**DEVELOPMENT OF HPLC METHOD FOR DETERMINATION OF VENLAFAXINE DURING CONCOMITANT USE OF METOPROLOL**

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**Abstract.** Venlafaxine, one of the most commonly prescribed antidepressants, very often is in a displayed medicinal therapy together with the selective β₁ receptor blocker metoprolol. In this regard, the research and development of reliable analytical methods for self-determination, as well as mixtures in chemical and biological samples is essential to provide fast and reliable information to the institutions of the type of substance concentration, the presence of metabolites and other chemical characteristics. Research teams in this field work in several directions - creating analytical HPLC methods for identification, purity and assay tests, their validation and testing them to extend their application to more than one substance and determination in respect of the time. In this connection there were optimized chromatographic and analytical parameters such as retention time, resolution, column efficiency as number of theoretical plates, capacity factors, specificity, repeatability, LOD, LOQ, linearity and system suitability test.

**Introduction**

Antidepressants are included in the top ten therapeutic classes of drugs currently available on the international market. Their increased use is due to the increased incidence of depression and related illnesses as a result of urbanization and stressful lifestyle. Another important factor is a breakthrough in the treatment of depression since the discovery of inhibitors of the reuptake of mediators responsible for the clinical effects of a number of depression - serotonin, norepinephrine, dopamine, and the like. Drugs including the monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), tetracyclic antidepressants (TeCAs), selective serotonin reuptake inhibitors (SSRIs), and serotonin-norepinephrine reuptake inhibitors (SNRIs) are most commonly associated with the term.¹² These medications are among those most commonly prescribed by psychiatrists and other physicians, and their effectiveness and adverse effects are the subject of many studies and competing claims.³ Many drugs produce an antidepressant effect, but restrictions on their use have caused controversy and off-label prescription a risk, despite claims of superior efficacy. Serotonin–norepinephrine reuptake inhibitors (SNRIs) are a class of antidepressant drugs used in the treatment of major depression and other mood disorders. They are sometimes also used to treat anxiety disorders, obsessive-compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD), chronic neuropathic pain, fibromyalgia syndrome (FMS), and for the relief of menopausal symptoms.⁴

SNRIs act upon and increase the levels of two neurotransmitters in the brain that are known to play an important part in mood, these being serotonin and norepinephrine. This can be contrasted with the more widely-used selective serotonin reuptake inhibitors (SSRIs) which only act on serotonin.⁵, ⁶

Selective serotonin reuptake inhibitors or serotonin-specific reuptake inhibitor (SSRIs) are a class of compounds typically used as antidepressants in the treatment of depression, anxiety disorders, and some personality disorders.⁷, ⁸

SSRIs are believed to increase the extracellular level of the neurotransmitter serotonin by inhibiting its reuptake into the presynaptic cell, increasing the level of serotonin in the synaptic cleft available to bind to the postsynaptic receptor. They have varying degrees of selectivity for the other monoamine transporters, with pure SSRIs having only weak affinity for the noradrenaline and dopamine transporter.⁹

Venlafaxine – (RS)-1-[2-dimethylamino-1-(4-methoxyphenyl)-ethyl]cyclohexanol (fig. 1), is licensed for the treatment of major depressive disorder (MDD), as a treatment for generalized anxiety disorder, and comorbid indications in certain anxiety disorders with depression.
Venlafaxine was the sixth most commonly prescribed antidepressant on the pharmaceutical market, with about 17 million prescriptions. Venlafaxine is very often is in a displayed medicinal therapy together with Metoprolol - (RS)-1-(isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol (fig. 2) which is a selective β₁ receptor blocker used in the treatment of several diseases of the cardiovascular system, especially hypertension. The active substance metoprolol is employed either as metoprolol succinate or metoprolol tartrate (where 100 mg metoprolol tartrate corresponds to 95 mg metoprolol succinate). The tartrate is an immediate-release and the succinate is an extended-release formulation.[10-13]

**Fig. 1. Chemical structure of Venlafaxine**

In the literature there was found predominantly HPLC methods for determination of venlafaxine or metoprolol at different chromatographic conditions. [14 – 19] These methods have an object the solution of analytical problems and goals but they do not give us sufficient data about pharmacokinetic profile of venlafaxine in presence of metoprolol in respect of some factors such as time and pH.

The present study includes the development of HPLC method for studying the effects of metoprolol on the pharmacokinetic profile of venlafaxine during concomitant use. There are identified a number of chromatographic and analytical parameters according to European Pharmacopoeia and ICH specifications for validation of analytical methods

**Experimental**

**Chromatographic system:**

The chromatographic procedure was carried out using:

- Liquid chromatograph Shimadzu LC – 10 Advp equipped with 4.6 x 150 mm column RP-18, ODS with particle size 5 µm;
- Detector SPD 10 A Vvp – UV-VIS with fixed analytical wavelengths.

**Chromatographic conditions:**

Isocratic mobile phase, prepared by mixing of filtered and degassed Acetonitrile : Water 70:30 v/v respectively;

- 230 nm analytical wavelengths;
- column temperature 25°C;
- flow rate about 1.5ml/min.

**Reagents:** Acetonitril HPLC grade, Water R (Reagents (R), European Pharmacopoeia 7.0), Methanol HPLC grade, CRS venlafaxinе HCl and CRS metoprolol tartrate.

**Test solutions and reference solutions:**

Test solutions were prepared by dissolving and mixing of adequate amounts of substances in the mobile phase to obtain solutions with concentration in ratio 9 μg – 2 mg/ml.

Reference solutions were prepared by the same manner from CRS.

**Validation of HPLC method in respect of venlafaxine:**

1. Specificity
   
   Specificity in respect of reagents – “Placebo” solution containing all reagents without active substances was prepared. There no peaks in the chromatogram obtained from this solution with Rt of Venlafaxine HCl and Rt of Metoprolol tartrate.

2. Repeatability

   Six (6) equal solutions from homogenous samples containing Venlafaxine HCl and Citalopram were analyzed by HPLC method. Standard deviation (SD) in AU and relative SD (RSD) in % were found to be 1398.20 AU and +/- 3.57%.

3. Limit of detection - LOD:

   10 µg for Venlafaxine HCl, established on the base of ratio noise – signal – 1:3.

4. Limit of quantitation - LOQ:

   40 µg for Venlafaxine HCl, established on the base of ratio noise – signal – 1:10.

5. Linearity:

   The analytical parameter linearity was studied in concentration ratio 9 µg – 2 mg. The accordance between the Area of peaks, measured in absorption units (AU) and concentrations in g/ml is propor-
tional in the intervals. The correlation coefficients was found to be about 0.99148 for Venlafaxine HCl – table 1.

Table 1. Data from linearity of venlafaxine HCl.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6017.7613</td>
<td>531.96082</td>
</tr>
<tr>
<td>B</td>
<td>5.68138E6</td>
<td>430910.5837</td>
</tr>
<tr>
<td>R</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>0.99148</td>
<td>5</td>
<td>9.42645E-4</td>
</tr>
</tbody>
</table>


For system suitability test determination some chromatographic parameters such as retention time, resolution and column efficiency as number of theoretical plates and capacity factors were appointed for optimization of conditions in respect of time. The results are shown on table 2. The resolution of binary mixture between venlafaxine HCl and metoprolol at mobile phase Methanol/Acetonitrile - 70 : 30 v/v (fig. 3) is suitable for pharmacokinetic investigations, assay and purity tests. The quantity of both compounds after 40 min is in the method tolerance – under +/- 2 % in respect of Area under peaks in AU. The chromatographic profile of venlafaxine HCl is not changed in presence of metoprolol tartrate. For identification of binary mixture more acceptable is flow rate about 1.5 ml/min at the same mobile phase.

Conclusion:

The created new HPLC method for quantitative determination of venlafaxine and metoprolol - pure compounds and in drug mixture are of great significance for analysis in a Pharmaceutical and a Clinical Pharmacy practice. The methods force the resolution that simultaneously analysis of binary mixtures of drug analytes are with satisfactory results for a complete studying of their pharmacokinetic profiles.

Table 2. Results of chromatographic parameters from system suitability test from 0 min (1) and after time interval of 40 min (2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Venlafaxine HCl (1)</th>
<th>Metoprolol tartrate (1)</th>
<th>Venlafaxine HCl (2)</th>
<th>Metoprolol tartrate (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>4.89</td>
<td>4.88</td>
<td>11.07</td>
<td>10.99</td>
</tr>
<tr>
<td>Resolution</td>
<td>3.43</td>
<td>-</td>
<td>3.39</td>
<td>-</td>
</tr>
<tr>
<td>Column efficiency as N</td>
<td>226.29</td>
<td>1619.84</td>
<td>225.23</td>
<td>1596.94</td>
</tr>
<tr>
<td>Capacity factor</td>
<td>2.26</td>
<td>-</td>
<td>2.25</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 3. Chromatograms of venlafaxine and metoprolol at first (1) and after 40 min time interval (2).
Development of HPLC method for determination of ...

References:


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