Suporting information

Table S1. Data pre-processing parameters

(a) Pre-processing paremeters with MarkerView™ software

Enhance Peak Finding:			
subtraction offset (scans)	4	subtraction offset	1.3
Min. retention time (scans)	3	Max. retention time (scans)	100
Min. spectral width (amu)	0.03 (+) / 0.01 (-)	noise threshold (cps)	20 (+) / 10 (-)
Peak Alignment, Filtering:			
retention time tolerance (min)	0.20 (+) / 0.15 (-)	mass tolerance (amu)	0.06 (+) / 0.10 (-)
retention time correction			
positive ion mode	*m/z* 309.20/1.81 min, *m/z* 367.17/5.76 min, *m/z* 265.12/8.80 min, *m/z* 281.21/12.92 min, *m/z* 326.21/15.92min, *m/z* 158.15/19.50 min		
negative ion mode	*m/z* 195.05/1.94 min, *m/z* 365.14/5.64 min, *m/z* 263.11/8.73 min, *m/z* 269.15/12.26 min, *m/z* 331.18/17.48 min		
Filtering:			
Number required samples	32	Max. number of peaks	10000

(b) Pre-processing parameters with XCMS under R statistical software

```
rm(list=ls(all=TRUE))
library(Biobase)
library(xcms)
library(multtest)
library(CAMERA)
sessionInfo()
xs<-xcmsSet(profmethod = "binlin",method="centWave",ppm = 80, peakwidth=c(6,30),
prefilter=c(5,150),snthresh =50, mzdiff =0.05,scanrange=c(60,1260))
xs <-group(xs,bw=5,minfrac=0.4)
save(xs,file="xs.Rda")
ret.xs.obiwarp <-retcor(xs,method="obiwarp",plottype="deviation")
ret.xs.obiwarp<-group(ret.xs.obiwarp, bw =5,minfrac=0.4)
save(ret.xs.obiwarp, file="ret.xs.obiwarp.Rda")
fill.ret.xs.obiwarp<-fillPeaks(ret.xs.obiwarp)
save(fill.ret.xs.obiwarp, file="fill.ret.xs.obiwarp.Rda")
report.fill.ret.xs.obiwarp<-diffreport(fill.ret.xs.obiwarp,"NC","GJ",eicmax=5000,file="gongjing
pos")
save(report.fill.ret.xs.obiwarp, file="report.fill.ret.xs.obiwarp.Rda")
```

(c) Pre-processing parameters applied in MZmine.

Settings		(+)ESI-MS	(–)ESI-MS
Centroid mass detector	Noise level (counts)	100	100
High data point chromatogram construction	Minimum time span (s)	1	1
	Minimum height(counts)	20	20
	m/z tolerance size	0.1	0.1
Baseline peak recognizer	Minimum acceptable height (counts)	50	50
	Minimum peak duration (s)	2	2
	absolute baseline level (counts)	700	700
Alignment	*m/z* tolerance size	0.1	0.1
	RT tolerance size (s)	6	6
Gap-filling	Intensity tolerance (%)	20	20
	m/z tolerance size	0.1	0.1
	RT tolerance size (s)	6	6

Table S2. The average retention time and peak area variation of seven standard compounds in test compounds mixture during the beginning, middle, and end of the run analyzed in positive and negative ion mode. (n = 5)

compound	Positive ion mode				Negative ion mode			
	RT (min)	RSD (%)	Peak Area ($\times 10^4$cps)	RSD (%)	RT (min)	RSD (%)	Peak Area ($\times 10^3$cps)	RSD (%)
cytidine	2.17 ± 0.01	0.46	4.54 ± 0.22	4.84	2.13 ± 0.00	0.00	7.41± 0.05	0.67
Phe.	6.08 ± 0.02	0.33	22.20 ± 1.50	6.76	5.95 ± 0.01	0.17	0.85± 0.03	3.53
Trp.	7.51± 0.02	0.27	7.83± 0.30	3.83	7.40 ± 0.01	0.14	4.85 ± 0.24	4.95
HA	8.84± 0.01	0.11	1.53± 0.11	7.19	8.79± 0.01	0.11	137.00 ± 2.00	1.46
scutellarin	9.99± 0.01	0.10	3.89± 0.08	2.06	9.92± 0.01	0.10	55.30±4.80	8.80
luteolin	14.12± 0.03	0.21	19.40± 0.80	4.12	14.03± 0.03	0.21	408.00± 14.00	3.43
rhein	——	——	——	——	20.32± 0.02	0.10	288.00± 11.00	3.82

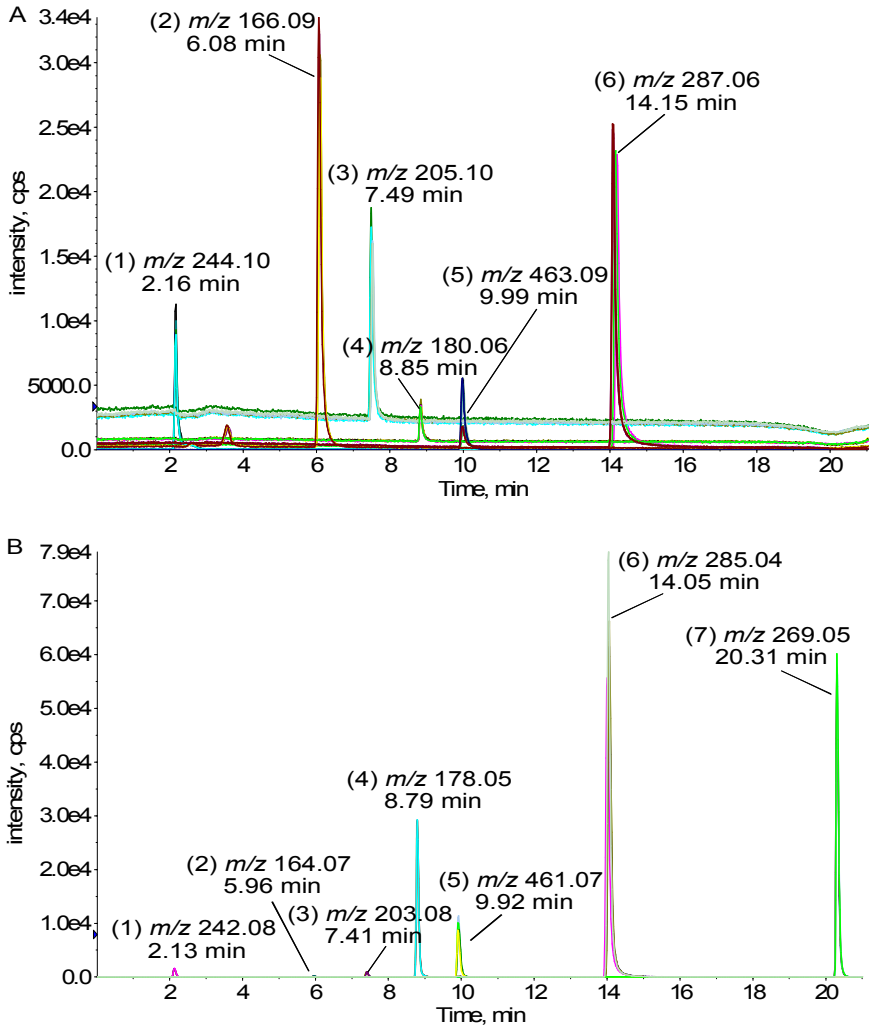

Figure S1. Overlaid XICs of test compounds mixture during the whole run by using Q-TOF MS/MS instrument in (A) positive ion mode and (B) negative ion mode. They give an overall idea of the system stability during the run. (1) cytidine; (2) phenylalanine(phe.); (3) tryptophan(trp.); (4) hippuric acid (HA); (5) scutellarin; (6) luteolin; (7) rhein.

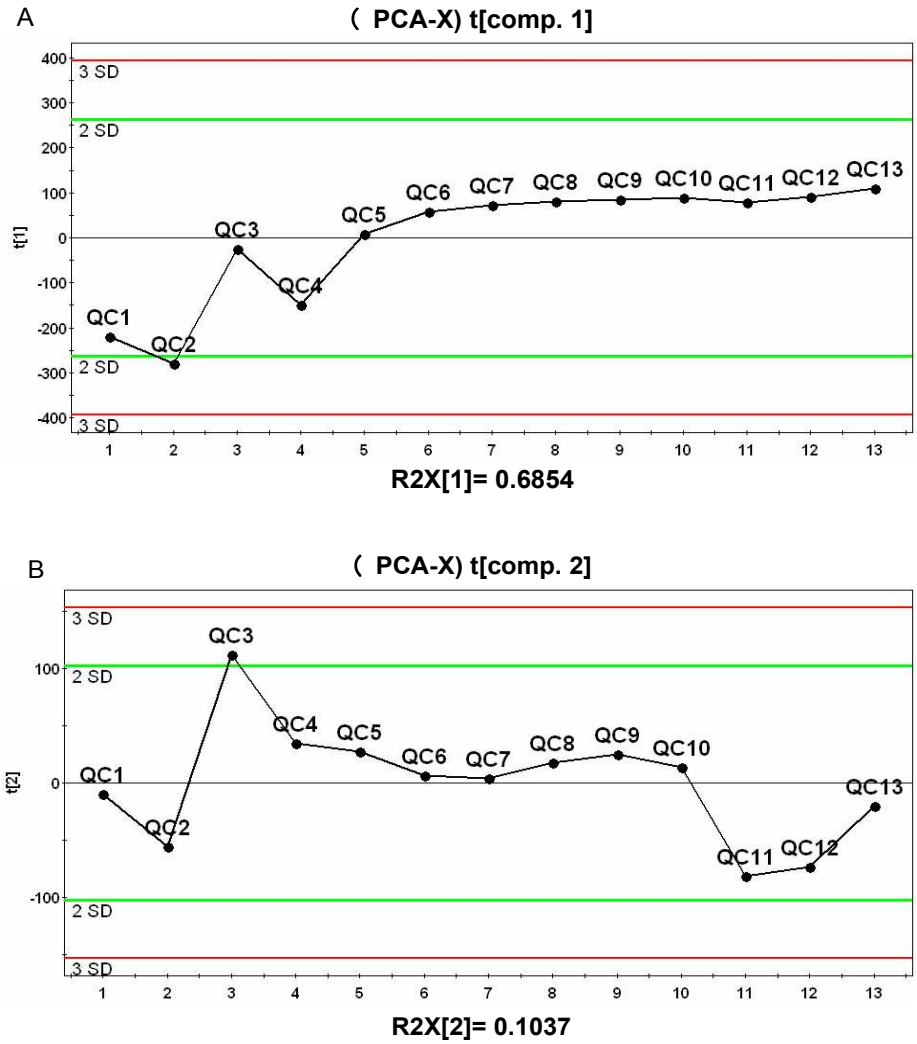

Figure S2. Quality control (QC) plots of thirteen repeater runs of RRLC-MS analyzed by using Q-TOF MS/MS instrument in positive ion mode generated by PCA using component 1 and 2. Peak area deviation could be evaluated by distribution of the runs. *X*-axis: run order; *Y*-axis: standard deviation. (A) QC plot for the first component; (B) QC plot for the second component.

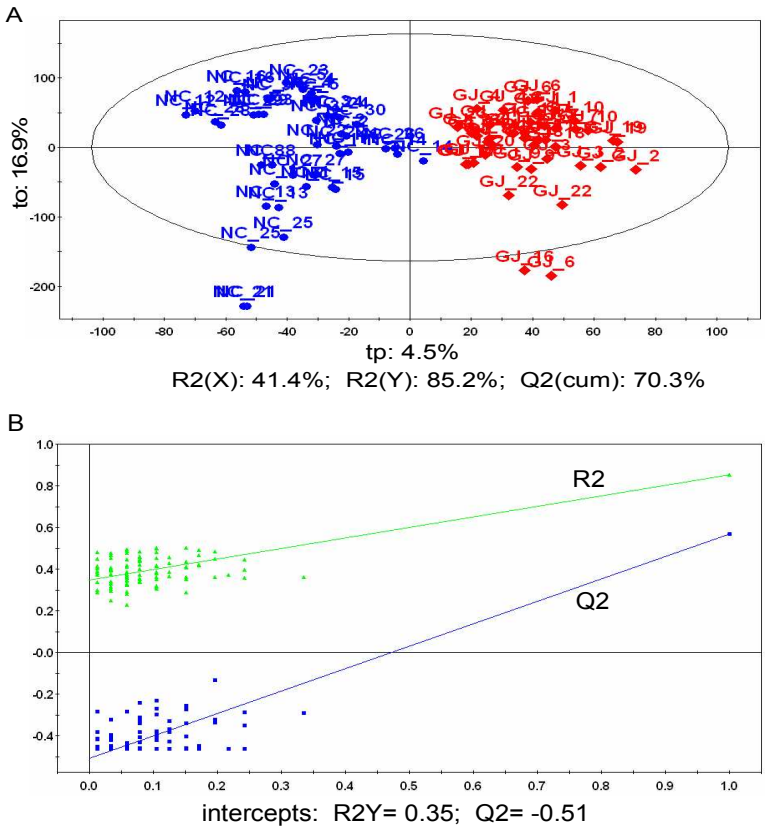

Figure S3. (A) Score plot of OPLS-DA of NC vs. GJ in negative ion mode in two components. (◆NC and ◆BC) (B) Validate plots (after 100 permutaions) of the PLS-DA model of NC vs. GJ in (-) RRLC-MS data set.