PHARMACOKINETIC DRUG-DRUG INTERACTIONS
EVALUATION OF MELOXICAM WITH SELECTED
CO-PRESCRIBED DRUGS

PhD Thesis
By
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DEPARTMENT OF PHARMACY
UNIVERSITY OF PESHAWAR, PAKISTAN
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EVALUATION OF MELOXICAM WITH SELECTED
CO-PRESCRIBED DRUGS

AMANULLAH

A THESIS SUBMITTED TO THE UNIVERSITY OF PESHAWAR IN
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DEPARTMENT OF PHARMACY
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# TABLE OF CONTENTS

1. INTRODUCTION 1

1.1. Drug-Drug Interaction 1

1.1.1. Classification of Drug-Drug Interaction 1

1.1.1.1. Pharmacokinetic Drug–Drug Interactions 3

1.1.1.2. Pharmacodynamic Drug–Drug Interactions 17

1.1.2. Factors Affecting Drug Interactions 19

1.1.2.1. Drug Associated Factors 19

1.1.2.2. Patient Associated Factors 20

1.1.3. Evaluation of DDIs 22

1.1.3.1. In Vitro Metabolic Models 22

1.1.3.2. In Vivo Metabolic Models 23

1.2. Meloxicam 25

1.2.1. Mode of Action 26

1.2.2. Pharmacokinetics of Meloxicam 26

1.2.2.1. Absorption 26

1.2.2.2. Distribution 27

1.2.2.3. Metabolism 27

1.2.2.4. Excretion 28

1.2.3. Indications and Dosage 28

1.2.4. Adverse Effects 28

1.2.5. Interactions 29

1.2.6. Population Pharmacokinetic of Meloxicam 31

1.3. Fluconazole 33

1.3.1. Mode of Action 33

1.3.2. Pharmacokinetics of Fluconazole 33

1.3.2.1. Absorption 33

1.3.2.2. Distribution 33

1.3.2.3. Metabolism 34

1.3.2.4. Excretion 34

1.3.3. Indications 34

1.3.4. Adverse Effects 34

1.3.5. Interactions 35

1.3.6. Population Pharmacokinetic of Fluconazole 37
1.4. Omeprazole  
1.4.1. Mode of Action  
1.4.2. Pharmacokinetic of Omeprazole  
1.4.2.1. Absorption  
1.4.2.2. Distribution  
1.4.2.3. Metabolism  
1.4.2.4. Excretion  
1.4.3. Indications  
1.4.4. Adverse Effects  
1.4.5. Interactions  
1.5. Aims and objective  

2. EXPERIMENTAL  
2.1. Chemicals  
2.1.1. Reference Standards  
2.1.2. Chemicals and Solvents  
2.2. Instrumentation  
2.2.1 HPLC System  
2.2.2 Column’s  
2.2.3 Balance  
2.2.4 Centrifuge Machine  
2.2.5. Vortex Mixer  
2.2.6. Distillation Unit  
2.2.7. Filtration Assembly  
2.2.8. Cartridges  
2.2.9. Spectrophotometer  
2.3. HPLC Method Development  
2.3.1. Preparation of Standard Solutions  
2.3.2. Sample Collection and Preparation  
2.3.3. Extraction of Samples from Plasma  
2.3.4. Chromatographic Conditions Optimizations  
2.3.4.1. Selection of HPLC Column  
2.3.4.2. Mobile Phase Composition
# TABLE OF CONTENTS

2.3.4.3. Flow Rate Composition 50  
2.3.4.4. Detection Wavelength 50  
2.3.4.5. Injection Volumes 50  
2.3.4.6. Internal Standard Selection 50  
2.3.4.7. Selection of Extraction Solvent 50  
2.3.4.8. Column Oven Temperature 50  
2.3.5. Method Validation 50  
2.3.5.1.Specificity 51  
2.3.5.2. Linearity 51  
2.3.5.3. Accuracy 51  
2.3.5.4. Precision 51  
2.3.5.5. Sensitivity 52  
2.3.5.6. Stability 52  
2.3.5.7. Robustness/ Ruggedness 52  
2.4. Pharmacokinetics and Pharmacokinetic Drug-Drug Interactions Study of Meloxicam and its Metabolites 52  
2.4.1. Study Design and Study Protocol 53  
2.4.2. Selection of Volunteer’s 53  
2.4.2.1. Inclusion Criteria 53  
2.4.2.2. Exclusion Criteria 54  
2.5. Pharmacokinetics of Meloxicam and its Metabolites 54  
2.5.1. Administration of Drug and Collection of Blood Samples 54  
2.5.2. Preparation of Blood Samples 54  
2.5.3. Quantification of Meloxicam 54  
2.5.4. Pharmacokinetic Data Analysis 55  
2.6. Pharmacokinetic Drug-Drug Interaction Studies 55  
2.6.1. Pharmacokinetic Drug-Drug Interaction of Single Dose of Meloxicam and Omeprazole 56  
2.6.1.1. Selection of Subjects 56  
2.6.1.2. Study Design 56  
2.6.1.3. Administration of Drug 57  
2.6.1.4. Collection of Plasma Sample 58  
2.6.1.5. Preparation and Analysis of Plasma Sample 58
2.6.1.6. PK-DDIs Data Analysis

2.6.2. Pharmacokinetic Drug-Drug Interaction of Meloxicam Using Multiple Dose of Omeprazole
2.6.2.1. Study Design
2.6.2.2. Selection of Volunteers
2.6.2.3. Administration of Drug
2.6.2.4. Collection of Plasma Sample
2.6.2.5. Preparation and Analysis of Plasma Sample
2.6.2.6. PK-DDIs Data Analysis

2.6.3. Pharmacokinetic Drug-Drug Interaction of Single Dose of Meloxicam and Fluconazole
2.6.3.1. Study Design
2.6.3.2. Selection of Volunteers
2.6.3.3. Administration of Drug
2.6.3.4. Collection of Plasma Sample
2.6.3.5. Preparation and Analysis of Plasma Sample
2.6.3.6. PK-DDIs Data Analysis

2.6.4. Pharmacokinetic Drug-Drug Interaction of Meloxicam with Multiple Dose of Fluconazole
2.6.4.1. Study Design
2.6.4.2. Selection of Volunteers
2.6.4.3. Administration of Drug
2.6.4.4. Collection of Plasma Sample
2.6.4.5. Preparation and Analysis of Plasma Sample
2.6.4.6. PK-DDIs Data Analysis

3. RESULTS and DISCUSSIONS

3.1. Development of HPLC-UV Method for the Meloxicam, 5-Hydroxy Meloxicam and 5-Carboxy Meloxicam
3.1.1. Sample Preparation
3.1.2. HPLC/UV Method Chromatographic Conditions Optimizations
3.1.2.1. HPLC Column Selection
3.1.2.2. Mobile Phase
3.1.2.3. Flow Rate 70
3.1.2.4. Wavelength 70
3.1.2.5. Column Oven Temperature 71
3.1.2.6. Selection of the Internal Standard 74
3.1.2.7. Injection Volume 74
3.1.3. Method Validation 74
3.1.3.1. Linearity 74
3.1.3.2. Selectivity 75
3.1.3.3. Precision 77
3.1.3.4. Accuracy 79
3.1.3.5. Sensitivity 79
3.1.3.6. Robustness 81
3.1.3.7. Stability 81
3.1.4. Conclusion 81

3.2. Pharmacokinetics of Meloxicam and its Metabolites 5-Hydroxy Meloxicam and 5-Carboxy Meloxicam 82
3.2.1. Selection of Volunteer’s 82
3.2.2. Results 85
3.2.2.1. Maximum Plasma Concentration (C_{max}) 87
3.2.2.2. Time of Peak Plasma Concentration (T_{max}) 88
3.2.2.3. Area under Curve [Bioavailability] (AUC) 88
3.2.2.4. Area under Moment of Plasma Concentration (AUMC) 88
3.2.2.5. Mean Residence Time (MRT) 88
3.2.2.6. Volume of Distribution (Vd) 88
3.2.2.7. Clearance (Cl) 88
3.2.2.8. Absorption Half-Life (t_{1/2-A}) 89
3.2.2.9. Distribution Half-Life (t_{1/2-D}) 89
3.2.2.10. Elimination Half-Life (t_{1/2-E}) 89
3.2.2.11. Rate Constant K_{10} 89
3.2.3. Discussion 90

3.3. Pharmacokinetic Interaction Studies of Meloxicam 92
3.4. PK-DDI of Meloxicam with Omeprazole Single Dose 92
## TABLE OF CONTENTS

3.4.1. Results of PK-DDI between Meloxicam and Omeprazole Single Dose Study

3.4.1.1. Maximum Plasma Concentration ($C_{\text{max}}$) 93
3.4.1.2. Time of Peak Plasma Concentration ($T_{\text{max}}$) 94
3.4.1.3. Area Under Curve [Bioavailability] (AUC) 94
3.4.1.4. Area Under Moment of Plasma Concentration (AUMC) 96
3.4.1.5. Mean Residence Time (MRT) 97
3.4.1.6. Volume of Distribution (Vd) 98
3.4.1.7. Clearance (Cl) 99
3.4.1.8. Elimination Half-Life ($t_{1/2}$-E) 100

3.5. PK-DDI of Meloxicam with Omeprazole Multiple Dose

3.5.1. Results of PK-DDI between Meloxicam Single and Omeprazole Multiple Dose

3.5.1.1. Maximum Plasma Concentration ($C_{\text{max}}$) 103
3.5.1.2. Time of Peak Plasma Concentration ($T_{\text{max}}$) 104
3.5.1.3. Area Under Curve [Bioavailability] (AUC) 104
3.5.1.4. Area Under Moment of Plasma Concentration (AUMC) 106
3.5.1.5. Mean residence time (MRT) 107
3.5.1.6. Volume of Distribution (Vd) 108
3.5.1.7. Clearance (Cl) 109
3.5.1.8. Elimination Half-Life ($t_{1/2}$-E) 110

3.6. Discussion of PK-DDI between Meloxicam and Omeprazole

3.7. PK-DDI of Meloxicam with Fluconazole Single Dose Study

3.7.1. Results of PK-DDI between Meloxicam and Fluconazole Single Dose Study

3.7.1.1. Maximum Plasma Concentration ($C_{\text{max}}$) 116
3.7.1.2. Time of Peak Plasma Concentration ($T_{\text{max}}$) 116
3.7.1.3. Area Under Curve [Bioavailability] (AUC) 117
3.7.1.4. Area Under Moment of Plasma Concentration (AUMC) 119
3.7.1.5. Mean Residence Time (MRT) 120
3.7.1.6. Volume of Distribution (Vd) 121
3.7.1.7. Clearance (Cl) 122
3.7.1.8. Elimination Half-Life ($t_{1/2}$-E) 123
3.8. PK-DDI of Meloxicam Single with Fluconazole Multiple Dose 125
3.8.1. Results of PK-DDI between Meloxicam Single and Fluconazole Multiple Dose Study 126
3.8.1.1. Maximum Plasma Concentration ($C_{\text{max}}$) 126
3.8.1.2. Time of Peak Plasma Concentration ($T_{\text{max}}$) 127
3.8.1.3. Area Under Curve [Bioavailability] (AUC) 127
3.8.1.4. Area Under Moment of Plasma Concentration (AUMC) 129
3.8.1.5. Mean Residence Time (MRT) 130
3.8.1.6. Volume of Distribution (Vd) 131
3.8.1.7. Clearance (Cl) 132
3.8.1.8. Elimination Half-Life ($t_{1/2}$-E) 133
3.9. Discussion of PK-DDI between Meloxicam and Fluconazole 135

4. CONCLUSION 137

5. FUTURE PERSPECTIVE 138

6. REFERENCES 0
DEDICATED
To My Father
Noor Rehman (Late)
A strong support for me throughout my life
Table-1.1: Transporter Mediated Clinical Drug–Drug Interactions

Table-1.2: Drug-Drug Interactions of Meloxicam

Table-1.3: Pharmacokinetic Parameters of Meloxicam (15 mg) given as Single Oral Dose in Healthy Human Volunteers (mean ± SD)

Table-1.4: Drug-Drug Interactions of Fluconazole

Table-1.5: Pharmacokinetic Parameters of Fluconazole (150 mg) Given as Single Oral Dose in Healthy Human Volunteers

Table-1.6: Drug-Drug Interactions of Omeprazole

Table-1.7: Pharmacokinetic Parameters of Omeprazole (20 mg) Given as Single Oral Dose in Healthy Human Volunteers

Table-2.1: Pharmacokinetic Parameters

Table-2.2: Study Design for DDI of Single Dose of Meloxicam and Omeprazole

Table-2.3: Details of the drug products used in the DDI study

Table-2.4: Study Design for DDI of Meloxicam with Multiple Dose Omeprazole

Table-2.5: Details of the Drug Products Used in the Multiple Dose DDI Study of Meloxicam and Omeprazole

Table-2.6: Study design for DDI of Single Dose of Meloxicam and Fluconazole

Table-2.7: Details of the Drug Products Used in the Single Dose DDI Study of Meloxicam and Fluconazole
Table-2.8: Study Design for DDI of Meloxicam with Multiple Dose Fluconazole

Table-3.1: Percent Recoveries of Meloxicam, 5-Hydroxy Meloxicam and 5-Carboxy Meloxicam with Different Solvents

Table 3.2: System Suitability Tests of Various Experimental Parameters

Table-3.3: Accuracy, Calibration, Linearity, Repeatability and Sensitivity of the Current RP- HPLC Method

Table-3.4: The Data of the Intra-day and Inter-days Precision (n = 3)

Table-3.5: Biochemical Tests Results of the Volunteers Enrolled in the Studies

Table-3.6: Demographic Profile of the Volunteers Enrolled in the Studies

Table-3.7: PK Parameters of Meloxicam Following Oral Administration of 15 mg Tablet by Using Non-compartmental Pharmacokinetic Model

Table-3.8: PK Parameters of Meloxicam Following Oral Administration of 15 mg Tablet by Using One-compartmental Pharmacokinetic Model

Table-3.9: The Pharmacokinetic Parameters of Meloxicam 15 mg Observed in this Study and Some of the Reported Studies

Table-3.10: The Pharmacokinetic Parameters of Meloxicam Tablet (15 mg) Alone and with Omeprazole Capsule (40 mg)

Table-3.11: Pharmacokinetic Parameters of Meloxicam Tablet (15 mg alone) and with Multiple Doses of Omeprazole Capsule (40 mg i.e., single dose for 3 days)

Table-3.12: The Pharmacokinetic Parameters of Meloxicam Tablet (15 mg) Alone and with Fluconazole Capsule (150 mg)
**Table-3.13**: Pharmacokinetic Parameters of Meloxicam Tablet (15 mg alone) and with Multiple Doses of Fluconazole Capsule (150 mg i.e., single dose for 3 days)
Figure-1.1: Classification of drug-drug interaction

Figure-1.2: Classification of CYP450

Figure-1.3: CYP450 distribution in human intestine

Figure-1.4: CYP450 distribution in human liver

Figure-1.5: Drug transporters, A: human hepatocytes, B: enterocytes of human small intestine, C: human renal proximal tubular cells, D: human brain capillary endothelial cells. SLC transporters are depicted by shaded circles and ABC transporters by shaded ovals. Solid line indicates the direction of drug transport

Figure-1.6: Classification of transporters

Figure-1.7: Mode of action of meloxicam

Figure-1.8: Metabolism of meloxicam; A: meloxicam, B: 5-hydroxy meloxicam, C: 5-carboxy meloxicam

Figure-3.1: The chromatogram of standard sample on supelco/discovery C18 column spiked with 100 ng/mL of meloxicam (peak -4), 75 ng/mL of each metabolite i.e 5-hydroxy meloxicam (peak-1); 5-carboxy meloxicam (peak-3) and 1000ng/mL of internal standard (peak-2)

Figure-3.2: Chromatograms showing the effect of different columns on the analytes i.e. 5-hydroxy meloxicam (peaks A), internal standard (peaks B), 5-carboxy meloxicam (peaks C) and meloxicam (peaks D)

Figure-3.3: Effect of columns on the retention factors (k) of the analytes i.e., 5-hydroxy meloxicam (A); internal standard (B); 5-carboxy meloxicam (C) and meloxicam (D)
Figure-3.4: RP-HPLC chromatograms showing mobile phase (methanol: 0.05% trifluoracetic-acid) in different composition.

Figure-3.5: RP-HPLC chromatograms showing impact of various flow rates of the mobile phase (methanol: 0.05% trifluoracetic-acid). Chromatogram, a; 0.8 mL/min, b; 0.9 mL/min, c; 1.0 mL/min, d; 1.3 mL/min, e; 1.5 mL/min

Figure-3.6: Effect of temperature on the retention factors ($k$) of the analytes i.e., 5-hydroxy meloxicam (A); internal standard (B); 5-carboxy meloxicam (C) and meloxicam (D)

Figure-3.7: Calibration curves of meloxicam, 5-hydroxy meloxicam, 5-carboxy meloxicam

Figure-3.8: RP-HPLC chromatograms of different samples, Peak-1; 5-hydroxy meloxicam, 2; internal standard, 3; 5-carboxy meloxicam, 4; meloxicam. Chromatograms a; blank solvent, b; blank plasma, c; plasma sample spiked with 1000 ng/mL of meloxicam, 500 ng/mL of 5-hydroxy meloxicam, 500 ng/mL of 5-carboxy meloxicam and 1000 ng/mL of internal standard, d; plasma sample

Figure-3.9: RP-HPLC chromatogram showing peaks of 5-hydroxy meloxicam (peak-1), 5-carboxy meloxicam (peak-3) and meloxicam (peak-4) at the lower limit of detection

Figure-3.10: RP-HPLC chromatogram showing peaks of 5-hydroxy meloxicam (peak-1), 5-carboxy meloxicam (peak-3) and meloxicam (peak-4) at the lower limit of quantification

Figure-3.11: Mean ± SD Plasma Drug Concentration Vs Time of Healthy Human Volunteers (n = 24) following oral administration of meloxicam tablet (15 mg), A: Normal plot and B: Semi-log scale
**Figure-3.12:** Mean ± SD Plasma Drug Concentration Vs Time of Healthy Human Volunteers (n = 24) following oral administration of meloxicam tablet (15 mg) alone and concurrently with single dose omeprazole capsule (40 mg) A: Normal plot and B: Semi-log scale

**Figure-3.13:** Graphical representation of the $C_{max}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers (n = 24)

**Figure-3.14:** Graphical representation of the $[AUC]^7_{0}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers (n = 24)

**Figure-3.15:** Graphical representation of the $[AUC]_{0}^{\infty}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers (n = 24)

**Figure-3.16:** Graphical representation of the $AUMC$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers (n = 24)

**Figure-3.17:** Graphical representation of the $MRT$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers (n = 24)

**Figure-3.18:** Graphical representation of the $Vd$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent
administration with single dose omeprazole capsule (40 mg) in healthy human volunteers (n = 24)

**Figure-3.19:** Graphical representation of the $Cl$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule 40 mg in healthy human volunteers (n = 24)

**Figure-3.20:** Graphical representation of the $t_{1/2-E}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers (n = 24)

**Figure-3.21:** Mean ± SD Plasma Drug Concentration Vs Time of Healthy Human Volunteers (n = 24) following oral administration of meloxicam single tablets 15 mg alone and concurrently with multiple dose omeprazole capsule 40 mg *i.e.*, single dose for 3 consecutive days and on third day meloxicam 15 mg was administered with it A: Normal plot and B: Semi-log scale

**Figure-3.22:** Effect of omeprazole 40 mg capsule o.d. for 3 days on the $C_{max}$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

**Figure-3.23:** Effect of omeprazole 40 mg capsule o.d. for 3 days on the $[AUC]_{0}^{72}$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

**Figure-3.24:** Effect of omeprazole 40 mg capsule o.d. for 3 days on the $[AUC]_{0}^{\infty}$ of the meloxicam following simultaneous administration of single dose
meloxicam tablets (15 mg) on 3\textsuperscript{rd} day in healthy human volunteers (n = 24)

**Figure-3.25:** Effect of omeprazole 40 mg capsule o.d. for 3 days on the $[\text{AUMC}]_0^\infty$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3\textsuperscript{rd} day in healthy human volunteers (n = 24)

**Figure-3.26:** Effect of omeprazole 40 mg capsule o.d. for 3 days on the $\text{MRT}$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3\textsuperscript{rd} day in healthy human volunteers (n = 24)

**Figure-3.27:** Effect of omeprazole 40 mg capsule o.d. for 3 days on the $\text{Vd}$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3\textsuperscript{rd} day in healthy human volunteers (n = 24)

**Figure-3.28:** Effect of omeprazole 40 mg capsule o.d. for 3 days on the $\text{Cl}$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3\textsuperscript{rd} day in healthy human volunteers (n = 24)

**Figure-3.29:** Effect of omeprazole 40 mg capsule o.d. for 3 days on the $t_{1/2}-E$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3\textsuperscript{rd} day in healthy human volunteers (n = 24)

**Figure-3.30:** Mean ± SD Plasma Drug Concentration Vs Time of Healthy Human Volunteers (n = 24) following oral administration of meloxicam tablet (15 mg) alone and concurrently with single dose fluconazole capsule (150 mg) A: Normal plot and B: Semi-log scale
Figure-3.31: Graphical representation of the $C_{max}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

Figure-3.32: Graphical representation of the $[AUC]_0^{72}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

Figure-3.33: Graphical representation of the $[AUC]_0^\infty$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

Figure-3.34: Graphical representation of the $[AUMC]_0^\infty$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

Figure-3.35: Graphical representation of the $MRT$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

Figure-3.36: Graphical representation of the $Vd$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

Figure-3.37: Graphical representation of the $Cl$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent
administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

**Figure-3.38:** Graphical representation of the $t_{1/2-E}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

**Figure-3.39:** Mean ± SD Plasma Drug Concentration Vs Time of Healthy Human Volunteers (n = 24) following oral administration of meloxicam single tablets 15 mg alone and concurrently with multiple dose fluconazole capsule 150 mg *i.e.*, single dose for 3 consecutive days and on third day meloxicam 15 mg was administered with it A: Normal plot and B: Semi-log scale

**Figure-3.40:** Effect of fluconazole 150 mg capsule o.d. for 3 days on the $C_{max}$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

**Figure-3.41:** Effect of fluconazole 150 mg capsule o.d. for 3 days on the $[AUC]_{0-24}$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

**Figure-3.42:** Effect of fluconazole 150 mg capsule o.d. for 3 days on the $[AUC]_{0-\infty}$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

**Figure-3.43:** Effect of fluconazole 150 mg capsule o.d. for 3 days on the $[AUMC]_{0-\infty}$ of the meloxicam following simultaneous administration of single dose
meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

Figure-3.44: Effect of fluconazole 150 mg capsule o.d. for 3 days on the MRT of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

Figure-3.45: Effect of fluconazole 150 mg capsule o.d. for 3 days on the Vd of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

Figure-3.46: Effect of fluconazole 150 mg capsule o.d. for 3 days on the Cl of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

Figure-3.47: Effect of fluconazole 150 mg capsule o.d. for 3 days on the t1/2-E of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADME</td>
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</tr>
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<td>Area Under Curve</td>
</tr>
<tr>
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<td>Peak Plasma Concentration</td>
</tr>
<tr>
<td>CL</td>
<td>Clearance</td>
</tr>
<tr>
<td>DDI</td>
<td>Drug Drug Interaction</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tri Acetic acid</td>
</tr>
<tr>
<td>Fig</td>
<td>Figure</td>
</tr>
<tr>
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<td>Fluconazole</td>
</tr>
<tr>
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<td>Gastro Esophageal Reflux Disease</td>
</tr>
<tr>
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<td>Gastro Intestinal Tract</td>
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<td>K10</td>
<td>Elimination Rate Constant</td>
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<tr>
<td>L</td>
<td>Liter</td>
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<tr>
<td>LOD</td>
<td>Limit of Detection</td>
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<tr>
<td>LLOQ</td>
<td>Lower Limit of Quantification</td>
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<td>Meloxicam</td>
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<td>Minutes</td>
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<td>mL</td>
<td>Milliliter</td>
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<td>MRT</td>
<td>Mean Residence Time</td>
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<tr>
<td>OME</td>
<td>Omeprazole</td>
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<tr>
<td>Pgp</td>
<td>P-glycoprotein</td>
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<tr>
<td>PK</td>
<td>Pharmacokinetic</td>
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<tr>
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<td>Revolution Per Minute</td>
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<td>Relative Standard Deviation</td>
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<tr>
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<td>Signal to Noise Ratio</td>
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<td>Standard Deviation</td>
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<td>Time to Achieved Cmax</td>
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<tr>
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<td>Elimination Half Life</td>
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<td>Trifluoroacetic acid</td>
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<tr>
<td>U.V.</td>
<td>Ultra Violet</td>
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<tr>
<td>Vd</td>
<td>Volume of Distribution</td>
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AMAN ULLAH
In the present study, pharmacokinetic profile of meloxicam in healthy human volunteers from Pakistan was evaluated and then potential pharmacokinetic drug-drug interaction of meloxicam with selected co-prescribed drugs i.e. omeprazole and fluconazole was investigated. The study was conducted in four phases. In the first phase, high-performance liquid chromatography coupled with ultraviolet-visible method was developed and validated for the quantification of meloxicam and, 5-hydroxy meloxicam and 5-carboxy meloxicam, the major metabolites of meloxicam, in human plasma. In the second phase, pharmacokinetics profile of meloxicam was evaluated in healthy human volunteers. In third phase, single oral dose pharmacokinetic drug-drug interactions of meloxicam with omeprazole and fluconazole, was investigated in normal human volunteers and in the fourth phase the impact of multiple dose administration (single dose for 3 days) of omeprazole and fluconazole on the pharmacokinetics of meloxicam was studied in human volunteers.

A simple, rapid and cost-effective method was developed and validated for analysis of meloxicam and its major metabolites (5-hydroxy meloxicam and 5-carboxy meloxicam) in human plasma using reverse phase high performance liquid chromatography (RP-HPLC) coupled with UV detector. The samples were analyzed by injecting 20 µL into the HPLC system using Supelco C18 (150 mm × 4.6 mm, 5 µm) analytical column, protected by a Perkin Elmer C18 (30 mm × 4.6 mm, 10 µm) guard column. The mobile phase methanol: TFA (0.05% aqueous solution) in 60:40% v/v was pumped at flow rate of 1.3 mL/min at 28 °C and the eluents were monitored at 353 nm using piroxicam as internal standard. Meloxicam and the metabolites were extracted from plasma using dichloromethane. The method was linear over the concentration range of 10- 2000 ng/mL. The limits of detection of meloxicam, 5-hydroxy meloxicam and 5-carboxy meloxicam were 3 ng, 10 ng and 8 ng, whereas limit of quantification were 9 ng, 30 ng and 25ng, respectively. The method was successfully applied for the pharmacokinetic studies in plasma samples of the healthy human Pakistani volunteers.

The PK study was conducted using open-label, two-sequence, cross-over study with a washout period of two weeks. This study was carried out in normal healthy male volunteers (n = 24) aged between 20 to 23 years, they were examined for various physical and biochemical tests and inclusion-exclusion criterion was strictly followed. Meloxicam (15 mg) with a full glass of water (250 mL) was administered orally by each of the volunteer and plasma samples were collected at predetermined time points and analyzed using the developed analytical method. Various pharmacokinetic parameters like $t_{1/2}$, $AUC$, $AUMC$, $Vd$, $Cl$, $MRT$ and $K_{i0}$ were assessed using PK-Summit® software and Microsoft Excel®, while some parameters like $C_{max}$ and $T_{max}$ obtained directly from the data /plot.

The mean ± SD $C_{max}$, $T_{max}$, [AUC]$_0^\infty$, $Vd$ and $Cl$ of meloxicam in the current study was 1235.11 ± 241.553 ng/mL, 5.833 ± 0.577 hr, 29.237 ± 4.615 µg.hr/mL, 30.795 ± 4.675 µg.hr/mL, 8.924 ± 1.969 L and 7.315 ± 1.064 mL/min, respectively.

The co-administration of single dose of meloxicam and omeprazole in local healthy human volunteers showed an increase in $C_{max}$ of meloxicam from 1235.11 ± 241.553 ng/mL to 1281.85 ± 124.38 ng/ mL and $T_{max}$ from 5.833 ± 0.577 hr to 6.166 ± 0.577
hr. The $[\text{AUC}]_0^\text{t}$ and $[\text{AUC}]_0^\infty$ also increased from 29237 ± 4615.21 ng.hr/mL to 32689.67 ± 4648.48 ng.hr/mL and 30795 ± 4675.53 ng.hr/mL to 34049.84 ± 4331.91 ng.hr/mL, respectively. The $Vd$ and $Cl$ decreased from 8.924 ± 1.969 L to 8.542 ± 1.449 L and from 7.315 ± 1.064 mL/min to 6.530 ± 1.295 mL/min, respectively, whereas elimination half-life increased from 13.547 ± 1.361 hr to 14.554 ± 1.129 hr. Though in multiple dose study meloxicam $C_{\text{max}}$, $T_{\text{max}}$ and elimination half-life increased from 1235.11 ± 241.553 ng/mL to 1515.66 ± 52.568 ng/mL, 5.833 ± 0.577 hr to 7.333 ± 0.942 hr and 13.547 ± 1.361 hr to 16.439 ± 1.347 hr, respectively. The $Vd$ and $Cl$ also increased from 29237 ± 4615.21 ng.hr/mL to 41875.75 ± 2470.93 ng.hr/mL and 30795 ± 4675.53 ng.hr/mL to 44639.77 ± 2585.13 ng.hr/mL, respectively, while $Vd$ and $Cl$ decreased from 8.924 ± 1.969 L to 7.596 ± 1.203 L and from 7.315 ± 1.064 mL/min to 5.032 ± 1.200 mL/min, respectively. The PK-DDI study between simultaneous administration of single oral dose of meloxicam and omeprazole did not significantly affect the pharmacokinetics of meloxicam and can safely be used. However, due to significantly changes in PK parameters of meloxicam with multiple doses of omeprazole, dosage modification of meloxicam may be required in maintaining steady state concentration.

The PK-DDI of single dose of meloxicam and fluconazole showed increase in the $C_{\text{max}}$, $T_{\text{max}}$, $[\text{AUC}]_0^\text{t}$ and $[\text{AUC}]_0^\infty$ values of meloxicam from 1235.11 ± 241.553 ng/mL to 1390.43 ± 184.223 ng/mL, 5.833 ± 0.577 hr to 7.166 ± 1.337 hr, 29237 ± 4615.21 ng.hr/mL to 37911 ± 5796.27 ng.hr/mL and 30795 ± 4675.53 ng.hr/mL to 40348 ± 6942 ng.hr/mL, respectively. The elimination half-life also increased from 13.547 ± 1.361 hr to 17.349 ± 1.961 hr, where $Vd$ and $Cl$ decreased from 8.924 ± 1.969 L to 8.570 ± 1.703 L and 7.315 ± 1.064 mL/min to 5.695 ± 1.679 mL/min, respectively. However, multiple dose administration of fluconazole significantly increased the $C_{\text{max}}$, $T_{\text{max}}$, $[\text{AUC}]_0^\text{t}$, $[\text{AUC}]_0^\infty$ and elimination half-life of meloxicam from 1235.11 ± 241.553 ng/mL to 1571.137 ± 179.742 ng/mL, 5.833 ± 0.577 hr to 7.833 ± 0.577 hr, 29237 ± 4615.21 ng.hr/mL to 59853.01 ± 5819.403 ng.hr/mL, 30795 ± 4675.53 ng.hr/mL to 67794.04 ± 8573.399 ng.hr/mL and 13.547 ± 1.361 hr to 20.899 ± 3.066 hr, respectively, while $Vd$ and $Cl$ significantly decreased from 8.924 ± 1.969 L to 6.735 ± 0.822 L and 7.315 ± 1.064 mL/min to 3.612 ± 0.498 mL/min, respectively. This PK-DDI study indicates a potential pharmacokinetic drug-drug interaction between meloxicam and fluconazole single and multiple doses at the level of metabolism, when administered concomitantly, as all the pharmacokinetic parameters are of clinical significance, therefore, dose adjustment may be required for maintaining steady state concentration.

The present studies evaluated the PK-DDI between the meloxicam and fluconazole or omeprazole in single or multiple oral drug administration, these findings are helpful for the patients using the meloxicam over the period for the treatment of different ailments.
1. INTRODUCTION

1.1. Drug-Drug Interaction

Drug-drug interaction (DDI) is a condition when one drug influences the activity of another co-administered drug, result in enhanced and diminished effects or increased toxicity of the involved drugs (Hines & Murphy, 2011). In general, the precipitant drug affects the activity of the object drug. Drug-drug interaction is a serious issue related to individual safety, during treatment (Tatonetti et al., 2011). The goal of therapy is to maximize beneficial effects, while trying to minimize the detrimental effects. Using at least one drug that interact may change this risk–benefit ratio (Byrne, 2003). Coexistence of numerous health conditions, need and practice of polypharmacy are the main causes of drug-drug interactions (Roughead et al., 2010). It is anticipated that prevalence of clinical drug interactions in patients, taking 2 drugs ranges from 10 to 15% and increases to 40% in patients with 5 drugs and to an alarming 80% for taking 7 or more (Moffa Jr et al., 2010). Comparatively older individuals are particularly prone to the prevalence of potential drug–drug interactions (Merlo et al., 2001). Understanding the drug interactions mechanisms has developed the idea to reexamine the drug use, in various populations under varying degree of conditions (Benet & Hoener, 2002).

The impact of drug-drug interactions ranges from no effect to life threatening and is taken into consideration clinically significant while results require alteration of dose one of the mediators or medical intervention (Williams & Feely, 2002). Clinically significant DDIs can minimize therapeutic benefits, leading to increase in the duration of the patient’s stay at the hospital and enhanced the cost of therapy (Oglu et al., 2016). Knowing how drug–drug interactions occur and how to accomplish them is a significant part of clinical practice. Drug can also interact with foods, beverages, herbs and herbal medicines or may with environmental chemical substances (Roughead et al., 2010).

1.1.1. Classification of Drug-Drug Interaction

The classifications of DDIs are presented in Fig-1.1.
Figure-1.1: Classification of drug-drug interaction
1.1.1.1. Pharmacokinetic Drug–Drug Interactions

PK-DDI involve alteration in one or more of the object drugs basic parameters of the absorption, distribution, metabolism or excretion. Changing ‘how much’ and for ‘how long’ it is present at the place of action (Snyder et al., 2012). These interactions can be measured from the pharmacokinetic parameters like plasma concentration, half-life, amount of free drug and drug excreted. Such an interaction is also called as ADME interactions. These are classified as

Absorption Interactions
Mucous membranes of the gastrointestinal tract, particularly inside the small intestine is responsible for the oral absorption of the drug, the non-ionized and lipid soluble form of the drug is absorbed. The rate and total amount of absorption depends on the pKa of the drug, its lipid solubility, pH of the stomach and factors like protein carriers i.e., Pgp. At absorption site one drug alters the absorption of another drug and as a result altered bioavailability (Buxton & Benet, 2011). The DIs because of alteration in absorption does not significantly produce adverse or toxic effects rather causing small changes in pharmacokinetic parameters, however drug with short half-life or those which require rapid peak plasma concentration for therapeutic efficacy may cause significant absorption interaction. Drug with long half-life are usually unaffected (Romac & Albertson, 1999). Increasing motility causes increase in rate of absorption not extent of total absorption. Extreme increase in motility with slow release formulation results decrease in rate and extent of absorption. Slowing motility decreases the rate of absorption not extent and slowing motility with slow release formulation increases rate as well as extent of total absorption. Changes in gastrointestinal motility may affect the rate but rarely change the extent of total absorption. In most cases changes in the motility does not lead to significant drug interactions. Increase gastric emptying, intensify the absorption rate by allowing the drug to rapidly come in contact with the larger absorptive surface area i.e., small intestine (Nies & Spielberg, 1996). Metoclopramide increases absorption of the cyclosporine and reduces digoxin absorption, by increasing gastrointestinal motility and emptying time, as digoxin is slowly dissolve and absorbed from gastrointestinal tract and cyclosporine absorbed mainly from small intestine (Remington et al., 2006; van Boxtel et al., 2008). Microbial flora in the GI tract may alter the absorption of the drug. Antibiotics reduces GI flora and may cause changes in the amount of drug being absorbed. Intestinal flora is responsible for digoxin metabolism in GI tract, tetracycline’s suppress this and enhances its bioavailability (Susla, 2001).
Drugs are weak acids or weak bases; pH of the GI tract can influence the degree of their absorption. Changing the acidic environment of the stomach by H2- blockers, PPIs and antacids affect the bioavailability of the drugs that rely on acidic pH for their dissolution and absorption for example ketoconazole results 90% reduction in its bioavailability when given with cimetidine (Albengres et al., 1998). This type of DI can be minimized by the separation of the administration time of the drugs by at least two hours.
Protein carriers cause influx or efflux of the drug from the cell and blood stream and affect the bioavailability. Rifampin and digoxin when used concurrently, digoxin elimination is enhanced because of stimulation of Pgp by rifampin, as a resultant oral bioavailability of digoxin is decreased. In contrast, Pgp is inhibited by grapefruit juice and hence increases the absorption of terfenadine, atorvastatin, cyclosporine, carbamazepine, midazolam, cisapride, estrogens, calcium-channel blockers (nifedipine, felodipine, amlodipine), astemizole and alprazolam (Mutalik & Sanghavi; Tripathi, 2013).

Certain drugs forms non-absorbable chelates and complexes in the GI tract and may account the DDIs. For instance, activated charcoal goes about as an adsorbing agent inside GIT for the management of drug overdose or drug toxicity by preventing its contact with the absorption sites within the stomach and small intestine. The antibacterial properties of tetracycline could possibly be lowered, as it forms poor soluble chelates with divalent and trivalent metallic ions, such as iron, aluminum, calcium and bismuth (Pleuvry, 2005). Similarly, efficacy and absorption of levodopa, methyl dopa and carbidopa may be reduced when concomitant use with iron supplements (Pazzucconi et al., 1996). Simultaneous administration of aluminum or magnesium containing antacids lowers serum levels of ciprofloxacin and other quinolones (Arayne et al., 2005). This type of DDIs is avoided by administering the object drug to four hours before or after the precipitant drug.

**Distribution Interactions**

Distribution interaction involves alteration of drug concentration at the site of action (Snyder et al. 2012). Plasma and tissues proteins via binding are normally responsible for the transport of drugs, of numerous plasma proteins, the most important are albumin, α1-acid glycoprotein, and lipoproteins (Palleria et al., 2013). Drug bound to plasma proteins are pharmacologically inert and only unbound drugs are pharmacologically active (Tripathi, 2013; Wilkinson et al., 2001). Drug having higher protein binding affinity displaces a low protein bound drug, thereby increase free concentration of the low bound drug, thus the drug unbound portion is not just available more at the site of action but also at the elimination site. This principle has been employed upon drugs that have protein binding capability (> 90%) and to drugs with narrow therapeutic window, where a smaller change in the concentration of drug transformed a substantial change with pharmacological effect and result in toxicity. NSAIDs, sulfonylureas, phenytoin, oral contraceptives and warfarin are usually recognized highly protein bound agents. Phenylbutazone, a highly protein bound drug displaces warfarin a low protein bound drug resulting in excessive bleeding. Previously, it had been considered as a vital mechanism for major interactions, now, these kind of interactions have come under more detailed study with the assumptions that many are clinically insignificant, apart from where the substrate drug elimination shall also be slowed by the displacing drug (Benet & Hoener, 2002; Patane et al., 2013) e.g. ibuprofen not simply displace methotrexate from protein binding site but decrease methotrexate clearance through 50% by inhibiting OAT1 (Tracy et al., 1992; Uwai et al., 2000).
**Metabolic Interactions**

Altered metabolism is the most clinically important cause of drug interaction. Drug metabolism mainly occur in the liver. Numerous other organs like intestine, lungs and skin are also involved in metabolism. Drug metabolism or biotransformation results a lipid soluble compound becomes more polar and water soluble with each metabolic step (Boothe, 2011). Biotransformation makes these metabolites biologically active or inactive and facilitates their excretion either by way of urine or bile. Thus, DIs that increases the metabolism of the object drug will decrease the degree or length of biologic activity of the parent compound, but if the metabolite or bio transformed compound is active, the overall pharmacological activity potentially increases or remain the same (Romac & Albertson, 1999). Drug metabolism occur in the microsomes of the liver and involves a series of interlinked reactions that are catalyzed by special proteins called liver microsomal enzymes. Some non-microsomal hepatic enzymes such as alcohol and aldehyde dehydrogenase, monoamine oxidase, esterase’s and amidases are also important in biotransformation. Variability in drug metabolism depend on the activity and the amount of enzymes (Pang et al., 2014).

**CYP 450 Enzymes**

This enzyme system is found in all life systems and is responsible for the detoxification of any xenobiotics or endogenous compounds (Gu & Manautou, 2012). CYP chemically is a complex of heme, protein having iron. They have an unusual spectrum at 450 nm in the presence of carbon monoxide, so they are given the name of cytochrome P450, P stand for pigment. This family of enzymes catalyzes a series of reaction using NADPH and oxygen as a source of electrons (Guengerich et al., 2009). There is a large variability in the content and composition of CYP450 enzymes among individuals because of differences in sex, age, diet and liver disease and this can result in variations in drug metabolism leading to drug–drug interactions (Cederbaum, 2015).

**Classification of CYP 450 Enzymes**

The major drug metabolizing CYPs are discussed in Fig-1.2 with respect to substrates, inhibitors and inducers.
Figure 1.2: Classification of CYP450

**Distribution of CYP 450 Enzymes**
The estimated proportions of the major drug-metabolizing CYP enzymes in the intestine and liver are given in Fig-1.3 and 1.4.

**Figure-1.3: CYP450 distribution in human intestine**

**Figure-1.4: CYP450 distribution in human liver**

These various isoenzymes mediate common DDIs primarily by two mechanisms including enzyme induction and enzyme inhibition.

**CYP450 Enzyme Induction**

Induction of drug metabolism usually occurs by gene transcription following prolonged exposure to an inducing agent (Mohutsky et al., 2010). The onset and offset of enzyme induction is variable and depends on the time to reach steady state for the inducer and the rate of synthesis of new enzymes. This adaptive process is relatively slow and can take days to months to occur (Romac & Albertson, 1999). The outcomes
CHAPTER-1

of enzyme induction are, increased rate in metabolism, decrease plasma drug concentration and reduce bioavailability and increase clearance of drug. In contrast drugs metabolized to active or toxic metabolites, induction result in increased effect or toxicity (Maronpot et al., 2010). Rifampin a strong inducer of CYP3A4, CYP2C9 and CYP2C19 induces the metabolism of statins, warfarin, glucocorticoids, cyclosporine, ketoconazole and verapamil. (Bibi, 2008; Jiezhong Chen & Raymond, 2006). CYP3A subfamily can be induced by anticonvulsant agents, rifamycins, glucocorticoids, and some macrolide antibiotics through post-transcriptional regulation. These increased enzymes lead to the development of DDIs (Thummel & Wilkinson, 1998).

**CYP450 Enzyme Inhibition**

It is a process which involves inhibition of the metabolism of drugs by enzymes, leading to drug accumulation and toxicity. This inhibition is of two types’ competitive inhibition and non-competitive inhibition.

In competitive inhibition, there is competition between the drugs for the enzymes, one will occupy the enzyme and inhibit the metabolism of another e.g. ciprofloxacin competitively inhibit the enzyme CYP1A2 and CYP3A4 and alter the metabolism of clozapine and ropivacaine, resulting high concentration in blood and lead to toxicity (Jokinen et al., 2003; Raaska & Neuvonen, 2000). Omeprazole is a CYP2C19 inhibitor drug that inhibits the biotransformation or metabolism of clopidogrel pro-drug into its active metabolite and decreases their antiplatelet effect (Norgard et al, 2009). This inhibition can be reversible. While in non-competitive inhibition, the inhibitor and substrate do not compete for the same active site, but at the allosteric site. Once a ligand binds the allosteric site the conformation of the active site changes, its ability to bind the substrate decreases and the product formation tails off. The duration of this type of inhibition may be longer if new enzymes have to be synthesized after the inhibitor drug is discontinued. e.g. fluoxetine non-competitively binds to the enzyme’s site increases the effect of sertraline by inhibiting its metabolism (Sproule et al., 1997).

CYP450 isoform inhibition is substrate independent. Thus, if a drug inhibits CYP3A4, it will probably inhibit any drug metabolized by that enzyme. This inhibition results in prolongation of half-life and an increase in the object drug concentration. Some inhibitors can affect more than one isoform. As an example, CYP1A2 and CYP3A4 are inhibited by the macrolide erythromycin (Hansten, 1998). High concentration of an inhibitor is required for inhibition of different isoforms. Fluconazole inhibits CYP2C9 at 100 mg/day, but 400 mg/day doses are required to inhibit CYP3A4. The higher the dose of the inhibitor, the faster the onset and the greater the magnitude of the inhibition. The onset of enzyme inhibition is seen more rapidly than with enzyme induction. Variations in drug response after an exposure to an inhibitor is common because of genetic differences in isoforms, environmental exposures, age, disease states, and the fact that substrates can be metabolized by more than one CYP-450 isoform (Romac & Albertson, 1999). Western populations are genetically deficient in
the CYP2D6 isoform used to metabolize beta-blockers, neuroleptics, and antidepressants. These patients would fail to demonstrate quinidine-induced inhibition of this isoform, which is seen in the rest of the population that has this isoform (Zanger & Schwab, 2013).

**Non-Microsomal Enzyme Inhibition (other than CYP450)**

Non-microsomal enzymes produce significant drug interactions and may become inhibited by several drugs. Allopurinol is a xanthine oxidase inhibitor, decrease the uric acid synthesis and used in the management of gout. So, if it is used concomitantly with the azathioprine, mercaptopurine and anticancer drugs, their metabolism will be inhibited as they are metabolized by the same enzyme, and their action is potentiated (Pizzorno et al., 2003). Amphetamine or foods containing tyrosine/tyramine/tryptophan when used along with MAO inhibitors such as selegiline, amines breakdown is inhibited and lead to hypertensive crisis by entering systemic circulation and releasing norepinephrine at the nerve endings (Fiedorowicz & Swartz, 2004).

**Elimination Interactions**

Highly polar parent compounds or the more water soluble metabolites of lipid soluble compounds are mainly excreted from the body through kidney, liver, lungs, feaces, saliva, milk, sweat and tear (Caterina et al., 2013). Renal tubular excretion i.e., glomerular filtration, active tubular secretion and blockade of tubular reabsorption are mainly responsible for the excretion of drugs (Flepisi et al., 2014). Drug like furosemide decreases the GFR and limit passive filtration of aminoglycoside by decreasing filtration pressure often by intravascular volume depletion leading to increased level and toxicity (Paige & Nagami, 2009). Furthermore, excretion of various water soluble organic acids and bases and their metabolites are dependent on the organic anions and cations transport systems (El-Sheikh et al., 2013). In inhibition of these transport systems by precipitant drugs can resulted increase in the concentration of the object drug. For example, penicillin’s and cephalosporin’s serum concentration increased by probenecid via blocking an organic anion transporter in renal tubules and delay their excretion (Caterina et al., 2013; Wu et al., 2010). Similarly, ionization is the pre-determined phase considering renal tubules drug’s reabsorption. The ionization of weak acids and bases, and the degree to which these agents are reabsorbed and excreted are greatly influenced by change in the urinary pH. Unionized and highly lipid soluble drugs reabsorbed from urine back into the blood (Delafuente, 2003). If the urine pH is acidic, the absorption of basic drugs is reduced, while in the presence of basic pH, acidic drug absorption is reduced and their urinary excretion is elevated. The reason is, it becomes ionized in acidic environment and become less lipid soluble, which may cause a fall in the quantity that is passively reabsorbed following filtration, on the other hand renal excretion of weak acids is preferred via more alkaline conditions (Caterina et al., 2013). Change in the urinary pH does not contribute a vital role in DDIs, however, they are useful in drug overdose as detoxification process. For example, amphetamine poisoning may be reversed by enhancing its removal through urine acidification with ammonium
chloride (Llera & Volmer, 2006). Use of sodium bicarbonate alkalinate the distal tubule causing ion trapping of aspirin or salicylic acid and thus facilitate its excretion (Proudfoot et al., 2003).

**Transporter Based Drug-Drug Interactions**

Transporters are increasingly recognized to be essential in drug absorption, distribution and excretion. They play a crucial role in pharmacokinetic drug interactions (Wang et al., 2007). In addition to drugs, they transport endogenous substances such as glucose, lipids, amino acids, hormones, steroids and bile acids (Ho & Kim, 2005). Drugs could possibly compete for binding the transporter, thus resulting in sudden changes in serum and tissue drug levels and probable harmful side effects.

**Location of Transporters**

Transporters are located on basolateral and apical membranes of tissues such as intestine, kidneys, brain and on sinusoidal and canalculus membranes of the liver (Russel, 2010; Arya & Kiser, 2016). Transporters along with their tissue localization are represented in the Fig-1.5.
**Figure-1.5**: Drug transporters, A: human hepatocytes, B: enterocytes of human small intestine, C: human renal proximal tubular cells, D: human brain capillary endothelial cells. SLC transporters are represented by circles while ABC transporters by ovals. Direction of the drug transport is represented by solid line and the movement of driving ions by dashed line.

**Transporter Types**

In the last 2 decades, several important human drug transporters have been identified. The two most important are solute carrier (SLC) transporters and ATP-binding cassette transporters (ABC) (P. Hansten, 2000; Schinkel & Jonker, 2003). Both reveal an extensive distribution within the body and are involved in the transport of a wide range of substrates. Several of them could probably contribute to the permeability of drugs into cells and the processes through which drugs access their pharmacological and toxicological targets (Dobson & Kell, 2008). Reckoning on the direction by which carrier proteins translocate the substrate across the cell membrane, they will be classified as influx or efflux transporters (Russel, 2010). Transporters classification is presented in Fig-1.6.
**CHAPTER-1**

**INTRODUCTION**

P-glycoprotein

It is a well-described ATP dependent carrier glycoprotein within the cytomembrane and is accountable for the active transport of drugs across numerous membranes within the intestines, brain, proximal tubules of the kidneys and testes (Bendayan et al., 2002; Carson et al., 2001; Preiss, 1998). Pgp causes efflux of xenobiotic from cytoplasm to extracellular fluid, it decreases intestinal absorption along with entry and penetration of substances in brain, fetal tissues, testes, and encourages their biliary, and renal elimination (Mutalik & Sanghavi, 2014). Pgp is a natural defensive process mainly against hydrophobic, amphipathic, uncharged or basic substances. It is the main transporter that is involved in the drug interactions (Finch & Pillans, 2014).

**BCRP**

It is half ABC transporter located in the GI tract, kidney, brain, liver, mammary tissues, placenta and testes, and having a role in mediating resistance in cell lines of breast cancer and limits the oral bioavailability and entry of certain drugs across the

---

**Figure-1.6: Classification of transporters**

**Efflux Transporters**
ABC transporters are by description efflux transporters since they utilize energy obtained from ATP hydrolysis to intervene the primary active export of drugs from the intracellular to the extracellular milieu, usually against a high diffusion gradient (Russel, 2010).

- **P-glycoprotein**
- **BCRP**
blood brain barrier, blood testis barrier and blood placenta barrier. It is also called ABCG2 as it belongs to G-protein subfamily (Maliepaard et al., 2001). Statins i.e. rosvuavastin is a substrate of BCRP and its concomitant use with itraconazole and cyclosporine (BCRP inhibitors) is avoided as it increases statin systemic oral exposure by inhibiting its efflux (Gupta et al., 2007).

**Influx Transporter**

Many of the SLC family members aid the cellular uptake or influx of substrates possibly through facilitated diffusion down the electrochemical gradient working as a channel or uniporter or by secondary active transport against a diffusion gradient coupled to the symport or antiport of inorganic or small organic ions to provide the driving force (Russel, 2010).

- **OAT/OCT**

They are responsible for the transportation of a variety of organic anions and cations, independent of protons or sodium gradients in kidney. They have secretory role while in hepatocytes and intestinal epithelia they have absorptive role (Koepsell & Endou, 2004).

- **OATP**

OATPs transport amphiphilic substances, which include a few endogenously synthesized metabolites for example thyroid hormones, bile acids and steroid conjugates (zu Schwabedissen & Kroemer, 2011). They are absorptive in hepatocytes and excretory for anionic and cationic drugs in kidney. Moreover, several drugs including chemotherapeutic agents, anti-histaminic drugs, diuretics and antibiotics have been recognized as substrates of OATPs. This transporter seems to be associated with clinically related transporter DDIs which are of biggest magnitude (van de Steeg et al; Zimmerman et al., 2013).
### Table-1.1: Transporter Mediated Clinical Drug–Drug Interactions

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Perpetrator</th>
<th>Victim</th>
<th>Pharmacokinetic impact</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-gp</td>
<td>Quinidine</td>
<td>Digoxin</td>
<td>Digoxin renal CL decreases 34–48%</td>
<td>(Giacomini et al., 2010)</td>
</tr>
<tr>
<td>P-gp</td>
<td>Ritonavir</td>
<td>Digoxin</td>
<td>Digoxin AUC increases 85%</td>
<td>(Ding et al., 2004)</td>
</tr>
<tr>
<td>P-gp</td>
<td>Dronedronone</td>
<td>Digoxin</td>
<td>Digoxin AUC increases 157% Cmax increases 75%</td>
<td>(Khojasteh et al., 2011)</td>
</tr>
<tr>
<td>P-gp</td>
<td>Ranolazine</td>
<td>Digoxin</td>
<td>Digoxin AUC increases 60% Cmax increases 46%</td>
<td>(Jetting, 2006)</td>
</tr>
<tr>
<td>P-gp</td>
<td>Verapamil</td>
<td>Digoxin</td>
<td>Digoxin plasma concentration increases</td>
<td>(Koda et al., 2016)</td>
</tr>
<tr>
<td>OATs</td>
<td>Ketoconazole</td>
<td>Fexofenadine</td>
<td>Fexofenadine plasma concentration increases</td>
<td>(Aytoun &amp; Morgan, 2001)</td>
</tr>
<tr>
<td>BCRP</td>
<td>Quinidine</td>
<td>Loperamide</td>
<td>Increase central adverse effects</td>
<td>(Sadegh et al., 2000)</td>
</tr>
<tr>
<td>BCRP</td>
<td>GF120918</td>
<td>Topotecan</td>
<td>Topotecan AUC increases 147%</td>
<td>(Kruitzer et al., 2002)</td>
</tr>
<tr>
<td>OCTs</td>
<td>Probenecid</td>
<td>Ciclosporin</td>
<td>Ciclosporin renal Cl decreases 22%</td>
<td>(Cundy, 1999)</td>
</tr>
<tr>
<td>OCTs</td>
<td>Acyclovir</td>
<td>Furosemide</td>
<td>Furosemide renal Cl decreases 66%</td>
<td>(M. Li et al., 2006)</td>
</tr>
<tr>
<td>OCTs</td>
<td>Acyclovir</td>
<td>Acyclovir renal Cl decreases 32% and AUC increase 40%</td>
<td>(Laskin et al., 1982)</td>
<td></td>
</tr>
<tr>
<td>OCTs</td>
<td>Penicillin</td>
<td>Penicillin</td>
<td>Penicillin renal clearance decrease</td>
<td>(Burckhardt, 2012)</td>
</tr>
<tr>
<td>OCTs</td>
<td>ACE inhibitors</td>
<td>ACE inhibitors</td>
<td>ACE renal clearance decrease, half-life increase</td>
<td>(Aytoun &amp; Morgan, 2001)</td>
</tr>
<tr>
<td>OCTs</td>
<td>Metformin</td>
<td>Metformin renal Cl decreases 27% and AUC increases 50%</td>
<td>(Somogyi et al., 1987)</td>
<td></td>
</tr>
<tr>
<td>OCTs</td>
<td>Pindolol</td>
<td>Pindolol</td>
<td>Pindolol renal Cl decreases approximately 54%</td>
<td>(A. Somogyi et al., 1992)</td>
</tr>
<tr>
<td>OCTs</td>
<td>Dofetilide</td>
<td>Dofetilide</td>
<td>Dofetilide renal Cl decreases 33%</td>
<td>(Abel et al., 2000)</td>
</tr>
<tr>
<td>OCTs</td>
<td>Levoxyfoxcin</td>
<td>Levoxyfoxcin</td>
<td>Increase in AUC; decrease in renal clearance of levoxyfoxcin</td>
<td>(Bauer et al., 2005)</td>
</tr>
<tr>
<td>OCTs</td>
<td>Cetirizine</td>
<td>Fexofenadine</td>
<td>Fexofenadine renal Cl decreases 41%</td>
<td></td>
</tr>
<tr>
<td>OCTs</td>
<td>Ciclosporin</td>
<td>Ciclosporin AUC increases 890% and Cmax increase by 678%</td>
<td>(Hedman et al., 2004; Neuvonen et al., 2006)</td>
<td></td>
</tr>
<tr>
<td>OCTs</td>
<td>Rosuvastatin</td>
<td>Rosuvastatin</td>
<td>Rosuvastatin AUC increase 610%</td>
<td>(Simonson et al., 2004)</td>
</tr>
<tr>
<td>OCTs</td>
<td>Efavamcin</td>
<td>Glyburide</td>
<td>Glyburide AUC increases 125%</td>
<td>(Zheng et al., 2009)</td>
</tr>
<tr>
<td>OCTs</td>
<td>Ritonovir</td>
<td>Rosuvastatin</td>
<td>Rosuvastatin AUC increases 107% and Cmax increases 365%</td>
<td>(Kiser et al., 2008)</td>
</tr>
</tbody>
</table>
1.1.1.2. Pharmacodynamic Drug–Drug Interactions

Pharmacodynamic is ‘what the drug does to the body’. In this the object drug activity at its site of action or at receptor is altered by the precipitant. Pharmacodynamic DDIs have tended to be less extensively studied than pharmacokinetic ones. In these, patients response to drug change without affecting the drugs ADME kinetics (Snyder et al., 2012). Pharmacodynamics drug interactions may be categorized into synergistic and antagonistic interactions:

**Synergistic Interactions**

In this resultant drug activity or effect is larger compared to the sum of the individual drug. This type of interaction could be beneficial or harmful and can occur at the same or different cellular receptor sites e.g. simultaneous use of two different benzodiazepine agonists such as midazolam and diazepam synergies at the same receptor site (Cottagnoud et al., 2003). These two agonists are additive in their effects. Similarly, synergism at different cellular receptors would include the simultaneous use of diazepam a benzodiazepine agonist with phenobarbital a barbiturate exhibit pronounced central nervous system depressive effects because of additive effect on the same chloride channel (Romac & Albertson, 1999). Similarly, additive effect of losartan and enalapril on blood pressure (Azizi et al., 1997).

**Antagonistic Interactions**

In antagonistic interactions, drugs can antagonize the pharmacological action of other drugs by specific receptor mechanisms or by nonspecific physiologic or cellular mechanisms that do not involve a single receptor site (Tripathi, 2013). Therapeutic specific receptor antagonism is the benzodiazepine receptor antagonist flumazenil that can rapidly reverse the central nervous system depressant effects of diazepam (Donaldson et al., 2007). Similarly, the opioid receptor antagonist naloxone reverse the analgesic effects of an opioid agonist such as morphine (Boom et al., 2012).

Non-receptor-mediated antagonism occurs at a physiological and physiochemical level. In physiological antagonism drugs act on two different types of receptors and antagonize action of each other. For example a thiazide diuretic (hydrochlorothiazide) and a potassium sparing diuretic (triamterene) don’t intervene one another at the exact same receptors site but result in opposite effects on the potassium urinary excretion, also insulin and glucagon or adrenaline and insulin, inverse effects on blood sugar level (Quinn & Day, 1995; Tripathi, 2013). Physiochemical interaction can be seen in case of alkaloid poisoning, where potassium permanganate chelates i.e., oxidizes the unabsorbed alkaloid in the stomach and prevent further absorption of alkaloid.
Similarly in arsenic poisoning, dimercaprol is used to chelate the heavy metal arsenic (Quinn & Day, 1995; Tripathi, 2013).
1.1.2. Factors Affecting Drug Interactions

Various other factors should be taken under consideration when assessing the potential drug-drug interaction. These factors include

1.1.2.1. Drug Associated Factors

Drugs mostly prone to create interaction problems are those having (Herman, 1999; Thummel & Wilkinson, 1998):

- Drug showing steep dose response curve.
- Drugs exhibiting first pass metabolism *i.e.*, the loss of drug because it goes through the liver for the first time.
- Drug with a single, inhabitable route of elimination.
- Drug having high plasma protein binding capability.
- Inhibitors or inducers of cytochrome P450 (CYP450) enzyme system.
- Drugs having active metabolites.
- Drugs possessing multiple pharmacological effects.
1.1.2.2. Patient Associated Factors

Factors that might raise the chances of the incidence of DDIs in patients comprise of:

- **Age**

  ADRs are associated with almost all the drugs, however the types and level may vary from patient to patient. Age may be an essential element, that stimulates the incidence of ADRs (Alomar, 2014). Very young children, infants, and neonates are at extraordinary risk of ADRs as they have underdeveloped systems including blood brain barrier, renal tubular, their capacity to absorb and metabolize the drug, and baroreceptor functions are less predictable and more variable (Clavenna & Bonati, 2009; De Gregori et al., 2009; Schoderboeck et al., 2009). Geriatric patients using multiple drugs for multiple health issues are at very high risk for ADRs. With the aging processes, liver, and kidneys loses the potential to eliminates the drugs (Budnitz et al., 2007). Also, the quantity of fat tissue to relative to water increases in the body, as significant decrease in the water observed with the age. Hence, the water soluble drugs reaches higher concentrations as there is a lesser amount of water to dilute while the fat soluble drugs acquire more, while there is moderately greater amount of fat tissues to store them (Jose & Rao, 2006).

- **Gender**

  The physiological variations of females and males influence the actions of numerous drugs. The biological and anatomical variations are human body structure, liver metabolic process and GI factors. Male in contrast to female have high body weight and organ size, less body fat, higher glomerular filtration rate and different gastric motility. These modifications may change the way the body handles drugs by varying their pharmacodynamics and pharmacokinetics (Alomar, 2014). Similarly the enzyme CYP3A4 is less active in males than females, whereas other enzymes are decreased in females which results in altered effects on drug metabolism (El-Eraky & Thomas, 2003). In Chinese people, midazolam metabolism is less likely in men than women because of CYP3A4 (Labbe et al., 2000). Specific female issues including menstruation, menopause and pregnancy may possibly have intense drug-effects in individuals (Mitchell et al., 2009). For example in the elimination of anti-epileptics increases in pregnancy leading to diminished effects. (Sit et al., 2008).

- **Race and Ethnicity**

  Ethnicity employs an ample impact on the drug metabolism and is restrained by various genetic factors that make the differences between individuals. Variations in genetic factor coding in enzymes for drug receptors, transporters and metabolizing enzymes (Sexton et al., 2000). Recent development proposes that ADRs might be evaded by changing the therapeutic plan in accordance with genetics. In a latest cohort study, the risk factors for ADRs are accompanied with ACE inhibitors were
assessed, African-Americans were discovered to be more susceptible to ACE associated angioedema than any other ethnic group (Morimoto et al., 2004).

- **Body Mass Index**

Drugs penetrate to various tissues at variable speed, due to the capability of drug to cross different membranes. Like, thiopental an anesthetic drug with high fat solubility, quickly enters the brain, while penicillin does not, which is water soluble drug. Overall, fat soluble drugs cross cell membranes quickly than water soluble drugs (Anderson & Holford, 2008). Drugs, which are fat soluble, leave the tissues very slowly thus accumulates in fatty tissues, such drugs circulate within the blood stream for a number of days even an individual has stopped using the drug (Zhao et al., 2009). Drug distribution may also differ from individual to individual. For example, older and overweight individual might store relatively big quantities of fat-soluble drug, as the proportion of their body fat escalates with aging, while thin people may store comparatively little (Rhodin et al., 2009).

- **Life Style**

Smoking is also among the threatening factors of several diseases like cancer, cardiovascular diseases and peptic ulcer (Woo et al., 2009). It alters the metabolic process by exciting different CYP450 isoenzymes 1A2, 1A1, and 2E1 (Tomlinson et al., 2005). Drugs substrates for CYP1A2 and their metabolism can be induced in smokers following a clinically significant reduction in pharmacologic effects. Theophylline, beta-blockers, insulin, oral contraceptives, H2 blockers, thiothixene and flecainide therapeutic responses could be afflicted with smoking (Himmelmann et al., 2003). One clinical study revealed that insulin dependent diabetic smokers required 15–20% more insulin than non-smokers, and as much as 30% more when they smoked heavily (Kroon, 2007).

Alcohol affects the metabolism of many drugs and changes the depth of ADRs rendering it more hazardous or harmful. Using alcohol with specific drugs may cause ADRs like nausea, vomiting, drowsiness, fainting, headaches, loss of coordination, hypotension (Krupski et al., 2009). An individual with peptic ulcer or gastritis, concurrent use of alcohol with NSAIDs produce internal bleeding as a result of severe ulceration (Kim et al., 2009). Persiant alcohol usage triggers enzymes, changes some drugs into hazardous substances that may damage the liver causing liver cirrhosis and liver hepatitis which in turn influence their capability to metabolize drugs. For example, beta blockers toxicity increases with liver problems (Reuben, 2006).

- **Multiple Diseases**

Multiple diseases make individuals more susceptible to ADRs because of usage of many drugs. Drugs useful in one disease are hazardous in another. Many drugs including NSAIDS when prescribed, in patients with peptic ulcer disease may lead to serious medical problems. Similarly beta blockers might worsen asthma when taken
for high B.P or heart disease and make it difficult for those who have diabetes to inform when their blood glucose is too low (Daneshtalab et al., 2004).

- Allergy

Drug independent cross reactive antigens may produce sensitizations that may manifest as a drug allergy (Chung et al., 2008). Following primary sensitization to a causative agent, another exposure triggers the affected T cells and antibodies to enter the elicitation stage, like the type I - IV immune reactions. All the drug allergies seen are type I or IV reactions; type II and III reactions are merely experienced infrequently. The drugs involved in hypersensitivity reactions are sulfa and β-lactam antibiotics (Harboe et al., 2007).

1.1.3. Evaluation of DDIs

ADME reports are imperative for the drug discovery and development. The ADME parameters acquired from these studies assist in the prediction of drug behaviors in individuals and are essential for the decision to improve, maintain or cancel a drug prospect. Nevertheless, imperfect ADME studies or misconception of the ADME data could cause problems in drug development. ADME studies are achieved through in vitro or in vivo designs (Zhang et al., 2012).

1.1.3.1. In Vitro Metabolic Models

In vitro models for drug metabolic interactions study comprised of various models including, expressed enzyme, whole organ, intact cells, tissue slices, and subcellular fractions (Lecluyse et al., 2005).

Expressed enzyme is a useful tool to examine enzymatic activity in a controlled atmosphere of enzyme content, buffer, and cofactors most of which is often quickly manipulated. Enzymes are independently expressed as recombinant enzymes through cDNA-directed expression technique in a variety of host cell lines (Hayes et al., 1995; Waterman & Johnson, 1991). The most generally employed single enzyme family could be the CYP450 family of isozymes that have been used to evaluate metabolic stability, human hepatic clearance, drug-drug interaction potential (inhibition) and reaction phenotyping (Carlson & Fisher, 2008; Tang et al., 2005). In certain occasions, single expressed enzyme system may not be valid in complex metabolic pathways i.e. metabolism using multiple enzymes. There are many examples where mechanism based inactivation has been observed in recombinant enzyme preparations although not in other in-vitro models such as liver microsomes probably as a result of more effective formation of reactive metabolites in the targeted individual enzyme process (Polasek & Miners, 2007).

Using tissue homogenization and differential centrifugation, numerous subcellular fractions like cytosol, S9 and microsomes are obtained. The predominate subcellular
fraction among these is the microsomal fraction containing the smooth endoplasmic reticulum because drugs generally metabolized by the CYP450 or by glucuronidation, both of which are present in the microsomal fraction. Once the major enzyme associated with metabolism or inhibition (e.g., sulfotransferase or aldehyde oxidase) of a drug is in the cytosol, then cytosolic design becomes extremely helpful and for complex metabolic pathways i.e., CYP hydroxylation followed by sulfation or carbonyl reduction and finally by oxidative conjugation, S9 fraction is suitable. However, for too complex metabolic pathways, sub-cellular fraction is not an appropriate model and it is way better to employ a more appropriate process like the hepatocyte model (M. A. Sinz, 2012).

Cellular systems i.e. hepatocytes are the principal liver cells where drug metabolism occurs. Hepatocytes membrane includes numerous uptake or efflux transporters. Drugs could passively diffuse through the hepatocyte cell membrane or be taken up by transporters (Shitara et al., 2003). The primary cultures of hepatocytes are to carry enzymes, co-factors at physiological concentrations, and provide a drug metabolism environment that strongly mimics the in-vivo conditions. Freshly prepared hepatocytes are a good model for drug metabolism and transporter studies, but they have restrictions as they are not easily available from humans and other higher animals. Furthermore the preparation is for once use, making it hard to repeat or compare with other studies (A. P. Li et al., 1999; Ruegg et al., 1997). However like subcellular fractions, hepatocytes may also be cryopreserved and kept for extended period of time, making them extremely ideal for routine or as well as high output assays to evaluate metabolic stability, metabolite recognition, induction, or cytotoxicity (Lake et al., 2009; Lecluyse et al., 2005). With respect to the study type, the hepatocytes can be utilized as a cell suspension usually for 2–3 hr or in cell culture for several days (M. W. Sinz, 1999).

In suspension, the hepatocytes are no more attached with an extracellular matrix or to one another that will be an improper environment for the cells. Thus, the viability of the cells will quickly decrease around 2–3 hr, restraining the length of incubation time and consequently the extent or degree of metabolism. Hepatocytes in culture will survive for several days as well as weeks due to the artificial atmosphere developed in culture having an extracellular matrix and cell-to-cell contact. However, even in culture the hepatocytes will start to dedifferentiate, and the phenotypic qualities of the cells might begin to change within 24–48 hr, most significant of these changes are those of the CYP enzymes. Tissue slices have been prepared from numerous organs, mostly the liver, intestine, kidney, brain, lung and signify more architectural integrity and structure comprising most of the organ cell types, cellular parts to basement membranes and cell to cell contacts. Tissue slices aren’t amenable to cryopreservation, thus, tests should be conducted on freshly separated and sliced organs that makes tissue slices not as useful for routine studies, particularly when slices are needed from species other than the rat (Sinz, 2012).

1.1.3.2. In Vivo Metabolic Models

In vivo models results are multi-factorial, provided and deliver a quantifiable set of pharmacokinetic parameters and toxicological endpoints. Animal studies are required to measure drug exposures and to determine potential toxicities. The current trend is to use rats as the first animal species for testing drug exposure because they are inexpensive and require small amount of test compound. Pharmacokinetics of a
compound in preclinical species with different dose paths i.e., oral, intravenous, subcutaneous, transdermal, intraperitoneal and infusion is an important section for selection, optimization and medical prospect nomination and development. Extensive PK studies are essential to evaluate the dose proportionality, bioavailability or food effects in single or multiple dose administration (Zhang et al., 2012). Compartmental and non-compartmental models were used by these studies to determine the PK-parameters of a compound, including maximum plasma concentration, time to reach maximum concentration, area under curve, volume of distribution, clearance, elimination half-life and bioavailability (Brown et al., 2006).
1.2. Meloxicam

Meloxicam[4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide] is an NSAID from oxicam group having molecular formula (C_{14}H_{13}N_{3}O_{4}S_{2}), molecular weight 351.4 and melting point 254 °C (Ahmed et al., 2005). It is a crystalline yellow powder, soluble in dimethylformamide, slightly soluble in ethanol, acetone and methanol and insoluble in water (O’Neil et al., 2001; Yazdanian et al., 2004). Being a Class-II drug, dissolution rate of meloxicam is the limiting step for its oral absorption. Solid dispersions of meloxicam using hydrophilic polymers (PEG-6000 and HPMC) has shown improved dissolution rate with subsequently enhanced oral bioavailability (Chaudhary et al., 2012).

Meloxicam is zwitterion, when pH is within 1 to 4 and behaves as an anion, when pH is above 4 (Davies & Skjodt, 1999). Meloxicam was soluble at neutral pH but became quickly insoluble with lowering pH (2.1 mg/L at pH = 5 and 0.5 mg/L at pH = 4) (Vignaduzzo et al., 2010). At very low pH its solubility enhanced, suggesting a second pK value and the presence of a cation. This indicates, it has low gastrointestinal side-effects as a result of low solvency in acidic media (Nasr, 2015). It has low ulcerogenic property compared with the other NSAIDs could possibly be, because of low affinity for COX-1 and generally employed in the management of arthritis, rheumatoid arthritis, ankylosing spondylitis and in other rheumatologic conditions (Dasandi et al., 2002; Gates et al., 2005b).
1.2.1. Mode of Action

Meloxicam exert its effects via selective inhibition of cyclooxygenase-2 (COX-2) enzyme, responsible for the conversion of arachidonic acid into prostaglandin, the arbitrator of inflammation (Turini & DuBois, 2002) as shown in Fig-1.7. Meloxicam concentrates in synovial fluid and signifies its use in the management of arthritis (Engelhardt et al., 1995).

![Mode of action of meloxicam](image)

**Figure-1.7:** Mode of action of meloxicam

1.2.2. Pharmacokinetics of Meloxicam

1.2.2.1. Absorption

The absolute bioavailability of meloxicam is 89% when administered orally, so not distinctly affected by concomitant intake of food (Aboelwafa & Fahmy, 2012; Davies & Skjodt, 1999; Turck et al., 1995). Its absorption is independent around the dose range 7.5 – 30 mg, resulting in dose - linear increase in plasma concentration (Liew et al., 2014). The peak plasma concentration ($C_{max}$) is attained within 5 – 6 hours after oral dose of 7.5 mg tablet under fasted conditions, indicating prolonged drug absorption (Ahmed et al., 2005). In conditions i.e. lower back pain, sciatica and acute
osteoarthritis, where rapid analgesic effect is desirable, a parenteral form of meloxicam is available, attaining $C_{\text{max}}$ within 1.5 hours (Davies & Skjodt, 1999). It undergoes gastrointestinal recycling and therefore does not submit to faster elimination, but with repeated cholestyramine administration sequestered meloxicam from the intestine and increases its clearance by 50% (Kaplan-Machlis & Klostermeyer, 1999; Turck et al., 1996).

1.2.2.2. Distribution

The meloxicam apparent volume of distribution following an oral administration is small i.e., 10 – 15 L (0.1 to 0.2 L/kg) because of extensive plasma protein binding i.e., >99% (Gates et al., 2005a; Hynninen et al., 2009; Schmid et al., 1995). Meloxicam readily enters the synovial fluid, with the free fraction twice the plasma, due to the lower albumin content (Degner et al., 1994). However this distribution of meloxicam in synovial fluid is affected by inflammation (Lapicque et al., 2000).

1.2.2.3. Metabolism

Meloxicam undergoes equally renal and fecal elimination, with 0.25% excreted unchanged in urine and 1.6% in feces. It experiences extensive hepatic phase-I biotransformation and no conjugated derivatives have been detected (Chesne et al., 1998). CYP2C9 plays a significant role in their metabolism (Bae et al., 2011). Four different metabolites, the main metabolite 5-carboxymeloxicam (60% of dose), a middle metabolite 5-hydroxymethylmeloxicam (9% of dose) were recognized in urine (Turck et al., 1997; Turck et al., 1996). The peroxidase action might be responsible for another two metabolites. All metabolites are pharmacologically inactive (Bae et al., 2007). The metabolism of meloxicam into its metabolites by CYP2C9 is given in Fig-1.8.
1.2.2.4. Excretion

Meloxicam excretion is in the form of metabolites via urine and feces, so, they are not detectable in the plasma. Only minimal amounts of parent drug can be detected in urine and feces (Regalado & Leon, 2007). The elimination half-life ($t_{1/2}$) and clearance ($Cl$) of meloxicam is 13 - 20 hours and 0.42 – 0.7 L/hr, respectively. Steady-state plasma concentration is attained within 3 - 5 days but a significant variance between subjects i.e., interindividual coefficient of variation 30% has been noticed (Elbary et al., 2001; Turck et al., 1997).

1.2.3. Indications and Dosage

Meloxicam is administered as a single dose (7.5 mg or 15 mg) per day with or without meal in the management of rheumatoid arthritis, osteoarthritis of the hip and knee, ankylosing spondylitis, postoperative pain after abdominal hysterectomy (Ambrus et al., 2009; Braun & Sieper, 2002; Zochling et al., 2006).

1.2.4. Adverse Effects

Meloxicam is a safer drug as compared to other NSAIDs, because of its selectivity towards COX-2, though some of the reported adverse effects are gastrointestinal (GI) effects experienced in 28% patients, 21% experienced musculoskeletal disorders, 15% respiratory disorders, 18% skin disorders, and severe gastrointestinal effects, for example ulcer and internal bleeding, happened in 0.8% patients (Junmin Chen et al., 2006).
1.2.5. Interactions

Meloxicam is a substrate of CYP2C9 and CYP3A4 makes it a potential candidate for DDI’s. Extensive work has been done by the researchers on the impact of other drugs on the pharmacokinetic of meloxicam, by considering its metabolism and plasma protein binding. Some of the interactions are described below in Tab-1.2.
### Table-1.2: Drug-Drug Interactions of Meloxicam

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Mechanism</th>
<th>Pharmacological Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin</td>
<td>MEL inhibits its metabolism by inhibiting CYP2C9 and displace it from protein binding sites</td>
<td>↑ anticoagulant effect of Warfarin</td>
<td>(Turck et al., 1997)</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>Fluvoxamine inhibits CYP2C9, ↓ MEL metabolism</td>
<td>↑ serum level of MEL</td>
<td>(Zullino &amp; Khazaal, 2005)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Inhibition of renal cyclooxygenase by MEL result impaired renal perfusion</td>
<td>Digoxin half-life reduced by 12%</td>
<td>(FL Degner et al., 1995)</td>
</tr>
<tr>
<td>Furosemide</td>
<td>MEL inhibit renal cyclooxygenase and impaired renal perfusion</td>
<td>Reduced natriuretic effect of Furosemide</td>
<td>(Muller et al., 1997)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Displace MEL from its plasma protein binding site</td>
<td>Serum level of MEL ↑</td>
<td>(Ulrich Busch et al., 1996)</td>
</tr>
<tr>
<td>Lithium</td>
<td>MEL inhibit renal COX-2 enzyme and affect the renal clearance of lithium</td>
<td>↑ Lithium plasma concentration</td>
<td>(Turck et al., 2000)</td>
</tr>
<tr>
<td>Cholestryamine</td>
<td>↑ clearance of cholestyramine by MEL</td>
<td>↓ level of cholestyramine</td>
<td>(U Busch et al., 1995)</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Inhibition of MEL metabolism by voriconazole through CYP2C9 inhibition</td>
<td>MEL serum level ↑</td>
<td>(Hynninen et al., 2009)</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Itraconazole impaired gastrointestinal absorption of MEL by inhibiting some transport system in the gut wall</td>
<td>Plasma level of MEL ↓</td>
<td>(Hynninen et al., 2009)</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>MEL displace cisplatin from protein binding sites</td>
<td>Increase serum level and anticancer effect of Cisplatin</td>
<td>(Naruse et al., 2007; Wolfesberger et al., 2006)</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Same mechanism as of Cisplatin</td>
<td>Doxorubicin serum level ↑</td>
<td></td>
</tr>
<tr>
<td>4-hydroperoxy ifosfamide</td>
<td>Displace 4-hydroperoxy ifosfamide from protein binding sites by MEL</td>
<td>↑ Serum level of 4-hydroperoxy ifosfamide</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Paracetamol enhances the affinity of MEL for its respective COX</td>
<td>Synergistic anti-nociception</td>
<td>(Miranda et al., 2006)</td>
</tr>
<tr>
<td>Dexketoprofen</td>
<td>Same mechanism as for Paracetamol</td>
<td>Synergistic anti-nociception</td>
<td>(Gonzalez et al., 2011)</td>
</tr>
</tbody>
</table>
1.2.6. Population Pharmacokinetic of Meloxicam

Meloxicam is primarily metabolized by CYP2C9 (major) and CYP3A4 (minor). CYP2C9 is polymorphic and is involved in the oxidation of meloxicam. Thirty-four alleles variants of the CYP2C9 gene display variations in enzyme expression and activity, ultimately causing differences in pharmacokinetics parameters. In Chinese male individual CYP2C9*13 allele was first identified and is responsible for decrease in enzymatic activity in individuals with the CYP2C9*3/*13 genotype. The CYP2C9*13 allele has also been found in Japanese and Koreans population and is not detected in other populations, however Chinese have higher CYP2C9*13 allele frequency than Korean populations, resulting in very low enzymatic activity of CYP2C9 substrate (Bae et al., 2011). In India and Pakistan CYP2C9*2 and CYP2C9*3 allelic form is common, CYP2C9*3 occur at a very high frequency than Germans and Egyptians while CYP2C9*2 is absent in Chinese, resulting poor metabolism and better pharmacokinetics parameters, as shown in Tab-1.3 (Dorado et al., 2011). However, CYP2C9*13 allele is not detected in populations like Germans and Egyptians (Bae et al., 2011; Chaudhary et al., 2016; Van Booven et al., 2010).
Table 1.3: Pharmacokinetic Parameters of Meloxicam (15 mg) given as Single Oral Dose in Healthy Human Volunteers (mean ± SD)

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Koreans (mean ± SD)</th>
<th>Germans (mean ± SD)</th>
<th>Indians (mean ± SD)</th>
<th>Egyptians (mean ± SD)</th>
<th>Pakistanis (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (ng/mL)</td>
<td>1445.7 ± 305.5</td>
<td>910 ± 23.0</td>
<td>1376 ± 0.25</td>
<td>683 ± 29.0</td>
<td>1051 ± 376.2</td>
</tr>
<tr>
<td>$T_{max}$ (hr)</td>
<td>4.1 ± 0.3</td>
<td>7</td>
<td>2.91 ± 2.02</td>
<td>2.5</td>
<td>3.125 ± 1.004</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>22.0 ± 4.9</td>
<td>21.2</td>
<td>30.5 ± 9.60</td>
<td>2.135 ± 0.407</td>
<td>13.694 ± 0.568</td>
</tr>
<tr>
<td>$AUC_0-t$ (µg-hr/mL)</td>
<td>37.5 ± 9.8</td>
<td></td>
<td></td>
<td></td>
<td>28.385 ± 0.411</td>
</tr>
<tr>
<td>$AUC_0-\infty$ (µg-hr/mL)</td>
<td>42.4 ± 13.2</td>
<td>35.09</td>
<td>35.79 ± 8.53</td>
<td>4.18 ± 0.135</td>
<td>28.667 ± 0.414</td>
</tr>
<tr>
<td>$V_d$ (L)</td>
<td>13.5</td>
<td>19.04 ± 7.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Cl$ (L/hr)</td>
<td>0.46</td>
<td>0.44 ± 0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References:
(Bae et al. 2007)  (Rani et al. 2004)  (Elbary et al. 2001)  (Ahmad et al. 2011)
1.3. Fluconazole

Fluconazole, chemically 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-2-propanol, is a relatively low molecular-weight broad spectrum synthetic triazole antifungal agent, possesses a hydroxyl group that’s make it water soluble compare to other imidazoles (Latimer et al., 2001). It is used both orally and intravenously in the treatment of vaginal or systemic candidacies, oesopharyngeal, esophageal skin infections and is the drug of choice in cryptococcosis and coccidioidal meningitis (Brammer et al., 1990; Egunsola et al., 2013; Martindale, 1996). It can also be a preliminary and maintenance treatment intended to be used in AIDS individuals with osyptococcal meningitis (Longo et al., 2011). It has lower morbidity rate than intrathecal amphotericin B.

1.3.1. Mode of Action

Fluconazole inhibits 14-α-sterol demethylase (microsomal CYP450 enzyme), as a result ergo sterol biosynthesis is impaired due to the accumulation of 14-α-methyl sterols (Ratzlaff et al., 2011). Methyl sterols disrupts the close packing of phospholipids acyl chain and inhibit the function of particular membrane bound enzyme system like ATPase and of the electron transport system, as a result fungal growth is inhibited (Georgopapadakou, 1998). It is fungi static, however, against certain organisms it may be fungicidal, in a dose-dependent manner (Longley et al., 2008).

1.3.2. Pharmacokinetics of Fluconazole

1.3.2.1. Absorption

Fluconazole is a white crystalline powder, soluble in water, having bioavailability 90% and peak plasma level (C_max) i.e. 6.72 µg/mL is attained within 2 hours after administration of oral 400 mg dose (Martin, 1999). The presence of food and stomach acid have no effect on the bioavailability of fluconazole but delayed the T_max for 4 hours.

1.3.2.2. Distribution

Plasma protein binding and the apparent volume of distribution of fluconazole is 12% and 0.7 L/kg, respectively (Bruggemann et al., 2009; Zonios & Bennett, 2008). It diffuses easily into the cerebrospinal fluid, sputum, saliva and concentrates in urine and skin. This property significances its use in treating systemic fungal infections.
The half-life of fluconazole is 32 hours and once daily dosing increases the plasma level 2.5 folds (Pacifici, 2016).

### 1.3.2.3. Metabolism

Fluconazole after absorption undergo little metabolism during the first pass in liver. Only three metabolites, one identified as a 1, 2, 4-triazole compound were recognized in urine. In case of hepatic dysfunctioning, no alteration in the pharmacokinetics of the drug is anticipated, so no dosage modification is needed (Debruyne & Ryckelynck, 1993).

### 1.3.2.4. Excretion

It is mainly cleared through renal excretion. In normal volunteers, when administered 80% is excreted as unchanged drug and 11% as metabolites in urine (Egunsola et al., 2013; Jura & Hillenbrand, 2006).

### 1.3.3. Indications

It is mainly used in candida, cryptococci and in nonmeningeal-coccidioidal infections as first choice therapy. A single-dose (150 mg) is beneficial for treating vaginal candidiasis, while a dose of 100 - 200 mg daily intended for oropharyngeal and esophageal candidiasis for 14 - 21 days (Zonios & Bennett, 2008). Patients undergoing bone marrow transplantation or having non-neutropenic candidemia, fluconazole found to be as effective as amphotericin B and has been observed to reduce the prevalence of superficial and systemic fungal infection prophylactically (Slavin et al., 1995).

Fluconazole can also be indicated in HIV and non-HIV individuals for the management of cryptococcal-meningitis infection, a dose of 400 mg daily for 2 months is given in HIV infection (Mussini et al., 2004) and 400 mg daily for 6 to 12 months can be used for treating non-meningeal cryptococcosis in both HIV and non-HIV patients.

### 1.3.4. Adverse Effects

Fluconazole is generally well tolerated. Gastrointestinal disturbances are the most common complaint and consist primarily of nausea, vomiting, diarrhea, headache and abdominal pain. Adverse effects like skin rashes, hepatic dysfunction i.e., elevation of serum transaminase level and exfoliate skin reactions have also been reported in 5% of subjects (Wells & Lever, 1992). Prolonged use can cause headache, anorexia and
alopecia, however, alopecia is reversible and dose dependent and generally occur with chronic doses *i.e.*, above 400 mg daily (Pappas et al., 1995). Fluconazole is a category C drug so must be avoided in pregnancy because of teratogenicity in animals and several reports of organ deformities in humans (Pursley et al., 1996).

1.3.5. Interactions

Fluconazole is a highly discerning inhibitor of CYP2C9 and CYP2C19 and a modest inhibitor of CYP3A4 (Saari et al., 2008). Several interactions about fluconazole that decreases or increases the concentration of drugs metabolized by these enzymes have been reported and are presented below in Tab-1.4.
## Table-1.4: Drug-Drug Interactions of Fluconazole

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Mechanism</th>
<th>Pharmacological Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampin</td>
<td>Rifampin an inducer of CYP2C9 induces FLU metabolism</td>
<td>Serum level of FLU ↓</td>
<td>(Lazar &amp; Wilner 1990)</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>The absorption of FLU reduces as Cimetidine raises the gastric pH</td>
<td>↓ serum level of FLU</td>
<td>(Lazar &amp; Wilner 1990)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Inhibition of CYP2C9 and CYP3A4 by FLU result altered metabolism of warfarin</td>
<td>Warfarin serum level ↑</td>
<td>(Black et al., 1996)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Inhibition of CYP2C9 and CYP3A4 by FLU result altered metabolism of Phenytoin</td>
<td>Raised serum Phenytoin level</td>
<td>(Howitt &amp; Oziemski, 1989)</td>
</tr>
<tr>
<td>Rosuvastatin</td>
<td>FLU inhibits CYP2C9 and CYP3A4, ↓ Rosuvastatin metabolism</td>
<td>Serum level of Rosuvastatin ↑</td>
<td>(Cooper et al., 2002)</td>
</tr>
<tr>
<td>Fluvastatin</td>
<td>FLU inhibits CYP2C9 and CYP3A4, ↓ Fluvastatin metabolism</td>
<td>↑ Fluvastatin plasma concentration</td>
<td>(Miller &amp; Carthan, 2003)</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>↓ Ibuprofen metabolism because of inhibition of CYP2C9 and CYP3A4 by FLU</td>
<td>↑ level of Ibuprofen</td>
<td>(Hynninen et al., 2006)</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>FLU inhibits its metabolism by inhibiting CYP2C9 and 3A4</td>
<td>Cyclosporin serum level ↑</td>
<td>(Sharif &amp; Ali, 1994)</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>Inhibition of CYP3A4 and 2C19 based Terfenadine metabolism by FLU</td>
<td>Plasma level of Terfenadine increases by 52%</td>
<td>(Cantilena et al., 1995)</td>
</tr>
<tr>
<td>Midazolam</td>
<td>FLU inhibits metabolism of Midazolam by inhibiting CYP2C19 and CYP3A4</td>
<td>Increase serum level of Midazolam</td>
<td>(Olkkola et al., 1996)</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>Inhibition of CYP2C19 and CYP3A4 based Omeprazole metabolism by FLU</td>
<td>Omeprazole serum level ↑</td>
<td>(Ge et al., 2010)</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>Inhibition of CYP2C19 and CYP3A4 by FLU</td>
<td>↑ serum level of Nelfinavir</td>
<td>(Garazzino et al., 2006)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Inhibition of CYP2C19 and CYP3A4 by FLU</td>
<td>↑ serum level of Diazepam</td>
<td>(Roßkamp et al., 1996)</td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td>FLU ↓ Sulfonylureas metabolism via CYP inhibitory mechanism</td>
<td>↑ oral hypoglycemic effect</td>
<td>(Albengres et al., 1998; Lazar and Wilner 1989; Niemi et al., 2001)</td>
</tr>
</tbody>
</table>
1.3.6. Population Pharmacokinetic of Fluconazole

Fluconazole is a highly discerning inhibitor of CYP2C9 and CYP2C19 and to a lesser extent CYP3A4 (Saari et al., 2008). CYP2C9 have various allelic variants, among them CYP2C9*2 and CYP2C9*3 is very common in different populations. Brazilians have the highest frequency of CYP2C9*2 allele than Caucasians, while have frequency somewhat lower than Mexicans. CYP2C9*3 allele is found in Italians and Iranians; however, their frequency was found to be higher in Italians than Iranians (Chaudhary et al., 2016). These allelic variants are responsible for the interindividual variability of pharmacokinetics and can result in drug toxicity or therapeutic failure (Dorado et al., 2011). Population pharmacokinetic of fluconazole is shown in Tab-1.5.
Table 1.5: Pharmacokinetic Parameters of Fluconazole (150 mg) Given as Single Oral Dose in Healthy Human Volunteers

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iranians (mean ± SD)</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>2220 ± 460</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>2.62 ± 0.40</td>
</tr>
<tr>
<td>t½ (hr)</td>
<td>30.86 ± 7.44</td>
</tr>
<tr>
<td>AUC 0–t (µg-hr/mL)</td>
<td>73.26 ± 20.29</td>
</tr>
<tr>
<td>AUC0–∞ (µg-hr/mL)</td>
<td>91.18 ± 28.54</td>
</tr>
<tr>
<td>References</td>
<td>(Safaei et al., 2015)</td>
</tr>
</tbody>
</table>

|                             | Italians (mean ± SD)        |
| Cmax (ng/ml)                | 2691 ± 430.2                |
| Tmax (hr)                   | 2.53 ± 0.23                 |
| t½ (hr)                     | 29.99 ± 4.34                |
| AUC 0–t (µg-hr/mL)          | 137.30                      |
| AUC0–∞ (µg-hr/mL)           | 114.15 ± 19.73              |
| References                  | (Ripa et al., 1993)         |

|                             | Brazilians (mean ± SD)      |
| Cmax (ng/ml)                | 3752 ± 759                  |
| Tmax (hr)                   | 2.79                        |
| t½ (hr)                     | 33.72 ± 11.50               |
| AUC 0–t (µg-hr/mL)          | 92.45 ± 27.97               |
| AUC0–∞ (µg-hr/mL)           | 154.45 ± 36.81              |
| References                  | (Porta et al., 2005)        |

|                             | Caucasians (mean ± SD)     |
| Cmax (ng/ml)                | 2659 ± 808                  |
| Tmax (hr)                   | 2.38 ± 1.06                 |
| t½ (hr)                     | 33.72 ± 11.50               |
| AUC 0–t (µg-hr/mL)          | 104.13 ± 31.24              |
| AUC0–∞ (µg-hr/mL)           | 175.13 ± 48.98              |
| References                  | (Jovanovic et al., 2005)    |

|                             | Mexicans (mean ± SD)       |
| Cmax (ng/ml)                | 4440 ± 799                 |
| Tmax (hr)                   | 2.59 ± 1.03                |
| t½ (hr)                     | 33.72 ± 11.50              |
| AUC 0–t (µg-hr/mL)          | 152.21 ± 28.89             |
| AUC0–∞ (µg-hr/mL)           | 175.13 ± 48.98             |
| References                  | (Palma-Aguirre et al., 2010) |
1.4. Omeprazole

Omeprazole, 5-methoxy -2-[(4-methoxy -3, 5-dimethyl -2-pyridinyl) methyl] sulphonyl IH- benz-imidazole is a proton pump inhibitor (Wood et al., 2003). It exists in S- and R-enantiomer forms (Cohen et al., 2005; Mears & Kaplan, 1996). It is acid labile, when given orally, so must be protected from exposure to gastric acidic juice. The solubility in water is very low. Nowadays omeprazole was formulated as enteric-coated granules (Shi & Klotz, 2008).

1.4.1. Mode of Action

Omeprazole causes reduction in gastric acid secretion and increases gastric pH, by inhibiting the gastric acid proton pump (H,K-ATPase) at the secretory membrane of the parietal cell, consequently hydrogen ions release in the gastric lumen is inhibited (Solana & Lopez-Herce, 2010). This inhibition is dose related and effects inhibition of equally basal and stimulated acid secretion irrespective of the stimulus (Bowlby & Dickens, 1994).

1.4.2. Pharmacokinetic of Omeprazole

1.4.2.1. Absorption

Omeprazole is a lipophilic weak base with a pKa around 4.0 and exhibit quick absorption when given orally (Olbe et al., 2003). $C_{max}$ is achieved within 0.5 - 2 hours and the bioavailability after an oral dose was 50% due to first-pass effect (Mostafavi & Tavakoli, 2004). Its half-life is 0.5 to 1.0 hours (Saha et al., 2014). It is a substrate as well as an inhibitor of Pgp, so its bioavailability is considerably affected by this efflux pump (Pauli-Magnus et al., 2001).

1.4.2.2. Distribution

Omeprazole is a well distributed drug with a volume of distribution of 0.3 L/kg because of extensive plasma protein binding i.e. 95% capability (Ogilvie et al., 2011). Its concentration level found to be high in duodenal and gastric cells after oral administration, however following IV administration it was quantified highly in liver, stomach, thyroid gland, duodenum and renal cells (Regardh, 1985; Regardh et al., 1985). It is also present in lactating mother’s milk (Nava-Ocampo et al., 2006).
1.4.2.3. Metabolism

Omeprazole experiences first-pass metabolism in liver. CYP2C19, CYP3A4 and CYP2C9 is responsible for omeprazole metabolization into hydroxy-omeprazole and omeprazole-sulphone, which are the primary major metabolites, both are inactive and do not contribute any pharmacological effect (Kearns & Winter, 2003). The formation of other minor metabolites 5-O-desmethyl-omprazole is also dependent on these CYP isoforms (Abelo et al., 2000).

1.4.2.4. Excretion

After complete metabolization 80% of omeprazole is excreted as metabolites in urine, while remaining in feces.

1.4.3. Indications

It is employed in the management of dyspepsia, gastric and duodenal ulcer, peptic ulcer, gastro-esophageal-reflux disease (GERD), helicobacter pylori infection and in zollinger-ellison syndrome (Klotz, 2000; Lassen, 2007).

1.4.4. Adverse Effects

Omeprazole is generally safe and well tolerated but still it has some unwanted side effects that are

- Atrophic gastritis (Kuipers et al., 1996).
- Community acquired pneumonia (Canani et al., 2006).
- DIARRHEA (DIAL ET AL., 2005).
- Hyponatremia (Bebarta et al., 2008).
- Polymyositis (Clark & Strandell, 2006).
- Hypomagnesemia and hypo calcemic seizures (Hess et al., 2012).
- Interstitial nephritis (Myers et al., 2001).
1.4.5. Interactions

Omeprazole when co-administered with other drugs may affect the bioavailability in two ways, it selectively inhibits CYP2C19, CYP3A4 and CYP2C9, therefore the drugs metabolized by these isoenzymes will be affected, and secondly it increases gastric pH, so the drug i.e. absorbed from the stomach may alter its absorption. Some of the interactions of omeprazole with drugs are as follows in Tab-1.6.
### Table-1.6: Drug-Drug Interactions of Omeprazole

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Mechanism</th>
<th>Pharmacological Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin</td>
<td>OME inhibits Warfarin metabolism by inhibiting CYP2C9</td>
<td>↑ anticoagulant effect of Warfarin</td>
<td>(Holbrook et al., 2005)</td>
</tr>
<tr>
<td>Citalopram</td>
<td>OME inhibits CYP2C19, ↓ Citalopram metabolism</td>
<td>↑ serum level of Citalopram</td>
<td>(Malling et al., 2005)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Inhibition of CYP2C19 and CYP3A4 by OME result altered metabolism of Diazepam</td>
<td>Diazepam serum level ↑</td>
<td>(Jones et al., 2004)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Metabolism of Phenytoin inhibited by OME through CYP2C9 inhibition</td>
<td>Raised serum Phenytoin level</td>
<td>(Stormer et al., 1993)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>OME inhibits CYP2C19 and CYP2C9, ↓ Clopidogrel metabolism</td>
<td>Serum level of Clopidogrel ↓</td>
<td>(Sibbing et al., 2009)</td>
</tr>
<tr>
<td>Erolotinib</td>
<td>OME reduces the absorption of Erolotinab by increasing the gastric pH</td>
<td>↓ Erolotinib plasma concentration</td>
<td>(Duong &amp; Leung, 2010)</td>
</tr>
<tr>
<td>HIV Medications</td>
<td>↓ HIV drugs absorption from the GIT by OME by altering gastric pH</td>
<td>↓ level of HIV medications</td>
<td>(Bista et al., 2007; Burger et al., 1998; Falcon &amp; Kakuda, 2008)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>OME reduces Ampicillin absorption</td>
<td>Ampicillin serum level ↓</td>
<td>(Bista et al., 2007)</td>
</tr>
<tr>
<td>Iron</td>
<td>Iron absorption is reduced by OME</td>
<td>Plasma level of Iron ↓</td>
<td>(Golubov et al., 1991)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>OME induces hypomagnesemia of diuretics by altering their absorption from intestine</td>
<td>Increase serum level of magnesium</td>
<td>(Gerson &amp; Triadafilopoulos, 2001; Melville et al., 1994)</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Inhibition of CYP3A4 based ketoconazole metabolism by OME</td>
<td>Ketoconazole serum level ↑</td>
<td>(Ge et al., 2010)</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Inhibition of CYP2C19 by OME</td>
<td>↑ serum level of Tacrolimus</td>
<td>(Moreau et al., 2006)</td>
</tr>
<tr>
<td>Cilostazol</td>
<td>Inhibition of CYP2C19 by OME</td>
<td>↑ serum level of Cilostazol</td>
<td>(Suri &amp; Bramer, 1999)</td>
</tr>
</tbody>
</table>
1.4.6. Population Pharmacokinetic of Omeprazole

Omeprazole metabolism and the formation of its major and minor metabolites are dependent on the CYP isoforms including CYP2C19, CYP3A4 and CYP2C9, however among them CYP2C19 is the major contributor (Abelo et al., 2000; Kearns & Winter, 2003). Allelic variants CYP2C19*1*2*3 are the most important detrimental alleles of this isoenzyme and are responsible for the individual variations in the pharmacokinetic parameters (Zand et al., 2005). The frequency of CYP2C19*1 allele in Japanese peoples is higher from Bangladeshis, almost similar with Koreans and lower from Iranians (Payan et al., 2015). However, CYP2C19*2 allele frequency found to be higher in Japanese than Koreans, resulting in lower metabolism and increase in drug toxicity (Saeed & Mayet, 2013) as depicted in Tab-1.7.
### Table-1.7: Pharmacokinetic Parameters of Omeprazole (20 mg) Given as Single Oral Dose in Healthy Human Volunteers

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iranians (mean ± SD)</td>
</tr>
<tr>
<td><strong>Cmax</strong> (ng/mL)</td>
<td>401 ± 179</td>
</tr>
<tr>
<td><strong>Tmax</strong> (hr)</td>
<td>2.0 ± 1.03</td>
</tr>
<tr>
<td><strong>t½</strong> (hr)</td>
<td>2.39 ± 0.79</td>
</tr>
<tr>
<td><strong>AUC 0–t</strong> (µg·hr/mL)</td>
<td>1.094 ± 0.439</td>
</tr>
<tr>
<td><strong>AUC 0–∞</strong> (µg·hr/mL)</td>
<td>1.094 ± 0.439</td>
</tr>
</tbody>
</table>

**References**
- (Noubarani et al., 2010)
- (Saha et al., 2014)
- (Watanabe et al., 2006)
- (Kang et al., 2002)
1.5. Aims and objective

The aims and objectives of this study are to

- Develop and validate high pressure liquid chromatography linked with ultraviolet-visible (HPLC-UV), analytical method for the quantification of meloxicam and its metabolites.
- Apply the developed method in the pharmacokinetic DDI study of meloxicam with selected co-prescribed drugs such as fluconazole and omeprazole in normal healthy Pakistani human volunteers.
- Study the influence of the fluconazole and omeprazole on the pharmacokinetics of meloxicam and its metabolites by analyzing volunteer’s blood samples.
- Calculate the pharmacokinetic parameters such as $C_{\text{max}}$, $t_{\text{max}}$, $t_{1/2}$, AUC and interpretation of the data by applying suitable statistical tools.
- Provide useful data to the health care professionals i.e. clinicians and pharmacists to safeguard from the potential risks associated with these drugs and provide proper dose adjustment if required.
2. EXPERIMENTAL
The evaluation of pharmacokinetic drug-drug interaction of meloxicam, 5-hydroxy meloxicam and 5-carboxy meloxicam with selected co-prescribed drugs such as fluconazole and omeprazole was studied in healthy human volunteers in the following three phases. In the first phase, a high-performance liquid chromatography coupled with ultraviolet-visible (HPLC-UV/Vis) analytical method was developed and validated for the quantification of meloxicam and its metabolites. In phase-II the pharmacokinetics profile of meloxicam and its metabolites in healthy human volunteers was studied and in the phase-III the pharmacokinetic drug-drug interaction of meloxicam with fluconazole and omeprazole was investigated in normal healthy human volunteers. The clinical trial was conducted according to Helsinki declaration (Association, 2013).
The pharmacokinetic data was interpreted after analyzing the blood samples of human volunteers through applying appropriate statistical tools to evaluate any potential pharmacokinetic DDI. Pharmacokinetic parameters like $C_{max}$, $T_{max}$, $t_{1/2}$ and $AUC$ were assessed from PK-Summit® software, using both compartmental and non-compartmental models.

2.1. Chemicals

2.1.1. Reference Standards
Meloxicam (purity 99.1%) was purchased from Sigma-Aldrich, 5-carboxy meloxicam (purity 99.9%) and 5-hydroxy meloxicam (purity 99.8%) was procured from Toronto Research Chemicals (Canada). Piroxicam (purity 98%), naproxen sodium (purity 98.3%) was gifted by Medicraft Pharmaceuticals Pvt. Ltd., (Peshawar, Pakistan) and lumefantrine (purity 97.3%), diclofenac-sodium (purity 99%) from Ferozson Laboratories Ltd., (Nowshera, Pakistan), respectively. Meloxicam tablets (15 mg; Trade name-MITS; B.No. 1248) of Shaigan Pharmaceuticals Pvt. Ltd (Rawalpindi, Pakistan), Omeprazole capsules (40 mg; Trade name-Risek; B.No. 1643C2) of Getz Pharmaceuticals Pvt. Ltd (Karachi, Pakistan) and Fluconazole capsules (150 mg; Trade name-Diflucan; B.No. 1813) of Pfizer laboratory. Ltd., (Karachi, Pakistan) were procured from the local market.

2.1.2. Chemicals and Solvents
The analytical grade HPLC solvents methanol (purity 99.9%), trifluoracetic-acid (TFA) (purity 99.9%), acetonitrile (purity 99.9%), diethyl-ether, dichloro-methane, n-hexane, DMF (dimethylformamide), concentrated HCl and glacial acetic acid were acquired from Sigma Aldrich (Oslo, Norway). Freshly, double distilled and deionized HPLC grade water was obtained in the laboratory from Millipore distillation apparatus (Milford, USA).
2.2. Instrumentation

2.2.1 HPLC System

The analysis of meloxicam and its metabolites were performed using HPLC system (Perkin Elmer Series-200, Norwalk, USA) consisting of a pump, column oven, UV detector, a vacuum degasser, a rheodyne manual injector with 20 μL loop linked by Pe Nelson network chromatography interface (NCI) 900. The chromatographic setup was run by software known as Perkin Elmer Total Chrome Workstation (version 6.3.1) for the solicitation and proceeding of data.

2.2.2 Column’s

The column used for analysis of the drug were Supelco C18 reverse phase HPLC column (150 mm x 4.6 mm, 5 µm), Perkin Elmer Brownlee® analytical C18 column (150 mm x 4.6 mm, 5 µm; Shelton, USA), Phenomenex Gemini® C18 column (150 mm x 4.6 mm, 5 µm; California, USA), Thermo Quest Hypersil® BDS C8 (150 mm x 4.6 mm, 5 µm), Thermo Quest Hypersil® BDS C18 (250 mm x 4.6 mm, 5 µm) and guarded by RP Perkin Elmer C18 (30 mm × 4.6 mm, 10 µm; Norwalk, USA) column.

2.2.3 Balance

A Schimadzu (AX 200) electronic balance was used for the weighing of the standards and chemicals.

2.2.4 Centrifuge Machine

Temperature controlled centrifuge machine (model: k-2080, Centurion plasma, UK) range 20 – 80 °C was used for the separation of samples.

2.2.5. Vortex Mixer

The samples and solvents mixing were carried out on a Touch Vortex mixer (Fisher Scientific Model: 232, Germany).

2.2.6. Distillation Unit

Freshly, double distilled and deionized HPLC grade water was obtained from Millipore distillation apparatus (Milford, USA) for chromatographic analysis.
2.2.7. Filtration Assembly

The filtration of the water and other organic solvents for chromatographic analysis was carried out on 0.45µm polyvinylidene fluoride (PVF) membrane filters by using an assembly that consists of 1.0 L glass flask (Sigma-Aldrich®) fitted with a vacuum pump (Taiwan).

2.2.8. Cartridges

Supelclean LC-18 SPE cartridges (1 mL; particle size 56.2 µm; Bellefonte, USA) with the Visiprep™ Vacuum Manifold were used for solid-phase extraction.

2.2.9. Spectrophotometer

Reference standard was scanned in Schimadzu UV/Vis Spectrophotometer (UV-1601) for the optimization of the wavelength.

2.3. HPLC Method Development

A simple, rapid and cost-effective method for the analysis of meloxicam, 5-hydroxy meloxicam and 5-carboxy meloxicam in human plasma was developed and validated using reverse phase high performance liquid chromatography (RP-HPLC) coupled with UV detector. Piroxicam was used as an internal standard. The method was validated according to standard guidelines, various experimental parameters and chromatographic conditions were optimized. The method was successfully applied in the “Pharmacokinetics and pharmacokinetic drug-drug interactions studies of meloxicam, 5-hydroxy meloxicam and 5-carboxy meloxicam with commonly selected co-prescribed drugs.

2.3.1. Preparation of Standard Solutions

The standard stock solutions of meloxicam (0.5 mg/mL) were prepared in methanol containing 20 µL of glacial acetic acid. The standard stock solution of 5-hydroxy meloxicam, 5-carboxy meloxicam and piroxicam (0.5 mg/mL) were also prepared in methanol, DMF (dimethylformamide) and methanol, respectively and further dilutions were prepared using mobile phase. The working standard solutions of meloxicam (10, 50, 100, 250, 500, 1000, 1500, 2000 ng/mL) were prepared by diluting the standard secondary stock solution of meloxicam (10 µg/mL). The working standard solutions of 5-carboxy meloxicam (25, 50, 75, 100, 250, 500, 750, 1000 ng/mL), 5-hydroxy meloxicam (30, 50, 75, 100, 250, 500, 750, 1000 ng/mL) and that of piroxicam (1000 ng/mL) were prepared from their respective standard secondary stock solution (10 µg/mL) and were stored at refrigerator temperature. Calibration standards of meloxicam and its metabolite, at eight different concentration levels in the range 10-2000 ng/mL were prepared in the mobile phase.
2.3.2. Sample Collection and Preparation

A single oral dose of the meloxicam (15 mg) was administered orally with glass of water (≈ 250 mL) and blood samples (2 mL) was collected periodically in heparinized gel tubes, centrifuged at 3000 rpm for 10 min; plasma was transferred into Eppendorf tubes and stored at -20 °C till analysis.

The blank plasma (collected before administration of the drug) were thawed at room temperature at the time of analysis and spiked with meloxicam, 5-hydroxy meloxicam, 5-carboxy meloxicam and piroxicam (internal standard).

2.3.3. Extraction of Samples from Plasma

Liquid-liquid extraction was assessed for the recovery of parent drug and its metabolites from the plasma samples. Plasma (200 µL) was mixed with internal standard (100 µL = 100 ng) in eppendorf tube, acetonitrile (600 µL) was added and vortexed for 3 min. Then dichloromethane (1100 µL) was added, further vortexed for 5 min and centrifuged for 15 min at 9000 rpm, organic layer was collected and was evaporated under the gentle stream of nitrogen. The residue was re-dissolved in methanol (2 mL) and injected into HPLC for analysis.

2.3.4. Chromatographic Conditions Optimizations

Different chromatographic parameters like HPLC columns, mobile phase composition ratio, flow rate, wavelengths of detector, injection volumes, internal standard and extraction solvents were optimized for the quantitative determination of meloxicam and its metabolites by using RP-HPLC method in an isocratic mode.

2.3.4.1. Selection of HPLC Column

The chromatographic analysis of meloxicam and its metabolite was carried out on five columns: Supelco C18 reverse phase HPLC column (150 mm x 4.6 mm, 5 µm), Perkin Elmer Brownlee® analytical C18 column (150 mm x 4.6 mm, 5 µm; Shelton, USA), Phenomenex Gemini® C18 column (150 mm x 4.6 mm, 5 µm; California, USA), Thermo Quest Hypersil® BDS C8 (150 mm x 4.6 mm, 5 µm) and Thermo Quest Hypersil® BDS C18 (250 mm x 4.6 mm, 5 µm). The column with the best peak resolution, retention and shape was selected.

2.3.4.2. Mobile Phase Composition

Several organic solvents such as acetonitrile, methanol, trifluoracetic-acid (TFA), phosphate buffer, pH adjusted distilled water were tried in different combinations both in an isocratic and in gradient mode for the determination of meloxicam and its
metabolites. The mobile phase composition which demonstrated good resolution and separation was selected for analysis.

2.3.4.3. Flow Rate Composition

The flow rate significantly affect the sample elution, resolution, separation and peak shape. In this method, different flow rates in the range of 0.8-1.5 mL/min were evaluated and the one that gave best resolution was selected as optimum flow rate.

2.3.4.4. Detection Wavelength

A Perkin Elmer UV lambda (Nowak, USA) was used to determine the maximum wavelength of maximum absorbance ($\lambda_{\text{max}}$) of meloxicam. Effect of wavelength on analytes response was studied by varying wavelength in the range of 330 – 355 nm.

2.3.4.5. Injection Volumes

Different injection volumes in the ranges of 10 µL - 50 µL were assessed using Rheodyne manual injector.

2.3.4.6. Internal Standard Selection

Different internal standards were tried such as naproxen sodium, lumefantrine, diclofenac-sodium, and piroxicam were tried. In present studies, the Piroxicam showed maximum response and better chromatographic properties, therefore it was selected as internal standard.

2.3.4.7. Selection of Extraction Solvent

Various solvents such as diethyl-ether, dichloromethane, acetonitrile and n-hexane were evaluated as extraction solvents. Optimum percent recovery was achieved using dichloromethane as extraction solvent.

2.3.4.8. Column Oven Temperature

The effect of column oven temperature in the range of 25 - 40 °C on the analytes was studied to evaluate changes in the peak shape and retention time.

2.3.5. Method Validation

Validated of the developed method was carried out according to ICH guidelines with special emphasis on specificity, linearity, accuracy, precision, robustness, selectivity, limit of detection (LOD) and limit of quantification (LOQ).
2.3.5.1. Specificity

The specificity of the developed method was judged from the complete peak resolution of the meloxicam and its metabolites in mobile phase, spiked plasma sample in 1:1 mixture (i.e., 1 µg/mL of the sample as well as internal standard).

2.3.5.2. Linearity

The linearity was evaluated by scheming calibration curves at eight concentration level of meloxicam (10, 50, 100, 250, 500, 1000, 1500 and 2000 ng/mL), 5-hydroxy meloxicam (30, 50, 75, 100, 250, 500, 750 and 1000 ng/mL), 5-carboxy meloxicam (25, 50, 75, 100, 250, 500, 750 and 1000 ng/mL) and piroxicam (1000 ng/mL). Following analysis, the ratio of peak area of the analyte and internal standard was plotted as a function of concentration of the analyte using the squares linear least regression equation and the correlation co-efficient.

2.3.5.3. Accuracy

The accuracy of the established method was calculated from the percent recovery of the spiked sample. Blank human plasma sample was spiked with analytes of different concentration levels keeping the concentration of I.S. constant. The similar concentrations levels of the standards were also prepared in mobile phase. Samples were then injected in triplicate and percent recoveries were calculated by the following equation:

\[
\text{Percent recovery} = \frac{A}{B} \times 100
\]

Where

- \(A\) = Response ratio of the peak of spiked plasma sample (analyte/I.S)
- \(B\) = Response ratio of the peak of sample in standard solution (analyte/I.S)

2.3.5.4. Precision

Precision of the developed method was determined by injection and the analysis repeatability. The injection precision repeatability was determined by injecting the biological sample spiked with 1000 ng/mL of meloxicam, 5-carboxy meloxicam and 5-hydroxy meloxicam for 10 times into HPLC. The retention time and the peak area was obtained and articulated as mean, standard deviation (±SD), and covariance (% RSD). The analysis repeatability was analyzed by spiking six biological samples with 1000 ng/mL of meloxicam, 5-carboxy meloxicam and 5-hydroxy meloxicam, independently.

Intermediate precision (the intra-day and inter-day studies) was determined by injecting plasma sample spiked with appropriate standard concentration of the analyte and internal standard on 8.00, 16.00 and 24.00 hours for three consecutive days. The
outcome was depicted as mean, standard deviation (±SD) as well as covariance (%RSD). The following equation was employed to determine the concentration

\[ C = \frac{x}{y} \times \frac{A}{B} \times C_{S} \times D_{F} \]

Where

- \( x \) = Peak area of the analyte in biological sample
- \( y \) = Peak areas of the I.S in biological sample
- \( A \) = Peak area of the I.S in 1:1 mixture
- \( B \) = Peak area of the analyte in 1:1 mixture
- \( C_{S} \) = Concentration of analyte in the 1:1 mixture
- \( D_{F} \) = is the dilution factor

2.3.5.5. Sensitivity

The developed method was assessed in terms of LOD and LOQ of the analytes. The LOD was determined from the peak of the analyte by the software at which signal-to-noise ratio (S/N) is 3 and LOQ was the concentration of analyte at which signal-to-noise ratio is equal to 10.

2.3.5.6. Stability

Stability studies of the meloxicam and its metabolites were assessed in spiked biological samples and standard stock solution at room temperature (25 °C), refrigerator temperature (2 - 8 °C) and at -20 °C for one month. Each sample was then analyzed (n = 3) and the following equation was used to evaluate the stability:

\[ \text{Percent Stability} = \frac{S_{t}}{S_{0}} \times 100 \]

Where

- \( S_{t} \) = Stability of sample at that time
- \( S_{0} \) = Initial stability of sample

2.3.5.7. Robustness/ Ruggedness

The robustness/ruggedness of the method was investigated by bringing deliberately small alterations in various chromatographic conditions, like composition of the mobile phase (± 2%), temperature of the column oven (± 5 °C), mobile phase flow rate (± 0.2 mL/min) and detection wavelength (± 5%).

2.4. Pharmacokinetics and Pharmacokinetic Drug-Drug Interactions Study of Meloxicam and its Metabolites

Pharmacokinetic study of meloxicam was carried out in three steps
First pharmacokinetics profile of meloxicam was evaluated in healthy human Pakistani volunteers.

Second pharmacokinetic drug-drug interactions of meloxicam with omeprazole and fluconazole after a single dose of administration, was investigated in normal human Pakistani volunteers.

Then pharmacokinetic drug-drug interactions of meloxicam after multiple doses of omeprazole and fluconazole were evaluated in normal healthy human Pakistani volunteers.

2.4.1. Study Design and Study Protocol

It was a two-sequence, open-label, cross-over study with a washout period of two weeks. This study was carried out in normal healthy male volunteers according to “World Medical Association’s Declaration of Helsinki - ethical principles for medical research involving human subjects”. The protocol of the study was approved by the ethical committee of the Department of Pharmacy, University of Peshawar, Pakistan.

2.4.2. Selection of Volunteer’s

Healthy male human volunteers (n = 24) aged between 20 to 23 years, weight in the range of 50 to 70 kg and BMI in the range of 23-30 were recruited in this study. A detail description regarding the clinical study was explained and those agreed signed the informed consent form, then they were examined for various physical and biochemical tests like renal function tests (RFTs), liver function tests (LFTs), blood screening, blood-pressure, low-density-lipoproteins (LDL), high-density-lipoproteins (HDL) and electrocardiography (ECG) were performed to investigate whether they were not suffering from any disease or any abuse/addictive substances.

2.4.2.1. Inclusion Criteria

Only healthy human volunteers were recruited in this study, they were divided into two groups i.e., n = 12 in each group. The volunteers having no recent past medication history of hypertension, diabetes, heart disease, cancer and with normal biochemical results were enrolled in the trail. The volunteer was advised to avoid any medicine for two weeks prior to the study or during clinical trial.
2.4.2.2. Exclusion Criteria

Volunteers having any systemic abnormality like AIDS or suffer from any cardiac, hepatic, renal and diabetic diseases or having high blood pressure were excluded from the trial. Smokers, obese and volunteers using snuff or any other addictive stuff were debarred from the study.

2.5. Pharmacokinetics of Meloxicam and its Metabolites

An open-label, single dose study of meloxicam (15 mg) was conducted in healthy male Pakistani volunteers (n = 24), before the administration of the drug they were fasted overnight and only water was allowed.

2.5.1. Administration of Drug and Collection of Blood Samples

The volunteers were directed to record any sickness as well as uncomfortable side effects with the medicine, to the doctor or principal investigator present in the trial. Volunteers at early morning at 8 o’clock meloxicam (15 mg) was administered. Breakfast and lunch was served to them after 2 and 6 hours of dosing. The blood sample (3 mL) from these volunteers were collected at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 24, 36, 48, and 72 hours’ time points in the EDTA tubes. The blood samples were centrifuged at 3000 rpm for 10 min; plasma was transferred into Eppendorf tubes and stored at -20 °C in a freezer till analysis.

2.5.2. Preparation of Blood Samples

Plasma samples were thawed at room temperature and spiked with piroxicam (I.S). The drug was extracted from plasma samples with dichloromethane as described above in section 2.3.3. The residue was redissolved in methanol (2 mL) and 20 µL was injected into HPLC for analysis.

2.5.3. Quantification of Meloxicam

The concentration of meloxicam in the volunteer’s plasma samples was quantified by using the following formula

\[ C = \frac{x}{y} \times \frac{A}{B} \times C_s \times D_f \]

Where

- \( x \) = Peak area of the analyte in biological sample
- \( y \) = Peak areas of the I.S in biological sample
- \( A \) = Peak area of the I.S in 1:1 mixture
- \( B \) = Peak area of the analyte in 1:1 mixture
- \( C_s \) = Concentration of meloxicam in the 1:1 mixture
D<sub>F</sub> = is the dilution factor
The outcome i.e. concentration of meloxicam, was determined in the form mean, standard deviation (±SD) and %RSD using Minitab<sup>®</sup>.

### 2.5.4. Pharmacokinetic Data Analysis

The concentration of meloxicam was plotted on a linear and semilog graph paper as a function of time. Pharmacokinetic parameters were processed from the data using PK Summit<sup>®</sup> software. Some parameters are presented below in **Tab-2.1**.

**Table-2.1: Pharmacokinetic Parameters**

<table>
<thead>
<tr>
<th>S/No</th>
<th>Pharmacokinetic parameters</th>
<th>Symbols</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Peak plasma concentration</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>ng/mL</td>
</tr>
<tr>
<td>2</td>
<td>Time of peak plasma concentration</td>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Hr</td>
</tr>
<tr>
<td>3</td>
<td>Area under curve (bioavailability) from zero to time point</td>
<td>AUC&lt;sub&gt;(0-t)&lt;/sub&gt;</td>
<td>ng.hr/mL</td>
</tr>
<tr>
<td>4</td>
<td>Area under curve (bioavailability) from zero to infinity</td>
<td>AUC&lt;sub&gt;(0-∞)&lt;/sub&gt;</td>
<td>ng.hr/mL</td>
</tr>
<tr>
<td>5</td>
<td>Area under first moment of plasma conc. vs time curve</td>
<td>AUMC</td>
<td>ng.hr*hr/mL</td>
</tr>
<tr>
<td>6</td>
<td>Absorption half life</td>
<td>t&lt;sub&gt;1/2-A&lt;/sub&gt;</td>
<td>Hr</td>
</tr>
<tr>
<td>7</td>
<td>Distribution half life</td>
<td>t&lt;sub&gt;1/2-D&lt;/sub&gt;</td>
<td>Hr</td>
</tr>
<tr>
<td>8</td>
<td>Elimination half life</td>
<td>t&lt;sub&gt;1/2-E&lt;/sub&gt;</td>
<td>Hr</td>
</tr>
<tr>
<td>9</td>
<td>Volume of distribution</td>
<td>Vd</td>
<td>mL/kg</td>
</tr>
<tr>
<td>10</td>
<td>Clearance</td>
<td>Cl</td>
<td>mL/hr/kg</td>
</tr>
<tr>
<td>11</td>
<td>Mean residence time</td>
<td>MRT</td>
<td>Hr</td>
</tr>
<tr>
<td>12</td>
<td>Elimination rate constant from central compartment</td>
<td>K&lt;sub&gt;10&lt;/sub&gt;</td>
<td>1/hr</td>
</tr>
</tbody>
</table>
2.6. Pharmacokinetic Drug-Drug Interaction Studies

The volunteers participated in the above study i.e., pharmacokinetics of meloxicam, play a part in the pharmacokinetic DDI study. They were briefed about the study before the clinical trial and a written consent was obtained from them. The wash out period between the two studies was 2 weeks and their inclusion and exclusion standards were similar as described in section 2.4.2.1 and 2.4.2.2.

The pharmacokinetic drug-drug interaction of meloxicam with selected co-prescribed drugs i.e., omeprazole and fluconazole was conducted in four phases.

- In first phase, drug-drug interaction of meloxicam with omeprazole after a single dose was evaluated.
- Second phase, drug-drug interaction of meloxicam with multiple doses of omeprazole i.e., single dose for 3 days was evaluated.
- Third phase, DDI of meloxicam with fluconazole after a single dose was studied.
- DDI of meloxicam with fluconazole multiple doses i.e., single dose for 3 days was investigated in the fourth phase.

2.6.1. Pharmacokinetic Drug-Drug Interaction of Single Dose of Meloxicam and Omeprazole

Pharmacokinetic drug-drug interaction of meloxicam and omeprazole, single dose administration was conducted in healthy male volunteers (n = 24). The study protocol was according to the Helsinki Declaration guidelines and approved by the ethical committee (Department of Pharmacy, University of Peshawar). Their inclusion and exclusion standards were same as described in section 2.4.2.1 and 2.4.2.2.

2.6.1.1. Selection of Subjects

The same criteria for the selection of the volunteers were adopted as discussed earlier in section 2.4.2.

2.6.1.2. Study Design

A randomized crossover, two sequence, two periods, open label, single dose model was used for the evaluation of pharmacokinetic drug-drug interaction of meloxicam
and omeprazole. The volunteers were grouped into two consists of 12 volunteers in each group as shown in the Tab-2.2.

**Table-2.2: Study Design for DDI of Single Dose of Meloxicam and Omeprazole**

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Group I (n = 12)</th>
<th>Group II (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1</td>
<td>Meloxicam alone (Mel tablet, dose: 15mg)</td>
<td>Meloxicam + Omeprazole (Mel tablet, dose: 15mg; Ome capsule, dose: 40mg)</td>
</tr>
<tr>
<td></td>
<td>Two-week washout period</td>
<td></td>
</tr>
<tr>
<td>Treatment 2</td>
<td>Meloxicam + Omeprazole (Mel tablet, dose: 15mg; Ome capsule, dose: 40mg)</td>
<td>Meloxicam alone (Mel tablet, dose: 15mg)</td>
</tr>
</tbody>
</table>

2.6.1.3. Administration of Drug

The volunteers were advised not to take any medicine two weeks prior to the clinical study. They were fastened overnight and the breakfast and lunch was given 2.0 and 6.0 hours intervals, respectively, after the administration of the drugs. No extra food or juices were allowed during the study.

In the first phase/sequence, group-I received meloxicam 15 mg and group-II received meloxicam 15 mg along with omeprazole capsule 40 mg. After two weeks’ washout period, in the second sequence, group-I took meloxicam and omeprazole simultaneously and group-II took only meloxicam 15 mg with a full glass of water (250 mL). The drug products details and their specified doses and dosage forms administered to the volunteers are given in Tab-2.3.

**Table-2.3: Details of the Drug Products Used in the DDI Study**

<table>
<thead>
<tr>
<th>Drug (Trade Name)</th>
<th>Manufacturer</th>
<th>Batch Number</th>
<th>Manufacture Date</th>
<th>Expiry Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam (Mits; 15mg Tablet)</td>
<td>Shaigan Pharmaceuticals Pvt. Ltd., (Rawalpindi, Pakistan)</td>
<td>1248</td>
<td>April, 2013</td>
<td>April, 2016</td>
</tr>
<tr>
<td>Omeprazole (Risek; 40mg Capsule)</td>
<td>Getz Pharmaceuticals Pvt. Ltd., (Karachi, Pakistan)</td>
<td>1643C2</td>
<td>April, 2013</td>
<td>April, 2016</td>
</tr>
</tbody>
</table>
2.6.1.4. Collection of Plasma Sample

The blood sample (3mL) from the volunteers were collected at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 24, 36, 48 and 72 hours’ time points in the EDTA tubes as described in section 2.5.1.

2.6.1.5. Preparation and Analysis of Plasma Sample

The plasma samples were prepared by the same procedure described in section 2.3.3 and analysis of these plasma samples were performed by the method developed explained in section 2.3.

2.6.1.6. PK-DDIs Data Analysis

PK-DDI of meloxicam and omeprazole was determined in the plasma samples of the volunteers after a single administration of drug. Compartmental and non-compartmental pharmacokinetic parameters were assessed or analyzed with PK-Summit® software. The plasma concentration of meloxicam as a function of time was plotted on linear and semi log graph papers.
2.6.2. Pharmacokinetic Drug-Drug Interaction of Meloxicam Using Multiple Dose of Omeprazole

Pharmacokinetic DDI of meloxicam with omeprazole multiple doses was also investigated in healthy human volunteers, following the same protocol and with the same inclusion and exclusion standards as described above in the section 2.4.2.1 and 2.4.2.2.

2.6.2.1. Study Design

A two-sequence, two-periods, crossover, multiple-dose, open-label study was performed in the evaluation of pharmacokinetic drug-drug interaction of meloxicam when co-administered with omeprazole. The study design and drug product details were depicted in Tab-2.4 and 2.5.

Table-2.4: Study Design for DDI of Meloxicam with Multiple Dose Omeprazole

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Group I (n = 12)</th>
<th>Group II (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1</td>
<td>Meloxicam alone (Mel tablet, dose: 15mg)</td>
<td>Meloxicam + Omeprazole (Mel tablet, dose: 15mg; Ome capsule, dose: 40mg for 3 consecutive days)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Two-week washout period</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>Meloxicam + Omeprazole (Mel tablet, dose: 15mg; Ome capsule, dose: 40mg for 3 consecutive days)</td>
<td>Meloxicam alone (Mel tablet, dose: 15mg)</td>
</tr>
</tbody>
</table>

Table-2.5: Details of the Drug Products Used in the Multiple Dose DDI Study of Meloxicam and Omeprazole

<table>
<thead>
<tr>
<th>Drug (Trade Name)</th>
<th>Manufacturer</th>
<th>Batch Number</th>
<th>Manufacture Date</th>
<th>Expiry Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam (Mits; 15mg Tablet)</td>
<td>Shaigan Pharmaceuticals Pvt. Ltd., (Rawalpindi, Pakistan)</td>
<td>1248</td>
<td>April, 2013</td>
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</tr>
<tr>
<td>Omeprazole (Risek; 40mg Capsule)</td>
<td>Getz Pharmaceuticals Pvt. Ltd., (Karachi, Pakistan)</td>
<td>1643C2</td>
<td>April, 2013</td>
<td>April, 2016</td>
</tr>
</tbody>
</table>
2.6.2.2. Selection of Volunteers

The criteria for the volunteer’s selection were same as discussed earlier in section 2.4.2.

2.6.2.3. Administration of Drug

The volunteers were divided into two groups, group-I and group-II, they were advised to fast overnight and not to take any medicine or juices before the study or during the study. Group-I received meloxicam 15 mg and group-II received omeprazole capsule 40 mg for three consecutive days and on third day meloxicam 15 mg was taken concomitantly with omeprazole. Followed by a two weeks of washout period, group-I received meloxicam (15 mg) with omeprazole (40 mg, capsule + 3 days) simultaneously and group-II took only meloxicam (15 mg) with a glass of water (250 mL). After the administration of the drugs, breakfast and lunch was given at time of 2.0 and 6.0 hours, respectively.

2.6.2.4. Collection of Plasma Sample

Blood samples (3 mL) were collected in the EDTA tubes from the volunteers following the same design and time points as presented in section 2.5.1.

2.6.2.5. Preparation and Analysis of Plasma Sample

The same preparation and analysis protocol outlined in section 2.3.3 and 2.3, respectively was pursued for the plasma samples.

2.6.2.6. PK-DDIs Data Analysis

The concentration of meloxicam in the plasma samples were determined by plotting on normal and semilog graph papers as concentration versus time. PK-Summit® software was used for the calculations of pharmacokinetic parameters \( C_{\text{max}} \), \( AUC \), \( T_{\text{max}} \), \( t_{1/2} \) etc after the administration of meloxicam alone or concomitantly with omeprazole multiple doses. Statistical analysis of data was done with Minitab 14.0 and MS-excel 2007.

2.6.3. Pharmacokinetic Drug-Drug Interaction of Single Dose of Meloxicam and Fluconazole

The effect of fluconazole on the pharmacokinetic of meloxicam after a single dose was evaluated in normal healthy human volunteers (n = 24), followed with
volunteer’s inclusion and exclusion criterion explained in the section 2.4.2.1 and 2.4.2.2. The protocols of the study “Declaration of Helsinki, World medical associations” was approved by the ethical committee (Department of Pharmacy, University of Peshawar).

2.6.3.1. Study Design

The study design of a single dose of meloxicam tablet (15 mg) alone or with co-administration of fluconazole was described in the Tab-2.6. The drug product details used in this study was given in Tab-2.7.

**Table-2.6: Study Design for DDI of Single Dose of Meloxicam and Fluconazole**

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Group I (n = 12)</th>
<th>Group II (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1</td>
<td>Meloxicam alone (Mel tablet, dose: 15mg)</td>
<td>Meloxicam + Fluconazole (Mel tablet, dose: 15mg; Flu capsule, dose: 150mg)</td>
</tr>
<tr>
<td>Two-week washout period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 2</td>
<td>Meloxicam + Fluconazole (Mel tablet, dose: 15mg; Flu capsule, dose: 150mg)</td>
<td>Meloxicam alone (Mel tablet, dose: 15mg)</td>
</tr>
</tbody>
</table>

**Table-2.7: Details of the Drug Products Used in the Single Dose DDI Study of Meloxicam and Fluconazole**

<table>
<thead>
<tr>
<th>Drug (Trade Name)</th>
<th>Manufacturer</th>
<th>Batch Number</th>
<th>Manufacture Date</th>
<th>Expiry Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam (Mits; 15mg Tablet)</td>
<td>Shaigan Pharmaceuticals Pvt. Ltd., (Rawalpindi, Pakistan)</td>
<td>1248</td>
<td>April, 2013</td>
<td>April, 2016</td>
</tr>
<tr>
<td>Fluconazole (Diflucan; 150mg Capsule)</td>
<td>Pfizer laboratory Ltd., (Karachi, Pakistan)</td>
<td>1813</td>
<td>April, 2013</td>
<td>April, 2015</td>
</tr>
</tbody>
</table>

2.6.3.2. Selection of Volunteers

The volunteer’s selection criteria were same as talk over earlier in section 2.4.2.
2.6.3.3. Administration of Drug

A brief instruction was given to the volunteers i.e. fastened overnight, not to take medicines or juices before or during the study, to avoid interaction with the drug. Before administration of the drugs two groups of the volunteers were made. Firstly, Group-I received meloxicam 15 mg alone and group-II was administered with meloxicam + fluconazole at a dose of 15 mg and 150 mg, respectively. After a washout period of two weeks, in second phase, group-I received meloxicam + fluconazole in a dose of 15 mg and 150 mg, respectively, while group-II received meloxicam alone. All the drugs were given with a full glass (250 mL) of water. Volunteers were provided with a breakfast 2.00 hours and lunch 6.00 hours after administration of the drug.

2.6.3.4. Collection of Plasma Sample

Blood samples (3 mL) from the volunteers were collected according to the format as described in section 2.5.1.

2.6.3.5. Preparation and Analysis of Plasma Sample

The samples were prepared as described in section 2.3.3 and then analyzed by using HPLC/UV method presented in section 2.3.

2.6.3.6. PK-DDIs Data Analysis

Compartmental and non-compartmental pharmacokinetic parameters of the study were determined using the software PK-Summit®. A normal and semilog graph paper was used for the plotation of the plasma concentration of meloxicam as a function of time and MS-office 2007 and Minitab-14.0 was used for the statistically evaluation of the pharmacokinetic data.

2.6.4. Pharmacokinetic Drug-Drug Interaction of Meloxicam with Multiple Dose of Fluconazole

The protocol of the study “pharmacokinetic drug-drug interaction of meloxicam co-prescribed with fluconazole multiple doses” was according to world Helsinki declaration of medical research in human subjects. The volunteers were selected after the inclusion and exclusion criteria presented in the section 2.4.2.1 and 2.4.2.2.
2.6.4.1. Study Design

Pharmacokinetic drug-drug interaction of meloxicam with fluconazole was an open label, multiple, crossover, two sequences and two periods study. The washout period between two sequences was two weeks and two groups were created i.e. 12 volunteers in each group as presented in Tab-2.8.

Table-2.8: Study Design for DDI of Meloxicam with Multiple Dose Fluconazole

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Group I (n = 12)</th>
<th>Group II (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment 1</td>
<td>Meloxicam alone (Mel tablet, dose: 15mg)</td>
<td>Meloxicam + Fluconazole (Mel tablet, dose: 15mg; Flu capsule, dose: 150mg for 3 consecutive days)</td>
</tr>
<tr>
<td></td>
<td>Two-week washout period</td>
<td></td>
</tr>
<tr>
<td>Treatment 2</td>
<td>Meloxicam + Fluconazole (Mel tablet, dose: 15mg; Flu capsule, dose: 150mg for 3 consecutive days)</td>
<td>Meloxicam alone (Mel tablet, dose: 15mg)</td>
</tr>
</tbody>
</table>

2.6.4.2. Selection of Volunteers

The criteria for the volunteer’s selection was adopted same as considered earlier in section 2.4.2.

2.6.4.3. Administration of Drug

In first sequence, meloxicam 15 mg was taken by group-I and fluconazole 150 mg for three consecutive days followed by concomitantly meloxicam 15 mg on the third day to group-II. In second sequence, group-I was administered with meloxicam tablet and fluconazole capsule i.e. 15 mg and 150 mg (3 days), respectively and group-I received meloxicam 15 mg alone. The washout period between the two sequences was 14 days. Before administration of the drug the volunteers were strictly instructed to avoid any medicine or juices before or during the study. They were only allowed by water and supplied with breakfast 2.00 hours and lunch 6.00 hours after administering drug.

2.6.4.4. Collection of Plasma Sample

Samples were collected according to the section 2.5.1.
2.6.4.5. Preparation and Analysis of Plasma Sample

The samples of the volunteers were prepared and analyzed as presented in section 2.3.3 and 2.3, respectively.

2.6.4.6. PK-DDIs Data Analysis

PK-Summit® software was used for the evaluation of pharmacokinetic parameters $C_{\text{max}}$, $AUC$, $T_{\text{max}}$, $t_{1/2}$ etc and data was statistically analyzed with MS-office 2007 and Minitab-14.0. The concentration of meloxicam vs function of time was plotted on a normal and semi log graph paper.
3. RESULTS and DISCUSSIONS

The pharmacokinetic drug-drug interactions of meloxicam and its metabolites with omeprazole and fluconazole were investigated in healthy human volunteers in following phases:

- High-performance liquid chromatography coupled with ultraviolet-visible (HPLC-UV/Vis) method was developed and validated for the quantification of meloxicam and its metabolites in human plasma.
- Evaluation of the pharmacokinetic profile of meloxicam in human volunteers from Pakistan.
- Investigation of the pharmacokinetic drug-drug interaction of single dose of meloxicam with omeprazole and fluconazole, when administered concomitantly in healthy human volunteers.
- Study of pharmacokinetic drug-drug interaction of multiple dose of omeprazole and fluconazole with meloxicam.

3.1. Development of HPLC-UV Method for the Meloxicam, 5-Hydroxy Meloxicam and 5-Carboxy Meloxicam

A simple, rapid and cost-effective method was developed and validated for analysis of meloxicam and its major metabolites (5-hydroxy meloxicam and 5-carboxy meloxicam) in human plasma using reverse phase high performance liquid chromatography (RP-HPLC) coupled with UV detector. The samples were analyzed by injecting 20 µL into the HPLC system using Supelco C18 (150 mm × 4.6 mm, 5 µm) analytical column, protected by a Perkin Elmer C18 (30 mm × 4.6 mm, 10 µm) guard column. The mobile phase methanol: TFA (0.05% aqueous solution) in 60:40% v/v was pumped at flow rate of 1.3 mL/min at 28 °C and the eluents were monitored at 353 nm using piroxicam as internal standard. Meloxicam and the metabolites were extracted from plasma using dichloromethane and the percent recovery for meloxicam, 5-hydroxy meloxicam and 5-carboxy meloxicam was 98.8%, 97.3%, 97%, respectively. The retention time of meloxicam, 5-hydroxy meloxicam, 5-carboxy meloxicam and piroxicam were checked over at 6.16, 4.36, 2.72, 3.45 min, respectively. The method was linear over the concentration range of 10- 2000 ng/mL. The limits of detection of meloxicam, 5-hydroxy meloxicam and 5-carboxy meloxicam were 3 ng, 10 ng and 8 ng, whereas limit of quantification were 9 ng, 30 ng and 25 ng, respectively. All the peaks were resolved within 7 min. The method was
successfully applied for the pharmacokinetic studies in plasma samples of the healthy human Pakistani volunteers.

3.1.1. Sample Preparation

The standard stock solution of meloxicam, 5-hydroxy meloxicam and piroxicam were prepared in methanol, while of 5-carboxy meloxicam in dimethylformamide. Their consequent working standard solution was prepared in the mobile phase methanol: TFA (0.05% aqueous solution) as 60:40% v/v. Various solvents such as diethyl-ether, dichloromethane, acetonitrile and n-hexane alone or in different proportions, were tried for the extraction of drug from plasma of the volunteers. Best recovery was achieved with dichloromethane, when used at least five times to that of plasma volume, while other compounds were having poor recovery. Both liquid-liquid and solid-phase extraction methods were evaluated for the extraction of parent drug and its metabolites from the plasma samples of the healthy human volunteers, however, liquid-liquid extraction was found to have a slight better percent recovery. Meloxicam 15 mg orally was administered with full glass of water (≈ 250 mL) and blood samples (2 mL) were collected in the EDTA tubes, and then centrifuged at 3000 rpm for 10 min; plasma was transferred into Eppendorf tubes and stored at -20 °C till analysis. Plasma (200 µL) was mixed with internal standard (100 µL = 100 ng) in eppendorf tube, acetonitrile (600 µL) was added and vortexed for 3 min, to precipitate proteins present in the plasma. Then dichloromethane (1100 µL) extraction solvent was added, further vortexed for 5 min and centrifuged for 15 min at 9000 rpm, organic layer was collected and evaporated under the gentle stream of nitrogen. The residue was re-dissolved in mobile phase (2 mL) and injected into HPLC for analysis. The percent recoveries with various organic solvents and extraction methods are presented in Tab-3.1.

<table>
<thead>
<tr>
<th>Solvent for Extraction</th>
<th>Plasma Volume (µL)</th>
<th>Solvent Volume (µL)</th>
<th>% Recovery (Meloxicam)</th>
<th>% Recovery (5-OH Meloxicam)</th>
<th>% Recovery (5-CO Meloxicam)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl ether</td>
<td>200</td>
<td>1100</td>
<td>79.13</td>
<td>68.34</td>
<td>65.07</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>200</td>
<td>1100</td>
<td>88.46</td>
<td>83.66</td>
<td>81.28</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>200</td>
<td>1100</td>
<td>98.80</td>
<td>97.32</td>
<td>97</td>
</tr>
<tr>
<td>n-hexane</td>
<td>200</td>
<td>1100</td>
<td>90.51</td>
<td>89.43</td>
<td>86.66</td>
</tr>
</tbody>
</table>

3.1.2. HPLC/UV Method Chromatographic Conditions Optimizations

In an isocratic HPLC method different chromatographic conditions like mobile phase ratio (± 2%), flow rate (± 0.3 mL/min), wavelengths of detector (± 10%), column oven temperature (± 5 °C) and injection volumes were evaluated. To select optimum
chromatographic condition for the quantitative determination of meloxicam and its metabolites in plasma of healthy human Pakistani volunteer’s system suitability tests were carried out as presented in Tab-3.2.

3.1.2.1. HPLC Column Selection

Analysis of meloxicam and its metabolites has been executed with five columns included C8 and C18 having 150 mm and 250 mm length of different manufacturers. Thermo Quest C8 (150 mm x 4.6 mm, 5 µm; Runcorn, UK), Supelco C18 reverse phase HPLC column (150 mm x 4.6 mm, 5 µm), Perkin Elmer Brownlee® analytical C18 column (150 mm x 4.6 mm, 5 µm; Shelton, USA), Phenomenex Gemini® C18 column (150 mm x 4.6 mm, 5 µm; California, USA), Thermo Quest C18 (250 mm x 4.6 mm, 5 µm; Runcorn, UK). System suitability test is carried out, keeping other parameters constant for all the five columns. Among all the columns the best resolution and other chromatographic properties were obtained with supelco discovery analytical C18 column (150 mm x 4.6 mm, 5 µm). Thermo Quest C8 column had a poor retention factor and a shorter analysis run time, while C18 column had a poor peak asymmetry and larger analysis run time, i.e., more than 13 mins. In case of Perkin Elmer and Phenomenex C18 columns both shows good retention, separation, peak asymmetry and analysis run time but resolution and efficiency was not good in compare of Supelco C18 column. The representative chromatogram of the standard sample spiked with meloxicam, 5-hydroxy, 5-carboxy meloxicam and I.S on the supelco/discovery C18 column and on the other various columns is shown in Fig-3.1 and Fig-3.2, respectively. Retention factor plotted against various columns were shown in Fig-3.3.
Figure-3.1: The chromatogram of standard sample on supelco/discovery C18 column spiked with 100 ng/mL of meloxicam (peak -4), 75 ng/mL of each metabolite i.e 5-hydroxy meloxicam (peak-1); 5-carboxy meloxicam (peak-3) and 1000 ng/mL of internal standard (peak-2)

Figure-3.2: Chromatograms showing the effect of different columns on the analytes i.e. 5-hydroxy meloxicam (peaks A), internal standard (peaks B), 5-carboxy meloxicam (peaks C) and meloxicam (peaks D)

Chromatogram 1 = Thermo Quest Hypersil® BDS C8 (150 mm x 4.6 mm, 5 µm)
Chromatogram 2 = Perkin Elmer Brownlee® Analytical C18 (150 mm x 4.6 mm, 5 µm)
Chromatogram 3 = Supelco Reverse Phase C18 (150 mm x 4.6 mm, 5 µm)
Chromatogram 4 = Phenomenex Gemini® C18 (150 mm x 4.6 mm, 5 µm)
Chromatogram 5 = Thermo Quest Hypersil® BDS C18 (250 mm x 4.6 mm, 5 µm)
3.1.2.2. Mobile Phase

For the determination of meloxicam and its metabolites, different organic solvents such as acetonitrile, methanol, trifluoracetic-acid (TFA), phosphate buffer, pH adjusted distilled water alone or in different ratios were tried both in an isocratic and in gradient mode. Best resolution and separation of the analytes was seen with mobile phase methanol: TFA (0.05% aqueous solution) as 60:40% v/v used. The resolution was also good, when acetonitrile was used in place of methanol but produced a slight broader peak. The retention of the analytes changed with the water, pH adjusted water in the range of 2 - 4 and with phosphate buffer, and phosphate buffer may affect the column life, so TFA was selected in place of these solvents. Further increase in the ratio of methanol resulted in poor separation of the analytes, whereas increase in TFA ratio resulted in broader shape of the peaks of the analytes and longer analysis run time. The effect of different mobile phase compositions on the resolution of peaks is depicted in the Fig-3.4.

Figure-3.3: Effect of columns on the retention factors (k) of the analytes i.e., 5-hydroxy meloxicam (A); internal standard (B); 5-carboxy meloxicam (C) and meloxicam (D)
3.1.2.3. Flow Rate

The flow rate is an important factor which may significantly affect the sample elution, resolution, separation and peak shape. Different flow rates in the range of 0.8-1.5 mL/min were investigated for the analysis of the analytes. In this method 1.3 mL/min was the one that gave best resolution as optimum flow rate. Below this analyte peak shape was affected and above this resulted in high column pressure. The effect of different flow rates on the retention times of the analytes is shown in Fig-3.5.
3.1.2.5. Column Oven Temperature

The effects associated with column oven temperature on the evaluation of meloxicam and its metabolites had been additionally examined inside range of 25 – 40 °C. The increase and decrease in temperature resulted had no effect on the resolution, peak asymmetry and separation of the analytes as shown in Tab-3.2. Retention factor plotted against various temperatures were shown in Fig-3.6.

![Graph showing retention factor vs temperature](image-url)
Figure-3.6: Effect of temperature on the retention factors \(k\) of the analytes i.e., 5-hydroxy meloxicam (A); internal standard (B); 5-carboxy meloxicam (C) and meloxicam (D)
### Table 3.2: System Suitability Tests of Various Experimental Parameters

<table>
<thead>
<tr>
<th>Experimental Parameters</th>
<th>Retention Factor ($k$)</th>
<th>Separation Factor ($\alpha$)</th>
<th>Peak Asymmetry ($A_s$)</th>
<th>Resolution ($Rs$)</th>
<th>Efficiency ($N$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column oven temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 °C</td>
<td>1.44</td>
<td>2.25</td>
<td>2.98</td>
<td>4.57</td>
<td>1.64</td>
</tr>
<tr>
<td>28 °C</td>
<td>1.45</td>
<td>2.27</td>
<td>3.09</td>
<td>4.81</td>
<td>1.60</td>
</tr>
<tr>
<td>32 °C</td>
<td>1.43</td>
<td>2.22</td>
<td>3.02</td>
<td>4.34</td>
<td>1.63</td>
</tr>
<tr>
<td>35 °C</td>
<td>1.45</td>
<td>2.26</td>
<td>2.88</td>
<td>4.76</td>
<td>1.53</td>
</tr>
<tr>
<td>40 °C</td>
<td>1.44</td>
<td>2.24</td>
<td>3.17</td>
<td>4.64</td>
<td>1.54</td>
</tr>
<tr>
<td>Stationary phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermo Quest C8 (150 mm x 4.6 mm, 5 µm)</td>
<td>1.18</td>
<td>1.90</td>
<td>2.54</td>
<td>4.09</td>
<td>1.30</td>
</tr>
<tr>
<td>Perkin Elmer C18 (150 mm x 4.6 mm, 5 µm)</td>
<td>1.36</td>
<td>2.00</td>
<td>2.72</td>
<td>4.18</td>
<td>1.50</td>
</tr>
<tr>
<td>Supelco C18 (150 mm x 4.6 mm, 5 µm)</td>
<td>1.45</td>
<td>2.27</td>
<td>3.09</td>
<td>4.81</td>
<td>1.60</td>
</tr>
<tr>
<td>Phenomenex C18 (150 mm x 4.6 mm, 5 µm)</td>
<td>1.50</td>
<td>2.36</td>
<td>3.13</td>
<td>4.90</td>
<td>1.65</td>
</tr>
<tr>
<td>Thermo Quest C18 (250 mm x 4.6 mm, 5 µm)</td>
<td>3.09</td>
<td>4.18</td>
<td>6.63</td>
<td>10.54</td>
<td>3.40</td>
</tr>
</tbody>
</table>
3.1.2.6. Selection of the Internal Standard

Various compounds were assessed as internal standard that include naproxen sodium, diclofenac sodium, lumefantrine and piroxicam. Among these piroxicam showed good resolution, sensitivity, specificity, recovery, stability, and while other naproxen has long retention time and diclofenac and lumefantrine show poor resolution and poor recovery, respectively.

3.1.2.7. Injection Volume

Different injection volumes in the ranges of 10 µL - 50 µL were assessed using Rheodyne manual injector. Among them 20 µL was selected as optimum injection volume, above this volume resulted poor and broader peak shape of the analytes.

3.1.3. Method Validation

Validation of the developed method was carried out per ICH guidelines with special emphasis on specificity, linearity, precision, accuracy, robustness, selectivity, limit of detection (LOD), and limit of quantification (LOQ).

3.1.3.1. Linearity

The calibration curves of meloxicam (10 - 2000 ng/mL), 5-hydroxy meloxicam (30 - 1000 ng/mL) and 5-carboxy meloxicam (25 - 1000 ng/mL) in spiked biological samples demonstrated excellent linearity ($r^2 = 0.999$) results are depicted in Tab-3.3 and overlay of the chromatograms of the spiked plasma samples signifying linearity is shown in Fig-3.7.
3.1.3.2. Selectivity

The selectivity of the method for the meloxicam and its metabolites was determined from a) mobile phase, b) blank plasma c) plasma sample spiked with meloxicam, 5-hydroxy meloxicam, 5-carboxy meloxicam and piroxicam as I.S, d) plasma sample spiked with I.S. The Fig-3.8 confirms the good resolution of the peaks and no other exogenous or endogenous peaks interfere with the chromatograms.
Figure 3.8: RP-HPLC chromatograms of different samples, Peak-1: 5-hydroxy meloxicam, 2; internal standard, 3; 5-carboxy meloxicam, 4; meloxicam. Chromatograms a: blank solvent, b: blank plasma, c: plasma sample spiked with 1000 ng/mL of meloxicam, 500 ng/mL of 5-hydroxy meloxicam, 500 ng/mL of 5-carboxy meloxicam and 1000 ng/mL of internal standard, d: plasma sample
3.1.3.3. Precision

The precision results include injection repeatability in terms of area and retention time of the peak by injecting sample 10 times, and that of analysis repeatability by separately preparing, evaluating 6 samples of meloxicam and its metabolites and the quantity recovered was calculated. Intermediate precision (inter-day, intra-day studies) are investigated in terms of recovery. The precision results are summarized in Tab-3.3 and 3.4. The method demonstrated good precision as the % RSD value for both inter-day and intra-day was less than 2%. 
Table-3.3: Accuracy, Calibration, Linearity, Repeatability and Sensitivity of the Current RP-HPLC Method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plasma</th>
<th>Meloxicam</th>
<th>5-Hydroxy Meloxicam</th>
<th>5-Carboxy Meloxicam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy (% recovery)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spiked concentration level 1&lt;sup&gt;a&lt;/sup&gt;(mean ± SD)</td>
<td>99.12 ± 0.2193</td>
<td>97.24 ± 0.3117</td>
<td>95.54 ± 0.2984</td>
<td></td>
</tr>
<tr>
<td>Spiked concentration level 2&lt;sup&gt;a&lt;/sup&gt;(mean ± SD)</td>
<td>98.49 ± 0.3735</td>
<td>97.91 ± 0.5663</td>
<td>97.62 ± 0.4716</td>
<td></td>
</tr>
<tr>
<td>Spiked concentration level 3&lt;sup&gt;a&lt;/sup&gt;(mean ± SD)</td>
<td>98.83 ± 0.5291</td>
<td>96.75 ± 0.7588</td>
<td>98.11 ± 0.8839</td>
<td></td>
</tr>
<tr>
<td>Accuracy (% RSD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spiked concentration level 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.70</td>
<td>0.59</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Spiked concentration level 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.54</td>
<td>0.41</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Spiked concentration level 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38</td>
<td>0.29</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Calibration range (ng. mL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>10.0-2000.0</td>
<td>30.0-1000.0</td>
<td>25.0-1000.0</td>
<td></td>
</tr>
<tr>
<td>Linearity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spiked samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.002</td>
<td>0.0006</td>
<td>0.0012</td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0102</td>
<td>0.0031</td>
<td>0.0015</td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>0.9997</td>
<td>0.9991</td>
<td></td>
</tr>
<tr>
<td>Repeatability</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection repeatability (mean; %RSD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spiked concentration level 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.616; 0.53</td>
<td>2.72; 0.33</td>
<td>4.36; 0.24</td>
<td></td>
</tr>
<tr>
<td>Analysis repeatability (mean; %RSD)</td>
<td>162652; 1.25</td>
<td>49475; 0.87</td>
<td>79263; 0.93</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limit of detection, LLOD (ng.mL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3.0</td>
<td>10.0</td>
<td>8.0</td>
<td></td>
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<td>Lower limit of quantification, LLOQ (ng.mL&lt;sup&gt;-1&lt;/sup&gt;)</td>
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Spiked concentration level 1= meloxicam: 50.0 ng.mL<sup>-1</sup>, 5-hydroxy meloxicam: 50.0 ng.mL<sup>-1</sup> and 5-carboxy meloxicam: 50.0 ng.mL<sup>-1</sup>; Spiked concentration level 2 = meloxicam: 100.0 ng.mL<sup>-1</sup>, 5-hydroxy meloxicam: 100.0 ng.mL<sup>-1</sup> and 5-carboxy meloxicam: 100.0 ng.mL<sup>-1</sup>; Spiked concentration level 3 = meloxicam: 1000.0 ng.mL<sup>-1</sup>, 5-hydroxy meloxicam: 1000.0 ng.mL<sup>-1</sup> and 5-carboxy meloxicam: 1000.0 ng.mL<sup>-1</sup>. <sup>a</sup>n = 5, <sup>b</sup>n = 10, <sup>c</sup>Retention time (min), <sup>d</sup>Peak area, <sup>e</sup>Amount recovered
Table-3.4: The Data of the Intra-day and Inter-days Precision (n = 3)

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<th>Known concentration spiked (ng.mL⁻¹)</th>
<th>Plasma (Concentration found) (ng.mL⁻¹)</th>
<th>Intra-day (mean ± S.D)</th>
<th>% RSD</th>
<th>Inter-day (mean ± S.D)</th>
<th>% RSD</th>
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<td>Meloxicam</td>
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<td>49.552 ± 0.2607</td>
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<tr>
<td>5-Hydroxy meloxicam</td>
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<td>48.698 ± 0.2152</td>
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<td>5-Carboxy meloxicam</td>
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3.1.3.4 Accuracy

The developed method accuracy was assessed from the percent recovery, the mean percent recovery of meloxicam, 5-hydroxy meloxicam, 5-carboxy meloxicam were 98.8%, 97.3%, 97% in plasma by using liquid-liquid extraction and 92%, 84%, 77.8% by using solid-phase extraction showing %RSD value less than 1% represents that the developed method was accurate as shown in Tab-3.3. Percent recovery of all the analytes was higher with liquid-liquid extraction than SPE.

3.1.3.5 Sensitivity

The LOD for meloxicam, 5-hydroxy meloxicam and 5-carboxy meloxicam were 3 ng, 10 ng and 8 ng, respectively. The LOQ were 9 ng, 30 ng and 25 ng, respectively. Chromatograms of LLODs and LLOQs are shown in Fig-3.9 and 3.10.
**Figure-3.9:** RP-HPLC chromatogram showing peaks of 5-hydroxy meloxicam (peak-1), 5-carboxy meloxicam (peak-3) and meloxicam (peak-4) at the lower limit of detection

**Figure-3.10:** RP-HPLC chromatogram showing peaks of 5-hydroxy meloxicam (peak-1), 5-carboxy meloxicam (peak-3) and meloxicam (peak-4) at the lower limit of quantification
3.1.3.6 Robustness

The robustness of the developed method was verified by doing minor changes in chromatographic conditions such as composition of the mobile phase (± 0.3%), flow rate of the mobile phase (± 0.2 mL/min), wavelength (± 3 nm), did not substantially affect peak shape and the retention time of the sample.

3.1.3.7 Stability

The spiked plasma samples were stable for I week under all the conditions; room temperature (25 °C), refrigerated (2 – 8 °C) or frozen at (-80 °C), while the standard solutions were stable for about a month when stored at 2 – 8 °C.

3.1.4. Conclusion

Several methodical approaches have been reported for the analysis of meloxicam in the plasma samples; these techniques include HPLC and LCMS. Reported methods applied advanced detectors like diode array detector and mass spectrometry (Velpandian et al., 2000; Ji et al., 2005; Medvedovici et al., 2005), which are not readily and easily available. Moreover, mobile phase composition used in all the methods is complex, consisting of buffers which can affect the column life and processing time (Velpandian et al., 2000; Nemutlu & Kir, 2003; Rani et al., 2004). Extraction of meloxicam from biological samples is a tedious job and most of the methods have used large volumes of plasma without any proper extraction procedure (Dasandi et al., 2002; Medvedovici et al., 2005). Not a single method of HPLC for the simultaneous analysis of meloxicam and its metabolites (5-hydroxy meloxicam and 5-carboxy meloxicam) has been reported in plasma. There is a need to develop a validated, sensitive, easy and rapid method for the estimation of meloxicam and its metabolites in biological samples, particularly for the pharmacokinetic studies. In the present method, suitable extraction procedure has been developed for extraction of meloxicam and its metabolites from biological samples. The developed method is a novel one with respect to mobile phase composition and shorter analysis. The method was simple, rapid, easy, accurate, precise and economical. Various chromatographic conditions were optimized for the analysis of these analytes according to standard guidelines. The effects of composition of the mobile phase, flow rate, temperature of the column oven, detection wavelength were studied. In this method meloxicam and its metabolites recovery was excellent from biological sample by using two step LLE procedures. All the peaks appear within 7 min in a single chromatographic run. Also, different system suitability tests were performed, calculated and designed against experimental parameters. This developed method can also be employed in various pharmaceutical dosage forms, in biological matrices, for routine laboratory analysis and for evaluating the pharmacokinetics and drug-drug interaction studies of meloxicam and its metabolites with selected other commonly co-prescribed drugs.
3.2. Pharmacokinetics of Meloxicam and its Metabolites 5-Hydroxy Meloxicam and 5-Carboxy Meloxicam

Meloxicam undergoes extensive hepatic Phase-I biotransformation and no conjugated derivatives have been detected. CYP2C9 is mainly responsible for its metabolism into four pharmacologically inactive metabolites. The 5-carboxymeloxicam (60% of dose), a middle metabolite 5-hydroxymeloxicam (9% of dose) were recognized as major metabolites. These metabolites are not detectable in plasma because they are highly polar (Engelhardt 1996; Busch et al., 1998), so for this reason the PK of meloxicam metabolites in plasma has not been discussed by the researchers, only ADME of parent drug in plasma has been discussed.

3.2.1. Selection of Volunteer’s

Healthy male human volunteers (n = 24) aged between 20-23 years having mean ± SD age 21.58 ± 1.05, height 64.25 ± 2.23, weight 61.20 ± 6.15 and BMI, 26.03 ± 1.92. All the volunteers were non-smokers, non-obese, having no systemic abnormality, no recent past medication history of hypertension, diabetes, heart disease, cancer or any other disease and have signed the informed consent form. They were examined for various physical and biochemical tests like renal function tests (RFTs), liver function tests (LFTs), blood screening, blood pressure, low density lipoproteins (LDL), high density lipoproteins (HDL) and electrocardiography (ECG) were performed to investigate whether they were not suffering from any disease or any abuse/addictive substances. The detail results of biochemical tests and clinical investigations of all the 24 volunteers participated in these studies are presented in Tab-3.5 and their demographic profile in Tab-3.6.
### Table-3.5: Biochemical Tests Results of the Volunteers Enrolled in the Studies

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83
### RESULTS AND DISCUSSIONS

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Table-3.6: Demographic Profile of the Volunteers Enrolled in the Studies

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<td>60-68</td>
<td>50-70</td>
<td>21.5-29.5</td>
</tr>
</tbody>
</table>

3.2.2. Results

The plasma concentration of meloxicam was plotted on a linear and semi log graph scale as function of time. The data and plot revealed that meloxicam follows one compartment model. The plasma concentration of meloxicam at various time intervals
on a linear and semi log graph paper was shown in Fig-3.11. Various pharmacokinetic parameters like $t_{1/2}$, $AUC$ $AUMC$, $Vd$, $Cl$, $MRT$ and $K_{10}$ were assessed using PK-Summit® software and Microsoft Excel®, while some parameters like $C_{max}$ and $T_{max}$ obtained directly from the plot/data. The PK parameters of meloxicam were calculated using both non-compartmental and one compartmental approach. The pharmacokinetic data of all the 24 volunteers is shown in Tab-3.7 and 3.8.

Figure-3.11: Mean ± SD Plasma Drug Concentration Vs Time of Healthy Human Volunteers (n = 24) following oral administration of meloxicam tablet (15 mg), A: Normal plot and B: Semi-log scale

Table-3.7: PK Parameters of Meloxicam Following Oral Administration of 15 mg Tablet by Using Non-compartmental Pharmacokinetic Model

<table>
<thead>
<tr>
<th>S/No</th>
<th>Pharmacokinetic parameters</th>
<th>Meloxicam alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± S.D</td>
</tr>
<tr>
<td>1</td>
<td>$C_{max}$ (ng/mL)</td>
<td>1235.110 ± 241.553</td>
</tr>
<tr>
<td>2</td>
<td>$T_{max}$ (hr)</td>
<td>5.833 ± 0.577</td>
</tr>
<tr>
<td>3</td>
<td>$AUC^{0-t}$ (ng.hr/mL)</td>
<td>29237.064 ± 4615.218</td>
</tr>
<tr>
<td>4</td>
<td>$AUC^{0-\infty}$ (ng.hr/mL)</td>
<td>30795.001 ± 4675.532</td>
</tr>
<tr>
<td>5</td>
<td>$AUMC$ (ng.hr*hr/mL)</td>
<td>681925.970 ± 137052.451</td>
</tr>
<tr>
<td>6</td>
<td>$t_{1/2-A}$ (hr)</td>
<td>2.862 ± 1.016</td>
</tr>
<tr>
<td>7</td>
<td>$t_{1/2-D}$ (hr)</td>
<td>9.583 ± 2.779</td>
</tr>
<tr>
<td>8</td>
<td>$t_{1/2-E}$ (hr)</td>
<td>13.547 ± 1.361</td>
</tr>
<tr>
<td>9</td>
<td>$Vd$ (L)</td>
<td>8.924 ± 1.969</td>
</tr>
</tbody>
</table>
Table 3.8: PK Parameters of Meloxicam Following Oral Administration of 15 mg Tablet by Using One-compartmental Pharmacokinetic Model

<table>
<thead>
<tr>
<th>S/No</th>
<th>Pharmacokinetic parameters</th>
<th>Meloxicam alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± S.D</td>
</tr>
<tr>
<td>1</td>
<td>(C_{\text{max}}) (ng/mL)</td>
<td>1235.11 ± 241.553</td>
</tr>
<tr>
<td>2</td>
<td>(T_{\text{max}}) (hr)</td>
<td>5.83 ± 0.577</td>
</tr>
<tr>
<td>3</td>
<td>(AUC_{(0-\infty)}) (ng.hr/mL)</td>
<td>31035.964 ± 4840.760</td>
</tr>
<tr>
<td>4</td>
<td>(\text{AUMC} ) (ng.hr*hr/mL)</td>
<td>687497.508 ± 131878.793</td>
</tr>
<tr>
<td>5</td>
<td>(MRT) (hr)</td>
<td>25.143 ± 4.345</td>
</tr>
<tr>
<td>6</td>
<td>(V_d) (L)</td>
<td>10.736 ± 2.397</td>
</tr>
<tr>
<td>7</td>
<td>(Cl) (mL/min)</td>
<td>7.501 ± 1.032</td>
</tr>
<tr>
<td>8</td>
<td>(K_{10}) (L/hr)</td>
<td>0.051 ± 0.006</td>
</tr>
</tbody>
</table>

3.2.2.1. Maximum Plasma Concentration \(C_{\text{max}}\)

The peak plasma concentration of meloxicam in the current study was 1235.11 ± 241.553 ng/mL (mean ± SD). While in some reported studies it was 1445.7 ng/mL (Bae et al., 2007), 910 ng/mL, 1376 ng/mL (Rani et al., 2004), 1549 ng/mL (Tan et al., 2000) and 683 ng/mL (Elbary et al., 2001).
3.2.2.2. Time of Peak Plasma Concentration ($T_{\text{max}}$)

The time taken by the meloxicam to reach $C_{\text{max}}$ (mean ± SD) was $5.833 \pm 0.577$ hr, whereas in other reported studies it was 4.1 hr (Bae et al., 2007), 7 hr, 2.91 hr (Rani et al., 2004), 2.5 hr (Elbary et al., 2001) and 5.6 hr (Tan et al., 2000).

3.2.2.3. Area Under Curve [Bioavailability] ($AUC$)

Area under the plasma drug concentration was determined from the trapezoidal method by calculating $[\text{AUC}]_0^{T_{\text{max}}}$ and $[\text{AUC}]_0^{\infty}$. In the current study (mean ± SD) of meloxicam $[\text{AUC}]_0^{T_{\text{max}}}$ and $[\text{AUC}]_0^{\infty}$ were $29.237 \pm 4.615 \mu g/hr/mL$ and $30.795 \pm 4.675 \mu g/hr/mL$, respectively, as plasma concentration was assessed up to 72 hours. Though in previous studies $[\text{AUC}]_0^{T_{\text{max}}}$ values were $37.5 \mu g/hr/mL$ (Bae et al., 2007) and $44 \mu g/hr/mL$ (Xu et al., 2001), while $[\text{AUC}]_0^{\infty}$ were $42.4 \mu g/hr/mL$ (Bae et al., 2007), $35.09 \mu g/hr/mL$, $35.7 \mu g/hr/mL$ (Rani et al., 2004), $4.18 \mu g/hr/mL$ (Elbary et al., 2001) and $53.5 \mu g/hr/mL$ (Tan et al., 2000).

3.2.2.4. Area Under Moment of Plasma Concentration ($AUMC$)

The AUMC value of meloxicam (mean ± SD) was calculated as $681925.970 \pm 137052.451 \text{ng.hr}^4\text{hr/mL}$.

3.2.2.5. Mean Residence Time ($MRT$)

The mean ± SD of MRT for meloxicam was determined as $22.633 \pm 2.19$ hr, whereas in previous studies it was $31.3$ hr (Tan et al., 2000) and $35$ hr (Xu et al., 2001).

3.2.2.6. Volume of Distribution ($Vd$)

The value in the present study for the volume of distribution of meloxicam (mean ± SD) was calculated as $8.924 \pm 1.969 \text{L}$, while in reported studies it was $13.5 \text{L}$, $19.4 \text{L}$ (Rani et al., 2004) and $9.3 \text{L}$ (Xu et al., 2001).

3.2.2.7. Clearance ($Cl$)

Total clearance of meloxicam by all routes was calculated as $7.315 \pm 1.064 \text{mL/min}$ (mean ± SD). However, in other studies it was $7.66 \text{mL/min}$, $7.33 \text{mL/min}$ (Rani et al., 2004) and $5.39 \text{mL/min}$ (Xu et al., 2001).
3.2.2.8. Absorption Half-Life ($t_{1/2} - A$)

The absorption half-life value (mean ± SD) of meloxicam determined was 2.862 ± 1.016 hr.

3.2.2.9. Distribution Half-Life ($t_{1/2} - D$)

The value of distribution half-life for meloxicam was 9.583 ± 2.779 hr (mean ± SD).

3.2.2.10. Elimination Half-Life ($t_{1/2} - E$)

The mean ± SD value for $t_{1/2} - E$ of meloxicam calculated was 13.547 ± 1.361 hr in the present study, though in previous reported studies it was 21.5 hr, 30.5 hr (Rani et al., 2004), 2.1 hr (Elbary et al., 2001).

3.2.2.11. Rate Constant $K_{10}$

The $K_{10}$ i.e., elimination rate constant for meloxicam from central compartment (mean ± SD) was calculated as 0.051 ± 0.006 L/hr.
Table-3.9: The Pharmacokinetic Parameters of Meloxicam 15 mg Observed in this Study and Some of the Reported Studies

<table>
<thead>
<tr>
<th>S/No</th>
<th>Pharmacokinetic Parameters</th>
<th>Meloxicam 15 mg (Present study)</th>
<th>Meloxicam 15 mg (Reported Studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>1235.110 ± 241.553</td>
<td>1445.7 (Bae et al., 2007), 910, 1376 (Rani et al., 2004), 683 (Elbary et al., 2001), 1051 (Ahmed et al., 2011), 1549 (Tan et al., 2000)</td>
</tr>
<tr>
<td>2</td>
<td>$T_{\text{max}}$ (hr)</td>
<td>5.833 ± 0.577</td>
<td>4.1 (Bae et al., 2007), 7, 2.91 (Rani et al., 2004), 2.5 (Elbary et al., 2001), 3.12 (Ahmed et al., 2011), 5.6 (Tan et al., 2000)</td>
</tr>
<tr>
<td>3</td>
<td>$AUC^{(0-t)}$ (µg.hr/mL)</td>
<td>29.237 ± 4.615</td>
<td>37.5 (Bae et al., 2007), 28.38 (Ahmed et al., 2011), 44 (Xu et al., 2001)</td>
</tr>
<tr>
<td>4</td>
<td>$AUC^{(0-\infty)}$ (µg.hr/mL)</td>
<td>30.795 ± 4.675</td>
<td>42.4 (Bae et al., 2007), 35.09, 35.7 (Rani et al., 2004), 4.18 (Elbary et al., 2001), 28.6 (Ahmed et al., 2011), 53.5 (Tan et al., 2000)</td>
</tr>
<tr>
<td>5</td>
<td>$AUMC$ (ng.hr*hr/mL)</td>
<td>681925.97 ± 137052.45</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>$t_{1/2-A}$ (hr)</td>
<td>2.862 ± 1.016</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$t_{1/2-D}$ (hr)</td>
<td>9.583 ± 2.779</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>$t_{1/2-E}$ (hr)</td>
<td>13.547 ± 1.361</td>
<td>18.29 (Gschwend et al., 2007), 21.5, 30.5 (Rani et al., 2004), 2.1 (Elbary et al., 2001), 13.70 (Ahmed et al., 2011)</td>
</tr>
<tr>
<td>9</td>
<td>$Vd$ (L)</td>
<td>8.924 ± 1.969</td>
<td>13.5, 19.4 (Rani et al., 2004), 9.3 (Xu et al., 2001)</td>
</tr>
<tr>
<td>10</td>
<td>$Cl$ (mL/min)</td>
<td>7.315 ± 1.064</td>
<td>7.66, 7.33 (Rani et al., 2004), 9.36 (Turck et al., 1997), 5.39 (Xu et al., 2001)</td>
</tr>
<tr>
<td>11</td>
<td>$MRT$ (hr)</td>
<td>22.633 ± 2.190</td>
<td>31.3 (Tan et al., 2000), 35 (Xu et al., 2001)</td>
</tr>
<tr>
<td>12</td>
<td>$K_{10}$ (L/hr)</td>
<td>0.051 ± 0.006</td>
<td>0.034 hr$^{-1}$ (Xu et al., 2001)</td>
</tr>
</tbody>
</table>

3.2.3. Discussion

The literature revealed that little work has been carried out to investigate the PK studies of meloxicam in local population. Therefore, in first phase of the study the pharmacokinetics of the meloxicam was studied in local healthy human volunteers. Meloxicam is completely metabolized into inactive metabolites i.e., 5-hydroxymethylmeloxicam (9% of dose) an intermediate metabolite, which is further
metabolize to 5-carboxymeloxicam (60% of dose) the major metabolite. CYP2C9 isozyme is the major contributor, while CYP3A4 play a minor role in this metabolization. Meloxicam equally undergoes renal and fecal elimination predominantly in the form of metabolites and only 0.25% excreted unchanged through urine and 1.6% in feces. The extensive metabolism of meloxicam makes it a potential candidate for the enzyme based metabolism drug-drug interaction studies. The metabolites of meloxicam are pharmacologically inactive and are not detectable in plasma (Engelhardt 1996). This is the reason in this study; the interaction of meloxicam was based on the determination of the parent drug only.

High inter-population and intra-populations variations have been observed in the meloxicam pharmacokinetic parameters (Bae et al., 2011; Zhang et al., 2014), which may be due to the differences in the genotypic variation in CYP2C9. Based on phenotyping, races containing CYP2C9 phenotype are considered as poor metabolizers of drugs like meloxicam etc. (Zhou et al., 2009; Ahmed et al., 2016). Different compartmental models have been reported in the literature for the evaluation of the pharmacokinetics of meloxicam. In some literature it reveals that meloxicam follows two-compartment model (Khawaja et al., 2011; Coetzee et al., 2012), while others proposed to follow one-compartment model (Del Tacca et al., 2002; Meineke & Turck, 2003; Gopu et al., 2013). However, in our study a single dose of meloxicam 15 mg administered to healthy volunteers follows one compartment model. The pharmacokinetics parameters of meloxicam depicted in Tab-3.7 and 3.8.

The pharmacokinetic parameters of meloxicam in the present study i.e., $C_{max}$, $T_{max}$, $[AUC]_0^\infty$, $MRT$, $Vd$, $Cl$ and $t_{1/2}$-E, all these values were in close resemblance to other reported studies of meloxicam as presented in Tab-3.9. The difference in the PK parameters may be due to the inter and intra population enzyme diversity and interethnic variability that plays a role in the meloxicam disposition and differences in the PK parameters. As it is primarily metabolized by CYP2C9 (major) and CYP3A4 (minor). CYP2C9 is polymorphic and is involved in the oxidation of meloxicam. Thirty-four allelic variants of the CYP2C9 gene show differences in enzyme expression and activity, leading to variation in pharmacokinetics parameters. The 13 allele of CYP2C9 have been reported in Chinese, Japanese and Koreans and are not identified in other populations. There frequency was higher (1.02%) in Chinese than Koreans population (0.54%), therefore they show decrease enzymatic activity towards CYP2C9 substrate i.e., meloxicam (Bae et al., 2011), so their $C_{max}$, $T_{max}$, $[AUC]_0^\infty$, $[AUC]_0^{72}$, $MRT$, $Vd$, $Cl$ will be high, while their $Vd$ and $CI$ will be low (Tan et al., 2000; Xu et al., 2001) from other reported studies. Similarly, Koreans have the second highest $C_{max}$, $[AUC]_0^{72}$ and $[AUC]_0^{\infty}$ (Bae et al., 2007). In India and Pakistan CYP2C9*2 and CYP2C9*3 allelic form is common and CYP2C9*3 occur at a very high (0.080%) frequency than Germans (0.06%) and Egyptians (0.06%) (Dorado et al., 2011), subsequently they have high $C_{max}$, $[AUC]_0^{\infty}$, $t_{1/2}$, $Vd$ and low $Cl$ values than Germans (Rani et al., 2004) and Egyptians (Elbary et al., 2001). Germans have somewhat high PK parameters than Egyptians although they have similar CYP2C9*3 allelic frequency, the reason behind this is CYP2C9*2 allele, whose frequency found to be higher in Germans (0.140%) than Egyptians (0.120%) and secondly their average bodyweight i.e., 86 Kg was more than Egyptians in the reported study (Booven et al., 2010).
3.3. Pharmacokinetic Interaction Studies of Meloxicam

PK-DDI of meloxicam with omeprazole and fluconazole was investigated in healthy human Pakistani volunteers. In first phase PK-DDI of meloxicam, after a single dose concomitant administration with omeprazole in healthy human volunteers, was investigated.

In the second phase the impact of multiple dose administration (single dose for 3 days) of omeprazole on the pharmacokinetic of single dose of meloxicam on last day was evaluated.

In the third phase, pharmacokinetic interaction of meloxicam was studied with single dose of fluconazole and in the last phase the impact of multiple dose administration (single dose for 3 days) of fluconazole on the pharmacokinetic of meloxicam single dose was investigated.

3.4. PK-DDI of Meloxicam with Omeprazole Single Dose

The plasma meloxicam concentration as a function of time was plotted both on normal and semi-log graph scales (Fig-3.12). The PK parameters using compartmental and non-compartmental models determined by means of Microsoft Excel-2007 and PK Summit® software as depicted in Tab-3.10.

Figure-3.12: Mean ± SD Plasma Drug Concentration Vs Time of Healthy Human Volunteers (n = 24) following oral administration of meloxicam tablet (15 mg) alone and concurrently with single dose omeprazole capsule (40 mg) A: Normal plot and B: Semi-log scale
Table-3.10: The Pharmacokinetic Parameters of Meloxicam Tablet (15 mg) Alone and with Omeprazole Capsule (40 mg)

<table>
<thead>
<tr>
<th>S/No</th>
<th>Pharmacokinetic parameters</th>
<th>Mel alone Mean ± S.D</th>
<th>Mel plus Ome single Mean ± S.D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$C_{max}$ (ng/mL)</td>
<td>1235.11 ± 241.553</td>
<td>1281.85 ± 124.38</td>
<td>0.557</td>
</tr>
<tr>
<td>2</td>
<td>$T_{max}$ (hr)</td>
<td>5.833 ± 0.577</td>
<td>6.166 ± 0.577</td>
<td>0.171</td>
</tr>
<tr>
<td>3</td>
<td>$AUC^{(0-t)}$ (ng.hr/mL)</td>
<td>29237 ± 4615.21</td>
<td>32689.67 ± 4648.48</td>
<td>0.082</td>
</tr>
<tr>
<td>4</td>
<td>$AUC^{(0-∞)}$ (ng.hr/mL)</td>
<td>30795 ± 4675.53</td>
<td>34049.84 ± 4331.91</td>
<td>0.090</td>
</tr>
<tr>
<td>5</td>
<td>$AUMC$ (ng.hr*hr/mL)</td>
<td>681925.97 ± 137052</td>
<td>851250.30 ± 109867.92</td>
<td>0.003</td>
</tr>
<tr>
<td>6</td>
<td>$t_{1/2-A}$ (hr)</td>
<td>2.862 ± 1.016</td>
<td>3.365 ± 0.467</td>
<td>0.134</td>
</tr>
<tr>
<td>7</td>
<td>$t_{1/2-D}$ (hr)</td>
<td>9.583 ± 2.779</td>
<td>4.903 ± 1.488</td>
<td>0.000</td>
</tr>
<tr>
<td>8</td>
<td>$t_{1/2-E}$ (hr)</td>
<td>13.547 ± 1.361</td>
<td>14.554 ± 1.129</td>
<td>0.061</td>
</tr>
<tr>
<td>9</td>
<td>$Vd$ (L)</td>
<td>8.924 ± 1.969</td>
<td>8.542 ± 1.449</td>
<td>0.594</td>
</tr>
<tr>
<td>10</td>
<td>$Cl$ (mL/min)</td>
<td>7.315 ± 1.064</td>
<td>6.530 ± 1.295</td>
<td>0.115</td>
</tr>
<tr>
<td>11</td>
<td>$MRT$ (hr)</td>
<td>22.633 ± 2.190</td>
<td>24.607 ± 1.808</td>
<td>0.025</td>
</tr>
<tr>
<td>12</td>
<td>$K_{10}$ (L/hr)</td>
<td>0.051 ± 0.006</td>
<td>0.052 ± 0.016</td>
<td>0.816</td>
</tr>
</tbody>
</table>

3.4.1. Results of PK-DDI between Meloxicam and Omeprazole Single Dose Study

3.4.1.1. Maximum Plasma Concentration ($C_{max}$)

A non-significant increase ($p > 0.557$) in the value of meloxicam plus omeprazole (mean ± SD) i.e., $1281.85 ± 124.38$ ng/mL has been studied as compared to meloxicam alone i.e., $1235.11 ± 241.553$ ng/mL. Graphical results were represented in Fig-3.13.
3.4.1.2. Time of Peak Plasma Concentration ($T_{\text{max}}$)

$T_{\text{max}}$ of meloxicam (mean ± SD) alone was 5.833 ± 0.577 hr, which non-significantly increases ($p > 0.171$) when meloxicam was given concurrently with omeprazole i.e., 6.166 ± 0.577.

3.4.1.3. Area Under Curve [Bioavailability] ($AUC$)

In a single dose PK-DDI study of meloxicam with omeprazole, the $[AUC]_{0}^{\infty}$ (mean ± SD) value 32689.67 ± 4648.48 ng.hr/mL and $[AUC]_{0}^{\infty}$ (mean ± SD) 34049.84 ± 4331.91 ng.hr/mL was non-significantly increased ($p > 0.082$ and $p > 0.090$, respectively) when compared to meloxicam alone $[AUC]_{0}^{\infty}$ and $[AUC]_{0}^{\infty}$ i.e., 29237 ± 4615.21 ng.hr/mL and 30795 ± 4675.53 ng.hr/mL, respectively. Results are graphically depicted in Fig-3.14 and 3.15.

Figure-3.13: Graphical representation of the $C_{\text{max}}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers (n = 24)
Figure-3.14: Graphical representation of the $\text{[AUC]}_0^{\text{24}}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers ($n = 24$)
Figure-3.15: Graphical representation of the $[\text{AUC}]_0^{\infty}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers ($n = 24$)

3.4.1.4. Area Under Moment of Plasma Concentration ($AUMC$)

The $AUMC$ (mean ± SD) value of meloxicam alone calculated was $681925.970 \pm 137052.451$ ng.hr*hr/mL, in combination with omeprazole was $851250.30 \pm 109867.92$ ng.hr*hr/mL. This shows a significant increase ($p < 0.003$) after the administration of meloxicam concomitantly with omeprazole, as shown in Fig-3.16.
3.4.1.5. Mean Residence Time (MRT)

A significance decrease ($p < 0.025$) in the value of $MRT$ (mean ± SD) of meloxicam alone $i.e.$, $22.633 ± 2.190$ hr has been observed as compared to meloxicam along with omeprazole $i.e.$, $24.607 ± 1.808$ hr, as described in Fig.-3.17.
Figure-3.17: Graphical representation of the MRT values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers (n = 24)

3.4.1.6. Volume of Distribution (Vd)

The volume of distribution (mean ± SD) of meloxicam with omeprazole (8.542 ± 1.449 L) was non-significantly decreased ($p > 0.594$) when compared to meloxicam alone (8.924 ± 1.969 L), as represented in Fig-3.18.
3.4.1.7. Clearance ($Cl$)

The (mean ± SD) in the clearance value of meloxicam non-significantly decreases ($p > 0.115$) when a single dose administration of meloxicam with omeprazole i.e., $6.530 \pm 1.295$ mL/min was compared to meloxicam alone i.e., $7.315 \pm 1.064$ mL/min, as shown in Fig-3.19.
3.4.1.8. Elimination Half-Life ($t_{1/2}$-E)

A non-significant increase ($p > 0.061$) in the meloxicam elimination half-life after concurrent administration with omeprazole [(mean ± SD) 14.554 ± 1.129 hr] was observed as compared to meloxicam alone i.e., 13.547 ± 1.361 hr, as presented in Fig-3.20.
Figure-3.20: Graphical representation of the $t_{1/2}-E$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose omeprazole capsule (40 mg) in healthy human volunteers (n = 24)
3.5. PK-DDI of Meloxicam with Omeprazole Multiple Dose

In the PK-DDI study of meloxicam single dose with omeprazole multiple dose, the meloxicam plasma concentration was plotted both on normal and semi-log graph scales as a function of time (Fig-3.21). The PK parameters were determined by compartmental and non-compartmental models using Microsoft Excel-2007 and PK Summit® software as presented in Tab-3.11.

**Table-3.11: Pharmacokinetic Parameters of Meloxicam Tablet (15 mg alone) and with Multiple Doses of Omeprazole Capsule (40 mg i.e., single dose for 3 days)**

<table>
<thead>
<tr>
<th>S/No</th>
<th>Pharmacokinetic parameters</th>
<th>Mel alone</th>
<th>Mel plus Ome multiple</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$C_{max}$ (ng/mL)</td>
<td>Mean 1235.11 ± 241.553</td>
<td>Mean 1515.66 ± 52.568</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>$T_{max}$ (hr)</td>
<td>Mean 5.833 ± 0.577</td>
<td>Mean 7.333 ± 0.942</td>
<td>0.000</td>
</tr>
<tr>
<td>3</td>
<td>$AUC^{(0-t)}$ (ng.hr/mL)</td>
<td>Mean 29237 ± 4615.21</td>
<td>Mean 41875.75 ± 2470.93</td>
<td>0.000</td>
</tr>
</tbody>
</table>
### Results and Discussions

#### 3.5.1. Results of PK-DDI between Meloxicam Single and Omeprazole Multiple Dose

#### 3.5.1.1. Maximum Plasma Concentration ($C_{\text{max}}$)

A significant increase ($p < 0.001$) in the value of meloxicam single and omeprazole multiple doses *i.e.*, $1515.66 \pm 52.568 \text{ ng/mL}$ has been observed as compared to meloxicam alone *i.e.*, $1235.11 \pm 241.553 \text{ ng/mL}$, as shown in Fig-3.22.
3.5.1.2. Time of Peak Plasma Concentration ($T_{\text{max}}$)

In this study the $T_{\text{max}}$ of the meloxicam significantly increased ($p < 0.000$) following oral administration of the omeprazole (o.d.) for 3 days, on 3$^{rd}$ day meloxicam tablet and omeprazole capsule were administered concurrently.

3.5.1.3. Area Under Curve [Bioavailability] ($AUC$)

A significant increase ($p < 0.000$) in $[AUC]^2_0$ of meloxicam after the administration of multiple doses of omeprazole was observed. Similarly, the (mean ± SD) of $[AUC]^2_0$ was significantly increased ($p < 0.000$) from $30795 ± 4675.53$ ng.hr/mL to $44639.77 ± 2585.13$ ng.hr/mL. The results are shown in Tab-3.11 and Fig-3.23, 3.24.
Figure-3.23: Effect of omeprazole 40 mg capsule o.d. for 3 days on the \([\text{AUC}]_0^{22}\) of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3\textsuperscript{rd} day in healthy human volunteers (n = 24)
3.5.1.4. Area Under Moment of Plasma Concentration ($AUMC$)

The (mean ± SD) $AUMC$ value of meloxicam was significant increase ($p < 0.000$) from 681925.970 ± 137052.451 ng.hr*hr/mL to 1195540.35 ± 129958.67 ng.hr*hr/mL after the multiple doses administration of omeprazole, as depicted in Fig-3.25.
3.5.1.5. Mean residence time (MRT)

A significance increase ($p < 0.000$) has been observed in the MRT value of meloxicam with multiple doses of omeprazole i.e., from (mean ± SD) $22.633 ± 2.190$ hr to $27.454 ± 2.596$ hr, as presented in Fig-3.26.
**Figure-3.26:** Effect of omeprazole 40 mg capsule o.d. for 3 days on the *MRT* of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

### 3.5.1.6. Volume of Distribution (*Vd*)

The (mean ± SD) *Vd* of meloxicam along with multiple dose omeprazole (7.596 ± 1.203 L) was non-significantly decreased (*p > 0.061*) when compared to meloxicam alone (8.924 ± 1.969 L), as described in **Fig-3.27**.
3.5.1.7. Clearance (Cl)

The clearance value (mean ± SD) of meloxicam become significantly decreased ($p < 0.000$) after omeprazole multiple dose administration, as shown in Tab-3.11 and Fig-3.28.
Figure-3.28: Effect of omeprazole 40 mg capsule o.d. for 3 days on the CI of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

3.5.1.8. Elimination Half-Life ($t_{1/2-E}$)

A significant increase ($p < 0.000$) in meloxicam $t_{1/2-E}$ was observed, when administered with multiple doses of omeprazole (mean ± SD) 16.439 ± 1.347 hr as compared to meloxicam alone i.e., 13.547 ± 1.361 hr. Results are graphically depicted in Fig-3.29.
Figure-3.29: Effect of omeprazole 40 mg capsule o.d. for 3 days on the $t_{1/2}$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)
3.6. Discussion of PK-DDI between Meloxicam and Omeprazole

Omeprazole is an acid labile, benz imidazole drug. It is a lipophilic basic drug with a pKa around 4.0 and extensive plasma protein bound i.e., 95%. It is a substrate as well as an inhibitor of Pgp, primarily metabolized by CYP2C19, CYP3A4 and CYP2C9 and is also a strong inhibitor of these enzymes (Pauli-Magnus et al., 2001; Kearns & Winter, 2003). Meloxicam being a class II and pH dependent soluble drug i.e., soluble at neutral pH but became quickly insoluble with lowering pH (Vignaduzzo et al., 2010). Its plasma protein binding capability is 99% and CYP2C9 plays a significant role in their metabolism. So, by considering its pH dependent solubility, protein binding capability and metabolism, research was carried out to find the impact of omeprazole both in a single and multiple dose on the pharmacokinetic of meloxicam. The co-administration of meloxicam with omeprazole results a non-significant increase (\( p > 0.557 \) (single dose)) i.e., increase by 4\% in the \( C_{\text{max}} \) value of meloxicam and a significant increase (\( p < 0.001 \) (multiple dose)) by 23\%, when meloxicam administered with multiple doses of omeprazole. This significant increase in the \( C_{\text{max}} \) value is evident from the significant increase i.e., by 62\% in the \( t_{1/2} \) and from the \( T_{\text{max}} \) value increase by 26\%, suggest an increase in meloxicam absorption across the intestinal epithelium when co-administered with omeprazole multiple doses. As omeprazole is a basic drug and with multiple doses it may have raised the gastrointestinal pH or it may have inhibited the metabolism of meloxicam, since omeprazole is an inhibitor of CYP2C9 as well. Raised GIT pH increases the dissolution of meloxicam, which has pH dependent solubility (i.e., 1.93 at pH = 7, 1.87 at pH = 6.8, 0.59 at pH = 5.8, 0.30 at pH = 4 and 0.11 at pH = 1.2) and was more soluble at neutral pH, so raised GIT pH increases the dissolution and absorption of meloxicam (Kotha et al., 2012). Similarly, it has been reported that omeprazole altered the absorption of ampicillin and erlotinib by varying gastric pH (Bista et al., 2007; Duong & Leung, 2010). However, in a single dose study, the absorption half-life was insignificantly affected result in a small increase in \( C_{\text{max}} \). Secondly, meloxicam is a substrate of CYP2C9 and its metabolism may have inhibited by omeprazole that is evident from increase in \( C_{\text{max}} \) and \( AUC \) values. Like omeprazole inhibited the metabolism of warfarin and phenytoin and raised their plasma level through CYP2C9 inhibition (Holbrook et al., 2005; Stormer et al., 1993).

The \([AUC]_0^{72}\) and \([AUC]_0^{60}\) values after single dose were non-significantly increased (11\% and 10\%, respectively), while in multiple doses the values were significantly increased by 44\% and 45\%, respectively, exhibit that large amount of the drug has been absorbed as \( t_{1/2} \) increase by 62\% and reached the systemic circulation after concurrent administration of meloxicam and omeprazole. The high \([AUC]_0^{72}\) and \([AUC]_0^{60}\) resulted in a significant high MRT value of meloxicam both in single and multiple dose study i.e., increase by 8\% and 22\% accordingly after treatment with omeprazole.

The volume of distribution of meloxicam non-significantly decreases after co-administration with single and multiple dose omeprazole. This decrease in \( Vd \) also resulted in a low clearance value, however this decrease in \( Cl \) was non-significant (\( p > 0.115 \)) after single dose concurrent administration with omeprazole and become significant (\( p < 0.000 \)) with multiple dose. The decrease in \( Vd \) and \( Cl \) resulted from increase in the elimination half-life of meloxicam that was 8\% (single dose) and 22\% (multiple dose) or decrease in the elimination rate i.e., 11\% (single dose) and 27\% (multiple dose), respectively. The alteration in the protein binding may also be
responsible for the decrease in the $V_d$ and $Cl$ values as both meloxicam and omeprazole are highly protein bound drugs, meloxicam 99%, while omeprazole 95% protein bound.

In conclusion, simultaneous administration of single oral dose of meloxicam and omeprazole did not substantially affect the pharmacokinetics of meloxicam and can safely be given without need in dose adjustment, however dosage modification in meloxicam is required when multiple doses of omeprazole are used concurrently.
3.7. PK-DDI of Meloxicam with Fluconazole Single Dose Study

The plasma samples of all the volunteers after the administration of meloxicam alone and with fluconazole was investigated by using HPLC/UV method and the pharmacokinetic parameters were obtained by applying Microsoft Excel-2007 and PK Summit® software as summarized in Tab-3.12. The plasma concentration of meloxicam was plotted as function of time on a normal and semi log graph scale, as presented in Fig-3.30.

![Figure 3.30: Mean ± SD Plasma Drug Concentration Vs Time of Healthy Human Volunteers (n = 24) following oral administration of meloxicam tablet (15 mg) alone and concurrently with single dose fluconazole capsule (150 mg) A: Normal plot and B: Semi-log scale](image)

**Table 3.12:** The Pharmacokinetic Parameters of Meloxicam Tablet (15 mg) Alone and with Fluconazole Capsule (150 mg)

<table>
<thead>
<tr>
<th>S/No</th>
<th>Pharmacokinetic parameters</th>
<th>Mel alone Mean ± S.D</th>
<th>Mel plus Flu single Mean ± S.D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>1235.11 ± 241.553</td>
<td>1390.43 ± 184.223</td>
<td>0.090</td>
</tr>
<tr>
<td>2</td>
<td>$T_{\text{max}}$ (hr)</td>
<td>5.833 ± 0.577</td>
<td>7.166 ± 1.337</td>
<td>0.006</td>
</tr>
<tr>
<td>3</td>
<td>$AUC_{(0-\infty)}$ (ng.hr/mL)</td>
<td>29237 ± 4615.21</td>
<td>37911 ± 5796.27</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>$AUC_{(0-t)}$ (ng.hr/mL)</td>
<td>30795 ± 4675.53</td>
<td>40348 ± 6942</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Parameter</td>
<td>Symbol</td>
<td>Mean Value (SD)</td>
<td>95% CI</td>
</tr>
<tr>
<td>---</td>
<td>-------------</td>
<td>--------</td>
<td>-----------------</td>
<td>--------</td>
</tr>
<tr>
<td>5</td>
<td>AUMC (ng*hr/mL)</td>
<td>AUMC</td>
<td>681925.97 (137052)</td>
<td>1009399 (241652)</td>
</tr>
<tr>
<td>6</td>
<td>t_{1/2}-A (hr)</td>
<td>t_{1/2}-A</td>
<td>2.862 (1.016)</td>
<td>2.928 (0.438)</td>
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<td>t_{1/2}-D (hr)</td>
<td>t_{1/2}-D</td>
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<td>6.473 (1.843)</td>
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<tr>
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<td>t_{1/2}-E (hr)</td>
<td>t_{1/2}-E</td>
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<td>17.349 (1.961)</td>
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<td>Vd (L)</td>
<td>Vd</td>
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<td>8.570 (1.703)</td>
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<td>10</td>
<td>Cl (mL/min)</td>
<td>Cl</td>
<td>7.315 (1.064)</td>
<td>5.695 (1.679)</td>
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<td>MRT (hr)</td>
<td>MRT</td>
<td>22.633 (2.190)</td>
<td>25.359 (2.306)</td>
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<td>K_{10}</td>
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3.7.1. Results of PK-DDI between Meloxicam and Fluconazole Single Dose Study

3.7.1.1. Maximum Plasma Concentration \((C_{\text{max}})\)

The \(C_{\text{max}}\) (mean ± SD) value of meloxicam alone was 1235.11 ± 241.553 ng/mL, which shows a non-significant decrease \((p > 0.090)\) when compared with meloxicam and fluconazole concurrent administration \(i.e.,\) 1390.43 ± 184.223 ng/mL, as shown in Fig-3.31.

![Graphical representation of the \(C_{\text{max}}\) values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers \((n = 24)\)](image)

**Figure-3.31**: Graphical representation of the \(C_{\text{max}}\) values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers \((n = 24)\)

3.7.1.2. Time of Peak Plasma Concentration \((T_{\text{max}})\)

The \(T_{\text{max}}\) (mean ± SD) value of meloxicam was significantly increased \((p < 0.006)\) from 5.833 ± 0.577 hr to 7.166 ± 1.337 hr, when meloxicam and fluconazole were administered concomitantly.
3.7.1.3. Area Under Curve [Bioavailability] ($AUC$)

A significant increase in the $[AUC]_0^2$ and $[AUC]_0^\infty$ value of meloxicam ($p < 0.001$ and $p < 0.001$) with single dose of fluconazole concomitantly was observed. The results are presented in Tab-3.12 and Fig-3.32, 3.33.
Figure-3.32: Graphical representation of the $[\text{AUC}]_0^{72}$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers ($n = 24$)
3.7.1.4. Area Under Moment of Plasma Concentration (AUMC)

The (mean ± SD) AUMC value of meloxicam was calculated as $681925.970 \pm 137052.451 \text{ ng.hr*hr/mL}$, shows a significant increase ($p < 0.001$) after concomitantly administration with fluconazole i.e., $1009399 \pm 241652 \text{ ng.hr*hr/mL}$, as depicted in Fig-3.34.
Figure-3.34: Graphical representation of the $[\text{AUMC}]_0^\infty$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

3.7.1.5. Mean Residence Time (MRT)

The value of $MRT$ (mean ± SD) was significantly increases ($p < 0.007$) when meloxicam was concomitantly administered with fluconazole i.e., $25.359 \pm 2.306$ hr as compared to meloxicam alone i.e., $22.633 \pm 2.190$ hr, as described in Fig.-3.35.
3.7.1.6. Volume of Distribution (Vd)

The value (mean ± SD) Vd of meloxicam decreased non-significantly ($p > 0.643$) when meloxicam and fluconazole were administered together i.e., $8.570 ± 1.703$ L, as compared to meloxicam alone i.e., $8.924 ± 1.969$ L. Graphical results are shown in Fig-3.36.
Figure-3.36: Graphical representation of the Vd values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

3.7.1.7. Clearance (CI)

The total clearance value of meloxicam alone was 7.315 ± 1.064 mL/min, a significant decrease (p < 0.009) was observed after concurrent administration with fluconazole i.e., 5.695 ± 1.679 mL/min, as represented in Fig-3.37.
Figure-3.37: Graphical representation of the Cl values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers (n = 24)

3.7.1.8. Elimination Half-Life ($t_{1/2} - E$)

The (mean ± SD) $t_{1/2} - E$ of a single oral dose of meloxicam increased significantly ($p < 0.000$) with fluconazole concurrent administration, as presented in Fig-3.38.
Figure-3.38: Graphical representation of the $t_{1/2}$-$E$ values of meloxicam after the oral administration of meloxicam tablet (15 mg) alone and after concurrent administration with single dose fluconazole capsule (150 mg) in healthy human volunteers ($n = 24$)
3.8. PK-DDI of Meloxicam Single with Fluconazole Multiple Dose

PK parameters were obtained using Microsoft Excel-2007 and PK Summit® software as summarized in **Table 3.13**. The plasma concentration of meloxicam as function of time was plotted on a normal and semi log graph scale, as presented in **Fig-3.39**.

**Figure-3.39**: Mean ± SD Plasma Drug Concentration Vs Time of Healthy Human Volunteers (n = 24) following oral administration of meloxicam single tablets 15 mg alone and concurrently with multiple dose fluconazole capsule 150 mg *i.e.*, single dose for 3 consecutive days and on third day meloxicam 15 mg was administered with it A: Normal plot and B: Semi-log scale

**Table 3.13**: Pharmacokinetic Parameters of Meloxicam Tablet (15 mg alone) and with Multiple Doses of Fluconazole Capsule (150 mg i.e., single dose for 3 days)

<table>
<thead>
<tr>
<th>S/No</th>
<th>Pharmacokinetic parameters</th>
<th>Mel alone Mean ± S.D</th>
<th>Mel plus Flu multiple Mean ± S.D</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>1235.11 ± 241.553</td>
<td>1571.137 ± 179.742</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>$T_{\text{max}}$ (hr)</td>
<td>5.833 ± 0.577</td>
<td>7.835 ± 0.577</td>
<td>0.000</td>
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<tr>
<td>3</td>
<td>$AUC^{(0-24)}$ (ng.hr/mL)</td>
<td>29237 ± 4615.21</td>
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### Results and Discussions

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<td>0.006</td>
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</tr>
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</table>

#### 3.8.1. Results of PK-DDI between Meloxicam Single and Fluconazole Multiple Dose Study

##### 3.8.1.1. Maximum Plasma Concentration \((C_{max})\)

The \((\text{mean} \pm \text{SD})\) \(C_{max}\) value of meloxicam alone was 1235.11 \(\pm\) 241.553 ng/mL, which was significantly increased \((p < 0.001)\) after giving with fluconazole multiple doses \(i.e.,\) 1571.137 \(\pm\) 179.742 ng/mL, as shown in Fig-3.40.
Figure-3.40: Effect of fluconazole 150 mg capsule o.d. for 3 days on the $C_{\text{max}}$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers ($n = 24$)

3.8.1.2. Time of Peak Plasma Concentration ($T_{\text{max}}$)

The $T_{\text{max}}$ (mean ± SD) value of meloxicam was significantly increased ($p < 0.000$) from 5.833± 0.577 hr to 7.833 ± 0.577 hr after administration with multiple doses of fluconazole.

3.8.1.3. Area Under Curve [Bioavailability] ($AUC$)

A significant increase in the $[AUC]_0^2$ and $[AUC]_0^\infty$ value of meloxicam ($p < 0.000$ and $p < 0.000$, respectively) was observed after concomitantly administration with multiple dose of fluconazole, as described in Tab-3.13 and Fig-3.41, 3.42.
Figure-3.41: Effect of fluconazole 150 mg capsule o.d. for 3 days on the [AUC]_{0}^{2} of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)
3.8.1.4. Area Under Moment of Plasma Concentration (AUMC)

The \textit{AUMC} (mean ± SD) value of meloxicam shows a significant increase ($p < 0.000$) after administration with fluconazole multiple doses \textit{i.e.}, $2401980.03 ± 497122.693$ ng.hr\textsuperscript{2}hr/mL, when compared to meloxicam alone administration, which was calculated as $681925.970 ± 137052.451$ ng.hr\textsuperscript{2}hr/mL, as presented in \textbf{Fig.-3.43}.
CHAPTER-3

RESULTS AND DISCUSSIONS

Figure-3.43: Effect of fluconazole 150 mg capsule o.d. for 3 days on the [AUMC]∞ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

3.8.1.5. Mean Residence Time (MRT)

The MRT (mean ± SD) value of meloxicam given concomitantly with multiple doses of fluconazole i.e., 22.633 ± 2.190 hr was significantly increase (p < 0.000) as compared to meloxicam alone i.e., 35.379 ± 3.552 hr. The results are depicted in Fig-3.44.
Figure-3.44: Effect of fluconazole 150 mg capsule o.d. for 3 days on the MRT of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers (n = 24)

3.8.1.6. Volume of Distribution (Vd)

The Vd value (mean ± SD) of meloxicam alone was 8.924 ± 1.969 L, which decreased significantly (p < 0.003) after multiple doses of fluconazole i.e., 6.735 ± 0.822 L. Graphical results are represented in Fig-3.45.
Figure-3.45: Effect of fluconazole 150 mg capsule o.d. for 3 days on the Vd of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3\textsuperscript{rd} day in healthy human volunteers (n = 24)

3.8.1.7. Clearance (Cl)

A significant decrease ($p < 0.000$) in the total clearance value of meloxicam was observed after fluconazole multiple doses, as shown in Fig-3.46.
Figure-3.46: Effect of fluconazole 150 mg capsule o.d. for 3 days on the Cl of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3\textsuperscript{rd} day in healthy human volunteers (n = 24)

3.8.1.8. Elimination Half-Life ($t_{1/2-E}$)

The $t_{1/2-E}$ mean ± SD value of meloxicam along with multiple doses of fluconazole was determined as 20.899 ± 3.066 hr, shows a significant increase ($p < 0.000$) in comparison to meloxicam alone value i.e., 13.547 ± 1.361 hr, as presented in Fig-3.47.
**Figure-3.47**: Effect of fluconazole 150 mg capsule o.d. for 3 days on the $t_{1/2}$-$E$ of the meloxicam following simultaneous administration of single dose meloxicam tablets (15 mg) on 3rd day in healthy human volunteers ($n = 24$)
3.9. Discussion of PK-DDI between Meloxicam and Fluconazole

Meloxicam is extensively metabolized drug mainly by CYP2C9 isozymes. Fluconazole is a bis-triazole water soluble antifungal agent and a selective inhibitor of CYP2C9, that interacts significantly with many drugs metabolize by this enzyme (Saari et al., 2008). So, meloxicam extensive metabolization and fluconazole discerning inhibition of CYP2C9, provide the basis of evaluating this metabolic pharmacokinetic interaction studies.

In the present study, the results reveal a non-significant increase in the $C_{\text{max}}$ value by 13% after the concurrent administration of a single dose of meloxicam with fluconazole, but shows a significance increase by 27% when fluconazole was administered for three days and on the third day meloxicam was administered concomitantly, exhibiting an increase in the absorption or decrease in the metabolism of meloxicam when administered with fluconazole. The $T_{\text{max}}$ of meloxicam in the presence of fluconazole was significantly higher both in a single and multiple dose i.e., increased by 23% and 35%, respectively, may be due to increase in the absorption of meloxicam, which is evident from the single and multiple dose high absorption half-life i.e., increase by 3% and 85%, respectively. It is probable both meloxicam and fluconazole share same transporters or fluconazole alter the transport system responsible for the transport of meloxicam but both meloxicam and fluconazole have no such roles in transporters and there is no evidence of the participation of transporters in the absorption of meloxicam, so it may likely be due to the inhibition of CYP2C9 mediated meloxicam metabolism by fluconazole.

The slight increase in the meloxicam $C_{\text{max}}$ (13%) after a single dose study reflects that the CYP2C9 enzyme was not fully inhibited by fluconazole but when fluconazole administered for three days it gets strongly inhibited and resulted a high significance increase in the $C_{\text{max}}$ value (27%) of meloxicam, which is of clinical importance. The increase in plasma concentration and increase in $T_{\text{max}}$ also indicates the decrease in the metabolism of meloxicam after co-administration with single and multiple doses of fluconazole. These results ensued a significant increase in the $[AUC]_0^{72}$ and $[AUC]_0^{\infty}$ values after a single dose by 30% and 31%, after multiple doses of fluconazole by 104% and 120%, respectively, prolonged the $t_{1/2}$-$E$ from 13 to 21 hours and raised MRT value by 12% (single dose) and 57% (multiple dose), indicates that the drug accumulate and stays for a longer period of time in the body that is evident from the significant decrease in the $t_{1/2}$-$D$ value by 32% (single dose) and 18% (multiple dose). Together these observations indicate that the meloxicam and fluconazole interaction occurs at the elimination level. In another study, decrease in the metabolism, increase in the plasma concentration and bioavailability of meloxicam by voriconazole based on the CYP2C9 inhibition has been assessed, which is also of clinical significance (Hynninen et al., 2009).

The value of $V_d$ of meloxicam after a single dose study non-significantly decrease by 4% and after multiple dose significantly by 25%, similarly the $Cl$ values after co-administration with fluconazole both in a single as well as in multiple doses significantly decreases by 23% and 50% respectively. These changes in the $V_d$ and $Cl$ values is because of decrease in the elimination rate value and may be due to alteration in the protein binding, as fluconazole is 12% and meloxicam is 99% bound. The significance decrease in $Cl$ value also resulted a significance increase in the $t_{1/2}$-$E$ and MRT value.
Overall outcomes from this study indicate a potential pharmacokinetic drug-drug interaction between single oral dose meloxicam plus fluconazole single and multiple doses at the level of metabolism, when administered concomitantly, as all the pharmacokinetic parameters are of clinical significance therefore, dose adjustment may be required for maintaining steady state concentration.
4. CONCLUSION
This study, the evaluation of pharmacokinetic drug-drug interaction of meloxicam with selected co-prescribed drugs such as omeprazole and fluconazole was studied in healthy human Pakistani volunteers and was conducted per Helsinki declaration, both in a single and multiple dose administration (single dose for 3 days) impact of omeprazole and fluconazole on meloxicam was evaluated. Novel RP-HPLC-UV/Vis method for the quantification of meloxicam and its metabolites was developed and validated according to ICH guidelines. This method was then successfully employed in the pharmacokinetic drug-drug interaction study between meloxicam and selected co-administered drugs omeprazole and fluconazole. The pharmacokinetic parameters of meloxicam in the current study were in accordance to other reported studies. The differences may be due to the inter and intra population enzyme diversity and interethnic variability that plays a role in the meloxicam disposition and differences in the PK parameters.

Co-administration of single dose of meloxicam and omeprazole attenuated the pharmacokinetic profile of meloxicam by a non-significant increase in the $C_{\text{max}}$, $T_{\text{max}}$, $t_{1/2}$, [AUC]$^\text{0}$, and [AUC]$^\text{00}$, while a non-significant decrease in the $Vd$ and $Cl$ values. However, co-administration of single dose of meloxicam with omeprazole multiple doses i.e., single dose for 3 consecutive days resulted a significant increase in the $C_{\text{max}}$, $T_{\text{max}}$, $t_{1/2}$, [AUC]$^\text{0}$, [AUC]$^{\text{00}}$, [AUMC]$^{\text{00}}$, MRT and a significant decrease in the $Cl$, whereas a non-significant decrease in the $Vd$ values of the pharmacokinetic profile of meloxicam.

A single dose fluconazole concurrent administration with meloxicam influenced the pharmacokinetics of meloxicam resulting a non-significant increase in the $C_{\text{max}}$ and a significant increase in the $T_{\text{max}}, t_{1/2}, [\text{AUC}]^0, [\text{AUC}]^{\text{00}}, [\text{AUMC}]^{\text{00}}, \text{MRT}$, although a significant decrease in the $Cl$ and a non-significant decrease in the $Vd$ values of meloxicam.

Though, the effect of fluconazole multiple dose administration i.e., single dose for 3 consecutive days affected significantly the pharmacokinetic profile of meloxicam i.e., $C_{\text{max}}, T_{\text{max}}, t_{1/2}, [\text{AUC}]^0, [\text{AUC}]^{\text{00}}, [\text{AUMC}]^{\text{00}}, \text{MRT}$ shows a very significant increase and $Vd, Cl$ a significant decrease.

Lack of significant pharmacokinetic drug-drug interaction between single dose meloxicam and omeprazole has been observed, so single dose omeprazole does not likely to enhance meloxicam toxicity, however concomitant administration of meloxicam with omeprazole multiple doses significantly affected the pharmacokinetics of meloxicam resulted an increase in the plasma drug exposure, so dosage adjustment is warranted or alternatively replacing omeprazole with another PPI class drug, which will not be a substrate of CYP2C9.

The meloxicam and fluconazole co-administration both in a single and multiple dose administration resulting a clinically significant pharmacokinetic drug-drug interaction and enhances meloxicam toxicity. Therefore, based on the results of the present study patients should be monitored carefully while prescribing either drugs or on the other hand another drug from the antifungal class must be considered which have less potential of interacting with meloxicam.
5. FUTURE PERSPECTIVE
Meloxicam is an NSAID from oxicam class and generally employed in the management of arthritis, rheumatoid arthritis, ankylosing spondylitis and in other rheumatologic conditions. Recently this group of NSAIDs gained attentions because of low ulcerogenic potential, so a comprehensive pharmacokinetic drug-drug interaction study of meloxicam both in a single and multiple dose is required to safeguard against potential risks of drugs co-administered with it.
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