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Validated HPLC-DAD method for stability study of sulbutiamine HCl

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Sulbutiamine (SUL) is a synthetic thiamine derivative widely used for treatment of memory disorders. In this study, a newly developed gradient HPLC-DAD method demonstrating no interference from the different degradation products of SUL has been optimized and validated. The drug was subjected to variable stress conditions including hydrolysis (at different pH values), oxidation, photolysis and dry heat. The drug was found to be labile to hydrolysis, oxidation and photolysis but stable in thermal and neutral hydrolytic degradation. Successful chromatographic separation of SUL from all degradation products with significantly different t_R values was achieved on a ZORBAX Eclipse Plus C18 column using a mobile phase containing a gradient mixture of solvent A (50 mM KH_2PO_4 (pH 3.6 ± 0.2)) and solvent B (methanol). UV detection was performed at 254 nm using a photodiode array detector (DAD). The reliability of the method was assessed by evaluation of accuracy, precision, specificity, robustness and ruggedness according to USP guidelines. The linear regression analysis data for the calibration curve showed a good relationship in the range of 2–40 μg mL^{-1}. System suitability tests were performed, and selectivity (α) and resolution (R_s) factors were found to be greater than 1.5 and 2, respectively. The assay method was successfully used to estimate SUL in Arcalion Forte® tablets and good percentage recoveries were obtained. The developed method compared favorably with the reported spectrophotometric method.

1. Introduction

Sulbutiamine HCl (SUL), is a nonpharmacopoeial drug, which is chemically known as *N*,*N*′-{dithiobis[2-(2-isobutyryloxyethyl)-1-methylvinylene]}bis[*N*-(4-amino-2-methyl pyrimidin-5-yl-methyl)-formamide].[1] It is a more efficient version of vitamin B_1, which mimics the effect of thiamine at a more drastic level. It is more efficient than thiamine in crossing the blood–brain barrier and can be used alone or stacked with other nootropics. It is used to treat asthenia, improve memory and improve erectile dysfunction.[2] In addition, many athletes use SUL as a legal performance-enhancing drug to enhance their performance in sports.[3]

Only two methods have been found in the literature for determination of SUL in biological fluids and pharmaceutical dosage form. The first method was a kinetic spectrophotometric method, which depended on the catalytic effect on the reaction between sodium azide and iodine in aqueous solution, and then it measures the decrease in absorbance of iodine at 348 nm.[4] The second reported method was HPLC with fluorescence detection for analysis of SUL along with other thiamine disulphides.[5] It depended on gradient elution using methanol and 0.011 M phosphate buffer, pH 4.5 (from 10% to 62% methanol) with an analysis time of 50 minutes using λ_{ex} = 365 nm and λ_{em} = 433 nm.

During manufacturing, storage and distribution, many drugs are susceptible to different environmental factors such as light, temperature and humidity; thus, forced degradation studies are necessary for providing information about chemical and physical factors that result in drug instability and hence help in selecting suitable packing and storage conditions. Moreover, results of stability studies are necessary for setting expiration dates for the API (active pharmaceutical ingredients) or drug products.[6–10] According to ICH guidelines Q1AR2,[10] the stability testing of an active ingredient should be performed under variable accelerated conditions (hydrolysis under different pH values, oxidation, photolysis and thermal degradation). A stability-indicating method is a method that can selectively analyze the parent constituent (API) from the pharmaceutical product, and it is developed to separate and determine the active drug in the presence of its impurities and degradation products.[11]

On reviewing the current literature, none of the known pharmacopoeias described any method of analysis for SUL. Moreover, the two published methods are time consuming and cannot be used to analyze SUL samples in the presence of its different degradation products.

Due to the importance of drug stability studies and the absence of information about SUL stability, the work in this

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manuscript aimed to perform a stability study for SUL according to ICH guidelines[10] through a validated stability indicating gradient HPLC-DAD method. The newly developed method has the advantages of being the first stability-indicating method for SUL with high sensitivity, precision and accuracy. Moreover, minimum sample preparation is required and the analysis can be performed within 10 minutes.

2. Experimental

2.1. Instruments

The HPLC (Agilent 1260 Infinity, Germany) instrument was equipped with an Agilent 1260 Infinity preparative pump (G1361A), Agilent 1260 Infinity Diode array detector VL (G131SD), Agilent 1260 Infinity Thermostated column compartment (G1316A) and Agilent 1260 Infinity preparative Autosampler (G2260A). Separation and quantitation was performed on a ZORBAX Eclipse Plus C18 column (250 × 4.6 mm i.d, 5 μm particle size) (USA).

2.2. Samples

2.2.1. Pure samples. Pharmaceutical grade SUL was provided as a gift from SIGMA Pharmaceutical Industries S.A.E, Quesna City, Egypt. Its purity was verified and found to be 99.90% ± 1.404% according to a previously reported method.[4]

2.2.2. Pharmaceutical preparation. Arcalion Forte® tablets (Batch no. 18255) were manufactured by SIGMA Pharmaceutical Industries S.A.E, Quesna City, Egypt and labeled as containing 400 mg per tablet.

2.3. Chemicals and solvents

- Methanol [(Sigma-Aldrich, Chromasolv®, Germany), and (Fisher Scientific, UK)].
- Deionized water (SEDICO Pharmaceuticals Co., Cairo, Egypt).
- Potassium dihydrogen phosphate, sodium hydroxide orthophosphoric acid, hydrochloric acid and hydrogen peroxide were of analytical grade and were purchased from El-NASR Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt.

2.4. Solutions

Stock standard solution of sulbutiamine (1 mg mL^{-1}) was prepared by accurately weighing 0.1 g SUL in a 100 mL volumetric flask and dissolving in methanol.

50 mM KH_2PO_4 buffer (pH = 3.6) was prepared by dissolving 6.8 g KH_2PO_4 in 1 L deionized water and then adjusting the pH to 3.6 ± 0.2 with aqueous phosphoric acid.

Working standard solution of sulbutiamine (0.1 mg mL^{-1}) was prepared by transferring 10 mL from SUL stock standard solution (1 mg mL^{-1}) into a 100 mL volumetric flask and then diluting with methanol : 50 mM KH_2PO_4 buffer (pH = 3.6) (50 : 50, v/v).

2.4.1. Pharmaceutical dosage form solution. Ten Arcalion Forte® tablets were crushed and triturated well in a mortar. An accurately weighed amount of the powdered tablet equal to 100 mg of SUL was transferred into a 100 mL volumetric flask. Then, 75 mL methanol was added, and the solution was ultra-sonicated for 15 minutes and filtered, and then the volume was completed with methanol.

3. Procedure

3.1. Chromatographic conditions

Chromatographic separation was performed on a ZORBAX Eclipse Plus C18 column using a gradient mixture of methanol : 50 mM KH_2PO_4 buffer (pH = 3.6 ± 0.2) as a mobile phase. The gradient elution program is given in Table 1. The injection volume was 50 μL, and detection was carried out at 254 nm using DAD, maintaining the column temperature at 25 °C. The run time was 10 min, and the peak area ratio (using an area of 20 μg mL^{-1} SUL as an external standard) was used to quantify SUL.

3.2. Validation of the method

Validation of the method was performed with respect to USP guidelines.[12]

3.2.1. Linearity. Linearity test solutions for the assay method were prepared from SUL working standard solution (0.1 mg mL^{-1}) at different concentration levels (2–40 μg mL^{-1}). Triplicate 50 μL samples of each solution were injected into the HPLC system. The integrated relative peak area (using the area of 20 μg mL^{-1} SUL as an external standard) of SUL was obtained, and the regression equation was computed.

3.2.2. Accuracy. The accuracy of the method was evaluated by analyzing nine concentrations of pure SUL in its linearity range. The relative peak area for each concentration was obtained, and the mean percentage recoveries were then calculated.

3.2.3. Precision. Precision was expressed as the percentage relative standard deviation (%RSD) of the percentage assay, and it was evaluated by testing intra-day and inter-day variations.

Repeatability (intra-day variation) was checked by analyzing three concentrations of pure SUL (16, 20 and 38 μg mL^{-1}) three times within the same day using the previously mentioned procedure under chromatographic conditions, and the %RSD values were then calculated.

Intermediate precision was verified on three different days using the previously chosen concentrations in the same laboratory using the specifications under chromatographic conditions, and the %RSD values were then calculated.

3.2.4. Limits of detection and quantitation. In order to determine detection and quantification limits, SUL

Table 1 Details of gradient elution program

Time (minutes)	% Methanol	Flow rate (minutes)
0	55	1.5
2	55	1.5
4	85	2
8	85	2
9	55	1.5
10	55	1.5

concentrations in the lower part of the linear range of the calibration curve (0.5, 1 and 2 μg mL^{-1}) and the equations LOD = 3.3 × N/B and LOQ = 10 × N/B were used, where N is the standard deviation of the response and B is the slope of the corresponding calibration curve.

3.2.5. Specificity. Specificity was determined by exposing SUL samples to different stress conditions and then calculating the resolution factors (R_s) of the drug peak from the nearest peak. Specificity was also established through determination of SUL in Arcalion Forte® tablets and comparing the t_R value of SUL in the sample with that of pure SUL. Moreover, the peak purity was checked by using a DAD.

3.2.6. Robustness. Robustness is expressed as %RSD, and it was checked by small deliberate alternation in experimental conditions. The relative peaks areas of SUL were calculated, from which percentage recoveries and %RSD values were also obtained. The altered parameters were changes in the mobile phase composition (±2% methanol) and pH of the buffer (±0.2 pH).

3.2.7. Ruggedness. Ruggedness evaluates the degree of reproducibility of the results obtained under variety of conditions, such as performing the analysis with two different analysts or using methanol from different manufactures [(Sigma-Aldrich, Chromasolv®, Germany), and (Fisher Scientific, UK)]. In each variation, the relative peaks areas of SUL and %RSD values were calculated.

3.3. System suitability testing parameters

In all chromatographic systems, system suitability testing parameters should be checked before starting sample analysis. The peak symmetry, capacity, selectivity and resolution factors, number of theoretical plates and height equivalent to theoretical plates were calculated for the principle peak.

3.4. Assay of pharmaceutical preparation

A SUL sample with a concentration of 16 μg mL^{-1} was prepared from Arcalion Forte® working solution (0.1 mg mL^{-1}) and injected in triplicate following the procedure described for the chromatographic conditions. The concentration of SUL in the prepared solution was then calculated from the constructed regression equation. A standard addition technique was carried out to prove the accuracy of the suggested method, and it was performed by spiking the pre-analyzed SUL sample (16 μg mL^{-1}) with an extra 80%, 100% and 120% of standard SUL.

3.5. Forced degradation studies

Sulbutiamine stock standard solution (1 mg mL^{-1}) was used during forced degradation studies, and each degraded sample with a concentration of 25 μg mL^{-1} was prepared in a mixture of methanol : 50 mM KH_2PO_4 buffer (pH = 3.6) (50 : 50, v/v). Then, the procedure described under chromatographic conditions was followed. From the relative peak area of SUL in each chromatographed sample, and the SUL % degradation was then calculated.

3.5.1. Hydrolytic degradation. Acidic hydrolysis was carried out at 80 °C for 3 hours by using solutions of 0.1 and 1 N HCl, while basic hydrolysis was performed at room temperature for half an hour using 0.1 N NaOH. For neutral hydrolysis, deionized water was used at 80 °C for 5 hours.

A separate 5 mL SUL stock standard solution (1 mg mL^{-1}) was transferred to four separate 25 mL volumetric flasks, and then mixed with 5 mL of either 0.1 N HCl, 1 N HCl, 0.1 N NaOH or deionized water. The prepared solutions were kept away from light to prevent possible photodegradation and at 80 °C except for 0.1 N NaOH, which was maintained at room temperature. The samples were cooled and neutralized with an amount of acid or base equivalent to that of the previously added amount, and then the volume was completed to the mark with a mixture of methanol : 50 mM KH_2PO_4 buffer (pH = 3.6) (50 : 50, v/v) to prepare sample working solutions of 200 μg mL^{-1} each.

3.5.2. Oxidative degradation. Solutions of 3% and 30% H_2O_2 were used to carry out oxidative degradation of SUL. After mixing 5 mL of SUL stock standard solution (1 mg mL^{-1}) with 5 mL of either 3% or 30% H_2O_2 in two separate 25 mL volumetric flasks, the solutions were maintained at 80 °C for 5 hours away from light to prohibit the possible effect of light. Samples were then evaporated on a water bath to expel the remaining H_2O_2, and the volume was adjusted using a mixture of methanol : 50 mM KH_2PO_4 buffer (pH = 3.6) (50 : 50, v/v) to prepare sample working solutions of 200 μg mL^{-1} each.

3.5.3. Photolytic degradation. The effect of light was studied on SUL solid and liquid samples. First, 5 mL of SUL stock standard solution (1 mg mL^{-1}) and 5 mg SUL powder were transferred separately to two 25 mL volumetric flasks. Samples were subjected to UV light for 3 hours (liquid sample) or 5 hours (solid sample). Then, 5 mL methanol was added to each flask, and the volume was then adjusted with methanol : 50 mM KH_2PO_4 buffer (pH = 3.6) (50 : 50, v/v) to prepare sample working solutions of 200 μg mL^{-1} each.

3.5.4. Thermal degradation. Sulbutiamine (5 mg) was stored at 80 °C for 3 hours in an oven. The powder was transferred to a 25 mL volumetric flask and dissolved in 5 mL methanol, and then the volume was completed with methanol : 50 mM KH_2PO_4 buffer (pH = 3.6) (50 : 50, v/v) to obtain sample solutions of 200 μg mL^{-1}.

4. Results and discussion

Because of the complex nature of separating multiple components during analysis of stability samples, chromatographic methods have taken priority over the conventional methods of analysis.[13–15] They possess greater accuracy and sensitivity for even small quantities of degradation products produced. The popularity of HPLC in stability studies is due to its high-resolution capacity, sensitivity and specificity.[16] Stability-indicating methods are traditionally performed using gradient elution, in order to ensure that degradants of various chemical compositions are all detected.[17]

To date, no stability-indicating method was found in the literature for determination of SUL. In this manuscript, we aimed to perform a stability study for SUL and to develop a novel stability-indicating HPLC-DAD method for its analysis. Complete separation of the analyte was accomplished in less

than 10 minutes, and the method can be successfully applied to perform long-term and accelerate stability studies of SUL.

4.1. Method development and optimization

The initial method development was conducted on pure SUL samples, and the samples were obtained from different degradation conditions in order to select conditions that achieve good resolution between the drug and degradation products. The suitability of the mobile phase was determined on the basis of suitability for stability studies, time required for analysis and SUL peak broadening.

4.1.1. Optimization of mobile phase. Initially, samples were analyzed using an isocratic elution of a mobile phase consisting of water : acetonitrile (30 : 70, v/v) at a flow rate of 1.0 mL min^{-1}. Under these conditions, the SUL peak was very broad. Replacing acetonitrile with methanol slightly improved the peak broadening. Several trials were performed on the mobile phase consisting of water : methanol (in the ratio of 30 : 70, v/v) in order to enhance the chromatographic resolution and decrease SUL peak broadening such as the addition of 0.25% triethyl amine (pH 5 adjusted with H_3PO_4 acid), 0.5% trifluoroacetic acid (pH 5 adjusted with NaOH) or the addition of 0.15 mM heptane sulfonic acid. Unfortunately, neither the chromatographic resolution nor the peak shape were improved.

The second step was to replace water with 50 mM KH_2PO_4 buffer at different pH values (3–8). It was noticed that changing the buffer pH greatly affected the t_R of SUL, and hence the resolution between SUL and the closest eluted degradation product. Extremely acidic pH resulted in decreasing SUL t_R, leading to bad chromatographic resolution, whereas an alkaline pH increased SUL t_R, which gives rise to a broader SUL peak and increases the analysis time.

After extensive trials, gradient elution was tested by using a gradient solvent mixture consisting of methanol : 50 mM KH_2PO_4 (pH 3.6 ± 0.2). Different gradient elution programs were tried until optimum system suitability testing (SST) parameters were obtained with a symmetric SUL peak. The details of the gradient elution program used are given in Table 1.

4.1.2. Selection of stationary phase. Different stationary phases were also tried such as ZORBAX Eclipse Plus C18 and C8 columns, and both these stationary phases gave almost the same results.

4.1.3. Selection of detection wavelength. The photodiode array detector was set at different wavelengths, including 230, 254 and 365 nm. Using 254 nm as a detection wavelength provided the best results with respect to sensitivity and peak shape.

4.1.4. Optimization of column temperature. The thermostated column compartment was adjusted to different temperatures (20, 25 and 30 °C). Note that the column temperature neither affected the chromatographic separation nor the peak shape.

4.2. Application of the method

After optimization of all factors affecting method selectivity and sensitivity, the method was applied for determination of SUL in its pharmaceutical formulation. Firstly, a calibration curve relating the integrated relative peak area of SUL (using 20 μg mL^{-1} SUL as an external standard) to its corresponding concentration was constructed. Good linear calibration was achieved in the range of 2–40 μg mL^{-1}, and the calibration equation was as follows:

$$A = 0.0520C + 0.0265,\ r = 0.9999$$

where A is the integrated relative peak area, C is the concentration in μg mL^{-1} and r is the correlation coefficient. The calibration curve parameters are given in Table 2.

Secondly, and in order to evaluate the suitability of the method, the method was applied to Arcalion Forte® tablets. A single peak at $t_R = 5.55 \pm 0.03$ minutes was observed in the chromatogram of the drug samples extracted from tablets, indicating no interference from the excipients that routinely occur in tablets. The mean % recovery of the drug content was found to be 102.75 ± 0.799 as shown in Table 3. The recovery studies were carried out at 80%, 100%, and 120% of the test concentration. The % recovery of SUL at all three levels was found to be acceptable (Table 3).

The suggested method compared favorably with the reported spectrophotometric method[4] as shown from the values of the calculated Student's t and F-ratio, confirming that there was no significant difference within a probability of 95% between the two methods (Table 4).

4.3. Method validation

4.3.1. Linearity. The linearity of the developed method was estimated, and the linear regression data for the calibration curve ($n = 9$) showed good linearity ($r = 0.9999$) over the concentration range of 2–40 μg mL^{-1} with respect to relative peak area (Table 2).

4.3.2. Accuracy. The accuracy was calculated as the % recovery of pure SUL and was found to be 100.76 as shown in Table 2. Moreover, when the proposed method was applied for estimation of SUL in its pharmaceutical dosage form after

Table 2 Regression and analytical parameters of the proposed HPLC-DAD method for determination of sulbutiamine (SUL)

Parameters	SUL
Linearity	
Range	2–40 μg mL^{-1}
Slope	0.0520
Intercept	0.0265
Correlation coefficient (r)	0.9999
Accuracy (mean ± %RSD)	100.76 ± 0.997
Precision (%RSD)	
Repeatability[a]	0.533
Intermediate precision[b]	1.831
LOD	0.50 μg mL^{-1}
LOQ	1.51 μg mL^{-1}

[a] The intra-day ($n = 9$) average of three different concentrations (16, 20 and 38 μg mL^{-1}) repeated three times within 1 day. [b] The inter-day ($n = 9$) average of three different concentrations (16, 20 and 38 μg mL^{-1}) repeated three times in 3 successive days.

Table 3 Determination of sulbutiamine (SUL) in Arcalion Forte® tablets by the proposed HPLC-DAD method and results of standard addition technique

				Standard addition technique	
Pharmaceutical formulation	Taken	Found	% Found[a], ±%RSD	Pure added (μg mL^{-1})	% Found[b]
Arcalion Forte® tablets, (B. N. 18255), claimed to contain 400 mg SUL per tablet	16.00	16.44	102.75 ± 0.799	12.00	96.88
				16.00	99.65
				20.00	99.25
	Mean ± %RSD				98.59 ± 1.497

[a] Average of 6 determinations. [b] Average of 3 determinations.

Table 4 Statistical comparison of the results obtained by applying the proposed HPLC-DAD method and the reported spectrophotometric for determination of sulbutiamine (SUL) in pure form

Items	HPLC method	Reported method[b,4]
Mean	100.76	99.90
%RSD	0.997	1.404
Variance	3.683	1.968
n	9	7
Student's *t*-test	1.411, (2.145)[a]	
F-value	1.953, (3.581)[a]	

[a] Figures between parenthesis represent the corresponding tabulated values of t and F at $P = 0.05$. [b] Spectrophotometric method that depended on measuring the decrease in absorbance of I_2 at 348 nm.

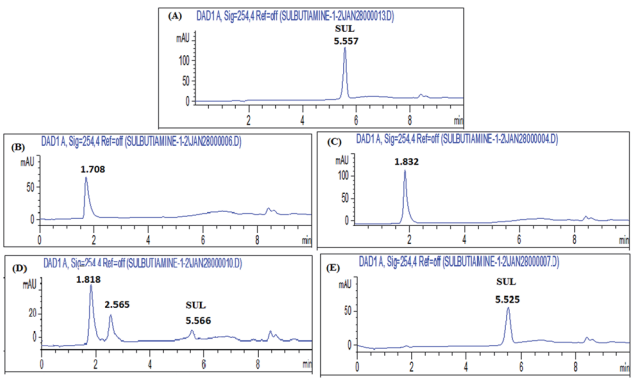

Fig. 1 HPLC chromatograms of (A) sulbutiamine, and its hydrolytic degradation products by (B) 1 N HCl, (C) 0.1 N NaOH, (D) 0.1 N HCl and (E) water.

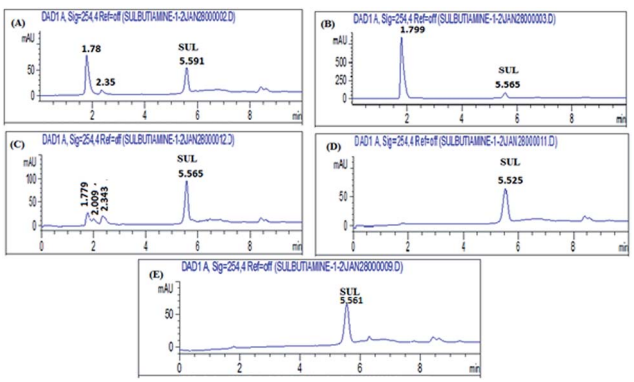

Fig. 2 HPLC chromatograms showing (A) oxidative degradation of sulbutiamine using 3% hydrogen peroxide and (B) 30% hydrogen peroxide, (C) photolysis of liquid sample, (D) photolysis of solid sample and (E) thermal degradation.

spiking with 80%, 100%, and 120% of additional pure SUL, good mean % recovery was obtained, and the results obtained are listed in Table 3.

4.3.3. Precision. The precision of the developed method was checked by testing intra-day (repeatability) and inter-day (intermediate precision) variations, and it was represented in terms of % relative standard deviation (%RSD). The obtained values of %RSD (Table 2) were <2%, confirming the high precision of the developed method.

4.3.4. Limits of detection and quantitation. Limits of detection and quantitation for SUL were found to be 0.5 and 1.51 μg mL^{-1}, respectively. This showed the adequate sensitivity of the developed method.

4.3.5. Specificity. The specificity of the developed method was assessed by its ability to resolve the major compound from possible degradation products as shown from the chromatograms in Fig. 1 and 2. The results revealed that the proposed method was able to completely discriminate the SUL from all degradation products, confirming the selectivity of the method. Moreover, acceptable results were obtained upon applying the method to Arcalion Forte® tablets (Table 3) to assess the method's specificity and confirm that the tablets' excipients did not interfere. On the other hand, when the peak purity was checked using a DAD, the purity factor was found to be 650.17 and the purity threshold was 146.59. The purity factor was more than the purity threshold, indicating that no additional peaks were co-eluted with the parent drug, which thus confirms the ability of the method to determine the analyte of interest in the presence of different degradation products.

4.3.6. Robustness. The percent relative standard deviation (%RSD) of peak areas was calculated for each studied parameter. It was found to be 0.467% for varying the mobile phase composition and 1.458 for varying the pH of the buffer used. The low values of %RSD (Table 5) indicate the robustness of the method.

4.3.7. Ruggedness. The ruggedness was evaluated by applying the method using two different analysts and methanol from different manufacturers. The values of the obtained %RSD

Table 5 Robustness and ruggedness studies of the developed HPLC-DAD method

	Factor
Robustness (%RSD)	
0.467	1-Mobile phase composition (±2% methanol)
1.458	2-pH of phosphate buffer (±0.2 pH)
Ruggedness (%RSD)	
0.914	1-Different methanol manufacturer
1.975	2-Two analysts

were reasonably low (<2.0%), confirming the good reproducibility of the suggested method (Table 5).

4.4. System suitability testing parameters

System suitability testing was carried out during method development and optimization as well as through the validation procedure.[18] The resolution (R_s) and selectivity (α) factors were calculated between SUL and the nearest eluted peak and were found to be >1.5 and 2, respectively, in all degradation conditions. Moreover, the symmetry factor was calculated for the basic component and was equal to 1. Other parameters such as capacity factor, number of theoretical plates and height equivalent to theoretical plates were calculated, and their values were within the acceptable limits (Table 6).

4.5. Results of forced degradation studies

The results of SUL stability studies are given in Table 7.

4.5.1. Hydrolytic degradation. The drug was found to be very sensitive to hydrolysis by 0.1 N NaOH and 1 N HCl, and it was completely degraded with an additional peak at $t_R = 1.83$ minutes for the basic degraded sample and at $t_R = 1.71$ minutes for the acid degraded sample (Fig. 1). On subjecting SUL to hydrolysis with 0.1 N HCl, the peak height of the parent drug was reduced, and new peaks at 1.81 and 2.55 minutes were produced (Fig. 1). On the other hand, the drug was not affected by hydrolysis under neutral conditions as seen in the chromatogram in Fig. 1.

4.5.2. Oxidative degradation. Oxidative degradation was tested using 3% and 30% H_2O_2. The height of the SUL peak was significantly reduced when treated with 30% H_2O_2 with the appearance of a degradation product peak at 1.78 minutes,

Table 6 System suitability testing parameters of the developed HPLC-DAD method

Parameters		SUL	Reference value[13]
t_R		5.55 ± 0.03 min	
Peak a symmetry		1	<1.5–2 or <2
K' (capacity factor)		2.36	1–10 acceptable
N (number of theoretical plates)		9417.44	Increases with increasing efficiency of separation
H (in cm) (Height equivalent to theoretical plates)		2.65×10^{-3}	The smaller the value the higher the column efficiency
Resolution (R_s)	0.1 N NaOH	14.25	$R < 2$
	0.1 N HCl	8.67	
	1 N HCl	14.25	
	3% H_2O_2	11.27	
	30% H_2O_2	14.22	
	Light	10.77	
Selectivity (α)	0.1 N NaOH	3.04	$\alpha < 1.5$
	0.1 N HCl	4.16	
	1 N HCl	3.04	
	3% H_2O_2	5.70	
	30% H_2O_2	27.93	
	Light	5.75	

Table 7 Summary of forced degradation studies

Stress conditions		Number of degradates (t_R)	Time of degradation (hours)	Degradation%
0.1 N NaOH at room temperature		1-(1.83)	1/2	100%
0.1 HCl at 80 °C		2-(1.81, 2.55)	3	93.6%
1HCl at 80 °C		1-(1.71)	3	100%
H_2O at 80 °C		No degradation	5	0%
3% H_2O_2 at 80 °C		2-(1.78, 2.35)	5	38.14%
30%H_2O_2 a 80 °C		1-(1.78)	5	93.13%
Photolysis	On liquid sample	3-(1.78, 2.01, 2.34)	3	26.8%
	On solid sample	No degradation	5	0%
Dry heat at 80 °C		No degradation	3	0%

whereas oxidation with 3% H_2O_2 resulted in the appearance of two degradation products at 1.78 and 2.35 minutes (Fig. 2).

4.5.3. Photolytic degradation. The chromatogram in Fig. 2 showed that SUL is photolabile and degraded by UV light, producing three different degradation products at t_R = 1.78, 2.01, and 2.34 minutes when it was in solution form, while it was found to be photo-stable when it was in a solid state.

4.5.4. Thermal degradation. Sulbutiamine was found to be thermally stable, as no additional peaks were observed when the drug was subjected to dry heat as shown in Fig. 2.

The chromatograms (Fig. 1 and 2) verified the stability indicating properties of the proposed method and assessed its ability to resolve the peak of the studied drug from all degradation products.

5. Conclusion

An accurate and reproducible HPLC-DAD method has been developed and validated for determination of SUL in pure form and marketed tablets. It is the first developed stability indicating method, in which all SUL degradation products were completely resolved from the parent drug. The short chromatographic run time of only 10 minutes makes this method suitable for processing many samples in a limited time, which is very important in quality control analysis of any drug.

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