Mass Spectrometry Facility

DIVISION OF BIOLOGICAL SCIENCES
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Date: _____

Name: _____ Supervisor/ PI: _____

E-mail ID:_____Phone No. _____

Affiliation:_____

Solubility of sample:_____No. of samples: _____

Sample Label:_____(please write behind the form for more number of samples)

Quantity:_____Concentration:_____Storage condition: _____

Objectives and Sample preparation procedure (reference paper if any) briefly:

LCMS	☐	GCMS	☐	MALDI	☐
Orbitrap	☐	Liquid	☐	GC FID	☐
Q-TOF	☐	Headspace	☐	GC ECD	☐
		SPME	☐		

Fill up and submit this form only after successful E-mail/ Phone confirmation from msfacilityiisc@gmail. com.
Debit Head (IISc Users) _____

For External Users:
Billing address: _____
GST number:_____Transaction ID Number: _____
To streamline the accounting process, we request that you utilize the NEFT/RTGS transactions or UPI payments for the payment of usage charges.

_____ Signature of the user

_____ Signature of the supervisor

Important Notes:

1. Samples will not be processed, if any of the above column and check list (next page) is not filled.
2. Please provide relevant LCMS/ GCMS/ MALDI reference paper similar to samples submitted.
3. The sample submission dates should be informed in advance after successful E-mail/ Phone confirmation from MS team (to allot the slots).
4. The slots and pricing are as of "December 2021" and will be revised periodically.
5. Please refer appendices page for detailed information on sample submission.
6. Samples will be discarded after the analysis.
7. Allowed timings for sample submission & brief discussion on samples are 12.30pm-1.30pm (for IISc users), 2.30pm–3.30pm (for External users) only.
8. Except trypsin digestion (for external users only), no other sample preparation/isotopic labelling services are available.
9. Please refer attached representative paper under each application category in appendices page or research on best LCMS/ GCMS/ MALDI article for sample preparation methods.
10. Please refer page number 3 for contact and sample submission address.

Check list:

GC-MS/ MALDI/ LCMS:

1. Sample concentration minimum 0.1mg (lyophilized form)/ 0.1mg/mL with 100µL (liquid form).
2. Storage temp.
3. Reference paper related to sample
4. Solubility
5. Derivatization (for GC-MS only)
6. No water content in sample (for GC-MS only)
7. Solubility in organic solvents (for GC-MS only)
8. Zip Tip/ Filtration
9. Non-volatile additives
   (If present specify additive & its percentage)
10. Proteomics sample information
    (Organism, staining used, complexity of sample, enzyme used for digestion, etc.)
11. Expected Mass (for MALDI samples)

Please note:
Next pages (3 and 4) are for user reference only, no need to take print for submission.

Guidelines for sample Submission:

LCMS/MALDI samples:

1. Sample concentration should be 1 to 0.1mg (lyophilised powder form), if liquid 1 to 0.1mg/ Ml with min. 100µL volume.
2. For sample work up and purification compatible solvents are water, ammonium hydrocarbonate, ammonium acetate, ammonium formiate,0.1% trifluoroacetic acid, 0.2% formic acid, methanol, acetonitrile, ethanol etc.
3. Avoid the use of non-volatile agents like salts (NaCl, CaCl2, KH2PO4), detergents (Tween, Triton, SDS), chaotropic agents (Urea, Guanidinium salts) and non-volatile solvents like DMSO, DMF, or Glycerol.
4. If you can't avoid these agents, purify. Dialysis, Zip Tips, and RP-HPLC are good purification methods. After purification, lyophilize if possible. Ion exchange beads may work well for salt removal.
5. Use highest purity reagents available to prepare samples for mass spectrometry
6. Look for any undissolved particles in your sample solution. If not sure, filter through 0.22 um filters
7. Research and attach reference paper on best LCMS/GCMS/HPLC/ MALDI method for your sample
8. Submit concentrated sample if possible (we can always dilute it if necessary)
9. For proteomics samples, please provide relevant information (Enzyme used/ Organism/ specific protein sequence/ modifications etc.

GCMS Samples:

1. Samples should be Volatile and Thermally Stable below 300deg.
2. Solvent extracts like DMSO, DMF & H2O are not acceptable without derivatization (mention suitable derivatization reagent)
3. Sample concentration/ volume should be 1 to 0.1mg (lyophilized powder form), if liquid 1 to 0.1mg/ mL with min. 100µL.
4. For unique derivatization, reagent should be provided by user.

Reasons for sample rejection:

1. No labels or illegible labels on samples.
2. Illegible handwriting on sample submission form or incomplete form
3. Sample forms turbid, insoluble or colloidal suspension or in-homogeneous solution in the user-advised solvent
4. Lack of detailed sample preparation information [please provide relevant LCMS/ GCMS/ MALDI reference papers similar to samples submitted).

Data analysis:

LCMS:

Proteomics:

List of Proteins will be provided by searching against in-house MASCOT/ Proteome discoverer software's/ server or MGF files of proteomics data can be sent to users

Metabolomics:

1. Bruker Impact HD-Q-TOF: MS chromatogram and mass spectral (MS and MS2) data will be provided along with demo version of Bruker compass data analysis software with manual compound search training video.
2. Thermo Orbitrap Fusion: List of compounds will be provided using Compound discoverer software (searching against comprises thermo metabolites library and open metabolomics data base)

GCMS:

List of compounds will be provided by using Agilent's mass hunter/ AMDIS software against NIST library for metabolites (version 2017).

Data storage:

The raw data with any available analyzed results will be sent to the user right after the analysis. The users are advised to keep the raw data and all other results with them safe. The facility doesn't provide any guarantee of safe storage of the data. Data will be stored for a maximum of 2 years in the operating computer. After this period, it will be removed from the storage without prior intimation.

Charges:

There is no provision for discounts or concessions.
If sample has to be repeated, same charges will apply (with additional slot booking).

Contact/ Sample submission address:

Mass Spectrometry Facility,
Room No: FC02/ 03, 1st Floor, C Wing,
BC department, New Biological Science building,
Indian Institute of Science, Bangalore-560012.
Ph.: 080 2293 3053.
Email: msfacilityiisc@gmail.com

Appendices page:

Services available at MS Facility, IISc

Liquid Chromatography Mass Spectrometry (LC-MS):

A. Molecular Weight Determination:

1. **Intact Protein:** (Turnaround Time - 2 days)
   - LCMS run (30-45 min) for single/ mid complex of proteins using intact protein column

   Result:
   - Deconvoluted data with list of proteins
2. **Small molecules:** (Turnaround Time - 2 days)
   - LCMS run using trap column (10-15 min) for single purified compounds only (mixtures will not be resolved)

B. Bottom-up Proteomics: (Reference paper link)

3. **Tryptic digest of few/complex mixture of proteins:** (Turnaround Time - 3 days)
   - LCMS run of tryptic digest using reverse phase column

   Result:
   - List of identified proteins or mgf file

C. Metabolomics

4. **Qualitative:** (Turnaround Time - 4 days) (Reference paper link)
   - LCMS run of sample extract using reverse/ HILIC column

   Result:

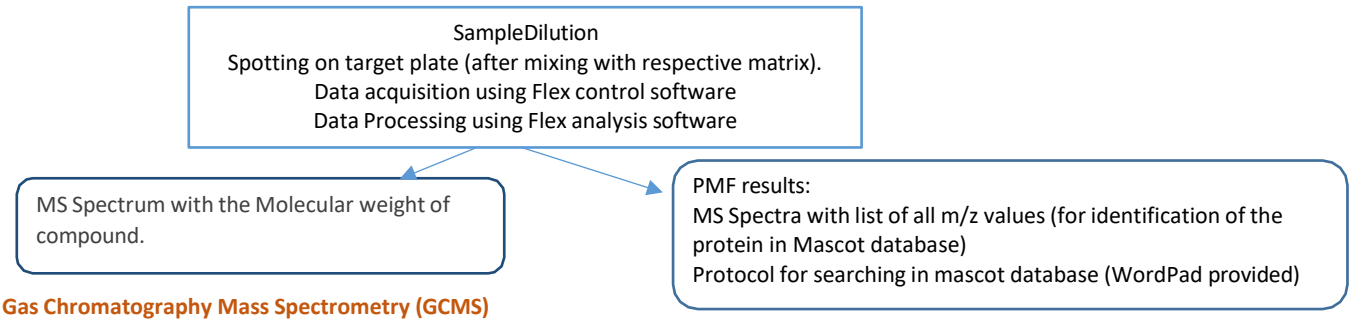
   Identification of compounds manually (using ms /msms data) for Bruker Q-TOF and instruction video for compound search will be provided.

   List of identified compounds provided (using thermo compound discoverer software) for Thermo Orbitrap fusion
5. **Quantitative:** (Turnaround Time - 5 days) (Reference paper link)
   - LCMS standards run for LOQ/ LOD determination
   - Calibration curve generation (minimum 5 dilutions)
   - Compound concentration determination in sample
   - (Internal standard should be as mentioned in referred paper)

   Result:
   - Calibration curve and sample concentration in excel format

Matrix Assisted Laser Desorption Ionization (MALDI):

1. Molecular weight determination (Proteins, Peptides, Polymers, Oligonucleotides, peptide mass fingerprinting (PMF), etc...) (Turnaround Time - 2 days)



Gas Chromatography Mass Spectrometry (GCMS)

1. **Qualitative: (**Turnaround Time - 3 days) (Reference paper link)
   - Sample dilution
   - Acquisition with given/ referred method
   - Analysis with Mass hunter & NIST database
   - List of compounds with chromatogram, peak area percent will be sent through mail.
2. **Quantitative: (**Turnaround Time - 5 days) (Reference paper link)
   - GCMS standards run for LOQ/ LOD determination
   - Calibration curve generation (minimum 5 dilutions)
   - Compound concentration determination in sample
   - Refer attached paper/discuss with MS team
   - (Internal standard should be as mentioned in referred paper)

   Result:
     - Calibration curve and sample concentration in excel format

Note:

1. Turnaround time mentioned above is for ≤5 samples (for similar type of samples).

2. Turnaround time will vary depending on sample types.