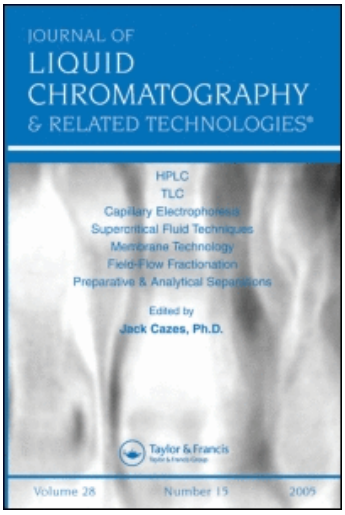


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# Monitoring of Impurity Level of Valsartan and Hydrochlorothiazide Employing an RP-HPLC Gradient Mode

Darko Ivanović[a]; Anđelija Malenović[a]; Biljana Jančić[a]; Mirjana Medenica[b]; Marija Mašković[c]
[a] Faculty of Pharmacy, Department of Drug Analysis, Belgrade, Serbia [b] Faculty of Pharmacy, Department of Physical Chemistry, Belgrade, Serbia [c] Medicines and Medical Devices Agency of Serbia, Serbia

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# Monitoring of Impurity Level of Valsartan and Hydrochlorothiazide Employing an RP–HPLC Gradient Mode

**Darko Ivanović, Anđelija Malenović, and Biljana Jančić**
Faculty of Pharmacy, Department of Drug Analysis, Belgrade, Serbia

**Mirjana Medenica**
Faculty of Pharmacy, Department of Physical Chemistry, Belgrade, Serbia

**Marija Mašković**
Medicines and Medical Devices Agency of Serbia, Serbia



**Abstract:** The multi-component preparation Co–Diovan® is indicated for the hypertension treatment in patients whose blood pressure is not adequately controlled by monotherapy. Its active ingredients are valsartan and hydrochlorothiazide.

The reversed–phase high performance liquid chromatographic method (RP–HPLC) for the determination of valsartan and hydrochlorothiazide, as well as their impurities level, was developed and described in this paper. As the investigated substances were structurally different with extremely different lipophilicity and polarity, isocratic elution was not possible, so an optimal gradient mode was settled on.

The chromatograms were recorded using the Agilent 1100 Series chromatographic system with DAD detector. Separations were performed on a Hypersil 120–5 ODS column (250 mm × 4.6 mm; 5 μm particle size) at 25°C column temperature. The gradient high performance liquid chromatographic system was developed, and the following mobile phases were used: A) mixture acetonitrile–water (10:90 V/V); pH of the mobile phase was adjusted to 2.5 with 85% orthophosphoric acid, and B) mixture acetonitrile–water (90:10 V/V); pH of the mobile phase was

Address correspondence to Prof. Dr. Mirjana Medenica, Faculty of Pharmacy, Department of Physical Chemistry, Vojvode Stepe 450, 11000 Belgrade, Serbia. E-mail: medenica@pharmacy.bg.ac.yu

adjusted to 2.5 with 85% orthophosphoric acid. Injection volume was 50 μL, flow rate 1 mL $min^{-1}$ and UV detection was performed at 256 nm. Methyl parahydroxybenzoate was used as an internal standard and acetonitrile–water (40:60 V/V) as a solvent.

Afterwards, the developed method was subjected to the method validation. The investigated validation parameters (selectivity, linearity, precision, accuracy, LOQ, and LOD) proved the suitability of the method for the simultaneous determination of valsartan, hydrochlorothiazide, and their impurities in appropriate tablets.

**Keywords:** High performance liquid chromatography, Validation, Valsartan, Hydrochlorothiazide, Impurities



## INTRODUCTION

Valsartan, chemically, is (S)-N-valeryl-N-((2′-(1H-tetrazole-5-yl)-biphenyl-4-yl)-methyl)-valine, and its impurity, CGP 55 390, which was tracked, is (S)-N-butyryl-N-((2′-(1H-tetrazole-5-yl)-biphenyl-4-yl)-methyl)-valine. The hydrochlorothiazide is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide and its impurity, SU 5683, is 5-chloroaniline-2,4-disulfonamide. There is no monograph for valsartan in Ph. Eur., USP or JP.

The literature contains only a few references concerning quantitative analysis of valsartan and hydrochlorothiazide in pharmaceutical dosage forms. The micellar electrokinetic chromatographic method[1] and capillary zone electrophoretic method[2] were optimized and validated for the analysis of six angiotensin–II–receptor antagonists, as well as for their simultaneous determination with hydrochlorothiazide.[3] Derivative spectrophotometric methods for the determination of valsartan, as well as valsartan in the presence of hydrochlorothiazide were described.[4–6] Ratio derivative spectrophotometry and the inverse least square technique were applied to a binary mixture of valsartan and hydrochlorothiazide in tablets without a separation procedure.[7] Spectrophotometry[8] for the pKa determination was developed and applied to five angiotensin–II–receptor antagonists. The HPLC method for the simultaneous determination of valsartan and hydrochlorothiazide in tablets were described in literature.[5,6,9] Many papers have been published concerning analysis of valsartan in biological fluids. In some of them, HPLC methods for valsartan determination in human plasma were described.[10,11] Also, efforts were made to develop an adequate HPLC method with fluorimetric and UV detection for the determination of four angiotensin–II–receptor antagonists in human urine.[12,13] The spectrophotometric method for the determination of two angiotensin–II–receptor antagonists in human urine was used.[14]

In this paper, the RP-HPLC novel method for the simultaneous analysis of valsartan, hydrochlorothiazide, and their impurities, CGP 55 390 and SU 5683 is described (Figure 1). Analyzed dosage forms should not contain more than 0.2% of the impurity CGP 55 390 relative calculated the valsartan content, and

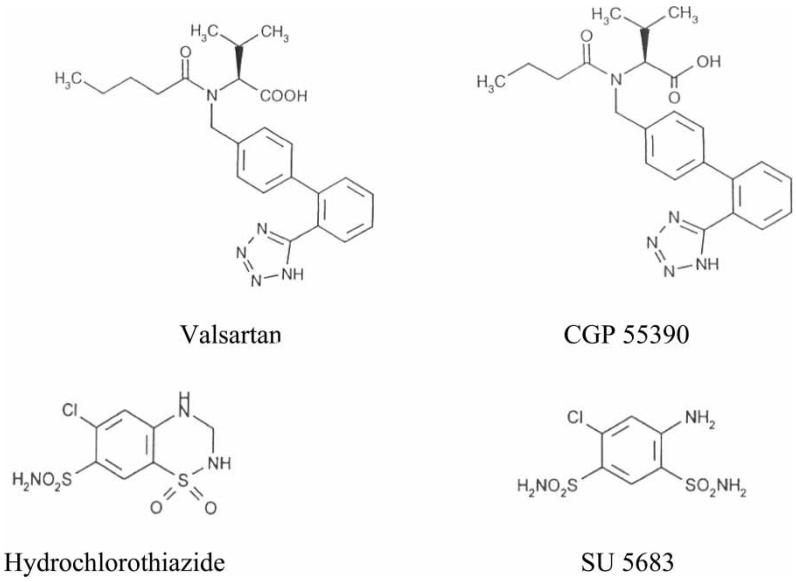


***Figure 1.*** Structural formulas of the analyzed compounds.



not more than 0.4% of the impurity SU 5683 relative calculated to the hydrochlorothiazide content.

Because of the importance of reliable methods for impurity profiling in pharmaceutical dosage forms, the RP–HPLC method, for the simultaneous determination of valsartan, hydrochlorothiazide, and their impurities in tablets, was developed and validated. There are no references concerning quantitative analysis of valsartan, hydrochlorothiazide, and their impurities in pharmaceuticals, so the proposed method could be considered as the improvement in chromatographic separation and determination of the above mentioned substances.

## EXPERIMENTAL

### Reagents and Samples

All the reagents used in experimental work were of an analytical grade. Acetonitrile, gradient grade (*Sigma–Aldrich*, Germany), water, HPLC grade, and orthophosphoric acid (*Carlo Erba*, Italy) were used for preparing a mobile phase and solvent. Co–Diovan® tablets were manufactured by *Novartis Pharma AG* (Switzerland) as well as the working standards of valsartan, hydrochlorothiazide, and their impurities CGP 55 390 and SU 5683.

## Chromatographic Conditions

The chromatographic system HP 1100 Series (*Agilent Technologies*, Germany) consisted of HP 1100 binary pump, HP 1100 DAD detector, HP 1100 column and autosampler thermostat, HP 1100 degasser, and HP Chem-Station integrator.

Separations were performed on a 4.6 mm × 250 mm, 5 μm particle size, Hypersil 120–5 ODS column at 25°C column temperature. Injection volume was 50 μL. Standard solutions, sample solutions, and laboratory mixtures were prepared using a mixture of 400 mL of acetonitrile and 600 mL of water as a solvent.

Separation and simultaneous determination of valsartan, hydrochlorothiazide, and their impurities CGP 55 390 and SU 5683 were performed using the gradient elution mode. The mobile phase A was a mixture of acetonitrile and water (10:90 V/V); pH of the mobile phase was adjusted to 2.5 with 85% orthophosphoric acid, and the mobile phase B was a mixture of acetonitrile and water (90:10 V/V); pH of the mobile phase was adjusted to 2.5 with 85% orthophosphoric acid. The gradient program is presented in Table 1.

The mobile phases as well as the solvent solution were filtered through a 0.2 μm Millipore filter. Methyl parahydroxybenzoate was used as an internal standard. The flow rate was 1.0 mL $min^{-1}$ and UV detection was performed at 256 nm.



## Stock Solutions

Stock solutions were prepared by dissolving the respective working standard substance in solvent to obtain the concentration of 2 mg $mL^{-1}$ for valsartan, 250 μg $mL^{-1}$ for hydrochlorothiazide, 20 μg $mL^{-1}$ for impurity CGP 55 390, 10 μg $mL^{-1}$ for impurity SU 5683, and 0.4 mg $mL^{-1}$ for methyl parahydroxybenzoate (internal standard).

***Table 1.*** Gradient program

| Time (min) | Mobile phase A (%) | Mobile phase B (%) |
|---|---|---|
| 0 | 95 | 5 |
| 6 | 30 | 70 |
| 7 | 10 | 90 |
| 15 | 10 | 90 |
| 15.1 | 95 | 5 |

Re-equilibration time was set up at 10 minutes.

### Solutions for the Method Linearity Estimation

For the calibration curves, a series of eight solutions were prepared in the concentration ranges 5–120 $\mu g\ mL^{-1}$ for valsartan; from 1.25–17.5 $\mu g\ mL^{-1}$ for hydrochlorothiazide; from 0.05–0.7 $\mu g\ mL^{-1}$ for impurity SU 5683, and a series of seven solutions were prepared in the concentration range 0.2–2.4 $\mu g\ mL^{-1}$ for impurity CGP 55 390. Methyl parahydroxybenzoate was used as an internal standard at a concentration of 20 $\mu g\ mL^{-1}$.

### Solutions for the Method Precision Estimation

To check the precision of the proposed RP–HPLC method, three series of standard solutions (60 $\mu g\ mL^{-1}$, 80 $\mu g\ mL^{-1}$, and 100 $\mu g\ mL^{-1}$ for valsartan; 10 $\mu g\ mL^{-1}$, 12.5 $\mu g\ mL^{-1}$, and 15 $\mu g\ mL^{-1}$ for hydrochlorothiazide; 1.2 $\mu g\ mL^{-1}$, 1.6 $\mu g\ mL^{-1}$, and 2 $\mu g\ mL^{-1}$ for impurity CGP 55 390; 0.4 $\mu g\ mL^{-1}$, 0.5 $\mu g\ mL^{-1}$, and 0.6 $\mu g\ mL^{-1}$ for impurity SU 5683) were prepared, with ten solutions for each concentration. Methyl parahydroxybenzoate was used as an internal standard with a concentration of 20 $\mu g\ mL^{-1}$.

### Solutions for the Method Accuracy Estimation

For the accuracy testing, two laboratory mixtures were prepared: i) The first, containing placebo components, valsartan and hydrochlorothiazide in the ratio of their content corresponding to that in tablets; ii) the second laboratory mixture containing placebo components, impurities CGP 55 390 and SU 5683 in the ratio of their content corresponding to that in tablets, as well.

Three series of standard solution dilutions calculated as 80%, 100%, and 120%, with the concentrations which correspond to those in tablets (64 $\mu g\ mL^{-1}$, 80 $\mu g\ mL^{-1}$, and 96 $\mu g\ mL^{-1}$ for valsartan; 10 $\mu g\ mL^{-1}$, 12.5 $\mu g\ mL^{-1}$, and 15 $\mu g\ mL^{-1}$ for hydrochlorothiazide; 1.28 $\mu g\ mL^{-1}$, 1.60 $\mu g\ mL^{-1}$, and 1.92 $\mu g\ mL^{-1}$ for impurity CGP 55 390; 0.4 $\mu g\ mL^{-1}$, 0.5 $\mu g\ mL^{-1}$, and 0.6 $\mu g\ mL^{-1}$ for impurity SU 5683) were prepared with three solutions for each concentration. In all solutions, methyl parahydroxybenzoate was added as an internal standard in a concentration of 20 $\mu g\ mL^{-1}$.

### Solutions for the Method Selectivity Estimation

To prove specificity/selectivity of the proposed RP–HPLC method, a mixture consisting of placebo components was prepared in the ratio which corresponded to their content in tablets and than treated in the same manner as the tablet mass used for sample solution.

A standard solution mix containing all the components in the ratio corresponding to the content in tablets (0.8 mg $mL^{-1}$ for valsartan; 125 μg $mL^{-1}$ for hydrochlorothiazide; 1.6 μg $mL^{-1}$ for impurity CGP 55 390 and 0.5 μg $mL^{-1}$ for impurity SU 5683) was prepared and used for specificity/selectivity assessment. Methyl parahydroxybenzoate was used as an internal standard in a concentration of 20 μg $mL^{-1}$.

### Sample Solution 1

Ten tablets were accurately weighted and powdered. The quantity of the powdered tablets containing 160 mg for valsartan and 25 mg for hydrochlorothiazide were transferred to the 100 mL volumetric flask and extracted with the solvent using a ultrasonic bath and mechanical stirrer, and then centrifuged. The supernatant was used to prepare a series of ten solutions containing 80 μg $mL^{-1}$ for valsartan, 12.5 μg $mL^{-1}$ for hydrochlorothiazide, and 20 μg $mL^{-1}$ of methyl parahydroxybenzoate as an internal standard.

The solutions were used for the valsartan and hydrochlorothiazide assay in tablets.



### Sample Solution 2

Ten tablets were accurately weighted and powdered. The quantity of the powdered tablets containing 160 mg of valsartan and 25 mg of hydrochlorothiazide was transferred to the 100 mL volumetric flask and extracted with the solvent using a ultrasonic bath and mechanical stirrer, and then centrifuged. The supernatant was used to prepare a series of ten solutions containing 800 μg $mL^{-1}$ of valsartan, 125 μg $mL^{-1}$ of hydrochlorothiazide, and 20 μg $mL^{-1}$ of methyl parahydroxybenzoate as an internal standard.

The solutions were used for the impurity profile estimation in the analyzed tablets.

## RESULTS AND DISCUSSION

Structural, physical, and chemical properties of analyzed substances are very important factors in establishing appropriate chromatographic conditions. According to that, non-polar HPLC columns were chosen for the preliminary study. Retention behaviour of the investigated substances was analyzed using different HPLC columns such as LiChrocart Purosphere STAR RP −18 endcapped, 150 mm × 4.6 mm, 5 μm; Zorbax SB–C18, 250 mm × 4.6 mm, 5 μm, and Hypersil 120–5 ODS, 250 mm × 4.6 mm, 5 μm.

It was noticed that the optimal retention of hydrochlorothiazide and its impurity SU 5683 requested mobile phases with a low percent of organic

solvent, i.e., less than 30%. However, on the other hand, valsartan and its impurity CGP 55 390, as more liphophilic substances were retained for almost 60 min under the same chromatographic conditions. Because of that, isocratic elution was not found to be economical to assay the mixture, and efforts were aimed at settling on an optimal gradient mode. A range of preliminary experiments, were conducted to establish some initial gradient elution conditions.

Up until now, the mobile phases containing organic solvent and different buffer solutions were used in most of the HPLC methods for the analyses of valsartan and hydrochlorothiazide combination. Therefore, in order to establish an economical HPLC method, which could be applied in the routine analysis of pharmaceuticals containing valsartan, hydrochlorothiazide, and their impurities, it was decided to use mobile phases without buffer solution.

Critical pairs in separation were hydrochlorothiazide and its impurity SU 5683, as well as valsartan and its impurity CGP 55 390. Hydrochlorothiazide and its impurity have a common substitute benzene ring. Compared to the hydrochlorothiazide, SU 5683 has one more free sulfonamide group and primary aromatic amine, so the key difference is in polarity and acidity/alkalinity. In the reverse phase mode, as a more polar compound, the impurity was eluted earlier than hydrochlorothiazide with low pH mobile phases (lower than 5.0). Increasing the pH had a greater influence on the impurity's retention than on hydrochlorothiazide, leading to non-acceptable selectivity. Having in mind structure and chromatographic behavior, it was concluded that a mobile phase with low pH values and low content of acetonitrile are appropriate for the separation of these two substances. On the other hand, valsartan and its impurity, as more lipophilic compounds in mixture, differ in one methyl group on the chain side. It was expected that valsartan, which has one methyl group more than CGP 55 390, will be retained more in the reverse phase mode. As the free carboxylic group in their structure will be in molecular form in acidic solutions, the mobile phase should have low pH values. On the basis of preliminary experiments, a high percent of acetonitrile is necessary for optimal run time. Such mixtures (compounds with high and low lipophility) demand gradient mode starting with a low percent of acetonitrile and gradual increasing of organic solvent content to achieve an optimal separation and retention time.

After settling on an appropriate gradient program, which enables satisfactory resolution between hydrochlorothiazide and SU 5683, as well as valsartan and CGP 55 390, the necessity of improving the peak shape and symmetry of hydrochlorothiazide and SU 5683 appeared. The addition of TEA had no significant influence on separation and peak shape, so it was excluded from further investigation. Because of hydrochlorothiazide and SU 5683 peak tailing, it was decided to keep the Hypersil 120–5 ODS, 250 mm $\times$ 4.6 mm, 5 $\mu$m particle size, on which the best separation and peak symmetry was achieved. All the experiments conducted in order to asses the most appropriate conditions for separating investigated substances enabled setting the gradient mode program given in Table 1.

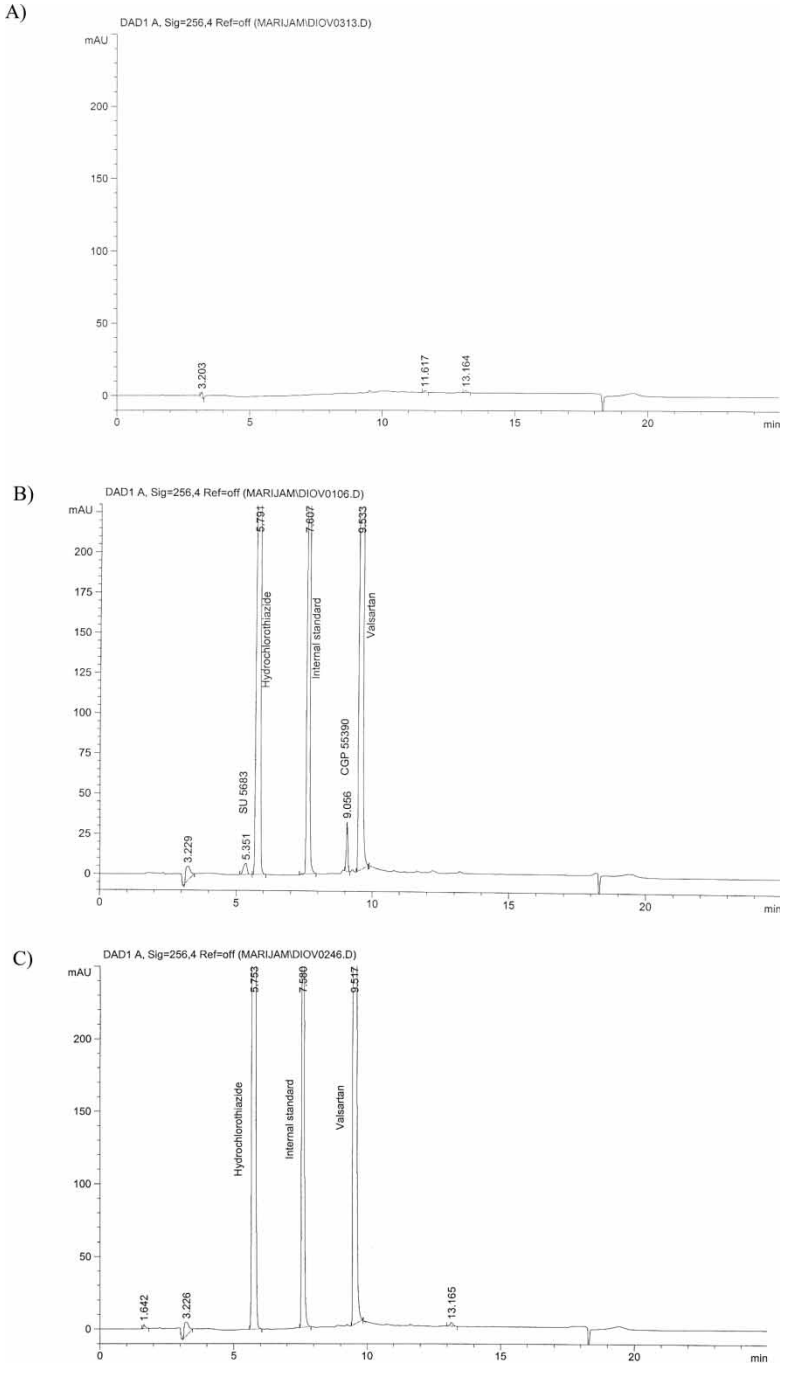


***Figure 2.*** The chromatogram of: A) Placebo, B) Laboratory mixture, and C) Sample 2.

Determination of statistical parameters in terms of selectivity/specificity, linearity, precision, and accuracy followed establishment of the optimal separation conditions.

The chromatograms of standard solution mix for estimating the method selectivity, placebo mixture, and the representative chromatogram of the sample solution 2, are presented in Figure 2. The chromatograms indicate that the proposed method is selective, as there are no significant interfering peaks at the retention times of valsartan, hydrochlorothiazide, impurity CGP 55 390, impurity SU 5683, and internal standard methyl parahydroxybenzoate. All excipients were eluated at different times and did not interfere with the analyzed compounds. The retention times are 5.37 min for SU 5683, 5.81 min for hydrochlorothiazide, 7.64 min for methyl parahydroxybenzoate, 9.05 min for CGP 55 390, and 9.58 min for valsartan.

Linear relationships between response and concentration was obtained from 5–120 $\mu g\ mL^{-1}$ for valsartan; from 1.25–17.5 $\mu g\ mL^{-1}$ for hydrochlorothiazide; from 0.05–0.7 $\mu g\ mL^{-1}$ for impurity SU 5683, and from 0.2–2.4 $\mu g\ mL^{-1}$ for impurity CGP 55 390. The obtained regression data are given in Table 2.

The results for precision of the proposed RP–HPLC method are given in Table 3. Statistical values, such as standard deviation (S), coefficient of variation (CV), and recoveries indicate that the proposed method is precise.

Accuracy investigation results for the evaluated RP–HPLC method are presented in Table 4. Statistical values, such as standard deviation (S), coefficient of variation (CV), and recoveries indicate that the proposed method is accurate.

Being important for the quantitative analysis, values of the limits of detection (LOD) and limit of quantification (LOQ) were experimentally determined (Table 5).

Results from the quantitative analysis of valsartan and hydrochlorothiazide in tablets are given in Table 6. Results are within the required limits of $\pm 5\%$, established in the MA dossier.

***Table 2.*** Calibration curves parameters

| Substance | Concentration range ($\mu g\ mL^{-1}$) | a | b | r | $S_b$ | $t_b$ |
|---|---|---|---|---|---|---|
| Valsartan | 5–120 | 1.3383 | 4.5675 | 0.9975 | 2.59 | 1.736 |
| Hydrochlorothiazide | 1.25–17.5 | 1.0413 | 0.3624 | 0.9995 | 0.1436 | 2.523 |
| CGP 55 390 | 0.2–2.4 | 13.089 | −0.5611 | 0.9996 | 0.2401 | 0.1028 |
| SU 5683 | 0.05–0.7 | 17.953 | 0.3092 | 0.9994 | 2.337 | 3.007 |

$y = ax + b$; a–Slope; b–Intercept; r–Correlation coefficient; $S_b$–Standard deviation of the intercept; $t_{tab.} = 3.707$ ($n = 8$, $p = 0.995$).

***Table 3.*** Precision of the RP–HPLC method

| Compound | Injected | Found ($\mu g\ mL^{-1}$) | R (%) | CV (%) |
|---|---|---|---|---|
| Valsartan ($\mu g\ mL^{-1}$) | 60 | $63.55 \pm 0.74^a$ | 105.9 | 1.16 |
| | 80 | $82.99 \pm 0.45$ | 103.7 | 0.55 |
| | 100 | $101.41 \pm 0.32$ | 101.4 | 0.31 |
| Hydrochlorothiazide ($\mu g\ mL^{-1}$) | 10 | $10.05 \pm 0.09$ | 100.5 | 0.91 |
| | 12.5 | $12.41 \pm 0.08$ | 99.2 | 0.64 |
| | 15 | $14.85 \pm 0.25$ | 99.0 | 1.70 |
| CGP 55 390 ($\mu g\ mL^{-1}$) | 1.2 | $1.191 \pm 0.005$ | 99.3 | 0.43 |
| | 1.6 | $1.573 \pm 0.011$ | 98.3 | 0.69 |
| | 2.0 | $1.980 \pm 0.019$ | 99.0 | 0.95 |
| SU 5683 ($\mu g\ mL^{-1}$) | 0.4 | $0.399 \pm 0.002$ | 99.8 | 0.58 |
| | 0.5 | $0.498 \pm 0.006$ | 99.5 | 1.20 |
| | 0.6 | $0.600 \pm 0.011$ | 100.1 | 1.79 |

[a]S–Standard deviation (n = 10).



According to the chromatograms obtained with the sample solution 2, which were used for the impurity profile estimation in the analyzed tablets, neither CGP 55 390 nor SU 5683 impurity were detected. It surely indicates a high quality of the raw materials used, namely active pharmaceutical ingredients valsartan and hydrochlorothiazide, as well as the good quality of the analyzed Co–Diovan® tablets.

***Table 4.*** Accuracy of the RP–HPLC method

| Compound | Injected | Found ($\mu g\ mL^{-1}$) | R (%) | CV (%) |
|---|---|---|---|---|
| Valsartan ($\mu g\ mL^{-1}$) | 64 | $67.42 \pm 0.76^a$ | 105.3 | 1.12 |
| | 80 | $83.92 \pm 0.42$ | 104.9 | 0.50 |
| | 96 | $99.51 \pm 0.40$ | 103.7 | 0.40 |
| Hydrochlorothiazide ($\mu g\ mL^{-1}$) | 10 | $10.05 \pm 0.03$ | 100.5 | 0.33 |
| | 12.5 | $12.64 \pm 0.04$ | 101.1 | 0.33 |
| | 15 | $15.04 \pm 0.07$ | 100.3 | 0.49 |
| CGP 55 390 ($\mu g\ mL^{-1}$) | 1.2 | $1.299 \pm 0.006$ | 101.5 | 0.48 |
| | 1.6 | $1.627 \pm 0.018$ | 101.7 | 1.09 |
| | 2.0 | $1.952 \pm 0.026$ | 101.7 | 1.33 |
| SU 5683 ($\mu g\ mL^{-1}$) | 0.4 | $0.404 \pm 0.001$ | 101.0 | 0.28 |
| | 0.5 | $0.502 \pm 0.003$ | 100.4 | 0.52 |
| | 0.6 | $0.610 \pm 0.014$ | 100.7 | 2.34 |

[a]S–Standard deviation (n = 3).

*Table 5.* Limits of detection (LOD) and limit of quantification (LOQ)

| Compound | LOQ[a] ($\mu g\ mL^{-1}$) | LOD[a] ($\mu g\ mL^{-1}$) |
|---|---|---|
| Valsartan | 0.02 | 0.004 |
| Hydrochlorothiazide | 0.04 | 0.01 |
| CGP 55390 | 0.02 | 0.008 |
| SU 5683 | 0.025 | 0.01 |

[a]Experimentally determined values.

*Table 6.* Quantitative analysis of Co–Diovan® tablets

| Compound | Injected ($\mu g\ mL^{-1}$) | Found ($\mu g\ mL^{-1}$) | Found ($mgtbl.^{-1}$) | R (%) | CV (%) |
|---|---|---|---|---|---|
| Valsartan | 80 | $79.6 \pm 0.8$[a] | 79.6 | 99.5 | 1.0 |
| Hydrochlorothiazide | 12.5 | $12.7 \pm 0.1$ | 12.7 | 101.7 | 0.8 |

[a]Standard deviation S ($n = 10$).



## CONCLUSION

The only possible way for monitoring impurities level of valsartan and hydrochlorothiazide was the gradient mode RP–HPLC, because of the drastically difference in structure, lipophilicity, and polarity of the investigated substances. The proposed method is selective enough and enables simultaneous qualitative and quantitative analysis of valsartan, hydrochlorothiazide, and their impurities CGP 55 390 and SU 5683, respectively. This is of great importance, having in mind the restrictive requirements for the impurities level in pharmaceuticals established by Pharmacopoeias and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Statistical parameters values of the proposed RP–HPLC method validation are within the required criteria. No excipients interference is present; method results are precise and accurate. Therefore, the evaluated RP–HPLC method presents a significant improvement in chromatographic analysis, compared to the other methods used mainly for the assay of valsartan and hydrochlorothiazide in pharmaceuticals, with no reference to their impurity profiles.

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