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Application of LC and HPTLC-densitometry for the simultaneous determination of benazepril hydrochloride and hydrochlorothiazide

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Abstract

Two methods are described for the simultaneous determination of benazepril HCl and hydrochlorothiazide in binary mixture. The first method was based on HPTLC separation of the two drugs followed by densitometric measurements of their spots at 238 and 275 nm for benazepril HCl and hydrochlorothiazide, respectively. The separation was carried out on Merck HPTLC aluminum sheets of silica gel 60 $F_{254,}$ using ethyl acetate–methanol–chloroform (10:3:2 v/v) as mobile phase. Second order polynomial equation was used for the regression line in the range 2–20 and 2.5–25 μg/spot for benazepril HCl and hydrochlorothiazide, respectively. The second method was based on HPLC separation of the two drugs on reversed phase, ODS column at ambient temperature using a mobile phase consisting of acetonitrile and water (35:65 v/v) and adjusting to pH 3.3 with acetic acid. Quantitation was achieved with UV detection at 240 nm based on peak area with linear calibration curves at concentration ranges 10–60 and 12.5–75 μg ml^{-1} for benazepril HCl and hydrochlorothiazide, respectively. The two proposed methods were successfully applied to the determination of both drugs in laboratory prepared mixtures and in commercial tablets. No chromatographic interference from the tablets excipients was found.

Keywords: Benazepril HCl; HPTLC; Hydrochlorothiazide; Reversed phase HPLC

1. Introduction

Benazepril HCl, {(3S)-3-[(1S)-1-Ethoxycarbonyl-3-phenylpropyl amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepin-1-yl}acetic acid hydrochloride, is a potent angiotensin converting

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enzyme inhibitor that is used in the treatment of essential hypertension and congestive heart failure [1]. Various spectrophotometric methods have been reported for the determination of benazepril HCl in pharmaceutical tablets using bromocresol green, 3-methylbenzothialozone hydrazone [2], first and second derivative spectrophotometry [2,3]. The chromatographic techniques of analysis: capillary [4], Electron capture gas chromatography [5] and HPLC using a LiChrosorb RP-18 column with phosphate buffer of pH 2.4-acetonitrile (7:3, v/v) as a mobile phase [6] or a Hypersil ODS column with 20 mM sodium heptansulfonate (pH 2.5)-acetonitrile containing 5% THF (63:37, v/v) as mobile phase [3] was employed.

Hydrochlorothiazide, 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide, is one of the oldest and widely used thiazide diuretics. The drug is official in both BP 1998 [7] and USP 24 [8] either alone or in binary mixtures with other drugs. The analytical methods for its determination have been reviewed [9].

The binary mixture of benazepril HCl and hydrochlorothiazide is widely used in the treatment of hypertension. Several analytical procedures have been described for the simultaneous determination of the two drugs including HPLC using a C18 micro-bore column with 0.025 M sodium dihydrogen phosphate (pH 4.8)-acetonitrile (55:45, v/v) as a mobile phase [10], absorbency ratio [11] and Vierordt's [12] spectrophotometry. Other spectrophotometric methods using first [11], second [2,13] derivative and ratio spectra first derivative [12] were applied. However, no planner chromatographic method has been reported for the simultaneous determination of benazepril HCl and hydrochlorothiazide in binary mixture.

Thin layer chromatography (TLC), which is one of the oldest chromatographic methods, is commonly used in medical–biochemical analysis, food analysis, and environmental pollutant analysis [14]. By comparison with HPLC, high performance thin layer chromatography (HPTLC) still preserves its advantages as rapid, reliable and economical analysis method. The main application of TLC in the pharmaceutical industry is intermediate quality control during the development and production of pharmaceutically active substances and testing of optically pure substances [15–17]. Although TLC is mainly used as a drug screening and confirmation tool [18], quantitative pharmaceutical analysis by TLC has recently attracted considerable interest due to improved technologies with HPTLC. In recent years, the HPTLC technique has been improved to incorporate the following features: HPTLC grade stationary phase, automated sample application devices, controlled development environment, automated developing chamber, computer-controlled densitometry and quantitation, and fully validated procedures. These features result in methods that are not only convenient, fast, robust, and cost efficient, but also reproducible, accurate and reliable [19]. Further optimization of all aspects of the separation process in TLC are HPTLC plates for quantitative determination because these new layers require smaller sample sizes and shorter development distances to reveal their separation potential and to provide faster separation and better resolution [20]. Applications of HPTLC to the quantitative analysis of drug substances in biological and formulation matrices have been reported [21].

The present work presents two new methods for simultaneous determination of benazepril HCl and hydrochlorothiazide in tablets using HPTLC-densitometry and reversed phase HPLC. The two proposed methods are simple, reduce the duration of the analysis and suitable for routine determination of the two drugs.

2. Experimental

2.1. Instrumentation

HPTLC plates (20×10 cm, aluminum plates precoated with Silica gel 60 F_{254}) were purchased from E. Merck (Darmstadt, Germany). The samples were applied to the plates using 10 μl Hamilton microsyringe. A Shimadzu dual wavelength flying spot densitometer model CS-9000 was used. The experimental conditions of measurements were $\lambda = 238$ and 275 nm for benazepril HCl and hydrochlorothiazide, respectively, photomode = reflection, scan mode = zigzag, swing width = 12.

The HPLC (Perkin-Elmer, Norwalk, Connecticut) instrument was equipped with a model series 410 LC Pump, Rheodyne 7125 injector with a 20 μl loop and a LC-235 diode array detector (Perkin-Elmer), Separation and quantitation were made on a 150×4.6 mm (i.d) Phenomenex®. Prodigy 5 μ ODS, (5 μm particle size). The detector was set at $\lambda = 240$ nm. Data acquisition was performed on a model 1022 PE Nelson (Perkin-Elmer).

2.2. Materials and reagents

Pharmaceutical grade of benazepril HCl and hydrochlorothiazide (Ciba-Geigy, Switzerland) were kindly supplied by Swisspharma®, Cairo, Egypt, and certified to contain 99.70 and 99.99%, respectively. The water for HPLC was prepared by double glass distillation and filtration through a 0.45 μm membrane filter. The acetonitrile and methanol used were HPLC grade (BDH, Poole, UK). Chloroform, ethyl acetate, glacial acetic acid and hydrochloric acid were analytical grade.

Commercial Cibadrex tablets used (Batch No. 007) were manufactured by SWISSPHARMA®, Cairo, Egypt, under license from Ciba-Geigy, Basle Switzerland. Each tablet contains 10 and 12.5 mg of benazepril hydrochloride and hydrochlorothiazide, respectively, in addition to tablet excipients consisting of hydrogenated castor oil, lactose, crospovidone, hydroxypropyl mythylcellulose, macrogol 8000, talc, titanium dioxide and red iron oxide.

2.3. Chromatographic conditions

The HPTLC plates were developed in ethyl acetate–methanol–chloroform (10:3:2 v/v) as a mobile phase. For detection and quantification, 10 μl of test and 10 μl of different concentrations of the standard solution within the quantitation range were applied as separate compact spots 15 mm apart and 10 mm from the bottom of the plate using 10 μl Hamilton microsyringe. The plate was developed up to the top (over a distance of 8 cm) in the usual ascending way. The chromatographic tank was saturated with mobile phase in the usual mode. After elution the plate was air dried and scanned at 238 nm for benazepril HCl and 275 nm for hydrochlorothiazide as under the described instrumental parameters.

The mobile phase of HPLC was prepared by mixing acetonitrile and water in a ratio of 35:65 v/v. the apparent pH was adjusted to 3.3 using glacial acetic acid (2 ml per 100-ml mobile phase). The mobile phase was filtered using a 0.45-μm membrane filter (Millipore, Milford, MA) and degassed by ultrasonic vibrations prior to use. The samples were also filtered using 0.45-μm disposable filters. The flow rate was 1.6 ml min^{-1}. All determinations were performed at ambient temperature. The injection volume was 20 μl.

2.4. Standard solutions and calibration graphs

Stock standard solution was prepared by dissolving 100 mg of benazepril HCl or 125 mg of hydrochlorothiazide in 50 ml methanol.

2.4.1. For HPTLC-densitometric method

The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 0.2–2 mg ml^{-1} for benazepril HCl and 0.25–2.5 mg ml^{-1} for hydrochlorothiazide. A total of 10 μl of each standard solution was applied to the HPTLC plate. Triplicate applications were made for each solution. The plate was developed on previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graph.

2.4.2. For HPLC method

The standard solutions were prepared by dilution of the stock solution with mobile phase to reach a concentration range 10–60 μg ml^{-1} for benazepril HCl and 12.5–75 μg ml^{-1} for hydrochlorothiazide. Triplicate 20 μl injections were made for each concentration and chromatographed under the conditions described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph.

2.5. Sample preparation

A total of 20 tablets were weighed and finely powdered. A portion of the powder equivalent to about 50 mg of benazepril HCl and 62.5 mg hydrochlorothiazide was weighed accurately, dissolved in and diluted to 50 ml with methanol. The sample solution was then filtered using 0.45-μm filters (Millipore, Milford, MA).

2.5.1. For HPTLC-densitometric

A total of 10 μl of the sample solution were applied to the HPTLC plate. The plate was developed in the previously described chromatographic conditions. The peak area of the spots were measured at 238 nm for benazepril HCl and 275 nm for hydrochlorothiazide and their concentrations in the sample were determined using multilevel calibration developed on the same plate under the same conditions, using second order polynomial regression equations.

2.5.2. For HPLC

Further dilution of the sample solution was carried out with mobile phase to provide a solution of 40 μg ml^{-1} for benazepril HCl and 50 μg ml^{-1} for hydrochlorothiazide. A 20-μl volume was injected into the HPLC, in triplicate, under the conditions described above. The peak area was used for determination of the two drugs in the sample, using the regression equation.

2.6. Percent recovery study

This study was performed by adding benazepril HCl and hydrochlorothiazide to a known concentration of the commercial tablet (standard addition method). The resulting mixtures were assayed and the results obtained were compared with expected results (Table 5).

3. Results and discussion

3.1. Assay parameters

3.1.1. HPTLC-densitometric methods

Experimental conditions, such as mobile phase, scan mode and wavelength of detection were optimized to provide accurate, precise and reproducible results for simultaneous determination of benazepril HCl and hydrochlorothiazide. The chosen scan mode was the zigzag mode and the wavelengths of scanning were chosen to be 238 nm for benazepril HCl and 275 nm for hydrochlorothiazide. The greatest differences between the R_f values of the two compounds (0.22 for benazepril HCl and 0.60 for hydrochlorothiazide), with minimum tailing were obtained by using the mobile phase consisting of ethylacetate–methanol–chloroform in ratio of 10:3:2 v/v, respectively. The separated spots of the two drugs were scanned separately on the same plate at the specified wavelengths.

The shape of calibration curves in thin layer chromatography is generally inherently non-linear due to scattering of light. Calibration curves generally comprise a pseudolinear region at low sample concentration and then departure from linearity begins at higher sample concentrations. The extent of individual ranges of the calibration curves is frequently very different for different substances. In some instances, the pseudolinear range may be adequate for most analytical purposes, in others no reasonable linear range may exist [22]. Scattering of light is highly dependent on the type of the TLC plate, measuring wavelength, measuring mode, molar absorptivity and concentration of the sample. The use of HPTLC plates is therefore advantageous since they are less scattering than conventional TLC plates [23].

Several transformation techniques have been suggested to linearize the normally non-linear calibration curve by conversion of the sample concentration and/or signal into reciprocals, logarithms or squared terms [22,24]. However errors in the original data are also transferred in the above methods leading to inhomogeneous variances in the transformed data [22]. Hence with the ready availability of computers in laboratories, there is increasing interest in applying non-linear curve fitting techniques to experimental data [25].

The Y-intercept for linear regressions of HPTLC methods are often not zero as would be

expected with HPLC methods. Unlike HPLC methods, for which linearity of detector response over a wide range of concentrations of analyte can be obtained, the calibration curve of UV detector response versus a wide range of concentrations for HPTLC often does not follow linear regression but rather polynomial regression. With HPTLC, the analyte interact with the layer surface of the stationary phase where scattering and absorption tend to take place, especially with high concentration of analyte [19]. These combined processes are not adequately described by Beer-Lambert law, but the Kubelka Munk model [26].

The relationship between the concentration of each of benazepril HCl and hydrochlorothiazide; and peak area of the spot was investigated. The linear relationship was tested and found to be unaccepted due to the presence of some curvature in the residuals (Fig. 1(a), Fig. 2(a)), indicating poor correlation. So the linear model for fitting the data can not be used. The second order polynomial fit was found to be more suitable, its residuals plot showed a much better scatter (Fig. 1(b), Fig. 2(b)) than that of linear model, indicating good correlation. The calibration graphs were constructed in the range of 2–20 μg/spot for benazepril HCl and 2.5–25 μg/spot for hydrochlorothiazide. The second order polynomial regression equations were found to be: $A = -2.60 \times 10^2 \ C^2 + 12.12 \times 10^3 \ C + 49.17 \times 10^3$ for benazepril HCl and $A = -3.30 \times 10^2 \ C^2 + 20.82 \times 10^3 \ C + 71.41 \times 10^3$ for hydrochlorothiazide, where A is the peak area of the spot and C is the concentration of drug in μg/spot. The characteristic parameters of the second order polynomial regression equation of the two drugs are shown in Table 1.

3.1.2. HPLC method

The developed HPLC method has been applied for the simultaneous determination of benazepril HCl and hydrochlorothiazide. To optimize the HPLC assay parameters, the effect of acetonitrile composition of the mobile phase on the capacity factor (K') was studied. A satisfactory separation was obtained with a mobile phase consisting of

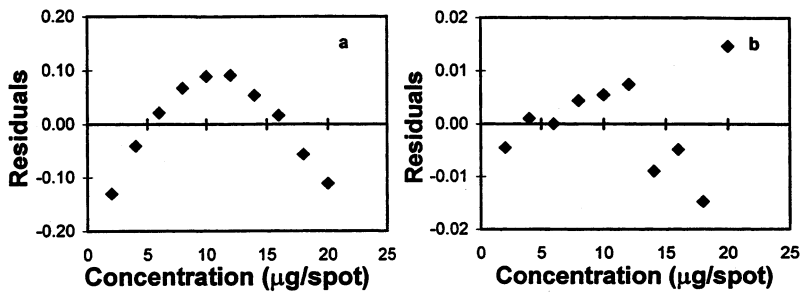

Fig. 1. Residual plot for the calibration of HPTLC method for determination of benazepril HCl using the linear fit (a) and the second order polynomial fit (b).

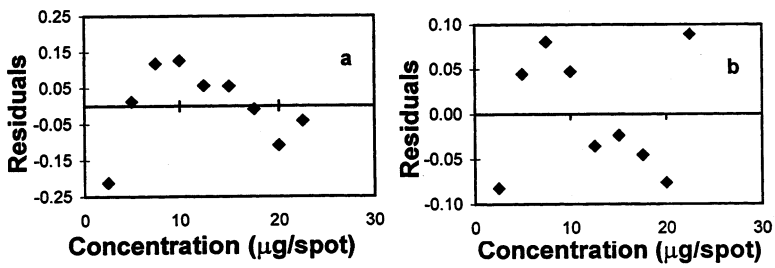

Fig. 2. Residual plot for the calibration of HPTLC method for determination of hydrochlorothiazide using the linear fit (a) and the second order polynomial fit (b).

Table 1
Characteristic parameters for the second order polynomial regression equation of the HPTLC-densitometric method

Parameters	Benazepril HCl	Hydrochlorothiazide
Linearity range (μg/spot)	2–20	2.5–25
Regression coefficient 1	-2.60×10^{2}	-3.30×10^{2}
Standard deviation of the coefficient 1	10	20
Confidence limit of the coefficient 1[a]	-2.90×10^{2} $-(-2.28\times10^{2})$	-3.77×10^{2} $-(-2.80\times10^{2})$
Regression coefficient 2	12.12×10^{3}	20.82×10^{3}
Standard deviation of the coefficient 2	2.4×10^{2}	3.6×10^{2}
Confidence limit of the coefficient 2[a]	11.08×10^{3} -12.80×10^{3}	19.48×10^{3} -22.16×10^{3}
Intercept	49.17×10^{3}	71.41×10^{3}
Standard deviation of the intercept	11.4×10^{2}	34.0×10^{2}
Confidence limit of the intercept[a]	45.62×10^{3} -52.85×10^{3}	63.37×10^{3} -79.44×10^{3}
Correlation coefficient	0.9997	0.9998
Standard error of estimation	0.01	0.03

[a] 95% confidence limit.

acetonitrile–water mixture (35:65, v/v). Increasing acetonitrile concentration to $>60\%$ led to inadequate separation of the two drugs. At lower acetonitrile concentration, separation occurred but with excessive tailing and increased retention time for benazepril HCl peak. Variation of apparent pH of the mobile phase resulted in maximum k' value at apparent pH 6.5, with loss of peak symmetry for benazepril HCl peak. At apparent pH 3–4 improved resolution for the two drugs was observed, however at apparent pH 3.3 optimum resolution with reasonable retention time was affected.

The specificity of the HPLC method is illustrated in Fig. 3 where complete separation of benazepril HCl and hydrochlorothiazide was noticed. The average retention time $\pm$ standard deviation for benazepril HCl and hydrochlorothiazide were found to be 4.3 ± 0.022 and 1.6 ± 0.013 min, respectively, for 10 replicates. The peaks obtained were sharp and have clear base line separation.

To determine the linearity of HPLC detector response, calibration standard solutions of benazepril HCl and hydrochlorothiazide were prepared as described in the text. Linear correlation was obtained between peak area versus concentration of each drug.

Characteristic parameters for regression equations of the HPLC method and correlation coefficient obtained by least squares treatment of the results were given in Table 2.

3.2. Accuracy of the proposed methods

The accuracy of the two proposed methods were checked by analyzing five laboratory-prepared mixtures of benazepril HCl and hydrochlorothiazide at various concentration ratios (Tables 3 and 4). Satisfactory recoveries with small relative standard deviations (RSD) were obtained, which indicated the high repeatability and accuracy of the two methods.

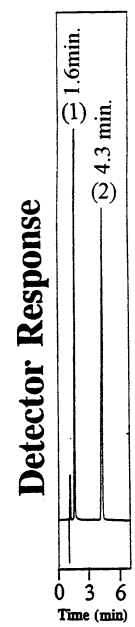

Fig. 3. A typical HPLC chromatogram of 20 μl injection of tablet solution of 40 μg ml^{-1} of benazepril HCl (2) and 50 μg ml^{-1} of hydrochlorothiazide (1).

Table 2
Characteristic parameters for the regression equation of HPLC method

Parameters	Benazepril HCl	Hydrochlorothiazide
Linearity range (μg ml^{-1})	10–60	12.5–75
Regression equation (Y)[a]		
Slope (b)	64.02×10^4	60.56×10^4
Standard deviation of the slope (S_b)	1.26×10^3	2.85×10^3
Relative standard deviation of the slope (%)	0.20	0.47
Confidence limit of the slope[b]	63.70×10^4 -64.34×10^4	59.77×10^4 -61.35×10^4
Intercept (a)	1.00×10^2	1.36×10^4
Standard deviation of the intercept (S_a)	4.54×10^4	13.88×10^4
Confidence limit of the intercept[b]	11.69×10^4 $-(-11.67\times10^4)$	39.91×10^4 $-(-37.19\times10^4)$
Correlation coefficient (r)	0.9999	0.9999
Standard error of estimation	6.59×10^4	1.49×10^5

[a] $Y=a+bC$, where C is the concentration of drug in μg ml^{-1} and Y is the peak area.
[b] 95% confidence limit.

The stability of benazepril HCl and hydrochlorothiazide during the analytical procedures of HPTLC and HPLC methods were studied and found to be stable.

The inter-day relative standard deviation were calculated at different concentration levels of benazepril HCl and hydrochlorothiazide for each method and found to be 0.75, 0.81% ($n=6$) for HPTLC method and 0.44, 0.49% ($n=6$) for HPLC method, respectively.

The non-instrumental methods for determination of the detection limit and the quantitation limit were applied [8], the limit of detection is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. While the limit of quantitation is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision. The detection limits of the two proposed methods were found to be 0.3 and 0.8 μg ml^{-1} for benazepril HCl; 0.4 and 0.9 μg ml^{-1} for hydrochlorothiazide, detected by HPLC and HPTLC methods, respectively. The relative sensitivity, based on detection limit, was calculated. The HPLC method was found to be more sensitive than the HPTLC method.

Table 3
Determination of benazepril HCl (I) and hydrochlorothiazide (II) in laboratory prepared mixtures using HPTLC-densitometric method

Mixture no.	Concentration (μg/spot)				% Recovery	
	Added		Found			
	I	II	I	II	I	II
1	10.0	12.5	10.01	12.56	100.1	100.5
2	10.0	10.0	9.90	10.10	99.0	101.0
3	10.0	15.0	9.97	15.12	99.7	100.8
4	20.0	5.0	20.08	4.97	100.4	99.5
5	4.0	20.0	4.02	19.80	100.6	99.0
Mean					100.0	100.2
RSD (%)					0.64	0.87

Table 4
Determination of benazepril HCl (I) and hydrochlorothiazide (II) in laboratory prepared mixtures using HPLC method

Mixture no.	Concentration (μg ml^{-1})				% Recovery	
	Added		Found			
	I	II	I	II	I	II
1	40	50	39.84	50.2	99.6	100.4
2	40	40	39.72	39.88	99.3	99.7
3	40	60	39.96	59.82	99.9	99.7
4	60	15	60.18	15.15	100.3	101.0
5	12	60	12.12	60.30	101.0	100.5
Mean					100.0	100.3
RSD (%)					0.66	0.56

3.3. Tablet analysis

The two proposed methods were applied to the determination of benazepril HCl and hydrochlorothiazide in commercial tablets of Cibadrex. Five replicates determinations were made. Satisfactory results were obtained for both drugs and were in a good agreement with the label claims (Table 5). Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding benazepril HCl and hydrochlorothiazide to the previously analysed tablets. The recovery of each drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analysis of the commercial tablets and the recovery study (standard addition method) of both drugs (Table 5) suggested that there is no interference from any excipients, which are normally present in tablets.

The results of determination of benazepril HCl and hydrochlorothiazide in tablets obtained from the HPTLC method were compared with those of the HPLC method. Statistical comparison of the results was performed with regard to accuracy and precision using Student's t-test and the F ratio at 95% confidence level (Table 5). There is no significant difference between the two methods with regard to accuracy and precision.

4. Conclusion

The proposed HPTLC and HPLC methods provide simple, accurate and reproducible quantitative analysis for simultaneous determination of benazepril HCl and hydrochlorothiazide in tablets. The HPLC method was found to be more

Table 5
Determination of benazepril HCl and hydrochlorothiazide in commercial tablets by the two proposed methods

	Mean found ± S.D.[a]	
	HPTLC	HPLC
Commercial tablets		
For benazepril HCl	100.4 ± 0.56	100.5 ± 0.73
	$t^b = 0.24$	
	$F^b = 1.69$	
For hydrochlorothiazide	99.7 ± 0.75	99.6 ± 0.71
	$t^b = 0.22$	
	$F^b = 1.12$	
Recovery[c]		
For benazepril HCl	100.1 ± 0.61	99.9 ± 0.23
For hydrochlorothiazide	100.0 ± 0.75	100.2 ± 0.68

[a] Mean and S.D. for five determinations; percentage recovery from the label claim amount.

[b] The theoretical t- and F-values are equal to 2.31 and 6.39, respectively.

[c] For standard addition of 50% of the nominal content ($n = 5$).

sensitive than the HPTLC method. While HPTLC method has the advantages of short run time, large sample capacity and use minimal volume of solvent. Both of HPTLC and HPLC methods reduce the duration of the analysis and appears to be equally suitable for routine determination of benazepril HCl and hydrochlorothiazide in their formulation.

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