July - Sep © 2015 Transactions; 2(7): 337-345

International Journal of Research and Applications

ISSN (online): 2349-0020 ISSN (print): 2394-4544 http://www.ijraonline.com/

Research Article

Validation and stability studies of developed HPLC method for estimation of amlodipine and perindopril in bulk and pharmaceutical dosage form

[1] Jagadeesh Kumar Ega and [2] Ravinder Vadde *

Corresponding Author:
jkjagadeeshkumare@gmail.com

DOI:
http://dx.doi.org/
10.17812/IJRA.2.7(58)2015

Manuscript:
Received: 18th July, 2015
Accepted: 31st Aug, 2015
Published: 20th Sep, 2015

Publisher:
Global Science Publishing Group, USA
http://www.globalsciencepg.org/

ABSTRACT

A HPLC method has been developed for simultaneous estimation of Amlodipine and Perindopril using Hiber Purosper 150 x 4.6 mm, 5, mobile phase Buffer and Acetonitrile (65:35), detection wavelength at 211 nm, at flow rate of 0.8 ml/min at retention time 4.5min for Amlodipine and Perindopril. The HPLC method developed was validated by performing the various method validation parameters like LOD, LOQ, linearity and range, precision, specificity, accuracy, robustness and system suitability parameters. Since the HPLC method has been developed, validation of method by using various parameters was performed to ensure that the performance characteristic of the method meets the requirements for the intended analytical applications. Amlodipine and Perindopril are used to treat hypertension and coronary artery disease. Perindopril also used in congestive heart failure, to prevent diabetic nephropathy.

Keywords: Calcium channel blocker, home blood pressure, left ventricular hypertrophy, Amlodipine, coronary artery disease, cost effectiveness, Perindopril and diabetic nephropathy.

[1] Assistant Professor, [2] Professor, [1 2] Dept., of Chemistry,
[1] Christu Jyothi Institute of Technology and Sciences,
[1] Affiliations: NBA, NAAC & JNTUH, Jangaon, Warangal, Telangana – 506 167.
[2] Kakatiya University, Warangal, Telangana, India – 506 009.

IJRA - Year of 2015 Transactions:
Month: July - September
Volume – 2, Issue – 7, Page No's:337-345
Subject Stream: Chemistry

Paper Communication: Author Direct

Paper Reference Id: IJRA-2015: 2(7)337-345

DOI: 10.17812/IJRA.2.7(58)2015

International Journal of Research and Applications
July-Sep © 2015 Transactions 2(7):337-345

eISSN : 2349 - 0020
pISSN: 2394 - 4544

CHEMI STRY RESEARCH A RTICLE

Validation and stability studies of developed HPLC method for estimation of amlodipine and perindopril in bulk and pharmaceutical dosage form

[1] Jagadeesh Kumar Ega and [2] Ravinder Vadde *

[1] Assistant Professor, [2] Professor , [1 2] Dept of Chemistry,
[1] Christu Jyothi Institute of Technology and Sciences,
[1] Affiliations: NBA, NAAC & JNTUH, Jangaon, Warangal, Telangana – 506 167.
[2] Kakatiya University, Warangal, Telangana, India – 506 009.
[1] jkjagadeeshkumare@gmail.com, [2] ravichemku@yahoo.co.in

ABSTRACT

A HPLC method has been developed for simultaneous estimation of Amlodipine and Perindopril using Hiber Purosper 150 x 4.6 mm, 5, mobile phase Buffer and Acetonitrile (65:35), detection wavelength at 211 nm, at flow rate of 0.8 ml/min at retention time 4.5min for Amlodipine and Perindopril. The HPLC method developed was validated by performing the various method validation parameters like LOD, LOQ, linearity and range, precision, specificity, accuracy, robustness and system suitability parameters. Since the HPLC method has been developed, validation of method by using various parameters was performed to ensure that the performance characteristic of the method meets the requirements for the intended analytical applications. Amlodipine and Perindopril are used to treat hypertension and coronary artery disease. Perindopril also used in congestive heart failure, to prevent diabetic nephropathy.

Keywords: Calcium channel blocker, home blood pressure, left ventricular hypertrophy, Amlodipine, coronary artery disease, cost effectiveness, Perindopril and diabetic nephropathy.

1. INTRODUCTION

Analytical method is a specific application of a technique to solve an analytical problem. The use of instrumentation is an exciting and fascinating part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied science. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air. The manufacture of materials, whose composition must be known precisely, is to be monitored by analytical instruments.

The methods (HPLC, GLC, NMR and Mass Spectroscopy) of choice for assay involve sophisticated equipment that are very costly and pose problems of maintenance. Hence they are not in the reach of most laboratories and small-scale industries, which produce bulk drugs and pharmaceutical formulations. Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C, and generally non-polar atomic bonds, such as S-S and others) results in a longer retention time because it increases the molecule's non-polar surface area, which is non-interacting with the water structure. On the other hand, polar groups, such as -OH, $-NH_2$, COO^- or $-NH_3^+$ reduce retention as they are well integrated into water. Very large molecules, however, can result in an incomplete interaction between the large analyte surface and the ligand's

alkyl chains and can have problems entering the pores of the stationary phase.

2. DRUG PROFILE OF AMLODIPINE AND PERINDOPRIL

A) AMLODIPINE

Amlodipine is a long-acting calcium channel blocker dihydropyridine (DHP) class used as an antihypertensive and in the treatment of angina pectoris. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance there by reducing blood pressure in angina, amlodipine increases blood flow to the heart muscle , as the cardiac calcium channels are not of the dihydropyridine-type. Amlodipine is used in the management of hypertension and coronary artery disease.

Amlodipine is a white to off-white, crystalline powder that is very slightly soluble in water, sparingly soluble in acetone and soluble in methanol.

B) PERINDOPRIL

Perindopril is a long-acting ACE inhibitor. It is used to treat high blood pressure, heart failure or stable coronary artery disease in form of perindopril arginine or perindopril erbumine. According to the Australian government's Pharmaceutical Benefits Scheme website, based on data provided to the Australian Department of Health and Aging by the manufacturer, *perindopril arginine* and *perindopril erbumine* are therapeutically equivalent and may be inter changed without differences in clinical effect.

Solubility Perindopril is a white to off-white, crystalline powder that is very slightly soluble in water, sparingly soluble in acetone and soluble in methanol.

Dosage and Administration

For Perindopril as treatment for hypertension, the initiation dose is 5 mg perindopril arginine (or 4 mg perindopril erbumine) once daily, then the dose may be increased to 10 mg perindopril arginine (or 8 mg perindopril erbumine) after 1 month of treatment to improve blood pressure control or in case of concomitant stable coronary artery disease. The *Anglo-Scandinavian Cardiac Outcomes Trial* showed the benefits of taking the two drugs Coversyl and Amlodipine together. The 9000 British patients aged 40 to 79 were involved in the five-year trial. Half were given the new drug combination, the rest were given traditional drugs. Coversyl and Amlodipine were found to be so effective that the trial was stopped early so that all patients could receive the combination.

Contraindications Pregnancy, Lactation, Situations where a patient has a history of hypersensitivity to Coversyl (perindopril) and renal failure

Precautions Assess renal function before and during treatment where appropriate, Reno vascular hypertension, Surgery/anesthesia. Symptomatic hypotension is rarely seen, but is more likely in volume-depleted patients, those receiving diuretics, or with the first two doses. A diuretic may later be given in combination if necessary; potassium-sparing diuretics are not recommended. Combination with neuroleptics or imipramine-type drugs may increase the hypotensive effect. Serum lithium concentrations may rise during lithium therapy.

Side effects Cough, fatigue, asthenia , headache and disturbances of mood and/or sleep.

Equipment and Apparatus used

HPLC with PDA detector (Waters 2695 Separations Module Equipped with 2996 PDA), Sonicator (Ultrasonic sonicator) , P^H meter (Thermo scientific) , Micro balance (Sartorial) , Vacuum filter pump , UV Stability chamber and Hot Air Oven.

Reagents used

Methanol HPLC Grade , Acetonitrile HPLC Grade , HPLC grade Water , Orthophosphoric acid , Potassium Dihydrogen Orthophosphate , Hydrochloric Acid , Sodium Hydrogen Peroxide and Sodium Hydroxide. Amlodipine and Perindopril reference Standard 5mg of Amlodipine and 4mg of Perindopril.

3. AIM AND OBJECTIVE OF PRESENT WORK

The drug analysis plays an important role in the development of drugs, their manufacture and the therapeutic use. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw materials used and the final product obtained meets the required specification. These drugs or formulation may be either in the new entities in the market or partial structural modification of the existing drugs or novel dosage forms or multi component dosage forms. The multi

component dosage form proves to be effective due to the combined mode of action on the body. The complexity of dosage forms including the presence of multiple drug entities posses' considerable challenge to the analytical chemist during the development of assay procedure. The estimation of individual drugs in these multi component dosage forms becomes difficult due to cumbersome extraction or isolation procedures. In the present study the drug Amlodipine and Perindopril was selected. The extensive literature survey carried out and revealed that there are very few methods reported for the estimation of these drugs of Amlodipine and Perindopril in Pharmaceutical dosage by different methods like U.V and HPTLC and in also Human Plasma reported. The existing methods use some costly solvents and not reliable methods for the estimation of Amlodipine and Perindopril.

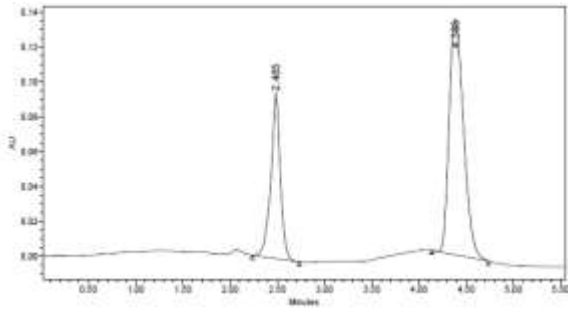
4. RESULTS AND DISCUSSIONS

Amlodipine is not only cost effective, but predicted to be cost saving when compared with usual care, warranting its consideration as an agent of choice in patients with CAD. Based on drug solubility and P^{ka} value following conditions has been used to develop the method for estimation of Amlodipine and Perindopril**.** The Tailing Factor for Amlodipine and Perindopril peak from standard solution should not more than 2.0. The theoretical plates for Amlodipine and Perindopril peak from standard solution should not less than 6000. % RSD of Amlodipine and Perindopril areas from five replicate injections of Standard solution not more than 2.0.

Method Development with Chromatographic conditions

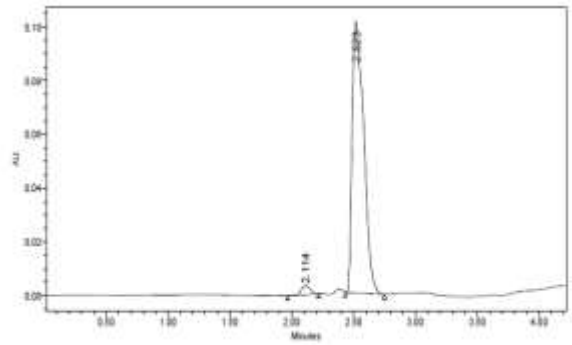
Chromatogram 1

S. No.	Retention Time	Area	% Area	USP Plate Count	USP Tailing
1	2.495	652676	31.62	2906	0.92
2	4.363	1411278	68.38	3577	1.41



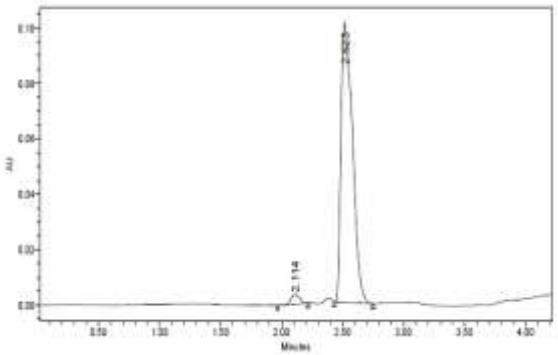
Chromatogram 2

S. No.	Retention Time	Area	% Area	USP Plate Count	USP Tailing
1	2.485	659995	31.57	3091	0.95
2	4.385	1430770	68.43	3858	1.34



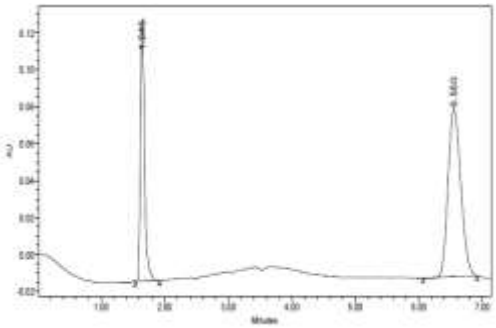
Chromatogram: 3

S. No	Retention Time	Area	% Area	USP Plate Count	USP Tailing
1	2.114	16397	2.43	4362	0.88
2	2.523	657466	97.57	3089	1.58



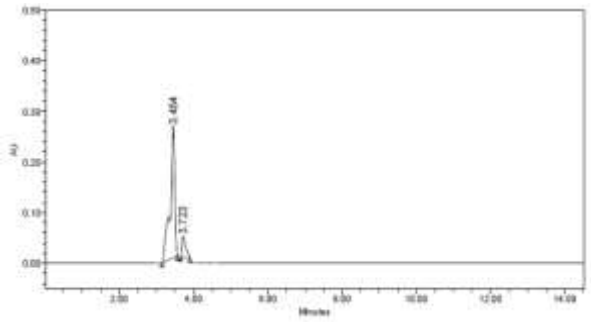
Chromatogram 4

S. No.	Retention Time	Area	% Area	USP Plate Count	USP Tailing
1	2.114	16397	2.43	4362	0.88
2	2.523	657466	97.57	3089	1.58



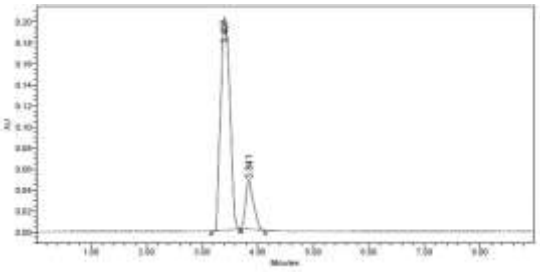
Chromatogram 5

S. No.	Retention Time	Area	% Area	USP Plate Count	USP Tailing
1	1.646	643731	33.73	3291	1.40
2	6.550	1264960	66.27	4980	1.17



Chromatogram 6

S. No.	Retention Time	Area	% Area	USP Plate Count	USP Tailing
1	3.454	2186580	87.99	7599	0.68
2	3.723	298382	12.01	6614	1.72



Chromatogram 7

S. No.	Retention Time	Area	% Area	USP Plate Count	USP Tailing
1	3.407	2350614	83.84	1585	1.14
2	3.841	453179	16.16	2787	1.35

METHOD FOR ESTIMATION OF AMLODIPINE AND PERINDOPRIL IN TABLET

Formulation used – Tablet

Brand Name –COVERSYL AM Tab Label Claim: 5mg of Amlodipine and 4mg Perindopril.

Sample preparation

Tablets were weighed and crushed into powder, in order to calculate the average weight of each tablet. From that powder weight equivalent to 5mg of Amlodipine and 4mg Perindopril were transferred into a 100 ml volumetric flask, 70ml of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.5ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Linearity: Linearity Graph for Amlodipine and Perindopril

S. No.	Pipette from stock (mL)	Volume of flask (mL)	Concentration in ppm(Amlo)	Concentration in ppm(Perin)	%Linearity Level
1	0.25	10	12.5	10	50
2	0.5	10	25	20	75
3	0.75	10	37.5	30	100
4	1.0	10	50	40	125
5	1.25	10	62.5	50	150
6	1.5	10	75	60	

Table 1

Sample Preparation:

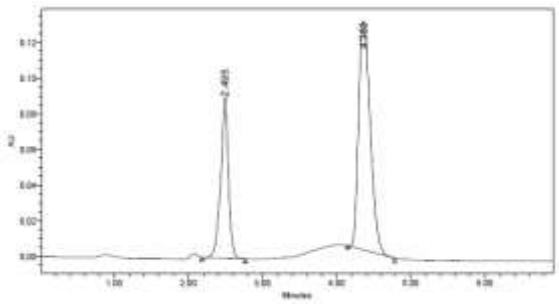
5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 60mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 2ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

EXPERMENTAL SECTION

DEVELOPMENT AND OPTIMIZATION OF THE HPLC METHOD

Flow rate	0.8 ml/min
Column	Hibar Purospher 150 x 4.6 mm, 5.
Detector wave length	211nm
Column temperature	30°C
Injection volume	10L
Run time	11 min
Diluent	water: Acetonitrile (30:70)
Mobile phase	Buffer:Acetonitril (65:35)

Table 2



Chromatogram 8

S. No.	Retention Time	Area	% Area	USP Plate Count	USP Tailing
1	2.495	652676	31.62	2906	0.92
2	4.363	1411278	68.38	3577	1.41

Linearity: Table for Amlodipine

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
10	25	424327
20	50	794536
30	75	1202654
40	100	1601293
50	125	1985992
60	150	2416445

Table 3

Linearity: Table for Amlodipine

Optimized characteristics for linearity of mlodipine by RP-HPLC

Parameters	Observed values
Linearity concentration	25-150PPM
Slope	27146
Intercept	44375
Correlation coefficient	0.9998

Table 4

Linearity: Table for Perindopril

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
10	25	215974
20	50	411769
30	75	591887
40	100	810118
50	125	995719
60	150	1205229

Table 5

Optimized characteristics for linearity of Perindopril by RP-HPLC

Parameters	Observed values
Linearity concentration	25-150PPM
Slope	27146
Intercept	44375
Correlation coefficient	0.9996

Table 6

METHOD PRECISION

Six Sample solutions were prepared individually from Amlodipine and Perindopril stock solution, as per methodology and injected each solution Into HPLC. Acceptance Criteria: % RSD Should not is more than 2.0%.

Table for Amlodipine Table 7

Sample No	Sample Areas	%Assay
1.	1609991	99.15285
2.	1597107	99.96647
3	1603022	99.13456
4	1597801	99.32159
5	1600471	99.07316
6	1589103	99.57761
AVG	1599582	99.37
SD	6929.2	0.694747
%RSD	0.40	0.35

SYSTEM PRECISSION FOR AMLODIPINE

Table 8

SYSTEM PRECISSION	AREAS
1	1617661
2	1626137
3	1620949
4	1625821
5	1629385
6	1631454
AVG	1625235
SD	5153.4
%RSD	0.30

Table for Perindopril Table 9

Sample No	Sample Areas	%Assay
1	779827	100.19
2	780041	99.26
3	781127	98.76
4	781246	99.34
5	781862	99.22
6	795396	99.20
AVG	783250	99.33
SD	5999.8	0.4680
%RSD	0.80	0.47

SYSTEM PRECISSION FOR PERINDOPRIL

Table 10

SYSTEM PRECISSION	AREAS
1	799109
2	796488
3	803844
4	795922
5	806056
6	800382
AVG	800300
SD	4020.4
% RSD	0.50

System suitability parameter for Amlodipine

Table 11

S. No.	Retention Times	Peak Area	Tailing Factor	Theoretical Plates
1	8.267	1617661	1.20	5444
2	8.395	1626137	1.18	5613
3	8.584	1620949	1.18	5563
4	8.629	1625821	1.16	5671
5	8.746	1629385	1.15	5818
6	8.850	1631454	1.15	5705
Mean	8.848	1625235	1.14	5701
SD	8.846	5153.4	1.13	5689
%RSD	8.843	0.3	1.12	5690

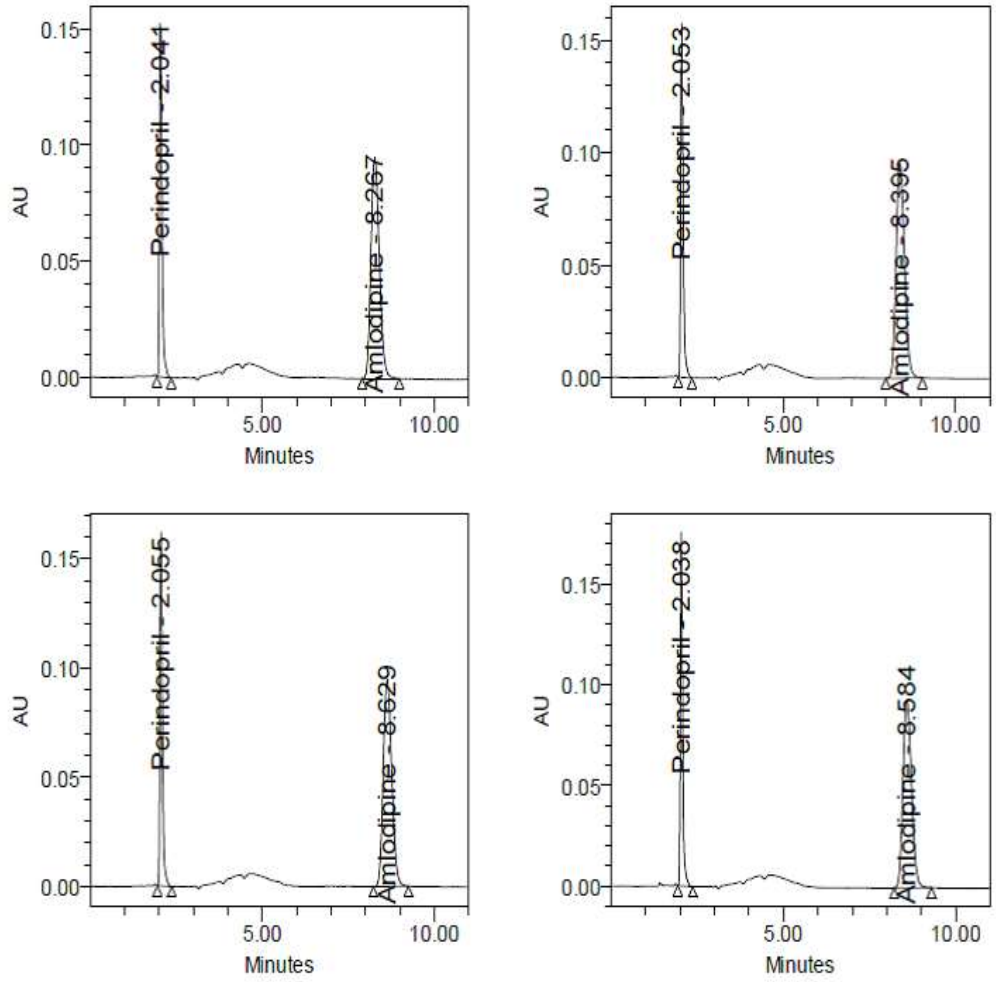
System Suitability Parameters for Perindopril

Table 12

S. No.	Retention Times	Peak Area	Tailing Factor	Theoretical Plates
1	2.038	799109	1.44	4760
2	2.041	796488	1.44	3954
3	2.051	803844	1.44	3744
4	2.053	795922	1.43	3883
5	2.055	806056	1.49	3859
6	2.055	800382	1.43	4266
Mean	2.053	800300	1.42	4267
SD	2.052	4020.4	1.43	4268
%RSD	2.054	0.5	1.43	

Peak Name:Perindopril Table 13

S. No.	Peak Name	Retention Time	Area	USP Plate Count	USP Tailing
1	Perindopril	2.030	813895	2172	1.38
2	Perindopril	2.032	798719	1943	1.30
3	Perindopril	2.037	797121	1954	1.26



Peak Name: Amlodipine Table 14

S. No.	Peak Name	Retention Time	Area	USP Plate Count	USP Tailing
1	Amlodipine	8.771	1654154	4776	1.14
2	Amlodipine	8.798	164088	4790	1.12
3	Amlodipine	8.913	1662734	4554	1.13

REFERENCES

1. Arauz-Pacheco C, Parrott MA, Raskin P. The treatment of hypertension in adult patients with diabetes. Diabetes Care 2002; 25: 134–147.

2 Pahor M, Psaty BM, Furberg CD. Treatment of hypertensive patients with diabetes. Lancet 1998; 351: 689–690.

3. Pylypchuk GB. ACE inhibitor—versus angiotensin II blocker—induced cough and angioedema. Ann Pharmacother 1998; 32: 1060– 1066.

4. Gradman AH. AT (1)-receptor blockers: differences that matter. J Hum Hypertens 2002; 16 (Suppl. 3): S9–S16.

5. The Expert Committee on the diagnosis and classification of diabetes mellitus. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003; 26 (Suppl. 1): 5–20.

6. Fogari R *et al*. Losartan and perindopril effects on plasma plasminogen activator inhibitor-1 and fibrinogen in hypertensive type 2 diabetic patients. Am J Hypertens 2002; 15: 316–320.

7. Bonora E *et al*. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity. 2000; 23: 57–63.

8. Trenkwalder P, Lehtovirta M, Dahl K. Long-term treatment with candesartan cilexetil does not affect glucose homeostasis or serum lipid profile in mild hypertensive with type II diabetes. J Hum Hypertens 1997; 11 (Suppl. 2): S81–S83.

9. Higashiura K, Ura N, Miyazaki Y, Shimamoto K. Effect of an angiotensin II receptor antagonist, candesartan, on insulin resistance and presser mechanisms in essential hypertension. J Hum Hypertens 1999; 13 (Suppl. 1): S71–S74.

10. Podoleanu, C. Moldovan, I. Barsan, D. Moncea, G. Naftali and S. Stolnicu. (2015) late onset of gingival hyperplasia in a patient undergoing fixed-dose combination antihypertensive therapy. *European Geriatric Medicine* 6, 368-370.

11. Verica Pavlic, Nina Zubovic, Sanja Ilic and Tijana Adamovic. (2015) Untypical Amlodipine-Induced Gingival Hyperplasia. *Case Reports in Dentistry* 2015, 1-4.

12. R Livada and J Shiloah. (2014) Calcium channel blocker-induced gingival 13 enlargement. *Journal of Human Hypertension* 28, 10-14.

14. Antush Mittal and Shoyab Khan. (2013) Phenytoin-induced gingival overgrowth: A case report. *Journal of Pierre Fauchard Academy (India Section)* 27, 102-105.

15 Sanjeev Joshi and Sucheta Bansal. (2013) A Rare Case Report of Amlodipine-Induced Gingival Enlargement and Review of Its Pathogenesis. *Case Reports in Dentistry* 2013, 1-3.

16. N. Charles, V. Ramesh, S.P.K. Kennedy Babu and B. Premalatha. (2012) Gene Polymorphism in Amlodipine Induced Gingival Hyperplasia: A Case Report. *Journal of Young Pharmacists* 4, 287-289.

17. Rohit Karnik, K. Mahalinga Bhat and G. Subraya Bhat. (2012) Prevalence of gingival overgrowth among elderly patients under amlodipine therapy at a large Indian teaching hospital. *Gerodontology* 29:10.1111/ger.2012.29.issue-3, 209-213.

18. Zohreh Rostami, Behzad Einollahi, Mohammad Javad Einollahi and Simin lessan. (2012) the Impact of Amlodipine on Gingival Enlargement after Kidney Transplantation. *Nephro-Urology Monthly* 4, 565-570.

19. Sung Jin Lee, Young Kuk Chung, Hae Lim Lee, Su Jin Choi, Sung Yeon Cho, Hyun Joo Choi and Hyung Wook Kim. (2012) A Case of Severe Gingival Overgrowth Caused by Long-Term Amlodipine Treatment. *Korean Journal of Medicine* 82, 623.

20. Milton Packer, Christopher M. O'Connor, Jalal K. Ghali, Milton L. Pressler, Peter E. Carson, Robert N. Belkin, Alan B. Miller, Gerald W. Neuberg, David Frid, John H. Wertheimer, Anne B. Crop and David L. DeMets for the Prospective Randomized Amlodipine Survival Evaluation Study Group N Engl J Med 1996; 335:1107-1114.