ABBREVIATIONS AND ACRONYMS

ACN	Acetonitrile
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine transaminase
Anti-TB	Anti-tuberculosis
ARV	Antiretroviral
AST	Aspartate transaminase
AUC_{0-24}	Area under the concentration-time curve from zero to 24 hours
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from zero to infinity
BCH	Brooklyn Chest Hospital
Cl_{tot}	Total clearance
C_{max}	Maximum concentration
Cp_{last}	Last measurable concentration
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOT	Directly observed treatment
ELISA	Enzyme-linked immuno-sorbent assay
EMB	Ethambutol
ETH	Ethionamide
GFR	Glomerular filtration rate
GGT	Gamma glutamyl transpeptidase
GIT	Gastro intestinal Tract
HAART	Highly active anti-retroviral therapy
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
INH	Isoniazid
KAN	Kanamycin
K_e	Elimination rate constant

KFT	Kidney function test
LC	Liquid Chromatography
LFT	Liver function test
LLD	Low limit of detection
LLQ	Low limit of quantification
MDRD	Modification of diet in renal disease
MDR-TB	Multi-drug resistant tuberculosis
MIC_{90}	Minimum inhibitory concentration for 90%
MRT	Mean residence time
MS	Mass spectrometry
MTB	*Mycobacterium tuberculosis*
NCA	Non compartmental analysis
NNRT	Non nucleoside reverse transcriptase inhibitor
PI	Protease inhibitor
UV	Ultra violet
PK	Pharmacokinetic
PZA	Pyrazinamide
RIF	Rifampicin
RSD	Relative standard deviation
SD	Standard deviation
T½	Half-life
TB	Tuberculosis
TCA	Trichloroacetic acid
TDM	Therapeutic drug monitoring
HPLC	High performance liquid chromatography
TFA	Trifluroacetic acid
T_{max}	Time to reach maximum concentration
UCB	Un-conjugated bilirubin
V_d	Volume of distribution
WHO	World health organization
XDR-TB	Extensively drug resistant tuberculosis

The CD4 cell counts mean (±SD) for the patient co-infected with HIV was 144±113.09 cell/mm^3, whereas the mean (±SD) of GFR rate for all patients was 109±28.89 ml/min. Regarding the liver enzymes results, the mean (±SD) of ALT and AST were 34.37±25.60 u/l and 75±46.31 u/l respectively. The mean (±SD) of GGT is 68.37±81.98 u/l and for UCB was 14.12±10 µmol/L.

4.3 Ofloxacin plasma concentrations and pharmacokinetic parameters

4.3.1 Ofloxacin plasma concentrations

Ofloxacin plasma concentrations at different time points after ofloxacin administration, over 24 hour's period are listed in Table 4.4. There are some patients with no concentration results at 1 and 2 hours because the sample tubes were broken during the centrifugation. Patients with missing 24 hour's concentrations left the hospital before their blood samples were taken.

Table 4.4: Ofloxacin plasma concentrations

Patient	Baseline (0 hr) (µg/ml)	Time (hours) after ofloxacin administration				
		1 hr (µg/ml)	2 hrs (µg/ml)	4 hrs (µg/ml)	8 hrs (µg/ml)	24 hrs (µg/ml)
1	3.53	2.73	2.84	7.87	7.51	4.13
2	BDL	**NA**	3.90	5.24	3.34	0.73
3	BDL	**NA**	2.46	3.11	3.80	**NA**
4	BDL	4.95	4.50	4.44	3.35	1.22
5	BDL	5.50	6.85	6.16	4.68	1.87
6	BDL	0.86	1.32	2.98	2.54	0.51
7	BDL	2.68	**NA**	3.90	2.65	**NA**
8	1.17	0.82	0.96	1.20	0.63	0.62
Mean	**2.35**	**2.92**	**3.26**	**4.36**	**3.56**	**1.51**
SD	**1.66**	**1.97**	**2.02**	**2.07**	**1.98**	**1.37**

NA= not available.

BDL= below detectable level (0.05 µg/ml)

Ofloxacin plasma concentration-time profiles for each patient and mean (±SD) plasma concentration-time profile for all patients are shown in Figures 4.3 and 4.4 respectively.

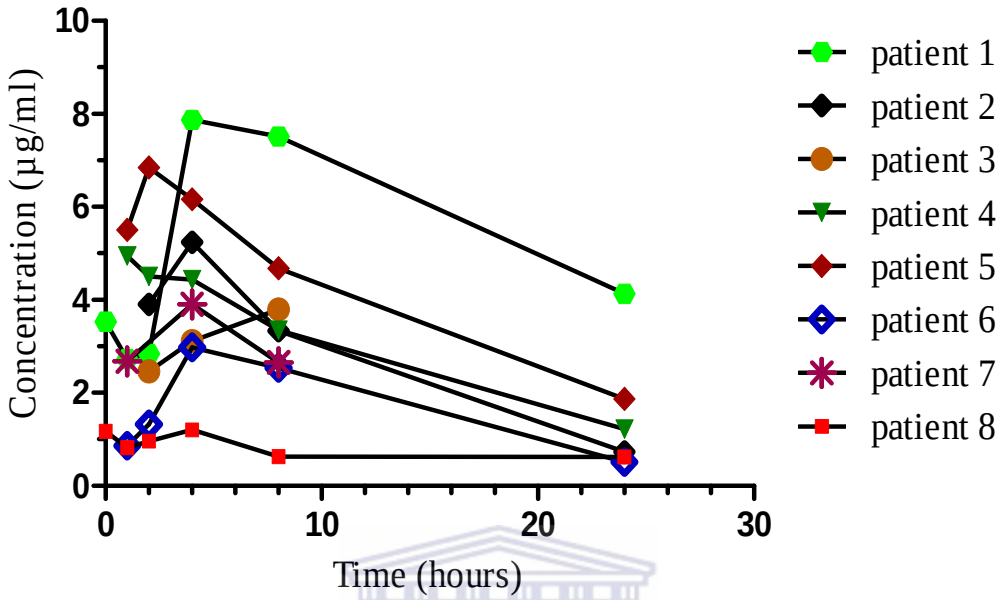

Figure 4.3: Ofloxacin plasma concentration-time profile for each patient.

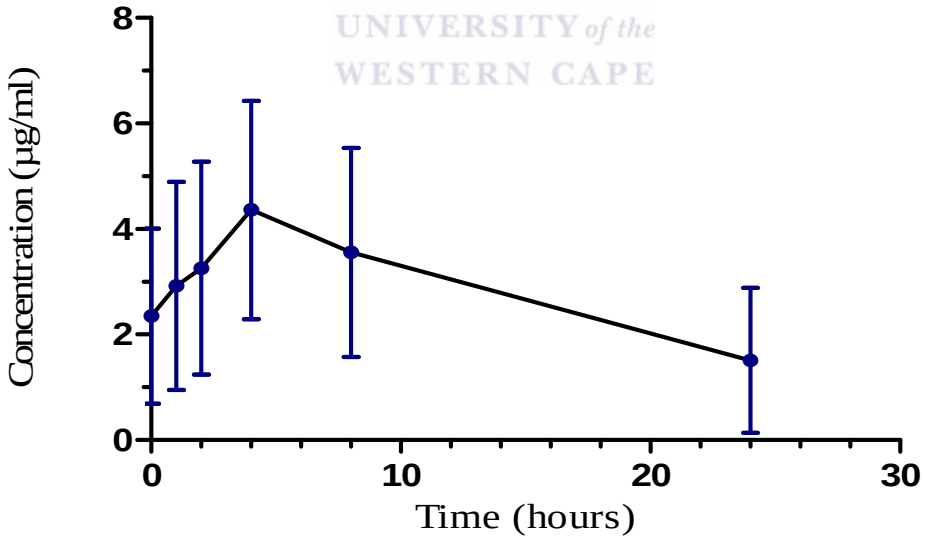

Figure 4.4: Ofloxacin mean (±SD) plasma concentration-time profile for all patients.

4.3.2 Ofloxacin pharmacokinetic parameters

Table 4.5 shows ofloxacin pharmacokinetic parameters in patients, who had been on treatment for two weeks. The parameters were calculated using the NCA method. The pharmacokinetic parameters for all the patients were expressed as mean (±SD). The range (minimum value-maximum value) for each parameter is also given. The PK parameters for patient 3 were excluded, as ofloxacin concentration at the elimination phase could not be estimated due to the 24 hour missing sample.

As reflected in Table 4.5, the mean (±SD) obtained for C_{max}, T_{max}, T½, K_e, MRT, V_d, CL_{tot}, AUC_{0-24} and $AUC_{0-\infty}$ were4.7±2.27 µg/ml, 3±1.29 hr, 9.55±4.69 hr, 0.08 ± 0.04,15.12±6.59 hr, 2.77 ± 1.1624 L/kg, 0.27 ± 0.25 L/hr/kg, 68.8 ± 42.61 µg/ml.hr and 91.9 ± 76.8 µg/ml.hr respectively. Related data from other studies in addition to the present one are included in Table 4.6 for comparison.

Table 4.5: Ofloxacin pharmacokinetic parameters

Patient	Pharmacokinetic parameters								
	T_{max} (hr)	**C_{max} (µg/ml)**	**AUC_{0-24} (µg/ml.hr)**	**K_e**	**T½ (hr)**	**MRT (hr)**	**$AUC_{0-\infty}$ (µg/ml.hr)**	**Cl_{tot} (L/hr/kg)**	**V_d (L/kg)**
1	4	7.87	137.4	0.037	18.54	29.14	249.22	0.053	1.44
2	4	5.24	58.86	0.095	7.28	11.2	66.54	0.2	2.13
4	1	4.95	65.81	0.064	10.81	10.7	84.87	0.17	2.68
5	2	6.85	93.27	0.059	11.74	17	125.51	0.14	2.49
6	4	2.98	41.16	0.100	6.90	11.1	46.26	0.28	2.88
7	2	3.90	*NA	0.097	7.17	12	50.57	0.24	2.6
8	4	1.2	16.71	0.161	4.38	14.7	20.56	0.83	5.18
Mean	**3**	**4.71**	**68.8**	**0.08**	**9.55**	**15.12**	**91.9329**	**0.27329**	**2.77143**
SD	**1.29**	**2.27**	**42.61**	**0.04**	**4.69**	**6.59632**	**76.8682**	**0.25606**	**1.1629**
Range	**1-4**	**1.2-7.87**	**16.71-140.5**	**0.037-0.16**	**4.38-18.54**	**10.7-29.14**	**20.56-249.22**	**0.053-0.83**	**1.44-5.18**

C_{max} = maximum plasma concentration, T_{max} = time to attain C_{max}; T½ = elimination half-life; AUC_{0-24} = area under the concentration-time curve from zero to 24 hrs; $AUC_{0-\infty}$= area under the plasma concentration-time curve from zero to infinity Cl_{tot} = total plasma clearance; V_d = volume of distribution; K_e = elimination rate constant; MRT = mean residence time.*For patient 7, the AUC_{0-24} is not available, as the last plasma concentration available was at 8 hrs.

Table 4.6: Ofloxacin pharmacokinetic parameters obtained in the present study and in previous studies.

Study conditions	Dose (mg)	C_{max} (µg/ml)	T_{max} (hr)	T ½ (hr)	K_e	AUC_{o-24} (µg/ml.hr)	$AUC_{o-\infty}$ (µg/ml.hr)	V_d (L/kg)	Cltot (L/hr/kg)	Reference
TB patients	800 (600-1000)	10.5 (8-14.3)	1.03 (0.5-6)	7.34 (3.53-28.3)	0.094 (0.025-0.196)	NA	NA	1.28 (0.78-2.83)	0.12 (0.02-0.32)	Zhu et al., 2002
MDR-TB patients	590.9	9.61±2.17	1.68±1.21	8.03±3.37	NA	70.57±26.4	82.45±43.64	1.37±0.24	NA	Chulavatnatol et al.,2003
Healthy volunteers	800	9.8 (8.2-11.4)	1.9 (1.6-2.2)	6.5 (6.1-6.9)	NA	85.3 (69.4-101.2)	93.1 (79.7-106.5)	NA	NA	Immanuel et al.,2002
Present study	800	4.71±2.27	3±1.29	9.57±4.69	0.08±0.04	69.55±42.93	92.54±77.69 (21.63-252.12)	2.73±1.12	0.26±0.24	_

C_{max} = maximum plasma concentration, T_{max} = time to attain C_{max}; T½ = elimination half-life; AUC_{0-24} = area under the concentration-time curve from zero to 24 hrs; $AUC_{0-\infty}$= the area under the plasma concentration-time curve from zero to infinity; Cl_{tot} = total plasma clearance; V_d = volume of distribution; K_e = elimination rate constant, NA= not available.

4.4 Discussion

4.4.1 The LC/MS analysis method

In this study, LC coupled with MS was used for the determination of ofloxacin concentrations in patients' plasma samples. The MS settings used (Table 3.1) similar to those with that previously reported in the literature, which provide high sensitivity, specificity, selectivity and rapid determination of ofloxacin in biological samples (van Vyncht et al., 2002; Ballesteros et al., 2003; Sinnaeve et al., 2003).

Good separation of ofloxacin at 9.6 minutes as a retention time with a high recovery percentage (97.6%) was obtained. Under the described chromatographic conditions, ofloxacin peak was well resolved and endogenous plasma components did not interfere with ofloxacin peak as indicated in the chromatogram (Figure 4.2, B). This revealed high specificity of the analysis method.

In summary, although other ofloxacin PK studies have used other HPLC methods coupled with UV or fluorescence detection, our validation results indicate that the LC/MS analysis method is successful and able to detect and quantify ofloxacin in plasma.

4.4.2 Demographic characteristics and concomitant infections

The numbers of patients in this study (8) (5 male and 3 female) close to the patients' numbers involved in other similar studies. The study conducted by Chulavatnatol et al. (2003) had11 patients (8 males and 3 females). Zhu et al. (2002) had 11 patients (9 males and 2 females) and Immanuel et al. (2002) study had7 patients (all males). The relatively small number of patients was due to the difficulty in obtaining consent from patients with HIV and TB. Most of the patients were also severely sick, and their inclusion in the study would have been unethical, even if they gave consent.

The mean age and weight of study group (33.12 yrs and 52.28 kg respectively), were comparable to those reported in the studies done before. For example, the mean age and weight of the patients in Chulavatnatol et al. (2003) study were 38.09 yrs and 59.34 kg respectively. For the study, which was done by Zhu et al. (2002), the mean age was 42 yrs;

and the mean weight was 64 kg. In the study conducted by Immanuel et al. (2002), the age and the weight were 34.5 yrs and 59.2 kg respectively.

Although 5 patients were co-infected with HIV, no other opportunistic infections present except MDR-TB and HBV was found in one patient only.

4.4.3 Laboratory results

The GFR is the best indicator for the kidney functions, as the kidney dysfunction stages can be evaluated using the GFR value. Stages of kidney function range from normal kidney function with a GFR more than 90 ml/min to renal failure where the GFR is less than 15 ml/min. In this study, 6 out of 8 patients (75%) had normal kidney function. Three out of the six patients were HIV-positive with CD4 cell counts 53, 48, and 327cell/mm^3. Two patients had mild decrease in their GFR values (60-89 ml/min). Therefore, they are classified as presenting mild renal failure. Both patients were HIV-positive with a CD4 cell counts 137 and 155 cell/mm^3.

With regard to the liver enzymes levels, the mean ALT value was within the normal range, however, some patients had abnormal levels. On the other hand, the AST and GGT mean values were above the upper limit of the normal range and the UCB levels within the normal range. Three patients had mild elevations in both ALT and AST (less than 5 times the upper limit of normal value, which is 200 u/l). Regarding GGT, three patients had high levels of this enzyme. One patient was co-infected with hepatitis and this might explain the patient's abnormal liver enzymes level, especially the GGT, which its elevation is an indicator of viral hepatitis. However, another patient is also co-infected with hepatitis, but the liver enzymes level within the normal range.

ALT, AST and GGT liver enzymes are markers for liver injury and alcohol abuse. Many conditions such as hepatitis, cirrhosis, drug-induced injuries and alcohol abuse can cause the elevation of these enzymes level. However, abnormal levels of these enzymes do not necessarily reflect how severely the liver is damaged (Giannini et al., 2005). The liver enzymes levels for patients in this study indicate some sort of liver up normality. Co-

infection with HBV explains this up normality in patient 2; however, for other patients it might be due to alcohol abuse or other liver disease that is not apparent.

4.4 4 Ofloxacin pharmacokinetics

The C_{max} and AUC_{0-24} results obtained in this study were lower than those reported in the literature by Zhu et al. (2002) and Immanuel et al. (2002). The mean $AUC_{0-\infty}$ was similar to that obtained by Immanuel et al. (2002) (Table 4.6). Furthermore, ofloxacin was absorbed with a longer T_{max} compared with that reported in the literature (Table 4.6). These two parameters are higher than those reported in the literature (Table 4.6). In the present study, ofloxacin T½ was longer than the one reported in previous studies (Table 4.6). Finally, K_e was similar to that reported by Zhu et al. (2002).

Ofloxacin has a high absorption rate and oral bioavailability more than 90%; therefore, its peak serum concentration was achieved within 1-2 hours (Table 2.3). The pharmacokinetic parameters of ofloxacin are dose independent; however, the absorbed amount increases linearly with increasing dose, which leads to an increase in C_{max} and AUC (Lode et al., 1987; Immanuel et al., 2002). This explains why not comparing our C_{max}, AUC_{0-24} and $AUC_{0-\infty}$ with the values obtained in Chulavatnatol et al. (2003) study.

The low C_{max} and low AUC_{0-24} in addition to the prolonged T_{max} indicate a reduction in the extent and the rate of ofloxacin absorption in our patients. Ofloxacin absorption may be blocked by divalent cations included in nutritional supplements or antacid. Co-administration of didanosine leads to a decrease of ofloxacin absorption and as a consequence, its AUC decreases (Walker, 1999; Katzung, 1998; Goodman et al., 2005). Furthermore, co-ingestion with food delays ofloxacin absorption and the time to reach T_{max} by an hour (Walker, 1999; Katzung, 1998). This suggests the influence of food or drug co-administration effect on ofloxacin absorption. None of our patients is taking a nutritional supplement or antacid, however, we cannot control the food intake in our patients. Another factor that could lead to long T_{max} is delayed gastric-emptying phenomenon, due to taking efavirenz by patients co-infected with HIV (Villani et al., 2001). This may explain the long T_{max} observed in patients 1 and 2 who were on ARVs

APPENDICES

Appendix A

Patient consent form (written consent only)

Patient study number: ……

Patient statement

This study and related procedures have been explained to me to my satisfaction, I have received a copy of the patient information sheet and have been given the opportunity to ask questions which have been answered to my satisfaction. I hereby agree to voluntarily participate in this program.

I understand that if I choose to withdraw from this study, I will need to inform my Doctor in order to enable him to evaluate my status, to review the consequences of my decision, as well as to allow him to perform procedures for an orderly termination of my participation.

…….. …….

Signature of patient Date (to be completed by patient)

Witness

……..