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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 aldosteron 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, HPLCMS/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 (3-7 %); Zolasartan (20 %); Enoltasosartan (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 ensyme 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];

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 plasminogen [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 ensyme inhibitors [44]; a) hypertension: Benazepril: Valsartan [45]; b) chronic heart failure [46]: Candesartan [47, 48]; c) diabetic nephropathy [49]; d) proteinuria [50]
4) thiazide diuretic Hydrochlorthiazide 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].

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 hihgher selectivity and specificity in separation and detection. Defferent 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.

SARTAN	Empirical structure	Molecular mass	Melting point
Abitesartan	$C_{26}H_{31}N_5O$	461.556	154-156
Azilsartan	$C_{30}H_{24}N_4O_8$	568.5	157-159
Candesartan	$C_{24}H_{20}N_6O_3$	440.45	183-185
Candesartan Cilexetil	$C_{33}H_{34}N_6O_6$	610.66	163
Elisartan	$C_{27}H_{28}ClKN_6O_5$	591.1	188-190
Eprosartan	$C_{23}H_{24}N_2O_4S$	424.52	260-261
Eprosartan Mesylate	$C_{23}H_{24}N_2O_4S.$ CH_3SO_3H	520.625	248-250
Embusartan	$C_{25}H_{24}FN_5O_3$	461.488	250-252
Fimasartan	$C_{27}H_{31}N_7OS$	501.65	260-263
Irbesartan	$C_{25}H_{28}N_6O$	428.23	180-181
Losartan	$C_{22}H_{23}ClN_6O$	422.92	183.5-184.5
Losartan Potassium	$C_{22}H_{22}ClKN_6O$	461.01	183.5-184.5
Milfasartan	$C_{30}H_{30}N_6O_3S$	554.667	192-195
Olmesarta Medoxomil	$C_{29}H_{30}N_6O_6$	558.585	175-180
Pomisartan	$C_{31}H_{30}N_4O_2$	490.596	176-178
Pratosartan	$C_{24}H_{25}N_6O$	413.499	184-187
Ripisartan	$C_{23}H_{22}N_8O$	426.476	235-238
Tazosartan	$C_{23}H_{21}N_7O$	411.459	243-246
Telmisartan	$C_{33}H30N_4O_2$	514.617	261-263
Saprisartan Potassium	$C_{25}H_{22}BrF_3N_4O_4SK$	611.431	272-275
Valsartan	$C_{24}H_{29}N_5O_3$	435.519	116-117
Zolasartan	$C_{24}H_{20}BrClN_6O_3$	555.814	421.5

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).

TABLE 2. Related substances of Candesartan.

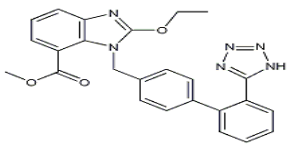
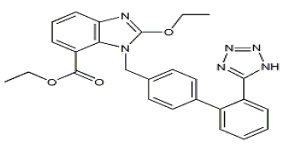
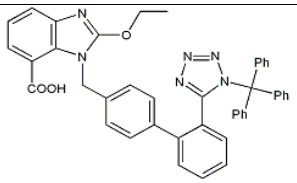
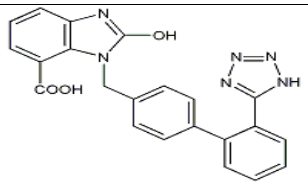
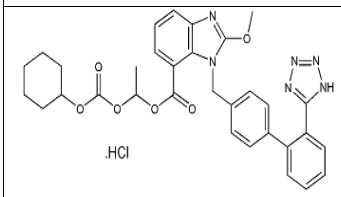
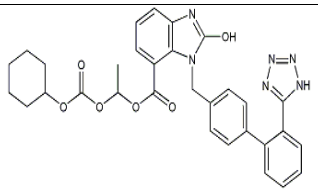
 Candesartan Methyl Ester	 Candesartan Ethyl Ester
 Trityl Candesartan	 Desethyl Candesartan
 Candesartan Cilexetil HCl Methoxy Analog	 Desethyl Candesartan Cilexetil

TABLE 3. Impurities of Losartan.

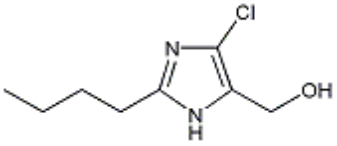
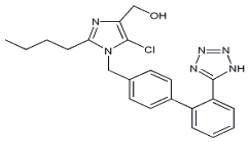
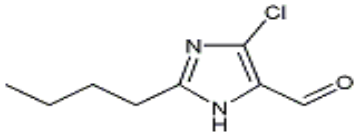
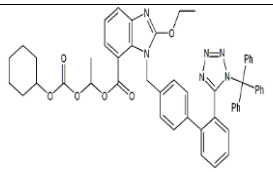
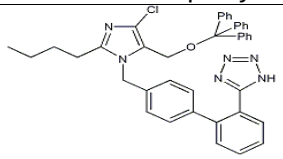
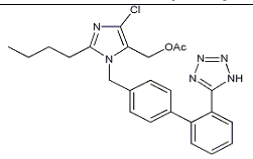
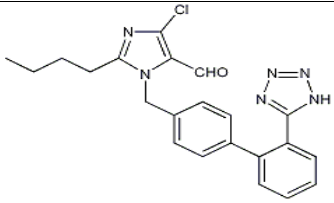
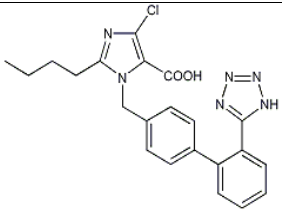
 Losartan EP Impurity A	 *Losartan EP Impurity C*
 Losartan EP Impurity D	 Losartan EP Impurity H
 Losartan EP Impurity I	 Losartan EP Impurity J
 Losartan EP Impurity K Losartan Aldehyde	 Losartan Carboxylic Acid

TABLE 4. Impurities of Losartan, Candesartan, Olmesartan.

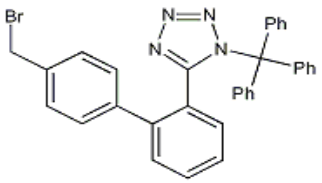
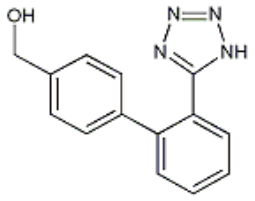
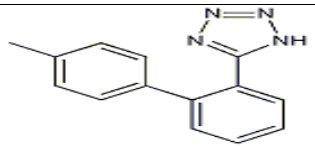
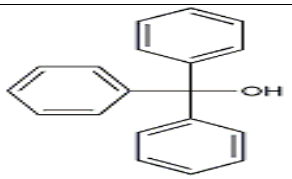
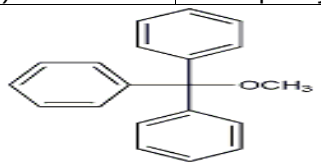
 5-(4'-(Bromomethyl)(1,1'-bi-phenyl)-2-yl)-1-trityl-1H-tetrazole	 p-(o-1H-tetrazol-5-ylphenyl)benzyl alcohol
 p-(o-1H-tetrazol-5-ylphenyl)toluene	 Triphenylmethanol
 Triphenylmethyl Methyl Ether	

TABLE 5. Impurities of Telmisartan.

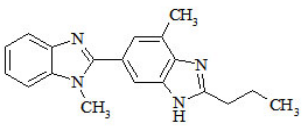
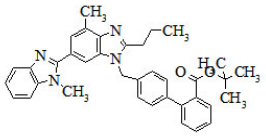
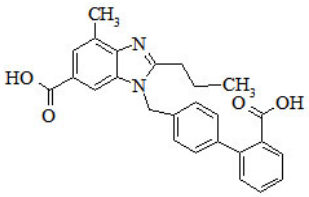
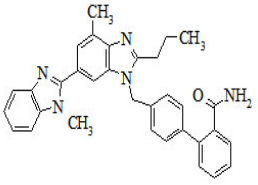
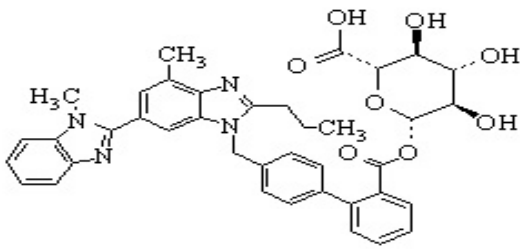
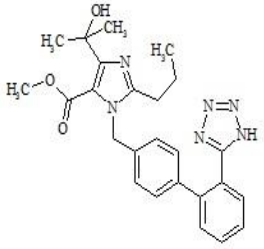
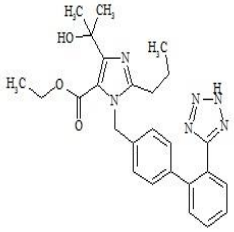
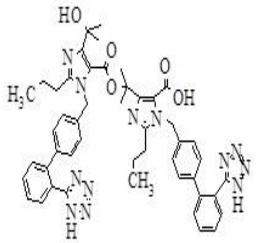
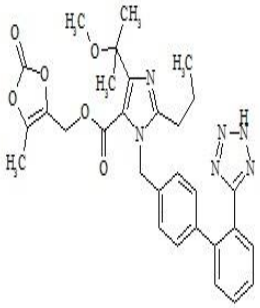
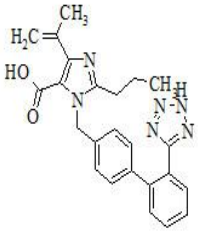
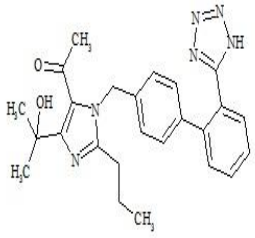
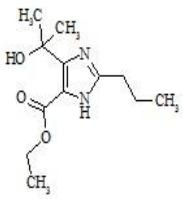
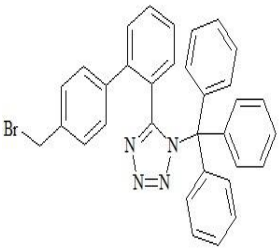
 Telmisartan Impurity A	 Telmisartan Impurity C
 Telmisartan Impurity E	 Telmisartan Impurity F
 Telmisartan acyl glucuronide	

TABLE 6. Related substances of Olmesartan.

 Olmesartan Methyl Ester	 Olmesartan *Ethyl Ester*
 Olmesartan Dimer Ester	 Olmesartan Methyl Ether
 Olmesartan Medoxomil Dehydro Impurity	 Olmesartan Methyl Ketone
 Ethyl 4-(1-Hydroxy-1-methylethyl)-2-Propyl-Imidazole-5-Carboxylate)	 Olmesartan (N-(Triphenyl-methyl)-5-(4'-bromomethyl-biphenyl-2-yl)tetrazole)

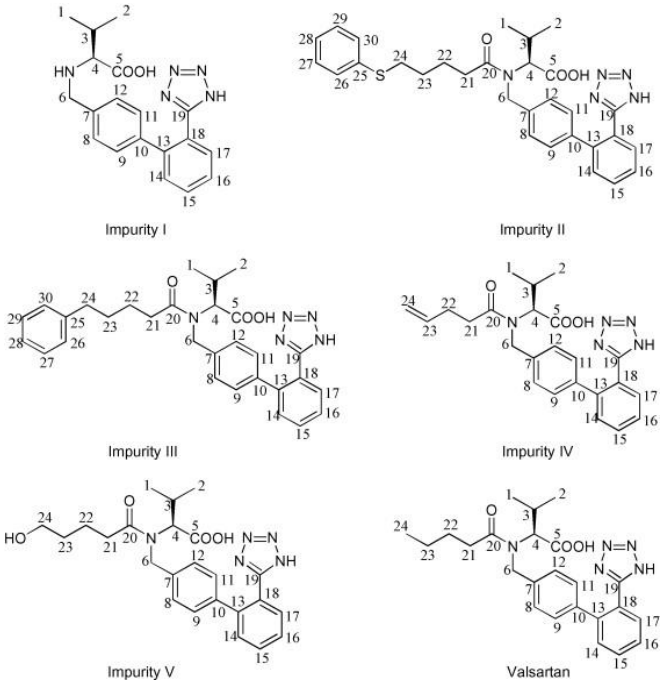


Fig. 1. Related substances of Valsartan.

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-5yl)biphenyl-4-yl]-methyl}valine benzyl ester.

Imp III, Imp IV and Imp V 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].

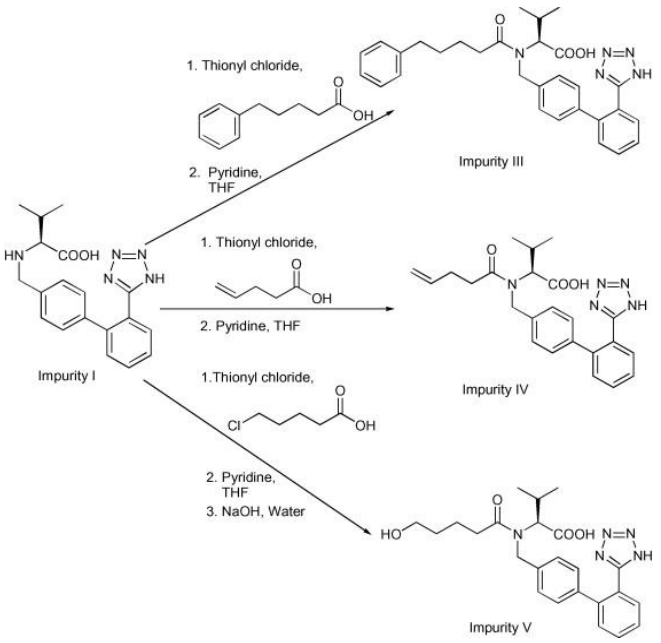


Fig 2. Impuirities of Valsartan.

Imp II (Fig. 2) is formed at the last step of the synthesis of Valsartan in a reductive cleavage of the phenyl sulfide group in the presence of Raney nickel in aqueous sodium hydroxide solution. Imp III and IV may result from desulphurization or free radical processes. 5-Chlorovaleric acid is a precursor of 5-phenylthiovaleric acid, from which is synthesized Imp II. Unreacted 5-chlorovaleric acid and 5-phenylthiovaleric acid reacted with Imp I and the product after alkaline hydrolysis gives Imp V (Fig. 3.) [71].

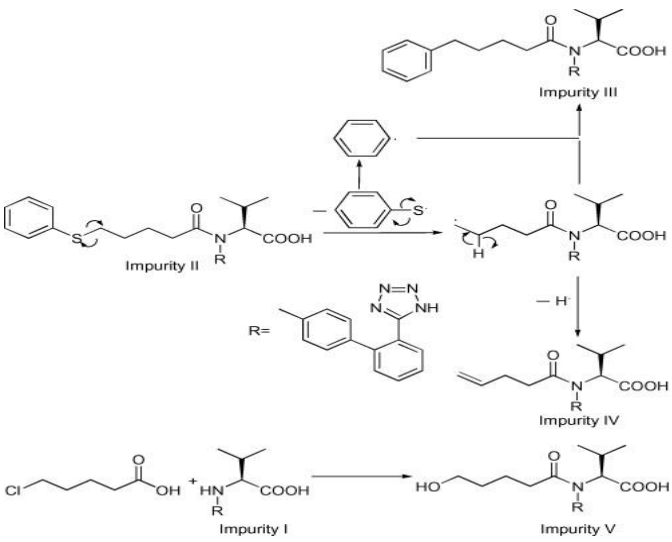


Fig. 3. Valsartan Imp III and Imp IV, obtained from Imp I and Imp II.

III. Methods for analysis of impurities of sartans.

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 kamedoxomil on YMC-Pack C_{18} 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].

Is is reported that Candesartan impurity: 2-ethoxy-1-[[2′-(1-ethyl-1H-tetrazol-5-yl)biphenyl-4-yl] me-thyl]-1H-benzimidazole-7-carboxylic acid ethyl ester is determined by HPLC with mas-specrometry detectioin with electrospray ionisartion (MS/ESI) [77].

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_{18} 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 C_{18} 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 C_{18} 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-diyl)-bis-(2-butyl-1H-imidazole-5,1-diyl)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 C_{18} 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 C_{18} column, (4.6 mm × 150 mm, 3 µm), column temperature: 25°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 100-5C_{18} column (250 x 4.6 mm x and 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 potassium 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-methylethyl)oxy]methyl]-1H-imidazol-1-yl]methyl]biphenyl-2-yl]-1H-tetrazole)

I 5-[4′-[[2-butyl-4-chloro-5-[[(triphenylmethyl]oxy]methyl]-1H-imidazol-1-yl]methyl]biphenyl-2-yl]-1H-tetrazole)

G (triphenylmethanol) [87].

The reported chromatographic system is: ACCHROM ODS-C_{18} 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 C_{18} column (150 mm x 4.6 mm x 3.5 µm), mobile phase: 25 mM potassium dihydrogen phosphate : 10 mM 1-hexaneusulphonic 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 Aquity BEH C_{18} 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 Chlorthalidone impurities in their formulations. Chromatographic separation is carried out on an Acquity BEH Shield-RP_{18} column (100 x 2.1 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-hexanesulphonic 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 Valsatan:
(*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine
(*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-(5-phenylthio) pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl] amine
(*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-(5-phenyl)pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4yl-methyl] amine
(*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-4-pentenoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine
(*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-(5-hydroxy) pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]amine [92].

HPLC condition are: RP_{18}, (250 mm x 4.6 mm x 5 µm); mobile phase A: 0.01 M KH_2PO_4 : 0.005 M K_2HPO_4; 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].

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

TABLE 7. Pharmacokinetic parameters of sartans.

There are the following features in the pharmacokinetic behavior of sartans:
1) suitability for oral administration as of physico-chemical 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 (I phase, non-synthetic chemical changes: oxidation, reduction, hydrolysis and hydratetion) in which in molecules are introduced active groups: -OH; -COOH; NH_2; 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 P_{450} 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 acti-vity 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 (acetylators).

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 inder 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 AT_1 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].

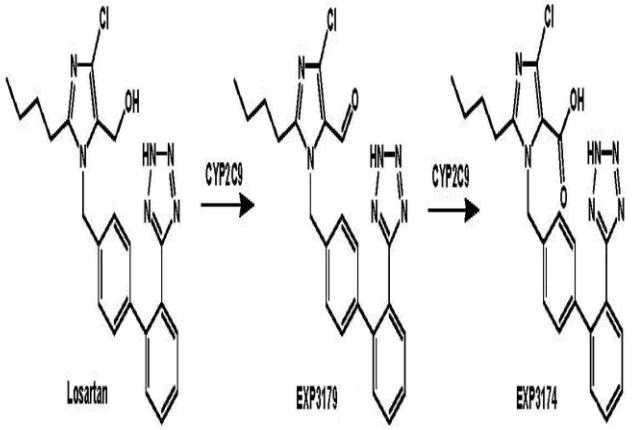


Fig. 4. Oxidation of Losartan to EXP3179 and EXP3174.

Candesartan cilexetil, Embusartan, 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 moe 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) SeO_2, dioxane/water to the aldehyde derivative (6), which is oxidated from b): benzeneseleninic acid/H_2O_2 in THFtetrahydrogurane to a carboxyl derivative (Fig.4.).The major metabolite of Tasosartan e Enoltasosartan, which is active [109].

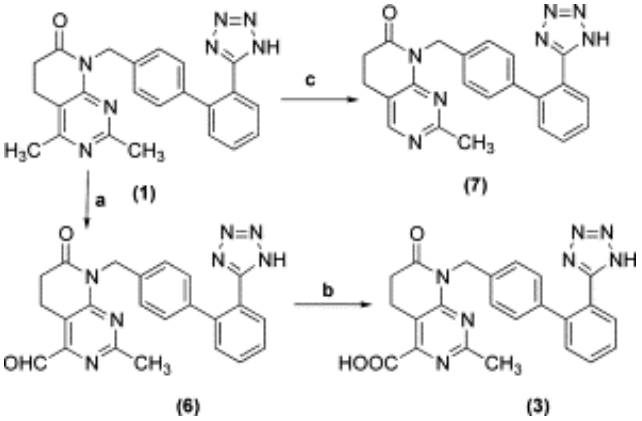


Fig. 4. Oxydation of Tasosartan.

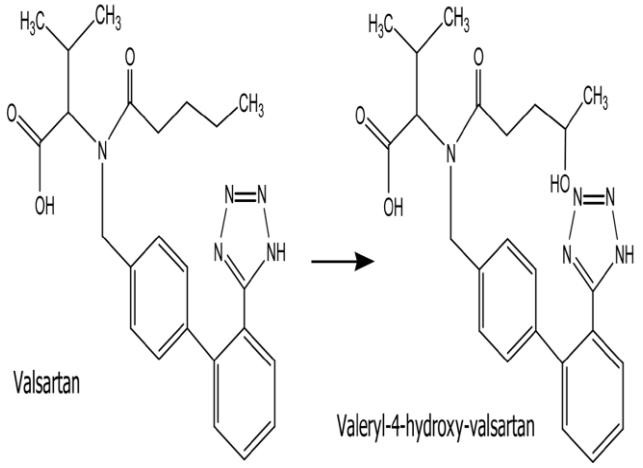


Fig. 5. Oxydation of Valsartan to valeryl-4-hydroxy-valsartan.

Valsartan is oxydazed from CYP2C9 to non - acticve metabolite 4-hydroxyvaleryl-Valsartan (Fig. 5.) [42].

Olmesartan medoxomil is hydrolysed [110] by carboxymethylenebutenolidase 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-cycloheptimidazol-4-one; 8- and 5-hydroxylated metabolite.

Candesartan cilexetil and its metabolite in human plasma and urine are quantified on Spherisorb 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 ULTREMEX 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 C_{18} 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 λ excitation = 250 nm and λemission = 370 nm [116]. Yan Other applied HPLC method is with U1tremex CN

column (250 mm x 4.6 mm x 5 µm), column temperature: 35 °C, mobile phase: isophosphoric acid (pH = 2.3; 0.015 M) : acetonitrile = 75 : 25 v/v; flow rate: 1.25 ml min., 35 °C, fluorescence detection at λ excitation = 250 nm and λ emission = 370 nm [117].

HPLC/MS with electrospray ionization with pneumatically-assisted nebulization is reported for the simultaneous determination of Losartan and EXP-3174 in human plasma, after extraction under acidic conditions by solid-phase extraction on a sorbent of copolymer, poly(divinylbenzene-co-N-vinylpyrroli-done). The analytes are separated on RP C_{18} Phenomenex Synergy 4 POLAR-RP 80A column (50 mm x 2 mm), column temperature: t = 40 ^{o}C, isocratic mobile phase: 0.1 % triethylamine/0.1 % acetic acid (pH =7.1) : acetonitorile = 65 : 35 v/v, flow rate: 0.7 ml/min. [118].

Losartan and Losartan acid in human plasma are estimated by LC-MS/MS method, after extraction from human plasma by solid phase extraction technique using Oasis HLB cartridge and following separationd on Zorbax SB C_{18} column [119].

In human plasma isocratic LC-MS-MS is applied by mobile phase: 0.1 % triethylamine: 0.1% acetic acid (pH = 7.1) : acetonitorile = 65 : 35 v/v [120].

HPLC-MS/MS for simultaneous assay of Losartan, EXP-3174 and Hydrochlorothiazide in human plasma is developed after liquid-liquid extraction, elution from C_{18} column and detectioin with an API 3000 triple-quadrupole mass spectrometer using negative electrospray ionization and multiple reaction monitoring [121].

UPLC/MS/MS with multiple reaction monitoring in the negative ionization mode is described for Losartan, EXP-3174 and Hydrochlorothiazide in human plasma, after solid-phase extraction on Oasis HLB and analysis on BEH C_{18} column, mobile phase: 1 % formic acid : acetonitrile = 15 : 85 v/v [122].

Losartan and its active metabolite EXP 3174 in human plasma and urine with limited plasma sample after liquid-liquid extraction are separated bt RP HPLC/MS [123].

For analysis of Valsartan and its metabolite in human plasma is proposed solid phase extraction, followed by HPLC separation on RP C_{18} Atlantis C18 column (100 mm x 3.9 mm), column temperature was kept to 40 ^{o}C, mobile phase: acertinitril :phosphate buffer (5 mM, pH = 2.5) in gradient mode at flow rate: 1.30 ml/min., fluorescence detector at λemission = 234 and λexcitation = 378 nm UV-detection at λ = 254 nm. Gradient elution mode is applied by the initial percentage of acetonirile 32 % with a stepness of 4.5 %/min. to reach the 50 % [124].

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.

Telmisartan is with the most high oral bioavailability and with the longest half-life.

Sartans are metabolized in the liver by the microsomal liver enzymes tsitihrom P450 2C9 (CYP2C9) and P450 3A4 (CYP3A4), but not by CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A5 and 4A11.

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