ORIGINAL ARTICLE

QUANTITATION OF BUCLIZINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS AND HUMAN SERUM BY RP-HPLC

M. SAEED ARAYNE, NAJMA SULTANA* AND FARHAN AHMED SIDDIQUI

Department of Chemistry, University of Karachi, Karachi-75270, Pakistan

**Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi*

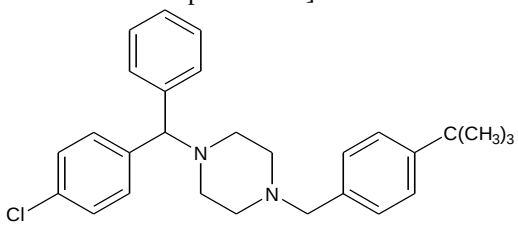
ABSTRACT

An isocratic reversed phase high-performance liquid chromatographic (HPLC) method with ultraviolet detection at 230 nm has been developed for the determination of buclizine hydrochloride in human serum and dosage formulation. Methylparaben was successfully used as an internal standard. Good chromatographic separation between buclizine and internal standard peaks was achieved by using a stainless steel analytical column Nucleosil, C_{18} (10μm, 25 cm x 0.46 cm). The system was operated at room temperature using a mobile phase consisting of acetonitrile–water (1:1) (pH 2.6) with phosphoric acid 85% at a flow rate of 2 ml/min. The calibration curve for buclizine hydrochloride in human serum was linear over the tested concentration range of 10, 3, 1.5, 0.5, 0.15, 0.05, and 0.025 μg/ml with a correlation coefficient of 0.9999. The intra- and inter-run precision and accuracy results were 98.07 to 100.34. The proposed method was validated for selectivity, linearity, accuracy, and precision. The method was found to be suitable for the quality control of buclizine hydrochloride in bulk drug as well as in human serum.

Keywords: Buclizine, methylparaben, UV detection, RP-HPLC, dosage formulation, method validation.

INTRODUCTION

Buclizine hydrochloride or (RS)-1-(4-tert-butylbenzyl)-4-(4-chlorobenzhydryl)piperazine dihydrochloride, is white or slightly yellowish, crystalline powder. It is piperazine derivative having antihistaminic, antimuscarinic, antiemetic, sedative properties and is used in motion sickness [The Merck Index (2001), Martindale, The Extra Pharmacopoeia. 1996, British Pharmacopoeia 2003].



Buclizine

In literature any single liquid chromatography method for the analysis of buclizine has not been reported, only one method of LC was reported (El Walily *et al*., 1999) in which buclizine was used as internal standard, but quantification was not done. The main purpose of this study was to develop a simple and reliable method to quantitate buclizine hydrochloride in a relatively short time with high linearity using a commercially available internal standard. Therefore, this study focused on the development of simple and rapid isocratic RP-HPLC method which can be employed for the routine analysis of buclizine hydrochloride in bulk drug formulations and in human serum. The established method was validated with respect to specificity, linearity, precision, accuracy and ruggedness.

Corresponding author: e-mail: arayne@gawab.com

EXPERIMENTAL

Materials and reagents

All chemicals and reagents were of analytical grade quality. Buclizine hydrochloride was supplied from AGP (Private) Limited. A stock solution of 100 ppm buclizine hydrochloride was prepared in acetonitrile and water (1:1) and stored in the dark at 4 °C. More dilute solutions were prepared daily with same solvent just before use.

Pharmaceutical dosage form

Longifene tablet, AGP (Private) Limited labeled to contain 25 mg buclizine hydrochloride drug.

Chemicals

HPLC grade acetonitrile and phosphoric acid were purchased from Merck Germany. Methylparaben was purchased from market. Other chemicals used were analytical grade. Longifene™ tablets were purchase from market.

Instrumentation and chromatographic conditions

An HPLC system consisted of an LC-10 AT VP Shimadzu pump, SPD-10AV VP Shimadzu UV visible detector, a Nucleosil, C_{18} (10μm, 25 cm x 0.46 cm) column was used for separation. The chromatographic and integrated data were recorded using a CBM-102 communication Bus Module Shimadzu. The separation was carried out under isocratic elution with mobile phase was acetonitrile and

water (80:20). The pH of this mobile phase was adjusted to 2.6 with phosphoric acid (85 %). The flow rate was 2.0 ml min^{-1}, the wavelength was monitored at 230 nm, and the injection volume was 10 µl. The assay procedure was performed using internal standard method with methylparaben as internal standard.

Preparation of standard and sample solutions

Standard preparation

Standard stock solutions of 100 ppm of buclizine hydrochloride and methylparaben in acetonitrile and water (1:1) were prepared in separate volumetric flasks. Working solutions were prepared by diluting the stock solutions with the same solvent to contain 10, 3, 1.5, 0.5, 0.15, 0.05, and 0.025 µg/ml. The resolution should not be less than 1.5 according to the USP 2005.

Procedure for longifene tablets

The drug content of twenty tablets was weighed, finely powdered and mixed. The average mass per tablet was determined. A quantity of the powder equivalent to 10 mg of buclizine hydrochloride was transferred accurately into a 100-ml calibrated dark flask containing acetonitrile and water mixture (1:1). The content of the flask was shaken for about 60 min and diluted to volume with same solvent. The solution was then filtrated through a 0.45-µm milli-pore filter (Gelman, Germany), that to separate out the insoluble excipients, rejecting the first portion of the filtrate. The desired concentration for the drug was obtained by accurate dilution and the analysis was followed up as in the general analytical procedure.

Procedure for human serum

Plasma samples, obtained from healthy volunteers, were collected and stored frozen to 1.0 ml of plasma, 10.0 ml of acetonitrile was added, the mixture was vortexed for one minute and than centrifuged for 10 minutes at 10,000 rpm. Obtained supernatant was filtered by 0.45-micron pore size membrane filter. An aliquot serum sample was fortified with buclizine hydrochloride to achieve final concentration 10, 3, 1.5, 0.5, 0.15, 0.05, and 0.025 µg/ml. These solutions were stored at –20°C and analyzed regularly at interval of two days up to eight days for serum drug analysis. 10 µl of this solution was injected and chromatographed. Calibrated glassware's from Pyrex were used for the solution and mobile phase preparation.

RESULTS AND DISCUSSION

Development and optimization of isocratic HPLC conditions

A reversed-phase assay was deemed most appropriate for initial testing. A UV scan of buclizine hydrochloride showed a maximal absorbance at or near 230 nm. Initial method development was conducted on a Nucleosil, C_{18} (10µm, 25 cm x 0.46 cm) column was used for separation at room temperature. This column provides efficient and reproducible separations of nonpolar compounds while minimizing solvent usage. Conse-quently, it was selected for method development and remains the column utilized in the validated assay.

Preliminary method development of suitable isocratic conditions to resolve buclizine hydrochloride on the C_{18} column was conducted with acetonitrile-water as the mobile phase. A mobile phase of acetonitrile-water (80:20 v/v) adjusted pH with phosphoric acid to 2.6 was found to provide a reproducible, baseline resolved peak. These conditions allowed for separation of buclizine hydro-chloride from internal standard.

The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. The specificity of the method was established through the study of resolution factor of buclizine hydrochloride peak from the internal standard peak. Peaks were identified using retention times compared with those of standards.

For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use [ICH 1996] and [USP 2002] have recommended the accomplishment of accuracy tests, precision, specificity, linearity, work strip and robustness of the method. The type of method and its respective use determine the parameters to be evaluated, especially, when the samples are complex biologic matrices, the calibration curve of buclizine was 10, 3, 1.5, 0.5, 0.15, 0.05, and 0.025 µg/ml were linear in the range mentioned as above. The representative linear equation was $y = 1x + 0.006$ ($n = 6$; $r = 0.9999$) where y represents injected concentration and *x* the recovered concentration. The detection limit, taken as the lowest absolute concentration of analyte in a sample, which can be detected but not necessary quantified under the stated experimental condition, was, 0.005 µg/ml. The limit of quantitation, taken as the lowest concentration of analyte in a sample, which can be determined with acceptable precision and accuracy, was 0.025.

System suitability

The HPLC system was equilibrated with the initial mobile phase composition, followed by 10 injections of the same standard. These 10 consecutive injections were used to evaluate the system suitability on each day of method validation.

The system suitability parameters including capacity factor >2, resolution > 3 and asymmetric factor < 2. All parameters were satisfactory with good specificity for the stability assessment of buclizine hydrochloride. Theoretical plates of the column were > 3000.

Table 1: Recovery and regression characteristics of buclizine, in human serum

Concentration	<——————— CV % (n=6) ———————>				
ug/ml	Day 1	Day 2	Day 3	Day 4	Recovery (%)
0.025	1.7	1.4	3.5	2.3	98.07
0.05	1.5	1.3	1.1	2.4	98.48
0.15	1	1.4	1.1	1.6	99.51
0.5	0.9	1.4	1	1.6	100.25
1.5	1.7	1.6	1	1.6	100.34
3	0.9	1.4	1	1.5	100
10	1	2	0.9	0.8	99.94
Correlation coefficient (r)			0.9999		
Standard error of estimate			0.0025		
Standard error			0.0013		
Intercept			0.006		
Slope			1		

Linearity

Linearity was tested by assaying serum spiked with known volumes of buclizine hydrochloride stock solution at concentrations 10, 3, 1.5, 0.5, 0.15, 0.05, and 0.025 μg/ml. Injected concentration versus recovered concentration were plotted and the correlation coefficients were calculated.

Precision and accuracy

Assay precision was determined by spiking human serum with varying concentrations of buclizine hydrochloride and fixed concentration of internal standard. Buclizine hydrochloride concentrations were determined in batches of three on the same run and on different runs. Intra and inter-run assay precision was determined by calculating coefficient of variation (CV) of the obtained data shown as table 1.

Intraday and inter-day accuracy and precision

The precision of the method was investigated with respect to repeatability. For intra-day precision, ten samples of seven concentrations were analyzed on the same day. Table 1 summarizes the correlation coefficient, standard error, standard error of estimate, and slope. Generally acceptable repeatability of the results with in one day and day-to-day was observed.

The statistical summary includes coefficient of variance (C.V) and recovery (%). Inter and intraday accuracy of the method ranged from (98.07% to 100.34%), (CV%, 0.9 to 3.5±) as shown in table 1.

The recovery of buclizine hydrochloride in dosage formulation was performed at three concentration levels (60, 90, and 120%) in dosage formulation, accuracy of the method in dosage formulation ranged from 98.66 % to 101.6% as shown in table 2.

Relative accuracy was determined by calculating the percent accuracy using the equation:

% Recovery = (observed concentration/nominal concentration) potency of standard

Table 2: Recovery characteristics of buclizine in dosage formulation

% label claim	Buclizine	
	% Recovery	% Error (±)
60	102	2.00
80	98.66	1.34
100	101.6	1.6
120	99	1.0

Label claim: Buclizine HCl 25 mg

Limit of quantitaition and detection

The limit of detection (LOD) and limit of quantitation (LOQ) of this method were determined from the coefficient of variation of a known concentration of buclizine hydrochloride, as per (ICH Q2B guidelines 1997) and (FDA, Guidance for Industry 2000).

The LOD for this assay, calculated from three times the noise level of the response, is 0.005 μg/ml. The LOQ for this assay calculated from ten times the noise level of the response, is 0.025 μg/ml.

Specificity

The specificity of the chromatographic method was determined to ensure separation of internal standard and buclizine hydrochloride as shown in figure 1. Specificity was also determined by screening four different samples of controlled human serum, which were free from interfering endogenous plasma components. This is evidenced by the

lack of interfering peaks in the chromatograms of serum samples. Figure 2 represents a typical chromatogram of blank serum and serum containing drug respectively. Solutions of buclizine hydrochloride containing aspirin, acetaminophen, caffeine and nicotine were prepared and then injected to check for interference from these commonly used drugs**.** The method demonstrated good resolution between the peaks and found to be free of interferences.

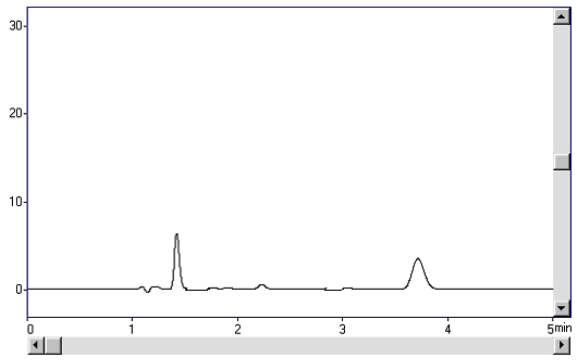


Figure 1: Representative chromatogram of buclizine reference standard with internal standard, Peak 1- Internal Standard, Peak 2- Buclizine

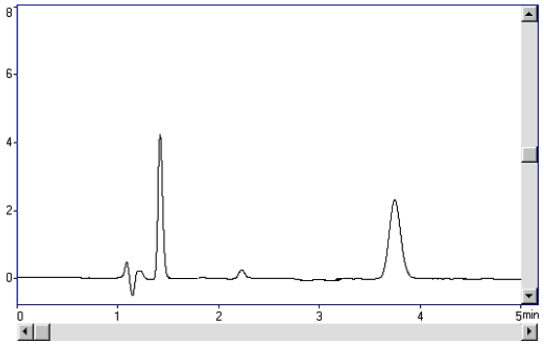


Figure 2: Representative chromatogram of and buclizine in human serum with internal standard.
Peak 1- Internal Standard, Peak 2- Buclizine

Ruggedness

The ruggedness was established by determining buclizine hydrochloride in dosage formulation and in human serum using the same chromatographic system and the same column by two analysts on a different day. The assay result indicated that the method was capable with high precision (table 1). Additionally, good separations were always achieved which suggested that the method was selective for all components under the test.

Robustness

Stability of standard solution and sample solutions were determined by assay after 24 and 48 hours at –20°C temperature against fresh standard solutions. It shows that all the drugs are stable and does not show much variation in the time span of 48 hours.

CONCLUSION

A simple and reliable HPLC method for measuring buclizine hydrochloride in human serum and pharmaceutical dosage formulation has been developed. A fully validated RP-HPLC procedure for the assay of buclizine hydrochloride drug in bulk, tablets and human serum is described for the first time. Hence, it can be recommended for the routine quality control of this antibiotic drug. Methylparaben could be successfully used as an internal standard. The low volume of blood or plasma needed, the availability of the internal standard, the simplicity of the separation procedure, the short run time and the low volume of injection make this method suitable for quick and routine analysis. The intra-run and inter-run variability and accuracy results were in acceptable limit.

REFERENCES

British Pharmacopoeia (2003). The Stationary office under license from the controller of Her Majesty's Stationary Office for the Department of Health on behalf of the Health Ministers, p.272.

El Walily AF, El GINDY A and Bedair MF (1999). Application of first-derivative UV-spectrophotometery, TLC-densitometery and liquid chromatography for the simultaneous determination of mebeverine hydrochloride and sulpiride. *J. Pharm. Biomed. Anal.* **21**(3): 535-48.

FDA: Guidance for Industry, Analytical Procedures and Methods Validation, August 2000.

ICH Q2B: Validation of Analytical Procedures: Methodology May 1997.

International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) Q2B (1996). Validation of Analytical Procedures, Methodology.

Martindale, The extra pharmacopoeia (1996). Thirty-first Edition. Royal Pharmaceutical society of Great Britain, 1 Lambeth High Street London SE17 Jn England, pp.435-36.

The Merck index. (2001) An Encyclopedia of Chemical, Drugs and Biologicals, 13th Ed., Merck Research Laboratories. Division of Merck & Co Inc. Whitehouse Station, NJ. p.245.

United States Pharmacopeia (2002). Validation of Compendial Methods, United States Pharmacopeia Convention: Rockville.

United States Pharmacopoeia (2005). 28th Review, U.S. Pharmacopoeial Convension Inc., Rockville, MD, pp.1196-1198.

Received: 09-10-06 – Accepted: 31-10-2006