# A validated HPTLC method for estimation of moxifloxacin hydrochloride in tablets

*Abstract*

A simple HPTLC method having high accuracy, precision and reproducibility was developed for the routine estimation of moxifloxacin hydrochloride in the tablets available in market and was validated for various parameters according to ICH guidelines. moxifloxacin hydrochloride was estimated at 292 nm by densitometry using Silica gel 60 $F_{254}$ as stationary phase and a premix of methylene chloride: methanol: strong ammonia solution and acetonitrile (10:10:5:10) as mobile phase. Method was found linear in a range of 9-54 nanograms with a correlation coefficient >0.99. The regression equation was: AUC = 65.57 × (Amount in nanograms) + 163 ($r^2$ = 0.9908).

**Key words:** HPTLC, moxifloxacin hydrochloride and Validation

## INTRODUCTION

Moxifloxacin hydrochloride, a fluoroquinolone, is slightly yellow crystalline monohydrochloride salt of 1-Cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-[(4aS, 7aS)-octahydro-6H-pyrrolo [3, 4-b] pyridin-6-yl]-4-oxo-3-quinoline carboxylic acid. It has been found to be effective in acute bacterial sinusitis[1] acute bacterial exacerbation of chronic bronchitis,[2] community acquired pneumonia,[3] skin and skin structure infections.[4] Commercially, it is available as ophthalmic solutions, oral tablets and I.V. diffusions in plastic containers. Various methods reported for its estimation are based on microbiological assays,[5] capillary electrophoresis,[6] voltametric determination,[7] liquid chromatography with UV detection,[5] atomic absorption spectrometry[8] and electrospray-ionization tandem mass spectrometric detection[9] For a cheaper and less time consuming routine analysis of moxifloxacin hydrochloride in bulk and formulations, the above-stated methods may stay tedious, time consuming and expertise requiring. Hence, a simple and validated HPTLC method for the estimation of the drug in marketed tablets can be of much significance and ease the treatment.

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## MATERIALS AND METHODS

### Instrumentation

Camag HPTLC system (Muttenz, Switzerland) with Linomat 5 sample applicator, TLC scanner 3, HPTLC plate heater III, UV cabinet, 100 µl Hamilton Syringe (Bonaduz, Schweiz), twin trough development chambers (for 10 cm x 10 cm sheets), and winCATS 1.3.4 software was used for the analytical purpose. Merck KGa A coated HPTLC aluminum sheets with Silica gel 60 $F_{254}$ (Darmstadt, Germany) were used as stationary phase. Mettler Toledo balance (Ohio, USA) model XP 205 was used for weighing the chemicals and reagents.

### Materials

Moxifloxacin hydrochloride working standard was obtained as a gift sample from Ranbaxy Research Laboratories (Gurgaon, India). Moxifloxacin hydrochloride

tablets (Claiming 400 mg of Moxifloxacin per tablet) of three different brands [Moxicip, Cipla Limited; Avelox, Bayer Healthcare; Moxif, Torrent Pharmaceuticals Limited] were collected from market and analyzed for the Moxifloxacin content by the proposed method. All the other chemicals and reagents were of analytical grade.

### Thin layer chromatography development

A premix of methylene chloride: methanol: strong ammonia solution and acetonitrile in a ratio of 10:10:5:10; respectively was optimized for thin layer chromatography plate development. A run distance was kept about 70 mm and 10 ml of the mobile phase was used for single development. The Rf value of Moxifloxacin peak was observed about 0.51. The dosing speed of nitrogen applicator was kept 150 nl/sec with a pre-dosage volume of 0.2 µl. Samples were applied as bands of 6 mm width with the gaps of 10 mm in between. Developed plates were dried at 40°C for 5 min. Detection was done at 292 nm using deuterium lamp in absorption-re-emission mode. The slit dimension of detection was kept 6.00 mm x 0.45 mm, scanning speed 20 mm/sec and data resolution 100 µm/step. The various statistical reports were generated according to the standard formulae and parameters were validated as per ICH[10] guidelines.

### Specificity and selectivity

The absence of any secondary spot having spectra different from moxifloxacin hydrochloride in the typical constituted placebo chromatogram of the tablet preparation, which may interfere with Moxifloxacin peak, indicates the specificity of the analytical method [Figures 1 and 2].

### Calibration standards, linearity and range

Moxifloxacin hydrochloride solution (4.5 µg/ml) was prepared in methanol and its 2, 4, 6, 8, 10 and 12 µl volumes were applied on the HPTLC plate as separate spots. The plate was developed, dried and analyzed at 292 nm by densitometry. The calibration data was generated [Table 1] and regression analysis [Table 2] was performed.

### Precision and formulation analysis

Precision was demonstrated by analyzing the tablet preparations in six replicates. Three different moxifloxacin hydrochloride tablet samples - Moxicip, Avelox and Moxif were prepared by sonicating the tablets in methanol. % Assay calculations (as Moxifloxacin hydrochloride) were based on the calibration curve. % Relative standard deviation of the % w/w assay values were reported [Table 3].

### Accuracy

Pre analyzed tablet sample preparations were spiked with moxifloxacin hydrochloride at three different levels (29.5 ng, 34.4 ng and 44.0 ng) and were analyzed

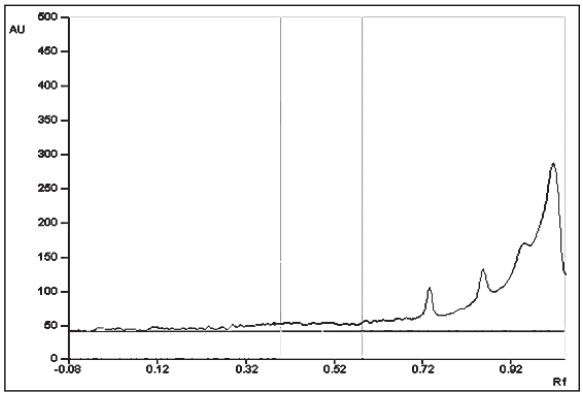


**Figure 1:** HPTLC chromatogram of the constituted tablet placebo.

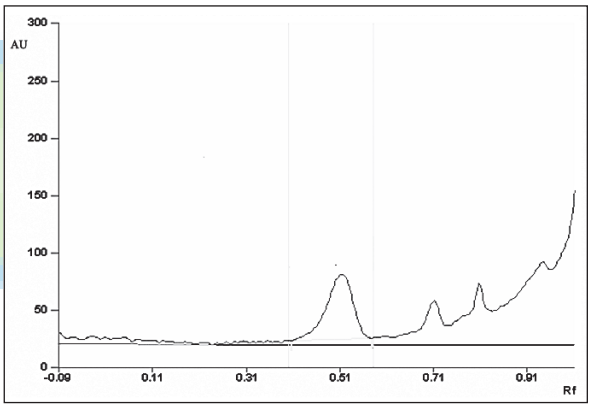


**Figure 2:** Chromatogram of the moxifloxacin hydrochloride Tablet

**Table 1: Calibration data for linearity**

| Amount in nanograms per spot | AUC at 292 nm (% RSD) ( n = 3) |
|---|---|
| 9.0 | 640 (0.79) |
| 18.0 | 1,339 (1.07) |
| 27.0 | 2,039 (0.88) |
| 36.0 | 2,642 (1.08) |
| 45.0 | 3,139 (1.08) |
| 54.0 | 3,570 (0.82) |

**Table 2: Regression analysis**

| Parameters | Results (n = 3) |
|---|---|
| Equation of the regression line | AUC = 65.57 × (Amount in nanogram) + 163 |
| Regression coefficient ($r^2$) | 0.9908 |
| Correlation coefficient | 0.9954 |

**Table 3: Method precision of the analytical method**

| Sample/Spot | Assay (%w/w) Moxicip | Assay (%w/w) Avelox | Assay (%w/w) Moxif |
|---|---|---|---|
| Sample/Spot-1 | 99.0 | 101.4 | 100.7 |
| Sample/Spot-2 | 98.7 | 98.4 | 100.9 |
| Sample/Spot-3 | 101.1 | 100.1 | 101.1 |
| Sample/Spot-4 | 98.1 | 99.5 | 101.6 |
| Sample/Spot-5 | 101.1 | 98.6 | 101.7 |
| Sample/Spot-6 | 101.8 | 100.8 | 100.5 |
| Mean | 100.0 | 99.8 | 101.1 |
| Standard deviation | 1.541 | 1.193 | 0.467 |
| RSD (%) | 1.54 | 1.19 | 0.46 |

**Table 4: Recovery study**

| Formulation spiked | Moxicip | Avelox | Moxif |
|---|---|---|---|
| Level of spiking (ng) | 29.5 | 34.4 | 44.0 |
| | | Recovery (%) | |
| Spot-1 | 100.6 | 97.9 | 100.1 |
| Spot-2 | 101.5 | 100.2 | 99.1 |
| Spot-3 | 98.7 | 103.8 | 98.0 |
| Spot-4 | 100.6 | 100.9 | 97.0 |
| Spot-5 | 103.4 | 101.2 | 98.6 |
| Spot-6 | 102.5 | 100.7 | 99.0 |
| Mean % recovery | 101.2 | 100.8 | 98.6 |
| SD | 1.661 | 1.901 | 1.057 |
| RSD (%) | 1.64 | 1.89 | 1.07 |

in six replicates. Accuracy was reported as % recovery [Table 4] based on actual and estimated concentrations.

### Ruggedness

Ruggedness of the proposed method was determined by changing the duration of the chamber saturation i.e., 30 ± 10 min. Assay (%) was determined.

## RESULTS AND DISCUSSION

The proposed analytical method for assay determination of moxifloxacin hydrochloride in Tablets was found suitable and applicable to different tablet formulations in market. Method was found linear in the range of ~9–54 ng with a good correlation of 0.99. A lower % RSD (below 2%) of % assay values, observed during replicate analysis of different tablets as the part of precision, indicate the suitability of the method. A % recovery ranging within 98-102 (%) demonstrated good accuracy of the analytical method. Additionally, the method was found rugged for chamber saturation time. The proposed method can be extended for assay of moxifloxacin hydrochloride in other formulations like parenteral preparations or ophthalmic solutions.

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