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**R E S E A R 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 $p^H$ 3.0 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/ml of 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. For opioid addiction it is typically only started when withdrawal symptoms have begun and for the first two days of treatment under direct observation of a health care provider. For longer term treatment of addiction a combination formulation of buprenorphine/naloxone is recommended to prevent misuse by injection. Maximum pain relief is generally within an hour with effects up to 24 hours.

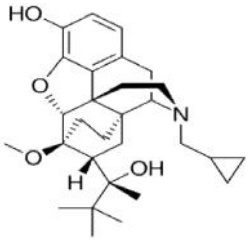


**Fig 1:** Structure of Buprenorphine

Naloxone, sold under the brandname 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.

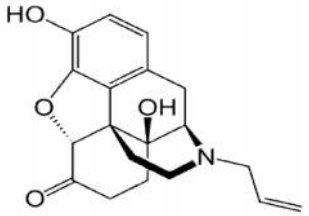


**Fig 2:** Structure of Naloxone

## 2. Materials and Methods

**Chemicals**

Buprenorphine and Naloxone, KH2PO4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

**Instrumentation**

HPLC Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. UV/VIS spectrophotometer LABINDIA UV 3000+pH meter, Adwa – AD 1020 Weighing machine.

**Chromatographic conditions**

| | |
|---|---|
| Column | :InertsilC18(4.6*250mm) 5µm |
| Mobile phase ratio | :Phosphate buffer pH 3.0: Methanol (30:70%v/v) |
| Detection wavelength | : 260 nm |
| Flow rate | : 0.8 ml/min |
| Injection volume | :10µl |
| Column Temperature | :Ambient |
| Auto sampler temp | :Ambient |

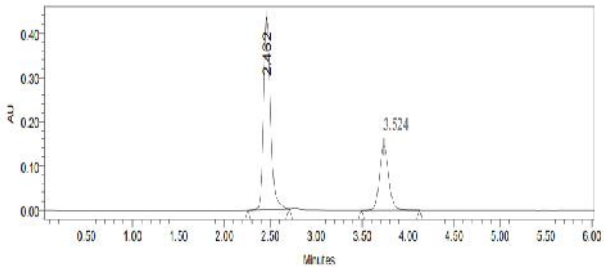


**Fig 3:** Optimized Chromatogram Buprenorphine and Naloxone

**Observation:** The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

**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 of Buprenorphine 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 Naloxone 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.

**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 of Buprenorphine 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 Detection:**

Accurately weigh and transfer 10mg of Naloxone working standard into a 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.

**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.

**Limit of Quantification:**

Accurately weigh and transfer 10mg of Naloxone working standard into a 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.

**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 Discussion

**Linearity:**

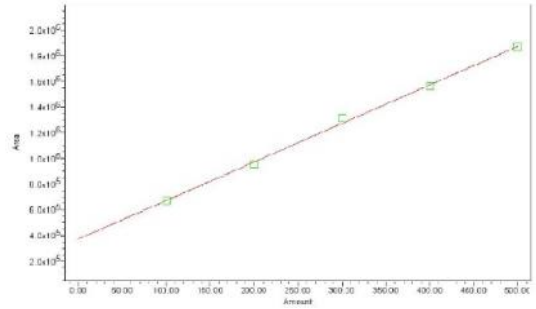

**Fig 4:** Calibration graph for Buprenorphine at 260 nm

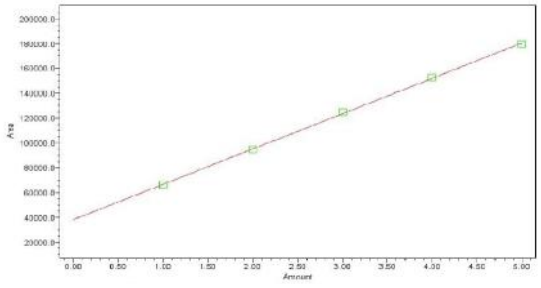

**Fig 5:** Calibration graph for Naloxone at 260 nm

The standard and samples of Buprenorphine and Naloxone 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.

**Variation in Flow:**

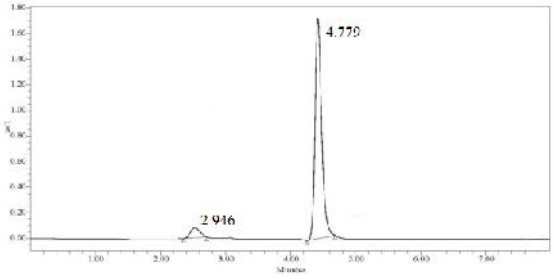


**Fig 6:** Chromatogram snowing less flow of 0.6ml/min

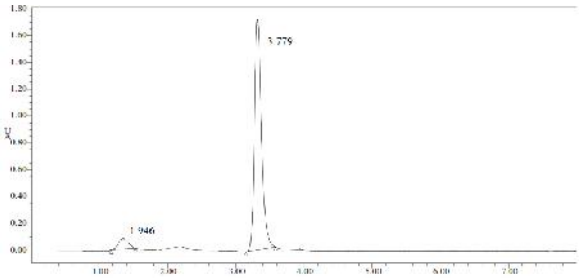


**Fig 7:** Chromatogram snowing more flow of 1.0ml/min

**Mobile Phase:**

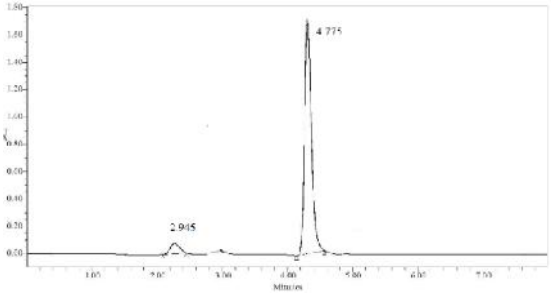


**Fig 8:** Chromatogram showing less organic composition

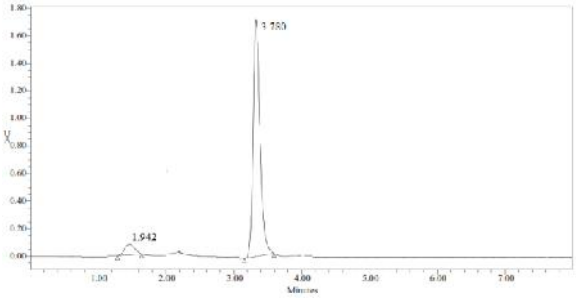


**Fig 9:** Chromatogram showing more organic composition

**Robustness:**

**Table 1:** 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 2:** Results of method precession for Buprenorphine and Naloxone

| Injection | Peak Area | |
|---|---|---|
| | **Buprenorphine** | **Naloxone** |
| 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 |

**Table 3:** Results of Intermediate precision for Buprenorphine and Naloxone

| Injection | Peak Area | |
|---|---|---|
| | **Buprenorphine** | **Naloxone** |
| 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 4:** 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 5:** 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 6:** 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 7:** 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 8:** 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 |

**Table 9:** Results of LOD

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

**Table 10:** 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 11:** 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 12:**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 13:** 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 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 $p^H$ 3.0 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/ml of 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.

## 5. References


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