# Simultaneous quantitation of aspirin, amlodipine and simvastatin in a fixed dose combination of encapsulated tablet formulation by HPLC-UV method

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**Abstract**: A high-pressure liquid chromatography (HPLC-UV) based simple and specific method for simultaneous quantitative determination of aspirin, amlodipine besylate and simvastatin in a capsule formulation has been developed and validated according to ICH guidelines. Chromatographic separation of the three drugs was carried out by aSpherisorbODS2 reverse phase column (4.6 x 250 mm; 5 µm) using amobile phase, which consisted of 70: 30 (v/v) mixture of acetonitrile and triethylamine phosphate buffer (pH 3; 0.015 M) with final pH adjusted to 2.5 using dilute ortho-phosphoric acid, at a flow rate of 1mL/min. The eluents were detected at UV wavelength of 237 nm and the retention times for aspirin, amlodipine besylate and simvastatin were ~2.7 mins, ~6.1 mins and ~10.5mins, respectively. This method is suitable and specific for the three drugs and was found to be linear ($R^2 \geq 0.995$), accurate, specific, reproducible and robust in the concentration range of 375 to 1125mcg/ml for aspirin, 25 to 75mcg/ml for amlodipine besylate and 50 to 150mcg/ml for simvastatin. This simple and convenient method could be easily utilized for the characterization and quantitation of the three drugs in a single formulation for combination therapy of cardiovascular diseases.

**Keywords**: Aspirin, amlodipine besylate, simvastatin, HPLC-UV, analysis, validation.

## INTRODUCTION

Cardiovascular disease (CVD) is a class of various diseases, which are manifested by a variety of structural and physiological abnormalities in the cardiovascular system (Gaziano *et al.,* 2006). The major types of CVDs include hypertension, is chemic heart disease (IHD) and coronary artery disease (CAD), which usually precede many other forms of cardiovascular dysfunctions. These are collectively one of the leading causes of morbidity and mortality worldwide anda whopping80% of annual CVD associated deaths are being reported in the developing countries alone(Gaziano, 2008, Gaziano *et al*., 2006, Yach *et al*., 2005). Worldwide, several combination drug regimens are usually employed for the appropriate management of these diseases and the number of patients using multiple drugs to manage these diseases specially hypertension has dramatically increased 7 times in last 14 to15 years (Gaziano *et al.,* 2006). It has been suggested that the 'polypill' approach which was first introduced in the year 2003; consisting of multiple drugs at reduced doses in a single formulation; could greatly help in reducing the risks of primary and secondary CVDs, minimize prescription gaps and increase the patient adherence by means of cost-effective therapy and ease of administration (Lafeber *et al*., 2012, Pharmacotherapy, 2005, Wald and Law, 2003).

Multiple studies including a few meta-analyses have shown that the use of aspirin and a statin along with one or two anti-hypertensive drugs, specially a calcium channel blocker, may greatly reduce the risk of primary and secondary cardiovascular events. These three classes of drugs have been the mainstay of many therapeutic regimens employed for various CVDs (Lafeber *et al*., 2012, Law *et al*., 2009, Soliman *et al*., 2011,Volpe *et al*., 2010).

### *Aspirin*

2-Acetoxybenzoic acid, is a potent antiplatelet agent and is classified as a non-steroidal anti-inflammatory drug (NSAID). Aspirin is also known as acetyl salicylic acid ($C_9H_8O_4$) with molecular weight of 180.157 g/mol (structural formula shown in fig. 1) (Pawar *et al.,* 1998).

### *Amlodipine*

Besylate,2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine benzenesulfonate ($C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$), is a long acting dihydropyridinecalcium channel blocker, and used as an anti-hypertensive medication. It also significantly reduces the rate of unstable angina due to adirect effect on vascular smooth muscles (Pitt *et al.,* 2000). Amlodipine besylate has a molecular mass of 567.10 g/mol (structure formula shown in fig. 1).

### *Simvastatin*

(1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-Hexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-

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yl]ethyl]-1-naphthalenyl-2,2-dimethyl-butanoate ($C_{25}H_{38}O_5$), belongs to a class of anti-lipidemic drugs known as 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors (statins). It has a molecular mass of 418.566g/mol (structural formula shown in fig. 1). It helps to reduce the plasma concentration of LDL cholesterol and thereby hampers the phenomena of atherosclerosis (Elevated *et al*., 2001, Pasternak *et al*., 2002).

In the present study, an HPLC-UV based simple, accurate, precise and robust analytical method has been developed and validated for a combination formulation (capsule) consisting of aspirin (75 mg; delayed release), amlodipine besylate (6.9 mg) and simvastatin (10 mg). To our knowledge, this is the first study, which reports an analytical method for a simultaneous determination of these aforementioned drugs.

## EXPERIMENTAL

### *Chemicals and reagents*

Aspirin was received as a gift from Shandong Xinhua Pharmaceutical Company (Zibo City, Shandong, China). (R,S)-Amlodipine besylate and simvastatin were generously presented by Cadila Pharmaceuticals (Ahmedabad, India) and Biocon Limited (Bangalore, India), respectively. Acetonitrile (HPLC grade) was purchased from Fischer Scientific (Pittsburgh, PA, USA) and triethylamine and ortho-phosphoric acid were obtained from Merck (Darmstadt, Germany).

### *Instrumentation and chromatographic conditions*

The separation of drugs and subsequent analyses of eluents were performed on two chromatographic systems. The first system had SIL-10ADVP auto sampler (Shimadzu Scientific Instruments (SSI), Kyoto, Japan) linked to LC-10AT chromatographs (SSI, Kyoto, Japan) with attached aSPD-10AVP UV-vis detector (SSI, Kyoto, Japan). Controlling of the chromatographic parameters and recording were performed via'Liquid Chromatography (LC) Solutions' software (SSI, Kyoto, Japan). The second chromatographic system had same instruments (chromatograph and detector) as that of the first system except that instead of autosampler, the samples were injected manually and the parameters were recorded by 'Chromatography Station for Windows (CSW)' (Watrex, USA). A Spherisorb ODS-2 (Waters Corporations, MA, USA) reverse phase column (4.6 x 250 mm; 5 µm) was used as a stationary phase for the separation of the drugs. A70: 30 (v/v) mixture of acetonitrile and triethylamine phosphate buffer (pH 3; 0.015 M) with final pH adjusted to 2.5 using dilute ortho-phosphoric acid was used as mobile phase. The mobile phase was degassed in an ultrasonicator (Ultrasonic LC-10 H, Elma, Germany) for 15-20 mins before the separation of the drug mixtures was carried out under ambient conditions at a flow rate of 1mL/min. Eluents were detected by an UV-vis detector set at wavelength of 237 nm. The results were statistically tabulated using data analysis and graphing software, Origin Pro8 (Origin Labs, Northampton, MA, USA).

### *Preparation of Standard and Sample Solutions*

Standard solution of aspirin, amlodipine (besylate) and simvastatin was prepared in diluent (70% acetonitrile in DI water) with final concentrations of 750µg/ml, 69µg/ml and 100µg/ml for individual drugs, respectively. Sample solutions were prepared by crushing and mixing the content of one capsule (containing amlodipine besylate, simvastatin and aspirin) in the diluent and then filtering off through 0.2µm membrane filter (Milipore, England) before analysis. The expected concentrations of individual drugs in the sample solution were expected to be same as that of standard solution. For preparing the placebo (controlled) drug solution, all the excipients were weighed separately, mixed in the diluent and then finally the solution was filtered off through 0.2µm membrane filter. Each solution (volume 10µL) was injected automatically by the auto sampler each time in the column for analysis.

### *Method validation*

The newly developed HPLC-UV based analytical procedure for simultaneous determination of aspirin, amlodipine besylate and simvastatin in the formulation was evaluated and validated according to guidelines of International Conference on Harmonization (ICH). The parameters included system suitability and specificity, linearity, accuracy, precision, reproducibility, robustness and range (Guideline, 2005).

### *System suitability and specificity*

The suitability of the system for performing the analysis was determined by running 5 replicates each of blank (mobile phase) and standard solutions. The area under curves of replicates of individual drugs in the standard solution were calculated for percentage relative standard deviation (% RSD; $\leq$2 %), theoretical plates ($\geq$1000) and tailing factor ($\leq$2.0) as per USP.

To evaluate the system specificity for the objective analytes, placebo (formulation constituents without active drug ingredients), blank (mobile phase only) and standard solutions were injected separately and respective chromatograms were observed for any interference between the active drug ingredients and excipients present in the finished formulation.

### *Linearity and accuracy*

To estimate the linear proportionality of the yields with the concentration of analytes, system linearity was calculated after 5 concentrations (50%, 80%, 100%, 120%, and 150%)of standard solutions were prepared by diluting the stock standard solution and run on HPLC.

**Table 1**: System suitability parameters

| Analyte (n = 5) | Area under the curve of eluent peaks | | | | | | % RSD* | Theoretical plates* | Tailing Factor* |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Mean Area | | | |
| Aspirin | 10060851 | 10047394 | 10087730 | 10093102 | 10079802 | 10073775.8 | 0.190 | 2361.654 | 1.334 |
| Amlodipine | 1110239 | 1101589 | 1104637 | 1105297 | 1114861 | 1107324.6 | 0.472 | 2707.808 | 1.432 |
| Simvastatin | 2825772 | 2809543 | 2828383 | 2826907 | 2830942 | 2824309.4 | 0.300 | 4785.047 | 1.217 |

USP-NF 29

**Table 2**: Calculated parameters for method linearity

| Analyte (n = 3) | Recovered amount of various percent strength's standard solutions injected | | | | | Correlation coefficient | Residual sum of square |
|---|---|---|---|---|---|---|---|
| | 50 % | 80 % | 100 % | 120 % | 150 % | | |
| Aspirin | 375 mcg/ml | 600 mcg/ml | 750 mcg/ml | 900 mcg/ml | 1125 mcg/ml | 0.999 | 0.997 |
| Amlodipinebesylate | 25 mcg/ml | 40 mcg/ml | 50 mcg/ml | 60 mcg/ml | 75 mcg/ml | 0.998 | 0.995 |
| Simvastatin | 50 mcg/ml | 80 mcg/ml | 100 mcg/ml | 120 mcg/ml | 150 mcg/ml | 0.998 | 0.997 |

**Table 3**: Calculated parameters for method accuracy

| Analyte (n = 3) | Percent mean recovery of the of percent strength's standard solution injected | | | | Over all standard deviation | Over all % RSD |
|---|---|---|---|---|---|---|
| | 50 % | 100 % | 150 % | Over all recovery | | |
| Aspirin | 100.299 | 100.059 | 99.945 | 100.099 | 0.271 | 0.271 |
| Amlodipinebesylate | 99.951 | 100.024 | 99.999 | 99.991 | 0.719 | 0.719 |
| Simvastatin | 100.102 | 100.140 | 99.645 | 99.962 | 0.460 | 0.460 |

**Table 4**: Calculated parameters for precision reproducibility and precision intermediate of the method

| Analyte (n = 6) | Mean drug recovered (%) | | | | Over all % mean | Over all % RSD |
|---|---|---|---|---|---|---|
| | Analyst 1 | | Analyst 2 | | | |
| | Mean | % RSD | Mean | % RSD | | |
| Aspirin | 100.475 | 0.790 | 99.8 | 0.797 | 100.137 | 0.834 |
| Amlodipine | 100.236 | 1.243 | 100.636 | 1.106 | 100.436 | 1.141 |
| Simvastatin | 99.576 | 1.038 | 100.756 | 1.007 | 100.166 | 1.153 |

**Table 5**: Method robustness

| Analytes (n = 3) | Mean percent recovery of analyte detected at two different wavelengths. | | Percent recovery of analyte at two different pH of Mobile phase | |
|---|---|---|---|---|
| | 232nm | 242nm | pH 2.0 | pH 3.0 |
| Aspirin | 99.814 | 100.976 | 100.083 | 99.857 |
| Amlodipine | 99.371 | 100.448 | 99.075 | 99.566 |
| Simvastatin | 99.493 | 100.896 | 100.588 | 100.233 |

The accuracy of the system was determined by evaluating the percent recovery of three concentrations (50%, 100% and 150% of standard solution) in triplicate.

***Precision and intermediate precision***

The precision of the system was carried out by comparing the percentage assay of six independently prepared samples with standard (100%) solution.

The intermediate precision of the systems was carried out by performing the assay tests each on two separate but similar instruments by two individual analysts on two different days. All the other procedures and factors were kept same as mentioned above for precision testing.

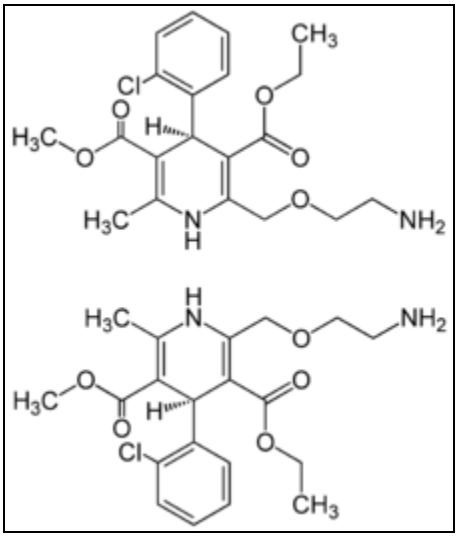
***Robustness***

To test the capacity of this newly developed analytical procedure to withstand small changes in the method, samples of standard solution were run in mobile phases with different pH values (pH 2 and 3). Moreover the estimation of assay was also carried out at two different wavelengths (232 nm and 242 nm).

***Range***

To determine the range of this analytical procedure, various concentrations prepared for linearity testing were calculated to determine highest and lowest possible concentrations with acceptable accuracy and precision.

***Stereoisomerism[edit]***



***Enantiomers of amlodipine***

Amlodipine is a chiral calcium antagonist, currently on the market and in therapeutic use as a racemate [1:1 mixture of (*R*)-(+)- and (*S*)-(-)-amlodipine][12] A method for the semi-preparative chromatographic purification of the enantiomers (*S*)-(-)-amlodipine and (*R*)-(+)-amlodipine has been reported.[13] Both enantiomers have different channel blocking activity.[14]

## RESULT

To develop a robust HPLC based analytical method for simultaneous determination of aspirin, amlodipine besylate and simvastatin, preliminary investigations were made in the light of reported literature (Barrett *et al*., 2006, Dongre *et al*., 2008, Mohammadi *et al*., 2007). The final detection was carried out at 237nm with good selectivity and sensitivity after determining the eluents at different wavelengths in the range of 230 to 245nm. After different runs, 70% (v/v) acetonitrile solution in DI water was selected as the best possible composition for mobile phase. The pH of the mobile phase also affected the quality of eluent's peaks and after multiple runs at different pH values; pH 2.5 (adjusted with dilute ortho-phosphoric acid) was finally selected. The retention times for the three eluents were found to be ~2.7 mins for aspirin, ~6.1 mins for amlodipine besylate and ~10.5 mins for simvastatin in the standard and sample solutions (see fig. 2 and 3). Moreover, to increase the stability of mobile phase over the period of time, triethylamine phosphate buffer was used instead of simple DI water to maintain the pH of mobile phase at 2.5.

## DISCUSSION

***Method validation***

The validation of this newly developed analytical procedure for simultaneous quantitative determination of aspirin, amlodipine (besylate) and simvastatin was successfully demonstrated following the criteria set as per ICH guidelines (Guideline, 2005).The validation of this procedure, included all the parameters ascribed in USP for validation of compendial methods under category-I (Chapter, 2007).

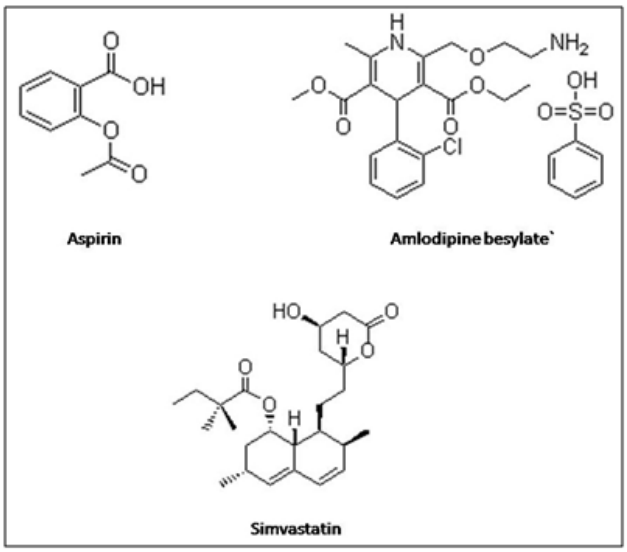



**Fig. 1**: Structural formula of aspirin, amlodipine besylate and simvastatin.

***System suitability and specificity***

Evaluation of this analytical procedure for its suitability with the system was undertaken by calculating % RSD for peak areas, theoretical plates and tailing factor of 5 replicate runs of the standard solution. All the parameters were satisfactory as per USP requirements for % RSD ($\leq$ 2%), theoretical plates ($\geq$ 1000) and tailing factor ($\leq$2.0). See table 1 for the parameters calculated for system suitability of aspirin, amlodipine (besylate) and simvastatin.

To investigate whether any excipient interferes with the elution of objective analytes; placebo (excipients without active ingredients), standard solution and blank were run on HPLC. There was no chromatographic interference observed in the developed method due to any additive material found in the formulation (see figs. 2 and 3).

***Linearity and accuracy***

The estimation of degree of proportionality between the concentration of analytes and their respective amounts recovered after analyses was made by performing linearity testing. Five working concentrations (50%, 80%, 100%, 120%, and 150%) of standard solution were run and their respective amounts recovered were found to be linearly correlated ($R^2 \geq 0.995$) for all the three drugs which were all above the USP limit of $\geq$0.99 (see figs. 4, 5, 6 and table 2).

The accuracy of the method was determined by 3 replicate runs of three different concentrations (50%,

100% and 150%) of standard solution. The system was found to be very accurate as the mean recovery of analytes obtained were found well within the range of 98% to 102% with % RSD calculated ≤2.0 as per USP requirements (see table 3).

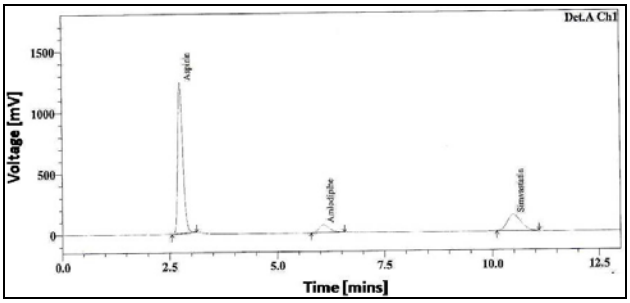



**Fig. 2**: HPLC-UV chromatogram of standard solution showing resolution of peaks of aspirin, amlodipine besylate and simvastatin.

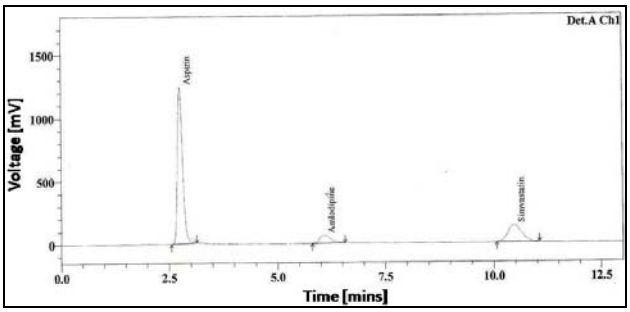



**Fig. 3**: HPLC-UV chromatogram of sample solution showing resolution of peaks of aspirin, amlodipine besylate and simvastatin.

***Precision and intermediate precision***

The newly established method was highly precise as the % RSD of mean recovery of 6 independently prepared standard solutions were 0.790%, 1.243% and 1.038% for aspirin, amlodipine and simvastatin, respectively (see table 4). These values were within the USP range of ≤2.0%.

Intermediate precision of the method was also observed to be satisfactory as the overall mean % RSD of 6 assays of standard solution on two separate instruments by two individual analysts on two different days were 0.834, 1.141 and 1.153 for aspirin, amlodipine (besylate) and simvastatin, respectively which were again well within the USP range of ≤2.0% (see table 4).

***Robustness***

The introduction of slight deliberate changes of pH of mobile phase and detection wavelength did not result in any significant difference in the recovery of eluents (see table 5). The % RSD of mean recovery of the three drugs was found to be within the acceptable range of ≤2%.

***Range***

The range for this analytical procedure was established after estimating the accuracy, precision and linearity for the highest and lowest possible concentrations. These parameters were found to be within the satisfactory limits as per pharmacopoeial standards. The concentration ranges for the three drugs were calculated as 375 to 1125 mcg/ml for aspirin, 25 to 75mcg/ml for amlodipine (besylate) and 50 to 150mcg/ml for simvastatin (see table 2). This implies that the newly developed analytical procedure is valid over a wide range of concentrations for the objective drugs.

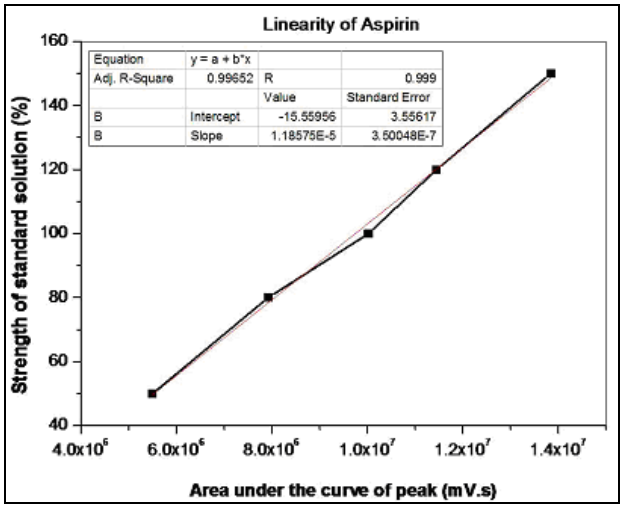



**Fig. 4**: Graph showing linearity of aspirin.

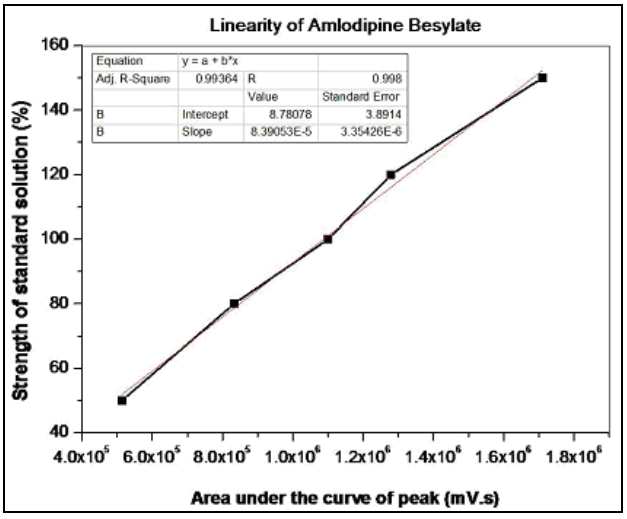



**Fig. 5**: Graph showing linearity of amlodipine (besylate). You measure amlodipine

## CONCLUSION

This study describes a new facile HPLC based analytical method for the simultaneous determination of aspirin (75mg), amlodipine (besylate) (6.9mg) and simvastatin (10mg) in a single formulation. This method offers

various advantages including easily to constitute with shorter run time and high resolution of the analytes's peaks. The method has been validated according to ICH guidelines and is found to be simple and convenient to perform; sensitive and specific for the objective drugs; and, accurate, precise and robust over a wide range of analysts' concentration. Therefore, the proposed method can be used for routine analysis of combined formulation of aspirin, amlodipine and simvastatinin any analytical setting of either a pharmaceutical industry or research organization or any academic institution which houses an HPLC-UV instrument.

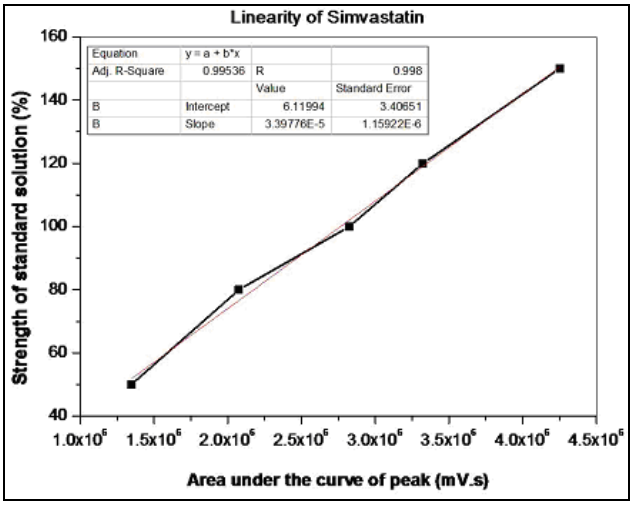



**Fig. 6**: Graph showing linearity of simvastatin.

## REFERENCES


Barrett B, Huclova J, Borek-Dohalsky V, Nemec B and Jelinek I (2006). Validated HPLC-MS/MS method for simultaneous determination of simvastatin and simvastatin hydroxy acid in human plasma. *J. Pharm. Biomed. Anal.,* **41**(2): 517-526.

Chapter G (2007). 1225>, Validation of compendial methods. *United States Pharmacopeia.* p.27.

Dongre VG, Shah SB, Karmuse PP, Phadke M and Jadhav VK (2008). Simultaneous determination of metoprolol succinate and amlodipine besylate in pharmaceutical dosage form by HPLC. *J. Pharm. Biomed. Anal.,* **46**(3): 583-586.

Elevated L, Low H and High H (2001). Effective use of statins to prevent coronary heart disease. *Am. Fam. Physician*., **63**(2): 309-321.

Gaziano TA (2008). Economic burden and the cost-effectiveness of treatment of cardiovascular diseases in Africa. *Heart,* **94**(2): 140-144.

Gaziano TA, Opie LH and Weinstein MC (2006). Cardiovascular disease prevention with a multidrug regimen in the developing world: A cost-effectiveness analysis. *Lancet,* **368**(9536): 679-686.

Guideline IHT (2005). Validation of analytical procedures: Text and methodology Q2 (R1). *IFPMA*: *Geneva*: 6-13.

Lafeber M, Grobbee DE, Spiering W, van der Graaf Y, Bots ML and Visseren FL (2012). The combined use of aspirin, a statin and blood pressure-lowering agents (polypill components) in clinical practice in patients with vascular diseases or type 2 diabetes mellitus. *European journal of preventive cardiology*: 771-778.

Law MR, Morris JK and Wald NJ (2009). Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *BMJ.,* **338**: 1665.

Mohammadi A, Rezanour N, Ansari Dogaheh M, Ghorbani Bidkorbeh F, Hashem M and Walker RB (2007). A stability-indicating high performance liquid chromatographic (HPLC) assay for the simultaneous determination of atorvastatin and amlodipine in commercial tablets. *Journal of chromatography. B, ,* **846**(1-2): 215-221.

Pasternak RC, Smith SC, Bairey-Merz CN, Grundy SM, Cleeman JI and Lenfant C (2002). ACC/AHA/NHLBI clinical advisory on the use and safety of statins12. *Journal of the American College of Cardiology,* **40**(3): 567-572.

Pawar D, Shahani S and Maroli S (1998). Aspirin-the novel antiplatelet drug. *Hong Kong Medical Journal,* (4): 415-418.

Pharmacotherapy C (2005). Combination pharmacotherapy for cardiovascular disease. *Annals of Internal medicine,* **143**(8): 593-599.

Pitt B, Byington RP, Furberg CD, Hunninghake DB, Mancini GJ, Miller ME and Riley W (2000). Effect of amlodipine on the progression of atherosclerosis and the occurrence of clinical events. *Circulation,* **102**(13): 1503-1510.

Soliman EZ, Mendis S, Dissanayake WP, Somasundaram NP, Gunaratne PS, Jayasingne IK and Furberg CD (2011). A Polypill for primary prevention of cardiovascular disease: A feasibility study of the World Health Organization. *Trials,* **12**: 3.

Volpe M, Chin D and Paneni F (2010). The challenge of polypharmacy in cardiovascular medicine. *Fundamental & Clinical Pharmacology,* **24**(1): 9-17.

Wald NJ and Law MR (2003). A strategy to reduce cardiovascular disease by more than 80%. *Bmj.,* **326**(7404): 1419.

Yach D, Leeder S, Bell J and Kistnasamy B (2005). Global chronic diseases. *Science* (New York, NY). **307**(5708): 317.