VALIDATION OF UV – SPECTROPHOTOMETRIC METHOD FOR IDENTIFICATION AND DETERMINATION OF ANGIOTENSIN II RECEPTOR ANTAGONIST LOSARTAN POTASSIUM

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Received: 6 Sep 2011, Revised and Accepted: 2 Dec 2011

INTRODUCTION

Losartan [ 2 - butyl - 5 - chloro - 3 - [ [ 4 - [ 2 - ( H - tetrazol - 5 - yl) phenyl ] phenyl ] methyl ] imidazol - 4 - yl ] methanol] is non - peptide drug with gradual and long - lasting antihypertensive effect 1, 2, exerts it's action by specific blockade of angiotensin II receptors [1, 3] and is prescribed for the treatment of frequent chronic peptide drug with gradual and long - lasting antihypertensive effect 1, 2, exerts it's action by specific blockade of angiotensin II receptors [1, 3] and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderate – to – severe essential hypertension – and is prescribed for the treatment of frequent chronic diseases such as: 1) moderat...
RESULTS AND DISCUSSION

I) Validation of spectrophotometric method for analytical parameter selectivity.

At the same manner like solutions of reference standard Losartan Potassium is prepared “placebo” solution, containing all labeled in tablets supplements (colloidal anhydrous silica dioxide, crospovidone, magnesia stearate, talc, mannitol, microcrystalline cellulose, starch, talc) in dosage formulations (tablets) without active ingredient Losartan Potassium. The selectivity of the applied UV – spectrophotometric method is proved by the lack of measured absorption of “placebo” solution at specific for Losartan Potassium wavelength λ = 208 nm.

<p>| Table 2: Obtained quantity (C) of Losartan Potassium in MM, recovery R (%) for C (RC) and Chauvenet’s criterion for C (UC) |</p>
<table>
<thead>
<tr>
<th>N:</th>
<th>C40</th>
<th>RC40</th>
<th>UC40</th>
<th>C50</th>
<th>RC50</th>
<th>UC50</th>
<th>C62.5</th>
<th>RC62.5</th>
<th>UC62.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>40.24</td>
<td>101.36</td>
<td>1.07</td>
<td>50.29</td>
<td>101.39</td>
<td>1.15</td>
<td>62.35</td>
<td>99.76</td>
<td>0.78</td>
</tr>
<tr>
<td>2.</td>
<td>39.88</td>
<td>99.2</td>
<td>0.21</td>
<td>49.75</td>
<td>98.91</td>
<td>0.48</td>
<td>63.12</td>
<td>101.97</td>
<td>0.35</td>
</tr>
<tr>
<td>3.</td>
<td>39.69</td>
<td>98.0</td>
<td>0.89</td>
<td>49.7</td>
<td>98.61</td>
<td>0.64</td>
<td>65.79</td>
<td>105.94</td>
<td>1.13</td>
</tr>
<tr>
<td>X</td>
<td>40.94±</td>
<td>99.52±</td>
<td>0.33</td>
<td>50.91±</td>
<td>99.64±</td>
<td>0.33</td>
<td>63.75±</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>R [%] ± SD [%]</td>
<td>99</td>
<td>1.71</td>
<td>1.54</td>
<td>1.82</td>
<td>3.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.28</td>
<td>1.7</td>
<td>0.33</td>
<td>1.53</td>
<td>3.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD [%]</td>
<td>0.7</td>
<td>1.71</td>
<td>0.66</td>
<td>1.54</td>
<td>2.82</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S X</td>
<td>0.16</td>
<td>0.98</td>
<td>0.19</td>
<td>0.88</td>
<td>1.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P [%]</td>
<td>95.0</td>
<td>95.0</td>
<td>95.0</td>
<td>95.0</td>
<td>95.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>4.3</td>
<td>4.3</td>
<td>4.3</td>
<td>4.3</td>
<td>4.3</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>t.S X</td>
<td>0.69</td>
<td>4.21</td>
<td>0.82</td>
<td>3.78</td>
<td>4.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X – t.S X</td>
<td>39.25</td>
<td>95.31</td>
<td>49.09</td>
<td>95.86</td>
<td>59.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X + t.S X</td>
<td>40.63</td>
<td>103.73</td>
<td>50.73</td>
<td>103.42</td>
<td>68.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E [%]</td>
<td>0.40</td>
<td>0.98</td>
<td>0.38</td>
<td>0.88</td>
<td>1.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R [%] ± SD [%]</td>
<td>102.56±</td>
<td>3.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For all of the obtained by UV – method results for the absorption in every sample is necessary to estimate the Chauvenet’s criterion (U), because when U for one value is higher than the relevant statistical requirements for Chauvenet’s criterion: U  < 1.68 (N = 3), the data must be removed as unexpected. The results for UA on Table 1. show the relations: UA<sub>ARS</sub> < 1.68; UA<sub>L40</sub> < 1.68; UA<sub>L50</sub> < 1.68, which confirm that it isn’t necessary to remove any of values as unexpected.

II) Validation of spectrophotometric method for analytical parameters accuracy and precision (repeatability)

On Table 1. are summarized data for: 1) added quantity of reference standard Losartan Potassium in model mixtures: L40, L50, L62.5; 2) weighed quantity (W) of model mixtures for analysis: WL40, WL50, WL62.5; 3) values for absorbance (A) of solutions of model mixtures with Losartan Potassium in distilled water at λ = 208 nm: A<sub>L40</sub>, A<sub>L50</sub>, A<sub>L62.5</sub> 4) Chauvenet’s criterion for absorbance (UA): UA<sub>L40</sub>, UA<sub>L50</sub>, UA<sub>L62.5</sub>

Absorptions of solutions of reference standard Losartan Potassium are correspondingly: L40 (ARS = 0.50270); L50 (ARS = 0.66003); L62.5 (ARS = 0.79494).

The content of Losartan Potassium is obtained by method of reference standard (RS). On Table 2. are indicated:

N – number of the individual measurements (1 + 3); C – obtained quantity of Losartan Potassium (C<sub>L40</sub>, C<sub>L50</sub>, C<sub>L62.5</sub>), after application of UV – spectrophotometric method (test); UC – Schievenou’s criterion for obtained quantity (UC<sub>L40</sub>, UC<sub>L50</sub>, UC<sub>L62.5</sub>); R (%) – degree of recovery (RC<sub>L40</sub>, RC<sub>L50</sub>, RC<sub>L62.5</sub>); X – arithmetical mean; SD – standard deviation; RSD – relative standard deviation (%); S X – mean quadratic deviation; P – confidence possibility (%); t – coefficient of Student; X ± t.S X = X ± t.S X + t.S X – confidence interval); E (%) – relative error [16].
For all values for UC (Table 2.) are shown relations: UCL40 < 1.68; UCL50 < 1.68; UCL62.5 < 1.68, which confirm, that all experimental data suit standard requirement [16].

For the assessment of accuracy and precision is calculated sample standard deviation (SD), by the applying of the Bessel’s correction, in which the denominator N − 1 (degrees of freedom) is used instead of N and in this case (S(X̄)²) is an unbiased estimator for (SD)².

1) Accuracy

Analytical parameter accuracy is presented by the degree recovery R(%) ± RSD (%)[16]:

- RСL40: 95.31 ± 103.73
- RСL50: 95.86 ± 103.42
- RСL62.5: 94.78 ± 110.34

For all model mixtures mean quadratic error and relative error are lower than 2.0.

2) Precision

For the estimation of an analytical parameter precision (repeatability) is used the uncertainty of the result, which is determined by: SD, RSD and (X̄ ± tS(X̄)) ± t.S(X̄). At confidence possibility P = 95% (t = 4.3) all data for the obtained quantity of Losartan Potassium correspond to the relevant confidence interval: СL40: 39. 25 ± 0.28; RSD = 0.7; СL50: 49.09 ± 50.73 (SD = 0.33; RSD = 0.66); СL62.5: 59.28 ± 68.22 (SD = 1.8; RSD = 2.82).

All values for SD are lower than 2.0 and for RSD are lower than 3.0.

III) Validation of analytical parameter linearity: application of method of linear regression analysis.

The prepared solutions with decreasing concentration of RS Los were analyzed by the written UV – spectrophotometric method. For every concentration (C) in g/µl was measured the respective value of the absorption (A) in absorption units (AU) at λ = 208 nm. The experimental results are putted into linearity regression analysis. The regression calibration curve is built. The obtained regression equation: y = 1275.x - 0.008, shows the proportional accordance А = f (C) in linear concentration range: 6.75.10⁻⁴ g/µl ÷ 3.10⁻⁴ g/µl, where the Buge – Lambert – Beere Law is valid. Coefficient of regression (R) is calculated: R² = 0.991. On Table 3. are pointed out data for concentrations and absorbances for linearity and the calibration curve for А > 0.2 at λ = 208 nm is illustrated on Fig. 1.

### Table 3: Concentrations and absorbances for linearity for Losartan Potassium

<table>
<thead>
<tr>
<th>N</th>
<th>Concentration [g / µl]</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.75.10⁻⁴</td>
<td>0.84961</td>
</tr>
<tr>
<td>2</td>
<td>6.25.10⁻⁴</td>
<td>0.79494</td>
</tr>
<tr>
<td>3</td>
<td>6.0.10⁻⁴</td>
<td>0.73892</td>
</tr>
<tr>
<td>4</td>
<td>5.5.10⁻⁴</td>
<td>0.67903</td>
</tr>
<tr>
<td>5</td>
<td>5.0.10⁻⁴</td>
<td>0.66003</td>
</tr>
<tr>
<td>6</td>
<td>4.5.10⁻⁴</td>
<td>0.57045</td>
</tr>
<tr>
<td>7</td>
<td>4.3.10⁻⁴</td>
<td>0.54944</td>
</tr>
<tr>
<td>8</td>
<td>4.0.10⁻⁴</td>
<td>0.5027</td>
</tr>
<tr>
<td>9</td>
<td>3.7.10⁻⁴</td>
<td>0.47047</td>
</tr>
<tr>
<td>10</td>
<td>3.5.10⁻⁴</td>
<td>0.42397</td>
</tr>
<tr>
<td>11</td>
<td>3.0.10⁻⁴</td>
<td>0.36386</td>
</tr>
</tbody>
</table>

**CONCLUSION**

All data for UA and UC suit the standard requirement: U < 1.68. At confidence possibility P = 95% all results for R correspond to the relevant CI: RСL40: 99.52 ± 1.71; RСL50: 99.64 ± 1.54; RСL62.5: 102.56 ± 3.05. Precision is estimated by data, which suit the respective confidence interval: СL40: 39.94 ± 0.28; СL50: 49.91 ± 0.33; СL62.5: 63.75 ± 1.8. Linearity is estimated by R² > 0.99. The applied UV – spectrophotometric method is appropriate for determination of Losartan Potassium in dosage pharmaceutical products – tablets.

**REFERENCES**


