Microwave assisted extraction of multiple trace levels of intermediate metabolites for camptothecin biosynthesis in *Camptotheca acuminate* and their simultaneous determination by HPLC-linear ion trap-orbitrap-MS/MS

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MAE-based multiple metabolites analysis by HPLC-LTQ-Orbitrap-MS/MS

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Supporting Information

Table S1 Analysis of variance (ANOVA) for the MAE of strictosidinic acid

Factor	Sum of Squares	Degree of Freedom	Mean of Squares	F-value	p	Significance
Solid liquid ratio	4.086	2.00	2.043	1292.81	0.02	*
Microwave Power	51.698	2.00	25.849	2229.31	0.015	*
Extraction Time	1.651	2.00	0.825	862.81	0.02	*
Error	1.859	2.00	0.929			
Total	59.293	8.00				

Table S2 Analysis of variance (ANOVA) for the MAE of strictosamide

Factor	Sum of Squares	Degree of Freedom	Mean of Squares	F-value	p	Significance
Solid liquid ratio	18.151	2.00	9.076	18.901	0.05	*
Microwave Power	174.811	2.00	87.405	182.027	0.005	*
Extraction Time	4.756	2.00	2.378	4.952	0.168	
Error	0.960	2.00	0.480			
Total	198.678	8.00				

Table S3 Analysis of variance (ANOVA) for the MAE of the occurrence of compounds

Factor	Sum of Squares	Degree of Freedom	Mean of Squares	F-value	p	Significance
Solid liquid ratio	8.222	2.00	4.11	37.00	0.03	*
Microwave Power	4.222	2.00	2.11	19.00	0.05	*
Extraction Time	0.222	2.00	0.11	1.00	0.50	
Error	0.222	2.00	1.11E-01			
Total	12.89	8.00				

Table S4 Comparison of MAE method and other extraction methods under the optimal conditions

Metabolites	**Matrix**	**Extraction method**	**Extraction time**	**Extraction solvent**	**Yield/ Extraction efficiency**	**References**
CAM	Samara	ME	24 h	Ethanol (85%)	242.6 μg/g DW	[16]
CAM	Samara	MAE	8 min	IL	674.5 μg/g DW	[16]
CAM	Samara	UAE	35 min	IL	00%	[13]
CAM analogues	Fruits	SPE	60 min	Methanol	A total of 30 compounds identified	[12]
LA, STR, CAM	Shoot apex	MAE	4 min	Acetonitrile (70%)	9.42, 16.21, 18,08 mg/g DW	Our study

Metabolites: LA: Loganic acid; STR: Strictosamide; CAM: Camptothecin.
Method: ME: Maceration extraction; UAE: Ultrasonic-assisted extraction; SPE: Bilayer solid-phase extraction
Solvent: IL: Ionic liquid-aqueous solution;
Yield: DW: Dry weight;

Fig. S1

A1-A4: The TIC chromatogram of *C. acuminata* extract obtained by MAE method based on raw data from HPLC-LTQ-Orbitrap-MS/MS. Each chromatographic peak of 1-17 in figure A1-A4 is corresponding to the chemical structures of 1-17 in the following figure B.

B: Chemical structures of metabolite compounds identified in shoot apex of *C. acuminate*

Fig. S2 MS spectra data for the identification of putative compounds.

A: Loganic acid; B: Strictosamide diol; C: Tryptamine; D: Secologanic acid;

E, I, O: Strictosamide Ketolactam I, II, III; F, J: Stricyosidinic acid I,II;

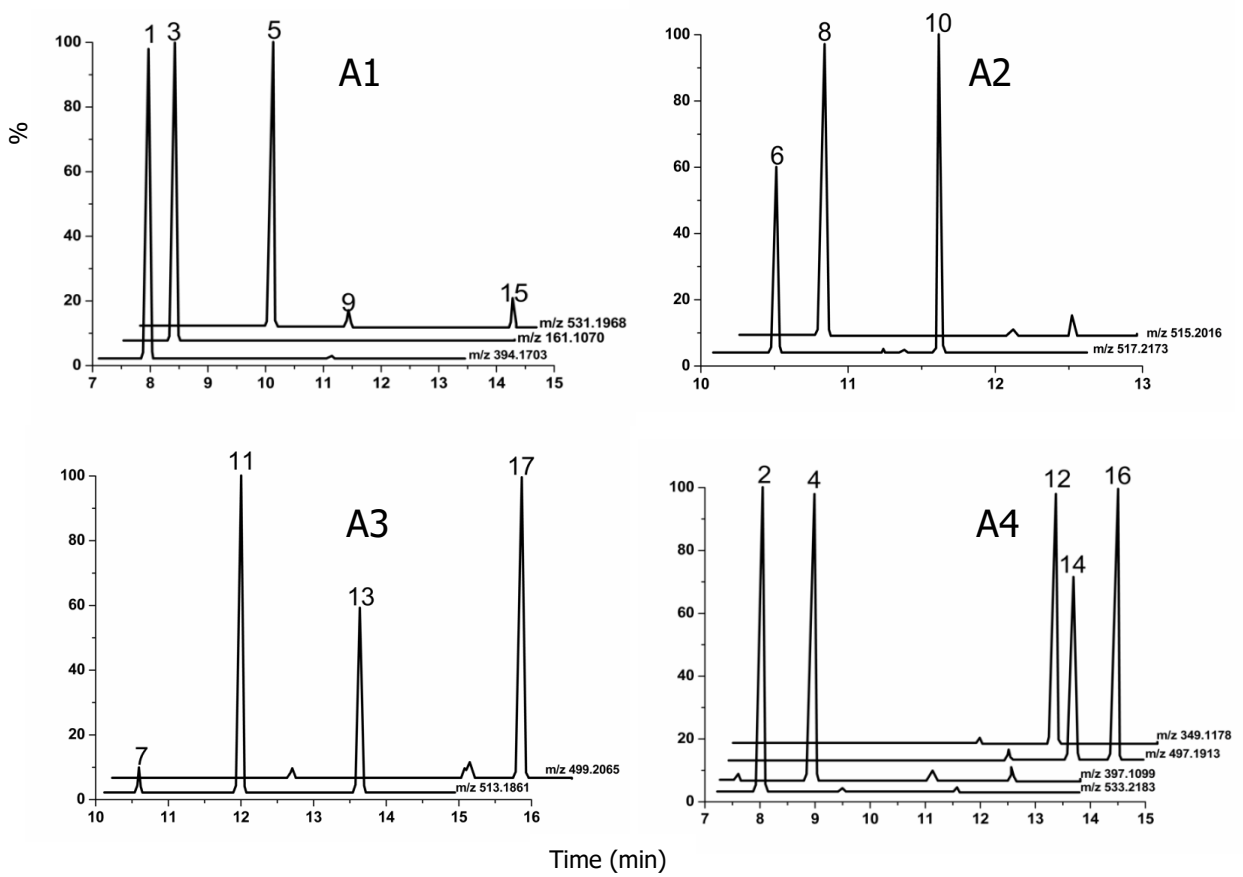
G, K, M: Pumiloside I, II, III; H: Strictosamide epoxide; L: Camptothecin;

N, P: Deoxypumiloside I, II; Q: Strictosamide.

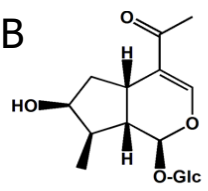
Fig. S3 Chromatograms (Fig.S3) for compounds quantitative analysis in plant tissues.

A: Root; B: Shoot apex; C: Young leaf; D: Mature leaf.

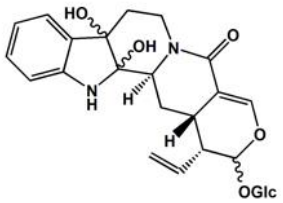
Fig.S1



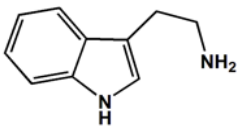
B



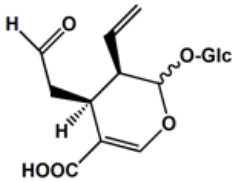
Peak1: Loganic acid



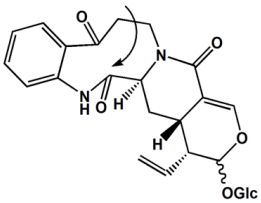
Peak2: Strictosamide diol



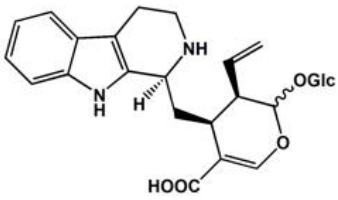
Peak3: Tryptamine



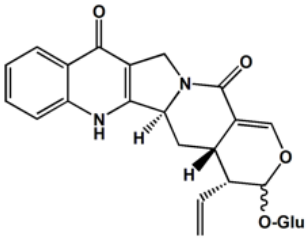
Peak4: Secologanic acid



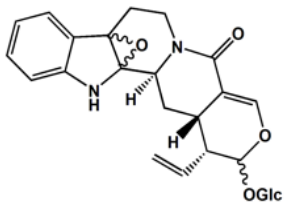
Peak5, 9, 15: Strictosamide Ketolactam I II III



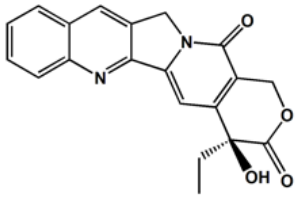
Peak6, 10: Stricyosidinic acid I II



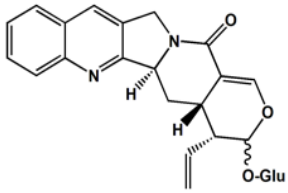
Peak7, 11, 13: Pumiloside I II III



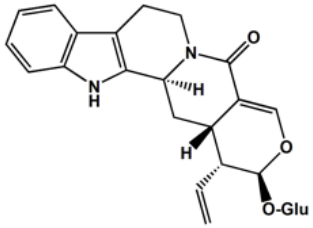
Peak8: Strictosamide epoxide



Peak12: Camptothecin

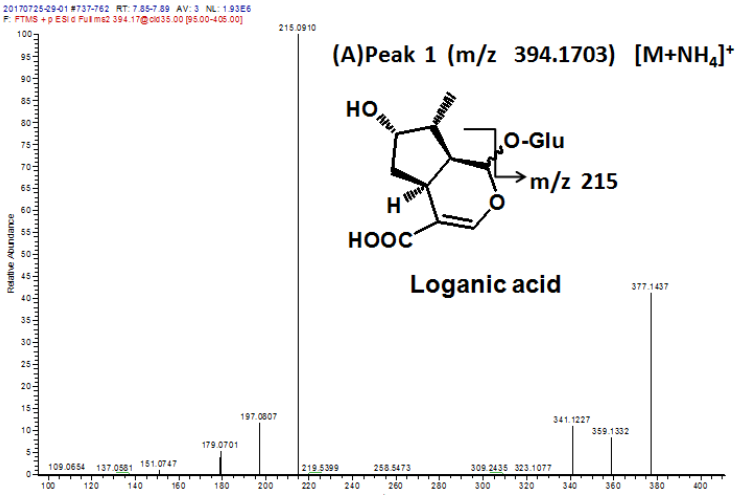


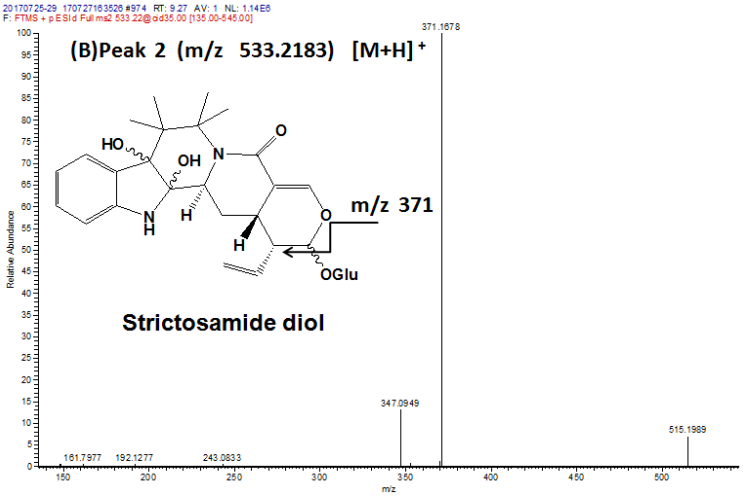
Peak14, 16: Deoxypumiloside I II

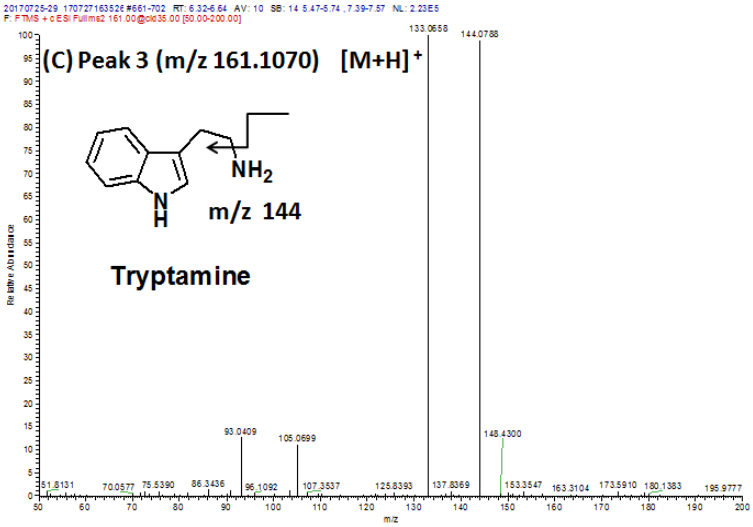


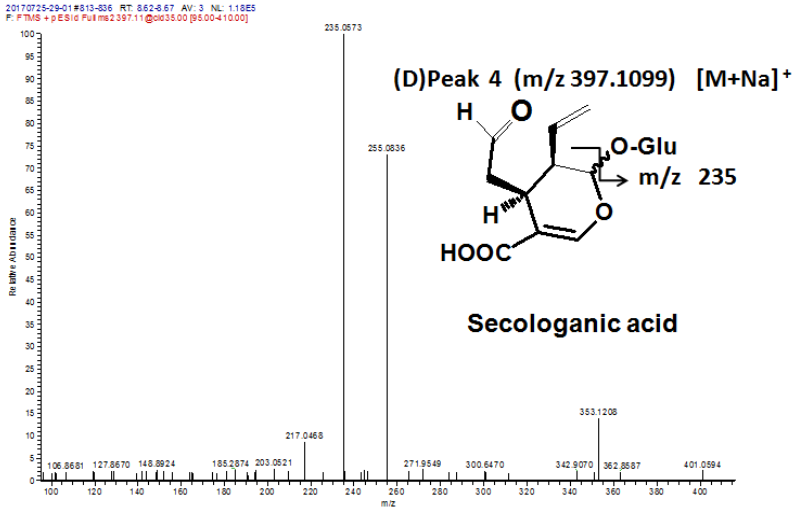
Peak17: Strictosamide

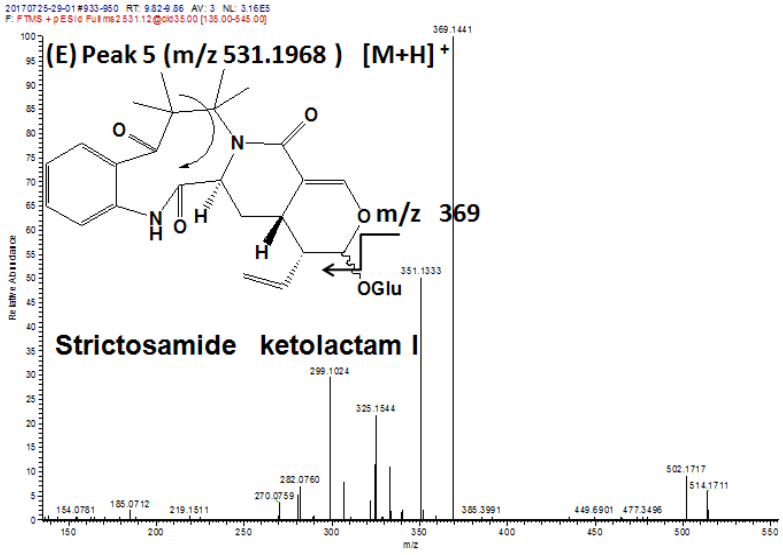
Fig.S2

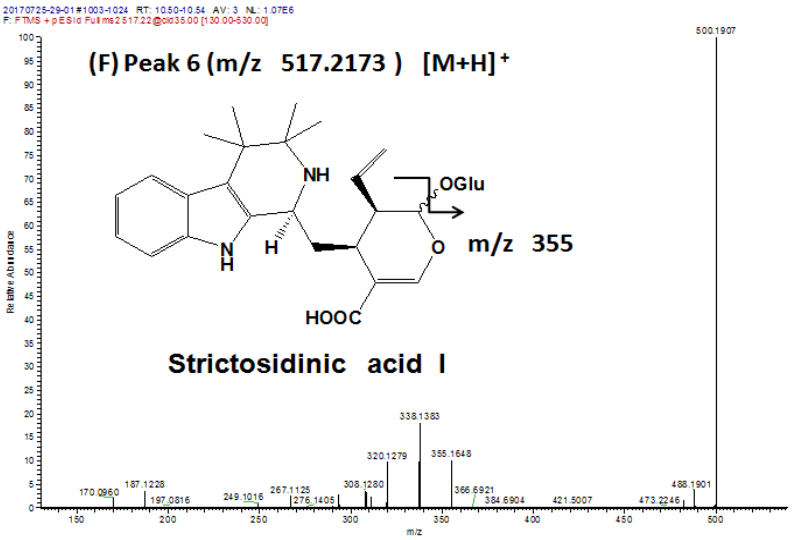

(A)Peak 1 (m/z 394.1703) [M+NH4]+
HO
O-Glu
m/z 215
H
O
HOOC
Loganic acid
Relative Abundance

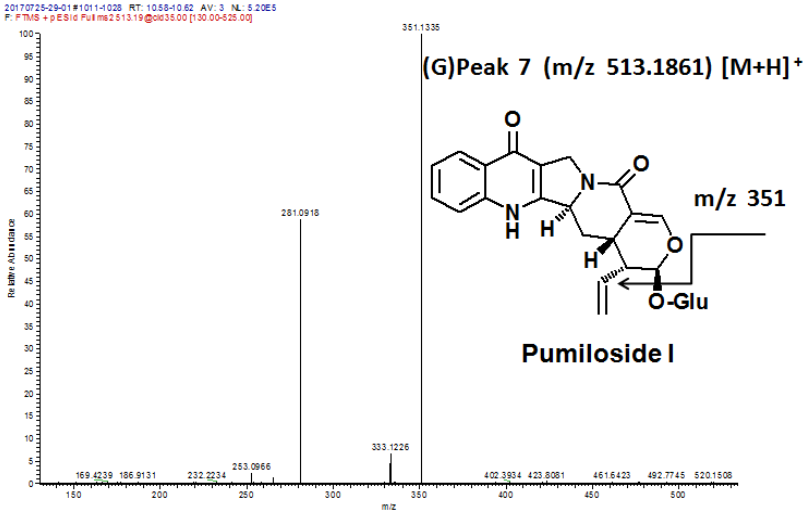

(B)Peak 2 (m/z 533.2183) [M+H]+
HO
OH
N
O
m/z 371
H
O
OGlu
Strictosamide diol
Relative Abundance
m/z

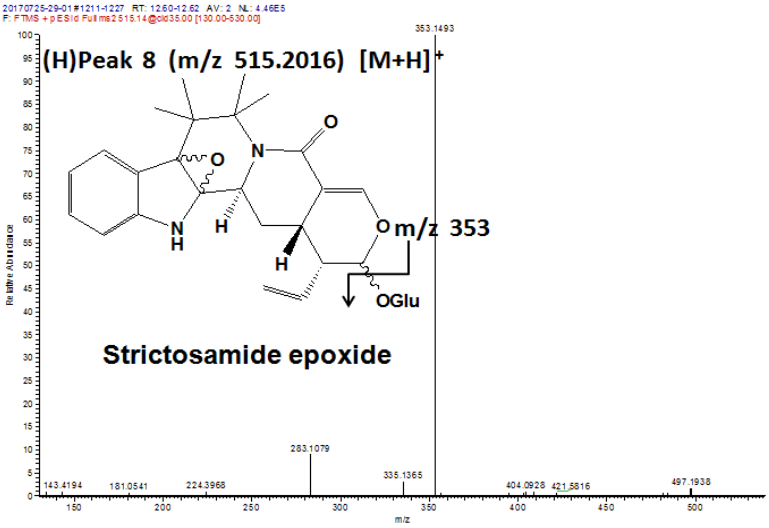

(C) Peak 3 (m/z 161.1070) [M+H]+
NH2
m/z 144
Tryptamine
Relative Abundance
m/z

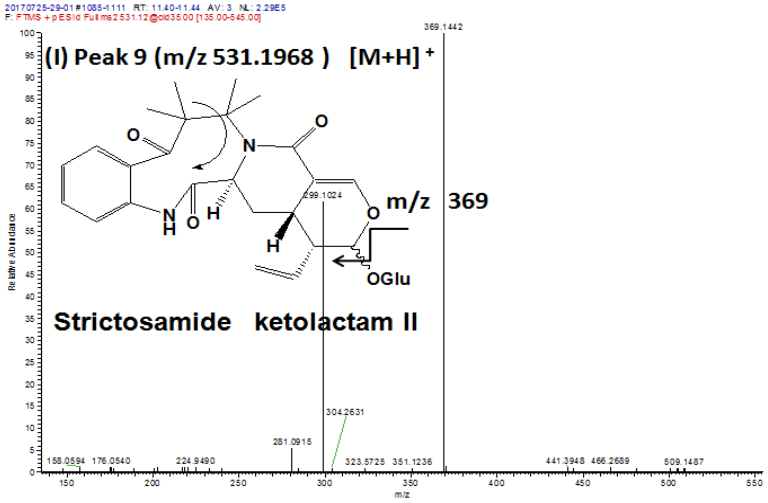

20170725-29-01#813-836 RT: 8.62-8.67 AV: 3 NL: 1.18E5
F: FTMS + p ESI d Full ms2 397.11@cid35.00 [95.00-410.00]
(D)Peak 4 (m/z 397.1099) [M+Na]+
O-Glu
m/z 235
Secologanic acid
Relative Abundance
m/z

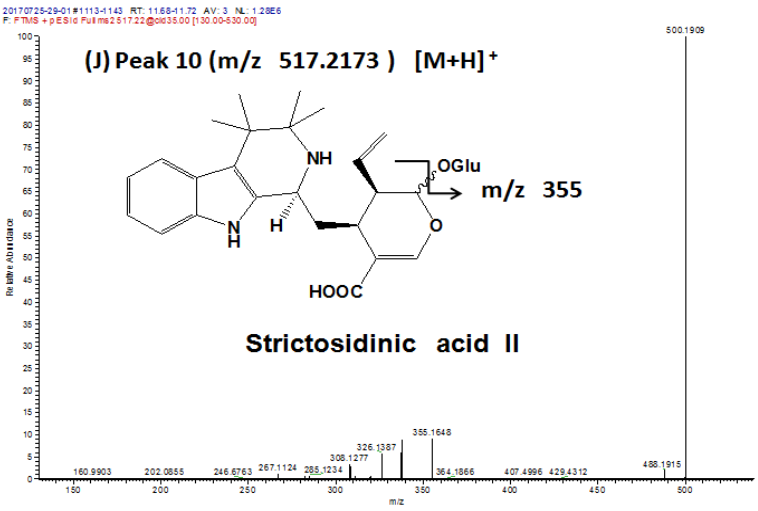

20170725-29-01#933-950 RT: 9.82-9.86 AV: 3 NL: 3.16E5
F: FTMS + p ESI d Full ms2 531.12@cid35.00 [135.00-545.00]
(E) Peak 5 (m/z 531.1968) [M+H]+
m/z 369
OGlu
Strictosamide ketolactam I
Relative Abundance
m/z

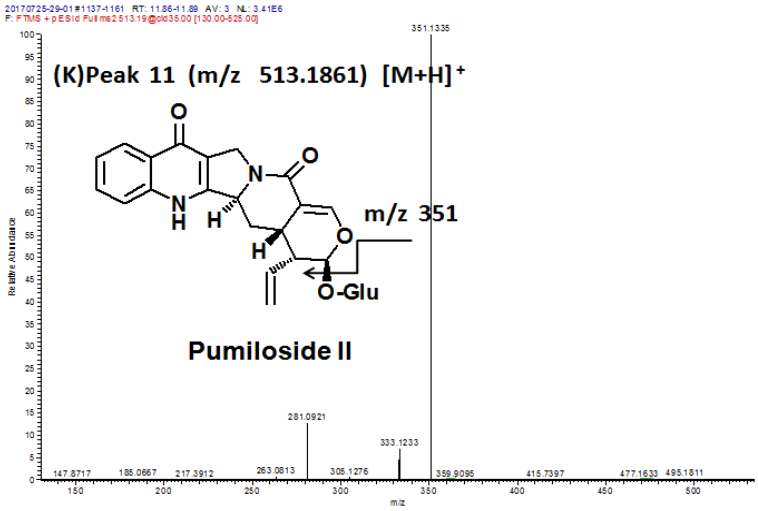

20170725-29-01#1003-1024 RT: 10.50-10.54 AV: 3 NL: 1.07E6
F: FTMS + p ESI d Full ms2 517.22@cid35.00 [130.00-530.00]
(F) Peak 6 (m/z 517.2173) [M+H]+
OGlu
m/z 355
Strictosidinic acid I
Relative Abundance
m/z

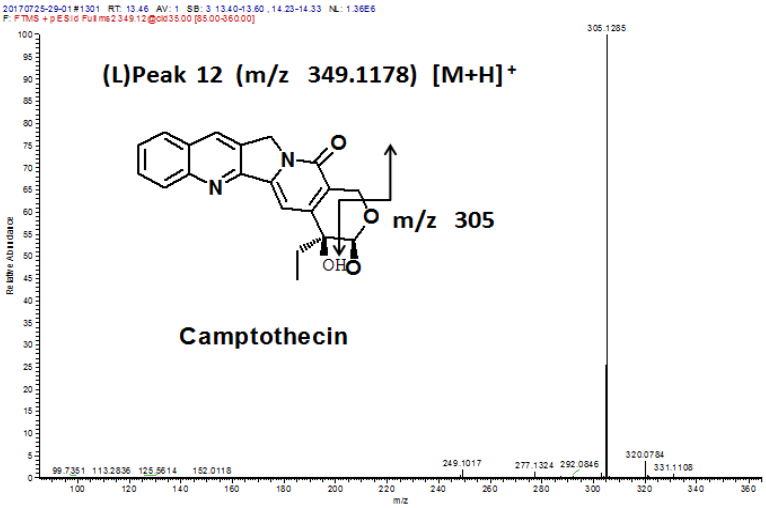

(G)Peak 7 (m/z 513.1861) [M+H]+
m/z 351
O-Glu
Pumiloside I
Relative Abundance
m/z

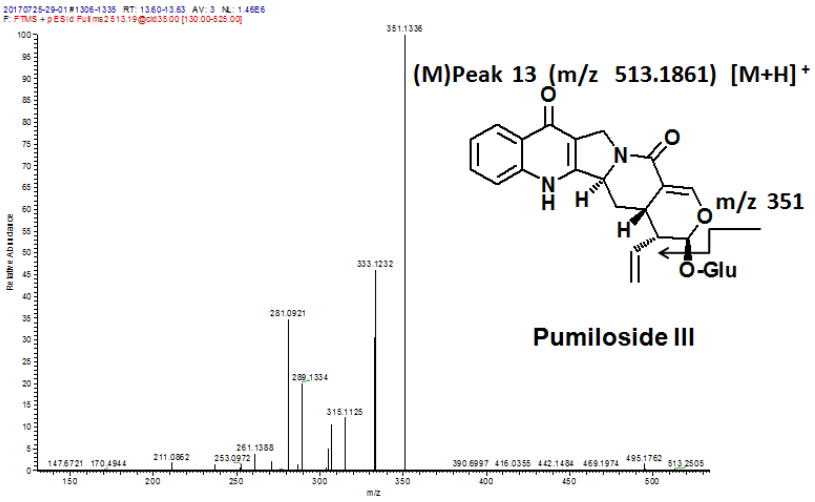

(H)Peak 8 (m/z 515.2016) [M+H]+
m/z 353
OGlu
Strictosamide epoxide
Relative Abundance
m/z

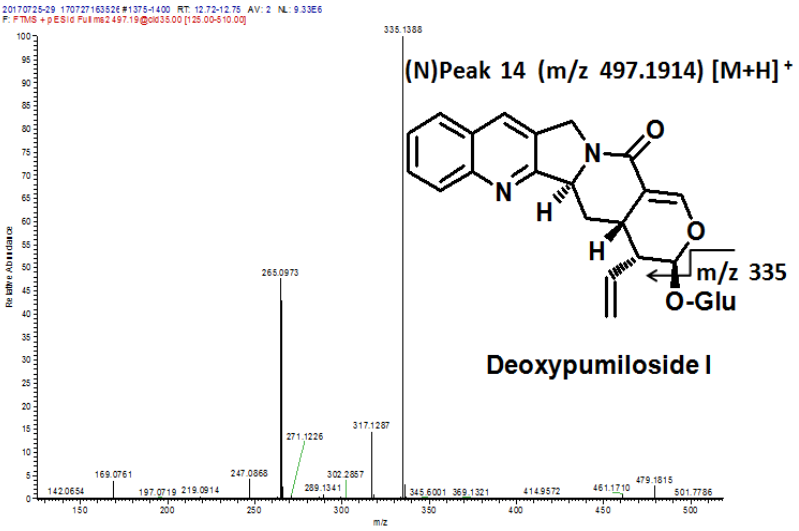

(I) Peak 9 (m/z 531.1968) [M+H]+
m/z 369
OGlu
Strictosamide ketolactam II
Relative Abundance
m/z

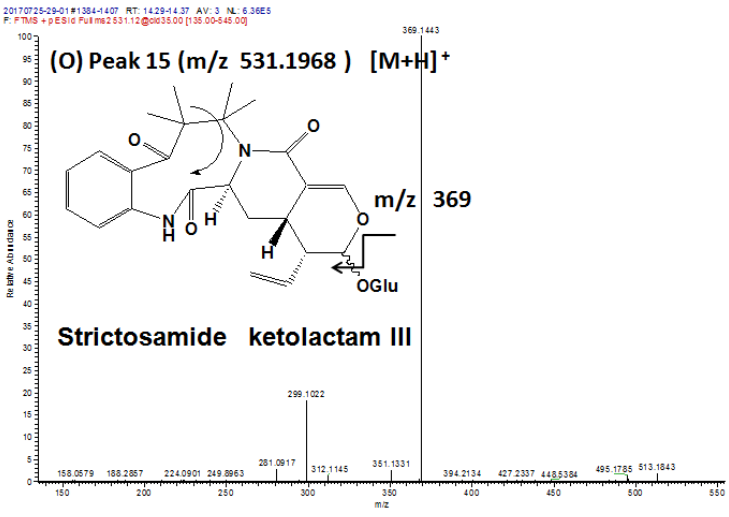

(J) Peak 10 (m/z 517.2173) [M+H]+
NH
OGlu
m/z 355
N
H
H
O
HOOC
Strictosidinic acid II
Relative Abundance
m/z


(K)Peak 11 (m/z 513.1861) [M+H]+
O
N
O
N
H
H
m/z 351
H
O
O-Glu
Pumiloside II
Relative Abundance
m/z


(L)Peak 12 (m/z 349.1178) [M+H]+
N
O
N
O
m/z 305
OH
O
Camptothecin
Relative Abundance
m/z


(M)Peak 13 (m/z 513.1861) [M+H]+
m/z 351
O-Glu
Pumiloside III
Relative Abundance
m/z


(N)Peak 14 (m/z 497.1914) [M+H]+
m/z 335
O-Glu
Deoxypumiloside I
Relative Abundance
m/z


(O) Peak 15 (m/z 531.1968) [M+H]+
m/z 369
OGlu
Strictosamide ketolactam III
Relative Abundance
m/z

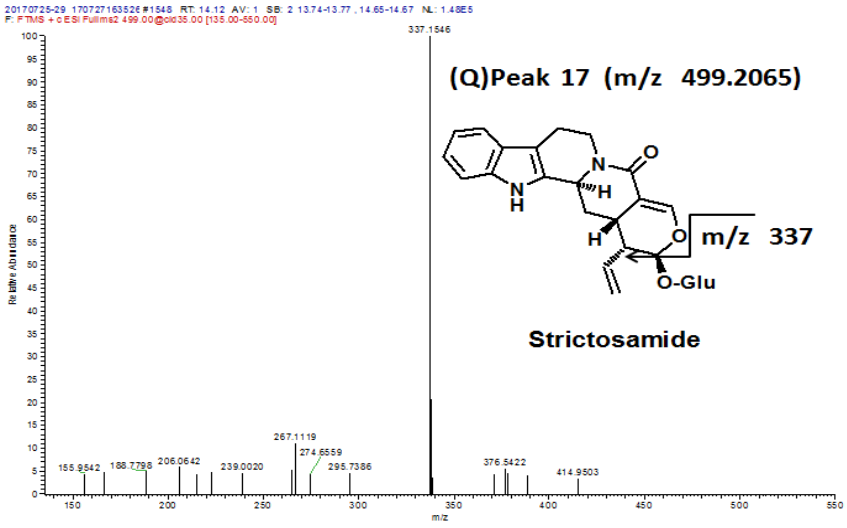
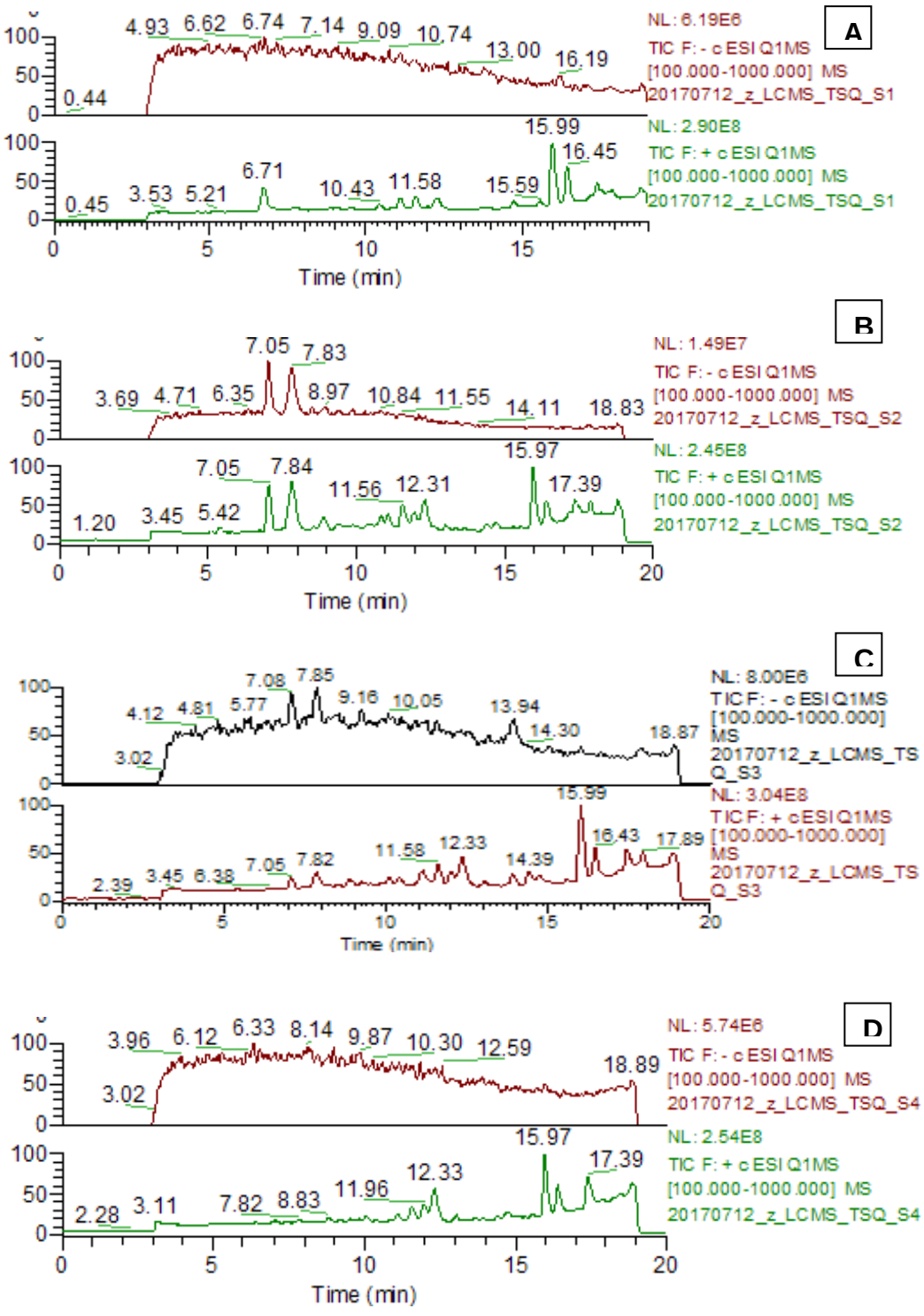

20170725-29 170727163526 #1548 RT: 14.12 AV: 1 SB: 2 13.74-13.77 , 14.65-14.67 NL: 1.48E5
F: FTMS + c ESI Full ms2 499.00@cid35.00 [135.00-550.00]
337.1546
(Q)Peak 17 (m/z 499.2065)
m/z 337
O-Glu
Strictosamide
Relative Abundance
m/z
267.1119
274.6559
295.7386
155.9542
188.7796
206.0642
239.0020
376.5422
414.9503

Fig. S3


A
NL: 6.19E6
TIC F: - c ESI Q1MS
[100.000-1000.000] MS
20170712_z_LCMS_TSQ_S1
NL: 2.90E8
TIC F: + c ESI Q1MS
[100.000-1000.000] MS
20170712_z_LCMS_TSQ_S1
Time (min)
B
NL: 1.49E7
TIC F: - c ESI Q1MS
[100.000-1000.000] MS
20170712_z_LCMS_TSQ_S2
NL: 2.45E8
TIC F: + c ESI Q1MS
[100.000-1000.000] MS
20170712_z_LCMS_TSQ_S2
Time (min)
C
NL: 8.00E6
TIC F: - c ESI Q1MS
[100.000-1000.000]
MS
20170712_z_LCMS_TS
Q_S3
NL: 3.04E8
TIC F: + c ESI Q1MS
[100.000-1000.000]
MS
20170712_z_LCMS_TS
Q_S3
Time (min)
D
NL: 5.74E6
TIC F: - c ESI Q1MS
[100.000-1000.000] MS
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NL: 2.54E8
TIC F: + c ESI Q1MS
[100.000-1000.000] MS
20170712_z_LCMS_TSQ_S4
Time (min)