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Development and validation of rp-hplc method for the simultaneous estimation of buprenorphine and naloxone in bulk and pharmaceutical dosage form

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ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Buprenorphine and Naloxone in bulk and pharmaceutical formulations. SeparationofBuprenorphine and Naloxone wassuccessfullyachievedonaPhenomenex Luna C18 (4.6×250mm, 5µm) particle size or equivalentin an isocratic mode utilizingAcetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)ataflowrateof1.0mL/minandeluates wasmonitoredat245nm,witharetentiontimeof2.102and 3.537 minutes for Buprenorphine and Naloxone respectively. The method was validated and the response was found to be linearinthe drug concentration range of 6µg/mLto 14µg/mL for Buprenorphine and 18µg/mLto 42µg/mL for Naloxone. The values of the slopeand the correlation coefficient were found to be 77824 and 0.999 for Buprenorphine and 10515 and 0.999 for Naloxone respectively. The LOD and LOQ for Buprenorphine were found to be 0.6µg/mL and1.8µg/mL respectively. The LOD and LOQ for Naloxone were found to be 0.8µg/mL and 2.4µg/mL respectively. This method was found to be good percentage recovery for Buprenorphine and Naloxone were found to be 100.351 and 100.93 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analytes in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

Keywords: Buprenorphine and Naloxone, High performance liquid chromatography, Validation.

INTRODUCTION

Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products.

Method development

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use" [1-2]. Understanding of the physical and chemical characteristics of drug allows one to select the most appropriate high performance liquid chromatography method development from the available vast literature. Information concerning the sample, for example, molecular mass, structure and functionality, pKa values and UV spectra, solubility of compound should be compiled. The requirement of removal of insoluble impurities by filtration, centrifugation, dilution or concentration to control the concentration, extraction (liquid or solid phase), derivatization for detection etc. should be checked. For pure compound, the sample solubility should be identified whether it is organic solvent soluble or water soluble, as this helps to select the best mobile phase and column to be used in HPLC method development.

Method development in HPLC can be laborious and time consuming. Chromatographers may spend many hours trying to optimize a separation on a column to accomplish the goals. Even among reversed phase columns, there is astonishing diversity, owing to differences in both base silica and bonded phase characteristics. Many of these show unique selectivity. What is needed is a more informed decision making process for column selection that may beused before the chromatographer enters the laboratory. The method of column selection presentedhere involves a minimal investment in time initially, with the potential of saving many hours in the laboratory.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods that support safety and characterization studies or evaluations of drug performance are also to be evaluated. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate the potential degradation of the API in the presence of formulation excipients [3,4].

The three critical components for a HPLC method are: sample preparation (% organic, pH, shaking/sonication, sample size, sample age) analysis conditions (%organic, pH, flow rate, temperature, wavelength, and column age), and standardization (integration, wavelength, standard concentration, and response factor correction). During the preliminary method development stage, all individual components should be investigated before the final method optimization. This gives the scientist a chance to critically evaluate the method performance in each component and streamline the final method optimization[5]. The percentage of time spent on each stage is proposed to ensure the scientist will allocate sufficient time to different steps. In this approach, the three critical components for a HPLC method (sample preparation, HPLC analysis and standardization) will first be investigated individually [6-8]. The degraded drug samples obtained are subjected to preliminary chromatographic separation to study the number and types of degradation products formed under various conditions [9]. Scouting experiments are run and then conditions are chosen for furtheroptimization [10]. Resolving power, specificity, and speed are key chromatographic method attributes to keep in mind during method development [11]. Selectivity can be manipulated by combination of different factors like solvent composition, type of stationary phase, mobile phase, buffers and pH. Changing solvents and stationary phases are the most comfortable approaches to achieve the separation. The proper range of pH is an important tool for separation of ionizable compounds. Acidic compounds are retained at low pH while basic compounds are more retained at higher pH. The neutral compounds remain unaffected. The pH range 4-8 is not generally employed because slight change in pH in this range would result in a dramatic shift in retention time. However, by operating at pH extremes (2-4 or 8-10), not only is there a 10-30 fold difference in retention time that can be exploited in method development but also the method can be made more robust which is a desirable outcome with validation in minutes [12,13]. Various steps for HPLC method development are given below.

MATERIALS AND METHOD

Buprenorphine from Sura labs, Naloxone from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck.

HPLC METHOD DEVELOPMENT TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Buprenorphine and Naloxone working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1ml of the above Buprenorphine and 0.3ml of the Naloxone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol. Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol:Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Bufferin proportion 45:55 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Phenomenex Luna C18 (4.6×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used: Waters HPLC with auto sampler and PDADetector 996 model.

Temperature: 35ºC

Column: Phenomenex Luna C18 (4.6×250mm, 5µm) particle size

Buffer: Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.

pH: 4.6

Mobile phase: Acetonitrile: Phosphate Buffer(45:55 v/v)

Flow rate: 1ml/min

Wavelength: 245 nm

Injection volume: 10 µl

Run time : 7 min

VALIDATION

Preparation Of Buffer And Mobile Phase

Preparation of Potassium dihydrogen Phosphate (KH_2PO_4) buffer (pH-4.6)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication.

Preparation of mobile phase

Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Flow rate : 1 ml/min
Wavelength : 245 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 7 minutes

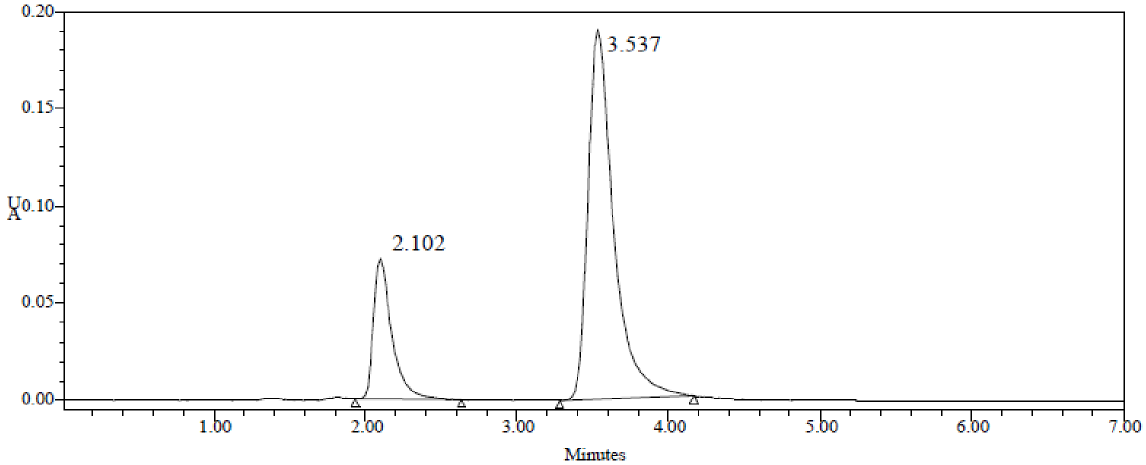

Fig 1: Optimized Chromatogram (Standard)

Table 1: Peak results for optimized

S.No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Buprenorphine	2.102	765789	69584		0.97	5587.0
2	Naloxone	3.537	2532158	190049	2.97	1.26	5398.0

From the above chromatogram it was observed that the Buprenorphine andNaloxone peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

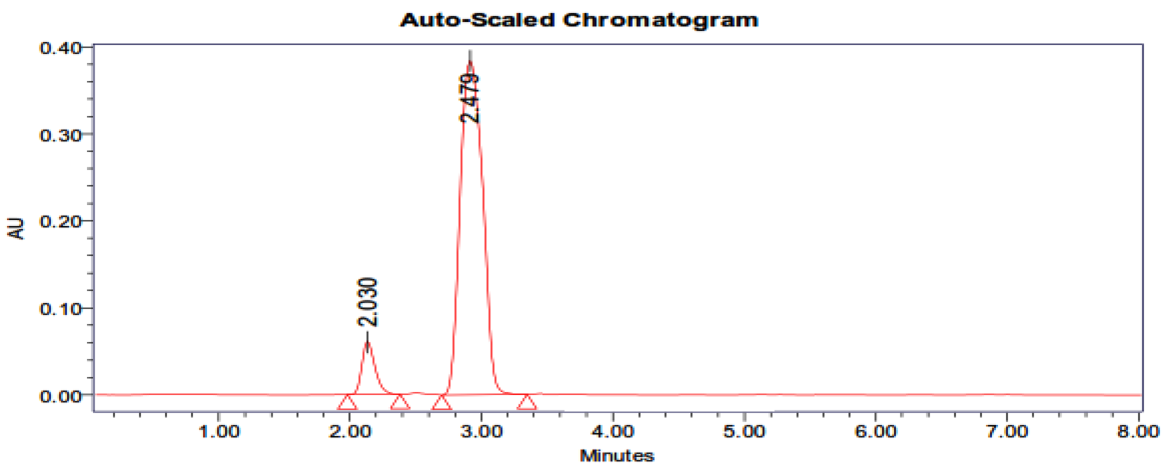

Fig 2: Optimized Chromatogram (Sample)

Optimized Chromatogram (Sample)

Mobile phase : Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size

Flow rate	:	1 ml/min
Wavelength	:	245 nm
Column temp	:	35ºC
Injection Volume	:	10 µl
Run time	:	7 minutes

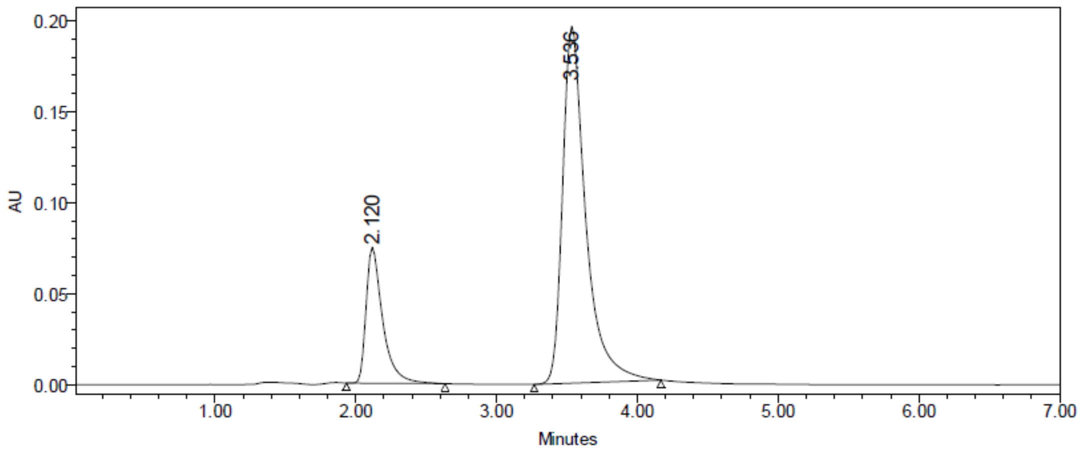

Fig 3: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Buprenorphine	2.120	775684	13124		0.99	6365.0
2	Naloxone	3.536	2658478	937405	5.06	1.23	7458.0

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Table 3: Results of system suitability for Buprenorphine

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Buprenorphine	2.117	765843	69587	5589	1.9
2	Buprenorphine	2.118	766594	69854	5576	1.6
3	Buprenorphine	2.116	765487	70211	5658	1.6
4	Buprenorphine	2.109	765928	69213	5642	1.7
5	Buprenorphine	2.102	765426	69558	5685	1.6
Mean			765855.6			
Std. Dev			466.6522			
% RSD			0.060932			

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample)

Table 4: Peak results for Assay sample

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Buprenorphine	2.120	756985	68958		0.98	7253	1
2	Naloxone	3.536	2569856	198564	2.06	1.23	8836	1
3	Buprenorphine	2.120	758745	69857		1.05	6530	2
4	Naloxone	3.537	2598654	195682	2.04	0.99	7270	2

5	Buprenorphine	2.102	756848	69588		1.7	7586	3
6	Naloxone	3.537	2587454	192541	2.04	1.6	8371	3

$$\%\text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Buprenorphine andNaloxone in pharmaceutical dosage form was found to be 99.8%.

Linearity

Chromatographic data for linearity study

Buprenorphine

Concentration µg/ml	Average Peak Area
6	205035
8	381239
10	561128
12	740162
14	909922

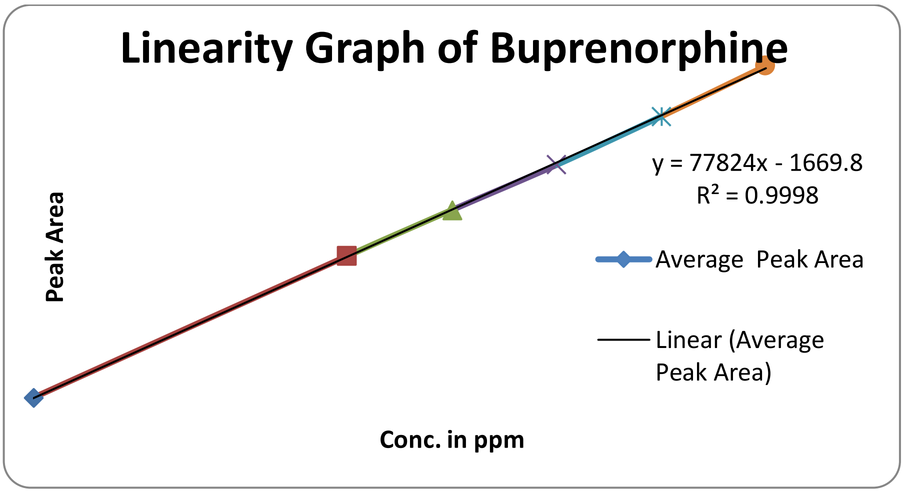

Fig 4: Calibration Graph for Buprenorphine

REPEATABILITY

Table 5: Results of Repeatability for Buprenorphine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Buprenorphine	2.108	766854	702564	5685	1.6
2	Buprenorphine	2.105	765884	698789	5584	1.4
3	Buprenorphine	2.113	765842	701235	5521	1.6
4	Buprenorphine	2.109	768985	700124	5525	1.9
5	Buprenorphine	2.109	765845	698986	5578	1.7
Mean			766682			
Std. Dev			1357.973			
% RSD			0.177123			

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 6: Results of method precision for Naloxone

S.no.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Naloxone	3.552	2569865	2231111	5365	1.6
2	Naloxone	3.550	2578474	2674210	5425	1.6

3	Naloxone	3.564	2568985	2231261	5368	1.5
4	Naloxone	3.564	2586845	2421301	5359	1.5
5	Naloxone	3.565	2545898	2324710	5498	1.6
Mean			2570013			
Std. Dev			15309.45			
% RSD			0.595695			

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 7: Results of Intermediate precisionDay 1 for Glipizide

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glipizide	2.066	242721	11323	5272	1.21
2	Glipizide	2.066	240155	11564	5168	1.16
3	Glipizide	2.066	240945	11887	5310	1.14
4	Glipizide	2.065	240385	11938	5275	1.19
5	Glipizide	2.069	249920	11652	5078	1.10
6	Glipizide	2.067	240820	11750	5225	1.17
Mean			243991			
Std. Dev			4641.97			
% RSD			1.5			

- %RSD of six different sample solutions should not more than 2

Table 8: Results of Intermediate precision for Buprenorphine

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Buprenorphine	2.108	758955	68986	5785	1.6
2	Buprenorphine	2.105	759869	68957	5698	1.4
3	Buprenorphine	2.113	758985	68545	5689	1.6
4	Buprenorphine	2.109	756894	68952	5781	1.9
5	Buprenorphine	2.109	759854	68595	5785	1.7
6	Buprenorphine	2.102	756985	68952	5693	1.6
Mean			758590.3			
Std. Dev			1339.793			
% RSD			0.176616			

- %RSD of Six different sample solutions should not more than 2.

Table 9: Results of Intermediate precision for Naloxone

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Naloxone	3.552	2659852	190025	5485	1.5	2.04
2	Naloxone	3.550	2648574	190048	5421	1.6	2.03
3	Naloxone	3.564	2659865	190054	5468	1.6	2.01
4	Naloxone	3.564	2658547	190078	5487	1.6	2.05
5	Naloxone	3.565	2648981	190016	5492	1.6	2.02
6	Naloxone	3.537	2654652	190057	5463	1.6	2.03
Mean			2655079				
Std. Dev			5242.086				
% RSD			0.197436				

- %RSD of Six different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged

Table 10: Results of Intermediate precision Day 2 for Buprenorphine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Buprenorphine	2.102	766895	69858	5586	1.5
2	Buprenorphine	2.105	765988	69854	5636	1.6
3	Buprenorphine	2.112	766532	69824	5432	1.6
4	Buprenorphine	2.113	766214	69875	5468	1.6
5	Buprenorphine	2.109	765897	69854	5546	1.9
6	Buprenorphine	2.109	765245	69848	5507	1.7
Mean			766128.5			
Std. Dev			567.7234			
% RSD			0.074103			

- %RSD of Six different sample solutions should not more than 2.

Table 11: Results of Intermediate precision for Naloxone

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Naloxone	3.537	2653254	190110	5428	1.6	7.98
2	Naloxone	3.552	2648985	190058	5452	1.6	6.4
3	Naloxone	3.560	2658213	190142	5498	1.6	8.9
4	Naloxone	3.564	2653652	190031	5442	1.5	8.3
5	Naloxone	3.564	2648978	190058	5489	1.5	7.5
6	Naloxone	3.565	2658985	190047	5463	1.6	5.3
Mean			2653678				
Std. Dev			4313.355				
% RSD			0.162543				

- %RSD of Six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Accuracy

Table 12: The accuracy results for Buprenorphine

%Concentration	Area	Amount	Amount	% Recovery	Mean
50%	392891.7	5	5.027	100.540%	100.351%
100%	781996	10	10.026	100.260%	
150%	1171988	15	15.038	100.253%	

Table 13: The accuracy results for Naloxone

%Concentration	Area	Amount	Amount	% Recovery	Mean
50%	204962	15	15.156	101.040%	100.93%
100%	365018	30	30.378	101.260%	
150%	521064.3	45	45.218	100.484%	

- The percentage recovery was found to be within the limit (98-102%).
- The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 14: Results for RobustnessBuprenorphine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	765789	2.102	5587	1.7
Less Flow rate of 0.9 mL/min	758698	2.330	5458	1.7
More Flow rate of 1.1 mL/min	7689584	1.950	5696	1.7
Less organic phase	758412	2.290	5586	1.4
More organic phase	769852	1.998	5355	1.5

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Naloxone

Parameter used for sample analysis	Peak Area	Retention	Theoretical plates	Tailing
Actual Flow rate of 1.0 mL/min	2532158	3.537	5398	1.6
Less Flow rate of 0.9 mL/min	2458692	3.885	5329	1.7
More Flow rate of 1.1 mL/min	2658642	3.263	5256	1.7
Less organic phase	2452148	4.435	5214	1.2
More organic phase	2653894	3.009	5524	1.0

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

A new method was established for simultaneous estimation of Buprenorphine and Naloxone by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Buprenorphine and Naloxone by using Phenomenex Luna C18 (4.6×250mm, 5µm) particle size, flow rate was 1ml/min, mobile phase ratio was (45:55 v/v) Acetonitrile: Phosphate Buffer (pH-4.6 was adjusted with orthophosphoric acid),detection wave length was 245nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.102mins and 3.537mins. The % purity of Buprenorphine and Naloxone was found to be 99.8%.The system suitability parameters for Buprenorphine and Naloxone such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Buprenorphine and Naloxone was found in concentration range of 6µg-14µg and 18µg-42µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.351% and 100.93%, %RSD for repeatability was 0.177 and 0.595. The precision study was precise, robust, and repeatable. LOD value was 0.6 and 0.8, and LOQ value was 1.8 and 2.4 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Buprenorphine and Naloxone in API and Pharmaceutical dosage form.

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