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Method development and validation for estimation of risperidone in novel liquisolid formulation by RP-HPLC

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ABSTRACT

The aim of the present work is to develop and validate a simple, rapid and precise reverse phase high performance liquid chromatography method for determination of Risperidone (RIS) in novel liquisolid tablets. A Chemisil® octadecylsilane (ODS) C_{18} column (250 mm x 4.6 mm i.d) with 5 µ particle size was utilized as a stationary phase. Methanol : buffer (0.2% v/v OPA in HPLC water) in the ratio of 80 : 20 v/v was used as mobile phase, at a flow rate of 0.6 mL/min. Detection was carried out at 235 nm, using PDA detector. The total runtime and retention time was found to be 10 and 3.72 min respectively. The developed method was validated (according to ICH) with respect to linearity, accuracy (recovery), precision, specificity and robustness. Obtained results observed that all the parameters were within the limit. Linearity range and correlation co-efficient (r^2) were 500 to 10000 ng/mL and 0.9969 respectively. The LOD and LOQ for RIS were found to be 364.07 ng/mL and 1103.26 ng/mL respectively. The developed method was simple, rapid, accurate, precise and sensitive for determination of RIS in novel liquisolid tablets and can be routinely employed inthe quality control.

Keywords: Risperidone, RP-HPLC, Validation, Liquisolid Tablets.

INTRODUCTION

Risperidone (RIS) is an atypical antipsychotic drug. It is used in the treatment of psychosis. It is used primarily in the management of schizophrenia, inappropriate behavior in severe dementia and manic episodes associated with bipolar I disorder. This is an atypical antipsychotic, a combined $5HT_{2A}$ and dopamine (D_2) antagonist. It blocks D_2 receptors in the limbic system and $5HT_{2A}$ receptors in the misocortical system. It belongs to the class of benzisoxazole. The chemical structure of RIS is 3-[2-[4-(6-flouro-1, 2-benzisoxazol-3-yl)-1piperidinyl] ethyl] -6, 7, 8, 9- tetra hydro 2 methyl –4H-pyrido [1,2-a] pyrimidin-4-one (Fig. 1). The molecular weight is 410.85 g/mol and molecular formula is $C_{23}H_{27}FN_4O_2$.

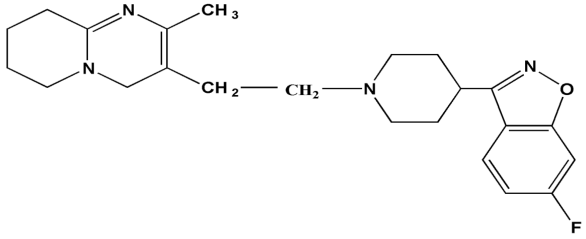

Fig. 1 structure of risperidone

Literature review revealed thatseveral analytical methods have been reported for estimation of RIS individually or in combination of other drugs in plasma, saliva, urine and formulations which includes LC-MS/MS [1-2], UV spectrophotometry [3-4], Column-Switching and Spectrophotometric Detection [5], High Performance Liquid Chromatography (HPLC) [6-12], High Performance Thin Layer Chromatography (HPTLC) [13], Differential Pulse Polarographic Study [14], Sensitive liquid chromatography/tandem mass spectrometry [15], LC-MS [5, 16], Thin Layer Chromatography (TLC) [17], Sensitive chemiluminence assay [18], Simultaneous determination of RIS by HPLC with Colorimetric method [19], capillary electrophoresis and 1H NMR spectroscopy [20]. These analytical methods were complicated, costly and time consuming.Mostof the estimations of the drug in pharmaceutical formulations are performed by HPLC for the reasons of specificity and robustness.There were no simple and reproducible official methods so far reported for determination of RIS in liquisolid tablets by RP-HPLC. Attempts were made to develop a simple, precise, accurate HPLC method for determination of RIS in liquisolid tablets. The proposed RP-HPLC method describes the validation parameters in accordance with ICH guidelines, by assessing its selectivity, specificity, linearity, precision, accuracy, and limit of quantification, limit of detection, robustness and system suitability.The developed method is of simple, precise and economical RP-HPLC methods for the determination of RIS in liquisolid tablet formulation and marketed formulation.

MATERIALS AND METHODS

Chemicals and reagents

Risperidone pure drug sample was kindly provided as a gift sample by Aurobindo Laboratories Ltd, Bangalore. HPLC grade methanol and water was purchased from Merck Specialists Private Ltd, Mumbai. HPLC grade ortho phosphoric acid procured from Fischer Scientific, Qualigens, Mumbai.RIS tablets (Respidon-2) were obtained from local pharmacy, Hyderabad, India.

Instrumentation and chromatographic conditions

A binary gradient high-performance liquid chromatograph from Shimadzu (Nakagyo Ku, Kyoto, Japan), HPLC Class VP series with two LC-20AD pumps, SPD-M20A PDA detector, CBM-20A VP system controller and Shimadzu Class VP version 6.12 SP2 data station system was used. The chemisil® ODS C_{18} analytical column (250 mm × 4.6 mm i.d., 5µ particle size, Phenomenex®, USA) was used.

Chromatographic analysis was carried out on a chemisil® ODS C_{18} analytical column (250 mm×4.6 mm i.d., 5 µ particle size, Phenomenex®, USA) column maintained at ambient temperature. The compounds were separated gradiently with a mobile phase consisting of methanol: buffer (0.2% v/v OPA in HPLC water) 80: 20 v/v ratio. Mobile phase was filtered through a 0.45 µm nylon membrane (Millipore Pvt. Ltd., Bangalore, India) and degassed in a CD-4820, electronic ultrasonic bath (Citizen Scale (I) Pvt. Ltd., Mumbai, India). The flow rate of the mobile phase was adjusted to 0.6 mL /min and the injection volume was 20 µl. Detection was performed at 235 nm.

Preparation of standard solutions

Weighed amount (5 mg) of RIS was transferred in to a clean 5 mL volumetric flask. To this few mL of methanol was added and sonicated for 5 min to dissolve. Make up the volume with methanol up to 5 mL and marked it as standard stock solution. It gives the concentration of 1000 µg/mL. Appropriate amount of stock solutions were diluted with mobile phase to obtain required concentrations viz. 500, 1000, 2000, 3000, 4000, 5000, 10000 ng/mL.

Formulation of liquisolid compacts

Weighed amount of RIS was initially dispersed in the calculated amount of non-volatile solvent (Span 20) system. To the above liquid medication, mixture of carrier (Micro crystalline cellulose) and coating material (Aerosil® 200) were added under continuous mixing in a mortar. Super disintegrating agent i.e., crospovidone was added to the above mixture and mixed thoroughly for 10 to 20 min. The final mixture was compressed using table top pilot scale 10-Station rotary tablet RimekMinipress-I. (M/S Karnavati Engineering Ltd., Gujarat, India.)

Method Validation

Linearity

Standard stock solution of RIS was diluted to prepare solutions containing 500, 1000, 2000, 3000, 4000, 5000 and 10000 ng/mL. The solutions were injected in triplicate into the HPLC column using methanol: buffer, 0.2% v/v OPA in water (80:20 v/v) as the mobile phase and keeping the injection volume constant (20 µl) throughout the experimental conditions.

System suitability

System suitability test was carried out on freshly prepared standard stock solution of RIS of concentration 2000 ng/mL and evaluated by making 6 replicate injections.

Specificity
To assess the method specificity, sample and placebo solutions of both marketed tablets and liquisolid compacts were prepared and analyzed to check the peak purity of RIS peak in sample solution. Placebo solution withoutRIS was prepared for liquisolid compacts with the same excipients (microcrystalline cellulose, Aerosil® 200, crospovidone and span 20) as those in the liquisolid compact formulations and for marketed formulations with commonly used excipients for tablets (magnesium stearate, talc, microcrystalline cellulose, starch and lactose.) and solutions were injected into the HPLC system following test conditions. Chromatogram was recorded and the responses of the peaks were measured.

Accuracy
To ascertain accuracy of the method, known amount of RIS was added to placebo preparation. Recovery of the method was evaluated at two different concentration levels (1000 and 2000 ng/mL). Percent recovery was calculated by comparing the area before and after the addition of working standard. The recovery studies were performed in triplicate.

Precision
Three injections of two different concentrations (500 and 2000 ng/mL), were given on the same day and the values of relative standard deviation (RSD) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

Limit of detection (LOD) and Limit of quantification (LOQ)
LOD can be defined as the smallest level of analyte that gives a measurable response whereas LOQ is the smallest concentration of analyte which gives a response that can be accurately quantified. For determination of LOD and LOQ standard stock solution of RIS (1 mg/mL) was prepared. Then 500, 1000, 2000, 3000, 4000, 5000, 10000 ng/mL of RIS standard solutions were prepared by diluting the standard stock solutions with methanol.

The L.O.D. was estimated from the set of five calibrationcurves.

LOD =3.3 X (S.D./Slope)

Where, S.D. = Standard deviation of the Y-intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

The L.O.Q. was estimated from the set of five calibrationcurves.

LOQ = 10 X (S.D./Slope)

Where, S.D. = Standard deviation of the Y-intercepts of the 5 calibrationCurves.
Slope = the mean slope of the 5 calibration curves.

Robustness
Robustness of the chromatographic method was a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters. Robustness of the method was checked by making slight changes in chromatographic conditions like mobile phase ratio andflow rate.

Preparation of sample solution
Assay of liquisolid compacts
Ten liquisolid tablets each containing 2 mg of RIS were weighed averaged and finely powdered. A portion of powder equivalent to the weight of one liquisolid tablet was accurately weighed and dissolved in 8 mL methanol in 10 mL volumetric flask. The volumetric flask was sonicated for 20 min to effect complete dissolution of RIS in the solution and make up volume up to marc with methanol and filtered through 0.45 µm membrane filter. After appropriate dilutions, test solution was injected into HPLC and chromatogram was recorded for the same and amount of drug wascalculated.

Assay of Marketed formulation
Ten marketed tablets of each containing 2 mg of RIS were accurately weighed, averaged and finely powdered. A quantity of powder equivalent to the weight of one tablet was accurately weighed and dissolved in 8 mL methanol in 10 mL volumetric flask. The volumetric flask was sonicated for 20 min to effect complete dissolution of RIS in the solution and make up volume up to marc with methanol and filtered through 0.45 µm membrane filter. After

appropriate dilutions, test solution was injected into HPLC and chromatogram was recorded for the same and amount of drug was calculated.

In vitro dissolution studies

Drug release studies were performed using a USP dissolution test apparatus-II at 50 rpm and bath temperature maintained at 37 ± 0.5 °C. 900 mL of freshly prepared 0.1N HCl was used as dissolution medium. Dissolution samples were collected at 5, 10, 15, 30, 45, 60, 90 and 120 min. At each time point, aliquots of samples were withdrawn from each vessel and replaced with equal volume of 0.1N HCl to maintain sink conditions. The samples were filtered through a 0.45 µm membrane filter into labeled glass tubes and further analyzed by HPLC.

RESULTS AND DISCUSSION

Method development and optimization of HPLC method

A new RP-HPLC method requires a series of preliminary investigations which are useful in establishing the optimal chromatographic conditions and provide maximum information for analyzing the chromatogram. In this study, a new RP-HPLC method for the estimation of RIS in liquisolid compacts and marketed formulation was developed and validated.

The analytical conditions were selected after testing the different conditions effecting HPLC analysis, for example buffer composition, buffer concentration, buffer to organic solvent ratio and other chromatographic conditions. Preliminary trials were done with different mobile phase compositions consisting of acetonitrile or methanol with HPLC water and different pH phosphate buffers. Based on these entire trials methanol : 0.2% v/v OPA in HPLC water in the ratio of 80:20 v/v at a flow rate of 0.6 mL/min showed symmetrical peaks with good resolution and was found to be ideal for the work. Methanol was chosen as organic constituent of mobile phase to reduce retention times and buffer was chosen to reduce peak asymmetry. A wavelength of 235nm was selected for present study. The retention time of RIS was found to be 3.72 min. Standard chromatogram of RIS was presented in Fig. 2 and optimized chromatographic conditions were tabulated in Table 1.

Table 1. Optimized chromatographic conditions

Stationary phase(column)	ChemisilODS C_{18} (250 mm×4.6 mm i.d., 5µ particle size, Phenomenex®, USA)
Mobile phase	Methanol ,buffer (0.2%v/v OPA in HPLC water)
Detection wavelength(nm)	235
Runtime (min)	10
Flow rate (mL/min)	0.6
Volume of injection loop (mL)	20
Column temperature	Ambient
RIS R_t (min)	3.72

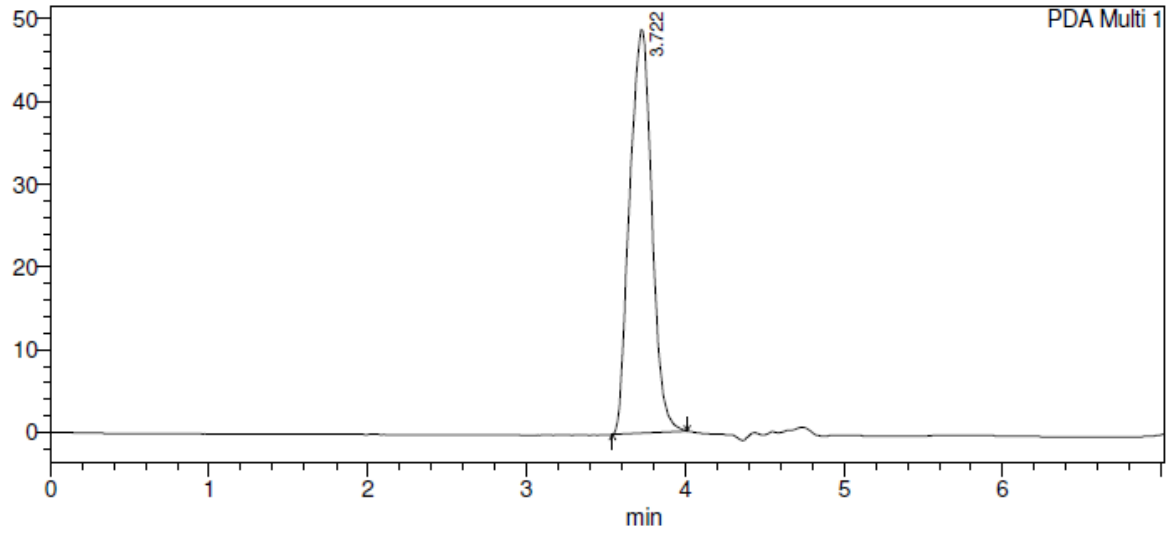

Fig. 2 Standard chromatogram of Risperidone

Linearity

Calibration curve wasconstructed by plotting average peak area against concentration and regression equation was computed. The equation was Y=113.01x-12468. The slope, intercept and correlation coefficient values were found to be 113.01, 12468 and 0.9969. The results were tabulated in Table 2. Graphically represented in Fig. 3 and it was

observed that the linearity was in the concentration range from 500 to 10000 ng/mL. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range.

Table 2. Linearity parameters of Risperidone

Conc.(ng/mL)	Area
500	72030
1000	102694
2000	222514.67
3000	296072
4000	421991.67
5000	545042.33
10000	1134235.33

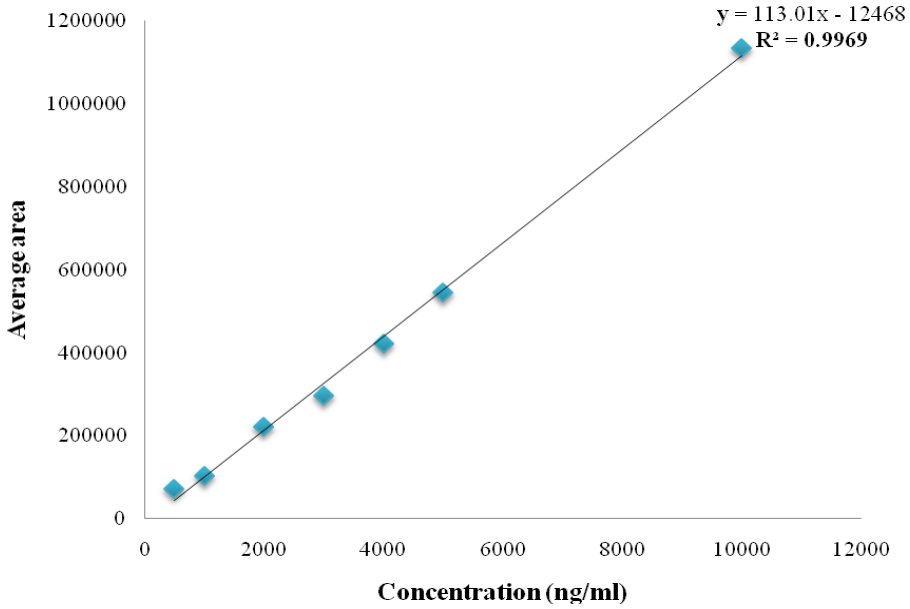

Fig. 3 Linearity curve of Risperidone

System suitability

System suitability test was carried out during the validation study to ensure that the optimized conditions of the method can produce the results of acceptable accuracy and precision. The system suitability parameters like Theoretical plates (N), Tailing factor (T) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of RIS in liquisolid compacts was validated or not. All the system suitability parameters were met during the entire course of validation study, tailing factor was less than 2 and theoretical plates were greater than 2000. The percent relative standard deviations (% RSD) of peak area and retention time of six standard solutions were less than 2 %. The results obtained were within the limit. The results were represented in Table 3.

Specificity

The excipients used in formulation and marketed formulations did not interfere in the estimation of RIS, no peaks were found at the retention time of RIS. Specificity of the method was checked for by injecting the placebo solution.

Table 3. System suitable parameters for analysis of Risperidone

Conc.	Injection	Area	R_t
	Inj-1	196951	3.65
2000ng/mL	Inj-2	198142	3.64
	Inj-3	197236	3.65
	Inj-4	198832	3.69
	Inj-5	196785	3.64
	Inj-6	197432	3.66
Mean		197563	3.655
SD		780.963	
%RSD		0.395	
Tailing factor		0.980	
Plate count		2342.87	

The peak purity indices for liquisolid compacts and marketed tablets were graphically represented in Fig. 4 and 5 respectively. From the results the method was said to be specific since specificity was tested against standard compounds and possible interference peaks in the presence of placebo under optimized test conditions. Therefore, it can be concluded that the method is specific and can assess unequivocally the analyte of the interest in the presence of possible interferences.

Precision

Intra-day and inter-day studies results were obtained in terms of percent relative standard deviation (%RSD). The developed chromatographic analytical method has % RSD values for both intra-day and inter-day precision were found to be less than 2. The results were tabulated in Table 4. The peak area values were reproducible and the statistical values (% RSD) were within the range, which confirm that the developed method was precise.

Limit of detection (LOD) and Limit of quantification (LOQ)

The parameters LOD and LOQ were determined using the signal-to-noise (S/N) method by comparing results of the test of samples with known concentrations of analyte to blank samples. The LOD and LOQ of the drug were found to be 364.07 ng/mL and 1103.26 ng/mL respectively. A signal-to-noise ratio of 3:1 is used for LOD whereas a signal-to-noise ratio of 10:1 is used for LOQ.

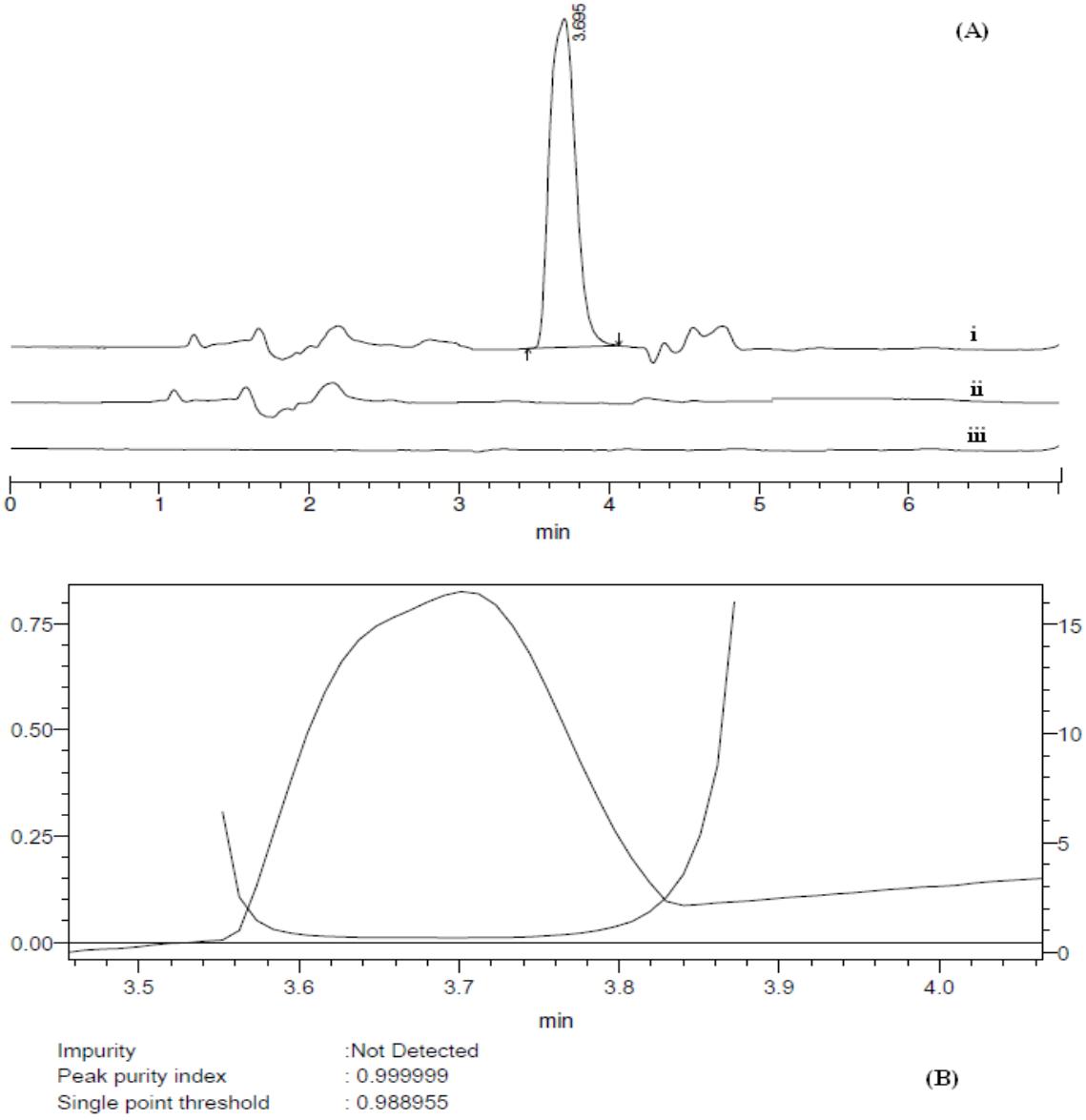

Fig. 4 chromatograms of liquisolid compacts (A) specificity (B) peak purity index

Table 4. Intra-day, inter-day study of Risperidone

Conc. ng/mL	Intra-day(n=3)		Inter-day(n=3)	
	Mean Conc. ± SD	%RSD	Mean Conc. ± SD	%RSD
500	500.08± 0.16	0.033	500.3±0.60	0.11
2000	2000.05±0.18	0.008	2000.1±0.12	0.006

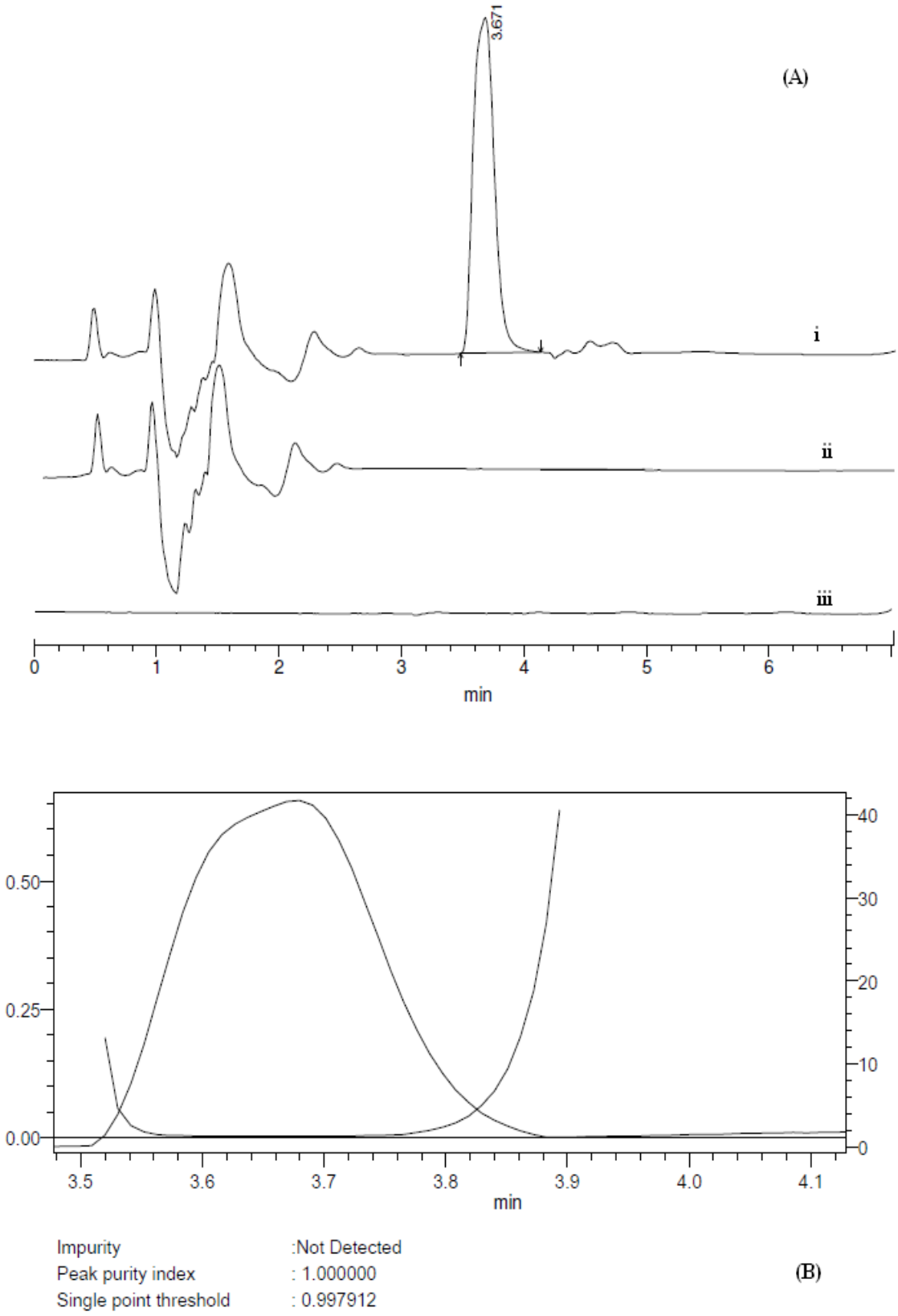

Fig. 5 chromatograms of marketed formulation (A) specificity (B) peak purity index

Robustness

The results indicate that changing the flow rate (± 0.1 mL/min) had no significant effect on the chromatographic behavior of RIS. Even a small change of mobile phase ratio (± 2 %), did not show a notable change in the peak area of the drug. The results were represented in Table 5. From the results, the chromatographic method was found to be robust, although small deliberate alterations in optimized method conditions did have a negligible effect on the chromatographic behavior of the solute.

Table 5. Robustness study

System suitability parameters (variations)		% RSD Peak area (n=6)	Mean Tailing factor (min) (n=6)	Mean R_t (n=6)
Varied flow rate (±0.1%) (mL/min)	0.7	0.79	1.03	3.33
	0.5	0.58	1.15	3.99
Varied mobile phase Composition (±2%)	78	0.43	1.69	3.71
	82	0.52	1.16	3.69

Assay

The drug in the liquisolid tablet and marketed formulation (Rispidon-2 mg) was 100.27 ± 4.6 % and 98.31 ± 2.03 %. The results were shown in Table 6. The results of assay studies were suggest sensitivity of the developed method.

Table 6. Assay of Risperidone in marketed formulations and liquisolid compacts

Formulation	% Assay
Rispidon-2 mg	98.31 ± 2.03
Liquisolid Compacts	100.27 ± 3.46

In vitro dissolution study

Dissolution for pure RIS, liquisolid compacts and marketed formulations was performed and the samples were analyzed by the developed RP-HPLC method as described in specificity section. The drug release at 15th min from liquisolid compact formulation and marketed formulation and pure drug were found to be 98.23 %, 67.29 % and 35.75 % respectively. Drug release profile was demonstrated in Fig. 6. This demonstrated the comparison of drug release from liquisolid compact formulation and marketed formulation to that of the pure drug. Release pattern reveal that RIS release from liquisolid tablet was 98 % at 15th min as compared to that of marketed formulation and pure drug.

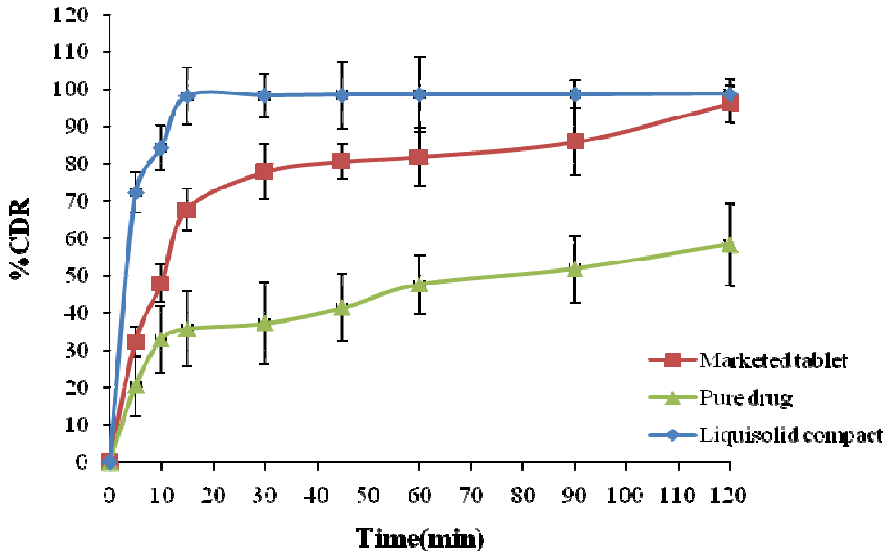

Fig. 6 In-vitro drug release profile of marketed tablet, pure drug and liquisolid compact of risperidone

CONCLUSION

The developed method was simple, rapid, accurate, precise and sensitive for determination of RIS in novel liquisolid tablet. The method includes simple working procedure with minimum use of organic solvents hence the developed method iscost effective and can be routinely employed in quality control tests for determination of RIS in novel liquisolid and marketed tablets.

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