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Validation and Stability indicating Reverse Phase-High Performance Liquid Chromatography for the Determination of Duloxetine in Tablets

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Abstract: A Simple, selective, accurate reverse phase high performance liquid chromatography (RP-HPLC) was developed for estimation of duloxetine HCl in pharmaceutical formulations. Chromatographic separation achieved isocratically on a C_{18} column (Use Kromasil, C_{18}, 5µ, 250×4.6 mm i.d. with mobile phase containing 0.5M TFA buffer: acetonitrile (65:35 v/v) and final pH adjust to 5.5±0.02 with phosphoric acid was used. The flow rate was 1ml/min and effluent was monitored at 232 nm. The retention time was 5.43 min. The method was validated in terms of linearity, accuracy and precision. The linearity curve was found to be linear over 0.25-4 µg/ml. The limit of detection and limit of quantification were found to be 2.44 and 8.16 ng/ml respectively. The proposed method was successfully used to determine the drug content of marketed formulations
Key words: Method Validation, Stability, Duloxetine, Tablets, RP-HPLC

Introduction

Duloxetine HCl is chemically, 2(+)-(S)-N - m e t h y l - (gamma) - (1 - n a p h t h y l o x y) – 2 thiophenepropylamine hydrochloride[1] (Fig-1). Duloxetine hydrochloride is a newer selective serotonin and norepinephrine reuptake inhibitor (SSNRI) used for major depressive disorders[2, 3]. Duloxetine molecular formula and weight is $C_{18}H_{19}NOS$, 297.41, soluble in water. Duloxetine is not official in any pharmacopoeia. A few methods in literature were reported for the determination of DLX and its key intermediate, desmethyl duloxetine in human serum by HPLC method[4-5]. Literature reported the characterization of phenolic impurities in duloxetine HCl samples by MS, NMR, X-rayanalysis and impurities formed by interaction of duloxetine HCl with various enteric polymers[6,7]. Simple UV Spectrophotometric method for estimation of duloxetine in formulations is reported but calibration range is from 5-50 µg/ml that shows the method is less sensitive.

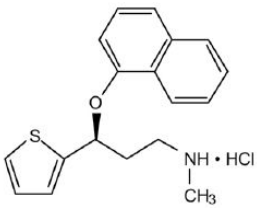

Fig 1: Structure of Duloxetine HCl

Experimental

A high performance liquid chromatograph system, with LC solutions data handling system (Shimadzu-LC 2010) with an auto sampler was used for the analysis. The data was recorded using LC 2010 solutions software. The purity determination performed on a stainless steel column 250 mm long, 4.6 mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5 µm diameter (Use Kromasil, C_{18}, 5µ, 250×4.6 mm i.d) with the mobile phase containing 0.5M TFA buffer: Acetonitrile (65:35 v/v) at ambient temperature. Flow rate was kept at 1.0 ml/min, and the elution was monitored at 232 nm.

Duloxetine working standard used from Nosch Laboratories Limited. For the estimation of Duloxetine in bulk and commercial formulations of duloxetine brand (DUTIN Ranbaxy), 20 tablets were obtained from retail pharmacies.

Each tablet was labeled contain 60 mg of duloxetine and had an expiry of not less than 365 days at the time of study. HPLC grade Trifluoro acetic acid, Acetonitrile-procured from Merck, India. High pure water was prepared by using Millipore Milli Q plus purification system

Preparation of mobile phase

Mobile phase was prepared by mixing 350 mL of acetonitrile with 650 mL of TFA buffer and its pH adjusted to 5.5. The mobile phase was sonicated for 15

min and then it was filtered through a 0.45 µ membrane filter paper

Preparation of Stock and Standard solutions

The standard stock solution of duloxetine HCl was prepared by dissolving 10 mg of drug in 50 ml volumetric flask separately using methanol. Final working standard solution of 50 µg/ml of duloxetine HCl was prepared by diluting 5 ml of the above solution to 10 ml with methanol. 0.20, 0.40, 0.70, 1.0, 2.0, 3.0 and 4.0 µg/ml concentration of solutions were prepared and injected under operating chromatographic conditions. Calibration curves were constructed by plotting peak area versus concentration of duloxetine HCl and the regression equation were calculated.

Assay in formulations

In case of marketed formulations, five accurately weighed tablets were crushed to a fine powder and an amount equivalent to 10 mg of duloxetine was added into different 100 mL volumetric flasks and volume was made up with methanol. The samples were filtered through a 0.45-µm-membrane filter; different serial dilutions (3, 6.2, 12.3, 25.4 µg/mL) were made from this solution in 25 mL volumetric flask and were injected for HPLC analysis.

Results and Discussion

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][8] have recommended the accomplishment of accuracy tests, precision, specificity, linearity of the method

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 Duloxetine. Theoretical plates of the column were >3000.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to true value. In case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte has been added within the range of method. If it is not possible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product (*i.e*. "to spike").In our studies, the later technique was adopted and duloxetine was spiked in drug product. The result of accuracy given in (Table 1) revealed that the method was found accurate for all above purposes.

Precision

Precision is the degree of reproducibility or repeatability of the analytical method under normal operating conditions. The method passed the test for repeatability as determined by %RSD of the area of the peaks of six replicate injections at 100% test concentration. The results of intra-and inter-day variation are shown in (Table 2).

Range and linearity

The linearity of an analytical method is its ability to elict test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity of the method was observed with in the expected concentration range demonstrating its suitability for analysis. The correlation coefficient (r2) was found to be 0.999 and value of intercept was less than 25 of the response of 100% of the test concentration in all the cases indicating functional linear relationship between the concentration of analyte and area under the peak.

Limits of detection and quantitation

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1.The lower limit of detection for Duloxetine is 2.44 ng/mL in reference material and formulation.Limit of Quantitation (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method (USP 2004). The LOQ values were found to be 8.16 ng/mL for raw material, formulations.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram. The excipiants used in different formulation products did not interfere with the drug peak and thus, the method is specific Duloxetine. To further confirm the specificity of the method, UV scans of spiked drug were taken in the range 200-400 nm and no significant change was found by comparing the absorbance of pure drug and spiked drug at the analytical wavelength of drug.

Stability Studies

Stability of sample in mobile phase

The stability of sample solution (Duloxetine) in mobile phase was demonstrated by injected the sample solution at different time intervals *viz*. 0, 3, 6, 9, 12 and 24 hours of time intervals. Up to 9 hours, no degradants were observed in the chromatogram. However, after 9 hours the chromatographic peak area of duloxetine decreased insignificantly. Hence, the sample solution was stable at least for 9 hours after its preparation. Interestingly, after 9 hours, when samples were stored at 20^0C under

laboratory light conditions, significant rise in the peak areas were observed. Thus, it would be preferable that the sample solution is to be injected before 9 hours of its preparation.

Hydrolysis

Individually, 5 mL of the standard solution was transferred to a 10 mL distillation flask and boiled for 1 h at 80°C after adding : (a) 5 mL of water for neutral hydrolysis (b) 5 mL of 0.1N HCl for acid hydrolysis (c) 5 mL of 0.1 N NaOH for basic hydrolysis. Before the analysis, (b) and (c) solutions were neutralized. For chemical oxidation to 5 mL of the standard solution, 100 µL of 30 % H_2O_2 solution (*v/v*) were added and mixed. The solution was left at room temperature for 1 hour in the dark.

Photochemical degradation

The photochemical stability of the Duloxetine was studied by exposing the methanolic stock solution to direct sunlight for 8 h (from 9 AM to 5 PM, at 20°C).

Thermal stress (test sample exposed to sunlight)

Transfer about 2 to 3 gm of sample into a clean dry watch glass and spread evenly. Expose to sunlight for 10 hours. After the sample got exposed to prescribed time, weigh accurately 25 mg of sample into a clean dry 50 mL volumetric flask, dissolve and dilute to the mark with mobile phase and exposed to sunlight.

Stability-indicating methods have received considerable attention for the determination of a vast number of drugs [9-12]. The international Conference on Harmonization (ICH) guideline entitled "Stability Testing of New Drug Substances and Products" requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substances18. Susceptibility to oxidation is one of the required tests. The hydrolytic and photolytic stabilities are also required. An ideal stability-indicating method is one that quantifies the drug present and also resolves its degradation products. This study was carried out by employing the following tests: hydrolysis (neutral, acidic and basic), chemical oxidation, photolysis and thermolysis. No decomposition was observed when the duloxetine was exposed to sunlight, temperature, UV; whereas significant change *i.e.*, decrease of assay about 25 to 30% observed when sample was treated with 0.1N NaOH and 0.1N HCl. The sample treated with 3% H_2O_2 was almost completely degraded.

Table 1: Accuracy/recovery of Duloxetine

Parameters	Conc µg/ml	%Recovery	%RSD
Assay	6.2	99.06	1.20
	12.3	99.28	1.46
	25.4	100	1.78
Assay in Spiking method	10	98.48	1.94
	20	101.02	1.26
	25	98.38	1.38

Table 2: Intermediate Precision of the method

Conc µg/ml	Assay in Formulation	
	Intra-day variation %RSD	Inter-day variation %RSD
0.099	0.98	0.96
0.20	0.89	0.85
0.50	0.78	0.75
1.50	0.90	0.92
3.10	0.88	0.89
15	0.75	0.74
25	0.76	0.77

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