RISPERIDONE WAS DEVELOPED AND VALIDATED BY REVERSE PHASE METHOD

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Abstract

A simple and specific liquid chromatographic method was developed and validated to the analysis of Risperidone API content using the RP-HPLC. All the parameters were determined according to ICH guidelines. The detection wavelength of eluent is 254 nm, MeoH and ammonium formate (Ph: 3.0 with OPA) in the ratio 90:10 was used as mobile phases by isocratic elution and C18 150 × 4.6 mm 3 µm part number 68315-15P column is used for separation with a flow rate 1.0 mL/min at ambient temperature to obtain good chromatographic separation results. The linearity data obtained for the concentration ranges from 20 µg to 100 µg obtained a linear curve. It is clear that the response of the risperidone is linear at 20% to 100%. The correlation coefficient is greater than 0.99. The developed method is validated as per ICH guidelines for the parameters of system suitability, linearity, precision, LOD & LOQ and robustness.

Keywords: *Risperidone, Isocratic elution, ambient temperature.*

INTRODUCTION:

Risperidone is chemically 3-[2-[4-(6-flouro-1,2-benzisoxazol-3-yl)-1-piperidinyl]-6,7,8,9-tetrahydro-2-methyl4H-pyridol[1,2-a] pyrimidin-4-one. having chemical formula $C_{23}H_{27}FN_4O_2$ and molecular weight 410.5 g/mol. The brand names of the risperidone in multiple names, Risperdal, Risperdal consta, Risperdal M-Tab. The generic name of the medicine is risperidone. Risperidone is an antipsychotic drug, belonging to the class of medicines known as alternate generation (second generation) of antipsychotics (SGA).

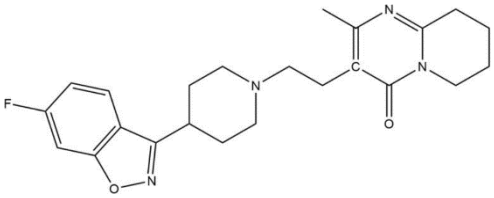
Mode of action:

Risperidone workshop by blocking dopamine and serotonin receptors in the brain. This helps to reduce the symptoms of schizophrenia and bipolar complaint, that delusions, hallucinations, and thought disorder. Several analytical techniques have been described for the estimation risperidone in bulk and pharmaceuticals. Bhavana A. kokane [3] conducted a method validation study in tablet bulk and dosage forms. This study utilized C18 column and mobile phase water: methanol (pH adjusted 5.5 aq. phase), the ratio used as 35: 65 v/v. and the flow rate can be used as 0.900 mL/min. and the detection was carried out in 276 nm. Another simple and consistent method report for determination of risperidone Krishna sanka [6] by using mobile phase composed of methanol: 0.2 % of buffer with OPA in HPLC water in the ratio 80:20 v/v. at a mobile phase flow rate 0.6 mL/min, detection was carried out 235 nm, using PDA detector. From in this two risperidone HPLC-developments we analysed a simple make through and accurately. Therefore, in the present research a simple and accurate method was developed and validated for determination Risperidone API. [2]

Materials and methods

Chemicals & reagents:

Risperidone API, methanol, HPLC grade water, ammonium formate, OPA (ortho phosphoric acid), and formic acid



Structure:

Figure: molecular structure of Risperidone.

Instruments:

A high-performance liquid chromatography- waters alliance with a compact photo diode array detector with an auto injector. The chromatogram was recorded using empower software. 0.45 µm filter paper, electronic analytical balance, Ph meter and ultrasonic bath were used.

PREPARATION OF ANALYTICAL SOLUTIONS:

Diluent preparation:

The diluent of ratio 80:20 (v/v) was prepared by mixing accurately, measured volumes of 80 mL of methanol and 20 mL of water in a 100 mL beaker. Stirred well, sonicated for 5 minutes and stored well.

Preparation of blank: Diluent taken as blank.

Preparation of buffer:

Preparation of 20 mM of Ammonium formate, weighed accurately 0.126 grams of Ammonium formate is taken in to 100 ml volumetric flask and make up to the mark, sonicated for 5 minutes and filtered after that maintain a pH 3.0 is with OPA (ortho phosphoric acid).

Preparation of risperidone API stock solution (1.0 mg/mL);

Transferred 10 mg of risperidone accurately weighed in to 10 mL of volumetric flask. It was dissolves in diluent stirred well and sonicated for 5 min After make up to the mark.

Preparation of test sample solution (100 µg):

Taken one ml of 1.0 mg/mL concentrated Solution in to a 10 mL of volumetric flask then after make up to the mark with diluent.

Method development work flow:

Trail: 1

Mobile phase A: HPLC grade water

Mobile phase B:100% methanol

Sample solution: from the stock solution (1.0 mg/mL) 100 µg concentration of the sample solution is prepared.

Chromatography conditions:

Column : ABX C18 (250 X 4.8)mm 3 µm

Wavelength : 254 nm

Flow rate : 1.0 ml/min

Injection vol. : 10 µL

Column Temp. : 40°C

Run time : 7.5 min

Diluent : Methanol: water (80:20 v/v)

Elution : Isocratic

Observation:

the peak shape is good but noises are observed. The trail 1 chromatogram result was shown in fig.1

Fig.1: typical chromatogram of sample trail 1

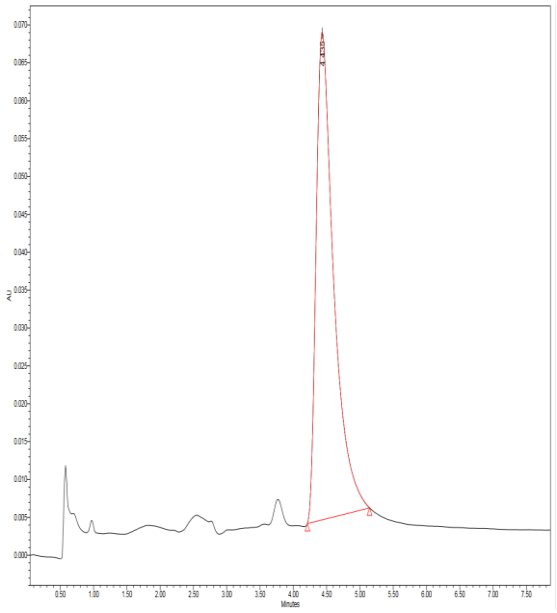


Table:1 results of trail 1

s.no	Compound name	Column	RT	Area	USP Plate count	USP tailing
1	Risperidone	ABX C18 (250 X 4.8) 3 µm	4.43	1134410	3500	1.18

Trail: 2

Mobile phase-A: A buffer solution is 0.1 N of formic acid is prepared, that is 100 µl of formic acid is taken in to a 100 mL of volumetric flask then it make-up to a mark with water.

Mobile phase-B: 100% Methanol

Sample solution: from the stock solution (1mg/mL) 100 µg concentration of the sample solution is prepared.

Chromatographic conditions;

Column : Cogent Bidentate C18 (250 x 4.6) mm 4 µm (P/N: 40018-25P)

Wavelength : 254 nm

Flow rate : 1.0 mL/min

Injection volume : 10 µL

Column temp. : 40^{o}C

Run time : 5.5 min

Diluent : Methanol : Formic acid (90:10)

Elution : Isocratic

Observation; noises are observed. The trail 2 chromatogram result was shown in fig.2

Fig 2: chromatogram of trail 2:

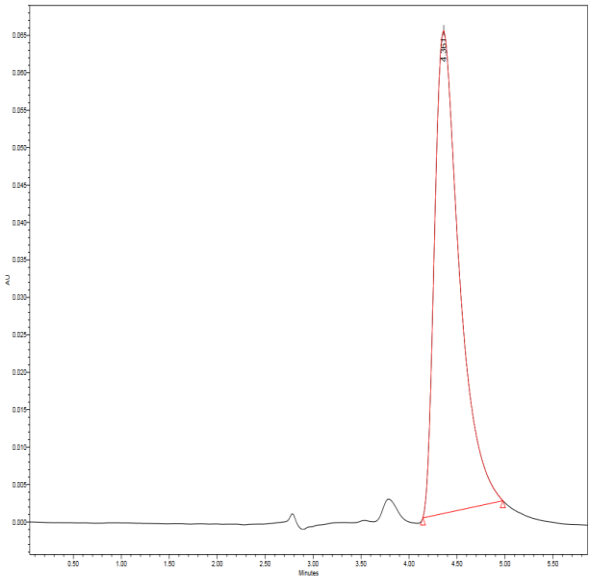


Table2: results of trail2

s.no	Compound name	Column	RT	Area	USP plate count	USP tailing
1	Risperidone	bident c18 (25	4.36	1437 74	4602	1.23

		0 x 4.6 mm) 3 µm				

Trail: 3. Finalized method:

Mobile phase A: A buffer solution is 20 mM of ammonia formate is prepared, for this 0.126 grams of ammonia formate is taken in to a 100 mL volumetric flask dissolves with water and fill up to the mark, and sonicated, filtered after that maintain a PH of 3.0 with OPA.

Mobile phase B: 100% Methanol

Chromatographic conditions;

Column : Cogent RP C18 (150x 4.6)mm 3 µm

Wavelength : 254 nm

Flow rate : 1.0 mL/min

Injection volume : 10 µL

Column temp. : 40°C

Run time : 5 min

Diluent : Methanol : Ammonia formate (90:10 v/v)

Elution : Isocratic

Observation: In this trail we observed that the peak is separated well and no noises, peak shape is good, This trail chromatogram result was shown in fig.3

Fig 3: chromatogram of trail 3

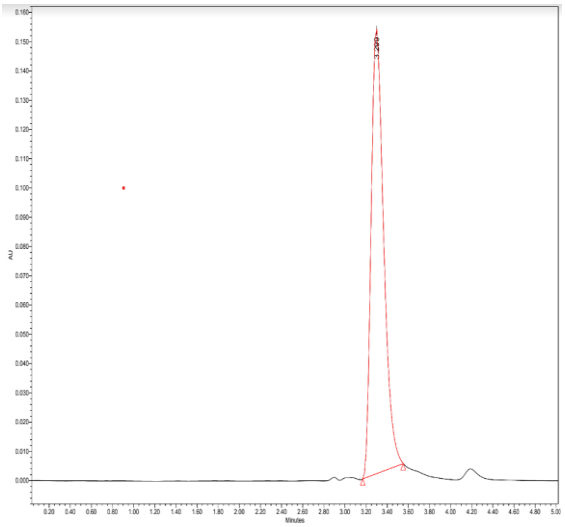
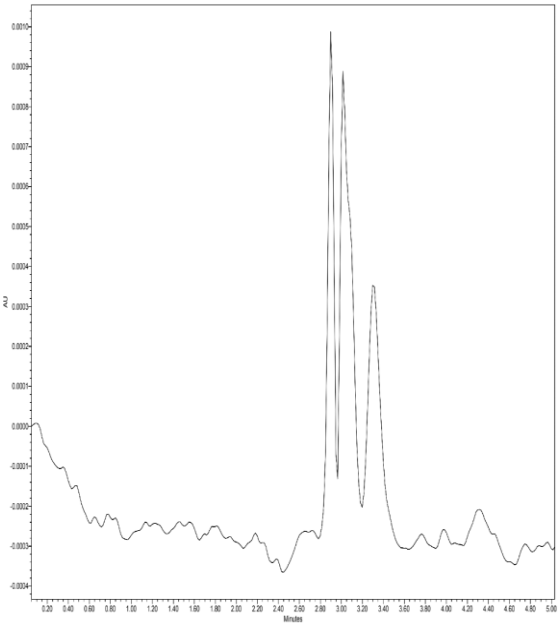


Table3: results of trail3

s. no	**Compound name**	**column**	**RT**	**USP plate count**	USP tailing
1	Risperidone	RP C18 (150 x 4.6) mm 3 µm	3.35	5800	1.08

Fig 4: Blank



Method validation

The process of method validation involves providing documented evidence to ensure a high level of confidence that an analytical method will consistently produce results that accurately reflect the quality characteristics of the products. This is achieved through the validation of analytical procedures, which determines the suitability of the methodology for providing useful analytical data.

Validation is the formal and systematic proof that a method complies with the requirements for testing a product when observing a defined procedure. [1, 2 & 9]

The method validation was done according to the following parameters:

- System suitability
- Linearity
- System precision
- Limit of detection (LOD)
- Limit of quantification (LOQ)
- Robustness

System suitability;

The working solution was prepared in accordance with the prescribed procedure. Under the given chromatographic conditions, six injections were carried out repeatedly. The system suitability testing was conducted as per the ICH guidelines Q2R1, and the recorded results included retention time, area, theoretical plates, and tailing factor. [3,4]

Table 4: system suitability results

s.no	Peak area	RT	Theoretical plate count	Tailing factor
1	1267410	3.30	5750	1.09
2	1276824	3.32	5880	1.10
3	1254724	3.31	5700	1.11
4	1252741	3.30	5780	1.09
5	1252741	3.29	5896	1.08
6	1240636	3.30	5791	1.08
Mean	**1257513**	**3.30**	**5799.5**	**1.09**
SD	**12720**	**0.010**	**-**	**0.011**
%RSD	**1.0**	**0.31**	**-**	**1.07**

Acceptance criteria;

- The % RSD for peak areas of risperidone not more than 2.0
- The % RSD for retention time of risperidone is not more than 0.5
- The theoretical plate count of risperidone is consisting more than 2000
- The tailing factor of risperidone peaks is not more than 2.0

Linearity;

Linearity in HPLC method validation is the ability of the method to produce a response that is directly proportional to the concentration of the analyte over a given range. This is an important parameter to assess because it ensures that the method is reliable and accurate for the quantitative determination of the analyte.

To evaluate linearity during the validation of an HPLC method, a set of standard solutions containing varying concentrations of the analyte must be created and analysed. The response, such as peak area, for each standard solution is then graphed against its respective concentration. The resulting graph should exhibit a linear relationship. If the graph is not linear, the method is deemed non-linear and should not be utilized for quantitative analysis.

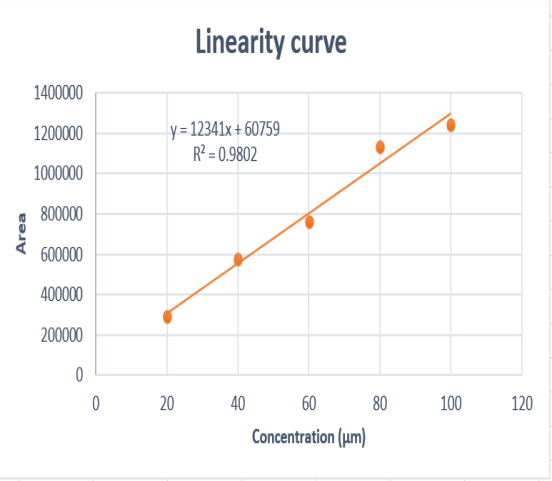
Aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 mL were extracted from the stock solution and subsequently diluted to a final volume of 10 mL, this process yielded resultant solutions with concentrations of 20, 40, 60, 80, and 100 µg/mL, respectively. A calibrated curve was then generated by plotting the concentrations against the corresponding peak areas. The equation of the line, correlation coefficient, and intercept were subsequently determined and recorded as the results. [8 & 9]

Table5: linearity results

S. no	name	Sample conc.	Level (%)	RT	Area
1	Risperidone	20 µg	20 %	3.289	290745
2	Risperidone	40 µg	40 %	3.29	574897
3	Risperidone	60 µg	60 %	3.299	760899
4	Risperidone	80 µg	80 %	3.296	1134487
5	Risperidone	100 µg	100 %	3.301	1245042

Correlation coefficient(r)	0.990051
Regression coefficient	0.9802
Slope	12340.92
Intercept	60758.8

Linearity plot of concentration in µg against area of risperidone



Acceptance criteria;

- The correlation coefficient (r) should be not less than 0.999

Conclusion;

Based on the above data, the experimental results meeting the acceptance criteria. Hence the method is linear.

System precision:

System precision is a specific measure of the reproducibility of the system response. It is calculated by injecting the standard solution multiple times and calculating the RSD of the peak area or peak height.

Six injections of standard solution for system precision and evaluated the system performance as per the protocol requirement. [5]

Table 6: system precision results.

Number of injections (Risperidone)	Area
1	1267410
2	1276824
3	1254724
4	1252741
5	1252741
6	1240636
AVG.	**1257513**
SD	**12720**
%RSD	**1.0**

Acceptance criteria:

- The % RSD for peak areas of risperidone from (n=6) replicate injections of standard solution should be not more than 2.0

LOD and LOQ:

➢ The limit of detection (LOD) refers to the minimum concentration of an analyte that can be detected, although it may not be accurately quantified. This value is commonly determined as the concentration of the analyte that generates a signal three times higher than the standard deviation of the blank signal (3:1).

LOD =Sample containing low concentration of analyte, Ex. dilution of lowest concentration calibration

LOD can be calculated from the formula;

LOD= 3.3σ /s

where σ is the standard deviation of the response at the low end of the calibration curve and S is the slope of the calibration curve.

➢ **LOQ** is the lowest concentration of an analyte that can be reliably quantified with an acceptable level of accuracy and precision. It is typically defined as the concentration of the analyte that produces a signal that is 10 times the standard deviation of the blank signal (10:1).

LOQ= sample with concentration at or above the LOD that is limit of quantified. LOQ ≥ LOD

LOQ can be calculated from the formula;

LOQ= 10σ /s

where σ is the standard deviation of the response at the low end of the calibration curve and S is the slope of the calibration curve. [7, 8]

Table7: LOD &LOQ results

parameter	RT	Linearity (µg/mL)	Value(% RSD)
LOD	3.289	20-100	2.97
LOQ	3.289	20-100	9.01

Robustness:

Robustness in HPLC validation is the ability of an HPLC method to remain unaffected by small, deliberate variations

in method parameters. It is a measure of the reliability of the method and its ability to produce consistent results under different conditions. [7, 8]

Table8: robustness results

Flow rate variation (-0.2 mL)/ decreasing:

Parameter	**Modified**	**Modified %RSD**	**Actual**	**Actual %RSD**
Flow	0.8ml/min	-	1.0 ml/min	-
RT	4.2 min	0.27	3.30 min	0.31
Tailing factor	1.2	0.86	1.10	1.07

Column temperature variation(-3^{0}C)/ decreasing

Parameter	**Modified**	**Modified %RSD**	**Actual**	**Actual %RSD**
Temperature	37	-	40	-
RT	3.27 min	0.15	3.30 min	0.31
Tailing factor	1.10	0.73	1.10	1.07

Column temperature variation (+3)/increasing

Parameter	**Modified**	**Modified %RSD**	**Actual**	**Actual %RSD**
Temperature	43	-	40	-
RT	3.27 min	0.12	3.30 min	0.31
Tailing factor	1.16	0.47	1.10	1.07

Summary and Conclusion:

The study of various parameters led to the development of the analytical method. First of all, maximum absorbance was found to be at 254 nm for risperidone Injection volume was selected to be 10 µl which gave a good peak area. The column used for study was RP C18 (150 x 4.6) mm 3 µm part number 68315-15P chosen good peak shape. 40°C temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0 mL/min because of good peak area and satisfactory retention time. Different pH and ratios of mobile phase were studied, mobile phase- A and Mobile Phase-B As per Optimized method fixed due to good symmetrical peak. This proposed study utilized mobile phase for analysis. Diluent is methanol: Ammonia formate (maintain 3 pH with OPA) (90:10) % v/v was selected because of which all the drug particles were completely soluble and showed good recovery.

The conclusion of HPLC method development and validation of risperidone is that the proposed method is simple, specific, accurate, precise, and robust. The method was validated according to ICH guidelines for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, and robustness. The method was found to be suitable for the

routine analysis of risperidone in bulk and dosage forms.

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