Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

APPLICATION OF ABSORPTION CORRECTION METHOD AND FIRST ORDER DERIVATIVE SPECTROPHOTOMETRY FOR SIMULTANEOUS DETERMINATION OF ANTI-HYPERTENSIVE COMBINATION (AMLODIPINE AND BENAZEPRIL)

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Received: 28 Mar 2013, Revised and Accepted: 11 May 2013

ABSTRACT

Objective: To develop simple, accurate, precise, reproducible UV Spectrophotometric methods for the simultaneous estimation of Amlodipine besylate (AM) and Benazepril HCl (BZ) in pure and Fixed Dose Combination (FDC).

Methods: First method, Absorption Correction Method, involves direct estimation of Amlodipine besylate at 366 nm and Benazepril at 259.4 nm. For estimation of Benazepril, corrected absorbance was calculated at 259.4 nm due to the interference of Amlodipine besylate at this wavelength. The second method was first order derivative spectrophotometry, wavelengths selected for quantification were 390 nm for Amlodipine besylate (zero crossing point for Benazepril) and 228 nm for Benazepril (zero crossing point for Amlodipine besylate).

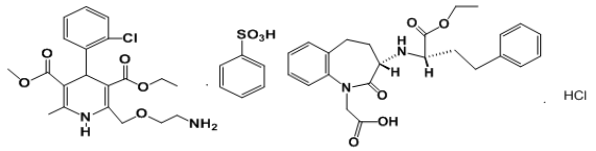
Results: In both methods linearity was observed in the concentration range of 5-50 µg/ml for both the drugs. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per International Conference on Harmonization (ICH) Q28 guidelines. The proposed method was successfully applied for the simultaneous estimation of both drugs in pharmaceutical dosage form as well as for dissolution study. The results of the marketed formulation assay were found to be 101.4 ± 2.05 % and 98.7 ± 1.94 % for AM and BZ respectively by absorption correction method and 98 ± 2.19 % and 100.9 ± 1.92 % for AM and BZ respectively by first order derivative spectrophotometry.

Conclusion: Simple, accurate and precise spectrophotometric methods have been developed and validated for the simultaneous determination of AM and BZ in API and tablet dosage forms without any interference. The methods can be routinely adopted for quality control of these drugs in tablet.

Keywords: Amlodipine besylate (AM), Benazepril hydrochloride (BZ), Absorption correction method, First order derivative spectroscopy method, Zero crossing point (ZCP).

INTRODUCTION

Amlodipine besylate (AM) (Fig.1A), chemically 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 1,4-dihydropyridine derivative L-type calcium channel blocker and used as an anti-hypertensive agent [1, 2].Benazepril HCl (BZ) (Fig.1B), chemically (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 angiotensin converting enzyme (ACE) inhibitors groups [2].Combination of both drugs into fixed dose has been an essential constituent in treatment of hypertension and is recommended by American Hypertension Society.



A B

Fig. 1: It shows chemical structures of Amlodipine besylate (A) and Benazepril HCl (B)

Several analytical methods like RP-HPLC, HPTLC, and LC-MS have been reported for determination of AM individually or in combination with other drugs [3-17]. A number of analytical methods have been reported for the quantitative determination of BZ such as RP-HPLC, HPTLC-densitometry and LC-MS in alone and in combination [11-13, 16, 18]. However, no analytical method is reported for simultaneous estimation of AM and BZ in combined dosage form by absorption correction method and derivative spectroscopy. So in present work, an attempt was made to develop simple, accurate, precise and reproducible spectrophotometric methods for simultaneous estimation of AM and BZ in fixed dose combination.

MATERIAL AND METHOD

Reagents and chemicals

AM (Torrent Pharma Ltd.) and BZ (Astron Research Ltd.) were received as gift sample. Marketed formulation (AMACE-BP tablet, Madras pharmaceuticals Ltd., India), containing 5 mg of AM and 10 mg of BZ was procured from local market. All other reagents employed were of high purity analytical grade. Double distilled water was used throughout the experiment.

Instrumentation

UV method was performed on SHIMADZU double beam spectrophotometer (Model: UV-1800) with 2 nm spectral bandwidth using 10 mm matched quartz cuvettes. Data acquisition was done by using UV-probe software version 2.42.

Preparation of Stock Solutions (AM, BZ and Binary mixture)

Aqueous solution (100 µg/ml) of AM, BZ and its binary mixture were prepared by adding accurately weighed 10 mg of AM and BZ and binary mixture of both drugs in 50 ml of 0.01 N HCl, then sonicated for 10 min and diluted up to 100 ml.

Absorption correction method (Method I)

Six standard solutions of each drug having concentration in the range of 5-50 µg/ml were prepared in 0.01N HCl for AM and BZ. Mixed standards containing AM and BZ were also prepared (5-50 µg/mL). After scanning standard solution (10µg/ml) of AM, two points were identified at which AM shows same absorbance and thepoints were 366 nm (absorbance maxima of AM) and 259.4 nm with minimum %RSD in their absorbance (<2%) at different concentrations (5-50 µg/ml). Hence it was proved that AM has same absorbance at 366 nm and 259.4 nm. Similar way linearity curve was also prepared for BZ at 259.4 nm. In binary mixture, direct

quantification of AM was done at 366 nm as BZ doesn't show any absorbance at this wavelength and quantification of BZ was done at 259.4 nm by subtracting absorbance of AM at 259.4 nm (which is equal to absorbance at 366nm).

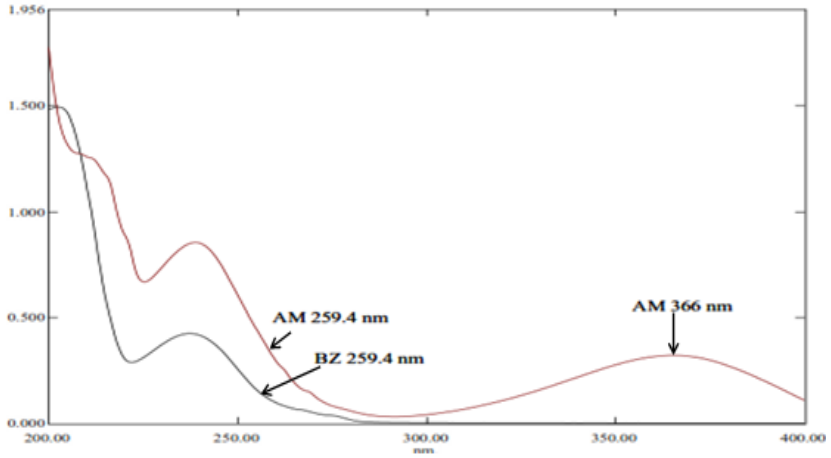


Fig. 2: It shows overlain spectra of AM and BZ

First order derivative spectroscopy method (Method II)

Aqueous solutions (10 µg/ml) of AM and BZ were scanned separately in the range of 200-400 nm. These spectrums were converted to first derivative spectra by using derivative mode with 8 delta point. The two spectra were overlain and it was observed that AM showed zero crossing point (ZCP) at 228 nm, while BZ showed ZCP at 390 nm respectively. At ZCP of AM (228 nm), BZ showed a first-derivative absorbance, whereas at the ZCP of BZ (390 nm), AM showed a first derivative absorbance. Hence 390 nm and 228 nm were selected as analytical wavelengths for determination of AM and BZ respectively. These two wavelengths can be employed for the determination of AM and BZ without any interference from the other drug in their combined dosage formulations.

A series of standard solutions were prepared having concentration range of 5-50 µg/ml for AM and BZ in 0.01N HCl using working standard solution (100 µg/ml). The first order derivative absorbance of resulting solution was measured at 390 nm and 228 nm, and calibrationcurves were plotted at these wavelengths. Linearity was observed for both the drugs individually and in combination within the concentration range of 5-50 µg/ml.

Method validation

The methods were validated according to International Conference on Harmonization Q2B guideline for validation of analytical procedures in order to determine the linearity, sensitivity, precision, accuracy, LOD and LOQ for each analyte [19, 20]. Calibration curves were generated with appropriate volumes of working standard solutions for both spectrophotometric methods with the range of 5-50 µg/ml. The linearity was evaluated by the least square regression method.

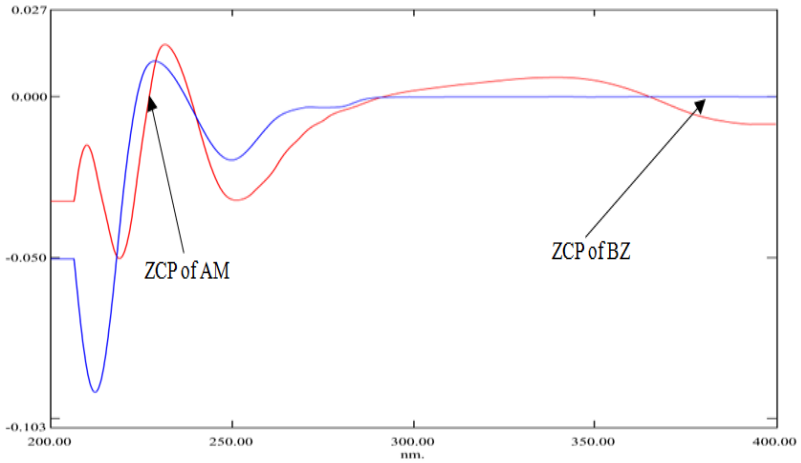


Fig. 3: It shows overlain first order derivative spectra of AM (red) and BZ (blue)

Analysis of Marketed tablet formulation

Accurately weighed powdered sample (132 mg) of marketed formulation (AMACE-BP) equivalent to 5 mg of AM and 10 mg of BZ was dissolved in 100mL volumetric flask containing 0.01 N HCl. The solution was kept for sonication for 20 minutes, filtered through nylon syringe filter (0.45 µ). Aliquot of this solution was diluted to produce the concentration of 5 µg/ml for AM and 10 µg/ml for BZ (n=6). The absorbance of sample solutions at 259.2 nm and 366 nm were measured and amount of drug present in the sample solution was calculated in the same manner as that of pure mixed standard solution.

***In-vitro* dissolution study**

In-vitro dissolution study was performed on marketed formulation AMACE-BP (6 tablets) by using USP type II (paddle) apparatus. Samples were taken at specific time intervals as per FDA guideline and were analysed by both proposed spectrophotometric methods. Results were plotted as cumulative percentage release (CPR) vs. time for both methods and results were compared.

RESULT AND DISCUSSION

Two simple, accurate, precise spectrophotometric methods (Absorption correction method and derivative spectrophotometric method) were developed for simultaneous estimation of AM and BZ which can be conveniently employed for routine analysis in pharmaceutical dosage forms. Figure-2 shows overlain spectra of both drugs using absorption correction method and it was found that at 259.4 nm and 366 nm, AM showed same absorbance at different concentrations. Results were tabulated in Table-1 which clearly indicates AM has similar absorbance at 366 nm and 259.4 nm with minimum % RSD. This method was validated by using binary mixture of both drugs and results were tabulated in Table-2. Figure-3 shows overlain spectra of AM and BZ using first order derivative spectroscopy. ZCP of AM and BZ were found to be 228nm and 390nm respectively. The regression coefficients of the AM and BZ calibration curves were greater than 0.99 for both methods. All the method validation parameters were well within the limits as specified in the ICH Q2B guideline as shown in Table 3. Table 4 lists the percent recovery (content uniformity) of both drugs by both spectrophotometric methods at three different levels 50 %, 100 % and 150 %. Moreover the % R.S.D. (less variation) shows good precision of both developed methods. Hence, the methods were suitably employed for assaying both the drugs in commercial marketed formulation (Table-5). The results of the marketed formulations were found to be 101.4 ±2.05% and 98.7 ± 1.94% for AM and BZ respectively by absorption correction method and 98 ± 2.19% and 100.9 ± 1.92% for AM and BZ by First order derivative spectrophotometry. Linearity was determined at different concentrations, AM and BZ show linearity in the concentration range of 5-50 µg/ml. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by standard deviation of response and slope of calibration curve.The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive (Table-3). Both methods were successfully applied for *in-vitro* dissolution study and results were compared and it was found that there is no significant difference in the CPR (cumulative percentage release) obtained from both spectrophotometric methods (Figure-4).

Table 1: It shows selection of wavelength of AM for absorption correction method

Concentration (µg/ml)	Absorbance at 366 nm*	% RSD**			
		259.3 nm*	259.4 nm*	259.5 nm*	259.6 nm*
5	0.087	0.948	**0.191**	0.578	0.967
10	0.174	0.854	**0.095**	0.191	0.481
20	0.340	0.195	**0.048**	0.442	1.138
30	0.515	0.354	**0.002**	0.487	1.377
40	0.679	0.122	**0.024**	0.418	0.916
50	0.823	0.161	**0.040**	0.141	0.529

*Average of six determinations **Relative standard deviation

Table 2: It shows validation of absorption correction method by using binary mixture

Concentration(µg/ml)	Absorbance of AM at 366 nm*	Absorbance of binary mixture at 259.4 nm*	Corrected absorbance of BZ at 259.4 nm	Absorbance of BZ at 259.4 nm from calibration curve*	% RSD
5	0.087	0.121	0.034	0.033	1.51
10	0.174	0.241	0.067	0.066	0.50
20	0.34	0.451	0.110	0.112	0.89
30	0.515	0.681	0.165	0.160	1.66
40	0.679	0.892	0.212	0.211	0.39
50	0.823	1.087	0.263	0.267	0.62

*Average of six determinations

Table 3: It shows validation parameters for Method I and Method II

Parameters	Method-1**		Method-2**	
	AM	BZ	AM	BZ
Working λ_{max}	366 nm	259.4 nm	390 nm	228 nm
Beer's law limit	5-50 µg/mL	5-50 µg/mL	5-50 µg/mL	5-50 µg/mL
Regression equation*	y = 0.0165x + 0.01	y = 0.0051x - 0.0106	y = 0.0004x+0.0007	y = 0.0005x+0.0004
Regression coefficient(r^2)*	0.999	0.998	0.997	0.998
Absorptivity*	0.017	0.0058	0.00046	0.00055
LOD* (µg/mL)	0.48	1.6	0.77	0.31
LOQ* (µg/mL)	1.48	0.52	2.35	0.94
% RSD intra-day precision*	1.38	1.15	1.63	0.89
% RSD inter-day precision*	1.71	1.63	1.39	0.67

*Average of six determinations **Absorption correction method ***First-order derivative spectrophotometric method

Table 4: It shows results of recovery study

Method	Drug	Amt. present (µg/ml)	Amt. added (µg/ml)	Amt. found (µg/ml)*	Amt. recovered*	% Recovery*	Avg. % recovery	% RSD**
Method-I	AM	20	10	29.67	9.67	96.7	98.52	2.6
		20	20	39.33	19.33	96.6		
		20	30	50.67	30.67	102.2		
	BZ	20	10	29.67	9.67	96.7	100.19	2.47
		20	20	40.33	20.33	101.6		
		20	30	50.67	30.67	102.2		
Method-II	AM	20	10	29.9	9.9	99	100.41	1.46
		20	20	39.96	19.96	99.8		
		20	30	50.73	30.73	102.4		
	BZ	20	10	30.13	10.13	101.3	99.72	2.48
		20	20	40.33	20.33	101.6		
		20	30	48.87	28.87	96.2		

*Average of three determinations **Relative Standard Deviation

Table 5: It shows assay results of Marketed formulation (AMACE-BP)

Assay	Drug	Label claimed (mg/tab)	Amt. found (mg/tab)*	% Label claimed	SD**	% RSD***
Method I	AM	5	5.07	101.4	2.05	2.02
	BZ	10	9.87	98.7	1.94	1.96
Method II	AM	5	4.9	98	2.19	2.23
	BZ	10	10.09	100.9	1.92	1.90

*Average of six determinations ** Standard Deviation ***Relative Standard Deviation

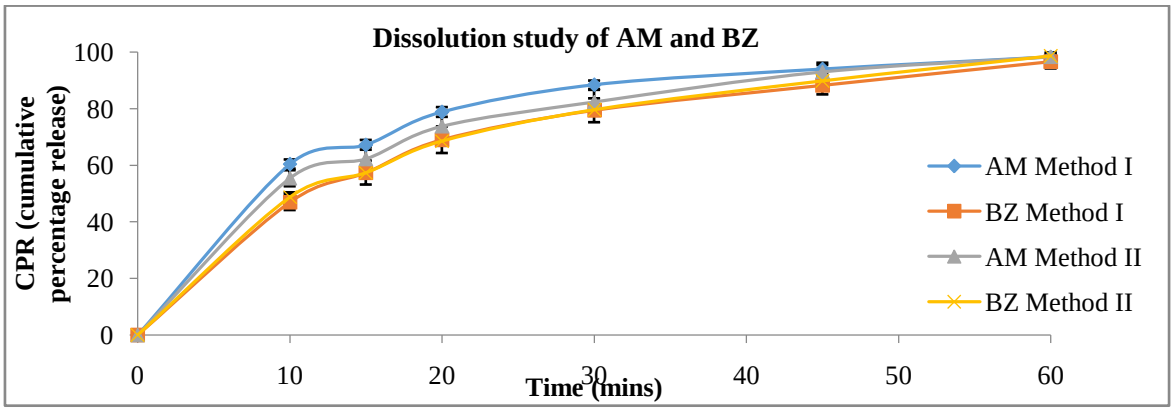


Fig. 4: It shows dissolution profile of AM and BZ by Absorption correction method and Derivative spectroscopic method

CONCLUSION

Simple, accurate and precise spectrophotometric methods have been developed and validated for the simultaneous determination of AM and BZ in API and tablet dosage forms without any interference. The developed spectrophotometric methods are recommended for routine analysis of the drugs in two component pharmaceutical preparations. The developed methods can also be successfully applied for dissolution testing of AM and BZ in combined dosage form.

ACKNOWLEDGEMENT

Abhi Kavathia and Kishan Suchak would like to acknowledge Department of Pharmaceuticals, Ministry of Chemical and Fertilizer, Govt. of India for providing financial assistance during this project and also would like to acknowledge Mr. Arpit Patwari for his technical support.

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