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METHOD DEVELOPMENT AND VALIDATION PROCESS OF BUPROPION (A NOVEL ANTI-DEPRESSANT DRUG) BY RP-HPLC (REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY)

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ABSTRACT

Product simple and precise RP-HPLC process was developed for the estimation osbervation of novel antidepressant drug bupropion with Waters X – Bridge C-18 5µm, 4.6 X 150 mm columnusing mobile phase Acetonitrile: Ammonium bicarbonate (5mM) pH-9 adjusted with 1% ALOH (25:25, %v/v). A rapid, simple and accurate RP-HPLC method was developed for the estimation of bupropion hydrochloride, using C18 column 250 x 4.6 mm i.d, 5 µm particle size in gradient mode, with mobile phase comprising of phosphate buffer (pH 4.0) and alcoholic solvent methanol. The flow rate was 1 mL min -1 and the detection wavelength was 252 nm. The retention time for bupropion hydrochloride was found to be 11.52 min. The validation was carried out in the light of ICH guidelines with respect to parameters linearity, specificity, accuracy, the limit of detection (LOD) and limit of quantification (LOQ). The proposed method showed linearity in the concentration range of 50 to 250 ppm for the drug Bupropion. The linear regression equation of Bupropion was found to be y = 6E+06x + 91344 and correlation coefficient value was found to be 0.997 indicating a high degree of linearity for the drug. The limit of detection (LOD) of bupropion was 0.5 ppm and limit of quantification (LOQ) was 2.0 ppm. The low values of %recovery and %C.V. showed that the method is precise within the acceptance limit of 5% (according to ICH guidelines).

KEYWORDS: Range, Bupropion, Quantification, RP-HPLC, PDA detector, ICH.

INTRODUCTION

Bupropion was first patented in 19741 and released onto the planet market in 1985. It had been briefly withdrawn thanks to seizures incidences but reintroduced in 1989 after the daily recommended dose was reduced to lower seizure likelihood. Bupropion may be a dopamine and norepinephrine reuptake inhibitor. Literature survey revealed that several chromatographic methods were reported for the estimation of bupropion hydrochloride in human plasma using its formulation. There exist reports on LC, LC-MS-MS, GC, TLC and HPTLC for analysis of the drug. There also exists report on chiral separation of bupropion employing a chiral AGP column. No method is published thus far for the determination of bupropion in bulk drugs. The most purpose of this work was to develop simple, rapid and accurate method which might be applied to research bupropion HCl in bulk form and to validate the tactic as per ICH (Q2R1) guideline.[5,6,7,8,9]

MATERIAL AND METHOD

MATERIAL

Chemicals and Reagents

Pure sample of bupropion hydrochloride was obtained as gratis from Wockhardt Ltd. (Aurangabad, India) and was used as without further purification. HPLC grade methanol was procured from Merck specialties Pvt. Ltd. (Mumbai, India). Analytical reagents (AR) grade ortho phosphoric acid and potassium dihydrogen phosphate were procured from Qualigens fine chemicals Pvt. Ltd. (Mumbai, India). Ultra pure water obtained from Millipore water purification system (Molsheim, France) was used throughout the study.

Equipments

UV-visible spectrophotometer (PerkinElmer, Shelton, CT, USA) was used for the determination of detection wavelength. A high-performance liquid chromatography (HPLC) system from PerkinElmer (Shelton, CT, USA) was used for the analysis of samples, which consisted of online degasser, sample injector (Rheodyne sample loop 20 µL), UV-visible detector (Series 200), pump (Reciprocating, series 200) and computing system loaded with Total Chrome Navigator (version 6.3.1) software. Other equipment used were, pH meter (Labindia, Mumbai, India), weighing machine (Shimadzu, AUX220, Kyoto, Japan) and a micro-pipette (Erba Biohit, Mannheim, Germany). All the samples were analyzed by using

Kromasil C-18 column 250mm x 4.6mm i.d., particle size 5µm; (Eka Chemicals AB, Bohus, Sweden).

Analytical Method Validation

Method validation is defined because the process of defining and proving an analytical method acceptable for its intended use. Recent guidelines for methods development and validation for brand spanking new noncompendial test methods are provided by the FDA draft document, "Analytical Procedures and Methods Validation Chemistry, Manufacturing, and Controls Documentation". In recent years, an excellent deal of effort has been devoted to the harmonization of pharmaceutical regulatory requirements within the us , Europe, and Japan. As a part of this initiative, the International Conference on Harmonization (ICH) has issued guidelines for analytical method validation.

METHOD

Preparation of sample for HPLC analysis

Stock solution of 1000 µg mL-1 was prepared by dissolving 10 mg bupropion HCl in 10 mL methanol. Sample solution was prepared by dissolving stock solution mobile phase as per the specified concentrations.

Selection of detection wavelength

Appropriate dilutions of the bupropion HCl were prepared using water as diluent. Solution was scanned using double beam UV-visible spectrophotometer between the ranges of 400 to 200 nm. The detection wavelength was found to be 252 nm.

Preparation of buffer

Potassium dihydrogen orthophosphate buffer (10 mM) was prepared by dissolving 1.36 gm of potassium dihydrogen ortho phosphate in 1000 ml of Milli-Q water and therefore the pH was adjusted to 4.0 with ortho phosphoric acid.

HPLC method development and validation:

Detection wavelength for the drug was found to be 252 nm and hence methanol was used as an organic phase. UV spectra of bupropion HCl is shown in Figure 2. Various trials were conducted to attain adequate peak symmetry and response factor by varying the concentration of organic phase and pH of the buffer system. Finally methanol and buffer (phosphate buffer 10 mM) with pH 4.0 was selected to investigate bupropion HCl samples by using gradient

HPLC mode. The chromatogram of the drug is shown in Figure 3. The rate of flow, injection volume and detection wavelength were 1 mL min-1, 20 µL and 252 nm, respectively.

The developed method was validated with reference to various parameters outlined within the International Conference on Harmonization (ICH) guidelines Q2 (R1). A stock solution containing 1 mL min-1 drug was prepared in MeOH and therefore the linearity was established by using the concentration within the range of 20-120 µg mL-1. The solutions were prepared in triplicate and analyzed by injecting 20 µL into HPLC. The intra-day and inter-day precision were established by analyzing 40 µg mL-1, 60 µg mL-1 and 80 µg mL-1 drug solutions 3 times on an equivalent day and therefore the next day, respectively. Accuracy decided by spiking three known concentrations of the drug, viz., 80%, 100% and 120% during a 10 µg mL-1 of measured standard stock solution in triplicate then determining the percent recovery of the added drug. Limit of detection and limit of quantitation were assessed by determining the signal to noise ratio of the injected concentration of the analyte. Robustness of the developed method was also determined by with reference to different parameters**.**

RESULTS AND DISCUSSION

Chromatographic experiment

Different chromatographic conditions were tried to optimize the method, which include the following:

Column: - X- BRIDGE C-8 3.5µ, 4.6 X 50 mm

Flow: - 1 ml/min

Detector U.V.:- 214 nm

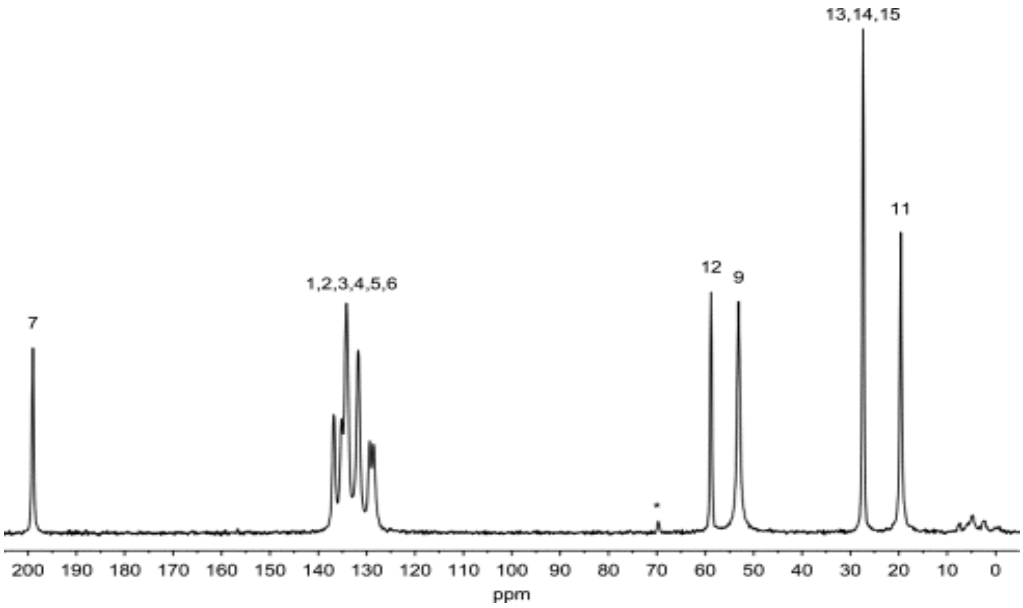
Injection Volume: - 20µl

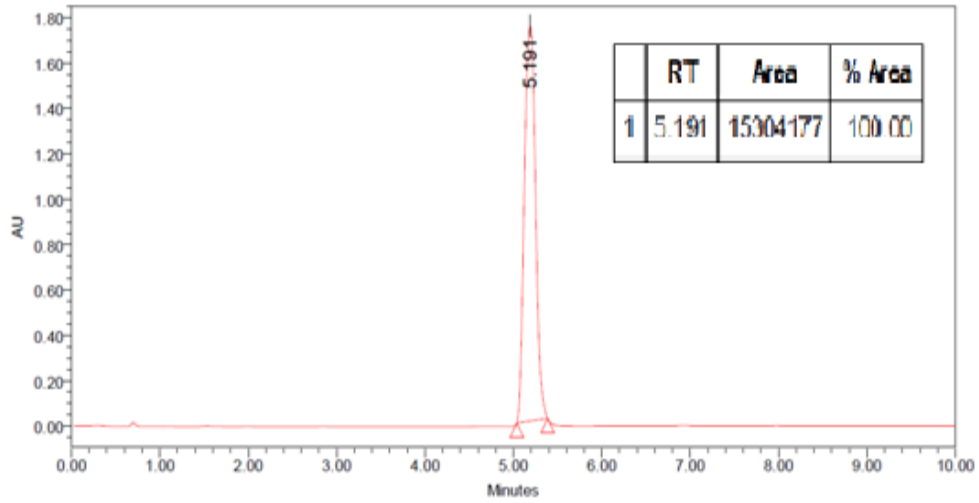
Run time:- 10 min

Column temp.:- 300C

Buffer:- 0.5 mm Ammonium acetate

Mobile Phase:- ACN : 0.5 mm Ammonium acetate (25:25 % v/v)





This study describes a highly sensitive, accurate and reproducible HPLC method for the determination of Bupropion because no such method was developed.

Instrumentation

A method has been developed by experimentation based on the literature survey and ascertained by statistical parameter of sampling using a Alliance Waters e2695 Separations Module from USA which is equipped with a Waters 2774 pump, Waters 2998 Photodiode Array Detector and a injector loop made of Rheodyne, Model No. 2767, Made in USA having a injection volume of 20 µl.

METHOD DEVELOPMENT

Drugs Solubility17

Solubility in different organic and aqueous solvents determined the best composition of the sample solvent. Bupropion was freely soluble in methanol, soluble in ACN and in mobile phase and slightly *soluble* in water.

Mobile Phase Selection

Different mobile phase were tested but adequate separation of drugs was found in acetonitrile: 5mM Ammonium bicarbonate buffer (25:25 %v/v).

pH Selection

By altering the pH of mobile phase separation of peak was observed. The pH of mobile phase was adjusted to 7.0, 8.0, 9.0 and 10.0. At pH 9.0 satisfactory separation of the drug with good resolution and short run time was achieved. At pH 7.0, 8.0 and 10.0 low retention time of Bupropion and poor separation.

Wavelength Selection

Maximum absorbance of Bupropion at 242.6nm was determined in mobile phase by utilizing Waters 2998 Photodiode Array Detector. Maximum peak height of drug was obtained at 242 nm by injecting the 3µg/ml concentration of sample of the drug and allows running at different wavelength.

CONCLUSION

Developed assay method is simple, rapid, accurate, precise, economical, specific and reproducible for the qualitative and quantitative determination of Bupropion with good resolution in short time and high sensitivity.

In the present projected work the analyte was separated in a short run. Optimization of the method showed that apart from the mobile phase pH and composition, flow rate is an important crucial parameter.

The selection of gradient mobile phase and flow rate, cut down over all time of sample analysis and thereby made the method most cost effective and rapid. Wavelength selection made the method more sensitive.

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