Estimation of melitracen HCl in single dosage form by RP-HPLC method

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ABSTRACT
A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the estimation Melitracen Hydrochloride in pharmaceutical dosage forms. The method was carried out on a Eurospher C$_{18}$ (250 cm×4 mm) column with precolumn and a mobile phase consisting of Acetonitrile:Water(pH 3.0) (50:50 v/v) at a flow rate of 1 ml/min. Detection was carried out at 229nm. Was used as an internal standard. The retention time of Melitracen HCl was found out to be 4.717. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantization and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Keywords: Estimation , Melitracen HCl , RP-HPLC method

INTRODUCTION
Melitracen HCl is a white to off White Powder. Amorphous in nature, sensitive to light and moisture. Chemically it 3-(10,10-dimethyl anthracen-9-ylidene)-N,N-dimethylpropan-1-amine. It is a Tricyclic Antidepressant. And work by inhibiting the re-uptake of the neurotransmitters norepinephrine and serotonin by neurons. Tricyclics may also possess an affinity for muscarinic and histamine H1 receptors to varying degrees. Although the pharmacologic effect occurs immediately, often the patient’s symptoms do not respond for 2 to 4 weeks used in treatment of Trigeminal Neuralgia and severe depression state. However, there is no HPLC method reported for the simultaneous estimation of these drugs in single as well as combined dosage form. Fixed dose containing Melitracen (0.5 mg is available in the tablet form in the market. The aim of this work was to develop an RP-HPLC method with ultraviolet detection for estimation of Melitracen HCl in pharmaceutical dosage form. The present RP-HPLC method was validated following the ICH guidelines.

Experimental Procedure
Methods:
1. Determination of \( \lambda_{\text{max}} \) of MEL:
The stock standard solutions of MEL were prepared by dissolving ~ 50.0 mg of drug in 100.0 ml of selected mobile phase Acetonitrile:Water(50:50v/v). \( \pH \) 3.0. The aliquot portions of stock standard solution were diluted appropriately to obtain a concentration of 50µg/ml of both MEL. The \( \lambda_{\text{max}} \) was determined on Shimadzu UV-Visible spectrophotometer (Model UV-2409) in the range 200-400 nm using mobile phase as blank.

2. Selection of mobile phase:
a) Preparation of standard solutions:
MEL standard solution: Accurately weighed quantity ~ 50.0 mg of MEL and diluted to 100.0 ml with mobile phase and 5.0 ml solution was pipetted out and the solution was further diluted to 50.0 ml with water.

b) Procedure:
The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing MEL was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each mobile phase was filtered through Whatman filter paper No.42.

Various mobile phases tried for the HPLC study are as follows

ACETONITRILE AND WATER TRIED AT DIFFERENT CONCENTRATIONS:

- Acetonitrile:Water(50:50v/v) \( \pH \) 3.0
- Acetonitrile:Water(60:40v/v) \( \pH \) 3.0
- Acetonitrile:Water(40:60v/v) \( \pH \) 3.0

From various mobile phases tried, mobile phase containing Acetonitrile: Water (50:50v/v) \( \pH \) 3.0 was selected, since it gives sharp
peak, well resolved peaks with symmetry within limits and significant reproducible retention time for MEL.

Chromatographic conditions:
The following chromatographic conditions were established by trial and error and were kept constant throughout method.
- Column: Eurospher 100-5 C18
- Dimension: 250x4 mm with precolumn
- Practical size Phases: 10µm
- Stationary Phases: C18 intersil
- Mobile Phases: Acetonitrile:Water(50:50v/v) pH 3.0
- Detection Wavelength: 229nm
- Flow Rate: 1ml/min
- Temperature: Ambient
- Sample Size: 50µL

3. Preparation of Calibration Curve:
   i) Standard solutions:
   - MEL standard stock solution:
     Accurately weighed quantity ~ 50.0 mg of MEL and diluted to 100.0 ml with mobile phase and 5.0 ml solution was pipetted out and the solution was further diluted to 50.0 ml with water.

   ii) Procedure:
     The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The various concentration from 10-100 µg/ml of drug solution were injected and peak area was obtained and was recorded.

4. System suitability test:
   System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standards solutions.

   A) Preparation of standard drug solution:
   - MEL standard solution:
     Accurately weighed quantity ~ 50.0 mg of MEL and diluted to 100.0 ml with mobile phase and volume was made up to the mark. Standard stock solution was diluted further with mobile phase to get final concentration 50µg/ml.

   B) Procedure:
     The previously filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. A 20µL std. drug solution was injected which was made in five replicates.

6. Application of Proposed method for estimation of MEL:
   Preparation of standard solution:
   Accurately weighed quantity of MEL ~ 50.0 mg was transferred to 100.0 ml volumetric flask, shaken vigorously for five minutes and volume was made up to the mark. The stock standard solution of MEL were mixed and diluted properly to obtain solutions of concentration 50µg/ml of MEL.

   Preparation of sample solution:
   Five different solutions of MEL were prepared by appropriately weighing the quantities of drug sample so as to get the concentrations of 50µg/ml. The peak area of standard laboratory solution and sample laboratory solutions was compared to obtain the concentration.

   The amount of each drug estimated in solution was calculated using following formula:

   \[
   \% \text{Estimation} = \left( \frac{A_t \times D_s \times W_s}{A_s \times D_t \times W_t} \right) \times 100
   \]

   At = Area count for sample solution.
   As = Area count for standard solution.
   Ds = Dilution factor for sample.
   Dt = Dilution factor for standard.
   Ws = Weight of standard (mg)
   Wt = Weight of sample (mg)

7. Application of proposed method for estimation of MEL in tablet formulation:
   Standard stock solution:
   Accurately weighed quantity of MEL ~ 50.0 mg was dissolved separately in 100.0 ml mobile phase. The stock solution of drug was mixed and further dilution was done appropriately with mobile phase to get concentration of 50µg/ml of MEL.

   Sample solution preparation:
   Twenty tablets were weighed and content emptied. The average weight determined. It was finely powdered and mixed thoroughly. Accurately weighed tablet powder ~ 50.0 mg of MEL was transferred in a 100.0 ml volumetric flask and mobile phase was added. It was shaken vigorously for 5 to 10 minutes. Later the volume was made up to mark with mobile phase. The solution was filtered through Whatman filter paper No.42. Further dilution was done with mobile phase to get concentration of 50µg/ml of MEL.
Procedure:

Equal volume (20µL) of standard and sample solution were injected separately equilibrium of stationary phase. The chromatograms were recorded and the response i.e., peak area of major peaks were measured. The content of MEL was calculated by comparing a sample peak with that of standard.

Amount of drug in tablet was calculated using formula-

\[
\% \text{ Label Claim} = \frac{At \times Ds \times Ws \times A \times 100}{As \times Dt \times Wt \times Lc}
\]

At = Area count for sample solution.
As = Area count for standard solution.
Ds = Dilution factor for sample.
Dt = Dilution factor for standard.
Ws = Weight of standard (mg)
Wt = Weight of sample (mg)
Lc = Label claim
A = Average weight

8. Validation parameters:

a) Accuracy:

It was ascertained on the basis of recovery studies performed by standard addition method.

Preparation of standard solution:

An accurately weighed quantity of preanalysed tablet powder ~ 50.0 mg of MEL was taken in 100.0 ml volumetric flask; to it standard solution of MEL was added in different proportions. Then volume was adjusted up to the mark with the solvent. Solution was then filtered through Whatman No. 42. The aliquot portion of the filtrate was diluted to get final concentration. The amount of drug contributed by tablet powder was deduced from the total amount of respective drugs estimated and the resultant quantities were assumed to be recovered from the added pure drugs. The content of drug was calculated using same formula as in the marketed formulation.

The %Recovery was then calculated by using formula-

\[
\% \text{ Recovery} = \frac{T-C \times 100}{P}
\]

Where,

T= total drug estimated
C=drug contributed by preanalysed powder
P=weight of pure drug added

b) Precision:

Precision of analytical method is expressed as S.D. or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

c) Ruggedness:

The studies of ruggedness were carried out under two different condition-

1) Days
2) Analyst

RESULT AND DISCUSSION

HPLC has gained valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature.

This technique is commonly used for the quantitative estimation of the drug from their formulation as well as for studying their metabolites of drugs and their estimation in their biological fluids. This method offers advantages of estimating the constituents for the multi component system without prior separation and even nano quantities can be estimated.

This technique was employed in the present investigation for estimation of Melitracen HCl in Tablet dosage form. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish HPLC method, the parameters studies are discussed in the following paragraph.

HPLC (Knauer) system with Eurospher 100-5 C_{18} (4x 250mm) column with Pre-column and UV2401 detector was used for the study. The standard and sample solution of MEL was prepared in mobile phase. Different pure samples of varying polarity (viz.Methanol, Ac-
observed that the retention of the analyte is decreased with increased in the proportion of organic modifier like acetonitrile in the mobile phase. The sharpness of the peak is achieved with increasing the proportion of acetonitrile whereas the increased proportion of aqueous resulted in broadening of the peak. The mobile phase pH across

During selection and optimization of the mobile phase it was

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### Table No.1: Result and statistical data for estimation of MEL in Laboratory Solution.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Weight of Std. (gm)</th>
<th>Weight of Sample</th>
<th>Peak area of Std. MEL</th>
<th>Peak area of sample MEL</th>
<th>% Drug estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.0501</td>
<td>0.0502</td>
<td>3660.340</td>
<td>3658.414</td>
<td>99.72</td>
</tr>
<tr>
<td>2.</td>
<td>0.0502</td>
<td>3659.414</td>
<td>100.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>0.0504</td>
<td>3659.814</td>
<td>99.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>0.0500</td>
<td>3659.819</td>
<td>99.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>0.0501</td>
<td>3659.819</td>
<td>99.78</td>
<td>Mean 99.85</td>
<td>±S.D. 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.V. 0.20</td>
<td></td>
</tr>
</tbody>
</table>

### Table No.2: Result and statistical data for estimation of MEL in Marketed Formulation.

#### Sample solution (marketed study)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Weight of Std. (gm)</th>
<th>Weight of Sample</th>
<th>Peak area of Std. MEL</th>
<th>Peak area of sample MEL</th>
<th>% Drug estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.0501</td>
<td>0.0502</td>
<td>3660.340</td>
<td>3659.890</td>
<td>99.84</td>
</tr>
<tr>
<td>2.</td>
<td>0.0502</td>
<td>3659.891</td>
<td>99.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>0.0504</td>
<td>3658.924</td>
<td>98.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>0.0500</td>
<td>3659.898</td>
<td>99.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>0.0501</td>
<td>3660.300</td>
<td>100.12</td>
<td>Mean 99.93</td>
<td>±S.D. 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.V. 0.11</td>
<td></td>
</tr>
</tbody>
</table>

### Table No.3: Result and statistical data for estimation of MEL for Recovery Study

#### Recovery Study

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Weight of Tablet (gm)</th>
<th>Peak area of Std. MEL</th>
<th>Amount of Pure drug added (µg/ml)</th>
<th>Peak area of sample MEL</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.0503</td>
<td>6867.470</td>
<td>0.0502</td>
<td>6866.898</td>
<td>98.84</td>
</tr>
<tr>
<td>2.</td>
<td>0.0505</td>
<td>6866.902</td>
<td>0.0502</td>
<td>6867.466</td>
<td>99.97</td>
</tr>
<tr>
<td>3.</td>
<td>0.0507</td>
<td>6866.902</td>
<td>0.0504</td>
<td>6867.469</td>
<td>99.97</td>
</tr>
<tr>
<td>4.</td>
<td>0.0509</td>
<td>6867.469</td>
<td>0.0500</td>
<td>Mean 99.63</td>
<td>±S.D. 0.6272</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.V. 0.62</td>
<td></td>
</tr>
</tbody>
</table>

### Table No.4: Result and statistical data for estimation of MEL for Interday study

#### Interday:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Weight of Tablet (gm)</th>
<th>Peak area of Std. MEL</th>
<th>Peak area of sample MEL</th>
<th>% Label Claim*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.0505</td>
<td>3660.340</td>
<td>3660.346</td>
<td>100.06</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>3660.298</td>
<td>99.85</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td>3660.312</td>
<td>99.97</td>
<td>Mean 99.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±S.D. 0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.V. 0.10</td>
</tr>
</tbody>
</table>

*Results are mean of three replicates

### Table No.5: Result and statistical data for estimation of MEL for Intraday Study

#### Intraday:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Weight of Tablet (gm) powder</th>
<th>Peak area of Std. MEL</th>
<th>Peak area of sample MEL</th>
<th>% Label Claim*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.0505</td>
<td>3660.340</td>
<td>3660.347</td>
<td>100.06</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>3660.306</td>
<td>99.75</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td>3660.339</td>
<td>99.97</td>
<td>Mean 99.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±S.D. 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C.V. 0.15</td>
</tr>
</tbody>
</table>

*Results are mean of three replicates

Etionitrile and Water) and buffers (viz Phosphate buffer) in different proportions were tried as mobile phase for development of the chromatogram.

During selection and optimization of the mobile phase it was
Table No.6: Result and statistical data for estimation of MEL for Different Analyst Study.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Weight of Tablet(gm) powder</th>
<th>Peak area of Std. MEL</th>
<th>Peak area of sample MEL</th>
<th>% Label Claim*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.0505</td>
<td>3660.340</td>
<td>3660.348</td>
<td>100.06</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>3660.326</td>
<td>99.87</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td>3660.339</td>
<td>99.93</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>99.95</td>
<td></td>
</tr>
<tr>
<td>±S.D.</td>
<td></td>
<td></td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>C.V.</td>
<td></td>
<td></td>
<td>0.65</td>
<td></td>
</tr>
</tbody>
</table>

*Results are mean of three replicates

Figure 1: Standard Solution for Flupenthixol HCl

Figure 2: Sample Solution for Flupenthixol HCl

Figure 3: Recovery study for Flupenthixol HCl

Figure 4: Linearity and Range for MEL

The mobile phase that was found to be most suitable was Acetonitrile: water (60:40 v/v) pH 3.0 The wavelength 229nm was selected for the evaluation of the chromatogram of both drugs. The selection of the wavelength was based on the ?max obtained by scanning of standard laboratory mixture in mobile phase. This system gave good resolution and optimum retention time with appropriate tailing factor <2).

After establishing the chromatographic conditions, standard laboratory mixture prepared and analyzed by following procedure described under experimental and results. It gave accurate, reliable results and therefore was extended for estimation of drugs in market tablet formulation.

The above results clearly indicate that HPLC technique can be successfully applied for the estimation of above-mentioned drugs in single dosage formulation.

Validation:

Validation of these methods was performed as per the ICH guidelines for these following parameters-

Accuracy:

Accuracy of the proposed method was ascertained from the recovery studies by standard addition method.

Precision:

Replicate estimation of tablet analysed by the proposed method has yielded quite consistent result indicating repeatability of method. Study showed ±S.D. <2.
Specificity:

Studies shows that there is no interference of peak from the component of matrix showing retention time for MEL 4.17min.

Ruggedness:

Studies were carried out only for the two different parameters like different time, different days and different analyst. Result of estimation by proposed method are very much similar under variety of conditions. This study signifies the ruggedness of the method under varying condition of its performance.

Linearity and Range- MEL in marketed formulation was found to be linear in the range of 80% to 120 % of test concentration with R$^2$ ~ 1 at selected wavelength fm hath the methods. Same procedure as described in USP was followed.

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REFERENCES:


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