2. LITERATURE REVIEW:

Literature review showed a number of analytical methods like LC-MS/MS, orthogonal HPLC, UPLC for the analysis of APIs. Few methods like HPLC and UPLC have been reported for the estimation of APIs in bulk drug and its formulations. Works regarding the study of impurity profile in APIs are being carried out. But literature survey showed no HPLC method for the determination of impurity profile.

Considering the foregoing, I undertook to develop high performance liquid chromatography (HPLC) method to determine the following:

1. The overall percentage purity of Active Pharmaceutical Ingredients.
2. The percentage individual related substance impurities and degradation products.

1. **Dell D et al (1981)** reported a high-performance liquid chromatographic method is described for the analysis of the anti-bacterial agent cefotaxime and desacetylcefotaxime in physiological fluids. Plasma or serum samples were mixed with chloroform--acetone to remove proteins and most lipid material. The aqueous phase was then freeze-dried, reconstituted in mobile phase and chromatographed on a reversed-phase column using UV detection at 262 nm. Urine was analysed directly after centrifugation to remove particulate matter. Two additional metabolites, which are lactones in which the beta-lactam ring has been opened, could be separated by this method.

2. **Dokladalova J et al (1983)** reported a gradient high-performance liquid chromatographic (HPLC) procedure has been developed for the determination of microgram amounts of cefoperazone in human serum and urine. The method employs a muBondapak C18 column and gradient elution with two mobile phases. Excellent separation of the drug from potential degradation products as well as from representative penicillins (sodium ampicillin, sodium methicillin, potassium penicillin G) and aminoglycosides (tobramycin, gentamicin, kanamycin) has been demonstrated. The HPLC method can be used to analyze cefoperazone in the presence of penicillins and aminoglycosides which can potentially be co-administered with cefoperazone.
3. **Yost RL et al (1985)** reported a reversed-phase high-performance liquid chromatographic assay for the simultaneous determination of cefotaxime and its metabolite desacetylcefotaxime in plasma and urine was developed. Plasma was deproteinized with small amounts of acetonitrile. After separation of the proteins the supernatant was extracted with a mixture of chloroform and 1-butanol. A phase separation was obtained leaving the cephalosporin and its metabolite in the aqueous part and extracting most of the interfering endogenous material. The aqueous phase was injected directly into the chromatograph. The assay was applied to study the pharmacokinetics of cefotaxime and its metabolite in a healthy volunteer. In a similar way this deproteinization and extraction method was also applied to assay for ceftazidime, cephalexin, cephalosporin and cefoxitin.

4. **Silber Michael B, et al. (1987)** reported a simple, accurate and precise isocratic reversed-phase high performance liquid of a new cephalosporin in human serum and urine. Mobile phase was prepared fresh on the day of analysis. Mobile phase A was prepared by combining 170ml of acetonitrile, 1.36g of monobasic sodium phosphate, 2ml of 85% phosphoric acid and 828ml of distilled water. Mobile phase B (used for urine assay) was prepared by combining 200ml of acetonitrile, 1.36g of monobasic sodium phosphate, 2ml of 85% phosphoric acid and 798ml of distilled water at pH 2.7 with flow rate of 2.0ml/min.

5. **Castillo M, et al. (1988)** reported the degradation rate constants for ampicillin and for dicloxacillin in the suspension filtrate, and their solubility coefficients (at 25°C) by spectrophotometry employing a multicomponent computer program.

6. **Okamoto Y et al (1990)** reported hydrolytic degradation products of cefdinir were studied in acidic (pH 1), neutral (pH 6), and basic (pH 9) solutions. Seven major degradation products were isolated by preparative and/or high-performance liquid chromatography and characterized by UV, IR, 1H-NMR, and mass spectra. To clarify degradation pathways in each pH solution, kinetic and product analyses during hydrolysis of cefdinir were carried out along with the followup reaction of representative degradation products. Cefdinir was shown to degrade via two major degradation routes: beta-lactam ring-opening and pH-dependent isomerizations.
(lactonization, epimerization at C-6 or C-7, syn-anti isomerization of N-oxime function).

7. **F Camus et al (1994)** reported a selective HPLC method is described for the determination of cefpodoxime levels in plasma and sinus mucosa. Sample preparation included solid-phase extraction with a C8 cartridge. Cefpodoxime and cefaclor (internal standard) were eluted with methanol and analyzed on an optimised system consisting of a C18 stationary phase and a ternary mobile phase (0.05 M acetate buffer pH 3.8-methanol-acetonitrile, 87:103, v/v) monitored at 235 nm. Linearity and both between- and within-day reproducibility were assessed for plasma and sinus mucosa samples. The method was used to study the diffusion of cefpodoxime in sinus mucosa.

8. **Okamoto Y et al (1996)** reported the validation of the HPLC method used for the determination of cefdinir and its related substances is described. The developed method was specific and stability-indicating and provided a linear response with concentration. The system and method precision, expressed as relative standard deviations, were not greater than 1%, and the reproducibilities within and between laboratories were acceptable for the assay method. The procedure can quantitate related substances greater than approximately 0.05% of the principal cefdinir peak.

9. **Kees F et al (1996)** reported in a cross-over study on twelve healthy volunteers cefpodoxime proxetil (CAS 87239-81-4) and acetylcysteine (CAS 616-91-1) were evaluated for possible pharmacokinetic interactions. The narrow range of 90% confidence intervals for the quotient test/reference for Cmax and AUC indicate reliable bioavailability of cefpodoxime proxetil independent of co-administered acetylcysteine.

10. **Martinez LG et al (1998)** reported Cefotaxime was derivatised with 1,2-naphthoquinone-4-sulphonate (NQS), extracted into solid-phase cartridges (C18) and detected using a UV-visible detection system. Optimum conditions for this new procedure were: hydrogencarbonate-carbonate buffer, pH 10.5, 5-min reaction time at 25 degrees C and an NQS concentration of 7.1x10(-3) mol l(-1). The accuracy and the precision of the liquid-solid procedure were tested. The procedure was used to measure cefotaxime in pharmaceutical and urine samples. The results obtained were contrasted with those reported for a HPLC method for urine samples. The
generalized H-point standard additions method was used to measure cefotaxime in urine samples.

11. **Nuevas L et al (1998)** reported Cefotaxime sodium is a broad spectrum third generation antibiotic. It is obtained by reaction of 7-aminocephalosporanic acid (7-ACA) and S-(2-benzothiazolyl)2-amino-alpha-(methoxyimino)-4-thiazoleethanethioate. 2-Mercaptobenzothiazole is a by-product of this reaction. This technique is accurate, precise (RSD = 0.4%), and has a sensitivity of 1.2% (differences in analytical response of 0.74 microg ml(-1) could be detected). Recovery experiments of cefotaxime from reaction mixtures include 100% for all assayed concentrations. For these reasons, this technique is found valid for the intended purposes.

12. **Eric-jovanovic S, et al. (1998)** reported a HPTLC method for the determination of ceftriaxone, cefixime and ceftaxime, on a pre-coated silica gel HPTLC plates with concentrating zone (2.5x10cm) by developing mobile phase ethyl-acetone-methanol-water (5:2.5:2.5:1.5 v/v/v/v). at 270nm.


14. **Li FS et al (2000)** reported A reversed-phase, isocratic high performance liquid chromatographic method with acid mobile phase can separate sulbactam and cefoperazone within 12 minutes. Column packed with Hypersil ODS2(250 mm x 4.6 mm i.d., 5 microns) was manufactured by Dalian Elite Company. Mobile phase is composed of water (adjusted to pH 4.0 with 1% phosphoric acid) and acetonitrile (80:20, V/V). The detection was performed at 210 nm and the injection volume was 2 microL. Cefoperazone and sulbactam have good linearity in the ranges of 100 mg/L to 800 mg/L and 100 mg/L to 1,000 mg/L with the correlation coefficients of 0.9991 and 0.9997 respectively. This method is easily to be operated and can be applied for manufacturing and medicinal study.

15. **Scanes T et al (2001)** reported A simple and sensitive HPLC method for the simultaneous determination of cefotaxime (I) and desacetylcefotaxime (II) in human plasma and cerebrospinal fluid (CSF) is described. The assay involves deproteinisation and subsequent separation on a reversed-phase HPLC column, with
ultraviolet detection at 262 nm. Retention times were 6.8 and 2.2 min for cefotaxime and desacetylcefotaxime, respectively. Average recoveries for the analytes were 78% (I) and 88% (II) from both matrices. Linear responses were observed over a wide range (0.58-940 microg/ml for (I) in plasma, 0.80-55.8 microg/ml for (I) in CSF, 0.54-148 microg/ml for (II) in plasma and 0.50-36.0 microg/ml for (II) in CSF).

16. T. Sendo (2002) reported a novel reversed-phase HPLC method was developed and validated for the assay of tetracycline hydrochloride and the limit of 4-epianhydrotetracycline hydrochloride impurity in tetracycline hydrochloride commercial bulk and pharmaceutical products. The method employed L1 (3 µm, 150 × 4.6 mm) columns, a mobile phase of 0.1% phosphoric acid and acetonitrile at a flow rate of 1.0 mL/min, and detection at 280 nm. The separation was performed in HPLC gradient mode. Forced degradation studies showed that tetracycline eluted as a spectrally pure peak and was well resolved from its degradation products.

17. T. Madhusudana Reddy et al (2003) reported Electrochemical reduction behavior of cephalosporins, Cefixime (CF) and Cefpodoxime Proxetil (CP) have been studied using different voltammetric techniques in Britton-Robinson buffer system. Two well defined cathodic waves are observed for both the compounds in the entire pH range. Number of electrons transferred in the reduction process was calculated and the reduction mechanism is proposed. The results indicate that the process of both the compounds is irreversible and diffusion-controlled. A differential pulse voltammetric method has been developed for the determination of these drugs in pharmaceutical formulations and urine samples.

18. Ling SS et al (2003) reported a high-performance liquid chromatographic method with ultraviolet (UV) detection was developed for measuring cefotaxime in rat and human plasma. The method used direct injection of the plasma supernatant after deproteinization with 70% perchloric acid. The speed, sensitivity, specificity and reproducibility of this method make it particularly suitable for the routine determination of cefotaxime in human plasma. Moreover, only a relatively small sample plasma volume (100 microliter) is required, allowing this method to be applied to samples taken from neonates.
19. Gehad G. Mohamed, et al. (2006) reported a simple rapid and accurate spectrophotometric method for the determination of β-lactum drugs, flucloxacillin and dicloxacillin in pure form and different preparations. The absorption of Fluclox and Diclox are recorded in different pH values ranged from 2 to 12 and the curves at pH 2-12 are characterized by two absorption bands at 225 - 270, and 225 - 274nm. for Fluclox and Diclox respectively.

20. Nanda RK, et al. (2009) reported accurate, precise, rapid and economical methods for the estimation of cefixime and ornidazole in tablet dosage form. It is based on simultaneous equation and wavelengths selected for analysis were 290.0nm for cefixime and 312.0nm for ornidazole.


22. N. H. Vadia et al (2009) reported new, simple, cheap, fast, accurate, sensitive and precise colorimetric methods that can be used for the determination of cefetamet pivoxil hydrochloride. The developed methods were successfully applied to the determination of this drug in synthetic mixtures and commercially available tablets.

23. RavichandranV(2010) reported validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, ruggedness, etc. When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by same or different persons, in same or different laboratories, using different reagents, different equipments, etc. In this review article we discussed about the strategy and importance of validation of analytical methods.

24. Dhoka Madhura V, et al. (2010) reported a simple, precise, accurate and sensitive Reverse phase high liquid chromatographic method for simultaneous estimation of Cefixime trihydrate and Erdosteine in combined capsules dosage form. Drugs were resolved on a HiQ Sil C8 column (25x4.6mm) utilizing mobile phase of TetraButyl
Ammonium Hydroxide (0.1N) pH adjusted to 6.5 with Orthophosphoric acid (10% aqueous) in a ratio of 2:1. Flow rate 1.0ml/min. at 254nm.

25. Kumudhavalli M.K, et al. (2010) reported a reversed phase High performance liquid chromatographic for simultaneous estimation of cefixime and potassium clavanate in tablet dosage form by mobile phase consisting of 0.03M phosphate buffer and methanol in the ratio of 84:16 with wavelength is 220nm and flow rate is 1ml/min.


29. Kamalakkannan, et al (2011) reported Analytical method development and validation for Candesartan Cilexetil as bulk drug and in pharmaceutical dosage forms by HPLC.

30. R.R.Yadav (2012) reported an isocratic reversed-phase UPLC method with UV detector has been developed for the determination of Methyl Tosylate, Ethyl tosylate and Isopropyl Tosylate. These are potential genotoxic impurities and hence need to be controlled in Sorafenib Tosylate. These are potential genotoxic impurities and hence need to be controlled in Sorafenib Tosylate. The analyst was performed using RRHD Eclipse Plus C18 UPLC column(50 x 2.1mm, 1.8um) as a stationary phase with column oven temperature 40ºC, and UV detection at 264nm. The method was optimized based on the peak shapes and resolution between Methyl Tosylate, ethyl tosylate, propyl tosylate and Sorafenib Tosylate.

32. **Prakash Katakam (2014)** reported Impurity profiling has become an important phase of pharmaceutical research where both spectroscopic and chromatographic methods find applications. Proper peak shapes and satisfactory resolution with good retention times suggested the suitability of the method for impurity profiling of valacyclovir-related drug substances.

33. **Lakkireddy Praksh (2014)** reported a simple, short and stability-indicating reverse phase-ultra-performance liquid chromatography method was developed and validated for the quantitative determination of related impurities of halobetasol propionate in halobetasol propionate 0.05% cream formulation. The proposed method was developed on an ACQUITY UPLC™ BEH Phenyl (2.1 \( \times \) 100 mm, 1.7 \( \mu \)m) column at 40°C with a mobile phase containing a gradient mixture of potassium hydrogen phosphate buffer and acetonitrile and methanol as modifiers with a runtime of 13.0 min at a monitored wavelength of 242 nm. A simple preparative method and liquid chromatography–mass spectrometry-compatible UPLC method also were developed for the isolation and identification of impurities and degradation products.

34. **Suganithi, (2014)** reported Development and Validation of UV Spectroscopic and HPTLC Methods for the Determination of Bosentan from Tablet Dosage Form and Low relative standard deviation and good % recovery values of both the methods showed that the developed methods were highly precise, accurate and free from interference present in formulation.

35. **K.S.Nataraj (2015)** reported a novel liquid chromatographic method has been developed and validated for the determination of Bosentan monohydrate (BOS), together with its four related substances (Styrene, Hydroxy, Bosentan stage-3 and Dimer) in a laboratory mixture as well as in marketed formulation. Efficient chromatographic separation was achieved on an Inertsil ODS-3V (450mm×4.6mm i.d., 5.0 \( \mu \)m particles), containing Mobile phase A – Buffer : Acetonitrile (50:50) and Mobile phase B – Buffer : Acetonitrile (20:80). Mobile phases were used in gradient combination for about 45 min at a flow rate 1.0ml/min and the eluant was monitored at 225nm. The method has shown good and consistent recoveries for BOS (93.79- 98.67%) and also for its four known impurities (97.2–101.3%). The proposed analytical method has been validated in accordance with ICH guidelines.
36. **Shashi daksh (2015)** reported the development of sound Analytical method(s) is of supreme importance during the process of drug discovery, release to market and development, culminating in a marketing approval. The objective of this paper is to review the method development, optimize and validation of the method for drug product from the development stage of the formulation to commercial batch of the product. Method development for the interested component in the finished product in process test and the sample preparation of drug product.

37. **Ashish Chauhan (2015)** reported that the analytical method development and validation are the continuous and inter-dependent task associated with the research and development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product-specific acceptance criteria. Design of experiment is a powerful tool for the method characterization and validation. Analytical professional should be comfortable to use it to characterize and optimize the analytical method.