Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 2, 2015

Original Article

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SERTRALINE AND DOXOFYLLINE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

B. VICTORIA RANI*[1], C. PARTHIBAN[1], M. SUDHAKAR[2]

[1]Department of Pharmaceutical Analysis and Quality Assurance, Malla Reddy College of Pharmacy, Osmania University, Secunderabad 500014, India, [2]Department of Pharmaceutics, Malla Reddy College of Pharmacy, Osmania University, Secunderabad 500014, India. Email: victoria_b189@yahoo.com

Received: 29 Sep 2014 Revised and Accepted: 20 Oct 2014

ABSTRACT

Objective: The aim of this work was to develop and validate a simple Reversed Phase - High Performance Liquid Chromatography method for the simultaneous estimation of Sertraline and Doxofylline in pharmaceutical dosage form.

Methods: The mobile phase consists of Phosphate buffer and Acetonitrile in the ratio of (30:70) with gradiant programming, Kromasil C_{18}(150×4.6 mm,5µ) column used as stationary phase with a flow rate of 1 ml/min, injection volume 10 µl and the run time was 7 min. Detection wavelength was at 234 nm by using Photo Diode Array detector.

Results: The retention times of Doxofylline and Sertraline retention were found to be 2.82 min and 3.93 min, respectively. The method was validated according to ICH guidelines. Validation parameters like accuracy, precision, linearity, range, limit of detection, limit of quantification and robustness all were within the limits. The linearity responses of Doxofylline and Sertraline were found to be in the concentration ranges of 100-600 ppm and 12.5-75 ppm. The percentage recovery for both drugs were found in the range of 99-100%. The LOD & LOQ values for Doxofylline were found to be 0.58 and 1.77µg/ml and Sertraline were found to be 0.27 and 0.82 µg/ml, respectively.

Conclusion: The results obtained are accurate and within the limits. Hence this method can be applicable for the estimation of Doxofylline and Sertraline in pharmaceutical dosage forms.

Keywords: Doxofylline and Sertraline, RP-HPLC, Validation.

INTRODUCTION

Doxofylline (also known as Doxophylline) is a xanthine derivative drug used in the treatment of asthma. It has antitussive and bronchodilator effects, and acts as a phosphodiesterase inhibitor. Chemically, Doxofylline is 7-(1, 3-dioxolan-2-methyl)-1,3-dimethyl purine-2,6-dione and the structure shown in figure-1. Sertraline is an antidepressant of the selective serotonin reuptake inhibitor. It is primarily prescribed for major depressive disorder in adult as well as obsessive-compulsive, panic and social anxiety disorders in both adults and children. Chemically, Sertraline is (1S, 4S)-4-(3,4-dichlorophenyl)-N-methyl - 1, 2, 3, 4 tetrahydronaphthalen-1-amine and the structure shown in fig. 2.

The literature survey reveals that the few HPLC method was developed and validated for the estimation of Doxofylline and Sertraline combination with other drugs. Lijuan He etal., Determined Sertraline in Human plasma by HPLC- Electrosray Ionisation Mass Spectrometry[3]. HR Joshi etal., estimated Doxofylline in tablets by Spectrophotometric and RP-HPLC[4]. Hence, it is necessary to develop a rapid,accurate and validated RP-HPLC method for the determination of Doxofylline and Sertraline from combined dosage form.

The developed method validated according to ICH guidelines. Since there is no reported method on simultaneous estimation of Doxofylline and Sertraline in combined tablet dosage forms. The main objective of this study was to develop and validate the assay method of Doxofylline and Sertraline in tablet dosage forms.

METHODS AND METHODS

Materials

Acetonitrile and water of HPLC grade were procured from Rankem lab ltd. Doxofylline and Sertraline standards were received as gift samples from Hetero Drugs Limited, Hyderabad, India. Ortho phosphoric acid A. R grade was purchased from E. Merck chemicals, Mumbai, India. Tablet DOXODER having combination of Doxofylline (400mg) and Sertraline (50mg) was used.

Instrumentation

The High performance liquid chromatography system consists of Waters 2695 with 2996 module Photo Diode Array detector equipped with a quaternary solvent delivery pump, automatic sample injector and column thermostat. The system was controlled by Empower 2 software and it is used for the analysis.

S. No.	Name	Model
1	Weighing Balance	Deniver
2	pH meter	Poloman
3	Sonicator	Ulta sonicator
4	HPLC	Water 2695 with 2996 PDA detector

Method development

Standard stock solution preparation

Weigh and transfer 40mg of Doxofylline working standard and 5mg of Sertraline working standard into a 10 ml clean dry volumetric flask, add 7 ml of diluent, sonicated for 5 minutes and make up to the final volume with diluent.

Standard preparation

Transfer 1 ml from the above stock solution was taken into a 10 ml volumetric flask and dilute to volume with diluent. The standard solution consists of 400µg/ml of Doxofylline and 50µg/ml of Sertraline, respectively.

Sample preparation

Finely grind pre-weighed twenty tablets. Transfer grinded sample quantitatively equivalent to 40mg Doxofylline and 5mg of Sertraline into a 100 ml volumetric flask, add 70 ml of diluent, sonicate to

dissolve for 25 min, and the dilute to volume with diluent. Further filter the solution through filter paper. From the filtered solution 1 ml was pipetted out into a 10 ml volumetric flask and the volume made up to 10 ml with diluent then the solution consist of 400µg/ml of Doxofylline and 50µg/ml of Sertraline.

RESULTS AND DISCUSSION

System Suitability

The system suitability tests were conducted before performing the validation and the parameters were within the acceptance criteria like retention times of Doxofylline and Sertraline were 2.82 minutes and 3.93 minutes, respectively. The plate count was >2000, peak tailing was <2 and the %RSD of peak areas of standard were ≤ 2.(Table 1), (fig. 3). Hence the proposed method was successfully applied to routine analysis without any problems.

Linearity

The linearity of Doxofylline and Sertraline were prepared in the range of 100-600µg/ml and 12.5-75µg/ml. These were represented by linear regression equation (Doxofylline) y=9208.7x+1630.4 (r=0.999), (Sertraline)y=7411.1x+606.64 (r=0.999). From the calibration curve the regression line for both drugs was linear (Table 2).

Precision

Injected standard preparation six times in same concentration in to the system. The precision of analytical method expresses closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous under the prescribed conditions. Reproducibility and Repeatability for Doxofylline and Sertraline was shown in (Table 3). This indicated the method was highly precise.

Accuracy

The percentage recoveries for Doxofylline and Sertraline was found to be 98-120% and the %RSD for Doxfylline and Sertraline was found to be 0.34 and 0.63. The results of recovery studies was shown in (Table 4).

Robustness

Robustness data for Doxofylline and Sertraline by changing the parameters like flow rate, temperature and mobile phase ratio. It was shown in (Table 5).

Limit of detection and limit of quantification

The values of LOD and LOQ were calculated by using slope and Y-intercept. The LOD and LOQ values for Doxofylline was found to be 0.58 and 1.77µg/ml and Sertraline was found to be 0.27 and 0.82µg/ml, respectively (Table 6).

Assay

The content of Doxofylline and Sertraline in the pharmaceutical dosage forms by using the developed method. The percentage purity of Doxofylline and Sertraline was found to be 99.85 % and 99.96% and %RSD values for both Doxofylline and Sertraline was within limit of ≤2 (Table 7).

Table 1: System suitability of Doxofylline and Sertraline

S. No.	Doxofylline				Sertraline			
	Rt(min)	Area	USP Plate count	USP tailing	Rt (min)	Area	USP Plate count	USP tailing
1	2.803	3670119	8104	1.17	3.924	362952	7009	1.16
2	2.807	3624766	8093	1.17	3.932	363964	7387	1.21
3	2.808	3639058	8158	1.17	3.932	363266	7404	1.19
4	2.818	3645506	8217	1.16	3.934	363258	7235	1.15
5	2.823	3599764	8210	1.16	3.949	366194	7413	1.14
Mean		3635842				363927		
SD		25994.94				1320.58		
%RSD		0.71				0.36		

SD=Standard deviation; RSD= Relative Standard deviation; Rt= Retention Time

Table 2: Linearity of Doxofylline and Sertraline

Doxofylline		Sertraline	
Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
100	959750	12.5	92562
200	1805512	25	188687
300	2765110	37.5	278100
400	3624173	50	364097
500	4700041	62.5	475030
600	5494993	75	551182

Table 3: Repeatability and Intermediate Precision of Doxofylline and Sertraline

Drug	Repeatability			Intermediate precision		
	Peak area (N = 6)	Std. Dev	%RSD	Peak area (N = 6)	Std. Dev	%RSD
Doxofylline	3634622	24031.21	0.66	3642856	21886.5	0.6
Sertraline	364706	2675.235	0.73	358767	5311.7	1.5

Doxofylline

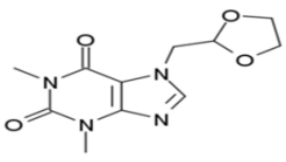

Fig. 1: Structure of Doxofylline Sertralline

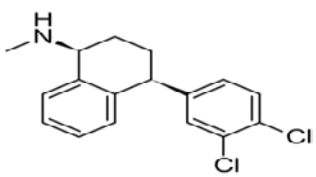

Fig. 2: Structure of Sertraline hydrochloride.

Table 4: Accuracy of Doxofylline and Sertralline

Accuracy level (%)	Doxofylline				Sertraline			
	Sample conc (µg/ml)	Added Conc (µg/ml)	Found Conc (µg/ml)	% Recovery	Sample Conc (µg/ml)	Added Conc (µg/ml)	Found Conc (µg/ml)	% Recovery
	400	200	202.22	101.11	50	25	24.81	99.27
50%	400	200	202.08	101.04	50	25	25.13	100.54
	400	200	201.46	100.73	50	25	25.26	101.07
	400	400	411.84	101.47	50	50	50.68	101.37
100%	400	400	401.36	100.34	50	50	50.6	101.20
	400	400	401.12	100.28	50	50	50.04	100.09
	400	600	600.72	100.12	50	75	74.70	99.61
150%	400	600	602.76	100.46	50	75	75.41	100.55
	400	600	599.82	99.97	50	75	75.60	100.81
Mean				100.61				100.50
SD				0.5053				0.7198
%RSD				0.50				0.71

Table 5: Robustness of Doxofylline and Sertraline

Parameters		Doxofylline			Sertraline		
		Mean Area	SD	%RSD	Mean Area	SD	%RSD
Flow rate	0.9 ml/min	3634346	183091.1	0.5	354211	4409.3	1.2
	1.0 ml/min	3635842	25994.9	0.7	363927	1320.5	0.3
	1.1 ml/min	3598463	14749.0	0.4	351526	4316.3	1.2
Temperature	25^{0}C	3524817	8670.3	0.2	346874	1168.8	0.3
	30º C	3635842	25994.9	0.7	363927	1320.5	0.3
	35^{0}C	3559288	4145.0	0.1	348047	3457.3	1.0
Mobile phase	29:71	3526011	10358.5	0.3	347571	182.4	0.1
	30:70	3635842	25994.9	0.7	363927	1320.5	0.3
	31:69	3555832	25306.5	0.7	348190	3463.4	1.0

Table 6: Limit of Detection and Limit of Quantification

S. No.	Parameters	Doxofylline	Sertralline
1	LOD	0.58	0.27
2	LOQ	1.77	0.82

Table 7: Assay of Doxofylline and Sertraline

	Label Claim		Amount found	
Doxoder	Doxofylline 400mg	Sertralline 50mg	Doxofylline 399.42 ± 0.28	Sertralline 49.98 ± 0.44

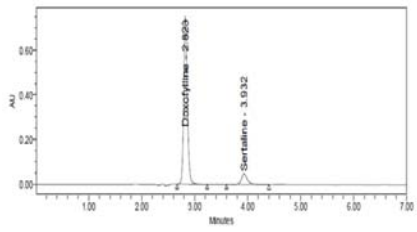

Fig. 3: HPLC chromatograph of standard solution (Doxofylline and Sertraline)

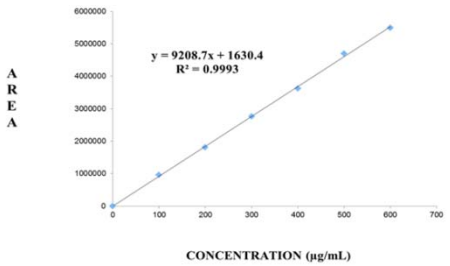

Fig. 4: Linearity graph of Doxofylline

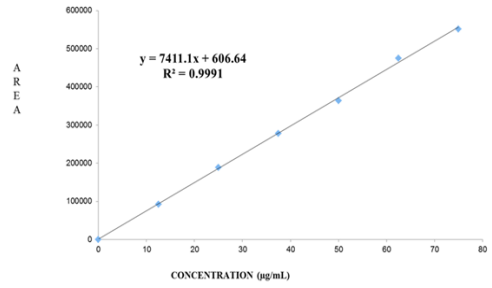

Fig. 5: Linearity graph of Sertraline

CONCLUSION

The proposed analytical technique of RP-HPLC is simple, accurate and extensively used method for the simultaneous estimation of Doxofylline and Sertraline in pharmaceutical dosage forms has been developed. The method was validated as per ICH guidelines. Statistical analysis proves that method is repeatable, sensitive for the analysis of Doxofylline and Sertraline in pharmaceutical dosage forms.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Giriraj, Shajan A. Simultaneous estimation and Method validation of Montelukast sodium and Doxofylline in Solid Dosage form by RP-HPLC. Int J Chem Pharm Sci 2011;2(1);31-6.
2. K Venkateswarlu, RK Venisetty, NR Yellu, S Keshetty, MG Pai. Develpoment of HPTLC-UV absorption densitometry method for the analysis of alprazolam and sertraline in combination and its application in the evaluation of marketed preparations. J Chromatogr Sci 2007;45(2);537-9.
3. Lijuan He, Fang Feng, Jie Wu. Determination of sertraline in Human plasma by HPLC-Electrosray Ionisation mass spectrometry and method validation. J Chromatogr Sci 2005;2(3);43.
4. HR Joshi, AH Patel, AD Captain. Spectrophotometric and RP-HPLC method for the determination of Doxofylline in pharmaceutical formulations. J Young Pharm 2010;2(3);289-96.
5. R Revathi, T Ethiraj, V Ganesan. HPLC method development for the simultaneous analysis of Doxofylline and Montelukast sodium in combined form. Pharm Methods 2011;2(4);223-8.
6. G Samanthula, Krishnaveni Yadhiki, KV Surendranath. Stability indicating RP-HPLC method for the simultaneous estimation of Doxofylline and Terbutalinesulphate in Pharmaceutical formulations 2013;81(4);969-82.