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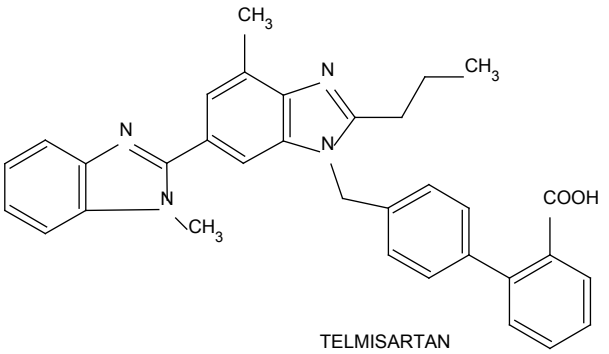
Thin layer chromatography (TLC) and video-scanning in a new method for the analysis of angiotensin AT_1 receptor antagonists, telmisartan and valsartan, in pharmaceuticals

Chromatografia cienkowarstwowa (TLC) i wideoskanowanie w nowej metodzie oznaczania antagonistów receptora angiotensynowego AT_1, telmisartanu i walsartanu w preparatach farmaceutycznych

INTRODUCTION

Non-peptidergic angiotensin AT_1 receptor antagonists (sartans) were introduced in the treatment of cardiovascular diseases more than two decades ago. So far, they have acquired and maintained an important position in the treatment of hypertension and congestive heart failure. Two sartans being examined in the present paper, telmisartan and valsartan (Figs. 1–2), appear to have more interesting pharmacological properties. Telmisartan acts also as an insulin sensitizer, probably by the activation of peroxisome-proliferator activated receptor subtype γ (PPARγ). Also, the treatment of hypertensive patients with valsartan, is associated with significantly lower incidences of new diabetes [8].



TELMISARTAN

Fig. 1. Chemical structure of telmisartan

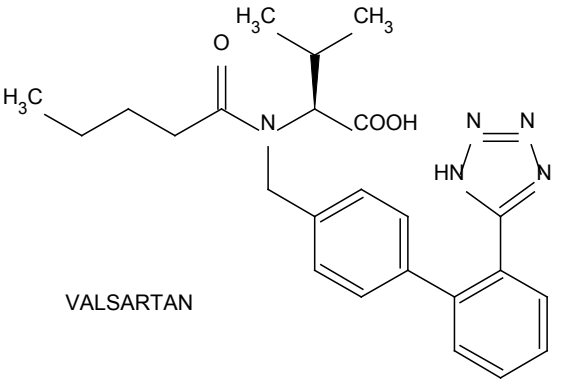


Fig. 2. Chemical structure of valsartan

So far, a few thin layer chromatographic (TLC) methods have been developed for determination of telmisartan and valsartan, alone or in different combinations. Generally, these methods are characterized by sufficient precision and accuracy [1–6]. However, there is not any paper including TLC determination of these drugs with video-scanning procedure. Probably, the reason for this is that many analysts are afraid of the great difference between quantification with a densitometer and quantification with a camera, which are two completely different systems [7]. Therefore, the objective of the present paper was to develop a simple TLC method with video-scanning procedure and to examine its usefulness for the quantitation of telmisartan and valsartan in respective pharmaceuticals.

MATERIAL AND METHODS

S a m p l e s a n d s o l u t i o n s . Telmisartan and valsartan pure substances from Toppharman (China) and respective tablets: Micardis® (Boehringer Ingelheim, Germany) containing 80 mg of telmisartan and Diovan® (Novartis Pharma, Germany) containing 160 mg of valsartan were used. 1,4-Dioxane and hexane for chromatography from E. Merck (Germany) and formic acid (99%) from POCh Gliwice (Poland) were applied. The stock solutions of the drugs at the concentration of 1 mg/mL were prepared by dissolving the appropriate amounts of the drugs in methanol.

C h r o m a t o g r a p h y. One-dimensional TLC was performed on 10 × 20 cm glass–backed silica gel $60F_{254}$ plates from E. Merck. They were dried in an oven at 105ºC for 15 min before analysis. Sample solutions in respective volumes were applied onto the plates as sharp 2 mm spots, 10 mm apart and 10 mm from the lower edge of the plate, by means of an AS 30 HPTLC applicator from Desaga (Germany) equipped with a Hamilton (USA) syringe. Chromatograms were developed to a distance of 8 cm in an unsaturated horizontal Teflon DS chamber from Chromdes Lublin (Poland). After the development at room temperature (20ºC), the plates were dried in the air in the same conditions. Video-scanning procedure was performed with a Desaga VD40 Video system comprising the Cab UV-Vis, in conjunction with a high-resolution Mitsubishi color-video CCD camera (mode CP 700D), with a horizontal resolution of 430 TV lines and a standard sensitivity of 1 lx. A Desaga video-documentation system ProViDoc, version 3.02, was applied.

Robustness of the method. Chromatograms were run with eluents containing 0.05, 0.1 and 0.15 parts of formic acid in the mobile phase containing 1,4-dioxane and hexane in the ratio 5:5 (v/v) while the amount of the mobile phase was varied in the range ± 0.5%. The temperature for the plates development was varied in the range ± 5%. The time from the sample application to chromatography and from chromatography to scanning was also changed (10, 20 and 30 min). The robustness of the method was checked by spotting onto the plates 0.8 μg of telmisartan and 1.8 μg of valsartan and determining the respective peak areas. Then, the results obtained were estimated by ANOVA.

Procedure for calibration. The working solutions of telmisartan and valsartan were prepared by dilution of the respective stock solutions ten times with methanol. They were spotted onto the plate covering the range 0.2–1.4 μg per spot for telmisartan and 0.5–3 μg per spot for valsartan. Six analyses were performed and then the calibration curves based on the ratio of the peak areas and the concentrations of the drugs were determined by a linear least squares regression.

Instrumental precision. Repeatibility was evaluated by analyzing the respective samples of both drugs three times at three levels: 0.3, 0.7 and 1.1 μg per spot for telmisartan and 0.8, 1.8 and 2.8 μg per spot for valsartan. Intermediate precision was assessed by analyzing similar levels during three separate days (one sample at each level once daily).

Precision and accuracy in tablets. Twenty Micardis® or Diovan® tablets were powdered in a glass mortar. Then, respective solutions were obtained by vortex–mixing the weighted amounts containing 2.5 mg of telmisartan and 5 mg of valsartan in methanol for 20 min, diluting with methanol to 25 mL and filtering through nylon membrane filters (0.45 μm). The solutions containing ca. 0.1 mg/mL of both drugs were applied onto the plates in volumes of 8 μL for telmisartan and 10 μL for valsartan. The plates were developed, dried, and scanned in conditions described above. The assay was done for six individually weighed amounts of the powdered tablets. The concentrations of the drugs in the sample zones were determined using calibration plots obtained by chromatography of standard solutions on the same plate. Finally, the results were estimated by t-Student test.

Recovery testing. Accuracy of the method was proved by preparing the model mixtures at three levels. The weighed portions of respective tablets containing 2.5 mg of telmisartan or 5 mg of valsartan were transferred to 25 mL flasks containing ca. 15 mL of methanol and vortex-mixed. The solutions were diluted to volume with methanol and filtered. Then, 5 mL volume of each filtrate was fortified with 50, 100 or 150% amounts of respective drug and diluted to 10 mL with methanol. Equal 8 μL volumes for telmisartan and 10 μL volumes for valsartan, together with standard solutions, were applied onto the plates. This procedure was repeated three times for each level of addition and then recoveries of the drugs were calculated.

Stability in sample solutions. Solutions at the concentration of 0.1 mg/mL were stored at room temperature (20 ºC) for 3, 6, 12 and 24 h in tightly capped, volumetric flasks. They were applied in volumes of 10 μL onto TLC plates which were then developed, dried and analyzed for the presence of any spot other than of the respective drug.

RESULTS AND DISCUSSION

Selection and optimization of the method. The TLC method was developed and optimized in view to developing a simple and reliable method with video-scanning detection. Several easily available solvents in different combinations were tried. Lastly, the mobile phase consisting of 1,4-dioxane-hexane-formic acid (99%) (5:5:0.1, v/v/v) was selected as optimal to obtain sharp and well-defined spots without tailing at R_F 0.27 ± 0.01 (mean ± SD) for telmisartan and R_F 0.39 ± 0.01 for valsartan.

Robustness of the method. Total RSD for the peak areas was found to be 0.97 % for telmisartan and 2.77% for valsartan. These relatively low values, obtained after introducing some small changes in the developed method, indicate that the robustness of the proposed procedure is sufficient. The results of ANOVA confirm that the proposed changes did not affect the peaks areas (Table 1).

Table 1. Results from the robustness study and statistical evaluation by ANOVA

Parameter	Mean area ± SD (n = 3)	
	Telmisartan	Valsartan
Formic acid in the mobile phase 1,4-dioxane-hexane (5:5, v/v) (0.05, 0.1, 0.15)	8138 ± 89.72	744.2 ± 20.57
Temperature (19, 20, 21°C)	8151 ± 75.43	730.6 ± 26.39
Time from sample application to chromatography (10, 20, 30 min)	8080 ± 108.7	737.5 ± 27.20
Time from chromatography to scanning (10, 20, 30 min)	8081 ± 48.51	734.1 ± 16.82
F	0.2512	0.1876
p	0.859	0.902

Linearity. Results from regression analysis showed that the linear relationships between peak areas and concentrations of the drugs were good over the tested concentration ranges (Table 2).

Table 2. Linear regression data of calibration plots and statistical evaluation by Fisher test (n = 6)

Drug	F	p	r	Mean slope ± SD	Mean intercept ± SD
Telmisartan	782.7	1.092E-06	0.9968	8795 ± 382.6	1078 ± 262.5
Valsartan	578.6	1.772E-05	0.9966	319.9 ± 22.06	150.0 ± 29.34

Precision of the system. Repeated sample application and measurement of peak areas were used to assess the repeatability and intermediate precision of the system at three different levels. The results from these experiments expressed by RSD for the respective response factors (relationships between the peak areas and concentration of the drugs) are given in Tables 3–4. Intermediate precision expressed as the total RSD was equal 4.34% for telmisartan and 5.14% for valsartan.

Table 3. Precision of the system for telmisartan and valsartan in the standard solutions

Amount examined (µg per spot)	Repeatability (n = 3)		Intermediate precision		
			(n = 3)		(n = 9)
	Response factor[a] mean ± SD	RSD (%)	Response factor[a] mean ± SD	RSD (%)	Total RSD (%)
Telmisartan					
0.3	10658 ± 364.9	3.42	10563 ± 406.3	3.85	
0.7	10300 ± 98.73	0.96	10550 ± 412.9	3.91	4.34
1.1	10118 ± 292.8	2.89	9981 ± 272.4	2.73	
Valsartan					
0.8	347.3 ± 16.45	4.74	356.0 ± 17.70	4.97	
1.8	373.2 ± 15.14	4.06	355.8 ± 19.61	5.51	5.14
2.8	358.5 ± 19.41	5.41	353.3 ± 19.49	5.52	

[a]Relationship between the peak area and concentration of the drug

Table 4. Precision and accuracy of the method for telmisartan and valsartan in powdered tablets (n = 6)

Detection	Amount expected (mg)	Amount found mean ± SD (mg)	Confidence interval (mg)	RSD (%)	t	p
Telmisartan	80.0	79.55 ± 3.52	78.98 – 80.13	4.42	-1.521	0.131
Valsartan	160.0	161.6 ± 7.12	159.3 – 163.9	4.41	1.339	0.189

Precision and accuracy in tablets. The results from this experiment are given in Table 5 and Figs. 3–4. The mean RSD values obtained were 4.42% for telmisartan, and 4.41% for valsartan. The results were homogenic and t-Student test did not show significant differences between them and the declared contents. The accuracy of the results was checked by calculating the 95% confidence intervals and checking if the obtained amounts were within them. For both drugs, the obtained contents were always within the confidence intervals, so the procedure for both drugs was proved to be sufficiently accurate.

Table 5. Accuracy data for telmisartan and valsartan in the model mixtures (n = 3)

	% of Addition			% of Addition		
	Telmisartan			Valsartan		
	50	100	150	50	100	150
Mean amount determined (mg)	60.42	80.84	101.4	121.9	159.4	203.1
RSD (%)	3.51	3.59	4.17	4.90	5.10	3.71
Recovery (%)	100.7	101.1	101.4	101.6	99.62	101.54

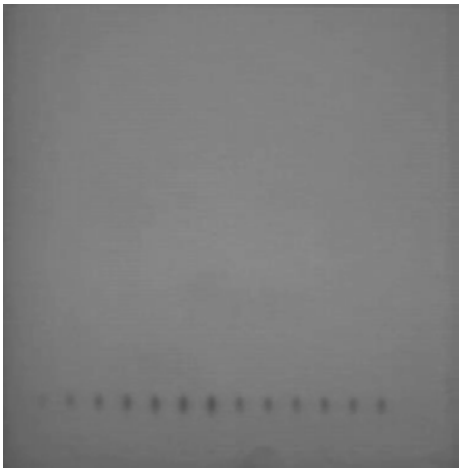


Fig. 3. Video-chromatogram recorded for standard samples of telmisartan in the calibration range 0.2-1.4 µg per spot and for samples obtained from Micardis® tablets

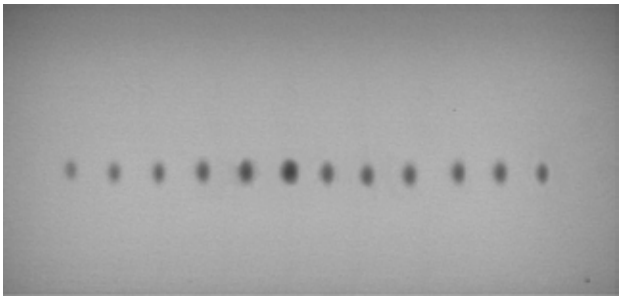


Fig. 4. Video-chromatogram recorded for standard samples of valsartan in the calibration range 0.5-3.0 µg per spot and for samples obtained from Diovan® tablets

Recovery. For telmisartan the proposed method afforded recoveries of 100.69–101.37%. For valsartan determination, the respective values were 99.62–101.59%.

Stability in sample solution. The drugs were stable in methanolic solutions stored at the temperature 20 ºC for 48 h and no additive spots were observed on the chromatograms.

CONCLUSIONS

New TLC method was developed for determination of telmisartan and valsartan in tablets using video-scanning procedure. To the best of our knowledge, it is the first TLC procedure using the mentioned technique for quantitation of angiotensin AT_1 receptor antagonists. The proposed procedure is simple, rapid and clearly less expensive than other chromatographic method. Although not all official validation requirements are fulfilled, the system is suitable for quantitative determination

of these drugs for different pharmaceutical purposes. For example, the method can be useful for the analysis of falsified drugs, which is a big problem in the global world. It may be also adapted for the "cleaning validation" in pharmaceutical industry.

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SUMMARY

Video-scanning procedure was performed for a new TLC method of quantitative analysis of two angiotensin AT_1 receptor antagonists, telmisartan and valsartan, in pharmaceuticals. Determination was carried out using silica gel adsorbent, a mobile phase consisting of 1,4-dioxane-hexane-formic acid (99%) (5:5:0.1, v/v/v) and detection at 254 nm for the both drugs. Compact spots were obtained for telmisartan with R_F 0.27 ± 0.01 (mean ± SD) and for valsartan with R_F 0.39 ± 0.01. Calibration plots were linear with good correlation coefficients equal 0.9968 and 0.9966 for telmisartan and valsartan. The method was verified for robustness, precision and accuracy.

Keywords: thin layer chromatography, video-scanning, angiotensin AT1 receptor antagonists, determination, pharmaceuticals

STRESZCZENIE

Opracowano metodę oznaczania dwóch antagonistów receptora angiotensynowego AT_1, telmisartanu i walsartanu w preparatach farmaceutycznych przy użyciu metody TLC z techniką

wideoskanowania. Oznaczenie przeprowadzono przy użyciu żelu krzemionkowego jako adsorbentu, fazy ruchomej złożonej z 1,4-dioksanu, heksanu i 99% kwasu mrówkowego (5:5:0.1 v/v/v) i detekcji przy 254 nm dla obydwu leków. Otrzymano zwarte plamy o wartości R_F 0.27 ± 0.01 (średnia ± SD) dla telmisartanu i 0.39 ± 0.01 dla walsartanu. Uzyskano liniowe wykresy kalibracyjne o wartościach współczynnika regresji 0.9968 dla telmisartanu i 0.9966 dla valsartanu. Metodę zweryfikowano pod względem odporności na niewielkie zmiany operacyjne, precyzji i dokładności.

Słowa kluczowe: chromatografia cienkowarstwowa, wideo-skanowanie, antagoniści receptora AT1, oznaczanie, preparaty farmaceutyczne