Summer School on Pharmaceutical Analysis
Analytical methods to achieve efficiency in the hit-to-lead process

SSPA 2021

Abstract book draft

ToC

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PROGRAM

22 September 2021			
9.00-9.20	Opening ceremony		
9.20-10.20	From hits to drugs: an overview of the process		**Tiziano Bandiera** IIT, PharmaChemistry Line, Genova, Italy
10.20-10.30	Virtual coffee break		
10.30-11.30	Ensuring data integrity and quality in drug discovery		**Barbara Vaccarini** Aptuit, an Evotec Company, Verona, Italy
11.30-12.30	Analytical techniques to support the medicinal chemistry		**Antonio Triolo** Menarini Ricerche S.p.A., Firenze, Italy
12.30-14.30	Lunch break		
14.30-15.30	Hit-to-lead: potency and selectivity	NMR-based screening principles and applications in the hit-to-lead phase of drug discovery projects	**Claudio Dalvit** Scientific Consultant, Lavis (TN), Italy
15.30-16.30	Hit-to-lead: potency and selectivity	The road towards the optimal lead candidate: potency and selectivity profiling by biochemical and biophysical approaches	**Edoardo Fabini** Nerviano Medical Sciences, Nerviano (MI), Italy
16.30-17.30	Hit-to-lead: potency and selectivity	Design follows function in early drug discovery: in vitro assays for hit-to-lead programs	**Lia Scarabottolo** Axxam S.p.A., Bresso (MI), Italy
23 September 2021			
9.00-11.00	Pre-ADMET studies	Approaches for ADMET parameters optimization during the drug discovery phase: advances and caveats	**Giulio M. Dondio** Aphad S.r.l., Buccinasco (MI), Italy
11.00-11.10	Virtual coffee break		
11.10-12.10	Pre-ADMET studies	Mass spectrometric strategies in drug metabolism and pharmacokinetics (DMPK) studies	**Giancarlo Aldini** Università di Milano, Milan, Italy
12.10-13.10	Pre-ADMET studies	The benefits of ion mobility mass spectrometry for metabolite identification	**Simona Scarpella** Waters S.p.A., Sesto San Giovanni (MI), Italy
13.10-14.30	Lunch break		
14.30-15.30	Bioavailability and toxicology	In vitro and in vivo drug discovery quantitation of small molecules by LC-MS/MS: a tiered approach	**Rossella Pisano** Accelera S.r.l, Nerviano (MI), Italy
15.30 -16.30	Bioavailability and toxicology	Bioanalysis in drug discovery: challenges and approaches	**Marco Michi** Aptuit, an Evotec Company, Verona, Italy
16.30-17.30	PhD presentations		
17.30-18.30	**Ceremony for the 25th anniversary**		
24 September 2021			
9.00-10.00	PhD presentations		
10.00-11.00		Analytical Supporting developability aspects of new chemical entities for early formulation screening	**Emanuela Del Vesco** Aptuit, an Evotec Company, Verona, Italy
11.00-11.10	Virtual coffee break		
11.10-12.10	Case studies	Enabling chemical technologies to automate process optimization and medicinal chemistry	**Antimo Gioiello** University of Perugia, Perugia, Italy
12.10-13.10	Case studies	"Chromatographic Isotope Effect": Retention time changes for polydeuterated laquinimod in reverse phase HPLC	**Vladimir Ioffe** Scientific expert, Kfar Saba, Israel
13.10-13.30	Closing remarks		

FROM HITS TO DRUGS: AN OVERVIEW OF THE PROCESS

TIZIANO BANDIERA

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The discovery of a new drug is a challenging process that develops through a series of subsequent stages both in the discovery and development phase. In the discovery phase, for projects aiming at small molecule therapeutics, the process starts with the identification of hits that can be evolved to quality lead compounds, which will be the starting point for obtaining one or more preclinical development candidates. In the process referred to as Hit-to-Lead, hits are usually modified in order to increase potency on the target and build selectivity against possible off-targets. In the last two decades, however, it has been increasingly recognized that quality leads should also possess drug-like properties in order to avoid problems, or even failure, in the development phase. To achieve this goal, it is important that the compounds undergo characterization of drug-like properties such as solubility, stability in biological fluids and metabolic stability already at the Hit-to-Lead stage. More extensive evaluation of drug-likeness will be conducted during Lead Optimization in order to identify the most suitable compound(s) to progress to preclinical development. The assessment of compounds' drug-likeness requires testing them in a number of different in vitro assays, followed eventually by dosing in animals to determine pharmacokinetics. To do so, it is necessary to develop proper analytical methods to detect compounds and measure their concentration in various media and biological fluids. The results of those assays will help the medicinal chemists in the discovery of compounds with the highest chance to become a drug. The lecture will provide an overview of the process from hits to drugs with a focus on the discovery stages.

ENSURING DATA INTEGRITY AND QUALITY IN DRUG DICOVERY

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The primary goal of any quality system is to provide the framework and methodology to ensure that the data are fit for purpose and to give assurance that the process under which the data have been generated are completely accountable, transparent, and reproducible.

There has been increasing evidence in recent years that research in life sciences is lacking in reproducibility and data quality. This raises the need for effective systems to improve data integrity in the evolving non-GxP research environment. The entire landscape of research is changing radically. Innovation, the lifeblood of scientific endeavour, is very costly and standards in research that were the norm yesterday will not be acceptable in the increasingly regulated and competitive world of tomorrow.

Thus, a non-GxP Research standard should focuses on data integrity and research reproducibility. The rigor and frequency of its application need to be adapted to the research phase to which it is applied: in early discovery, focus is laid on innovation, protection of intellectual property and data integrity.

The aim of this presentation is to provide the basics for implementing a quality management system in a non-regulated research organisation in order to guarantee Data Integrity and Data Quality. The critical elements that need to be considered to ensure a successful implementation of research quality standards in both industry and academia are presented and discussed. The quality standard proposed is founded on data integrity principles named ALCOA+ and good research practices and contains basic quality system elements, which are common to most laboratories. A pragmatic and risk-based quality system and associated assessment process is presented to ensure reproducibility and data quality of experimental results while making best use of the resources.

ANALYTICAL TECHNIQUES TO SUPPORT THE MEDICINAL CHEMISTRY

ANTONIO TRIOLO

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The entire process of introducing a new drug to the market can be considered as a race against time: it typically takes more than 10 years for its discovery and development, and only a few years are available for the return of the investments, i.e. the time lapse from market introduction to expiry of the patent application.
In addition, the total cost for the development of a new drug is very high (averaging to 2.6 million USD, with an increase of 8.5% per year),[1] and so is the risk of failure for a candidate drug entering in clinical trials: the overall likelihood of approval starting from Clinical Phase I over the last decade is only 7.9%.[2]
The above aspects take to a very competitive landscape among companies involved in drug discovery, and to the need for methods and technologies aimed at improving overall efficiency and reducing risks.
Perhaps the main issue is the still unmet need to reduce the attrition rate of potential new drugs entering the clinic, mainly dealt with the careful selection of the strongest candidates during the phases of lead generation and optimization, and the acquisition of early knowledge of the potential critical points of a research program. This in fact contributes in preventing the high costs of a drug candidate failure during clinical phase, and in concentrating resources on the projects with the most probable success.
In this effort, after a period starting from the early 2000s, during which the expectations from introduction of combinatorial chemistry and high throughput screening had not been followed by significant results, Medicinal Chemistry has essentially returned to more traditional synthetic methods, but with improvements gained from the combinatorial experience.[3] The latter consist not only in changes of synthesis philosophy (use of robust chemical reactions amenable to diverse functionalizations, more attention to drug-like physico-chemical properties, synthesis of single compounds or small and more focused libraries), but also, end even more, in product purification and analysis, often constituting a previous significant time constrain. The main changes introduced can be summarized as follows:

- Use of information-rich and specific analytical techniques, to minimize analytical workload while maintaining full information on sample identity and purity directly from the crude reaction products: LC-MS (or-high resolution MS), high-field NMR.
- Increased use of open access instrumentation: the synthetic chemist analyzes his own products with standardized methods and automated software, minimizing the return time of results.
- Use of automated and integrated platforms for sample analysis and purification, such as mass-directed preparative HPLC.

All these aspects significantly contribute in speeding up the delivery of products for pharmacological screening, as well as generation of lead compounds and small-scale drug candidates.
This presentation will give an overview of the current analytical techniques in Medicinal Chemistry, particularly LC-MS, largely considered as the workhorse in this field, and its integration with other techniques, with practical examples and applications.

1. A. DiMasi et al., J. Health Economics, 47 (2016) pp. 20-33
2. D. Thomas, D. Chancellor, S. Chaudhuri et al., Clinical Development Success Rates and Contributing Factors 2011–2020, https://pharmaintelligence.informa.com/~/media/informa-shop-window/pharma/2021/files/reports/2021-clinical-development-success-rates-2011-2020-v17.pdf, accessed 15/07/2021.
3. I. B. Campbell et al., Medicinal chemistry in drug discovery in big pharma: past, present and future, Drug Discovery Today 23 (2018) pp. 219-234.

NMR-BASED SCREENING: PRINCIPLES AND APPLICATIONS IN THE HIT-TO-LEAD PHASE OF DRUG DISCOVERY PROJECTS

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Scientific Consultant

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NMR spectroscopy is now well recognized as a powerful tool in the hit identification and hit-to-lead optimization phases of drug discovery projects. Although currently NMR cannot compete with other biophysical techniques in term of throughput, NMR has some distinctive features that make it very appealing. The rich information content obtained with NMR, combined with the reliable identification and binding affinity measurement of the hits, avoid squandering time and resources in the pursuit of unsuitable compounds. Furthermore, the possibility of detecting very weak affinity ligands, makes it particularly suitable in Fragment Based Drug Discovery (FBDD) projects and in tackling difficult biological targets with low ligandability. Several observable NMR parameters can be used for identifying and characterizing the interactions of a ligand with the receptor. Most of the experiments utilize ^{1}H NMR spectroscopy. However, it has been shown that improvements in the detection and efficiency can be achieved utilizing ^{19}F NMR spectroscopy.[1] These ^{19}F NMR-based screening methodologies can be classified in three groups based on the detected signals. (i) The protein-based method, known as PrOF (Protein Observed Fluorine),[2] utilizes proteins labelled with selected fluorinated amino acids and monitors chemical shift perturbations of the protein ^{19}F NMR signals in the presence of a binder. (ii) The ligand-based method, known as FAXS (Fluorine, chemical shift Anisotropy and eXchange for Screening),[1] monitors changes in transverse relaxation and/or chemical shift of the ligand ^{19}F NMR signal(s). It can be performed in direct or competition format. The former requires a library of fluorinated molecules whereas the latter requires only one fluorinated suitable reporter molecule. (iii) The substrate-based method, known as n-FABS (n Fluorine Atoms for Biochemical Screening),[3] is a functional assay and utilizes substrates carrying a fluorinated group. It measures the area of the ^{19}F NMR signals of the substrate and product of the enzymatic reaction. Its flexibility allows a broad range of screening applications, from purified enzymes to intact living cells. The principles of these methodologies will be presented along with selected applications in drug discovery projects.

1. C. Dalvit and A. Vulpetti *J. Med. Chem*. 62, 2218-2244 (2019).
2. K.E. Arntson and W.C.K. Pomerantz *J. Med. Chem.* 59, 5158–5171 (2016).
3. C. Dalvit, M. Veronesi and A. Vulpetti *J. Biomol. NMR* 74, 613-631 (2020).

THE ROAD TOWARDS THE OPTIMAL LEAD CANDIDATE: POTENCY AND SELECTIVITY PROFILING BY BIOCHEMICAL AND BIOPHYSICAL APPROACHES

EDOARDO FABINI

Research Scientist

Nerviano Medical Sciences, Nerviano (MI), Italy

Protein kinases are enzymes that regulate biological activities by pinpoint phosphorylation of specific amino acid residues of key proteins. Through this process, they modulate multiple cellular functions including growth and differentiation. Abnormal activities in this set of enzymes can result in diseases and can constitute the driver for tumor insurgence and progression. Kinases share high homology in the ATP-binding pocket; however, the presence of subtle differences can be exploited to obtain chemical entities with high potency and selectivity toward a single member within a kinase family. In recent years, several kinase inhibitors reached clinical approval in oncological target therapy thanks to a vast plethora of assays that have been developed to accelerate the discovery of new lead candidates.

At NMS, the road towards the development of the optimal lead candidate begins with the identification of a target kinase acting as a driver in a specific tumor context. The selected target protein is then expressed, purified and characterized for its activity. A crucial step of the process consists in the development of a screening assay that can reliably quantify target inhibition. The assay is then fully automated and a high-throughput screening (HTS) campaign on proprietary chemical compound libraries is run to identify a primary hit for the target. After the initial hit identification, a careful work of chemical optimization is needed to improve compound potency, selectivity and ADME properties. During development, a special focus is given to the selectivity within the kinome tree. Small molecules are routinely screened against a platform encompassing over 100 kinases to determine cross-reactivity and define a comprehensive selectivity profile. Orthogonal methodologies such as enzyme activity and inhibition studies, homogeneous time-resolved fluorescence assay (HTRF), surface plasmon resonance (SPR), differential scanning fluorimetry (DFS) and bioluminescent resonance energy transfer (nanoBRET) are all used to gain information on the compound under investigation and to characterize different properties of the lead candidate. In this presentation different examples of how hit compounds are developed will be presented, focusing especially on how selectivity within the kinase enzyme family is explored and how different technologies are applied to attain a lead candidate that can further advance in the drug development process.

DESIGN FOLLOWS FUNCTION IN EARLY DRUG DISCOVERY: IN VITRO ASSAYS FOR HTS AND HIT-TO-LEAD PROGRAMS

LIA SCARABOTTOLO

Director Discovery Services

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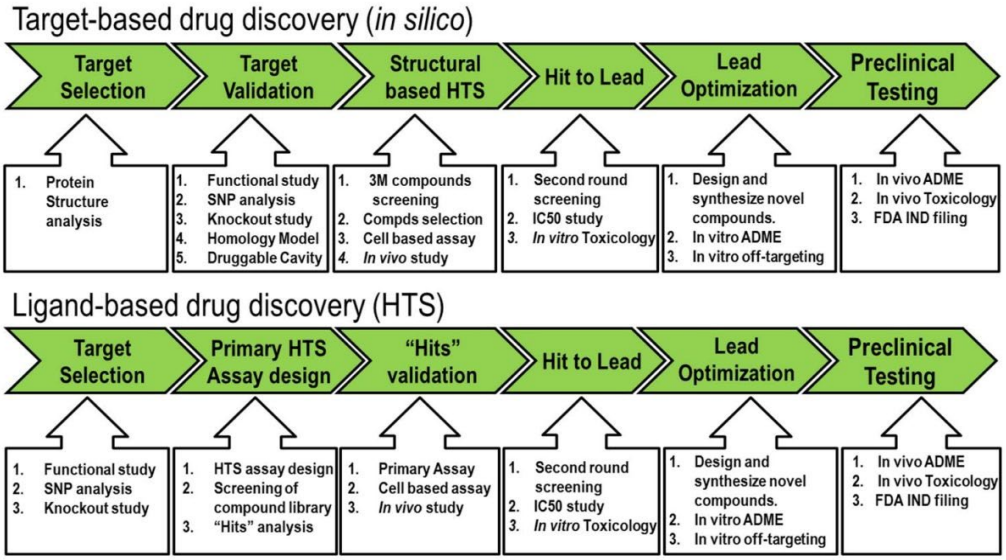
The early process of drug discovery can be simplified in two major approaches, depicted in the figure below:

- a target-based in silico approach, which requires reliable structural information on the protein target.
- a ligand based HTS approach, which uses chemical compound collections



At Axxam we have implemented a state-of-the art facility for the HTS-based approach, with several diversified automated screening stations, including robotic devices for liquid handling and plate assembling and readers for different types of measurements, such as: luminescence, fluorescence, FRET, current amplitude, gene expression, image analysis. With both the in-silico and HTS approaches, the Hit-to-Lead phase begins once the screening campaign has been completed along with the identification and validation of the most promising positive hits.

The aim of the Hit-to-Lead phase is to refine each hit series towards more potent and selective compounds, possessing sufficient properties to be able to examine their efficacy in the next step of *in vivo* preclinical testing. This preclinical phase typically consists of intensive Structure-Activity Relationship (SAR) investigations around each core compound structure, with assays carried out to establish the magnitude of activity (efficacy and efficiency) and selectivity of each compound.

The effectiveness of an HTS campaign substantially depends on the quality of the assays and the compound collection used: biology and chemistry must work in harmony! Axxam has a strong and consolidated expertise in the early drug discovery phases from HTS to Hit-to-Lead, concerning the biology aspects.

The primary assay, used in HT and focused on the molecular target, must achieve the quality parameters of meaningfulness, reliability, and robustness, but should also be low cost and suitable for miniaturization, to allow for a reliable and sustainable screening of hundreds of thousands of molecules. On the other side, the assays used in the Hit-to-Lead phase can be more expensive and adaptable to a lower throughput, but they should be as close possible to the native situation and should provide as much information as possible, to validate the pharmacological improvement of the chemical entities.

In my lecture I will describe examples of cell-free and cell-based *in vitro* assays, developed in our laboratories, for different classes of targets and different final purposes, used in the HTS and Hit-to-Lead phases, as well as how we handle the flow of activities, to guarantee the best turnaround for generating the results.

APPROACHES FOR ADMET PARAMETERS OPTIMIZATION DURING THE DRUG DISCOVERY PHASE: ADVANDAGES AND CAVEATS

GIULIO M. DONDIO

COO

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The high attrition rate during the drug development phase is still representing a key challenge to address for the pharmaceutical industries.
Poor pharmacokinetic (ADME) profiles of the lead compounds was one of the major causes of failure in Phase I clinical trials. In the recent years it seemed that ADME issues were significantly addressed although the lack of efficacy and/or toxicity of the novel chemical entities is nevertheless the primary source of trial failure.
In the last two decades we assisted in a profound paradigmatic shift in the drug discovery process which looked at the optimization of most of the ADMET parameters very early throughout the discovery process by using several surrogate in vitro assays leading to the so called multi-parametric lead optimization strategy.
There are several assays that can be run in different formats during the lifetime of a drug discovery project. However, there is no a rule of thumb indicating which tests and at which stage have to be applied. Nevertheless, there are set of physico-chemical and biochemical assays that are generally though essential to be incorporated very early in most of the drug discovery projects.
A review of the largely used in vitro ADMET assays will be discussed describing advantages and possible issues associated with the interpretation of the associated data.
Suggestions on ADME assays to be employed during lead optimization and pre-IND phase will be discussed also in the light of the most recent FDA and EMA guidelines.

MASS SPECTROMETRIC STRATEGIES IN DRUG METABOLISM AND PHARMACOKINETICS (DMPK) STUDIES

GIANCARLO ALDINI

Full Professor

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Drug Metabolism and Pharmacokinetics (DMPK) is a fundamental aspect within the drug discovery and development process. The key role of DMPK in drug discovery is to participate in the design and selection of the lead compound by balancing the DMPK properties associated with potency, selectivity and safety. Furthermore, DMPK gives additional insight into the processes that control the absorption, distribution, metabolism and excretion (ADME) of the drug candidate (and its metabolites) with the aim of providing a mechanistic understanding of the pharmacokinetics, pharmacodynamics and safety of the drug candidate.

Liquid chromatography/mass spectrometry (LC/MS) is the reference analytical tool used in DMPK studies thanks to its selectivity, low sample volume requirements, sensitivity, and speed. Since the introduction of Atmospheric Pressure Ionization (API) sources such as Electrospray Ionization (ESI), LC-MS is the most used analytical tool for both quantitative and qualitative DMPK analyses. The aim of the seminar is to go over the main methodological and instrumental MS solutions adopted in recent years in DMPK studies. In particular, analytical strategies adapted for metabolite identification and characterization based on triple quadrupole MS analyzers such as precursor ion and neutral-loss experiments will be shown, together with the applications of ion traps for MS^n and high-resolution MS systems (HRMS). Furthermore, chemical strategies based on derivatization, isotopic fingerprinting and H/D exchange experiments will be presented.

LC-MS strategies for absolute- and semi-quantitative analysis will also be discussed. In particular, applications of LC-Triple quadrupoles (TQMS), which became the workhorse for quantitative bioanalysis in the 1990s, will be considered, together with the most recent advantage of hybrid systems such as quadrupole-TOF and quadrupole-orbitrap which, beside being the gold standard for qualitative analyses, now also offer the right performance for quantitative analyses, thanks to sensitivity, dynamic range, resolution, accuracy and
scan-to-scan reproducibility, making them a worthwhile alternative to TQMS systems.

Finally, analytical methods to predict compounds which can potentially induce idiosyncratic reactions by forming protein covalent adducts will be discussed. Also in this regard different MS strategies will be discussed such as the neutral loss scanning based on a triple quadrupole mass spectrometer which was the first LC/MS method developed for detecting reactive metabolites trapped by GSH in studies of bioactivation of drugs or other xenobiotics

Methodological insights together with application and case studies will be considered in the seminar.

THE BENEFIT OF ION MOBILITY MASS SPECTROMETRY FOR METABOLITE IDENTIFICATION

SIMONA SCARPELLA

Mass Spectrometry Sales Specialist Italy

Waters S.p.A, Sesto San Giovanni (MI), Italy

Metabolite identification plays a crucial role in drug development, leading to a constant need to evaluate new technologies that can improve data quality or increase efficiencies. The metabolite identification challenges that technologies such as IMS can address depend on the drug development stage.

Liquid chromatography–mass spectrometry (LC–MS) helps to address these challenges by separating compounds in mixtures using two dimensions, namely retention time (RT) and mass-to-charge ratio (m/z). Careful and reproducible analytical measurement of both properties usually allows the DMPK scientist to determine what overall biotransformations have occurred and what the structures look like over many samples or studies. Different acquisition methods are also employed, such as data dependent and data-independent acquisition (DDA and DIA, respectively). DIA methods, such MS^E rapidly alternates between full scan low collision and high collision energies, allowing both precursor (drug and metabolite) and product ion high resolution accurate mass measurements to be obtained. IMS has now evolved to afford an added dimension of separation to metabolite identification, based on an ion's size and shape, along with an accurate mass-to-charge ratio, as it passes through a gas such as nitrogen. This property can be used to generate a collision cross section (CCS) value, correlated to its drift time (DT), that describes the ion's overall size (influenced by structure, charge, conformation, and interaction with the gas). The development of IMS is decades-old, but its availability in combination with commercialized LC–MS instruments has led to increased interest in its relevance for a range of applications, including metabolite identification. Ion mobility supported on commercial LC–MS platforms now includes traveling wave ion mobility (TWIMS), drift tube ion mobility (DTIMS), and trapped ion mobility (TIMS), all of which generate CCS values that can be stored in customized libraries. Spectral clarity is improved by using DT to align retention time data and filter out nonpertinent ions, which is especially important when looking for metabolite fragment ions in biological matrices of preclinical toxicity models. In addition, the precise CCS value, which is matrix and ion-concentration independent, can be used to track isomeric metabolites across chromatographic conditions and resolve coeluting compounds throughout the entire drug development life cycle. This ability to more confidently detect, characterize, and track metabolites across time and geographies without worrying about varying chromatographic conditions greatly eases the burden of the biotransformation scientist.

LC–MS in combination with IMS generates high-quality data that directly impacts the confidence of later conclusions; however, software is needed to easily manage, analyze, and store these complex data sets efficiently. Fortunately, several solutions have been developed that incorporate IMS into software packages at each stage of development and across molecule types.

Conclusion. New technologies for metabolite identification workflows, such as ion mobility, are needed to generate the best data, within the needed time frame, at the appropriate phase of the drug discovery and development process. IMS delivers this capability by adding another separation dimension that increases confidence in identification and structural elucidation, distinguishes coeluting metabolites, and tracks metabolites across different matrices and chromatographic conditions using CCS values. When combined with the effective software, ion mobility can improve efficiencies and workflows to deliver real value to the metabolite identification laboratory.

IN VITRO AND IN VIVO DRUG DISCOVERY QUANTITATION OF SMALL MOLECULES BY LC-MS/MS: A TIERED APPROACH

ROSSELLA PISANO

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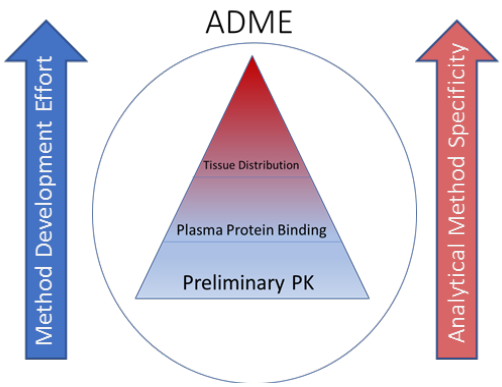
In the hit-to-lead drug discovery stage, several experiments for ADME assessment such as preliminary PK, tissue distribution in rodents and plasma protein binding require compound quantitation in biological matrices. The preferred analytical technique for small molecules is LC-MS/MS.
The method should be developed with adequate sensitivity, selectivity and linearity even though often without the use of Internal Standard (IS).
A tiered approach is adopted based on the method application.

A dedicated time-saving workflow supports the high-throughput preliminary PK assessments service available at Accelera: a generic protein precipitation sample preparation combined with a column switching/mobile phase test is used for chromatographic method development. Chromatographic methods with isocratic/ gradient elution and gradient wash, run times< 5 min (or <4 min with UHPLC) and k'>2 are selected. Moreover, the use of surrogate matrix and reduced matrix volume for extraction comply with the 3Rs rules.



The LC-MS/MS quantitative analytical methods that support ADME in vitro studies such as plasma Protein Binding (PPB) are often in the latest lead optimization stage: the LLOQ of the method must be adjusted to be suitable to determine the free drug at the lowest concentration tested in multispecies equilibrium dialysis experiments.
LC-MS/MS quantitative analytical methods are also applicable to tissue distribution studies performed in rodents during the discovery phase.
Homogenizers, sonicators, microdismembrators, hydrolysis are used for sample preparation of matrices such as brain, lung, liver, eyes, intestine and tumor.
At Accelera a bead beaten homogenizer (Precellys Evolution) is used to prepare 24 samples in parallel, in a rapid (few minutes) and easy way. This technique is routinely applied for mouse and rat brain drug quantification alongside plasma preliminary PK. It is also applied successfully to tissues like kidney, skin, heart and liver.
Complex tissue matrices might need a higher specificity of the analytical method to prevent potential interferents from affecting the determination of the analyte.

Case studies of methods developed in our laboratories to support potential anti-cancer drug candidates in PK, PPB and tissue distribution experiments are presented. Triple quadrupole instruments API5000, API5500 and API6500 coupled with HPLC were used for analyte determination within different concentration ranges.
The whole bioanalytical workflow for sample and data management, from the study design to reporting, was supported by Watson LIMS software.

BIONALYSIS IN DRUG DISCOVERY: CHALLENGES AND APPROACHES

MARCO MICHI

Senior Research Leader

Aptuit (Verona) Srl, an Evotec Company, Discovery DMPK, Verona, Italy

Bioanalysis in support to the drug discovery processes has specific peculiarities that are different from those related to the drug development support. Rapid turnaround, hundreds of compound to be tested per month, and the absence of any previous information on stability, in vivo exposure, analytical challenges and metabolism represent a unique challenging situation where speed of analysis should meet reliability of the data. The lack of a proper internal standard represent another important limitation for LC-MSMS methods that may be more susceptible of matrix effect issues. In this presentation it will be described a general overview of the sample preparation techniques, the matrix effect common issues and how to deal with them, the method development approach and metabolism aspect to be considered when using different ionization techniques.
In the last decades the number of biologics and multi domain drugs such as ADCs in the discovery pipeline of BioTech and Pharma companies increased significantly posing new challenges for better driving the drug design process. At the same time LCMS technology continued to evolve improving the instrument sensibility and enabling the application of this technology platform to the bioanalysis of PK studies as an alternative to the well-established ELISA. In particular the possibility to develop generic assays without the need of specific reagents together with the multiplexing capability of the LCMS is an attractive approach in the Drug Discovery environment. An overview of the therapeutic proteins quantitative analysis using LC-MS/MS will be also discussed.

ANALYTICAL SUPPORTING DEVELOPABILITY ASPECTS OF NEW CHEMICAL ENTITIES FOR EARLY FORMULATION SCREENING

EMANUELA DEL VESCO

SVP Pharmaceutical Development and manufacturing

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Designing and selecting appropriate lead candidates is key in reducing clinical attrition and costs associated with the development of new drugs. Early formulation work is performed across these phases ensuring a smooth transition between Discovery and Development phases. In this contest analytical support is focused mainly into solid form optimization screening, solubility and stability profiling and on overall biopharmaceutical evaluation to select the appropriate attributes for identified best lead candidates. This includes not only experimental protocols, appropriate criteria selection but also reinforced interactions and close communication between department experts for data cross –review and ensure key decision making and mitigation plans established at the stage of candidate entry into development“.

ENABLING CHEMICAL TECHNOLOGIES TO AUTOMATE PROCESS OPTIMIZATION AND MEDICINAL CHEMISTRY

ANTIMO GIOIELLO

Associate Professor

Affiliation: University of Perugia, Department of Pharmaceutical Sciences, Perugia, Italy

Small-molecule drug discovery remains an enormous challenge in which various compounds characteristics, as the potency, selectivity, in vivo efficacy and safety, need to be fine-tuned to provide novel active pharmaceutical ingredients. To this aim, the ability to rapidly prepare compound collections for medicinal chemistry learning cycles and the capacity to optimize processing methods of lead products for clinical studies, are crucial for the success rate of drug discovery programs.[1] In this context, continuous flow chemistry and related technologies are considered valuable tools to solve synthesis limitations in terms of compound throughput, quality and scalability, and have shown great potential in uncovering and developing novel drug candidates.[2]

In this lesson, case studies on the use of enabling chemical technologies to expedite hit-to-lead explorations and optimize chemical processes are discussed. The scope is to illustrate how innovative approaches as flow chemistry and automation can be exploited for streamlined compound synthesis and characterization. Particular emphasis will be given to the integration of flow synthesizers with process control devices, in-line analysis, and downstream operations (Figure 1) to realize systems capable of reaction optimization, multistep continuous synthesis, and parallel/sequential compound library preparation, that can be coupled with bio-assays and predictive computational tools and accelerate medicinal chemistry discovery cycles.

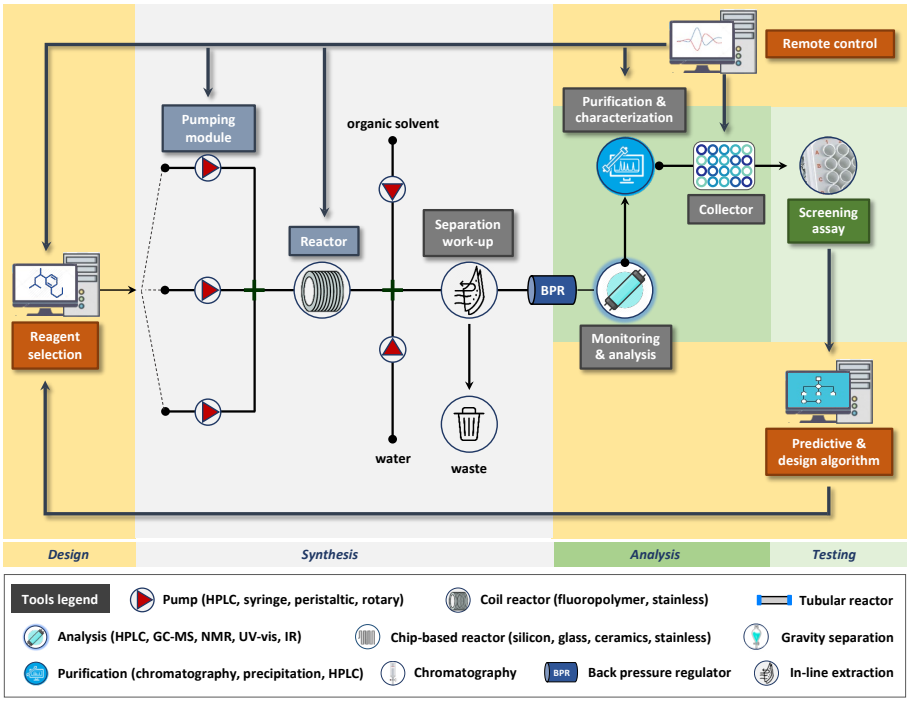

Figure 1.

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"CHROMATOGRAPHIC ISOTOPE EFFECT": RETENTION TIME CHANGES FOR POLYDEUTERATED LAQUINIMOD IN REVERSE PHASE HPLC

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Laquinimod is a medicine developed by Active Biotech and Teva for the treatment of multiple sclerosis (MS) and additional indications of other autoimmune and neurodegenerative diseases.
As a part of development, several polydeuterated analogues of Laquinimod were prepared. During analytical method development we observed noticeable reduction of retention time with increase of deuterium atoms in the molecule.D_5-Laquinimod (five hydrogen atoms replaced with deuterium) elutes about 0.2 min earlier than the non-deuterated (RT is about 15.7 min), while D_{16}-Laquinimod (sixteen hydrogen atoms replaced with deuterium) elutes more than 1.0 min earlier.
The difference of chromatographic behavior of deuterated compounds, as compared to non-deuterated prototypes, is discussed in relation with the differences between C-H and C-D bonds. Deuterium atom is smaller than hydrogen; consequently, C-D bonds are shorter than C-H, which results in smaller "molar volume" and reduced lipophilicity of polydeuterated molecules. RP chromatography is based, mainly, on hydrophobic interactions. Therefore retention of compounds with lower lipophilicity should be weaker. As a result, in RP LC, molecules containing heavier isotopes of hydrogen elute earlier. This was suggested as a major contribution to the observed "Isotope effect".