

Handbook for users of UCL Chemistry Mass Spectrometry

Service General

UCL Chemistry Mass Spectrometry Facility is located in UCL Chemistry Department, LG11. Please fill the submission form which found on <https://www.ucl.ac.uk/chemistry/mass-spec>

Place samples in the trays provided outside of the mass spec lab, LG11.

If you have requested that samples be returned after analysis, this is also where you pick them up.

(Please note: **If samples have not been collected within 2 weeks they will be disposed of**).

Telephone number for the service is 0207 679 4605.

Instruments

The service currently has 5 mass spectrometers:-

Thermo MAT900: This instrument is a magnetic sector mass spectrometer and has Electron Ionisation (EI), Chemical Ionisation (CI) ion sources with an insertion probe for a sample loading. Mass measurements accurate to <5ppm can be carried out on this instrument.

Micromass LCT Premier: This instrument has an Electrospray (ESI) ion source, coupled to a time-of-flight (ToF) analyser, which can be operated in V- or W- reflectron modes. Mass measurements accurate to <5 ppm can be carried out on this instrument with a direct infusion of a sample. A Waters 1525 LC pump is used to deliver the mobile phase to the source, and samples are selected and injected using a Waters 2777 autosampler.

Micromass MALDI-TOF: This instrument has a laser desorption (MALDI) ion source, coupled to a time-of-flight (ToF) analyser, which can be operated in either linear or reflectron modes. This instrument is very simple to operate, and users with sufficient demand can be trained to prepare and run their own samples.

Waters UPLC-MS: An Acquity Ultra Performance LC Waters system connected to a SQ Detector also from Waters. Samples injected through the autosampler and components of the mixture separated on a reversed-phase column and the effluent direct a single quadrupole mass spectrometer. The instrument could be used by staff/students after the training. If you have not used the instrument for more than 3 months follow-up training is necessary.

Accela LC- Finnigan LTQ: An Accela 1100 HPLC system is coupled to a Thermo Finnigan LTQ two-dimensional ion-trap mass spectrometer. This system provides capillary LC-MS and -MSⁿ analyses in both electrospray (ESI) and atmospheric pressure chemical ionisation (APCI) modes with mass range m/z 100 - 2,000.

Sample Submission

Samples should be attached to a completed copy of the current submission form, and labelled with a legible reference which must be the same as that specified on the form. Samples should not be submitted in tubes greater than 12.5 mm in diameter, nor in crimp top autosampler vials, nor in NMR tubes.

The vials and caps are supplied by UCL Chemistry Mass Spectrometry Facility. Please collect them from LG11.



Ideally, samples should be submitted neat (not in solution), and a suitable solvent for the compound must be given on the form.

An approximate sample quantity should also be given.

If submission of a sample in solution is unavoidable, the concentration and solvent used should be given on the form.

The final dilution of samples for ESI will normally be done using acetonitrile or methanol; and for EI/CI analyses DCM.

A freezer is available for storage of thermally unstable samples.

It is important that samples submitted for ESI do not contain any traces of trifluoroacetic acid (TFA).

Even at very low levels, TFA causes severe reduction in sensitivity in an ESI source, especially in negative ion mode.

Preferred solvents are those which are least polar and most volatile. DCM, ether, acetone, methanol, THF, ethyl acetate and acetonitrile are all acceptable.

Solvents such as water, DMF and DMSO can be used if necessary but are best avoided if possible.

Sample submission form

The form should be filled in as fully as possible.

In particular, it is essential that a suitable solvent be specified for the sample if the sample is not in solution.

If any service requirement is not selected, defaults are used. These are indicated on the form.

Some idea of the sample quantity or concentration is also useful, particularly when samples are submitted for Electrospray (ESI).

Five ionisation methods are available; some notes on how to select an appropriate method are given below. There is also an option "No preference". See below for notes about this option.

Samples will not be returned by default. If remaining sample is wanted, the "Sample to be returned" box on the form must be checked.

Results

If the "Email results" box is checked, a PDF file of the results will be sent to the user, and no printed output produced

If the "Email results" box on the form is not checked, then printed output will be produced and placed on the same table as used for sample submission.

If the "Email results" box is checked and the sample is to be returned, it will be placed in the tray labelled "Returned Samples", next to the trays where samples are submitted.

Samples not collected after 2 weeks will be disposed of.

The sender name on all emailed results will be "Mass Spectrometry Service", and the subject line will include the name of the attached file.

However, if any comment on the result is necessary, it will be written in the body of the email. Any enquiries should be sent to the service manager.

If you have not received the results within 5 days please enquire to UCL Chemistry Mass Spectrometry Facility (020 7679 4605) on a progress for your sample analysis.

Ionisation Methods

The following five ionisation methods are available:-

Electrospray Ionisation (ESI)

Principles

In electrospray ionisation (ESI) a fine spray of charged droplets is created by the application of a high voltage (typically 1-3 kV) to a capillary containing a stream of solvent (the mobile phase). The process is assisted by use of a heated co-axial nebuliser gas, such as nitrogen. The charged droplets - expelled from the tip of the capillary - evaporate until the Rayleigh limit is reached (this is the point at which the Coulomb repulsion equals the surface tension). Beyond this limit the droplets are unstable and explode to form micro-droplets. This process is repeated until individual solvated ions are formed. Evaporation of the solvent results in the generation of isolated gas-phase ions.

Electrospray ionisation occurs at atmospheric pressure, and the ions formed in positive mode are of the form $[M+H]^+$ and $[M+Na]^+$ ions being observed. Dimers of the form $[2M+H]^+$ and $[2M+Na]^+$ can also be formed.

Ions of the form $[M+NH_4]^+$ are sometimes observed, notably if ammonium buffers have been used in the sample preparation.

Solvent adducts can also be formed with acetonitrile, resulting in peaks $[M+H+MeCN]^+$ and/or $[M+Na+MeCN]^+$.

Solvent adducts can also be formed with methanol, resulting in peaks $[M+H+MeOH]^+$ and/or $[M+Na+MeOH]^+$.

Larger molecules can form multiply-charged ions, of the form $[M+nH]^{n+}$

The ions formed in negative mode are most commonly of the form $[M-H]^-$. Negative ion adducts with sodium $[M-H+Na]^-$ and formate $[M-H+HCO_2]^-$ are also sometimes formed.

Samples are introduced by injection of about 10 μ L of a very low concentration (about 1ng/L) solution into the mobile phase.

If LC-MS is required, the sample solution is passed through an LC column before entering the source.

The mobile phase used is usually 50:50 water (0.1% formic acid):methanol.

Applications

- ESI is suitable for the analysis of **organic compounds with medium - high polarity**.
- Since positive ionisation is dependent on protonation, molecules containing basic functional groups work well in this mode.

- Negative ionisation, in contrast, functions by deprotonation, thus the presence of acidic functional groups is a prerequisite for production of negative ions. Most relatively small organics are amenable to ESI providing they contain sufficient functionality.
- In general, compounds containing amino, amide, ester, aldehyde/keto or hydroxyl functional groups are suitable for positive ion ESI, and compounds containing carboxylate, hydroxyl/phenol or imide functional groups are suitable for negative ion ESI.
- **Accurate mass measurements** can be carried out on using ESI up to a mass of ~1,400 Da. Such measurements are carried out under the same instrument conditions as for nominal mass spectra, so that a nominal mass spectrum does not need to be run first.
- A full range spectrum with all the ions accurately mass measured is generated. It is strongly recommended that, if the primary purpose in submitting the sample is to confirm the elemental formula by accurate mass measurement, then electrospray is made the first choice of ionisation method.
- Since electrospray is carried out on a dedicated instrument, and a separate spectrum does not need to be acquired before an accurate mass measurement is made, turnaround time can be very short.
- Almost all samples submitted for electrospray are run within two-three days of submission.

Electron Ionisation (EI)

Principles

Electron ionisation (EI) is the classical ionisation method in mass spectrometry. Sample molecules in the gas phase interact in the ion source with electrons at an energy of 70eV.

Some of the molecules are ionised by removal of an electron, extracted from the source by a high voltage and separated in the mass analyser of the mass spectrometer.

The ions formed are radical cations of the form M^+ . The internal energy of the ions created is relatively high, and so some compounds may not yield molecular ion information under these conditions.

Samples are introduced into the source in a small crucible, which may then be heated to a temperature from 200°C up to 300°C in order to volatilise the compound.

Applications

- The advantages of EI are the production of diagnostically useful fragment ions for structure elucidation and highly reproducible spectra, permitting spectral database searching.
- EI is suitable for a wide range of small (MW < ~1,200) organic compounds.

- The likelihood of observing a molecular ion in general decreases with increasing saturation, especially if heteroatoms are present.
- If a molecular ion is not observed using EI, one of the "soft" ionisation techniques described can be used.
- Since electron ionisation is a gas phase technique, it may not be suitable for very volatile, polar or thermally labile compounds.
- Accurate mass measurements can be carried out on individual ions using EI up to a mass of ~ 900 Da, although since these measurements are carried out under different conditions to those used to record nominal mass spectra, a nominal mass spectrum must have been run first to ensure that the ion which is to be measured is observable.
- **GC-MS** can also be carried out using EI, though accurate mass measurement is not possible.

Chemical Ionisation (CI)

Principles

In this technique, a reagent gas is introduced into the ion source and ionised by an electron beam. This results in various reagent gas ions being formed in the source. The sample is then introduced into the source in the same way as for EI, and undergoes ion-molecule reactions with the reagent gas ions.

The most common ionisation mechanisms which occur are proton transfer, in which a compound with a proton affinity greater than that of the reagent gas is ionised to form a $[M+H]^+$ ion. Electrophilic addition, in which a cation produced by the ionisation of the reagent gas attaches to the sample molecule can produce ions of the form $[M+NH_4]^+$ ions from many compounds when ammonia is used as the reagent gas.

Both these mechanisms create ions with less internal energy than is the case in EI, resulting in less fragmentation, and a more intense molecular ion.

The departmental service usually uses methane and ammonia as the reagent gas for CI.

Applications

- CI can be used for small ($MW < \sim 1,200$) organic compounds which do not yield molecular ion information using EI, and for which such information is required.
- Accurate mass measurements can be carried out on individual ions using CI up to a mass of ~ 800 Da, although since these measurements are carried out under different conditions to those used to record nominal mass spectra, a nominal mass spectrum must have been run first to ensure that the ion which is to be measured is observable.
- **GC-MS** can also be carried out using CI, though in general EI is to be preferred for GC/MS.

Matrix Assisted Laser Desorption Ionisation (MALDI)

Principles

In this technique, the analyte is dissolved, mixed with a solution of a matrix material, and this mixture is deposited on a target, where it is dried down to a solid. Pulsed laser energy in the UV range (337 nm) is then directed at the target to desorb sample molecules from the matrix. The main function of the matrix in MALDI is to absorb energy at the wavelength of the laser, and thus transform the laser energy into excitation energy leading to ablation and ionisation. Ions observed are of the form $[M+H]^+$, with $[M+Na]^+$ or $[M+K]^+$ ions also observed.

Typical matrixes used are alpha-cyano 4-hydroxy cinnamic acid for peptides, proteins and large organic compounds and dithranol (1,8,9-anthracenediol) for synthetic polymers. Polymers often give enhanced spectra with ions of the form $[M+Na]^+$ if sodium trifluoroacetate is added to the sample/matrix mixture.

Applications

- The major application of MALDI is the analysis of large molecules, particularly proteins, peptides and synthetic polymers, although many large (MW >1,000) organic and organometallic compounds are also amenable to the technique.
- MALDI should be the first method of choice for compounds with molecular weights greater than ~1,000. It is not suitable for compounds with molecular weights less than 500.
- Accurate mass measurement for the purpose of elemental composition determination is not possible with MALDI, although in the mass ranges for which MALDI is generally used, such measurements have little meaning.

“No preference” ionisation

If this option is selected, the service manager will select an ionisation method appropriate to the type of compound. This is useful if you are analysing your sample for the first time and we have not established which ionisation method is suitable for your compound.

It can also be specified if your main aim in submitting the sample is confirming the elemental formula of your compound by accurate mass measurement of the molecular ion, and you do not mind how the sample is ionised.

Since many organic compounds can be ionised using more than one method, the decision can be made partly on the basis of instrument availability and backlogs.

Mass Measurement Two types of mass measurement are available:-

Nominal Mass Measurement

In this mode of mass measurement, masses are reported as integer values.

For example, the molecular ion from methyl stearate ($C_{19}H_{38}O_2$) would be reported as

$$(19 \times 12 + 38 \times 1 + 2 \times 16) = 298.1$$

Accurate Mass Measurement

Here, sample ions are measured against ions of known m/z value from a reference present in the source with the sample.

Using the example of methyl stearate as above, the exact mass of the molecular ion is

$$(19 \times 12.00000 + 38 \times 1.007825 + 2 \times 15.994915) = 298.2872.$$

Accuracy is typically better than 5 ppm.

Note:- that this value is the monoisotopic mass of the compound, not its molecular weight.

- Elemental composition calculations are supplied for ions accurately mass measured.
- For ESI, accurate mass measurements are carried out on the full range of the spectrum, so that a nominal mass spectrum does not need to be run first.
- A full spectrum will be produced with all the ions accurately measured.
- Instrument conditions are fixed, and do not have to be changed between acquiring nominal or accurate mass spectra.
- This means that sample turn-round time is the same regardless of which type of mass measurement is required.
- For EI, and CI accurate mass measurements are carried out on specific ions under different instrument conditions to those used for recording full spectra, so that a nominal mass spectrum needs to be run first to ensure that the ion of interest is present.

Gas Chromatography - Mass Spectrometry (GC-MS)

The instrument is fitted with a Trace 1300 gas chromatograph and a TriplePlus RSH autosampler and connected to an ISQ single quadrupole mass spectrometer. Due to the requirements for a sample development method, GC-MS samples are normally run overnight.

The GC column normally used is a TR-5MS universal, 30 m id 0.25 mm, 5% Phenyl Polysilphenylene-siloxane. This column is equivalent and is suitable for a wide range of compounds. Helium is used as the mobile phase.

Either EI or CI ionisation methods may be used with GC-MS, although EI is greatly to be preferred. Accurate mass measurement is not possible with GC-MS.

Liquid Chromatography / Mass Spectrometry (UPLC/MS)

UPLC-MS can be carried out on the Acquity Ultra Performance LC Waters system connected to a SQ Detector also from Waters, using ESI at the ionisation method. The LC column normally used is a Thermo Hypersil GOLD, C4 reverse-phase column of length 50mm, inner diameter 2.1mm and particle size 1.9 μ . Mobile phase is water (0.1% formic acid in water and 95% acetonitrile-5% water. 0.1% formic acid.

Note that since electrospray ionisation (ESI) is the ionisation method used for LC-MS, compounds which do not ionise under ESI conditions will not be detectable using LC-MS.

Use of LC-MS should be confined to high molecular weight, thermally unstable or polar compounds which are not suitable for GC-MS.

Unlike GC-MS, accurate mass measurement is possible with an Agilent LC connected to a 6510 Q-TOF mass spectrometer.

Limitations

There are certain limits both on mass ranges and the combinations of techniques which are possible.

The limitations are as follows:-

- Accurate mass measurements cannot be carried out in CI mode on masses above 800 Da, and in some circumstances not even as high as that.
- For EI mode the limit is closer to 900 Da, but, as noted above, electrospray ionisation (ESI) should be the method of choice for accurate mass measurement, and this is particularly true if the ion to be measured has a mass greater than ~500 Da.
- The upper limit on accurate mass measurement using ESI is 1,200Da, although accurate mass measurement becomes an increasingly meaningless exercise with increasing mass above about 1,000 Da. This is because the increasing number of possible elemental formulae makes it impossible to assign an unambiguous formula to a measured mass.
- Accurate mass measurement cannot be carried out at all using MALDI, although in the mass range at which MALDI is most useful, the comment above about accurate mass measurement of high masses holds true.
- The LCT Premier instrument on which all ESI is carried out will not observe ions with masses less than ~100 Da. This is not a limitation of the ESI technique, but of the type of mass analyser.
- Negative ion EI is not possible. This is not an instrument limitation, but a reflection of the fact that stable negative ions are not generated in EI.
- Negative ion CI is possible, but the method of choice for negative ion work should be ESI (or MALDI for higher masses).
- If GC-MS is required, the only ionisation methods available are EI or CI.
- EI is the preferred option, particularly if identification of unknown compounds is required.
- Accurate mass measurement is not possible on GC-MS runs.
- Spectral database searching is only available for EI spectra.
- If LC-MS is required, the only ionisation method available is ESI.

Other facilities available from the service are as follows:-

Library Search

The NIST02 mass spectral database is installed on Thermo GC-MS computer. This database contains 175,214 EI spectra.

The database may be searched for a match with any EI spectrum acquired.

This is routinely carried out on compounds detected during GC-MS runs.

In addition, if an EI spectrum of a known compound is required, the database can be searched by a range of criteria, such as compound name, formula, molecular weight, CAS number, etc.

Theoretical Isotope Distribution Patterns

The service has facilities for calculating theoretical isotope distributions for any combination of elements.

There is, however, a good free program for doing this available for download from <http://www.wsearch.com.au>

Elemental Composition Calculations

Calculations of possible elemental compositions may be carried out for any given mass. These calculations are supplied routinely with accurate mass results, but may be carried out on any given mass at any time.

A free program for carrying out such calculations is available for download from <http://www.wsearch.com.au>

Output in electronic format: Spectra (or any other output) can be supplied in a range of graphics formats, for inclusion in theses or reports. The default format for results sent by email is Portable Document Format (PDF).

Data Archiving

Data acquired on all instruments are archived, and may be retrieved at any time.

Data are stored on Chemistry Network <http://storage.chem.ucl.ac.uk/ms>

Data acquired from 2006 are also still available.

Charges

A charge is levied on all work carried out by the service.

Charging is carried out monthly against the account of whoever is named as "Supervisor" on the form.

If no supervisor's name is given, the sample will not be run.

Charges for samples submitted from the Chemistry Department are as follows:

Nominal Mass spectrum:	£7.00
Accurate Mass measurement:	£10.00
GC-MS run:	£20.00 per hour or £7 per sample
LC-MS run:	£20.00 per hour

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