STABILITY INDICATING RELATED SUBSTANCES METHOD FOR BISOPROLOL AND CILNIDIPINE IN BULK AND PHARMACEUTICAL DOSING TYPES AND ITS DEGRADENTS WERE CHARACTERISED BY USE OF LC-MS/MS

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Abstract

A reliable, simple yet consistent RP-HPLC method has been developed and verified to combined dosage form of Bisoprolol, Cilnidipine, their relevant substances and characteristic of the degradants by LC-MS/MS. The process used was optimized by using X-Bridge phenyl column (150mmx4.6mm, 3.5 μ) with a gradient elution mode of mobile phase consists of methanol and 0.1% tri ethyl amine of pH-2.5. The developed RP-HPLC method shows strong linearity in the range of concentrations 2-30 μ g/ml of Bisoprolol, 4-60 μ g/ml of Cilnidipine and 0.1-1.5 μ g/ml of Bisoprolol and Cilnidipine impurities. Percentage of regeneration and percentage RSD values were found to be within the allowable limit. The developed method was found to be applicable for routine analysis and utilized for the calculation of both active ingredients and their impurities in tablet dosage form.

Key words: Bisoprolol, Cilnidipine, RP-HPLC, Forced degradation, LC-MS/MS

1. Introduction

Bisoprolol is used to medicate for cardiovascular disease which involve the heart or blood vessels which may include high blood pressure. Bisoprolol marketed under the trade name Zebeta, among others is a beta blocker drug^[1-2] most commonly used for heart disease^[3-4]. This includes in particular high blood pressure^[5-6], chest pain^[7-8] from insufficient blood flow to the heart, and heart failure^[9]. It is taken by the mouth. Common side effects include headache^[10], feeling tired, diarrhea^[11] and swelling of the legs. More serious side effects include worsening asthma^[12-13], blocking the ability to recognise low blood sugar^[14] and worsening

heart failure. There are concerns that use during pregnancy may be harmful to the child. Bisoprolol is a selective type in the beta blocker family of drugs.

Cilnidipine is a blocker of the calcium channel^[15]. Cilnidipine is approved for the treatment of hypertension^[16-17]. It is a calcium antagonist, followed by an L-type and N-type calcium channel blocking mechanism. Cilnidipine, unlike most calcium antagonists, can work on the N-type calcium channel^[18-19] in addition to its role on the L-type calcium channel^[20].

2. Materials and Method

Materials and Reagents

Ortho phosphoric acid, water, methanol, acetonitrile, Tri ethyl amine were obtained from Merck India Ltd, Worli, Mumbai, India. Bisoprolol, Cilnidipine and its impurities are acquired from Glenmark pharmaceuticals Company, Bombay.

Equipment

HPLC (Waters alliance e-2695 consisting of quaternary pump) equipped with a photo diode array detector was used in the isolation, detection and quantitative estimation of bisoprolol and Cilnidipine and it was associated with four impurities. Stationary phase of the column used is X-bridge phenyl (150mmx4.6mm, 5 microns). The out put signal has been measured and evaluated using Empower 2.0 software. Ultrasonic bath has been used in the preparation of sample purchased in Labman, India. Samples and the weighing standards have been carried out on the endeavour analytic balance.

LC-MS/MS terms and conditions

The molecular weight of newly developed degradation products were detected using HPLC e-2695 machine fitted with 2998 PDA detector and an MS mass detector. AB SCIEX software has been used for data acquisition and processing. A splitter was put in front on the ESI source, allowing only 35% of the data to be entered. Standard operating source conditions for bisoprolol and Cilnidipine scanning in positive ESI mode were optimised as follows; fragmentor voltage was set at 80V, capillary voltage at 3000V, skimmer at 60V, nitrogen was used as a drying and nebulizing gas (45 psi). Ultra high pure nitrogen gas has been used as a collision gas.

Buffer preparation

1 ml of tri ethyl amine is dissolved in 1 lt of HPLC grade water and change pH-2.5 with ortho phosphoric acid. Filter the solution with 0.45µ filter paper.

Diluent

Methanol was used as diluent.

Preparation of Regular stock solution

Weigh exactly 20 mg of bisoprolol and 40 mg of Cilnidipine standards and transfer to a 100 ml volumetric flask, then add 70 ml of methanol diluent and sonicate for 15 min to dissolve the content and make up the diluent mark.

Preparation of Stock solution for Impurity

Weigh accurately 5 mg each of bisoprolol impurity-A, bisoprolol impurity-B and Cilnidipine impurity-1, Cilnidipine impurity-2 standards and transfer into 100 ml flask, then add 70 ml of methanol diluent and sonicate for 20 min to dissolve the content and make up the mark with same diluent. Further dilute 2 ml of the above solution to 10 ml.

Preparation of standard solution

Transfer 5 ml of the standard stock and 5 ml of impurity stock solution to 50 ml volumetric flask and made up to the mark with diluent. Filter with 0.45 micron filter paper.

Conditions of chromatography

An instrument of the HPLC system (waters alliance e-2695) connected to the LC-MS/MS (Qtrap 5500 triple quadrupole) was used to develop and validate the method. The compound and its associated impurities were separated by using X-bridge phenyl column with dimensions of 150mmx4.6mm, 3.5 microns. The peaks were separated by using gradient elution mode with a flow 1 ml/min of mobile phase containing methanol and tri ethyl amine. UV detection was captured at 224 nm, injection volume was fixed at 10 µl. Table 1 shows the gradient programme.

Table 1. Gradient programme

Time (min)	Mobile phase-A (Methanol)	Mobile phase-B (buffer)
0	70	30
5	60	40
10	40	60
12	70	30
17	80	20

Method validation

System suitability

Machine fitness parameters have been measured to check the efficiency of the model. Parameters including USP plate count, USP tailing and percent RSD were calculated and found to be within the limits.

Specificity

Specificity is the capacity to test the analyte unmistakably in the absence of many other materials that might be required to have been present in a sample and the standard. It was checked by examining chromatograms of blank samples and samples spiked with bisoprolol and Cilnidipine and their impurities.

Accuracy

Accuracy is the proximity to the true value of the test results obtained by the method. The recovery studies were evaluated at three different levels of concentration. At each level a minimum of three injections were given and the number of the drug existing, that proportion of recovery and the associated standard deviation were measured.

Precision

The consistency of the analytical process is the degree of consensus between the actual testing results. Analysis of the multiple sampling of the homogeneous sample was studied. The reliability of the present procedure was tested in terms of reproducibility, intraday and interday variations. It was checked by analyzing the samples at different time intervals on the same day as well as on different days.

Linearity and range

Linearity of the test technique has been its ability to obtain results which were inversely proportional to the quantity of the analyte in a sample within a given range. The calibration curve was prepared using peak area versus regular solution concentration and the regression

equations was determined. The least square formula was used to measure the slope, intercept and coefficient of correlation.

Robustness

The robustness of an analytical technique is a measure of its ability to remain uninvolved yet deliberate changes in process parameters and to show its reliability during regular use. The robustness analysis was conducted by injecting standard solution into the HPLC system and by altering chromatographic conditions like flow rate (± 0.2 ml/min), organic matter content with in mobile phase ($\pm 10\%$). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

Degrading stress

The peaks in the chromatogram should agree. ICH guidance Q1(A) R2 was performed in conjunction with stress degradation experiments. The peaks of degradation should be well distanced and at least 1.0 resolution between peaks. For the largest peaks to go over, a separation must occur. A degradation of around 20 percent has been attained via several various stress conditions like acid, alkali, peroxide, reduction, thermal and photo in what is known as a forced degradation experiment.

3. Results and Discussion

Optimization of method

Development pathways have been under taken to achieve good resolution between bisoprolol, Cilnidipine and its impurities. Several proportions of buffer and water in the mobile phase with isocratic and gradient modes have been tested to optimize chromatographic conditions. However, at each trial the mobile phase composition was modified in order to improve resolution and also to achieve acceptable retention times. In the end 0.1 percent tri ethyl amine and methanol in gradient elution was selected as the result of a higher response to the production of pharmaceutical ingredients and their impurities and the use of methanol as diluent. Various stationary phases such as C₈, C₁₈, Cyano and phenyl columns have been used during the optimization of the method. On the basis of these trials it was found that the peak shapes of the active ingredients and all impurities were relatively good on the X-bridge phenyl 150mmx4.6mm, 3.5 μ column connected to a PDA detector. The mobile phase flow rate was 1.0 ml/min. UV detection was performed at 224 nm to achieve sufficient sensitivity between the analyte and all impurities. Under these conditions, the best response between impurities and analyte was achieved when impurity spiked solution was injected. The resolution, theoretical plate count, tailing factor and percent RSD of bisoprolol and Cilnidipine and its impurities are within an acceptable range indicate that the method is accurate. The method developed was validated in accordance with the ICH guidelines, with all results within the limit.

Method validation

System suitability

System suitability parameters such as USP plate count of bisoprolol was 3652 and Cilnidipine was 5984, USP tailing of bisoprolol was 1.01 and Cilnidipine was 1.06, percent RSD values of bisoprolol was 0.24 and Cilnidipine was 0.49. The measured values were found to be within the limit. Figure 1 represents the chromatogram of standard.

Figure 1. Chromatogram for Standard

Specificity

Sample and standard solutions were analysed individually to examine the interference according to the placebo test method. The following figures show that the active ingredients were well separated from the blanks and their excipients, and there was no placebo interference with the main peak, so the method is specific. Figure 2 shows the chromatogram of blank.

Figure2. Chromatogram for Blank

Linearity

The linearity plots of bisoprolol, Cilnidipine and its related impurities were drawn by taking area of the peak on Y-axis and their respective concentrations on X-axis. Test solutions were prepared for the related impurity stock solution substance method at different concentration levels. The linearity range was found to be 2 µg/ml to 30 µg/ml for bisoprolol, 0.1 µg/ml to 1.5 µg/ml of bisoprolol imp-1, 0.1-1.5 µg/ml of bisoprolol imp-2, 4 µg/ml to 60 µg/ml of Cilnidipine, 0.1 µg/ml to 1.5 µg/ml of Cilnidipine imp-1 and 0.1 µg/ml to 1.5 µg/ml of Cilnidipine imp-2. Under optimal chromatographic conditions, linear relations between the peak areas and the corresponding concentrations were found. Correlation coefficients for all components were under the limit. Figure 3 shows the linearity plots of bisoprolol, Cilnidipine

and its related impurities. Table 2 gives the linearity data of bisoprolol, Cilnidipine and their related impurities.

Table 2. Results of Linearity

Linearity	Area						Conc. (µg/ml)		
	Bis	Cil	Bis I-1	Bis I-2	Cil I-1	Cil I-2	Bis	Ciln	All Imps
Lin-1	723654	821510	55412	65177	43510	39812	2	4	0.1
Lin-2	1859642	2179127	126534	172712	99912	97837	5	10	0.25
Lin-3	3532987	4225672	252301	338507	222567	182790	10	20	0.5
Lin-4	7023654	8296959	523695	679429	400695	386985	20	40	1
Lin-5	8763251	10325244	631254	807194	502524	471081	25	50	1.25
Lin-6	10425961	12338079	754210	1021180	615807	572775	30	60	1.5

Bis-Bisoprolol

Cil-Cilnidipine

Bis I-1-Bisoprolol Impurity-1

Cil I-1-Cilnidipine Imp-1

Bis I-2-Bisoprolol Impurity-2

Cil I-2-Cilnidipine Imp-2

All Imps-Bisoprolol Imp-1, Bisoprolol Imp-2, Cilnidipine Imp-1, Cilnidipine Imp-2

Figure 3. Linearity Chromatograms For Bisoprolol, Cilnidipine and their Impurities

Accuracy

Accuracy was conducted by triplicate by analyzing active pharmaceutical ingredients (bisoprolol and Cilnidipine) sample solution spiked with known amounts of all related impurities at three concentration levels of 50, 100 and 150 percent of each at a specified limit. The percentage recovery for all impurities was calculated and ranges from 99.8- 100.9 percent. Table 3 represents the accuracy data of bisoprolol and Cilnidipine.

Table 3. Results of accuracy

S. No	% Level	% Recovery	
		Bisoprolol	Cilnidipine
1	50	100.4	99.9
2	100	100.8	99.6
3	150	99.5	100.5

Precision

The precision of the analytical technique is the degree of proximity of a series of measurements derived from multiple samples of a homogeneous mixture. Precision of the related substance method was achieved by injecting six individual determinations of bisoprolol and Cilnidipine spiked with that of their related impurities and the percent RSD

was calculated for each impurity and these results showed that the method is precise under the specified experimental conditions. Table 4 represents the precision data of bisoprolol and Cilnidipine.

Table 4. Precision results

Sample Number	% Purity			
	Intra day Precision		Inter day Precision	
	Bisoprolol	Cilnidipine	Bisoprolol	Cilnidipine
1	94.62	94.13	94.35	94.23
2	94.54	94.16	94.33	94.25
3	94.55	94.18	94.37	94.26
4	94.61	94.15	94.32	94.21
5	94.58	94.11	94.36	94.27
6	94.57	94.12	94.31	94.28

LOD and LOQ

LOD and LOQ of the compounds were carried out by progressively injecting lower concentrations of the standard solutions. The LOD concentrations of bisoprolol, its impurities 1, 2 were 0.061, 0.003, 0.003 µg/ml and their s/n values were 6, 4, 3 and Cilnidipine, its impurities were 0.12, 0.003, 0.003 µg/ml and their s/n values were 7, 3, 3. LOQ values for bisoprolol, its impurities were 0.2, 0.01, 0.01 µg/ml and their s/n values were 25, 23, 23 and the LOQ values for Cilnidipine, its impurities were 0.4, 0.01, 0.01 and their s/n values were 27, 23, 23 respectively.

Robustness

In order to measure the robustness of the developed method, the conditions of the experiment have been deliberately changed, such as flow rate, organic percentage of the mobile phase. In all these varied conditions, the resolution of impurities between active pharmaceutical ingredients were not significantly affected and there was no significant influence on retention time, plate count and tailing factor. Therefore the method was robust. The results of robustness was shown in table 5.

Table 5. Results of Robustness

Parameter name	% RSD for purity	
	Bisoprolol	Cilnidipine
Flow (0.8 ml/min)	0.64	0.86
Flow (1.2 ml/min)	0.38	0.41
Org Phase (-10%)	0.57	0.78
Org Phase (-10%)	0.29	0.34

Degradation studies

Forced degradation samples under different conditions were analysed at an initial concentration of 20 µg/ml of bisoprolol and 40 µg/ml of Cilnidipine by spiking with known impurities using a PDA detector to ensure homogeneity of bisoprolol and Cilnidipine peaks. Degradation studies were conducted as follows: hydrolysis in acidic medium (1N HCl at 60°C for 15 min.), hydrolysis in basic medium (1N NaOH at 60°C for 15 min.), peroxide degradation (30% H₂O₂ at 30 min), reduction degradation (30 percent NaHCO₃ at 60°C for 15 min.). Results were provided in table 6. Using LC-MS/MS, the degradants were characterized. Figure 4 represents the degradation schemes of bisoprolol and Cilnidipine and the mass spectras of DPs were shown in figure 5-11.

Table 6. Results of Forced degradation

Degradation condition	% purity of Bisoprolol	% purity of Cilnidipine
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Acid degradation	78.62	75.19
Alkali degradation	75.36	78.32
Peroxide degradation	72.17	73.65
Reduction degradation	98.17	97.56
Thermal degradation	96.34	95.19
Photolytic degradation	82.45	81.58
Hydrolysis degradation	77.36	75.36

Chromatograms For Bisoprolol, Clinidipine and their Impurities

4(A)

4(B)

Figure 4. Forced Degradation Schemes

Characterization of DPs by LC-MS/MS:

Scheme-1:

Bisoprolol degradation product-1 was fragmented under acid degeneration conditions as shown in Scheme 1. Extensive spectra can be seen for the loss of C_3H_8O at m/z -244.09, for

the loss of C_3H_8O at m/z -186.05, and for the loss of $C_3H_7ClO_2$ at m/z -78.05 (both from m/z 244.09). The suggested approach has been validated by MS/MS studies and precise mass measurements.

Degradation Product-1 fragmentation pathway

Scheme-2:

Bisoprolol degradation product -2 fragmentation pathway is shown in Scheme 2 under Alkali degeneration conditions. At m/z -182.09 (loss of C_3H_9N) and m/z -108.05 (loss of $C_3H_8O_2$ from m/z 182.09), the spectrum shows copious product ions. The suggested approach has been validated by MS/MS studies and precise mass measurements.

Degradation product-2 fragmentation pathway

Scheme-3:

Under the conditions of Peroxide degeneration, the fragmentation mechanism of Bisoprolol degradation product -3 was observed. C_3H_8O , C_3H_8O , and $C_3H_8O_3$ are all lost at m/z -226.12, m/z -168.08, and m/z -78.05, respectively, according to the spectrum. With the use of MS/MS studies and precise mass measurements, the suggested methodology has been proven.

Degradation product-3 fragmentation pathway

Scheme-4:

Depicts the breakdown of Bisoprolol degradation product -4 under reduction degeneration circumstances. A large number of product ions can be seen in the spectrum at m/z-250.16 (loss of C_3H_9N), m/z-194.13 (loss of C_3H_6O), and m/z-78.05 (loss of $C_6H_{14}O_2$). The suggested approach has been validated by MS/MS studies and precise mass measurements.

Degradation product-4 fragmentation pathway

Scheme-5:

When Cilnidipine was degraded in acidic and alkaline settings, the fragmentation mechanism of -5 was seen. $C_6H_4N_2$ is lost at m/z-431.05, m/z-390.16 (loss of $C_2H_2O_2Cl_3$), m/z-269.14 (loss of $C_6H_4NO_2$ from m/z 390.16), m/z-193.11 (loss of C_6H_5 from m/z 269.14), m/z-109.09 (loss of $C_4H_4O_2$ from m/z 193.11). The suggested approach has been validated by MS/MS studies and precise mass measurements.

Degradation product-5 fragmentation pathway

Scheme-6:

Cilnidipine degradation product -6 was fragmented under Peroxide degeneration conditions, as seen in Scheme 6. It's clear that the m/z -332.14 (loss of $C_4H_8O_3$), m/z -230.11 (loss of $C_4H_8O_3$), m/z -109.09 (loss of $C_6H_4NO_2$), and m/z -313.15 (loss of $C_6H_4NO_2$ from m/z 434.17) are the most common product ions in the spectrum. With the use of MS/MS studies and precise mass measurements, the suggested methodology has been proven.

Degradation product-6 fragmentation pathway

Scheme-7:

Under reduced degeneration conditions, the fragmentation process of Cilnidipine degradation product -7 is shown in Scheme 7. m/z -328.14 (loss of C_6H_5), m/z -207.13 (loss of $\text{C}_6\text{H}_4\text{NO}_2$), m/z -123.11 (loss of $\text{C}_4\text{H}_4\text{O}_2$), m/z -244.12 (loss of $\text{C}_{10}\text{H}_9\text{O}_2$), are the most common product ions. With the use of MS/MS studies and precise mass measurements, the suggested methodology has been proven.

Degradation product-7 fragmentation pathway

Figure 5. Mass spectra of DP-1

Figure 6. Mass spectra of DP-2

Figure 7. Mass spectra of DP-3

Figure 8. Mass spectra of DP-4

Figure 9. Mass spectra of DP-5

Figure 10. Mass spectra of DP-6

Figure 11. Mass spectra of DP-7

4. Conclusion

This paper presents a simple, selective, validated and well defined stability indicating gradient RP-HPLC method for the quantitative determination of bisoprolol and Cilnidipine, as well as their chromatographic impurities. All degradation products formed during stress conditions and associated impurities of active pharmaceutical ingredients are well separated and the peaks have been well resolved and separated with an appropriate retention time, indicating that the proposed method has been shown to be fast, simple, feasible and affordable for robustness. This developed method can therefore be used for routine analysis of the production samples and to check the quality of the drug samples during stability studies.

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Conflicts of interest

Authors declare that there was no conflicts of interest.

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5. References

- [1] Zipursky, JS., Macdonald, EM., Luo, J., Gomes, T., Mamdani, MM., Paterson, JN., Jurrlink, DN. 2017. Lipophilic β -blockers and suicide in the elderly. *Journal of clinical psychopharmacology*, 37, 381-384.
- [2] Morales, Daniel R., Jackson, Cathy., Lipworth, Brian J., Donnan, Peter T., Guthrie, Bruce. 2014. Adverse respiratory effect of acute β -blocker exposure in asthma. *Chest*. Elsevier BV, 145, 779-786.
- [3] Kwok, CS., Pradhan, A., Khan, MA., Anderson, SG., Keavney, BD., Myint, PK., et al. 2014. Bariatric surgery and its impact on cardiovascular disease and mortality, a systematic review and meta analysis. *International journal of cardiology*, 173, 20-8.

- [4] Liu, Wei., Cao, Yubin., Dong, Li., Zhu, Ye., Wu, Yafei., Lv, Zongkai., Ihezor-Ejiofor, Zipporah., Li, Chunjie. 2019. *Peridontal therapy for primary or secondary prevention of cardiovascular disease in people with periodontitis. The cochrane database of systematic reviews*,12, CD009197.
- [5] Poulter, NR., Prabhakaran, D., Caulfield, M. 2009. *Hypertension. Lancet*,386, 801-12.
- [6] Palatini, P., Julius, S. 2009. *The role of cardiac autonomic function in hypertension and cardiovascular disease. Current hypertension reports*,11,199-205.
- [7] Schey, R., Villarreal, A., Fass, R. 2007. *Noncardiac chest pain. Current treatment. Gastroenterology & Hepatology*,3, 255-62.
- [8] Backus, BE., Six, AJ., Kelder, JC., Bosschaert, MA., Mast, EG., Mosterd, A., et al. 2013. *A prospective validation of the HEART score for chest pain patients at the emergency department. International journal of cardiology*,168, 2153-8.
- [9] Mahtani, KR., Heneghan, C., Onakpoya, I., Tierney, S., Aronson, JK., Roberts, N., et al. 2018. *Reduced salt in take for Heart failure. A systematic review. JAMA internal medicine*, 178, 1693-1700.
- [10] Charles, A. 2013. *Vasodilation out of the picture as a cause of migraine head-ache. Lancet Neurol*,12, 419-420.
- [11] Das, S., Jayaratne, R., Barrett, KE. 2018. *The role of iron transporters in the pathophysiology of infectious diarrhea. Cellular and molecular gastroenterology and hrpatology*,6, 33-45.
- [12] Van de Loo, KF., van Gelder, MM., Roukema, J., Roeleveld, N., Merkus, PJ., Verhaak, CM. 2016. *Prenatal maternal psychological stress and childhood asthma and wheezing, a meta analysis. A European respiratory journal*,47, 133-46.
- [13] Martinez, FD. 2007. *Genes, Environments, development and asthma: a reappraisal. The European respiratory journal*, 29, 179-84.
- [14] Yanai, H., Adachi, H., Katsuyama, H., Moriyama, S., Hamasaki, H., Sako, A. 2015. *Causative antidiabetic drugs and the underlying clinical factors for hypoglycemia in patients with diabetes. World journal of diabetes*, 6, 30-6.
- [15] Sica, Domenic A. 2003. *Calcium channel blocker related peripheral edema. Can it be resolved. The journal of clinical hypertension Wiley*,5, 291-295.
- [16] Hernandorena, I., Duron, E., Vidal, JS., Hanon, O. 2017. *Treatment options and considerations for hypertensive patients to prevent dementia. Expert opinion on pharmacotherapy (review)*,18, 989-1000.
- [17] Go, AS., Bauman, MA., Coleman King, SM., Fonarow, GC., Lawrence, W., Williams KA., Sanchez, E. 2014. *An affective approach to high blood pressure control; a science advisory from the American Heart Association, the American college of cardiology, and the centers for disease control and prevention. Hypertension*,63, 878-85.
- [18] Molderings, GJ., Likungu, J., Gothert, M. 2000. *N-type calcium channels control sympathetic neurotransmission in human heart atrium. Circulation*,101, 403-7.
- [19] Adams David, J., Berecki, Geza. 2013. *Mechanisms of conotoxin inhibition of N-type calcium channels. Biochimica et Biophysica Acta (BBA) Biomembranes*,1828,1619-1628.
- [20] Shaw, RM., Colecraft, HM. 2013. *L-type calcium channel targetting and local signalling in cardiac myocytes. Cardiovascular research*,98, 177-86.