SUMMARY

The subject of Pharmaceutical sciences is a broad one and embraces the knowledge of the source, physical and chemical properties, compounding, physiological actions, absorption and excretion and therapeutic uses of drugs. With the advent of newer drug molecules either partially 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 products, metabolites and above all their biological features. The quality of the drugs encompasses the potency, uniformity, purity, pharmacological action, stability etc. It is the responsibility of the manufacturer to maintain the quality and produce effective, safe and non-toxic forms of the drug. Quality assurance and control of Pharmaceutical chemicals and formulations is essential for ensuing the availability of safe and effective drug formulations to consumers. Hence, pharmaceutical analysis occupies a vital role in statutory certification of drugs and their formulations either by the industry or by the regulatory authorities.

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 scientific manner for the determination of drug in its pharmaceutical formulations. The various problems encountered in pharmaceutical analysis coupled with the importance of achieving results in new methods of analysis being quickly adopted by the pharmaceutical industry and chemical laboratories depending upon the facilities available.

Among several instrumental methods (HPLC, GC, fluorimetry, NMR, Mass Spectroscopy, Spectrophotometry (covering IR, UV and visible regions) available for the assay of drugs, visible spectrophotometric techniques are considered to be simple and less expensive. The sensitivity and selectivity of the colorimetric methods depend on the nature of the chemical reactions involved in color development and not on the sophistication of the equipment.

Visible spectrophotometric techniques have been extensively used in the present investigations. Five drugs namely 7-Aminodesacetoxy cephalosporanic acid (7-ADCA), Rifabutin (RFB), Azelaic acid (AZA), Capecitabine (CPTB), Clostebol acetate (CSB) have been chosen for the development of new analytical methods with the selection of appropriate chromogenic reagents for their assay by exploiting their characteristics, (physical and chemical properties) based upon the basic moieties and functional groups present in them.

The choice of the chromogenic regent for color development on a particular method is still a challenging problem. It depends upon the careful consideration of factors such as the scale of economics of the reaction, the presence of other functional groups besides the chosen one that might be adversely affected by the reagents, the instability or high reactivity of desired colored product, the rate of reaction and other related factors. The objective is to get the best yields possible: The selection of a chromogenic reagent for the determination of particular drug is made after a literature survey for the methods that have been under consideration.

This Ph.D thesis attempts to provide the use of Twenty four chromogenic reagents such as Bromo thymol blue (BTB, M_{1a} for RFB), Bromocresol green (BCG, M_{1b} for RFB), Bromocresol purple (BCP, M_{1c} for RFB), Alizarin Red – S (ARS, M_{1d} for RFB); Safranine-O (SFNO, M_{2a} for ADCA&AZA); Methylene blue (MB, M_{2b} for ADCA&AZA), Methylene violet (MV, M_{2c} for ADCA&AZA); Iodine / P-N-methyl amino phenol sulphate – Para sulphanilic acid (I_2 /PMAP- SAc, M_3 for RFB & CPTB); Tannic acid /Metol – Potassium dichromate (TA / Metol –Cr (VI), M_4 for RFB & CPTB); Citric acid – Acetic anhydride (C-A, M_5 for RFB) ;1,2-Naphthoquinone sulphonate (NQS, M_6 for ADCA) ; Vanillin (VN, M_7 for ADCA) ; Ninhydrin-ascorbic acid (NIN/AA,M_8 for ADCA); 4 Amino- phenazone (4 AP, M_9 for CSB); Isonicotinic acid hydrazide (INH, M_{10} for CSB); β-Naphthol, Hydrogen peroxide, Dinitrophenyl hydrazine (β-NPT,H_2O_2-DNPH, M_{11} for ADCA); N-Bromo succinimide/ Metol-sulphanilic acid (NBS/PMAP- SAc M_{12} for RFB, ADCA); Haematoxylin - chloramine T, (Haet-CAT, M_{13} for ADCA); Sodium meta periodate / Sodium

molybdate/p-N-methylaminophenol sulphate- Sulphanilamide (IO_4^-/ Mo(VI)/PMAP-SA, M_{14} for CPTB); N-Bromo succinimide – cellistine blue (NBS/CB, M_{15} for RFB&ADCA); Chloramine T- Gallocyanine (CAT/GC, M_{16} for ADCA&RFB); Potassium permanganate – fast green FCF (MnO_4^- / FGFCF, M_{17} for ADCA&RFB); Folin ciocalteau reagent (FC reagent, M_{18} for ADCA&RFB); Ammonium Molybdate – H_2SO_4, (AM/ H_2 SO_4, M_{19} for ADCA&RFB); Ferric chloride / o-phenanthroline (Fe^{3+} / o-Phen, M_{20} for ADCA&RFB); Ferric chloride / Potassium ferricyanide (Fe^{3+} /$K_3[Fe(CN)_6]^{3-}$, M_{21} for ADCA&RFB); Sodium meta periodate / 3-Methyl benzothiazolinone (IO_4^-/MBTH, M_{22} for ADCA&RFB); Chloranil (CL, M_{23a} for ADCA&RFB); Chloranil-Acetaldehyde (CL-CH_3CHO, M_{23b} for ADCA&RFB); Sodium Nitroprusside-Acetone (SNP-CH_3COCH_3, M_{24a} for ADCA); Sodium Nitroprusside- Hydroxylamine (SNP–HA,M_{24b} for RFB); for the assay of selected drugs in pure form and pharmaceutical formulations. The analytical utility of these chromogenic reagents has been reviewed separately.

Under the proposed experimental conditions, the methods M_1 to M_{24} refer to the serial number. The alphabets 'a' to 'd' refer to the actual dye (acidic, M_1; basic, M_2; in ion association complex formation); aromatic primary amine (M_{12} in redox /charge transfer complex formation) or variation under experimental conditions (Complex formation with chloranilic acid, M_{23} or Sodium nitroprusside, M_{24}) in the developed methods.

Methods (M_3, M_4, M_{12}, M_{14}-M_{17}, M_{20}, M_{21}) are indirect ones (drugs is not involved in color formation) in which the procedures involve two steps. The first step in methods (M_{12}, M_{14}, M_{19}-M_{21}, M_{20}, M_{21}) is the oxidation of the drug with excess oxidant (NBS in M_{12}& M_{14}; IO_4^- in M_{14}, CAT in M_{16} $KMno_4^-$ in M_{17}, Fe (III) in M_{20}&M_{21}) giving the products (inclusive of the reduced form of oxidant IO_3^- in M_{14}, Fe (II) in M_{20}&M_{21}) besides the unreacted oxidant. In methods (M_3& M_4), the drug is precipitated in the form of an adduct with the excess reagent (I_2 in M_3, TA in M_4). The second step in methods (M_{12}, M_{14}, M_{20}, M_{21}) is the reaction of either the reduced form of oxidant [Fe (II) with o-Phenanthroline in M_{20} or $K_3Fe(CN)_6$, M_{21}, IO_3^- with PMAP-SA after masking IO_4^- with Mo(VI), M_{14}] with color producing agent to develop color. In methods (M_{15}, M_{16}& M_{17}), the second step is the reaction between the unreacted oxidant and dye thereby diminishing

the intensity of dye color (CB in M_{15}, GC in M_{16}, FGFCF in M_{17}). In all these methods, the oxidant reacted with the drug in the first step, which corresponds to the amount of the drug, is calculated by substracting the unreacted oxidant from the oxidant initially taken. The second step in methods (M_3&M_4) is the color development of the precipitant existing in the filtrate after the separation of adduct through filtration (I_2, M_3; TA, M_4) followed by color development with PMAP-Cr (VI) (M_4) or PMAP –SAc (M_3) by formation of charge transfer complex.

The rest among the developed methods are direct ones in which either the drug (M_1, M_2, M_5, M_6 – M_{11}, M_{13}, M_{18}, M_{19}, M_{23}& M_{24}) or its oxidation product (M_{22}) reacts with the reagent (acid dye, M_1; basic dye, M_2; NQS,M_6;Vanillin, M_7;Ninhydrin-ascorbic acid, M_8; 4-AP,M_9;INH, M_{10};FC, M_{18};AM-H_2SO_4, M_{19};CA, M_{23};SNP,M_{24}) or its initial reaction product with another reagent (aconitic anhydride,M_5;Haematin, M_{13}) or insitu formed intermediate from MBTH and aldehyde formed from drug through oxidation (M_{22}) to produce color.

Chapter – I opens with the introduction giving a brief account of various aspects to be considered for the development of new visible spectrophotometric methods for the assay of selected drugs. They include classification, official status, chemical names, structures, analytically useful functional groups in drugs, analytical utility of chromogenic reagents in general and in the investigation, chemistry of the colored species formed for the methods developed and the general methodology for developing new visible spectrophotometric methods [spectral characteristics of the colored species, optimisation of experimental conditions (effect of pH, reagent concentration, order of addition, keeping time and temperature during each addition, effect of solvent, rate of color formation and stability), optical characteristics (Beer's law limits, sandell's sensivity, optimum photometric range and molar absorptivity useful for sensitivity studies), interference studies (selectivity), precision (standard deviation, percent range of error, testing of significance by F-test), accuracy (comparision of the proposed and reference methods of pharmacentical formulations, testing of significance by t-test) and reaction sequence in color formation in the investigations].

Chapter – II begins with the introduction giving a brief account of chemical name, structures, analytically useful functional groups, therapeutic importance, commercially available formulations and literature on physico-chemical methods reported for ADCA. As little attention was paid in developing visible spectrophotometric methods by exploiting thoroughly the analytically useful groups in ADCA, the author has developed Nineteen visible spectrophotometric methods for the estimation of ADCA in bulk samples and pharmaceutical formulations. Formulation sample solutions were prepared in such a way that any interference caused by active or inactive ingredients accompanying the selected drug in one or more among the proposed methods was avoided based on their differences in their solubility behaviour (inorganic solvents, acid or base).

The decreasing order of sensitivity (ϵ_{max}) among the proposed methods are M_7>M_{2a}>M_{2b}>M_{2c}>M_{13}>M_{23a}>M_6>M_{15}>M_{23b}>M_{16}>M_8>M_{21}>M_{17}>M_{12}>M_{20}>M_{11}>M_{19}>M_{18}>M_{24a}.

Chapter –III starts with the introduction giving a brief account of chemical name, structures, analytically useful functional groups, therapeutic importance, commercially available formulations and literature on physico-chemical methods reported for RFB. As little attention was paid in developing visible spectrophotometric methods by exploiting thoroughly the analytically useful groups in RFB, the author has developed eighteen visible spectrophotometric methods for the estimation of RFB in bulk samples and pharmaceutical formulations. Formulation sample solutions were prepared in such a way that any interference caused by active or inactive ingredients accompanying the selected drug in one or more among the proposed methods was avoided basing on their differences in their solubility behaviour (inorganic solvents, acid or base).

The decreasing order of sensitivity ϵ_{max} among the proposed methods are M_{20}> M_{23b}> M_{17}> M_{21}> M_{1b}> M_4> M_{15}> M_3> M_{24b}> M_{1c} > M_{1a}= M_{16}> M_{1d}> M_{23a}> M_5>M_{18}> M_{19}> M_{12} respectively.

Chapter IV starts with the introduction giving brief account of therapeutic importance and physico-chemical methods reported for AZA. The author has made some efforts and succeeded in developing three visible spectrophotometic methods. (MethodsM_{2a}, M_{2b}, M_{2c}). Methods M_{2a}, M_{2b}, M_{2c} exploits the Ion association complex reaction of AZA with basic dyes such as SFNO, M_{2a}; MB, M_{2b}; MV, M_{2c}.

The decreasing order of sensitivity ($\in_{max}$) among the proposed methods are $M_{2b}>M_{2a}>M_{2c}$.

Chapter V commences with the introduction giving brief account of therapeutic importance and physico chemical methods reported for CPTB. There is no report on the present work of the spectrophotometric determination of CPTB. The author has made some attempts and succeeded in developing four visible spectrophotometric methods by exploiting various functional groups of CPTB (Methods M_3, M_4, $M_{14}$and M_{22}).

The decreasing order of sensitivity ($\in_{max}$) among the proposed methods are $M_4>M_3>M_{14}>M_{22}$.

Chapter – VI begins with the introduction giving brief account of therapeutic importance and physico-chemical methods reported for CSB. Exploiting thoroughly the analytically useful groups in CSB developed visible spectro photometric methods. The author has made some efforts and succeeded in developing two visible spectrophotometric Methods (M_9, M_{10}).

The decreasing order of sensitivity ($\in_{max}$) among the proposed methods are $M_{10}>M_9$.

The analytical data concerning optimum conditions fixation, selectivity, sensitivity, precision and accuracy studies given in chapters II to VI reveal that the proposed methods are simple, selective (through interference studies of usually existing additives and active ingredients in formulations) and accurate with reasonable precision and accuracy. Hence the proposed methods can be used as alternative methods to reported ones and provide a wide choice for the routine assay of selected drugs existing in bulk form or pharmaceutical formulations depending upon the availability of chemicals and situations arising due to the presence of concomitants. The part of the work was communicated to reputed national and international journals for publications.