A new, rapid and sensitive reverse phase HPLC method was developed and validated for the determination of benzhexol hydrochloride in tablet formulations and urine. The mobile phase used is acetonitrile and water, (50 % v/v) and the pH was adjusted to 5 using phosphoric acid. The separation was achieved on C18 reversed-phase column (250 mm x 4 mm i.d.). The flow rate was 0.6 ml/min and UV detection is at 254nm. The retention time for benzhexol hydrochloride was 7.4 min. The calibration curve was linear up to 40 µg/mL. The mean recovery for benzhexol hydrochloride is 101.16. The assay was precise within day and between days. The method provided excellent sensitivity, recovery, accuracy and reproducibility in therapeutic or toxic concentrations. Common excipients do not interfere.

**Keywords**: Benzhexol hydrochloride, Reverse phase HPLC and C18 reversed-phase column.

### Introduction

Benzhexol stops salivation and reduces skeletal muscle tone and rigidity in parkinsonian syndrome in which condition it is used to produce symptomatic relief and improvement of patients condition [1]. Chemically, Benzhexol hydrochloride is 1-cyclohexyl-1-phenyl-3-piperidinopropan-1-ol hydrochloride, which has molecular formula C\textsubscript{20}H\textsubscript{31}NO, HCl and molecular weight 337.9. It is a white or yellowish white crystalline powder of melting point about 250 ºC. It is slightly soluble in water, sparingly soluble in alcohol and in dichloromethane, a 1% solution in water has a pH of 5.2 to 6.2 [2].

Several high performance liquid chromatographic (HPLC) methods have been reported for the determination of benzhexol hydrochloride [3,4] and its major metabolites. The present paper focuses on the use of an HPLC procedure based on a fast isocratic elution.

**Experimental**
Chemicals

Benzhexol hydrochloride (raw material) and Parkinol tablets (5 mg/tab) provided from El-nile company (provided from El-nile company, Egypt), phosphoric acid were purchased from Merck and acetonitrile HPLC Grade, methanol and chloroform were provided from Sigma-Aldrich. Human urine was obtained from Medico-Legal Organization (Egypt).

Instrumentation and chromatographic procedure

A Hewlett-Packard “HP-1050 HPLC” instrument equipped with UV detector was used for determination of benzhexol. An octadecyl silica “Li Chrosper 100 RP 18” column with 5 µm particle size and ID of 250 mm x 4 mm was used. The pH adjustment was carried out using Jenway pH-meter. The mobile phase of acetonitrile and water, (50 % v/v) was adjust to pH 5 using phosphoric acid.

Sample preparation

Standard solutions

Ten tablets were weighted and the average tablet weight determined. The tablets were finely powdered and a portion of powder equivalent to one average tablet weight was weighted and quantitatively transferred into a 50 ml volumetric flask. 25 ml of methanol were added and the dispersion was shaken for 20 minutes. Ultrasonication followed for another 20 minutes and then the solution was diluted to volume with methanol shaked well and left to precipitate. Appropriate dilutions were made from the clear supernatant solution with acetonitrile so that in the middle of the standard solution range [5]. These solutions were stored at 4°C. Urine standard samples were prepared by dilution of the stock solutions with drug free urine.

Extraction

A 0.5 mL urine (containing benzhexol in concentration 0.5-40 µg/mL) in glass tube was mixed with 5 mL chloroform. The tube was capped tightly. After vertical agitation for 2 min and centrifugation at 5000 rpm for 10 min, the lower organic phase was transferred to a clean conical tube and evaporated. The residue was reconstituted by adding 50 µL of the mobile phase [6]. A total of 20 µL was injected into the chromatographic system.

Results and discussion
Fig. 1 shows a typical chromatogram obtained following analysis of benzhexol in tablets. Sharp and symmetrical peak was obtained with minimal tailing, thus facilitating accurate measurement of the peak area ratio. No interfering peaks were found in the chromatogram due to tablet excipients.

![Chromatogram of benzhexol (1 µg/mL)](image)

*to = 2.1 min

where to is Void time (can be interpreted as part of the total analyte retention time that the analyte actually spends in the mobile phase moving through the column, and for the rest of the retention time the analyte sits on the stationary phase surface).

**Linearity**

Detector response linearity was performed by preparing five triplicate calibration samples (0.5-40 µg/mL) covering the range between therapeutic and toxic concentrations [7]. Calibration curves were obtained.
Fig. 2. standard calibration curve of benzhexol

Table 1: Calibration data for benzhexol (n=6)

<table>
<thead>
<tr>
<th>Range (µg/mL)</th>
<th>Coefficient of correlation r</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5-40 µg/mL</td>
<td>0.999</td>
<td>6.009</td>
<td>0.023</td>
</tr>
</tbody>
</table>
RP HPLC DETERMINATION OF BENZHEXOL

Six replicate commercial tablets analyzed for Precision and accuracy. For the analysis of benzhexol in Parkinol tablets 5 mg, twenty tablets of each drug were weighed and finally powdered. A portion of the powder corresponding to 100 µg/mL of the drug was weighed and dissolved in methanol and the flask was mechanically shaken for 5 min. The solution was removed into a centrifuge tube and centrifuged at 2500 rpm for 5 min, Filter the solution and diluted by mobile phase. 20 µL was injected. The injection occurred from the same solution during four days.

Table 2. Analysis of variance for intra- and inter day

<table>
<thead>
<tr>
<th>Day/ Assay</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.99</td>
<td>5.02</td>
<td>5.07</td>
<td>5.05</td>
<td>4.96</td>
<td>5.03</td>
</tr>
<tr>
<td>2</td>
<td>5.01</td>
<td>5.03</td>
<td>5.05</td>
<td>5.02</td>
<td>4.95</td>
<td>5.04</td>
</tr>
<tr>
<td>3</td>
<td>5.03</td>
<td>5.02</td>
<td>4.97</td>
<td>5.03</td>
<td>5.01</td>
<td>5.02</td>
</tr>
<tr>
<td>4</td>
<td>4.94</td>
<td>5.01</td>
<td>4.99</td>
<td>4.93</td>
<td>4.96</td>
<td>4.92</td>
</tr>
</tbody>
</table>

Mean = 5.002

The average recovery shown in Table 2 was 100.04 % with the coefficient of variation of 0.889 % Thus it was concluded that there was no significant difference for the assay which was tested within day and between days.

Assay detection limits

Limits of detection (LOD)

The limit of detection (LOD), defined as the lowest concentration of the analyte that can be clearly detected above the baseline signal, is estimated as three time the signal to noise ratio [8] LOD was determined as 0.02 µg/mL.

Limit of quantification (LOQ)

The LOQ was obtained by the same procedure used for LOD, but estimated as ten times the signal to noise ratio. LOQ values were determined as 0.06 µg/mL.

Ruggedness

The method ruggedness was tested by varying several chromatographic parameters and studying the effect on column efficiency (represented by the number of theoretical plates N).
Mobile phase composition

Varying the acetonitrile percent ( % ACN ) from 0 to 100 % to choose the better composition which give better column efficiency. The results showed that the composition 50 % acetonitrile to 50 % water gave the more column efficiency as in Fig.3. Where also varying the acetonitrile percent from 50 to 55 % did not significantly alter column efficiency. N is the number of the theoretical plates in the column.

![Graph showing the effect of variation of acetonitrile composition on column efficiency]

**Fig.3. Effect of the variation of acetonitrile composition on column efficiency**

Mobile phase pH

Varying the mobile phase pH between 3 to 7 to obtain better column efficiency. The results showed that at pH 5 column efficiency be higher and from pH 5 to pH 3 no significantly alter column efficiency, Fig. 4.
Flow rate

Variation of the flow rate from 0.3 to 1 mL/min, showed that column efficiency decreased when the flow rate increased. As the flow rate increased the retention time decreased so column efficiency decreased. However, 0.6 mL/min seems to be a good compromise when considering the chromatographic system and solvent economy.
Temperature

Varying the temperature between 10 and 50 °C significantly altered column efficiency Fig. 6. Therefore a controlled temperature of 30 °C was chosen.

Application
Four replicate determinations at different concentration ranges, were carried out for spiked urine. The recovery values almost reach 100%, revealing a high accuracy of the result (Table 3). The mean values obtained and the calculated standard deviations are compared with those obtained by the pharmacopoeia method [9], by applying the t-test and F-value [10] (Table 3). Such comparison showed that there is no significance difference, at proposed and the pharmacopoeia methods. The present methods are accurate, precise, highly sensitive, rapid, and simple in technique and in good agreement with the pharmacopoeia method.

Table 3. Chromatographic determinations of benzhexol in spiked urine samples

<table>
<thead>
<tr>
<th>Added (µg/mL)</th>
<th>found (µg/mL)</th>
<th>S.D.</th>
<th>Recovery (%) ± R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9.50</td>
<td>0.06</td>
<td>95.00 ± 1.90</td>
</tr>
<tr>
<td>20</td>
<td>19.3</td>
<td>0.13</td>
<td>96.50 ± 3.61</td>
</tr>
<tr>
<td>30</td>
<td>28.5</td>
<td>0.07</td>
<td>95.00 ± 4.50</td>
</tr>
</tbody>
</table>

Conclusion

This HPLC procedure appears rapid, simple, and suitable for routine analysis. Satisfactory validation data were collected for linearity, precision, recovery and ruggedness, LOQ values allowed to measure therapeutic and toxic concentrations. Its results were in agreement with those of reference methods.

References

7. D. M. Bliesner, Validating chromatographic methods, 2006