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Analytical Method Development and Validation for Simultaneous Determination of Simvastatin and Mupirocin by Reverse Phase Liquid Chromatographic Method

Analitik Yöntem Geliştirme ve Validasyon İçin Eşzamanlı Tayini onun Simvastatin ve Mupirosin tarafından Ters Fazlı Sıvı Kromatografik Yöntem

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ABSTRACT
Objectives: The proposed study is aimed towards development and validation of reverse phase high performance liquid chromatographic method for the simultaneous estimation of Simvastatin and Mupirocin.
Materials and methods: The chromatographic method developed is optimized for flow rate with column, solvent and buffer used, ratio of mobile phase, molarity and pH. The validation of method optimized and the forced degradation studies of both drugs (for acidic, alkaline, oxidation, heat, light, and neutral conditions) were carried out following the guidelines of the International Harmonization Conference (ICH).
Results: Kromasil C-18 column (250mm X 4.6mm, 5µm) with UV detection at 224 nm and acetonitrile: phosphate buffer (30 mM) (70:30 v/v) pH 3.5, adjusted with orthophosphoric acid as mobile phase at flow rate of 1.1mL/min were found to give good resolution of Mupirocin and Simvastatin at retention times of 2.32 ± 0.008 min and 13.55 ± 0.254 min, respectively, with high accuracy (% recovery was 99.69 ± 0.82 for Mupirocin, and 101.10 ± 0.02 for Simvastatin) and linearity in the range 5-30 µg/mL (r^2=0.9969 for Mupirocin and r^2=0.9959 for Simvastatin). The diagnostic limit and the lower limit of determination were 0.771±0.234 and 2.338 ±0.246 µg/mL for Mupirocin, 0.595±0.282 and 1.803±0.334 µg/mL for Simvastatin, respectively. The validated method was used to understand degradation behavior of both the drugs after forced degradation studies.
Conclusion: Developed method is found to be specific, sensitive, precise and accurate for the simultaneous estimation of Mupirocin and Simvastatin for combined dosage form.
Keywords: Simvastatin, Mupirocin, Validation, Stability indicating

ÖZ

Giriş ve Amaç: Önerilen çalışma, Simvastatin ve Mupirosin eşzamanlı tahmini için ters faz yüksek performanslı sıvı kromatografik yöntemgeliştirilmesi ve doğrulanması hedeflenmektedir.

Yöntem ve Gereçler: 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 Uluslararası Harmonizasyon Konferansı (ICH) yönergelerine göre gerçekleştirilmiştir.

Bulgular: UV saptaması 224 nm'deki Kromasil C-18 kolon (250mm X 4.6mm, 5µm) ve mobil fazda 1,1 mL / dk akış hızında ortofosforik asit ile ayarlanmış pH 3,5 asetonitril: fosfat tamponunun (30 mM) (70:30 v/v) Mupirosin ve Simvastatin çözünürlüğünü sırasıyla 2,32 ± 0,008 dk ve 13,55 ± 0,254 dk tutma sürelerinde Yüksek doğsusallık (% geri kazanım, Mupirosin için 99.69 ± 0.82 ve Simvastatin için 101.10 ± 0.02) ve 5-30 ug / mL aralığında doğrusallık ile (Mupirosin için $r^2 = 0.9969$ ve Simvastatin için $r^2 = 0.9959$) sağladığı bulunmuştur. Tespit limiti ve kantitasyon limiti sırasıyla Mupirocin için 0.771 ± 0.234 ve 2.338 ± 0.246 µg / mL, Simvastatin için 0.595 ± 0.282 ve 1.803 ± 0.334 µg / mL olarak bulunmuştur. Validasyon yöntem, zorunlu bozunma çalışmalarından sonra her iki ilacın bozunma davranışını anlamak için bir şekilde kullanılmıştır.

Sonuç: Geliştirilen yöntemin, kombine dozaj formunda Mupirosin ve Simvastatinin eşzamanlı tahmini için spesifik, duyarlı, kesin ve doğru olduğu bulunmuştur.

Anahtar Kelimeler: Simvastatin, Mupirosin, Validasyon, Kararlılık

INTRODUCTION:

Mupirocin [MUP], chemically known as (α*E*,2*S*,3*R*,4*R*,5*S*)-5-[(2*S*,3*S*,4*S*,5*S*)-2,3-epoxy-5-hydroxy-4-methylhexyl]tetrahydro-3,4-dihydroxy-β-methyl-2*H*-pyran-2-crotonic acid, ester with 9- hydroxynonanoic acid, calcium salt (2:1), dihydrate is the most commonly used topical antibiotics.[1,2] It is known to be active against aerobic Gram-positive cocci such as *S. aureus, S. epidermidis* as well as some Gram-negative cocci including methicillin-resistant *S. aureus.*[3] Topical ointment of 2% MUP is used as antimicrobial agent for prophylaxis use in ulcers, operative wounds, burns and 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 decreases cholesterol by inhibiting rate limiting step in synthesis of cholesterol.[6] Other than this traditional application, recently wound healing activities of SIM have explored as it acts by improving vascular endothelial growth factor (VEGF) production thereby stimulating angiogenesis, reduces oxidative stress, improves micro vascular function and also enhances 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 are reported for analysis of individual drug as well as in combination with other drugs including UV spectrophotometry as well as HPLC. Abu-Nameh ESM *et.al.*[9] has proposed a simple and rapid HPLC method for the analysis of simvastatin in commercial tablet formulation using a C18-Hypersil column and mobile phase with composition of acetonitrile, phosphate buffer and methanol in 5: 3: 1, v/v/v ratio and UV detection at 230 nm. Bana A. et.al[10] has reported development of UV spectroscopy and HPLC method for simultaneous estimation of halobetasol propionate and mupirocin. Souri E. et.al.[11] has proposed development and validation of derivative spectrophotometric method for analysis of simvastatin and ezetimibe by simultaneous estimation whereas Dixit RP et. al.[12] has proposed development of RP-HPLC method in stability indicating studies for the same drug combination. Patel KG [13] et.al. have developed RP-HPLC method for simultaneous

determination of aspirin in combination with simvastatin by using Analytical Quality by Design (AQbD)" approach.

Literature search for prior art of the method for simultaneous analysis of SIM and MUP indicated no information reported for simultaneous analysis of these two drugs by HPLC. The present study is focused on development of simple, precise and accurate RP-HPLC method for simultaneous analysis of SIM in combination with MUP in developed dosage form.[14]

MATERIALS AND METHODS

Materials

Pure Simvastatin and Mupirocin were procured as a gift sample from SAVA Healthcare Ltd. with its assay values. HPLC grade solvents and chemicals purchased from Merck were used for the study.

Instrument

The instrument used in proposed method for HPLC was Agilent 1120 Compact LC system connected to UV detector for analysis purpose. All the data were acquired and processed using EZ-Chrom Elite compact software. Analytical balance [Shimadzu] with 1 mg sensitivity was used for weighing the samples.

Development and optimization of the method for chromatographic conditions

Method development and optimization for the chromatographic condition was done by varying molarity (25, 30 mM), pH (3.0, 3.5. 4.0), volume of acetonitrile (55, 65, 75 mL) of mobile phase composition and flow rate (1.0, and 1.1, 1.2 mL/min).

Formulation of Mobile phase

Mobile phase was prepared by mixing acetonitrile and phosphate buffer solution followed by adjustment of pH 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.

Formulation of 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 volumetric flask in mobile phase (1000 µg/mL). Aliquot of 1 mL from this solution was diluted to 10 mL of mobile phase to get final concentration 100 µg/mL SIM. Same procedure was repeated for MUP to get solution of 100 µg/mL MUP.

Formulation of sample solution

Combined dosage form which is in the form of topical spray is composed of 1.0% w/v of each SIM and MUP.[14] Accurately measured quantity of 1.0 mL of formulation consisting of SIM (10 mg) and MUP (10 mg) , respectively was transferred to 100.0 mL volumetric flask, followed by addition of 30 mL mobile phase, ultrasonication of this solution for 20 minutes at room temperature and volume make up to the mark with mobile phase to get concentration of 100 µg /mL for SIM and 100 µg /mL MUP, respectively.

Optimization of chromatographic conditions

Various preliminary trials were conducted during method development to understand the effect of parameters on their response. For mobile phase, selection of solvent was done from ACN and methanol and selection of buffer was done from ammonium acetate buffer and phosphate buffer based on the chromatographic responses received. Mobile phase was further optimized for molarity of buffer used and variation of pH from 3.0 to 4.5 adjusted using orthophosphoric acid.

Chromatographic method for analysis

After baseline stabilisation (for about 30 min), using optimized chromatographic conditions, standard solution of 20 µg /mL was successively injected into the system to record the chromatogram for satisfactory reproducibility of the peak areas. This procedure was repeated

for the sample solution and the peak areas of the standard and sample was acquired to determine concentration of SIM and MUP in the sample. This analysis was repeated six times.

Method validation of optimized method

ICH Q2 (R1) guidelines[15] were used for the validation of developed analytical method. The validation parameters include system suitability testing, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ), and stability.

System suitability test

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

Specificity

Specificity of the method was evaluated by comparing the chromatogram of solution of placebo solution and sample solution prepared using formulation having SIM and MUP to check the interference of excipients. Placebo formulation which has same composition as that of that of sample solution except SIM and MUP was used for preparation of placebo solution.

Accuracy

For determination of accuracy of the method, three different concentration levels of 80%, 100% and 120% for SIM and MUP, respectively were selected. These samples were analyzed in six replicates by using the proposed method. The recovery studies for SIM was done by addition of standard Simvastatin at concentration of 8 µg/mL, 12 µg/mL and 24 µg/mL to the known concentration of sample (10 µg/mL) to be analyzed, by standard addition method. Content of SIM recovered was analyzed. Similar procedure was repeated for recovery studies of MUP as well. The percent recovery for both the drugs was calculated separately.

Precision

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

Linearity and Range

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

LOD and LOQ

The LOD and LOQ of SIM and MUP was calculated by injecting standard solution in its lower concentrations. LOD and LOQ for both the drugs was determined based on 3.3s/n and 10s/n rule where s/n indicates signal to noise ratio.

Robustness

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

Forced degradation studies

Standard samples of SIM and MUP were exposed to different stress conditions like acidic, alkaline, oxidative, thermal, photostability, and neutral conditions for forced degradation. In

case of acidic and alkaline degradation, samples were treated with 0.1 M HCl and 0.1M NaOH at 40°C for 24 hr. Oxidative degradation was done using 3% v/v H_2O_2 at 30°C for 24 hr. Thermal degradation was conducted by placing powder sample in an oven at 60°C for 24 hr. Photostability was checked by exposing sample to UV light by keeping the sample in a UV chamber for 24 hrs; for degradation in neutral condition, the drugs were treated with water for 2 hrs at 80°C. After stipulated time, all the samples were cooled to room temperature and analysed by using optimised chromatographic conditions to assess stability of the drugs.

Stability

Stability of stock solution and sample solution for short-term and long-term duration, was checked by storing respective solutions for 24 hrs and 3 days at room temperature. Each solution was observed individually for appearance of solution after storage and analyzed in six replicates by HPLC using optimized conditions. Retention time, peak shape and assay of active substances were compared with freshly prepared solutions statistically.

Statistical analysis

All the samples used for analysis were in six replicates, values of RSD were computed for all the samples.

Ethics committee approval or patient informed consent is not required for proposed study.

RESULTS AND DISCUSSION

RP-HPLC method was selected for development of analytical method for estimation of SIM and MUP simultaneously due to its simplicity and suitability. It 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

C8 and C18 columns were checked for their performance in proposed study. It was found that drugs eluted earlier in C18 Column with satisfactory peak shape as compared to C8 column. Therefore, Kromasil C-18 column (250mm X 4.6mm, 5µm) was selected for the proposed study of simultaneous estimation of SIM and MUP.

Mobile phase composition

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

By taking trials of different compositions of mobile phases, the optimised mobile phase composition for simultaneous estimation of SIM and MUP was ACN: 30 mM pH 3.5 phosphate buffer (70: 30, v/v) with flow rate 1.1 mL/min These optimized parameters were selected based on factors like theoretical plate (should be more than 2000), 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

Detection wavelength for the proposed study was determined by analysing SIM and MUP for their isobasic points. Standard solutions of both the drugs were prepared using stock solution (20 µg/mL each), scanning was done in UV range of 400-200 nm by using UV-Visible Spectrophotometer (Shimadzu 1800) and overlaid spectra was recorded (Figure 1). Isobasic point of the overlaid spectra where the two substances absorb light of that specific wavelength (224 nm) to the same extent at same analytical concentration was selected as wavelength for detection during the analysis of MUP and SIM in combination formulation.

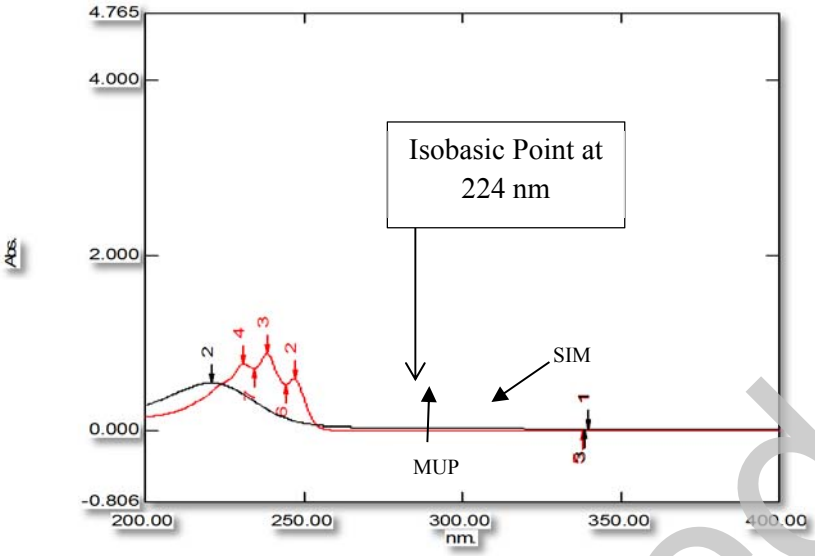

Figure 1. Overlaid spectra of MUP and SIM

Validation of Optimized Method

System suitability test

System suitability test is often used as control strategy as an essential component of HPLC method. It is normally conducted to verify if developed method has adequate resolution and reproducibility for the analysis to be carried out. For 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 (5162 ± 0.02 for MUP and 8518 ± 0.07 for SIM), tailing factor (0.976 ± 0.02 for SIM and 1.16 ± 0.02 for MUP) were found to be satisfactory.

Specificity

Result for specificity of proposed method are mentioned as figure 2. It indicates no interference in retention time of SIM and MUP with clear separation of peaks.

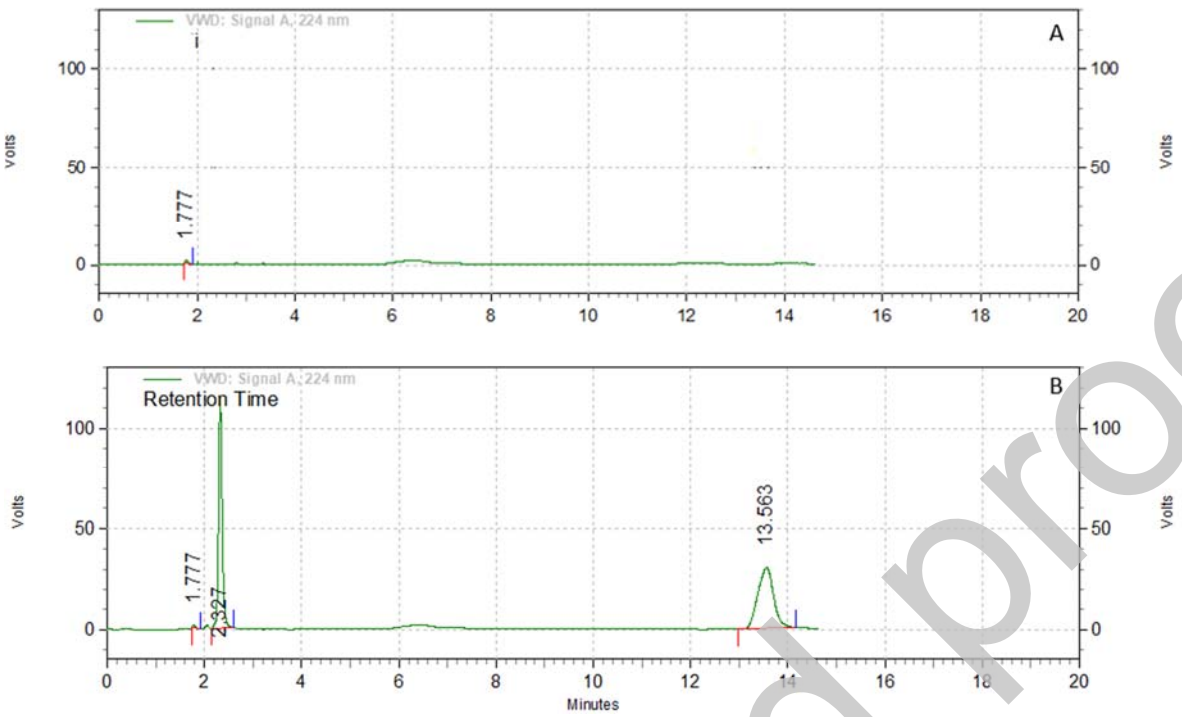

Figure 2. A. Chromatogram for placebo solution. B. Chromatogram of formulation showing simultaneous estimation of MUP at 2.33 min and SIM at 13.56 min of retention time.

Accuracy

Accuracy of the proposed method was determined by conducting recovery experiments. Results for accuracy at different levels are mentioned in table 1.

Table 1. Percent recovery studies at three different level

Active ingredients	Analyte conc. (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

Percent recovery of SIM and MUP was found to be close to 100% and RSD values are below 2% which fulfils the acceptance criteria for accuracy as indicated in ICH guidelines. Hence method developed can be considered reliable for analytical application.

Precision

Results of intraday and interday precision using concentration of 8 µg/mL, 12 µg/mL and 24 µg/mL of both SIM and MUP are shown in table 2.

Table 2. Results of precision study for proposed method

Actual conc. (µg/mL)	Measured mean conc. (µg/mL)±SE	RSD (%)	Measured mean conc. (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

SE: Standard Error

Percent RSD values lower than 2% indicated acceptable precision of developed method.

Linearity and range

Developed method was assessed for linearity at concentration range of 5-30 µg/mL. Calibration curve analysis indicated good correlation coefficient (r^2=0.9969 for MUP and r^2=0.9959 for SIM) was observed for SIM and MUP in the given concentration range for both the drugs, respectively. The regression equations for SIM was found to be y=431.45x + 228.54 and for MUP, it was found to be y=449.25x – 101.93 (y: peak area, x: concentration)

LOD and LOQ

The LOD and LOQ of the proposed method was found 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.

Robustness

The results of robustness of the developed method evaluated by changing flow rate, mobile phase ratio, wavelength, pH and molarity of buffer with respective RSD% values are shown in table 3. It was observed that the calculated assay (%) values were in an acceptable range of 95.00% to 105.00% with RSD% lower than 2 which showed no significant effect on the results of analysis. These results indicated that the developed method is robust to minor variations in system parameters.

Table 3. Results of robustness study for 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
	3.70	97.67	0.94	97.67	0.79
Molarity of Buffer (mM)	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

Forced degradation study

The analytical results after forced degradation of samples are mentioned in table 4 followed by respective figures of the peaks after degradation. SIM was found to degrade completely in alkaline conditions, followed by oxidative conditions (10.64%), thermal conditions (9.89%) and acidic conditions (4.85%). Degradation in neutral condition and photodegradation was

found to be less than 1%. Dixit RP et.al [12] have reported similar results for degradation behaviour of SIM. In case of MUP, maximum degradation was found to be in acidic, oxidative and neutral conditions (approx. 7%) followed by thermal conditions (6.6%). Degradation in alkaline condition and photodegradation was found to be less than 2%. The ability of the proposed method to show degradation peaks after forced degradation studies indicates potential of the method as stability indicating analytical method. Degradation peak did not show any interference with the retention times of respective drugs indicating specificity of the analytical method developed.

Table 4. Results of forced degradation study for proposed method

Stress Conditions	Amount of SIM recovered		Amount of SIM degraded		Amount of MUP recovered		Amount of MUP degraded	
	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
Photo Degradation	99.01	1.24	0.99	0.02	98.57	1.59	1.43	0.09

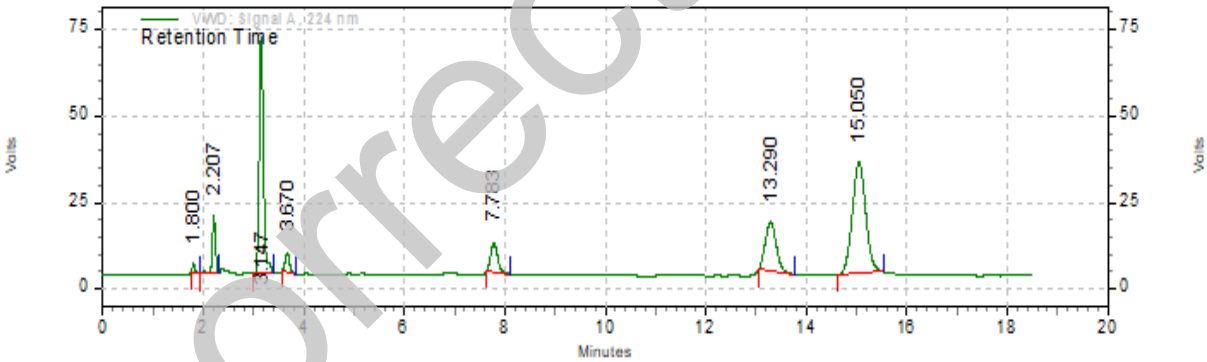

Figure 3. Degradation pattern of MUP and SIM after acid degradation

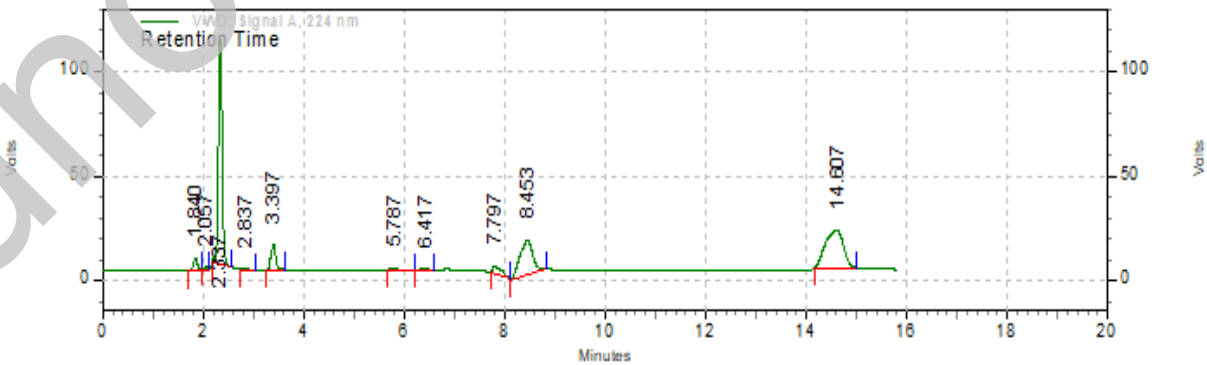

Figure 4. Degradation pattern of MUP and SIM after alkali degradation

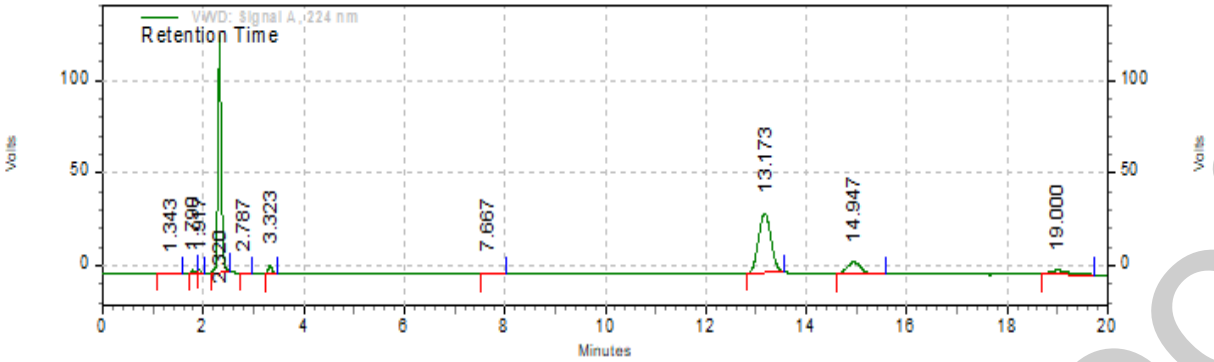

Figure 5. Degradation pattern of MUP and SIM after oxidative degradation

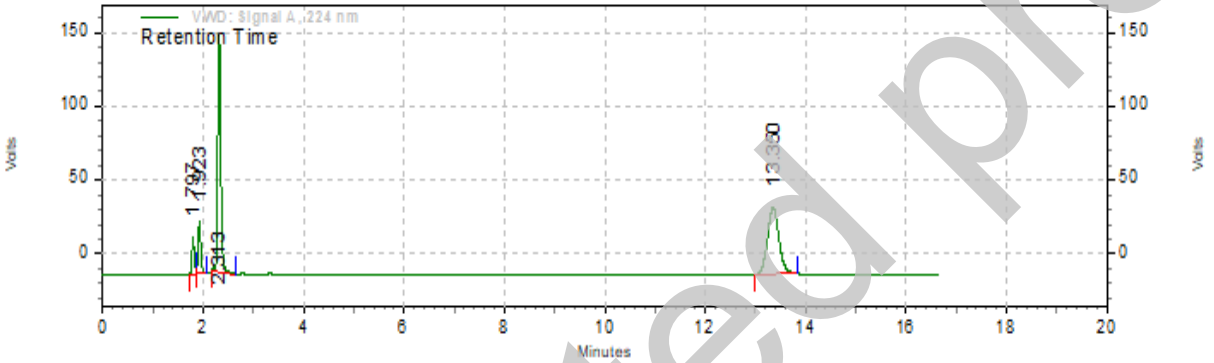

Figure 6. Degradation pattern of MUP and SIM after neutral degradation

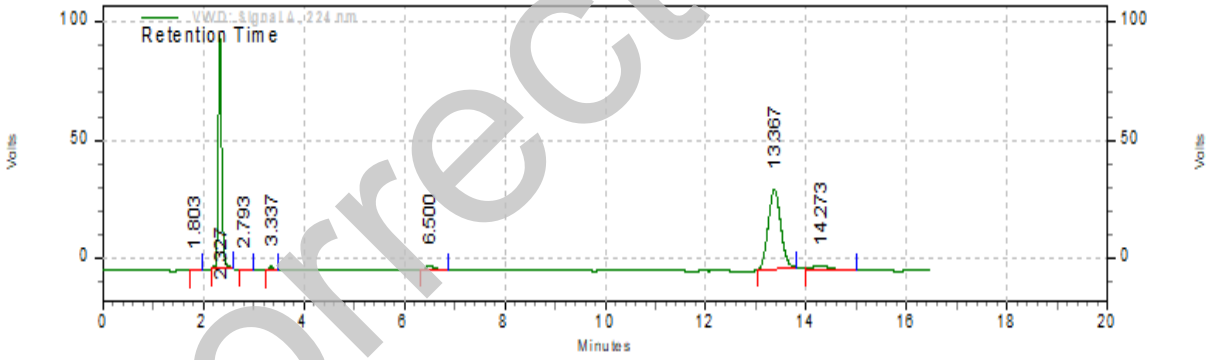

Figure 7. Degradation pattern of MUP and SIM after thermal degradation

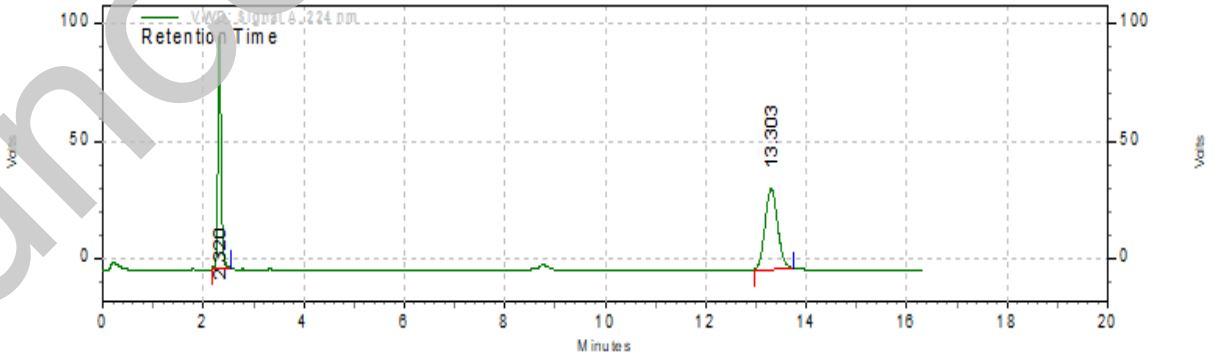

Figure 8. Degradation pattern of MUP and SIM after photo degradation

Stability

Stock solutions and sample solutions kept for short term stability (24h) and long-term stability (3 days) did not show any precipitation or any other changes in appearance of solution. It also

did not show any significant difference in chromatographic responses such as peak shape and retention time. Percent deviation between assay of freshly prepared and stored stock solution was found to be 1.12% for SIM and 0.91% for MUP in case of short-term stability and 1.02 % for SIM and 1.24% of MUP for long term stability. Whereas percent deviation between assay of freshly prepared and stored sample solution was found to be 1.21% for SIM and 0.45% for MUP in case of short-term stability and 0.07 % for SIM and 1.45% of MUP for long term stability. These results indicate stability of stock solution as well as sample solutions of the proposed method for 3 days at room temperature.

Analysis of developed formulation

From the current study developed HPLC method was applied for quantification of the SIM and MUP in developed formulation. Percent amount of drug was found to be 97.80±0.31 for MUP and 97.80±0.45 for SIM. Hence the developed method can be applied for routine analysis of SIM and MUP in topical spray formulation which has combination of these two drugs.

CONCLUSION

The simple, convenient, RP-HPLC method for analysis of SIM and MUP in combination dosage form was developed and validated. Kromasil C-18 column (250mm X 4.6mm, 5µm) with UV detection at 224 nm and mobile phase with composition of ACN: 30 mM pH 3.5 phosphate buffer (70: 30, v/v), at flow rate of 1.1mL/min was found to give good resolution of MUP and SIM. The method was validated as per ICH guidelines and proposed method was found to be specific, accurate, precise and robust for quantitation of SIM and MUP and can be applied for routine analysis of developed topical spray formulation which has combination of both the drugs.

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