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Spectrophotometric methods for the determination of benazepril hydrochloride in its single and multi-component dosage forms

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Abstract

Three sensitive and accurate methods are presented for the determination of benazepril in its dosage forms. The first method uses derivative spectrophotometry to resolve the interference due to formulation matrix. The second method depends on the color formed by the reaction of the drug with bromocresol green (BCG). The third one utilizes the reaction of benazepril, after alkaline hydrolysis, with 3-merthylbenzothialozone (MBTH) hydrazone where the produced color is measured at 593 nm. The latter method was extended to develop a stability-indicating method for this drug. Moreover, the derivative method was applied for the determination of benazepril in its combination with hydrochlorothiazide. The proposed methods were applied for the analysis of benazepril in the pure form and in tablets. The coefficient of variation was less than 2%.

Keywords: Benazepril hydrochloride; Spectrophotometry; Hydrochlorothiazide

1. Introduction

Benazepril hydrochloride (BN.HCl), {(3S)-3-[(2S)-1-Ethoxycarbonyl-3-phenylpropylamino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepin-1-yl} acetic acid hydrochloride, is a potent angiotensin converting enzyme inhibitor that is used in the treatment of mild and moderate essential and renovascular hypertension [1]. The literature reveals only few papers concerning the determination of BN.HCl in plasma and urine using capillary gas chromatography [2], an electron capture gas chromatographic technique [3] and a HPLC for its determination in pharmaceuticals [4]. However, until now, no spectrophotometric procedure has been reported for its determination in its dosage forms.

Derivative spectrophotometry offers enhancement of the qualitative features, thus increasing the finger printing utility of UV spectrophotometry for the identification of organic compounds [5]. The selectivity of derivative spectrophotometry has been used in pharmaceutical analysis to assay drugs with poorly developed maxima or

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when spectral overlapping existed from the excipients or other active ingredients in dosage forms [6–8].

Many pharmaceutical compounds have been determined by the formation of an ion-pair complex [9,10]. This technique depends on the reaction of a drug that has a basic cationic nitrogen and an anionic dye at a suitable pH, where a highly colored ion-pair complex is formed.

3-methylbenzothiazolone hydrazone (MBTH) has been used as a chromogenic reagent for qualitative and quantitative determination of aromatic amines and amino compounds [11]. MBTH was used for the determination of cholesterol in serum [12], tricyclic antidepressants [13] and benzodiazepines [14].

The aim of this work was the development of simple and sensitive analytical methods for the assay of BN.HCl dosage forms by the use of derivative spectrophotometry and utilizing its reaction with bromocresol green (BCG) and MBTH. Moreover, the MBTH methods was used for stability studies of the drug. Furthermore, the derivative technique was adopted to develop a method for the determination of BN.HCl in combination with hydrochlorothiazide (HCT) in dosage forms.

2. Experimental

2.1. Apparatus

A Perkin-Elmer model 550S UV-VIS spectrophotometer with 1 cm matched quartz cells and a Hitachi model 560 recorder was used.

2.1.1. Materials and reagents

All materials used were analytical reagent grade. BN.HCl and HCT were of pharmaceutical grade. Cibacin tablets (Ciba, Egypt) were labelled to contain 10 mg BN.HCl per tablet. Cibadrex tablets (Ciba, Switzerland) were labelled to contain 20 mg BN.HCl and 25 mg HCT per tablet. Bromocresol green (BCG) 0.1% w/v was prepared in distilled water; Mcllvaine buffer solution: citric acid and di-sodium hydrogen phosphate pH 2.2 was used.

MBTH 0.2% w/v was prepared in 0.1 N HCl; Ferric chloride hexahydrate reagent ($FeCl_3$) 1% w/v was prepared in 0.1 N HCl.

2.1.2. Reference drug solutions

(a) Solutions for derivative and BCG methods: Stock solution of BN.HCl containing 0.1 mg ml^{-1} was prepared in distilled water.

(b) Solution for MBTH method: $\sim$40 mg of BN.HCl were accurately weighed, transferred into 50-ml volumetric flask and dissolved in a minimum amount of distilled water. Subsequently, 15 ml of 10 N NaOH solution was added and heated in boiling water bath for 75 min. After cooling, 15 ml 10 N HCl solution was added and the volume was completed with distilled water.

(c) Solutions for mixture analysis: $\sim$80 mg of BN.HCl was weighed and dissolved in 100 ml of 0.1 N HCl. Into another 100-ml volumetric flask, $\sim$10 mg of HCT was transferred and dissolved in methanol.

2.1.3. Sample solutions

(a) Single-component dosage form: An accurately weighed amount of powdered Cibacin tablets, equivalent to 10 mg BN.HCl, was transferred into a 25-ml volumetric flask using distilled water. The contents of the flask was shaken for $\sim$45 min, and the volume was completed. The contents were mixed and filtered.

With the MBTH method, the coat of the tablets was firstly removed and a quantity of the powdered tablets, equivalent to 10 mg BN.HCl, was transferred into a 50-ml volumetric flask using 10 ml of distilled water. The contents of the flask was shaken for 45 min and filtered. The filtrate was subjected to the same procedure as for the MBTH method.

(b) Two-component dosage form: 20 Cibadrex tablets were weighed and finely powdered. For BN.HCl content: A quantity of the tablet powder, equivalent to 20 mg BN.HCl, was transferred into a 25-ml volumetric flask using 0.1 N HCl. The contents of the flask was shaken for 30 min, the volume was completed, mixed and filtered.

For HCT content: A quantity of the powder, equivalent to 10 mg HCT, was transferred into a 50-ml volumetric flask using methanol. The con-

tents of the flask were shaken for 20 min, the volume was completed, mixed and filtered.

2.1.4. Drug assay

2.1.4.1. Derivative spectrophotometric method. Different aliquots of the standard solution or tablet extract, within the range shown in Table 1, were transferred into 25-ml volumetric flasks and diluted to volume with 0.1 N HCl solution. The D_1 and D_2 values were recorded at the specified wavelengths (Table 1).

2.1.5. BCG method

Various portions of the standard solution or tablet extract, within the range shown in Table 1, were transferred into 60-ml separating funnels. 5 ml of Mcllvaine buffer solution (pH 2.2) and 4 ml of BCG reagent were added. The contents of each separator were mixed and the produced color was extracted with chloroform (3 × 3 ml). The chloroformic extract was transferred into 10-ml volumetric flask and the volume was completed with chloroform. The absorbance of the solution was measured at 412 nm against a reagent blank.

2.1.6. MBTH method

Different volumes of standard solution or tablet extract, within the range cited in Table 1, were transferred into 10-ml volumetric flasks and 1 ml each of ethanol, MBTH and $FeCl_3$ solutions were added. The contents were mixed and completed to 10 ml using ethanol. The absorbance was measured against a reagent blank at 593 nm.

2.1.7. BN.HCl–HCT binary mixture

For BN.HCl content: Various aliquots of standard solution or tablet extract, within the specified range (Table 1), were transferred into 25-ml volumetric flasks and diluted to volume with 0.1 N HCl. The D_2 values were measured at 243 nm.

For HCT content: Different volumes of standard solution or tablet extract, within the specified range (Table 1), were transferred into 10-ml volumetric flasks and completed to volumes with methanol. The D_1 values were measured at 326 nm.

3. Results and discussion

3.1. Derivative method

The direct A_{max} method for the analysis of drugs in dosage forms is often subjected to spectral interference from formulation matrix. Such a problem becomes serious in the assay of tablets of weakly absorbing compounds as BN.HCl, which are formulated at a relatively low dosage level. The drug shows a relatively low absorption in the UV region with no characteristic bands. On the other hand, the D_1 and D_2 spectra in 0.1 N HCl (Fig. 1) showed characteristic and distinct maxima at 240 and 244 nm, respectively. These two analytical wavelengths were selected for the analysis of BN.HCl in its dosage forms on the basis of reasonable sensitivity and reproducibility. Moreover, the ratios of the D_2 maxima (D_2 222 nm/D_2 244 nm) of solutions of seven different concentrations of BN.HCl (Table 1) were calculated. They were independent of concentration and were reasonably reproducible ($1.56 \pm 8.7 \times 10^{-3}$). These ratios were used for the detection of the presence of interference [15].

3.2. BCG method

Containing a basic cationic nitrogen, BN.HCl reacts with BCG, an acid dye, to form a colored

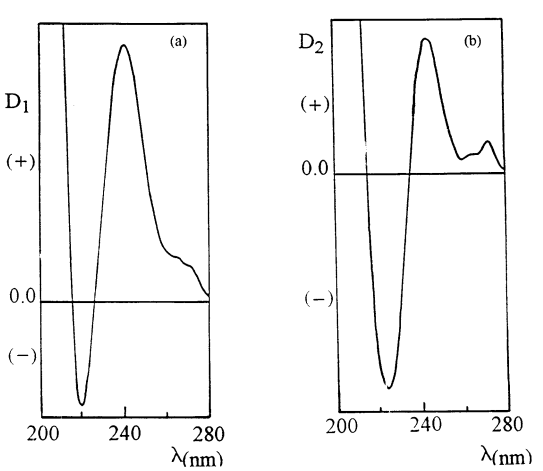
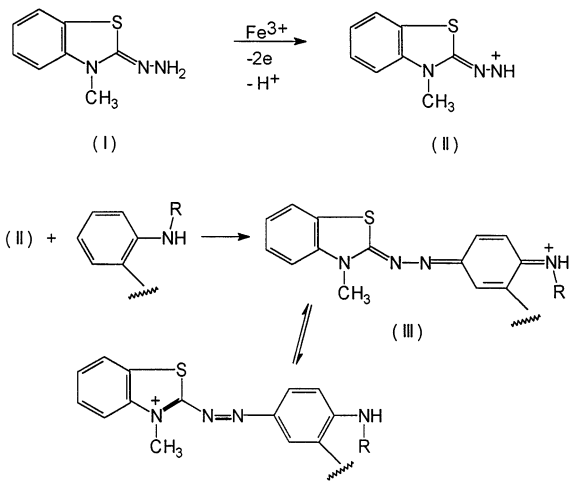


Fig. 1. (a) First and (b) second derivative spectra of 1.6 mg% BN.HCl in 0.1 N HCl.

Table 1
Analytical parameters for the determination benazepril hydrochloride using the proposed methods

Method	Concentration range (μg ml^{-1})	Wavelength (nm)	Solvent	Linear regression			RSD* (%)	Apparent molar absorptivity (l·mol^{-1} cm^{-1})
				Intercept (a)	Slope (b)	Correlation coefficient (r)		
D_1	8–24	240	0.1 N HCl	1.3489	38.113	0.9998	0.60	—
D_2	8–24	244	0.1 N HCl	1.2122	19.879	0.9999	0.46	—
BCG	4–24	412	Distilled water	0.0212	0.4018	0.9994	1.39	18522.98
MBTH	4–40	593	Distilled water	0.0134	0.2399	0.9999	0.43	11059.39
BN.HCl–HCT mix								
BN.HCl D_2	8–24	243	0.1 N HCl	0.8884	20.076	0.9994	1.01	—
HCT D_1	4–10	326	Methanol	−1.535	56.071	0.9994	1.15	—

* Intraday RSD ($n = 5$).



Scheme 1. Scheme 1

product at a suitable pH. The yellow color of the resulting ion-pair complex was extracted with chloroform and measured at 412 nm. The effect of the pH on the development of the color was investigated. The maximum color intensity was achieved by using a buffer solution of pH 2.2. a study on the effect of the concentration of BCG reagent revealed that 4 ml of the reagent gave maximum sensitivity. The produced yellow color was found to be stable for at least 30 min.

3.3. MBTH method

BN.HCl is susceptible to hydrolysis in an alkaline medium with subsequent ring opening. The process of hydrolysis was found to be accelerated by elevating the temperature and increasing the basicity of the medium. It was found that 10 N NaOH and heating for 75 min gave complete hydrolysis of BN.HCl. The product of hydrolysis contains an aromatic amino group with free ortho- and para-positions which can be coupled with MBTH in the presence of $FeCl_3$, as oxidant, to yield a highly colored product. The mechanism of the reaction can be interpreted as in Scheme 1. The experimental conditions of the reaction were studied to establish the most favorable conditions with respect to maximum sensitivity and obedience to Beer's law. It was found that 1 ml of MBTH reagent was enough to develop the color to its full intensity. Several oxidizing agents, e.g. $FeCl_3$, cerric ammonium sulphate and ammonium iron(III) sulphate were investigated. $FeCl_3$ gave maximum color. The effect of diluting solvents, e.g. 0.1 N HCl, water, methanol and ethanol, were tested. Ethanol gave maximum sensitivity. The blue color produced reached its maximum sensitivity after 20 min at room temperature and was found to be stable for at least 30 min.

Regression analysis of the results obtained by three methods using the method of the least squares to calculate the slope (b), intercept (a) and correlation coefficient (r) are presented in Table 1. The high values of correlation coefficients and very small values of the intercepts prove the linearity of the calibration graph and the adherence to Beer's law. Replicate determinations at different concentration levels of the drug were carried out to test the precision and reproducibility of the proposed methods. The intraday relative standard deviations (RSD) were found to be less than 2% (Table 1). Moreover, the RSD values of the slopes and the intercepts of the calibration graphs were calculated (Table 2). The results shown in Table 2 indicate that the proposed methods are highly reproducible.

3.4. Stability-indicating method

The absorption spectra of BN.HCl and its degradation products are nearly similar and completely overlapped. This means that the determi-

Table 2
Intraday precision of BN analysis

Method	Conc. Range ($\mu g\ ml^{-1}$)	RSD*	
		Slope (b)	Intercept (a)
D_1	8–24	0.44	4.35
D_2	8–24	0.23	1.45
BCG	4–24	0.14	6.17
MBTH	4–40	0.073	3.14
BN–HCT mixture			
BN.HCl D_2	8–24	0.15	4.47
HCT D_1	4–10	0.18	2.11

* Five determinations.

Table 3
Assay results for the analysis of degraded benazepril in synthetic mixtures with intact drug by the proposed method

No.	Added conc. (μg ml^{-1})		Ratio		%Recovery of Deg. BN	
	BN	Deg. BN	BN	Deg. BN	A_{max} method	Proposed method
1	32	32	1	1	217.07	101.78
2	64	32	2	1	334.15	98.53
3	64	16	4	1	568.29	98.86
4	80	16	5	1	685.37	98.08
5	24	4	6	1	802.43	100.60
Mean						99.57
SD						1.56
RSD (%)						1.57

nation of the intact BN in the presence of its degradation product is impossible due to the high level of spectral interference that cannot be resolved by a derivative technique.

Since the intact BN does not react with MBTH, while its hydrolytic product reacts giving a blue color, the proposed MBTH method was extended to develop a stability-indicating assay for this drug. Thus, five synthetic mixtures of the intact BN and its degradation product in different proportions were made and analyzed using the proposed method. The mean percentage recovery for the degraded product was 99.75 ± 1.56 (Table 3). These data indicated that the method is accurate and selective, as the presence of the intact drug did not interfere. The use of the A_{max} method for the analysis of these mixtures gives erroneous results due to the spectral overlapping of the intact drug with its degradation product. The error in each result decreases with the increase of concentration of degraded BN relative to the concentration of the intact BN (Table 3).

3.5. BN.HCl–HCT mixture

The combination of BN with HCT has a wide application for the treatment of hypertension. This mixture represents a combination of a weakly absorbing minor component (BN) with a strongly absorbing major component (HCT). The main problem in the analysis of such a mixture is the precise, specific and easy measurement of BN which possesses only a low absorption in the UV region while HCT exhibits a high absorption in this region. Moreover, the problem is further complicated because BN is present as a minor component in that combination and HCT is a major one.

Preliminary attempts to analyze this mixture using a derivative technique showed that this technique cannot cope with the level of interference of HCT in the absorption spectrum of BN due to its low absorptivity and low concentration. Therefore, the use of difference in solubilities of the components of the mixture in different solvents was proposed. It was found that BN is completely soluble in 0.1 N HCl while HCT is partially soluble. However, the part of HCT that dissolves in 0.1 N HCl is high enough to overlap with the absorption spectrum of BN and hence prevented its determination by A_{max} method (Fig. 2). On the other hand, the application of the second derivative to the absorption spectra of both drugs can resolve the interference due to HCT (Fig. 2). Therefore, from Fig. 2, BN can be assayed in presence of HCT by measuring its D_2 values at 243 nm where the latter shows no contribution (zero-crossing point of HCT). On the other hand, HCT was determined in this combination using methanol as a solvent and measuring its D_1 amplitude at 326 nm (Fig. 3).

Under the described experimental conditions, the graphs obtained by plotting the derivative values of each drug in this mixture versus concentration, in the range stated in Table 1, show linear relationships. A critical evaluation of the pro-

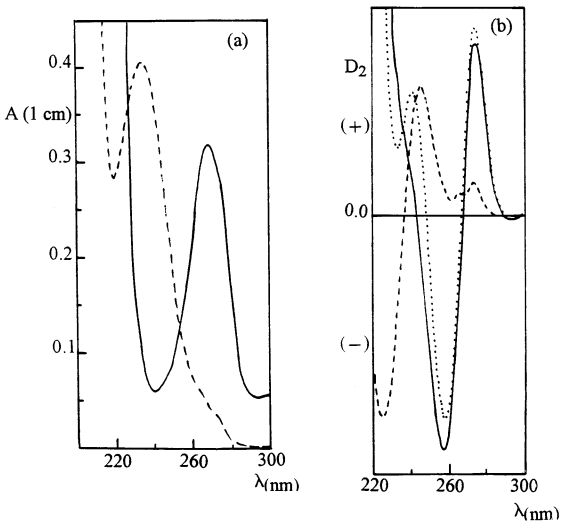


Fig. 2. (a) Zero-order and (b) second derivative spectra of 1.2 mg% BN.HCl (---), 2.0 mg% HCT (—) and their mixture (. . . . .) in 0.1 N HCl.

posed method was performed by the statistical analysis of the data, where slopes intercepts and correlation coefficients were shown in Table 1.

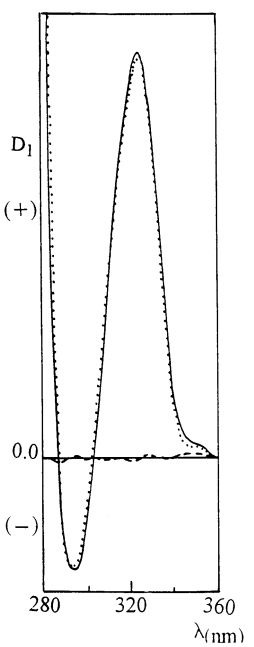


Fig. 3. First derivative spectra of 0.64 mg% BN.HCl (---), 0.8 mg% HCT (—) and their mixture (. . . . .) in methanolic HCl.

High values of correlation coefficients and small values of intercepts (Table 1) validated the linearity of he calibration graphs and the obedience to Beer's law. The RSD values of the slope and intercepts of the calibration graphs (Table 2) indicated the high reproducibility of the proposed method.

To prove the validity of the proposed method, six synthetic mixtures of BN and HCT were made in different proportions and analyzed according to the above procedures. The results obtained (Table 4) show high reproducibility.

3.6. Dosage form analysis

The proposed methods were applied for the determination of BN in its single-component dosage form (Cibacin tablets). The developed derivative method was applied for the analysis of the BN–HCT binary mixture in pharmaceutical preparations (Cibadrex tablets). The results obtained were both precise and accurate (Table 5). The exact composition of the pharmaceutical preparations studied (Cibacin and Cibadrex tablets) is not known. Therefore, the proposed methods were applied for the analysis of the drug in presence of most of the excipient that were used during the manufacture of the tablets. Lactose, starch, talc, and magnesium stearate were not found to interfere with the assay of BN. This implies that the proposed methods can be applied for the analysis of BN in tablets where the components of tablet formulation were not found to interfere with the assay (Table 5).

In conclusion, the derivative method was found to be simple and accurate, and was applied for the determination of BN in its tablet forms either single or in combination with HCT, while the BCG method is a more general and simpler method for the determination of BN. On the other hand, the MBTH method is a specific method for the determination of the degradation product of BN. Hence, it can be utilized for the stability studies of the drug.

The proposed methods are accurate, rapid, sensitive, providing a simple solution for the problem of low absorptivity of the drug in the UV region and can be applied for the routine analysis of BN in its single and multi-component dosage forms.

Table 4
Assay results for the analysis of benazepril and hydrochlorothiazide synthetic mixtures by the proposed derivative method

BN content			HCT content		
Added concentration (μg ml^{-1})		% Recovery of BN	Added concentration (μg ml^{-1})		% Recovery of HCT
BN.HCl	HCT		BN.HCl	HCT	
16.0	8.0	98.41	16.0	8.0	99.28
16.0	12.0	99.96	32.0	8.0	100.39
16.0	16.0	98.41	48.0	8.0	100.39
16.0	20.0	98.41	64.0	8.0	100.39
16.0	24.0	99.96	80.0	8.0	101.51
24.0	30.0	98.82	96.0	8.0	99.28
Mean		99.0			100.2
SD		0.78			0.84

Table 5
Determination of benazepril hydrochloride in tablets using the proposed spectrophotometric methods.

Tablets	Method	Found ± SD*	T**	F**
Cibacin	D_1	98.62 ± 0.96	0.693	1.458
	D_2	100.1 ± 1.21	1.341	1.078
	BCG	98.83 ± 0.49	0.455	5.726
	MBTH	99.3 ± 1.28	0.271	1.218
Cibadrex				
BN.HCl	D_2	99.69 ± 1.39		
HCT	D_1	99.5 ± 0.5		

* Average of five determinations.
** Theoretical values: $t = 2.31$ and $F = 6.39$ at $P = 0.05$.

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