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Original Article

SIMULTANEOUS DETERMINATION OF FLUPENTIXOL AND NORTRIPTYLINE HCl USING RP-HPLC WITH PDA DETECTOR

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ABSTRACT

Objective: In the current investigation, to separated and validate the cancer healing drugs (Nortriptyline HCl and Flupentixol) through the HPLC (e-2695) instrument containing a PDA detector.

Methods: A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Nortriptyline HCl and Flupentixol. The chromatographic strategy utilized Agilent eclipse XDB column of dimensions 250x4.6 mm, 5 micron, using isocratic elution with a mobile phase of Methanol and 0.1% orthophosphoric acid (40:60). A flow rate of 1 ml/min and a detector wavelength of 250 nm utilizing the PDA detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.

Results: LOD and LOQ concentrations for Flupentixol were 0.015 µg/ml, 0.05 µg/ml and for Nortriptyline HCl were 0.3 µg/ml, 1.0 µg/ml. The calibration charts plotted were linear with a regression coefficient of R^2>0.999. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range.

Conclusion: The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

Keywords: Nortriptyline HCl, Flupentixol, RP-HPLC, Development, Validation

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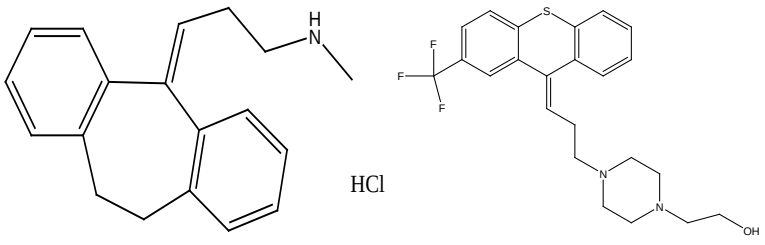
INTRODUCTION

Flupentixol (INN), also known as flupenthixol (former BAN), marketed under brand names such as Depixol and Fluanxol is a typical antipsychotic [1, 2] drug of the thioxanthene class. Flupentixol's main use is as a long-acting injection given once in every two or three weeks to individuals with schizophrenia [3, 4] who have poor compliance with medication and suffer frequent relapses of illness, though it is also commonly given as a tablet. There is little formal evidence to support its use for this indication but it has been in use for over fifty years [5]. Flupentixol is also used in low doses as an antidepressant [6-8]. There is tentative evidence that it reduces the rate of deliberate self-harm among those who repeatedly self-harm [9].

Nortriptyline, sold under the brand name Pamelor, among others, is a medication used to treat depression. This medicine is used for: neuropathic pain [10, 11], attention deficit hyperactivity disorder (ADHD) [12, 13], smoking cessation [14] and anxiety [15]. As with many antidepressants [16, 17], its use for young people with depression and other psychiatric disorders [18] may be limited due to increased suicidality in the 18-24 population initiating treatment. Nortriptyline is a less preferred treatment for ADHD and stopping smoking. It is taken by mouth. Common side effects include dry mouth, constipation, blurry vision, sleepiness, low blood pressure with standing, and weakness. Serious side effects may include seizures [19], an increased risk of suicide in those less than 25 y of age, urinary retention [20], glaucoma [21], mania [22], and a number of heart issues. Nortriptyline may cause problems if taken during pregnancy. Use during breastfeeding appears to be relatively safe. It is a tricyclic antidepressant (TCA) [23, 24] and is believed to work by altering levels of serotonin [25] and norepinephrine. This medication is in capsule or liquid and is taken by the mouth one to four times a day, with or without food. Usually, people are started on a low dose and it is gradually increased. A level between 50 and 150 ng/ml of nortriptyline in the blood generally corresponds with an antidepressant effect [26]. Both drug structures are shown in fig. 1. The aim of the study is to separate the pharma ingredients Nortriptyline HCl and Flupentixol by using RP-HPLC.



A **B**

Fig. 1: Structure of (A) Nortriptyline HCl and (B) Flupentixol

Till today there are no HPLC method was reported in the literature. Hence we developed a method for the simultaneous quantification of Nortriptyline HCl and Flupentixol. The developed HPLC method was utilized for the estimation of the combined drugs by *in vitro* method.

MATERIALS AND METHODS

Chemicals

Methanol, HPLC-grade orthophosphoric acid, water were purchased from Merck India Ltd, Mumbai, India. APIs of Nortriptyline HCl and Flupentixol standards were procured from Glenmark, Mumbai.

The instrumentation

Waters alliance liquid chromatography (model 2695) [27, 28] was monitored with empower 2.0 data handling system and a detector of photodiode array (model 2998) [29, 30] was used for this study.

Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic and gradient mode was tested. However, the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally, 0.1% OPA buffer and methanol with isocractic elution was selected because it results in a greater response of active pharmacy ingredients. During the optimization of the method, various stationary phases such as C_8, C_{18} phenyl and amino, Symmetry C_{18} columns were tested. From these trials the peak shapes were relatively good with a agilent eclipse XDB column of 250 x 4.6 mm, 5 µ with a PDA detector. The mobile phase flow rate has been done at 250 nm in order to obtain enough sensitivity. By using the above conditions we get retention times of Nortriptyline HCl and Flupentixol were about 2.717 and 4.123 min with a tailing factor of 1.04 and 1.15. The number of theoretical plates for Nortriptyline HCl and Flupentixol were 3812, 4499, which indicate the column's successful output. The proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Validation procedure

According to ICH Q2 (R1) guidelines [31, 32], analytical parameters were validated [33, 34] such as system appropriateness, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced deterioration, and stability.

Preparation of buffer

In 1 Lt of HPLC Water, 1 ml of orthophosphoric acid was dissolved and filtered through 0.45 µ filter paper.

Chromatographic conditions

The HPLC analysis was performed on a reverse phase HPLC system with isocratic elution mode using a mobile phase of methanol and 0.1% OPA and agilent eclipse XDB column (250x4.6 mm, 3.5 µ) column with a flow rate of 1 ml/min.

Diluent: Mobile phase was used as diluent.

Preparation of the standard stock solution

Standard stock solution of Nortriptyline HCl (100 mg) and Flupentixol (5 mg) were prepared in 100 ml volumetric flask. It was filtered through a 0.45µ syringe filter. Standard stock solution concentrations of Nortriptyline HCl (1000µg/ml) and Flupentixol (50µg/ml) were obtained.

Preparation of the sample stock solution

Five Flupentixol and Nortriptyline HCl tablets were accurately weighed and triturated to get a fine powder. A 100 mg Nortriptyline HCl and 5 mg Flupentixol equivalent weight tablet powder was transferred into a 100 ml volumetric flask and dissolved in diluent. The solution was ultra-sonicated for 10 min and made the volume with diluent. The tablet sample stock solution was then filtered through 0.45 micron syringe filter and utilized for preparing sample solution for the assay.

RESULTS AND DISCUSSION

The main analytical challenge during development of a new method was to separate active Pharma ingredients. In order to provide good performance, the chromatographic conditions were optimized.

System suitability

In System suitability [35-38], injecting standard solution and reported USP tailing and plate count values are tabulated in table 1.

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Nortriptyline HCl		Flupentixol	
		Mean	Std dev	Mean	Std dev
USP Plate Count	NLT 2000	3845	10.264	4485	9.457
USP Tailing	NMT 2.0	1.06	0.482	1.12	0.647
USP Resolution	NLT 2.0			7.65	2.248
Retention time	NLT 2.0	2.726	1.254	4.134	1.149

mean±SD (n=6)

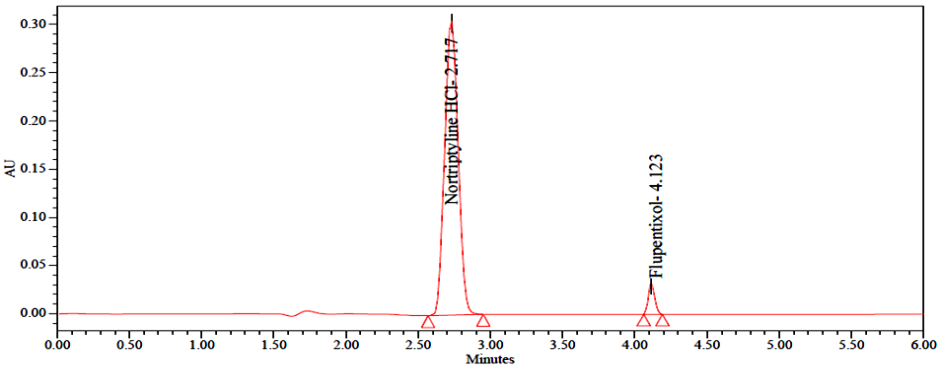


Fig. 2: Chromatogram of system suitability

Specificity

In this test method, placebo, sample and standard solutions were analyzed individually to examine the interference [39, 40].

The below fig. shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

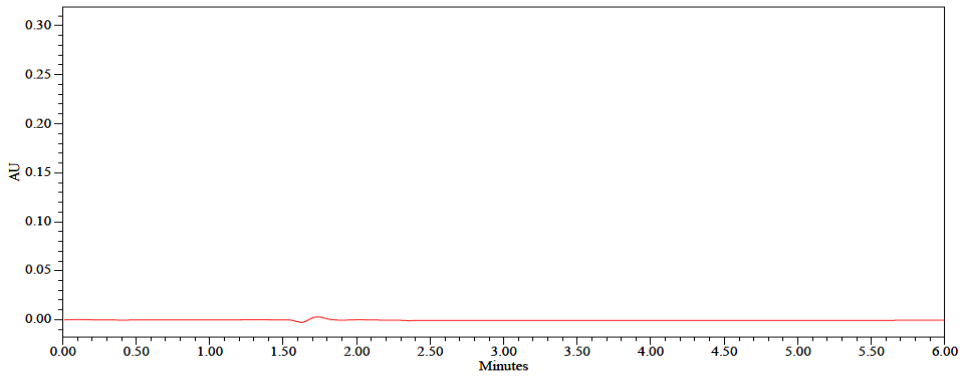


Fig. 3: Chromatogram of blank

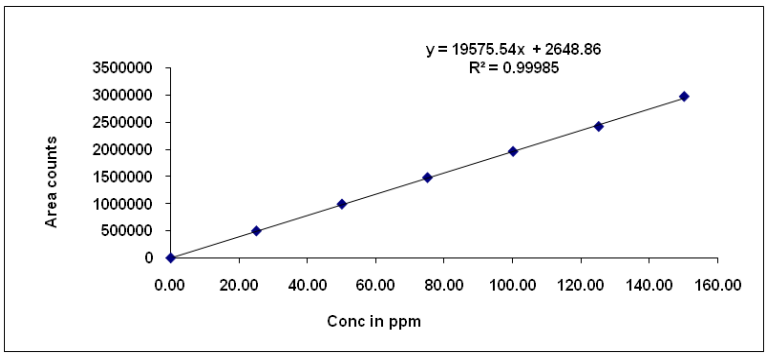
Linearity

The area of the linearity peak versus different concentrations has been evaluated for Nortriptyline HCl, Flupentixol as 10, 25, 50, 100, 125, 150 percent, respectively [41-43]. Linearity was performed in the range of 5-75µg/ml of Nortriptyline HCl and 2-30µg/ml of Flupentixol. The correlation coefficients achieved greater than 0.999 for all.

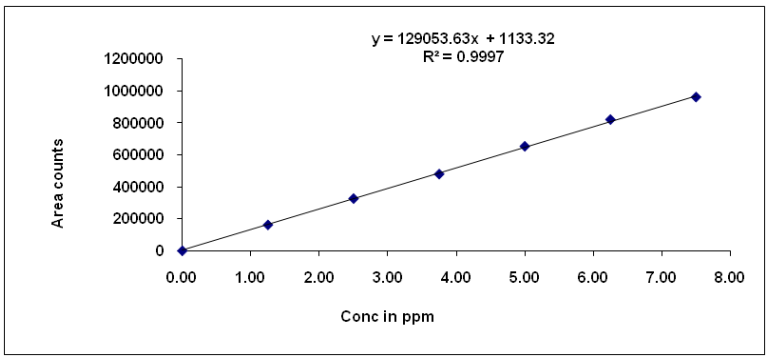
Table 2: Linearity of nortriptyline HCl and flupentixol

S. No.	Conc µg/ml	Nortriptyline HCl area count	Conc. µg/ml	Flupentixol area count
1	25.00	495068	1.25	161527
2	50.00	988745	2.50	325949
3	75.00	1475496	3.75	478598
4	100.00	1956477	5.00	651899
5	125.00	2414459	6.25	818873
6	150.00	2965454	7.50	958745
Correl coef		0.99985		0.99979
Slope		19575.54		129053.63
intercept		2648.86		1133.32

(n=3)



A



B

Fig. 4: Calibration plots of (A) Nortriptyline HCl (B) Flupentixol

Accuracy

In this method, Accuracy was conducted in triplicate by analyzing active pharma ingredient sample solution at three kinds of concentration levels of 50, 100 and 150% of each at a specified limit. Percentage recoveries were measured and found to be within the limit. The accuracy and reliability of the developed method were established. The results are given in table 3.

Table 3: Results of accuracy

S. No.	% Level	Nortriptyline HCl		Flupentixol	
		Mean % recovery	Std dev	Mean % recovery	Std dev
1	50	99.8	0.633	100.2	0.29
2	100	98.9	0.27	98.8	0.591
3	150	99.2	1.062	100.3	0.178

(n=3)

Precision

In the method precision study prepare six different samples in the concentration of Nortriptyline HCl (100 ppm) and Flupentixol (5 ppm) are injected into HPLC system.

Intraday precision

Six replicates of a sample solution containing Nortriptyline HCl (100µg/ml) and Flupentixol (5µg/ml) were analysed on the same day. Peak areas were calculated, which were used to calculate mean, SD and %RSD values. These results are given below table 4 [44, 45].

Inter-day precision

Also called Intermediate precision. In this, six replicates of a sample solution containing Nortriptyline HCl (100µg/ml) and Flupentixol (5µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values [46]. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%.

LOD and LOQ

The LOD concentrations for Nortriptyline HCl are 0.3 µg/ml and s/n values is 3 and Flupentixol 0.015 µg/ml and s/n value 3. The LOQ concentration for Nortriptyline HCl 1.0 µg/ml and its s/n value is 10 and Flupentixol 0.05 µg/ml and s/n value is 10. The method is validated as per the ICH guidelines [47, 48].

Robustness

The conditions of the experiment were designed to test the robustness of the established system intentionally altered, such as flow rate, mobile phase in organic percentage in all these varied conditions [49-51]. Robustness results for Nortriptyline HCl and Flupentixol were found to be within the limit and results are tabulated in table 7.

Table 4: Precision results of nortriptyline HCl and flupentixol

S. No.	% Assay			
	Nortriptyline HCl		Flupentixol	
	M. P.	I. P.	M. P.	I. P.
1	100.1	100.1	99.8	99.9
2	100.2	100.0	100.0	99.8
3	100.1	100.0	100.1	100.2
4	100.0	100.1	100.2	100.1
5	100.0	100.1	100.7	100.5
6	100.2	100.1	100.1	100.7
Mean (n=6)±SD	100.1±0.084	100.1±0.051	100.1±0.316	100.2±0.336
%RSD (n=6)	0.10	0.05	0.32	0.34

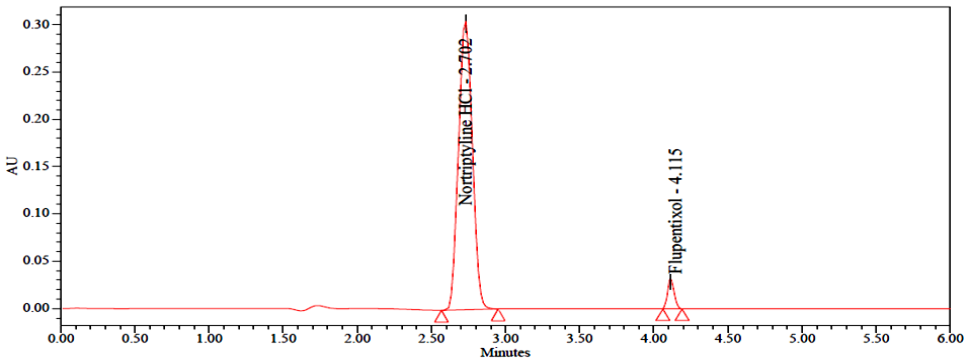


Fig. 5: Chromatogram of method precision

Table 5: LOD and LOQ for nortriptyline HCl and flupentixol

Nortriptyline HCl				Flupentixol			
LOD		LOQ		LOD		LOQ	
Concentration	s/n	Concentration	s/n	concentration	s/n	Concentration	s/n
0.3 µg/ml	3	1.0 µg/ml	10	0.015 µg/ml	3	0.05 µg/ml	10

Table 6: Robustness data of nortriptyline HCl and flupentixol

Parameter name	Nortriptyline HCl % assay		Flupentixol % assay	
	Mean	Std dev	Mean	Std dev
Flow minus (0.8 ml/min	99.9	0.04	99.9	0.006
Flow plus (1.2 ml/min)	100.1	0.049	99.8	0.124
Organic minus (36:64)	99.0	0.032	99.5	0.072
Organic plus (44:56)	100.1	0.026	99.8	0.107

RSD-Relative standard deviation; (n=3)

Degradation studies

The Flupentixol and Nortriptyline HCl sample was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation [52, 53] have carried out to find out that the method is suitable for products of degradation [54, 55]. In addition, the studies provide details about the conditions during which the drug is unstable, in order that the measures are often taken during formulation to avoid potential instabilities.

Acid degradation

Acid degradation was done at ^{1}N HCl and degradation was formed 13.3% for Nortriptyline HCl and 13.4% for Flupentixol.

Alkali degradation

Alkali degradation was done at ^{1}N NaOH and degradation was formed 13.7% for Nortriptyline HCl and 13.9% for Flupentixol.

Peroxide degradation

Peroxide degradation was done at 20% hydrogen peroxide and degradation was formed 14.4% Nortriptyline HCl and 15.1% for Flupentixol.

Reduction degradation

In reduction degradation, 4.6% Nortriptyline HCl and 8.7% Flupentixol degradation were observed.

Thermal degradation

In thermal degradation the standard was degraded to 5% of Nortriptyline HCl and 4.2% of Flupentixol.

Hydrolysis degradation

In hydrolysis degradation, the standard was degraded to 4.4% of Nortriptyline HCl and 2.9% of Flupentixol.

All degradation results are tabulated in table 9.

Table 9: Forced degradation results of nortriptyline HCl and flupentixol

Degradation condition	Nortriptyline HCl % deg		Flupentixol % deg	
	Mean	Std dev	Mean	Std dev
Control degradation	0.1	0.026	0.2	0.074
Acid degradation	13.3	0.452	13.4	0.245
Alkali degradation	13.7	0.247	13.9	0.361
Peroxide degradation	14.4	0.196	15.1	0.289
Reduction degradation	4.6	0.153	8.7	0.524
Thermal degradation	5	0.187	4.2	0.521
Hydrolysis degradation	4.4	0.241	2.9	0.183

(n=3)

CONCLUSION

We present in this article simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Nortriptyline HCl and Flupentixol. All the products of degradation formed during the stress conditions and active pharma ingredients are well separated and peaks were well resolved from each other and separate with an appropriate retention time, indicating that the proposed method to be fast, simple, feasible and affordable in assay condition. Therefore the developed method during stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

Declared none

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