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RP-HPLC method for simultaneous determination of escitalopram oxalate and flupentixol HCl in tablet dosage form

Wrushali A. Panchale [1], Shivrani W. Nimbokar [1], Bhushan R. Gudalwar [2], Ravindra L. Bakal [1] and Jagdish V. Manwar [2,*]

[1] *IBSS's Dr. Rajendra Gode Institute of Pharmacy, Amravati-444 602, MS, India.*
[2] *IBSS's Dr. Rajendra Gode College of Pharmacy, Amravati-444 602, MS, India.*

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Abstract

RP-HPLC method was developed for simultaneous determination of escitalopram oxalate (ESC) and flupentixol HCl (FLU) in tablet dosage form. Mobile phase consisting of mixture of acetonitrile and potassium phosphate buffer (pH 7.0 with 0.1% triethylamine) in the ratio 60: 40 at flow rate of 1ml/min using C18 Grace (250mmX 4.6mm) column at 231 nm. The retention time of ESC with FLU was found to be 2.96 min and 6.98 min, respectively. The linearity range for ESC with FLU observed was 5-25 µg/ml and 10-50 µg/ml, respectively. Method was validated as per ICH guidelines. Validation parameters studied were linearity and range, recovery study, precision, LOD, LOQ and robustness. Statistical data obtained was found to satisfactorily.

Keywords: RP-HPLC; Escitalopram oxalate; Flupentixol HCl; Validation

1. Introduction

Escitalopram oxalate (ESC) is used to treat depression and anxiety. It works by helping to restore the balance of a certain natural substance (serotonin) in the brain [1-2]. It is official in IP and BP [3-4]. Flupentixol HCl is used to relieve the symptoms of schizophrenia and other similar mental health problems [5-6]. It is also official in IP and BP [3-4]. There are number of analytical methods for the analysis of pharmaceutical drugs from different formulations [7-27]. Literature survey revealed various analytical methods have been reported for estimation of ESC alone and in combination with other drugs [28-34]. Similarly, there are also various methods for determination of FLU alone and in combination with other drugs [35-36]. Likewise, in literature there is one UV-spectroscopic method and one RP-HPLC available for simultaneous analysis of ESC and FLU in combined dosage form [37-38]. However, nobody has enclosed the complete validation as per ICH guidelines. Therefore, attempts were made to develop new RP-HPLC method for simultaneous determination of (ESC) with FLU in tablet dosage form.

2. Material and methods

2.1. Instrumentation and chemicals

Chromatography was performed with Youngline ACME 9000 (Autochro-3000 software) system coupled with Grace (4.6 mm I.D x 250 mm) C18 column and UV 730 detector. A Rheodyne injector (manual loading) with a 20 µL external loop was used. All chemicals and reagents used in method were of HPLC grade. Standard drugs were obtained as gift samples

*Corresponding author: Dr. Jagdish V. Manwar
IBSS's Dr. Rajendra Gode College of Pharmacy, Mardi Road, Amravati- 444602, MS, India..

from Emcure Pharmacecals, Pune and tablet formulations (Galop FX®, contents- ESC - 10 mg & FLU- 0.5 mg) were purchased from pharmacy.

2.2. Selection of wavelength and chromatographic conditions

Wavelength for analysis of both the drugs was selected by scanning the individual drug's standard solutions in methanol (i.e. ESC 40 µg/ml, FLU 2 µg/ml). From overlain spectra, wavelength 231 nm was selected for further experimental work. Mobile phase for separation of drugs from mixed standard solution (containing ESC 40 µg/ml & FLU- 2 µg/ml) was consists of mixture of acetonitrile and potassium phosphate buffer (pH 7.0 with 0.1% triethylamine) (60:40 v/v) in isocratic mode with flow rate 1 ml/min using 20 µl injection volume.

2.3. Evaluation of system suitability parameters

The system suitability test was performed by collecting data from five replicate injections (20 µl) of mixed standard solution (containing ESC 40 µg/ml & FLU- 2 µg/ml in methanol) at selected chromatographic conditions. The studied parameters includes retention time, resolution, AUC, HETP and tailing factor.

2.4. Tablet assay

Average weight of 20 tablets was determined and were then crushed to fine powder. Average power equivalent to 40 mg of ESC (also contain 2 mg of FLU) was weighed accurately and was transferred to 100 ml volumetric flask. To this 20 ml of methanol was added and shaken for 30 min and sonicated for 10 min. Final volume was added up to 100 ml with same solvent. The solution was filtered the whatman filter paper. About 10 ml of above solution was diluted to 100 ml with methanol. The contained 40 µg/ml of ESC and 2 µg/ml of FLU. About 20µl sample solution was injected into the system and concentration of each drug was calculation from respective regression equation prepared for individual drug using AUC.

2.5. Validation of method

Studied validation parameters includes accuracy and precision, linearity & range, LOD (limit of detection) & LOQ (limit of quantitation) and robustness [39].

2.6. Accuracy & precision

To study the accuracy and precision, recovery study was carried out by addition of standard drugs solutions to preanalysed sample. Recovery study was undertaken at three levels i.e. 80%, 100% and 120%.

2.7. Linearity & range

Linearity was studied by injecting a series of dilutions of mixed standard stock solution in the concentration range 20-100 µg/ml (ESC) and 1-5 µg/ml (FLU) into the HPLC system using 20µl volume. Calibration graph was plotted as concentration versus AUC.

2.8. LOD & LOQ

The LOD & LOQ were confirmed by diluting known concentrations of drug until the average AUC were approximately 3 or 10 times the standard deviation of AUC of the blank for five replicate determinations. The signal/noise ratios 3:1 and 10:1 were taken as the LOD and LOQ, respectively.

2.9. Robustness

Robustness was studied by making changes in the chromatographic conditions, such as slight change in change in mobile phase flow rate (±0.1 ml/min), mobile phase composition ((±1%), and change in wavelength (±1 nm). Percent contents of drugs were measured in preanalysed tablet formulation

3. Results and discussion

On the basis of literature survey, combination of ESC and FLU was selected for RP-HPLC method development for simultaneous estimation of both from tablet dosage form. Solvent methanol was used to prepare standard and sample solutions as it dissolved both the drugs at selected concentration. Wavelength for detection selected was 231 nm because at this wavelength both the drug showed better sensitivity. Concentration selected were 40 µg/ml for ESC and 2 µg/ml for FLU. At selected chromatographic conditions i.e. mobile phase consisting of mixture of acetonitrile and

potassium phosphate buffer (pH 7.0 with triethylamine) in the ratio 60: 40 at a flow rate of 1 ml/min with Grace C18(4.6 mm I.D x 250 mm) column at ambient temperature, retention time obtained for ESC and FLU was 2.96 and 6.98 min, respectively (Figure 1). 0.1% triethylamine was used to correct the pH so as to get sharp peak with minimum tailing and fronting. Herein, ESC elutes first because of it is more polar in nature followed by less polar FLU [40-41].

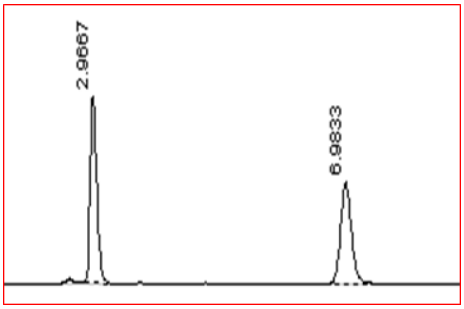

Figure 1 Chromatogram of ESC (t_R 2.96 min)and FLU(t_R 6.98 min) (mixed standard solution)

The validation study was performed as per ICH guidelines. Linearity and range was studied by using the series of dilution of each drug solution. Both the drugs shows linear response over the studied range. From this, concentration for ESC and FLU were selected. The LOD & LOQ were checked by diluting known concentration of standard drug until the mean responses were approximately 3 or 10 times the standard deviation of the responses of the blank for five replicate measurements. The signal/noise ratios 3:1 and 10:1 were considered as the LOD and LOQ, respectively. LOD and LOQ values obtained are given in Table 1. Precision of the method was checked by measuring system suitability parameter by replicate injection of mixed standard solution. The results are expressed % RSD.

Table 1 Results of the validation of method.

Study	**Parameters**	**Result**	
		ESC	**FLU**
Label claim*		10 mg	0.5 mg
% Recovery♣	80% level	99.25	99.48
	100% level	99.56	101.85
	120% level	100.05	100.75
Linearity and range	Range♣♣	15-75	1-5
	% RSD**	0.66	0.98
	R^2	0.992	0.999
	LOD	0.509	0.305
	LOQ	0.975	0.004
System suitability parameters	t_R	2.96	6.98
	Resolution	-	14.81
	AUC	475.63	422.16
	HETP	2683	14778
	Tailing Factor	1.33	1.62
Robustness#			
(i) Flow rate	0.9 ml/min	99.33	101.25
	1.1 ml/min	99.58	101.69
(ii)Mobile phase (a:b)##	61:39	101.18	99.28
	69:41	101.22	98.98
(iii) Intra- & Inter-day variation	Intra-day	100.34	99.15
	Inter-day	100.62	100.52

* Amount in mg/tablet; ** Mean of three results;
# %Contents of drugs were measured in preanalysed tablet formulation;
a- Acetonitrile, b- potassium phosphate buffer;
♣ Results are expressed in percentage, w/w;
♣♣ Concentration in µg/ml.

Recovery study was performed to determine the recovery of pure drugs from sample solution. Recovery study by standard addition method at three levels i.e. 80,100 and 120 %. The percentage recovery for both the drug was closed

to 100% w/w for both drugs. The percent contents of drugs were measured in preanalysed tablet formulation (Table 1). Precision was determined by studying system suitability parameters by injecting standard solution (Table 1).

The capacity of developed method was checked by performed robustness study. The conditions changed deliberately were change in flow rate (± 0.1), mobile phase composition (± 1), and wavelength (± 1), Intraday and inter-day variation and percent contents in formulation were estimated. The result showed develop method remain unaffected. Results of robustness study is represented in Figure 2.

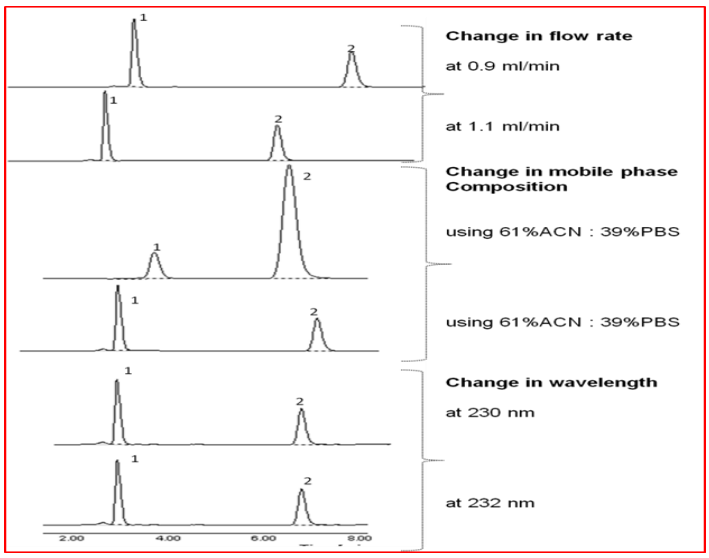

Figure 2 Chromatograms of robustness study (1- ESC, 2- FLU)

4. Conclusion

Novel RP-HPLC method for simultaneous analysis of escitalopram oxalate and flupentixol HCl from combined dosage form is simple, accurate and precise. It does not get affected upon smaller variation in experimental condition. Thus, It be used for routine quality control analysis of bulk drugs and marketed tablet dosage forms.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest

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