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Comparison of capillary electrophoresis (CE) and classical spectrophotometry (UV) for determination of two sartans in pharmaceutical formulations

Porównanie metody elektroforezy kapilarnej(CE) i klasycznej spektrofotometrii (UV) do oznaczania dwóch sartanów w preparatach farmaceutycznych

INTRODUCTION

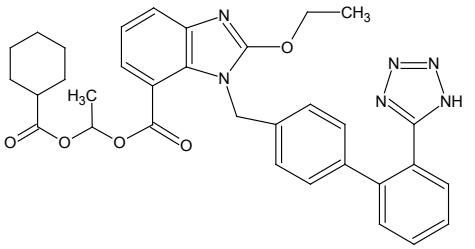
AT_1 receptor antagonists (sartans) display a great therapeutic promise in the field of cardiovascular medicine and are currently being exploited as antihypertensive agents. Even with the availability of many other antihypertensive medications, sartans are very attractive from the stand point of their general tolerability and mechanism of action [2].

In recent years, capillary electrophoresis (CE) is more and more frequently applied as a highly effective analytical procedure. It has many advantages such as lower sampling volume than HPLC method and equally high separation efficiency, allowing to separate possible impurities present in tablets. On the other hand, there are only a few publications on CE determination of some AT_1 receptor antagonists [1, 5, 8] while to the best of our knowledge there is not any report about CE determination of candesartan and valsartan. Therefore, the aim of the present study was to elaborate and to validate a new CE method for determination of these two sartans in pharmaceutical formulations and to compare it with classical spectrophotometric procedure (UV). Spectrophotometry was proposed for its simplicity and less time and fewer reagents than HPLC or CE methods. As for spectrophotometry, some reports exist concerning determination of candesartan [4] and valsartan [3, 6, 7], generally in combination with different diuretics, especially with hydrochlorothiazide.

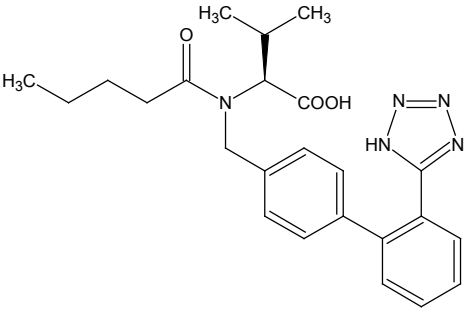
MATERIAL AND METHODS

Chemicals and apparatus. Candesartan cilexetil, telmisartan and valsartan pure substances from Topharman (China), and respective tablets Atacand® (Astra Zeneca, Sweden) and Diovan® (Novartis Pharma, Germany) were used. All used chemicals were of analytical grade and were purchased from E. Merck (Germany). Purified water was obtained using a deionizer SolPure 7 from Poll. Lab. (Poland). Capillary electrophoresis was carried out using a PrinCE CE system

(PrinCE Technologies, Netherland) equipped with a UV Lambda 1010 detector. Data acquisition and signal processing were performed using Dax Data Acquisition and Analysis software. A fused-silica capillary tubing (Polymicro Technologies, AZ, USA) of 75 µm i.d. and 72 cm effective length (total length 94 cm) were used. Sample loading was achieved by hydrodynamic injection (10 mbar, 6 s). The running buffer was prepared by mixing 50 mmol/l Na_2HPO_4 with 1 mol/l NaOH. For pH controlling, a pH meter HI-9024 from Hanna Instruments (Germany) was applied. Prior to use, the buffer was filtered through a 0.45 µm nylon membrane filter. Before start-up, the capillary was preconditioned with 0.1 mol/l NaOH and deionized water, each for 10 min regular sequence, and finally with a running buffer for 5 min. Between runs, the capillary was rinsed with deionized water and finally with a running buffer, all for 5 min. The capillary was left filled with water between analysis and when not in use. The analysis was carried out at 30°C and a potential of 25 kV. The autosampler was kept at room temperature (approximately 21ºC). The UV detection at 233 and 217 nm for candesartan and valsartan, respectively, was applied. For spectrophotometric method, a Perkin-Elmer Lambda 15 UV double-beam spectrophotometer (MA, USA) with quartz cells of 1 cm was used. The UV spectra were recorded using 2 nm slit and 240 nm/min scanning speed. The assay was performed at different analytical wavelengths, for candesartan at 211 and for valsartan at 206 nm.



CANDESARTAN CILEXETIL



VALSARTAN

Fig. 1. Chemical structures of candesartan and valsartan

Preparation of solutions. For both drugs, the stock solutions of 1 mg/ml were prepared in methanol. They were stored at 4°C and were found to be stable for at least 2 weeks. The working solutions of the drugs were prepared in water-methanol mixture (1:1) for CE or in methanol for UV method.

Preparation of tablets. Twenty tablets were weighed and the average mass values were calculated. For each determination, an independent tablet powder was weighed.

Calibration for CE method. For both drugs, the linearity was assessed using standard solutions in a 40–140 μg/ml concentration range, each containing 150 μg/ml of internal standard (valsartan was applied for candesartan and telmisartan for valsartan determinations).

Calibration for UV method. For both drugs, linearity was assessed using standard solutions in a 2–12 μg/ml concentration range.

Precision of the systems. To determine precision of both systems, the respective solutions at three different concentrations were measured three times daily (intra-day precision). Intermediate precision was assessed by analyzing similar concentrations on three separate days (one sample at each concentration once daily). In CE method, the response factor was determined as the relationship between the ratio of peak areas (the drug versus internal standard) and concentration of the respective drug.

Accuracy and precision of CE method. For both drugs, the samples were obtained by weighing the tablet powders equivalent to 12.5 mg of candesartan and valsartan. The samples were placed in 25 ml volumetric flasks containing 15 ml of methanol, sonicated for 10 min to dissolve the active ingredient, diluted to the mark and filtered by nylon membrane filters (0.45 μm). Then, 3.0 ml volumes were transferred to 10 ml flask together with 1.5 ml of the internal standard solution at concentration of 100 μg/ml and 0.5 ml of methanol. Finally, they were diluted to the mark with water and transferred directly to CE vials. The assay was repeated six times, individually weighing the tablet powders.

Accuracy and precision of UV method. The samples were obtained by weighing the tablet powders equivalent to 4 mg of candesartan and 20 mg of valsartan. The samples were placed in 25 ml volumetric flasks containing ca. 15 ml of methanol, sonicated for 10 min to dissolve the active ingredient, diluted to the mark and filtered by nylon membrane filters (0.45 μm). Respective volumes of 0.1 ml for candesartan and 0.5 ml for valsartan were transferred to 10 ml volumetric flasks and diluted to volume with methanol.

Specificity. The specificity of both methods was checked by a simple comparison of electropherograms or UV absorption spectra obtained from the standards and these for respective tablet samples.

RESULTS AND DISCUSSION

Optimalization of C E method. In order to improve the migration times and peak shapes, borate, phosphate and citrate buffers were employed. In addition, the effect of concentration of the buffer was examined. Finally, 50 mmol/l phosphate buffer at pH 9.0 showing the nicest peak shapes and a stable baseline current was chosen. The separation voltage was set at 25 kV, which affords sufficient migration time and acceptable current generation.

Robustness study. For robustness study in CE method, the influence of deliberate small changes in the pH buffer (9.0±0.5) and in the wavelength of detection (233±2nm for candesartan and 217±2nm for valsartan) on the results was tested. In addition, the robustness of UV method was tested by changing the wavelength (211±2 nm for candesartan and 206±2 nm for valsartan, respectively). Despite small changes in the migration times or absorbance values, stability towards these changes was proved.

Linearity in CE method. The six-point calibration curves were found to be linear as least squares regression gave excellent correlation coefficient (r), which was 0.9992 for candesartan and 0.9999 for valsartan (Table 1). The typical electropherograms for respective candesartan and valsartan solutions are shown in Figs. 2–3.

Table 1. Linear regression equations for capillary electrophoresis (CE) and spectrophotometric (UV) method

Method	Drug	Linearity range (μg/ml)	Regression equation y = ax + b (n=6)	r
CE		40-140		
	candesartan		y=4.6291(±0.3287)x–0.1155(±0.0247)	0.9992
	valsartan		y=6.6424(±0.0518)x+0,0475(±0.0145)	0.9999
UV		2-12		
	candesartan		y=0.0942(±0.0021)x+0.0475(±0.0382)	0.9999
	valsartan		y=0.0940(±0.0035)x+0.0588(±0.0403)	0.9998

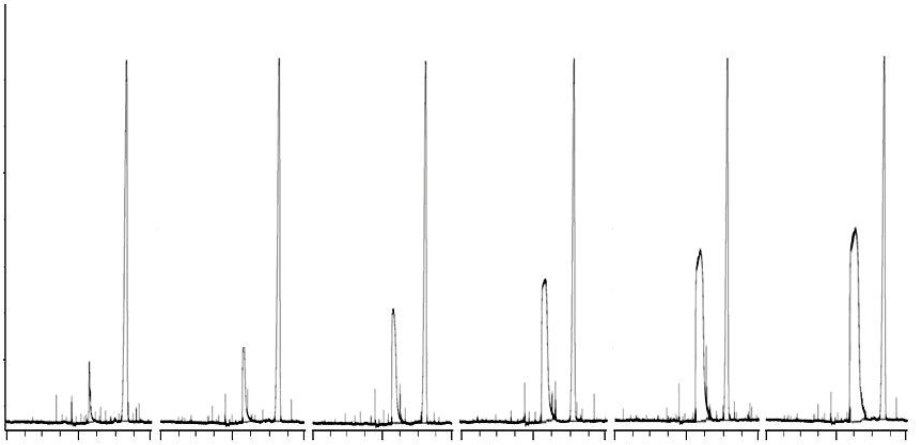


Fig. 2. Electropherograms recorded for standard solutions of candesartan in the calibration range (40–140 μg/ml) in the presence of valsartan (internal standard)

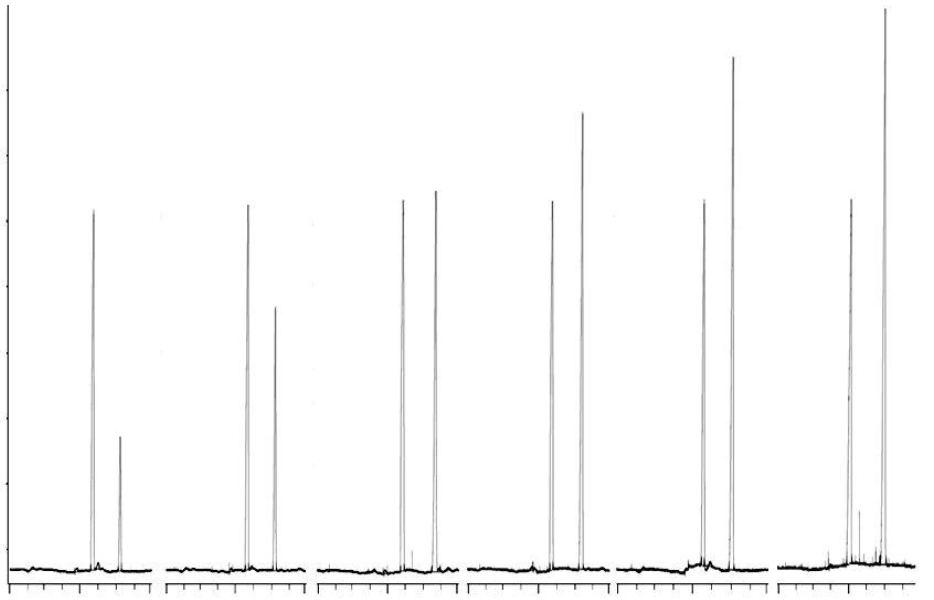


Fig. 3. Electropherograms recorded for standard solutions of valsartan in the calibration range (40–140 μg/ml) in the presence of telmisartan (internal standard)

Linearity in UV method. The six-point calibration curves were found to be linear with good correlation coefficient (r), which was 0.9999 for candesartan and 0.9998 for valsartan (Table 1). The typical spectra for respective candesartan and valsartan solutions are shown in Figs. 4–5.

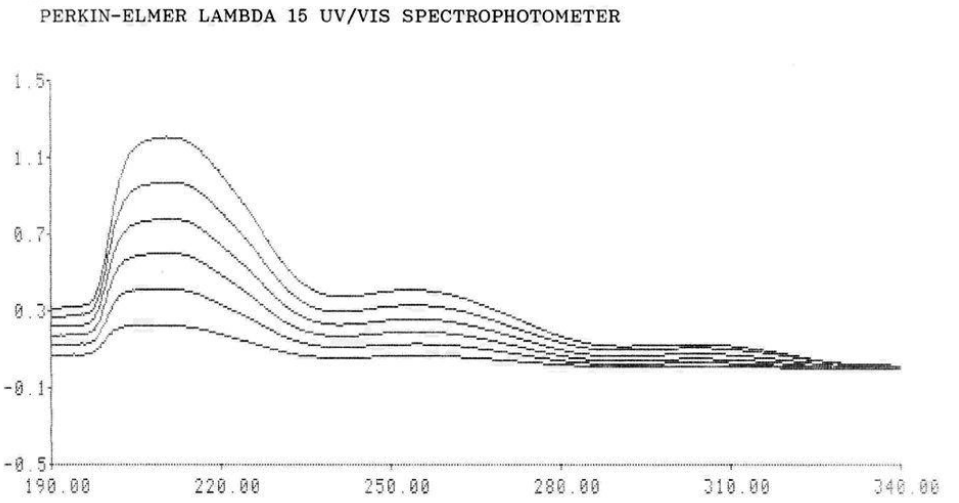


Fig. 4. Spectra of standard solutions of candesartan in the calibration range 2–12 μg/ml

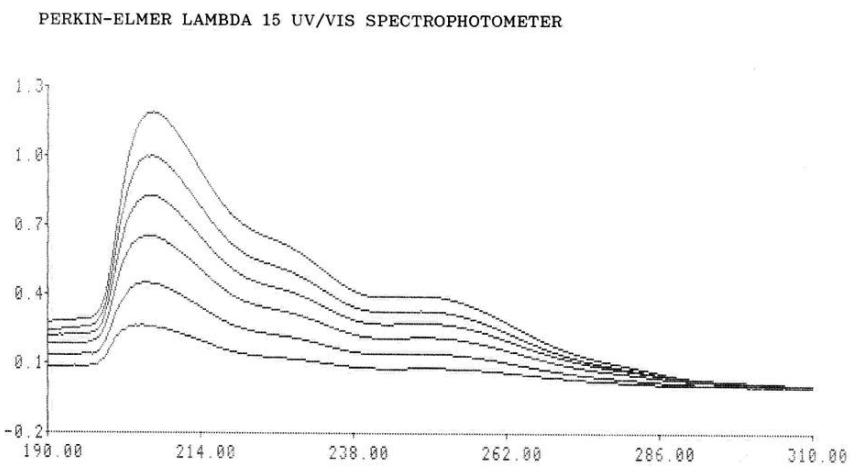


Fig. 5. Spectra of standard solutions of valsartan in the calibration range 2–12 µg/ml

Precision of the systems. The RSD values for inter day and the intermediate precision are shown in Table 2.

Table 2. Precision of the system (data obtained for the standard solutions of the drugs)

		Intra-day precision (n=3)		Intermediate precision		
				(n=3)		(n=9)
Method	Range [µg/ml]	Response factor* Mean ± SD	RSD [%]	Response factor* Mean ± SD	RSD [%]	Total RSD [%]
CE						
Candesartan	60	3.7152±0.0972	2.62	3.6978±0.0744	2.01	3.02
	140	3.7723±0.0650	1.72	3.8335±0.0838	2.18	
	220	3.9290±0.0781	1.99	3.9090±0.0705	1.80	
Valsartan	60	6.8489±0.2049	2.99	6.8828±0.2008	2.92	2.14
	140	6.8311±0.0351	0.51	6.8328±0.0891	1.30	
	220	6.7171±0.1286	1.92	6.7181±0.0826	1.23	
UV						
Candesartan	3	0.1045±1.89E-03	1.81	0.1057±2.52E-03	2.39	3.24
	7	0.1037±6.86E-04	0.66	0.1024±2.32E-03	2.27	
	11	0.1001±7.32E-04	0.73	0.0993±1.05E-03	1.06	
Valsartan	3	0.1020±1.17E-03	1.14	0.1039±2.49E-03	2.40	2.84
	7	0.1009±2.68E-03	2.66	0.1034±3.47E-03	3.36	
	11	0.1032±1.92E-03	1.86	0.1013±2.46E-03	2.43	

*Relationship between the ratio of peak areas (the drug versus internal standard) and concentration of the respective drug

Precision of the methods. All results except those obtained for candesartan in CE procedure, were homogenic and t test showed no significant differences between them and the declared amounts (Table 3).

Table 3. Precision and accuracy of the assays (the data obtained for the powdered tablets; n=6)

Method	Mean content (mg)	SD (mg)	RSD (%)	Confidence interval (mg)	Recovery (%)	t-Student test
CE						
Candesartan	15.44	0.39	2.53	15.26-15.62	96.50	*t=-6.0370; p=1.3332E-05*
Valsartan	158.6	4.01	2.52	157.0-160.7	99.28	t=-1.2195; p=0.2393
UV						
Candesartan	15.97	0.37	2.32	16.22-16.46	99.84	t=-0.4257; p=0.6729
Valsartan	159.3	2.24	1.40	158.8-160.3	99.54	t=-1.9830; p=0.0553

Table 4. Statistical comparisons between capillary electrophoresis (CE) and spectrophotometric (UV) method

Method	F–Snedecor test		t–Student test		Wilcoxon test	
	F	p	t	p	W	p
Candesartan CE-UV	1.12	0.76	*-4.87*	*1.08E-05*	*10**	*1.01E-3*
Valsartan CE-UV	*3.21*	*3.44E-03*	-0.49	0.63	73*	0.59

*n<25

Accuracy in tablets. As was shown in Table 3, for both methods all the obtained values lie in respective confidence interval, so all assays were proved to be sufficiently accurate.

Specificity. To assess potent interferences from degradation products, we investigated the stability of analytes during 24 h. We compared the peak-area ratios or absorption of the drugs after 1, 3, 6, 12 and 24 hrs, and monitored the presence of additional peaks in electropherograms and in respective spectra. For both methods, no observable changes were noted.

Statistical comparison. A pair-wise comparison of precision by F-Snedecor test and accuracy by t-Student or Wilcoxon tests was performed. For candesartan, higher accuracy in UV method than in CE method was achieved. It was probably because of some problems when candesartan had been dissolved in methanol-water mixture. For valsartan, the UV method showed to be more precise than CE procedure.

CONCLUSIONS

The proposed CE and classical UV spectrophotometric methods demonstrated sufficient stability and reproducibility with RSD of less than 3.4% for both intra-day and intermediate precision. They were successively used for the determination of the mentioned drugs in respective formulations. Both methods only require a simple extraction of the drugs from the tablets before analysis. However, some statistically significant differences between the methods were stated. The elaborated UV method seems to be more accurate and more precise than CE procedure. Spectrophotometry is also clearly less expensive and requiring a shorter analysis time. On the other hand, electrophoretic analysis could be more selective and more useful in the case when some possible impurities are present in tablets.

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SUMMARY

Two new methods, capillary electrophoresis (CE) and classical spectrophotometry (UV), were developed and validated for determination of two AT_1 receptor antagonists, candesartan and valsartan in pharmaceutical formulations. CE was performed using a 75 μm x 94 cm fused silica capillary (72 cm effective length). The phosphate buffer at pH 9.0 (50 mmol/l), 25 kV voltage, 30°C temperature and hydrodynamic injection (10 mbar, 6 s) were chosen as CE parameters. The solutions of both drugs were prepared in water-methanol mixture (1:1). Detection was done spectrophotometrically at 233 nm for candesartan and 217 nm for valsartan. UV spectrophotometry was carried out at 211 and

206 nm for methanolic solutions of both drugs. The precision and accuracy of both methods were compared. Despite some differences, the results showed that they both are suitable for quantitative determination of these drugs for different pharmaceutical purposes.

Key words: candesartan, valsartan, determination, capillary electrophoresis, spectrophotometry

STRESZCZENIE

Opracowano i zwalidowano dwie nowe metody oznaczania leków z grupy antagonistów receptora angiotensynowego AT_1, kandesartanu i walsartanu przy użyciu elektroforezy kapilarnej (CE) oraz klasycznej spektrofotometrii (UV). W metodzie CE zastosowano niemodyfikowaną kapilarę kwarcową o wymiarach 75 µm x 94 cm (72 cm do celi pomiarowej), 50 mmol/l bufor fosforanowy o pH 9.0, napięcie 25 kV, temp. 30°C i nastrzyk hydrodynamiczny (10 mbar, 6 s). Roztwory obydwu leków przygotowano w mieszaninie metanol-woda (1:1). Detekcję prowadzono spektrofotometrycznie przy 233 nm dla kandesartanu oraz 217 nm dla walsartanu. W metodzie spektrofotometrycznej (UV) mierzono absorbancję roztworów metanolowych przy 211 nm dla kandesartanu i 206 nm dla walsartanu. Obydwie metody porównano pod względem precyzji oraz dokładności. Pomimo pewnych różnic potwierdzonych statystycznie wyniki badań pokazują, że obydwie metody można wykorzystać do oznaczeń farmaceutycznych.

Słowa kluczowe: kandesartan, walsartan, oznaczanie, elektroforeza kapilarna, spektrofotometria