RECENT APPLICATIONS OF SPECTROPHOTOMETRIC AND THIN LAYER CHROMATOGRAPHIC METHODS FOR QUALITY CONTROL OF FIXED DOSAGE COMBINATIONS OF SARTANS WITH HYDROCHLOROTHIAZIDE

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ABSTRACT

The aim of current study is to summarize the application of spectrophotometric and thin layer chromatographic methods for simultaneous determination of sartans and thiazide diuretic Hydrochlorothiazide in fixed dosage preparations. Hypertension is one of the most significant social disease, leading to stroke, myocardial infarction and high mortality. Combination therapy with antihypertensive drugs from different classes: beta-blockers, calcium antagonists, angiotensine-converting enzyme inhibitors, angiotensine II receptor antagonists (sartans) and diuretics improves blood pressure by synergistic effect. The treatment with fixed-dose formulations in a single-dosage form allows to optimize the blood pressure control. The very modern trends in hypertension therapy is the application of fixed dosage preparations of sartans with a thiazide diuretic Hydrochlorothiazide (HCTZ): Candesartan (Atacand HCT), Irbesartan (Avapro HCT), Olmesartan medoxomil (Benicar HCTZ), Valsartan (Co-Valsacor).

For improvement of blood pressure control in hypertension current trends include triple fixed-dose combinations with an angiotensin II receptor blocker, a calcium channel blocker,
and a thiazide diuretic. For simultaneous determination of sartans in fixed-dosage formulations with Hydrochlorothiazide are developed different types of spectrophotometric methods: UV, first, second and fourth-derivative and first derivative of ratio spectrophotometry; zero-crossing difference spectrophotometry, simultaneous equation method, absorbance ratio (Q-analysis) and chemometric methods. HPTLC methods with different mobile and stationary phases are reported for simultaneous estimation of fixed dosage forms. Other chromatographic method like TLC-densitometry is also applied.

INTRODUCTION

Hypertension is one of the most significant social disease, leading to dysfunction of heart, kidneys and brain blood vessels and increased eye pressure.\(^1\) For blood pressure control are applied alternative therapeutic groups of drugs: beta-blockers, calcium antagonists, vasodilators and diuretics.

Development of sartans (angiotensin II receptor antagonists), facilitates the study of the role of renin-angiotensin-aldosterone system on the control of the blood pressure and the pathogenesis of hypertension, chronic heart failure and chronic renal failure diseases.\(^2\)

For therapy of hypertension the very often applied fixed combinations are of sartans with thiazide diuretic Hydrochlorothiazide (HCTZ): Candesartan (Atacand HCT)\(^3\), Irbesartan (Avapro HCT)\(^4\), Olmesartan medoxomil (Benicar HCTZ)\(^5\), Valsartan (Co-Valsacor)\(^4\) or with Chlorthalidone: Azilsartan (Edarbyclor).\(^6\)

Structures of sartans are summerized in Table. 1. and Table. 2.

**Table. 1. Chemical structures of sartans.**

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<td>Irbesartan</td>
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Table 2. Chemical structures of sartans.
Combination therapy with fixed-dose formulations in a single-dosage form allows to optimize the blood pressure control.[7] Current perspectives for the rational therapy for improvement of blood pressure control in hypertension[8] include triple fixed-dose formulations with an angiotensin II receptor blocker, a calcium channel blocker, and a thiazide diuretic.[9]


For analysis of sartans in monocomponent pharmaceutical dosage forms are described different methods:
1) spectrophotometry[13-15];
2) high performance liquid chromatography (HPLC)[13,14];
3) TLC-densitometry for simultaneous quality and quantity analysis of Losartan, Telmisartan and Valsartan in mixtures: Silicagel G60F254, mobile phase: 7.5 : 1.5 : 5 : 5 : 0.01 : 0.03 = CHCl3 : CH3OH : acetone : toluene : CH3COOH, detection at $\lambda = 254$ nm [16-18];
5) HPTLC with fluorescence densitometry for simultaneous determination of some angiotensin II receptor blockers in tablets: Losartan potassium, Irbesartan, Olmesartan
medoxomil and Valsartan: Silicagel HPTLC plates, developed using chloroform: glacial acetic acid = 7.5 : 2.5 as mobile phase, reflectance/ fluorescence mode at excitation wavelengt $\lambda = 255 \text{ nm}$\(^{[23]}\); 
6) head-space capillary gas chromatography (GC) with flame ionization detector for the determination of residual solvents: methanol, dichloromethane, n-hexane, acetidin, chloroform, cyclohexane, toluene in Valsartan\(^{[24]}\); 
7) micellar electrokinetic chromatography in tablets and capsules\(^{[25]}\); 
8) super-critical fluid chromatography for quantification of Losartan potassium in Cozaar® tabl.\(^{[26]}\); 
9) capillary zone electrophoresis for Losartan potassium in Cozaar® tabl.\(^{[26]}\) and for sartans alone\(^{[27]}\) and in capsules in combitations with Hydrochlorothiazide (HCTZ)\(^{[28]}\); 
10) electrochemical methods: 
a) linear sweep polarography: for Telmisartan in tabl. by dripping mercury electrode\(^{[29]}\) and Valsartan in caps.\(^{[30]}\); 
b) differential sweep and square-wave voltammetry: for Vasartan in caps.\(^{[30]}\); c) anodic adsorptive stripping voltammetry at a glassy carbon electrode\(^{[31]}\) and square-wave adsorptive stripping voltammetry for Candesartan cilexetil.\(^{[32]}\) 

For determination of sartans in tablets are developed the following spectrophotometric methods:

1) UV-spectrophotometry: Azilsartan medoxomil: 249 nm\(^{[33]}\); Candesartan cilexetil: $\lambda = 253 \text{ nm}$\(^{[34]}\); Eprosartan mesylate: $\lambda = 233 \text{ nm}$\(^{[35]}\); Irbesartan: $\lambda = 246.4 \text{ nm}$\(^{[36]}\); Losartan potassium: \(^{[37]}\) $\lambda = 208 \text{ nm}$\(^{[38]}\); Olmesartan medoxomil: \(^{[39]}\) $\lambda = 250 \text{ nm}$\(^{[40]}\), $\lambda = 256 \text{ nm}$\(^{[41]}\); Telmisartan: $\lambda = 234 \text{ nm}$\(^{[42]}\), $\lambda = 297 \text{ nm}$\(^{[43]}\); Valsartan: $\lambda = 221.6 \text{ nm}$ and $\lambda = 231.2 \text{ nm}$\(^{[44]}\), $\lambda = 250 \text{ nm}$\(^{[45]}\); 
2) first derivative spectrophotometry: in tablets: Candesartan\(^{[46]}\); Losartan potassium\(^{[47=50]}\) 
3) second-derivative UV spectrophotometry in tablets: Losartan potassium at $\lambda = 219.6 \text{ nm}$ and $\lambda = 228.8 \text{ nm}$\(^{[47]}\), $\lambda = 234 \text{ nm}$\(^{[50]}\), Valsartan\(^{[44]}\); 
4) ratio derivative spectrophotometry of losartan potassium and hydrochlorothiazide\(^{[51]}\); 
5) zero order spectra of Telmisartan $\lambda_{\text{max}}$ at $\lambda = 234.0 \text{ nm}$\(^{[52]}\); 
6) difference spectrophotometry: Telmisartan: by calculation the difference between the absorbance values of the solution in 0.01 M NaOH at $\lambda = 295 \text{ nm}$ and in 0.01 N HNO$_3$ at $\lambda = 327 \text{ nm}$\(^{[53]}\); 
7) zero-crossing difference spectrophotometric determination olmesartan medoxomil\(^{[54]}\);
8) spectrophotometry in visible area after derivativate reaction for Losartan with calmagite 491 and orange-II (λ = 486 nm)\(^{55}\); 2.3-dichloro-5.6-dicyano-1.4-benzoquinone and measuring the complex at λ = 460 nm\(^{56}\) or with bromothymol blue\(^{57}\); Telmisartan with congo-red at λ = 593 nm\(^{58}\); Olmesartan: bromocresol green at λ = 409 nm and bromophenol blue ar λ = 412 nm\(^{59}\).

The most applied method is reversed phase (RP C18) HPLC with:

I) UV-detection:

1) Azilsartan medoxomil in tabl.: λ = 248 nm, Hypersil BDSC column, mobile phase: 0.05 M potassium hydrogen phosphate : acetonitrile = 60 : 40 v/v, flow rate: 1.0 ml/min\(^{60}\);

2) Candesartan cilexetil in tabl.: λ = 254 nm, Inertsil ODS-3 column, mobile phase: 0.02 M monobasic potassium phosphate buffer : acetonitrile : triethylamine = 40 : 60 : 0.2 v/v, flow rate: 2 ml/min.\(^{61}\);

3) Irbesartan in tabl.: λ = 260 nm, Inertsil ODS column, mobile phase: methanol : acetonitrile: 2 % orthophosphoric acid = 40 : 40 : 20 v/v, flow rate: 1.5 ml/min.\(^{62}\);

4) Losartan Potassium in tabl.: λ = 225 nm, CLC-C8 column, mobile phase: triethylamine : acetonitrile = 60 : 40 v/v, flow rate: 1.0 ml/min.\(^{63}\); λ = 235 nm, Shimpack column, mobile phase: acetonitrile : phosphate buffer = 40 : 60 v/v, flow rate: 1.1 ml/min.\(^{64}\); Spherisorh C18 column, flow rate: 1.5 ml/min., λ = 254 nm\(^{65}\);

5) Losartan Potassium in caps: λ = 254 nm, LiChroSpher column, mobile phase: potassium phosphate buffer : acetonitrile = 65 : 35 v/v, flow rate: 1.0 ml/min.; t = 35°C\(^{66}\);

6) Olmesartan medoxomil in tabl.: λ = 260 nm, mobile phase: acetonitrile : 5 mM ammonium

7) Telmisartan in tabl.\(^{69}\)

a) λ = 230 nm, mobile phase: methanol : acetonitrile = 30 : 70 v/v, flow rate: 1 ml/min.\(^{70}\)

b) λ = 230 nm, Waters column in gradient mode, mobile phase:10 mM potassium dihydrogen phosphate: acetonitrile = 64 : 40 v/v, flow rate: 1.0 ml/min.\(^{71}\)

c) λ = 243 nm, mobile phase: potassium dihydrogen phosphate : acetonitrile = 60 : 40 v/v, flow rate: 1 ml/min., column temperature 45°C\(^{72}\);

d) λ = 254 nm, Supelco Discovery RP Amide column, mobile phase: potassium phosphate buffer : acetonitrile = 55 : 45 v/v in an isocratic mode, flow rate: 1 ml/min.\(^{73}\);

e) λ = 256 nm, Chromosil column, mobile phase: methanol : orthophosphoric acid : acetonitrile = 80 : 5 : 15 v/v, flow rate: 1.5 ml/min.\(^{74}\)
f) $\lambda = 295$ nm, Luna C$_1$ column, mobile phase: phosphate buffer : acetonitrile = 60: 40 v/v, flow rate: 1 ml/min.$^{[75]}$; $\lambda = 296$ nm, Phenomenex column, mobile phase: 10 mM potassium dihydrogen phosphate buffer : methanol = 20: 80 v/v, flow rate: 0.8 ml/min.$^{[76]}$;

8) Valsartan$^{[77]}$ in tabl. and caps.: mobile phases: phosphate buffer : methanol = 50 : 50 v/v; flow rate: 1 ml/min; $\lambda = 210$ nm$^{[78]}$ or phosphate buffer : acetonitrile = 55 : 45 v/v; flow rate: 1.3 ml/min., $\lambda = 265$ nm$^{[44]}$; Valsartan in tablets: $\lambda = 269$ nm mobile phase: methanol : water : THF = 60 : 35 : 5 v/v, flow rate: 1 ml/min.$^{[79]}$;

9) Valsartan and impurities: $\lambda = 210$ nm, mobile phase: water : acetonitrile = 1 : 4 v/v; flow rate: 0.8 ml/min.$^{[80]}$; Valsartan and degradation products: $\lambda = 250$ nm, mobile phase: methanol : water = 70 : 30 v/v$^{[81]}$;

II) HPLC with fluorescence detection: Valsartan: Eurospher-100 C18 PF column: mobile phase: methanol: sodium dihydrogen phosphate = 10: 30 v/v, flow rate: 1 ml/min; $\lambda_{\text{excitation}} = 265$ nm and $\lambda_{\text{emission}} = 395$ nm$^{[82]}$; 3) HPLC with MS detection: Eprosartan and related substances$^{[83]}$; Irbesartan and degradation products: LC/MS/TOF (time of flight)$^{[84]}$.

The very often applied for simultaneous assay of sartans in fixed-dosage combinations with Hydrochlorothiazide (HCTZ) are spectrophotometric methods and TLC.

I. Application of spectrophotometric methods for simultaneous determination of sartans in combinations with Hydrochlorothiazide.

The following different types of spectrophotometric methods are developed for simultaneous determination of sartans in fixed-dosage combinations with Hydrochlorothiazide (HCTZ):

1) UV-spectrophotometry: Telmisartan/HCTZ$^{[85]}$, Valsartan ($\lambda = 264$ nm)/HCTZ ($\lambda = 270.5$ nm)$^{[86]}$

2) first derivative spectrophotometry:
   a) Candesartan cilexetil/HCTZ$^{[87]}$; E
   b) Eprosartan/ HCTZ$^{[88-89]}$ and Irbesartan/HCTZ$^{[89-90]}$;
   c) Olmesartan medoxomil/HCTZ$^{[91]}$, Telmisartan/HCTZ$^{[92]}$, Valsartan/HCTZ$^{[93]}$ in 0.1 N NaOH at $\lambda = 227.8$ nm in methanol at $\lambda = 276.5$ nm;

3) second-derivative method: Irbesartan/HCTZ$^{[90]}$;
4) fourth-derivative spectrophotometry: Irbesartan/HCTZ\textsuperscript{[95]}; Losartan potassium/HCTZ: \( \lambda = 280\text{-}290 \text{ nm}\textsuperscript{[96]}; \\
5) first derivative of ratio spectrophotometry: Candesartan cilexetil/ HCTZ\textsuperscript{[88]}; Olmesartan medoxomil/HCTZ\textsuperscript{[91]}
6) zero-crossing difference spectrophotometry: Olmesartan medoxomil/HCTZ\textsuperscript{[91]}
7) simultaneous equation method: Irbesartan (250nm)/HCTZ (270.6 nm)\textsuperscript{[97]}; Telmisartan (TEL) and Hydrochlorothiazide (HCTZ)\textsuperscript{[98-99]}
8) absorbance ratio (Q-analysis) method: Irbesartan HCTZ\textsuperscript{[97]}; Telmisartan/HCTZ\textsuperscript{[98]}
9) Chemometric Methods\textsuperscript{[100]}

For simultaneous determination of Candesartan cilexetil and Hydrochlorothiazide in tablet dosage forms are developed: UV spectrophotometric\textsuperscript{[101]}, first derivative: spectrophotometry\textsuperscript{[87,102]}, Q-Analysis\textsuperscript{[103]}; PLS method\textsuperscript{[104]}.

For simultaneous quantification of Candesartan cilexetil and Hydrochlorothiazide are described first derivative and ratio derivative spectrophotometry by zero-crossing method. The first derivative amplitudes at \( \lambda = 270.1 \text{ nm} \) (Candesartan cilexetil) and \( \lambda = 255.5 \text{ nm} \) (Hydrochlorothiazide) are selected. The first derivative of the ratio spectra is reported by division of the absorption spectrum of the binary mixture by a normalized spectrum of one of the components. The first derivative of the ratio amplitudes at \( \lambda \): 236 nm, 250 nm, 232 nm, 267 nm, and 280 nm are selected.\textsuperscript{[87]}

Method based on difference and derivative-difference spectrophotometry with a zero-crossing measurement technique are applied by obtaining of linear calibration graphs of absorbance difference values at \( \lambda = 292 \text{ nm} \) (Candesartan cilexetil) and \( \lambda = 338 \text{ nm} \) (HCTZ). Linear second derivative difference values at \( \lambda = 296 \text{ nm} \) for (Candesartan cilexetil) and first derivative difference values at \( \lambda = 299 \text{ nm} \) (HCTZ) are used.\textsuperscript{[101]}

The first derivative method for assay of Candesartan cilexetil and Hydrochlorothiazide in tablet dosage forms is based on the measurement of absorbance of one drug at the zero crossing point of another drug. Candesartan cilexetil and Hydrochlorothiazide are determined at two different wavelengths: \( \lambda = 222.69 \text{ (zero crossing point of Hydrochloro-thiazide)} \) and \( \lambda = 254.63 \text{ nm (zero crossing point of Candesartan cilexetil)} \) from the derivative spectra.\textsuperscript{[102]}
A first-derivative spectrophotometric method for the determination of Irbesartan and in the presence of Hydrochlorothiazide is described. Measurements are made at the zero-crossing wavelength at $\lambda = 263.0$ nm for Irbesartan.$^{[90]}$

The simultaneous determination of Irbesartan and Hydrochlorothiazide in a binary mixture without previous separation is carried out by a compensation technique: derivative spectrophotometric analysis with overlapping spectra by using the ratios of the derivative maxima or the derivative minimum. Other method for obtaining the content of drugs is using of the first derivative of the ratio spectra, obtained by dividing the absorption spectra of the binary mixture by that of one of the components. The amplitudes in the first derivative of the ratio spectra at $\lambda$: 231 nm, 238 nm, 248 nm, 266 nm, 279 nm are selected. Absorbance ratio method is performed by the absorbances at $\lambda$: 272 nm/263 nm and 241 nm/263 nm in the zero-order spectra of their mixture.$^{[105]}$ Other applied methods is a second-derivative spectrophotometry by zero-crossing wavelengths at $\lambda = 230.1$ nm for Irbesartan and $\lambda = 232.7$ nm for Hydrochlorothiazide$^{[94]}$, ratio method coupled with constant multiplication and ratio difference method.$^{[106]}$

For the simultaneous determination of Losartan and Hydrochlorothiazide in fixed combinations in tablets are developed the following methods: first-derivative$^{[107-110]}$, fourth-derivative at $\lambda$: 280 nm-290 nm$^{[96]}$, Q-analysis method$^{[110]}$, partial least squares (PLS) algorithm of absorbances at $\lambda$: 220-274 nm.$^{[111]}$ For quantification of components in Hyzaar® filmtablets: Losartan/HCTZ is developed by The ratios of the minimum and maxima and of the first derivative spectra is are calculated in compensation technique by for Losartan/HCTZ in Hyzaar® filmtablets: 218 nm/236 nm (Los), 230 nm/261nm (HCTZ)$^{[107]}$.

For simultaneous spectrophotometric estimation of Losartan potassium and Hydrochlorothiazide in tablet dosage form is proposed first-derivative by measurement of the absorption at $\lambda_{\text{max}}$ Losartan = 271.6 nm (zero crossing wavelength for HCTZ) and $\lambda_{\text{max}}$ HCTZ = 335.0 nm (zero crossing point for Losartan)$^{[109]}$. The same method is used at $\lambda_{\text{max}}$ Losartan = 222 nm and $\lambda_{\text{max}}$ HCTZ = 332 nm.$^{[110]}$

In simultaneous UV equation method in tablets are solved simultaneous equations by measurement the absorbance at: $\lambda_{\text{max}}$ Losartan = 218 nm, $\lambda_{\text{max}}$ HCTZ = 272 nm and $\lambda$(isosbestic point) = 266.5 nm. Components of this fixed combination in tablets are assayed by absorbance ratio method (Q-analysis) by measurement of absorbances at isosbestic
wavelength ($\lambda = 266.5$ nm) and wavelength of maximum absorption of Hydrochlorothiazide ($\lambda = 272$ nm).\[^{[110]}\]

Chemometric algorithm methods as UV partial least squares with variable selection of the UV-zero order spectra between $\lambda = 220$ nm and $\lambda = 300$ nm is described for mixture Losartan/HCTZ in tablets.\[^{[111]}\] The difference of absorbance values at $\lambda = 206.6$ nm and $\lambda = 261.4$ nm is used for the estimation of Losartan content and absorbance values at 270.6 nm is applied for the quantification of estimation of Hydrochlorothiazide.\[^{[64]}\]

For quantitative analysis of Olmesartan medoxomil and Hydrochlorothiazide in fixed dosage preparations are reported different spectrophotometric methods:\[^{[112-114]}\]: first derivative of ratio spectrophotometry; at $\lambda = 231.0$ and $\lambda = 271.0$ nm; zero-crossing difference spectrophotometry: $\lambda = 257.8$ (OLM) and $\lambda = 240.2$ nm (HCT) \[^{[91]}\]; simultaneous equation method, absorbance ratio method, three wavelength method 115Hemke Q-analysis method (absorbance ratio).\[^{[116]}\]

UV-spectrophotometric determination of Olmesartan medoxomil and Hydrochlorothiazide in fixed pharmaceutical formulations includes simultaneous equation method at $\lambda = 271.5$ nm and $\lambda = 257.0$ nm, absorbance ratio method: $\lambda = 261.5$ nm for Hydrochlorothiazide an isoabsorptive wavelength and $\lambda = 257.0$ nm for Olmesartan medoxomil. In three wavelength method, two wavelengths are selected: $\lambda = 263.8$ and $\lambda = 278.4$ nm), where Hydrochlorothiazide give same absorbances, third wavelength ($\lambda = 316.5$ nm) is where Olmesartan gives nearly zero absorbance.\[^{[115]}\]

For simultaneous estimation of Olmesartan medoxomil and Hydrochlorothiazide in combined tablet dosage form has been developed method with the application of Q-analysis method (absorbance ratio), which involves the formation of Q-absorbance equation at $\lambda = 264$ nm (isobestic point) and at $\lambda = 271$ nm, the maximum absorption of hydrochlorothiazide.\[^{[116]}\]

The following different types of spectrophotometric methods are developed for simultaneous determination of Telmisartan in fixed-dosage combinations with Hydrochlorothiazide: UV-spectrophotometry:\[^{[85]}\], first derivative spectrophotometry:\[^{[92]}\], simultaneous equation method\[^{[98-99]}\], absorbance ratio (Q-analysis) method\[^{[98]}\], chemometric methods.\[^{[100]}\]

Sensitive method is first derivative spectrophotometry using a zero-crossing technique of measurement at $\lambda = 241.6$ for Telmisartan and $\lambda = 227.6$ nm for telmisartan and
Hydrochlorothiazide. Otwer method is the first derivative of ratio spectrophotometry, where the amplitudes are measured at $\lambda = 242.7$ nm for Telmisartan and $\lambda = 274.9$ nm for Hydrochlorothiazide $^{[117]}$.

The described UV-spectrophotometric method for simultaneous estimation of Telmisartan and Hydrochlorothiazide from their tablet dosage form involves multiwavelength spectrophotometric estimation, where interference due to Hydrochlorothiazide at $\lambda = 286$ nm (wavelength for estimation of Telmisartan) are eliminated by recording absorbance difference at $\lambda = 286$ nm and $\lambda = 308$ nm whereas interference of Telmisartan at $\lambda = 262$ nm (wavelength for estimation of Hydrochlorothiazide) is removed by recording absorbance difference at $\lambda = 262$ nm and $\lambda = 282$ nm.$^{[118]}$

The wavelength selected for UV-spectrophotometric simultaneous determination pharmaceutical dosage form. are $\lambda = 296$ nm for Telmisartan and $\lambda = 270$ nm for Hydrochlorothiazide.$^{[85]}$ In combined dosage form the method for quantitation is based on simultaneous equation method absorptivity values at selected wavelengths Telmisartan ($\lambda = 295$ nm) Hydrochlorothiazide ($\lambda = 273$ nm).$^{[99]}$

Different spectrophotometric methods for simultaneous estimation of Telmisartan and Hydrochlorothiazide in two component solid dosage forms have been developed. One method includes the simultaneous equation method and multicomponent mode of analysis at $\lambda = 295$ nm (Telmisartan) and $\lambda = 273$ nm (Hydrochlorothiazide). Other method is based on the absorbance ratio (Q-analysis) method at $\lambda = 283.0$ nm (isoabsorptive point) and $\lambda = 293.0$ nm (absorbance maxima of Telmisartan). The first order derivative spectrophotometry at $\lambda = 273.0$ nm for Telmisartan (zero crossing point for HCTZ) and $\lambda = 295.0$ nm for Hydrochlorothiazide (zero crossing for TEL) is proposed.$^{[98]}$

For simultaneous determination of Valsartan and Hydrochlorothiazide in fixed combinations are applied the following methods:$^{[119-121]}$: UV-spectrophotometry in tablets: $\lambda_{\text{max}}$ Valsartan = 264 nm, $\lambda_{\text{max}}$ HCTZ = 270.5 nm$^{[86]}$; first derivative ratio method.$^{[121]}$ in 0.1 N NaOH at $\lambda = 227.8$ nm in methanol at $\lambda = 276.5$ nm$^{[93]}$; second derivative method$^{[122]}$; UV-absorption ratio method$^{[123]}$: $\lambda_{\text{max}}$ HCTZ = 270.5 nm, $\lambda_{\text{isoabsorptive point}} = 231.5$ nm$^{[124]}$; H-point standard addition method and partial least square regression.$^{[125]}$

First-derivative ultraviolet spectrophotometry is used to determine Valsartan and Hydrochlorothiazide simultaneously in combined pharmaceutical dosage forms. The
derivative procedure is based on the linear relationship between the drug concentration and the first derivative amplitudes at $\lambda = 270.6$ (Valsartan) and $\lambda = 335$ nm (Hydrochlorothiazide).\[93\]

For simultaneous estimation of Valsartan and Hydrochlorothiazide in a tablet dosage form are developed two UV-spectrophotometric methods: 1) solving of simultaneous equations, based on the measurement of absorbance at two wavelengths: $\lambda = 249.4$ nm for Valsartan and $\lambda = 272.6$ nm for Hydrochlorothiazide; 2) absorbance ratio method, which involves formation of $Q$-absorbance equation at $\lambda = 258.4$ nm (isoabsorptive point) and at $\lambda = 272.6$ nm ($\lambda_{\text{max}}$ Hydrochlorothiazide).\[123\]

Simultaneous determination of Valsartan and Hydrochlorothiazide by the H-point standard additions method and partial least squares calibration is described. Absorbances at a pair of wavelengths at $\lambda = 216$ nm and $\lambda = 228$ nm, are monitored with the addition of standard solutions of Valsartan.\[125\]

Spectrophotometric methods have been developed for determination of Losartan potassium and Irbesartan in their pharmaceutical dosage forms. The methods were based on coloured complexes that measured either bromcresol purple at $\lambda = 415$ nm or cresol red $\lambda = 435$ nm.\[126\]

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II. Application of thin layer chromatographic methods for simultaneous quantification of sartans in combinations with Hydrochlorothiazide.

For simultaneous determination of sartans in combinations with Hydrochlorothiazide in tablets are developed different HP TLC methods. HPTLC methods with different chromatographic system are reported for simultaneous estimation of Candesartan cilexetil and Hydrochlorothiazide on Silicagel $G_{60}F_{254}$ using mobile phase: chloroform: methanol (8:2, v/v).\[127-129\]

In one of methods is described normal phase (NP) TLC system: stationary phase: TLC plate Silicagel $G_{60}F_{254}$; mobile phase: acetone: chloroform : ethylacetate : methanol $= 3 : 3 : 3 : 0.5$ v/v; detection at $\lambda = 280$ nm. The obtained in this chromatographic system data for analytical parameter $R_f$ are: $R_f = 0.27$ (Candesartan cilexetil), $R_f = 0.45$ (Hydro-chlorothiazide).\[127\] HPTLC is applied for separation of the two drugs followed by densitometric measurements of their spots $\lambda = 270$ nm. The separation is carried out on Merck HPTLC aluminium sheets of
Silicagel G<sub>60</sub>F<sub>254</sub>, using chloroform : methanol = 8 : 2 v/v as mobile.<sup>[128]</sup> HPTLC technique is performed on Silicagel G<sub>60</sub>F<sub>254</sub> aluminum plates with mobile phase: of toluene : ethyl acetate: formic acid (85%) in the ratio of 6:4:1 v/v.<sup>[129]</sup>

Simultaneous analysis of Eprosartan and hydrochlorothiazide in tablets by high performance thin layer chromatography with Ultra violet absorption densitometry.<sup>[130=127. Patel]</sup>

HPTLC method for simultaneous estimation of Irbesartan and Hydrochlorothiazide using TLC plates precoated with Silicagel G<sub>60</sub>F<sub>254</sub> and the mobile phase: acetonitrile : chloroform in the ratio of 5 : 6 v/v, λ = 270 nm is proposed.<sup>[131]</sup> For analysis of these combination other HPTLC systems on Silicagel G<sub>60</sub>F<sub>254</sub> and at λ = 260 nm include: mobile phase: acetonitrile : chloroform : glacial acetic acid = 7 : 3 : 0.1 v/v<sup>[132]</sup> or acetonitrile : ethyl acetate = 8 : 2 v/v.<sup>[133]</sup>

The content of Losartan and Hydrochlorothiazide in combined dosage form is estimated by NP TLS system: TLC plate Silicagel G<sub>60</sub>F<sub>254</sub>; mobile phase: chloroform: methanol: acetone: formic acid = 7.5: 1.5 : 0.5 : 0.03 v/v; detection at λ = 254 nm. The obtained results for analytical parameter Rf are: Rf = 0.61 (Losartan), Rf = 0.41 (Hydrochlorothiazide).<sup>[109]</sup>

Olmesartan medoxomil and Hydrochlorothiazide are simultaneously determined by HPTLC: plate Silicagel G<sub>60</sub>F<sub>254</sub>; mobile phase: acetonitrile : ethyl acetate : glacial acetic acid = 7 : 3 : 0.4 v/v at 254 nm.<sup>[134]</sup> Other applied method is densitometric HPTLC: Silicagel G<sub>60</sub>F<sub>254</sub>, mobile phase: chloroform : methanol : toluene = 6 : 4 : 5 v/v at λ= 258 nm.<sup>[135]</sup>

For this fixed combination is described densitometric HPTLC on Silicagel G<sub>60</sub>F<sub>254</sub>, mobile phase, chloroform : methanol : formic acid = 8 : 1.5 : 0.5 v/v and detection at λ = 260 nm for Olmesartan medoxomil and λ = 272 nm for Hydrochlorothiazide.<sup>[136]</sup>

For Telmisartan and Hydrochlorothiazide is applied densitometry - HPTLC: precoated Silicagel G<sub>60</sub>F<sub>254</sub>, mobile phase: ethyl acetate: chloroform: methanol = 10: 3: 1 v/v, λ = 270 nm<sup>[137]</sup> or chloroform : methanol : toluene = 2 : 5 : 5v/v at λ = 272 nm.<sup>[133a] shah</sup>

Other method is based on TLC separation of the two drugs followed by the densitometric measurements of their spots at λ = 295 nm Telmisartan and λ = 225 nm and for Hydrochlorothiazide<sup>[117]</sup>
For simultaneous quantification of Valsartan and Hydrochlorthiazide in tablet dosage form is carried out NP TLC system: TLC plate Silicagel G_{60}F_{254}; mobile phase: chloroform : methanol : toluene : glacial acetic acid = 6 : 2 : 1 : 0.1 v/v; detection at $\lambda = 260$ nm.\textsuperscript{[139]} For assay of Valsartan and Hydrochlorthiazide is reported TLC-densitometry system: Camag TLC densitometer; stationary phase: TLC plates, precoated with Silicagel Silicagel G_{60}F_{254}, mobile phase: chloroform: methanol: acetone: toluene : acetic acid = 7.5: 1.5: 5: 5: 0.01: 0.03 v/v; detection at $\lambda = 254$ nm.\textsuperscript{[140]}

CONCLUSION
Recent trends in simultaneous determination of sartans and thiazide diuretic Hydrochlorothiazide in fixed dosage preparations are connected with the application of different spectrophotometric and chromatographic methods: layer chromatography and high performance layer chromatography. From spectrophotometric methods the very often used are UV and derivative methods.

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