Journal of Chromatographic Science Advance Access published January 11, 2016

Journal of Chromatographic Science, 2016, 1–9
doi: 10.1093/chromsci/bmv226
Article

Article

Stability-Indicating HPTLC Method for Studying Stress Degradation Behavior of Sulbutiamine HCl

Nehal F. Farid and Nada S. Abdelwahab*

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Benisuef University, Benisuef, Egypt

Author to whom correspondence should be addressed. Email: nadasayed2003@yahoo.com

Received 25 September 2014; Revised 14 October 2015

Abstract

Sulbutiamine (SUL) is an ester of thiazides with neurotropic action. A new stability indicating HPTLC method has been developed and validated for the determination of SUL in the presence of different degradation products. The drug was subjected to different stress conditions following ICH strategy such as hydrolytic degradation (neutral, alkaline and acidic hydrolysis), oxidation, photodegradation and dry heat degradation. The drug demonstrated degradation under all decomposition conditions except neutral hydrolysis and dry heat, where the drug was completely degraded with 0.1 N NaOH, 1 N HCl and 30% H_2O_2 while it was partially degradaed by 0.1 N HCl, 3% H_2O_2 and UV light. Structure elucidation of the resulting degradation products was performed using ESI-Q-MS–MS. A well-defined peak for SUL was obtained at $R_f = 0.46$ and was completely separated from all obtained degradation products. Chromatographic separation was carried out on HPTLC aluminum plates precoated with silica gel 60 F_{254} using acetone–methylene chloride–ammonia buffer (pH 8.5 ± 0.2) (7:3:0.5, v/v) as a developing system. Densitometric scanning of the separated peaks was performed at 254 nm. System suitability testing parameters were calculated to ascertain the quality performance of the developed method. The method was validated with respect to USP guidelines regarding accuracy, precision, specificity, robustness and ruggedness. Good correlation coefficients were achieved in the range of 0.4–5.0 µg/band, and the limit of detection and limit of quantitation were found to be 0.11 and 0.33 µg/band, respectively. The utility of the suggested method was verified by application to Arcalion forte® tablets where no interference from additives was found.

Introduction

Sulbutiamine HCl (SUL) is a synthetic molecule, which consists of two thiamine (vitamin B_1) molecules bound together by a sulfur group. As a dimer of two thiamine molecules, it is a lipophilic compound that crosses the blood–brain barrier more readily than thiamine. It appears to be somewhat useful in treating asthenia and alleviating fatigue (1). It is chemically known as [4-[(4-amino-2-methyl-pyrimidin-5-yl) methyl-formyl-amino]-3-[2-[(4-amino-2-methyl-pyrimidin-5-yl) methyl-formyl-amino]-5-(2-methylpropanoyloxy)pent-2-*en*-3-yl]disulfanyl-pent-3-enyl]-2-methylpropanoate (2).

Reviewing the literature, few methods were found for the analysis of SUL. The drug was analyzed by a kinetic spectrophotometric method, which depended on the catalytic effect on the reaction between sodium azide and iodine in aqueous solution, by measuring the decrease in absorbance of iodine at 348 nm (3). Furthermore, it was determined along with other thiamine disulfides by the HPLC method, which depended on fluorescence detection (λ_{ex} = 365 nm; λ_{em} = 433 nm) using gradient elution with methanol and 0.01 M phosphate buffer, pH 4.5 (from 10% methanol to 62%, analysis time was 50 min) (4). All these reported methods determined SUL in the absence of its degradation products. Recently, the authors published a RP-HPLC method for studying the stability of the cited drug under different conditions (5). To our knowledge, no stability indicating HPTLC determination for SUL has been described in the literature.

Generally, many drugs are sensitive to different environmental factors such as light, temperature and humidity which often vary during manufacturing, storage and distribution, so stability indicating testing of active substance is necessary to provide information about potential degradation products, physical and chemical factors that result in drug instability. Hence by controlling these factors, shelf life and drug efficacy could be improved (6, 7).

According to FDA guidelines (8), a stability indicating method (SIM) is defined as a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences such as degradation products, process impurities, excipients or other potential impurities. During stability studies, liquid chromatography (LC) is used routinely to separate and quantify the analytes of interest (9–11). There are three components necessary for implementing a SIM: sample generation, method development and method validation (8, 12). ESI-Q-MS–MS permits rapid identification and characterization of drug degradation products and impurities, hence it is widely used in stability studies of many drugs (13, 14).

Due to the importance of stability indicating testing methods, our work aimed to develop and validate a sensitive, selective and accurate stability indicating HPTLC method for determination of SUL in the presence of different degradation products resulting from different stress conditions. The study was further extended for identification of resulting degradation products using ESI-Q-MS–MS.

Experimental

Instrumentation

HPTLC method

In this study, scanning was carried out by using CAMAG TLC Densitometric Scanner 3S/N 130319 with WINCATS software (CAMAG, Muttens, Switzerland). A TLC CAMAG Linomat V autosampler was used, which was equipped with a 100-mL syringe (Camag, Switzerland). Following up drug degradation during degradation studies was carried out by using an ultraviolet (UV) lamp with a short wavelength of 254 nm (VL-6.LC; Marne la Vallee, France). HPTLC plates (20 × 10 cm) coated with 60 F254 silica gel (Merck, Darmstadt, Germany) with 0.2 mm thickness were used as a stationary phase. During TLC scanning, the scanning mode was absorbance, source of radiation was deuterium lamp. Chromatographic development was carried out in a glass chamber (Macherey-Nagel, Germany).

LC–MS

The LC system consisted of a Thermo Fisher Scientific (San José, USA) Accela HPLC 1200 LC-10AD pumping system, coupled with an Accela autosampler and a Hypersil Gold C18 column (50 × 2.0 mm, 2.1 µm, Phenomenex). The used mobile phase was 1% formic acid–methanol (90:10, v/v) with a flow rate of 200 µL/min.

Mass spectrometric analysis was performed using a TSQ Quantum Access MAX triple stage quadrupole mass spectrometer (Thermoscientific, New York, USA). The instrument was equipped with an electrospray ionization (ESI) source. The data acquisition was under the control of Xcalibur software version 2.2. SUL was individually tuned by direct injection of SUL solution (1 mg/mL).

Reagents and chemicals

Pure samples

SUL was kindly supplied by SIGMA Pharmaceutical industries (Quesna City, Egypt, SAE). The purity was checked and found to be 99.90 ± 1.404 according to the reported spectrophotometric method (3).

Pharmaceutical dosage form

Arcalion forte® tablets (batch no. 18255) were manufactured by SIGMA Pharmaceutical industries (Quesna City, Egypt, SAE) and labeled to contain 400 mg SUL per tablet.

Chemicals and reagents

Acetone, methylene chloride, ammonia solution 33%, ammonium chloride, sodium hydroxide, conc. HCl, 30% H_2O_2 and phosphoric acid were of analytical grade (El Nasr Pharmaceutical Chemicals Co., Abu- Zabaal, Cairo, Egypt). Furthermore, 0.1 M ammonia buffer was prepared by dissolving 0.68 g ammonium chloride in 50-mL distilled water and then 20 mL ammonia solution were added and the volume was adjusted to 100-mL by distilled water.

Solutions

Stock standard solution of SUL (2 mg/mL)

Stock standard solution of SUL (2 mg/mL) was prepared by dissolving 50 mg SUL in 25 mL methanol.

Pharmaceutical dosage form solution

Ten tablets were crushed and triturated well in a mortar. The average tablet weight was determined and a powder sample equivalent to 100 mg of SUL was transferred into a 100-mL volumetric flask. About 75-mL methanol was added, and the flask was sonicated for 15 min. The volume was completed to the mark with methanol to obtain sample stock solution of 1 mg/mL, and the solution was then filtered using Whatman® qualitative filter paper (185 mm, China).

Procedure

Chromatographic conditions

Samples were applied in the form of bands of 6 mm width with a 100 µL sample syringe on aluminum plates precoated with silica gel $60F_{254}$ (20 × 10 cm), using the autosampler. A constant application rate of 0.1 µL/s was used, and the space between bands was 8.9 mm. The slit dimension was 6.0 × 0.3 µm, and the scanning speed was 20 mm/s. The mobile phase consisted of methylene chloride–acetone–ammonia buffer (3:7:0.5, v/v, pH 8.5 ± 0.2). Linear ascending development was carried out in a glass chamber saturated with the mobile phase. Development of the plates was left till the mobile phase migrates 8 cm. Following the development, the plates were air dried, bands were visualized under an UV lamp at 254 nm and densitometric scanning was performed using a CAMAG TLC scanner in the reflectance–absorbance mode at 254 nm and operated by WINCATS software. The radiation source was a deuterium lamp.

For LC–MS system, the used mobile phase was 1% formic acid–methanol (90:10, v/v) with a flow rate of 200 µL/min, and the stationary phase was a Hypersil Gold C18 column (50 × 2.0 mm, 2.1 µm, Phenomenex).

Mass spectrometric conditions

ESI-Q-MS–MS studies were performed in positive ESI and single reaction monitoring (SRM) mode using the following parameters:

(i) Turbo ion spray temperature: 400°C—capillary temperature: 270°C
(ii) Sheath gas (N_2): 20 psi—auxiliary gas (N_2): 2 psi
(iii) Ion spray voltage: 3.6 kV—collision energy: 27 and 36 V
(iv) SRM transition (*m/z*): 333.3 and 123.3

Construction of the calibration curve

In this method, 0.2–2.5 mL from SUL stock standard solution (2 mg/mL) were transferred accurately into a series of 10-mL volumetric flasks, and the volumes were completed to the mark with methanol. Then, 10-µL from each flask were spotted in triplicates on HPTLC plates. The procedure under chromatographic conditions was

performed. The mean integrated relative peak areas (using 1.2 µg/band as external standard) were plotted against concentrations to obtain the calibration graph.

Analysis of dosage form

Concentration of 150 µg/mL was prepared by accurately transferring 1.5 mL from pharmaceutical dosage form stock (1000 µg/mL) solution into a 10-mL volumetric flask, and the volume was completed with methanol. Then, 10-µL of the prepared sample was spotted (n = 6) on the HPTLC plate to obtain a concentration of 1.5 µg/band and the chromatographic method was then continued as mentioned previously. The relative peak area of each spot was determined, and the concentration was calculated from the previously computed regression equation.

Accelerated degradation studies

SUL stock standard solution (2 mg/mL) was used during forced degradation studies. Furthermore, concentration of 5 µg/band of each degraded sample was then applied on HPTLC plates in replicates. The procedure under chromatographic conditions was then followed. From the relative peak area of SUL in each chromatographed sample, SUL % degradation was then calculated.

Hydrolytic degradation

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

Separate 2.5-mL of SUL stock standard solution (1 mg/mL) was transferred to four separate 25-mL volumetric flasks and then mixed with 5-mL of 0.1 N HCl, 1 N HCl, 0.1 N NaOH and distilled water. The prepared solutions were kept away from light to exclude the possible photodegradation at 80°C except for 0.1 N NaOH, which was kept at room temperature. 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 methanol to prepare samples working solutions of 200 µg/mL each.

Oxidative degradation

In oxidative degradation of SUL, 3 and 30% H_2O_2 were used. By mixing 2.5-mL of SUL stock standard solution (2 mg/mL) with 5 mL of either 3 or 30% H_2O_2 in two separate 25-mL volumetric flasks, the solutions were kept at 80°C for 5 h away from light to prohibit the possible effect of light. Samples were then cooled and evaporated on a water bath to remove the remaining H_2O_2, and then the volume was adjusted using methanol to prepare samples working solutions of 200 µg/mL each.

Photolytic degradation

The effect of light on SUL solid and liquid samples was studied. Then, 2.5 mL from SUL stock standard solution (2 mg/mL) and 5 mg SUL powder were transferred separately into two 25-mL volumetric flasks. Samples were subjected to UV light for 3 h (liquid sample) or 5 h (for solid sample). Five milliliters of methanol were added to each flask, and the volume was then adjusted with methanol to prepare samples working solutions of 200 µg/mL each.

Thermal degradation

SUL 5 mg was stored at 80°C for 3 h in an oven. The powder was transferred to a 25-mL volumetric flask, dissolving in 5-mL methanol, and then the volume was completed with methanol to obtain samples solutions of 200 µg/mL.

Results

Method validation

Validation was performed according to USP (15).

Calibration and range

The relative peak area (using 1.2 µg/band as external standard) was plotted versus the corresponding concentration (n = 9). The calibration curve showed good relationship over the concentration range of 0.4–5 µg/band.

The regression equation is:

$$A = -0.0157X^2 + 0.7569X + 0.1245 \quad r = 0.9998,$$

where A is the relative peak area (using 1.2 µg/band as external standard), C is the concentration in µg/band and r is the correlation coefficient. The calibration curve parameters are given in Table I.

Accuracy

Accuracy is the measure of exactness of an analytical method. Accuracy was checked by determining nine different concentrations of SUL in

Table I. Regression and Analytical Parameters of the Proposed HPTLC Method for Determination of SUL and Statistical Comparison with the Reported Spectrophotometric for Determination of SUL in Pure Form

Parameters	SUL	Reported method [3]
Linearity		
Range (µg/band)	0.4–5	
Slope		
Coefficient 1[a]	−0.0157	
Coefficient 2[b]	0.7569	
Intercept	0.1245	
Correlation coefficient (r)	0.9998	
Precision (% RSD)		
Repeatability*	1.636	
Intermediate precision**	2.174	
LOD	0.11 µg/band	
LOQ	0.33 µg/band	
Accuracy (mean ± %RSD)	100.57 ± 1.908	99.90 ± 1.404
N	9	7
Student's t-test	0.772 (2.145)[a]	
F-value	1.871 (4.147)[a]	

Following a polynomial regression $A = aX^2 + bX + C$, where A is the peak area ratio, X is the concentration in µg/band, a and b are coefficients 1 and 2, respectively, and c is the intercept.

Figures between parenthesis represent the corresponding tabulated values of t and F at P = 0.05. Spectrophotometric method that depended on measuring the decrease in absorbance of I_2 after its reaction with sodium azide in the presence of SUL at 348 nm.

*The intraday (n = 9), average of three different concentrations (1, 2 and 3 µg/band) repeated three times within day.

**The interday (n = 9), average of three different concentrations (1, 2 and 3 µg/band) repeated three times in three successive days.

the calibration range. The relative peak area was determined, and the concentrations were obtained from the regression equation. The mean recovery was found to be 100.57 ± 1.908% (Table I).

Precision

Precision is the degree of repeatability of an analytical method under normal operating conditions. This was verified by testing the repeatability and intermediate precision. Repeatability was tested by analysis of three different concentrations (1, 2 and 3 µg/band) by the proposed HPTLC method three times on the same day. Intermediate precision was checked by applying the proposed method for the analysis of the chosen concentrations on three different days. In each case, the % RSD was determined and was found to be 1.636 and 2.174, respectively (Table I).

LOD and LOQ

To determine detection and quantification limits, SUL concentrations in the lower part of the linear range of the calibration curve and the equations, limit of detection (LOD) = $3.3 \times N/B$ and limit of quantitation (LOQ) = $10 \times N/B$, where N is the standard deviation of the response and B is the slope of the corresponding calibration curve, were used.

The LOD and LOQ were 0.11 and 0.33 µg/band, respectively, indicating the high sensitivity of the method (Table I).

Specificity

Specificity is the capability to determine precisely and specifically the analyte in the presence of additional components that may be anticipated to be there in the sample matrix. It is the level of interference from impurities, excipients, active ingredients and degradation products, and it guarantees that a peak response is only due to a single analyte (16).

It was verified by complete resolution of SUL from all its degradation products (Figures 1 and 2). Also, the good results obtained by applying the method to Arcalion forte® tablets proved that additives did not interfere (Table II).

Robustness

It is the capacity of the method to remain unaffected by small deliberate variations in the method parameters. In our method, this was done by applying small changes in developing system composition (±2% acetone), small changes in amount of buffer used ((±0.03 mL) and varying the saturation time (±5 min). In each case, the retardation factor (R_f) value and then %RSD were determined and %RSD was found to be <2%, which means that the method is robust. The results are shown in Table III.

Ruggedness

It is the degree of reproducibility of the results obtained under variety of conditions; it was tested by using HPTLC plates from different manufacturers (Merck, Germany and Fluka, Germany) and also by examining the method by two different analysts. The %RSD was determined and was found to be 0.094 and 1.483, respectively. The low values of %RSD indicate the ruggedness of the method. The results are summarized in Table III.

Analysis of Arcalion forte® tablets

The concentration of SUL in Arcalion forte® tablets were found to be 101.60 ± 1.060%, suggesting that there is no interference from any of the excipients which are normally present in tablets. The method was further validated by applying the standard addition technique by addition of known samples of pure SUL to known concentrations of pharmaceutical preparation and the resulting mixtures were analyzed by the proposed method. The results are summarized in Table II.

Degradation behavior of sSUL HCl

ESI-Q-MS–MS were used to identify SUL degradation products obtained under different stress conditions. All the compounds showed abundant protonated molecular ions under positive ESI conditions. Collision-induced dissociation (CID) spectra of MH^+ were recorded to identify the structure of each degradate. The mass spectra of the obtained degradation products and the expected degradation scheme are given in Figures 3 and 4.

Hydrolytic degradation

SUL was completely degraded when treated with 0.1 N NaOH and 1 N HCl, where an additional peak at $R_f = 0.11$ (Deg 1) was observed (Figure 1). On the other hand, 0.1 N HCl resulted in reduction in SUL peak height with additional two peaks at $R_f = 0.06$ (Deg 2) and 0.11 (Deg 1) (Figure 1). The drug was found to be stable under neutral hydrolysis (Figure 1).

Oxidative degradation

In the case of oxidative degradation of SUL using 3% H_2O_2, the peaks of the degradation products were observed at $R_f = 0.08$ (Deg 3) and $R_f = 0.72$ (Deg 4) (Figure 2) with molecular masses of m/z 671 and 739.87, respectively; whereas 30% H_2O_2 resulted in complete degradation of the drug giving a peak at $R_f = 0.64$ with the same molecular mass as that of (Deg 4).

Photolytic degradation

Irradiation of SUL solution with UV light yielded three different degradation products at $R_f = 0.08$ (Deg 3), $R_f = 0.13$ (Deg 5) that could not be identified by ESI-Q-MS–MS and $R_f = 0.64$ (Deg 4). The drug in the solid state was found to be photo stable (Figure 2).

Thermal degradation

SUL was found to be thermally stable as it gave no additional peaks (Figure 4).

From the chromatograms (Figures 1 and 2), the developed method was able to resolve the peak of the studied drug from all degradation products, proving the stability indicating properties of the proposed methods. The results of SUL stability studies are summarized in Table IV.

System suitability testing

System suitability testing is done after validation of the method to ensure the performance of the developed method. System suitability was checked by calculating parameters including resolution (R_s), peak symmetry, capacity factor (K') and selectivity (α), and the results in Table V show that all the degradation peaks were completely separated from the (SUL) peak, where R_s and selectivity (α) were found to be more than 1.5. The results are summarized in Table V.

Statistical analysis

Statistical analysis of the results obtained by the proposed HPTLC method and the reported method (3) for determination of pure SUL was carried out, and the calculated t and F values were less than the

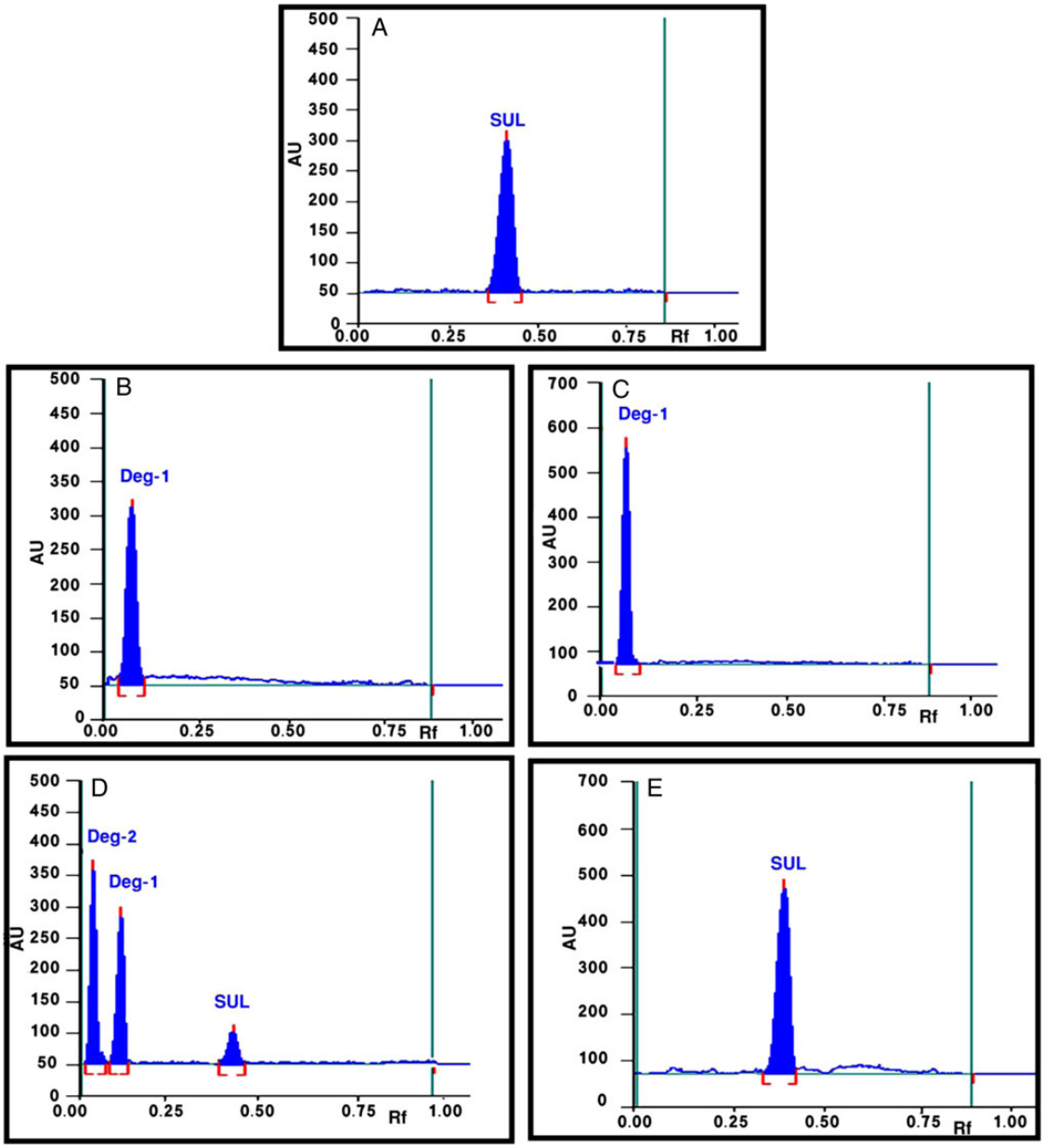

Figure 1. HPTLC-chromatograms of (A) sulbutiamine, (B) 1 N HCl, (C) 0.1 N NaOH, (D) 0.1 N HCl and (E) neutral hydrolysis. This figure is available in black and white in print and in color at *JCS* online.

theoretical ones (using a probability of 95%), which indicates that there is no significant difference between the proposed method and the reported one (Table I).

Discussion

During the development of a SIM, a quantitative analytical procedure is used to detect a decrease in the amount of the active pharmaceutical ingredient present due to degradation products (18). Separation of a multicomponent mixture in a single run is difficult to achieve, and therefore liquid chromatographic methods were taking preference over traditional methods as spectrophotometric methods in the stability study (12). The HPTLC-densitometric method has advantages of low operating costs, high sample output, the need for minimal sample preparation and mobile phase having pH 8 or more can be used (19). The main object of the developed stability indicating HPTLC was to completely separate the peaks of the degradation products from that of the drug.

SUL is a non-pharmacopoeial drug and up to date there is one reported SIM (5) for determination of SUL. Hence, our work concerned with studying SUL stability under different stress conditions following ICH guidelines (20) and developing a validated stability indicating HPTLC-densitometric method.

Initial trials were conducted, with the aim of selecting a suitable developing system for accurate determination of SUL and for achieving optimum resolution between SUL and its degradation products. Several developing systems such as methylene chloride–acetone–ammonia solution (3:7:0.4, v/v), methylene chloride–acetone–acetic acid (3:7:0.4, v/v),

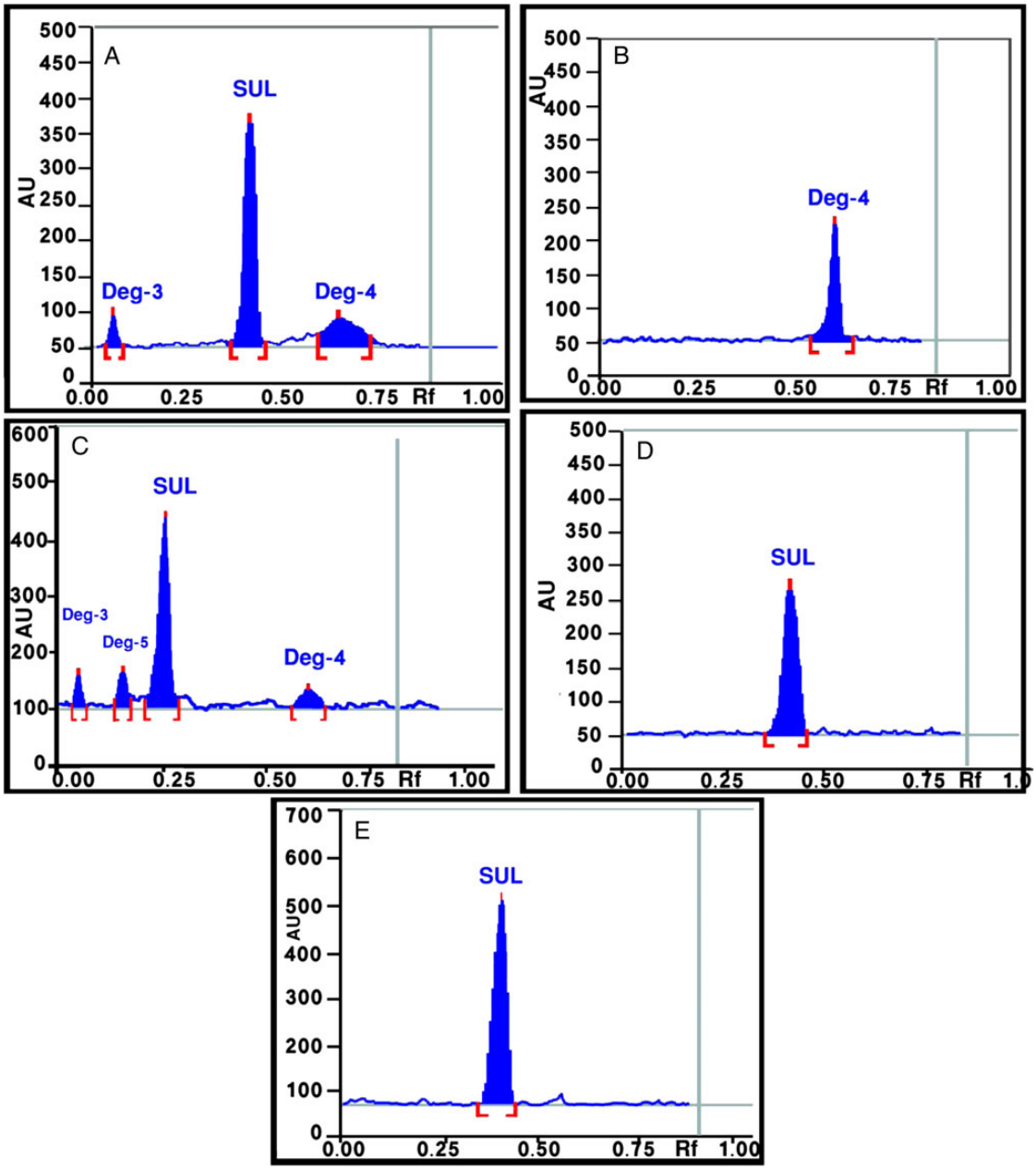

Figure 2. HPTLC-chromatograms of (A) 3% H_2O_2, (B) 30% H_2O_2, (C) UV-light [liquid sample], (D) UV-light [solid sample] and (E) thermal degradation. This figure is available in black and white in print and in color at *JCS* online.

Table II. Determination of SUL in Arcalion forte® Tablets By the Proposed HPTLC Method and Results of the Standard Addition Technique

Pharmaceutical formulation	Taken	Found	% Found[a] ± % RSD	Standard addition technique	
				Pure added (µg/band)	% Found[b]
Arcalion® tablets (B. N. 18255) claimed to contain 400 mg SUL/tablet	1.500	1.524	101.60 ± 1.060	1.00	100.11
				1.50	103.33
				2.00	103.00
Mean ± % RSD					102.15 ± 1.772

[a]Average of six determinations.
[b]Average of three determinations.

hexane–acetone–ammonia solution (3:7:0.4, v/v), hexane–acetone–acetic acid (3:7:0.4, v/v), ethyl acetate–acetone–ammonia solution (10:2:0.4, v/v), ethyl acetate–acetone–ammonia solution (3:7:0.4, v/v), ethyl acetate–acetone–acetic acid (10:2:0.4, v/v) were tried, and it was observed that all systems almost gave the same chromatographic separation. Addition of ammonia was found to be necessary for giving compact, nondiffused SUL spots and for moving the spot of NaOH degradation product above the baseline; however, bad resolution between SUL and H_2O_2 spots were observed. On the other hand, acetic acid was essential for achieving good separation among SUL and H_2O_2 spots, but all separated spots were tailed.

Table III. Robustness and ruggedness studies of the developed HPTLC method

Factor	Robustness (% RSD)
1. Mobile phase composition (±2% acetone)	1.843
2. Amount of ammonia buffer (±0.03 mL)	1.143
3. Saturation time (±5 min)	1.711
	Ruggedness (% RSD)
1. Different HPTLC plates manufacturer	0.094
2. Two analysts	1.483

Combinations of acetic acid and ammonia solution in different ratios resulted in phase separation in all tested systems. Replacing ammonia solution and acetic acid with ammonia buffer (pH 8.5 ± 0.2) resulted in the desired resolutions and compact spots with reasonable R_f values (Figures 1 and 2).

Finally, methylene chloride–acetone–ammonia buffer (3:7:0.5, v/v) was the developing system of choice. Scanning was tried at 254 and 230 nm in order to enhance method sensitivity, and using 254 nm as a detection wavelength was better regarding the signal-to-noise ratio.

Regarding the stability study of SUL, ICH recommendations (20) have been followed. The drug was subjected to hydrolytic degradation (with water, 0.1 N NaOH, 0.1 and 1 N HCl), oxidative degradation (3 and 30% H_2O_2), photodegradation (UV lamp (254 nm)) and dry heat (80°C).

It was found that the rate of the alkaline hydrolysis was faster than acidic hydrolysis, and it was found that complete degradation was observed by using 0.1 N NaOH while it was observed in acidic medium by using 1 N HCl. Furthermore, the drug was completely degraded when subjected to 30% H_2O_2 while partially degraded by using either 0.1 N HCl, 3% H_2O_2 and photodegradation. On the other hand, the drug was found to be stable in neutral hydrolysis and dry heat.

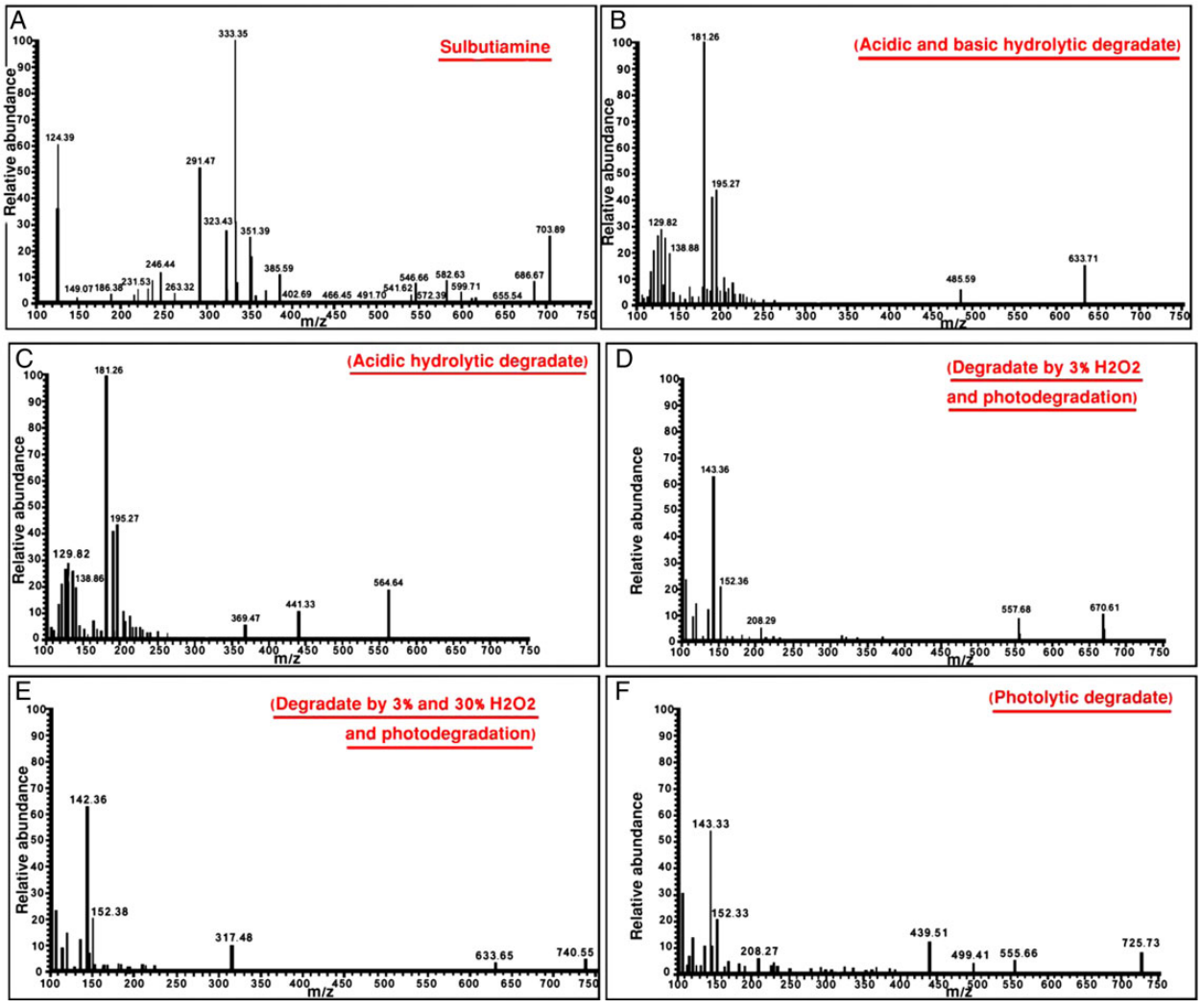

Figure 3. Mass spectra of (A) SUL, (B) Deg 1, (C) Deg 2, (D) Deg 3, (E) Deg 4 and (F) Deg 5. This figure is available in black and white in print and in color at *JCS* online.

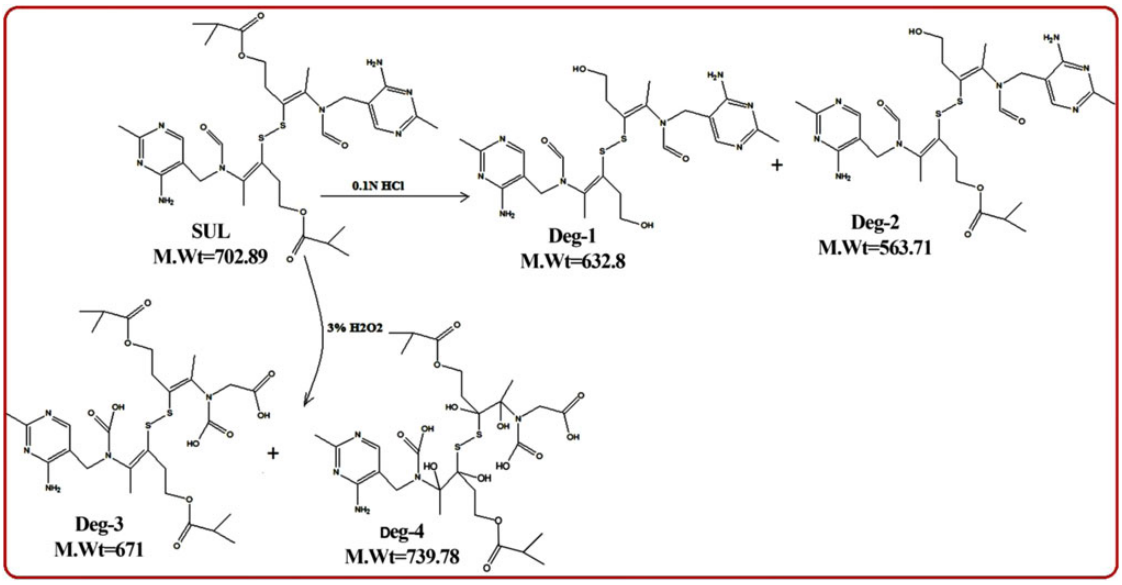

Figure 4. Expected degradation pathway of sulbutiamine determined by ESI-Q-MS–MS profile. This figure is available in black and white in print and in color at *JCS* online.

Table IV. Summary of forced degradation studies of SUL

Stress conditions	Time of degradation (h)	Number of degradates (R_f)	Degradation (%)
0.1 N NaOH at room temperature	½	1—(0.08)	100
0.1 N HCl at 80°C	3	2—(0.06, 0.11)	93.2
1 N HCl at 80°C	3	1—(0.11)	100
H_2O at 80°C	7	No degradation	0
3% H_2O_2 at 80°C	7	2—(0.08, 0.72)	45.4
30% H_2O_2 at room temperature	½	1—(0.64)	100
Photolysis			
On liquid sample	3	3—(0.04, 0.13, 0.18)	21
On solid sample	7	No degradation	0
Dry heat at 80°C	3	No degradation	0

Table V. System Suitability Parameters for the Determination of SUL by the Proposed HPTLC Method

Parameters	SUL	Reference value (17)
R_f	0.46 ± 0.03	
Peak asymmetry	0.92	1
K' (capacity factor)	0.54	1–10 acceptable
Resolution (R_s)		
0.1 N NaOH	6.40	$R > 2$
0.1 N HCl	6.57	
1 N HCl	5.55	
3% H_2O_2	7.11, 2.22	
30% H_2O_2	3.27	
Light	5.00	
Selectivity (α)		
0.1 N NaOH	7.40	>1
0.1 N HCl	10.86	
1 N HCl	5.69	
3% H_2O_2	9.25, 1.55	
30% H_2O_2	1.51	
Light	2.85	

Figure 4 shows the chemical structure of SUL, which contains two ester groups and therefore it is highly susceptible to hydrolysis. Exposing SUL to 0.1 N and 1 N HCl flowed by ESI-Q-MS–MS showed that using 0.1 N HCl led to cleavage of one of the two ester groups with the production of a degradate with $m/z = 633.71$ (Figure 3) and by increasing the acidic strength to 1 N the two ester groups were broken with the production of a new degradate with $m/z = 564.64$ (Figure 3). On the other hand, hydrolysis using 0.1 N NaOH led to direct cleavage of the two ester groups indicating that the drug is highly sensitive to alkaline degradation.

On oxidation with 3% H_2O_2, the two aldehyde groups along with one of the two pyrimidine rings and the two C=C were oxidized, which was confirmed by ESI-Q-MS–MS that showed molecular ion peaks at $m/z = 670.61$ and 740.55 (Figure 3).

The photodegradation of SUL led to production of three degradates, two of them with the same molecular mass and nearly the same R_f values as those obtained with 3% H_2O_2 (Degs 3 and 4) and a third new degradate (Deg 5) with a molecular mass of $m/z = 740$. In most cases, photodegradation may lead to free radical mechanisms and hence it is difficult to predict the structure of Deg 5.

Conclusion

The developed HPTLC method is simple, precise, accurate and specific for the determination of SUL as bulk drug and in pharmaceutical dosage form without any interference from excipients. The method also succeeded to be a stability indicating one, where the peaks of the degradation products were completely separated from that of the drug. The chromatographic method was further validated according to USP guidelines. The proposed HPTLC method also has the advantage of being suitable for routine analysis in quality control laboratories as several samples can be run at a time and hence reduces the analysis time.

Acknowledgments

The authors express their appreciation and thanks to Drs Mohamed Abdelwahab and Heba Atef (associate professors of pharmaceutical organic chemistry) for their effort in elucidation of the chemical structures of the degradation products.

References

1. Battendorff, L., Weekers, L., Shoffeniels, E.; Injection of sulbutiamine induces an increase in thiamine triphosphate in rat tissues; *Biochemical Pharmacology*, (1990); 40: 2557–2560.
2. Through website http://en.wikipedia.org/wiki/Sulbutiamine (accessed February 22, 2014).
3. Metwally, M.E.S., El Shabrawy, Y.; Kinetic spectrophotometric determination of sulbutiamine in pharmaceutical preparations and in human serum; *Analytical Sciences*, (2000); 16: 633–636.
4. Gele, P., Boursier- Neyret, C., Lesourd, M., Sauveur, C.; Determination of sulbutiamine and its disulphide derivatives in human plasma by HPLC using online post column reactors and fluorimetric detection; *Chromatographia*, (1993); 36: 67.
5. Abdelwahab, N.S., Farida, N.F.; Validated HPLC-DAD method for stability study of sulbutiamine HCl; *RSC Advances*, (2014); 4: 30523–30529.
6. Athak, A.P., Rajput, S.J.; Development of a stability indicating HPLC method for simultaneous determination of olanzapine and fluoxetine in combined dosage forms; *Journal of Chromatographic Sciences*, (2009); 47: 605–611.
7. Darwish, I.A., Askal, H.F., Khedr, A.S., Mahmoud, R.M.; Stability indicating TLC method for quantitative determination of rebaverine; *Journal of Chromatographic Sciences*, (2008); 46: 5–11.
8. U.S. Department of Health and Human Services FDA, August (2000) Guidance for industry, analytical procedures and method validation, www.fda.gov/guidance/index.htm.
9. Ravi, T.K., Gandhimathi, M., Suganthi, A., Sarovar, S.; Forced-degradation study of valdecoxib as bulk drug and in tablet formulation by HPTLC; *Journal of Separation Sciences*, (2006); 29: 1647–1652.
10. Beebee Fauzee, A.F., Walker, R.B.; Forced degradation studies of clobetasol 17-propionate in methanol, propylene glycol, as bulk drug and cream formulations by RP-HPLC; *Journal of Separation Sciences*, (2013); 36: 849–856.
11. Fayed, A.S., Shehata, M.A., Hassan, N.Y., El-Weshahy, S.A.; Validated HPLC and HPTLC stability-indicating methods for determination of alfuzosin hydrochloride in bulk powder and pharmaceutical formulations; *Journal of Separation Sciences*, (2006); 29: 2716–2724.
12. Bakshi, M., Singh, S.; Development of validated stability-indicating assay methods—critical review; *Journal of Pharmaceutical and Biomedical Analysis*, (2002); 28: 1011–1040.
13. Ramisetti, N.R., Kuntamukkala, R., Lakshetti, S., Sripadi, P.; Identification and characterization of stress degradants of lacosamide by LC-MS and ESI-Q-TOF-MS/MS: development and validation of stability indicating RP-HPLC method; *Journal of Pharmaceutical and Biomedical Analysis*, (2014); 95: 256–264.
14. Raju, B., Ramish, M., Srimivas, R., Raju, S.S., Venkatswarlu, Y.; Identification and characterization of stressed degradation products of prolifloxacin using LC-ESI-MS/Q-TOF, MS^n experiments: development of a validated specific stability indicating LC–MS method; *Journal of Pharmaceutical and Biomedical Analysis*, (2011); 56: 560–568.
15. The United States Pharmacopeia, National Formulary 35, 30th ed. United States Pharmacopeia Convention Inc., (2012).
16. Through website http://shodhganga.inflibnet.ac.in/bitstream/10603/4560/11/11_chapter%201.pdf (accessed 13 March, 2014).
17. Center for Drug Evaluation and Research, U.S. Food and Drug Administration, *Reviewer Guidance, Validation of Chromatographic Methods*, FDA, Rockville, MD, November, (1994).
18. Through website http://www.chromatographyonline.com/lcgc/Column%3A+Validation+Viewpoint/Developing-and-Validating-Stability-Indicating-Met/ArticleStandard/Article/detail/165201 (accessed February 21, 2014).
19. Chavhan, M.L., Shirkhedkar, A., Surana, S.J.; Development and validation of a stability indicating RP-TLC densitometric method for determination of loratadine in bulk and in tablets; *Arabian Journal of Chemistry*, (2013). http://dx.doi.org/10.1016/j.arabjc.2012.12.014.
20. ICH Q1A (R2) Stability testing of new drug substances and products. Proceedings of the International Conference on Harmonization, 2003. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2__Guideline.pdf (accessed February 22, 2014).