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DEVELOPMENT AND VALIDATION OF LC-MS/MS METHOD FOR SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND TELMISARTAN IN TABLET AND CHARACTERIZATION OF DEGRADANT BY LC- MS/MS

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Key words

Azelnidipine, Telmisartan, LC-MS/MS method, Validation, ICH Q2 (R1) guidelines.

ABSTRACT

The objective of this study was to explore the degradation behavior of Azelnidipine and Telmisartan under acidic, basic, oxidative, photolytic and thermal stress conditions as per prescribed International Conference on Harmonization (ICH) guidelines. Azelnidipine was found to be liable under acidic and oxidative stress conditions, whereas it was stable under basic, photolytic and thermal stress conditions. Similarly, Telmisartan was found to be liable under acidic and oxidative stress conditions, whereas it was stable under basic, photolytic and thermal stress conditions. A total of two degradation products (DPs) were characterized for azelnidipine and two degradation products (DPs) were characterized for Telmisartan, and their chromatographic separation was accomplished on Hypersil, BDS, C18, (150mm x 4.6mm, 5µm) column using a mobile phase consisting buffer (pH-5) : methanol in isocratic elution mode. The ion transitions were quantified in positive mode with MRM transition of 583.300→496.200 Da for Azelnidipine and 515.100→499.500 Da for Telmisartan. All the stressed sample were subjected to ESI-MS/MS and LCMS/MS analysis. Azelnidipine and Telmisartan and its degradation products were characterized based on MRM scan mode and fragmentation patterns were obtained from ESI-MS/MS spectra. Structural elucidation of DPs of Azelnidipine and Telmisartan was achieved by comparing their fragmentation patterns with that of Azelnidipine and Telmisartan. The developed method has been validated for specificity, linearity, accuracy, precision and robustness as per ICH guideline. The method provided good linearity over the range of 0.4-1.2 µg/ml for Azelnidipine and 2.0-6.0 µg/ml

for Telmisartan with short run time of 10 min. The proposed method was successfully applied for the estimation of Azelnidipine and Telmisartan in its pharmaceutical dosage form. The LC-MS/MS method were found to be simple, accurate, robust and reproducible. The assay can can be successfully applied for routine QC analysis.

INTRODUCTION

High blood pressure (hypertension) is a common condition in which the long-term force of the blood against your artery walls is high enough that it may eventually cause health problems, such as heart disease. Blood pressure is determined both by the amount of blood your heart pumps and the amount of resistance to blood flow in your arteries. The more blood your heart pumps and the narrower your arteries, the higher your blood pressure. A blood pressure reading is given in millimeters of mercury (mm Hg).

Blood pressure is the force against blood vessel walls as the heart pumps blood. When the heart squeezes and pushes blood into the vessels, blood pressure goes up. It comes down when the heart relaxes. Blood pressure changes from minute to minute. It's affected by activity and rest, body temperature, diet, emotions, posture, and medicines. High blood pressure, or hypertension, is when the force of the blood pushing on the blood vessel walls is too high. When someone has high blood pressure: • The heart has to pump harder. • The arteries (blood vessels that carry the blood away from the heart) are under greater strain as they carry blood.

Antihypertensive are a class of drugs that are used to treat hypertension (high blood pressure). Antihypertensive therapy seeks to prevent the complications of high blood pressure, such as stroke and myocardial infarction. Evidence suggests that reduction of the blood pressure by 5 mmHg can decrease the risk of stroke by 34%, of ischaemic heart disease by 21%, and reduce the likelihood of dementia, heart failure, and mortality from cardiovascular disease. There are many classes of antihypertensive, which lower blood pressure by different means. Among the most important and most widely used medications are thiazide diuretics, calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists (ARBs), and beta blockers. Which type of medication to use initially for hypertension has been the subject of several large studies and resulting national guidelines. The fundamental goal of treatment should be the prevention of the important endpoints of hypertension, such as heart attack, stroke and heart failure. Patient age, associated clinical conditions and end-organ damage also play a part in determining dosage and type of medication administered. The several classes of antihypertensive differ in side effect profiles, ability to prevent endpoints, and cost. The choice of more expensive agents, where cheaper ones would be equally effective, may have negative impacts on national healthcare budgets. As of 2018, the best available evidence favors low-dose thiazide diuretics as the first-line treatment of choice for high blood pressure when drugs are necessary.

LC-MS/MS method [8]

In present research work, an attempt was made for development and validation of stability indicating LC-MS/MS method for characterization of forced degradation products of azelnidipine and telmisartan in bulk and pharmaceutical dosage form.

Drug identification:

The identification of azelnidipine and telmisartan standard API for experimental

work had done for confirmation of its identity, standard, quality and purity. The identification was done by taking IR, solubility, melting point determination and mass spectra.

Procedure for preparation of reagents for force degradation study:

- 0.1 N Hydrochloric acid: 0.85 ml of concentrated HCl was taken and added to 100 ml volumetric flask. Volume was made up to the mark with water
- 0.1 N NaOH: 0.4 gm. NaOH was taken and added to 100 ml flask. 75 ml water was added in flask and NaOH was dissolved. Volume was made up to the mark with water.
- 3% H2O2: 10 ml of 30%H2O2 was taken in 100 ml volumetric flask. Volume was made up to the mark with water0.1 N Hydrochloric acid: 0.85 ml of concentrated HCl was taken and added to 100ml volumetric flask. Volume was made up to the mark with water.

Procedure for Preparation of Forced Degradation Solution in Bulk to find out Degradants Compounds of Azelnidipine

1. **Acid Degradation -** Acid decomposition studies were performed by refluxing 1.0 ml of stock solution was transferred in to 100 ml of volumetric flask. One ml of 0.1 N HCl solutions was added and mixed well and put for 4 hours at 60ºC on water bath. After time period the content was cooled to room temperature. Then the volume was adjusted with diluent to get 0.8 µg/ml solution of Azelnidipine. After making final solutions, it is infused into the mass spectrometer by syringe method to identified and characterized the degradation compounds of Azelnidipine and its fragmentation pathway is identified by MRM scan (Q1→Q3). Mass spectra and its fragmentation pathway of Azelnidipine.
2. **Base Degradation** - Base decomposition studies were performed by refluxing 10 ml of stock solution was transferred in to 100 ml of volumetric flask. One ml of 0.1 N NaOH solutions was added and mixed well and put for 3 hours at 60ºC on water bath. After time period the content was cooled to room temperature. Then the volume was adjusted with diluent to get 0.8 µg/ml solution of Azelnidipine. After making final solutions, it is infused into the mass spectrometer by syringe method to identified and characterized the degradation compounds of Azelnidipine and its fragmentation pathway is identified by MRM scan (Q1-> Q3). Mass spectra and its fragmentation pathway of Azelnidipine.
3. **Oxidative Degradation** - Oxidative decomposition studies were performed by refluxing 1.0 ml of stock solution was transferred in to 100 ml of volumetric flask. One ml of 3% H2O2 solutions was added and mixed well and put for 6 hours at 60ºC on water bath. After time period the content was cooled to room temperature. Then the volume was adjusted with diluent to get 0.8 µg/ml solution of Azelnidipine. After making final solutions, it is infused into the mass spectrometer by syringe method to identified and characterized the degradation compounds of Azelnidipine and its fragmentation pathway is identified by MRM scan (Q1◊Q3). Mass spectra and its fragmentation pathway of Azelnidipine.
4. **Thermal Degradation** - Put about 10.0mg of Azelnidipine standard into petridish and place the petridish into hot air oven at 1050C for 5 days. After 5 days weigh and transfer about 10.0mg of azelnidipine powder into a 100ml volumetric flask and make up volume with diluent. Transfer 1.0ml solution into a 100ml volumetric flask and make up volume with diluent to get 0.8 µg/ml solution of Azelnidipine. After making final solutions, it is infused into the mass spectrometer by syringe method to identified and characterized the degradation compounds of Azelnidipine and its fragmentation pathway is identified by MRM scan (Q1→Q3). Mass spectra and its fragmentation pathway of Azelnidipine.
5. Photolytic Degradation Put about 10.0mg of Azelnidipine standard into petridish and place the petridish into photo stability chamber for 5 days After 5 days weigh and transfer about 10.0mg of azelnidipine powder into a 100ml volumetric flask and make up volume with diluent. Transfer 1.0ml solution into a 100ml volumetric flask and make up volume with diluent to get 0.8 µg/ml solution of Azelnidipine. After making final solutions, it is infused into the mass spectrometer by syringe method to identified and characterized the degradation compounds of Azelnidipine and its fragmentation pathway is identified by MRM scan (Q1→Q3). Mass

spectra and its fragmentation pathway of Azelnidipine.

Procedure for Preparation of Forced Degradation Solution in Bulk to find out Degradants Compounds of Telmisartan

1. **Acid Degradation** - Acid decomposition studies were performed by refluxing one ml of stock solution was transferred in to 100 ml of volumetric flask. One ml of 0.1 N HCl solutions was added and mixed well and put for 2 hours at 60ºC on water bath. After time period the content was cooled to room temperature. Then the volume was adjusted with diluent to get 4.0 µg/ml solution of Telmisartan. After making final solutions, it is infused into the mass spectrometer by syringe method to identified and characterized the degradation compounds of Telmisartan and its fragmentation pathway is identified by MRM scan (Q1→Q3). Mass spectra and its fragmentation pathway of Telmisartan.
2. **Base Degradation** - Base decomposition studies were performed by refluxing one ml of stock solution was transferred in to 100 ml of volumetric flask. One ml of 0.1 N NaOH solutions was added and mixed well and put for 4 hours at 60ºC on water bath. After time period the content was cooled to room temperature. Then the volume was adjusted with diluent to get 4.0µg/ml solution of Telmisartan. After making final solutions, it is infused into the mass spectrometer by syringe method to identified and characterized the degradation compounds of Telmisartan and its fragmentation pathway is identified by MRM scan (Q1→Q3). Mass spectra and its fragmentation pathway of Telmisartan.
3. **Oxidative Degradation** -Oxidative decomposition studies were performed by refluxing one ml of stock solution was transferred in to 100 ml of volumetric flask. One ml of 3% H2O2 solutions was added and mixed well and put for 5 hours at 60ºC on water bath. After time period the content was cooled to room temperature. Then the volume was adjusted with diluent to get 4.0 µg/ml solution of Telmisartan. After making final solutions, it is infused into the mass spectrometer by syringe method to identified and characterized the degradation compounds of Telmisartan and its fragmentation pathway is identified by MRM scan (Q1◊Q3). Mass spectra and its fragmentation pathway of Telmisartan.
4. Thermal Degradation Put about 100.0mg of Telmisartan standard into petridish and place the petridish into hot air oven at 1050C for 5 days. After 5 days weigh and transfer about 5mg of Telmisartan powder into a 100ml volumetric flask and make up volume with diluent. Transfer 1.0ml solution into a 100ml volumetric flask and make up volume with diluent to get 4.0µg/ml solution of Telmisartan. After making final solutions, it is infused into the mass spectrometer by syringe method to identified and characterized the degradation compounds of Telmisartan and its fragmentation pathway is identified by MRM scan (Q1→Q3). Mass spectra and its fragmentation pathway of Telmisartan.
5. . Photolytic Degradation Put about 100.0mg of Telmisartan standard into petridish and place the petridish into photo stability chamber for 5 days. After 5 days weigh and transfer about 5mg of Telmisartan powder into a 100ml volumetric flask and make up volume with diluent. Transfer 1.0ml solution into a 100ml volumetric flask and make up volume with diluent to get 4.0µg/ml solution of Telmisartan. After making final solutions, it is infused into the mass spectrometer by syringe method to identified and characterized the degradation compounds of Telmisartan and its fragmentation pathway is identified by MRM scan (Q1→Q3).

Chromatographic Trials

Mobile Phase	Ratio (v/v)	Retention Time	Remarks
Telmisartan and Azelnidipine in Water: Methanol	50:50	1.82 (Telmisartan)	Only Telmisartan peak is observed and peak shape is not sharp.
Telmisartan and Azelnidipine in Water: Methanol: 0.1% formic acid	50:50	1.82 (Telmisartan) 9.20 (Azelnidipine)	Both Telmisartan and Azelnidipine peak is observed. Azelnidipine peak show tailing.
Telmisartan and Azelnidipine in Water: Acetonitrile	50:50	2.0 (Telmisartan) 10.15 (Azelnidipine)	Both Telmisartan and Azelnidipine peak is observed. Still Azelnidipine peak show tailing.
Telmisartan and Azelnidipine in Water: Acetonitrile: 0.1% formic acid	50:50	1.99 (Telmisartan) 5.60 (Azelnidipine)	Both Telmisartan and Azelnidipine peak is observed. Still Azelnidipine peak show tailing.
Telmisartan and Azelnidipine in Buffer, pH 5.0: Methano	50:50	1.76 (Telmisartan) 6.74 (Azelnidipine)	Both Telmisartan and Azelnidipine peak is observed. Both the peak is sharp and does not show tailing.

CONCLUSION:

- There is no analytical work has been available regarding LC-MS/MS method for Azelnidipine and Telmisartanin a literature. Data regarding behavior of drug in chromatographic conditions and other relevant analytical properties are not available.
- The objective of this study was to study the degradation behaviour of Azelnidipine and Telmisartan under acidic, basic, oxidative, photolytic and thermal stress conditions as per prescribed International Conference on Harmonization (ICH) guidelines.
- Azelnidipine and Telmisartan was degraded under acidic and oxidative stress conditions.
- Azelnidipine was degraded under acidic and oxidative stress condition and Telmisartan was degraded under acidic and basic stress condition.
- A total of two degradation products (DPs) were characterized for azelnidipine and two degradation products (DPs) were characterized for Telmisartan, and their chromatographic separation was accomplished on Hypersil, BDS, C_{18}, (150mm x 4.6mm, 5µm) column using a mobile phase consisting buffer (pH-5) : methanol in a isocratic elution mode.
- The ion transitions were quantified in positive mode with MRM transition of 583.300→496.200 Da for Azelnidipine and 515.100→499.500 Da for telmisartan.
- Retention time of Azelnidipine and Telmisartan were found to be 6.8 min and 1.7 min respectively with a flow rate of 1.0 ml/min.

REFERENCES

1. *Mendis S, Puska P, Norrving B.World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization.38 from the original on 2014,38.*
2. Adamsson Eryd S, Gudbjornsdottir S, Manhem K, Rosengren A, Svensson AM, Miftaraj M Blood pressure and complications in individuals with type 2 diabetes and no previous cardiovascular disease: National population based cohort study. BMJ. 2016,354,4070.
3. Arima H, Chalmers J, Woodward M, Anderson C, Rodgers A, Davis S et al. Lower target blood pressures are safe and effective for the prevention of recurrent stroke: The Progress trial. Journal of Hypertension. 2006,24(6),1201–8
4. Arnold J, Yusuf S, Young J, Mathew J, Johnstone D, Avezum A et al. Prevention of heart failure in patients in the Heart Outcomes Prevention Evaluation study. Circulation. 2003,107(9),1284–1290
5. Asayama K, Ohkubo T, Yoshida S, Suzuki K, Metoki H, Harada A et al. Stroke risk and antihypertensive drug treatment in the general population, The Japan arteriosclerosis longitudinal study. Journal of Hypertension. 2009, 27(2),357–64
6. Baker S, Priest P, Jackson R. Using thresholds based on risk of cardiovascular disease to target treatment for hypertension: Modelling events averted and number treated. BMJ. 2000,320(7236),680–685
7. Tripathi KD. Pharmacological Classification of Drugs; 6th Edition, Jaypee Brothers Medical Publishers, India, 2021, pp 1170-1179.