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A validated method for the determination and purity evaluation of benazepril hydrochloride in bulk and in pharmaceutical dosage forms by liquid chromatography☆

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Abstract

A gradient liquid chromatographic (LC) method has been developed for the determination and purity evaluation of benazepril hydrochloride in bulk and pharmaceutical dosage forms. The method is simple, rapid and selective. 5-Methyl-2-nitro phenol has been used as internal standard. The method is linear in the range of 50–800 μg. The precision for inter and intra-day assay variation of benazepril hydrochloride is below 1.6% RSD. The accuracy determined as relative mean error (RME) for the intra-day assay is within ± 2.0%. The method is stability indicating, and is useful in the quality control of bulk manufacturing and also in pharmaceutical formulations.

Keywords: Benazepril HCl; Related compounds; Gradient mode; Purity; Assay; 5-Methyl-2-nitro phenol; HPLC

1. Introduction

Benazepril hydrochloride is an angiotensin converting enzyme (ACE) inhibitor with the official chemical name 3-[[1-(ethoxycarbonyl)-3-phenyl-(1S) propyl] amino]-2,3,4,5-tetrahydro-2-oxo-1-(3S)-benzazepin-1-acetic acid hydrochloride (I) (Fig. 1), and is used in the treatment of hypertension.

Only a few liquid chromatographic (LC) methods have been reported for the determination of benazepril and its pro-drug benazeprilat [1–5]. Furthermore, the determination of benazepril in dosage forms by UV detection [1], a semi-quantitative procedure for benazepril and benazeprilat in plasma and urine by radiometric detection [2], determination of benazepril in pharmaceutical dosage forms by derivative UV spectroscopy and by ion-pair LC [3] or by photo diode array detection [4] have been described. These LC methods can be used for the determination of benazepril in pharmaceutical formulations and biological fluids, but so far no data on specificity of the methods with respect to impurities is available.

The purpose of this paper is to describe a procedure for the separation and quantification of benazepril and benazepril-related compounds, by-

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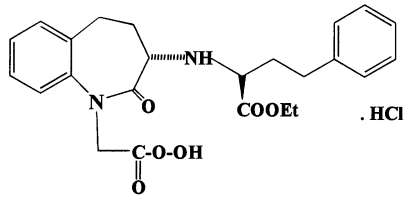
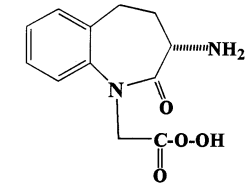
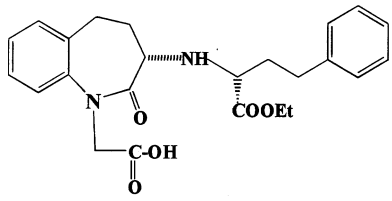
products formed during synthesis or degradation products. The chemical structures of these compounds are listed in Fig. 1. For the purity evaluation and quantitative determination of benazepril, a gradient liquid chromatographic method has been used. The chromatographic method involves a linear gradient programme wherein the strength of solvent A is 100% for first 15 min after which linear gradient programme starts. The initial 15-min run serves the purpose of quantitative determination of benazepril while the entire gradient programme is useful for the determination and



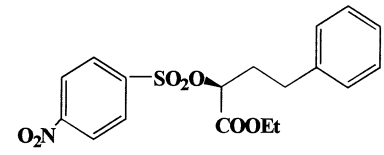
Benazepril HCl (**I**)



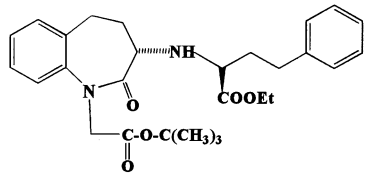
1-ter-butyloxy carbonyl methyl-3(S)-amino-2,3,4,5-tetrahydro-1H-[1]-benzazepin-2-one (**V**)



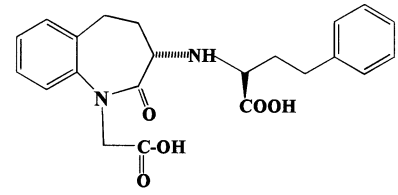
Benazepril isomer (**II**)



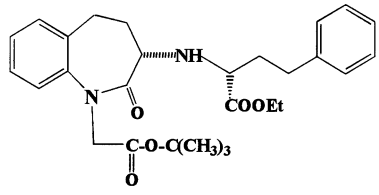
Ethyl (+) R-2-(4 nitrobenzene sulfonyloxy) -4-phenylbutyrate (**VI**)



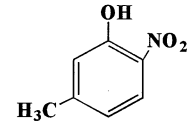
Benazepril prefinal (**III**)



Benazeprilat (**VII**)



Isomer of Benazepril pre-final (**IV**)



5-Methyl 2-Nitro Phenol (**Internal standard**)

Fig. 1. Structures of benazepril HCl and its related compounds including internal standard. (II and **IV** are by-products; **VII** is degradation product; **III**, **V** and **VI** are intermediates).

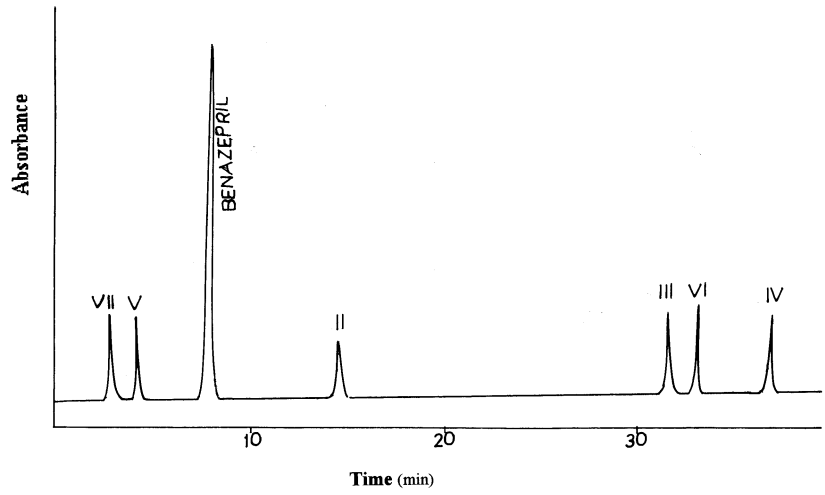


Fig. 2. HPLC chromatogram of benazepril and its related compounds.

Table 1
System suitability report

Compound	Capacity factor[a]	Selectivity[a]	Tailing factor[a]	Resolution[a]	Theoretical plates[a]
I	1.8	2.8	1.2	9.9	10 546
II	3.9	2.2	1.1	12.9	12 114
III	10.7	2.8	1.2	44.1	169 260
IV	12.5	1.1	1.1	8.1	127 050
V	0.7	4.0	1.4	5.2	4474
VI	11.6	1.1	1.3	7.6	284 128
VII	0.2	–	1.8	1.9	2645

[a] Number of samples analysed is three.

quantification of impurities present in it. 5-Methyl-2-nitro phenol was used as an internal standard for the quantitative determination of benazepril. This method can also be used to determine benazepril in pharmaceutical dosage forms. Finally the method has been validated for the determination of benazepril HCl.

2. Experimental

2.1. Chemicals

Samples of benazepril and its impurities were received from Process R&D division of Dr. Reddy's Research Foundation, Hyderabad, India. HPLC grade acetonitrile and methanol were obtained from Merck, USA. Analytical reagent grade sodium dihydrogenphosphate was obtained from Pro analysi, E Merck (India). Orthophosphoric acid was obtained from Loba Chemie, India. Tablets of Lotensin (40 mg) were procured from Ciba-Geigy. High purity water was prepared by using Waters Milli-Q plus purification system.

2.2. Equipment

The LC system consisted of a Perkin-Elmer Series 200 lc pump, a Rheodyne injector equipped with a 20 μl sample loop, and a SPD M6A Photodiode detector (Shimadzu.) The Waters HPLC system LC Module I Plus consisted of a pump and an UV-Visible tunable variable wavelength detector and was used in laboratory B.

Analysis was carried out at ambient temperature (27°C) using a Hichrom RPB C18, 5 μm particle size, 250 × 4.6 mm (Hichrom make). The output signal was monitored and integrated using CLASS 10A (Shimadzu) software in lab A and Millennium 2010 Chromatography Manager software (Waters) in lab B.

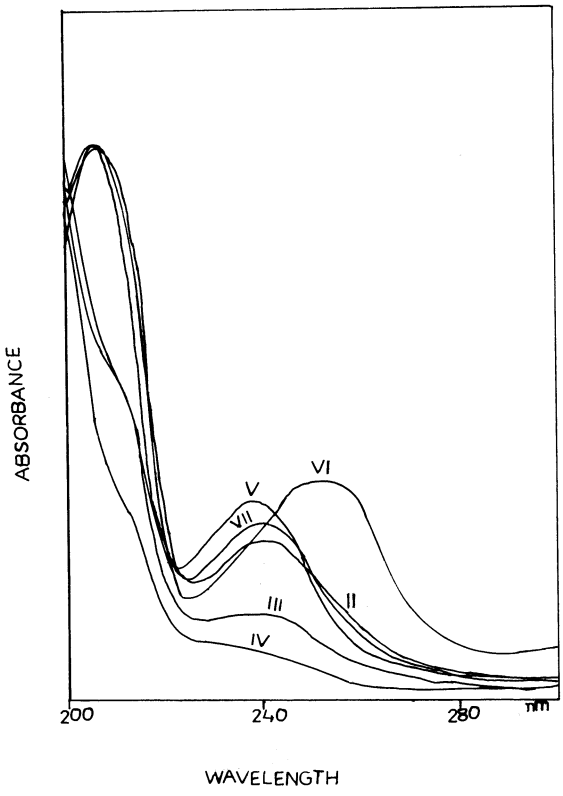


Fig. 3. Absorption spectra of benazepril related compounds.

Table 2
RRF values of benazepril related compounds

Compound	RRF[a]
Benazepril HCl	1.000
II	1.000
III	0.992
IV	0.990
V	1.050
VI	0.630
VII	0.985

[a] Relative response factor.

2.3. Sample preparation

Sample solutions of impurities and benazepril were prepared with solvent A of the eluent.

The concentration of the Internal standard was 500 μg/ml in each solution of benazepril and was used for validation studies.

2.4. Preparation of solvent A

Five hundred millilitres of aqueous 0.025 M sodium dihydrogenphophate buffer, adjusted pH to 2.8 with concentrated orthophospharic acid was mixed with 300 ml of acetonitrile and 200 ml of methanol. After mixing, the solvent was filtered and degassed through 0.45 μ Nylon filter using vacuum pump.

2.5. Preparation of solvent B

Degassed and filtered acetonitrile was used as solvent B.

2.6. Chromatographic condition

A linear gradient programme was used for determination of the purity and the assay of Benazepril. The ratio of solvents (A:B) was kept at 100:0 for the first 15 min and subsequently linearly decreased to 60:40 during 15 min and maintained at this ratio for another 15 min. The flow rate was kept constant at 1.2 ml/min. The detection was performed at 210 nm. A Hichrom RPB C18 (250 × 4.6 mm, 5 μm) column was used.

3. Results and discussion

3.1. Separation of impurities

To develop a suitable and robust LC method for the determination of benazepril and its impurities, different mobile phases and columns were employed to achieve the best separation and resolution. Initial reversed-phase (RP) separations indicated that all the impurities could not be separated adequately in a reasonable amount of time using isocratic conditions. This is consistent

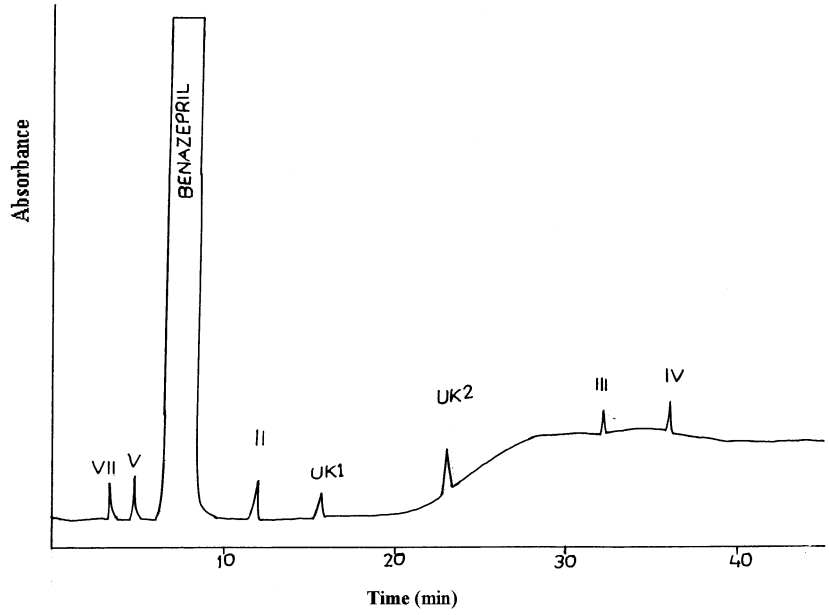


Fig. 4. Chromatogram of benazepril HCl using High–Low chromatography technique.

with the wide range of polarities of the various impurities (Fig. 1). Using gradient RP-LC, base line separation between all impurity pairs was obtained in a short chromatographic run. The separation of a sample containing all impurities as well as Benazepril is shown in Fig. 2. System suitability results of the method are presented in Table 1.

3.2. Quantification of impurities

Absorption spectra of the Benazepril impurities are shown in Fig. 3. UV detection was carried out at 210 nm for the quantification of the impurities and benazepril. Relative response factors (RRFs) were determined to allow quantification of the impurities [6]. RRF is the ratio of the peak response per unit concentration for the each impurity to the peak response per unit concentration for the reference compound (benazepril) under the given analytical conditions. The RRF values of benazepril related compounds are given in Table 2. For the quantification of impurities a High-Low chromatographic [7] technique was used. In this technique a concentrated (3.5 mg/ml) sample of benazepril was injected and the response of each impurity was recorded. A typical chromatogram of High-Low chromatography of benazepril is shown in Fig. 4. The impurities can be identified by matching the UV spectrum and the retention times with standards. Weight percentages of each impurity present in benazepril sample (in 3.5 mg/ml) were calculated using its peak response and RRF values. The impurities namely UK1 and UK2 eluted at retention times 15 and 22 min, respectively, were not identified (Fig. 4). UV absorption spectra of these impurities shown in Fig. 5, were similar to that of II and III, respectively. Hence the RRF of II and III were used for calculating weight percentages of UK1 and UK2 present in the benazepril sample.

3.3. Standard addition and recovery of impurities

Standard addition and recovery experiments were conducted to determine the accuracy of the present method for the quantification of impurities. As per ICH guide lines the range of addition levels of impurities used in this study is 0.2–1.0% of the concentration (0.5 mg/ml) of the benazepril and the recovery of each impurity added to the benazepril sample was calculated from the slope

and intercept of the calibration graph drawn in the concentration range 0.25–10 μg (0.05–2%) [8]. The equation for calibration curve is $y = bx + a$. The values of slope, intercept and correlation coefficient for calibration plot of each impurity were given in Table 3. The intercept values were found to be less than the 10% of area response produced by 2% concentration level [9]. The mean recovery of all the impurities were found to be in the range of 94–105%. The recovery data is presented in Table 4.

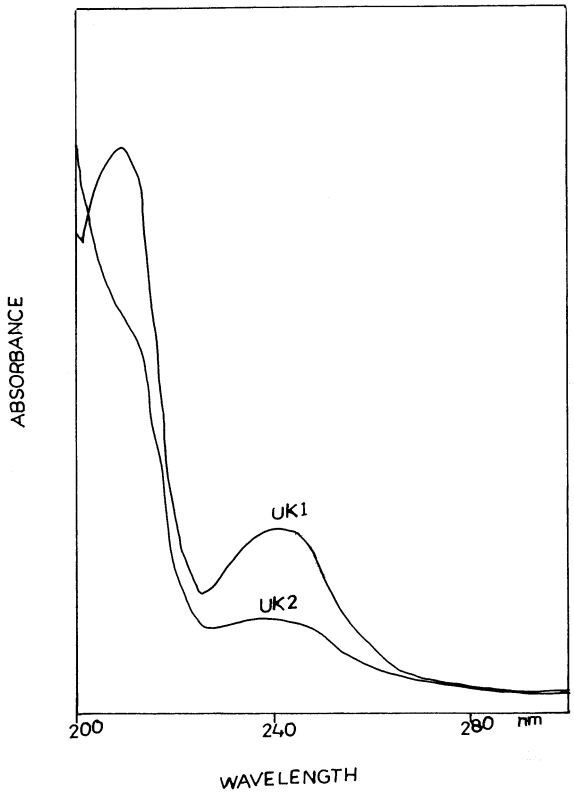


Fig. 5. Absorption spectra of unknown impurities UK1 and UK2 using PDA detection.

Table 3
Parameters of calibration plot for impurities

Impurity	Slope	Intercept	Correlation coefficient
II	29 527 ± 1089	−2751.8 ± 252	0.998
III	28 784 ± 1233	2179.5 ± 193	0.997
IV	23 251 ± 989	−819.7 ± 089	0.995
V	23 751 ± 1672	1607.9 ± 202	0.990
VI	25 989 ± 1134	6316.9 ± 755	0.989
VII	27 684 ± 1205	12 551.0 ± 1583	0.993

Table 4
Recovery of benazepril related compounds

Compound	Added (μg) ($n = 3$)	Recovered (μg)	% of recovery
II	1.57	1.48	94.2
	2.75	2.71	98.5
	3.93	4.07	103.6
III	1.43	1.38	96.7
	2.50	2.58	103.3
	3.58	3.75	104.7
IV	1.39	1.36	96.8
	2.43	2.31	95.1
	3.48	3.42	98.2
V	1.52	1.59	104.5
	2.66	2.64	99.3
	3.8	3.68	96.8
VI	1.62	1.70	105.0
	2.84	2.92	102.8
	4.05	3.99	98.5
VII	1.35	1.27	94.5
	2.36	2.38	100.7
	3.37	3.43	101.8

3.4. Assay determination of benazepril

Benazepril can be determined with 5-methyl 2-nitro phenol (Fig. 1), as the internal standard with UV detection at 210 nm. The absorption spectra of both benazepril and the internal standard are shown in Fig. 6. Using the following parameters the assay method was extensively validated.

3.4.1. Specificity

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components.

All the possible known impurities discussed above were added to pure benazepril and the mixture was analysed and the results were compared with the analysis of pure benazepril sample. Reproducible results were observed in both cases (Table 5).

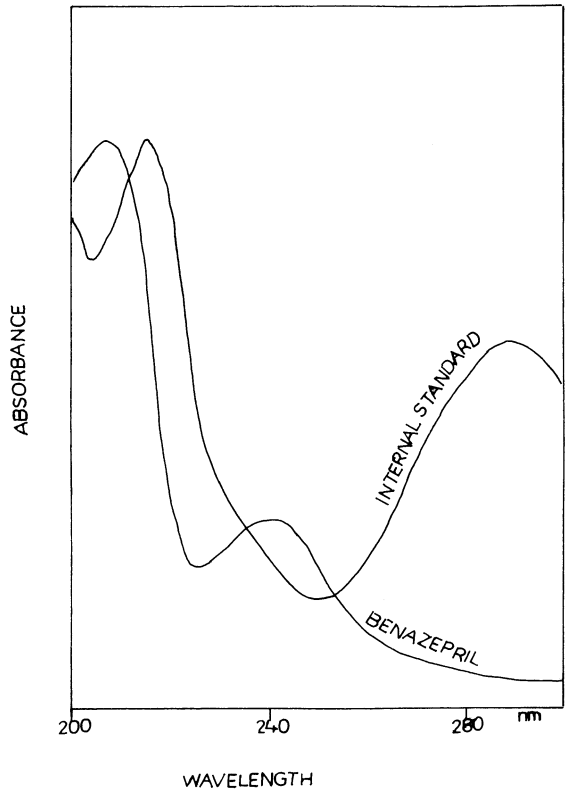


Fig. 6. Absorption spectra of benazepril HCl and internal standard (5-methyl 2-nitro phenol).

Table 5
Test for specificity of the method

Sample spiking with all the impurities	Pure sample
98.79	100.05
99.15	99.72
98.91	98.85
Mean: 98.95	Mean: 99.54
S.D.: 0.183	S.D.: 0.62
RSD: 0.185%	RSD: 0.62%

3.4.1.1. Assay determination of benazepril from lotensin tablets. The specificity of the method was also checked for a dosage form of benazepril. Therefore 20 Lotensin (40 mg) tablets were extracted into methanol and centrifuged. The supernatant was diluted with mobile phase to required concentrations and analysed. The results of the extracted samples at four different concentrations were ($99 \pm 1.5\%$) comparable with the claimed values. Benazepril and the internal standard were well separated from the exceipient peaks in the formulation sample. A typical LC chromatogram is shown in Fig. 7.

3.4.2. Linearity

Linearity was checked by preparing standard solutions at seven different concentration levels ranging from 50 to 800 μg per ml. The linearity was also checked for three consecutive days for the solutions of same concentrations prepared from the stock solution. The precision for inter day linearity is below 1.5% RSD. The equation for calibration curve is $y = 4.97x + 0.001$. The RSD values for the slope and intercept are 1.36 and 36.2%, respectively. The correlation coefficient was found to be more than 0.999, indicating good linearity.

3.4.3. Accuracy

The accuracy of the method was checked at three concentration levels i.e. at 250, 400 and 600 μg each in triplicate. The assay accuracy was tested for 3 days at the same concentration levels in triplicate. Solutions for the standard curves were prepared fresh every each day. The assay accuracy variation shown in terms of relative mean error (RME), total error (TE) and % recovery are tabulated in Table 6 [10]. The RME and TE values are below ± 2.0 for the intra-day assay experiments.

3.4.4. Precision

The assay precision was studied for the parameters viz. repeatability, intermediate precision and reproducibility (ruggedness).

Repeatability is the intra-day variations in assay values obtained at different concentration levels of benazepril and are expressed in terms of

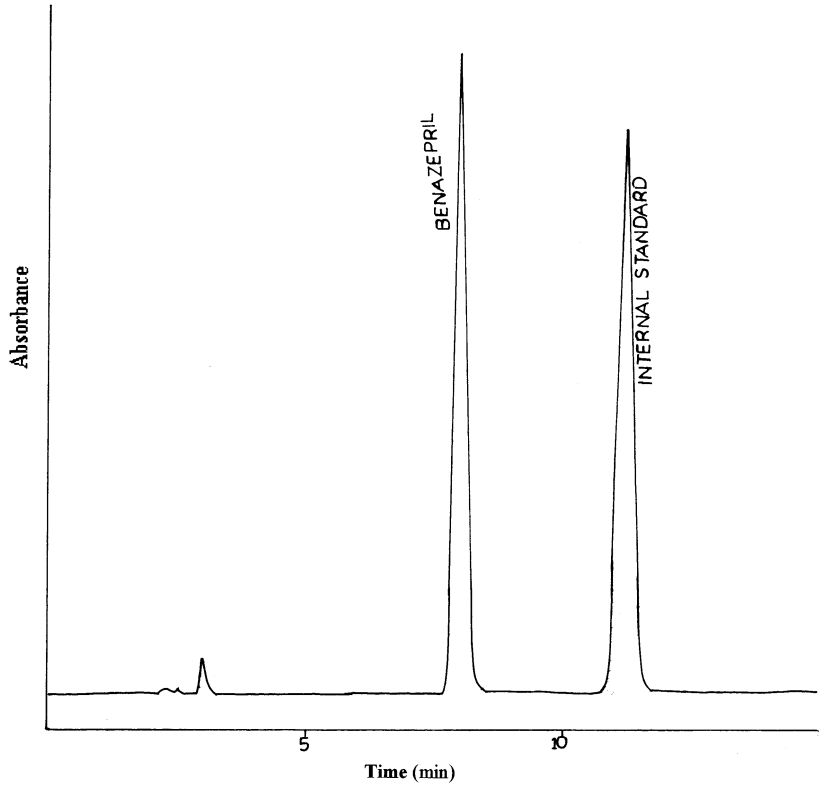


Fig. 7. Chromatogram of formulated benazepril (lotensin 40 mg) with internal standard.

Table 6
Accuracy in the assay determination of benazepril HCl[a]

Day of analysis	Taken (mg)	Recovery (mg) ($n = 3$)	% Recovery	RME (%)	TE (%)
0 day	0.246	0.247	100.4	0.4	1.1
	0.431	0.437	101.4	1.4	1.7
	0.616	0.625	101.5	1.5	2.0
1 day	0.246	0.2415	98.2	−1.8	−1.5
	0.431	0.434	100.7	0.8	0.9
	0.616	0.611	99.2	−0.8	−0.8
2 day	0.246	0.2413	98.1	−1.9	−1.7
	0.431	0.424	98.4	−1.8	−1.1
	0.616	0.6086	98.8	−1.3	−0.8

[a] % Recovery = % recovery of benazepril from the sample against taken. RME, relative mean error; TE, total error. RME = {(MEAN − TCONC)/TCONC}*100. TE = {2S.D. + (MEAN − TCONC)/TCONC}*100, in which S.D. is the standard deviation of the mean of triplicate of each concentration level and TCONC the theoretical concentration of the analyte of each level.

RSD values calculated from the data of each day of three days i.e. 0, 1, 2 days. RSD values were found to be well below 2.0%, indicating a good repeatability (Table 7).

The intermediate precision is the inter-day variation at the same concentration level, is determined on successive days. The inter-day variations calculated for each concentration level from three days data are expressed in terms of RSD values. At each concentration level the RSD values were well below 2% indicates a good intermediate precision (Table 7).

The ruggedness of an assay method is defined as the degree of reproducibility of the results obtained by analysis of the same sample under a variety of normal test conditions such as different labs, different analysts, different instruments and different lots of reagents. The same samples of three concentration levels in triplicate of day 2 were analysed at laboratory B with a different instrument (LC Module I plus HPLC system containing Pump and a UV-Visible detector) by a different analyst with different lots of reagents. The data obtained from laboratory B were within 2% RSD when compared with the data of parent lab.

Table 7
Inter and intra-day assay variation of benazepril HCl

Intra-day			
0 Day			
Mean of conc. (mg/ml) $n=3$	0.247	0.437	0.625
S.D.	0.0036	0.00173	0.003
RSD (%)	1.46	0.40	0.48
1 Day			
Mean of conc. ($n=3$)	0.245	0.434	0.611
S.D.	0.00153	0.0007	0.0081
RSD (%)	0.64	0.16	0.61
2 Day			
Mean of conc. ($n=3$)	0.2413	0.424	0.6086
S.D.	0.001154	0.0040	0.0023
RSD (%)	0.48	0.95	0.38
Inter-day			
Mean (of mean conc. of 3 days)	0.2444	0.4316	0.6148
S.D.	0.00289	0.0068	0.0088
RSD (%)	1.18	1.58	1.44

3.4.5. Stability

In routine testing in which many samples are prepared and analysed every day, it is essential that solutions are stable enough to allow for delays such as instrument breakdowns or overnight analysis using auto samplers. The stability of the benazepril in solution containing the mobile phase and the internal standard was determined for the samples stored in refrigerator and at room temperature. The samples were checked after three successive days of storage and the data were compared with freshly prepared samples. In each case the RSD values of assay were found to be below 2.0%. This indicates that the benazepril is stable in the solution and compatible with internal standard.

4. Conclusion

An analytical LC method developed and validated for the purity and quantitative determination of benazepril in bulk and as well as in a pharmaceutical dosage form has been described. The developed method has found to be selective, sensitive, precise and stability indicating for the quantification of benazepril and its impurities. This method can be employed for purity and assay monitoring in the bulk manufacturing.

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