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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF NALOXONE IN BULK AND PHARMACEUTICAL FORM BY USING RP-HPLC

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Abstract:
In the current work, an endeavour be situated made to give a fresher, delicate, basic, precise and minimal effort RP-HPLC technique. It is effectively applied for the assurance of Naloxone in drug arrangements without the obstructions of other component in the details. In HPLC strategy, HPLC conditions were upgraded to get, a sufficient detachment of eluted mixes. At first, different portable stage pieces were attempted, to come to be great ideal outcomes. Portable stage and stream rate choice be subject to on top boundaries (tallness, resulting, imaginary plates, limit factor), run time and so on the milieu with Buffer: acetonitrile (43:57) with 1 ml/min river rate is very strong.The ideal frequency for identification was 254 nm at which better indicator reaction for drug was gotten. The normal conservation time for Naloxone were discovered to be 2.2. Framework appositeness tests are an essential piece of chromatographic strategy. They are applied to check the duplicability of the chromatographic framework. To discover its adequacy, framework reasonableness tests were done on newly arranged stock activities. The alignment was direct in focus scope of 60 – 100 mg/ml. The low estimations of % R.S.D. show the strategy is exact and precise. The mean recuperations be situated found in the scope of 99.8%– 101.4%. Sample worth and quality of the sample were assessed using three illustrations of five and three different concentrations, respectively, prepared and analysed on the same day. Variability on a day-to-day basis was measured using three concentrations analysed over three separate days over a three-day period. The precision also reproducibility of the assay are seen in these data.

Keywords: *Naloxone, RP-HPLC, Method development, Validation*

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INTRODUCTION:

Naloxone nasal sprays are indicated for the emergency treatment of an opioid overdose or suspected opioid overdose.[1] Intramuscular, intravenous, and subcutaneous injections are indicated for complete or partial reversal of opioid depression, diagnosis of known or suspected opioid overdose, and as an adjunct therapy in the treatment of septic shock.[2] Naloxone is a competitive inhibitor of the μ-opioid receptor. Naloxone antagonizes the action of opioids, reversing their effects.[3] If a patient has not taken opioids, naloxone does not have a significant effect on patients. IUPAC name is (4*R*,4*aS*,7*aR*,12*bS*)-4*a*,9-dihydroxy-3-prop-2-enyl-2,4,5,6,7*a*,13-hexahydro-1*H*-4,12-methanobenzofuro[3,2-e] isoquinolin-7-one. Molecular formula $C_{19}H_{21}NO_4$. Molecular Weight is 327.4.

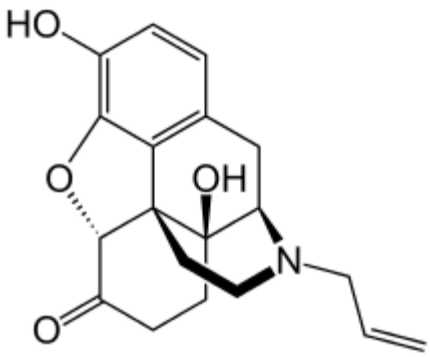

Figure 1: Structure of Naloxone

The literature survey revealed that There are very few methods reported in the literature for analysis of Naloxone alone or in combination with other drugs in the pure form and pharmaceuticals formulations. Naloxone was quantified in microparticles [4], dosage forms [5], transdermal formulations [6], human plasma, human urine, and human liver microsomes [7] using spectrophotometry, HPLC [8], and liquid chromatography-mass spectrometry [9]. In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Naloxone estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Naloxone. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the estimation of Naloxone in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Naloxone were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 254 nm with column Symmetry C8 Symmetry C8 (150mm x4.6,3.5 µm), dimensions at Ambient temperature. The optimized mobile phase consists of pH 4.5 buffer: Acetonitrile (43:57). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of Triethylamine buffer

5ml of triethylamine in 1000ml of water and its pH was maintained at by using orthophosphoric acid.

Mobile Phase

A mixture of 50 volumes of Triethylamine pH 3.5 & 50 volumes of Acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases.

Preparation of orthophosphoric acid

3ml orthophosphoric acid is diluted in 10ml water

Preparation of standard stock solution of Naloxone

10mg of Naloxone was weighed and transferred in to 10ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg/ml of solution by diluting 1ml to 10ml with methanol.

Preparation of mixed standard solution

Weigh accurately 10 mg of Naloxone in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 250µg/ml of Naloxone is prepared by diluting 2.5 ml of Naloxone to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

5 Capsules (each Capsules contains 250 mg of Naloxone) were weighed and taken into a mortar and make it fine powder and uniformly mixed.

Capsules stock solutions of 250µg/ml were prepared by dissolving weight equivalent to 10 mg of Naloxone dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 10 ml with mobile phase. Further dilutions are prepared in 5 replicates of 250 µg/ml of Naloxone was made by adding 2.5 ml of stock solution to 10 ml of mobile phase.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 30 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Symmetry C8 (150mm x4.6, 3.5µm) column, the mobile phase of composition pH 4.5 buffer: Acetonitrile (43:57) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Naloxone in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 5-25 mg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%,150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Naloxone and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

LOD = $3.3\sigma/S$ and

LOQ = $10\ \sigma/S$, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION:

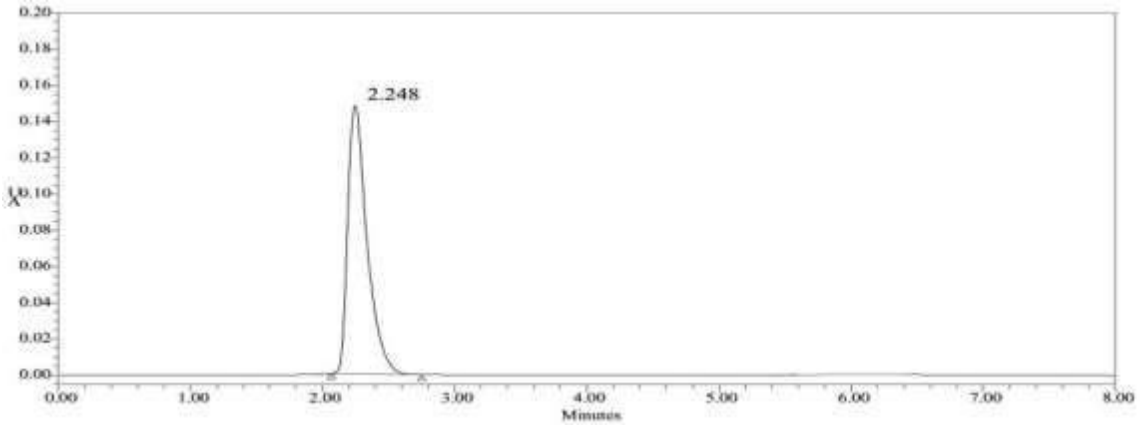

Figure 2: Standard chromatogram

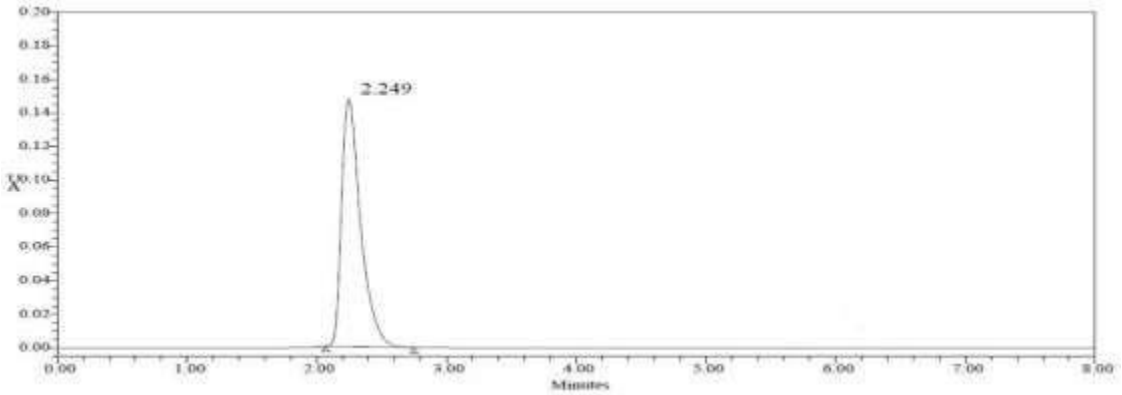

Figure 3: Sample chromatogram

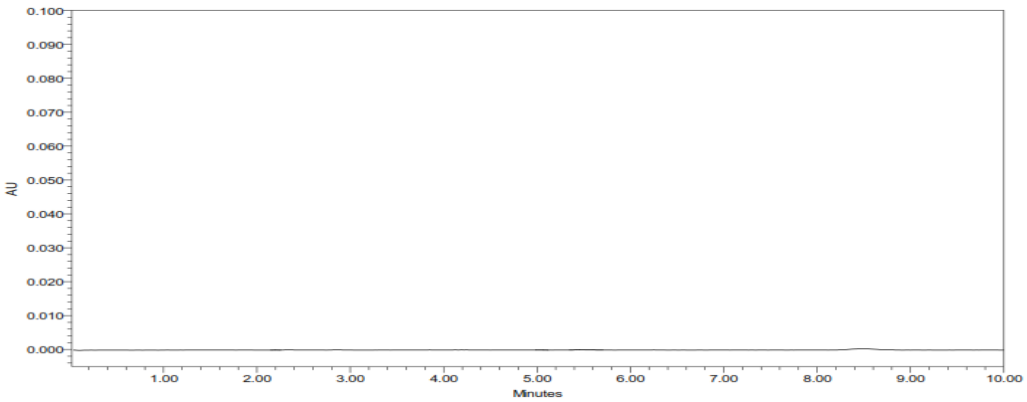

Figure 4: Blank chromatogram

Table 1: System suitability parameters

Drug name	USP tailing	USP theoretical plates
Naloxone	1.3	2203.7

Table 2: Assay results for Naloxone

S.No.	Naloxone	
01	Spl. Area	1525384
02	Std. Area	1532594
03	Std. Wt	10mg
04	Spl. Wt	16.98mg
05	LC	500mg
06	Avg. Wt	993.7mg
07	Std. Purity	99.8
08	Assay %	98.8

Table 3: Linearity results of Naloxone

Parameters	Results observed Naloxone
Slope	34029.38
Intercept	-838359
Correlation	0.9993

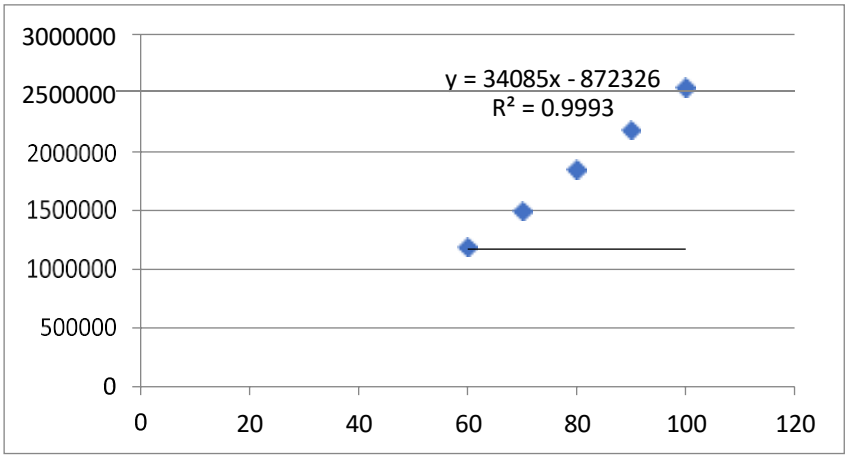

Figure 5: Linearity graph for Naloxone

Table 4: Showing accuracy results for Naloxone

%Concentratio	Area	Amount Added	Amount Found	Recovery	Mean Recovery
50%	984243	5	5.07	101.40%	100.56%
100%	1567396	10	9.98	99.80%	
150%	2497228	15	15.08	100.50%	

Table 5: Precision results for Naloxone

S.No.	**Injection number (80 mcg/ml)**	**Retention Time of Naloxone**	**Area ofNaloxone**
1	Injection-1	2.412	1549491
2	Injection-2	2.259	1530248
3	Injection-3	2.259	1530713
4	Injection-4	2.261	1527834
5	Injection-5	2.257	1537667
	AVG		1535191
	STD		8792.8
	%RSD		0.57

Table 6. Ruggedness results of Naloxone

Number of injections	**Retention Time of Naloxone**	**Area of Naloxone**
Injection-1	2.293	1976857
Injection-2	2.291	1971778
Injection-3	2.290	1970279
Injection-4	2.290	1979007
Injection-5	2.286	1970631
AVG		1973711
STD		3966.9
%RSD		0.20

Table 7: Robustness results for Naloxone

Proposed variations		**USP Plate Count**	**USP Tailing**
Variation in mobile phase composition	10% less	2963.1	1.2
	*Actual	2203.7	1.3
	10% more	2268.8	1.3
Variation inflow rate	**0.8ml/min**	2519.4	1.3
	1ml/min	2203.7	1.3
	1.2ml/min	2474.4	1.3

Table 8: LOD, LOQ of Naloxone

Drug	LOD	LOQ
Naloxone	3.20	9.86

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Naloxone in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Naloxone in pure and its pharmaceutical dosage forms.

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