# Cleaning and verification procedure of LC/ESI-MS systems

## 1. Objective

The purpose of this document is to describe the cleaning and verification procedures to be applied after the use of mass spectrometers, namely ThermoFinnigan LCQ and LTQ mass spectrometers models and coupled HPLC systems, in order to ensure that they are in proper condition for the next users.

The rules defined in this document are mandatory for all the equipment users, namely UniMS technicians and any other person using the equipment.

## 2. Training

Before operating on the mass spectrometers independently, the user must be fully trained and approved. This training shall be given by the UniMS technicians.

Non trained users must request assistance from the UniMS technicians.

## 3. General rules

- The use of the equipment must be pre-booked (section 4) and registration of the effective time used should be performed (section 5) afterwards.
- A spectrum from a standard must be saved at the beginning and end of the work to ascertain the equipment condition (section 6).
- After use, the equipment is to be left cleaned (section 7).
- The user must leave the work area clean, taking all the materials from his self use
- Always use LC-MS water or water from a routinely maintained milli-Q, for the preparation of all solutions.

## 4. Booking

Anyone wishing to use the equipment, should pre-book of equipment in the calendar available at

For LTQ: http://unims.itqb.unl.pt/LTQ/month.php

For LCQ: http://unims.itqb.unl.pt/LCQ/month.php

selecting events> add new event

Fill the different fields, so that the person who performed the booking is easily identifiable.

In the Tab “participants”, choose:

Research IBET - if it is a project ibet

Research ITQB - if it is a project itqb

Mass group - if it is a project from Ana Coelho's work group

External Academic Services - if it is a service for an external academic client

Industry services - if it is a service for an industrial client

**Concomitantly** always select "Public access" and also select the name of a technician if you need help with the analysis. The simultaneous selection is made by pressing CTRL.

The booking is only considered effective after confirmation by the responsible UniMS technician and only then the pre-booking appears on the public calendar. Please do not consider that the booking is confirmed before this.

**5. Records**

The use of the equipment must be recorded in Annex 3. All fields must be filled.
Identification of the files where the results of the equipment's verification tests are, shall be made in Annex 3 as well.
Both spectra collected before and after the assay, should be stored in the folder "verification tests" on the computer.
If a problem or error message is detected during operation, these should be recorded and fully described in the logbook (hard bound book) of the equipment.

**6. Verification of LC-MS system performance**

This verification is to ascertain that the equipment is in good operating conditions thus ensuring the possibility of following users carry out their analyzes. This verification should be done always, after use of mass spectrometers:

1. Before the verification of the mass spectrometer, clean it with the aid of mobile phase solvent, using an injection syringe or HPLC system (without column).
2. After that, inject the standard used in the optimization of your method and verify if the mass spectrum obtained is equal to that obtained at the start of the assay. In the case of not having a standard, this type of operation should be done with the

standard reserpine, which is a standard routinely used to verify the sensitivity for assays of small molecules. In Annex 1 of this document there is a table with the methodology for preparing reserpine solution. Its mass spectrum and fragmentation spectrum are in Annex 2 of this document.

3. After the verification test, clean the mass spectrometer as above describe in point 1.

If the peaks *m /z* of the standard are inconsistent with the expected, cleaning should be carried out as described in the next section.

**7. Cleaning**

This more thorough cleaning, should be performed whenever necessary or if suspected that the equipment is contaminated, i.e., if the *m/z* peaks of the standard assay are still observed with high intensity (> $10^3$).

This cleaning procedure aims to eliminate components/contaminants of capillaries and capillary transfer of ions.

**ESI Source**

Attention should be made to the solvents, the sample and the buffers used in the assays, in order to prepare a suitable mixture for cleaning that better solubilizes the non-volatile components.

This cleaning methodology consists on the following steps:

- Disassemble the syringe;
- Disassemble the capillary transfer of the sample;
- Remove the fittings from the capillaries;
- Remove the source of ionization. During the cleaning of the ionization source and the capillary ion transfer, caution is required, because some components may be hot;
- Place the capillary transfer ion in a beaker with 5% nitric acid;
- Sonicate the beaker for 20 minutes, then repeat this step with methanol LC-MS;
- Wash the inside surface of the ionization source with laboratory tissue soaked in LC-MS methanol;
- Allow the ion transfer capillary to dry and cover it with laboratory tissue in order to prevent dust contamination;

- Replace the capillary ion transfer
- If contamination persists, check if the capillary ion transfer shows signs of discoloration or deposit that has not been removed by normal cleaning; if necessary, proceed to a cleaning with sandpaper.
- Finally, assemble the syringe and the capillary transfer sample.

**Cleaning of the injection system between different assays**

- Wash three times the syringe with the sample solvent previously used and once with the solvent of the new sample, if the two solvents are different;

**Clean the internal components of the mass spectrometer with the help of different solvents:**

The washes described below may be used with the help of an HPLC pump if a higher solvent flow is needed, always without a column present.

These washes intend to eliminate persisting contamination of the equipment. Several solvents with different polarities should be used.

For example:

- Acetonitrile 50%
- Acetonitrile
- Methanol 50%
- Methanol

If the previous wash methodology is not effective to eliminate the contaminants, another type of wash methodology should be used:

- Solvent: isopropanol 100%
- Pump Flow: 5-10µL/min
- Drying gas: 0-3 (arb)
- Capillary temperature: 350ºC
- Gas Pressure: 15 – 20 (arb)

**Cleaning the HPLC system**

The cleaning of the HPLC system consists in the cleaning of the chromatographic column and pre-column, when in use, according to the manufacturer's instructions. Each user is responsible for the maintenance/cleaning of its columns and pre-columns. When cleaning the

column or preparing the column for storage, the column should be disconnected to the MS thereby avoiding contamination of the mass spectrometer.

Always remove the eluents of the mobile phase after the assay and replaced them with acetonitrile LC-MS (or another solvent recommended for that particular column). Remember to purge the lines of the HPLC pump.

In the case of the nanoLC, the mobile phase is always the same, there is no need to replace the eluents.

Remove the vials from the sample holder and clean the holder with a tissue soaked in acetonitrile.

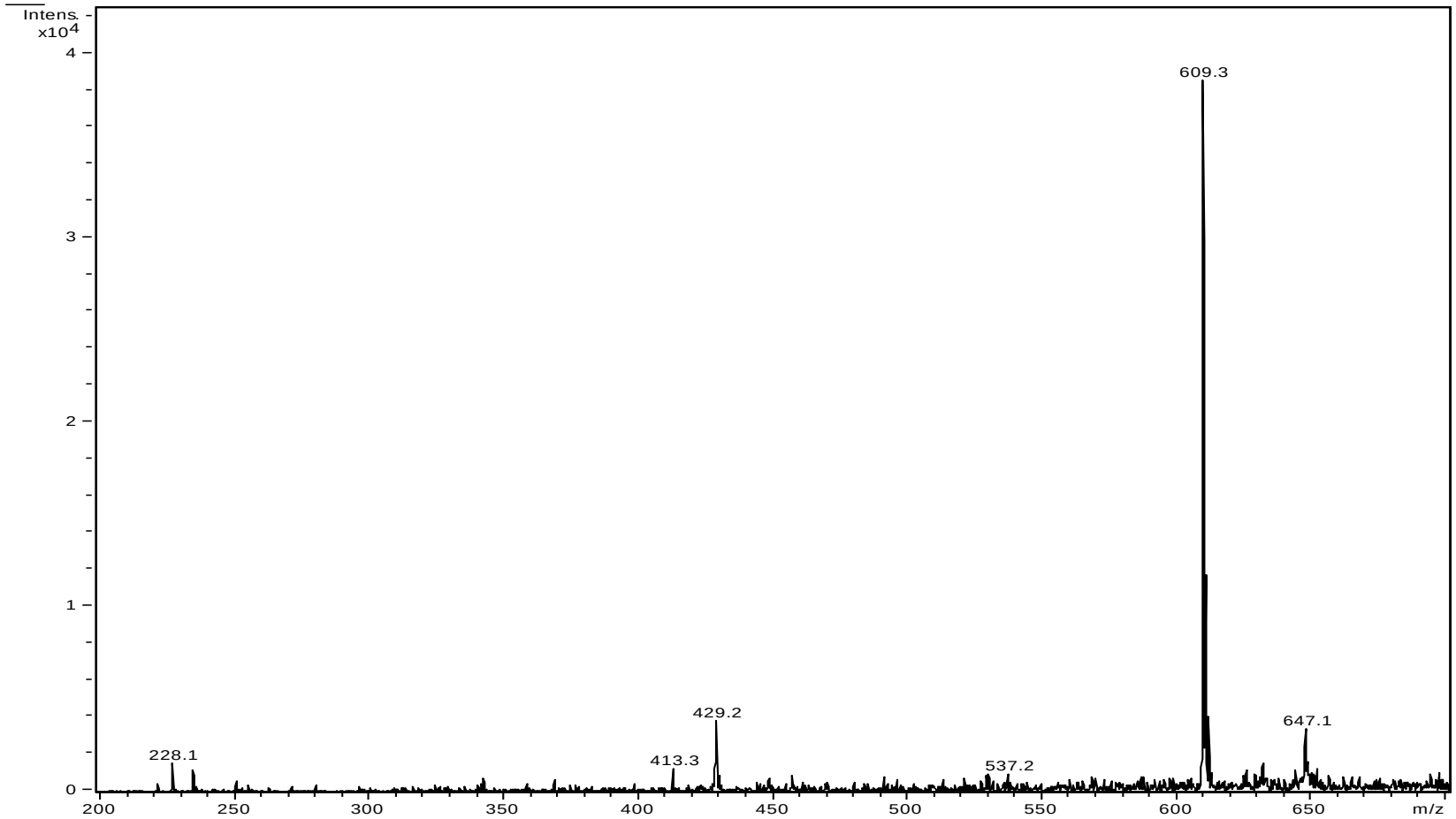
The nanoLC system, is usually used 96 well plates and each user shall have their own

Purge the sample needle and injection system least 10x with acetonitrile.

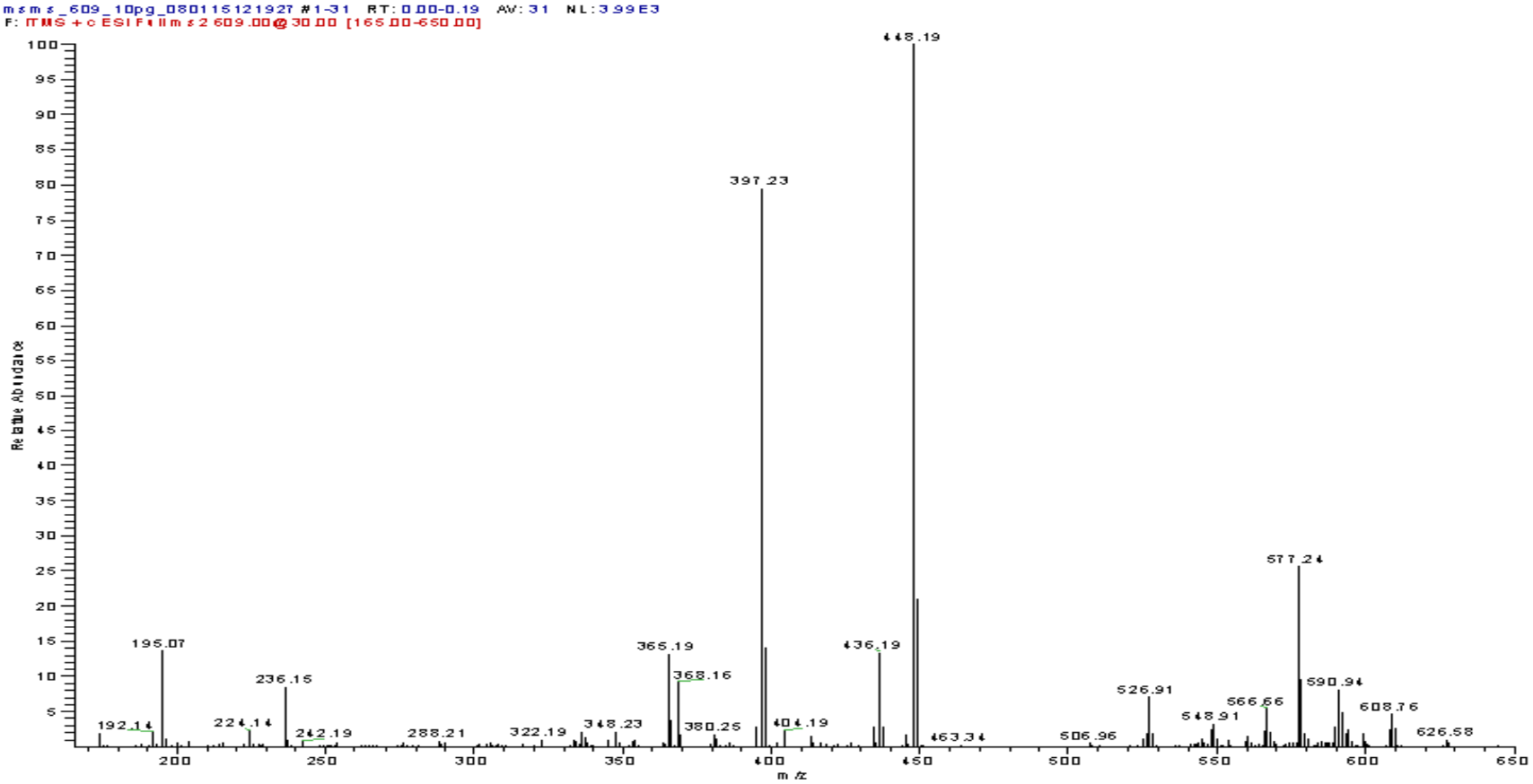
Write in the of the logbook equipment that everything is OK.

## Sensitivity assay parameters

| Standard/ Reagent | Mode / Ionization source | Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Concentration** | **Capillary Temp (ºC)** | **Drying Gas (Psi/ arb)** | **Pressure gas (l/min / arb)** | **Injection flow (µl/min)** | **Capillary voltage (kV)** | **Parent ion *(m/z)*** | **Solvent Blank solution** | **Solvent** |
| Reserpine | MS/MS / ESI | 10 pg/ ul (LCQ)<br>125 fg /ul (LTQ) | 300 | 10-20 | 0-4 | 1-25 | 3-4.5 | 609 | Isopropanol 50% (LCQ e LTQ) | Methanol 0.1% formic acid (LCQ e LTQ) |




Mass spectrum for Reserpine solution




Fragmentation spectra for Reserpine solution

Annex 3

| Date | Source | | HPLC System | | | Utilization Time | | Samples injections | | FileIdentification of MS Standard spectrums | Results (observed m/z)/ Observations* | Project Responsible / Group | Name/ Ext. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ESI | Nano | Normal | Micro | Nano | Start | End | Type | Number | | | | |
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* Whenever you detect any anomaly or some sort of damage, record it in the logbook of the equipment.
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