SOCIAL AND HEALTH IMPORTANCE AND ANALYTICAL CHARACTERISTICS OF LOSARTAN

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Summary. The aim of current study is to summarize and comprise the different developed methods for determination of angiotensin receptor blocker Losartan Potassium alone and in combination with calcium antagonists, ACE – inhibitors and diuretics (Hydrochlorothiazide) in dosage pharmaceutical preparations (tablets, capsules) and in biological materials (plasma, urine). The reported techniques for the determination of Losartan Potassium include: 1) high performance liquid chromatography (HPLC) with UV – detection (tablets, capsules, plasma, urine); 2) HPLC with fluorescence detection (plasma, urine); 3) HPLC with mass detection (tablets, plasma); 4) supercritical fluid chromatography (tablets); 5) high performance thin layer chromatography (HPTLC) (tablets); 6) capillary zone electrophoresis (tablets); 7) capillary electrophoresis; 8) micellar electrokinetic capillary chromatography (tablets); 9) UV – derivative spectrophotometry (tablets); 10) spectrofluorimetry (urine). From the results is obvious, that for determination of Losartan Potassium, HPLC is the most widely used method and spectrofluorimetry is specific for analysis in urine.

Key Words: Losartan Potassium, analytical methods, analysis, HPLC, HPTLC, UV – spectrophotometry.

Hypertension is one of the most important social disease, from which are suffering more than 1 billion people all over the world. In the regulation of blood pressure the rennin – angiotensin system plays very important role. The cascade begins with the cleavage of angiotensinogen by renin to the decapptide angiotensin I. Angiotensin converting enzyme produces the powerful vasoconstrictor angiotensin II by clipping a dipeptide unit [1].

Sartans are angiotensin II receptor antagonists which reduce the pressor effects of vasoconstrictor angiotensin II. The therapy with sartans is with higher social importance because offers a good quality of life for hypertensive patients due to the absence of side effects [2]. In comparison with monotherapy the combination therapy is more effective in reducing the blood pressure due to synergistic effects [3]. Because of their variety of pharmacological activities sartans are widely applied in clinical practice alone or in combinations with other antihypertensive drugs such as beta blockers, calcium antagonists, diuretics, angiotensin – converting enzyme inhibitors for treatment of hypertension (mild, severe and associated with diabetes mellitus) [4, 5]. For therapy of hypertension sartans are very often applied in fixed combinations with thiazide diuretic Hydrochlorothiazide (HCTZ): Candesartan [6]; Eprosartan [7]; Irbesartan [8, 9]; Losartan Potassium [6]; Olmesartan, Valsartan, Telmisartan [9]. Losartan is non – peptide antihypertensive agent with a gradual and long – lasting effect and exerts its effective blood pressure lowering action by specific blocking of angiotensin II receptors. Losartan is prescribed alone or combined with diuretic Hydrochlorothiazide (HCTZ) for therapy of essential hypertension [10, 11], stroke prevention [10, 12, 13], moderate heart failure [11, 14], atrial fibrillation [12, 15, 16], hypertensive patients with type 2 diabetes mellitus [17]. Losartan improves endothelial function in non insulin dependent diabetes [18] and possesses antialbuminuric effect in hypertensive patients with type 2 diabetes mellitus [19]. Oxidative stress is involved in the initiating of pathogenesis of hypertension [20], stroke [21], vascular disease [22] and atherothrombosis [23]. Important antioxidants are alfa lipoic acid for diabetic neuropathy and flavonoids diosmin and hesperidin for chronic venous insufficiency, coumarins [24], Galantamine [25], peptide esters of Galantamine [26]. In this connection it is reported one new role of Losartan as antioxidant in renal transplant recipients with renin – angiotensin system polymorphisms [27]. Losartan is applied in combination with inhibitors of angiotensin – converting enzyme (Enalapril, Lisino-
Several methods have been reported for analysis of Losartan Potassium (Los) and its degradation products in drugs and biological materials [31]: 1) spectrophotometry (SFM); 2) spectrophotofluorimetry; 3) high performance thin layer chromatography (HPTLC); 4) capillary zone electrophoresis (CZE); 5) multisyringe chromatography (MSC); 6) supercritical fluid chromatography (SFC); 7) HPLC [32]. Literature review reveals that UV spectrophotometry [33, 34, 35, 36, 37, 38, 39], HPTLC [40] and HPLC [41, 42] methods are very often applied for the estimation of Losartan in pharmaceutical dosage forms. Different spectrophotometric methods for the determination of Losartan Potassium in tablets or in capsules are developed. UV method at \( \lambda = 208 \) nm in tablets is one of the most simple [43]. A first - derivative UV quantitative analytical method is used for the determination of Losartan [44] by measurement of the absorption at \( \lambda = 232.5 \) nm in Cozaar tablets 25 mg [45] or at \( \lambda = 234 \) nm in tablets [46] and in capsules [47]. Second - derivative UV spectrophotometry is described for analysis of Losartan in tablets [48, 49], by measuring the distance between two extremum values at \( \lambda = 219.6 \) nm and \( \lambda = 228.8 \) nm (in Cozaar tablets 50 mg) [48]. Two - wavelength ratio UV spectrophotometry (206.6 nm/270.6 nm and 236 nm/270 nm) [50] and dual - wavelength UV spectrophotometry: (A261.4 nm - A206.6 nm) are also applied [50]. Spectrophotometry in visible area for analysis of Losartan in bulk and in synthetic mixture for solid dosage forms is proposed. Method is based on measurement of the absorption of derivative an orange – red colour complexes of Losartan in acidic medium (pH = 1.2) after reaction with Calmagite at \( \lambda = 491 \) nm or Orange - II at \( \lambda = 486 \) nm [51]. In other similar methods are used derivative reaction between Losartan and 2.3 - dichloro - 5.6 - dicyano - 1.4 - benzoquinone and measuring the complex at \( \lambda = 460 \) nm [52] or with bromothymol blue and phosphate buffer [34]. For Losartan Potassium and Atenolol is described extractive spectrophotometric method based on the formation of coloured complexes in the presence of buffer of pH = 7 with different reagents: the purple complex between Losartan Potassium and ferroin solution and the yellow complex from Atenolol with methyl orange solution. After quantitatively extraction with chloroform the absorbances of complexes in the organic layers are measured at \( \lambda = 571 \) nm and \( \lambda = 426.0 \) nm for Losartan Potassium and \( \lambda = 426 \) nm for Atenolol [53]. For the simultaneously determination of Losartan in combination with HCTZ or Amlodipine (Aml) or Enalapril are described different spectrophotometric methods. First derivative UV spectrophotometry is reported for analysis of Losartan and HCTZ in tablets [54] by measurement of the absorption at \( \lambda_{\text{max Los}} = 271.6 \) nm (zero crossing wavelength for HCTZ) and \( \lambda_{\text{max HCTZ}} = 335.0 \) nm (zero crossing point for Losartan [55] or at \( \lambda_{\text{Los}} = 222 \) nm and \( \lambda_{\text{max HCTZ}} = 332 \) nm [56]. First derivative UV method is also used for Losartan and Amlodipine in tablets [57, 58] and for Amlodipine 5 mg/Losartan 12.5 mg/HCTZ 50 mg in Trilopace® tablets by measurement of the absorption at \( \lambda_{\text{max Aml}} = 236.5 \) nm, \( \lambda_{\text{Los}} = 254 \) nm and \( \lambda_{\text{max HCTZ}} = 271 \) nm [59]. First derivative spectrophotometry of the ratio spectrum is described for quantification of components in Hysaaz® filmtablets: Losartan 50.0 mg/HCTZ 12.5 mg at \( \lambda = 238.36 \) nm [60] and for combination from Losartan Potassium and Atorvastatin in tablets [61]. Compensation technique by calculation the ratios of the maxima and minimum of the first derivative spectra is applied for Losartan 50.0 mg and HCTZ 12.5 mg in Hysaaz® filmtablets: 218 nm/236 nm (Los), 230 nm/261 nm (HCTZ) [60]. Absorbance ratio (Q – analysis method) for analysis of Losartan and Amlodipine in tablets is based on measurement of absorption at an iso - absorptive point \( \lambda = 242.5 \) nm and \( \lambda_{\text{max Aml}} = 237.5 \) nm [58]. The same method is appropriate for Losartan and HCTZ in tablets by measurement of absorbances at isosbestic wavelength (\( \lambda = 266.5 \) nm) and wavelength of maximum absorption of HCTZ (\( \lambda = 272 \) nm) [56]. Simultaneous UV equation method in tablets employs formation and solving of simultaneous equation by measurement the absorbance at: 1) \( \lambda = 208 \) nm (Amlodipine and Losartan) and at absorbance maximum of Amlodipine \( \lambda = 237.5 \) nm [58]. 2) \( \lambda_{\text{Los}} = 250 \) nm and \( \lambda_{\text{Enalapril}} = 222 \) and [62] in tablets Envas (25 mg Losartan/5 mg Enalapril); 3) \( \lambda_{\text{Los}} = 251.6 \) nm, \( \lambda_{\text{Atenolol}} = 224.2 \) nm, \( \lambda_{\text{HCTZ}} = 271.6 \) nm [63]; 4) \( \lambda_{\text{Los}} = 218 \) nm, \( \lambda_{\text{HCTZ}} = 272 \) nm and \( \lambda_{\text{isosbestic point}} = 266.5 \) nm [56]; 3) \( \lambda_{\text{Los}} = 236 \) nm and \( \lambda_{\text{HCTZ}} = 270 \) nm [64]; 4) \( \lambda_{\text{Los}} = 206.6 \) nm and \( \lambda_{\text{HCTZ}} = 276.6 \) nm [50]. Dual wavelength method uses the difference of absorbance values at \( \lambda = 206.6 \) nm and \( \lambda = 261.4 \) nm for the estimation of Losartan and absorbance values at 270.6 nm for the estimation of HCTZ [50]. AUC method for Losartan, Amlodipine and HCTZ is based of measurement of absorbations at: 266 nm –
HPTLC method is described for simultaneously de- 
e: methanol : triethylamine = 6.5 : 4 : 0.5 v/v [71]; acetone : formic acid = 7.5 : 1.5 : 5 : 0.03 v/v; toluene : CH₃COOH, detection at λ = 254 nm [73, 74, 75]. Capillary zone electrophoresis is applied for: 1) Losartan in Cozaar® tablets [76]; 2) Losartan/HCTZ in tablets [77] and in capsules [78]; 3) Losartan/Chlorthalidone in capsules [59]; 4) Losartan/irbesartan/telmisartan in urine with internal standard Candesartan [79]; 5) Losartan/candesartan/eprosartan/irbesartan/telmisartan/valsartan in tablets [80]; 6) Losartan/candesartan/eprosartan/irbesartan/telmisartan/valsartan/hctz in tablets [81]; 7) Losartan/sartans [82]. Other reported methods are: I) capillary electrochromatography [76]; II) Micellar Electrokinetic Capillary Chromatography (MEKC): 1) Losartan in Cozaar® tablets [76], by system equipped with a variable – wavelength UV detector, fused silica capillaries Phoenix, coated with a UV – transparent polymer; 2) Losartan and several angiotensin – II – receptor antagonists [83]; III) Multi – syringe chromatography (MSC): for Losartan/HCTZ in superficial water, groundwater and wastewater outlet samples, flow rate = 0.8 ml/min., UV at λ = 226 nm [84]; IV) Supercritical fluid chromatography (SFC) for Losartan in Cozaar® tablets with UV detector [76]. HPLC is the most common analytical technique for the determination of different drugs: 1) vitamins (B₆ and C): stationary phase: column RP – C₁₈ ODS (4.6 mm x 250.0 mm, 5 μm), column temperature: 40°C, mobile phase: acetonitrile : distilled water = 60 : 40 v/v; flow rate:1.0 ml/min., UV – detection at λ = 254 nm [85]; 2) Amino acids on stationary phase: column Spherisorb ODS RP – C₁₈, column temperature 25°C, mobile phase: methanol : distilled water = 1 : 1 v/v; flow rate: 1.0 ml/min., analytic alwavelength λ = 210 nm [86]; 3) Lisinopril on column Spherisorb RP C₁₈ ODS – 2 (4.6 mm x 250.0 mm, 5 μm), column temperature 40°C, mobile phase: 0.125 % solution of sodium 1–hexanesulfonate in phosphate solution (pH = 2) : acetonitrile = 800 : 200, isocratic flow rate: 1.5 ml/min., detection: λ = 215 nm [87, 88]; 4) combination of: Atenolol (1): t_R=10.6 min., Metoprolol (3): t_R=7.6 min., Bisoprolol (4): t_R=8.3 min., Propranolol (5): t_R=8.7 min., Diltilzem (6): t_R=9.2 min., Flecainide (7): t_R=9.3 min., Amlodipine (8): t_R=9.5 min., Carvedilol (9): t_R=9.6 min., Verapamil (10): t_R=9.9 min., Losartan (11): t_R=10.2 min., Irbesartan (12): t_R=10.7 min., Telmisartan (13): t_R=10.8 min., Valsartan (14): t_R=11.1 min., internal standard Diazepam (15): t_R=11.5 min. (Fig. 1 ) [89].

Losartan is analysed by HPLC: 1) in substance: 1 ml/min., t = 35°C, methanol as diluent, λ = 254 nm

Fig. 1. HPLC for determination of beta – blockers and sartans [70].
For the determination of Losartan in tablets are used the following HPLC methods: A) isocratic RP HPLC on: 1) Novapack ODS (4.0 mm x 150 mm, 5 μm), phosphate buffer (pH = 6.2): acetonitrile = 60 : 40 v/v, 1 ml/min.; λ = 254 nm [44]; 2) Shim-pack C18 (4.6 mm x 250 mm, 10 μm), acetonitrile : phosphate buffer (pH = 3.8): 40 : 60 v/v, 1.1 ml/min., λ = 235 nm [50]; 3) Shimadzu CLC – C8 (4.6 mm x 150 mm, 5 μm) or LiChrospher 100 RP – C8 (4.6 mm x 150 mm, 5 μm); triethylamine solution (0.5%) (pH = 2.4) : acetonitrile 60 : 40 v/v; 1.0 min./min.; 20 °C; λ = 225 nm [92]; 4) Spherisorb C18 (4.6 mm x 250.0 mm, 5 μm), 1.5 ml/min., λ = 254 nm [95]; c) 1.0 ml/min., t = 20°C, λ = 225 nm in Cozaar® tablets [96]; 2) gradient RP HPLC [28], t = 40°C, 1.5 ml/min. in Cozaar® tablets [97]. For analysis of Losartan in capsules are described isocratic RP HPLC methods [92] with column temperature: t = 35°C; 1.0 ml/min. at λ = 254 nm [3] on LiChroSpher RP-C18 (4.6 mm x 100 mm, 5 μm), 58 mM potassium dihydrogen phosphate (pH = 6.2): acetonitrile = 65 : 35 v/v [31]. For Losartan and its degradation products is used HPLC with mass detection [97]. In tablets Losartan and HCTZ are analysed by gradient RP HPLC on Microbondapak C18 (3.9 mm x 300 mm, 10 μm), 25°C, methanol : 0.05 potassium hydrogen phosphate, 1.0 ml/min.; at λ = 270 nm [98] or isocratic RP HPLC, 1.0 ml/min. and UV – detection [99, 100, 101] at λ = 215 nm [102]: 1) on Erbasil C18 column (4.6 mm x 250 mm, 5 μm) [65] or Erbasil C18 column (4.0 mm x 125 mm, 5 μm), 0.1 M phosphate buffer (pH = 4.0): acetonitrile = 65: 35 v/v; λ = 230 nm [103]; 2) on RP-YMC ODS A-132 C18 (6.0 mm x 150 mm, 5 μm), column temperature 35°C, 0.01 M sodium dihydrogen phosphate : methanol : acetonitrile = 8 : 2 : 1 v/v, λ = 265 nm [60]; 3) on C18 column; (4.6 mm x 150 mm, 5 μm), 0.01 M potassium dihydrogen phosphate : acetonitrile = 65 : 35 v/v, 1.0 ml/min. at λ = 265 nm [104]; 4) Bondapak C18 (3.9 mm.x 300 mm, 10 μm), 10 mmol/l potassium dihydrogen phosphate (pH = 3.1): acetonitrile = 65 : 35 v/v, 1.5 ml/min., λ = 226 nm [105]. Losartan HCTZ and their degradation products are analysed by: HPLC with UV – detection [106]. For Losartan, HCTZ and Ramipril is applied isocratic HPLC methods: with UV – detection at λ = 215 nm, internal standard Amlodipine besylate [107] and with UV – detection at λ = 210 nm on RP C18 (4.6 mm x 250 mm, 5 μm), buffer: acetonitrile = 60 : 40 v/v, 1.0 ml/min. [108]. Losartan Potassium, Hydrochlorothiazide and Amlodipine Besilate in tablets are determined by isocratic HPLC on Kromasil C18 column (4.6 mm x 250 mm, 5 μm), 0.02 M phosphate buffer (pH = 3.7): acetonitrile = 57 : 43 v/v, λ = 232 nm [59]. For analysis of Losartan and Enalapril in tablets is reported gradient RP HPLC: on Kromasil C18 (4.6 mm x 250 mm, 5 μm); methanol : water : acetonitrile = 45 : 35 : 20 v/v, 1 ml/min. detection at λ = 224 nm [62]. For quantification of Losartan and Ramipril in tablets is developed isocratic HPLC method on Hypersil ODS C18 (4.6 mm x 250 mm, 5 μm), acetonitrile : methanol : tetrabutyl ammonium hydrogen sulfate = 10 mM : 30 : 40 v/v, 1.0 ml/min., UV – detection at λ = 210 nm [109]. For Losartan and Atenolol in tablets is described isocratic RP – HPLC method: 1) 1.2 ml/min., UV – detection at λ = 235 nm, tLos = 3.767 [110=88]; 2) in Losar beta® tablets: Supelcosil ODS (4.6 mm.x 250 mm, 5 μm), acetonitrile and 25 mM potassium dihydrogen phosphate = 45 : 55 v/v, pH = 3.0, 1.2 ml/min., UV – detection at λ = 227 nm, internal standard: Chlorzoxazone [111]. Losartan and Metolazone are determined on Thermo Hypersil BDS C18 (250 mm × 4.6 mm, 5.0 μm) with isocratic conditions, acetonitrile:water (60:40), 0.8 ml/min., UV – detection at 237 nm [112]. Losartan and Amlodipine in tablets are analysed by HPLC with UV – detection [113].

For simultaneous analysis of Losartan in combinations with other sartans are reported gradient [114] and isocratic HPLC with UV – detection: tLos = 9.0 min., tCandesartan = 10.3 min., tIbesartan = 11.5 min.; tOlmesartan = 12.0 min.; tTelmisartan = 13.4 min. Valsartan = 19.9 min. [49]. For mono – and multicompontent analysis for isolation from plasma and urine is used solid phase microextraction, combined with HPLC. For the quantification in plasma is used RP HPLC [52, 115] with UV – detection at λ = 225 nm, 0.9 ml/min., internal standard Diclofenac
sodium [115] and $\lambda = 254$ nm, column Chromolith RP-18 (4.6 mm x 100 mm), mobile phase: 0.01 mol/l disodium hydrogenphosphate buffer : Acetonitrile = 60 : 40 v/v [116].

The following methods have been reported for analysis in plasma of Losartan and EXP 3174 [51]: isocratic HPLC [117, 118, 119] on ULTREXEMEX CN (5 μm), UV – detection at $\lambda = 245$ nm, LOQ = 5 ng/ml [120]; Intersil ODS-2 (4.6 mm x 250 mm, 5 μm), 50 mM ammonium acetate : acetonitrile = 74 : 26 v/v, 1.0 ml/min. at $\lambda = 245$ nm [121]; RP C$_{18}$ column and mas detection [117]; or on column Capcellpak C$_{18}$ (1.5 mm x 150 mm, 5 μm), 40°C; mobile phase: 0.1 % trietanolylamine : acetonitrile = 650 : 350 v/v; flow rate = 1.5 ml/min., mas detection; 117. In plasma Losartan and EXP 3174 are determined also by mas detection, internal standard: Valsartan [127].

Quantification of Losartan, Candesartan, Irbesatran and Telmisartan is by HPLC with mas detection [129]. For analysis of Losartan, Candesartan, Irbesatran and Valsartan [130] is reported RP HPLC on Betasil C$_{18}$ (4.6 mm x 250 mm, 5 μm), acetonitrile : 5 mM sodium acetate = 40 : 60 v/v, 1.0 ml/min., UV – detection at $\lambda = 250$ nm, and fluorescent detection at $\lambda_{\text{excitation}} = 250$ nm, $\lambda_{\text{emission}} = 380$ nm [128]. Atenolol, Bisoprolol, Cardevalidol, Propanolol, Metoprolol, Sotalol, Dilizem, Flecainame, Amlodipine, Verapamil, Losartan, Irbesatran, Telmisatran, Valsartan: internal standard Diazepam are determined by gradient RP HPLC: Atlantis C$_{18}$ (2.1 mm x 150 mm, 5 μm), column temperature 25°C, 10 mM ammonium acetate (pH = 3.1 with formic acid): acetonitrile, 0.3 ml/min., mas detection [89].

Losartan in combination with sartans is determined by gradient HPLC fluorescent method [88, 131] on Bondapak C18 column, (3.9 mm x 300 mm, 10 μm) 5 mM acetonitrile buffer : acetonitrile, $\lambda_{\text{excitation}} = 250$ nm, $\lambda_{\text{emission}} = 375$ nm [131] or with HPLC with mas detection [132, 133]. In urine Losartan and EXP 3174 are quantified by gradient HPLC with UV – detection [98], with fluorescent detection: $\lambda_{\text{excitation}} = 250$ nm, $\lambda_{\text{emission}} = 370$ nm on Ultremex CN (4.6 mm x 250 mm, 5 μm), 0.015 M phosphoric acid: acetonitrile = 79 : 21 v/v, 35°C, 1.25 ml/min. [124] or with mas detection MS [118]. For analysis in urine of Losartan in combinations with sartans are reported the following methods: I) Losartan, Telmisatran, Valsartan: Chromolith (4.6 mm x 250 mm), 5 mM phosphate buffer (pH = 3.8): acetonitrile : methanol = 65 : 20 : 15 v/v, 3 ml/min., $\lambda_{\text{excitation}} = 259$ nm, $\lambda_{\text{emission}} = 399$ nm [134]; II) Losartan, Irbesatran, Valsartan, Candesaratan cilexitel and its metabolite Candesaratan M1: gradient HPLC with UV – detection on Bondapak C$_{18}$ (3.9 mm x 300 mm, 10 μm), 5 mM acetonitrile buffer : acetonitrile [135]; III) Losartan, Candesartan, Irbesatran, Telmisatran [134]; HPLC with UV – detection at $\lambda = 250$ nm, 1.0 ml/min., and HPLC with $\lambda_{\text{excitation}} = 250$ nm, $\lambda_{\text{emission}} = 380$ nm [123]; IV) HPLC with UV – detection at $\lambda = 232$ nm, 1 ml/min., t = 35°C [136]. For Losartan and HCTZ in superficial water, groundwater and wastewater is described HPLC at $\lambda = 226$ nm, 0.8 ml/min. [47]. For simultaneous Losartan and its metabolite in influent wastewater is reported RP HPLC/MS [137].

Conclusion

First-derivative spectrophotometric method is found to be an excellent alternative to an HPLC method for the determination of Losartan in tablets. For analysis of Losartan and HCTZ in binary mix-
tures methods based on HPLC with UV – detection, fluorimetric or mas detection and first-derivative spectrophotometry are most applied [32].

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