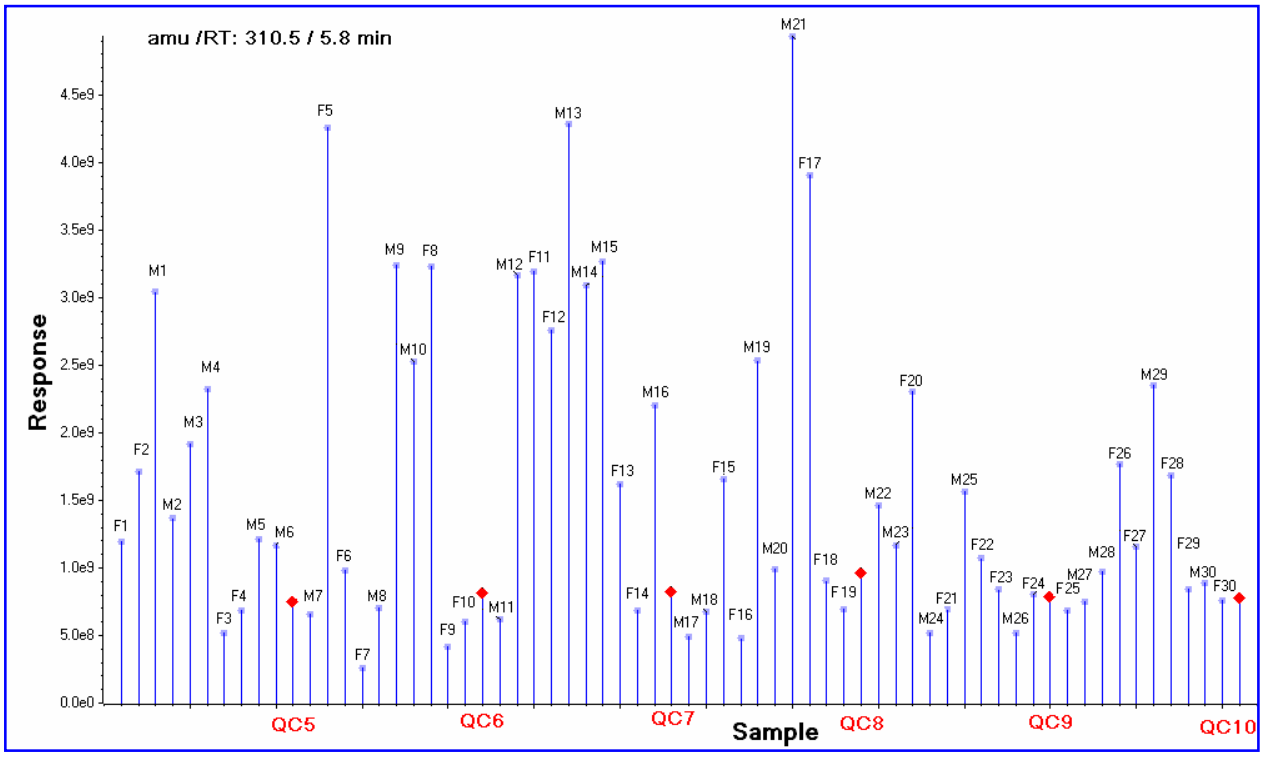
Research method and work plan

Drug Discovery represents one of the strongest drives in the pharmaceutical market. Drug Discovery is characterised by strong competition, lengthy procedures and high cost. The pace for the development of new drugs requires multidisciplinary cooperation and large scale state of the art facilities.

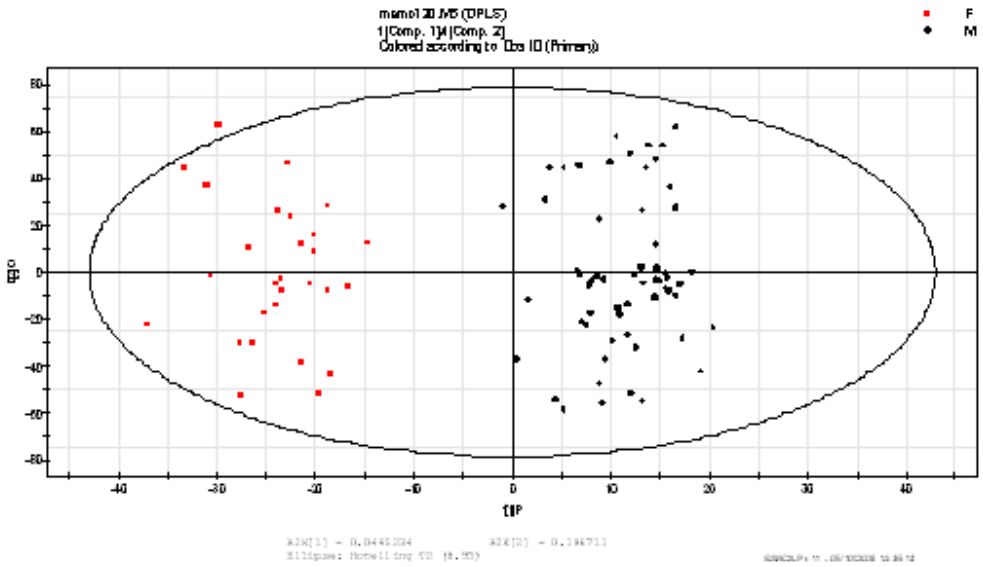


The main research aim of our project is to characterise and identify as many as biomarkers as possible using novel bioanalytical technologies. Compounds are detected in different types of biofluids (urine, blood plasma, cerebrospinal fluid etc) by HPLC-MS and newly introduced techniques: very high pressure and high resolution UPLC-MS and ultra temperature liquid chromatography. Metabolic profiling of body fluids and tissues by unbiased spectroscopic detection of endogenous and drug related metabolites allow the extraction of comprehensive

biochemical information, which is of diagnostic and prognostic value. The nature of the data reflects actual biological events (physiological phenotype). Metabonomic analyses of body-fluids can be used in early drug development for predicting general organ toxicity (such as liver toxicity or kidney toxicity) but also for monitoring more specific effects. Thus changes of single biomarkers can be monitored, but also simultaneous regulations of several metabolites within pathways are investigated. Databases are built to complement the various databases for proteomic and genomic information. The generation of this data is an essential precursor to the efficient use of metabonomics for the discovery of biomarkers of both human disease and in understanding the relevance of pre-clinical models of disease to drug discovery.

Samples of urine and plasma obtained from control and test groups are characterised by (UP)LC-MS to obtain a picture of the mammalian metabonome. The compounds present in the samples may be identified by a variety of means, including the use of authentic standards and isolation and identification using classical techniques such as NMR spectroscopy and mass spectrometry. Where isolation is required prior to identification techniques such as preparative scale HPLC using a range of different types of stationary phase to maximise selectivity may be employed. Selected fractions from the preparative scale separations are further purified by chromatography on a smaller scale with identification using e.g. HPLC-NMR or, if the fraction is pure, by NMR alone. Whilst the aim is to provide a general "map" of the plasma and urinary global metabolite profiles specific biomarkers related to disease is given priority.

As mentioned mass spectrometry (MS) will be a major analytical tool. MS is widely used now in the pharmaceutical industry and it is in fact the demands of the life sciences sector that have lead to the remarkable improvements in MS technologies and the rapid growth of new instruments. Other spectroscopic techniques to be used include NMR (alone or coupled on-line to HPLC and LC-MS).



The generated raw data is fed to specially developed informatics platforms: MarkerView, MarkerLynx, MzMine and others. In this process the complex LC-MS data is "thinned"; the algorithm incorporated in these software finds and aligns the chromatographic peaks in the three dimensional data matrix. The multi-dimensionality of the data matrix collapses thus resulting to a peak table that features the variables to be analysed in multivariate statistics. As a first step we perform Principal Component Analysis. Advnanced Statistical tools such as PLS-DA and OPLS-DA, OSC are used also at a later stage. These high efficiency tools are used to highlight the differences "hidden in the grass" within the massive dataset, build data banks, correlate the findings and reach lead finding. The "leads" (i.e. the possible active ingredients or substances) may be identified using data mining tools and existing web based databanks (e.g. human metabolome project) .

The goal of this process is the identification and elucidation of disease, drug efficacy or toxicity biomarkers. Biomarkers research is a premier topic in MS technology with application in the understanding of disease pathway, the discovery and development of new drugs and the development of diagnostic tools. Biomarkers are often defined as any peptide, protein or metabolite that is reproducibly differentially expressed between two or more samples. Such differences usually require analysis by MS and confirmation by MS/MS. Samples to be analysed are typically very complex, thus technologies to reduce the

complexity of the sample are essential. Hence the exploitation of advanced separation technologies remains vital. In the project high end spectroscopic technologies are combined with the newest modes of high performance chromatography. This combination offers unique advantages such as speed, higher efficiency and resolution power. In addition new dimensions for optimisation are studied (e.g. separation temperature).