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Simultaneous determination of Amlodipine Besilate and Valsartan in pharmaceutical formulation using high performance liquid chromatography

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ABSTRACT

A simple, selective, sensitive, precise, simultaneous high performance liquid chromatographic analysis of commercial tablets formulation containing amlodipine besilate and valsartan are reported. Good chromatographic separation was achieved using a Zarbax ODS (4.6 cm x 250 mm, 5 µm). Past Number: 880952-702. S.N. VSF 0060920 and a mobile phase consisting of acetonitrile-phosphate buffer (50/50, v/v) at a flow rate 1.0 mL/min. The ultraviolet detector was set a wavelength of 254 nm. amlodipine besilate and valsartan were eluted at 1.5 and 4.2 min, respectively. No extraneous materials were found to interfere. The recoveries of amlodipine besilate and valsartan in pharmaceutical preparation were all greater than 98% and their relative standard deviations were less than 2.0%.

Key Word: high performance liquid chromatography (HPLC); amlodipine; besilate; valsartan; pharmaceutical formulations.

INTRODUCTION

Amelodipine besilate (AM) is a dihydropyridine calcium-channel blocker. It is used in the management of hypertension and angina pectoris (1). It produces peripheral arterial and coronary vasodilatation 920. While valsartan (VL) is an angiotensin II receptor antagonist, used in the management of hypertension and may be used in patients with heart failure (1). It also regulates the cardiovascular functions (2). AM and VL are co-formulated together in commercial tablets for their anti-hypertensive effect.

Determination of AM is described in British pharmacopoeia (3) by HPLC method. It is also determined by several methods involving spectrophotometric methods (12), HPLC (13-16) and chemometric methods (17).

This work deals with the determination of the mixture of the two drugs AM and VL by three different techniques without previous separation.

EXPERIMENTAL SECTION

Apparatus

Agilent 1200 series, vacuum degasser, thermostatted column compartment G1316A/G1316B, diode arrayand multiple wavelength detector SL, quaternary pump (Germany).

Chemicals and reagents

HPLC grade acetonitrile was purchased from Merck (Germany).

HPLC procedure

Chromatographic conditions

The analytical column was a Zorbax ODS (4.6 cm x 250 mm, 5 µm). Past Number: 880952-702. S.N. VSF 0060920 and a mobile phase consisting of acetonitrile and phosphate buffer (50/50, v/v) at a flow rate of 1.0 mL/min and at room temperature. The ultraviolet detector was set a wavelength of 210 nm. Solutions and mobile phase were freshly prepared at the time of use.

Standard solution preparation

Stock standard solutions of amlodipine besilate and valsartan were prepared separately by dissolving 50 mg of each in 100 mL mobile phase. Working standard solutions were prepared individually by diluting the stock solutions with the same mobile phase to obtain a concentration range of 2-20 µg mL^{-1} for amlodipine besilate and 5-50 µg mL^{-1} for valsartan.

Preparation of spiked serum sample

An aliquot of serum (200 µL) and standard solution (200 µL) was pipetted out into 10 mL tapered bottom centrifuge tube, and the volume was made up by acetonitrile. The mixture was mixed briefly and after standing for 5 min at room temperature, the mixture was centrifuged at 4000 r/min for 20 min. The final concentration of the spiked serum sample containing of 2-20 µg mL^{-1} for amlodipine besilate and 5-50 µg mL^{-1} for valsartan. was obtained by further dilution with mobile phase.

Preparation of pharmaceutical dosage sample

Pharmaceutical formulations of the two different brands, containing and valsartan, commercially available in many countries were evaluated. The contents of twenty tablets were individually weighed, mixed and finely powdered in a mortar. Portions of the powder of amlodipine besilate and valsartan were accurately weighed and diluted with mobile phase to get the final concentration of 2-20 µg mL^{-1} for amlodipine besilate and 5-50 µg mL^{-1} for valsartan µg/mL.

Calibration and linearity

Calibration curves were constructed in the ranges of 2-20 µg mL^{-1} for amlodipine besilate and 5-50 µg mL^{-1} for valsartan, to encompass the expected concentrations in the measured samples. Curves were obtained by plotting the peal area against concentrations of these drugs.

Accuracy

Absolute recoveries of six different concentrations of amlodipine besilate and valsartan in serum and in placebo of respective dosage forms were determined by assaying the samples as described above. Mean recoveries and the relative standard deviations were calculated.

Precision

The precision of the assay method was considered on two levels; repeatability and intermediate precision. These levels were ascertained based on the analysis of spiked serum samples and spiked placebo sample of respective dosage forms. RSD and confidence interval for both levels were calculated.

RESULTS AND DISCUSSION

Chromatograms of samples

The aim of this research was to develop a new, simple, more accurate, reproducible, sensitive HPLC method for the simultaneous determination of amlodipine besilate and valsartan in human serum and pharmaceutical dosage form. A satisfactory separation of each drug from biological endogenous components and pharmaceutical excipients was obtained. To optimize the appropriate HPLC conditions for separation of the examined drugs, various reversed-phase columns, isocratic and gradient mobile phase systems were tried. The optimum wavelength for detection was 254 nm at which much better detector responses for the two drugs were obtained. The mobile phase was found to be suitable to improve the sharpness and thinness of the amlodipine besilate and valsartan peaks. The retention times for the investigated drugs were found to be 1.5 min (amlodipine besilate) and 4.2 min (valsartan) (Figure 1). No endogenous serum components and pharmaceutical excipients eluted at the retention times of the peaks of interest.

Method validated

The method was validated with regard to specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and robustness. Peak areas of amlodipine besilate and valsartan of calibration standards were proportional to the concentration in serum and dosage forms over the ranges tested 2-20 and 5-50 µg/mL., respectively. Each concentration was tested in triplicate. The slope and intercept values were calculated. The standard deviations on slope were calculated and similarly standard deviations of an intercept were calculated, for amlodipine besilate and valsartan. The calibration curves were fitted by linear least-square regression and showed correlation coefficients not less than 0.999. The LODs and LOQs of amlodipine besilate and valsartan were calculated on the peak area using the following equations:

$LOD = 3 \times N/B,$

$LOQ = 10 \times N/B,$

where N, the noise estimate, is the standard deviation of the peak areas (three injections) of the drugs and B is the slope of the corresponding calibration curve (Tables 1- 3).

Application to pharmaceutical dosage form

The proposed method are successfully applied for the simultaneous determination of amlodipine besilate and valsartan in tablets without interference of the excipients present and without prior separation (Table 4).

Application to the serum samples

In order to check the applicability of the proposed HPLC method to biological materials, the recovery studies were performed on human serum samples. Analysis of drugs in serum by HPLC technique always suffers from being tedious and expensive. In our proposed method, simple extraction procedure was applied using acetonitrile, then after centrifugation, supernatant is

directly injected and analyzed. Atypical chromatogram was obtained for the serum spiked with amlodipine besilate and valsartan, showing no interference form endogenous substance present in serum (Table 5).

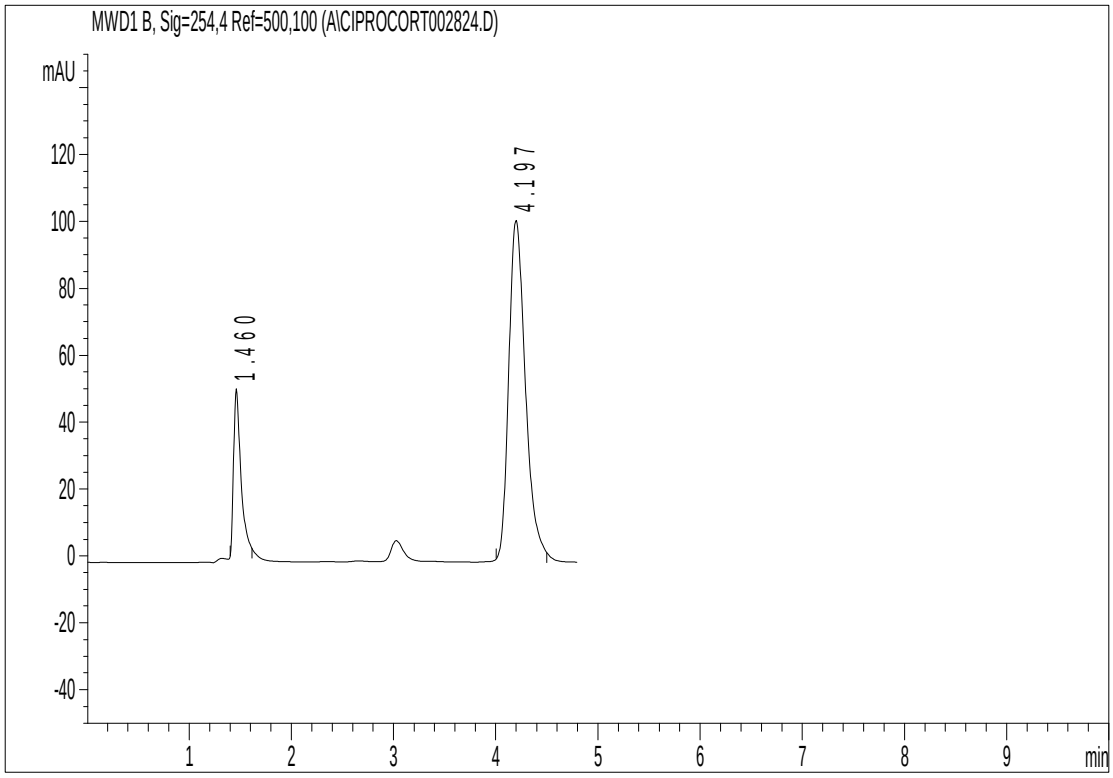

Fig. 1. HPLC chromatogram of amlodepine besylate and valsartan.

Table 1 Statistical data for calibration graphs of amlodipine besilate and valsartan by HPLC

	amlodipine besilate	valsartan
Selected wavelength	254 nm	254 nm
Linearity range, µg mL^{-1}	2-20	5-50
Slope (b)	33.429	26.984
SD of slope (Sb)	0.08954	0.05890
Intercept (a)	1.948	-10.530
SD of intercept (Sa)	0.4564	2.2985
Corre. coefficient (r)	0.9999	0.9998
Coeff. determination (r^2)	0.9999	0.9999
LOD, µg mL^{-1}	0.08	0.36
LOQ, µg mL^{-1}	0.22	1.28

Table 2: Determination and statistical analysis of the results obtained for assay of authentic amlodipine besilate and valsartan compared with reported method [18].

Reported method Recovery %	Amlodipine besilate Taken µg mL^{-1} -	Amlodipine besilate Recovery %	Valsartan Taken µg mL^{-1}	Valsartan Recovery %
100.9	2	99.2	5	102.0
100.3	6	100.0	5	100.6
100.1	10	100.5	7.5	100.8
98.5	14	99.9	10	100.6
100.7	18	100.2	12.5	100.4
	20	99.8	50	100.4
X^{-}	100.10	99.93		100.59
±SD	0.95	0.44		0.91
RSD%	0.95	0.44		0.90
N	5	6		6
t*		0.387 (2.26)		0.951
F*		4.720 (5.19)		1.093

The values between parenthesis are the corresponding theoretical values of t and F at the 95% confidence level.

Table 3: Determination of amlodipine besilate and valsartan in laboratory prepared mixtures by the proposed HPLC method

Mix	Ratio AML:VLS	Amlodipine besilate Taken (µg mL^{-1}) AML	Amlodipine besilate Taken (µg mL^{-1}) VLS	Valsartan Recovery (%) AML	Valsartan Recovery (%) VLS
1	1:1	10	10	98.8	99.8
2	1:2	10	20	99.4	100.2
3	1:3	10	30	99.9	99.3
4	1:4	10	40	100.3	100.6
5	1:5	10	50	100.0	99.7
X^{-}				99.68	99.92
±SD				0.59	0.50
RSD%				0.59	0.50

Table 4: Determination of amlodipine besilate and valsartan in pharmaceutical dosage forms by the proposed HPLC procedure

BN		Amlodipine besilate	Valsartan
7601001	Recovery (%)	100.63	100.48
	±SD	0.96	0.43
	RSD%	0.96	0.43

Table 5: Determination of amlodipine besilate and valsartan spiked serum by the proposed HPLC procedure

	Amlodipine besilate	Valsartan
Recovery (%)	97.93	96.11
±SD	0.78	0.82

CONCLUSION

The chromatographic method described is adequate for quantitation of amlodipine besilate and valsartan in human serum and pharmaceutical dosage forms at different concentration levels. It is very simple, accurate and effective and provided no interference peaks for endogenous components and pharmaceutical excipients. In spite of the complex matrix analyzed, acceptable values of precision and accuracy have been obtained all levels by this method regarding the guidelines for assay validation. The separation of these drugs takes 5 min in one chromatogram, so a large number of samples can be analyzed in a short period of time. The method uses simple mobile phase and is very beneficial for column life. In summary, the method can be successfully applied to samples of pharmaceutical dosage form and clinical and pharmacokinetic studies.

REFERENCES

[1] Sweerman, S.C. (**2004**) Martindale, The Complete Drug Reference, D., The Pharmaceutical Press, London, UK, pp 862, 1018.
[2] Garrisor, J.C., Goodman, &Gillman's, (**2001**) The Pharmacological Basis of Therapeutics. Hardman, J.G., Limbird, L.E. 10th Ed., McGraw-Hill, Dallas, Taxas, USA, pp 857, 894.
[3] British Pharmacopoeia, Her Majesty's Stationary office, , London, 2604 (**2004**), pp 124-125.
[4] Malesuik, M. D.; Cardoso, S.G.; Bajerski; L.;Lanzanova, A.; J. AOAC, 89 (2), (**2006**), 359-364.
[5] Khopade S.A, Jain N.K.; *Indian Drugs* 37 (**2000**) 351-353.
[6] Jain H.K., Agrawal R.K.; *Indian Drugs* 37 (**2000**) 196-199.
[7] Sane R.T., Phadke M., Hijii P.S.,. Shah M &Patel P.H.; *Indian Drugs* 35 (**1998**) 79–85.
[8] Singh S., Jain R.; *Indian Drugs* 34 (**1997**) 678-679.
[9] Bhatt, J., Singh, S., Subbaiah, Sgah, B., Kambli, S. & Ameta S.; Biomedical Chromatography, 21 (2) (**2007**) 169-175.
[10] Li, C.P., Yan, X.P., Shan, W.G.; Yaowu Fenxi Zazhi, 26 (12)(**2006**) 1878-1879.
[11] Ma, Y.Y., Qin, F., Sun, X.H., Li, F.M.; *J. Pharm. Biomed. Anal*., 43 (4)(**2007**) 1540-1545.
[12] Dinc E., Uslu B., Ozkan S.A.; *Anal. Lett.*, 37 (4)(**2004**) 679-693.
[13] Santana E., Altinay S., Goger N. & Sentruk Z.; *J. Pharm. Biomed. Anal*., 25 (5-6)(**2001**) 1009.
[14] Ferreiros N., Iriarte G., Alonso R. M.; *J. of Separation Science*, 31 (4)(**2008**) 667-676.
[15] Kocyigit, Kaymakcoglu, B., Unsalan, S. & Rollas, S.; *Die Pharmazie*, 61 (7)(**2006**) 586-589.
[16] Ivanovic D., Malenovic A., Janaic B. & Medenica; *J. of Liquid Chromatography*, 30 (17-20)(**2007**) 2879-2890.
[17] Erk N.; *Anal. Lett*., 35 (2)(**2002**) 238.
[18] Nesrin K., Heba M., Mohamed M. and Azza A.; *Bull. Fac. Pharm.*Cairo University, 47(2), (**2009**) 35-47.