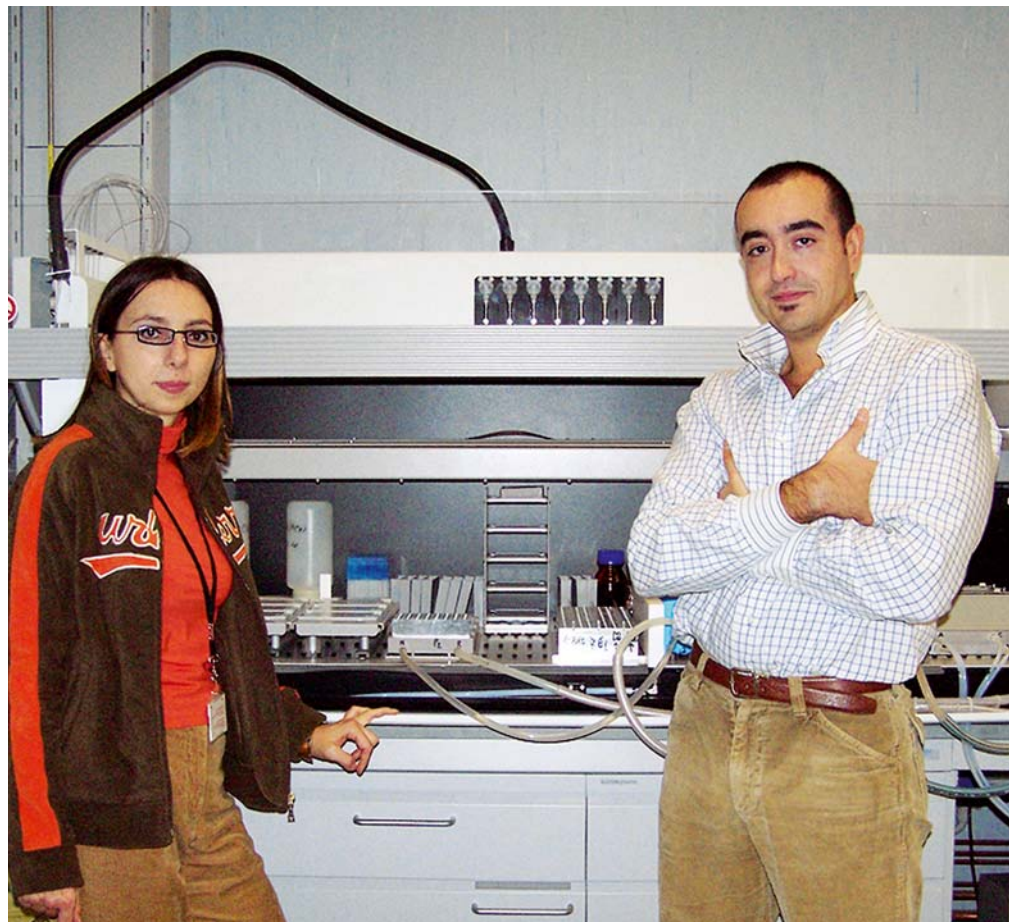


High throughput microsomal stability assays for analyzing candidate drugs

Drug discovery researchers from the Istituto di Ricerche di Biologia Molecolare (IRBM) "P. Angeletti", the Italian site of Merck Research Laboratories, have developed automated methods for performing high throughput microsomal stability assays using a Tecan Genesis RWS™ 200 Workstation. Automating these assays allows the researchers to predict the pharmacokinetics of multiple candidate drugs in just two days, instead of four, and vastly increases the throughput. Pharmacokinetic analysis of candidate drugs is a critical part of the drug development process, and helps to eliminate compounds with inappropriate bioavailability, toxicity, effective concentrations or duration or persistence of action. Such compounds would fail at later stages in drug development, so eliminating these earlier in the process saves a great deal of money, time and labor.

Dr Massimiliano Fonsi, DMPK Chemist at IRBM, has developed these microsomal stability assays using the Tecan workstation equipped with an eight tip liquid handling (LiHa) arm and robotic manipulator (RoMa) arm, Thermomixer and heater/cooler system. This set-up performs all the liquid handling steps, including sample pooling. The assay can be automated on other Tecan liquid handling workstations, including from the Freedom EVO® series. The protocol includes 1,200 LC/MS/MS runs that represent single incubation steps and, using the novel sample pooling method based on cassette analysis, all of these data can be acquired within 48 hours (as opposed to 96). This system has a number of advantages, as Dr Fonsi explained.



Dr Fonsi at the Istituto di Ricerche di Biologia Molecolare

“My aim is to be able to screen as many candidate drugs as possible. LC/MS is a serial technique, so you cannot read an entire plate in one go - each well has to be analyzed with a single chromatography run. At the moment, the chromatography takes about two minutes and I generate 600 samples, which would take about 48 hours in total. Doubling the samples would take 96 hours, but the high selectivity of mass spectrometry means that it is possible to pool two (or more) samples for analysis, using the Tecan Genesis platform, and each drug can then be analyzed with a specific transition in the mass spectrometer. At the moment our analysis time has been halved; increasing the velocity of the RoMa arm will make it possible to increment the assay throughput by running multiple incubations at the same time and pooling more samples in a final plate for analysis in a single run. Pooling the samples in this way has been used previously for *in vivo* pharmacokinetics but, as far as I know, nobody else has set up this automated system for HTS microsomal stability *in vitro* screening.”

“Each of our projects focuses on a specific class of compound, but IRBM is working on different projects, with several different classes of drugs. This means that I have to customize the assay and the analysis for each specific class of compound using the Gemini™ software, and Tecan’s flexibility is fundamental in this respect. We also have to be able to customize the protocol to suit various experimental conditions, such as different concentrations of proteins, different species models and different cofactors. This is very important and, currently, other laboratories using these methods do not have so much flexibility and are only able to run the assay under fixed conditions. We developed this protocol in order to assay different cofactors within the same experiment. We have also introduced control samples that do not contain any cofactors in order to obtain further information about unexpected phenomena that could overlap with the microsomal metabolism, such as precipitation, hydrolysis or other chemical reactions.”

“The method that we have developed also allows us to measure multiple time points, which means that we can calculate the intrinsic clearance of the drug as well as measure the residual percentage. With our method, 20 different compounds can be screened in duplicate, in three different species, collecting six time points over 90 minutes’ incubation. The same basic procedure can also be modified to screen 32 compounds with cofactors but without duplicates, or 10 compounds in duplicate with two different separated cofactors (e.g. NADPH and UDGPA).”

“In the future, we are hoping to get a Freedom EVO 200 Workstation with a Te-MO™ 96 head, which will allow us to increase our throughput even further as well as provide better prediction of a drug’s pharmacokinetics.”