LITERATURE REVIEW:

S. K. Dubey et al ³ (2010) developed and validated the HPLC Method for quantification of trandolapril using UV detection. Column used was Merck LiChroCART - RP C18 (250 x 4.0, 5 µm). Mobile phase composition was mixture of acetonitrile: methanol: phosphate buffer (0.025mM) pH 3.0 (40:35:25) having flow rate of 1 ml/min. The wavelength used was 220 nm. Peak area obtained were linearly related to concentration of drug in samples in range of 2.5-17.5 µg/mL with correlation coefficient of 0.999. The method was validated as per ICH guidelines for various parameters. The results for accuracy, precision and robustness were found to be within accepted limits.

S. K. Dubey ⁴ (2010) developed and validated a new UV- Spectrophotometric method for determination of Trandolapril Maleate. Wavelength of 207.5nm was used in the study. The method provides a linear response across a quantitation range of 5µg/ml to 30µg/ml in phosphate buffer pH 6.8. The method gave satisfactory results in terms of repeatability and intermediate precision. Also accuracy values were very good, the recovery being between 97.87 to 101.64%. The method was validated and proved to be robust and rugged. The results showed that this method can be used for rapid determination of Trandolapril maleate.

D. Kowalczuk ⁵ (2005) made the HPTLC method and densitometric analysis for simultaneous measurement of trandolapril and verapamil in 2-component mixtures and in their combination capsules. The active substances were extracted from capsules with methanol and chromatographed on TLC plates coated with silica gel 60 F254 in horizontal chambers with ethyl acetate-ethanol-acetic acid (8 + 2 + 0.5, v/v) mobile phase. Chromatographic separation of these components was followed by ultraviolet densitometric quantification at 215 nm. The calibration graphs were constructed over the concentration range from 0.5 to 1.5 microg/microL (corresponding to 5.0-15.0 microg/spot) for both drugs with good correlation (r > or = 0.990).

Gumieniczek et al ⁶ (2001) developed and validated a liquid chromatographic method for determination of trandolapril and verapamil in capsules. LiChrosorb RP18
column is used for the analysis with a mobile phase composition of acetonitrile-methanol-phosphate buffer pH 2.7 (40:40:20) and UV detection at 220 nm. Peak height ratios were linearly related to amounts of the drugs in the range 4–20 μg/mL.

**Gumieniczek et al** 7 (2000) established a high-performance liquid chromatographic assay of trandolapril in capsules. Samples were chromatographed on a LiChrosorb RP-18 column and the mobile phase was acetonitrile -0.067 M phosphate buffer pH 2.7 (7:3, v/v). The UV detection at 220 nm and benazepril as an internal standard were used. The method was tested for linearity (over the range 4-20 micrograms/ml), precision and accuracy and was successfully applied for the quantitative determination of trandolapril in capsules.

**Vikas, Rao J.R. et al** 8 (2010) established Stability Indicating HPTLC method for Trandolapril Estimation in the Bulk Drug and Tablet Dosage Form. Aluminum foil TLC plates precoated with silica gel 60F 254 were used as stationary phase and toluene: ethyl acetate: methanol: formic acid (2.5:8:1:0.5) as mobile phase. A compact band (Rf 0.51) was obtained for trandolapril. Densitometric analysis is performed in absorbance mode at 220 nm. The method was validated for precision, recovery and robustness. The drug was subjected to acid and base hydrolysis, oxidation, photochemical and thermal degradation and undergoes degradation under all these conditions.

**I. cendrowska et al** 9 (2002) studied the stereo chemical purity of trandolapril and octa hydro H indole 2 carboxylic acid by HPLC method. HPLC conditions for identification of stereo isomers and stereo chemical purity of key intermediate in trandolapril synthesis, octa hydro H indole 2 carboxylic acid and final drug were elaborated. The chemical and stereo chemical purity of synthetic trandolapril was proved to be as high as 99.2 – 99.8 % on both chiral and non chiral RP columns.

**V. Bhaskara Rajul et al** 10 (2011) estimated trandolapril in bulk and tablet dosage form by RP HPLC method. C18 column used for separation, a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 35:65 v/v was used with flow rate of 0.8 ml/min and the detection wavelength was 210 nm. The linearity was observed in the range of 20-60 μg/ml with a correlation coefficient of 0.999. The
The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of trandolapril in tablet dosage forms.

**C. Rambabu et al** (2010) developed a simple and sensitive RP HPLC method for trandolapril in bulk and formulation. Chromatographic determination is performed on a Hypersil gold C18 (100mm, 4.6mm-ID; 5μm) with mobile phase consisting of buffer: acetonitrile 50:50 v/v, flow rate - 1.0 ml/min, wavelength used was 215nm. The standard curve is linear over a concentration range of 25.0-150μg/ml (r²=0.9999). The developed method was validated successfully, indicating that the method was accurate and precise.

**N. Sreekanth et al** (2010) developed and validated HPTLC method for trandolapril in bulk and pharmaceutical dosage forms. Quantification of trandolapril was carried out with percolated silica gel 60F254 as stationary phase using mobile phase consisting of Chloroform: Methanol: Acetic acid (8:1.5:0.5 v/ v/ v) and scanned in Absorbance Reflectance mode at 212 nm using Camag TLC scanner 3 with WinCAT software. The Rf value of trandolapril was found to be 0.54 (±0.03). The proposed method has permitted the quantification of trandolapril over the linearity range of 25150 ng/spot and its percentage recovery was found to 99.7%. The proposed method can be successfully applied for the estimation of drug content of different marketed formulations simultaneously on a single plate and provides a faster and cost effective quality control tool for routine analysis of trandolapril as bulk drug and in tablet dosage forms.

**J. N. Harlikar et al** (2003) determined Perindopril, Indapamide, Ramipril, Trandolapril simultaneously in Pharmaceutical formulations using Reverse Phase Liquid Chromatography. Mobile phase used was 0.05M ammonium acetate (pH 2.5) and acetonitrile in volume ratio of 70:30 at a flow rate of 1.0 ml/ minute. A supelco C-18, (3μ, 33 x 4.6 mm) column was used as stationary phase. Quantitation was performed using UV detector at 215 nm. The method shows good resolution between all peaks. The method is sensitive, accurate and precise excipients present in the dosage forms did not interfere with the assay method.
D. D. Rao et al (2009) studied Stress Degradation Studies and developed a Stability-Indicating HPLC Assay Method for Pharmaceutical Dosage Form. A stability-indicating HPLC method was developed for the quantitative determination of trandolapril in pharmaceutical dosage forms in the presence of degradation products. The column was X Terra RP18 150 mm, 4.6 mm, 5 µm. The separation was achieved on gradient method. The mobile phase A contains a mixture of pH 3.0, 10 mM Na₂HPO₄ buffer: acetonitrile (65:35, v/v) and the mobile phase B contains a mixture of pH 3.0, 10 mM Na₂HPO₄ buffer: acetonitrile (45:55, v/v). The flow rate was 1.2 mL min⁻¹ and the detection wavelength was 210 nm. The retention time of trandolapril is 5.7 min. The total runtime was 20 min within which drug and degradation products were separated. Trandolapril was subjected to different ICH prescribed stress conditions. Degradation was found to occur in hydrolytic and oxidative stress condition, while drug was stable to thermal and photolytic stress conditions. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The method developed was successfully applied to the determination of trandolapril in pharmaceutical preparations. The developed RP-HPLC method was validated with respect to linearity, accuracy, precision and ruggedness.

O. H. Jhee et al (2005) determined verapamil in rat plasma by coupled column microbore-HPLC method. This report describes an automated coupled column microbore-high-performance liquid chromatography (HPLC) with fluorescence detection for direct determination of verapamil in small volume of rat plasma. They used HPLC system consisting of three columns such as precolumn, intermediate and analytical column and six-port switching valve and injected small volume of rat plasma to the system without sample preparation. An aliquot of sample was directly injected into Capcell Pak MF Ph precolumn for clean-up and enrichment, 35mm Capcell Pak C18, intermediate column for concentration of compounds and 250mm Capcell Pak C18 analytical column for separation of compounds and two mobile phases are used as mobile phase A (50mM ammonium phosphate, pH 4.5) and B (50mM ammonium phosphate: acetonitrile = 70:30 v/v). Analysis of verapamil and internal standard, propranolol was performed with direct injection of 10 µl of rat plasma to the system and were eluted at 22 and 12 min, respectively, at a mobile
phase flow rate of 0.5 (mobile phase A) and 0.15 ml/min (mobile phase B). The peaks of verapamil and internal standard were good shapes and well separated from any interfering endogenous peaks during a total run time of 25 min.

P. A. Hynlng et al 16 (1988) performed Liquid-Chromatographic quantification compared with Gas-Chromatographic-Mass-Spectrometric determination of Verapamil and Norverapamil in plasma. The plasma samples were extracted at alkaline pH with hexane containing butanol and then back extracted in to phosphate buffer. Supelcosil LC 18 DB column with mobile phase phosphate buffer:ACN (70:30) was used. Fluorescence detection was used. The assay was accurate and precise.

Dionex application note 17 revealed the determination of Verapamil Hydrochloride Purity Using the Acclaim PA Column. In this application note, they describe a new method for the fast determination of verapamil hydrochloride and verapamil-related compounds A, B, and D, using a polar-embedded reversed-phase column, the Acclaim® PolarAdvantage (PA). Column used was Acclaim PA, 5 μm, 4.6 × 250 mm , Temperature: 35 °C, Mobile Phase: A: 20 mM KH₂PO₄ adjust pH to 3.0 with H₃PO₄ , B: CH₃CN, wavelength at 278 nm was used. Gradient run of 35 min was applied. The new method requires only about half the time of the proposed USP monograph method, provides significant eluent and therefore cost savings, and meets the resolution requirement.

U. Huber 18 (1998) analysed antianginal drugs by HPLC. Column was 4.6 x 75 mm Zorbax SB-C18, 3.5 μm, Mobile phase was A = 0.025 M KH₂PO₄ in water (pH = 3 with H₂SO₄), B = acetonitrile Flow rate 1.0 ml/min, Gradient applied 20 % B to 80 % B in 10 min UV detector was variable wavelength detector: 204 nm, standard cell fluorescence detector. Column temperature 25°C. The method presented showed an easy, reliable and precise analysis of the antianginal drug verapamil. The values for LOD, precision of RT, precision of area and linearity show the good performance of the analysis.

A. Bright1 et al 19 (2010) established application of RP-HPLC and UV-Visible spectroscopy for the estimation of atenolol and verapamil in tablets before and after the expiry period. Chromatography was carried out on a C-18 column using a mobile
phase of 0.02M KH2PO4 buffer solution: methanol: acetonitrile (60:30:10 v/v) for
atenolol. The flow rate was 1ml/min with detection at 275nm. For Verapamil, a
mobile phase of 0.15M sodium acetate buffer: acetonitrile (70:30 v/v) was used. The
flow rate was 2ml/min with detection at 278nm. The calibration curves obtained using
HPLC method was linear in the range 640 – 960 μg/ml-1 for atenolol and 160 – 240
μg/ml-1 for Verapamil. The calibration curves obtained using UV-Visible
spectroscopy was linear in the range 50-150 μg/ml-1 for atenolol and 20-100 μg/ml-1
for Verapamil. The assays of atenolol found using HPLC technique before expiry was
49.87 mg/tablet and after expiry was 44.75mg/tablet. For Verapamil before expiry it
was 39.80 mg/tablet and after expiry 33.41mg/tablet. This was substantiated using
UV-Visible spectroscopy. Recovery studies have been carried out to ensure the
accuracy of the procedure adopted in each case. The high recovery percentage
highlights the accuracy of the method followed.

E. M. Koves et al\textsuperscript{20} (1992) evaluated a photodiode array/HPLC-based system for the
detection and quantitation of basic drugs in postmortem blood. Using selective
extraction, that is, back extraction into 0.2N sulfuric acid and 6N hydrochloric acid
after the initial extraction with toluene under basic conditions (from 2 mL of blood),
basic and weakly basic drugs, such as propranolol and diazepam, can be
simultaneously quantitated and identified with a high degree of confidence. A
microcomputer-based photodiode array detector was used to evaluate peak purity and
facilitate peak identification. The chromatographic conditions developed are suitable
for the screening of several basic, acidic, amphoteric, and neutral drugs. Retention
data and ultraviolet spectral data for 119 drugs on two reversed-phase columns, using
acidic mobile phases, were also presented.

V. Ivanova et al\textsuperscript{21} (2008) developed determination method for verapamil in human
plasma after solid-phase extraction using HPLC. The clean up of the plasma samples
was tested using several adsorbents for solid-phase extraction and recovery obtained
by mixed-mode cartridges (HLB - hydrophilic-lipophilic balance) ranging between
94.70 and 103.71%. HPLC separation was performed with isocratic elution on
Lichrospher 60 RP-select B column (250 mm x 4 mm I.D., 5 microm). The mobile
phase was 40% acetonitrile and 0.025 mol/L KH2PO4 with pH 2.5 having flow rate
of 1 mL/min. Diltiazem was used as internal standard and the detection wavelength
was 200 nm. The calibration curves were linear in the range of 10-500 ng/mL. The developed method was suitable for routine analysis of verapamil in human plasma.

**G. Venkatesh et al** (2007) simultaneously determined buparvaquone, atenolol, propranolol, quinidine and verapamil using RP HPLC UV method. The method was applicable in rat in situ intestinal permeability study to assess intestinal permeability of BPQ, a promising lead compound for Leishmania donovani infections. The method was validated on a C-4 column with mobile phase comprising ammonium acetate buffer (0.02M, pH 3.5) and acetonitrile in the ratio of 30:70 (v/v) at a flow rate of 1.0ml/min. This method was simple, reliable and can be routinely used for accurate permeability characterization.

**Ph. Hubert et al** (1994) determined HPLC analysis of verapamil and norverapamil in plasma using automated solid phase extraction for sample preparation and fluorometric detection. Solid phase extraction (SPE) of the analytes from plasma was done on disposable extraction cartridges (DECs) and reversed phase HPLC with fluorescence detection was used. The DEC filled with endcapped cyanopropyl silica (50 mg) was first conditioned with methanol and phosphate buffer of pH 7.4. A 1.0-mL volume of plasma sample containing the internal standard was then applied on the DEC. The washing step was performed with the same buffer. The analytes were eluted with 0.24 mL of methanol containing 0.2 % of 2-aminoheptane. A 0.41-mL volume of acetate buffer of pH 3.0 was then passed through the DEC and 0.25 mL of the resultant extract was directly introduced into the HPLC system. The absolute recoveries of the drugs were around 95 % and the limit of detection of verapamil was 1.0 ng/mL.

**D. J. Saville et al** (2000) simultaneously analysed S- and R-verapamil and metabolites, S- and R-norverapamil in human plasma by HPLC. Chromatographic separation was achieved using a Chiralcel® OD-RH column (5 μm, 4.6 mm i.d. x 15 cm, Daicel Chemical Industries Ltd., Tokyo, Japan) and a mobile phase consisting of 30 mM hexafluorophosphate and acetonitrile (66: 34, v/v, pH 4.6). Detection of S- and R-verapamil and the metabolites, S- and R-norverapamil was accomplished with a fluorescence detector. The wavelengths of excitation and emission were set at 280
nm and 315 nm respectively. The method was simple because it does not require a column-switching system or organic solvent as a component in the mobile phase, and the sample preparation is more economic as solid-phase extraction was not required. Thus, the assay described was suitable for studying the pharmacokinetics and metabolism of verapamil.

Wahid et al \textsuperscript{25} (2011) developed HPLC method for verapamil in presence of NSAID. The mobile phase consist of acetonitrile : water (55:45) of ph 2.7 at flow rate 2.0 ml/min at 230 nm. The method was cost effective, rapid, simple and precise for simultaneous analysis of verapamil and NSAID.

Brandsteterova E et al \textsuperscript{26} (1999) developed Achiral and chiral HPLC method for monitoring norverapamil and its two metabolites in in-vitro microsomal samples. Norverapamil is a chiral drug with the same ability as verapamil in reversing multi-drug resistance against some cytostatics. R(+) norverapamil is less cardiotoxic as S(-) enantiomer and so it could be applied in higher concentrations. HPLC conditions were worked out for both achiral and chiral assays and solid phase extraction as a preseparation procedure was used for microsomal sample. The total concentrations of norverapamil and its metabolites were calculated from achiral chromatograms and the ratio of enantiomers were determined from chiral separations.

Marina Stefova et al \textsuperscript{27} (2008) developed HPLC method for the assay of verapamil in human plasma. HPLC separation was performed with isocratic elution on Lichrospher 60 RP-select B column (250 mm × 4 mm I.D., 5 μm particle size). The mobile phase was 40% acetonitrile and 0.025 mol/L KH2PO4 with pH 2.5 at flow rate of 1 mL/min. The clean up of the plasma samples was done by using mixed-mode cartridges (HLB - hydrophilic-lipophilic balance) Diltiazem was used as internal standard and the detection wavelength was 200 nm. The calibration curves were linear in the range of 10–500 ng/mL. The developed method is convenient for routine analysis of verapamil in human plasma.

Wieslaw Sawicki et al \textsuperscript{28} (2011) developed sensitive analytical method to determine verapamil and its metabolite norverapamil in human plasma by using HPLC system with fluorescence detection, followinng extraction of the investigated compounds.
Internal standard used was propranolol. The method had very good precision which was supported by validation study. The method was fast with run time of about 8 minutes. It was used successfully in pharmacokinetic and bioavailability studies of verapamil administration in drug formulations.