QUANTIFICATION OF TESTOSTERONE LEVELS IN DIFFERENT AGE PEOPLE BY H.P.L.C

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ABSTRACT

Testosterone is a sexual hormone. In order to identification of age factor on Testosterone releasing has estimated. Analysis has performed for human plasma samples by using the HPLC analysis and concluded that at the age of 25 years testosterone releasing is very high than other age group people.

Key words: Testosterone, HPLC, And AGE: 25, 35, 45, Plasma
INTRODUCTION

Testosterone is a steroid hormone from the androgen group and is found in mammals, reptiles,[1] birds,[2] and other vertebrates. In mammals, testosterone is primarily secreted in the testes of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid.

In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair.[3] In addition, testosterone is essential for health and well-being[4] as well as the prevention of osteoporosis.[5] On average, an adult human male body produces about ten times more testosterone than an adult human female body, but females are more sensitive to the hormone.[6] Testosterone is observed in most vertebrates. Fish make a slightly different form called 11-ketotestosterone.[7] Its counterpart in insects is ecdysone.[8] These ubiquitous steroids suggest that sex hormones have an ancient evolutionary history.[9]

![Structure of Testosterone](image)

**Figure 1** Structure of Testosterone

IUPAC name of Testosterone is \((8R,9S,10R,13S,14S,17S)\)-17-hydroxy-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17- \(\text{dodecahydrocyclopenta[a]phenanthren-3-one}\), Formula is \(\text{C}_{19}\text{H}_{28}\text{O}_{2}\) Mol. mass is 288.42

METHODS AND MATERIALS

All samples are collected from local villages from different age, different places. All chemicals i.e. water, methanol, Acetonitriel, Formic acid are purchased from Merck. From plasma Testosterone was extracted by SPE method. Solid Phase Extraction (SPE) procedure The solvent volumes shown below are for a 30 mg bed mass. The solvent volumes will need to be adjusted for a smaller or larger bed mass.

**Final Prep and Analysis:** Liquid Chromatography Analysis was performed using an HP 1100 LC system from Agilent, equipped with a gradient pump, in-line degasser, multi-wavelength detector. PEAK software was used to analyze the data. The HPLC column used was a Gemini 5 um, C18, 50 x 2.0 mm.
**Table.1** Conditions for sample preparation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Load:</th>
<th>Elute</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol 400 µL at flow 1 mL/min; Water 400 µL at flow 1 mL/min</td>
<td>500 µL of Human plasma was diluted with 1000 µL of water (1:2 dilution)</td>
</tr>
<tr>
<td>2</td>
<td>Water 800 µL at 1 mL/min Methanol:Water (50:50) 800 µL at 1 mL/min</td>
<td>1-2 mins at 10&quot; of Hg</td>
</tr>
</tbody>
</table>

**Table.2** Chromatographic condition

<table>
<thead>
<tr>
<th>1</th>
<th>Mobile phase</th>
<th>0.1% formic acid in water (pH 2.7, mobile phase A). 0.1% formic acid in acetonitrile (mobile phase B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Mobile phase</td>
<td>A/B 30/70</td>
</tr>
<tr>
<td>3</td>
<td>Flow rate</td>
<td>0.5 mL/min</td>
</tr>
<tr>
<td>4</td>
<td>Run time</td>
<td>1 min</td>
</tr>
<tr>
<td>5</td>
<td>Detector:</td>
<td>API 3000 LC</td>
</tr>
<tr>
<td>6</td>
<td>Column:</td>
<td>Gemini 5 µm C18 110 Å LC Column 50 x 2 mm</td>
</tr>
<tr>
<td>7</td>
<td>Inject Volume</td>
<td>5 µL</td>
</tr>
<tr>
<td>8</td>
<td>Temp</td>
<td>Ambient</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

Testosterone has monitored in plasma samples by using RP-HPLC method. Plasma samples were collected from 25, 35 and 45 years age subjects. Male samples were collected process with the proposed method and analysed with the proposed method. Results were tabulated in table-3. Higher quantity of testosterone has found in 25 years plasma samples higher than 35 and 45 years age samples.

**Table.3** Amount of Testosterone in Human Plasma

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Sex /AGE</th>
<th>Amount of Testosterone found in Blood (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male/25 Years</td>
<td>0.097, 0.084, 0.076, 0.083, 0.092</td>
</tr>
<tr>
<td>2</td>
<td>Male/35 Years</td>
<td>0.053, 0.057, 0.049, 0.055, 0.058</td>
</tr>
<tr>
<td>3</td>
<td>Male/45 Years</td>
<td>0.047, 0.049, 0.045, 0.046, 0.043</td>
</tr>
</tbody>
</table>

From above data clearly at the age of 25 Testosterone levels are very high than age 35, 45. May this difference depended on age factor, food, living style. But finally from above results the hormone releasing levels depended on age only.

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CONCLUSION

The above results reveals that, testosterone is releasing at 25 age is higher than other age period. Proposed RP-HPLC method also has applicable for the estimation of testosterone in plasma sample. The method is accurate and ruggedness.

REFERENCES


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Graph. 1 Testosterone levels in Human Plasma


