



## Studies on structural elucidation of delphinium alkaloids by using LC-ESI-MS technique.

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### ABSTRACT

1. A rapid, sensitive and specific liquid chromatography-electron spray-mass spectrometry (LC-ESI-MS) method to identify the different diester-diterpenoid and monoester type alkaloids from *delphinium cashmerianum* collected from Sopi Kargil. Chromatographic separation was achieved on C-18 column and peaks determined by mass spectrometry in positive and negative modes. The validated method led to tentative identification of eight alkaloids on the basis of their retention times and fragmentation patterns. Results showed that the positive mode response was much higher than the negative ion mode. Chromatographic conditions were optimized to obtain high resolution and short run time.

### Indexing terms/Keywords

delphinium cashmerianum, LC-ESI-MS, Sopi.

### Academic Discipline and Sub-Disciplines

Applied material Sciences.

### SUBJECT CLASSIFICATION

Phytochemistry subject classification.

### TYPE (METHOD/APPROACH)

Experimental. Analytical liquid chromatographic and mass spectrometric method.

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## INTRODUCTION

It is the genus belonging to family renunculaceae comprising of more than 300 species of perennial flowering plants distributed in Asia, North America, Europe and tropical Africa<sup>1</sup>. The members of this genus are toxic to humans and livestock<sup>2</sup>. The perennial species of *Delphinium* along with the annual species of consolidate are commonly called as Larkspur<sup>3,4</sup>. In the flowering season of June – July the plant has raceme of colored flowers varying from purple to blue and red and white. In most species the flower has five petal like sepals joined together with a spur (hence common name) enclosing four similarly colored petals. They possess small black and shiny seeds.

*Delphinium cashmerianum* Royle (Kashmir Larkspur) is a perennial herb found in the Himalayas, from Pakistan to Uttarakhand, at altitudes of 2700-4500 m. It is characterized by woolly-haired, conspicuously veined bluish-purple flowers, 2-3 cm across clustered in a dense flat-topped head. Flowers have a stout spur 1.5 cm long. Leaves are rounded in outline, deeply lobed, 3-5 cm across. It is quite similar to Musk Larkspur which is found only above altitudes of 4500 m, and has larger, more inflated flowers.

The majority of phytochemical investigation on *Delphinium* species (Renunculaceae) have been carried out for diterpenoid alkaloid. Alkaloids like Neoline, Chasmanine, Homochasmanine and Delphisine were reported by pelletier<sup>5</sup> in *Delphinium staphisagria* in 1975. Warnock<sup>6</sup> reported benzoylated quercetin glycosides from *Delphinium carolinianum* in 1982. Jong-C Park and S. W Pelletier<sup>7</sup> isolated hetisane type of alkaloids like davisinol, 18 benzoyldavisinol and davisin from *Delphinium davisii*. Gabriel De La Fuente<sup>8</sup> reported isolation of six new norditerpenoid alkaloids Nudicaulidines from *Delphinium cardiopetalum*. Ayhan Ulubelen<sup>9</sup> et al have reported norditerpenoid 8-acetyl condelphine, condelphine and senbusine from *Delphinium pyramdale*. Delavaine, deoxylycoctinine and methyllycaconitine from *Delphinium dissectum* were reported by Nyamdari Batbayar et al<sup>10</sup>. Werner Herz et al<sup>11</sup> have reported flavonol glycosides from ethanolic extract of aerial parts of *Delphinium staphisagria*. Yang-Qing He et al<sup>12</sup> have also reported flavanoids from *Delphinium albocœruleum* along with diterpenoids and one triterpenoid.

## MATERIALS AND METHODS

### Plant Material

The root part of *Delphinium cashmerianum* (15 Kg) were collected from SOPI, Kargil Ladakh (J&K, India) in august 2010. The specimen was identified by Akhtar H. Malik, Curator, Centre for Biodiversity & Taxonomy, University of Kashmir (Specimen deposited under accession No. 341320 and Collection No. 1301 - Bilal, Kash).

### Extraction

The air dried, finely powdered root material (5Kg) was extracted for 72 hours with methanol to afford the respective extract, which was concentrated under reduced pressure and was coded as DEL.

### Reagents and chemicals

HPLC grade acetonitrile, methanol and formic acid were purchased from Sigma Aldrich. HPLC grade water (18.2MΩ) was procured from Milli-Q water system (USA). other chemicals were of analytical grade and are available commercially.

### LC System

LC analysis was carried out by an Agilent 1260 infinity series consisting of a pump, detector, an auto sampler and a column component. The samples were separated on chromolith RP-18e column (4.6 mm ID, 50mm length) Merck at room temperature. The mobile phase consisted of (A) aqueous formic acid (0.1%) and (B) methanol and the elution gradient was set as follows : 0–8 min, linear gradient from 12% to 25% of B; 8–12 min, isocratic conditions at 25% of B;

12–16 min, linear gradient from 25% to 40% of B; 16–40 min, linear gradient from 40 to 50% of B, 40–50 min, linear gradient from 50 to 100% of B. Flow rate: 1 ml/min.

## LIQUID-CHROMATOGRAPHY TANDEM-MASS-SPECTROMETRY (LC-ESI-MS/MS)

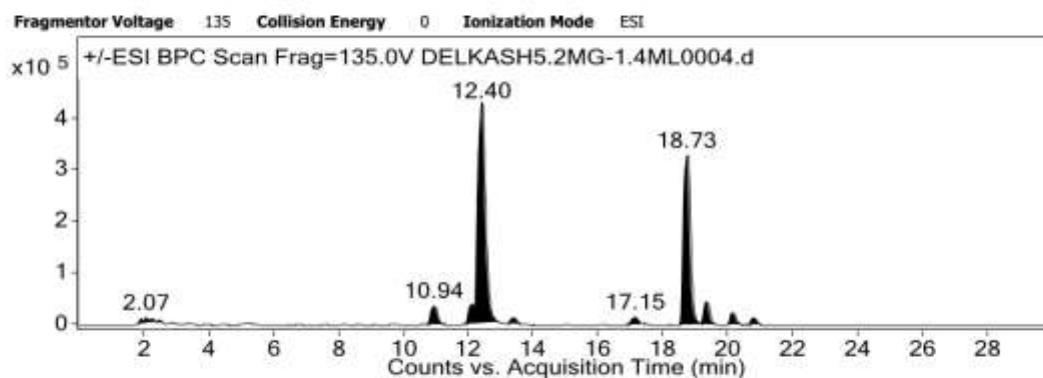
For LC-ESI-MS<sup>n</sup> experiments, LC-MS QqQ-6410B equipment (Agilent Technologies) comprising a chromatographic system 1260 Infinity (Agilent Technologies) coupled with an Agilent Triple Quad mass spectrometer fitted with an ESI source was used. The conditions of ESI source were set as follows: sheath and auxiliary gases flow, 25 and 3 arbitrary units, respectively; spray voltage, 4KV; capillary temperature 325°C and tube length voltage: 120 V. The samples were analysed in both positive and negative modes and full scan mass range was set between m/z 100-1200 with the acquisition of centroided-type mass spectra. Accurate mass analyses were calibrated according to manufacturers guidelines. In the MS<sup>n</sup> experiments, data dependent MS<sup>n</sup> scanning was performed to minimize total analysis time as it can trigger fragmentation spectra of target ions and prevent repetition by dynamic exclusion settings. The software of the Agilent technologies was used for data analysis. After the automated removal of noise and baseline signals, the theoretical extract chromatogram of main alkaloids was shown in the plot (fig 3)



**Data Filename** DELKASH5.2MG-1.4ML0004.d **Sample Name** DELKASH5.2MG-1.4ML  
**Sample Type** Sample **Position** Vial 47  
**Instrument Name** SG11351102 **User Name** IIIM  
**Acq Method** **Acquired Time** 5/4/2013 1:32:24 PM  
**IRM Calibration Status** Not Applicable **DA Method** ARTEMISIN-MS2.m  
**Comment** 50MM+100MMX4.9MM CHROMO

**Sample Group** **Info.**  
**User Defined 1** **User Defined 2**

### User Chromatograms



#### Integration Peak List

Start	RT	End	Height	Area
10.73	10.94	11.32	35880	487068
11.87	12.11	12.18	39047	410807
12.18	12.4	12.93	429546	6816676
13.21	13.36	13.37	10283	50384
13.37	13.39	13.42	10318	30032
17.06	17.15	17.31	14325	142471
18.5	18.73	19.13	330205	4569394
19.17	19.33	19.63	44140	510437
19.99	20.15	20.41	22409	249431
20.62	20.78	21.01	12586	139651

fig-3 Total chromatogram of Delphinium cashmerianum.

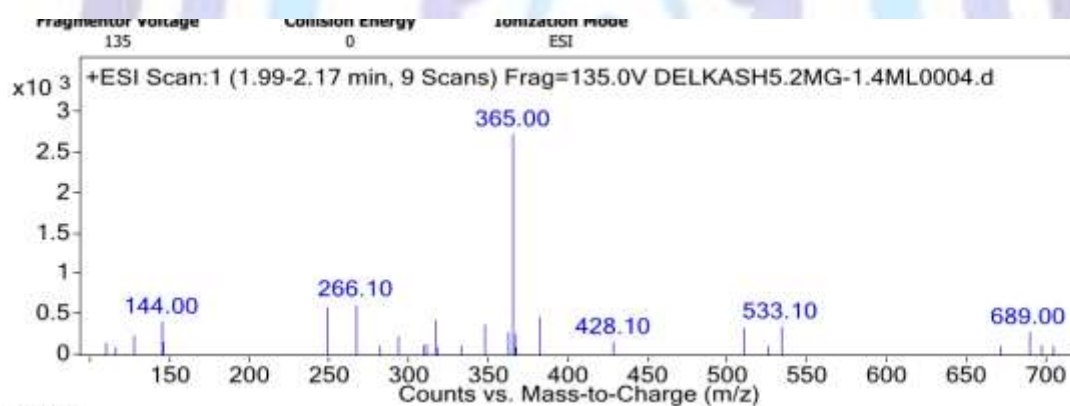
## RESULTS AND DISCUSSION

For LC-MS experiments different fragmentation measurements were conducted in independent LC-MS<sup>n</sup> experiments. In the first measurement the survey scan was performed in analyser at (R=30,000) followed by MS<sup>2</sup> scan at (R=15,000). The accurate tandem mass spectra can provide strong evidences for identification of fragments and neutral loss. In the separate measurement in which ESI MS<sup>2</sup> and MS<sup>3</sup> experiments using dynode detection provided more fragment ions and order of fragmentation could be determined to show evidence of structural identification. Fifteen alkaloids were picked from the chromatogram at the retention times from 2 -20 min. Among them nine compounds were identified as 14-2-methylbutyl nudicaudiline, 14-cis cinnamoyl nudicaudiline, Davisinol, uncinitine, condelphine, Peregrine, Karakoline and 10- hydroxy methyllyoaconitine. by comparing their retention times and on the basis of their fragmentation patterns in MS-MS data. The other compounds were tentatively identified by comparing their mass data with those reported in literature. The Retention time values and mass data of deduced compounds from peaks are summarised in table 3. The fragmentation patterns of the identified compounds is shown in (fig 4).



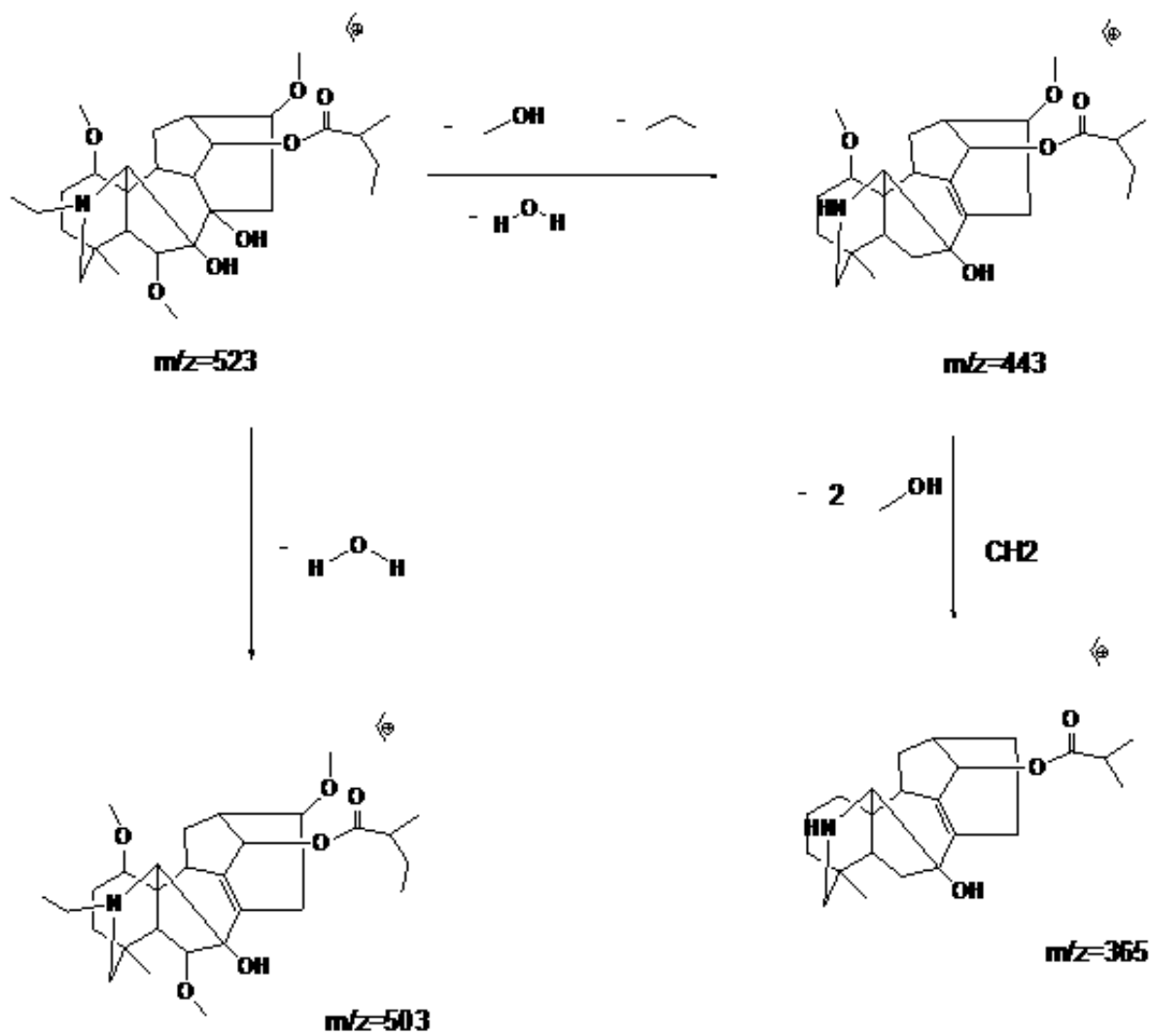
Table-3

S No	Tr in minutes	m/z	Adduct peaks	Fragmentation peaks	Identification
1	2.0-2.10	507[C <sub>28</sub> H <sub>45</sub> NO <sub>7</sub> ]	[M+2]509, [M+2H+Na]533	MS <sup>2</sup> [533]428, 365	14-Isobutylnudicaudiline
2	2.29-2.36	521[C <sub>29</sub> H <sub>47</sub> NO <sub>7</sub> ]	[M+2]523, [M+3]524	MS <sup>2</sup> [523]443,365	14-(2-methylbutyl)nudicaudiline.
3	10.80-11.13	313[C <sub>20</sub> H <sub>27</sub> NO <sub>7</sub> ]	[M+1]314,[M+2]315, [M+3]316, [M-1]312	MS <sup>2</sup> [315]271	Davisinol
4	12.24-12.63	359[C <sub>22</sub> H <sub>33</sub> NO <sub>3</sub> ]	[M-1]358],[M+2H+Na]386	MS <sup>2</sup> [359]341,297	Uncinitine
5	13.26-13.37	445[C <sub>26</sub> H <sub>39</sub> NO <sub>5</sub> ]	[M+H+Na]469],[M+CAN+Na]510	MS <sup>2</sup> [448]360,342,308	Peregrine
6	13.42-13.55	449[C <sub>25</sub> H <sub>39</sub> NO <sub>6</sub> ]	[M+19]468	MS <sup>2</sup> [449]359,341,314	Condorphine
7	17.06-17.53	374[C <sub>22</sub> H <sub>32</sub> NO <sub>4</sub> ]	[M+2]376, [M-18]356	MS <sup>2</sup> [374]356, 324,	Karakoline
8	19.21-19.50	698[C <sub>37</sub> H <sub>50</sub> N <sub>2</sub> O <sub>11</sub> ]	[M+1]699,[M+2]700,[M+24]723	MS <sup>2</sup> [700]683,587	10-Hydroxymethyllyoaconitine

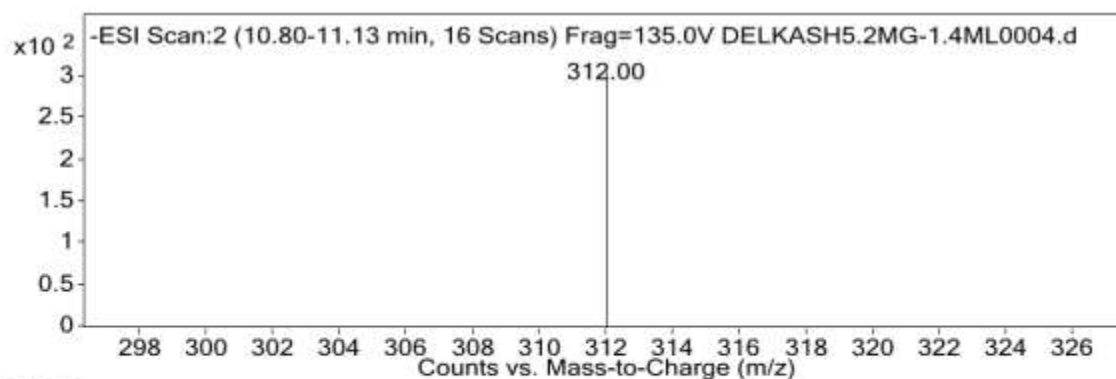


Peak List

m/z	Abund
144	411.9
247.9	594.5
266.1	625.7
316	435.1
347	387.1
365	2733.6
381.1	469.6
509	330.1
533.1	349.9
689	282.7



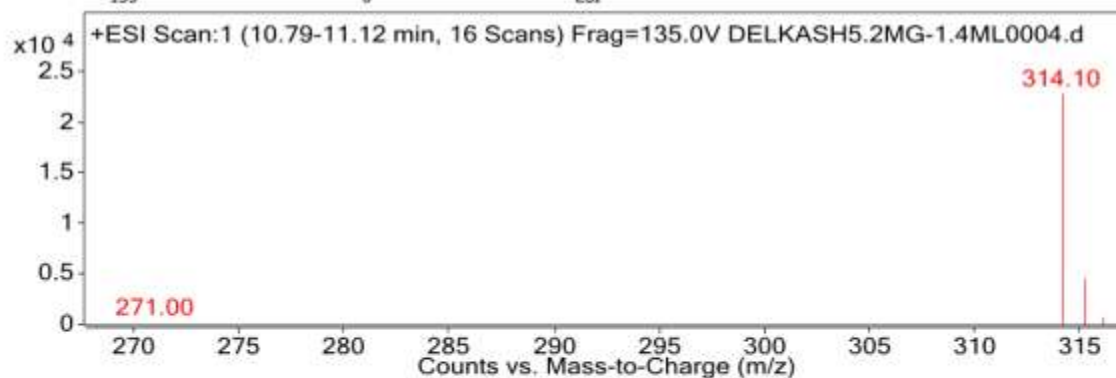
Fragmentation pattern of 14-(2-methylbutyl nudicaudiline)



## Peak List

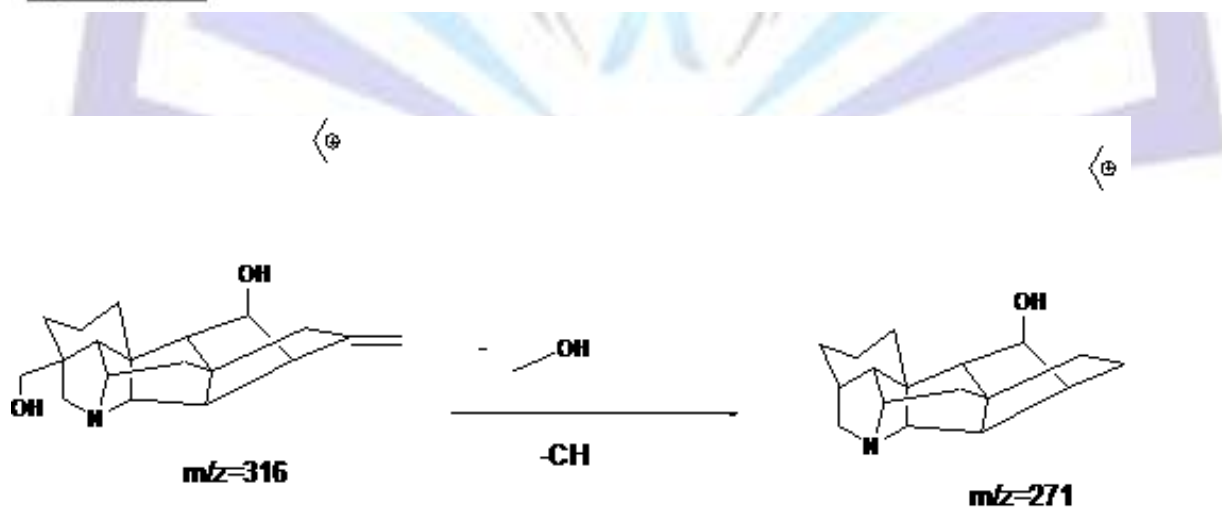
m/z	Abund
312	304.8

Fragmentor Voltage 135 Collision Energy 0 Ionization Mode ESI

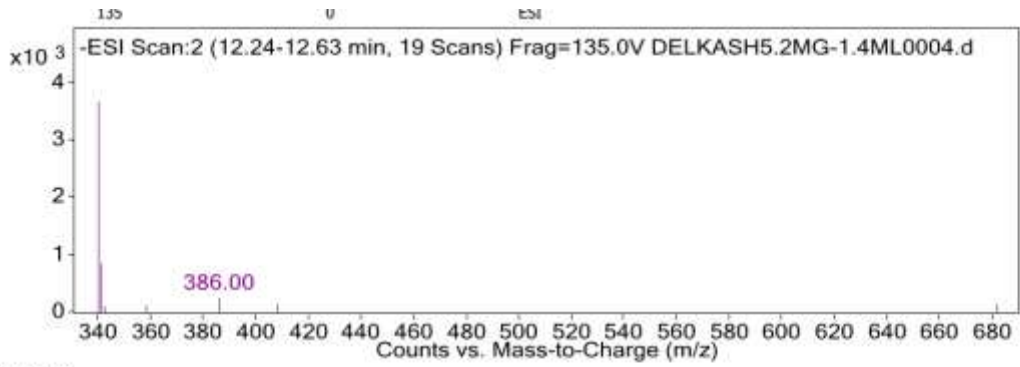


## Peak List

m/z	Abund
268.9	136.6
271	156.8
313	142.3
314.1	22814.2
315.1	4681.3
316	801.5

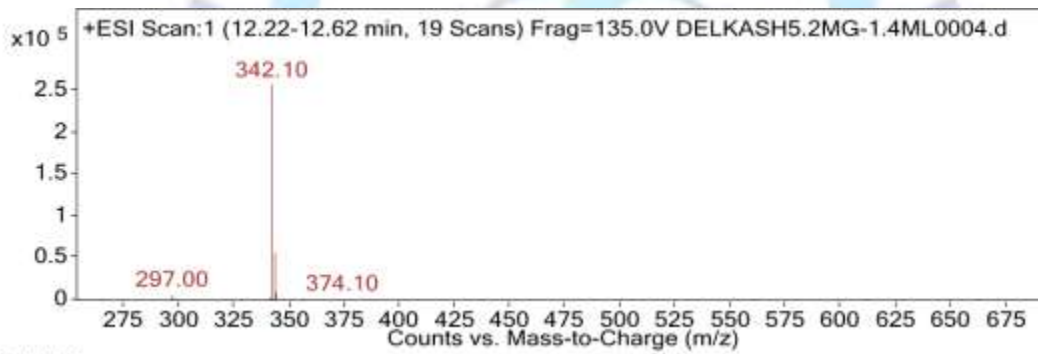


Fragmentation pattern of Davisinol



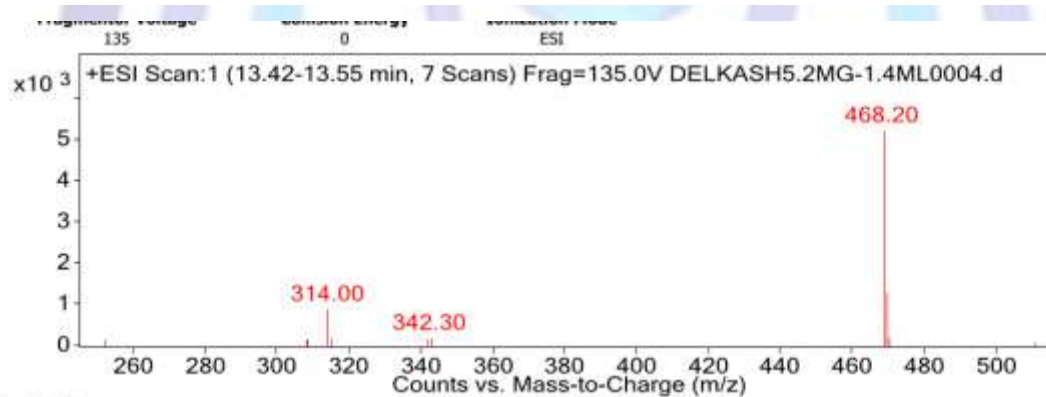
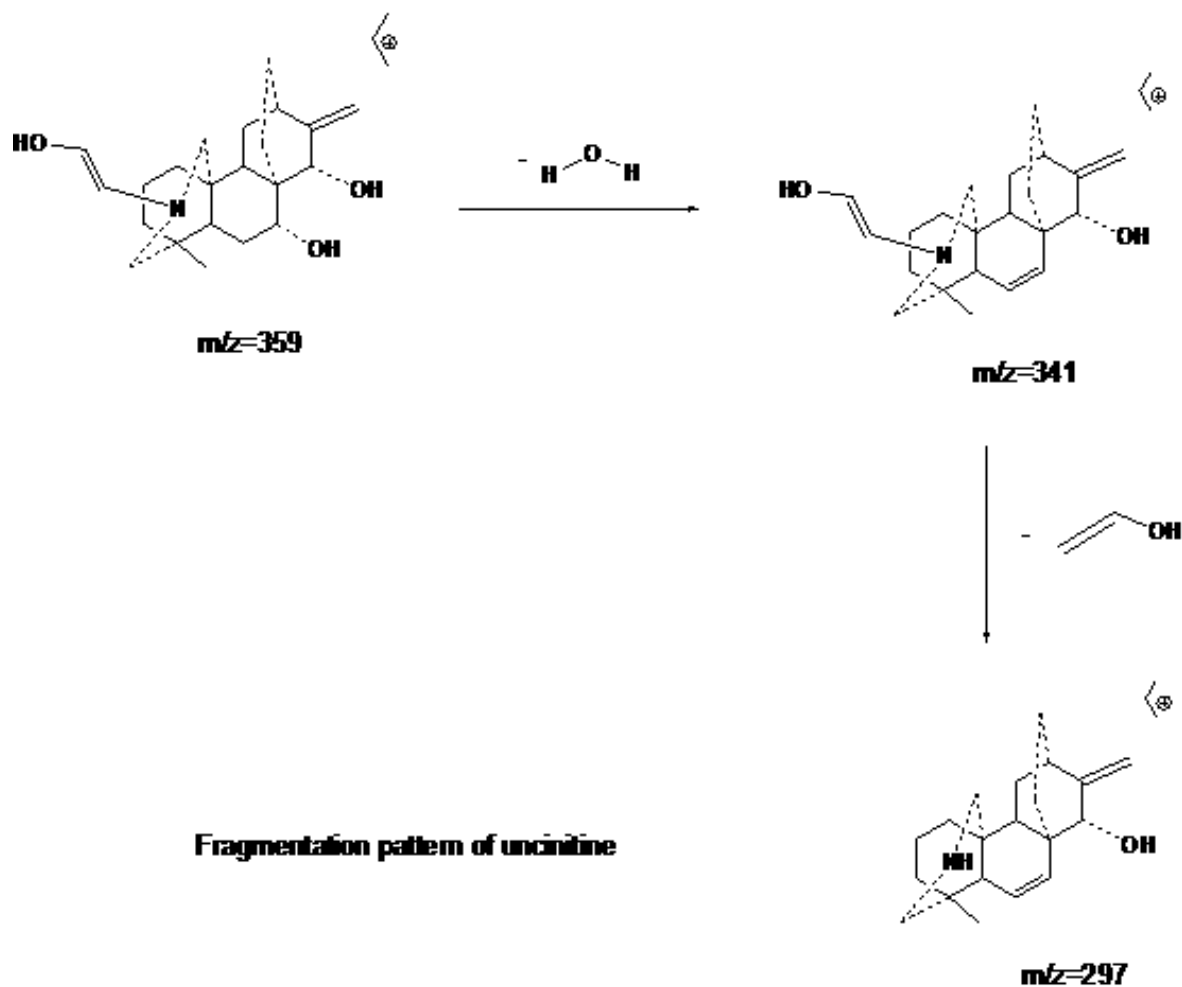
**Peak List**

<i>m/z</i>	Abund
340	3675.3
341	851.3
342	123
357.9	145.9
386	264.4
407.9	163.7
681.1	168.8



**Peak List**

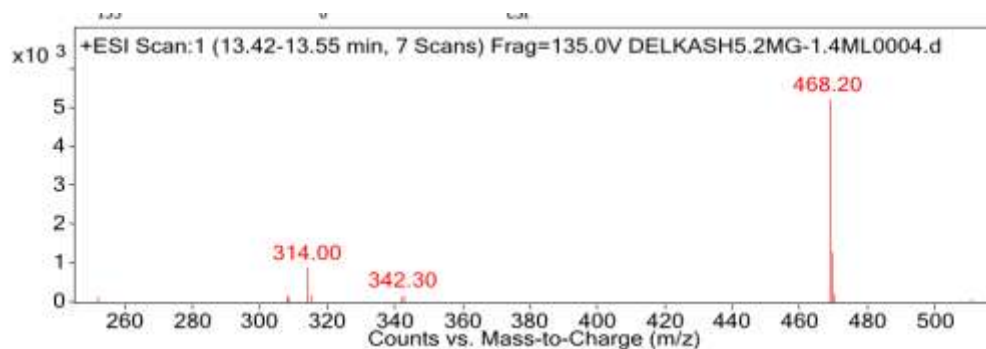
<i>m/z</i>	Abund
265	1806.4
297	6495
328.1	2011.4
341	2608.7
342.1	256497.7
343.1	56885.6
344.1	9041.3
356	1433.3
374.1	1587.8
683.1	1467.8



## Peak List

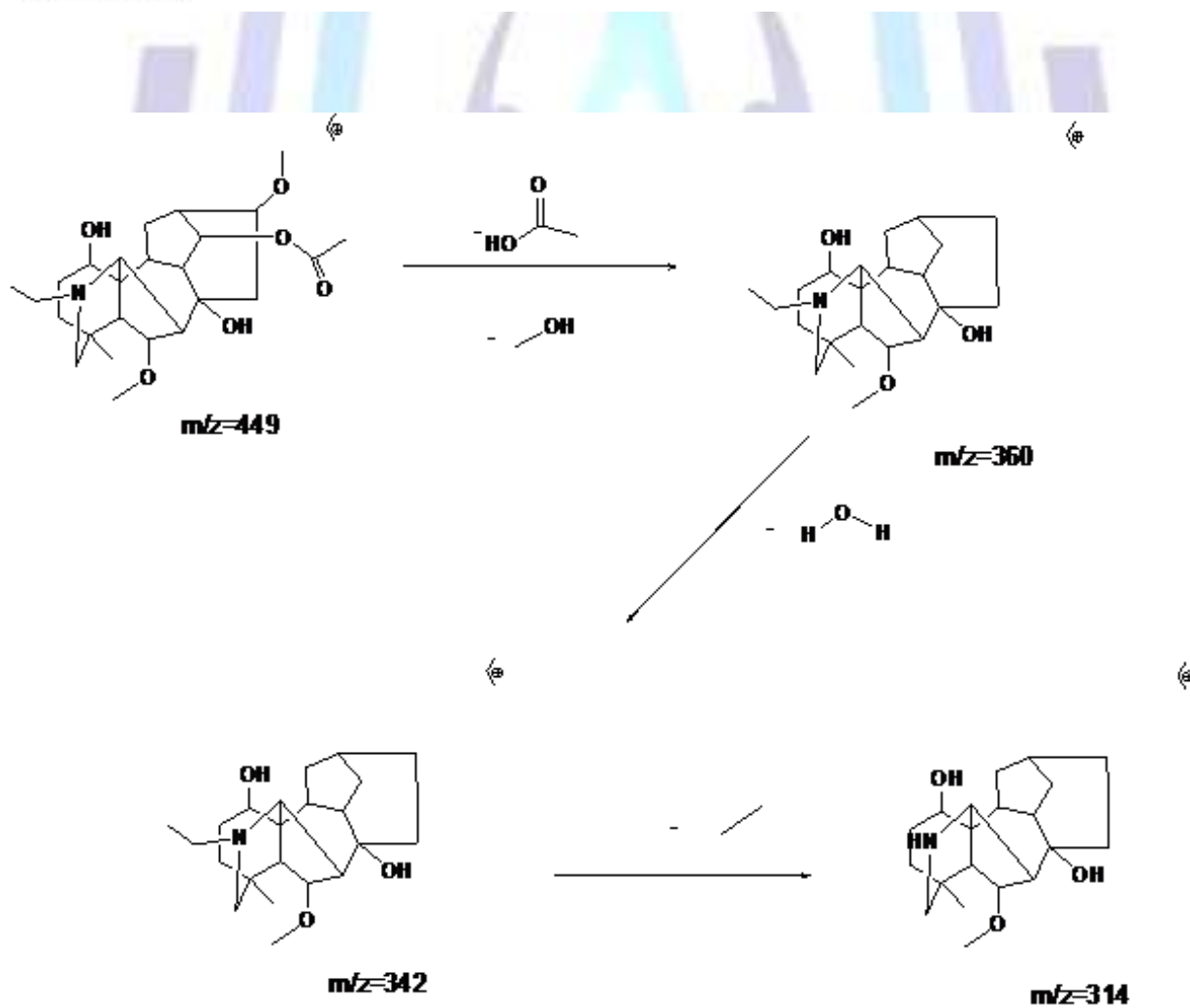
$m/z$	Abund
252	146.4
307.8	161.5
308.3	146.9
314	902
315	199.6
341.8	169.9
342.3	202.1
468.2	5234.4
469.2	1329
470	222.2



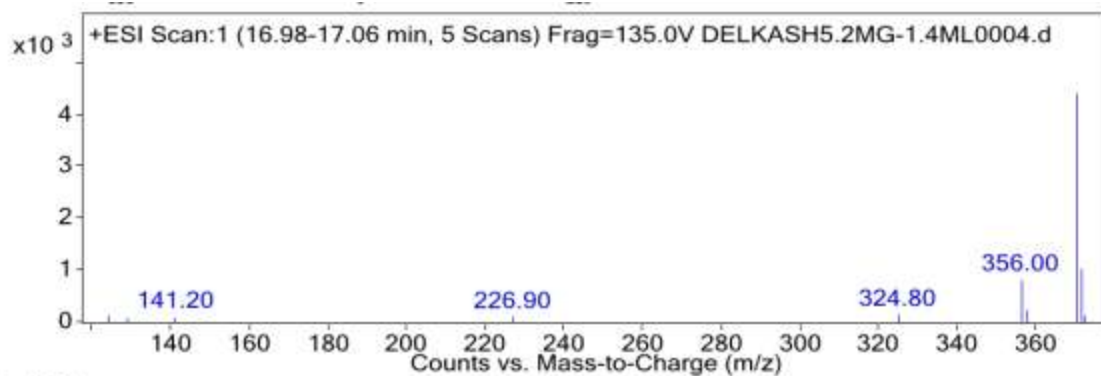


Peak List

m/z	Abund
252	146.4
307.8	161.5
308.3	146.9
314	902
315	199.6
341.8	169.9
342.3	202.1
468.2	5234.4
469.2	1329
470	222.2

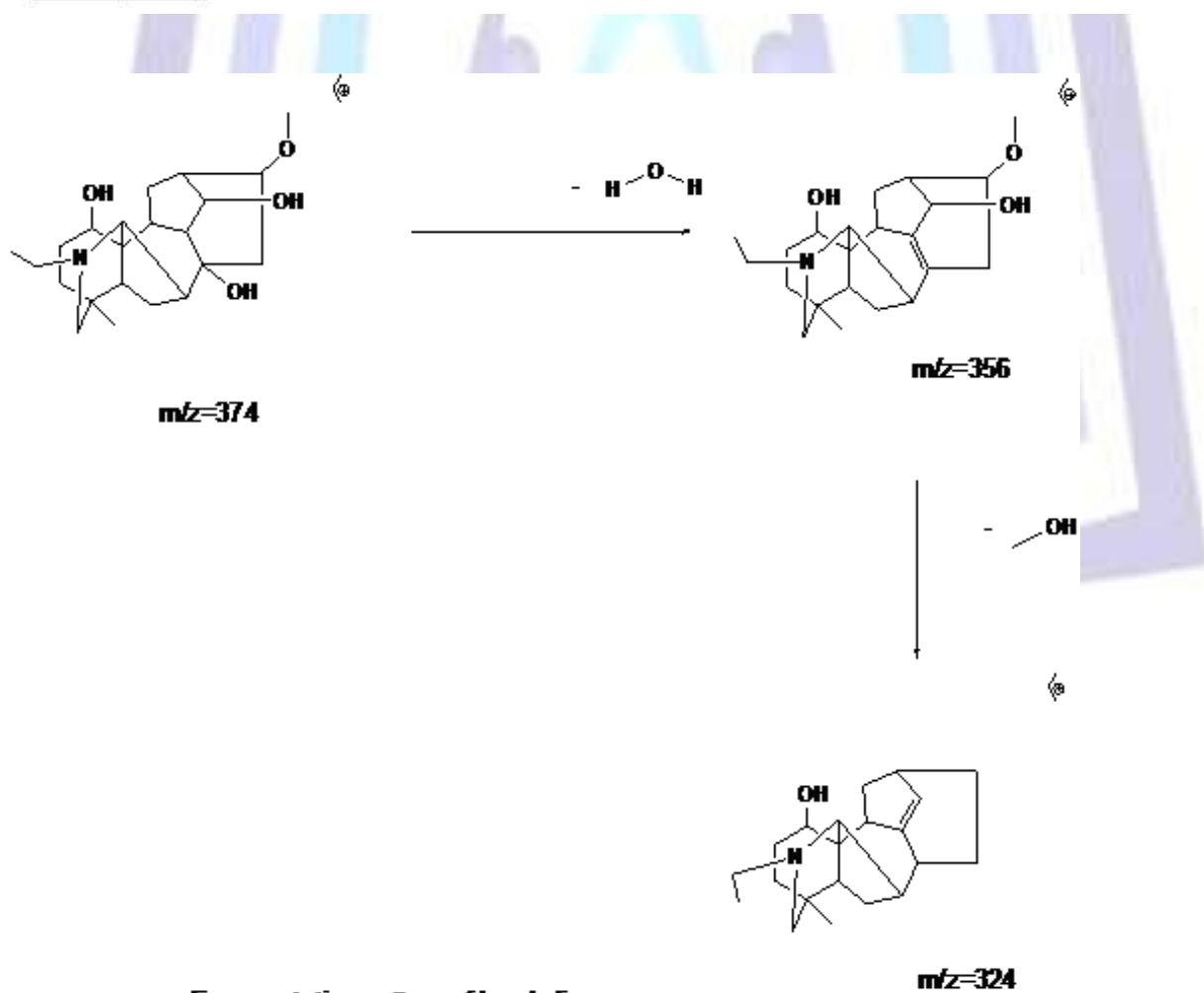


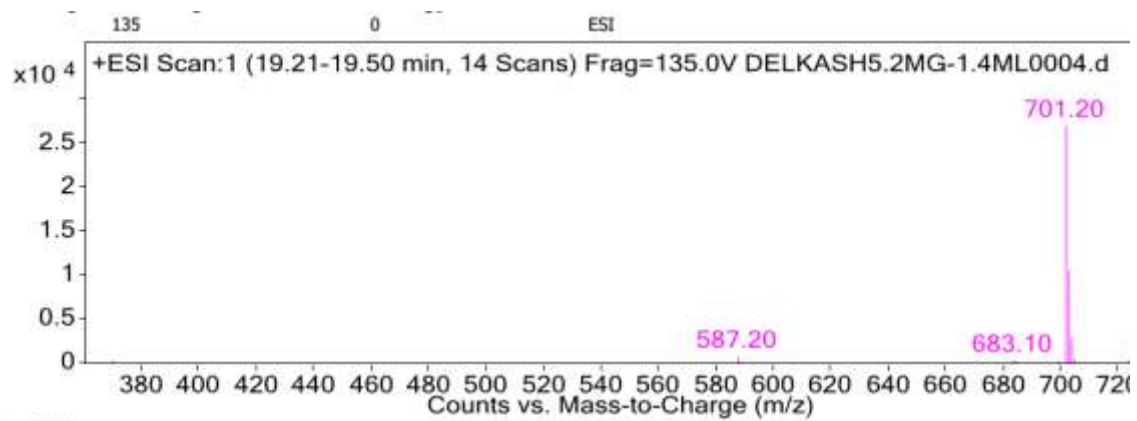
Fragmentation pattern of condelfine



## Peak List

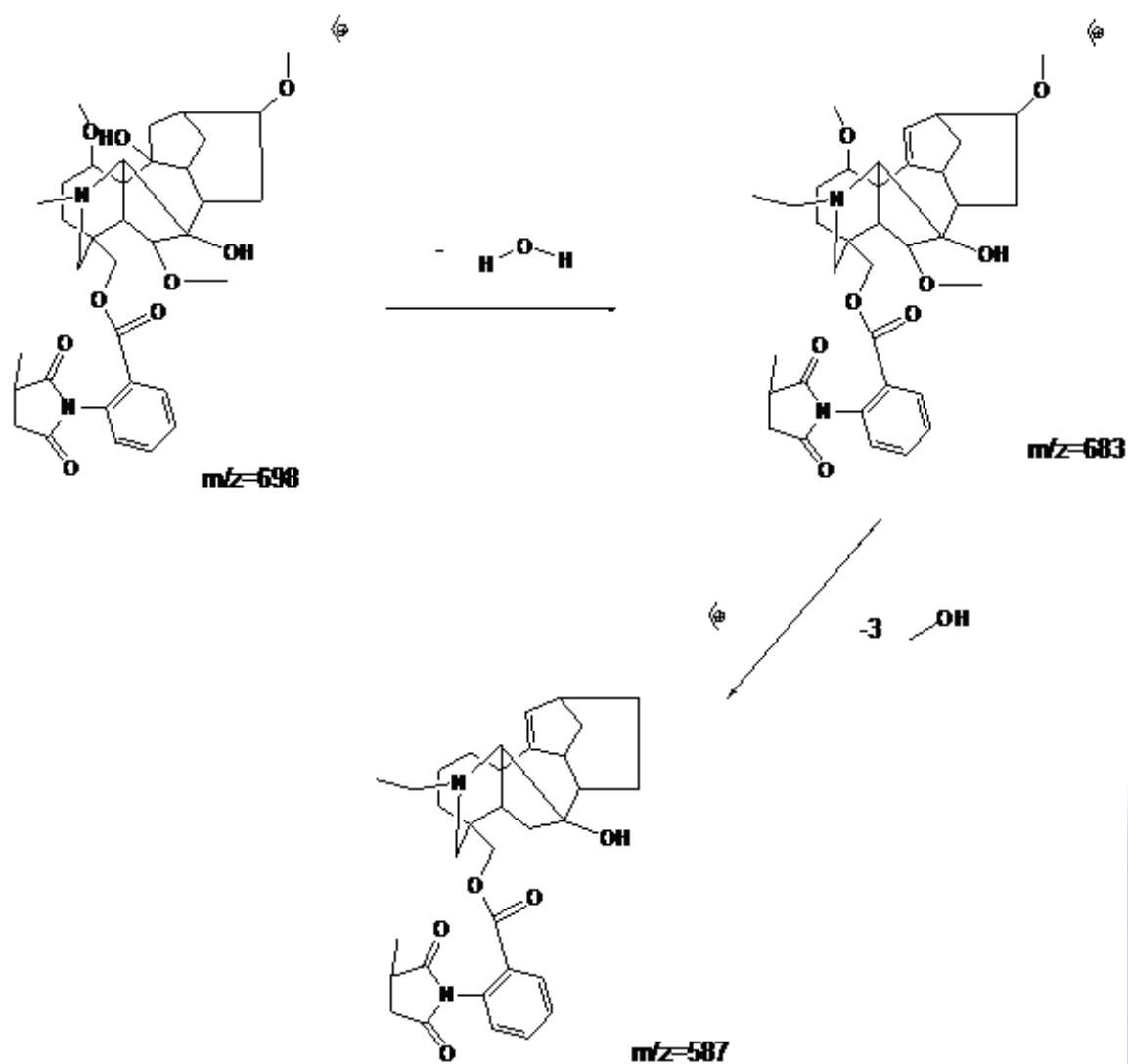
m/z	Abund
124.2	123.2
128.9	109.5
141.2	119.1
226.9	112.1
324.8	157.6
356	834.7
357.1	246.2
370.1	4413
371.1	1027.8
371.9	135.9



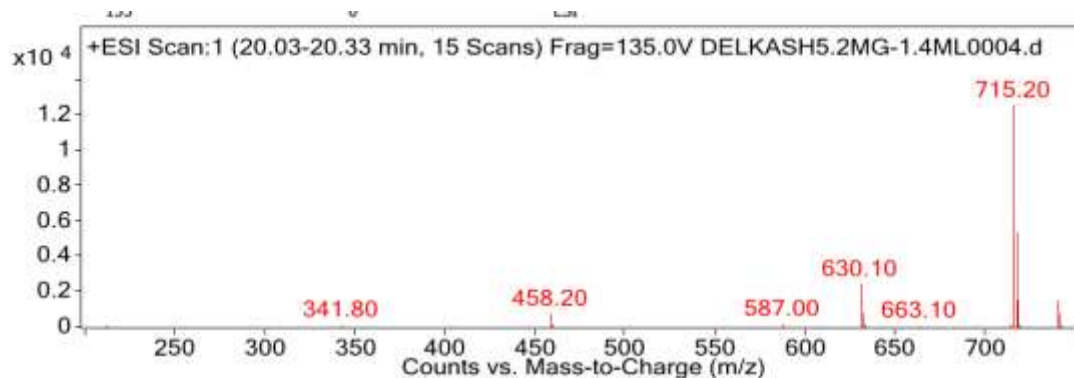
**Peak List**

m/z	Abund
370.1	375
587.2	729.1
683.1	256.9
684.3	247.5
699.1	370.7
701.2	26960.8
702.2	10627.9
703.2	2835.8
704.2	451.5
723.1	302





**Fragmentation pattern of 10-hydroxymethyllycoconitine**



Peak List

m/z	Abund
458.2	770.8
459.1	230.2
630.1	2448.9
631.2	848.8
713.1	240.8
715.2	12593.5
716.3	5459.3
717.2	1569.1
739.2	1556.7
740.2	828.4

## CONCLUSION

In this study a simple and efficient LC-ESI-MS<sup>n</sup> method has been developed to identify alkaloids from roots of *Delphinium cashmerianum*. Moreover the LC-ESI-Mass spectrometry has been demonstrated to be an effective tool for analysis of the components and searching of novel compounds in plant extracts. It also provided the essential data for further pharmacological and toxicological studies on *Delphinium* plants.

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