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Development and Validation of Spectrophotometric Method for the Determination of Risperidone in Bulk Drug and Pharmaceutical Formulation by UV And HPLC

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Abstract

Pharmaceutical chemistry encompasses all the major areas of chemistry; analytical, physical, organic and radiochemistry. It is a science that applies general laws of chemistry to come up with chemical nature, physiochemical composition, structures, and properties of drugs. The proposed spectrophotometric method was systematically in accordance with ICH guidelines, and the findings confirmed its reliability, sensitivity, and reproducibility. The λmax of risperidone was established at 202 nm, and from the optical characteristics (Table 2), the drug was shown to range of 2.5–20 µg/mL. Sensitivity of the method was further demonstrated through the calculation of LOD and LOQ, which were determined as 1.05 µg/mL and 3.34 µg/mL, respectively, using standard formulae (k = 3.3 for LOD and k = 10 for LOQ). These values highlight the method's capacity to detect and quantify risperidone even at low concentration levels. Precision was confirmed through intra-day and inter-day analyses (Table 2), where %RSD values consistently remained below 2.0, ensuring reproducibility. Accuracy was validated by recovery studies (Table 3), with percentage recovery values exceeding 100%, demonstrating robustness and the absence of interference from formulation excipients. Stability testing further supported the method's reliability, as both standard and sample solutions remained stable for at least 24 hours, with %RSD below 2.0 and no significant degradation observed. Finally, application of the method to tablet formulations (analyzed in triplicate) showed excellent agreement with the labeled claim (Table 4), confirming the suitability of the proposed method. Detecting even low quantities of risperidone is a beneficial application for quality control, stability studies, and regulatory compliance.

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Keywords: Physiochemical composition, stability studies, ICH guidelines, spectrophotometric method.

Introduction

Pharmaceutical chemistry encompasses all the major areas of chemistry; analytical, physical, organic and radiochemistry. It is a science that applies general laws of chemistry to come up with chemical nature, physiochemical composition, structures, and properties of drugs. It also includes a study of influence of drug on an organism, the conditions of their quality control and ways of storage. In the case of a drug, only a thorough knowledge of physical and colloid chemistry is able to comprehend the total action of that drug. There is the lack of any possibility to study the molecular structure of a drug as well as to create methods of its synthesis and analyzing without the sound knowledge of organic and analytical chemistry.

Risperidone

Risperidone is a widely prescribed psychotropic agent primarily used in the management of schizophrenia, and its therapeutic action is based on a dual mechanism involving blockade of both receptors.

Spectrophotometric Method

The spectrophotometric method is an analytical technique that quantifies the interaction of light with a chemical substance by measuring the extent of light absorption or transmission at a defined wavelength. The principle is based on the fact that molecules absorb light of specific wavelengths depending on their structural characteristics. By recording the intensity of absorbed light relative to a blank or reference solution, the method enables accurate determination.

HPLC

HPLC is a method used in analysis in which components within a mixture are separated, identified and quantified. These mixtures can contain objects of food, chemicals, pharmaceuticals, biological samples, environmental materials, and agricultural products, chemical substances in solutions. It uses high pressure pumps that operate to propel a as the mobile phase. The mobile phase moves the mixture of the samples through the commonly known as stationary phase.

The molecules of a single sample react differently with the adsorbent matter in the aspect of various kinds and motions, and, accordingly, their relocation movements of each molecule are not identical. These disparities in rate results in the separation since all the species are washed out of the column to one particular detector, like the UV detectors. The result of the detector is in a graphical form also known as chromatogram. Peaks representing tract of the sample may be represented by graphical representation of signal intensity as a function of time or volume and are called chromatograms. The flow receiver all the samples at their respective retention time and area under graph will bear an inversely proportional relation to the quantity of the sample.

Aim

To develop and validate UV or HPLC spectrophotometric methods for the determination of risperidone in pure and tablet dosage forms.

Objectives

Development of UV or HPLC spectrophotometric methods for the determination of Risperidone. Validation of the method developed. Application of the developed methods in determination of risperidone in its pure form and in tablet dosage forms. The objective of the present investigations was to develop a simple, accurate and economical spectrophotometric methods for estimation of Risperidone in tablet formulations.

Methodology

Risperidone tablet from Banglesh, Orion Laboratories Ltd, Analytical grade Hydrochloride acid from local Market, A UV–Visible double-beam spectrophotometer (UV-1601 PC, Shimadzu Ltd., Japan), a variable-volume micropipette (10–1000 µL, Gene Pete Co.), and a digital balance were employed for the study. The chromatographic method was using an HPLC system equipped.

UV Spectroscopy

Selection of Wavelength (λmax) Determine Ethanol as a reagent Determination of Wavelength of Maximum Absorption (lambda max). The standard solutions of risperidone were prepared by means of 5 0g/mL and 10 0g/mL of 0.1 N HCl. UV-Visible spectrophotometer was utilized to scan them at a wavelength of 200-380nm with methanol as the blank.

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Preparation of Working Solution to Calibrate

A 10 mg portion of pure risperidone was measured accurately weighed and mixed with 0.1 N HCl and diluted to volume with water in a 100 mL volumetric flask, to give the primary stock solution. Out this, 5 ml was then diluted to 100 ml using a 0.1 N HCl to obtain the working stock solution. This working solution was serially diluted to obtain a concentration of 2.5, 5, 10, 15 and 20 g/mL. These solutions were later applied in making the calibration graph.

The Preparation of Sample Solution

The valorization of risperidone in the commercial dosage forms was estimated by weighing twenty tablets properly, pulverising and homogenizing them. A volume referable to 10 mg of risperidone ow was taken into a 100 mL volumetric flask. To it 40 mL of 0.1 N HCl was added and the solution was sonicated (5 min) to ensure total dissolution. Absorbance of this solution was taken at 202 nm against blank. All the analyses were repeated in triplicate (Table 1).

The Method is Validated

Based on the specifications set by the International Council of Harmonisation (ICH), the methodology devised was validated on the aspects of linearity, precision, accuracy, stability, limit of detection (LOD), and limit of quantitation (LOQ) (Table 2).

The linearity of risperidone was evaluated using five concentration levels, being 2.5 to 20 µg/ml. At this range, a calibration plot of absorbance versus the concentration established the linearity of the parameter (Figure 2), and the demonstrates that Beer B statist's law was obeyed. The standard regression equation was estimated as $Y = 0.0508X + 0.0112$ and the correlation value (r) was calculated as 0.9991 (Table 2), and both of them show that the relationship between the variables is quite well-correlated.

The evaluation of the level of precision was based on the variability that took place within the same day (repeatability) and over a series of days (intermediate precision). On calculation the relative standard deviation as a percentage (% RSD) was calculated within the acceptable limit which is below 2.0. This observation justifies that the strategy can be repeated.

Recovery tests were conducted to identify the accuracy of procedure. In these analyses, pure risperidone was added to original formulations whose composition had been previously analysed. Recovery experiments were performed at three various concentration levels,

relative to a formulation concentration of 5 1g/mL. These concentrations were 80%,100%, and 120 percent. With the retrieved amount of medicine, the recovery was calculated, and the result demonstrated that the procedure was precise, although there was a slight interference of the excipients (Table 3).

LOD & LOQ: In accordance with the definitions prescribed by the ICH, the LOD (k= 3.3) and LOQ (k= 10) of the technique were determined. Table 2 contains for the approach mentioned. Using the following formulae, the limits of detection (LOD) and limits in this investigation of the concomitant curve:

$$LOD = 3.3\ S/M;\ LOQ = 10\ S/M$$

Stability Study: Both solutions were analysed for a period of twenty-four hours at room temperature, and the RSD of absorbance for both solutions was computed. This was done in order to demonstrate that both the sample solution was stable during the analysis process.

For HPLC Estimation

Chromatographic Condition

Mobile Phase

The mobile phase was optimized in reversed-phase HPLC, the mobile phase is typically and different buffer systems in varying ratios. Optimization was carried out by altering solvent compositions until suitable separation was achieved. Both isocratic and gradient techniques were employed for the delivery of the mobile phase.

Wavelength Selection

The individual was first scanned in the UV range to absorption (λmax). The wavelength corresponding to maximum absorbance was selected for analysis. In simultaneous estimation methods, the isosbestic point was utilized as the analytical wavelength.

Method Validation Procedure

Method considering the following parameters:

Suitability: Standard preparations were made and details injected into the chromatographic system. Tailing factor, retention time and theoretical plate counts were used to determine the suitability parameter of the suitability parameters on the basis of the chromatograms. Five injections of each standard solution were made and the retention time noted.

Specificity was set as the capability of the method to quantify the analyte in the presence of anticipated contamination, intermediates or excipients. Standard solutions were injected and chromatograms visually assessed to determine the ability of interference.

Precision: Five multiple injections of standard solutions were run and the retention times and the peak heights of the key peaks were recorded. Peak area and retention time repeatability were determined by the calculation of the % relative standard deviation (%RSD).

Linearity: Appropriate stock solution dilutions were performed to have varied hormone concentrations. These were transferred to the chromatograph where the number of areas of peaks were recorded. A calibration curve was plotted by Y-axis on the peak area versus the X-axis on the concentration (ppm). Slope, intercept, correlation coefficient (r), and regression coefficient (R2) were assessed, and the intercept set at zero was tested.

Accuracy: It was assessed using recovery studies which entailed spiking of known quantities of standard drug into previously analyzed samples at three concentrations levels (80%, 100% and 120%). The analyses at each level were triplicated. The percentage recovery and range parameters were computed to ensure that the accuracy of approach is confirmed.

Robustness: Robustness was defined as the ability of an analyte to be perturbed by small but deliberate changes in the chromatographic conditions such as the composition of the mobile phase, flow rate (0.1 mL/min), buffer pH (+/- 0.2 pH units) and detection wavelength (+- 2 nm). Standard and sample solutions, and spiked solutions, were tested at these different conditions to ensure that assay performance was within acceptable limits at the different conditions.

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD was determined as the lowest concentration of analyte that could be confirmed but not quantified, a signal-to-noise ratio of 3:1. The limit of quantitation (LOQ) was determined as the concentration with signal-to noise ratio of 10: 1. These values show high sensitivity of the method.

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

Where , SD = Standard deviation of the response

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

S = Slope of the calibration curve

Preparation of Stock Solution

In order to generate a standard stock solution with the same concentration of 200 µg/mL, an adequate quantity of risperidone was weighed and then transferred to a separate flask. The risperidone was then dissolved and added to methanol until it reached the desired concentration. From the stock solution, a volume of 0.25 millilitres was extracted and transferred to a flask. The same amount of 0.25 millilitres was then transferred from the stock solution to a volumetric flask with a capacity of 10 millilitres. In order to obtain a solution that contained 5 µg/mL of RIS, the volume was brought up to the mark, as shown in Table 1.

Preparation of Sample

In the assay of risperidone in tablet dosage form, 20 accurate-weighed tablets were homogenized and their coarse powder was made homogeneous. A weight of powdered tablet representing equivalent amount of 4 mg of risperidone (RIS) was weighed on a precision calibrated digital analytical balance. The weighing was transferred to 100 mL volumetric flask and 50 mL methanol was added. Sonication of the solution was carried out to achieve maximum dissolution of the drug taking 20 minutes in total. Subsequent to this the solution was transferred through Whatman filter paper No. 41 to eliminate any insoluble excipients or any particulate material. Then the volume was added to the mark with methanol to provide the stock solution.

With this source stock solution, 4 mL was pipetted into a 10 mL volumetric flask and diluted-up to volume with methanol to obtain the desired working concentration. This solution was then taken in preparation to further analysis.

Calibration Curves

Calibration curves of risperidone were built by plotting peak area versus rising drug concentration and the regression equation was calculated. There was preparation of standard working solutions of risperidone over a certain range of concentrations. In particular, using an accurate volumetric pipette, 0.25, 0.5, 0.75, 1.0, 1.25, mL aliquots of the standard stock solution were pipetted into 10 mL volumetric flasks, each being diluted to volume with the mobile phase.

A 20 -mL aliquot of each of the prepared concentrations was injected into the HPLC system using the optimized chromatographic conditions. The results were taken as areas of the peaks obtained, and calibration curves were calculated. These curves indicated that there was a high

linear relationship between concentration and response, and therefore the technique is appropriate to quantitatively determine risperidone.

Results and Discussion

- The proposed spectrophotometric method was systematically in accordance with ICH guidelines, and the findings confirmed its reliability, sensitivity, and reproducibility.
- The λmax of risperidone was established at 202 nm, and from the optical characteristics (Table 2), the drug was shown to range of 2.5–20 µg/mL.
- Sensitivity of the method was further demonstrated through the calculation of LOD and LOQ, which were determined as 1.05 µg/mL and 3.34 µg/mL, respectively, using standard formulae (k = 3.3 for LOD and k = 10 for LOQ).
- These values highlight the method's capacity to detect and quantify risperidone even at low concentration levels.
- Precision was confirmed through intra-day and inter-day analyses (Table 2), where %RSD values consistently remained below 2.0, ensuring reproducibility.
- Accuracy was validated by recovery studies (Table 3), with percentage recovery values exceeding 100%, demonstrating robustness and the absence of interference from formulation excipients.
- Stability testing further supported the method's reliability, as both standard and sample solutions remained stable for at least 24 hours, with %RSD below 2.0 and no significant degradation observed.
- Finally, application of the method to tablet formulations (analyzed in triplicate) showed excellent agreement with the labeled claim (Table 4), confirming the suitability of the proposed method.

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TABLE 1: DATA FOR STANDARD CURVE

Concentration (µg/ml)	Absorbance
0.00	0.000
2.5	0.134
5	0.278
10	0.525
15	0.785
20	1.014

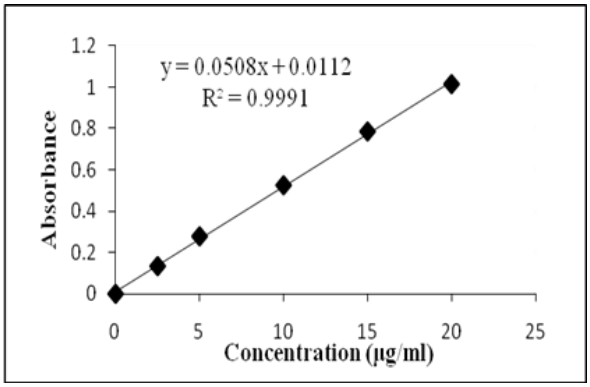

FIG. 2: STANDARD CURVE OF RISPERIDONE

TABLE 2: VALIDATION PARAMETERS

Parameters	Results	
Absorption maxima(nm)	202	
Linearity range (µg/ml)	2.5to 20	
Standard Regression equation	Y = 0.0508X + 0.0112	
Correlation coefficient	0.9991	
LOD (µg/ml)	1.05	
LOQ (µg/ml)	3.34	
Stability (hrs)	48	
Precision	Intraday Concentration	Interday (%RSD)
	(%RSD)	
	5(µg/ml) 0.385	0.313

TABLE 3: RECOVERY STUDY

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Level of Addition (%)	Formulation (µg/ml)	Addition of pure drug (µg/ml)	% Recovery of pure drug	Recovery (%)±S.D.
80	5	4	100.5	
100	5	5	100.5	100.67±0.29
120	5	6	101.0	

TABLE 4: DETERMINATIONS OF ACTIVE INGREDIENTS IN TABLETS

Sample	Label claimed	Amount found	% Labelled Claim*
Risperidone	2 mg/tablet	2.01±0.008	100.5

* Average of three determinations

HPLC Method Development

To attain the best chromatographic performance and accurate risperidone quantification, a number of mobile phase compositions were thoroughly assessed during the technique development process. Peak symmetry, retention time, resolution, and repeatability were among the factors that were specifically examined during the testing of various combinations of organic modifiers and aqueous buffers. The combination of methanol and acetate buffer produced the best results out of all the systems that were investigated.

Method validation

Linearity

The conducted studies on linearity in the present study proved that the method developed is quite reliable and acceptable in quantitative estimation of drug. To get a calibration curve, a plot was drawn between the concentration and the proportional corresponding area under the peak. The best wavelength to use was 214 nm. The calibration data showed an excellent linearity over the ranges of the selected concentrations thus meeting the validation requirement of one of the ICH requirements.

Risperidone The method is shown to be linear over the range 2-20ug/mL with a correlation coefficient (r 2) of 0.9957 indicating a very strong linear relationship between concentration

and registered signal. In the case of trihexyphenidyl, calibration was performed between the range of 1-10 ug/mL with the correlation coefficient even higher (0,9994) indicating practically perfect linearity. These findings demonstrate the precision and consequently the reproducibility of the technique within the ranges tested.

Precision

The precision was assessed as repeatability and indicated as relative standard deviation (RSD). Intra-day accuracy (n = 6) of risperidone (RIS) was 0.11-0.62, whereas that of trihexyphenidyl (THP) was 0.25-0.35. Inter-day precision (n = 6) was within acceptable limits (RSD values 0.20 - 1.50 for RIS and 0.25 - 0.67 for THP) and indicated the reproducibility of the method.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The sensitivity of the methodology was tested by determining the LoD and LoQ. The determination of LoD was determined to be 0.23 and 0.56 mcg/g and LoQ as 0.04 and 0.21 mcg/ml, respectively, of risperidone and trihexyphenidyl. These findings indicate the sensitivity of the technique to the sensitivity of the low concentration of the drugs.

Accuracy

Reliability was checked by the conventional method of addition The recovery percentage values recorded were 99.93 and 100.12 which was as expected with minimal interference by the excipients in the case of RIS and THP respectively.

Robustness

Robustness experiments were developed to assess the repeatability of the procedure up to small, intentional changes in the analytical conditions, like alteration of mobile phase composition and flow rate as well as in detection wavelength. These findings showed that there were little variations that did not have significant implications in the performance of the assay and, therefore, the method was satisfactory.

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Table 5: Risperidone examination information and outline of approval parameters

Parameters	Absorption correction UV and HPLC Spectrophotometry method	
	RIS at 202 nanometer	**RIS at 214 nanometer**
Concentration range (microgram per milliliter)	2–40	4–20
Sandell's sensitivity (μg/cm^2 absorbance unit)	0.097	0.0175
Slope	0.0101	0.0533
Intercept	0.0069	0.0026
Correlation coefficient	0.999	0.997
LOD	0.477	0.266
LOG	2.386	1.328
% Recovery (Accuracy, n = 5)	101.52 ± 1.29	100.55 ± 0.82
Repeatability (n = 6), %	1.365	1.348
Precision		
Interday	0.832–1.032	1.01–1.18
Intraday	0.924–1.15	0.75–1.29
Assay± S.D	99.81 ± 1.60	100.42 ± 0.92

Conclusion

The analytical approach that was devised turned out to be straightforward, sensitive, exact, and extremely dependable. It also consistently provided accurate results regardless of the experimental circumstances that were being used. The fact that it was effectively used to the estimate of risperidone in commercial pharmaceutical formulations without any observable interference from frequently used excipients, preservatives, or other formulation additives is

one of its most important characteristics. Its specificity is one of its significant features. Its simplicity, combined with robust validation outcomes, makes it highly suitable for adoption in quality control laboratories, research settings, and pharmaceutical industries where rapid and accurate analysis is essential.

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