Acquity UPLC™ system**
Column	Acquity UPLC™ HSS T3 column
Mobile Phase	Acetonitrile and Water with 0.1% formic acid
Temperature	45 ºC
Xevo TQ-S MS	
MS	**TQ-MS**
Ionization mode	ESI Positive and Negative
Source temp.	150°C
Desolvation temp.	300 °C

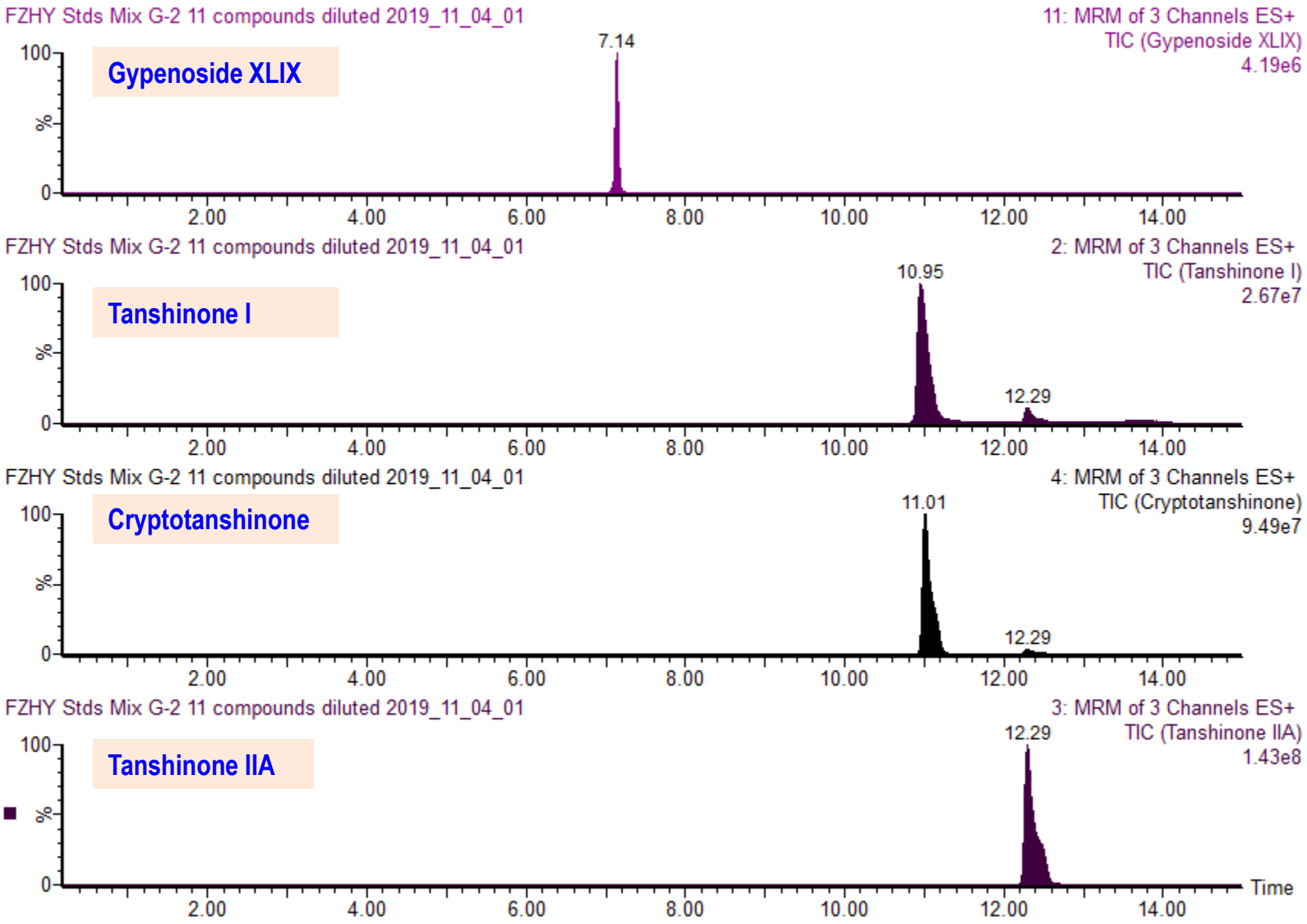
LC-MS/MS Analysis of Standard Compounds (Group-1)



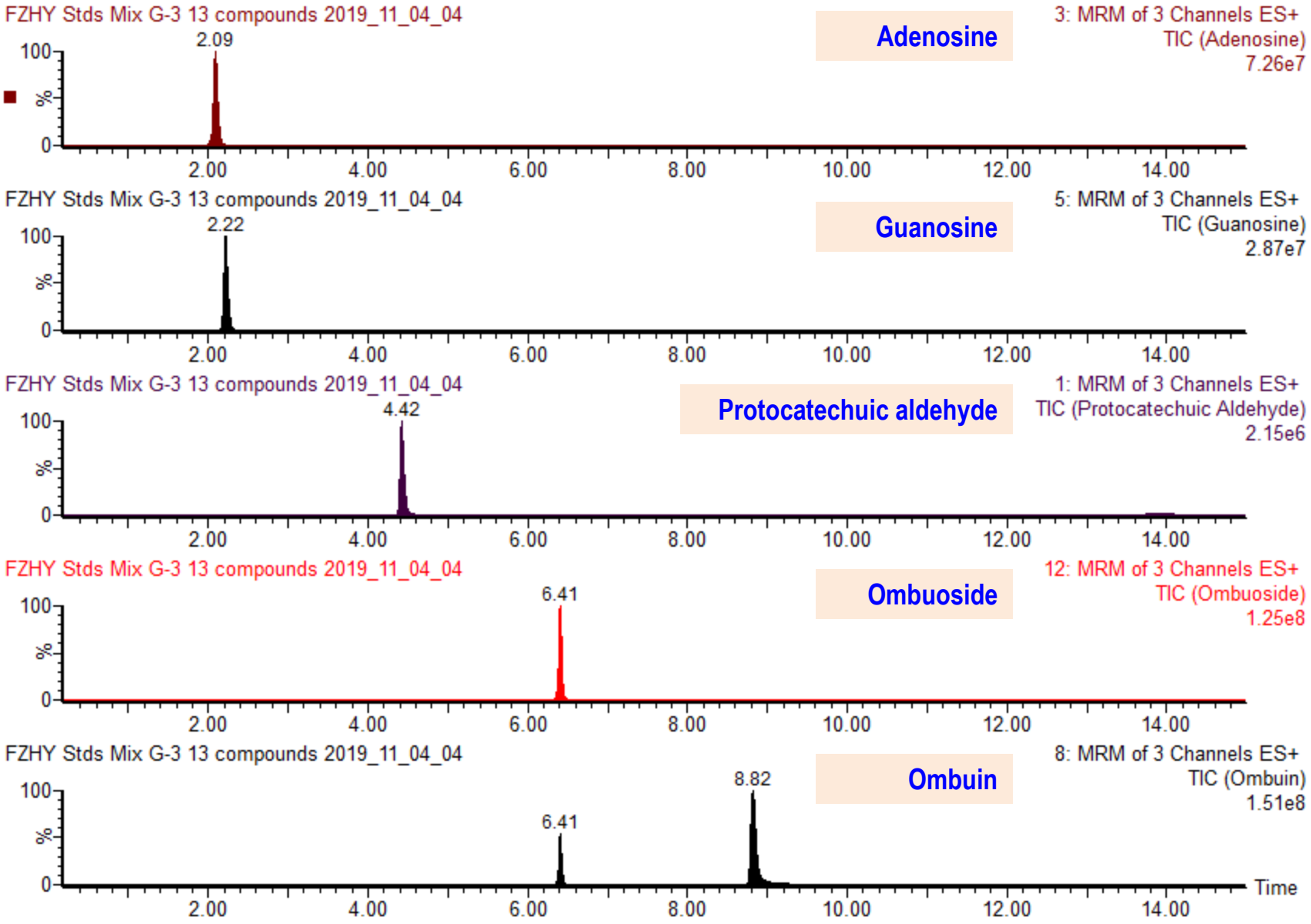
LC-MS/MS Analysis of Standard Compounds (Group-2)



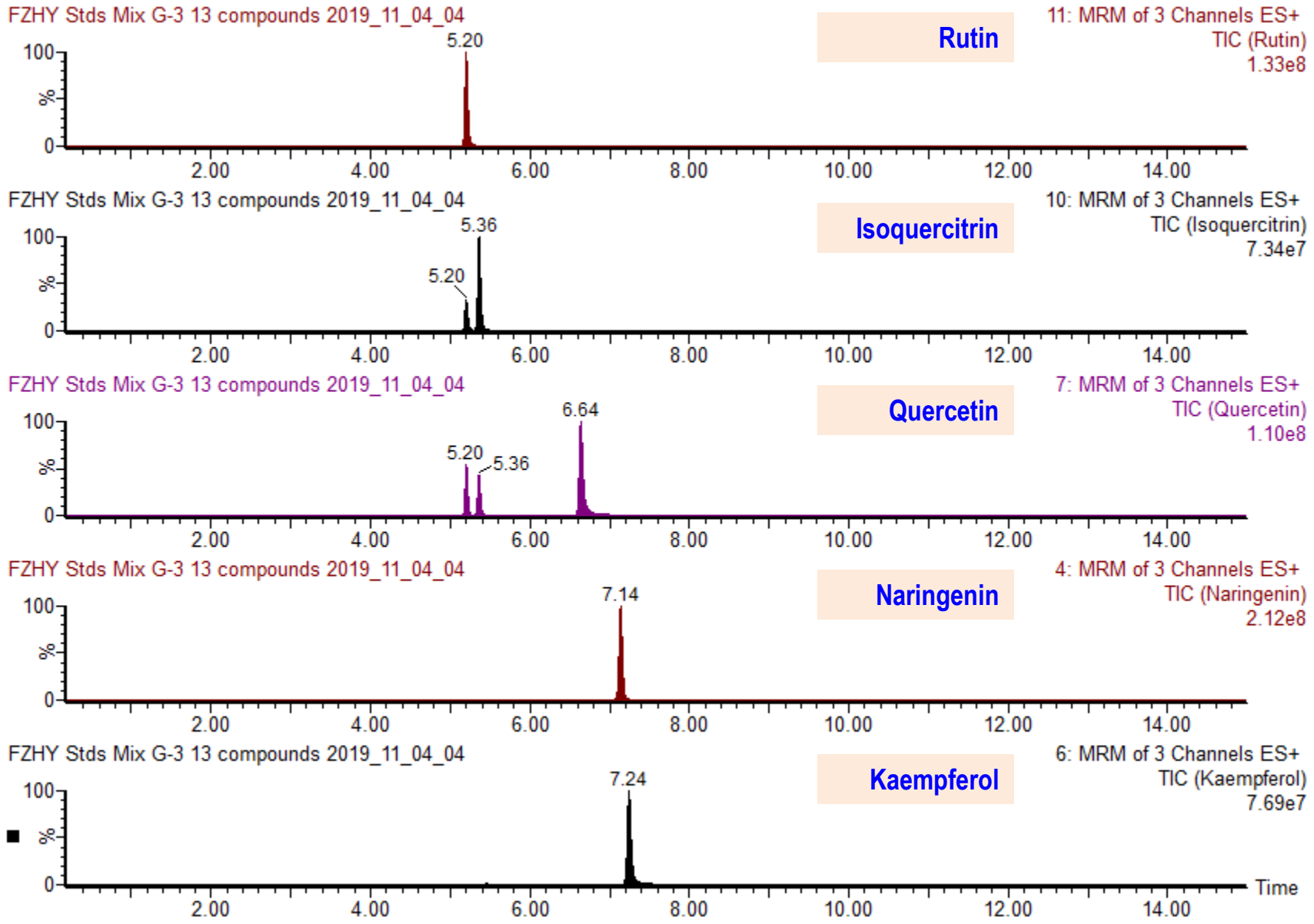
LC-MS/MS Analysis of Standard Compounds (Group-2) (*continued*)



LC-MS/MS Analysis of Standard Compounds (Group-3)



LC-MS/MS Analysis of Standard Compounds (Group-3) *(Continued)*

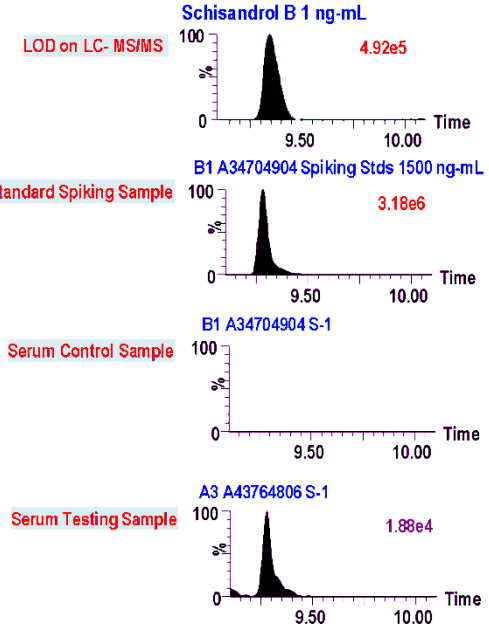


Serum Samples

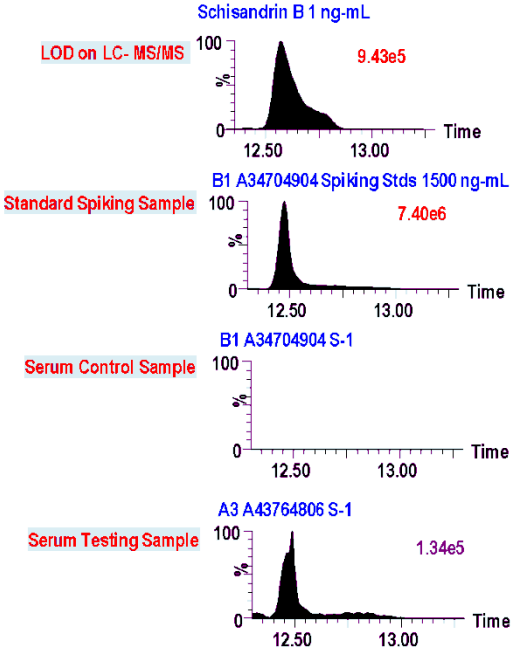
Group A		Group B	
A1 A41131604	Collected on 10/21/11	**B1 A34704904**	Collected on 02/22/11
A2 A43732106	Collected on 12/19/11	**B2 A41883706**	Collected on 03/24/11
A3 A43764806	Collected on 01/19/12	**B3 A41884506**	Collected on 04/19/11
A4 A44740206	Collected on 03/13/12	**B4 A34707506**	Collected on 06/07/11
A5 A44741406	Collected on 06/04/12	**B5 A43763406**	Collected on 09/15/11
A6 A44832006	Collected on 08/31/12	**B6 A43732906**	Collected on 02/29/12
A7 A44514904	Collected on 11/06/12		
A8 A44515701	Collected on 11/19/12		
A9 A44831606	Collected on 02/12/13		

Typical Chromatograms of Marker Compounds Detected on LC-MS/MS

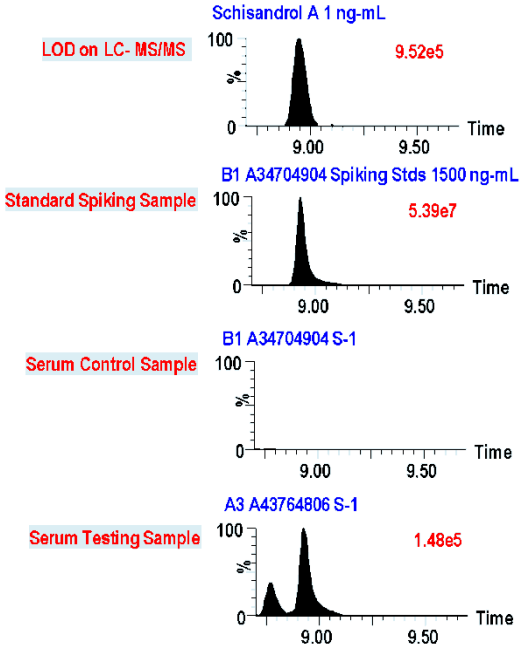
Schisandrol B



Schisandrin B



Schisandrol A



Results

- **Control and Group A Samples (Y = detected by LC-MS/MS)**

Category	Marker Compound	Control	Group A								
		B1 A34704904 Spiking Stds	A1 A41131604	A2 A43732106	A3 A43764806	A4 A44740206	A5 A44741406	A6 A44832006	A7 A44514904	A8 A44515701	A9 A44831606
Not Detected	Protocatechuic aldehyde	Y									
	Naringenin	Y									
	Tanshinone I	Y									
	Kaempferol	Y									
	Tanshinone IIA	Y									
	Cryptotanshinone	Y									
	Quercetin	Y									
	Schisandrin C	Y									
	Rutin	Y									
	Ombuoside	Y									
	Gypenoside XLIX	Y									
	Protocatechuic acid	Y									
	Danshansu	Y									
	Uridine	Y									
	Prunasin	Y									
	Rosmarinic acid	Y									
	Amygdalin	Y									
	Salvianolic acid B	Y									
	Massoia lactone										
	Isoquercitrin										
Detected in All Samples	Adenosine	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	Guanosine	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Detected in Group A Samples	Ombuin	Y	Y	Y	Y	Y	Y	Y	Y	Y	
	Schisandrol B	Y			Y	Y	Y	Y		Y	
	Schisandrin B	Y			Y	Y	Y	Y	Y	Y	
	Schisantherin A	Y			Y	Y	Y	Y		Y	
	Schisandrol A	Y			Y	Y	Y	Y		Y	
	Schisandrin A	Y			Y	Y	Y	Y		Y	

Results

- **Control and Group B Samples (Y = detected by LC-MS/MS)**

Category	Marker Compound	Control	Group B					
		B1 A34704904 Spiking Stds	B1 A34704904	B2 A41883706	B3 A41884506	B4 A34707506	B5 A43763406	B6 A43732906
Not Detected	Protocatechuic aldehyde	Y						
	Naringenin	Y						
	Tanshinone I	Y						
	Kaempferol	Y						
	Tanshinone IIA	Y						
	Cryptotanshinone	Y						
	Quercetin	Y						
	Schisandrin C	Y						
	Rutin	Y						
	Ombuoside	Y						
	Gypenoside XLIX	Y						
	Protocatechuic acid	Y						
	Danshansu	Y						
	Uridine	Y						
	Prunasin	Y						
	Rosmarinic acid	Y						
	Amygdalin	Y						
	Salvianolic acid B	Y						
	Massoia lactone							
	Isoquercitrin							
Detected in All Samples	Adenosine	Y	Y	Y	Y	Y	Y	Y
	Guanosine	Y	Y	Y	Y	Y	Y	Y
Detected in Group A Samples	Ombuin	Y						
	Schisandrol B	Y						
	Schisandrin B	Y						
	Schisantherin A	Y						
	Schisandrol A	Y						
	Schisandrin A	Y						

Conclusion

Total 15 serum samples (Group A: 9 samples; Group B: 6 samples) were analyzed. The results showed that schisandrol A, schisandrol B, schisandrin A, schisandrin B, schisantherin A, and ombuin were found in some of Group A samples, but not detected from Group B samples. Adenosine and guanosine were identified in all serum samples. Apart from this, other 22 marker compounds (see Results part) were not detected from any of serum samples.

In conclusion, marker compounds **schisandrol A, schisandrol B, schisandrin A, schisandrin B, schisantherin A,** and **ombuin** were identified in serum samples collected from a patient in FZHY Phase II study.