Supplementary information

Investigating 11 withanosides and withanolides by UHPLC-PDA and mass fragmentation study from Ashwagandha (*Withania somnifera*)

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Table S1. A-System suitability (SST) and **B**- specificity parameters for UHPLC-PDA methodology

Table S2.: Quantification results in the total content of compounds (1-11) in WSE by external standard **A**-with eleven standards **B**- with three standards withanoside-IV for withanosides (1, 2 & 7), withaferin-A for withaferin-A (6) and withanolide-A for withanolides (3-5, 8-11)

Figure S1. Linearity and residual plots of validation by UHPLC-PDA method for compounds (**1-11**)

Figure S2 ESI-MS/MS data for eleven withanosides and withanolides in ESI (+ve and -ve) mode

Table S1. A-System suitability (SST) and **B**-specificity parameters for UHPLC-PDA methodology

Table S1. A-System suitability (SST) parameters for UHPLC-PDA methodology

Injection no.	Retention time	Response	No. of theoretical plates	Tailing factor (Tf)	Resolution factor (R_s)	Capacity factor (K')
1	21.268	1854293	158157	1.001	114.596	22.420
2	21.274	1854519	159209	0.997	115.865	22.386
3	21.280	1855196	159456	0.995	118.361	22.423
4	21.287	1852488	159850	0.999	120.113	22.431
5	21.278	1852462	159874	0.993	117.336	22.390
6	21.294	1848726	160190	1.000	119.146	22.262
Mean	21.280	1852947	159456	0.998	117.570	22.385
Results	Retention time (RSD %,0.044)	Response, (RSD %, 0.127)	Average No. of theoretical plates (159456)	Tailing factor (Average-0.998)	Resolution factor (Rs-117.57)	Capacity factor (K'-22.385)

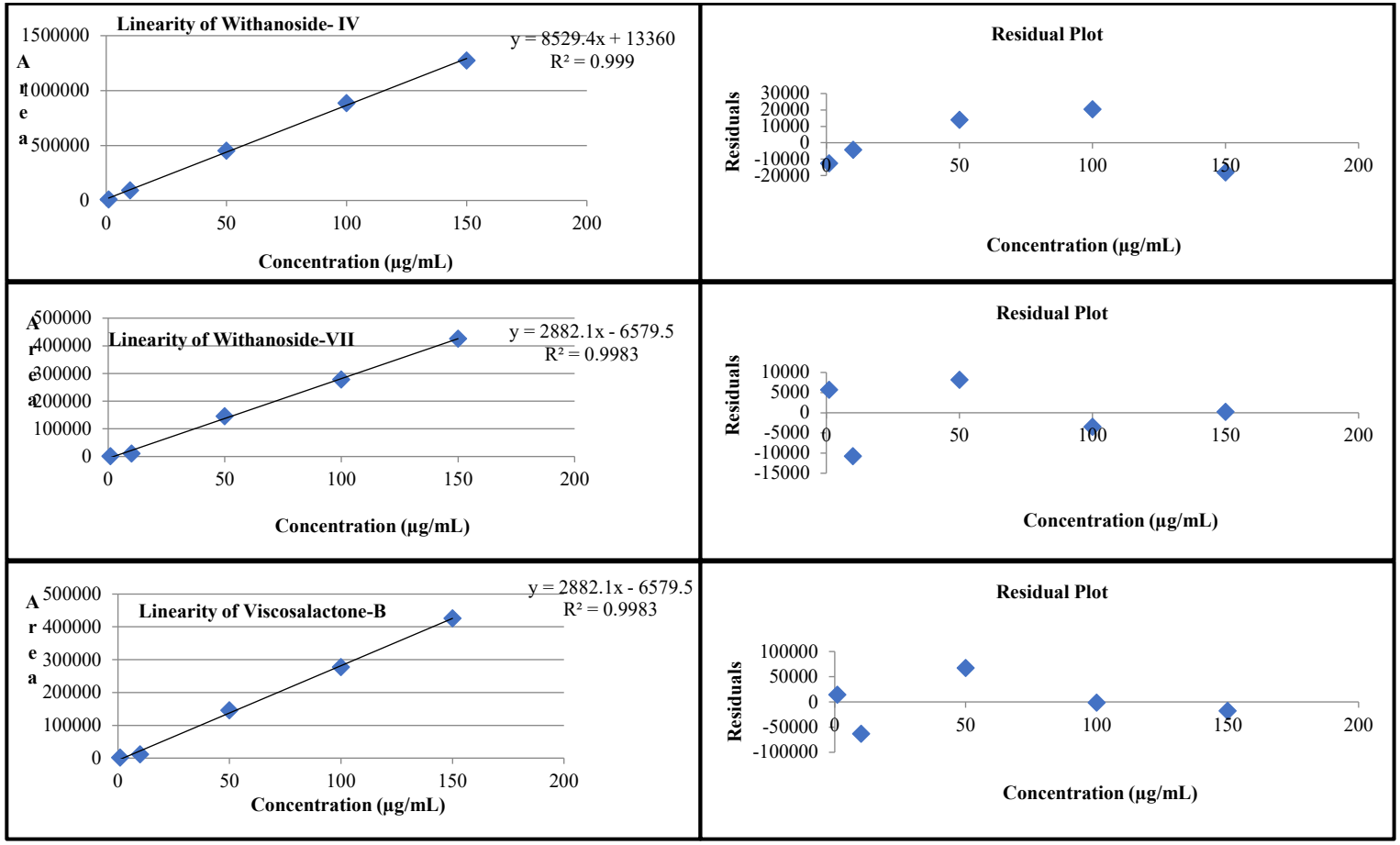
Table S1. B-Specificity prameters for the sample UHPLC-PDA methodology

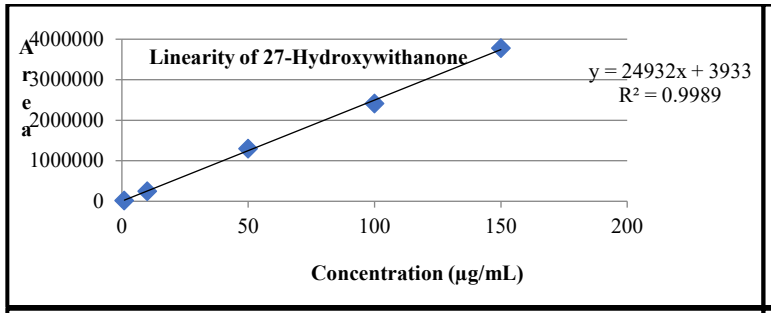
Sr. No	Compounds	Peak Purity index	Five point Peak Purity
1	Withanoside-IV	0.9905	0.9942
2	Withanoside-VII	0.997	0.9997
3	Viscosalactone-B	0.9899	0.9899
4	27-Hydroxy withanone	0.9889	0.997
5	Dihydro Withaferin A	0.9679	0.9916
6	Withaferin-A	0.9922	0.9975
7	Withanoside- V	0.996	0.9983
8	12-Deoxywithastramonolide	0.9925	0.9991
9	Withanolide-A	0.9955	0.9996
10	Withanone	0.9832	0.9977
11	Withanolide-B	0.9929	0.9994

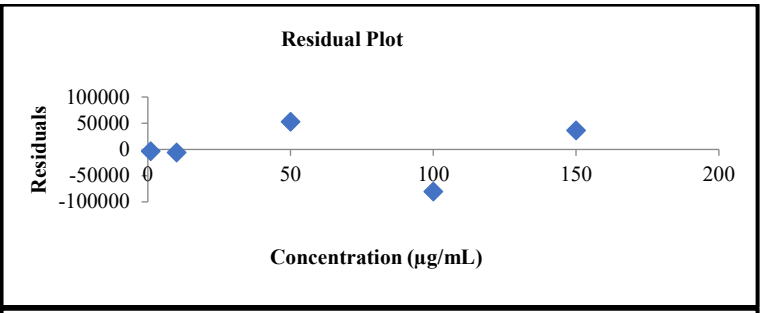
Table S2.: Quantification results in the total content of compounds (**1-11**) in WSE by external standard **A**-with eleven standards **B**- with three standards withanoside-IV for withanosides (**1, 2 & 7**), withaferin-A for withaferin-A (**6**) and withanolide-A for withanolides (**3-5, 8-11**)

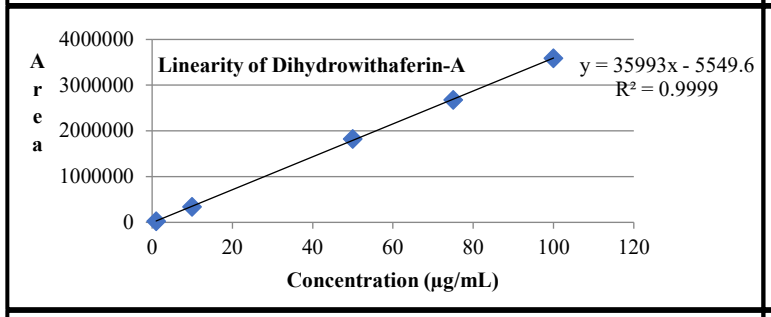
SAMPLES	A	B	% RE
Batch 1	4.18	3.95	5.50
Batch 2	4.13	3.89	5.81
Batch 3	4.10	3.86	5.85
Batch 4	4.11	3.89	5.35
Batch 5	4.19	3.99	4.77
Batch 6	4.09	3.89	4.89
Batch 7	4.02	3.83	4.73
Batch 8	4.10	3.90	4.88
Batch 9	4.11	3.90	5.11
Batch 10	4.22	4.01	4.98
Batch 11	4.12	3.90	5.34
Batch 12	4.27	4.05	5.15
Mean	4.14	3.92	5.20
SD	0.07	0.06	-
RSD	1.62	1.66	-

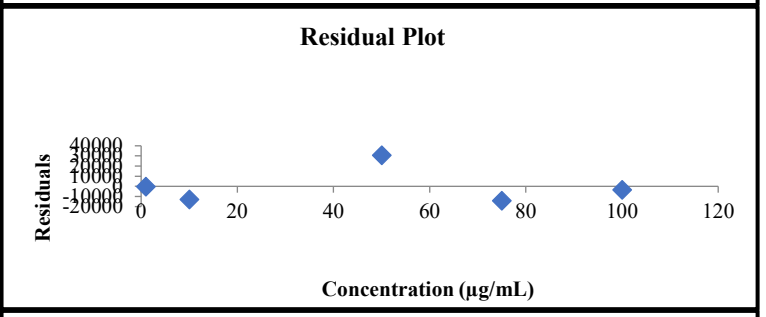
Figure S1. Linearity and residual plots of validation by UHPLC-PDA method for compounds (**1-11**)

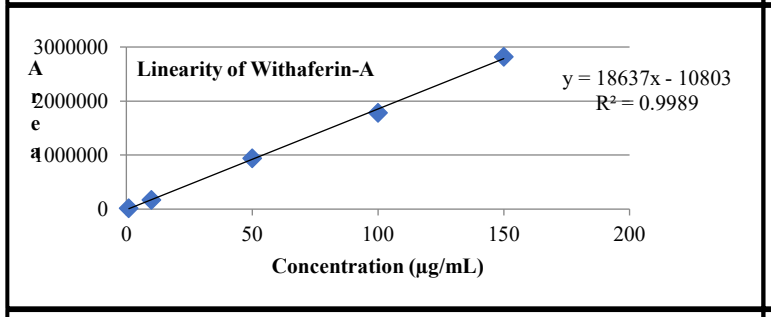


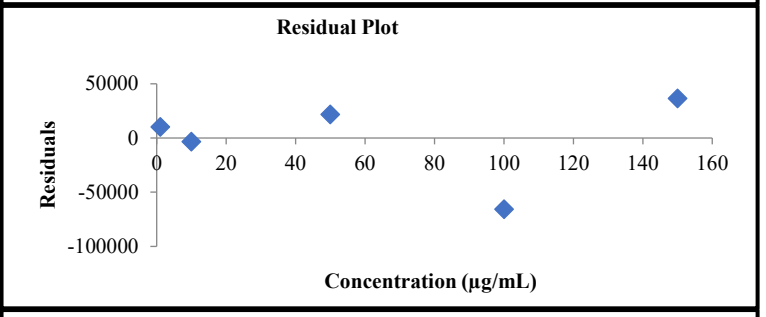

Linearity of 27-Hydroxywithanone
y = 24932x + 3933
R² = 0.9989
Area
Concentration (µg/mL)

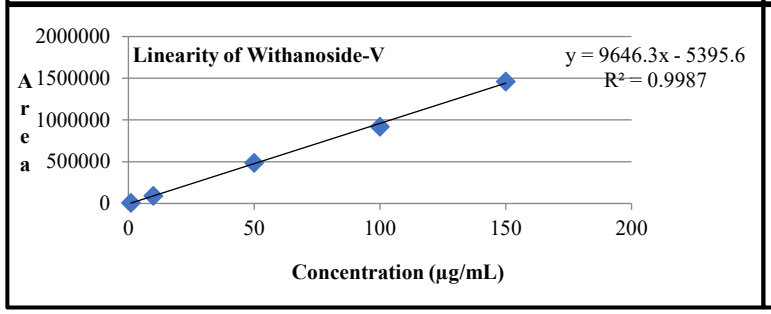

Residual Plot
Residuals
Concentration (µg/mL)

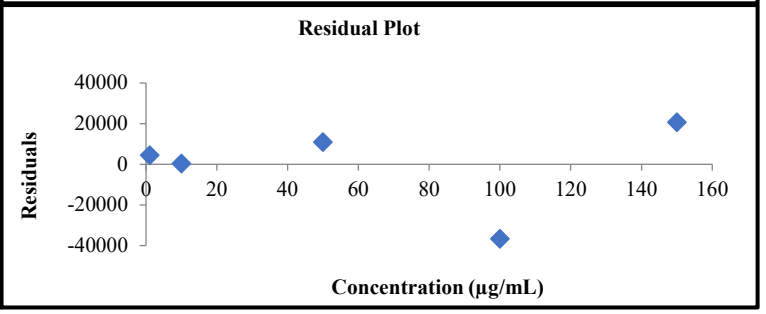

Linearity of Dihydrowithaferin-A
y = 35993x - 5549.6
R² = 0.9999
Area
Concentration (µg/mL)

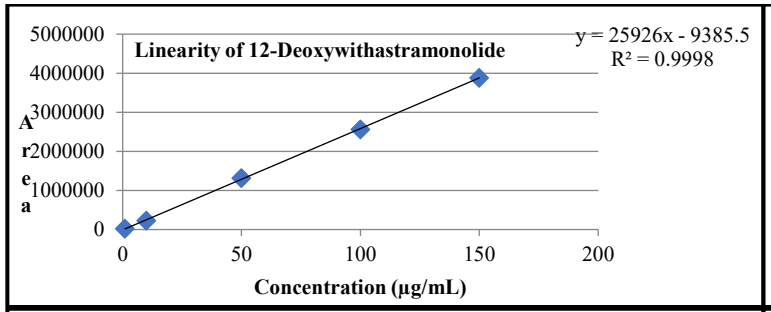

Residual Plot
Residuals
Concentration (µg/mL)

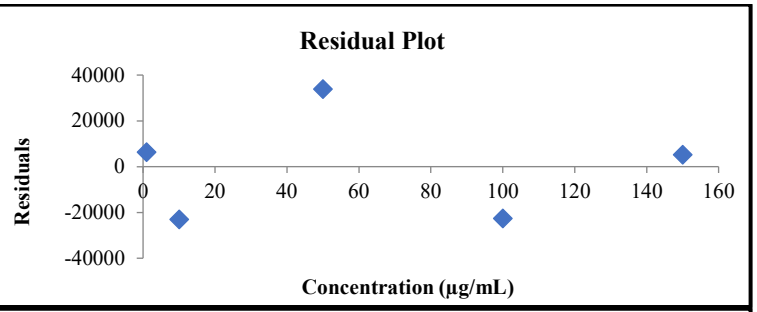

Linearity of Withaferin-A
y = 18637x - 10803
R² = 0.9989
Area
Concentration (µg/mL)

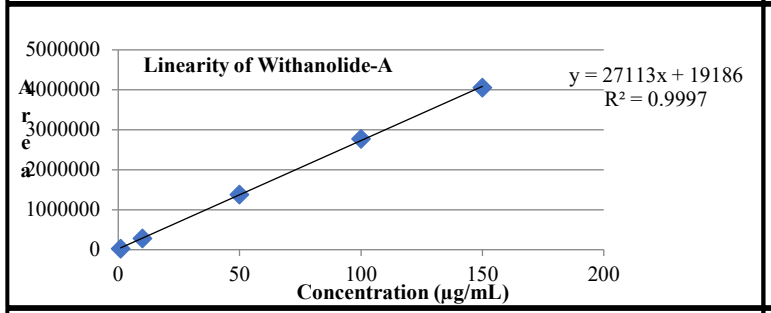

Residual Plot
Residuals
Concentration (µg/mL)

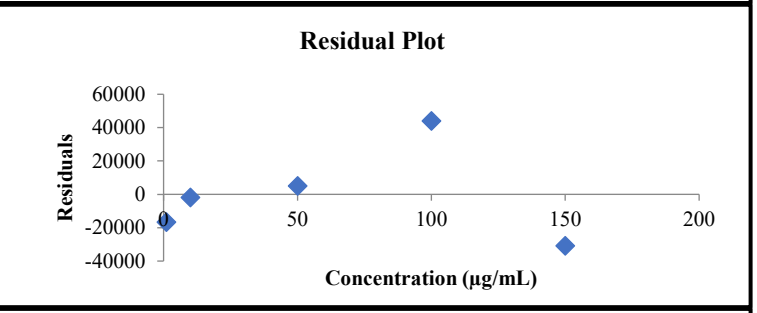

Linearity of Withanoside-V
y = 9646.3x - 5395.6
R² = 0.9987
Area
Concentration (µg/mL)

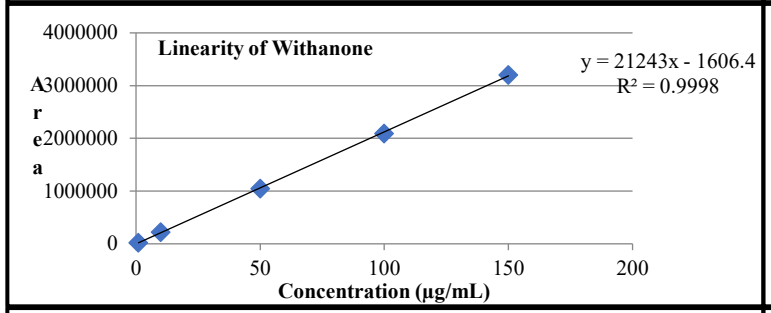

Residual Plot
Residuals
Concentration (µg/mL)

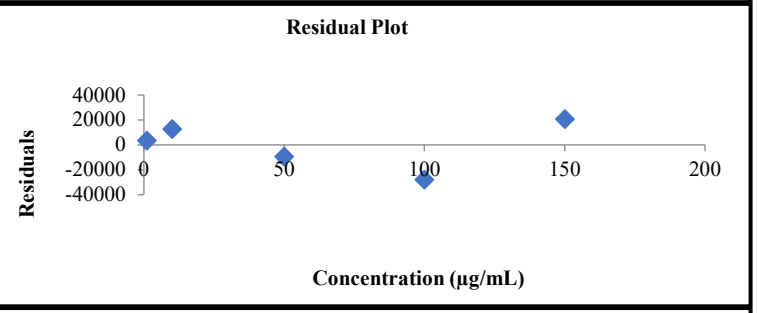

Linearity of 12-Deoxywithastramonolide
y = 25926x - 9385.5
R² = 0.9998
Area
Concentration (µg/mL)

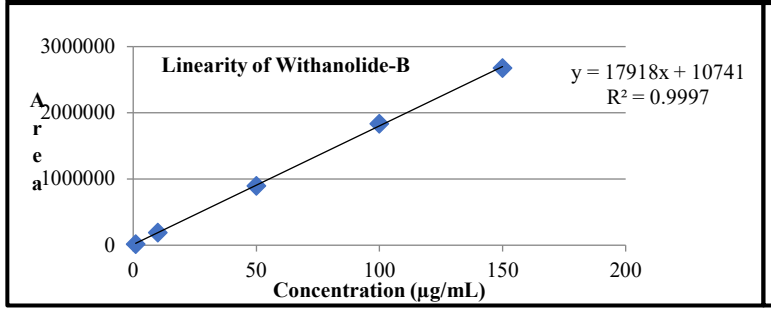

Residual Plot
Residuals
Concentration (µg/mL)


Linearity of Withanolide-A
y = 27113x + 19186
R² = 0.9997
Area
Concentration (µg/mL)


Residual Plot
Residuals
Concentration (µg/mL)


Linearity of Withanone
y = 21243x - 1606.4
R² = 0.9998
Area
Concentration (µg/mL)


Residual Plot
Residuals
Concentration (µg/mL)


Linearity of Withanolide-B
y = 17918x + 10741
R² = 0.9997
Area
Concentration (µg/mL)

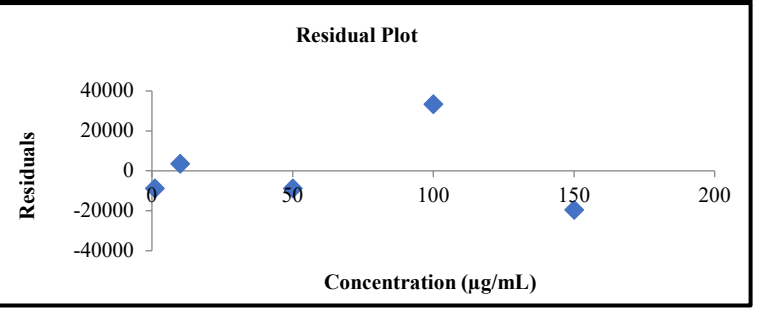
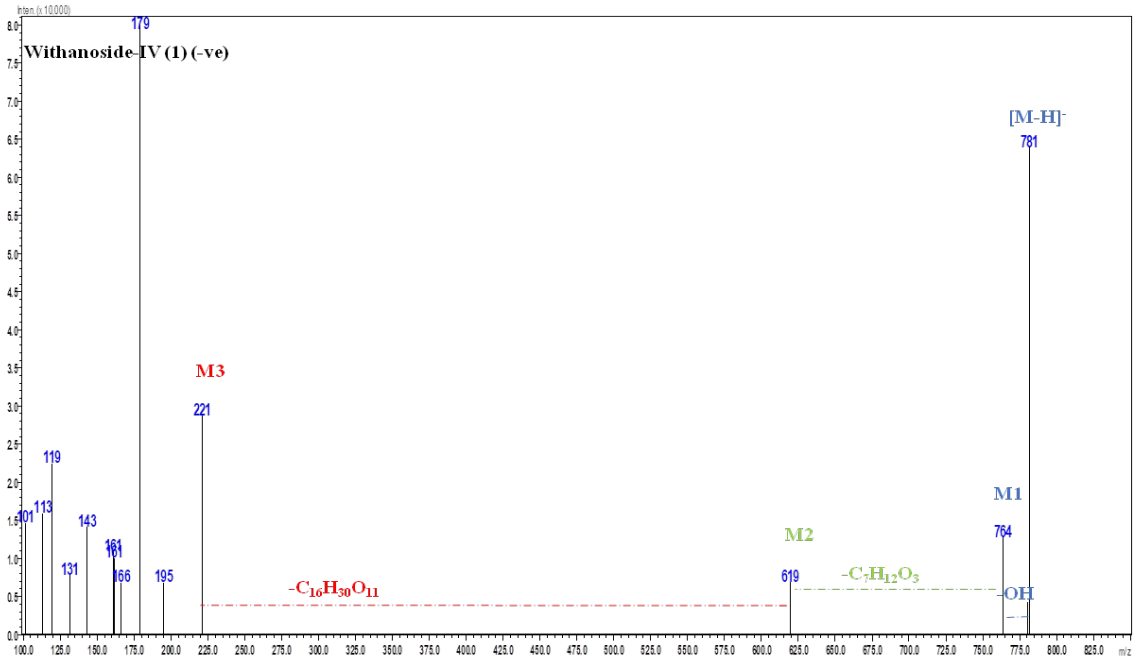
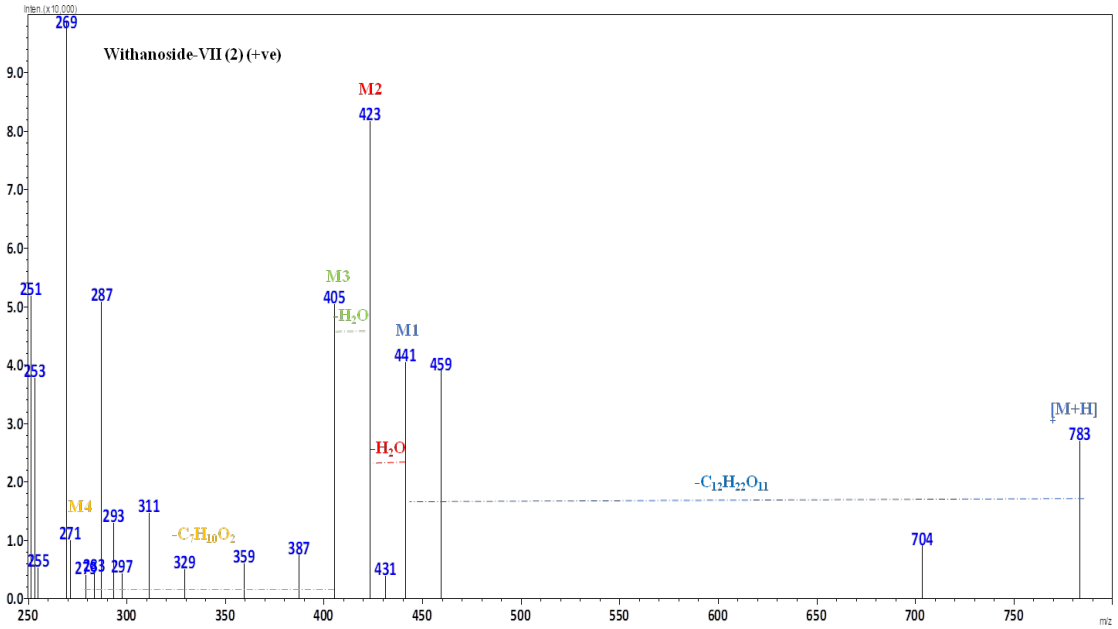
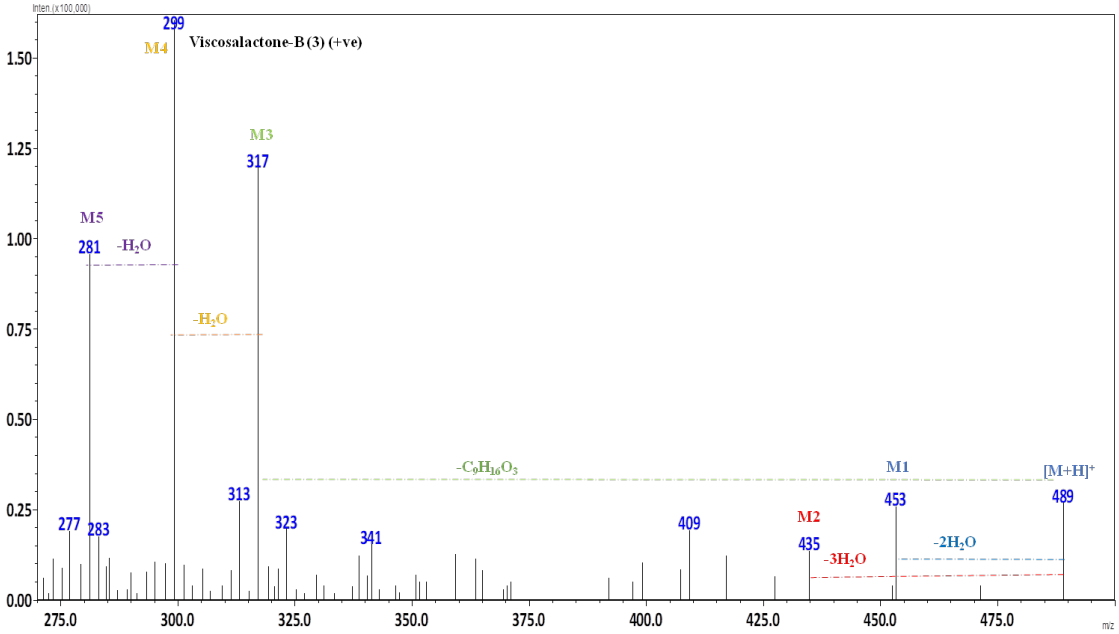
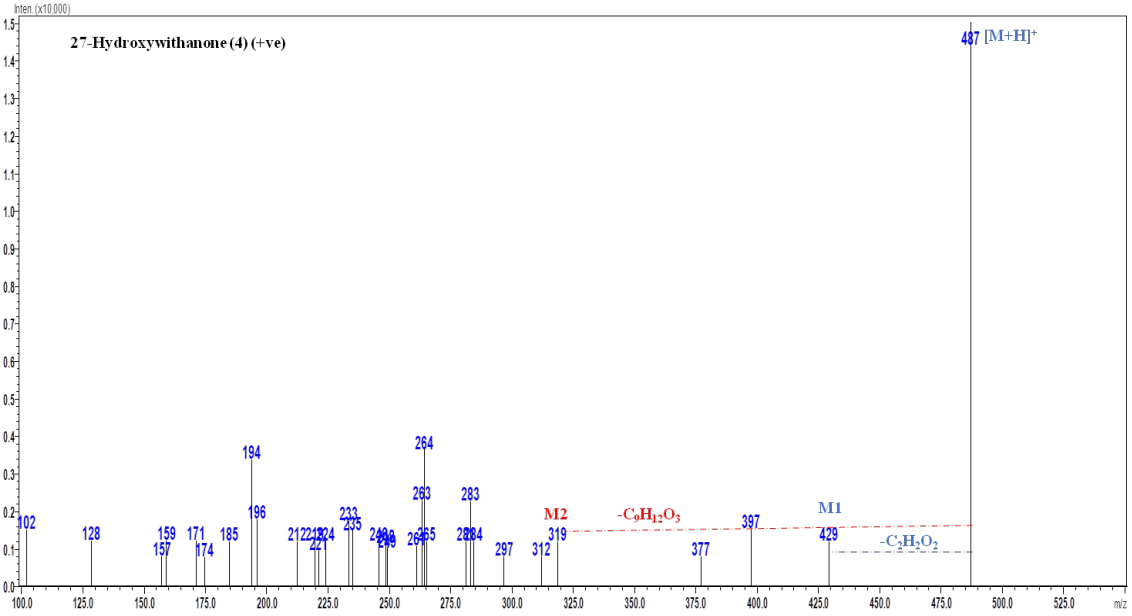

Residual Plot
Residuals
Concentration (µg/mL)

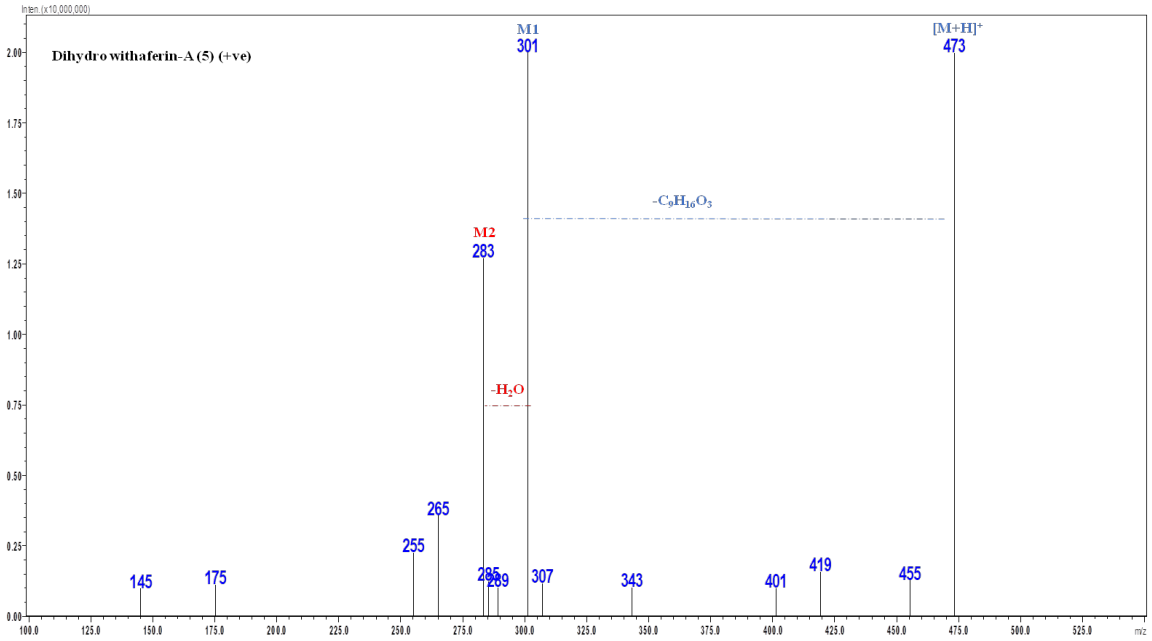
Figure S2. ESI-MS/MS data for eleven withanosides and withanolides (**1-11**) in ESI (+ve and -ve) mode

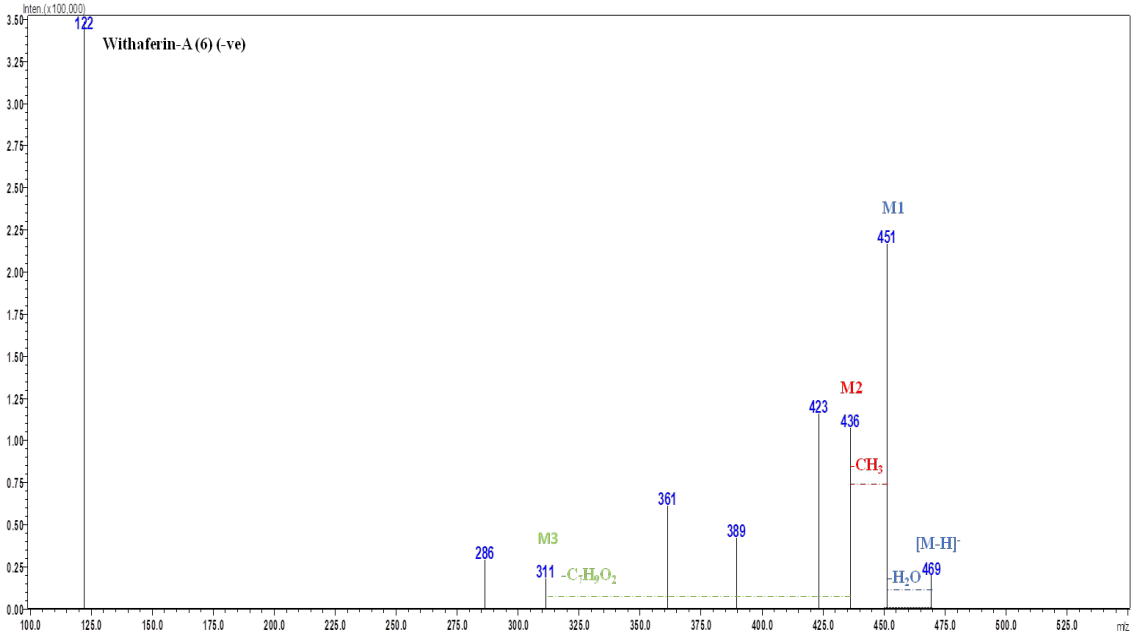


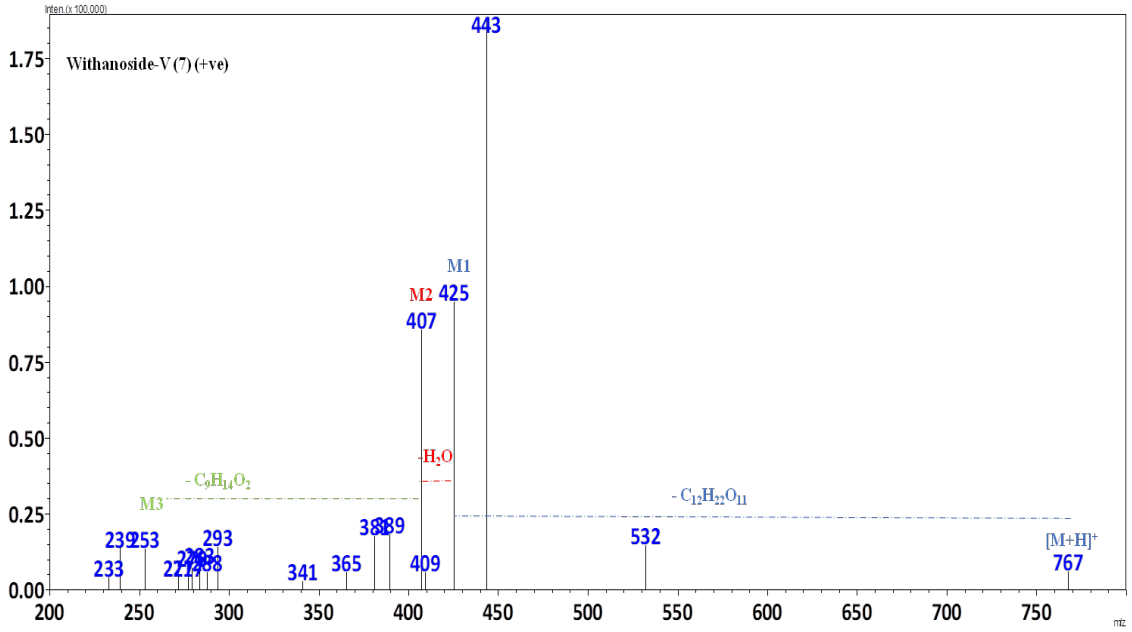


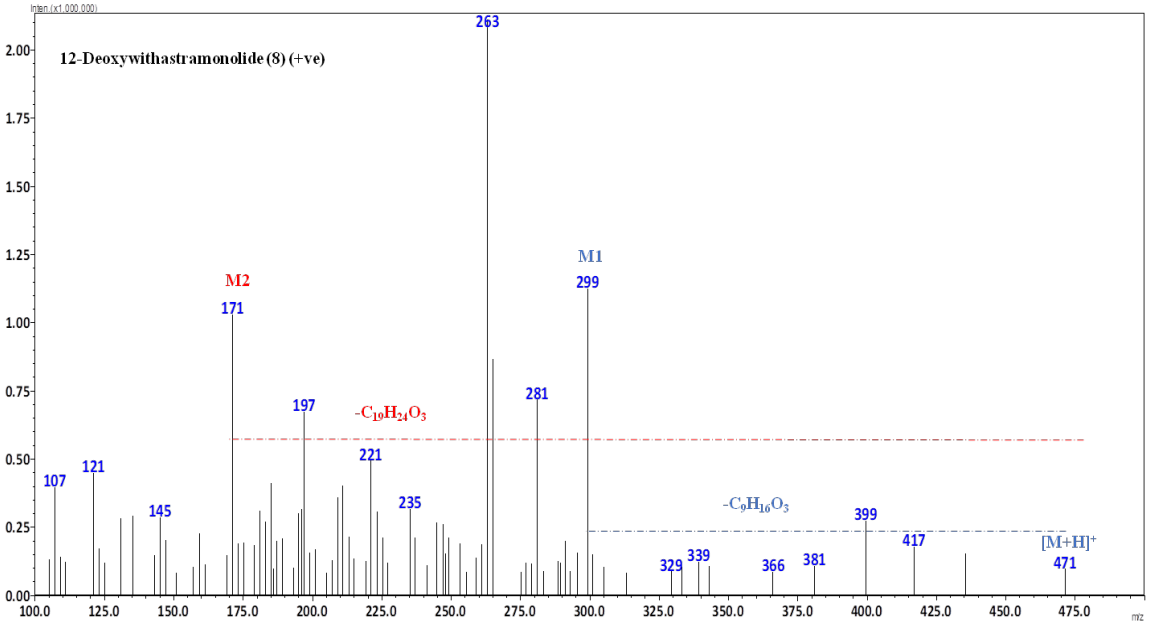

Inten.(x100,000)
Viscosalactone-B (3) (+ve)
299
M4
M3
317
M5
281
-H2O
-H2O
-C9H16O3
M1
[M+H]+
489
453
313
323
277
283
341
409
M2
435
-3H2O
-2H2O
m/z

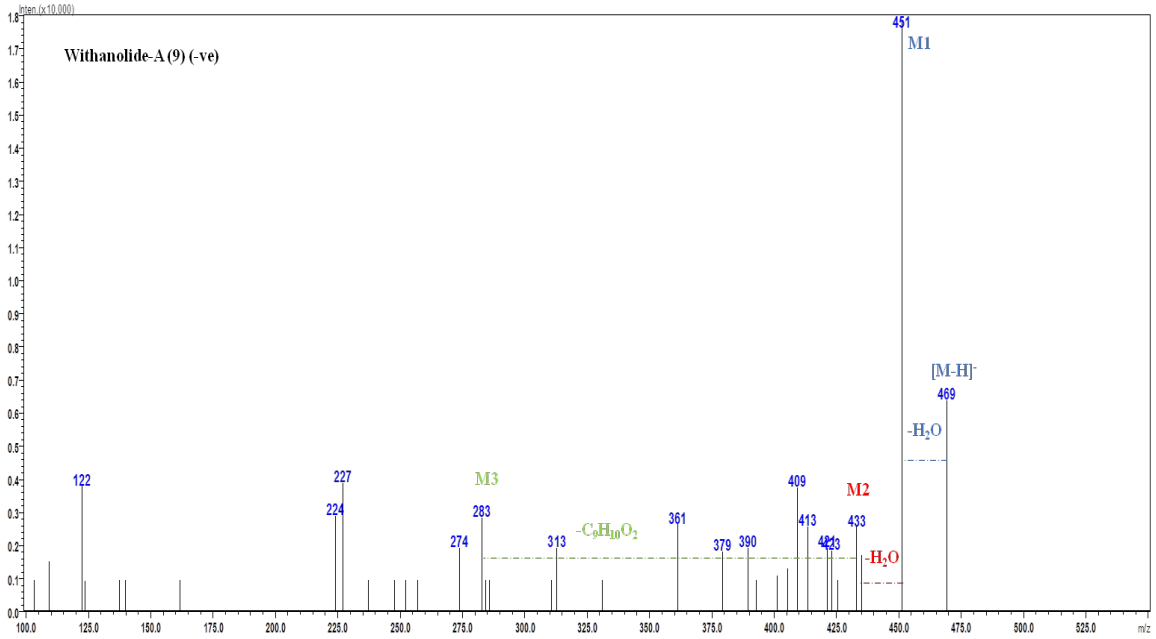

Inten.(x10,000)
27-Hydroxywithanone (4) (+ve)
487 [M+H]+
264
194
263
283
M2
-C9H12O3
M1
397
-C2H2O2
233
196
235
102
128
159
171
185
157
174
297
312
319
377
429
m/z

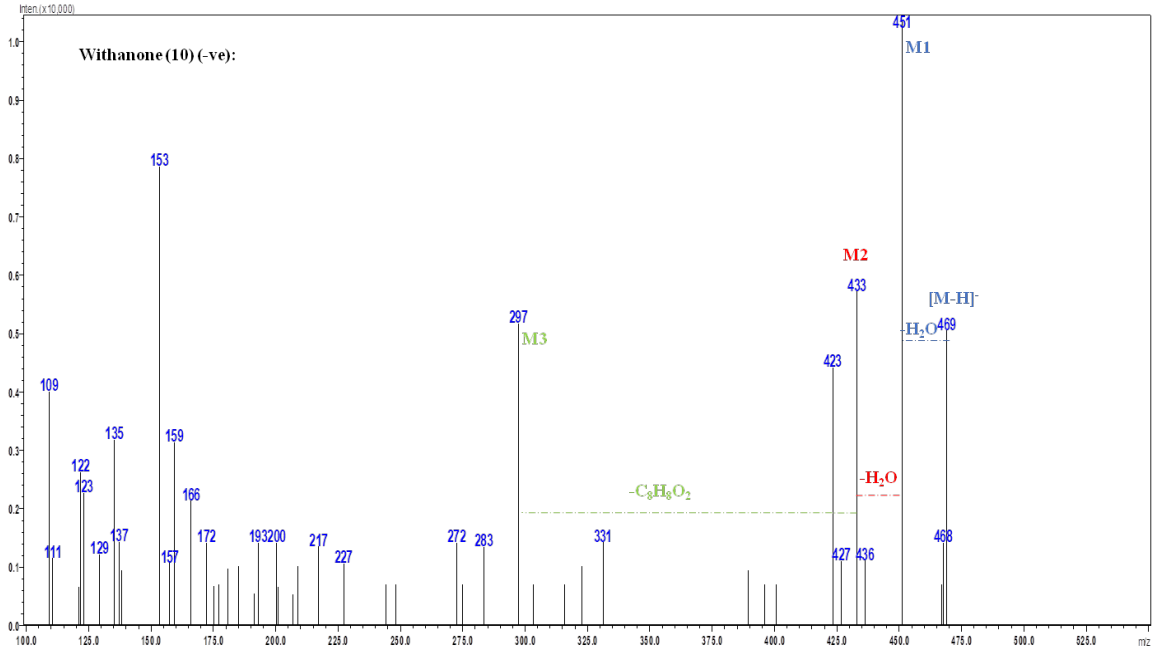

Inten.(x10,000,000)
Dihydrowitha ferin-A (5) (+ve)
M1
301
[M+H]+
473
-C9H16O3
M2
283
-H2O
265
255
145
175
285
289
307
343
401
419
455
m/z

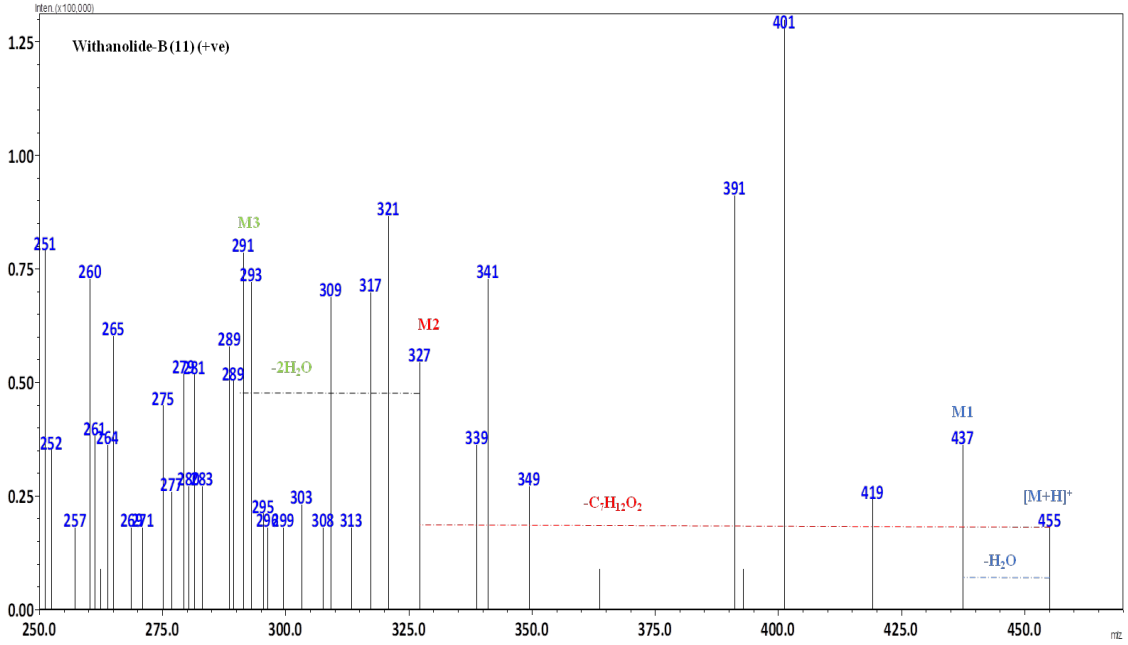

Inten.(x100,000)
122
Withaferin-A (6) (-ve)
M1
451
M2
423
436
-CH3
361
389
M3
286
311
-C7H9O2
[M-H]-
-H2O
469
m/z


Withanoside-V (7) (+ve)
443
M1
M2
425
407
-H2O
- C9H14O2
M3
- C12H22O11
532
[M+H]+
767
409
365
341
293
253
233
Intsn (x 100,000)
mz


12-Deoxywithastramonolide (8) (+ve)
263
M1
299
M2
171
197
-C10H24O3
281
221
121
107
145
235
-C9H16O5
399
417
[M+H]+
471
329
339
366
381
Intsn (x1,000,000)
mz


Inten.(x10,000)
Withanolide-A (9) (-ve)
451
M1
[M-H]-
469
-H2O
122
227
224
M3
283
274
313
-C9H10O2
361
379
390
409
413
M2
433
-H2O
100.0
125.0
150.0
175.0
200.0
225.0
250.0
275.0
300.0
325.0
350.0
375.0
400.0
425.0
450.0
475.0
500.0
525.0
m/z


Inten.(x10,000)
Withanone (10) (-ve):
451
M1
153
M2
433
[M-H]-
-H2O
469
297
M3
423
109
135
159
122
123
166
-C8H8O2
-H2O
137
111
129
157
172
193200
217
227
272
283
331
427
436
468
100.0
125.0
150.0
175.0
200.0
225.0
250.0
275.0
300.0
325.0
350.0
375.0
400.0
425.0
450.0
475.0
500.0
525.0
m/z


Inten.(x100,000)
Withanolide-B (11) (+ve)
1.25
1.00
0.75
0.50
0.25
0.00
250.0
275.0
300.0
325.0
350.0
375.0
400.0
425.0
450.0
m/z
401
391
321
M3
291
251
293
260
341
309
317
M2
265
289
327
-2H2O
289
275
M1
437
339
252
264
349
-C7H12O2
419
[M+H]+
455
303
257
313
-H2O