SUMMARY

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Modern medicines for human use are required to meet exact standards which relate to their quality, safety and efficacy .The evolution of safety and efficacy and their maintenance in practice is dependent upon the existence of adequate methods for quality control of the product. 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 the pharmaceutical analysis coupled with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, precision and accuracy results in new methods of analysis being quickly adopted by the pharmaceutical industry and chemical laboratories depending upon the facilities available.

Among several instrumental techniques [HPLC, GC, fluorimetry, NMR, mass spectrophotometry, IR, UV and visible regions] available for the assay of drugs, visible spectrophotometric technique is considered to be simple and less expensive. The selectivity and sensitivity of the visible spectrophotometric method depends only on the nature of chemical reactions involved in color development and not on the sophistication of equipment.

The modern method of choice for assay of drugs is high performance liquid chromatography [HPLC] that requires highly sophisticated equipment, trained personnel, high purity chemicals and proper maintenance. HPLC technique has been

regarded as best among various techniques in spite of its heavy cost and proper maintenance. The development of highly efficient micro particulate bonded phase has increased the versatility of the technique and has greatly improved the analysis of multi component mixtures. The systems used are often described as belonging to one of four mechanistic types, adsorption, partitions, ion - exchange, size exclusion. Absorption and partition system can be normal phase (stationary phase more polar than eluent) or reversed phase (stationary phase less polar than eluent).

Visible spectrophotometry and **HPLC techniques** have been used in the present thesis work. Using visible spectrophotometry, twenty new analytical methods are developed for the assay of five selected drugs (TABLE-A) such as **Alfuzosin hydrochloride [AFZ], Cefprozil [CEF], Mirtazapine [MIRT], Betamethasone [BMS]** and **Eplerenone [EPL]** by exploiting their characteristics, physical and chemical properties depending upon functional groups present in each drug. In addition two HPLC procedures have been developed for the assay of CEF and MIRT in pharmaceutical formulations.

The content of the thesis has been divided into six chapters and appropriate references have been placed at the end of the each chapter.

Chapter-I deals with an introduction giving a brief account of various aspects to be considered for the development of new visible spectrophotometric (part-A) and HPLC (part-B) methods for the assay of five selected drugs. The introduction includes a brief account on selected drugs and their formulations in the present investigation and general information and methodology for the development of new methods using visible spectrophotometry (part-A) and HPLC (part-B). The information given under part-A classification includes analytically useful functional groups in drugs, chemistry of

chromogenic reagents, reactions used in the present investigation, and general methodology for developing new visible spectrophotometric methods (spectral characteristics of the colored species) optimization of experimental conditions (effect of pH, reagent concentration and order of addition, keeping time and temperature during each addition, effect of solvent, color development and stability) optical characteristics (Beer's law limits, Sandell's sensitivity, optimum photometric range and molar absorptivity useful for sensitivity), selectivity, precision, standard deviation, percent range of error, testing of significance by F-test, accuracy(comparison of the proposed and reference methods of pharmaceutical formulation, testing of significance by t-test and recovery experiments in the present investigations.

The information given under part-B, includes HPLC system components (solvent delivery systems, solvent degassing systems, gradient elution devices, sample introduction systems liquid chromatography detectors, column packing materials inclusive of bonded phase, derivatization, gradient elution), performance calculations (relative retention, theoretical plates, plates per meter, height equivalent to theoretical plate, capacity factor, resolution, peak asymmetry), linear fit properties of solvents used in chromatography and validation of analytical methods(recovery, response function, sensitivity, precision and accuracy) in the present investigations.

In HPLC (part-B), the choice of stationary and mobile phases, internal standard, column conditions and detecting devices are important. The author has developed HPLC methods for the determination of CEF and MIRT and is included in their respective chapters.

Chapter-II begins with the introduction giving brief account of chemical name, structure, and mode of action, characteristics, analytically useful functional groups,

commercially available formulations and literature on physicochemical methods reported for Alfuzosin hydrochloride [AFZ]. There are few visible spectrophotometric methods for the assay of AFZ. Existing analytical methods reveal that relatively little attention was paid in developing visible spectrophotometric methods by exploiting thoroughly useful functional groups in AFZ. The chemical features of analytically useful functional groups in AFZ offer a lot of scope for the development of new methods, hopefully with better sensitivity precision and accuracy, which prompted the author to carry out investigations in this accord. The author has developed nine versatile spectrophotometric methods.

Alfuzosin hydrochloride [AFZ] possesses different functional groups such as aromatic primary amine, tertiary amine, and amide and methoxy groups of varied reactivity. Aromatic primary amine in Alfuzosin hydrochloride was responsible for the development of diazo coupling product with Phloroglucinol and Resorcinol [M_4 and M_5] in presence of $NaNo_2$; oxidative coupling product with 3-methyl-2-benzathiazolinone hydrazone (MBTH) in presence of Fe (III) salt [M_9], 4-amino phenazone (4-AP) in presence of IO_4^- [M_{10}], and Brucine in presence of IO_4^- [M_{11}]; Redox/Charge transfer complex formation reaction with p-Dimethylamino phenol sulphate (PMAP) in the presence of Cr (VI) [M_{13}]; charge transfer complex with Chloramine-T- PMAP-Sulphanilamide (SA) combination [M_{14}]; Redox reactions based on reducing property with FC reagent- Na_2CO_3 [M_{16}] and Fe(III) – $[Fe(CN)_6]^{-3}$ [M_{17}].The results are incorporated in chapter II.

Chapter-III focuses on the introduction giving brief account of chemical name, structures, therapeutic importance, commercially available formulations and analytically useful functional groups of cefprozil [CEF]. There are very few

physicochemical methods reported in the literature, hence there is a need for sensitive, accurate and flexible visible spectrophotometric methods for its determination in a wide variety of pharmaceutical formulations. The author has made some attempts in this direction and succeeded in developing nine visible spectrophotometric methods based on the analytically useful functional groups present in CEF.

Cefprozil [CEF] possesses β-lactam ring, phenolic hydroxyl, sulfide, amino, carboxylic acid and amide functional groups. The author has developed nine versatile spectrophotometric methods. Presence of 1° amine permits the nucleophilic substitution reaction with 1,2-Naphthaquinone sulphonic acid sodium salt (NQS) [M_2]; Based on phenolic hydroxyl group, diazo coupling reaction with diazotized p-nitro aniline in presence of alkali [M_6];Oxidative coupling reactions with MBTH in presence of Fe (III) oxidant [M_9] and with 4-AP in the presence of $NaIO_4$ oxidant [M_{10}]; Based on reducing property (phenolic hydroxyl, sulphides etc) charge transfer complex reaction with N-bromosuccinamide (NBS)-PMAP-SA combination [M_{15}]; Redox reactions with FC regent-Na_2CO_3[M_{16}], Ammonium Molybdate(AM)-H_2SO_4[M_{18}], $KMnO_4$-Fast green FCF [M_{19}]and Fe(III)-o-Phenanthroline [M_{20}].The results are incorporated in part-A.

Part-B of this chapter reveals a brief note on the chemical properties and the literature survey of the HPLC methods of cefprozil. A very few HPLC methods for the assay of CEF in pharmaceutical formulations were reported in the literature. Taking all these views of the drug into consideration, the author has developed as simple HPLC method for the quantitative estimation of CEF by using stationary phase [a stainless steel column 250mm long, 4.6mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5μm diameter][use (Inertisil ODS-3V, 5μ(250mmx4.6mm)] and mobile phase combination of phosphate buffer of pH 4.2 and

acetonitrile in the ratio of 50:50 v/v. The detection was carried at 220nm. The results of this investigation are presented in this part.

Chapter –IV opens with the introduction giving or brief account of chemical name, structure, therapeutic importance, analytically useful functional groups, commercially available formulations and the literature on the physicochemical methods reported so far for Mirtazapine [MIRT]. As there are very few visible spectrophotometric methods for the assay of MIRT, there is a need to develop few more visible spectrophotometric methods for its determination in a wide variety of pharmaceutical formulations. The author proposed seven visible spectrophotimetric methods by exploiting the functional groups present in MIRT.

Mirtazapine [MIRT] possesses tertiary amine group. The seven methods proposed by the author are based on reactivity of tertiary amine. Internal salt formation reaction with aconitic anhydride (dehydration product of citric acid) [M_7]; Ion-association complex formation with acid dyes such as Bromocresol green (BCG) [M_{8a}] and Bromocresol purple (BCP) [M_{8b}]; Redox reactions with FC reagent-Na_2CO_3 [M_{16}], with Fe(III) - $[Fe(CN)_6]^{-3}$ [M_{17}], with $KMnO_4$-FastGreen FCF [M_{19}] and with Fe(III)-o-Phenanthroline [M_{20}].

Part-B of this chapter reveals a brief account on the chemical properties and the literature survey of the HPLC method of Mirtazapine. A very few HPLC methods for the assay of MIRT were reported in the literature. The author has developed a simple HPLC method for the quantitative estimation of MIRT with a better sensitivity by using stationary phase [a stainless steel column 250mm long, 4.6mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5μm diameter] [symmetry C_{18}, 5μ(250mmx4.6mm)] and mobile phase combination of [solution A and

solution B in the ratio of 70:30v/v], where solution A is prepared by dissolving 6.8grams of potassium dihydrogen orthophosphate in 1000mL of water. Adjust pH to 7.4±0.05 with triethylamine and solution B is prepared by mixing acetonitrile and tetrahydrofuran in the ratio of 60:40v/v]. The detection was carried out at 292m. The results of investigation are incorporated in this part.

Chapter-V begins with the introduction giving a brief account of chemical name(s), therapeutic importance, structure, analytically useful functional groups, commercially available pharmaceutical formulations and literature on the physicochemical methods reported for Betamethasone [BMS]. $\Delta^{1,4}$- 3- Keto group, and α-ketol group present in BMS were exploited in the present investigation. The author developed five visible spectrophotometric methods for BMS.

Based on the reactivity of α-ketol group in BMS produces a Meisenheimer like σ-complex with m- dinitrobenzene (MDNB) in alkaline medium [M_1]; $\Delta^{1,4}$-3-Keto steroidal moiety give condensation product with Iso nicotinic acid hydrazide (INH) [M_3]; α-ketol group responsible for oxidative coupling product with MBTH in the presence of Fe(III)[M_9], and with $NaIO_4$/phenylhydrazine hydrochloride (PHH)+$[Fe(CN)_6]^{-3}$ (hexacyano ferrate (III)) in acid medium [M_{12}]; α-ketol probably responsible for the redox reaction with ammonium molybdate (AM) - H_2SO_4 [M_{18}] .

Chapter-VI deals with the introduction giving a brief account of chemical structure, chemical name, therapeutic importance, commercially available formulations and analytically useful functional groups in Eplerenone [EPL]. There are few visible spectrophotometric methods for the assay of EPL. This prompted the author to develop simple and sensitive visible spectrophotometric methods by exploiting the functional

groups present (Δ^4-3-keto steroidal moiety, 9,11- epoxide moiety, methyl ester and a γ - lactone) in EPL. The efforts led to the development of two visible spectrophotometric methods. Δ^4-3-keto moieties responsible for σ-complex formation with MDNB in alkaline medium [M_1]; and condensation reaction with INH [M_3] are presented.

The data and information of selected drugs, reagents and techniques given in chapters [II-VI] (TABLE-B); reveals that the proposed methods are simple, selective, sensitive (some are superior to most of the reported visible spectrophotometric methods) and accurate with reasonable precision and accuracy. In addition, selectivity to each selected drug and its formulations was achieved by selecting the appropriate combination of solvent systems, acids or bases in the sample solution preparation and exploring specific functional groups exclusively present in the drug but not in the exciepients, additives or other active ingredients present in the formulations, to the extent possible. In any visible spectrophotometric method, if the exploited functional group and solubility characteristics of selected drug and another active ingredient are similar it is not possible to estimates the selected drug in combined dosage forms, unless they are separated initially. The proposed methods can be used as alternative methods to reported ones and provide wide choice for the routine determination of the above mentioned drugs depending upon the availability of chemicals and situation arising due to the presence of concomitants. The order of the absorption maxima and sensitivity for the selected drugs that were discussed in the present thesis are given in the (TABLE-C). Five papers were published (including one supporting paper), one paper was in press (supporting paper) and much of the work has been communicated to reputed national and international journals.