Validated spectrofluorimetric method for determination of sulpiride in commercial formulations using Hantzsch condensation reaction

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Abstract: A simple, sensitive, selective and cost effective spectrofluorimetric method has been established for the quantification of sulpiride after their complete alkaline hydrolysis. The method is based on the condensation of the primary amino group of alkaline hydrolytic product of sulpiride with acetyl acetone and formaldehyde in acidic medium (0.25 M HCl) to form a fluorescent product. The reaction product formed shows maximum fluorescence intensity at 483 nm after excitation at 431 nm. The different reaction conditions influencing the condensation reaction were carefully optimized and a linear range of 0.1-3.5 μg mL^{-1} with good correlation coefficient between florescent intensity and concentration of sulpiride was found at optimum parameters. The LOD and LOQ were found to be 11 and 39 ng mL^{-1} respectively. The proposed method was successfully used for the quantification of sulpiride in bulk powder and commercial formulations. The effect of common pharmaceutical excipients and co-administered drug was also studied and no interferences were observed. The validity of the method was tested by analyzing sulpiride in bulk powder, and pharmaceutical formulations through recovery studies. Recoveries (%) were obtained from 98.62 to 100.24% for bulk powder, and 97.09 to 100.57 % for commercial formulations. The results were validated statistically with those obtained by reference literature high performance liquid chromatographic method.

Keywords: Sulpiride; acetyl acetone; formaldehyde; spectrofluorimetric.

INTRODUCTION

Sulpiride, 5-(aminosulfonyl)-N- [(1-ethyl-2-pyrrolidinyl) ethyl] -2-methoxy-benzamide is a substituted benzamide antipsychotic reported to be a selective antagonist of central dopamine (D_2, D_3, and D_4) receptors. Sulpiride has antidepressant activities, and found for the treatment of acute and chronic schizophrenia as well as other similar mental disorders, such as depression and hallucination with a low frequency of extrapyramidal side effects (Tokunaga *et al*., 1997; Liu *et al*., 2002; Li *et al*., 2006).

Sulpiride is absorbed poorly and slowly from the gastrointestinal tract and maximum level in serum found in 2-6 h (Wiesel *et al*., 1980). Its oral bioavailability is only 25-35% with marked inter individual differences. The usual half-life is 6-8 hours. Sulpiride does not completely metabolized and excreted unchanged in the urine with about 70-90 % of an intravenous dose and 15-25% of an oral dose (Gorazza and Tonini, 2000; Bressolle *et al*., 1984). It is usually given in two or three divided doses. The suggested oral dose of sulpiride in the handling of schizophrenia is 200-300 mg three times a day with a regular increase to a maximum of 1200 mg daily (Tang, 1997).

In the literature, various analytical methods have been reported for the determination of sulpiride. These methods include gas chromatography (GC) with mass spectrometric (Frigerio and Pantarotto, 1977; Rop *et al*., 1999) or oscillopolarographic detection (Zeng and Song, 1997), HPLC with UV (Bressolle and Bres, 1985; El-Walily *et al*., 1999; Chiba *et al*., 2003), fluorescence (Tokunaga *et al*., 1997; Nicolas *et al*., 1986; Huang *et al*., 2001; Cho and Lee, 2003) or MS detection (Peak *et al*., 2004), capillary electrophoresis with electrochemi-luminescence (Liu *et al*., 2002), or ultraviolet (Xu and Stewart, 2000) and spectrophotometric (El-Walily *et al*., 1999; Attia *et al*., 2003; Radwan 2003; Zayed 2005; Shah *et al*., 2008) methods.

Limited numbers of methods based on fluorescence measurement have been reported in the literature for determination of sulpiride in commercial dosages and biological samples. The reported methods require lengthy processes, extensive heating time, have narrow range of calibration, use of expensive reagents and suffer from interference effect (Abdelal *et al*., 2009; Walash *et al*., 2010; Nie *et al.,* 2007).

The aim of the current work is to develop a simple, accurate, sensitive, low-cost and proficient spectrofluorimetric method for the quantification of sulpiride in pharmaceutical preparations. The proposed method is based on the reaction of acetyl acetone and formaldehyde as a fluorogenic reagent which undergoes Hantzsch condensation reaction with the primary amine of sulpiride.

MATERIALS AND METHODS

Instruments

Excitation and emission measurements of fluorescent compounds were carried out on an RF-5301 PC

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Spectrofluorophotometer (Shimadzu Japan) using 1 cm quartz cell. A thermostatic water bath (YuJia China) was used for heating purposes.

Material and chemicals

All chemicals used were of analytical reagent or of high grade purity. Acetyl acetone (ACROS-01 Geel, Belgium), formaldehyde 37% and hydrochloric acid (Merck Darmstadt, Germany) were used. Standard sulpiride powder was supplied by Hang Zhou Pharma and Chem. Co China. Commercial formulations of sulpiride (sulpiride-50, 50 mg caps manufactured by Combitic Global D-2 industrial area Sonepat-131001 (Hr) India, Sulpiride-50, 50 mg caps manufactured by Xier kangtai Pharmaceutical Co. Ltd North zone, High-New Technology Industrial Zone, Pingxiang, Jiangxi, P.R.China. Sulpiride Capsules 50 mg manufactured by ARBRO Pharmaceuticals Ltd., 6/14 Kirti Nagar, Industrial Area, New Delhi (India), Sulpiride-50, 50 mg capsule manufactured by Unicure (India) Pvt. Ltd were purchased from local market.

Preparation of standard solutions (100 µg mL^{-1})

Standard solution of sulpiride (100 µg mL^{-1}) was prepared by dissolved 0.005 gram of authentic standard in 5 mL of 2 M NaOH solution in a 50 mL conical flask and heated on a boiling water bath for 30 minutes. After cooling it was transferred to 50 mL volumetric flask and diluted to the mark with distilled water. Working standard solutions were prepared freshly by proper dilution with distilled water.

Preparation of reagents solutions

Acetyl acetone solution

A standard solution of acetyl acetone (1.4 M) was prepared by adding 7.19 mL of acetyl acetone to distilled water and diluted to 50 mL.

Formaldehyde solution

Formaldehyde solution (15%) was prepared by mixing 20.2 mL of 37 % formaldehyde to distilled water in 50 mL volumetric flask and diluted up to the mark.

Hydrochloric acid solution

Hydrochloric acid (0.25 M) solution was prepared by diluting 2.2 mL of (11.65 M) HCl to 100 mL with distilled water.

General recommended procedure

Proper volumes of sulpiride stock solution, equal to concentration of 0.1-3.5 µg mL^{-1} were added to a set of test tubes followed by the addition of 1 mL formaldehyde (15%) solution, 1 mL of hydrochloric acid (0.25 M) and 2.5 mL of acetyl acetone (1.4 M) solution in a sequence. Each test tube was moderately stirred and heated for 30 min in a boiling water bath. The contents were cooled under tape water and finally transferred to volumetric flasks and diluted to 25 mL with distilled water. The resultant fluorescence product intensity was measured at 483 nm after excitation at 431 nm (fig. 1). A blank solution was prepared simultaneously in the same way except addition of the sulpiride.

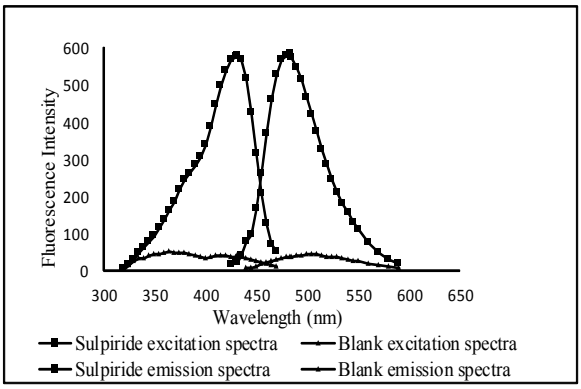


Fig. 1: Excitation and emission spectrum of sulpiride after condensation reaction.
1mL (100 µg mL^{-1}) sulpiride, 1 mL of 25% formaldehyde, 1 mL of 0.25 M HCl, 2.5 mL of 1.4 M acetyl acetone, heated at 100^{o}C for 30 min, diluted to 25 mL

Procedure for commercial formulations

Filling of four capsules comprising 50 mg of active constituents were weighed and average mass in one capsule was calculated. An accurately weighed amount of the sample equivalent to 0.005 g of sulpiride was dissolved in 5 mL of 2 M NaOH, heated on boiling water bath for 30 min and after cooling filtered, and diluted to 50 mL with distilled water. Sample solution of 10 µg mL^{-1} was prepared from 100 µg mL^{-1} sample solution by diluting proper volume with distilled water.

RESULTS

Optimization of reaction conditions

Different experimental factors affecting the fluorophore formation were carefully studied by changing one factor individually and keeping the others constant. These factors include formaldehyde solution concentration and volume, acetyl acetone solution concentration and volume, hydrochloric acid concentration and volume, heating temperature, heating time and effect of different solvents used for dilution.

Effect of concentration and volume of formaldehyde

The effect of concentration and volume of formaldehyde effecting the Hantzsch condensation reaction were thoroughly studied. The effect of formaldehyde concentration was studied from 5-25%. It was noted that fluorescence intensity of the condensation product increased with increase in concentration upto 15% further increase in concentration results in the formation of water insoluble product (fig. 2). The effect of volume of formaldehyde was also studied by adding different mL keeping the concentration of formaldehyde and others

reagents constant. It was observed that by using 1 mL of 15% formaldehyde maximum product formation occurred.

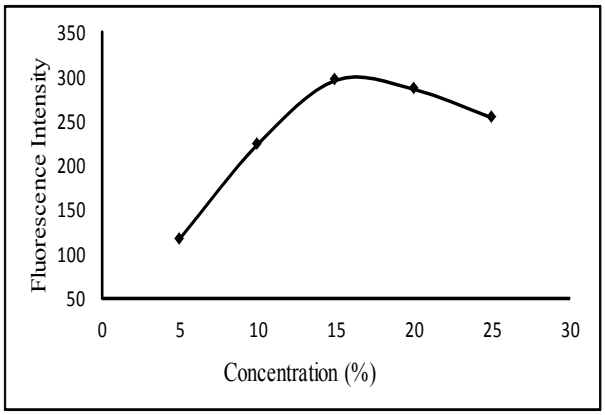


Fig. 2: Effect of concentration of formaldehyde on the condensation reaction.
1 mL (100 µg mL^{-1}) sulpiride, 1 mL of 5-25% formaldehyde, 1 mL of 0.25 M HCl, 1 mL of 1 M acetyl acetone, heated at 100 ^{0}C for 20 min, diluted to 25 mL

Effect of concentration and volume of acetyl acetone
The effect of concentration of acetyl acetone was also studied from 0.6-1.8M. It was observed that maximum fluorophore formation was occurred with 1.4 M acetyl acetone (fig. 3). The effect of volume of 1.4 M acetyl acetone was also studied and it was found that 2.5 mL was found to be optimum for maximum product formation.

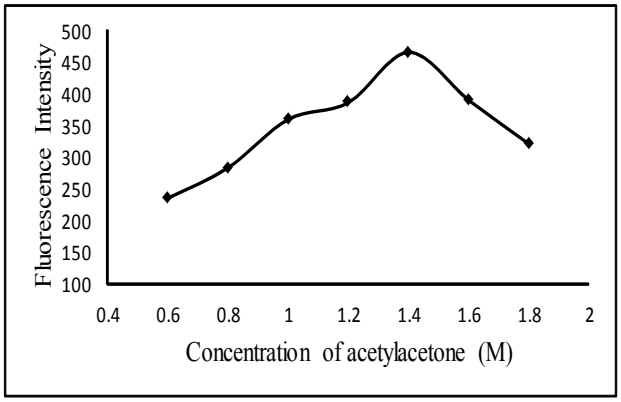


Fig. 3: Effect of concentration of acetyl acetone on the condensation reaction.
1 mL (100 µg mL^{-1}) sulpiride, 1 mL of 15% formaldehyde, 1 mL of 0.25 M HCl, 1 mL of 0.6-1.8 M acetyl acetone, heated at 100^{o}C for 20 min, diluted to 25 mL.

Effect of concentration and volume of hydrochloric acid
The condensation reaction was found to acidity dependent; therefore the concentration of hydrochloric acid was studied from 0.05 M to 0.5 M. The fluorescence intensity increases with HCl concentration upto 0.25 M (fig. 4). The effect of HCl volume was also examined and maximum fluorescent product formation produced with 1 mL of 0.25 M hydrochloric acid.

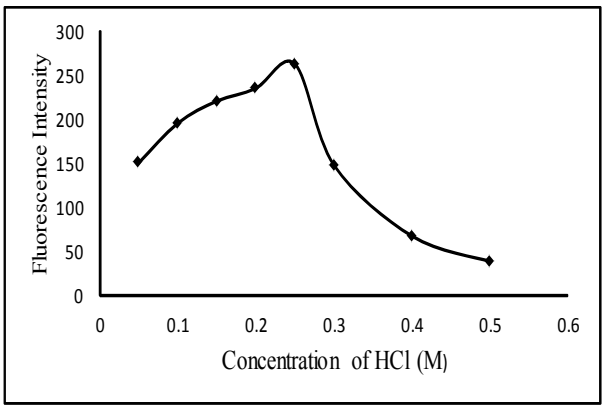


Fig. 4: Effect of HCl concentration on the condensation reaction
1 mL (100 µg mL^{-1}) sulpiride, 1 mL of 15% formaldehyde, 1 mL of 0.05-0.5 M HCl, 2.5 mL of 1.4 M acetyl acetone, heated at 100^{o}C for 20 min, diluted to 25 mL.

Effect of heating temperature and heating time
The effect of temperature in the range of 60-100^{o}C and heating time in the range of 10-40 min on the condensation reaction of acetyl acetone-formaldehyde and sulpiride was studied. It was observed that maximum fluorescent product was formed when the reagents were heated for 30 minutes at 100^{o}C (figs. 5 and 6).

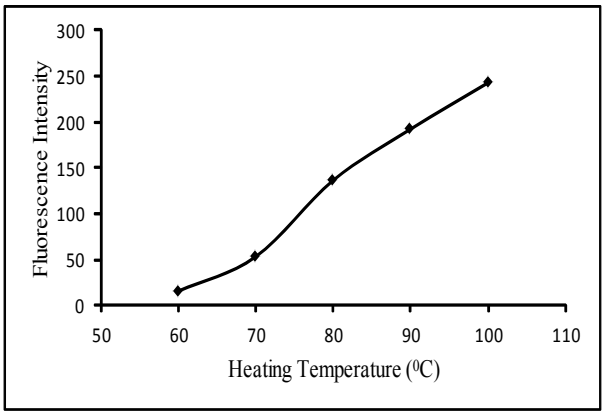


Fig. 5: Effect of heating temperature on the condensation reaction
1 mL (100 µg mL^{-1}) sulpiride, 1 mL of 15% formaldehyde, 1 mL of 0.25 M HCl, 2.5 mL of 1.4 M acetyl acetone, heated at 60-100^{o}C for 20 min, diluted to 25 mL

Stability of the reaction product
Stability of the fluorophore was studied by evaluating the fluorescence intensity after 10 minutes interval up to 90 minutes. It was investigated that no change in fluorescence intensity of the reaction product was

observed. This indicates that the fluorescent reaction product is stable and will not affect the result of analysis even if the analysis is performed up to 90 minutes of the dilution.

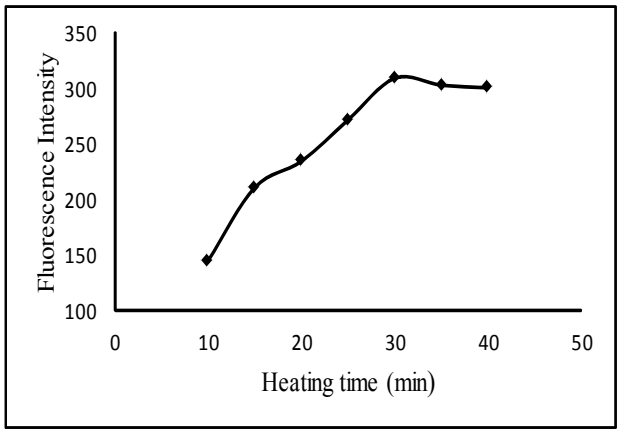


Fig. 6: Effect of heating time on the condensation reaction. 1 mL (100 µg mL^{-1}) sulpiride, 1 mL of 15% formaldehyde, 1 mL of 0.25 M HCl, 2.5 mL of 1.4 M acetyl acetone, heated at 100°C for 10-40 min, diluted to 25 mL

Effect of diluting solvents

Different solvents were used for dilution to select the most appropriate one for maximum fluorophore formation. The studied solvents include; distilled water, methanol, ethanol and acetonitrile. Maximum fluorescence intensities were obtained by using distilled water for dilution.

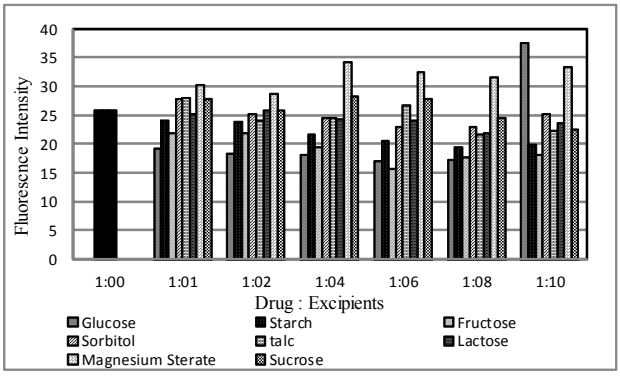


Fig. 7: Effect of excipients on the fluorescence intensity of the fluorescent condensation product.

Effect of Interferences

The effect of interferences from the commonly used excipients such as glucose, lactose, starch, talc, sorbitol, magnesium stearate, sucrose and fructose in the assay of sulpiride were carefully studied (fig. 7). Under the optimized experimental condition, to a known amount of drug (sulpiride 0.2 µgmL^{-1}), excipient in different concentration in the ratio of 1:1, 1:2, and 1:4 were added and investigated by the developed method. No interferences effect was noted in the determination of sulpiride from these common excipients and percent recoveries were in the range of 95.68% to 101.05% (table 1). The effects of common co-administered drugs like mefenamic acid, paracetamol, diclofenac sodium and metronidazole, were also studied. It was found that there is no interference of these drugs in the determination of sulpiride by the proposed method (fig. 8).

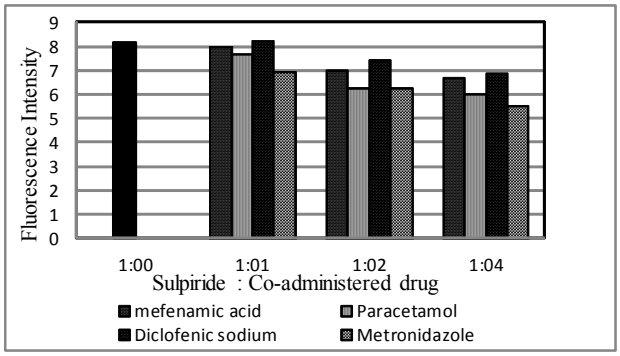


Fig. 8: Interference effect of co-administered drugs on the determination of sulpiride by the proposed method.

Table 1: Percent recoveries of Sulpiride (0.2 µg mL^{-1}) in the presence of excipients

Excipients	Excipients added (mg/L)	Drug: Excipient	% Recovery ± RSD
Glucose	0.2	1:01	95.70 ±2.07
	0.4	1:02	95.68 ± 7.94
	0.8	1:04	101.05± 3.33
Sorbitol	0.2	1:01	98.68 ± 7.45
	0.4	1:02	97.38 ± 5.85
	0.8	1:04	96.62 ± 3.02
Fructose	0.2	1:01	98.59 ± 5.03
	0.4	1:02	96.23 ± 0.72
	0.8	1:04	99.86 ±2.21
Talc	0.2	1:01	96.20 ± 1.13
	0.4	1:02	99.68 ± 5.14
	0.8	1:04	96.40 ± 2.76
Starch	0.2	1:01	98.13 ± 4.18
	0.4	1:02	97.88 ± 2.51
	0.8	1:04	98.96 ± 2.55
Lactose	0.2	1:01	99.33 ± 4.99
	0.4	1:02	99.22 ± 3.44
	0.8	1:04	95.94 ± 2.37
Magnesium stearate	0.2	1:01	98.31 ± 2.96
	0.4	1:02	100.93±3.87
	0.8	1:04	96.52 ± 3.18
Sucrose	0.2	1:01	98.96 ± 4.84
	0.4	1:02	100.75± 2.77
	0.8	1:04	97.42 ± 1.14

Each result is the average of separate triplicate analysis

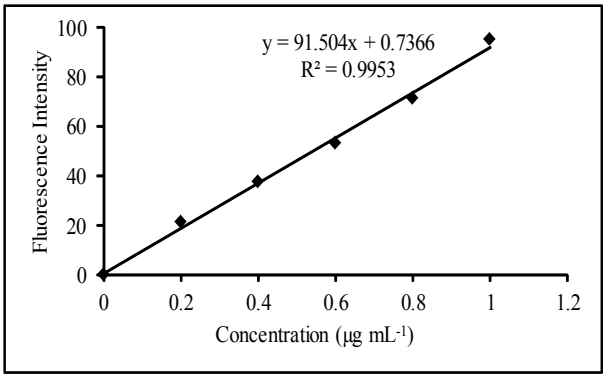


Fig. 9: Linear range of the fluorescence product of sulpiride with acetyl acetone-formaldehyde sulpiride (0.2-1.0 µg mL^{-1}), 1 mL of 15% formaldehyde, 1 mL of 0.25 M HCl, 2.5 mL of 1.4 M acetyl acetone, heated at 100°C for 30 min, diluted to 25 mL

DISCUSSION

Hantzsch reaction is known as condensation reaction that was reported in the literatures as a useful pathway for payroll and pyridine synthesis (Bratlon and Olis, 1979). In the same way, acetyl acetone together with formaldehyde reacts with aliphatic amines by Hantzsch condensation reaction forming a fluorescent product. Sulpiride do not contain primary amino group, while upon hydrolysis with alkali the cleavage of weak amide group occurs with production of primary amine. This primary amine of sulpiride used for determination through Hantzsch condensation reaction using acetyl acetone as β-diketone and formaldehyde as an aldehyde to form a fluorescent condensation product. In the first step formaldehyde reacts with acetyl acetone and form 3, 5-diacetylheptane-2, 6-dione. In the second step the 3, 5-diacetylheptane-2, 6-dione undergoes condensation with amino group of the sulpiride and produces a fluorescent product (Scheme 1) which exhibited maximum fluorescent intensity at λeme 483 nm after excitation at λexc 431 nm.

Analytical figures of merit

At optimum experimental conditions, a linear relationship between concentration of sulpiride and fluorescence intensity in the range of 0.1-3.5 µg mL^{-1} was observed. A minimum of three replicates were used for each concentration of the standard prepared to construct the calibration curve. The regression equation for the calibration curve was $\Delta F = 91.504C + 0.7366$ with a good correlation coefficient of 0.9953 (fig. 9). The detection limit (LOD) was calculated with concentration of sulpiride corresponding to fluorescence intensity which is equal to blank mean (Y_B) plus three times the standard deviation of the blank (S_B). Similarly the quantification limit (LOQ) was calculated with concentration of sulpiride corresponding to fluorescence intensity which is equal to blank mean (Y_B) plus ten times the standard deviation of the blank (S_B). The detection limit was found to be 1.1×10^{-2} µg mL^{-1} and quantification limit of 3.9×10^{-2} µg mL^{-1}. The optical characteristic such as the linear regression equation, slope, intercept, correlation coefficient, and relative standard deviation are given in table 2.

Table 2: Analytical parameter for spectrofluorimetric determination of sulpiride

Parameter	Value
λ_{ex}(nm)	431
λ_{em} (nm)	483
Linear range (µgmL^{-1})	0.1-3.5
Limit of detection 3S (µg mL^{-1})	1.1×10^{-2}
Limit of quantification 10S (µg mL^{-1})	3.9×10^{-2}
Regression equation (y)	Y= 91.504X + 0.7366
Slope (b)	91.504
Intercept (a)	0.7366
Correlation coefficient (r)	0.9953
Standard deviation (µg mL^{-1})	3.9×10^{-3}
Relative standard deviation (%)	3.81 %

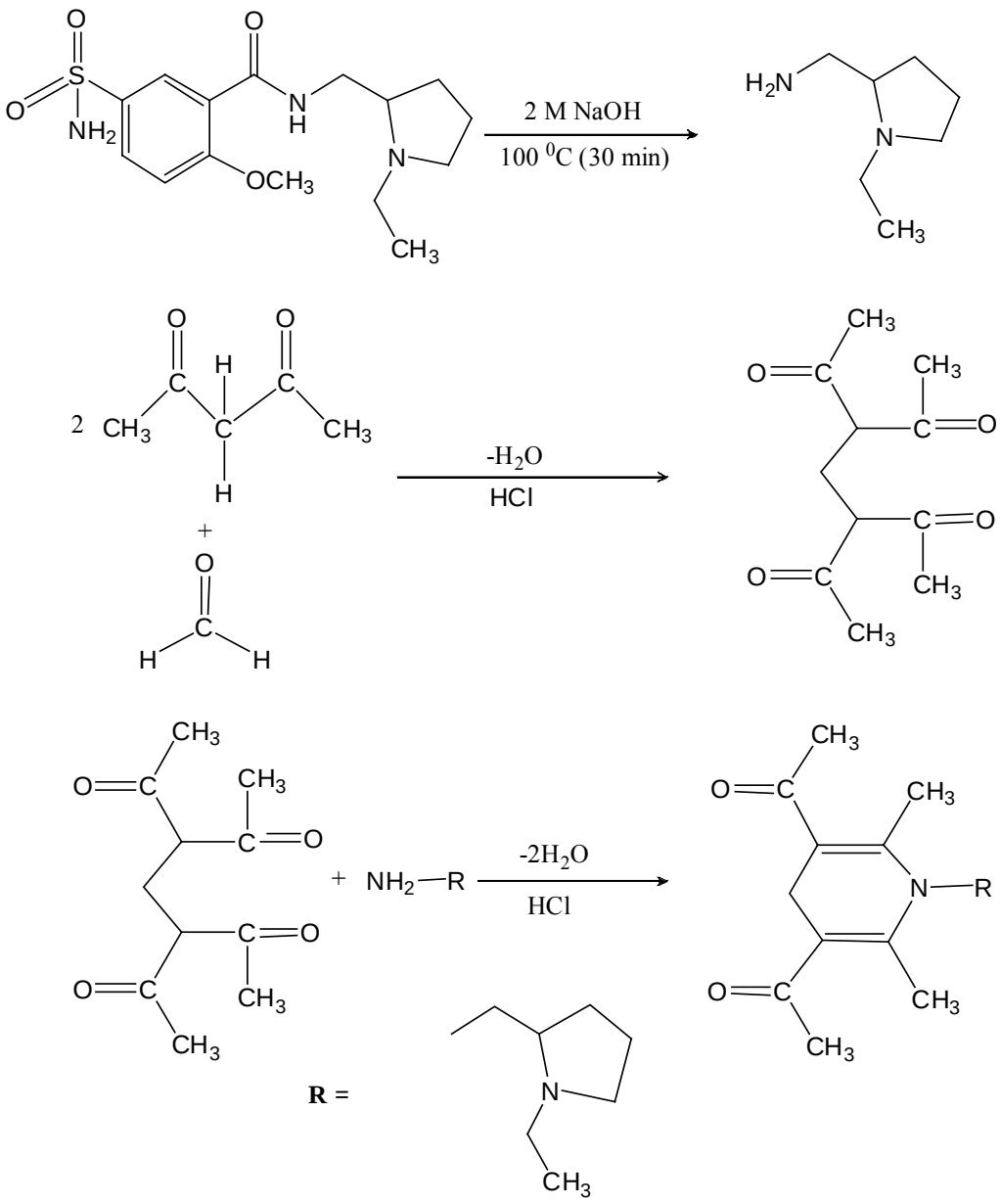
Accuracy, precision and recovery studies

The accuracy and precision of the developed method was evaluated by determining the sulpiride in pure form and commercial formulations using (0.2, 0.4 and 0.6 µg mL^{-1}) in triplicate. The results for pure form are given in table 3 and for dosage form in table 4. The standard analytical error, relative standard deviation (RSD) and recoveries obtained for the proposed method was found to be acceptable. The percent recoveries for pure form range from 98.88 % to 100.24 % and 95.01% to 101.36 % for commercial formulations with good RSD values, showing high reproducibility of the proposed method for sulpiride determination. The accuracy of the proposed method was also checked using standard addition method. For this purpose, a known amount of pure sulpiride was added to four different kinds of pre-analyzed dosage forms and then determined by the recommended procedure. The percent recoveries were found to be 97.09% to 100.55% (table 5). This indicates that the developed method has high accuracy and suitable for the determination of sulpiride in commercial formulations.

Table 3: Accuracy and precision of the proposed method for sulpiride determination in pure form

µg taken	µg found	% Recovery±RSD	Error (%)
0.2	0.200	100.24 ± 0.46 %	0
0.4	0.394	98.62 ± 1.75 %	0.6
0.6	0.593	98.88±1.67%	0.7
X'		99.25%	
±SD		0.870	
t-test		2.03 (4.303)	

Each result is the average of separate triplicate analyses



Scheme 1: Proposed reaction mechanism for condensation of sulpiride with acetyl acetone and formaldehyde

Application

The developed proposed method was effectively used for the analysis of sulpiride in four different products of capsules and the results were statistically evaluated with a reference literature high performance liquid chromatographic method (Tokunaga *et al*., 1997, Bressolle and Bres, 1985). The student's t-test was used to check the accuracy and variance ration F-test was used for precision determination. The evaluated values of t- and F- at 95% confidence level were found less than the tabulated values as revealed by the result summarized in table 6.

CONCLUSION

The investigated spectrofluorimetric method is simple, precise, accurate, linear, and selective and offer advantages of reagent availability, stability and minimum time consumption with high sensitivity. It does not require any pretreatment and tedious extraction procedure and has wide linear range with good accuracy and precision. Thus it can be extended for routine analysis of sulpiride in pharmaceutical industries and research laboratories. Compared with the LC/MS technique and HPLC procedure, the developed spectrofluorimetric method is simple, low cost, and easy to operate.

Table 4: Accuracy and precision of the proposed method for sulpiride determination in dosage form

Pharmaceutical preparation	Amount taken ($\mu g\ mL^{-1}$)	Amount found ($\mu g\ mL^{-1}$)	%Recovery ± RSD
1, 50 mg capsule	0.2	0.202	101.36 ± 5.12
	0.4	0.380	95.01 ± 1.93
	0.6	0.607	101.2 ±1.16
2, 50 mg capsule	0.2	0.200	100.00 ± 2.10
	0.4	0.397	99.44 ± 2.01
	0.6	0.608	101.46 ± 0.63
3, 50 mg capsule	0.2	0.200	100.29 ±1.34
	0.4	0.396	99.14 ± 2.48
	0.6	0.589	98.32 ± 1.80
4, 50 mg capsule	0.2	0.199	99.75 ± 3.85
	0.4	0.397	99.45 ±1.84
	0.6	0.603	100.57 ± 0.59

Each result is the average of separate triplicate analysis

Table 5: Evaluation of recovery test of sulpiride in commercial formulation (capsules) by the proposed method (Standard addition method)

Pharmaceutical preparation	Amount taken ($\mu g\ mL^{-1}$)	Amount found ($\mu g\ mL^{-1}$)	%Recovery ± RSD
1, 50 mg capsule	0.204	0.200	98.03 ± 6.22
	0.401	0.395	98.47 ± 2.84
	0.608	0.594	97.58 ±2.31
2, 50 mg capsule	0.200	0.199	99.35 ± 4.22
	0.403	0.396	98.20 ± 4.55
	0.620	0.616	99.41 ± 3.30
3, 50 mg capsule	0.200	0.201	100.29 ± 3.07
	0.403	0.405	100.55 ± 2.76
	0.620	0.602	97.09 ± 0.56
4, 50 mg capsule	0.200	0.199	99.54 ± 2.81
	0.403	0.400	99.28 ± 4.44
	0.620	0.606	97.80 ± 2.28

Table 6: Determination of sulpiride in commercial formulation and statistical comparison with reference method

S. No.	Name of commercial formulation	Labeled amount	Amount determined	
			Proposed method	Reference method (1)
1	1, 50 mg capsule	50 mg/tab.	49.59 mg/tab.	46.68 mg/cap
				F-test=0.106 (19)
				t-test=0.38 (4.303)
2	2,50 mg capsule	50 mg/tab.	50.15 mg/tab.	49.11 mg/cap
				F-test=1.43 (19)
				t-test=1.23 (4.303)
3	3, 50 mg capsule	50 mg/tab.	49.62 mg/tab.	46.74 mg/cap
				F-test=0.003 (19)
				t-test=1.313 (4.303)
4	4, 50 mg capsule	50 mg/tab.	49.96 mg/tab.	48.39 mg/cap
				F-test=0.089 (19)
				t-test=0.23 (4.303)

Each result is the average of separate triplicate analysis

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