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Smart Spectrophotometric Methods for Stability Indicating Assay of Risperidone

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ABSTRACT

Three smart, simple, sensitive, accurate and precise methods were developed for the determination of risperidone in bulk powder, in pharmaceutical preparation and in presence of its degradate. Method (A) Dual Wavelength method is based on measuring the difference in the amplitude of intact risperidone in presence of its degradation product at two different wavelengths, this is done at 265 nm and 275 nm in the range of 5 – 30 µg ml^{-1} with LOD of 0.074 µg ml^{-1} and LOQ of 0.245 µg ml^{-1}. Method (B) Bivariate method; the method was applied for analysis of risperidone in presence of its degradation product this is done at 240 nm and 280 nm in the range of 5 – 35 µg ml^{-1} with LOD of 0.045, 0.291 µg ml^{-1} and LOQ of 0.149, 0.970 µg ml^{-1}, respectively. Method (C) First Derivative method (1D); is used for the determination of intact risperidone in presence of its degradation product at 274.2 nm in the range of 5 – 35 µg ml^{-1} with LOD of 0.089 µg ml^{-1} and LOQ of 0.297 µg ml^{-1}. The obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95% confidence level and no significant difference was observed regarding accuracy and precision.

INTRODUCTION

Risperidone **(Figure 1)** is 4-[2-[4-(6-fluorobenzo [d] isoxazol-3-yl)-1-piperidyl]ethyl]-3-methyl-2,6-diazabicyclo[4.4.0]deca-1,3-dien-5-one, belongs to the chemical class of benzisoxazole derivatives. It is an atypical antipsychotic agent and acts through selective antagonism of serotonin $5HT_2$, dopamine D_2 receptors[1]. Clinically, it is used in the treatment of schizophrenia and other psychoses[2]. The therapeutic importance of the drug has promoted the development of several analytical methods for its quantitative determination. The British Pharmacopeia adopts a non-aqueous titrimetric method for the determination of risperidone[3]. Other analytical techniques include several spectrophotometric[4-10], polarographic[11] and chromatographic[12-18] methods for determination of risperidone in pure form, pharmaceutical preparations and/or biological fluids have been reported.

Under computer-controlled instrumentation, dual wavelength, bivariate and first derivative methods are playing a very important role in the analysis of risperidone in presence of its degradation product without previous separation by UV–VIS spectrophotometry[19-25].

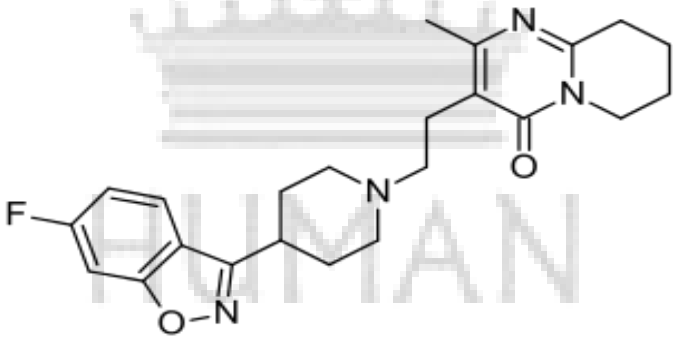

Figure 1. Structural formula of Risperidone

MATERIALS AND METHODS

Apparatus

- Shimadzu UV-Vis. 1650 Spectrophotometer (Japan).

Materials and reagents

Pure sample

Risperidone was kindly supplied by multi-apex company, Egypt, B. No. (RN0030612).

Pharmaceutical preparation

Sigmadone® tablets: product of Sigma Company, Egypt, Batch No. (40708), labeled to contain 3 mg of Risperidone per tablet purchased from local pharmacies.

Reagents and solvents

All chemicals and reagents used throughout the work were of analytical grade.

- Methanol (Sigma–Aldrich, USA).
- 3% Hydrogen peroxide.

Standard solution

- Stock solution of risperidone (0.1 mg ml^{-1}) was prepared by dissolving 10 mg of risperidone in 100 ml methanol and this is the working standard solution.

Degraded sample

100 mg of risperidone sample was taken in 100 mL round bottom flask, 10 mL of 3% hydrogen peroxide solution was added, and contents were mixed well at room temperature. After 4 hr, 1 mL of this solution was diluted to 100 mL with methanol. The obtained solution was claimed to contain (0.1 mg ml^{-1})[18].

Procedures

Construction of the calibration curves (General procedures)

a. Dual wavelength method: Aliquots of risperidone standard solution (100 µg/ml) ranging from (50–300) µg were transferred to 10 ml volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to 400 nm) of these solutions were recorded using methanol as a blank. The difference in the absorbance was measured at 265 and 275 nm and plotted against the concentration to construct the calibration curve.

b. Bivariate method: Aliquots of risperidone standard working solution and its degradate equivalent to (50 - 350) µg were transferred separately into two sets of 10 ml volumetric flasks,

then diluted to volume with methanol. Calibration curves at different wavelengths 240, 245, 250, 255, 260, 270, and 280 nm were constructed and the regression equation at each wavelength was calculated. From both sets of regression equations, the sensitivity matrices K was calculated[26-27]; **Table 2** from which the optimum pair of wavelengths to carry out the determination (240 and 280 nm) were investigated. Calibration curves were constructed by plotting the absorbance versus the corresponding drug or degradate concentration in µg ml^{-1}.

c. First derivative method: Aliquots of standard risperidone solution in methanol containing (50– 350) µg of the drug were added to a series of 10 ml volumetric flasks and then diluted to the mark with methanol. First - derivative (^1D) spectra of the drug were recorded against methanol as blank. The peak amplitude at 274.2 nm was measured for each drug concentration.

Calibration curve relating peak amplitude to drug concentration in µg ml^{-1} was constructed, the regression equation was derived.

Analysis of laboratory prepared mixtures

Aliquots of intact risperidone containing (250 - 100) µg were transferred into a series of 10 ml volumetric flasks containing risperidone degradate (50 - 200) µg for method A and (300 - 100) µg were transferred into a series of 10 ml volumetric flasks containing risperidone degradate (50 - 250) µg for method B & C; the spectra of laboratory prepared mixtures were scanned from 200-400 nm and stored in the computer, then analyzed as described under "General Procedure" for each method.

The intact drug concentrations were calculated from the corresponding regression parameters presented in **Table 1** and the results were shown in **Table 4**.

Analysis of pharmaceutical preparation

Ten Sigmadone® 3 mg tablets were accurately weighed and finely powdered, then a quantity equivalent to 10 mg of risperidone was shaken three times with 25 ml methanol 10 minutes then filtered into 100 ml volumetric flask and the volume was adjusted to the mark with methanol to obtain a concentration of (0.1 mg ml^{-1}). Proceed as described under "General Procedure" for each method.

RESULTS AND DISCUSSION

Dual wavelength method:

The zero-order absorption spectra of risperidone **(Figure 2)** show an overlaping, which does not permit direct determination of risperidone in presence of degradate.

In this method, the interference between risperidone and its degradate can be removed by measuring the difference in absorbance at 265 and 275 nm. This difference is zero for risperidone degradate, while it is directly proportional to the concentration of risperidone.

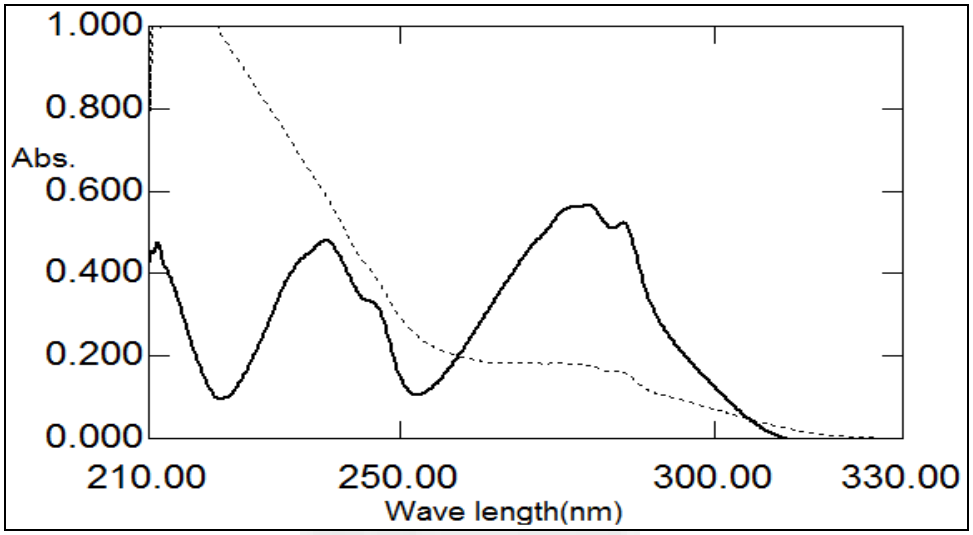

Figure 2. UV- Spectra of Intact risperidone (25µg ml^{-1})(—), Degradate Risperidone (25 µg ml^{-1})(…..).

Bivariate method:

In the present study, stability indicating bivariate calibration spectrophotometric method was developed aiming to selective quantitation of risperidone in its bulk powder, in pharmaceutical formulation or in presence of its degradation product. The principle of bivariate calibration is the measurement of two components (A and B) at two selected wavelengths (λ_1 and λ_2) to obtain two equations [26-27]:

$$A_{AB1} = m_{A1}C_AC_B + e_{AB1}$$

$$A_{AB2} = m_{A2}C_AC_B + e_{AB2}$$

The resolution of each equation set allows the evaluation of C_A and C_B values:

$$C_A = (A_{AB1} – e_{AB1} - m_{B1}C_B) / m_{A1}$$

$$C_B = [m_{A2} (A_{AB1} – e_{AB1}) + m_{A1} (e_{AB2} – A_{AB2})] / m_{A2}m_{B1} – m_{A1}m_{B2}$$

Where C_A, C_B are the concentration of component A and component B; m_{A1}, m_{A2} are the slope values of A at λ_1, λ_2; m_{B1}, m_{B2} are the slope values of B at λ_1, λ_2; A_{AB1}, A_{AB2} are the absorbance of the binary mixture at λ_1, λ_2; e_{AB1}, e_{AB2} are the sum of the intercepts of A and B at λ_1 and λ_2 respectively.

In order to apply the bivariate method in the resolution of risperidone and its degradate, the absorbance of the two component at seven different selected wavelengths was recorded in the region of overlapping; 240, 245, 250, 255, 260, 270 and 280 nm. The calibration curve equations and their respective linear regression coefficients were obtained directly with the aim of ensuring that there was a linear relationship between the absorbance and the corresponding concentration.

According to Kaiser method[27], the slope values of the linear regression equations for both intact drug and its degradate at the selected wavelengths were used to calculate the sensitivity matrices **K** to find out the optimum pair of wavelength at which the binary mixtures were recorded, **Table 2**.

$$K = \begin{bmatrix} m_{A_1} & m_{B_1} \\ m_{A_2} & m_{B_2} \end{bmatrix}$$

For the bivariate determination of risperidone and its degradate, 240 and 280 nm were found to give the maximum value of K and thus can be used for the analysis; Table (2).

First derivative method:

It is clear from the spectra in **Figure 2** that, there is a band overlapping between the risperidone and its degradation product. Such overlapping was eliminated by the first derivative (^{1}D) scanning of risperidone and its degradation product in methanol, risperidone has a peak at 274.2 nm which shows no interference from the degradation product. Thus it would be possible to adopt the (^{1}D) spectrophotometry at 274.2 nm for direct determination of risperidone in presence of its degradation product as seen in **Figure 3**.

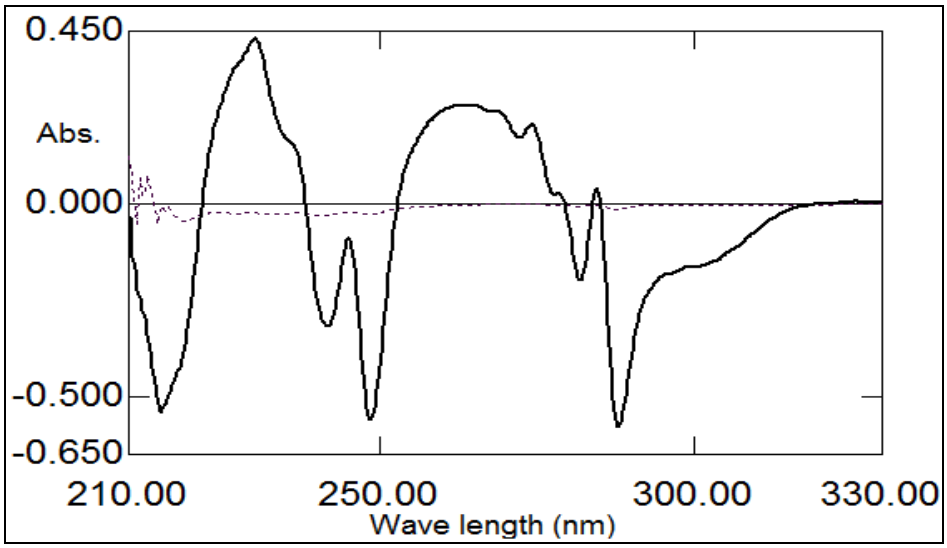

Figure 3. First-derivative Spectra of Intact Risperidone (20 µg ml^{-1}) (—) and its Degradation Product (20 µg ml^{-1}) (…..) in Methanol

Validation of the methods

Linearity and range

For dual wavelength method: Linear correlation was obtained between the differences in amplitudes at 265 and 275 nm, against the corresponding concentration of risperidone. Good linearity is obtained in the concentration range of 5 - 30 µg ml^{-1}. The corresponding regression equation was computed to be:

$$\Delta P_{275-265} = 0.0108\ C - 0.0022 \qquad (r = 0.9996)$$

Where ΔP is the amplitude difference at the selected wavelengths, C is the concentration in µg ml^{-1} and r^2 = the correlation coefficient as shown in **Table 1**.

For bivariate method: Calibration graphs were constructed for the determination of risperidone by the proposed procedure, where Beer's law was obeyed in the range of 5 -35 µg ml^{-1}. The linear regression of the graphs were as follows.

$$A_{240} = 0.0236\ C - 0.0237 \qquad (r=0.9996)$$

$$A_{280} = 0.0289\ C - 0.0120 \qquad (r=0.9998)$$

Where A is the absorbance, C is the drug concentration in µg ml^{-1}.

For first derivative method: At the described wavelength linear relationship was obtained between the peak amplitude and the risperidone concentration in the range (5 - 35µg ml^{-1}).The linear regression equation was:

$$A_{274.2} = 0.0103\ C + 0.0027 \qquad (r = 0.9997)$$

Where A is the peak amplitude at 274.2 nm and C is the drug concentration in µg ml^{-1}.

Linearity range, regression equation, intercept, slope and correlation coefficient for the calibration data were presented in **Table 1**.

Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines[28] from the following equations:

$$LOD = 3.3\ S_a / slope$$

$$LOQ = 10\ S_a / slope$$

Where S_a is the standard deviation of y-intercepts of regression lines.

LOD and LOQ values of lornoxicam for each method were listed in **Table 1**. The small values of LOD and LOQ indicate good sensitivity.

Accuracy and precision

According to the ICH guidelines[28], three replicate determinations of three different concentrations of the studied drugs in pure form within their linearity ranges were performed in the same day (intra-day) and in three successive days (inter-day) for each method. Accuracy as recovery percent (%R) and precision as percentage relative standard deviation (%RSD) were calculated and results are listed in **Table 3**. The small values of %RSD indicate high precision of the methods. Morever, the good %R confirms excellent accuracy.

Specificity

The specificity of the proposed methods were assured by applying the laboratory prepared mixtures of the studied drug and its degradate. The results are listed in **Table 4** .

Pharmaceutical Applications

The proposed methods were applied to the determination of the studied drug in Sigmadone® 3 tablets. The results were validated by comparison to a previously reported method[10].

No significant differences were found by applying t-test and F-test at 95% confidence level[29], indicating good accuracy and precision of the proposed methods for the analysis of the studied drugs in their pharmaceutical dosage form **Table 5**.

Table 1. Spectral data for determination of the studied drug by the proposed methods

Parameters	**Dual wavelength**	**Bivariate**		**First Derivative**
Wavelength (nm)	275 & 265	240	280	274.2
Linearity range (µgml^{-1})	5-30	5 — 35	5-35	5 — 35
LOD (µgml^{-1})	0.073	0.045	0.291	0.089
LOQ (µgml^{-1})	0.245	0.149	0.970	0.297
Regression equation*				
Slope (*b*)	0.0108	0.0236	0.0289	0.0103
Intercept (*a*)	0.0022	0.0237	0.0120	0.0027
Correlation coefficient (*r*)	0.9996	0.9996	0.9998	0.9997

* $y = a + bx$ where y is the response and x is the concentration.

Table 2. Values of the sensitivity matrix determinants calculated according to Kaiser's method (k x 10^6) for the mixture of risperidone and its oxidative degradate by the proposed bivariate method

λ/λ	240	245	250	255	260	270	280
240	0	35.48	136.64	121.36	38.68	359.64	**535.82**
245		0	86.4	78.4	44.4	239.34	429.42
250			0	6.4	76.8	253.92	348.96
255				0	66.4	208.68	284.84
260					0	130.26	202.38
270						0	77.25
280							0

Table 3. Intraday and interday accuracy and precision for the determination of the risperidone by the proposed methods

Method	Conc. µg.ml^{-1}	Intraday			Interday		
		Found Conc. ± SD	Accuracy (%R)	Precision (%RSD)	Found Conc. ± SD	Accuracy (%R)	Precision (%RSD)
Dual wavelength	15	14.86±0.0006	99.09	0.365	14.89±0.0012	99.30	0.728
	20	19.71±0.0012	98.55	0.548	19.74±0.0017	98.70	0.821
	25	24.71±0.0015	98.84	0.577	25.05±0.0021	100.19	0.776
Bivariate	15	14.76±0.024	98.41	0.166	14.94±0.185	99.63	1.236
	20	20.04±0.149	100.22	0.742	20.04±0.171	100.22	0.854
	25	24.68±0.107	98.71	0.432	24.73±0.153	98.93	0.618
First derivative	15	15.14 ± 0.0015	100.95	0.963	15.01 ± 0.0015	100.09	0.971
	20	20.13 ± 0.001	100.63	0.476	19.96 ± 0.0015	99.82	0.733
	25	24.98± 0.001	99.92	0.385	24.92 ± 0.00058	99.66	0.223

Table 4. Determination of risperidone and its degradate in their laboratory mixtures by the proposed methods

	Intact in (µg ml^{-1})	Degradate in (µg ml^{-1})	Percent of degradate	Intact found in (µ ml^{-1})	Recovery % of intact
Dual wavelength difference	25	5	16.67	25.02	100.07
	20	10	33.33	19.65	98.24
	15	15	50	15.02	100.12
	10	20	66.67	9.93	99.26
	Mean ± SD%				99.42±0.883
Bivariate	30	5	14.29	29.98	99.93
	25	10	28.57	25.25	101.00
	20	15	42.86	19.83	99.15
	15	20	57.14	14.97	99.80
	10	25	71.43	9.81	98.10
	Mean ± SD%				99.60±1.068
First derivative	30	5	14.29	29.83	99.45
	25	10	28.57	24.59	98.37
	20	15	42.86	19.83	99.17
	15	20	57.14	15.08	100.52
	10	25	71.43	9.83	98.35
	Mean ± SD%				99.17±0.896

Table 5. Determination of risperidone in sigmadone® tablets by the proposed and reported methods

	Dual wavelength	Bivariate	First derivative	Reported method[10]
N*	7	7	7	6
$\bar{X}$	99.67	99.63	100.47	100.16
SD	0.434	0.969	0.647	0.631
RSD%	0.435	0.973	0.644	0.631
t*	1.604 (1.7959)	1.184 (1.7959)	1.368 (1.7959)	———
F**	2.114 (4.39)	0.424 (4.95)	1.360 (4.39)	———

* No. of experimental.

** The values in the parenthesis are tabulated values of t and F at (p= 0.05).

CONCLUSION

The proposed methods are simple, rapid, accurate, precise and can be used for the determination of risperidone in pure form and in pharmaceutical dosage form as well as in presence of its degradation product.

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