GUIDELINE FOR LC-MS 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.

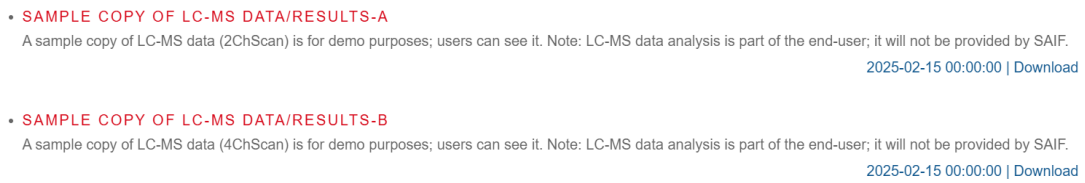
   Objective: Determination of molecular weights of compounds/analytes separated by liquid chromatography in LC-MS system with In-source fragmentation.

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



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.

LC-MS/MS Instrument

Developed for integrated HPLCMS/MS qualitative and quantitative applications, the **Waters ACQUITY® TQD features** the highest levels of **tandem quadrupole** MS selectivity, robustness, speed, and accuracy.

Specification: **Waters Acquity TQD LC/MS/MS System equipped with Waters Alliance HPLC System.**

1.ZSprayTM dual-orthogonal API source
2.Mass scan range: *m/z* 50-2000 Th
3.Acquisition speed: 10 scan/second
4.Polarity switching(ES+/ ES-): 20 ms
5.ESCi mode switching: 20 ms
6.MRM Sensitivity : 1 pico gram (Reserpine S/N 2000:1)

LC-MS SAMPLE SUBMISSION REQUIREMENTS

1. Sample Quantity & Quality

- Submit **5–10 mg** of clean, properly prepared **solid or lyophilized sample**.
- Samples must be free from **salts, detergents, polymers, and other MS-incompatible contaminants**.

2. Buffer & Solvent Compatibility

- Avoid high concentrations of **non-volatile buffers**, particularly **phosphate and Tris**.
- Use **LC-MS-grade solvents and reagents** wherever applicable.

3. Sample Identification

- Clearly label every sample with a unique **Sample ID / Code**.
- The **same Sample ID** must appear on both the **submission form and sample container**.

4. Sample Container

- Submit samples in **properly sealed, clean, and compatible vials/tubes**.
- Prevent leakage, evaporation, and contamination during transport.

5. User Responsibility

- Users are responsible for providing **accurate and complete sample information**.
- Inadequately labelled, contaminated, or improperly prepared samples may be **returned for re-preparation**.

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SOP – PREPARATION OF PLANT EXTRACTS

PROTOCOL – I: 70% ETHANOL EXTRACTION & LIQUID–LIQUID FRACTIONATION

MASS SPECTROMETRY & PHYTOCHEMISTRY

STARTING MATERIAL
250 g DRY WEIGHT PLANT MATERIAL

PLANT PART USED
Root / Leaves / Stem / Seeds / Bark / Whole plant / Flower

- Dry completely
- Pulverize to coarse/fine powder
- Store in clean, dry, airtight container

1. EXTRACTION WITH 70% ETHANOL

1. Transfer 250 g powdered plant material into extraction vessel.
2. Add 2.5 L of 70% ethanol (1:10, w/v). Mix thoroughly.
3. Macerate for 24–48 h at room temperature with occasional stirring/shaking.
4. Filter through suitable filter paper or Büchner funnel.
5. Repeat extraction 2–3 times with fresh 70% ethanol. Combine all filtrates.
6. Concentrate combined extract under reduced pressure (Rotary evaporator) at ≤40 °C.
7. Remove residual solvent under vacuum, if required.
8. **Aq-EtOH CRUDE EXTRACT** Record weight and calculate extraction yield.
9. Extraction yield (%) = (Weight of dried crude extract (g) / 250 g plant material) × 100

2. PREPARATION OF Aq-EtOH CRUDE EXTRACT

- Dissolve/suspend the dried crude extract in minimum volume of water or aqueous ethanol.
- Transfer to a separatory funnel.
- Ensure the solution is sufficiently aqueous for efficient partitioning.

3. LIQUID–LIQUID EXTRACTION

Partition successively with solvents of increasing polarity

Aq-EtOH Crude Extract → n-Hexane Fraction → Chloroform / DCM Fraction → Ethyl Acetate Fraction → Aqueous Fraction

1. Add appropriate volume of solvent to separatory funnel.
2. Shake gently with frequent venting.
3. Allow layers to separate completely.
4. Collect organic layer.
5. Repeat extraction 2–3 times with fresh solvent.
6. Combine organic layers.
7. Dry over anhydrous sodium sulfate (as appropriate).
8. Filter and concentrate under reduced pressure at suitable temperature.
9. Record weight of dried fraction.
10. Store fractions in labelled containers at ≤4 °C, protected from light.

4. FRACTION LABELLING

Fraction	Suggested Code
70% EtOH crude extract	Aq-EtOH-CE
n-Hexane fraction	HEX
Chloroform/DCM fraction	DCM/$CHCl_3$
Ethyl acetate fraction	EtOAc
Remaining aqueous fraction	AQ

5. DOCUMENTATION (Record for each batch)

- Plant name & voucher/specimen no.
- Plant part used
- Collection location & date
- Starting dry weight (250 g)
- Solvent & solvent volume
- Number of extraction cycles
- Crude extract weight
- Weight of each fraction
- % Yield of crude extract & fractions
- Batch/Extraction number
- Date & operator initials

SAFETY FIRST

Perform all solvent extraction and concentration in a fume hood.
Follow institutional chemical safety procedures.
Consult SDS for each solvent before use.

Eye Protection | Hand Protection | Lab Coat | Use Fume Hood

Maintain clean records for reproducibility and quality assurance – Essential for Phytochemical & Mass Spectrometry Workflow.

Protocol I: Preparation of 70% Ethanol Extract and Liquid–Liquid Fractions

1. Starting Material

Plant material: 250 g dry weight

Plant part used: Root / Leaves / Stem / Seeds / Bark / Whole plant / Flower

1. Dry the plant material completely and record the **dry weight (250 g)**.
2. Pulverize the material to a coarse/fine powder using a suitable grinder.
3. Store the powdered material in a clean, dry, airtight container until extraction.

2. Extraction with 70% Ethanol

Solvent: 70% EtOH in water (v/v)

1. Transfer **250 g powdered plant material** into a suitable extraction vessel.
2. Add approximately **2.5 L of 70% ethanol** (1:10, w/v).
3. Mix thoroughly and keep for **24–48 h** at room temperature with occasional stirring/shaking.
4. Filter through suitable filter paper or a Büchner funnel.
5. Repeat the extraction **2–3 times** with fresh 70% ethanol to maximize extraction.
6. Combine all filtrates.
7. Concentrate the combined extract under reduced pressure using a rotary evaporator at a temperature preferably **≤40 °C**.
8. Remove residual solvent under vacuum, if required.
9. Record the weight of the resulting **Aq-EtOH crude extract** and calculate the extraction yield.

3. Preparation of Aq-EtOH Crude Extract

1. Dissolve/suspend the dried crude extract in a minimum suitable volume of **water or aqueous ethanol**.
2. Transfer the solution/suspension to a separatory funnel.
3. Ensure that the extract is sufficiently aqueous to permit efficient liquid–liquid partitioning.

4. Liquid–Liquid Extraction

Partition the aqueous extract successively with solvents of increasing polarity.

Suggested sequence:

Aq-EtOH crude extract → n-Hexane fraction → Chloroform/DCM fraction → Ethyl acetate fraction → Aqueous fraction

For each solvent:

1. Transfer the aqueous extract into a separatory funnel.
2. Add an appropriate volume of the selected organic solvent.
3. Shake gently with frequent venting.
4. Allow the layers to separate completely.
5. Collect the organic layer.
6. Repeat the extraction **2–3 times** with fresh solvent.

7. Combine the corresponding organic layers.
8. Dry the organic fraction over anhydrous sodium sulfate, where appropriate.
9. Filter and concentrate under reduced pressure at a suitable temperature.
10. Record the weight of each dried fraction.
11. Store fractions in appropriately labelled containers, preferably at **4 °C or below**, protected from light.

5. Fraction Labelling

Fraction	Suggested Code
70% EtOH crude extract	Aq-EtOH-CE
n-Hexane fraction	HEX
Chloroform/DCM fraction	DCM/$CHCl_3$
Ethyl acetate fraction	EtOAc
Remaining aqueous fraction	AQ

6. Documentation

For every extraction, record:

- Plant name and voucher/specimen number
- Plant part used
- Collection location and date
- Starting dry weight: **250 g**
- Solvent and solvent volume
- Number of extraction cycles
- Crude extract weight
- Weight of each solvent fraction
- Percentage yield of crude extract and fractions
- Batch/extraction number
- Date and operator initials

Safety: Perform solvent extraction and concentration in an appropriate fume hood. Follow institutional chemical-safety procedures and consult the SDS for each solvent before use.