



UniPi-AOUP LSM laboratory: 2023 activity report

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1. Laboratory organization

The *Laboratorio di Spettrometria di Massa* (LSM) is a joint venture between University of Pisa (UniPi) and Azienda Ospedaliero Universitaria Pisana (AOUP), and is included in the network of Center for Instrument Sharing of the University of Pisa (CISUP). It was officially instituted on January 8th, 2020, incorporating the pre-existing mass spectrometry facility, created in 2013 at the St. Chiara Hospital of Pisa under the auspices of Regione Toscana, with equipments already available at the University of Pisa. The agreement between UniPi and AOUP expired in January 2023 and UniPi is still awaiting renewal by AOUP.

LSM was organized to carry out both research and development and assistance to patients. The lab includes two rooms with LC-MS and ICP-MS systems, one sample preparation lab, and a small lab with sample preparation devices.

1.1. Location. UniPi-AOUP LSM is located in Pisa, at St. Chiara Hospital, building 13, entrance B, ground floor. The laboratory includes 2 sample preparation labs, 2 instrumental labs, 1 meeting room, and 3 offices (320 m²).

1.2. Mass Spectrometers. LSM is equipped with the following mass spectrometers:

- Agilent 7900, ICP-MS (UniPi - DAM)
- Sciex QTRAP 6500⁺, LC-MS/MS (UniPi – CISUP)
- Sciex API 4000, LC-MS/MS (UniPi - DAM)
- Sciex API 3000, LC-MS/MS (UniPi - DAM)
- Thermo Scientific Q Exactive, LC-Hybrid Quadrupole-Orbitrap-MS (AOUP)
- Waters Synapt XS HDMS 4K, HR LC-Q-IMS-TOF (UniPi – CISUP)



Figure 1. MS systems in the UniPi-AOUP LSM lab: Waters Synapt XS HR LC-Q-IMS-TOF mass spectrometer (upper-left) and Sciex QTrap 6500+ LC-Hybrid Tandem Quadrupole (upper-right), Sciex API 3000 LC-triple quadrupole mass spectrometer (middle-left), Sciex API 4000 LC-triple quadrupole mass spectrometer (middle-right), Thermo Scientific Q Exactive LC-Hybrid Quadrupole-Orbitrap-MS (lower-left), and Agilent 7900 ICP-MS (lower-right).

1.3. CISUP's Mass Spectrometers.

The **Sciex QTRAP 6500+** (picture top right in Figure 1) was installed on March 15th, 2019. It was bought by UniPi with funding provided by the Department of Clinical and Experimental Medicine, Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, and Department of Translational Research and of New Surgical and Medical Technologies (all together contributed for 60%), and by the University Central Administration (40%). It is now framed in the Center for Instrument Sharing of the University of Pisa (CISUP). Its technology is unique and based on a triple quadrupole

mass analyzer configuration, but the third quadrupole can be configured as a Linear Ion Trap (LIT). In practice, the system can be operated as a conventional triple quadrupole instrument, with all its typical scan functions such as MRM, product ion and neutral loss, or as a QTRAP, with the third quadrupole that works as a LIT. This configuration provides additional qualitative scan functions, with the simultaneous structure confirmation or identification. The QTRAP® 6500+ is one of the fastest and most sensitive LC-MS available on the market. It provides the very good LOQs to enable the detection and quantification of chemical compounds at trace level in very complicated matrices. Improved polarity switching and MRM³ speeds allow faster chromatography and, consequently, better throughput. The system is ideal to perform assays with high specificity and accuracy in clinical chemistry, pharmaceutical drug discovery, food testing, environmental monitoring, protein quantitation, forensic drug testing, as well as for basic research purposes.

The **Waters Synapt XS HDMS 4K** (picture upper-left in Figure 1) was installed on December 17th, 2023. It is a high-resolution (HR) mass spectrometer with quadrupolar analyzer (Q), ion mobility separator (IMS) and time of flight analyzer (TOF), as shown in Figure 2.

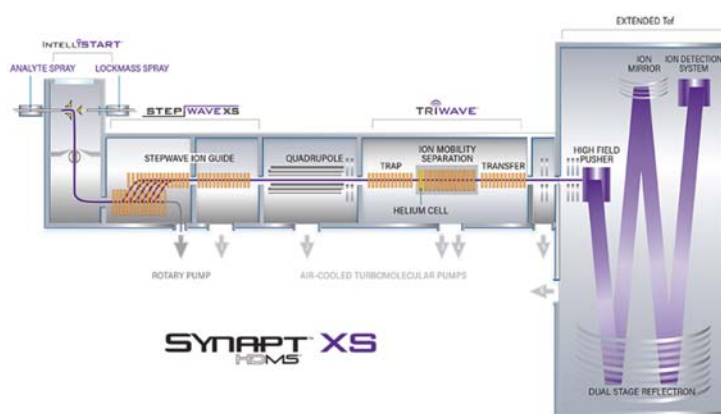


Figure 2. Schematic diagram of the Synapt XS HDMS 4K mass spectrometer

Differently from the mass spectrometers with “traditional” analyzers, where ion separation is based just on the different m/z values, IMS discriminates ions according to their size and shape or, more precisely, to their collision cross section (collision cross section, CCS). IMS, therefore, constitutes an additional separation dimension, extremely useful when the analytes of interest are inside complex mixtures, such as those of metabolomic interest. IMS is located between two electrostatic sectors, which can be used as collision cells. So that, MS³ experiments are also possible. The coupling of Synapt XS with an ultra-high performance liquid chromatograph (UPLC) increases the separative dimensions up to 5: chromatography, MS, MS², IMS, and MS³. All this makes the instrument suitable for untargeted and targeted metabolomic investigations.

2. Staff

The mass spectrometry research activity is carried out just by UniPi postdoc fellows and PhD students, under the supervision of Prof. Alessandro Saba (Full Professor of Biochemistry, serving also at AOUP till the end of 2023). At present, Prof. Saba is the only UniPi employee serving at the LSM; no UniPi technician or researcher is assigned to LSM.

3. Research Collaborations

The LSM has research collaborations with several Departments of UniPi and of some national and international research institutions. In particular, LSM performs mass spectrometry experiments for research projects in collaboration with UniPi researchers from 5 departments of the University of Pisa (Department of Clinical and Experimental Medicine, Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, Department of Translational Research and of New Surgical and Medical Technologies, Department of Biology, and Department of Pharmacy), national research institutions, such as CNR and Foundation Edmund Mach, and international, such as the University of Miami Health System, the University of Chicago, and the University Hospital La Paz in Madrid.

4. Research products.

The peer-reviewed papers published in 2023 and reporting data partially or totally acquired in the LSM laboratory are listed below. In some of them CISUP was acknowledged, since those studies were partially carried out with the CISUP's Sciex QTrap 6500⁺ mass spectrometer. Those papers are the result of collaborations between LSM and other departments of UniPi (Pharmacy, Translational Medicine, Biology), University of Florence, CNR, University of Madrid, University of Miami, and University of Chicago.

1. Rutigliano G, Bertolini A, Grittani N, Frascarelli S, Carnicelli V, Ippolito C, Moscato S, Mattii L, Kusmic C, Saba A, Origlia N, Zucchi R. Effect of Combined Levothyroxine (L-T4) and 3-Iodothyronamine (T1AM) Supplementation on Memory and Adult Hippocampal Neurogenesis in a Mouse Model of Hypothyroidism. *Int J Mol Sci.* 2023;24(18):13845. <https://doi.org/10.3390/ijms241813845>. **CISUP acknowledged.**
2. Cuffaro D, Bertolini A, Bertini S, Ricci C, Cascone MG, Danti S, Saba A, Macchia M, Digiacomo M. Olive Mill Wastewater as Source of Polyphenols with Nutraceutical Properties. *Nutrients.* 2023;15(17):3746. <https://doi.org/10.3390/nu15173746>. **CISUP acknowledged.**
3. Cuffaro D, Pinto D, Silva AM, Bertolini A, Bertini S, Saba A, Macchia M, Rodrigues F, Digiacomo M. Insights into the Antioxidant/Antiradical Effects and In Vitro Intestinal Permeation of Oleocanthal and Its Metabolites Tyrosol and Oleocanthalic Acid. *Molecules.* 2023;28(13):5150. <https://doi.org/10.3390/molecules28135150>. **CISUP acknowledged.**
4. Raffaelli A, Saba A. Ion scanning or ion trapping: Why not both? *Mass Spectrom Rev.* 2023;42(4):1152-1173. <https://doi.org/10.1002/mas.21746>.
5. González-Guerrero C, Borsò M, Alikhani P, Alcaina Y, Salas-Lucia F, Liao XH, García-Giménez J, Bertolini A, Martin D, Moratilla A, Mora R, Buño-Soto A, Mani AR, Bernal J, Saba A, de Miguel MP, Refetoff S, Zucchi R, Moreno JC. Iodotyrosines Are Biomarkers for Preclinical Stages of Iodine-Deficient Hypothyroidism in Dehal1-Knockout Mice. *Thyroid.* 2023 Jun;33(6):752-761. <https://doi.org/10.1089/thy.2022.0537>. **CISUP acknowledged.**
6. Bellini E, Bandoni E, Giardini S, Sorce C, Spanò C, Bottega S, Fontanini D, Kola A, Valensin D, Bertolini A, Saba A, Paoli L, Andreucci A, Mingai L, Varotto C, Sanità di Toppi L. Glutathione and phytochelatins jointly allow intracellular and extracellular detoxification of cadmium in the liverwort *Marchantia polymorpha*. *Environ Exp Bot.* 2023 May;209:105303. <https://doi.org/10.1016/j.envexpbot.2023.105303>.

7. Lettieri M, Scarano S, Caponi L, Bertolini A, Saba A, Palladino P, Minunni M. Serotonin-Derived Fluorophore: A Novel Fluorescent Biomaterial for Copper Detection in Urine. *Sensors (Basel)*. 2023 Mar 10;23(6):3030. <https://doi.org/10.3390/s23063030>.
8. Shareef R, Furman A, Watanabe Y, Bruellman R, Abdullah MA, Dumitresu AM, Refetoff S, Bertolini A, Borsò M, Saba A, Zucchi R, Weiss RE. Congenital Hypothyroidism in Two Sudanese Families Harboring a Novel Iodotyrosine Deiodinase Mutation (IYD R279C). *Thyroid*. 2023 Feb;33(2):261-266. <https://doi.org/10.1089/thy.2022.0492>. **CISUP acknowledged.**
9. Calvigioni M, Bertolini A, Codini S, Mazzantini D, Panattoni A, Massimino M, Celandroni F, Zucchi R, Saba A, Ghelardi E. HPLC-MS-MS quantification of short-chain fatty acids actively secreted by probiotic strains. *Front Microbiol*. 2023 Mar 3;14:1124144. <https://doi.org/10.3389/fmicb.2023.1124144>. **CISUP acknowledged.**
10. Poli G, Demontis GC, Sodi A, Saba A, Rizzo S, Macchia M, Tuccinardi T. An in silico toolbox for the prediction of the potential pathogenic effects of missense mutations in the dimeric region of hRPE65. *J Enzyme Inhib Med Chem*. 2023 Dec;38(1):2162047. <https://doi.org/10.1080/14756366.2022.2162047>.

5. Students activity

Most of this research activity in LSM is carried out by the PhD students and fellows. In particular:

2020-2023 PhD student: Beatrice Campi (discussed in 2024)

PhD program: Biochemistry and Molecular Biology (Regione Toscana) – administrative office: University of Siena – cycle XXXVI

Title: **Development and validation of accurate HPLC-MS-MS based methods to investigate the vitamin D status involved in COVID-19** (reg. IRIS 11365/1265594).

Note: ***CISUP acknowledged in the PhD thesis.***

2020-2023 PhD student: Andrea Bertolini (discussed in 2024)

PhD program: Biochemistry and Molecular Biology (Regione Toscana) – administrative office: University of Siena – cycle XXXVI

Title: **Investigation on the role of thyroid hormones and their derivatives in different physiopathogenetic mechanisms using HPLC-MS/MS** (reg. IRIS 11365/1258876).

Note: ***CISUP acknowledged in the PhD thesis.***

2021-2024 PhD student: Valentina Vitelli (thesis in progress)

PhD program: Biochemistry and Molecular Biology (Regione Toscana) – administrative office: University of Siena – cycle XXXVII

Title: **Phytomonitoring and phytodecontamination of heavy metals with plants for environmental sustainability and the green economy.**

Research Fellowship PON “Ricerca e Innovazione” 2014-2020, Asse IV “Istruzione e ricerca per il recupero” Azione IV.4 Azione IV.5 “Dottorati su tematiche green”.

2022-2026 PhD student: Elena Fortin (thesis in progress)

PhD program in Medical Sciences, Karolinska Institutet, Solna, Svezia

Title: **Novel biomarkers in cardiovascular disease and dysglycaemia.**

2022-2023 Fellow: Agnese Giamborino (research work concluded)
University of Pisa – Department of Pathology
Title: **Development of mass spectrometer-based analytical methods to evaluate vitamin D status as a risk factor for susceptibility to SARS-COV2.**

2023-2026 PhD student: Agnese Giamborino (thesis in progress)
PhD program: Biochemistry and Molecular Biology (Regione Toscana) –
administrative office: University of Siena – cycle XXXIX
Title: **Translational study of factors modulating vitamin D availability in obesity.**

2023-2026 PhD student: Benedetta Nardi (thesis in progress)
PhD program: Biochemistry and Molecular Biology (Regione Toscana) –
administrative office: University of Siena – cycle XXXIX
Title: **Biochemical markers of social anxiety disorder in bipolar subjects.**

6. Assistance to patients

The *Laboratorio di Spettrometria di Massa* carries out also diagnostic activity in clinical pathology, endocrinology, pharmacology, and forensic toxicology, under the responsibility of AOUP. At present, no CISUP's instrument is involved in this activity. The instrumentations used for the assistance to patients are:

- Agilent 7900, ICP-MS (UniPi)
- Sciex API 4000, LC-MS/MS (UniPi)
- Thermo Scientific Q Exactive, LC-Hybrid Quadrupole-Orbitrap-MS (AOUP).

7. Working hours of CISUP's Sciex QTRAP 6500+

Sciex QTRAP 6500+ was involved in several research projects, most of them not supported by specific grants. In 2023 the instrument worked approximately 1360 hours that are equivalent to 5.5 hours/day (if we consider 249 working days in 12 months). The main topics of the national and international collaborations are listed below. Most of them are documented by the publications listed in section 4:

1. Investigation on the extraskeletal effects of vitamin D and of its metabolites in humans and animals (grants: PRA_2022_71, PRIN 2022 BIODTECT-CP).
2. Investigation on the thyroid endocrine system.
3. Investigation on the beneficial effect on human health of some components of olive oils.

8. Future developments of the LSM's activity

The main development plan includes analytical activities on behalf of third parties, which is fundamental to support the running costs and guarantee equipment service contracts, CISUP's instruments included. This can be achieved by the recruitment of one technician, who has been recently requested to UniPi.

Sincerely,

Prof. Alessandro Saba