2. REVIEW OF LITERATURE

A method has already been developed in Ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) and also validated in quantifying a protease inhibitor darunavir by taking darunavir-d9 as internal standard (IS) by Gupta et al.\textsuperscript{62}. On an Acquity UPLC BEH C18 (50 mm × 2.1 mm, 1.7 µm particle sizes) analytical column the chromatographic separation was attained. The routine estimation of plasma darunavir conc. by application of this method was illustrated by a bioequivalence study that conducted in forty healthy Indian subjects for a 600 mg tablet formulation also 100 mg ritonavir as booster under fast and fed conditions.

Naser et al.,\textsuperscript{63} have illustrated development and validation of a novel LC–MS method for the simultaneous estimation of currently FDA-approved protease inhibitor and also non-nucleoside reverse transcriptase inhibitor. The evaluation of this method relevance was done with clinical samples and exterior quality assurance capability testing samples.

Fayet et al.,\textsuperscript{64} reported a sensitive and accurate LC–MS/MS method for the estimation of plasma drug levels. Single-step extraction of RAL, MVC, DRV, ETV and RTV from plasma (100 µl) was carried out by protein precipitation in taking of 600 µl of acetonitrile, following the addition of 100 µl darunavir-d9 (DRV-d9) at 1000 ng/ml in MeOH/H2O 50/50 as internal standard (I.S.). The Chromatographic separations are carried out in using a gradient program with 2 mM ammonium acetate with 0.1% formic acid and acetonitrile containing 0.1% formic acid.

A LC–tandem MS assay for simultaneous determination of the plasma conc. of eleven antiretroviral agents (including darunavir) was developed by Martin et al\textsuperscript{65}. As per authors this method, with its easy sample preparation affords sensitive, precise and accurate estimation of plasma conc. of antiretroviral drugs that may be employed in therapeutic drug monitoring in HIV patients.

A new method using HPLC–MS was developed and validated by Antonio et al, for the determination of plasma conc. of the new protease inhibitors
darunavir (DRV) \textsuperscript{66} and also other eleven antiretroviral agents. This new HPLC–MS methods gives an accurate, sensitive and suitable estimation of darunavir also other eleven antiretrovirals.

Laura et al., reported a easy, rapid and highly sensitive HPLC–MS/MS method for estimation of Darunavir, \textsuperscript{67} NNRTI, as well as the more new antiretrovirals, the CCR5 antagonist maraviroc and the “second generation” non-nucleoside reverse transcriptase inhibitor etravirine and rilpivirine. The method illustrated is being favorably used to detect plasma antiretroviral conc. from samples elicited from clinical pharmacokinetic studies.

A simple, rapid, reliable and highly sensitive on-line 2D-LC/MS/MS method to determine antiretroviral drugs \textsuperscript{68} was developed and validated by Rao et al. The developed method showed better selectivity, accuracy and precision for estimation of the antiretroviral drugs.

A particular RP-HPLC method for separation and quantification of darunavir \textsuperscript{69} from its process related substances has been developed and validated by Rao et al. The separation was accomplished on RP-select B, C(8) column attached to a PDA detector in use of 10mM phosphate buffer containing 0.1% of triethylamine acetonitrile as a mobile phase in gradient elution. The method was validated in basis of accuracy, precision, linearity, robustness, LOD and LOQ.

A novel stability-indicating R-HPLC method has been developed for darunavir ethanolate, \textsuperscript{70} by Reddy et al. The chromatographic separation was attained taking an X-Bridge C18 HPLC column using 0.01M ammonium formate buffer at 265 nm. The HPLC method was validated as per ICH guidelines in regards to specificity, precision, linearity, accuracy and robustness.

Heine et al., \textsuperscript{71} have clinically evaluated DBS sampling for the estimation of conc.of plasma for the new antiretroviral drugs etravirine, darunavir/ritonavir and raltegravir. DBS are correlated reproducibly to plasma levels and it can be applied for determining antiretroviral drug found in patients who are HIV-infected.

A sensitive and accurate HPLC-MS method for intracellular estimation of 14 antiretroviral drugs \textsuperscript{72} in PBMCs for HIV+ patients was validated by D'Avolio et al. The method was accurate, in a range of intraday/inter-day % S.D. within 2.6-14.8%.

Hirano et al., \textsuperscript{73} reported the use of HPLC with UV detection for simulataneous assay of darunavir (DRV), atazanavir, ritonavir and lopinavir. The obtained results
reports that HPLC-UV method may be applied for regular estimation of concentrations of plasma of ETV and 4 PIs in clinically.

Heine et al., 74 described that in a lesser sensitive assay the change of a method to a presumed lesser sensitive detector did not certainty result, and reported a method that may be used in laboratory where the availability of earlier mass spectrometers are there.

Else et al., 75 described an easy, rapid and precise HPLC-MS/MS method for quantification of protease inhibitors. The method reported is being favorably applied to detect plasma antiretroviral conc. from samples elicited from clinical pharmacokinetic review.

A bioanalytical method developed by D'Avolio et al., 76 for the estimation of darunavir, modifying their earlier HPLC-MS chromatographic run, then validated. The method was validated taking the concentration ranges detected in clinical trials and the conventional clinical practice into consideration. The developed and validated method provides a simple and economical sample shipment for therapeutic drug monitoring and the pharmacokinetic studies.

A LC-MS/MS method was developed by Quaranta et al., 77 for the quantification of darunavir in plasma at the conc. correlated with therapy. This method gives a finest linearity for the all calibration curves.

The development and validation of a new LC-MS method for simultaneous estimation of darunavir was described by Rezk et al.78 The evaluation of this method relevance was done with clinical samples and exterior quality assurance capability testing samples.

A sensitive and precise LC-tandem mass spectrometry technique for the estimation of plasma drug levels by Fayet et al.79 This is the 1st analytical method that acknowledges the simultaneous assay of antiretroviral agents designed to 4 different steps of HIV replication.

A novel HPLC method was developed and validated for Darunavir by D'Avolio et al.80 Calibration curves were advanced to desired ranges of drug conc. in patients. Better extraction ability and lower limit of detection allow it an adoptable method for applicable in clinical trials and therapeutic drug monitoring of darunavir.

D'Avolio et al., 81 have optimized a new method using HPLC-MS was developed and validated. This new HPLC-MS technique provides a definite, precise
Takahashi et al., have validated the determination of darunavir concentrations using the HPLC method. The calibration curve was linear. The average accuracy ranged from 100.7 to 105.6%. Both the interday and intraday coefficients of variation were less than 6.7%, which was similar to or much lower than previously reported values by the LC/MS/MS method. It is concluded that HPLC can be used to determine plasma DRV concentrations and routinely in the clinical study; thus, this HPLC method enables further study of DRV pharmacokinetics in conventional hospital laboratories.

A sensitive and precise HPLC method with UV detection has been optimized and validated for Darunavir by Goldwirt et al. This method allows an applicable kit for therapeutic drug monitoring in patients infected by HIV.

An assay was developed by Heine et al., using LC-MS. The method was validated over a range of 0.1 to 20 microg/mL on basis of yield conc. ranges in patients who treated by these drugs. The method is now favorably used for regular therapeutic drug monitoring and pharmacokinetic studies in HIV patients.

Degradation products of paliperidone formed under different forced conditions have been optimized through LC–UV–PDA and LC–MS studies by Sawant et al. Here paliperidone was lead to forced degradation. Three degradation products were formed and separated. Validation of the LC–DAD method was performed in accord to guidelines of ICH. The method met all required criteria and was applied for analysis of commercially available tablets. All the products were characterized through LC–MS analyses and their fragmentation pathways were proposed.

Bocato et al., have reported a novel LC–MS/MS method utilizing the polar organic method for analysis of risperidone and chiral metabolites of risperidone. They have optimized a SPME procedure for extraction of these analytes from liquid culture medium. The method was validated and SPME unveiled to be an essential kit that to be employed in biotransformation studies. The biotransformation results unveiled that it is feasible in getting a drug with enantiomeric pure form.

Two LC–MS/MS methods were illustrated, for the quantification of risperidone and paliperidone by Meulder et al. Method validation results indicates the method is adequately specific to enantiomers of 7-hydroxyrisperidone and able to estimate the analytes with better accuracy and precision in conc. range of
Danel et al., have studied the HPLC semipreparative enantioseparation of paliperidone by optimization of different experimental conditions. This separation was favorably achieved in use of the polysaccharide Chiralcel OJ chiral stationary phase and n-hexane/ethanol/methanol ternary mobile phase.

A LC–MS/MS method has been developed for the simultaneous quantification of seventeen antipsychotic drugs by Sampedro et al. The method was favorably used to the definite identification and precise estimation of antipsychotic drugs in human postmortem brain tissues: thus, the method can be applied for forensic evaluations.

An enantioselective HPLC method with electrochemical detection was optimized and validated for simultaneous quantification of paliperidone and risperidone by Locatelli et al. The method was accurate and sensitive also favorably used in a clinical study evaluating the stereoselectivity of risperidone 9-hydroxylation.

The stability of thirty common antipsychotics in spiked whole blood was evaluated over 10 weeks in a preliminary experiment by Saar et al. Eight APs were integrated to the F experiment. All desired drugs in the F experiment reported remarkable reduction after twenty weeks of at least one storage condition. Therefore, necessary to be aware of potential changes in drug conc. throughout storage when elucidating analytical results.

A easy, linear gradient, fast, accurate and stability-indicating analytical method was designed for quantification of paliperidone related substances and degradants of API and also tablets by Bindu et al. The method was validated in regards to precision, accuracy, linearity, robustness, ruggedness, LOQ and LOD.

A pre-column dansylated UPLC-MS/MS method for simultaneous quantification of risperidone (RIP) & paliperidone (9-OH-RIP) was developed by Cai et al. The developed method was allows in analysis of first morning urine samples of schizophrenic patients treated by risperidone and healthy volunteers.

A rapid and sensitive LC/MS/MS method has been optimized and validated for simultaneous determination of risperidone and its active metabolite (paliperidone) in plasma of human by Bhatt et al. The developed method was used for the quantification of the parameters of pharmacokinetic of RSP and paliperidone.

Cutroneo et al., have reported a chemometric method to develop the separation of some drugs. The reason of the method is the explicit determination and
estimation of drug candidates in biological fluids. The procedure gives a chromatogram of well separated solutes.

Moody et al.,\textsuperscript{96} have developed a LC-APCI-MS-MS method for quantification of risperidone and paliperidonewith improved sensitivity, selectivity, and dynamic range.

A reversed-phase HPLC method for quantification of risperidone and paliperidone was established by Xiao et al.\textsuperscript{97} The linear range was 2-600 micrograms/L, \( r = 0.996 \) for risperidone, and 2-800 micrograms/L, \( r = 0.998 \) for 9-hydroxyrisperidone. The average recovery was \((98.2 +/− 3.5)\%\) for risperidone, and \((97.8 +/− 3.8)\%\) for 9-hydorxyrisperidone. The method has been used to esimate risperidone and paliperidonemass concentration in patient plasma.

A HPLC with UV detection method for the simultaneous quantification of risperidone and paliperidone has been optimized by LLerena et al.\textsuperscript{98} Studies of analytical interference unveiled that the most commonly co-administered antidepressants and benzodiazepines did not interfere.

Titier et al.,\textsuperscript{99} have optimized a HPLC assay for the simultaneous estimation of risperidone and paliperidone. This rapid method (run time <5 min) is currently used for poison management.

An easy and sensitive HPLC method with UV absorbance detection is reported for the estimation of risperidone and paliperidone, using clozapine as internal standard by Avenoso et al.\textsuperscript{100} it has favorbly been used for pharmacokinetic studies and therapeutic drug monitoring.

A HPLC method has been designed for the simultaneous estimation of risperidone and paliperidone by Nagasaki et al.\textsuperscript{101} Risperidone, paliperidone and trazodone as internal standard were detected by uv absorbance at 280 nm.

A method for the simultaneous determination of paroxetine, risperidone and paliperidone in human plasma has been optimized by Schatz et al.\textsuperscript{102} The precision, accuracy and specificity found acceptable, and show method is applicable for clinical studies and routine drug monitoring.

Balant-Gorgia et al.,\textsuperscript{103} have studied interindividual variability factors affecting risperidone conc. under regular therapeutic drug monitoring conditions. Therapeutic drug monitoring data, that was collected in specimen of the population for which the drug was deliberated, made allow us to estimate the dose decrase required in aged
patients and so gave important data in addition to the one obtained during premarketing studies carried out with strict inclusion and exclusion criteria.

An easy, sensitive and precise method for the simultaneous estimation of risperidone (RSP) and paliperidone in plasma of human was described by Aravagiri et al.\textsuperscript{104} The calculation of the total active moiety in plasma of schizophrenic patients may be essential in evaluating the relationship between dose and plasma conc. and dose and clinical outcome of patients rather than measuring RSP only.

A HPLC method was developed for estimation of risperidone and paliperidone in serum by Olesen et al.\textsuperscript{105} Some traditional low dose neuroleptics and clozapine did inhibit, but this is less important, because risperidone is deliberated as a substitute to these drugs.

A method for the estimation of risperidone and paliperidone in plasma of human has been developed by Moing et al.\textsuperscript{106} The precision, accuracy and specificity have been checked, and show that the method is applicable for clinical studies.

A HPLC method has been optimized by Woestenborghs et al.,\textsuperscript{107} for the simultaneous quantification of the new antipsychotic risperidone and paliperidone in plasma, urine and animal tissues. The method was used to pharmacokinetic studies in experimental animals, human volunteers and patients.

A LC–MS-MS method with a fast and easy sample preparation was optimized and validated by Koehler et al.,\textsuperscript{108} for quantification of ropivacaine. A good quadratic response over the range of 1.0–200.0 ng/ml was reported. This method is applicable for pharmacokinetic studies.

The rapid and simple UPLC–MS method was optimized and validated for determination ropivacaine by Romana et al.\textsuperscript{109} The method was fully validated and suitable for routine analyses.

A liquid chromatographic method using cellulose tris(4-chloro-3-methylphenylcarbamate) coated on silica as chiral stationary phase was favorably optimized for S-ropivacaine by Dossou et al.\textsuperscript{110} The LOD and LOQ were obtained to be about 0.2 and 1.0 µg/mL, respectively, with respect to 0.02 and 0.1% of the enantiomeric impurity in S-ropivacaine.

A simple and sensitive HPLC-UV method has been optimized and validated for estimation of ropivacaine by Qin et al.\textsuperscript{111} This method is suitable for simultaneous estimation of bupivacaine, ropivacaine, lidocaine, procaine, tetracaine and for
therapeutic drug monitoring and pharmacokinetic study.

Sawaki et al.,\textsuperscript{112} studied the viability of HPLC/MS as a selective and precise analytical method for determining blood conc. of ropivacaine. The results of the this study revealed that LC/MS method was specific enough and sensitive for the determination of ropivacaine, showing that it gives a suitable kit for monitoring therapeutic effects and evaluating pharmacokinetic parameters of drug in blood.

A selective HPLC assay coupled with UV detection has been optimized and validated for the simultaneous estimation of ropivacaine and bupivacaine in plasma of human by Gaudreault et al.\textsuperscript{113} The method was sensitive, reproducible and accurate and was suitable for study the pharmacokinetics of ropivacaine and bupivacaine in a perioperative theme.

A HPLC-diode array detection method was developed and validated to simultaneously estimate ropivacaine by Salmerón-Garcia et al.\textsuperscript{114} The developed method was suitable to evaluate chemical stability of ropivacaine.

The quantification of ropivacaine and its major metabolites in urine was carried out by Abdel-Rehim et al.,\textsuperscript{115} using microextraction in a packed syringe as an on-line sample preparation method with LC and MS/MS. The method is miniaturized and fully automated suitable for pharmacokinetic and pharmacodynamic studies.

Dong et al.,\textsuperscript{116} set a R-HPLC method for determination of plasma ropivacaine in dog. The linear range was 0.1-25 microg/ml ($r = 0.9982$). The recovery of ropivacaine was 91.2%-93.6%, RSD were 2.10%-3.40% (n=6). The detection limit was 0.05 microg/ml. This method is simple, fast, precise and suitable for estimation of concentration of ropivacaine in dog plasma.

A HPLC method has been optimized for ropivacaine by Tanaka et al.\textsuperscript{117} The absorbance of the eluate was monitored at 210 nm. This method suitable for clinical and forensic applications for estimation of the local anesthetic drug ropivacaine.

A simple and reliable RP-HPLC method has been developed by Zuo et al.,\textsuperscript{118} for the simultaneous estimation of ropivacaine and antipyrine. The method has been validated to be precise, sensitive and linear.

An easy and fast R-HPLC method was optimized for estimation of ropivacaine by Zhang et al.\textsuperscript{119} The results reported that it is an easy, fast and safe method for drug monitoring in clinical research.

A sensitive HPLC method and microdialysis were optimized to investigate the
pharmacokinetics of ropivacaine in blood and brain of rat by Kau et al.\textsuperscript{120}

The method shows no endogenous intrusion and sensitivity of method was adequate in estimation of biological samples.

The authors, Rifai et al.,\textsuperscript{121} developed a HPLC assay for simultaneous quantification of plasma ropivacaine and bupivacaine conc. taking ultraviolet detection and a simple solid-phase extraction method. The method reported here is preferably applicable for the therapeutic monitoring of plasma ropivacaine and bupivacaine conc.

A sensitive HPLC method has been optimized for quantification of ropivacaine in plasma by Reif et al.\textsuperscript{122}. The LOD of ropivacaine in plasma samples were 0.9 ng/ml.

Sinha et al.,\textsuperscript{123} have developed and validate an easy and fast isocratic RP-HPLC for the simultaneous quantification of amlodipin and telmisartan in integrated dosage form. This method was got to be effective, sensitive, precise, suitable and cheap and is applicable for regular Q.C. analyses.

A specific LC–electrospray ionization mass spectrometric method was optimized and validated for rapid quantification of telmisartan in plasma of human by Ben-mei et al.\textsuperscript{124}.

An easy and fast RP-LC method for separation and estimation of the related substances of telmisartan (TLM) was optimized and validated by Rao et al.\textsuperscript{125} The method was suitable to estimate related substances and assay of TLM in bulk drugs and marketed tablets.

A fast and specific LC–MS/MS assay method has been optimized and fully validated for the simultaneous estimation of telmisartan and amlodipine in plasma of human by Vasu et al.\textsuperscript{126} The proposed method was elicited to applicable for clinical studies.

A specific, sensitive and rapid method based on HPLC was optimized for the simultaneous estimation of telmisartan and hydrochlorothiazide in plasma of human by Salama et al.\textsuperscript{127} The method utilizes proteins precipitation with acetonitrile as only sample preparation prior to RP-HPLC.

A fast and specific method using LC–MS/MS has been optimized for the simultaneous quantification of telmisartan and hydrochlorothiazide in plasma of human by Tingting et al.\textsuperscript{128} The assay was suitable for pharmacokinetic study in 09
healthy patient given a single oral dose of tablet containing combined telmisartan 80 mg and hydrochlorothiazide 12.5 mg.

A HPLC method with fluorimetric detection has been optimized for the quantification of telmisartan by Torrealday et al.\textsuperscript{129} Separation was performed at room temperature. This method applicable for quantification of the active drug in urine samples obtained from hypertensive patients.

A fast and specific LC tandem mass spectrometry method has been optimized and validated for the simultaneous quantification of ramipril, telmisartan and ramiprilat in plasma of human by Gupta et al.\textsuperscript{130} The solid-phase extraction method was applied for the extraction of ramipril, telmisartan and ramiprilat from plasma of human. The method allows a simple reversed isocratic chromatography condition and mass spectrometry detection that permits detection at sub-nanogram levels.

Column-switching HPLC method has been developed and validated for estimation of losartan, telmisartan, and valsartan in human urine by Maria et al.\textsuperscript{131} The optimized column-switching method was favorably used for the estimation of the analytes in urine samples of human of patients submitted at ARA-IIIs therapy.

A fast HPLC method taking a monolithic column with fluorescence detection has been optimized for quantification of telmisartan in plasma of human by Zhang et al.\textsuperscript{132} The assay was big turnout, specific and accurate, and it was favorable used to a bioequivalence study of 2 formulations of telmisartan.

Shah et al.,\textsuperscript{133} carried out degradation studies under conditions recommended by ICH such as hydrolysis (acidic, basic and neutral), photolysis, thermal stress and oxidation.

A fast, sensitive and specific method for quantification of the angiotensin II receptor antagonist, telmisartan, in plasma of human has been optimized by Pengfei et al.\textsuperscript{134} The assay was used for pharmacokinetic study of telmisartan administer as a single oral dose (80 mg) to healthy subject.

Janardhanan et al.,\textsuperscript{135} developed and optimized a validated isocratic reverse phase HPLC separation of Rosuvastatin, Telmisartan, Ezetimibe and Atorvastatin in pharmaceutical preparation employing response surface methodology. The separation was performed by taking phenomenex C18 column (15 cm × 4.6 mm id, 5 µm particle sizes) and UV detection at 239 nm. The ranges of the separate variables used for the development were MeCN: 33–38%, buffer conc.: 10–20 mM and flow rate: 1–2
ml/min. The developed assay condition was validated in accord to ICH guidelines and used for the quantitative analysis of various tablet. The developed method was simple, accurate and precise. Thus, it can be applied for the regular analysis in Q.C. laboratories.

A new LC/APCI-MS/MS method with on-line sample acquire for the estimation of telmisartan in blood plasma of human was reported by Hempen et al.\textsuperscript{136} The methods for quantification of telmisartan in blood plasma of human showed to give comparable results for amount of analyte.

Shewiyo et al.,\textsuperscript{137} have surveyed HPTLC method for optimization and validation of HPTLC methods to assay active ingredients in pharmaceuticals reported in year 2005–2011. The method and proposals for optimization, validation and quantitative assays were compared with the standard modes of conducting them.

Hillaert et al.,\textsuperscript{138} optimized a capillary zone electrophoretic method for separation of 6 angiotensin-II-receptor antagonists including telmisartan. The method applicable for the quantitative estimation these compounds, but only for the more soluble ones.

A capillary zone electrophoretic method optimized by Hillaert et al.,\textsuperscript{139} . A face-centred central composite designed for the study. The method applicable for the quantitative determination of the more stable angiotensin-II-receptor antagonists.

A novel and simple titrimetric method for estimation of usually used angiotensin-II-receptor antagonists (ARA-IIs) is developed and validated by Patil et al.\textsuperscript{140} The proposed titrimetric method is easy, fast, convenient and sufficiently precise for Q.C. purposes.

A MRM based bioanalytical method was optimized for estimation of PEG 400 in rat plasma by Vijaya Bhaskar et al.\textsuperscript{141} In literature there was no MRM formed bioanalytical method optimized and validated for the analysis of PEG 400 in plasma.

Gonzalez et al.,\textsuperscript{142} reported the chemometrical optimization and the validation of a quantitative HPLC-PDA-Fluo method for the simultaneous analysis of drugs usually combined in cardiovascular therapy. This method applicable for estimation of these drugs in human plasma samples obtained from patients under cardiovascular treatment.

An easy, rapid, precise and highly selective spectrofluorimetric method has been optimized for estimation of some angiotensin II receptor antagonists by El-
Shaboury et al.,\textsuperscript{143} in pure forms along with in their pharmaceutical dosage forms. All the variables affecting the relative fluorescence intensity were studied and optimized. Good accuracy and precision were successfully obtained for the analysis of tablets containing each drug alone or combined with hydrochlorothiazide (HCTZ) without interferences from the co-formulated HCTZ or the additives commonly present in tablets.

A noval, precise, selective, sensitive, fast, RP-HPLC method was optimzed for quantification of related substances of Telmisartan and Hydrochlorthiazide in tablet dosage form by Mukhopadhyay et al.\textsuperscript{144} It sutable for the regular analysis of the related substances of both Telmisartan and Hydrochlorthiazide in this combination.

A high fast exection LC method has been optimized and validated for quantification of ramipril and telmisartan simultaneously in integrateddosage form by Kurade et al.\textsuperscript{145} The method was suitable and can be favorably used used to estimate drug content of marketed formulations.

An easy, fast, and accurate method was optimized for the simultaneous estimation of telmisartan and hydrochlorothiazide in integrated pharmaceutical dosage form by Rane et al.\textsuperscript{146} The suggested method allowed to be applicable and precise for estimation of telmisartan and hydrochlorothiazide in preparation of pharmaceuticals.

A precise and selective LC–APCI-MS method has been optimized and validated for the determination of zaleplon by Zhang et al.\textsuperscript{147} The validated LC–APCI-MS method has suitable to study zaleplon pharmacokinetic, bioavailability and bioequivalence in eighteen adult subjects.

The silylating reagents were evaluated to achieve optimal derivatization conditions for analyzing different benzodiazepines based on GC–EI–MS by Gunnar et al.\textsuperscript{148} The method was completely validated in regards of accuracy, intra- and interday precision, LOD, LOQ, linearity, selectivity and extraction efficiency.

A Sciex API 150 EX method was optimized for estimation of zolpidem and zaleplon in whole blood by Giroud et al.\textsuperscript{149} The method was favorably used for forensic cases.

Bharathi et al.,\textsuperscript{150} have identified impurities in zaleplon bulk drug at levels below 0.1% by RP-HPLC. The mol.wt. of impurities was estimated by LC-MS analysis. These impurities were isolated from crude samples of zaleplon taking reverse-phase preparative HPLC.
Kintz et al.,\textsuperscript{151} have screened and reported seventeen benzodiazepines and hypnotics in oral fluid after getting with the Intercept\textsuperscript{®} device by LC-MS/MS. This method applicable for monitoring subjects under the influence.

Villain et al.,\textsuperscript{152} have screened sixteen benzodiazepines and hypnotics in hair of human by LC–MS/MS. The yield results were applicable to screen for sixteen benzodiazepines in hair and identify them at lower conc., making the method usable to monitor single dose.

A selective LC tandem mass spectrometry method was optimized and validated for simultaneous identification of API in urine and whole blood by Verplaetse et al.\textsuperscript{153} The developed method was fully validated.

UPLC-MS Remane et al.,\textsuperscript{154} have developed method for blood plasma analysis after simple liquid-liquid extraction. The applicability of method was favorably exhibit for many of the drugs by analysis genuine plasma samples and exterior Q.C. samples.

Metwally et al.,\textsuperscript{155} have reported spectrophotometric, spectrodensitometric and HPLC stability indicating methods for quantification of Zaleplon in both pure and dosage forms. The unimportance variation of the suggested procedure results with those of the reference one allowed their accuracy and precision.

Aten easy, accura and selective HPLC method with ultraviolet detection (230 nm) was optimized and validated for estimation of zaleplon in human plasma by Nirogi et al.\textsuperscript{156} This validated method is sensitive, simple and repeatable suitable to be used in pharmacokinetic studies.

A sensitive and rapid chromatographic method in combination with a simple and effective sample preparation step was reported estimation of zaleplon in plasma of human by Feng et al.\textsuperscript{157} The use of this method is illustrated for the analyzing zaleplon plasma samples in a phase-I human pharmacokinetic study.
2.1 AIM OF OBJECTIVE

In the past era of pharmaceutical chemistry, there were various challenges to face for development of new methods for analysis of active pharmaceutical ingredients singly or in combination of two or three, due to limited available facilities and instrumentation. It is very much difficult to develop new and economical method for evaluation of new profile drugs. To overcome these problems analyst are now dealing with modern analytical techniques which have wide scope for application and its utility. This is because of new instrumentations like HPLC, HPTLC, Gas Chromatography, Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography, Liquid Chromatography-Mass Spectrometry (LC-MS) and others. Definitely these modern methods have various advantages over the conventional analytical techniques; to use the merits of these modern analytical techniques we are trying to develop some new precise and economical analytical methods for commercial benefits.

Once a drug candidate attains for use in human, all stability storage and testing must be carried out accord to cGMP. Stability studies are important to each phase of drugs in life cycle. They not only confers that a product will maintain its potency during its defined shelf life, but it will do so in several of storage conditions.

The ICH stability guidelines Q1A (R2) describes stress testing for noval drug substances and drug products, for elucidation the intrinsic stability of drug substance and drug products. The stress testing may also give details about degradation pathways and selectivity of the employed analytical method. The ICH guideline Q1A (R2) defines stress testing of the features that are suitable to alter during storage and are expected to effect quality, safety and efficacy, should be carried out by validated stability indicating testing method.

A survey of literature unveiled that different assay methods like simple chromatographic and spectrophotometric techniques are reported for given drugs Darunavir (DN), Paliperidone (PP), Ropivacaine (RP), Telmisartan (TR) & Zaleplon (Zpl) alone or for formulations. But it was observed that no any R-HPLC and HPTLC method especially using chromophore has not yet stated for the given drugs neither as bulk drug nor in formulation. Even stability indicating assay method for DN, PP, RP, TR & Zpl is not found by R-HPLC & HPTLC with chromogenic reactions, and also no SIAM developed for the same drugs for it.
Keeping all above fact in mind, a valuable thought was made to develop method using HPLC & HPTLC for these drugs in their pure and formulation. Also the aim was to achieve degradation sample for developing stability indicating method for drug substance and drug product.

2.2 PLAN OF WORK
To achieve the objectives of the planned work in time keeping the quality statement intact, we have formatted a schedule of plan which is as follows;
1. Referencing and Selection of Drugs:
2. Experimental
   2.1. Materials
      2.1.1. Chemicals
      2.1.2. Solutions
   2.2. Method Development
      2.2.1. Selection and detection of wavelength
      2.2.2. Selection of mobile phase
      2.2.3. Selection & chromonisation by chromophoric agent
      2.2.4. Preparation of standard calibration of curve
      2.2.5. Estimation of drug in marketed formulation by proposed method
   2.3. Studying effect
      2.3.1. Effects of sequence of reagents added
      2.3.2. Influence of organic solvents
      2.3.3. Influence of surfactants
      2.3.4. Influence on standing time
      2.3.5. Effect of temperature
      2.3.6. Influence of chromophore concentration
      2.3.7. Influence of absorption of product.
   2.4. Stress Studies
      2.4.1. Hydrolysis studies
         2.4.1.1. Acid Hydrolysis
         2.4.1.2. Base Hydrolysis
         2.4.1.3. Neutral Hydrolysis
      2.4.2. Oxidation studies
      2.4.3. Photo stability studies
2.4.4. Thermal studies

2.5. Analysis of stressed samples by HPLC & HPTLC

2.6. Method Validation
   2.6.1. Validation for Accuracy, sensitivity & reproducibility
   2.6.2. Recovery study
   2.6.3. Precision Linearity and range
   2.6.4. Ruggedness
   2.6.5. Application of proposed method

Data Compilation and Thesis Writing