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# Development and validation of a highly sensitive spectrophotometric method for the estimation of risperidone in pure and in dosage forms

**Hemavathi N.Deepakumari, Hosakere D.Revanasiddappa***
**Department of Chemistry, University of Mysore, Manasagangothri, Mysore-570 006, (INDIA)**
**E-mail : hdrevanasiddappa@yahoo.com**

## ABSTRACT

The UV spectroscopic method of analysis is widely applicable for routine analytical procedure for the determination of chemical compounds. The present paper describes a simple, highly sensitive and validated UV- spectrophotometric method for the estimation of risperidone (RSP) in both pure and in pharmaceutical preparations. The proposed method is based on the measurement of the absorbance of RSP in THF, which shows maximum absorbance at 285 nm. Calibration graph is linear in the concentration range of 0.5 – 5 µg/ml with correlation coefficient (r) 0.9994. The apparent molar absorptivity is $6.497 \times 10^4$ l/mol/cm. The method gave satisfactory results in terms of repeatability and intermediate precision (RSD < 3.0 %). The method developed was validated and proved to be robust and rugged. The results showed that this method was successfully applied to the determination of risperidone in tablets, and the results were statistically compared with those of the literature method by employing Student's *t*-test and *F*- test.


## KEYWORDS

UV-Spectrophotometry;
Validation; Risperidone;
Pharmaceutical preparations.

## INTRODUCTION

Risperidone (RSP), 3-{2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidin]ethyl}-6,7,8,9-tetra hydro-2-methyl-4H-pyrido-[1,2-]pyrimidine-4-one (Fig. 1) is a atypical neuroleptic drug, reported to have fewer adverse effects than traditional agents, is effective in psychoses[1], such as schizophrenia and other psychiatric illnesses in adults and children[2]. Among the second generation antipsychotics, risperidone (RSP) is commonly used to treat patients with schizophrenia. In 2003, the FDA approved RSP for the short-term treatment of the mixed and manic states associated with bipolar disorder. It is also approved in 2006 European Pharmacopoeia for the treatment of irritability in children and adolescents with autism. The drug is official in 2005 European Pharmacopoeia and the official method of its determination is high-performance liquid chromatography[3].

Chemical literature reveals that, several methods have been used to determine RSP in biological samples including HPLC with electrochemical detection[4,5], RP-HPLC with UV detection[6], electrophoresis[7] and MEPS-LC-UV[8]. Most extensively used technique for its determination is LC-MS/MS but, several procedures using this technique are confined to biological fluids like human plasma[9-12], urine[13] and serum[14]. A limited number of analytical methods for the quantitative estimation of RSP in pharmaceutical samples are known. Procedures based on high performance liquid chromatography and thin layer densitometric methods[15], RP-

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HPLC[16,17], chemiluminescence assay[18], gas chromatographic[19] and spectrophotometry[20-25] methods are available in the literature. The reported chromatographic techniques[4-6,8-17,19] require expensive experimental set-up, whereas the cited spectrophotometric method[20] is less sensitive, lack of selectivity[21], requires extraction step[22] for RSP determination. The reported method[23] is indirect one and methods[24,25] require costly chemicals for RSP analysis. Thus, there is a need to develop sensitive, accurate and cost-effective methods for its determination. The comparison of the performance characteristics of the proposed methods with the existing spectrophotometric methods is shown in TABLE 1.

The aim of the present investigation is to develop a simple, highly sensitive, accurate, reproducible and economically viable or inexpensive method that could be used to determine RSP in bulk drug and in pharmaceutical dosage forms. The proposed method was validated as per the current ICH guidelines[26].

## MATERIALS AND METHODS

### Apparatus

Systronics model 118 UV-vis spectrophotometer provided with 1-cm matched quartz cells were used for spectral and the absorbance measurements.

**TABLE 1 : Comparision of the Performance characteristic of the existing visible spectrophotometric methods with the proposed methods**

| Sl No | Reagent/s used | Methodology | Linear range, µg/ml and molar absorptivity, l/ mol/cm | Remarks | Ref |
|---|---|---|---|---|---|
| 1. | 0.1 N HCl | UV-Spectrophotometry | 2 - 6 | Lack of selectivity | 20 |
| 2. | 0.1 N HCl | UV-Spectrophotometry | 2 - 20 | Less sensitive | 21 |
| 3. | Methyl orange | Ion-pair complex showed absorption maximum at 423 nm | 0.1– 12 | Sensitive but requires tedious extraction procedures. | 22 |
| | Orange G | Ion-pair complex showed absorption maximum at 498 nm | 0.6 – 11 | Less sensitive and requires extraction procedure | |
| | Cobalt thiocyanate | Ternary complex formed was measured at 625 nm | 4 - 80 | | |
| 4. | Chloramine T-Xylene cyanol FF<br>Chloramine T-Malachite green | The absorbance of colored chromogen was measured at<br>612 nm<br>619 nm | 2 - 26<br>2 -18 | Less sensitive and time consuming (< 20 min) | 23 |
| 5. | Bromophenol blue (BPB) in method A<br>phenol red (PR) in method B | Ion-pair complex measured at<br>410 nm<br>400 nm | 0.5 – 10<br>0.5 – 25 | Sensitive but requires costly reagents<br>Less sensitive | 24 |
| 6. | *p*-chloranilic acid (*p*-CA) in method A and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in method B | bright pink colored charge transfer complex measured at 530 nm<br>orange-colored charge transfer complex measured at 460 nm | 0.0 – 25<br>0.0 – 50 | Both the methods are less sensitive | 25 |
| 7. | THF | UV-spectrophotometry measured at 285 nm | 0.0 – 5.0 | Highly sensitive and selective | Developed method |

### Reagents and standards

All chemicals and reagents used were of analytical reagent grade and THF was purchased from Merck (Mumbai, India).

### Preparation of standard RSP solution

Pharmaceutical grade RSP certified to be 99.99 % pure was received from Cipla India Ltd., Mumbai, India, as a gift sample and was used as received. A stock standard solution equivalent to 100 µg/ml of RSP was prepared by dissolving 10 mg of the pure drug in 100 ml THF (Merck, Mumbai, India). Working solutions were prepared as required by dilution.

A pharmaceutical formulation of RSP such as Respidon [Torrent (Mind)], Rispond (Micro Synapse) and Rozidal [Ranbaxy (Solus)] were purchased from

local markets.

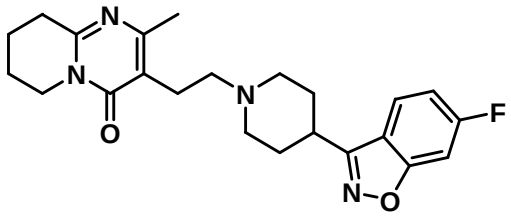

**Figure 1 : Structure of risperidone**

## Procedures

### Preparation of calibration curve

Aliquots of standard solution containing 0.5, 1.0, 2.0……..5 mL of RSP (10 µg/ml) were transferred into a series of 10 ml calibrated flasks using micro burette and the volume was made up to the mark with THF. Absorbance of each solution was measured at 285 nm against THF (blank). Calibration curve was prepared by plotting absorbance *vs* concentration of drug. Concentration of unknown was read from the concurrent calibration curve or the regression equation using Beer's law data.

### Procedure for tablets

For analysis of the tablet dosage forms, twenty tablets from each brand (Respidon [Torrent (Mind)], Rispond (Micro Synapse) and Rozidal [Ranbaxy (Solus)]) were ground into fine powder and quantity of the each powder equivalent to 10 mg of RSP was weighed accurately into a three separate 100 ml calibrated flasks and 10 ml of THF was added. The content was shaken for about 30 min; the volume was diluted to the mark with THF and mixed well and filtered using a Whatman No.41 filter paper. The filtrate containing RSP was at a concentration 100 µg/ml was subjected to analysis by the procedure described above after suitable dilution step.

### Analysis of Placebo blank and synthetic mixture

A placebo blank containing starch 10 mg, methyl cellulose 15 mg, gum acacia 10 mg, talc 10 mg, magnesium stearate 10 mg and sodium alginate 15 mg was prepared by combining all these components to form a homogenous mixture. 10 mg of placebo blank was accurately weighed and its solution was prepared as that described under "*Procedure for tablets*" and then subjected to analysis procedure described under *"Preparation of calibration curve".*

Synthetic mixture was prepared by adding 10 mg of pure RSP to placebo blank. Then the extraction procedure for tablets was applied by taking required quantity of synthetic mixture to get 100 µg/ml RSP solutions. The above mixture was subjected to analysis at three different concentrations equivalent to 0.5, 2 and 4 µg/ml. The results of the study indicate that the common excipients did not interfere in the assay.

## RESULTS AND DISCUSSION

### Absorption spectra

Ultraviolet (UV) spectroscopy is one of the most frequently employed techniques in pharmaceutical analysis. The proposed UV method allows rapid and eco-nomical quantitation of RSP in tablets without any time-consuming sample preparation. Moreover, the spec-trophotometric method involves simple instrumentation compared with other instrumental techniques. Solubility of RSP was checked in different organic solvents and the drug gave good spectral characteristics with the solvent THF. RSP solutions (stock solutions and working standards) made in THF showed absorption maximum at 285 nm. THF did not show any significant absorbance at this wavelength. The absorption spectrum of RSP in THF is presented in Figure 2.

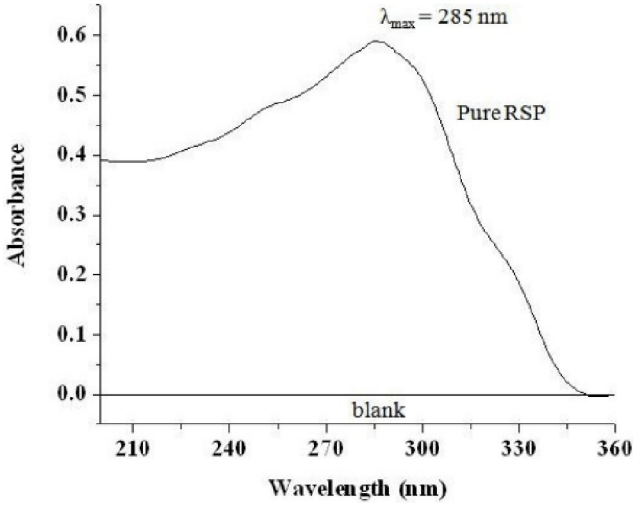


**Figure 2 : Absorption spectrum for RSP**

### Method validation

The method developed was validated as per ICH/ USP guidelines for parameters like linearity and sensi-

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tivity, limits of detection (LOD) and quantitation (LOQ), precision, accuracy, selectivity and recovery.

Linearity, sensitivity, limits of detection and quantitation

A linear correlation was found between the absorbance at 285 nm and concentration of RSP in the ranges is given in TABLE 2. Regression analysis of the calibration curve (Figure 3) using the method of least-squares was made to calculate the slope (b), intercept (a) and correlation co-efficient (r) and the values are presented in TABLE 2. The optical characteristics such as absorption maxima, Beer's law limit, molar absorptivity and Sandell's sensitivity values[27] are also given in TABLE 2.

The limits of detection (LOD) and quantification (LOQ) evaluated as per ICH guidelines using the formulas:

$$LOD = \frac{3.3 \times \sigma}{s}$$

$$LOQ = \frac{10 \times \sigma}{s}$$

where σ is the standard deviation (n=5) of reagent blank determination and s is the slope of the calibration curve.

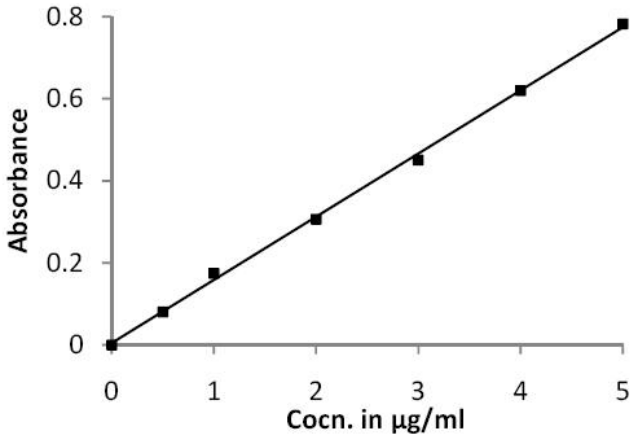


**Figure 3 : Calibration curve for RSP**

## Precision and accuracy

The intra and inter-day precision and accuracy of the proposed method was established by replicate analysis of RSP samples at three different concentrations (low, medium and high) (TABLE 3) within the working limits, each being repeated five times. The relative error, RE (%) and relative standard deviation, RSD (%) values of both intra- and inter-day studies were satisfactory and showed that the best appraisal of the procedures in daily use. The analytical results obtained from this investigation are summarized in TABLE 3. The values of percentage relative error between the concentrations of RSP for taken and found showed the high accuracy of the method. The results obtained are presented in TABLE 3 and show that the accuracy is good.

**TABLE 2 : Analytical and regression parameters of the proposed method**

| Parameter | Method |
|---|---|
| $\lambda_{max}$ nm | 285 |
| Beer's law range (µg/ml) | 0 .5 – 5.0 |
| Molar absorptivity (*ε*), (l/mol/cm) | 6.497 x $10^4$ |
| Sandell sensitivity (µg/$cm^2$) | 0.0063 |
| Intercept (a) | 0.0035 |
| Slope (b) | 0.1542 |
| Correlation coefficient (r) | 0.9994 |
| $S_a$ | 0.0141 |
| $S_b$ | 0.0031 |
| LOQ ( µg/ml) | 0.2336 |
| LOD ( µg/ml) | 0.0771 |

***y*=a+b*x*, where *x* is the concentration of RSP in µg/ml and y is the absorbance at 285 nm., $S_a$ is the standard deviation of the intercept, $S_b$ is the standard deviation of the slope.**

**TABLE 3 : Evaluation of accuracy and precision**

| | RSP taken, µg/ml | Intra-day accuracy and precision | | | Inter-day accuracy and precision | | |
|---|---|---|---|---|---|---|---|
| | | RSP found*, µg/ml | RE, % | RSD, % | RSP found**, µg/ml | RE, % | RSD, % |
| Developed method | 0.5 | 0.493 | 1.34 | 2.85 | 0.503 | -0.56 | 2.40 |
| | 2 | 1.967 | 1.64 | 1.76 | 2.016 | -0.78 | 2.40 |
| | 4 | 3.928 | 1.79 | 2.26 | 3.986 | 0.36 | 1.89 |

**RE: Relative error; RSD: Relative standard deviation; * Mean value of 5 determinations; ** Mean value of 3 determinations**

## Ruggedness

Ruggedness was examined by evaluating the analysis performed by four analysts, and also by a single analyst performing analysis on a three different instruments in same laboratory. These variations do not have any significant affect on the recovery of the method. The result of method ruggedness is shown in TABLE 4.

## Application to analysis of pharmaceutical samples

The method was applied successfully to the determination of RSP in dosage forms. To check the validity of the proposed method, RSP was determined in Respidon [Torrent (Mind)], Rispond (Micro Synapse)

and Rozidal [Ranbaxy (Solus)] and the results are presented in TABLE 5. The results of an assay of tablets were statistically compared with those of the reference method[20] by applying the Student's *t*- test for accuracy and *F*- test for precision. The results in the TABLE 5 showed that there is no significant difference between the proposed and reference methods[20] at the 95 % confidence level with respect to accuracy and precision. The calculated *t*- and *F*- values (TABLE 5) did not exceed the tabulated values (t=2.77 and F=6.39).

**TABLE 4 : Method ruggedness expressed as intermediate precision (% RSD)**

| | RSP taken, µg/ml | Ruggedness | |
|---|---|---|---|
| | | Inter-analysts (% RSD), n=3 | Inter-instruments (% RSD), n=3 |
| Developed method | 0.5 | 1.45 | 2.17 |
| | 2 | 1.58 | 1.66 |
| | 6 | 1.79 | 2.27 |

**TABLE 5 : Results of determination of RSP in tablets and statistical comparison with the reference method**

| Tablet brand Name* | Nominal amount mg per tablet | Found** (% of nominal amount ± SD) | |
|---|---|---|---|
| | | Reference Method [20] | Proposed method |
| Respidon[a] | 1 mg | 102±0.688 | 100.39 ± 0.82 t=1.59, F=2.09 |
| Rispond[b] | 1 mg | 101.5±0.549 | 99.98 ± 0.85 t=1.71, F=2.40 |
| Rozidal[c] | 1 mg | 101.2±0.262 | 100.16 ± 0.44 t = 2.35, F = 2.76 |

***Marketed by: a. Torrent (Mind); b. Micro Synapse; c. Ranbaxy (Solus); **Mean value of five determinations; Tabulated *t*- and *F*-values at 95 % confidence level are 2.77 and 6.39, respectively.**

## Recovery study by standard addition technique

The accuracy and precision of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure drug at three different concentrations and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that frequently encountered common ingredients of formulations were found not to interfere. The results of recovery study are compiled in TABLE 6.

**TABLE 6 : Results of recovery experiments via the standard addition technique**

| Tablet brand name | RSP tablet added, µg/ml | Pure RSP added, µg/ml | Total found µg/ml | Pure RSP recovered* % ± SD |
|---|---|---|---|---|
| Respidon | 1 | 1 | 1.99 | 99.35 ± 0.66 |
| | 1 | 2 | 3.03 | 101.37 ± 0.82 |
| | 1 | 3 | 4.01 | 100.48 ± 0.99 |
| Rispond | 1 | 1 | 2.01 | 100.84 ± 0.83 |
| | 1 | 2 | 2.98 | 99.19 ± 0.20 |
| | 1 | 3 | 3.99 | 99.92 ± 0.85 |
| Rozidal | 1 | 1 | 1.99 | 99.06 ± 0.32 |
| | 1 | 2 | 3.01 | 100.11 ± 0.44 |
| | 1 | 3 | 4.04 | 101.32 ± 0.55 |

*** Mean value of three measurements**

## CONCLUSIONS

The present study describes a fully validated UV-spectrophotometric method for the determination of RSP in pure and in pharmaceutical formulations with enhanced selectivity. The proposed method is simple and not required expensive experimental set up like HPLC and other chromatographic methods. Moreover, in terms of simplicity, rapidity, sensitivity and free from interference by common additives and excipients, the reagents utilized in the proposed method is cheaper, readily available and the procedures do not involve any stringent reaction conditions or tedious sample preparation. On the other hand, in terms of simplicity and expense, the method could be considered superior in comparison with the chromatographic European Pharmacopoeia method and the previously reported spectrophotometric methods, especially with those based on non-aqueous medium. The proposed method is sensitive enough to determine micro amounts of the drug; therefore, it can be used for quality control and routine estimation of RSP in pharmaceutical tablets where precision, time and cost effectiveness of analytical methods are important.

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