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Research Article

VALIDATED AND STABILITY INDICATING HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF SULFADIAZINE SODIUM, SULFATHIAZOLE SODIUM AND SULFADIMIDINE SODIUM IN WATER SOLUBLE POWDER DOSAGE FORM

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ABSTRACT

Objective: The main objective of this study was to develop a simple and validated stability-indicating Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method for the simultaneous determination of Sulfadiazine sodium (SDZS), Sulfathiazole sodium (STZS) and Sulfadimidine sodium (SDMS) in water soluble powder dosage form.

Method: The direct separation was achieved on C18 chemically bonded column (250 mm ×4.6 mm i.d.,5 µm particle) at room temperature using an isocratic solvent mixture of water:methanol:glacial acetic acid (750:249:1, v/v/v). The mobile phase was fixed at 1.0 ml/min and the analytes were monitored at 270 nm using photodiode array detector. Forced degradation experiments were carried out by exposing SDZS, STZS and SDMS standards and the water soluble powder formulation for thermal, photolytic, oxidative and acid-base hydrolytic stress conditions.

Results: The observed results shows that degradation products were well resolved from the main active ingredients peaks and thus proving a reliable stability-indicating method. The method was validated as per ICH and USP guidelines (USP34/NF29).

Conclusion: The method was found to be adequate for the routine quantitative estimation of SDZS, STZS and SDMS in commercially available water soluble powder dosage form.

Keywords: Sulfadiazine sodium, Sulfathiazole sodium, Sulfadimidine sodium, Water soluble powder dosage form, Validation, Stability indicating method.

INTRODUCTION

Sulphanur® 3 forte water soluble powder is a veterinary drug that contains three types of Sulpha drugs with a broad spectrum activity against bacteria, Sulfadiazine sodium (SDZS), Sulfathiazole sodium (STZS) and Sulfadimidine sodium (SDMS). It is ideal against coccidia, well absorbed from the G.I.T. It is indicated in treatment of coccidia in poultry and large animals, also in bacterial pneumonia, enteritis and bacterial diarrhea [1]. Figure 1 shows the structure of the three active ingredients present in the Sulphanur® 3 forte water soluble powder dosage form. The SDZS and STZS are official in BP and USP [2-5] whereas SDMS is only official in BP [6]. All these pharmacopeial methods are based on the titration of a single sulfonamide drug with sodium nitrite titrant. Nitrite titration is used particularly for the assay of primary aromatic amines. It is only suitable for the determination of single component drug assay. The combined simultaneous analysis of the three active ingredients has not been adopted in any official pharmacopoeia.

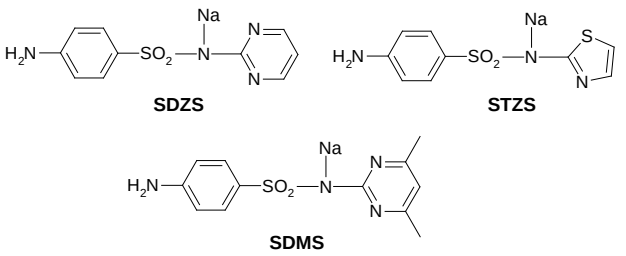

Fig. 1: Structures of SDZS, STZS and SDMS active ingredients

In the literature, there are many HPLC and LC-MS papers describing the determination of multi veterinary drugs including SDZS, STZS and SDMS residues in meat, bovine, porcine, chicken tissues, whole egg and milk [7-11]. However, no stability-indicating HPLC method has been reported for the determination of single or combined SDZS, STZS and SDMS drugs.

Therefore, there is a need to develop a validated stability-indicating quality control method to allow the simultaneous determination of SDZS, STZS and SDMS in their water soluble powder dosage form. The proposed method is aimed to separate the three active drugs from each other and from the unknown degradation products and excipients. Subsequently, this method was validated as per ICH/USP guidelines validation norms [12-13].

MATERIAL AND METHODS

Materials and Reagents

Reference standards of Sulfadiazine sodium (SDZS), Sulfathiazole sodium (STZS) were purchased from Sigma-Aldrich (Germany). Reference standard of Sulfadimidine sodium (SDMS) was purchased from Nanhai Beisha Pharmaceutical Co., ltd (China). Glacial acetic acid, HPLC grade methanol (MeOH), hydrochloric acid fuming (37%), sodium hydroxide pellets and hydrogen peroxide (30%), were purchased from Merck (Germany). High purified water was prepared by using a Millipore Milli-Q plus water purification system. Sulphanur® 3 forte water soluble powder (Labeled claim: each one g contains 250 mg SDZS, 250 mg STZS and 250mg SDMS) samples, and all the active ingredients and excipients (Dextrose Monohydrate) usually used in manufacturing the pharmaceutical combination, were kindly supplied by Pharmacare pharmaceutical company (Palestine). ZORBAX Eclipse XDB-C18 chemically bonded column (250 mm ×4.6 mm i.d., 5 µm particle) purchased from Agilent Technologies (USA).

Equipments

The HPLC system consisted of LaChrom (Merck-Hitachi) equipped with model L-7100 pump, L-7200 autosampler, L-7300 column oven, DAD L-7450 photo diode array (PDA) detector and D-7000 software HSM version 3.1 (Merck Hitachi, England). A double beam ultraviolet-visible spectrometer (PG Instruments, United Kingdom) was used. UV-Chamber, Spectroline Fluorescence Analysis Cabinet CM-10 (USA) was also used.

Chromatographic conditions

The optimum mobile phase was prepared by mixing high purified water with methanol and glacial acetic acid (750:249:1;v/v/v) .The mobile phase was filtered by using 0.45 μm microporous filter and was degassed by sonication prior to use. A wavelength of 270 nm was chosen since it was found the most appropriate for the determination of the three active ingredients. The flow rate used was 1.0 ml/minute. The injection volume was 20 μl and the temperature of the column was at room temperature. Total run time of last eluting SDMS was about 15 minutes.

Preparation of standard solutions

The standard solution for all drugs was prepared by dissolving 50 mg SDZS reference standard, 50 mg STZS reference standard and 50 mg SDMS reference standard in 80 ml of high purified water, shaken by mechanical means for 3 minutes, sonicated for two minutes and then diluted up to 100 ml with the same solvent. Using volumetric pipette, 5 ml of this solution was transferred to 25 ml volumetric flask and completed to volume using the high purified water. This solution was filtered using 0.45 μm membrane filter before analysis. The obtained final solution contained 100μg/ml SDZS, 100μg/ml STZS and 100μg/ml SDMS.

Preparation of sample solution

Sample solution was prepared by transferring 200 mg of the Sulphanur® 3 forte water soluble powder to 100 ml volumetric flask and then dissolved in 80 ml of high purified water, shaken by mechanical means for 3 minutes, sonicated for two minutes and then diluted up to 100 ml with the same solvent. Using volumetric pipette, 5 ml of this solution was transferred to 25 ml volumetric flask and completed to the volume using high purified water. This solution was filtered using 0.45 μm membrane filter before analysis. The obtained final solution contained 100μg/ml SDZS, 100μg/ml STZS and 100μg/ml SDMS.

Forced degradation study

ICH prescribed stress conditions such as acidic, basic, oxidative, thermal and photolytic stresses were carried out.

Standard drug stock solutions

Forced degradation study was conducted on solutions that were prepared by transferring 50 mg SDZS reference standard into five different 100 ml volumetric flasks. Also 50 mg STZS reference standards were transferred separately into another five different 100ml volumetric flasks and finally 50 mg SDMS reference standards were transferred separately into another five different 100ml volumetric flasks. Then 80 ml of high purified water was added in each flask and shaken by mechanical means for 3 minutes, sonicated for two minutes until completely dissolved. These stock solutions were kept at room temperature protected from light and used for forced degradation studies.

Acid hydrolysis

Ten ml of 1.0 N HCl was added into one of the flasks containing SDZS stock solution and another 10 ml was added into one of the flasks containing STZS stock solution and also another 10 ml was added into one of the flasks containing SDMS stock solution, then diluted to 100 ml with purified water and kept at room temperature for 60 minutes in a dark place. Five ml of this solution was transferred into 25 ml volumetric flask, neutralized with 0.1 N NaOH and completed to volume using the purified water. This solution was filtered using 0.45 μm membrane filter before analysis. The obtained final solutions contained 100 μg/ml SDZS, 100 μg/ml STZS and 100 μg/ml SDMS respectively.

Base hydrolysis

Ten ml of 1.0 N NaOH was added into one of the flasks containing SDZS stock solution and another 10 ml was added into one of the flasks containing STZS stock solution and also another 10 ml was added into one of the flasks containing SDMS stock solution, then diluted to 100 ml with purified water and kept at room temperature for 60 minutes in a dark place. Five ml of this solution was transferred into 25 ml volumetric flask, neutralized with 0.1 N HCl and completed to volume using the purified water. This solution was filtered using 0.45 μm membrane filter before analysis. The obtained final solutions contained 100 μg/ml SDZS, 100 μg/ml STZS and 100 μg/ml SDMS respectively.

Oxidative hydrolysis

Ten ml of 30% H_2O_2 was added into one of the flasks containing SDZS stock solution and another 10 ml was added into one of the flasks containing STZS stock solution and also another 10 ml was added into one of the flasks containing SDMS stock solution, then diluted to 100 ml with purified water and kept at room temperature for 24 hours in a dark place. Five ml of this solution was transferred into 25 ml volumetric flask and completed to volume using the purified water. This solution was filtered using 0.45 μm membrane filter before analysis. The obtained final solutions contained 100 μg/ml SDZS, 100 μg/ml STZS and 100 μg/ml SDMS respectively.

Thermal degradation

One of the flasks containing SDZS stock solution, another one containing STZS stock solution and another one containing SDMS stock solution were studied separately for their thermal degradation by keeping them at 70˚C in a water bath protected from light for 72 hours and then diluted to 100 ml with water. Five ml of this solution was transferred into 25 ml volumetric flask and complete to volume using purified water. This solution was filtered using 0.45 μm membrane filter before analysis. The obtained final solutions contained 100 μg/ml SDZS, 100 μg/ml STZS and 100 μg/ml SDMS respectively.

Photo degradation

One of the flasks containing SDZS stock solution, another one containing STZS stock solution and another one containing SDMS stock solution were studied separately for their photo degradation by exposing them to UV light at 254 nm in UV-Chamber for 48 hours and then diluted to 100 ml with purified water. Five ml of this solution was transferred into 25 ml volumetric flask and complete to volume using purified water. This solution was filtered using 0.45 μm membrane filter before analysis. The obtained final solutions contained 100 μg/ml SDZS, 100 μg/ml STZS and 100 μg/ml SDMS respectively.

Forced degradation study on Sulphanur® 3 forte water soluble powder

The sample stock solutions were prepared by separately transferring 200 mg of the Sulphanur® 3 forte water soluble powder into series of five different 100 ml volumetric flasks. The very same procedure adopted for the standards solutions was used in the Sulphanur® 3 forte water soluble powder. The obtained final solution contained 100 μg/ml of each of SDZS, STZS and SDMS.

RESULTS AND DISCUSSION

Method development and Optimization

Our main focus was to design a durable HPLC method that is simple enough to use in routine quality control laboratory. The developed method should also have the capability to separate all the active ingredients from their degradants in compliance to ICH specifications.

First the wavelength was selected at 270 nm since the overlaid ultraviolet absorption spectra of SDZS, STZS and SDMS (0.05 mg/ml each) revealed that they shared this wavelength. Afterward, various mobile phases have been tried, namely methanol (MeOH) strength, acetic acid percentage and temperature were all investigated. Firstly H_2O: MeOH (50:50 v/v) was tried on ZORBAX Eclipse XDB-C18 chemically bonded column (250 mm ×4.6 mm i.d., 5 μm particle) and the resolution (R_s) between the compounds was poor even when the percent of MeOH was decreased upto 5%. Different percentages of acetic acid (0.05%, 0.1% and 0.15%) were tested and 0.1% was chosen during the entire study. The effect of MeOH strength at 15, 20, 25, 30 and 35% using 0.1% acetic acid was also explored. At

15%, SDMS eluted at 37 minutes while at 35%, SDZS and STZS peaks were eluted at close by proximity and the R_s value was less than 1.2. The best resolution values were attained at 25% MeOH. Different temperatures of 15, 20, 25, 30, and 35°C were evaluated. It turned out that varying the temperatures had no significant influence on R_s or on tailing factor (T_f) and therefore, room temperature was used during the entire investigation. Figure 2 shows typical chromatogram of the placebo at the optimized conditions. Figure 3 also shows a typical HPLC chromatogram of freshly prepared mixture of SDZS, STZS and SDMS (100 µg/ml each) using the optimized conditions of MeOH:H_2O:acetic acid, 24.9%:75%:0.1%, v/v/v.

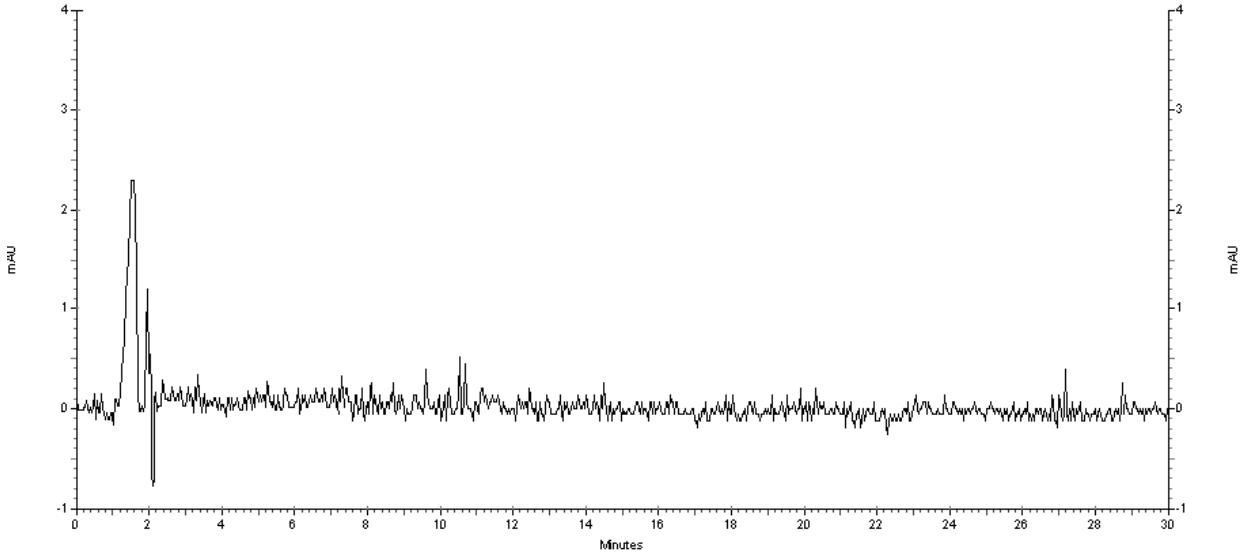

Fig. 2: Zoomed view of typical placebo chromatogram.

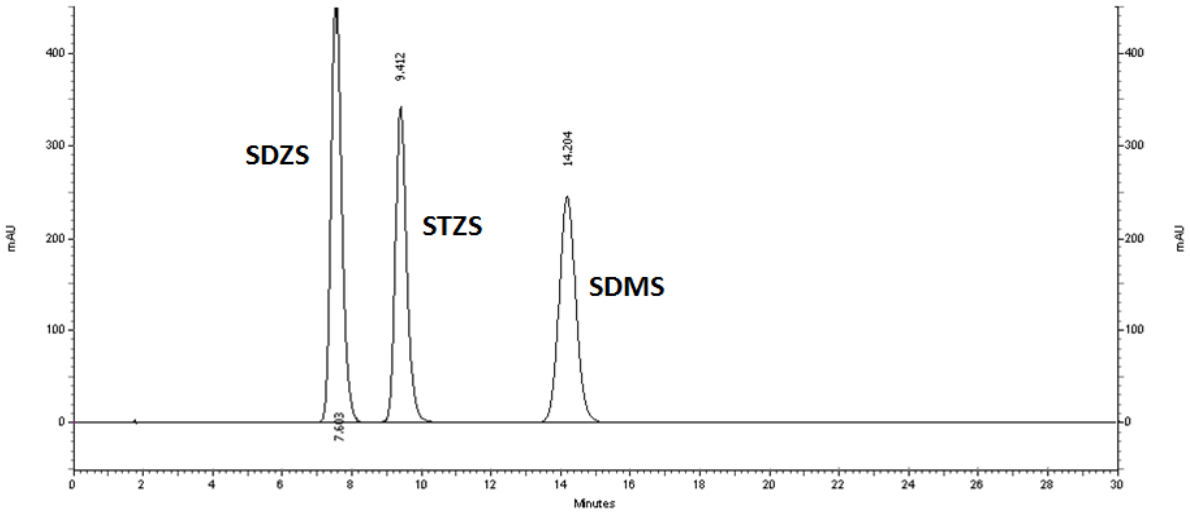

Fig. 3: Chromatogram of a standard mixture of SDZS, STZS and SDMS (100 µg/ml each).

Method Validation

After the successful optimization of the RP-HPLC-PDA method, it was validated in accordance to the ICH/USP guidelines [12, 13]. Parameters such as system suitability, specificity (placebo and forced degradation interferences), sensitivity (LOD and LOQ), linearity, range, accuracy (recovery), precision (repeatability and intermediate precision), robustness and stability indicating capability were all validated.

System suitability

The system suitability was determined by injecting six replicates of the same standard solution and analyzing each active ingredient for its peak area, peak USP tailing factor, resolution, number of theoretical plates and capacity factor. The system suitability results for a combined solution of SDZS, STZS and SDMS (100 µg/ml each) revealed RSD % of less than 0.8% for the three peaks areas. This method meets the accepted requirements as set by the Palestinian Ministry of Health Registration Department (table 1).

Table 1: Summary of the system suitability study

Parameter	SDZS	STZS	SDMS	Accepted limit
% RSD	0.68	0.71	0.79	≤ 2.0%
Tailing factor (T_f)	1.17	1.21	1.14	≤ 2.0
Resolution (R_s)	---	3.2	7.3	≥2.0
Number of theoretical plates (N)	4267	4896	7589	≥2000
Capacity factor (k')	2.9	3.8	6.3	≥2.0

Specificity (placebo and forced degradation interference)

Generally the specificity of a method is its suitability for the analysis of a compound in the presence of potential impurities. Placebo, standard and sample test solutions were all injected at the same wavelength of 270 nm to assure the specificity of the optimized method. A comparison of the retention times of SDZS, STZS and SDMS in sample solution and in the standard solution were exactly the same. Figures 2 and 3 showed that there are no interferences at the retention time of SDZS, STZS and SDMS due to the placebo. Therefore, the proposed method is suitable for the quantification of the active ingredients in the water soluble powder dosage form.

The specificity of the method on SDZS, STZS and SDMS has been determined in the presence of their stress impurities. It was assessed by performing forced degradation studies on pure standards of the three active ingredients separately to indicate the initial results and on samples of the Sulphanur® 3 forte water soluble powder dosage form in presence of their potential degradants. The stress conditions studied are UV-light (254 nm), heat (70°C), acid hydrolysis (0.1 N HCl), base hydrolysis (0.1 N NaOH) and oxidation (3% H_2O_2). The sample stress solutions were analyzed against freshly prepared standards and sample. The degradation % and purity check for the stressed standards and sample solutions were calculated as summarized in Table 2.

Table 2: Summary of the forced degradation study

Name	Stress condition	Degradation%	Purity index*
SDZS standard	Acidic/0.1 N HCl / 60 min at RT	2.3	1.0000
	Alkaline/0.1 N NaOH / 60min at RT	2.5	1.0000
	Oxidative/3 % H_2O_2 /24 hours at RT	10.8	1.0000
	Thermal/70 °C/72 hours	2.7	0.9999
	Light/ UV-254nm /48 hours	4.1	0.9999
SDZS sample	Acidic/0.1 N HCl / 60 min at RT	2.6	1.0000
	Alkaline/0.1 N NaOH / 60min at RT	2.4	1.0000
	Oxidative/3 % H_2O_2 /24 hours at RT	10.4	1.0000
	Thermal/70 °C/72 hours	2.8	0.9999
	Light/ UV-254nm /48 hours	3.9	0.9999
STZS standard	Acidic/0.1 N HCl / 60 min at RT	3.3	1.0000
	Alkaline/0.1 N NaOH / 60min at RT	2.6	1.0000
	Oxidative/3 % H_2O_2 /24 hours at RT	12.3	1.0000
	Thermal/70 °C/72 hours	2.7	1.0000
	Light/ UV-254nm /48 hours	3.2	1.0000
STZS sample	Acidic/0.1 N HCl / 60 min at RT	3.1	1.0000
	Alkaline/0.1 N NaOH / 60min at RT	2.4	0.9998
	Oxidative/3 % H_2O_2 /24 hours at RT	11.8	0.9999
	Thermal/70 °C/72hours	2.5	1.0000
	Light/ UV-254nm /48 hours	3.3	1.0000
SDMS standard	Acidic/0.1 N HCl / 60 min at RT	4.3	1.0000
	Alkaline/0.1 N NaOH / 60min at RT	2.6	1.0000
	Oxidative/3 % H_2O_2 /24 hours at RT	8.5	1.0000
	Thermal/70 °C/72 hours	3.1	1.0000
	Light/ UV-254nm /48 hours	3.4	1.0000
SDMS sample	Acidic/0.1 N HCl / 60 min at RT	4.1	1.0000
	Alkaline/0.1 N NaOH / 60min at RT	2.5	1.0000
	Oxidative/3 % H_2O_2 /24 hours at RT	8.8	1.0000
	Thermal/70 °C/72hours	3.1	0.9999
	Light/ UV-254nm /48 hours	3.3	0.9999

* Accepted when > 0.990, the purity index is a measure of spectral heterogeneity of a peak.

Table 2 revealed that the oxidative stress result showed extensive degradation in comparison to other stress conditions. Peak purity index for all active ingredients was found to be not less than 0.9998, a higher value than the accepted limit (0.990). Therefore, there was no interference between the main active ingredients with any other stress impurity peaks in the chromatogram. Almost the same pattern of degradation was obtained for SDZS, STZS and SDMS in their water soluble powder dosage form samples. Figures (4-8) show the chromatographic profiles of the active ingredients and the degradation products after exposing the water soluble powder dosage form to different stress conditions as in Table 2.

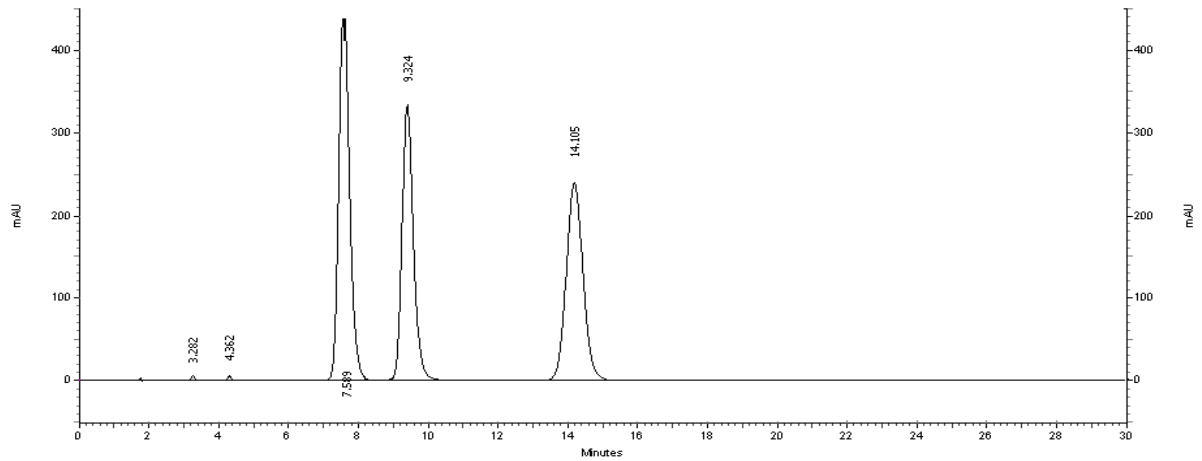

Fig. 4: Chromatogram of Sulphanur® 3 forte powder upon exposure to UV-light

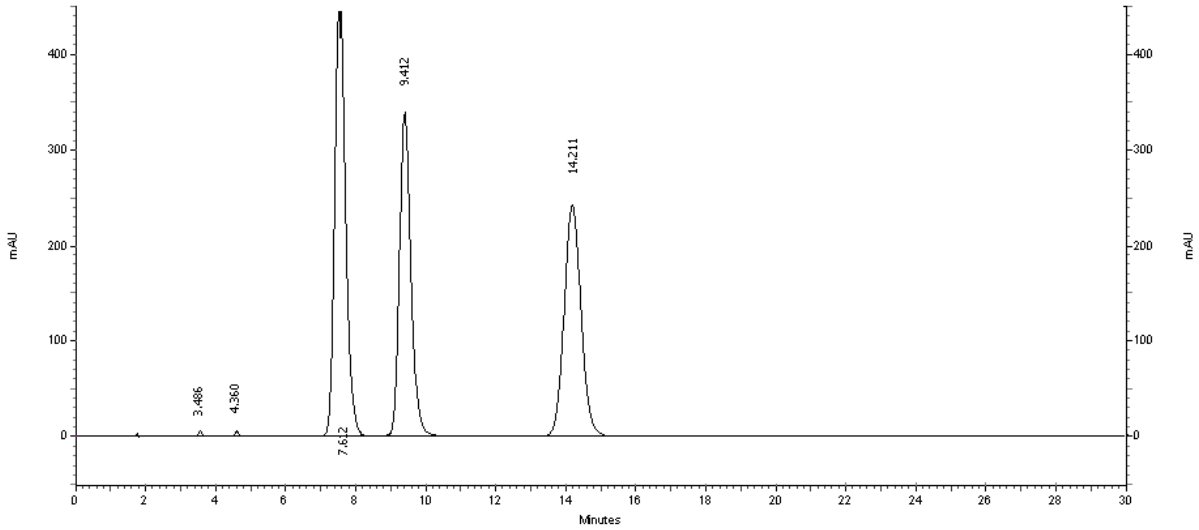

Fig. 5: Chromatogram of Sulphanur® 3 forte powder upon exposure to heat

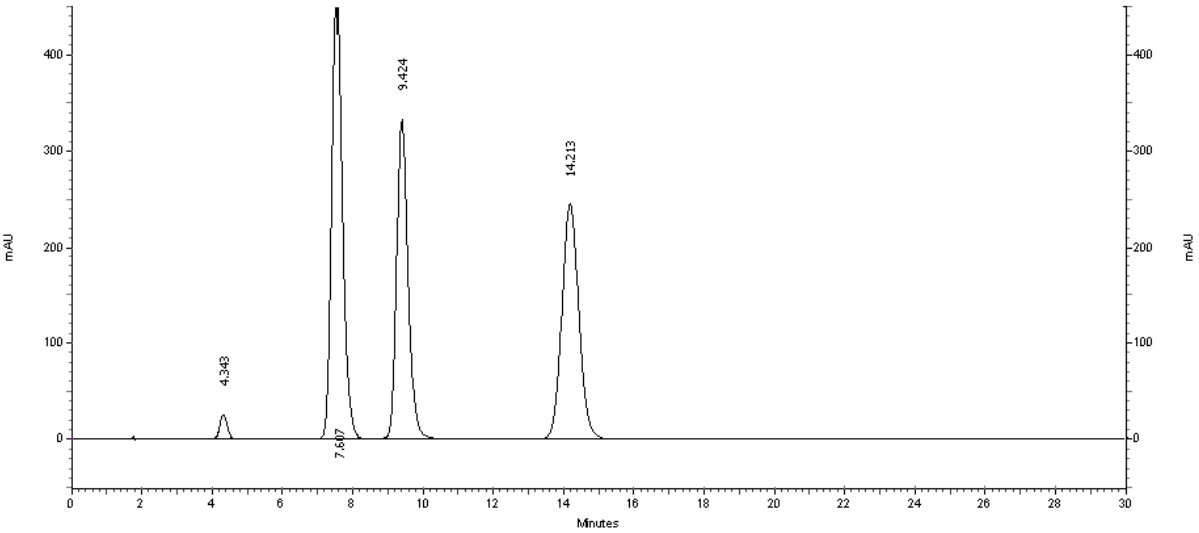

Fig. 6: Chromatogram of acidic degradation of the Sulphanur® 3 forte powder

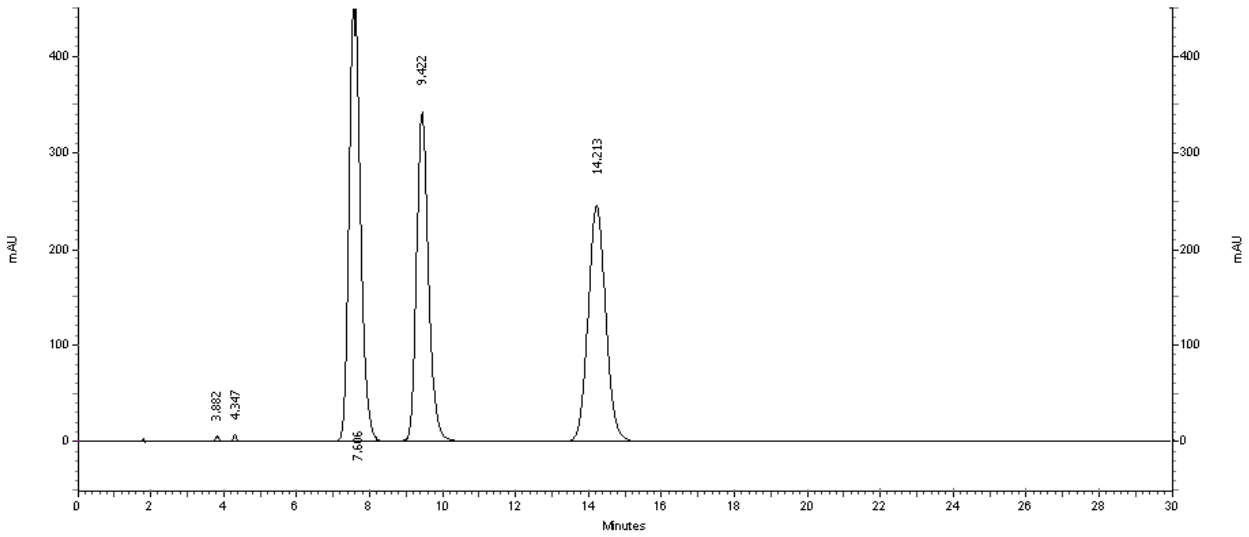

Fig. 7: Chromatogram of basic degradation of the Sulphanur® 3 forte powder

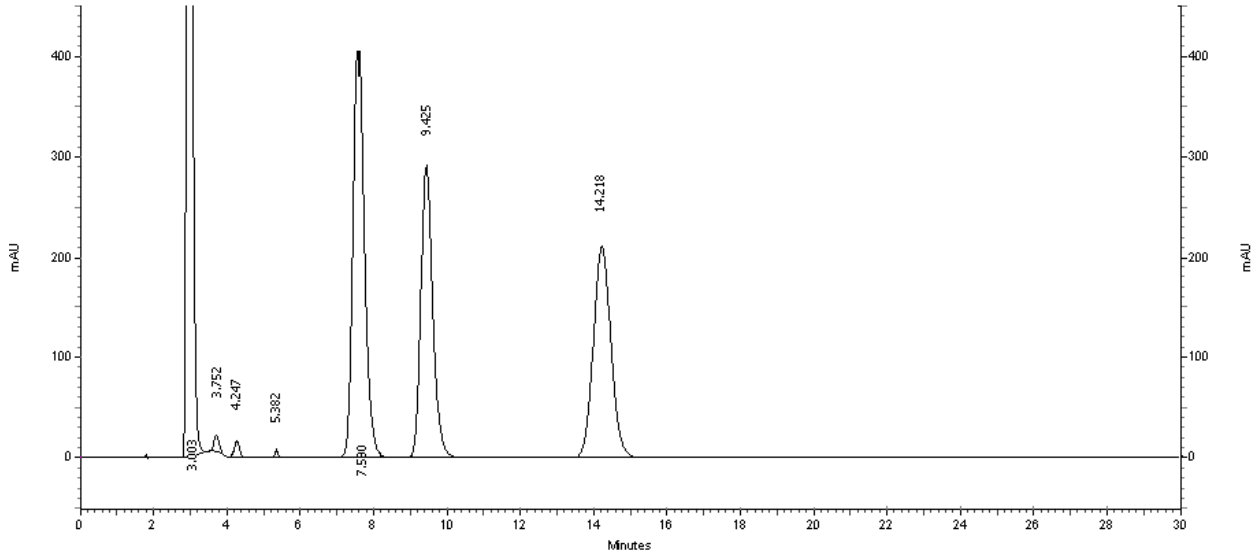

Fig. 8: Chromatogram of oxidative degradation of the Sulphanur® 3 forte powder, the first peak at 3.003 minutes is due to H_2O_2.

Sensitivity

The sensitivity of the method was explored via measuring the limit of detection (LOD) and the limit of quantitation (LOQ) for SDZS, STZS and SDMS at a signal-to-noise ratio of 3 and 10 respectively. It has been achieved by injecting a series of diluted solutions with known concentrations. LOD was found to be 0.3, 0.4 and 0.4 µg/ml for SDZS, STZS and SDMS respectively. LOQ was found to be 1.0, 1.3 and 1.3 µg/ml for SDZS, STZS and SDMS respectively with RSD of 3.4%, 3.9% and 3.7% for SDZS, STZS and SDMS respectively.

Linearity and range

Different amounts of SDZS, STZS and SDMS in the range of 50% to 150% of the labeled amount (5 concentration levels) were spiked to placebo. The linearity in the range of 50-150 µg/ml of each of SDZS, STZS and SDMS was investigated. The regression lines demonstrated linearity in the tested range. The regression analysis confirmed that the deviation of the y-intercept from zero is not significant; and the regression lines were linear with R^2 of 0.9999 for each of SDZS, STZS and SDMS.

Table 3: Summary of regression statistics

Active ingredient	Linearity range (µg/ml)	(R^2)	Linearity equation*	Y-intercept (%) %
SDZS	50-150	0.9999	Y=307990X+20033	0.07%
STZS	50-150	0.9999	Y=231302X+58480	0.25%
SDMS	50-150	0.9999	Y=264385X+24518	0.09%

**Y is the dependent variable and X is the independent variable*

Accuracy (recovery)

Accuracy was determined by the recovery study of known amounts of SDZS, STZS and SDMS standards added to a placebo matrix of Sulphanur® 3 forte water soluble powder dosage form. Different concentrations of the three active ingredients were added to placebo matrix and the recovery was measured. The accuracy as reflected from recovery data for the three active ingredients is listed in Table 4. The average recovery data of SDZS, STZS and SDMS showed results between 98.5% and 101.6% with % RSD of less than 1.0%, which are within the acceptable limit of (98.0 to 102.0% recovery and %RSD of not more than 2.0%).

Table 4: Average recoveries at five concentration levels of spiking of SDZS, STZS and SDMS

Active ingredient	Amount added (level %)	Average recovery (%) ± S.D *(n=3)*	RSD (%) *(n=3)*
SDZS	50 µg /ml (50%)	100.6 ± 0.87	0.86
	75 µg /ml (75%)	100.4 ± 0.84	0.84
	100 µg /ml (100%)	100.7 ± 0.93	0.92
	125 µg /ml (125%)	99.6 ± 0.94	0.94
	150 µg /ml (150%)	98.6 ± 0.59	0.60
STZS	50 µg /ml (50%)	101.5 ± 0.53	0. 52
	75µg /ml (75%)	100.1 ± 0.98	0.98
	100 µg /ml (100%)	101.4 ± 0.76	0.75
	125 µg /ml (125%)	99.2 ± 0.89	0.90
	150 µg /ml (150%)	99.7± 0.92	0.92
SDMS	50 µg /ml (50%)	101.6 ± 0.49	0.48
	75 µg /ml (75%)	100.4 ± 0.96	0.96
	100 µg /ml (100%)	101.6 ± 0.52	0.51
	125 µg /ml (125%)	99.3 ± 0.86	0.87
	150 µg /ml (150%)	98.5 ± 0.58	0.59

Precision

Repeatability

One laboratory analyst carried out the assay of SDZS, STZS and SDMS on six determinations of homogeneous sample of Sulphanur® 3 forte water soluble powder dosage form at 100% level of the test concentration with the same analytical equipment at the same day. The assay results and statistical evaluation for assay of the three active ingredients showed %RSD values of 0.66%, 0.79% and 0.63% for SDZS, STZS and SDMS respectively, which are within the acceptable limit of 2.0%.

Intermediate Precision (ruggedness)

Two laboratory analysts carried out the assay of SDZS, STZS and SDMS on twelve homogeneous samples of the Sulphanur® 3 forte water soluble powder at 100% level of the final test concentration with two different analytical equipments on two different days. The assay results and statistical evaluation for assay of the three active ingredients reveals % RSD values of 1.34%, 1.46% and 1.38% for SDZS, STZS and SDMS respectively, which are within the acceptable limit of 2.0%. The results of the assay of the three ingredients proved that the method is repeatable and rugged enough for day to day use.

Robustness

Premeditate variations were performed in the experimental conditions of the RP-HPLC-PDA method to assess its robustness. The three variations imposed to the chromatographic method are summarized in Table 5. The modifications include different mobile phase flow rates, three different column temperatures and different Methanol percentages in mobile phase. The % RSD values showed no significant change in the final assay results of each of the above three ingredients using the three variations (Table 5).

Table 5: Robustness testing of the three active ingredients of SDZS, STZS and SDMS

Active ingredient	Parameter	Modification	Average assay% ± S.D (n=3)
SDZS	Mobile phase ratio (v/v) Water: Methanol	760:240	100.8 ± 0.62
	Flow rate (ml/min)	750:250	101.4 ± 0.48
	Temperature (°C)	740:260	100.5 ± 0.92
		0.9	100.2 ± 0.25
		1.0	100.7 ± 0.28
		1.1	100.4 ± 0.21
		15°C	100.1 ± 0.83
		25°C	99.7 ± 0.79
		35°C	99.4 ± 0.78
STZS	Mobile phase ratio (v/v) Water: Methanol	760:240	101.5 ± 0.48
	Flow rate (ml/min)	750:250	101.8 ± 0.27
	Temperature (°C)	740:260	100.5 ± 0.97
		0.9	100.7 ± 0.33
		1.0	100.4 ± 0.54
		1.1	100.6 ± 0.32
		15°C	100.3 ± 0.46
		25°C	100.1 ± 0.73
		35°C	99.8 ± 0.58
SDMS	Mobile phase ratio (v/v) Water: Methanol	760:240	101.3 ± 0.61
	Flow rate (ml/min)	750:250	101.6 ± 0.43
	Temperature (°C)	740:260	100.7 ± 0.83
		0.9	100.8 ± 0.64
		1.0	101.8 ± 0.25
		1.1	101.1 ± 0.57
		15°C	99.5± 0.92
		25°C	99.4 ± 0.84
		35°C	100.3 ± 0.97

Applicability of the method to marketed products

It is evident from the results obtained that the validated method gave satisfactory results with respect to the analysis of the three drugs. The validated method is applied to a commercially available package (Sulphanur® 3 forte) as shown in Table 6.

This acceptable value indicated the applicability of the proposed method for the routine quality control of Sulphanur® 3 forte water soluble powder without interference with the excipients. This was evidenced from the good labeled claim percentages as well as the absence of any peaks in the chromatogram of the water soluble powder.

Table 6: Result of market product (Sulphanur® 3 forte water soluble powder)

Product Name	Labeled claim (mg/g)	SDZS (mg/g)	STZS (mg/g)	SDMS (mg/g)
Sulphanur® 3 forte water soluble powder	SDZS (250), STZS (250), SDMS (250)	251.3	248.8	249.2

CONCLUSION

The developed HPLC method was validated for the quantitative quality control determination of SDZS, STZS and SDMS in Sulphanur® 3 forte water soluble powder. It was evaluated over system suitability, specificity, sensitivity, linearity, range, accuracy (recovery), precision (repeatability and intermediate precision) and robustness. All the validation results were within the allowed specifications of ICH/USP guidelines. The developed method is proved to be rapid, accurate, and stability indicating for the simultaneous determination of the combined SDZS, STZS and SDMS in Sulphanur® 3 forte water soluble powder in the presence of the excipients and the degradation products. There was always a complete separation of all ingredients from their degradation products and from the placebo. As a result, the proposed HPLC method could be adopted for quantitative quality control and routine analysis of Sulphanur® 3 forte water soluble powder.

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