QBD BASED ANALYTICAL DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING METHOD FOR ESTIMATION OF RITODRINE IN PHARMACEUTICAL DOSES FROM RP–UPLC

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Abstract:

A simple, Accurate, precise method was developed for the estimation of the Ritodrine in bulk and pharmaceutical dosage form. Chromatogram was run through Hibar100 x 2.1 mm, 2µ. Mobile phase containing 0.1N OPA: Acetonitrile taken in the ratio 55(%v/v) and 45 was pumped through column at a flow rate of 0.9 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was ACQUITY TUV ChA 220.0 nm. Retention time of Ritodrine was found to be 1.065 min. %RSD of the Ritodrine was found to be 1.5. %Recovery was obtained as 99.95% for Ritodrine. Regression equation of Ritodrine is y = 69931x + 4571.9. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Ritodrine, QbD Approach, Method development, RP-UPLC

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1. Introduction

The conventional approach for analytical method development involves changing one factor at a time (OFAT), commonly known as OFAT technique. The OFAT is very time consuming and lengthy process which generates voluminous data, resulting in a tedious approach. Also, once the method is developed, the method still may need additional efforts when validated[1]. The modern pharmaceutical analysis and regulatory scenario, demands to use novel chemometric tools which control many variables simultaneously and helps to provide desired results with minimum experimental trials. This can be achieved by implementing Quality-by-Design (QbD) approach in analytical method development. In International Conference on Harmonization (ICH) guideline Q8(R2) for Pharmaceutical development, QbD is defined as "A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management"[1,2]. The elements of QbD can be extended for analytical method development. The Analytical QbD utilizes statistical modelling and design of experiments (DoE) to arrive at a method operable design region (design space), the robust area, where the developed method provides the desired results[3].

UPLC is an emerging area of analytical separation science which retains the practicality and principles of UPLC while increasing the overall interlaced attributes of speed, sensitivity and resolution. Speed and peak capacity can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC by using fine particles. UPLC takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates for increased speed, sensitivity and superior resolution.

In this article we explored the potential of UPLC to improve the analysis of the samples that are encountered during pharmaceutical development and manufacturing. Particular emphasis has been placed on determining whether UPLC can reduce analysis times without compromising the quantity and quality of the analytical data generated compared to UPLC.

Ritodrine is a phenethylamine derivative with tocolytic activity. Ritodrine binds to and activates beta-2 adrenergic receptors of myometrial cells in the uterus, which decreases the intensity and frequency of uterine contractions. Specifically, ritodrine probably activates adenyl cyclase, thereby increasing production of cyclic adenosine monophosphate (cAMP), which in turn enhances the efflux of calcium from vascular smooth muscle cells. A lack of intracellular calcium prevents uterine myometrial contractions. In addition, this agent may directly inactivate myosin light chain kinase, a critical enzyme necessary for the initiation of muscle contractions.it is chemically called as 4-[(1S,2R)-1-hydroxy-2-{[2-(4-hydroxyphenyl)ethyl]amino}propyl]phenol [4-5].

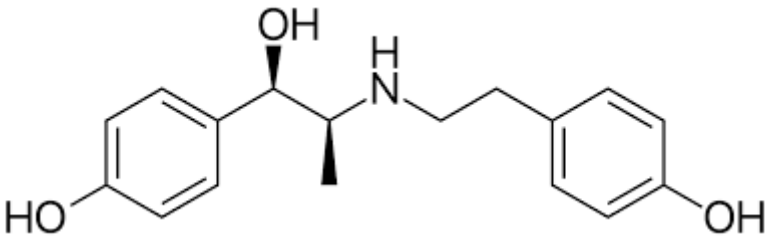

Figure1: Chemical structure of Ritodrine

2. MATERIALS AND METHOD [6-7]

5.1. Chemicals and reagents

Pure Ritodrinewas procured from Spectrum pharma lab (Hyderabad). Hydrochloric acid AR grade (HCL) and sodium hydroxide AR grade (NAOH) were obtained from rankem, India. Hydrogen Peroxide (H_2O_2) was purchased from Qauligens. Acetic acid AR grade was purchased from Fisher scientific, India and S.D. Fine chem Ltd. Respectively. Potassium dihydrogen orthophosphate and orthophosphoric acid were obtained from S.D. Fine chem Ltd and Merck India Pvt Ltd. Respectively. UPLC grade Acetonitrile (ACN) and methanol (MeOH) were purchased from Fischer scientific. UPLC grade water used throughout analysis was obtained from the Merck milli-Q water purification unit.

5.2. Apparatus and Equipment

- UPLC studies were carried out on WATERS UPLC 2965 SYSTEM with a Photo diode array detector (PDA) set at 220 nm for uv detection. columns, viz; Agilent C18 (150×4.6 mm, 5 µm), Discovery C18(150*4.6mm,5 µm), Zodiac (150*4.6mm,5 µm) ,BDS (150*4.6mm,5 µm)and Phenomenex(150*4.6mm,5 µm)column were utilized in the study. Design Expert® (11.0.0) modeling software (Stat-Ease Inc., Minneapolis, MN, USA) was used for generation of contour plots and 3D space.
- pH meter (Eutech instruments pH tutor, pH meter, India) was used to check the pH of all solutions.
- Other equipment sonicator (ePEI ultrasonic generator), Analytical balance (Mettler Toledo), vortex meter (IKA Vortex), Hot air oven (Yorco scientific).

Simple and Robust RP-UPLC method development by DOE approach.

Preparation of drug solution

Preparation of Standard stock solutions: Accurately weighed 10mg of Ritodrine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Ritodrine)

Preparation of Standard working solution (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Ritodrine).

Preparation of Sample stock solutions: **10**mg of tablet of were Taken, and then Weight equivalent to 1tablet was transferred into a 100mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. (100µg/ml of Ritodrine)

Preparation of Sample working solution: From the filtered solution 2 ml was pippeted out into a 10 ml volumetric flask and made upto 10ml with diluent. (20µg/ml of Ritodrine).

Methodology for Validation Parameters [8-12]

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Ritodrine (20ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Standard stock solutions: Accurately weighed 10mg of Ritodrine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Ritodrine)

Preparation of Standard working solution (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Ritodrine).

Preparation of Sample stock solutions: **10**mg of tablet of were Taken, and then Weight equivalent to 10 tablets was transferred into a 100mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. (100µg/ml of Ritodrine)

Preparation of Sample working solution: From the filtered solution 2 ml was pippeted out into a 10 ml volumetric flask and made up to 10ml with diluent. (20µg/ml of Ritodrine).

Linearity:

Preparation of Standard stock solutions: Accurately weighed 10mg of Ritodrine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Ritodrine)

25% Standard solution: 0.25ml each from two standard stock solutions was pipette out and made up to 10ml. (5µg/ml of Ritodrine)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (10µg/ml of Ritodrine)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Ritodrine,)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (20µg/ml of Ritodrine)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Ritodrine)

150% Standard solution: 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (30µg/ml of Ritodrine)

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 10mg of Ritodrine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Ritodrine)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System

suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml Standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.25ml Ritodrine, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.9ml Ritodrine of, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies: [13-19]

Oxidation:

To 1 ml of stock solution of Ritodrine, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the result antsolution was diluted to obtain 20µg/ml solutionand10µlwereinjectedintothe system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution Ritodrine, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c.The resultant solutionwasdilutedtoobtain2 0 µg/ml solution and10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Ritodrine, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The result ant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placedinovenat105°C for1h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 20µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 200µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1hrs or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

5.5.2. Preparation of buffer

5.5.2.1. 0.01N Potassium dihydrogen ortho phosphate

Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.0 with dil. Orthophosphoric acid solution

5.5.2.2. 0.1% Ortho phosphoric acid buffer

1ML of Ortho phosphoric acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water

Initial UPLC runs of Ritodrine

Initial UPLC runs of Ritodrine of 20 µg/mL concentration were performed using

Different buffer viz, Potassium dihydrogen ortho phosphate and Ortho phosphoric acid.

Different organic modifier viz, acetonitrile and methanol

Different columns such as Symmetry C18 (150×4.6 mm, 5 µm), Agilent C18 (150×4.6 mm, 5 µm), Discovery C18(150*4.6mm,5 µm), Zodiac (150*4.6mm,5 µm),BDS (150*4.6mm,5 µm)and Phenomenex (150*4.6mm,5 µm)column

Optimization of method

The method was optimized using central composite design (CCD). The initial trials are needed to optimize the final method. Total Three factors viz; % Organic concentration, Flow rate and column temperature were needed to be optimized. So CCD was used to optimize these parameters which were varied over three level (high, mid and low).different ranges of four parameters ranging from 36.59-53.41%, 0.01N potassium dihydrogen orthophosphate, column temperature26.64 and 33.36^0C and 0.6636-1.34ml/min flow rate respectively were taken and counter and 3D surface plot showing the effect of each parameter on Retention Time, Area, Theoretical plates and Asymmetry (CQA) were generated. A desirability function applied to the optimized conditions to predict retention time, asymmetry, theoretical plates and peak area.

Table 1. Design summary of CCD

Design Summary					
File version: DX 11.0.0 Study Type: Response surface Design Type: central composite design Design Model: Quadratic			ATP: Robustness CQA: Retention time, Area, Theoretical plates and Asymmetry Runs: 24		
CMPs	Unit	Type	Subtype	Min.	Max.
column temperature	^{0}C	Numeric	Continuous	26.64	33.36
Flow rate	ml/min	Numeric	Continuous	0.6636	1.34
%Org ratio	%	Numeric	Continuous	36.59	53.41

Method validation

The final optimized chromatographic analytical method was validated as per the International Conference on Harmonization (ICH) Q2(R1) guidelines for system suitability, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. Standard stock solution was prepared by dissolving 10mg of Ritodrine in 50 mL of diluents to a final concentration of 200µg/ml. then 1ml stock solution is transferred into 10 v/f and made upto the volume to get 20µg/ml.

Linearity

Standard calibration curves were generated with seven different concentrations including the LOQ by making serial volume to volume dilution of stock solution I over the range of 25-150 µg/ml. Linear calibration curves were generated between peak area and drug concentration. The linearity was examined using linear

regression, which was calculated by the least square regression method.

Accuracy

The accuracy of developed analytical method was analyzed by developed method, accuracy experiments were carried out using standard addition method. Three different level concentrations (50%, 100%, and 150%) of standards were added to pre-analyzed samples in triplicate. The percentage accuracy of Ritodrine at each level and each triplicate were calculated and the mean of percentage accuracy (n=9) and the relative standard deviation was determined.

Precision

The precision of the developed analytical method was determined by repeatability (intraday) and intermediate precision (inter-day). Repeatability defines the use of the analytical procedure within a laboratory over a short period of time that was examined by assaying the samples during the same day. Intermediate precision was evaluated by comparing the assays on different days. SD and %RSD were determined.

Limits of detection and quantitation

Limits of detection (LOD) and limit of quantitation (LOQ) were determined from the signal-to-noise ratio. The detection limit was refer to as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantitation limit was refer to as the lowest concentration level that provided a peak area with a signal-to-noise ratio higher than ten.

System suitability

The system suitability was determined by taking six replicates of the drug at same concentration of 100µg/ml. The acceptance criteria was ± 2% for the percent coefficient of variation (% CV) for the peak area, retention time of drug, USP Plate Count, and asymmetry.

Robustness

The Robustness is one of the validation parameter, it measure of method capacity to remain unaffected by small, deliberate changes in chromatographic conditions was studied by testing the influence of small changes in the organic content of mobile phase (±10%), flow rate (±10%) and pH (±10%) and.

Stress study

Generation of stress samples of Ritodrine

Acid Hydrolysis

To 1 ml of stock solution of 1ml of 2N HCl solution was added. These degradation samples were kept in Radley apparatus (Veego) with continuous stirring at 70º C for 1 hrs in 2N HCl. These samples were neutralized to pH 7, diluted and analyzed by the UPLC system.

Base hydrolysis

To 1 ml of stock solution of 1ml of 2N NAOH solution wasadded. These degradation samples were kept in Radley apparatus (Veego) with continuous stirring at 70º C for 1 hrs in 2N NaOH solution. These samples were neutralized to pH 7, diluted and analyzed by the UPLC system.

Neutral hydrolysis

10 mg of Ritodrine was weighed and dissolved in 50 ml of water. These degradation samples were kept in Radley apparatus (Veego) with continuous stirring at 70º C for 24 hrs. These samples were diluted and analyzed by the UPLC system.

Oxidative study

To 1 ml of stock solution of 1ml of 20% H2O2 solution was added. These degradation samples were kept in dark area without disturbance at room temperature for 24 hrs. These samples were diluted and analyzed by the UPLC system.

Thermal degradation

10mg Ritodrine was kept in a Petri dish and kept in hot air oven at 70ºC for 1 day. Sampling was done at multiple time points. Samples were dissolved in methanol, diluted 10 times and analyzed by the UPLC system.

Photo degradation

10mg Ritodrine was uniformly spread in a Petri dish and was exposed to directly sunlight for 24 hrs. Sampling was done at multiple time points and analyzed by the UPLC system.

3. Results and Discussion

Authentication and identification of received API

Authentication by UV-VIS spectra

After scanning from 400 to 200nm in UV-VIS spectrophotometer, Ritodrine was showed absorption maxima at 220 nm in 0.1NHCl. UV spectra of drug given in figure 3**.**

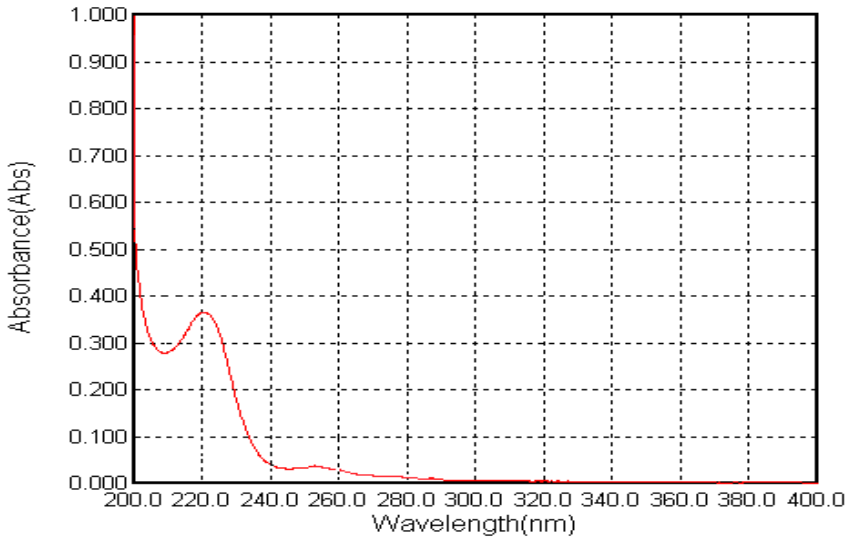

Figure 2. UV spectra of Ritodrine Simple and Robust RP-UPLC method development by DOE approach

Parameter selection

Various preliminary UPLC trials were carried out for selection of Column and organic modifier. The choice of C18 column based on the preliminary investigation was done using Symmetry C_{18} (150×4.6 mm, 5µm), Azilent C_{18} (150×4.6 mm, 5µm), Discovery (150×4.6 mm, 5µm) and BDS(150×4.6 mm, 5 µm) columns. Symmetry C_{18} column having less tailing, higher theoretical plate and good shape of drug peak as compare to the Azilent, BDS and Discovery column. (Appendices 1) Selection of a suitable organic modifier is also important to get better selectivity with adequate separation of all analytes. Commonly used organic solvents for the reversed phase UPLC include Acetonitrile and Methanol, from that trials Acetonitrile showed to be an ideal and suitable organic modifier compared to acetonitrile, because Ritodrine was solubilized in acetonitrile compare to methanol. Therefore, acetonitrile was selected and finalized as the organic modifier for further optimization study.

Optimization of method

The method was optimized using central composite design (CCD). The initial trials are needed to optimize the final method. Organic concentration, Flow rate and column temperature were needed to be optimized. So, CCD was used to optimize these parameters which were varied over three level (high, mid and low). different ranges of four parameters ranging from 40-50%, 0.01N potassium dihydrogen orthophosphate, column temperature 28^0Cand 32^0C and 0.8-1.20ml/min flow rate respectively were taken and counter and 3D surface plot showing the effect of each parameter on Retention Time, Theoretical plates and Asymmetry (CQA)

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CMPs	Unit	Type	Subtype	Min.	Max.
Temp	-	Numeric	Continuous	28 ^{0}C	32 ^{0}C
Flow rate	ml/min	Numeric	Continuous	0.8	1.20
%Org ratio	-	Numeric	Continuous	40	50

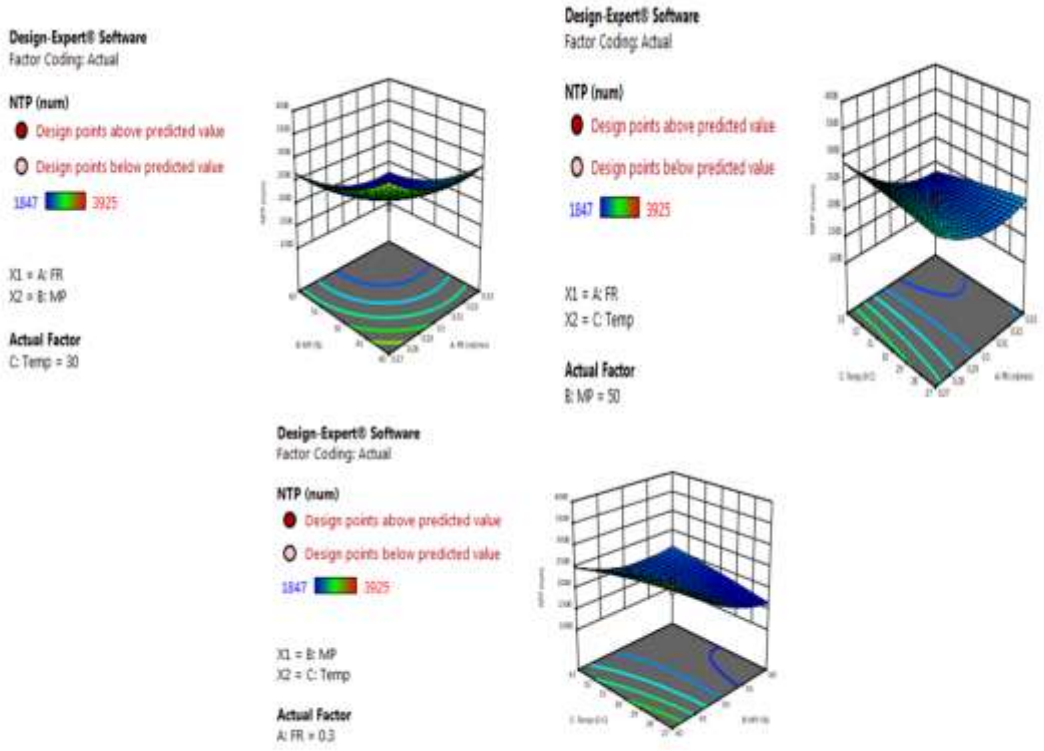

Figure 3:3D contour plots of asymmetry as a function of Column temperature, Flow rate and organic ratio.

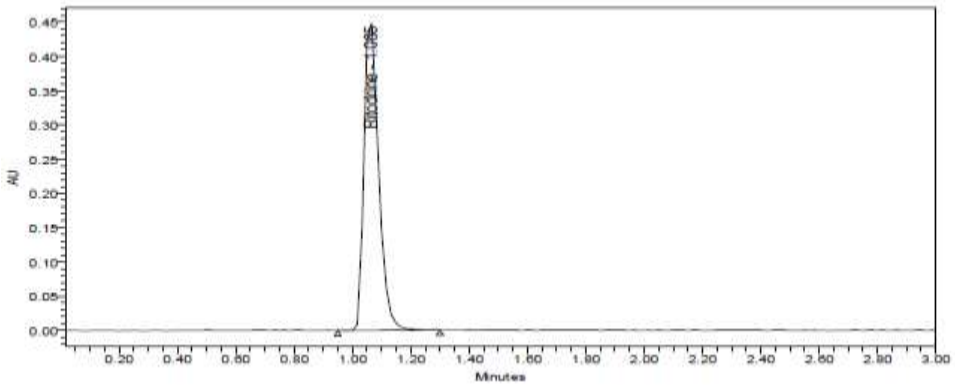

Figure 4 optimized method chromatogram

Property	**Value**
Column	Hibar100 x 2.1 mm, 2µ.
Mobile phase	0.1% OPA: Acetonitrile (55:45)
Temp	30.7424
Flow rate	0.300293

Method validation

Specificity:

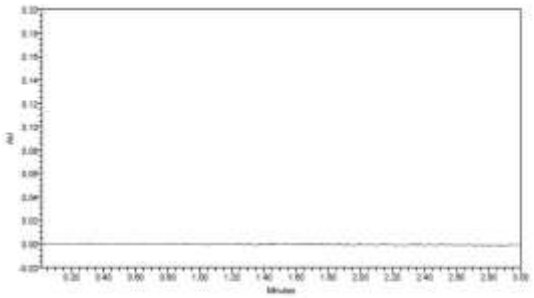

Figure 5 blank Chromatogram

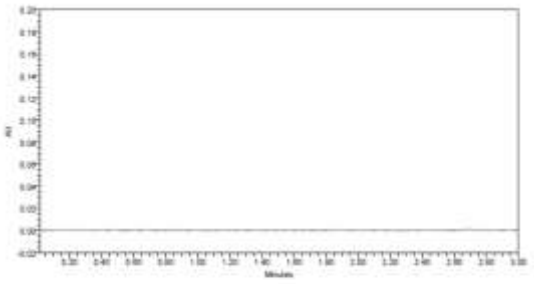

Figure 6 Placebo Chromatogram

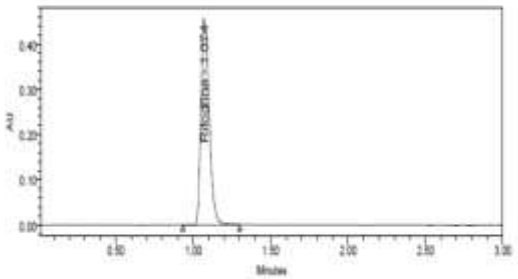

Figure 7 Standard Chromatogram

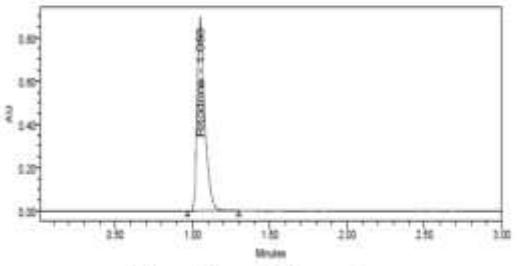

Figure 8 Sample Chromatogram

Precision:

System Precision:

Table 3 Repeatability data of Ritodrine

S.No	**Peak Area**
1	1614492
2	1628791
3	1633785
4	1622511
5	1665909
6	1672190
AVG	1639613
STDEV	23779.7
%RSD	1.5

Method precision:

Table 4 Method precision data of Ritodrine

S.No	Peak Area
1	1651826
2	1644750
3	1666124
4	1626204
5	1624602
6	1643084
AVG	1642765
STDEV	15722.3
%RSD	1.0

Intermediate precision:

Table 5 Intermediate precision data of Ritodrine

S.No	Peak Area
1	1615090
2	1625364
3	1632131
4	1655599
5	1656995
6	1602481
AVG	1631277
STDEV	21817.0
%RSD	1.3

LINEARITY:

Table 6 Linearity Concentration and Response of Ritodrine

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	5	384852
50	10	698731
75	15	1036294
100	20	1385235
125	25	1735394
150	30	2134249

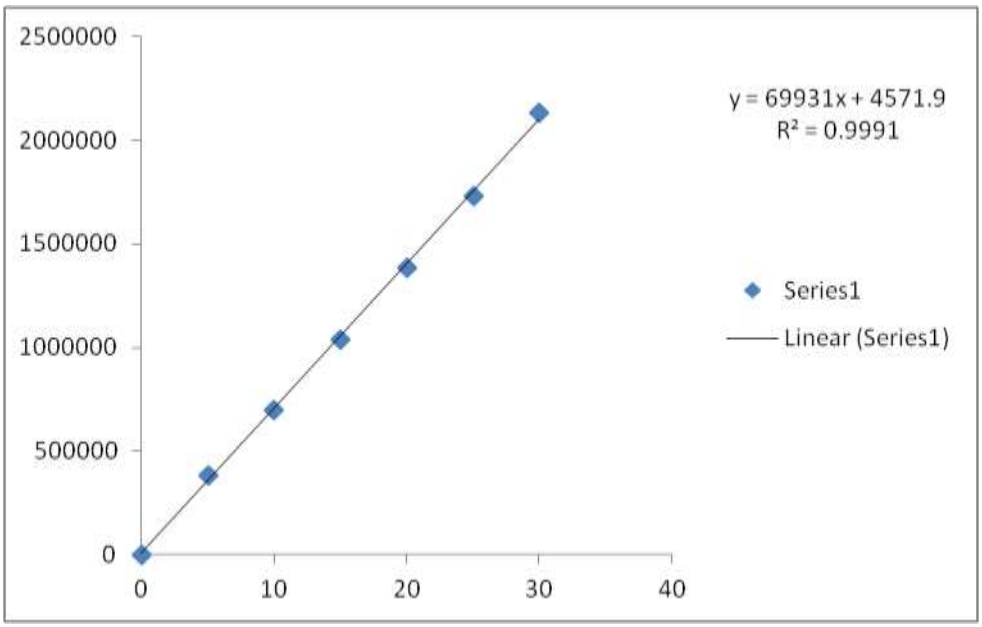

Figure 9: Linearity graph of Ritodrine

Accuracy:

Table 7 Accuracy data

% Level	**Amount Spiked (µg/mL)**	**Amount recovered(µg/mL)**	**% Recovery**	Mean %Recovery
50%	10	10.10	100.96	99.95%
	10	10.06	100.64	
	10	9.90	99.00	
100%	20	19.89	99.43	
	20	20.08	100.41	
	20	19.83	99.16	
150%	30	29.90	99.65	
	30	30.24	100.78	
	30	29.84	99.47	

Robustness:

Table 8 Robustness Data

Parameter	**%RSD**

Flow Minus	0.5
Flow Plus	0.4
Mobile phase Minus	1.1
Mobile phase Plus	1.1
Temperature minus	0.6
Temperature plus	0.8

ASSAY OF MARKETED FORMULATION

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula.

Table 9 Assay of Formulation

Sample No	**Standard Peak Area**	**Sample Peak Area**	**%Assay**
1	1614492	1651826	100.54
2	1628791	1644750	100.11
3.	1633785	1666124	101.41
4.	1622511	1626204	98.98
5.	1665909	1624602	98.89
6.	1672190	1643084	100.01
AVG	1639613	1642765	99.99
STDEV	23779.7	15722.3	0.96
%RSD	1.5	1.0	0.96

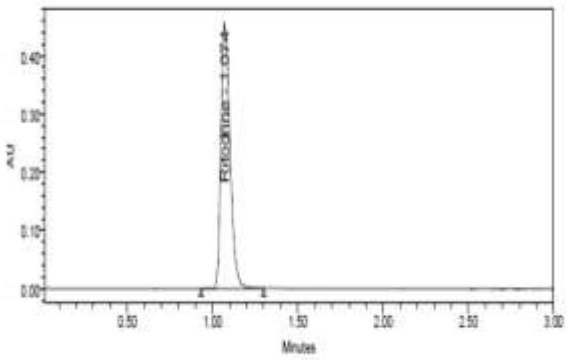

Figure 15 Assay Standard chromatogram

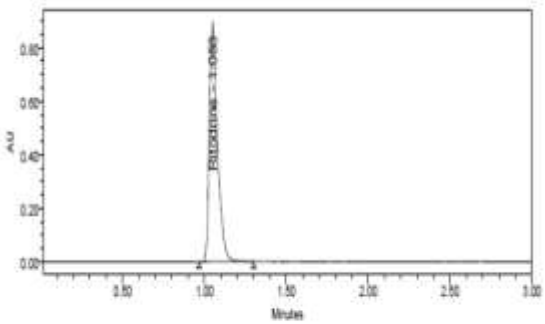

Figure 16 Sample chromatogram

Stability study:

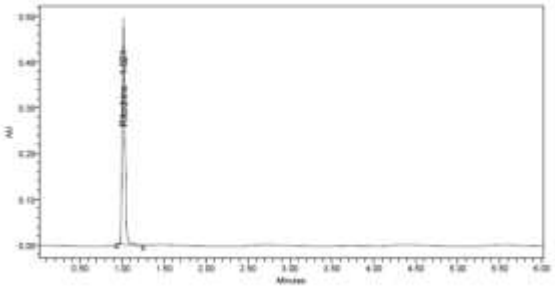

Figure 9 Chromatogram of drug in 2N HCl at 70 °C temperature for 8 hrs

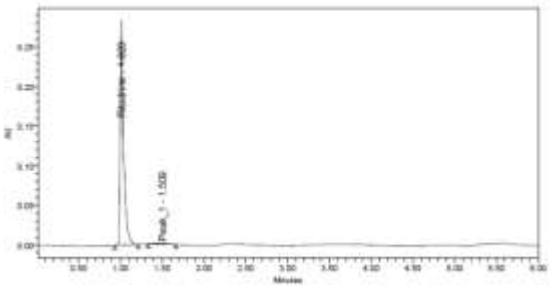

Figure 10 Chromatogram of drug in 2N NaOH at 70 °C temperature for 8 hrs

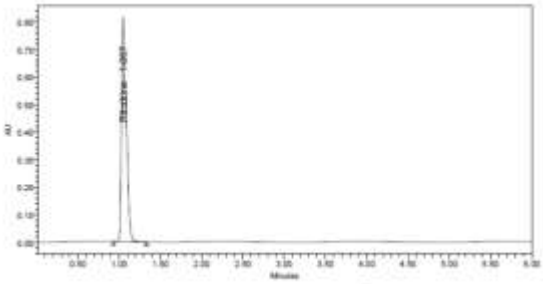

Figure 11 Chromatogram of drug in water at 70 °C temperature for 24 hrs

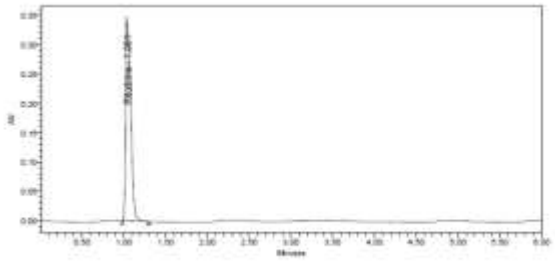

Figure 12 Chromatogram of drug in 20% H_2O_2 at room temperature for 24 hrs

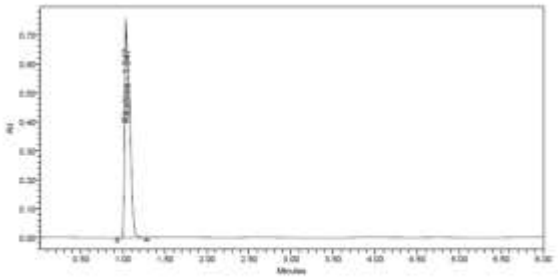

Figure 13 Chromatogram of drug at 70 °C temperature for 24 hrs

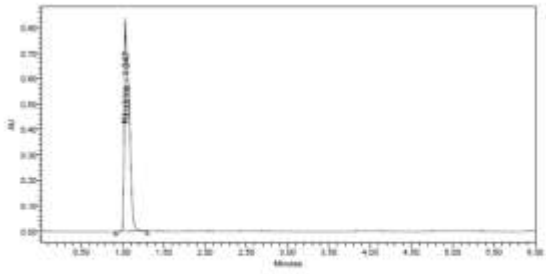

Figure 14 Chromatogram of drug after 24hrs exposure in direct sunlight

Table 10. Summary of degradation study

S.No	Condition of degradation study	% of drug degraded	Retention time of degradant
1.	2N HCl, 8 hrs	4.12	-
2.	2N NaOH, 8hrs	6.63	1.509
3.	Oxidative degradation, 24 hrs	0.62	-
4.	Thermal degradation, 1 days	4.01	-
5.	Photo degradation, 24 hrs	1.56	-
6.	Neutral hydrolysis, 24 hrs	3.73	-

Summary:
The method was optimized using central composite design (CCD). The initial trials are needed to optimize the final method. Total Three factors viz; % Organic concentration, Flow rate and column temperature were needed to be optimized. So CCD was used to optimize these parameters which were varied over three level (high, mid and low).different ranges of four parameters ranging from 36.59-53.41%, 0.01N potassium dihydrogen orthophosphate, column temperature26.64 and 33.36^0C and 0.6636-1.34ml/min flow rate respectively were taken and counter and 3D surface plot showing the effect of each parameter on Retention Time, Area, Theoretical plates and Asymmetry (CQA) were generated. A desirability function applied to the optimized conditions to predict retention time, asymmetry, theoretical plates and peak area. After scanning from 400 to 200nm in UV-VIS spectrophotometer, Ritodrine was showed absorption maxima at 220 nm in 0.1NHCl. Retention time of Ritodrine was 1.065min. We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Six working sample solutions of 20ppm are injected and the % Amount found was calculated and %RSD was found to be 1.5. Six working sample solutions of 20ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 1.0. Six working sample solutions of 20ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 1.3. To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 5ppm to 30ppm of Ritodrine. Plot a graph to concentration versus peak area. Slope obtained was y = 69931x + 4571.9and Correlation Co-efficient was found to be 0.999. Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 99.95%. Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions is calculated.

4. Conclusion

A simple analytical and robust UPLC method was developed for the determination of Ritodrine by using QbD approach using Design Expert® software. Validated stability indicating UPLC method for Ritodrine was developed which is capable to separate drug substance from the degradation products. Stress degradation studies have been performed for drug by using various stress conditions. No degradation products were found in case of Peroxide hydrolysis, neutral hydrolysis, thermal degradation and UV degradation. One significant degradation product was found in 2N HCl and 2N base hydrolysis. Results which were obtained from the validation of developed analytical method were within limit as per ICH guidelines.

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