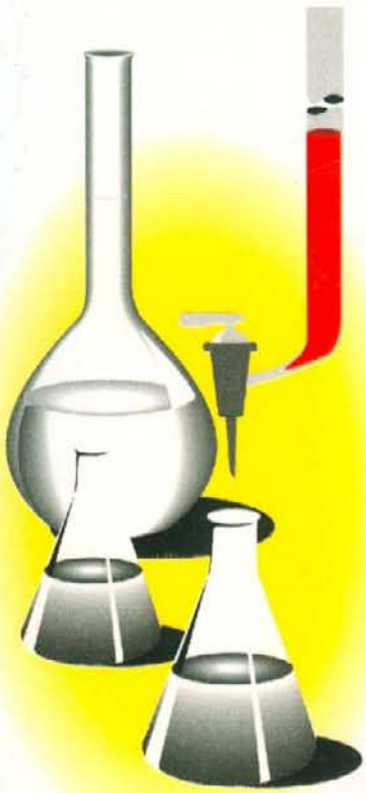
Revised Second Edition

PHARMACEUTICAL DRUG ANALYSIS



Ashutosh Kar

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PHARMACEUTICAL DRUG ANALYSIS

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Methodology-Theory-Instrumentation
Pharmaceutical Assays-Cognate Assays

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Preface to the Second Edition

Modern **Pharmaceutical Drug Analysis** essentially involves as a necessary integral component even greater horizons than the actual prevalent critical analysis of not only the active pharmaceutical substances but also the secondary pharmaceutical product(s) *i.e.,* the dosage forms having either single or multi-component formulated product. The fundamental reasons for this sudden legitimate surge in the newer evolving methodologies in the **'analysis of drug substances'** are perhaps due to the tremendous growth in the progress of 'medicinal chemistry' towards achieving one ultimate objective which is to obtain **'better drugs for a better world'.**

With the advent of computer-aided-drug modeling (CADM) the critical, scientific and faster approach to newer drug entities based on the biologically active prototypes, combinatorial chemistry, chiral chemistry and biotechnology has paved the way towards more specific, potent and above all less toxic **'drugs'** to improve the ultimate quality of life in humans.

Keeping in view the above astronomical growth in the design of complicated, specific and highly active drug molecules an equally viable, rigorous, accurate and precise analytical methods have been evolved with the passage of time which have now occupied pivotal and vital positions in most of the **Official Compendia** *viz*., USP, BP, Int.P., Eur. P, IP etc., for the analysis of such compounds both in pure and dosage forms.

The articulated developments in the fields of science and technology being utilized as on date, amalgamated with relatively stringent new regulations, namely: Federal Drug Authority (FDA); International Conference on Harmonization (ICH); Current Good Manufacturing Practices (cGMP); Pre-Approval Inspections (PAIs) and the like are now serving as a **'legal binding'** specifically for the pharmaceutical drug analysis even much more complicated in comparison to the situation prevailing almost two decades ago.

The present revised textbook on **'Pharmaceutical Drug Analysis'** caters for the much needed handbook and reference book, which is absolutely current with regard to the esteemed philosophy of analytical chemistry, an obvious solid support towards drug discovery, development, stability studies, bioavailability and pharmacokinetic studies, and above all the quality assurance of pure drugs together with their respective dosage forms.

The *thirty-two different chapters* meticulously divided into *six parts* invariably covers up analytical techniques being used in most of the **Official Compendia.** Each chapter categorically and explicitly deals with the introduction, theoretical aspect(s), instrumentation, typical examples of pharmaceutical analysis and cognate assays.

The textbook on **'Pharmaceutical Drug Analysis'** would enormously serve the undergraduates, postgraduates, researchers, analytical chemists working in the Quality Assurance Laboratories, new drug development, production and control, teaching, or regulatory authorities.

ASHUTOSH KAR

Preface to the First Edition

The ever expanding and broad horizon of Pharmaceutical Sciences invariably emphasizes one cardinal aspect that basically they are nothing but 'Applied Sciences.' With the advent of newer drug molecules either partially synthesized, totally synthesized or isolated from naturally occurring microbial and plant products—it has become absolutely necessary to ascertain and examine critically their physical characteristics, chemical equivalence, chemical impurities and their prescribed limits, degradation of products, metabolites and above all their biological features. All these salient features of a '*drug*' help a researcher not only in planning a precise experimental design but also in the interpretation of data in a logical and scientific manner. Pharmaceutical scientists ought to have a good command over the wide-spectrum of chemical analysis so as to achieve completeness in their scientific pursuit of knowledge. Unfortunately, such information is either found scattered in various available literatures or appears as an extremely specific work on a rather scanty and limited subject area.

The main objective of **'Pharmaceutical Drug Analysis'** is to offer not only a ready reference handy textbook but also an intermediate level of coverage for the convenient analysis of pure pharmaceutical substances and their respective dosage forms wherever applicable. The present copious textual compilation of information is solely intended to narrow down the apparently wide gap existing between the available basic texts and the extremely specific research papers from various scientific journals. The contents of this textbook have been meticulously designed to provide fundamentals of various disciplines embodying pharmaceutical drug analysis specifically for the under-graduate students. It will also be useful to the graduate students studying modern methods of pharmaceutical analysis to a great extent. Particular emphasis has been laid on the pharmaceutical substances that are specially found in the *Official Compendia*. It will also cater to scientists and investigators, working in other fields of pharmaceutical sciences who wish to update their personal wealth of knowledge and understanding of the intricacies of modern methods of Pharmaceutical Drug Analysis. Enough literature have been cited at the end of each chapter under 'Recommended Readings' so as to enable the reader to follow up a particular topic with ease.

Part—I has three chapters that exclusively deal with 'General Aspects' of pharmaceutical analysis. Chapter 1 focuses on the pharmaceutical chemicals and their respective purity and management. Critical information with regard to description of the finished product, sampling procedures, bioavailability, identification tests, physical constants and miscellaneous characteristics, such as : ash values, loss on drying, clarity and color of solution, specific tests, limit tests of metallic and non-metallic impurities, limits of moisture content, volatile and non-volatile matter and lastly residue on ignition have also been dealt with. Each section provides adequate procedural details supported by ample typical examples from the *Official Compendia*. Chapter 2 embraces the theory and technique of quantitative analysis with specific emphasis on volumetric analysis, volumetric apparatus, their specifications, standardization and utility. It also includes biomedical analytical chemistry, colorimetric assays, theory and assay of biochemicals, such as : urea, bilirubin, cholesterol; and enzymatic assays, such as alkaline phosphatase, lactate dehydrogenase, salient features of radioimmunoassay and automated methods of chemical analysis. Chapter 3 provides special emphasis on errors in pharmaceutical analysis and their statistical validation. The first aspect is related to errors in pharmaceutical analysis and embodies classification of errors, accuracy, precision and makes

an attempt at minimizing systematic errors. The second aspect is mainly devoted to statistical validation and comprises of statistical treatment of finite samples, distribution of random errors, significant errors, comparison of results, method of least squares and criteria for rejection of an observation.

The various modern techniques involved in Pharmaceutical Drug Analysis mostly covered in the *official compendia* have been adequately dealt with in Part II through Part VI and systematically spread over from Chapter 4 through Chapter 32 in the present textual compilation.

Each chapter has its unique style of presentation that essentially comprises of the following vital features, namely : brief introduction, theory with necessary details and relevant reactions, instrumentation, assay methods—with typical appropriate examples invariably selected from the *Official Compendia* including brief theoretical treatment of individual pharmaceutical substance and dosage form, materials required, procedures, calculations wherever applicable, cognate assays and lastly citation of relevant literature under 'Recommended Readings'.

Part—II contains twelve chapters under the broad category of 'Chemical Methods'. Section—A deals on treatment by 'titrimetric methods' based on acidimetry and alkalimetry. The first arm of this section deliberates on aqueous titrations (Chapter 4), while the second on non-aqueous titrations (Chapter 5). Section—B relates to 'redox methods' with specific reference to permanganate, dichromate and ceric sulphate titration methods (Chapter 6); and also the iodimetric and iodometric titrations (Chapter 7). Section—C concerns with the 'precipitation methods' and focuses on argentometric methods (Chapter 8). Section—D comprises the 'complexometric methods' using organic ligands, such as EDTA. Particular stress has been laid on the effect of pH on complexation, stability of complexes, usage of pM indicators and masking and demasking agents (Chapter 9). Section—E solely embodies the conventional 'gravimetric methods'. The topic has been treated with respect to Law of Mass Action, reversible reactions, principle of solubility of product and common-ion effect. Typical examples have been included of pharmaceutical substances assayed after conversion to free acid, or free base, or free compound and lastly to derivatives or substitution products (Chapter 10). Section—F is entirely devoted to 'thermoanalytical methods' consisting mainly of thermogravimetric analysis (TGA), differential thermal analysis (DTA) and lastly thermometric titrations (TT) (Chapter 11). Section—G particularly embodies the 'miscellaneous methods' which do not fall into the regimen of Section—A through Section—F. It deals with diazotization (Chapter 12), estimation of phenols and related compounds (Chapter 13) using bromine or potassium bromate, potassium iodate solutions; Karl Fischer method for determination of water (Chapter 14); and lastly tetrazolium assay of steroids (Chapter 15).

Part—III exclusively treats 'Electrochemical Methods' invariably and extensively used in the analysis of pharmaceutical substances in the *Official Compendia*. Two important methods, namely; potentiometric methods (Chapter 16) deal with various types of reference electrodes and indicator electrodes, automatic titrator; besides typical examples of nitrazepam, allopurinol and clonidine hydrochloride. Amperometric methods (Chapter 17) comprise of titrations involving dropping-mercury electrode, rotating—platinum electrode and twin-polarized microelectrodes (*i.e*., dead-stop-end-point method).

Part—IV has been entirely devoted to various 'Optical Methods' that find their legitimate recognition in the arsenal of pharmaceutical analytical techniques and have been spread over nine chapters. Refractometry (Chapter 18) deals with refractive index, refractivity, critical micelle concentration (CMC) of various important substances. Polarimetry (Chapter 19) describes optical rotation and specific optical rotation of important pharmaceutical substances. Nephelometry and turbidimetry (Chapter 20) have been treated with sufficient detail with typical examples of chloroetracyclin, sulphate and phosphate ions. Ultraviolet and absorption spectrophotometry (Chapter 21) have been discussed with adequate depth and with regard to various vital theoretical considerations, single-beam and double-beam spectrophotometers; besides typical examples amoxycillin trihydrate, folic acid, glyceryl trinitrate tablets and stilbosterol. Infrared spectrophotometry (IR) (Chapter 22) essentially deals with a brief introduction of group-frequency

region and fingerprint region followed by detailed theoretical aspects covering molecular vibrations and factors influencing vibrational frequencies. Having described the single monochromator infrared spectrophotometers, the applications of IR-spectroscopy have been discussed with respect to pharmaceutical substances and pharmaceutical dosage forms. Analytical aspects of IR-spectroscopy have also been treated adequately for the determination of *cis-trans* isomer ratio in clomiphene citrate, distinction of pri-, sec- and tert-amine salts from one another, studying complex formations and quantitative reaction sequences, identification of functional groups and fingerprinting.

Nuclear resonance spectroscopy (NMR) (Chapter 23) treats the subject with regard to the NMR-phenomenon and proton-NMR. Various theoretical aspects viz., orientations of magnetic nucleus under external-magnetic field (Bo), precessional frequency, saturation of the signal, absorption positions in NMR-spectrum, chemical shift, spin-spin interactions, ^{3}H-NMR, ^{13}C-NMR and 2D-NMR. Special emphasis has been given to the interpretation of a NMR-spectrum, chemical shift, relative peak area, multiplicity of the signal and coupling constant. Instrumentation has been dealt adequately. Applications of NMR-spectroscopy in pharmaceutical analysis, identification testing and assay of drugs have been treated so as to justify their vital importance in modern methods of analysis.

Emission spectroscopy (Chapter 24) provides a brief introduction, theory and instrumentation with regard to its excitation sources, electrodes, sample handling, monochromators, detectors, spectrographs and its applications. Flame spectroscopy (Chapter 25) widely used in the quantitative estimation of various elements *e.g*., Na, K, Ca, Ba has also been included. Both simple flame photometer and internal-standard flame photometer have been discussed in sufficient detail. The assay of Na, K and Ca in blood serum and water; assay of Ba, K and Na in calcium lactate have been described followed by cognate assays. Atomic absorption spectroscopy (AAS) (Chapter 26) treats this versatile aspect of analytical technique at length. It deals with the merits of AAS Vs Flame Emission Spectroscopy (FES) specifically treats both the single-beam and the double-beam AAS followed by the dements of AAS. Instrumentation specifically treats both the single-beam and the double-beam AAS. The various aspects of AAS *e.g*., analytical techniques, detection limit and sensitivity, and interference have been duly covered. A few typical examples of AAS in pharmaceutical analysis *e.g.*, Zn in Insulin-Zinc Suspension, Pd in Carbenicillin Sodium have been described followed by cognate assays.

Part—V is solely confined to the 'Assay Methods based on Separation Techniques' and is spread over five chapters. Liquid-liquid extraction (Chapter 27) mostly treats the subject theoretically and is supported by appropriate examples. Errors due to the volume change and effectiveness of an extraction have been dealt with adequately. Various factors that influence solvent extraction, such as : temperature and inert solutes, pH ion-pair formation and synergistic extraction have been described. A number of typical assay methods, for instance : Cu (I) as the neo-cuproin complex, Fe (III) as the 8-hydroxy quinolate complex, Pb (I) by the dithizone method, Mo (VI) by the thiocyanate method and Ni (II) as dimethylglyoxime complex and by synergistic extraction have been discussed. Thin-layer chromatography (TLC) (Chapter 28) illustrates the versatility of this technique over paper and column chromatography. Various aspects of experimental techniques of TLC viz., preparation of thin layers on plates, choice of adsorbents and solvent systems, activation of adsorbent, purification of adsorbent layers, spotting of components, development of thin layers, special techniques in TLC, chemical reactions on TLC plates, combination of TLC with other techniques and finally detection and evaluation of chromatograms have been expatiated profusely with examples. Applications of TLC in pharmaceutical analysis have been discussed in sufficient length. Gas-liquid chromatography (GLC) (Chapter 29) exclusively treats the subject with regard to various theoretical aspects, namely: plate theory, rate theory, random walk and nonequilibrium theory. Instrumentation comprises mainly different vital components. The working techniques for quantitative analysis *e.g.*, area normalization and internal standard method have been described. Lastly the applications of GLC in pharmaceutical analysis have been described with suitable examples from the *Official Compendia*.

High Performance Liquid Chromatography (HPLC) (Chapter 30) gives an elaborate discussion of theoretical aspects. Instrumentation encompasses the various important components *e.g*., solvent reservoir and degassing system; pressure, flow and temperature; pumps and sample injection system;

columns; detectors; strip-chart recorder; data-handling device and microprocessor control. Another important aspect to facilitate HPLC known as the 'derivatization' has been discussed. The applications of HPLC in the assay of drugs, such as: cephalosporins, frusemide, theophylline, corticosteroids, dichlorphenamide, Human Insulin and lastly cognate assays have been fully elaborated.

Size Exclusion Chromatography (Chapter 31) has also been included as a means of analysis for substances that undergo separation more or less as per their molecular size, viz., insulin and human insulin—for proteins of higher molecular weight; corticotrophin—for impurities of higher molecular weights; and plasma-protein solution—for polymers and aggregates.

Part—VI has been solely devoted to 'Miscellaneous Assay Methods' wherein radioimmunoassay (RIA) (Chapter 32) has been discussed extensively. Various arms of theoretical aspects viz., hapten determinants and purity; importance of antigenic determinants; and analysis of competitive antibody binding of isotopically labeled compounds. The applications of RIA in pharmaceutical analysis, such as : morphine, hydromorphone and hydrocordone in human plasma; clonazepam, flurazepam in human plasma; chlordiazepoxide in plasma; barbiturates, flunisolide in human plasma have been described elaborately. Lastly, the novel applications of RIA-techniques, combined RIA-technique-isotope dilution and stereospecificity have also been included to highlight the importance of RIA in the analytical armamentarium.

It is earnestly believed that **'Pharmaceutical Drug Analysis'** will fulfill the entire requirements of both penultimate and final year students of B. Pharm., for their various courses in analytic chemistry in the universities. It may also help the post-graduate students in their compulsory paper on 'Modern Analytical Techniques' to a great extent.

'Pharmaceutical Drug Analysis' will prove to be a valuable and indispensable guide to those working in Research & Development Laboratories, Quality Assurance Laboratories as well as Drug Testing Laboratories where either new products are being developed or routine analyses are carried out. Academicians and researchers engaged in the evaluation of pharmaceutical drug substances either in pure or dosage forms will also enormously benefit from **'Pharmaceutical Drug Analysis'** by virtue of its ultimate goal of maintaining very high standards of quantitative analysis.

Finally, I wish to record here my special thanks to the numerous colleagues and friends who have not only extended their invaluable help by providing me with relevant sources of material but also by taking an active participation in the discussion of various chapters.

It is hoped that **'Pharmaceutical Drug Analysis'** will soon prove to be an invaluable guide to both undergraduate and postgraduate students and to my esteemed colleagues in the teaching profession. Those working in Research & Development Laboratories, Quality Assurance Laboratories and Drug Testing laboratories will also find the book helpful in solving many of their intricate problems.

ASHUTOSH KAR