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# CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF SIMVASTATIN AND AMLODIPINE BESYLATE

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**ABSTRACT**

An isocratic RP-HPLC method was developed and validated for the quantitation of Simvastatin and Amlodipine besylate in combined tablet dosage forms. Quantitation was achieved using a reversed-phase Hypersil silica BDS (250x4.6mm with 5µ particle size) column at ambient temperature with mobile phase consisting of 0.05M ammonium acetate buffer (pH-4) and acetonitrile in the ratio (40 + 60, v/v). The flow rate was 1.0 mL/min. Measurements were made at a wavelength of 240nm. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay method was found to be linear from 30.0-70.0 µg/mL for Amlodipine besylate and 60.0-140.0 µg/mL for Simvastatin. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of Simvastatin and Amlodipine besylate in combined dosage forms.

**Keywords:** Simvastatin, Amlodipine besylate, RP-HPLC.

## INTRODUCTION

Simvastatin is antihyperlipidic agent. Simvastatin is structural analog of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme). Like other agents, it inhibits the enzyme hydroxymethylglutaryl-CoA (HMG-CoA) reductase. It has an extremely high affinity for this enzyme and was considered the most potent agent of the HMG-CoA class until Simvastatin was approved (Anonymous 1). Simvastatin is inactive lactone prodrug and hydrolyzed in the gastrointestinal tract to the active ß hydroxy derivative. It was approved by the FDA in December 1991. It decreases total cholesterol, LDL cholesterol, triglycerides, and apolipoprotein B, while increasing HDL. This paper now describes an HPTLC method for the determination of Simvastatin and ezetimibe in tablets. The method is rapid, accurate and precise (Anonymous 2). The method was validated by following the analytical performance parameters suggested by the International Conference on Harmonization. The aim of the present study was to develop and validate a simple and fast LC method, through evaluation of the parameters of linearity, precision, accuracy, detection and quantitation limits, robustness, and specificity, to determine Simvastatin and Amlodipine besylate in pharmaceutical formulations.

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***Chemicals and Reagents***

AVC reference (assigned purity, 99.9%) and AML (assigned purity, 99.8%) were obtained from Chandra labs (Hyderabad, India). The purity of the AVC and AML were evaluated by obtaining their melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drugs were used without further purification. Tablets were purchased from local market and the labeled amount was 10 mg AVC and 5mg AML each. HPLC-grade acetonitrile was from Merck. Ammonium Acetate (AR grade) and Glacial Acetic acid (A R grade) were from Merck. Solvents were filtered through a 0.45µm nylon membrane filter and degassed by using ultrasonicator.

***Instrumentation and Chromatographic Conditions***

The developed method used a Shimadzu LC system consisting of a Model LC-10 AT pumps, an SPD-10 AVP UV-VIS detector, an SCL-10 AVP system controller; data were acquired and processed by Shimadzu class-VP 5.0 software. The separation was carried out at ambient temperature by using a Hypersil silica BDS column (250 X 4.6 mm id X 5 µm particle size) prepacked with 5 µm RP-18. The mobile phase consisting of 0.05M ammonium acetate buffer (pH was adjusted to 4.0 with 10% glacial acetic acid) and acetonitrile (40 + 60, v/v) was used. The flow rate was 1.0 mL/min. The injection volume was 20 µL. For all standards and samples, triplicate injections were made. External standards with measurement of peak areas were used for quantitation.

***Preparation of Standard Solutions***

Standard stock solutions of AVC and AML were prepared separately at a concentration of 2mg/ml and 1mg/ml by dissolving the appropriate amount of standard into the mobile phase.

***Preparation of Sample Solutions***

An accurately weighed amount of powdered tablets equivalent to 10 mg AVC and 5mg AML were transferred to a 50 mL volumetric flask with 30 mL mobile phase, the flask was sonicated for 15 min, and the contents of the flask were diluted to volume with mobile phase. After filtration through a 0.2µm nylon membrane (25mm disposable filter), an aliquot amount was transferred to a 10ml volumetric flask to get a final concentration of 60 µg/mL AVC and 30 µg/mL AML (Farmacopea, 2006).

**METHOD VALIDATION**

***Linearity, limit of quantitation (LOQ), and limit of detection (LOD)***.

The linearity of the calibration curves was determined for intra- and interday precision on 3 different days. Aliquots of 0.3, 0.4, 0.5, 0.6, and 0.7 mL of a 2000.0 µg/mL standard solution of AVC and 1000 µg/mL standard solution of AML were transferred to 10mL volumetric flasks and diluted to volume with mobile phase. The final concentrations obtained were 60.0, 80.0, 100.0, 120.0, and 140.0 µg/mL AVC and 30.0, 40.0, 50.0, 60.0, and 70.0µg/mL AML, respectively. The calibration curves were constructed by plotting the absolute peak area (*y*) versus the concentration (*x*), by using linear regression analysis. The LOQ (defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy) and the LOD (defined as the lowest absolute concentration of analyte in a sample that can be detected but not necessarily quantified) were calculated according to the ICH specifications.

***System suitability test***.—Relative standard deviation (RSD) values for the peak areas, tailing factors, theoretical plates and retention times were the chromatographic parameters selected for the system suitability test.

***Accuracy***

This parameter was evaluated by the recovery studies at concentration levels of 80, 100, and 120%, which consisted of adding known amounts of AVC and AML reference materials to the samples. Aliquots of 0.24, 0.3, and 0.36 mL of 2.0 mg/mL AVC and 1mg/mL AML standard solution were transferred to 10mLvolumetric flasks containing 60µg/mL AVC and 30µg/mL AML sample. The contents were mixed and diluted with mobile phase to give final concentrations of 108.0, 120.0, and 132.0 µg/mL of AVC and 54.0, 60.0 and 66.0 µg/mL of AML, respectively. Each solution was prepared in triplicate and each was injected in triplicate. The amount of AVC and AML recovered were calculated in relation to the average from the intermediate precision study.

***Precision***

Repeatability (intraassay) and intermediate precision (interassay) were determined by assaying samples of tablets, at the same concentration (80µg/mL AVC and 40µg/mL AML), under the same experimental conditions, during the same day and on 3 different days, respectively. The intermediate precision (interassay) was evaluated by comparing the assays on these 3 different days. The relative standard deviation (RSD) was determined.

***Robustness***

Robustness was tested by changing the following

parameters of the LC method: (*a*) mobile phase proportion— 0.05M ammonium acetate buffer – acetonitrile (between 40 + 60 and 45 + 55, v/v), pH 4 as the mobile phase; (*b*) stationary phase—reversed-phase MetaSil octadecylsilane (250 X 4.6 mm, 5 µm; MetaChem Technologies, Torrance, CA); and (*c*) another liquid chromatography—quantitation was performed in a Shimadzu liquid chromatography equipped with a Model LC-10AS pump, Rheodyne injector with a 20 µL loop, and Model SD-10A UV detector.

***Specificity***

Specificity of the method was evaluated by preparing a placebo tablet containing the same excipients as in the commercial product. The solution was prepared by using the procedure described in *Preparation of Sample Solutions* and injected 3 times. Moreover, it was used as the chromatographic peak purity tool, which is another way to verify the specificity of the method (International Conference on Harmonization, 2005).

**Table 1. Experimental values obtained for the standard curves of Simvastatin and Amlodipine besylate by the LC method**

| Conc, µg/mL | | Absolute area[a] | | Mean area | | RSD, %[b] | |
|---|---|---|---|---|---|---|---|
| **AVC** | **AML** | **AVC** | **AML** | **AVC** | **AML** | **AVC** | **AML** |
| 60 | 30 | 612.840<br>618.743<br>617.978 | 258.419<br>261.886<br>271.018 | 617.127 | 258.134 | 0.11 | 0.28 |
| 80 | 40 | 778.329<br>780.480<br>779.527 | 342.283<br>343.655<br>343.877 | 787.437 | 323.224 | 0.14 | 0.27 |
| 100 | 50 | 994.192<br>993.779<br>995.153 | 412.886<br>440.758<br>441.635 | 986.337 | 431.737 | 0.08 | 0.26 |
| 120 | 60 | 1110.712<br>1119.577<br>1112.586 | 520.757<br>529.725<br>520.947 | 1189.937 | 520.532 | 0.13 | 0.14 |
| 140 | 70 | 1415.175<br>1416.745<br>1417.524 | 611.737<br>612.837<br>611.734 | 1447.448 | 612.122 | 0.10 | 0.11 |

[a] Each value is the mean of 3 injections.
[b] RSD = Relative standard deviation.

**Table 2. Experimental values of AVC and AML obtained for a commercially available sample by using the LC method**

| Sample | Intraday precision | | | | | |
|---|---|---|---|---|---|---|
| | AVC | | | AML | | |
| | **Day 1** | **Day 2** | **Day 3** | **Day 1** | **Day 2** | **Day 3** |
| 1 | 101.55 | 100.07 | 100.04 | 101.55 | 102.47 | 100.36 |
| 2 | 101.57 | 100.37 | 101.57 | 101.57 | 100.46 | 102.25 |
| 3 | 102.47 | 101.13 | 100.00 | 100.07 | 101.13 | 100.31 |
| 4 | 100.46 | 100.31 | 101.15 | 100.37 | 100.31 | 100.85 |
| 5 | 102.25 | 100.25 | 100.05 | 100.04 | 100.00 | 101.15 |
| 6 | 102.88 | 100.03 | 101.70 | 101.57 | 101.25 | 100.05 |
| Mean | 101.86 | 100.36 | 100.75 | 100.86 | 100.94 | 100.83 |
| RSD[a], % | 0.85 | 0.40 | 0.81 | 0.77 | 0.88 | 0.80 |
| Intraday precision | 100.99 | | | 100.86 | | |
| RSD, % | 0.68 | | | 0.82 | | |

[a] RSD = Relative standard deviation.

**Table 3. Experimental values obtained in the recovery test for AVC and AML by using the LC method**

| Nominal value, % | Added amount, µg/mL | | Found amount, µg/mL | | Recovery, % | | Average recovery, (n=3) | | RSD, % | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AVC | AML | AVC | AML | AVC | AML | AVC | AML | AVC | AML |
| 80 | 48 | 24 | 47.89 | 23.89 | 99.77 | 99.54 | 100.24 | 100.25 | 0.56 | 0.92 |
| 80 | 48 | 24 | 48.41 | 23.98 | 100.85 | 99.92 | | | | |
| 80 | 48 | 24 | 48.04 | 24.31 | 100.08 | 101.29 | | | | |
| 100 | 60 | 30 | 60.05 | 29.89 | 100.08 | 99.63 | 100.27 | 100.38 | 0.42 | 0.89 |
| 100 | 60 | 30 | 59.98 | 30.04 | 99.97 | 100.13 | | | | |
| 100 | 60 | 30 | 60.45 | 30.41 | 100.75 | 101.37 | | | | |
| 120 | 72 | 36 | 72.78 | 36.03 | 101.08 | 100.08 | 100.33 | 99.97 | 0.66 | 0.24 |
| 120 | 72 | 36 | 71.89 | 36.05 | 99.85 | 100.14 | | | | |
| 120 | 72 | 36 | 72.05 | 35.89 | 100.07 | 99.69 | | | | |
| Mean (n=9) | | | | | | | 100.28 | 100.20 | 0.54 | 0.68 |

**Figure 1. Simvastatin**

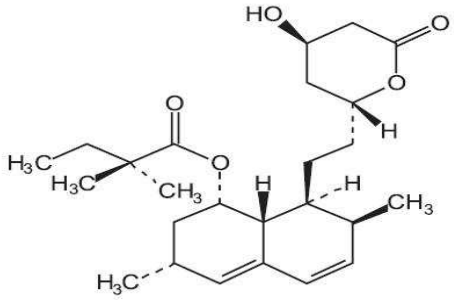



**Figure 2. Amlodipine besylate**

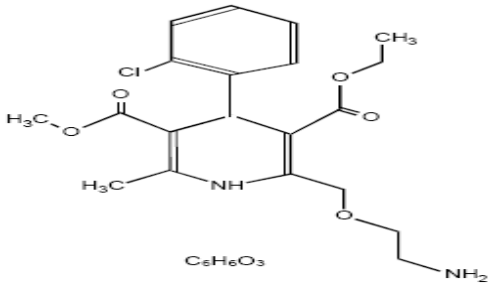



**Figure 3.** Chromatogram of AML (40µg/mL) and SIM (80µg/mL) sample solution. Chromatography conditions: 0.05M Ammonium acetate buffer – Acetonitrile (40 + 60, v/v), at pH 4.0, mobile phase; flow rate of 1.0 mL/min; Hypersil silica BDS column RP-18 (250 X4.6 mm id X 5 µm) stationary phase; UV detection at 240 nm; ambient temperature; injection volume of 20 µL.

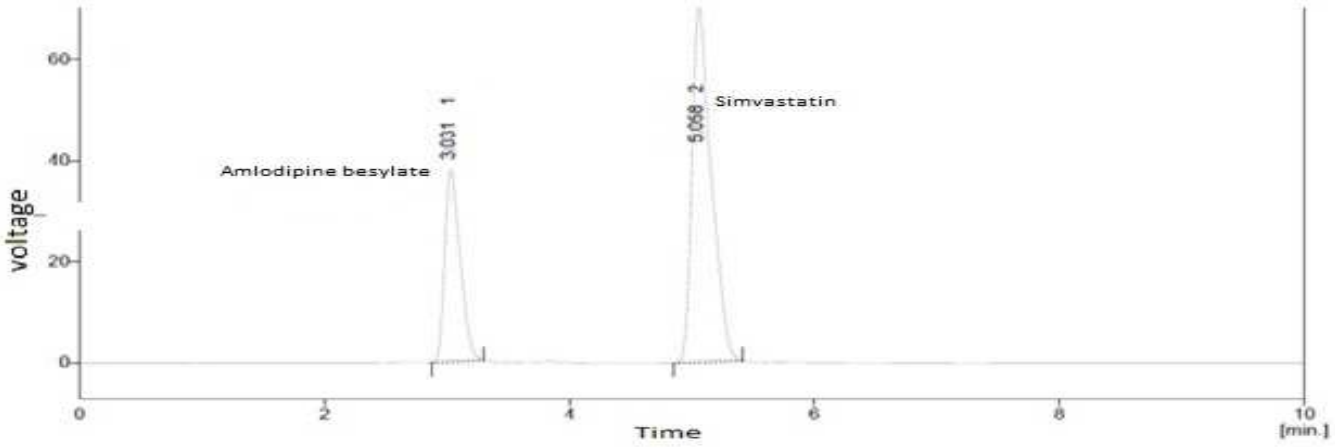


**Figure 4.** Chromatogram of AML (40µg/mL) and SIM (80µg/mL) standard solution. Chromatography conditions: 0.05M ammonium acetate buffer – acetonitrile (40 + 60, v/v), at pH 4.0, mobile phase; flow rate of 1.0 mL/min; Hypersil silica BDS column RP-18 (250 X4.6 mm id X 5 µm) stationary phase; UV detection at 240 nm; ambient temperature; injection volume of 20 µL.

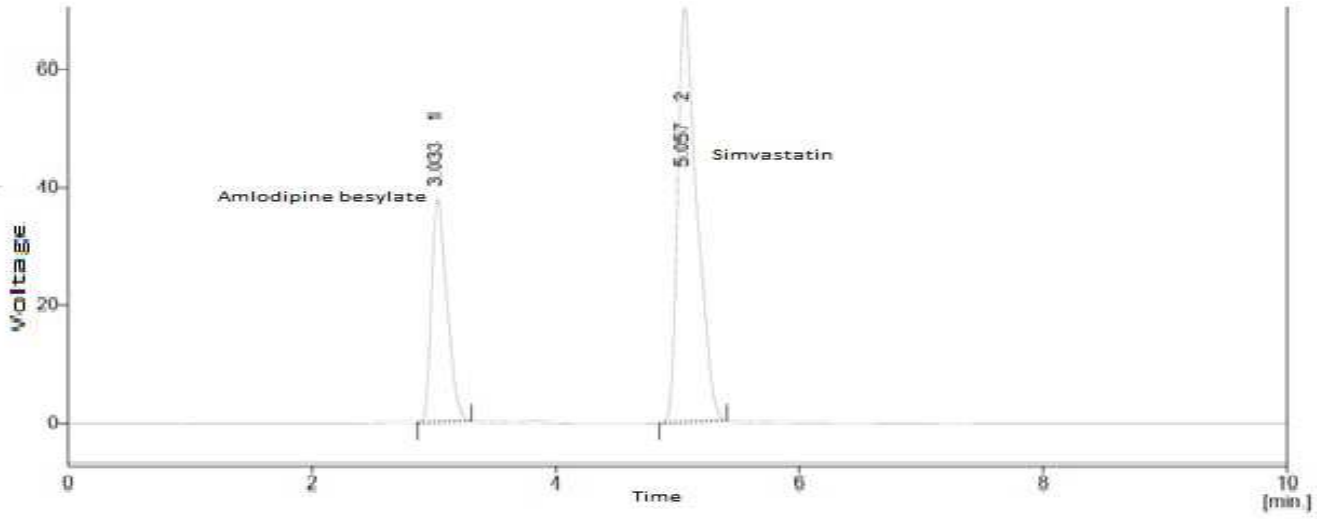
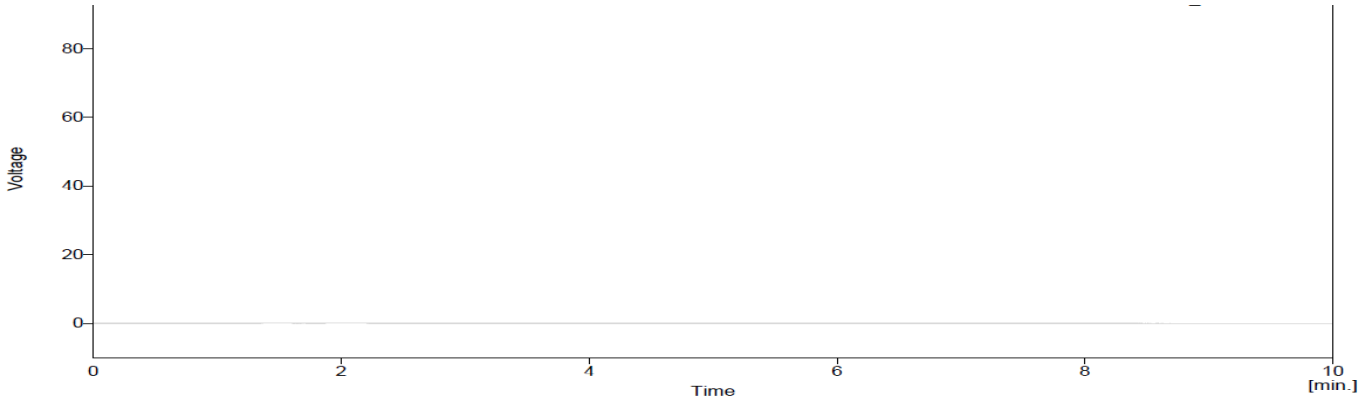



**Figure 5.** Chromatogram for placebo solution. Chromatography conditions: 0.05M ammonium acetate buffer – acetonitrile (40 + 60, v/v), at pH 4.0, mobile phase; flow rate of 1.0 mL/min; Hypersil silica BDS column RP-18 (250 X4.6 mm id X 5 µm) stationary phase; UV detection at 240 nm; ambient temperature; injection volume of 20 µL.




## RESULTS AND DISCUSSION

In this work, a method based on reversed-phase LC, using UV detection, was developed and validated for Simvastatin and Amlodipine besylate in a tablet dosage form. The experimental conditions were selected after different stationary and mobile phases were tested. Reversed-phase CN, C8, and C18 columns were used. However, best results were observed when the Hypersil silica BDS RP-18 (250X4.6mm with 5µ particle size) column was used. The mobile phase, 0.05M ammonium acetate buffer – acetonitrile (40 + 60, v/v**)**, pH 4.0, was selected. To avoid the use of buffer solutions, acetonitrile–water, methanol–water and acetonitrile–methanol–water mixtures were tested (Juyal *et al.*, 2008). However, the resulting peaks were asymmetrical and the number of theoretical plates was unsatisfactory. Then acetonitrile–ammonium acetate buffer mixtures were tested. Although it was possible to obtain good chromatographic conditions, a higher number of theoretical plates were obtained with the mobile phase chosen.

Before an analytical method is applied to quality control, it is necessary to validate the method. The validation ensures that the procedure is suitable for its intended purpose. The guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 1996) and the United States Pharmacopeia describe the analytical parameters that should be evaluated in a method validation. The type of method and its respective use determine which parameters should be evaluated. It is the responsibility of the analyst to select the parameters considered relevant for each method (Riahi Siavash *et al*., 2007).

To assess linearity, standard curves for AVC and AML were constructed by plotting concentration (µg/mL) versus absolute area and showed good linearity in the 30.0-70.0µg/mL for Amlodipine besylate and 60-140µg/mL for Simvastatin**.** The representative linear equations for these drugs were

y =10.39x-41.16, AVC

y = 8.592x - 3.862, AML

where *x* is concentration and *y* is the peak absolute area. The correlation coefficients were found to be 0.998 AML and 0.995 AVC indicating good linearity. The mean absolute area values are presented in Table 1. The detection and quantitation limits determined were 0.3957 and 1.199 µg/mL; AVC and 0.3469 and 1.051µg/mL; AML, respectively. These low values indicated the high sensitivity of the proposed method. The experimental values obtained for the determination of AVC and AML in samples are presented in Table 2. The low RSD values of 0.85, 0.40, 0.81%; AVC and 0.77, 0.88, 0.80%; AML (intraday precision), and 0.68% AVC and 0.82% AML (interday precision) showed the good precision of the method. Figure 3 shows chromatograms of a commercial sample solution and figure 4 shows AVC and AML standard solution. The retention time of AML and AVC were 3.033 (RSD = 0.11%) and 5.057 min (RSD = 0.10%), which are good values for routine quality control. The specificity test demonstrated that there was no interference in the drug peak. The chromatogram obtained through the injection of the placebo solution did not contain any other peak at the retention time of AML and AVC. The chromatographic peak purity tool shows that the peak was 100% pure. Thus, it was shown that the peaks at 3.033 and 5.057 min were not due to any interference from the excipients in the formulation (United States Pharmacopeia, 2003).

The accuracy expresses the agreement between the accepted value and the value found. The mean recovery was found to be 100.28% AVC and 100.20% AML for the tablets (Table 3). This value shows the good accuracy of the proposed method. The robustness of the method, evaluated by changing the mobile phase proportion—0.05M ammonium acetate buffer – acetonitrile (between 40 + 60 and 45 + 55, v/v, pH 4), demonstrated an increase in the retention time of the drug. The effect of using MetaSil octadecylsilane (250 X 4.6 mm, 5 µm) as the stationary phase increased the retention time by 1.5 min. Even so, the method was robust. The last experiment was quantitation by using another liquid chromatography (Shimadzu equipped with a Model LC-10AS pump, Rheodyne injector with a 20 µL loop, and Model SD- 10A UV detector) in which the retention time suffered a small increase (to 3.323 and 5.537); however, it was possible to quantify the drug satisfactorily, and this confirmed the robustness of the method. At that rate, it was possible to demonstrate that the developed method was robust with all the changes employed.

The results indicate that the reversed-phase LC assay demonstrates linearity, precision, and accuracy at concentrations ranging from 30.0-70.0µg/mL for Amlodipine besylate and 60-140µg/mL for Simvastatin**.** In addition, the developed method is simple, fast, specific, robust, and sensitive and is an acceptable method for the routine quality control of Amlodipine besylate and Simvastatin in the formulation studied.

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