SUMMARY

Analytical chemistry may be defined as the science and art of developing sensitive, reliable and accurate method for determining the composition of materials in terms of elements or compounds which they contain. Analytical techniques or methods are applied mainly in two areas, viz qualitative analysis and quantitative analysis for identifying or quantifying constituents in a sample.

The best way to characterize the quality of a bulk drug is to determine its purity. There are two possible approaches to reach this goal. The determination of the active ingredient content with a highly accurate and precise specific method or the determination of its impurities. It involves the introduction of more refined and sensitive methods of physiochemical analysis such as colorimetry, spectrophotometry covering UV, visible and IR regions, fluorimetry or turbidimetry, NMR and Mass, and chromatography (GLC, HPLC, TLC) that enables one to assay of drugs more accurately and with the smallest consumption of the analyte, reagent and time.

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 **four** selected drugs {**Gemcitabine [GEMC], Aceclofenac [ACFN], Dorzolamide [DORZ], Fexofenidine [FEFD]**} **[TABLE -A,P.294]** by exploiting their functional groups present in them with the use of appropriate chromogenic reagents in pure form and pharmaceutical formulations **[TABLE -B,P.295 - 298]**. In addition to visible spectrophotometric methods, the author has developed **four** new HPLC procedures for the assay of above **four** selected drugs **{GEMC, ACFN, DORZ, FEFD}** in pharmaceutical formulations **[TABLE - C, P.299]**.

The content of the thesis has been divided into **five 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 four selected drugs. 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 **four** new HPLC procedures for the assay of above **four** selected drugs{ **GEMC,ACFN, DORZ, FEFD**}in pharmaceutical formulations 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 Gemcitabine [GEMC]. Very few visible spectrophotometric methods for the assay of GEMC inpharmaceutical formulations. Existing analytical methods reveal that relatively little attention was paid in developing visible spectrophotometric methods by exploiting thoroughly useful functional groups in GEMC. The chemical features of analytically useful functional groups in GEMC 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 **eleven** versatile visible spectrophotometric methods for the assay of GEMC in pure and Pharmaceutical formulations which is included in **Part-A.**

Gemcitabine [GEMC] possesses different functional groups such as aromatic primary amine, tertiary amine, and keto groups of varied reactivity. Aromatic primary amine in gemcitibine was responsible for the development of **ion-association complex formation** with **acid dyes** such as **Bromocresol purple (BCP) [M_{1a}]** and **Bromocresol green (BCG) [M_{1b}], condensation reaction** with **Isatin - H_2SO_4 [M_3]; Xanthydrol-H_2SO_4 [M_4]** and **Vanillin-H_2SO_4 [M_5]; PDAB [M_6]; PDAC [M_7]; ninhydrin** in presence of **ascorbic acid[M_8]; ß-**

naphthol with H_2O_2 [M_{15}] and **diazo coupling product** with **Phloroglucinol** and **Resorcinol** [M_{12} and M_{13}].

Part-B of this chapter reveals a brief note on the chemical properties and the literature survey of the HPLC methods of **Gemcitabine.** 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 GEMC 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 (**BDS-C_{18}, 150mmx4.6mm**)] and **mobile phase** in the combination of [**solution A** and **solution B** in the ratio of **60:40 v/v**], where **solution A** is prepared by dissolving 5.16grams of **Sodium acetate** in 950mL of water and adjusted to 1000mL with 50mL **Methanol, solution B** is **Acetonitrile.** The detection was carried at **268nm.** The results of this investigation are presented in this part.

Chapter-III focuses on the introduction giving brief account of chemical name, structures, therapeutic importance, commercially available formulations and analytically useful functional groups of **Aceclofenac [ACFN].** 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 **eleven** visible spectrophotometric methods based on the analytically useful functional groups present in ACFN which is included in **part -A.** As **aceclofenac [ACFN]** possesses carboxylic acid, secondary nitrogen

and keto functional groups of varied reactivity the author has developed **eleven** versatile spectrophotometric methods.

Methods (M_{2a} and M_{2b}) are based on the formation of **ion association complex** of **carboxylic acid groups** of **Aceclofenac** with **basic dyes (Safranin-O, Methylene blue)**. More over the ketone moiety present in **Aceclofenac** undergoes condensation reaction with **4 -AP** and **INH [M_{10} & M_{11}].** The Presence of secondary amine in **Aceclofenac** permits oxidative coupling reactions with **MBTH** in presence of **Fe (III) oxidant [M_{14}]** ,with **4-AP** in the presence of **$[Fe(CN)_6]^{-3}$ oxidant [M_{15}],** with **DCQC [M_{16}]** and with **$NaIO_4$/phenylhydrazine hydrochloride (PHH)+$[Fe(CN)_6]^{-3}$ (hexacyano ferrate (III))** in acid medium **[M_{17}]** that produces colored product; **complex formation reaction** with **CTC [M_{18}]; charge tranfer complex formation** with **DDQ [M_{19}]** and **inner color complex reactions** with **(SNP - HA) [M_{20}].**

Part-B of this chapter reveals a brief note on the chemical properties and the literature survey of the HPLC methods of **aceclofenac [ACFN].** A very few HPLC methods for the assay of ACFN 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 ACFN by using stationary phase a stainless steel column **[Phenomenex,5μ(250mmx4.6mm)]** and **mobile phase** combination of **phosphate buffer** of **pH 4.2** and **acetonitrile** in the ratio of **45:55 v/v.** The detection was carried at **275nm.** 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 **Dorzolamide [DORZ].**

Part -A of this chapter describes the authors attempts in developing visible spectrophotometric methods as there are very few visible spectrophotometric methods for the assay of **Dorzolamide [DORZ]** in the literature survey and hence, there is a need to develop few more visible spectrophotometric methods for its determination in various pharmaceutical formulations. Based on this the author proposed **seven** visible spectrophotimetric methods by exploiting the functional groups present in **Dorzolamide [DORZ].**

Dorzolamide [DORZ] possesses tertiary amine group. The **seven** methods proposed by the author are based on reactivity of tertiary amine. **Ion-association complex formation** with **acid dyes** such as **Bromocresol purple [M_{1a}]** and **Bromocresol green (BCG) [M_{1b}]; oxidative couping reactions** with **MBTH** in presence of **Fe (III) oxidant [M_{14}]**, with **4-AP** in the presence of **$[Fe(CN)_6]^{-3}$ oxidant [M_{15}]** and with **DCQC[M_{16}]; complex formation reaction** with **CTC [M_{18}]** and **charge transfer complex formation** with **DDQ [M_{19}].**

Part-B of this chapter reveals a brief account on the chemical properties and the literature survey of the HPLC method of **Dorzolamide [DORZ].** A very few HPLC methods for the assay of DORZ were reported in the literature. The author has developed a simple HPLC method for the quantitative estimation of DORZ 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] **[BDS, C_{18}, 5μ(250mmx4.6mm)]** and **mobile phase** combination of **Phosphate buffer of**

pH 2.2 and **Methanol** in the ratio of **92:8 v/v.**. The detection was carried out at **254nm**. 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 **Fexofenidine [FEFD]. Carboxylic** and **tertiary nitrogen group** present in **FEFD** were exploited in the present investigation. The author developed **three** visible spectrophotometric methods for **FEFD.**

Part -A of this chapter is focused on the author attempts in developing suitable visible spectrophotometric methods with better sensitivity, selectivity, precision and accuracy by exploiting the analytically useful groups in FEFD, The **carboxylic acid group** of **Fexofenidine** forms ion association reaction with **basic dyes (Methylene blue and Methylene violet) that are studied under proposed methods (M_{2b} and M_{2c})** and more over the **tertiary nitrogen** of **FEFD** forms **complex formation reaction** with **CTC [M_{18}].**

Part -B of this chapter reveals a brief note on the chemical properties and the literature survey of the HPLC methods on **Fexofenidine (FEFD).** As there are very few HPLC (especially in pharmaceutical formulations) methods for the assay of **Fexofenidine (FEFD)**reported in the literature the author has attempted to developed a simple HPLC method for the quantitative estimation of **FEFD** with a better sensitivity by using stationary phase **[Intersil;BDS;3V,250x4.6mm,5µ column]** and **mobile phase** combination of **[solution A** and **solution B** in the ratio of **40:60 v/v]**, where **solution A** is 0.05M **Ammonuim acetate** solution, **solution B** is **Methanol**

. The detection was carried at **230nm**. The results of this investigation are incorporated in this part of this chapter

The data and information of selected drugs, reagents and techniques given in chapters [II-V] (**TABLE - B,P.295-298**); 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. 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-D,P.300**). Three papers were published (including one supporting paper), three papers were in press (including two supporting papers) and much of the work has been communicated to reputed national and international journals.

TABLE - A

STRUCTURAL FEATURES OF SELECTED DRUGS

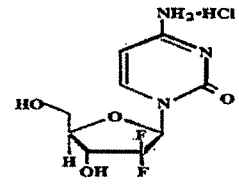
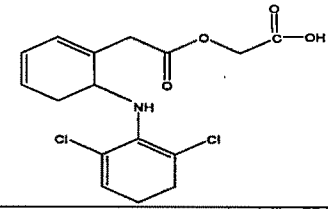
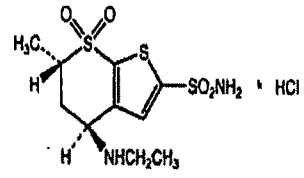
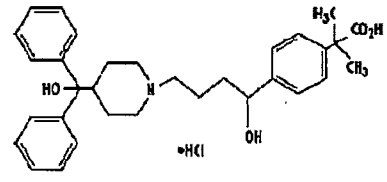
Sl. No	Generic Name	Drug Category	Chemical Name, Molecular formula& Molecular weight	Structure	Analytical important moieties/functional groups
1	**Gemcitabine**	**Anti cancer**	**(2,2´-difluorodeoxy-cytidine, dFdC)** **Molecular Formula:** $C_8H_{14}N_2O_2$ **Molecular weight:** 170.21 g mol^{-1}		Primary,Tertiary nitrogen and keto group
2	**Aceclofenac**	**Anti-inflammatory**	[2-[(2,6-Dichlorophenyl)amino]phenyl]acetyl] oxy]acetic acid. **Molecular Formula:** $C_{16}H_{13}Cl_2NO_4$ **Molecular weight: 354.2 g mol^{-1}**		Keto group, Aliphatic Carboxylic and secondary nitrogen group
3	**Dorzolamide hydrochloride**	**Anti-glaucoma**	(4S-trans)-4-(ethylamino)-5,6-dihydro-6-methyl4H-thieno[2,3-b]thiopyran-2-sulfonamide7,7-dioxide monohydrochloride **Chemical Formula:** $C_{10}H_{16}N_2O_4S_3 \cdot HCl$ **Molecular weight:** 360.9		secondary nitrogen group
4	**Fexofenadine hydrochloride**	**Anti -histamine**	(±)-4-[1 nylmethyl)-1-piperidinyl]-butyl]- α, α-dimethylhydroxy-4-benzeneacetic acid hydrochloride **Chemical Formula:** $C_{32}H_{39}NO_4 \cdot HCl$ **Molecular weight:** 538.13		Carboxylic and Tertiary nitrogen group

TABLE - B

LIST OF PROPOSED AND REPORTED VISIBLE SPECTROPHOTOMETRIC METHODS

Drug Estimated	Functional group/moiety in the drug exploited	Reagent used for the exploitation of functional group/ moiety	Method proposed in the thesis	Type of reaction involved	Chapter no in which the method incorporated
Gemcitibine (GEMC)	Aliphatic primary amine group	BCP	M_{1a}	Ion association complex	II
	---- do---	BCG	M_{1b}	---- do---	II
	---- do---	Isatin - H_2SO_4	M_3	Condensation	II
	---- do---	Xanthydrol - H_2SO_4	M_4	Condensation	II
	---- do---	Vanillin	M_5	Condensation	II
	---- do---	PDAB	M_6	Condensation	II
	---- do---	PDAC	M_7	Condensation	II
	---- do---	Ninhydrin - Acsorbic acid	M_8	Condensation	II
	---- do---	β-Naphthol,H_2O_2,2,4-DNPH	M_9	Condensation	II
	Aliphatic primary amine group	$NaNO_2$-phloglucinol	M_{12}	Diazo coupling	II
	---- do---	$NaNO_2$-resorcinol	M_{13}	---- do---	II

TABLE - B

LIST OF PROPOSED AND REPORTED VISIBLE SPECTROPHOTOMETRIC METHODS

Drug Estimated	Functional group/moiety in the drug exploited	Reagent used for the exploitation of functional group/ moiety	Method proposed in the thesis	Type of reaction involved	Chapter no in which the method incorporated
Aceclofenac (ACFC)	Carboxylic group	SFNO	M_{2a}	Ion association complex	III
	Carboxylic group	MB	M_{2b}	---- do---	III
	Keto group	4-AP	M_{10}	Condensation	III
	Keto group	INH	M_{11}	Condensation	III
	Secondary amine group	MBTH-Fe (III)	M_{14}	Oxidative coupling	III
	---- do---	4AP-K_3 $[Fe(CN)]_6$	M_{15}	---- do---	III
	---- do---	DCQC	M_{16}	---- do---	III
	---- do---	IO_4^-/PHH/$[Fe(CN)_6]^{-3}$	M_{17}	---- do---	III
	Secondary amine group	CTC	M_{18}	Complex formation	III
	---- do---	DDQ	M_{19}	Charge transfer complex formation	III
	---- do---	SNP - HA	M_{20}	Inner complex formation	III

TABLE - B

LIST OF PROPOSED AND REPORTED VISIBLE SPECTROPHOTOMETRIC METHODS

Drug Estimated	Functional group/moiety in the drug exploited	Reagent used for the exploitation of functional group/ moiety	Method proposed in the thesis	Type of reaction involved	Chapter no in which the method incorporated
Dorzolamide (DZLD)	Aromatic primary amine group	BCP	M_{1a}	Ion association complex	IV
	---- do---	BCG	M_{1c}	---- do---	IV
	Secondary amine group	MBTH-Fe (III)	M_{14}	Oxidative coupling	IV
	---- do---	4AP-K_3 $[Fe(CN)]_6$	M_{15}	---- do---	IV
	---- do---	DCQC	M_{16}	---- do---	IV
	Secondary amine group	CTC	M_{18}	Complex formation	IV
	---- do---	DDQ	M_{19}	Charge transfer complex formation	IV

TABLE - B

LIST OF PROPOSED AND REPORTED VISIBLE SPECTROPHOTOMETRIC METHODS

Drug Estimated	Functional group/moiety in the drug exploited	Reagent used for the exploitation of functional group/ moiety	Method proposed in the thesis	Type of reaction involved	Chapter no in which the method incorporated
Fexofenidine (FEFD)	Carboxylic group	MB	M_{2b}	Ion association complex	V
	Carboxylic group	MV	M_{2c}	---- do---	V
	Teritary amine group	CTC	M_{18}	Complex formation	V

TABLE - C

RP - HPLC METHODS

Drug responded	Method proposed in the thesis	Chromatographic column used	Mobile phase composition	Detection limit	Linearity range	Reference
Gemcitibine	M_{21}	**BDS-C_{18}, 5μ(150mmx4.6mm)**	Combination of [**solution A** and **solution B** in the ratio of **60:40 v/v**], where **solution A** is prepared by dissolving 5.16grams of **Sodium acetate** in 950mL of water and adjusted to 1000mL with 50mL **Methanol, solution B is Acetonitrile**	268	3-25.4μg.ml^{-1}	**Chapter - II**
Aceclofenac	M_{22}	**Phenomenex, 5μ(250mmx4.6mm)**	Combination of **phosphate buffer** of **pH 4.2** and **acetonitrile** in the ratio of **45:55 v/v**	275	4-20μg.ml^{-1}	**Chapter - III**
Dorzolamide	M_{23}	**BDS-C_{18} 5μ (250mmx4.6mm)**	**Phosphate buffer of pH 2.2** and **Methanol** in the ratio of **92:8 v/v**	254	20-80μg.ml^{-1}	**Chapter - IV**
Fexofenidine	M_{24}	**Intersil, 5μ(250mmx4.6mm)**	Combination of [**solution A** and **solution B** in the ratio of **40:60 v/v**], where **solution A** is 0.05M **Ammonuim acetate** solution, **solution B** is **Methanol**	230	1-5μg.ml^{-1}	**Chapter - V**

TABLE - D

THE ORDER OF SENSITIVITY (ε_{max}) OF THE SELECTED DRUGS

Drug	ε_{max} (Sensitivity)
Gemcitabine	$M_8 > M_{1b} > M_9 > M_{1a} > M_{13} > M_{12} > M_6 > M_7 > M_3 > M_4 > M_5$
Aceclofenac	$M_{20} > M_{14} > M_{2b} > M_{2a} > M_{19} > M_{15} > M_{16} > M_{17} > M_{11} > M_{18} > M_{10}$
Dorzolamide hydrochloride	$M_{19} > M_{1b} > M_{1a} > M_{16} > M_{14} > M_{18} > M_{15}$
Fexofenadine hydrochloride	$M_{18} > M_{2b} > M_{2c}$