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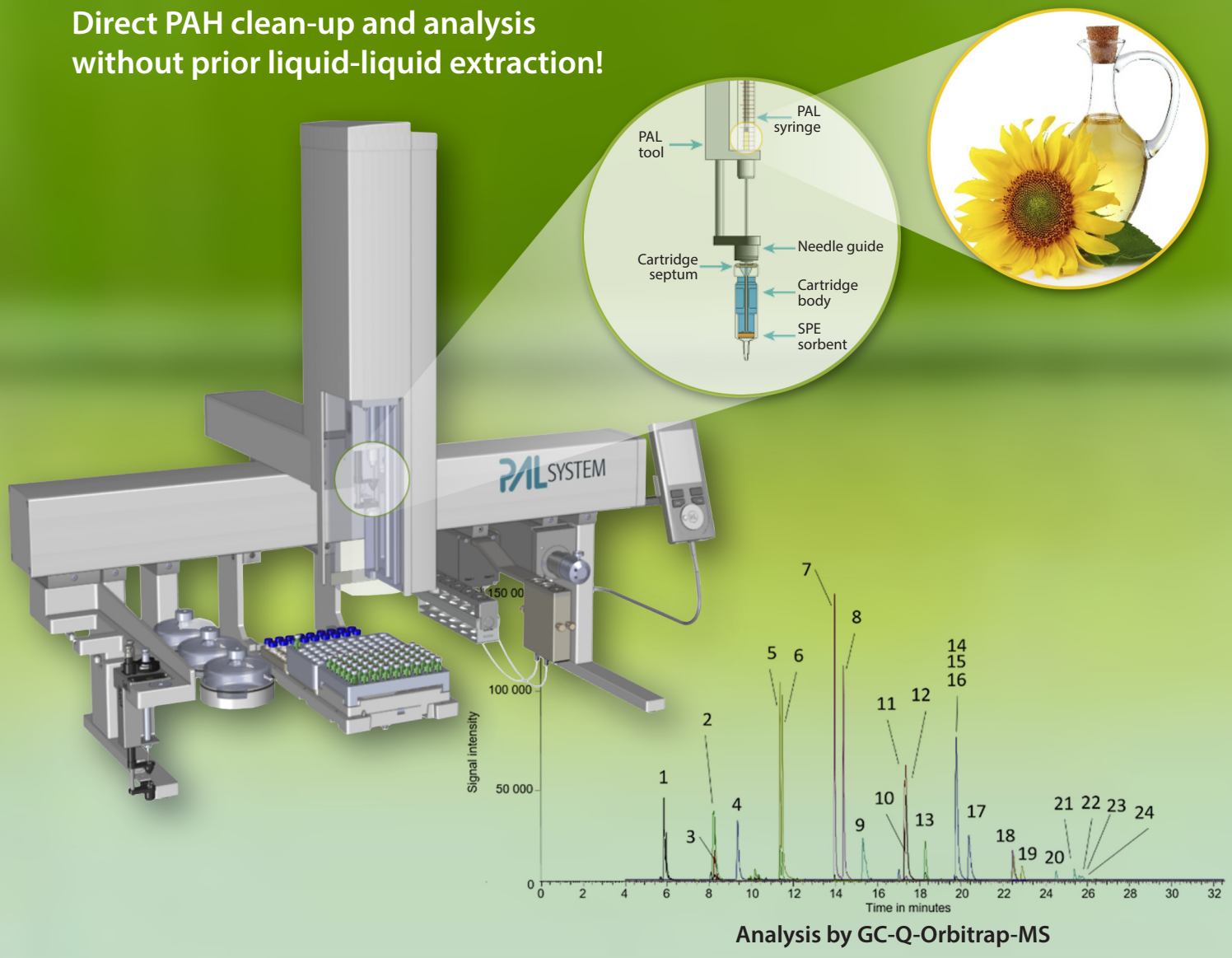

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RESEARCH ARTICLE

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New liquid chromatography assays for simultaneous quantification of antihypertensives atenolol and valsartan in their dosage forms

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Nowadays, various single-pill combinations are used as the best choice in hypertension management. However, these pills made a high challenge to analysts in terms of quality control assays. We have developed three sensitive, selective, fast, simple, green, accurate, precise, and robust isocratic high-performance liquid chromatography methods for simultaneous determination of valsartan and atenolol in dosage forms. To find the appropriate high-performance liquid chromatography conditions for the separation of the examined drugs, various columns, isocratic mobile phase systems were tried, and successful attempts were performed. The used columns proved to be indispensably applicable and gave a shorter analysis time and peak symmetries. This reduction in total run time leads to low solvent consumption and makes all methods more economical. The linearity, accuracy, and precision remained within the acceptable limits. Therefore, all developed methods are suitable for the routine quality control analysis of any pharmaceutical preparation containing the two tested drugs with the proposed chromatographic methods advantages for checking quality during stability studies of their pharmaceutical preparations.

KEYWORDS
Atenolol, liquid chromatography, single-pill combinations, valsartan

1 | INTRODUCTION

Hypertension is usually very difficult to be treated with only a single medicine management. Therefore, a lot of single-pill combinations are used to assure the management of hypertension. That type of treatment makes it more important to get suitable assay methods for the simultaneous analysis of the co-administered drugs [1]. The main aim of any antihypertensive therapy is to normalize blood pressure without intolerable side effects [2]. This can be done by combining antihypertensive with various mechanisms of action. For such purposes, effective and reliable methods for the analysis of the determination of active pharmaceutical ingredients in bulk, model mixtures, drugs, and biological fluids should be developed. In this study, valsartan and atenolol were chosen as representative examples.

Valsartan is an orally active nonpeptide triazole-derived antagonist of angiotensin II with antihypertensive properties. Several techniques have been reported in the literature for the determination of valsartan individually and in combination with other drugs other than atenolol

Article related abbreviations: CA, C8 chromatographic columns LiChrospher® 60 RP-select B (4 mm i.d. X 125 mm, 5 μm); CB, LiChrospher® 60 RP-select B (4 mm i.d. X 250 mm, 5 μm); MA, mobile phase A; MB, mobile phase B; MC, mobile phase C; XDB, eXtra Dense Bonding

[3–23] in pharmaceutical dosage forms or human serum samples.

Atenolol is used as an antihypertensive and antiarrhythmic drug by acting as beta-blocker specific for beta-1 adrenergic receptors. Numerous analytical methods were reported [24–35] for the determination of atenolol in bulk and in combination with other drugs other than valsartan. However, to the best of our knowledge, the only published method [35] for the simultaneous analysis of valsartan and atenolol was developed this year by our group in order to introduce the in vitro dissolution profiles of their commercial tablets. It is first and simple method and in moving deeply in method development we found some new concepts of columns, buffer choicen and composition, pH of buffer, percentage of organic modifier, column oven temperature, flow rate, and injection volumes. In previously published articles, we worked on Zorbax XDB-C8 column, which is quite different from RP Select B column, in carbon load, active surfaces, number of theoretical plates, pore volumes, metal cation residuals, and column dimensions. This two-column Zorbax XDB-C8 and RP Select B showed differences in column efficiency and analytes peak symmetries when flow rate was changed. In decreasing flow rate, Zorbax XDB-C8 increases peak asymmetry with decreasing resolution, while lower flow rate with the use of RP Select B showed increased peak symmetries with decreased tailing and increased resolution of analytes. In this work, we demonstrated peak elution reverting due to change of type of phosphate buffer, or dependence of the type of phosphate buffer cation on the eluting profile of the different molecules. This governing the elution profile, might have great importance in method choice for impurities, degradation products, or metabolite studies in research of drugs related compounds, stability products, and bioequivalence or pharmacokinetic studies.

Compared with that, in this article, we decided to choose and test quite different C8 octylsilane based column, RP Select B with two dimensions of 125 and 250 mm, with 5 μm particles. In short comparison features, Zorbax XDB-C8 with 5um particles is a matrix with lower carbon load of 7.6%, with the lower active surface of 180m2/g, double end-capped, base deactivated metal cations, with pH range 2–9, and 80Angstrom pore size and with about 100.000 number of theoretical plates per meter for toluene, while RP Select B (C8) is a matrix is an older generation of single end-capped particles with 60Angstrom pore sizes, with almost twice higher carbon load of 12.6% and 360 m^2/g, but with halved number of theoretical plates to 55.000, and pH usable range 2–8, without base deactivation of metal cations in silica gel. All these tremendous differences in chromatographic matrixes prompted us to check the usability and applicability of older, cost-effective column RP Select B which can be purchased in cartridge type. We expected quite a different situation in chromatogram profile in comparison between Zorbax XDB-C8 and RP Select B. Our target in comparing with previous our paper is chromatographic column 4 mm id × 250 mm, 5 μm that can provide better chromatogram with better peak symmetries, double more number of theoretical plates, because the column is twice more loaded with chromate matrix. As will be shown below, with this column, we do not need to work with a lower flow rate of 0.5–0.6 mL used for 125 mm columns, since peaks are well balanced due to the doubled column filling matrix.

Atenolol and valsartan due to their chemical structure have quite different solubilities in water and organics, Log P = 0.16 and Log P = 1.499, respectively. This variation complicates creating a rapid successful and robust chromatographic method for their simultaneous determination. Their official pharmacopoeial assay methods were using quite different percentages of organic modifiers in the mobile phase, which suggests the inevitable use of gradient elution for their simultaneous determination. Uses of ion-pairing reagents do not promise a lot, long-lasting equilibrations with expensive chemicals and dedicating columns, without warranty of successful reasonable chromatogram.

Therefore, new analytical methods for their separation and quantification in pharmaceutical formulations were needed for their quality control assays especially in a combined mixture.

The aim of our work was to develop new simple and faster, isocratic HPLC methods for the determination of valsartan and atenolol. The main target for our newly developed methods was classical, routine quality control assay in all types of pharmaceutical quality control laboratories worldwide, with less sophisticated equipment and budgets.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Valsartan (purity 99.9%) was purchased from Jubilant Generics (India), atenolol (purity 98.9%) was purchased from Sigma-Aldrich (Switzerland). 40 mg valsartan (standard sample) and 50 mg atenolol (standard sample) were put in 100 mL measuring flasks and dissolved in 50 mL 50% v/v methanol, ultrasound crushed and treated for 2 min, and shaken for 15 min with an orbital shaker. The final concentrations were 0.5 mg/mL for atenolol and 0.4 mg/mL for valsartan. Samples were filtered through RC 0.45 μm syringe filters and injected.

Methanol used in experiments was HPLC gradient grade and potassium dihydrogen phosphate and potassium dihydrogen phosphate were of Ph. Eur. reagent grade and purchased from Merck Darmstdat, Germany. Analytical Balance Mettler Toledo MPC227, pH–meter Metrohm 827, deionized water from TKA Micro system, with final conductivity <0.05 μS/cm. IKA orbital shaker KS4000i was used for sample agitation. The nylon and regenerated cellulose RC 0.45 μm syringe filters were purchased from Agilent Technologies.

2.2 | Instrumental and conditions

The chromatography equipment used was a product of Varian, model Varian Pro Star PDA 330 with Varian Star software version 6.81 and Varian LC 920 PDA controlled by Galaxy software version 1.98. Three mobile phases were examined. Mobile phase A (MA) composed of potassium dihydrogen phosphate (25 mM, pH 7.3) and methanol (50:50, v/v); mobile phase B (MB) composed of ammonium dihydrogen phosphate (50 mM, pH 7.25) and methanol (50:50, v/v). Both A and B were pumped at 0.5 mL/min. Peak elution is reverting depending of type and molarity of phosphate buffer. The temperature of column oven was set at 40°C. The UV detector was adjusted at 225 nm wavelength; and mobile phase C (MB) composed of ammonium dihydrogen phosphate (50 mM, adjusted pH to 7.2 with 33% NH_4OH) and methanol (45:55, v/v), pumped with 1.0 mL/min at 42°C set temperature of column oven, with UV detector set to 225 nm and 237 nm wavelength. Analysis performed on CA: C8 chromatographic columns LiChrospher® 60 RP-select B (4 mm i.d. X 125 mm, 5 μm) and CB: LiChrospher® 60 RP-select B (4 mm i.d. X 250 mm, 5 μm). The injection volume was 5uL.

The used columns LiChrospher® 60 RP-select B (4 mm i.d. x 125 mm, 5 μm) and LiChrospher® 60 RP-select B (4 mm i.d. x 250 mm, 5 μm), purchased from Merck Darmstdat, Germany.

2.3 | Sample preparation

Twelve tablets of each preparation were studied to obtain statistically significant results. The tablets with declared contents of 80 mg valsartan and 100 mg of atenolol were purchased from a local drug store, pharmacy. The tablets were put in 100 mL measuring flasks and dissolved in 50 mL 50% v/v methanol, ultrasound crushed and treated for 2 min, and shaked 15 min with an orbital shaker. After that measuring flasks were filled to mark for 100 mL, the final concentrations were 0.5 mg/mL for atenolol and 0.4 mg/mL for valsartan. Samples were filtered by RC Agilent 0.45uM syringe filters and injected. These concentrations were used in analysis using CA, while in experiments performed on CB, the working concentrations were reduced twice with adding mobile phase in ratio 1:1, and achieving working concentration of 0.25 mg/mL atenolol and 0.2 mg/ml valsartan. After filtration through the above filters, 5 μL were injected on the working column.

3 | RESULTS

The emerging of new pharmaceutical formulations provokes the necessity for simple, accurate, economical, green and fast analytical techniques to be applied in quality control laboratories where time and cost are critical [36]. Moreover, minimizing toxicity with retaining method efficacy may be one of the challenging aspects in developing a safer methodology. To find the appropriate HPLC conditions for the separation of the examined drug, various columns, isocratic mobile phase systems were tried, and successful attempts were performed using a CA and CB is a versatile reversed-phase sorbent optimized for HPLC separations of basic compounds. Based on spherical silica particles, the sorbent prevents secondary interactions with basic substances and ensures that they are eluted as highly symmetrical peaks. Besides offering excellent separation properties for basic compounds, LiChrospher® 60 RP-Select B is also suitable for the determination of neutral and acidic substances. Method development was initiated by trying several mobile phases with various compositions to attain optimum separation and resolution. In this study, the combination of MA, MB, and MC provided the most suitable results. The selected flow rates of 0.5 mL/min and 1.0 mL/min provided optimum resolution. The UV–VIS detector and column oven were set at 225 nm and 40 and 42°C, respectively. Conversely, relatively longer wavelengths, i.e., 237 and 273 nm were tried by us for peak detection. The applicability of the mobile phase concept was tested on chromatographic systems and columns with different performances, and the obtained chromatograms are shown in Figures 1–3.

Chromatograms were obtained with satisfactory retention factors and very good peaks symmetry of both analyte peaks (tailing factors according to USP of around 1.2–1.4), with resolution better than required ($R > 7$). This was accomplished under the following chromatographic conditions: HPLC column was CA, column temperature 40°C, flow rate 0.5 mL/min, MA or MB and eluent monitored at 225 nm; HPLC column was CB, column temperature 42°C, flow rate 1.0 mL/min, MC and eluent monitored at 225 nm and 237 nm. The chromatograms showed that there is no interference between the principal peaks of valsartan and

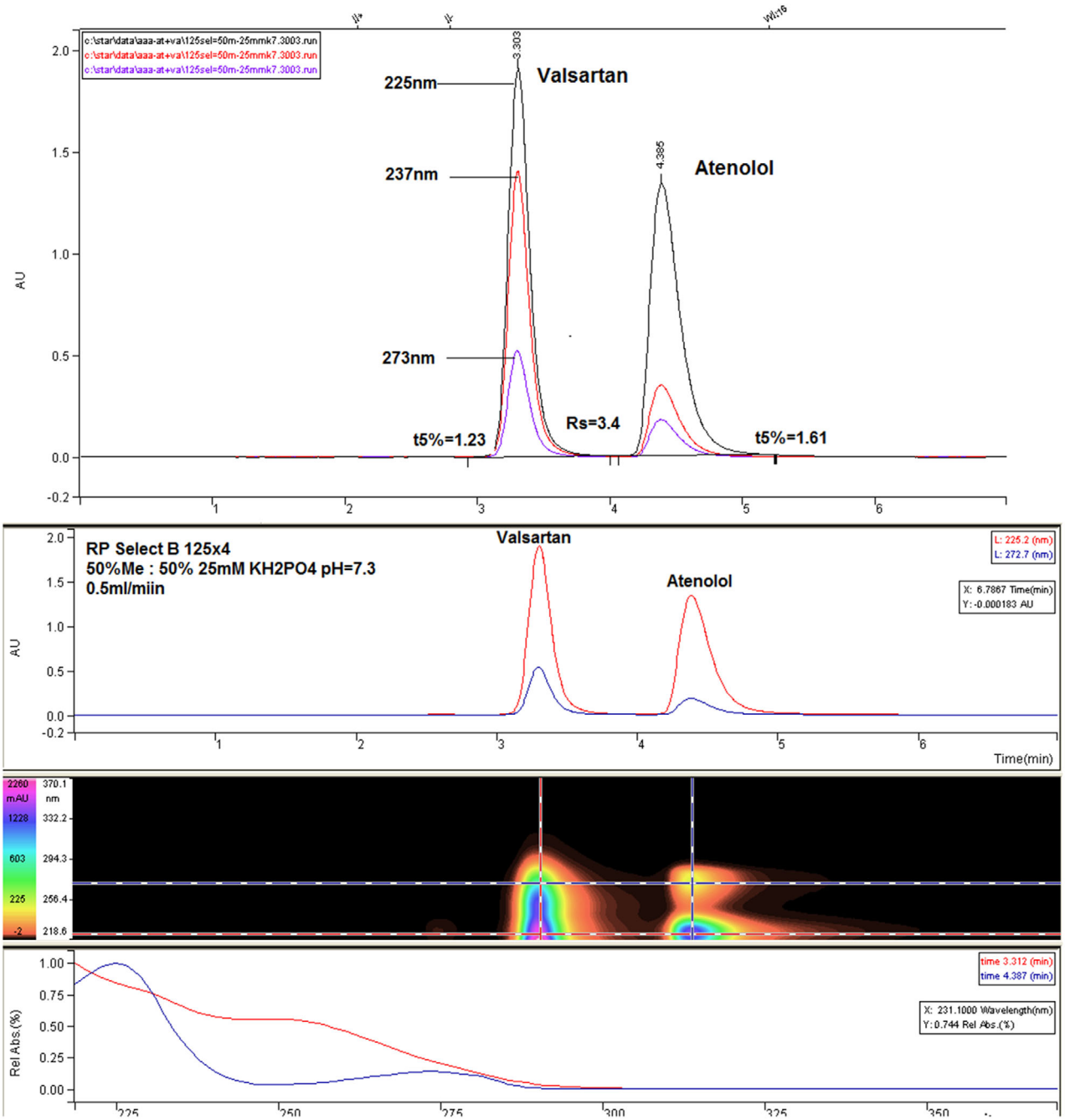


FIGURE 1 Chromatogram obtained using Varian Pro Star 330 HPLC system and mobile phase potassium dihydrogen phosphate (25 mM, pH 7.3) and methanol (50:50, v/v), C8 chromatographic column LiChrospher® 60 RP-select B (4 mm id × 125 mm, 5 μm) (MA, CA). Top view presents chromatogram of analytes at 3selected wavelengths. Down part illustrates full 3D UV-contour diagram extracted from eluted peak analytes with their recognizing UV spectra at the bottom

atenolol with the components of the placebo and the used solvent, and also good resolution. The results of linearity showed that a phenomenal relationship between obtained peak areas and used concentrations of the tested drugs and also indicated high sensitivity of the proposed HPLC methods.

All proposed methods were validated according to The International Conference on Harmonisation guideline for the Validation of analytical procedures. The specificity of the proposed methods was determined with an evaluation of the obtained chromatograms of the blank, placebo solutions, test solutions, and standard solutions. For comparison purposes, the chromatogram of solvent was added, which should be almost identical to placebo, which confirms selectivity of the proposed methods (Figures 1–3). In triplicate run from which the linear

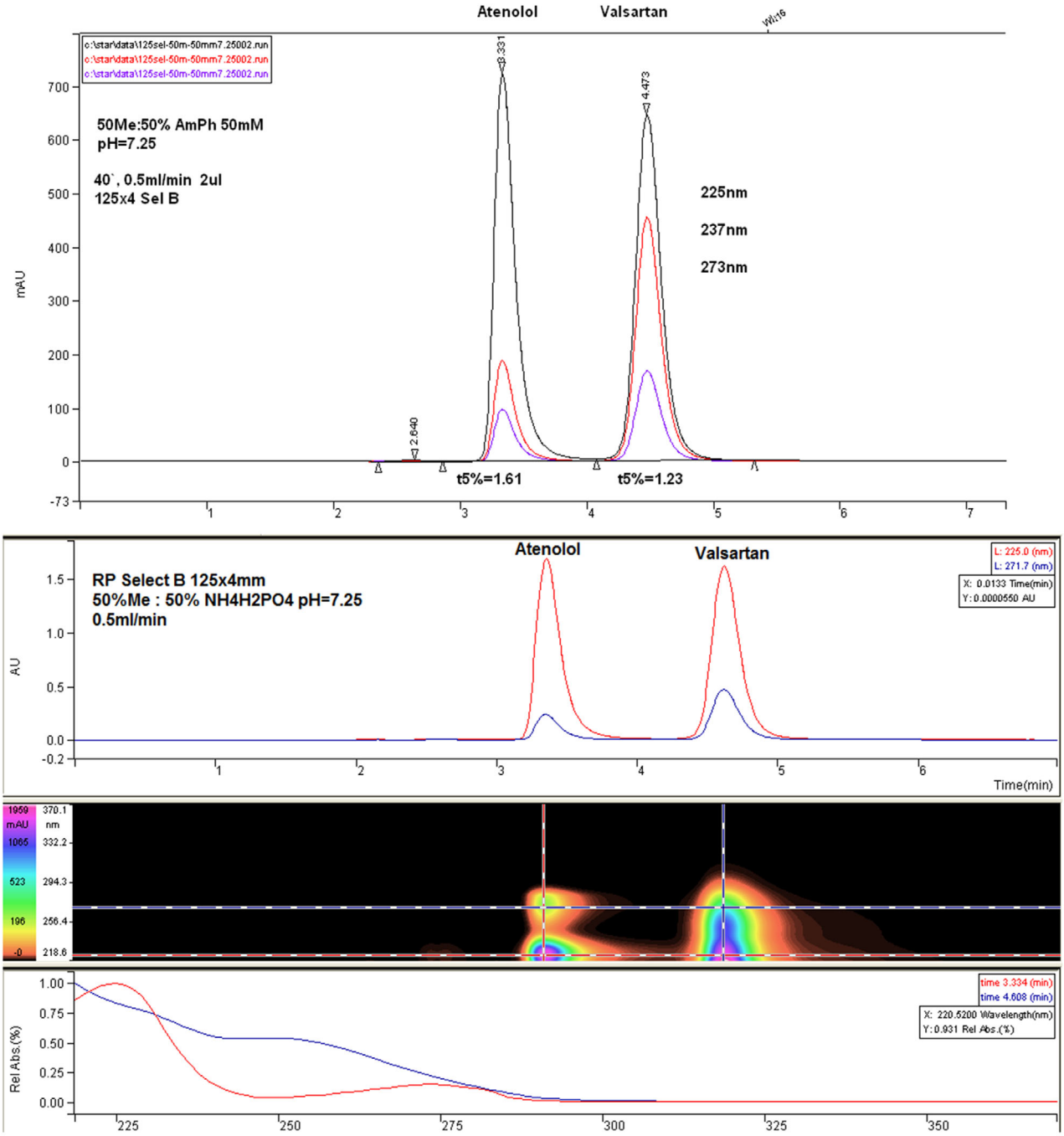
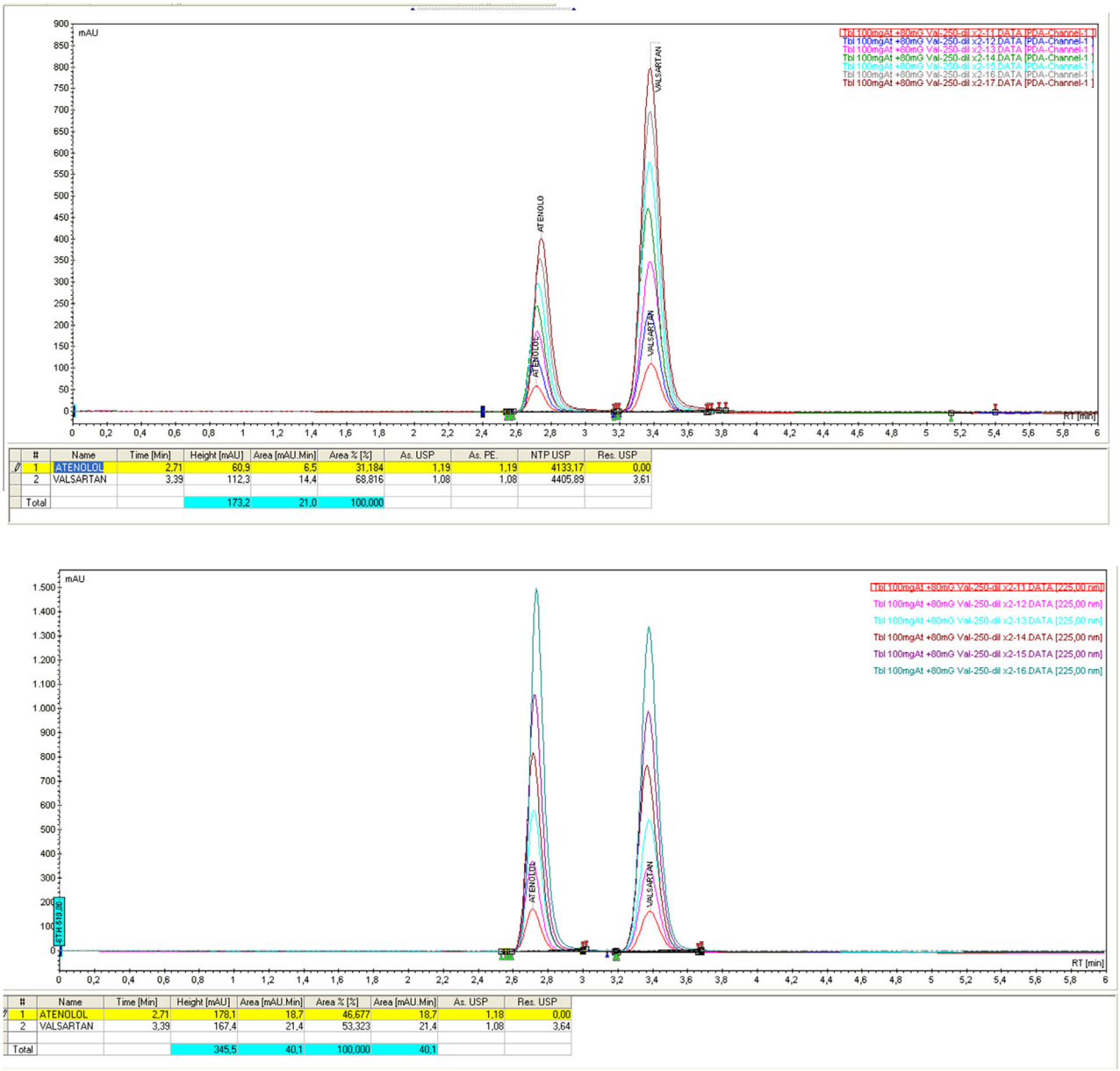


FIGURE 2 Chromatogram obtained using Varian Pro Star PDA 330 HPLC system and mobile phase ammonium dihydrogen phosphate (50 mM, pH 7.25) and methanol (50:50, v/v), C8 chromatographic column LiChrospher® 60 RP-select B (4 mm id × 125 mm, 5 μm) (MB, CA). The top view presents chromatogram of analytes at 3 selected wavelengths. Down part illustrates full 3D UV-contour diagram extracted from eluted peak analytes with their recognizing UV spectra at the bottom, appearing in reverted appearing from the column compared with the previous situation

regression equations were calculated. Linearity was examined and proven at different concentration levels in the range of working concentration of valsartan (0.1-0.7 mg/mL) and atenolol (0.1-0.7 mg/mL) under all chromatographic conditions used. Varian Pro Star 330 PDA, CA and MA or MB, flow rate 0.5 mL/min, column temperature 40°C, and signal monitoring at a wavelength of 225 nm. Linearity was examined and proven at different concentration levels in the range of working concentration of valsartan (0.08–0.48 mg/mL) and atenolol (0.1–0.6 mg/mL) under both chromatographic conditions Varian LC 920 HPLC system, CB and MC, flow rate 1.0 mL/min, column



#	Name	Time [Min]	Height [mAU]	Area [mAU.Min]	Area % [%]	As. USP	As. PE.	NTP USP	Res. USP
1	ATENOLOL	2,71	60,9	6,5	31,184	1,19	1,19	4133,17	0,00
2	VALSARTAN	3,39	112,3	14,4	68,816	1,08	1,08	4405,89	3,61
Total			173,2	21,0	100,000				

#	Name	Time [Min]	Height [mAU]	Area [mAU.Min]	Area % [%]	Area [mAU.Min]	As. USP	Res. USP
1	ATENOLOL	2,71	178,1	18,7	46,677	18,7	1,18	0,00
2	VALSARTAN	3,39	167,4	21,4	53,323	21,4	1,08	3,64
Total			345,5	40,1	100,000	40,1		

FIGURE 3 Elution profiles obtained for test samples prepared of valsartan (80 mg) and atenolol (100) mg using mobile phase potassium dihydrogen phosphate (50 mM, adjusted pH to 7.2 with 33% NH4OH) and methanol (45:55, v/v). Chromatographic conditions: Varian LC 920 HPLC system, C8 chromatographic column LiChrospher® 60 RP-select B (4 mm id × 250 mm, 5 μm) (MC, CB), flow rate 1.0 mL/min, column temperature 42°C, upper at UV = 237 nm, bottom at UV = 225 nm

temperature 42°C, and signal monitoring at wavelengths of 225 and 237 nm.

Under chromatographic conditions with MA and eluent monitored at 225 nm, flow rate 0.5 mL/min, column temperature 40°C, chromatographic column CA. Valsartan linearity regression equation $y = 152.5x - 0.8429$ and an obtained correlation coefficient of $R^2 = 0.9998$, atenolol linearity regression equation $y = 243.36x - 10.243$ and an obtained correlation coefficient of $R^2 = 0.9993$; signal monitoring at a wavelength of 237 nm, flow rate 0.5 mL/min, column temperature 40°C, chromatographic column LiChrospher® 60 RP-select B (4 mm id × 125 mm, 5 μm) valsartan linearity regression equation $y = 259.46x - 6$ and an obtained correlation coefficient of $R^2 = 0.9996$, atenolol linearity regression equation $y = 64.75x + 0.2714$ and an obtained correlation coefficient of $R^2 = 0.9995$. The values of LOD were 0.6 μg/mL, LOQ was 3 μg/mL for atenolol, and LOD was 0.8 μg/mL and LOQ was 4 μg/mL for valsartan.

Under chromatographic conditions with MB and eluent monitored at 225 nm, flow rate 0.5 mL/min, column temperature 40°C, chromatographic column CA. valsartan

linearity regression equation $y = 136x - 3$ and an obtained correlation coefficient of $R^2 = 0.9998$, atenolol linearity regression equation $y = 1181.8x - 43$ and an obtained correlation coefficient of $R^2 = 0.9989$; and eluent monitored at 237 nm, flow rate 0.5 mL/min, column temperature 40°C, chromatographic column LiChrospher® 60 RP-select B (4 mm id × 125 mm, 5 μm) valsartan linearity regression equation y = 171.11x-5.9714 and an obtained correlation coefficient of $R^2 = 0.9997$, atenolol linearity regression equation $y = 134.57x - 2.2857$ and an obtained correlation coefficient of $R^2 = 0.9996$. LOD was 0.6 μg/mL and LOQ was 3 μg/mL for atenolol, and LOD was 0.8 μg/mL and LOQ was 4 μg/mL for valsartan.

Under chromatographic conditions with MC and eluent monitored at 225 nm, flow rate 1.0 mL/min, column temperature 42°C, chromatographic column CB. Valsartan linearity regression equation $y = 53.064x - 8.7429$ and an obtained correlation coefficient of $R^2 = 0.9975$, atenolol linearity regression equation $y = 48.936x - 10.357$ and an obtained correlation coefficient of $R^2 = 0.9952$; and eluent monitored at 237 nm, flow rate 1.0 mL/min, column temperature 42°C, chromatographic column LiChrospher® 60 RP-select B (4 mm id × 250 mm, 5 μm) valsartan linearity regression equation $y = 30.5x - 0.8429$ and an obtained correlation coefficient of $R^2 = 0.9998$, atenolol linearity regression equation $y = 12.95x + 0.4$ and an obtained correlation coefficient of $R^2 = 0.9994$. LOD was 0.3 μg/mL and LOQ was 1 μg/mL for atenolol, and LOD was 0.4 μg/mL and LOQ was 1.3 μg/mL for valsartan.

Intraday and interday %RSD values <2% clearly assuring that this method was found to be fairly precise and reproducible (Table 1). Regarding accuracy, a known amount of the standard drug was added to the fixed amount of preanalyzed sample solution. %Recovery was calculated by comparing the area before and after addition of the standard drug. The high value of recoveries obtained for valsartan and atenolol indicated that the proposed methods were found to be accurate. The linearity, accuracy, and precision remained within the acceptable limits. The robustness of the developed methods was evaluated by small deliberate changes in method parameters such as flow rate and temperature of the column. The results of the robustness study results are shown in Table 2. The %RSD values of robustness which was <2 % revealed that the proposed methods are robust. Furthermore, any small changes in the examined conditions did not significantly affect the retention times of valsartan and atenolol. Since we could not find on the market tablets with incorporated both antihypertensive drugs, we checked the method on binary mixtures of two tablets in testing solutions. Table 3 presents the determination of analytes in different vendors with the three described methods.

4 | DISCUSSION

The concept of method mobile phase composition was developed on shorter CA for reducing run time analyses during numerous experimental variables in compositions. Salt buffer choice and composition, pH of the buffer, percentage of organic modifier, column oven temperature, flow rate, and injection volumes were optimum for the tested drugs analysis. Summary conclusions were successfully applied on twice longer column 250 mm × 4mm 5um (CB) (Figure 3) with identical column matrix with the increased power of separation, stronger retentions with doubling number of theoretical places, and better peak shapes at almost identical run time.

According to all results presented above, we can conclude that switch to testing from the previous Zorbax XDB-C8 column to RP Select B, provided us with an abundance of new interesting experimental results, facts, and benefits, in method development approach for our tandem of interesting analytes valsartan and atenolol. In direct comparison, Zorbax XDB-C8 yielded sharper peaks due to better matrix chemistry with twice higher theoretical plates per meter, which achieves non-essential resolution between atenolol and valsartan about 7.2. This column showed higher peak asymmetry with a reducing flow rate. With the use of RP Select B reducing the flow rate was beneficial to peak symmetry by reducing their tailings. Use of shorter 125 mm Select B column was better choice in numerous experiments with changes of mobile phase compositions and other experimental variables, temperature, flow rate, injection volumes, and showed resolution value about 3, and a number of theoretical plates about 2000. Exactly with shorter 125 mm RP Select B column, reverting of elution profile of atenolol and valsartan was notified, after unexpectedly "insignificant" changes in the type of phosphate buffer used in mobile phases. After establishing a good direction of selecting optimal mobile phase composition, sample preparation, and other mentioned variables, the final conclusion summary set of variables was applied and tested to 250 × 4 mm column RP Select B, which enables much better instant visible peak symmetries almost regardless of flow rate, a higher resolution between peaks about 4, and double theoretical plates for peaks (4100-4440), which might be essential in some cases where better resolution and improved selection would appear to be necessary.

5 | CONCLUSION

The main target of the work was to conduct and compare the different chromatographic methods for the analysis of valsartan and atenolol binary mixture in bulk and in

TABLE 1 Intra-and Inter-day accuracy and precision results for valsartan and atenolol

Chromatographic conditions	Analyte	Intra-day precision[a]		Inter-day precision[b]	
		Mean (%)	RSD (%)	Mean (%)	RSD (%)
MA, CA, 225 nm wavelength	Valsartan	99.76	0.429	100.81	0.379
		98.95	0.593	99.94	0.335
		100.11	0.319	100.23	0.615
	Atenolol	99.95	0.384	100.28	0.348
		100.83	0.517	99.49	0.364
		100.91	0.391	100.93	0.493
MB, CA, 225 nm wavelength	Valsartan	98.76	0.429	99.81	0.379
		100.95	0.593	99.94	0.335
		100.11	0.319	100.23	0.615
	Atenolol	99.94	0.384	100.35	0.428
		101.16	0.693	98.84	0.338
		100.94	0.237	100.93	0.619
MC, CB, 225 nm wavelength	Valsartan	99.84	0.165	100.81	0.138
		100.27	0.203	99.47	0.184
		100.35	0.195	100.29	0.139
	Atenolol	99.59	0.139	100.35	0.149
		100.19	0.138	99.17	0.106
		99.98	0.104	100.36	0.113
MC, CB, 237 nm wavelength	Valsartan	100.01	0.124	100.04	0.108
		100.14	0.186	99.67	0.125
		100.34	0.147	100.29	0.145
	Atenolol	99.84	0.134	100.21	0.111
		100.49	0.197	99.94	0.110
		100.47	0.135	100.34	0.159

[a] $n = 6$.
[b] $n = 18$.

a pharmaceutical dosage form. New fast, simple, green, selective, accurate, precise, and robust isocratic HPLC methods were developed for simultaneous determination of valsartan and atenolol in dosage forms. The developed methods were essential for the quality control of a large number of samples in short time intervals. The concept of the mobile phase's composition was evaluated and confirmed on different chromatographic systems. Reliability was proved by examining different validation parameters of the suggested methods and the successful application to the pharmaceutical dosage form. The main benefit of the suggested HPLC methods was the short analysis time (chromatographic run time < 6 min), better control of elution profile of analytes due to quite different chromatographic characteristics of RP Select B column 125–250 × 4 mm 5um, compared to column used in our previous publication 150 × 4.6 mm Zorbax XDB-C8 5um. The lower overall resolution between atenolol and valsartan gives an advantage to RP Select B column, since this resolution is unnecessarily high in Zorbax XDB-C8 column. The octylsilane C8 columns proved to be indispensably applicable and gave a shorter analysis time and peak symmetries compared to C18. This reduction in total run time, leads to low solvent consumption, and makes all methods more economical. Therefore, all suggested methods are suitable for the routine quality control analysis of any pharmaceutical preparation containing the two tested drugs with the proposed chromatographic methods advantages for checking quality during stability studies of their pharmaceutical preparations.

TABLE 2 Results of the study of robustness for valsartan and atenolol with system suitability parameters calculated for resolution and peak symmetries

Chromatographic conditions	Conditions of analysis	Retention time of valsartan, min	Retention time of atenolol, min	Resolution	Atenolol-valsartan (tailing at 5% of height)
MA, CA, 225 nm wavelength	flow rate 0.6 mL/min flow rate 0.4 mL/min temperature of column 38° C temperature of column 42° C	3.32	4.17	3.40	1.61—1.23
		2.80	3.49	3.07	1.68 – 1.28
		3.95	4.93	3.51	1.58 – 1.24
		3.37	4.21	3.30	1.63 – 1.24
		3.21	4.08	3.41	1.69 – 1.25
MB, CA, 225 nm wavelength	flow rate 0.6 mL/min flow rate 0.4 mL/min temperature of column 38° C temperature of column 42° C	4.42	3.22	3.71	1.60 – 1.28
		3.71	2.71	3.49	1.68 – 1.32
		5.28	3.75	3.78	1.55 – 1.21
		4.51	3.29	3.85	1.63 – 1.26
		4.33	3.15	3.60	1.57 – 1.31
MC, CB, 225 nm wavelength	flow rate 1.1 mL/min flow rate 0.9 mL/min temperature of column 40° C temperature of column 44° C	3.39	2.71	3.64	1.18 – 1.08
		3.09	2.51	3.59	1.19 – 1.09
		3.71	2.97	3.71	1.14 – 1.09
		3.48	2.81	3.66	1.17 – 1.08
		3.19	2.63	3.57	1.19 – 1.09
MC, CB, 237 nm wavelength	flow rate 1.1 mL/min flow rate 0.9 mL/min temperature of column 40° C temperature of column 44° C	3.36	2.75	3.65	1.17 – 1.08
		3.09	2.48	3.54	1.18 – 1.08
		3.77	3.02	3.71	1.15 – 1.07
		3.44	2.88	3.69	1.18 – 1.07
		3.25	2.59	3.51	1.19 – 1.09

TABLE 3 Determination of analytes in different vendors with the three described methods*

	Tablet vendor	Content of analytes under chromatographic conditions, % (at 225 nm)		
		MA, CA	MB, CA	MC, CB
Sample origin 100 mg declared content of atenolol	1	97.97 ± 0.05	98.71 ± 0.04	98.52 ± 0.05
	2	96.76 ± 0.09	96.23 ± 0.03	95.87 ± 0.04
	3	102.11 ± 0.02	101.89 ± 0.09	101.46 ± 0.08
	4	99.04 ± 0.05	99.42 ± 0.07	99.67 ± 0.04
Sample origin 80 mg declared content of valsartan	5	99.28 ± 0.05	98.71 ± 0.09	98.52 ± 0.04
	6	98.42 ± 0.07	97.81 ± 0.05	98.87 ± 0.03
	7	100.64 ± 0.04	101.14 ± 0.04	100.85 ± 0.02
	8	98.12 ± 0.09	98.96 ± 0.02	99.22 ± 0.06

*$n = 3$.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHOR CONTRIBUTIONS

Marjan Piponski and Liliya Logoyda were associated with conceptualization, methodology, formal analysis, writing - original draft, writing - review and editing, and project administration. Kateryna Peleshok, Sergiy Kovalenko, Hytham Ahmed, Ahmed Abdel-Megied, and Obianuju Florence Ezike were associated with methodology, formal analysis, and writing - review and editing.

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REFERENCES

1. Gradman AH, Basile JN, Carter BL, Bakris GL. American Society of Hypertension Writing, G., Combination therapy in hypertension. JASH. 2010;4:90–8.
2. Acelajado MC, Hughes ZH, Oparil S, Calhoun DA. Treatment of resistant and refractory hypertension. Circ Res. 2019;124:1061–70.
3. Macek J, Klíma J, Ptácek P. Rapid determination of valsartan in human plasma by protein precipitation and high-performance liquid chromatography. J Chromatogr B. 2006;832:169–72.
4. Tian DF, Tian XL, Tian T, Wang ZY, Mo FK. Simultaneous determination of valsartan and hydrochlorothiazide in tablets by RP-HPLC. Indian J Pharm Sci. 2008;70:372–4.
5. Satana E. S Altinay, N G Göğer, S A Ozkan, Sentürk, Z., Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first-derivative ultraviolet spectrophotometry and LC. JPBMAL. 2001;5:1009–13.
6. Patel NR, Patel SK. First Derivative Spectrophotometric Method for The Simultaneous Estimation of Valsartan And Hydrochlorthiazide in Their Combined Dosage Form. IJPLS. 2012;3:1828–32.
7. Nataraj KS, Ramakrishnama Charya SV, Goud ES, S.Saigeethika R. K., Simple Quantitative Method Development and Validation of Valsartan in Pureform And Pharmaceutical Dosage Forms By UV–Spectroscopy. IJPBS. 2011;1:67–73.
8. Kalaimagal A, Jerad SA, Niraimathi V. Spectrophotometric methods for the estimation of valsartan in bulk and oral dosage form. Int J Pharm Pharm Sci. 2012;4:481–3.
9. Gupta KR, Wadodkar AR, Wadodkar SG. UV-Spectrophotometric methods for estimation of Valsartan in bulk and tablet dosage form. Int J Chemtech Res. 2010;2:985–9.
10. Rao GS, Rao GV, Vardhan S, Ramachandran D. Development and validation of new UV-spectrophotometric assay method for valsartan in pure and in formulations. J chem pharm. 2013;5:229–32.
11. Ramachandran S, Mandal BK, Navalgund SG. Simultaneous spectrophotometric determination of valsartan and ezetimibe in pharmaceuticals. Trop J Pharm Res. 2011;10:809–15.
12. Nikam MB, Dhamane H, Aligave A, Kondawar MS. Simultaneous estimation of valsartan, amlodipine besylate and hydrochlorothiazide by first order derivative UV spectrophotometric method. Int J Pharm Technol. 2010;2:642–50.
13. Selvan PS, Gowda KV, Mandal U, Solomon WDS, Pal TK. Simultaneous determination of fixed dose combination of nebivolol and valsartan in human plasma by liquid chromatographic-tandem mass spectrometry and its application to pharmacokinetic study. J Chromatogr B. 2007;858:143–50.
14. Sabi-mouka EMB, Agbokponto JE, Zhang R, Li Q, Ding L. Simultaneous determination of a fixed-dose combination of

lercanidipine and valsartan in human plasma by LCâ€“MS-MS: Application to a pharmacokinetic study. J Chromatogr Sci. 2016;54:1553–9.
15. Koseki N, Kawashita H, Hara H, Niina M, Tanaka M, Kawai R, Nagae Y, Masuda N. Development and validation of a method for quantitative determination of valsartan in human plasma by liquid chromatography-tandem mass spectrometry. J Pharm Biomed. 2007;43:1769–74.
16. Chitlange SS, Bagri K, Sakarkar DM. Stability Indicating RP-HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation. AJRC. 2008;1:15–8.
17. Galande VR, Baheti KG, Indraksha S, Dehghan MH. Estimation of amlodipine besylate, valsartan and hydrochlorothiazide in bulk mixture and tablet by UV spectrophotometry. Indian J Pharm Sci. 2011;74:18.
18. Liu F, Zhang J, Xu Y, Gao S, Guo Q. Simultaneous determination of hydrochlorothiazide and valsartan in human plasma by liquid chromatography/tandem mass spectrometry. Anal Lett. 2008;41:1348–65.
19. Thanusha G, Jose C, Babu G, Basavaraj KPC, Panditi VR, Sharadha C. Validated RP-HPLC method for the quantitative estimation of valsartan in bulk and pharmaceutical dosage forms. Int J Chemtech Res. 2010;2:1194–8.
20. Kumar PVS, Sahu M, Prasad KD, Shekhar MC. Development and validation of analytical method for the estimation of valsartan in pure and tablet dosage form by RP-HPLC method. Int J Res Pharm Chem. 2011;1:945–9.
21. Vinzuda DU, Sailor GU, Sheth NR. RP-HPLC method for determination of valsartan in tablet dosage form. Int J Chemtech Res. 2010;2:1461–7.
22. Kendre MD, Banerjee SK. Precise and accurate RP-HPLC method development for quantification of valsartan in tablet dosage form. Int. J Pharm Sci Drug Res. 2012;4:137–9.
23. Gonzalez L, Lopez JA, Alonso RM, Jimenez RM. Fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high-performance liquid chromatography with fluorimetric detection. J Chromatogr A. 2002;949:49–60.
24. Attia KA-SM, Nassar MW, Abolmagd E. Simultaneous spectrophotometric determination of amlodipine and atenolol in pharmaceutical preparations using chemometric techniques. Anal Chem Ind J. 2016;16:205–10.
25. Lalitha G, Salomi P, Ravindra RK. Development of an analytical method and its validation for the analysis of atenolol in tablet dosage form by UV-Spectrophotometry. Int j pharm pharm sci. 2013;5:197–9. .
26. Lalitha KV, Kiranjyothi R, Padma B. UV Spectrophotometric method development and validation for the determination of Atenolol and Losartan Potassium by Q-analysis. Int bull drug res. 2013;3:54–62.
27. Agarwal R, Gfadnis A. Kinetic spectrophotometric determination of atenolol in perchloric acid medium. Int j pharm pharm sci. 2012;4:350–2.
28. Basavaiah K, Chandrashekar U, Nagegowda P. Titrimetric, spectrophotometric and kinetic methods for the assay of atenolol using bromate-bromide and methyl orange. J Serb Chem Soc. 2006;71:553–63.
29. Pai NR, Patil SS. Development and validation of liquid chromatographic method for atenolol and its related substance. Der Pharm Sin. 2013;4:76–84.
30. Naikini P, Akula A, Ajitha A, Rao VUM. RP-HPLC method development and validation for the simultaneous estimation of amlodipine and atenolol in bulk and tablet dosage forms. Int j pharm pharm sci. 2014;6:390–4.
31. Chaudhari V, Hussian S, Ubale M. A newer validated and stability indicating HPLC method forthe estimation of Atenolol and Hydrochlorothiazide in bulk drug and dosage form. Int J Chem Stud. 2013;1:93–101.
32. Belal F, Sharaf M, Aly F, Hefnawy M, Awady M. Stability-indicating HPLC Method for the Determination of Atenolol in Pharmaceutical Preparations. J Chromat Separation Techniq. 2013;4:1–7.
33. Yilmaz B, Arslan S. Determination of atenolol in human urine by gas chromatography-mass spectrometry method. J Chromatogr Sci. 2011;49:365–9.
34. Tengli AR, Gurupadayya BM. Method development and validation of tablet dosage form containing losartan, atenolol and hydrochlorthiazide using internal standard by RP-HPLC. J Chromat Separation Techniq. 2013;4:1–5.
35. Piponski M, Peleshok K, Logoyda L, Kravchuk L, Piatnochka V, Zakharchuk U. Efficient Validated HPLC/UV Method for Determination of Valsartan and Atenolol in Dosage Form And In Vitro Dissolution Studies. BRIAC. 2020;10:6669-75.
36. Piponski M, Stoimenova TB, Stefov S, Balkanov T, Serafimovska GT, Logoyda L. Development of a novel, fast, simple, non-derivative HPLC method with direct UV measurment for quantification of memantine hydrochloride in tablets. J Sep Sci. 2020;43:3482–90.

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