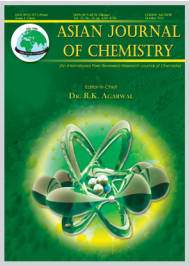
Asian Journal of Chemistry; Vol. 24, No. 4 (2012), 1883-1884

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



NOTE

RP-HPLC Estimation of Clebopride in Pure and Tablet Dosage Form

R. KALAICHELVI[1,*] and E. JAYACHANDRAN[2]

[1]K.C. Reddy Institute of Pharmaceutical Sciences, Jangamguntla Palem, Medikonduru Mandal, Guntur-522 348, India
[2]S.C.S. College of Pharmacy, Devanagari Dist, Harapanahalli-583 131, India

*Corresponding author: E-mail: rkselvi123@rediffmail.com

(*Received*: 2 May 2011; *Accepted*: 30 November 2011) AJC-10784

A rapid, accurate, sensitive and precise reverse phase high performance liquid chromatographic method was developed for the estimation of clebopride in pure and tablet dosage form. Separation of the drug was carried out on a reverse phase C_{18} column using a mobile phase consisting of 0.1 % phosphoric acid and acetonitrile in the ratio of 70:30 v/v. The flow rate was 1 mL/min. The detection of clebopride has done at a wavelength of 272 nm. The linearity was found in the range of 25-200 µg/mL with a correlation coefficient of 0.999. The proposed method was validated for its sensitivity, linearity, accuracy and precision. The developed RP-HPLC method was successfully applied for the quantitative determination of clebopride in tablets.

Key Words: Clebopride, RP-HPLC, Tablets.

Clebopride, N-(1'-benzyl-4'-piperidyl)-2-methoxy-4-amino-5-chlorobenzamide, is a dopamine antagonist drug with antiemetic and prokinetic properties used to treat functional gastrointestinal disorders. Detailed investigation at several centres has demonstrated its encouraging antiemetic, gastrokinetic and anxiolytic properties[1-3]. Literature survey reveals that the drug can be estimated by thin-layer chromatography and high-performance liquid chromatography[4-6], UV spectrophotometry[7], gas chromatography-mass spectrometry and radioimmunoassay in both animals[8] and man[9,10]. In this study a rapid, accurate, sensitive and precise reverse phase high performance liquid chromatographic method was developed for the estimation of clebopride in pure and in pharmaceutical dosage form.

The analysis of the drug was carried out on a Shimadzu HPLC system equipped with a reverse phase C_{18} column (250 mm × 4.6 mm; 5 µm), a LC-20AD pump, a 10 µL injection loop and a SPD-M20A detector and running on LC-solution software. A mixture of 0.1 % phosphoric acid and acetonitrile in the ratio of 70:30 v/v (pH 4.5) was found to be the most suitable mobile phase for ideal separation of clebopride. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. The column was maintained at 25 ± 2 ºC temperature. The detection of the drug was monitored at 272 nm. The run time was set at 10 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 3.96 min.

Preparation of standard solution: A standard stock solution of 1000 µg/mL was prepared by dissolving 100 mg of clebopride in 100 mL of solvent mixture 0.1 % phosphoric acid and acetonitrile (70:30 v/v). The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 500 µg/mL as a working standard solution of the drug.

Calibration plot: From this working standard solution, the dilutions ranging from 25-200 µg/mL were prepared in 10 mL volumetric flasks using the above solvent mixture. 10 µL of each dilution was injected 6 times into the column at a flow rate of 1.0 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 25-200 µg/mL of clebopride. This calibration curve was later used to estimate the amount of clebopride in tablets dosage form.

Validation of the proposedn method

System suitability parameters: System suitability tests were carried out on six replicate injections of the standard solution containing clebopride. The relative standard deviation for replicate injections, theoretical plates per meter and tailing factor were obtained.

Linearity: The calibration curve was obtained with five concentrations of standard solution (25-200 µg/mL). The

TABLE-1
ASSAY RESULTS AND PRECISION STUDIES

Sample	Labeled amount (mg/tablet)	Amount found (mg/tablet)	Label claim[a] ± SD (%)	Precision[b]	
				Inter-day	Intra-day
Clebopride tablets	0.5	0.499	99.99 ± 0.0003	0.1106	0.1104

a: Average of six determinations. b: RSD (%) of six determinations.

TABLE-2
RECOVERY STUDY

Sample	Label claim (mg/tablet)	Amount of drug added (µg/mL)	Amount of drug recovered (µg/mL)	Percentage recovery ± SD[a]
Clebopride tablets	0.5	25	25.13	100.53 ± 0.3314
		50	49.96	99.92 ± 0.1342
		75	75.01	100.01 ± 0.0555

a: Mean of six determinations.

solutions were prepared in six times and analyzed. The linearity was evaluated by linear regression analysis.

Precision: The precision of the assay was determined by repeatability and intermediate precision. Repeatability was evaluated by recording the chromatograms of 50 µg/mL concentration of the drug solution for six times. The intermediate precision was studied by assaying samples at same concentration and during the same day and comparing the assay on three different days.

Accuracy: Accuracy of the method was studied by recovery experiments. Recovery studies were carried out by adding a known quantity of pure drug to a pre-analyzed formulations and the proposed method was followed. From the amount of drug found, percentage recovery was calculated.

Limit of detection and limit of quantitation: The parameters LOD and LOQ were determined using the standard deviation of y-intercepts of regression lines and the slope.

Estimation of clebopride in tablet dosage form: Twenty tablets of clebopride were weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 5 mg of clebopride was dissolved in sufficient quantity of diluent, sonicated for 20 min, the volume was made up 25 mL with the same and the solution was filtered through a 0.45 µ membrane filter. From the filtrate, 5 mL of aliquot was taken in a separate 10 mL volumetric flask, the contents made up to the volume. The solution was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the calibration plot obtained for the pure drug.

In the proposed method, the retention time of clebopride was found to be 3.96 min. Linearity range was observed in concentration range of 25-200 µg/mL. The linearity curve was plotted by taking concentration (µg/mL) of clebopride in X axis and its peak area ratio in Y axis The regression equation was found to be Y = 35449X + 27486 (r = 0.999). The use of 0.1 % phosphoric acid and acetonitrile in the ratio of 70:30 v/v resulted in peak with good shape and resolution. The asymmetry factor was found to be 1.36, which indicated asymmetric nature of peak. The number of theoretical plates was found to be 6056, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.617 and 1.868 ng/mL, indicates the sensitivity of the method. Estimation of clebopride in tablet dosage form was carried out (Table-1). The proposed RP-HPLC method was also validated for intra-day and inter-day precision. No interfering peaks were found in the chromatogram within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by proposed RP-HPLC method. The high percentage of recovery of clebopride (Table-2) ranging from 99.92-100.53 % indicates that the proposed method is highly accurate.

Conclusion

The developed method was found to be simple, sensitive, precise, accurate and rapid for determination of clebopride from pure and tablet dosage forms. The mobile phase used in this method is simple to prepare and none of excipients present in clebopride tablets interfered in the estimation.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to the management, K.C.Reddy Institute of Pharmaceutical Sciences, Guntur for providing the necessary facilities to carry out the research work.

REFERENCES

1. J. Prieto, J. Moragues, R.G. Spickett, A. Vega, M. Colombo, W. Salazar and D.J. Roberts, *J. Pharm. Pharmacol*., **29**, 147 (1977).
2. P.N.C. Elliot, P. Jenner, G. Huizing, C.D. Marsden and R. Miller, *Neuropharmacology*, **16**, 333 (1977).
3. D.J. Roberts, *Curr. Thu. Res., (Suppl. IS)*, 31 (1982).
4. G. Huizing, A.H. Beckett and J. Segura, *J. Chromatogr*., **172**, 227 (1979).
5. J. Segura, I. Garcia, L. Borja, E. Tarrus and O.M. Bakke, *J. Pharm. Pharmacol*., **33**, 214 (1981).
6. D.J. Roberts, Laboratorios Almirall, Barcelona, Personal Communication (1985).
7. B. Thangabalan, A.E. Prabahar, R. Kalaichelvi and P. Vijayaraj Kumar, *Rasayan J. Chem*., **2**, 712 (2009).
8. Y. Hayasaka, S. Murata and K. Umemura, *Chem. Pharm. Bull*., **29**, 1478 (1981).
9. M. Yano, K. Nakamichi, T. Yamaki, T. Fukami, K. Ishikawa and I. Matsumoto, *Chem. Pharm. Bull*., **32**, 1491 (1984).
10. P.R. Robinson, M.D. Jones and J. Maddock, *J. Chromatogr*., **432**, 153 (1988).