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Development of analytical method for Risperidone by UV Spectrophotometry using methanol as a solvent

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Abstract

A simple, sensitive, specific, spectrophotometric method has been developed for the detection of Risperidone in pure form and pharmaceutical dosage forms. The optimum condition for the analysis of the drug were established. Risperidone exhibiting absorption both at 240 and 280 nm and obeyed beers law in the concentration range of 20 to 60µg/ml. The lower limit of detection was found to be 2.788 µg/ml and the limit of quantification was found to be 8.45 µg/ml. The regression equation was y = 0.0284 x and y = 0.028 x for 240 nm and 280 nm respectively. The precision of the method was found to be 1.9083 at 240 nm and 1.9583 at 280 nm against the label claim of 2 mg. The percentage recovery was found to be 2.12 ± 0.242 and 2.19 ± 0.196 for 240 nm and 280 nm respectively. The sample solution was stable up to 24 hours. The assay results were found to be in good agreement against the label claim. The proposed method was simple sensitive, precise, accurate and easy for routine quality control analysis.

Keywords: Spectrophotometry, Risperidone, Methanol

INTRODUCTION

Risperidone (RIS) is belonging to the chemical class of Benzisoxazole derivatives and chemically it is 4-(2-(4-(6- Fluro benzo[d] isoxazd-3yl] - 1- piperidyl] ethyl]-3-methyl-2, 6-diazabicyclo deca-1, 3-dien- 5-one [1] with molecular formula C_{23} H_{27} FN_4O_2. 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]. Structure of RIS is shown in Fig.1. 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, insoluble in NaOH and Acetonitrile. The λ_{max} was found to be both at 240 nm and 280 nm.

Literature review for RIS analysis revealed that several methods were found based on different technique such as HPLC with UV detection [6], Visible spectrophotometric methods [7-8], LC-MS and HPLC ESI/MS assay for its quantification in plasma and serum [9-12], Chiral Chromatography [13], Pulse Polarography [14], Chemiluminescence assay [15] and LC with coulometric Detection [16]. 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 develop a simple, sensitive, specific, spectrophotometric method for the detection of RIS in pure form and in pharmaceutical dosage form.

MATERIALS AND METHODS

Experimental

Instrumentation:

A double – beam spectrophotometer, Shimadzu, Japan was used for the detection of absorbance, Mettler Tremedo as weighing balance, Bronson sonicator and Borosil glass apparatus were used for the experimental purpose.

Chemicals and Reagents:

Risperidone working standard was supplied by M/S Orchid chemicals and Pharmaceuticals Chennai as a gift sample. Risperidone (2mg tablet) procured from the pharmacies which were manufactured by M/S Torrent Pharmaceutical Ltd Baddi, Solan (HP), India. All other chemicals used in the analysis were AR grade.

Procedure

Preparation of stock solution:

100 mg of pure drug was weighed and transferred to a 100 ml volumetric flask 50 ml methanol was added to the above flask and dissolved, the volume was made up with the methanol. Further dilutions were made to get 40 µg/ml in the final concentration.

Preparation of sample solution:

The average weight of the tablets were determined by weighing 10 tablets and were powdered. Tablet powder equivalent to 2 mg of RIS was weighed and transferred to a 100 ml volumetric flask. About 20 ml of methanol was added and sonicated for 5 min for the complete dissolution of drug, the volume was made up with methanol and mixed well. Then the above solution was filtered through whatmann filter paper. Dilutions were made with methanol to attain a concentration of 40 µg/ml. Six replicates of analysis were carried out with sample weighed individually. The average weight of tablet was found to be 0.202 g.

Method validation

Various methods for analysis of RIS in Pure form and in tablet dosage form was carried out as per ICH guidelines.

Linearity:

The method was validated according to ICH Q2B guidelines [17] for the validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy of the analyte [18-23]. For RIS, five point calibration curves were generated with the appropriate volumes of the working standard solutions for the method. 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 (intra-day) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements [17]. 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 of drug. 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-

$$LOD = 3.3\ s/m;\ LOQ = 10\ s/m$$

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 [17-19].
Stability:

The stability of RIS in methanol solution was studied by the UV method. Sample solutions were prepared in triplicate and stored at 4°C and 25°C for 30, 60 and 90min, after 12 hrs and 24hours.

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 labour. 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 absorption spectrum of RIS in methanol solution is shown in Fig. 2.

It was found that two λ_{max} were observed in which the drug was placed for absorption analysis in UV – spectrophotometer, they are 240 nm and 280 nm, hence these two wavelengths has been taken into account for the further development of the method.

Calibration curves:
Calibration curve data were constructed in the range of the expected concentrations of 20 µg/mL to 60 µg/mL. Beer's law was obeyed over this concentration range. The regression equation was $y = 0.0284\ x$ and $y = 0.028\ x$ for 240 nm and 280 nm respectively. The correlation coefficient (r) of the standard curve was found to be greater than 0.99. The stock solutions and working standards were made in Methanol. The λ_{max} of the drug for the analysis was determined by taking scan of the drug sample solution in the entire UV region. It was found to be that two peaks were observed in the method at the wavelengths of 240 and 280 nm. Calibrated data was presented in table 1 and fig.3a and 3b.

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 methanol and analyzed. With the relevant calibration curves to determine the intra and inter-day variability were determined. The intra and inter-day precision were determined by using RSD %. The precision, accuracy and reproducibility of the results are given in Table 2, 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 label claim of 2 mg. 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 obtained result presented in the table 2 demonstrates the validity and accuracy of the proposed method for the determination of all drugs in pharmaceutical dosage forms. These results reveal that the developed method have an adequate precision and accuracy and consistency. Consequently, it can be applied to the determination of RIS in pure form and pharmaceuticals without any interference from the excipients.

The values of LOD and LOQ were determined by the statistical method and presented in Table 2**.** The stability of the drug solutions which was taken from the linearity study was used for the stability studies and found that it is stable up to 24 hrs. The data were presented in table 3 for 240 nm and 280 nm.

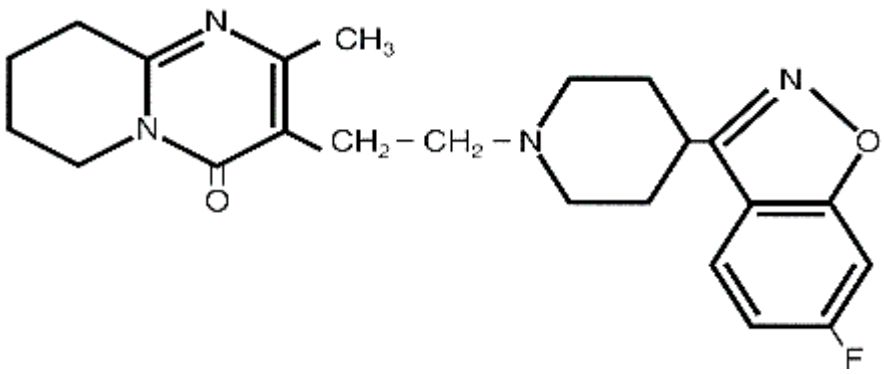

Fig. 1: Structure of Risperidone

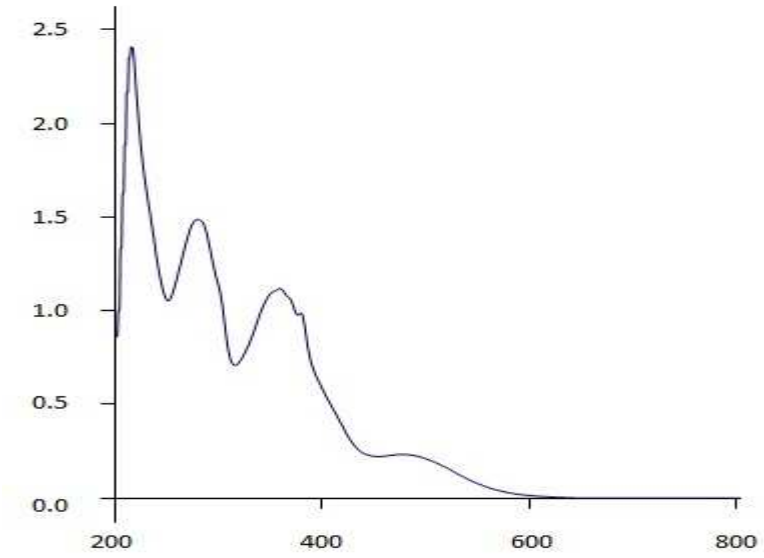

Fig. 2: Absorption spectrum for RIS

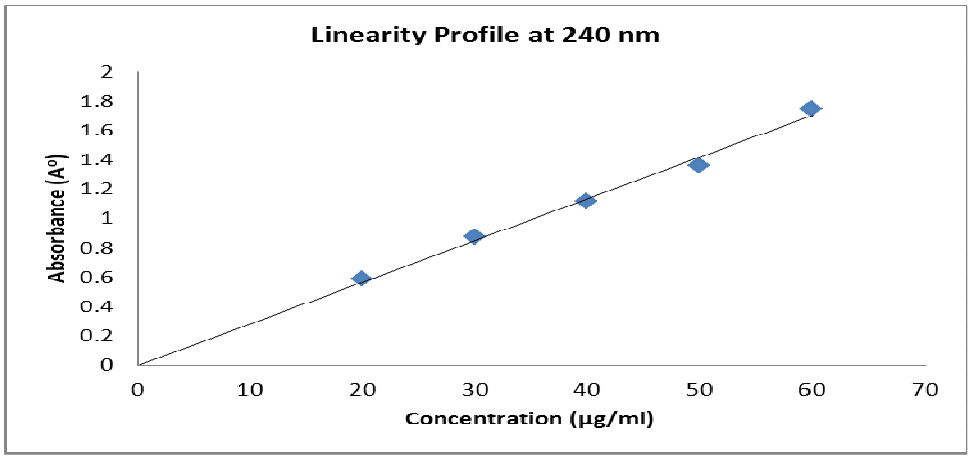

Fig. 3a: Linearity for RIS at 240 nm

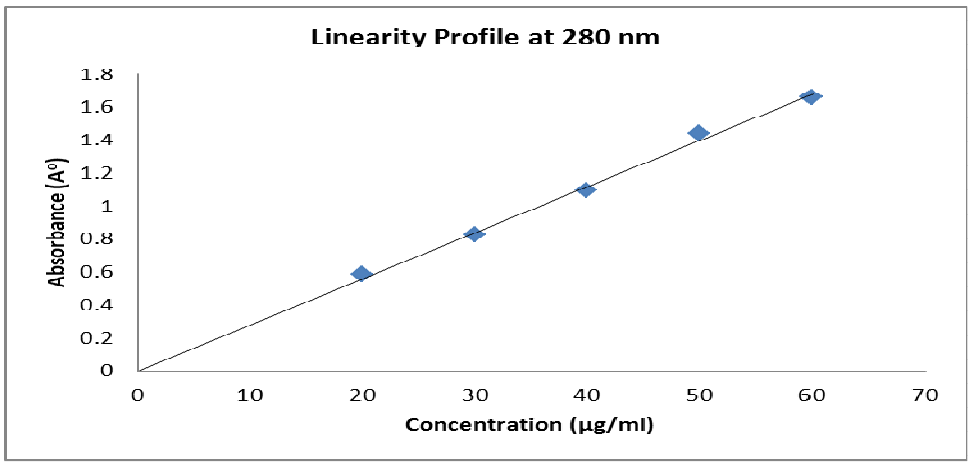

Fig. 3b: Linearity for RIS at 280 nm

Table 1: Linearity data for RIS

Concentration (µg /ml)	Absorbance at 240nm	Absorbance at 280nm
20	0.588	0.584
30	0.872	0.826
40	1.115	1.097
50	1.359	1.443
60	1.746	1.661

Table 2: Validation parameters

Parameters	Values
Linearity range (µg/ml)	20-60
Precision at 240 nm	1.9083 ± 0.2916
Precision at 280 nm	1.9583 ± 0.3416
Accuracy at 240 nm	2.12 ± 0.242
Accuracy at 280 nm	2.19 ± 0.196
LOD (µg/ml)	2.788
LOQ (µg/ml)	8.45
Std deviation	0.044

Table 3: Stability study data

Time	Absorbance at 240nm		Absorbance at 280nm	
	4^oC	25^oC	4^oC	25^oC
0min	0.159	0.159	0.116	0.116
30min	0.160	0.162	0.117	0.112
60min	0.158	0.160	0.116	0.118
90min	0.159	0.157	0.115	0.111
After 12 hrs.	0.158	0.139	0.116	0.0978
After 24 hrs	0.157	0.125	0.114	0.0865

CONCLUSION

The developed spectrophotometric method was simple, sensitive, and specific, for the detection of RIS in pure form and pharmaceutical dosage form. It could be precisely quantify and LOD was found to be 2.788 µg/ml and the limit of quantification to be 8.45 µg/ml. All the calibration curves shows the linear relationship with the absorbance and concentration and coefficient correlation was higher than 0.99. Precision of the method was found to be 1.9083 and 1.9583 at 240 and 280 nm respectively against the label claim of 2 mg. The percentage recovery was found to be 106 ± 0.242 and 109 ± 0.098 at 240 and 280 nm. Since at 240 nm the absorbance of the compound is high. We can use this wavelength for the determination of the RIS, because at this wavelength the concentration requirement is low. The sample solution was stable up to 24 hrs.
The proposed method will be suitable for the analysis of RIS in pure and pharmaceutical dosage form.

REFERENCES

[1] The Merck Index, Merck Research Laboratories division of Merck and company, 13th ed, NJ, USA, **2001**, 1627.
[2] British Pharmacopoeia, British Pharmacopoeia Commission, The Stationary office on behalf of the medicines and health care products Regulatory Agency, Vol.1, 4th ed., **2002**, 1,1500.
[3] Hardman, G., Limbid, L.E. and Gilman, A.G., The Pharmacological Basis of Therapeutics, McGraw Hill. 10th ed., **2001**, 279.
[4] Tripathi K.D, Essentials of Medical Pharmacology, 5th ed., Jaypee Brothers, Medical publishers, New Delhi, **1998**, 391- 397.
[5] ICH, QIA Stability Testing of New Drug Substances and Products, *Int. Con. on Harmonization*, Geneva, November **1996**.
[6] Baldaniya S L, Bhatt K K, Mehta R S, Shah D A, *Ind. J. Pharm. Sci*., **2008**, 70 (4), 494-497.
[7] Singhvi I, Goyal A, *Pharmainfo.net*, accessed on www.pharmainfo.net on 25/04/08.
[8] M.Sravan Kumar, A.Anton Smith, G.Alagumani Vasagam, A.Kottai Muthu and R.Manavalan, *International Journal of Pharma Sciences and Research*, **2010**, 1(2), 122-126.
[9] Huang MZ, Shentu J Z, Chen J C, Liu J, Zhou H, *J Zhejiang Univ Sci B.*, **2008**, 9(2), 114-120.
[10] Zhou Z, L xin, Kunyan L, Zhihong X, Zeneng C, Wenxin P, Wang F, Zhu R, Huande L, *J Chrom. B*, **2004**, 802 (2), 257-262.
[11] Bartlett MG, Zhang G, Terry Jr.A V, *J. Chrom. B*, **2007**, 856(1-2), 20-28
[12] Subbaiah G, Singh S, Bhatt J, *Rapid Comm. Mass Spectro.* **2006**, 20(14), 2109-2114.
[13] Danel C, Barthelemy C, Azarzar D, Robert H, Bonte J P, Odou P, Vaccher C, *J. Chrom. A.*, **2007**, 1163(1-2), 228-36/
[14] Joshi A, Jeyaseelan C, Jugade R, *Croat. Chem. Acta*, **2006**, 79(4), 541-544.
[15] Song Z, Wang C, *J. Pharm. Biomed. Anal.*, **2004**, 36 (3), 491-494.
[16] Schatz D S, Saria A, *Pharmacology*, **2000**, 60, 51-56.
[17] International Conference of Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Methodology, Adopted in Geneva, **1996**.
[18] F.W. Fifield and D. Kealey, 5th ed., Black Well Science Ltd., **2000**, 270 – 276.
[19] G.C. Hokanson, A Life Cycle Approach to the Validation of Analytical Methods During Pharmaceutical Product Development, Part – II: Changes and the Need for Additional Validation, Pharm. Tech., **1994**, 92 -100.
[20] J.M. Green., *Anal. Chem. News and Features*, **1996**, 305A – 309A.
[21] Wegscheider, Validation of Analytical Methods, in: Accreditation and Quality Assurance in Analytical Chemistry, H. Guenzler, Springer (ed.) Verlag, Berlin, **1996**.
[22] J. Vessman, *J. Pharm. and Biomed. Anal*., **1996**, 14, 867 – 869.