Development of Analytical Method for Risperidone by UV Spectrophotometry

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Abstract:

A simple, sensitive, specific, spectrophotometric method developed for the detection of Risperidone in bulk drug and Pharmaceutical formulation. The optimum conditions for the analysis of the drug were established. The λ max of the Risperidone was found to be 280 nm. The method shows high sensitivity with linearity 2 to 6μ g/ml. The lower limit of detection and the limit of quantification was found to be 1.012 and 3.036 respectively. All the calibration curves shows a linear relationship between the absorbance and concentration and coefficient correlation was higher than 0.99. The regression of the curve was Y = 0.039*x* - 0.002. Precision of the method was found to be 2.0325 ± 0.044 against the label claim of 2mg. The percentage recovery was found to be 102 ± 0.188. The sample solution was stable up to 2 hours. The proposed method will be suitable for the analysis of RIS in bulk and pharmaceutical formulation.

Key words: *Risperidone, Spectroscopy, Estimation*

Introduction:

Risperidone (RIS) is belonging to the chemical class of Benzisoxazole derivatives and chemically It is 4-(2-(4-(6-Flurobenzo[d]isoxazd-3yl]1-piperidyl] ethyl]-3-methyl-2,6 diazabicyclodeca-1,3-dien-5-one [1] with molecular formula C_{23} H_{27} FN_4O_2 was presented in fig 1. RIS is an antipsychotic agent [2], which acts through selective antagonism of serotonin $5HT_2$, dopamine D_2 receptors, used in the treatment of schizophrenia and other psychoses [3]. It is mostly metabolized by alicyclic hydroxylation and oxidative N- dealkylation [4]. An ideal stability indicating method is one that quantifies the drug and also resolves its degradation products [5]. RIS is soluble in 0.1N HCL and methanol and insoluble in sodium hydroxide and acetonitrile. The λ max was found to be at 280nm.

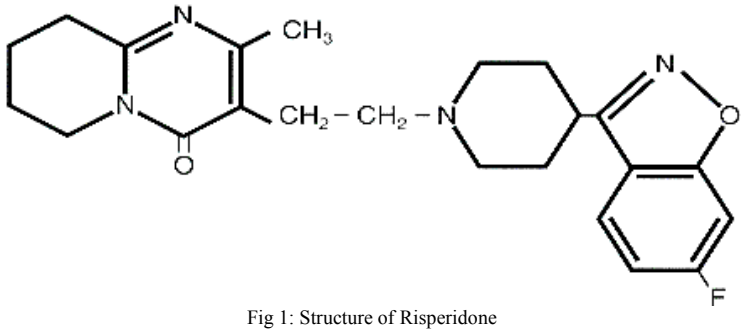

Fig 1: Structure of Risperidone

Literature review for RIS analysis revealed several methods based on different technique such as HPLC with UV detection [6].Visible spectrophotometric methods [7], LC-ms and HPLC ESI/MS assay for its quantification in plasma and serum [8-11], Chiral Chromatography [12], Pulse Polarography [13], Chemiluminescence assay [14], LC with coulometric Detection [15]. However there is no method reported for the detection of RIS in bulk and pharmaceutical formulation by UV spectrophotometry..

The aim of present work is to find out a simple, sensitive, specific, spectrophotometric method developed for the detection of RIS in bulk drug and pharmaceutical formulation.

Materials and Methods:

Reagents & Materials

RIS working standard was supplied by M/S Orchid chemicals and Pharmaceuticals, Chennai. RIS (Label claim: 2mg tablet) was manufactured by M/S Torrent Pharmaceutical Ltd Baddi, Solan (HP), India. All other chemicals used in the analysis were AR grade.

Apparatus

A double – beam spectrophotometer Perkin Elmer (Lambda 25) was used for the detection of absorbance, Mettler Tremedo (weighing balance) and Bronson sonicator were used for experimental purpose.

Method

Preparation of stock solution

100mg of the pure drug was weighed and transferred to a 100ml volumetric flask, 50ml 0.1N HCL was added to the above flask and dissolved, the volume was made up with the 0.1N HCL.

Preparation of sample solution

The average weight of the tablets were determined by weighing 10 tablets and these were powdered. Tablet powder equivalent to 2mg of RIS was weighed and transferred to a 100ml volumetric flask. About 20ml of 0.1N HCL was added and sonicated for 5 min for complete dissolution of drugs, the volume was made up with 0.1N HCL and mixed well, and then the above solution was filtered through Whatmann filter paper. Dilutions were made with 0.1N HCL to attain a concentration of 4μg/ml. Six replicates of analysis were carried out with sample weighed individually. The average weight of tablet was found to be 0.21g.

Method validation

Various methods for analysis of RIS in bulk and pharmaceutical formulation was carried out as per ICH guideline.

Linearity

The method was validated according to ICH Q2B guidelines [16] for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy of the analyte [17-22]. For RIS, five point calibration curves were generated with the appropriate volumes of the working standard solutions for UV methods. The linearity was evaluated by the least-square regression method using unweighted data.

Precision and accuracy

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicate at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements [16]. The intermediate precision was studied by comparing the assays on three different days and the results are documented as the standard deviation and RSD %. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained.

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$$LOD = 3\ s/m;\ LOQ = 10\ s/m$$

Where s, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability [16-18]. The values of LOD and LOQ are given in Table 1**.**

Table 1: Linearity study of RIS

Concentration (μg /ml)	Absorbance
2	0.0799
3	0.1101
4	0.1519
5	0.198
6	0.232

Stability

The stability of RIS in 0.1N HCL solution was studied by the UV method. Sample solutions were prepared in triplicate and stored at 4 and 25°C for 30, 60, 90 and 120min. The stability of these solutions was studied by performing the experiment.

Recovery study

Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method. The same range of concentrations, as employed in the linearity studies, was used. To study the accuracy, precision and reproducibility of the proposed method and dosage forms, recovery experiments were carried out using the standard addition method. These studies were performed by the addition of known amounts of pure RIS to the pre-analyzed tablet formulation and the mixtures were analyzed using the proposed techniques. After parallel analyses, the recovery results were calculated using the related calibration equations.

Results and Discussion:

The development of a simple, rapid, sensitive and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations and the cost of materials and labor. RIS is a UV-absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The λ_{max} of the drug for analysis was determined by taking scans of the drug sample solutions in the entire UV region.It was found to be that only one peak was observed in this method at the wavelength of 240nm.

Calibration curves

Calibration curve data were constructed in the range of the expected concentrations of 2 µg/mL to 6 µg/mL. Beer's law was obeyed over this concentration range. The regression equation was found to be Y = 0.039*x* - 0.002. The correlation coefficient (r) of the standard curve was found to be greater than 0.995. The stock solutions and working standards were made in 0.1N HCL. Calibration curve was presented in table 1 and fig 2.

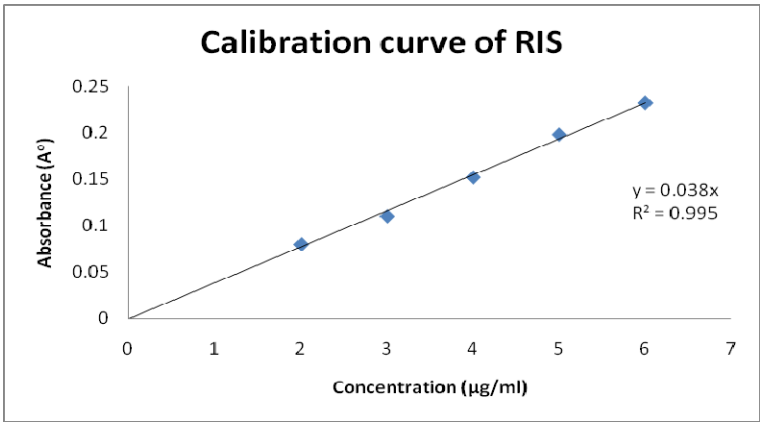

Fig 2: Calibration curve of RIS

The analytical characteristics and necessary validation parameters for the UV techniques for RIS are presented in Table 2.

Table 2: Validation parameters

Parameters	Values
Linearity range (µg/ml)	2-6
Precision (%)	2.0325 ± 0.044
Accuracy (%)	102 ± 0.188
LOD (µg/ml)	1.012
LOQ (µg/ml)	3.036
Stability (h)	2
Std Deviation (SD ±)	0.044

Performing replicate analyses of the standard solutions was used to assess the accuracy precision and reproducibility of the proposed methods. The selected concentration within the calibration range was prepared in 0.1N HCL and analyzed with the relevant calibration curves to determine the intra- and inter day variability. The intra- and inter day precision were determined as the RSD %. The precision, accuracy and reproducibility of the results are given in Tables 1, which demonstrate a good precision, accuracy and reproducibility.

The proposed methods can be successfully applied for RIS assay in tablet dosage forms without any interference. The assay showed the drug content of this product to be in accordance with the labeled claim 2mg. The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method. In order to check the accuracy and precision of the developed method and to prove the absence of interference by excipients, recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulations of all drugs. The application of this procedure is explained in the experimental section. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of all drugs in tablets. These results reveal that the developed method have an adequate precision and accuracy and consequently, can be applied to the determination of RIS tablet in pharmaceuticals without any interference from the excipients.

The stability of RIS in 0.1N HCL solution was evaluated to verify whether any spontaneous degradation occurs, when the samples were prepared. The stability profile for 30, 60, 90 and 120min. the results were expressed as a percentage of the drug remaining. The obtained data showed that the sample solutions were stable during 2 h.

Conclusion:

The developed spectrophotometric method was simple, sensitive, and specific, for the detection of RIS in bulk & pharmaceutical formulation. It could be precisely quantify and LOD was found to be 1.012 and the limit of quantification to be 3.036. All the calibration curves shows a linear relationship between the absorbance and concentration and coefficient correlation was higher than 0.99. Precision of the method was found to be 2.0325 ± 0.044 against the label claim of 2mg. The percentage recovery was found to be 102 ± 0.188 and the sample solution was stable for up to 2 hours. The proposed method will be suitable for the analysis of RIS in bulk and pharmaceutical formulation.

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