HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF OFLOXACIN IN PLASMA

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ABSTRACT

A new simple High Performance Thin Layer Chromatographic (HPTLC) method for determination of Ofloxacin in plasma has been developed and validated. A Simple precipitation method was carried out by using Trichloroacetic acid and a known amount of supernatant solution was spotted on precoated silica gel 60 F254 plates using a Camag Linomat IV autosampler. Detection and quantitation were performed without using an internal standard. The mobile phase selected was n-butanol: Methanol: Ammonia (6:1.3 v/v/v) with UV detection at 295 nm. The calibration curves of Ofloxacin in methanol and in plasma were linear in range 100-600 ng. The limit of quantization for Ofloxacin in human plasma was 100 ng and no interference was found from endogenous compounds. The recovery of Ofloxacin from human plasma using the described precipitation procedure was about 88.104%. The method provides a direct estimate of the amount of Ofloxacin present in human plasma.

Key words: Ofloxacin, High Performance Thin Layer chromatography, Trichloroacetic acid, Densitometry.

INTRODUCTION

The fluoroquinolones are a series of synthetic antibacterial agents which are used for the treatment of a variety of bacterial infections. They have demonstrated activity against a wide range of Gram-positive and Gram-negative organisms and have proved useful against micro-organisms that are resistant to other antibacterial agents. Ofloxacin, a fluorinated carboxyquinolone, is the racemate, (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid.

Ofloxacin exhibits a marked bactericidal effect by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. In vitro studies on bacterial activity against Mycobacterium tuberculosis have suggested that ofloxacin is likely to be the most useful drug in the early stages of treatment and in preventing the emergence of resistance to other drugs. Its favorable pharmacokinetic features include good oral absorption and lack of metabolism resulting in less drug interactions. There is favorable pharmacokinetic features include good oral absorption and lack of metabolism resulting in less drug interactions.

HPLC seperation & quantitation of ofloxacin enantiomers in rat microsomes and Determination of the antibacterial ofloxacin in human urine and serum samples by solid-phase spectrofluorimetry.

EXPERIMENTAL

Reagents

A reference standard of Ofloxacin was obtained from CIPLA (Nasik, Maharashtra). N-butanol, methanol, ammonia, (all AR grade) were used for for developing TLC plates. Trichloro acetic acid was used for precipitation.

Preparation of standard solutions

5 mg of Ofloxacin was diluted with methanol final volume of 10 ml in volumetric flask. Standard solutions were obtained by diluting the stock solutions to concentrations ranging from 2 to 120 ug/ml.

Preparation of plasma samples

To 0.5 ml of plasma, 0.5 ml of Ofloxacin working standard (Final concentrations: 100,200,300,400,500,600 ng), 0.5 ml methanol and 0.5 ml of trichloro acetic acid (10 % w/v) were added to a glass tubes. Each sample was vortex mixed for 3 min and centrifuged (2500 rpm for 15min). Unknown plasma samples were prepared in an identical manner except for the addition of Ofloxacin.

Instrumentation and chromatographic conditions

A remi cyclomixer was used for mixing and vortexing the samples. The 20-µl aliquots of the samples were spotted onto TLC plate in the form of bands of width of 6 mm with space between bands of 5 mm, with a 100 µl sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F254 (10 x 10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm x 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using mobile phase n-butanol: Methanol: Ammonia (6:1.3 v/v/v). The optimized chamber saturation time for mobile phase was 10 min. The length of chromatogram run was 9 cm and development time was approximately 20 min. The drug had an Rf value of 0.40 and
was separated from other components in plasma. TLC plates were
dried in a current of air with the help of a hair drier. Densitometric
scanning was performed on CAMAG thin layer chromatography
scanner at 295 nm for all developments operated by WINCATS
software version 1.4.2. The source of radiation utilized was
deuterium lamp emitting a continuous UV spectrum between 200 to
400 nm.

Quantitation
The quantization of the chromatograms was performed using the
ratio of the peak area to that of a standard. A representative
standard curve of Ofloxacin was obtained by plotting
the area under the peak of Ofloxacin against the concentration
of Ofloxacin. The data were best fitted by a linear equation
\( y = mx + b \) The recovery
linearity of the detector response was tested by spotting standards
solutions containing different concentrations
of Ofloxacin in human plasma samples was 100 ng/spot.

Method validation
Linearity of HPTLC method was constructed by analysis of six
samples in triplicate spiked with 200, 300 and 400 ng/spot with the peak areas obtained from
spiking plasma samples having concentrations of 100 and 400 ng. Freeze thaw stability of the spiked quality control
samples was determined after three freeze thaw cycles stored at -28
\( ^\circ \)C ± 5 \( ^\circ \)C. Short term stability of the spiked quality control samples was
determined for a period of 5 hours stored at room temperature.

RESULTS AND DISCUSSION
The peak area was observed to be dependent on the amount of the
standard, ofloxacin and a linear relationship \( r^2=0.996 \) was found
between the peak areas of ofloxacin at various concentrations over
the range 100-600 ng (fig 1). The solvent system used for
development of the plates produced no interference peaks in the
area under the curve, and all other compounds were distinctly
separated (fig 2). The RF value of ofloxacin under the conditions
used was found to be 0.40±0.05 and spots were quantified at a
wavelength of 295 nm. The accuracy, precision and reliability of the
procedure were ascertained by adding known concentrations of
drug to drug-free plasma and analyzing five samples of each
concentration by the method described for precipitation (Table 1).
The recovery of ofloxacin in the precipitation procedure from 0.5 ml
of plasma was found to be 96.66 % (n=3) given in (Table 2). The
intra- and inter- day precisions are given in (Table 3, 4.)
ofloxacin was shown to be stable through three freeze-thaw cycles,
during storage at -28°C ± 5°C, and in the short term stability 5hr at
room temperature and stock solution stability 3hr and 30 min.,
results obtained were precise and accurate 5.

### Table 1: Accuracy and precision of a HPTLC method for the determination of Ofloxacin in plasma.

<table>
<thead>
<tr>
<th>Concentration Added [ng/spot]</th>
<th>Concentration detected (mean±SD, n=5) [ng/spot]</th>
<th>C.V. A (%)</th>
<th>Accuracy B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>189.03 ± 14.63</td>
<td>7.74</td>
<td>94.51</td>
</tr>
<tr>
<td>300</td>
<td>280.88 ± 11.99</td>
<td>4.27</td>
<td>93.62</td>
</tr>
<tr>
<td>400</td>
<td>378.77 ± 15.07</td>
<td>3.98</td>
<td>94.69</td>
</tr>
</tbody>
</table>

A. Coefficient of variation.
B. After correction for recovery.

### Table 2: Recovery study data of a HPTLC assay for Ofloxacin in plasma.

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration [ng spot⁻¹]</th>
<th>Mean amount [ng spot⁻¹] (Mean ± S.D.)</th>
<th>Mean recovery [%]</th>
<th>RSD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma (Mean ± S.D.)</td>
<td>Solution (Mean ± S.D.)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>200</td>
<td>181.41 ± 10.113</td>
<td>185.27 ± 11.920</td>
<td>97.91</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>288.68 ± 15.311</td>
<td>301.11 ± 14.798</td>
<td>95.87</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>386.61 ± 16.502</td>
<td>401.85 ± 19.192</td>
<td>96.21</td>
</tr>
<tr>
<td>Average mean recovery [%]</td>
<td></td>
<td>96.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Intra-day analysis of Ofloxacin.

<table>
<thead>
<tr>
<th>Concentration Added (ng/spot)</th>
<th>Mean amounts of drug found (Mean ± S.D.) [ng/spot]</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>174.21 ± 7.863</td>
<td>4.51</td>
</tr>
<tr>
<td>300</td>
<td>275.51 ± 10.433</td>
<td>3.79</td>
</tr>
<tr>
<td>400</td>
<td>376.74 ± 13.625</td>
<td>3.62</td>
</tr>
</tbody>
</table>

### Table 4: Inter-day analysis of Ofloxacin

<table>
<thead>
<tr>
<th>Concentration Added (ng/spot)</th>
<th>Mean amounts of drug found [ng/spot] (Mean ± S.D.)</th>
<th>Mean from 3 days ± S.D.) [ng/spot]</th>
<th>RSD [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>176.75</td>
<td>171.39 ± 6.811</td>
<td>3.97</td>
</tr>
<tr>
<td>300</td>
<td>266.68</td>
<td>269.55 ± 7.690</td>
<td>2.85</td>
</tr>
<tr>
<td>400</td>
<td>397.19</td>
<td>384.81 ± 10.886</td>
<td>2.83</td>
</tr>
</tbody>
</table>

### Table 5: Stability study data of HPTLC assay for ofloxacin in plasma.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Concentration of ofloxacin [ng spot⁻¹]</th>
<th>Mean R.S.D.</th>
<th>Mean R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze and thaw (n=5)</td>
<td>100</td>
<td>91.84</td>
<td>93.58</td>
</tr>
<tr>
<td>Short term (n=3)</td>
<td>200</td>
<td>92.71</td>
<td>93.85</td>
</tr>
<tr>
<td>Stock solution (n=3)</td>
<td>400</td>
<td>94.74</td>
<td>91.54</td>
</tr>
</tbody>
</table>
CONCLUSIONS
This HPTLC method for quantification of ofloxacin in human plasma is accurate, precise, rapid, and selective. It is a simple, practical, and economical alternative for studies of the bioavailability, bioequivalence, and pharmacokinetics of this drug in human plasma. The advantages of the method are that it uses a small amount of sample (20 μL), the volume of mobile phase used is approximately 10 mL per plate, detection of the plates takes approximately 7 min.

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REFERENCES