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Simultaneous spectrophotometric estimation of Amlodipine Besylate and Benazepril HCl in pure and pharmaceutical dosage form

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ABSTRACT

Two simple, accurate, precise, reproducible, requiring no prior separation and economical procedures for simultaneous estimation of amlodipine besylate and benazepril HCl in capsule dosage form have been developed. First method is simultaneous equation method; in this method 366 nm and 238 nm were selected to measure the absorbance of drugs at both wavelengths. The second method is Q value analysis based on measurement of absorbance at 213 nm (as an iso absorptive point) and 238 nm (λ max of benazepril HCl). At selected wavelength both drugs show linearity in a concentration range of 10 50 µm/ml. Both methods are validated as per ICH guideline. The proposed methods are recommended for routine analysis since it is rapid, simple, accurate, and sensitive and specific as it does not required heating and organic solvent for extraction.

Key words: Amlodipine besylate, benazepril HCl, Simultaneous equation method, Q analysis.

INTRODUCTION

Amlodipine besylate 2-[(2-amino ethoxy) - methyl]-4-(2-cholophenyl)-1, 4-dihydro-6-methyl-3, 5- pyridine dicarboxylic acid 3-ethyl-5-methyl ester, benzosulfonate, is a dihydro calcium channel blocker.

benazepril HCl (3S)-3-[(1S) - 1-ethoxycarbonyl-3 phenylpropylamino]-2, 3, 4, 5- tetrahydro-2-oxo-1H-1-benzazepin-1-yl] acetic acid hydrochloride is an antihypertensive drug, which belongs to the group of angiotensin convertase inhibitors. It acts on the renin-angiotensin-aldosterone system by inhibition of the conversion of the inactive angiotensin I to the highly potent vasoconstrictor angiotensin II. It also reduces the degradation of bradykinin. benazepril HCl is applied in pharmacotherapy as a first choice drug for treatment of arterial hypertension, ischemic heart disease, hypertrophy of the left heart ventricle and post infarction heart dysfunction.

Literature survey revealed that stability indicating RP-HPLC [1], UV spectrophotometric[2,3] HPLC in human serum[4], HPLC determination of Atorvastatin Calcium with Amlodipine

Besylate[5], LC-MS[6] are reported for the estimation of amlodipine besylate alone or in combination. Several analytical methods have been reported for the quantitative determination of benazepril HCl such as VIS-spectrophotometer[7], high performance liquid chromatography (HPLC) [8], and HPTLC-densitometry [9], LC-MS [10].

As no method is reported for amlodipine besylate and benazepril HCl in combination, the aim of the present study was to develop accurate, precise and selective UV spectrophotometric procedure for the analysis of amlodipine besylate and benazepril HCl in pure and pharmaceutical dosage form.

MATERIALS AND METHODS

Instrumentation and chemical

Spectral runs were made on a Double beam UV-Visible spectrophotometer, model-Jasco-V 630 with spectral bandwidth of 1.5 nm and automatic wavelength corrections with a pair of 10 mm quartz cell. Double distilled water was used in entire experiment. Hydrochloric acid purchased from Merck Ltd., Mumbai, India.

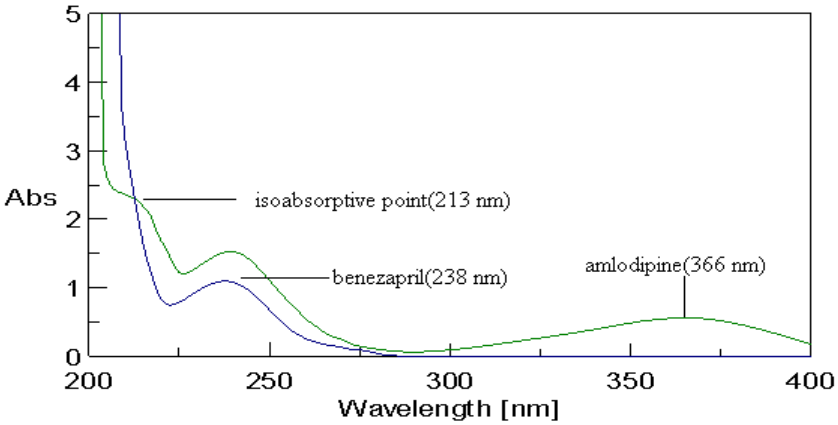
Preparation of standard drug solutions

An accurately weighed 100mg of each of amlodipine besylate and benazepril HCl was dissolved in 100 ml of 0.1N HCl in a concentration of 1000µg/mL each. From this 10ml solution was taken and made to 100 ml with 0.1N HCl to obtain a concentration of 100µg/mL each. Daily working standard solutions of amlodipine besylate and benazepril HCl was prepared by suitable dilution of the stock solution with 0.1N HCl.

Determination of maximum wavelength and Isoabsorptive point

Of the stock solution 5ml was diluated upto 10ml to get concentration of 50µg /ml of amlodipine besylate and 50µg /ml of benazepril HCl.These solution were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both the drugs. Amlodipine besylate showed absorbance maxima at 366nm (λ1) and benazepril HCl showed absorbance maxima at 238 nm (λ2). The overlain spectra shows isoabsorptive points at 213 nm (Fig 1).

Fig1. Overlain spectra of amlodipine and benazepril



Method I (Simultaneous equation method)

Two wavelengths selected for the method are 366 nm and 238 nm that are absorption maxima of amlodipine besylate and benazepril HCl respectively in 0.1N HCl. The stock solutions of both

the drugs were further diluted separately with 0.1N HCl to get a series of standard solutions of 10- 50 µg /mL concentrations. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations-

$$Cx = \frac{A2 \times 0.021636 - A1 \times 0.00006948}{0.0006647} \quad \text{.....equation (1)}$$

$$Cy = \frac{A1 \times 0.03076 - A2 \times 0.01132246}{0.0006647} \quad \text{.....equation (2)}$$

Where, A1 and A2 are absorbance's of mixture at 366nm and 238 nm respectively, ax1 and ax2 are absorptivities of amlodipine besylate at λ1 and λ2 respectively and ay1 and ay2 are absorptivities of benezapril HCl at λ1 and λ2 respectively. *Cx* and *Cy* are concentrations of amlodipine besylate and benazepril respectively.

Method II (Absorption ratio or Q Analysis method)

From the overlain spectrum of amlodipine besylate and benazepril HCl, two wavelengths were selected one at 213 nm which is the isoabsorptive point for both drugs and the other at 238 nm which is λ max of benazepril HCl. The absorbances of the sample solutions are prepared in a similar manner as in the previous method, were measured at selected wavelength. The absorbance ratio values for both the drugs were calculated. The method employs Q-values and the concentrations of drugs in sample solution were determined by using the following formula**,**

Conc. of amlodipine besylate:

$$Cx = \frac{Qm - Q1}{Q2 - Q1} \times \frac{A}{a} \quad \text{.....equation (3)}$$

Conc. of benazepril HCl:

$$Cy = \frac{Qm - Q2}{Q1 - Q2} \times \frac{A}{a} \quad \text{.....equation (4)}$$

A = Absorbance of sample at isoabsorptive point, a = Absorptivities of amlodipine besylate and benazepril HCl respectively at isoabsorptive point. Qm is absorbance ratio of mixture, Q1 and Q2 are amlodipine besylate and benazepril HCl at Iso-absorptive point to maximum wavelength of one of the component (selected wavelength).

Method Validation

Method was validated accordance to ICH guidelines [11] for system suitability, linearity, precision, accuracy, limit of detection, limit of quantification and specificity.

Linearity

The linearity of this method was evaluated by Linear Regression Analysis, which was calculated by Least Square method and the drug was linear in the concentration range of 10-50 µg/ml for both the drugs. Calibration standards were prepared by spiking required volume of working standard (100µg/mL) solution into different 10 ml volumetric flasks and volume made with 0.1N HCl to yield concentrations of 10, 20, 30, 40 and 50µg/ml. The resultant absorbances of the drugs were measured. Calibration curve was plotted between absorbance of drug against concentration of the drug [Fig 2.]. Calibration curve of absorptivity of drug against concentration

of the drug was plotted [Fig 3]. These results shown there was an excellent correlation between absorbance and analyte concentration.

Fig2: Linearity graph of Amlodipine and benazepril (Absorbance).

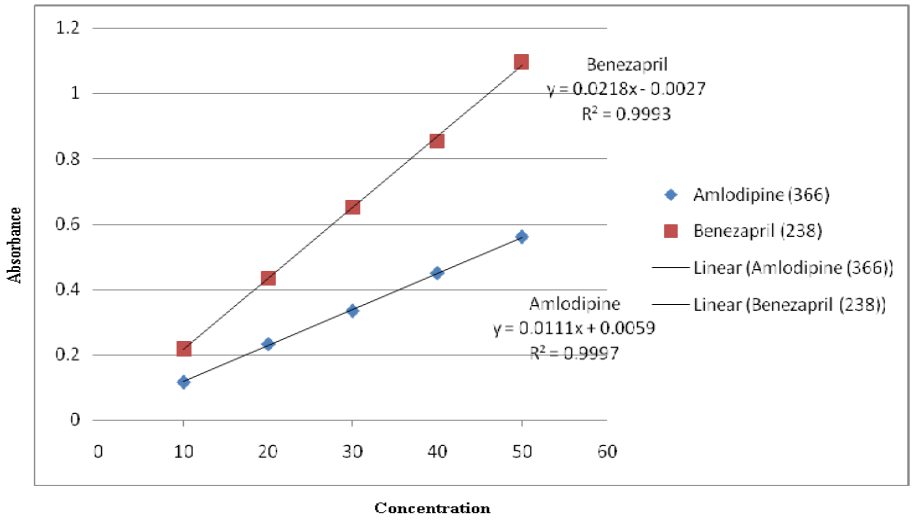


Fig3: Linearity graph of Amlodipine and benazepril (Absorptivity).

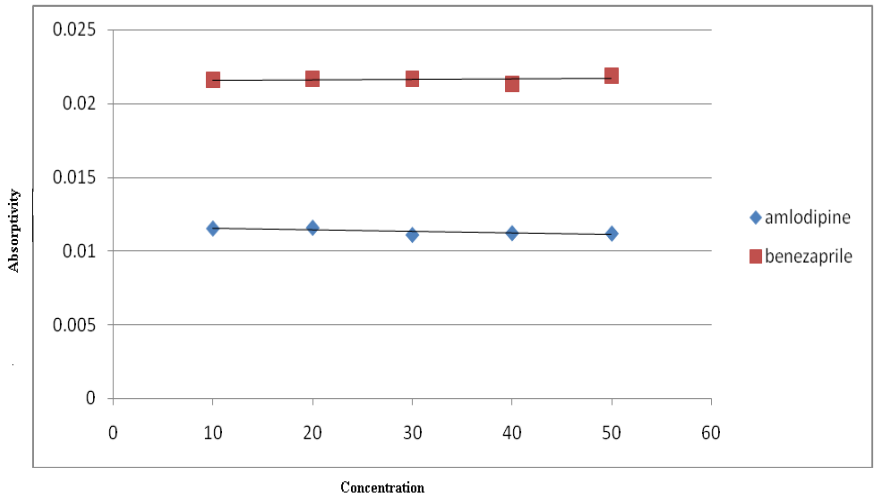


Table1:- Result of recovery studies

Method	Recovery Level (Added amount)	Percent recovery ± SD #	
		Amlodipine besylate	benazepril HCl
I	80%	98.99 ±0.856	101.20±1.355
	100%	99.85 ± 1.203	98.90+0.756
	120%	99.50±1.032	99.90±1.152
I	80%	100.40±1.341	100.5±0.876
	100%	101.10±0.679	99.30±1.585
	120%	99.50±0.945	98.90±1.204

S.D.: Standard deviation, #: Average of three estimation at each level of recovery.

Accuracy

Accuracy was confirmed by recovery study as per ICH norms at three different concentration levels 80%, 100%, 120% by replicate analysis (n=3). Here to a pre analysed sample solution,

standard drug solutions were added and then percentage of drug content was calculated. The result of accuracy study was reported in Table 1. From the recovery study it is clear that the method is accurate for quantitative estimation of amlodipine besylate and benazepril HCl in tablet dosage form as the statistical parameters are within the acceptance range (S.D. < 2.0).

Intra-day and Inter-day Precision

Intra-day precision was studied by six replicate measurements at three concentration levels in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. Accuracy of the method was determined by calculating recovery studies. Statistical evaluation revealed that relative standard deviation of the drug at different concentration levels for six injections were less than 2. Precision and accuracy data were shown in [Table 2 and 3.].

Table 2:- Intra-day precision and accuracy of amlodipine and benazepril

Drug	conc. (µg/mL)	Method I		Method II	
		Accuracy	%RSD	Accuracy	%RSD
Amlodipine besylate	10	101.70±1.29	1.293	99.21±1.48	1.481
	15	99.82±1.18	1.179	98.63±0.92	0.916
	20	99.25±0.82	0.824	99.75±1.07	1.068
Benazepril HCl	10	100.14±1.54	1.541	101.23±1.46	1.462
	15	99.66±0.98	0.977	99.74±0.86	0.855
	20	98.83±0.81	0.809	100.89±0.92	0.924

Table 3:-Inter-day precision and accuracy of amlodipine and benezapril

Drug	conc. (µg/mL)	Method I		Method II	
		Accuracy	%RSD	Accuracy	%RSD
Amlodipine besylate	10	98.99± 0.98	0.976	100.57± 0.78	0.782
	15	99.48± 1.17	1.172	98.82± 1.32	1.317
	20	100.20± 0.97	0.974	99.78 ±1.54	1.544
Benezapril HCl	10	101.88± 0.94	0.939	98.76 ±1.09	1.091
	15	99.74± 1.45	1.446	99.64±1.47	1.469
	20	101.02± 0.84	0.841	99.49 ±0.96	0.958

Table 4:- Optical characteristics data and validation parameters

Parameters	Value	
	Amlodipine besylate	Benazepril HCl
Working λmax	366	238
Beer's law limit (µg/mL)	10-50 µg/ml	10-50 µg/ml
Absorptivity*	0.011322	0.02163
Correlation coefficient*	0.9997	0.9993
Intercept*	0.0059	0.0027
Slope*	0.0111	0.0218

*COV: Coefficient of variation, * Average of six determination.*

Application of methods to capsule dosage forms

For the estimation of drugs in the commercial formulations, twenty capsules were weighed and average weight was calculated. For analysis of drug, quantity of powder equivalent to 10 mg of amlodipine besylate was weighed and dissolved in 60 mL of 0.1N HCl and sonicated for 10 minutes in a 100ml volumetric flask and this solution was filtered through Whatmann No.1 filter paper. The residue was washed with 10ml 0.1N HCl three times and volume made upto 100ml with 0.1N HCl. The solution obtained was diluted with the 0.1N HCl so as to obtain a

concentration in the range of linearity previously determined. All determinations were carried out in six replicates. In Method I, the concentration of both amlodipine besylate and benazepril HCl were determined by measuring the absorbance of the sample at 366 nm and 238nm. For Method II, the concentration of both amlodipine besylate and benazepril HCl were determined by measuring absorbance of the sample at 213 nm and 238 nm and values were substituted in the respective formula to obtain concentrations. Results of tablet analysis are shown in [Table 5.].

Table 5:- Analysis data of capsule formulation

Method	Drug	Lable claim (mg/tab)	Amount found* (mg/tab)	Lable claim (%)	SD*	%COV
I	Amlodipine besylate	10	9.88	98.8	1.159	1.1730
	Benazepril HCl	20	19.78	98.92	0.9015	0.9113
II	Amlodipine besylate	10	10.02	100.21	1.612	1.6086
	Benazepril HCl	20	19.97	99.85	0.9975	0.9989

*S.D.: Standard deviation, COV: Coefficient of variation, *Average of six estimation of tablet formulation.*

RESULTS AND DISCUSSION

Drug content in capsule (amount found) was directly found from equations for both the methods. Standard deviations and Coefficient of variation was calculated (Table 2and 3). The low standard deviation values indicated repeatability, accuracy and reproducibility of the methods. Reproducibility, reliability and interference were also confirmed by recovery studies. Thus, it can be concluded that the methods developed were simple, accurate, sensitive and precise. Statistical analysis and drug recovery data showed that both methods are sensitive, accurate and precise. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully in simultaneous estimation of amlodipine besylate and benazepril HCl in marketed formulations

CONCLUSION

The above proposed methods are simple, economical rapid, accurate, precise, and can be used for a routine quantitative analysis of amlodipine besylate and benazepril HCl in pure drug and capsule dosage form.

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