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Development and Validation of Spectrophotometric Methods for Simultaneous Determination of Azelnidipine and Telmisartan in Mixture

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Abstract

The manuscript describes two simple and sensitive spectrophotometric methods for simultaneous determination of Azelnidipine and Telmisartan from mixture. First method is based on simultaneous equations. (Vierodt's method). The wavelengths selected were 253 and 295 nm for estimation of Azelnidipine and Telmisartan, respectively. Second method involves 1st order derivative spectrophotometry. The wavelengths selected were 235 nm (ZCP of Azelnidipine) for estimation of Telmisartan and 226 nm (ZCP of Telmisartan) for analysis of Azelnidipine without any interferences of sample matrix. Both methods are validated as per ICH Q2 (R1) guideline for various parameters. The linearity was found in the concentration range of 2 - 16 µg/ml for both drugs. The methods are observed to be simple and can be applicable for the routine quality control testing of Azelnidipine and Telmisartan in their combination.

Keywords: *Azelnidipine, Telmisartan, Simultaneous equations, First order derivative, mixture, Validation*

INTRODUCTION:

Azelnidipine (AZL) (Figure 1) is a dihydropyridine calcium channel blocker having a gradual onset and long-lasting hypoglycaemic effect, with little increase in heart rate (Tripathi, 2003). Telmisartan (TEL) (Figure 2) is a benzimidazole derivative and a non-peptide angiotensin II receptor antagonist with antihypertensive property (Goyal, 2009). AZL and TEL are official in Indian Pharmacopoeia (The Indian, 2018). Literature survey reveals HPLC and spectrophotometric methods for the estimation of AZL (Prabhakar, 2018; Shah, 2016 & Rele, 2014) and TEL (Patel, 2013; Jadhav, 2018) in dosage forms separately. Literature survey also reveals HPLC and spectrophotometric methods for the estimation of AZL and TEL in combination (Vangallu, 2022; Yuvasri, 2021). The present manuscript describes an alternative simple and sensitive spectrophotometric methods for the simultaneous estimation of AZL and TEL in mixture.

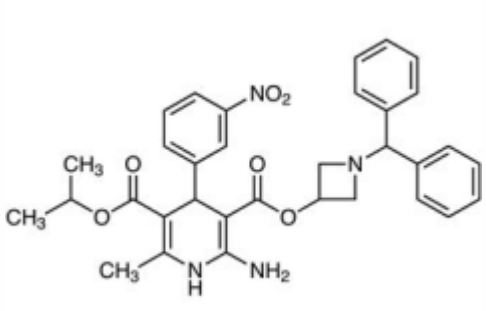

Figure 1. Structure of Azelnidipine

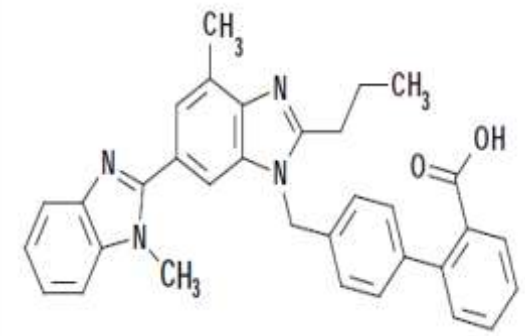

Figure 2. Structure of Telmisartan

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EXPERIMENTAL

Apparatus

A shimadzu model 1700 (Shimadzu, Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Materials and reagents

Azelnidipine (AZL) and Telmisartan (Tel) pure drug powder were supplied by Shiva Healthcare Ltd., Mehsana, Gujarat, India. Methanol (AR Grade) was procured from S.D. Fine Chemicals Ltd., Mumbai, India. Whatman filter paper no 41 (Millipore, USA) was used for filtering. The marketed tablets were purchased from the local pharmacy.

Preparation of standard solutions and synthetic mixture

Standard stock solution (500 µg/ml) & working standard solution (100 µg/ml)

Accurately weighed AZL (50 mg) and TEL (50 mg) was transferred to two different volumetric flasks (100 ml). Approximately 50 ml of methanol was added in both the flasks and sonicated for 5 min. The final volume was adjusted up to the mark in both the flasks with methanol to prepare 500 µg/ml standard stock solution of both AZL and TEL. For working standard solutions, accurately 20 ml of above solutions was shifted to two different 100 ml volumetric flasks and diluted up to the mark with methanol to prepare 100 µg/ml working standard solutions.

Synthetic mixture

The laboratory mixture was prepared by taking accurately weighed AZL (10 mg) and TEL (50 mg) along with excipients (200 mg) such as starch, magnesium stearate, lactose, talc and triturated with the help of mortar-pastel.

Method development

a) Simultaneous Equation method

It is based on the absorption of the two drugs at their absorbance maxima wavelengths. The working standard solutions were scanned in the wavelength range of 200-400 nm using methanol as blank. The satisfactory absorbance was found to be at 253 nm & 295 nm for AZL and TEL, respectively.

b) First Derivative Method

It is based on the absorption of one drug at the zero-crossing point (ZCP) of other drug and vice – versa. The working standard solutions were scanned in wavelength range of 200-400 nm using methanol as blank. The overlain UV spectra are converted to first order derivative spectra using transformation tool of the UV probe 2.0 software. The overlain derivative spectra showed ZCP of 235 and 226 nm for ALZ and TEL, respectively. Hence the wavelengths selected were 226 and 235 nm, respectively for estimation of AZL and TEL.

VALIDATION OF THE PROPOSED METHOD

Validation of the methods for parameter like linearity, precision, accuracy, limit of detection and quantification was carried out as per ICH Q2 (R1) guidelines. (ICH, 2005)

Linearity

Standard solutions were made ranging 2-16 µg/ml and calibration curves were plotted as concentration v/s absorbance at the maxima of both the drugs. As the calibration curves were plotted, the linear regression equations were calculated.

Precision (Repeatability and Reproducibility)

The precision is a measure to check the degree of reproducibility or repeatability of analytical method.

Method precision (Repeatability/Precision on replication)

Repeatability was checked by precision studies done under same conditions (same analyst, same apparatus, identical reagents and short interval of time) with same sample. The study was performed by making standard solution of AZL (2 µg/ml) and TEL (10 µg/ml) for six times and analysed as per the proposed method.

Intermediate precision (Reproducibility/interday and intraday)

Interday precision was done by assaying freshly prepared solutions of AZL and TEL in triplicate on 3 different days while intraday precision was evaluated by assaying freshly prepared AZL and TEL solutions in triplicate on the same day of 3 different concentrations.

Limit of Detection (LOD) and Limit of quantification (LOQ)

The sensitivity of the method can be shown as LOD and LOQ. The values of LOD and LOQ can be found by the following formula given in ICH guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response

S= slope of the calibration curve.

Accuracy (Recovery study)

The accuracy of the method was determined by calculating the recoveries of AZL and TEL by the standard addition method. Known amounts of standard solutions of AZL and TEL were added at 50, 100 and 150 % level to prequantified sample solutions of AZL and TEL (1 µg/ml and 5 µg/ml). The amounts of AZL and TEL were estimated by applying obtained values to the respective regression line equations.

Assay of synthetic mixture

The synthetic mixture amounts identical to 2 mg AZL and 10 mg TEL were precisely weighed and shifted to 100 ml volumetric flask. About 45 ml of methanol was added to the flask and sonicated for 10 - 15 min. The final volume was

made up with methanol and filtered by the Whatman paper. The resulting solution was suitably diluted to get final sample solution (AZL, 2µg/ml & TEL, 10µg/ml). This sample was analysed as per the developed methods and unknown concentration were computed using respective simultaneous equations in method I and calibration curves in method II.

Assay of tablets

The tablets (20) were weighed accurately and average was determined. The tablet powder equivalent to 2 mg AZL and 10 mg TEL were precisely weighed and shifted to 100 ml volumetric flask. About 45 ml of methanol was added to the flask and sonicated for 10 - 15 min. The final volume was made up with methanol and filtered by the Whatman paper. The resulting solution was suitably diluted to get final sample solution (AZL, 2µg/ml & TEL, 10µg/ml). This sample was analysed as per the developed methods and unknown concentration were computed using respective simultaneous equations in method I and calibration curves in method II.

RESULTS AND DISCUSSION

The standard solution of AZL and TEL were scanned separately between 200-400 nm against methanol as blank. Method I is based on simultaneous equations. (Vierodt's method). The wavelengths selected were 253 and 295 nm for estimation of AZL and TEL, respectively (Figure 3). Method II is based on 1st order derivative spectrophotometry. The wavelengths selected were 235 nm (ZCP of AZL) for estimation of TEL and 226 nm (ZCP of TEL) for estimation of AZL (Figure 4).

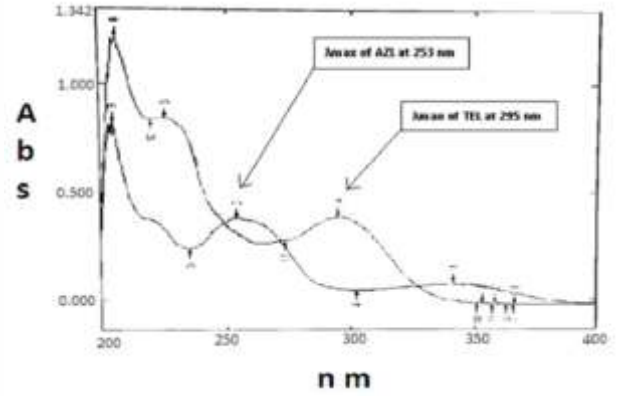

Figure 3. Overlain UV spectra of AZL and TEL in methanol

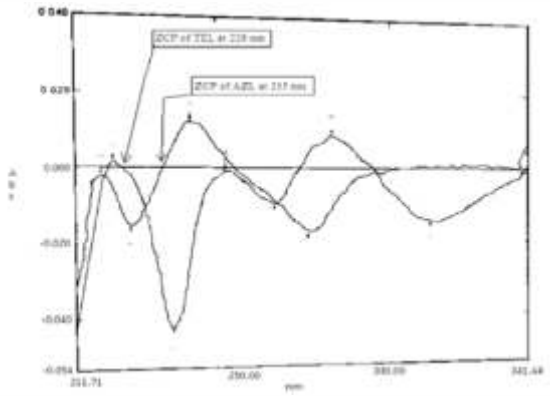

Figure 4. Overlain first order derivative spectra of AZL and TEL in methanol

The linearity is found to be linear in the range of 2 - 16 µg/ml for AZL and TEL in both methods. Repeatability was studied by calculating the %RSD for six determinations of the concentration of 2 µg/ml and 10 µg/ml for AZL and TEL, respectively under same experimental conditions. The % RSD for AZL and TEL are found to be less than 1% in both methods showed that the methods are repeatable. Intraday and inter day variations were determined by analysing three different solutions of both AZL and TEL within the same day and three different days over a period of week. The %RSD for AZL and TEL are found to be less than 2.0% in both methods indicate that the methods are precise. LOD and LOQ for both drugs were calculated theoretically using equation as per ICH guidelines. The data shows that the methods are sensitive. The assay results obtained from analysis are in good range of the drugs label claim with high reproducibility indicates suitability of methods for the simultaneous estimation of AZL and TEL from mixture and tablets without interferences of excipients. The results of recovery study with low value of standard deviations indicate that the developed methods are accurate. The overall summary of validation parameters for both methods are shown in Table 1 and 2, respectively.

Table 1: Summary of validation parameters for simultaneous equations method

Parameters	Azelnidipine		Telmisartan	
Wavelength Range (nm)	253	295	253	295
Beer's law limit (g/ml)	2-16	2-16	2-16	2-16
Regression equation (Y=mx+c)	Y = 0.060x – 0.009	Y = 0.010x +0.004	Y = 0.068x + 0.005	Y = 0.079x –0.012
Slope (m)	0.06	0.010	0.068	0.079
Intercept (c)	00.009	0.004	0.005	0.012
Correlation coefficient (r2)	0.9990	0.9980	0.9980	0.9990
Repeatability (n=6) (% RSD)	0.5745	0.3396	0.1311	0.1135
Intraday (n=3) (% RSD)	0.121 – 0.323	0.254 – 0.431	0.211- 0.354	0.321 – 0.562
Interday (n=3) (% RSD)	0.424 – 0.657	0.485 – 0.795	0.498 – 0.687	0.435 – 0.644
LOD (g/ml)	0.18	0.15	0.62	0.13
LOQ (g/ml)	0.55	0.44	1.50	0.39
Accuracy (n=3) (% Recovery ± S.D)	99.42 ± 0.78		99.74 ± 0.25	
% Assay ± S.D (n=6)	99.33 ± 0.27		99.86 ± 0.16	
% Assay ± S.D (n=6) Tablets	98.79 ± 0.43		101.3 ± 1.03	

Table 2: Summary of validation parameters for derivative spectrophotometric method

Parameters	Azelnidipine	Telmisartan
Wavelength Range (nm)	226	235
Beer's law limit (g/ml)	2-16	2-16
Regression equation (Y=mx+c)	Y = 0.005x + 0.004	Y = 0.002x + 0.001
Slope (m)	0.005	0.002
Intercept (c)	0.004	0.001
Correlation coefficient (r2)	0.9990	0.9990
Repeatability (n=6) (% RSD)	0.80	0.54
Intraday (n=3) (% RSD)	0.115 – 0.235	0.148 – 0.294
Interday (n=3) (% RSD)	0.249 – 0.495	0.561 – 1.02
LOD (g/ml)	0.67	0.58
LOQ (g/ml)	2.00	1.77
Accuracy (n=3) (% Recovery ± S.D)	99.30 ± 1.52	99.90 ± 0.32
% Assay ± S.D (n=6)	100.0 ± 0.56	99.90 ± 0.36
% Assay ± S.D (n=6) Tablets	99.12 ± 0.68	100.8 ± 0.94

CONCLUSION

The results obtained by applying the suggested procedures, have proved that the proposed methods are simple, sensitive, accurate, highly reproducible, reliable and rapid. The developed methods have been validated as per ICH guidelines and results are found to be satisfactory. The methods can be practicable applied successfully in routine analysis and quality control test for the estimation of AZL and TEL in combination without any interruption of the excipients.

Acknowledgement

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Conflict of interest

The authors have no conflicts of interest regarding this investigation.

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