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Development and Validation of Spectrophotomtric Method for the Determination of Risperidone

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ABSTRACT

A simple and sensitive method for the spectrophotometric determination of risperidone (RIS) is proposed. The method is based on the oxidation of the drug using chloramine-T (CAT) and the excess oxidant is determined by either xylene cyanol FF (XCFF) or malachite green (MG), the absorbances of which are measured at 612 or 619 nm for RIS-XCFF and RIS-MG respectively. Under the proposed optimum conditions Beer's law is obeyed in the concentration range of 2.00-26.00 and 2.00-18.00 μgmL^{-1} and the molar absorptivities are calculated to be 4.37×10^4 and 3.93×10^4 $Lmol^{-1}cm^{-1}$ for RIS-XCFF and RIS-MG systems. The limit of detection and quantification for RIS-XCFF and RIS- MG are found to be 0.891, 2.702 μgmL^{-1} and 0.529, 1.605 μgmL^{-1} respectively. The proposed method is applied successfully for the pharmaceutical formulations.

Key words: Spectrophotometry, Risperidone, Chloramine T, Xylene cyanol FF, Malachite green.

INTRODUCTION

Risperidone, chemically 3-[2-[4-(6-fluro-1,2-benzisoxazol-3-yl) - 1-piperidinyl] ethyl]-6,7,8,9-tetra hydro –2-methyl-4H-pyridol[1,2-a] pyrimidin-4-one[1] is an antipsychotic drug [2], which acts through selective antagonism of serotonin $5HT_2$, dopamine D2 receptors, used in the treatment of schizophrenia and other psychoses [3]. It is effective in the treatment of schizophrenia and other psychiatric illnesses in adults and children including pervasive developmental disorders, autism and attention-deficit disorder [4, 5]. Usually, oral doses of RIS in the treatment of chronic schizophrenia are 2~6 mg/d. After oral administration, RIS is rapidly and completely absorbed from the gastro-intestinal tract and mainly metabolised via hydroxylation and N-dealkylation [6, 7]. RIS is especially useful in the treatment of severe

schizophrenia that is resistant to other drugs. It also acts on harmful symptoms of the illness. However, it induces extra pyramidal side effects and hyperprolactinemia [8]. The maximal daily dose of risperidone is 4–8 mg and the therapeutic concentration of the drug in serum is 10 to 90 ng/mL.

Several analytical techniques such as visible spectrophotometric method [9], tandem mass spectrometry [10-11], LC-MS and HPLC-ESI/MS assay for its quantification in plasma and serum [12-15], chiral chromatography [16], pulse polarography [17], chemiluminescence assay [18] and LC with coulometric detection [19] have been reported for the determination of RIS. The only one reported spectrophotometric is based on the ion pair complex formation of the drug with bromocresol green, bromophenol blue and bromothymol blue and the method requires tedious extraction procedure. The present report gives a simple and sensitive method for the determination of RIS based on the oxidation of the drug by CAT and the excess oxidant is determined by either XCFF or MG.

MATERIALS AND METHODS

Apparatus

A UV-VIS 2550 (Shimadzu, Japan) spectrophotometer with 1cm matched quartz cell was used for all the measurements.

Reagents and materials

A 0.0028 M CAT was prepared by dissolving 0.2 g CAT in distilled water and made up to the volume in a 250 mL standard flask to get 800 µg mL^{-1} CAT concentration and the solution was standardized iodometrically [20]. Stock solution of XCFF (s d fine chem. Ltd., India) and MG (Merck India Ltd., Mumbai) equivalent to 1000 µg mL^{-1} were prepared separately and diluted to obtain working concentration of 250 µg mL^{-1}.

Standard drug solution

A 1000 µg mL^{-1} drug solution was prepared by dissolving an accurately weighed amount of the drug in ethanol and made up to the mark with the same. The standard solution was diluted using ethanol in a 100 mL standard flask to get 100 µg mL^{-1} as the working concentration.

Procedure

Different concentrations containing 2.00-26.00 µg $mL^{-1}$of RIS were transferred into a series of 10 mL calibrated flasks using a microburette. One mL of H_2SO_4 (2 M) was added followed by 2.0 mL of CAT (0.0028 M), shaken well and kept aside for 15 min. Then either 1 mL of 250 µg mL^{-1} XCFF or 1.5 mL of 250 µg mL^{-1} MG were added and diluted up to the volume with distilled water, mixed well and the absorbance of each was measured at 612 nm and 619 nm for RIS-XCFF and RIS-MG respectively.

Absorption spectra

The method is based on the reaction of surplus CAT with the corresponding dye solution in acidic medium, which bleaches the coloured dye solution to colourless leucoform, the decolouration being caused by the oxidative destruction of the dyes, which was measured at 612 and 619 nm for RIS-XCFF and RIS-MG respectively (Figure 1).

Assay of formulation
Twenty tablets of Rispond (2 mg) were ground into fine powder and an amount equivalent to labeled amount is dissolved in ethanol by stirring for 10 min and filtered into a 100 mL standard flask. An aliquot of the sample was taken and analyzed according to the proposed procedure.

RESULTS AND DISCUSSION

In the present method, two dyes XCFF and MG have been used for the determination of RIS. The determinations of RIS are indirect and are based on the determination of surplus CAT after the oxidation reaction of RIS by CAT. The drug undergoes oxidation and the reaction is found to be complete and quantitative in 15 min.

RIS when added in increasing concentration to a fixed concentration of CAT, RIS will get oxidized and there will be a concomitant decrease in CAT concentration. A concomitant increase in the concentration of dye resulted when a fixed concentration of the dye is added to decreasing concentration of CAT. The use of excess of reagent produced no further increase in absorbance.
Preliminary investigation showed that sulphuric acid is better than hydrochloric, nitric or acetic acid because of the turbidity that formed during the addition of HCl and the reaction was very slow in the presence of nitric acid and acetic acid. Different concentrations of H_2SO_4 were tested for the proposed reaction, one mL of 2 M sulphuric acid was found to be ideal for the oxidation step and the same quantity of acid was employed for the estimation of the dye.

Quantification
A linear correlation was found between absorbance at λmax and concentration of RIS. The graphs showed negligible intercept and are described by the equation: Y = a + bX (where Y = absorbance; a = intercept; b = slope and X = concentration in µg mL^{-1}) .Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 1. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values of both methods are also given in Table 1.

Detection and quantitation limits
According to ICH guidelines [21] limit of detection (LOD) is the smallest concentration of a solution of an element that can be detected with 95 % certainity and the quantitation limit is generally determined by the analysis of samples with known concentrations of analyte with those of blank samples and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. Based on the standard deviation of the reagent blank and the slope of the calibration curve of the analyte, the detection limit may be expressed as D_L= (3.3 σ) / S and quantitation limit can be expressed as Q_L= (10 σ) / S where σ= standard deviation of the reagent blank, S= slope of the calibration curve. The values for LOD and LOQ are presented in Table 1 and reveal good sensitivity of the spectrophotometric method.

Validation of the method
The validity of the method for the assay of RIS was examined by determining the precision and accuracy. Accuracy was checked at four concentration levels within the specified range, five replicate measurements were recorded at each concentration levels. The results were recorded as

standard deviation. Precision was checked at four concentration levels, five replicate measurements were recorded at each concentration level. The results are summarized in Table 2. The calculated standard deviations and relative standard deviations were all below 2.0 % indicating good precision of the proposed procedure.

Interference study

In the pharmaceutical analysis, it is important to test the selectivity towards the excipients and fillers added to the pharmaceutical preparations. Species that can occur in the real samples together with the drug were investigated. To investigate the effect of tablet fillers on the measurements involved in the methods, standard addition method was carried out. Under the optimum reaction conditions, to a known amount of the drug, excipients such as starch, glucose, cellulose, lactose and talc were added in different concentrations and analyzed. It was found that the excipients did not interfere at the levels normally found in dosage forms.

Table 1: Analytical parameters

	Using XCFF	Using MG
λmax nm	612	619
Beer's Law Limit (µg mL^{-1})	2.00-26.00	2.00-18.00
Molar Absorptivity ($Lmol^{-1}cm^{-1}$)	4.37×10^4	3.93×10^4
Sandell's Sensitivity (µg cm^{-2})	0.0093	0.0105
Limit of Detection** (µg mL^{-1})	0.891	0.529
Limit of Quantification** (µg mL^{-1})	2.702	1.605
Regression Equation*	Y=a+ bX	Y=a+ bX
Slope (b)	0.0372	0.0623
Intercept (a)	0.1433	-0.0140
Correlation coefficient (r)	0.9966	0.9828

** Y is the absorbance and X concentration in µg mL^{-1}*

** * Calculated according to ICH guidelines*

Table 2: Evaluation of accuracy and precision

Using XCFF

Concentration taken (µg mL^{-1})	*Concentration found (µg mL^{-1})	RE (%)	SD (µg mL^{-1})	RSD (%)
6.00	5.973	0.450	0.052	0.870
8.00	7.992	0.100	0.028	0.350
10.00	10.003	-0.030	0.026	0.259
12.00	12.010	-0.083	0.031	0.258

Using MG

Concentration taken (µg mL^{-1})	*Concentration found (µg mL^{-1})	RE (%)	SD (µg mL^{-1})	RSD (%)
2.00	1.998	0.100	0.018	0.900
4.00	3.997	0.075	0.012	0.300
6.00	6.007	-0.116	0.029	0.482
8.00	8.011	-0.137	0.036	0.449

** Average of 5 determinations*

Applications

The proposed method was applied successfully to determine RIS in tablets. Table 4 gives the result of the determination from which it is clear that there is a close agreement between the results obtained by the proposed method and label claim. In student's t-test no significant difference was found between the calculated and tabulated values in respect to accuracy and precision.

Table 4: Results of assay of formulations

	Brand name	Labeled amount (mg)	Amount found (mg)	% Label claim± SD
Using XCFF	Rispond[a]	2.00	1.990	99.5± 0.027 t-test= 1.65
Using MG	Rispond[a]	2.00	1.998	99.9± 0.044 t-test= 0.203

[a]*Micro labs Ltd., India.; * Mean value of four determinations; Value of t at 95 % confidence level is 3.182.*

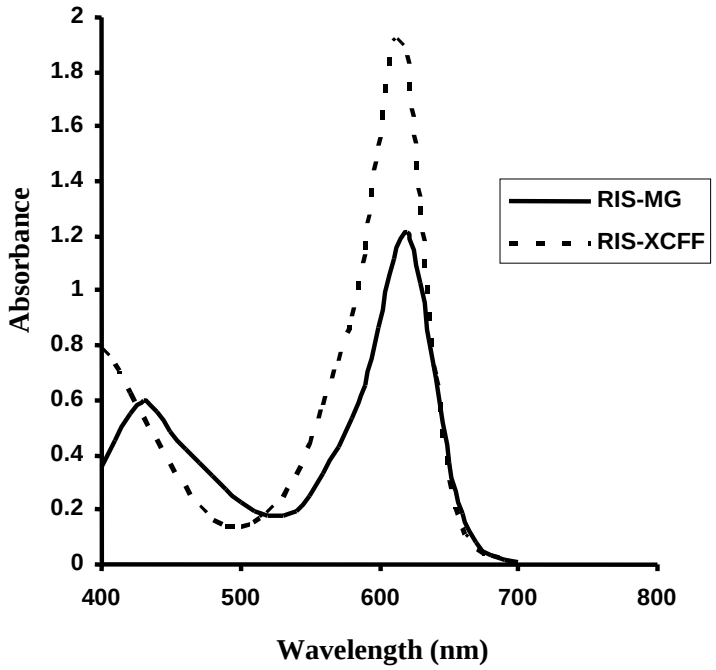

Figure 1: Absorption spectra for RIS-XCFF and RIS-MG.

CONCLUSION

A simple and accurate method for the determination of RIS has been developed. Method is easy to perform and do not contain any stringent experimental variables which effect the reliability of the results. The commonly used excipients and additives in the preparation of tablets were found not to interfere in the analysis. The methods thus can be used for the determination of RIS in pure and dosage forms.

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