A comprehensive summary of the work to be incorporated in the thesis entitled “Analytical study of pharmaceutical substances: Method development and validation study: Few case studies” has been described as under,

PART [A]: HPLC METHOD DEVELOPMENT AND VALIDATION OF SOME PHARMACEUTICAL FORMULATION.

The research work undertaken in these studies mainly addresses analysis, development of stability indicating HPLC methods and validation protocol, according to ICH guidelines.

Section-1 Deals with the development and validation of a stability-indicating high performance liquid chromatographic method for assay of lercanidipine hydrochloride in tablets and for determining content uniformity.

A simple, precise and accurate HPLC method has been developed and validated as per ICH guidelines. An isocratic separation was achieved using a Chromasil YMC Pack C8, 150×4.6 mm i.d., 5μm particle size columns with a flow rate of 1 ml/min and using a UV detector to monitor the elute at 240 nm. The mobile phase consisted of 0.02 M ammonium dihydrogen phosphate buffer: methanol (35:65, v/v) with pH 3.5 adjusted with phosphoric acid. The method was validated for specificity, linearity, limit of detection, limit of quantitation, precision, accuracy, robustness and solution stability. The specificity of the method was determined by assessing interference from the placebo and by stress testing of the drug (forced degradation). The method was linear over the concentration range of 20-80 μg/ml ($r^2 = 0.9992$) with a limit of detection and quantitation of 0.1 and 0.3 μg/ml respectively. Intraday and interday system and method precision were determined and accuracy was between 99.3-101.9 %. The method was found to be robust and suitable for assay of lercanidipine hydrochloride in a tablet formulation and for determination of content uniformity. Degradation products resulting from the stress studies did not interfere with the detection of lercanidipine hydrochloride and the assay is thus stability-indicating. Hence, the method is useful for routine quality control analysis and also for determination of stability.
Section-2  Deals with the stability-indicating high performance liquid chromatographic assay for the simultaneous determination of atenolol and lercanidipine hydrochloride in tablets.

The chromatographic separation was achieved on phenomenex Gemini C18 (250×4.6mm, 5 μm) column using a mobile phase consisting of acetonitrile and buffer (20 mM potassium dihydrogen phosphate pH 3.5) in the ratio of (55:45, v/v) at a flow rate of 1.0 ml/min and UV detection at 235 nm. The linearity of the proposed method was investigated in the range of 40-160 μg/ml (r² = 0.9995) for atenolol and 8-32 μg/ml (r² = 0.9993) for lercanidipine. Degradation products produced as a result of stress studies did not interfere with the detection of atenolol and lercanidipine and the assay can thus be considered stability-indicating.

The developed procedure has been evaluated for the specificity, linearity, accuracy, precision, limit of detection, limit of quantification and robustness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate and robust and stability indicating. Hence, the method is useful for routine quality control analysis and also for determination of stability.

PART [B]: UPLC METHOD DEVELOPMENT AND VALIDATION OF SOME PHARMACEUTICAL FORMULATION.

To develop a rapid, simple and reliable ultra performance liquid chromatographic method for the estimation of some active pharmaceutical ingredients from their single and combine pharmaceutical dosage forms by UPLC and to perform the validation procedure for same.

Section-1  Deals with the simultaneous estimation of aspirin (ASP), clopidogrel bisulphate (CLP) and atorvastatin calcium (ATV) from capsule dosage form. Chromatography was carried out at 25°C on a 50 × 2.1 mm i.d., 1.7 μm Acquity BEH C₁₈ column with isocratic mobile phase 0.1% orthophosphoric acid and acetonitrile (55:45, v/v) at a flow rate of 0.35 mL/min. The detection was carried out at 230 nm. The retention times were about 0.59, 1.04 and 2.89 min for ASP, CLP and ATV, respectively. The total runtime was less than 4 min. The method was validated according to ICH guidelines and the acceptance criteria for
accuracy, precision, linearity, specificity and system suitability were met in all cases. The method was linear in the range of 12-48 µg/mL for ASP, 12-48 µg/mL for CLP and 3.2-12.8 µg/mL for ATV. Limit of detection obtained were 0.03 µg/mL for ASP, 0.06 µg/mL for CLP and 0.07 µg/mL for ATV. With the developed method, only this mobile phase is sufficient for quantification of ASP, CLP and ATV either in combination (i.e., ASP + CLP, ASP + ATV, ATV + CLP) or in single dosage form as per availability of formulation for many pharmaceutical industries. It can be successfully used for routine analysis of ASP, CLP and ATV in combined dosage form without any interference from common excipients and impurity.

Section-2 Deals with the RP-UPLC method for the simultaneous estimation of orally administered hypertension drugs (atenolol, hydrochlorothiazide, amlodipine besylate, indapamide, nifedipine and lercanidipine hydrochloride) of which atenolol is administered with anyone of the other five drugs in combined hypertension therapy. Chromatography was carried out at 25°C on a 2.1 × 50 mm i.d., 1.7 µm Acquity BEH C18 column with the isocratic mobile phase of 0.01 M, 4.0 pH, aqueous phosphate buffer and acetonitrile (50: 50, v/v) at a flow rate of 0.35 mL/min. All drugs were separated in less than 4 min with good resolution and minimal tailing, without interference of excipients. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all case. The column effluent was monitored at 230 nm. The detector response was linear in the range of 1-20 µg/mL of these drugs. Limit of detection obtained were 0.04 µg/mL for atenolol, 0.02 µg/mL for hydrochlorothiazide, 0.03 µg/mL for amlodipine besylate, 0.03 µg/mL for indapamide, 0.02 µg/mL for nifedipine and 0.01 µg/mL for lercanidipine hydrochloride. The suggested method has advantage that all the drugs can be quantified alone or in combination with atenolol using single mobile phase.

Many pharmaceutical industries manufacture their formulation of all mentioned drugs either in combination or in single dosage form. Most of the pharmaceutical industries use time consuming method and different mobile phases for different dosage form of drugs. But with the proposed method developed, time and cost required for changing different mobile phases could be saved, because only one mobile phase can be used for six drugs and their combinations. This makes the method suitable for routine analysis in quality control laboratories.