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ORIGINAL RESEARCH ARTICLE

Analytical Method Development and Validation for the Simultaneous Estimation of Buprenorphine Hydrochloride and Naloxone Hydrochloride in Pharmaceutical Dosage Forms By RP-HPLC

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ABSTRACT

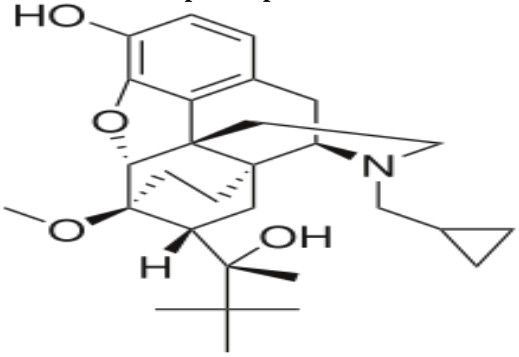
A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Buprenorphine hydrochloride and Naloxone hydrochloride in pharmaceutical dosage forms. Isocratic elution at a flow rate of 1ml min^{-1} was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Water:Methanol:0.1% Ortho phosphoric acid 60:20:20(v/v/v). The UV detection wavelength was at 273 nm. The retention time for Buprenorphine hydrochloride was 3.85 min and Naloxone hydrochloride was 5.85. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Buprenorphine hydrochloride and Naloxone hydrochloride in pharmaceutical dosage forms.

Key words: Buprenorphine hydrochloride and Naloxone hydrochloride, HPLC, Development, 273nm.

INTRODUCTION

Buprenorphine hydrochloride is a Schedule V synthetic opiate partial agonist with analgesic and opiate antagonist activities. Numerous studies have supported the safety and efficacy of buprenorphine or buprenorphine with naloxone for the treatment of opioid dependence. Currently, buprenorphine is only available commercially as a parenteral injection (0.3 mg/mL). Reckitt and Benckiser Pharmaceuticals has submitted a New Drug Application to use buprenorphine products in the treatment of opiate or opioid dependence, and the application is in the final stages of FDA review.

Fig 1: Structure of Buprenorphine



Buprenorphine hydrochloride bind to opiate receptors in CNS, altering perception of and response to painful stimuli while causing generalized CNS depression. Also has partial antagonist properties, which may lead to opioid withdrawal effects in patients with physical drug dependence.

Buprenorphine hydrochloride exerts its analgesic effect via high affinity binding to μ subclass opiate receptors in the central nervous system. Although buprenorphine hydrochloride may be classified as a partial agonist, under the conditions of recommended use it behaves very much like classical μ agonists such as morphine. One unusual property of buprenorphine hydrochloride observed in in-vitro studies is its very slow rate of dissociation from its receptor. This could account for its longer duration of action than morphine, the unpredictability of its reversal by opioid antagonists, and its low level of manifest physical dependence.

The most severe and serious adverse reaction associated with opioid use in general is respiratory depression, the mechanism behind fatal overdose. Buprenorphine behaves

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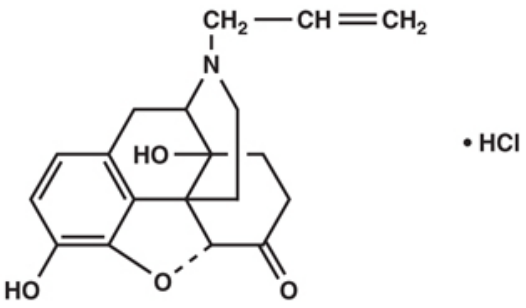
differently than other opioids in this respect, as it shows a ceiling effect for respiratory depression. Moreover, former doubts on the antagonisation of the respiratory effects by naloxone have been disproved: Buprenorphine effects can be antagonised with a continuous infusion of naloxone. Concurrent use of buprenorphine and CNS depressants (such as alcohol or benzodiazepines) is contraindicated as it may lead to fatal respiratory depression. Benzodiazepines, in prescribed doses, are not contraindicated in individuals who are tolerant to either opioids or benzodiazepines.

Naloxone hydrochloride is an opioid antagonist, is a synthetic congener of oxymorphone. In structure it differs from oxymorphone in that the methyl group on the nitrogen atom is replaced by an allyl group. Chemically it is known as 17-allyl-4,5α-epoxy,3-14-dihydroxymorphinan-6-one hydrochloride. It has a molecular weight of 363.84. Naloxone hydrochloride occurs as a white to slightly off-white powder, and is soluble in water, in dilute acids, and in strong alkali; slightly soluble in alcohol; practically insoluble in ether and in chloroform.

Naloxone hydrochloride injection is available as a sterile solution for intravenous, intramuscular, and subcutaneous administration. Each mL contains 0.4 mg of naloxone hydrochloride. Each mL contains 8.9 mg of sodium chloride. The pH is adjusted between 3.0 to 6.5 with hydrochloric acid or sodium hydroxide. The air in the cartridges has been displaced by nitrogen gas.

Fig 2: Naloxone hydrochloride



The precise mechanism by which naloxone reverses most of the effects of opioid analgesics has not been fully determined. It has been proposed that there are multiple subtypes of opioid receptors within the central nervous system (CNS), each mediating different therapeutic and/or side effects of opioid drugs. At least two of these types of receptors (mu and kappa) mediate analgesia as well as side effects. A third type of receptor (sigma) may not mediate analgesia; actions at this receptor may produce the subjective and psychotomimetic effects characteristic of opioids with mixed agonist/antagonist activity (i.e., butorphanol, nalbuphine, and pentazocine). Naloxone apparently displaces previously administered opioid analgesics from all of these types of receptors and competitively inhibits their actions. Antagonism of opioid actions may precipitate withdrawal symptoms in patients who are physically dependent on opioid drugs (except for buprenorphine). Naloxone has no opioid agonist activity of its own.

Naloxone hydrochloride injection is indicated for the complete or partial reversal of opioid depression, including respiratory depression, induced by natural and synthetic opioids including propoxyphene, methadone, and certain mixed agonist-antagonist analgesics: nalbuphine, pentazocine, butorphanol, and cyclazocine.

Naloxone hydrochloride is also indicated for the diagnosis of suspected or known acute opioid overdosage. Naloxone hydrochloride injection may be useful as an adjunctive agent to increase blood pressure in the management of septic shock.

Experimental

Chemicals and Reagents

All HPLC solvents used like Acetonitrile, ammonium acetate which are of HPLC grade were purchased from E.Merck,

Instrumentation and Analytical conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Gemini C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with, Water:Methanol:0.1% Ortho phosphoric acid 60:20:20(v/v/v) was selected with a flow rate of 1ml min^{-1}. The detection wavelength was set at 273nm with a runtime of 10 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through

the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock, working standard solutions and Sample solutions

100mg of Buprenorphine hydrochloride and Naloxone hydrochloride was weighed separately and transferred (working standard) into a 100ml volumetric flask. The diluent methanol was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 1ml of the above stock solution was pipette into a 10ml volumetric flask and diluted up to the mark with diluent. The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 2 to 12ppm working standard solutions. Calibration solutions were prepared and analyzed immediately after preparation.

The formulation tablets of Buprenorphine hydrochloride and Naloxone hydrochloride were crushed to give finely powdered material. Powder equivalent to 10 mg of drug was taken in 10 ml of volumetric flask containing 5 ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 6 ppm.

Fig 3: Standard chromatogram of Buprenorphine hydrochloride and Naloxone hydrochloride

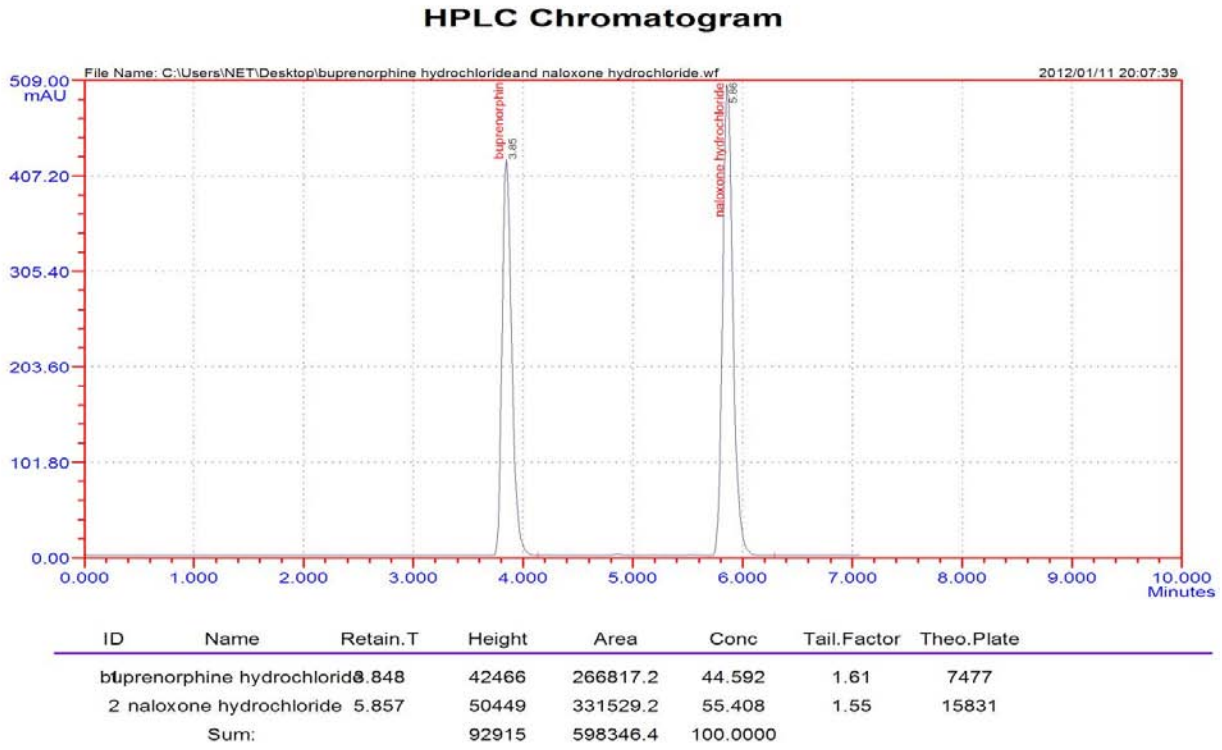


Table 1: Chromatographic conditions

S.No	Test H.P.L.C Conditions	Result
1	Elution	Isocratic
2	A.P.I Concentration	6 ppm
3	Mobile Phase	Water: Methonal: 0.1% Ortho phosphoric acid 60:20:20(v/v/v).
4	P^H	4.4
5	Column	C18
6	Wave Length	273 nm
7	Flow	1 ml\min
8	Runtime	10 mins
9	Retention Time	Buprenorphine hydrochloride 3.85 Naloxone hydrochloride 5.85
10	Area	Buprenorphine hydrochloride: 266817 Naloxone hydrochloride: 331529
11	Tailing Plates	Buprenorphine hydrochloride: 7477 Naloxone hydrochloride: 15831
12	Tailing Factor	Buprenorphine hydrochloride: 1.61 Naloxone hydrochloride: 1.55
13	Pump Pressure	11.9 psi

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

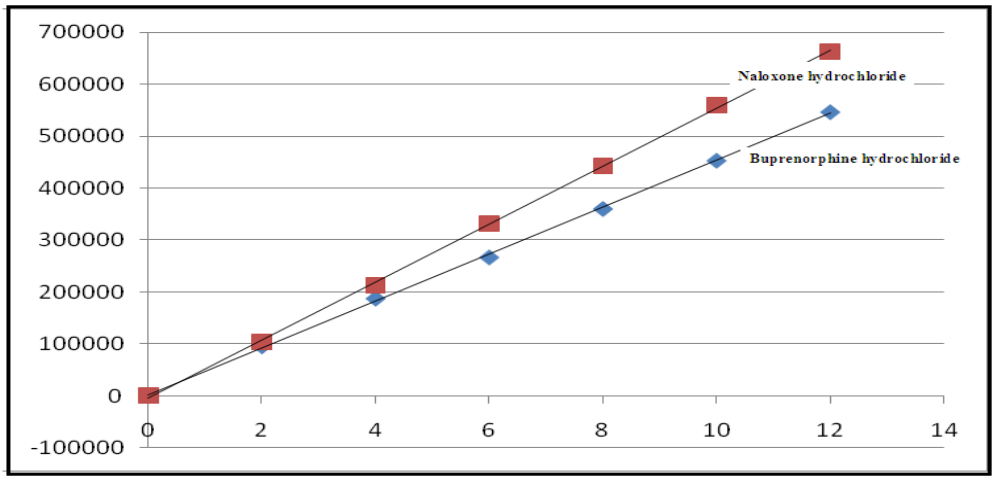
Linearity

The developed method has been validated as per ICH guidelines (Zucman D, 2007). Working standard solutions of Buprenorphine hydrochloride and Naloxone hydrochloride in the mass concentration range of 2 ppm to 12 ppm was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Buprenorphine hydrochloride and Naloxone hydrochloride was obtained by plotting the peak area ratio versus the applied concentrations. The linear correlation coefficient was found to be 0.999.

Table 2: Linearity of Buprenorphine hydrochloride and Naloxone hydrochloride

S.No	Conc (PPM)	Area of Buprenorphine HCl	Area of Naloxone HCl
1	2	95393	103687
2	4	187365	212368
3	6	266817	331529
4	8	360262	442364
5	10	453287	559743
6	12	546872	663272
Correlation coefficient:		0.9998	0.9998
Slope:		45166	55927
Intercept:		1866	-5140

Fig 4: Calibration curve of Buprenorphine hydrochloride and Naloxone hydrochloride



Precision

Repeatability of the method was checked by injecting replicate injections of 6 ppm of the solution for six times on the same day as intraday precision study of Buprenorphine hydrochloride and Naloxone hydrochloride and the RSD was found to be for 0.3 Buprenorphine hydrochloride and 0.32 for Naloxone hydrochloride

Table 3: Precision parameters of Buprenorphine hydrochloride and Naloxone hydrochloride

Injection	Concentration (ppm)	Buprenorphine HCl	Naloxone HCl
1	6	266817	331529
2	6	266975	331168
3	6	265871	331157
4	6	266813	328961
5	6	265169	331728
6	6	266820	330165
	RSD	0.3	0.32

Accuracy

The accuracy of the method was determined by calculating recovery of Buprenorphine hydrochloride and Naloxone hydrochloride by the method of standard addition. Known amount of Buprenorphine hydrochloride and Naloxone hydrochloride (2ppm, 4ppm and 8ppm) was added to a pre quantified sample solution and the amount of Buprenorphin e hydrochloride and Naloxone hydrochloride

were estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Buprenorphine hydrochloride and Naloxone hydrochloride was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery was calculated and the average recovery was found to be 99.575 for Buprenorphine hydrochloride and 99.7 for Naloxone hydrochloride .

Table 4 :Recovery results

Recovey	Conc. of sample	Recovery of Buprenorphine HCl	Recovery of Naloxone HCl	% Recovery of Buprenorphine HCl	% of recovery of Naloxone HCl
50%	2 ppm	1.989	1.978	99.45	99.05
100%	4 ppm	3.991	3.982	99.775	99.55
150 %	8 ppm	7.96	8.04	99.5	100.5

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as per ICH guide-lines. Results are shown in Table 5.

Table 5: Results of LOD and LOQ.

Parameter	Measured for Buprenorphine HCl	Measured for Naloxone HCl
LOD	0.02ppm	0.005ppm
LOQ	0.06ppm	0.017ppm

Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied. Results of Robustness are shown in Table 6.

Table 6: Robustness results

Parameter	Modification	Peak Area of Buprenorphine	Peak Area of Naloxone	% of change Buprenorphine	% of change Naloxone
Standard	No change	266817	331529	…….	……
M.PHASE	Water: Methonal: 0.1% Ortho phosphoric acid 65:15:20(v/v/v).	265921	332319	0.34	0.24
P^H	5.2	264547	330975	0.85	0.18
Wavelength	279 nm	263265	335871	1.33	1.31

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drugs Buprenorphine hydrochloride and Naloxone hydrochloride preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the Water:Methonal were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Water:Methanol:0.1% Ortho phosphoric acid 60:20:20(v/v/v). The retention time of Buprenorphine hydrochloride was 3.85 and Naloxone hydrochloride was found to be 5.86 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability and validation parameters are given in Table 7. The average recovery of Buprenorphine hydrochloride was found to be 99.575 and Naloxone hydrochloride was found to be 99.7 indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Buprenorphine hydrochloride and Naloxone hydrochloride in tablet formulation. The result for Buprenorphine hydrochloride and Naloxone hydrochloride was comparable with a corresponding labeled amount. The absence of

additional peaks indicates no interference of the excipients used in the tablets.

Table 8: Formulation results of Buprenorphine hydrochloride and Naloxone hydrochloride

Formulation	Dosage	Sample concentration	%Estimation of Buprenorphine HCl
SUBOXONE (Tablet)	8 mg Buprenorphine	6 ppm	99.94% (Buprenorphine HCl**)**
SUBOXONE (Tablet)	2 mg Naloxone	6 ppm	99.87% (Naloxone HCl)

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Buprenorphine hydrochloride and Naloxone hydrochloride in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Buprenorphine hydrochloride and Naloxone hydrochloride in pharmaceutical dosage form.

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