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Analytical method development and validation of droperidol and fentanyl citrate in bulk and pharmaceuticl dosage form by RP-HPLC

V. Nagasravani*, A. Srujana, K. Sujatha, G. Sumana, G. Sunitha, V. Suresh

Department of pharmaceutical Analysis and Quality assurance, Teegala Ram Reddy College of Pharmacy, Telangana, India

Corresponding author: V. Nagasravani

ABSTRACT

A new method was established for simultaneous estimation of Droperidol and fentanyl citrate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Droperidol and fentanyl citrate by using Agilent C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer ph 4.0: ACN (30:70%v/v), detection wave length was 254nm.

Keywords: Droperidol, Simultaneous, RP-HPLC, Fentanyl citrate.

INTRODUCTION

Droperidol causes a CNS depression at subcortical levels of the brain, midbrain, and brains. It may antagonize the actions of glutamic acid within the extrapyramidal system. It may also inhibit cathecolamine receptors and the reuptake of neurotransmiters and has strong central antidopaminergic action and weak central anticholinergic action. It can also produce ganglionic blockade and reduced affective response. The main actions seem to stem from its potent Dopamine(2) receptor antagonism with minor antagonistic effects on alpha-1 adrenergic receptors as well.

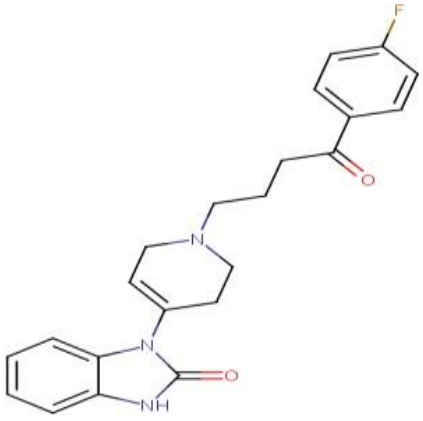

Fig 1: Structure of droperidol

Fentanyl's citrate analgesic activity is, most likely, due to its conversion to morphine. Opioids close N-type voltage-operated calcium channels (OP2-receptor agonist) and open calcium-dependent inwardly rectifying potassium

channels (OP3 and OP1 receptor agonist). This results in hypopolarization and reduced neuronal excitability. It is primarily a mu-opioid agonist. Fentanyl is also used as an adjunct to general anesthetics, and as an anesthetic for induction and maintenance

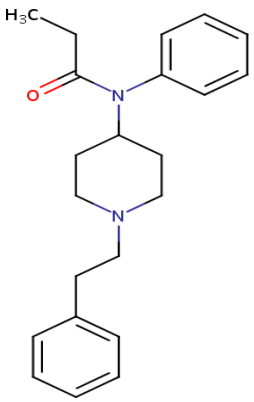

Fig 2: Structure of fentanyl citrate

MATERIALS AND METHODS

Instruments are used

Alliance, Model No. BSA224SC LC+20AD HPLC, Software- UV WIN5, Solution, LABINDIA UV 3000+ UV/VIS spectrophotometer, Adwa – AD 102U pH meter

Drug sample

Droperidol and fentanyl citrate Active pharma ingredients and Marketed samples of droperidol and fentanyl citrate Tablet.

Chemicals and Reagents

Potassium di hydrogen ortho phosphate (Make: Merck and Grade: Empata ACS), Orthophosphoric acid (Make : Merck and Grade: Emparta ACS), Acetonitrile and Methanol (Make :Merckand Grade: HPLC)

Mobile Phase Optimization

Phosphate buffer : ACN (70:30%v/v) has been selected as mobile phase. If any buffer selected buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved.

If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved.

Wave length of Selection

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Droperidol and Fentanyl citrate was obtained and the isobestic point of Droperidol and Fentanyl citrate

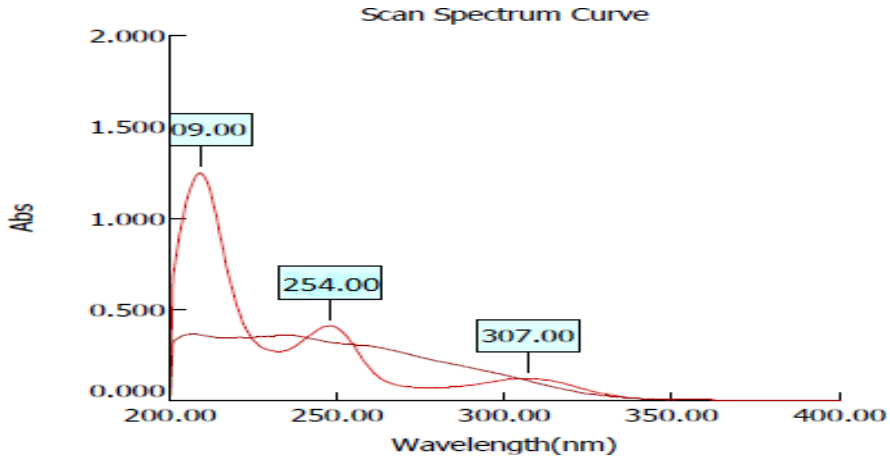

Fig 3: Overlay spectrum of Droperidol and Fentanyl citrate

Optimization of Chromatographic conditions

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5 m) was found to be ideal as it gave good peak shape and resolution at

0.8ml/min flow at 260nm with 10µL injection volume.

Preparation of Phosphate buffer

Accurately weighed 6.8 grams of KH2PO4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase

A mixture of Phosphate buffer pH 4.0 3 0 0 m l (30%), 700 mL of ACN (30%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

Mobile phase is used as Diluent**.**

Preparation of the standard solution

10mg of Droperidol and fentanyl working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution).

Preparation of Sample Solution (Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Fentanyl citrate and Droperidol (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent.

Optimized Chromatographic conditions

Column	Agilent C18 54m (4.6*250mm)
Mobile Phase and Composition	pH 4.0 Phosphate Buffer: ACN (30:70%v/v)
Flowrate	1 mL/min
Column oven Temperature	Ambient
Injection volume	10µL
Detection wavelength	254nm
Auto sampler Temperature	Ambient

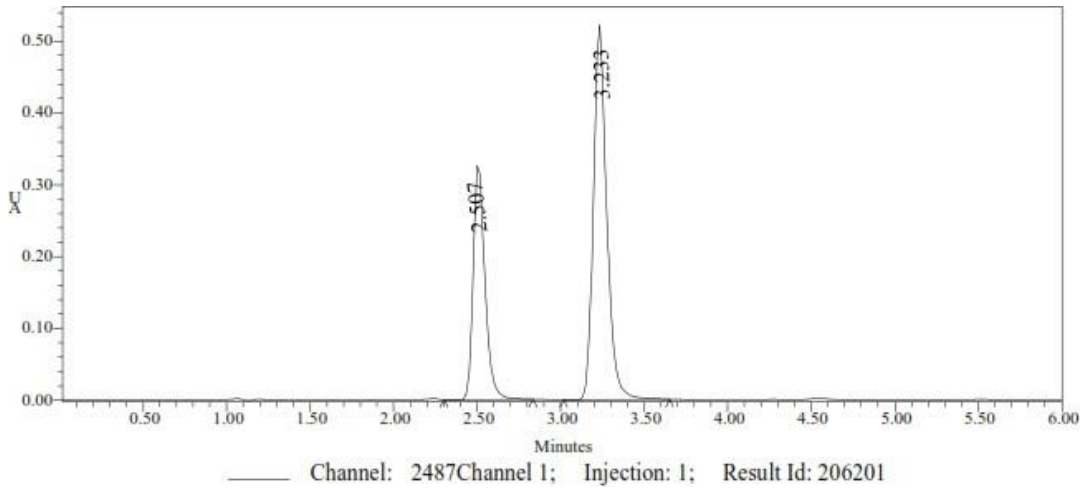

Fig 4: Chromatogram of droperidol and fentanyl citrate

Method Validation[3-4]

By using Optimised condition Analytical Method of Assay carried out by ICH Guideline Q2B.[3-4] The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

System Suitability and System Precision[3-4]

According to ICH guidelines[3-4]System suitability checking out is an integral a part of many analytical procedures. The tests are based on the idea that the equipment, analytical operations and samples to be analyzed represent an integral system that can be evaluated as such. System suitability check parameters so be established for a particular procedure depend on the type of procedure being validated. System Precision Specification:%RSD for Area and Retention time for the six replicate injections should not be more than 2.0 of each analyte and system suitability results were shown in table 2 and system precision were summarized in Table 3.

Table 1: Results of System suitability and system precision

S.No	Droperidol			Fentanyl citrate			Resolution
	Rt in min	Plate count	Tailing Factor	Rt in min	Plate count	Tailing Factor	
1	2.569	4668	1.3	3.842	6090	1.3	4.0
%RSD for Area and Retention time for the six replicate injections was not more than 2.0							

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank.

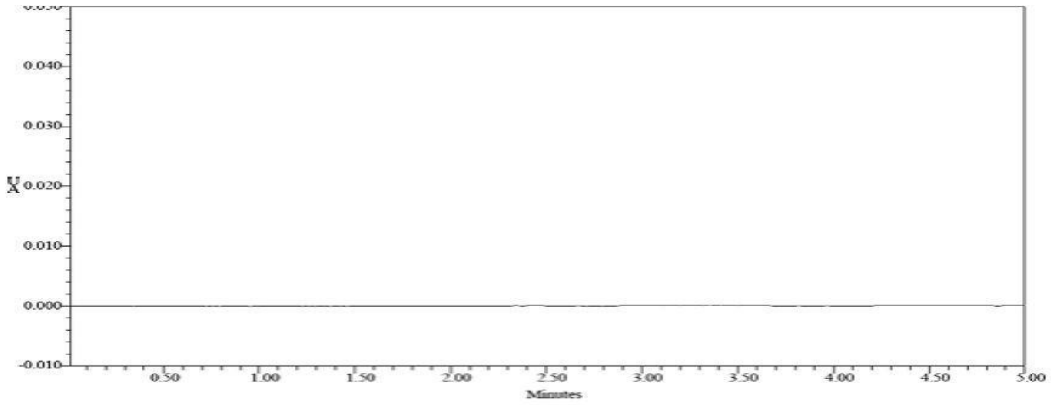

Fig 5: Blank of droperidol and fentanyl citrate

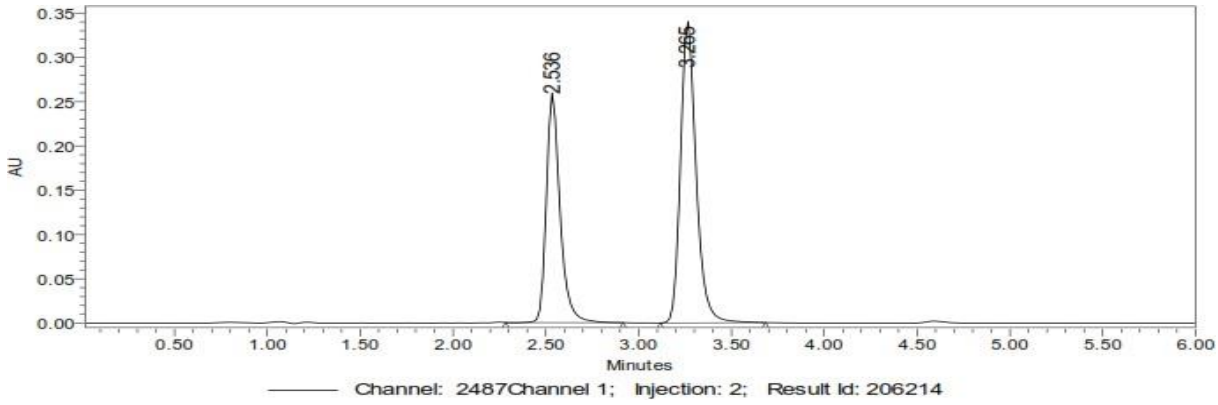

Fig 6: Chromatogram sample of droperidol and fentanyl citrate

Blank solution and Placebo solution should not be interfered at the retention time of the three main Analyte peaks. No Blank and Placebo interference was observed at the retention times of the two main Analyte peaks.

Linearity

The linearity study was performed for the concentration of 100ppm to 500ppm and1ppm to 5ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The chromatograms and results are tabulated in Table. 2-3 Calibration graph for Droperidol and Fentanyl citrate are shown in Fig. 5,6.

Table 2: Linearity Results Fentanyl citrate

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

Table 3: Linearity Results Droperidol

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

Correlation coefficient should be not less than 0.999

Plotting of calibration graphs

The resultant areas of linearity peaks are plotted against Concentration

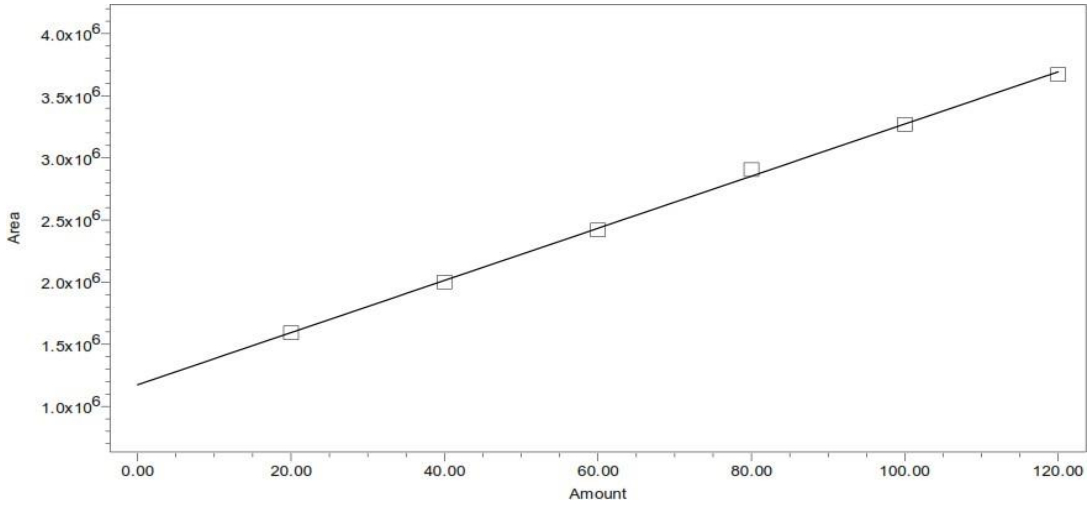

Fig 7: Calibration curve of Fentanyl citrate

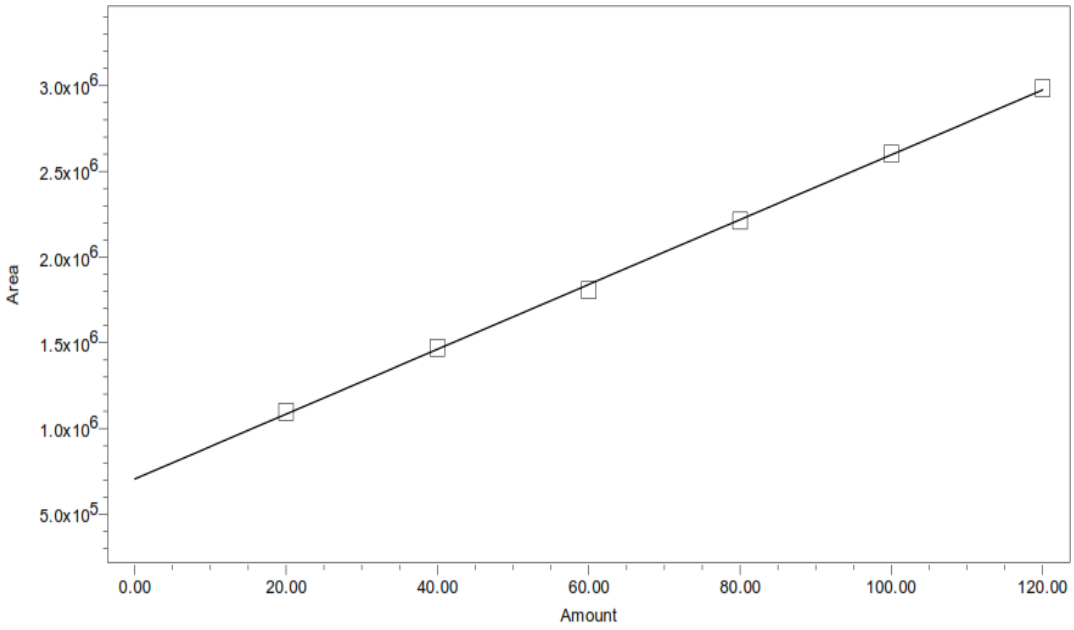

Fig 8: Calibration curve of Droperidol

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.

Table 4: System suitability results For Fentanyl citrate(Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	3483	1.26
2	1.0	2936	1.3
3	1.2	2832	1.1

Table 5: System suitability results for Droperidol (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	6645	1.3
2	1.0	5824.4	1.3
3	1.2	6059.0	1.2

The results are summarized. On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

Table 6: System suitability results for Fentanyl citrate(Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	3254.5	1.1
2	Actual	3516	1.2
3	10% More	3215	1.2

Results for actual Mobile phase composition (55:45Water : Methanol) have been considered from Accuracy standard

Table 7: System suitability results for Droperidol (Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	6691	1.3
2	Actual	6532.1	1.2
3	10% More	6557	1.3

Results for actual Mobile phase composition (55:45Water : Methanol) have been considered from Accuracy standard.

CONCLUSION

A new method was established for simultaneous estimation of Droperidol and fentanyl citrate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Droperidol and fentanyl citrate by using Agilent C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer ph 4.0 : ACN (30:70%v/v), detection wave length was 254nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.The retention times were found to be 3.503 m ins and 2.577 mins.

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