PO-CON1234E

SHIMADZU
Excellence in Science

Identification of antioxidants in Fructus aurantii using a new on-line combination of analytical techniques

IMSC 2012 PTh-031

Zongtao LIN[1], Jing DONG[2], Hong WANG[1], Ying XU[1], Yuki HASHI[2], Shizhong CHEN[1*]
[1]School of Pharmaceutical Sciences, Peking University, Beijing, China
[2]Shimadzu(China), China

Identification of antioxidants in Fructus aurantii using a new on-line combination of analytical techniques

Introduction

A new on-line method for simultaneous identification and monitoring of antioxidants in Fructus aurantii was established by coupling high performance liquid chromatography-diode array detector-electrospray ionisation-ion trap-time of flight-mass spectrometry with post-column derivatisation and luminol-potassium ferricyanide chemiluminescence (HPLC-DAD-ESI-IT-TOF-MS-PCD-LPFCL). The HPLC fingerprint, structural identification and radical scavenging profile were rapidly obtained by an on-line system using ultraviolet (UV) absorption, MS and LPFCL. Details of the precise substitution patterns of various structures were achieved through UV absorption shift using PCD. Twenty-six flavonoids were identified by either their PCD and MS data or comparison with reference substances. The results showed that this method was rapid and precise, and therefore would be an effective and sensitive method for bioactive components analysis and quality evaluation for complex medicinal samples.

Experimental

Sample preparation

1.0 g of *F.* aurantii powder (60 mesh) was accurately weighed and extracted with 50 mL methanol in an ultrasonic water bath for 30 min.

HPLC conditions

Column: Diamonsil C_{18} column (250 mm × 4.6mm i.d.; 5 μm)
Oven temperature: 40°C
Wavelength range: 200-400nm
A: ACN+0.02%FA (%, v/v)
B: Water+0.02% FA (%, v/v)
Injection volumn: 10 μL
Flow rate: 1.0 mL/min

Table 1 Gradient Program

Time(min)	Solution A (%)	Solution B (%)
0.00	95	5
20.00	75	25
50.00	10	90

Post Column Derivisation system solutions

Table2 Experimental conditions for the post column addition of UV shift reagents

Shift reagent	Pump 1	Flow 1 (mL·min-1)	Pump 2	Flow 2 (mL·min-1)	pH	Temp (°C)
$AlCl_3$	NaOH[a]	0.8	$AlCl_3$[b]	0.8	5.0	90
$AlCl_3$/ HCl	NaOH[a]	0.8	$AlCl_3$[b]/ HCl	0.8	3.5	90
NaOAc	NaOH[a]	0.8	NaOAc[c]	0.8	8.0	50
NaOAc/H_3BO_3	NaOH[a]	0.8	NaOAc[d]/H_3BO_3[d]	0.8	6.0	50

a. 0.01mol·L^{-1}NaOH aqueous solution
b. 0.3mol·$L^{-1}$$AlCl_3$ aqueous solution; c. 0.5mol·L^{-1}NaOAc aqueous solution;
d. 0.1mol·L^{-1}NaOAc/0.7 mol·$L^{-1}$$H_3BO_3$=1:1(v/v)

Post column derivisation techniques were used to give additional structural information, such as the linkage of sugar moieties, free phenolic groups, by inducing a shift of the UV absorption maxima of compounds.

Identification of antioxidants in Fructus aurantii using a new on-line combination of analytical techniques

ESI-IT-TOF-MS analysis

The MSn experiments were performed by using a LCMS-IT-TOF system (Shimadzu Corporation) equipped with an ESI source. All of the MS^n data were acquired in both positive and negative ion modes, CDL temperature 200°C, heat block temperature 200°C. The collision energies for each compound ranged from 20-50%. Spectra were acquired over a mass range of *m/z* 100-800.

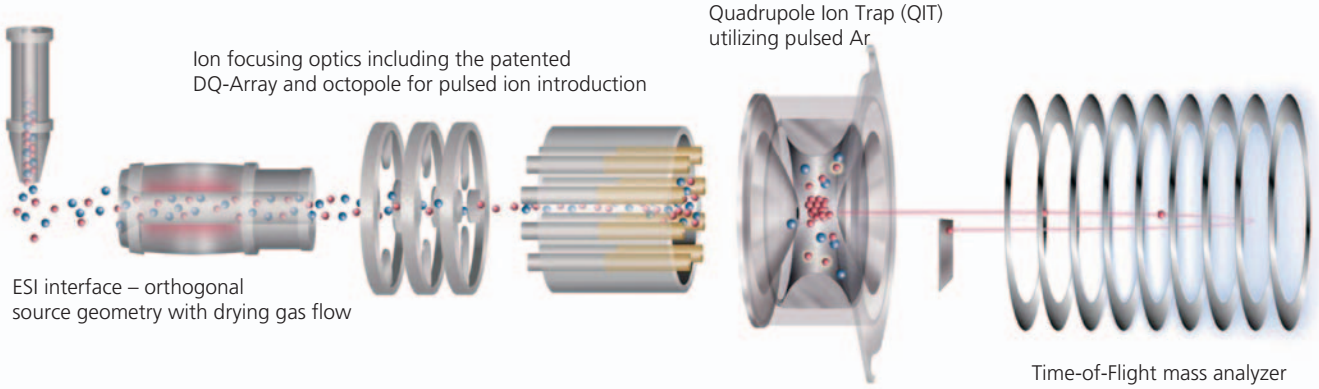

Fig. 2 Schematic representation of the LCMS-IT-TOF

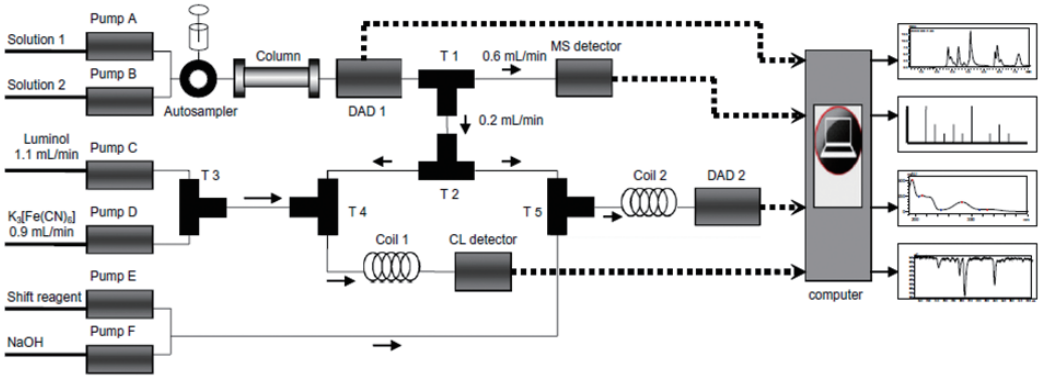

Fig. 3 HPLC-DAD-ESI-MS-PCD-LPFCL detection apparatus (pumps E and F were only used during PCD analysis; T: T-piece).

*3.0 ×10^{-4} M luminol solution (containing 10^{-4} M EDTA, pH 13.0)
*3.0 ×10^{-4} M $K_3Fe(CN)_6$ solution (pH13.0)
*Temp. room temperature

Identification of antioxidants in Fructus aurantii using a new on-line combination of analytical techniques

LPFCL detection method

Free radical *in vivo* like superoxide radicals ($O_2^{\cdot -}$) are known to be involve in various disease processes. Natural compounds possessing free radical scavenging properties were considered to be safe antioxidant agents for prevention and treatment of those diseases.
Luminol-potassium ferricyanide chemiluminesence (LPFCL) involving a superoxide radical mechanism were thus considered to evaluate radical scavenging activity of plant extracts or individual compounds. A on-line system was developed (Fig. 3) to screen the potential antioxidants in plant extracts and identify their structures.
One quarter of the eluate stream (0.2 ml/min) was added to a mixed solution of luminol (1.1 mL/min) and $K_3Fe(CN)_6$ (0.9 ml/min) at a T-piece, then immediately introduced into a reaction coil (10 m, 0.25 mm) maintained at 25°C throughout the detection. The mixture finally arrived at a fluorescence spectrophotometer for recording the intensity of emission light at 425 nm.

Results and discussion

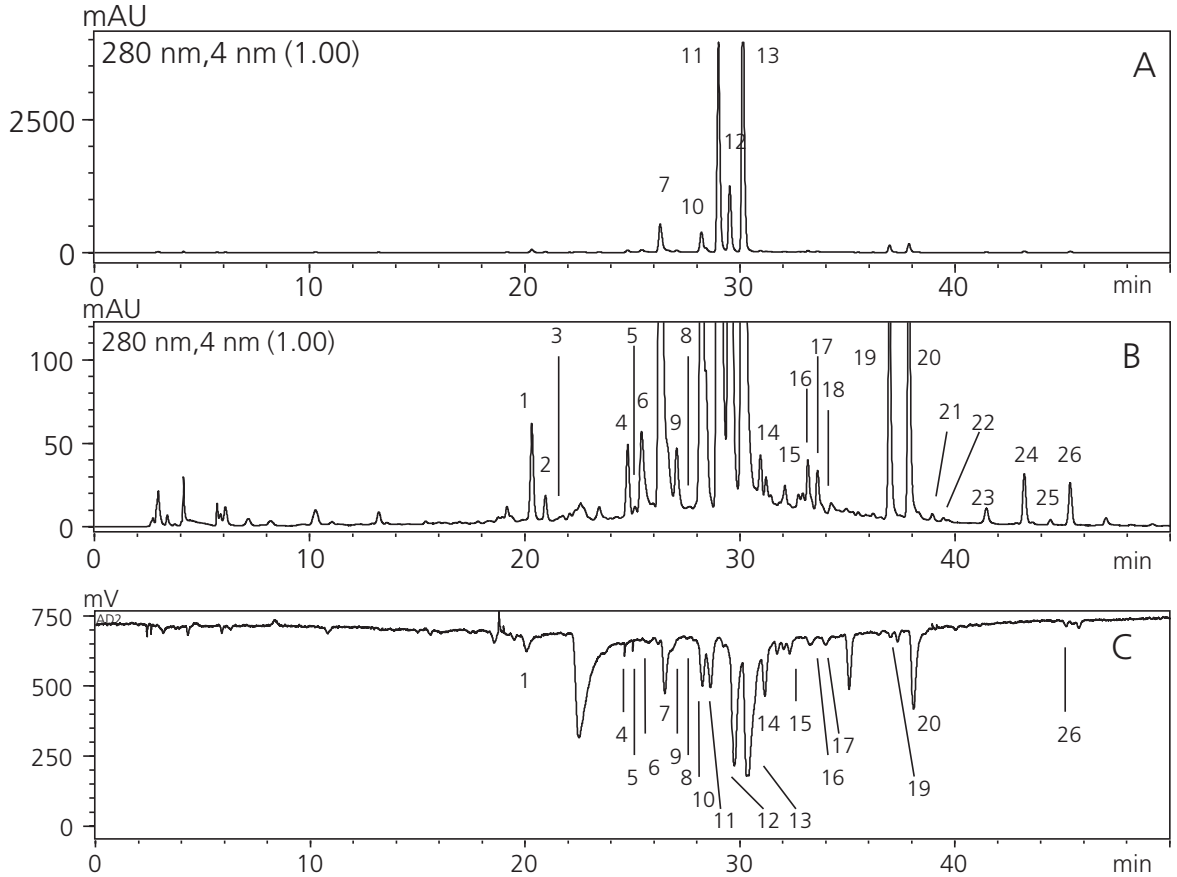

Fig. 4 HPLC chromatograms (A and B) and LPFCL inhibition profile of Fructus Aurrantii

Identification of antioxidants in Fructus aurantii using a new on-line combination of analytical techniques

Table3 UV shift data

No.	RT (min)	UV spectra		$AlCl_3$		$AlCl_3$/HCl		NaOAc		NaOAc/H_3BO_3	
		II	I	II	I	II	I	II	I	II	I
1	20.32	271	334	300	379	301	378	282	333	281	343
2	20.96	275	339	281	352	278	378	284	371	296	369
3	21.77	284	332	306	374	305	381	284	363	284	347
4	24.78	284	325	302	375	301	377	285	361	283	325
5	25.11	265	351	276	392	275	376	272	372	266	389
6	25.43	284	325	302	372	303	370	288	350	282	325
7	26.29	276	330	276	324	275	370	281	364	279	325
8	27.67	273	325	278	325	275	370	278	365	279	325
9	27.06	285	330	302	376	304	370	287	349	283	389
10	28.21	283	329	303	376	303	376	284	356	282	325
11	29.00	282	328	303	376	303	376	284	356	283	329
12	29.52	284	326	302	376	301	376	285	356	283	327
13	30.17	283	326	302	375	301	374	285	356	283	325
14	30.96	275	342	303	374	304	374	274	-	278	388
15	32.09	282	326	282	324	304	370	282	354	281	332
16	33.16	282	330	303	366	301	370	282	360	282	325
17	33.60	282	330	303	373	301	376	282	360	282	331
18	35.49	287	324	305	370	307	367	324	-	281	325
19	36.95	284	324	308	375	311	367	319	-	286	324
20	37.85	271	336	270	331	270	330	270	332	270	334
21	38.94	276	324	275	324	273	324	-	325	275	324
22	39.45	278	324	277	325	273	330	272	330	-	325
23	41.45	270	333	269	336	269	335	269	336	269	336
24	43.22	254	343	255	344	254	344	254	344	254	342
25	44.43	271	323	269	326	270	324	270	324	269	325
26	45.35	281	341	289	354	289	355	288	-	280	345

A variety of bioactive flavonoids were separated and detected by the on-line system. A number of peaks displayed their antioxidative ability in the corresponding inhibition profile (See Fig. 4 chromatogram C).

Multi-stage MS analysis were performed in both positive and negative modes. Based on the MSn data and UV shift information (Table 3), 26 compounds were identified (See Table 4).

Table4 MS^n data and identified results

No.	RT (min)	Compound Name	(+)ESI-MSn data (Observed)
1	20.32	6,8-Di-C-glucopyranocylapigenin	595.1626→577.1532→457.1126
2	20.96	6,8-Di-C-glucopyranocyldiosmetin	625.1751→607.1669→487.1220
3	21.77	Naringenin -7-O-triglycoside	743.2382→581.1871→273.0840
4	24.78	Eriocitrin	597.1817→289.0772→
5	25.11	Rutin	611.1623→465.0877→303.0487
6	25.43	Neoeriocitrin	597.1817→289.0772→
7	26.29	Isovitexin	433.1190→397.0895→283.0635
8	27.67	3′ -Methoxyl isovitexin	463.1277→397.0973→313.0698
9	27.06	Naringenin-7-O-sophorose	597.1855→435.1314→273.0794
10	28.21	Narirutin	581.1841→419.1372→273.0774
11	29.00	Naringin	581.1851→419.1388→273.0792
12	29.52	Hesperidin	611.1957→449.1494→303.0900
13	30.17	Neohesperidin	611.1952→449.1444→303.0875
14	30.96	Neohesperidin	653.1718→347.0767→332.0532
15	32.09	7-O-6′′ - Malonylnaringin	667.1808→521.1370→359.1181
16	33.16	Poncirin	595.2099→433.1513→287.0937
17	33.60	Neoponcirin	595.2090→433.1496→287.0947
18	35.49	Naringenin	273.0761→147.0457→
19	36.95	Hesperitin	303.0870→177.0566→145.0322
20	37.85	Isosinensetin	373.1287→343.0818→163.0759
21	38.94	Gossypetin hexamethyl	403.1393→373.1287→358.0689
22	39.45	Auranetin	373.1287→343.0818→163.0759
23	41.45	Nobiletin	403.1393→373.1287→358.0689
24	43.22	3′ ,4′ ,3,5,6,7,8-Hexa-methoxyflavone	433.1499→403.1393→373.1287
25	44.43	Tangeritin	373.1287→343.0818→168.0059
26	45.35	7-Hydroxyl-4′ ,3,5,6,8-Pentamethoxyflavone	389.1236→359.0767→341.0661

Identification of antioxidants in Fructus aurantii using a new on-line combination of analytical techniques

Conclusions

An on-line system based on the combination of HPLC, MS, PCD and LPFCL was established and investigated to screen and identify multiple active constituents in Fructus Aurantii.
This method was rapid and effective for screening and identification of antioxidative compounds with superoxide scavenging activity in complicated herbal extracts and thus it can offer a potential approach for components analysis and quality control.

First Edition: September, 2012

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/