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Determination of Duloxetine Hydrochloride in Pharmaceutical Formulation by HPLC with UV Detection

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Abstract: A rapid and accurate liquid chromatographic method has been developed for the determination of duloxetine hydrochloride in solid dosage form. Chromatographic separation was achieved on a BDS- Hypersil Phenyl C_{18} column (200mm×4.6 mm, 5µm). The mobile phase consisting of a mixture 25 mM phosphate buffer (pH 3.0) and acetonitrile in the ratio of (60:40) was delivered at a flow rate of 1.0 ml/min. Detection was performed at 217 nm using UV detector. The retention time for duloxetine hydrochloride was around 8.1; separation was complete in less than 15 min. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range of 0.20 – 10 µg/ml. The method was validated for accuracy, precision and recovery studies. Statistical analysis proved the method was precise, reproducible, selective, specific, and accurate for analysis of duloxetine hydrochloride. The wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase imply the method is suitable for routine quality control of formulation products.

Key wards: Duloxetine hydrochloride, Reverse-phase liquid chromatography, Solid dosage form.

Introduction

Duloxetine hydrochloride, (+)-(S)-N-methyl-gamma-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride[1], although the exact mechanisms of the antidepressant and central pain inhibitory action of duloxetine in humans are unknown, the antidepressant and pain inhibitory actions are believed to be related to its potentiation of serotonergic and noradrenergic activity in the CNS. Preclinical studies have shown that duloxetine is a potent inhibitor of neuronal serotonin and nor epinephrine reuptake and a less potent inhibitor of dopamine reuptake[2].

A survey of literature revealed that the following analytical methods were reported for determination of duloxetine hydrochloride and its metabolites by spectrofluorimetric method[3] and chromatographic methods *viz;* high performance liquid chromatography[4-15] and tandem mass spectrometry detector[16-17].

Since this drug is being marketed in domestic and international market, there is a need to develop a simple assay procedure for the determination of this drug, particularly in its pharmaceutical formulations for quality control purpose. Liquid chromatography with UV detection is often preferred in ordinary laboratories because of its wide suitability and availability. The present paper describes a rapid and accurate LC method for the determination of duloxetine hydrochloride in solid dosage formulation.

Experimental

Chemicals and reagents

Duloxetine hydrochloride was given as a gift sample from Zydus Cadila, Ahmedabad, India. HPLC grade acetonitrile and water were purchased from Ranbaxy Fine Chemicals Limited, SAS Nagar, India and Qualigens Chemicals, India respectively. Potassium dihydrogen phosphate was purchased from SD Fine Chemicals, Mumbai.

Instrumentation

Chromatography was performed on a (Shimadzu HPLC Class 10A Series) equipped with two LC-10AT pumps with a variable UV-Vis detector SPD-10A. Samples (20 µl) were injected by means of a Rheodyne injector fitted with a 20 µl loop. Class LC-10AT series, version 5. 03 was employed for data collecting and processing. A BDS- Hypersil Phenyl RP-C18 column (250 mm X 4.6 mm i.d.; Particle size 5µ) was used for separation. The mobile phase consisting of 25 mM phosphate buffer (pH 3.0) and acetonitrile in the ratio of 60: 40 % v/v was delivered at a flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45 µm membrane and degassed for 30 min in an ultrasonic bath. Analysis was performed at ambient temperature and detection was performed at 217 nm. The injection volume was 20 µl. Before to injection of the drug solutions, the column was equilibrated for 45 min with the mobile phase flowing through the systems.

Calibration curve

A stock solution of the drug was prepared by dissolving 50 mg of duloxetine hydrochloride in a 50 ml volumetric flask containing acetonitrile, sonicated for about 10 min and then made up to the volume with same. Aliquots of these stock solutions were suitably diluted with mobile phase to get the working standard solution of drug in the concentration range between 0.2 and 10 µg/ml.

Analysis of pharmaceutical preparation

Twenty capsule contents were weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 20 mg was transferred to a 100 ml volumetric flask. The contents were ultrasonicated for 15 min with 50 ml of HPLC acetonitrile and made up to the mark with same. The resulting solution was allowed to settle for about an hour, and the supernatant was suitably diluted to give the desired concentration with mobile phase. The solution was filtered using 0.2µm membrane filter. The drug content per capsule (on an average weight basis) was calculated.

Precision

Intra-day and inter-day accuracy and precision of the assay samples containing (2, 5 and 10 µg/ml) for were analyzed six times in the same day (intra-day) and for three consecutive days by different analysts (inter-day).

Specificity

The specificity of the method was assessed by analyzing standard drug, pharmaceutical product and placebo; compared the Retention time of the standard with that of the sample to determine whether the pharmaceutical product and placebo led to interfere.

Recovery studies

Recovery studies were done at three different levels. The pre-analyzed samples were spiked with 1, 2 and 3 µg/ml, and the mixtures were reanalyzed by the proposed method. Percentage recovery was calculated from the amount of drug found in the solution.

Robustness

By introducing small but deliberate changes in the mobile phase pH (± 0.1), mobile phase composition (± 2.0%), detection wavelength (± 5.0 nm), flow rate (± 10.0 % of absolute value) robustness of the described method was studied.

Results and Discussion

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The goal of this study was to develop a simple, precise and rapid RP-HPLC method for the analysis of duloxetine hydrochloride in its pharmaceutical dosage form, with UV detection.

The suitability of the mobile phase was decided on the basis of the various trials. After several trials, finally a mobile phase consisting of a mixture of 25 mM phosphate buffer (pH 3.0) and acetonitrile in the ratio of (60:40 % v/v) at flow rate of 1 ml/min was adopted; this produces good acceptable peak shape. The retention time for was around 8.1 min. The chromatogram is shown in Figure 1.

A system suitability test was performed to check various parameters such as number of theoretical plates and peak tailing. Theoretical plates and peak tailing were found to be 13917 and 1.37 respectively. The calibration curve of duloxetine hydrochloride was constructed by plotting the peak area against the concentration between 0.2 and 10 µg/ml, the correction coefficient was found to be 0.998. The results show that an excellent correlation existed between peak area and concentration of drug within the concentration range tested. This method was also validated for its intra-day and inter-day precision at concentrations of 2, 5 and 10 µg/ml of duloxetine hydrochloride, which was expressed as relative standard deviation. The intra-day precision was found to be between 0.41 % and 0.91 % and inter-day precision was found to be between 0.29 % and 0.56 %. The results indicated the good precision of the developed method.

The present study was used to quantify duloxetine in capsule form. Duloxetine capsules (20 mg) were analyzed, average drug content was found to be 99.41% of the labeled amount. The specificity of the method was confirmed by comparing the Retention time of standard with that of the marketed formulation. There is no interference from the excipients commonly

present in the capsule, which indicates that the capsule excipients did not interfere with the estimation of the drug in the proposed HPLC method. Hence the developed method is specific and selective. Recovery studies were performed by adding known amount of drug solutions to the pre-analyzed sample solutions and the recovery ranged from 99.05 % to 99.33% (Table 1). The obtained results suggested the accuracy of the developed method for the determination of the duloxetine hydrochloride in the formulation.

The standard deviation of peak areas was calculated for each parameter such as small changes in the variations of pH of the mobile phase (± 0.1), mobile phase composition (± 2.0%), wavelength of detection (± 5.0 nm) and flow rate (± 10.0% of absolute value). The % R.S.D. was found to be less than 2%. The low values of % R.S.D. indicated robustness of the method.

Conclusions

The results of our study indicate that the proposed RP-HPLC method is simple, rapid, precise and accurate. The developed HPLC method was found suitable for determination of duloxetine hydrochloride in bulk drug and in marketed solid dosage formulation without any interference from the excipients. Statistical analysis proves that, the method is repeatable and selective for the analysis of duloxetine hydrochloride. It can therefore be concluded that use of the method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range.

Table 1: Intra and inter day precision of HPLC method[a]

Actual Conc. (µg/ml)	Intra-day precision			Inter-day precision		
	Observed Conc. (µg/ml)	S.D	% R.S.D.	Observed Conc. (µg/ml)	S.D	% R.S.D.
2	1.985	0.02	0.91	1.98	0.01	0.56
5	4.90	0.03	0.58	4.90	0.02	0.48
10	9.94	0.04	0.41	9.88	0.03	0.29

[a] n=6

Table 2: Recovery studies [a]

Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)
1	0.993	99.33
2	1.985	99.25
3	2.972	99.05

[a] n= 6

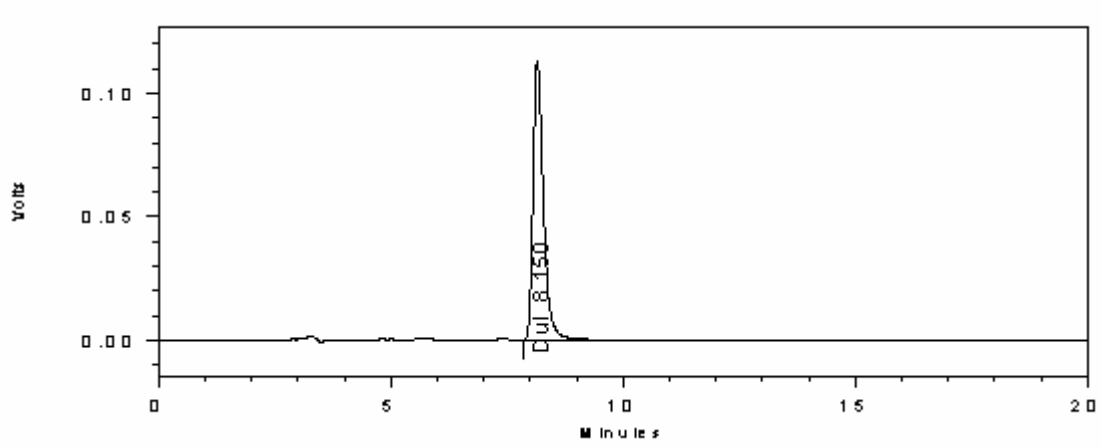

Figure 1. Typical HPLC chromatogram of duloxetine hydrochloride

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