Sarvani V. *et al.* / *International Journal of Pharmacy & Therapeutics*, 4(3), 2013, 148-153.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

ROLE OF LC-MS IN DRUG DISCOVERY PROCESS

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ABSTRACT

The combination of liquid chromatography and mass spectrometry (LC–MS) is a powerful analytical tool and is widely applied in these days. The use of LC-MS is increasing and is proven to be useful in many areas of drug discovery like identification of lead molecule, identification of impurities and study of pharmacokinetic and metabolism profiles. High-throughput LC-MS-systems are used in the "accelerated drug discovery" process studies. The current developments in liquid chromatography-mass spectrometry (LC-MS) and its applications to the drug discovery process are reviewed in the present article.

Key Words:- Liquid Chromatography(LC), Mass Spectroscopy, Drug discovery.

INTRODUCTION

Chromatographic process can be defined as separation technique involving mass-transfer between stationary and mobile phase. High Performance Liquid Chromatography (HPLC) is one mode of chromatography, one of the most used analytical techniques. HPLC utilizes a liquid mobile phase to separate the components of a mixture. The stationary phase can be a liquid or a solid phase. These components are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure. In the column, the mixture separates into its components. The amount of resolution is important, and is dependent upon the extent of interaction between the solute components and the stationary phase. The stationary phase is defined as the immobile packing material in the column. The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases. As a result, HPLC acquires a high degree of versatility not found in other chromatographic systems and it has the ability to easily separate a wide variety of chemical mixtures (Chen G *et al*., 2007).

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster It also allows you to use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture. The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive (Chen G *et al*., 2007)

Introduction of Mass Spectrometry

Mass spectrometry is an analytical tool used for measuring the molecular mass of a sample.

A mass spectrometer determines the mass of a molecule by measuring the mass-to-charge ratio (*m/z*) of its ion. Ions are generated by inducing either the loss or

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gain of a charge from a neutral species. Once formed, ions are electro statically directed into a mass analyzer where they are separated according to *m/z* and finally detected. The result of molecular ionization, ion separation, and ion detection is a spectrum that can provide molecular mass and even structural information (Kyranos JN *et al*., 2001).

In order to measure the characteristics of individual molecules, a mass spectrometer converts them to ions so that they can be moved about and manipulated by external electric and magnetic fields (Clarke NJ *et al*., 2001). The three essential functions of a mass spectrometer, and the associated components, are:

- A small sample is ionized, usually to cations by loss of an electron. The Ion Source
- The ions are sorted and separated according to their mass and charge. The Mass Analyzer
- The separated ions are then measured, and the results displayed on a chart. The Detector

Each of the three tasks listed above may be accomplished in different ways. In one common procedure, ionization is effected by a high energy beam of electrons, and ion separation is achieved by accelerating and focusing the ions in a beam, which is then bent by an external magnetic field (Ackermann BL *et al.,* 2002). The ions are then detected electronically and the resulting information is stored and analyzed in a computer. A mass spectrometer operating in this fashion is outlined in the following diagram.

The heart of the spectrometer is the ion source. Here molecules of the sample (black dots) are bombarded by electrons (light blue lines) issuing from a heated filament. This is called an EI (electron-impact) source. Gases and volatile liquid samples are allowed to leak into the ion source from a reservoir (as shown). Non-volatile solids and liquids may be introduced directly. Cations formed by the electron bombardment (red dots) are pushed away by a charged repellor plate (anions are attracted to it), and accelerated toward other electrodes, having slits through which the ions pass as a beam. Some of these ions fragment into smaller cations and neutral fragments (Ermer J *et al*., 1948). A perpendicular magnetic field deflects the ion beam in an arc whose radius is inversely proportional to the mass of each ion. Lighter ions are deflected more than heavier ions. By varying the strength of the magnetic field, ions of different mass can be focused progressively on a detector fixed at the end of a curved tube (also under a high vacuum).

Combination of LC-MS

The combination of liquid chromatography and mass spectrometry (LC–MS) is a powerful and indispensable analytical tool that is widely applied in many areas of chemistry, medicine, pharmaceutics and biochemistry. The power of MS, especially when coupled to LC, is recognized by clinical laboratories worldwide and the growing versatility of these systems puts clinical laboratories in a position where they can provide a rapid response to changing clinical needs. The principle of MS is the production of ions from analyzed compounds that are separated or filtered on the basis of their mass-to charge ratio (m/z). Innovative and successful research efforts in the past decades on the design of an effective interface connection between LC (operated under atmospheric pressure) and MS (operated under a high-vacuum environment) have made LC congenial with MS. This technique provides a higher level of sensitivity and specificity. Besides specificity and sensitivity the ability of these techniques to measure multiple analytes simultaneously is a tremendous benefit of LC coupled to MS methods since many other techniques are limited to determine one analyte at a time. Liquid chromatography is a fundamental separation technique in the life sciences and related fields of chemistry. Liquid chromatography can safely separate a very wide range of organic compounds, from small-molecule drug metabolites to peptides and proteins. Mass spectral data add specificity that increases confidence in the results of both qualitative and quantitative analyses. Some mass spectrometers have the ability to perform multiple steps of mass spectrometry on a single sample. They can generate a mass spectrum, select a specific ion from that spectrum, fragment the ion, and generate another mass spectrum; repeating the entire cycle many times. Such mass spectrometers can literally deconstruct a complex molecule piece by piece until its structure is determined. Application areas for LC–MS or LC–MS/MS in the clinical laboratory are therapeutic drug monitoring, neonatal screening, reference methods, and toxicology (Geoghegan KF and Kelly MA, 2005).

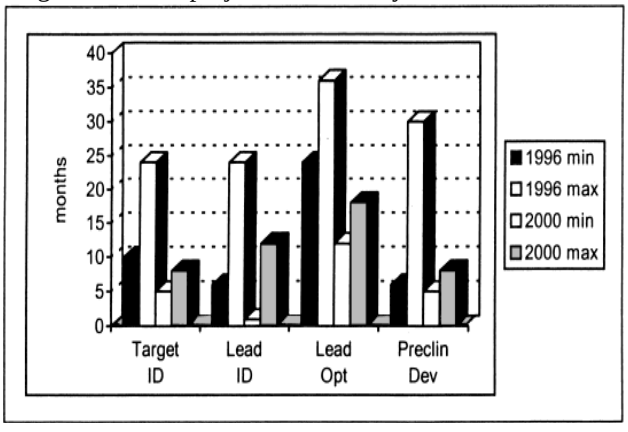
Drug development consists of four distinct stages:
(1) Drug discovery;
(2) Preclinical development;
(3) Clinical development; and
(4) Manufacturing

Drug Discovery

- The goal of the drug discovery stage is to generate a novel lead candidate with suitable pharmaceutical properties (i.e., efficacy, bioavailability, toxicity) for preclinical evaluation (Lim C.K. and Lord G *et al*., 2002).
- Potential lead compounds contained in natural product sources or from the extensive database of a synthetic compound library are screened for activity.

- Lead compounds identified from screening efforts are optimized in close collaboration with exploratory metabolism programs and drug safety evaluations.
- In 1997, it was estimated that the synthesis and screening of ca. 100,000 compounds is typically required for the discovery a single quality lead compound.
- The process of identifying a lead compound can take up to 2-4 years. Optimization of the resulting lead may take an additional 1-2 years.
- The drug discovery stage involves three primary analysis activities: target identification; lead identification; and lead optimization.

The maximum and minimum development times for a drug in 1996 and projections for the year 2000



PRECLINICAL DEVELOPMENT

- The preclinical stage of drug development focuses on activities that are necessary for filing an Investigational New Drug (IND)/Clinical Trail Application (CTA).
- Process research, formulation, metabolism, and toxicity are the major areas of responsibility in this development stage (Janiszewski JS *et al*., 2001).
- Analysis activities that feature LC/MS primarily focus on the identification of impurities, degradants, and metabolites.
- Preclinical development activities are completed in 10-15 months.
- During preclinical development, the structure, physical, and chemical characteristics, and stereochemical identity of the IND/CTA candidate are fully characterized.
- Appropriate bioanalytical methods are developed for the evaluation of pharmacokinetics, typically a series of studies focusing on absorption, distribution, metabolism, and excretion (ADME) in toxicology species, as well as systemic exposure and metabolism in toxicological and clinical studies (Ackermann BL *et al*., 2002).

CLINICAL DEVELOPMENT

- The clinical development stage is comprised of three distinct components or phases (I-III), and culminates in the filing of the New Drug Application (NDA)/Marketing Authorization Application (MAA). Each phase involves process scale-up, pharmacokinetics, drug delivery, and drug safety activities.
- During phase I clinical development, the compound's safety and pharmacokinetic profile is defined.
- The determination of Cmax, AUC, elimination half-life, volume of distribution, clearance and excretion, and potential for drug accumulation is made in addition to studies that provide estimates of efficacious doses.

Experiments to Assess ADME Characteristics (Eddershaw PJ *et al*., 2000)

Absorption

- Caco-2 cells, PAMPA, PgP-transport
- In vivo PK profiling

Distribution

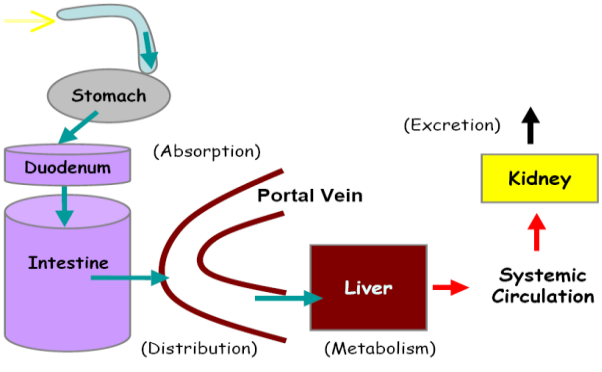
- In vitro protein binding, in vivo tissue distribution studies

Metabolism (Korfmacher W *et al*., 2005)

- Metabolic stability in microsomes, S9 fractions, hepatocytes
- P450 Inhibition: microsomes and/or rCYPs, co-administration
- P450 Induction: Gene Chips, PXR, multiple dosing studies
- Metabolite characterization (Cox K *et al*., 2005).

Excretion

- Quantitation of drugs and metabolites in biological fluids



- After acceptable safety and pharmacokinetic data are observed in phase I trials, phase II studies are initiated with the goal of establishing efficacy, determining the effective dose range, and obtaining safety and tolerability data (Janiszewski JS *et al*., 2001).

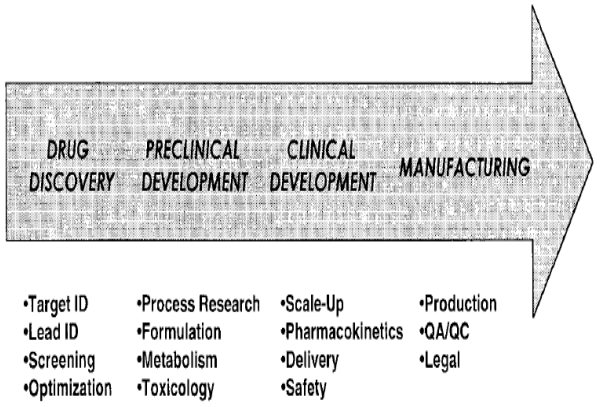
- In phase II, the dose and dosing interval to be used in the patient population are defined as well as the estimated no-effect dose.
- Phase II studies may require 1-1.5 years to complete.
- The goal of phase III is to complete the human safety and efficacy programs and to secure approval.
- Typical pharmacodynamic evaluations include blood glucose monitoring and blood pressure.
- Safety evaluations include physical examinations and clinical laboratory tests (i.e., liver function tests) performed before dosing and before discharge.

MANUFACTURING (Clarke NJ *et al*., 2001)

- Once formulated, the drug is packaged and readied for distribution to pharmacies.
- Manufacturing processes and facilities undergo a preapproval regulatory review and periodic inspections once production is in progress.
- This information and technology are formally transferred for routine monitoring and release by quality control (QC) scientists in manufacturing groups.
- During the manufacturing stage, comparisons are made to other drug products in the same category, including stability, bioavailability, and purity.
- Manufacturing interruptions occur due to contamination by packaging materials or unexpected impurities that exceed product specfications.
- Also, with the growing use of outsourced services for the product manufacturing of intermediates, drug substance and drug product, out-of-specfication results must be immediately addressed

INTEGRATION OF LC/MS INTO DRUG DEVELOPMENT (Geoghegan KF and Kelly MA, 2005)

- Liquid chromatography/mass spectrometry (LC/MS)-based techniques provide unique capabilities for pharmaceutical analysis.
- LC/MS methods are applicable to a wide range of compounds of pharmaceutical interest, and they feature powerful analytical figures of merit (sensitivity, selectivity, speed of analysis, and cost effectiveness).
- The growth in LC/MS applications has been extensive, with retention time and molecular weight emerging as essential analytical features from drug target to product.
- LC/MS-based methodologies that involve automation, predictive or surrogate models, and open access systems have become a permanent fixture in the drug development landscape.



APPLICATIONS OF LC-MS IN DRUG DISCOVERY PROCESS

- In the drug discovery stage the lead candidate is analyzed for protein identification, natural protein identification and metabolite stability profile which are done by using LC-MS (Chen G *et al*., 2007).
- During drug discovery process molecular weight determination for combinational/medicinal chemistry support LC-MS is used for analysis of lead candidate.
  - In target identification step peptide mapping in protein identification and glycoprotein mapping in glycoprotein identification is done by using LC-MS.
  - In lead identification step the natural products de replication and bio affinity screening analysis is done by using LC-MS (Eddershaw PJ *et al*., 2000).
  - In lead optimization step the pharmacokinetic screening and metabolic stability analysis are done by using LC-MS.

- In the preclinical development stage for the evaluation of impurity, degradant and metabolite identification LC-MS is used (Lim CK and Lord G, 2002).
  - In metabolite identification the standard methods and databases can be prepared by using LC-MS.
  - In impurity identification the natural products and the standard methods and databases can be prepared by using LC-MS.
  - In degradant identification predictive models for chemical degradants and predictive models for biomolecule degradants can be done by using LC-MS.
- In the clinical development stage the quantitative bioanalysis and structure identification analysis are done by using LC-MS.
  - In quantitative bioanalysis the selected ion monitoring, selected reaction monitoring, automated

offline solid phase extraction, automated on-line extraction can be done by using LC-MS.

- In metabolite identification the template structure identification can be done by using LC-MS.
- In manufacturing stage the analysis of impurity and degradant identification are done by using LC-MS.
- In impurity identification the data dependent analysis can be done by using LC-MS.

Figure 1. HPLC Instrumentation

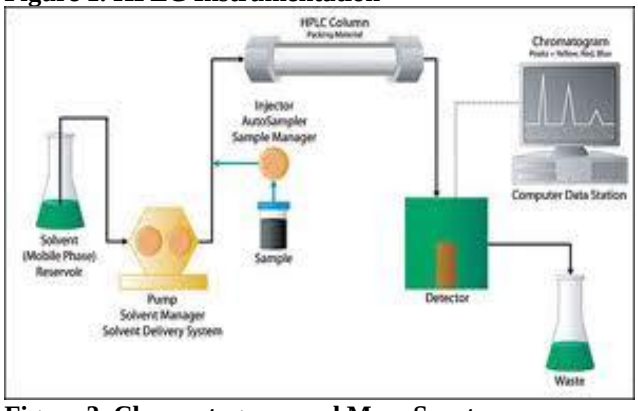


Figure 2. Diagram of Mass spectrum

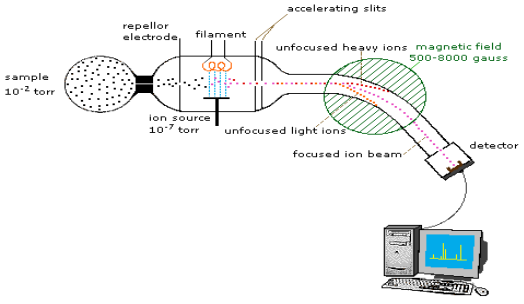
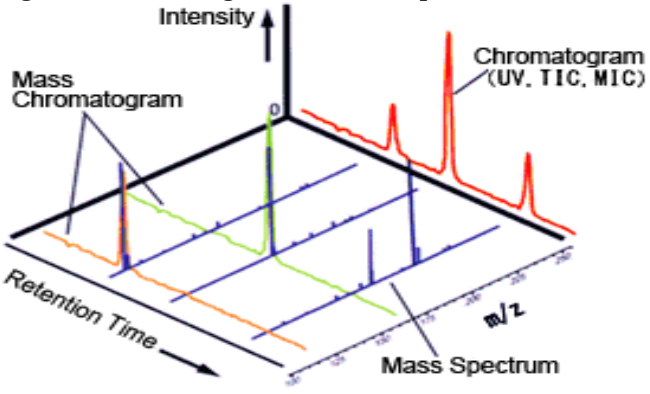


Figure 3. Chromatogram and Mass Spectrum



DRUG DISCOVERY PROCESS

Figure 4. Stages in Drug Discovery Process

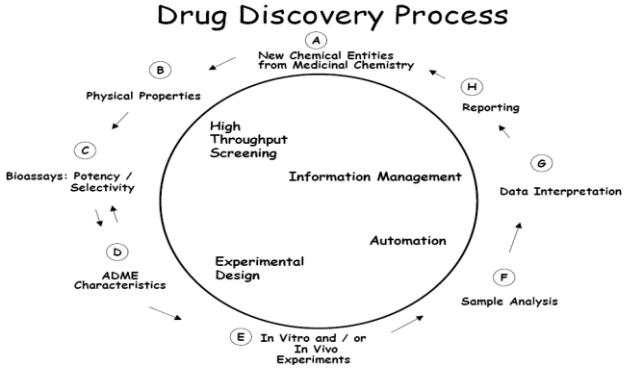


Figure 5. Stages of new drug discovery from the initial synthesis to Phase I in humans.

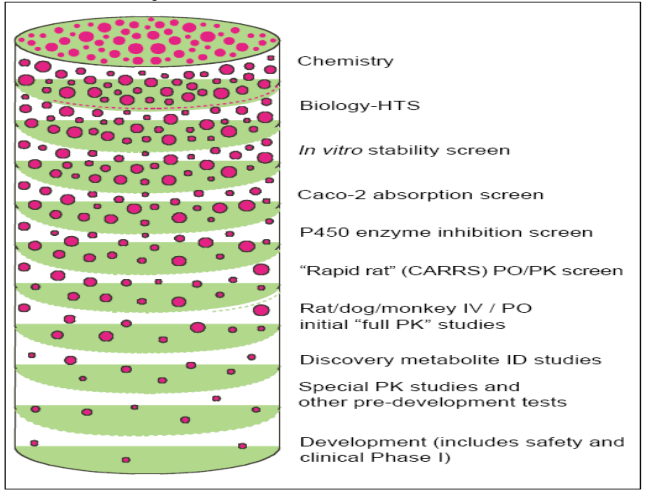
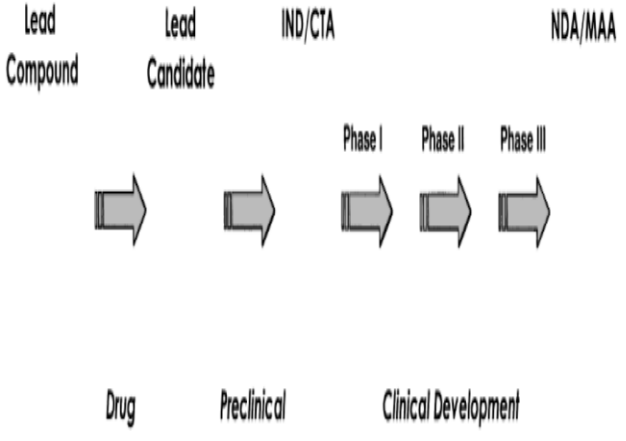


Figure 6. The stages of drug development and associated pharmaceutical analysis activities



CONCLUSION

The combination of liquid chromatography and mass spectrometry (LC–MS) is a powerful analytical tool and is widely applied in these days. The use of LC-MS is increasing and is proven to be useful in many areas of drug discovery like identification of lead molecule, identification of impurities and study of pharmacokinetic and metabolism profiles, quantitative bioanalysis and structure identification analysis, analysis of impurity and degradant identification are done by using LC-MS. High-throughput LC-MS-systems are used in the "accelerated drug discovery" process studies.

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