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Research

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.

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.

Chromatography [2]

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

" *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing component of the system, or mobile phase, is either a liquid or a gas. Concurrent with development of the different adsorbent materials has been the development of methods more specific to particular classes of analytes. In general, however, the trend in development of chromatography has been toward faster, more efficient.

"In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system. Chromatography has progressed considerably from Tswett's time and now includes a number of variations on the basic separation process".

"Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"

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

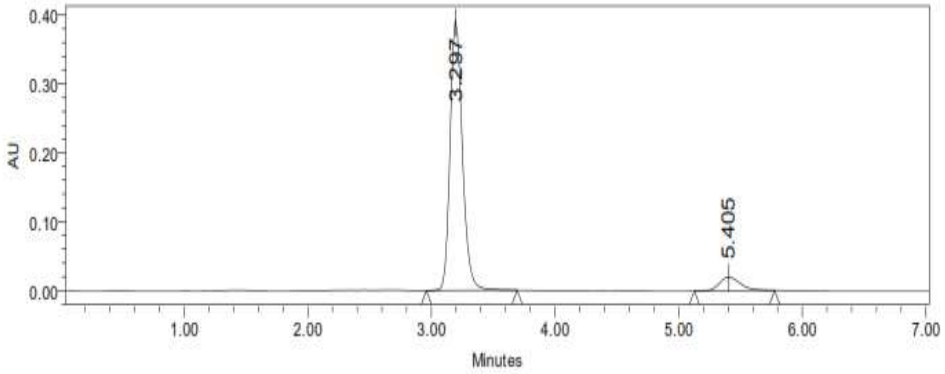


Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Buprenorphine	3.297	859856	42569	1.24	7896
2	Naloxone	5.405	5698	3652	1.36	6582

Optimized Chromatogram (Sample)

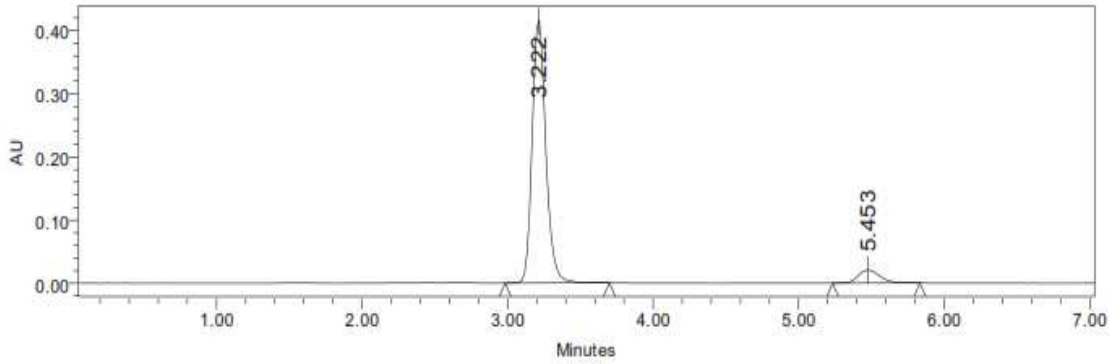


Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Buprenorphine	3.222	865898	43659	1.26	7985
2	Naloxone	5.453	5789	3785	1.38	6659

- *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 3: Peak results for assay standard

Buprenorphine

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Buprenorphine	3.211	859785	42598	1.25	7856
2	Buprenorphine	3.222	859865	42895	1.24	7859
3	Buprenorphine	3.254	857849	42578	1.25	7869

Naloxone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Naloxone	5.414	5699	3685	1.36	6598	6.9
2	Naloxone	5.453	5687	3659	1.37	6537	6.9
3	Naloxone	5.424	5689	3649	1.36	6582	7.0

Assay (Sample)

Table 4: Peak results for Assay sample

Buprenorphine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Buprenorphine	3.297	865985	43659	1.26	7985
2	Buprenorphine	3.294	865798	43875	1.26	7925
3	Buprenorphine	3.295	865456	43659	1.27	7946

Naloxone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Naloxone	5.435	5789	3659	1.37	6659	6.9
2	Naloxone	5.417	5798	3684	1.38	6689	7.0
3	Naloxone	5.434	5749	3695	1.38	6648	6.9

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

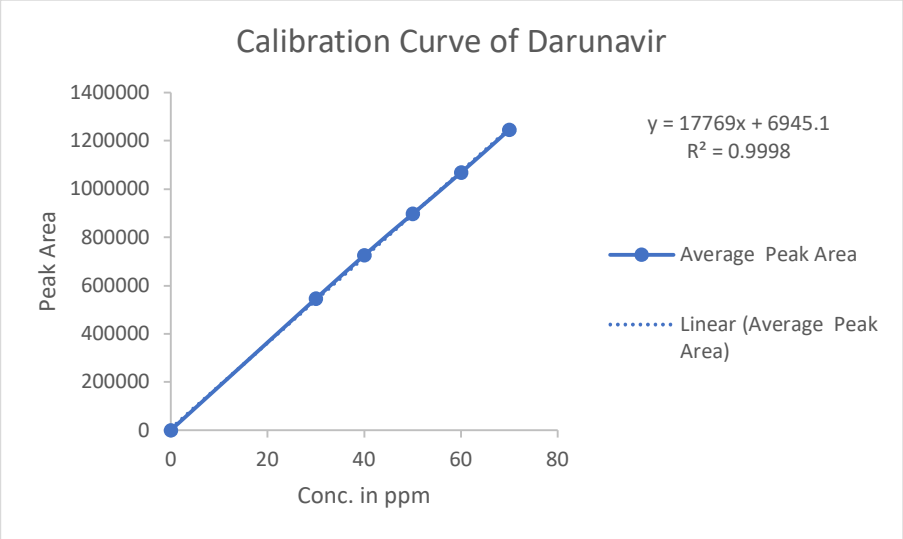
The % purity of Buprenorphine and Naloxone in pharmaceutical dosage form was found to be 99.82%.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

Buprenorphine

Concentration µg/ml	Average Peak Area
30	545894
40	725985
50	897856
60	1068594
70	1245698



Naloxone

Concentration	Average
10	2038
20	3859
30	5698
40	7489
50	9218

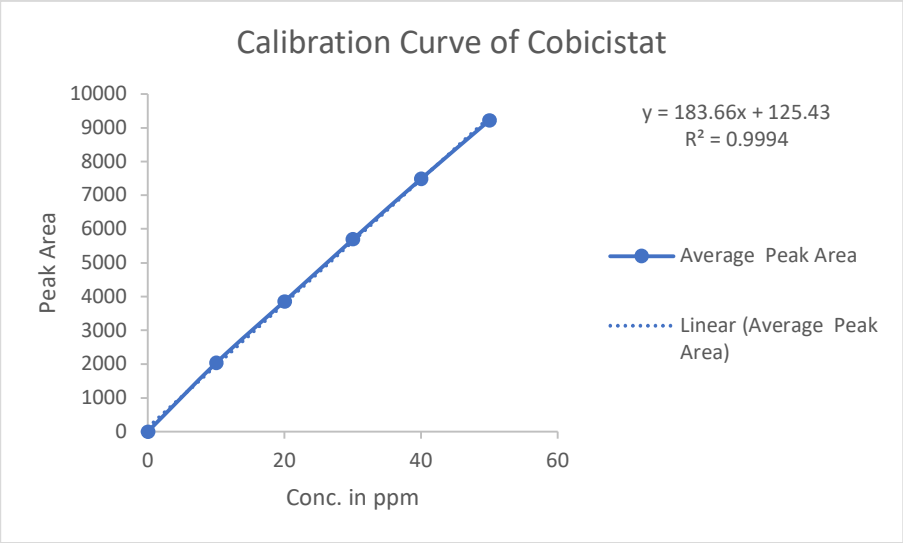


Fig 3: Chromatogram showing linearity level

REPEATABILITY

Table 5: 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	859856	42659	7859	1.24
2	Buprenorphine	3.253	857985	42598	7869	1.24
3	Buprenorphine	3.297	856984	42587	7846	1.25

4	Buprenorphine	3.215	856987	42569	7819	1.25
5	Buprenorphine	3.254	859878	42894	7856	1.24
Mean			858338			
Std.dev			1454.222			
%RSD			0.169423			

- *%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 Repeatability for Naloxone

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Naloxone	5.441	5697	3659	6592	1.36
2	Naloxone	5.442	5689	3648	6539	1.36
3	Naloxone	5.409	5698	3692	6584	1.37
4	Naloxone	5.520	5639	3648	6579	1.36
5	Naloxone	5.424	5688	3689	6549	1.36
Mean			5682.2			
Std.dev			24.57031			
%RSD			0.432408			

Intermediate precision

Table 7: Results of Intermediate precision day1 for Buprenorphine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Buprenorphine	3.211	868956	43659	7985	1.26
2	Buprenorphine	3.211	869857	43985	7954	1.27
3	Buprenorphine	3.210	865983	43879	7946	1.26
4	Buprenorphine	3.212	866587	43865	7963	1.27
5	Buprenorphine	3.211	864256	43875	7964	1.26
6	Buprenorphine	3.297	868974	43562	7942	1.26
Mean			867435.5			
Std. Dev.			2167.095			
% RSD			0.249828			

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

Table 8: 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			

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

Table 9: 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	845985	44585	8025	1.27
2	Buprenorphine	3.233	847895	44895	8069	1.28
3	Buprenorphine	3.244	848985	44758	8046	1.27
4	Buprenorphine	3.297	847859	44548	8094	1.28
5	Buprenorphine	3.297	845984	44865	8042	1.28
6	Buprenorphine	3.202	847898	44254	8076	1.27
Mean			847434.3			

Std. Dev.	1201.345
% RSD	0.141763

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

Table 10: 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	5898	3986	6852	1.39
2	Naloxone	5.410	5884	3955	6864	1.39
3	Naloxone	5.420	5863	3956	6829	1.40
4	Naloxone	5.405	5845	3945	6874	1.39
5	Naloxone	5.409	5896	3925	6829	1.39
6	Naloxone	5.463	5874	3962	6825	1.40
Mean			5876.667			
Std. Dev.			20.39281			
% RSD			0.347013			

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

ACCURACY

Table 11: The accuracy results for Buprenorphine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	451144.3	25	24.998	99.992%	100.1873%
100%	897248.3	50	50.104	100.208%	
150%	1344562	75	75.278	100.362%	

Table 12: The accuracy results for Naloxone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2895	15	15.084	100.560%	100.748%
100%	5685.333	30	30.282	100.940%	
150%	8449	45	45.335	100.744%	

Robustness

Table 13: Results for Robustness - Buprenorphine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	859856	3.297	7896	1.24
Less Flow rate of 0.9mL/min	915847	3.639	7251	1.20
More Flow rate of 1.1mL/min	842564	2.859	7415	1.21
Less organic phase	825498	3.460	7365	1.23
More organic phase	814578	3.022	7258	1.22

Table 14: Results for Robustness- Naloxone

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.1mL/min	5698	5.405	6582	1.36
Less Flow rate of 0.9mL/min	6452	6.250	6785	1.32
More Flow rate of 0.8mL/min	5254	4.863	6365	1.34
Less organic phase	5487	6.196	6254	1.38
More organic phase	5369	5.010	6298	1.33

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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