SIMULTANEOUS DETERMINATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN TABLETS BY THIN-LAYER CHROMATOGRAPHY-DENSITOMETRIC METHOD

Dobrina D. Tsvetkova\textsuperscript{1*}, Danka P. Obreshkova\textsuperscript{1}, Valentina B. Petkova\textsuperscript{2}, Stefka A. Pankova\textsuperscript{3}, Peter J. Atanasov\textsuperscript{4}, Petja S. Kasnakova\textsuperscript{5}

\textsuperscript{1}Medical University-Sofia, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 2 Dunav Str., Sofia 1000, BULGARIA.
\textsuperscript{2}Medical University-Sofia, Faculty of Pharmacy, Department of Sotial Pharmacy, 2 Dunav Str., Sofia 1000, BULGARIA.
\textsuperscript{3}Medical University-Plovdiv, Faculty of Pharmacy, Department of Pharmacognosy and Pharmaceutical Chemistry, Plovdiv, BULGARIA.
\textsuperscript{4}UMHATEM “N. I. Pirogov”, Clinic of Internal Diseases - Sofia, BULGARIA.
\textsuperscript{5}Medical University-Plovdiv, Medical College, Plovdiv, BULGARIA.

ABSTRACT

The aim of current study was to validate a thin-layer chromatography-densitometric method for simultaneous determination of Valsartan and thiazide diuretic Hydrochlorothiazide in tablets. TLC-densitometric method with system: Camag TLC densitometer; stationary phase: precoated with Silicagel \textsubscript{G60F254} plates, 10 mm x 20 mm; mobile phase: chloroform : methanol : toluene : acetic acid = 30:10:5:0.5 v/v; detection at \( \lambda = 262 \) nm was applied. The content of Valsartan and Hydrochlorothiazide in tablets was obtained by method of reference standard. For all results Chauvenet's criterion is lower than standard requirements: \( U \text{ St.} < 1.73 \) (\( N = 6 \)). Accuracy is presented by the degree of recovery \( R \) [\%], which suit relevant confidence interval: Valsartan: 93.51 % \( \div \) 100.19 %; Hydrochlorothiazide: 85.57 % \( \div \) 111.21 %. The content of compounds in tablets correspond to the relevant confidence interval: Valsartan: 149.60 mg \( \div \) 160.32 mg; Hydrochlorothiazide: 10.69 mg \( \div \) 13.91 mg. The applied method is appropriate for an estimation of Valsartan and HCTZ in fixed combination in
tablets for treatment of hypertension.

**KEYWORDS:** Valsartan, Hydrochlorothiazide, hypertension, thin-layer chromatography, densitometry.

**INTRODUCTION**

Hypertension is one of the most important social disease, from which are suffering more than 1 billion people all over the world. Because of their variety of pharmacological activities angiotensin II receptors antagonists (blockers, sartans) are widely used in treatment of hypertension and other different related diseases such as diabetes mellitus. The combinations between sartans and other antihypertensive drugs such as beta blockers, calcium antagonists, diuretics, angiotensin-converting enzyme inhibitors are applied very often in clinical practice for therapy of different forms of hypertension (mild, severe and associated with diabetes mellitus).[1]


Valsartan is used in fixed combinations with Amlodipine Besylate[7], Benazepril[8], Captopril[9], Ezetimibe[10], Simvastatin.[11] In observation of 1668 patients with hypertension is proved that in comparison with monotherapy with Valsartan (Val), in application of fixed combination Val/HCTZ in most % of cases are reached the required values of RR < 140/90 mm Hg as follows: 1) Val: women – 30.5 %; patients < 65 years – 29.7 %; patients > 65 years – 24.5 %; patients with height weight – 31.2 %; 2) Val/HCTZ: women – 47.8 %; patients < 65 years – 44.6 %; patients > 65 years – 43.9 %; patients with height weight – 49.0 %.[12] In clinical trial with 126 patients with mild to moderate essential hypertension in northeast China is observed significant reduction of systolic RR in application of Val 80 mg/HCTZ 12.5 mg, in comparison with monotherapy with Val 80 mg.[13] After 8 weeks therapy of 1313 hypertensive patients is approached RR < 140/90 mm Hg. In this study fixed combination Val 160 mg/HCTZ 12.5 mg lowers RR with 15 mm Hg (72 %), which is greater,
compared with monotherapy with Val 160 mg (11 mm Hg, 61 %) and HCTZ 25 mg (10 mm Hg, 50 %).[14] In 8 weeks study with 1346 patients the reduction of RR is significantly higher with Val 320 mg/HCTZ 25 mg in comparison with Val 160 mg/HCTZ 12.5 mg.[15] Fixed combination therapy with Val 80 mg-320 mg/Amlodipine (Aml) 2.5 mg-5 mg daily is more effective than: 1) Valsartan and Amlodipine monotherapy in reducing of RR in patients with mild to moderate hypertension[7]; 2) Amlodipine monotherapy in decreasing of RR in patients with moderate to severe hypertension.[7] In cases with moderate to severe hypertension Val 320 mg/HCTZ 25 mg triple therapy is highly effective in reducing of systolic RR with 40 mm Hg and diastolic RR with 25 mm Hg, compared with dual components Val 320 mg/HCTZ 25 mg; Val 320 mg/Aml 10 mg; Aml 10 mg/HCTZ 25 mg.[16,17]

For the simultaneous determination of Hydrochlorothiazide and Metoprolol Succinate in a solid dosage pharmaceutical formulation is applied HPLC with C18 column; temperature of 24 °C; mobile phase : 50 mM disodium hydrogen phosphate : methanol : acetonitrile = 525 : 225 : 250 v/v; flow rate – 1 ml/min., UV detection at λ = 222 nm.[18]

For simultaneous determination of Valsartan and HCTZ in fixed combinations are applied the following methods: I) spectrophotometry in tablets: 1) UV: λ_{Val} = 264 nm, λ_{HCTZ} = 270.5 nm[19]; 2) UV 1st derivative ratio method[20]: λ = 227.8 nm/λ = 276.5 nm[21]; 3) UV 2nd derivative method[22]; 4) UV absorption ratio method: λ_{HCTZ} = 270.5 nm, λ_{isosbbsontive point} = 231.5 nm[23]; II) HPLC: 1) tablets[21, 24-25], 2) plasma: a) ion pair HPLC UV: HIQ sil ODS (250 mm x 4.6 mm); methanol : 0.0025 M orthophosphoric acid = 70 : 30 v/v, pH = 4.6 with 0.1% hexanesulfonic acid, λ = 259 nm[22]; III) HPLC/ESP/MS[26]: 1) Phenomenex Kromasil C8, water : methanol = 27 : 73 v/v[27]; 2) C8, acetonitrile : methanol : 0.001% ammonia = 75 : 15 : 10 v/v.[28]

High performance thin-layer chromatography (HPTLC)-densitometry on Silicagel G60F254 is described for simultaneous determination of components of fixed combinations: Val/Amlodipine (tablets, plasma): ethylacetate : methanol : ammonium hydroxide = 55 : 45 : 5 v/v, λ = 237 nm[29]; Val/Nebivolol Hydrochloride (Neb) (tablets): ethylacetate : methanol : acetic acid, λ_{Neb} = 280 nm, λ_{Val} = 240 nm[30], Candesartan Cilexetil/HCTZ in (tablets).[31]

For angiotensin II receptor antagonists Valsartan, Losartan and Telmisartan: 1) TLC densitometric method for simultaneous quality and quantity analysis is validated[32] and is applied for simultaneous determination of the same sartans in tablets[33]; 2) methods of
reference standard and calibration curve for estimation of accuracy and precision by validated TLC densitometric method are compared.\cite{34-38}

MATERIALS AND METHODS
Materials.
I) Reference standards (RS): Valsartan (Val), Hydrochlorothiazide (HCTZ)
II) Tablets: Co-Diovan®
III) Reagents with analytical grade quality: chloroform, methanol, acetone, toluene, formic acid, acetic acid
IV) TLC plates, precoated with Silicagel $G_{60}F_{254}$, 10 mm x 20 mm (Merck).

Methods.
TLC-densitometry. System: Camag TLC densitometer; stationary phase: TLC plates, precoated with Silicagel $G_{60}F_{254}$, 10 mm x 20 mm (Merck); mobile phase (MPh): chloroform: methanol: acetone: toluene: acetic acid = 7.5: 1.5: 5: 5: 0.01: 0.03 v/v; detection at $\lambda = 254$ nm.

Preparation of sample.
An accurately weighted quantity of powdered tablets was dissolved in volumetric flask of 10.0 ml with 95 % ethanol.

Preparation of reference standards.
An accurately weighted quantity of reference standards of Valsartan (160 mg) and Hydrochlorothiazide were separately dissolved in volumetric flask of 10.0 ml with 95 % ethanol. All solutions were filtered and chromatograms were recorded.

RESULTS AND DISCUSSION
In Table 1. (Val) and in Table 2. (HCTZ) are pointed out the data for.
1) spot peak area (AUC): AUC Val (AUC St.Val = 15059); AUC HCTZ (AUC St.HCTZ = 1934);
2) Chauvenet’s criterion (U): U AUC Val, U AUC HCTZ.
Table 1. AUC and Chauvenet's criterion for AUC (U AUC) for Valsarta in Co-Diovan tabl.

<table>
<thead>
<tr>
<th>N.</th>
<th>AUC Val</th>
<th>U AUC Val</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>14329</td>
<td>0.70</td>
</tr>
<tr>
<td>2.</td>
<td>14350</td>
<td>0.66</td>
</tr>
<tr>
<td>3.</td>
<td>14356</td>
<td>0.65</td>
</tr>
<tr>
<td>4.</td>
<td>14545</td>
<td>0.28</td>
</tr>
<tr>
<td>5.</td>
<td>14921</td>
<td>0.47</td>
</tr>
<tr>
<td>6.</td>
<td>15009</td>
<td>0.64</td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>14685</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>505</td>
<td></td>
</tr>
<tr>
<td>RSD [%]</td>
<td>3.44</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. AUC and Chauvenet's criterion for AUC for Hydrochlorothiazide in Co-Diovan tabl.

<table>
<thead>
<tr>
<th>N.</th>
<th>AUC HCTZ</th>
<th>U AUC HCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1773</td>
<td>0.86</td>
</tr>
<tr>
<td>2.</td>
<td>1797</td>
<td>0.70</td>
</tr>
<tr>
<td>3.</td>
<td>1824</td>
<td>0.52</td>
</tr>
<tr>
<td>4.</td>
<td>1833</td>
<td>0.46</td>
</tr>
<tr>
<td>5.</td>
<td>2077</td>
<td>1.15</td>
</tr>
<tr>
<td>6.</td>
<td>2114</td>
<td>1.40</td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>1903</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>151</td>
<td></td>
</tr>
<tr>
<td>RSD [%]</td>
<td>7.93</td>
<td></td>
</tr>
</tbody>
</table>

For the development of the validation procedure for TLC-densitometry the analytical parameters selectivity, accuracy, precision, linearity were studied.

1) Selectivity.

At the same manner like solutions of RS were prepared "placebo" solutions, containing all labeled in tablets supplements, without the active substance. The selectivity of the applied TLC- method is confirmed by the fact, that on chromatograms with "placebo" preparations aren’t exist spots with Rf, corresponded to Rf of the respective reference standard.[32]

2) Linearity.

An accurately weighed quantity (1 mg, 2 mg, 3 mg, 5 mg, 7 mg, 9 mg) of reference standard of Val was dissolved in volumetric flasks to 10.0 ml with mobile phase to obtain solutions with concentrations: $10^{-4}$ g/ml, $210^{-4}$ g/ml, $310^{-4}$ g/ml, $510^{-4}$ g/ml, $710^{-4}$ g/ml, $910^{-4}$ g/ml. The obtained regression equation, showing the proportional accordance 3D volume =
f(C) in concentration range $1.10^{-4}$ g/ml $\div 9.10^{-4}$ g/ml is: $y = 2.10^{11}.x + 4.10^{6}$, $R^2 = 0.9986$ (Val).\[32\]

3) Accuracy and precision (repeatability).

For the assessment of accuracy and precision a sample standard deviation (SD) is calculated, by applying the Bessel’s correction in which the denominator $N - 1$ (degrees of freedom) is used instead of N. For the estimation of the analytical parameter precision (repeatability) is used the uncertainty of the result, which is determined by: SD, RSD and confidence interval.\[35\]

Chromatograms for model mixtures I, II, III, containing respectively – I: 120 mg (75 %); II: 160 mg (100 %); III: 200 mg (125 %) of theoretical concentration of Vasartan in tablets were recorded. From the assessment of precision, all values for the content of Valsartan in model mixtures lie in the relevant confidence interval: method of reference standard: 118.57 mg $\div$ 122.19 mg (I); 160.4 $\div$ 161.86 (II); 192.32 $\div$ 205.56 (III); method of calibration curve: 113.81 $\div$ 120.01 (I); 156.81 $\div$ 165.85 (II); 195.07 $\div$ 211.85 (III).\[33\]

In Table 3, are indicated: N – number of the individual measurements (1 ÷ 6); [Val], [HCTZ] – obtained quantity of Valsartan and Hydrochlorothiazide; U[Val], U [HCTZ] – Chauvenet's criterion for content of Val and HCTZ; R[Val], R[HCTZ] – recovery; $\bar{x}$ – arithmetical mean; standard (SD) and relative standard deviation (RSD) (%); S $\bar{x}$ – mean quadratic error; P – confidence possibility (%); t – coefficient of Student; $\bar{x}$ ± t.S $\bar{x}$ – confidence interval. The content of Valsartan and Hydrochlorothiazide in Co-Diovan tabl. is obtained by method of reference standard (AUC St.Val = 15059); AUC HCTZ (AUC St.HCTZ = 1934).

Table 3. Obtained quantity of Valsartan (Val) and Hydrochlorothiazide (HCTZ) in Co-Diovan tabl.

<table>
<thead>
<tr>
<th>N:</th>
<th>[Val] [mg]</th>
<th>R [Val][%]</th>
<th>U [Val]</th>
<th>[HCTZ] [mg]</th>
<th>R [HCTZ] [%]</th>
<th>U [HCTZ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>152.24</td>
<td>95.15</td>
<td>0.84</td>
<td>11.46</td>
<td>91.68</td>
<td>0.87</td>
</tr>
<tr>
<td>2.</td>
<td>152.47</td>
<td>95.29</td>
<td>0.77</td>
<td>11.61</td>
<td>92.88</td>
<td>0.71</td>
</tr>
<tr>
<td>3.</td>
<td>152.53</td>
<td>95.33</td>
<td>0.75</td>
<td>11.79</td>
<td>94.32</td>
<td>0.53</td>
</tr>
<tr>
<td>4</td>
<td>154.54</td>
<td>96.59</td>
<td>0.13</td>
<td>11.85</td>
<td>94.80</td>
<td>0.46</td>
</tr>
<tr>
<td>5.</td>
<td>158.53</td>
<td>99.08</td>
<td>1.10</td>
<td>13.42</td>
<td>107.36</td>
<td>1.15</td>
</tr>
<tr>
<td>6.</td>
<td>159.47</td>
<td>99.67</td>
<td>1.39</td>
<td>13.66</td>
<td>109.28</td>
<td>1.40</td>
</tr>
</tbody>
</table>

$\bar{x}$ ± SD 154.96 ± 3.25 12.30 ± 0.97

$\bar{x}$ R [%] ± RSD [%] 96.85 ± 2.1 98.39 ± 7.93
For all experimental data Chauvenet's criterion is lower than standard requirements: $U_{St.} < 1.73$ ($N = 6$) and it isn’t necessary to remove anyone of them as unexpected. Accuracy is presented by recovery $R$ (%) and RSD (%).\cite{35} Data show that at respective $P$ all results for $R$, suit relevant confidence interval ($\bar{X} \pm t.S \bar{X}$): 1) Val: $93.51 \pm 100.19$ (SD = 2.03, RSD = 2.10); HCTZ: $85.57 \pm 111.21$ (SD = 7.8, RSD = 7.93). From the assessment of precision\cite{35} is obvious, that all values for the obtained content of compounds in tablets correspond to the relevant confidence interval: Val: $149.60 \pm 160.32$ (SD = 3.25, RSD = 2.10); HCTZ: $10.69 \pm 13.91$ (SD = 0.97, RSD = 7.89).

**CONCLUSION**

TLC-densitometric method for simultaneous analysis of Valsartan and Hydrochlorothiazide in tablets was applied. All data for $R$ [%] $\pm$ RSD [%] suit standard requirements. The obtained quantities by the applied method are: $154.96 \pm 3.25$ (Val); $12.30 \pm 0.97$ (HCTZ). The applied TLC-method can be used for the simultaneous identification and determination of Valsartan and Hydrochlorothiazide in formulated drug products tablets.

**REFERENCES**


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