



Analytical Method Development and Validation for Simultaneous Determination of Simvastatin and Mupirocin Using Reverse-Phase High-pressure Liquid Chromatographic Method

Ters Faz Yüksek Basıncılı Sıvı Kromatografik Yöntem Kullanılarak Simvastatin ve Mupirosin Eşzamanlı Belirlenmesi için Analitik Yöntem Geliştirme ve Validasyon

© Rupali KALE^{1*}, © Pratiksha SHETE², © Dattatray DOIFODE¹, © Sohan CHITLANGE²

¹Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Department of Pharmaceutics, Pimpri-Chinchwad, India

²Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Department of Quality Assurance, Pimpri-Chinchwad, India

ABSTRACT

Objectives: This study was aimed to develop and validate the use of reverse-phase high pressure liquid chromatographic method for the estimation of simvastatin (SIM) and mupirocin (MUP) simultaneously.

Materials and Methods: The chromatographic method developed is optimized for flow rate with the column, solvent, and buffer used and mobile phase ratio, molarity, and pH. The validation of the optimized method and the forced degradation studies of both drugs (under acidic, alkaline, oxidation, heat, light, and neutral conditions) were conducted following the The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines.

Results: Kromasil C18 column (250 mm × 4.6 mm, 5 µm) with ultraviolet detection at 224 nm and acetonitrile/phosphate buffer (30 mM, 70:30 v/v, pH 3.5; adjustment done using orthophosphoric acid) as mobile phase at a flow rate of 1.1 mL/min were observed to provide a good resolution for MUP and SIM at retention times of 2.32±0.008 and 13.55±0.254 min, respectively, with high accuracy (percent recovery was 99.69±0.82 for MUP and 101.10±0.02 for SIM) and linearity in the range of 5-30 µg/mL (r^2 : 0.9969 for MUP and r^2 : 0.9959 for SIM). The diagnostic limit and the lower limit of determination were 0.771±0.234 and 2.338±0.246 µg/mL for MUP and 0.595±0.282 and 1.803±0.334 µg/mL for SIM, respectively. The validated method was used to understand the degradation behavior of both drugs after the forced degradation studies.

Conclusion: The analytical method developed is determined to be specific, sensitive, precise, and accurate for the estimation of MUP and SIM simultaneously in the combined dosage form.

Key words: Simvastatin, mupirocin, validation, stability

ÖZ

Amaç: Bu çalışmanın amacı, simvastatin (SIM) ve mupirosin (MUP) tahmininde eş zamanlı olarak ters fazlı yüksek performanslı sıvı kromatografik yöntemin kullanımını geliştirmek ve doğrulamaktır.

Gereç ve Yöntemler: Geliştirilen kromatografik yöntem kolon, çözücü ve tampon kullanılan akış hızı, mobil faz oranı, molarite ve pH için optimize edilmiştir. Optimize edilmiş yöntemin doğrulanması ve her iki uyuşturucunun (asidik, alkali, oksidatif, termal, ışık ve nötr koşullar için) zorunlu bozunma çalışmaları İnsan Kullanımına Yönelik Farmasötikler için Teknik Gereksinimlerin Uyumlaştırılması Uluslararası Konseyi yönergelerine göre gerçekleştirilmiştir.

Bulgular: Ultraviyolele saptaması 224 nm'deki C18 kolon (kromasil; 250 mm × 4,6 mm, 5 µm) ve mobil fazda 1,1 mL/dk akış hızında ortofosforik asit ile ayarlanmış pH 3,5 asetoneitril: fosfat tamponunun (30 mM) (70:30 v/v) MUP ve SIM çözünürlüğünü sırasıyla 2,32±0,008 dk ve 13,55±0,254 dk

*Correspondence: rupalikale07@gmail.com, Phone: +9376927834, ORCID-ID: orcid.org/0000-0001-9777-5133

Received: 14.05.2020, Accepted: 16.10.2020

©Turk J Pharm Sci, Published by Galenos Publishing House.

tutma sürelerinde yüksek doğruluk (% geri kazanım, MUP için $99,69 \pm 0,82$ ve SIM için $101,10 \pm 0,02$) ve 5-30 $\mu\text{g/mL}$ aralığında doğruluk ile (MUP için $r^2: 0,9969$ ve SIM için $r^2: 0,9959$) sağladığı bulunmuştur. Tespit limiti ve kantitasyon limiti sırasıyla MUP için $0,771 \pm 0,234$ ve $2,338 \pm 0,246$ $\mu\text{g/mL}$, SIM için $0,595 \pm 0,282$ ve $1,803 \pm 0,334$ $\mu\text{g/mL}$ olarak bulunmuştur. Valide yöntem, zorunlu bozunma çalışmalarından sonra her iki ilacın bozunma davranışını anlamak için kullanılmıştır.

Sonuç: Geliştirilen analitik yöntemin, kombine dozaj formunda MUP ve SIM'nin aynı anda tahmini için spesifik, hassas, kesin ve doğru olduğu belirlenmiştir.

Anahtar kelimeler: Simvastatin, mupirosin, validasyon, stabilite

INTRODUCTION

Mupirocin (MUP), chemically known as (α E,2S,3R,4R,5S)-5-[(2S,3S,4S,5S)-2,3-epoxy-5-hydroxy-4-methylhexyl] tetrahydro-3,4-dihydroxy- β -methyl-2H-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid, calcium salt (2:1), dihydrate, is the most commonly used topical antibiotics.^{1,2} MUP is known to be active against *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are aerobic Gram-positive cocci, and methicillin-resistant *S. aureus*, which is Gram-negative cocci.³ Topical ointment of 2% MUP is used as an antimicrobial agent for prophylaxis use in burns, operative wounds, and ulcers, as well as for the treatment of skin infections.^{4,5} Simvastatin (SIM), chemically known as (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, is traditionally used in the treatment of various types of hypercholesterolemia, which decrease cholesterol by inhibiting the rate-limiting step in the synthesis of cholesterol.⁶ Other than this traditional application, the wound-healing activity of SIM has been recently explored as it improves vascular endothelial growth factor production, thereby stimulating angiogenesis, reducing oxidative stress, improving microvascular function, and enhancing endothelial function, which ultimately improves the wound-healing activity.⁷ These properties make SIM a drug of choice to be used in combination with antimicrobial agents.⁸ Various analytical methods for the analysis of individual drugs, as well as in combination with other drugs, have been reported, including ultraviolet (UV) spectrophotometry and high-performance liquid chromatography (HPLC). Abu-Nameh et al.⁹ proposed a simple HPLC method for the rapid analysis of SIM in commercial tablet formulation using a C18 Hypersil column and mobile phase composed of acetonitrile (ACN), phosphate buffer, and methanol with the ratio of 5:3:1 v/v/v and UV detection at 230 nm. Bana et al.¹⁰ reported the development of the UV spectroscopy and HPLC methods for the simultaneous estimation of halobetasol propionate and MUP. Effat and Masoud¹¹ proposed the development and validation of the derivative spectrophotometric method for the analysis of SIM and ezetimibe by simultaneous estimation. Meanwhile, Dixit et al.¹² proposed the development of the reverse-phase (RP)-HPLC method for the stability-indicating studies of the same drug combination. Patel et al.¹³ developed the RP-HPLC method for the simultaneous determination of aspirin in combination with SIM using the analytical quality by design approach.

Literature search for prior articles on methods for the simultaneous analysis of SIM and MUP indicated no information reported for the simultaneous analysis of these two drugs

by HPLC. The present study focused on the development of a simple, precise, and accurate RP-HPLC method for the simultaneous analysis of SIM and MUP in the combined dosage form.¹⁴

MATERIALS AND METHODS

Materials

Pure SIM and MUP were procured as gift samples from SAVA Healthcare Ltd. with their assay values. HPLC-grade solvents and chemicals purchased from Merck were used in this study.

Instrument

The instrument used in the proposed HPLC method was Agilent 1120 Compact LC System connected to a UV detector for analysis purposes. All of the data were acquired and processed using the EZChrom Elite Compact software. An analytical balance (Shimadzu) with 1 mg sensitivity was used to weigh the samples.

Development and optimization of the method for chromatographic conditions

Method development and optimization for chromatographic conditions were conducted by varying the molarity (i.e., 25 and 30 mM), pH (i.e., 3.0, 3.5, and 4.0), and volume of ACN (i.e., 55, 65, and 75 mL) of the mobile phase and flow rate (1.0, 1.1, and 1.2 mL/min).

Mobile phase

The mobile phase was prepared by mixing ACN and phosphate buffer, and its pH was adjusted using orthophosphoric acid. The mobile phase was filtered through 0.45 μm membrane filter paper after 15 min of sonication at room temperature before using it for analysis.

Standard stock solution

The standard stock solution of SIM and MUP was prepared individually. Accurately weighed 10 mg of SIM was dissolved in 10 mL of the mobile phase in a volumetric flask (1,000 $\mu\text{g/mL}$). A 1 mL aliquot from this solution was diluted with 10 mL of the mobile phase to obtain the final concentration of 100 $\mu\text{g/mL}$ SIM. The same procedure was repeated for MUP to obtain a solution of 100 $\mu\text{g/mL}$ MUP.

Sample solution

The combined dosage form, which is in the form of a topical spray, is composed of 1.0% w/v each of SIM and MUP.¹⁴ Accurately measured quantity of 1.0 mL of the formulation consisting of SIM (10 mg) and MUP (10 mg) was transferred to a 100.0 mL volumetric flask, followed by the addition of 30

mL mobile phase, ultrasonication of this solution for 20 min at room temperature, and volume make up to the mark with the mobile phase to obtain a concentration of 100 µg/mL for SIM and 100 µg/mL MUP.

Optimization of chromatographic conditions

Various preliminary trials were conducted during method development to understand the effect of certain parameters on their responses. For the mobile phase, the solvent was selected from ACN and methanol and the buffer was selected from ammonium acetate buffer and phosphate buffer based on the chromatographic responses received. The mobile phase was further optimized by varying the molarity of the buffer used and adjusting the pH from 3.0 to 4.5 using orthophosphoric acid.

Chromatographic method for analysis

After baseline stabilization (for approximately 30 min), using the optimized chromatographic conditions, a standard solution of 20 µg/mL was successively injected into the system to record the chromatogram of the satisfactory reproducibility of the peak areas. This procedure was repeated for the sample solution, and the peak areas of the standard and sample were acquired to determine the concentrations of SIM and MUP in the sample. This analysis was repeated six times.

Validation of the optimized method

The International Harmonization Conference (ICH) Q2 (R1) guidelines¹⁵ were used to validate the developed analytical method. The validation parameters include system suitability test, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ), and stability.

System suitability test

The developed method was checked for system suitability using standard solutions (100 µg/mL) of SIM and MUP. Parameters of the resolution, tailing factor (T), capacity factor, and theoretical plates (N) were evaluated as results of system suitability with percent relative standard deviation (% RSD) of six replicate injections.

Specificity

Method specificity was evaluated by comparing the chromatograms of the placebo and sample solutions prepared using the formulation consisting of SIM and MUP to check the interference of excipients. The placebo formulation has nearly the same composition as that of the sample solution, except that SIM and MUP were used to prepare the sample solution.

Accuracy

To determine the accuracy of the method, three different concentration levels of 80%, 100%, and 120% for SIM and MUP were selected. These samples were analyzed in six replicates using the proposed method. The recovery studies for SIM were done by adding standard SIM at concentrations of 8, 12, and 24 µg/mL to the known concentration of the sample (10 µg/mL) to be analyzed through the standard addition method. The content of the recovered SIM was analyzed. A similar procedure was

repeated for the recovery studies of MUP. The percent recovery for both drugs was calculated separately.

Precision

The proposed method was evaluated for precision by checking its performance with intraday and interday variations of 8, 12, and 24 µg/mL of SIM and MUP. For intraday precision, six replicates of each concentration of the standard and sample solutions were injected consecutively on the same day. For interday precision, the same standard and sample solutions were injected on three different days. The % RSD was calculated by determining the percent content of each sample injected.

Linearity and range

Six standard solutions of 5, 10, 15, 20, 25, and 30 µg/mL, with accuracy between 98% and 102% and precision of less than 2% RSD, were selected to assess linearity. These solutions were injected using the optimized method conditions, and the response acquired in terms of peak area was plotted against the respective concentrations. The linear relationship between peak area and concentration was evaluated by calculating the correlation coefficient, intercept, and slope.

LOD and LOQ

To calculate the LOD and LOQ of SIM and MUP, the lower concentrations of the standard solution were injected. The LOD and LOQ for both drugs were determined based on the 3.3 s/n and 10 s/n rule where s/n is the signal-to-noise ratio.

Robustness

The robustness of the proposed method is evaluated to check its potential to resist small but intentional variations in the optimized parameters. For the robustness studies of the proposed chromatographic conditions, the mobile phase was varied as follows: ACN/phosphate buffer ratio by ±2%, flow rate by ±0.1 mL/min, wavelength by ±1 nm, pH by ±0.2, and molarity of the buffer by ±2 mM.

Forced degradation studies

Standard samples of SIM and MUP were exposed to different stress conditions, such as acidic, alkaline, oxidative, thermal, photostability, and neutral conditions, for the forced degradation studies. In the case of acidic and alkaline degradation, samples were treated with 0.1 M HCl and 0.1 M NaOH at 40°C for 24 h. Oxidative degradation was done using 3% v/v H₂O₂ at 30°C for 24 h. Thermal degradation was conducted by exposing the powder sample to 60°C for 24 h in an oven. Photostability was checked by exposing the sample to UV light by placing the sample in a UV chamber for 24 h. For degradation under neutral conditions, the drugs were treated with water for 2 h at 80°C. After the stipulated time, all of the samples were cooled to room temperature and analyzed using the optimized chromatographic conditions to assess the stability of the drugs.

Stability

The stability of the stock and sample solutions for the short-term and long-term durations was checked by storing the solutions for 24 h and 3 days at room temperature, respectively.

Each solution was observed individually for the appearance of solution after storage and analyzed in six replicates by HPLC using the optimized conditions. The retention time, peak shape, and assay of active substances were compared with freshly prepared solutions statistically.

Statistical analysis

All of the samples were analyzed in six replicates, and the RSD values were computed for all of the samples. Ethics committee approval or patient informed consent is not required for the proposed method.

RESULTS AND DISCUSSION

The RP-HPLC method was selected for the development of the analytical method for the estimation of SIM and MUP simultaneously because of its simplicity and suitability. The analytical method was developed and optimized to determine suitable chromatographic conditions for obtaining sharp and well-resolved peaks of SIM and MUP with minimal tailing.

Column selection

The C8 and C18 columns were checked for their performance in the proposed method. The drugs eluted earlier in the C18 column exhibited a satisfactory peak shape compared with the C8 column. Therefore, the Kromasil C18 column (250 mm×4.6 mm, 5 μm) was selected for the proposed method of simultaneous estimation of SIM and MUP.

Mobile phase composition

Mobile phase selection was done based on the polarity, pKa, and solubility of SIM and MUP, as well as the available literature data. Phosphate buffer (pH 3.5) or ammonium acetate buffer was selected as the aqueous phase and methanol or ACN was selected as the organic phase for mobile phase composition. Because ammonium acetate buffer did not show any peak for SIM and methanol was observed to increase column pressure and peak tailing, both were eliminated from the study and the combination of phosphate buffer and ACN was optimized further. Variation in the ACN/phosphate buffer ratio led to substantial changes in the chromatographic responses. A decrease in the ACN/phosphate buffer ratio resulted in increased retention time, as well as peak split; hence, the ACN/phosphate buffer ratio of 70:30 (v/v) was selected for further optimization. Decreasing the molarity of the buffer from 30 mM to 10 mM resulted in a tailing factor of more than 2. The mobile phase pH of less than 3.0 resulted in peak split, and the mobile phase pH of more than 4.0 resulted in increased retention time. The flow rate was optimized based on the peak properties (i.e., peak shape and symmetry) and retention time.

By conducting trials of different compositions of the mobile phase, the optimized mobile phase composition for the simultaneous estimation of SIM and MUP was ACN/30 mM pH 3.5 phosphate buffer (70:30, v/v) with a flow rate of 1.1 mL/min. These optimized parameters were selected based on several factors, namely, theoretical plate (should be more than 2,000), retention time of both drugs (RSD should be less than 2), and tailing factor of both drugs (should be less than 2).

Wavelength selection for detection

The detection wavelength for the proposed method was determined by analyzing the isobasic points of SIM and MUP. Standard solutions of both drugs were prepared using stock solution (20 μg/mL each), scanning was done in the UV range of 400–200 nm using a UV-visible spectrophotometer (Shimadzu 1800), and overlaid spectra were recorded (Figure 1). The isobasic point of the overlaid spectra where the two substances absorb light of that specific wavelength (224 nm) to the same extent at the same analytical concentration was selected as the wavelength for detection during the analysis of MUP and SIM in the combined dosage form.

Validation of the optimized method

System suitability test

System suitability test is often used as the control strategy, which is an essential component of the HPLC method. The system suitability test is normally conducted to verify if the developed method has adequate resolution and reproducibility for the analysis to be conducted. For the proposed method, system suitability results viz. resolution (29.66±1.20), capacity factor (1.15±0.01 for MUP and 11.31±0.01 for SIM), retention time (RSD values of 0.01 for MUP and 0.25 for SIM), theoretical plate (5.162±0.02 for MUP and 8.518±0.07 for SIM), and tailing factor (0.976±0.02 for SIM and 1.16±0.02 for MUP) were determined to be satisfactory.

Specificity

The results for the specificity of the proposed method are shown in Figure 2. The figure shows no interference in the retention time of SIM and MUP with a clear separation of peaks.

Accuracy

The accuracy of the proposed method was determined by conducting recovery experiments. The results for accuracy at different levels are listed in Table 1.

The percent recovery of SIM and MUP was determined to be close to 100%, and the RSD values are less than 2%, which

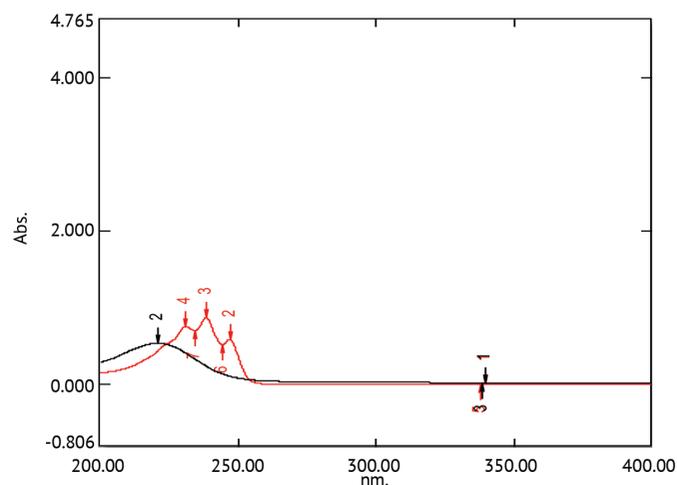


Figure 1. Overlaid spectra of MUP and SIM
MUP: Mupirocin, SIM: Simvastatin

fulfills the acceptance criteria for accuracy as indicated in the ICH guidelines. Hence, the method developed can be considered reliable for analytical application.

Precision

The results of intraday and interday precision using 8, 12, and 24 µg/mL concentrations of both SIM and MUP are listed in Table 2.

Linearity and range

The developed method was assessed for linearity at the concentration range of 5–30 µg/mL. Calibration curve analysis indicated good correlation coefficients (r^2 : 0.9969 for MUP and r^2 : 0.9959 for SIM) for SIM and MUP in the given concentration range for both drugs. The regression equation for SIM was expressed as $y = 431.45x + 228.54$ and the regression equation for MUP was expressed as $y = 449.25x - 101.93$, where y denotes the peak area and x denotes the concentration.

LOD and LOQ

The LOD and LOQ of the proposed method were determined to be 0.595 ± 0.282 and 1.803 ± 0.334 for SIM and 0.771 ± 0.234 and 2.338 ± 0.246 µg/mL for MUP, respectively.

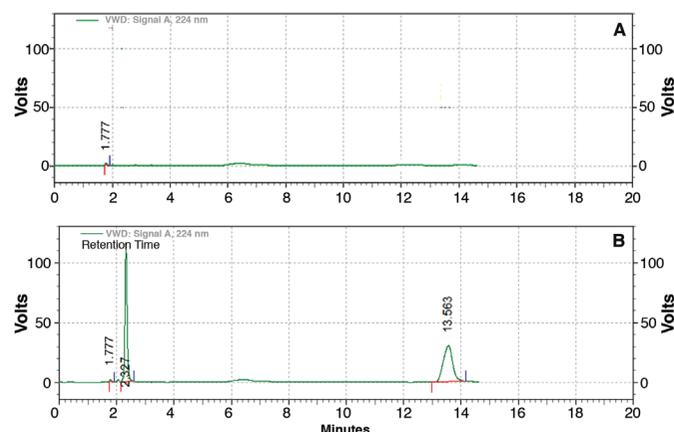


Figure 2. (A) Chromatogram of the placebo solution. (B) Chromatogram of the formulation showing simultaneous estimation of MUP at 2.33 min and SIM at 13.56 min of retention time

MUP: Mupirocin, SIM: Simvastatin

Table 1. Percent recovery studies at three levels

Active ingredients	Analyte concentration (µg/mL)	Mean recovery (%) ± SE	RSD (%)
SIM	8.00	101.10 ± 0.01	0.02
MUP		99.67 ± 0.02	0.04
SIM	12.00	101.08 ± 0.16	0.39
MUP		99.69 ± 0.35	0.82
SIM	24.00	100.29 ± 0.01	0.90
MUP		99.41 ± 0.01	0.81

SE: Standard error, RSD: Relative standard deviation, MUP: Mupirocin, SIM: Simvastatin

Robustness

The results of the robustness of the developed method evaluated by changing the flow rate, mobile phase ratio, wavelength, pH, and molarity of the buffer with the respective % RSD values are listed in Table 3. The calculated assay (%) values were in the acceptable range of 95.00% to 105.00% with % RSD lower than 2, which indicated no significant effect on the results of

Table 2. Intraday and interday precision results of the proposed method

Actual concentration (µg/mL)	Measured mean concentration (µg/mL) ± SE	RSD (%)	Measured mean concentration (µg/mL) ± SE	RSD (%)
	SIM		MUP	
Intraday				
8.00	7.98 ± 0.42	1.02	8.12 ± 0.47	1.15
12.00	11.87 ± 0.62	1.51	12.12 ± 0.52	1.27
24.00	23.46 ± 0.04	0.19	23.27 ± 0.04	0.20
Interday				
8.00	8.10 ± 0.49	1.21	7.61 ± 0.11	0.06
12.00	11.97 ± 0.40	0.97	12.02 ± 0.39	0.95
24.00	23.76 ± 0.05	0.28	23.48 ± 0.03	0.74

%RSD values lower than 2% indicate acceptable precision of the developed method. SE: Standard error, RSD: Relative standard deviation, MUP: Mupirocin, SIM: Simvastatin

Table 3. Robustness results of the proposed method

Factor	Variation	SIM		MUP	
		Mean amount (%)	RSD (%)	Mean amount (%)	RSD (%)
Flow rate (mL/min)	1.0	98.86	1.28	98.54	1.25
	1.1	98.60	1.03	98.32	0.98
	1.2	98.31	1.43	98.13	1.63
ACN/ phosphate buffer (70:30, v/v)	68:32	98.38	1.54	98.12	1.39
	70:30	98.73	0.98	99.10	1.34
	72:28	98.67	1.27	98.78	1.28
Wavelength (nm)	223	97.59	1.50	97.30	1.23
	224	98.60	0.97	99.21	1.32
	225	98.01	1.38	98.45	1.30
pH	3.30	97.67	0.58	97.84	1.01
	3.50	97.50	0.76	97.19	0.51
Molarity of the buffer (mM)	3.70	97.67	0.94	97.67	0.79
	28.00	97.39	0.82	97.67	0.78
	30.00	97.70	0.78	97.70	1.18
	32.00	97.51	0.77	97.38	1.13

RSD: Relative standard deviation, MUP: Mupirocin, SIM: Simvastatin

the analysis. These results showed that the developed method is robust to minor variations in system parameters.

Forced degradation studies

The analytical results after forced degradation of the samples are listed in Table 4, followed by the respective figures of the peaks after degradation. SIM was observed to degrade completely in alkaline conditions, followed by oxidative conditions (10.64%), thermal conditions (9.89%), and acidic conditions (4.85%). Degradation in neutral conditions and photodegradation was observed to be less than 1%. Dixit et al.¹² reported similar results for the degradation behavior of SIM. In the case of MUP, maximum degradation was observed in acidic, oxidative, and neutral conditions (approximately 7%), followed by thermal conditions (6.6%). Degradation in alkaline conditions and photodegradation was observed to be less than 2%. The capability of the proposed method to reveal the degradation peaks after forced degradation studies indicates the potential of the method as a stability-indicating analytical method. The degradation peaks did not show any interference in the retention times of the respective drugs, indicating the specificity of the analytical method developed (Figure 3-8).

Stability

The short-term stability (24 h) and long-term stability (3 days) of the stock and sample solutions did not show any precipitation or any other change in the appearance of the solution. The stock and sample solutions also did not show any significant difference in chromatographic responses, such as peak shape and retention time. The percent deviation between assay of freshly prepared and stored stock solutions was determined to be 1.12% for SIM and 0.91% for MUP in the case of short-term stability and 1.02% for SIM and 1.24% for MUP in the case of

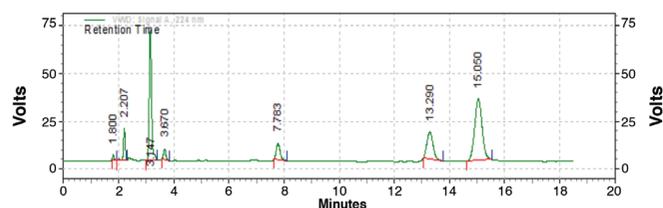


Figure 3. Degradation patterns of MUP and SIM under acidic conditions
MUP: Mupirocin, SIM: Simvastatin

long-term stability. By contrast, the percent deviation between assay of freshly prepared and stored sample solutions was determined to be 1.21% for SIM and 0.45% for MUP in the case of short-term stability and 0.07% for SIM and 1.45% for MUP

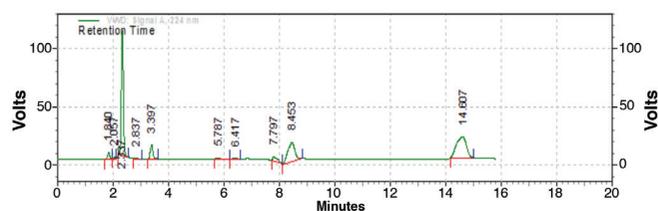


Figure 4. Degradation patterns of MUP and SIM under alkali conditions
MUP: Mupirocin, SIM: Simvastatin

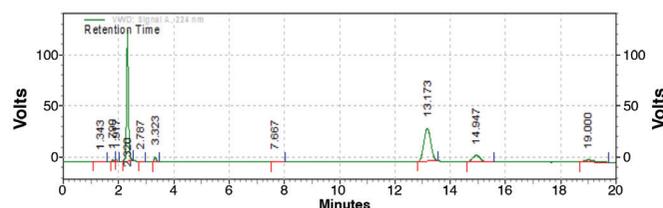


Figure 5. Degradation patterns of MUP and SIM under oxidative conditions
MUP: Mupirocin, SIM: Simvastatin

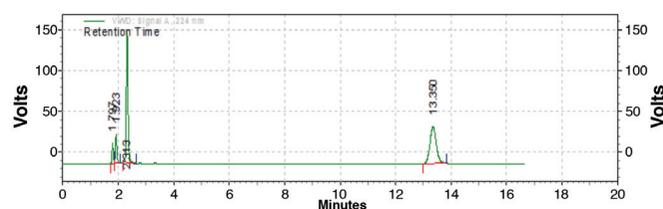


Figure 6. Degradation patterns of MUP and SIM under neutral conditions
MUP: Mupirocin, SIM: Simvastatin

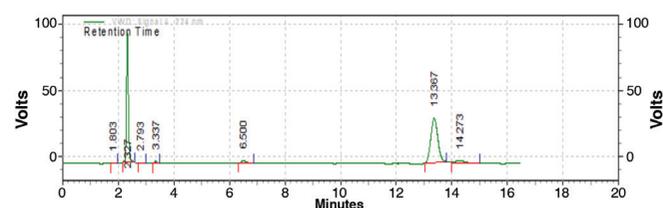


Figure 7. Degradation patterns of MUP and SIM under thermal conditions
MUP: Mupirocin, SIM: Simvastatin

Table 4. Results of the forced degradation studies of the proposed method

Stress conditions	Amount of recovered SIM		Amount of degraded SIM		Amount of recovered MUP		Amount of degraded MUP	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Acidic	95.14	1.51	4.85	0.08	92.79	1.82	7.21	0.18
Alkali	-	-	100.00	1.02	99.91	1.71	0.09	0.03
Oxidative	89.32	1.21	10.64	0.15	92.65	0.91	7.35	0.30
Neutral	99.14	1.32	0.85	0.01	92.65	1.35	7.35	0.26
Thermal	90.81	1.77	9.89	0.07	93.40	1.43	6.60	0.07
Photodegradation	99.01	1.24	0.99	0.02	98.57	1.59	1.43	0.09

RSD: Relative standard deviation, MUP: Mupirocin, SIM: Simvastatin

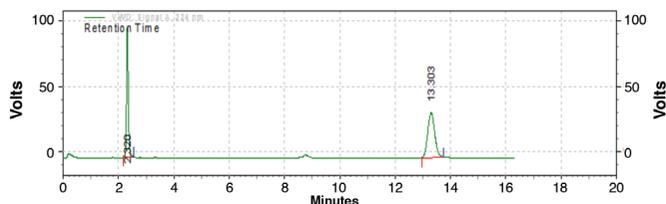


Figure 8. Degradation patterns of MUP and SIM after photodegradation
MUP: Mupirocin, SIM: Simvastatin

in the case of long-term stability. These results indicate the stability of the stock and sample solutions for 3 days at room temperature.

Analysis of the developed formulation

The developed HPLC method was applied to the quantification of SIM and MUP in the developed formulation. The percent content of each drug was determined to be 97.80 ± 0.31 for MUP and 97.80 ± 0.45 for SIM. Hence, the developed method can be applied to the routine analysis of SIM and MUP in topical spray formulation, which has a combination of these two drugs.

CONCLUSION

A simple and convenient RP-HPLC method for the analysis of SIM and MUP in the combined dosage form was developed and validated. Kromasil C18 column (250 mm \times 4.6 mm, 5 μ m) with UV detection at 224 nm and mobile phase composed of ACN/30 mM pH 3.5 phosphate buffer (70:30, v/v) at a flow rate of 1.1 mL/min were observed to provide a good resolution for MUP and SIM. The method was validated as per ICH guidelines, and the proposed method was determined to be specific, accurate, precise, and robust for the quantitation of SIM and MUP and can be applied to the routine analysis of the developed topical spray formulation, which has a combination of both drugs.

ACKNOWLEDGMENTS

The authors would like to thank SAVA Healthcare Ltd. for supplying the samples of MUP and SIM.

Conflict of interest: No conflict of interest was declared by the authors. The authors are solely responsible for the content and writing of this paper.

REFERENCES

1. Sujata S. Recent advances in topical wound care. *Indian J Plast Surg.* 2012;45:379-387.

2. Thornton SC, Taylor SC, Weinberg JM. Topical antimicrobial agents in dermatology. *Clin Dermatol.* 2003;21:70-77.
3. Dumville JC, Lipsky BA, Hoey C, Cruciani M, Fison M, Xia J. Topical antimicrobial agents for treating foot ulcers in people with diabetes. *Cochrane Database Syst Rev.* 2017;14:6.
4. Dai T, Huang YY, Sharma SK, Hashmi JT, Kurup DB, Hamblin MR. Topical antimicrobials for burn wound infections. *Recent Pat Antiinfect Drug Discov.* 2010;5:124-151.
5. Sarabahi S. Recent advances in topical wound care. *Indian J Plast Surg.* 2012;45:379-387.
6. Tripathi KD. *Essentials of Medical Pharmacology*, (6th ed). New Delhi: Jaypee Brothers Medical Publishers Ltd. 2008;636:776.
7. Bitto A, Minutoli L, Altavilla D, Polito F, Fiumara T, Marini H, Galeano M, Calò M, Lo Cascio P, Bonaiuto M, Migliorato A, Caputi AP, Squadrito F. Simvastatin enhances VEGF production and ameliorates impaired wound healing in experimental diabetes. *Pharmacol Res.* 2008;57:159-169.
8. Akira E. A historical perspective on the discovery of statins. *Proc Jpn Acad Ser B Phys Biol Sci.* 2010;86:484-493.
9. Abu-Nameh ESM, Shawabkeh RA, Ali A. High-performance liquid chromatographic determination of simvastatin in medical drugs. *Am J Anal Chem.* 2006;61:63-66.
10. Bana A, Sathe MA, Rajput SJ. Analytical Method Development and Validation for Simultaneous Estimation of Halobetasol Propionate and Mupirocin in the ratio 1:40 by U.V. Spectroscopy And RP- HPLC Method. *Int J Pharm Sci Res.* 2019;10:1392-1401.
11. Effat S, Masoud A. Development and validation of a derivative spectrophotometric method for simultaneous determination of simvastatin and ezetimibe. *J Chem.* 2010;7:S197-S202.
12. Dixit RP, Barhate CR, Padhye SG, Viswanathan CL, Nagarsenker MS. Stability indicating RP-HPLC method for simultaneous determination of simvastatin and ezetimibe from tablet dosage form. *Indian J Pharm Sci.* 2010;72:204-210.
13. Patel KG, Patel AT, Shah PA, Gandhi TR. Multivariate optimization for simultaneous determination of aspirin and simvastatin by reverse phase liquid chromatographic method using AQbD approach. *Bull Fac Pharm Cairo Univ.* 2017;55:293-301.
14. Kale R, Doifode D, Shete P. Film forming, antimicrobial and growth promoting wound healing spray formulation. *Int J Pharm Invest.* 2020;10:320-325.
15. ICH Q2 (R1). Validation of analytical procedures: Text and Methodology. International conference on harmonization of technical requirements for the registration of pharmaceutical for human use. Geneva, Switzerland: 2005. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf