

## Mass spectrometry laboratory General Instructions

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### General instructions for Lab YO309 users

Follow precisely the instructions given here, and the most recent user guides of the instruments. Most recent versions can be found in JYU Infrabooking. **If instructions are not followed or any damage is caused to instruments, the permission to use MS laboratory and its instrumentation will be cancelled.**

The samples should be prepared by using the solvents and consumables found in lab YO309. Other consumables and vials might contain impurities and extractables that can contaminate the instruments. All samples have to be filtered prior the HPLC analysis to protect the columns.

To avoid contaminations, keep the laboratory clean. Leave clean equipment and tidy fume hood for the next user. Wash your own dishes and dispose samples and consumables that are for single use.

Lab O309 has air conditioning to ensure stable temperature for all instruments. Changes in the temperature affect to the performance (calibration and mass accuracy) of the instruments. For this reason, it is important to keep the lab doors closed.

**All persons working in the MS laboratory must read and sign the Organic Chemistry SOP (ORG-SOP) and pass the MS Lab induction (MS-USER, given by Lab Operator).** The above is necessary also if you use laboratory for preparation of the samples. Only persons with appropriate training (instrument specific training) can use the MS instruments independently. To request training, contact Lab Manager Elina Kalenius, [elina.o.kalenius@jyu.fi](mailto:elina.o.kalenius@jyu.fi) or send an email to [jyu-mslab@jyu.fi](mailto:jyu-mslab@jyu.fi).

### Price list

To cover the costs of consumables used for sample preparation, a small fee is charged from each sample.

	Independent use	Service by operator
JYU Chemistry department users	3 €	4 €
JYU Other departments (research collaboration)	5 €	7 €

For external and service measurements, ask quotation from Lab Manager ([elina.o.kalenius@jyu.fi](mailto:elina.o.kalenius@jyu.fi)).

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## MS and Chromatography instruments available in MS laboratory

Details about the instruments are shown in JYU Infrabooking (<https://infrabooking.jyu.fi/>).

<b>Agilent 6530 UHPLC-QTOF MS</b>	ESI-QTOF MS equipped with UHPLC inlet and UV detector. High-resolution mass spectrometer capable for < 1ppm mass accuracy and MS/MS experiments (targeted, all ions). Mainly used for accurate mass and HPLC-MS measurements for organic synthesis products (no metals allowed).
<b>Agilent 6460 UHPLC-TQ MS</b>	ESI-TripleQuad MS equipped with UHPLC inlet and UV detector. Used for targeted analysis of small molecules.
<b>Agilent 6560 ESI-IM-QTOF MS</b>	Ion mobility mass spectrometer for structural chemistry studies for biomolecules, supramolecules and metal coordination compounds as well as to HR-MS, IM-MS and MS/MS experiments.
<b>Agilent 1260 Infinity II SFC/UHPLC</b>	Chromatographic instrument for SFC and HPLC chromatography equipped with DAD detector.
<b>ThermoScientific Orbitrap OE 120 Vanquish UHPLC</b>	Ultra-high resolution Orbitrap mass spectrometer equipped with Vanquish UHPLC and DAD detector. Used for untargeted and targeted analysis.

## Safety instructions

Protective gloves, protective goggles and laboratory coat must always be used when preparing samples. Sample preparation takes place only in fume hood. The safety glass of the fume hood is kept as low as possible while working, and after the use safety glass is closed immediately. Each user must take care of disposing samples properly. The sample solutions are poured into an appropriate waste container and the used consumables (vials, Eppendorf tubes, pipette tips) are left in the fume hood to evaporate before disposal. The laboratory has an eye wash and an emergency shower in case of chemical spills/accidents. Please also check that you know the locations of fire extinguisher, fire blanket and the nearest first aid kit. More detailed safety information is available at organic chemistry SOP and during MS lab user induction (MS-USER).

When using syringe needles, take care of correct disposal of the needles. The needles are disposed to the container of the sharp waste, so that they do not cause a danger to other users. The needles must be placed in the waste container so that they point inside of the container.

Non-harmful gases are used in the laboratory. However, these can be life-threatening if they erupt uncontrollably and displace oxygen. If you notice an uncontrolled gas release, leave the laboratory immediately. The nearest exits are through the corridor or through the student laboratory.

## Internal service and ordering MS analysis from operator:

**All users of the MS lab must have passed the MS-lab induction (MS-USER). This applies also to persons preparing samples for service analysis.** Mass spectrometry internal service analysis can be ordered for covalent compounds with molecular weight < 3000 Da. The analyses of larger compounds, complexes, and larger data sets (metal coordination, hydrogen bonded, biomolecules, supramolecules) should be first discussed with Lab Manager Elina Kalenius ([elina.o.kalenius@jyu.fi](mailto:elina.o.kalenius@jyu.fi), SK517).

Experiment types available for orders are:

<b>Profile mass spectrum</b>	Provides nominal mass accuracy broad scan from sample
<b>Accurate mass</b>	Provides accurate mass (< 5ppm, <0.3 mDa) for a certain ion
<b>CID</b>	Provides MS/MS fragmentation spectrum for a pre-selected ion
<b>HPLC-MS</b>	Provides a RP-HPLC-MS data using general HPLC method

MS analysis can be ordered from the operator by using form: *MS analysis order form 2024.pdf*, which can be found in <https://www.jyu.fi/fi/tutkimusryhmat/kiertotalouden-orgaaninen-analytiikka#toc-ms-lab>. The form should be filled in detail. The analysis will not be executed if the form is not submitted prior the analysis or the necessary details are missing. Submitted the completed form to the MS lab email [jyu-mslab@jyu.fi](mailto:jyu-mslab@jyu.fi)

## Sample preparation

Sample can be in a solid or solution form. Samples MUST be prepared using the semimicrobalance, ultrapure solvents and consumables available in the MS laboratory. Prepare the sample into the chromatography vials which can be found next to the balance, samples are not accepted in any other vials. Weighed amount should be 0.2-0.5 mg (max. 0.5 mg). Report the accurate weigh in the order form. If you want to submit the sample in solution, use the ultrapure solvents found in the MS lab (Fridge 2 → Solvents for general use). Suitable concentration for samples is between 0.5 and 10 mM. All samples must have a label with your name and sample code. The same information should be marked into the order form. Measured spectra are emailed to orderer and samples are disposed after measurement if not otherwise requested.

**If you are submitting the sample for the first time ask instructions and guidance from lab operator.**



**Figure 1.** Reserved locations for submitting samples in YO309. Mark the storage place of your compound also to order form.

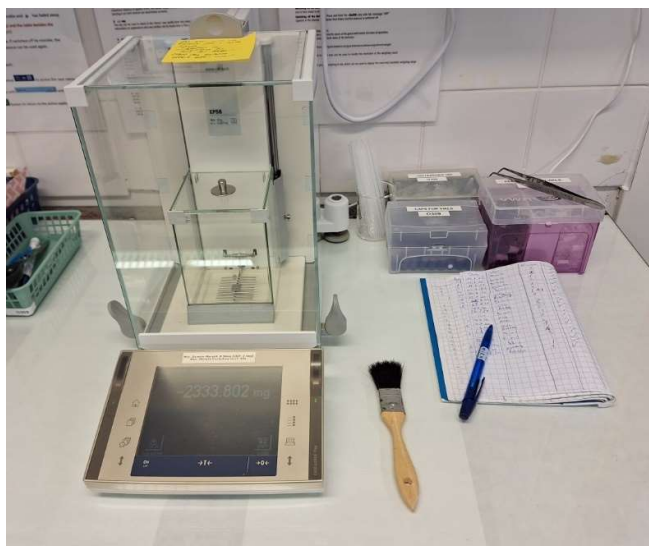
## Using Semimicrobalance

Semimicrobalance (Mettler Toledo XP56) should be used only for preparation of MS samples. For other use, please consult Lab Manager or Lab Operator in advance. Semimicrobalances are sensitive and expensive devices and should not be used without proper training. Ask instructions from Lab Operator before use.

The following instructions have to be followed when using the balance:

1. Fill your vial OUTSIDE the balance. After zeroing, lift your container outside the balance by using tweezers or gloves and fill it there. Do not fill the container on the balance plate in any circumstances.
2. Clean the balance after every use with a brush. Wipe also the table beside the balance.
3. Mark the number of samples in the log book.
4. Leave the balance on, do not turn it off.

More detailed instructions on balance use can be found next to the balance. If you notice shortage in accessories (vials, spatulas, Eppendorfs), please inform the lab operator.



**Figure 2.** Balance station and semimicro balance in YO309. Clean the balance and station every time after use. Mark the use to logbook.

## Independent use of instruments

Independent use of MS equipment is allowed for persons with appropriate training. Independent use of instruments requires following trainings / courses:

Passed grade from KEMS534 Basic Principles in Mass Spectrometry

Signed Organic Chemistry SOP

MS laboratory user orientation (MS-USER)

Instrument specific training

Required training is given by Lab Operator or Lab Manager. To obtain permission to use, please contact Lab Manager Elina Kalenius ([elina.o.kalenius@jyu.fi](mailto:elina.o.kalenius@jyu.fi)). Students can also get the user permission for Agilent 6530 UHPLC-ESI-QTOF after passing course KEMS535 Practical methods in Mass Spectrometry.

The instruments are reserved through **electronic reservation system** (JYU Device Booking system, [infrabooking.jyu.fi](http://infrabooking.jyu.fi)). The reservation should be made for all experiments (also when there are no previous reservations and the instruments are otherwise free to use). In reservation form, you also need to state the project where expenses are charged from.

Minimum reservation time slot is 2 hours and maximum reservation time 2 days per week. If a longer time slot is needed, please contact the Lab Manager. If you don't need the reserved time slot, cancel the time from [infrabooking](http://infrabooking.jyu.fi) and inform the next user, if possible.

Each instrument has a **logbook**, that **has to be filled** every time when using the instrument. You also need to state the number of measured samples in the logbook. The experimental information (instrument parameters, sample preparation etc.) should be recorded at personal note books or lab diaries. User manuals for each instrument are available in [infrabooking](http://infrabooking.jyu.fi).

## Data PC and storage of data

The independent user can store their data either to their own data storage media (hard drive, USB drive) or use MS Lab S-drive. In YSK building 5th floor, there is a Data PC for data analysis needs. Data PC can also be accessed remotely from JYU workstations after reservation. You can reserve the Data PC and find *DataPC Instructions* in [Infrabooking.jyu.fi](http://infrabooking.jyu.fi) (Data PC page). If you request a user permission for S-drive and/or Data PC, please send a HelpJYU ticket for that (<https://help.jyu.fi/jp>).

## Solvents and accessories for measurements

Lab YO309 uses a phased solvent system. The storage bottles are located in secured solvent cabinet, intermediate bottles (blue cap) in Fridge 2 and solvent bottles used for actual sample preparation (brown small glass bottles) in the fridge marked as "Solvents for general use". Brown small solvent bottles are filled from intermediate bottles and intermediate bottles from storage bottles.

**DO NOT EVER take solvent with syringe or pipette directly from storage or intermediate bottle.** Change solvent immediately if you suspect any solvent contamination.



Figure 3. Phased solvent system bottles.

Contamination should be avoided in handling of pipettes and syringes. If you are uncertain how to use the automatic pipettes, please ask instructions from Lab operator. The automatic pipettes must not be moved to other labs.

Accessories and vials needed in sample preparation (syringes, spatulas, Eppendorf-tubes etc.) are found in marked drawers and cupboards. Make sure that cupboards and drawers are kept tidy and accessories in their marked places. If you notice shortage in accessories, please inform Lab Operator immediately. Accessories are reserved only for MS samples preparation. Do not use them for other purposes.

## Liquid chromatography (HPLC)

### Sample preparation for HPLC

All samples have to be weighed using semimicrobalance of MS lab, and then dissolved to ultrapure solvents available in MS Lab. Prepare the stock solution first, with concentration 1-5 mM or 1mg/ml. Then prepare the sample by diluting the stock solution to appropriate concentration. Suitable starting concentrations to HPLC-MS is 10-50  $\mu$ M. Concentration needed depends remarkably from sample properties. If you notice saturation of the detector (in HPLC-MS, marked with \* in the spectrum), lower the concentration immediately. Suitable solvents for stock and sample solution are MeCN and H<sub>2</sub>O when measuring with Agilent 6530, for other instruments also MeOH can be used, if that is used as an eluent.

Samples need to be filtered before measuring. Always use the consumables and filters available in MS lab (DO NOT bring your own!). The filters are for single use only, and should be discarded immediately after use. Use of inappropriate filter causes unnecessary background to your measurement and might hinder the detection of your compound. In the worst case, use of inappropriate filter can cause contamination to the instrument which will take days or weeks to clean. For these reasons different filters are used for different purposes.



**Figure 4.** Different filters. Left: spin filter, Middle and right: two different syringe filters.

### Selecting appropriate filter:

#### HPLC-MS

Sample in H<sub>2</sub>O → PTFE syringe filter

Sample in organic solvent → PTFE spin filter

#### SFC/HPLC 1260:

Sample in H<sub>2</sub>O → Nylon syringe filter

Sample in organic solvent → PTFE syringe filter

If you use needles, please take care to dispose them appropriately to the waste container of sharp objects. Needles should never stick out from container. The needles should be dropped horizontally to the waste. Shake the bin gently, if necessary, but don't put your fingers inside the sharp waste. If the needle waste gets full, ask for new one from the lab operator.

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## Measurements using HPLC

Prior the HPLC (and SFC) measurements always check following:

1. The level of solvents in eluent bottles and record solvent levels to the software.
2. Check the levels in washing bottles (needle wash, injection flush, pump seal wash). Add solvents if needed.
3. Empty the waste bottle if needed. Note that when using SFC there has to be some solvent on the bottom of HPLC waste bottle, because of the necessary backpressure.
4. Before starting the measurements, check the gases and mark their pressures into the logbook. Check also other details asked in the logbook. If the gas pressures are insufficient for your measurements, inform lab personnel.

When you change the eluents, remember to flush the glass filter first in a beaker with small amount of the new solvent. This prevents the contamination of the eluent bottle. Purge the glass filter and solvent lines starting from the beaker. Remember to purge all solvent lines you are using. Check also the condition of the glass filters. If they need to be changed, inform the Lab Operator.

## Buffers and columns (only for advanced HPLC user)

Always vacuum filter all buffers through 0.25 µm filter paper before use. Pure solvent needs not to be filtered. Filtration system can be found in marked box under the fume hood. See the correct assembling from Fig. 5. Sonicate the buffers and solvents 15-30 min to remove the air bubbles.

After using buffers flush it out from the solvent lines and column with water before changing to organic solvents. Ready buffers are stored in fridge or chemical cabinet.

When installing a column, please make sure that it is tightly fitted. Columns should not be stored at the instrument. If you are not continuing your experiments on following day, please run storage solvent inside the column, remove the column and store it in its box. Mark to the column box which solvent you stored the column with. Fill in the column logbook used eluents, flow rate and operating pressure.

Ultrasonicator can be found in the fume hood. Use water until max level.



Fig.5. Buffer Filtration system.