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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND METOPROLOL SUCCINATE FROM THE SYNTHETIC MIXTURE BY THREE DIFFERENT UV SPECTROPHOTOMETRIC METHODS

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ABSTRACT

Three new UV spectrophotometric methods namely simultaneous equation, Q-absorbance ratio, and first derivative (zero-crossing) spectroscopic methods were developed and validated for simultaneous estimation of Azelnidipine and Metoprolol Succinate in tablet formulation which was simple, sensitive, precise and accurate. In the simultaneous equation method, absorbance was measured at 223 and 257 nm for both drugs. Azelnidipine and Metoprolol Succinate showed an iso-absorptive point at 233 nm in the Q- absorbance ratio method. The second wavelength used was 257 nm which is λ max of Azelnidipine in Methanol: Water (80:20) solvent. The first derivative (zero-crossing) method was based on the transformation of UV spectra into first derivative spectra followed by measurement of the first derivative signal at 241 and 234 nm for Azelnidipine and Metoprolol Succinate, respectively using 2 nm as wavelength interval (Δλ) and 1 as scaling factor. Developed methods were validated according to ICH guidelines including parameters viz., specificity, linearity and range, precision, accuracy, the limit of detection, and quantification. All three methods showed a linear response in the concentration range of 1-5 µg/ml and 6.25-31.25 µg/ml for Azelnidipine and Metoprolol succinate respectively. Results of method validation parameters follow ICH guidelines is in acceptable limits. Based on the assay results obtained, methods were compared using one-way ANOVA. The outcome of the statistical analysis proved that there was no considerable dissimilarity between all the developed methods. Methods were found to be simple, fast, highly sensitive, cost-effective, and hence can be useful for

simultaneous estimation of Azelnidipine and Metoprolol Succinate which showed good applicability of the developed method.

KEYWORDS: Azelnidipine (AZE), Metoprolol Succinate (MET), simultaneous equation, Q-absorbance ratio, first derivative (zero-crossing) spectroscopic methods.

1. INTRODUCTION

1.1 Azelnidipine was synthesized by Ube Industries, Ltd. and developed by Sankyo Co., Ltd. (currently known as Daiichi Sankyo Co., Ltd., Tokyo, Japan) and was launched into the market as CALBLOCK in Japan in 2003. Azelnidipine (AZE) (3-[1- (diphenyl methyl) azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1, 4- dihydropyridine-3, 5-dicarboxylate) is a new dihydropyridine derivative with calcium antagonistic activity. Azelnidipine inhibits Trans membrane Ca^{+2} influx through the voltage dependent channels of smooth muscle in vascular walls. They enter the cells through cell membrane, lower peripheral vascular resistance and arterial pressure. It is used for treatment of essential hypertension and angina pectoris. The drug has renoprotective effects (such as reducing proteinuria by dilating efferent arterioles), as well as cardioprotective, insulin resistance-improving, cerebroprotective, and antiatherosclerotic effects. Azelnidipine occurs as two enantiomers due to an asymmetric carbon at the 4-position of the 1, 4-dihydropyridine ring(1).

A literature survey revealed that few methods are reported for determination of AZE, either alone or in combination by spectrophotometric(2–6), and HPLC(7–10) and HPTLC(11).

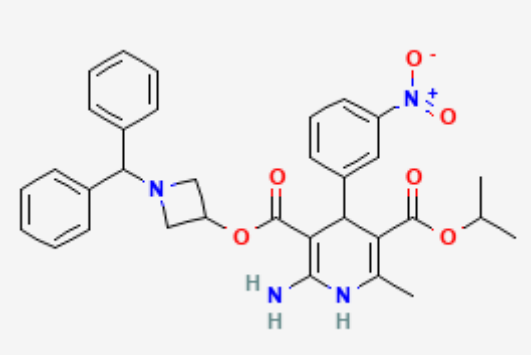

Figure 1: Chemical structure of Azelnidipine.

1.2 Metoprolol is a propanol amine that is 1-(propan-2-ylamino) propan-2-ol substituted by a 4-(2-methoxyethyl) phenoxy group at position 1. Iupac name of metoprolol succinate is

butanedioic acid; 1-[4-(2-methoxyethyl) phenoxy]-3-(propan- 2-ylamino) propan-2-ol. It is a propanol amine, aromatic ether, secondary alcohol, and a secondary amino compound. Metoprolol is a selective beta-1 blocker commonly employed as the succinate and tartrate derivatives. Metoprolol is a cardioselective beta-blocker that is widely used in the treatment of hypertension and angina pectoris. It has a role as a beta-adrenergic antagonist, an antihypertensive agent, a xenobiotic, an environmental contaminant, and a geroprotector. Metoprolol has been linked to rare cases of drug-induced liver injury. Metoprolol is indicated for the treatment of angina, heart failure, myocardial infarction, atrial fibrillation, atrial flutter, and hypertension(12).

A literature survey revealed that few methods are reported for determination of MET, either alone or in combination by spectrophotometric(13–17), and HPLC(18,19), HPTLC(20).

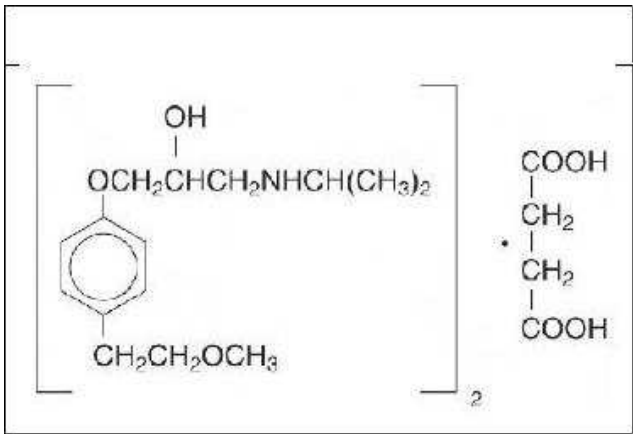

Figure 2: Chemical structure of Metoprolol Succinate.

The aim of the present work was to develop a 3 spectrophotometric method because there is no single method reported for the combination.

2. MATERIALS AND METHODS

2.1 Apparatus

A double beam UV/Visible spectrophotometer (1700). A Shimadzu electronic analytical balance was used for weighing the sample. Azelnidipine (Pure chem PVT. Ltd. Ankleshwar, Gujarat), Metoprolol Succinate (CTX Life science, Sachin GIDC, Surat).

2.3 Solvent

Methanol: Water (80:20) %v/v.

2.2 Preparation of standard stock solution

Accurately weighed quantities of 10 mg of AZE and 10 mg of MET were transferred into a 10 mL volumetric flask individually and dissolved in a solvent. Prepared stock solutions are 1000 µg/mL of AZE and 1000 µg/mL of MET.

3. Procedure

3.1 Simultaneous Equation and Q-Absorbance Ratio Method

Standard stock solutions containing 1000 µg/ml of AZE and MET were suitably diluted separately with solvent to obtain the drug solutions containing 5µg/ml and 10µg/ml concentration respectively. Both the solutions were scanned in the UV region (200 - 400 nm) and spectra were recorded. Based on the spectral pattern, SE (simultaneous equation) and AR (Q-absorbance ratio) methods were chosen for the estimation of both the drugs. From the overlain spectra (Figure 3), 223 nm (λ max of MET) and 257 nm (λ max of AZE) were selected for SE method. In case of AR method, 233 nm (isosbestic point) and 257 nm (λ max of AZE) was selected, which showed excellent linearity and therefore used for simultaneous determination.

Varying concentrations ranging from 1-5 µg/ml of AZE and 6.25-31.25 µg/ml MET were prepared by diluting respective stock solutions. All the solutions were scanned in the UV region and absorbances were noted at 223 and 257 nm for SE; 233 and 257 nm for AR method. Absorptivity values were calculated for AZE and MET at their relevant wavelengths by applying following formula:

Absorptivity = absorbance / concentration (gm/100 ml)

Absorptivity value of individual solution at their respective wavelength was calculated and average absorptivity value (Table 1) at specific wavelength of particular drug was used for calculating concentration of drugs.

Table 1: Average absorptivity values for SE and AR method.

SE				**AR**			
Avg. absorptivity*				**Avg. absorptivity***			
MET		AZE		MET		AZE	
223nm	257nm	223nm	257nm	257nm	233nm	257nm	233nm
0.0333	0.00188	0.0474	0.0562	0.00188	0.00472	0.0562	0..0297
*(n = 6) Average of six determinations.							

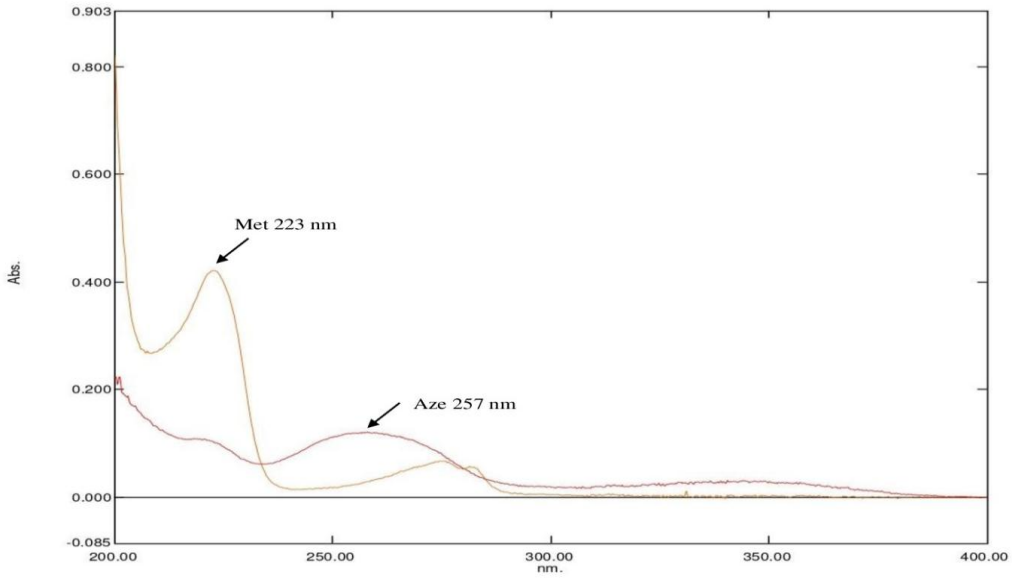

Figure 3: Overlain UV spectra of AZE and MET.

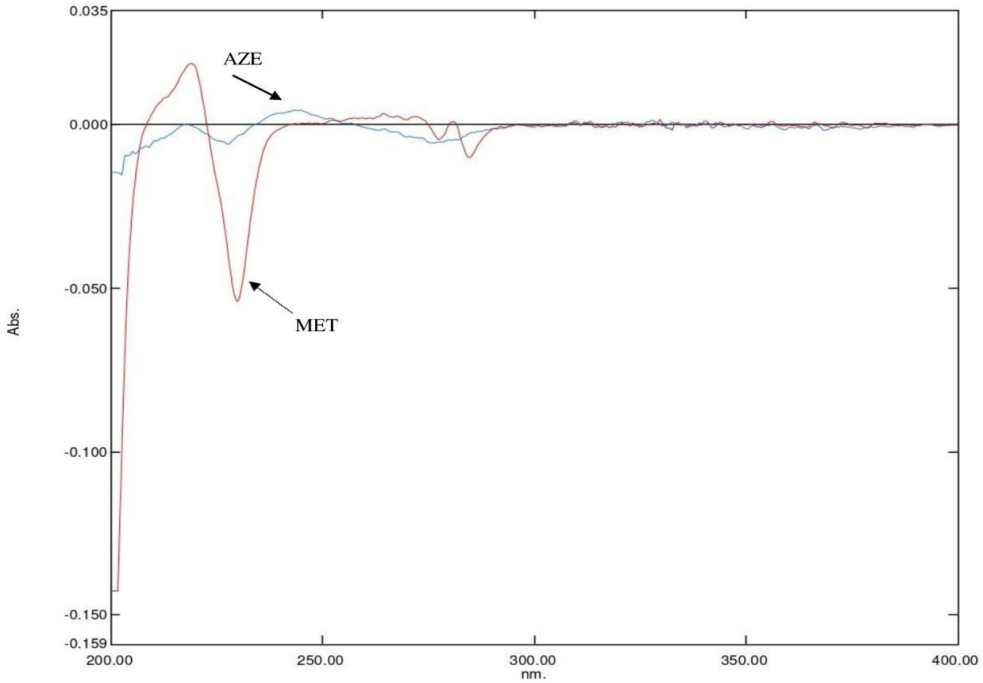

Figure 4: Overlain 1st derivative (zero crossing) UV spectra of AZE and MET

3.2 First Derivative (zero crossing) Method

The normal UV spectra of AZE and MET were transformed into first and second derivative spectra. Based on the spectral pattern and zero crossing points, first DR (derivative spectroscopic) method was chosen for the study. First derivative spectra showed typical zero-

crossing points at 234 nm for AZE and 241 nm for MET applying 2 nm as wavelength interval (Δλ) and 1 as scaling factor. After assessing overlain spectra, 234 nm and 241 nm were selected for further studies (Figure 4). Calibration curve was plotted for both AZE and MET in the concentration range of 1 to 31.25 µg/ml. Results were subjected to regression analysis by least square method to determine the values of slope, intercept and correlation coefficient.

4. Analysis of Sample Solution

4.1 Simultaneous Equation Method

After scanning the sample solution (formulation) between 200 to 400 nm, responses were noted at 223 and 257 nm. The unknown concentration of drugs present in the sample solution was estimated by solving following formula (Sen et al., 2016):

$$Cx = \frac{A_2\, a_{y1} - A_1\, a_{y2}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$Cy = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

Where and are the concentrations of AZE and MET, ax1 and ax2 are absorptivity's of AZE at 223 nm and 257 nm, respectively. ay1 and ay2 are absorptivity's of MET at 223nm and 257 nm, respectively. A1 and A2 are the absorbances of sample solution at 223 and 257 nm.

4.2 Absorbance Ratio Method

The unknown concentration of drugs in the sample solution was estimated by AR method applying following formula:

$$\mathrm{Cx} = \frac{Qm - Qy}{\mathrm{Qx} - \mathrm{Qy}} \times \frac{\mathrm{A1}}{ax1}$$

$$\mathrm{Cy} = \frac{Qm - Qx}{\mathrm{Qy} - \mathrm{Qx}} \times \frac{\mathrm{A1}}{ay1}$$

Where, ax1 and ax2 are absorptivity's of AZE at 257 and 233nm, respectively. ay1 and ay2 are absorptivity's of MET at 257 and 233 nm. A1 and A2 are the absorbances of sample solution at 257 and 233 nm. Cx and Cy are the concentrations of AZE and MET, respectively in sample solution.

$$Qm = \frac{A2}{A1} \qquad Qx = \frac{ax2}{ax1} \qquad Qy = \frac{ay2}{ay1}$$

4.3 First Derivative (zero crossing) Method

Sample solution was scanned in the UV region (200-400 nm) and spectrum was recorded and transformed into their 1st derivative spectra and amplitude was measured at 234 or 241 nm. The unknown concentration of drugs present in the sample solution was estimated by using regression equation.

5. Validation of Spectroscopic Methods

The developed methods were validated in accordance with "International Conference on Harmonization" guidelines (ICH, 2005).

5.1 Specificity

To check the interference between tablet excipients used in the formulation and drug substance, specificity study was carried out. All the tablet excipients (as per marketed formulation) were mixed in proportion and diluted using methanol and filtered using Whatman filter paper no 41. All the solutions (Placebo and standard) were scanned in the UV region and compared to assess the interference among excipients and drugs.

5.2 Linearity and Range

Linearity and range of all the three methods were checked by analysing all the standard solutions separately, containing AZE (1, 2, 3, 4, and 5 µg/ml) and MET (6.25, 12.5, 18.75, 25.0, and 31.25 µg/ml) in solvent and absorbances were noted at 223 and 257 nm for SE method; 223 and 257 nm and 233 nm (isobestic point) for AR Water; 234 and 241 nm for 1st DR method. Calibration graphs were constructed using absorbances of standard drug solutions versus concentration in SE and AR method; 1st derivative signal of standard drug solutions versus concentration in DR method. Regression analysis was performed by least squares method to determine the values of slope, intercept and correlation coefficient.

5.3 Precision

Precision of the methods were evaluated by performing repeatability, intra-day and inter-day precision studies. Repeatability of the methods were evaluated by analysing sample solutions (AZE and MET: 1 & 6.25 µg/ml) six times by measuring the absorbances of both the drugs solution at 223 and 257 nm in SE method; 223nm, 257nm and 233 nm (Isosbestic point) for AR method; 234nm, 241nm for 1st DR method, respectively and % RSD was calculated. Intra-day precision was performed by analysing sample solutions (AZE and MET: 1 & 6.25 µg/ml) in triplicate at two different concentration levels for three times on the same day

within the linearity range and % RSD was calculated. Inter-day precision was evaluated by repeated analysis of sample solutions (AZE and MET: 1: 6.25 µg/ml) in triplicate at two different concentration levels within the linearity range on three different days and the percentage RSD was calculated.

5.4 Accuracy

In order to ensure the suitability and reliability of the projected methods, recovery studies were performed by the standard addition method. To an equivalent quantity of pre-analyzed sample solution (AZE and MET:: 1: 6.25 µg/ml), a known concentration of standard AZE and MET were added at 80, 100, and 120% levels and the resulting solutions were reanalyzed by projected methods and % recoveries were calculated. The outcome of accuracy studies was assessed based on the percentage of standard MET and MET recovered from the formulation by applying the following formula: -

%Recovery = (Amount of drug found after addition of standard drug – Amount of drug found Before the addition of standard drugs)/ (Amount of standard drug added)*100

5.5 LOD and LOQ

The sensitivity of the proposed methods was determined in terms of LOD and LOQ. The limit of detection and limit of quantification of AZE and MET were calculated applying the following equation as per ICH guidelines.

LOD = 3.3 * (□/s)

LOD = 10 * (□/s)

Where, □ = the standard deviation of the response, S = the slope of the calibration curve.

5.6 Stability

The stability of the solutions were checked by observing any changes in terms of absorbance and spectral pattern which was compared to freshly prepared solutions by keeping the solutions at room temperature and analyzing them at frequent intervals.

6. RESULTS AND DISCUSSION

Three UV spectroscopic methods namely SE, AR and 1st DR spectroscopic methods were developed and validated for simultaneous estimation of AZE and MET in Synthetic mixture form which are simple, sensitive, precise and accurate. In SE method, absorbance was measured at 223 and 257 nm for both the drugs. In AR method 257 and 233 nm was used for the detection and quantification of AZE and MET. 1st DR method was based on the

transformation of UV-spectra in to first derivative spectra and followed by measurement of first derivative signal at 234 and 241 nm for AZE and MET, respectively using 2 nm as wavelength interval (Δλ) and 1 as scaling factor.

Comparative overlain spectra of placebo and drug solutions indicate that there was no interference between excipients and standard drugs (Figure 5 & 6). Linear relation was established for AZE and MET in the concentration range of 1- 31.25 µg/ml for all the methods. Overlain spectra of AZE and MET are shown in Figure 5& 6. Calibration graphs were plotted using absorbance of standard drug solution versus concentration for SE and AR method. 1st derivative signal of standard drug solution versus concentration was used to plot calibration curve for 1st DR method.

Regression analysis was performed by applying least square method for calculating values of slope, intercept and correlation coefficient for AZE and MET at their relative wavelengths. Outcome of precision studies were evaluated in terms of % RSD, follows ICH guideline with acceptable limits (<2), which shows good repeatability, low intra and interday variability, indicating an excellent precision of the developed methods (Table 2). The outcome of recovery studies ranged from 98-102% for both the drug suggests suitability of the proposed methods (Table 3). Percentage recovery indicates that there was no interference from tablet excipients. Moreover, low LOD and LOQ values prove the sensitivity of the proposed methods (Table 2). Solution stability was checked at room temperature and it was found to be stable up to three days.

The projected methods were successfully applied for the quantitative determination of AZE and MET in synthetic mixture (1 mg of AZE and 6.25 mg of MET). Sample solutions were analysed six times and experimental values were found to be within 96 and 100 % for both the drugs and hence the developed methods can be used for the simultaneous determination of both the drugs in combined tablet formulation. Statistical analysis was performed to check the effect of all three developed methods based on assay results obtained.

Statistical significance between all the three methods were tested using one-way ANOVA. Significance level was set at $p<0.05$for all the tests. Results of ANOVA are presented in Table 5. The results of assay proved that there was no considerable dissimilarity between all the developed methods.

Table 2: Summary of linear regression and method validation data for the proposed methods.

Parameter	SE		AR		DR	
Drug	**AZE**	**MET**	**AZE**	**MET**	**AZE**	**MET**
Wavelengths (nm)	257 nm	223 nm	257 nm	233 nm	241 nm	234 nm
Linearity range (µg/ml)	1 - 10 (µg/ml)	6.25 – 31.25 (µg/ml)	1 – 10 (µg/ml)	6.25–31.25 (µg/ml)	1 - 10 (µg/ml)	6.25 - 31.25 (µg/ml)
Correlation coefficient	0.996	0.999	0.997	0.998	0.992	0.996
Slope	0.0508	0.0352	0.062	0.257	0.0096	0.0017
Intercept	0.0124	0.0238	0.009	0.027	0.0212	0.0158
LOD (µg/ml)	0.088	1.875	0.092	1.770	0.085	1.825
LOQ (µg/ml)	0.264	5.625	0.276	5.31	0.255	5.475
Precision (% RSD) Intra-day (n=3) *	1.10	0.93	1.17	1.25	1.08	1.33
Inter-day (n=3) *	1.02	0.88	1.35	0.97	1.16	1.17

n = number of determinations, % RSD (Percentage relative standard deviation).

Table 3: Recovery data of the proposed methods.

Drugs	% MEAN±SD*			
	Levels (%)	**SE**	**AR**	**DR**
AZE	80%	99.33±0.04	99.7±0.005	100.12 ± 0.82
	100%	100.5±0.005	101±0.006	100.26 ± 0.46
MET	120%	103.4±0.04	101.5±0.005	99.84 ± 0.49

*Mean ± SD (n = 3), SD (Standard deviation)

Table 5: Results of statistical comparison using one way ANOVA for SE, AR and DR spectroscopic methods.

Drugs	Simultaneous Equation Method	Absorbance Ratio Method	First Derivative Method
AZE	99.87	98.77	99.85
MET	99.75	98.60	98.05

All values are expressed in Mean ± SD (n=6).

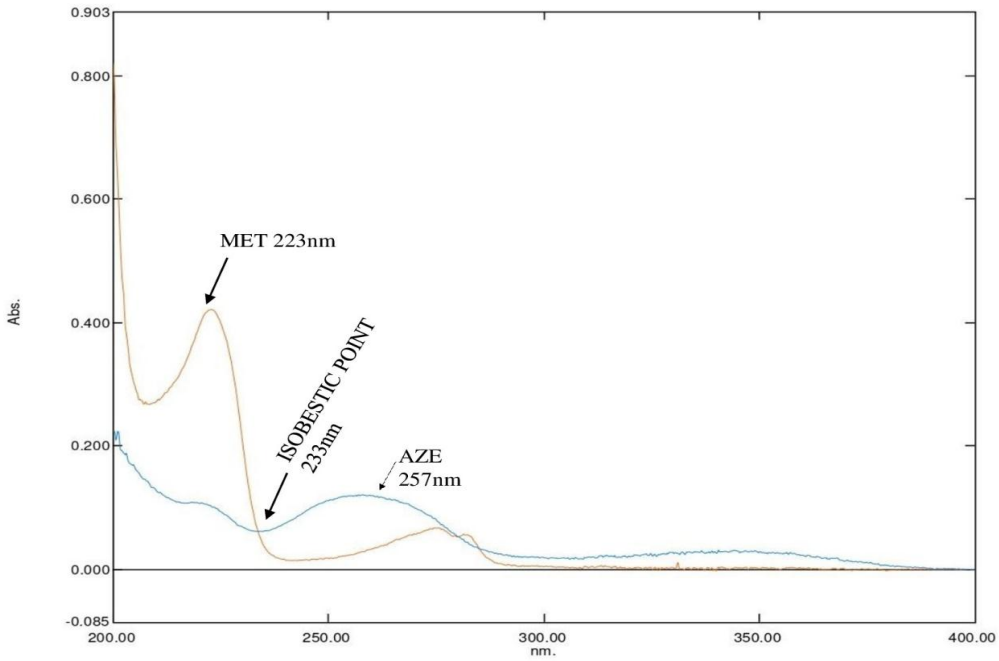

Figure 5: Overlain UV spectra of formulation excipients and standard drugs for SE and AR methods.

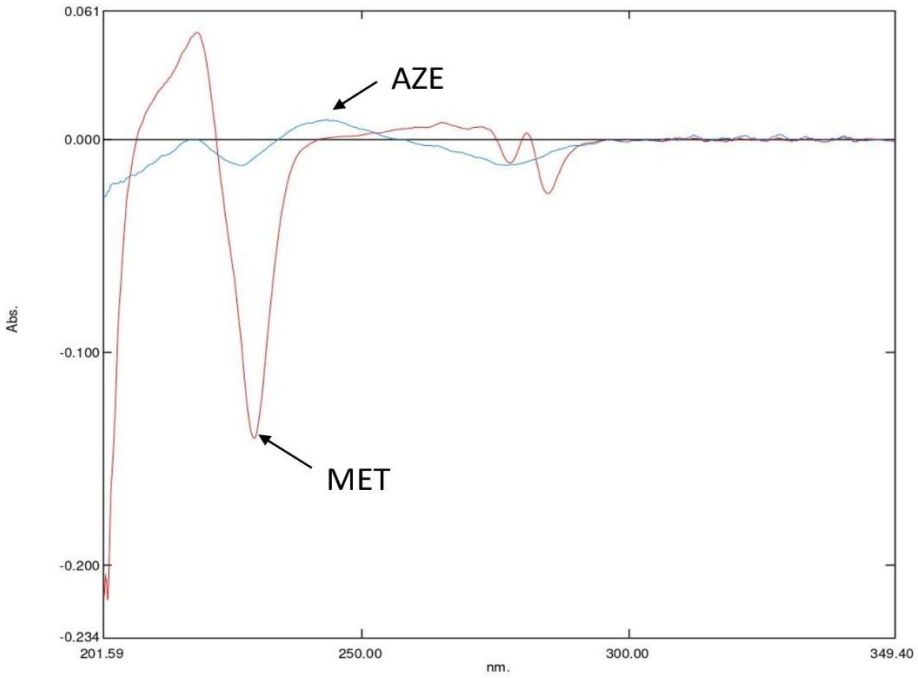

Figure 6: Overlain first derivative (zero crossing) UV spectra of formulation excipients and standard drugs for 1st DR method.

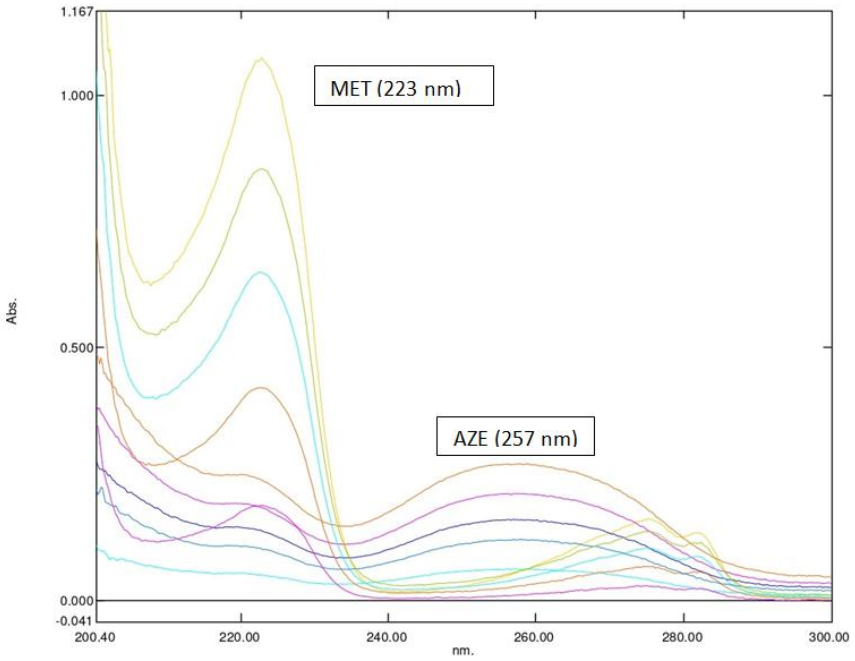

Figure 7: Overlain UV spectra of AZE and MET (1- 31.25 µg/ml) for SE and AR methods.

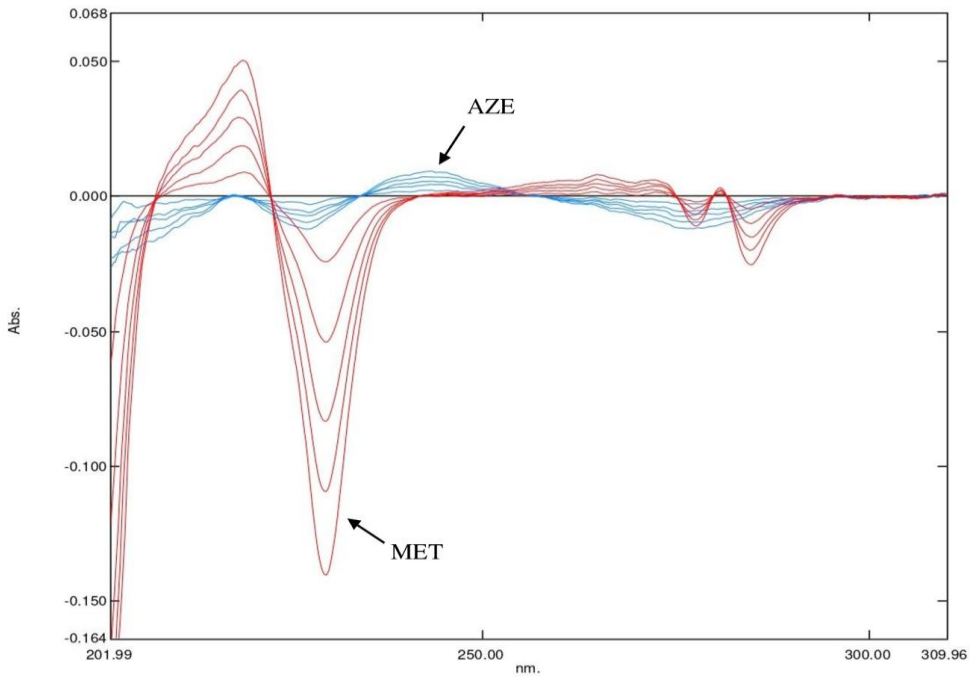

Figure 8: Overlain 1st derivative (zero crossing) UV spectra for 1st DR method.

CONCLUSION

Three different methods namely SE, AR, and 1st DR spectroscopic methods were developed for simultaneous estimation of AZE & MET in bulk. Developed methods were validated according to ICH guidelines. Projected methods were found to be simple, sensitive, precise,

accurate and cost effective. Moreover, all the developed Spectrophotometric methods require little sample preparation procedure and have wide concentration range with high sensitivity. Statistical data reveals that there is no statistically significant dissimilarity among all the three methods. Therefore, all the developed methods can be used successfully for routine quality control analysis of AZE and MET in combined tablet dosage form.

REFERENCE

1. Wellington K, Scott LJ. Azelnidipine. Drugs, 2003; 63(23): 2613–21.
2. Rele Ranjan V. Spectrophotometric estimation of azelnidipine in bulk and pharmaceutical dosage form by second order derivative methods. Journal of Chemical and Pharmaceutical Research, 2014; 6(8): 198–202.
3. Shah K, Patel M, CAPTAIN A. UV-spectrophotometric method development and validation for determination of azelnidipine in pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 2011 Jun; 4: 238–40.
4. Shah K, Patel M, CAPTAIN A. UV-spectrophotometric method development and validation for determination of azelnidipine in pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 2011 Jun; 4: 238–40.
5. Jenisha Modi, Shivangi. K.Patel, Namarata Parikh, Shreya Shah, Prasanna K. Pradhan, U.M.Upadhyay. Stability indicating Analytical method development and validation for estimation of azelnidipine. World Journal of Pharmaceutical Research, 2015; 5(2): 831–47.
6. Shah K, Patel M, CAPTAIN A. UV-spectrophotometric method development and validation for determination of azelnidipine in pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 2011 Jun; 4: 238–40.
7. Suneetha G, Venkateswarlu P, Prasad PSS. Sensitive Analysis of Azelnidipine and Related Derivative in Human Plasma by Ultra-Performance Liquid Chromatography-Tandam Mass Spectrometry. Asian Journal of Chemistry, 2013; 25(18): 10319–21.
8. Megha G. Gore *, Pratap S. Dabhade. RP-HPLC Method Development And Validation Of Azelnidipine. International Journal Of Pharmaceutical Sciences And Research, 7(12).
9. Jian-jun Z, Hong-Jian ji, Xiao-Hua Z. Determination of azelnidipine by LC-ESI-MS and its application to a pharmacokinetic study in healthy chinese volunteers. Die Pharmaze - An international Journal of Pharmaceutical Sciences, 2008; 63(8): 568–70.

10. Selvadurai Muralidharan*, Subramaniya Parasuraman, Vijayan Venugopal. Simple Validation of Azelnidipne by RP-HPLC Method. Rapports De Pharmcacie, 2015; 1(1): 43–5.
11. Rane AS, Mahajan SK. Validation And Forced Stability-Indicating Hptlc Method For Determination Of Azelnidipine. Rane et al World Journal of Pharmaceutical Research World Journal of Pharmaceutical Research SJIF Impact Factor 6 [Internet], 2016; 5(9): 1053–62. Available from: www.wjpr.net
12. Plosker GL, Clissold SP. Controlled Release Metoprolol Formulations. Drugs, 1992 Mar; 43(3): 382–414.
13. Patel DM, Patel D, Patel A, Sheth A, Shah UJ. Method Development and Validation for Simultaneous Estimation of Benidipine Hydrochloride and Metoprolol Succinate in Tablet. Journal of Drug Delivery and Therapeutics, 2019 Dec 15; 9(6-s): 28–33.
14. Seema M. Dhole*, Dinesh R. Chaple, Madhulika T. Harde. Validated UV Spectrophotometric Method for Simultaneous Estimation of Metoprolol Succinate and Amlodipine Besylate in Their Combined Tablet Dosage Form. International Journal of Analytical and Bioanalytical Chemistry, 2013; 3(3): 82–5.
15. Hinge M. Development and validation of UV spectrophotometric method for simultaneous estimation of cilnidipine and metoprolol succinate in bulk drugs and combined dosage form. Pharm Lett., 2015 Jun; 7: 299–306.
16. Kulkarni M, Vishwanathrao K, Sakarkar D. Development and validation of spectrophotometric method for determination of metoprolol succinate. International Journal of ChemTech Research, 2009 Jun; 1.
17. Patel D, Patel M. Simultaneous Estimation of Metoprolol Succinate and Olmesartan Medoxomil in Pharmaceutical Dosage Form by UV Spectroscopy. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012 Jun; 3: 935–9.
18. Brijesh S, Patel D, Ghosh S. Development of Reverse-Phase HPLC Method for Simultaneous Analysis of Metoprolol Succinate and Hydrochlorothiazide in a Tablet Formulation. Tropical Journal of Pharmaceutical Research, 2010 Jan 13; 8(6).
19. Dongre VG, Shah SB, Karmuse PP, Phadke M, Jadhav VK. Simultaneous determination of metoprolol succinate and amlodipine besylate in pharmaceutical dosage form by HPLC. Journal of Pharmaceutical and Biomedical Analysis, 2008 Feb; 46(3): 583–6.
20. Jain P, Patel M, Bari S, Surana S. Development and validation of HPTLC method for simultaneous determination of amlodipine besylate and metoprolol succinate in bulk and tablets. Indian Journal of Pharmaceutical Sciences, 2012; 74(2): 152.