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Development of stability-indicating methods for determination of Sulpiride in presence of its degradation products in bulk and dosage forms

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ABSTRACT

Simple, accurate, sensitive, and precise UV spectrophotometric and chemometric methods were developed for determination of Sulpiride (SLP) in presence of its degradation products. Two spectrophotometric methods were developed, namely, double divisor ratio spectra (DDRD) and ratio subtraction (RS); the linearity range was 20-200 ug.ml^{-1} for both spectrophotometric methods, with mean percentage recoveries of 99.51±0.24 and 99.73±0.32 for the double divisor ratio spectra and ratio subtraction method respectively. The developed chemometric-assisted spectrophotometric methods were the principle component regression (PCR) and partial least squares (PLS) methods. The linearity range was also 20-100 ug.ml^{-1} for both methods while the mean percentage recoveries were found to be 99.63 ±0.22 and 99.60 ±0.26 for the PCR and PLS methods respectively. The developed methods were successfully applied for determination of SLP in bulk powder, laboratory-prepared mixtures and in dosage form. The results obtained were compared to the reported spectrophotometric method; there was no significant difference between the proposed methods and the reported method.

KEYWORDS

Sulpiride;
Double divisor ratio spectra;
Ratio subtraction;
Chemometrics;
Dosage form;
Stability study.

INTRODUCTION

Sulpiride (SLP), chemically [(RS)-N-[(1-Ethylpyrrolidin-2-yl) methyl]-2-methoxy-5-sulphamoylbenzamide] (Figure 1) is a substituted benzamide used mainly in the treatment of psychosis (e.g. schizophrenia) and depression. SLP is a selective antagonist at dopamineD_2 and D_3 receptors[1]. Also It was found to activate the endogenous gamma-hydroxybutyrate receptor in vivo at therapeutic concentrations[2].

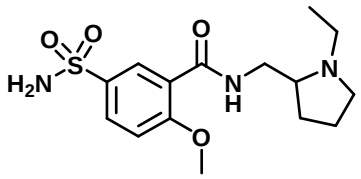

Figure 1 : Chemical structure of Sulpiride

Many analytical methods reported for the estimation of SLP in pharmaceutical preparations and in biological fluids. These methods include titrimetry[3] high performance liquid chromatography[4-26], thin layer chromatography[27-29], capillary electrophoresis[30-32],

spectrophotometry[33-42] and electrochemical methods[43-45].

Investigation on the chemical stability is an essential matter to the quality control of pharmaceuticals. An ideal stability indicating method is one that quantifies the standard drug alone and also resolves its degradation products.

A second derivative synchronous fluorescence spectroscopic (SDSFS) technique was developed for the simultaneous determination of SLP and its alkaline degradates[40].

The aim of the present work is to develop simple, sensitive and selective stability- indicating methods for the quantitative determination of SLP in presence of its acidic and alkaline degradates. This was achieved by developing different techniques including double divisor ratio spectra, ratio subtraction, PCR and PLS mathematical methods.

EXPERIMENTAL

Instrumentation

A double beam UV-VIS spectrophotometer (UV Probe -1800 version 2.32 Shimadzu, Kyoto, Japan) with matched 1-cm quartz cells, connected to IBM compatible personal computer (PC). Bundled, UV-PC personal spectroscopy software version 3.7 was used to process the absorption and the derivative spectra. The chemometric calculations were performed in Matlab for Windows™ version 7 Mathworks Inc.2004. The PLS procedures was taken from PLS Toolbox 2.1, Eigenvector Research Inc.2001 created by B.M. Wise, N.B. Gallagher for use with Matlab.

Materials

(A) Pure samples

Sulpiride was kindly supplied by national organization for drug control and research (NODCAR). Its purity was found to be 99.79%±0.19 (n=5) according to the reported method[3].

(B) Pharmaceutical dosage form

Dogmatil® capsules, each containing 50 mg SLP Batch No. 2EG004 manufactured by Memphis Chemical Co. For Pharmaceutical and Chemical Industries, Cairo, Egypt, obtained from local market.

(C) Reagents

Sodium hydroxide and hydrochloric acid, 1 and 5 mol L^{-1} (BDH, UK), aqueous solutions. Methanol (Merck, Darmstadt, Germany).

All chemicals and reagents used are of analytical grade.

Preparation of standard solutions for the drug

Stock solution of SLP was prepared by dissolving 100.0 mg in 100 ml of methanol to obtain to obtain a stock solution of (1mg.ml^{-1}). This solution was further diluted with the same solvent as appropriate to obtain the working standard solutions.

Preparation of degradation products

(A) Preparation of acid-induced degradation products

Methanolic Sulpiride solution containing 50 mg was mixed with 25 ml of 5 mol L^{-1} HCl was refluxed for 5 hours. The solution was cooled, neutralized with 5 mol L^{-1} NaOH and transferred to 50 ml volumetric flask and diluted to the mark with methanol to obtain a stock solution of 1mg ml^{-1}. This solution was further diluted with the same solvent as appropriate to obtain the working standard solutions.

(B) Preparation of alkali-induced degradation products

Methanolic SLP solution containing 50 mg was mixed with 25 ml of 1 mol L^{-1} NaOH was refluxed for 4 hours. The solution was cooled, neutralized with 1 mol L^{-1} HCl and transferred to 50 ml volumetric flask and diluted to the mark with methanol to obtain a stock solution of 1mg.ml^{-1}. This solution was further diluted with the same solvent as appropriate to obtain the working standard solutions.

Complete degradation of the studied drug was confirmed by an HPLC method, using a Zobrax C_{18} column (5um, 150 mm x 4.6mm i.d). The mobile phase was a mixture of methanol: water: acetic acid (60:30:1, by volumes) and UV detection 290 nm, where no peaks corresponding to intact drug were detected in case of the degraded samples[4].

Structural elucidation of the drug and the obtained degradation product was achieved by IR spectrophotometry (Figure 2-4).

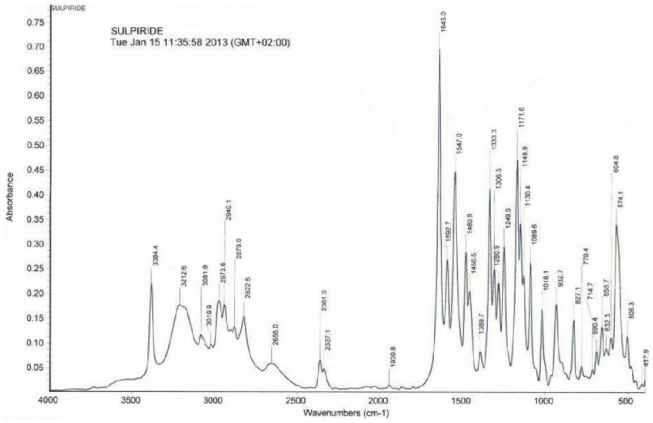

Figure 2 : IR of intact Sulpiride

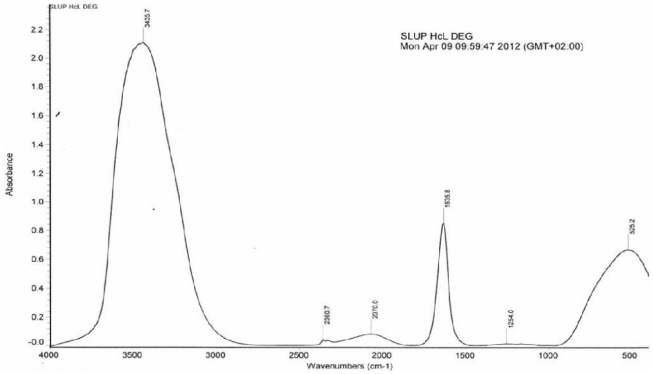

Figure 3 : IR of acidic degradation product

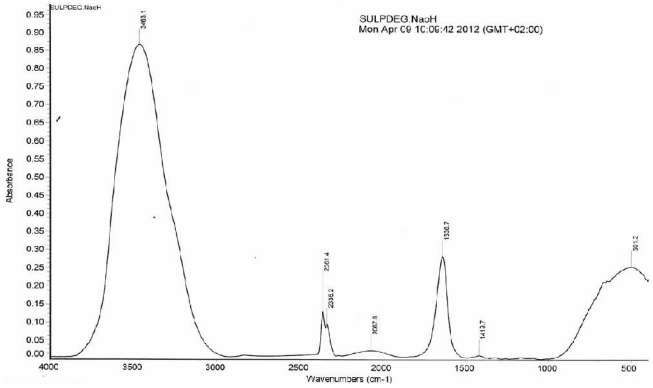

Figure 4 : IR of alkaline degradation product

PROCEDURES

Calibration curve for double divisor ratio spectra spectrophotometric method

Accurately measured volumes of SLP working solution were transferred into a series of 10- mL volumetric flasks and then diluted to the mark with methanol to provide concentration from 20-200 ug.ml^{-1}. The zero-order spectrum of each dilution was recorded against methanol as blank, the previous spectrum of each dilution was divided by the double divisor spectrum of (acidic degradates and alkaline degradates 20 ug.ml^{-1}). The first derivative of the obtained spectra was then computed, using $\Delta\lambda$=8 and scaling factor 10. The amplitude for the resulted spectra were recorded at 293.2 nm and plotted against the corresponding concentration. The regression equations were then computed.

Calibration curve for ratio subtraction spectrophotometric method

Into a series of 10mL volumetric flasks, aliquots equivalent to 0.2 -2 mg and 1mg SLP and its acidic degradation product, respectively were accurately transferred from their standard working solutions (SLP, 400 ug.ml^{-1}, and acidic degradation product 200 ugml^{-1}) and the volume was completed with methanol. The spectra of the prepared solutions from 220-370nm were scanned and stored in the computer. The spectra of the laboratory prepared mixtures were divided by the spectrum of 40 ug.ml^{-1} of acidic degradation product, then the absorbance in the plateau region at 315nm (the constant) was subtracted. The obtained curves were then multiplied by the spectrum of 40 ugml^{-1} of acidic degradation product. The peak amplitudes of the obtained curves were measured at 287nm and plotted against the corresponding drug concentrations. The regression equation was then computed.

Training and validation sets for the PCR and PLS method

Different 25 mixtures of SLP, its acidic and alkaline degradation products were prepared by transferring different volumes of their corresponding working solutions into 10 ml measuring flasks, completing the volume with methanol. Sixteen samples were used for calibration and the other nine samples were used as validation set. The concentration ranges and the composition of the calibration and validation samples are given in TABLE 1.

The absorbances of these solutions were scanned and exported to MATLAB® 7 for subsequent data manipulation. The suggested model was applied to predict the concentration of SLP in the validation samples.

Laboratory prepared mixtures

Solutions containing different ratios of SLP and its acidic and alkaline degradates were prepared to obtain

mixture solutions containing different ratios of the three components in case of the double divisor ratio spectra method. While adding calculated volumes of each of SLP and its acidic degradate working standard solutions, to obtain mixtures containing 10-60% of the acidic degradation product was achieved in case of ratio subtraction method.

TABLE 1 : The concentration of mixtures of Sulpiride, its alkaline and acidic degradate in the training set.

Sample No.	Sulpiride	Alkaline degradate ($ug.ml^{-1}$)	Acidic degradate ($ug.ml^{-1}$)
1	60	6	6
2	60	2	2
3	20	10	4
4	100	4	10
5	100	6	4
6	60	4	4
7	40	8	10
8	80	10	8
9	80	6	10
10	60	10	10
11	100	2	8
12	20	8	2
13	20	6	8
14	60	8	8
15	80	4	2
16	40	2	4

Application to pharmaceutical preparation

The contents of 10 capsules were emptied and mixed well. A portion equivalent to 50.0 mg of SLP was accurately weighed, sonicated in 20 ml methanol and filtered into 50-ml volumetric flask. The residue was washed with methanol three times each with 8ml methanol and completed to the volume with the same solvent. The general procedures under the construction of calibration curve were followed and the concentration of SLP was calculated.

RESULTS AND DISCUSSION

The International Conference on Harmonization (ICH) guideline entitled "stability testing of new drugs substances and products "requires the stress testing of new substances and products, also requires the stress testing to be carried out to elucidate the inherent stability, characteristics of the active substance[46]. An ideal stability indicating method is one that quantifies the standard drug alone and also resolves its degradation products.

The zero-order absorption spectra of SLP and its acidic and alkaline degradation products (Figure 5) showed sever overlapping which makes the direct determination of SLP in presence of its degradation products very difficult.

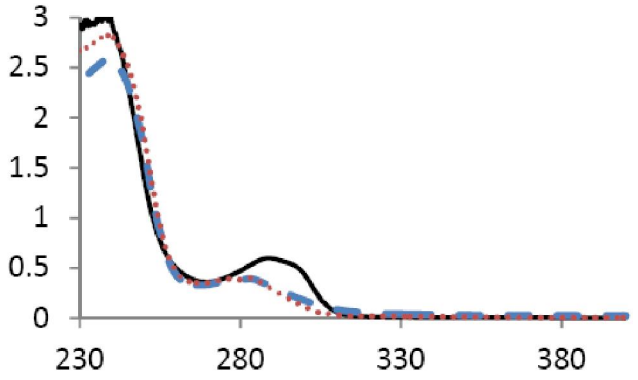

Figure 5 : Zero- order absorption spectra of Sulpiride (——), its acidic degradate (-----) and its alkaline degradate (.....), (80 $ugml^{-1}$ each) using methanol as a solvent.

DDRD spectrophotometric method

The technique of double divisor ratio spectra (DDRD) method was proposed as a sensitive, rapid and selective spectrophotometric method for the determination of SLP in presence of different ratios of both its acidic and alkaline degradation products, using a double divisor spectrum of (acidic and alkaline degradates $20ug.ml^{-1}$ each).

The main instrumental parameter conditions were optimized for reliable determination of the intact drug. Correct choice of the double divisor concentration plays an important role, regarding selectivity and sensitivity. Different double divisor spectra were tried; it was found that a spectrum of both acidic and alkaline degradates $20ugml^{-1}$ each was the best one which gives highest sensitivity and lowest peak noise. Furthermore to optimize DDRD method for determination of SLP in presence of its degradation products, different smoothing and scaling factors were also tested where a smoothing factor $\Delta\lambda$=8nm, and scaling factor=10 were suitable to enlarge the signals of SLP to facilitate its measurement and to diminish error in reading the signal.

Dividing the absorption spectra of SLP in range of 20-200 ug.ml^{-1} by the absorption spectrum of a mixture of both its acidic and alkaline degradate, 20ugml^{-1} each (as a double divisor; the first derivative of the obtained spectra (Figure 6) was computed and measured at a maximum of 293.2 nm. DDRD method showed good linearity and reproducibility at the selected wavelength without interference from its acidic and alkaline degradates.

Linearity of the peak amplitudes of the obtained spectra at 293.2nm was obtained in range 20-200ug.ml^{-1} (Figure 7), and the regression equation was computed (TABLE 3).

The proposed methods are valid for the determination of SLP in presence of both its acidic and alkaline degradates in different laboratory prepared mixtures containing different ratios of the three components as presented in TABLE 5A.

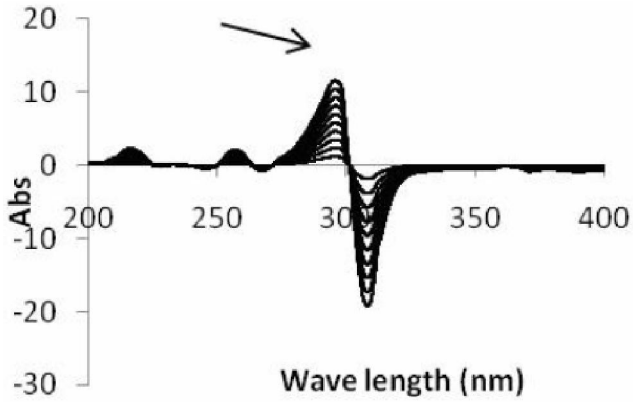

Figure 6 : First derivative of the double divisor ratio spectra for different Sulpiride concentrations (20-200 ug.ml^{-1}) using a double divisor of its acidic and alkaline degradates, 20 ug.ml^{-1}, each.

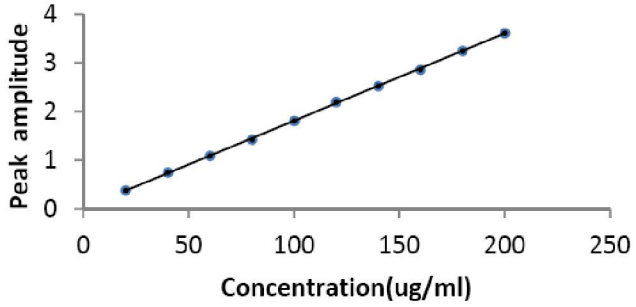

Figure 7 : Linearity of the peak amplitude of the first derivative of the double divisor ratio spectra at 293.2 nm to the corresponding Sulpiride concentration using (its acidic and alkaline degradation products, 20 ug.ml^{-1} each) as double devisor.

Ratio subtraction method

Ratio subtraction method is an innovating spectrophotometric method[73] used to solve the problem of overlapping spectra in binary mixtures; it was applied to the mixture of SLP (X) and its acidic degradation product (Y) this was achieved by dividing the spectrum of the mixture by a known concentration of 40 ug.ml^{-1} acidic degradation product (Y). The division will give a new curve (Figure 8) that represents

$$\frac{X}{Y'} + constant$$

If we subtract this constant (Figure 9), then multiply the new curve obtained after subtraction by Y' (the divisor), we can obtain the original curve of the drug (X) in the mixture (Figure 10).

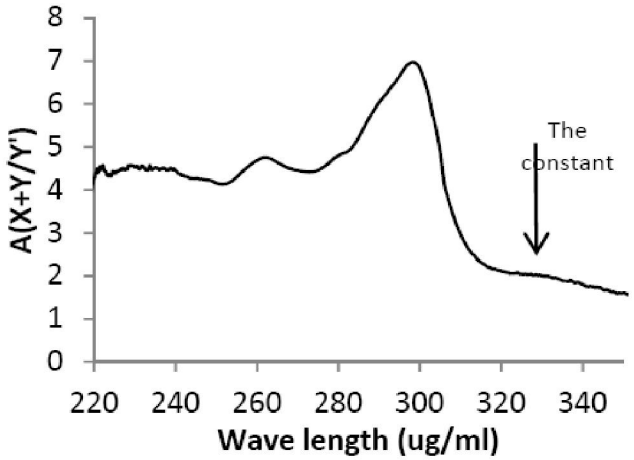

Figure 8 : Division spectra of laboratory-prepared mixture of 80 ug.ml^{-1} Sulpiride(X) and 100 ug.ml^{-1} of its acidic degradate(Y) using 40 ug.ml^{-1} acidic degradate(Y') as a divisor and methanol as a solvent.

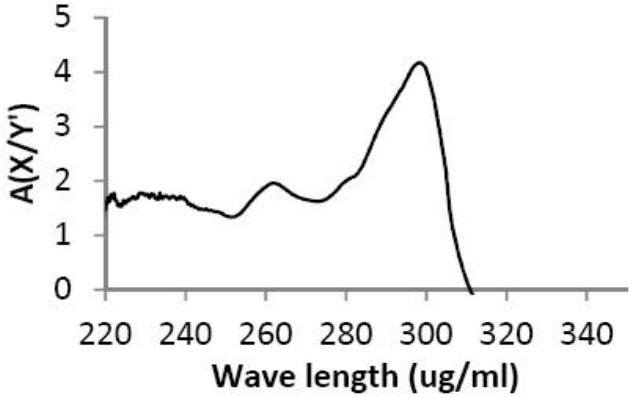

Figure 9 : Division spectra of laboratory-prepared mixture of 80 ug.ml^{-1} Sulpiride(X) and 100 ug.ml^{-1} of its acidic degradate(Y) using 40 ug.ml^{-1} acidic degradate(Y') as divisor and methanol as a solvent after subtraction of the constant.

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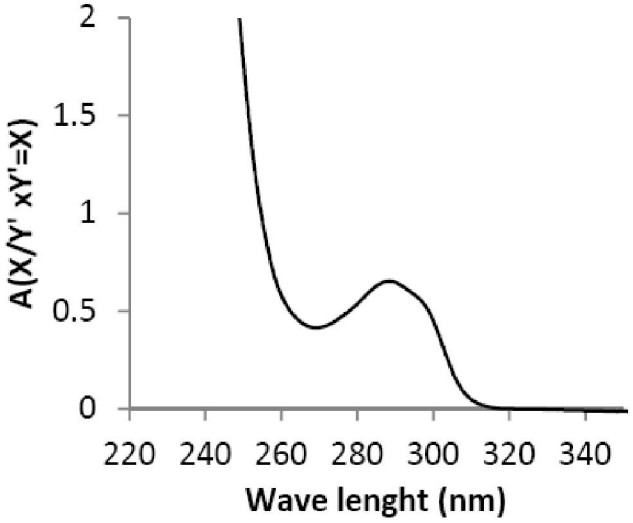

Figure 10 : The obtained absorption spectra of Sulpiride in laboratory-prepared mixture of 80 ug.ml^{-1} Sulpiride (X) and 100 ug.ml^{-1} of its acidic degradate using the proposed method.

Linearity of the peak amplitudes of the obtained spectra at 287 nm was obtained in range 20-200ugml^{-1} as shown in (Figure 11), and the regression equation was computed (TABLE 3).

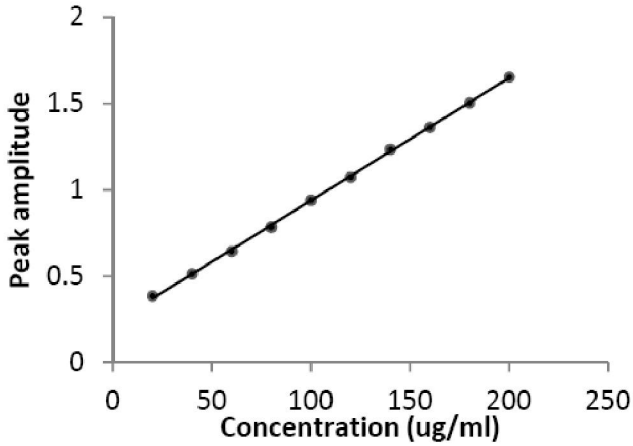

Figure 11 : Linearity of the peak amplitude of the absorption spectra at 287 nm to the corresponding Sulpiride concentration.

The proposed methods are valid for the determination of SLP in presence of both its acidic and alkaline degradates in different laboratory prepared mixtures containing different ratios of the three components as presented in TABLE 5B.

Chemometric method

A stability indicating method based on multivariate calibration models namely Partial least squares (PLS) and principle component regression (PCR) methods was investigated for the selective determination of SLP in the presence of both its acidic and alkaline degradation products and in pharmaceutical dosage forms. A calibration set was designed with sixteen calibration samples containing SLP and its degradation products. Another nine samples were used for validation set in the ranges and concentrations shown in TABLE 1. The UV spectra of the prepared solutions were recorded over the range 230–370 nm. Wavelengths (200–229 nm) dominated by noise and non informative spectral region after 370 nm are not included. Spectra were digitized each at 0.1 nm interval and the experimental data points were exposed to MATLAB- version 7.0 for calculations. The selection of the optimum number of factors for the PLS and PCR technique was a very important step before constructing the models because if the number of factors retained was more than the required, more noise will be added to the data. On the other hand, if the number retained was too small meaningful data that could be necessary for the calibration might be discarded. In this study the leave one out cross validation method was used[74,75].

TABLE 2 : The concentration of mixtures of Sulpiride, its acidic and alkaline degradate in the validation set.

Sample No.	Sulpiride	Alkaline Degradate (ugml^{-1})	Acidic degradate (ugml^{-1})
1	20	2	10
2	40	10	6
3	40	4	8
4	100	8	6
5	100	10	2
6	80	2	6
7	80	8	4
8	20	4	6
9	40	6	2

TABLE 2 shows different concentrations of SLP and both its acidic and alkaline degradates used in the validation set. To validate the prediction ability of the suggested models, they were used to predict the concentration of SLP in laboratory prepared mixtures containing different ratios of them. Satisfactory results were obtained as shown in TABLE 5C

The predicted concentrations of the intact drug in the validation samples were plotted against the known concentration values, (Figure 14, 15). This was used to

determine whether the model accounted for the concentration variation in the validation set. Plots were expected to fall on a straight line with a slope of one and zero intercept. SLP in all samples lay on a straight line. All plots had a slope of nearly one and an intercept close to zero.

Also, the concentration residuals were plotted against the actual concentrations for the validation samples (Figure 16, 17). This tool was used to determine whether the model accounted for the concentration variation in the validation set and it also provided information about how well the method would predict future samples. The residuals for all samples appeared to be randomly distributed around zero.

Statistical comparison between the results obtained by the proposed methods and those obtained by the reported spectrophotometric method[3] showed no significant differences, as seen in TABLE 6.

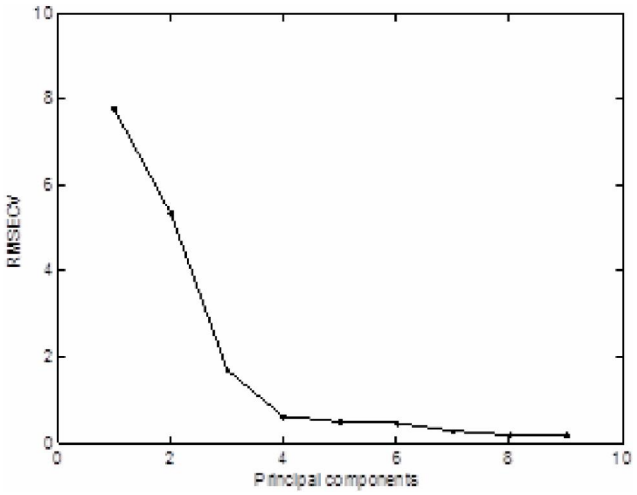

Figure 12 : RMSECV plot of a training set prediction using cross validation (PCR).

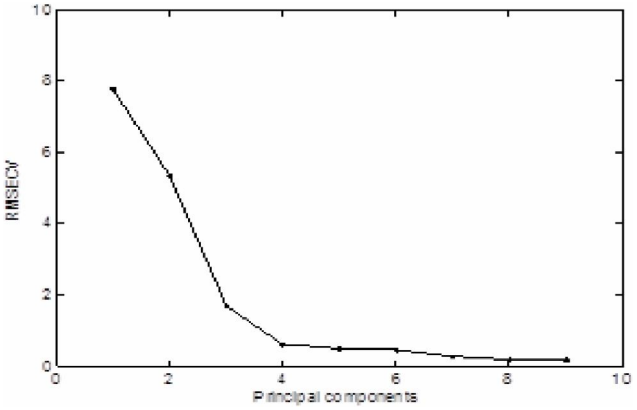

Figure 13 : RMSECV plot of a training set prediction using cross validation (PLS).

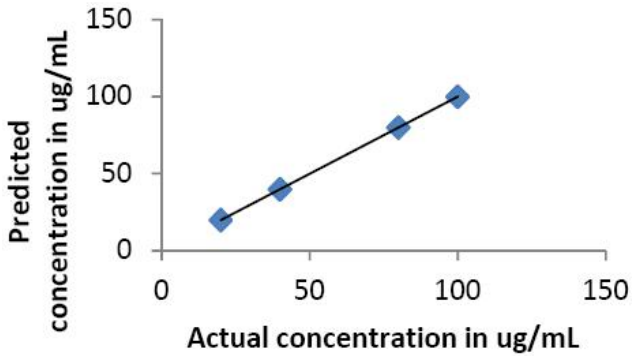

Figure 14 : Predicted concentration versus actual concentration of Sulpiride in the validation set using PCR method.

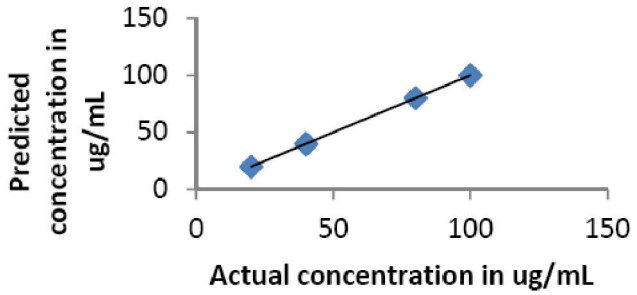

Figure 15 : Predicted concentration versus actual concentration of Sulpiride in the validation set using PLS method.

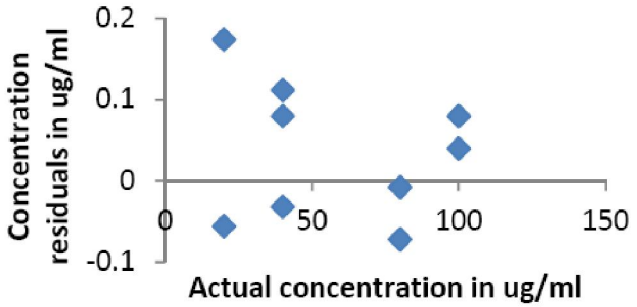

Figure 16 : Concentration residuals versus actual concentration of Sulpiride in the validation set using PCR method.

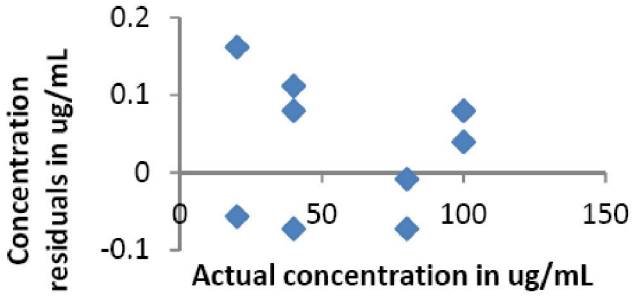

Figure 17 : Concentration residuals versus actual concentration of Sulpiride in the validation set using PLS method.

The proposed method was also successfully applied to the analysis of SLP and in the pharmaceutical preparation (capsule dosage form) presented in TABLE 7. The accuracy of the proposed methods

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was assessed by applying the standard addition technique (TABLE 7).

TABLE 3 : Validation parameters for the proposed stability-indicating spectrophotometric methods.

Validation parameters		DDRD method	RS method
Linearity (μgml^{-1})		20-200	20-200
Slope		0.0227	0.0071
Intercept		0.00511	0.228
Correlation coefficient (r)		0.9999	0.9998
LOD (μgml^{-1})		1.56	4.46
LOQ (μgml^{-1})		4.74	13.51
Precision	Intra-day[a]	0.43	1.23
	Inter-day[b]	0.70	1.17

TABLE 4 : Results of assay validation obtained by applying the proposed chemometric methods for the determination of Sulpiride in presence of both its acidic and alkaline degradation products.

Validation Parameters	PCR	PLS
Predicted versus actual concentration plot		
Slope	1.000	1.000
Intercept	-0.06309	-0.04926
Correlation coefficient(r)	0.9999	0.9999
RMSEP	0.086	0.086

TABLE 5 : Specificity of the proposed stability-indicating methods (A) Specificity of the proposed DDRD method; (B) Specificity of the for RS method; (C) Specificity of the proposed multivariate calibration methods.

(A)

Laboratory prepared mixtures			% Recovery*
Intact (ugml^{-1})	Acidic degradate (ugml^{-1})	Alkaline Degradate (ugml^{-1})	DDRD
80	40	20	98.67
80	20	40	98.45
80	40	40	98.12
80	60	40	102.30
80	20	80	101.04
Mean			100.23
SD			0.51

(B)

Laboratory prepared mixtures		% Recovery*
Intact (ugml^{-1})	Acidic degradation product (ugml^{-1})	RS
80	20	98.59
80	40	98.06
80	60	98.23
80	80	99.11
80	100	98.59
80	120	100.00
Mean		98.76
SD		0.70

(C)

Sr. No.	Concentration taken (ugml^{-1})			Recovery%*	
				Sulpiride	
	Sulpiride	Alkaline degradate	Acidic degradate	PCR	PLS
1	20	2	10	100.28	100.28
2	40	10	6	99.80	99.80
3	40	4	8	99.72	99.72
4	100	8	6	99.92	99.92
5	100	10	2	99.96	99.96
6	80	2	6	100.09	100.09
7	80	8	4	100.01	100.01
8	20	4	6	99.13	99.19
9	40	6	2	100.08	100.18
Mean				99.88	99.90
SD				0.36	0.32
RMSEP				0.086	0.086

***The average of three separate determinations.**

TABLE 6 : Statistical comparison of the results obtained by the proposed methods and the reported method for the determination of Sulpiride in pharmaceutical preparation.

Items	DDRD	RS	PCR	PLS	Reported method*
Mean	99.51	99.73	99.63	99.60	99.79
SD	0.24	0.32	0.22	0.26	0.19
RSD%	0.24	0.32	0.22	0.26	0.19
N	5	5	5	5	5
Variance	0.059	0.062	0.044	0.068	0.036
Student's t-test (2.306)	2.02	0.4	1.26	0.72	
F-test (6.388)	1.62	1.68	1.34	1.87	

The values between parenthesis are the theoretical values of t-test and F-test at P=0.05.

***Direct Spectrophotometric method where SLP is measured in 0.1M NaOH at 291nm[3].**

TABLE 7 : Determination of SLP in the pharmaceutical preparation and application of standard addition technique by the proposed stability –indicating methods; (A) Determination of SLP in the pharmaceutical preparation and application of standard addition technique by the proposed DDRD and RS methods. (B) Determination of SLP in the pharmaceutical preparation and application of standard addition technique by the proposed chemometric methods.

(A)

Pharmaceutical preparation	DDRD Method				RS method			
	Found% ±SD*	Standard addition technique			Found% ±SD*	Standard addition technique		
		Pure added ($ugml^{-1}$)	Pure found ($ugml^{-1}$)	Recovery%**		Pure added ($ugml^{-1}$)	Pure found ($ugml^{-1}$)	Recovery%**
Dogmatil® capsules 50.0 mg B.N. 2EG004.	99.51 ± 0.24	40	39.86	99.65	99.72 ± 0.32	40	40.96	102.38
		60	59.26	98.77		60	60.40	100.66
		80	80.26	100.33		80	80.20	100.25
		100	100.57	100.57		100	102.30	102.30
		120	121.82	101.51		120	122.50	102.08
		Mean ± SD		100.17±1.02		Mean ± SD		101.53±1.00

(B)

Pharmaceutical preparation	PCR Method				PLS method			
	Found% ±SD*	Standard addition technique			Found% ±SD*	Standard addition technique		
		Pure added ($ugml^{-1}$)	Pure found ($ugml^{-1}$)	Recovery%**		Pure added ($ugml^{-1}$)	Pure found ($ugml^{-1}$)	Recovery%**
Dogmatil® capsules 50.0 mg B.N. 2EG004.	99.63 ± 0.22	40	39.60	99.00	99.60 ± 0.26	40	39.80	99.50
		60	59.70	99.50		60	59.65	99.42
		80	80.96	101.21		80	80.96	101.21
		100	99.00	99.00		100	99.50	99.50
		Mean ± SD		99.68±1.04		Mean ± SD		99.91±0.86

***The average of five separate determinations; **The average recovery of three separate determinations.**

CONCLUSION

The proposed methods are simple, very sensitive, precise, and can be easily applied in QC laboratories for the determination of SLP in presence of its acidic and alkaline degradation products. The proposed methods could also be successfully applied for the routine analysis of SLP presence of its acidic and alkaline degradation products either in its pure bulk powder or in a dosage form in QC laboratories, without any preliminary separation step.

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