

# Research & Reviews: Journal of Pharmaceutical Analysis

## A Short Commentary on Bioanalytical Techniques

N Venkateswarlu\*

Department of Pharmacy, Vagdevi Institute of Pharmacy, Nellore, Andhra Pradesh-524001, India

### Short Commentary

Received: 03/03/2015

Revised: 26/03/2015

Accepted: 31/03/2015

#### \*For Correspondence

N Venkateswarlu, Department of Pharmacy, Vagdevi Institute of Pharmacy, Nellore, Mobile: +91 9000187511, E-mail: [nussum.venkatesh@gmail.com](mailto:nussum.venkatesh@gmail.com)

Keywords: Xenobiotics, bioanalysis, bioequivalence, Mass spectroscopy

#### ABSTRACT

The development of the bioanalytical techniques brought a progressive discipline that the long run holds several exciting opportunities to any improvement. The most impact of bioanalysis within the pharmaceutical trade is to get a quantitative live of the drug and its metabolites. The aim is to perform the pharmacological medicine, toxicokinetics, and bioequivalence and exposure response like pharmacokinetic/pharmacodynamic studies. Numerous bioanalytical techniques area unit performed in bioanalytical studies like combined techniques, activity techniques, and matter binding assays. This review extensively highlights the role of bioanalytical techniques and combined instruments in assessing the bioanalysis of the medicine.

#### INTRODUCTION

The field of bioanalysis has matured considerably from early studies in drug metabolism victimization several easy and advanced techniques, and in today's Bioanalyst is well equipped to cope with the trendy challenges. A bioanalytical technique may be a set of procedures concerned within the assortment, processing, storage, and analysis of a biological matrix for a substance. Bioanalytical technique validation (BMV) is that the method won't to establish that a quantitative analytical technique is appropriate for organic chemistry applications. Bioanalysis covers the quantitative activity of Xenobiotics of medicine like their metabolites, and biological molecules in unnatural locations or concentrations and Biotics like macromolecules, proteins, DNA, giant molecule medicine, metabolites in biological systems. Bioanalysis<sup>[1-6]</sup> may be a progressive discipline that the long run holds several exciting opportunities to any improve sensitivity, specificity, accuracy, efficiency, assay outturn, information quality, information handling and process, analysis value and environmental impact. The most impact of bioanalysis within the pharmaceutical trade is to get a quantitative live of the drug or its metabolites for the study of pharmacological medicine, toxicokinetics, bioequivalence and exposure-response like pharmacokinetic/pharmacodynamic studies. The main target of bioanalysis within the pharmaceutical trade is to produce a quantitative live of the active drug and/or its metabolite(s) for the aim of pharmacological medicine, toxicokinetics, bioequivalence and exposure-response (pharmacokinetics/pharmacodynamics studies). The dependableness of analytical findings may be a matter of nice importance in rhetorical and clinical materia medica, because it is in fact a requirement for proper interpretation of pharmacology findings. Unreliable results won't solely be oppose in court, however may conjointly cause unwarranted legal consequences for the litigant or to wrong treatment of the patient. Within the last decade, similar discussions are happening within the closely connected field of pharmacokinetic (PK) studies for registration of prescribed drugs.

As per Bioanalytical technique Validation (BMV)<sup>[7-11]</sup> pointers for trade, these pointers area unit applied to bioanalytical strategies that area unit used for the quantitative determination of medicine and their metabolites in biological matrices like plasma, body waste and diagnosis studies. Bioanalytical technique validation includes all of the procedures that demonstrate that a specific technique developed and used for quantitative activity of analytes in an exceedingly given biological matrix is reliable and reproducible. Validation of a bioanalytical technique is that the method by that it's established that the

performance characteristics of the strategy meet the wants for the meant bioanalytical application. This performance characteristics area unit expressed in terms of bioanalytical technique validation parameters. The elemental bioanalytical technique validation <sup>[11-15]</sup> parameters embody preciseness, accuracy and sensitivity.

### **Bioanalytical Techniques**

Some techniques normally utilized in bioanalytical studies embody

Hyphenated techniques

- LC-MS (liquid chromatography-mass spectrometry)
- GC-MS (gas chromatography-mass spectrometry)
- CE-MS (capillary electrophoresis-mass spectrometry)

Chromatographic strategies

- HPLC (high performance liquid chromatography)
- Gas activity

### **LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS)**

Bioanalytical liquid activity-mass spectroscopic analysis may be a technique that uses liquid chromatography with the mass spectroscopic analysis. LC-MS <sup>[16-25]</sup> is often utilized in laboratories for the quantitative and chemical analysis of drug substances, drug product and biological samples. LC-MS has competed a major role in analysis and interpretation of bioavailability, bioequivalence and pharmacokinetic information. Through LC-MS biological samples area unit determined throughout all phases of technique development of a drug in analysis and internal control.

### **Method Development**

Method of study area unit being habitually developed, improved, validated, collaboratively studied and applied. Activity separations area unit chiefly needed that rely upon the samples to be analyzed. The activity procedure is vital for the general approach to LC-MS/MS technique development. In most cases as desired separation is achieved simply with solely some experiments. In alternative cases a substantial quantity of experimentation could also be required.

#### ***Procedure for technique Development***

- Collect the chemistry properties of drug molecules from the literature.
- confirm solubility profile
- MS scanning and optimization
- Mobile section choice
- Choice of extraction technique and optimization
- Choice of activity technique (based on solubility study, retention of compound)

#### ***Reversed section Chromatography***

Reversed section packing's like C<sub>18</sub>, C<sub>8</sub> area unit the foremost widespread and most generally used for reversed section <sup>[26-30]</sup>. Additionally to those C<sub>4</sub>, C<sub>2</sub> and phenyl secured are on the market. Reversed section sorbents usually involves learning with Associate in nursing organic solvent (e.g. methanol) followed by Associate in nursing binary compound solvent (e.g. water).

#### ***Normal section Chromatography***

Traditional section packing's embody oxide, amino and aluminium oxide. Traditional section packing usually needs learning with a non-polar solvent and extraction is carried with polar solvents.

Compounds that area unit with basic hydrogen ion concentration practical team's area unit maintained by oxide. However, polar compounds area unit irreversibly maintained on a oxide surface and during this case amino could also be used.

### **Steps in LC-MS/MS technique Development**

Proper information regarding the sample is critical for an efficient technique development. Some data relating to the analyte is critical like

- Variety of compounds gift
- Molecular weights of compound
- Sample Solubility
- Drug Stability
- Concentration vary of compounds in samples of interest

### **Method Optimization**

During the optimization stage, the initial sets of conditions that were evolved throughout the strategy development area unit improved and maximized in terms of resolution and peak form, plate counts imbalance, capacity, extraction time, detection limits, limit of quantization, and overall ability to quantify the particular analyte <sup>[31-35]</sup> of interest. Optimization of a way will follow either of 2 general approaches like manual or laptop driven. The manual approach includes variable one independent variable at a time, whereas holding all others constant, and recording the changes in response. The variables may embody flow rates, mobile or stationary section composition, temperature etc.

### **Mode of Separation Technique**

Since most of the pharmaceutical compounds area units polar in nature thus reverse section activity is generally tried initial within which a non-polar stationary section <sup>[35-38]</sup> is employed. The mobile section consists of water or buffer and organic section (acetonitrile or methanol). Thus polar compounds get eluted initial and non-polar compounds area unit maintained for an extended time. The stationary sections utilized in reverse phase activity area unit n-octadecyl (RP-18), n-octyl (RP-8), ethyl group (RP-2), phenyl, cyano, alcohol and hydrophobic polymers. It's the primary alternative for many samples; particularly neutral or un-ionized compounds that dissolve in water-organic mixtures. Traditional section is tried if reverse section fails wherever the sample could also be powerfully maintained with 100% acetonitrile as mobile section.

### **Selection of Stationary Phase/Column**

Prior to choice of column it's necessary to grasp the properties of column packing. oxide tends to dissolve higher than hydrogen ion concentration eight and cross-linked compound particles, for instance, phenylethylene or poly methacrylates area unit used for separation of bases, which may face up to powerfully basic mobile section. Oxide particles have surface silanol teams, -SiOH that area unit used for chemical bonding of stationary phases by silanization reactions with chlorosilanes. Regarding 1/2 the silanol team's area unit with chemicals secured and also the rest area unit capped with tri methyl radical silyl teams to render them inert. The foremost normally used non-polar secured sections (for reversed phase chromatography) area unit C18 and C8 with C18 being the foremost widespread (known as ODS for octadecylsilane); C8 is intermediate in property, wherever C18 is non-polar.

### **Selection of Mobile section**

The main criterion in choice and optimisation of mobile section is to attain optimum separation of all the individual impurities and degradants from one another and from the analyte peak. The parameters which require to be thought-about whereas choosing and optimizing the mobile section area unit buffer, hydrogen ion concentration of the buffer and mobile section composition.

### **Mass chemical analysis Detection and information system**

Liquid activity/mass spectroscopic analysis (LC-MS) <sup>[39-41]</sup> is promptly turning into the well-liked tool of liquid chromatography. It's powerful analytical technique that mixes the resolution of liquid activity with the detection specificity of mass spectroscopic analysis. Liquid activity separates the sample parts and so introduced them to the mass spectroscopic analysis. Mass spectroscopic analysis creates and detects charged ions. The LC-MS information could also be wont to offer the data regarding molecules weight, structure, identification, amount of specific sample parts. Structural data can even be generated by victimization sure sort of mass spectrometers typically those that area unit used with multiple analyzers that are called tandem bicycle mass spectrometers. This could be achieved by fragmenting the sample within the instrument and analyzing the product generated.

### **Mass spectroscopic analysis**

Mass spectrometers area unit divided into 3 elementary elements like ionization supply, instrument and detector.

### **Sample Introduction**

The samples are inserted directly into the ionization supply or can even endure some sort of activity to the ionization supply. This technique typically involves the LC-MS technique within which prism spectroscopy is coupled on to (HPLC) or (GC).

### **Methods of Sample Ionization**

Many ionization strategies area unit on the market every having its own benefits and drawbacks. The ionization technique used depends on the sort of sample beneath investigation and also the prism spectroscopy <sup>[42-45]</sup> on the market. Ionization strategies area unit of the many varieties and embody the following:

- a) Air pressure chemical ionization (APCI)
- b) Electro spray ionization (ESI)
- c) Quick atom bombardment (FAB) and,
- d) Matrix aided optical maser activity ionization (MALDI)

### **Steps of MS/MS Analysis**

1. Q1 (first quadrupole acts as a mass filter)
2. Q2 (Acts as a collision cell wherever chosen ions area unit broken into fragments)
3. Q3- The ensuing fragments area unit analyzed by third quadrupole.

### **Detection and Recording of Sample ions**

The detector detects the particle current, amplifies it and so the signal is transmitted to the info system wherever it's recorded within the variety of mass spectra. The  $m/z$  values of the ions area unit planned against their intensities to indicate the quantity of parts within the sample, the molecular mass of every element, and also the relative abundance of the varied parts within the sample. The varied varieties of detectors area unit provided to suit the sort of instrument and also the most ordinarily used embody photomultiplier tube <sup>[46-50]</sup> and micro-channel plate detectors.



Figure 1: LC/MS/MS Instrument

## REFERENCES

1. Hewavitharana AK et al. Simple Screening Method for Staurosporine in Bacterial Cultures using Liquid Chromatography-Tandem Mass Spectrometry. *J Bioanal Biomed.* 2009; 1:001-004.
2. Moreno RA et al. Cimetidine Quantification in Human Plasma by Highperformance Liquid Chromatography Coupled to Electrospray Ionization Tandem Mass Spectrometry. Application to a Comparative Pharmacokinetics Study. *J Bioanal Biomed.* 2009; 1:005-013.
3. Alam P et al. Estimation of Swertiamarin in *Enicostemma Littorale* and Marketed Formulations Using HPLC-UV Method. *J Bioanal Biomed.* 2009; 1:022-027.
4. Venkatesh DN et al. Bioavailability Studies on Developed Prochlorperazine Maleate Sustained Release Tablets by HPLC. *J Bioanal Biomed.* 2009; 1: 054-057.
5. Musmade PB et al. High Performance Liquid Chromatographic Method for the Determination of Clobetasol in Rat Plasma and its Application to Skin Penetration. *J Bioanal Biomed.* 2010; 2:001-007.
6. El-Maghraby SI et al. High Performance Liquid Chromatographic Method for the Determination of Clobetasol in Rat Plasma and its Application to Skin Penetration. *J Bioanal Biomed.* 2010; 2: 008-012.
7. Wang R et al. The Pharmacokinetics Evaluation and Bioequivalence of new Docetaxel Injections and Taxotere using Healthy Rats. *J Bioanal Biomed.* 2010; 2:023-027.
8. Cañas-Alonso RC et al. Pharmacokinetics of Casiopeína IIgly in Beagle Dog: A Copper Based Compound with Antineoplastic Activity. *J Bioanal Biomed.* 2010; 2:028-034.
9. Zufía L et al. Validation of an LC Method for Therapeutic Drug Monitoring of Voriconazole in Patients. *J Bioanal Biomed.* 2010; 2:035-043.
10. Singh V et al. Effect of Induced Mastitis on Disposition Kinetics of Gatifloxacin Following Intravenous Administration in Goats. *J Bioanal Biomed.* 2010; 2:044-048.
11. Silva Solon LG et al. Comparative Bioavailability of a Generic and Two Compounded Naproxen Sodium Suspensions Administered to Rats. *J Bioanal Biomed.* 2010; 2:048-054.
12. Mohammad A et al. Sodium Deoxycholate Micelles Activated Separation of Coexisting Five-nucleobases by High-performance Thin-layer Chromatography. *J Bioanal Biomed.* 2010; 2:055-059.
13. Moses PF et al. Simple and Validated Method for Estimation of Amlodipine by LC-MS (ESI) Using Healthy Indian Human Volunteers: and Evaluation of Pharmacokinetic Parameters. *J Bioanal Biomed.* 2010; 2:069-074.
14. Baxla SL et al. Pharmacokinetics of Gentamicin and its Interaction with Paracetamol after i.v. Administration in Buffalo Calves (*Bubalus bubalis*). *J Bioanal Biomed.* 2010; 2:065-068.
15. Srinivasa Rao M et al. Quantification of 4-Oxiranyl Methoxy-9h-Carbazole a Genotoxic Impurity in Carvedilol Drug Substances by Lc-Ms. *J Bioanal Biomed.* 2010; 2:091-095.
16. Kuchekar SR et al. Rapid and Specific Approach for Direct Measurement of Topiramate in Human Plasma by LCMS/MS: Application for Bioequivalence Study. *J Bioanal Biomed.* 2010; 2: 107-112.
17. Zhao Y et al. Apolipoprotein E-Deficient Lipoproteins Induce Foam Cell Formation by Activation of PERKEIF- 2a Signaling Cascade. *J Bioanal Biomed.* 2010; 2:113-120.
18. Afergan E et al. <sup>31</sup>P-NMR and Differential Scanning Calorimetry Studies for Determining Vesicle's Drug Physical State and Fraction in Alendronate Liposomes. *J Bioanal Biomed.* 2010; 2: 125-131.
19. Nuchnoi P et al. The Identification of Functional Non-Synonymous SNP in Human ATP-Binding Cassette (ABC), Subfamily Member 7 Gene: Application of Bioinformatics Tools in Biomedicine. *J Bioanal Biomed.* 2011; 3:026-031.
20. Siva Rao TS et al. Quantification of Tamsulosin in Human Plasma Using LC-MS/MS. *J Bioanal Biomed.* 2011; 3:055-058.
21. Yi SJ et al. Quantification of Ticlopidine in Human Plasma Using Protein Precipitation and Liquid Chromatography Coupled with Tandem Mass Spectrometry. *J Bioanal Biomed.* 2011; 3:059-063.

22. Ram M et al. Nicotinamide Phosphoribosyltransferase in Rheumatoid Arthritis. *J Bioanal Biomed.* 2011; 3:081-084.
23. Franceschi L et al. Development and Validation of a Liquid Chromatography-Tandem Mass Spectrometry Method for the Determination of Voriconazole and Posaconazole in Serum Samples. *J Bioanal Biomed.* 2011; 3:092-097.
24. Kim B et al. Liquid Chromatography Tandem Mass Spectrometry Determination of Bicalutamide in Human Plasma and Application to a Bioequivalence Study. *J Bioanal Biomed.* 2011; 3:098-102.
25. Breindahl T et al. Quantification of Fentanyl in Human Serum by Column-Switching Liquid Chromatography and Tandem Mass Spectrometry. *J Bioanal Biomed.* 2011; 3:103-107.
26. Toppo R et al. Effects of Concurrent Administration of Meloxicam on Pharmacokinetic Parameters of Enrofloxacin in Turkeys. *J Bioanal Biomed.* 2011; 3:108-112.
27. Udristoiu A et al. Relation between LDH and Mg as Factors of Interest in the Monitoring and Prognoses of Cancer. *J Bioanal Biomed.* 2012; 4:001-005.
28. Harmita et al. Optimization and Validation of Analytical Method of Cotrimoxazole in Tablet and Plasma In vitro by High Performance Liquid Chromatography. *J Bioanal Biomed.* 2012; 4:026-029.
29. Ramani AV et al. Study of Pharmacokinetics and Tissue Distribution of BITS-17 in Rat Plasma and Tissue Homogenate Using a Validated LC Method. *J Bioanal Biomed.* 2012; 4:079-084.
30. Brown KJ et al. Accurate Quantitation of Dystrophin Protein in Human Skeletal Muscle Using Mass Spectrometry. *J Bioanal Biomed.* 2012; 5:001.
31. El-Habibi ESM et al. Efficacy of Curcumin in Reducing Risk of Cardiovascular Disease in High Fat Diet-Fed Rats. *J Bioanal Biomed.* 2013; 5:066-070.
32. Hashemian AR and Mansoori GA. Cancer Nanodiagnosics and Nanotherapeutics through the Folate-Conjugated Nanoparticles. *J Bioanal Biomed.* 2013; 5:061-064.
33. Chandra P. Advances in Clinical Diagnosis through Electrochemical Aptamer Sensors. *J Bioanal Biomed.* 2013; 5:e119.
34. Ghosh S and Mukhopadhyay R. Nucleic Acid Sensing onto Peptide Nucleic Acid (PNA) Modified Solid Surfaces. *J Bioanal Biomed.* 2013; 5:e120.
35. Yuan L et al. Host Factors to Predict Treatment Response in HCV Patients: Implications for Individualized Therapy and Translational Medicine for HCV Management. *J Bioanal Biomed.* 2013; 5:e121.
36. Loudon JA. Repurposing Amlexanox as a 'Run the Red Light Cure- All' with Read-through – a 'No-Nonsense' Approach to Personalised Medicine. *J Bioanal Biomed.* 2013; 5:079-096.
37. Patelia EM. Current Research on Biomedicine. *J Bioanal Biomed.* 2013; 5:097-098.
38. Soundarapandian P et al. Mineral Composition of Edible Crab, *Charybdis Natator* Herbst (Crustacea: Decapoda). *J Bioanal Biomed.* 2013; 5:099-101.
39. Sarkar P et al. Niche Induces Dedifferentiation. *J Bioanal Biomed.* 2013; 5:102-107.
40. Nicolini C et al. From Nanobiotechnology to Organic and Biological Monitoring of Health and Environment for Biosafety. *J Bioanal Biomed.* 2013; 5:108-117.
41. Bandyopadhyay A. Complexities of Biosimilar Product. *J Bioanal Biomed.* 2013; 5:118-121.
42. Chandra P. Miniaturized Multiplex Electrochemical Biosensor in Clinical Bioanalysis. *J Bioanal Biomed.* 2013; 5:e122.
43. Sultana N. Synthesis and Spectrophotometric Determination Ibuprofen Charge Transfer Complexes with P-Chloranil, 7, 7, 8, 8-Tetracyanoquinodimethane, Bromothymol Blue, Methyl Orange and Picric Acid. *J Bioanal Biomed.* 2013; 5:122-129.
44. DiPaola M et al. Development of Biosimilars: Analysis of Etanercept Glycosylation as a Case Study. *J Bioanal Biomed.* 2013; 5:180-186.
45. Zhao W et al. MicroRNA and its Potential Use for the Treatment of Hepatitis C Virus Infection. *J Bioanal Biomed.* 2014; 6:e125.
46. Nazem A and Mansoori GA. Nanotechnology Building Blocks for Intervention with Alzheimer's Disease Pathology: Implications in Disease Modifying Strategies. *J Bioanal Biomed.* 2014; 6:009-014.

47. Fusco DN and Chung RT. Review of Current Dengue Treatment and Therapeutics in Development. J Bioanal Biomed. 2014; 58:002.
48. Lenjisa JL et al. Hypertensive Urgency: The Role of Clinical Pharmacist in Identifying and Managing Drug Therapy Problems: The Case of Ambo Hospital Medical Ward, Ethiopia. J Bioanal Biomed. 2014; 6:015-018.
49. Lenjisa JL and Fereja TH. A Retrospective Analysis of Prescribing Practices through WHO Prescribing Indicators at Four Selected Hospitals of West Ethiopia. J Bioanal Biomed. 2014; 6:029-032.
50. Shintani H. How to Recovery of Damaged Microorganisms by Supplying Several Sorts of Nutrients. J Bioanal Biomed. 2014; 6:024-028.