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Simultaneous determination of several angiotensin-II receptor antagonists by liquid chromatography

S.G.Hiriyanna[1,2]*, K.Basavaiah[2], V.Dhayanithi[1], A.Bindu[1], Sudhaker Pujari[1], Hari N.Pati[1]

[1]Department of Analytical Chemistry, Advinus Therapeutics Pvt. Ltd., Bangalore-560058, (INDIA)

[2]DOS in Chemistry, Manasagangothri, University of Mysore, Mysore-570006, (INDIA)

E-mail: hiriyanna.g@gmail.com

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ABSTRACT

We optimized a simple rapid and elegant HPLC method for the simultaneous quantification of six angiotensin-II-receptor antagonists: Losartan, Olmesartan, Irbesartan, Valsartan, Telmisartan and Candesartan. The quantification is carried out using HPLC with UV detection. Combination of the studied parameters permitted the separation of the six ARA-IIs, which was best carried out using 10mM Potassium dihydrogen phosphate buffer (pH 3.5) with acetonitrile, methanol and commercially available ODS stationary phase column. The main feature of the method developed is that it can separate and unequivocally quantify chemically similar compounds in a single run utilizing a gradient elution profile. The retention time of Losartan, Olmesartan, Irbesartan, Valsartan, Telmisartan and Candesartan were 9.0, 10.3, 11.5, 12.0, 13.4 and 19.9 min, respectively. Some parameters (linearity, limit of detection and quantitation, Stability of solution, Precision and accuracy) were validated. The method was accurat, precise and suitable for the intended purpose.

KEYWORDS

Sartans;
Separation;
Validation;
ARA-IIs;
HPLC.

INTRODUCTION

Angiotensin-II receptor antagonists (ARA-IIs) are safe and most effective molecule in the rennin-angiotensin system and exerts a wide range of cardiovascular, renal and endocrine actions. Angiotensin-II receptor antagonists are used either alone, or in conjunction with hydrochlorothiazide, a thiazide diretic[1,2]. The angiotensin type 2 (AT_2) receptor, which is thought to have cardio protective effects and inhibitor effects on growth, is left unaffected[3-5]. Until now, there have been seven ARA-IIs available on the market namely; Lorsartan, Olmesartan, Irbesartan, Valsartan, Telmisartan, Candesartan and Eprosartan. Contain a biphenyltetra zole moiety, where as telmisartan contains a structurally related biphenyl carboxylic acid moiety and the structure of eprosartan differs from the others. Candesartan is orally administered as a pro-drug candesartan cilexetil, which is completely converted to an active compound candesartan during absorption from the gastrointestinal tract. All other ARA-IIs are active on their own and do not require metabolism in to active molecules[6,7]. Compared with placebo, angiotensin II type-1 receptor blockers significantly improved the hyperemia and reduced plasma levels of malondialdehyde[8].

Hypertension is one of the single major reasons for cardiovascular disease observed in developed and developing nations. Earlier hypertensive therapy involved the use of compounds which would as function of β blocker, e.g. Celiprolol and Bisoprolol etc. The recent trend in treating hypertension is to develop drugs that will act on specific targets such as cell surface recep

tors[9-11]. Separation of the compounds has gained a vital role in all stages of drug development. Therefore, the development of these separations is carried out using high performance liquid chromatography (HPLC), capillary electrophoresis (EC) and gas chromatography (GC). High-performance liquid chromatography (HPLC) has been the major technique used for the determination of different ARA-IIs, but these studies have usually been limited to the determination of either one or two of the sartan compounds[12-17]. There are reports in literature of quantification of sartans by capillary zone electrophoresis[18-21] and LC-MS[22-24].The aim of the present study was therefore to develop a Selective, simple, accurate and capable of separating the six ARA-IIs: Losartan, Olmesartan, Irbesartan, Valsartan, Telmisartan and Candesartan, and the most important parameters for quantitative analysis were validated.

EXPERIMENTAL

Materials

HPLC grade acetonitrile and methanol were procured from Spectrochem, Mumbai, India. Analytical grade potassium dihydrogenphosphate and ortho phosphoric acid ware obtained from Merck, Mumbai, India. Olmesartan drug substance was obtained from Advinus Therapeutics Pvt. Ltd, Bangalore, India. Losartan, Irbesartan, Valsartan, Telmisartan and Candesartan drug substance ware obtained from Cipla Ltd., Bangalore, India. HPLC grade water was obtained from a Milli-Q water purification system (Millipore, MA, USA). Inertsil ODS (150mm×4.6mm, 5µ) column was procured from G L Sciences Inc., Japan. The commercially available drugs Telmisartan CRESAR 20 mg tablet from Cipla, Olmesartan medoxamil 20 mg tablet OLMAT from Micro Carsyon, Valsartan VALZAR 40 mg capsules from Torrent Pharma, Irbesartan 150 mg tablets IROVEL from Sun Pharma, Losartan 25 mg tablets LOSAR from Unichem, and Candesartan 4 mg tablets CANDESAR from Ranbaxy were used for quantitative determinations.

Instrumentation

The pH of the solutions was measured on a calibrated Seven Multi pH meter (Mettler Toledo, Schweraenbach, Switzerland). Mobile phase was sonicated in a sonicator (S.V.Scientific, India).

Agilent 1200 Series system consisting of an on-line degasser, G1311A quaternary pump, G1329A auto liquid sampler, G 1316A thermostat column controller and G1314B variable wavelength UV detector (VWD). The data were processed through the use of Chemstation software version, B-02-01-SR1 (260).

Chromatographic conditions

Chromatographic separation and validation ware performed on an Inertsil ODS-3V (150 mm ×4.6 mm, 5µ) column. Mobile phase A consisted of 10 mm potassium dihydrogenphosphate, pH adjusted to 3.5 with orthophosphoric acid and mobile phase B contained acetonitrile: methanol (60:40 v/v). Separation was carried out by gradient elution program, where the initial mobile phase consisted of mobile phase A and mobile phase B in a ratio of 50:50 (v/v) for 3 min. Subsequently, the percentage of mobile phase B was increased from 50 to 85 up to 15 min. The same ratio was held for 5 min, and brought back to initial condition within 5 min. The column was allowed to get equilibrated for 5 min. before performing the next injection. Chromatography was performed at room temperature using a flow rate of 1.0 ml min^{-1}. The injection volume was 10µl. The column eluent was monitored at 230 nm.

Preparation of standard solution

The diluent was prepared by mixing mobile phase A and mobile phase B in the ratio of 50:50 (v/v) and degassed using a sonicator before use. A stock solution of each ARA-IIs in diluent was prepared at a concentration of 0.5 mg/ml. Further solutions were obtained by serial dilutions of stock solution. The volume was made up to mark with the diluent and the solutions were filtered through 0.2 µm syringe filters. For the chromatographic analysis, a mixture of standards has been utilized.

RESULTS AND DISCUSSION

Method development by reverse phase HPLC

The pH of the buffer plays an important role because it influences the separation by affecting the polarity of the compounds. The most important character of the amphoteric nature of the ARA-IIs (figure 1), their retention is greatly influenced by pH, which determines whether these compounds are negatively or positively charged. Different concentrations of the buffer were tested to optimize the separation. Selection of the ex

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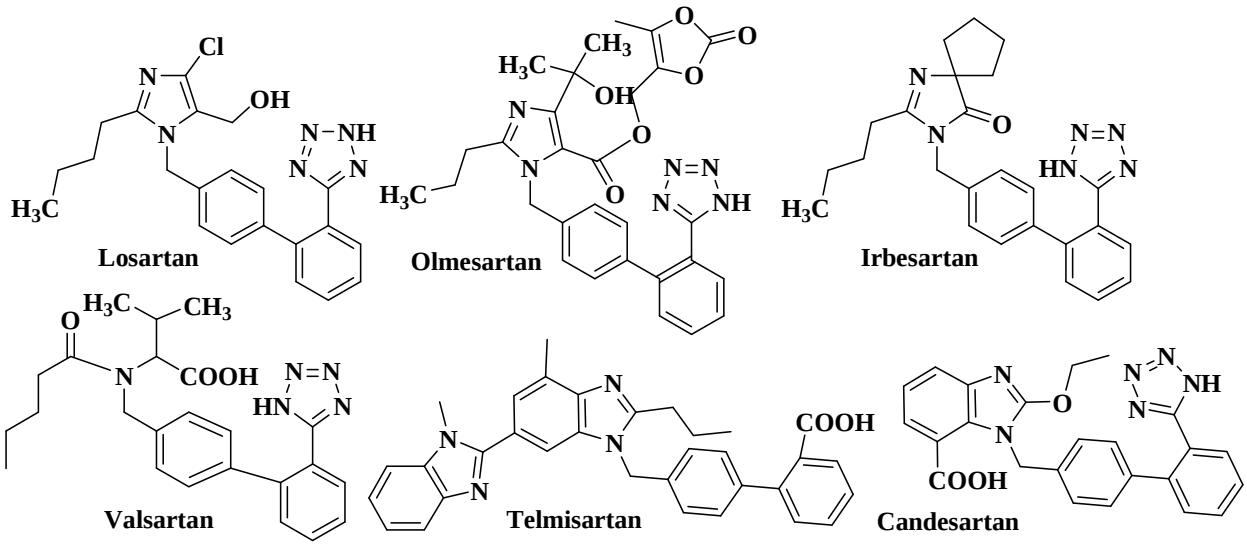

Figure 1: The chemical structure of losartan, olmesartan, irbesartan, valsartan, telmisartan, candesartan

perimental domain was made from prior experience and knowledge of the separation system. This offers the possibility of using either an acidic or a basic buffer. While running with different concentrations of basic buffer, there was no base line separation between the Valsartan and Irbesartan. The basic medium trials were given up because the aim of this study was to develop a method to separate these six ARA-IIs.

The acidic medium was investigated and the best peak shapes and best analysis time were obtained while using buffer of pH 3.5. Therfore, the measurements were performed at different acidic buffer concentrations. In this method, a 10 mM potassium dihydrogen phosphate buffer was prepared by adjusting the pH of the solution to 3.5 by the addition of 5% v/v phosphoric acid solution. Alone and various combinations of acetonitrile and methanol were used as the mobile phase B in our initial efforts in the reverse phase separation. The pH 3.5 was chosen as optimal pH value in the aqueous component of mobile phase A and acetonitrile : methanol (60:40 v/v %) was chosen as the best option for organic modifier of mobile phase B. Using these conditions, slightly differential retention of the six analytes was achieved on the column support with a gradient elution mode.

Attempts to separate the six ARA-IIs on octadecyl silane (C18) was chosen as packing of the analytical column that accomplished an efficient separation. The effect of buffer, acetonitrile and methanol concentration, temperature and flow rate on resolution (Rs), peak shape, retention time(t_R) and tailing factor were examined and most optimum conditions were found to be a mobile phase A consisting of 10 mm potassium dihydro genphosphate and mobile phase B consisting of acetonitrile:methanol (60:40v/v%) at the flow rate of 1.0 ml/min with the column maintained at ambient temperature. A typical chromatogram obtained applying this optimized condition is depicted in figure 2. The retention times, tailing factor and resolution for Losartan, Olmesartan, Irbesartan, Valsartan, Telmisartan and Candesartan are given in TABLE 1.

TABLE 1: System suitability parameters (n=6)

Name of the compound	Retention time (t_R, min)	Tailing factor	Number plates (N)	Resolution
Losartan	9.0	1.06	17780	-
Olmesartan	10.3	1.06	23360	4.61
Irbesartan	11.5	1.08	27832	3.93
Valsartan	12.0	1.14	32071	2.43
Telmisartan	13.4	1.01	28532	2.98
Candesartan	19.9	1.10	49884	22.56

Quantitative determination in pharmaceutical formulations

The same method can be applied in the quantitative determination of the combination of ARA-IIs in tablets. Consequently, there are no problems for determining the concentration of single ARA-II or combined ARA-IIs, namely; Losartan, Olmesartan, Irbesartan, Valsartan, Telmisartan and Candesartan in the pharmaceutical formulation. Using different placebo mixtures, it has been demonstrated that these excipients do not adversely affect the results.

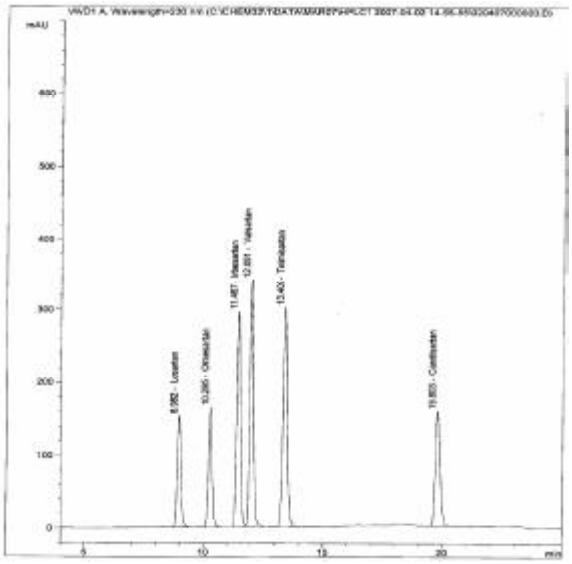

Figure 2 : A typical chromatogram of the six ARA-IIs obtained applying the optimized chromatographic conditions

Linearity

The linear range of the standard curves for the validated assay were 9 µg/ml to 300 µg/ml for Lorsartan, Olmesartan and Valsartan, 3 µg/ml to 400 µg/ml for Irbesartan and Candesartan, 1.5 µg/ml to 250 µg/ml for Telmisartan. These calibration curves are obtained by plotting the peak area obtained for these component against their respective concentration. Each of the component exhibited good linear dependence in the concentration chosen linear regression analysis was used to calculate the slope, intercept and linear regression coefficient (r^2) for each of the component. The results are summarized in TABLE 2.

Precision (repeatability and reproducibility)

The repeatability of the method was evaluated by calculating the relative standard deviation of the estimation of each component for six replicate injections of the same sample and the reproducibility was expressed in terms of relative standard deviation of the estimation of each component obtained for analyses performed on two consecutive days, six times each day. The precision studies for each component were performed, the mean value of the concentration found in % w/w and the relative standard deviation are summarized in TABLE 3 .These results were conformed that the method were precise for estimation of the ARA-IIs.

Accuracy

TABLE 2: Linearity

Name of the compound	Concentration range (µg/mL)	Correlation coefficient(r2)
Losartan	9.0 to 300	0.9999
Olmesartan	9.0 to 300	0.9992
Irbesartan	3.0 to 400	0.9997
Valsartan	9.0 to 300	0.9998
Telmisartan	1.5 to 250	0.9997
Candesartan	3.0 to 400	0.9997

TABLE 3: Precision (repeatability and reproducibility) (n=6)

Name of the compound	Inter-day precision	Intra-day precision
	Amount found in % w/w (RSD %)	Amount found in % w/w (RSD %)
Losartan	100.9 (0.40)	100.4 (0.81)
Olmesartan	101.2 (0.92)	99.3 (0.59)
Irbesartan	101.0 (1.02)	99.2 (0.72)
Valsartan	101.5 (1.11)	101.4 (0.63)
Telmisartan	101.2 (0.85)	100.5 (1.11)
Candesartan	100.7 (0.96)	99.2 (1.05)

TABLE 4: Accuracy (n=6)

Name of the compound	Recovery			
	LOQ	80%	100%	120%
Losartan	101.8±0.4	100.3±0.6	100.9±0.5	100.8±0.4
Olmesartan	101.3±0.9	99.3±0.5	101.4±0.2	100.2±0.4
Irbesartan	101.2±0.8	99.1±0.9	100.7±0.6	101.5±0.4
Valsartan	101.4±0.7	101.7±0.2	101.6±0.4	100.3±0.6
Telmisartan	98.2±0.6	101.1±0.5	101.2±0.8	99.8±0.4
Candesartan	101.8±0.8	99.8±0.7	100.7±0.3	100.5±0.5

The accuracy of the method was evaluated with known amount of the each component at four levels ranging from LOQ to 120 % in triplicate. Absolute recoveries for each of the component in the mixture were done. The recoveries were excellent and ranged from 97.5 % to 103 %, refer TABLE 4 for details.

Limit of detection and limit of quantitation

The limits of detection and quantitation were estimated by obtaining the detector signal for a standard solution of fixed dilutionand then performing serial dilution.The limits of detection were found to be 4.0 µg/ml for Losartan, Olmesartan and Valsartan, 1.3 µg/ml for Irbesartan and Candesartan, 0.4 µg/ml for Telmesartan. The limit of quantitation were found to be 9.0 µg/ml for Lorsartan, Olmesartan and Valsartan , 3.0 µg/ml for Irbesartan and Candesartan, 1.5 µg/ml for Telmesartan.The peak signal to noise ratios of about 2-3 at LOD and 10-12 at LOQ level (figure 3).

Stability in solution

The stability of the stock solutions of these compounds ware checked and proved to stable for at least

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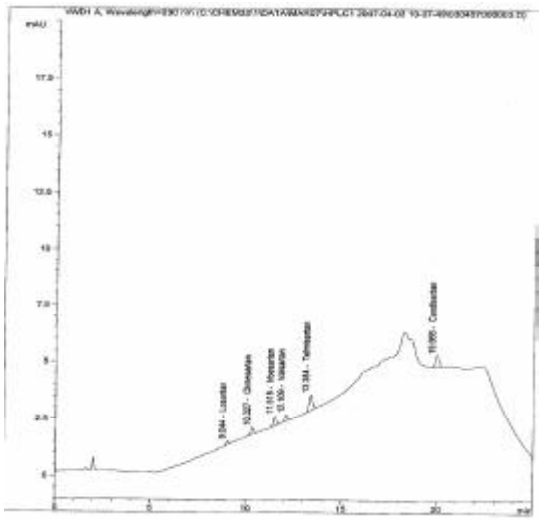

Figure 3: A typical chromatogram of six ARA-IIs LOQ level concentration

48hrs at ambient temperature when protected from light. Each standard stock solution was analyzed immediately after preparation and solution was stored at bench top in tightly capped volumetric flasks. The stored solution of the each compound were reanalyzed after 48hrs. The area obtained for each compound after 48hrs did not show any significant change compared with the area of initial anaysis.This indicates that the each compound was stable in the diluent for at least 48hrs when stored at room temperature.

CONCLUSIONS

A selective, accurate and precise high performance liquid chromatography assay coupled to UV detection was developed for the detection of some angiotensin II receptor antagonists (ARA-IIs).The above results presented here reveal that the separation of six ARA-IIs can be achieved using a 10 mM potassium dihydrogen phosphate buffer solution at pH 3.5.The possibility of simultaneous quantification and identification of the active ingredient in the finished product is therefore very attractive.

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