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*Research Article*

# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DROPERIDOL AND FENTANYL CITRATE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP- HPLC

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**ABSTRACT**

The purpose of the study is to develop and validate a simple, accurate, precise and rapid isocratic reverse-phase high-performance liquid chromatographic method for simultaneous determination of Droperidol and Fentanyl citrate in bulk and tablet formulations. Chromatographic separation was supported on Agilent C18 5µm (4.6×250mm) column with a blend of phosphate buffer pH 4.0: ACN (30:70%v/v) as mobile phase at a flow rate of 1 ml/min. UV detection was performed at 254 nm using HPLC Alliance Waters® separation module 2695 with Empower-software. The retention times were found to be 3.503 mins and 2.577 mins for droperidol and fentanyl citrate, respectively. Calibration plots were linear ($r^2$=0.999) over the concentration range 20-100 µg/ml for Droperidol and Fentanyl citrate. Theoretical plates and tailing factor for Droperidol and Fentanyl citrate were found to be 1.3, 5824.4 and 1.2, 2936.0 respectively and the resolution was found to be 9.4. The optimized method was validated in accordance with ICH guidelines(IC(R1)) for accuracy, precision, specificity, linearity, and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. As the method shows high recovery and low relative standard deviation which confirms the suitability of the method for routine determination of Droperidol and Fentanyl citrate in bulk drug and tablet dosage forms.

**Keywords**: Droperidol, fentanyl citrate, simultaneous, RP-HPLC method and validation, ICH.

## INTRODUCTION

Fentanyl citrate is chemically N-phenyl-N-[1-(2-phenylethyl) piperidin-4-yl] propenamide and a potent narcotic analgesic, abuse of which leads to habituation or addiction. It is primarily a mu-opioid agonist, it also used as an adjunct to general anesthetics, and as an anesthetic for induction and maintenance. Fentanyl is a compound with molecular formula $C_{22}H_{28}N_2O$ and molecular weight of 336 gm/mol with Pka 4.05 and is soluble in water[1].

Droperidol is chemically 1-{1-[4-(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydropyridin-4-yl}-2,3-dihydro-1H-1,3-benzodiazol-2-one and often used in conjunction with fentanyl to maintain the patient in a calm state of neuroleptanalgesia, also used as antipsychotic, Antiemetic and dopamine antagonists. Droperidol is a compound with molecular formula $C_{22}H_{22}FN_3O_2$ and molecular weight 379 gm/mol with pKa 7.46 and is soluble in water[2].

The literature survey indicates that droperidol[3-5] and fentanyl citrate[6-12] estimation were carried by UV, TLC and HPTLC methods in different pharmaceutical dosage forms. Hence, there is no specific method reported for estimation of droperidol and fentanyl citrate using HPLC, hence this study performed using RP-HPLC method.

## MATERIALS AND METHODS

All the chemicals and reagents procured and used were of AR/HPLC grade. Pure standards of droperidol, fentanyl citrate and HPLC grade methanol, acetonitrile as well as AR grade Potassium dihydrogen orthophosphate were obtained from Merck India (Mumbai, India). Chromatographic analysis was done using Waters 2695 HPLC system with empower software.

### Preparation of mobile phase

Isocratic mixture of 300ml Phosphate buffer pH 4.0 (30%) and 700 mL of ACN (70%) were taken and degassed for 5 minutes in ultra sonicator then vaccum filtered through 0.45 µ filter.

### Preparation of the individual Droperidol standard preparation

Accurately 10mg of Droperidol standard was weighed and transferred into a 10ml volumetric flask and about 2ml of mobile phase was added. Then it was sonicated to dissolve the drug completely and made volume upto the mark with mobile phase. Further 10.0 ml from the above stock solution is pipette into a 100ml volumetric flask and then volume made upto the mark with mobile phase.

### Preparation of the individual Fentanyl citrate standard preparation

Accurately 10mg of Fentanyl citrate standard was weighed and transferred into a 10ml volumetric flask and about 2ml of mobile phase was added. Then it was sonicated to dissolve the drug completely and then volume made upto the mark with the mobile phase. Further 10.0 ml from the above stock solution is pipette into a 100ml volumetric flask and diluted upto the mark with mobile phase.

### Preparation of tablet sample solution

Accurately 10 tablets are weighed and transferred into clean dry mortar and crushed using clean pestle. From that, weight equivalent to 10 mg of Fentanyl citrate and Droperidol was into a 10mL volumetric flask and about 7mL of mobile phase was added and sonicated to dissolve completely and volume made up to the mark with mobile phase. Further dilution was made by pipetting 3 ml of above solution into a10ml volumetric flask and volume made up to the mark with mobile phase.

### Procedure

20µl of the prepared standard and sample solutions were injected into the chromatographic system and the areas of Fentanyl citrate and Droperidol peaks were measured.

### System Suitability

Tailing factor and theoretical plates for Fentanyl citrate and Droperidol standard solution were calculated.

## ANALYTICAL METHOD VALIDATION[13-15]

### Preparation of standard solution (Fentanyl citrate and Droperidol)

Accurately weighed each 10 mg of Fentanyl citrate and Droperidol working standards were transferred into a 10mL and 100ml of clean dry volumetric flasks respectively. About 7mL and 70ml of mobile phase was added separately and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further 3ml and 0.3ml of each the above Fentanyl citrate and Droperidol stock solutions were pipetted into a 10ml volumetric flasks and diluted up to the mark with diluent.

### Accuracy

### Preparation of 50%, 100%, 150% solutions (With respect to target Assay concentration)

Accurately 5mg, 10mg, 15mg of Fentanyl citrate and 5mg, 10mg, 15mg of Droperidol working standards were weighed separately and transferred each into a 10mL and 100ml volumetric flasks and about 7mL and 70ml of mobile phase was added and sonicated to completely dissolve and made volume up to the mark with the mobile phase. Further 3ml and 0.3ml of each the above Fentanyl citrate and Droperidol stock solutions were pipetted into a 10ml volumetric flasks and diluted up to the mark with mobile phase.

### Procedure

20µl standard solution and 50%, 100%, 150% solutions and were injected into the chromatographic system. The Amount found and Amount added as well as individual recovery and mean recovery values for Fentanyl citrate and Droperidol were calculated.

### Precision (Repeatability)

### Procedure

The standard solution was injected repeatedly for five times and the areas for all five injections in HPLC were measured and %RSD for the area of five replicate injections was calculated.

### Specificity

Specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak.

The specificity was performed by injecting blank.

**LOD and LOQ**

LOD and LOQ values were calculated by diluting known concentrations of Droperidol and Fentanyl citrate til the normal response was approximately 3 to 10 times the standard deviation (SD) of response (peak area) for the three replicate determinations.

**Linearity**

Linearity was calculated by preparing aliquots of standards to obtain final concentrations of 20-100 µg/ml of Droperidol and Fentanyl citrate then 20 µl of the standard solutions were injected and chromatogram areas were recorded. Calibration plots were constructed with average peak areas *versus* concentrations and regression equations were figured for both the drugs.

**Robustness**

Robustness evaluate the impact of deliberate change in the flow rate and mobile phase composition of the method.

a) The flow rate was varied at 0.8ml/min and 1.2 ml/min. Standard solution of Droperidol and Fentanyl citrate was analyzed using the varied flow rates along with method flow rate.

b) The organic composition of mobile phase was changed from 70% to 60% and 80%. Standard solution of Droperidol and Fentanyl citrate was analyzed using the varied mobile phase composition along with the actual mobile phase composition of the method.

**RESULTS AND DISCUSSION**

The objective was to develop a unique HPLC method for the simultaneous detection and quantitation of Droperidol and Fentanyl citrate. The isobestic point was measured by preparing 10µg/ml of individual and mixed standards and the solution was scanned in U.V region from 200-400nm. The intersection spectrum of Droperidol and Fentanyl citrate was obtained and the isobestic point was observed at 254 nm. Many trails were performed with various column and several mobile phases compositions under gradient and isocratic conditions to optimize appropriate conditions for the detection and quantification of Droperidol and Fentanyl citrate. The effects of pH of mobile phase and column oven temperature on resolution between the components and tailing factors were also studied. Both oven temperature and mobile phase pH were found to have an immense effect on the resolution and peak shapes. The results indicate a good resolution between the compounds with appropriate peak shapes and retention times were achieved on a Agilent C18 5µm (4.6×250mm) column using a linear gradient of mobile phase consisting of a mixture of Phosphate buffer pH 4.0 and acetonitrile in the ratio of 70:30%v/v at a flow rate of 1.0 ml/min and ambient column temperature.

A demonstrative chromatogram showing resolution between Droperidol and Fentanyl citrate is shown in Figure 1.

The LOD for Droperidol and Fentanyl citrate were found to be 3.1 µg/ml and 3.02 µg/ml respectively. The LOQ for Droperidol and Fentanyl citrate were found to be 10.1 µg/ml and 10 µg/ml respectively. The results for validation and system suitability test parameters are summarized in Table 1. Results for robustness evaluation for both the drugs are presented in Table 2. Insignificant differences in peak areas and less variability in retention times were observed.

A validated HPLC method for the simultaneous quantification of Droperidol and Fentanyl citrate has been established as per ICH guidelines. It has shown that the developed method achieved accuracy, reproducibility, repeatability, linearity, precision, and selectivity, which prove the reliability of the method. The method enabled accurate, sensitive, and reproducible quantification of tablet formulation in routine analysis. The result shows that the method could find practical application as a quality control tool for the simultaneous estimation of two drugs from their combined dosage form in a quality control laboratory.

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**Table 1: Summary of validation and system suitability parameters**

| Parameter (Units) | Droperidol | Fentanyl citrate |
|---|---|---|
| Linearity range (µg/ml) | 20-100 µg/ml | 20-100 µg/ml |
| Correlation coefficient | 0.999 | 0.999 |
| LOD (µg/ml) | 3.1 µg/ml | 3.02 µg/ml |
| LOQ (µg/ml) | 10.1 µg/ml | 10 µg/ml |
| Recovery (%) | 102.0% | 101.0% |
| Precision (%RSD)<br>Interday (n=3)<br>Intraday (n=3) | <br>1.76<br>0.741 | <br>1.33<br>0.604 |
| Retention time ± allowable time (min.) | 3.2±0.2 | 2.5±0.2 |
| % Purity | 100.3% | 101.1% |
| Theoretical Plates | 5824.4 | 2936.0 |
| Tailing Factor (asymmetry factor) | 1.3 | 1.2 |

**Table 2: Robustness of droperidol and fentanyl citrate**

| Parameter | USP Plate count | | USP Tailing | |
|---|---|---|---|---|
| | Droperidol | Fentanyl citrate | Droperidol | Fentanyl citrate |
| **Flow Rate(ml/min)** | | | | |
| 0.8 | 6645 | 3483 | 1.3 | 1.2 |
| 1.0 | 5824 | 2936 | 1.3 | 1.3 |
| 1.2 | 6059 | 2832 | 1.2 | 1.1 |
| **Organic phase composition in mobile phase** | | | | |
| 10% less | 6691 | 3254 | 1.3 | 1.1 |
| Actual | 6532.1 | 3516 | 1.2 | 1.2 |
| 10% more | 6557 | 3215 | 1.3 | 1.2 |

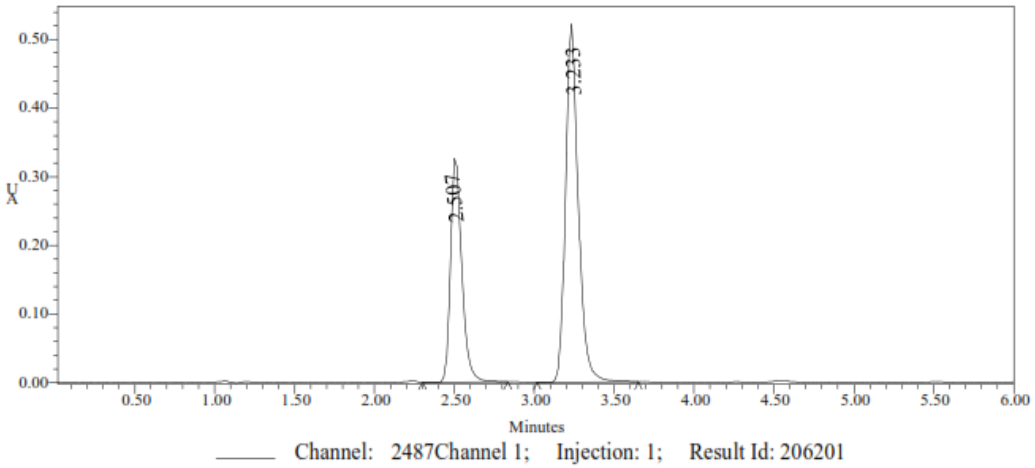


**Fig. 1: Chromatogram of Droperidol and Fentanyl citrate using Phosphate buffer pH 4.0: ACN (70: 30%v/v)**

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