Chapter 3                                                                                         Objectives and Plan of Work

3.1 RATIONALE OF THE STUDY

1. Literature survey on analytical methods of FDC revealed that though a large number of analytical methods for simultaneous estimation of drugs in FDC are available but still there are number of combinations which have no published analytical methodology for simultaneous estimation.

2. No analytical methods are available for simultaneous estimation of drugs in fixed dose combination in any pharmacopoeia, thereby making it essential to develop sensitive, reliable and reproducible methods for simultaneous estimation of drugs in FDCs.

3. Method development by HPLC-UV for two or more compounds and their related impurities becomes very complex if the UV profiles are not similar. Hence need to be analyzed by other sophisticated techniques such as LC-MS, UPLC-PDA, UPLC/Q-TOF-MS etc.


5. Application of ICH validation parameters to developed analytical methods.

6. Chemical and physicochemical compatibility of the APIs in an FDC with one another as well as with possible excipients.

7. Stress testing of individual drugs as per ICH guidelines.


9. Identification and characterization of impurities and degradation products in the bulk drugs and in their dosage forms.

10. On-going stability studies on marketed formulation to confirm the established shelf lives and stability of individual drugs in fixed dose combinations.

3.2 RATIONALE OF SELECTION OF FDCs

Fixed dose combination tablets containing 100 mg of aceclofenac and 500 mg of paracetamol has been approved by Drug Controller General of India, in the year 2004 for acute painful condition in adults for relief from various diseases related with pain and inflammation, such as acute pain in osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, low back pain, dental pain, fracture, painful pharyngitis and tonsillitis. Simultaneous determination of aceclofenac and paracetamol is also reported by stability-indicating HPLC (Natarajan and Raman, 2007; Jamil et al., 2008), which described the separation of some unknown degradation products formed when the pure drugs were subjected to forced degradation studies. But these methods are still only for
the determination of aceclofenac and paracetamol without demonstrating its separation from their major known degradation products, diclofenac and para-aminophenol.

Fixed dose combination tablets containing 40 mg of telmisartan and 12.5 mg of hydrochlorothiazide has been approved by Drug Controller General of India, in the year 2003. The combination of telmisartan and hydrochlorothiazide is reported to have an additive effect in lowering blood pressure compared with each of the respective monotherapies. Hypertension is a risk factor for cardiovascular disease and continues to be a major health problem in many areas of the world. The blood pressure is not adequately controlled with monotherapy. A widely used, effective and well-tolerated combination is a selective angiotensin II type 1 receptor blockers and a low dose diuretic agent. Telmisartan is a potent, highly selective and orally active antagonist belonging to the family of angiotensin II receptor antagonist. Hydrochlorothiazide is a diuretic and antihypertensive agent that often used in combination with other antihypertensive drugs such as beta blockers, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers. Simultaneous determination of telmisartan and hydrochlorothiazide is also reported by HPLC (Bhat et al., 2007; Wankhede et al., 2007; Rane et al., 2008) and LC/MS (Yan et al., 2008), which described the determination of these drugs without describing the separation their major known degradation products. No method is available for simultaneous determination of these drugs in any pharmacopoeias. Hence the widespread use of these pharmaceutical combination products has stimulated the development of analytical methods for simultaneous determination of these drugs along with their degradation products.

3.3 OBJECTIVES OF THE STUDY

1. To develop stability-indicating analytical methods for simultaneous estimation of drugs in fixed dose combination formulations.

2. To validate the proposed analytical methods as per ICH guidelines.

3. To perform stress testing/forced degradation studies on individual drugs.

4. Identification of impurities and degradation products in the bulk drugs and in their pharmaceutical formulations.

5. To establish the degradation mechanism.

6. To perform stability studies on marketed formulations as per ICH guidelines to confirm the shelf lives of products.
3.4 DRUG PROFILES

ACECLOFENAC (BP, 2008)

Structure

![Aceclofenac structure](image)

**Chemical Name:** 2-[2-[2-(2, 6-Dichlorophenyl) aminophenyl] acetyl] oxyacetic acid

**Molecular Formula:** C\(_{16}\)H\(_{13}\)Cl\(_2\)NO\(_4\)

**Molecular Weight:** 354.19

**Category:** Analgesic and non-steroidal anti-inflammatory drug (NSAIDs)

**PHYSICAL PROPERTIES**

**Appearance:** A white or almost white crystalline powder

**Solubility:** Practically insoluble in water, soluble in methanol and other alcohols

**Melting point:** 149-155°C

**Storage:** To be stored in well-closed, light resistant containers.

**IDENTIFICATION**

1. IR spectrophotometry- The infra-red absorption spectrum should be concordant with the reference spectrum of aceclofenac.
2. Mass spectrometry- By comparing with the spectrum obtained with the reference spectrum.

3. Dissolve 50.0 mg in methanol and dilute to 1000 ml with the same solvent. Dilute 2.0 ml of the solution to 50 ml with methanol. Examine between 220 nm and 370 nm, the solution shows an absorption maximum at 275 nm.

MODE OF ACTION
It is an effective analgesic and anti-inflammatory agent with a good tolerability profile. Through its analgesic and anti-inflammatory properties, aceclofenac provides relief from a variety of painful conditions. The mode of action of aceclofenac is largely based on the inhibition of prostaglandin synthesis. Aceclofenac is a potent inhibitor of enzyme cyclooxygenase, which is involved in the production of prostaglandins.

THERAPEUTIC USES
Osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, dental pain, and postoperative pain.

ADVERSE REACTIONS
The major side effects are of gastro-intestinal system such as dyspepsia, abdominal pain, nausea, diarrhoea, ulcerative stomatitis.

PARACETAMOL (IP, 1996; USP, 2000; BP, 2008)

Structure

![Paracetamol Structure](image)
Chapter 3

Objectives and Plan of Work

Chemical Name: N-(4-hydroxyphenyl)-acetamide, 4-hydroxyacetanilide, p-acetaminophen

Molecular Formula: C₈H₇NO₂

Molecular Weight: 151.20

Category: Analgesic and antipyretic

PHYSICAL PROPERTIES

Appearance: White crystals or white, crystalline powder having a bitter taste.

Solubility: Freely soluble in alcohols, sparingly soluble in water

Melting point: 168-172°C

Storage: To be stored in well-closed, light resistant containers.

IDENTIFICATION (IP, 1996)

1. Test A: The infra-red absorption spectrum should be concordant with the reference spectrum of paracetamol.

2. Test B: Dissolve 50 mg in sufficient methanol to produce 100 ml. To 1 ml of this solution, add 0.5 ml of 0.1 M hydrochloric acid and dilute to 100 ml with methanol. Protect the resulting solution from the light and immediately measure the absorbance at 249 nm.

3. Test C: Melts between 168°C and 172°C.

MODE OF ACTION

Paracetamol is a clinically proven analgesic and antipyretic drug. The site and mechanism of analgesic effect is unclear. It produces analgesia by elevation of the pain threshold and antipyretic through action on the hypothalamic heat regulating center.

THERAPEUTIC USES

Relief from mild to moderate pain, reduction of fever, substitute to aspirin.
ADVERSE REACTIONS
Liver damage may occur with therapeutic dose, overdose causes hepatic failure from acute hepatic necrosis and renal failure from acute tubular necrosis.

Marketed FDC tablets containing aceclofenac and paracetamol

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Brand Name</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aceclo Plus</td>
<td>Aristo, Mumbai, India</td>
</tr>
<tr>
<td>2</td>
<td>Aceroc-P</td>
<td>Wockhardt, Mumbai, India</td>
</tr>
<tr>
<td>3</td>
<td>Arflur-P</td>
<td>FDC, Mumbai, India</td>
</tr>
<tr>
<td>4</td>
<td>Topnac-P</td>
<td>Systopic, Delhi, India</td>
</tr>
<tr>
<td>5</td>
<td>Dolowin Plus</td>
<td>Micro labs, Bangalore, India</td>
</tr>
<tr>
<td>6</td>
<td>Flexidol-P</td>
<td>Cipla, Bangalore, India</td>
</tr>
<tr>
<td>7</td>
<td>Morcet</td>
<td>Moraceae, Uttarakhand, India</td>
</tr>
<tr>
<td>8</td>
<td>Aceclofen Plus</td>
<td>Ind Swift, Mumbai, India</td>
</tr>
</tbody>
</table>

TELMISARTAN (BP, 2008)

Structure

![Telmisartan](image)
Chemical Name: 4'-[[4-Methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl] methyl] biphenyl-2-carboxylic acid.

Molecular Formula: C_{33}H_{30}N_{4}O_{2}

Molecular Weight: 514.63

Category: Antihypertensive

PHYSICAL PROPERTIES

Appearance: White or off-white, crystalline powder.

Solubility: Practically insoluble in water, but freely soluble in organic solvents

Melting point: 170°C.

Storage: Store in well-closed containers and protected from light.

IDENTIFICATION

1. IR spectrophotometry- By comparing the spectrum obtained with the reference spectrum.
2. Mass spectrometry- Examine by mass spectrometry comparing with the spectrum obtained with the reference spectrum.
3. Melts between 168°C and 172°C.

MODE OF ACTION

It is a benzimidazole derivative antagonist of subtype 1 angiotensin II receptors (AT1). Mechanism of action is based on inhibition of of the renin-angiotensin system to the treatment of hypertension. It can be achieved by inhibiting the angiotensin-converting enzyme (ACE) that converts angiotensin I into its active form angiotensin II (AGII), or by blockade of angiotensin II (type AT1) receptors.

THERAPEUTIC USES

Treatment of essential hypertension in adults and coronary heart disease.
HYDROCHLOROTHIAZIDE (IP, 1996; USP, 2000; BP, 2008)

Structure

![Chemical Structure of Hydrochlorothiazide](image)

**Chemical Name:** 6-chloro-1, 1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide

**Molecular Formula:** C7H8ClN3O4S2

**Molecular Weight:** 297.72

**Category:** Diuretic and antihypertensive

**PHYSICAL PROPERTIES**

**Appearance:** White or almost white

**Solubility:** Slightly or very slightly soluble in water, sparingly soluble in alcohol, freely soluble in solutions of dilute alkali hydroxides, and in methanol.

**Melting point:** 270-275°C

**Storage:** Store in well-closed containers and protected from light.

**IDENTIFICATION**

1. IR spectrophotometry- By comparing the spectrum obtained with the reference spectrum.
2. Mass spectrometry- By comparing the spectrum obtained with the reference spectrum.
3. Melts between 265°C and 270°C.
MODE OF ACTION
Hydrochlorothiazide acts directly on the kidney, increasing the excretion of sodium, chloride, potassium, and consequently water mainly in the renal tubules.

THERAPEUTIC USES
Hydrochlorothiazide is a diuretic which reduces the reabsorption of electrolytes from the renal tubules. Used to treat hypertensive disease and to manage the oedema due to mild-to-moderate congestive heart failure. Oedema due to chronic hepatic or renal disease may also respond favourably. It may be used in the treatment of hypercalciuria in patients who have recurrent urinary calculi composed of calcium salts.

ADVERSE EFFECTS
Hydrochlorothiazide may induce hyperglycaemia and may aggravate pre-existing diabetes mellitus. The use of hydrochlorothiazide may be associated with electrolyte imbalance including hypochloraeemic alkalosis, hyponatraemia, and hypokalaemia. Renal and/or hepatic insufficiency may be aggravated by hydrochlorothiazide.

Marketed FDC tablets containing telmisartan and hydrochlorothiazide

Table 6: List of some marketed FDC tablets containing telmisartan (40 mg) and hydrochlorothiazide (12.5 mg)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Brand Name</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Telma-H</td>
<td>Glenmark, Mumbai, India</td>
</tr>
<tr>
<td>2</td>
<td>Newtel-H</td>
<td>Systopic, Delhi, India</td>
</tr>
<tr>
<td>3</td>
<td>Telday-H</td>
<td>Torrent, Ahmedabad, India</td>
</tr>
<tr>
<td>4</td>
<td>Teli-H</td>
<td>Cadila, Ahmedabad, India</td>
</tr>
<tr>
<td>5</td>
<td>Telista-H</td>
<td>Lupin, Mumbai, India</td>
</tr>
<tr>
<td>6</td>
<td>Arbitel-H</td>
<td>Micro Cardicare, Bangalore, India</td>
</tr>
<tr>
<td>7</td>
<td>Telmistat-H</td>
<td>Biocon, Bangalore, India</td>
</tr>
<tr>
<td>8</td>
<td>Telvas-H</td>
<td>Aristo, Mumbai, India</td>
</tr>
</tbody>
</table>
3.5 PLAN OF WORK

1. Selection of fixed dose combination (FDC) formulations.
   - FDC tablet containing aceclofenac and paracetamol
   - FDC tablet containing telmisartan and hydrochlorothiazide

2. Physical characterization and identification of drugs.

3. Selection of appropriate analytical technique
   - UPLC-QTOF-MS (Ultra performance liquid chromatography-Quadrupole time-of-flight mass spectroscopy) have been employed for the analysis of drugs in the selected fixed dose combinations.

4. Analytical method development
   - Selection and optimization of MS/MS parameters (UPLC-QTOF-MS)
   - Selection and optimization of UPLC conditions (UPLC-QTOF-MS)

5. Validation of analytical methods as per ICH guidelines.
   - Linearity and range
   - Limit of detection
   - Limit of quantitation
   - Precision
   - Accuracy
   - Robustness
   - Specificity


7. Identification of impurities and degradation products.

8. Establishment of degradation mechanisms.


10. Stability studies on marked formulations as per ICH guidelines to confirm the shelf life and stability profiles of individual drugs in FDCs.