Chapter II

Survey of literature and objectives of the present investigation
Section (i): Survey of literature of selected drugs

(a) Aciclovir (ACV):

Various spectrophotometric method\textsuperscript{1-4}, spectrophotometric and spectrofluorimetric method\textsuperscript{5,6}, Flow Injection Chemiluminescence method\textsuperscript{7}, polarographic determination\textsuperscript{8}, Fourier transform (FT) Raman spectroscopy\textsuperscript{9}, liquid chromatography-spectrofluorimetric detection\textsuperscript{10}, HPLC-fluorescence\textsuperscript{11}, electrochemiluminescence method\textsuperscript{12}, fluorimetric method\textsuperscript{13,14} and HPLC methods\textsuperscript{15-27}, are reported in the literature for the estimation of acyclovirin tablets formulations.

Sultan, M\textsuperscript{1}. Proposed a simple and reliable spectrophotometric method for the determination of acyclovir in pharmaceutical formulations. The method is based on its oxidative coupling reaction with 3-methylbenzothiazolin-2-one hydrazone (MBTH) in the presence of FeCl\textsubscript{3} as an oxidant to produce deep-green colored species measurable at 616 nm. The absorbance-concentration plot is linear over the range 20–200 µg ml\textsuperscript{-1} with minimum detectability of 1.06 µg ml\textsuperscript{-1} (4.71×10\textsuperscript{-6} M). The molar absorptivity was 9.41×10\textsuperscript{2} l mol\textsuperscript{-1} cm\textsuperscript{-1} with correlation coefficient (n=7) of 0.9998. The different experimental parameters affecting the development and stability of the color were studied carefully and optimized. The proposed method was applied successfully to the determination of acyclovir in its dosage forms. The percentage recoveries ±SD (n=9) were 98.63±0.34, 99.61±0.58, 99.35±0.58 and 99.72±0.86 for tablets, ophthalmic ointment and cream respectively. A proposal of the reaction pathway was presented.

Basavaiah, K and Prameela, H.C\textsuperscript{2}, proposed a simple and cost effective spectrophotometric method is described for the determination of acyclovir in bulk drug and in formulations. The method is based on the formation of blue coloured chromogen when the
drug reacts with Folin–Ciocalteu (F–C) reagent in alkaline medium. The coloured species has an absorption maximum at 760 nm and obeys Beer's law in the concentration range 50–450 \( \mu g \text{ ml}^{-1} \). The absorbance was found to increase linearly with increasing concentration of acyclovir, which is corroborated by the calculated correlation coefficient value of 0.9998 \((n=9)\). The apparent molar absorptivity and Sandell sensitivity were \( 1.65 \times 10^2 \text{ l mol}^{-1} \text{ cm}^{-1} \) and 1.36 \( \mu g \text{ cm}^{-2} \), respectively. The slope and intercept of the equation of the regression line are \( 6.87 \times 10^{-4} \) and \( 8.33 \times 10^{-3} \), respectively. The limit of detection was 5.68 \( \mu g \text{ ml}^{-1} \) and the limit of quantification was 18.95 \( \mu g \text{ ml}^{-1} \). The proposed method was successfully applied to the determination of acyclovir in pharmaceutical formulations. The reliability of the assay method was established by parallel determination by standard-addition method, and by recovery studies. The results demonstrated and the procedure is at least as accurate, precise and reproducible \((\text{RSD}<2\%)\) as the official method, while being simple and less time consuming. A statistical analysis indicated that there was no significant difference between the results obtained by the proposed procedure and those of the official method.

El-Din, M.K\(^3\), have developed two simple, accurate, and reliable spectrophotometric methods for the determination of 2 antiviral drugs, acyclovir (ACV) and ribavirin (RBV), in their pharmaceutical formulations. These methods are based on oxidation of the 2 drugs with either cerium (IV) ammonium sulfate (Method A) or potassium persulfate (Method B). The products of oxidation in both methods are coupled with 3-methylbenzothiazolin 2-one hydrazone, producing a deep blue color with a maximum absorption wavelength at 630 nm. In Method A, the absorbance-concentration plots were linear over the ranges of 5-50 and 10-60 microg/mL with detection limits of 0.18 microg/mL \((8 \times 10^{-7} \text{ M})\) and 0.63 microg/mL \((2.58 \times 10^{-6} \text{ M})\) for ACV and RBV, respectively. In Method B, the ranges were 5-45 and 20-50 microg/mL with detection limits of 0.11 microg/mL \((4.88 \times 10^{-7} \text{ M})\) and 1.40 microg/mL \((5.73 \times 10^{-6} \text{ M})\) for the 2 drugs, respectively. The molar absorptivities were 4.1
x \(10^3\) and 3.65 \(10^3\) L/mol/cm in Method A and 5.03 \(10^3\) and 3.97 \(10^3\) L/mol/cm in Method B for the 2 drugs, respectively. The proposed methods were applied successfully for the determination of the 2 drugs in their pharmaceutical formulations. The percentage recoveries +/- standard deviation were 99.57 +/- 0.86 and 100.82 +/- 0.46 for ACV; 99.41 +/- 1.08 and 100.35 +/- 1.03 for RBV. The results obtained were compared statistically with those given by official methods and showed no significant differences regarding accuracy and precision.

Hesham Salem\(^4\), have developed simple, rapid and sensitive spectrophotometric procedures were developed for the analysis of atenolol, timolol maleate, propranolol hydrochloride, metoprolol tartarate, betaxolol hydrochloride, levobunolol hydrochloride and bisprolol fumarate in pure form as well as in their pharmaceutical formulations. The methods are based on the reaction of these drugs as n-electron donors with the sigma-acceptor iodine, and the pi-acceptors: 7,7,8,8-tetracyanoquinodimethane, tetracyanoethylene, 2,3,5,6-tetrabromo-1,4-benzoquinone (bromanil) and 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil). The obtained charge-transfer complexes were measured at 365 nm for iodine (in 1,2-dichloroethane), at 840, 420, and 470 nm for 7,7,8,8-tetracyan-oquinodimethane, tetracyanoethylene and 2,3-di-chloro-5,6-dicyano-1,4-benzoquinone (in acetonitrile), respectively, and at 450 and 440 nm for bromanil and chloranil (in ethanol), respectively. Due to the rapid development of colors at ambient temperature, the obtained results were used on thin-layer chromatograms for the detection of the investigated drugs. Beer's plots were obeyed in a general concentration range of 4–120 μg ml\(^{-1}\) with correlation coefficients not less than 0.9991. The proposed procedures could be applied successfully to the determination of the investigated drugs in tablets and ophthalmic solutions with good recovery; percent ranged from 98.03±0.98 to 100.30±0.90. The association constants and standard free energy changes using Benesi–Hildebrand plots were studied.
Abdellatef, H.E et al\textsuperscript{5}, have developed simple and sensitive spectrophotometric and spectrofluorimetric methods for analysis of acyclovir and acebutolol hydrochloride. The proposed methods are based on oxidation of the selected drug with cerium(IV) ion in acidic medium with subsequent measurement of either the decrease in absorbance at 320 nm or the fluorescence intensity of the produced cerous(III) ion at 363 nm (excitation at 250 nm). Beer's law obeyed from 1.0-7.0 microg ml\textsuperscript{-1} and 0.25-2.5 microg ml\textsuperscript{-1} acebutolol hydrochloride, using the spectrophotometric and spectrofluorimetric method, respectively. The proposed methods were successfully applied for determination of the selected drug in its pharmaceutical preparation with good recoveries.

Ayad, M.M et al\textsuperscript{6}, proposed a simple and sensitive spectrophotometric and spectrofluorimetric methods are described for analysis of acyclovir and acebutolol hydrochloride. The proposed methods are based on oxidation of the selected drugs with cerium(IV) ion in acidic medium with subsequent measurement of either the decrease in absorbance at 320nm or the fluorescence intensity of the produced cerous(III) ion at 361-363nm (excitation at 250nm). Beer's law obeyed from 2 to 8, 0.25 to 2.5microgcm\textsuperscript{-1} acyclovir, 1 to 7 and 0.25 to 2.5microgml\textsuperscript{-1} acebutolol hydrochloride, using the spectrophotometric and spectrofluorimetric method, respectively. The proposed methods were successfully applied for determination of the selected drugs in their pharmaceutical preparations with good recoveries.
(b) Amiloride (AMI):

Various spectrophotometric method, capillary zone electrophoresis method and HPLC methods, are reported in the literature for the estimation of amiloride tablets formulations.

Martín, E. et al, have developed three methods for the simultaneous determination of amiloride (AMI) and hydrochlorothiazide (HCT): zero-crossing, derivative quotient spectra with normalized divisor and multiple linear regression (MULTIC) methods. The two first methods use the derivative spectrophotometry, and the last one uses the absorbance measurement. The three methods were used to determine both compounds in synthetic mixtures and pharmaceutical preparations with errors less than 5% and 15%, respectively.

Mohamed Ael-M and Salem H et al, proposed a simple multivariate calibration method for analysis of two types of hypotensive mixture. The mixtures are composed of chlorthalidone with atenolol or chlorthalidone with both amiloride hydrochloride and atenolol. The components of the mixtures result in substantial spectral overlap—between 87.5 and 91.0%. Resolution of the mixtures under investigation has been accomplished mainly by using CLS and PCR methods. The components in each mixture have been simultaneously determined in three commercial dosage forms with high accuracy and without interference from commonly encountered excipients and additives. Good recoveries were obtained with both synthetic mixtures and commercial tablets. The results obtained were compared with those from pharmacopeial methods and found to be in good agreement. The results obtained from CLS and PCR were also compared with those obtained from a 1D spectrophotometric method.

Ferraro, M.C et al, have developed a multivariate spectrophotometric calibration for the simultaneous analysis of synthetic samples and commercial tablet preparations containing...
hydrochlorothiazide (HCT) and amiloride hydrochloride (AMH) is reported. Partial least squares (PLS-1) analysis of electronic absorption spectral data allowed the rapid and accurate resolution of mixtures in which the analyte ratios were approximately 10:1, without the need of a previous separation step and without interference from other sample constituents. The method, validated by the analysis of synthetic mixtures of both drugs, where accuracy over the linear working range as well as inter- and intra-assay precision were determined, was used in the concentration ranges of 21.7-30.4 mg l(-1) for HCT and 1.8-3.0 mg l(-1) for AMH. The proposed method was successfully applied to the evaluation of the stability of the stock solutions of the analytes in MeOH-H(2)O and to the elaboration of drug dissolution profiles of commercial tablets, results being concordant with those furnished by the USP technique. The method was also employed for the determination of drug content in two different pharmaceutical formulations, providing results that were in excellent agreement with those obtained by HPLC.

Dinç, E and Ustündag, O et al31, describes four chemometric techniques, classical least squares (CLS) and inverse least squares (ILS) and principal component regression (PCR) and partial least squares regression (PLSR) were applied to the absorption and derivative spectrophotometric determinations of amiloride and hydrochlorothiazide in a pharmaceutical preparation. Four chemometric calibrations for both zero-order and first derivative spectra were constructed by measuring the absorbance and their dA/dlambda values at 34 points in the wavelength range 205-395 nm for a training set containing 2-10 microg/ml amiloride and 4-28 microg/ml hydrochlorothiazide corresponding to 25 point mixture design. The building chemometric calibrations were confirmed by using the synthetic mixtures containing two drugs. The results obtained by the proposed techniques based on the use of the measurements at the absorption spectra and at the first derivative spectra were statistically compared with each other.
Ferraro, M.C et al, proposed a numerical method, based on the use of spectrophotometric data coupled to PLS-1 multivariate calibration, is reported for the simultaneous determination of furosemide and amiloride hydrochloride in synthetic samples and commercial tablets. The method was applied in the concentration ranges of 8.0-13.0 mg l(-1) for furosemide and 1.0-1.6 mg l(-1) for amiloride hydrochloride. Its accuracy and precision were determined, and it was validated by the analysis of synthetic mixtures of both drugs. The method was successfully applied to the quantitation of furosemide and amiloride hydrochloride in three different pharmaceutical formulations, providing results in agreement with those obtained by HPLC. It allowed the rapid, accurate and precise simultaneous estimation of the concentration of both analytes of interest in spite of their important spectral overlap, high concentration relationship and the presence of small amounts of different, unmodelled, absorbing excipients.

Inés Toral, M et al, proposed a simple and fast method for the simultaneous determination of amiloride and furosemide by digital derivative spectrophotometry. HCI 1 x 10(-2) mol/l dissolved in ethanol was used as solvent and to extract drugs from formulations. Subsequently the samples were evaluated directly by first digital derivative spectrophotometry, using a smoothing factor of 8 and scale factor of 1 x 10(-4). The simultaneous determination of furosemide and amiloride can be carried out at 241.4 and 343.6 nm, respectively. In both cases, the zero crossing approach was used. When both compounds are present together in a sample, it is possible to quantify one in the presence of the other, without mutual interference. The determination range was found to be of 6.9 x 10(-8) to 16 x 10(-5) and 6.8 x 10(-8) to 8 x 10(-5) mol/l, for amiloride and furosemide, respectively. A good level of repeatability (RSD) of 0.9 and 0.6% was observed for amiloride and furosemide, respectively. The ingredients commonly found in commercial
pharmaceutical formulations do not interfere. The proposed method was applied to the
determination of these drugs in pharmaceutical formulations.

(c) Chloroquine (CQ):

Various methods are reported in the literature for the estimation of chloroquine which
includes, spectrophotometric method43-48, spectrophotometric and conductometric method49,
Fluorimetric method50 and HPLC method51.

Shaban M et al43, proposed a sensitive spectrophotometric method for the
determination of some antimalarial drugs such as chloroquine (CQ) and pyrimethamine
(PYM). The method involves the formation of ion-pairs between the two drugs under
investigation and inorganic complexes of Molybdenum(V)-thiocyanate followed by its
extraction with methylene chloride. The optimum conditions for the ion-pairs formation are
established. The method permits the determination of chloroquine and pyrimethamine over a
concentration range of 2.0–42.0 and 2.0–43.0 μg ml⁻¹, respectively. The Sandell sensitivity is
found to be 0.027 and 0.042 μg cm⁻² for chloroquine and pyrimethamine, respectively. The
method was simple, rapid, reproducible and accurate within ±1%. The method is applicable
to the assay of the two drugs under investigation in different dosage forms and the results are
in good agreement with those obtained by the official method.

Zayed, M.A et al44, have developed two simple and accurate spectrophotometric
methods for the determination of anti-malarial drugs, chloroquine phosphate (CQP) and
pyrimethamine (PYM), in pure and in different pharmaceutical preparations. The charge
transphere (CT) reactions between CQP and PYM as electron donors and 2,3-dichloro-5,6-
dicyano-p-benzoquinone (DDQ) π-acceptor and iodine σ-acceptor reagents to give highly
coloured complex species have been spectrophotometrically studied. The optimum
experimental conditions have been studied carefully. Beer’s law is obeyed over the
concentration range of 1.0–15 μg ml\(^{-1}\) for CQP and 1.0–40 μg ml\(^{-1}\) for PYM using I\(_2\) and at 5.0–53 μg ml\(^{-1}\) for CQP and 1.0–46 μg ml\(^{-1}\) for PYM using DDQ reagents, respectively. For more accurate results, Ringbom optimum concentration range is calculated and found to be 10–53 and 8–46 μg ml\(^{-1}\) for CQP and PYM using DDQ, respectively and 5–15 and 8–40 μg ml\(^{-1}\) for CQP and PYM using iodine, respectively. The Sandell sensitivity is found to be 0.038 and 0.046 g cm\(^{-2}\) for DDQ method and 0.0078 and 0.056 g cm\(^{-2}\) for I\(_2\) method for CQP and PYM, respectively which indicates the high sensitivity of both methods. Standard deviation (S.D. = 0.012–0.014 and 0.013–0.015) and relative standard deviation (R.S.D. = 0.09–1.4 and 1.3–1.5\%) \((n = 5)\) for DDQ and I\(_2\) methods respectively, refer to the high accuracy and precision of the proposed methods. These results are also confirmed by between day precision of percent recovery of 99–100.6%, and 98–101% for CQP and PYM by DDQ method and 99–102% and 99.2–101.4% for CQP and PYM by I\(_2\) method respectively. These data are comparable to those obtained by British and American pharmacopoeias assay for the determination of CQP and PYM in raw materials and in pharmaceutical preparations.

Alaa S et al\(^{45}\), proposed a simple, rapid, accurate and sensitive spectrophotometric method for the microdetermination of some pharmaceutically important aminoquinoline antimalarials, namely amodiaquine dihydrochloride (I), chloroquine phosphate (II) and primaquine phosphate (III). The method is based on the interaction of these drugs with calmagite indicator to give highly coloured ion-pair complexes which exhibit maximum absorption at 663, 665 and 666 nm, respectively, Beer's law is obeyed in the concentration ranges 1.0–25.0, 1.0–28.0 and 1.0–33.0 μg/ml for the drugs I, II and III, respectively. For more accurate analysis, the Ringbom optimum concentration ranges are 2.5–22.5, 2.0–26.0 and 3.0–30.0 μg/ml, respectively. The apparent molar absorptivities were calculated. Statistical treatment of the experimental results indicates that the method is sufficiently
accurate and precise. The accuracy of the method is indicated by the recovery (99.8±1.4%) and the precision by the relative standard deviation (>1.5%). The proposed method has been applied to the determination of these drugs in certain formulations, with results that compared favourably with those obtained by the official methods.

Abdel-Gawad et al\textsuperscript{46}, have developed a spectrophotometric method for the determination of trace amounts of chloroquine and mebeverine in pharmaceuticals. The method was based on the use of Rose Bengal.

Raghuveer et al\textsuperscript{47}, proposed a spectrophotometric method for the determination of chloroquine phosphate in dosage form. In this method orthogonal polynomial coefficient was employed to correct for irrelevant absorption arising due to the presence of pharmaceutical excipients in the assay of chloroquine phosphate tablets.

Abdel salam et al\textsuperscript{48}, developed a sensitive and simple spectrophotometric method for the determination of some antimalarials. The method is based on the formation of mol., complexes between iodine as a acceptor and the basic drug in chloroform solution.

Alaa S et al\textsuperscript{49}, have developed two methods for the determination of amodiaquine hydrochloride, chloroquine phosphate and primaquine phosphate, based on the formation of their ion-associates with [Cd\textsuperscript{2+}, Co\textsuperscript{2+}, Mn\textsuperscript{2+} and Zn\textsuperscript{2+}] thiocyanate, ammonium reineckate and/or sodium cobaltinitrite. The molar combining ratio reveal that (1:1) (drug:reagent) ion associates are formed for all reagents except for ammonium reineckate which form (1:2) ion associates with all studied drugs. The optimum conditions for the ion-association have been studied. Conductometric method was applied for the direct determination of the suggested drugs in bulk powders, whereas indirect atomic absorption spectrometric method, depending on the measurement of the excess metal ion present in supernatant solutions after precipitation of the ion associates is used to calculate the drug concentration. Optimum
concentration ranges for the determination of aminoquinoline antimalarial drugs under consideration were 0.46–12.90 and 0.155–3.87 mg using conductometric and indirect atomic absorption spectral methods, respectively. The proposed procedures have been applied successfully to the analysis of these drugs in certain formulations and the results are favourably comparable to the official methods.

(d) Clomipramine(CPH):

Literature survey reveals spectrophotometric method\textsuperscript{52-56}, chemiluminometric determination\textsuperscript{57}, electrophoresis method\textsuperscript{58} and HPLC method\textsuperscript{59}, for estimations of clomipramine in its formulations.

Padmarajiah et al\textsuperscript{52}, proposed a new, simple, and sensitive spectrophotometric method for the determination of certain tricyclic antidepressants, belonging to the dibenzazepine class of drugs. The proposed method is based on the reaction of imipramine hydrochloride (IPH), desipramine hydrochloride (DPH), clomipramine hydrochloride (CPH), trimipramine maleate (TPM) or opipramol (OPP) with iron(III), and subsequent reaction with ferricyanide in an acetic acid medium, to yield a blue product, with maximum absorption at 720–730 nm.

Samiha, A et al\textsuperscript{53}, have developed two sensitive spectrophotometric methods for the determination of imipramine hydrochloride, clomipramine hydrochloride, desipramine hydrochloride, and trimipramine maleate in bulk and in dosage forms. The first method is based on the interaction of diazotized $p$-nitroaniline (DPNA) with the dibenzazepine drug in $5M$ hydrochloric acid. The second is based on the oxidative coupling of the dibenzazepine drug with 3-methylbenzothiazolin-2-one hydrazone (MBTH) in the presence of ammonium iron(III) sulphate in $0.1M$ hydrochloric acid. The resulting chromophores are measured at 575 nm (for the DPNA method) or at 620–630 nm (for the MBTH method), and are stable for at least 24 hr. The commonly encountered excipients and additives do not interfere with the
determinations. Results from the analysis of pure drugs, commercial tablets and laboratory-prepared tablets by these methods agree well with those of official methods.

Nagaraja, P\textsuperscript{54}, have proposed a simple, sensitive and selective spectrophotometric procedure was developed for the determination of imipramine hydrochloride, desipramine hydrochloride, clomipramine hydrochloride and trimipramine maleate belonging to dibenzazepine class of drugs. The method is based on the interaction of diazotized p-phenylenediamine dihydrochloride with the drug in sulphuric acid medium. The resulting chromophore was measured at 565 nm, and was stable for about 2.5 hr. The commonly encountered excipients and additives do not interfere with the determination. Dibenzazepine drugs can be determined in the range of 0.1-4.0 µg/ml, with a relative standard deviation of 1.92% for ten replicate measurement of 2.0 µg/ml dibenzazepine drugs. Results from the analysis of preformulations and commercial tablets by this procedure agree well with those of the official method.

Ayesha Syeda et al\textsuperscript{55}, proposed a new reagent 2,2'-bipyridine for the determination of certain dibenzazepine class of tricyclic antidepressants by spectrophotometric method. The spectrophotometric method is based on the reaction of imipramine hydrochloride (IPH), desipramine hydrochloride (DPH), clomipramine hydrochloride (CPH), trimipramine maleate (TPM) and opipramol (OPP) with iron (III) and subsequent reaction with 2,2'-bipyridine in an acetic acid medium to yield a pink color with maximum absorption at 530 nm. The color developed was stable over 3–4 h at room temperature (27 °C). The commonly encountered excipients and additives did not interfere with the determination. Results from the analysis of pure drugs and commercial tablets agreed well with those of the official method (United States Pharmacopoeia, 24, USP Convention, Rockville 2000, pp. 505–506, 865-867.).
José, L.F.C et al\textsuperscript{56}, have developed an automated multicommutated flow system for the spectrophotometric determination of clomipramine in pharmaceutical preparations. The method was based on the oxidation of clomipramine by ammonium monovanadate in acidic medium yielding a coloured product with an absorbance maximum at 620 nm. The reaction development was enhanced when the binary sampling approach was exploited to insert the sample into the carrier stream. This approach, consisting of the intercalation of small sample and reagent aliquots, led to a more efficient sample zone homogenisation. The determinations were carried out at two distinct sampling times (6 and 12 s) in order to evaluate the system’s performance under different dispersion conditions, which also showed the versatility of the multicommutation time-based sample insertion. Beer’s law was verified for clomipramine concentrations of up to 50 mg l\textsuperscript{-1}. R.S.D. \((n=10)<2.0 \) and 1.3\% were attained for sampling times of 6 and 12 s, respectively. The results were in agreement with those obtained by the reference procedure, with R.D.<2.6\%.

(e) Levobunolol (LV):

Various methods are reported in the literature for the estimation of levobunolol which includes, spectrophotometric method\textsuperscript{60,61}.

Salem H\textsuperscript{60}, have developed simple, rapid and sensitive spectrophotometric procedures for the analysis of atenolol, timolol maleate, propranolol hydrochloride, metoprolol tartrate, betaxolol hydrochloride, levobunolol hydrochloride and bisprolol fumarate in pure form as well as in their pharmaceutical formulations. The methods are based on the reaction of these drugs as n-electron donors with the sigma-acceptor iodine, and the pi-acceptors: 7,7,8,8-tetracyanoquinodimethane, tetracyanoethylene, 2,3,5,6-tetrabromo-1,4-benzoquinone (bromanil) and 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil). The obtained charge-transfer complexes were measured at 365 nm for iodine (in 1,2-dichloroethane), at 840, 420,
and 470 nm for 7,7,8,8-tetracyan-oquinodimethane, tetracyanoethylene and 2,3-di-chloro-5,6-dicyano-1,4-benzoquinone (in acetonitrile), respectively, and at 450 and 440 nm for bromanil and chloranil (in ethanol), respectively. Due to the rapid development of colors at ambient temperature, the obtained results were used on thin-layer chromatograms for the detection of the investigated drugs. Beer's plots were obeyed in a general concentration range of 4-120 microg ml(-1) with correlation coefficients not less than 0.9991. The proposed procedures could be applied successfully to the determination of the investigated drugs in tablets and ophthalmic solutions with good recovery; percent ranged from 98.03+/-0.98 to 100.30+/-0.90. The association constants and standard free energy changes using Benesi-Hildebrand plots were studied.

Nesrin, K et al61, have developed five poly(vinyl chloride) (PVC) matrix membrane electrodes responsive to the β-blockers atenolol (AT), bisoprolol fumarate (BI), timolol maleate (TI), and levobunolol HCl (LV) were characterized. A precipitation-based technique with ammonium reineckate anion as an electroactive material in PVC matrix with AT, BI, TI, and LV cations was used for fabrication of Electrodes 1-4, respectively. Electrode 5 fabrication was based on precipitation of LV cation with tungstophosphate anion as an electroactive material. Fast and stable Nernstian responses at 1 x 10^{-2}-1 x 10^{-7} M for different β-blockers over the pH range of 2-8 were found for these electrodes, which were evaluated according to International Union of Pure and Applied Chemistry recommendations. The method was successively applied for the determination of β-blockers in their pharmaceutical formulations. Validation of the method according to quality assurance standards showed the suitability of the proposed electrodes for use in the quality control assessment of these drugs. The recoveries for the determination of the β-blocker drugs by the 5 proposed selective electrodes were 100.1 ± 0.7, 99.9 ± 0.8, 100.0 ± 1.1, 100.5 ± 1.1, and 100.6 ± 0.7% for Sensors 1-5, respectively. Statistical comparison between the results
obtained by this method and the official method of the drugs was performed and no significant difference was found.

(f) Metoprolol:

Various methods are reported in the literature for the estimation of Metoprolol which includes, spectrophotometric method

A selective adrenergic beta-1-blocking agent with no stimulatory action. It's binding to plasma albumin is weaker than alprenolol and it may be useful in angina pectoris, hypertension, or cardiac arrhythmias.

Du oudes auteurs have developed a sensitive spectrophotometric method for the determination of metoprolol in tablets and ampoules is presented. Using spectrophotometric measurements, it was found that metoprolol and benzyl orange form a chloroform soluble ion-pair complex with an absorption maximum at 401 nm. The composition of the ion-pair complex was determined by applying Job's method to equimolar solutions of metoprolol: benzyl orange (1:2); molar absorptivity 7.39x103 mol-1 cm-1. Extraction of the ion-pair complex in chloroform was accomplished easily at a Britton-Robinson's buffered optimum pH=5.2, μ=0.1mol/dm3. The relative stability constant, calculated according to the method of Sommer and Job's non-equimolar solutions, was log K=9.72 (avg.value). Beer's law was obeyed up to 3.42μg/ml of metoprolol (the detection limit was also 3.42 μg/ml). The precision of the method was checked at three different concentrations. The RSD (n=7) varied from 0.51 to 2.03%. Reproducibility was examined by analysing Lopresor tablets and ampoules. Recoveries varied from 99-101%. the reported method, applied to the assay of metoprolol in tablets and ampoules, gives precise and reproducible results.
Sensitive and specific atomic adsorption spectroscopy (AAS) and spectrophotometric methods have been developed for the determination of beta adrenergic blocking drug, metoprolol. The method is based on the formation of Cu(II) dithiocarbamate complex by derivatization of the secondary amino group of metoprolol with CS2 and CuCl2 in the presence of ammonia. The copper-bis(dithiocarbamate) complex was extracted into chloroform and the concentration of metoprolol was determined directly by spectrophotometric and indirectly by AAS measurement of copper. The two methods developed were applied to the assay of metoprolol in commercial tablet formulations. The methods were compared statistically with each other and with the high performance liquid chromatography (HPLC) method of USPXXII using t- and F-tests.

Nafisur Rahman, Habibur Rahma and Syed Najmul Hejaz Azmi have developed a kinetic spectrophotometric method for the determination of metoprolol in pharmaceutical formulations. The method is based on reaction of the drug with alkaline potassium permanganate at 25±1 °C. The reaction is followed spectrophotometrically by measuring the change in absorbance at 610 nm as a function of time. The initial rate and fixed time (at 15.0 min) methods are utilized for constructing the calibration graphs to determine the concentration of the drug. Both the calibration graphs are linear in the concentration range of 1.46x10⁻⁶—8.76x10⁻⁶ M (10.0—60.0 µg per 10 ml). The calibration data resulted in the linear regression equations of log (rate)=3.634+0.999 log C and A=6.300x10⁻⁴+6.491x10⁻² C for initial-rate and fixed time methods, respectively. The limits of quantitation for initial rate and fixed time methods are 0.04 and 0.10 µg ml⁻¹, respectively. The activation parameters such as Ea, ΔH‡, ΔS‡ and ΔG‡ are also evaluated for the reaction and found to be 90.73 kJ mol⁻¹, 88.20 kJ mol⁻¹, 84.54 J K⁻¹ mol⁻¹ and 63.01 kJ mol⁻¹, respectively. The results are validated statistically and through recovery studies. The method has been successfully applied to the determination of metoprolol in pharmaceutical formulations.
Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.
Section (ii): Objectives of the present investigations:

Quality is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer goods, there can be and there is no "second" quality in drugs. Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. As a matter of fact, it is built in from the time of inception of the thought to make a product, to the time, it is finally made and send out with an OK quality report. In popular practice, the quality of medicines or pharmaceutical products is assured through quality control. It is, therefore, essential that quality assurance department must adopt "Good Laboratory Practice" to ensure reliability of pharmaceuticals together with their careful control are our moral obligations arising from the humanism towards the sick human beings. Consequently, the manufactures and the control of drugs are very responsible and they need substantial knowledge of the science. The decision to release or reject a product is based upon one or two types of control action or combination of both. If the product is a single entity of high purity, the analytical data is the basis for decision but most of the time, the formulation is a physical mixture of several potent drugs. With the growth of pharmaceutical analysis involving complex instrumentations, providing simple analytical procedures for complex formulations is a matter of foremost importance.

Drugs and pharmaceuticals play a very significant role in the present days for the prevention, control and curing of different kinds of human diseases. It is a common observation and the practical truth that a single drug of a particular composition is marketed in various brand names by different manufactures. The possibility of minor changes in the chemical composition and standard of the drug will have a profound effect on the physiological and biological activities of the patient. It is very much painful for the present days scientist in general and to the analytical pharmaceutical chemist in particular to note in
the various dailies about the entry of the spurious and substandard drugs into market, which definitely will have an adverse effect on the human beings at large.

It is with this challenge in mind, the author has taken up her thorough investigations to evaluate the purity of the various drugs released into the market. The author has made an extensive survey of the chemical and biochemical literature to know whether the reports involving simple experimental techniques such as the spectrophotometric techniques are available for ascertaining the assay and purity of the drugs. It is the observation of the author that not much attention is paid to simple and rapid spectrophotometric methods for the assay of drugs available in literature.

Various instrumental techniques (HPLC, GC, Fluorimetry, NMR, IR, UV and Visible regions) are available in the literature for the assay of drugs. These methods are either expensive or do not give reproducible. Usually spectrophotometric technique is simple and less expensive. The selectivity and sensitivity of the spectrophotometric methods depend only on the nature of chemical reactions involved in colour development and not on the sophistications of the experiment.

UV and Visible spectrophotometric methods are highly versatile, sensitive and reproducible. This made the author to develop new spectrophotometric methods for the estimation of selected drugs having varying used in pharmaceutical preparations.

Section (iii): Preparation of Reagents and Solutions

AR Grade Chemicals are used for preparation of Reagents and solutions in the present investigation

Hydrochloric acid (0.1 N):

Hydrochloric acid solution (0.1N) is prepared by diluting the requisite volume of concentrated AR hydrochloric acid (Ranbaxy make) with distilled water and standardized by usual procedure.
**NaOH solution (0.1N):**

It is prepared by dissolving 4 gms of sodium hydroxide (Merck) to 1000 ml with distilled water.

**Ceric ammonium sulphate (0.05M)**

3.1628 g of AR ceric Ammonium Sulphate is dissolved in double distilled water and the resulting solution is made up to the mark in the 100 ml standard flask with double distilled water.

**Ammonium ferrous sulphate Solution (0.02M)**

0.7842 g of AR Ammonium Ferrous Sulphate is dissolved in distilled water and the solution is made up to the mark in the 100 ml standard flask with distilled water.

**Ammonium Thio-Cyanate (1M)**

7 g of AR Ammonium thio-cyanate is dissolved in double distilled water and the resulting solution is made up to the mark in the 100 ml standard flask with double distilled water.

**Hydrochloric Acid Solution (5N)**

Hydrochloric acid solution (5N) is prepared by diluting the requisite volume of concentrated AR hydrochloric acid (Ranbaxy make) with distilled water.

**Buffer solution (pH 1.5):**

Buffer solution is prepared by mixing 289 ml of glycine solution (37.52 gm of glycine and 29.24 gm of sodium chloride are dissolved in 500ml of distilled water) with 711ml of 0.1 M HCl.

**Buffer solution pH 3.5 (potassium acid phthalate – HCl):**

It is obtained by diluting a mixture of 50 ml of 0.2M potassium acid phthalate and 8.4 ml of 0.2M HCl to 200 ml with distilled water and the pH is adjusted to 3.5.
Wool fast blue solution (WFB): (0.2% W/V):

Wool fast blue solution is prepared by dissolving 200 mg of wool fast blue (Flaka) in 100 ml of distilled water.

Bromocresolgreen solution (BCG): (0.5% w/v):

It is prepared by dissolving 500 mg of bromocresol green (Loba) in 100 ml of distilled water.

DDQ (0.1% w/v)

DDQ (2,3-dichloro 5,6-dicyano-p-benzoquinone) (Loba Chem., India) solution is prepared by dissolving 100 mg in 100 ml of methanol.

Levobunolol solution:

An accurately weighed 50 mg of levobunolol is dissolved in methanol and the volume is adjusted to 50 ml with methanol. Further dilution is made to obtain the working concentration.

Chloroquine solution:

An accurately weighed 50 mg of chloroquine is dissolved in methanol. The volume is adjusted to 50 ml with methanol in 50 ml standard flask. One ml of this solution contains 1 mg/ml. The stock solution is further diluted to get desired concentration.

Amiloride solution:

An accurately weighed 50 mg of amiloride is dissolved in methanol. The volume is adjusted to 50 ml with methanol in 50 ml standard flask. One ml of this solution contains 1 mg/ml. The stock solution is further diluted to get desired concentration.

Aciclovir solution:

50 mg of pure aciclovir is dissolved in methanol and the volume is adjusted to 50 ml with methanol. The stock solution is further diluted to get working concentration of 100 µg/ml.
Clomipramine solution:

Pure clomipramine (50 mg) is dissolved in 50 ml methanol to obtain a stock solution of 1 mg/ml. The final concentration of clomipramine is brought to 100 µg/ml with methanol.

Metoprolol solution:

Pure Metoprolol (50 mg) is dissolved in 50 ml methanol to obtain a stock solution of 1 mg/ml. The final concentration of Metoprolol is brought to 100 µg/ml with methanol.