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

Analytical Method Development and Validation For the Simultaneous Estimation of Buprenorphine and Naloxone By RP- HPLC Method

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A B S T R A C T

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Buprenorphine and Naloxone was done by RP-HPLC. The Phosphate buffer was p^H3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Buprenorphine and Naloxone were found to be from 100-500 µg/mlof Buprenorphine and 1-5µg/ml of Naloxone . Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Buprenorphine and naloxone . LOD and LOQ were found to be within limit.The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Key words: Methanol: Phosphate buffer,Inertsil C_{18} column,Buprenorphine and Naloxone

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Buprenorphine, sold under the brand name Subutex, among others, is an opioid used to treat opioid addiction, acute pain, and chronic pain. It can be used under the tongue, by injection, as a skin patch, or as an implant. When used for opioid addiction it is recommended that a health care provider observe the person while they take the medication. For longer term treatment of addiction a combination formulation of buprenorphine/naloxone is recommended in some countries. Maximum pain relief is generally within an hour with effects up to 24 hours. Side effects may include respiratory depression (decreased breathing), sleepiness, adrenal insufficiency, QT prolongation, low blood pressure, allergic reactions, and opioid addiction. Among those with a history of seizures, there is a risk of further seizures. Opioid withdrawal following stopping is generally mild. It is unclear if use during pregnancy is safe and use while breastfeeding is not recommended. Buprenorphine affects different types of opioid receptors in different ways. Depending on the type of receptor it may be an agonist, partial agonist, or antagonist.

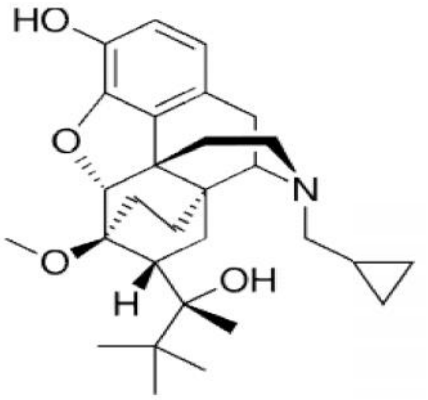

Fig 1: Structure of Buprenorphine

Naloxone, sold under the brand name Narcan, among others, is a medication used to block the effects of opioids, especially in overdose. Naloxone may be combined with an opioid (in the same pill) to decrease the risk of misuse. When given intravenously, naloxone works within two minutes, and when injected into a muscle, it works within five minutes; it may also be sprayed into the nose. The effects of naloxone last about half an hour to an hour. Multiple doses may be required, as the duration of action of most opioids is greater than that of naloxone. Administration to opioid-dependent individuals may cause symptoms of opioid withdrawal, including restlessness, agitation, nausea, vomiting, a fast heart rate, and sweating. To prevent this, small doses every few minutes can be given until the desired effect is reached.[1] In those with previous heart disease or taking medications that negatively affect the heart, further heart problems have occurred. It appears to be safe in pregnancy, after having been given to a limited number of women. Naloxone is a non-selectiveand competitive opioid receptor antagonist. It works by reversing the depression of the central nervous system and respiratory system caused by opioids.

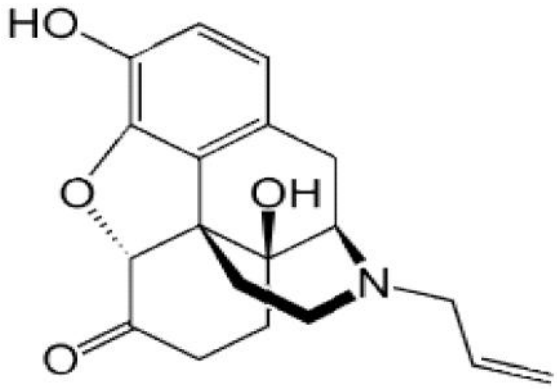

Fig 2: Structure of Naloxone

2. Materials and Methods

Chemicals:

Water and Methanol for HPLC,Acetonitrile for HPLC,Ortho phosphoric Acid,KH2PO4, Methanol,Ortho phosphoric acid,Potassium dihydrogen ortho phosphate,Tri ethyl amine,Water.

Instrumentation: HPLC-auto sampler –UV detector, Separation module2695, UV. Detector 2487, Empower-software version-2, Waters, U.V double beam spectrometer, UV 3000+, U.V win software, Lab India.

Chromatographic conditions

Table No 1: Optimized chromatographic conditions

Parameter	Description
Flow rate	0.8 ml/min
Column	Inertsil C18 Column(250mm x 4.6mm)5µm.
Mobile phase	Phosphate buffer pH 3.0: Methanol (30:70%v/v)
Detector	PDA
Column temperature	Ambient
Sample temperature	Ambient
Wavelength	260 nm
Injection volume	10µl

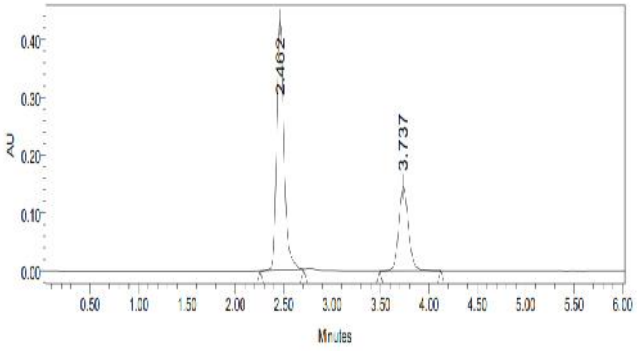

Fig 3: Optimized Chromatogram

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Buprenorphine and Naloxone 10mg of working standard into a 10mL& 100ml

clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3ml& 0.3ml 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 10 mg ofBuprenorphine and Naloxone (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3 ml of Buprenorphine and Naloxone of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

Precision:

Accurately weigh and transfer 25 mg of Buprenorphine and Naloxoneworking standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Accuracy:

Accurately weigh and transfer 10 mg of Buprenorphine and Naloxone 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Linearity:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg ofBuprenorphine and Naloxone (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Detection:

Accurately weigh and transfer 10 mg of Buprenorphine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Quantification:

Accurately weigh and transfer 10 mg of Buprenorphine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

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.

3. Results and Discussions

Linearity:

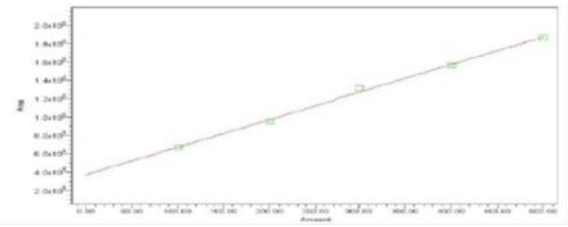

Fig 4: Calibration graph for Buprenorphine

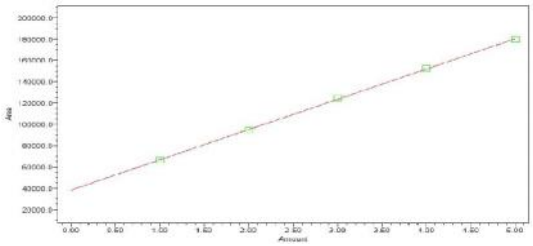

Fig 5: Calibration graph for Naloxone

Robustness:

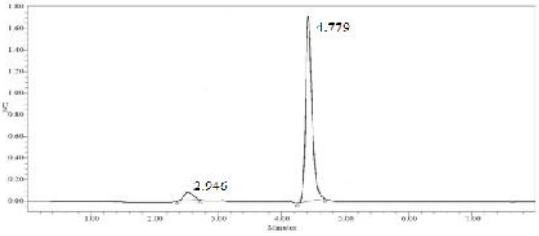

Fig 6: Chromatogram snowing less flow of 0.6ml/min

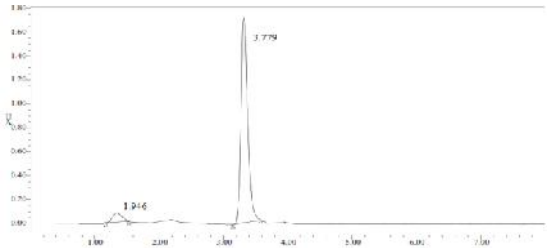

Fig 7: Chromatogram snowing more flow of 1.0ml/min

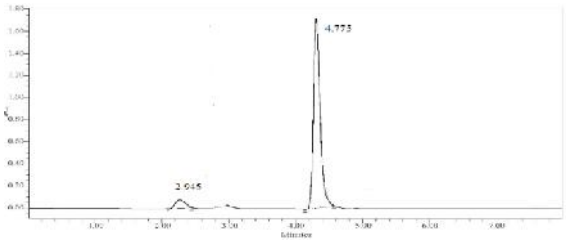

Fig 8: Chromatogram showing less organic composition

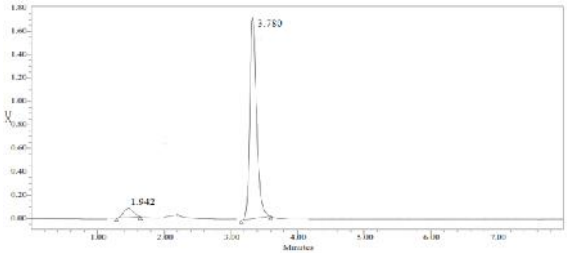

Fig 9: Chromatogram showing more organic composition

Table No 2: Results of system suitability parameters for Buprenorphine and Naloxone

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Buprenorphine**	2.5	124505	213642		1.2	4673.4
2	**Naloxone**	3.9	1308495	154566	6 0	1.3	6090.3

Table No 3: Results of method precession for B

Injection	Bupernorphine	Naloxane
	Area	
Injection-1	1302729	123149
Injection-2	1302947	123766
Injection-3	1303236	124271
Injection-4	1303977	124691
Injection-5	1309759	124956
Average	1304529.8	124162.7
Standard Deviation	2961.1	725.6
%RSD	0.2	0.6

Uprenorphine and Naloxone

Table No 4: Results of Intermediate precision for Buprenorphine and Naloxone

Injection	Buprenorphine	Naloxone
	Area	
Injection-1	1300148	122487
Injection-2	1304520	122626
Injection-3	1305937	122632
Injection-4	1306476	122702
Injection-5	130871	122962
Average	1305070.2	122681.8
Standard Deviation	3061.8	174.8
%RSD	0.2	0.1

Table No 5: Accuracy (recovery) data for Buprenorphine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table No 6: Accuracy (recovery) data for Naloxone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table No 7: Area of different concentration of Buprenorphine

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table No 8: Area of different concentration of Naloxone

S.No	Linearity Level	Concentration	Area
1	I	1ppm	66510

2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

Table No 9: Analytical performance parameters of Buprenorphine and Naloxone

Parameters	Buprenorphine	Naloxone
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria: Correlation coefficient (R2) should not be less than 0.999

Table No 10: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Buprenorphine	52	152	2.9
Naloxone	52	156	3

Table No 11: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Buprenorphine	52	522	10.03
Naloxone	52	524	10.1

Table No 12: Flow Rate (ml/min) data for Buprenorphine

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table No 13: Flow rate (ml/min) data for Naloxone

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table No 14: Change in Organic Composition in the Mobile Phase for Buprenorphine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table No 14: Change in Organic Composition in the Mobile Phase for Naloxone

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Buprenorphine and Naloxone was done by RP-HPLC. The Phosphate buffer was pH3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C18 column C18 (4.6 x 150mm, 5mm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Buprenorphine and Naloxone were found to be from 100-500 µg/ml of Buprenorphine and 1-5mg/ml of Naloxone.

Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Buprenorphine and naloxone . LOD and LOQ were found to be within limit.The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

5. References

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