CHAPTER – 7

DETERMINATION OF MANIDIPINE
Manidipine dihydrochloride\textsuperscript{1} (1, 4-Dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridine dicarboxylic acid-2-[4-(diphenylmethyl)-1-piperazinyl] ethyl methyl ester hydrochloride) is a dihydropyridine type, long-acting antihypertensive and calcium antagonist vasodilator drug.

It is a lipophilic, third generation, vasoselective, dihydropyridine calcium channel antagonist. It is used to treat hypertension, chronic stable angina pectoris. Manidipine shows long lasting calcium channel blocking action in vascular smooth muscle cells and antihypertensive actions in various types of hypertensive modes. It inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes.

Manidipine has high selectivity for resistance vessels and inhibits renal vascular constrictions induced by norepinephrine in spontaneously hypertensive rats. It increases renal blood flow and has a prominent natriuretic action without changing glomerular filtration rate.

Manidipine prevents the development of cerebrovascular lesions and inhibits the progression of vascular damage in the brain and kidneys of hypertensive rats. It has neutral effects on glucose and lipid metabolism. The structure, chemical name and analytically important functional groups of the drug have been shown in table 7.01.

**Part-I**

**Visible Spectrophotometry**

The literature survey reveals only a few spectrophotometric\textsuperscript{2-4}, HPLC\textsuperscript{5}, liquid chromatographic\textsuperscript{6-7}, LC-MS\textsuperscript{8-10} and other\textsuperscript{11-14} techniques for the determination of MND in biological fluids. Hence the author has made an attempt to develop few more simple, sensitive spectrophotometric methods for the determination of Manidipine in bulk and synthetic mixtures.
In these methods $K_3\text{Fe(CN)}_6/\text{FeCl}_3$, $\text{NaNO}_2/\text{HCl}$, $\text{NaNO}_2/\text{HCl}$/resorcinol and VN (table 7.04) reagents are used to produce colored species of reasonable stability for spectrophotometric determination of MPN in bulk and synthetic mixtures. A simple and sensitive UV-spectrophotometric method has also been developed to use it as a reference method for comparing accuracy of the results obtained in the proposed methods.

**Table 7.01**

Structural features of active functional groups of selected drug

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Chemical Name</th>
<th>Structure</th>
<th>Analytically important functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manidipine (MND)</td>
<td>1, 4-Dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridine dicarboxylic acid-2-[4-(diphenylmethyl)-1-piperazinyl] ethyl methyl ester hydrochloride.</td>
<td><img src="image" alt="Structure" /></td>
<td>Imino group, $3^0$ amino group, Nitro group…etc.</td>
</tr>
</tbody>
</table>
**Table 7.02**

Characteristics and therapeutic importance of the selected anti-hypertensive drug

<table>
<thead>
<tr>
<th>Pharmocodynamic / Therapeutic category</th>
<th>Characteristics</th>
<th>Therapeutic importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-hypertensive</td>
<td>分子式：C\textsubscript{35}H\textsubscript{38}N\textsubscript{4}O\textsubscript{6}.2HCl</td>
<td>显示持续的钙通道阻断作用于血管平滑肌细胞，并在各种类型的高血压模式下具有降压作用。它抑制外流的钙离子进入心肌和血管平滑肌细胞的膜。</td>
</tr>
<tr>
<td></td>
<td>分子量：683.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>描述：浅黄色结晶粉末</td>
<td></td>
</tr>
<tr>
<td></td>
<td>溶解性：易溶于二甲基亚砜，稀溶于甲醇。</td>
<td></td>
</tr>
</tbody>
</table>

**Table 7.03**

Particulars of chemically available formulations of selected anti-hypertensive agent

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Brand Name</th>
<th>Pharmaceutical Concern</th>
<th>Formulation Type</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iperten</td>
<td>Chiesi Hellas</td>
<td>Tablet</td>
<td>10 mg</td>
</tr>
<tr>
<td>2</td>
<td>Madiplot</td>
<td>Takeda Chemical Industries</td>
<td>Tablet</td>
<td>10 mg, 20 mg</td>
</tr>
</tbody>
</table>
Results:

The optical characteristics such as absorption maxima, Beer’s law limits, molar absorptivity and Sandell’s sensitivity of the proposed methods are reported in table 7.04. The precision was found by analyzing five replicate samples containing same amount of the drug and the results are presented in table 7.04.

Results pertaining to the determination of MND in synthetic mixtures are summarized in table 7.05. The accuracy of these methods were ascertained by comparing the results obtained with the proposed and reference methods in case of synthetic mixtures and are summarized in table 7.05.

As an additional check on the accuracy of these methods, recovery experiments were performed by adding known amounts of pure drug to pre-analyzed synthetic mixture and percent recovery values obtained are listed in table 7.05. Recovery experiments indicated the absence of interference from the commonly associated pharmaceutical additives and excipients.

The results relating to absorption spectra are graphically presented in figs. 7.01 to 7.04. The Beer’s law plots of the systems are illustrated as figs. 7.05 to 7.08. The optimum photometric range for MND using the reagents mentioned are recorded as figs. 7.09 to 7.12. The results relating to reference method are presented graphically in figs. 7.13 and 7.14. The reaction mechanisms relating to the proposed methods are summarized in schemes 7.01 to 7.04.
Table 7.04
Optical, regression characteristics, precision and accuracy of proposed methods for MND

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>(M_{sd})</th>
<th>(M_{sg})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(\lambda) max, nm</td>
<td>760</td>
<td>392</td>
</tr>
<tr>
<td>2</td>
<td>Color of the chromogen</td>
<td>Bluish green</td>
<td>Pale Yellow</td>
</tr>
<tr>
<td>3</td>
<td>Beer’s law limits ((\mu g/ml))</td>
<td>8.0 – 24.0</td>
<td>20.0 – 100.0</td>
</tr>
<tr>
<td>4</td>
<td>Molar absorptivity (l mol(^{-1}) cm(^{-1}))</td>
<td>2.9259×10(^3)</td>
<td>1.1293×10(^3)</td>
</tr>
<tr>
<td>5</td>
<td>Sandell’s sensitivity ((\mu g) cm(^{-2}))</td>
<td>0.2336</td>
<td>0.6053</td>
</tr>
<tr>
<td>6</td>
<td>Regression equation ((Y = mX + b))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slope (m)</td>
<td>0.0422</td>
<td>0.0025</td>
</tr>
<tr>
<td></td>
<td>Intercept (b)</td>
<td>0.0066</td>
<td>0.0034</td>
</tr>
<tr>
<td>7</td>
<td>Standard deviation on slope ((S_m))</td>
<td>4.5644×10(^{-4})</td>
<td>7.0238×10(^{-3})</td>
</tr>
<tr>
<td>8</td>
<td>Standard deviation on intercept ((S_b))</td>
<td>7.7459×10(^{-5})</td>
<td>4.6590×10(^{-5})</td>
</tr>
<tr>
<td>9</td>
<td>Standard error of estimate ((S_e))</td>
<td>5.7735×10(^{-3})</td>
<td>4.4422×10(^{-3})</td>
</tr>
<tr>
<td>10</td>
<td>Correlation coefficient ((r))</td>
<td>0.9998</td>
<td>0.9988</td>
</tr>
<tr>
<td>11</td>
<td>Relative Standard Deviation ((%))*</td>
<td>0.5232</td>
<td>1.5433</td>
</tr>
<tr>
<td>12</td>
<td>% Range of error (confidence limits)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05 level</td>
<td>± 0.6495</td>
<td>± 1.9159</td>
</tr>
<tr>
<td></td>
<td>0.01 level</td>
<td>± 1.0772</td>
<td>± 3.1775</td>
</tr>
<tr>
<td>13</td>
<td>Limit of Detection</td>
<td>0.5507</td>
<td>5.5908</td>
</tr>
<tr>
<td>14</td>
<td>Limit of Quantification</td>
<td>1.8355</td>
<td>18.6362</td>
</tr>
</tbody>
</table>

* Mean of five determinations
### Table 7.04 (continued)

Optical, regression characteristics, precision and accuracy of proposed methods for MND

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>$M_{Si}$</th>
<th>$M_{Sm}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\lambda$ max, nm</td>
<td>480</td>
<td>410</td>
</tr>
<tr>
<td>2</td>
<td>Color of the chromogen</td>
<td>Orange Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td>3</td>
<td>Beer’s law limits (µg/ml)</td>
<td>6.0 – 30.0</td>
<td>10.0 – 60.0</td>
</tr>
<tr>
<td>4</td>
<td>Molar absorptivity (1 mol$^{-1}$ cm$^{-1}$)</td>
<td>$1.8298\times10^3$</td>
<td>$1.2073\times10^3$</td>
</tr>
<tr>
<td>5</td>
<td>Sandell’s sensitivity (µg cm$^{-2}$)</td>
<td>0.3736</td>
<td>0.5662</td>
</tr>
<tr>
<td>6</td>
<td>Regression equation ($Y= mX+b$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slope (m)</td>
<td>0.0338</td>
<td>0.0108</td>
</tr>
<tr>
<td></td>
<td>Intercept (b)</td>
<td>0.0027</td>
<td>0.0142</td>
</tr>
<tr>
<td>7</td>
<td>Standard deviation on slope (Sm)</td>
<td>$8.0188\times10^{-4}$</td>
<td>$1.7519\times10^{-4}$</td>
</tr>
<tr>
<td>8</td>
<td>Standard deviation on intercept (Sb)</td>
<td>$1.5957\times10^{-2}$</td>
<td>$6.8227\times10^{-3}$</td>
</tr>
<tr>
<td>9</td>
<td>Standard error of estimate (Se)</td>
<td>$1.5215\times10^{-2}$</td>
<td>$7.3287\times10^{-3}$</td>
</tr>
<tr>
<td>10</td>
<td>Correlation coefficient (r)</td>
<td>0.9992</td>
<td>0.9995</td>
</tr>
<tr>
<td>11</td>
<td>Relative Standard Deviation (%)*</td>
<td>0.9462</td>
<td>0.5068</td>
</tr>
<tr>
<td>12</td>
<td>% Range of error (confidence limits)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05 level</td>
<td>± 1.1747</td>
<td>± 0.6292</td>
</tr>
<tr>
<td></td>
<td>0.01 level</td>
<td>± 1.9482</td>
<td>± 1.0434</td>
</tr>
<tr>
<td>13</td>
<td>Limit of Detection</td>
<td>1.4163</td>
<td>1.8952</td>
</tr>
<tr>
<td>14</td>
<td>Limit of Quantification</td>
<td>4.7211</td>
<td>6.3173</td>
</tr>
</tbody>
</table>

* Mean of five determinations
Table 7.05
Assay and recovery of MND in synthetic mixtures

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labeled Amount (mg)</th>
<th>Proposed Methods</th>
<th>Reference method</th>
<th>% Recovery by proposed methods**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method</td>
<td>Amount found*</td>
<td>t-Value</td>
</tr>
<tr>
<td>Mixture-I</td>
<td>10</td>
<td>M$_{5d}$</td>
<td>9.96 ± 0.023</td>
<td>0.6874</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M$_{5g}$</td>
<td>9.94 ± 0.053</td>
<td>0.8949</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M$_{5i}$</td>
<td>9.96 ± 0.051</td>
<td>0.3100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M$_{5m}$</td>
<td>9.95 ± 0.028</td>
<td>1.1293</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation of five determinations. The t-test and F-test values refer to comparison of the proposed methods with the reference method. Theoretical values at 95% confidence limits, t=2.306, F=6.39.

**Mean ± standard deviation of three determinations.
Table 7.05 (continued)
Assay and recovery of MND in synthetic mixtures

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labeled Amount (mg)</th>
<th>Proposed Methods</th>
<th>Reference method</th>
<th>% Recovery by proposed methods**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method</td>
<td>Amount found*</td>
<td>t-Value</td>
</tr>
<tr>
<td>Mixture-II</td>
<td>10</td>
<td>M5d</td>
<td>9.90 ± 0.077</td>
<td>0.1673</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M5g</td>
<td>9.90 ± 0.072</td>
<td>0.4392</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M5i</td>
<td>9.91 ± 0.047</td>
<td>1.0092</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M5m</td>
<td>9.92 ± 0.055</td>
<td>1.1499</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation of five determinations. The t-test and F-test values refer to comparison of the proposed methods with the reference method. Theoretical values at 95% confidence limits, t=2.306, F=6.39.

**Mean ± standard deviation of three determinations.
Discussion:

The characteristics of the proposed methods summarized in table 7.04 clearly indicate that the reagents $K_3\text{Fe(CN)}_6/\text{FeCl}_3$, $\text{NaNO}_2/\text{HCl}$, $\text{NaNO}_2/\text{HCl}/\text{resorcinol}$ and $\text{VN}$, can be successfully employed for the determination of Manidipine.

The molar absorptivity of the colored species shows that the sensitivity of the proposed methods is good. The above inference is substantiated by low values of Sandell’s sensitivity. Correlation coefficients are very close to unity. This shows that the absorbance depends on the concentration of the drug. The low standard deviation and high percent recovery values show that precision and accuracy of the methods are good. The recovery experiments (table 7.05) are more precise and therefore these methods can be applied for the determination of MND in bulk and pharmaceutical dosage forms in any laboratory.

Part-II

High Performance Liquid Chromatography

For the determination of MND in biological fluids only a few chromatographic methods$^5$-$^7$ have been reported in the literature. In the present investigation, the author has successfully developed a sensitive and precise HPLC method for the determination of MND in bulk and synthetic mixtures by using kromosil C18 column as stationary phase and acetonitrile, methanol, tetrahydrofuran in proportion of 90:05:05 (v/v) as mobile phase without use of internal standard (table 7.06 ).
Table 7.06
Chromatographic conditions for MND (M₅₃)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stationary Phase</th>
<th>Mobile Phase</th>
<th>Flow Rate (ml/min.)</th>
<th>Run Time (min.)</th>
<th>Column Temperature (°C)</th>
<th>Volume of injection loop (µl)</th>
<th>Detection Wave length (nm)</th>
<th>Retention Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MND (M₅₃)</td>
<td>C18 Kromosil</td>
<td>ACN + Methanol + THF</td>
<td>1.0</td>
<td>10</td>
<td>Ambient</td>
<td>20</td>
<td>239</td>
<td>3.74</td>
</tr>
</tbody>
</table>
Results:

The optimum chromatographic conditions such as stationary phase, mobile phase, flow rate, column temperature, detection wave length and retention time of proposed method are reported in table 7.06.

The system suitability parameters like number of theoretical plates, height equivalent to theoretical plate, linearity range, resolution, peak asymmetry, limit of detection and limit of quantification have been calculated using the observed readings and these results are presented in table 7.07. Results pertaining to the determination of MND in synthetic mixtures are summarized in table 7.08.

The precision of the method was ascertained from the peak area of MND obtained by the determination of five replicates of fixed amount of MND and results are presented in table 7.07. The accuracy of this method was ascertained by comparing the results obtained with the proposed method and the reference method in case of synthetic mixtures and are reported in table 7.08.

The chromatogram of MND (M5) was recorded and presented in fig. 7.15. The linear fit of the system is illustrated graphically in fig. 7.16. The results relating to reference method are presented graphically in figs. 7.17 and 7.18.
Table 7.07

System suitability parameters and precision of the proposed method (M₁₅) for MND

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>MND (M₁₅)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wave length (nm)</td>
<td>239</td>
</tr>
<tr>
<td>2</td>
<td>Retention Time (t), (min.)</td>
<td>3.75</td>
</tr>
<tr>
<td>3</td>
<td>Theoretical plates (n)</td>
<td>11520</td>
</tr>
<tr>
<td>4</td>
<td>Plates per meter (N)</td>
<td>46080</td>
</tr>
<tr>
<td>5</td>
<td>Height Equivalent to Theoretical Plate (HETP), (mm)</td>
<td>2.1701×10⁻⁵</td>
</tr>
<tr>
<td>6</td>
<td>Peak Asymmetry</td>
<td>1.16</td>
</tr>
<tr>
<td>7</td>
<td>Resolution factor</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Linearity range (µg/ml)</td>
<td>30.0 – 70.0</td>
</tr>
<tr>
<td>9</td>
<td>Regression equation (Y=mX+b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slope (m)</td>
<td>1563.14</td>
</tr>
<tr>
<td></td>
<td>Intercept (b)</td>
<td>602.6</td>
</tr>
<tr>
<td>10</td>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
</tr>
<tr>
<td>11</td>
<td>Relative Standard Deviation (%) *</td>
<td>1.4217</td>
</tr>
<tr>
<td>12</td>
<td>Percent range of error (confidence limits)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05 level</td>
<td>± 1.7650</td>
</tr>
<tr>
<td></td>
<td>0.01 level</td>
<td>± 2.9273</td>
</tr>
<tr>
<td>13</td>
<td>Limit of Detection</td>
<td>1.1276</td>
</tr>
<tr>
<td>14</td>
<td>Limit of Quantification</td>
<td>3.7586</td>
</tr>
</tbody>
</table>

*Mean of five determinations
Table 7.08
Assay and recovery of MND in synthetic mixtures

<table>
<thead>
<tr>
<th>Method</th>
<th>Formulation</th>
<th>Labeled Amount (mg)</th>
<th>Proposed Method</th>
<th>Reference method</th>
<th>% Recovery by proposed method**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amount found*</td>
<td>t-Value</td>
<td>F-Value</td>
</tr>
<tr>
<td>M&lt;sub&gt;5t&lt;/sub&gt;</td>
<td>Mixture-I</td>
<td>10</td>
<td>9.95 ± 0.031</td>
<td>1.8244</td>
<td>1.4215</td>
</tr>
<tr>
<td></td>
<td>Mixture-II</td>
<td>10</td>
<td>9.89 ± 0.030</td>
<td>1.0541</td>
<td>1.9600</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation of five determinations. The t-test and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limits, t=2.306, F=6.39.

**Mean ± standard deviation of three determinations.
Discussion:

This method is based on the use of a C18 column with a suitable mobile phase without use of internal standard. It can be seen from the results (table 7.07) that the proposed method has good sensitivity. Statistical analysis of the results shows that the proposed procedure has good precision and accuracy. The analysis of synthetic mixtures (table 7.08) reveals that the proposed method is suitable for their analysis with virtually no interference of the usual additives present in pharmaceutical formulations.
Fig. 7.01 Absorption spectrum of MND with $K_3Fe(CN)_6/FeCl_3$ system ($M_{5d}$)

Fig. 7.02 Absorption spectrum of MND with $NaNO_2/HCl$ system ($M_{5g}$)
Fig. 7.03 Absorption spectrum of MND with NaNO₂/HCl/Resorsinol system (M₅ᵢ)

Fig. 7.04 Absorption spectrum of MND with VN (M₅₉₇)
Fig. 7.05 Beer's law plot of MND with $K_3\text{Fe(CN)}_6/\text{FeCl}_3$ system ($M_{5d}$)

Absorbance

Concentration ($\mu$g/ml)

0 5 10 15 20 25 30

0 0.2 0.4 0.6 0.8 1 1.2

Fig. 7.06 Beer's law plot of MND with NaNO$_2$/HCl system ($M_{5g}$)

Absorbance

Concentration ($\mu$g/ml)

0 20 40 60 80 100 120

0 0.05 0.1 0.15 0.2 0.25 0.3
Fig. 7.07 Beer's law plot of MND with NaNO₂/HCl/Resorsinol system (M₅₁)

Fig. 7.08 Beer's law plot of MND with VN (M₅₆)
Fig. 7.09 Ringbom plot of MND with $K_3\text{Fe(CN)}_6/\text{FeCl}_3$ system ($M_{5d}$)

Fig. 7.10 Ringbom plot of MND with $\text{NaNO}_2/\text{HCl}$ system ($M_{5g}$)
Fig. 7.11 Ringbom plot of MND with NaNO₂/HCl/Resorsinol system ($M_{S_1}$)

Fig. 7.12 Ringbom plot of MND with VN ($M_{S_m}$)
Fig. 7.13 Absorption spectrum of MND in methanol medium (UV reference method)

Fig. 7.14 Beer's law plot of MND in methanol medium (UV reference method)
Fig. 7.15 The chromatogram of MND (M_{5t})

Fig. 7.16 Linear fit of MND chromatographic system (M_{5t})
Fig. 7.17 Absorption spectrum of MND in methanol medium (UV reference method)

Fig. 7.18 Beer's law plot of MND in methanol medium (UV reference method)
Reduced Manidipine + Fe$^{+3}$ → Fe$^{+2}$

Fe$^{+2}$ + [Fe(CN)$_6$]$^{3-}$ → Fe$_3$ [Fe(CN)$_6$]$_2$

Colored Complex

Scheme 7.01 Reaction of MND with K$_3$Fe(CN)$_6$ / FeCl$_3$ system (M$_{5d}$)
Scheme 7.02 Reaction of MND with NaNO₂ / HCl system (M₅₈)
Scheme 7.03 Reaction of MND with NaNO₂ / HCl / Resorcinol system (M₅ᵢ)
Scheme 7.04 Reaction of MND with VN
References: