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*Research Article*

# Stability-Indicating HPTLC Method for Determination of Duloxetine Hydrochloride in Bulk Drug and Tablet Formulation

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A simple, accurate, precise, sensitive, selective, and stability-indicating high-performance thin-layer chromatographic method was developed and validated for determination of duloxetine hydrochloride both in bulk drug as well as in tablet formulation. The stationary phase used in our method consisted of HPTLC aluminum plates precoated with silica gel 60F-254, while, chloroform : methanol (8 : 2, v/v) was used as binary mobile phase. These chromatographic conditions eluted the drug effectively, and distinct compact spots were seen, (Rf, retardation factor, value (0.42 ± 0.20). Densitometric determination of duloxetine hydrochloride was carried out in the absorbance mode at a wavelength of 217 nm. The mean value of corelation coefficient; slope and intercept were 0.9962 ± 0.0015, 121.54 ± 0.61, and 987.3 ± 6.17, in the amount range of 600–2000 ng (nanogram) per spot, respectively. Stress testing validation were performed as per the guidelines of International Conference on Harmonization (ICH) and drug was subjected to stress conditions like acid-hydrolysis, alkali-hydrolysis, oxidation, and thermal degradation. As the method effectively separated the active drug from its degradation products, it can be employed as a stability-indicating assay method (SIAM) for identification and quantitative determination of duloxetine HCl in bulk drug and tablet dosage formulation.

## 1. Introduction

Duloxetine hydrochloride designated in IUPAC as (+)-(S)-N-methyl-gamma-(1-naphthyloxy)-2- thiophenepropylamine hydrochloride (Figure 1) is an agent indicated for the treatment of major depressive disorder (MDD), [1]. The years from 1990 through 1999 were termed the "Decade of the Brain" by the Library of Congress and the National Institute of Mental Health. Since that decade, our understanding of depression has grown enormously. It is estimated that Major Depressive Disorder (MDD) affects 18 million people in the United States and 340 million people worldwide.

It is also indicated for the management of neuropathic pain associated with diabetic peripheral neuropathy [2]. Duloxetine has also been thoroughly experimented to be associated with smoking cessation, and research is going on [3]. A significant reduction in the frequency of incontinence episodes and a resulting improvement in quality of life were found when duloxetine was given to a group of women with stress urinary incontinence. Duloxetine 80 mg, dosed at 40 mg BID, reduced the frequency of episodes by 64% to 100% in half of the women who took the drug, compared with a 41% median reduction seen in a group of women on placebo [4]. Duloxetine is a potent reuptake inhibitor of both neuronal serotonin as well as nor-epinephrine but a less potent inhibitor of dopamine. Duloxetine has no significant affinity for dopaminergic, adrenergic, cholinergic, histaminergic, opioid, glutamate, and GABA receptors in vitro. Duloxetine does not inhibit monoamine oxidase (MAO) [5].

Literature survey revealed many analytical methods for the estimation of duloxetine hydrochloride [6, 7], however, no HPTLC method has so far been developed for bulk and tablet formulations.

HPTLC method is becoming a routine analysis technique due to its advantages. The major advantage is that, several

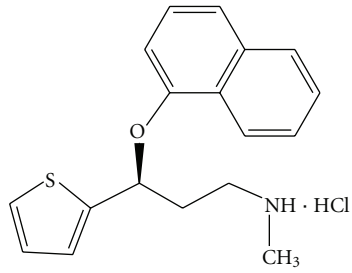


Figure 1: Structure of Duloxetine HCl.

samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thereby minimizing the analysis cost and time. The aim of this work was to develop an accurate, specific, repeatable, and stability-indicating method for the determination of duloxetine HCl in the presence of its degradation products and related impurities as per ICH guidelines [8].

## 2. Experimental

*2.1. Materials.* Duloxetine HCl was received as a gift from Zydus Cadila, Ahmedabad, India. The other chemicals and reagents used were of analytical grade and were purchased from Rankem and Qualigens, India.

*2.1.1. HPTLC Instrumentation.* HPTLC instrumentation consisted of Camag Linomat IV, and samples were spotted in the form of bands of width 5 mm using Camag microlitre syringe (constant application rate of 1 $\mu$L s$^{-1}$) on Merck silica gel precoated HPTLC aluminum plate 60 F254, (20 $*$ 10 cm, 250 $\mu$ thickness). The slit dimensions of 5 mm $\times$ 0.45 mm and a scanning speed of 5 mm s$^{-1}$ were found to be optimum. The solvent system employed consisted of chloroform : methanol ($CHCl_3 : CH_3OH$, 80 : 20 v/v). Ascending development technique was carried out in twin trough glass chamber previously saturated with the mobile phase. The saturation time for mobile phase was found to be 40 min at room temperature. The length of solvent front allowed to move along the vertical length of HPTLC plate was 8.5 cm. The HPTLC plates were dried in a current of hot air with the help of an air-drier. The densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at a wavelength of 217 nm, employing deuterium lamp.

*2.1.2. Calibration Curves of Duloxetine Hydrochloride.* A stock solution of duloxetine HCl (100 $\mu$g mL$^{-1}$) was prepared in methanol, and from this 6, 8, 10, 12, 16, and 20 $\mu$L were spotted on TLC plate to obtain a concentration of 600, 800, 1000, 1200, 1600, and 2000 ng per spot, respectively. Linearity was also determined over the range of 600–2000 ng per spot, and data obtained was subjected to linear least-square regression analysis.

*2.2. Method Validation*

*2.2.1. Precision.* Six replicates of same concentration (600 ng per spot of Duloxetine HCl) were checked for repeatability of the sample application and determination of peak area. The results qualify the requirements of %RSD. of peak area of Duloxetine HCl. The intraday and interday variation for the determination of Duloxetine HCl was carried out at three different concentration levels of 600, 800, and 1000 ng per spot.

*2.2.2. Robustness of the Method.* The mobile phase volume and temperature were varied, and effect on the quality of chromatograms was recorded. The mobile phase ratios of chloroform:methanol was deliberately changed (2 : 8, 4 : 6, and 6 : 4 v/v), and its effect on results was seen at different concentrations of 600, 800, and 1000 ng per spot.

*2.2.3. Limit of Detection and Limit of Quantification.* The background noise of blank sample was determined by analyzing pure methanol, and then signal-to-noise ratio, limit of detection (LOD) and limit of quantification (LOQ) were determined by spotting a minimum concentration of duloxetine HCl (starting from 20 ng per spot, and then concentration was gradually increased).

*2.2.4. Recovery Studies.* The preanalysed samples were spiked with additional 80, 100, and 120% of the standard Duloxetine HCl, and the mixtures were reanalyzed by this method. The experiment was repeated in triplicate, and percentage recoveries in formulations were determined.

*2.2.5. Analysis of the Tablet Formulation.* The drug content in duloxetine HCl in tablet formulation (Tablet, Symbal, label claim: 30 mg per tablet) was determined. Twenty tablets were powdered, and then a powder equivalent to 20 mg was weighed and extracted with methanol. To ensure complete extraction, it was sonicated for 15 min, and then solution was serially diluted with methanol to get a concentration of 0.1 $\mu$g $\mu$L$^{-1}$. The resulting solution was filtered, and then 10 $\mu$L, that is, 1000 ng per spot solution was applied on TLC plate followed by development and scanning.

*2.2.6. Forced Degradation of Duloxetine Hydrochloride.* A stock solution was prepared by dissolving 10 mg of Duloxetine HCl in 100 mL of methanol, and 20 mL of this solution was utilized for forced degradation studies.

*2.2.7. Preparation of Acid-Induced and Alkali-Induced Degradation Product.* To 20 mL of methanolic stock solution, 10 mL each of 2 M NaOH (for 8 hours) and 0.5 M HCl (for 4 hours) were added separately at room temperature. A sample was taken every hour and was neutralized (using standard pH paper). The concentration of drug equivalent to 1000 ng per spot of duloxetine was immediately subjected to HPTLC determination to find out the effect of stress conditions. The forced degradation was performed in the dark to prevent the possible degradation by light.

Table 1: Linear regression data (ng per spot) for the calibration curves[a].

| Linearity range | $r \pm$ SD | Slope ± SD | Confidence limit of slope[b] | Intercept ± S.D. | Confidence limit of intercept[b] |
|---|---|---|---|---|---|
| 600–2000 | 0.9962 ± 0.0015 | 121.54 ± 0.61 | 120.98–122.08 | 987.31 ± 6.17 | 982.14– 992.92 |

[a] $n = 3$, [b]95% confidence limit.

Table 2: Intraday and interday precision of HPTLC method.

| Amount (ng per spot) | Intraday precision ($n = 6$) | | | | Interday precision ($n = 6$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean area | SD | %RSD | SE | Mean area | SD | %RSD | SE |
| 600 | 5403.23 | 24.43 | 0.45 | 9.97 | 5356.38 | 25.23 | 0.47 | 10.30 |
| 800 | 7209.17 | 22.69 | 0.31 | 9.26 | 7154.16 | 24.29 | 0.34 | 9.91 |
| 1000 | 9015.28 | 21.79 | 0.24 | 8.89 | 8928.81 | 22.16 | 0.25 | 9.04 |

SD: standard deviation; SE: standard error.

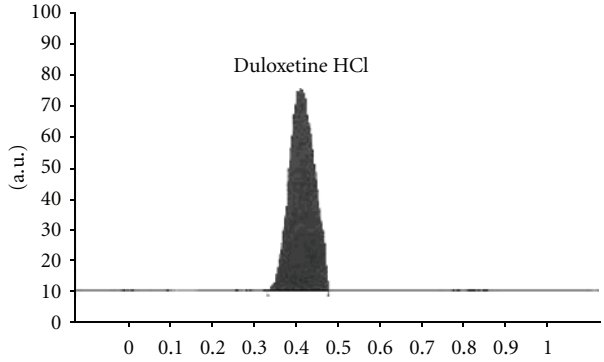


Figure 2: A typical HPTLC chromatogram of duloxetine hydrochloride ($R_f = 0.42$).

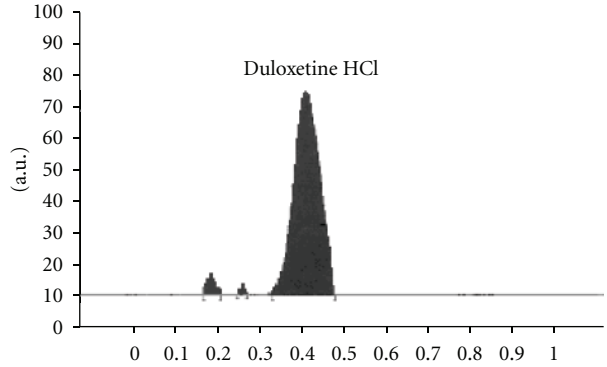


Figure 3: A HPTLC chromatogram of alkali-treated duloxetine hydrochloride.

*2.2.8. Preparation of Hydrogen Peroxide-Induced Degradation Product.* To 20 mL of methanolic stock solution, 10 mL of hydrogen peroxide (30%, v/v) was added at the room temperature. This solution was then heated in boiling water bath for 8 hours to remove the excess of hydrogen peroxide. The resultant solution (1000 ng per spot) was applied on TLC plate, and the chromatograms were recorded.

*2.2.9. Thermal Degradation Product.* The thermal degradation of the drug was carried out by heating the stock solutions at 70°C for 5 hours. The resultant solutions were appropriately diluted every hour, 1000 ng per spot was applied on TLC plate, and chromatograms were recorded.

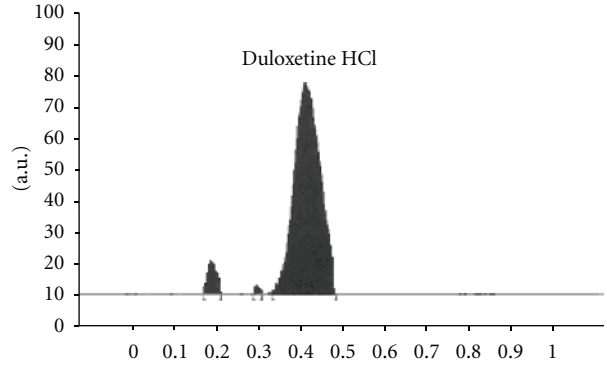


Figure 4: A HPTLC chromatogram of hydrogen peroxide-treated duloxetine hydrochloride.

## 3. Results and Discussion

*3.1. Development of the Optimum Mobile Phase.* The main purpose of our HPTLC method development and validation was to devise a stability-indicating assay method. The analysis of both pure drug as well as of degraded products were tried in different solvent systems. Initially combinations of butanol, hexane, toluene, and acetic acid with varying proportions were tried, but finally, the mobile phase consisting of chloroform : methanol (8 : 2, v/v) gave well-defined spots having good resolutions. The optimum development time was found to be 40 min at room temperature.

*3.2. Calibration Curves.* The linear regression data for the calibration curves ($n = 3$) as shown in Table 1 correlated a

Table 3: Recovery studies of duloxetine hydrochloride.

| Excess drug spiked to Preanalyzed drug (%)* | Drug content (ng per spot) | Recovery (%) | %RSD | SE |
|---|---|---|---|---|
| 0 | 600 | 99.36 | 0.55 | 0.45 |
| 80 | 1080 | 100.49 | 0.78 | 0.64 |
| 100 | 1200 | 100.45 | 0.65 | 0.53 |
| 120 | 1420 | 100.41 | 0.62 | 0.51 |

* ($n = 6$).

Table 4: Stress degradation of duloxetine hydrochloride.

| Stress conditions* | Degradation products ($R_f$ value) | Recovery (%) |
|---|---|---|
| 0.5 M HCl RT (4 h) | 2, (0.12, 0.21) | 83.13% |
| 2.0 N NaOH RT (8 h) | 2, (0.19, 0.28) | 92.28% |
| 30% $H_2O_2$ RT (8 h) | 2,(0.19, 0.31) | 91.11% |
| Heat (70°C) (5 h) | 1, (0.24) | 94.45% |

* ($n = 3$), RT (room temperature), h (hours).

Table 5: Summary of validation parameters.

| Parameter | Data |
|---|---|
| Linearity range | 600–2000 (ng per spot) |
| Limit of detection | 100 (ng per spot) |
| Limit of quantification | 350 (ng per spot) |
| Correlation coefficient | 0.9962 ± 0.0015 |
| Recovery (%) | 99.85 ± 0.60 |
| Precision (%RSD) | |
| Interday | 0.35 |
| Intraday | 0.33 |

good linear relationship over the concentration range 600–2000 ng per spot. No significant differences were observed in the slopes of standard curves.

### 3.3. Validation of the Method

*3.3.1. Precision.* The repeatability of sample application and measurement of peak area were expressed in terms of relative standard deviation (%RSD) and was found to be less than 0.1%. The results depicted in Table 2 revealed intraday and interday variation of duloxetine hydrochloride at three different concentration levels of 600, 800, and 1000 ng per spot.

*3.3.2. Robustness of the Method.* Our method was robust, since %RSD was found to be less than 2.0% after introducing deliberate small changes in temperatures and mobile phase ratio.

*3.3.3. LOD and LOQ.* As per the ICH guidelines, signal-to-noise ratio having values of 3 and 10 were considered as LOD and LOQ, respectively. The LOD and LOQ were found to be 100 and 350 ng per spot, respectively.

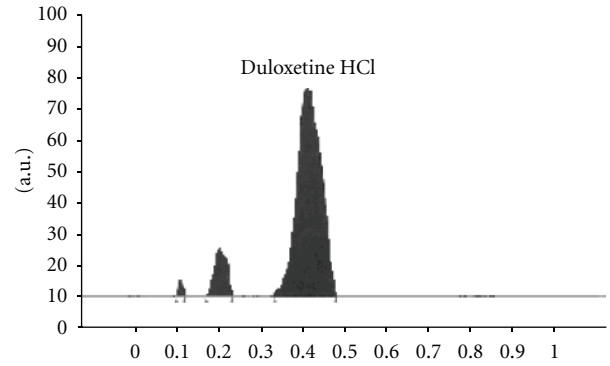


Figure 5: A HPTLC chromatogram of acid-treated duloxetine hydrochloride.

*3.3.4. Recovery Studies.* The proposed method was utilized for extraction studies, and subsequent estimation of duloxetine hydrochloride in pharmaceutical dosage forms after spiking a preanalysed sample with 80, 100, and 120% of additional drug. The recoveries were found to be between 99–101% as listed in Table 3.

*3.3.5. Analysis of the Tablet Formulation.* A single peak was observed in the chromatogram obtained in the analysis of drug samples extracted from tablets, and no additional interfering peak(s) were observed due to the presence of excipients in the tablets. The drug content was found to be 99.28% with a %RSD of 0.48%.

*3.3.6. Stability-Indicating Property.* The method distinctively separated the degraded products of duloxetine HCl due to acid, base, oxidative, and thermal treatments. The chromatograms of degraded products were well resolved from the chromatograms of drug as shown in Figures 2, 3, 4, 5 and 6. The degradation product(s) formed, $R_f$, and percentage recoveries calculated are shown in Table 4. The summary of validation parameters is shown in Table 5.

## 4. Conclusion

The proposed HPTLC method was developed and validated for precision, specificity, and accuracy. The method was applicable both for determination of bulk drug as well as the tablet formulation. The method separates the drug from its degradation products, and as such it can be described as a stability-indicating assay method (SIAM).

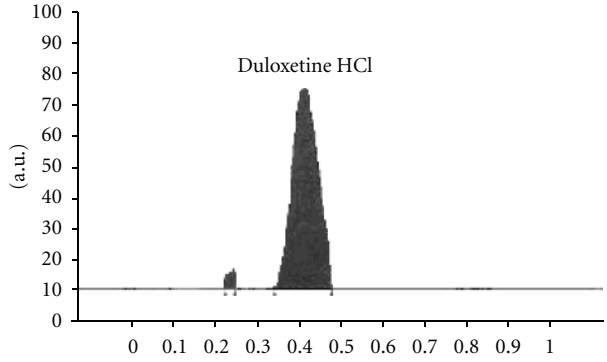


Figure 6: A HPTLC chromatogram of thermally degraded duloxetine hydrochloride.

## Acknowledgment

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