PREFACE

Analytical chemistry is defined as “The science and the art of determining the composition of materials in terms of the elements or compounds contained”. This branch of chemistry, which deals with both theoretical, practical science and practiced in a large number of laboratories in many diverse ways. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. In analytical chemistry it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species that is to find out what substance is composed and exactly how much.

The primary goal of pharmaceutical analysis is to assure drug quality. It is well known that quality can not be test into a product; however, well plan testing with suitable methodology and instrumentation can help build quality into a drug product. It is essential to understand potential degradation reaction that may occur in the the formulated product under various stress conditions that might be encountered during storage and inshipment of final package.

In view of the foregoing discussion, forced degradation (FD) study is a process in which the natural degradation rate of a pharmaceutical product is increased by the application of an additional stress. FD studies (i) help to identify reactions that cause degradation of pharmaceutical product, (ii) are part of the development strategy and an integral component of validating analytical methods that indicate stability and detect impurities which are formed during manufacture, storage, or use and their properties are different from the desired product with respect to activity, efficacy and safety and (iii) are designed to generate product-related variants and develop analytical methods to
determine the degradation products formed during accelerated and longterm stability studies. Any significant degradation product should be evaluated for characterization and quantization for its potential hazard.

Impurities in pharmaceuticals are the unwanted chemicals that can develop during synthesis or with aging of active pharmaceutical ingredient (API). Presence of impurity even in small quantity may influence the efficacy and safety of pharmaceutical products. Hence, there is increasing interest in impurities present in APIs. Recently, impurity profile has become essential components of the drug development process. Administration of two or more drugs at a time becomes imperative for several therapeutic reasons. The multi-component formulations have gained lot of importance now-a-days due to greater patient acceptability, increased potency, multiple action, fewer side effects and quicker relief. The combined dosage form are complex in nature during process of estimation, it is important to confirm that one component does not interfere with estimation of other.

There is a plethora of analysis of such formulations without prior separation. For the estimation of multi-component formulation, the instrumental techniques, which are commonly employed, are spectrophotometry, GLC, HPTLC and HPLC etc., these methods are based upon the measurement of specific and nonspecific physical properties of the substances.

Literature review has revealed that several techniques like HPLC, UPLC, GC, NMR, and UV-Visible spectrophotometry are available for the assay of drugs and impurities but very limited assay methods were available for stability indicating studies. In some cases, no precise, accurate analytical methods are reported and quite often the
reported methods need improvement or changes because of lack of selectivity, specificity and sensitivity with existing methods.

The author attempted for proposed thesis work of stability indicating RP-HPLC method for determination of selected drugs in bulk and pharmaceutical dosage form. The proposed methods were simple, selective, specific and economical than existing methods and also were used for routine analysis in pharmaceutical laboratories.

In the present study, the author has developed and validated seven HPLC methods for the estimation of twelve different drugs, including three impurities of Esomeprazole magnesium trihydrate in bulk samples and pharmaceutical formulations in single and in combined dosage form. The following twelve drugs used in the treatment of different ailments were selected for the present study.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the drug</th>
<th>Chemical structure</th>
<th>Therapeutic use</th>
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<tbody>
<tr>
<td>1</td>
<td>Telmisartan</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>Anti-hypertensive agent</td>
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<tr>
<td>2</td>
<td>Rosuvastatin calcium</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>Anti-lipidemic agent</td>
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<tr>
<td>3</td>
<td><strong>Capecitabine</strong></td>
<td>Anti-cancer agent</td>
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<tr>
<td>4</td>
<td><strong>Gemcitabine hydrochloride</strong></td>
<td>Anti-cancer agent</td>
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<tr>
<td>5</td>
<td><strong>Metformin hydrochloride</strong></td>
<td>Anti-diabetic agent</td>
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<td>6</td>
<td><strong>Saxagliptin</strong></td>
<td>Anti-diabetic agent</td>
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<td>7</td>
<td><strong>Sitagliptin phosphate</strong></td>
<td>Anti-diabetic agent</td>
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<td>8</td>
<td>Lamivudine</td>
<td>Anti-retroviral agent</td>
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<tr>
<td>9</td>
<td>Efavirenz</td>
<td>Anti-retroviral agent</td>
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<td>Tenofovir disoproxil fumarate</td>
<td>Anti-retroviral agent</td>
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<td>Esomeprazole magnesium trihydrate</td>
<td>Anti-ulcer agent</td>
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<tr>
<td>11.2. EP Impurity- E</td>
<td><img src="image1.png" alt="Image" /></td>
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<td>11.3. EP Impurity- C</td>
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<td>12</td>
<td>Propiveriene</td>
<td>Anti-cholinergic agent</td>
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<td></td>
<td>hydrochloride</td>
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In the thesis entitled “Stability Indicating RP-HPLC Method Development and Validation for Determination of Selected Drugs in Bulk and Pharmaceutical Dosage Form” has been described in eight chapters as under,

**CHAPTER-I** includes importance of pharmaceutical analysis in estimation of drugs in bulk and pharmaceutical dosage form and also described the chromaohraphy and introduction of HPLC instrumentation. It includes the method development and validation in terms of accuracy, precision, linearity, range, robustness, ruggedness and specificity by force degradation as per ICH guidelines. It also includes aim and objective.

**CHAPTER-II** deals with the stability indicating RP-HPLC method development and validation for determination of Telmisartan and Rosuvastatin calcium in bulk and pharmaceutical dosage form. Telmisatran is a chemically 4’-([4-methyl-6-(1-methyl-lH-benzimidazol-2yl)-2-propyl-lH-benzimidazol-l-yl][methyl]-2-biphenylcarboxylic acid and
used as anti-hypertensive agent. Rosuvastatin calcium is chemically \((E)-(3R,5S)-7-\{4-(4-fluoro \text{ phenyl})-6-isopropyl-2-\{methyl(methylsulphonylamino)pyrimidin-5-yl\}-3,5\text{-dihydroxy hepten-6-oicacid}\]\text{ calcium and used as anti-lipidemic agent. Only few RP-HPLC methods were reported in literature survey for simultaneous determination of Telmisartan and Rosuvastatin calcium but no stability-indicating HPLC assay method was available in literature for the determination of Telmisartan and Rosuvastatin calcium in pharmaceutical dosage form.}

The author developed stability indicating RP-HPLC method development and validation for determination of Telmisartan and Rosuvastatin calcium in bulk and pharmaceutical dosage form by using column Zodiac C18 (150 mm x 4.6 mm, 5 µm) with mobile phase containing mixture of buffer and acetonitrile in the ratio of (60 : 40% v/v) in an isocratic pump mode. The detection of the drugs was monitored at 241 nm. The retention time for Telmisartan and Rosuvastatin calcium was 3.094 min and 5.404 min respectively.

The method was found to be linear in the range of 8-240 µg/ml and 2-60 µg/ml for Telmisartan and Rosuvastatin calcium with correlation coefficient \(r^2=0.999, \ r^2=0.999\) respectively. The result of forced degradation studies reveals that it was the stability indicating method. Hence the proposed RP-HPLC method was simple, economical, rapid used for routine analysis in quality control laboratories.

**CHAPTER-III** deals the stability indicating RP-HPLC method development and validation for determination of Capecitabine and Gemcitabine hydrochloride in bulk and pharmaceutical dosage form. Capecitabine is chemically Pentyl \(1-(5\text{-deoxy}\beta,D-\text{ribofuranosyl})\)-5-fluoro-1, 2-dihydro-2-oxo-4-pyrimidin, carbamate and used as anti-
cancer agent. Gemcitabine hydrochloride is chemically Cytidine, 2-deoxy-2,2-difluoro monohydrochloride and used as anti-cancer agent. The literature survey revealed that HPLC methods for determination of Capecitabine and Gemcitabine hydrochloride were reported individually. One method was reported for simultaneous analysis but no stability method was reported.

The author developed a stability indicating method was accurate and economical for the determination of Capecitabine and Gemcitabine hydrochloride in bulk and pharmaceutical dosage form. An Inertsil ODS-3v C18 column having dimensions of (250 × 4.6 mm, 5 μm) with mobile phase containing a mixture of 50 mM orthophosphoric acid adjusted to pH 5.50, buffer and acetonitrile (40 : 60 v/v) was used. The pH of mobile phase was adjusted to 5.50. The flow rate was 1.2 ml/min and the column effluents were monitored at 241 nm. The retention time for Gemcitabine hydrochloride and Capecitabine was found to be 2.94 and 4.97 min respectively. The proposed method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. The method was found to be linear in the range of 24-56 μg/ml and 60-140 μg/ml for Gemcitabine hydrochloride and Capecitabine hydrochloride, with regression coefficient $r^2=0.999$ and $r^2=0.999$.

The limit of detection and limit of quantification 6.85 μg/ml, 20.7 μg/ml for Capecitabine and 2.77 μg/ml, 6.85 μg/ml for Gemcitabine hydrochloride respectively. Especially the specificity by force degradation study was conducted for indicating stability of method. The proposed method is simple, rapid and accurate so that the method will be useful for routine quality control analysis.
CHAPTER-IV deals with the stability indicating RP-HPLC method development and validation for determination of Metformin hydrochloride and Saxagliptin in bulk and pharmaceutical dosage form. Metformin hydrochloride is chemically 1,1-dimethyl biguanide hydrochloride and used as anti-diabetic agent. Saxagliptin is chemically \((1S,3S,5S)-2-((2S)-\text{Amino-(3-hydroxytricyclo(3.3.1.13,7)dec-1-yl)acetyl}-2-\text{azabicyclo(3.1.0)hexane-3-carbonitrile}}\) and used as anti-diabetic agent. Various publications were available regarding determination of Saxagliptin and Metformin hydrochloride but most of the methods are less accurate and economical.

The author developed stability indicating RP-HPLC method development and validation for determination of Metformin hydrochloride and Saxagliptin in bulk and pharmaceutical dosage form. Chromatographic separation of Metformin hydrochloride and Saxagliptin was achieved on Inertsil C8 (150 mm x 4.6 mm, 5 µm) and the mobile phase containing pH 2.5 buffer and acetonitrile in the ratio of 70 : 30 v/v. The flow rate was 1.0 ml/min, detection was carried out by absorption at 229 nm using a photodiode array detector at ambient temperature. The retention time of Metformin hydrochloride and Saxagliptin was found to be 2.8 and 5.2 min. The drugs were exposed to thermal, photolytic, hydrolytic, acid, alkali and oxidative stress and the stressed samples were analyzed by use of the proposed method and chromatograms from the stressed samples, obtained by use of the photodiode-array detector. The linearity of the method was excellent over the range 70-750 µg/ml and 0.7-7.5 µg/ml for Metformin hydrochloride and Saxagliptin respectively.
The correlation coefficient was 0.999. The proposed method was validated according to ICH guidelines. The method was found to be suitable, precise and accurate method for quantitative analysis of dosage form and study of its stability.

CHAPTER-V reveals the stability indicating RP-HPLC method development and validation for determination of Metformin hydrochloride and Sitagliptin phosphate in bulk and pharmaceutical dosage form. Sitagliptin phosphate is chemically (R)-4-oxo-4-[3 (trifluoromethyl)5, dihydro[1,24]triazolo[4,3a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2 amine used as anti-diabetic agent. Literature survey revealed that few analytical methods for the determination of Metformin hydrochloride and Sitagliptin phosphate in bulk and pharmaceutical dosage form.

The author developed a new accurate, precise and economical stability indicating RP-HPLC method development and validation for determination of Metformin hydrochloride and Sitagliptin phosphate in bulk and pharmaceutical dosage form. Chromatographic separation of Metformin hydrochloride and Sitagliptin phosphate was achieved on Zodiac C18 (150 mm x 4.6 mm, 5 µm) and the mobile phase containing TEA buffer and methanol in the ratio of 80 : 20 v/v. The flow rate was 1.0 ml/min, detection was carried out by absorption at 224 nm using a photodiode array detector at ambient temperature. The RT of Metformin hydrochloride and Sitagliptin phosphate is found to be 3.9 and 6.3 min. The drugs were exposed to thermal, photolytic, hydrolytic, acid, alkali and oxidative stress and the stressed samples were analyzed by use of the proposed method and chromatograms from the stressed samples, obtained by use of the photodiode-array detector. The linearity of the method was excellent over the range 80-730 µg/ml and 8-70 µg/ml for Metformin hydrochloride and Sitagliptin phosphate.
respectively. The correlation coefficient was 0.999. The proposed method was validated according to ICH guidelines.

**CHAPTER-VI** comprises the stability indicating RP-HPLC method development and validation for determination of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz in bulk and pharmaceutical dosage form. Lamivudine is chemically (2R,5S)-4-amino-L-[2-(hydroxymethyl)-1,3-oxathiolan-5yl]-2(1H)-pyrimidinone and used as anti-retroviral agent. Tenofovir disoproxil fumarate is chemically bis (isopropoxy-carbonyloxy methyl ester of (R)-9-(2-phosphonomethoxy-propyl) adenine with fumaric acid and used as anti-retroviral agent. Efavirenz is chemically (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2, 4-dihydro-1H-3, 1-benzoxazin 2-one and used as anti-retroviral agent.

After literature survey the author attempted an accurate, precise, reproducible, economical, gradient and stability indicating Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the estimation of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz in pharmaceutical dosage form. In this method Waters C18 (75 x 4.6 mm, 5 μm) column with gradient mobile phase containing methanol and water in different ratios were used. The flow rate was 1.0 ml/min. and the detection wavelength was 260 nm. The linearity was observed in the range of 60-450 μg/ml, 60-450 μg/ml and 120-900 μg/ml for Lamivudine, Tenofovir disoproxil fumarate and Efavirenz with correlation coefficient of 0.9992, 0.9992 and 0.9994 respectively. Retention times were 2.394, 5.827 and 9.700 min for Lamivudine, Tenofovir disoproxil fumarate and Efavirenz. Therefore the proposed method can be
applied for routine quality control analysis of pharmaceutical dosage form used for multidrug therapy.


The author attempted simple, precise, accurate and economical stability indicating method was developed and validated as per ICH guidelines. An Phenomenex Prodigy ODS (250 x 4.6 mm, 3 µm) column with mobile phase containing pH 7.6 buffer and methanol in an gradient mode at the flow rate of 1ml/min. at the temperature 40°C with 20 µl volume of injection was used for chromatographic separation. The overall run time of the method was 60 min. The detection was carried out at the wavelength of 300 nm. The established method found to be good linear over the range of 0.1-4 µg/ml for Esomeprazole magnesium trihydrate, 0.1-4 µg/ml EP Impurity-A, EP Impurity-E and EP Impurity-C and the percentage of recovery of Esomeprazole and Impurities was found to
be in the range of 98-102%. The retention time of Esomeprazole magnesium trihydrate, EP Impurity-A, EP Impurity-E and EP Impurity-C was found to be 13.05, 22.67, 26.35 and 38.05 min. The drugs were exposed to thermal, photolytic, hydrolytic, acid, alkali, and oxidative stress and the stressed samples were analyzed by use of the proposed method and chromatograms from the stressed samples, obtained by use of the UV detector.

**CHAPTER-VIII** it deals with the stability indicating RP-HPLC method development and validation for determination of Propiveriene hydrochloride in bulk and pharmaceutical dosage form. Propiveriene hydrochloride is chemically 1-methyl-4-piperidinyl- diphenylpropoxyacetate hydrochloride and used as anticholinergic agent. Literature survey revealed that no method were available for determination of Propiveriene hydrochloride in bulk and pharmaceutical dosage form.

The author developed a new simple, rapid, accurate, precise, sensitive and selective method for the estimation of Propiveriene hydrochloride in bulk and pharmaceutical dosage form. The chromatographic separation was achieved by using column Agilent CN (250 mm x 4.6 mm, 5 µm) and the mobile phase containing OPA buffer : methanol in the ratio of 50 : 50 v/v in an isocratic mode at flow rate of 1 ml/min and detection was carried out at 218 nm. The retention time was 5.22 min The developed method was found to good linear over the range of 18-162 µg/ml. Accuracy studies revealed that mean recoveries were between 98-102%. The results obtained by the forced degradation studies were good enough to say that developed method was stable.

The work represented in chapter II to VI and VIII of the thesis describes the development and validation of new analytical stability indicating methodologies for assay
of some important drugs in bulk and pharmaceutical dosage form and chapter VII describes stability indicating methodology for determination of impurities in bulk and pharmaceutical dosage form by RP-HPLC.

The developed analytical methods are useful for determination of selected drugs in routine analysis in quality control laboratories.

The author published five papers in reputed national and international journals from the entire research work.