Method Development and Validation of Simultaneous Estimation of Buprenorphine and Naloxone in Bulk and Pharmaceutical Dosage Forms by RP-HPLC Method

K Niharika[1], Dr. P V Madhavi Latha[2], K Suvarna[3], Dr. P Uma Devi[4]
[1,2,3,4]*Viswanadha Institute of Pharmaceutical Sciences*

***Abstract*—A rapid, sensitive, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous quantification of Buprenorphine and Naloxone using a Waters HPLC system. Chromatographic separation was achieved on an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm particle size) maintained at ambient temperature. The mobile phase consisted of methanol and acetonitrile in the ratio of 85:15 (v/v), which was filtered through a 0.45 µm membrane filter prior to use. The flow rate was maintained at 1.0 mL/min, and detection was carried out at 247 nm using the PDA detector.**

***Index Terms*—Buprenorphine, and Naloxone, RP-HPLC.**

I. INTRODUCTION

Suboxone® is a combination drug containing buprenorphine and naloxone. Buprenorphine (13-14) acts as a partial agonist at the μ-opioid receptor and an antagonist at the κ-opioid receptor. It exhibits a high affinity for the μ-opioid receptor but has lower intrinsic activity compared to full μ-opioid agonists such as heroin, oxycodone, or methadone. This property allows buprenorphine to alleviate withdrawal symptoms and cravings in opioid-dependent individuals while reducing the risk of respiratory depression and abuse.

Naloxone, on the other hand, is a pure opioid antagonist. It is included in the formulation to deter misuse; when taken sublingually as prescribed, naloxone (15-16) has minimal systemic absorption and therefore little clinical effect. However, if the combination is misused parenterally (e.g., injected), naloxone precipitates opioid withdrawal by blocking opioid receptor activity.

In opioid-naïve patients (those who have not taken opioids), Suboxone® produces minimal euphoric or analgesic effects due to buprenorphine's partial agonist nature and ceiling effect on receptor activation.

II. MATERIALS AND METHODS

Preparation of Stock solution: 100 mg of Buprenorphine and 100 mg of Naloxone API standards were accurately weighed and are transferred into two separate 100 ml volumetric flasks and dissolved in mobile phase, then sonicated for 20 minutes to obtain 1000µg/ml.

Preparation of working standard solution: From the above standard stock solution, 4 ml from each solution was pipetted out and transferred into 100ml volumetric flasks, made upto the volume with mobile phase to get 40µg/ml of Buprenorphine and Naloxone.

III. RESULTS AND DISCUSSION

Method validation: Validation parameters include specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness and assay (1-6).

Specificity: Specificity is the ability to assessing equivocally the analyte in the presence of components which may be expected to be present. Typically, these components include impurities, degradants, matrix etc. Blank solution and standard solutions of Buprenorphine (40µg/ml) and Naloxone (40µg/ ml) were injected into the HPLC system. The peak purity data of Buprenorphine and Naloxone were compared. There should not be any interference at the retention time of the main peaks.

Linearity: Linearity for the drugs Buprenorphine and Naloxone (17-27) was determined by preparing the standard solutions at six concentrations levels in six replicates in the range of 20-70µg/ml Buprenorphine and 20-70µg/ml for and Naloxone from stock solution. The linearity charts of Buprenorphine and Naloxone was shown in the figure no 2&3. The correlation coefficient was found to be 0.9998 and 0.9999 for Buprenorphine and Naloxone respectively. Linearity results were tabulated in table 2.

Accuracy: Accuracy was performed by spiking known amounts of standard solution to sample solution at three different concentrations levels (50%, 100%, 150%) and there by analyzed for %RSD which should not be more than 2.0. The % recovery was calculated and the results was reported in table no. 3 & 4.

Precision: The precision (7-12) of the analytical method was studied by injecting six replicates of standard containing 40µg/ml of Buprenorphine and 40µg/ml of Naloxone which were injected into HPLC system. The % RSD was calculated and the results were reported in the table no.5 & 6.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection was defined as the concentration which yields a signal - to – noise ratio 3:1 whereas the limit of quantification was calculated to be the lowest concentration that could be measured with signal - to – noise ratio10:1. LOD and LOQ were calculated from slope and standard deviation. The results were tabulated in table no. 7.

Robustness: The smallest deliberate changes in method like change in flow rate are made but there were no predictable changes in the results and are in the range as per ICH guidelines. Conditions like decrease in flow rate (0.8 ml/min), increase in flow rate (1.2 ml/min) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was found to be within the limits and results were tabulated in table no.8.

Assay: Assay was conducted on marketed formulation and mean % assay was found. The results were tabulated in table no. 9.

Table1: Optimised Chromatographic conditions

Parameters	Method
Stationary Phase(column)	Inertsil -ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol and Acetonitrile
Flow rate (ml/min)	1.0 ml/min
Run time(minutes)	8 min
Temperature in the	Ambient
Volume of	20
Wavelength of	247 nm
Drug RT (min)	3.213 min for Buprenorphine and 5.031 for Naloxone.

Figure 1: Optimized chromatogram

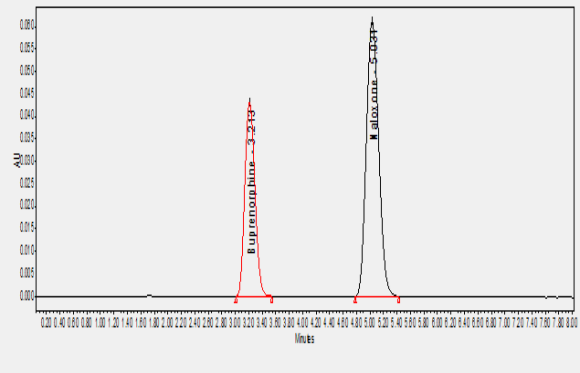

Table 2: Linearity data of Buprenorphine and Naloxone

Buprenorphine		Naloxone	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
20	230828	20	386907
30	357053	30	581164
40	469514	40	779814
50	596111	50	986354
60	719969	60	1181597
70	825875	70	1374407

Figure 2: Calibration Curve of Buprenorphine

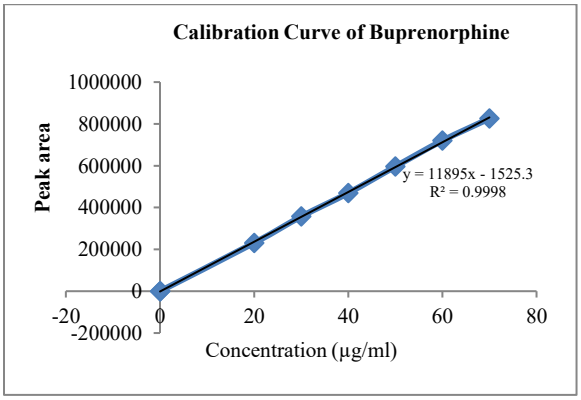

Figure 3: Calibration Curve of Naloxone

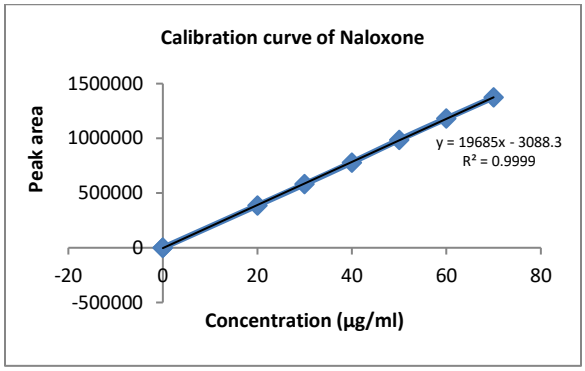

Table 3: Accuracy data of Buprenorphine

Concentration	Amount added	Amount found	% Recovery	Statistical Analysis of % Recovery
% of spiked level	(ppm)	(ppm)		
50% - 1	20	19.92	99.78	99.87
50% - 2	20	19.96	99.89	
50% - 3	20	19.88	99.78	0.68
100 % - 1	40	39.94	98.92	99.84
100 % - 2	40	39.86	99.75	
100% - 3	40	39.76	99.08	0.657
150% - 1	60	59.97	99.96	100.07
150% - 2	60	60.02	100.08	
150% - 3	60	60.01	100.01	0.345

Table 4: Accuracy data of Naloxone

Concentration	Amount added	Amount found	% Recovery	Statistical Analysis of % Recovery
% of spiked level	(ppm)	(ppm)		
50% - 1	20	19.89	99.87	99.97
50% - 2	20	19.72	99.87	
50% - 3	20	20.08	100.03	0.874
100 % - 1	40	39.92	99.88	99.97
100 % - 2	40	40.01	100.07	
100% - 3	40	40.05	100.08	0.687
150% - 1	60	59.95	98.87	99.94
150% - 2	60	59.97	99.88	
150% - 3	60	59.98	99.98	0.97

Table 5: System Precision data of Buprenorphine and Naloxone

S. No	Buprenorphine	Naloxone
1	474086	779124
2	475127	779053
3	473951	779324
4	473672	779381
5	474236	779182
Mean	474214.4	779212.8
SD	550.7652	139.9559
% RSD	0.116143	0.017576

Table 6: Method Precision data of Buprenorphine and Naloxone

S. No	Buprenorphine	Naloxone
1	470326	779204
2	471012	779637
3	472294	779084
4	470214	779247
5	470857	779134
6	472235	779352
Mean	471156.3	779276.3
SD	910.5696	199.5742
% RSD	0.193263	0.02561

Table 7: LOD and LOQ data of Buprenorphine and Naloxone

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
Buprenorphine	0.34	1.03
Naloxone	0.19	0.59

Table 8: Robustness data of Buprenorphine and Naloxone

S No	Drug Name	Condition	Peak area	% RSD
1	Buprenorphine	Decreased Flow rate of 0.8 ml/min	469387	0.052
2		Increased Flow rate of 1.2 ml/min	471343	0.877
3	Naloxone	Decreased Flow rate of 0.8 ml/min	778524	0.042
4		Increased Flow rate of 1.2 ml/min	780462	0.022

Table 9: Assay data Buprenorphine and Naloxone

S. No	Peak area of Buprenorphine	% Assay	Peak area of Naloxone	% Assay
1	470326	99.38	779204	99.41
2	471012		779637	
3	472294		779084	
4	470214		779247	

IV. CONCLUSION

The developed RP-HPLC method was validated as per ICH guidelines. All the system suitability parameters were within the range as stated by ICH guidelines. Interference peaks were not observed in blank, standard and sample chromatogram. Hence simple, precise and accurate, sensitive, specific and robust method was developed and validated. This can be used in quality control department with respect to routine analysis.

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