

## Research Article



## A Validated HPTLC Method for the Determination of Doripenem in Pharmaceutical Dosage Forms

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### ABSTRACT

A simple, selective and precise high performance thin layer chromatographic method was developed and validated for the determination of Doripenem in bulk drug and in formulation. The method uses aluminium plates pre-coated with silica gel 60<sub>F-254</sub> as the stationary phase and butyl alcohol: glacial acetic acid: water (4:2:2, v/v/v) as solvent system. This system gave compact spot for Doripenem ( $R_f$ : 0.45 ± 0.02). Densitometric analysis of Doripenem was performed in the absorbance mode at 294nm. The linear regression analysis data for the calibration plot showed good linear relationship over a concentration range of 1 – 5 µg spot<sup>-1</sup>. The values of correlation coefficient, slope and intercept were 0.9991, 1915.4 and 2222.4 respectively. The method was validated for precision, robustness and recovery. The limit of detection and limit of quantification were 0.031 and 0.095 µg spot<sup>-1</sup>, respectively.

**Keywords:** Doripenem, Dosage forms, HPTLC, Validation.

### INTRODUCTION

Doripenem is chemically (+)-(4R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-4-methyl-7-oxo-3-[[3S,5S]-5-[(sulfamoylamino)-methyl]-3-pyrrolidinyl]thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid. It is a broad spectrum carbapenem antibiotic with antibacterial activity against a broad range of gram positive and gram negative bacteria, as well as against a variety of anaerobes.<sup>1,2</sup> Doripenem appears to be a potent carbapenem with a spectrum resembling currently marketed antipseudomonal carbapenems, but with greater activity when tested against some non-fermentative bacillary strains. Furthermore, Doripenem has a 1-β-methyl group, hindering attack by human renal dehydropeptidase-1 and thus, unlike imipenem, its use does not require co-administration of a dehydropeptidase-1 inhibitor such as cilastatin. It has been recently approved in the European Union and the United States for clinical use in complicated intra-abdominal and urinary tract infections, joining imipenem, meropenem and ertapenem.<sup>3,4</sup> In comparison to other carbapenems, it has equal or greater activity against β-lactam nonsusceptible Enterobacteriaceae, including strains with extended spectrum β-lactamases.<sup>5</sup> Doripenem was also found to be the most active carbapenem against *Pseudomonas aeruginosa*.<sup>1,4</sup>

The Literature survey reveals few studies regarding determination of Doripenem in pharmaceutical dosage forms and biological fluids. These works include HPLC, UV Spectrophotometric and potentiometric methods to determine Doripenem in pharmaceutical dosage forms.<sup>6-9</sup> The assay of Doripenem in the human plasma and mouse serum are also reported by HPLC methods.<sup>10-14</sup>

Doripenem is not official in any Pharmacopoeia and there is no monograph containing methods to characterize or quantify Doripenem. Such methods could offer official parameters to guarantee the validity of the assay. Hence, there is a need for simple, rapid and reproducible method for the routine analysis of Doripenem in pharmaceutical dosage forms.

The present study describes the development and validation of an HPTLC method for assay of Doripenem.<sup>15,16</sup>

Today TLC is rapidly becoming a routine analytical technique due to its low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase and thus reducing the analysis time and cost per sample as compared to HPLC. The aim of the present study was to develop a simple, validated and rapid HPTLC method for routine analysis of Doripenem in injections. The HPTLC method was studied following official guidelines, evaluating the main parameters and the procedures and validated according to ICH guidelines.

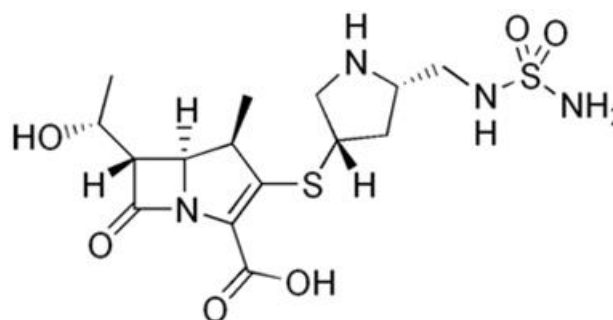


Figure 1: Structure of Doripenem



## MATERIALS AND METHODS

### Chemicals and Reagents

Doripenem monohydrate Reference Standard was supplied by Akorn India Private Limited, Himachal Pradesh, India. Doripenem Injection was purchased from local market. All the chemicals and reagents used were of Analytical grade and were purchased from Merck Chemicals, India.

### HPTLC Instrumentation

The samples were spotted in the form of 6mm width with a Camag microlitre syringe on pre-coated silica gel aluminium plates 60 F<sub>254</sub> (10 × 10 cm with 250 mm thickness, E. Merck), using a Camag Linomat 5 applicator. The plates were pre-washed with methanol and activated at 60°C for 5 min prior to chromatography. The slit dimension was kept at 6.00 × 0.45 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of butanol: glacial acetic acid: water 4:2:2, (v/v/v), and 10 ml of mobile phase was used. Linear ascending development was carried out in a 10 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25°C±2). The length of the chromatogram run was approximately 8 cm, subsequent to development; the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by WINCats software.

### Preparation of standard solution and linearity study

An accurately weighed quantity of 10 mg of Doripenem was transferred to 10 ml volumetric flask, dissolved in methanol and made up to mark with the same solvent to obtain concentration 1 µg/µl. Standard solutions of 1, 2, 3, 4, 5 and 8 µl of Doripenem was applied on TLC plate with the help of microlitre syringe, using Linomat 5 sample applicator to obtain the concentration of 1, 2, 3, 4, 5 and 8 µg spot<sup>-1</sup>. The standard curves were evaluated for within day and day-to-day reproducibility. Each experiment was repeated six times.

### Method validation

#### Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (3 µg spot<sup>-1</sup> of Doripenem). The intra and inter-day variation for the determination of Doripenem was carried out at three different concentration levels of 2, 3 and 4 µg per spot.

#### Limit of detection (LOD) and limit of quantification (LOQ)

In order to determine detection and quantification limit, Doripenem concentrations in the lower part of the linear range of the calibration curve were used. Doripenem solutions of 1, 2, 3, 4, 5 and 8 were prepared and applied

in triplicate. The LOQ and LOD were calculated using equation  $LOD=3.3 \times N/B$  and  $LOQ=10 \times N/B$ , where, N is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

#### Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot of Doripenem in sample was confirmed by comparing the R<sub>f</sub> values and spectra of the spot with that of standard. The peak purity of Doripenem was assessed by comparing the spectra at three levels, i.e., peak start(S), peak apex(M) and peak end(E) positions of the spot.

#### Ruggedness

Ruggedness of the method was performed by spotting 3 µg spot<sup>-1</sup> of Doripenem by two different analyst keeping same experimental and environmental conditions.

#### Accuracy

The analyzed samples were spiked with extra 80, 100 and 120% of the standard Doripenem and the mixtures were analyzed by the proposed method. At each level of the amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations.

#### Robustness

By introducing small changes in the mobile phase composition, the effects of the results were examined. Mobile phases having different compositions of butanol: glacial acetic acid: water was tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ±5%. The plates were prewashed by methanol and activated at 60±5°C for 2, 5 and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20 and 40 min.

### Application of proposed method to Injection formulation

To determine the concentration of Doripenem in injections, a quantity of Doripenem powder for injection (INAREM 500mg) equivalent to 10 mg of Doripenem was dissolved in methanol, sonicated for 20 minutes and made up to volume in a 10ml volumetric flask. After filtration through 0.41 µm filter (millifilter, Milford, MA), 3 µl of the solution was spotted followed by development and scanning as described in standard preparation. The analysis was repeated in triplicate.

## RESULTS AND DISCUSSION

### Development of optimum mobile phase

TLC procedure was optimized with a view to develop a sensitive and reproducible assay method for Doripenem. Initially, butanol:water 6:4, (v/v) gave good resolution for



Doripenem, but typical peak nature was missing. Finally, the mobile phase consisting of butanol: glacial acetic acid: water 4:2:2, (v/v/v) gave a sharp and well defined peak at R<sub>f</sub> value of 0.45 (Fig. 3). Well defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature (Figure 4).

**Calibration curve**

The linear regression data for the calibration curves showed good linear relationship over the concentration range 1-5µg spot<sup>-1</sup> (Figure 2) Linear regression equation was found to be Y=1915.4x+2222.4 (r<sup>2</sup>=0.9991).

**Table 1:** Results of intraday and inter-day precision studies

Drug	Conc. µg/spot	Intraday		Inter-day	
		% Amount found*±SD	% RSD	% Amount found*±SD	% RSD
Doripenem	2	99.96±0.38	0.38	99.79±0.34	0.34
	3	100.26±0.28	0.28	100.67±0.54	0.54
	4	99.99±0.21	0.21	100.09±0.21	0.21

\*n=6

**Table 2:** Results of recovery studies of Doripenem

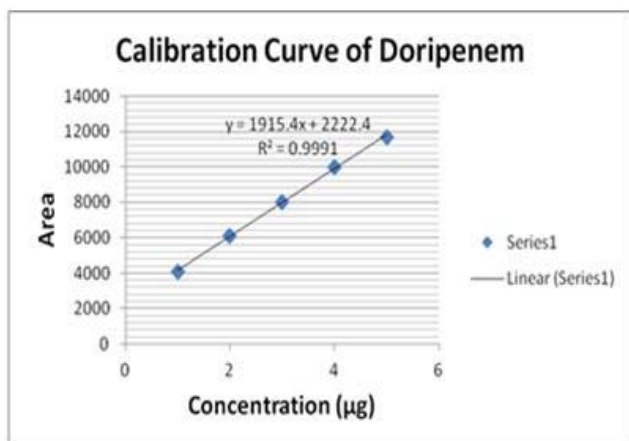
Label claim of Doripenem Injection (INAREM) (mg/vial)	Amount of Standard drug added (%)	Drug recovered * (%)	% RSD
500	0	100.17	0.44
500	80	100.97	0.34
500	100	99.97	0.16
500	120	101.47	0.23

\*n=6

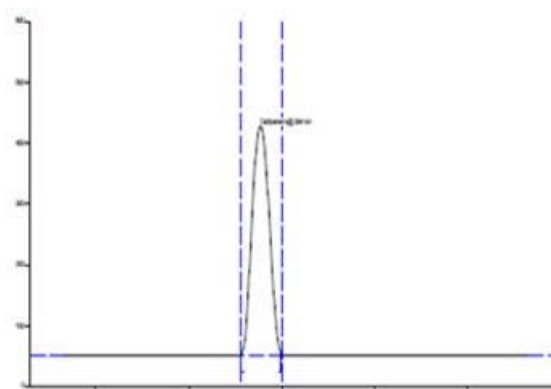
**Table 3:** Results of robustness studies\*

Parameter	S.D. of peak area	% RSD
Mobile phase composition	18.65	0.23
Mobile phase volume	19.72	0.25
Development distance	23.72	0.30
Activation of TLC plate	21.04	0.26
Duration of saturation	19.02	0.24
Time from spotting to chromatography	21.54	0.27
Time from chromatography to scanning	21.72	0.27

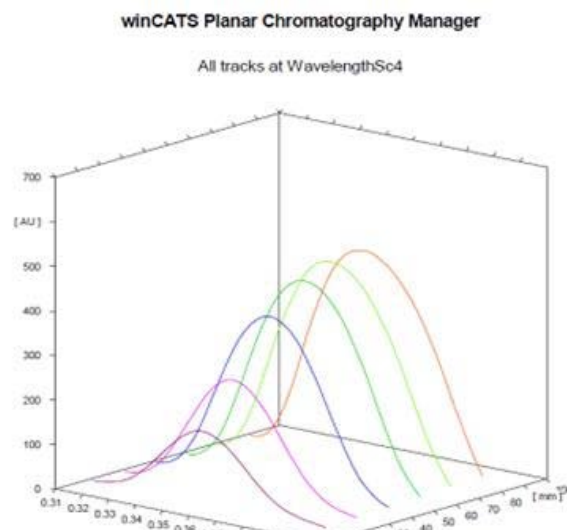
\* n=6



**Figure 2:** Calibration Curve of Doripenem



**Figure 3:** Chromatogram of Doripenem



**Figure 4:** Scan of all tracks of spots

— 1µg — 2µg — 3µg — 4µg — 5µg — 8µg spots.

**Table 4:** Results of analysis of Doripenem Injection by proposed method\*

Label Claim	Amount found ± SD	% of Label claim ± SD
500mg	502.30mg ± 2.90	100.46 ± 0.58

\* n=6

## Validation of method

### Precision

The precision of the developed HPTLC method was expressed in terms of % relative standard deviation (% RSD). The results depicted revealed high precision of the method presented in Table 1.

### LOD and LOQ

Detection limit and quantification limit was calculated by the method described above. The LOQ and LOD were found to be 0.031 and 0.095  $\mu\text{g spot}^{-1}$  respectively. This indicates the adequate sensitivity of the method.

### Recovery studies

The proposed method when used for extraction and subsequent estimation of Doripenem from the pharmaceutical dosage form after spotting with 80, 100 and 120% of additional drug; afforded good recovery of Doripenem. The amount of drug added and the % recovery are listed in Table 2.

### Specificity

The peak purity of Doripenem was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot, i.e.,  $r^2$  (S, M)=0.998 and  $r^2$  (M,E)=0.999. Good correlation ( $r^2=0.99$ ) was also obtained between standard and sample spectra of Doripenem.

### Robustness of the method

The standard deviation of peak areas was calculated for each parameter and %RSD was found to be less than 2%. The low values of %RSD values as indicated in Table 3 indicated robustness of the method.

### Analysis of the marketed formulation

A single spot at  $R_f$  0.45 was observed in the chromatogram of the drug samples applied from the injections. There was no interference from excipients. The % drug content and %RSD were calculated (Table 4). The low %RSD value indicated the suitability of this method for the routine analysis of Doripenem in pharmaceutical dosage forms.

## CONCLUSION

The developed HPTLC technique was simple, precise, accurate and stability indicating. As the carbapenems are sensitive to degradation, selectivity is an important validation parameter. The low value of %RSD of precision, accuracy and robustness studies of the method shows that it is selective and reproducible for the analysis of Doripenem in pharmaceutical dosage forms. It can be used to determine the purity of the drug available from various dosage forms.

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