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Method Development and Validation of Stability Indicating Assay for Risperidone in Solid Dosage Form by Using HPTLC

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A simple, selective, rapid and precise stability indicating HPTLC method was developed for the quantitative estimation of risperidone in tablet dosage form and validated. The method was performed by using of HPTLC plates (Merck) pre-coated with silica gel 60F-254 on aluminium sheets and a mobile phase comprising of acetonitrile:triethylamine (5:0.2 v/v). Densiometric analysis of the drug was performed in the absorbance mode at 279 nm. The drug was subjected to acid-alkali hydrolysis, oxidation and photolytic degradation and was found to be susceptible to these methods. Linearity of risperidone was found to be 100-700 ng/spot (r^2 = 0.996). The limit of detection and limit of quantitation was found to be 46.56 and 141.12 ng/spot respectively for risperidone. The percentage recovery of risperidone was 99.08-99.95 %. The % RSD values for intraday precision study were < 1.0 % and for inter-day study were < 2.0 %, confirmed that the method was sufficiently precise. The validation studies were fulfil International Conference on Harmonization (ICH) requirements. The method was validated for the precision, robustness and recovery. Results revealing effective separation of the drug from its degradatied products confirm that it can be employed as a stability indicating method.

Keywords: HPTLC, Estimation, Validation, Risperidone, ICH.

INTRODUCTION

Risperidone (Fig. 1) 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 is antipsychotic drug used for the treatment of schizophrenia, the mixed and manic states associated with bipolar disorder and irritability in children with autism [1]. Blockade of dopaminergic D2 receptors in the limbic system alleviates positive symptoms of schizophrenia such as hallucinations, delusions and erratic behaviour and speech [2]. The techniques include chemiluminescence [3], RP-HPLC [4], TLC [5] and visible spectrophotometry [6] other methods to quantify risperidone in bulk dosage form, including HPLC combined with capillary electrophoresis [7], HPLC-DAD [8], HPLC-MS/MS [9], RP-HPLC [10], LC-MS/MS [11-13], affinity capillary electrophoresis and ^{1}H NMR spectroscopy [7]. These methods [7,11-13] are time consuming in comparison to a simple HPLC and UV method. Previous methods are not directly applicable for this issue and need more investigation for method development and validation. So, an approach was made to develop a simple, precise, accurate, specific and robust stability-indicating HPTLC method for the quantitative determination of risperidone in solid dosage forms.

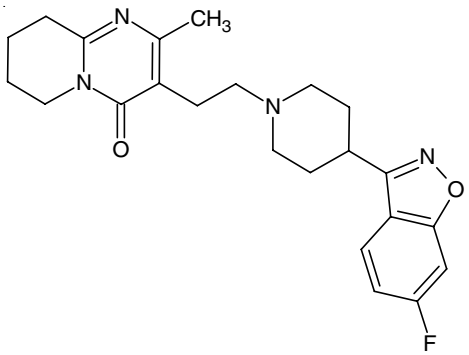

Fig. 1. Chemical structure of risperidone

EXPERIMENTAL

Methanol was used as a solvent for preparing drug solutions. Risperidone was obtained from Torrent Pharmaceuticals Ltd. Ahmedabad India and used as working standards. Acetonitrile and triethylamine (HPLC grade, S.D.Fine. Chem. Ltd., Mumbai) as mobile phase.

Experimental work was performed on (20 cm × 10 cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany) aluminium backed silica gel 60 F254 HPTLC plates.

Selection of stationary phase: Separation and identification of the drug was successfully performed on (20 cm × 10 cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany) aluminium backed silica gel 60 F_{254} HPTLC plates.

Selection and optimization of the mobile phase: Firstly, single solvents were tried on the basis of their polarity to resolve the spot. Then the mixtures of solvents or modifiers were used to resolve the spot of risperidone (Table-1). The spot was resolved in mixture of acetonitrile and as a modifier triethylamine. The spot was resolved in single solvent acetonitrile but slight tailing was observed. Hence, to reduce the tailing modifier which acts as an ion pair reagent or ion suppressing reagent triethylamine was added in the solvent system. Thus, the mobile phase consisted of acetonitrile:triethylamine in ratio 5:0.2 (v/v).

TABLE-1
OPTIMIZATION OF MOBILE PHASE

Solvent System	Composition (v/v)	Polarity of solvents	R_f (risperidone)
Chloroform	2	4.4	3.2
Ethyl acetate	4	4.3	5.2
Acetonitrile	5	6.2	2.5
Acetonitrile:triethylamine	5:0.2	6.2	3.5

Detector and detection wavelength selection: UV spectra of risperidone were recorded in methanol and shows maxima at 279 nm.

Preparation of standard stock solution and calibration curve: Standard stock solution was prepared by dissolving 10 mg of risperidone in methanol and volume was adjusted to 10 mL with solvent, to give a solution containing 1000 ng/µL of risperidone. From that 1 mL was withdrawn and mix in 10 mL of methanol, to give a solution concentration of 100 ng/µL. Linearity was carried by applying the stock solution on plate by microliter syringe with the applicator to give concentrations of 100-700 ng/spot of risperidone. All concentration was spotted six times on the plate. Calibration curve was established by plotting peak area on ordinate and corresponding concentration on abscissa (Fig. 2).

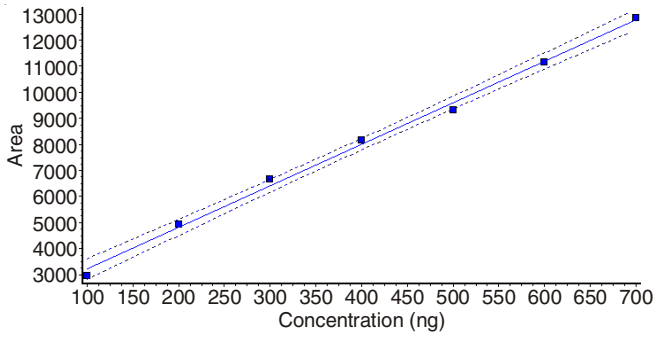

Fig. 2. Calibration curve of risperidone

Preparation of physical mixture: Risperidone (4 mg) was accurately weighed and transferred to 10 mL volumetric flask, dissolved in methanol and volume was made up to mark with same solvent. The solution (1 µL, containing 400 ng of risperidone) was spotted; the plate was developed and scanned (Table-2). The concentration was determined by regression equation.

TABLE-2
RESULTS OF ANALYSIS OF PHYSICAL MIXTURE

Component	Amount taken (ng)	Amount found (ng) ± SD	RSD (%) (n = 6)
Risperidone	400	400.24 ± 1.39	0.3472

Application of method to estimate the drugs in tablet formulations: The determination of risperidone in tablet dosage form, twenty Sizodone-4 tablets were accurately weighed, their average weight calculated and powdered it. Twenty tablets was used for the assay. Six replicates of solutions, containing 4 mg of risperidone was obtained by dissolving the powdered material in methanol, which was sonicated for 0.5 h. The solutions was filtered and diluted to 100 mL with methanol and applied on the plate to give 400 ng/spot of risperidone (Table-3). HPTLC plate was developed as described above and the spots were scanned at 279 nm.

TABLE-3
ASSAY OF RISPERIDONE IN TABLETS

Component	Label claim (mg)	Amount found mg ± SD (n = 6)	Label claim (%)	RSD (%)
Risperidone	4	3.985 ± 0.199	99.64	0.199

RESULTS AND DISCUSSION

Linearity: The linearity was studied in the concentration range of 100-700 ng/spot and for risperidone. Linear regression data is shown in Table-4.

TABLE-4
RESULTS OF LINEARITY STUDIES

Parameter	Risperidone
Linearity range (ng/spot)	100-700
Slope	15.968
Y-intercept	1616.6
Correlation coefficient (r)	0.9965

Precision: Repeatability of the developed method was performed by analyzing six samples of same drug concentrations on a TLC plate. Chromatograms were recorded and area of each spots was measured. Results of this determination are reported in Table-5.

TABLE-5
RESULTS OF REPEATABILITY OF METHOD

S. No.	Area of risperidone [400 ng/spot]
1	8172.7
2	8157.3
3	8169.2
4	8183.9
5	8178.4
6	8178.2
Mean	8172.3
SD	10.08
RSD (%)	0.1233

Intra-day and inter-day precision: Intra-day precision was determined by analyze by three different concentrations and each concentration for three times, on the day. Inter-day precision was determined by three different concentration from 3 different day. The results are summarized in Tables 6 and 7.

TABLE-6
RESULTS OF INTRA-DAY PRECISION

Risperidone		
Concentration (ng/spot)	Amount found ng/spot ± SD (n = 3)	RSD (%)
300	399.70 ± 5.091	1.2737
400	400.45 ± 7.536	1.8818
500	498.35 ± 4.455	0.8939

TABLE-7
RESULTS OF INTER-DAY PRECISION

Risperidone		
Concentration (ng/spot)	Amount found ng/spot ± SD	RSD (%) (n = 3)
300	301.20 ± 2.899	0.962
400	397.35 ± 5.586	1.405
500	495.35 ± 9.687	1.955

Accuracy (recovery studies): Recovery studies were performed by standard addition method at 80, 100 and 120 % concentration levels of the claims, to the pre-analyzed sample and their contents was re-analyzed, using the proposed method. The results are given in Table-8.

Sensitivity: The sensitivity of the method was analyzed in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by using the formula, LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is residual standard deviation of regression line and S is slope of corresponding regression line. The LOD and LOQ were found to be 46.56 ng/spot and 141.11 ng/spot for risperidone.

Specificity: The risperidone was resolved with good resolution by the developed solvent system as shown in the Fig. 3. The R_f value for risperidone was found to be 0.55. The typical absorption spectrum of risperidone is shown in Fig. 4. The peak-purity of risperidone was determined by comparing

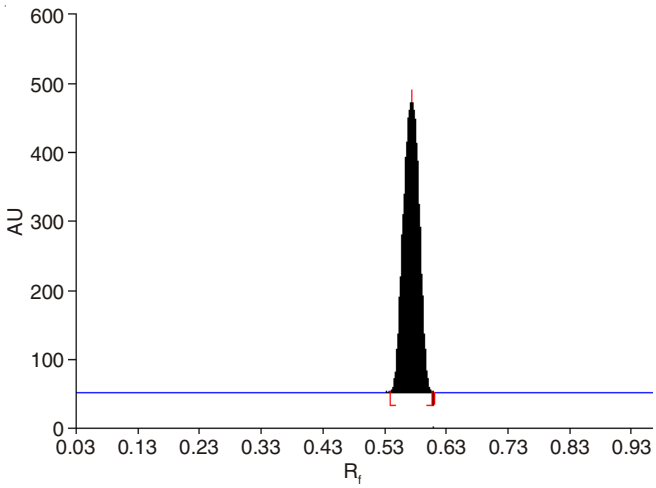

Fig. 3. A typical chromatogram of risperidone (R_f 0.55) measured at 279 nm. Mobile phase-acetonitrile:triethylamine (5: 0.2 v/v)

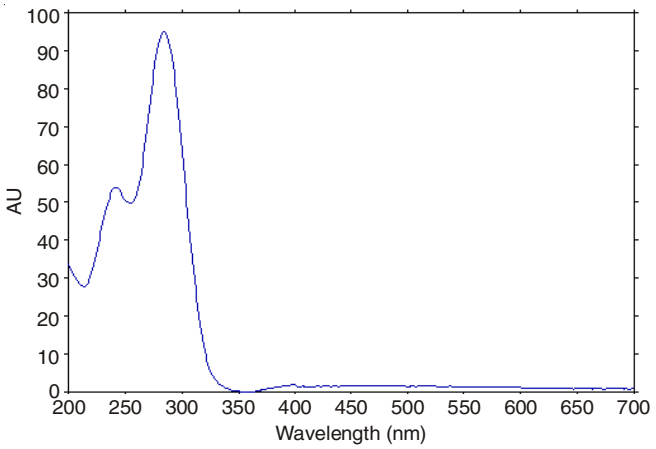

Fig. 4. A typical absorption spectrum of risperidone

their respective spectra at the peak start (S), peak apex (M) and at peak end (E) positions of the spot. Correlation r (S, M) = 0.9997, r (M, E) = 0.9998 for risperidone was observed (Fig. 5).

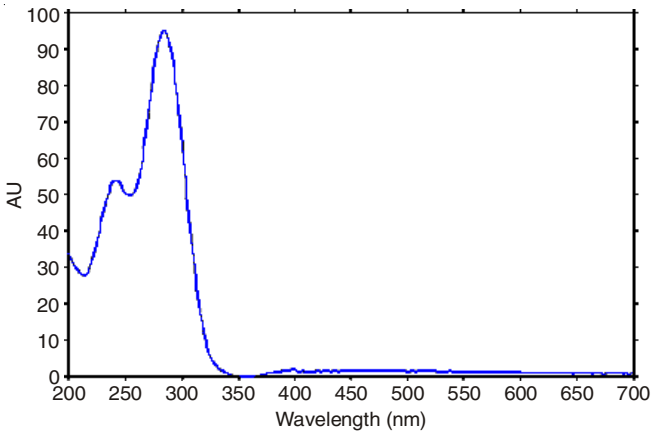

Fig. 5. Peak purity spectrum of risperidone scanned at peak-start, peak-apex and peak-end positions of the spot (correlation > 0.99)

Ruggedness: Ruggedness of the developed method was established by two different analysts, who assayed the formulation, using similar operational and environmental conditions. The results are summarized in Table-9.

Forced degradation of risperidone (Table-10)

Acid and base induced degradation of risperidone: To 20 mL of 1 M methanolic HCl and 1 M methanolic NaOH 8 mg of standard risperidone was mixed. This mixture was condence for 1 h at 65 °C. After interval of 1 h 1 mL sample was withdrawn and neutralized. Resultant solution (400 ng/spot of risperidone) was put on HPTLC plate and the chromatograms were developed (Figs. 6 and 7). Acid degradation of risperidone observed after 4 h and base degradation of risperidone also observed after 4 h.

TABLE-8
RESULTS OF RECOVERY STUDIES

Component	Initial concentration (ng/spot)	Excess drug added (ng)	Drug recovered ng ± SD (n = 3)	Recovery (%)	RSD (%)
Risperidone	400	320	318.70 ± 1.215	99.08	0.3812
	400	400	400.34 ± 2.054	99.05	0.5130
	400	480	479.90 ± 1.754	99.55	0.3654

TABLE-10
FORCED DEGRADATION OF RISPERIDONE

S. No.	Sample exposure condition	Number of degraded products with (R_f value)	Drug recovered (%)	Drug degraded (%)
1	Acid degradation	1 (0.75)	92.19	7.81
2	Acid induced degradation without reflux	1 (0.30)	84.25	6.24
3	Base degradation	1 (0.80)	90.50	9.5
4	H_2O_2 degradation	1 (0.72)	88.90	11.10
5	UV degradation	2 (0.80, 0.90)	89.91	10.09, 9.89
6	Dry heat degradation	No degradation	96.42	–

TABLE-9
RESULTS OF RUGGEDNESS STUDIES

Component	Analyst I		Analyst II	
	Label claim (%)	RSD (%)	Label claim (%)	RSD (%)
Risperidone	100.08	0.2797	100.13	0.2787

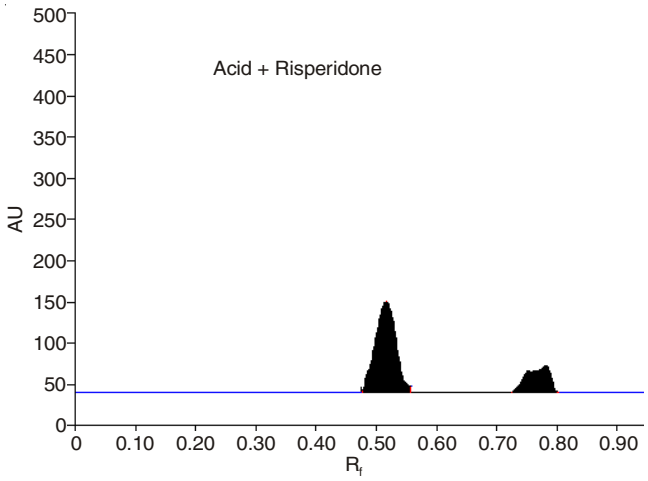

Fig. 6. Acidic degradation of risperidone

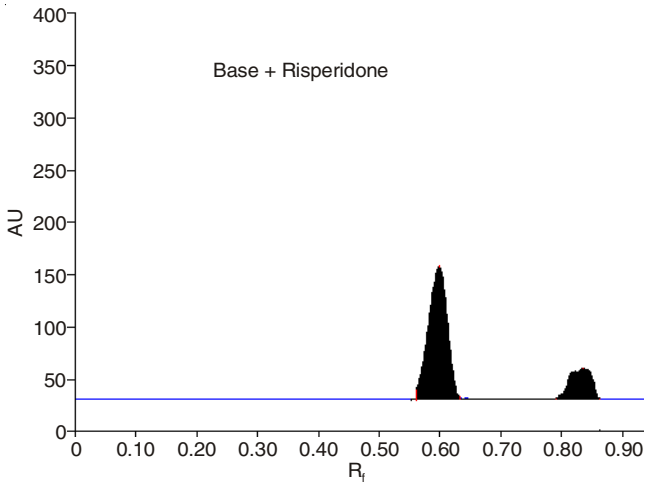

Fig. 7. Basic degradation of risperidone

Acid induced degradation of risperidone without reflux: The 8 mg of risperidone was separately dissolved in 20 mL of 1 M of methanolic HCl. Solution was placed for 72 h at room temperature in the dark in order to exclude the possible degradative effect of light. Resultant solution (400 ng/spot of risperidone) was applied on HPTLC plate and the chromatograms were developed (Fig. 8). Acid degradation without reflux observed after 72 h.

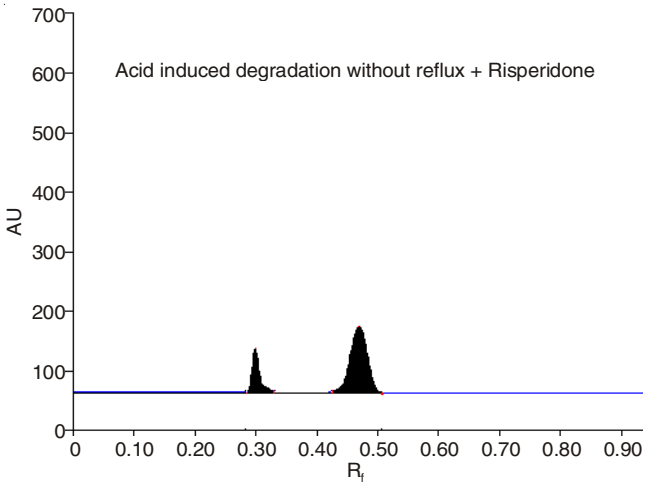

Fig. 8. Acid induced degradation without reflux of risperidone

Hydrogen peroxide-induced degradation: Risperidone (8 mg) was added to 20 mL of hydrogen peroxide (30 % v/v). This mixture was refluxed for 4 h at 65 °C. After interval of 1 h 1 mL sample was withdrawn. Resultant solution (400 ng/spot of risperidone) was applied on HPTLC plate and the chromatograms were run as described. Hydrogen peroxide degradation of risperidone observed after 4 h (Fig. 9).

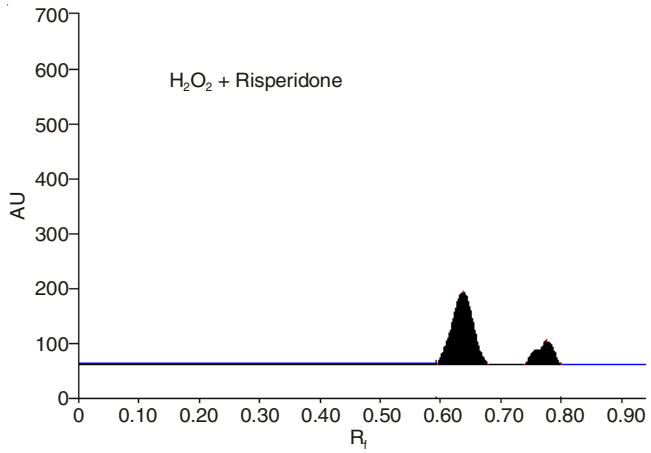

Fig. 9. Hydrogen peroxide induced degradation of risperidone

Dry heat degradation of risperidone: Powdered drug was stored at 55 °C for 3 h in dry heat condition showed significant degradation. The dry heat stability of the drug was also studied by exposing stock solution of 400 ng/µL of risperidone to direct dry heat for 6 h. The resultant solution was 400 ng/spot of risperidone was applied on HPTLC plate and chromatograms were run as described. No degradation is observed in dry heat (Fig. 10).

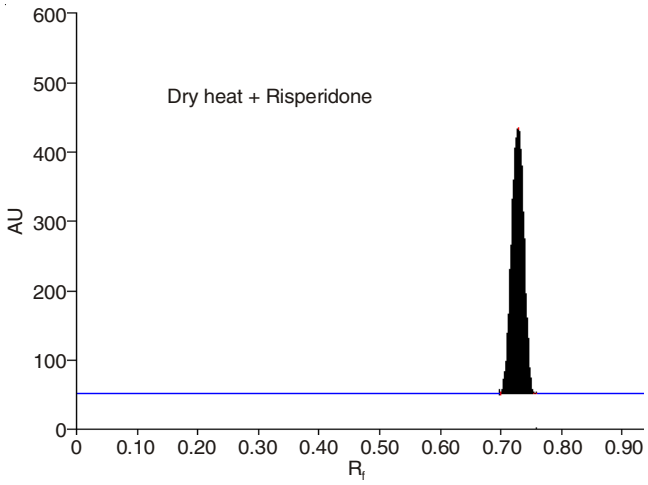

Fig. 10. Dry heat degradation of risperidone

Photochemical degradation of risperidone: The photochemical stability of the drug was also carried by exposing the stock solution of 400 ng/µL of risperidone to open sunlight for 24 h. The solution was 400 ng/spot of risperidone was applied don HPTLC plate and chromatograms were run. Degradation of risperidone was observed after 3 days in UV light (Fig. 11).

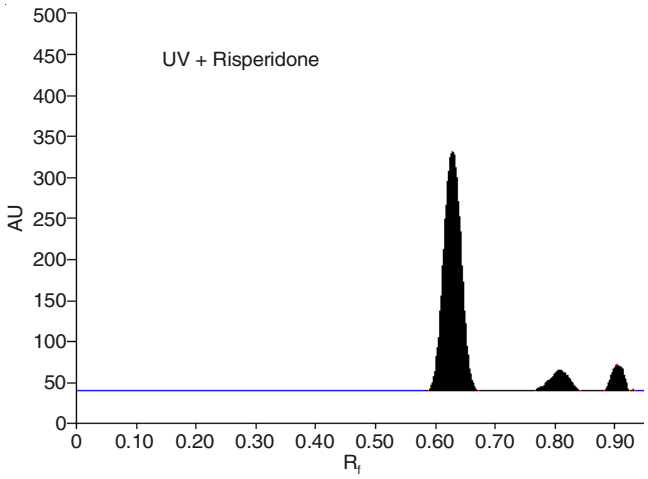

Fig. 11. Photochemical degradation of risperidone

Conclusion

The stability indicating estimation was maticulus, simple, precise, sensitive and rapid. Method was applied for the reckoming of risperidone in bulk dosage form. The relative standard deviation (RSD) for all parameters was less than one, indicate that validity of method was also within the limit so the proposed method can be used for routine quantative estimation of risperidone.

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