PART–IV
CHAPTER – I

SIMULTANEOUS DETERMINATION OF SALBUTAMOL SULPHATE AND BROMHEXINE HYDROCHLORIDE IN FORMULATIONS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

SALBUTAMOL SULPHATE (SS)
A) Chemical Name: 2-tert.-Butylamino-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol sulfate

B) Formula:
   i) Structural:
   ii) Molecular: \((C_{13}H_{21}NO_3)_2, H_2SO_4\)

C) Molecular Weight: 567.7

D) Description: A white crystalline powder.

E) Solubility: Freely soluble in water, soluble in alcohol and in ether.

F) Category: Beta adrenceptor antagonist.

BROMHEXINE HYDROCHLORIDE (BH)
A) Chemical Name: 2-Amino-3,5-dibromo-N-cyclohexyl-N-methylbenzylamine hydrochloride
B) Formula:
   i) Structural:

   ![Chemical Structure Diagram]

   ii) Molecular: $C_{14}H_{21}Br_{2}N_{2}.HCl$.

C) Molecular Weight: 412.64

D) Description: A white crystalline powder.

E) Solubility: Soluble in ethanol (95%), methanol and chloroform.

F) Melting point: 237.5–238°C.

G) Category: An antimucolytic.

   Both the drugs are official in I.P.\(^{(2)}\) & B.P.\(^{(3)}\). Literature survey reveals that there are some spectrophotometric methods for the individual determination of SS\(^{(4,5)}\) and BH\(^{(6,7)}\) and some HPLC methods for the simultaneous determination of SS and Theophylline\(^{(8,9)}\). BH and Chlorprenaline hydrochloride and Dechloxine hydrochloride\(^{(10–17)}\) are reported. But there is no HPTLC method available for the simultaneous determination of SS and BH. So an attempt to develop a HPTLC method for the simultaneous determination of SS and BH from formulations is made and presented in this chapter.

4.1.2 EXPERIMENTAL

1. Instrumentation

   Camag Linomat IV samples applicator, with TLC Scannar II controlled by Cats 3.15 software and twin trough chamber were used.

2. Solvents and Chemicals
Working standard of SS was procured from Tata Pharma, Patalganga, India and BH was procured from IPCA Laboratories Ltd., Mumbai, India. Their purities were 99.91% and 99.85% respectively.

Methanol, Chloroform and Triethylamine were of A.R. grade from S.D. Fine Chemicals Ltd., Thane, India.

3. **Chromatographic Condition**

Mobile phase consisted a mixture of Methanol: Chloroform: Triethylamine (5.5:4.5:0.05; v/v), was used as stationary phase. Precoated 60F254 silica gel on aluminium sheets with thickness of 0.25 mm. Detection was carried out using a UV detector at 276 nm.

4. **Preparation of Working Standard Solution**

**Standard Stock Solution**

Working Standard stock solutions of 2.0 mg/ml of SS and 4.0 mg/ml of BH were prepared separately by dissolving 200 mg of standard SS and 400 mg of standard BH in 100 ml of methanol.

**Working Standard Solution**

For Brand I, 1 ml each of SS and BH solutions are pipette to 10 ml in methanol. This gave 200 µg/ml of SS and 400 µg/ml of BH.

For Brand II, 1 ml of SS and 2 ml of BH solutions are diluted to 10 ml in methanol. This gave 200 µg/ml SS and 800 µg/ml of BH.

5. **Sample Solution**

An amount of sample equivalent to label claim was transferred to 50 ml volumetric flask and diluted with methanol such that the final concentrations of SS and BH were 200 µg/ml and 400 µg/ml for Brand I or 200 µg/ml and 800 µg/ml for Brand II respectively.
6. **Calibration procedure**

Aliquots of Standard stock solution of SS and BH were taken in volumetric flasks and diluted up to the mark such that final concentration of SS and BH were in the range of 20–1000 µg/ml. Evaluation of both drugs were performed with a densitometer at 276 nm. Peak areas were recorded for all the peaks.

7. **Evaluation**

Peak areas for all the peaks were recorded. From the peak areas the amounts of SS and BH were computed as,

\[ W = \frac{RA \times C \times D}{RB \times W^*} \]

where,

- RA = Areas of sample.
- RB = Areas of standard.
- C = Concentration of standard in mg/ml.
- D = Dilution factor.
- W* = Exact weight of the sample taken (syrup/tablet) in ml or mgs.
- W = Wt. of sample per 5 ml of syrup or per tablet in mgs.

4.1.3 **RESULTS AND DISCUSSION**

1. **Chromatography**

The mobile phase resolved SS and BH very efficiently as shown in *Fig. 4.1*. The Rf values were 0.45 and 0.82 for SS and BH respectively. The wavelength of 276 nm was selected because at this wavelength the difference of absorbance between SS and BH was found to be maximum (*Fig. 4.2*).
2. **System Suitability**

To ascertain the resolution and reproducibility of the chromatography method, system suitability tests were carried out using working standard solution of SS and BH. This solution was spotted 5 times. Parameters such as tailing factor, resolution factor and R & D are shown in Table 4.1.

**TABLE 4.1**  
**SYSTEM SUITABILITY AND PARAMETERS FOR SS AND BH (N=5)**

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SS</th>
<th>BH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tailing Factor</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Resolution</td>
<td>–</td>
<td>1.8</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>1.65</td>
<td>1.30</td>
</tr>
</tbody>
</table>

3. **Linearity, Limit of Detection & Quantification**

Plots of peak areas versus the concentration of SS and BH were found to be linear in the range of 20–1000 µg/ml. *Fig. 4.3* They are represented by following linear equation.

\[
Y_{SS} = 0.3800 + (-) 0.2895 \quad (\gamma = 0.999)
\]

\[
Y_{BH} = 0.4195 + (-) 12.4551 \quad (\gamma = 0.999)
\]

The limit of detection (LOD) and limit of quantification (LOQ) of SS and BH were found to be 5 µg/ml, 15 µg/ml and 8 µg/ml, 30 µg/ml, respectively.

4. **Assay**

The content of two drugs found in syrup and tablet analysed by the proposed method are tabulated in Table 4.2. The low values of RSD indicate that the method is precise and accurate.
5. **Accuracy and Precision**

The accuracy and precision of the proposed method were further confirmed by recovery experiments. Three different levels of standards were added to the preanalysed sample and each level was repeated 3 times.

Mean percentage recoveries of SS and BH for Brand 1 and Brand 2 were 99.17% and 99.33% and 99.49% and 99.82% respectively as shown in table 4.3.

**TABLE 4.2**

**RESULTS OF ASSAYS OF SS AND BH**

<table>
<thead>
<tr>
<th>BRAND</th>
<th>SALBUTAMOL SULPHATE</th>
<th>BROMHEXINE HCL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LABEL CLAIM</td>
<td>AMOUNT FOUND</td>
</tr>
<tr>
<td>BRAND 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Syrup)*</td>
<td>2 mg/5ml</td>
<td>1.98 mg/5ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(RSD = 1.4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=5</td>
</tr>
<tr>
<td>BRAND 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Tablet)*</td>
<td>2 mg/TAB</td>
<td>1.99 mg/TAB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(RSD=1.62%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=5</td>
</tr>
</tbody>
</table>

*1 = Bronkotus Syrup by Biddle Sawyer Ltd. Mumbai (INDIA).

*2 = Grilinctus BM Tablet by Wardex Pharmaceuticals Ltd., Madras (INDIA).

**TABLE 4.3**

**RESULTS OF RECOVERY ANALYSIS**

<table>
<thead>
<tr>
<th>NO.</th>
<th>SALBUTAMOL SULPHATE (%)</th>
<th>BROMHEXINE HYDROCHLORIDE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>98.67</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>99.25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>99.60</td>
</tr>
<tr>
<td>---</td>
<td>----</td>
<td>-------</td>
</tr>
<tr>
<td>MEAN</td>
<td>99.17</td>
<td>99.33</td>
</tr>
<tr>
<td>R.S.D.</td>
<td>1.85</td>
<td>1.30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II</th>
<th>1</th>
<th>99.17</th>
<th>99.83</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>99.50</td>
<td>99.80</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>99.80</td>
<td>99.82</td>
<td></td>
</tr>
<tr>
<td>MEAN</td>
<td>99.49</td>
<td>99.82</td>
<td></td>
</tr>
<tr>
<td>R.S.D.</td>
<td>1.70</td>
<td>1.45</td>
<td></td>
</tr>
</tbody>
</table>
4.1.4 REFERENCES

7. N.M.Sanghavi; M.M.Samarth; R.Singh, P.S.Mathur, Indian Drugs, 27(9), (1990); 486–488.
8. S.Ray; A.Bandopadhyay, Indian Drugs, 27(9), (1990), 313–316.
PART–IV

CHAPTER – II

SIMULTANEOUS DETERMINATION OF CINNARIZINE AND DOMPERIDONE MALEATE FROM PHARMACEUTICAL PREPARATIONS

BY HPTLC

4.2.1 DRUG PROFILES

CINNARIZINE (Cinn)

A) Chemical Name: 1-Benzhydryl-4-cinnamyl-piperazine

B) Formula:
   i) Structural:

   ![Chemical Structure of Cinnarizine]

   ii) Molecular: C_{26}H_{28}N_{2}

C) Molecular Weight: 368.50

D) Description: A white pale yellow crystalline powder.

E) Solubility: Practically insoluble in water, slightly soluble in alcohol, soluble in ether and freely soluble in methylene chloride.

F) Melting Point: 192°C with decomposition (For hydrochloride).

G) Category: An antihistamine.

DOMPERIDONE MALEATE (DoM)

A) Chemical Name: 5-Chloro-1-[1-[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)propyl]piperidin-4-yl]-1,3-dihydro-2H-benzimidazol-2-one maleate
B) Formula:

i) Structural:

![Chemical structure diagram]

ii) Molecular: C_{22}H_{24}ClN_{3}O_{2}

C) Molecular Weight: 266.3

D) Description: A white to almost white powder.

E) Solubility: Sparingly soluble in water, soluble in dehydrated alcohol, slightly soluble in methylene chloride and practically insoluble in ether.

F) Melting Point: 242.5°C.

G) Category: anti-emetics.

Cinn & DoM are official in B.P.\(^{(2)}\). Literature survey reveals that Cinn has been determined by spectrophotometrically\(^{(3-7)}\) GC\(^{(8)}\), and HPLC\(^{(9)}\) where as DoM has been determined by spectrophotometrically\(^{(10-11)}\) and HPTLC\(^{(12)}\) methods. A potentiometric method\(^{(13)}\) for the simultaneous determination of Cinn and Ficilin has been reported.\(^{(14-18)}\) Recently an HPLC\(^{(19,20)}\) method for simultaneous determination of Cinn and DoM has been reported. However there is no method reported so far for the simultaneous determination of these two drugs from formulations by HPTLC. An attempt to develop an HPTLC method for the
simultaneous determination of Cinn & DoM from formulations is therefore made and presented in this chapter.

4.2.2 EXPERIMENTAL

1. Instrumentation

Camag Linomet IV samples applicator, with TLC Scanner II controlled by Cats 3.15 Software and twin trough chamber was used.

2. Solvents and Chemicals

Working standard of Cinn was procured from S.G. Chemicals Mumbai, India and that of DoM was procured from Vasudha Pharmachem. India with purities 99.95% and 99.92% respectively.

Methanol, Dichloromethane and Formic acid were of AR grade from S.D. Fine Chemicals Ltd., Thane, India.

3. Chromatographic Condition

Mobile phase consisted of a mixture of Methanol: Dichloromethane: Formic acid, (1:9:0.05; v/v). A Precoated 60F254 silica gel HPTLC plate with thickness of 0.2 um was used as stationary phase. Detection was carried out at 276 nm.

4. Preparation of Working Standard solution

Standard Stock Solution

Standard stock solutions of 4.0 mg/ml of Cinn and 3.0 mg/ml of DoM was prepared by dissolving 400 mg of standard Cinn and 300 mg of standard of DoM in 100ml of methanol.

Working Standard Solution

1 ml of the standard stock solution was diluted to 10 ml with methanol. This gave concentration of 400 µg/ml of Cinn and 300 µg/ml of DoM. This solution was used as working standard.
5. **Sample Preparation**

Twenty tablets were weighed and crushed to fine powder. Powder equivalent to 20 mg of Cinn and 15 mg of DoM was weighed in a 50 ml volumetric flask. 40 ml of methanol was added, after sonication for 10 mins, the solution was cooled and diluted up to the mark with methanol. The solution was centrifuged and the supernatant was used for the analysis.

6. **Calibration Procedure**

From above stock solutions various dilutions were made to get solutions of 60–1000 µg/ml of Cinn and 50–1000 µg/ml of DoM.

Microsoft Excel software was used to plot the peak area v/s concentration in µg/ml.

7. **Evaluation**

Peak areas for all the peaks were recorded. From the peak areas respective amounts were computed as follows:

\[ W = \frac{R_A \times C \times D}{R_B \times W^*} \]

where,

\( R_A \) = Areas of sample.

\( R_B \) = Areas of standard.

\( C \) = Conc. of standard in mg/ml.

\( D \) = Dilution Factor.

\( W^* \) = Exact weight of the sample taken

\( W \) = Amount of sample per tablet.

### 4.2.3 RESULTS AND DISCUSSION

1. **Chromatography**

The mobile phase resolved Cinn and DoM very efficiently as shown in
**Fig. 4.4.** The Rf values were 0.48 & 0.80 for Cinn & DoM respectively. The wavelength of 276 nm was selected because the absorption spectra of these two drugs overlap at this wavelength. (**Fig. 4.5**).

2. **System Suitability**

To ascertain the resolution and reproducibility of the chromatographic method, system suitability tests was carried out using working standard solution of Cinn and DoM. Table 4.4

<table>
<thead>
<tr>
<th>TABLE 4.4</th>
<th>SYSTEM SUITABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARAMETERS</td>
<td>Cinn</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>1.0</td>
</tr>
<tr>
<td>Resolution Factor</td>
<td>–</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>1.15</td>
</tr>
</tbody>
</table>

3. **Linearity, Limit of Detection and Limit of Quantification**

Cinn showed linearity of response between 60–1000 µg/mcl and DoM showed linearity of response between 50–1000 µg/mcl. (**Fig. 4.6**). These linearities were represented by linear regression equation as follows:

\[ Y_{\text{Cin.}} = 1.16 \times + (-60.49) \quad (\gamma = 0.993) \]

\[ Y_{\text{DoM}} = 1.73 \times + (-107.32) \quad (\gamma = 0.992) \]

The limit of detection (LOD) & limit of quantification (LOQ) of Cinn and DoM were found to be 29 µg/mcl, 87 µg/mcl & 30 µg/mcl, 95 µg/mcl respectively.

4. **Assay**
The content of Cinn and DoM found in tablets by the proposed method are tabulated in Table 4.5. The low values of RSD indicate that the method is precise & accurate.

5. **Accuracy and Precision**

The accuracy and precision of the proposed method were further confirmed by recovery experiments. Three different levels of standards were added to the preanalysed sample and each level was repeated three times. These results are shown in Table 4.6. The % recoveries for Cinn is ranges from 96.85–98.57%. The % recoveries for DoM is ranging from 97.40–99.00% respectively.

**TABLE 4.5**

**RESULTS OF ASSAYS OF Cinn. & DoM.**

<table>
<thead>
<tr>
<th>Brand</th>
<th>Cinn (n=5)</th>
<th>DoM (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Label claim mg/tab.</td>
<td>Amt. Found mg/tab.</td>
</tr>
<tr>
<td>Stugil Tab. Johnson &amp; Johnson</td>
<td>20.00</td>
<td>19.98</td>
</tr>
</tbody>
</table>

**TABLE 4.6**

**RESULTS OF RECOVERY ANALYSIS**

<table>
<thead>
<tr>
<th>No.</th>
<th>Cinn (n=5) (Label claim: 20 mg/tab.)</th>
<th>DoM (n=5) (Label claim: 15 mg/tab.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added</td>
<td>Found</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>19.71</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>29.52</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>38.80</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>48.43</td>
</tr>
<tr>
<td>MEAN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.2.4 REFERENCES

3. G.A.Saleh; H.F.Askal; Pharmazie, 45(3), (1990), 220.
5. B.P.Zorya; S.G.Solomonova; Farm. Zh. (Kiev), 6 (1991), 69–70. (Ukrain)
12. S.S.Zarapkar; B.B.Salunkhe; Indian Drugs, 27(10), (1990) 537–540.
13. Q.Wu;R.Yu;H.Xu;Nanjing Yaoxue Yuan Xuebao, 16(2),(1985)64–66 (Ch.).
PART–IV

CHAPTER–III

SIMULTANEOUS DETERMINATION OF DILOXANIDE FUROATE AND TINIDAZOLE IN PHARMACEUTICAL PREPARATIONS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

4.3.1 DRUG PROFILES

DILOXANIDE FUROATE (DF)

A) Chemical Name: 4-[(dichloroacetyl)(methyl)amino]phenyl furan-2-carboxylate

B) Formula:

   i) Structural:

   ii) Formula: C\textsubscript{14}H\textsubscript{11}Cl\textsubscript{2}NO\textsubscript{4}

C) Molecular weight: 328.15

D) Description: White or almost white, crystalline powder.

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

F) Melting Point: 114°–116°C.

G) Category: An antiamoebic.

TINIDAZOLE (TZ)

A) Chemical Name: 1-(2-Ethylsulfonylethyl)-2-methyl-5-nitro-imidazole

B) Formula:

   i) Structural:
ii) Formula: C₈H₁₃N₃O₄S

C) Molecular weight: 247.26

D) Description: Pale yellow crystals or crystalline powder.

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

F) Melting Point: 125°–128°C.

G) Category: Antiprotozoal.

The drugs are official pharmacopoeial⁽¹⁻³⁾. Determination of DF & TZ by spectrophotometry⁽⁴⁻⁷⁾, GLC⁽⁸⁻¹⁰⁾, HPLC⁽¹¹⁻¹³⁾ and HPTLC⁽¹⁴⁻¹⁶⁾ from fixed dose combination tablets have been reported. HPTLC methods reported have the limitations such as need of costly RP–C18 plates in reverse phase HPTLC and low Rf values (0.12 for TZ) in normal phase HPTLC. This paper describes a new HPTLC method, which is simple, sensitive, and rapid and stability indicating for DF & TZ from tablet forms.

4.3.2 EXPERIMENTAL:

1. Instrumentation

   A Camag Linomat IV sample applicator with TLC Scanner II controlled by Cats 3.15 v software and twin trough chamber were used.

2. Solvents and Chemicals
Working standards of DF and TZ were procured from Merind Ltd. Bhandup, Mumbai India, having purity 99.81% & 99.31% respectively. Methanol and Methylene chloride used were of AR grade supplied by S.D. Fine Chemicals Ltd., Thane, India.

3. **Chromatographic Condition**

   Mobile phase consisted of a mixture of Methylene chloride; Methanol (9.6: 0.25 v/v). Stationary phase was used of Merck HPTLC plates coated with 60F254 Silica gel on aluminium sheets were used as stationary phase. Plates were scanned at 280 nm.

4. **Preparation of working standard solution**

   **Standard stock solution**

   Accurately weighed about 100 mg of DF and TZ working standard and transferred in 100 ml of volumetric flask, dissolved and diluted with methanol.

   **Working Standard Solution**

   4.0 ml of the standard stock solution was diluted to 10 ml volumetric flask with methanol to give a concentration of 400 mcg ml\(^{-1}\) each of DF and TZ.

5. **Sample Preparation**

   Twenty tablets were weighed and crushed to fine powder. Powder equivalent to 375 mg of DF and 300 mg of TZ was weighed and transferred it into 100 ml volumetric flask. 70 ml methanol was added and dissolved by sonication for 30 minutes and diluted upto the mark with methanol. This solution was filtered through Whatman paper no. 1. An aliquot (5 ml) of the filtrate was taken in a 50ml volumetric flask and diluted upto the mark with methanol.

6. **Calibration procedure**

   From above stock solutions eight dilutions were made to get solutions of DF and TZ in the range of 40–500 µg/ml.
7. **Evaluation**

Peak areas for all the tracks were recorded. From the peak areas respective amounts were computed as follows:

\[
W = \frac{R_{spl} \times C \times D}{R_{std} \times w^*} \times \text{Avg. wt. tablet}
\]

where,

- \( R_{spl} \) = Area of DF and TZ in sample solution.
- \( R_{std} \) = Area of DF and TZ in standard solution.
- \( C \) = Conc. of std. DF and TZ mg ml\(^{-1}\).
- \( D \) = Dilution Factor
- \( W \) = Amount of DF and TZ per tablet in mgs.
- \( w^* \) = Weight of a tablet powder taken for analysis in mgs.

4.3.3 **RESULTS AND DISCUSSION**

1. **Chromatography**

The mobile phase of Methylene chloride: Methanol (9.6 : 0.25; v/v) was selected because it was found to ideally resolve the two components of DF and TZ with Rf values 0.45 and 0.28 respectively (*Fig. 4.7*). A wavelength of 280 nm was selected on the basis of UV maxima of the drugs (*Fig. 4.8*).

2. **System Suitability**

To ascertain the resolution & reproducibility of the chromatographic method, system suitability tests were carried out standard stock solution of DF and TZ values for different parameters are as shown in Table 4.7.
### TABLE 4.7
PARAMETERS OF SYSTEM SUITABILITY

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
</tr>
<tr>
<td>Retention Factor</td>
<td>0.45</td>
</tr>
<tr>
<td>Resolution</td>
<td>2.6</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>1.2</td>
</tr>
<tr>
<td>RSD (n=5)</td>
<td>1.1</td>
</tr>
</tbody>
</table>

3. **Linearity, Limit of Detection & Limit of Quantification**

Peak areas versus the respective concentration of DF and TZ were found to be linear in the concentration range of 40–500 µg/ml (*Fig. 4.9*). They were represented by the following linear regression equation:

\[
Y_{DF} = 0.86 \times + 11.13 \quad (\gamma = 0.9992)
\]

\[
Y_{TZ} = 1.97 \times + 22.68 \quad (\gamma = 0.9991)
\]

The limit of detection (LOD) and limit of quantification (LOQ) for DF and TZ were found to be 15 µg/ml, 45 µg/ml & 12 µg/ml, 40 µg/ml respectively.

4. **Assay**

The contents of DF and TZ from the commercial brand manufactured by CFL Pharma, Delhi India was analysed by the proposed method the results obtained were in Table 4.8. Low values of RSD indicate that the method is precise and accurate.
5. **Accuracy and Precision**

To confirm the accuracy and precision of the proposed method, recovery experiments were carried out by standard addition technique, by adding known standards at three different levels to the preanalysed sample. Each level was repeated thrice. These results are shown in Table 4.9. % recoveries for DF is ranging from 97.42 to 97.87% and that for TZ is ranging from 96.68 to 99.00%.

### TABLE 4.8
**RESULTS OF ASSAY**

<table>
<thead>
<tr>
<th>Brand</th>
<th>Sr.No.</th>
<th>DF Label Claim (750 mg/tablet)</th>
<th>TZ Label Claim (600 mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg %</td>
<td>mg %</td>
</tr>
<tr>
<td>Amibactin–BD</td>
<td>1</td>
<td>749.12 99.88</td>
<td>600.15 100.034</td>
</tr>
<tr>
<td>CFL Pharma</td>
<td>2</td>
<td>749.95 99.99</td>
<td>599.88 99.98</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>749.88 99.98</td>
<td>599.77 99.96</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>749.65 99.95</td>
<td>599.92 99.99</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>750.65 100.09</td>
<td>598.83 99.81</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>749.85 99.98</td>
<td>599.71 99.95</td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td>1.10</td>
<td>1.30</td>
</tr>
</tbody>
</table>

### TABLE 4.9
**RESULTS OF RECOVERY ANALYSIS OF DF & TZ**

<table>
<thead>
<tr>
<th>Brand</th>
<th>Drug</th>
<th>Amt. of Drug added (mg)</th>
<th>Amt. Found* in mg.</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
</table>

6. **Ruggedness and Robustness**

The results of ruggedness testing (Two analysts) and robustness studies (deliberate variation in method condition) for the assay of the brand drug are as shown in Table 4.10 & Table 4.11.

<table>
<thead>
<tr>
<th>% Recovery</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DF</strong></td>
<td><strong>TZ</strong></td>
</tr>
<tr>
<td>Analyst I</td>
<td>99.36</td>
</tr>
<tr>
<td>Analyst II</td>
<td>99.25</td>
</tr>
</tbody>
</table>
TABLE 4.11
RESULTS OF ROBUSTNESS STUDIES

<table>
<thead>
<tr>
<th>Development Distance (cm)</th>
<th>% Assay DF</th>
<th>% Assay TZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>99.36</td>
<td>99.65</td>
</tr>
<tr>
<td>8.0</td>
<td>99.25</td>
<td>99.30</td>
</tr>
<tr>
<td>9.0</td>
<td>99.45</td>
<td>99.15</td>
</tr>
</tbody>
</table>

7. Solution Stability

The standard and sample solutions were prepared and stored at room temperature for 10 mins to 240 mins and then applied on the same chromatoplate. After development it was observed that there were no additional spots in final chromatogram compared to initial chromatogram indicating the solution is stable upto 4 hrs.

8. Force degradation study

The fine powdered samples of the brand drugs containing 375 mg and 300 mg of DF & TZ were taken in 5 different 100 ml standard flasks. 10 ml each of 0.1N HCl, 0.1N NaOH and 30% H₂O₂ were added to three of the above flasks and kept aside for a week. The 4th and 5th flasks were kept in sunlight and in the dark respectively also for a week. After the completion of a week, the samples were diluted upto the mark with methanol and filtered through whatman filter paper no.1 1 ml of each filtrate was further diluted to 10 ml with methanol and 5µl of each of these solutions were spotted on a plate, developed and scanned as per the proposed method. It was observed that there were no additional peaks other than DF and TZ at 0.45 and 0.28 Rf values and there were considerable decrease in the
peak areas and % assays as shown in Table 4.12. This study shows that the proposed method is stability indicating.

**TABLE 4.12**

**RESULTS OF DEGRADED STUDY**

<table>
<thead>
<tr>
<th>Brand</th>
<th>DF</th>
<th>TZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Label Claim</td>
<td>Label Claim</td>
</tr>
<tr>
<td></td>
<td>750 mg/tab</td>
<td>600 mg/tab</td>
</tr>
<tr>
<td>Sunlight</td>
<td>742.12 (98.18%)</td>
<td>592.51 (98.38%)</td>
</tr>
<tr>
<td>Dark</td>
<td>748.10 (99.75%)</td>
<td>598.8 (99.8%)</td>
</tr>
<tr>
<td>HCL (0.1N)</td>
<td>721.8 (96.24%)</td>
<td>580.10 (96.68%)</td>
</tr>
<tr>
<td>NaOH (0.1N)</td>
<td>702.7 (93.69%)</td>
<td>579.10 (96.52%)</td>
</tr>
<tr>
<td>H$_2$O$_2$ (30%)</td>
<td>718.0 (95.73%)</td>
<td>580.10 (96.68%)</td>
</tr>
</tbody>
</table>
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4.3.4 REFERENCES


PART – IV
CHAPTER – IV

SIMULTANEOUS DETERMINATION OF ATENOLOL AND AMLODIPINE
IN PHARMACEUTICAL PREPARATIONS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

4.4.1 DRUG PROFILES

ATENOLOL (ATL)

A) Chemical Name: (R,S)-4-[2-Hydroxy-3-[(1-methylethyl)amino]propoxy]benzeneacetamide

B) Formula:
   i) Structural:

   ![Structural formula of Atenolol]

   ii) Molecular: C_{14}H_{22}N_{2}O_{3}

C) Molecular Weight: 266.3

D) Description: A white to almost white powder.

E) Solubility: Sparingly soluble in water; soluble in dehydrated alcohol; slightly soluble in methylene chloride and practically insoluble in ether.

F) Melting point: 146–148°C.

G) Category: Beta-adrenoceptor antagonist.

AMLODIPINE BESYLATE (AMLO)

A) Chemical Name: (R,S)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate

B) Formula:
iii) Structural:

iv) Molecular: C_{20}H_{25}ClN_{2}O_{5}.C_{6}H_{5}SO_{3}H

C) Molecular Weight: 567.1

D) Description: A white to pale yellow powder.

E) Solubility: Sparingly soluble in water and in alcohol; Slightly soluble in chloroform and in ether.

F) Category: Antianginal.

ATL is official in Indian Pharmacopoeia (IP\(^{(1)}\)) and British Pharmacopoeia (BP\(^{(2)}\)). Literature survey reveals that few methods were reported such as UV–spectrophotometry\(^{(3–5)}\), spectrofluorimetry\(^{(6)}\), HPLC\(^{(7–8)}\) and GLC\(^{(9)}\) for the determination of ATL in tablets.

AMLO is not official in any Pharmacopoeia. Few HPLC methods\(^{(10–14)}\) were reported in the literature\(^{(15–17)}\) for its determination in tablets and biological fluids.

The combined dosages containing these two drugs available in the market from Cipla Ltd (India) and Lyka Laboratories Ltd. Mumbai (India). Simultaneous determination of ATL and AMLO by HPLC have been reported\(^{(18–24)}\). However, there is no analytical method for the simultaneous determination\(^{(25–28)}\) of these two drugs by HPTLC. High Performance Thin Layer Chromatography (HPTLC) is a more effective technique for simultaneous determination of ATL and AMLO for routine analysis. The aim of the present investigation is to develop an HPTLC
method for the simultaneous determination of ATL and AMSO. This has been achieved using methylene chloride: methanol: ammonia (25% NH₃) (8.8:1.3:0.1; v/v) as mobile phase and silica gel 60F₂₅₄ HPTLC plates as stationary phase. Quantitative estimation was accomplished by densitometric scanning with UV detector at 230 nm wavelength.

**4.4.2 EXPERIMENTAL**

1. **Instrumentation**
   
   A Camag Linomat IV sample applicator, with TLC Scanner II controlled by Cats 3.15 v software and twin trough chamber were used.

2. **Solvents and Chemicals**
   
   Working standards of ATL & AMLO were procured from Qualirex Chemicals Pvt. Ltd. Aurangabad, and TATA Pharma Ltd., Patalganga, India respectively. These working standards were of 99.81% & 99.31% pure respectively.

   Methylene chloride, methanol and ammonia solution (25% NH₃) used were of AR grade supplied by S.D. Fine Chemicals Ltd., Thane, India.

3. **Chromatographic Condition**
   
   Mobile phase consisted of a mixture of methylene chloride: Methanol: ammonia solution (25% NH₃) in the proportion of (8.8: 1.3: 0.1; v/v). Merck HPTLC plates coated with silica gel 60F₂₅₄ on aluminum sheets were used as stationary phase. Detection was carried out at 230 nm. 10µl of working standard and sample solutions were spotted on HPTLC plate.

4. **Working standard Preparation**

   **Stock solution**
Accurately weighed about 250 mg of ATL and 25 mg of AMLO working standard and transferred in 25 of volumetric flask, dissolved & dilute with methanol.

**Working Standard Solution**

4.0 ml of each of the standard stock solution was diluted to 100 ml with methanol to give a concentration of 400 µg/ml of ATL and 40 µg/ml of AMLO. This solution was used as the working standard for analysis of samples.

5. **Sample Preparation**

Two brands of this dosage form was procured from the market and assayed by using proposed method.

Twenty tablets were weighed and crushed to a fine powder and appropriate amounts of each one, corresponding to about 100 mg ATL and 10 mg AMLO were weighed and transferred in a 50 ml volumetric flask. 40 ml of methanol was added for dissolve the powder using sonicator for 30 min, the solution were made upto the volume with methanol and filtered through a Whatman paper (No. 1). An aliquot (2 ml) of the filtrate solution was taken in a 10 ml volumetric flask and diluted upto the mark with methanol and used for the analysis.

6. **Calibration Procedure**

From above stock solutions eight various dilutions were made to get solutions of ATL & AMLO in the range of 10–500 µg/ml.

7. **Evaluation**

Peak areas for all the peaks were recorded. From the peak areas respective amounts were computed as follows:

\[
W = \frac{Rsp1 \times C \times D \times \text{Avg. wt. of tablets}}{Rstd \times w} \times \text{Factor} \]

where,
RSPL = Area of ATL/AMLO in sample solution.
RSTD = Area of ATL/AMLO in standard solution.
C = Conc. of std. ATL/AMLO mg ml\(^{-1}\).
D = Dilution Factor
W = Amount of ATL/AMLO per tablet in mgs.
w* = Weight of a tablet powder taken for analysis in mgs.
Factor = Conversion Factor for amlodipine besylate to AMLO base = 0.78

4.4.3 RESULTS AND DISCUSSION

1. **Chromatography**

   UV spectrum of ATL and AMLO (Conc. 10 µg/ml each in methanol) is as shown in *Fig. 4.10*. A wavelength of 230 nm was chosen as a common wavelength to match the concentration ratio of the drugs present in the formulation. The mobile phase was prepared by mixing methylene chloride, methanol and Ammonia solution (25% NH\(_3\)) in the proportion of (8.8: 1.3:0.1; v/v) because it gave highest resolution, minimum tailing and Rf values between 0.2 and 0.8 for the peaks of interest (*Fig. 4.11*).

2. **System Suitability**

   To ascertain the resolution & reproducibility of the chromatographic method, system suitability tests were carried out on standard stock solution of ATL & AMLO. Values for these parameters are as shown in Table 4.13.
3. **Linearity, Limit of Detection & Limit of Quantification**

Plots of peak areas versus the respective concentration of ATL and AMLO were found to be linear in the concentration range of 10–500 µg/ml (Fig. 4.12). They were represented by the linear regression equation:

\[
Y_{ATL} = 4.54 \times + 63.46 \quad (\gamma = 0.9991)
\]

\[
Y_{AMLO} = 5.71 \times + 178.85 \quad (\gamma = 0.9994)
\]

The limit of detection (LOD) and limit of quantification (LOQ) of ATL & AMLO were found to be 1 µg/ml, 3 µg/ml & 2 µg/ml, 6 µg/ml respectively.

4. **Assay**

The content of ATL and AMLO found in tablets by the proposed method is tabulated in Table 4.14. Low values of RSD indicate that the method is precise.

5. **Accuracy and Precision**

To confirm the accuracy and precision of the proposed method, recovery experiments were carried out by standard addition technique, by adding known standards at three different levels to the pre analysed sample. Each level was repeated thrice, the results are summarized in Table 4.15.
The % recoveries for ATL are ranging from 98.28 to 99.86%. The % recoveries for AMLO are ranging from 99.12 to 99.71%.

### TABLE 4.14

RESULTS OF ASSAYS OF ATL AND AMLO

<table>
<thead>
<tr>
<th>Brand</th>
<th>S.No.</th>
<th>Amt of ATL Label Claim: 50 mg/Tablet</th>
<th>Amt of AMLO Label Claim: 5 mg/Tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/tablet</td>
<td>%</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amlopres – AT</td>
<td>1</td>
<td>49.97</td>
<td>99.94</td>
</tr>
<tr>
<td>Cipla Ltd.</td>
<td>2</td>
<td>49.73</td>
<td>99.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>49.82</td>
<td>99.64</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>49.89</td>
<td>99.78</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>49.92</td>
<td>99.84</td>
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<tr>
<td>Mean Assay</td>
<td></td>
<td>49.87</td>
<td>99.70</td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td>1.30%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stamlo Beta</td>
<td>1</td>
<td>48.98</td>
<td>99.78</td>
</tr>
<tr>
<td>Lyka Labs</td>
<td>2</td>
<td>49.12</td>
<td>98.24</td>
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<td></td>
<td>3</td>
<td>49.15</td>
<td>98.30</td>
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<tr>
<td></td>
<td>5</td>
<td>49.30</td>
<td>98.60</td>
</tr>
<tr>
<td>Mean Assay</td>
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<td>49.27</td>
<td>98.59</td>
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<tr>
<td>RSD</td>
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<td>1.12%</td>
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</table>
## TABLE 4.15
RESULTS OF RECOVERY ANALYSIS

<table>
<thead>
<tr>
<th>Brand</th>
<th>Drug</th>
<th>Amt of Drug added (mg)</th>
<th>Amt found* in mg</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>ATL</td>
<td>0</td>
<td>49.85</td>
<td>99.70</td>
<td>1.30</td>
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<td>Amlopres–AT</td>
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<td>58.97</td>
<td>98.28</td>
<td>1.40</td>
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<td>20</td>
<td>69.82</td>
<td>99.74</td>
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<td>78.80</td>
<td>98.50</td>
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<td>99.00</td>
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</tr>
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<td>1.32</td>
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<td>1.08</td>
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<td>34.90</td>
<td>99.71</td>
<td>1.32</td>
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<td>1.23</td>
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<td>ATL</td>
<td>0</td>
<td>49.89</td>
<td>99.78</td>
<td>1.30</td>
</tr>
<tr>
<td>Stamlo Beta</td>
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<td>58.98</td>
<td>98.3</td>
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<td>Mean</td>
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<td>99.27</td>
<td>99.15</td>
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<tr>
<td></td>
<td>AMLO</td>
<td>0</td>
<td>4.90</td>
<td>98.00</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>14.89</td>
<td>99.27</td>
<td>1.32</td>
</tr>
<tr>
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<td>24.92</td>
<td>99.68</td>
<td>1.42</td>
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<td></td>
<td>30</td>
<td>34.87</td>
<td>99.63</td>
<td>1.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
<td>99.15</td>
<td>1.41</td>
</tr>
</tbody>
</table>

*Average of three experiments.
4.4.4 REFERENCES

5. S.V. Erram, and H.P. Tipnis, Indian Drugs, 30(9), 1993, 460–467.


PART–IV

CHAPTER–V

SIMULTANEOUS DETERMINATION OF BROMHEXINE HYDRO–
CHLORIDE AND ORCIPRENALINE SULPHATE IN PHARMACEUTICAL
PREPARATION BY HPTLC

4.5.1 DRUG PROFILES

BROMHEXINE HYDROCHLORIDE (BH)

H) Chemical Name: 2-Amino-3,5-dibromo-N-cyclohexyl-N-
methylbenzylamine hydrochloride

I) Formula:

iii) Structural:

iv) Molecular: C_{14}H_{21}Br_{2}N_{2}\cdot\text{HCl}.

J) Molecular Weight: 412.64

K) Description: A white crystalline powder.

L) Solubility: Soluble in ethanol (95%), methanol and chloroform.

M) Melting point: 237.5–238°C.

N) Category: An antimucolytic.

ORCIPRENALINE SULPHATE (OS)

A) Chemical Name: 5-[1-hydroxy-2-(propan-2-ylamino)ethyl]benzene-1,3-diol
sulfate
B) Formula:

v) Structural:

vi) Molecular: \((C_{11}H_{17}NO_3)_2H_2SO_4\)

C) Molecular Weight: 520.6

D) Description: A white crystalline powder.

E) Solubility: Soluble in water, ethanol and methanol.

F) Melting point: 202–203°C

G) Category: Beta adrenoceptor agonist.

Both drugs are official in pharmacopoeias\(^{(1-3)}\). Literature survey\(^{(4-10)}\) reveals that there were few methods available to estimate these drugs individually but no method available to determine these drugs\(^{(11-14)}\) simultaneously. This paper describes a new HPTLC method which is simple, sensitive, rapid and stability indicating for determination of BH and OS from syrup.

4.5.2 EXPERIMENTAL

1. Instrumentation

A Camg Linomat IV applicator, TLC Scanner II controlled by Cats 3.15 v software and twin trough chamber were used.

2. Solvents and Chemicals

Working standards of BH and OS were procured from IPCA Laboratories, and German Remedies Mumbai, India respectively, having purity 99.85% &
99.96% respectively. Methanol, Chloroform and Diethylamine used were of AR grade and were supplied by S.D. Fine Chemicals Ltd., Thane, India.

3. Chromatographic Condition

Mobile phase consisted of a mixture of Methanol : Chloroform : Diethylamine (7:3:0.1; v/v). Merck HPTLC plates coated with silica gel 60F254 on aluminum sheets were used as stationary phase. Detection was carried out with \( \lambda_{\text{max}} \) at 310nm wavelength.

4. Preparation of Working Standard Solution

Standard Stock Solution

Accurately weighed about 100 mg of BH and OS working standards and transferred in 100 ml volumetric flask, dissolved and diluted with methanol. (1mg/ml).

Working Standard Solution

4.0 ml of BH and 5.0 ml of OS were diluted to 50 ml volumetric flask with the methanol to give a concentration of 80 mcg/ml and 100 mcg/ml each of BH and OS. This solution was used as working standard for the analysis of the sample.

5. Sample Preparation

Five ml of syrup sample equivalent to 4 mg of BH and 5 mg of OS was pipette out in a 100 ml volumetric flask. Seventy ml methanol was added and sonicated for 30 minutes, the solution was diluted upto the mark with the methanol with constant shaking. This solution was centrifuged for 10 minutes at 5000 rpm and the supernatant liquid was used for the analysis.

6. Calibration Procedure

From above standard stock solutions various dilutions were made to get solutions of 20–120 µg/ml of BH and OS for calibration curve.
7. **Evaluation**

Peak areas for all the peaks were recorded. From the peak areas respective amounts were computed as follows:

\[ W = \frac{R_{spl} \times C \times D}{R_{std} \times w^*} \]

where,

- \( R_{spl} = \) Area of BH and OS in sample solution.
- \( R_{std} = \) Area of BH and OS in standard solution.
- \( C = \) Conc. of std. BH and OS mg/ml
- \( D = \) Dilution Factor
- \( W = \) Amount BH and OS per 5 ml in syrup
- \( w^* = \) Syrup taken for analysis in ml.

### 4.5.3 RESULTS AND DISCUSSION

1. **Chromatography**

   The mobile phase of Methanol : Chloroform : Diethylamine (7:3: 0.1; v/v) was selected because it was found to ideally resolve the two peaks of BH and OS with Rf values 0.75 and 0.32 respectively ([Fig. 4.13](#)). A wavelength of 310 nm was selected on the basis of UV maxima these drugs. ([Fig. 4.14](#))

2. **System Suitability**

   To ascertain the resolution & reproducibility of the chromatography method, system suitability tests were carried out on standard stock solution of BH and OS. Values obtained for different parameters are as shown in Table 4.16.
TABLE 4.16
PARAMETERS OF SYSTEM SUITABILITY TEST

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>BH 1.4</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>BH 1.2</td>
</tr>
<tr>
<td>RSD (n=5)</td>
<td>BH 1.1</td>
</tr>
</tbody>
</table>

3. **Linearity, Limit of Detection & Quantification**
   
   Plots of peak areas versus the respective concentration of BH and OS were found to be linear in the concentration range of 20–120 µg/ml (Fig. 4.15). They were represented by the linear regression equation:
   
   \[
   Y_{BH} = 5.53 \times -1.39 \quad (\gamma = 0.9979)
   \]
   
   \[
   Y_{OS} = 6.78 \times -5.82 \quad (\gamma = 0.9997)
   \]
   
   The limit of detection (LOD) and quantification (LOQ) for BH and OS were found to be 4 µg ml\(^{-1}\), 5µg ml\(^{-1}\) & 14 µg ml\(^{-1}\), 18 µg ml\(^{-1}\) respectively.

4. **Assay**
   
   The content of BH and OS found in the syrup by the proposed method are tabulated in Table 4.17. Low values of RSD indicate that the method is precise.

5. **Accuracy and Precision**
   
   To confirm the accuracy and precision of the proposed method, recovery experiments were carried out by standard addition technique, by adding known standards at three different levels to the preanalysed sample. Each level was repeated thrice, these results are summarized in Table 4.18. The % recovery for BH is ranging from 99.40 to 99.50%. The % recovery for OS is ranging from 99.71 to 99.88%. Results indicate good precision and lack of interference from the excipients present in the formulations.
6. **Solution Stability**

Standard and sample solutions were prepared and stored at room temperature for four hours and then applied on the same chromatoplate. After development it was observed that there were no additional spots in final chromatogram compared to initial chromatogram indicating the solution is stable upto 4 hrs.

**TABLE 4.17**

**RESULTS OF ASSAY OF BH AND OS**

<table>
<thead>
<tr>
<th>Brand</th>
<th>S.No.</th>
<th>BH</th>
<th>Label Claim 4 mg/5ml</th>
<th>OS</th>
<th>Label Claim 5 mg/5ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIOSELPENT German Remedies</td>
<td>1</td>
<td>4.05</td>
<td>101.25</td>
<td>4.99</td>
<td>99.80</td>
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<tr>
<td>India</td>
<td>2</td>
<td>3.90</td>
<td>97.50</td>
<td>4.95</td>
<td>99.00</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.95</td>
<td>98.75</td>
<td>5.03</td>
<td>100.60</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.01</td>
<td>100.25</td>
<td>4.98</td>
<td>99.60</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.99</td>
<td>99.75</td>
<td>4.85</td>
<td>97.00</td>
</tr>
<tr>
<td>MEAN</td>
<td></td>
<td>3.98</td>
<td>99.50</td>
<td>4.96</td>
<td>99.20</td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td>1.70</td>
<td></td>
<td>1.20</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4.18**

**RESULTS OF RECOVERY ANALYSIS**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>BH</th>
<th>Label Claim 4 mg/5ml</th>
<th>OS</th>
<th>Label Claim 5 mg/5ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added</td>
<td>Found</td>
<td>%</td>
<td>Added</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>3.97</td>
<td>99.40</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>4.08</td>
<td>99.50</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>4.15</td>
<td>99.43</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>4.25</td>
<td>98.85</td>
<td>30</td>
</tr>
<tr>
<td>MEAN</td>
<td></td>
<td>99.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
7. **Force Degradation Study**

The well shaked syrup sample containing 4 mg and 5 mg per 5 ml of BH & OS were taken in 5 different 100 ml standard flasks. 10 ml each of 0.1N HCl, 0.1N NaOH and 30% H₂O₂ were added to three of the above flasks and kept aside for a week. The 4<sup>th</sup> and 5<sup>th</sup> flasks were kept in sunlight and in the dark respectively also for a week. After the completion of a week, the samples were diluted up to the mark with methanol. The solution was centrifuged for 10 minutes at 5000 rpm and the supernatant was used for the analysis. 3 µl of each of these solutions was spotted on a plate, developed and scanned as per the proposed method. It was observed that there were no additional peaks other than BH and OS at 0.75 and 0.32 Rf values and there were considerable decrease in % assays as shown in *Table 4.19.*

**TABLE 4.19**

RESULTS OF DEGRADED STUDY OF BH AND OS

<table>
<thead>
<tr>
<th>CONDITIONS</th>
<th>BH Label Claim 4 mg/5 ml</th>
<th>OS Label Claim 5 mg/5 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunlight</td>
<td>3.82 (95.50%)</td>
<td>4.89 (97.80%)</td>
</tr>
<tr>
<td>Dark</td>
<td>3.95 (98.75%)</td>
<td>4.97 (99.40%)</td>
</tr>
<tr>
<td>HCl (0.1N)</td>
<td>3.88 (97.005)</td>
<td>4.78 (95.60%)</td>
</tr>
<tr>
<td>NaOH (0.1N)</td>
<td>3.01 (75.25%)</td>
<td>4.31 (86.20%)</td>
</tr>
<tr>
<td>H₂O₂ (30%)</td>
<td>7.18 (95.75%)</td>
<td>4.63 (92.60%)</td>
</tr>
</tbody>
</table>
4.5.4 REFERENCES


PART–IV

CHAPTER–VI

STABILITY INDICATING HPTLC METHOD FOR THE DETERMINATION OF CINNARIZINE IN PHARMACEUTICAL PREPARATIONS

4.6.1 DRUG PROFILE

CINNARIZINE (Cinn)

A) Chemical Name: 1-Benzhydryl-4-cinnamyl-piperazine

B) Formula:

i) Structural:

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

ii) Molecular: $C_{26}H_{28}N_2$

C) Molecular Weight: 368.40

D) Description: A white pale yellow crystalline powder.

E) Solubility: Practically insoluble in water; Slightly soluble in alcohol; soluble in ether and freely soluble in methylene chloride.

F) Melting point: 192°C with decomposition (For hydro chloride).

G) Category: H₁ Receptor and Calcium channel blocker.

Cinn is official in B.P.(2) Literature survey reveals that spectrophotometry(3–7), HPLC(8), GC(9) and Potentiometry(10–11) analytical methods can determine(12–16), it in formulations. However, there is no HPTLC method reported so far for its determination. Therefore an HPTLC method was develop and results obtained are presented in this chapter.
4.6.2 EXPERIMENTAL

1. Instrumentation

Camag Linomat IV samples applicator, with TLC Scanner II controlled by Cats 3.15 software and twin trough chamber were used.

2. Solvents and Chemicals

A working standard of Cinn was procured from F.D.C. Ltd. Mumbai, India with purity 99.98%. Methanol, Chloroform and Formic acid of AR grade were obtained from S.D. Fine Chemicals Ltd., Thane, India.

3. Chromatographic Condition

Mobile phase consisted a mixture of Methanol: Chloroform: Formic acid (1:9:0.05; v/v). Merck HPTLC plates (0.2mm thickness) precoated with 60F$_{254}$ Silica gel on aluminum sheet were used as stationary phase. Detection carried out $\lambda$ maximum at 254 nm.

4. Standard Preparation

Standard Stock Solution

Accurately weighed about 100 mg of working standard Cinn. and transferred in a 100 ml volumetric flask, dissolved and diluted with methanol. (1mg/ml).

Working Standard Solution

5 ml of standard stock solution was pipette in a 10 ml volumetric flask and diluted with methanol (500 µg/ml).

5. Sample Preparation

Twenty tablets were weighed and crushed to fine powder. Powder equivalent to 25 mg of Cinn was weighed in a 25 ml volumetric flask. 20 ml of methanol was added, and sonication was done for 30 mins, and diluted upto the mark with methanol. The solution was centrifuged at 3000 rpm and 5 ml of
supernatant solution was pipette out in to 10 ml volumetric flask and diluted upto mark with in methanol (500 µg/ml).

6. **Calibration Procedure**

   From above stock solutions eight various dilutions were made to get solutions of 60–1000 µg/ml of Cinn.

7. **Evaluation**

   Peak areas for all the peaks were recorded. From the peak areas respective amounts were computed as follows:

   \[ W = \frac{R_A \times C \times D}{R_B \times W^*} \]

   where,

   \( R_A \) = Area of sample
   \( R_B \) = Area of standard
   \( C \) = Conc. of std. in mg/ml.
   \( D \) = Dilution factor.
   \( W^* \) = Exact weight of sample taken.
   \( W \) = Amt of Cinn per tablet.

4.6.3 **RESULTS AND DISCUSSION**

1. **Chromatography**

   A typical chromatogram obtained is as shown in *Fig. 4.16*. The Rf value was 0.8. The wavelength of 254 nm was selected for the detection (*Fig. 4.17*).

2. **System Suitability**

   System suitability test was carried out by freshly prepared working standard solution to ascertain the resolution, tailing factor and reproducibility of the chromatographic method. Values for the preparation are as shown in Table 4.20.
TABLE 4.20
SYSTEM SUITABILITY PARAMETERS

<table>
<thead>
<tr>
<th>No.</th>
<th>PARAMETERS</th>
<th>Cinn</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>TAILING FACTOR</td>
<td>1.0</td>
</tr>
<tr>
<td>2.</td>
<td>R.S.D. (n=5)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

3. **Linearity, Limit of Detection and Limit of Quantification**

   Linearity response for the drugs was obtained by plotting a graph of detector response in terms of area under the peak v/s concentration *Fig. 4.18*. The graph was linear in the range of 60–1000 µg/ml and the linear regression equation is,

   \[ Y_{\text{Cinn}} = 0.54 \times + 34.33 \quad (\gamma = 0.999) \]

   LOD & LOQ for Cinn. were found to be 15 µg/ml and 45 µg/ml, respectively.

4. **Assay**

   Three brands of tablets were studied for the content of Cinn. Results are tabulated in Table 4.21. Low values of RSD indicate that the method is precise and accurate.

5. **Accuracy and Precision**

   The accuracy of the proposed method was confirmed by recovery experiments. Three different levels of standards were added to the preanalysed sample and each level was repeated three times. Percentage recoveries of Cinn. For brand I, II and III were 99.7–99.82%, 99.67–99.93% & 99.69–99.935 respectively (Table 4.22). These results indicate good accuracy and lack of interference from the excipients in the tablets.
### TABLE 4.21

**RESULTS OF ASSAY OF TABLETS**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Cinn (Label Claim: 25 mg/tablet)</th>
<th>Found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>BRAND 1</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>24.89</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>24.92</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>24.98</td>
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<tr>
<td>4</td>
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<td>25.02</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>24.92</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td></td>
<td>24.95</td>
</tr>
<tr>
<td><strong>RSD</strong></td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td><strong>BRAND II</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>24.89</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>25.08</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>24.94</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>24.90</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>24.95</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td></td>
<td>24.95</td>
</tr>
<tr>
<td><strong>RSD</strong></td>
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<td>0.75</td>
</tr>
<tr>
<td></td>
<td><strong>BRAND III</strong></td>
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<td>24.99</td>
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<td>2</td>
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<td>24.88</td>
</tr>
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<td>24.98</td>
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<tr>
<td>5</td>
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<td>24.93</td>
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<td><strong>MEAN</strong></td>
<td></td>
<td>24.97</td>
</tr>
<tr>
<td><strong>RSD</strong></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>

Brand I– Wallace Pharmaceuticals Ltd. India (Cintigo Tablet)
Brand II– Johnsons & Johnsons India (Stugil Tablet)
Brand III– Geno Pharmaceuticals Ltd., India (Vertigon Tablet)
TABLE 4.22
RESULTS OF RECOVERY ANALYSIS

<table>
<thead>
<tr>
<th>BRAND</th>
<th>Cinn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAND I</td>
<td>99.81</td>
</tr>
<tr>
<td></td>
<td>99.71</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>99.82</td>
</tr>
<tr>
<td></td>
<td>99.78</td>
</tr>
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<td>BRAND II</td>
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<td>99.67</td>
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<tr>
<td>AVERAGE</td>
<td>99.93</td>
</tr>
<tr>
<td></td>
<td>99.78</td>
</tr>
<tr>
<td>BRAND III</td>
<td>99.88</td>
</tr>
<tr>
<td></td>
<td>99.69</td>
</tr>
<tr>
<td>AVERAGE</td>
<td>99.93</td>
</tr>
<tr>
<td></td>
<td>99.83</td>
</tr>
</tbody>
</table>

6. **Forced Degradation Study**

Five ml of stock solution of Cinn (1 mg/ml) was mixed with five ml of 1N NaOH, 1N HCl and 30% H₂O₂ in different 10 ml volumetric flasks, kept in daylight and in the dark for a week and then diluted with methanol up to the mark. Cinn. In sodium hydroxide and Hydrochloric acid was found stable without showing any degraded product, however in 30% H₂O₂, there was one unknown impurity detected. However, it was found that Cinn as well as the impurity are nicely separated from each other. The Rf value of the degraded product is 0.45. The percentage amount of Cinn and its respective impurity found in 30% H₂O₂ by external standard method and in dark as well as in light by the area normalization
The peak purities of the chromatograms of Cinn.

**TABLE 4.23**

DEGRADATION STUDY OF CINNARIZINE IN 30% H₂O₂

<table>
<thead>
<tr>
<th>Peaks</th>
<th>Rf</th>
<th>Imp (%)</th>
<th>Assay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>Cinn</td>
<td>0.84</td>
<td>90.43</td>
<td>88.88</td>
</tr>
<tr>
<td>Impurity</td>
<td>0.45</td>
<td>9.57</td>
<td>11.12</td>
</tr>
</tbody>
</table>

1. Impurity % by area normalization.
2. Assay % (Cinn) by external standard method.

7. **Stability of the sample solution**

Standard solution injected after 24 hours did not show any appreciable change.
4.6.4. REFERENCES