ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
21 (1999) 1017–1024

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

www.elsevier.com/locate/jpba

Simultaneous determination of benazepril hydrochloride and hydrochlorothiazide by micro-bore liquid chromatography

I.E. Panderi *, M. Parissi-Poulou

Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopolis Zografou, GR-157 71, Athens, Greece

Received 15 March 1999; received in revised form 23 July 1999; accepted 30 July 1999

Abstract

A micro-bore liquid chromatographic method was developed for the simultaneous determination of benazepril hydrochloride and hydrochlorothiazide in pharmaceutical dosage forms. The use of a BDS C-18 micro-bore analytical column, results in substantial reduction in solvent consumption and increased sensitivity. The mobile phase consisted of a mixture of 0.025 M sodium dihydrogen phosphate (pH 4.8) and acetonitrile (55:45, v/v), pumped at a flow rate of 0.40 ml min^{-1}. Detection was set at 250 nm using an ultraviolet detector. Calibration graphs are linear (r better than 0.9991, $n = 5$), in concentration range 5.0–20.0 μg ml^{-1} for benazepril hydrochloride and 6.2–25.0 μg ml^{-1} for hydrochlorothiazide. The intra- and interday R.S.D. values were $< 1.25\%$ ($n = 5$), while the relative percentage error (E_r) was $< 0.9\%$ ($n = 5$). The detection limits attained according to IUPAC definition were 0.88 and 0.58 μg ml^{-1} for benazepril hydrochloride and hydrochlorothiazide, respectively. The method was applied in the quality control of commercial tablets and content uniformity test and proved to be suitable for rapid and reliable quality control.
© 1999 Elsevier Science B.V. All rights reserved.

Keywords: Benazepril hydrochloride; Hydrochlorothiazide; Liquid chromatography; Micro-bore columns

1. Introduction

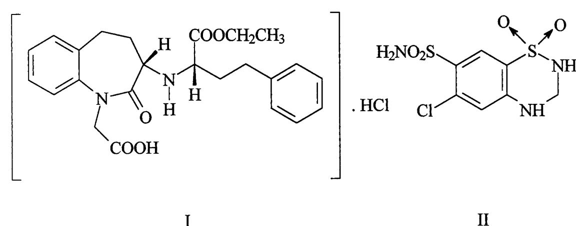
Benazepril hydrochloride (3-[1-(ethoxycarbonyl)-3-phenyl-(1*S*)-propylamino]-2,3,4,5-tetrahydro-2-oxo-1H-1-(*3*S)-benzazepine-1-acetic acid monohydrochloride) [1], is a new an giotensin-converting enzyme (ACE) inhibitor which is shown to be effective in the treatment of hypertension and congestive heart failure. The compound, a dicarboxylic acid monoethylester, is a prodrug which is hydrolysed in vivo to its active carboxylic acid metabolite, benazeprilat [2]. Hydrochlorothiazide (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide) [3], is an antihypertensive diuretic agent which is indicated in the management of hypertension. Its combination with benazepril hydrochloride increases the antihypertensive effects. Structures of the compounds are presented in Scheme 1.

Several reports have been described for the determination of benazepril alone, in formula-

* Corresponding author. Tel.: +30-1-7284527; fax: +30-1-7238297.

0731-7085/99/$ - see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0731-7085(99)00195-8

tions or in biological fluids. These methods include the use of high performance liquid chromatography, [4,5] derivative spectrophotometry, [6] and gas chromatography–mass spectrometry [7]. Hydrochlorothiazide has also been determined using near-IR spectroscopy [8], differential spectrophotometry [9], derivative spectrophotometry [10,11], thin layer chromatography [12], and high performance liquid chromatography [13–15]. To our knowledge, only a derivative UV–Vis spectrophotometric method and an absorbancy ratio method have been described for the simultaneous



I II

Scheme 1. Structure of benazepril hydrochloride I, and hydrochlorothiazide, II.

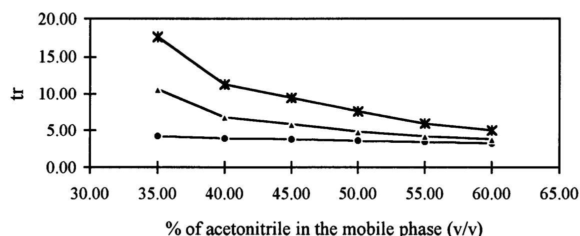


Fig. 1. Plots of the retention time, tr, versus acetonitrile concentration in the mobile phase of hydrochlorothiazide ●, benazepril hydrochloride ▲, and naproxen *.

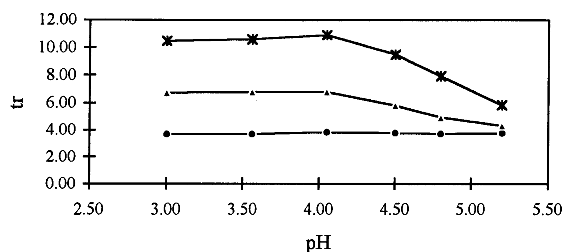


Fig. 2. Plots of the retention time, tr, versus pH of the phosphate buffer in the mobile phase of hydrochlorothiazide ●, benazepril hydrochloride ▲, and naproxen *.

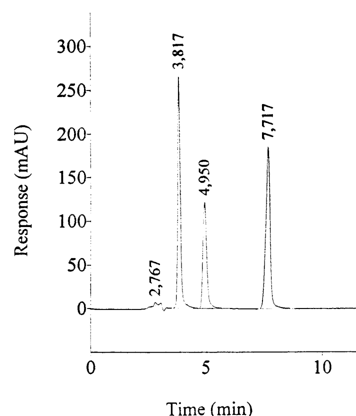


Fig. 3. Representative chromatogram of a mixture of hydrochlorothiazide (12.5 μg ml^{-1}), benazepril hydrochloride (10.0 μg ml^{-1}), and naproxen (15.0 μg ml^{-1}) at retention times 3.82, 4.95 and 7.72 min, respectively. Chromatographic conditions: reversed-phase HPLC on a micro-bore C-18 BDS column; mobile phase, 0.025 M potassium dihydrogen phosphate (pH 4.8) and acetonitrile (55:45, v/v); flow rate, 0.40 ml min^{-1} and a UV detector at 250 nm.

determination of both of these compounds in pharmaceutical dosage [16].

As the combination of these two compounds in antihypertensive therapy has become popular in recent years, we thought that it would be of particular interest to develop a simple, sensitive and reliable method for their simultaneous determination. Thus, our purpose was to develop an environmentally friendly high performance liquid chromatographic method using micro-bore cartridges. In recent years, there has been an increasing interest in columns with reduced internal diameter and packed with smaller particles. Several advantages emerge from using micro-bore columns, the most important is the reduction of solvent usage, as they operate at lower flow rates (0.30–0.50 ml min^{-1}). Another very practical aspect of the choice of micro-bore columns is the much reduced peak volumes, which are related to their low volumetric flow rates. For an equal injected mass, the solute concentration is greater for micro-bore columns due to decreased volumetric dilution, which results in increased sensitivity [17–19].

In this work, a high-performance liquid chromatographic technique using micro-bore columns

Table 1
Calibration equations for the determination of benazepril hydrochloride and hydrochlorothiazide by high-performance liquid chromatography

Sample (μg ml^{-1})[a]		Regression equations[b]	r[c]	S.D.[d]		S_r[e]
Bz	Hy			Slope	Intercept	
5.00–20.0	6.25–25.00	$R_{Bz} = 0.048\ C_{Bz} - 0.008$	0.9991	6.9×10^{-4}	0.014	0.009
5.00–20.0	6.25–25.00	$R_{Hy} = 0.118\ C_{Hy} - 0.050$	0.9998	1.0×10^{-3}	0.023	0.020

[a] Bz, Benazepril hydrochloride; Hy, hydrochlorothiazide.
[b] Ratios of peak area of each compound to that of the internal standard, R, versus the appropriate concentration, C, in μg ml^{-1}; five standards.
[c] Correlation coefficient.
[d] Standard deviation of slope (μg ml^{-1}) and intercept.
[e] Standard error of the estimate.

Table 2
Accuracy and precision of within- and between-run analysis for the determination of benazepril hydrochloride and hydrochlorothiazide by high-performance liquid chromatography

Nominal concentration (μg ml^{-1})		Assayed concentration (μg ml^{-1})					
Benazepril	Hydrochlorothiazide	Benazepril			Hydrochlorothiazide		
		Mean ± S.D.	R.S.D. (%)[a]	E_r (%)[b]	Mean ± S.D.	R.S.D. (%)[a]	E_r (%)[b]
Intra-day ($n = 5$)							
3.25	4.00	3.21 ± 0.04	1.25	−0.9	3.97 ± 0.02	0.05	−0.7
5.00	6.25	5.04 ± 0.05	0.99	0.8	6.19 ± 0.04	0.65	−0.2
10.00	12.50	9.93 ± 0.04	0.40	−0.7	12.55 ± 0.07	0.56	0.1
20.00	25.00	20.09 ± 0.13	0.65	0.4	24.98 ± 0.16	0.64	−0.1
Inter-day ($n = 5$)							
5.00	6.25	5.10 ± 0.03	0.59	2.0	6.31 ± 0.04	0.32	1.0
20.00	25.00	20.21 ± 0.20	0.99	1.0	25.12 ± 0.23	0.92	0.5

[a] Percentage relative standard deviation.
[b] Relative percentage error.

Table 3
Determination of benazepril hydrochloride and hydrochlorothiazide in commercial formulations by high-performance liquid chromatography

Commercial formulation	Benazepril hydrochloride; found (mg/tablet)[a]		Hydrochlorothiazide; found (mg/tablet)[a]	
	Mean ± S.D. ($n = 10$)	Recovery (%)	Mean ± S.D. ($n = 10$)	Recovery (%)
Cibadrex (10/12.5)	9.95 ± 0.04	99.5	12.45 ± 0.11	99.6
Cibadrex (20/25)	19.86 ± 0.19	99.3	25.02 ± 0.07	100.1

[a] The indicated values are the mean of 10 different analyses of the same commercial batch.

Table 4
Recoveries of benazepril hydrochloride and hydrochlorothiazide in spiked commercial samples

Drug	Amount added (mg)	Amount found (mg)	m[a]	Recovery[b] (%)
Benazepril hydrochloride	5.0	9.03	1.005	100.5
	8.0	11.93		
	10.0	14.10		
	12.5	16.59		
Hydrochlorothiazide	6.25	10.30	0.992	99.2
	10.0	14.57		
	12.5	17.07		
	15.6	20.75		

[a] m is the slope the linear regression analysis of the amount found versus the amount added.
[b] Recovery (%) $= m \times 100$.

has been utilised successfully for the determination of benazepril hydrochloride and hydrochlorothiazide in pharmaceutical tablets. The methods offers an enhancement in sensitivity and is more selective than the previously mentioned spectrophotometric methods [16].

2. Experimental

2.1. Materials

Solvents were of HPLC grade and were purchased from Lab-Scan Science (Ireland). Sodium dihydrogen phosphate monohydrate (pro-analysi) and hydrochloric acid (analytical-reagent grade) were purchased from Merck (Darmstadt, Germany). Water was deionised and further purified by means of a Milli-Q Plus Water Purification System (Millipore). Benazepril hydrochloride and hydrochlorothiazide of pharmaceutical purity grade were kindly provided by Novartis Pharma (Basle, Switzerland), while naproxen of pharmaceutical purity grade was purchased from Minerva Hellas. All substances were used without any further purification. Cibadrex (10 + 12.5) and Cibadrex (20 + 25) tablets are products of Novartis Pharma; each tablet was labelled to contain 10.0 and 20.0 mg of benazepril and 12.5 and 25.0 mg of hydrochlorothiazide, respectively. The excipients present in tablets are: Hydroxypropyl methylcellulose (hypromellose), hydrogenated castor oil, lactose, polyvinylpyrrolidone XL, iron oxide red, E172, macrogol 8000, talc and titanium oxide.

2.2. Instrumental and chromatographic conditions

The high-performance liquid chromatographic system consisted of a GBC Model LC1126 pump and a Rheodyne Model 7725i injector with a 5-μl loop, which were coupled to a GBC Model LC1210 UV–Vis detector operating at 250 nm. The analytical column was a reversed phase BDS C-18 micro-bore column (25 × 0.30 cm i.d.) containing 5-μm packing, (Shandon Scientific,Cheshire, UK). The mobile phase consisted of 0.025 M sodium dihydrogen phosphate (pH 4.8) and acetonitrile (55:45, v/v). The mobile phase was filtered through a 0.20-μm nylon membrane filter (Gelman Sciences) and degassed under vacuum prior to use. All chromatographic experiments were carried out at a flow rate 0.40 ml min^{-1}. Data acquisition were performed using WinChrom chromatography software package: ChemWin, version 1.2. A pH meter (Metrohm, model 654 Herisau) was used for all pH measurements.

2.3. Stock standard solutions

Stock standard solutions of benazepril hydrochloride (Bz), 1.0 mg ml^{-1}, hydrochlorothiazide (Hy), 1.0 mg ml^{-1}, and naproxen (Np), 1.0 mg ml^{-1}, were prepared by

dissolving appropriate amounts of the compounds in methanol. These solutions were stored in the dark under refrigeration at 4°C and were found to be stable for several weeks.

A series of mixed standard solutions were prepared by the appropriate dilution of the above mentioned stock standard solutions in mobile phase to reach concentration ranges of 5.0–20.0 and 6.25–25.0 μg ml^{-1} for Bz and Hy, respectively. In each sample 15.0 μg ml^{-1} of the internal standard Np was added. Standard solutions were found to be stable during the analysis time.

2.4. Assay of pharmaceutical preparations

Twenty tablets were weighed and finely pulverised. An appropriate portion of this powder, equivalent to 10.0 mg of Bz and 12.5 mg of Hy, was transferred to a 25-ml volumetric flask with 20 ml of methanol. The solution was sonicated for 10 min, followed by shaking by mechanical means for 20 min and finally diluted to volume with methanol. A portion of this solution was centrifuged at 4000 rev min^{-1} (2890 g) for 15 min. A 5-ml aliquot was transferred to a 25-ml volumetric flask and diluted to volume with mobile phase. Consequently a 2-ml aliquot of this solution and a 150-μl aliquot of the stock solution of Np were further diluted to 10 ml of mobile phase; 5 μl sample was injected into the HPLC system. Peak area ratios of each compound to that of the internal standard were then measured for the determinations. The same procedure was followed for the content uniformity test, using one tablet per sample.

2.5. Calibration procedure

Two calibration curves were constructed by assaying the above mentioned mixed standard solutions of Bz and Hy in mobile phase. The concentration range covered was 5.0–20.0 μg ml^{-1} for Bz and 6.25–25.0 μg ml^{-1} for Hy. Triplicate 5-μl injections were made of each solution and the peak area ratio of each drug to that of the internal standard was plotted against the corresponding concentration to obtain the calibration graph.

The overall precision and accuracy of the assay was evaluated by analysing three series of mixed standard solutions of Bz and Hy, at concentrations of 3.25, 5.0, 10.0 and 20.0 μg ml^{-1} for Bz and 4.00, 6.25, 12.5 and 25.0 μg ml^{-1} for Hy. In each sample 15.0 μg ml^{-1} of the internal standard Np was added. The relative standard deviation (% R.S.D.) was determined in order to assess the precision of the method, while the accuracy was expressed by the relative percentage error (E_r %).

In order to determine the effect of the excipients used in the formulation of tablets on the determination of Bz and Hy, the standard addition method [20] was used. Thus, five equal amounts of powdered tablets equivalent to 4.0 mg of Bz and 5.0 mg of Hy, were spiked with different amounts of reference standards of Bz and Hy. The samples were analysed as mentioned in the assay procedure, while in each sample, 15.0 μg ml^{-1} of the internal standard Np was added. Peak area ratios of each drug to that of the internal standard were measured for the determination of both compounds.

3. Results and discussion

3.1. Chromatographic characteristics

Chromatographic separations were carried out on a micro-bore BDS C-18 column. The effect of composition and pH of the mobile phase on the retention time (tr) of Hy, Bz and Np (internal standard), were investigated. A satisfactory separation was obtained with a mobile phase consisting of 0.025 M sodium dihydrogen phosphate and acetonitrile (55:45, v/v). Increasing acetonitrile concentration to more than 60% led to inadequate separation of the compounds (Fig. 1). At lower acetonitrile concentration, separation occurred but with excessive tailing and increased retention time for Np peak. Retention times of the compounds show no significant difference at pH values ranging from 3.0 to 4.0 (Fig. 2). A pH value of 5.0 led to inadequate resolution of Hy and Bz. Thus, a pH value of 4.8 was found to be the optimum, as at this pH value the analyte peaks were well resolved and almost free from tailing.

Peak asymmetry or tailing factor, T, was calculated as: $T = W_{0.05}/2f$; where $W_{0.05}$ is the distance from the leading edge to the tailing edge of the peak, measured at a point 5% of the peak height from the baseline and f is the distance from the peak maximum to the leading edge of the peak. Peak asymmetries were found to be 1.21, 1.07 and 1.06 for Hy, Bz, and Np, respectively. The optimum wavelength for detection was at 250 nm, at which the best detector responses for all the substances were obtained.

The specificity of the HPLC method is illustrated in Fig. 3 where complete separation of the compounds was observed. Hy was eluted at 3.82 min, Bz appeared at 4.95 min, while the internal standard Np was eluted at 7.72 min.

3.2. Statistical analysis of chromatographic data

Under the experimental conditions described above, linear relationships were observed between the peak area ratio of each compound to that of the internal standard and the corresponding concentration, as shown by the equations presented in Table 1. The correlation coefficient (r) and the standard error of the estimate (S.E.) of the calibration lines are also given, along with the S.D. of the slopes and the intercepts.

In order to further evaluate the linearity of the proposed method, five calibration equations, were constructed over a period of four weeks. The average regression equation for Bz was:

$$R_{Bz} = 0.049(\pm 1.3 \times 10^{-3})\ C_{Bz} - 0.008(\pm 8.4 \times 10^{-4})$$

and for Hy was:

$$R_{Hy} = 0.119(\pm 1.3 \times 10^{-4})\ C_{Hy} - 0.050(\pm 3.1 \times 10^{-3})$$

where R is the ratio of peak area of each compound to that of the internal standard and C is the appropriate concentration expressed in μg ml^{-1}.

The slopes of the calibration equations of Bz and Hy had R.S.D. values of 2.6 and 1.1%, respectively while the R.S.D. values of the intercepts of these calibration equations were found to be 10.5 and 6.2%, respectively. The correlation coefficient of each standard curve constructed invariably exceeded 0.9991.

The limits of detection LOD attained, as defined by IUPAC [21], $LOD_{(k=3)} = k \times S_a/b$ (where b is the slope of the calibration graph and S_a is the S.D. of the blank signal) were found to be 0.88 and 0.58 μg ml^{-1} for Bz and Hy, respectively. The limits of quantitation LOQ were also attained according to the IUPAC definition, $LOQ_{(k=10)} = k \times S_b/b$, and were found to be 2.92 and 1.95 μg ml^{-1} for Bz and Hy, respectively.

Intra-day data for the precision and accuracy of the method given in Table 2, indicate for Bz R.S.D.% = 0.40–1.25 and $E_r\% = -0.9$–0.8 and for Hy R.S.D.% = 0.50–0.64 and $E_r\% = -0.7$–0.1. Moreover, the inter-day R.S.D.% values (Table 2) for the determination of Bz and Hy were ranged from 0.59 to 0.99 and 0.32 to 0.92, respectively.

The influence of small deliberate variations of the method parameters in the retention times of the analytes was examined for the evaluation of the robustness of the proposed method [22,23]. Thus, replicate injections ($n = 3$) of a mixed standard solution containing 10.0 μg ml^{-1} of Bz, 12.5 μg ml^{-1} of Hy and 15.0 μg ml^{-1} of Np, were performed under small changes of three chromatographic parameters (factors). The factors selected to be examined were the pH of the buffer, the flow rate and the percentage of the acetonitrile in the mobile phase; each factor was changed at three levels (-1, 0 and 1). One factor at the time was changed to estimate the effect. Results presented in Table 5, indicate that retention times, capacity factors and tailing factors of the analytes remained unaffected by small variations of these parameters.

The statistical evaluation of the HPLC method revealed its good linearity and reproducibility and led us to the conclusion that it could be used for the simultaneous determination of Bz and Hy in tablets, reliably.

3.3. Assay of pharmaceutical formulations

The proposed method was evaluated in the assay of commercially available tablets containing

Table 5
Robustness evaluation of the high-performance liquid chromatographic method

Chromatographic changes		Hydrochlorothiazide			Benazepril hydrochloride			Naproxen		
Factor[a]	Level	tr[b]	k'[c]	T[d]	tr[b]	k'[c]	T[d]	tr[b]	k′[c]	T[d]
A: pH of the buffer										
4.70	−1	3.77	0.37	1.36	4.96	0.79	1.08	7.83	1.82	0.80
4.80	0	3.82	0.38	1.21	4.95	0.79	1.07	7.72	1.79	1.06
4.90	1	3.88	0.41	1.33	4.87	0.76	1.24	7.69	1.78	1.10
Mean ± S.D. ($n=3$)		3.82 ± 0.06	0.39 ± 0.02	1.30 ± 0.08	4.93 ± 0.05	0.78 ± 0.02	1.13 ± 0.10	7.75 ± 0.07	1.80 ± 0.02	0.99 ± 0.16
B: Flow rate (ml min^{-1})										
0.37	−1	3.83	0.41	1.15	4.86	0.76	1.03	7.76	1.80	1.03
0.40	0	3.82	0.38	1.21	4.95	0.79	1.07	7.72	1.79	1.06
0.43	1	3.77	0.39	1.27	4.92	0.77	1.06	7.66	1.76	1.09
Mean ± S.D. ($n=3$)		3.81 ± 0.03	0.39 ± 0.02	1.21 ± 0.06	4.91 ± 0.04	0.77 ± 0.02	1.05 ± 0.02	7.71 ± 0.05	1.78 ± 0.02	1.06 ± 0.03
C: % of acetonitrile in the mobile phase (v/v)										
44	−1	3.85	0.41	1.25	4.99	0.80	1.11	7.78	1.81	0.95
45	0	3.82	0.38	1.21	4.95	0.79	1.07	7.72	1.79	1.06
46	1	3.80	0.39	1.17	4.83	0.74	1.01	7.63	1.78	1.04
Mean ± S.D. ($n=3$)		3.82 ± 0.02	0.39 ± 0.02	1.21 ± 0.04	4.92 ± 0.08	0.80 ± 0.01	1.06 ± 0.05	7.71 ± 0.08	1.79 ± 0.02	1.01 ± 0.08

[a] Three factors (A, B and C) were slightly changed at three levels (1, 0, −1); each time a factor was changed from level (0) the other factors remained at level (0).
[b] Retention time.
[c] Capacity factor.
[d] Tailining factor.

a mixture of Bz and Hy in the proportion 0.8:1.0. Ten replicate determinations were carried out on an accurately weighted amount of the pulverised tablets equivalent to 10.0 mg of Bz and 12.5 mg of Hy. The results obtained gave a mean of 9.98 ± 0.22 with a % R.S.D. of 2.20 for Bz, and mean of 12.52 ± 0.17 with a % R.S.D. of 1.36 for Hy.

The method proved to be suitable for the content uniformity test, where a great number of assays on individual tablets is required. Commercially available tablets containing a mixture of Bz and Hy in proportion 0.8:1.0 were analysed using the proposed methodology and the results are given in Table 3. Recoveries achieved were in accordance with the actual content of Bz and Hy in tablets.

Recovery studies were also performed, by analysing spiking sample powders with appropriate amounts of the reference standard of both compounds. Two calibration curves were then constructed by plotting the amount of the drug found (mg) versus the amount of the drugs added (mg) for each one of the two compounds. The following linear regression equations were obtained through regression analysis of data:

$$C^{f}_{Bz} = 1.005(\pm 0.0019) \times C^{a}_{Bz} + 3.94(\pm 0.18),\ r = 0.9996 \quad (1)$$

$$C^{f}_{Hy} = 0.992(\pm 0.021) \times C^{a}_{Hy} + 5.05(\pm 0.24),\ r = 0.9995 \quad (2)$$

where C^{f}_{Bz} and C^{f}_{Hy} are the amounts (mg) found for Bz and Hy, respectively, while C^{a}_{Bz} and C^{a}_{Hy} are the amounts (mg) added for Bz and Hy, respectively; r is the correlation coefficient of the calibration equation.

The y-axis intercept of the above mentioned linear regression equations indicate the amount (mg) of the drug found in the powdered tablets, while the percentage recoveries were calculated as: % recovery = slope × 100. The results presented in Table 4 indicate that there is no interference from the excipients used in the formulation of the tablets.

In conclusion, high-performance liquid chromatography proved to be a suitable technique for the reliable analysis of commercial formulations containing combinations of hydrochlorothiazide and benazepril hydrochloride. The micro-bore HPLC method is a selective, reliable and sensitive method, which offers the advantage of lower solvent consumption over the conventional chromatographic methods. Moreover, the proposed method offers a short analytical run time of 10.0 min and achieved a good resolution between Hy, Bz and Np.

References

[1] J.W.H. Watthey, J.L. Stanton, M. Desai, J.E. Babiarz, B.M. Finn, J. Med. Chem. 28 (1985) 1511–1516.
[2] J.R. Wade, D.M. Hughes, A.W. Kelman, A. Howie, P.A. Meredith, J. Pharm. Sci. 82 (1993) 471–474.
[3] H.P. Deppeler, in: J. Smith (Ed.), Analytical Profile of Drug Substances, vol. 10, Academic Press, New York, 1982, pp. 405–416.
[4] F. Barbato, P. Morrica, F. Quaglia, Farmaco 49 (1994) 457–460.
[5] A. Gumieniczek, L. Przyborowski, J. Liq. Chromatogr. 20 (1997) 2135–2142.
[6] D. Bonazzi, R. Gotti, V. Andrisano, V. Carvini, J. Pharm. Biomed. Anal. 16 (1997) 431–438.
[7] A. Sioufi, F. Pommier, G. Kaiser, J.P. Dubois, J. Chromatogr. 434 (1988) 239–246.
[8] D.J. Wargo, J.K. Drennen, J. Pharm. Biomed. Anal. 14 (1996) 1415–1423.
[9] M.A. Elsayed, Y.M. Elsayed, H. Abdine, Analyst 105 (1980) 222–229.
[10] I. Panderi, M. Parissi-Poulou, Int. J. Pharm. 86 (1992) 99–106.
[11] A.F.M. El Walily, S.F. Belal, E.A. Heaba, A. El Kersh, J. Pharm. Biomed. Anal. 13 (1995) 851–858.
[12] S.M. Brown, K.L. Busch, J. Planar Chromatogr. 4 (3) (1991) 189–193.
[13] J. Kirschbaum, S. Perlman, J. Pharm. Sci. 73 (1984) 686–689.
[14] J.Y.K. Hsieh, C. Lin, B.K. Matuszewski, M.R. Dobrinska, J. Pharm. Biomed. Anal. 12 (1994) 1555–1562.
[15] D. Farthing, H. Fakhry, E.B.D. Ripley, D. Sica, J. Pharm. Biomed. Anal. 17 (1998) 1455–1459.
[16] N. Erk, F. Onur, Analusis 25 (1997) 161–163.
[17] K.J. Wilson, P.M. Yuan, T.D. Schlabach, Recept. Biochem. Methodol. 14 (1989) 17–19.
[18] D.N. Mallet, B.J. Law, J. Pharm. Biomed. Anal. 9 (1991) 53–56.
[19] U.D. Neue, HPLC columns. Theory, Technology, and Practise, Wiley, New York, 1997, pp. 49–53.
[20] J.N. Miller, Analyst 116 (1991) 1–14.
[21] G.L. Long, G.L. Winefordner, Anal. Chem. 55 (1983) 712A–724A.
[22] D.R. Jenke, J. Liq. Chromatogr. 19 (12) (1996) 1873–1891.
[23] Y.V. Heyden, K. Luypaert, C. Hartmann, D.L. Massart, J. De Beer, Anal. Chim. Acta 312 (1995) 245–262.