Guideline for LC-MS data analysis for facility users

Liquid chromatography–mass spectrometry (**LC-MS**, or alternatively **HPLC-MS**) is an analytical tool that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). LC-MS is a powerful technique that has very high sensitivity and selectivity and so is useful in many applications.

Experiment

1. **LC-ESI/MS (LC-MS)2ChScan****: It is a two channel fullscan data acquisition experiment with polarity ES positive and ES negative at cone voltage 30, simultaneously.

   Objective: Determination of molecular weights of compounds/analytes separated by liquid chromatography in LC-MS system.

2. **LC-ESI/MS (LC-MS)4ChScan****: It is a four channel fullscan data acquisition experiment with polarity ES positive and ES negative at cone voltage 30 and 60, simultaneously.

   Objective: Determination of molecular weights of compounds/analytes separated by liquid chromatography in LC-MS system with In-source fragmentation.

   Key Aspects of ESI In-Source Fragmentation (4ChScan)
   Structural information: It can provide characteristic fragments for identification, often used when MS/MS is not available. It serves as an alternative to tandem MS (MS/MS) for structural elucidation.

   https://www.saiflucknow.org/download_file
   SAMPLE COPY OF LC-MS DATA/RESULTS-A

   - SAMPLE COPY OF LC-MS DATA/RESULTS-A
     A sample copy of LC-MS data (2ChScan) is for demo purposes; users can see it. Note: LC-MS data analysis is part of the end-user; it will not be provided by SAIF.
     2025-02-15 00:00:00 | Download

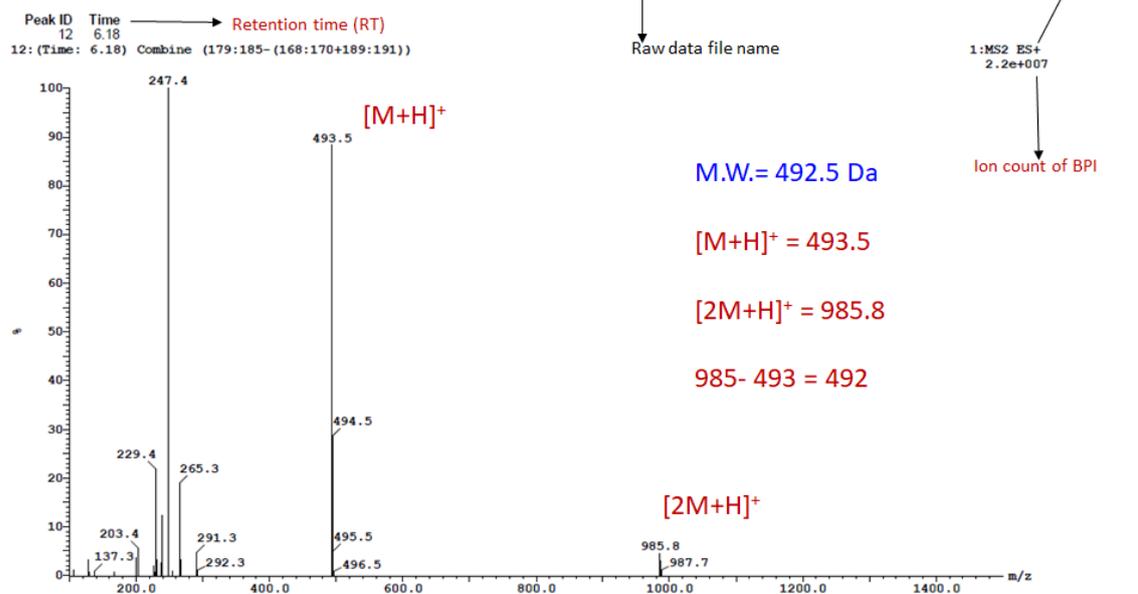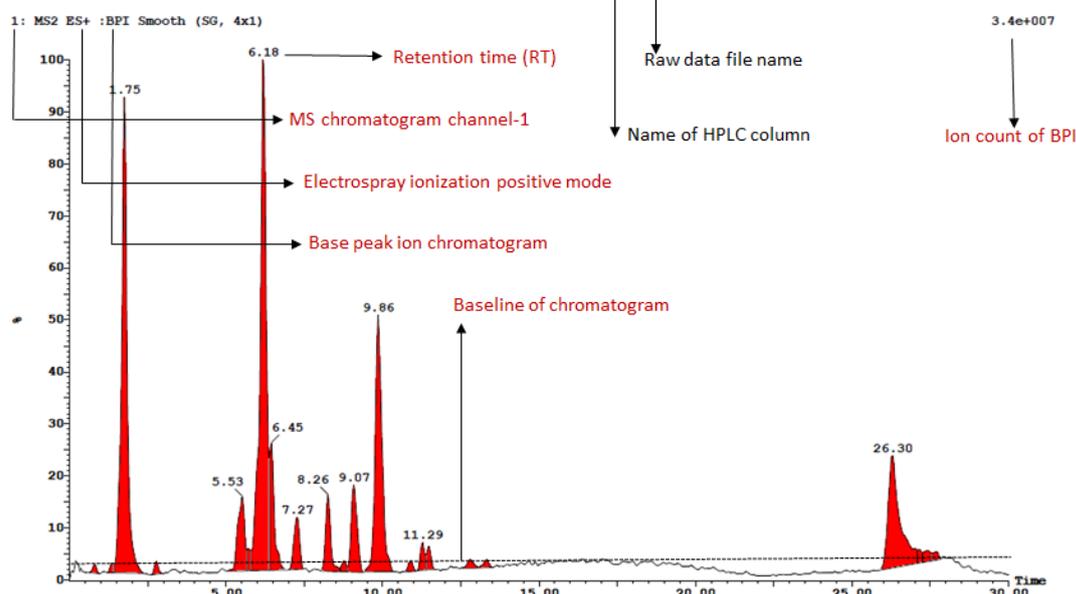   - SAMPLE COPY OF LC-MS DATA/RESULTS-B
     A sample copy of LC-MS data (4ChScan) is for demo purposes; users can see it. Note: LC-MS data analysis is part of the end-user; it will not be provided by SAIF.
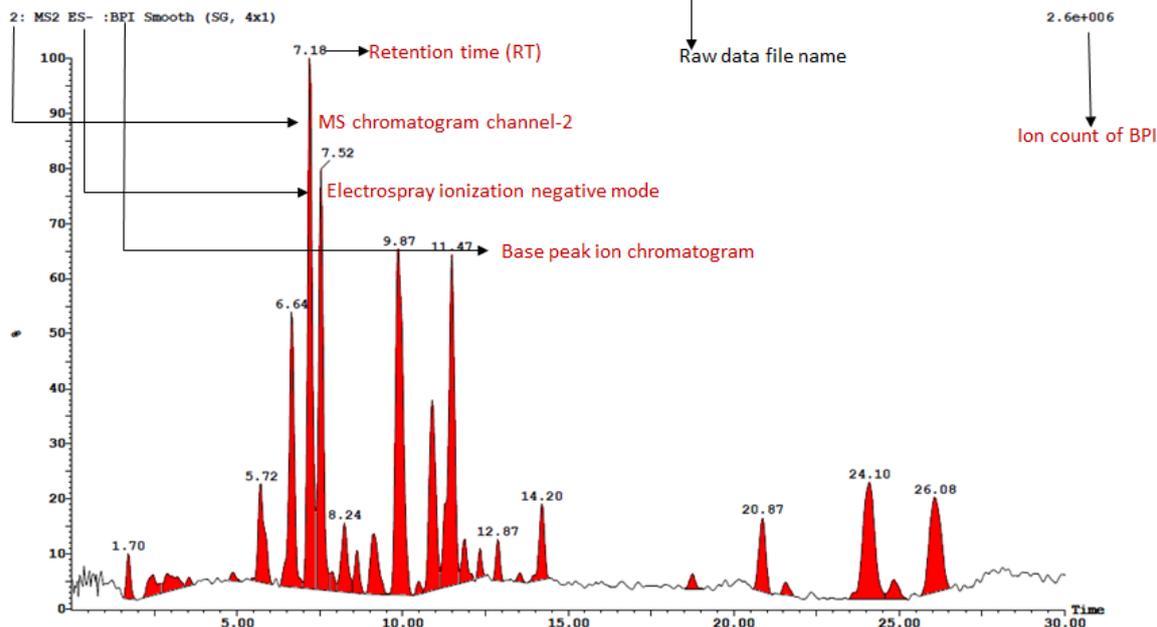   2025-02-15 00:00:00 | Download

Note:

- The results of the aforementioned LC-MS experiment will be a processed data file in PDF format, and only manual data analysis is possible.
- Data analysis is part of the end-user; it will not be provided by SAIF.
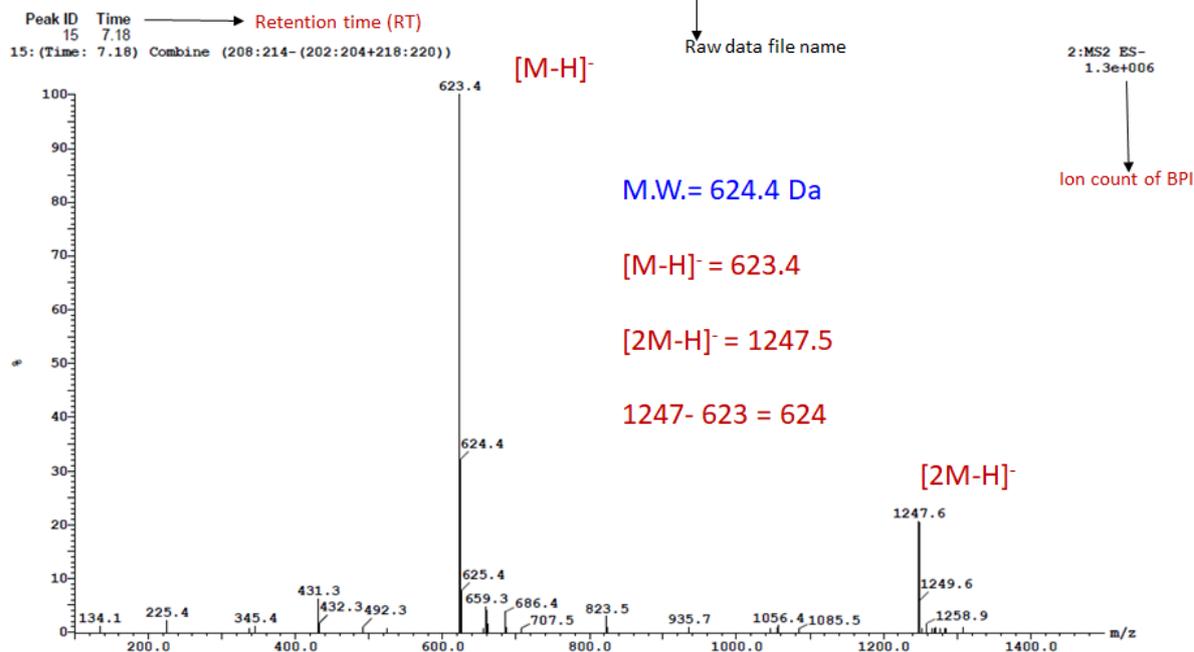
Data interpretation/analysis: LC-ESI (+) MS mode

Sample: 7
Vial:1:57
ID:DKMR60
File:19IDKMAUG07
Date:07-Aug-2019
Time:22:29:44
Description:BETASIL C8 250 X4.6, 5um

Printed: Thu Aug 08 09:24:04 2019

1: MS2 ES+ :BPI Smooth (SG, 4x1)   3.4e+007

→ Retention time (RT)

→ Raw data file name

→ MS chromatogram channel-1

→ Name of HPLC column

→ Ion count of BPI

→ Electrospray ionization positive mode

→ Base peak ion chromatogram

Baseline of chromatogram

6.18
1.75
9.86
6.45
5.53
8.26
9.07
7.27
11.29
26.30

Sample: 7
Vial:1:57
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Printed: Thu Aug 08 09:24:04 2019

Electrospray ionization positive mode

Peak ID   Time
12   6.18   → Retention time (RT)
12: (Time: 6.18) Combine (179:185- (168:170+189:191))

→ Raw data file name

1:MS2 ES+
2.2e+007

→ Ion count of BPI

247.4

$[M+H]^+$

493.5

M.W.= 492.5 Da

$[M+H]^+ = 493.5$

$[2M+H]^+ = 985.8$

$985- 493 = 492$

$[2M+H]^+$

229.4
265.3
494.5
203.4
291.3
495.5
985.8
137.3
292.3
496.5
987.7

Data interpretation/analysis: LC-ESI (-) MS mode

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Sample: 7
File:19IDKMAUG07
Description:BETASIL C8 250 X4.6, 5um
Vial:1:57
Date:07-Aug-2019
ID:DKMR60
Time:22:29:44
Printed: Thu Aug 08 09:24:04 2019

2: MS2 ES- :BPI Smooth (SG, 4x1)
2.6e+006

Retention time (RT)

MS chromatogram channel-2

Electrospray ionization negative mode

Base peak ion chromatogram

Raw data file name

Ion count of BPI

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Sample: 7
File:19IDKMAUG07
Description:BETASIL C8 250 X4.6, 5um
Vial:1:57
Date:07-Aug-2019
ID:DKMR60
Time:22:29:44
Printed: Thu Aug 08 09:24:04 2019

Peak ID   Time   Retention time (RT)
15   7.18
15: (Time: 7.18) Combine (208:214-(202:204+218:220))

$[M-H]^-$

Raw data file name

2:MS2 ES-
1.3e+006

Ion count of BPI

M.W.= 624.4 Da

$[M-H]^- = 623.4$

$[2M-H]^- = 1247.5$

1247- 623 = 624

$[2M-H]^-$

Electrospray ionization (ESI) source adduct formation occurs when analyte molecules bind with ions (e.g. H^+, Na^+, NH_4^+) in the spray plume, creating adduct ions like $[M+H]^+$, $[M+Na]+$ $[M+NH4]+$ or $[M-H]^-$, $[M+Cl]^-$, $[M+CH_3COO]^-$. These adducts are crucial for determination of molecular weights of analytes. User may use https://tmsdatabase.cdri.res.in/AductIonCalculator for possible adduct formation and make a table for as shown below.

Example-1

Table -1: Chromatographic retention time and detection of compound with adduct formation.

S.No.	RT	M.W.	ESI (+)		ESI (-)		
			$[M+H]_+$	$[2M+H]_+$	$[M-H]_-$	$[2M-H]_-$	$[M+Cl]_-$
1	6.18	**492**	493	985	-	-	-
2	7.18	**624**	-	-	623	1247	659

Note-

- Users can anticipate the probable chemical compounds in a sample based on previously known compounds and their molecular weight information in the literature (written papers on similar samples such as plants, microorganisms, and animals).

- The user should do extensive literature search so that the maximum number of compounds can be identified in sample. Without literature support, LC-MS data analysis is not possible.

- After obtaining LC-MS data and molecular weight information, the user need LC-HRMS and LC-HRMS/MS data to determine chemical formula and identify the chemicals detected in MS.

- The user can utilize the ESI/APCI ion calculator to confirm the molecular weight of chemicals observed during the LC-MS run. https://tmsdatabase.cdri.res.in/AductIonCalculator

- Data analysis is part of the end user and will not be provided by SAIF.

Reference:

- Singh A, Ali M, Mishra DK, Kanojiya S. Comparative Profiling of Withanolides in Tissues of Withania somnifera and Datura metel Using UHPLC-MS/MS. Chem Biodivers. 2025 Dec;22(12):e01319. doi: 10.1002/cbdv.202501319. Epub 2025 Aug 22. PMID: 40845291.

- Singh Y, Nimoriya R, Rawat P, Mishra DK, Kanojiya S. Structural Analysis of Diastereomeric Cardiac Glycosides and Their Genins Using Ultraperformance Liquid Chromatography-Tandem Mass Spectrometry. J Am Soc Mass Spectrom. 2021 May 5;32(5):1205-1214. doi: 10.1021/jasms.1c00017. Epub 2021 Apr 5. PMID: 33818079.