**DEPARTMENT OF PHARMACY**

**MIT MUZAFFARPUR**

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**AFFILIATED TO**

**ARYA BHATT KNOWLEDGE UNIVERSITY,**

**MITHAPUR, PATNA**

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**Name of Course: PHARMACEUTICAL ANALYSIS-II**

**Course code (T): 091304**

**Course code (P): 091304P**

**Semester : III**

**Academic year : 2018-2019**

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**1. Vision**

**To generate competent Pharmaceutical human resources to serve society, industry and nation through personal development and professional excellence.**

**2. Mission**

* To inspire and in still the ethical values, leadership, and entrepreneurship skill in the students for betterment of the society and hence the nation.
* To offer state-of-art-undergraduate, postgraduate and the doctoral program.
* To excel in Pharmacy education, patient centered care, community engagement and research.

**3. Programme Educational Objectives (PEO’S)**

PEO-1 Students will be able to use their fundamental concepts and technical competence in field of pharmaceutical Analysis as and when required in Pharmaceutical Industry and /or institutes to achieve professional excellence

PEO-2 Students will acquire strong and well defined concepts in of Pharmaceutical analysis as per requirements of Pharmaceutical Industries, Community and Hospital Pharmacy.

PEO-3 They will be able to work in a team while being competent enough in solving complex problems in the area of Pharmaceutical Sciences.

PEO-4 Be ethical, professional and conscious of their environmental and social responsibilities.

PEO-5 Possess an attitude for continuous learning and practicing in the field of work.

4. Program Objectives

Pharmaceutical analysis is the subject which deals mainly with the quantitative analysis of those chemicals and dosage forms associated with the practice of pharmacy. It provides training ground for the accuracy expected from pharmacy graduates. The graduates of the program will acquire:

1. Knowledge and understanding outcome:

* Understand the significance of Pharmaceutical Analysis in the profession.
* Learn the various tools and techniques available for the analysis of drugs.
* Principles of various conventional analytical techniques

2. Practical outcome

* Expression of various concentrations and preparations.
* Application of Pharmacopoeial purity and identity tests for real life samples.
* Proper handling of laboratory equipments and glassware.

3. Intellectual outcome:

* Selection of an optimum analytical technique for a given sample.
* Converting the observations to meaningful results and drawing the inferences.
* Comparing various methods of analysis and their outcomes

5. Course Outcomes (COs)

On the completion of the course, students will be able to:

* Demonstrate adequate knowledge on basic principles and techniques of complexometric titration and non aqueous titration
* Execute Diazotisation titrations, Karl-Fischer titrations and Kjeldahl method of titration as when required.
* Discuss the fundamental of different techniques of separation such as TLC. Paper Chromatography, HPLC and GLC volumetric analysis, and significance of quality control in pharmaceutical.
* Learn Potentiometry, Conductometry, Coulometry, Polarography and Amperometry and to use the methods as when required.
* Identify and apply the knowledge of Pharmaceutical Analysis as when required

**6. Mapping of CO’S & PO’S**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| PO | CO1  | CO2 | CO3 | CO4 | CO5 |
| PO1 | √ | √ | √ | √ | √ |
| PO2 | √ | √ | √ | √ | √ |
| PO3 | √ | √ | √ | √ | √ |
| PO4 | √ | √ | √ | √ | √ |
| PO5 | √ | √ | √ | √ | √ |

**7. Academic Calendar**

**Calendar (Odd Semesters): 2018-19**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| S.No | Events | B.Pharm 1stsem | B.Pharm 3 rdsem | B.Pharm 5thsem | B.Pharm 7thsem |
| 1 | Class Start Date |  | **16.7.2018** | **16.7.2018** | **16.7.2018** |
| 2 | First Sessional Exam start date |  |  |  |  |
| 3 | First Sessional Exam End date |  |  |  |  |
| 11 | Theory exam Date |  | **Dec -2018** | **Dec-2018** | **Dec- 2018** |
| 12 | Practical Exam Start Date/Final Presentation |  | **Jan-2019** | **Jan-2019** | **Jan-2019** |
| 13 | Practical Exam End Date/ Final Presentation |  | **Jan-2019** | **Jan-2019** | **Jan-2019** |

**8. List of Holidays**

|  |  |  |  |
| --- | --- | --- | --- |
| S.No | Holiday | Date | Day |
| 1 | Independence Day | 15.08.2018 | Wednesday |
| 2 | Bakrid | 22.08.2018 | Wednesday |
| 3 | Sri Krishna Janmasthami | 03.09.2018 | Monday |
| 4 | Muharam | 21.09.2018 | Friday |
| 5 | Gandhi Jayanti | 02.10.2018 | Tuesday |
| 6 | Durga Puja | 13.10.2018-21.10.2018 | Saturday to Saturday |
| 7 | Chehallum | 30.10.2018 | Tuesday |
| 8 | Deepabali | 07-11,2018 -16.11.2018 | Wednesday to Friday |
| 9 | Hazarat Mohamad sahib Birthday | 21.11.2018 | Wednesday |
| 10 | Christmas | 25-12-2018 -31.12.2018 | Tuesday To Monday |

|  |
| --- |
| MUZAFFARPUR INSTITUTE OF TECHNOLOGY |
| ODD SEM (JULY- DEC 2018) TIME TABLE FOR 3 rd , 5 th&& 7 th SEMESTER, B.PHARM, WITH EFFECT FROM 16.07.2018. |
| DAY | SEMESTER |  9 AM TO 10 | 10 -11 AM | 11- 12 AM | 12 -1 PM | 2 TO 5 PM |
| MON | THIRD SEM | APHE II SK | PHARM ANAL II GT | PHARMACEUTICS III AB | PHARMACOGNOSY II NRB | CLASS TEST |
| FIFTH SEM | PHARMACEUTICS V RKC | PHARMACEUTICS V LAB RKC | CLASS TEST |
| SEVENTH SEM | PHARMA. BIOTECH SNS | PHARM CHEM VII RP | PHARMA. INDUST. MANAG. | PHARMACOLOGY III RP | CLASS TEST |
| TUES | THIRD SEM | PHARMACEUTICS III AB | PHARM CHEM IV SW | PHARMACEUTICS III AB(T) | PHARM ANAL II GT(T) | PHARMACEUTICS III LAB AB |
| FIFTH SEM | PHARM CHEM V SNS | PHARMACEUTICS VI AB | PHARMA CEUTICS V RKC | PHARMACOLOGY I SK | PHARM CHEM V LAB SNS |
| SEVENTH SEM | PHARMACEUTICS VIII RKC | PHARM CHEM VII RP | PHARMACOLOGY III RP | PHARMACEUTICS VIII RKC(T) | PHARMACOLOGY III LAB RP |
| WED | THIRD SEM |   | PHARMACOGNOSY II NRB(T) | PHARMACOGONOSY II NRB | PHAR ANAL II GT | PHARMACOGONOSY II LAB NRB |
| FIFTH SEM | PHARMACOLOGY I SK | PHARM CHEM V SNS  | PHARMACEUTICS VI AB | PHARMACOLOGY I SK(T) | PHARMACOLOGY I LAB SK |
| SEVENTH SEM | PHARM CHEM VII RP(T) | PHARMACEUTICS VIII RKC | PHARM CHEM VII RP | ELECTIVE OPT | PHARM CHEM VII RP |
| THURS | THIRD SEM | APHE II SK(T) | PHARM CHEM IV SW | APHE II SK | PHARM CHEM IV SW(T) | PHARM ANAL II LAB GT |
| FIFTH SEM | PHARM CHEM V SNS | PHARMACEUTICS VI AB | PHARMACOGONOSY IV SW |   | PHARMACOGONOSY IV LAB SW |
| SEVENTH SEM | PHARMACEUTICS VIII RKC | PHARMA. BIOTECH SNS(T) | PHARMACOLOGY III RP  | ELECTIVE OPT | ELECTIVE LAB-OPT |
| FRI | THIRD SEM | APHE II SK | PHARMACUTICAL CHEMISTRY IV LAB SW | APHE II LAB SK |
| FIFTH SEM | PHARMACOGONOSY IV SW | PHARMACEUTICS V RKC | PHARMACOGONOSY IV SW(T) | PHARMACEUTICS V RKC(T) | PHARMACEUTICS VI LAB OPT AB |
| SEVENTH SEM |   | ELECTIVE OPT (T) | ELECTIVE OPT  | PHARMA. BIOTECH.SNS | PHARMACEUTICS V III RKC LAB |
| SAT | THIRD SEM | PHARMACOGONOSY II NRB | PHARM CHEM IV SW | PHAR ANAL II GT | PHARMACEUTICS III AB |   |
| FIFTH SEM | PHARM CHEM V SNS(T) | PHARMACOLOGY I SK | PHARMACEUTICS VI AB | PHARMACOGONOSY IV SW |   |
| SEVENTH SEM | PHARMACOLOGY III RP(T) | PHARMA. INDUST. MANAG. | PHARMA. BIOTECH SNS |   |   |

9. Sample Time Table

**10. COURSE DESCRIPTION: PHARMACEUTICAL ANALYSIS-II : B. PHARM –THIRD SEMESTER**

1. Course Syllabus

Module -1

 Theoretical considerations and application in drug analysis and quality control of the following analytical techniques. Non-aqueous titrations and Complexometric titrations

 Module -2

Miscellaneous Methods of Analysis: Diazotisation titrations, Kjeldahl method of nitrogen estimation, Karl-Fischer titration, Oxygen flask combustion, Gasometry ; Extraction procedures including separation of drugs from excipients

Module -3

 Chromatography: The following techniques will be discussed with relevant examples of Pharmacopoeialproducts.TLC, HPLC, GLC, HPTLC, Paper Chromatography and Column Chromatography.

Module -4

 Potentiometry; Introduction; Theory; Instrumentation; Applications

Module -5

 Conductometry; Introduction; Theory; Instrumentation; Applications

Module -6

 Coulometry; Introduction; Theory; Instrumentation; Applications

Module -7

 Polarography; Introduction; Theory; Instrumentation; Applications

Module -8

 Amperometry; Introduction; Theory; Instrumentation; Applications

**11. TEXT BOOKS/ REFERENCE BOOKS**

1. R. M. VERMA, ANALYTICAL CHEMISTRY THEORY AND PRACTICE, C.B.S. PUBLICATIONS.

 2. DR. A.V. KASTURE, PHARMACEUTICAL ANALYSIS, VOL-I, NIRALI PUBLICATION.

3. PHARMACEUTICAL ANALYSIS: VOL-I ; ASHOTOSH KAR: CBS PUBLISHERS ANA DISTRIBUTORS

4, PHARMACEUTICAL DRUG ANALYSIS:; ASHOTOSH KAR: NEW AGE INTERNATIONAL (P) LIMITED PUBLISHERS

5.MEITES, L., ED. HANDBOOK OF ANALYTICAL CHEMISTRY, NEW YORK, MCGRAW-HILL, 1963.

6.PIETRZYK, DJ, AND CW FRANK, ‘ANALYTICAL CHEMISTRY’, LONDON, ACADEMIC PRESS, 2ND ED., 1979.

7.HARGIS, L.G., ‘ANALYTICAL CHEMISTRY’, NEW JERSEY, PRENTICE HALL, 1988.

8.JEFFREY, G.H. J. BASSETT., J. MENDHAM AND R.C. DENNEY, **‘**VOGEL’S TEXTBOOK OF QUANTITATIVE CHEMICAL ANALYSIS**\*,’**

5TH ED., NEW YORK, LONGMAN SCIENTIFIC AND TECHNICAL, 1989.

9.SCHIRMER, R.E., **‘**MODERN METHODS OF PHARMACEUTICAL ANALYSIS**,** 2ND. VOL. 1, BOSTON, CRC PRESS 1991.

**12. Assessment Methods for CO’S; Theory & Practical**

12.1. Theory

|  |  |  |  |
| --- | --- | --- | --- |
| S.No | Assessment Tools | Marks | Outcomes |
| 1 | Sessional Examination | 20 | CO1 CO2 CO3 CO4 |
| 2 | Assignment  | 02 | CO1 CO2 CO3 CO4 |
| 3 | Presentation | 02 | CO1 CO2 CO3 CO4 |
| 4 | Quizzes | 01 | CO1 CO2 CO3 CO4 |
| 5 | Attendance | 05 | NA |
| 6 | University Examination | 70 | NA |

12.2. Practical

|  |  |  |  |
| --- | --- | --- | --- |
| S.No | Assessment Tools | Marks | Outcomes |
| 1 | Attendance | 05 | CO1 CO2 CO3 CO4 |
| 2 | Experiment valuation  | 10 | CO1 CO2 CO3 CO4 |
| 3 | Internal Viva- voce | 05 | CO1 CO2 CO3 CO4 |
| 4 | University Practical Exam | 30 | CO1 CO2 CO3 CO4 |

**13 Delivery Methodologies**

|  |  |  |
| --- | --- | --- |
| Outcomes | Methods | Supporting Tools |
| CO 1 | Chalk-Talk, Interactive classroom, ICT usage, Case study discussion about diseases, Group discussions, , Web based learning | Board, Laptop, Projector, You Tube, Whatsapp, Google,  |
| CO2 | Chalk-Talk, Interactive classroom, ICT usage, Case study discussion about diseases, Group discussions, , Web based learning | Board, Laptop, Projector, You Tube, Whatsapp, Google,  |
| CO3 | Chalk-Talk, Interactive classroom, ICT usage, Case study discussion about diseases, Group discussions, , Web based learning | Board, Laptop, Projector, You Tube, Whatsapp, Google,  |
| CO4 | Chalk-Talk, Interactive classroom, ICT usage, Case study discussion about diseases, Group discussions, , Web based learning | Board, Laptop, Projector, You Tube, Whatsapp, Google,  |
| CO5 | Chalk-Talk, Interactive classroom, ICT usage, Case study discussion about diseases, Group discussions, , Web based learning | Board, Laptop, Projector, You Tube, Whatsapp, Google,  |

**14. Lesson Plan: Theory & practical**

14.1. Theory

|  |  |
| --- | --- |
| Lecture No. | Contents |
| 1 | Lesson -1 Non-aqueous titrations; Introduction :Solvents used |
| 2 | Lesson -2 Non-aqueous titrations; Methodology: preparation of 0.1 n perchloric acid; Standardization of 0.1 N Perchloric Acid,; Choice of Indicators, and Effect of Temperature on Assays |
| 3 | Lesson-3 Assay by non-aqueous titrations; Acidimetry in Non-aqueous Titrations;  |
| 4 | Lesson-4 Alkalimetry in Non-aqueous Titrations |
| 5 | Lesson-5 Complexometric titrations; Introduction; Theory; |
| 6 | Lesson -6 Effect of pH on complexation; Stability of complexes; Colouration of complexes; Titrability of polyvalent metal ions employing disodium acetate;  |
| 7 | Lesson -7 Usage of pM indicators in complexometric titrations |
| Class Test |
| 8 | Lesson -8 Assay methods; Direct titration methods; Masking and demasking agents; Residual titration methods |
| 9 | Lesson -09 Diazotisation titrations; Introduction; Theory; Assay methods. |
| 10 | Lesson -10 Assay methods; Preparation of 0.1 M sodium nitrite solution; Standardization of 0.1 M sodium nitrite solution with sulphanilamide; |
| 11 | Lesson 11 Calcium aminosalicylate; Isocarboxazid; Phthalylsulphathiazole; Cognate assays |
| 12 | Lesson -12 Karl Fischer method for determination of water;Introduction;Theory |
| 13 | Lesson -13 Instrumentation; Automated electrochemical Karl Fischer analysis |
| 14 | Lesson – 14 Applications of Karl Fischer Method for Determination of Water in Pharmaceutical Analysis; Prednisolone sodium phosphate; Cognate assays |
| 15 | Lesson -15 Kjeldahl method of nitrogen estimation |
| 16 | Lesson- 16 Oxygen flask combustion |
| 17 | Lesson -17 Gasometry |
| Class Test |
| 18 | Lesson -18 Extraction procedures including separation of drugs from excipients |
| 19 | Lesson -19 Chromatography; Theory; Classification; column chromatography, Paper Chromatography |
| 20 | Lesson-20 Thin layer chromatography; Theory; Versatility of TLC over paper and columnchromatography |
| 21 | Lesson-21 Experimental techniques of TLC, Steps of TLC |
| 22 | Lesson-22 Applications of TLC in pharmaceutical analysis |
| 23 | Lesson-28 Gas Liquid Chriomatography; Introduction; Theory; Plate theory; Rate theory Random walk and nonequilibrium theory |
| 24 | Lesson-29 Instrumentation; Carrier gas pressure regulator and flow meter; Sample injection system; Separation column; Thermal compartment; Detectors; Recording of signal current; Integrator |
| 25 | Lesson-30 Working techniques for quantitative analysis; Applications of GLC in pharmaceutical analysis |
| 26 | Lesson-32 HPLC; Introduction;Theory, instrumentation; Applications of HPLC in pharmaceutical analysis  |
| 27 | Lesson -33 HPTLC |
| 28 | Lesson-34 Electrochemical methods: Potentiometry: Introduction: Theory; General considerations ; End-point determination |
| 29 | Lesson -35 Instrumentation; Applications of potentiometric titrations in pharmaceutical analysis |
| Class Test |
| 30 | Lesson -37 Conductometry; Introduction; Theory; Instrumentation; Applications of Conductometry |
| 31 | Lesson -38 Coulometry; Introduction; Theory; Instrumentation; Applications of Coulometry |
| 32 | Lesson -39 Polarography: Introduction; Theory; Instrumentation; Applications of Polarography |
| 33 | Lesson -40 Amperometry; Introduction; Theory; Instrumentation; Applications of amperometric titrations in pharmaceutical substances |
| 34 | Class Test |

14.2. Practical

|  |  |
| --- | --- |
| Exp. No | Experiment |
| 1 | EXPERIMENT 1. DETERMINATION OF HARDNESS IN WATER SAMPLES WITH EDTA |
| 2 | EXPERIMENT 2. THIN-LAYER CHROMATOGRAPHIC SEPARATIONS OF AMINO ACIDS |
| 3 | EXPERIMENT 3ANALYSIS OF A NON-PRESCRIPTION MEDICINE (OTC DRUG) |
| 4 | EXPERIMENT 4. PROCEDURE FOR AMINO ACIDS IN ORANGE AND LEMON JUICE |
| 5 | EXPERIMENT 5. PROCEDURE FOR PIGMENTS IN GREEN PLANTS. |
| 6 | EXPERIMENT 6; PROCEDURE FOR A MIXTURE OF INDICATOR DYES |
| 7 | EXPERIMENT 7: REQUIREMENTS FOR THE TITRATION OF WEAK BASES WITH PERCHLORIC ACID  |
| 8 | EXPERIMENT 8: TITRATION OF HALOGEN ACID SALTS OF BASES WITH PERCHLORIC ACID |
| 9 | EXPERIMENT 9:-TO SEPARATE THE AMINO ACID MIXTURE BY PAPER CHROMATOGRAPHY |
| 10 | . EXPERIMENT 10:-DETERMINATION OF ALUM BY COMPLEXOMETRIC TITRATION |
| 11 | EXPERIMENT 11:-PREPARE AND STANDARDIZATION OF NaNO2 BY DIAZOTIZATION TITRATION |

**15. Sample Theory Handouts**

|  |  |
| --- | --- |
| Institute  | M.I.T., MUZAFFARPUR |
| Program Name | B.PHARM |
| Course Code | 091304 P |
| Course Name | PHARMACEUTICAL ANALYSIS -II |
| Labs (per week) | 1 | **Course Credits** | 2 |
| Course Coordinator Name | Dr G Thakur |

1. **Scope and Objectives of the Course**

Pharmaceutical analysis is the subject which deals mainly with the quantitative analysis of those chemicals and dosage forms associated with the practice of pharmacy. It provides training ground for the accuracy expected from pharmacy graduates. The graduates of the programme will acquire knowledge and skill the various tools and techniques available for the analysis of drugs.

SAMPLE LESSON : HPLC

1. INTRODUCTION

* Chromatography is a technique to separate mixtures of substances into their components on the basis of their molecular structure and molecular composition.
* This involves a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas).
* The mobile phase flows through the stationary phase and carries the components of the mixture with it.
* Sample components that display stronger interactions with the stationary phase will move more slowly through the column than components with weaker interactions.
* This difference in rates causes the separation of various components.
* Chromatographic separations can be carried out using a variety of stationary phases, including immobilized silica on glass plates (thin-layer chromatography), volatile gases (gas chromatography), paper (paper chromatography) and liquids (liquid chromatography).
* High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid 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.

All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation

**2. TYPES OF HPLC**

**1.NormalPhaseHPLC**:
This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these. Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

2.**ReversePhaseHPLC:**
The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.

**3.Size-exclusionHPLC:**
The column is filled with material having precisely controlled pore sizes, and the particles are separated according to its their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

**4.Ion-ExchangeHPLC:**
The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

**3. SCHEMATIC INSTRUMENTATION OF HPLC**

1. **Solvent Reservoir**: Mobile phase contents are contained in a glass resorvoir. The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.
2. **Pump**: A pump aspirates the mobile phase from the solvent resorvoir and forces it through the system’s column and detecter. Depending on a number of factors including column dimensions, particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.
3. **Sample Injector**: The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).
4. **Columns** : Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 µm. Columns with internal diameters of less than 2 mm are often referred to as microbore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.
5. **Detector**: The HPLC detector, located at the end of the column detect the analytes as they elute from the chromatographic column. Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.
6. **Data Collection Devices**: Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

**Application of HPLC**

The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include

**Pharmaceutical Applications**

1. To control drug stability.
2. Tablet dissolution study of pharmaceutical dosages form.
3. Pharmaceutical quality control.

**Environmental Applications**

1. Detection of phenolic compounds in drinking water.
2. Bio-monitoring of pollutants.

**Applications in Forensics**

1. Quantification of drugs in biological samples.
2. Identification of steroids in blood, urine etc.
3. Forensic analysis of textile dyes.
4. Determination of cocaine and other drugs of abuse in blood, urine etc.

**Food and Flavor**

1. Measurement of Quality of soft drinks and water.
2. Sugar analysis in fruit juices.
3. Analysis of polycyclic compounds in vegetables.
4. Preservative analysis.

**Applications in Clinical Tests**
1. Urine analysis, antibiotics analysis in blood.
2. Analysis of bilirubin, biliverdin in hepatic disorders.
3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.

**16.** Sample Lab. Course Handouts

|  |  |
| --- | --- |
| Institute  | M.I.T., MUZAFFARPUR |
| Program Name | B.PHARM |
| Course Code | 091304 P |
| Course Name | PHARMACEUTICAL ANALYSIS -II |
| Labs (per week) | 1 | **Course Credits** | 2 |
| Course Coordinator Name | Dr G Thakur |

1. **Scope and Objectives of the Course**
* To understand the importance of analysis in pharmaceutical industry
* To understand the knowledge about assay of pharmaceutical substance and product
* To develop basic practical skills using instrumental techniques
* To inculcate theoretical knowledge on various instrumental techniques adopted for analysis of pharmaceuticals
* To develop various methodologies for assay of drugs and pharmaceuticals with the skills and knowledge gained
* To understand and gain knowledge on trouble shooting in adopting various methodologies using instrumental techniques

**Sample EXPERIMENT NO. 01**

**Aim: To determine the Rf value of given sample of paracetamol and compare with standard paracetamol by TLC.**

**Requirement:** TLC plate, TLC chamber, TLC development chamber, Solvents for mobile phase, Silica gel G, Forceps, Detecting reagents/UV chamber, Paracetamol.

**References**: Sethi PD and Charegaonkar D. “Identification of Drugs in Pharmaceutical Formulations by Thin Layer Chromatography”, Second Edition, 2005, CBS Publishers and Distributors, New Delhi, page: 1-27, 83.

**Principle:**

Thin layer chromatography is a method of analysis in which the stationary phase (a finely divided solid) is spread as a thin layer on a solid rigid supporting plate; and the mobile phase: a liquid is allowed to migrate across the surface of the plate by the capillary action and separation takes places due to adsorption phenomenon and gives different Rf values for each sample compound.

Rf= Distance travel by solute/Distance travel by solvent

**Stationary Phase**

Silica gel, the most commonly used stationary phase, has the empirical formula SiO2. However, at the surface of the silica gel particles, the dangling oxygen atoms are bound to protons. The presence of these hydroxyl groups renders the surface of silica gel highly polar.Thus, polar functionality in the organic analyte interacts strongly with the surface of the gel particle and non polar functionality interacts only weakly.

**Mobile Phase**

For silica gel chromatography, the mobile phase is an organic solvent or mixture of organic solvents. As the mobile phase moves past the surface of the silica gel it transports the analyte past the particles of the stationary phase. However, the analyte molecules are only free to move with the solvent if they are not bound to the surface of the silica gel. Thus, the fraction of the time that the analyte is bound to the surface of the silica gel relative to the time it spends in solution determines the retention factor of the analyte.The ability of an analyte to bind to the surface of the silica gel in the presence of a particular solvent or mixture of solvents can be viewed as a the sum of two competitive interactions.

First, polar groups in the solvent can compete with the analyte for binding sites on the surface of the silica gel. Therefore, if a highly polar solvent is used, it will interact strongly with the surface of the silica gel and will leave few sites on the stationary phase free to bind with the analyte. The analyte will, therefore, move quickly past the stationary phase.

Similarly, polar groups in the solvent can interact strongly with polar functionality in the analyte and prevent interaction of the analyte with the surface of the silica gel. This effect also leads to rapid movement of the analyte past the stationary phase.

**Method:**

**Preparation of TLC plate**

Suspend 100 g of silica gel G in 200-250 ml of water, mix with a stirrer to get homogeneous slurry.

Take the air dried TLC glass plates or dried in oven at 1100C and pour the silica gel G slurry into the glass plate. (Thickness should be around 250 μm) Slurry should be used within 2 minute of preparation otherwise slurry will dry and needs more water to maintain the fluidity.

Dry the plate in a TLC chamber until complete drying occurs. Dried TLC plates are activated in oven at 1100C for 30 minutes and immediately used for development after cooling.

**Pre-coated plates:**

With the availability of pre-coated plates commercially, the use of laboratory hand-made plates is on decline. The pre-coated plates with different support material (glass, aluminium, plastic) and with different sorbent layers are available in different format and thickness by various manufacturers.

Usually plates with sorbent thickness of 100-250μm are used for qualitative and quantitative analysis. Pre-coated plates also require activation.

**Sample Preparation**

For TLC on silica gel, the use of least polar solvent which allows quantitative dissolving and spotting of sample and there is no preliminary development and separation within the initial spot at the origin, is recommended.

**Application of sample**

Sample application is most critical step for obtaining good resolution. The sample should be completely transferred to the layer, however, under no circumstances; the application process should damage the layer, as damaged layer results in unevenly shaped spots.

Wherever possible, use of automatic application device is recommended. The sample should be applied through clean smaller diameter capillary.

**Selection of mobile phase**

**First level**: A neat solvent from different selectivity areas is tested. Within a selectivity area, solvents may give similar separation. Usually diethyl ether, ethanol, methanol, tetrahydrofuran, dimethyl formamide, dichloromethane, ethylacetate, acetonitrile, methyl-ethyl ketone, toluene and chloroform are used as neat solvents.

If acceptable resolution and medium Rf value range is achieved, the analyst can directly third level, if first level does not yield satisfactory result, then proceed with second level.

**Second level**: From first level, solvents which leave the main fraction/component of the analyte near the starting point or close to the solvent front, are required to adjusted. If Rf values are too high, solvent strength should be decreased by adding non polar solvents and polar solvents for too low Rf values.

**Third level**: Mixtures of solvents from different selectivity group are investigated; the strength is adjusted, if required. These mixtures can be binary, tertiary or even quaternary, but binary mixtures are preferred one.

At this level, addition of small amount of acidic (acetic acid) or basis (triethyl amine) modifiers significantly enhance the separation efficiency of mobile phase.

**Fourth level**: At this level, final optimization of mobile phase to be used for a particular separation is made. To get the best separation, small variations in the proportions of different solvents may have to be made.

**Preconditioning**

Chamber saturation has pronounced influence on the separation profile. When the plate is introduced into an unsaturated chamber, during the course of development, the solvent evaporates from the plate mainly at the solvent front. Therefore larger quantity of the solvent shall be required for a given distance; hence resulting is increase in Rf values. If the tank is saturated (by lining with filter paper) prior to development, solvent vapours soon get uniformly distributed throughout the chamber. As soon as the plate is placed in such a saturated chamber, it soon gets preloaded with solvent vapours, hence less solvent shall be required to travel a particular distance, resulting in lower Rf values.

**Development and drying**

Develop the chromatogram in twin trough chamber or other TLC development chamber until and unless solvent reaches the three fourth distance of the plate. Dry the developed plate in chromatographic drying chamber.

**Evaluation of thin layer chromatogram**

First of all spots of TLC are detected by using suitable detecting reagent or physical methods. The evaluation depends on the purpose of a chromatographic analysis. For quantitative determination often localization of substances is sufficient. This can be easily achieved by parallel runs with reference substances.

Rf values: A parameter often used for qualitative evaluation is the Rf value (retention factor). The Rf value is defined as follows:

Rf =Distance travel by solute (spot) / Distance travel by solvent front i.e. Rf values are between 0 and 1, best between 0.1 and 0.8.

**Sample Experiment No-2**

**Aim:-Chromatographic condition for Paracetamol**

**Standard solution**: Dissolve appropriate quantity of paracetamol (i.e. approximate 10μg/ml) in methanol for spotting.

**Sample solution**: Suspend powdered sample in methanol, sonicate for 5 minute, filter through whatman filter paper and use the filtrate for spotting.

**Mobile phase**: Chloroform: Acetone: Toluene (6.5: 2.5: 1.0)

Chamber saturation time: 30 minutes.

Migration distance: 70 mm (If 10x10 cm plate is used)

**Detection**: UV light (254nm) or iodine vapours.

**Method**:

1. Take the given mobile phase ration in a TLC chamber and saturate the chamber for 30 min.

2. Take a dried and activated TLC plate.

3. Take the prepared standard solution and apply the spot in TLC plate around 1 cm above from the bottom.

4. Take the selected test solution and apply the spot in same TLC plate parallel to the standard spot.

5. Wait for drying of spot and kept the TLC plate tilted in the saturated TLC chamber.

6. Develop the TLC plate up to 80-90% of the stationary phase.

7. Remove the TLC plate from the chamber and kept for air drying of mobile phase.

8. View the spot(s) under UV chamber or Iodine chamber.

9. Calculate the Rf values of the selected paracetamol standard and sample.

Results: Rf value of the standard and sample paracetamol was found to be ........ and ......... respectively.

**17. Sample Assignment: I, II & III**

Sample Assignment -1

1. Attempt the following aspect of ‘*Thin-layer Chromatography*’ (TLC):

 (*a*) Importance of TLC,

 (*b*) Theory of TLC, and

 (*c*) Versatility of TLC over paper and column chromatography.

**2.** Discuss comprehensively the various *experimental techniques* of TLC:

 (*i*) Preparation of TLC plates,

 (*ii*) Choice of ‘adsorbents’,

 (*iii*) Choice of ‘solvent system in TLC’, and

 (*iv*) Activation of ‘adsorbent’.

3. Explain how one may use HPLC to accomplish the following :

 (*a*) Isolation of alkaloid and glycoside,

 (*b*) Control of microbiological processes.

 Give suitable examples in support of your answer

4. Based on ‘**acidimetry in non-aqueous titrations**’, how do we carry out the assay of the following ‘**drugs**’

 along with their theory, procedure and calculations:

 (*i*) Methyldopa;

 (*ii*) Adrenaline

 (*iii*) Metronitazole ;

 (*iv*) Salbutamol sulphate

5. How would your carry out complexometric titrations by the direct titration method? Discuss the assay of the

 Following pharmaceutical drugs explicitly :

 (*i*) Magnesium sulphate

 (*ii*) Calcium carbonate

 (*iii*) Dibasic calcium phosphate

 (*iv*) Zine undecylenate.

Sample Assignment -2

1. Potentiometric titration curves between 25 ml of 0.01 M NaF and 0.01 M La (NO3)3 may be obtained as thefollowing **three** predominant variants, namely :

 (*a*) Sigmoid (Regular) Curve,

 (*b*) First Derivative Curve, and

 (*c*) Second Derivative Curve.

With the help of a diagramatic neat-sketch of each curve explain and affirm which one gives the most reliable‘**equivalence point**’ and why

2. How would you carry out the assay of the following ‘**drugs**’ ?

 (*i*) Nitrazepam,

 (*ii*) Allopurinol,

 (*iii*) Bendrofluazide,

 (*iv*) Cimetidine,

 (*v*) Lomustine, and

 (*vi*) Ethinyloestradiol

**3.** (*a*) Give a plausible explanation of the theoretical aspects of amperometric methodof analysis with specificreference to both Fick’s Law and Nernst Equation.

(*b*) Give a brief account of the various salient features of amperometry.

4. How would you assay the following medicinal compounds amperometrically :

(*i*) Procaine hydrochloride,

 (*ii*) Procainamide hydrochloride,

(*iii*) Presence of Ni with dimethylglyoxime, and

(*iv*) Presence of Pb with K2Cr2O7 solution

Sample Assignment -3

1. Based on the ‘**diazotization reaction**’ how would you carry out the assay of the following ‘**drug substances**’ :

 (*i*) Isocarboxazid

 (*ii*) Phthalylsulphathiazole

 (*iii*) Sulphamelthoxazole

 (*iv*) Primaquine phosphate.

2. How would you assay the following medicinal compounds by Karl Fischer Method?

 (*i*) Prednisolone sodium phosphate

 (*ii*) Rifamycin sodium

 (*iii*) Sodium methyl hydroxybenzoate

 (*iv*) Triamcinolone acetonide.

3. What are the various detectorsused in GLC equipment ? Describe the following two commonly used detectors

in an elaborated manner :

 (*a*) Thermal Conductivity Detector (TCD)

 (*b*) Flame Ionization Detector (FID).

4. Give details for the assay of:

 (*a*) Water present in mentrophin, and

 (*b*) Chloroform present in colchicine.

**18. Students List**

|  |
| --- |
| STUDENT OF B.PHARMACY ;2017 BATCH |
| S.NO | SESSION | NAME | ROLL NO. | AKU REG.NO |
| 15 | 2017-21 | PRATIKA KUMARI | 17P01 | 17109107001 |
| 16 | 2017-21 | FARHEEN ALIA | 17P02 | 17109107002 |
| 17 | 2017-21 | RAJ NANDINI NAYAN | 17P03 | 17109107003 |
| 18 | 2017-21 | SUBHASH KUMAR | 17P04 | 17109107004 |

**19. Previous Arya Bhatt Knowledge University Questions**

Pharmaceutical Analysis-II (Exam- 2016)

091304

Time- 3 Hours Full Marks: 70

Instructions:

(I)There are nine questions in this paper. All questions carry equal marks.

(II)Attempt any five questions in all

(III) Question No-1 is compulsory.

1. Answer any seven 2 X 7

True or False

1. A coulomb is the quantity of electricity given by the flow of one ampere of current for one second (T/F)

2. The principle of separation is mainly partition rather than adsorption in paper chromatography. (T/F)

3. Cadmium ions can be called electro- reducible material (T/F)

Fill in the blanks:

4. Masking agent Triethanolamine for……………

5. Saturated calomel electrode is a ………………..

6. A complexing agent is an electron……………..ion.

7. Paper chromatography can be considered to be type of ………….. Chromatography.

MCQ

8. In TLC the Rf value is ratio of

 (a) Distance traveled by solute of solvent

 (b) Distance traveled by solute of solute

 (c) Both of above

 (d) None of above

9. Pyrolysis in GLC is done at a temperature between

 (a)100-200 0 C

 (b) 1000-2000 0 C

 (c) 2000-4000 0 C

 (d) 500-1000 0 C

10. Confirmation of the end point by adding a drop or two of the titrant is called

 (a)Fleeting end point

 (b)Floating end point

 (c) Flooding end point

 (d) 500-1000 0 C

2. Discuss chromatographic techniques andprincile involved in TLV and paper Chromatography.

3. What is difference between reference and indicator electrodes. Classify different types of electrodes used in potentiometry and discuss the principle underlying potentiometry.

4. Write short notes on:

(a) Principle and application of non-aqueous titration

(b) Nernst equation and glass electrode

5. Give an account on basic system suitability parameters used in high performance liquid chromatography (HPLC).

6. Write Short notes on

(a) Gasometry

(b) Instrumentation of GLC

7. Write Short notes on

(a) Complex metric titrations

(b) GLC

8. Discuss in detail polarography, its theory, principle and methods of analysis.

9. Write a detailed note on theory , principle and application of Amperometric titration.