Quality Control Of Impurities And Comparison Of Pharmacokinetic Parameters Of Angiotensin Receptor Antagonists

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Abstract—The major risk for mortality from chronic heart and kidney disease worldwide is an inadequate treatment of hypertension. Therapy of hypertension becomes successfully by the developed in recent years a new class compounds – sartans (angiotensin II-receptor antagonists), specifically blocking renin angiotensin aldosterone system.

Benefits of sartans in hypertensive patients include reduction in left ventricular hypertrophy, improvement of diastolic function, decrease of ventricular arrhythmias, reduction of microalbuminuria, improvement of renal function, cardioprotective effect in patients with heart failure.

The impurity profiling of active pharmaceutical ingredients is an important quality control parameter.

Impurities are analysed by high performance liquid chromatography, thin layer chromatography, capillary electrophoresis and spectrophotometry.

For estimation of impurities of sartans the most applied methods, due to highest selectivity and specificity in separation and detection, are: HPLC-MS, HPLC-MS/MS, HPLC-ESI/MS and HPLC-TOF/MS.

The important pharmacokinetic data of sartans are:
1) suitability for oral administration as to have physico-chemical properties, determining good pharmacoki-netic behavior
2) suitability to the criteria of the rule of Christopher A. Lipinski (Rule 5): a) Mr < 500; b) H – donors (NH, OH) < 5; c) H – acceptors (N, O) < 10; d) logP<5: LogP = 4.9 (Candesartan); LogP = 3.58 (Eprosartan); LogP = 4.52 (Irbesartan); LogP = 4.68 (Losartan); LogP= 4.31 (Olmesartan); LogP = 4.66 (Telmisartan); LogP = 3.68 (Valsartan)
3) high plasma protein binding, which provide to be obtained once daily
4) Telmisartan is with the highest oral bioavailability and with the longest half-life
5) the bioavailability of other sartans is: Candesartan (3-11 %); Tasosartan (37 %); Zolasartan (20 %); Enolatasosartan (36-72 %).

Keywords—sartans; impurities; pharmacokinetic parameters; metabolism.

I. INTRODUCTION.

Arterial hypertension is a widespread disease with more than 1 billion cases worldwide. The major risk for mortality from chronic heart and kidney disease is an inadequate treatment of hypertension. Stages in the development of arterial hypertension are: 1) I: Phase A (prehypertensive); Phase B (transient); 2) II: defined as SBP ≥ 160 mm Hg or DBP ≥ 100 mm Hg: Phase A (unsustainable); Phase B resistant; 3) III: Phase A (compensated); Phase B (uncompensated). Antihypertensive drugs are: beta-blockers, calcium antagonists, angiotensin-converting enzyme inhibitors, vasodilators and diuretics [1].

I. Pharmacological applications of sartans.

Sartans are applied in a number of heart diseases [2, 3];
1) heart failure [4]; 2) prevention of atrial fibrillation in heart failure [5]; 3) myocarditis [6]; 4) hypertension with vascular hypertrophy [7]; 5) hypertension with left ventricular dysfunction [8]; 6) acute coronary syndrome [9]; 7) protection of the vascular endothelium [10]; 8) platelet aggregation [11]; 9) prevention of stroke [12]; 10) cerebral ischemia [11, 13]; 11) prevention of dementia [14]; 12) Alzheimer – sartans reduce the development of disease by 40 % compared to other antihypertensive agents [15] due to prevention of beta-amyloid-induced cognitive impairment [16];
Impurities are analysed by liquid and thin layer chromatography, capillary electrophoresis and spectrophotometry [64].

HPLC coupled with mass spectroscopy (MS) is the most applied method due to higher selectivity and specificity in separation and detection. Different HPLC methods are applied: LC-MS, LCMS/MS, LC-ESI/MS and LC-TOF/MS [70].

On TABLE 1. are presented data for empirical structures, molecular mass and melting point of sartans.

<table>
<thead>
<tr>
<th>SARTAN</th>
<th>Empirical structure</th>
<th>Molecular mass</th>
<th>Melting point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abitesartan</td>
<td>C_{26}H_{31}N_5O_5</td>
<td>461.556</td>
<td>154-156</td>
</tr>
<tr>
<td>Azilsartan</td>
<td>C_{30}H_{29}N_5O_5</td>
<td>568.5</td>
<td>157-159</td>
</tr>
<tr>
<td>Candesartan</td>
<td>C_{28}H_{20}N_5O_3</td>
<td>440.45</td>
<td>183-185</td>
</tr>
<tr>
<td>Candesartan Cilexetil</td>
<td>C_{33}H_{34}N_6O_6</td>
<td>610.66</td>
<td>163</td>
</tr>
<tr>
<td>Elosartan</td>
<td>C_{22}H_{26}ClKIN_6O_5</td>
<td>591.1</td>
<td>188-190</td>
</tr>
<tr>
<td>Eprosartan</td>
<td>C_{23}H_{24}N_5O_4S</td>
<td>424.52</td>
<td>260-261</td>
</tr>
<tr>
<td>Eprosartan Mesylate</td>
<td>C_{23}H_{32}N_6O_5S.S. CH_3SO_H</td>
<td>520.625</td>
<td>248-250</td>
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<tr>
<td>Embusartan</td>
<td>C_{28}H_{24}F_2N_5O_3</td>
<td>461.488</td>
<td>250-252</td>
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<tr>
<td>Fimasartan</td>
<td>C_{22}H_{31}N_5O_5</td>
<td>501.65</td>
<td>260-263</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>C_{25}H_{28}N_6O_4</td>
<td>428.23</td>
<td>180-181</td>
</tr>
<tr>
<td>Losartan</td>
<td>C_{22}H_{23}ClIN_5O</td>
<td>422.92</td>
<td>183.5-184.5</td>
</tr>
<tr>
<td>Losartan Potassium</td>
<td>C_{22}H_{22}ClKIN_6O</td>
<td>461.01</td>
<td>183.5-184.5</td>
</tr>
<tr>
<td>Milfasartan</td>
<td>C_{30}H_{30}N_6O_3S</td>
<td>554.667</td>
<td>192-195</td>
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<tr>
<td>Olmesartan Medoxomil</td>
<td>C_{29}H_{30}N_6O_6</td>
<td>558.585</td>
<td>175-180</td>
</tr>
<tr>
<td>Pomisartan</td>
<td>C_{19}H_{30}N_6O_2</td>
<td>490.596</td>
<td>176-178</td>
</tr>
<tr>
<td>Pratosartan</td>
<td>C_{24}H_{23}N_5O_4</td>
<td>431.499</td>
<td>184-187</td>
</tr>
<tr>
<td>Ripisartan</td>
<td>C_{22}H_{22}N_5O_4</td>
<td>426.476</td>
<td>235-238</td>
</tr>
<tr>
<td>Tazosartan</td>
<td>C_{23}H_{21}N_5O_4</td>
<td>411.459</td>
<td>243-246</td>
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<tr>
<td>Telmisartan</td>
<td>C_{33}H_{30}N_5O_2</td>
<td>514.617</td>
<td>261-263</td>
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<td>Saprisartan Potassium</td>
<td>C_{28}H_{30}BrF_3N_6O_3</td>
<td>611.431</td>
<td>272-275</td>
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<tr>
<td>Valsartan</td>
<td>C_{29}H_{32}N_5O_3</td>
<td>435.519</td>
<td>116-117</td>
</tr>
<tr>
<td>Zolasartan</td>
<td>C_{22}H_{20}BrClN_6O_3</td>
<td>555.814</td>
<td>421.5</td>
</tr>
</tbody>
</table>

TABLE 1. Empirical structure, molecular mass and melting point of sartans.

Related substances of some sartans are given in the following tables: Candesartan (TABLE 2), Losartan (TABLE 3); equal related substances for Losartan, Candesartan and Olmesartan. (TABLE 4), Telmisartan (TABLE 5), Olmesartan (TABLE 6), Valsartan (Fig. 1).

13) Parkinson [17]; 14) migraine (Candesartan) [18]; 15) headache [19]; 16) inflammation: an antiinflammatory effect [20]; 17) type 2 diabetes: a) prevention of diabetes [21]; b) symptomatic hypertension in type 2 diabetes [22]; 18) kidney: symptomatic hypertension: a) renoprotective effect in the absence of diabetes [23]; b) renoprotective effect in type 2 diabetes [24, 25]; c) diabetic nephropathy [26]; d) prevention of progression of renal failure [27]; e) glomerulonephritis [28]; g) impaired renal function and hyperuricemia [29]; h) improvement of renal perfusion with hypercholesterolemia [30]; 19) liver fibrosis [31, 32] and portal hypertension [32]; 20) diabetic retinopathy [33]; 21) colitis [34]; 22) prevention of prostate cancer [35]; 23) inactivation of plasinomugen [36].

Due to the synergistic effect the combination therapy of sartans with other antihypertensive drugs is more effective than the respective monotherapies. Sartans are used in combination with:
1) beta-blockers in hypertension: Propranolol [37]
2) calcium channel blockers in hypertension: Amlodipine besylate: Olmesartan Medoxomil [38], Telmisartan [39], Valsartan [40, 41]; Felodipine [42]; Nilvadipine [43]
3) angiotensin-converting enzyme inhibitors [44]; a) hypertension: Benazepril: Valsartan [45]; b) chronic heart failure [46]: Candesartan [47, 48]; c) diabetic nephropathy [49]; d) proteinuria [50]
4) thiadaza diuretic Hydrochlorothiazide in hypertension: Candesartan cilexetil [51], Eprosartan, [52], Irbesartan [53, 54], Losartan [55, 56], Olmesartan Medoxomil [57], Telmisartan [58], Valsartan [59, 60]
5) statins in hypertension [61]: Rosuvastatin: Olmesartan, Irbesartan, Telmisartan [61], Simvastatin: Valsartan [62, 63].

II. Impurities of sartans.

During all stages of the pharmaceutical manufacturing process: development, production, stability testing and regulatory expectation of pharmaceutical formulations, the impurity profiling of active pharmaceutical ingredients is an important quality control parameter [64]. The importance of analysis of potentially toxic impurities is in order to increase the safety of drug therapy, quality and efficacy of pharmaceuticals [65].

Active ingredient related impurities include stereochemistry and functional group of active drug.
Process related impurities include chemicals, reagents, catalysts, residual solvents, synthetic intermediate products [66].

Stability related impurities are result of degradation or transformation of active pharmaceutical ingredient. International Conference of Harmonization have specified various limits for impurities in drug substances [67] and in drug products [68] as they may influence bioavailability, safety and efficacy of drugs [66].

Drug impurity profiling include identification and quantitative determination of related substances in drug substances and in pharmaceutical formulations [69].
### TABLE 2. Related substances of Candesartan.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Structure</th>
</tr>
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<tbody>
<tr>
<td>Candesartan Methyl Ester</td>
<td><img src="image1" alt="Candesartan Methyl Ester" /></td>
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<tr>
<td>Candesartan Ethyl Ester</td>
<td><img src="image2" alt="Candesartan Ethyl Ester" /></td>
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<tr>
<td>Trityl Candesartan</td>
<td><img src="image3" alt="Trityl Candesartan" /></td>
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<tr>
<td>Desethyl Candesartan</td>
<td><img src="image4" alt="Desethyl Candesartan" /></td>
</tr>
<tr>
<td>Candesartan Cilexetil HCl Methoxy Analog</td>
<td><img src="image5" alt="Candesartan Cilexetil HCl Methoxy Analog" /></td>
</tr>
<tr>
<td>Desethyl Candesartan Cilexetil</td>
<td><img src="image6" alt="Desethyl Candesartan Cilexetil" /></td>
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</table>

### TABLE 3. Impurities of Losartan.

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Structure</th>
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</thead>
<tbody>
<tr>
<td>Losartan EP Impurity A</td>
<td><img src="image7" alt="Losartan EP Impurity A" /></td>
</tr>
<tr>
<td>Losartan EP Impurity C</td>
<td><img src="image8" alt="Losartan EP Impurity C" /></td>
</tr>
<tr>
<td>Losartan EP Impurity D</td>
<td><img src="image9" alt="Losartan EP Impurity D" /></td>
</tr>
<tr>
<td>Losartan EP Impurity H</td>
<td><img src="image10" alt="Losartan EP Impurity H" /></td>
</tr>
<tr>
<td>Losartan EP Impurity I</td>
<td><img src="image11" alt="Losartan EP Impurity I" /></td>
</tr>
<tr>
<td>Losartan EP Impurity J</td>
<td><img src="image12" alt="Losartan EP Impurity J" /></td>
</tr>
<tr>
<td>Losartan EP Impurity K</td>
<td><img src="image13" alt="Losartan EP Impurity K" /></td>
</tr>
<tr>
<td>Losartan Aldehyde</td>
<td><img src="image14" alt="Losartan Aldehyde" /></td>
</tr>
<tr>
<td>Losartan Carboxylic Acid</td>
<td><img src="image15" alt="Losartan Carboxylic Acid" /></td>
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</table>

### TABLE 4. Impurities of Losartan, Candesartan, Olmesartan.

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-(4’-(Bromomethyl)(1,1’-bi-phenyl)-2-yl)-1-trityl-1H-tetrazole</td>
<td><img src="image16" alt="5-(4’-(Bromomethyl)(1,1’-bi-phenyl)-2-yl)-1-trityl-1H-tetrazole" /></td>
</tr>
<tr>
<td>p-(o-1H-tetrazol-5-ylyphenyl)benzyl alcohol</td>
<td><img src="image17" alt="p-(o-1H-tetrazol-5-ylyphenyl)benzyl alcohol" /></td>
</tr>
<tr>
<td>p-(o-1H-tetrazol-5-ylyphenyl)toluene</td>
<td><img src="image18" alt="p-(o-1H-tetrazol-5-ylyphenyl)toluene" /></td>
</tr>
<tr>
<td>Triphenylmethanol</td>
<td><img src="image19" alt="Triphenylmethanol" /></td>
</tr>
</tbody>
</table>

### TABLE 5. Impurities of Telmisartan.

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telmisartan Impurity A</td>
<td><img src="image20" alt="Telmisartan Impurity A" /></td>
</tr>
<tr>
<td>Telmisartan Impurity C</td>
<td><img src="image21" alt="Telmisartan Impurity C" /></td>
</tr>
<tr>
<td>Telmisartan Impurity E</td>
<td><img src="image22" alt="Telmisartan Impurity E" /></td>
</tr>
<tr>
<td>Telmisartan Impurity F</td>
<td><img src="image23" alt="Telmisartan Impurity F" /></td>
</tr>
<tr>
<td>Telmisartan acyl glucuronide</td>
<td><img src="image24" alt="Telmisartan acyl glucuronide" /></td>
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</tbody>
</table>
TABLE 6. Related substances of Olmesartan.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Structure</th>
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</thead>
<tbody>
<tr>
<td>Olmesartan Methyl Ester</td>
<td><img src="image1" alt="Olmesartan Methyl Ester" /></td>
</tr>
<tr>
<td>Olmesartan Ethyl Ester</td>
<td><img src="image2" alt="Olmesartan Ethyl Ester" /></td>
</tr>
<tr>
<td>Olmesartan Dimer Ester</td>
<td><img src="image3" alt="Olmesartan Dimer Ester" /></td>
</tr>
<tr>
<td>Olmesartan Methyl Ether</td>
<td><img src="image4" alt="Olmesartan Methyl Ether" /></td>
</tr>
<tr>
<td>Olmesartan Medoxomil</td>
<td><img src="image5" alt="Olmesartan Medoxomil" /></td>
</tr>
<tr>
<td>Dehydro Impurity</td>
<td><img src="image6" alt="Dehydro Impurity" /></td>
</tr>
<tr>
<td>Ethyl 4-(1-Hydroxy-1-methyl)ethyl-2-Propyl-Imidazole-5-Carboxylate</td>
<td><img src="image7" alt="Ethyl 4-(1-Hydroxy-1-methyl)ethyl-2-Propyl-Imidazole-5-Carboxylate" /></td>
</tr>
<tr>
<td>Olmesartan Medoxomil</td>
<td><img src="image8" alt="Olmesartan Medoxomil" /></td>
</tr>
<tr>
<td>Olmesartan Methyl Ketone</td>
<td><img src="image9" alt="Olmesartan Methyl Ketone" /></td>
</tr>
<tr>
<td>Impurity I</td>
<td><img src="image10" alt="Impurity I" /></td>
</tr>
<tr>
<td>Impurity II</td>
<td><img src="image11" alt="Impurity II" /></td>
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<tr>
<td>Impurity III</td>
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<tr>
<td>Impurity IV</td>
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<tr>
<td>Impurity V</td>
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</tr>
<tr>
<td>Valsartan</td>
<td><img src="image15" alt="Valsartan" /></td>
</tr>
</tbody>
</table>

Fig. 1. Related substances of Valsartan.

Initially the impurities of Valsartan were investigated by Nie, as it is isolated (S)-N-valeryl (N-[[2'-{(1-methyl-tetrazol-5-yl)biphenyl-4-yl]-methyl}]valine.

In US Pharmacopeia are included: (R)-N-valeryl-N-[[2'-{(1H-tetrazole-5-yl)biphenyl-4-yl]-methyl}]valine; (S)-N-butyryl-N-[[2'-(1H-tetrazole-5-yl)biphenyl-4-yl]-methyl]valine; (S)-N-valeryl-N-[[2'-{(1H-tetrazole-5-yl)biphenyl-4-yl]-methyl]valine benzyl ester.

Imp I, Imp II and Imp III are obtained in the synthesis of Valsartan of Fig. 2. Imp III is obtained in pyridine and tetrahydrofurane at -5-0 °C from condensation of Imp I with 5-phenylvaleroyl chloride, formed by 5-phenylvaleric acid, by reaction with thionyl chloride at room temperature.

Imp IV and Imp V are formed from Imp I in the same manner from 4-pentenoic acid and 5-chlorovaleric acid, respectively, and Imp V is obtained after alkaline hydrolysis of the condensation product with sodium hydroxide in an aqueous medium [67].
Sodium azide is the impurity as is the precursor in the synthesis of Irbesartan, Candesartan and Valsartan and is determined by gradient HPLC on Hydro RP column (250 x 4.6 mm x 4 μm), room temperature and UV-detection at λ = 205 nm [72]. In Irbesartan substance sodium azide is analysed by ion chromatography [73].

Azilsartan impurities are investigated by HPLC method, using Inertsil ODS-3 column (250 x 4.6 mm x 5 μm) in gradient mode with mobile phase: mixture of acetonitrile and potassium dihydrogen orthophosphate buffer [74]. Gradient reverse-phase HPLC is developed for the quantitative determination of related compounds of Azilsartan kamedoxomin on YMC-Pack C18 column (150 mm x 4.6 mm x 3 μm) [75].

Related substances Desethyl Candesartan cilexetil, 1N-Ethyl Candesartan cilexetil; 2N-Ethyl Candesartan cilexetil, 1N-Ethyl Oxocandesartan cilexetil; 2N-Ethyl Oxocandesartan cilexetil are analysed by HPLC/MS [76].

Ultra High-Pressure Liquid Chromatography (UPLC) method is proposed for determination of Candesartan cilexetil impurities in tablet formulation. The chromatographic separation is performed on Waters Acquity UPLC system and BEH Shield RP, column using gradient elution of mobile phase A: 0.01 M phosphate buffer adjusted pH 3.0 with orthophosphoric acid and mobile phase B: acetonitrile: water = 95 : 5 % v/v, UV-detection at λ = 254 nm for Candesartan Ethyl Ester and Desethyl Candesartan Cilexetil and at λ = 210 nm for Tritylalcohol and Triphenylmethyl Methyl Ether impurities [78].

For assay of impurities of Eprosartan mesylate is reported HPLC method at Phenomenex Gemini C18 column (250 mm x 4.6 mm x 5.0 μm), gradient elution with mobile phase: A: 10 mM ammonium acetate buffer (pH to 3.0) : acetic acid; B: acetonitrile, UV-detection [79].

Reversed-phase liquid chromatography method for the simultaneous determination of Eprosartan mesylate and its six impurities is applied. Separation is achieved in less than 7 min using a fused-core C18 column (100 mm x 2.1 mm x 2.6 μm), gradient elution with mobile phase: 10 mM ammonium formiate (pH = 3.0) : acetonitrile [80].

Eprosartan and related substances 4,4′-(5,5′-(1E,1′E)-3,3′-(4,4′-methylene-bis (thiophene-4,2-diyl))-bis-(2-carboxyprop-1-ene-3,1-diyli)-bis-(2-buty1-1H-imidazole-5,1-diyli)bis-(methylene) are estimated by HPLC/MS [81].

Stability indicating UPLC method for simultaneous determination of Eprosartan mesylate.
Hydrochlorothiazide and their impurities in tablets is developed by performing of chromatographic separation on Acquity HSS C18 column (150 x 2.1 mm x 1.7 μm), gradient elution using acetonitrile and 10 mM disodium hydrogen phosphate buffer (pH = 5.5, adjusted with phosphoric acid), flow rate: 0.3 ml/min., UV-detection at λ = 274 nm [82].

Isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method is used for the determination of 2-cyano-4'-bromomethyl biphenyl and 2-n-butyl-1,3-diazaspiro[4,4]-non-1-ene-4-one impurities of Irbesartan substance and in pharmaceutical dosage forms [83].

In RP-HPLC method for the estimation of process related impurities of Irbesartan the separation is carried out on Hypersil Octadecylsilyl C18 column, (4.6 mm x 150 mm, 3 μm), column temperature: 35°C, mobile phase A: 0.55 % v/v orthophosphoric acid, pH = 3.2 with triethylamine); mobile phase B: solvent A : acetonitrile = 5 : 95 v/v, flow rate: 1.2 ml/min., UV-detection at λ = 220 nm. The gradient program is: time (min.) / % mobile phase B = 0/40, 10/40, 22/50, 26/50, 28/40 and 35/40 [84].

HPLC is developed for Losartan related substances by using of Kromasil 100-5C18 column (250 x 4.6 mm x 5 μm particle size), column temperature: 35 °C, mobile phase: 0.1 % phosphoric acid in water : acetonitrile = 50 : 50 v/v, UV-detection at λ = 220 nm [85].

Gradient HPLC method is developed for simultaneous quantitative determination of Losartan impurities and its included in European pharmacopoeia 7.0 related impurities [86]:

B ([2′-[1H-tetrazol-5-yl]biphenyl-4-yl)methanol]
C ([2-butyl-5-chloro-1-[2′-[1H-tetrazol-5-yl]biphenyl-4-yl][methyl]-1H-imidazol-4-yl)methanol]
D (2-butyl-4-chloro-1H-imidazole-5-carbaldehyde)
E (5-(4′-methylbiphenyl-2-yl)-1H-tetrazole)
F (5-(4′-[2-butyl-4-chloro-5-[[1-(methylene)oxy]methyl]-1H-imidazol-1-yl]methyl)biphenyl-2-yl)-1H-tetrazole)
I (5-(4′-[2-butyl-4-chloro-5-[[triphenylmethylene]oxy]methyl]-1H-imidazol-1-yl]methyl)biphenyl-2-yl)-1H-tetrazole)
G (triphenylmethanol) [87].

The reported chromatographic system is: ACCHROM ODS-C18 column (250 mm x 4.6 mm x 5 μm), column temperature: 35 °C, mobile phase: acetonitrile : 0.1 % phosphoric acid under a gradient elution, flow rate: 1.0 ml/min., UV-detection at λ = 220 nm [87].

HPLC method is described for the estimation of Telmisartan related impurities in tablets formulation by using X-Bridge C18 column (150 mm x 4.6 mm x 3.5 μm), mobile phase: 25 mM potassium dihydrogen phosphate : 10 mM 1-hexaneusulfonic acid [88].

For the assay of Telmisartan related substances in tablets formulation, a gradient reverse phase ultra-performance liquid chromatographic (RP-UPLC) method is developed by using Waters Acquity BEH C18 column (100 mm x 2.1 mm x 1.7 μm) and UV-detection at λ = 290 nm [89].

HPLC method for simultaneous quantification of low level impurities of Telmisartan and Hydrochlorothiazide in tablet dosage forms is reported [90].

UPLC method has been developed for simultaneous determination of Telmisartan impurities and Chlorothalidone impurities in their formulations. Chromatographic separation is carried out on an Acquity BEH Shield-RP18 column (100 mm x 1.7 μm), column temperature: 25°C, mobile phase A: pH = 4.5 buffer : acetonitrile = 90 : 10 v/v: mobile phase B: pH = 4.5 buffers : acetonitrile = 20 : 80 v/v, flow rate: 0.3 ml/min, UV-detection at λ = 290 nm, injection volume: 3 μl.

pH = 4.5 buffer is prepared using 0.025 M potassium dihydrogen phosphate, 0.0027 M 1-hexaneusulfonic acid sodium salt and 1 ml of triethylamine in milli-Q water. The gradient program is reported as follows: time (min.) / % mobile phase B: 0/20, 2/30, 5/45, 8/55, 10/80, 14/80, 14.1/20 and 18/20 [91].

Gradient HPLC with mas-spectrometry is used for the detection of 5 impurities of Valsartan: (S)-N-(1-carboxy-2-methylprop-1-yl)-N'-[2′-(1H-tetrazol-5-yl)-biphenyl-4-yl]amine
(S)-N-(1-carboxy-2-methylprop-1-yl)-N-(5-phenylthio) pentanoyl-N'=[2′-(1H-tetrazol-5-yl)-biphenyl-4-yl] amine (S)-N-(1-carboxy-2-methylprop-1-yl)-N-(5-phenyl)pentanoyl-N'=[2′-(1H-tetrazol-5-yl)-biphenyl-4-yl]-methyl amine (S)-N-(1-carboxy-2-methylprop-1-yl)-N-(5-phenyl)pentanoyl-N'=[2′-(1H-tetrazol-5-yl)-biphenyl-4-yl]-methyl amine

HPLC condition are: RP18, (250 mm x 4.6 mm x 5 μm); mobile phase A: 0.01 M KH2PO4 : 0.005 M K2HPO4; mobile phase B: water : acetonitrile = 1:4 v/v; gradient elution: A:B: 0/50, 20/70, 30/70, 40/80, 50/50, 60/50, 0.8 ml/min., λ = 210 nm [92].

Capillary zone electrophoresis is applied for chiral purity of Valsartan in tablets, where R-enantiomer of Valsartan is an impurity. Separations is carried out in a 50 μm, 64/56 cm fused-silica capillary, 25 mM phosphate buffer (pH = 8), containing 10 mM
acetyl-β-cyclodextrin as a chiral selector, applied voltage: 30 kV and temperature: 30 °C [93].

IV. Pharmacokinetic parameters of sartans.

Data on the pharmacokinetic parameters of sartans are summarized on TABLE 7. [94]: Candesartan [95], Irbesartan [96, 97], Olmesartan medoxomil [98], Valsartan [99].

<table>
<thead>
<tr>
<th>Sartan</th>
<th>Biological half-life [h]</th>
<th>Protein binding [%]</th>
<th>Bioavailability [%]</th>
<th>Renal clearance [%]</th>
<th>Hepatic clearance [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candesartan cilexetil</td>
<td>4-9</td>
<td>&gt;99</td>
<td>15-42</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Eprosartan</td>
<td>5-9</td>
<td>97-98</td>
<td>13</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>11-15</td>
<td>90-95</td>
<td>70</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>Losartan</td>
<td>2</td>
<td>98.7</td>
<td>33</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>EXP 3174</td>
<td>6-9</td>
<td>99.8</td>
<td>-</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Olmesartan</td>
<td>14-16</td>
<td>&gt;99</td>
<td>29</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>24</td>
<td>&gt;99</td>
<td>42-58</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Valsartan</td>
<td>6</td>
<td>95</td>
<td>25</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>

TABLE 7. Pharmacokinetic parameters of sartans.

There are the following features in the pharmacokinetic behavior of sartans: 1) suitability for oral administration as of physicochemical properties, determining good pharmacokinetic behavior 2) suitability to the criteria of the rule of Christopher A. Lipinski (Rule 5): a) Mr < 500; b) H – donors (NH, OH) < 5; c) H – acceptors (N, O) < 10; d) logP<5: LogP = 4.9 (Candesartan); LogP = 3.58 (Eprosartan); LogP = 4.52 (Irbesartan); LogP = 4.68 (Losartan); LogP= 4.31 (Olmesartan); LogP = 4.66 (Telmisartan); LogP = 3.68 (Valsartan) [100]. 3) high plasma protein binding, which provide to be obtained once daily.

Telmisartan is with the most high oral bioavailability and with the longest half-life. The bioavailability of other sartans is: Tasosartan (3-7%); Zolasartan (20%); Enoltasosartan (36-72%) [100].

V. Metabolism of sartans.

Drug metabolism (biotransformation) is the major mechanism for maintaining homeostasis in the body exposure to the effects of drugs, pesticides, environmental contaminants. In biotransformation drug molecules are converted into metabolites which are with large polarity (by the addition of ionizable groups), with high degree of ionization at physiological pH, reduced capacity for protein binding, high molecular weight and larger sized molecules [101].

Under the influence of specific enzymes: cytochrome P450; microsomal aminooxidase with mixed function; Z-uridilphosphoglucuronil transferase; glutathione-S-transferase is going the phase of functionalization (1 phase, non-synthetic chemical changes: oxidation, reduction, hydrolysis and hydration) in which in molecules are introduced active groups: -OH; -COOH; NH2; SH, which leads to increased hydrophilicity [101].

Factors affecting the metabolism are:

I. Chemical: lipophilicity, molecular size, degree of ionization.

II. Biological: gender, body type, age, hormones, diseases:

a) chronic liver diseases: reduce the processes of conjugation
b) chronic renal insufficiency: decreases the levels of cytochrome P450 and slows down the metabolic changes
c) infectious diseases: increase the level of endogenous interferon component that inhibits certain metabolic pathways
d) acute alcohol impairment: inhibition of enzyme activity by contacting of ethanol with cytochrome P 450
e) chronic alcohol damage: induction of enzyme activity in I and II biotransformation phase [64].

III. Genetic: fast and slow metabolizers (acyetylators).

Metabolites are excreted or participate in the phase II (conjugation): detoxifying reactions: acetylation, methylation, conjugation of a drug molecule or its metabolites from the first phase to the highly polar, water-soluble natural endogenous substances: glucuronic acid (O-, N-, S-, C- glucuronide); glutathione; amino acids: glycine, taurine, glutamic acid [101].

75% of the drug metabolism is under an influence of Cytochrome P450 enzymes. CYP3A4 is responsible for 50% of drug metabolism [101]. CYP2C is the second expressed P450 subfamily in human liver and CYP2C9 is the most highly expressed isoform [102].

The differences observed in lipid solubility, absorption/distribution, plasma protein binding, bioavailability, biotransformation, plasma half-life, and systemic elimination influence the duration of action, and efficacy of sartans. On the basis of the daily mg dose, the antihypertensive potency of sartans follows the sequence: Candesartan cilexetil > Telmisartan = Losartan > Irbesartan = Valsartan > Eprosartan [103]. Sartans are metabolized in the liver by the microsomal liver enzymes Citochrome P450 2C9 (CYP2C9) and P450 3A4 (CYP3A4), but not by CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A5 and 4A11.

After oral administration approximately 14% of Losartan dose is converted to the pharmacologically active metabolite: 5-carboxylic acid, designated as EXP 3174 [104]. Metabolite is long-acting (6 to 8 h) and is 10-40 times more potent in blocking AT1 receptors than Losartan [105]. Both Losartan and EXP 3174 are more than 98% bound to plasma proteins [106]. The major metabolic pathway for Losartan is by the Cytochrome P450 (CYP) 3A4, 2C9 and 2C10 isoenzyme [104]. The two-step oxidation of Losartan to EXP 3174 is catalyzed by CYP3A4 [107, 108] and CYP2C9 in human liver microsomes [108]. Hydroxymethyl group at C5 is oxidized to a carboxyl group of the active metabolite EXP 3174 (Fig. 4) [107].

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Candesartan cilexetil, Emhusartan, Losartan, Olmesartan medoxomil and Tasosartan are ester prodrugs and after absorption through the gastrointestinal tract are hydrolysed by esterases to its respective active metabolite: Candesartan-7-carboxylic acid, BAY 10-6735, EXP 3174, Olmesartan and Enoltasosartan. The metabolites are more active than the corresponding prodrug: Candesartan-7-carboxylic acid: 30-100 times more than Candesartan cilexetil. Candesartan slowly is biotransformed to a very small extent by oxidation by CYP2C9 [95].

Irbesartan is metabolized in the liver by oxidation of P450 CYP2C9 and less by CYP3A4 and subsequent glucuronidation to Irbesartan main metabolite glucuronide (6 %) [96].

Tasosartan is biotransformed by oxidation of one or both methyl groups. Experimental oxidation is carried out by: a) SeO₂, dioxane/water to the aldehyde derivative (6), which is oxidated from b): benzeneseleninic acid/H₂O₂ in THFtetrahydrogruane to a carboxyl derivative (Fig.4.). The major metabolite of Tasosartan e Enoltasosartan, which is active [109].

Valsartan is oxydazed from CYP2C9 to non-active metabolite 4-hydroxyvaleryl-Valsartan (Fig.5.) [42]. Olmesartan medoxomil is hydrolysed [110] by carboxymethylenebutenolodinase in the liver to the active metabolite RNH-6270 FK9 [111].

Paraoxonase 1 as a major bioactivating hydrolase for olmesartan medoxomil in human blood circulation [112].

Pratosartan is biotransformed to: (S)-(+)2-(1-hydroxypropyl)-3-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-5,6,7,8-tetrahydro-3H-cycloptimadazol-4-one; 8- and 5-hydroxylated metabolite.

Candesartan cilexetil and its metabolite in human plasma and urine are quantified on Sphersorb S3P (phenyl) column (100 mm x 4.6 mm x 3 μm), mobile phase A: phosphate buffer (pH = 2.8) : acetonitrile : water = 20 : 20 : 60 v/v; phase B: phosphate buffer (pH = 2.8) : acetonitrile : water = 20 : 60 : 20 v/v, flow-rate was 1.0 ml/min., λexcitation = 265 an λemission = 395 nm. The acetonitrile gradient A/B is: 0-9 min.: 80 %/20 %; 9-14 min.: 68 %/32 %, 14-25 min.: 80 %/20 % [113].

Losartan and its metabolite EXP 3174 are determined in biological material by HPLC, after liquid-liquid extraction on column ULTREXEM CN and UV-detection at λ = 245 nm [114]. Other HPLC with UV-detection method include analysis in human plasma, urine and dialysate. For plasma gradient method with phenyl analytical column, mobile phase: 25 mM potassium phosphate : acetonitrile = 70 : 30 v/v and fluorescence detection is used. For urine and dialysate an isocratic mobile phase: 25 mM potassium phosphate : acetonitrile = 60 : 40 v/v (pH = 2.2) is applied [115].

After extraction of Losartan and EXP 3174 from human plasma with ether, HPLC separation is carried out on Diamonsil C18 column, mobile phase: 0.02 M sodium dihydrogen phosphate buffer (pH = 2.35 with phosphoric acid) : acetonitrile = 57 : 43 v/v, flow-rate: 0.5 ml/min., fluorescence detection at λexcitation = 250 nm and λemission = 370 nm [116].

Yan Other applied HPLC method is with U1tremex CN.
CONCLUSION

The most applied methods for analysis of impurities of sartans, due to highest selectivity and specificity in separation and detection, are: HPLC-MS, HPLCMS/MS, HPLC-ESI/MS and HPLC-TOF/MS.

The important pharmacokinetic data of sartans are: suitability to the criteria of the rule of Christopher Lipinski: LogP = 4.9 (Candesartan); LogP = 3.58 (Eprosartan); LogP = 4.52 (Irbesartan); LogP = 4.68 (Losartan); LogP = 4.31 (Olmesartan); LogP = 4.66 (Telmisartan); LogP = 3.68 (Valsartan) and high plasma protein binding.

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