DEVELOPMENT AND VALIDATION: RP-HPLC METHOD FOR ESTIMATION OF ASSAY OF NALBUPHINE HYDROCHLORIDE IN INJECTION FORM

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ABSTRACT

Nalbuphine is an analgesic antagonist. It is roughly weight-based to morphine when the parenteral administration of 'normal doses is applied in analgesic practices. Even reasonable anesthesia is evident when used as an 'equilibrium' anesthesia procedure, though its utility in this sense is constrained by a relative 'ceiling' effect in minimizing anesthesia requirements with nalbuphine. Like most analgesics' agonists/antagonists, there is also an effect of 'ceiling' of nalbuphine-induced respiratory depression that does not appear readily. Chromatography is mainly used in chemical analytics, but mainly a separation method, with high-performance liquid chromatography

(HPLC) being a highly flexible technique in that analytes are isolated by a column containing micrometer particles. Now the most widely used HPLC separation method is reverse-phase day chromatography. The explanations are basic, flexible and the scope of the reversed-phase approach because compounds of a varied polarity and molecular mass can be handled. Analytical and preparational applications in biochemical separation and purification were established both in reversed-phase chromatography. The reversed-phase chromatography of molecules with excellent recovery and resolution will distinguish them with some hydrophobic characteristics, such as proteins, peptides, and nuclear acids.

KEYWORDS: Nalbuphine, Analgesic, HPLC, Placebo.

INTRODUCTION

Analytical chemistry is a science of calculation composed of strong concepts and techniques which are useful in all areas of science and methodology.[1] The chemical composition,

structure, and compound behavior were used to study.[2] The aim of the analysis is to integrate and understand chemical knowledge which is a broad variety of contexts that can be of use to society. It includes the use of collecting and analyzing the qualitative, quantitative, and systemic knowledge of components by a broad variety of techniques and methodologies.[3,4]

Qualitative analysis is the detection of sample components and compounds. A quantitative analysis is used to determine absolute or approximate concentrations of compounds or elements found in a sample. Structural analysis implies the recognition in an entity, molecule, or characteristic groups or atoms of the special arrangement of atoms.[5,6]

The fastest-growing analytical tool for the study of drugs is high-performance liquid chromatography. Its versatility, high specificity, and strong sensitivity allow it the perfect solution for the study of a vast variety of drugs both in dosage and in biological fluids. In the late 1960s and 1970s, HPLC was developed.[7] Currently, the separation procedure is commonly known for sample analysis and purification in many areas.
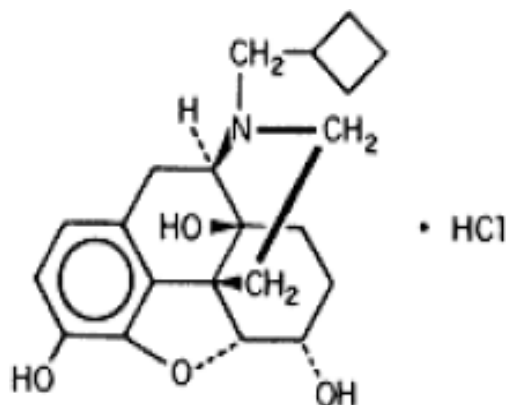

Figure 1: Nalbuphine structure.

Molecular formula: $C_{21}H_{27}NO_4 \cdot HCl$

Chemical name: 17-(cyclobutyl methyl)- 4,5α-epoxy morphinan- 3, 6α, 14-triol hydrochloride

Description: White to slightly off-white powder

MATERIALS AND METHODS

Instruments used

- System
- Pump

- Detector

- Column

- pH meter

- vacuum pump

- Digital balance

- Sonicator

    Reagents and Chemicals

- Sodium Octane Sulphonate

- Sodium Acetate Trihydrate

- Glacial acetic acid

- Methanol

- Water

- Nalbuphine hydrochloride - 94.1 % purity

Method development

Solubility: Nalbuphine HCl water soluble solution (35.5 mg/mL at 25°C and ethanol, chloroform insoluble, ether insoluble).

Selection of chromatographic condition: The proper selection of the system depends on the sample's composition, molecular weight and solubility (ionic/ionizable/neutral molecule). The medicines chosen in the sample are polar in nature and thus can be used in reverse or ion-pair or in ion exchange. Due to its simplicity and convenience, the reversed HPLC process was chosen for separation.[8]

Wavelength selection: The choice of detection wavelength was based on the scanned absorption for the Nalbuphine HCl when the conditions were developed for developing the test procedure. The spectrum was estimated between 190 and 400 nm and was accomplished by means of the measurement of the absorption from stock solution of 1.0 mg/ml Nalbuphine HCl solution in water. The spectrum was created using the water comparison solution by using a quartz cell of 1 cm. The spectrum was extracted using the water comparison solution by using 1cm quartz cell. Around 280 was the $\square_{max}$ of misoprostol. This is why 280 nm was chosen for the prediction.[9]

RESULTS AND DISCUSSION

Validation of rp-hplc method

Validation of the system was performed after the development of HPLC for the evaluation of the single-component dosage types. The process for validating the established method has been followed in this section.[10]

Specificity: For demonstration of precision of the HPLC method, the following methods were used. The first method was introduced to adjust the HPLC method conditions, namely the mobile phase percentage of the organic solvent, pH of the mobile phase, flow rates, etc in HPLC and where appropriate, observation was made of additional peaks. The second method consists of the diode array detector method of the peak purity test. The normal and sample drug peaks were reported and compared with the derived spectra and derivative columns of the diode array. The third technique was based on calculating the drug's absorption level in varying wavelengths.

Table 1: Specificity.

Sample ID	Interference		
	Nalbuphine Hydrochloride RT	Purity Angle	Threshold angle
Blank	Nil	NAP	NAP
Placebo	Nil	NAP	NAP
Standard + Placebo	6.123	0.15	0.304
Standard	6.122	0.109	0.291
Test Sample	6.123	0.15	0.304

Accuracy: Recovery studies have established the method's accuracy. The comparison criteria for each substance have been applied to the formula at 100%. This have been further diluted in the formulation calculation by method. HPLC have been analyzing the resulting sample solutions. Each medication was measured with the sum present, percent recovery, percent RSD deviation.

Table 2: Accuracy studies.

Series	No of Sample	Added in mg	Found in mg	Recovery in %	Average in %
50%	01	26.65	26.35	98.9	99.3
	02	26.58	26.41	99.4	
	03	26.42	26.35	99.7	
75%	01	39.52	39.79	100.7	100.5
	02	39.57	39.89	100.8	
	03	39.66	39.70	100.1	
	01	51.32	52.18	101.7	101.2

100%	02	51.63	52.15	101.0	
	03	51.86	52.32	100.9	
125%	01	65.16	65.51	100.5	
	02	64.84	65.54	101.1	100.8
	03	65.08	65.65	100.9	
150%	01	78.16	78.56	100.5	
	02	77.80	78.54	100.9	100.7
	03	77.79	78.35	100.7	
			Mean	100.5	
			Std dev.	0.72	
			% RSD	0.7	
			Confidence Interval	100.2 & 100.9	

Precision

Table 3: Nalbuphine HCl system precision.

No of injections	RT	Response
1	6.599	2328334
2	6.595	2329430
3	6.597	2325803
4	6.595	2326326
5	6.595	2323824
6	6.593	2326955
Mean	6.596	2326779
SD	0.00	1968.66
RSD %	0.0	0.1

Table 4: System suitability parameters.

System Suitability Parameters for Nalbuphine hydrochloride	Observed value	Acceptance criteria
Tailing factor of the 1st inj of normal preparation	1.5	NMT 2.0
Theoretical plates from the 1st inj of normal preparation	3853	NLT 1500
The RSD% of 6 replicate inj of standard preparation.	0.1	NMT 2.0
The RSD% of RT for 6 inj of standard solution.	0.0	NMT 1.0

Test preparation

Table 5: Test preparation.

| No. of Sample | Method Precision |
	Nalbuphine Hydrochloride
1	99.2
2	99.2
3	99.6

4	99.8
5	99.5
6	99.6
Mean	99.5
SD	0.15
RSD%	0.2
Confidence Interval	99.2 & 99.5

Linearity and Range

A diagram of concentrations on the X-axis and peaks on the Y-axis was calculated by drawing the linearity and the correlation coefficient. The research method was used to generate and evaluate 7 distinct concentrations of Nalbuphine HCl, ranging from LOQ, 50%, 75%, 125%, 150% to 200% in relation to the working concentration. In the table below the results are summarized.

Table 6: Linearity and range.

Level in percentage	Concentration in µg/ml (ppm)	Peak Response of Nalbuphine Hydrochloride
10	20	226810
25	50	568280
50	100	1133346
75	150	1719579
100	200	2290891
125	250	2868421
150	300	3440804
Slope		11495
Y intercept		-7150
Coefficient of correlation		1.0000
Coefficient of regression (r2)		1.0000
Y intercept should be ±2.0% of the active response at 100% concentration		-0.3

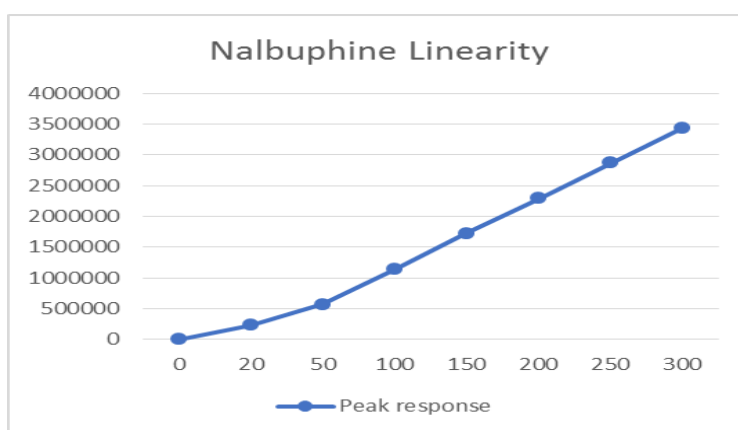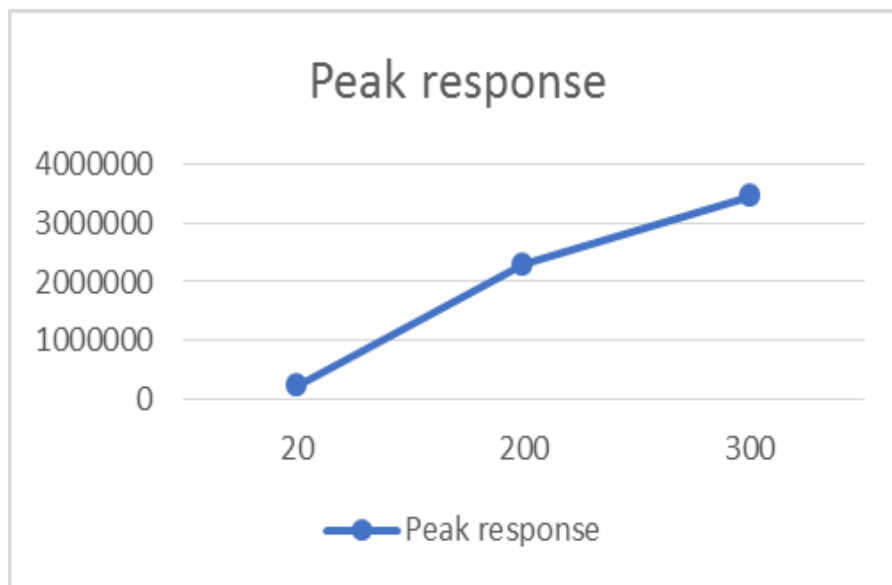**Figure 2: Linearity with peak response.**

Range

Table 7: Range with concentration and % RSD.

Level	Concentration in µg/ml	%RSD
Lower 10%	20.00	0.2
Middle100%	200.00	0.1
Higher 150%	300.00	0.1

Figure 3: Peak response.

Ruggedness: The USP describes the degree of reproducibility of findings generated in a range of environments, including labs, analysts, equipment, environmental conditions, operators and materials. Ruggedness is a measure of reproducibility of research outcomes, from laboratory to laboratory and from analyst to analyst, under regular, planned operating conditions.

Robustness: The subsequent optimized conditions were significantly different to show the robustness of the technique.

1. Impact of variance in variation in wavelength.
2. Column Oven Temperature Variance Impact.

Table 8: Effect of wavelength variation.

System Suitability Parameters for Nalbuphine Hydrochloride	Observed value at Wavelength			Acceptance criteria
	278 nm	280 nm	282 nm	
Tailing factor of the 1st inj. of normal preparation	1.5	1.5	1.5	NMT 2.0
Theoretical plate count from 1st inj by standard preparation.	3676	3854	3604	NLT 1500
The RSD% of 6 replicate injections of standard preparation.	0.3	0.1	0.2	NMT 2.0

Table 9: Comparison between System -1 & System-2.

No. of Samples	System -1 Nalbuphine Hydrochloride	System-2 Nalbuphine Hydrochloride
1	99.2	99.5
2	99.2	99.4
3	99.6	99.2
4	99.8	99.3
5	99.5	99.2
6	99.6	99.3
Mean	99.5	99.3
SD	0.15	0.20
% RSD	0.2	0.2
Confidence Interval	99.2 & 99.5	99.2 & 99.5

Column oven temperature variance impact

Check the strength parameters of the system by injecting regular preparations into the HPLC system at 35°C and 45°C column-oven temperature to show the robustness of the test process. Assess the parameters of the device suitability and tabulate the effects in the following table.

Table 10: Column oven temperature variance impact.

System Suitability Parameters for Nalbuphine hydrochloride	Observed value with Column Oven Temperature			Acceptance criteria
	20°C	25°C	30°C	
Theoretical plate count after 1st inj of the normal preparation	1.6	1.5	1.6	NMT 2.0
Theoretical plate count from 1st inj for standard preparation.	3080	3854	3510	NLT 1500
The RSD% of 6 replicate inj. of standard preparation.	0.1	0.1	0.1	NMT 2.0

Stability studies: For reproducible and consistent findings the stability of a sample and norm used by the HPLC process is important in a reasonable period. The drug-spiked stability of the sample was short-stable after eight hours at room temperature.

Table 11: Stability studies.

Time in hours	Sample area	Standard area
Initial	2378740	2399730
4th Hours	2378740	2409545
8th Hours	2374540	2401026
12th Hours	2381951	2405910
16th Hours	2388626	2412545
20th Hours	2385760	2414695
24th Hours	2380471	2415555
28th Hours	2392170	2423669
32nd Hours	2393143	2426931
36th Hours	2388383	2412868
40th Hours	2390248	2422430
44th Hours	2394084	2417134
48th Hours	2384840	2406560
Mean	2385516	2412970
SD	6239.7	8400.2
% RSD	0.3	0.3

Filter validation: Conduct filter validation using three separate filter, namely 0.45 μm nylon, 0.45 μm PVDF and Whatman No.1, relative to the unfiltered sample. Prepare the regular approach according to the test methodology and prepare the test preparation in the same way. Filter the pre-test by way of a single filter. Inject standard solution and a filtered HPLC chromatographic test solution into the HPLC method. Calculate the percentage difference between the measurement in various filters.

Table 12: Filter validation & assay percentage.

Filters	Assay in %	Difference from Initial
Unfilter	99.8	NAP
0.45 μm nylon	98.1	1.5
0.45 μm PVDF	99.8	0.0
Whatman No.1	100.5	0.7

Few methods of evaluating Nalbuphine HCl were included in the literature published. It has been concluded that the above-selected component dosage type has been evaluated using a few methods which support the continuation of this work. The scope and aim of this study are

to establish and validate a new simple HPLC process for estimating Nalbuphine HCl in the form of an injection dose.

The predicted columns for Thermo Hypertsil C 18 (4.6 X 250 mm) were used in the development of RP-HPLC methods with a particle size of 5 microns. The moving step Sodium Acetate Filler, Methanol with a ratio of 55:45, which is injected at a rate of 1.0 ml/min, is used to inject and elude injection volume of 50µl. Detection at 280 nm was performed. The measurements were made with the above optimized chromatographic condition by means of a calibration curve process. Nalbuphine HCl was observed to have 6.109 minutes in symmetric peak form, decent resolution, and fair retention periods.

Nalbuphine HCl's 20 µg/ml to 200µg/ml. For nalbuphine HCl, 11497, 7149, 1,0000 were found to have slope-intercept and correlation coefficient(s), which suggests an excellent vs correlation factor in standard solutions.

Precision and precision of the systems developed were studied by system precision. The percentage of RSDs for accuracy were found below the appropriate limit that indicated that the system established was accurate. The method developed has proven robust. The percentage of RSD values were considered to be below the appropriate parameters for Nalbuphine HCl recovery percentages. The findings show that the process of calculating the above drugs is appropriate.

Therefore, Nalbuphine HCl chromatography is rapid, quick, precise, sensitive, accurate, and effective. The HPLC-RP was simple and does not suffer from typical excipients in the preparation of pharmaceuticals and very useful in drug research.

CONCLUSION
The accuracy of the methods developed has been studied according to system accuracy and process precision. The RSD value was found to be within the appropriate range for precision, which indicated that the system developed was correct. The method developed has proven robust. The RSD percentage values for the Nalbuphine HCl recovery percentage were included in the appropriate criterion. This means that the procedure for calculating the drugs alluded to above is satisfactorily correct.

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