# Simultaneous Estimation of Simvastatin and Metformin by RP-HPLC Method

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## Abstract

In the current study precise and accurate reverse phase liquid chromatographic method has been developed and validated for simultaneous estimation of simvastatin and metformin as the reversed phase HPLC method is most appropriate for the assessment of both the ingredient simultaneously. The method was developed using Jasco Auto sampler HPLC of 50mM Sodium Dihydrogen Phosphate Monohydrate at pH 3.0: Acetonitrile (30:70 v/v). Analysis was performed using UV-Visible detector and the eluents were monitored at 235 nm. The retention time of simvastatin and metformin was observed at 7.650min and 2.858min respectively. The method was validated with respect to linearity, robustness, precision and accuracy. The projected method was successfully applied for the simultaneous quantitative determination.

### Keywords

Simvastatin, Metformin, RP-HPLC, ICH guidelines, simultaneous.

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## INTRODUCTION

Simvastatin is one of the well-known statins which is a HMG-COA reductase inhibitor. HMG-COA reductase, is a vital enzyme involved in the synthesis of cholesterol in liver. Thus, by inhibiting the enzyme, simvastatin is widely used for the treatment of hyperlipidemia and related cardiovascular diseases. Simvastatin is a prodrug which gets converted into β-hydroxy to inhibit the enzyme. The IUPAC nomenclature is [(1$S$,3$R$,7$S$,8$S$,8a$R$)-8-[2-[(2$R$,4$R$)-4-hydroxy-6-oxoo-xan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]2, 2dimethylbutanoate.[1]

Metformin hydrochloride (3-(diaminom ethylidene)-1, 1-dimethylguanidine hydrochloride) is a well-known oral antihyperglycemic drug, largely prescribed for the management of type 2 diabetes. It shows its action by reducing the production of the hepatic glucose, absorption of glucose from intestine and by improving the insulin sensitivity. [2]

Diabetic and hyperlipidemic generally are detected commonly together in many patients. The combination of these drugs gains great importance, demand and a single formulation and target two different disorders. [3,4]

The analytical evaluation of this combination is not very much explored. There are reports of estimation of simvastatin and metformin singly or in combination with different drugs by UV spectrophotometry, RP-HPLC, HPTLC and LC-MS/ MS. Very few methods were developed in recent years, thus there is lot of scope to explore and develop

accurate, simple and rapid analytical techniques. [5,6]

The present paper attempts to develop and validate the RP-HPLC method for the determination of the content of simvastatin and metformin.

## Chemicals

Simvastatin (SIMV) and metformin (MET) API procured as gift sample from Ipca Laboratories Limited Mumbai. Other chemicals and reagents of analytical grade were purchased from Merck (India).

## Instrumentation

The study was carried out on Jasco Auto sampler HPLC (AS-5040, PU- 2080, UV-2075). The column used was Thermosil C18 (250*4.6mm,5μ).

## Chromatographic conditions

The chromatographic separation was carried out on Thermosil C18 (250*4.6mm,5μ), at 25°C of column temperature with mobile phase comprising of 50mM Sodium Dihydrogen Phosphate Monohydrate at pH 3.0: Acetonitrile (30:70 v/v) and filtered before used through a 0.45 μ membrane and degassed for 15 minutes. The flow rate was fixed at 1 mL/min, the injection volume was 20 μl and the analysis was performed using UV-Visible detector and the eluents were monitored at 235 nm.

## Preparation of standard stock solution

Standard stock solutions of SIMV and MET were prepared separately by dissolving 10 mg of each in separate 10 ml volumetric flask with small quantity of mobile phase. The mixture was sonicated for 5 min and the volume made up 10 ml with mobile phase to give a concentration of 1000 μg/ml. Nylon membrane of 0.45 μm was used to filter the final standard solution.

## Preparation of standard solutions for calibration

Aliquots stock solutions SIMV and MET were accurately transferred in to 10 ml volumetric flasks and diluted to mark with mobile phase to yield a concentration range of 1 - 20 μg/ml for SIMV and MET.

## Preparation of test

Twenty tablets were weighed and crushed. The powdered drug equivalent to 500 mg of MET, SIMV of 20 mg was transferred in a single vial. 5 ml of methanol was used to dissolve the powder completely and the volume was made to 10 ml.

## Selection of analytical wave length

The lambda max of both API was determined by spectral analysis under UV region (200-400 nm). The results showed the absorption maximum for the drugs in the given solvent was at 235 nm.

## METHODOLOGY [7,8,9,10]

To optimize the RP-HPLC chromatographic parameters several mobile phase compositions (several trials) were tried. An excellent resolution and peak symmetry for SIMV and MET was obtained with a mobile phase mixture of 50 mM Sodium Dihydrogen Phosphate Monohydrate at pH 3.0: Acetonitrile (30:70 v/v) at a flow rate of 1ml/min. Quantification was carried out at 235 nm based on peak area. System suitability was evaluated for the proposed method. The system suitability test was carried out on freshly prepared standard stock solution of SIMV and MET. Parameters such as resolution, peak tailing HETP were studied to evaluate the suitability of the system.

## Method validation

The validation parameters described in ICH guidelines like limit of detection, limit of quantitation, linearity, accuracy, precision, robustness were evaluated.

## Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were separately determined based on the signal to noise ratio. For LOD the S/N ratio was 3:1 and for LOQ the ratio was 10:1.

## Linearity

Under the optimized conditions, a calibration curve was prepared for SIMV and MET. Based on LOD and LOQ the concentration ranges 1-20 μg/ml was prepared to determine the linearity. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

## Accuracy

Recovery studies were evaluated by standard addition method. In this studies, the known amount of standard drugs was added to a pre-analyzed sample at three different levels (80 %, 100 % and 120 %). This mixed solutions were analyzed in triplicate at every level using developed method. The percent of individual recovery and % RSD at each level for the both drugs were measured.

## Precision

The precision was determined by repeatability and intermediate precision through intra-day precision and inter-day precision study of the method. The intra-day precision was examined by replicating the assay thrice for two levels in the same day whereas the inter-day precision was analyzed by determining the assay on two different days of two different concentrations in triplicates. The results of precision study were stated in terms of % RSD.

## Robustness

To ascertain the robustness of the method, the deliberate changes in optimized conditions were carried out. In the present study robustness of the method was examined by deliberate modifications in chromatographic conditions namely flow rate (± 0.1

ml/min) and detection wavelength (± 5 nm). The effect of these variables on the AUC was determined.

**Analysis of marketed formulation**

The content of both the drugs was estimated in tablet formulation by the developed and validated HPLC method.

**RESULTS AND DISCUSSION**

Good resolution, peak shape, theoretical plates, retention time and asymmetry are the main parameters which determine the optimization of any chromatographic method for analysis. Thus, to attain all these parameters, various chromatographic conditions were investigated and optimized for the estimation of SIMV and MET, such as mobile phase with different composition, flow rate, stationary phases. It was observed that good symmetrical, sharp, well resolved SIMV and MET peaks were obtained in a short run time (15 min) with mobile phase which consisted of 50mM Sodium Dihydrogen Phosphate Monohydrate at pH 3.0: Acetonitrile (30:70 v/v), with flow rate of 1 mL/minute at the analytical wavelength 235 nm. The retention time of SIMV and MET was observed at 7.650 min and 2.858 min respectively (Fig 1).

The method developed for the simultaneous estimation of SIMV and MET was found to be suitable as the resolution factor and symmetry were within the acceptable range. The resolution (Rs) was 13.841 and symmetry was 1.57 and 1.38 for SIMV and MET respectively.

The calculated LOD and LOQ were found to be 0.0024 µg/ml and 0.0073 µg/ml for SIMV and 0.0044 µg/ml and 0.0132 µg/ml for MET showed that the method is specific and sensitive to estimate these drugs at low concentration level.

The calibration curve obtained for a series of concentration in the range of 1-12 µg/ml for SIMV and MET was found to be linear. The calculated regression coefficient was 0.9997 and 0.9999 for SIMV and MET respectively depicting good linearity (Fig 2 and Fig 3).

Recovery studies of the drug were carried out for the accuracy parameter at three different concentrations levels i.e. multiple level recovery studies. A known amount of standard drug was added into pre-analyzed sample and subjected them to the proposed HPLC method. The % recovery was found to be within the limits. (Table 1 and 2)

Precision was studied to find out intra and inter day variations in the test method of SIMV and MET. The values of % RSD (< 2.0) indicate that the proposed method is quite precise, reproducible and results are shown in Tables 3,4,5 and 6.

Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate and wavelength. It was observed that there were no marked changes in the chromatograms. The AUC obtained in the changed condition was within the limit. (Table7,8,9 and 10)

Finally, the developed validated method was applied for quantitative estimation of the marketed formulation tablets. The developed and validated HPLC method was used to estimate the content which was found to be 98 % for SIMV 99.68% for MET. (Table 11)

**CONCLUSION**

The developed and validated method is very rapid as the total run time is 15 min. The method can detect the lower concentration accurately which make its wide applicability. The method can be definitely applied for routine quality control testing as well as for other analysis like bioavailability studies.

**Table-1: Recovery data of SIMV**

| Level (%) | Drug Conc (mg) | Amt added (mg) | Total Amt (mg) | Amt recovered (mg) | % Recovery |
|-----------|----------------|----------------|----------------|--------------------|------------|
| 80% | 20 | 16 | 36 | 36.65 | 102 |
| 100% | 20 | 20 | 40 | 40.12 | 100.40 |
| 120% | 20 | 24 | 44 | 44.04 | 100.12 |

Conc= Concentration, Amt= Amount

**Table- 2: Recovery data of MET**

| Level (%) | Drug Conc (mg) | Amt added (mg) | Total Amt (mg) | Amt recovered (mg) | % Recovery |
|-----------|----------------|----------------|----------------|--------------------|------------|
| 80% | 500 | 400 | 900 | 898.3 | 99.70 |
| 100% | 500 | 500 | 1000 | 999.6 | 99.96 |
| 120% | 500 | 600 | 1100 | 1098 | 99.81 |

Conc= Concentration, Amt= Amount

**Table- 3: Precision study (inter- day) of SIMV**

| Conc µg/ml | Area | AVG | SD | %RSD |
|---|---|---|---|---|
| 4 | 210153 | | | |
| 4 | 209963 | 210090.70 | 110.57 | 0.05 |
| 4 | 210156 | | | |
| 8 | 297684 | | | |
| 8 | 305410 | 301747.00 | 3878.50 | 1.29 |
| 8 | 302147 | | | |

Conc= Concentration, AVG= average, SD= Standard deviation, RSD= Relative standard deviation

**Table- 4: Precision study (inter- day) of MET**

| Conc µg/ml | Area | AVG | SD | %RSD |
|---|---|---|---|---|
| 4 | 76102 | | | |
| 4 | 75803 | 75973.00 | 153.66 | 0.20 |
| 4 | 76014 | | | |
| 8 | 146415 | | | |
| 8 | 146863 | 145898.00 | 1302.85 | 0.89 |
| 8 | 144416 | | | |

Conc= Concentration, AVG= average, SD= Standard deviation, RSD= Relative standard deviation
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**Table-5: Precision study (intra-day) of SIMV**

| Conc µg/ml | Area | AVG | SD | %RSD |
|---|---|---|---|---|
| 4 | 165811 | 166024.00 | 2749.69 | 1.66 |
| 4 | 168874 | | | |
| 4 | 163387 | | | |
| 8 | 302684 | 299747.00 | 3833.77 | 1.28 |
| 8 | 295410 | | | |
| 8 | 301147 | | | |

Conc= Concentration, AVG= average, SD= Standard deviation, RSD= Relative standard deviation

**Table-6: Precision study (intra-day) of MET**

| Conc µg/ml | Area | AVG | SD | %RSD |
|---|---|---|---|---|
| 4 | 75166 | 75031.00 | 190.92 | 0.25 |
| 4 | 74896 | | | |
| 4 | 74112 | | | |
| 8 | 145941 | 121858.67 | 222.86 | 0.18 |
| 8 | 145523 | | | |
| 8 | 145866 | | | |

Conc= Concentration, AVG= average, SD= Standard deviation, RSD= Relative standard deviation

**Table-7: Robustness study with change in flow rate of SIMV**

| Conc µg/ml | Area | AVG | SD | %RSD |
|---|---|---|---|---|
| 4 | 161863 | | | |
| 4 | 159863 | 160227.33 | 1487.35 | 0.93 |
| 4 | 158956 | | | |
| 8 | 292684 | | | |
| 8 | 296710 | 293513.67 | 2872.80 | 0.98 |
| 8 | 291147 | | | |

Conc= Concentration, AVG= average, SD= Standard deviation, RSD= Relative standard deviation

**Table- 8: Robustness study with change in flow rate of MET**

| Conc µg/ml | Area | AVG | SD | %RSD |
|---|---|---|---|---|
| 4 | 75226 | | | |
| 4 | 74636 | 74624.67 | 607.08 | 0.81 |
| 4 | 74012 | | | |
| 8 | 146941 | | | |
| 8 | 146523 | 146426.67 | 568.65 | 0.39 |
| 8 | 145816 | | | |

Conc= Concentration, AVG= average, SD= Standard deviation, RSD= Relative standard deviation

**Table-9: Robustness study with change in wavelength of SIMV**

| Conc µg/ml | Area | AVG | SD | %RSD |
|---|---|---|---|---|
| 4 | 161473 | | | |
| 4 | 158463 | 159297.33 | 1901.16 | 1.19 |
| 4 | 157956 | | | |
| 8 | 292684 | | | |
| 8 | 295410 | 296413.67 | 4319.85 | 1.46 |
| 8 | 301147 | | | |

Conc= Concentration, AVG= average, SD= Standard deviation, RSD= Relative standard deviation

**Table-10: Robustness study with change in wavelength of MET**

| Conc µg/ml | Area | AVG | SD | %RSD |
|---|---|---|---|---|
| 4 | 75112 | | | |
| 4 | 74213 | 74879.67 | 586.12 | 0.78 |
| 4 | 75314 | | | |
| 8 | 146925 | | | |
| 8 | 146123 | 146254.67 | 615.16 | 0.42 |
| 8 | 145716 | | | |

Conc= Concentration, AVG= average, SD= Standard deviation, RSD= Relative standard deviation

**Table-11: Assay Results of Tablet Dosage Form**

| Parameter | MET | SIMV |
|---|---|---|
| Label claim amount (mg) | 500 | 20 |
| Amount found (mg) | 498.4 | 19.7 |
| % Purity | 99.68 | 98 |
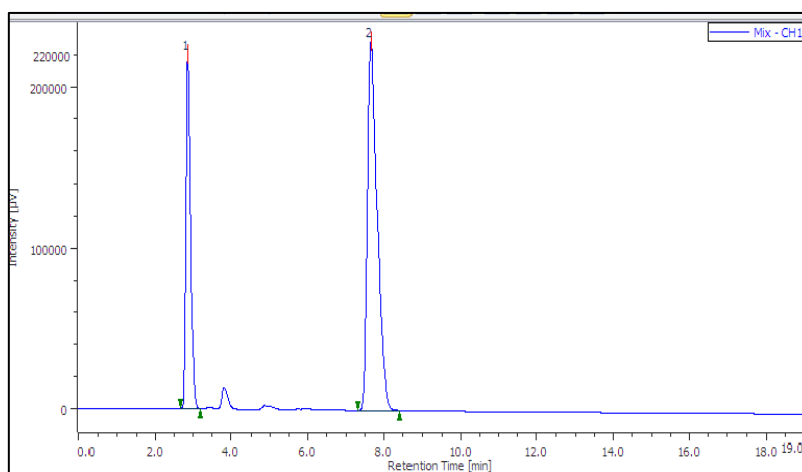


**Figure-1: HPLC Chromatogram with three resolved peak of MET and SIMV**

$y = 40649x - 2232.6$
$R^2 = 0.9997$
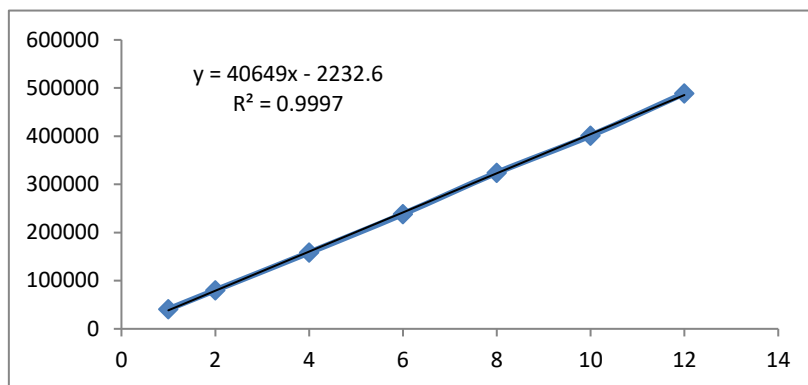
**Figure-2: Linearity curve of standard SIMV**
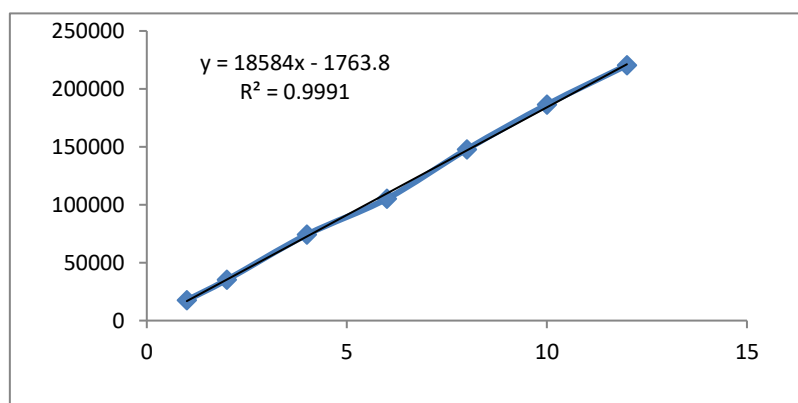


$y = 18584x - 1763.8$
$R^2 = 0.9991$

**Figure-3: Linearity curve of standard MET**

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