RESEARCH ARTICLE

Method Development and Validation by UV Spectrophotometric Analysis and RP-HPLC Method for Simultaneous Estimation of Risperidone and Trihexyphenidyl

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ABSTRACT

Risperidone is of the chemical class of benzisoxazole derivatives. Risperidone chemically name is 3-[2-[4-(6-fluoro-1, 2-benzisoxazol-3-yl)-1- piperidinyl] ethyl]-6, 7, 8, 9-tetrahydro- 2-methyl-4H-pyrido [1, 2-a] pyrimidine-4-one. It is effective in treating positive and negative schizophrenic symptoms with high effectiveness on 5HT and D2 receptors with less incidence of EPS.[1] Trihexyphenidyl chemically name as 1-cyclohexyl-1-phenyl-3-(1- piperidyl)-1-propanol.[2] It treats Parkinson's disorder and acts on a muscarinic receptor in the central nervous system.[3] Both Risperidone and Trihexyphenidyl are official in IP.[4]

RP- HPLC and UV-Visible Spectrophotometer, simultaneous estimating Risperidone and Trihexyphenidyl, has developed a validation method

Keywords: EPS, Risperidone, Trihexyphenidyl.

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INTRODUCTION

In this study, the literature survey shows that different methods of HPLC and UV have been reported for the determination of Risperidone and Trihexyphenidyl individually or simultaneously for bulk drug or formulation,[5-17] the literature survey shows that no simultaneous estimation of stability of both drug have been determined in different mobile phase. The current study used the RP-HPLC and UV double spectrophotometric method using the ratio of mobile phase to determine both the drugs' pure and pharmaceutical formulation.

MATERIALS AND METHODS

Instruments

A double beam UV-visible spectrophotometer with a spectrum width of 2 nm, the accuracy of wavelength is 0.5 nm, and 1 cm quartz cells. The chromatography method was developed by the HPLC series equipped with a UV-visible detector was used.

Chemicals

Sun Pharmaceutical Limited Jammu gifted the pure RIS, and TRI bulk powder and pharmaceutical formulation were purchased from local market.

For HPLC Estimation

Chromatographic Condition

Mobile Phase

The mobile phase will be selected based on the hit and trial method. In reverse phase, HPLC, generally mobile phase, is polar and prepared by mixing different ratio of polar solvents such as water, methanol, acetonitrile, and various types of buffers are used and trialed by changing the composition of these various solvents. The isocratic and gradient two techniques are used for pumping of the mobile phase.

Wavelength

The single drug or combination of drugs will be scanned first to select the wavelength maxima, and the wavelength is fixed

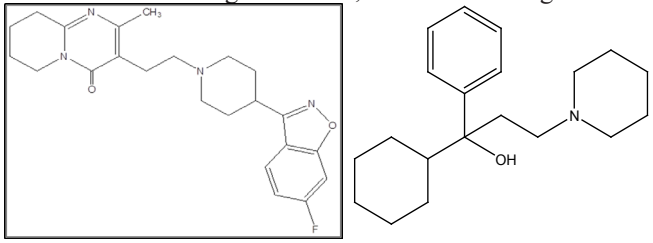

Figure 1: (a) Structure of Risperidone (b) Structure of Trihexyphenidyl

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for the highest absorbance of the drugs. The isosbestic point is used for simultaneous methods of drug analysis.

Method Validation Procedure

System suitability: Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters were evaluated from tailing factor, retention times, and theoretical plates of standard chromatograms. Standard solution preparations were injected five times into the chromatographic system, and retention times were recorded.

Specificity: Specificity is the ability to assess the analyte unequivocally in the presence of components that might be expected to be present, such as impurities, degradation products, and matrix components. Standard solution preparations were injected into the chromatographic system, and the chromatograms were checked visually for any interference

System precision: Standard solution preparations were injected five times into the chromatographic system, and retention times were recorded. The peak responses for major peaks were recorded. The retention time and area of five determinations are measured, and %relative standard deviation should be calculated.

Linearity: Different levels of standard solutions were prepared by pipetting out a known volume of stock solution and made up to volumes with the diluent to get different analyte concentrations. The above solutions were injected into the chromatograph. The area response for each level was recorded and slope, intercept, correlation coefficient, and regression coefficient (R square) were calculated. Intercept for statistical equivalence to zero was tested, and a graph of concentration (ppm) on the X-axis and area on the Y-axis was plotted.

Accuracy: Spike known quantity of standard drugs at specification level into the sample, and these samples were injected triplicate for each level. From the results obtained, accuracy and range parameters were calculated. The study was performed by making three different standard concentrations of known amounts of the studied drugs. Finally, the final volume is made up of solvent (mobile phase) and mixed well. The resulting mixtures were analyzed by the proposed HPLC method.

Robustness: The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like mobile phase composition, flow rate, and temperature, which may differ, but the responses were still within the specified limits of the assay. The standard solution, sample solution, and sample solution spiked with impurities were injected into the chromatograph at varied flow conditions ± 0.1 milliliter/min, mobile phase buffer pH ± 0.2 units, and wavelength by + or -2nanometer.

Limit of Detection and Quantification: The limit of detection (LoD) is the lowest amount of an analyte in a sample that can be detected but not necessarily quantized under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 3:1. The limit of Quantification (LoQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as the LoQ of the method. These can represent that the sensitivity of the method is high. The detection limit (LoD) and quantitation limit (LoQ) may be expressed as:

$$LoD. = 3.3\ (SD/S)$$

Where SD = Standard deviation of the response

$$LoQ. = 10(SD/S)$$

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte

Preparation of Stock Solution

Weighed an equal amount of Risperidone and Trihexyphenidyl and was transferred to a separate flask and dissolved and diluted to the mark with methanol to obtain a standard stock solution having the same concentration of 200 µg/mL. A 0.25 mL was taken from the stock solution and transferred to a flask; obtained from the above stock solution, 0.25 mL was transferred to a volumetric flask of 10 mL capacity. Volume was made up to the mark to give a solution containing 5 µg/mL of RIS and TRI (Table 1).

Preparation of Sample

Twenty tabs were considered and powdered finely. 4miligram of RIS and 2 µg of THP equivalent powder were taken. Then transferred it into a volumetric flask of 100milliliter capacity. By use of methanol (50milliliter), the volume was made up and sonicated for 20 minutes for maximum dissolution of the drug. Filtration was carried out by Whatman filter No.41. After that rest of the volume was built up with solvent. 4 mL from it was taken and diluted with methanol for 10 mL volumetric flask. The medicines risperidone and trihexyphenidyl were taken first on the butter paper and then weighed through the digital analytical balance that is calibrated properly.

Calibration Curves

Calibration curves were constructed by plotting peak areas vs. concentrations of RIS and TRI, and regression equations were calculated. Calibration curves were plotted over a different concentration range. Accurately measured standard working solutions 0.25, 0.5, 0.75, 1.0, and 1.25 mL were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase. Each solution's aliquots (20 µL) were injected under the operating chromatographic conditions.

For UV Spectrophotometry

Absorbance Ratio Method

The samples were taken in the cuvette and put in the UV-visible double beam spectrophotometer by fixing the wavelength at 206 nanometers for trihexyphenidyl and 239 nanometers for risperidone to measure the absorbance of different dilutions. The calibration curve was prepared for each drug by plotting a graph between absorbance and concentration.

Table 1: Relapse examination information and outline of approval parameters

	Absorption correction UV Spectrophotometry method		
Parameters	*RIS at 239 nanometer*	*THP at 206 nanometer*	*RIS at 206 nanometer*
Concentration range (microgram per milliliter)	2–40	4–20	8–40
Sandell's sensitivity (μg/cm^2 absorbance unit)	0.097	0.0175	0.0576
Slope	0.0101	0.0533	0.0196
Intercept	0.0069	0.0026	0.0375
Correlation coefficient	0.999	0.997	0.9977
LOD	0.477	0.266	0.242
LOG	2.386	1.328	1.209
% Recovery (Accuracy, n = 5)	101.52 ± 1.29	100.55 ± 0.82	--------
Repeatability (n = 6), %	1.365	1.348	0.844
Precision			
Interday	0.832–1.032	1.01–1.18	0.64–1.24
Intraday	0.924–1.15	0.75–1.29	0.60–0.94
Assay± S.D	99.81 ± 1.60	100.42 ± 0.92	

The validation parameters like linearity, range, accuracy, precision, limit of quantification and detection, system suitability, and specificity are validated with proper testing according to ICH and US-FDA guidelines.

RESULTS AND DISCUSSION

HPLC Method Development

Several mobile phase compositions measured the HPLC parameters. The optimized peak was obtained with the mobile phase composition of methanol and acetate buffer. A satisfactory separation and good correlation were obtained with the selected mobile phase at 214 nanometer

Method validation

Linearity

The linearity data obtained in the present study is acceptable for both drugs.

Calibration curves of risperidone and trihexyphenidyl at 214nanometer, respectively, with a correlation of 0.9957 for RIS and 0.9994 for THP at a concentration range from 2-20 microgram/ml and 1-10 microgram/ml.

Precision

The precision was measured as repeatability and was studied by RSD value. The interday (n=6) and intraday (n=6) in which intra-day precision for RIS was 0.11-0.62 and THP were .25 -0.35 while interday precision of RSD value of RIS was .20-1.50 and THP was 0.25-067.

LoD and LoQ

For risperidone and trihexyphenidyl drug samples, LoD values were 0.23 micro-gram per milliliter and 0.04 microgram per milliliter while LoQ values were 0.56 microgram per milliliter and 0.21 microgram per milliliter.

Accuracy

The accuracy was performed by the addition method. The value obtained was 99.93 for RIS and for THP is 100.12

Robustness

% relative standard deviation was less than 2%.

UV Analysis Method Development and Validation Parameter

Regression parameters are mentioned in Table 1, and calibration curves of RIS at 239 nanometer.

CONCLUSION

RP- HPLC and UV- Visible Spectrophotometer, simultaneous estimating Risperidone and Trihexyphenidyl, has developed a validation method. The method was estimated as specific, and no interference was seen from impurities and excipients. The method developed was simple, precise, and accurate for determination and can be further used for the analysis of Risperidone and Trihexyphenidyl for dosage formulation.

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