SUMMARY

DEVELOPMENT OF SPECTROPHOTOMETERIC METHODS FOR THE
QUANTITATIVE ESTIMATIONS OF ANTINEOPLASTIC DRUGS

GENERAL INTRODUCTION:

Drug (French – Drouge, a dry herb). It is the single active chemical entity present in a medicine that is used for diagnosis, prevention, treatment, cure of a disease. “A drug may be define as an agent intended for use in the diagnosis, mitigation, treatment cure of prevention of disease in man or in other animals.”

The anticancer or antineoplastic drugs either kill cancer cells or modify their growth. However selectivity of majority of drugs is limited & they are one of the most toxic drug’s used in therapy.

The term cancer malignant, neoplasm, & malignant tumors are synonymous & are distinguished from benign tumors by the properties of differentiation, invasiveness & the ability of metastasis (spread to other part of the body).

Treatment of malignant disease with drugs is a rather recent development started after 1940 when nitrogen mustard was used but progress has been rapid both in revealing pathobiology of disease & discovery of new drugs.

Types of drug’s used in cancer chemotherapy

(i) Alkylating agent & related compound.
(ii) Vincalkaloids.
(iii) Antimetaboilites.
(iv) Cytotoxic antibiotics.
(v) Hormone.
(vi) Radioactive isotopes.
(vii) Miscellaneous agents.
LITERATURE REVIEW AND PAST WORK:

Seven different antibiotics now are established clinical anticancer agents, and several other antibiotics are undergoing clinical development. Most of these agents have been approved within the last few years. Thus, the recognition of antibiotics as an important class of antineoplastic drugs is very recent. However, some of the compounds in this class have been known for a long time. For example, dactinomycin (actinomycin D) was first isolated in 1940 by Waksman and Weedruff, although its activity against neoplasms was not described until 1958. Furthermore, plicamycin, originally discovered as aureolic acid in 1953, had to be rediscovered twice before its antitumor activity was established in 1962, these compounds were originally rejected as antibacterial agents because of their cytotoxicity. Only later it was found that this toxicity could be turned to an advantage in the chemotherapy of cancer to an discovery of antitumor activity is much simpler today, and some laboratories routinely screen extracts of microorganism cultures for cytotoxicity and lysogenic phage induction in bacteria (a predictor of potential antitumor activity), Such assays can be performed on small quantities of the extracts.

The production of antitumor agents from microbial fermentations has some special advantages and disadvantages over chemical synthesis. Occasionally, the biosynthesis can be controlled to afford novel analogues. This has been true for actinomycin and bleomycins. Strain selection and fermentation conditions can optimize the formation of a particular component of an antibiotic mixture. Thus, streptomyces parvullus produces dactinomycin almost, exclusively, in contrast with other species that form complex mixtures of actinomycins the fermentation in S. casespitosus has been developed similarly, to produce almost all mitomycin. In some cases, such as with doxorubicin, improvement of the antibiotic yield has
been difficult this results in an expensive product and intensive research on chemical synthesis.

The actionmycins comprise numerous closely related structures. All of them contain the same chromophore, a substituted 3-phenoxazone-1, 9-dicarboxylic acid known as actinooin. Each of the carboxyl groups is bonded to pentapeptide lactose by way of the amino group of an L-threonine unit of this pentapeptide. The hydroxyl group of the L-threonine forms part of the lactose, along with L-methylvaline, the fifth amino acid from the chromophore. D-Valine or D-alloisoleucine is the second amino acid, and the fourth amino acid usually is sarcosine. The third amino acid is more variable, consisting of L-proline, L-hydroxy-proline, L-oxoproline, or others, produced by called isoactinomycins, whereas those with different pentapeptide lactones are called anisoactinomycins. The individual pentapeptide lactones are designated A and B, depending on the or attachment to the 9-0H 1-carboxylic acid, respectively. Dactinomycinomycin (actinomycin D, actinomycin C₁) with an amino acid sequence of L-threonine, D-valine, L-proline, sarcosine and L-N-methylvaline. Actinomycin C³ which is used in Germany, differs from actinomycin D by a D-alloisoleucine unit instead of D-valine in both the α and β chains.

The mode of action of actinomycins has been studied extensively, and it now is generally accepted that they intercalate into double-helical DNA. In the intercalation process, the helix unwinds partially to permit the flat phinoxazone chromophore to fit in between successive base pairs. Adjacent G – C pairs are especially suitable because the 2-amino groups of the guanines can hydrogen bond with the carbonyl groups of threonines in the actinomycin. This bonding reinforces the bonding between the heterocyclic chromophores. Additional
stability is conferred by the interaction between the pentapeptide lactone chains and DNA. These chains lie in the minor groove of the double helix, running in opposite directions, and they make numerous vander Waals interactions with the DNA.

Interaction into DNA changes its physical properties in characteristic way. Thus, the length, viscosity, and melting temperature increase, whereas the sedimentation coefficient decreases. Changes in substituents on the actinomycine influence their binding to DNA, usually by making it less effective. Opening of a lactone ring or changing the stereochemistry of an amino acid abolishes activity, and replacement of the 4 and 6–methyl groups by other substituents reduces it. Replacement of the 2–amino group also reduces activity.

**PLANNING OF WORK:**

**Category of the Drugs Selected:**

The drug is defined as any substance or product that is used to modify or explore physiological systems or pathological states for the benefit of the recipient. The drugs are first grouped according to their therapeutic action and then subdivided according to the chemical structure of drugs. Category of the drugs selected for the work are as enumerated below:

**Antineoplastic:**

(i) **Alkylating Drugs**

Procarbazine (Matulane); Dacarbazine (DTIC) Altretamine (Hexalen).

(ii) **Purine Antagonists**

Mercaptopurine (6–MP); Fludarabine Phosphate.

(iii) **Pyrimidine Antagonists**

Cytarabine (ARA–C); Azacitidine

(iv) **Plant Alkaloids**
Vinblastine (Velban); Vincristine (Oncovin); Etoposide (VP-16, Ve Pe–Sid); Teniposide (Vumon); Paclitaxel (Taxol); Docetaxel (Taxotere); Dicloxacillin Sodium; Epirubicin; Epirubicin HCl; Epirubicin Benzoate; Mitoxantrone.

(v) **Sulphonamides**
Sulphamethoxy Pyridazine (SMPZ)

(vi) **Diuretic and Anti hypertensive**
Frusemide; 4–chloro–5–sulphamoylanthranilic acid (CSAA) (Decom–position Product of Frusemide)
It is a potent diuretic, and is also used in the treatment of hypertension.

(vii) **Keratolytic**
4–methyl benzoic acid; 4–methyl salicylic acid. They have bacteriostatic and Fungicidal properties.

(viii) **Antimalarial**
Pyrimethamine; 5–(4–chlorophenyl)–6–ethyl pyrimidine–2,4–diamine.

(ix) Methyclo thiazide and candesartan cilexetil


(xi) Moxonidine;4–chboro–N–(Imidazolidin–2–ylidene)–6–methoxy–2–methyl pyrimidine–5–Amine (C_{9}H_{12}ClN_{3}O) and Amlodipine.

(xii) Gatifloxacin and propyphenazone.

**Categorisation of work:**
In the present work an attempt has been made to develop quick and reliable methodology for control analysis of the selected drugs, available in formulations as single component, binary or ternary mixtures. Literature survey reveal no such work, so far has been done on these formulations using the applied techniques. Hence it was considered worth while to undertake this project. The methodology applicable to the selected formulations are summarized and are categorized in nine different chapters as enumerated below:

Chapter–II, Section–A: Simultaneous spectrophotometric analysis, using absorbance absorbitivity ratio techniques of the following binary mixture of drugs:
(A) Dicloxacillin Sodium– Docetaxel, Epirubicin–Epirubicin salt– Mitoxantrone; Mercaptopurine; Fludarabine phosphate; cytarabine; Azacitidine; Viblastine; Vincristine, Etoposide; Teniposide, Procarbazine; Dacarbazine; Paclitaxel; Altretamine; Sulphamethoxy Pyridazine; Frusemide; 4–chloro–5–sulphamoyl anthranilic acid; 4–methyl benzoic acid; 4–methyl salicylic acid; pyrimethamine; methyclo, Thiazide, candesarten; cilexetil; cycloxacillin; oxacillin; Moxonidine; Amlodipine; Gatigloxacin and propyphenazone and thiabenzazone.

Chapter–II, Section–B: Spectrophotometric estimation of total sulphonamides, using isoabsorptive point in a ternary mixture of trisulphadrugs.

Chapter–II, Section–C: Determination of isoabsorptive point, of an intact molecule and its decomposition product of an acid labile drug thia benzazone.

Chapter–III, Section–A: Simultaneous spectrophotometric analysis using difference absorbance/difference absorbance ratio technique based on pH–induced spectral changes of the following binary component drug formulations:
Epirubicin/Epirubicin benzoate–Mitoxantrone; Mercaptopurine; Fudarabine phosphate; viblastine; vincristine; Paceitaxel; Altretamine; Sulphamethoxy Pyridazine in presence of pyrimethamine.

**Chapter–III, Section–B:** Simultaneous spectrophotometric analysis using difference absorbance/difference absorbance ratio technique based on pH–induced spectral changes, of a ternary mixture of salicylamide, propyphenazone and pyrithyldione in presence of caffeine.

**Chapter–III, Section–C:** Difference spectrophotometric analysis, based on reaction–induced spectral changes of Frusemide in presence of its degradation product.

**Chapter–IV, Section–A:** First derivative spectrophotometric analysis of the following drugs. Dicloxacillin sodium–Docetaxel, Mercaptopuyrine–Epirubicin/ Fudarabinephosphate; Dacarbazine–Epirubicin; Epirubicinbenzoate; Dacarbazine–Mitoxantrone.

**Chapter–IV, Section–B:** Second derivative spectrophotometric analysis of the following drugs. Procarbazine–Epirubicin/Epirubicin benzoate; Frusemide in presence of its degradation products; 4–methyl Benzoic acid and 4–methyl salicylic acid.

**CHAPTER–V: VALIDATED SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF METHYCLO THIAZIDE AND CANDESARTAN CILEXETIL IN TABLET DOSAGE FORM:**

A method for the simultaneous determination of methyclothiazide and candesartan cilexetil in bulk and tablet dosage form was developed. The method employs formation and solving of simultaneous equations using 225.8 and 255 nm as two analytical wavelengths. The absorbance maxima of methyclo thiazide and
candesartan cilexetil were found to be 225.8 nm and 255 nm respectively in methanol. The linearity range lies between 1–7 µg/ml for methyclo thiazide and 2–10µg/ml for candesartan cilexetil at their respective wavelength. Both the drugs obey Beer’s law. The molar absorptivity and Sandell’s sensitivity were found to be 4.11×10⁴ and 0.0073 respectively for methyclo thiazide and for candesartan cilexetil 6.21×10⁴ and 0.0098 respectively. The recovery studies confirmed the accuracy of the developed method.

CHAPTER–VI: DETERMINATION OF DEGRADATION PRODUCT FOR COMBINATION CONTAINING CYCLOXACILLIN AND OXACILLIN IN CAPSULE DOSAGE FORM BY LC–MASS SPECTROSCOPY

A study was carried out using LC–mass spectroscopy to determine the degradation products of formulation (Capsules) containing cycloxacillin and Dicloxacillin. The degradation product of cycloxacillin was determined by using LC–mass spectroscopy methods. The one part of the degraded product was found to be penicilloic acid of cycloxacillin with molecular weight of 452 and the other part of the degraded product was found to be penilloic acid of cycloxacillin with molecular weight of 408. The degradation of cycloxaccin is so rapid and it could be because of presence of small amount of water, which could have been present in the alcohol used for granulation process, because these types of degradation occur normally as a result of hydrolysis.

CHAPTER–VII: QUANTITATIVE DETERMINATION OF PACEITAXEL FIRST ORDER DERIVATIVE UV–SPECTROPHOTOMETRY USING AREA UNDER CURVE: Paceitaxel (PCT) is used as antiulcer and mucosal protective. A new simple economical and rapid UV–spectrophotometric first order derivative method using “Area under curve” (AUC) technique has been developed
for the quantitative determination of Paclitaxel (PCT) in bulk and tablets. Methanol (20% v/v) was used as a solvent. Zero order spectrum of PCT was derivatised into first order using UV–probe software of the UV–vis spectrophotometer and the AUC was determined between the two selected wavelengths 276.80 nm to 306.00 nm. PCT followed linearity in the concentration range of 10–80 µg/mL with \( r^2 > 0.99 \). The quantity of drug estimated by this method was in good accord with label claimed. This method was found to be accurate precise and ruggedness as shown by low value of \% RSD.

CHAPTER–VIII: SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF MOXONIDINE AND AMLODIPINE IN TABLET DOSAGE FORM: Four new simple, accurate and precise spectrophotometric methods have been developed for simultaneous determination of moxonidine and amlodipine in pharmaceutical dosage form. Method (A) involves formation and solving of simultaneous equation using 299 nm and 364 nm as two wavelengths. Method(B) involves formation of Q–absorbance equation at 339 nm (iso absorptive point) and at 299 nm (\( e_{\text{max}} \) of moxonidine).

Method (C) involves first order derivative method for simultaneous estimation of these two drugs. Method (D) involves the area under curve (AUC) for first order derivative spectrum. Both the drugs obey the Beer’s law in the range 5–50 µg/mL for amlodipine and 5–40 µg/ml for moxonidine. The results of analysis have been validated statistically and by recovery studies.

CHAPTER–IX: SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF GATIFLOXACIN AND PROYPHENAZONE IN BULK DRUG AND IN OPHTHALMIC DOSAGE FORM: Three simple accurate, economical and reproducible spectrophotometric methods have been developed for the simultaneous estimation of gatifloxacin (GTX) and proyphenzone (PRZ) in ophthalmic dosage form. The
first method is Q-analysis method based on absorbance ratio at two selected wavelengths 303 nm (iso-absorptive point) and 292 nm ($\lambda_{\text{max}}$ of Gatifloxacin). The second method is simultaneous equation method based on measurement of absorbance at two wavelength 292 ($\lambda_{\text{max}}$ of Gatifloxacin) and 319 ($\lambda_{\text{max}}$ of propyphenazone) and the third method is area under curve method and wavelength range selected were 290–294 nm ($\lambda_1–\lambda_2$) for gatifloxacin and 317–321 nm ($\lambda_3–\lambda_4$) for propyphenazone. The linearity lies between 1–12 µg/mL for (GTX) and 1–12 µg/mL for propyphenazone for all the three methods. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guideline. As the % RSD was found less than 2, all the methods were proved to be precise and accurate and can be successfully applied for the simultaneous determination of both the drugs in bulk and ophthalmic dosage form.