Development and validation of a HPTLC method for Estimation of Duloxetine Hydrochloride in Bulk Drug and in Tablet Dosage Form

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Duloxetine hydrochloride is a potent dual reuptake inhibitor of serotonin and norepinephrine used to treat major depressive disorders. The present work describes a simple, precise and accurate HPTLC method for its estimation as bulk and in tablet dosage form. The chromatographic separation was carried out on precoated silica gel 60 F254 aluminium plates using mixture of chloroform:methanol (8:1 v/v) as mobile phase and densitometric evaluation of spots was carried out at 235 nm using Camag TLC Scanner-3 with win CAT 1.3.4 version software. The experimental parameters like band size of the spot applied, chamber saturation time, solvent front migration, slit width etc. were critically studied and optimum conditions were evolved. The drug was satisfactorily resolved with Rf value 0.11±0.01. The accuracy and reliability of the proposed method was ascertained by evaluating various validation parameters like linearity (40-200 ng/spot), precision (intra-day RSD 0.46-0.75%, inter-day RSD 0.46-1.59%), accuracy (98.72±0.20) and specificity according to ICH guidelines. The proposed method can analyse ten or more formulation units simultaneously on a single plate and provides a faster and cost-effective quality control tool for routine analysis of duloxetine hydrochloride as bulk drug and in tablet formulation.

Key words: Duloxetine hydrochloride, HPTLC, densitometric estimation, method development and validation

Duloxetine hydrochloride (DH), chemically (+)-(S)-N-methyl-3-(1-naphthyloxy)-3-(thiophen-2-yl)-propan-1-amine is a drug that primarily targets major depressive disorders and pain related to diabetic neuropathy.1-3. It is a potent dual reuptake inhibitor of serotonin and norepinephrine. Literature review reveals that several HPLC4-6 and LC-MS7 methods have been reported for estimation of duloxetine hydrochloride in single and combined form with other drugs, but no HPTLC method is reported so far. The present study illustrates development and validation of a simple, accurate, economical and reproducible procedure for determination of duloxetine hydrochloride by HPTLC as bulk and in tablet dosage form.

Pharmaceutical grade duloxetine hydrochloride working standard was a generous gift from Hetero Drugs Ltd. Erragadda, Hyderabad. Fixed dose tablets (Duvanta-20) containing 20 mg of duloxetine hydrochloride were procured from Intas Pharmaceuticals Ltd. Ahmedabad. Silica gel 60 F254 TLC plates (20×20 cm, layer thickness 0.2 mm, E.

Duloxetine hydrochloride (20 mg) was weighed accurately and transferred to 10 ml volumetric flask. The volume was made upto 10 ml with methanol to obtain concentration of 2 μg/μl. 0.1 milliliter of the above solution was further diluted with methanol to obtain the concentration 0.02 μg/μl of duloxetine hydrochloride. For analysis in tablet dosage form, twenty tablets were weighed (each containing 20 mg of DH) and their average weight was calculated. The tablets were finely powdered and powder equivalent to 20 mg of duloxetine hydrochloride was accurately weighed and dissolved in 10 ml of methanol to obtain the concentration of 2 μg/μl. The solution was centrifuged for 15 min at 600 rpm. The solution

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was filtered through Whatman filter paper no. 41, the residue was washed with methanol and volume was adjusted to 10 ml with the same solvent. This solution was further diluted with methanol so as to have concentration same as that of final standard solution.

TLC plates were pre-washed with methanol. Activation was done in oven at 105° for 20 min. The plates were allowed to cool at room temperature. The chromatographic estimations were performed using following conditions: stationary phase, precoated silica gel 60 F254 aluminium plates (20 cm×20 cm×250 µm); mobile phase, chloroform:methanol (8:1 v/v); chamber saturation time, 20 min; wavelength of scanning, 235 nm; slit dimensions, 6.00×0.30 mm; spotting parameters used were, band width, 8 mm and space between two bands, 15.4 mm.

Four microlitres of standard solution of duloxetine hydrochloride (0.02 µg/µl) was applied on pre-washed and activated plate under nitrogen stream using semiautomatic spotter. It was developed at constant temperature in a Camag twin-trough chamber previously saturated for 20 min with mobile phase. The plate was removed from the chamber and air dried. Densitometric measurements were performed at 235 nm in reflectance mode with Camag TLC Scanner 3 using win CAT software version 1.3.4 incorporating track optimization position. For the preparation of a calibration curve, aliquots of 2, 4, 6, 8, 10 µl of standard solutions of duloxetine hydrochloride (0.02 µg/µl) were applied on the TLC plate using semiautomatic spotter under nitrogen stream. TLC plates were dried, developed and densitometrically analysed as described earlier.

The method was validated as per the ICH guidelines in terms of linearity, accuracy and specificity, intra-day and inter-day precision, repeatability of measurement of peak area as well as repeatability of sample application.

Literature survey revealed that several HPLC and LC-MS methods have been reported for estimation of duloxetine hydrochloride which is sophisticated but costly and time consuming. As no HPTLC method has been reported so far for estimation of duloxetine hydrochloride, the present study was aimed at development of a versatile, speedy and cost effective HPTLC technique for determination of duloxetine hydrochloride as bulk and in tablet dosage form.

Since duloxetine hydrochloride is freely soluble in methanol, tablet powder was extracted with methanol. Centrifugation for 15 min at 600 rpm helped to completely extract the drug from tablet matrix. Various solvent systems like mixture of chloroform: acetic acid, chloroform:ethanol:formic acid, chloroform:toluene, chloroform:benzene:toluene, chloroform:benzene:toluene:acetic acid were tried to separate and resolve spot of duloxetine hydrochloride from its impurities and other excipients of formulations. The mixture of chloroform: methanol (8:1 v/v) could resolve DH spot with better peak shape (fig 1).

The proposed method was validated according to ICH guidelines in terms of linearity, accuracy, inter and intra-day precision, repeatability and specificity (Table 1). The method was found to be linear in the range of

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Result of duloxetine hydrochloride</th>
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<tbody>
<tr>
<td>Linearity range</td>
<td>40-200 ng</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.99981</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.70</td>
</tr>
<tr>
<td>Accuracy (n=6)</td>
<td>98.72±0.20</td>
</tr>
<tr>
<td>Repeatability of sample application (n=6)</td>
<td>2.08 %</td>
</tr>
<tr>
<td>Repeatability of measurement of peak area (n=6)</td>
<td>0.22 %</td>
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<tr>
<td>Precision</td>
<td></td>
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<tr>
<td>Inter-day (n=3)</td>
<td>0.46-1.59 %</td>
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<tr>
<td>Intra-day (n=3)</td>
<td>0.46-0.75 %</td>
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<td>Specificity</td>
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Different validation parameter of the proposed HPTLC method for estimation of duloxetine hydrochloride in tablet dosage form.
areas for six spots. The RSD for the peak area values was calculated and was found to be 2.08%. The RSD values for measurement of peak area and sample application were both below the instrumental specifications (1% and 3%, respectively), ensuring proper functioning of HPTLC system.

To confirm the specificity of the proposed method, duloxetine hydrochloride was spotted on TLC plate, developed and scanned as described earlier. It was observed that excipients present in formulation did not interfere with peak of duloxetine hydrochloride (Rf, 0.11±0.01). The UV spectrum of standard duloxetine hydrochloride (fig. 2) was also compared with spectrum of duloxetine hydrochloride extracted from tablet, which showed good correlation. The proposed HPTLC method was found to be rapid, specific, precise and accurate.

### ACKNOWLEDGEMENTS

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### REFERENCES

Simultaneous UV Spectrophotometric Estimation of Ambroxol Hydrochloride and Levocetirizine Dihydrochloride

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Prabu, et al.: Simultaneous estimation of ambroxol and levocetirizine

A novel, simple, sensitive and rapid spectrophotometric method has been developed for simultaneous estimation of ambroxol hydrochloride and levocetirizine dihydrochloride. The method involved solving simultaneous equations based on measurement of absorbance at two wavelengths 242 nm and 231 nm, the \( \lambda_{\text{max}} \) of ambroxol hydrochloride and levocetirizine dihydrochloride, respectively. Beer's law was obeyed in the concentration range 10–50 µg/ml and 8–24 µg/ml for ambroxol hydrochloride and levocetirizine dihydrochloride respectively. Results of the method were validated statistically and by recovery studies.

Key words: Ambroxol hydrochloride, levocetirizine dihydrochloride, \( \lambda_{\text{max}} \), spectrophotometric method

Ambroxol hydrochloride (AMB) is chemically, trans-4-(2-amino-3,5-dibromobenzyl) amino) cyclohexanol hydrochloride. Levocetirizine dihydrochloride (LEVC) is chemically, (RS)-2-\{4-[(R)-p-chloro-α-phenylbenzyl]-1-piperazinyl\} ethoxyacetic acid dihydrochloride. AMB reduces bronchial hyper-reactivity and acts as a mucolytic and cough suppressant. LEVC is usually used in allergic conditions including rhinitis. Combination of AMB and LEVC is used for the treatment of bronchitis. These two drugs are not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of AMB and LEVC in formulations. Capillary electrophoresis, spectrometry, gas chromatography, LC with potentiometric detection, MS detection and UV detection methods have been reported for the estimation of AMB. However, no references have been found for simultaneous determination of AMB and LEVC in pharmaceutical formulations. A successful attempt has been made to estimate these two drugs simultaneously by spectrophotometric analysis.

A Shimadzu UV/Vis spectrophotometer, model-1601 (Japan) was employed with spectral bandwidth of 0.1 nm and a wavelength accuracy of ±0.5 nm with automatic wavelength correction with a pair of 3 mm quartz cells. AMB and LEVC (Aristo Pharma Ltd.), methanol (Merck India Ltd., Mumbai) and distilled water were used in the present study.

Stock solutions (500 µg/ml) of AMB and LEVC were prepared by dissolving separately in 20 ml of water in a 100 ml clean volumetric flask, and the volume was made up to 100 ml with distilled water. The maximum absorbance of AMB and LEVC was obtained at 244 nm (\( \lambda_{1} \)) and 231 nm (\( \lambda_{2} \)), respectively. AMB and LEVC showed linearity with absorbance in the range of 10–50 µg/ml and 8–24 µg/ml at their respective maxima, which were validated by least square method. Coefficients of correlation were found to be 0.9992 for AMB and 0.9993 for LEVC. For simultaneous estimation of AMB and LEVC, a series of standard solutions in concentration range of 2 to 24 µg/ml, were prepared by diluting appropriate volumes of the standard stock solutions. The scanning of solutions of AMB and LEVC were carried out in