CHAPTER 2

RESEARCH ENVISAGED AND DRUG PROFILES

The Pharmacopoeias frequently specify spectrophotometric and high performance chromatographic methods for tests concerning identity and purity.\(^1\) The presence of impurities is an important criterion for evaluating the pharmaceutical quality and is necessarily done to determine the shelf-life after storage or stress tests.\(^2\)

The multi-drug therapy is an ancient phenomenon to combat interrelated symptoms of diseased status of human beings. Since it ensure timely and complete medication for disorder and it has patient compliance, as it reduces the number of formulations to be taken at a time. Therefore, the pharmaceutical formulations with combinations of drugs have shown an increasing trend to counteract other symptoms specific to one drug formulation, and hence analytical chemist will have to accept the challenge of developing reliable methods for estimation of drugs from marketed formulations.

Simultaneous analysis procedures are now being used more frequently for estimation of drugs in multi-component pharmaceutical formulations due to their inherent advantages viz. avoid time consuming extraction and separation, economical in the sense that use of expensive regents is minimized are equally accurate and precise. For the estimation of multi-component formulation, the instrumental techniques, which are commonly employed, are spectrophotometry, gas chromatography, high
performance thin layer chromatography (HPTLC), High performance liquid chromatography etc. The validation of methods has to validate by using same parameters as per ICH guidelines.

New drug and drug combinations are launched in the market day by day. Many new drugs are not official in Pharmacopoeias. Some analytical methods may be available having limitations of validation parameters or not economical. Estimation of drug components is applicable only after prior separation that involves tedious and time-consuming procedure.

Hence, the proposed work was undertaken with an aim to develop analytical methods for estimation of some marketed formulations. In the present work, emphasis is given to develop simple, accurate, precise and rapid methods for estimation of the components in the selected marketed formulations using spectrophotometry and HPLC. The work will lead to new methods which will serve adequately for routine drug analysis in pharmaceutical industries. The compilation of above research work will help the pharma sector by providing methods for routine economical analysis.

In the present study an attempt was made to develop spectrophotometric analytical method for estimation of drugs in bulk and marketed formulations by using simultaneous equations method, simultaneous equations method using area under the curve, graphical absorbance ratio (Q analysis) method, two-wavelength method and derivative spectroscopy. HPLC work was planned depending upon the availability of reported methods. In case a method has already been reported,
attempt was made to provide alternate procedures, which are time and cost efficient. Attempt was made to develop all the best possible and applicable methods to serve as an alternate to each other. The developed methods are validated as per the ICH guidelines.

The marketed formulations which are commonly prescribed or preferred in the treatment of different ailments and new drug or drug formulations as well as marketed formulation for whom simple, precise and economical analytical methods are not available are selected for estimation purpose.

After literature surveys following drug formulations were selected for development of spectrophotometric methods for the estimation of some marketed formulations.

Formulation containing single drug component:

1. Rabeprazole sodium in tablet formulation
2. Serratiopeptidase in tablet formulation

Formulation containing two drug components:

1. Ambroxol and Loratadine in tablet formulation
2. Amitriptyline hydrochloride and Chlordiazepoxide in tablet formulation
3. Amlodipine besylate and Atorvastatin calcium in tablet formulation
4. Aspirin and Atorvastatin calcium in tablet formulation
5. Chlordiazepoxide and Trifluoperazine hydrochloride in tablet formulation
6. Domperidone maleate and Rabeprazole sodium in tablet formulation
Formulation containing three drug components:

1. Ambroxol, Cetirizine dihydrochloride and Phenylephrine hydrochloride in tablet formulation
2. Ambroxol, Salbutamol sulphates and Theophylline in tablet formulation

Following drug formulations were selected for development of HPLC methods for the estimation of some marketed formulations.

Formulation containing single drug component:

1. Mupirocin in Ointment formulation
2. Rabeprazole sodium in tablet formulation

Formulation containing two drug components:

1. Amitriptyline hydrochloride and Chlordiazepoxide in tablet formulation
2. Amlodipine besylate and Atorvastatin calcium in tablet formulation
3. Chlordiazepoxide and Trifluoperazine hydrochloride in tablet formulation
4. Domperidone maleate and Rabeprazole sodium in tablet formulation

Formulation containing three drug components:

1. Ambroxol, Cetirizine dihydrochloride and Phenylephrine hydrochloride in tablet formulation

Formulation containing four drug components:

1. Ambroxol, Levocetirizine dihydrochloride, Phenylpropanolamine hydrochloride and Paracetamol in tablet formulation.
Literature survey for each of the drugs of selected marketed formulations revealed the following information on general properties and methods of analysis in bulk, in single and multicomponent formulations and in biofluids.
2.1. RESEARCH ENVISAGED

There are many single and multicomponent formulations available in the market but for very few drug formulations, official methods are prescribed by the pharmacopoeia. Almost all official methods available only after prior separation, which is tedious, time consuming and not economical. The proposed estimation procedures avoid time consuming extractions and separations and are economical in the sense that use of expensive reagent is minimized. Hence, the proposed work was undertaken with an aim to develop techniques for estimation of single and multicomponent drug combinations available in the market. The work will lead to new methods which will serve adequately for routine estimation of marketed formulations in pharmaceutical industries. The compilation of above scientific work will help drug industry by providing methods for routine economical analysis.

The emphasis was put on to develop simple accurate precise and rapid methods for estimation of the components in the selected marketed formulations using spectrophotometry or HPLC.

For spectrophotometry, techniques used for estimation are multicomponent spectroscopy, two wavelength method, derivative spectroscopy, simultaneous equation method (Vierordt’s method), simultaneous equation using area under the curve and graphical absorbance ratio (Q analysis) method. HPLC work was planned depending upon the availability of reported methods. In case a method
has already been reported, attempt was made to provide alternate procedure which are time and cost efficient. Attempt was made to develop all the best possible and applicable methods to serve as an alternate to each other.

Formulation excipients are the pharmaceutical necessities and may absorb UV radiation. Methods are available for separation or extraction of interfering components. In some marketed formulations, excipients were found interfering. It was not possible to eliminate the effect of interference by any such methods or proper selection of solvent. Hence, a generalized approach to overcome such type of unwanted interference has been proposed.

Following drug formulations were selected for development of analytical methods.

1. Mupirocin in ointment formulation.
2. Rabeprazole sodium in tablet formulation.
3. Serratiopeptidase in tablet formulation.
5. Amitriptyline hydrochloride and Chlordiazepoxide in tablet formulation.
6. Amlodipine besylate and Atorvastatin calcium in tablet formulation.
7. Aspirin and Atorvastatin calcium in tablet formulation.
8. Chlordiazepoxide and Trifluoperazine hydrochloride in tablet formulation.
9. Domperidone maleate and Rabeprazole sodium in tablet formulation.

10. Ambroxol, Cetirizine dihydrochloride and Phenylephrine hydrochloride in tablet formulation.

11. Ambroxol, Salbutamol sulphate and Theophylline in tablet formulation.

12. Levocetirizine dihydrochloride, Phenylpropanolamine hydrochloride, Paracetamol and Ambroxol in tablet formulation.

Literature review reveals that no analytic method is available for formulations containing mupirocin, serratiopeptidase, Ambroxol, Loratadine, Chlordiazepoxide, Trifluoperazine, Cetirizine dihydrochloride and Phenylephrine hydrochloride as one of the drugs of the formulation. Hence, few simple and rapid spectrophotometric methods, which are precise and accurate, were developed for formulations containing these drugs.

1. The Pharmacopoeia specifies liquid chromatographic method for estimation of mupirocin but no any analytical method is available for estimation of marketed formulation of mupirocin. In this proposed work presented a new way to determine mupirocin in pharmaceutical products (ointments) by RP-HPLC method. This technique was directly applied to determine mupirocin (without any pre-treatment) rapidly and precisely in marketed samples.
2. Rabeprazole is not official in Pharmacopoeia. Literature survey for rabeprazole revealed the availability of HPLC, HPTLC method in presence of its degradation products and HPLC method in human plasma by using solid-phase extraction. There is no any UV-spectrophotometric method and RP-HPLC method has been reported for routine quality control analysis. The proposed work describes a simple, selective, sensitive and reproducible difference spectrophotometric and RP-HPLC methods for the estimation of RPZ in marketed tablet formulations. The HPLC method could be utilized for more specific than the spectrophotometric methods, but it is a more costly method. However, the methods are presently considered more reliable and promising for the routine analysis of RPZ in pharmaceutical dosage forms.

3. Serratiopeptidase is a metalloenzyme derived from bacteria belonging to genus Serratia. Tablet preparations are available alone or in combination with other drugs in the market. Literature review reveals that no analytical method is available for formulations containing serratiopeptidase, as one of the drugs of the formulation. Hence, first order derivative spectrophotometric methods have been developed.

4. The commercially available marketed tablet dosage form Lorfast-AM® contains loratidine (LRT) and ambroxol (AMB) with the wide difference in concentrations (1:12) and different analytical procedures have been reported for the determination of each one of them alone. No methods have been reported for their simultaneous quantification in their mixtures.
Thus the proposed study is to establish simple methods for their simultaneous determination suitable for quality control purposes.

5. The combination of amitriptyline hydrochloride and chlordiazepoxide is used in therapeutics as antipsychotic. It is used to treat depressive illness accompanied by anxiety, agitation, restlessness and disturbances of sleep. For the formulations containing amitriptyline and chlordiazepoxide, no analytical method is cited in literature. Hence, rapid and accurate spectrophotometric and binary mixture resolution by HPLC methods was developed for formulations containing amitriptyline and chlordiazepoxide.

6. Various methods are available for estimation of amlodipine alone\textsuperscript{50,53-55,78}, or in combination with other drugs like felodipine\textsuperscript{51} enalapril maleate\textsuperscript{57,59} lisinopril\textsuperscript{58}, benazepril\textsuperscript{60,76,82}, atenolol\textsuperscript{77,83} but no any method is available in combination with atorvastatin. Hence some spectrophotometric and RP-HPLC methods are proposed for simultaneous estimation from marketed tablet formulation.

7. Aspirin is non steroidal anti-inflammatory agent and atorvastatin is antihyperlipidemic agent. The combination is prescribed to treat cardiovascular disorder like hypertension with angina pectoris. Aspirin combinations are available in market with other drugs like paracetamol, caffeine and phenobarbital\textsuperscript{104}, dipyridamol\textsuperscript{86}, isosorbide 5-mononitrate\textsuperscript{105}. Aspirin and atorvastatin is new combination available in market. The literature survey revealed that high-performance liquid-chromatography analytical method has been reported for aspirin and related compounds
simultaneously. No spectrophotometric method was available for estimation of marketed formulation containing both the drugs. Hence spectrophotometric methods are proposed for simultaneous estimation of both the drugs from marketed formulations.

8. Simultaneous determination of chlordiazepoxide and clidinium bromide in pharmaceutical formulations by derivative spectrophotometry is reported. Determination of chlordiazepoxide, diazepam and their major metabolites in blood or plasma by spectrophotodensitometry is reported. Determination of trifluoperazine with promethazine by bead injection spectroscopy-flow injection analysis is reported. There is no method reported for the simultaneous estimation of CDZ and TFP from a combined dosage form. The proposed investigation gives simple, accurate and economical procedures for simultaneous estimation of CDZ and TFP from tablet dosage form by using spectrophotometric and RP-HPLC methods.

9. Domperidone combination are available with drugs like cinnarazine, paracetamol, ranitidine, omeprazole, rabeprazole. Several methods for estimation have been reported in multi component drug formulations of domperidone with other drugs. There is no method reported for the simultaneous estimation of domperidone and rabeprazole from a combined dosage form. Attempt was made to improve sensitivity of measurements for this formulation by spectrophotometry and RP-HPLC.
10. Combinations of decongestant and antihistamine pharmaceutical preparations are widely used for cough and cold treatments. Phenylephrine hydrochloride, cetirizine dihydrochloride and ambroxol is bronchosecretolytic. The three component tablet formulation is advised as nasal decongestant in allergy with cough. The combination is not yet official in any pharmacopoeia. High performance thin layer chromatographic method is available for cetirizine and ambroxol in tablets but no any method is available for estimation of three component tablet formulation containing cetirizine dihydrochloride, phenyl ephrine hydrochloride and ambroxol. The proposed work was to investigate the utility of multicomponent spectrophotometry, derivative spectrophotometry, and RP-HPLC method for the estimation of these drugs from marketed formulation.

11. Salbutamol hydrochloride is anti-mucolytic drug and theophyllin is xanthine derivative that relaxes smooth muscles, relieves bronchospasm and has a stimulant effect on respiration Ambroxol is having expectorant and bronchosecretolytic effect. Typical anti-mucolytic drugs called salbutamol hydrochloride and theophylline and ambroxol hydrochloride encountered in tablets were determined simultaneously either by ultraviolet spectrophotometry method in solid dosage forms. No any method is available for estimation of three component tablet formulation containing salbutamol sulphates, theophylline and ambroxol combination hence, some spectrophotometric methods are proposed which are simple, precise, accurate and economical.
12. New marketed tablet formulation containing Levocetirizine dihydrochloride, phenylpropanolamine hydrochloride, paracetamol and ambroxol is indicated for seasonal allergic rhinitis, acute allergic reactions due to drugs, food or insect bites. No analytical method has been reported for the simultaneous determination of LCT, PPA, PML and AMB in a multicomponent tablet dosage forms. The proposed RP-HPLC method is simple, accurate and specific and can therefore be applied to the determination of the cited drugs in four-component pharmaceutical preparations.

2.2. PLAN OF WORK

2.2.1 General steps involved in spectrophotometric analysis:

(i) Literature survey- A detailed account of all analytical methods existing for analysis of the drugs is required to avoid duplication of method developed and solvents or reagents used. From the survey we get an idea about the solubility, absorbance maxima, and molar absorptivities in various solvents and stability profile of the drug, on the basis of which details for the new method can be deduced.

(ii) Defining- defining the analytical problem that needs to be solved.

(iii) Sampling-Procurement of representative samples of drugs or chemicals.

(iv) Establishment of Working Conditions:
(a) Type of instrument: A major factor which determines accuracy regarding reproducibility of results. Validation of instrument fulfills the purpose.

(b) Selection of proper solvent system for analysis: A solvent or solvent mixture in which the drug components of the formulation are soluble and stable are selected. Another point that needs consideration in the absorbance maxima of the components in the selected solvent. Greater the difference in absorbance maxima, better will be the results.

(c) Selection of sampling wavelength: Sampling wavelength are selected considering the peaks and valleys in the UV spectra of the individual components and the other wavelengths where the components show a difference in absorbance.

(d) Selection of optimum concentration range and absorbance value: A calibration curve is prepared from a series of standard solutions. These standards should approximate overall composition of the actual samples and should cover a reasonable concentration range of the analyte. Concentration range of the analyte should be selected such that it must obey Beer's law in that range.

(e) Selection of mixed standards: The selection of mixed standards and the concentration of each component in the mixed standard is also important criteria. The concentration of each component in the mixed standards is determined from the molar absorptivity values of the component and the ratio in which the different components are present in the formulation to be analysed. The number of mixed standards to be
used, is determined by carrying out the analysis using varying number of mixed standards and keeping into view the accuracy and reproducibility of the results.

(v) To develop Spectrophotometric analytical methods for estimation of drugs in marketed formulations by using multicomponent mode of analysis, simultaneous equation method, first order derivatives zero crossing method, absorbance ratio method, two wavelength method and simultaneous equation method using area under the curve.

(vi) To develop high performance liquid chromatographic method for estimation of drugs in marketed formulations.

(vii) To validate the developed methods as per the ICH guidelines- In order to validate, authentic laboratory samples are prepared and analyzed by the proposed method. This step is important because it ascertains the effect of interfering excipients. The marketed formulations are subjected to analysis by the same method.

2.2.3 General steps involved in HPLC analysis:

(i) Review of literature available for the analysis of selected drugs in single and multicomponent marketed formulations.

(ii) Critical examination of the structure of the drugs and their physicochemical properties to select the chromatographic parameters for resolution

(iii) Selection of the method for quantitative chromatographic analysis.

(iv) Common solvents or ratio of solvents in which the drug components are resolved separately and intensely.
(v) Analysis of formulations with the method developed.

(vi) Performing recovery studies and statistical evaluation of the results of formulation analysis.

(vii) Wherever HPLC and spectrophotometric methods have been developed for formulation, comparison of methods with each other.
2.3 Drug profiles

2.3.1 Ambroxol hydrochloride (AMB)

Ambroxol is official in British Pharmacopoeia\textsuperscript{1}. Drug is available in combination form with N acetyl cysteine as exclusive respiratory antioxidant as a co-prescription in pulmonary tuberculosis, AIDS, COPD. Marketed formulations contain combination of ambroxol with the drugs like terbutaline, guiphenesin, menthol, salbutamol, theophylline, cetirizine, Phenylephrine, phenylpropanolamine, paracetamol or loratadine.

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graph
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**Chemical Name:** trans-4- \([(2\text{-Amino-3, 5-dibromobenzyl}) \text{ amino}]\) cyclohexanol hydrochloride

**Molecular formula:** $C_{13}H_{18}Br_2N_2O \cdot HCl$

**Molecular weight:** 414.57

**Melting Point:** 235-240° C

**Description and solubility:** It is a white to yellowish crystalline powder. Slightly soluble in water, soluble in dimethylformamide, methanol, ethanol and insoluble in chloroform.

**Category:** Expectorant and bronchosecretolytic
Literature cited

Ambroxol is a compound with potent mucolytic activity, for which it is used as an expectorant and bronchosecretolytic in therapeutics\(^7\). Ambroxol stimulates the transportation of the viscous secretion in the respiratory organs and reduces the standstill ness of the secretion.

Ambroxol hydrochloride can be found in pharmaceutical preparations such as drops, granules, injections, syrups and tablets. Several methods have been used for the individual determination of ambroxol hydrochloride in pharmaceutical solutions and tablets including colorimetry\(^8\)-\(^9\), spectrophotometry\(^10\)-\(^15\), HPLC\(^16\)-\(^22\), HPLC-MS\(^23\), TLC\(^24\)-\(^25\), HPTLC\(^26\), capillary isotochophoresis\(^27\), flow injection\(^28\)-\(^29\) and capillary electrophoresis\(^30\). Some complex methods have been reported for ambroxol determination in biological fluids\(^7\)-\(^8\),\(^30\)-\(^31\) and by Raman spectroscopy\(^32\).

2.3.2 AMITRIPTYLINE HYDROCHLORIDE (AMT)

Amitriptyline is official in British Pharmacopoeia1, Indian Pharmacopoeia2, and United States Pharmacopoeia3. Tablet formulations are available in market containing 10, 25, 50 and 75 mg of drug. It is also given in combination with chlordiazepoxide and chlorpromazine.
Chemical Name: 3-(10,11-dihydro-5H-dibenzo[a,d]cycloheptene-5-ylidene)-N,N-dimethyl-1-propanamine hydrochloride

Molecular formula: C_{20}H_{23}N.HCl

Molecular weight: 313.87

Melting Point: 195-199°C

Description and solubility: Colourless crystals or white powder. Soluble 1 in 1 of water, 1 in 1.5 of ethanol, 1 in 56 of acetone, 1 in 1.2 of chloroform, and 1 in 1 of methanol; practically insoluble in ether.

Category: Amitriptyline hydrochloride is extensively used in the treatment of emotional and psychiatric disorders in which the major symptom is depression, particularly endogenous depression.

Literature cited for analysis:
The official methods of British Pharmacopoeia and United States Pharmacopoeia include non-aqueous titration and titrimetric method with perchloric acid for estimation of drug. Analytical methods for the determination of AMT and with other drugs includes Gas chromatography, colorimetry, HPLC, spectrophotometry, ion-pair chromatography, potentiometric method and atomic absorption spectroscopy.
2.3.3 AMLODIPINE BESYLATE (AML)

Amlodipine besylate is official in British Pharmacopoeia\textsuperscript{1}. Amlodipine besylate is calcium channel blocker, is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the trans membrane influx of calcium ions into vascular smooth muscle and cardiac muscle. It is used as an antihypertensive and antianginal drug\textsuperscript{6}. The tablets are formulated as white tablets equivalent to 2.5, 5 and 10 mg of amlodipine for oral administration. In addition to the active ingredient, amlodipine besylate, each tablet contains the following inactive ingredients: microcrystalline cellulose, dibasic calcium phosphate anhydrous, sodium starch glycolate, and magnesium stearate.

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\begin{array}{c}
\text{Chemical Name:} \quad 3\text{-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-2(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylatemono besylate.} \\
\text{Molecular formula:} \quad C_{26}H_{31}ClNO_2O_3S \\
\text{Molecular weight:} \quad 566.9 \\
\text{Melting Point:} \quad 178-179^\circ \text{C} \\
\text{Description and solubility:} \quad \text{Amlodipine besylate is a white crystalline powder. Soluble in aqueous acid, ethanol, ethyl acetate, slightly soluble in water and sparingly soluble in chloroform.}
\end{array}
\]
**Category:** Calcium channel blocker is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the trans membrane influx of calcium ions into vascular smooth muscle and cardiac muscle. It is used as an antihypertensive and antianginal drug.\(^6\)

**Literature cited for analysis:** Survey of literature reveals that AML is estimated by colorimetric\(^{48-51}\), spectrophotometric\(^{52-60}\), HPLC\(^{61-78}\), GC\(^{79}\), HPTLC\(^{80-83}\) and voltametry\(^{84}\) methods.

### 2.3.4 ASPIRIN (ASP)

Aspirin is official in British Pharmacopoeia\(^1\), Indian Pharmacopoeia\(^2\) and United States Pharmacopoeia\(^3\). Marketed tablet formulation contains 50, 60, 75, 150, 162.5 or 500mg of drug. Delayed release and micro fine form of tablet formulations are also available. It is given in combination with paracetamol, caffeine, clopidogrel or atorvastatin.

![Chemical Structure of Aspirin](attachment:image)

**Chemical Name:** 2-Acetoxybenzenecarboxylic acid

**Molecular formula:** \(C_9H_8O_4\)

**Molecular weight:** 180.57

**Melting Point:** 143°C
Description and solubility: Colorless or white crystals or white crystalline powder or granules. It is stable in dry air but gradually hydrolyses in contact with moisture to acetic and salicylic acids. Soluble 1 in 300 of water, 1 in 5 of ethanol, 1 in 17 of chloroform, and 1 in 10 to 15 of ether; soluble in solutions of acetates and citrates and, with decomposition, in solutions of alkali hydroxides and carbonates.

Category: Aspirin\textsuperscript{1-3,6} chemically known as acetyl salicylic acid is used as non-steroidal anti-inflammatory and analgesic drug. It also produces thrombolytic effect in long-term low doses.

Literature cited for analysis:
Dosage forms of acetylsalicylic acid and its combinations with other drugs have been listed in various pharmacopoeias\textsuperscript{1-3}. Several methods are described in these pharmacopoeias and literatures for the quantitative determination of acetylsalicylic acid and its combinations with other drugs including titrimetry\textsuperscript{1,3-4,6}, colorimetry\textsuperscript{5}, fluorimetry\textsuperscript{85-86}, spectrophotometry\textsuperscript{87-92}, high-performance liquid chromatography\textsuperscript{3,93-107} in pharmaceutical preparations. Among the various analytical techniques, high-performance liquid chromatography (HPLC) constitutes the most popular chromatographic method for separating mixtures of drugs and their degradation products.
2.3.5 ATORVASTATIN CALCIUM (ATV)

Atorvastatin is not official in Pharmacopoeia. It is prescribed along with antihypertensive or antiarrythmic drugs. A marketed tablet formulation contains 10mg and 20 mg of ATV. It is also recommended in combination with aspirin, fenofibrate, ezatimib or amlodipine.

![Chemical Structure of Atorvastatin Calcium](image)

**Chemical Name:** Atorvastatin calcium is [R-(R*, R*)]-2-(4-fluorophenyl)-βδ-dihydroxy-5- (1-methylethyl)-3-phenyl-4- [(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate

**Molecular formula:** \( C_{66}H_{68}CaF_2N_4O_{10}, 3H_2O \)

**Molecular weight:** 1209.4

**Melting Point:** 159.2-160.7°C

**Description and Solubility:** Atorvastatin calcium is a white to off-white crystalline powder that is insoluble in aqueous solutions of pH 4 and below, very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol.

**Category:** Atorvastatin calcium is a second generation HMG-CoA reductase inhibitor recently approved for clinic use as a cholesterol-lowering agent. Atorvastatin is a potent inhibitor of (HMG-CoA), the rate-limiting enzyme in cholesterol biosynthesis. This synthetic HMG-CoA reductase inhibitor induces a significant reduction in total cholesterol, low-
density lipoprotein cholesterol, and plasma triglycerides in clinical studies\textsuperscript{108-109}.

**Literature cited for analysis.**

Existing validated assays for ATV included an enzyme inhibition assay\textsuperscript{110} and a gas chromatography/mass spectrometry (GC/MS) assay\textsuperscript{111}. Reviewing the literature revealed that all the reported methods for the determination of atorvastatin biological matrices rely on the use of chromatographic techniques such as LC/MS/MS, microbore LC/ ESI-MS/MS and HPLC with electrospray tandem mass spectrometry\textsuperscript{112-117}, HPLC\textsuperscript{118-123} and spectrophotometric methods\textsuperscript{124}.

**2.3.6 CETIRIZINE DIHYDROCHLORIDE (CET)**

Cetirizine dihydrochloride is official in British Pharmacopoeia\textsuperscript{1}. Marketed tablet formulations contain 5 or 10mg of drug in combination with paracetamol, phenylephrine, pseudoephedrine, ambroxol or phenylpropanolamine.

![Chemical Structure of Cetirizine Dihydrochloride](image)

**Chemical Name:** (RS)-2-[2-[4-[(4-phenylmethyl] piperzin-1-yl] ethoxy] acetic acid dihydrochloride

**Molecular formula:** C\textsubscript{21}H\textsubscript{27}Cl\textsubscript{3}N\textsubscript{2}O\textsubscript{3}

**Molecular weight:** 461.8

**Melting Point:** 110-115°C
Description and solubility: A white or almost white powder, freely soluble in water, practically insoluble in acetone and in methylene chloride.

Category: Second-generation histamine H₁ antagonist possessing an effective treatment for a wide range of allergic diseases¹²⁵.

Literature cited for analysis:

Cetirizine is the carboxylated metabolite of hydroxyzine, and it has high specific affinity for histamine H₁ receptors¹²⁶. Recently, several HPLC³ and liquid chromatography-mass spectrum (LC/MS)¹²⁶,¹²⁸-¹²⁹ methods have been reported to detect cetirizine in plasma. Other methods are spectrophotometric¹³⁰ and HPLC¹³⁰-¹³⁶ is reported.

2.3.7 CHLORDIAZEPOXIDE (CDZ)

Chlordiazepoxide was the first benzodiazepine to be used clinically with general properties similar to those of diazepam¹³⁷. Chlordiazepoxide is official in B.P¹, I.P² and USP³. Tablet formulations are available in the market containing 10 and 20 mg of drug. It is also available in combination form along with trifluoperazine hydrochloride or amitriptyline hydrochloride.
Chemical Name: 7-Chloro-N-methyl-5-phenyl-3H-1,4-
benzodiazepin-2-amine 4-oxide

Molecular Formula: C_{16}H_{14}ClN_{3}O

Molecular weight: 299.8

Melting Point: 236-236.5°C

Description and solubility: A yellow crystalline powder and sensitive to
sunlight. Practically insoluble in water; soluble 1 in 50 of ethanol and 1 in
130 of ether; slightly soluble in chloroform.

Category: Anxiolytic.

Literature cited for analysis:
The methods for quantitative estimation of chlordiazepoxide include liquid
chromatography^{2-3,138-143}, spectrophotometric^{144-146}, TLC^{142-143,147-148},
spectrodensitometry^{149}, and voltametry^{150} have been reported.
2.3.8 Domperidone maleate (DOM)

Domperidone maleate is official in British Pharmacopoeia\textsuperscript{1}. It is potent dopamine antagonist with antiemetic properties. Tablet, syrup, suspension and drop formulations are available in market. Marketed tablet formulations contain 5 or 10 mg of drug in combination with pantoprazole, rabeprazole, ranitidine, paracetamol or famotidine.

![Chemical Structure of Domperidone Maleate](image_url)

**Chemical Name:** 5-chloro-1-\{1-\[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)propyl\]-4-piperidinyl\}-1,3-dihydro-2H-benzimidazol-2-one

**Molecular formula:** \( C_{22}H_{24}N_5O_2 \cdot Cl \)

**Molecular weight:** 425.92

**Melting Point:** 242°C

**Description and solubility:** White or almost white powder, practically insoluble in water, slightly soluble in methanol and ethanol. It is soluble in dimethylformamide and freely soluble in aqueous solution of alkali hydroxide and ammonia.

**Category:** Potent gastrokinetic and antinauseant drug that is apparently devoid of central sedative and autonomic effects\textsuperscript{151}.

**Literature cited for analysis:**

DOM has been determined by voltammetry\textsuperscript{152}, colorimetry\textsuperscript{153-154}, Spectrophotometry\textsuperscript{155-156}, HPLC\textsuperscript{157-163} and HPTLC\textsuperscript{164}. 
Radioimmunoassay\textsuperscript{165-167} was the only method reported previously for the determination of unchanged domperidone in biological samples.

2.3.9 LORATADINE (LRT)

Loratadine is official in United States Pharmacopoeia\textsuperscript{3}. Syrup, suspension and Tablet formulations are available in market. 10 mg tablets are indicated as H1 receptor antagonist, penetrates very poorly into the central system, hence is devoid of CNS depressant effects. It also suppresses mast cell mediator release. The drug is available in combination with ambroxol.

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\text{Chemical Name: } 4-(8\text{-chloro-5, 6 dihydro-11 H-benzo-} \\
[5,6] \text{cyclohepta[1,2-b]pyridin-11-yldene). 1-piperidine carboxylic acid ethyl ester}
\]

\textbf{Molecular formula: } C_{22}H_{23}ClN_{2}O_{2} \\
\textbf{Molecular weight: } 382.89 \\
\textbf{Melting Point: } 132-137^{\circ}C
Description and solubility: Loratadine occurs as white or white to off-white powder crystals, insoluble in water, but soluble in alcohol and ether, very soluble in acetone, alcohol, and chloroform.

**Category:** Potent long active tricyclic non sedating-histamine H1-receptor antagonist\(^{168-169}\).

**Literature cited for analysis:** Loratadine is a long acting non-sedating histaminic agent that was developed for the treatment of seasonal allergic rhinitis\(^{170}\), whose anti-histaminic action is more effective than the other anti-histaminic drugs available commercially.

Various analytical procedures have been reported for quantitative determination of loratadine both in dosage forms and in biological fluids, include spectrophotometry\(^{171-176}\), atomic absorption spectrometry\(^{177}\), colorimetry\(^{177}\), densitometry\(^{178}\), polarography\(^{179-180}\), HPLC\(^{181-183}\), HPLC-MS\(^{184-185}\), GLC\(^{186}\) and GC-MS\(^{187-188}\) and capillary electrophoresis\(^{189}\).

### 2.3.10 MUPIROCIN (MPR)

Ointment and cream formulation are available in market containing 2% w/w of mupirocin. The drug is official in British Pharmacopoeia\(^1\) and United States Pharmacopoeia\(^3\). It is very effective in the treatment of skin infection (pyoderma) with dermatoses as well as in dermatoses with secondary skin infection.
**Chemical Name:** (2E)-5,9-Anhydro-2,3,4,8-tetrahydroxy-8-[(2S,3S)-3-[1S,2S)-hydroxy-1-methyl-propyl]]oxiranyl[methyl]-3-methyl-1-talo-non-2-enonic acid, 8 carboxyoctyl ester.

**Molecular formula:** $C_{26}H_{44}O_9$

**Molecular weight:** 500.62

**Melting Point:** 77-78°C

**Description and solubility:** Major component of the pseudomic acids, an antibiotic complex produced by Pseudomonas fluorescens. An off-white crystalline solid. It is soluble in dehydrated alcohol, acetone and chloroform; slightly soluble in water and ether.

**Category:** Topical antibacterial\(^{190}\)

**Literature cited for analysis:** The analytical methods reported for the determination of mupirocin include high-performance liquid chromatographic analysis of mupirocin in polyethylene glycols 400 and 3350 using dual ultraviolet and evaporative light scattering detection is
Also use of ion chromatography as an alternative method for the analysis of calcium in calcium mupirocin is reported. 

### 2.3.11 PARACETAMOL (PML)

Paracetamol having synonym as acetaminophen is official in British Pharmacopoeia, Indian Pharmacopoeia, and United States Pharmacopoeia. Various formulations are available in market in combination with different categories of drugs like antihistaminics, antitussive and expectorants, non opoid analgesics, musculoskeletal relaxants.

![Chemical Structure of Paracetamol](image)

**Chemical Name:** 4-hydroxyl acetanilide.

**Molecular formula:** \( C_8H_9NO_2 \)

**Molecular weight:** 151.16

**Melting Point:** 168-172°C

**Description and Solubility:** White crystals or crystalline powder. Very slightly soluble in cold water, considerably more soluble in hot water; soluble in ethanol, methanol, dimethylformamide, ethylene dichloride, acetone, and ethyl acetate; very slightly soluble in chloroform; slightly soluble in ether; practically insoluble in petroleum ether, pentane, and benzene.
Category: Analgesic and antipyretic

Literature cited for analysis:

Indian Pharmacopoeia\(^2\) and British Pharmacopoeia\(^1\) specifies titrimetric method and USP specifies spectrophotometric method for the estimation. PML has been determined by colorimetry\(^{193}\), spectrophotometrically\(^{194-211}\), HPLC\(^{212-229}\), TLC\(^{230}\), HPTLC\(^{231}\), GC\(^{232}\), FTIR\(^{233-234}\), Potentiometric\(^{235}\) and by ion–pair chromatography\(^{236}\).

2.3.12 PHENYLEPHRINE HYDROCHLORIDE (PEP)

Phenylephrine is official in Indian Pharmacopoeia\(^2\) and Phenylephrine hydrochloride is official in British Pharmacopoeia\(^1\) and United States Pharmacopoeia\(^3\). It is a frequent constituent of nasal decongestant preparations. Injectable formulations are indicated for hypotension and to maintain adequate level of blood pressure during spinal and inhalation anesthesia. Tablet formulations are combined with cetirizine, paracetamol, ambroxol as decongestant.
**Chemical Name:** \((1R)-1-(3\text{-Hydroxyphenyl})-2\text{-}(methylamino)\text{ethanol hydrochloride}\)

**Molecular formula:** \(C_9H_{13}NO_2, \text{HCl}\)

**Molecular weight:** 203.7

**Melting Point:** 140-145°C

**Description and solubility:** A white or almost white, crystalline powder. It is freely soluble in water and in alcohol.

**Category:** Sympathomimetic, to relieve nasal congestion.

**Literature cited for analysis:**

Several methods have been described for the quantitative determination of phenylephrine hydrochloride in combination with other drugs, including spectrophotometry\(^{237}\) and HPLC\(^{238}\) in pharmaceutical preparations either separately or in combination with other drugs.

### 2.3.13 PHENYLPROPANOLAMINE HYDROCHLORIDE (PPA)

Phenylpropanolamine hydrochloride is official in British Pharmacopoeia\(^1\) and United States Pharmacopoeia\(^3\). The drug is given in combination with paracetamol, caffeine, chlorpheniramine maleate, bromhexine and guaiphenesin as expectorant, antitussives and mucolytics.

![Chemical Structure](image)

**Chemical Name:** \((\alpha S)-rel-\alpha\text{-}[\(1R\)-1-Aminoethyl]benzenemethanol}
Molecular formula: C₉H₁₃NOHCl

Molecular weight: 187.7

Melting Point: 194-197°C

Description and solubility: A white to creamy-white crystalline powder. Soluble 1 in 1 of water and 1 in about 7 of ethanol; practically insoluble in chloroform and ether.

Category: Sympathomimetic

Literature cited for analysis:
Raman spectroscopy²³⁹, Spectrophotometric method²⁴⁰-²⁴¹, HPLC²⁴²-²⁴⁵, Capillary electrophoresis²⁴⁶ and conductimetric²⁴⁷ method.

2.3.14 RABEPRAZOLE SODIUM (RPZ)

Rabeprazole sodium is not official in pharmacopoeia. Rabeprazole sodium is available for oral administration as delayed-release; enteric-coated and uncoated tablets containing 10 or 20 mg of drug.

Chemical Name: 2-[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl]-1H-benzimidazole sodium.

Molecular formula: C₁₈H₂₀N₃NaO₃S

Molecular weight: 381.43

Melting Point: 140-141°C
**Description and solubility:** Rabeprazole sodium is a white to slightly yellowish-white solid. It is very soluble in water and methanol, freely soluble in ethanol, chloroform and ethyl acetate and insoluble in ether and n-hexane.

**Category:** It is substituted benzimidazoles that suppresses gastric acid secretion by the specific inhibition of the (H⁺ / K⁺ AT-Pase) enzyme at the secretary surface of the gastric parietal cell and hence are called proton pump inhibitors. It is indicated for the treatment of active duodenal ulcer, active benign ulcer, symptomatic erosive or ulcerative gastro-oesophageal reflux disease (GERD), proton pump inhibitor (PPI) that inhibits gastric acid secretion via interaction with (H⁺/K⁺) AT-Pase in gastric parietal cells.

**Literature cited for analysis:** RPZ has been determined by colorimetrically, spectrophotometry, HPLC-MS, HPLC, voltametrically and by capillary electrophoresis method.

**2.3.15 SALBUTAMOL (SBT)**

Salbutamol is official in Indian Pharmacopoeia and salbutamol sulphate is official in British Pharmacopoeia. The drug is given in combination with theophylline, ambroxol or bromhexine as bronchodilator and mast cell stabilizer. The drug is also given in combination with steroidal drugs like beclomethasone dipropionate in different pharmaceutical dosage forms.
Chemical Name: α1-[(1,1-Dimethylethyl)amino]methyl]-4-hydroxy-1,3-benzenedimethanol

Molecular formula: C_{13}H_{21}NO_3_2 H_2SO_4

Molecular weight: 576.7

Melting Point: 157-158°C

Description and solubility: A white crystalline powder. Soluble 1 in 70 of water and 1 in 25 of ethanol; slightly soluble in ether.

Category: β adrenoreceptor agonist.

Literature cited for analysis:


2.3.16 SERRATIOPEPTIDASE (SER)

Serratiopeptidase is not official in Pharmacopoeia. Many studies of proteolytic enzymes over the years have demonstrated their effectiveness in relieving pain and inflammation independently of steroids or nonsteroidal anti-inflammatory drugs^{278}. Serratiopeptidase is a metalloenzyme derived from bacteria belonging to genus *Serratia*. Controlled fermentation of enterobacteria, *Serratia* secretes serratiopeptidase in medium. It makes a role of specific digestion of bradykinins and fibrins formed in the process of inflammation. So it can display anti-inflammatory effects efficiently with very low side effects.
compared with other steroidal or non-steroidal anti-inflammatory drugs. Tablet formulations containing 2.5, 5, 10 and 20 mg of serratiopeptidase are available in the market. The drug is also given in combination with non steroidal antiinflammatory drugs. Serratiopeptidase is also given along with minerals like zinc, cobalt and manganese sulphate.

**Chemical Name:** Serratiopeptidase is a metalloenzyme derived from bacteria belonging to genus *Serratia*

**Molecular formula:** (One unit of serratiopeptidase is defined as the enzymatic activity yielding a product equivalent to 1.0 microgram of Tyrosine per min at pH 9.0 and 37 °C on hammerstein casein as a substrate as per assay)

**Molecular weight:** An endopepdase having molecular weight about 52Kdalton.

**Description and solubility:** White or pale brownish powder. Serratiopeptidase is a proteolytic enzyme derived from the bacterium belong to genus *Serratia*. It has white or slightly brownish color and a unique odour and contains 1,600~2,600 Serratiopeptidase units per mg (according to Regulations of National Institute of Health in Korea).

**Category:** Anti-inflammatory and Anti-swelling effect. Enhancing effect on the transfer of antibiotic to the focal site, lysis and discharge of sputum and pus. Serratiopeptidase appears to decrease the weight and viscosity of nasal discharge in patients with chronic sinusitis.
Literature cited for analysis: Radioimmunossay\textsuperscript{279}, HPLC\textsuperscript{280} and HP-steric exclusion chromatography\textsuperscript{281} have been reported for the estimation of serratiopeptidase.

2.3.17 THEOPHYLLINE (THP)

Theophylline is official in British Pharmacopoeia\textsuperscript{1}, Indian Pharmacopoeia\textsuperscript{2}, and United States Pharmacopoeia\textsuperscript{3}. Theophylline, an alkaloid found in the leaves of the Camellia sinesis is used clinically as a bronchodilator in the management of chronic obstructive pulmonary disease\textsuperscript{282}. Conventional dosage forms of theophylline are administered 3–4 times a day to avoid large fluctuations in plasma concentrations\textsuperscript{283}.

![Chemical Structure of Theophylline]

Chemical Name: 1,3-Dimethyl-3,7-dihydro-1H-purine-2,6-dione.

Molecular formula: C\textsubscript{7}H\textsubscript{8}N\textsubscript{4}O\textsubscript{2}

Molecular weight: 180.2

Melting Point: 270-274\textdegree C

Description and solubility: A white crystalline powder. Soluble 1 in 120 of water, 1 in 80 of ethanol and 1 in 110 of chloroform; sparingly soluble in ether; soluble in dilute acids, ammonia and alkali hydroxide solutions.
**Category:** Xanthine bronchodilator, relaxes smooth muscles, relieves bronchospasm, stimulant effect on respiration.

**Literature cited for analysis:**
Salbutamol, theophylline and ambroxol in combination induce bronchodilation and assist the patient in coughing up viscid mucus, and have been used in the symptomatic treatment of bronchial asthma and other bronchospastic conditions.

A survey of the literature revealed that the analysis of salbutamol, theophylline and ambroxol either in single or multicomponent mixtures has been reported through titrimetric\(^1\)\(^-\)\(^3\) and spectrophotometric\(^2\)\(^8\)\(^4\) methods in pharmaceutical preparations.

**2.3.18 TRIFLUOPEARZINE HYDROCHLORIDE (TFP)**

![Chemical Structure of Trifluoperazine]

Trifluoperazine is official in British Pharmacopoeia\(^1\), Indian Pharmacopoeia\(^2\) and United States Pharmacopoeia\(^3\). It is high potency piperazine side chain phenothiazine. A marketed tablet formulation contains 1, 2 or 5 mg of drug given in combination with chlordiazepoxide, trihexyphenidyl or chlorpromazine.

**Chemical Name:** 10-\([3-(4\text{-}methyl\text{-}1\text{-}piperazinyl})\text{propyl\text{-}2-}
(trifluoromethyl)\text{-}10\text{H\text{-}phenothiazine dihydrochloride}\)
Molecular formula: \( \text{C}_{21}\text{H}_{24}\text{F}_{3}\text{N}_{3}\text{S}, 2\text{HCl} \)

Molecular Weight: 480.4

Melting Point: 242-243°C

Description and solubility: A white to pale yellow, crystalline powder, hygroscopic, freely soluble in water, soluble in alcohol.

Category: Antipsychotic, phenothiazine tranquilizer with anti-emetic effect.

Literature cited for analysis:
The official method for the determination of TFP is non-aqueous titration with perchloric acid, determining the end point potentiometrically\(^{285}\), colorimetric\(^{3,285-288}\), spectrophotometry\(^{1,289-290,293}\), HPLC\(^{291-293}\), Thin layer chromatography\(^{293}\), bead injection spectroscopy-flow injection analysis\(^{294}\).
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