

# Development and Validation of HPLC for Quantification of Remifentanyl Hydrochloride in Bulk and in Dried Powder for Injection

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**Abstract:** *Remifentanyl Hydrochloride is an ultra-short-acting opioid analgesic widely used in anesthesia. Due to its rapid metabolism and chemical structure, establishing a reliable quality control method is essential. This study describes the development and validation of a simple, rapid, accurate, and precise Reversed-Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the quantitative estimation of Remifentanyl Hydrochloride in bulk drug forms and dried (lyophilized) powder formulations for injection*

**Keywords:** Remifentanyl Hydrochloride, RP-HPLC, Method Validation, Injectable Formulation, ICH Guidelines, Quality Control

## I. INTRODUCTION

Analytical chemistry deals with methods for determining the chemical composition of samples of matter. Analytical Chemistry plays an important role in the resolution of a chemical compound into its proximate or ultimate parts, determination of its elements or of the foreign substances it may contain. Its application extends to all parts of an industrial society.<sup>1-6</sup>

## II. HISTORY OF ANALYTICAL CHEMISTRY

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and chemicals are present in the world around us. The first instrumental analysis was flame emissive spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered rubidium (Rb) and caesium (Cs) in 1860. Most of the major developments in analytical chemistry took place after 1900. During late 20<sup>th</sup> century analytical chemistry found wide application in forensic, environmental, industrial and medical field.

Importance of Analytical Chemistry:

It finds numerous applications in various disciplines of chemistry.

It finds wide applications in other fields of related sciences.

Analytical chemistry is concerned with chemical characterization of matter, both qualitative and quantitative.

**Qualitative analysis** deals with the identification of elements, ions or compounds present in the sample.

### Quantitative analysis

Quantitative analytical measurement plays a vital role in many research areas in chemistry, biochemistry, biology, geology and other sciences. It deals with the determination of how much amount of one or more constituents are present in the sample.<sup>1-6</sup>

## III. METHOD DEVELOPMENT

Method development is a challenging and time-consuming process requiring much experience, creativity, logical thinking, and experimentation. With all the software and automated systems available today, method development is



still very much a trial-and-error approach, expedited by a logical sequence of generic scouting runs and fine-tuning steps to achieve the requisite resolution and method performance.<sup>7</sup>

### EXPERIMENTAL WORK

#### Material and instruments:

#### Materials:

The drugs used for the present investigation were obtained from Arrow chem Mumabi.

Details of Pure drug:

**Table No. 8: Details of API**

Drug	Supplied by	Quantity	Purity (Assay)
Remifentanil Hydrochloride	Arrow Chem Mumbai.	10 g	99.9 % w/w

Marketed Preparation:

**Table No. 9: Details of marketed Preparation**

Brand Name	Mfd by	Content	Quantity
Ultiva®	Abbott Pharmaceuticals	1 mg	1 vial

The marketed preparation was obtained from local market and is referred here after in this thesis by the name as such.

#### Reagents and chemicals:

All reagents and chemicals used were of AR grade and HPLC grade.

Methanol (HPLC grade).

Acetonitrile (HPLC grade)

Disodium hydrogen phosphate (AR grade).

Distilled Water (HPLC grade).

Triethylamine (HPLC grade).

Ortho Phosphoric Acid (HPLC grade).

#### Instruments:

**Table No. 10: Instruments Used**

Sr. No	Instruments	Make	Model
1	UV-Visible Spectrophotometer	Shimadzu	UV 1900i
2	HPLC	Waters 600	996 PDA Detector
3	pH Meter	Hanna	-
4	Balance	Citizen	CY 104 (Micro Analytical Balance)
5	Ultra sonicator	-	1.5 L 50



**Study of Functional Group by Using Infrared Spectroscopy:**

**Remifentanil Hydrochloride API:** - Accurately weighed 3 mg of **Remifentanil Hydrochloride** API was mixed properly with 300 mg of dried KBr, then carefully triturated in a mortar pestle. Keep this mixture in a die and IR spectrum was taken using the Diffused Attenuated reflectance mode.

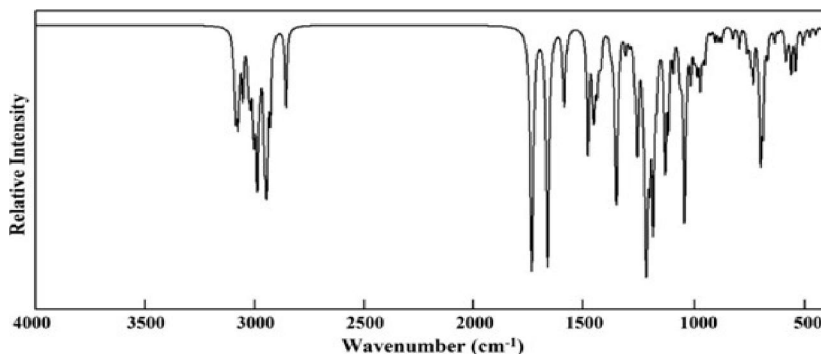


Fig. No. 8: IR Spectra of Remifentanil Hydrochloride

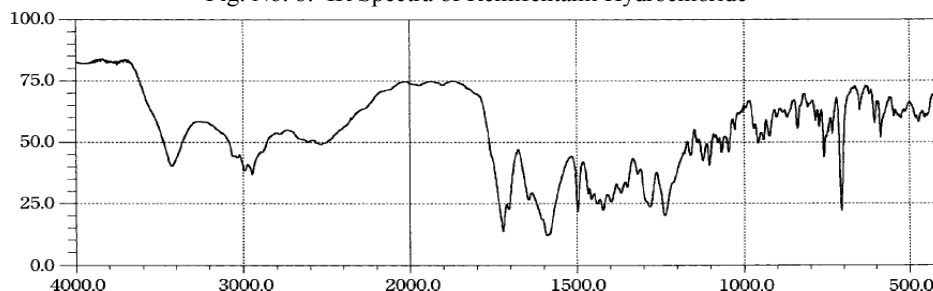


Fig. No. 9: Reference IR Spectra of Remifentanil Hydrochloride

**Conclusion:**

The IR spectra of the given test drugs is matches with the IR spectra of standard drugs.

**Determination of wavelength maxima**

**Remifentanil Hydrochloride standard stock solution:**

An accurately weighed quantity of **Remifentanil Hydrochloride** (RFH) 5 mg was transferred to the 10 ml volumetric flask and dissolved in HPLC grade ACN. The volume was made up to the mark with the same g/ml. □to make (500 The aliquot portions of stock standard solutions were diluted appropriately with HPLC grade g/ml of RFH. The solutions were scanned in the □ACN to obtain concentration 5 range of 400–200 nm in 1 cm cell against blank. The UV absorbance spectrum of RFH were recorded and found to be 210 nm.

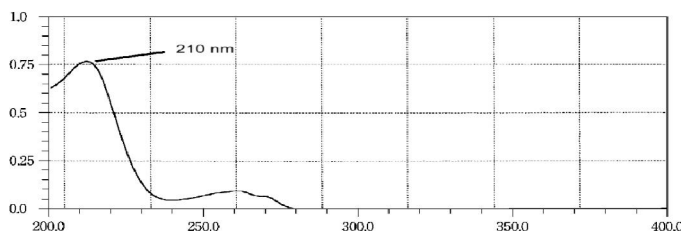


Fig. No. 10: Wavelength Maxima for Remifentanil Hydrochloride



### Development of HPLC method for estimation of Remifentanil Hydrochloride

#### Method Development Strategy:

#### Selection of Common Solvent (Diluents):

ACN of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in mobile phase. The selection was made after assessing the solubility of RFH in different solvents i.e Acetonitrile and water.

#### Preparation of standard stock solution:

Accurately weighted RFH 100 mg was dissolved in 100 ml ACN. This solution was used as standard stock solution.

#### Preparation of diluent:

ACN of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the Mobile phase.

#### Procedure:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing RFH was injected in different combinations of solvents, to get a stable peak with good peak characters. Each solution was filtered through Membrane filter (size 0.15 $\mu$ ). To achieve peaks with good symmetry various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

#### Chromatographic Parameters:

<b>Column:</b>	C18 (Thermo Hypersil gold) /4.6 x 250 mm, 5 $\mu$ particle size
<b>Flow Rate:</b>	1.0ml/min
<b>Wavelength:</b>	210 nm
<b>Injection volume:</b>	20 $\mu$ l
<b>Column oven Temperature:</b>	Ambient (25 <sup>0</sup> C)
<b>Run Time:</b>	10 minutes
<b>Mobile Phase:</b>	0.1% OPA and ACN (50:50)
<b>Preparation of 0.1% OPA:</b>	Dilute 1 ml ortho phosphoric acid in 1000 ml of volumetric flask and makeup the volume upto the mark with HPLC water.

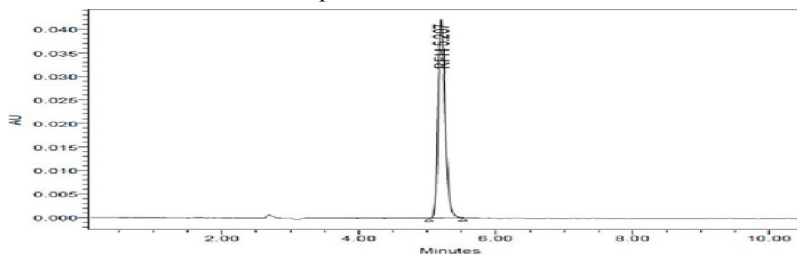


Fig. No. 11: Separation of RFH in selected mobile phase showing retention time at 5.207 min.

#### System suitability studies

System suitability is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from 5 replicate injections of standard solutions.



The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. Standard working solution of RFH was injected five times under optimized chromatographic conditions. System suitability parameters were recorded and reported.

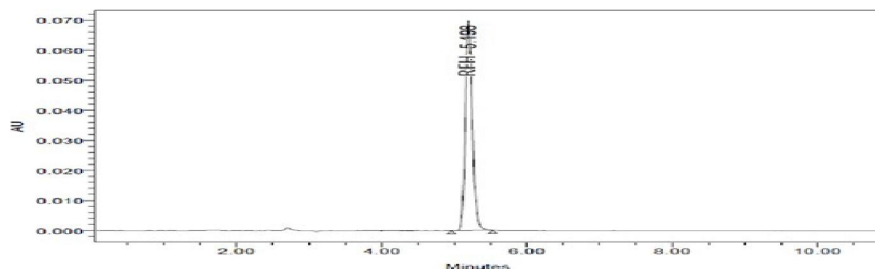
**B) Procedure:**

Filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline L std. drug solution was injected which was made in five □ was obtained. A 20 replicates and the system suitability parameters were recorded.

**Table No. 11: Result of System suitability test**

Sr.No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	RFH	RFH	RFH	RFH
1	340268	5.207	1.10	7526
2	338951	5.304	1.20	7588
3	335895	5.208	1.30	7645
4	342697	5.310	1.15	7600
5	337894	5.290	1.20	7550
<b>Mean</b>	339141	5.263	1.19	7582
<b>S.D</b>	1181	0.068	0.1	56.07
<b>%R.S.D.</b>	1.05	0.98	1.5	0.70

**Fig. No 12: Separation of RFH in selected mobile phase showing retention time at 5.19 min.**



**Application of proposed method for estimation of RFH in lyophilized injection formulation:**

**Standard stock solution:**

**a) Preparation of standard solutions:**

**Remifentanil Hydrochloride standard stock solution:** Accurately weighed quantity of 100 mg RFH was dissolved in ACN and volume was made up to 100 ml mark by same to obtain 1000 µg/ml stock solution.

**Remifentanil Hydrochloride standard working solution:** Pipette out 1 ml from standard stock solution and dilute it with 10 ml ACN to obtain 100 µg/ml of RFH. **Sample solution preparation:**

Entire content of **Ultiva®** injection vial (1 mg) was transferred to a 10 ml volumetric flask, the volume was made upto the mark with ACN, the resultant concentration was 100 µg/ml. The whole content was centrifuged at 5000 rpm for 10

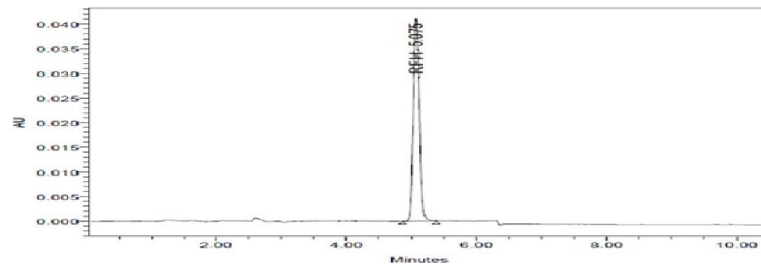


min followed by passing through 0.45  $\mu$  membrane filter. 1 ml of resultant was transferred to a 10 ml volumetric flask and the volume was made upto the mark with ACN, the concentration of working sample solution was 10  $\mu$ g/ml.

**Procedure:**

Equal volume L (20 of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content of RFH was calculated by comparing a sample peak with that of standard.

Fig. No.13: Chromatogram of marketed formulation showing retention time 5.075 min.



Brand name : Ultiva® injection vial

Sr. No.	Assay (mg)	% Purity
1	99.5	99.50
2	100	100.00
3	100	100.00
4	100	100.00
5	100	100.00
Average	0.99	99.99
SD	0.002	0.002
% RSD	0.22	0.22

Table No. 12: Results and statistical data for estimation of RFH in marketed formulation

**Validation parameters:**

- Accuracy
- Precision
- Ruggedness
- Robustness
- Linearity and range
- Specificity
- Placebo Interference study



**Accuracy:**

The accuracy samples were prepared by spiking the standard into the pre-analyzed formulation sample at different concentrations (80%,100% and 120%) and injected each in triplicate. The resultant mix was injected and recovery of standard spiked was calculated. The % Recovery was then calculated by using formula

$$\% \text{ Recovery} = \frac{A-B}{C} \times 100$$

Where- A = Total amount of drug estimated.  
B = Amount of drug found on pre analyzed basis.  
C = Amount of pure drug added.

Calculate the amount recovered, % recovery, average recovery, % RSD of triplicate sample preparation, overall recovery and overall % RSD. Record the observation into the following table.

	RFH		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt recovered (µg/ml)	79.9	100.0	119.9
	80.0	100.0	120.0
	80.0	99.9	120.0
% Recovery	99.87	100.00	99.91
	100.00	100.00	100.00
	100.00	99.90	100.00
Mean % recovery	99.96	99.97	99.98
% RSD	0.02	0.02	0.05

Table No 13: Accuracy studies by standard addition method Acceptance criteria:

The % RSD for the triplicate at each spike level shall be NMT 2.0.

The overall % RSD for % recovery for all spike levels shall be NMT 2.0.

The % recovery at each spike level shall be NLT 98.0 and NMT 102.0 of the added amount.

**Precision:**

**System precision**

Prepared the standard solution as per test method and inject into the HPLC system in three replicates. Calculate the % RSD for the area responses and record the observations into the following table

Sr. No.	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	1.210	NMT 2.0
2	Theoretical plates	7757.53	NLT 2000
3	Tailing factor	1.40	NMT 2.0

Table No. 14: Results for System Precision showing system suitability

Where, NMT - Not More Than NLT – Not Less Than



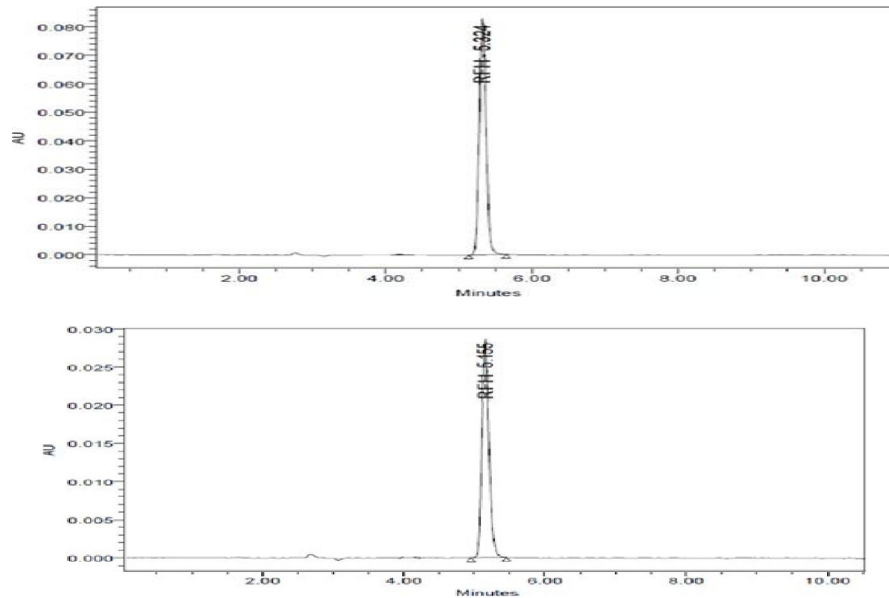


Fig.no 14: Chromatogram System precision Showing Repeatability

Injection No.	Area Response
	RFH
1	340298
2	341600
3	349500
Average	343799
SD	2979
% RSD	1.52

Table No. 15: System precision Showing Repeatability Acceptance criteria:

% RSD for replicate injections shall be NMT 2.0

**Method precision:**

Prepared three samples solutions as per the test method and injected into the HPLC system by following the conditions prescribed in the Test method.

**Procedure:**

Sample solution was prepared and injected into the HPLC system, the chromatograms were recorded for peak area response for the RFH. The assay and % label claim for RFH was calculated. Fig.no 15: Chromatogram of Method precision

Sr.no.	RFH	
	Assay (mg)	Assay % of LC
1	99.98	99.98
2	100.00	100.00
3	100.00	100.00
Average	99.99	99.99



SD	0.011	0.011
% RSD	0.011	0.011

Table No.16: Method Precision Studies Set – I

**Acceptance criteria:** The% RSD for the three determinations shall be NMT 2.0

**Ruggedness: Intermediate precision**

Prepared three sample solutions as per the test method. Injected into the different HPLC system (preferably with different manufacturer or same manufacturer with different configuration) by using the different column and by the different analyst at different date.

Sr.No.	RFH	
	Assay (mg)	Assay % of LC
1	99.9	99.9
2	99.9	99.9
3	100.00	100.0
Average	99.93	99.93
SD	0.05	0.05
% RSD	0.05	0.05

Table No. 17: Intermediate precision Studies (Ruggedness) Set – II Acceptance criteria: The % RSD for the three determinations shall be NMT 2.0

**Data analysis between method precision and Intermediate precision:**

Compared the data obtained in this section verses the data obtained in method precision and evaluate the overall average, overall SD and overall % RSD and recorded the observation into the following table

Sr.no.	% Assay of LC	
	RFH	
	Set – I	Set - II
1	99.98	99.9
2	100.00	99.9
3	100.00	100.0
Average	99.90	
SD	0.02	
% RSD	0.02	

Table No. 18: Intermediate precision (Ruggedness) evaluation of data SET – I: Method Precision data , SET – II: Intermediate Precision data

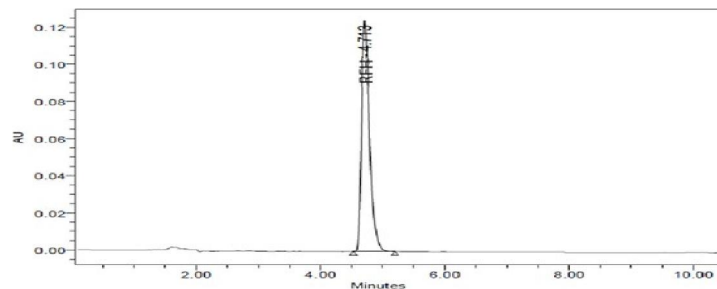
**Acceptance criteria:** The overall % RSD for the twelve determinations shall be NMT

**Robustness:**

**Effect of Variation in flow rate of mobile phase by  $\pm 10\%$ :**

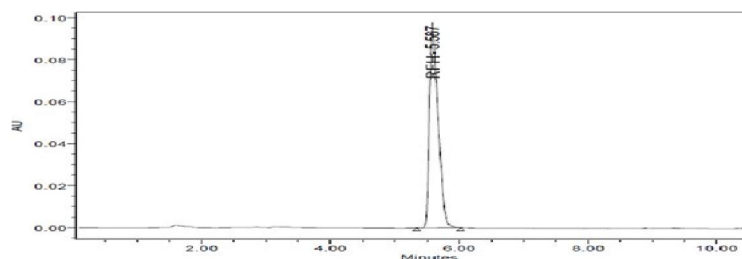
Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system at  $-10\%$  flow rate (0.9mL/min) and  $+10\%$  flow rate (1.1mL/min) when compared with the Test method flow rate.





**Procedure:** Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measured the peak response for the major peaks.

1.1 ml/min



0.9 ml/min

Fig. No. 16: Chromatograms of Change in Flow Rate

System suitability parameters were recorded and the results are presented in the table below.

Sr. No.	System Suitability parameter		Observations for flow rate			Limit s
			Unchanged	0.9 ml	1.1 ml	
1	The % RSD of peak area response for five replicate injections	RFH	1.22	1.52	1.55	NMT 2.0
2	Theoretical plates	RFH	7327	7455	7414	NLT 2000
3	Tailing factor	RFH	1.48	1.61	1.56	NMT 2.0
4	Retention Time (Min)	RFH	5.21	5.58	4.71	

**Table No. 19:** System suitability of change in Flow Rate

**Observation:** The allowable variation in flow rate of the method is from 0.9ml/min to 1.1 ml/min

**Acceptance criteria:** All the system suitability parameters shall pass

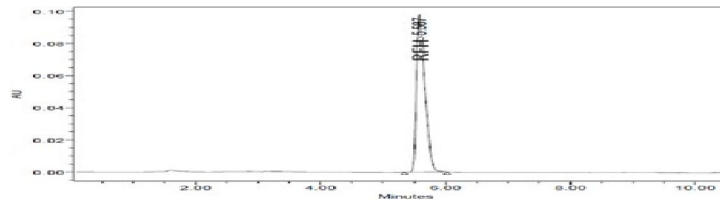
**Change in organic composition + 10% (0.1%OPA: ACN)**

System suitability dilution was prepared and injected into the HPLC system at -10% and + 10 % ACN (Organic phase) compared with the optimized method mobile phase concentration.

**Procedure:** Injected standard solution into the HPLC system in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks. Check the system suitability and record the results in the table.

-10% ACN: (0.1%OPA: ACN 55:45)





+10% ACN: (0.1%OPA: ACN 45:55)

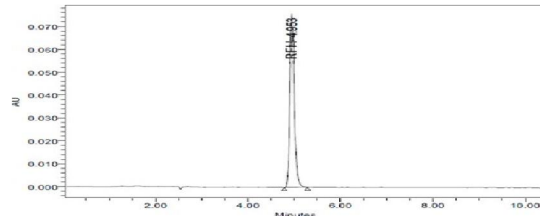


Fig. No. 17: Chromatograms of Change Organic Composition of mobile Phase

Sr. No.	System Suitability parameter		Observations			Limits
			Unchanged	- 10%	+ 10%	
1	The % RSD of peak area response for five replicate injections	RFH	1.17	1.55	1.46	NMT 2.0
2	Theoretical plates	RFH	7397	7496	7347	NLT 2000
3	Tailing factor	RFH	1.65	1.70	1.42	NMT 2.0
4	Retention Time (Min)	RFH	5.10	5.597	4.953	

**Table No. 20: System suitability of change in Organic Composition Observation:** The allowable variation in ACN composition of method is from 90% to

110%. **Acceptance criteria:** 1. All the system suitability parameters shall pass.

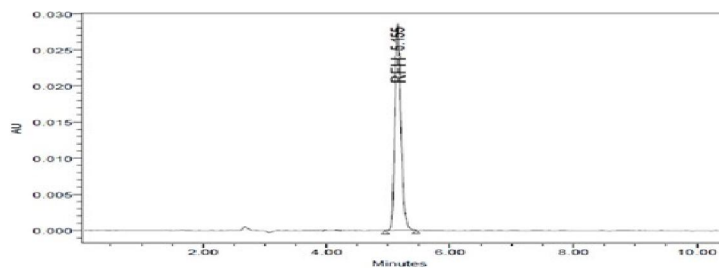
**Effect of Variation in Wavelength by  $\pm 2$  units:**

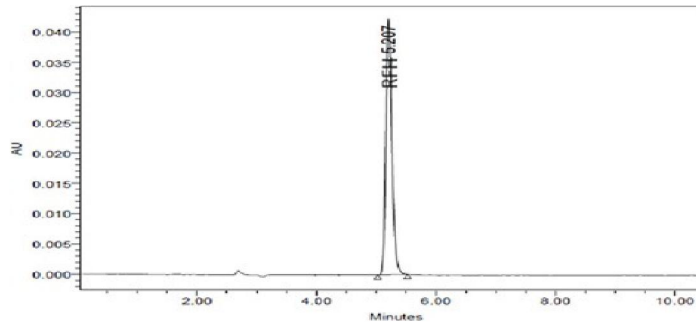
Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system. Measure the peak area response at different wavelengths at flow rate 1 ml/min.

**Procedure:**

Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks.

**At 208nm wavelength**





At 212 nm wavelength

Fig. No. 18: Chromatograms of Change in wavelength.

Sr. No.	System Suitability parameter		Observations for wavelength			Limits
			Unchanged	208nm	212nm	
1	The % RSD of peak area response for five replicate injections	RFH	1.50	1.36	1.22	NMT 2.0
2	Theoretical plates	RFH	7357	7587	7678	NLT 2000
3	Tailing factor	RFH	1.16	1.22	1.24	NMT 2.0
4	Retention Time (Min)	RFH	5.21	5.155	5.207	

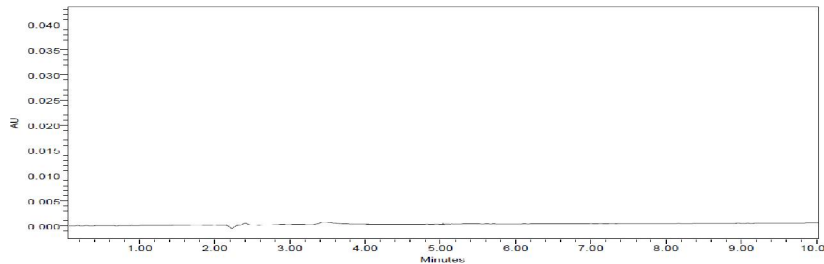
Table No.21: System suitability of change in wavelength

**Specificity:**

**Placebo Interference study:**

Prepared the placebo solution by weighing equivalent amount of placebo present in the sample to be taken for assay preparation in triplicate, diluted it as per the test method and injected into the HPLC system. Evaluate the % interference from placebo and recorded the observation.

**Sample matrix**



**Placebo Preparation**

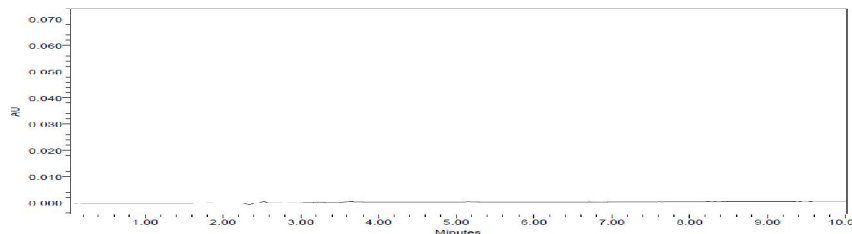


Fig. No. 19: Chromatograms of placebo interference study



Observation	Placebo prep.1	Placebo prep.2	Placebo prep.3
% Interference	No Interference	No Interference	No Interference

Table No. 22: Placebo Interference Acceptance criteria:

No interference should observe from placebo at the retention time of RFH.

**Linearity and range:**

Prepared the series of standard concentrations ranging from 50 % to 150 % of the targeted concentration of RFH. Each of the linearity dilution was injected into the HPLC system with optimized chromatographic parameters.

**Procedure:**

Separately inject standard preparation and linearity preparations into the HPLC system, record the chromatograms and measure the peak responses for RFH peaks.

The details of mean peak areas for linearity concentrations are presented in following table and plot the graph of concentration verses average area response for RFH, the correlation coefficient and equation of regression were recorded.

Sr. No.	% Level	RFH	
		Conc. (µg/ml)	Mean peak area
1	50	50	170500
2	80	80	275200
3	100	100	339141
4	120	120	406500
5	150	150	509050

Table No.23:- Observations of Linearity and range study for RFH Acceptance criteria:

The correlation coefficient shall be NLT 0.99

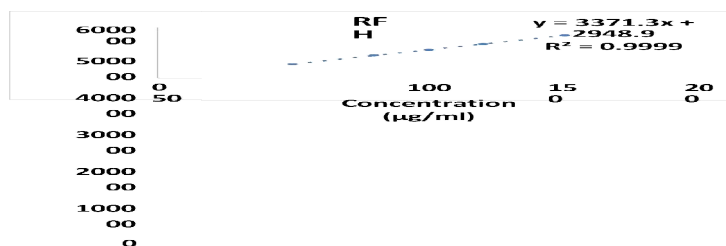


Fig. No. 20: Plot of linearity and range study for RFH

**III. RESULT AND DISCUSSION**

High Performance Liquid Chromatography which is a highly sophisticated technique, it is used for the determination of active molecules from their formulations.

Remifentanil Hydrochloride is a potent, ultra-short-acting synthetic opioid widely used in anesthesia due to its rapid onset and predictable recovery profile. The drug is highly labile and prone to degradation under varying pH, temperature, and enzymatic conditions, making the development of a reliable analytical method essential.

Although several analytical approaches including HPLC, UV spectrophotometry, and LC-MS/MS have been reported for RFH, majority of these methods focus on **bioanalytical applications**. Limited literature is available on validated chromatographic methods for **bulk drug analysis** and **pharmaceutical dosage forms**, particularly the **dried powder for injection** formulation.



The pure standard of **RFH** was procured from Arrow chem Mumbai. Percent purity of above-mentioned drug was reported by Supplier Company as follows-

Table No. 24: Details of API

Drug	Supplied by	Quantity	Purity (Assay)
Remifentanil Hydrochloride	Arrow Chem Mumbai.	10 g	99.9 % w/w

These were not analyzed in our study and the % purity stated by the suppliers was taken as standard for comparison studies.

**RP-High Performance Liquid Chromatography (HPLC) Method:**

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique is commonly used for the quantitative estimation of the drugs from their formulation as well as for studying their metabolites of drugs and their estimation in their biological fluids. This technique was employed in the present investigation for estimation of RFH in dried powder for injection formulation. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish RP-HPLC method the following parameters were studied.

**HPLC Column Selected:**

HPLC Waters 600 system with C18 (Thermo Hypersil gold) /4.6 x 250mm, 5µ particle size column and PDA detector were used for the study. The standard and sample solution of RFH were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

**Mobile Phase selected:**

Mobile phase composed of water (0.1 % OPA) and ACN (50:50 % v/v). An isocratic program was developed with a total run time of 10 min. The wavelength 210 nm was selected for the evaluation of the chromatogram of drugs. The selection of the wavelength was based on the λ max obtained by scanning of standard solution. This system gave good resolution and optimum retention time with appropriate tailing factor (<2). The mean values of system suitability test result are depicted in Table below. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

Table No. 25: Chromatographic Parameters:

Column	C18 (Thermo Hypersil gold) /4.6 x 250 mm
Flow Rate	1 ml/min
Wavelength	210 nm
Injection volume	20µl
Column oven Temperature	Ambient
Run Time	10 minutes
Mobile Phase	Mixture of ACN & water (0.1% OPA) in ratio 50:50 % v/v

**Mobile phase-preparation**

1. Dilute 1 ml ortho phosphoric acid in 1000 ml of volumetric flask and make up the volume upto the mark with HPLC water.



**Preparation of diluent:**

ACN of HPLC grade was selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the Mobile phase.

Sr.No	Peak area	Retention Time	Symmetry	No. of theoretical Plates
	RFH	RFH	RFH	RFH
1	340268	5.207	1.10	7526
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Mean	339141	5.263	1.19	7582
S.D	1181	0.068	0.1	56.07
%R.S.D.	1.05	0.98	1.5	0.70

Table No. 26: Summary of system suitability of Test results

After establishing the chromatographic conditions, Mix standard and marketed preparation solutions were prepared and analyzed by procedure described under experimental work. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation.

Amount of drug in tablet was calculated using following formula:

$$\text{Assay (mg/ml)} = \frac{A_t}{A_s} \times \frac{D_s}{D_t} \times \frac{W_s}{W_t} \times \frac{p}{100} \times W_t \text{ mg/ml of test sample}$$

$$\% \text{ Label claim} = \frac{\text{Assay (mg/ml)} \times 100}{\text{Label claim in mg/ml}}$$

Where,

$A_t$  =

Area count for standard solution.  $D_s$  =

$D_t$

Area count for sample solution.  $A_s$  = Area

Dilution factor for standard.

= Dilution factor for sample. P

= Potency of drug

**IV. VALIDATION**

Validation of these methods was performed as per the USP guidelines for these following parameters:

**Precision:**

**System Precision**

Prepared the standard solution as per test method and injected into the HPLC system in three replicates. It was found that all system suitability parameters are well within the limits.



**Method Precision**

Replicate estimation of tablet analyzed by the proposed method has yielded quite consistent result indicating repeatability of method. Study showed R.S.D. less than 2.

Table No. 27: Data showing system Precision

Sr. No.	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	1.210	NMT 2.0
2	Theoretical plates	7757.53	NLT 2000
3	Tailing factor	1.40	NMT 2.0

Table No.28: Method Precision Studies Set – I

Sr.no.	RFH	
	Assay (mg)	Assay (mg)
1	99.98	99.98
2	100.00	100.00
3	100.00	100.00
Average	99.99	99.99
SD	0.011	0.011
% RSD	0.011	0.011

**Linearity & Range:**

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was carried out for five levels in the range of 80% to 150%. A graph was plotted with concentration on X axis and mean peak areas on Y-axis. The R<sup>2</sup> value was found to be 0.999 for RFH. The result show that an excellent correlation exists between concentration and mean peak areas within the concentration range. Thus the method developed is accurate, precise, specific, & linear. Hence it can be said that, RP-HPLC is the most accurate, precise and reproducible among all methods.

**Accuracy:**

Accuracy of the proposed method was ascertained from the recovery studies by standard addition method. Recovery results werewell within the range 99.96-99.98%. Thus, the method was found to be accurate.

Table No. 29: Result of Accuracy Studies

	RFH		
	Levels		
	80%	80%	80%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt recovered (µg/ml)	79.9	100.0	119.9



	80.0	100.0	120.0
	80.0	99.9	120.0
% Recovery	99.87	100.00	99.91
	100.00	100.00	100.00
	100.00	99.90	100.00
Mean % recovery	99.96	99.97	99.98
% RSD	0.02	0.02	0.05

#### Robustness:

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters, the standard solution and test solutions were injected for each of the changes made to access the Robustness of proposed analytical method.

#### Following Parameters were covered under robustness parameter.

Effect of variation in flow rate of mobile phase by  $\pm 10\%$

Organic phase composition ( $\pm 10\%$ )

Change in Wavelength by  $\pm 2$  units

The results suggested all the system suitability parameters were within limits.

#### Specificity:

Is the ability to assess unequivocally the analyte in the presence of impurities, degradants, matrix etc. It is evaluated by injecting the blank, placebo and the control sample solution prepared as per the proposed method to check for the interference if any peak at the retention time of RFH. Thus, no interference was found at the Retention time of RFH.

## V. SUMMARY AND CONCLUSION

### SUMMARY

Dried powder for injection containing RFH is a potent, ultra-short-acting synthetic opioid widely used in anesthesia due to its rapid onset and predictable recovery profile. There are several analytical methods majorities of this focus on bioanalytical applications. Limited literature is available on validated chromatographic methods for **bulk drug analysis** and **pharmaceutical dosage forms**, particularly the **dried powder for injection** formulation that is routinely used in clinical practice. Existing bioanalytical methods often involve complex sample handling (acidification, protein precipitation, low-temperature stabilization), which are not directly applicable or practical for routine **quality control laboratories** in the pharmaceutical industry.

The present study was undertaken with an objective of developing suitable, sensitive and simple analytical RP-HPLC method for estimation of RFH in dried powder for injection.

In the developed RP-HPLC method the analyte was resolved using Mobile phase composed of water (0.1% OPA) and ACN in the ratio 50:50 % v/v. An isocratic program was developed contributing a total run time of 10 min. using HPLC auto-sampler system containing PDA detector with EMPOWER Software and C18 (Thermo Hypersil gold) /4.6 x 250 mm, 5 $\mu$  particle size column, the detection wavelength was 210 nm. The method gave the good resolution and suitable retention time.

The results of analysis in all the method were validated in terms of accuracy, precision, ruggedness, linearity and range. The methods were found to be sensitive, reliable, reproducible, rapid and economic also.



## VI. CONCLUSION

From the results of the study, it can be concluded that the present RP-HPLC technique was successfully used for the estimation of the RFH in the dried powder for injection formulation.

The method showed good reproducibility, it was accurate, precise, specific, reproducible and sensitive. The analysis of dried powder for injection formulation of RFH was done by the developed and validated RP-HPLC method.

The RP-HPLC method was also simple, accurate, precise, reproducible and economical too. It may be adopted for routine control analysis of RFH.

No interference of additives, matrix etc. was encountered in this method. Further studies on other pharmaceutical formulations would throw more light on these studies. Suitability of this method on biological samples needs to be studied.

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