

RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF ETIZOLAM AND PROPRANOLOL IN PURE AND ITS TABLET DOSAGE FORM

D. Umamaheshwari*, and B. Jayakar

Department of Pharmaceutical Analysis, Vinayaka Mission's College Of Pharmacy, Vinayaka Missions University, Salem –636 008, Tamilnadu, India.

ABSTRACT

A new reverse phase high performance liquid chromatography method has been developed for the simultaneous estimation of Etizolam and propranolol from their tablet formulation. The developed method was carried out on a C₁₈(250x4.6mm) 5.0 µm column using a mobile phase of 0.02M sodium phosphate w/v : acetonitrile. (60:40) pH 6.5 with triethylamine. The flow rate was 1.0 ml/min with detection at 240 nm. The retention time for Etizolam 3.183 mins and for propranolol hydrochloride 4.863 mins. Etizolam and propranolol showed a linear response in the concentration range 4-50 µg/mL, 4.5-50 µg/mL respectively. The result of analysis have been validated statistically and by recovery studies. The mean recoveries found for Etizolam was 99.98% and for propranolol was 100.09%. The developed method was found to be simple, accurate, precise and selective for simultaneous estimation of Etizolam and propranolol HCl in tablets.

Keywords: Etizolam, Propranolol HCl, RP-HPLC method validation.

INTRODUCTION

ETIZOLAM (Eti) is chemically 7-(2-chlorophenyl-4-ethyl-13-methyl-3-thia-1,8,11,12-tetraazatricyclo 8.3.0.0^{2,6}) trideca - 2(6), 4,7,10,12 pentane. Etizolam is one of the most widely prescribed drugs for the treatment of anxiety and has strong muscle relaxing properties. Propranolol (Pro) is chemically (2RS)-1-[(1-methylethyl)amino]-3-(naphthalen-1-yloxy)propan-2-ol.¹³ Propranolol Hydrochloride is a non-selective beta blocker, that is, it blocks the action of epinephrine on both β₁- and β₂-adrenergic receptors.

Literature survey reveals few analytical methods like UV⁹, HPLC¹⁰, HPTLC¹¹ and GC¹² are reported for the estimation of propranolol in single and other combined dosage forms.

For Etizolam, few analytical methods that have been reported for the determination of etizolam using solid phase extraction with GC-MS⁶, high performance liquid chromatography (HPLC)⁷ and capillary gas chromatography-mass spectrometry.⁸

The combination of Etizolam and Propranolol has recently been introduced into the market. Our study made an attempt to develop simple, accurate and reproducible RP-HPLC method for the routine analysis of these drugs simultaneously in tablet dosage form.

EXPERIMENTAL

Drug and reagents

Sample of Etizolam and propranolol was received from MMC Healthcare Pvt. Ltd Baddi (H.P) Tablet purchased from the local market, HPLC grade Acetonitrile and AR Grade sodium phosphate were provided from Mark spewloties Pvt. Ltd. Mumbai. Triethyl amine is obtained from S.D fine chemicals Ltd. Mumbai.

Buffer

0.02M sodium phosphate and adjusted to pH 6.5 with triethylamine and filtered through 0.45 µm membrane.

Standard

The stock solution containing 4-50 $\mu\text{g ml}^{-1}$ of Etizolam and propranolol were prepared using mobile phase.

Chromatographic equipments and conditions

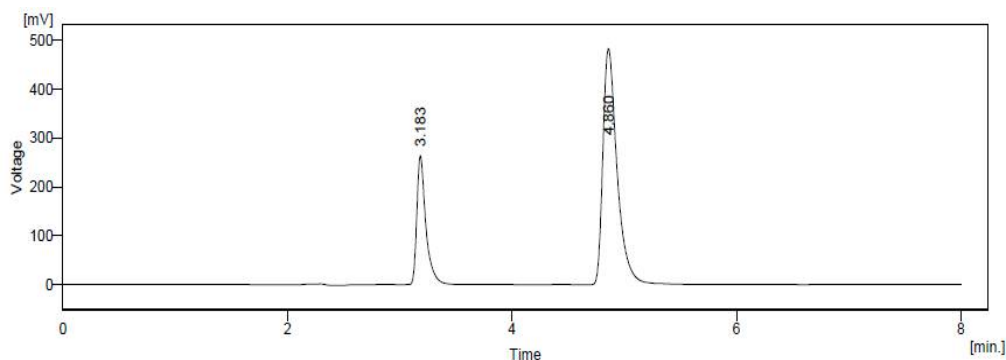
The liquid chromatogram was equipped with UV detector and Nuclosil C₁₈. (25cm x 4.6cm) column that contains 5 microns packing chemically bonded to porous particle; flow rate was 1.0 mL min⁻¹ and column temperature at 25^o C. the capacity of injected sample volume is 20 μl .

Mobile Phase

Filtered and degassed mixture of buffer and acetonitrile was prepared in the ratio 60:40% v/v, where the buffer was 0.02M sodium phosphate and adjusted to pH 6.5 using tri ethylene and it was filtered through 0.45 μm membrane.

Diluent-

Acetonitrile : Water(1:1 v/v) was used as a diluent.



Standard (I) chromatogram of Etizolam and Propranolol

Standard Preparations

The stock solution containing 80 to 120 $\mu\text{g mL}^{-1}$ of Etizolam and propranolol hydrochloride were prepared using mobile phase.

Table 2: Precision of HPLC method

Components	Concentration($\mu\text{g ml}^{-1}$)	N	System precision		Method precision	
			Peakarea	RSD%	Peakarea	RSD%
Etizolam	15	6	122247	0.375	122994	0.3
Propranolol	150	6	356904	0.322	353842	0.3

Table 3: Accuracy (Recovery Studies)

Parameters % recovery	Etizolam	Propranolol
80% level	99.98 %w/v	100.09 %w/v
100%level	100.1%w/v	98.31 %w/v
120%level	100.05%w/v	99.99 %w/v

Table 4: The determination of Etizolam and Propranolol Tablet dosageform

Components	Label claim (mg/tablet)	N	Amount present (mg/tablet)	Percentage Labelclaim (%w/v)
Etizolam	50	6	50.52	101%w/v
Propranolol	200	6	200.31	98.3%w/v

Linearity study

The calibration curves were obtained by diluting the stock solution in the mobile phase to furnish solution with final concentrations of 5, 10, 15 and 20 ml⁻¹ for Etizolam, 50, 100 and 150 µg ml⁻¹ for Propranolol standard and 20 µl injected individually, and response of concentration plotted against area obtained and the results were shown in Table-1.

Sample Preparation

Twenty tablet contents were weighed and crushed to form a fine powder. A quantity of powder equivalent to 10 mg of Etizolam, 800 mg of Propranolol in 100 ml of mobile phase. The powder was dissolved by sonication using sufficient amount of diluent and then made up to the mark with the same. The solution was filtered and 5 ml of the filtrate was diluted to 50 ml.

RESULTS AND DISCUSSION

The procedure for the simultaneous analysis of Etizolam and Propranolol using isocratic RP-HPLC has been reported. This work was focused on optimization of the conditions for the simple and rapid as well as low cost effective analysis including a selection of the proper column and mobile phase to obtain satisfactory results. To obtain satisfactory resolution and to avoid peak tailing of compounds an optimization of the proposed method was carried using the different mobile phases. Mobile phases of various compositions of acetonitrile-water were also tested. The best results were obtained using the mobile phase buffer-acetonitrile (60:40) adjusted to pH 6.5 with triethylamine.

The most reproducible results were obtained with C18 (250x4.6 mm) 5.0 µm detection was performed at 240 nm in the sensitivity range of 0.01.

Using buffer-acetonitrile (60:40 v/v) as a mobile phase at a flow rate of 1.0 ml min⁻¹. System suitability tests were performed and chromatographic parameters such as asymmetry factor, tailing factor, resolution, no. of theoretical plates and K_{prime} were calculated from experimental data were given in Table-1.

The validity of the liquid chromatographic assay was established through a study of linearity, sensitivity, system precision, method precision, accuracy, robustness and ruggedness.

The linearity was established with a series of working solution prepared by diluting the stock solution with dilution to the final concentration. Each concentration was injected in triplicate and the mean value of peak area was taken for the calibration curve.

A linear response in peak area ratios was observed over the concentration range 4-50 µg ml⁻¹ for Etizolam, 80-120 µg ml⁻¹ for Propranolol. The linear regression equations are $Y = 4395 - 42300$ ($r = 0.9997$) for etizolam, $Y = 19505 - 47000$ ($r = 0.9995$) for propranolol. The limits of detection were found to be 1.5 µg l for etizolam 0.7 µg ml⁻¹ for propranolol. The results of system precision and method precision were determined by 6 replicate injection of mixed standard solution of various concentrations was studied and the results were given in Table-2.

The accuracy of HPLC methods was confirmed by recovery by spiking 80, 100 and 120% of pure drugs to the preanalysed samples the recovery values for etizolam and propranolol were 99.98%-100.09, 100.1-98.31% w/v, and 100.05% -99.99% w/v respectively Table-3.

The robustness was performed by changing the detection wavelength ±5 nm, flow rate the flow rate ±10% and the results were interpreted by statistical analysis by calculating RSD values all the results were within the acceptance criteria of not more than 2%.

The ruggedness of the method was determined by the same assay by different instrument, different analyst and on different days and were within the acceptance criteria of not more than 2%.

The result is shown that the proposed RP-HPLC method provides better sensitivity in the assay, as well as significantly shorter analysis time.

CONCLUSION

RP-HPLC method is simple rapid and sensitive and therefore suitable for the routine analysis of etizolam and propranolol in its oral dosage form.

ACKNOWLEDGEMENTS

The authors are thankful to M/s The Madras Pharmaceuticals Ltd, Chennai for providing facilities for this work and they would also like to thank Biocon Ltd, Bangalore for providing gift sample for the study.

REFERENCES

1. Granville GR. Propranolol, Anxiety and the Central nervous system. *KBr J Clin Pharmacol.* 1974;1(5):361-3.
2. The Merck Index, an Encyclopedia of chemicals, drugs and biologicals. Fourteenth ed. NJ. 2006.
3. Patel VB, Chaudhary HR, Dave JB and Patel CN. Development and validation of spectrometric method for simultaneous estimation of escitalopram and etizolam in tablet dosage form by simultaneous

- equation method. *Inventi Rapid: Pharm Analysis & Quality Assurance*. Article ID- *Inventi:ppaqa/367/12*.
4. Sanghavi NM and Jivani NG. Estimation of propranolol hydrochloride. *Talanta*. 1980;27(7):591-2.
 5. Jayant BD, Vinay BP, Badmanaban R, Shital MP and Chhaganbahi N. Development and validation of UV-spectroscopic, HPTLC and RPHPLC methods for the estimation of etizolam in bulk and in tablet dosage form. *Inventi Rapid: Pharm Analysis & Quality Assurance*. 2012, Article ID-"*Inventi:ppaqa/433/12*."
 6. Casacchia M, Bolino F and Ecarl U. Etizolam in the treatment of generalized anxiety disorder. A double-blind study versus placebo. *Curr Med Res Opin*. 1990;12:215-23.
 7. Krishna R, William G and Richard EM. Assay of Total and Free Propranolol in Plasma by Liquid Chromatography with Fluorometric Detection. *Clin Chem*. 1985;31(1):131-4.
 8. Palak P and Kashyap KB. Development and Validation of HPTLC Method for Estimation of Propranolol Hydrochloride and Flunarizine Dihydrochloride in Combined Dosage Form. *International Scholarly Research Network*. 2012;2-6.
 9. Tulja RG, Gowri SD, Kadgpathi P and Satyanarayana B. A Validated RP HPLC Method for Simultaneous Determination of Propranolol hydrochloride and Alprazolam in Bulk and in Pharmaceutical formulations. *Journal of Pharmacy Research*. 2011;4:296-360.
 10. Salle ED, Baker KM, Bareggi SR, Watkins WD, Chidsey CA and Frigerio A. A sensitive gas chromatographic method for the determination of propranolol in human plasma. *Journal of Chromatography*. 1973;84:347-53.
 11. Prashanth KV and Basavaiah K. Simple and Rapid Spectrophotometric Determination of Propranolol Hydrochloride as Base Form in Pharmaceutical Formulation through Charge Transfer Complexation. *Chemical Sciences Journal*. 2012;CSJ-71.
 12. Suryakant B and Rajput SJ. RP-HPLC Method for simultaneous estimation of Escitalopram Oxalate and Etizolam in a tablet formulation. *J Pharm Biomed Sci*. 2012;19(19):2-4.
 13. Shivarkar NA, Dudhe PB, Nagras MA and Jain KS. Simultaneous Estimation of Flunarizinedihydrochloride and Propranolol hydrochloride in Bulk Drug and Capsule. *International Journal of ChemTech Research*. 2012;4(3):1007-12.
 14. Inoue H, Maeno Y, Iwasa M, Matoba R and Nagao M. Screening and determination Granville GR. Propranolol, Anxiety and the Central nervous system. *KBr J Clin Pharmacol*. 1974;1(5):361.