CHAPTER II
REVIEW OF LITERATURE
The scope of the review is to know and evaluate the existing methods in the literature based on the guidelines of International conference on Harmonization for the glucosamine and diacerein, donepezil hydrochloride, amiodarone hydrochloride and sildenafil citrate tablets, ear drops (composed of beclomethasone dipropionate-0.025%w/v, chloramphenicol-5.0%w/v, clotrimazole-1.0%w/v and lidocaine-1.73%w/v) and antiseptic solution (composed of chloroxylenol-4.8%w/v and terpineol-9.0%v/v). The evaluation criterion is based on the guidelines given by ICH for Analytical methods. If any method meets the criteria it will be taken as a benchmark and can be used in the analysis of the product. If it does not meet the criteria, the new method needs to be improved to fulfill the requirements of specificity, linearity, solution stability, accuracy, precision, ruggedness, robustness, limit of detection and limit of quantitation. If any method is meeting the requirements of ICH for an individual product, the same method may be taken for the combination of molecules determination.

II.a. Glucosamine sulphate Potassium chloride and diacerein Tablets

Glucosamine (C₆H₁₃NO₅) is a carbohydrate derivative of amino compound. Glucosamine is part of the structure of the polysaccharides, which compose the exoskeletons of crustaceans and other arthropods. Glucosamine is one of the most abundant mono-saccharides and produced commercially by the
hydrolysis of crustacean exoskeletons or less commonly by fermentation of a grain such as corn or wheat.

Diacerein (C_{19}H_{12}O_{8}), also known as diacetyl rhein and used in the treatment of osteoarthritis. It works by inhibiting interleukin-1. A Cochrane (cochrane collaboration is an international, independent, not-for-profit organization of over 28,000 contributors from more than 100 countries, dedicated to making up-to-date, accurate information about the effects of health care readily available worldwide) review found diacerein to be slightly more effective than placebo in diacerein has a small effect in improving pain and slowing the progress of osteoarthritis in the hip (Fidelix TS 2006).

As glucosamine has no UV absorbance and the diacerein has UV absorbance and glucosamine is generally estimated with refractive index detector (RID) method and pre-derivatization methods, diacerein is generally estimated using titrimetry and liquid chromatographic methods.

Iain Alasdair Donaldson (1985) has developed a micro assay method for the determination of glucosamine, galactosamine and mannosamine in mixtures containing amino acids. The procedure involves the reduction of these hexosamines in the presence of ^{14}\text{C}-labelled standards, followed by their reaction with ^{3}\text{H}-dansyl chloride. By a combination of thin layer electrophoresis and thin layer chromatography on a single plate, the dansylated hexosamine derivatives are both separated from those of the amino acids and resolved from one another. Trimethylamine buffer (100mM, pH=10), adjusted with formic acid, 80% (v/v) pH=4.5, buffer was stored at chromatography.
paper sleeved in broad visking tubing and were stored at 4°C in the pyridine/acetone buffer. The chromatography solvent was composed of cyclohexane:ethyl acetate: ethanol 24:4:3 (%v/v/v), was made up from “analar” reagents and stored as a stock solution. Phenyl boric acid (PBA) (5mg per mL), was added to the solvent immediately prior to use. Plastic backed polyamide thin layer chromatographic (TLC) plates, 20x20cm. The method will assay as little as 400pmoles of any one of these three hexosamines. The developed assay method was checked linearity, accuracy and ruggedness so the developed method as concluded as stability indicating.

Wayne Way et al., (2000) have developed a simple RP-HPLC method for the determination of glucosamine in nutritional supplements and validated the method based on United States Pharmacopoeia (USP) guidelines. Separations were performed using Inertsil ODS-3 column with an isocratic mobile phase consisting of 10% methanol, 90% 0.005M Na-octane sulfonate (pH=2.1). Glucosamine was detected using a refractive index (RI) detector. The octane sulfonate ion pairing agent allowed for retention on a C18 column. The method proved highly reliable with respect to standard performance characteristics. The method allows for convenient quality control for the regular analysis.

Ali Aghazadeh Habashi et al., (2002) have proposed a HPLC method for the determination of glucosamine (GlcN) in rat plasma. Galactosamine used as internal standard and added to 100μL of plasma containing GlcN followed by precipitation of plasma proteins with acetonitrile. Evaporation of the
decanted supernatant solution was accelerated by the addition of methanol. GlcN was derivatized by addition of a solution containing 1-naphthyl isothiocyanate. Sample cleanup included passage through an anion exchange cartridge. Analysis was accomplished by injection of 0.1mL of the sample solution into an isocratic HPLC system consisting of a C18 column, a mobile phase composed of acetonitrile: water: acetic acid: triethylamine in the ratio of 4.5: 95.5:0.1:0.05(%v/v), a flow rate of 0.9mL per min and UV detector set at 254nm. Galactosamine and GlcN appeared 26 and 29min post injection, respectively. The assay was linear over the range of 1.25-400μg per mL (CV<10%) with a detection limit of 0.63μg per mL and a limit of quantification of 1.25μg per mL. The method was applied to the determination of GlcN in rat plasma after oral administration of 350mg per kg of GlcN hydrochloride. The present assay method is specific, sensitive, precise and accurate and is suitable for pharmaco kinetic studies.

Yasser El Saharty and Ahmed Abdel Bary (2002) have proposed a simple, rapid, selective and specific HPLC method for quantitative determination of glucosamine and its (β-1-4)-D-polymeric form chitosan. The chromatographic separation was achieved using an amino phase column with refractive index (RI) detection. The mobile phase consisted of ACN: H₂O:CH₃COOH in the ratio of 50:50:0.02 (% of v/v) and pH was adjusted to 4.0. The standard curves for glucosamine sulphate showed linearity (r≥0.99) over the concentration range from 20 to 1000μg per mL for raw materials and dosage forms. The precision of the raw material assay expressed as the percent
relative standard deviation (%RSD), was less than 3% at all concentrations. Chitosan, poly-(β-1-4)-D-glucosamine compounds was hydrolyzed with 6M HCl at 100°C for 10 hours and the released glucosamine was determined by the same HPLC method. The proposed method showed linear relation in concentration ranges of 100–500 μg per mL. The suggested procedure was applied for the determination of glucosamine sulphate and chitosan in their dosage forms and the validity of the method was further checked by applying the standard addition technique. The method was found to be specific with good linearity, accuracy, precision and is well suited for quantitation of glucosamine sulphate and chitosan in raw materials and pharmaceutical formulations.

Xianhao Cheng and Louis Kaplan (2003) have developed a method for the determination of neutral amino sugars and a sugar alcohol in fresh waters using HPLC and pulsed amperometric detection with a single isocratic analysis. Arabinose, galactosamine and mannosamine are separated with a mobile phase of 22.8 mM NaOH /KOH at a temperature of 17°C. The HPLC column (Carbo Pac PA1 is an anion exchange column) was used for analysis and the resolutions are 0.73 and 0.64, respectively. The method separates closely eluting glucose, mannose and mannose, xylose peaks with resolutions of 0.85 and 0.71. Other sugars, fucose, rhamnose, galactose, fructose, ribose, glucosamine and mannitol are resolved completely.

Yu Shao et al., (2004) have developed a stability indicating HPLC method for the assay of glucosamine in active ingredients and solid dosage
formulations. The HPLC separation was achieved on a phenomenex Luna amino column (150mm×4.6mm, 5μ) using a mobile phase of acetonitrile: phosphate buffer the ratio of 75:25 (%v/v) with pH=7.50 at a flow rate of 1.5mL per min and UV detection at 195nm. The method was validated for specificity, linearity, solution stability, accuracy, precision, limit of detection and limit of quantitation. The detector response for glucosamine hydrochloride was linear over the selected concentration range from 1.88 to 5.62mg per mL with a correlation coefficient 0.9998. The accuracy was checked and found satisfactory results. The sample and standard solutions were stable for one week. The method was successfully used for analysis of active excipient compatibility samples used for development of a solid dosage formulation in our laboratory and subsequent stability studies. The method was also used for the analysis of glucosamine in several commercially available solid dosage forms.

Joseph ZiQi Zhou et al., (2005) have developed a single laboratory validation method for determination of glucosamine in raw materials and dietary supplements containing glucosamine sulfate and/or glucosamine hydrochloride by with HPLC FMOC Su derivatization. Glucosamine is an amino sugar (UV in active) so derivatization is necessary for HPLC-UV detection. In this method glucosamine salt (H₂SO₄/HCl) dissolved in aqueous solution and added triethylamine (TEA) to neutralize the H₂SO₄/HCl salts. Then glucosamine is free base derivatized with N-(9-fluorenethylmethoxycarbonyloyxy succinimide (FMOC-Su) at 50°C for 30min
and analyzed by HPLC with UV detection because glucosamine has 2 natural stereo isomers (α and β) and the inter conversion of these two, in aqueous solution is not preventable, two peaks are shown in the chromatogram. The sum of the areas of these two peaks is used for the quantification of the glucosamine free base. The method was applied to many forms like tablets, capsules and combination products with chondroitin, SAMe (S-adenosyl-L-methionine) and MSM (methyl sulfonyl methane), liquid drink or drink mix, chews and raw materials. Tests with two blank matrixes containing SAMe, vitamin C, citric acid, chondroitin sulfates, methyl sulfonyl methane, lemon juice concentrate and other potential interferents showed the method to be selective and specific. Eight calibration curves prepared over seven working days indicated excellent reproducibility with the linear range at least over 2.0–150 μg per mL and determination coefficients >0.9999. Average spike recovery from the blank matrix (n=8 over two days) was 93.5, 99.4 and 100.4% at respective spike levels of 15, 100 and 150% and from the sample matrix containing glucosamine (n = 3) was 99.9 and 102.8% at respective levels of 10 and 40%, with relative standard deviations <0.9%. The stability tests confirmed that glucosamine FMOC Su derivative once formed is stable at room temperature for at least five days. Limit of quantitation was 1.0 μg per mL and limit of detection was 0.3 μg per mL. The developed method is stability indicating and ready to use regular analysis.

Yunqui Wu et al., (2005) have proposed a simple spectrophotometric method for the determination of glucosamine release from sustained release
hydrophilic matrix tablet based on reaction with ninhydrin, optimized and validated. The purple color (ruhemann purple) resulted from the reaction was stabilized and measured at 570nm. The method optimization was essential as many procedural parameters influenced the accuracy of determination including the ninhydrin concentration, reaction time, pH, reaction temperature and purple color stability. Glucosamine tablets (600mg) with different hydrophilic polymers were formulated and manufactured on a rotary press. Dissolution studies were conducted using de ionized water at 37±0.2°C with paddle rotation of 50RPM and samples were removed manually at appropriate time intervals. Under given optimized reaction conditions that appeared to be critical, glucosamine was quantitatively analyzed and the calibration curve in the range of 0.202–2.020mg (r=0.9999) was constructed. The recovery rate of the developed method was 97.8–101.7% (n=6). Reproducible dissolution profiles were achieved from the dissolution studies performed on different glucosamine tablets. The developed method is easy to use, accurate and highly cost effective for routine studies relative to HPLC and other techniques.

Ali Aghazadeh Habashi et al., (2005) have developed a simple RP-HPLC method for determination in plasma of N-butyryl glucosamine (GLBU). Transferred 100μL of plasma containing GLBU was added fucose as internal standard. GLBU and fucose were derivatized using 1-phenyl-3-methyl-5-pyrazolone in the presence of Sodium hydroxide at 70°C for 30min. The solution was neutralized with hydrochloric acid and the excess derivatizing reagent was extracted with chloroform. The aqueous layer was injected into an
isocratic HPLC system consisting of an auto injector, a single pump and a UV detector set at 245nm. Two different 250mm reversed phase columns (10µm, C18) were used. The mobile phase was a mixture of phosphate buffer (pH7.0) and acetonitrile in the ratio of 80:20%v/v, pump at a flow rate of 1.0mL per min at ambient temperature. Derivatized fucose and GLBU appeared 24 and 28min and at 34 and 37min using 4 and 10µm columns, respectively. The assay was linear over the range of 0.2-200µg per mL with a limit of quantification of 0.2 and 1.0µg per mL for the 4 and 10µ columns, respectively. The method was applied to the determination of GLBU in rat plasma after oral administration of 233mg per kg of GLBU. The developed method was precise and accurate with sufficient sensitivity for pharmaceutical dosage forms.

Zhu et al., (2005) have developed a simple, rapid, selective and specific HPLC method for the quantitative determination of glucosamine and its application for estimating purity of chitin. The chromatographic separation was achieved using a reversed phase C8 column, pre column derivatization with 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl) and UV detection at 254nm. The mobile phase consisted of CH3CN and H2O. The optimum conditions of acid hydrolysis of chitin (concentration HCl, temperature and heating time) was obtained by performing the orthogonal array design (OAD) procedure and the released glucosamine was determined by the above HPLC method. The accuracy of the method was checked by the standard addition technique. The method was found to be specific with good linearity, accuracy, precision, well
suited for quantitation of glucosamine and determination of the purity of chitin in biological materials and food products.

Sullivan and Sherma (2005) have developed a quantitative method using silica gel HPTLC plates, automated band wise sample application, detection with ninhydrin chromomeric reagent solution and automated visible mode densitometry has been developed for determination of glucosamine in nutritional supplements containing a variety of other active and inactive ingredients. Analysis was performed on 200mm×100mm high performance silica gel 60F_{254} GLP plates. The plates were developed to the top with dichloromethane: methanol (1:1%v/v) and dried in a fume hood before use. Standard and sample solutions were applied to the plate by means of a camag linomat IV automated spray on band applicator equipped with a 100μL syringe and operated with the band length 6mm, application rate 4second per μL, table speed 10mm per second, distance between bands 4mm, distance from the plate edge 7mm and distance from the bottom of the plate 1.5cm. The volumes applied for each analysis were 2.00μL, duplicate 4.00μL and 8.00μL of the TLC standard (1.00–4.00μg). For each of the nine products, a different sample volume was applied in duplicate so that the sample zone scan areas matched the scan areas of the middle (2.00μg) glucosamine hydrochloride standards. Accuracy was validated by analysis of spiked blank and standard addition samples and precision by performing replicate analysis on a single day and different days. Recoveries of glucosamine hydrochloride from the spiked blank and standard addition samples were 100.0% and 101.5%, respectively. The
intermediate precision was 1.20% RSD for a sample analyzed in duplicate once per plate on five different days over a seven days period. A survey was made of free glucosamine content compared to the label values for nine commercial supplement products using the new method, which is shown to be suitable for routine use in nutritional supplement analysis for manufacturing quality control or governmental regulatory purposes.

Priya Gaonkar et al., (2006) have developed a rapid and sensitive method for the determination of glucosamine sulphate in tablets by UV spectrophotometry. In this pre derivatization method, glucosamine sulphate was reacted with phenyl iso-thiocyanate in presence of a base to yield phenylthiourea derivative. This derivative showed maximum absorbance at 240nm. Beer's law is obeyed in the concentration range of 5-25mg per mL. The method was validated in terms of linearity, precision (relative standard deviation 1.1%), accuracy and specificity. The proposed method is the only method available for spectrophotometric determination of the drug. It is simple, precise, accurate, sensitive and reproducible and can be used for the routine quality control testing of the marketed formulations.

Virginie Esters et al., (2006) have demonstrated a quantitative densitometric HPTLC method for the determination of glucosamine in a dietary supplement containing dried extracts of the main plants traditionally used for rheumatic disorders. The HPTLC method was chosen in order to circumvent the tedious and time consuming sample preparation steps necessarily performed before using HPLC methods when analyzing complex matrixes.
Glucosamine was separated from the plant extracts on a silica gel 60F (254) HPTLC plate using a saturated mixture of 2-propanol ethyl acetate ammonia solution (8%) (10:10:10, v/v/v). The plates were developed vertically up to a distance of 80mm. For visualization, the plate was dipped into a modified anisaldehyde reagent and heated at 120°C for 30min in a drying oven. Glucosamine appeared as brownish red chromatographic zones on a colorless background. Densitometric quantification was performed at lambda=415nm by reflectance scanning. The HPTLC method was successfully validated and the method showed good performance thereby fulfilling its objective of quantifying accurately. The relative standard deviations for repeatability and intermediate precision were between 4.9 and 8.6%. Moreover, the method was found to be accurate, did not exceed the acceptance limits of 85 and 115% on the whole analytical range (800-1,200ng of glucosamine).

Lopez Cervantes et al., (2007) have developed a HPLC method for the quantitation of glucosamine in chitin. The method includes an acid hydrolysis of chitin. The chromatographic separation is achieved using a hypersil ODS 5μm column (250x4.6mm) at 38°C, with pre column derivatization with 9-fluorenethylmethyl chloroformate, UV detection at 264nm. The mobile phase is a mixture of mobile phase-A [30mM ammonium phosphate (pH 6.5) in 15:85 methanol: water (%v/v)], mobile phase-B [methanol: water 15:85 (%v/v)] and mobile phase-C [90:10 acetonitrile: water (%v/v)] with a flow rate of 1.2mL per min. The HPLC method proposed showed adequate repeatability (percent RSD 5.8), accuracy (92.7% recovery) and sensitivity, with a detection limit of 65.
2μg per mL. The method is successfully applied to the quantitation of glucosamine for the determination of the purity of chitin from shrimp waste.

Michaela et al., (2008) have developed an HPTLC method for the quantification of glucosamine in nutritional supplements. TLC plates were amino plates, with simple heating of the plates for derivatization. On heating the plate glucosamine reacts to form a compound which strongly absorbs light between 305 and 330nm with weak fluorescence. The reaction product can be detected sensitively either by absorption of light or by fluorescence detection. The detection limit in absorption mode is approximately 25ng per spot. In fluorescence mode a detection limit of 15ng is achievable. A calibration plot for absorption detection is linear in the range 25 to 4000ng glucosamine. The derivative formed from glucosamine by heating is stable for months and the relative standard deviation is 1.64% for 600ng glucosamine. The amounts of glucosamine found in nutritional supplements were in agreement with the label declarations.

Massoud Amanlou, (2008) has developed a reproducible method for the determination of glucosamine and chondroitin contents of several such products in the market place and to determine if they significantly deviate from their label claim. A total of fourteen products containing glucosamine sulfate and nine products containing chondroitin sulfate were evaluated. The waters HPLC system with RP C18, 250mm x 4.6mm, 5μm and the isocratic mobile phase which was consisted of MeOH:H2O:CH3COOH (10:89.6:0.04 %v/v) was prepared daily, degassed by passing through a 0.45μm filter. The injection
volume was 25μL being pumped at a flow rate of 1.2mL per min and the wavelength for UV detection was 254nm. The amounts of glucosamine and chondroitin were found to be significantly different from the label claim in one product, ranging from as low as 59.00 % to over 112.14 % of the label claim for glucosamine and 77.69% to over 94.86% for chondroitin. Retail price of the product did not appear to be related to the quantity of active ingredients. The overall results of this study show that famous brands are better candidates for counterfeiting than expensive ones.

Xianhuo Wang et al., (2008) have proposed a sensitive and reliable HPLC method with fluorescence detection based on the pre column derivatization of glucosamine with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) was established for the quantitative determination of glucosamine in rat plasma. The plasma protein was precipitated by acetonitrile, followed by vortex mixing and centrifugation. The supernatant was divided into the organic layer and aqueous layer by adding Sodium chloride and then the aqueous layer was derivatized with AQC in 0.2M borate buffer of pH 8.8 before the HPLC analysis. An amino acid analysis column (3.9×150mm, 4μm) was applied, with 140mM Sodium acetate buffer (pH=5.25) and acetonitrile as mobile phase at a flow rate of 1.0mL per min. A linear correlation coefficient of 0.9987 was calculated within the range of 0.1-30μg per mL of the standard curve for glucosamine. The limit of detection was 30ng per mL. The intra-day and inter-day precisions (as (percent) %RSD) were less than 7.38 and 12.72%, respectively. The intra-day and inter-day accuracy ranged from 91.8 to 110.0%.
Extraction recoveries of glucosamine in plasma were more than 90%. The validated method was successfully applied for the quantitative determination of glucosamine in rat plasma and evaluation for pharmacokinetic study of glucosamine. It was also possible to be applied for the quantitative determination of other compounds containing amino group in biological samples.

Hou et al., (2008) have developed a reproducible HPLC method for the determination of N-acetyl, D-glucosamine (GlcNAc) was established to detect the raw material and capsules of GlcNAc. Alltima ODS column (5μm, 250mmx4.6mm) was adopted with 0.1M per L Potassium di-hydrogen phosphate solution. 10mM per L tetra-butyl ammonium hydrogen sulfate, adjusted pH to 6.50 with phosphoric acid as the mobile phase at the flow rate of 0.8mL per min under the column temperature at room temperature. The detection wavelength was at 220nm. A satisfactory separation obtained among, GluNAc, related impurities and excipient was obtained. The calibration curve was linear in the range of 0.4-8μg for GlcNAc. The average recoveries of low, middle and high concentrations from capsules were 99.9%, 100.1% and 100.2%, with RSD 0.46%, 0.03% and 0.28%, respectively. The method was simple, rapid, accurate and suitable for the determination of GlcNAc.

Silvia HM Borgmann et al., (2008) have developed and validated a dissolution test for diacerhein in capsules using spectrophotometric method. The dissolution established conditions were: 900mL of Sodium phosphate buffer pH7.0 with 0.75% of Sodium lauryl sulphate as dissolution medium,
using a basket apparatus at a stirring rate of 50RPM. The drug release was evaluated by UV spectrophotometric method at 258nm. The method was validated to meet requirements for a global regulatory filing. The validation included specificity, linearity, precision and accuracy. In addition, filter suitability and drug stability in medium were demonstrated.

Janhavi Rao et al., (2009) have developed a stability indicating HPLC method for the validation and quantitative determination of diacerein in capsule dosage forms. An isocratic separation was achieved using a ODS-3, 250×4.6mm, 5μm particle size columns with a flow rate of 1.0mL per min and using a UV detector to monitor the elute at 254nm. The mobile phase consisted of phosphate buffer: acetonitrile (40:60, %v/v) with pH4.0 adjusted with phosphoric acid. The drug was subjected to oxidation, hydrolysis, photolysis and thermal degradation. Diacerein was found to degrade in acidic, basic and oxidative stress and also under neutral condition. Complete separation of degraded products was achieved from the parent compound. All degradation products in an overall analytical run time of approximately 10min with the parent compound diacerein eluting at approximately 4.9min. The method was linear over the concentration range of 1-10μg per mL (r²=0.9996) with a limit of detection and quantitation of 0.01 and 0.05μg per mL respectively. The method has the requisite accuracy, selectivity, sensitivity, precision and robustness to assay diacerein in capsules. Degradation products resulting from the stress studies did not interfere with the detection of diacerein and the assay is thus stability indicating.
Ashwini Ojha et al., (2009) have developed a simple HPLC method with UV detection for simultaneous determination of rhein (the immediate metabolite of diacerein) and aceclofenac from human plasma samples. Sample preparation was accomplished through liquid-liquid extraction with ethyl acetate and chromatographic separation was performed on a reversed phase octa decylsilane (ODS) column. Mobile phase consisted of a mixture of acetate buffer and acetonitrile run under gradient at flow rate of 1.0mL per min. Wavelength was set at 258nm. The method was validated for linearity, accuracy, precision and stability. The calibration was linear over the range of 0.1–7.0μg per mL for rhein and 0.5–20μg per mL for aceclofenac using 500μL plasma samples. Extraction recoveries were 85% for rhein and 70% for aceclofenac. The method can easily be adopted for high throughput clinical and pharmacokinetic studies of above two drug fixed dose combination formulations.

Keddal Govindaram Lalitha (2010) has proposed a simple, economic and accurate isocratic RP-HPLC method for the quantitation of diacerein in tablet dosage form. The quantitation was carried out using Zorbax-CN (cyano) column. The mobile phase was ammonium acetate buffer (pH adjusted to 3.5): Acetonitrile (53:47%v/v). The LOD and LOQ are found to be 3.952μg per mL and 11.97μg per mL, respectively. The flow rate was 1.0mL per min with UV detection at 254nm. The method has been validated and proved to be accurate, precise, linear, rugged, robust, simple and rapid. The calibration curve was linear in the concentration range 25-150μg per mL with coefficient of
correlation 0.9994. The percentage (%) recovery of diacerein was found to be 101.60%. The method is useful in the quality for the estimation of diacerein in tablet dosage form.

Sarika Narade and Snehal Patil, (2010) have developed a spectrophotometric method for the estimation of diacerein in bulk and pharmaceutical dosage forms. Diacerein shows maximum absorbance at 258.5nm. Beer's law was obeyed in the concentration range of 1-10μg per mL. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.02μg per mL and 0.07μg per mL, respectively. The results of recovery studies (98.68-101.11%) indicated that proposed method is accurate and precise for the determination of diacerein in capsules.

Jagadeeswaran et al., (2010) have developed a simple reverse phase high performance liquid chromatographic method for the simultaneous determination of diacerein and aceclofenac in tablets. Chromatographic separations of the two drugs were analyzed on a phenomenex C18 column (250×4.60mm, 5μm). The mobile phase constituted of 0.01M Potassium dihydrogen phosphate and acetonitrile 60:40 (%v/v) and pH adjusted to 4.5 using glacial acetic acid was delivered at the flow rate 2.0mL per min. Detection was performed at 280nm. The retention time of diacerein and aceclofenac was 3.61 and 6.28min, respectively. Calibration curves were linear with correlation coefficient is 0.99 to 1.0 over a concentration range of 80-120μg per mL of diacerein and aceclofenac. The percent relative standard deviation (%RSD) was found to be < 2.0%.
Santosh Bhalerao et al., (2010) have proposed a novel, simple and robust high performance thin layer chromatographic method and validated for the determination of diacerein and aceclofenac in the combined pharmaceutical tablet dosage form. The developed method demonstrates extraction of diacerein and aceclofenac by solid liquid extraction and their densitometric determination. Paracetamol was used as an internal standard (IS). The pre coated silica gel $60F_{254}$ Aluminum plate was selected as the stationary phase and the mixture of ethyl acetate: methanol: glacial acetic acid in the ratio of (12: 0.5: 0.2 %v/v/v) was used as developing solvents. The detection of diacerein and aceclofenac was carried out at 268nm by TLC scanner-3 (Camag). The developed method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation and robustness parameters. The correlation coefficient of diacerein and aceclofenac were 0.9997 and 0.9996 observed respectively. The average percentage recovery of diacerein and aceclofenac were found to be 100.14±1.15 and 100.71±0.33 respectively. Intra and inter-day precision measured as coefficient of variation were less than 2% for both analytes. The proposed HPTLC method has potential applications for determination of diacerein and aceclofenac in combined tablet dosage form.

Sohan S Chitlange, (2010) has developed the three UV spectrophotometric methods for the simultaneous determination of diacerein (DIA) and aceclofenac (ACE) in tablets, in the present work. Method-I is simultaneous equation method, wavelength selected are 258.5nm ($\lambda_{\text{max}}$ of diacerein) and 274nm ($\lambda_{\text{max}}$ of aceclofenac). Method-II involves multi
component mode of analysis, wavelength selected are 258.5nm ($\lambda_{\text{max}}$ of diacerein) and 274nm ($\lambda_{\text{max}}$ of aceclofenac). Method-III is area under curve method, wavelength range selected is 263.5-253.5nm for diacerein and 279-269nm for aceclofenac respectively. All the methods were found linear between 2-14µg per mL for diacerein and 4-28µg per mL for aceclofenac. The accuracy and precision of the methods were determined and validated statistically which showed no significant difference between the results obtained by the three methods. The proposed methods are simple, accurate and can be used for its intended purpose.

II. b. Donepezil hydrochloride tablets

Donepezil is a centrally acting reversible acetyl cholinesterase inhibitor (increase neuromuscular transmission) its main therapeutic use is in the treatment of Alzheimer’s disease. It has been tested in other cognitive disorders including Lewy body dementia (Rojas-Fernandez, 2001) and vascular dementia (Malouf R, 2004) but it is not currently approved for these indications. Donepezil has been found to improve sleep apnea in Alzheimer’s patients (Moraes W). The studies found that speech of autistic children who were mild to moderately affect appeared to improve from the use of donepezil (pn.psychiatryonline.org) medication.

Norio Yasui Furukori et al., (2002) have developed a simple and sensitive HPLC/UV method for the quantification of donepezil, a centrally and selectively acting acetyl eholinesterase inhibitor, in human plasma. After
sample alkalinization with 0.5mL of NaOH (0.1M), the test compound was extracted from 1.0mL of plasma using isopropanol: hexane (3:97, %v/v). The organic phase was back extracted with 75μL of HCl (0.1M) and 50μL of the acid solution was injected into a C18 STR ODS-2 analytical column (5μ, 150x4.6mm). The mobile phase consisted of phosphate buffer (0.02M, pH4.6), perchloric acid (6M) and acetonitrile (59.5:0.5:40, %v/v) and was delivered at a flow rate of 1.0mL per min at 40°C. The peak was detected using a UV detector set at 315nm and the total time for a chromatographic separation was approximately 8min. The method was validated for the concentration range 3-90ng per mL. Mean recoveries were 89-98%. Intra and inter-day relative standard deviations were less than 7.3 and 7.6%, at the concentrations ranging from 3 to 90ng per mL. The method showing good specificity with respect to commonly prescribed psychotropic drugs and it could be successfully applied for pharmacokinetic studies and therapeutic drug monitoring.

Lu YH et al., (2003) have developed a sensitive and specific liquid chromatography mass spectrometry (time of flight) [LC-MS (TOF)] method for the determination of donepezil in human plasma after an oral administration of five mg donepezil hydrochloride tablet. Alkalized plasma was extracted with isopropanol: n-hexane (3:97 %v/v) and loratadine was used as internal standard. Solutes were separated on a C18 column with a mobile phase of methanol acetate buffer (pH4.0) (80:20 %v/v). Detection was performed on a time of flight mass spectrometry equipped with an ESI interface and operated in positive ionization mode. Donepezil quantitation was realized by computing
the peak area ratio (donepezil: loratadine) (donepezil m/z 380[M+H] + and loratadine m/z 383[M+H] +) and comparing them with calibration curve (r=0.9998). The linear calibration curve was obtained in the concentration range of 0.1-15μg per L. The detection limit of donepezil was 0.1μg per L. The average recovery was more than 90%. The intra and inter run precision was measured to be below 15% of percent (%) RSD. The method is sensitive, simple and rapid so it can meet the need of the studies on the pharmacokinetics and bioavailability of donepezil.

Lu Y et al., (2004) have developed a sensitive, simple and specific liquid chromatographic method coupled with electro spray ionization mass spectrometry for the determination of donepezil in plasma using loratidine as the internal standard, after extraction of the alkalized plasma by isopropyl alcohol and n-hexane (3:97, %v/v), solutes are separated on a C18 column with a mobile phase of methanol: acetate buffer (pH 4.0) (80:20, v/v). Detection is performed with a time of flight mass spectrometer equipped with an electro spray ionization source operated in the positive ionization mode. Quantitation is accomplished by computing the peak area ratio (donepezil [M+H]+ m/z 380 loratadine [M+H]+ m/z 383) and comparing them with the calibration curve (r=0.9998). The linear calibration curve is obtained in the concentration range 0.1-15ng per mL. The limit of quantitation is 0.1ng per mL. The mean recovery from human plasma is 99.4% ± 6.3% (ranging 93.4–102.6%). The inter and intra day relative standard deviation is less than 15%.
Sonemoto et al., (2006) have developed a HPLC/fluorescence method for determination of donepezil hydrochloride (DP) in rat urine. DP in rat urine was extracted with n-hexane following alkalization with 0.05M borate buffer (pH10). The separation of DP was achieved within 25min by a reversed phase C30 column (50x4.6mm). The calibration curve showed a good linearity over the range of 5-1000nM (r=0.998) with a detection limit of 1.74nM (S/N=3). The precision for intra and inter-day assay were 2.8 and 2.9% as percent (%) RSD, respectively. The method was applied to monitor DP after a single IP administration of DP to rat.

Mahasen A Radwan et al, (2006) have developed a new precise, sensitive and accurate stereo selective HPLC method for the simultaneous determination of donepezil enantiomers in tablets and plasma with enough sensitivity to follow its pharmacokinetics in rats up to 12hour after single oral dosing. Enantiomeric resolution was achieved on a cellulose tris (3, 5-dimethylphenyl carbamate) column known as chiralcel-OD, with UV detection at 268nm and the mobile phase consisted of n-hexane, isopropanol and triethylamine (87:12.9:0.1)% v/v. Donepezil enantiomers were well resolved with mean retention times of 12.8 and 16.3min, respectively. Linear response (r>0.994) was observed over the range of 0.05-2μg per mL of donepezil enantiomers, with detection limit of 20ng per mL. The mean relative standard deviation (%RSD) of the results of within day precision and accuracy of the drug were <or=10%. There was no significant difference (p>0.05) between inter and intra-day studies for each enantiomers which confirmed the
reproducibility of the assay method. The mean extraction efficiency was 92.6-
93.2% of the enantiomers. The proposed method was found to be suitable and
accurate for the quantitative determination of donepezil enantiomers in tablets.
The assay method also shows good specificity to donepezil enantiomers and it
could be successfully applied to its pharmacokinetic studies and to therapeutic
drug monitoring.

Samah Sayed Abbas et al., (2006) have developed a stability indicating
assay method for the determination of donepezil hydrochloride (I) in presence
of oxidative degradate (II) were developed and validated. The first three are
spectrophotometric methods depending on using zero order (D°), first order
(D₁) and second order (D²) spectra. The absorbance was measured at 315nm for
(D°) while the amplitude was measure at 332.1n for (D₁) and 340nm for (D²)
using de ionized water as a solvent. Donepezil hydrochloride can be
determined in the presence of up to 70% of its oxidative degradate (II) using
(D°) 80% using (D₁) and 90% using (D²). The linearity range was found to be
8-56µg per mL for (D°), (D₁) and (D²). These methods were applied for the
analysis of I in both powder and tablet form. A spectrophotometric method
depending on measuring the native fluorescence of I in de ionized water using
226nm and 391nm was suggested. The linearity range was found to be 0.32-
3.20µg per mL using this method, I was determined in the presence of up to
90% of II. The proposed method was applied for the analysis of I in tablet form
as well as in human plasma. The last method depends on using TLC separation
of I from its oxidative degradate II and I was determined spectr
densitometrically. The mobile phase was methanol: chloroform: 25% ammonia (16:64:0.1) %v/v. The linearity range was found to be 2-15µg per spot. This method was applied to the analysis of I in both powder and tablet form using acetonitrile as a solvent.

Zhiyong Xie et al., (2006) have developed a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method and validated for the determination of donepezil in human plasma samples. Diphenhydramine was used as the internal standard. The collision induced transition m/z 380 to 91 was used to analyze donepezil in selected reaction monitoring mode. The signal intensity of the m/z 380 to 91 transition was found to relate linearly with donepezil concentrations in plasma from 0.1–20.0ng per mL. The lower limit of quantification of the LCMS/MS method was 0.1ng per mL. The intra and inter-day precisions were below 10.2% and the accuracy was between ±2.3% and ±2.8%. The validated LC-MS/MS method was applied to a pharmacokinetic study in which healthy Chinese volunteers each received a single oral dose of 5mg donepezil hydrochloride. The non compartmental pharmacokinetic model was used to fit the donepezil plasma concentration time curve. Maximum plasma concentration was 12.3 W2.73ng per mL which occurred at 3.50W1.61h post dosing. The apparent elimination half life and the area under the curve were, respectively, LC-MS/MS is a rapid, sensitive and specific method for determining donepezil in human plasma samples.
Sangshetti et al., (2008) have developed two simple and sensitive methods for the determination of donepezil hydrochloride in bulk and tablets. Method-A and B describes simple UV spectrophotometric and colorimetric method in methanol, respectively. In method B orange colored complex was observed due to reaction of keto group of donepezil with 2, 4-dinitrophenyl hydrazine in dilute sulphuric acid. Molar absorptive of the drug was found to be at $1.38 \times 10^5$ and $3.077 \times 10^4$ for method-A and B, respectively. The Beer Lambert's law was obeyed in the concentration range of 5-40 and 10-60µg per mL for method-A and B, respectively. The proposed methods were simple, selective and accurate and giving reproducible results so these methods can be successfully applied for routine estimation of donepezil in bulk and pharmaceutical dosage forms.

Een Jeong Park et al., (2008) have developed a selective, sensitive and rapid hydrophilic interaction liquid chromatography with electro spray ionization tandem mass spectrometry method for the determination of donepezil in human plasma. Donepezil was twice extracted from human plasma using methyl tert-butyl ether at basic pH. The analytes were separated on an Atlantis HILIC Silica column with the mobile phase of acetonitrile: ammonium formate (50 mM, pH4.0) (85:15, %v/v) and detected by tandem mass spectrometry in the selective reaction monitoring mode. The calibration curve was linear ($r=0.9994$) over the concentration range of 0.10–50.0ng per mL and the lower limit of quantification was 0.1ng per mL using 200µL plasma sample. The coefficient of variation and relative error for intra and inter
assay at four QC levels were 2.7 to 10.5% and −10.0 to 0.0%, respectively. There was no matrix effect for donepezil and cisapride. The present method was successfully applied to the pharmacokinetic study of donepezil after oral dose of donepezil hydrochloride (10 mg tablet) to male healthy volunteers.

II. c. Amiodarone hydrochloride tablets

Amiodarone is an anti-arrhythmic agent (medication used for irregular heart beat) used for various types of tachyarrhythmia (fast forms of irregular heart beat), both ventricular and supra ventricular (atrial) arrhythmias. Despite relatively common side-effects, it is used in arrhythmias that are otherwise difficult to treat with medication. Related newer compounds, such as dronedarone, have lower efficacy but a reduced rate of side-effects. It is popular in Europe as a treatment for angina pectoris (Deltour 1962; Charlier 1962). Amiodarone resembles thyroid hormone and its binding to the nuclear thyroid receptor might contribute to some of its pharmacologic actions (Goodman and Gilman’s).

Susanto et al., (1986) have developed a method for the determination of amiodarone and its main metabolite (desethyl amiodarone) from plasma with silica column by using high performance liquid chromatography. The recovery of amiodarone and its metabolite was greater than 80% over an investigated range of 0.1−5μg per mL of plasma and the limit of quantitation by the assay was 50ng per mL of plasma. The column extraction of amiodarone and its metabolite coupled with the chromatographic versatility of the method make it
suited for both detailed pharmacokinetic studies and routine plasma analysis of amiodarone.

Kannan et al., (1987) have developed a reproducible high performance liquid chromatographic (HPLC) method for the measurement of amiodarone (AM) and its metabolite(s) in serum and tissues. The method uses a 5μm silica column, methanol containing 0.02% perchloric acid at pH4 as the mobile phase and ultraviolet detection at 240nm. The standard curves for AM and desethyl amiodarone (DAM) were linear for serum (range 0.025-6.0μgram per mL) and tissues (range 0.1-0.5μgram for 10-25mg wet weight). There was a significant decrease as a function of time in AM and DAM concentrations in patients' sera left at ambient temperature in the presence of light. This HPLC method was applied to studies on serum AM elimination kinetics in patients and on tissue uptake during chronic AM administration to rabbits. The elimination half life (5.8h) of AM after a 5mg per Kg intravenous dose to a patient was similar to that after acute oral doses AM, being lipophilic, accumulated maximally in the fat tissue (56μg per g wet weight), followed by lung and liver in rabbits injected with AM for six weeks. The latter two tissues also contained nearly equal quantities of DAM. The high concentrations of AM and DAM in the liver and lungs may be related to the hepatic toxicity and pulmonary fibrosis associated with chronic AM therapy. Two new metabolites were found in the lung and bile of AM treated rabbits, but these have not yet been identified.

Ching Nan OU et al., (1990) have developed a stable and simple NP-HPLC method for simultaneously determination of amiodarone and its
metabolite, n-desethyl amiodarone. The mobile phase composed of ammonium sulphate (17mM per L, pH6.8) and methanol in the ratio of 8:92 (%v/v) with flow rate of 1.8mL per min, chromatographic column was silica 150mmx3.9mm, 5μm and detection at 254nm. Relative recovery was 100% over the assay range of 0.1 to 20.0mg per mL for both compounds. Repeatability and reproducibility also validated and found the developed method was stability indicating and we can use for regular analysis.

Trivier et al., (1992) have developed a reversed phase HPLC assay using ultraviolet detection is described for determining the production of the major N-dealkylated metabolite of amiodarone in rat liver microsomes. The principal advantages of this method are its simple sample preparation (protein precipitation by acetonitrile), low detection limit for N-desethyl amiodarone (0.05μM per L) and relatively short analysis time (16min).

Dhawailie (1995) has developed a simple and rapid high performance liquid chromatographic (HPLC) method for the determination of amiodarone (AD) in plasma and tissues. The method involved deproteinization of plasma or homogenized tissue with acetonitrile containing an internal standard (N-cetylpyridinium chloride) followed by reversed phase chromatography using μ-bondapack C18 column (10μm) with a mobile phase consisting of acetonitrile: methanol: sodium di-hydrogen phosphate buffer (70:10:20%, v/v), the pH adjusted to 4.0 and pumped at flow rate of 1.0mL per min. The column effluent was monitored at 242nm. A linear relationship was obtained between peak height ratios (drug to internal standard) versus drug levels over the
concentration range of 50-750ng per mL. The detection limit of AD in plasma and tissues by this method was 20ng per mL.

Masaki Koichin et al., (1999) have developed a simple reverse phase high performance liquid chromatographic (HPLC) assay method for the measurement of amiodarone and its metabolite, desethylamiodarone in the serum. The ODS-2 column was used and the absorbance of the effluent from the column at 254nm was measured. The standard curves for amiodarone and desethylamiodarone were linear up to 5µg per mL. The coefficient of variation (CV) was within 10% at a concentration of 100ng per mL, and the CV of the intra and inter-day variation was within 10% at concentrations of 0.25, 0.5 and 1.0µg per mL, respectively. These results suggest that the limit of detection is 100ng per mL for amiodarone and des-ethyl amiodarone and this assay is thus considered to be a reliable method in clinical practice.

Andrew et al., (2001) have developed a HPLC method for the determination of amiodarone in rat plasma. After liquid-liquid extraction, the separation of amiodarone from internal standard and endogenous components was accomplished using reversed phase chromatography. The mobile phase, a combination of monobasic Potassium phosphate, methanol and acetonitrile, was run isocratically through a C8 analytical column. The UV detection was at 254nm for ethopropazine, the internal standard and subsequently changed to 242nm for amiodarone detection. Analytical run time was less than 13min. Mean recovery was 75% and 82% for lower (0.5µg per mL) and higher concentrations (5µg per mL), respectively. The assay exhibited excellent linear
relationships between peak height ratios and plasma concentrations; quantization limit was at least 0.035µg per mL. Accuracy and precision were <17% over the concentration range of 0.035 to 5µg per mL. The assay was applied successfully to the measurement of amiodarone plasma concentrations in rats given the drug orally.

Juenke Joetta et al., (2004) have proposed a rapid isocratic high performance liquid chromatographic method for the simultaneous measurement of the antiarrhythmic drug amiodarone and its potentially active metabolite N-desethylamiodarone (DEA). Liquid liquid extraction, amiodarone and its metabolite are quantitated (0.3-6.0mg per L) by on an HPLC-UV system. The analytical time was reduced by 50%, without compromising the assay performance. The assay limit of quantitation, linearity, imprecision and accuracy adequately covered the therapeutic range for appropriate patient monitoring. Amiodarone and DEA can be simultaneously and accurately quantitated in serum or plasma by HPLC-UV detection with imprecision < 6% at therapeutic concentrations and a quantitation range from 0.3 to 6.0mg per L.

LIN Dai et al., (2005) have developed a method for determining the concentration of amiodarone in serum by HPLC. The column was C18, the mobile phase was acetonitrile: methanol: 0.01M per L, ammonium acetate [100mL containing (0.2) mL triethylamine, pH value was adjusted to 4.0 with acetic acid], the detection wavelength was 240nm, the flow rate was 1.2mL per min and the temperature of column was at room temperature. The calibration curves were linear in the range (0.062 5)-(8.00) mg per L for amiodarone, the
recovery was (100.9%)-(107.9%), inter-day percent (%) RSD was less than 7% and intra-day percent (%) RSD was less than 8%. The method could be used for therapeutic amiodarone monitoring.

Rajendran et al., (2006) have developed a HPLC method for simultaneous estimation of amiodarone and its metabolite desethyl amiodarone in plasma. After precipitation with acetonitrile, the separation of amiodarone, desethyl amiodarone and internal standard was accomplished using reversed phase chromatography. The mobile phase, a combination of ammonium acetate (pH3.5 adjusted with ortho phosphoric acid) and acetonitrile was run isocratically through a C18 analytical column. The UV detection at 242 and 247nm for amiodarone and des-ethyl amiodarone, respectively. Analytical run time was 10min. Mean recovery was 84% for 0.5μg per mL concentrations. The assay exhibited good linear relationship between peak height ratios and plasma concentration. Quantification limit was at least 0.01μg per mL of amiodarone and des-ethyl amiodarone. Accuracy and precision were over the concentration range of 0.01-10μg per mL. Assay was successfully applied to the measurement of amiodarone and its metabolite des-ethyl amiodarone in human plasma of patients who were on long term oral therapy on amiodarone.

Kuhn et al., (2010) have developed a stable isotope dilution LC-MS/MS assay method to measure amiodarone, the most frequently used agent for maintaining sinus rhythm in patients with atrial fibrillation and its major metabolite des-ethyl amiodarone in human plasma and serum. Measurement of amiodarone and des-ethyl amiodarone was performed during a 4.0min run time
using amiodarone D (4) and des-ethyl amiodarone D (4) as internal standards. Calibration curves covering twelve calibrators measured in four replicates each for the analysis of both amiodarone and des-ethyl amiodarone were linear and reproducible in the range of 0.01-40.0mg per L. Limits of detection in plasma matrix were 2.7µg per L for amiodarone and 1.9µg per L for des-ethyl amiodarone and lower limits of quantification in plasma matrix were 7.5µg per L for amiodarone and 2.5µg per L for des-ethyl amiodarone. Inter assay imprecision and inaccuracy were <8% and <9% for both substances. Mean extraction yield was 99.6% (range 92.6-107.7%) for amiodarone and 90.2% (range 80.0-94.7%) for des-ethyl amiodarone. The present HPLC method with UV detection using a commercially available reagent set for the HPLC analysis of these drugs. This sensitive and interference free LC-MS/MS assay permits rapid and accurate determination of amiodarone and des-ethyl amiodarone in human plasma and other body fluids.

### III. d. Sildenafil citrate tablets

Sildenafil citrate is used to treat erectile dysfunction and pulmonary arterial hypertension (PAH). The mechanism of action of sildenafil citrate involves the release of nitric oxide (NO) in the corpus cavernosum of the penis. Sildenafil is metabolised by liver enzymes and excreted by both the liver and kidneys. If taken with a high-fat meal, absorption is reduced; the time taken to reach the maximum plasma concentration increases by around one hour and the maximum concentration itself is decreased by nearly one-third. Sildenafil
improves the sexual function in female patients on antidepressants (Numberg HG, 2008). Its use is now standard treatment for erectile dysfunction in all settings, including diabetes (Yoram Vardia, 2009). Sildenafil citrate major active metabolite is N-desmethyl sildenafil and it is quantitated in plasma, serum or whole blood in order to assess pharmacokinetic status in those receiving the drug therapeutically (Baselt R, 2008).

Segall et al., (2000) have developed a method for the determination of sildenafil citrate in the presence of its oxidative degradation products by RP-HPLC method. The method was validated as stability indicating by forced decomposition of sildenafil citrate in acid, base, oxidative, thermal and photochemical media. The chromatographic conditions employed with C18, 5μm, 250mmx4.6mm column, isocratic elution with 70mM Potassium phosphate monobasic containing 100mM triethylamine, adjust pH3.0 with ortho-phosphoric acid and acetonitrile in the ratio of 70:30 (%v/v) and ultraviolet (UV) detection at 225nm. The peak area versus sildenafil citrate concentration proved linear over the 10-160% range of the working analytical concentration of 0.5mg per mL. The absolute recovery of sildenafil citrate was 100.9 ±1.1%. The precision, expressed as percent (%) RSD of ten replicate injections of sildenafil citrate reference solution found to be within the limit.

Daraghmeh et al., (2001) have developed and validated an HPLC method for the assay of sildenafil citrate and its related substances in the drug commercial products and in tablets. A chromatographic system comprising a μBondapak C18 (10μm) column, a composition of mobile phase was 0.2M
ammonium acetate and adjust pH 7.0 with triethylamine and acetonitrile 50:50 (%v/v) a flow rate of 1.0 mL per min and a UV detector set at 240 nm has shown good chromatographic separation for sildenafil and the related substances. The degree of linearity of the calibration curves, the percent recoveries of sildenafil and related substances, the limit of detection (LOD) and limit of quantification (LOQ) have been determined and the HPLC method under study was found to be specific, precise, accurate, reproducible and stability indicating.

Reddy et al., (2002) have developed a simple extractive spectrophotometric method for the estimation of sildenafil citrate in both pure and pharmaceutical dosage forms. These methods are based on the formation of ion association-pair complexes of the drug with two acid dyes namely orange-II and erichrome black-T in acidic medium followed by their extraction in to chloroform. The absorbance of the chloroform layers was measured at their respective wave length of maximum absorbance against the corresponding reagent blank. The method has been statistically evaluated and is found to be precise and accurate.

Sager et al., (2002) have developed a simple, reliable and fast spectro photometric method for the estimation of sildenafil citrate in bulk samples and formulations. The linear dynamic range of 40-200 μg per mL, with linear regression of 0.9999. The method is based on the reaction between the sildenafil with sodium nitroprusside and hydroxylamine hydrochloric acid in
the presence of Sodium carbonate. The yellow coloured chromogen formed has an absorption maximum of 475nm.

Berzas et al., (2002) have developed a simple and rapid method for the analysis of sildenafil citrate in its pharmaceutical formulations in the presence of excipients using its metabolite as internal standard for quantification. The optimal separation conditions were established in presence of compounds in the pharmaceutical formulations and provided analysis times of less than 12min. the column conditions were 110Kpa pressure, injector and flame ionization detector (FID) temperatures (325 to 300°C), time and temperature for the split less step (0.75min and 70°C), samples size (2μL) and an oven temperature program. Aspects such as stability of the solutions, linearity, accuracy, reproducibility, specificity, LOD and LOQ are examined in order to validate the method in presence of all excipients. Some of these aspects such as specificity were also assessed by mass spectrometric detection in the SCAN mode. The highly satisfactory results obtained in both the validation and ruggedness rests show that the GC method could be a very valuable option as an official method for the determination of sildenafil citrate in its pharmaceutical formulations.

Mu Chang Tseng and Jer Huei Lin (2002) have developed a LC-MS/MS method to identify and quantify an adulterated sildenafil citrate in a dietary supplement capsule. The capsule is claimed to be an extract of animal organs, traditional Chinese herbs and indicated for enhancing sexual activity. At first, the sample was extracted with 50%v/v methanol in water and the extract was
injected directly into the LC-MS/MS with no separation and a full scan of positive ion electro spray (ESI+) analysis was performed. The analytical column was cosmosil 5C18-AR, 4.6x150mm; mobile phase was composed of methanol, acetonitrile and 1% acetic acid in the ratio of 25:17:58 (%v/v), capillary voltage 3kV, collision energy 25eV, source temperature 120°C and desolvation temperature 350°C. The detection limit was 40ng per mL. An excellent calibration curve of sildenafil citrate was obtained with the $r^2$ correlation coefficient of 0.9977. The percent (%) RSD of inter-day and intra-day were between 7.56–7.75% and 9.09–11.25%, respectively. This method is suitable for identification and measurement of sildenafil citrate in capsule.

Dinesh et al., (2002) have developed a RP-HPLC method for the quantitation of sildenafil citrate in pure form and its pharmaceutical formulations. Mobile phase composed with water and acetonitrile 48:52% (v/v) with flow rate of 1.0mL per min and the column was C18, 250mmX4.6mm, 5μm; UV detection was 245nm. It is an internal standard method using piroxicam as the internal standard is used. The linear dynamic range of sildenafil was found to be 0.05-7.5μg per mL. The excipients present in the formulations have no interference with the assay procedure so the developed method concluded as stability indicating.

Tracqui and Ludes (2003) have developed a LC-MS method for the determination of sildenafil in biological fluids. Liquid-liquid extraction was performed by chloroform: 2-propanol: n-heptane 25:10:65 (%v/v) at pH9.5 with 300 nano gram of buprenorphine-d₄ as the internal standard. After
agitation (10min) and centrifugation the organic phase was evaporated and the dry extract resuspended in 25μL methanol, from which 2μL was injected onto a novapak C18 (Waters) HPLC column. Separation was carried out by a gradient of (acetonitrile+10μg per mL trimethylamine) in 2mM NH₄COOH pH3.0 buffer (35–70% in 9min). Detection was done by a Perkin Elmer sciex API-100 single quadrupole mass analyzer with an ion spray interface operated in positive ion mode. The retention times of sildenafil and the internal standard were 4.2 and 5.0min, respectively.

Abd Elbary et al., (2004) have developed a rapid, specific and reliable HPLC method for the estimation of sildenafil citrate in pharmaceutical dosage forms using mobile phase of methanol: water: acetonitrile 60:20:20 (%v/v/v), pH6.1 with 0.1% glacial acetic acid and detection at 290nm. The recovery and coefficient of variation from six tablets containing 50mg of sildenafil were 100.90% and 0.45% respectively. It is suggested that the proposed HPLC method should be used for routine quality control and dosage form assay of sildenafil citrate. The proposed method was also used to study the stability of sildenafil citrate in different dosage forms of the drug.

Bulent Ergun et al., (2005) have developed a RP-HPLC method for the quantitative determination of sildenafil citrate in pharmaceutical dosage forms and spiked human plasma. Chromatography was carried out on a RP-C18 column, using a mixture of acetonitrile and water in the ratio of 45:55 (%v/v) as a mobile phase at a flow rate of 1.0mL per min. Phenobarbital Sodium was used as an internal standard and detected using a diode array detector at 220nm.
Retention times of sildenafil citrate and phenobarbital Sodium were 7.2min and 3.2min. The method was validated for its linearity, precision, accuracy, stability, robustness and ruggedness. The proposed method was applied to sildenafil citrate tablets and spiked human plasma.

Kuchekar et al., (2005) have developed two methods for the estimation of sildenafil citrate in health care medicines. The method-A is based on reduction of ferric ions to ferrous ions by the drug, which further in presence of potassium ferric cyanide as oxidizing agent produces blue colored complex measured at 715nm against reagent blank. The linearity over the range of 10 to 70μg per mL of drug. The method-B is based on reduction of ferric ions to ferrous ions by the drug, which further in presence of Potassium dichromate as oxidizing agent produces greenish blue colored complex measured at 700nm against reagent blank. The chromogen obeyed linearity over the range of 50 to 130μg per mL of drug.

Massoud Mahmoudian (2005) was determined the sildenafil citrate by various quantitative methods and in various media. The methods used consist of UV spectroscopy, TLC and HPLC. These methods were used to determine the amount of sildenafil in pharmaceutical preparations, soft drinks and biological liquids. HPLC was used for evaluation of pharmacokinetic parameters of sildenafil. It is concluded that HPLC is the most reliable and applicable method in this regard.

Giuseppe Carlucci et al., (2005) have developed a HPLC method with ultraviolet absorption detection at 230nm and validated for the determination of
a phosphor diesterase-V inhibitor and sildenafil citrate in seminal plasma. A single step liquid-liquid extraction procedure using ethyl acetate was performed to recover sildenafil citrate from 1.0mL of seminal plasma combined with 200μL of 0.1M NaOH. Symmetry C18 column (150x4.6mm, 5μm) was used and the mobile phase consisted of 32% acetonitrile and 68% of 0.016M phosphoric acid, at a flow rate of 1.0mL per min. The quantitation limit was 5ng per mL. Intra-day and inter-day relative standard deviation (RSD) did not exceed 6.6%.

Ehab et al., (2005) have developed an HPTLC method for determination of sildenafil citrate in commercial products. The compounds were separated on silica gel, with CHCl₃: MeOH: diethyl amine, 90:10:1 (%v/v), as mobile phase and the analyte spots were quantified denstiometrically at λ=305nm. Recovery of sildenafil citrate was 100.6 and 98.2%, for pure and spiked samples, respectively. Method precision and specificity were also validated. The method was used for determination of sildenafil in four pharmaceutical products and three aphrodisiac herbal preparations which were claimed to be totally natural. Sildenafil citrate was also detected in herbal preparations in a capsule form.

Milap Nahata et al., (2006) have developed a simple HPLC method for the determination of sildenafil citrate in oral dosage form, using C18 column with the mobile phase composition of 50% 0.2M ammonium acetate and 50% acetonitrile. The flow rate was 0.5mL per min. the detection was at 245nm and the injection volume was 10μL. The column was maintained at 25°C. The developed method was applicable for oral solutions.
Shao Pu Liu et al., (2006) have proposed highly sensitive resonance Rayleigh scattering (RRS) method for the determination of sildenafil citrate, based on the fact that sildenafil reacted with Evans Blue (EB) to form an ion association complex in pH1.1-4.6 aqueous solution. The wavelength of the maximum RRS was at 365nm and other scattering peaks were at 400, 442, 470 and 534nm, respectively. The intensity of RRS was directly proportional to the concentration of sildenafil citrate in the range 0-11.5μg per mL and the detection limit for sildenafil was 30.3ng per mL. The composition of the ion association complex was sildenafil and Evan blue 1:1, as established by Job's method. The method had good selectivity and could be applied to the determination of sildenafil citrate in the aqueous phase without using organic solvent extraction. The method was simple and rapid.

Sheshashena Reddy et al., (2006) have developed a HPTLC method for the quantitative determination of sildenafil citrate in herbal medicinal formulations. Chromatography was performed on silica gel 60F254 HPTLC place, pre washed with methanol. Mobile phase composed of toluene, acetone and methanol in the ratio of 6:2:2 (%v/v). The plates were developed vertically, to a distance of 8cm, in a saturated chamber and densitometric quantitation was performed detection at 312nm by reflectance scanning. Recovery from the herbal medicinal powders and tablets was 83.17% and 99.41%, respectively. The standard sildenafil citrate calibration plot was linear (r=0.9993) over the concentration range 100-600ng per spot and the quantitative results showed the sildenafil content of the herbal formulations analyzed was in the range 76.4 to
85.0mg (percent (%) RSD< 3%). This HPTLC method in complementary to other chromatographic methods and has potential use for routine quality control analysis.

Nora Al Shaalan (2007) has developed a method for the quantitative determination of sildenafil citrate through charge transfer complexes (CTC) formation with three electron acceptor reagents. The methods involve the reaction of sildenafil citrate as n-donor with either chloranil (tetra-chloro 1, 4-benzoquinone), tetra-cyanoethylene (TCNE) or 1, 7, 8, 8- tetra cyano quino dimethane (TCNQ) as n-acceptors, to give a stable and highly colored radical anion. The colored products were quantified spectrophotometrically. The condition ranges adhering to Beer’s law are 0.05-3.00mg per mL. The molar ratios of the reactants were ascertained. The different parameters were carefully studied and optimized. Statistical analysis of the results revealed equal precision and accuracy to the results of the reported method. The methods were applied for determination of sildenafil citrate in pure form and in viagra tablets.

Irena Baranowska et al., (2007) have developed a RP-HPLC method for the determination of sildenafil citrate (SC) and its N-desmethyl active metabolite (N-desmethyl sildenafil) in the presence of different drugs in human urine. The analysed drugs were extracted from urine by liquid-liquid extraction. Effective RP-HPLC separation of the examined drugs was performed using an analytical column (Purospher STAR RP18, 125mm×3mm, 5μm) with a gradient mobile phase system and diode array or fluorescence detector. Linear ranges of detection for sildenafil citrate and metabolite were found to be
0.03.8.5μg per mL ($r^2=0.9994$) for both compounds. Linear ranges for other drugs (analgesic, antibiotic, diuretic and demulcent), which could exist in urine from patients treated with sildenafil citrate also determined. Complete separation of all analytes was achieved below 25min. The retention times for all studied analytes ranged from 4.76 to 18.84min. The limits of detection and limits of quantification for both analysed compounds were calculated and recovery studies were also performed. The mean absolute recoveries of both were >94%. The new procedure was suitably validated and successfully applied for the analysis of sildenafil, its active metabolite and other drugs in urine samples of patients with pulmonary hypertension.

Prasanna Kumar Reddy and Ramanjaneya Reddy (2008) have developed a RP-HPLC method for the estimation of sildenafil citrate using inertsil C18, 5μm, 150mmx4.6mm with a mobile phase of acetonitrile and phosphate buffer in the ratio of 70:30 %v/v, pH7.0 at a flow rate of 0.8mL per min with UV detection at 228nm. The retention time was 4.0min. The method is accurate (99.15-101.85%), precise (intra-day variation 0.13-1.56% and inter-day variation 0.30-1.60%) and linear within range 0.1-30mg per mL ($R^2=0.999$) concentration and was successfully used in monitoring left over drug. The detection limit of sildenafil citrate at a signal to noise ratio of 3 was 1.80ng per mL in human plasma while quantification limit in human serum was 5.60ng per mL. The proposed method is applicable to stability studies and routine analysis of sildenafil citrate in pharmaceutical formulations as well as in human plasma samples.
Alaa Khedr et al., (2008) have developed a HPLC method for determination of phenytoin (PHN), para hydroxy metabolite of phenytoin (POH) and sildenafil (SIL) in rabbit plasma and validated. The mobile phase was isocratic elution with a mobile phase consist of 29% acetonitrile and 71% Sodium acetate solution (0.02M, pH4.6), detection at 220nm, column was zorbax extended C18 column (150mm×4.6mm). The method was fully validated for linearity and range, selectivity, precision, stability, recovery and robustness. The linearity of the method was in the range of 0.15 to 39µg per mL for PHN and 0.15 to 33µg per mL for both POH and SIL. Limits of detection (LOD) of PHN, POH and SIL were 0.15±0.01, 0.15±0.01 and 0.15±0.01µg per mL, respectively. The % recoveries of PHN, POH and SIL from rabbit plasma were 101.88±0.12, 99.16±0.25 and 99.49±0.33, respectively. The method was applied on plasma collected from rabbits at different time intervals after receiving 30mg per Kg PHN-Na with (and without) 8mg per Kg sildenafil citrate.

Pistos et al., (2008) have developed a simple HPLC method and validated for the determination of sildenafil and its active metabolite (N-desmethyl sildenafil) in human blood, using a hypersil ODS column. The chromatographic mobile phase was having the composition of 35:65 (%v/v) acetonitrile 0.015M di Sodium hydrogen phosphate, triethylamine 0.1%, pH7.4 at 1.0mL per min flow rate and detection at 230nm. The method is linear in the concentration range of 10-500ng per mL for each analyte, with percent (%) relative standard deviation was less than 5.05%. The limits of detection and
quantitation for both analytes were 5.0ng per mL (s/n>3) and 10.0ng per mL (s/n>10), respectively. The method was applied in two post mortem human blood samples, concerning two fatal cases from sildenafil citrate use, reported for the first time in Greece and the results were further confirmed with LC-MS. The method is proposed as supplementary to LC-MS when inadequate mass fragmentation does not provide information appropriate to meet confirmation criteria.

Harikrishna et al., (2008) have developed a simple, rapid and sensitive extractive spectrophotometric method for the assay of sildenafil citrate (SC) in pure and pharmaceutical formulations. These methods are based on the formation of chloroform soluble ion association complexes of SC with brilliant blue-G (BBG) and with bromo cresol purple (BCP) in KCl-HCl buffer of pH1.30 (for BBG) and in Na OAc-HCl buffer of pH3.5 (for BCP) with absorption maximum at 615nm and 406nm for BBG and BCP, respectively. Reaction conditions were optimized to obtain the maximum color intensity. The absorbance was found to increase linearly with increase in concentration of SC, which was corroborated by the calculated correlation coefficient values (0.9991 and 0.9993). The systems obeyed Beer's law in the range of 0.01-4.0 and 0.021-15.0μg per mL for BBG and BCP, respectively. Various analytical parameters have been evaluated and the results have been validated by statistical data. No interference was observed from common excipients present in pharmaceutical formulations.
Bregje et al., (2009) have proposed an UPLC method for quantitation of sildenafil and desmethyl sildenafil simultaneously in 50μL of plasma. Deuterated sildenafil was used as an internal standard. After liquid-liquid extraction, analytes were separated on an UPLC column and quantified via tandem mass spectrometry. The calibration range was linear, with acceptable accuracy and a precision of <15% for both compounds. The lower limits of quantification were 1.0ng per mL. Matrix effects were present, but inter plasma batch variability was under 12%. The method was successfully applied to samples from a pharmacokinetic study into sildenafil pharmacokinetics in neonates, making maximum use of the limited number and amount of plasma samples available.

Sungho Ahn et al., (2009) have developed a method for sildenafil and its analogues, which are used as illegal additives in several dietary supplements, were isolated by liquid-liquid extraction and column chromatography and analyzed by fast atom bombardment mass spectrometry (FABMS). Structures of sildenafil and its derivatives were elucidated by FAB tandem mass spectrometry (MS/MS) with exact mass measurement in the positive ion mode. To find structurally diagnostic ions for the sildenafil analogues, authentic sildenafil was preferentially analyzed by high energy collision induced dissociation (CID) MS/MS. The CID MS/MS spectra of [M+H]⁺ precursor ions resulted in the formation of numerous characteristic ions via a series of dissociative processes. The product ions formed by CID provided important information on the modification of the piperazine ring, the
phenyl sulfonyl group and the pyrazolopyrimidine moiety of sildenafil. By interpreting their MS/MS spectra, the chemical structures of sildenafil analogues isolated from dietary supplements could be elucidated and fragmentation patterns were proposed. To clearly identify the sildenafil derivatives in dietary supplements, some of the derivatives such as acetildenafil, homo sildenafil and hydroxy homo sildenafil which are not commercially available were synthesized and compared with their MS/MS spectra. In addition, high resolution mass measurements were conducted to obtain the elemental compositions of sildenafil and its analogues.

Kannappan et al., (2010) have developed a reproducible HPLC method for the analysis of tadalafil and sildenafil citrate (API). The column used was YMC Pack ODS AQ (150mmx4.6mm). The mobile phase used was phosphate buffer (10mM, pH3.0) acetonitrile gradient run at the flow rate of 1.0mL per min with UV (PDA) detector at 220nm at ambient temperature. Extraction of tadalafil and sildenafil citrate from tablet was carried out using methanol. Linearity was observed in the range from 50 to 150μg per mL for tadalafil with a correlation coefficient (R2) 0.99 and 10ng per mL as the limit of detection. The values of linearity range, correlation coefficient (R2), limit of detection were 50 to 150μg per mL, 0.99 and 20ng per mL respectively for sildenafil. Parameters of validation prove the precision and stability of the method and it's applicability for the assay of tadalafil and sildenafil citrate. The method is suitable for routine analysis of the drug.
Chien Chun Wang et al., (2010) have developed two simple and sensitive surfactant mediated spectrofluorimetric methods for the determination of sildenafil. The developed methods are based on the interaction of sildenafil with normal micelles of HTAB (hexa decyl tri-methyl ammonium bromide, method-A) and the formation of ion association complexes of sildenafil with SDS (sodium dodecyl sulfate, method-B). In both methods, the formed species produce considerable fluorescence enhancement, which allows sildenafil to be quantitatively determined. Linearity was obtained for sildenafil in the concentration range 0.004 to 25µg per mL with a detection limit of 0.0012µg per mL by method-A and a linearity range of 0.005 to 50.0µg per mL with a detection limit of 0.0016µg per mL by method-B. The proposed methods have been applied to the analysis of bulk drug, tablets, herbal medicine and beverages. Validation processes were performed by recovery studies and statistical analysis results found to be satisfactory results.

Issa et al., (2010) have developed two simple and highly sensitive spectrophotometric methods for the quantitative determination of the drug sildenafil citrate (SC) and in pharmaceutical formulations, through ion associate formation reactions with mono chromotropic acid azo dyes, chromotrope 2B (I) and chromotrope 2R (II) and ion pair reactions (method-B) with bi-chromotropic acid azo dyes, 3-phenylazo-6-o-carboxyphenylazo-chromotropic acid (III), bis-3,6-(o-hydroxyphenylazo) chromotropic acid (IV), bis-3,6-(p-N,N-dimethylphenylazo) chromotropic acid (V) and 3-phenylazo-6-o-hydroxyphenylazo-chromotorpic acid (VI). The reaction products,
extractable in methylene chloride, were quantitatively measured at 540, 520, 540, 570, 600 and 575 nm using reagents, I-VI, respectively. The reaction conditions were studied and optimized. Beer's plots were linear in the concentration ranges 3.3-87.0, 3.3-96.0, 5.0-115.0, 2.5-125.0, 8.3-166.7 and 0.8-15.0 μg per mL with corresponding molar absorptivities $1.02 \times 10^4$, $8.34 \times 10^3$, $6.86 \times 10^3$, $5.42 \times 10^3$, $3.35 \times 10^3$ and $2.32 \times 10^4$ L per mol per cm using reagents I-VI, respectively. The limits of detection and sandell's sensitivities were calculated. The methods were successfully applied to the analysis of commercial tablets and the recovery study reveals that there is no interference from the common excipients that are present in tablets. Statistical comparison of the results was performed with regard to accuracy and precision using student's t- and F-tests at 95% confidence level. There is no significant difference between the reported and proposed methods with regard to accuracy and precision.

Erica Ferreira Batista et al., (2010) have developed a method for the determination of sildenafil citrate using differential pulse voltammetry and a cathodically pre treated boron doped diamond electrode is described. The obtained analytical curve is linear in the sildenafil concentration range $7.3 \times 10^{-7}$-$7.3 \times 10^{-6}$ M per L in a 0.1 M per L H$_2$SO$_4$, with a detection limit of $6.4 \times 10^{-7}$ M per L. The proposed method, which is fast and simple to carry out, was successfully applied in the determination of sildenafil citrate in pharmaceutical formulations, with results in close agreement (at 95% confidence level) with those obtained using a comparative HPLC method.
II. e. Ear drops (composition of beclomethasone-0.025%w/v, chloramphenicol-5.0%w/v, clotrimazole-1.0%w/v and lidocaine-1.73%w/v)

Beclomethasone dipropionate is used for the treatment of rhinitis (e.g. heavy fever) and it treat to severe inflammatory skin disorders (e.g. eczema) unresponsive to less potent steroids, but it is generally avoided in the treatment of psoriasis due to the risk of rebound on withdrawal. Deposition on the tongue and throat may promote oral candidiasis which appears as a white coating, possibly with irritation (Willey R, 1976; Salzman, 1988 and Fukushima, 2003).

Chloramphenicol is considered a prototypical broad-spectrum antibiotic, alongside the tetracyclines. Chloramphenicol is effective against a wide variety of gram-positive and gram-negative bacteria, including most anaerobic organisms. The most serious adverse effect associated with chloramphenicol treatment is bone marrow toxicity, which may occur in two distinct forms: bone marrow suppression. The effect usually occurs weeks or months after chloramphenicol treatment has been stopped and there may be a genetic predisposition (Nagao, 1969). Chloramphenicol directly interferes with substrate binding; macrolides sterically block the progression of the growing peptide.

Clotrimazole is normally used for the treatment of fungal infections of both humans and animals such as vaginal yeast infections, oral thrush,
and ringworm. It is also used to treat athlete's foot and jock itch. It may alter the metabolism of other drugs.

Lidocaine is a commonly used local anesthetic and anti arrhythmic drug and used topically to relieve itching, burning and pain from skin inflammations, injected as a dental anesthetic or as a local anesthetic for minor surgery.

Jeffrey Koup (1978) has developed a new method for the analysis of serum chloramphenicol by reverse phase, high performance liquid chromatography (HPLC). The method involves a preliminary extraction of 0.1mL of serum with ethyl acetate containing an internal standard, chromatography with a reverse phase C18 micro particulate column with an acetonitrile: acetate buffer mobile phase and detection by measuring UV absorbance at 270nm. Assay performance was compared with an existing microbiological assay. The HPLC method demonstrated both increased precision and increased sensitivity. The specificity of the HPLC method was also evaluated. The new method presents an alternative approach to the analysis of clinical specimens.

Barry Sample et al., (1979) have developed a procedure for the determination of chloramphenicol in biological fluids. u-Bondapak C18 reverse phase column (300x4.0mm) is used. The eluting mobile phase is acetonitrile and 0.01M of Sodium acetate buffer, pH4.0 per liter (20:80v/v) pumped at a rate of 2.0mL per min. Detection is at 280nm. The chart speed is 5mm per min. Retention time for chloramphenicol is 10min under these circumstances.
Developed method was checked under inter-day and intra-day, reproducibility and accuracy and found satisfactory results so the method is applicable for routine analysis.

Flood et al., (1980) have developed a common methodology for determining three antiarrhythmic drugs, disopyramide, lidocaine and quinidine. Alkalized serum and internal standard (p-chlorodisopyramide) are extracted into dichloromethane, the organic phase is evaporated and the redissolved residue is injected onto a reverse phase column (µBondapack C18). Quantitation is via peak height ratios of analyte vs internal standard (as detected at 205nm) referenced to a serum based multiple drug standard. A mobile phase of 30mM per L phosphate buffer and acetonitrile (72:28 %v/v) is used. The developed method is applicable for routine analysis.

Hoogerheide et al., (1981) have developed a simple stability indicating high performance liquid chromatographic (HPLC) method which separates clotrimazole from impurities and decomposition products in bulk drugs, creams, tablets and solutions. Average recovery data for drug substance added to placebos were, tablet 99.8%, solution 99.5% and cream 100.0%. Average reproducibility's (%RSD) on drug substance and formulations were, drug substance 1.3%, tablets 1.8%, solutions 1.1% and creams 0.6%. The method allows for the simultaneous determination of (o-chlorophenyl) di-phenyl methanol hydrolysis product impurity.

Mauriece Smith and Noel O Nuessle (1981) have developed a reverse phase HPLC method by using a micro-particulate octa-decyl silane (ODS)
column and an acetonitrile: phosphate buffer mobile phase is employed to determine lidocaine in an admixture of lidocaine injection with 5 percent dextrose injection. The method is accurate over the range of 5 to 12.5µg on column, as employed in our laboratories, which corresponds to 2 to 5mg per mL of lidocaine hydrochloride in the admixture solution. It is precise (2.00 to 2.88% coefficient of variation) and has been used in stability studies of lidocaine. The method is also used to quantitate a degradation product of lidocaine, 2, 6-dimethylaniline, over the range of 5 to 25ng on column (corresponding to 2 to 10µg per mL in the admixture solution) with a precision of 1.82 to 4.50% coefficient of variation. Samples are prepared by dilution in methanol containing the internal standard, procaine hydrochloride and each chromatogram requires about 4 minutes.

Wiese et al., (1982) have developed an RP-HPLC method for the determination of chloramphenicol and its monosuccinate ester in piglet plasma. It involves precipitation of plasma proteins by addition of methanol to the plasma sample, followed by injection of the supernate onto the column. Chloramphenicol and its ester are separated using a lichrosorb R-18 column and phosphate buffer pH=4.9 containing 30% methanol as eluent. Determination of chloramphenicol and its monosuccinate ester in pig plasma was made with a precision of 2.6% and 2.4% (relative standard deviation), respectively.

Boer and Pijnenburg (1983) have developed HPLC method for the simultaneous determination of chloramphenicol and its most important
degradation products. A 150mm long C18 reverse phase column was used with a mobile phase consisting of boric acid solution: acetonitrile \((60:45\%v/v)\) adjusted to a pH3.0. The amount of degradation of chloramphenicol eye drop solutions containing boric acid and borax, at pH 4.7 and 7.2 respectively, is determined at 4, 21, 100 and 120°C. The solution of pH7.2 is more stable than that of pH4.7 at 4 and 21°C, but is less stable at 100 and 120°C. Preparation has to be accomplished in subdued light. The solutions have to be protected from light during storage.

Kushida (1983) has developed a method for the simultaneous analysis of chloramphenicol and four antiepileptic drugs (phenobarbital, phenytoin, carbamazepine and primidone) in plasma by high performance liquid chromatography (HPLC). The method involves a preliminary extraction of 0.1mL of plasma with diethyl ether containing phenacetin as an internal standard, chromatography with a reverse phase column with a methanol: water mobile phase and detection by measuring ultraviolet absorbance at 210nm. The method demonstrated sufficient precision, sensitivity and specificity. The recoveries of the drugs were >95% with the exclusion of primidone (80.3%), the maximum within day and day to day coefficients of variation for all drugs were <5%, the lower detection limits were 0.5μg per mL or less for all drugs analyzed and six other antibiotics, phenyl ethyl malondiamide, carbamazepine-10, 11-epoxide, and chloramphenicol esters did not interfere with the analysis. The HPLC method was tested for clinical applicability by analyzing plasma samples from a volunteer who received concurrent single doses of
chloramphenicol, phenobarbital and phenytoin. This method can be used for studying drug interactions between chloramphenicol and antiepileptic drugs and for monitoring the concentrations of these drugs in plasma when administered concurrently to prevent concentration related side effect(s) of each drug.

Kushida et al., (1984) have developed a simultaneous determination of lidocaine and its pharmacologically active metabolites, monoethylglycinexylidide and glycinexylidide, in plasma by high performance liquid chromatography method. By use of a bare (un-bonded) silica gel with aqueous eluents, separations of organic amines such as lidocaine and its metabolites, which are very difficult and have poor peak symmetry on bonded reverse phase packing, were easily accomplished with good peak symmetry. The method is sufficiently precise, sensitive and specific. Analytical recoveries of all compounds were greater than 90%; CVs for reproducibility were less than 5% for all compounds, the lower detection limits were 0.1mg per L or less. This method can be used to monitor the concentrations of these compounds in plasma.

Helle R Angelo et al., (1986) have developed a simple high performance liquid chromatographic method for the determination of disopyramide and lidocaine simultaneously with their dealkylated metabolites. After basic tert-butyl methyl ether extraction and back extraction with phosphoric acid, the drugs and their metabolites were injected into a supelcosil-CN column and the absorbance of the elutent was measured at 215nm. The sensitivity was 0.3μM
per L and the obtained precision, selectivity and stability during storage were adequate for the performed clinical studies in patients therapeutically treated with disopyramide and/or lidocaine.

Morris et al., (1988) have developed a simple and rapid assay of serum chloramphenicol which combines the specificity of the enzyme chloramphenicol acetyl transferase with the convenience of a colorimetric detection system. The assay is linear over the drug concentration range 5-200μM (1.5-65mg per L) and therefore is suitable for detection below and above the therapeutic range (31-62μM, 10-20mg per L with potential toxicity above 75μM, 24mg per L). This method does not detect the microbiologically inactive succinate or palmitate pro drugs of chloramphenicol and evidence suggest that the major metabolite, chloramphenicol glucuronide also is not detected. Good correlation with an HPLC method has been achieved (r=0.9860). The assay is based on a two reagent system with very simple methodology.

Sintov et al., (1989) have developed a sensitive high performance liquid chromatographic (HPLC) assay method using fluorescence detection for quantifying lidocaine levels in plasma (in the ng per mL range). This novel HPLC assay has made possible the simultaneous monitoring of lidocaine levels in coronary and peripheral plasma obtained after myocardial controlled release matrix administration (0.92mg per Kg during 4h) in the arrhythmic dog. The method employed extracts the drug from plasma using 1-chlorobutane and a subsequent derivatization with 9-fluorenylmethylchloroformate in acetonitrile.
at 110°C. The derivative was chromatographed on a C18 reverse phase column and measured with fluorescence detection (excitation 254nm, emission 313nm). N-Methylephedrine was found to be suitable as an internal standard, post derivatization. The derivatization product of lidocaine was identified and characterized by mass spectral analysis. It was found to have a unique and reproducible dicarbamate structure, which was stable for at least three days at room temperature. The method was tested with human plasma as well as on dog plasma. Analytical recoveries were 88.6 +/- 3.6 and 77.4 +/- 3.0% (mean +/- S.E.), respectively, at levels ranging from 25 to 200ng per mL. The lower detection limit was 1.0ng per mL lidocaine. In this rapid method was found to be suitable for the bioavailability pharmacokinetic assessment of lidocaine low dose regional drug administration.

Girault et al., (1991) have developed a new, simple and sensitive assay method for the simultaneous quantitative measurement of beclomethasone dipropionate and its hydrolysis products in human plasma and urine. Beclomethasone 17, 21-dipropionate, beclomethasone 17-monopropionate, beclomethasone and the internal standard, dexamethasone 21-acetate, were measured by combined liquid chromatography and negative ion chemical ionization mass spectrometry with methane as the reagent gas. A particle beam interface from Hewlett Packard was used. Under mild operating conditions, abundant and stable characteristic high mass ions were generated in the ion source of the mass spectrometer by a resonance electron capture mechanism. The fast extraction procedure requires 1.0mL of plasma or urine and the
quantification limit of the method is 1.0 ng per mL for the three tested compounds. The developed method was reproducible and stability indicating.

Osama H Abdelmageed and Pakinaz Y Khashaba (1993) have developed a simple, specific rapid and sensitive spectrophotometric method for the assay of clotrimazole, in bulk drug and its pharmaceutical preparations. This method is based on the ion pair complex reaction of clotrimazole and methyl orange in aqueous methanol and in the presence of citric acid. The chromogen, being extractable with chloroform, could be measured quantitatively at 422 nm. All variables were studied to optimize the reaction conditions. Regression analysis of beer's plot showed good correlation in a general concentration range of 2–14 μg per mL. The proposed method has been successfully applied for the analysis of the bulk drug and its dosage forms such as powder, vaginal tablets, topical solution and creams. No interference was observed from betamethasone dipropionate (Lotriderm cream) or dexamethasone acetate and azidamphenicol (Baycuten cream) or other common pharmaceutical adjuvants. In addition, this method was also found to be specific for the analysis of clotrimazole in the presence of its hydrolytic products as well as imidazole, as a possible impurity.

Lorec et al., (1994) have developed a gas liquid chromatographic method for the simultaneous measurement in serum of bupivacaine, lidocaine and their main metabolites, 2,6-pipecolylxylidide (PPX) and mono ethylglycine xylidide (MEGX), respectively. The procedure involves a one step extraction and injection of the extract into a gas chromatograph equipped with a capillary
column and nitrogen phosphorus detector under constant temperature conditions. Recovery of all components averaged 94%, with the lowest detection limit of 15ng per mL for the four drugs. The precision within series coefficients of variation ranged from 7.7% for bupivacaine, 8.6% for lidocaine, 10.2% for MEGX and 15.8% for PPX. The inter-day coefficients ranged from 0.7 to 6.5%. Concomitant use of caffeine and carbamazepine may interfere with MEGX and bupivacaine determination, respectively.

Kountourellis et al., (1995) have developed a simple and reliable HPLC method for the determination of bampipine, hydrocortisone, dexamethasone, betamethasone and beclomethasone. The procedure, which simultaneously resolves all five compounds, could be employed for the analysis of each component in different pharmaceutical formulations. Chromatographic separation was accomplished under isocratic conditions using a bondapak 10μm, C18 column 250x2.1mm and a mobile phase of acetonitrile: water (48:52 %v/v) and 0.65% acetic acid pumped at a rate of 1.0mL per min. Column effluent was monitored with an UV detector at 251nm. The compounds were eluted in the range from 2.5 to 8.7min. Linearity, reproducibility and recovery (% of labelled amount) were satisfactory for all compounds. The method has been successfully applied to the analysis of ointment, cream, gel and lotion.

Orsi et al., (1995) have developed a HPLC method for the simultaneous determination of beclomethasone dipropionate and its principal degradation products. The only sample treatment necessary for the analysis is its dilution
with methanol. The practical detection limit is 0.02% of each impurity in terms of the parent drug, whereas the absolute limit of detection is 2.5 ng of each injected compound. The method has been applied to the analysis of both pharmaceutical formulations and samples of bulk drug.

Rossi et al., (1997) have developed for the quantitation of lidocaine and its dominant metabolites in rat plasma, 3-hydroxy-lidocaine glucuronide and 3-hydroxy-MEG-X glucuronide. Frozen plasma samples (100-200 μL) were thawed and deproteinated by precipitation with acetonitrile, before the conversion of glucuronidated into their respective hydroxylated forms by acid hydrolysis. After extraction with solid phase C18 cartridge chromatography, the metabolites and parent drug were analyzed by capillary gas chromatography nitrogen phosphorus detection, without derivatization. A detection limit of 0.005 μg per mL for lidocaine and non glucuronidated metabolites and 0.01 μg per mL for glucuronidated metabolites was achieved. The method is specific and accurate.

Kang L et al., (1999) have developed and validated a sensitive and reliable method based on solid phase extraction and reverse phase liquid chromatography method for the quantitation of lidocaine (Lid) in dog plasma. Phenacemide was used as an internal standard (IS) in the extraction which employed C18 solid phase extraction cartridges. The washing and eluting solutions were 2 mL acetonitrile pH 9.0 phosphate buffer (10:90 %v/v) and 0.5 mL acetonitrile pH 4.0 phosphate buffer (40:60 %v/v) respectively. The eluent obtained from the cartridge was directly analyzed on a reverse phase
ODS column with UV detection at 210nm. A clean chromatogram and high sensitivity were achieved at this wavelength. The mobile phase was acetonitrile and pH5.9 phosphate buffer (20:80 %v/v). The retention times were 6.4 and 7.2 min for Lid and IS, respectively, at a flow rate of 1.0 mL per min. The mean absolute recovery was 96.6% (n=9) with a CV of 3.8% for Lid and 81.7% with CV of 2.5% (n=3) for IS. The limit of quantitation was 20 ng per mL, with the intra and inter-day precisions (n=5) of 4.4 and 3.4%, respectively and the intra and inter-day accuracies (n=5) of -4.3 and -5.0%, respectively. For the analyses of Lid in spiked plasma samples at 20, 100 and 200 ng per mL, the overall mean intra and inter-day precisions (n=15) were 3.9 and 4.9%, respectively and the overall mean intra and inter day accuracies (n=15) were -3.7 and -4.6%, respectively. The correlation coefficients for calibration plots in the range 20-1000 ng per mL in plasma were typically higher than 0.998. The suitability of the method was demonstrated by the study in a beagle dog receiving a low intravenous dose of Lid.

Somchart Kanjanawattana et al., (2001) have developed a method for lidocaine detection in dental pulp by high performance liquid chromatography. The amounts of lidocaine in dog pulps were quantitated after local injection to evaluate lidocaine recovery from pulp tissue with this technique. Comparison was also made between the amount of lidocaine found in upper and lower canines. The high performance liquid chromatography system was shown to be a reliable and reproducible tool for lidocaine determination. Lidocaine extraction from the tissue showed recovery of 90%. The amount of lidocaine
recovered from the upper canine (0.21 µg per mg) was higher than the lower canine (0.17 µg per mg).

Nakamura Katsuyuki et al., (2001) have developed a method for the determination of lidocaine and three metabolites. Lidocaine and thee metabolites were extracted simultaneously with CHCl₃ under alkaline condition with NH₄OH (pH9-10). Mobile phase of 15% acetonitrile in 80mM phosphate buffer (pH3.0) was able to separate all the drugs, especially GX from caffeine which closely adjacent each other. Large amount of MEGX and trace amount of GX and 3 hydroxy lidocaine were detected in a body who died by oral lidocaine overdose. In a case of death caused by intravenous lidocaine injection, little metabolites were detected which was considered that the death occurred in a short time after injection.

Markus et al., (2001) have developed an HPLC method for the quantification of oxycodone and lidocaine in a gel matrix. The mobile phase consisted of methanol: water: acetic acid (35:15:1 %v/v) and was delivered at 1.5mL per min through a 4.6×250mm C8 column. Oxycodone was detected at 285nm and lidocaine at 264nm. Linear calibration curves were obtained for oxycodone in the range of 0.05–1.5% (w/w) and for lidocaine in the range of 0.1–5.0% (w/w). Oxycodone and lidocaine were treated with hydrogen peroxide and the oxidation products were readily separated on the column. The method was applied to assess the stability of a gel containing oxycodone hydrochloride (0.3%w/w) and lidocaine (1.5%w/w). The gel was stored under
refrigeration in ready to use syringes and under these conditions oxycodone and lidocaine were stable for at least one year.

Gebauer MG et al., (2001) have developed a method for the quantification of oxycodone and lidocaine in a gel matrix by using HPLC. The mobile phase consisted of methanol: water: acetic acid (35:15:1 %v/v) and was delivered at 1.5mL per min through a 4.6x250mm C8 column. Oxycodone was detected at 285nm and lidocaine at 264nm. Linear calibration curves were obtained for oxycodone in the range of 0.05-1.5% (w/w) and for lidocaine in the range of 0.1-5.0% (w/w). Oxycodone and lidocaine were treated with hydrogen peroxide and the oxidation products were readily separated on the column. The method was applied to assess the stability of a gel containing oxycodone hydrochloride (0.3% w/w) and lidocaine (1.5% w/w).

Frank Streit et al., (2001) have developed a reliable, simple, sensitive and rapid procedure for determining MEGX in serum by LC-MS/MS. This procedure also allows the simultaneous measurement of serum lidocaine concentrations in the same sample. The internal standard was monopropylglycine xylidide (MPGX). To quantify lidocaine, we used a single point calibrator with a final concentration of 1.1mg per L. For sample preparation, 100μL of calibrator, quality control sample or patient sample was vortex mixed with 200μL of methanol containing the internal standard MPGX (50μg per L) polypropylene tubes. After centrifugation for 10min at 4000g, the supernatants were decanted and after re centrifugation for 1.0min, were placed in a Series 200 auto sampler (Perkin Elmer). The column was an oasis HLB
column (2.1x20mm; Waters), temperature maintained at 50°C with oven. The
LC-MS/MS system consisted further of a Series 200 binary pump from Perkin
Elmer, an M480 pump (Dionex) and a six port rheodyne valve. Sample
injection (20µL) was by a series 200 auto injector fitted with a 200µL sample
loop. The column was washed for 1.0min (flow rate, 800µL per min)
with methanol–30mM per L ammonium acetate (20:80 by volume),
followed by a 2.5min elution step (flow rate, 1000µL per min) with methanol:
30mM per L ammonium acetate (75:25% by volume). The column was then re
equilibrated for 0.5min (flow rate, 800µL per min) with methanol: 30mM per L
ammonium acetate (20:80 % by volume) in preparation for the next injection.
In our experience, a single oasis column can be used for 300 injections. For
detection a sciex API 2000 triple quadrupole mass spectrometer with a turbo
ion spray (heated electro spray) interface was used. The analytes that eluted
from the HPLC were introduced into the turbo ion spray source (heated to
450°C) at a split of 1:10. High purity argon was used as the collision gas.
Ionization was achieved in the positive ion mode with an ionization voltage of
2200V, an orifice voltage and collision energy of 19eV and a heater probe
temperature of 450°C. The first quadrupole was set to select the protonated ions
[M+ H+] of MEGX (m/z 207.0), MPGX (m/z 221.2) and lidocaine (m/z 235.2).
The second quadrupole was used as collision chamber. The third
quadrupole was used to select the characteristic product ions of MEGX
(m/z 58.0), MPGX (m/z 72.0) and lidocaine (m/z 86.0). The elution times for
MEGX, MPGX and lidocaine were 1.7, 1.7 and 1.8 min, respectively. The developed analytical procedure is widely applicable and stability indicating.

Eduardo Ricci Junior et al., (2002) have developed a simple high performance liquid chromatography method to determine the assay of lidocaine hydrochloride in aqueous receiving media. Lidocaine hydrochloride was analysed using a 5 mm Lichro CART RP18 column (125x4 mm). The mobile phase was acetonitrile: 0.05 M Sodium phosphate buffer, pH 6.0 (35:65) and 0.05% of diethylamine at a flow rate of 1.0 mL per min. The retention time was 7.9 min. Detection was carried out at 210 nm at room temperature (28°C). The method was found to be linear in the range 1.25 to 25 mg per mL, showing average intra assay and inter assay coefficients of variation below 3.5%. The proposed method was validated for linearity, specificity, precision and accuracy.

Solich et al., (2002) have developed a reverse phase high performance liquid chromatographic (RP-HPLC) methods with UV detection and validated for determination of clotrimazole, methylparaben and propylparaben compounds in a topical cream. The first method describes determination of the active component clotrimazole and two preservatives present in the cream. The second method describes determination of two degradation products of clotrimazole, imidazole and (2-chlorophenyl) di-phenyl methanol, in a topical cream after long term stability tests. Chromatographic separation was on a purospher RP-18e column, the mobile phase in Method-1 for separation of clotrimazole, methyl paraben and propyl paraben comprises acetonitrile and
water (70:30 %v/v). For determination of degradations products imidazole and (2-chlorophenyl) di-phenyl methanol, the optimum composition of mobile phase in Method-2 was acetonitrile and water (75:25 %v/v) apparent pH2.7. Analysis time was <10min for both methods. The methods were found to be applicable for routine analysis of the active compound clotrimazole, preservatives and degradation products in the pharmaceutical product.

Chukwuenweniwe J Eboka et al., (2003) have developed a simple, cheap, fast, accurate, sensitive and precise colorimetric method that can be used for the determination of chloramphenicol. Chloramphenicol was reduced in a mixture of glacial acetic acid and water using Titanium (II!) chloride at room temperature within 10min. The reduced product was then heated for 20min with p-dimethylaminobenzaldehyde to yield the final product whose absorbance was used for the determination of the concentration of chloramphenicol. This method was compared with those obtained with the microbiological assay of chloramphenicol. The final product of the two step reaction was greenish yellow in color absorbed strongly in the visible region and obeyed Beer's law at max=440nm. The method developed was sensitive and accurately determined chloramphenicol in the presence of common excipients and in different dosage forms. There was statistically no significant difference (p<0.05) between the results with the method developed and those obtained with the microbiological assay of chloramphenicol. This method routinely used for the determination of chloramphenicol in bulk drug and in
different dosage forms. The advantage of the method is its speed and simplicity.

Helena Kubala Drincic (2003) has developed method based on matrix solid phase dispersion (MSPD) for the gas chromatographic (GC) determination of chloramphenicol (CAP) residues in animal muscle tissue. Muscle tissue was blended with octa-decyl silyl derivatized silica (C18) column was washed with n-hexane and acetonitrile: water (5: 95 %v/v), after which CAP was eluted with acetonitrile: water (50:50 %v/v) and partitioned into ethyl acetate. The final extract was evaporated and a tri-methyl silyl derivative of CAP was prepared with sylon HTP and detected by GC with an electron capture detector (ECD) and a mass spectrometer. For quantitation, the internal standard used was the meta-isomer of CAP (m-CAP) for GC-ECD. Muscle tissue samples were fortified at three concentration levels. At 5, 10 and 15 µg per kg levels the respective mean recoveries were 93, 96 and 98%. The detection and quantitation limits with ECD were 1.6 and 4.0 µg per kg, respectively. No statistically significant difference was observed in the efficiency of CAP extraction from muscle tissue of various animals (bovine, porcine and poultry) by the MSPD technique.

Koitabashi Motoko et al., (2003) have developed a novel method for determining lidocaine using a flow injection analysis system with electrochemical detection (FIA-ECD). The detection was based on the voltammetric oxidation prepeak of α-tocopherol, due to lidocaine, where the prepeak current was proportional to the concentration of lidocaine. Using FIA-
ECD, the peak current height, which was proportional to the amount of lidocaine, ranged from 11.7ng to 585ng (r=0.998). Lidocaine was determined ten times with a relative standard deviation (RSD) of 1.9%. The detection limit (S/N=3) was 11.7ng. Both analytical results were essentially identical. The presented method is useful for the determination of lidocaine, because it requires only one hundredth of the amount of lidocaine and one twentieth of the measuring time and produces less waste compared to the titration method.

Wiberg Kent et al., (2003) have developed a new method for the rapid determination of lidocaine in pharmaceutical solutions. A conventional HPLC system with a diode array detector (DAD) was used with no chromatographic column connected. As eluent, purified water (Milli Q) was used. The pump and autosampler of the HPLC system were mainly utilized as an automatic and convenient way of introducing the sample into the DAD. The method was tested on the local anesthetic compound lidocaine. The UV spectrum (245-290nm) from the samples analyzed in the detector was used for multivariate calibration for the determination of lidocaine solutions. The content was determined with PLS regression. This comparison of the two methods was done twice on different occasions. The results show that in respect of accuracy, precision and repeatability the new method is comparable to the reference method. The main advantages compared with liquid chromatography are the much shorter time of analysis (<30s) as well as the automatic and simple analytical procedure and the low consumption of organic solvents.
Fuyu Guan et al., (2003) have developed a method involving the use of a liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), for the detection of the analytes in equine samples. Ammonium formate or acetate buffer and acetonitrile added to LC mobile phase and the analytes analyzed with electro spray ionization (ESI) and atmospheric pressure chemical ionization conditions, helium used as a damping gas. The developed method is simple, reproducible and accurate.

Abdel Moety et al., (2003) have developed high performance liquid chromatographic technique for the determination of clotrimazole (CZ), ketoconazole (KZ) and fluconazole (FZ), in pure forms and in pharmaceutical formulations. The proposed HPLC method can be successfully applied as a stability indicating method for the determination of CZ in presence of its acid degradation products [(2-chlorophenyl) di-phenyl methanol and imidazole], the analyzed drugs were separated on a reversed phase column [bondapak C18 (10μm, 250mm×4.6mm)] using a mobile phase containing acetonitrile: 25mM tris-hydroxy methyl amino methane in phosphate butter (pH7.0) ratio of 55:45 (%v/v), with UV detection at 260nm. The differences in the retention times of the three azoles permit their use as internal standard for each other. In addition, a coupled TLC densitometric method has been also applied as a stability indicating method to separate and quantify CZ alone or in presence of byproducts, impurities and/or its acid degradation products. The TLC fractionation was performed on a pre-coated silica gel F$_{254}$ plates using a solvent system consisting of chloroform: acetone: ammonia (25%) (7:1:0.1
%v/v), CZ was well separated from its acid degradation products and quantified by densitometric scanning at 260nm so the developed method is widely applicable for analysis.

Malenovic Andjelija et al., (2004) have developed an RP-HPLC method for the optimal separation of lidocaine, cetrimonium bromide and color in the pharmaceutical pellets. The selectivity factor values defined the optimal conditions, which were continued by analyzing the appropriate mathematical models with the aid of the response surface methodology (RSM) it was possible to anticipate to a certain degree and precisely select optimal experimental conditions. Separations were performed on a ODS 4.6x150mm, 5μm particle size column. Samples were introduced through an injector valve with a 20μL sample loop. UV detection was performed at 208nm. The optimization was performed within the pH range from 2.0 to 5.5, temperature range from 20°C to 55°C and composition of the mobile phase acetonitrile: water from 15:85 %v/v to 35:65 %v/v. The developed method is applicable for lidocaine and cetrimonium bromide in pharmaceutical preparations.

Jadwiga Piwowarska et al., (2004) have developed a high performance liquid chromatographic method for the determination of lidocaine (2-(dietyloamino)-N-(2, 6-dimetylofenylo) acetamid) and its metabolite monoethylglycine xylidide (MEGX), in human serum containing various concentration of bilirubin. Lidocaine and its metabolite were extracted from human serum using dichloromethane. After separation of the layers and freezing at 32 °C, the organic layer was decanted and evaporated under a
stream of nitrogen. The sample was dissolved in the mobile phase (12% acetonitrile in 15mM Potassium di-hydrogen orthophosphate, pH3.0) and after separation on a Supelcosil LC8-DB column; the analytes were measured by ultraviolet detection at 205nm. Trimethoprim (TMP) was used as the internal standard. The recovery of the examined analytes ranged from 95.7 to 97.9% for lidocaine and from 98.0 to 99.9% for MEGX. The lower limit of quantification (LLOQ) was established at 200µg per L for lidocaine and at 10µg per L for MEGX. The choice of suitable conditions for chromatographic separation of lidocaine and its metabolite MEGX allowed the elimination of the influence of endogenous bilirubin on the result of analysis.

Gupta Abhishek et al., (2004) have developed a sensitive and rapid, on line reversed phase high performance liquid chromatographic method for quantitation of compounds at low concentrations in pressurized metered dose inhaler (MDI) systems. Traditional methods for the quantitation of compounds in MDI formulations involve the opening of the MDI vial along with sample dilution prior to quantitation. The new method reported in this study, involves a direct injection from the MDI vial into the needle injector port of a manual injector. Since there is no dilution step involved, this method can be used to quantitate low concentrations of compounds in MDIs with excellent precision. In addition, since the method requires a small injection volume of 5µL, repeated analyses can be performed in order to generate multiple data points using the same MDI vial. Validation of the method was performed using ethanol-1, 11, 2-tetrafluoroethane (134a)-based MDIs. Beclomethasone
dipropionate (BDP), a corticosteroid used for the treatment of asthma, was used as a model compound. Phase separation studies were conducted to investigate the miscibility of the ethanol-134a mixtures with different mobile phase solvent compositions. For the MDI systems in this study, an acetonitrile water (90:10, %v/v) mobile phase at a flow rate of 0.9mL per min was found to give acceptable chromatography for BDP on a apollo C18 5μm, 150mmx4.6mm column (alltech associates, Deerfield, IL, USA). Ultraviolet detection was done at 240nm and the retention time of BDP was 2.7min. The on line HPLC method was characterized to be accurate, precise, sensitive and specific.

Sri Venkata et al., (2004) have developed a utility of near infrared spectroscopy (NIRS) for quantitative analysis of a model drug in hot melt extruded film formulations. Polyethylene oxide (PEO) films with clotrimazole (CT) as a model drug were prepared by hot melt extrusion (HME) incorporating drug concentrations ranging from 0–20% and analyzed using a fourier transform near infrared (FT-NIR) spectrophotometer in the reflectance mode. High performance liquid chromatography (HPLC) was the reference method used for this study. The NIR calibration model derived for CT was composed of 21 frequency ranges that were correlated to the values quantified using the HPLC reference method. The FT-NIR method developed resulted in an assayed CT amount in the film matrix to be within 3.5% of the quantity determined by the reference method. These studies clearly demonstrate that FT-NIR is a powerful method for the quantitation of active drug substances contained in films produced by HME.
Prodduturi et al., (2004) have developed and validated a stability indicating HPLC method for the determination of clotrimazole (CT), an antifungal agent and its degradation products, (o-chlorophenyl) diphenyl methanol (CPDM) and imidazole (IMZ), in hot-melt extruded films. This method utilized a solid phase extraction of clotrimazole from the polymeric films using 100% methanol. HPLC separation of clotrimazole and its degradants were carried out on waters novapak phenyl column (150x4.6mm, 3μm) at a flow rate of 1.0mL per min with an analysis time of 8minutes. Compounds were eluted using a mobile phase of methanol: 25mM dibasic Potassium phosphate, 75:25 %v/v, pH7.0. The analyte was detected using a waters 2996 photo diode array detector (PDA). The mean extraction recovery was 99.8±2.3%. No interference was observed from any of the components of the matrix. A very good separation of clotrimazole and its degradation products, CPDM and IMZ was achieved (elution time was 2.0min for IMZ, 4.2min for CPDM and 6.8min for CT) indicating the specificity of the method. The detector response was linear in the range of 0.1-500μg per mL (R2=0.999) for clotrimazole and 0.01-200 (R2=0.989) for CPDM. Limit of detection and quantitation for CT were calculated to be 0.01 and 0.1μg per mL, respectively. Excellent intra-day and inter-day assay precision (<3% CV) and accuracy (<5% diff.) were observed over a linear range of 1-300μg per mL. A stability indicating validated method, which was robust, sensitive, specific, accurate and reliable, was developed. Good separation between the drug and its degradants was achieved using a specific column (with phenyl end capping) based on the
structure of the degradation product. The method has been used to quantitate clotrimazole and its degradants in hot melt extruded polymeric films

Sherry K Cox et al., (2005) have developed a simple, accurate and sensitive HPLC method for the determination of mono ethyl glycine xylidide (MEGX) and lidocaine in porcine microsome. Lidocaine and MEGX were measured by direct injection after the addition of the internal standard. Chromatography was performed on a µBondapak C18 column using an isocratic mobile phase of 0.03M Potassium dihydrogen phosphate: acetonitrile (87:13 %v/v), pH5.9. UV absorbance was measured at 205nm. The procedure produced linear curves for the concentration range 50–1000ng per mL with a limit of detection of 10ng per mL. Recoveries for both compounds were greater than 90%. This assay produced accurate and repeatable results.

Karlaganis and Bircher (2005) have developed a method to measure bioavailability of lidocaine by simultaneous peroral and intravenous dosing. Lidocaine hydrochloride corresponding to 125mg base was given per orally. Simultaneously, 30mg of deuterated lidocaine-d₃ were injected intravenously. Blood samples were taken at intervals for 270min. Plasma samples were spiked with mepivacaine hydrochloride as internal standard, alkalinized to pH11.7 and extracted with diethyl ether. The extracts were analysed by capillary GC ammonia CI MS using a 15m×0.32mm glass capillary column coated with SE-54. The ion source pressure was 0.4Torr of ammonia as reagent gas. Quasi molecular ions were monitored at m/z 235, 238 and 247 for lidocaine, lidocaine-d₃ and mepivacaine, respectively. Calibration curves were linear from
0.2 to 5.0nM lidocaine per mL plasma. Inter-day reproducibility of this method was 6.9% for lidocaine-d₃ (n = 16; 1.90±0.13nM per mL). Bioavailability of lidocaine in five normal volunteers ranged from 26 to 36% (mean 31±SD 5%) and in a cirrhotic with an end to side portacaval shunt it approached 100%, as anticipated. The method is well suited for determination of bioavailability of lidocaine after simultaneous administration of rather small and safe doses both intravenously and per orally.

Mukasa T Bagonluri et al., (2005) have developed a sensitive liquid chromatographic (LC) method with UV detection for the determination of residues of lidocaine (LID) and its major metabolite, mono ethyl glycine xylidide (MEGX). Inertsil ODS-3 (3.0×250mm, 5µm) column is used for analysis. Isocratic mobile phase made up of 0.05M phosphate buffer (pH4.0): acetonitrile (88:12 %v/v) at a flow rate of 1.0mL per min. The limits of quantification for LID and its major metabolite, MEGX were 10 and 20ng per g, respectively. The method was validated and used to measure the concentration of residues of LID and MEGX in elk velvet antlers harvested after either LID anesthesia or application of a drug free control method.

Malenovic et al., (2005) have developed a simple and rapid RP-HPLC method for the simultaneous determination of lidocaine and cetrimonium bromide in the presence of pellet color corrigent. Separations were performed on a beckman ultrasphere ODS 4.6mmx150mm, 5µm particle column at 40°C. The mobile phase consisted of water phase and acetonitrile (72:28 %v/v), pH value of the mobile phase was adjusted to 2.0 with 85% ortho phosphoric acid.
Bisacodil was used as an internal standard. The flow rate was 1.0 mL per min and UV detection was performed at 208 nm. The proposed RP-HPLC method was validated. According to the obtained results, the developed method was found to be suitable and accurate for the determination of these drugs in commercial formulations.

Mira Cakar et al., (2005) have developed simple and reliable thin layer chromatography densitometry methods for determination of anti-mycotics (bifonazole, clotrimazole and miconazole) and preservatives (benzyl alcohol and benzoic acid). The following mobile phases were used: ethyl acetate: n-heptane: methanol: di-ethylamine (3:4.5:1:0.2 %v/v) for bifonazole and benzyl alcohol; n-butyl acetate: n-heptane: methanol: di-ethylamine (3:4.5:1:0.2 %v/v) for clotrimazole and benzyl alcohol and n-butyl acetate: carbon tetrachloride: methanol: diethylamine (3:6:2.5:0.5 %v/v) for miconazole and benzoic acid. The chromatographic zones on silica gel plates were scanned in the reflectance/absorbance mode at 230 nm (bifonazole, benzyl alcohol, miconazole and benzoic acid) and 210 nm (clotrimazole and benzyl alcohol). The recovery for all substances ranged from 98.7 to 100.7%. The limits of detection and quantitation were 0.03 to 0.2 μg and 0.1 to 0.5 μg per spot, respectively. The proposed methods were applied for determination of antimycotics and preservatives in commercially available pharmaceuticals.

Dalibor Satinsky et al., (2006) have developed a new separation method based on a reverse phase sequential injection chromatography (SIC) technique for simultaneous determination of chloramphenicol and
betamethasone in pharmaceutical eye drops. A short monolithic column coupled with a sequential injection analysis (SIA) system enabled separation of two compounds in one step. A Chromolith Flash RP-18e, 250×4.6mm column with a 5mm pre-column (Merck, Germany) and a FIAlab 3000 system (USA) with a 6-port selection valve and 5mL syringe were used for sequential injection chromatographic separations in this study. The mobile phase used was acetonitrile: water (30: 80 %v/v), flow rate 0.48mL per min; UV detection was at two wavelengths 241 and 278nm (absorption maxima of betamethasone and chloramphenicol, respectively). The basic validation parameters showed good results, linearity of determination for both compounds including internal standard (propylparaben) >0.999, repeatability of determination (percent (%) RSD) in the range 0.8-1.7% at two different concentration levels and detection limits in the range 0.5-1.0mg per mL. The chromatographic resolution between compound peaks was greater than 2.1 and the analysis time was less than 8min under optimal conditions. The developed sequential injection chromatography method was compared with the HPLC method and was found to be applicable for routine analysis of active compounds in pharmaceutical preparations.

Pan et al., (2006) have developed a HPLC method with a monolithic column and detection by mass spectrometry for determination of chloramphenicol in honey samples. Honey samples were dissolved in water containing sodium chloride and extracted with acetonitrile. Chloramphenicol (CAP) residues at ppb concentrations were detected by liquid chromatography mass spectrometry (LC-MS), with electro spray ionization, in negative ion
mode. The mobile phase was methanol 0.2% aqueous ammonium acetate solution, 45:55 (%v/v). Under these conditions CAP is eluted after 4 min and 8 min from a Merck RP-18e monolithic column and a conventional C18 column, respectively. Recovery at three fortification levels (0.2, 20 and 200 μg per Kg) was in the range 78–93% with RSD from 3.7 to 3.9%. The coefficient of determination, correlation coefficient was 0.9995 over a 0.1–100 μg per L linear range. This method used for determination of CAP in honey. The method was fit for the purpose of monitoring commercial products.

Vytautas Tamosiunas et al., (2006) have developed a high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) method for the determination of chloramphenicol (CAP) in milk products. Mass spectral acquisition was done using electro spray ionization in the negative ion mode applying multiple reactions monitoring of two diagnostic transition reactions for CAP (m/z 321→152 and m/z 321→257). Milk samples were extracted with ethyl acetate and evaporated to dryness, followed by a cleanup step using the liquid-liquid extraction with carbon tetrachloride: hexane (1:1 %v/v) mixture. The calibration curve showed a good linearity in the concentration range from 0.02 to 1.0 μg per kg with the correlation coefficient above 0.995. The method gave a decision limit and a detection capability of 0.050 and 0.066 μg per kg, respectively. The mean recoveries of CAP from the milk samples spiked at 0.1–0.45 μg per kg were in the range of 86–92%. The applicability of this technique was demonstrated by analysis of milk products.
Renata Hajkova et al., (2007) have developed and validated a novel simple isocratic HPLC method with UV detection for the determination of three compounds in spray solution (active component clotrimazole and two degradation products imidazole and (2-chlorophenyl) di-phenyl methanol) using ibuprofen as an internal standard, 3.5μm zorbax stable bond (SB)-Phenyl column (75mm×4.6mm). The optimal mobile phase is used for separation of clotrimazole and degradation products and imidazole, (2-chlorophenyl) di-phenyl methanol and ibuprofen (internal standard). Mobile phase consists of a mixture of acetonitrile and water (65:35 %v/v) with pH 3.5 with phosphoric acid. At a flow rate of 0.5mL per min and detection at 210nm, the total time of analysis was less than 6min. The method was applied for routine analysis (batch analysis and stability tests) in commercial spray solution.

Vikas V Vaidya et al., (2007) have developed and validated an HPTLC method for simultaneous determination of clotrimazole and tinidazole in a pharmaceutical formulation. The analytes were separated on silica gel 60F_{254} HPTLC plates with toluene: ethyl acetate: methanol: glacial acetic acid, 6.0:3.0:1.0:0.3 (%v/v) as mobile phase, after chamber saturation for 10min. The development distance was 8cm. The plate was then dried in air and scanned and quantified at λ=254nm. Response to clotrimazole and tinidazole was a linear function of concentration in the ranges 120 to 320μg per mL and 300 to 800μg per mL, respectively. The limits of detection for clotrimazole and tinidazole were 20 and 60μg per mL, respectively. The respective limits of
quantification were 120 and 200µg per mL. The method enables accurate, precise and rapid simultaneous analysis of clotrimazole and tinidazole.

Tomasz Sniegocki et al., (2007) have developed different analytical methods based on gas chromatography–mass spectrometry (GC–MS/MS) and liquid chromatography–mass spectrometry (LC–MS/MS) for the determination of chloramphenicol (CAP) in milk. GC-MS/MS has performed in negative chemical ionization (NCI) mode by monitoring the transitions m/z 466→304, 466→322 and the results were compared with LC–MS/MS in electro spray mode by monitoring the transitions of all the selected ions m/z 321→152, 321→257. The decision limit (CCα) for CAP determination by LC–MS/MS was established at a level of 0.11µg per Kg, while the corresponding value for GC–MS/MS was 0.083µg per kg. Detection capability (CCβ) for CAP by LC–MS/MS was 0.15µg per kg and for GC–MS/MS was 0.14µg per Kg. As it was found, both methods are useful for CA determination in milk. Helium and methane used as carrier gas. Chromatographic separation was performed in capillary column DB-1MS (30m x 0.25µm x 0.25mm, J&W Scientific). The injection and transfer line were kept at 280°C. The gas chromatography oven was programmed from 110°C to 200°C at the rate of 20°C per min and subsequently to 241°C at 8°C per min and finally to 280°C at 20°C per min and left to cool for 5min. Chromatographic separation was performed in the column luna C8 (150mmx2mm, 3µm) (Phenomenex). The mobile phase for LC analysis consisted of 5mM ammonium formate (A) and acetonitrile (B). The gradient mobile phase was pumped through the analytical column with the
gradient programmed. Separation of the analytes was accomplished at a flow of 0.2mL per min at an ambient temperature. Instrument API 3000 was operated in the electro spray negative ion mode m/z 321 as a precursor ion and ions m/z 152 and 257 as transition products for chloramphenicol.

Dhananjay Meshram et al., (2008) have developed a normal phase TLC method with densitometric detection at 220nm, for analysis of clotrimazole and metronidazole in combined dose tablets and cream. The compounds were separated on silica gel plates with toluene: ethyl acetate: methanol: acetic acid 5.5:2:0.6:0.1 %v/v as mobile phase. Regression analysis showed response was a linear function of the amounts of the drugs in the ranges 0.4–0.8 and 2–4μg per band for clotrimazole and metronidazole, respectively. Quantification was achieved by measurement of peak area and comparison with calibration standards. The method was successfully applied to pharmaceutical formulations. There was no chromatographic interference from common excipients present in the tablets and cream. The method was validated for accuracy, precision and specificity.

Dominic Storzinger et al., (2008) have developed and validated a sensitive and selective high performance liquid chromatography method for the precise determination of posaconazole and the internal standard clotrimazole in human plasma. Samples have extracted using solid phase extraction and separated on a reverse phase C8 column (150×4.6mm, 5μm) using phosphate buffer (pH6.7, 0.04M): acetonitrile: methanol (43:49:8 %v/v) as mobile phase. UV detection has performed at 260nm. This method showed that a lower limit
of quantification was 50ng per mL and the limit of detection 3ng per mL. Linearity was tested in the range from 50 to 5000ng per mL ($r^2=0.9998$), mean recovery was 86%. The method proved to be a useful tool for therapeutic drug monitoring and it is specific, precise and showed excellent reproducibility as well as a favorable accuracy.

Bahia Abbas Mousa et al., (2008) have proposed two chromatographic methods for the determination of some anti fungal drugs in the presence of either their degradation products or cortisone derivatives. The densitometric method determined mixtures of each of ketoconazole (KT), clotrimazole (CL), miconazole nitrate (MN) and econazole nitrate (EN) with the degradation products of each one. Mixtures of MN with hydrocortisone (HC) and EN with triamcinolone acetonide (TA) were also successfully separated and determined by this technique. For KT and CL, a mixture of methanol: water: triethylamine (70: 28:2 %v/v) was used as a developing system and the spots were scanned at 243nm and 220nm for KT and CL, respectively. For MN and EN, a mixture of hexane: isopropyl alcohol: triethylamine (80:17:3 %v/v) was used as a developing system and the spots were scanned at 225nm for both drugs. The HPLC method determined mixtures of CL or EN with their degradation products which were separated and quantified on a zorbax C8 column. Elution was carried out using methanol: phosphate buffer pH2.5 (65:35 %v/v) as a mobile phase at a flow rate of 1.5mL per min and UV detection at 220nm for CL and EN, a mixture of methanol: water containing 0.06mL triethylamine pH10.0 (75: 25 %v/v) was used as a mobile phase at a flow rate of 1.5mL per
min and UV detection at 225nm. The methods were also used to separate mixtures of CL with betamethasone dipropionate (BD) and EN with TA in a laboratory prepared mixture and in pharmaceutical preparations. The methods were sensitive, precise and applicable for determination of the drugs in pharmaceutical dosage forms.

Parul Parmar and Ankita Mehta (2009) have developed and validated a simple, precise, accurate and rapid high performance thin layer chromatographic method for the determination of clotrimazole in bulk drug and tablet dosage form. The stationary phase used was pre-coated silica gel 60F_{254}. The mobile phase used was a mixture of cyclo-hexane: toluene: methanol: tri-ethylamine (8:2:0.5:0.2 %v/v). The detection of spot was carried out at 262nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 200 to 1000ng per spot for clotrimazole. The limit of detection and the limit of quantification for clotrimazole were found to be 50ng per spot and 200ng per spot, respectively. The proposed method can be successfully used to determine the drug content in bulk drug and tablet formulation.

Ankam et al., (2009) have developed a specific, accurate and precise reverse phase high performance liquid chromatographic method for the simultaneous determination of butenafine hydrochloride and betamethasone in cream formulation. The determination was carried out on licrocart licrosphere RP select B (250x4.6mm, 5μm) column in isocratic mode, the mobile phase consisting of 50mM ammonium acetate buffer and acetonitrile in the ratio of
60:40 %v/v, pH 4.5 ±0.1 with glacial acetic acid. The flow rate was 2.0mL per min and eluent was monitored at 254nm. The retention times of butenafine hydrochloride and betamethasone were 4.7min and 7.7min, respectively. The resolution factor was greater than 4.0, linearity of butenafine hydrochloride and betamethasone were in the range of 100-300μg per mL and 5-15μg per mL, respectively. The proposed method is also found to be precise and robust for the simultaneous determination of butenafine hydrochloride and betamethasone in cream formulation.

Madhusudhana Reddy et al., (2009) have developed a common extraction procedure for the isolation of acidic, basic and neutral drugs from urine samples. Total of 28 doping drugs were analyzed on API 3200 Triple quadrupole mass spectrometer using C18 column in atmospheric pressure electro spray ionization. The mobile phase composition was a mixture of 1% formic acid and acetonitrile with gradient time period. The method developed was very sensitive for detection of 28 doping agents. The linearity was performed for each drug and the total recovery percentage ranged from 57 to 114. Limit of detection is found to be 0.5ng per mL for carboxy finasteride and 1-5ng per mL for other drugs. The method was successfully used to detect positive urine samples of 3-OH stanozolol, methyl phenidate, mesocarb, clomiphene metabolite and carboxy finasteride. The method developed based on controlled pH extraction method and HPLC mass spectrometry analysis allowed better identification and confirmation of gluco corticosteroids and a
few other drugs in different categories. The validated method has been used for regular analysis.

Wang Shu Dong et al., (2010) have developed a quantitative determination method for lidocaine hydrochloride and norfloxacin in chitosan hemostatic sponge by RP-HPLC. The determination was carried out with ODS-3 C18 column (4.6mm×250mm, 5μm). The mobile phase consisted of acetonitrile: 1% phosphoric acid solution (12:88 %v/v), with pH adjusted to 3.0±0.1 by using triethylamine. Detecting wavelength was at 254nm. The linear range for lidocaine hydrochloride and norfloxacin was both 0.025-0.100mg per mL. The average recovery and percent (%) RSD of norfloxacin were 98.84% and 1.72% and those of lidocaine hydrochloride were 100.24% and 1.86%. The method was simple instrumental operation, good stability and high accuracy.

Biljana Jancic Stojanovic et al., (2010) have optimized and validated an RP-HPLC method for the simultaneous determination of hydrocortisone acetate and of lidocaine in suppositories. For method optimization, response surface methodology was applied and the obtained model was tested using analysis of variance. The optimal separations were conducted on a beckman coulter 150×4.6mm 5μm, column at 20°C. The mobile phase was methanol: water (65:35 v/v), pH adjusted to 2.5 with 85% orthophosphoric acid, with a flow rate of 1.0mL per min. UV detection was performed at 250nm. Phenobarbital was used as an internal standard. The method was validated for selectivity, linearity, precision and robustness.
Morteza Moradi et al., (2010) have developed a method for the determination of clotrimazole in anti fungal drugs. Chromatographic data were recorded and analyzed using chromana software (version 3.6.4). An ODS-3 column (150mm×4.6mm, 3μm) was applied to separate the drugs under isocratic elution conditions. A mixture of acetate buffer (pH 4.5) and acetonitrile (25:75 %v/v) at a flow rate of 0.9mL per min was used as a mobile phase and the analytes were detected at 230nm. The linearity of the proposed method was very good with the correlation of determination (r²) being greater than 0.99 (n=9). The LOD based on S/N=3 were reported at the range of 0.9–4.0μg per L for different drugs. The pre concentration factors were determined as 133, 127.9 and 71 for KZ, CZ and MZ, respectively. The extraction percentage values were in the range of 17–32%. The intra-day and inter-day RSD for the drugs were less than 6.2% and 12.1%, respectively. The term inter-day percent (%) RSD refers to relative standard deviations of replicate measurements during several days while, the term intra-day percent (%) RSD refers to relative standard deviation of replicate measurements during one day. It is clear that inter-day RSDs are higher than intra-day RSD. Three phase hollow fiber liquid phase micro extraction was used to obtain high clean up and good pre concentration factors for the extraction of ketoconazole, clotrimazole and miconazole. Due to simplicity and low cost of the extraction device, the hollow fiber can be discarded after each extraction to avoid carry over cross contamination. This serves to maintain reasonable intra-day and inter-day percent (%) RSD. The developed method was applied for the extraction of
target drugs from the tap water, plasma and urine samples. Accordingly, it was concluded that the method is applicable for HPLC analysis.

II. f. Antiseptic solution (composed of chloroxylenol-4.8% w/v and terpineol-9.0% v/v)

Chloroxylenol is commonly used in antibacterial soaps, antiseptic solutions. Previous studies have shown a low antimicrobial activity which is enhanced by additives. Its antibacterial action is due to disruption of cell membrane potentials. It is not significantly toxic to humans and other mammals but is toxic to fish. Terpineol is a naturally occurring monoterpene alcohol. It has a pleasant odor similar to lilac and is a common ingredient in perfumes, cosmetics and flavors. α-terpineol is one of the two most abundant aroma constituents of lapsang souchong tea (Shan-Shan Yao, 2005).

Theresa et al., (1985) have developed a general high-performance liquid chromatographic profiling and separation method for the estimation of essential oils, especially those rich in mono and sesquiterpene. This method was applied to the semi-preparative fractionation of several types of lime oil. The oils were fractionated on three different silica columns in tandem using a mobile phase of 8% ethyl acetate in hexane: methylene chloride (1:1), with refractive index detection. Fractions were collected, concentrated and analyzed directly by capillary gas chromatography-mass spectrometry. The method was simple, reproducible, easily scaled up and requires no sample work-up. The results clearly demonstrate that normal phase high performance liquid chromatography
is valuable both as a profiling technique and as a pre-fractionation procedure prior to gas chromatographic-mass spectrometric identification. Twenty three new constituents were tentatively identified in cold pressed lime oil using this technique. At least 40 yet unidentified new constituents, including many sesquiterpene alcohols, were well resolved by gas chromatography-mass spectrometry of the high-performance liquid chromatographic fractions.

Gatti et al., (1997) have developed a pre-column derivatization HPLC method for the separation of chlorophenols. The compound reacts (50 min at 110°C) with 2- and 4-chlorophenols to give fluorescent ethers that can be separated by reversed-phase HPLC and detected at $\lambda_{\text{exc}}$ = 360 nm, $\lambda_{\text{em}}$ = 500 nm. The experimental conditions for derivatization and chromatographic separation are discussed. Applications for the determination of chlorocresol (4-chloro-3-cresol) and chloroxylenol (4-chloro-3, 5-xylenol) in pharmaceutical formulations (creams, ointments) are described.

Gatti et al., (2001) have proposed a RP-HPLC method for the analysis of phenol, thymol, chlorocresol and chloroxylenol in commercial pharmaceutical dosage forms. The use of 1-fluoro-2, 4-dinitrobenzene as UV labelling reagent in pre-column derivatization has been investigated. The compound reacts rapidly (2 min) under mild conditions (ambient temperature or 40°C) with phenols to give ethers that can be separated by RP-HPLC and detected at 292 nm. The other procedure involves a post-column on-line photochemical conversion. A photoreactor was arranged between the analytical column and the fluorescence ($\lambda_{\text{ex}}$ = 270 nm, $\lambda_{\text{em}}$ = 310 nm) and UV-diode array ($\lambda$ = 270 nm) detector to enhance the
performance of the methods. The methods showed good selectivity and sensitivity.

Liang M et al., (2002) have developed a method for the separation and determination of terpineol oil by temperature programming capillary gas chromatography. An OV-1 fused silica capillary column (30mx0.32mm, 0.25µm) was used with a temperature increase rates of 1.5°C per min from 70°C to 100°C, 5°C per min from 100°C to 160°C, 10°C per min from 160°C to 220°C and then hold for 2min. The main chemical components and their relative contents of the terpineol oil, terpinene oil, red and yellow oils of middle oils in the terpineol production and natural terpineol were analyzed by capillary gas chromatography-Fourier transform infrared spectrometry (GC-FTIR) and gas chromatography-mass spectrometry (GC-MS). The typical chromatograms of these oils are given. It has offered the information of the boiling points of the chemical compounds and it is helpful to reutilize them after their separation by rectification. The results showed that the main compounds of the heavy cut of terpineol were longifolene and beta-caryophyllene. They are from the raw material of terpinene oil. The main compounds in the natural terpineol oil were eucalyptol, trans-4-thujanol, p-isopropenyl toluene, cis-4-thujanol, linalool, camphor, borneol, 4-terpineol, alpha-terpineol and safrole. The contents of beta-terpineol and gamma-terpineol were not as in synthetic terpineol.

Uzi Ravid et al., (2006) have developed a natural enantiomerically pure (4R) (+)-α-terpineol was detected using a permethylated β-cyclodextrin chiral capillary GC column. For the first time, in the essential oil of micromeria
fruticosa (L) Druce high enantiomeric purities of the (+)-enantiomer (78-88%) were detected in seven other oils. Relatively high enantiomeric purities of (4S) (-)-α-terpineol (80%) were detected in the oils of cinnamon and laurus nobilis L lower enantiomeric purities of the (-)-enantiomer (64-69%) were detected in another four oils.

Anantha kumar et al., (2006) have developed a TLC method for glycosidically bound volatile compounds of nutmeg have identified as glycoconjugates of p-cymene-7-ol, eugenol, methoxyeugenol and alpha-terpineol. Using phenyl-beta-glucoside as external standard the contents of these glycosidic precursors were estimated based on the measurement of TLC spot density on a densitometer. p-cymene-7-ol rutinoside was the major aroma glycoside (3.15mg per 100g), followed by glucosides of methoxyeugenol (0.61mg per 100g), eugenol (0.50mg per 100g) and alpha-terpineol (0.51mg per 100g). A dose-dependent breakdown of these glycosidic precursors was observed during gamma-radiation processing. Among the four glycosides, alpha-terpineol glucoside was the most sensitive to radiation while p-cymene-7-ol rutinoside was the least sensitive. A reduction in the content of total glycosides by almost 50% was noted at a dose of 5kGy. Partitioning of aroma glycoside into n-butanol from aqueous extracts was found to result in rapid isolation of aroma glycosides, avoiding time consuming pre-purification on amberlite XAD-2 column. A routine method based on extraction into n-butanol and subsequent quantification of post-irradiation changes in aroma glycosides on a TLC plate using a densitometer was proposed.
Marion Kamphoff et al., (2007) have developed an application method for volatile substances by solid-phase dynamic extraction (SPDE) method. In this study, the influence of extraction and desorption parameters of an SPDE-GC method for the determination of the d-limonene degradation products, namely, α-terpineol, (-)-carveol and (S)-carvone, in an aqueous model system was evaluated using a factorial fractional design. The aim was to reduce the number of factors that should be considered for the optimization of an SPDE procedure for different applications. It could be shown that the extraction efficiency of α-terpineol, (-)-carveol and (S)-carvone is significantly influenced by the extraction parameters incubation temperature, number of extraction strokes and amount of added NaCl. All 3 parameters have a positive effect on the extraction and determination of the examined d-limonene degradation products. Due to the identification of significant factors on the basis of an experimental design, the results of this study can be very useful for further development of SPDE methods for different applications.

Roland JW Meesters et al., (2008) have developed a method for the determination of the monoterpenic alcohols verbenol, myrtenol, perillyl alcohol, α-terpineol, Δ3-carene-10-ol, thymol and p-α, α-trimethyl benzylalcohol in urine samples. After an enzymatic cleavage of their glucuronide and sulfate conjugates the monoterpenic alcohols were converted in the urine matrix with 7-diethylaminocoumarin-3-carbonylazide into monoterpen-[7-(diethylamino)coumarin-3-yl]-carbamate derivates prior to analyses. Enrichment of the monoterpenic alcohols from the urine matrix was achieved by online solid
phase extraction (SPE) with restricted-access material (RAM). After removal of excess derivatization reagent and urine matrix components, monoterpene derivatives were separated by HPLC in combination with fluorescence (FLD) detection and simultaneous mass spectrometric (MS) identification. Detection limits (LOD) for studied monoterpene alcohols ranged between 22 and 197ng per L. The method was validated and successfully applied to urine samples from human subjects orally exposed to monoterpenes through an intake of cough medication containing monoterpenes as active medicinal ingredients.

Wu et al., (2008) have developed an ultra performance liquid chromatography (UPLC) method for the simultaneous determination of 21 preservatives: 2-methyl-4-isothiazoline-3-ketone, bronopol, 5-chloro-2-methyl-4-isothiazoline-3-ketone, benzyl alcohol, 2-phenoxyethanol, methyl-p-hydroxybenzoate, ethyl-p-hydroxy benzoate, methyl benzoate, 4-hydroxybenzoic acid iso-propyl ester, propyl-p-hydroxy benzoate, 4-chloro-3-methylphenol, ethyl benzoate, 2-phenylphenol, 4-hydroxybenzoic acid iso-butyl ester, butyl-p-hydroxy benzoate, 4-chloro-3,5-dimethylphenol, phenyl benzoate, 2,4-dichloro-3,5-dimethylphenol, 2-benzyl-4-chlorophenol, triclocarban and triclosan in cosmetics. A Waters UPLC-C18 column was used with 0.1% formic acid solution as the mobile phase under the condition of gradient elution. Preservatives were extracted with methanol by ultra sonicator and then they were analysed by UPLC-PDA detector. All these preservatives were baseline separated in 8.5min. The pre-treatment method of samples and the chromatographic condition of analysis were critically examined in this study.
The recoveries ranged from 90.5 to 97.8%, with percent (%) RSD values below 3.2%, and all correlation coefficients (r) were no less than 0.9997. Thus, this method could be used for analyzing the preservatives in cosmetic products.

Based on the review of literature glucosamine and diacerein dosage forms have methods individually for each active ingredient estimation, The methods found in the literature are prederivatization, internal standard and highly expensive methods for determination of all actives which is not feasible in the routine analysis and there is no such a single method which can be used for the analysis of both the active ingredients.

Simultaneous HPLC methods are not available in literature for the determination of the constituents of ear drops formulation (composed of beclomethasone dipropionate, chloramphenicol, clotrimazole and lidocaine) and antiseptic solution formulation (composed chloroxylenol and terpineol).

In the case of donepezil hydrochloride, amiodarone hydrochloride and sildenafil citrate tablets, the literature suggest few methods but the analysis time and analysis cost is very high which is not feasible for routine analysis and the methods do not meet the ICH guideline requirments.