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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 novel, precise, accurate, rapid and cost effective isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method was developed, optimized and validated for the estimation of Buprenorphine and Naloxone in bulk and pharmaceutical dosage forms. The drugs were estimated using Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size column. A mobile phase composed of tri ethylamine buffer and methanol in proportion of 32:68 v/v, at a flow rate of 1.0 ml/min was used for the separation. Detection was carried out at 248 nm. The linearity range obtained was 30-70 µg/ml for Buprenorphine and 10-50 µg/ml for Naloxone with retention times (Rt) of 3.297 min and 5.405 min for Buprenorphine and Naloxone respectively. The correlation coefficient values were found to be 0.999 & 0.999. Precession studies showed % RSD values less than 2 % for both the drugs in all the selected concentrations. The percentage recoveries of Buprenorphine and Naloxone were found to be 100.1873% for Buprenorphine and 100.748% for Naloxone respectively. The assay results of Buprenorphine and Naloxone were found to be 99.82%. The limit of detection (LOD) and limit of quantification (LOQ) were 2.6µg/ml and 7.8µg/ml for Buprenorphine and 3.4µg/ml 10.2µg/ml for Naloxone respectively. The proposed method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated method was successfully used for the quantitative analysis of commercially available dosage form.

Keywords: *Buprenorphine and Naloxone, RP-HPLC, ICH Guidelines, Validation.*

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INTRODUCTION:

Analytical chemistry [1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

Qualitative analysis is the identification of elements, species and/or compounds present in sample.

Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Analytical techniques There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various *analytical techniques*. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte(s) in a sample that can be measured. *Atomic, molecular spectrometry* and *chromatography*, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis. *Spectrometric techniques* may involve either the *emission or absorption* of *electromagnetic radiation* over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels. *Chromatographic techniques* provide the means of separating the components of mixtures and simultaneous qualitative and quantitative analysis, as required. The linking of chromatographic and spectrometric techniques, called *hyphenation*, provides a powerful means of separating and identifying unknown compounds.

Analytical methods

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results. The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte(s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report. The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread. Many *standard analytical methods* have been published as papers in analytical journals and other scientific literature, and in textbook form. Collections by trades associations representing, for example, the cosmetics, food, iron and steel, pharmaceutical, polymer plastics and paint, and water industries are available standards organizations and statutory authorities, instrument manufacturer's applications notes, the Royal Society of Chemistry and the US Environmental Protection Agency are also valuable sources of standard methods. Often, laboratories will develop their own *in-house methods* or adapt existing ones for specific purposes.

Method development forms a significant part of the work of most analytical laboratories, and *method validation and* periodic revalidation is a necessity. Selection of the most appropriate analytical method should take into account the following factors:

- The purpose of the analysis, the required time scale and any cost constraints;
- The level of Analyte(s) expected and the detection limit required;
- The nature of the sample, the amount available and the necessary sample preparation procedure;
- The accuracy required for a quantitative analysis;
- The availability of reference materials, standards, chemicals and solvents, instrumentation and any special facilities;
- Possible interference with the detection or quantitative measurement of the analyte(s) and

the possible need for sample clean-up to avoid matrix interference;
- The degree of selectivity available − methods may be selective for a small number of analytes or specific for only one.
- Quality control and safety factors.

MATERIALS AND METHODS:

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

Procedure:

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.

Optimized chromatographic conditions:

Instrument used : Waters HPLC with auto sampler and PDA Detector 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 ultra sonication.
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 mobile phase:

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)

Column : Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size
Column temperature : 38˚C
Wavelength : 248nm
Mobile phase ratio : Methanol: TEA buffer pH 4.8 (32:68v/v)
Flow rate : 1ml/min
Injection volume : 20µl
Run time : 7minutes

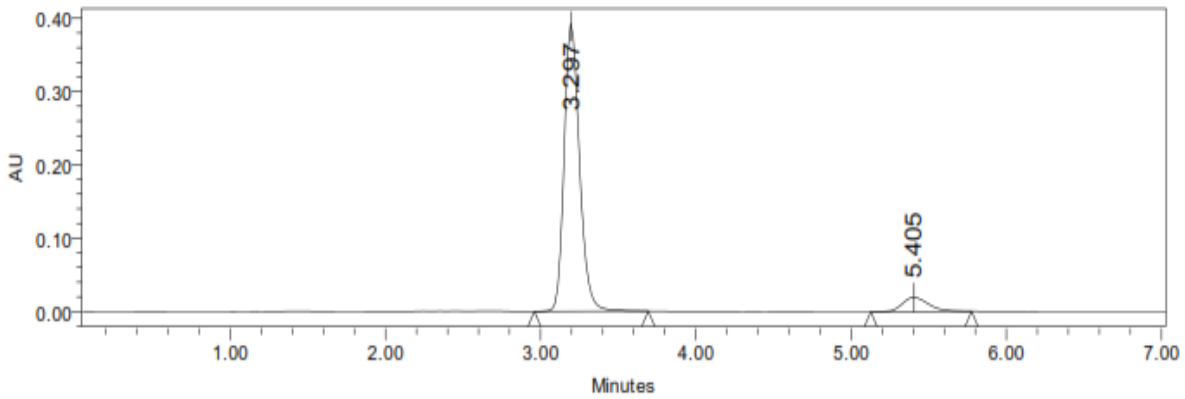


Figure-: Optimized Chromatogram (Standard)

Table No.18: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Buprenorphine	3.297	859857	42568	1.25	7897	
2	Naloxone	5.405	5699	3653	1.37	6583	6.9

Optimized Chromatogram (Sample)

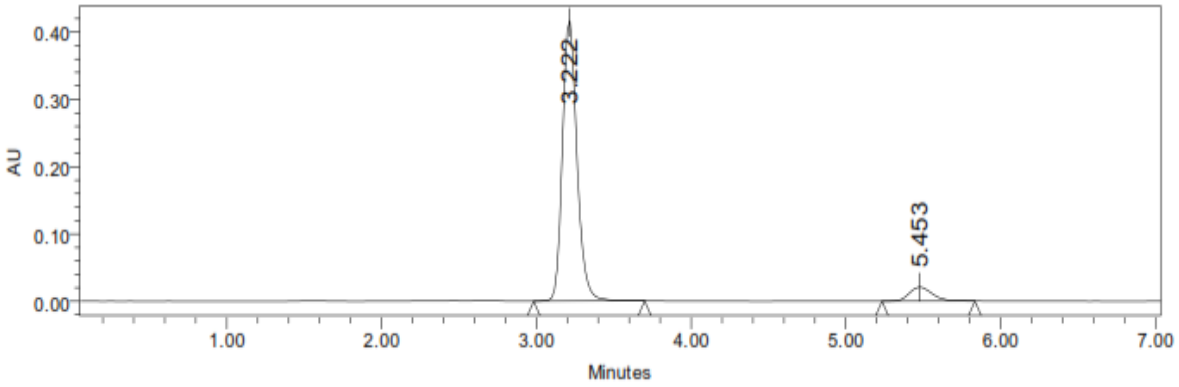


Figure-: Optimized Chromatogram (Sample)

Table No. 19: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Buprenorphine	3.222	865899	43658	1.27	7986	
2	Naloxone	5.453	5787	3786	1.39	6658	7.1

Acceptance Criteria:

- 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.

Assay (Standard):

Table-: Peak results for assay standard

Buprenorphine

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Buprenorphine	3.211	859786	42599	1.26	7857
2	Buprenorphine	3.222	859864	42895	1.25	7858
3	Buprenorphine	3.254	857848	42579	1.26	7868

Naloxone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Naloxone	5.414	5698	3686	1.36	6599	6.8
2	Naloxone	5.453	5686	3658	1.37	6538	6.7
3	Naloxone	5.424	5688	3647	1.36	6583	7.1

Assay (Sample):

Table-: Peak results for Assay sample

Buprenorphine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Buprenorphine	3.297	865986	43658	1.26	7986
2	Buprenorphine	3.294	865799	43876	1.26	7924
3	Buprenorphine	3.295	865457	43657	1.27	7948

Naloxone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Naloxone	5.435	5788	3658	1.37	6658	6.9
2	Naloxone	5.417	5797	3683	1.38	6698	7.0
3	Naloxone	5.434	5748	3694	1.38	6647	6.9

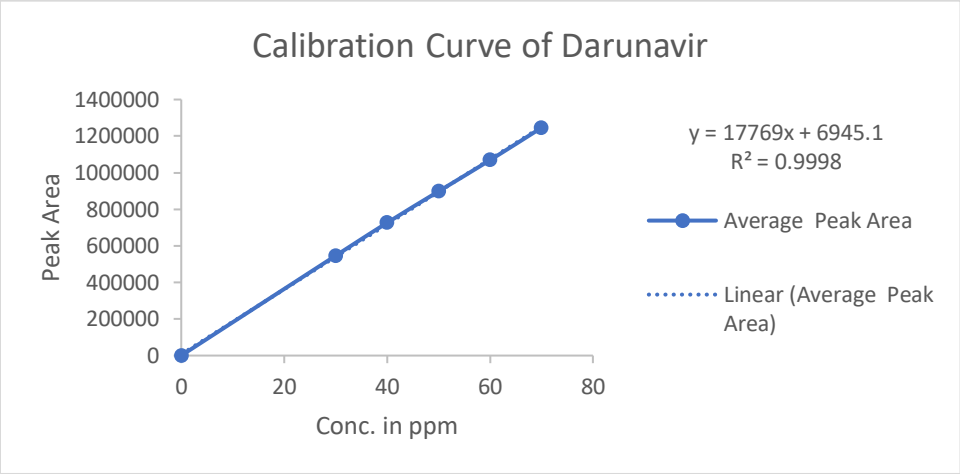
$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 99.89%

The % purity of Buprenorphine and Naloxone in pharmaceutical dosage form was found to be 99.82%.

Linearity
Chromatographic data for linearity study:
Buprenorphine:

Concentration µg/ml	Average Peak Area
30	545893
40	725986
50	897857
60	1068593
70	1245698



Naloxone:

Concentration µg/ml	Average Peak Area
10	2039
20	3858
30	5697
40	7488
50	9217

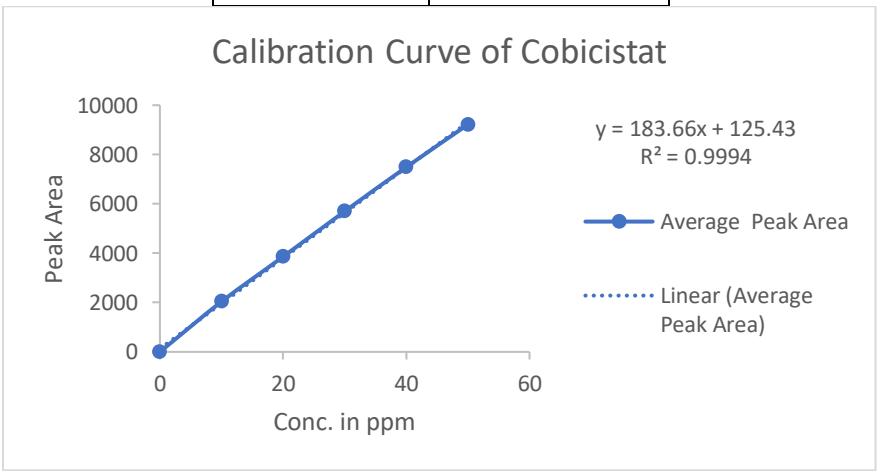


Fig: Chromatogram showing linearity level

Repeatability:

Table-: Results of Repeatability for Buprenorphine:

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Buprenorphine	3.213	859857	42658	7858	1.24
2	Buprenorphine	3.253	857986	42597	7867	1.24
3	Buprenorphine	3.297	856983	42586	7845	1.25
4	Buprenorphine	3.215	856986	42568	7818	1.25
5	Buprenorphine	3.254	859877	42893	7855	1.24
Mean			858338			
Std.dev			1454.222			
%RSD			0.169423			

Acceptance Criteria:

- %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 : Results of Repeatability for Naloxone:

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Naloxone	5.441	5698	3658	6593	1.36
2	Naloxone	5.442	5687	3649	6538	1.36
3	Naloxone	5.409	5688	3693	6585	1.37
4	Naloxone	5.520	5638	3649	6578	1.36
5	Naloxone	5.424	5687	3688	6542	1.36
Mean			5679.6			
Std.dev			23.71287			
%RSD			0.417509			

Intermediate precision: Buprenorphine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Buprenorphine	3.211	868957	43658	7986	1.26
2	Buprenorphine	3.211	869858	43986	7953	1.27
3	Buprenorphine	3.210	865984	43878	7945	1.26
4	Buprenorphine	3.212	866588	43866	7962	1.27
5	Buprenorphine	3.211	864257	43874	7963	1.26
6	Buprenorphine	3.297	868973	43563	7943	1.26
Mean			867435.5			
Std. Dev.			2167.095			
% RSD			0.249828			

Acceptance Criteria:

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

Table : Results of Intermediate precision day1 for Naloxone

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Naloxone	5.411	5785	3789	6659	1.37
2	Naloxone	5.410	5798	3758	6625	1.38
3	Naloxone	5.420	5766	3746	6649	1.38
4	Naloxone	5.423	5746	3795	6675	1.37
5	Naloxone	5.419	5782	3761	6653	1.38
6	Naloxone	5.409	5786	3752	6627	1.37
Mean			5777.167			
Std. Dev.			18.40018			
% RSD			0.318498			

Acceptance Criteria:

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

Table-: Results of Intermediate precision Day 2 for Buprenorphine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Buprenorphine	3.211	845986	44586	8026	1.27
2	Buprenorphine	3.233	847894	44897	8068	1.28
3	Buprenorphine	3.244	848987	44759	8047	1.27
4	Buprenorphine	3.297	847858	44547	8093	1.28
5	Buprenorphine	3.297	845985	44866	8041	1.28
6	Buprenorphine	3.202	847899	44253	8077	1.27
Mean			847434.3			
Std. Dev.			1201.345			
% RSD			0.141763			

Acceptance Criteria:

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

Table: Results of Intermediate precision Day 2 for Naloxone

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Naloxone	5.411	5899	3987	6853	1.39
2	Naloxone	5.410	5885	3956	6865	1.39
3	Naloxone	5.420	5864	3957	6828	1.40
4	Naloxone	5.405	5847	3946	6873	1.39
5	Naloxone	5.409	5898	3927	6828	1.39
6	Naloxone	5.463	5875	3963	6826	1.40
Mean			5876.667			
Std. Dev.			20.39281			
% RSD			0.347013			

Acceptance Criteria:

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

Accuracy:

Table-: The accuracy results for Buprenorphine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	451145.3	25	24.997	99.993%	100.1869%
100%	897249.3	50	50.103	100.209%	
150%	1344563	75	75.279	100.363%	

Table : The accuracy results for Naloxone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2896	15	15.083	100.561%	100.749%
100%	5686.333	30	30.284	100.942%	
150%	8448	45	45.336	100.745%	

Robustness

Table-: Results for Robustness
Results for Robustness - Buprenorphine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	859857	3.297	7895	1.24
Less Flow rate of 0.9mL/min	915848	3.639	7252	1.20
More Flow rate of 1.1mL/min	842563	2.859	7416	1.21
Less organic phase (about 5 % decrease in organic phase)	825499	3.460	7364	1.23
More organic phase (about 5 % Increase in organic phase)	814577	3.022	7259	1.22

Table : Results for Robustness- Naloxone

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.1mL/min	5699	5.405	6583	1.36
Less Flow rate of 0.9mL/min	6453	6.250	6786	1.32
More Flow rate of 0.8mL/min	5255	4.863	6364	1.34
Less organic phase (about 5 % decrease in organic phase)	5488	6.196	6253	1.38
More organic phase (about 5 % Increase in organic phase)	5367	5.010	6297	1.33

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

CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Buprenorphine and Naloxone was done by RP-HPLC.
The TEA buffer was p^H 4.8 and the mobile phase was optimized with consists of Methanol: TEA buffer mixed in the ratio of 32:68 % v/ v.
A Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size or equivalent chemically bonded to porous silica particles was used as stationary phase.
The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Buprenorphine and Naloxone were found to be from 30-70µg/ml, 10-50µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.
The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Buprenorphine and Naloxone. LOD and LOQ were found to be within limit.
The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear.
The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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