



# SYLLABUS

Ph.D.

## PHARMACEUTICAL ANALYSIS

**NATIONAL INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH  
GUWAHATI**

SilaKatamur (Halugurisuk), P.O.: Changsari  
Dist: Kamrup, Assam, Pin: 781101, Assam, India  
Website: [www.niperguwahati.ac.in](http://www.niperguwahati.ac.in)

# Ph.D. Syllabus

## PHARMACEUTICAL ANALYSIS

Course No.	Course Name	Credits
<b>Semester-I</b>		
PA-710	Chromatography in Pharmaceutical Analysis	2
PA-720	Advances in Chiral Chromatography	2
PA-730	Mass Spectrometry in Pharmaceutical Analysis	2
GE-711	Seminar and Assignments	0
CS -701	Research Methodology (Compulsory)	2

Course No.	Course Name	Credits
<b>Semester-II</b>		
PA-740	Omics studies by Mass Spectrometry	2
PA-750	Analytical Chemometrics	2
PA-760	Reference standard development and its applications	2
PA-770	Workshops/Presentations/Assignments	2
CS -801	Research and Publication Ethics (Compulsory)	2

**\*Detailed Syllabus is available at Page No. 39-40**

## Ph.D. Syllabus SEMESTER - I

### PA-710 :- Chromatography in Pharmaceutical Analysis (2 Credits)

1. **Sample preparation techniques:** Extraction of analytes from non-biological, biological, and plant-based matrices including Soxhlet extraction, Liquid-liquid extraction, solid-phase extraction, solid-phase microextraction, protein precipitation, Ultrafiltration, direct injection methods. Concept and need for derivatization in pharmaceutical analysis with specific examples.
2. **Theory and practice of HPLC in pharmaceutical research:** HPLC method development and validation approaches for analytical and bioanalytical applications including a selection of mobile phase, stationary phase, detectors. Applications of HPLC in impurity profiling, related substance, dissolution testing, entrapment efficiency, drug loading, and release kinetics. Scale-up of HPLC to preparative applications.
3. **HPLC Troubleshooting:** troubleshooting approaches to remedy chromatographic output like broad and unsymmetrical peaks, peak fronting and tailing, noisy and drifting baseline, ghost peaks. Instrument-related troubleshooting approaches like high back-pressure, system leakage, flow cell blockage, check valve-related problems.  
scale-up of HPLC method to preparative HPLC
4. **Advancements in Liquid chromatography:** Ultra and nano-HPLC, HPLC method development for biomolecules, monolithic stationary phases-applications, stationary phases packed by core-shell technology, molecularly imprinted polymers as sorbents for separation and extraction.
5. **Theory and practice of GC in pharmaceutical research:** GC method development and validation approach for analytical and environmental applications including the selection of carrier gas, columns, detectors. GC analysis with special emphasis given to headspace and SPME. Applications of GC in residual solvent and impurity analysis, forensic and environmental science, volatile organic content analysis. and GC Troubleshooting
6. Case studies

#### Recommended books:

1. Grinberg N, Carr PW, editors. Advances in Chromatography, Volume 57. CRC Press; 2020 Aug 9.
2. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. John Wiley & Sons; 2011 Sep 20.
3. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. John Wiley & Sons; 2012 Dec 3.
4. Sparkman OD, Penton Z, Kitson FG. Gas chromatography and mass spectrometry: a practical guide. Academic press; 2011 May 17.

### PA-720 Advances in Chiral Chromatography (2 Credits)

1. Fundamentals: Nomenclature and CIP rules, types of chirality, types of isomers, configurations, and classification, eutomer, distomer, enantiomeric excess, chiral switching and bridging strategies.
2. Regulatory guidelines about chiral pharmaceutical compounds
3. Techniques for identification of isomers and absolute configurations: NMR, Polarimeter,

XRD, DSC.

4. Separation techniques for chiral separation: HPLC, LC-MS, GC-MS, SFC, CE and other advanced techniques, Chiral derivatizing agents and mechanism.
5. HPLC- method development in various modes of separation (normal, reverse, PO, HILIC, preparative), chiral columns: coated vs immobilized, applications, chiral recognition/separation mechanism
6. Chirality in human body (proteins, receptors, enzymes, etc.) and role of chirality in pharmacokinetics, interconversion, dynamics, and toxicity of pharmaceuticals, natural products, and pesticides.
7. Practical's on separation of enantiomers by various chromatographic modes.

**Recommended books:**

1. Stereochemistry of organic compounds by Ernest L. Eliel
2. Chirality in biological nanospaces reactions in active sites by Nilashis Nandi
3. Chiral separations by liquid chromatography and related technologies by Hassan Y. Aboul-Enein
4. Chiral analysis by Kenneth W. Busch
5. Regulatory guidelines and review/research papers

**PA-730:- Mass Spectrometry in Pharmaceutical Analysis (2 Credits)**

## 1. Basic principles of Mass Spectrometry

Instrumentation: Ionization techniques: Electron ionization, Chemical ionization, Atmospheric pressure ionization (Electrospray ionization, APCI, and APPI), other sources: MALDI, ICP, etc.

3. Mass Analyzers: Quadrupole, Time of flight, Ion traps, LIT, FTICR, Orbitrap, High Resolution Mass Spectrometry

4. Hyphenated Mass Spectrometry: GC/MS, HPLC/UPLC-MS and Tandem Mass Spectrometry (Product ion scan, Precursor ion scan, neutral loss scan, SIM and MRM)

4. Interpretation of mass spectra: Isotopes and ion abundances, the Fragmentation pattern of organic molecules with different functional groups, Qualitative analysis, Quantitative analysis

5. Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) to the detection and determination of various drugs

5. Applications: Application of mass spectrometry in Pharmacology/Toxicology, Environmental Monitoring/Analysis and Organic chemistry (Structure elucidation of organic molecules, A brief outline of omics study including the scope of biomarkers study. Impurity profiling and drug metabolite profiling, reference standards development

6. Development, Validation, and transfer for high throughput bioanalytical LC-MS/MS Methods.

**Recommended books:**

1. A Textbook of Mass Spectrometry by Jürgen H Gross, ISBN: 978-3-319-54398-7
2. Mass Spectrometry: Principles and Applications, Book by Edmond de Hoffmann, Jean Joseph Charette, and Vincent Stroobant
3. Gas Chromatography and Mass Spectrometry: A Practical Guide, Book by Fulton G. Kitson, ISBN: 9780080920153
4. Liquid Chromatography-Mass Spectrometry *By Wilfried M.A. Niessen, ISBN 9780367577827*
5. Medical Applications of Mass Spectrometry, Editors: Karoly Vekey, Andreas Telekes, Akos Vertes, ISBN: 9780444519801

**GE-711: Seminar (0 Credit)**

**This subject is introduced to develop presentation skills of students**

# Ph.D. Syllabus

## SEMESTER - II

### PA-740 Omics study by Mass Spectrometry

**(2 Credits)**

#### A: Fundamentals and analytical methodologies in metabolomics

- (a) Metabolomics: Significance in clinical research
- (b) Collection and preparation of clinical samples for metabolomics
- (c) Preparation of external and internal standards for quality control
- (d) Targeted and untargeted Metabolomic profiling by gas chromatography/liquid chromatography-mass spectrometry
- (e) Data interpretation and statistical analysis
- (f) Application of metabolomics in clinical cases

#### B: Lipidomics

- (a) Basic fundamentals and importance of lipidomics
- (b) Sample preparation and internal standards for lipidomics
- (c) Lipids classification and characterization using mass spectrometry.
- (d) Shotgun lipidomics and LC-MS/MS based lipidomics
- (e) Targeted and global lipidomics.
- (f) Application of Lipidomics in Biomedical Research
- (g) Data interpretation and statistical analysis

#### C: Proteomics

- (a) Basics and importance of proteomics.
- (b) Strategies in proteomics: Gel based and gel-free proteomics
- (c) Database and search engines in proteomics.
- (d) Applications of proteomics: Understanding mechanism of pathogenesis, Drug discovery, Disease diagnosis, identification, and characterization of novel proteins
- (e) Quantitative proteomics: Labeled and label-free proteomics
- (f) PTM (post translational modifications) sample prep, enrichment and separation
- (g) Interactomics and its applications in biological sciences.
- (h) Advanced topics – Proteogenomics, Top-down proteomics
- (i) Data interpretation and statistical analysis
- (j) Bioinformatics for protein analysis: RAW files conversion (msconvert), analysis of MS data (PMF), analysis of MS/MS data, Quantitation tools and methods, DIA analysis tools, Blind PTM search, PTM site localization and annotation, Targeted protein, and PTM analysis

#### D. Phyto Metabolomics

- (a) Collection of Plant Material: Planning, Pressing, Drying, Poisoning, Mounting, Labeling, Storing
- (b) Extraction Techniques: Maceration, Infusion, Digestion, Decoction, Percolation, Soxhlet extraction, Microwave-assisted extraction, Ultrasound-assisted extraction, Supercritical fluid extraction, Pressurized hot water extraction, Pressurized fluid extraction, Membrane-assisted solvent extraction, Stir-Bar sorptive Extraction.
- (c) Derivatization Techniques: (1) Silylation, Alkylation/Methylation, Acylation, Esterification for GCMS analysis (2) Derivatization method of amine, carboxyl, phenols, hydroxyl, carbonyl, thiols functional group for LC-MS/MS analysis

- (d) Analytical Techniques: Phyto metabolomics analysis by NMR, GC-MS, GC-QTOF, LC-MS, LC-QTOF, CE-MS, FTICR-MS  
 (e) Data interpretation and statistical analysis

### Recommended books:

1. Metabolomics: From Fundamentals to Clinical Applications edited by Alessandra Sussulini. Cham : Springer International Publishing ISBN: 9783319476568
2. Metabolomics in Practice: Successful Strategies to Generate and Analyze Metabolic Data  
 Editors: Wolfram Weckwerth, Michael Lämmerhofer, Wiley-VCH, ISBN: 9783527330898
3. Metabolomics, Editors Paul L. Wood, ISBN: 978-1-0716-0864-7
4. Lipidomics: Current and Emerging Techniques (ISSN) 1st Edition, by William Griffiths (Editor, Contributor), Yuqin Wang (Editor, Contributor), Jonathan M Curtis (Contributor), Yu Xia (Contributor), Ruth Andrew (Contributor), Giuseppe Astarita (Contributor), Steven Wilson (Contributor), Jonas Abdel-Khalik (Contributor), J Griffin (Contributor), Royal Society of Chemistry, ISBN 978-1788011600
5. Mass Spectrometry-Based Lipidomics Methods and Protocols, Editors, Fong-Fu Hsu, ISBN: 978-1-0716-1410-5
6. Lipidomics in Health & Disease: Methods & Application (Translational Bioinformatics Book 14) 1st ed. 2018 Edition by Xiangdong Wang (Editor), Duoqiao Wu (Editor), Huali Shen (Editor), ISBN 978-9811306198
7. LC-MS/MS in Proteomics: Methods and Applications Book in Methods in molecular biology (Clifton, N.J.) · January 2010 DOI: 10.1007/978-1-60761-780-8

### PA-750 CHEMOMETRICS IN ANALYTICAL CHEMISTRY (2 Credits)

1. Descriptive Statistics: Normal Distribution, Lorentzian Distribution, handling Multivariate Data,
2. Pattern Recognition: Unsupervised Analysis: Choice of Variables, Measures between Objects, Clustering Techniques, Hierarchical Techniques, K-Means Algorithm, Principal Component Analysis (PCA) case studies
3. Pattern Recognition: Supervised Learning: Discriminant Functions, Bayes' Theorem, Linear Discriminant Function, Nearest Neighbours, Artificial Neural Networks
4. Calibration and Regression Analysis: Linear Regression, Errors, and Goodness of Fit, Polynomial Regression, Multivariate Regression
5. Uncertainty in pharmaceutical method development and validation, Process capability and approaches in uncertainty calculation
6. Software for chemometric based calculation: Case studies based interpretation

### Recommended books:

- 1.0 Mike J Adams, Chemometrics in Analytical Spectroscopy, 2nd edition, 2004, RSC Analytical Spectroscopy Series.
- 2.0 Alexey L. Pomerantsev, Chemometrics in Excel, first edition, 2009, Wiley publications.
- 3.0 Kurt Varmuza, Peter Filzmoser, Introduction to Multivariate Statistical Analysis in Chemometrics, first edition, 2009, CRC press.
- 4.0 James N. Miller, Jane C. Miller, Robert D. Miller. Statistics and Chemometrics for

Analytical Chemistry, Seventh edition, 2018, Pearson Publications.

5.0 Łukasz Komsta, Yvan Vander Heyden, Joseph Sherma. Chemometrics in Chromatography. first edition, 2009, CRC press.

6.0 Hardeo Sahai, Mario M. Ojeda. Analysis of Variance for Random Models, Volume 2: Unbalanced Data: Theory, Methods, Applications, and Data Analysis. volume 1 and 2. 2004. Birkhäuser Publications.

### **PA-760:- Reference Standard Development and Its Applications**

**(2 Credits)**

1. Introductions: Basics, purpose, importance, and sources of reference standards
2. Various types of reference standards (ARS/WS/authentic material) and quality control tests for certified reference material
3. Standardization procedures of ARS from WS, method validation, and uncertainty of method
4. Production and planning: selection of candidate material, measurement and testing procedures, validation of measurement procedures, verification and calibration of measuring equipment, homogeneity assessment, stability studies(long term/short term and transport), assigning property values based on results of measurements, the uncertainty of assigned values
5. Characterization of reference standards: physical test, purity test, assay development, HPLC, UV, FT-IR, DSC, TGA, LC-QTOF, Muffle furnace, and ICP-MS for the standardization of certified reference materials
6. Various methods for loss on drying, loss on ignition, potency, and purity assessments
7. Labeling, containers, and storage of reference materials
8. Various formats of certificate of analysis for RF/CRF/Analytical standards
9. NABL, WHO, and ISO guidelines: special focus on ISO 17034:2016 general requirements for the competence of reference material producers
10. Various pharmacopeia guidelines for the reference standards
11. Case studies (a) research article study of melamine reference material  
(b) POP and NEM reference material development for dope control research  
© Phytopharmaceuticals/herbal reference standard

#### **Recommended books:**

1. Zschunke A, editor. Reference materials in analytical chemistry: a guide for selection and use. Springer Science & Business Media; 2000 Jun 21.
2. Medvedevskikh SV, Kremleva ON, Vasil'eva IE, Sobina EP, editors. Reference Materials in Measurement and Technology: Proceedings of the Third International Scientific Conference. Springer Nature; 2020.
3. Stoepler M, Wolf WR, Jenks PJ, editors. Reference materials for chemical analysis: certification, availability and proper usage. John Wiley & Sons; 2008 Jul 11
4. ISO 17034:2016 general requirements for the competence and consistent operation of reference material producers.
5. Hon PY, Chu PW, Cheng CH, Lee TC, Chan PK, Cheung ST, Wong YC. Development of melamine-certified reference material in milk using two



different isotope dilution mass spectrometry techniques. Journal of Chromatography A. 2011 Sep 28;1218(39):6907-13.

6. WHO guidelines for the reference materials (<https://www.who.int/guidelines/quality-control>)
7. USP, IP, and BP guidelines

**PA-770:- Workshops/Presentations/Assignments (2 Credits)**

This course is designed to give training about research-based case studies and their presentations along with practical exposure.

**(Syllabus for Compulsory Courses)****Semester-I****CS- 701 :- Research Methodology****(2 Credits)**

Unit 1: **Objectives and types of research:** Motivation and objectives, research methods vs methodology. Types of research – descriptive vs analytical, applied vs fundamental, quantitative vs qualitative, conceptual vs empirical. Introduction to drug discovery & development research, objectives, flowchart from discovery to post-marketing research, overview of research methodology in various areas of drug discovery and development research.

Unit 2: **Research formulation and Literature review**– Defining and formulating the research problem, selecting the problem, the necessity of defining the problem, the importance of literature review in defining a problem, Literature review - primary and secondary sources, reviews, monographs, patents, research databases, web as a source, searching the web, critical appraisal of literature, identifying gap areas from literature review and research databases, and development of a working hypothesis.

Unit 3: **Research design and methods:** Research design – basic principles, need of research design, features of good design, important concepts relating to research design, observation and facts, laws and theories, prediction and explanation, research databases, development of models, developing a research plan – exploration, description, diagnosis, and experimentation.

Unit 4: **Execution of the research, data collection and analysis:** Aspects of method validation, observation and collection of data, methods of data collection, sampling methods, data processing and analysis strategies and tools, data analysis with statistical packages (GraphPad Prism, SPSS for Student t-test, ANOVA, etc), hypothesis testing, generalization, and interpretation.

Unit 5: **Safety measures in the laboratory: Handling of hazardous chemicals, incompatible chemicals, flammable solvents, toxic chemicals and forms of toxic materials.** Approaches for prevention and management of fire, electrical, chemical, biological, and gaseous hazards, good laboratory practices. General safety rules, waste minimization approaches and safety practices for disposal of chemical waste, biologicals and other laboratory waste.

**(Syllabus for Compulsory Courses)****Semester-II****CS- 801 :- Research and Publication Ethics****(2 Credits)****Unit 1: Research Ethics:**

- a) Ethics – ethical issues, ethical committees (human & animal)
- b) Ethics with respect to science and research
- c) Intellectual honesty and research integrity
- d) Scientific misconducts: Falsification, Fabrication, and Plagiarism
- e) What is plagiarism? Similarity report software like iThenticate/ Turnitin/ Urkund.
- f) Redundant publications: duplicate and overlapping publications, salami-slicing
- g) Selective reporting, and misrepresentation of data

**Unit 2: Publication Ethics:**

- a) Publication ethics: definition, introduction, and importance.
- b) Best practices / standards-setting initiatives and guidelines: COPE, WAME, etc.
- c) Conflicts of interest
- d) Publication and Research misconduct: definition, concept, problems that lead to unethical behavior and vice versa, types
- e) Violation of publication ethics, authorship, and contributorship
- f) Identification of publication misconduct, complaints, and appeals
- g) Predatory publishers and journals.
- h) Journal finder/journal suggestion tools.

**Unit 3: IPR and scholarly publishing:**

Intellectual Property Rights (IPR) and patent law, commercialization, copyright, royalty, trade-related aspects of intellectual property rights (TRIPS)

**Unit 4: Report and thesis writing:**

- a) Structure and components of scientific reports, types of reports, technical reports, and thesis.
- b) Thesis writing – different steps and software tools (Word processing, etc) in the design and preparation of the thesis, layout, structure (chapter plan), and language of typical reports, Illustrations and tables, bibliography, referencing, and footnotes.
- c) Oral presentation – planning, software tools, creating and making an effective presentation, use of visual aids, the importance of effective communication
- d) Writing a research proposal and research grant
- e) Scholarly publishing – IMRaD concept and design of research paper, citation and acknowledgment, reproducibility, and accountability.
- f) Graphical Abstract and Artwork preparation

**Unit 5: Databases and Research Metrics**

- a) Indexing databases: PubMed, Embase, etc.
- b) Citation databases: Web of Science, Scopus, etc.
- c) Impact Factor of the journal as per Journal Citation Report, SNIP, SJR, IPP, Cite Score, *etc.*
- d) Metrics: h index, g index, i10 index, altmetrics