STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR PENTAZOCIN HYDROCHLORIDE AND NALAXONE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A new method was established for estimation of Pentazocine HCl and Naloxone HCl by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Pentazocine HCl and Naloxone HCl by using Intersil ODS C_{18} column (250×4.6mm) 5.0µm, flow rate was 1.0 ml/min, mobile phase ratio was 30% OPA buffer: 70% Methanol (pH was adjusted 3.0 with NaoH), detection wavelength was 254 nm. The instrument used was Waters HPLC, UV detector 2450, Spinchrom -software version-2. The method shows linearity between the concentration range of 500µg/ml to 2500µg/ml for Pentazocine HCl and 5µg/ml to 25µg/ml of Naloxone HCl. The % recovery of Pentazocine Hcl and Naloxone HCl were found to be in the range of 100.1 % - 100.5 %.As there was no interference due to mobile phase, the method was found to be specific. The method was robust as observed from insignificant variation in the results of analysis by changes in flow rate and wavelength variation separately and analysis being performed by different analysts.

Keywords: *Pentazocine HCl, Naloxone HCl, RP-HPLC, Validation*

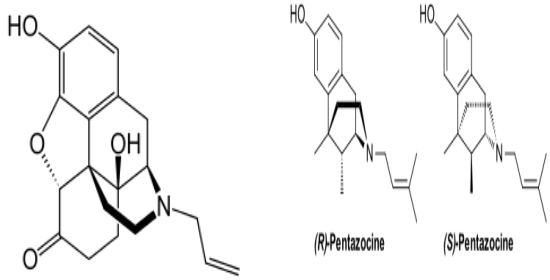
INTRODUCTION

Naloxone hydrochloride specific opiate antagonist that has no agonist activity. It is a competitive antagonist at mu, delta, and kappa opioid receptors. IUPAC name of Naloxone hydrochloride (4R,4aS,7aR,12bS)- 4a, 9 dihydroxy-3-prop-2-enyl-2,4,5,6,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2e]isoquinolin 7-one;hydrochloride [1]. While the mechanism of action of naloxone is not fully understood, the preponderance of evidence suggests that 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 [2].

The preponderance of evidence suggests that pentazocine antagonizes the opioid effects by competing for the same receptor sites, especially the opioid mu receptor. Pentazocine hydrochloride IUPAC name (1R, 9R, 13R)-1, 13-dimethyl-10-(3-methylbut-2-enyl)-10-azatricyclo [7.3.1.$0^{2,7}$] trideca-2(7),3,5-trien-4-ol;hydrochloride [3].Chemical structure of Naloxone hydrochloride and Pentazocine hydrochloride shows in figure-1

Correspondingly, this manuscript described the optimization of an isocratic RP-HPLC method for the routine quality

control analysis of naloxone hydrochloride and pentazocine hydrochloride in laboratory prepared binary mixture. In spite of that Development and optimization of isocratic RP-HPLC method is a tedious process that involves instantaneous determination of several factors. It is recognized to provide risk-based understanding of the analytical as well as major factors affecting the performance of analytical method. Furthermore, it provided thorough understanding of the possible risk and associated with interaction among the method variables, respectively [5-11]. Therefore, the aim of present study was to develop, optimize and validate sensitive and cost-effective RP-HPLC method for estimation of naloxone hydrochloride and pentazocine hydrochloride in laboratory prepared binary mixtures.



Naloxone hydrochloride **Pentazocine hydrochloride**

Figure-1: Chemical structure of Naloxone hydrochloride and Pentazocine hydrochloride.

MATERIALS and METHODS

Pentazocine HCl and Naloxone HCl bulk drugs was procured as a gift sample from spectrum lab Hyd Pvt. Ltd. OPA used for analysis was of AR Grade and Distilled Water. Waters UV-Vis (double beam) spectrophotometer was used for spectrophotometric analysis. It was connected to a personal computer having UV Probe Ver.2.10 software and provided with 1 cm quartz cells. HPLC used Waters (2695 separation module.2487 UV detector), Software: Spinchrom Detector: Prominence UV Vis detector.

Chromatographic conditions

Intersil ODS C_{18} column (250×4.6mm) 5.0µm, flow rate was 1.0 ml/min, mobile phase ratio was 30% OPA buffer: 70%Methanol (pH was adjusted 3.0 with NaOH), detection wavelength was 240 nm Injection volume was 20 µl and analysis was performed at ambient temperature.

Preparation of Phosphate buffer:

Accurately pippet out 1ml of OPA was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with NaOH

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.

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 254nm (figure-2). At this wavelength both the drugs show good absorbance.

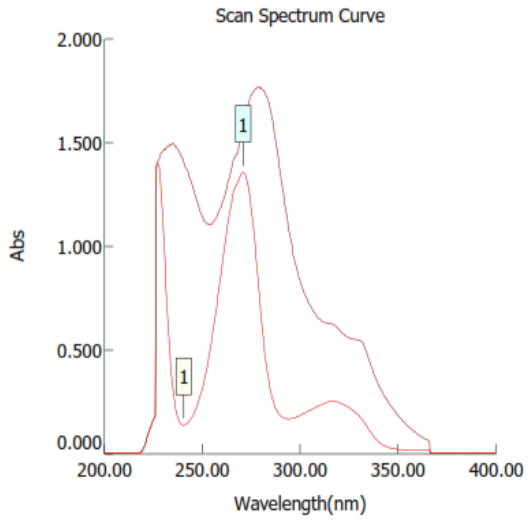


Figure-2: UV spectrum wavelength selection

Chromatographic Conditions:

Freshly prepared Buffer and acetonitrile 40:60 (v/v) adjust pH 3 were filtered through 0.45µ membrane filter and sonicate before use. Flow rate of Mobile phase was maintained at 1.5mL/min. the colume temperature was ambient temperature. The detection was carried out Dual i.e, 210 and 303nm, Injection Volume 20µL and total run time was20 min. Column was Symmetry C_{18}, 250 x 4.6 mm, 5µ particle size.

Assay procedure:

Separately inject 20µl of diluent, placebo, standard preparation (5 times) and sample preparation into the chromatographic system. Record the chromatogram and measure the peak responses. Examine the placebo chromatogram for any extraneous peaks observed in the chromatogram of sample preparation. Chromatograph the standard preparation and record the chromatograms and measure the peak responses. The tailing factor for the principal peak is not more than 2.0 and the number of the theoretical plates is not less than 5000. The % RSD (Relative Standard Deviation) is not more than 2.0. Separately inject 20 µl of standard preparation and assay preparation in the chromatograph, record the chromatograms and measure the responses for the major peaks.

Validation of proposed method:

The Proposed method was validated as per ICH guidelines [4].

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions during the analysis of basic drugs like naloxone hydrochloride and pentazocine hydrochloride, one of the well-known problems in pharmaceutical industry is peak tailing. Since these compounds strongly interact with polar ends of HPLC column packing materials, causing severe peak asymmetry and low separation efficiencies. High purity silica backbone and advances in bonding technology have alleviated the tailing problem of polar compounds in HPLC to a significant extent. During the optimization of the method, different columns (Inertsil C_{18} 150 mm×4.6 mm) and two organic solvents (acetonitrile and methanol) were tested. The chromatographic conditions were also optimized by using different buffers like phosphate, acetate and citrate for mobile phase preparation. After a series of screening experiments, it was concluded that mixer of phosphate buffers gave better peak shapes than their acetate and citrate counter parts. With methanol as solvent both the peaks shows less theoretical plates and more retention time compared to acetonitrile. The chromatographic separation was achieved on a Inertsil ODS C_{18} 150 mm×4.6 mm, 5µm column, by using a mixture of buffer and acetonitrile in proportion (55:45, v/v) as mobile phase, the detection was carried by using UV detector. At Ambient column temperature and pH 3.0 of mobile phase, the peak shape Naloxone hydrochloride

and Pentazocine hydrochloride was found symmetrical. The flow rate kept was 1 ml/min and wavelength 275 nm to achieve adequate retention time of peak (Figure 3).

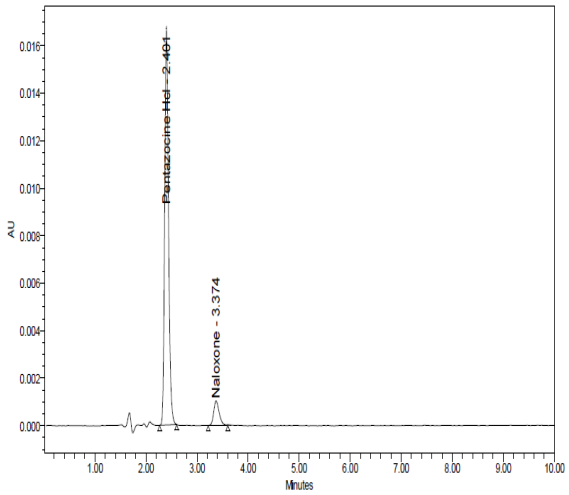


Figure-3: Optimized Chromatogram Pentazocine HCl and Naloxone HCl

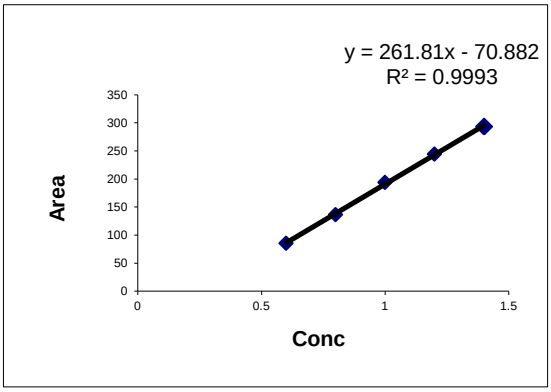
Validation of Method

Linearity and range

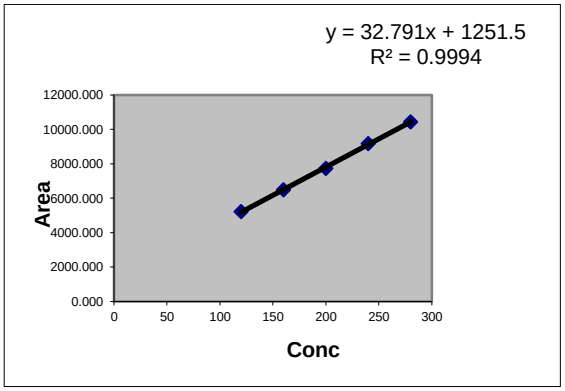
Linearity of pentazocine Hcl and naloxone HCl was observed in both methods the range of 500µg/ml to 2500µg/ml for Pentazocine Hcl and 5µg/ml to 25µg/ml 0f Naloxone Hcl Detection wavelength used was 240nm. Values are shown in table 1 and figure-4

Table 1: Linearity data

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



Naloxone HCl



Pentazocine HCl

Figure 4: Linearity graphs of Naloxone HCl and Pentazocine HCl

Precision

Intraday and Interday Precision studies on RP-HPLC and UV method for Pentazocine Hcl and Naloxone Hcl which shows the high precision % amount in between 98% to 102% indicates to analytical method that concluded [5]. Precision Values are shown in Table 2, figure-5 was shows Chromatogram of precision of Pentazocine HCl and Naloxone HCl

Table 2: Results of Precision for Pentazocine HCl and Naloxone HCl

Injection	Pentazocine HCl Peak Area	Naloxone HCl Peak Area
Injection-1	87799	7524
Injection-2	86973	7519

Injection-3	86232	7524
Injection-4	87604	7581
Injection-5	85975	7558
Injection-6	87018	7565
Average	86933.8	7545.2
Standard Deviation	723.5	26.2
%RSD	0.8	0.3

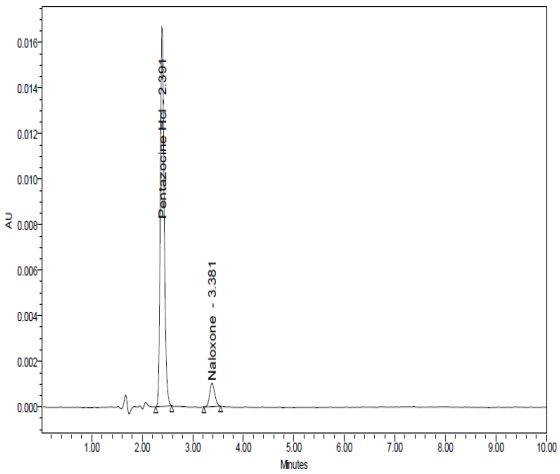


Figure-5 Chromatogram of precision of Pentazocine HCl and Naloxone HCl

Degradation Studies

Degradation studies of Pentazocine HCl and Naloxone HCl was shows in table-3 and Chromatogram of acid degradation figure-6

Table-3: 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	8128	5.54	7298	3.54
	5			
Peroxide	82049	4.66	7267	3.95
Thermal	82411	4.24	7245	4.24
Photo	82185	4.50	7264	3.99

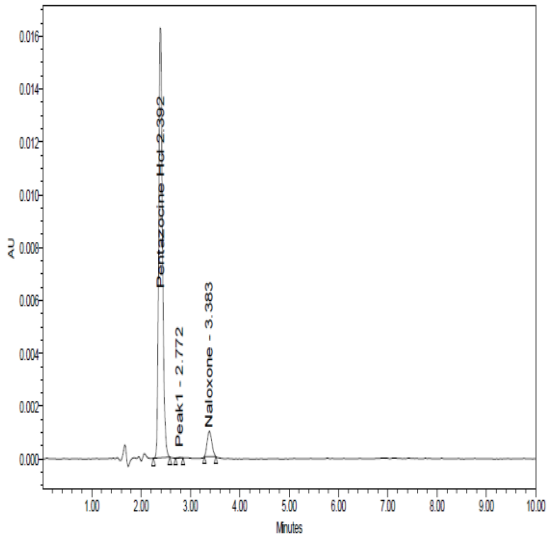


Figure-6: Chromatogram of acid degradation of Pentazocine HCl and Naloxone HCl

CONCLUSION

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of Pentazocine HCl and Naloxone HCl in new tablet formulation. The method is very simple and specific as both peaks are well separated from its impurities and excipient peaks with total runtime of 10min, which makes it especially suitable for routine quality control analysis work.

References

1. *Mostafavi A, Abedi G, Jamshidi A, Afzali D, Talebi M. Development and validation of a HPLC method for the determination of buprenorphine hydrochloride, naloxone hydrochloride and noroxymorphone in a tablet formulation. Talanta. 2009;77(4):1415-9.*

2. *Sime RL, Forehand R, Sime RJ. The crystal structure of a narcotic antagonist: naloxone hydrochloride dihydrate. Acta Crystallographica Section B: Structural Crystallography and Crystal Chemistry. 1975;31(9):2326-30.*
3. *Richards GC, Sitkowski K, Heneghan C, Aronson JK. The Oxford Catalogue of Opioids: A systematic synthesis of opioid drug names and their pharmacology. British journal of clinical pharmacology. 2021;87(10):3790-812.*
4. *Tawakkol MS, Mohamed ME, Hassan MM. Determination of naloxone hydrochloride in dosage form by high-performance liquid chromatography. Journal of liquid chromatography. 1983;6(8):1491-7.*
5. *Tawakkol MS, Mohamed ME, Hassan MM. Determination of naloxone hydrochloride in dosage form by high-performance liquid chromatography. Journal of liquid chromatography. 1983;6(8):1491-7.*
6. *Sams RA, Malspeis L. Determination of naloxone and naltrexone as perfluoroalkyl ester derivatives by electron-capture gas-liquid chromatography. Journal of Chromatography A. 1976 ;125(2):409-20.*
7. *Goldfrank L, Weisman RS, Errick JK, Lo MW. A dosing nomogram for continuous infusion intravenous naloxone. Annals of emergency medicine. 1986;15(5):566-70.*
8. *Kelly JW, Stewart JT, Blanton CD. HPLC separation of pentazocine enantiomers in serum using an ovomucoid chiral stationary phase. Biomedical Chromatography. 1994;8(5):255-7.*
9. *Liu SY, Woo SO, Koh HL. HPLC and GC–MS screening of Chinese proprietary medicine for undeclared therapeutic substances. Journal of pharmaceutical and biomedical analysis. 2001;24(5-6):983-92.*
10. *Liu SY, Woo SO, Koh HL. HPLC and GC–MS screening of Chinese proprietary medicine for undeclared therapeutic substances. Journal of pharmaceutical and biomedical analysis. 2001;24(5-6):983-92.*
11. *Noggle Jr FT. Liquid chromatographic analysis of pentazocine and tripelennamine in combination. Journal of liquid chromatography. 1983;6(11):2005-17.*