International Journal of Chemistry and Pharmaceutical Sciences
**ISSN: 2321-3132 | CODEN (USA): IJCPNH**
*Available online at:http://www.pharmaresearchlibrary.com/ijcps*

# Analytical Metthod Development and Vaidation for Felodipine and Simvastatin in combind dosage Form by RP-HPLC

Koduru Nikhitha*[1], SK. Salma[2]

[1]*PG Research Scholar, Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Nellore, A.P.*
[2]*Assistant Professor, Department of Pharmaceutical Chemistry, Ratnam Institute of Pharmacy, Nellore, A.P.*

## ABSTRACT

A new method was established for simultaneous estimation of Felodipine and Simvastatin by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Felodipine and Simvastatin by using Thermosil C18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.566 mins and 3.417mins. The % purity of felodipine and Simvastatin was found to be 101.27% and 99.97% respectively. The system suitability parameters for felodipine and Simvastatin such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Felodipine and Simvastatin was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient ($r^2$) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC

**Keywords:** Thermosil C18 column, Felodipine and Simvastatin, RP-HPLC

**ARTICLE HISTORY: Received** 11 January 2022, **Accepted** 10 February 2022, **Available Online** 31 March 2022

**©2022***Production and hosting by Pharma Research Library Publishers, All rights reserved.*

**Citation:** Koduru Nikhitha, *et al. Analytical Metthod Development and Vaidation for Felodipine and Simvastatin in combind dosage Form by RP-HPLC, Int. J. of Chem. and Pharm. Sci., 10(1), 2022: 21-26.*

*This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.*

**CONTENTS:**



***Corresponding author***
*Koduru Nikhitha*
*PG Research Scholar,*
*Department of Pharmaceutical Analysis,*
*Ratnam Institute of Pharmacy, Nellore, A.P.*

## 1. Introduction

Felodipine is a long-acting 1,4-dihydropyridine calcium channel blocker (CCB)b. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By

inhibiting the influx of calcium in smooth muscle cells, felodipine prevents calcium-dependent myocyte contraction and vasoconstriction. Felodipine is the most potent CCB in use and is unique in that it exhibits fluorescent activity.

**IUPAC Name :** 3-ethyl 5-methyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate

**Chemical formula :** $C_{18}H_{19}Cl_2NO_4$

**Molecular weight :** 384.254

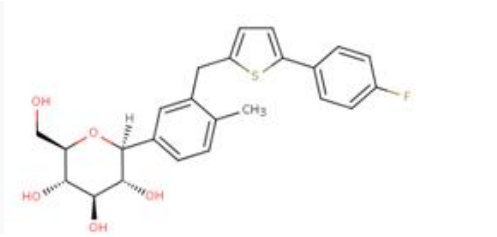

**Figure 1**

All statins act by inhibiting 3-hydroxy-3-methylglutaryl coenzyme. A HMG-CoA reductase, the rate-limiting enzyme of the HMG-CoA reductase pathway, the metabolic pathway responsible for the endogenous production of cholesterol. Statins are more effective than other lipid-regulating drugs at lowering LDL-cholesterol concentration, but they are less effective than the fibrates in reducing triglyceride concentration. However, statins reduce cardiovascular disease events and total mortality irrespective of the initial cholesterol concentration.

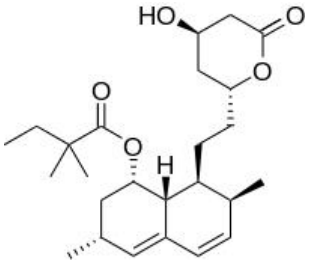

**Figure 2**

**IUPAC Name :** (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate

**Chemical formula :** $C_{25}H_{38}O_5$

**Molecular weight :** 418.566 g/mol

## 2. Methodology

**Preparation of phosphate buffer**

6.8 grams of sodium acetate was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with orthophosphoric acid. The resulting solution was sonicated and filtered.

**Preparation of mobile phase**

Mix a mixture of above buffer 30 ml (30%) and 70 ml of Methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

**Diluents preparation**

Mobile phase was used as the diluent.

**Preparation of the individual Felodipine standard preparation:** 10 mg of Felodipine working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.5 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

**Preparation of the individual Simvastatin standard preparation:** 10 mg of Simvastatin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2ml of diluent and sonicate to Dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 3 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

**Assay**

**Assay preparation of the Felodipine and Simvastatin standard and sample solution**

**Sample solution preparation:** 1mg of Felodipine and 10 mg Simvastatin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

**Standard solution preparation**

1mg Felodipine and 10 mg Simvastatin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

**Procedure**

10µL of the blank, standard and sample were injected into the chromatographic system and areas for the Felodipine and Simvastatin the peaks were used for calculating the % assay by using the formulae.

**Trial-1**

Chromatographic conditions

Column : Thermosil C18 4.6x150mm, 5µm

Mobile phase ratio: MeOH: H2O (60:40%v/v)

Detection wavelength: 252 nm

Flow rate: 1ml/min

Injection volume : 10µl

Column temperature: Ambient

Auto sampler temperature: Ambient

Run time: 10min

Retention time: 2.384 min&7.222 min

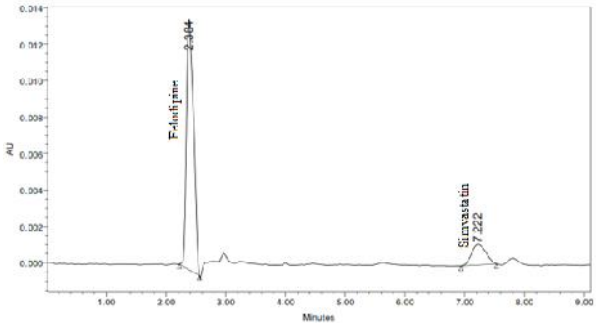


**Figure 3 Chromatogram showing trial-1 injection**

**Observation:** The trial shows no proper separation peaks in the chromatogram, so more trials were required for obtaining peaks.

**Trial - 2**

Chromatographic conditions
Column : Symmetry C18 4.6x150mm 5µm
Mobile phase ratio : ACN: Methanol (40:60%v/v)
Detection wavelength: 252 nm
Flow rate : 1ml/min
Injection volume : 20µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 8.0 min
Retention time: 4.015 min & 4.638 mins

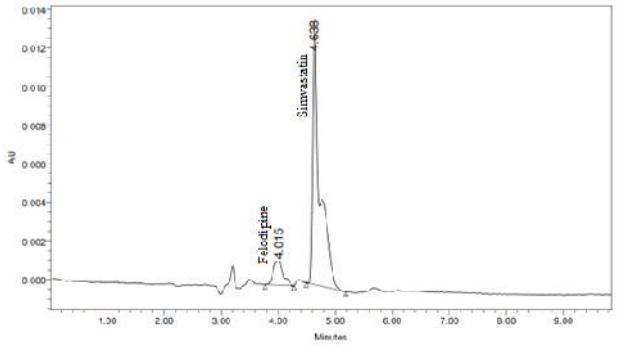


**Figure 4 Chromatogram showing trial-2 injection**

**Observation:** In this trial two peaks were separated but don't have proper resolution. Still more trials were required for proper peaks.

**Optimized method:**
**Chromatographic conditions**
Column : Thermosil C18 (4.0×125 mm) 5.0µm
Mobile phase ratio: Methanol: Sodium acetate buffer (70: 30 % v/v)
Detection wavelength: 252 nm
Flow rate: 0.7 ml/min
Injection volume : 10µl
Column temperature: Ambient
Auto sampler temperature: Ambient
Run time : 8min
Retention time: 2.449 & 3.191 mins

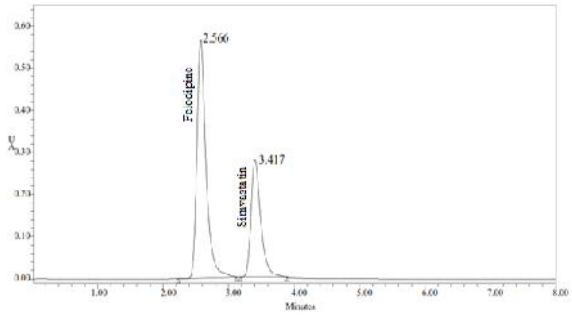


**Figure 5 Chromatogram showing trial-5 injection**

**Observation**
The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

## 3. Results and Discussion

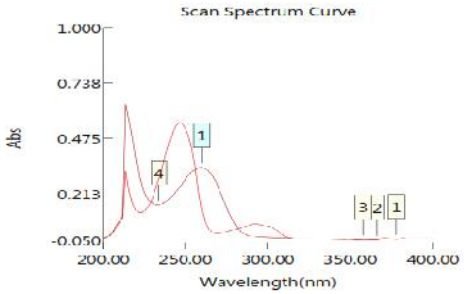


**Figure 6 Spectrum showing overlapping spectrum of Felodipine and Simvastatin**

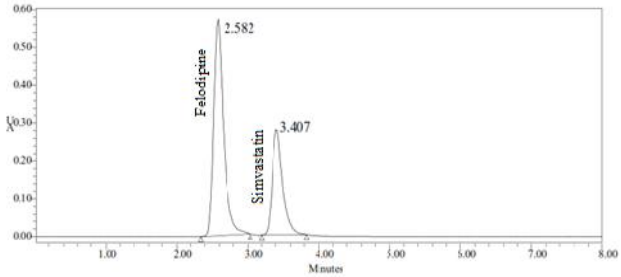


**Figure 7 Assay of sample injection**

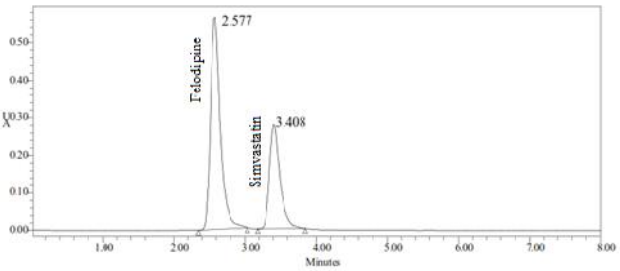


**Figure 8 Assay of Standared injection**

**Table1**

| S.No | Name of compound | Amount taken | %purity |
|---|---|---|---|
| 1 | Felodipine | 754.7 | 99.24 |
| 2 | Simvastatin | 735.6 | 101.04 |

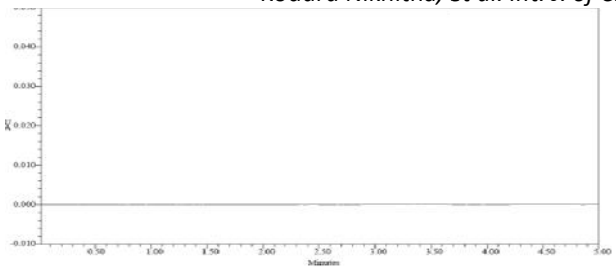

**Figure 9 Blank chromatogram**

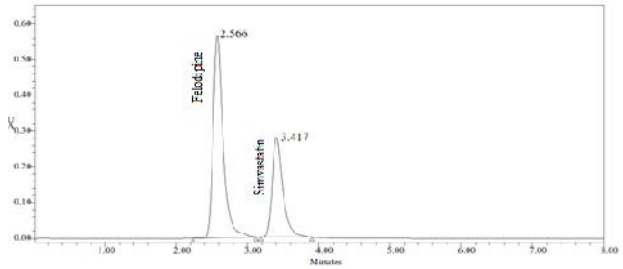


**Figure 10 Chromatogram showing standard injection**

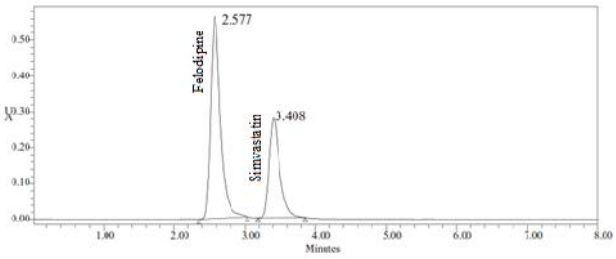


**Figure 11 Chromatogram showing sample injection**

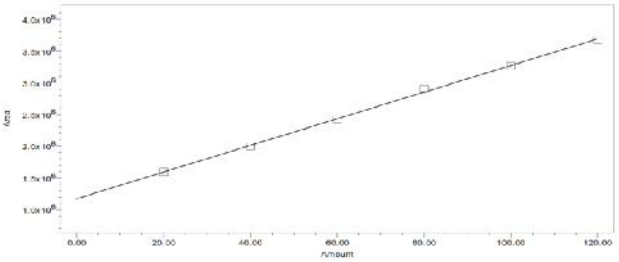

**Figure 12 Linearity results for felodipine**

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 20 ppm | 471543 |
| 2 | II | 40 ppm | 656277 |
| 3 | III | 60 ppm | 794999 |
| 4 | IV | 80 ppm | 946124 |
| 5 | V | 100 ppm | 1002139 |
| Correlation Coefficient | | | 0.999 |

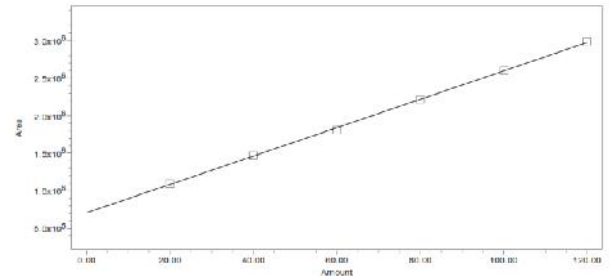

**Figure 13 Linearity results for simvastatin**

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 20ppm | 56472 |
| 2 | II | 40 ppm | 73841 |
| 3 | III | 60ppm | 92655 |
| 4 | IV | 80ppm | 111541 |
| 5 | V | 100ppm | 130567 |
| Correlation Coefficient | | | 0.999 |

**Table 4: Accuracy results of felodipine**

**Peak Name:Felodipine**

| | Peak Name | RT | Area | Height ( V) |
|---|---|---|---|---|
| 1 | Felodipine | 3.397 | 1365757 | 133891.1 |
| 2 | Felodipine | 3.413 | 1374036 | 133774.6 |
| 3 | Felodipine | 3.519 | 1360204 | 131701.0 |
| Mean | | | 1366666 | |
| Std. Dev. | | | 6960.2 | |
| % RSD | | | 0.51 | |

**Table 5: Accuracy results of simvastatin**

**Peak Name: Simvastatin**

| | Peak Name | RT | Area | Height ( V) |
|---|---|---|---|---|
| 1 | Simvastatin | 2.553 | 2629787 | 277036.4 |
| 2 | Simvastatin | 2.554 | 2641613 | 277483.8 |
| 3 | Simvastatin | 2.564 | 2619828 | 269170.9 |
| Mean | | | 2630409 | |
| Std. Dev. | | | 10906.0 | |
| % RSD | | | 0.41 | |

**Table 6: Intermediate precision of Simvastatin**

**Peak Name: Simvastatin**

| | Peak Name | RT | Area | Height (µV) |
|---|---|---|---|---|
| 1 | Simvastatin | 2.756 | 5698542 | 539568.1 |
| 2 | Simvastatin | 2.688 | 5682534 | 536985.4 |
| 3 | Simvastatin | 2.633 | 5695846 | 539584.1 |
| 4 | Simvastatin | 2.613 | 5689452 | 534569.8 |
| 5 | Simvastatin | 2.617 | 5636591 | 534985.5 |
| Mean | | | 5600593 | |
| Std. Dev. | | | 203577.3 | |
| % RSD | | | 0.44 | |

**Table 6: Intermediate precision of felodipine**

**Peak Name: Felodipine**

| | Peak Name | RT | Area | Height (µV) |
|---|---|---|---|---|
| 1 | Felodipine | 3.617 | 2624315 | 231325.6 |
| 2 | Felodipine | 3.635 | 2623598 | 231315.4 |
| 3 | Felodipine | 3.461 | 2623541 | 231250.1 |
| 4 | Felodipine | 3.447 | 2624987 | 231342.6 |
| 5 | Felodipine | 3.438 | 2635698 | 231765.2 |
| Mean | | | 2626428 | |
| Std. Dev. | | | 5215.78 | |
| % RSD | | | 0.19 | |

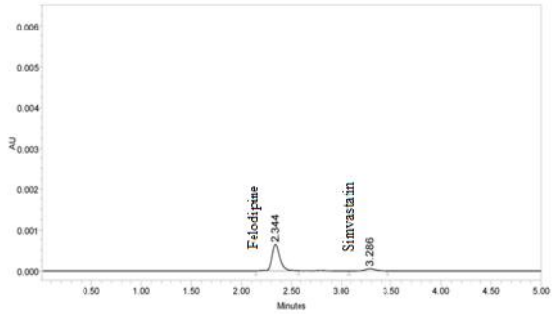


**Figure 14: Results for Limit of Detection**

**Table 7**

| Drug name | Standard deviation(σ) | Slope(s) | LOD(µg) |
|---|---|---|---|
| Felodipine | 373625.50 | 581075863 | 3.17 |
| Simvastatin | 5772.40 | 476579210 | 0.0172 |

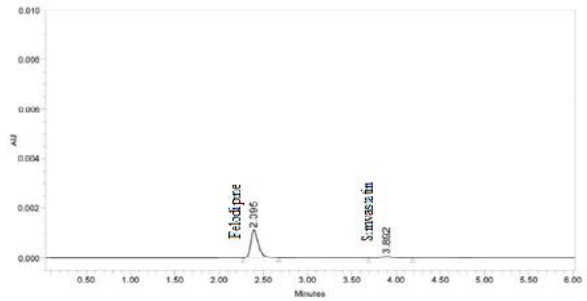


**Figure 15 Results for Limit of Quantification**

**Table 8**

| Drug name | Standard deviation(σ) | Slope(s) | LOQ(µg) |
|---|---|---|---|
| Felodipine | 372727.80 | 574265980 | 5.80 |
| Simvastatin | 5761.30 | 478828490 | 0.212 |

**Table 9: System suitability results for felodipine**

| S. No | Change in organic composition in the mobile phase | System suitability results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 5 % less | 6232 | 1.4 |
| 2 | ***Actual** | **4668** | **1.3** |
| 3 | 5 % more | 6387 | 1.4 |

**Table 10: Showing system suitability results for Simvastatin**

| S. No | Change in organic composition in the mobile phase | System suitability results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 5 % less | 5437 | 1.3 |
| 2 | ***Actual** | **6089** | **1.2** |
| 3 | 5 % more | 4817 | 1.2 |

## 4. Conclusion

A new method was established for simultaneous estimation of Felodipine and Simvastatin by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Felodipine and Simvastatin by using Thermosil C18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. The retention times were found to be 2.566 mins and 3.417mins. The % purity of Felodipine and Simvastatin was found to be 101.27% and 99.97% respectively. The system suitability parameters for Felodipine and Simvastatin such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Felodipine and Simvastatin was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient ($r^2$) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively.

## 5. References


[1] Douglas A.Skoog, F. James Holler & Stanley R. Crouch. Instrumental analysis, India edition, 2007, pg: 13-14.

[2] Gurdeep R. Chatwal & Sham K. Anand. Instrumental Methods Of Chemical Analysis (Analytical Chemistry) , pg: 2.566-2.567.

[3] Ahuja S & Dong MW. Handbook of Pharmaceutical Analysis by HPLC. 1st edition, Academic Press Publisher.UK 2005.

[4] Satinder Ahuja & Neil Jespersen. Modern Instrumental Analysis 47 (Comprehensive Analytical Chemistry) volume-47, pg-7-8.

[5] Willard HH, Merrit LL, Dean JA, Settle FA. Instrumental methods of analysis, CBS Publishers and Distributors, New Delhi, 6th edition, 1986, 1-15.

[6] Douglas A. Skoog, F. James Holler, Timothy A. Nieman. Principles of instrumental analysis, Saunders Golden Sun burst Series, Philadelphia, 2nd edition, 1980, 725-760.

[7] David G.Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists, Harcourt Publishers Limited, 2nd Edition, 1999, 221-232, 267-311.

[8] Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC Method Development, Wiley Inter Science, New York, 2nd Edition, 1997, 1-56, 234-289,685-712.

[9] Beckett A.H, J.B. Stenlake. Practical Pharmaceutical Chemistry, 4th edition. C.B.S. Publications, Pg. No.53-62.

[10] Remingtonn's The Science and Practise of Pharmacy,20th Edition, 2000.

[11] Connors KA. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc, New Delhi, 3rd Edition, 1994, 373-421.

[12] Rashmin.B.Patel, Mrunali R. Patel, An Introduction to Analytical method development for pharmaceutical formulations, Pharmainfo.net 2008; 17:19

[13] Sharma B.k Instrumental methods of chemical analysis. 19 ed: Goel Publishing House, 2003.

[14] Galen Wood Ewing, Instrumental methods of chemical analysis, 340-345.

[15] United States of Pharmacopeia, USP30-NF25, the official compendia of standards, official May 1, 2007.

[16] ICH topic Q2B, validation of analytical procedure & methodology, The European agency for evaluation of medicinal products, human medicines evaluation unit 1996.

[17] ICH: Q2A, Text on validation of analytical procedure (October 1994).

[18] Dipti B. Patel, N. J. Patel, S. K. Patel, A. M. Prajapati, and S. A. Patel, Rp hplc method for the estimation of Simvastatin in tablet dosage form. Indian Journal of Pharmaceutical Sciences, 2010 Jan-Feb; 72(1): 113–116.

[19] Kamepalli Sujana, D. Gowri Sankar, Konda Abbulu and O.Bala Souri, Simultaneous estimation of Simvastatin and Felodipine by reverse phase HPLC in bulk and pharmaceutical dosage form. International Journal of Pharmacy & Life sciences, Vol. 3, Issue 8: August: 2012, Pg.no:1905-1908.

[20] Chandan, M. Vasudevan World Academy of Science, Engineering and Technology International Journal of Medical, Health, Pharmaceutical and Biomedical Engineering Vol:7 No:12, 2013.

[21] P. Ravisankar1, G. Devala Rao et al Asian journal if pharmaceutical and clinical research Vol 6, Supply 3, 2013.

[22] Kullai Reddy Ulavapalli1 et al Indian Journal of Novel Drug delivery 3(2), Apr-Jun, 2011, 134-142

[23] Anil Waldiaa, Shubash Guptab et alJournal of Chemical Technology Vol. 15, November 2008, pp. 617-620.

[24] Shreya R. Shah′′, S. Dey, et alJournal of Taibah University for Science, Volume 8, Issue 1, January 2014, Pages 54–63.