www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN(USA): IJPRIF ISSN : 0974-4304
Vol.1, No.3, pp 568-574 , July-Sept 2009

Determination of Risperidone and forced degradation behavior by HPLC in tablet dosage form

A. P. Suthar[1]*, S. A. Dubey[2], S. R. Patel[1], A. M. Shah[1]

[1]K.N.V. Pharmacy College, Metoda, Rajkot, India

[2]Lachoo Memorial College of Science & Technology, Sector-A, Shastrinagar, Jodhpur.

Email: kumar4a@gmail.com

Abstract : A simple, specific, sensitive, precise stability-indicating high-performance liquid chromatography method for determination of Risperidone in tablet dosage form was developed and validated. A Waters Xterra RP8 column (250*4.6 mm, 5 µ) in isocratic mode, with mobile phase consisting of a mixture of solution (10 mM potassium dihydrogen phosphate, pH 3.5 ± 0.05): acetonitrile: methanol (65:20:15) was used. The quantitation performed at flow rate of 1.0 mL/min at 276 nm and run time was 12 min. The analytical method was validated as per ICH guideline for linearity, accuracy, precision, specificity, limit of detection, limit of quantification, robustness and stability and method can be extended to the analysis of Risperidone in tablet formulations. the relative standard deviation values for precision was less than 2%, and % recovery was greater than 98% for risperidone. The drug undergoes oxidative degradation only.

Key Words: Risperidone, RP-HPLC, Validation, Specificity

Introduction

Risperidone (RISP) is belonging to the chemical class of benzisoxazole derivatives and chemically, it is 4-[2-[4-(6-fluorobenzo[*d*]isoxazol-3-yl)-1-piperidyl] ethyl]-3-methyl-2, 6 diazabicyclo [4.4.0] deca-1, 3-dien-5-one with molecular formula $C_{23}H_{27}FN_4O_2$ and CAS number 106266-06-2 [(1)]; Risperidone is official in BP 2007 [(2)]. Risperidone is atypical psychotropic agent and used as an antipsychotic for bipolar disorder, borderline personality disorder, drug intoxication, brief drug-induced psychosis, and other schizophreniform and psychiatric disorder. Risperidone is mostly metabolized by alicyclic hydroxylation and oxidative *N*-dealkylation [(3)].

Literature review for risperidone analysis revealed several methods based on different technique, such as; Visible spectrophotometric methods [(4)]; HPLC with UV detection [(5)]; LC-MS and HPLC-ESI/MS assay for its quantification in plasma and serum [(6-9)]; Chiral Chromatography [(10)]; Pulse polarography [(11)]; Chemiluminescence assay [(12)]; LC with Coulometric Detection [(13)]. However, there is no method reported for quantification of RISP in tablet dosage forms in the literature.

Stress testing carried out to elucidate the inherent stability characteristic of the active substances and forms an important part of the API and drug product development. It suggests that degradation products that are formed under a variety of conditions should be identified and degradation pathways be established. The purpose of stress testing is to provide evidence on how the quality of drug substance varies with time under the effect of varieties of environmental factors such as temperature, humidity, light and presence of oxygen. An ideal stability-indicating method is one that quantifies the drug and also resolve its degradation products [(14)].

The aim of present work is to develop a simple, specific, sensitive, accurate and stability indicating HPLC analytical procedure for the analysis of risperidone in tablet dosage form in the presence of its degradation products and related impurities as per ICH guideline [(15)].

Experimentals

Reagent and Materials

Risperidone working standard was supplied by Torrent Pharmaceutical Ltd. and sample tablet (Label claim: 1 mg

and 4 mg; Respidon tablet; and manufacturer: Torrent Pharmaceutical Ltd.) were procured from the local market. Methanol, acetonitrile and water (HPLC grade), potassium dihydrogen phosphate, triethylamine, ortho-phosphoric acid (all AR grade) were used.

Apparatus

Shimadzu HPLC system equipped with LC-2010HT with SPD-10Avp UV and PDA detector, Waters Xterra RP8 column (250*4.6 mm, 5µ). Sartorius weighing balance, CP225D and PEI make ultra-sonicator were used for experimental purpose.

Methods

Diluting agent preparation

Solvent mixture of water and acetonitrile, in the ratio of 50:50 (v\v) was used as diluting agent.

Stock Solution

Accurately weighed 25.10 mg of Risperidone Working standard (Potency 99.73%) was transferred into 100 mL volumetric flask, dissolved and volume was made up to the mark with diluting agent. The final solution contained 250.3µg/mL of risperidone.

Standard Solution

10 mL of risperidone stock solutions was transferred to a 100 mL clean volumetric flask and the volume was made up with diluting agent and mix well. The solution was then filtered through 0.2 µm glass nylon filter. The final solution (25.03 µg/mL) was injected into the HPLC system.

Chromatographic condition

Chromatographic separation was achieved at 25°C on a reverse phase RP8 column using mobile phase consisting of potassium dihydrogen phosphate buffer, methanol and acetonitrile in the ratio of 65:15:20 (v/v/v). Potassium dihydrogen phosphate buffer was prepared by dissolving 0.34 gm buffer in 250 mL HPLC grade water and 0.25 mL of triethylamine was added to it and pH was adjusted to 3.5±0.05 with dilute ortho-phosphoric acid. The flow rate was kept at 1.0 mL/min and detection was carried out at 276 nm. The sample was injected using 10 µl fixed loop, and the total run time was 12 min.

Sample preparation

Twenty tablets were weighed accurately; the average weight was determined and then ground to a fine powder. The quantity of powder equivalent to 25 mg of Risperidone was transferred to 100 ml volumetric flask and mixed with 65 ml of diluting agent and solution ultrasonicated to dissolve content for 45 minutes then after volume was made up to mark with same diluting agent, filtered through 0.2 µm glass nylon filter. This solution was further diluted with same solvent to obtain concentration of 25 µg/mL. Working standard of risperidone was also prepared in the same way at that concentration level and 10 µl was injected.

Validation of method

Validation of the developed method was done according to ICH Q2B guideline 1996.

Stress Degradation of Risperidone

(i) Acid and base-induced degradation

25mg accurately weighed drug was transferred to 50 mL volumetric flask. To it, 50 mL of diluting agent was added and sonicated for 20-25 min with intermittent shaking. To it, 10 ml of 5N hydrochloric acid and 10 ml 5N sodium hydroxide was added separately and finally volume were made up to 100ml. Sample was refluxed on water bath for 10 hrs.; neutralized by alkali and acid respectively, and diluted 10 times with same solvent before injection and sample was analyzed with respect to unstressed sample.

(ii) Hydrogen peroxide-induced degradation

The method described above (i) was followed by except that 3.5 mL of 3% H_2O_2 was added in place of HCl/NaOH. The sample was kept at ambient temperature for 5 min. and analyzed with respect to unstressed sample.

(iii) Thermal degradation

The sample was kept at 105°C for 72 hrs. and method described in (i) followed here but, HCl/NaOH was not added and analyzed with respect to unstressed sample.

(iv) Photolytic degradation

The sample was kept in a photolytic chamber at 1.2 million/hour, followed by analysis as per proposed method.

Results and Discussion

Method development

The chromatographic condition were optimized with a view to develop a stability-indicating assay method for Risperidone in tablet dosage forms. Three different column, namely, Waters Symmetry (100 ×3 mm, 3.5µ),

Inertsil ODS 3 (250×4.6 mm, 5µ) and Waters XTerra RP8 (250 × 4.6 mm, 5µ) were tried as under chromatographic conditions. The column Waters XTerra RP8, gave good peak shape with response at affordable retention time with peak purity of Risperidone in presence its degradation products.

Also sodium salts of citric acid, sodium and potassium dihydrogen phosphate were tried at concentration level of 10 mM to 20 mM. The isocratic profile, methanol and acetonitrile in mobile phase varied from 10-40% (v/v), was also altered to give the best separation of the peaks. Using 10 mM potassium dihydrogen phosphate solution, 0.1% triethylamine, pH 3.5±0.05 by dilute ortho-phosphoric acid and Risperidone peak amongst degradation products peaks were found resolved. The final chromatographic system comprising e reverse-phase C8 column (250 × 4.6 mm, 5µ) with a mobile phase consisting of a mixture of solution (10mM potassium dihydrogen phosphate, 0.1% triethylamine, pH 3.5±0.05 adjusted with ortho-phosphoric acid), methanol and acetonitrile in a ratio of 65:15:20 at a flow rate 1.0 mL/min was found optimum. Detection was performed at 276 nm. (Table 1). A typical UV and PDA chromatogram are shown in Fig. 1.

Calibration curve

The linearity of the response for risperidone assay method was determined by preparing and injecting standard solutions with concentrations of 4.973 - 44.757 µg/ml Risperidone (Fig. 2). The linear regression data for the calibration curves indicate that the response is linear over the concentration range studied with correlation coefficient, r2 value as 0.998. The values of slope and intercept were 34018 and -54237, respectively. (Table 2)

Validation of the method

Precision

The precision of analytical method is determined by assaying a sufficient number of aliquots of homogenous sample to be able to calculate statistically valid estimate of % RSD (Relative Standard deviation). System precision and method precision of a standard sample were carried out using six replicate of same solution and six injections from six different solution (15 µg/ml), respectively. It showed RSD of 0.238 and 0.444, respectively. This shows method is precise as relative standard deviation is below 2.0%. Intermediate precision of the method was determined by same sample using different day, different analyst, different laboratory, different instrument, and different column at wavelength of 276 nm. Validation parameters for analysis of Risperidone is presented in Table 3.

Accuracy

The accuracy of the method was determined by spiking working standard into tablet solution. The recovery studies were performed by standard addition method, at 75%, 100%, 125% level. The resulting solutions were assayed in triplicate and the results obtained were compared with expected results and expressed as percentage. Recovery of Risperidone in the range of 98.27-101.00% shows method may be used for routine analysis of Risperidone in tablet dosage form. The percent recovery indicates the accuracy of the developed method.

Robustness

Robustness of the method was determined by analyzing standard solution at normal operating condition and also by changing some operating analytical condition such as flow rate, column oven temperature, detection wavelength, mobile phase acetonitrile content and buffer pH.

The parameters and results of normal operating condition (control) against changed conditions are included in Table 4.

These data were subjected to ANOVA test to see any significant difference between the data sets. No significant difference in mean %assay was found as the calculated value of F is lower than the critical value of F. Hence, the robustness of the method is established to the extent of variations applied to the experimental conditions.

System suitability

As per USP XXII16, system suitability tests were carried out at before performing each of validation parameters for risperidone by the proposed HPLC method summarized in Table 5.

Baldaniya S L et al. “RP-HPLC estimation of risperidone in tablet dosage forms” suggested only estimation of Risperidone in tablet dosage form with 50 mM KH2PO4: methanol:acetonitrile-10:10:80, as a mobile phase; but the proposed method performed at pH 3.5 with same solvent, difference in ratio for establishing stability indicating method of Risperidone in tablet dosage forms.

Analysis of marketed formulations

The developed method was applied to the analysis of Risperidone in tablet dosage from marketed as Respidon (Label claim 1 and 4 mg strength, Torrent Pharmaceutical Ltd.). The results of analysis are given in Table 6.

The contents of marketed tablet dosage form were found to be in the range of 100±2% with RSD less than 2% which indicate suitability for routine analysis of Risperidone in tablet dosage form.

Stability indicating property

The values of assay, degradation (%), peak purity index with stress condition are given in Table 7. The stressed condition samples are evaluated relative to the control sample with respect to assay and degradation (%). The degradation (%) of Risperidone indicate susceptibility of drug to peroxide degradation only. The peak purity index is a measure of spectral heterogeneity of a peak based on the comparison of spectra ovr the entire peak. The non-ideal effects are quantified and provided as a value of peak purity index. When the peak is pure, the peak purity index is greater than 0.990 as seen in Table 7.

Conclusion

The developed HPLC method is specific, accurate and stability indicating. Statistical analysis proves that method is precise, robust and selective for analysis of

Risperidone in tablet dosage form. The developed method is suitable for the quality control analysis of Risperidone in tablet dosage form.

Table 1 Optimized chromatographic condition of Risperidone for the proposed method

Conditions	Results
Mobile Phase	Buffer: Methanol: ACN – 65:15:20, Isocratic, Buffer: - 10mM KH_2PO_4, 0.1% TEA, pH 3.5 by dilute OPA
Diluting agent	Water: ACN – 50:50
Column	Waters XTerra RP8 (250 × 4.6 mm), 5µm particle size, pore size 125Å, Waters Corporation
Column Oven	25°C (ambient)
Flow rate	1.0 ml/min
Detector	UV at 276 nm
Injection Volume	10 µl
Sample Cooler temp.	15°C
Run time	12 minutes

Table 2 Regression analysis of the calibration curve for the proposed method

Parameters	Values
Calibration range (µg/ml)	4.973-44.757
Slope (m)	34018
Intercept (c)	- 54237
Correlation coefficient (r^2)	0.998

Table 3: Summary of validation parameters for the proposed method

Parameters	Values
Specificity	No interference was found to be w.r.t. excipients, impurities
Linearity range (µg/ml)	5-45
Precision (n=6)	
System Precision	0.238*
Method Precision	0.444
Intermediate precision	0.521
Accuracy (%)	98.27 – 101.00
LOD (µg/ml)	0.230
LOQ (µg/ml)	0.698
Robustness	Robust#

* % RSD

To the extent of variations applied in analytical conditions

Table 4: The influence of changes in chromatographic parameters on RP-HPLC analysis of Risperidone (Method Robustness)

Conditions	Results
Robustness (ANOVA)	$F_{stat} < F_{critical}$
a). Change in Flow rate	
• 1.2 ml/min	Pass
• 0.8 ml/min	Pass
b). Change in ACN	
• -2%	Pass
• +2%	Pass
c). Change in Column temperature	
• 20°C	Pass
• 30°C	Pass
d). Change in Wavelength	
• 274 nm	Pass
• 278 nm	Pass
e). Change in pH of buffer	
• 3.3	Pass
• 3.7	Pass

Table 5 System suitability parameters for Risperidone by the proposed method

Parameter		Theoretical Plates	Asymmetry	%RSD of replicate of injection
Limits:		***NLT 2000***	***NMT 2.0***	***NMT 2.0%***
Specificity		5568.03	1.21	0.699
Linearity		5503.96	1.13	0.138
Precision	System	5545.40	1.13	0.238
	Method	5510.32	1.12	0.496
Ruggedness*		5629.96	1.11	0.520
Accuracy		5502.37	1.12	0.783
Robustness*		5915.00	1.10	0.320
Solution Stability		5544.24	1.13	0.213
Forced Degradation*		5810.74	1.10	0.567

* mean of replicate determinations.

Table 6: Analysis of marketed formulations

Brand name	Label claim (mg)	Mean ± SD	% RSD
Respidon-1	1	99.14 ± 0.281	0.283
Respidon-4	4	100.73 ± 0.196	0.195

Table 7Stress study data for Risperidone

Conditions	Assay degradation	Resolution between Risperidone and Closely eluting peak	Peak purity
Control sample	-	-	0.999
Acid-5N/10ml, 10 hrs.	-	-	1.000
Alkali-5N/10ml, 10 hrs.	-	-	1.000
Peroxide-3%(w/v)H2O2, 3.5ml, 5 min	11.0	2.88	0.999
Thermal deg, 105°C/72hrs.	-	-	0.999
Photolytic deg, (1.2 million lux/hrs.)	-	-	0.999

Figure 1a) Typical UV chromatogram of standard solution

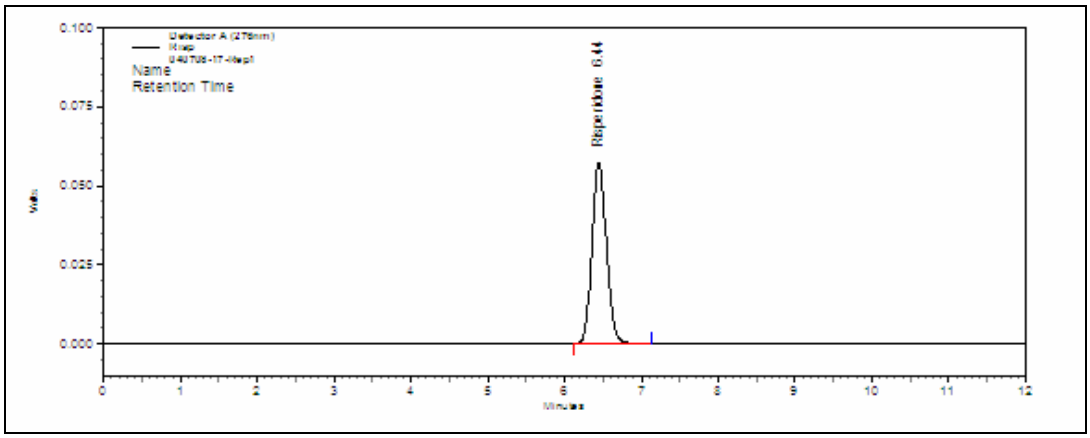


Figure 1b) PDA chromatogram of standard solution

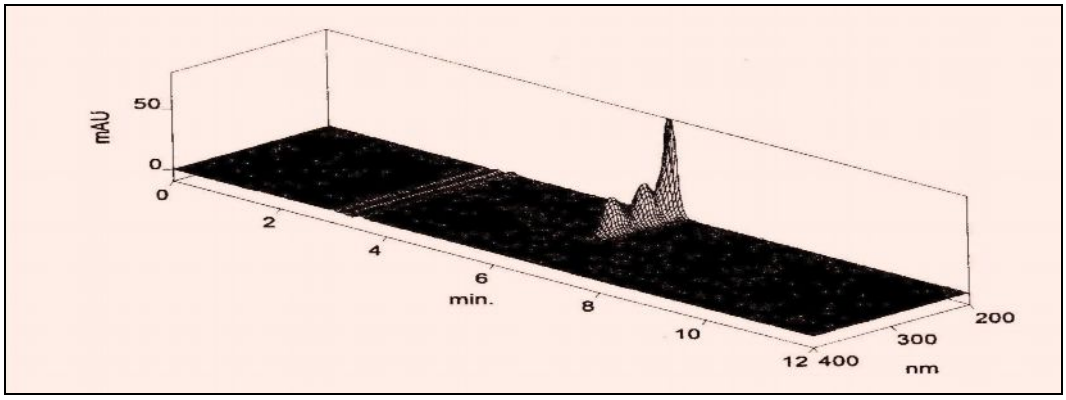
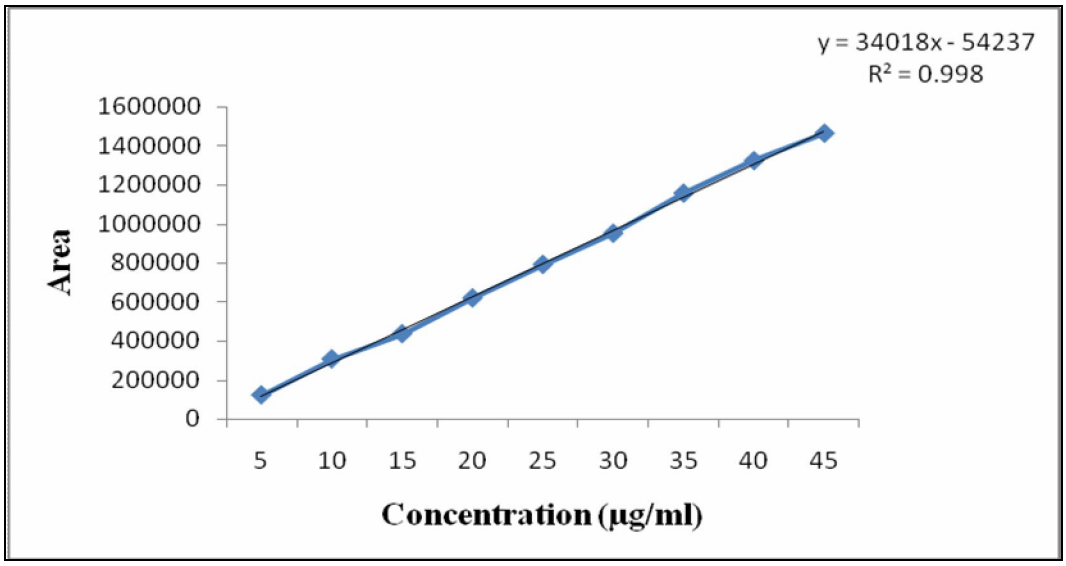


Figure 2 Linearity curve for Risperidone by the proposed method



References

1. The Merck Index, Merck Research Laboratories, division of Merck and company, 13th ed, NJ, USA, 2001, 1627.
2. British Pharmacopoeia, Medicines and Healthcare Products Regulatory Agency (M.H.R.A), (2007).
3. Tripathi K D, Essentials of Medical Pharmacology, 5th ed, Jaypee Brothers, Medical Publishers, New Delhi, 747, 150, 391, 394, 396, 397.
4. Singhvi I, Goyal A, "Visible spectrophotometric determination of risperidone in tablet formulations" accessed on www.pharmainfo.net on 25/04/08
5. Baldaniya S L, Bhatt K K, Mehta R S, Shah D A, "RP-HPLC estimation of risperidone in tablet dosage forms" Indian J. of Pharm. Sci., 2008, 70 (4), 494-497.
6. Huang M Z, Shentu J Z, Chen J C, Liu J, Zhou H "Determination of risperidone in human plasma by HPLC-MS/MS and its application to a pharmacokinetic study in Chinese volunteers" J Zhejiang Univ Sci B., 2008, 9(2), 114–120.
7. Zhou Z, L Xin, Kunyan L, Zhihong X, Zeneng C, Wenxin P, Wang F, Zhu R, Huande L, "Simultaneous determination of clozapine, olanzapine, risperidone and quetiapine in plasma by high-performance liquid chromatography–electrospray ionization mass spectrometry" Journal of Chromatography B, 2004, 802(2), 257-262.
8. Bartlett M G, Zhang G, Terry Jr. A V, "Simultaneous determination of five antipsychotic drugs in rat plasma by high performance liquid chromatography" Journal of Chromatography B, 2007, 856(1-2), 20-28.
9. Subbaiah G, Singh S, Bhatt J, "Liquid chromatography/tandem mass spectrometry method for simultaneous determination of risperidone and its active metabolite 9-hydroxyrisperidone in human plasma" Rapid Communications in Mass Spectrometry, 2006, 20(14) , 2109 – 2114.
10. Danel C, Barthelemy C, Azarzar D, Robert H, Bonte J P, Odou P, Vaccher C, "Analytical and semipreparative enantioseparation of 9-hydroxyrisperidone, the main metabolite of risperidone, using high-performance liquid chromatography and capillary electrophoresis. Validation and determination of enantiomeric purity", J. Chromatogr A., 2007, 1163(1-2), 228-36.
11. Joshi A, Jeyaseelan C, Jugade R,"Differential pulse polarographic studies of risperidone in pharmaceutical formulations", Croat. Chem. Acta, 2006, 79(4), 541-544.
12. Song Z, Wang C,"Sensitive chemiluminescence assay for risperidone in pharmaceutical preparations", J. Pharm.Biomed. Anal., 2004, 36(3), 491-494.
13. Schatz D S, Saria A, "Simultaneous Determination of Paroxetine, risperidone and 9-Hydroxyrisperidone in Human Plasma by High-Performance Liquid Chromatography with Coulometric Detection" Pharmacology, 2000(60), 51-56.
14. ICH, Q1A Stability Testing of New Drug Substances and Products, Int Conf on Harmonization, Geneva, November 1996.
15. ICH Q2B Validation of Analytical Procedure: Methodology, Int Conf on Harmonization. Geneva, November, 1996.
16. United States Pharmacopoeia, XXII edn (The US Pharmacopoeial Convention, Inc. 12601 Twinbrook Parkway, Rockville MD 20852), 1990, 1566.

*****