3.1 DRUG & HYDROTROPE PROFILE

3.1.1 ETORICOXIB\(^{(116,117)}\)

Molecular structure:

\[
\begin{align*}
\text{Molecular structure:} & \\
\text{Molecular weight} & : 358.842 \\
\text{Molecular formula} & : C_{18}H_{15}ClN_2O_2S \\
\text{Chemical name} & : 5\text{-chloro-6'}\text{-methyl-3-[4-(methylsulfonyl)phenyl]-2,3'-bipyridine} \\
\text{Category} & : \text{Selective cyclooxygenase – 2 (COX-2) Inhibitor.} \\
\text{Dose} & : \text{Osteoarthritis 60 mg once daily, Rheumatoid arthritis 90 mg} \\
\text{Acute Gout} & : 120 \text{ mg once daily for adult up to 8 days} \\
\text{Description} & : \text{White or almost white, crystalline powder or colorless crystals; odour, slight.} \\
\text{Solubility} & : \text{Freely soluble in acetone, in chloroform, in ethanol (95%) and in ether, sparingly soluble in water.}
\end{align*}
\]
Melting point: 134-135 °C

Therapeutic Use: Anti-inflammatory, analgesic and arthritis.

Table 3.1: List of Some Important Analytical Methods for Etoricoxib

<table>
<thead>
<tr>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titrimetric</td>
<td>i. Bulk drug(^{(118)})</td>
</tr>
<tr>
<td></td>
<td>ii. Formulations(^{(118,119)})</td>
</tr>
<tr>
<td>UV-Spectrophotometric</td>
<td>Formulations(^{(119)})</td>
</tr>
</tbody>
</table>

Figure 3.1: Infra Red Spectra of Etoricoxib
3.1.2 CARVEDILOL\(^{(120,121)}\)

Molecular Structure:

![Molecular Structure of Carvedilol](image)

Molecular weight : 406.47

Molecular formula : \(C_{24}H_{26}N_2O_2\)

Chemical name : 1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl]amino]- 2-propanol

Category : Non selective β –adrenergic blocker with \(\alpha_1\) – blocking activity

Dose : 50 mg; 100 mg

Description : A white or almost white crystalline powder; Colorless crystals from ethyl acetate

Solubility : Freely soluble in DMSO; soluble in methylene chloride (\(\text{CH}_2\text{Cl}_2\)); methanol; sparingly soluble in ethanol, isopropanol; slightly soluble in ethyl ether; practically insoluble in water & dilute acids

Melting point : 114 – 115\(^0\)C

Therapeutic use : Antihypertensive; in treatment of congestive heart failure.

Table 3.2: List of Some Important Analytical Methods for Carvedilol

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV-Visible</td>
<td>Bulk drug(^{(122,123)}), formulations(^{(122,123)})</td>
</tr>
<tr>
<td>2.</td>
<td>HPLC</td>
<td>Bulk drug(^{(124,125)}), formulations(^{(124,125)})</td>
</tr>
<tr>
<td>3.</td>
<td>Others</td>
<td>Bulk drug(^{(126)}), formulations(^{(126,127)})</td>
</tr>
</tbody>
</table>
3.1.3 CLOBAZAM\textsuperscript{128,129}

Molecular Structure:

\[
\begin{align*}
N & \quad 1 \quad 2 \\
\text{C} & \quad \text{H}_3 \quad \text{O} \\
\text{Cl} & \quad 6 \\
\text{N} & \quad 5 \\
\text{Cl} & \quad 7 \\
\text{C} & \quad 8 \\
& \quad \text{H}_2 \text{C} \\
& \quad \text{N} \\
& \quad \text{N} \\
& \quad \text{O} \\
& \quad \text{O} \\
& \quad \text{Cl} \\
& \quad 9 \\
& \quad \text{C} \\
& \quad \text{H}_2 \text{C} \\
& \quad \text{N} \\
& \quad \text{N} \\
& \quad \text{O} \\
& \quad \text{O} \\
& \quad \text{Cl} \\
& \quad 9 \\
& \quad \text{C} \\
& \quad \text{H}_2 \text{C} \\
& \quad \text{N} \\
& \quad \text{N} \\
& \quad \text{O} \\
& \quad \text{O} \\
& \quad \text{Cl} \\
& \quad 9 \\
& \quad \text{C} \\
& \quad \text{H}_2 \text{C} \\
& \quad \text{N} \\
& \quad \text{N} \\
& \quad \text{O} \\
& \quad \text{O} \\
& \quad \text{Cl} \\
& \quad 9 \\
& \quad \text{C} \\
& \quad \text{H}_2 \text{C} \\
& \quad \text{N} \\
& \quad \text{N} \\
& \quad \text{O} \\
& \quad \text{O} \\
& \quad \text{Cl} \\
& \quad 9 \\
\end{align*}
\]

Molecular weight : 300.74

Molecular formula : $C_{16}H_{13}ClN_2O_2$

Chemical name : 7-chloro-1-methyl-5-phenyl-1$H$-1,5-benzodiazepine-2,4(3$H,5H$)-dione

Category : 1, 5 – Benzodiazepine as minor tranquilizer
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**Dose** : 50 mg; 100 mg

**Description** : A white or almost white crystalline powder; crystals are obtained in 50 % ethanol

**Solubility** : Freely soluble in DMF; methylene chloride (CH₂Cl₂); sparingly soluble in alcohol; slightly soluble in water

**Melting point** : 166 – 168° C

**Therapeutic use** : Anxiolytic and Anticonvulsant.

Table 3.3: List of Some Important Analytical Methods for Clobazam

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.</td>
<td>UV-Visible</td>
<td>Bulk drug(^{(130)}), formulations(^{(130)})</td>
</tr>
<tr>
<td>5.</td>
<td>HPLC</td>
<td>Bulk drug(^{(131,132)}), formulations(^{(131,132)})</td>
</tr>
<tr>
<td>6.</td>
<td>Others</td>
<td>Bulk drug(^{(133)}), formulations(^{(133)})</td>
</tr>
</tbody>
</table>

![Figure 3.3: Infra Red Spectra of Clobazam](image-url)

**Figure 3.3: Infra Red Spectra of Clobazam**
3.1.4 FLUOXAMINE MALEATE\(^{(134,135)}\)

**Molecular Structure:**

![Molecular Structure of Fluvoxamine Maleate](image)

**Molecular weight**: 434.41

**Molecular formula**: \(C_{15}H_{21}F_3N_2O_2\)

**Chemical name**: 2-\{[(E)-5-methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene]amino\}oxy\} ethanamine maleate or (E)-5-methoxy-1-[4-trifluoromethyl]phenyl]-1-Pentanone-O-(2-aminoethyl) oxime

**Category**: Selective Serotonin Reuptake Inhibitor (SSRI)

**Dose**: 50 mg; 100 mg; 300 mg

**Description**: A white or off white crystalline powder; crystals are obtained from acetonitrile

**Solubility**: Freely soluble in alcohol, chloroform and methyl alcohol; sparingly soluble in water; Practically insoluble in diethyl ether.

**Melting point**: 120 – 121.5\(^{0}\)C

**Therapeutic use**: Anti-depressant and antiobessional.

**Table 3.4: List of Some Important Analytical Methods for Fluvoxamine Maleate**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV-Visible</td>
<td>Bulk drug(^{(136)}), formulations(^{(136)})</td>
</tr>
<tr>
<td>2.</td>
<td>HPLC</td>
<td>Bulk drug(^{(137)}), formulations(^{(137)})</td>
</tr>
<tr>
<td>3.</td>
<td>Others</td>
<td>Bulk drug(^{(138)}), formulations(^{(138)})</td>
</tr>
</tbody>
</table>
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Figure 3.4: Infra Red Spectra of Fluvoxamine Maleate

3.1.5 CLOZAPINE\textsuperscript{(139,140)}

Molecular Structure :

![Molecular Structure of Clozapine](image)

Molecular weight : 326.82
Molecular formula : C\textsubscript{18}H\textsubscript{19}ClN\textsubscript{4}
Chemical name : 8-chloro-11-(4-methylpiperazin-1-yl)-10,11-dihydro-5H-dibenzo\textsubscript{[b,e]}[1,4]diazepine
Category : Combined serotonin (5HT\textsubscript{2}) & Dopamine D\textsubscript{2} receptor antagonist
Dose : 50 mg; 100 mg
Description : A yellow or almost pale yellow crystalline powder; yellow crystals are obtained from acetone-petroleum ether
Solubility : Freely soluble in alcohol, chloroform and methyl alcohol; sparingly soluble in water.
Melting point : 183 – 184°C
Therapeutic use : Anti-psychotic.

Table 3.5: List of Some Important Analytical Methods for Clozapine

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UV-Visible</td>
<td>Bulk drug(^{(141)}), formulations(^{(141)})</td>
</tr>
<tr>
<td>2</td>
<td>HPLC</td>
<td>Bulk drug(^{(142,143)}), formulations(^{(142,143)})</td>
</tr>
<tr>
<td>3</td>
<td>Others</td>
<td>Bulk drug(^{(144,145,146)}), formulations(^{(144,145,146)})</td>
</tr>
</tbody>
</table>

Figure 3.5: Infra Red Spectra of Clozapine
3.1.6 EZETIMIBE

Molecular Structure:

![Molecular Structure of Ezetimibe](image)

- Molecular weight: 409.43
- Molecular formula: C\(_{24}\)H\(_{21}\)F\(_2\)NO\(_2\)
- Chemical name: [3R-[3α(s*),4β]]-1-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-Azetidinone
- Category: New class of lipid-lowering agents that inhibits the absorption of cholesterol from intestine
- Dose: 5 mg; 10 mg
- Description: A white or off white solid powder.
- Solubility: Freely soluble in methanol; ethanol or acetone; Practically insoluble in water.
- Melting point: 165 – 167\(^0\)C
- Therapeutic use: Lipid-lowering agent.

**Table 3.6: List of Some Important Analytical Methods for Ezetimibe**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UV-Visible</td>
<td>Bulk drug(^{(149)}), formulations(^{(149)})</td>
</tr>
<tr>
<td>2</td>
<td>HPLC</td>
<td>Bulk drug(^{(150)}), formulations(^{(150)})</td>
</tr>
<tr>
<td>3</td>
<td>Others</td>
<td>Bulk drug(^{(151)}), formulations(^{(151)})</td>
</tr>
</tbody>
</table>
3.1.7 FENOFIBRATE

Molecular Structure:

![Molecular Structure Diagram]

Molecular weight: 360.83
Molecular formula: $C_{20}H_{21}ClO_4$
Chemical name: propan-2-yl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate
Category: HMG-CoA Reductase Inhibitor
Dose: 50 mg; 100 mg
Description: A white or almost white crystalline powder; crystals are obtained from isopropanol.

Solubility: Soluble in acetone, ether chloroform and benzene; slightly soluble in methanol, ethanol; practically insoluble in water.

Melting point: 80 – 81°C

Therapeutic use: Anti-Lipidmic agent.

Table 3.7: List of Some Important Analytical Methods for Fenofibrate

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV-Visible</td>
<td>Bulk drug(^{154}), formulations(^{154})</td>
</tr>
<tr>
<td>2.</td>
<td>HPLC</td>
<td>Bulk drug(^{155}), formulations(^{155})</td>
</tr>
<tr>
<td>3.</td>
<td>Others</td>
<td>Bulk drug(^{156}), formulations(^{156})</td>
</tr>
</tbody>
</table>

Figure 3.7: Infra Red Spectra of Fenofibrate
3.1.8 ATENOLOL

Molecular Structure:

\[
\begin{align*}
\text{O} & \quad \text{NH} \quad \text{CH}_3 \\
\text{O} & \quad \text{NH} \quad \text{CH}_3 \\
\text{H}_2\text{N} & \quad \text{K} \\
\end{align*}
\]

Molecular weight : 266.34
Molecular formula : \( \text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3 \)
Chemical name : 2-{4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl}acetamide
Category : A selective \( \beta_1 \)-receptor antagonist (\( \beta_1 \)-Adrenergic blocker)
Dose : 5 mg; 10 mg
Description : A white or off white solid powder.
Solubility : Practically insoluble in water; sparingly soluble in strong acid; soluble in strong bases
Melting point : 147 – 149\(^0\)C
Therapeutic use : Antihypertensive agent, anti-anginal.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UV-Visible</td>
<td>Bulk drug(^{(159,160)}), formulations(^{(159,160)})</td>
</tr>
<tr>
<td>2</td>
<td>HPLC</td>
<td>Bulk drug(^{(161,162)}), formulations(^{(161,162)})</td>
</tr>
<tr>
<td>3</td>
<td>Others</td>
<td>Bulk drug(^{(163,164,165)}), formulations(^{(163,164,165)})</td>
</tr>
</tbody>
</table>
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3.1.9 TELMISARTAN^{166,167}

Molecular Structure:

![Molecular Structure of Telmisartan]

Molecular weight : 514.62
Molecular formula : C_{33}H_{30}N_{4}O_{2}
Chemical name : 4’-[1,4’-dimethyl-2-propyl[2,6’-bi-benzimidazole]-1’-yl]methyl 1,1’- biphenyl-2-carboxylic acid
Category : Angiotensin – II Antagonist at AT_{1} receptor

Figure 3.8: Infra Red Spectra of Atenolol
Dose : 5 mg; 10 mg
Description : A white or off white solid powder.
Solubility : Practically insoluble in water; sparingly soluble in strong acid;
soluble in strong bases
Melting point : 261 – 263°C
Therapeutic use : Antihypertensive agent.

Table 3.9: List of Some Important Analytical Methods for Telmisartan

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV-Visible</td>
<td>Bulk drug(^{[168]}), formulations(^{[168]})</td>
</tr>
<tr>
<td>2.</td>
<td>HPLC</td>
<td>Bulk drug(^{[169,170]}), formulations(^{[169,170]})</td>
</tr>
<tr>
<td>3.</td>
<td>Others</td>
<td>Bulk drug(^{[171,172]}), formulations(^{[171,172]})</td>
</tr>
</tbody>
</table>

Figure 3.9: Infra Red Spectra of Telmisartan

3.1.10 PIOGLITAZONE\(^{[173,174]}\)

Molecular Structure :
**MATERIALS AND METHODS**

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**Molecular weight** : 356.70

**Molecular formula** : C_{19}H_{20}N_{2}O_{3}S

**Chemical name** : 5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione

**Category** : Insulin sensitizer (acts through Proxisome Proliferators’ Activated \( \gamma \) -receptor)

**Dose** : 15 mg; 45 mg

**Description** : A white or odourless white crystalline powder.

**Solubility** : Soluble in DMF; slightly soluble in absolute ethanol; very slightly soluble in acetone or acetonitrile practically insoluble in water.

**Melting point** : 193 – 195\(^o\)C

**Therapeutic use** : Hypoglycemic agent.

*Table 3.10: List of Some Important Analytical Methods for Pioglitazone*

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV-Visible</td>
<td>Bulk drug(^{175}), formulations(^{175})</td>
</tr>
<tr>
<td>2.</td>
<td>HPLC</td>
<td>Bulk drug(^{176,177}), formulations(^{176,177})</td>
</tr>
<tr>
<td>3.</td>
<td>Others</td>
<td>Bulk drug(^{178,179}), formulations(^{178,179})</td>
</tr>
</tbody>
</table>

*Figure 3.10: Infra Red Spectra of Pioglitazone*
3.1.11 REPAGLINIDE\textsuperscript{(180,181)}

Molecular Structure:

\[ \text{Molecular structure diagram} \]

Molecular weight: 452.59
Molecular formula: \( C_{27}H_{36}N_2O_4 \)
Chemical name: \((+)-2\)-ethoxy-\(\alpha\)-\[\((S)\)-\(\alpha\)-isobutyl-o-piperidino-benzyl]carbamoyl]-p-Toluic acid
Category: Meglitinide as insulin activity stimulator
Dose: 0.5 mg; 4 mg
Description: A white or off white solid powder.
Solubility: Practically insoluble in water
Melting point: 129 – 131\(^0\)C
Therapeutic use: Antihypertensive agent.

Table 3.11: List of Some Important Analytical Methods for Repaglinide

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV-Visible</td>
<td>Bulk drug\textsuperscript{(182,183,184)}, formulations\textsuperscript{(182,183,184)}</td>
</tr>
<tr>
<td>2.</td>
<td>HPLC</td>
<td>Bulk drug\textsuperscript{(185,186,187,188)}, formulations\textsuperscript{(185,186,187,188)}</td>
</tr>
<tr>
<td>3.</td>
<td>Others</td>
<td>Bulk drug\textsuperscript{(189)}, formulations\textsuperscript{(189)}</td>
</tr>
</tbody>
</table>
**3.1.12 ROSIGLITAZONE MALEATE**

Molecular Structure:

![Molecular Structure Image]

**Molecular weight** : 473.50

**Molecular formula** : $C_{18}H_{19}ClN_3O_3S$. $C_4H_4O_4$

**Chemical name** : 5-(4-{2-[methyl(pyridin-2-yl)amino]ethoxy}benzyl)-1,3-thiazolidine-2,4-dione.Maleate
Category: Insulin sensitizer (acts through Proxisome Proliferators’ Activated γ-receptor)

Dose: 5 mg; 10 mg

Description: A white or off white solid powder; colorless crystals are obtained from methanol

Solubility: Soluble in acetone, ether chloroform and benzene; slightly soluble in methanol, ethanol; practically insoluble in water.

Melting point: 153 – 155°C

Therapeutic use: Hypoglycemic.

Table 3.12: List of Some Important Analytical Methods for Rosiglitazone Maleate

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV-Visible</td>
<td>Bulk drug[^192^], formulations[^192^]</td>
</tr>
<tr>
<td>2.</td>
<td>HPLC</td>
<td>Bulk drug[^193^], formulations[^193^]</td>
</tr>
<tr>
<td>3.</td>
<td>Others</td>
<td>Bulk drug[^194^], formulations[^194^]</td>
</tr>
</tbody>
</table>

Figure 3.12: Infra Red Spectra of Rosiglitazone Maleate
3.1.13 SIMVASTATIN\(^{195,196}\)

Molecular Structure:

![Molecular Structure of Simvastatin]

Molecular weight: 418.57
Molecular formula: C\(_{25}\)H\(_{38}\)O\(_5\)
Chemical name: 2,2–dimethyl butanoic acid (1S, 3R, 7S, 8S, 8aR) – 1, 2, 3, 7, 8, 8a-hexahydro-3,7-dimethyl-8-[2-[(2R,4R) tetrahydro -4- hydroxyl-6 -oxo-2H pyran-2-yl]ethyl]-1-naphthalenyl ester
Category: HMG-CoA Reductase Inhibitor
Dose: 5 mg; 10 mg
Description: A white or off white solid powder; colorless crystals are obtained from methanol
Solubility: Soluble in acetone, ether chloroform and benzene; slightly soluble in methanol, ethanol; practically insoluble in water.
Melting point: 135 – 138\(^0\)C
Therapeutic use: Hypolipidmic agent.

Table 3.13: List of Some Important Analytical Methods for Simvastatin

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV-Visible</td>
<td>Bulk drug(^{197}), formulations(^{197})</td>
</tr>
<tr>
<td>2.</td>
<td>HPLC</td>
<td>Bulk drug(^{198,199,200,201,202,203,204}), formulations(^{198,199,200,201,202,203,204})</td>
</tr>
<tr>
<td>3.</td>
<td>Others</td>
<td>Bulk drug(^{205}), formulations(^{205})</td>
</tr>
</tbody>
</table>
3.1.14 ACECLOFENAC\(^{206,207}\)

**Molecular Structure:**

![Molecular Structure of Aceclofenac]

- **Molecular weight**: 354.18
- **Molecular formula**: \(C_{16}H_{13}Cl_{2}NO_{4}\)
- **Chemical name**: \([\{(2\text{-}[(2\text{-}chlorophenyl)amino]phenyl}acetyl]oxy\]acetic acid
- **Category**: Cyclooxygenase Inhibitor
- **Dose**: 50 mg; 100 mg
- **Description**: A white or almost white crystalline powder; white crystals are obtained from cyclohexane
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Solubility : Freely soluble in alcohol, chloroform and methyl alcohol; sparingly soluble in water.

Melting point : 149 – 150°C

Therapeutic use : Anti-inflammatory and analgesic.

Table 3.14: List of Some Important Analytical Methods for Aceclofenac

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV-Visible</td>
<td>Bulk drug(^{(208,209,210)}), formulations(^{(208,209,210)})</td>
</tr>
<tr>
<td>2.</td>
<td>HPLC</td>
<td>Bulk drug(^{(211,212)}), formulations(^{(211,212)})</td>
</tr>
<tr>
<td>3.</td>
<td>Others</td>
<td>Bulk drug(^{(213)}), formulations(^{(213)})</td>
</tr>
</tbody>
</table>

Figure 3.14: Infra Red Spectra of Aceclofenac
3.1.15 KETOCONAZOLE\(^{(214,215)}\)

**Molecular Structure:**

![Molecular Structure of Ketoconazole](image)

**Molecular weight**: 531.44

**Molecular formula**: \(C_{26}H_{20}Cl_2N_4O_4\)

**Chemical name**: Cis-1-acetyl-4-\([2-(2,4-dichlorophenyl)-2H-imidazol-ylmethyl]-1,3-dioxolan-4-ylmethoxy]phenyl\]-piperazine

**Category**: An Imidazole derivative used in fungal infection

**Dose**: 5 mg; 10 mg

**Description**: A white or off white solid powder.

**Solubility**: Practically insoluble in water; sparingly soluble in strong acid; soluble in strong bases

**Melting point**: 146 – 148\(^0\)C

**Therapeutic use**: Anti-fungal

### Table 3.15: List of Some Important Analytical Methods for Ketoconazole

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Method</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV-Visible</td>
<td>Bulk drug(^{(216)}), formulations(^{(216)})</td>
</tr>
<tr>
<td>2.</td>
<td>HPLC</td>
<td>Bulk drug(^{(217,218,219,220,221,222,223)}), Formulations(^{(217,218,219,220,221,222,223)})</td>
</tr>
<tr>
<td>3.</td>
<td>Others</td>
<td>Bulk drug(^{(224)}), formulations(^{(224)})</td>
</tr>
</tbody>
</table>
Figure 3.15: Infra Red Spectra of Ketoconazole

3.1.16 SODIUM ACETATE\textsuperscript{(225,226)}

- **Molecular structure**: \(\text{CH}_3\text{COONa}, 3\text{H}_2\text{O}\)
- **Molecular weight**: 136.08
- **Molecular formula**: \(\text{C}_2\text{H}_3\text{NaO}_2, 3\text{H}_2\text{O}\)
- **Category**: Pharmaceutical aid (for peritoneal dialysis fluids)
- **Description**: Colourless crystals or white, crystalline powder; odourless.
- **Solubility**: Very soluble in water, soluble in ethanol (95%).
- **pH**: Between 7.5 and 9.0, determined in a 5% w/v solution.
- **Melting point**: 324°C with decomposition.
3.1.17 SODIUM BENZOATE

Molecular structure:

\[
\begin{align*}
\text{Molecular weight} & : 144.11 \\
\text{Molecular formula} & : \text{C}_7\text{H}_5\text{NaO}_2 \\
\text{Category} & : \text{Pharmaceutical aid (preservative).} \\
\text{Description} & : \text{White, crystalline or granular powder or flakes; odourless or with a faint odour; hygroscopic.} \\
\text{Solubility} & : \text{Freely soluble in water, sparingly soluble in ethanol (95%).} \\
\text{Acidity or alkalinity} & : \text{To 20 ml of a 5% w/v solution in carbon dioxide-free water add 0.2 ml of phenolphthalein solution. Not more than 0.2 ml of 0.1 M hydrochloric acid or 0.2 ml of 0.1 M sodium hydroxide is required to change the colour of the solution.} \\
\text{Melting point} & : > 300^\circ\text{C}
\end{align*}
\]

3.1.18 SODIUM CITRATE

Molecular structure:

\[
\begin{align*}
\text{Molecular weight} & : 294.10 \\
\text{Molecular formula} & : \text{C}_6\text{H}_5\text{Na}_3\text{O}_7\cdot2\text{H}_2\text{O}
\end{align*}
\]
**SODIUM SALICYLATE**

<table>
<thead>
<tr>
<th>Category</th>
<th>Systemic alkalinising agent.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose</strong></td>
<td>1 to 10 g.</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>White, granular crystals or white, crystalline powder, odourless; slightly deliquescent in moist air.</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>Freely soluble in water, practically insoluble in ethanol (95%) and in ether.</td>
</tr>
<tr>
<td><strong>Acidity or alkalinity</strong></td>
<td>Titrate 20 ml of a 10.0% w/v solution in carbon dioxide-free distilled water with 0.05 M sulphuric acid or 0.1 M sodium hydroxide using thymol blue solution as indicator, not more than 0.5 ml of 0.05 M sulphuric acid or 0.1 M sodium hydroxide is required.</td>
</tr>
<tr>
<td><strong>Melting point</strong></td>
<td>150°C</td>
</tr>
</tbody>
</table>

### 3.1.19 SODIUM SALICYLATE

**Molecular structure:**

![Molecular structure](image)

<table>
<thead>
<tr>
<th>Molecular weight</th>
<th>160.10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular formula</strong></td>
<td>C₇H₆NaO₃</td>
</tr>
<tr>
<td><strong>Category</strong></td>
<td>Anti-inflammatory, analgesic.</td>
</tr>
<tr>
<td><strong>Dose</strong></td>
<td>500 mg to 2 g, with food, in the treatment of rheumatism, 5 to 10 g daily, in divided doses.</td>
</tr>
</tbody>
</table>
Description : Colourless, small crystals or shiny flakes or white crystalline powder.

Solubility : Freely soluble in water (concentrated solutions are liable to deposit crystals of the hexahydrate) and in ethanol (95%), practically insoluble in ether.

Acidity : To 20 ml of a 10.0% w/v solution in carbon dioxide-free distilled water, add 0.1 ml of phenol red solution, the solution is yellow. Titrate with 0.01 M sodium hydroxide to a reddish violet colour, not more than 2.0 ml of 0.01 M sodium hydroxide is required.

Melting point : 159°C

3.1.20 UREA\(^{233,234}\)

Molecular structure :

\[ \text{H}_2\text{N} \overset{\text{O}}{\text{C}} \overset{\text{NH}_2}{} \]

Molecular weight : 60.06
Molecular formula : CH\(_4\)N\(_2\)O
Category : Keratolytic.
Dose : 5 to 15 g.
Description : White, crystalline powder or transparent crystals; odourless or almost odourless, but may gradually develop a slight odour of ammonia upon long standing, slightly hygroscopic.
Solubility: Freely soluble in water and in boiling ethanol (95%), soluble in ethanol (95%), practically insoluble in chloroform, in dichloromethane and in ether.

Acidity or alkalinity: To 10 ml of 5% w/v solution add 0.1 ml methyl red solution and 0.4 ml of 0.01 M hydrochloric acid, the resulting solution is red to orange.

Melting point: 132°C

3.1.21 NIACINAMIDE

Molecular structure:

\[
\begin{array}{c}
O \\
\text{NH}_2 \\
\end{array}
\]

Molecular weight: 122.13

Molecular formula: \(\text{C}_6\text{H}_6\text{N}_2\text{O}\)

Category: B-group vitamin.

Dose: Orally, prophylactic, 15 to 30 mg daily, therapeutic, 50 to 250 mg daily. By intravenous injection, 50 to 250 mg daily.

Description: Colourless crystals or white, crystalline powder; odour, faint and characteristic.

Solubility: Freely soluble in water and in ethanol (95%); slightly soluble in chloroform and in ether.

pH: Between 6.0 and 7.5, determined in a 5.0% w/v solution.

Melting point: 128-131°C
3.2 INSTRUMENTATION & PLAN OF WORK

1. Ultraviolet and visible spectroscopy methods for different drugs were developed and validated on the “Double beam UV-Visible Spectrophotometer, company-Shimadzu, Model(1800)” with a 1 cm matching quartz cell and “Double beam UV-Visible Spectrophotometer, company-Systronic, Model(2101)” with a 1 cm matching quartz cell. All analytical grade (company-Merck) chemicals were used in method development and validation.

2. HPLC methods were developed and validated on “Younglin HPLC model (Acme-9000)” All chemical used in HPLC method were HPLC grade (company-Merck).

    Table 3.16: List of Instruments and Equipments with their specifications

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Instrument/Equipment</th>
<th>Company</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Double Beam Spectrophotometer</td>
<td>Shimadzu</td>
<td>UV-1800</td>
</tr>
<tr>
<td>2.</td>
<td>Double Beam Spectrophotometer</td>
<td>Systronic</td>
<td>UV-2101</td>
</tr>
<tr>
<td>3.</td>
<td>High Performance Liquid Chromatography</td>
<td>Younglin</td>
<td>Acme-9000</td>
</tr>
<tr>
<td>4.</td>
<td>pH Meter</td>
<td>Labtronics</td>
<td>LT-11</td>
</tr>
<tr>
<td>5.</td>
<td>Electronics Balance</td>
<td>Ohaus</td>
<td>N-13123</td>
</tr>
<tr>
<td>7.</td>
<td>Sonicator</td>
<td>Khera Inst. Ltd.</td>
<td>K-3241</td>
</tr>
</tbody>
</table>
Table 3.17: List of Chemicals and Reagents with their specifications

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Chemical/Reagent</th>
<th>Company</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sodium benzoate Purified</td>
<td>Merck</td>
<td>Analytical</td>
</tr>
<tr>
<td>2.</td>
<td>Urea</td>
<td>Merck</td>
<td>GR</td>
</tr>
<tr>
<td>3.</td>
<td>Sodium salicylate Extrpure</td>
<td>Merck</td>
<td>Analytical</td>
</tr>
<tr>
<td>4.</td>
<td>Sod Acetate trihydrate</td>
<td>Merck</td>
<td>GR</td>
</tr>
<tr>
<td>5.</td>
<td>Sod Citrate dihydrate</td>
<td>Merck</td>
<td>GR</td>
</tr>
<tr>
<td>6.</td>
<td>Methanol</td>
<td>Merck</td>
<td>HPLC</td>
</tr>
<tr>
<td>7.</td>
<td>Chloroform</td>
<td>Merck</td>
<td>HPLC</td>
</tr>
<tr>
<td>8.</td>
<td>Acetone</td>
<td>Merck</td>
<td>HPLC</td>
</tr>
<tr>
<td>9.</td>
<td>K$_2$HPO$_4$</td>
<td>Ranchem</td>
<td>HPLC</td>
</tr>
<tr>
<td>10.</td>
<td>Sodium Hydroxide</td>
<td>Ranchem</td>
<td>GR</td>
</tr>
<tr>
<td>11.</td>
<td>Acetonitrile</td>
<td>Merck</td>
<td>HPLC</td>
</tr>
<tr>
<td>12.</td>
<td>Dichloromethane</td>
<td>Merck</td>
<td>HPLC</td>
</tr>
<tr>
<td>13.</td>
<td>Cyclohexane</td>
<td>Merck</td>
<td>HPLC</td>
</tr>
<tr>
<td>14.</td>
<td>Ethyl Acetate</td>
<td>Merck</td>
<td>HPLC</td>
</tr>
<tr>
<td>15.</td>
<td>Carbon tetrachloride</td>
<td>Merck</td>
<td>HPLC</td>
</tr>
<tr>
<td>16.</td>
<td>Diethyl Ether</td>
<td>Merck</td>
<td>HPLC</td>
</tr>
<tr>
<td>17.</td>
<td>Acetic Acid</td>
<td>Merck</td>
<td>HPLC</td>
</tr>
<tr>
<td>18.</td>
<td>Water</td>
<td>CDH/Merck</td>
<td>HPLC</td>
</tr>
</tbody>
</table>
Drug samples were obtained from different companies as gift samples for research and were authenticated by IR and melting point determinations.
3.2 PLAN OF WORK

a. UV spectrophotometric hydrotropic solubilization Method

Based on above mentioned facts, the proposed research work may proceed on the following lines-

- Literature survey.
- Selection of poorly water-soluble drugs from commonly used NSAID and antidepressant, antiseizures for the proposed research work.
- Identification of drug candidates.
- Determination of interference in spectrophotometric estimations in presence of hydrotropic agents and formulation additives.
- Preparation of most concentrated aqueous solutions of commonly used hydrotropic agents.
- Selection of hydrotropic agents for selected poorly water-soluble drugs based on approximate solubility determination.
- Determination of equilibrium solubilities of drugs in distilled water and in solutions containing hydrotropic agents.
- Spectrophotometric analysis of some poorly water-soluble bulk drug samples by pharmacopoeial methods (involving use of organic solvents).
- Spectrophotometric analysis of the same drugs using hydrotropic solutions (in place of organic solvents) & the validation of proposed Spectrophotometric methods of analyses.
- Quantitative spectrophotometric analysis of marketed solid dosage forms of some poorly water-soluble drugs using hydrotropic solubilization technique and validation of such methods by recovery studies.
- To evaluate the physical and chemical stability of prepared hydrotropic solid dispersions, liquid oral solutions (syrups), dry syrups (for reconstitution) and topical solutions at ambient temperature (room temperature), at a moderate temperature (40°C and 75% RH) and at a relatively high temperature (55 ± 1°C).
b. **Visible Spectrophotometric (colorimetry) Method**

- Literature survey.
- Selection of poorly water-soluble drugs from commonly used hypoglycemic, antihypertensive & antiseizure for the proposed research work.
- Identification of drug candidates.
- Determination of interference in spectrophotometric estimations in presence of color forming agent (Indicator) and formulation additives.
- Preparation of most stable drug-indicator complex commonly used indicators.
- Extraction of drug from different excipients using different solvents.
- Spectrophotometric analysis of some poorly water-soluble bulk drug samples by pharmacopoeial methods.
- Spectrophotometric analysis of the bulk drugs & the validation of proposed Spectrophotometric methods of analyses.
- Quantitative spectrophotometric analysis of marketed solid dosage forms of some poorly water-soluble drugs and validation of such methods by recovery studies.
- To evaluate the physical and chemical stability of prepared hydrotropic solid dispersions, liquid oral solutions (syrups), dry syrups (for reconstitution) and topical solutions at ambient temperature (room temperature), at a moderate temperature (40°C and 75% RH) and at a relatively high temperature (55 ± 1°C).
c. **HPLC method**

- Literature survey.
- Selection of poorly water-soluble drugs from commonly used antihyperlipidmic, and antifungal for the proposed research work.
- Identification of drug candidates.
  - Method development
    i) Selection of Column.
    ii) Selection of mobile phase.
    iii) Optimization of Chromatographic conditions.
    iv) System suitability studies.
    v) Study of linearity range.
    vi) Analysis of standard drug
    vii) Analysis of marketed formulation by proposed method.
    viii) Recovery studies.
    ix) Validation of proposed method.
- Method validation
  a. Selectivity
  b. Accuracy
  c. Precision
  d. Recovery
  e. Linearity
  f. Dilution integrity

5) References

- To evaluate the physical and chemical stability of prepared hydrotropic solid dispersions, liquid oral solutions (syrups), dry syrups (for reconstitution) and topical solutions at ambient temperature (room temperature), at a moderate temperature (40ºC and 75% RH) and at a relatively high temperature (55 ± 1º C).
3.3 EXPERIMENTAL WORK

3.3 (a) Hydrotropic & Colorimetric Methods

3.3.1. ETORICOXIB:-

Etoricoxib drug sample was supplied as gift sample by Sun Pharma Labs. Ltd., Jammu. Commercial tablets of etoricoxib were procured from the market (KINGCOX-60mg from Cadila Pharma., ETROBAX-90 mg from Ranbaxy Ltd., RETOZ-120 mg from Dr. Reddy’s Lab) All other chemicals used were of analytical grade.

Preliminary solubility studies of Etoricoxib: solubilities of Etoricoxib were determined in 2 M sodium benzoate solution, distilled water sufficient excess amount of drug was added to screw-capped glass vials of 20 ml capacity, containing distilled water, and 2 M sodium benzoate solution. The vials were shaken mechanically for 12 hours at in orbital shaker (Khera Instrument Pvt. Ltd., India). The solutions were allowed to equilibrate for next 24 hours and then centrifuged for 5 min at 2000 rpm. The supernatant of each vial was filtered through Whatman filter paper # 41. filtrates were diluted suitably and analyzed against corresponding solvent blanks.

Analysis of etoricoxib in tablets using 2 M sodium benzoate solution: Twenty tablets of formulation-I (KINGCOX) were weighed and powdered. Powder equivalent to 60 mg Etoricoxib was transferred to a 50 ml volumetric flask containing 40 ml of 2 M sodium benzoate solution. The flask was shaken for about 5 min to solubilize the drug. Then volume was made upto the mark with distilled water. Solution was filtered through Whatman filter paper # 41. filtrate was divided in two parts, A and B. part A was kept at room temperature for 48 hours to check the effect on stability of drug in presence of urea and also to note precipitation, if any, during this period. Part B filtrate was appropriately diluted with distilled water and absorbance was noted at 282 nm (\(\lambda_{\text{max}}\)) against solvent blank and the drug content was calculated (Table-3.18). After 48 hours, filtrate of part B was also appropriately diluted with distilled water and analyzed for drug content. There was no precipitation in the filtrate in 48 hours. Similar procedures were adopted in cases of formulation-II (ETROBAX) and formulation-III (RETOZ).
Recovery Studies:

Recovery studies are performed by adding extra bulk drug nearly forty percent of formulations. For recovery studies, tablet powder of formulation I ((KINGCOX) equivalent to 60 mg drug was taken in a 25 ml volumetric flask. In this flask 30 mg of pure drug (corresponding spiked drug) was transferred and 20 ml of 2.0 M sodium benzoate solution was added and the flask was shaken for about 10 min. Then volume was made up to the mark with distilled water and filtered through Whatman filter paper # 41. The solution was diluted appropriately with distilled water and analyzed for drug content. Similar procedures were adopted for formulation II (ETROBAX) & formulation III (RETOZ). The results of analysis of recovery studies are presented in (Table 3.19).

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated*(Mean ± S.D.)</th>
<th>% Coeff. Of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (KINGCOX)</td>
<td>60</td>
<td>99.370 ± 1.228</td>
<td>1.356</td>
<td>0.501</td>
</tr>
<tr>
<td>II (ETROBAX)</td>
<td>90</td>
<td>99.614 ± 1.495</td>
<td>1.516</td>
<td>0.641</td>
</tr>
<tr>
<td>III (RETOZ)</td>
<td>120</td>
<td>99.856 ± 1.327</td>
<td>1.236</td>
<td>0.595</td>
</tr>
</tbody>
</table>

*Average of six determinations

Table-3.19: Recovery studies of commercial tablets of Etoricoxib

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated*(Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (KINGCOX)</td>
<td>60</td>
<td>30</td>
<td>99.316 ± 1.484</td>
<td>1.494</td>
<td>0.665</td>
</tr>
<tr>
<td>II (ETROBAX)</td>
<td>90</td>
<td>40</td>
<td>100.514 ± 1.445</td>
<td>1.437</td>
<td>0.647</td>
</tr>
<tr>
<td>III (RETOZ)</td>
<td>120</td>
<td>60</td>
<td>99.288 ± 0.863</td>
<td>0.878</td>
<td>0.386</td>
</tr>
</tbody>
</table>

*Average of six determinations
3.3.2. CARVEDILOL:-

Carvedilol drug sample was supplied as gift sample by Sun Pharma Labs. Ltd., Jammu. Commercial tablets of Carbidilol were procured from the market (CARDITON-6.25 mg from M/s Kopran Pharma. Ltd., CARCA-12.50 mg from M/s Intas Pharma Ltd., CARLOC-25 mg from M/s Cipla Ltd.). All chemicals used were of analytical reagent grade.

Standard solution of Carvedilol was prepared by dissolving 6.25 mg of pure drug in methanol and diluting to 100 ml with methanol. A 20 ml aliquot of this solution was diluted to 50 ml with distilled water. Twenty tablets were weighed and powdered. An amount of the powder equivalent to 6.25 mg of the drug was weighed, transferred into a 50 ml volumetric flask, dissolved and diluted to 50 ml with methanol. It was sonicated for ten minutes and filtered through Whatman filter paper No. 42. A 20 ml aliquot of the filtrate was pipetted out and diluted to 50 ml with water.

An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml 0.1 N HCl, 3 ml of bromo phenol blue solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of chloroform and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with chloroform.

A reagent blank was prepared in a similar manner without adding the drug. The absorbance of yellow coloured chromogen was measured at 414 nm against the reagent blank. Similar procedures were adopted in cases of formulation-II (CARCA) and formulation-III (CARLOC). The drug content of the formulations were conducted. The results of such studies are presented in (Table 3.20). Carvedilol reacts with Bromo phenol blue in acidic solution to give chloroform soluble yellow coloured ion-association complex, which exhibits an absorption maximum at 414 nm. The optimum reaction conditions for the quantitative determination of the ion-pair complex were established through a number of preliminary experiments.

The optimum concentration of the reagent was studied. It was observed that 3 ml of 0.05% bromo phenol blue solution was sufficient for maximum colour development of the complex. The effect of pH was studied by extracting the coloured complex in presence of various acid solutions and buffers. The maximum and constant colour
intensity was observed when 0.1 N HCl was used. Several organic solvents such as methylene dichloride, chloroform and carbon tetrachloride were tried for extraction of the coloured complex from the aqueous phase. However, chloroform was found to be the most suitable solvent. The absorbance of the complex was found to be stable for more than 12 h. The proposed method of determination of Carvedilol shows molar absorptivity of $1.8 \times 10^4$. Linear regression of absorbance with concentration gave a correlation coefficient of 0.9995 and RSD was found to be less than two. The method developed in the present work was found to be sensitive, accurate, precise and reproducible and can be used for routine determination of Carvedilol in bulk and in dosage forms.

For recovery studies, tablet powder of formulation I ((KARDITON) equivalent to 6.25 mg drug was taken in a 50 ml volumetric flask. In this flask 2.5 mg of pure drug (corresponding spiked drug) was transferred and 20 ml of methanol was added and the flask was shaken for about 10 min. Then volume was made up to the mark with methanol and filtered through Whatman filter paper No. 42. An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml 0.1 N HCl, 3 ml of bromo phenol blue solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of chloroform and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with chloroform. The absorbance of yellow coloured chromogen was measured at 414 nm against the reagent blank. Similar procedures were adopted for formulation II (CARCA) and formulation III (CARLOC). The results of analysis of recovery studies are presented in (Table 3.21).

<table>
<thead>
<tr>
<th>Table-3.20: Results of analysis of commercial tablets of Carvedilol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet Formulation</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>I (KARDITON)</td>
</tr>
<tr>
<td>II (CARCA)</td>
</tr>
<tr>
<td>III (CARLOC)</td>
</tr>
</tbody>
</table>

*Average of six determinations
Table-3.21: Recovery studies of commercial tablets of Carvedilol

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated*(Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (KARDITON)</td>
<td>6.25</td>
<td>2.5</td>
<td>99.560 ± 1.595</td>
<td>1.601</td>
<td>0.718</td>
</tr>
<tr>
<td>II (CARCA)</td>
<td>12.50</td>
<td>5</td>
<td>100.404 ± 1.124</td>
<td>1.239</td>
<td>0.504</td>
</tr>
<tr>
<td>III (CARLOC)</td>
<td>25</td>
<td>10</td>
<td>99.696 ± 0.750</td>
<td>0.760</td>
<td>0.336</td>
</tr>
</tbody>
</table>

*Average of six determinations

3.3.3. CLOBAZAM:-

Clobazam drug sample was supplied as gift sample by Sun Pharma Labs. Ltd., Jammu. Commercial tablets of Clobazam were procured from the market (CLOBATOR-5 mg from M/s Torrent Pharma. Ltd., CLODUS-10 mg from M/s Zydus Cadila Healthcare Ltd., FRISIUM 20 mg from M/s Sanofi Aventis Pharma. Ltd.). All chemicals used were of analytical reagent grade.

Standard solution of Clobazam was prepared by dissolving 5 mg of pure drug in methanol and diluting to 100 ml with methanol. A 20 ml aliquot of this solution was diluted to 50 ml with distilled water. Twenty tablets of formulation-I (CLOBATOR) were weighed and powdered. An amount of the powder equivalent to 5 mg of the drug was weighed, transferred into a 50 ml volumetric flask, dissolved and diluted to 50 ml with methanol. It was sonicated for ten minutes and filtered through Whatman filter paper No. 42. A 20 ml aliquot of the filtrate was pipetted out and diluted to 50 ml with water.

An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml 0.1 N HCl, 3 ml of bromo phenol blue solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of chloroform and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with chloroform. A reagent blank was prepared in a similar manner without adding the drug. The absorbance of yellow coloured chromogen was measured at 413 nm against the reagent blank. Similar procedures were adopted in cases of formulation-II (CLODUS) and formulation-
III (FRISIUM). The drug content of the formulations was conducted. The results of such studies are presented in (Table 3.22). Clobazam reacts with Bromo phenol blue in acidic solution to give chloroform soluble yellow coloured ion-association complex, which exhibits an absorption maximum at 413 nm. The optimum reaction conditions for the quantitative determination of the ion-pair complex were established through a number of preliminary experiments.

The optimum concentration of the reagent was studied. It was observed that 3 ml of 0.05% bromo phenol blue solution was sufficient for maximum colour development of the complex. The effect of pH was studied by extracting the coloured complex in presence of various acid solutions and buffers. The maximum and constant colour intensity was observed when 0.1 N HCl was used. Several organic solvents such as methylene dichloride, chloroform and carbon tetrachloride were tried for extraction of the coloured complex from the aqueous phase. However, chloroform was found to be the most suitable solvent. The absorbance of the complex was found to be stable for more than 12 h. The proposed method of determination of Clobazam shows molar absorptivity of $1.8 \times 10^4$. Linear regression of absorbance with concentration gave a correlation coefficient of 0.9995 and RSD was found to be less than two. The method developed in the present work was found to be sensitive, accurate, precise and reproducible and can be used for routine determination of Clobazam in bulk and in dosage forms.

For recovery studies, tablet powder of formulation I ((CLOBATOR) equivalent to 5 mg drug was taken in a 50 ml volumetric flask. In this flask 2 mg of pure drug (corresponding spiked drug) was transferred and 20 ml of methanol was added and the flask was shaken for about 10 min. Then volume was made up to the mark with methanol and filtered through Whatman filter paper No. 42. An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml 0.1 N HCl, 3 ml of bromo phenol blue solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of chloroform and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with chloroform. The absorbance of yellow coloured chromogen was measured at 413 nm against the reagent blank. Similar procedures were adopted for
formulation II (CLODUS) and formulation III (FRISIUM). The results of analysis of recovery studies are presented in (Table 3.23).

Table-3.22: Results of analysis of commercial tablets of Clobazam

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (CLOBATOR)</td>
<td>5</td>
<td>98.236 ± 0.918</td>
<td>0.934</td>
<td>0.411</td>
</tr>
<tr>
<td>II (CLODUS)</td>
<td>10</td>
<td>101.064 ± 0.782</td>
<td>0.773</td>
<td>0.350</td>
</tr>
<tr>
<td>III (FRISIUM)</td>
<td>20</td>
<td>99.152 ± 1.069</td>
<td>1.089</td>
<td>0.479</td>
</tr>
</tbody>
</table>

*Average of six determinations

Table-3.23: Recovery studies of commercial tablets of Clobazam

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim(mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated*(Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (CLOBATOR)</td>
<td>5</td>
<td>2</td>
<td>99.560 ± 1.595</td>
<td>1.601</td>
<td>0.718</td>
</tr>
<tr>
<td>II (CLODUS)</td>
<td>10</td>
<td>4</td>
<td>100.404 ± 1.124</td>
<td>1.239</td>
<td>0.504</td>
</tr>
<tr>
<td>III (FRISIUM)</td>
<td>20</td>
<td>8</td>
<td>99.696 ± 0.750</td>
<td>0.760</td>
<td>0.336</td>
</tr>
</tbody>
</table>

*Average of six determinations

4. FLUVOXAMINE MALEATE:-

Fluvoxamine maleate drug sample was supplied as gift sample by Sun Pharma Laboratories, Ltd., Jammu. Commercial tablets of Fluvoxamine maleate were procured from the market as Fluvator-50 mg (Torrent Pharma) and Sorset-100 mg (Ranbaxy Ltd.). All other chemicals used were of analytical grade.

Solubilities of Fluvoxamine maleate were determined at 27±1°C in 10 M urea solution, distilled water and buffer of pH 10.0. Sufficient excess amount of drug was added to screw-capped glass vials of 30 ml capacity, containing distilled water, buffer of pH 10.0 and 10 M urea solution. The vials were shaken mechanically for 12 hours at 27±1°C in
orbital shaker (Khera Instrument Pvt. Ltd., India). The solutions were allowed to equilibrate for next 24 hours and then centrifuged for 5 min at 2000 rpm. The supernatant of each vial was filtered through Whatman filter paper No. 41. Filtrates were diluted suitably and analyzed against corresponding solvent blanks.

Twenty tablets of formulation-I (Fluvator) were weighed and powdered. Powder equivalent to 50 mg Fluvoxamine maleate was transferred to a 50 ml volumetric flask containing 40 ml of 10 M urea solution. The flask was shaken for about 5 min to solubilize the drug. Then volume was made up to the mark with distilled water. Solution was filtered through Whatman filter paper No. 41. filtrate was divided in two parts, A and B. part A was kept at room temperature for 48 hours to check the effect on stability of drug in presence of urea and also to note precipitation, if any, during this period.

Part B filtrate was appropriately diluted with distilled water and absorbance was noted at 271 nm ($\lambda_{\text{max}}$) against solvent blank and the drug content was calculated (Table 3.24). After 48 hours, filtrate of part B was also appropriately diluted with distilled water and analyzed for drug content. There was no precipitation in the filtrate in 48 hours. Similar procedure was adopted in case of formulation-II (Sorset) and drug content was calculated (Table 3.24).

In order to check the accuracy, reproducibility and the precision of the proposed method, recovery studies were performed by adding powder of bulk drug in dosage form. Pre analyzed tablet powder (Formulation-I) equivalent to 50 mg of Fluvoxamine maleate was transferred to 50 ml of volumetric flask. Pure Fluvoxamine maleate drug sample (20 mg) was added and flask was shaken for 5 min to solubilize the drug. The volume was made up to the mark with distilled water. Then solution was filtered through Whatman filter paper No. 41. The filtrate was diluted with distilled water appropriately and absorbance was noted at 271 nm against corresponding reagent blank. Drug content was calculated and percent recovery was calculated (Table 10). Similar procedure was repeated using same way. The drug content was determined and percent recovery was estimated (Table 3.25).
Table 3.24: Results of analysis of commercial tablets of Fluvoxamine Maleate

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of Variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Flvator)</td>
<td>50</td>
<td>99.63 ± 0.885</td>
<td>0.892</td>
<td>0.442</td>
</tr>
<tr>
<td>II(Sorset)</td>
<td>100</td>
<td>101.28 ± 0.987</td>
<td>0.974</td>
<td>0.403</td>
</tr>
</tbody>
</table>

*Average of six determinations

Table 3.25: Recovery studies of commercial tablets of Fluvoxamine Maleate

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated*(Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Flvator)</td>
<td>50</td>
<td>20</td>
<td>99.75 ± 1.486</td>
<td>1.505</td>
<td>0.607</td>
</tr>
<tr>
<td>II (Sorset)</td>
<td>100</td>
<td>40</td>
<td>99.96 ± 0.870</td>
<td>0.879</td>
<td>0.435</td>
</tr>
</tbody>
</table>

*Average of six determinations.

3.3.5. CLOZAPINE

Clozapine drug sample was supplied as gift sample by Sun Pharma Labs. Ltd., Jammu. Commercial tablets of Clozapine were procured from the market (SIZOPIN-25 mg from M/s Sun Pharma Labs. Ltd., SKIZORIL-50 mg from M/s Intas Pharma Ltd. and ZOPIN-100 mg from M/s PIL Ltd.). All chemicals used were of analytical reagent grade.

Standard solution of Clozapine was prepared by dissolving 25 mg of pure drug in methanol and diluting to 100 ml with methanol. A 20 ml aliquot of this solution was diluted to 50 ml with distilled water. Twenty tablets of formulation-I (SIZOPIN) were weighed and powdered. An amount of the powder equivalent to 25 mg of the drug was weighed, transferred into a 50 ml volumetric flask, dissolved and diluted to 50 ml with methanol. It was sonicated for ten minutes and filtered through Whatman filter paper No. 42. A 20 ml aliquot of the filtrate was pipetted out and diluted to 50 ml with water.
An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml 0.1 N HCl, 3 ml of alizarin red solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of chloroform and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with chloroform. A reagent blank was prepared in a similar manner without adding the drug. The absorbance of magenta coloured chromogen was measured at 514 nm against the reagent blank. Similar procedures were adopted in cases of formulation-II (SKIZORIL) and formulation-III (ZOPIN). The drug content of the formulations were conducted. The results of such studies are presented in (Table 3.26). Clozapine reacts with Alizarin red in acidic solution to give chloroform soluble yellow coloured ion-association complex, which exhibits an absorption maximum at 514 nm. The optimum reaction conditions for the quantitative determination of the ion-pair complex were established through a number of preliminary experiments.

The optimum concentration of the reagent was studied. It was observed that 3 ml of 0.05% alizarin red solution was sufficient for maximum colour development of the complex. The effect of pH was studied by extracting the coloured complex in presence of various acid solutions and buffers. The maximum and constant colour intensity was observed when 0.1 N HCl was used. Several organic solvents such as methylene dichloride, chloroform and carbon tetrachloride were tried for extraction of the coloured complex from the aqueous phase. However, chloroform was found to be the most suitable solvent. The absorbance of the complex was found to be stable for more than 12 h. The proposed method of determination of clozapine shows molar absorptivity of $1.8 \times 10^4$. Linear regression of absorbance with concentration gave a correlation coefficient of 0.9995 and RSD was found to be less than two. The method developed in the present work was found to be sensitive, accurate, precise and reproducible and can be used for routine determination of Clozapine in bulk and in dosage forms.

For recovery studies, tablet powder of formulation I ((SIZOPIN) equivalent to 25 mg drug was taken in a 50 ml volumetric flask. In this flask 12 mg of pure drug (corresponding spiked drug) was transferred and 20 ml of methanol was added and the flask was shaken for about 10 min. Then volume was made upto the mark with methanol.
and filtered through Whatman filter paper No. 42. An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml 0.1 N HCl, 3 ml of Alizarin red solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of chloroform and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with chloroform. The absorbance of magenta coloured chromogen was measured at 514 nm against the reagent blank. Similar procedures were adopted for formulation II (SKIZORIL) and formulation III (ZOPIN). The results of analysis of recovery studies are presented in (Table 3.27).

Table-3.26: Results of analysis of commercial tablets of Clozapine

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. Of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (SIZOPIN)</td>
<td>25</td>
<td>99.790 ± 1.0514</td>
<td>1.053</td>
<td>0.470</td>
</tr>
<tr>
<td>II (SKIZORIL)</td>
<td>50</td>
<td>100.492 ± 1.0445</td>
<td>1.0394</td>
<td>0.467</td>
</tr>
<tr>
<td>III (ZOPIN)</td>
<td>100</td>
<td>99.868 ±0.951</td>
<td>0.952</td>
<td>0.425</td>
</tr>
</tbody>
</table>

*Average of six determinations

Table-3.27: Recovery studies of commercial tablets of Clozapine

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated*(Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I(SIZOPIN)</td>
<td>25</td>
<td>13</td>
<td>99.52 ± 0.588</td>
<td>0.592</td>
<td>0.241</td>
</tr>
<tr>
<td>II (SKIZORIL)</td>
<td>50</td>
<td>25</td>
<td>99.36 ± 0.708</td>
<td>0.713</td>
<td>0.289</td>
</tr>
<tr>
<td>III (ZOPIN)</td>
<td>100</td>
<td>50</td>
<td>99.67 ±0.584</td>
<td>0.586</td>
<td>0.238</td>
</tr>
</tbody>
</table>

*Average of six determinations
3.3.6. EZETIMIBE

Ezetimibe drug sample was supplied as gift sample by Sun Pharma Laboratories Ltd., Jammu. Commercial tablets of Ezetimibe were procured from the market as EZEDOC-10 mg (Lupin Pinnacle) and EZTA-20 mg (Zydus Cadila). All other chemicals used were of analytical grade.

Solubilities of Ezetimibe were determined at 27±1°C in 2 M sodium benzoate solution, distilled water. Sufficient excess amount of drug was added to screw-capped glass vials of 30 ml capacity, containing distilled water and 2 M sodium benzoate solution. The vials were shaken mechanically for 12 hours at 27±1°C in orbital shaker (Khera Instrument Pvt. Ltd., India). The solutions were allowed to equilibrate for next 24 hours and then centrifuged for 5 min at 2000 rpm. The supernatant of each vial was filtered through Whatman filter paper No. 41. Filtrates were diluted suitably and analyzed against corresponding solvent blanks.

Twenty tablets of formulation-I (EZEDOC) were weighed and powdered. Powder equivalent to 10 mg Fluvoxamine maleate was transferred to a 50 ml volumetric flask containing 40 ml of 2 M sodium benzoate solution. The flask was shaken for about 5 min to solubilize the drug. Then volume was made upto the mark with distilled water. Solution was filtered through Whatman filter paper No. 41. filtrate was divided in two parts, A and B. part A was kept at room temperature for 48 hours to check the effect on stability of drug in presence of urea and also to note precipitation, if any, during this period.

Part B filtrate was appropriately diluted with distilled water and absorbance was noted at 324 nm ($\lambda_{\text{max}}$) against solvent blank and the drug content was calculated (Table 3.28). After 48 hours, filtrate of part B was also appropriately diluted with distilled water and analyzed for drug content. There was no precipitation in the filtrate in 48 hours. Similar procedure was adopted in case of formulation-II (EZTA) and drug content was calculated (Table 3.28).

In order to check the accuracy, reproducibility and the precision of the proposed method, recovery studies were performed by adding powder of bulk drug in dosage form. Preanalyzed tablet powder (Formulation-I) equivalent to 10 mg of Ezetimibe was
transferred to 50 ml of volumetric flask. Pure Ezetimibe drug sample (5 mg) was added and flask was shaken for 5 min to solubilize the drug. The volume was made up to the mark with distilled water. Then solution was filtered through Whatman filter paper No. 41. The filtrate was diluted with distilled water appropriately and absorbance was noted at 324 nm against corresponding reagent blank. Drug content was calculated and percent recovery was calculated (Table 5.12). Similar procedure was repeated using same way for formulation-II. The drug content was determined and percent recovery was estimated (Table 3.29).

Table 3.28: Results of analysis of commercial tablets of Ezetimibe

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of Variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (EZETA)</td>
<td>10</td>
<td>100.368 ± 0.613</td>
<td>0.611</td>
<td>0.274</td>
</tr>
<tr>
<td>II(EZEDOC)</td>
<td>20</td>
<td>99.738 ± 1.199</td>
<td>1.202</td>
<td>0.536</td>
</tr>
</tbody>
</table>

*AVERAGE OF SIX DETERMINATIONS*

Table 3.29: Recovery studies of commercial tablets of Ezetimibe

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated*(Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (EZETA)</td>
<td>10</td>
<td>5</td>
<td>99.38 ± 1.235</td>
<td>1.243</td>
<td>0.504</td>
</tr>
<tr>
<td>II(EZEDOC)</td>
<td>20</td>
<td>10</td>
<td>100.63 ± 0.503</td>
<td>0.500</td>
<td>0.205</td>
</tr>
</tbody>
</table>

*AVERAGE OF SIX DETERMINATIONS.*
3.3.7. FENOFIBRATE

Fenofibrate drug sample was supplied as gift sample by Sun Pharma Labs. Ltd., Jammu. Commercial tablets of Fenofibrate were procured from the market (FENOLIP-145 mg from Cipla Pharma., STANLIP-160 mg from Ranbaxy Ltd., LOTZL-200 mg from Grandix lab) All other chemicals used were of analytical grade.

**Preliminary solubility studies of Fenofibrate:** solubilities of Fenofibrate were determined in 4 M sodium acetate and 1.25 M sodium citrate solution, distilled water sufficient excess amount of drug was added to screw-capped glass vials of 20 ml capacity, containing distilled water, and 4 M sodium acetate and 1.25 M sodium citrate solution. The vials were shaken mechanically for 12 hours at in orbital shaker (Khera Instrument Pvt. Ltd., India). The solutions were allowed to equilibrate for next 24 hours and then centrifuged for 5 min at 2000 rpm. The supernatant of each vial was filtered through Whatman filter paper # 41. filtrates were diluted suitably and analyzed against corresponding solvent blanks. In this experiment mixed hydrotropy principle is applied in which to hydrotrops in different concentration were used for increasing the solubility of the drug for example 4 M sodium acetate and 1.25 M sodium citrate.

**Analysis of Fenofibrate in tablets using 4 M sodium acetate and 1.25 M sodium citrate solution:** Twenty tablets of formulation-I (FENOLIP) were weighed and powdered. Powder equivalent to 145 mg Fenofibrate was transferred to a 50 ml volumetric flask containing 40 ml of 4 M sodium acetate and 1.25 M sodium citrate solution. The flask was shaken for about 5 min to solubilize the drug. Then volume was made up to the mark with distilled water. Solution was filtered through Whatman filter paper # 41. filtrate was divided in two parts, A and B. part A was kept at room temperature for 48 hours to check the effect on stability of drug in presence of sodium benzoate and also to note precipitation, if any, during this period. Part B filtrate was appropriately diluted with distilled water and absorbance was noted at 296 nm ($\lambda_{\text{max}}$) against solvent blank and the drug content was calculated (Table-3.30). After 48 hours, filtrate of part B was also appropriately diluted with distilled water and analyzed for drug content. There was no precipitation in the filtrate in 48 hours. Similar procedures were adopted in cases of formulation-II (STANLIP) and formulation-III (LOTZL).
Recovery Studies:

Recovery studies are performed by adding extra bulk drug nearly forty percent of formulations or more. For recovery studies, tablet powder of formulation I (FENOLIP) equivalent to 145 mg drug was taken in a 25 ml volumetric flask. In this flask 70 mg of pure drug (corresponding spiked drug) was transferred and 20 ml of 4 M sodium acetate and 1.25 M sodium citrate solutions were added and the flask was shaken for about 10 min. Then volume was made up to the mark with distilled water and filtered through Whatman filter paper # 41. The solution was diluted appropriately with distilled water and analyzed for drug content. Similar procedures were adopted for formulation II (STANLIP) & formulation III (LOTZL). The results of analysis of recovery studies are presented in (Table 3.31).

Table-3.30: Results of analysis of commercial tablets of Fenofibrate

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (FENOLIP)</td>
<td>145</td>
<td>100.073 ± 0.7481</td>
<td>0.7476</td>
<td>0.3054</td>
</tr>
<tr>
<td>II (STANLIP)</td>
<td>160</td>
<td>99.90 ± 0.1008</td>
<td>0.1009</td>
<td>0.0411</td>
</tr>
<tr>
<td>III (LOTZL)</td>
<td>200</td>
<td>100.596 ± 0.6114</td>
<td>0.6078</td>
<td>0.2734</td>
</tr>
</tbody>
</table>

*Average of six determinations

Table-3.31: Recovery studies of commercial tablets of Fenofibrate

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (FENOLIP)</td>
<td>145</td>
<td>70</td>
<td>99.33 ± 1.762</td>
<td>0.1774</td>
<td>0.719</td>
</tr>
<tr>
<td>II (STANLIP)</td>
<td>160</td>
<td>80</td>
<td>100.61 ± 0.1322</td>
<td>1.314</td>
<td>0.540</td>
</tr>
<tr>
<td>III (LOTZL)</td>
<td>200</td>
<td>100</td>
<td>100.92 ± 1.702</td>
<td>1.686</td>
<td>0.695</td>
</tr>
</tbody>
</table>

*Average of six determinations
3.3.8. ATENOLOL

Atenolol drug sample was supplied as gift sample by Sun Pharma Labs. Ltd., Jammu. Commercial tablets of Atenolol were procured from the market (ATENEX-25 mg from Zydus Cadila., ATENOVA-50 mg from Lupin (Pinnacle), CADPRES-100 mg from Cadila Pharma.) All other chemicals used were of analytical grade.

Preliminary solubility studies of Atenolol: solubilities of Atenolol were determined in 2 M sodium salicylate solution, distilled water sufficient excess amount of drug was added to screw-capped glass vials of 20 ml capacity, containing distilled water, and 2 M sodium salicylate solution. The vials were shaken mechanically for 12 hours at in orbital shaker (Khera Instrument Pvt. Ltd., India). The solutions were allowed to equilibrate for next 24 hours and then centrifuged for 5 min at 2000 rpm. The supernatant of each vial was filtered through Whatman filter paper # 41. filtrates were diluted suitably and analyzed against corresponding solvent blanks.

Analysis of Atenolol in tablets using 2 M sodium salicylate solution: Twenty tablets of formulation-I (ATENEX) were weighed and powdered. Powder equivalent to 25 mg Atenolol was transferred to a 50 ml volumetric flask containing 40 ml of 2 M sodium salicylate solution. The flask was shaken for about 5 min to solubilize the drug. Then volume was made upto the mark with distilled water. Solution was filtered through Whatman filter paper # 41. filtrate was divided in two parts, A and B. part A was kept at room temperature for 48 hours to check the effect on stability of drug in presence of urea and also to note precipitation, if any, during this period. Part B filtrate was appropriately diluted with distilled water and absorbance was noted at 278 nm ($\lambda_{\text{max}}$) against solvent blank and the drug content was calculated (Table-3.32). After 48 hours, filtrate of part B was also appropriately diluted with distilled water and analyzed for drug content. There was no precipitation in the filtrate in 48 hours. Similar procedures were adopted in cases of formulation-II (ATENOVA) and formulation-III (CADPRES).
Recovery Studies:

Recovery studies are performed by adding extra bulk drug nearly forty percent of formulations or more.

For recovery studies, tablet powder of formulation I ((ATENEX) equivalent to 25 mg drug was taken in a 25 ml volumetric flask. In this flask 12 mg of pure drug (corresponding spiked drug) was transferred and 20 ml of 2.0 M sodium salicylate solution was added and the flask was shaken for about 10 min. Then volume was made upto the mark with distilled water and filtered through Whatman filter paper # 41. The solution was diluted appropriately with distilled water and analyzed for drug content. Similar procedures were adopted for formulation II (ATENOVA) & formulation III (CADPRES). The results of analysis of recovery studies are presented is (Table 3.33).

Table-3.32: Results of analysis of commercial tablets of Atenolol

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of Variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (ATENEX)</td>
<td>25</td>
<td>99.238 ± 0.6068</td>
<td>0.6115</td>
<td>0.2714</td>
</tr>
<tr>
<td>II (ATENOVA)</td>
<td>50</td>
<td>101.10 ± 1.113</td>
<td>1.101</td>
<td>0.4540</td>
</tr>
<tr>
<td>III (CADPRES)</td>
<td>100</td>
<td>100.278 ±0.6606</td>
<td>0.6588</td>
<td>0.2954</td>
</tr>
</tbody>
</table>

*Average of six determinations

Table-3.33: Recovery studies of commercial tablets of Atenolol

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (ATENEX)</td>
<td>25</td>
<td>13</td>
<td>100.61 ± 0.932</td>
<td>0.926</td>
<td>0.381</td>
</tr>
<tr>
<td>II (ATENOVA)</td>
<td>50</td>
<td>25</td>
<td>99.49 ± 0.799</td>
<td>0.803</td>
<td>0.326</td>
</tr>
<tr>
<td>III (CADPRES)</td>
<td>100</td>
<td>50</td>
<td>99.11 ±0.788</td>
<td>0.795</td>
<td>0.322</td>
</tr>
</tbody>
</table>

*Average of six determinations
3.3.9. TELMISARTAN

Telmisartan drug sample was supplied as gift sample by Oasis Test Laboratory Jaipur. Commercial tablets of Telmisartan were procured from the market (TETAN-20 mg from Alembic Ltd., TELDAY-40 mg from Torrent Lab. Ltd., and TELSAR-80 mg from Unichem Lab) All other chemicals used were of analytical grade.

**Preliminary solubility studies of Telmisartan:** solubilities of Telmisartan were determined in 10 M urea solution, distilled water sufficient excess amount of drug was added to screw-capped glass vials of 20 ml capacity, containing distilled water, and 10 M urea solution. The vials were shaken mechanically for 12 hours at in orbital shaker (Khera Instrument Pvt. Ltd., India). The solutions were allowed to equilibrate for next 24 hours and then centrifuged for 5 min at 2000 rpm. The supernatant of each vial was filtered through Whatman filter paper # 41. filtrates were diluted suitably and analyzed against corresponding solvent blanks.

**Analysis of Telmisartan in tablets using 10 M urea solution:** Twenty tablets of formulation-I (TETAN) were weighed and powdered. Powder equivalent to 20 mg Telmisartan was transferred to a 50 ml volumetric flask containing 40 ml of 10 M urea solution. The flask was shaken for about 5 min to solubilize the drug. Then volume was made upto the mark with distilled water. Solution was filtered through Whatman filter paper # 41. filtrate was divided in two parts, A and B. part A was kept at room temperature for 48 hours to check the effect on stability of drug in presence of urea and also to note precipitation, if any, during this period. Part B filtrate was appropriately diluted with distilled water and absorbance was noted at 315 nm ($\lambda_{\text{max}}$) against solvent blank and the drug content was calculated (Table-3.34). After 48 hours, filtrate of part B was also appropriately diluted with distilled water and analyzed for drug content. There was no precipitation in the filtrate in 48 hours. Similar procedures were adopted in cases of formulation-II (TELDAY) and formulation-III (TELSAR).
**Recovery Studies:**

Recovery studies are performed by adding extra bulk drug nearly forty percent of formulations or more.

For recovery studies, tablet powder of formulation I ((TETAN) equivalent to 20 mg drug was taken in a 25 ml volumetric flask. In this flask 10 mg of pure drug (corresponding spiked drug) was transferred and 20 ml of 10.0 M UREA solution was added and the flask was shaken for about 10 min. Then volume was made upto the mark with distilled water and filtered through Whatman filter paper # 41. The solution was diluted appropriately with distilled water and analyzed for drug content. Similar procedures were adopted for formulation II (TELDAY) & formulation III (TELSAR). The results of analysis of recovery studies are presented is (Table 3.35).

**Table-3.34: Results of analysis of commercial tablets of Telmisartan**

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of Variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (TETAN)</td>
<td>20</td>
<td>99.01 ±1.4459</td>
<td>1.4603</td>
<td>0.6466</td>
</tr>
<tr>
<td>II (TELDAY)</td>
<td>40</td>
<td>100.494 ± 0.7029</td>
<td>0.6995</td>
<td>0.3110</td>
</tr>
<tr>
<td>III (TELSAR)</td>
<td>80</td>
<td>100.121 ±0.9806</td>
<td>0.9888</td>
<td>0.2987</td>
</tr>
</tbody>
</table>

*Average of six determinations

**Table-3.35: Recovery studies of commercial tablets of Telmisartan**

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated*(Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (TETAN)</td>
<td>20</td>
<td>10</td>
<td>99.13 ± 1.103</td>
<td>1.113</td>
<td>0.450</td>
</tr>
<tr>
<td>II (TELDAY)</td>
<td>40</td>
<td>20</td>
<td>100.06 ± 1.139</td>
<td>1.138</td>
<td>0.465</td>
</tr>
<tr>
<td>III (TELSAR)</td>
<td>80</td>
<td>40</td>
<td>99.41 ±0.824</td>
<td>0.829</td>
<td>0.336</td>
</tr>
</tbody>
</table>

*Average of six determinations
3.3.10. PIOGLITAZONE

Pioglitazone drug sample was supplied as gift sample by Sun Pharma Labs. Ltd., Jammu. Commercial tablets of Pioglitazone were procured from the market (CLOBATOR-5 mg from M/s Torrent Pharma. Ltd., CLODUS-10 mg from M/s Zydus Cadila Healthcare Ltd., FRISIUM 20 mg from M/s Sanofi Aventis Pharma. Ltd.). All chemicals used were of analytical reagent grade.

Standard solution of Pioglitazone was prepared by dissolving 15 mg of pure drug in methanol and diluting to 100 ml with methanol. A 20 ml aliquot of this solution was diluted to 50 ml with distilled water. Twenty tablets of formulation-I (PIOCON) were weighed and powdered. An amount of the powder equivalent to 5 mg of the drug was weighed, transferred into a 50 ml volumetric flask, dissolved and diluted to 50 ml with methanol. It was sonicated for ten minutes and filtered through Whatman filter paper No. 42. A 20 ml aliquot of the filtrate was pipetted out and diluted to 50 ml with water.

An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml of phthalate buffer of pH 2.4, 3 ml of Bromocresol green solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of chloroform and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with chloroform. A reagent blank was prepared in a similar manner without adding the drug. The absorbance of pale orange coloured chromogen was measured at 419 nm against the reagent blank. Similar procedures were adopted in cases of formulation-II (GLITTER) The drug content of the formulations were conducted. The results of such studies are presented in (Table 3.36). Pioglitazone reacts with Bromocresol green in acidic solution to give chloroform soluble pale orange coloured ion-association complex, which exhibits an absorption maximum at 419 nm. The optimum reaction conditions for the quantitative determination of the ion-pair complex were established through a number of preliminary experiments.

The optimum concentration of the reagent was studied. It was observed that 3 ml of 0.05% Bromocresol green solution was sufficient for maximum colour development of the complex. The effect of pH was studied by extracting the coloured complex in
presence of various acid solutions and buffers. The maximum and constant colour intensity was observed when phthalate buffer of pH 2.4 was used. Several organic solvents such as methylene dichloride, chloroform and carbon tetrachloride were tried for extraction of the coloured complex from the aqueous phase. However, chloroform was found to be the most suitable solvent. The absorbance of the complex was found to be stable for more than 12 h. The proposed method of determination of Pioglitazone shows molar absorptivity of $1.8 \times 10^4$. Linear regression of absorbance with concentration gave a correlation coefficient of 0.9996 and RSD was found to be less than two. The method developed in the present work was found to be sensitive, accurate, precise and reproducible and can be used for routine determination of Pioglitazone in bulk and in dosage forms. The method was based on the formation of an ion association complex with indicator or colouring agent.

For recovery studies, tablet powder of formulation I ((PIOCON) equivalent to 15 mg drug was taken in a 50 ml volumetric flask. In this flask 7 mg of pure drug (corresponding spiked drug) was transferred and 20 ml of methanol was added and the flask was shaken for about 10 min. Then volume was made up to the mark with methanol and filtered through Whatman filter paper No. 42. An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml phthalate buffer of pH 2.4, 3 ml of Bromocresol green solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of chloroform and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with chloroform. The absorbance of pale orange coloured chromogen was measured at 419 nm against the reagent blank. Similar procedures were adopted for formulation II (GLITTER). The results of analysis of recovery studies are presented in (Table 3.37).
MATERIALS AND METHODS

Table-3.36: Results of analysis of commercial tablets of Pioglitazone

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of Variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (PIOCON)</td>
<td>15</td>
<td>100.254 ± 1.1237</td>
<td>1.1208</td>
<td>0.5025</td>
</tr>
<tr>
<td>II (GLITTER)</td>
<td>30</td>
<td>99.654 ± 0.7972</td>
<td>0.7999</td>
<td>0.3565</td>
</tr>
</tbody>
</table>

*Average of six determinations

Table-3.37: Recovery studies of commercial tablets of Pioglitazone

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (PIOCON)</td>
<td>15</td>
<td>7</td>
<td>99.431 ± 0.3776</td>
<td>0.3793</td>
<td>0.1542</td>
</tr>
<tr>
<td>II (GLITTER)</td>
<td>30</td>
<td>15</td>
<td>99.674 ± 0.5845</td>
<td>0.5862</td>
<td>0.2387</td>
</tr>
</tbody>
</table>

*Average of six determinations

3.3.11. REPAGLINIDE

Repaglinide drug sample was supplied as gift sample by Sun Pharma Labs. Ltd., Jammu. Commercial tablets of Repaglinide were procured from the market (RAPILIN-0.5 mg from M/s Sun Pharma. Ltd., REGAN-1mg from M/s Ranbaxy Ltd. & REPA 2 mg from M/s Orchid Pharma. Ltd.). All chemicals used were of analytical reagent grade.

Standard solution of Repaglinide was prepared by dissolving 0.5 mg of pure drug in methanol and diluting to 100 ml with methanol. A 20 ml aliquot of this solution was diluted to 50 ml with distilled water. Twenty tablets of formulation-I (RAPILIN) were weighed and powdered. An amount of the powder equivalent to 0.5 mg of the drug was weighed, transferred into a 50 ml volumetric flask, dissolved and diluted to 50 ml with methanol. It was sonicated for ten minutes and filtered through Whatman filter paper No. 42. A 20 ml aliquot of the filtrate was pipetted out and diluted to 50 ml with water.
An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml of 1,10-phenanthroline solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of chloroform and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with chloroform. A reagent blank was prepared in a similar manner without adding the drug. The absorbance of red coloured chromogen was measured at 520 nm against the reagent blank. Similar procedures were adopted in cases of formulation-II (REGAN) and formulation-III (REPA). The drug content of the formulations were conducted. The results of such studies are presented in (Table 3.38). Repaglinide reacts with 1,10 phenanthroline solution to give chloroform soluble greenish red coloured ion-association complex, which exhibits an absorption maximum at 520 nm. The optimum reaction conditions for the quantitative determination of the ion-pair complex were established through a number of preliminary experiments.

The optimum concentration of the reagent was studied. It was observed that 3 ml of 0.05% 1, 10-phenanthroline solution was sufficient for maximum colour development of the complex. The effect of pH was studied by extracting the coloured complex in presence of various acid solutions and buffers. The maximum and constant colour intensity was observed. Several organic solvents such as methylene dichloride, chloroform and carbon tetrachloride were tried for extraction of the coloured complex from the aqueous phase. However, chloroform was found to be the most suitable solvent. The absorbance of the complex was found to be stable for more than 12 h. The proposed method of determination of Repaglinide shows molar absorptivity of $0.565 \times 10^4$. Linear regression of absorbance with concentration gave a correlation coefficient of 0.9998 and RSD was found to be less than two. The method developed in the present work was found to be sensitive, accurate, precise and reproducible and can be used for routine determination of Repaglinide in bulk and in dosage forms.

For recovery studies, tablet powder of formulation I ((RAPILIN) equivalent to 0.5 mg drug was taken in a 50 ml volumetric flask. In this flask 0.25 mg of pure drug (corresponding spiked drug) was transferred and 20 ml of methanol was added and the flask was shaken for about 10 min. Then volume was made up to the mark with methanol.
and filtered through Whatman filter paper No. 42. An aliquot of 10 ml each of the 
standard and sample preparation was transferred to 125 ml separating funnel followed by 
3 ml 1, 10-phenanthroline solution and rest water to make the volume to 25 ml. The 
solution was extracted three times successively with 10, 5, 5 ml portions of chloroform 
and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted 
to 25 ml with chloroform. The absorbance of greenish blue coloured chromogen was 
measured at 520 nm against the reagent blank. Similar procedures were adopted for 
formulation II (REGAN) and formulation III (REPA). The results of analysis of recovery 
studies are presented in (Table 3.39).

Table-3.38: Results of analysis of commercial tablets of Repaglinide

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. Of Variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (RAPILIN)</td>
<td>0.5</td>
<td>100.231 ±0.1432</td>
<td>0.1421</td>
<td>0.0767</td>
</tr>
<tr>
<td>II (REGAN)</td>
<td>1</td>
<td>99.301 ± 0.7238</td>
<td>0.7154</td>
<td>0.2876</td>
</tr>
<tr>
<td>III (REPA)</td>
<td>2</td>
<td>99.068 ±0.1274</td>
<td>0.1283</td>
<td>0.0543</td>
</tr>
</tbody>
</table>

*Average of six determinations

Table-3.39: Recovery studies of commercial tablets of Repaglinide

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated*(Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (RAPILIN)</td>
<td>0.5</td>
<td>0.25</td>
<td>99.567 ± 0.4732</td>
<td>0.4755</td>
<td>0.1934</td>
</tr>
<tr>
<td>II (REGAN)</td>
<td>1</td>
<td>0.5</td>
<td>100.092 ± 0.4973</td>
<td>0.4963</td>
<td>0.2032</td>
</tr>
<tr>
<td>III (REPA)</td>
<td>2</td>
<td>1</td>
<td>99.671 ±0.5845</td>
<td>0.586</td>
<td>0.2382</td>
</tr>
</tbody>
</table>

*Average of six determinations
MATERIALS AND METHODS

3.3.12. ROSIGLITAZONE

Rosiglitazone drug sample was supplied as gift sample by Sun Pharma Labs. Ltd., Jammu. Commercial tablets of Rosiglitazone were procured from the market (SENZIA – 2 mg from M/s Cipla Ltd., ROSS – 4 mg from M/s Orchid Lab. Ltd., REZULT – 8 mg from M/s Sun Pharma. Ltd). All chemicals used were of analytical reagent grade.

Standard solution of Rosiglitazone was prepared by dissolving 2 mg of pure drug in methanol and diluting to 100 ml with methanol. A 20 ml aliquot of this solution was diluted to 50 ml with distilled water. Twenty tablets of formulation-I (SENZIA) were weighed and powdered. An amount of the powder equivalent to 5 mg of the drug was weighed, transferred into a 50 ml volumetric flask, dissolved and diluted to 50 ml with methanol. It was sonicated for ten minutes and filtered through Whatman filter paper No. 42. A 20 ml aliquot of the filtrate was pipetted out and diluted to 50 ml with water.

An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml 0.1 Britton Robinson (B-R) buffer of pH 4.5, 3 ml of Bromo thymol blue solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of methylene dichloride and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with methylene di chloride. A reagent blank was prepared in a similar manner without adding the drug. The absorbance of orange coloured chromogen was measured at 434 nm against the reagent blank. Similar procedures were adopted in cases of formulation-II (ROSS) and formulation-III (REZULT). The drug content of the formulations were conducted. The results of such studies are presented in (Table 3.40). Rosiglitazone reacts with Bromo thymol blue in acidic solution to give methylene di chloride soluble orange coloured ion-association complex, which exhibits an absorption maximum at 457 nm. The optimum reaction conditions for the quantitative determination of the ion-pair complex were established through a number of preliminary experiments.

The optimum concentration of the reagent was studied. It was observed that 3 ml of 0.05% bromo thymol blue solution was sufficient for maximum colour development of the complex. The effect of pH was studied by extracting the coloured complex in presence of various acid solutions and buffers. The maximum and constant colour...
intensity was observed when Britton Robinson (B-R) buffer of pH 4.5 was used. Several organic solvents such as methylene dichloride, chloroform and carbon tetrachloride were tried for extraction of the coloured complex from the aqueous phase. However, methylene di chloride was found to be the most suitable solvent. The absorbance of the complex was found to be stable for more than 12 h. The proposed method of determination of Rosiglitazone shows molar absorptivity of $1.7 \times 10^5$. Linear regression of absorbance with concentration gave a correlation coefficient of 0.9995 and RSD was found to be less than two. The method developed in the present work was found to be sensitive, accurate, precise and reproducible and can be used for routine determination of Rosiglitazone in bulk and in dosage forms.

For recovery studies, tablet powder of formulation I ((SENZIA) equivalent to 2 mg drug was taken in a 50 ml volumetric flask. In this flask 1 mg of pure drug (corresponding spiked drug) was transferred and 20 ml of methanol was added and the flask was shaken for about 10 min. Then volume was made up to the mark with methanol and filtered through Whatman filter paper No. 42. An aliquot of 10 ml each of the standard and sample preparation was transferred to 125 ml separating funnel followed by 2 ml Britton Robinson (B-R) buffer of 4.5, 3 ml of bromo thymol blue solution and rest water to make the volume to 25 ml. The solution was extracted three times successively with 10, 5, 5 ml portions of methylene di chloride and filtered through anhydrous sodium sulphate into 25 ml volumetric flask and diluted to 25 ml with methylene di chloride. The absorbance of orange coloured chromogen was measured at 434 nm against the reagent blank. Similar procedures were adopted for formulation II (ROSS) and formulation III (REZULT). The results of analysis of recovery studies are presented in (Table 3.41)
### Table-3.40: Results of analysis of commercial tablets of Rosiglitazone

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>% Label claim estimated* (Mean ± S.D.)</th>
<th>% Coeff. of Variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (SENZIA)</td>
<td>2</td>
<td>99.335 ±0.1082</td>
<td>01092</td>
<td>0.0443</td>
</tr>
<tr>
<td>II (ROSS)</td>
<td>4</td>
<td>99.446 ± 0.9754</td>
<td>0.9905</td>
<td>0.3983</td>
</tr>
<tr>
<td>III (REZULT)</td>
<td>8</td>
<td>100.453 ± 1.4328</td>
<td>1.4236</td>
<td>0.7958</td>
</tr>
</tbody>
</table>

*Average of six determinations

### Table-3.41: Recovery studies of commercial tablets of Rosiglitazone

<table>
<thead>
<tr>
<th>Tablet Formulation</th>
<th>Label claim (mg)</th>
<th>Drug added (mg)</th>
<th>% Label claim estimated*(Mean ± S.D.)</th>
<th>% Coeff. of variation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (SENZIA)</td>
<td>2</td>
<td>1</td>
<td>100.754 ± 1.3492</td>
<td>1.3395</td>
<td>0.5513</td>
</tr>
<tr>
<td>II (ROSS)</td>
<td>4</td>
<td>2</td>
<td>100.537 ± 1.14325</td>
<td>1.4214</td>
<td>0.7685</td>
</tr>
<tr>
<td>III (REZULT)</td>
<td>8</td>
<td>4</td>
<td>99.392 ± 2.0623</td>
<td>2.0752</td>
<td>0.8723</td>
</tr>
</tbody>
</table>

*Average of six determinations
3.3 (b) HPLC METHODS:

3.3.13. SIMVASTATIN

Table 3.42: HPLC instrumentation & chromatographic conditions for Simvastatin

<table>
<thead>
<tr>
<th>1. S. No.</th>
<th>Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Instrument</td>
<td>A HPLC instrument (Younglin series) with Model Acme-9000</td>
</tr>
<tr>
<td>3.</td>
<td>Column</td>
<td>Promosil C-18, (250 mm, 4.6 mm, 5µm)</td>
</tr>
<tr>
<td>4.</td>
<td>Mobile Phase</td>
<td>Different mobile phase used for Trial 1 to 6</td>
</tr>
<tr>
<td>5.</td>
<td>Flow Rate</td>
<td>1.0 mL/minute</td>
</tr>
<tr>
<td>6.</td>
<td>Detection wavelength</td>
<td>254 nm</td>
</tr>
<tr>
<td>7.</td>
<td>Injection Volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>8.</td>
<td>Run Time</td>
<td>20 minutes</td>
</tr>
</tbody>
</table>

Optimum condition of mobile phases were investigated in the development of an HPLC method suitable for analysis of Simvastatin in the bulk drug. These included Methanol: Acetonitrile: Buffer (50:20:30) (% v/v), Methanol: Acetonitrile: Buffer (60:20:20), Methanol: buffer (50:50), Methanol: Buffer (70:40), Methanol: Buffer pH 4.0 (90:10), and Methanol: Buffer pH 4.0 (96:4). The same solvent mixture was used for extraction of the drug from the formulation containing excipients.

Standard preparation of Simvastatin.

Accurately weigh and transfer about 20mg of simvastatin working standard into 100mL volumetric flask, and about 70 mL of diluents, sonicate to dissolve, dilute to volume with diluents and mix. Filter the solution through 0.45µ. 

SPECTROPHOTOMETRIC & HPLC METHODS FOR POORLY WATER SOLUBLE DRUGS  Chapter....3
TRIAL- 1

Chromatographic condition 1

1. Preparation of buffer solution:
Mix 5mL of glacial acid in 1000mL of water. To 1000mL of 5mL glacial acetic acid solution, add 0.94gm of 1-Hexane sulphonic acid anhydrous. sonicate to dissolve.

2. Preparation of mobile phase
The mobile phase is prepared by mixing Methanol: Acetonitrile: Buffer (50:20:30). Filtered and degas it.

3. Chromatographic Run:
Load the standard solution of antilipidmic drug simvastatin in the injector, enter the HPLC parameters as per Table: 3.42, save the method, inject and run for 20min.

The chromatogram obtained was shown in Figure: 3.1

![Figure-3.16 Mobile phase = Methanol: Acetonitrile: Buffer (50:20:30)](image)

Result: Three peaks were obtained with improper Tailing factor.

TRIAL- 2

Chromatographic condition 2

Here mobile phase ratio was changed.

1. Preparation of buffer solution:
Mix 5mL of glacial acid in 1000mL of water. To 1000mL of 5mL glacial acetic acid solution, add 0.94gm of 1-Hexane sulphonic acid anhydrous. sonicate to dissolve.
2. Preparation of mobile phase
The mobile phase is prepared by mixing Methanol: Acetonitrile: buffer in the ratio of (60:20:20). Filtered and degas it.

3. Chromatographic Run:
Load the standard solution of simvastatin in the injector, enter the HPLC parameters as per (Table: 3.42), save the method, inject and run for 20min.

The chromatogram obtained was shown in Figure: 3.17

![Chromatogram](image)

**Figure-3.17:** Mobile phase = Methanol : Acetonitrile : Buffer pH4.0 (60:20:20)

**Result:** Three peaks were separate with irregular separation.

**TRIAL- 3**

**Chromatographic condition 3**
Here mobile phase was changed.

1. **Preparation of buffer solution:**
Mix 5mL of glacial acid in 1000mL of water. To 1000mL of 5mL glacial acetic acid solution, add 0.94gm of 1-Hexane sulphonic acid anhydrous,sonicate to dissolve.

2. **Preparation of mobile phase**
The mobile phase is prepared by mixing Methanol: buffer pH4.5 in the ratio of 50:50. Filtered and degas
3. Chromatographic Run:

Load the standard solution of simvastatin in the injector, enter the HPLC parameters as per (Table: 3.42); save the method, inject and run for 20 min.

**Result:** Peaks were obtained with indefinite separation.

**TRIAL- 4**

**Chromatographic condition 4**

Here mobile phase was changed.

1. **Preparation of buffer solution:**

Mix 5mL of glacial acid in 1000mL of water. To 1000mL of 5mL glacial acetic acid solution, add 0.94gm of 1-Hexane sulphonic acid anhydrous. sonicate to dissolve.

2. **Preparation of mobile phase**

The mobile phase is prepared by mixing Methanol: Buffer in the ratio of (70:40). Filtered and degas.

3. **Chromatographic Run:**

Load the standard solution of simvastatin in the injector; enter the HPLC parameters as per (Table: 3.42), save the method, inject and run for 20min.
The chromatogram obtained was shown in Figure: 3.19

**Figure-3.19: Mobile phase =** Methanol: Buffer pH4.0 (70:30)

**Result:** Two peaks were separated with improper Tailing factor.

**TRIAL- 5**

**Chromatographic condition 5**

Here mobile phase was changed.

1. **Preparation of buffer solution:**
Mix 5mL of glacial acid in 1000mL water. To 1000mL of 5mL glacial acetic acid solution, add 0.94gm of 1-Hexane sulphonic acid anhydrous. sonicate to dissolve.

2. **Preparation of mobile phase**
The mobile phase is prepared by mixing Methanol: Buffer in the ratio of (90:10) Filtered and degas it.
3. Chromatographic Run:
Load the standard solution of simvastatin in the injector, enter the HPLC parameters as per (Table: 3.42), save the method, inject & run for 20 min. as shown below in fig.: 3.20

![Figure-3.20](image)

**Figure-3.20: Mobile phase = Methanol: Buffer pH 4.0 (90:10)**

**Result:** peak eluted at 10.109 min.

**TRIAL- 6**

**Chromatographic condition 6**

In this chromatographic condition mobile phase ratio was changed.

1. **Preparation of buffer solution:**
   Mix 5mL of glacial acid in 1000mL of milli Q water. To 1000mL of 5mL glacial acetic acid solution, add 0.94gm of 1-Hexane sulphonic acid anhydrous. sonicate to dissolve.

2. **Preparation of Mobile phase:**
   The mobile phase is prepared by mixing buffer: methanol in the ratio of 96:4 Filtered and degas it

3. **Chromatographic Run:**
Load the standard solution of simvastatin in the injector, enter the HPLC parameters as per (Table: 3.42), save the method, inject and run for 20min.
Figure-3.21: Mobile phase = Methanol: Buffer pH 4.0 (96:4)

Result: peak eluted at 9.546 min

Determination of stability of drug sample in the Optimized mobile phase

The stability of the drug in the solution during analysis was determined during the course of experiments on the same day and also after 48 h storage at laboratory bench conditions and in the refrigerator using concentration 10µg/ml (Table 3.43).

Table-3.43: Stability of the simvastatin in the optimized mobile phase

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Storage conditions</th>
<th>Mean area±SD (At zero hrs)</th>
<th>SE</th>
<th>Mean area±SD (At 48 hrs)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Room Temperature (25±0.5°C)</td>
<td>252952.3±285 9.82</td>
<td>3474.9</td>
<td>248844.7±3 650.63</td>
<td>2777.53</td>
</tr>
<tr>
<td>2</td>
<td>Refrigerator (4±0.5°C)</td>
<td>252952.3±285 9.82</td>
<td>3474.9</td>
<td>250011.3±3 653.10</td>
<td>2778.63</td>
</tr>
</tbody>
</table>

*Concentration of drug 10µg/ml in mobile phase.
➢ OPTIMIZED METHOD

1. **Preparation of buffer solution:**

Mix 5mL of glacial acid in 1000mL of water. To 1000mL of 5mL glacial acetic acid solution, add 0.94gm of 1-Hexane sulphonic acid anhydrous. sonicate to dissolve.

2. **Preparation of mobile phase**

The mobile phase is prepared by mixing buffer: methanol in the ratio of 96:4 Filtered and degas it.

3. **Standard preparation of simvastatin**

Accurately weigh and transfer about 20 mg of drug simvastatin working standard into 100mL volumetric flask, and about 70 mL of diluents, sonicate to dissolve, dilute to volume with diluents and mix. Filter the solution through 0.45µm.

4. **Preparation of system suitability solution.**

Accurately weigh and transfer about 10mg of working standard into 100ml volumetric flask. Add 25mL of 0.1N HCl and 25mL of Diluent. Sonicate to dissolve. Keep the sample at about 80 °C. for 4 hours. Use this solution as system suitability solution.

5. **Preparation of placebo solution**

Accurately weigh and transfer powdered content of placebo equivalent to 100mg of Drug into 100 mL volumetric flask. Add about 70 mL diluent and sonicate for about 15 min. dilute to the volume and mix. Filter the solution through 0.45µm filters.

6. **Preparation of diluent**

Use mobile phase as diluent

As in Figure-3.21 Mobile phase = Methanol: Buffer pH 4.0 (96:4)
### Table-3.44. Chromatographic conditions for the optimized method for Simvastatin

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Instrument</td>
<td>A HPLC instrument (Younglin series) with Model Acme-9000</td>
</tr>
<tr>
<td>2.</td>
<td>Column</td>
<td>Promosil C-18, (250 mm, 4.6 mm, 5µm)</td>
</tr>
<tr>
<td>3.</td>
<td>Mobile Phase</td>
<td>Mix 5ml of glacial acid in 1000ml of water. To 1000ml of 5ml glacial acetic acid solution, add 0.94gm of 1-Hexane sulphonic acid anhydrous. sonicate to dissolve. The mobile phase is prepared by mixing buffer: methanol in the ratio of 96:4 Filtered and degas it.</td>
</tr>
<tr>
<td>4.</td>
<td>Flow Rate</td>
<td>1.0 mL/minute</td>
</tr>
<tr>
<td>5.</td>
<td>Detection wavelength</td>
<td>254 nm</td>
</tr>
<tr>
<td>6.</td>
<td>Injection Volume</td>
<td>10µL</td>
</tr>
<tr>
<td>7.</td>
<td>Run Time</td>
<td>20 Minutes</td>
</tr>
<tr>
<td>8.</td>
<td>Retention time, min</td>
<td>9.546±0.234</td>
</tr>
<tr>
<td>9.</td>
<td>Tailing factor</td>
<td>1.048±0.274</td>
</tr>
<tr>
<td>10.</td>
<td>Asymmetry factor</td>
<td>1.139±0.864</td>
</tr>
<tr>
<td>11.</td>
<td>Theoretical plates</td>
<td>5642±0.426</td>
</tr>
<tr>
<td>1.</td>
<td>Resolution</td>
<td>3.344±0.628</td>
</tr>
</tbody>
</table>
Fig. 3.22: system suitability solution.

Result: The retention time of Simvastatin was peak eluted at 9.546 min. The peaks are well separated with a resolution of 13.344 and Tailing 1.136.

3.3.14. ACECLOFENAC

Table 3.45: HPLC instrumentation & chromatographic conditions for Aceclofenac

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Instrument</td>
<td>A HPLC instrument (Younglin series) with Model Acme-9000</td>
</tr>
<tr>
<td>2.</td>
<td>Column</td>
<td>Promosil C-18, (250 mm, 4.6 mm, 5µm)</td>
</tr>
<tr>
<td>3.</td>
<td>Mobile Phase</td>
<td>Different mobile phase used for Trial 1 to 6</td>
</tr>
<tr>
<td>4.</td>
<td>Flow Rate</td>
<td>1.0 mL/minute</td>
</tr>
<tr>
<td>5.</td>
<td>Detection wavelength</td>
<td>277 nm</td>
</tr>
<tr>
<td>6.</td>
<td>Injection Volume</td>
<td>10µL</td>
</tr>
<tr>
<td>7.</td>
<td>Run Time</td>
<td>10 minutes</td>
</tr>
</tbody>
</table>

Optimum condition of mobile phases were investigated in the development of an HPLC method suitable for analysis of in the bulk drug. These included Methanol:
Materials and Methods

Acetonitrile: water (50:20:30) (% v/v), Methanol: Acetonitrile: water (60:20:20), Methanol: water (50:50), Methanol: acetonitrile (70:30), water: acetonitrile (60:40), and Water: Acetonitrile (55:45). The same solvent mixture was used for extraction of the drug from the formulation containing excipients.

Standard preparation of Aceclofenac:-

Accurately weigh and transfer about 20 mg of Aceclofenac working standard into 100 mL volumetric flask, and about 70 mL of diluents, sonicate to dissolve, dilute to volume with diluents and mix. Filter the solution through 0.45 μ.

Trial-1

Chromatographic condition 1

1. Preparation of mobile phase

The mobile phase is prepared by mixing Methanol: Acetonitrile: Water (50:20:30). Filtered and degas it.

2. Chromatographic Run:

Load the standard solution of NSAID drug Aceclofenac in the injector, enter the HPLC parameters as per Table: 3.45, save the method, inject and run for 10 min.

The chromatogram obtained was shown in Figure: 3.23

![Chromatogram](image)

Figure-3.23: Mobile phase = Methanol: Acetonitrile: Water (50:20:30)

Result: five to six peaks were obtained with improper Tailing factor.
TRIAL- 2  

**Chromatographic condition 2**

Here mobile phase ratio was changed.

1. **Preparation of mobile phase**

The mobile phase is prepared by mixing Methanol: Acetonitrile: Water in the ratio of (60:20:20). Filtered and degas it.

2. **Chromatographic Run:**

Load the standard solution of Aceclofenac in the injector, enter the HPLC parameters as per (Table: 3.45), save the method, inject and run for 10 minute as shown in fig. 3.24

![Figure-3.24](image)

*Figure-3.24: Mobile phase = Methanol : Acetonitrile : Buffer pH4.0 (60:20:20)*

**Result:** Three peaks were separate with irregular separation.

TRIAL- 3

**Chromatographic condition 3**

Here mobile phase was changed.

1. **Preparation of mobile phase**

The mobile phase is prepared by mixing Methanol: Water in the ratio of 50:50. Filtered and degas

2. **Chromatographic Run:**

Load the standard solution of Aceclofenac in the injector, enter the HPLC parameters as per (Table: 3.45); save the method, inject and run for 10 minute as shown in fig. 3.25
Figure-3.25 Mobile phase = Methanol: water (50:50)

Result: Two peaks were obtained with indefinite separation.

TRIAL- 4

Chromatographic condition 4
Here mobile phase was changed.

1. Preparation of mobile phase
The mobile phase is prepared by mixing Methanol: Acetonitrile 70:40. Filtered & degas.

2. Chromatographic Run:
Load the standard solution of Aceclofenac in the injector; enter the HPLC parameters as per (Table: 3.45), save the method, inject and run for 10min.

The chromatogram obtained was shown in Figure: 3.26

Figure-3.26: Mobile phase = Methanol: Acetonitrile (70:30)

Result: Two peaks were separated with improper Tailing factor.
TRIAL- 5

Chromatographic condition 5

Here mobile phase was changed.

1. Preparation of mobile phase

The mobile phase is prepared by mixing Water : Acetonitrile (60 : 40) Filtered and degas it.

2. Chromatographic Run:

Load the standard solution of Aceclofenac in the injector, enter the HPLC parameters as per (Table: 3.45), save the method, inject and run for 10min.

The chromatogram obtained was shown in Figure: 3.27

Analysis

Sample Name: aceclofenac
Sample ID:
File: 0160.RAW
Date: 2010-07-15 PM 01:00:34
Channel: 1. UV730D A

Chromatogram

Figure-3.27: Mobile phase = Water : Acetonitrile (60 : 40)
Result:

<table>
<thead>
<tr>
<th>Area%</th>
<th>Name</th>
<th>RT[min]</th>
<th>Area[mV*s]</th>
<th>Height[mV]</th>
<th>Amount[]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.79</td>
<td></td>
<td>1.3167</td>
<td>20.9734</td>
<td>1.9008</td>
<td>0.0000</td>
</tr>
<tr>
<td>99.21</td>
<td></td>
<td>6.6667</td>
<td>2637.0259</td>
<td>256.7034</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

Result: peak eluted at 7.109 min.

**TRIAL- 6**

**Chromatographic condition 6**

In this chromatographic condition mobile phase ratio was changed.

1. **Preparation of Mobile phase:**
   The mobile phase is prepared by mixing Water : Acetonitrile (55 : 45). Filtered and degas it

2. **Chromatographic Run:**
   Load the standard solution of Aceclofenac in the injector, enter the HPLC parameters as per (Table: 3.45), save the method, inject and run for 10 min.

![Graph](image)

**Figure-3.28:** Mobile phase = Water : Acetonitrile (55 : 45)

Result: peak eluted at 6.667 min

**Determination of stability of drug sample in the Optimized mobile phase**
   The stability of the drug in the solution during analysis was determined during the course of experiments on the same day and also after 48 h storage at laboratory bench conditions and in the refrigerator using concentration 10µg/ml (Table 3.45)
Table 3.46: Stability of the Aceclofenac in the optimized mobile phase

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Storage conditions</th>
<th>Mean area± SD (At zero hrs)</th>
<th>SE</th>
<th>Mean area± SD (At 48 hrs)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Room Temperature (25±0.5 °C)</td>
<td>253045.3±2967.82</td>
<td>3235.9</td>
<td>248964.7±3345.63</td>
<td>2854.45</td>
</tr>
<tr>
<td>2.</td>
<td>Refrigerator (4±0.5 °C)</td>
<td>252834.3±2786.82</td>
<td>3546.9</td>
<td>24987.3±3234.10</td>
<td>2865.87</td>
</tr>
</tbody>
</table>

*Concentration of drug 10µg/ml in mobile phase.

➤ OPTIMIZED METHOD

1. Preparation of mobile phase
   The mobile phase is prepared by mixing Water : Acetonitrile (55 : 45).
   Filtered and degas it.

2. Standard preparation of Aceclofenac
   Accurately weigh and transfer about 20 mg of drug Aceclofenac working standard into 100mL volumetric flask, and about 70 mL of diluents, sonicate to dissolve, dilute to volume with diluents and mix. Filter the solution through 0.45µm.

4. Preparation of system suitability solution.
   Accurately weigh and transfer about 10mg of working standard into 100ml volumetric flask. Add 25mL of 0.1N HCl and 25mL of Diluent. Sonicate to dissolve. Keep the sample at about 80 °C. for 4 hours. Use this solution as system suitability solution.

5. Preparation of placebo solution
   Accurately weigh and transfer powdered content of placebo equivalent to 100mg of Drug Aceclofenac into 100 mL volumetric flask. Add about 70 mL diluent and sonicate for about 15 min. dilute to the volume and mix. Filter the solution through 0.45µm filters.

6. Preparation of diluent
   Use mobile phase as diluent
   As shown in Figure-3.27. Mobile phase = Water : Acetonitrile (55 : 45)
Table-3.47 Chromatographic conditions for the optimized method for Aceclofenac

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Instrument</td>
<td>A HPLC instrument (Younglin series) with Model Acme-9000</td>
</tr>
<tr>
<td>2.</td>
<td>Column</td>
<td>Promosil C-18, (250 mm, 4.6 mm, 5µm)</td>
</tr>
<tr>
<td>3.</td>
<td>Mobile Phase</td>
<td>The mobile phase is prepared by mixing Water : Acetonitrile (55 : 45). Filtered and degas it</td>
</tr>
<tr>
<td>4.</td>
<td>Flow Rate</td>
<td>1.0 mL/minute</td>
</tr>
<tr>
<td>5.</td>
<td>Detection wavelength</td>
<td>277 nm</td>
</tr>
<tr>
<td>6.</td>
<td>Injection Volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>7.</td>
<td>Run Time</td>
<td>10 Minutes</td>
</tr>
</tbody>
</table>

Table-3.48: System Suitability Test Parameters for Aceclofenac

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>RP-HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Retention time, min</td>
<td>6.60±0.132</td>
</tr>
<tr>
<td>3.</td>
<td>Tailing factor</td>
<td>1.048±0.274</td>
</tr>
<tr>
<td>4.</td>
<td>Asymmetry factor</td>
<td>1.123±0.472</td>
</tr>
<tr>
<td>5.</td>
<td>Theoretical plates</td>
<td>5859±0.774</td>
</tr>
<tr>
<td>6.</td>
<td>Resolution</td>
<td>2.895±0.431</td>
</tr>
</tbody>
</table>

**Result:** The retention time of Aceclofenac was 6.667 and 9.694. The peaks are well separated with a resolution of 2.895 and Tailing 1.048.
### 3.3.15. KETOCONAZOLE

**Table 3.49: HPLC instrumentation & chromatographic conditions for Ketoconazole**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Instrument</td>
<td>A HPLC instrument (Younglin series) with Model Acme-9000</td>
</tr>
<tr>
<td>2.</td>
<td>Column</td>
<td>Promsil C-18, (250 mm, 4.6 mm, 5µm)</td>
</tr>
<tr>
<td>3.</td>
<td>Mobile Phase</td>
<td>Different mobile phase used for Trial 1 to 6</td>
</tr>
<tr>
<td>4.</td>
<td>Flow Rate</td>
<td>1.0 mL/minute</td>
</tr>
<tr>
<td>5.</td>
<td>Detection wavelength</td>
<td>238 nm</td>
</tr>
<tr>
<td>6.</td>
<td>Injection Volume</td>
<td>10µL</td>
</tr>
<tr>
<td>7.</td>
<td>Auto Sampler Temperature</td>
<td>5°C</td>
</tr>
<tr>
<td>8.</td>
<td>Run Time</td>
<td>10minutes</td>
</tr>
</tbody>
</table>


**Standard preparation of Ketoconazole.**
Accurately weigh and transfer about 20mg of Ketoconazole working standard into 100mL volumetric flask, and about 70 mL of diluents, sonicate to dissolve, dilute to volume with diluents and mix. Filter the solution through 0.45µ.
TRIAL- 1

Chromatographic condition 1

1. Preparation of buffer solution:
Mix 7.601 gm of KH$_2$PO$_4$ in 1000mL of water. To 1000mL of 1.452 gm K$_2$H-PO$_4$ solution, add 4.8 gm of sodium chloride anhydrous. sonicate to dissolve.

2. Preparation of mobile phase
The mobile phase is prepared by mixing Methanol: Acetonitrile: Buffer (50:20:30). Filtered and degas it.

3. Chromatographic Run:
Load the standard solution of Ketoconazole drug Ketoconazole in the injector, enter the HPLC parameters as per Table: 3.49, save the method, inject and run for 10 min.
The chromatogram obtained was shown in Figure: 3.29

![Chromatogram](image)

**Figure-3.29 Mobile phase =** Methanol: Acetonitrile: Buffer (50:20:30)

Result: Two peaks were obtained with improper Tailing factor.

TRIAL- 2

Chromatographic condition 2

Here mobile phase ratio was changed.

1. Preparation of buffer solution:
Mix 7.601 gm of KH$_2$PO$_4$ in 1000mL of water. To 1000mL of 1.452 gm K$_2$H-PO$_4$ solution, add 4.8 gm of sodium chloride anhydrous. sonicate to dissolve.

2. Preparation of mobile phase
The mobile phase is prepared by mixing Water: Acetonitrile: Buffer (60:20:10). Filtered and degas it.
3. Chromatographic Run:

Load the standard solution of ketoconazole in the injector, enter the HPLC parameters as per (Table: 3.49), save the method, inject and run for 20min as shown in figure 3.30

![Figure-3.30: Mobile phase = Methanol : Acetonitrile : Buffer pH4.0 (60:20:20)](image)

Result: One peak with many minor peaks were separate with irregular separation.

TRIAL- 3

Chromatographic condition 3

Here mobile phase was changed.

1. Preparation of buffer solution:

Mix 7.601 gm of KH$_2$PO$_4$ in 1000mL of water. To 1000mL of 1.452 gm K$_2$H-PO$_4$ solution, add 4.8 gm of sodium chloride anhydrous. sonicate to dissolve.

2. Preparation of mobile phase

The mobile phase is prepared by mixing Water: Acetonitrile: Buffer (60:25:15). Filtered and degas

3. Chromatographic Run:

![Figure-3.31: Mobile phase = Water: Acetonitrile: Buffer (60:25:15).](image)
Load the standard solution of simvastatin in the injector, enter the HPLC parameters as per (Table: 3.49); save the method, inject and run for 10 min.

**Result:** Peaks were obtained with indefinite separation.

**TRIAL- 4**

**Chromatographic condition 4**

Here mobile phase was changed.

1. **Preparation of buffer solution:**
   Mix 7.601 gm of KH$_2$PO$_4$ in 1000mL of water. To 1000mL of 1.452 gm K$_2$HPO$_4$ solution, add 4.8 gm of sodium chloride anhydrous. sonicate to dissolve.

2. **Preparation of mobile phase**
   The mobile phase is prepared by mixing Water: Acetonitrile: Buffer (65:25:10). Filtered and degas.

3. **Chromatographic Run:**
   Load the standard solution of Ketoconazole in the injector; enter the HPLC parameters as per (Table: 3.49), save the method, inject and run for 10min.
   The chromatogram obtained was shown in Figure: 3.32

![Figure-3.32 Mobile phase](image)

**Figure-3.32 Mobile phase =** Water: Acetonitrile: Buffer (65:25:10),

**Result:** Two peaks were separated with improper Tailing factor.

**TRIAL- 5**

**Chromatographic condition 5**

Here mobile phase was changed.
1. Preparation of buffer solution:
Mix 7.601 gm of KH$_2$PO$_4$ in 1000mL of water. To 1000mL of 1.452 gm K$_2$HPO$_4$ solution, add 4.8 gm of sodium chloride anhydrous. sonicate to dissolve.

2. Preparation of mobile phase
The mobile phase is prepared by mixing Water: Acetonitrile: Buffer (55:45:10), Filtered and degas it.

3. Chromatographic Run:

![Chromatogram](image)

**Figure-3.33 Mobile phase** = Water: Acetonitrile: Buffer (55:45:10).

Load the standard solution of Ketoconazole in the injector, enter the HPLC parameters as per (Table: 3.49), save the method, inject and run for 10min.

The chromatogram obtained was shown in Figure: 3.33

**Result**: Result: peak eluted at 2.371 min.

**TRIAL- 6**

**Chromatographic condition 6**

In this chromatographic condition mobile phase ratio was changed.

1. Preparation of buffer solution:
Mix 7.601 gm of KH$_2$PO$_4$ in 1000mL of water. To 1000mL of 1.452 gm K$_2$HPO$_4$ solution, add 4.8 gm of sodium chloride anhydrous. sonicate to dissolve.

2. Preparation of Mobile phase:
The mobile phase is prepared by mixing Water: Acetonitrile: Buffer pH 6.8 (51:45:4). Filtered and degas it.
3. Chromatographic Run:
Load the standard solution of Ketoconazole in the injector, enter the HPLC parameters as per (Table: 3.49), save the method, inject and run for 10min.

Figure-3. 34 Mobile phase = Water: Acetonitrile: Buffer pH 6.8 (51:45:4).

Result: peak eluted at 2.713 min

Determination of stability of drug sample in the Optimized mobile phase
The stability of the drug in the solution during analysis was determined during the course of experiments on the same day and also after 48 h storage at laboratory bench conditions and in the refrigerator using concentration 10µg/ml (Table 3.50).

Table 3.50: Stability of the Ketoconazole in the optimized mobile phase

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Storage conditions</th>
<th>Mean area± SD (At zero hrs)</th>
<th>SE</th>
<th>Mean area± SD (At 48 hrs)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Room Temperature (25±0.5 °C)</td>
<td>256754.3±2654.85</td>
<td>3143.7</td>
<td>250032.5±3033.44</td>
<td>2567.53</td>
</tr>
<tr>
<td>2.</td>
<td>Refrigerator (4±0.5 °C)</td>
<td>2576455.3±2787.76</td>
<td>3342.6</td>
<td>251254.1±3834.23</td>
<td>2654.37</td>
</tr>
</tbody>
</table>

*Concentration of drug 10µg/ml in mobile phase.
OPTIMIZED METHOD

1. Preparation of buffer solution:
Mix 7.601 gm of KH₂PO₄ in 1000mL of water. To 1000mL of 1.452 gm K₂H₂PO₄ solution, add 4.8 gm of sodium chloride anhydrous. sonicate to dissolve.

2. Preparation of mobile phase
The mobile phase is prepared by mixing Water: Acetonitrile: Buffer pH 6.8 (51:45:4). Filtered and degas it.

3. Standard preparation of Ketoconazole
Accurately weigh and transfer about 20mg of Ketoconazole working standard into 100mL volumetric flask, and about 70 mL of diluents, sonicate to dissolve, dilute to volume with diluents and mix. Filter the solution through 0.45μm.

4. Preparation of system suitability solution.
Accurately weigh and transfer about 10mg of working standard into 100ml volumetric flask. Add 25mL of 0.1N HCl and 25mL of Diluent. Sonicate to dissolve. Keep the sample at about 80 °C. for 4 hours. Use this solution as system suitability solution.

5. Preparation of placebo solution
Accurately weigh and transfer powdered content of placebo equivalent to 100mg of Drug X into 100 mL volumetric flask. Add about 70 mL diluent and sonicate for about 15 min. dilute to the volume and mix. Filter the solution through 0.45μm filters.

6. Preparation of diluent
Use mobile phase as diluent.
As shown in Figure-3.34 Mobile phase = Water: Acetonitrile: Buffer pH 6.8 (51:45:4).
Table-3.51. Chromatographic conditions for the optimized method for ketoconazole

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Instrument</td>
<td>A HPLC instrument (Younglin series) with Model Acme-9000</td>
</tr>
<tr>
<td>2.</td>
<td>Column</td>
<td>Promosil C-18, (250 mm, 4.6 mm, 5µm)</td>
</tr>
<tr>
<td>3.</td>
<td>Mobile Phase</td>
<td>Mix 7.601 gm of KH₂PO₄ in 1000mL of water. To 1000mL of 1.452 gm K₂HPO₄ solution, add 4.8 gm of sodium chloride anhydrous. sonicate to dissolve.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The mobile phase is prepared by mixing Water: Acetonitrile: Buffer pH 6.8 (51:45:4). Filtered and degas it.</td>
</tr>
<tr>
<td>4.</td>
<td>Flow Rate</td>
<td>1.50 mL/minute</td>
</tr>
<tr>
<td>5.</td>
<td>Detection wavelength</td>
<td>238 nm</td>
</tr>
<tr>
<td>6.</td>
<td>Injection Volume</td>
<td>10μL</td>
</tr>
<tr>
<td>7.</td>
<td>Run Time</td>
<td>10 Minutes</td>
</tr>
</tbody>
</table>

Table-3.52: System Suitability Test Parameters for Ketoconazole

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>RP-HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Retention time, min</td>
<td>2.713±0.356</td>
</tr>
<tr>
<td>2.</td>
<td>Tailing factor</td>
<td>1.586±0.274</td>
</tr>
<tr>
<td>3.</td>
<td>Asymmetry factor</td>
<td>1.123±0.315</td>
</tr>
<tr>
<td>4.</td>
<td>Theoretical plates</td>
<td>5987±0.9807</td>
</tr>
<tr>
<td>5.</td>
<td>Resolution</td>
<td>2.235±0.543</td>
</tr>
</tbody>
</table>

**Result:** The retention time of Ketoconazole was 2.713. The peaks are well separated with a resolution of 2.235 and Tailing 1.586.