Method development and validation of an Isocratic High pressure Liquid chromatography technique for analysis of Risperidone in Bulk drug and Formulation

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Abstract:

Risperidone is a potent antipsychotic drug which is mainly used to treat schizophrenia and schizoaffective disorder. A new isocratic HPLC method was developed for analysis in formulations. The developed method was validated according to ICH guidelines. The method was developed with the mobile phase 0.1% OPA: Methanol:Acetonitrile in the ratio of 60:25:15(V/V/V). In this method the back pressure was very less because of OPA in mobile phase. In validation results the accuracy, precision, recovery are within the limits.

Keywords: Risperidone, accuracy, precision, recovery, LOQ and LOD

Introduction:

Risperidone is a potent antipsychotic drug which is mainly used to treat schizophrenia , schizoaffective disorder, the mixed and manic states associated with bipolar disorder, and irritability in people with autism.[1-3] Risperidone belongs to the class of atypical antipsychotics. It is a dopamine antagonist possessing antiserotonergic, antiadrenergic and antihistaminergicproperties.

Side effects of Risperidone might include significant weight gain and metabolic problems such as diabetes mellitus, as well as tardive dyskinesia and neuroleptic malignant syndrome. Risperidone and other antipsychotics also increase the risk of death in patients with dementia.[4-6]

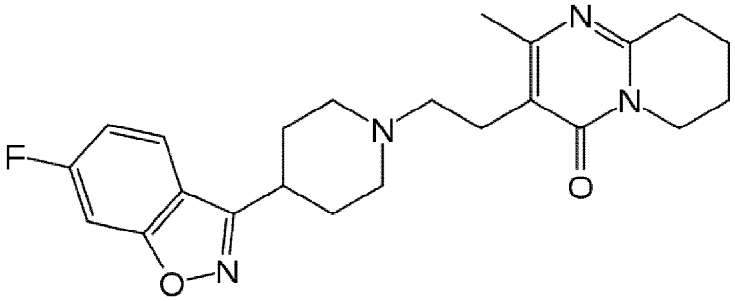

Figure.1 Structure of Respiridone

Applications of Respiridone

Risperidone is used for the treatment of schizophrenia, bipolar disorder and behavior problems in people with autism. Antipsychotic medications such as Risperidone have a slight benefit in people with dementia, they have been linked to higher incidences of death and stroke.

Instrumentation:

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Respiridone, an isocratic PEAK HPLC instrument with ChromosilC18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with, Rheodyne manual sample injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance. Electronic balance-denver (SI234)

Experimental conditions:

Respiridone (1mg/ml) standard stock solutions were prepared using methanol as a solvent. Aliquots of mixed standard solutions of Respiridone were diluted in mobile phase to get a final concentration of 2-12µg/ml.

Chemicals and solvents:
The API of Respiridone is obtained as gift sample from Hetero Drugs pvt Limited. Marketed formulation tablets were purchased from local market. All the solvents used were HPLC grade and were purchased from Merk chemicals private limited, Mumbai.

Preparation of Standard drug solution:
10mg of standard drug Respiridone were weighed separately and were dissolved in 10ml of methanol separately. Both the drugs were mixed well and then filtered through 0.45µm nylon membrane filter paper. From this required concentrations were prepared by proper dilution.

Analysis of dosage form

Pharmaceutical form containing 0.5 mg of Respiridone and was weighed and dissolved in 10 ml of mobile phase and sonicated for 15 min. Using methanol the 1 ml prepared sample solution was taken in 10 ml volumetric flask and twice diluted to prepare 0.5 µg/ml concentrated solution, and filtered through 0.45µ membrane filter.

HPLC Method Development For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

Detection wavelength:
The spectrum of diluted solutions of the Respiridone in methanol was recorded. The absorption spectrum of Respiridone obtained by scanning the sample separately on UV spectrophotometer in UV region (200-

400nm) in spectrum mode showed that the drug has maximum absorbance at 250nm. Analysis was carried out by adjusting the UV detector of the HPLC system at 250nm.

Choice of stationary phase:

Preliminary development trials have performed with octadecyl columns with different types, configurations and from different manufacturers. Finally the expected separation and shapes of peak was succeeded Analytical column Inertsil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size.

Selection of the mobile phase:

Several systematic trials were performed to optimize the mobile phase. Different solvents like Methanol, Water, Acetonitrile and 0.1% Ortho phosphoric acid (OPA) in different ratios and different P^H values of the mobile phase ratios by using different buffer solutions in order to get sharp peak and base line separation of the components and without interference of the excipients. Satisfactory peak symmetry, resolved and free from tailing was obtained in mobile phase 0.1% OPA:Methanol:Acetonitrile in the ratio of 60:25:15(V/V/V) in isocratic condition.

Selection of the mobile phase flow rate

Flow rates of the mobile phase were changed from 0.5 – 1.2 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1ml/min flow rate was ideal for the successful elution of the analyte.

Optimization of HPLC Method

After completion of several systematic trials to optimize the chromatographic conditions, a sensitive, precise and accurate RP-HPLC method was developed for the analysis of Respiridone in pharmaceutical dosage forms. The optimized chromatographic conditions were shown in table.1.The chromatograms of blank standard and formulation were shown in figure 2,3 and 4

Standard solution Concentration	**Respiridone 6µg/ml**
Mobile Phase	0.1%OPA:Methanol:Acetonitrile 60:25:15(V/V/V)
Wavelength	250nm
Column	C_{18} Column
Pump mode	Isocratic
Flow rate	1ml/min
Diluents	Mobile phase
Injection volume:	20µl
P^H	4.3
Concentration	8µg/ml
Retention Time	7.3
Run Time	12min
Area	405355

Th. Plates	5584
Tailing Factor	0.71
Pump Pressure	10 ±12MPa

Table 1: Optimized Conditions of Respiridone

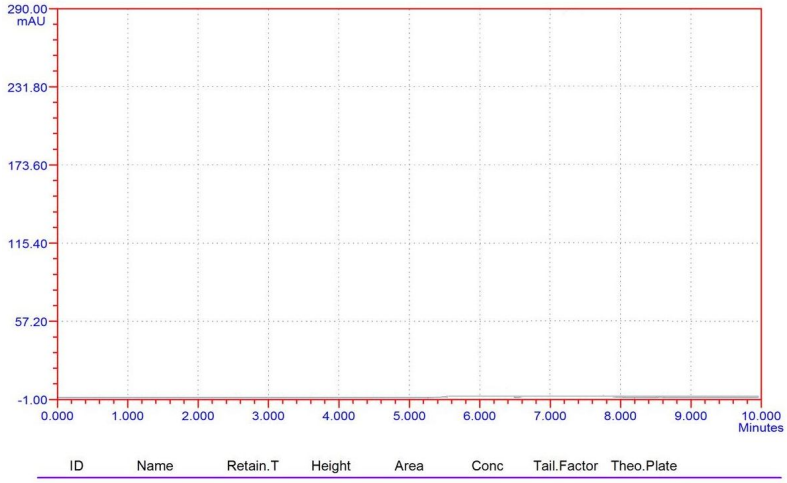

Figure:2 Chromatogram of Blank

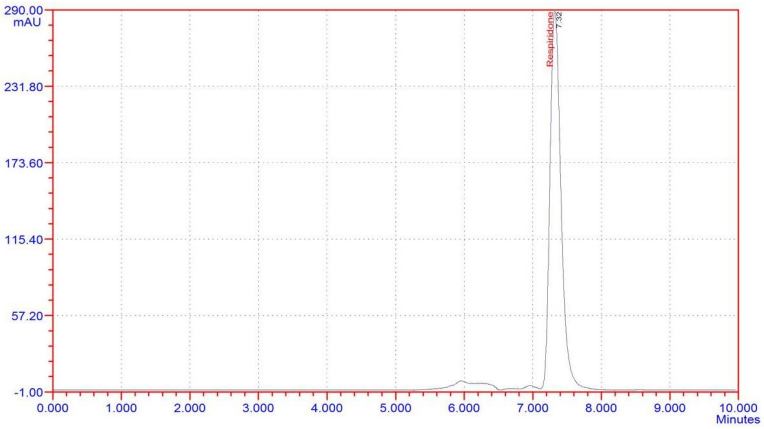

Figure:3 Chromatogram of Standard

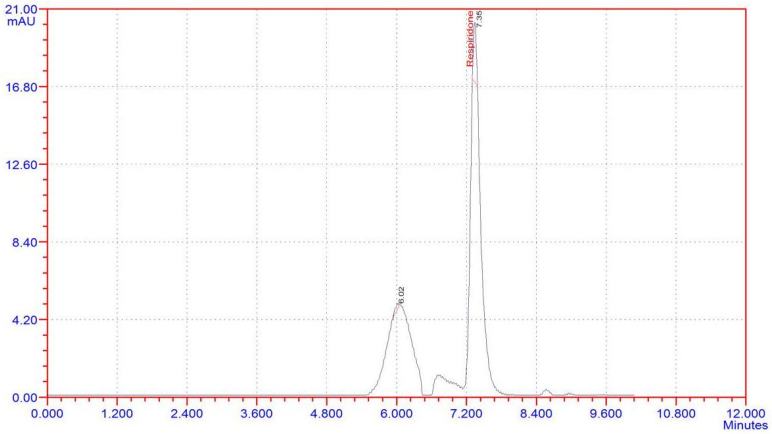

Figure 4 Chromatogram of formulation

For the s estimation of Respiridone in pharmaceutical dosage forms, a simple method was developed by change in the method conditions like mobile phase composition, wavelength, stationary phase and the flow rate of the mobile phase. Finally separate sharp peaks with high resolution and clean base line observed with Chromosil C18 column, mobile phase ratio of 1% opa:methanol:acetonitrile(60:25:15), Mobile phase P^H was adjusted to 4.3, detector wavelength at 250nm and 1ml/min Mobile phase flow rate.

In the developed conditions, the two compounds Respiridone was separate and sharp peaks were observed with low tailing factor, high number of theoretical plates and resolution factor was found to be very high. Hence the developed conditions obey the system suitability criteria. In this conditions there is no interference of excipients in the placebo solution indicates that the developed method is specific for Respiridone only. Hence the proposed method is specific.

The developed method show linearity between 2-12µg/ml with good correlation coefficient (r2 = 0.999 for Respiridone). Regression equation was found to be y= 36699X+81435 for Respiridone and Repeatability of the method was confirmed by precision studies. Precision was carried out a concentration of standard i.e. 8µg/ml. %RSD for intraday precision was found to be 0.50 for Respiridone . %RSD for intraday precision was found to be 0.32 for Respiridone. Results confirmed that the proposed method is precise.

The recovery technique was performed to study the accuracy and reproducibility of the proposed methods. For this, known quantities of the 4µg/ml solution were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed. The total amount of was determined by using the proposed methods and the amount of added drug was calculated by the difference. The % recovery was found to be within the acceptance criteria of 98-102%. Results were shown in table 5. This showed that the recoveries of the proposed methods are satisfactory. Hence the proposed method is accurate.

Robustness of the method was confirmed by change in the optimized chromatographic conditions and % change in each changed condition was calculated. % change in all changed condition was found to be well acceptance criteria of less than 2. This indicates that the proposed method is Robust.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a

measurable response (signal-to-noise ratio of 3). The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal-to-noise ratio of 10). LOD and LOQ for Respiridone were found to be 0.5ppm and 1.65ppm respectively.

Formulation study was carried out by using marketed formulation tablet of Respiridone (RISPERDAL). Standard concentration was prepared and the area of the peak response was used for the calculation of the % assay. It was found more than 99% accurately estimate Respiridone In pharmaceutical dosage forms.

Thus the method developed in the present investigation is simple, sensitive, accurate, rugged, robust, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Respiridone in tablet dosage forms.

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