

Novel RP-HPLC Method for Simultaneous Determination of Sitagliptin and Simvastation in Bulk and Tablet Dosage Form

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ABSTRACT: A simple, rapid, accurate, precise and novel high-performance liquid chromatographic method for simultaneous analysis of Sitagliptin (SITA) and Simvastation (SIMV) in pharmaceutical dosage form has been developed and validated. The chromatographic separation was accomplished on Welchrom RP-C₁₈ Column (250 mm X 4.6 mm; 5 μ m), Shimadzu LC-20AT Prominence Liquid Chromatograph and with a mixture of 10 mM Phosphate buffer: acetonitrile and methanol in the range of (45:35:20 v/v/v). The flow rate was fixed at 1 mL/minute and the analysis was performed using Shimadzu SPD-20A Prominence UV-detection was performed at 255 nm. The SITA and SIMV were separated within seven minutes. The retention time for SITA and SIMV was found to be 3.352 minutes and 5.402 minutes respectively. The calibration plots were linear over the concentration range of 10-50 μ g/ml for SITA ($r^2 = 0.9998$) and 4-20 μ g/ml for SIMV ($r^2 = 0.9999$). There was no interference due to commonly used excipients. The relative standard deviation for inter-day precision was lower than 2.0 % which obviously indicates that the present method was said to be highly precise. Regarding accuracy of the developed method the % RSD were also found less than 2 % which shows the method is completely accurate. The method was very sensitive with regard to LOD 0.681 μ g/ml, 0.116 μ g/ml and LOQ 2.250 μ g/ml, 0.384 μ g/ml respectively. The mean assay values for SITA and SIMV were determined in tablet dosage form were found to be within limits. The developed RP HPLC method was found to be simple, rapid, sensitive, highly precise and accurate highly suitable for routine analysis of drug samples containing SITA and SIMV.

Keywords: Sitagliptin and Simvastatin, RP-HPLC Method, Simultaneous estimation.

I. INTRODUCTION

The combination of SITA and SIMV is used together with a decorous diet and exercise to treat type 2 diabetes. It is also utilized together with a proper diet to treat high cholesterol and triglyceride (fats) levels in the blood. This medicine may help stop medical problems caused by clogged blood vessels such as heart attacks and strokes. SITA is a dipeptidyl peptidase - 4 inhibitor utilize to treat type 2 diabetes mellitus i.e., it helps to control blood sugar levels by increasing substances in the body that make the pancreas release more insulin. It also signals the liver to stop producing glucose when there is too much sugar in the blood and SIMV belongs to the group of medicines called statins and is used as HMG-CoA reductase inhibitor to lower the levels of LDL cholesterol. These two disorders commonly occur in patients at the same time, and have been typically treated with administration of these two drugs in separate tablets. The combination was approved in 2011 and is marketed as Juvisync by Merck. Currently, the combination tablet is approved in three doses: SITA/SIMV 100 mg/10 mg, 100 mg/20 mg, and 100 mg/40 mg.

Literature survey revealed that there were few analytical methods have been reported for the simultaneous estimation of above said drugs individually, tertiary or in combination with some other drugs in biological samples as well as pharmaceutical dosage forms by UV- spectrophotometry^[1-9], HPLC^[10-18], HPTLC^[19], LC-MS/MS^[20,21], Capillary zone electrophoresis^[22]. All the above reported methods were based on the estimation of SITA/SIMV alone or in combination with other drugs. However most of the available methods have limitations such as long run time, poor resolution, uneconomical and low sensitivity. So based on the above mentioned reasons, indeed an attempt has been made to develop a simple, precise, accurate, reproducible and robust RP-HPLC method for the simultaneous determination of SITA and SIMV in pharmaceutical dosage form. The structural formulas of SITA and SIMV are shown in Fig. 1 and Fig. 2.

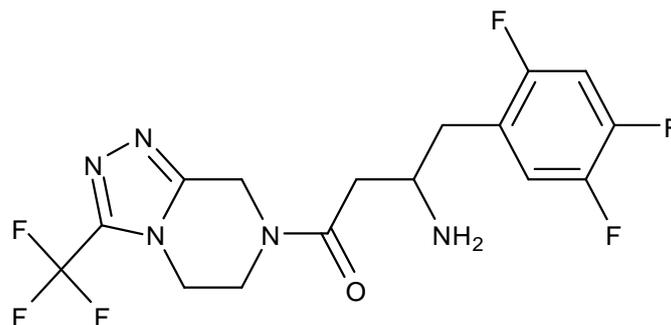


Figure 1: Chemical structure of SITA

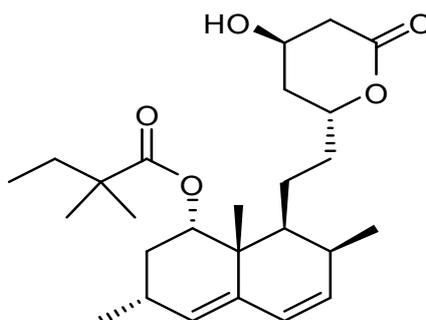


Figure 2: The chemical structure of SIMV

II. MATERIALS AND METHODS

2.1 Chemicals and reagents

Pharmaceutically pure samples of SITA and SIMV were obtained as a gift sample from Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade. Potassium dihydrogen orthophosphate and phosphoric acid purchased from Rankem Ltd., Mumbai, India, while acetonitrile (HPLC grade) and triethylamine (HPLC grade) purchased from Merck Pharmaceuticals Private Ltd., Mumbai, India. Ortho phosphoric acid utilized was of HPLC grade and obtained from Merck Specialties Private Ltd., Mumbai, India. Glassware used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid mixture, then rinsed thoroughly with triple distilled water and dried in hot air oven. Commercial formulation was procured from local drug market.

2.2 Instrument specifications

Quantitative HPLC was performed on an isocratic high performance liquid chromatograph (Shimadzu LC-20AT Prominence Liquid Chromatograph) with a LC-20AT VP pump, manual injector with loop volume of 20 μ L (Rheodyne), programmable variable wavelength Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C₁₈ Column (4.6 X 250 mm, 5 μ m particle size). The HPLC system was equipped with "Spinchrome" software. In addition an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40), UV-Visible Spectrophotometer (Systronics model-2203) were used in this present study.

2.3 Chromatographic conditions

The chromatographic separation was accomplished on Welchrom RP-C₁₈ Column (250 mm X 4.6 mm; 5 μ m), Shimadzu LC-20AT Prominence Liquid Chromatograph and mobile phase consisted of a mixture of 10 mM Phosphate buffer: acetonitrile and methanol in the range of (45:35:20 v/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 μ l and the analysis was performed using Shimadzu SPD-20A Prominence UV-Visible detector and the eluents were monitored at 255 nm.

2.4 Preparation of mobile phase

The appropriate composition of the mobile phase prepared contains phosphate buffer, acetonitrile and methanol in the ratio of 45:35:20 v/v/v. Prior to applying for separation, both the solvent and buffer solutions were filtered by passing through 0.45 μ m nylon filter and sonicated for about 15 min.

2.5 Preparation of 0.025 M phosphate buffer solution

0.025 M phosphate buffer solution was prepared by dissolving precisely weighed three of sodium dihydrogen phosphate in 1000 mL HPLC grade water. The solution was filtered through 0.45 mm membrane filter and sonicated for about 15 min. The pH was adjusted for 6.3 with triethyl amine.

2.6 Preparation of standard stock solution

Standard Stock solutions of SITA and SIMV pure drugs were separately prepared by dissolving 10 mg of each in separate 10 ml of volumetric flask with small quantity of mobile phase. The mixture was sonicated for 10 min and filled up the volume with mobile phase to give a concentration of 1000 µg/ml. These stock solutions were used for preparing working standards and calibration standards. The standard solution was filtered through 0.45 µm nylon membrane filter and degassed by sonicator.

2.7 Preparation of calibration standards

Aliquots stock solutions of SITA and SIMV were accurately transferred in to 10 ml volumetric flasks and diluted to mark with mobile phase to yield a concentration range of 10 - 50 µg/ml for SITA and 4 - 20 µg/ml for SIMV. The calibration line was obtained by plotting the mean peak area (n = 3) against concentrations of drug.

2.8 Preparation of test sample solution

20 tablets of juvisync containing SITA 100 mg and SIMV 20 mg were weighed and finely powdered. The powder equivalent to 100 mg of SITA and 20 mg of SIMV was weighed and transferred to a 100 ml volumetric flask containing 60 mL mobile phase as a diluent. After sonication for complete solubility, the volume was made up to the mark with mobile phase to obtain concentration of 1000 µg/ml. Resultant solution was filtered through whatman filter paper followed by 0.45 µ nylon filter. 1 mL of filtrate was taken in 10 mL volumetric flask and was made up to 10 mL with diluent to obtain a concentration of 100 µg/ml for SITA and 20 µg/ml for SIMV. From these solution aliquots concentrations are prepared and utilized for the sample analysis.

2.9 Selection of analytical wave length

The solution of SITA and SIMV was prepared with the diluents and the UV spectrum was recorded by scanning the sample in the UV region (200 - 400) in spectrum mode. The results showed that the absorption maximum for the drug in the given solvent was at 255 nm and hence the detector was set at 255 nm for monitoring the eluents.

2.10 METHODOLOGY

To optimize the RP-HPLC chromatographic parameters several mobile phase compositions (several trials) were tried. A satisfactory separation and excellent peak symmetry for SITA and SIMV was obtained with a mobile phase mixture of 10 mM Phosphate buffer: acetonitrile and methanol in the range of (45:35:20 v/v) at a flow rate of 1ml/min to get better reproducibility and repeatability. Quantification was carried out at 255 nm based on peak area. Complete resolution of the peak with clear baseline was obtained (Fig 3). system suitability test parameters and optimized chromatographic conditions for SITA and SIMV for the proposed method are reported in Table 1.

Table 1: Optimized chromatographic conditions and system suitability parameters

| Parameter | Chromatographic conditions | |
|---|--|----------------|
| Instrument | SHIMADZU LC-20AT Prominence liquid chromatograph | |
| Column | WELCHROM C ₁₈ Column (4.6 X 250mm, 5µm) | |
| Detector | SHIMADZU SPD-20A Prominence UV-Vis detector | |
| Diluents | 10 mM Phosphate buffer: Acetonitrile: methanol (45:35:20 v/v/v). | |
| Mobile phase | 10 mM Phosphate Buffer: Acetonitrile: methanol (45:35:20 v/v/v). | |
| Flow rate | 1mL/min. | |
| Detection wave length | UV at 255 nm. | |
| Run time | 8 minutes | |
| Column back pressure | 105-112 kgf | |
| Temperature | Ambient temperature (25 °C) | |
| Injection Volume | 20µL | |
| | SITA | SIMV |
| Retention time (t _R) | 3.352 minutes. | 5.402 minutes. |
| Theoretical plates [th.pl] (Efficiency) | 12,337 | 16,375 |
| Resolution | - | 14.808 |
| Tailing factor (asymmetry) | 1.112 | 1.130 |

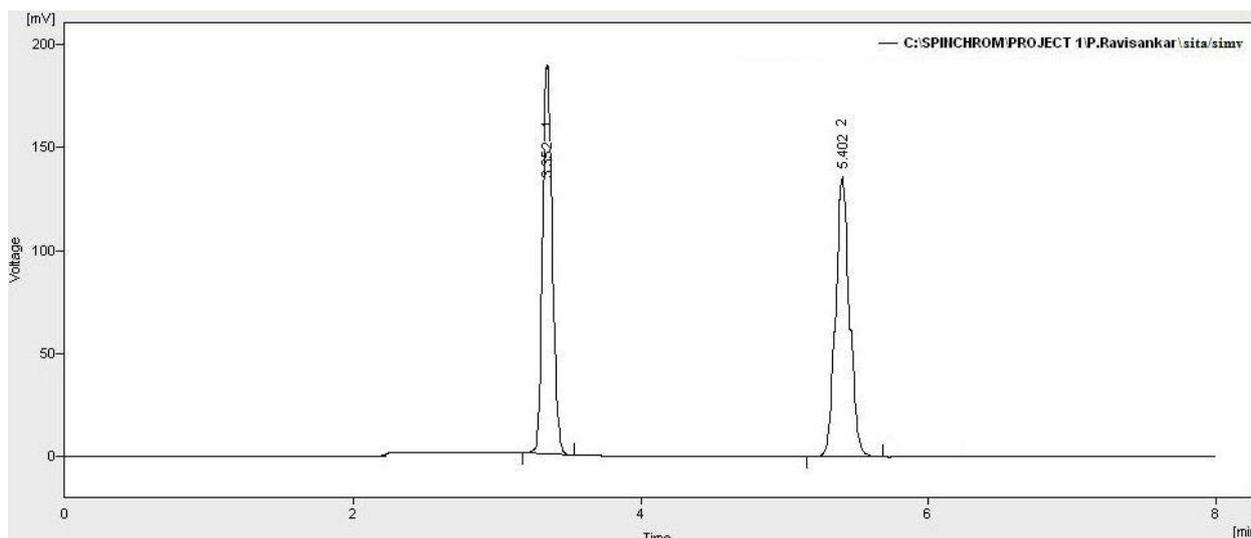


Figure 3: Chromatogram of the SITA and SIMV synthetic drug

2.11 Method validation^[23,24]

The method was validated for Specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and stability of the analytical solution as per the international conference on harmonization ICH Q2(R1)^[25] guidelines.

2.11.1 Specificity

Specificity of the method is performed by separate injections of SITA and SIMV standard and sample. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc., Specificity of the method was performed by comparing the chromatograms of blank, standard and sample. It was found that there is no interference due to excipients in the tablet formulation and also that there is good correlation between the retention times of standard and sample. The specificity results are tabulated in Table 2.

Table 2: Specificity study

| Name of the solution | Retention time, (t _R)min. |
|--|--|
| Mobile phase | No peaks |
| Placebo | No peaks |
| Solution containing a concentration of SITA and , 50 µg/ml and SIMV, 20 µg/ml. | Peaks at 3.352 min and 5.402 min for SITA and SIMV respectively. |

2.11.2 Precision

The precision (repeatability and intermediate precision) experiments were conducted by determining the intra-day precision and inter-day precision of the method. The intra-day precision was examined by replicating the assay thrice for the three levels in the same day where as the inter-day precision was studied by taking over the assay on three dissimilar days, three times on each day for the three concentration levels. The results of precision study were stated in terms of % RSD and it is presented in Table 3.

2.11.3 Accuracy (Recovery studies)

Accuracy of the method was determined by standard addition method. Recovery tests were carried out by analyzing mixtures of SITA and SIMV different compositions. Known amount of standard drugs were added to a pre-analyzed sample at three different levels (80 %, 100 % and 120 %) and the mixed standard solutions were analyzed in triplicate at every level as per the suggested method. The percent of individual recovery and % RSD at each level for the all drugs are given in Table 3.

2.11.4 Robustness

The method of robustness known as its capacity to remain unaltered due to the deliberate changes in optimized conditions set for the method. To ascertain the robustness of the method, the conditions of experiment were deliberately changed for their effect on the assay, peak tailing factor and number of theoretical plates. In

the present study robustness of the method was examined by carrying out slight but deliberate modifications in chromatographic conditions namely flow rate (± 0.1 ml/min), detection wavelength (± 5 nm) and Mobile phase composition (± 2 %). The effect of these variables on the developed method was determined. The results obtained are summarized in Table 3.

2.11.5 LOD and LOQ

As per ICH guideline both LOD and LOQ were ascertained by computing the standard deviation of the response of the working standard solution at lowest concentration near the y-intercept divided by the average of the slope. For each drug six replicates of the analyte at lowest concentration within the calibration range were measured and quantified. The LOD was decided by multiplying the ratio between the standard deviation to the slope with 3.3. The LOQ was assessed by multiplying the same ratio with 10. The results of LOD and LOQ obtained for the drugs are exhibited bellow in Table 3.

Table 3: Summary of validation parameters

| Parameters | SITA | SIMV | | |
|-----------------------------------|------------------------|-----------------------|-------|-------|
| Retention time (min) | 3.352 | 5.402 | | |
| LOD ($\mu\text{g/ml}$) | 0.6819 | 0.1165 | | |
| LOQ ($\mu\text{g/ml}$) | 2.250 | 0.384 | | |
| Linearity ($\mu\text{g/ml}$) | 10-50 $\mu\text{g/ml}$ | 4-20 $\mu\text{g/ml}$ | | |
| Correlation coefficient | 0.9998 | 0.9999 | | |
| Precision (inter-day) % RSD (n=6) | 0.280* | 0.290* | | |
| Precision (intra-day) % RSD (n=6) | 0.320* | 0.330* | | |
| Recovery (%) | | | | |
| 80 % analyte added | 98.0 | 0.35* | | |
| 100 % analyte added | 99.9 | 0.54* | | |
| 120 % analyte added | 98.95 | 0.42* | | |
| Robustness | Robust | Robust | | |
| Assay % (n = 6) | | | | |
| Bulk drug | 98.42 | 0.32* | 99.32 | 0.65* |
| Tablets | 99.82 | 0.12* | 99.55 | 0.32* |

RSD = Relative standard deviation, * = % RSD

2.12 Analysis of marketed formulation

20 μl of above prepared sample solution was injected into HPLC system and peak areas were measured under optimized chromatographic conditions. The result formulation analysis and its chromatogram is given in Table 4 and Fig.4.

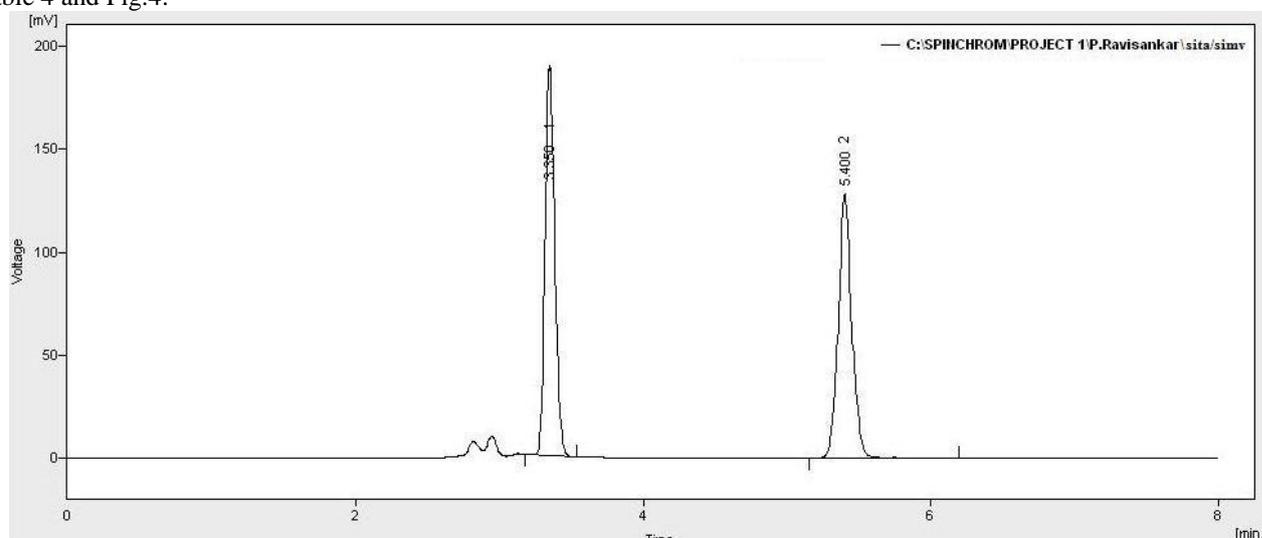


Figure 3: Chromatogram of Juvisync tablet formulation

Table 4: Assay results for SITA and SIMV

| Formulation | Label claim | | Amount found (mg) | | Assay (%) | | RSD (%) | |
|-------------|-------------|-------|-------------------|-------|-----------|-------|---------|------|
| | | | Mean±SD (n=3) | | | | | |
| Juvisync | SITA | SIMV | SITA | SIMV | SITA | SIMV | SITA | SIMV |
| | 50 mg | 20 mg | 49.91 | 19.91 | 99.82 | 99.55 | 0.12 | 0.32 |

RSD= Relative standard deviation

III. RESULTS AND DISCUSSION

The mobile phase and stationary phase play a prominent role on resolution, peak shape, theoretical plates, and asymmetry. To attain symmetrical peaks with decorous resolution, various chromatographic conditions was investigated and optimized for the estimation of SITA and SIMV, such as mobile phase with different composition, pH, and various stationary phases. For obtaining appropriate mobile phase for the analysis, various mobile phase consisting of mixtures of acetonitrile, methanol HPLC grade water, phosphate buffer were tested. Eventually it was demonstrated that highly symmetrical, sharp, well resolved SITA and SIMV peaks were obtained in a short time when utilized Welchrom C₁₈ column with mobile phase consisted of a mixture of 10 mM Phosphate buffer (pH 6.3): acetonitrile: methanol (45:35:20 v/v/v). with flow rate of 1mL/minute at the analytical wavelength 255 nm.

Chromatograms showed a peak of SITA at retention time of 3.352 min and peak of SIMV at retention time of 5.402 min respectively. The specificity was determined to test the interference of commonly used excipients. The comparison of standard and blank chromatograms indicates no co-eluting peak between the two main peaks in the chromatograms as well as it was properly resolved, well shaped peaks also shows the specificity of the method. Therefore, it was concluded that the method is specific. The specificity results are summarized in Table 2. All the system suitability parameters were evaluated. Since there are no marked changes in the system suitability parameters, the method was capable to remain unaffected by small variation of tested variables for robustness study. The calibration curve was obtained for a series of concentration in the range of 10-50 µg/ml for SITA and 4 - 20 µg/ml for SIMV respectively and it was found to be linear. The regression equation obtained from linearity plot for SITA was $Y = 27.239 x - 6.4173$ with $R^2=0.9998$ and for SIMV was $Y = 58.463 x + 0.5685$ with $R^2=0.9999$ which shows that this method had good linearity. The standard chromatograms of SITA and SIMV are shown in Figure 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. Precision was studied to find out intra and inter day variations in the test methods of SITA and SIMV for the six times on the same day and on different days. The values of % RSD (< 2.0) indicate that the proposed method is quite precise and reproducible and results are shown in Tables 3. Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate, temperature, mobile phase composition etc., It was observed that there were no marked changes in the chromatograms. Infact the parameters are within the limit which indicates that the method has robustness and suitable for routine use. The calculated LOD and LOQ are found to be 0.681 µg/ml and 2.250 µg/ml for SITA and 0.116 µg/ml and 0.384 µg/ml for SIMV showed that the method is specific and sensitive to estimate these drugs at low concentration level. Finally the developed validated method was applied for quantitative estimation of the marketed formulation tablets. 20 µL of sample solution was injected into liquid chromatograph and chromatogram was recorded and the mean assay value was found to be 99.82 ± 0.12 % for SITA and 99.55 ± 0.32 % for SIMV. Satisfactory results were achieved. The mean % found for all two drugs were in good agreement with the label claim and results are presented in Table 4. The sample chromatogram of SITA and SIMV is shown in Figure 4. Bench top solution stability was tested to the working solutions of the drugs and the results suggest that the solutions are stable up to 48 hours in the routine and operational and environmental conditions.

IV. CONCLUSION

The present proposed research study by the author describes the estimation of SITA and SIMV available as combination tablet dosage forms and was carried out by utilizing RP-HPLC. The linearity of the proposed method was in the range of 10-50 µg/ml for SITA and 4-20 µg/ml for SIMV respectively. The LOD and LOQ of SITA were 0.681µg/ml and 2.250 µg/ml and for the estimation of SIMV were 0.116 µg/ml and 0.384 µg/ml respectively. The total run time of the above titled drugs were 8 minutes. The developed method has several advantages like good linearity, decrease the time needed for analysis and low flow rate used in this

method consumes less solvent consumption, improved resolution as compared with the previous methods which makes the method more economical than the existing methods in practice. The developed RP-HPLC method for the quantification of SITA and SIMV was found to be simple, specific, highly sensitive, fast, economical, precise and extremely accurate with robustness. Therefore this method can be recommended for the routine analysis of SITA and SIMV in quality control and clinical laboratories.

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