ACTA CHROMATOGRAPHICA, NO. 19, 2007

SIMPLE AND SENSITIVE LC–UV METHOD FOR SIMULTANEOUS ANALYSIS OF HYDROCHLOROTHIAZIDE AND CANDESARTAN CILEXETIL IN PHARMACEUTICAL FORMULATIONS

S. S. Qutab[1], *S. N. Razzaq*[1], *M. Ashfaq*[1], *Z. A. Shuja*[1], and *I. U. Khan*[1,*]

[1]Schazoo Laboratories (Pvt) Ltd, 45 G.T. Road, Lahore-54000, Pakistan
[2]Department of Chemistry, Government College University, Lahore-54000, Pakistan

SUMMARY

A simple, sensitive, and inexpensive high-performance liquid-chromatographic method has been developed for simultaneous determination of hydrochlorothiazide and candesartan cilexetil in pharmaceutical formulations. Chromatographic separation was achieved on a Phenyl-2 column with a 25:75:0.2 mixture of 0.02 M potassium dihydrogen phosphate, methanol, and triethylamine, final pH 6.0 ± 0.1, as mobile phase. Detection was at 271 nm. Response was a linear function of concentration in the range 5–45 μg mL^{-1} for hydrochlorothiazide and 12–56 μg mL^{-1} for candesartan cilexetil; the correlation coefficients were 0.9993 and 0.9991, respectively. Total elution time for the two components was less than 5 min.

INTRODUCTION

Cartex-H tablets (Schazoo Laboratories Lahore Pakistan), which contain candesartan cilexetil and hydrochlorothiazide, are one of the most commonly used formulations for treatment of high blood pressure when one medicine (monotherapy) is not sufficiently effective. Hydrochlorothiazide (6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide; Fig. 1) is a diuretic. Several analytical methods, including LC–MS [1,2], voltametry [3], spectrophotometry [4–9], capillary electrophoresis [10], and HPLC [11–15], have already been reported for its determination, either alone or in combination with other drugs. Candesartan cilexetil ((±)-1-hydroxyethyl-2-ethoxy-

1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-7-benzimidazolecarboxylate cyclohexyl carbonate; Fig. 2) is a non-peptide angiotensin II receptor. The literature contains very few methods for analysis of candesartan cilexetil; those reported include HPLC with fluorimetric detection [16,17] and spectrofluorimetry [18]. HPLC and ratio derivative spectrophotometric methods have been used for simultaneous determination of the two compounds [19,20]. The HPLC method used diode-array detection for simultaneous quantification of hydrochlorothiazide and candesartan cilexetil. The retention time was more than 6.5 min.

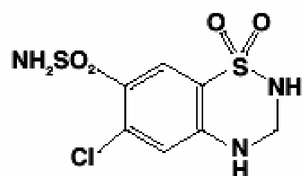

Fig. 1

Chemical structure of hydrochlorothiazide

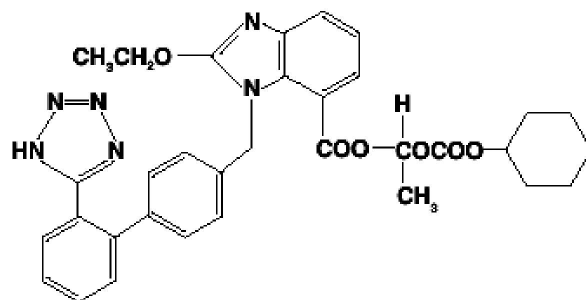

Fig. 2

Chemical structure of candesartan cilexetil

In this paper we describe a simple, inexpensive, sensitive, and validated HPLC method with elution time less than five minutes for simultaneous determination of candesartan cilexetil and hydrochlorothiazide in pharma-

ceutical formulations. The method has been successfully used for quality-control analysis of the drugs and for other analytical purposes.

EXPERIMENTAL

Chemicals and Reagents

Reference standards of candesartan cilexetil and hydrochlorothiazide were obtained from Schazoo Laboratories (Lahore, Pakistan). A pharmaceutical product (Catex-H tablets) containing the same drugs, obtained from the same laboratory, was used in the experiments. Cartex-H tablets were reported to contain 16 mg candesartan cilexetil and 12.5 mg hydrochlorothiazide. Methanol (HPLC grade), potassium dihydrogen phosphate, orthophosphoric acid, and triethylamine (analytical reagent grade) were purchased from Merck (Lahore, Pakistan). All excipients used were of pharmaceutical grade. Starch was purchased from Rafhan (Pakistan), lactose from Borculo (Holland), magnesium stearate from Coin Chen (China), and avicel from JRS Pharma (India). Deionized water was used throughout the experiment. Before use, mobile phase was filtered through 0.45-µm cellulose acetate filters from Millipore (USA). Whatman no. 41 filter papers (obtained commercially) were used in the preparation of sample solutions.

Apparatus and Chromatographic Conditions

A Shimadzu LC-10A system comprising a model LC-10AT pump, an SPD-10A variable-wavelength detector (operated at 271 nm), a CBM-10A interface module with class LC-10 HPLC software, and a Rheodyne injection valve with a 20-µL loop was used for development and evaluation of the method. Compounds were separated on a 250 mm × 4.6 mm i.d., 5-µm particle, Hypersil (UK) Phenyl-2 column. The mobile phase was a 25:75:0.2 mixture of 0.02 M potassium dihydrogen phosphate, methanol, and triethylamine, final pH 6.0 ± 0.1 (adjusted by addition of 10% orthophosphoric acid); the flow rate was 1 mL min^{-1}. The mobile phase was degassed by sonication before use. An external standard method was used. UV detection was performed at 271 nm. HPLC was performed at room temperature. Peak identity was confirmed by comparison of spectra and retention times with those of standards.

Preparation of Standard Solutions

A stock solution of candesartan cilexetil and hydrochlorothiazide reference standards (0.32 and 0.25 mg mL^{-1}, respectively) was prepared in methanol, because both drugs are soluble in this solvent. Working standard solution (32 µg mL^{-1} candesartan cilexetil and 25 µg mL^{-1} hydrochlorothiazide) was obtained by diluting the stock solution with the mobile phase.

Linearity

The linearity of the method was checked by analyzing five solutions in the range 5–45 µg mL^{-1} for hydrochlorothiazide (5, 15, 25, 35, and 45 µg mL^{-1}) and 12–52 µg mL^{-1} for candesartan cilexetil (12, 22, 32, 42, 52 µg mL^{-1}). Each solution was prepared in triplicate.

Limits of Detection and Quantification

The limit of detection (*LOD*) is defined as the lowest concentration of an analyte that can be readily detected but not necessarily quantified. It is usually regarded as the amount for which the signal-to-noise ratio (SNR) is 3:1. The limit of quantitation (*LOQ*) is defined as the lowest concentration of an analyte that can be quantified with acceptable precision and accuracy. It is usually regarded as the amount for which the SNR is 10:1. Two types of solution, i.e. blank and spiked with known progressively decreasing concentrations of each analyte, were prepared and analysed. The limits of detection (*LOD*) and quantification (*LOQ*) were then established by evaluating the minimum levels at which the analyte could be readily detected or accurately quantified, respectively.

Accuracy

Method accuracy was determined by addition of known amounts of hydrochlorothiazide and candesartan cilexetil to a sample solution of known concentration and comparing calculated and measured concentrations. A sample solution containing hydrochlorothiazide and candesartan cilexetil (0.25 and 0.32 mg mL^{-1}, respectively) was prepared by dissolving 12.5 mg hydrochlorothiazide and 16 mg candesartan cilexetil in 50 mL methanol. Samples (3 mL) of the filtered solution were transferred to 50-mL volumetric flasks containing 1.0, 2.0, 3.0, or 4.0 mL hydrochlorothiazide and candesartan cilexetil standard

solution (0.25 and 0.32 mg mL^{-1}, respectively). The solutions were diluted to volume with mobile phase so the final concentrations were 20.0, 25.0, 30.0, and 35.0 µg mL^{-1} for hydrochlorothiazide and 25.6, 32.0, 38.4, and 44.8 µg mL^{-1} for candesartan cilexetil. Each solution was prepared in triplicate.

Specificity

A synthetic mixture containing 16 mg candesartan cilexetil, 12.5 mg hydrochlorothiazide, and 30 mg each of starch, lactose, magnesium stearate, and avicel, which are present as excipients in the pharmaceutical formulation, was accurately weighed and transferred to a 50-mL volumetric flask. The mixture was shaken well with 30 mL methanol and then diluted to volume with methanol. After filtration, 5 mL of the filtrate was transferred to a 50-mL volumetric flask and diluted to volume with mobile phase, to furnish a final solution containing 32 µg mL^{-1} candesartan cilexetil and 25 µg mL^{-1} hydrochlorothiazide.

Application of the Method to Tablets

Twenty tablets were weighed to obtain the average weight. They were then were ground manually using a pestle and mortar made of china clay. An amount of powder equivalent to 16 mg candesartan cilexetil and 12.5 mg hydrochlorothiazide was transferred to a 50-mL volumetric flask with 30 mL methanol, shaken for 5 min, then diluted to volume with methanol to furnish a solution containing 0.32 mg mL^{-1} candesartan cilexetil and 0.25 mg mL^{-1} hydrochlorothiazide. After filtration the solution was diluted with mobile phase to give a final concentration of 32 µg mL^{-1} candesartan cilexetil and 25 µg mL^{-1} hydrochlorothiazide.

RESULTS AND DISCUSSION

Method Optimization

Conditions were optimized for simple, isocratic, accurate, and sensitive simultaneous HPLC determination of hydrochlorothiazide and candesartan cilexetil in tablet formulations. A large number of HPLC methods have been developed for analysis of hydrochlorothiazide but very few for candesartan cilexetil.

Method development was started with 80% methanol in water, but no peaks were observed. The mobile phase was then adjusted by mixing potassium dihydrogen phosphate (0.02 M) with methanol in the ratio 20:80. This resulted in distorted signals that were not well defined. Addition of 0.2 mL triethylamine and subsequent adjustment of the pH with phosphoric acid resulted in good separation and symmetrical peaks. Addition of triethylamine improves the separation by masking polar silanol groups on the stationary phase, thus enabling analyte molecules to move through the column without interference from the stationary phase.

The optimum mobile phase was, therefore, 0.02 M potassium dihydrogen phosphate–methanol–triethylamine 25:75:0.2 (*v*/*v*), final pH 6.0 ± 0.1. Under these experimental conditions sharp peaks were obtained for hydrochlorothiazide and candesartan cilexetil at the retention times 2.8 and 4.9 min, respectively, as shown in Fig. 3.

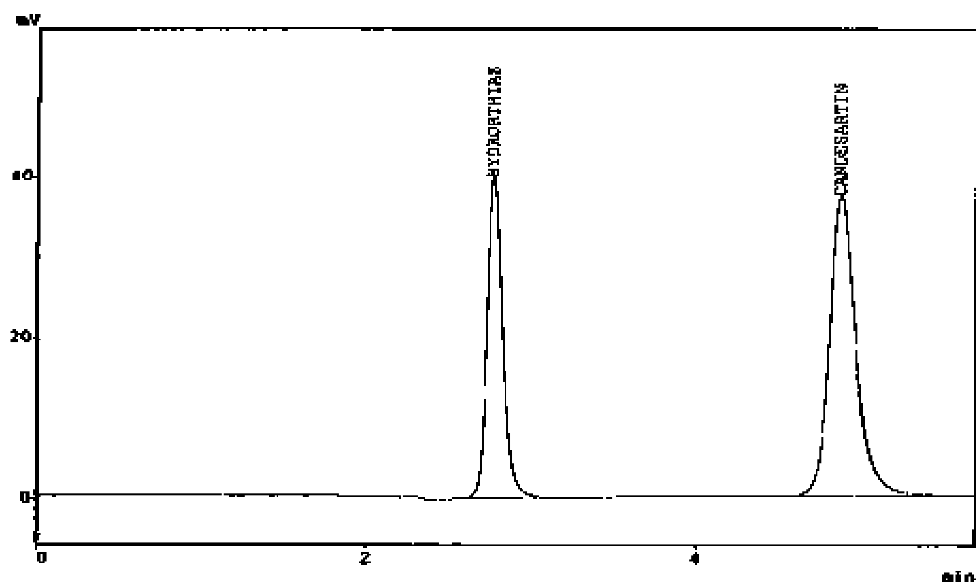

Fig. 3

Chromatogram obtained from hydrochlorothiazide and candesartan reference substances

Method Validation

The method was validated for linearity, accuracy, precision, limits of detection and quantification, and specificity.

Linearity

The linearity of the method was evaluated by analyzing five solutions in the concentration ranges 5–45 μg mL^{-1} for hydrochlorothiazide and 12–52 μg mL^{-1} for candesartan cilexetil. The peak areas obtained from different concentrations of the drugs were used to calculate linear regression equations. These were $Y = 1.36X - 0.085$ and $Y = 1.20X + 0.041$, with correlation coefficients of 0.9993 and 0.9991 for hydrochlorothiazide and candesartan, respectively The high values of the correlation coefficients were indicative of linear relationships between analyte concentration and peak area.

Limits of Detection and Quantification

The limits of detection (*LOD*) and quantification (*LOQ*) were established by evaluating the minimum level at which the analyte could be readily detected and quantified with accuracy, respectively. The *LOD* was 0.08 and 0.13 μg mL^{-1} for hydrochlorothiazide and candesartan cilexetil, respectively (SNR 3:1) and the *LOQ* was 0.19 and 0.22 μg mL^{-1} for hydrochlorothiazide and candesartan cilexetil, respectively (SNR 10:1).

Accuracy

Method accuracy was checked by preparing synthetic mixtures containing different amounts of hydrochlorothiazide and candesartan cilexetil and analyzing the mixtures by use of the method. Percentage recovery, relative standard deviation, and relative percentage error were then calculated. The results obtained (Table I) indicate that recoveries were excellent, not less than 99% and that relative standard deviations and relative percentage error were less than 2%.

Precision

Intra-day precision was calculated from results obtained from fivefold replicate analysis of samples at three different concentrations on the same day. Inter-day precision was calculated from results from the same samples analyzed on five consecutive days. The results obtained are listed in Table II.

Table I

Accuracy of the method

Drug	Concentration ($\mu g\ mL^{-1}$)	Amount recovered ($\mu g\ mL^{-1}$)	Recovery (%)	*RSD* (%)	*RPE*[a]
Hydrochlorothiazide	20.0	19.90	99.50	1.59	0.50
	25.0	25.18	100.72	1.98	0.72
	30.0	30.21	100.70	1.85	0.70
	35.0	34.51	98.60	1.86	1.40
Candesartan cilexetil	25.6	25.86	101.02	1.30	1.02
	32.0	31.82	99.44	0.56	0.56
	38.4	38.77	100.96	0.38	0.96
	44.8	45.23	100.96	1.40	0.96

[a]Relative percentage error

Table II

Intra-day and inter-day precision of the method

Compound	Concn ($\mu g\ mL^{-1}$)	*n*	Intra-day precision		Inter-day precision	
			Mean	*RSD* (%)	Mean	*RSD* (%)
Hydrochlorothiazide	6.25	5	6.24	1.60	6.26	2.08
	12.5	5	12.46	1.04	12.49	1.60
	25.0	5	25.16	0.91	25.21	0.71
Candesartan cilexetil	8.0	5	8.03	1.00	7.98	1.50
	16.0	5	16.12	1.12	16.09	1.37
	32.0	5	31.92	0.47	32.08	0.97

Specificity

The specificity of the method was tested by calculating the percentage recovery of each component in the presence of the other component and in the presence of possible interfering materials such as starch, lactose, magnesium stearate, and avicel. The results are presented in Table III, which shows separation of analytes from the excipients was complete.

Table III

Specificity of the method

Hydrochlorothiazide[a]			Candesartan cilexetil[a]		
Added ($\mu g\ mL^{-1}$)	Recovered ($\mu g\ mL^{-1}$)	Recovery (%)	Added ($\mu g\ mL^{-1}$)	Recovered ($\mu g\ mL^{-1}$)	Recovery (%)
25.00	25.28	101.12	32.00	32.32	101.0
25.00	25.56	102.24	32.00	32.61	101.91
25.00	25.44	101.76	32.00	31.75	99.22
25.00	25.12	100.48	32.00	32.13	100.41
Mean		101.4			100.63
RSD		0.76%			1.12%

[a]Each sample contained 60 $\mu g\ mL^{-1}$ each of starch, lactose, magnesium stearate, and avicel

Stability

The stability of candesartan cilexetil and hydrochlorothiazide in solution was checked by determining the percentage deviation of the amounts present in solution after 72 h at room temperature in comparison with the amount at zero time. The results obtained after 72 h showed no significant variation; the percentage deviation was less than 2% of the initial amount. This is indicative of good stability of each component in the mixture over a period of 72 h.

Application of the Method to Tablets

The method was used for determination of hydrochlorothiazide and candesartan cilexetil in tablet formulations. The results obtained (Table IV) showed percentage recoveries were high and *RSD* (%) values were low, which confirms the method is suitable for routine determination of these components in their pharmaceutical preparations. Figure 4 shows a typical chromatogram obtained from analysis of a tablet formulation.

CONCLUSION

A simple and accurate reversed-phase HPLC method has been established for simultaneous determination of candesartan cilexetil and hydrochlorothiazide. The method was validated by testing its linearity, accuracy, preci-

Table IV

Results from analysis of hydrochlorothiazide and candesartan cilexetil in tablets

Hydrochlorothiazide			Candesartan cilexetil		
Added ($\mu g\ mL^{-1}$)	Recovered ($\mu g\ mL^{-1}$)	Recovery (%)	Added ($\mu g\ mL^{-1}$)	Recovered ($\mu g\ mL^{-1}$)	Recovery (%)
25.00	25.45	101.80	32.00	32.18	100.56
25.00	25.52	102.08	32.00	32.56	101.75
25.00	25.13	101.52	32.00	32.48	101.50
Mean		101.47			101.27
RSD		0.82%			0.62%

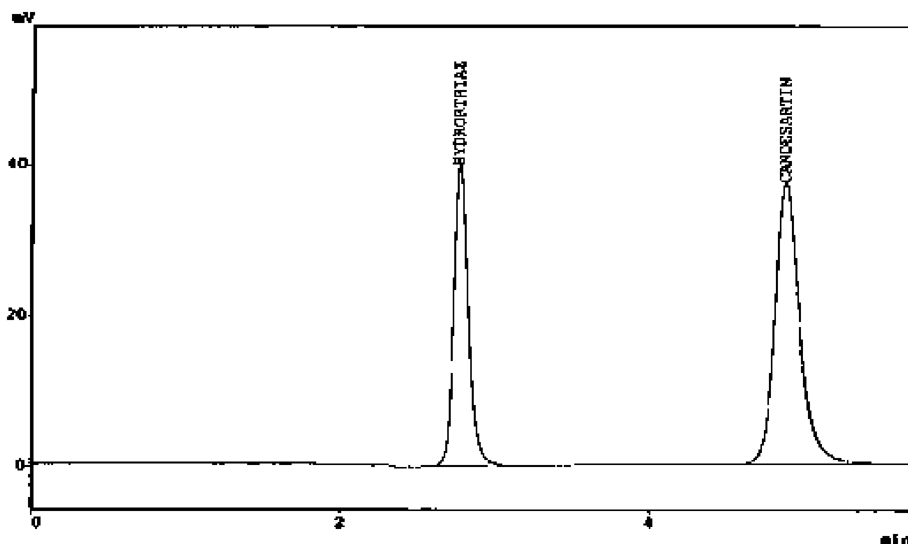

Fig. 4

Chromatogram obtained from hydrochlorothiazide and candesartan cilexetil in tablets

sion, limits of detection and quantitation, and specificity. The run time of less than five minutes enables its application for routine analysis of candesartan cilexetil and hydrochlorothiazide in pharmaceutical formulations.

REFERENCES

[1] A. Vonaparti, M. Kazanis, and I. Panderi, J. Mass. Spectrom., **41**, 593 (2006)

[2] N.V. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, S. Wishu, and D.P. Varma, Biomed. Chromatogr., **19**, 751 (2005)

[3] O. Abdel Razak, J. Pharm. Biomed. Anal., **34**, 433 (2004)

[4] M.C. Ferraro, P.M. Castellano, and T.S. Kaufman, J. Pharm. Biomed. Anal., **30**, 1121 (2002)

[5] E. Dinc and D. Baleanu, J. Pharm. Biomed. Anal., **30**, 715 (2002)

[6] A. Jończyk and Z. Nowakowska, Acta Pol. Pharm., **58**, 339 (2001)

[7] S.A. Shah, I.S. Rathod, B.N. Suhagia, S.S. Savale, and J.B. Patel, JAOAC Int., **84**, 1715 (2001)

[8] E. Satana, S. Altinay, N.G. Goger, S.A. Ozkan, and Z. Senturk, J. Pharm. Biomed. Anal., **25**, 1009 (2001)

[9] N. Erk, J. Pharm. Biomed. Anal., **24**, 603 (2001)

[10] M.G. Quaglia, E. Donati, G. Carlucci, P. Mazzeo, and S. Fanali, J. Pharm. Biomed. Anal., **29**, 981 (2002)

[11] D.L. Hertzog, J.F. McCafferty, X. Fang, R.J. Tyrrell, and R.A. Reed, J. Pharm. Biomed. Anal., **30**, 747 (2002)

[12] F. Belal, I.A. Al-Zaagi, E.A. Gadkariem, and M.A. Abounassif, J. Pharm. Biomed. Anal., **24**, 335 (2001)

[13] G. Carlucci, G. Palumbo, P. Mazzeo, and M.G. Quaglia, J. Pharm. Biomed. Anal., **23**, 185 (2000)

[14] J. Kirschbaum and S. Perlman, J. Pharm. Sci., **73**, 686 (1984)

[15] G.N. Menon and L.B. White, J. Pharm. Sci., **70**, 1083 (1981)

[16] L. Gonzalez, J.A. Lopez, R.M. Alonso, and R.M. Jimenez, J. Chromatogr. A, **949**, 49 (2002)

[17] H. Stenhoff, P.O. Lagerstrom, and C. Andersen, J. Chromatogr. B, **731**, 411 (1999)

[18] E. Cagigal, L. Gonzalez, R.M. Alonso, and R.M. Jimenez, J. Pharm. Biomed. Anal., **26**, 477 (2001)

[19] N. Erk, J. Liq. Chromatogr. Related Technol., **26**, 2581 (2003)

[20] N. Erk, Pharmazie, **58**, 796, (2003)