Programme: M. Sc. Part-II (Analytical Chemistry)
Course Code: ACC-503
Title of the Course: Separation Techniques
Number of Credits: 3 **Effective from AY:** 2019-20

Prerequisites for the course:	Should have knowledge of basic analytical techniques such as chromatography, electro-analytical techniques and data handling at MSc part-I level.	
Course Objectives:	1. Introduction of various statistical approach used in analytical data handling 2. Introduction of different separation techniques used for qualitative, quantitative estimation	
Course Outcomes:	1. Students should be in a position to understand principle behind different purification techniques. 2. Students should be in a position to select the separation techniques for purification of analytes from interferents. 3. To understand the HPLC method development and application in qualitative and quantitative analysis	
Content:	**1. Basic Separation Technique:** 1.1. General aspects of separation techniques-role of separation technique in analysis; 1.2. Separating the analyte from interferents 1.3. General theory of separation efficiency: Separation factor 1.4. Classifying separation techniques: Separations based on Size; Separations based on mass or density, Separations based on complexation reactions (Masking); Separations based on a change of state; Separations based on a partitioning between phases. (Note: Following techniques shall be discussed as representative example) 1.5. Basic principles of distillation; theory of vacuum, steam, azeotropic and fractional distillation. 1.6. Fractionation by solvent extraction: based on chemical nature and based on polarity of analyte. 1.7. Centrifugation techniques: Sedimentation velocity, Analytical and preparative centrifugation; Density gradient centrifugation; applications in separation. **2. Chromatographic Methods:** 2.1. Introduction to chromatography: definitions, theories, principles of chromatographic technique, terms and parameters used in chromatography, classification of chromatographic methods, Partition versus adsorption chromatography, development of chromatograms, qualitative and quantitative analysis by chromatography; 2.2. Planar Chromatography (Paper and thin layer): 2.2.1. Paper Chromatography- introduction, principle, theory, types (ascending, descending, circular, two dimensional paper chromatography); techniques; choice of solvent; multiple development, qualitative and quantitative measurement applications; 2.2.2 Thin Layer Chromatography (TLC)- definition; mechanism; efficiency of thin layer plates; methodology (technique); criteria	6 hrs 24hrs

	for selection of stationary and mobile phases (numerical to calculate elution strength of mixed solvents used as mobile phase); choice of adsorbents; preparation of plates; spotting (spot capacity); development of chromatogram; identification and detection using physical and chemical methods; reproducibility of Rf values and improving resolution; Two-dimentional TLC; comparison of TLC with paper chromatography, column chromatography, thin layer ionophoresis and electrophoresis; Qualitative, quantitative evaluation and applications; 2.3. High-performance TLC (HPTLC): introduction, principle, theory, classification (classical, high performance, ultra, preparative HPTLC); Difference between TLC and HPTLC with respects to the parameters; scanning densitometer; Quantitative analysis using TLC-densitogram and applications. 2.4. Gas Chromatography (GC): Instrumentation, selection of operating condition, choices of GC column, methods to prepare derivatives of samples (silylation, acylation, alkylation), working principle of GC detectors such as TCD, ECD, FID, Analysis of GC data and quantification methods such as normalizing peak area, internal std., external std, standard addition. 2.5. Column Chromatography- definition; types (conventional, flash, LPLC, Dry column vacuum chromatography); principle; packing, loading, eluting and collecting eluent in the column chromatography and experimental requirements; theory of development; migration rates of solutes; band broadening and column efficiency; variables that affect column efficiency; Van Deemeter equation and its modern version; scale-up and thump rule for conventional column, qualitative and quantitative analysis; applications. 2.6. Liquid-liquid partition chromatography (HPLC)- Introduction; selection of stationary and mobile phase; types of bonded phase chromatography-NPC and RPC and stationary phases used; reversed phase partition chromatography; steps in HPLC method development in partition chromatography- elution techniques (isocratic and gradient, ion pairing agents, buffer agents, organic modifiers); optimization of capacity factor, gradient selectivity factor and column plate numbers; numerical on method development using Snyder's polarity index. Preparative vs analytical HPLC; Chiral chromatography- Pirkle stationary phases, examples of enantiomer separation such as ibuprofen, calculation of enantiomeric excess. Choosing detectors- working principle of RI, UV-Vis, conductivity and ELSD. 2.7. Size Exclusion Chromatography: definition; theory; principle; types; stationary phases in gel chromatography; physical and chemical characteristics of gel, mechanism of gel permeation chromatography (GPC); instrumentation of GPC; applications of GPC- determination of molecular weight of polymer with numericals. 2.8. Supercritical-Fluid Chromatography: introduction; important properties of supercritical-fluids; instrumentation and variables, SFC column vs other column, applications.	

	3. Electrophoresis: 3.1. Theory of electrophoresis; Type of electrophoresis- Free solution and supporting medium electrophoresis, paper electrophoresis, capillary electrophoresis and gel electrophoresis. 3.2. Capillary electrophoresis-instrumentation, sample introduction in CE, types of CE methodology, electrophoretic mobility and electroosmatic mobility, total mobility, efficiency and resolution in CE column, numericals. 3.3. Gel electrophoresis - types of gel, Polyacrylamide gel electrophoresis PAGE, Agarose GE, factors affecting separation; 3.4. Staining and detecting electrophoresis band; 3.5. Separation of neutral molecule by MEKC; 3.6. Separation and determination of Vitamin B-complex by using CZE and MEKC.	6 hrs
Pedagogy:	Lectures/ tutorials/ seminars/ term papers/assignments/ presentations/ self-study or a combination of some of these. Sessions shall be interactive in nature to enable peer group learning.	
References/ Readings	1. G. D. Christian, *Analytical Chemistry*, John Wiley, New York, 2004, 6th Ed. 2. D. A. Skoog, D. M. West,F. J. Holler, *Fundamentals of Analytical Chemistry*, Sounders College Publishing, 2014, 9th Ed. 3. D. Harvey, *Modern Analytical Chemistry*, The McGraw-Hill, 2000, 1st Ed. 4. L. R. Snyder, J. J. Kirkland, J.W. Dolan, *Introduction to modern liquid chromatography*, John Wiley, New York, 2009, 3rd Ed. 5. H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle, Instrumental *methods of Analysis*, CBS Publishing New Delhi, 7th Ed. 6. G. H. Jeffery, J. Bassett, J. Mendham, R C. Denney, *Vogel's Text Book of Quantitative Chemical Analysis*, John Wiley, New York, 1989, 5th Ed. 7. H. Gunzler, A.Williams, *Handbook of analytical techniques*, John Wiley, New York, 2002, 1st Ed.	