CONTENTS

Abbreviations i
List of Figures iii
List of Tables vi
Abstract viii
Acknowledgement xv

Chapter/Section Page Number

Chapter 1: Importance of the research plan
1.1. Introduction 1
1.2. References 5

Chapter 2: Review of literature
2.1. Options and Issues in Antimalarial therapies
2.1.1. Introduction 7
2.1.2. Treatment of Malaria 8
2.1.3. Antimalarial therapeutic agents 9
2.1.4. Emergence of Artemisinin Resistance in P. falciparum 12
2.1.5. Global Pipeline of Antimalarial Drugs 14
2.2. Antimalarial Combination Therapies
2.2.1. Why combine antimalarials? 15
2.2.2. Why do combination regimens not always work? 15
2.2.3. What constitutes an ideal combination? 18
2.2.4. What combinations are available? 21
2.2.5. The future 28
2.3. Drug Discovery and Pharmacokinetics
2.3.1. Pharmacokinetics 31
2.3.2. Role of Pharmacokinetics 32
2.3.3. Key pharmacokinetic parameters: Theory and rationale 33
2.3.4. Pharmacokinetic screening of NCEs 35
2.4. Selected Drugs for Potential Antimalarial Combination Therapy
2.4.1. Lumefantrine 37
2.4.2. Piperaquine 40
2.4.3. CDRI Candidate Antimalarials 43

2.5. References 45

Chapter 3: Bioanalytical method development and validation
3.1. Regulatory Considerations for Method Development and Validation
3.1.1. Introduction 62
3.1.2. Validation Parameters 62
3.1.3. Chemicals and reagents 70

3.2. Development and Validation of LC-ESI-MS/MS Method for Quantification of Lumefantrine and CDRI trioxane 99-411 in rat plasma
3.2.1. Experimental 73
3.2.2. Results 74
3.2.3. Conclusion 79

3.3. Development and Validation of LC-ESI-MS/MS Method for Quantification of Lumefantrine and CDRI trioxane 97-78 in rat plasma
3.3.1. Experimental 80
3.3.2. Results 81
3.3.3. Conclusion 87

3.4. Development and Validation of LC-ESI-MS/MS Method for Quantification of Piperaquine and CDRI trioxane 97-78 in rat plasma
3.4.1. Experimental 88
3.4.2. Results 89
3.4.3. Conclusion 95

3.5. Development and Validation of LC-ESI-MS/MS Method for Quantification of Piperaquine and CDRI trioxane 99-411 in rat plasma
3.5.1. Experimental 96
3.5.2. Results 97
3.5.2. Conclusion 103

3.6. Conclusion 104
3.7. References 105
Chapter 4: *In-vivo* pharmacokinetic studies

4.1. Intravenous pharmacokinetics, oral bioavailability, dose proportionality and in situ permeability of anti-malarial lumefantrine in rats

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1.1. Introduction</td>
<td>106</td>
</tr>
<tr>
<td>4.1.2. Methods</td>
<td>108</td>
</tr>
<tr>
<td>4.1.3. Results and Discussion</td>
<td>113</td>
</tr>
<tr>
<td>4.1.4. Conclusions</td>
<td>121</td>
</tr>
</tbody>
</table>

4.2. Gender Differences in Pharmacokinetics of lumefantrine and its metabolite desbutyl-lumefantrine in Rats

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.1. Introduction</td>
<td>122</td>
</tr>
<tr>
<td>4.2.2. Materials and Methods</td>
<td>123</td>
</tr>
<tr>
<td>4.2.3. Results and discussion</td>
<td>125</td>
</tr>
<tr>
<td>4.2.4. Conclusion</td>
<td>129</td>
</tr>
</tbody>
</table>

4.3. Role of P-gp in Absorption of Lumefantrine

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3.1. Introduction</td>
<td>130</td>
</tr>
<tr>
<td>4.3.2. Methods</td>
<td>131</td>
</tr>
<tr>
<td>4.3.3. In-situ Single Pass Intestinal Perfusion study</td>
<td>131</td>
</tr>
<tr>
<td>4.3.4. In-vivo pharmacokinetic study</td>
<td>134</td>
</tr>
<tr>
<td>4.3.5. Results and Discussion</td>
<td>136</td>
</tr>
<tr>
<td>4.3.6. Conclusion</td>
<td>141</td>
</tr>
</tbody>
</table>

4.4. References

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4. References</td>
<td>142</td>
</tr>
</tbody>
</table>

Chapter 5: *In vitro* and in situ Studies

5.1. In vitro Protein Binding Study of Lumefantrine by charcoal adsorption assay

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1.1. Introduction</td>
<td>148</td>
</tr>
<tr>
<td>5.1.2. Experimental</td>
<td>151</td>
</tr>
<tr>
<td>5.1.3. Results and discussion</td>
<td>153</td>
</tr>
<tr>
<td>5.1.4. Conclusion</td>
<td>154</td>
</tr>
</tbody>
</table>

5.2. In vitro Metabolic Stability

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2.1. Introduction</td>
<td>155</td>
</tr>
<tr>
<td>5.2.2. Experimental</td>
<td>156</td>
</tr>
<tr>
<td>5.2.3. Results and discussion</td>
<td>157</td>
</tr>
<tr>
<td>5.2.4. Conclusion</td>
<td>158</td>
</tr>
</tbody>
</table>
5.3. Simulated gastric fluid (SGF)/Simulated Intestinal Fluid (SIF) Stability Study of Lumefantrine
5.3.1. Introduction 160
5.3.2. Experimental 160
5.3.3. Results and Discussions 162
5.3.4. Conclusion 164

5.4. Red Blood Cell (RBC) uptake study of Lumefantrine
5.4.1. Introduction 165
5.4.2. Experimental 167
5.4.3. Results and Discussions 169
5.4.4. Conclusion 170

5.5. Prediction of human absorption of candidate antimalarial (99-411) using in-house validated in-situ single-pass intestinal perfusion model
5.5.1. Introduction 171
5.5.2. Materials and Method 172
5.5.3. Results and Discussions 176
5.5.4. Conclusion 180
5.6. References 182

Chapter 6: Interaction Studies
6.1. Introduction 184
6.2. Materials and Method 185
6.3. Results 188
6.4. Conclusion 197
6.5. References 198

List of Publications 200