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R E S E A R C H A R T I C L E

Development of New simultaneous RP-HPLC method for the estimation of Pentazocine HCl and Naloxone HCl in tablet dosage form

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A B S T R A C T

The aim of present research work made to develop and validate RP-HPLC method for the simultaneous estimation of Pentazocaine and Naloxone in bulk and combined dosage form. The optimized mobile phase was consists of 30% OPA buffer: 70%Methanol, the pH was maintained at 3.0. The chromatographic separation was carried on Inertsil ODS C 18, column (4.6*150mm, 5µ). The detection of absorption maxima was monitored at 239 nm. The flow rate was maintained at 1.0 ml/min. The retention time of Pentazocaine and Naloxone were found to be from 2.401min & 3.374 min respectively. The values of % RSD are less than 2% indicating accuracy and precision of the method. The mean percentage recovery was found to be 100.43% of Pentazocaine and 100.50% of Naloxone. The proposed method is precise, simple and accurate to determine the amount of Pentazocaine and Naloxone in formulation. So the method can be useful in the routine quality control of these drugs.

Keywords: Pentazocaine and Naloxone, RP-HPLC, Mobile phase, Accuracy, Regression coefficient

A R T I C L E I N F O

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CONTENTS

1. Introduction

Naloxone is a specific opiate antagonist that has no agonist activity. It is a competitive antagonist at mu, delta, and kappa opioid receptors. It naloxone antagonizes the opioid effects by competing for the same receptor sites, especially the opioid mu receptor. Recently, naloxone has been shown to bind all three opioid receptors (mu, kappa and gamma) but the strongest binding is to the mu receptor. Naloxone has been shown to block the action of pain-lowering endorphins which the body produces naturally. These endorphins likely operate on the same opioid receptors that

naloxone blocks. It is capable of blocking a placebo pain-lowering response, both in clinical and experimental pain, if the placebo is administered together with a hidden or blind injection of naloxone. Pentazocine antagonizes the opioid effects by competing for the same receptor sites, especially the opioid mu receptor.

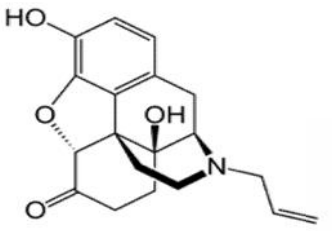

Fig 1: Structure of Naloxone

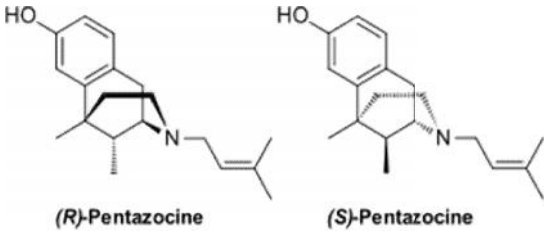

Fig 2: Structure of Pentazocaine

Pentazocine HCl and Naloxone HCl are existing drugs. Literature reveals different methods for their analysis in their formulations2,3. But our present plan is to develop a new, simple, precise& accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated.

2. Materials and Methods

Instruments used:

Table 1: List of Instruments

S. No	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector
2	UV/VIS spectrophotometer	LABINDIA UV 3000^{+}
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A

Chemicals used:

Table 2: List of Chemicals

S .No	Chemical	Brand
1	Pentazocine HCl	Supplied by Pharmatrain
2	Naloxone HCl	Supplied by Pharmatrain
3	Tri ethyl amine	FINAR chemical LTD
4	Water and Methanol for HPLC	Standard solutions Ltd
5	Acetonitrile for HPLC	Standard solutions Ltd
6	HCl, H_2O_2, NaOH	MERCK

HPLC Method Development

Wave length selection:

UV spectrum of 10 µg/ml Pentazocine HCl and Naloxone HCl in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 239nm. At this wavelength both the drugs show good absorbance.

Optimization of Column:

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

Optimized Chromatographic Conditions:

Instrument used **:**Waters HPLC with auto sampler and uv detector.
Temperature **:**Ambient
Column **:**Inertsil ODS (4.6 x 150mm, 5µm)
Buffer **:**OPA buffer
pH **:**3.0
Mobile phase **:**30% OPA buffer: 70%Methanol
Flow rate **:**1 ml per min
Wavelength **:**239 nm
Injection volume **:** 20 µl
Run time **:** 10 min

Preparation of mobile phase:

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Standard Solution Preparation:

Accurately weigh and transfer 500 mg of Pentazocine HCl and 5 mg of Naloxone HCl working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 500 mg of Pentazocine HCl and 5 mg of Naloxone HCl working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

System Suitability: System suitability is an integral part of many analytical procedures. The system suitability parameters such as theoretical plates, tailing factor and resolution. Tailing factor for the peaks due to Pentazocaine and Naloxane in Standard solution should not be more than 2.0. Theoretical plates should not be less than 2000. Resolution should not be less than 2.

Method Validation

Method validation was done for the according ICH guidelines Q2 (R1). The validation parameters like linearity, specificity, accuracy, precision, LOD & LOQ and robustness[11,12].

Linearity:
For determination of linearity five different concentrations were prepared from the standard stock solution and injected in triplicate. Then plotting the graph concentration Vs peak area and measure the correlation coefficient. It should not more than 0.999.

Precision:
The standard and sample solutions were injected into the five times in intraday and inter day, the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak area.

Accuracy:
For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same. Each solution was injected three times under optimized conditions and then calculates the mean percentage recovery.

LOD & LOQ:
The sensitivity of the proposed method for measurement of Pentazocaine and Naloxone were estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ).The LOD and LOQ were calculated by using the slope and SD of response (intercept).The mean slope value and SD of response were obtained after plotting six calibration curves.

Robustness:
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

The flow rate was varied at 0.9 ml/min to 1.1ml/min:
Standard solution 1500 & 15 µg/ml of Pentazocine HCl & Naloxone HCl prepared and analysed using the varied flow rates along with method flow rate.

The Organic composition in the Mobile phase was varied from ±10%:
Standard solution 1500 & 15 µg/ml of Pentazocine HCl & Naloxone HCl was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

Degradation Studies:
The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Pentazocine HCl and Naloxone HCl using the proposed method. The standard solutions are placed in various stress conditions like acid, base, peroxide, thermal and photolytic conditions and calculate the amount of drug degraded in given stress conditions.

3. Results and discussion

System Suitability:
The system suitability of the method was checked by injecting five different preparations of the Pentazocine HCl and Naloxone HCl standard. The parameters of system suitability were checked. It was found from above data that all the system suitability parameters for developed method were within the limit. The results were shown in table 3.

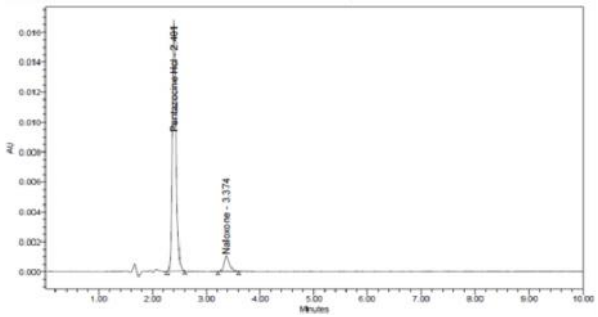

Fig 3: Chromatogram for System suitability

Assay:
Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown in table 4.

Linearity: The linearity range was found to lie from 500µg/ml to 2500µg/ml of Pentazocine HCl, 5µg/ml to 25µg/ml 0f Naloxone HCl and chromatograms are shown in table 5 and fig 4 & 5.

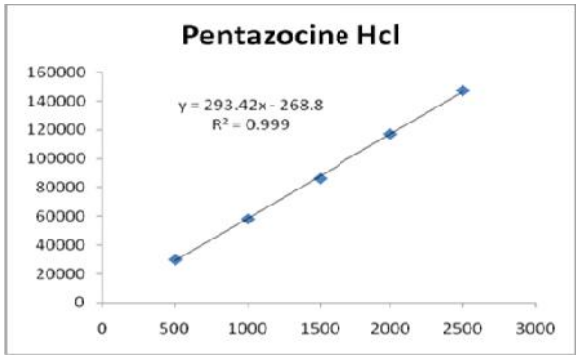

Fig 4: Calibration graph for Pentazocine HCl

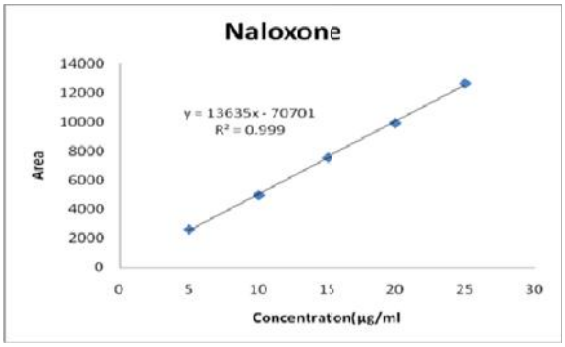

Fig 5: Calibration graph for Naloxone HCl

Precision: Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are given in table 6.

Accuracy: Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated. The data was given in table 7 & 8.

Robustness: The standard and samples of Pentazocine HCl and Naloxone HCl were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results were reported in table 10 & 11.

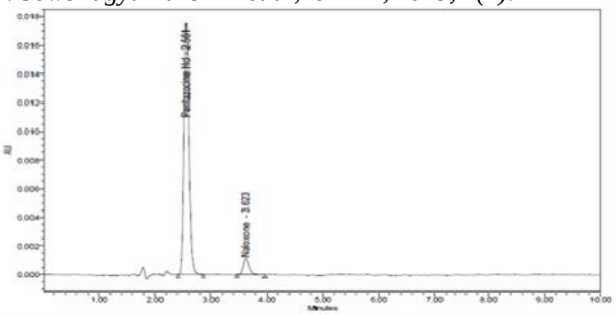

Fig 6: Chromatogram showing less flow

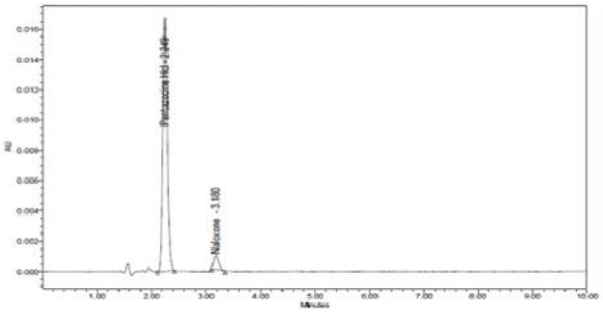

Fig 7: Chromatogram showing more flow

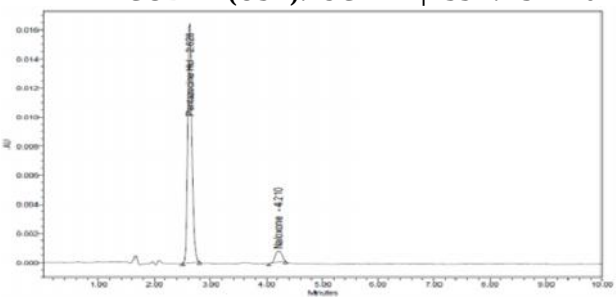

Fig 8: Chromatogram showing less organic composition

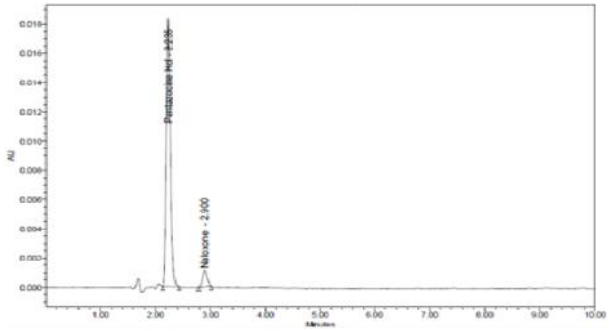

Fig 9: Chromatogram showing less organic composition

Table 3: Results of system suitability parameters

S. No	Name	RT (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Pentazocine HCl	2.401	86345	16547		1.18	4682.77
2	Naloxone HCl	3.374	7556	1033	5.75	1.20	4633.60

Table 4: Results of Assay for Pentazocine HCl and Naloxone HCl

Drug	Label Claim (mg)	% Assay
Pentazocine HCl	50	100.08
Naloxone HCl	0.5	100.04

Table 5: Area of different concentration of Pentazocine HCl and Naloxone HCl

S. No	Pentazocine HCl		Naloxone HCl	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	500	30018	5	2613
2	1000	58216	10	4969
3	1500	86174	15	7547
4	2000	117088	20	9909
5	2500	147293	25	12640

Table 6: Results of Precision for Pentazocine HCl and Naloxone HCl

Injection	Intraday precision		Inter day precision	
	Area for Pentazocaine	Area for Naloxone	Area for Pentazocaine	Area for Naloxone
Injection-1	87799	7524	86017	7508
Injection-2	86973	7519	86172	7587
Injection-3	86232	7524	86652	7576
Injection-4	87604	7581	86680	7534
Injection-5	85975	7558	86818	7558
Injection-6	87018	7565	86585	7517
Average	86933.8	7545.2	86933.8	7546.7
Std Dev	723.5	26.2	723.5	32.1
%RSD	0.8	0.3	0.8	0.4

Table 7: Accuracy (recovery) data for Pentazocine HCl

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	43148.6	250	250.20	100.08	100.43%
100%	86625.0	500	502.30	100.46	
150%	130313.3	750	755.63	100.75	

*Average of three determinations

Table 8: Accuracy (recovery) data for Naloxone HCl

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	3818.7	2.5	2.52	100.75	100.50%
100%	7587	5	5	100.08	
150%	11447	7.5	7.55	100.67	

*Average of three determinations

Table 9: Results of LOD & LOQ

Parameter	Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
LOD	Pentazocaine	58	175	3.02
	Naloxone	58	174	3.00
LOQ	Pentazocaine	58	579	9.98
	Naloxone	58	580	10.00

Table 10: Results for variation in flow for Pentazocine HCl and Naloxone HCl

Drug	Flow Rate (ml/min)	System Suitability Results		USP Resolution
		USP Plate Count	USP Tailing	
Pentazocaine	0.9	4531.39	1.20	5.90
Naloxone		4857.7	1.27	
Pentazocaine	1.0	4529.07	1.18	5.75
Naloxone		4633.60	1.20	
Pentazocaine	1.1	4072.7	1.15	5.97
Naloxone		5791.3	1.35	

* Results for actual flow (1.0ml/min) have been considered from Assay standard.

Table 11: Results for variation in mobile phase for Pentazocine HCl and Naloxone HCl

Drug	Variation in mobile phase	System Suitability Results		USP Resolution
		USP Plate Count	USP Tailing	
Pentazocaine	10 % less	4683	1.21	5.97
Naloxone		5278. 62	1.20	
Pentazocaine	Actual*	4529.07	1.18	5.75
Naloxone		4633.60	1.20	
Pentazocaine	10% more	4383	1.21	5.97
Naloxone		5201.62	1.20	

* Results for actual Mobile phase composition have been considered from Accuracy

Table 12: Degradation results for Pentazocine HCl and Naloxone HCl

Sample Name	Pentazocine HCl		Naloxone HCl	
	Area	% Degraded	Area	% Degraded
Standard	86056.0	-	7565.7	-
Acid	81872	4.86	7239	4.32
Base	81285	5.54	7298	3.54
Peroxide	82049	4.66	7267	3.95
Thermal	82411	4.24	7245	4.24
Photo	82185	4.50	7264	3.99

4. Conclusions

The estimation of Pentazocine HCl and Naloxone HCl was done by RP-HPLC. The assay of Pentazocine HCl and Naloxone HCl was performed with tablets and the % assay was found to be 100.08 and 100.04 which shows that the method is useful for routine analysis. The linearity of Pentazocine HCl and Naloxone HCl was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.3 for Pentazocine HCl and Naloxone HCl which shows that the method is precise. The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 100.43% and 100.50% for Pentazocine HCl and Naloxone HCl. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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