

<https://doi.org/10.46344/JBINO.2021.v10i02.30>

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PAROXETINE HYDROCHLORIDE AND ETIZOLAM IN PHARMACEUTICAL DOSAGE FORM

Radharapu Satchitkrishna* , Patel Dhara, Meshram Dhananjay.

Department of Quality Assurance, Pioneer Pharmacy Degree College, Vadodara, Gujarat, India.

(Received on Date: 22 February 2021

Date of Acceptance: 18 March 2021

Date of Publish: 19 March 2021)

Email address: patel.dhara.j@gmail.com

ABSTRACT

A simple, sensitive, precise, rapid and accurate reverse phase high performance liquid chromatography method has been developed and validated for simultaneous estimation of Paroxetine HCl and Etizolam in combined pharmaceutical dosage form. The chromatographic separation was performed on Hypersil BDS C18 column ((150 x 4.6 mm, 5 μ particle size). Mobile Phase consisted of mixture of 30 mM phosphate buffer pH 4.5 and ACN in ratio of 40: 60 % v/v at a flow rate 1.0 ml/min .The detection wavelength was set at 249 nm. The proposed method was validated for linearity, accuracy, precision, LOD and LOD and robustness. The calibration was linear over the concentration range of 12.5-75 μ g/ml for Paroxetine hydrochloride and 0.5-3.0 μ g/ml for Etizolam. The retention times were found to be 4.7 ± 0.002 min for Paroxetine hydrochloride and $8.8 \text{ min} \pm 0.015$ min for Etizolam. The mean recoveries were 100.03 ± 0.09 and 99.45 ± 1.25 for Paroxetine hydrochloride and Etizolam, respectively. The method can be easily adopted for quality control analysis.

Keywords: Paroxetine HCl, Etizolam, Reverse phase High performance liquid chromatography, Validation.

Abbreviations: PAR- Paroxetine HCl;ETZ- Etizolam;Nm- Nanometer;ICH- International council for harmonisation;RP-HPLC- Reverse phase high performance liquid chromatography.

INTRODUCTION

Chemically Paroxetine HCl (PAR) is known as (3S, 4R)-3-[(1, 3-benzodioxol-5-yl)methyl]-4-(4-fluorophenyl) piperidine hydrochloride. Pharmacological class of PAR is selective serotonin reuptake inhibitor and it is used as anti-depressant [1]. PAR is prescribed to treat various disorders such as depression, generalized anxiety disorder (GAD), panic disorders and post traumatic stress disorder (PTSD) [2]. Mechanism of action of PAR is that it inhibits the reuptake of serotonin [3, 4]. Literature survey shows that less analytical methods were reported for quantification of PAR. Quantification of PAR in biological fluids and finished products is done by using UV spectrometric method [5], HPLC [6-10], HPTLC [11] and UPLC [12]. Chemically Etizolam (ETZ) is known as 7-(2-chlorophenyl)-4-ethyl-1,3-methyl-3-thia-1,8,11,12-tetraza-tricyclo[8.3.0.0]trideca-

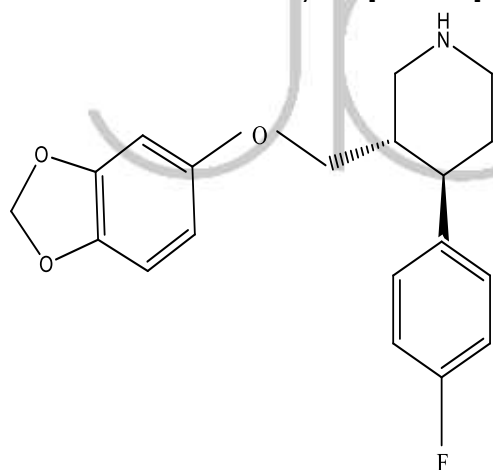


Figure 1: Chemical structure of PAR

MATERIALS AND METHODS

Apparatus

RP-HPLC instrument (Shimadzu, LC-2010CHT, Japan) equipped with a UV-Visible detector and a photodiode array detector, Hypercil BDS C18 column (150 x 4.6 mm, 5 μ particle size) was used.

2(6),4,7,10,12-pentaene. It belongs to chemical class of thienotriazolodiazepines. It is used to treat anxiety [13]. Many analytical methods are reported for estimation of ETZ individually or in combination with other drug some of them are HPLC [14], HPTLC [15], LC-MS [16, 17] and GC-MS [18, 19]. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for simultaneous estimation of PAR and ETZ in their capsule. Literature survey does not reveal any simple chromatographic method for simultaneous estimation of PAR and ETZ in synthetic mixture or dosage forms. The present paper describes simple, sensitive, rapid, accurate, precise and cost effective RP-HPLC method for simultaneous estimation of both drugs in capsule form.

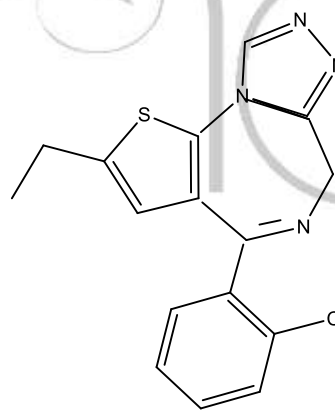


Figure 2: Chemical structure of ETZ

Chromatograms were automatically obtained by LC-Solution system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India), Nylon 0.45 μ m – 0.47 mm membrane filter. Whatman filter paper no. 41 (Millipore, USA), Digital

pH meter (Janki Impex, India) were used in the study.

Materials and Reagents

PAR bulk powder was kindly gifted by Zydus Cadila Healthcare pvt ltd, Dhabhasa, Vadodara, Gujarat. ETZ bulk powder was kindly gifted by Centaur Pharmaceuticals, Vakola, Mumbai, Maharashtra. HPLC grade acetonitrile (Finar Chemicals Ltd., Mumbai, India), NaH_2PO_4 and Na_2HPO_4 (S.D. Fine Chemicals Ltd., Mumbai, India) were used in the study. Water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 μm - 47 mm membrane filter.

Preparations of Mobile phase and Standard solutions (PAR and ETZ)

Acetonitrile and 30 mm phosphate buffer (pH adjusted to 4.5 using ortho phosphoric acid (OPA) solution in ratio of 60:40 v/v was use as mobile phase. The primary standard stock solution of PAR and ETZ were prepared by dissolving 100 mg drug in 100 ml HPLC grade methanol to obtain 1000 $\mu\text{g}/\text{ml}$ solution. By diluting 2.5 ml of PAR and 10 ml of ETZ from primary stock solution to obtain 250 $\mu\text{g}/\text{ml}$ PAR and 100 $\mu\text{g}/\text{ml}$ ETZ (secondary stock solutions). By diluting different aliquots from secondary stock solution of PAR (0.5, 1, 1.5, 2, 2.5 and 3 ml) and ETZ (0.05, 0.1, 0.15, 0.20, 0.25 and 0.30 ml) working solutions of different concentrations were obtained for both the drugs.

Preparations of Sample solutions

For estimation of both the drugs in the commercial formulations, twenty capsules were weighed and average weight was calculated. The powder equivalent to 12.5 mg PAR and 0.5 mg of ETZ were transferred to 100 ml volumetric flask consisting of 30 ml methanol. Then methanol was filled up to the mark of volumetric flask. Concentrations obtained were 125 $\mu\text{g}/\text{ml}$ (PAR) and 5 $\mu\text{g}/\text{ml}$ (ETZ).

Methodology

To optimize the RP-HPLC parameters, different mobile phase compositions were tried. An adequate separation and good peak symmetry for PAR and ETZ was obtained with a mobile phase consisting of acetonitrile and 30 mm phosphate buffer (pH adjusted to 4.5 by using ortho phosphoric acid (OPA) solution in ratio of 60:40 v/v at a flow rate 1 ml/min to get better reproducibility and repeatability. Quantification was carried out at 249 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Figure 3). Optimized chromatographic conditions and system suitability test parameters for PAR and ETZ for the proposed method are reported in Table 1 and 2, respectively. Overlain UV spectrum showed that both drugs showed good absorbance at 249 nm, hence the wavelength of 249 nm was selected for quantification of PAR and ETZ (Figure 4)

Table 1: Optimized chromatographic conditions for PAR and ETZ

Method Parameter		Optimized Value
Stationary phase		Hypersil BDS column C18 column (250mm × 4.6mm i.d., particle size 5mm)
Mobile phase		30 mm Phosphate buffer : ACN 40:60 v/v (4.5 pH)
Elution mode		Gradient
Flow rate		1ml/min
Run time		20 min.
Retention time(min)	PAR	4.7 min ± 0.02
	ETZ	8.8 min ± 0.15
Wavelength detection		249 nm
Injection Volume		20µl

METHOD VALIDATION

Specificity: By injecting the sample solutions, standard solutions, placebo and blank solution the specificity of the method was studied.

System suitability testing

For this study blank run of 20 µl ACN solution was given for 12 mins. Further 6 replicates of 20 µl standard solutions were injected and %RSD of the response peaks was calculated.

Calibration curve (Linearity of HPLC method)

Linearity of both the drugs was found out by plotting the graph between the concentration Vs peak area. For PAR graph was found linear over the concentration range of 12.5-75 µg/ml and for ETZ it was found from 0.5-3 µg/ml. For PAR and ETZ correlation coefficient was found to be 0.998 and 0.998 respectively while Retention time of PAR and ETZ was found to be 4.7 (± 0.04) min and 8.8 (± 0.15) min respectively.

Precision (Repeatability)

Under optimised parameters of proposed method peak area of six replicates of PAR (37.5 µg/ml) and ETZ (1.5 µg/ml) solution were recorded and the result was reported in %RSD.

Intermediate precision

It includes interday and intraday precision. In interday three concentrations were selected from calibration curve of PAR (12.5, 37.5 and 75 µg/ml) and ETZ (0.5, 1.5 and 3 µg/ml) and they were analyzed for 3 different days over a period of 1 week. In intraday three concentrations of PAR (12.5, 37.5 and 75 µg/ml) and ETZ (0.5, 1.5 and 3 µg/ml) were scanned for 3 times during the same day. The result of these parameters reported in terms of relative standard deviation.

Accuracy (% Recovery)

By using standard addition method accuracy of the method was studied at three different levels 80%, 100% and 120%. To achieve 80, 100 and 120% levels a known amount of standard solution of

PAR (20, 25, 30 µg/ml) and ETZ (0.8, 1, 1.2 µg/ml) were added to prequantified sample solutions. From these sample solutions recovery study of both the drugs were studied.

Robustness

This parameter was studied by making the deliberate changes in optimised chromatographic conditions. Flow rate was changed from 1 ml/min to 0.9 ml/min and from 1 ml/min to 1.1 ml/min. The ratio of mobile phase (Phosphate buffer (pH 4.5): ACN) was also changed from 40: 60 to 42: 58 and 38: 62. At three different wavelengths that are 248, 249 and 250 nm standard solution of both the drugs was scanned. All the above mentioned parameters were studied at three levels and it was concluded that the method is robust because no change in results was observed by changing the parameters.

Limit of Detection (LOD) and Limit of Quantification (LOQ).

As per ICH guidelines LOD and LOQ of PAR and ETZ was calculated by using the following equations.

$$\text{LOD} = 3.3 \times \sigma / S \quad \text{LOQ} = 10 \times \sigma / S$$

Where, σ = Standard deviation of response, S = Slope of regression equation.

RESULTS AND DISCUSSIONS

Different mobile phase compositions were tried to attain the good separation and symmetry peaks of both the drugs. By using mixture of 30 mm phosphate buffer (pH 4.5): ACN as mobile phase in ratio of 40: 60 (%V/V) good peak symmetry and separation was achieved. Quantification was done at 249 nm by

using UV detection. Retention time of PAR and ETZ was found to be 4.7 and 8.8 mins respectively. A representative chromatogram was shown in figure 3. From specificity studies it was assured that peak response produced by Paroxetine HCl and Etizolam was without any interference of excipients, impurities and degradation products (figure 5 and 6). By injecting 20 µl freshly prepared standard solution of PAR and ETZ system suitability parameters were studied and summarized in **Table 2**. Calibration curve of PAR and ETZ was plotted between peak area vs concentration and it was found to be linear over the concentration range of 12.5-75 µg/ml and 0.5-3 µg/ml respectively. All the linearity data were shown in Figure 7, 8 and 9. Interday and intraday precision values for PAR and ETZ was found to be within acceptance limit (**Table 3**). The proposed method was found to be accurate because mean %drug recovery of PAR and ETZ was found to be 100.06 and 99.45 respectively which was found to be in acceptance criteria (**Table 4**). Robustness of given method was studied by making deliberate changes in flow rate, mobile phase composition and wavelength. However, from results it was found that the method is robust (**Table 5**). The limit of detection of PAR and ETZ was found to be 0.09 and 0.04 while limit of Quantification of PAR and ETZ was found to be 0.27 and 0.13 respectively (**Table 6**). These values indicate the method is sensitive.

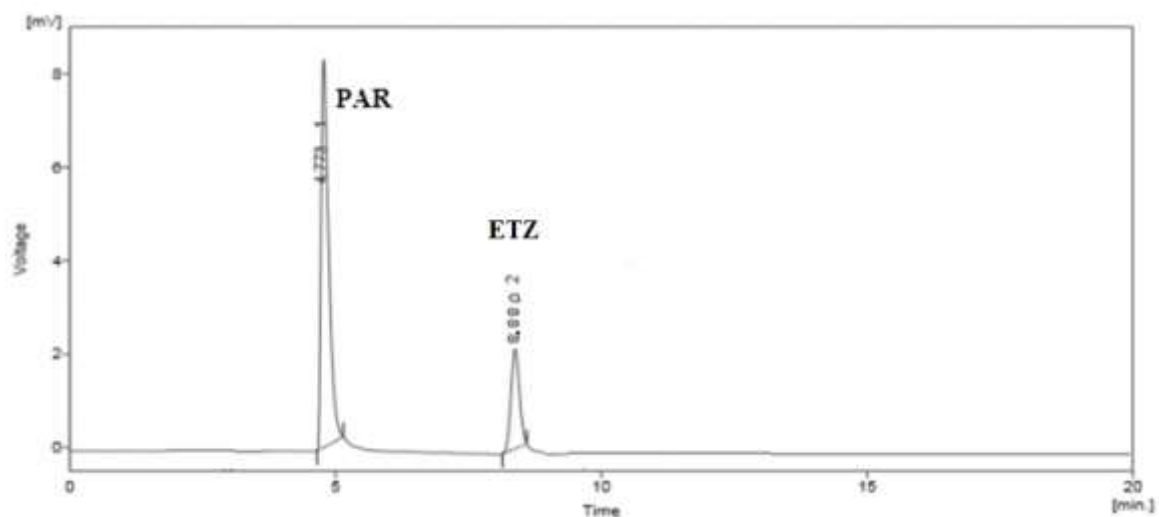


Figure 3: Chromatogram of standard solution of (PAR +ETZ) using optimized conditions

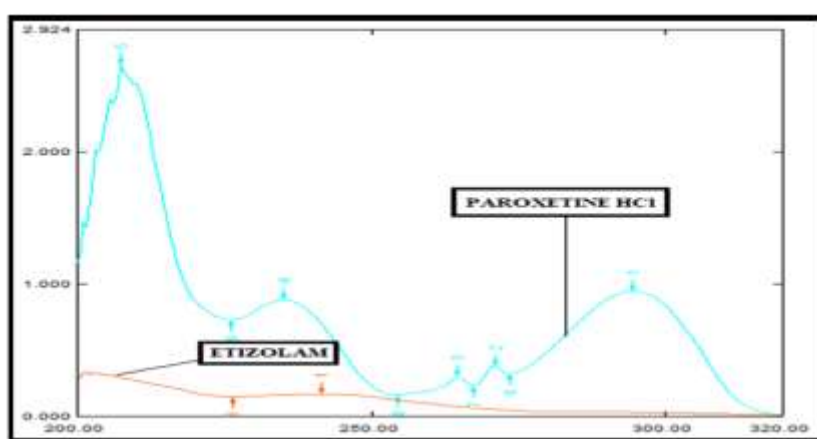


Figure 4: Overlain UV spectrum of PAR and ETZ

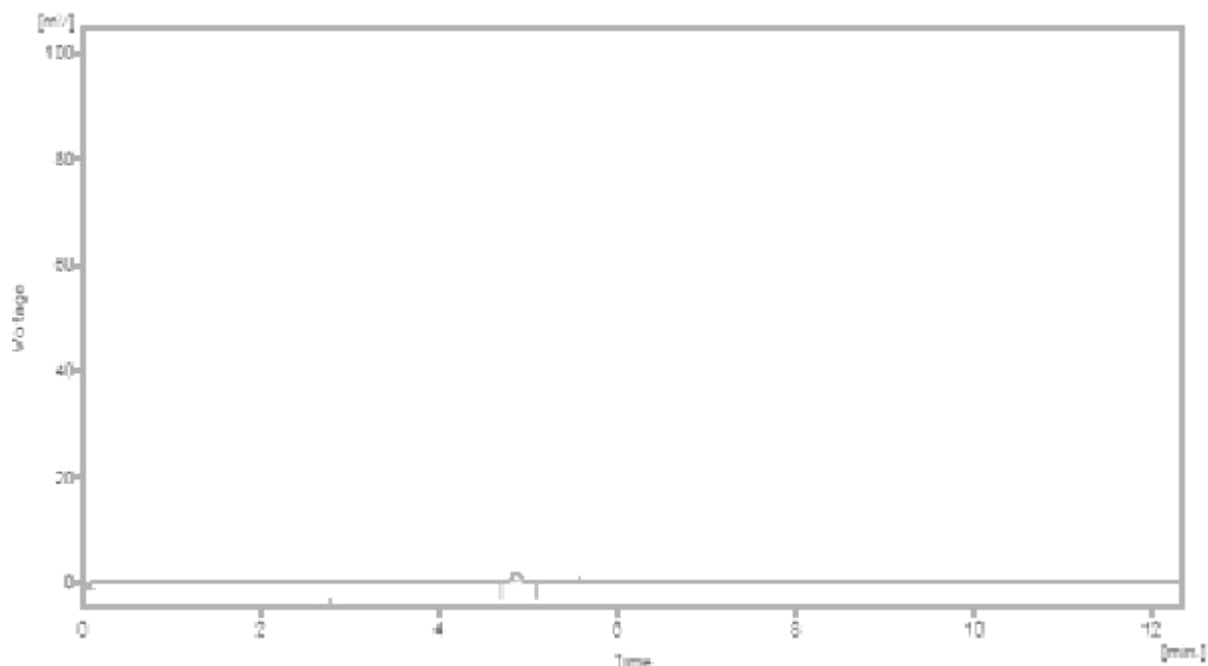


Figure 5: Chromatogram of Blank using optimized condition

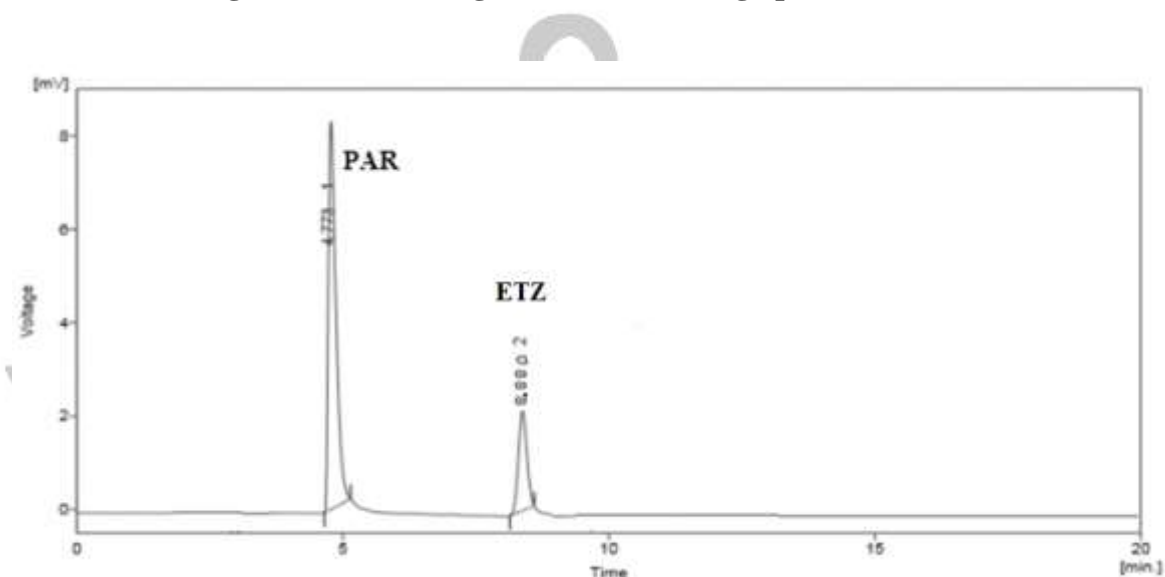


Figure 6: Chromatogram of sample solution containing PAR and ETZ (37.5 : 1.5 µg/ml)

Table 2: System suitability testing parameters

Parameters	Observed Results ± SD (n=6)		%RSD		Acceptance criteria
	PAR	ETZ	PAR	ETZ	
Retention time (Rt)	4.7 ± 0.002	8.8 ± 0.015	0.04	0.15	%RSD < 2
Peak area	906.41 ± 3.802	256.89 ± 3.00	0.41	1.17	%RSD < 2
Theoretical plates	6958 ± 83.34	2531 ± 6.11	1.68	0.24	>2000

(N)					
Tailing factor(N)	1.306 ± 0.012	1.30 ± 0.02	0.93	1.53	T ≤ 1.5
Resolution(Rs)	10.4		0.7		>2

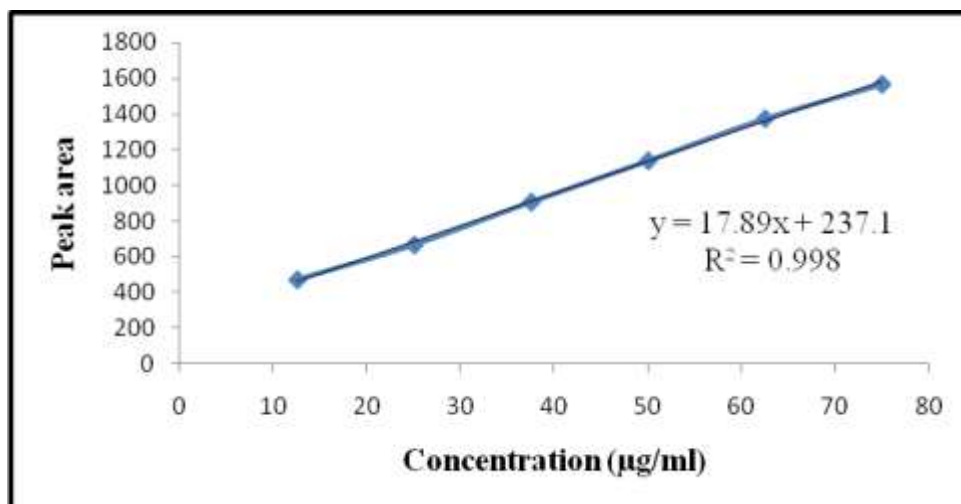


Figure 7: Calibration curve of PAR (12.5 -75 µg/ml)

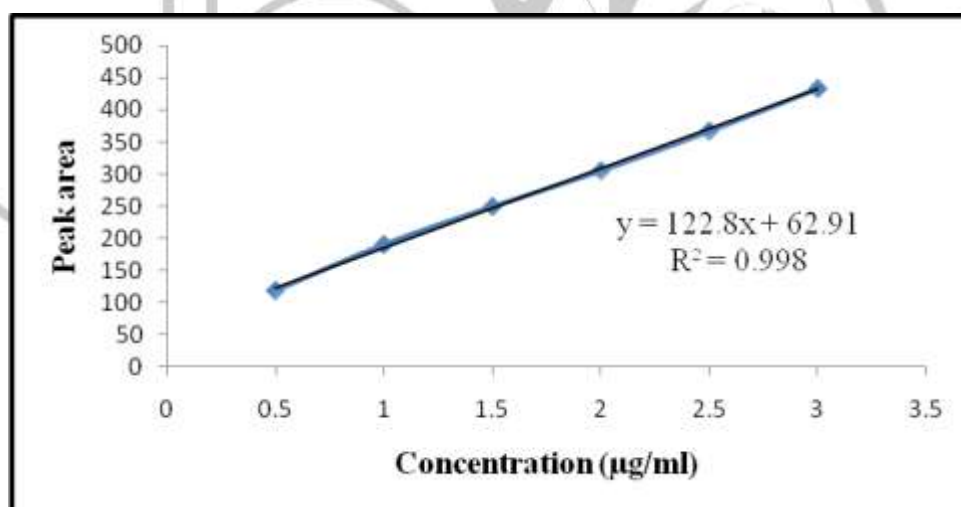


Figure 8: Calibration curve of ETZ (0.5-3.0)

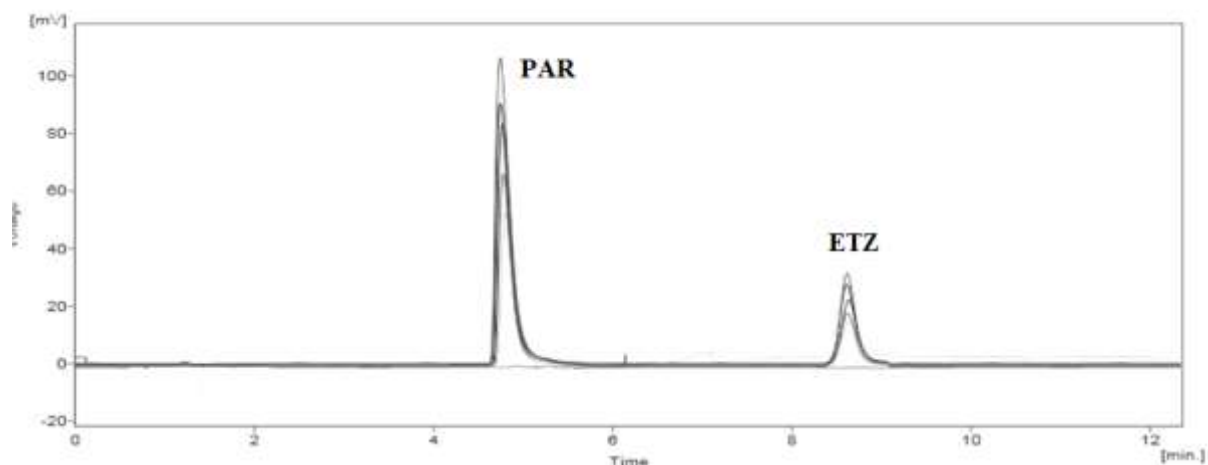


Figure 9: Overlain chromatogram of PAR (12.5 -75 µg/ml)and ETZ(0.5-3.0 (µg/ml)

Table 3: Repeatability of PAR and ETZ

Drug	Con.(µg/ml)	Peak area (n=6)	% RSD
PAR	37.5	902.57	0.60
ETZ	1.5	247.64	1.55

Table 4: Interday and Intraday precision of PAR and ETZ

Con.(µg/ml)		Interday		Intraday		% RSD			
		Mean of peak area		Mean of peak area		Interday		Intraday	
PAR	ETZ	PAR	ETZ	PAR	ETZ	PAR	ETZ	PAR	ETZ
12.5	0.5	456.325	119.051	482.700	119.968	0.87	1.34	1.58	1.95
37.5	1.5	903.737	242.037	902.33	250.131	1.15	1.53	0.83	1.33
75	3	1569.514	444.982	1574.969	433.568	0.37	1.25	0.79	1.25

Table 5: Recovery studies of PAR and ETZ

Drug	Spiked level %	Amount taken (µg/ml)	Amount found (µg/ml)	% Recovery	Mean recovery± SD
PAR	80	45	44.98	99.95	100.03± 0.09
	100	50	50.05	100.01	
	120	55	55.08	100.14	
ETZ	80	1.8	1.77	98.33	99.45±1.25
	100	2	2.01	100.5	
	120	2.2	2.19	99.54	

Table 6: Results of Robustness study

Sr no	Parameter	Conditions	Peak area		Tailing factor		Retention time	
			PAR	ETZ	PAR	ETZ	PAR	ETZ
1	Flow rate (1.0 ± 0.1 ml/min)	0.9	900	252	1.32	1.30	4.79	10.14
		1.0	910	255	1.34	1.32	4.77	9.90
		1.1	915	258	1.36	1.28	4.74	9.84
2	Mobile phase (± 2% V/V)	42:58	920	261	1.13	1.39	4.82	10.04
		40:60	925	265	1.15	1.37	4.77	9.91

		38:62	928	268	1.17	1.35	4.67	9.94
3	Wavelength(± 1 nm)	248	927	256	1.23	1.27	819	231.56
		249	930	259	1.25	1.28	825	239.54
		250	940	264	1.27	1.30	837	235.55

Table 7: LOD and LOQ values for PAR and ETZ

Drug	LOD ($\mu\text{g/ml}$)	LOQ ($\mu\text{g/ml}$)
PAR	0.09	0.27
ETZ	0.04	0.13

Table 8: Assay of marketed formulation

Capsule	Label claim (mg)	% Assay \pm SD (n=6)
PAR	12.5	100.08 \pm 0.0011
ETZ	0.5	100.38 \pm 0.0069

CONCLUSION

RP-HPLC method was developed for the simultaneous estimation of PAR and ETZ in combined dosage form without prior separation. During estimation of both the drugs from the formulation another excipients present in the formulation had not shown any interference. Developed method was also successfully applied to formulation. Result of all the validation parameters were found within limits. Proposed HPLC method is simple, accurate, precise and cost effective so it can be used for routine analysis of PAR and ETZ in combined dosage form.

ACKNOWLEDGEMENT

Authors are very thankful to Zydus Cadila healthcare pvt ltd, Dhabhasa, Vadodara and Centaur pharmaceuticals, Vakola, Mumbai for providing the gift sample of Paroxetine HCl and Etizolam respectively. Authors also extend their thanks to management of Pioneer Pharmacy Degree College, Vadodara for providing the necessary facilities.

REFERENCES

- Potter WZ and Hollister LE, (2004) Basic and Clinical Pharmacology. (9th Edn), McGraw-Hill company, New York.
- Tucker P, Zaninelli R, Yehuda R, Ruggiero L, Dillingham K, Paroxetine in the treatment of chronic post traumatic disorder: results of a placebo controlled, flexible dosage trial, J Clin Psychiatry, 2001; 11: 860–868.
- Brown JH, Taylor P, Robert LJ, Marrow, (2001) The Pharmacological Basis of Therapeutics. McGraw-Hill company, New York.
- Sharma MC, Sharma S, Validated Simultaneous spectrophotometric estimation of Paroxetine HCl bulk and tablet dosage form using Ferric Chloride, J. of Optoelectro. and Biomed. Materials, 2010; 4: 185–189.
- Syed MR, Hashmi S, Naik JB, UV Spectrophotometric method development and validation for determination of Paroxetine hydrochloride in pharmaceutical dosage form. International Journal of Pharmacy

- and Pharmaceutical sciences, 2010; 2: 43-45.
6. John Lambropoulos, George A Spanos, Nick V Lazaridis, Method development and validation for the HPLC assay (potency and related substances) for 20 mg Paroxetine tablets, Journal of Pharmaceutical and Biomedical Analysis, 1999; 19: 793-802.
 7. Agrawal N, Esteve-Romero J, Dubey NP, Durgbanshi N, Bose D, Determination of Paroxetine in pharmaceutical preparations using HPLC with electrochemical detection, Open Analytical Chemistry Journal, 2013; 7:1-5.
 8. Munigela N, Babu JM, Anjaneyulu Y, K Naveen Kumar, Vyas K, Spectral characterization of degradation impurities of paroxetine hydrochloride hemihydrate Scientia Pharmaceutica, 2008; 76: 653.
 9. Knoeller J, Vogt-Schenkel R, Brett MA, A simple and robust HPLC method for the determination of Paroxetine in human plasma. Journal of Pharmaceutical and Biomedical Analysis, 1995, 13: 635-38.
 10. H Juan, Z Zhiling, L Huande, Simultaneous determination of fluoxetine, citalopram, Paroxetine, venlafaxin in plasma by high performance liquid chromatography-electrospray ionization mass spectrometry, J. Chromatogr. B, 2005; 820: 33-39.
 11. Venkatachalam, Chatterjee Vidya S, Stability indicating high performance thin layer chromatography determination of Paroxetine hydrochloride in bulk drug and pharmaceutical formulations. Analytica chimica acta, 2007; 598: 312-17.
 12. R Udhaya, Y Petla, A Naidu, T Rajasekhara Reddy, V Vidya Sagar, Validation of an UPLC-MS/MS method for simultaneous Quantification of two selective serotonin and nor epinephrine reuptake inhibitors and one selective serotonin reuptake inhibitor in human plasma. Der. Pharma Chemica 3 ; 2012: 1164-1173.
 13. Kumar vikram, Singh HP and Rathore R.P.S, Method development and validation of escitalopram oxalate and Etizolam by UV-Spectrophotometry. International journal of institutional pharmacy and life sciences, 2015; 5: 12-42.
 14. Tanaka E, Masaru T, Misawa S and Wakasugi C, Simultaneous Determination Of Twelve Benzodiazepines In Human Serum Using A New Reversed phase Chromatographic Column on A 2 microns Porous microspherical Silica Gel. Journal of Chromatography B Biomedical Application, 1996; 682: 173-178.
 15. Masaru T, Shibamoto A, Ritsuko W and Sosuke M, Simultaneous Determination of Triazolam, Etizolam And Their Metabolites By High performance Thin Layer Chromatography. Japanese Journal of Forensic Toxicology, 2003; 21: 150-151.
 16. Masaru T, Hideki S, Tatsuo S and Eisuke T, Simultaneous Determination of Triazolam, Etizolam and Their Metabolites by LC/ESI/TOF/MS. Japanese Journal of Forensic Toxicology, 2005, 23: 174-175.
 17. Lee XP, Kumazawaa T, Satoa J, Shojia Y, Hasegawaa C, Simple Method For The Determination Of Benzodiazepines In Human Body Fluids By High performance Liquid Chromatography-mass Spectrometry. Analytica Chimica Acta, 2003; 492: 223-231.
 18. Masaru T, Watanabe R, Masui S, Matoba R, Shinozuka T et al. Simultaneous Determination Of Triazolobenzodiazepine

Drugs And Their Metabolites By Ion Trap Gas Chromatography Tandem Mass Spectrometry. Analytical Sciences, 2001; 17: 11283-11286.

19. Inoue H, Maeno Y, Iwasa M, Matobab R and Nagao M, Screening

and determination of benzodiazepines in whole blood using solidphase extraction and gas chromatography/mass Spectrometry. Forensic Science International, 2000; 113: 367-373.

