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Development and Validation of TLC-Densitometry Method for the Estimation of Anti-psychotic Drug in Bulk and Tablet Formulation

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Abstract: The present work describes a simple, precise, accurate and rapid HPTLC method for analysis of Risperidone in bulk and pharmaceutical dosage form. Precoated silica gel 60F $_{254}$ plate was used as stationary phase. The separation was carried out using Dichromethane: Methanol: Ethanol: Triethylamine (12: 12: 6: 0.1 v/v/v/v) as mobile phase. The densitometry scanning was carried out at 280 nm. The linearity was obtained in the range 4000–8000 ng /spot with correlation coefficient (r2 = 0.9989). The method was validated in terms of linearity, accuracy, precision and specificity. The limit of detection and the limit of quantification for Risperidone were found to be 197.69 ng/spot and 599.08 ng/spot, respectively. The proposed method can be successfully used to determine the drug content of bulk drug and marketed formulation of tablet.

Keywords: HPTLC method of estimation, Risperidone, Tablets.

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 [6,7,8,9]; Chiral Chromatography [10]; Pulse polarography [11]; Chemiluminescence's assay [12]; LC with Coulometric Detection [13]; and stability indicating HPLC method[14]. To the best of knowledge no HPTLC method has been developed for the estimation of risperidone in bulk and tablet formulation, hence we have developed a simple and economical HPTLC method for the estimation of risperidone in bulk and pharmaceutical formulation.

EXPERIMENTAL:

MATERIALS AND METHODS

Risperidone was obtained as a gift sample from Orchid Pharmaceuticals Ltd., Chennai. All the chemicals used were of analytical grade. Tablets containing risperidone (equivalent to 2 mg risperidone) were purchased from a local pharmacy.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

A Camag HPTLC system comprising of Camag linomat IV semiautomatic sample applicator, Hamilton syringe (100 µl), Camag TLC scanner-3, Camag CATS4 software, Camag Twin trough chamber (10 x 10 cm) and ultrasonicator were used during study. Silica gel 60F $_{254}$ TLC plates (20 x 20 cm, layer thickness 0.2 mm E. Merck, Germany) were used as the stationary phase. Dichromethane: Methanol: Ethanol: Triethylamine (12: 12: 6: 0.1 v/v/v/v) was used as mobile phase. Methanol was used as solvent.

STANDARD SOLUTIONS AND CALIBRATION GRAPHS

Working standard of RISP 25 mg was weighed accurately and diluted with methanol to obtain the final concentration of 1000 µg/ml. TLC plates were prewashed with methanol. Activation of plates was done in an oven at 50˚ for 5 min. The chromatographic conditions maintained were precoated silica gel 60F $_{254}$ aluminum sheets (10 x 10 cm) as stationary phase, Dichromethane: Methanol: Ethanol: Triethylamine (12: 12: 6: 0.1 v/v/v/v) as mobile phase, chamber and plate saturation time of 30 min, migration distance allowed was 90 mm, wavelength scanning was done at 280 nm., keeping the slit dimension at 2 x 0.2mm. Deuterium lamp provided the source of radiation. To prepare calibration curve, from standard solution of 1000 µg/ml RISP aliquots of 4, 5, 6, 7 and 8 µl were applied on the TLC plate. The TLC plate was dried, developed and analyzed photo metrically as described earlier. The R_f value of standard RISP was found to be 0.56(i.e., Distance traveled by solute is 50.5mm and the distance traveled by solvent front is 90 mm)

ANALYSIS OF MARKETED FORMULATION:

Twenty tablets were weighed and powdered, and the powder equivalent to 25 mg of RISP was dissolved in 10 ml methanol. The solution was sonicated for 15 min and filtered using Whatman filter paper No 42 and residue was washed with methanol. The extracts and washings were pooled and transferred to a 25 ml volumetric flask and volume was made with methanol. From the above filtrate, 6 µl was spotted and the plate was developed, scanned and densitogram was recorded. The content of the drug was calculated from the peak areas recorded. The R_f value of sample was found to be 0.56(i.e., Distance traveled by solute is 50.9mm and the distance traveled by solvent front is 90 mm).

METHOD VALIDATION

The method was validated in compliance with ICH guidelines.

SPECIFICITY

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for RISP in sample was confirmed by comparing the R_f and spectra of the spot with that of standard. The peak purity of RISP was assessed by comparing the spectra at three different levels, i.e., peak start (*S*), peak apex (*M*) and peak end (*E*) positions of the spot.

PRECISION

The precision was determined at two levels, i.e. repeatability and intermediate precision.

Repeatability was determined by six replicate applications and six times measurement of a sample solution at the analytical concentration. The intra and inter-day precision was determined at three different concentration levels of 4000, 7000 and 8000 ng/spot respectively.

RECOVERY STUDIES

The recovery studies were carried out by adding known amount of RISP on pre-analyzed sample as 80, 100 and 120% and analyzed by the proposed method, in triplicate. This was done to check the recovery of the drug at different levels in the formulations.

ROBUSTNESS

To study the robustness of the method, small but deliberate variations in mobile phase composition (± 2%), chamber saturation period (± 10%), development distance (80,85,90 mm) ,time from application to development (0, 10, 15, 20 min), time from development to scanning (0, 30, 60, 90 min) were carried out.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The LOD and LOQ were separately determined based on the calibration curves. The standard deviations of the y intercepts and slope of the regression lines were used.

RESULT AND DISCUSSION

OPTIMIZATION OF PROCEDURES

Different proportions of Dichloromethane, Methanol, Ethanol and Triethylamine, were tried while mobile phase selection. Ultimately Dichromethane: Methanol: Ethanol: Triethylamine (12: 12: 6: 0.1 v/v/v/v) was finalized as mobile phase. The spots developed were dense, compact and typical peak of RISP was obtained for both standard and sample is shown in fig 1 and 2.

LINEARITY

The analytical concentration ranges over which the drugs obeyed Beer Lambert's law was found to be 4000–8000 ng/spot. (r2 = 0.9989). The standard calibration data for RISP is given in table 1 and fig 3.

ANALYSIS OF THE MARKETED FORMULATION

The spot at R_f 0.56 was observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The RISP content was found to be close to 100% and the results are summarized in table2. The low %RSD value indicated the suitability of this method for routine analysis.

SPECIFICITY

The peak purity of RISP was assessed by comparing the spectra at peak start, peak apex, and peak end positions of the spot.

PRECISION

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. Results are shown in Table 3.

RECOVERY STUDIES

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard RISP was added to pre-analyzed samples and were subjected to the proposed HPTLC method. Results of recovery studies are shown in table 4.

ROBUSTNESS

Statistical analysis showed no significant difference between the results obtained by the developed method and those obtained by variations of some parameters. The method was found to be unaffected by small changes with % RSD for all the parameters less than 2% indicating that method is robust. The results are summarized in table 5.

LOD AND LOQ

Detection limit and Quantification limit were calculated and found to be 197.69 ng/spot and 599.08 ng/spot, respectively. This indicates the adequate sensitivity of the method.

Table 1: Linear regression data for calibration curves.

Detection Wavelength (nm)	280
Linearity range(ng/spot)	4000-8000
Regression equation(Y=mx+c) Slope(m) Intercept(c)	 2.8391 164.3
Correlation coefficient(r)	0.9989
Limit of detection (LOD)	197.69ng/spot
Limit of quantitation (LOQ)	599.08 ng/spot

Table 2: Results of marketed formulation analysis.

Marketed formulation	Label claim(mg)	Area * of densitogram	Amount of drug estimated(mg) ±S.D*	% Mean amount estimated ±S.D*
Respidon-2	2	16477.6	1.99±0.01	99.50±.0.46

***** Average of six determinations.

Table 3A: Statistical evaluation of repeatability of developed method

Drug- RISP	**Repeatability***
Conc(ng/spot)	6000
Mean area ± SD	16477.6±37.61
% Content ± SD	99.50±.0.46
RSD (%)	0.23
S.E	0.1877

*Average of six determinations.

Table 3B: Intra-day and Interday precision of risperidone by HPTLC method [a]

Drug	**Amount ng/spot**	**Intra- day precision**				**Inter-day precision**			
		Area	SD	%RSD	S.E.[b]	Area	SD	%RSD	S.E.[b]
RISP	4000	10985.4	9.65	0.42	3.94	10981.3	11.71	0.51	4.80
	7000	19223.8	17.87	0.26	0.14	19229.3	24.87	0.36	0.19
	8000	22434.4	31.87	0.31	0.08	22447.7	35.98	0.35	0.17

[a] n=3 [b] Standard error

Table 4: Recovery studies of risperidone

Excess drug added to the analyte(%)	**Theoretical content(ng)**	**Recovery (%)**	**% RSD**
80	4800	98.32	0.83
100	6000	99.27	0.18
120	7200	99.04	0.44

Table 5: Results of robustness studies

A: Chromatographic changes (% of Dichromethane in mobile phase)

% change in mobile phase	**R_f**	**Peak area**
+2%	0.55	16507.8
0%	0.56	16498.9
-2%	0.57	16401.3
Mean*±SD	0.56±0.01	16469.33±59.08

* Average of three determinations.

B: Chromatographic changes (Chamber saturation)

Chamber saturation (Time in min)	**R_f**	**Peak area**
33	0.58	16499.1
30	0.57	16513.2
27	0.56	16509.3
Mean*±SD	0.57±0.01	16507.2±7.2

* Average of three determinations.

C: Chromatographic changes (development distance)

Development distance(mm)	R_f	Peak area
80	0.54	16523.2
85	0.57	16472.4
90	0.55	16511.1
Mean*±SD	0.55±0.02	16502.2±26.53

* Average of three determinations.

D: Chromatographic changes (Time from application to development)

Time from application to development	R_f	Peak area
0	0.56	16499.2
10min	0.56	16478.4
20min	0.57	16523.7
30min	0.57	16510.3
Mean*±SD	0.56±0.01	16500.4± 15.92

* Average of three determinations.

E: Chromatographic changes (Time from development to scanning)

Time from development to scanning	R_f	Peak area
0	0.56	16498.3
10min	0.55	16493.4
20min	0.55	16499.7
30min	0.55	16497.7
Mean*±SD	0.55±0.005	16497.4±2.71

* Average of three determinations.

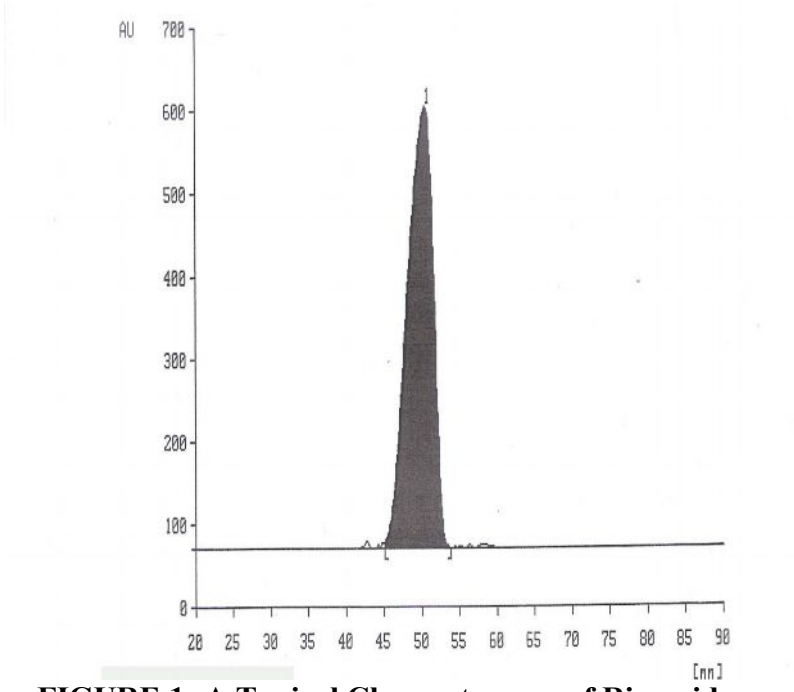

FIGURE 1: A Typical Chromatogram of Risperidone

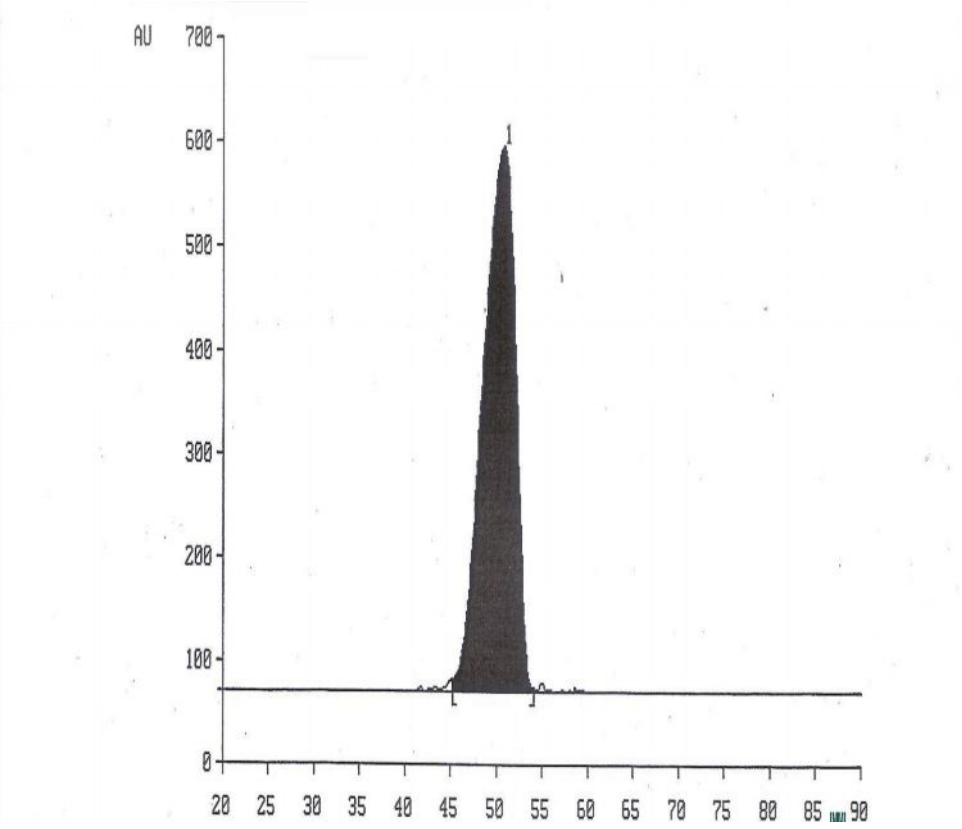

FIGURE 2: Chromatogram of Risperidone in Tablet Formulation

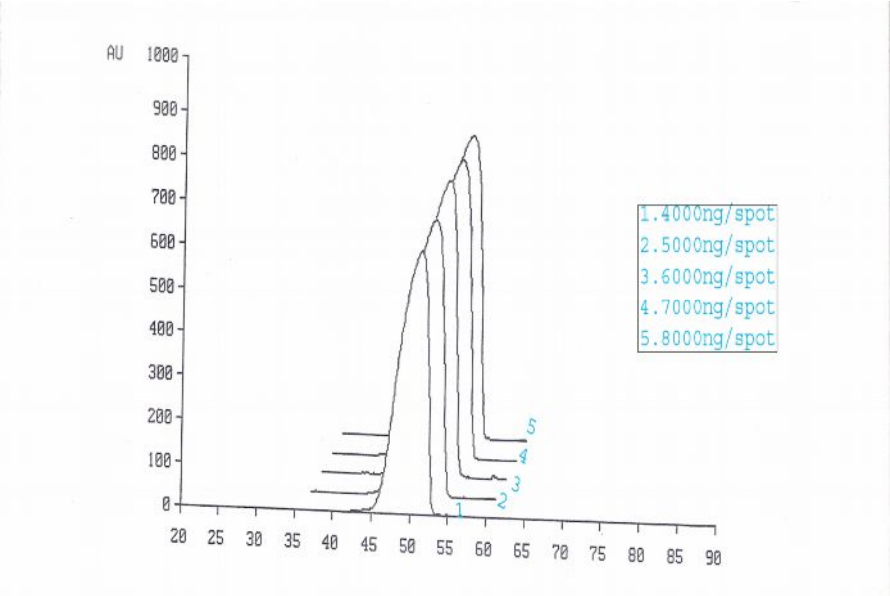

FIGURE 3: Chromatogram from peaks 1-5 shows 4000-8000ng/spot of standard risperidone.

CONCLUSION

The proposed HPTLC method was validated as per ICH guidelines. The standard deviation, %RSD and standard error calculated for the method are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Hence, it can be concluded that the developed HPTLC method is accurate, precise and selective and can be employed successfully for the estimation of Risperidone in tablet formulation.

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