

## Estimation of Flupenthixol HCl in single dosage form by RP-HPLC method

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**Abstract-** A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for estimation of Flupenthixol Hydrochloride in pharmaceutical dosage forms. The method was carried out on a Eurospher C<sub>18</sub> (250 cm×4 mm) column with precolumn and a mobile phase consisting of Acetonitrile:Water (pH 3.0) (50:50 v/v) at a flow rate of 1 ml/min. Detection was carried out at 229nm. Acetonitrile was used as an internal standard. The retention time of Flupenthixol HCl was found out to be 3.98. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantization and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms.

**Keywords-** Flupenthixol Hydrochloride, Acetonitrile, pH, Eurospher, intersil, recovery, validation

### Introduction

Flupenthixol HCl is a white to off White Powder [1-9]. Amorphous in nature, sensitive to light and moisture. Chemically it is 2-[4-[3-[2-(trifluoromethyl)-9-ylidene]propyl]piperazin-1-yl]Ethanol. It is a thioxanthene drug and acts by antagonism of D1 and D2 Dopamine receptors (as well as serotonin).it effectively relieves Hallucination disorders associated with schizophrenia. It is official in B.P[1],Martindale-the extra Pharmacoeipia. However, there is no HPLC method reported for the simultaneous estimation of these drugs in single as well as in combined dosage form. Fixed dose containing Flupenthixol (1mg is available in the tablet form in the market. The aim of this work was to develop an RP-HPLC method with ultraviolet detection for estimation of Flupenthixol in pharmaceutical dosage forms. The present RP-HPLC method was validated following the ICH guidelines.

### Material and reagents

Acetonitrile HPLC grade was procured from Qualigens fine chemicals, Mumbai. Phosphoric acid AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. Reference standard of Flupenthixol Hydrochloride are procured from Biocon Laboratories, Bangalore.

### Method and Material

#### 1. Determination of $\lambda_{max}$ of FLU:

The stock standard solutions of FLU were prepared by dissolving 50mg of drug in 100ml of selected mobile phase Acetonitrile:Water (50:50v/v).PH 3.0.The aliquot portions of stock standard solution were diluted appropriately to obtain a concentration of 50 $\mu$ g/ml of both FLU. The  $\lambda_{max}$  was determined on Shimadzu UV-Visible spectrophotometer (Model UV-2409) in the range 200-400 nm using mobile phase as blank.

#### 2. Selection of mobile phase:

##### a) Preparation of standard solutions:

FLU standard solution:

Accurately weighed quantity ~ 50.0 mg of FLU and diluted to 100.0 ml with mobile phase and 5ml solution was pipetted out and the solution was further diluted to 50.0 ml with water.

##### b) Procedure:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing FLU was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each mobile phase was filtered through Whatman filter paper No.42.

Mobile phases tried for the HPLC study are as follows:

ACETONITRILE AND WATER TRIED AT DIFFERENT CONCENTRATIONS:

- Acetonitrile:Water(50:50v/v) PH 3.0
- Acetonitrile:Water(60:40v/v) PH 3.0
- Acetonitrile:Water(40:60v/v) PH 3.0

From various mobile phases tried, mobile phase containing Acetonitrile: Water (50:50v/v) PH 3.0 was selected, since it gives sharp peak, well resolved peaks with symmetry within limits and significant reproducible retention time for FLU.

#### Chromatographic conditions [10-12]:

The following chromatographic conditions were established by trial and error and were kept constant throughout method.

Column : Eurospher 100-5 C18  
Dimension : 250x4 mm with precolumn  
Practical size Phases : 10 $\mu$ m  
Stationary Phases : C18 intersil  
Mobile Phases: Acetonitrile:Water(50:50v/v) PH 3.0  
Detection Wavelength : 229nm  
Flow Rate : 1ml/min  
Temperature : Ambient  
Sample Size : 50 $\mu$ L

### 3. Preparation of Calibration Curve:

#### i) Standard solutions:

FLU standard stock solution:  
Accurately weighed quantity ~ 50.0 mg of FLU and diluted to 100.0 ml with mobile phase and 5ml solution was pipetted out and the solution was further diluted to 50ml with water.

#### ii) Procedure:

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The various concentration from 10-100 µg/ml of drug solution were injected and peak area was obtained and was recorded..

#### 4. System suitability test:

System suitability is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standards solutions.

#### A) Preparation of standard drug solution:

FLU standard solution:  
Accurately weighed quantity ~ 50.0 mg of FLU and diluted to 100.0 ml with mobile phase and volume was made up to the mark. Standard stock solution was diluted further with mobile phase to get final concentration 50µg/ml.

#### B) Procedure:

The previously filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. A 20µL std.drug solution was injected which was made in five replicates.

Application of Proposed method for estimation of FLU:

Preparation of standard solution:

Accurately weighed quantity of FLU ~ 50.0 mg was transferred to 100.0 ml volumetric flask, shaken vigorously for five minutes and volume was made upto mark. The stock standard solution of FLU were mixed and diluted properly to obtain solutions of concentration 50µg/ml of FLU.

Preparation of sample solution:

Five different solutions of FLU were prepared by appropriately weighing the quantities of drug sample so as to get the concentrations of 50µg/ml. The peak area of standard laboratory solution and sample laboratory solutions was compared to obtained the concentration.

The amount of each drug estimated in solution was calculated using following formula-

$$\%c \text{ Estimation} = \frac{A_t}{A_s} \times \frac{D_s}{D_t} \times \frac{W_s}{W_t} \times 100$$

$A_t$  = Area count for sample solution.

$A_s$  = Area count for standard solution.

$D_s$  = Dilution factor for sample.

$D_t$  = Dilution factor for standard.

$W_s$  = Weight of standard (mg)

$W_t$  = Weight of sample (mg)

### 5. Application of proposed method for estimation of FLU in Marketed formulation:

#### Standard stock solution:

Accurately weighed quantity of FLU ~ 50.0 mg was dissolved separately in 100.0 ml mobile phase. The stock solution of drug was mixed and further dilution was done appropriately with mobile phase to get concentration of 50µg/ml of FLU.

#### Sample solution preparation:

Twenty tablets were weighed and content emptied. The average weight determined. It was finely powdered and mixed thoroughly. Accurately weighed tablet powder equivalent to 1mg of FLU was transferred in a 100.0 ml volumetric flask and mobile phase was added. It was shaken vigorously for 5 to 10 minutes. Later the volume was made up to mark with mobile phase. The solution was filtered through Whatman filter paper No.42. Further dilution was done with mobile phase to get concentration of 50µg/ml of FLU.

#### Procedure:

Equal volume (20µL) of standard and sample solution were injected separately equilibrium of stationary phase. The chromatograms were recorded and the response i.e., peak area of major peaks were measured. The content of FLU was calculated by comparing a sample peak with that of standard.

Amount of drug in tablet was calculated using formula-

$$\% \text{ Label Claim} = \frac{A_t}{A_s} \times \frac{D_s}{D_t} \times \frac{W_s}{W_t} \times \frac{A}{L_c} \times 100$$

$A_t$  = Area count for sample solution.

$A_s$  = Area count for standard solution.

$D_s$  = Dilution factor for sample.

$D_t$  = Dilution factor for standard.

$W_s$  = Weight of standard (mg)

$W_t$  = Weight of sample (mg)

$L_c$  = Label claim

$A$  = Average weight

Validation parameters:

#### a) Accuracy:

It was ascertained on the basis of recovery studies performed by standard addition method.

Preparation of standard solution:

An accurately weighed quantity of preanalysed tablet powder ~ 50.0 mg of FLU was taken in 100.0 ml volumetric flask; to it standard solution of FLU was added in different proportions. Then volume was adjusted up to the mark with the solvent. Solution was then filtered through Whatman No. 42. The aliquot portion of the filtrate was diluted to get final concentration. The amount of drug contributed by tablet powder was deduced from the total amount of respective drugs estimated and the resultant quantities were assumed to be recovered from the added pure drugs. The content of drug was calculated using same formula as in the marketed formulation. The %Recovery was then calculated by using formula-

$$\% \text{ Recovery} = \frac{T-C}{P} \times 100$$

Where,

T= total drug estimated

C=drug contributed by preanalysed powder

P=weight of pure drug added

#### b) Precision:

Precision of analytical method is expressed as S.D. or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

#### c) Ruggedness:

The studies of ruggedness were carried out under two different condition-

1) Days

2) Analyst

i) Interday (Different days):

Same procedure was performed was performed as under marketed formulation analysis on different days.

ii) Intraday:

It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. interval within a day.

iii) Different analyst:

The sample solution was prepared by two different analysts and the same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

#### d) Specificity:

Specificity was measured as ability of the proposed method to obtain well separated peak for FLU without any interference from components of matrix.

Mean retention time for—

FLU --- 3.98 min

The values obtained were very close to that in standard solution indicates no interference from the components of matrix.

#### e) Linearity and range:

According to BP tablet powder equivalent to 80, 90,100,110,120% of label claim was taken and dissolved in mobile phase, diluted appropriately to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions were recorded. FLU marketed formulation was found to be linear in the range + 20% of the test concentration of the respective drug.

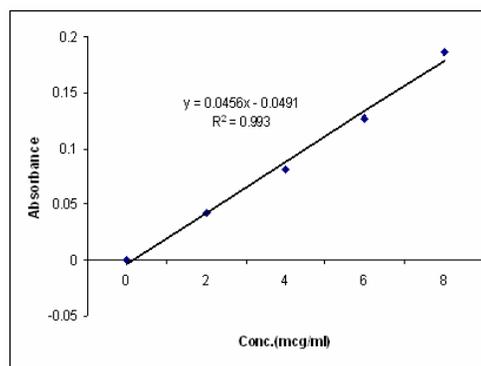


Fig 1: Graph Showing Linearity and Range of FLU

#### Result and Discussion

HPLC has gained valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique is commonly used for the quantitative estimation of the drug from their formulation as well as for studying their metabolites of drugs and their estimation in their biological fluids. This method offers advantages of estimating the constituents for the multi component system without prior separation and even nano quantities can be estimated. This technique was employed in the present investigation for estimation of Flupenthixol HCl in tablet dosage form. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish HPLC method, the parameters studies are discussed in the following paragraph. HPLC (Knauer) system with Eurospher 100-5 C18 {4x 250mm} column with Precolumn and UV-2401 detector was used for the study. The standard and sample solution of FLU was prepared in mobile phase. Different pure samples of varying polarity (*viz.*, Methanol, Acetonitrile and Water) and buffers (*viz.*, Phosphate buffer) in different proportions were tried as mobile phase for development of the chromatogram. During selection and

optimization of the mobile phase it was observed that the retention of the analyte is decreased with increased in the proportion of organic modifier like acetonitrile in the mobile phase. The sharpness of the peak is achieved with increasing the proportion of acetonitrile whereas the increased proportion of aqueous resulted in broadening of the peak. The mobile phase pH across 3.0 gave symmetry. The mobile phase that was found to be most suitable was Acetonitrile: water (60:40 v/v) pH 3.0. The wavelength 229nm was selected for the evaluation of the chromatogram of both drugs. The selection of the wavelength was based on the  $\lambda_{max}$  obtained by scanning of standard laboratory mixture in mobile phase. This system gave good resolution and optimum retention time with appropriate tailing factor «2). After establishing the chromatographic conditions, standard laboratory mixture prepared and analyzed by following procedure described under experimental and results. It gave accurate, reliable results and therefore was extended for estimation of drugs in market tablet formulation. The above results clearly indicate that HPLC technique can be successfully applied for the estimation of above-mentioned drugs in single dosage formulation.

**Validation:**

Validation of these of these methods was performed as per the ICH guidelines for lese following parameters-

**Accuracy:**

Accuracy of the proposed method was ascertained from the recovery studies by standard addition method.

**Precision:**

Replicate estimation of capsule analysed by the proposed method has yielded quite consistent result indicating repeatability of method. Study showed  $\pm$ S.D. <2.

**Specificity:**

Studies shows that there is no interference of peak from the component of matrix showing retention time for FLU 3.98min

**Ruggedness:**

Studies were carried out only for the two different parameters like different time, different days and different analyst. Results of estimation by proposed method are very much similar under variety of conditions. This study signifies the ruggedness of the method under varying condition of its performance.

**Linearity and Range-** FLU in marketed formulation was found to be linear in the range of 80% to 120 % of test concentration with  $R^2 \sim 1$  at selected wavelength fm hath the methods. Same procedure as described in USP was followed.

**Acknowledgments**

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**Table 1: Result and statistical data for estimation of FLU in Laboratory solution  
Standard Solution:**

Sr.No.	Weight of Std.(gm)	Weight of Sample	Peak area of Std.	Peak area of sample	% Drug estimation
	FLU	FLU	FLU	FLU	FLU
1	0.0501	0.0502	4252.75	4146.934	99.72
2		0.0502		4148.75	100.2
3		0.0504		4149.75	99.85
4		0.05		4150.75	99.72
5		0.0501		4151.75	99.78
<b>Mean</b>					99.85
<b>±S.D.</b>					0.20
<b>C.V.</b>					0.20

**Table 2: Result and statistical data for estimation of FLU in marketed formulation  
Sample solution :( marketed study)**

Sr.No.	Weight of Std.(gm)	Weight of Sample	Peak area of Std.	Peak area of sample	% Drug estimation
	FLU	FLU	FLU	FLU	FLU
1.	0.0501	0.0502	4252.750	4248.750	99.84
2.		0.0502		4249.750	99.85
3.		0.0504		4250.750	99.97
4.		0.0500		4251.750	99.89
5.		0.0501		4253.750	100.12
<b>Mean</b>					99.93
<b>±S.D.</b>					0.11
<b>C.V.</b>					0.11

**Table 3: Result and statistical data for system suitability Test of FLU  
System Suitability Test:**

Sr.No.	Peak Area	Height(mV)	Area(%)	Retention Time	Height(%)
	MEL	MEL	MEL	MEL	MEL
1	3660.252	260.340	100.0	4.717	100.0
2	3665.252	260.340	100.0	4.717	100.0
3	3660.254	260.344	100.0	4.719	101.0
4	3672.251	260.343	100.0	4.715	100.0
5	3669.252	260.342	100.0	4.717	100.0

**Statistical data**

<b>Mean</b>	3665.452	260.341	100.0	4.717	100.20
<b>± S.D</b>	6.14	0.1788	1.30	0.01483	1.10
<b>C.V.</b>	0.1675	0.687	1.3	0.3144	1.10

**Table 4: Result and statistical data for estimation of FLU for Recovery study**  
Recovery Study:

Sr.No.	Weight of Tablet(gm)	Peak area of Std	Amount of Pure drug added(µg/ml)	Peak area of sample	% Recovery
	FLU	FLU	FLU	FLU	FLU
1.	0.0503	4252.750	0.0502	4248.750	99.84
2.	0.0505		0.0502	4249.750	99.85
3.	0.0507		0.0504	4250.750	99.97
4.	0.0509		0.0500	4251.750	99.89
<b>Mean</b>					99.93
<b>±S.D.</b>					0.11
<b>C.V.</b>					0.11

**Table 5: Result and statistical data for estimation of FLU for Interday study**  
Interday:

Sr.No.	Weight of Tablet(gm)	Peak area of Std.	Peak area of sample	% Label Claim*
		FLU	FLU	FLU
1.	0.0505	4255.750	4255.750	100.06
2.			4253.750	99.85
3.			4254.750	99.97
<b>Mean</b>				99.96
<b>±S.D.</b>				0.10
<b>C.V.</b>				0.10

\*Results Are Mean of Three Replicates

**Table 6: Result and statistical data for estimation of FLU for Intraday study**

**Intraday:**

Sr.No.	Weight of Tablet(gm) powder	Peak area of Std.	Peak area of sample	% Label Claim*
		FLU	FLU	FLU
1.	0.0505	4257.750	4257.750	100.06
2.			4255.750	99.75
3.			4256.750	99.97
			<b>Mean</b>	99.92
			<b>±S.D.</b>	0.15
			<b>C.V.</b>	0.15

**\*Results Are Mean of Three Replicates**

**Table 7: Result and statistical data for estimation of FLU for different analyst study**

**Different Analyst:**

Sr.No.	Weight of Tablet(gm) powder	Peak area of Std.	Peak area of sample	% Label Claim*
		FLU	FLU	FLU
1.	0.0505	4253.750	4253.750	100.06
2.			4251.750	99.75
3.			4252.750	99.93
			<b>Mean</b>	99.91
			<b>±S.D.</b>	0.15
			<b>C.V.</b>	0.15

**\*Results Are Mean Of Three Replicates**

## List of Figures

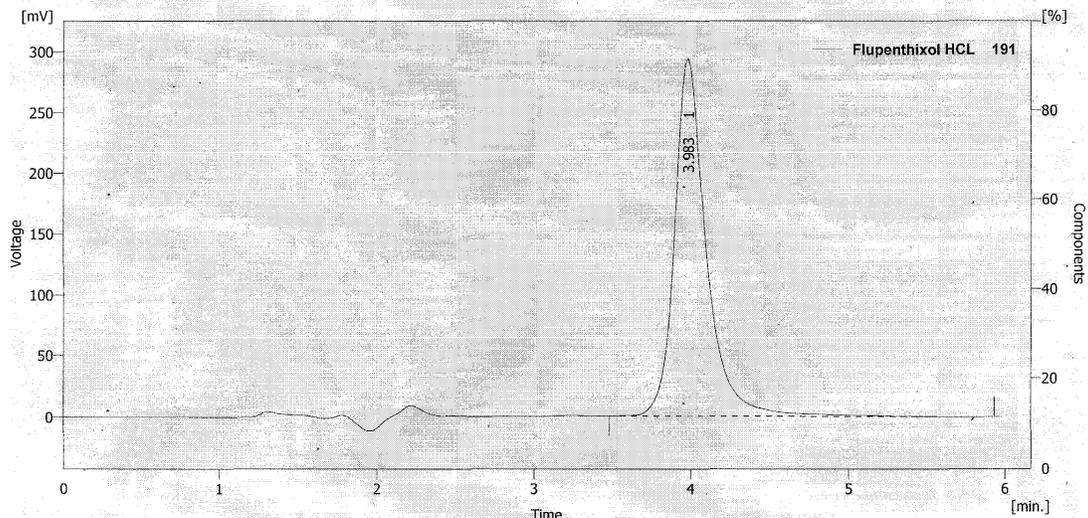


Fig 2- Chromatogram of Flupenthixol HCl as Pure Drug

Result Table (Uncal - Flupenthixol HCL 191)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.983	4146.934	293.800	100.0	100.0	0.22
	Total	4146.934	293.800	100.0	100.0	

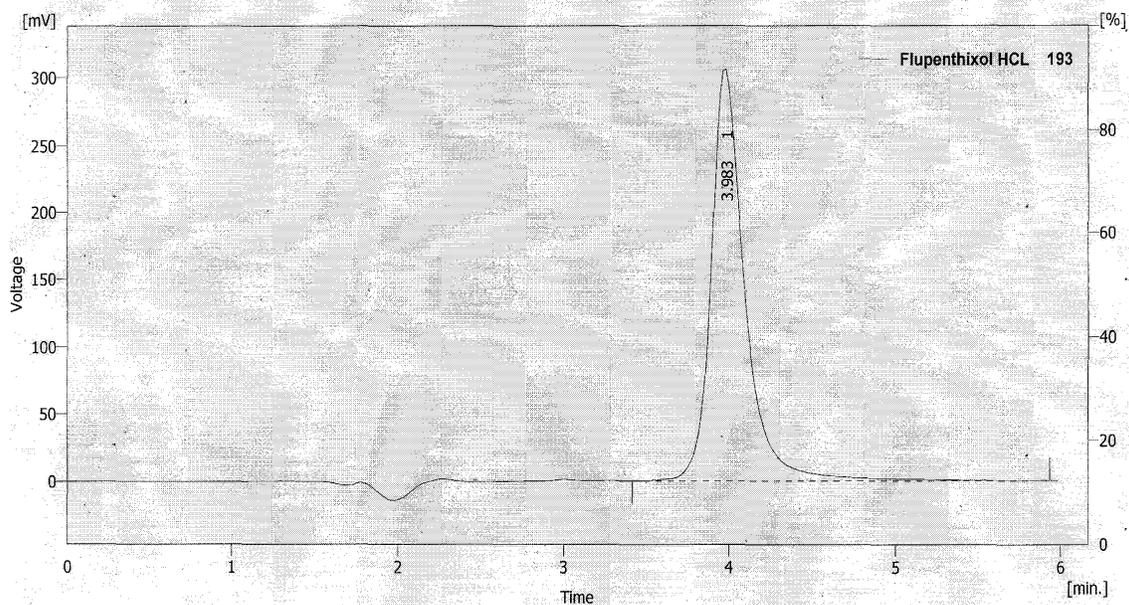


Fig 3- Chromatogram of Flupenthixol HCl in Marketed Formulation

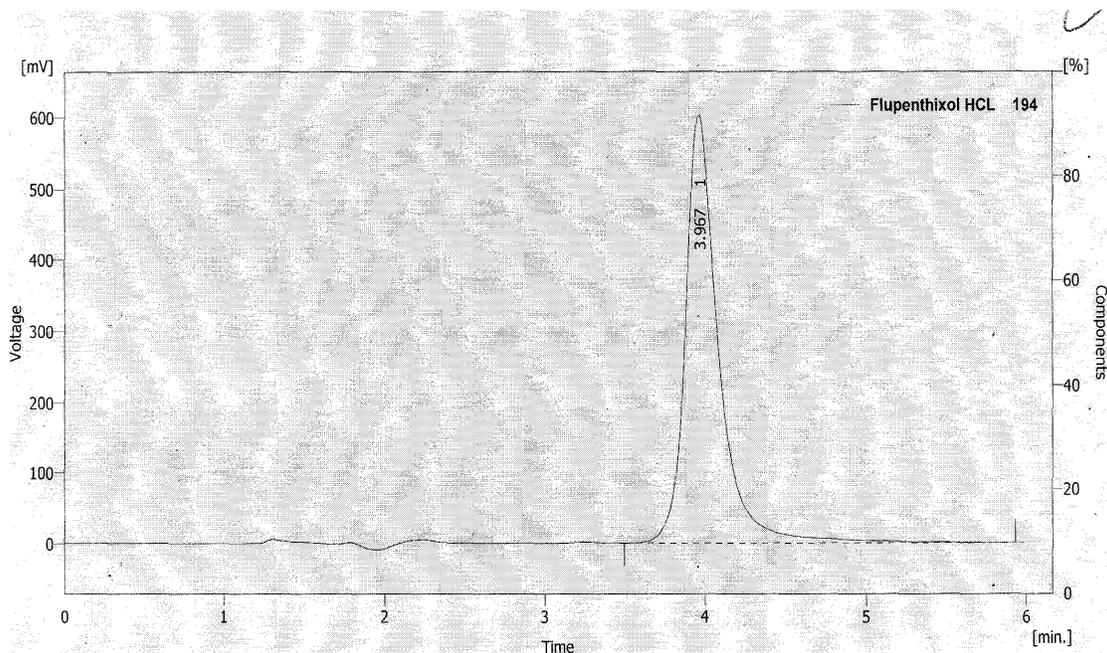


Fig 4- Chromatogram of Flupenthixol HCl as Validation parameter