THE CLINICAL PHARMACOLOGY OF

METHADONE INDUCTION

Erin Brooke Morton, BBiotech(Hons)

Discipline of Pharmacology
School of Medical Sciences
(Faculty of Health Sciences)
University of Adelaide

January 2007

A thesis submitted for the degree of Doctor of Philosophy
Table of Contents

List of Chapters ........................................................................................................................................... ii

List of Tables ................................................................................................................................................... xiv

List of Figures ................................................................................................................................................ xvi

List of Equations ........................................................................................................................................... xxi

List of Appendices ....................................................................................................................................... xxi

Abstract ....................................................................................................................................................... xxii

Declaration .................................................................................................................................................... xxv

Acknowledgements ....................................................................................................................................... xxvi

Publications and presentations in support of this thesis ............................................................... xxviii

Additional publications and presentations associated with the work contained in this thesis ........................................... xxviii

Abbreviations, prefixes and symbols .............................................................................................................. xxix
1. Methadone Pharmacology: A Review of the Literature; Hypotheses & Aims of the Project ................................................................. 1

1.1. Opioid Pharmacology................................................................................................................................. 1

1.1.1. Historical use of plant-derived medicines ................................................................................................. 1

1.1.2. Opium discovery and therapeutic use ......................................................................................................... 2

1.1.3. Opioid receptors ....................................................................................................................................... 2

1.1.3.1. Binding by the $\mu$ Opioid Receptor (MOP) ...................................................................................... 3

1.1.3.2. Regulation of the $\mu$ Opioid Receptor (MOP) .................................................................................. 6

1.1.3.3. $\mu$ Opioid Receptor (MOP), and its encoding gene ($OPRM1$) ......................................................... 7

1.1.4. Physiological Responses to Activation of the $\mu$ Opioid Receptor (MOP) ............................................. 8

1.1.5. Heroin synthesis, addiction, and current problems ................................................................................... 9

1.2. Dependence and addiction .......................................................................................................................... 10

1.3. Substitution Treatments ............................................................................................................................. 11

1.4. Methadone Maintenance Treatment ........................................................................................................ 12

1.4.1. History ................................................................................................................................................... 12

1.4.2. Objectives .............................................................................................................................................. 12

1.4.3. Recruitment and Retention ..................................................................................................................... 13

1.5. Methadone Pharmacology in Relation to MMT ....................................................................................... 13

1.5.1. Physiological Reactions to MMT ........................................................................................................ 14

1.5.2. Pharmacokinetic/Pharmacodynamic Relationships .............................................................................. 14

1.5.3. Withdrawal ............................................................................................................................................. 17

1.5.4. Tolerance ............................................................................................................................................... 18

1.5.5. Cross-tolerance ...................................................................................................................................... 19

1.5.6. Current Methodology for MMT ........................................................................................................... 20

1.5.6.1. Routes of Methadone Administration ............................................................................................. 20

1.5.6.2. Chronic Dosing in MMT to Target a Narrow Plasma Concentration Range ......................................................................................................................... 20
1.5.6.3. Single vs Divided Daily Dose ................................................................................................................................. 21
1.5.6.4. MMT Locale: Hospital/Clinic versus Private Practice ........................................................................................................... 22
1.5.7. Risks of MMT ......................................................................................................................................................... 23
1.5.7.1. Constipation ......................................................................................................................................................... 23
1.5.7.2. Nausea and Vomiting ......................................................................................................................................... 24
1.5.7.3. Drowsiness, Confusion, and Mood State ............................................................................................................. 24
1.5.7.4. Hyperalgesia ......................................................................................................................................................... 25
1.5.7.5. Respiratory depression, toxicity and death ............................................................................................................ 26
1.5.8. Starting Doses in MMT .............................................................................................................................................. 30
1.5.9. Factors that can Change the Outcome of MMT .............................................................................................................. 32
1.5.9.1. Social environment .............................................................................................................................................. 32
1.5.9.2. Therapy environment ........................................................................................................................................ 32
1.5.9.3. Family history ......................................................................................................................................................... 33
1.5.9.4. Genetic factors (pharmacogenetics) .................................................................................................................... 34
1.5.10. Continued heroin use during MMT ............................................................................................................................ 34
1.6. Methadone ................................................................................................................................................................. 35
1.6.1. Methadone Chemistry .............................................................................................................................................. 35
1.6.1.1. Physicochemical Properties and Australian Availability of Methadone ........................................................ 35
1.6.1.2. Stereoisomers of Methadone ............................................................................................................................. 36
1.7. Pharmacokinetics of Methadone in the Human Body ..................................................................................................... 37
1.7.1. Methadone Pharmacokinetics in Acute and Chronic Dosing .......................................................................................... 37
1.7.1.1. Stable Isotope Utilisation for Pharmacokinetic Studies ....................................................................................... 46
1.7.2. Pharmacokinetic Parameters and Causes of Change ................................................................................................. 48
1.7.2.1. Clearance via Metabolism ................................................................................................................................. 48
1.7.2.1.1. CYP3A4................................................................................................. 49
1.7.2.1.2. Auto-induction of CYP3A4 metabolism ........................................ 50
1.7.2.1.3. Methods for Measuring CYP3A4 Activity................................. 54
   1.7.2.1.3.1. Erythromycin Breath Test (EBT) ........................................... 55
1.7.2.1.4. CYP2D6 and genotyping ............................................................... 56
1.7.2.2. Renal Clearance....................................................................................... 57
1.7.2.3. Bioavailability ......................................................................................... 58
1.7.2.4. Volume of Distribution ........................................................................... 58
   1.7.2.4.1. Protein binding by α-1-acid glycoprotein (AAG) ......................... 59
1.7.2.5. Half-life ................................................................................................... 61
1.7.2.6. Stereoselective differences ...................................................................... 61
1.7.3. Factors that may alter Methadone Pharmacokinetics............................... 66
   1.7.3.1. P-glycoprotein (encoded by the MDR1 / ABCB1 gene).................... 66
   1.7.3.2. Pregnancy and lactation ................................................................. 67
   1.7.3.3. Liver, renal, and respiratory diseases ............................................... 67
   1.7.3.4. Drug Interactions .............................................................................. 69
1.8. Summary and Conclusions from Existing Literature ....................................... 70
1.9. Overview and Significance of the Current Research ........................................ 71
   1.9.1. Hypotheses ............................................................................................. 73
   1.9.2. Aims and Objectives ............................................................................. 74

2. Study Details, Subject Recruitment, Clinical Procedure, and Subject Characteristics ........................................................................................................ 75
   2.1. Study Design ............................................................................................. 75
   2.2. Study Overview ....................................................................................... 75
   2.3. Recruitment ............................................................................................. 76
2.3.1. Centres Involved ........................................................................................................ 76
2.3.2. Recruitment Procedure ............................................................................................ 78
2.3.3. Confidential Codes for Subjects ............................................................................. 78
2.3.4. Inclusion/Exclusion Criteria and Adherence to the Protocol ................................. 79
  2.3.4.1. Age, Gender, Suitability, and Consent ............................................................... 79
  2.3.4.2. Anomalous Methadone Intake in One Subject ................................................. 79
  2.3.4.3. Pregnancy and Liver Function ........................................................................... 80
  2.3.4.4. Mental Health .................................................................................................. 80
  2.3.4.5. Protocol Violations leading to Exclusion .......................................................... 81
2.3.5. Reasons for Non-completion of the Studies ............................................................ 82
  2.3.5.1. Subjects that failed the criteria ......................................................................... 82
  2.3.5.2. Subjects that withdrew from the study .............................................................. 82
2.4. Study Protocol ............................................................................................................. 82
  2.4.1. General ................................................................................................................ 82
  2.4.2. Study A: Pharmacokinetics and pharmacodynamics of methadone from the
time of induction to stable maintenance ......................................................................... 84
  2.4.3. Study B: Predicting Methadone Stabilisation Dose .............................................. 88
2.5. Subject Characteristics ............................................................................................... 89

3. Experimental Methods .................................................................................................... 92
  3.1. Chemicals and Reagents ........................................................................................... 94
  3.2. LC-MS Assay for Quantification of $^2$H$_6$ (d6) and $^2$H$_0$ (d0) R- and S-methadone
Concentrations in the Plasma Samples of MMT Subjects ............................................... 95
    3.2.1. Instrumentation and Chromatography Conditions .............................................. 95
    3.2.2. Sample Preparation ........................................................................................... 96
    3.2.3. Assay Calibration, Quality Control Samples, and Validation ............................. 96
3.2.4. Data Corrections........................................................................................................ 97
3.2.5. Data Analysis ........................................................................................................... 98
3.2.6. Validation Results and Discussion................................................................. 101
3.2.7. LC-MS Difficulties.......................................................................................... 104
3.2.8. Assay of subjects’ samples.............................................................................. 104
3.2.9. Pharmacokinetic Analyses .......................................................................... 105
3.2.10. Statistical Analyses....................................................................................... 106
3.2.11. Comparison to UV-HPLC............................................................................... 107

3.3. HPLC with UV Chromatography for Plasma Methadone Concentrations in
MMT subjects during Days 5-14 and 44-49 of chronic daily oral dosing .......... 107
3.3.1. HPLC Instrumentation and Chromatography Conditions ......................... 107
3.3.2. Sample Preparation......................................................................................... 108
3.3.3. Assay Calibration, Quality Control Samples, and Validation..................... 108
3.3.4. Data analysis.................................................................................................... 109
3.3.5. Validation Results and Discussion................................................................ 112
3.3.6. Assay of subjects’ samples........................................................................... 113

3.4. Morphine Concentrations...................................................................................... 114
3.4.1. Hair Samples .................................................................................................... 114
3.4.1.1. Procedures ............................................................................................... 114
3.4.1.2. Data Analysis.......................................................................................... 114
3.4.2. Plasma Morphine Concentrations ................................................................. 115
3.4.2.1. Instrumentation and Chromatography Conditions ................................ 115
3.4.2.2. Sample Preparation............................................................................... 115
3.4.2.3. Assay trouble-shooting.......................................................................... 116
3.4.3. Assay Calibration, Quality Control Samples, and Validation..................... 117
3.4.4. Data analysis.................................................................................................... 117
3.4.5. Validation Results and Discussion ............................................................... 120
3.4.6. Assay of subjects’ samples ................................................................. 121

3.5. Estimation of Concurrent Heroin Use during MMT ........................................... 121
3.5.1. Statistical Analyses .............................................................................. 122
3.5.2. Statistical Analyses using Multiple Linear Regression.............................. 122
3.5.3. Selection of Independent Variables and Subject Groups ......................... 123

3.6. Erythromycin Breath Test (EBT) ................................................................. 124
3.6.1. Equipment ............................................................................................ 124
3.6.2. Procedure ............................................................................................ 126
3.6.2.1. Subjects ......................................................................................... 126
3.6.2.2. Vials ............................................................................................... 126
3.6.3. Quantification of $^{14}$CO$_2$ ................................................................ 127
3.6.4. Validation of EBT .............................................................................. 127
3.6.5. Data analysis ....................................................................................... 128
3.6.6. Statistical Analysis ............................................................................. 130

3.7. Plasma AAG Concentration Measurement ................................................... 131
3.7.1. Instrumentation and Conditions .......................................................... 131
3.7.2. Sample Preparation ............................................................................ 131
3.7.3. Assay Calibration, Quality Controls, and Validation ......................... 131
3.7.4. Data analysis ....................................................................................... 131
3.7.5. Validation Results and Discussion ..................................................... 132
3.7.6. Assay of subject samples ................................................................. 132

3.8. Pharmacodynamics ..................................................................................... 133
3.8.1. Methadone Symptoms Checklist as a measure of withdrawal ............... 133
3.8.2. Respiratory rates and Oxygen Saturation ............................................. 135
3.8.3. Data analyses ..................................................................................... 136
3.8.3.1. Regression analyses ................................................................. 136
3.8.3.1.1. Total Plasma Methadone Concentrations .......................... 137
3.8.3.1.2. Pharmacodynamic Effects of Withdrawal Symptoms, Respiratory
Rate and Blood Oxygen Saturation, and Plasma Methadone
Concentration-Effect Relationships ............................................ 137
3.8.3.2. Power of the studies ................................................................. 137

4. Pharmacokinetics of Methadone during Induction and Stabilisation of MMT. 139
4.1. Introduction .................................................................................... 139
4.2. Results: Plasma d6-R- and d6-S-Methadone Concentrations as Measured by
LC-MS .................................................................................................... 141
4.3. Pharmacokinetics of IV d6-methadone: Calculated from LC-MS Results .... 143
4.4. Plasma AAG Concentrations on Day 1 and Day 40 for 24 MMT subjects .... 150
4.5. Discussion ....................................................................................... 153
4.5.1. Pharmacokinetics ........................................................................ 153
4.5.1.1. Methadone Pharmacokinetics and differences in routes of
administration ............................................................................. 153
4.5.1.2. Stereospecific methadone pharmacokinetics ......................... 155
4.5.1.3. Changes in Methadone Clearance in this Study ..................... 157
4.5.1.3.1. Presence of illicit drugs ....................................................... 158
4.5.1.4. A Reported Change in Half-Life between Methadone Isotopes .... 160
4.5.1.5. A Study Comparison of Methadone Pharmacokinetic Parameter
Changes ....................................................................................... 161
4.5.1.6. Summary ................................................................................. 165
5. Relationship between Cytochrome P450 CYP3A4 Activity as Measured by the EBT and Changes in Methadone Clearance during Induction and Stabilisation

5.1. Introduction

5.2. Results

5.2.1. Sampling

5.2.2. Modelling of Data

5.2.3. EBT Parameters

5.3. Correlation: Relationship between in vivo Hepatic CYP3A4 Activity and Methadone Enantiomer Clearance

5.4. Discussion

5.4.1. EBT in this study

5.4.2. CYP3A4 activity and Methadone Pharmacokinetics

5.4.3. CYP3A4 activity in general

5.4.3.1. CYP3A4 activity and Benzodiazepines and Liver Function

5.4.3.2. CYP3A4 activity and nutritional status

5.4.4. EBT in general

5.4.4.1. Comparisons with the EBT in the literature

5.4.4.2. Potential Limitations

5.4.4.3. EBT Advantages

5.4.5. Summary

6. Pharmacodynamics and Total Plasma Methadone Concentrations during Induction and Stabilisation of MMT

6.1. Introduction

6.2. Methodological Findings
6.2.1. HPLC with UV chromatography and Total (d0 + d6) Plasma Methadone Concentrations................................................................. 189

6.2.2. Accuracy and Reproducibility of the HPLC with UV Chromatography Assay .................................................................................. 190

6.2.3. Results and Analyses: Plasma Methadone Concentrations and Linear Regression against Dose......................................................... 191

6.2.4. Plasma Methadone Concentrations and Multiple Linear Regression .......... 198

6.2.5. Discussion of Methadone Dose and Plasma Methadone Concentrations .... 199

6.2.5.1. Plasma methadone concentrations during MMT ......................... 200

6.2.5.2. Plasma methadone concentrations in this study......................... 200

6.3. Pharmacodynamic Effects......................................................................................................................... 202

6.3.1. Withdrawal Symptoms measured by the Methadone Symptoms Checklist. 202

6.3.1.1. Categorical Withdrawal Score Results......................................... 203

6.3.1.2. Withdrawal Score and MMT Details ........................................... 206

6.3.1.3. MSC-Categorical Withdrawal Score and Methadone Concentration- Effect Relationship.............................................................. 207

6.3.1.4. Discussion of Withdrawal Symptoms during MMT ..................... 211

6.3.1.4.1. Withdrawal Scores from this study and their relationship with plasma methadone concentrations ........................................... 211

6.3.1.4.2. Summary.................................................................................. 213

6.3.2. Respiratory rate ....................................................................................... 213

6.3.2.1. Respiratory rate Results .................................................................. 214

6.3.2.2. Blood Oxygen Saturation Results .................................................. 217

6.3.2.3. Clinically Significant Respiratory Depression................................. 220

6.3.2.4. Respiratory rate and MMT Details.................................................... 221

6.3.2.5. Blood Oxygen Saturation and MMT Details .................................. 222
6.3.2.6. Respiratory Function and Methadone Concentration-Effect Relationships ................................................................. 224

6.3.2.7. Discussion of Respiratory Function during MMT ................................................. 234
   6.3.2.7.1. Respiratory function in general ............................................................. 234
   6.3.2.7.2. Respiratory function in relation to MMT ............................................. 234
   6.3.2.7.3. Respiratory function correlations with plasma methadone concentrations ......................................................... 236
   6.3.2.7.4. Respiratory function and chronic vs acute opioid treatment in other studies .................................................. 238
   6.3.2.7.5. Summary ......................................................................................... 239

6.3.3. Discussion of all Pharmacodynamics during MMT .................................... 240

7. Continued Heroin/Morphine Use during MMT ................................................. 242
   7.1. Introduction ................................................................................................. 242
   7.2. Heroin/Morphine Results ............................................................................ 242
       7.2.1. Prior to MMT Commencement: Self-Report, Urine, and Hair Sample Analysis ............................................................... 243
       7.2.2. Morphine Assay Performance ................................................................... 247
       7.2.3. Plasma Morphine Concentrations ............................................................ 248
       7.2.4. Comparison of Plasma Morphine Concentrations with Self-Report and Urinalysis ................................................................. 251
   7.3. Continued Heroin Use as a Function of Prior Use, Methadone Dose, and Plasma Methadone Concentrations ............................................. 252
   7.4. Results of Multiple Linear Regression Analyses ................................................. 253
   7.5. Discussion .................................................................................................. 257
       7.5.1. Drug use prior to MMT ........................................................................ 257
7.5.2. Methadone data and continued drug use during MMT ........................................... 258

7.5.3. Prediction of continued heroin or morphine use in 10 subjects during MMT
(Study A) .................................................................................................................. 259

7.5.4. Predictions of continued heroin or morphine use in 14 or 24 MMT subjects
(Studies A and B) .................................................................................................. 260

7.5.4.1. Study protocol comparison ........................................................................ 262

7.5.5. Summary ....................................................................................................... 262

8. Discussion ............................................................................................................. 265

8.1. Subject Retention and Representation of the MMT Population ......................... 265

8.2. Project Findings in Relation to the Project Aims ................................................. 266

8.3. Implications of my Results in Regards to MMT ............................................... 269

8.3.1. Clearance Differences only between Methadone Isomers .............................. 269

8.3.2. No CYP450 3A4 Activity Correlation with Methadone Clearance .............. 271

8.3.3. Characterisation of MMT Induction Phase Pharmacodynamics .................. 272

8.3.4. Continued Opioid Use during MMT .............................................................. 275

8.3.5. Study Limitations .......................................................................................... 277

8.4. What these implications mean to MMT overall .................................................. 278

8.5. Summary and Conclusions .............................................................................. 279

Appendices .............................................................................................................. 280

Appendix 1: Information for Clinics and Clients ...................................................... 280

Appendix 1-1: Poster for Studies A and B ................................................................. 280

Appendix 1-2: Information sheet for Study A Subjects ........................................... 281

Appendix 1-3: Consent Form for Study A Subjects ................................................. 286

Appendix 1-4: Information Sheet for Study B Subjects ........................................... 287

Appendix 1-5: Consent form for Study B Subjects .................................................. 291
Appendix 1-6: Project Summary Sheet for Clinic Staff Use ........................................292

**Appendix 2: Paperwork for Client Files** ................................................................. 293

Appendix 2-1: Case Report Form for Day 1 and Day 40 of Study A ...................... 293
Appendix 2-2: Case Report Form for Days 2-14 and Days 41-49 of Study A ...... 294
Appendix 2-3: Case Report Form for Day 1 and Day 40 of Study B ..................... 295
Appendix 2-4: Pharmacodynamics Notes Sheet ....................................................... 296
Appendix 2-5: Profile of Mood States Questionnaire ............................................. 297
Appendix 2-6: Methadone Symptoms Checklist ...................................................... 298

**Appendix 3: Documentation for Experimental Work** ........................................ 300

Appendix 3-1: LC-MS Methadone Assay Checklist ............................................. 300
Appendix 3-2: LC-MS Methadone Assay Sample Sheet ........................................ 301
Appendix 3-3: LC-MS Methadone Assay Run Sheet ............................................. 302
Appendix 3-4: HPLC Methadone Assay Checklist ............................................... 303
Appendix 3-5: HPLC Methadone Assay Sample Sheet .......................................... 304
Appendix 3-6: HPLC Methadone Assay Run Sheet ............................................... 305
Appendix 3-7: Erythromycin Injection Preparation Instructions for RAH Pharmacy 306
Appendix 3-8: Erythromycin Validation Information Sheet .................................. 307
Appendix 3-9: EBT Validation Report Form .......................................................... 309
Appendix 3-10: Morphine Assay Checklist ............................................................ 310
Appendix 3-11: Morphine Assay Sample Sheet .................................................... 311
Appendix 3-12: Morphine Assay Run Sheet .......................................................... 312

**Appendix 4: An Additional Publication Associated with the Work Contained in this Thesis** .................................................................................................................. 313

Bibliography ............................................................................................................. 321
List of Tables

Table 1-1: Summary of pharmacokinetic parameters (racemic) of intravenously administered methadone reported in the literature.................................................................39

Table 1-2: Summary of pharmacokinetic parameters (racemic) of orally administered methadone reported in the literature.................................................................41

Table 1-3: Summary of pharmacokinetic parameters of methadone enantiomers reported in the literature .................................................................................................62

Table 2-1: Recruitment of Subjects at MMT Centres.................................................................77

Table 2-2: Sampling schedules for Study A and Study B...........................................................83

Table 2-3: Study A Subject Demographics...............................................................................89

Table 2-4: Study B Subject Demographics...............................................................................90

Table 2-5: Demographics of the Total Subject Population.........................................................91

Table 3-1: Subjects’ samples (D* = Day * of MMT) tested by LC-MS for plasma methadone concentrations..............................................................................................93

Table 3-2: Intra-assay validation of plasma d0 and d6 R- and S-methadone concentrations by the LC-MS assay, using the LOQ and QCs from the large validation assay102

Table 3-3: Inter-assay validation of plasma d0 and d6 R- and S-methadone concentrations by the LC-MS assay, using the LOQ and QCs from 3 validation assays performed on separate days.................................................................................................103

Table 3-4: Intra-assay validation data for the HPLC-UV assay for plasma R- and S-methadone concentrations using the LOQ and QCs from the large validation assay .................................................................................................112

Table 3-5: Inter-assay validation data for the HPLC-UV assay for plasma R- and S-methadone concentrations using the LOQ and QCs from 3 validation assays performed on separate days.................................................................................................113
Table 3-6: Intra-assay validation data for the plasma morphine concentration assay using the QCs and two lowest standards from the large validation assay .................. 120

Table 3-7: Inter-assay validation data for the plasma morphine concentration assay using the QCs and two lowest standards from both validation assays .................. 120

Table 3-8: Intra-assay validation data of the plasma AAG concentration radioimmunoassay in a NOR-Partigen® plate.................................................................................. 132

Table 4-1: Ongoing inter-assay accuracy, precision, r² value and slope for assays (n = 11) of plasma d0 and d6 R- and S-methadone concentrations in subjects’ samples142

Table 4-2: Individual pharmacokinetic parameters for R-, S-, and rac-methadone during induction and steady state phases of MMT following IV-dose of 5 mg d6-methadone in 10 Study A subjects .............................................................. 147

Table 4-3: Comparison between R- and S-methadone pharmacokinetic parameters during induction and steady state phases of MMT in 10 subjects after IV-dose of 5 mg d6-methadone............................................................................................................ 149

Table 4-4: Ongoing inter-assay accuracy and precision for assays (n = 8) of plasma AAG concentrations in subjects’ samples. .................................................................150

Table 4-5: Individual plasma AAG concentrations (mg/dl) in 10 Study A subjects on Day 1 and Day 40 of MMT ......................................................................................... 151

Table 4-6: Individual plasma AAG concentrations (mg/dl) in 14 Study B subjects on Day 1 and Day 40 of MMT ......................................................................................... 151

Table 5-1: Erythromycin Breath Test parameters in 24 Subjects on a) Day 1 and b) Day 40 of MMT ............................................................................................................. 172

Table 5-2: Comparison of Day 1 and Day 40 Erythromycin Breath Test parameters in 24 MMT subjects ...................................................................................................... 173

Table 5-3: Correlations between erythromycin clearance and R-, S-, and rac-methadone clearances on Day 1 and Day 40 of MMT in 10 Study A subjects................. 176
Table 5-4: Correlations between EBT parameter CER$_{20\text{min}}$ and R-, S-, and rac-methadone clearances on Day 1 and Day 40 of MMT in 10 Study A subjects....................... 176

Table 5-5: Correlations between EBT parameter $T_{\text{max}}$ and R-, S-, and rac-methadone clearances on Day 1 and Day 40 of MMT in 10 Study A subjects....................... 177

Table 5-6: Erythromycin, R-, S-, and rac-methadone clearance change (%) from Day 1 to Day 40 of MMT in 10 Study A subjects.............................................................. 177

Table 5-7: Correlations between percentage and direction (positive or negative) of erythromycin clearance change and R-, S-, and rac-methadone clearance changes in 10 Study A subjects during MMT.................................................. 178

Table 6-1: Ongoing inter-assay accuracy, precision, $r^2$ value and slope for HPLC with UV chromatography assays (n = 17) of plasma R- and S-methadone concentrations in subjects’ samples........................................................................................................ 191

Table 6-2: Regression analysis of day of MMT, methadone dose (mg), and time since dose (h), as predictors of racemic plasma methadone concentrations (ng/ml) in 10 Study A subjects........................................................................................................ 199

Table 6-3: Regression analysis of day of MMT, methadone dose (mg), and time since dose (h), as predictors of categorical withdrawal scores (0-16) in 10 Study A subjects........................................................................................................ 206

Table 7-1: Percentage of a) Study A subjects, b) Study B subjects, and c) total subjects using illicit drugs prior to Day 1 (Day 1) and between Day 1 and Day 40 (Day 40), as measured by self-report and urinalysis.......................................... 244

Table 7-2: Day 1 hair sample analysis results......................................................................................................................... 246

Table 7-3: Ongoing inter-assay accuracy, precision, $r^2$ value and slope for assays (n = 8) of plasma morphine in subjects’ samples........................................................................ 248

Table 7-4: Plasma morphine concentrations for 10 Study A subjects on Days 1-14 and 40-49 of MMT........................................................................................................ 249
Table 7-5: Analysis of Variance (2-way) of median plasma morphine concentrations in 10 Study A subjects during induction and steady state phases of MMT .......... 250

Table 7-6: Regression analyses of [prior drug use (Day 1 hair morphine concentration, hair monoacetylmorphine (MAM) concentration, and hair heroin concentration (ng/mg)), mean methadone dose (mg), and mean plasma R-, S-, and rac-methadone concentration (ng/ml)], as predictors of continued opioid use during MMT as expressed by median plasma morphine concentration (ng/ml) in (1) 10 Study A subjects, (2) 14 non-incarcerated subjects, and (3) 24 Study A and Study B subjects. ................................................................. 254

Table 7-7: Regression analyses of prior drug use (Day 1 hair morphine concentration (ng/mg)), and mean plasma R-methadone concentration (ng/ml), as predictors of continued opioid use during MMT, expressed by median plasma morphine concentration (ng/ml) in (1) 10 Study A subjects, (2) 14 non-incarcerated subjects, and (3) 24 Study A and Study B subjects. ............................................. 255

Table 7-8: Regression analyses of prior drug use (Day 1 hair morphine concentration and hair monoacetylmorphine (MAM) concentration (ng/mg)), and mean plasma rac-methadone concentration (ng/ml), as predictors of continued opioid use during MMT as expressed by median plasma morphine concentration (ng/ml) in 24 Study A and Study B subjects......................................................... 256

Table 7-9: Regression analyses of prior drug use (Day 1 hair morphine concentration (ng/mg)), as a predictor of continued opioid use during MMT as expressed by median plasma morphine concentration (ng/ml) in (1) 10 Study A subjects, (2) 14 non-incarcerated subjects, and (3) 24 Study A and Study B subjects. ........... 257
List of Figures

Figure 1-1: Phases of μ opioid receptor (MOP) activation .......................................................... 4

Figure 1-2: Timeline of μ opioid receptor (MOP) activation represented on log scale ............ 5

Figure 1-3: Hyperexcitability and enhanced transmitter release during opioid withdrawal .. 17

Figure 1-4: Chemical Structure of Methadone with the chiral atom marked with an asterisk 35

Figure 1-5: Methadone Hydrochloride with stable-labels of \(^{(2H_6)}\)-methadone hydrochloride indicated in bold type, and the chiral carbon identified by an asterisk .......... 47

Figure 1-6: Methadone and metabolites EDDP and EMDP ..................................................... 49

Figure 1-7: Increase in plasma methadone concentration over time, predicted from Population Pharmacokinetic analysis of single dose and steady-state data .......... 52

Figure 3-1: Representative LC-MS chromatograms of a) drug-free plasma sample and
b) subject plasma sample containing both unlabelled and labelled methadone .. 99

Figure 3-2: Representative HPLC chromatograms of a) a drug-free plasma sample and b) a
subject plasma sample ........................................................................................................... 110

Figure 3-3: Representative Coulochem system chromatograms of an a) drug-free plasma
sample and a b) subject plasma sample ................................................................................. 118

Figure 3-4: Urea breath test device with mouthpiece and drinking straw with alkaline trapping solution. ............................................................................................................... 125

Figure 4-1: Mean (±SEM) plasma R- and S-\(^{2H_6}\)-methadone concentrations after a 5 mg IV

dose in 10 Study A subjects during a) induction and b) steady state phase of MMT ............................................................ 144

Figure 4-2: Changes in systemic plasma R- and S-methadone clearances (L/h) between induction and steady state phases of MMT in 10 Study A subjects (P = 0.41
and 0.37, respectively) ........................................................................................................ 149

Figure 4-3: Plasma AAG concentrations (mg/dl) on Day 1 and Day 40 of MMT for 24 subjects ......................................................................................................................... 152
Figure 5-1: CER profiles of a) the volunteer, and b) a representative subject on Day 1, and 
c) Day 40 of MMT, following a 4 μCi IV dose of 14C-erythromycin .......... 170

Figure 5-2: Changes in erythromycin clearance from Day 1 to Day 40 of MMT
(n = 24 subjects) ............................................................................................ 172

Figure 5-3: Correlations between erythromycin clearance (L/h) and methadone clearance 
(L/h) in 10 Study A MMT subjects: ............................................................. 175

Figure 5-4: Correlations between CER20min (% dose/min) and methadone clearance (L/h) in 
10 Study A MMT subjects: ........................................................................ 175

Figure 5-5: Correlations between Tmax (min) and methadone clearance (L/h) in 10 Study A 
MMT subjects: .................................................................................................. 176

Figure 6-1: Methadone dose (mg) changes during MMT in a) 10 Study A subjects and 
b) 14 Study B subjects .................................................................................. 192

Figure 6-2: Total Day 1 and Day 40 methadone doses in 24 MMT Subjects. .......... 193

Figure 6-3: Correlations (with 95 % CI) between mean Study A methadone dose (mg) and 
peak and trough plasma methadone concentrations (ng/ml) on 14 days of MMT 
(Day 1 to 14), where each point represents a single day ............................... 194

Figure 6-4: Correlations (with 95 % CI) between individual Study A Day 40 methadone 
dose (mg) and peak and trough plasma a) R-, b) S-, and c) rac-methadone 
concentrations (ng/ml) ................................................................................ 195

Figure 6-5: Mean plasma methadone concentrations (ng/ml) during MMT for Study A 
subjects ........................................................................................................... 197

Figure 6-6: Withdrawal symptom scores (Mean ± SEM) at time of trough and peak R-
Methadone Concentrations in 10 Study A MMT subjects  
(maximum score = 16) .................................................................................. 204

Figure 6-7: Pre- and post-dose withdrawal symptom score (0-16) in 24 subjects on Day 1 
and Day 40 of MMT. ..................................................................................... 205
Figure 6-8: Concentration-effect relationships between plasma R-methadone concentration (ng/ml) and MSC categorical withdrawal symptom score. 208

Figure 6-9: Plasma R-methadone concentrations and withdrawal scores (0-16) per day of induction phase of MMT in 10 Study A subjects. 210

Figure 6-10: Respiratory rates (mean ± SEM) at time of trough and peak plasma R-methadone concentrations in 10 Study A subjects during MMT. 215

Figure 6-11: Pre- and post-dose respiratory rates (breaths/min) in 24 subjects (Study A and Study B) on Day 1 and Day 40 of MMT. 216

Figure 6-12: Blood oxygen saturation (mean ± SEM) at time of trough and peak plasma R-methadone concentrations in 10 Study A subjects. 218

Figure 6-13: Pre- and post-dose blood oxygen saturation (%) in 24 subjects (Study A and Study B) on Day 1 and Day 40 of MMT. 219

Figure 6-14: Concentration-effect relationships between plasma R-methadone concentration (ng/ml) and respiratory rate (breaths/min). 225

Figure 6-15: Concentration-effect relationships between plasma R-methadone concentration (ng/ml) and blood oxygen saturation (%). 227

Figure 6-16: Concentration-effect relationships between peak plasma R-methadone concentration (ng/ml) and respiratory rate (breaths/min). 230

Figure 6-17: Concentration-effect relationships between peak plasma R-methadone concentration (ng/ml) and blood oxygen saturation (%). 231

Figure 6-18: Plasma R-methadone concentrations (ng/ml) and respiratory rate (breaths/min) at times of trough and peak sampling per day of induction phase of MMT in 10 Study A subjects. 232

Figure 6-19: Plasma R-methadone concentrations (ng/ml) and blood oxygen saturation (%) at times of trough and peak sampling per day per day of induction phase of MMT in 10 Study A subjects. 233
Figure 7-1: Median plasma morphine concentration and percentage of Study A subjects testing positive per day of MMT ................................................................. 251

List of Equations

Equation 3-1: ........................................................................................................................ 106
Equation 3-2: ........................................................................................................................ 106
Equation 3-3: ........................................................................................................................ 106
Equation 3-4: ........................................................................................................................ 106
Equation 3-5: ........................................................................................................................ 128
Equation 3-6: ........................................................................................................................ 128
Equation 3-7: ........................................................................................................................ 129

List of Appendices

Appendix 1: Information for Clinics and Clients ................................................................. 280
Appendix 2: Paperwork for Client Files ............................................................................ 293
Appendix 3: Documentation for Experimental Work ....................................................... 300
Appendix 4: An Additional Publication Associated with the Work Contained in this Thesis ............................................................................................................. 313
Abstract

- Methadone is the foremost, long-standing pharmacological treatment for opioid addiction. It has been shown to have considerable cost benefit to the community and to decrease mortality. Despite methadone’s decades-long use, much is still unknown regarding its clinical pharmacology, particularly during the induction phase of Methadone Maintenance Treatment (MMT).

- Contrary to previous reports, I found systemic methadone clearance does not increase significantly between induction and steady state phases of MMT, and did not approach the previously reported 3-fold increase. Clinical dose prescription based on the premise of metabolism auto-induction could increase risk of respiratory depression.

- Significant differences between R- and S-methadone pharmacokinetics showed the importance of stereoselective measurement in a clinical situation and significant plasma concentration-effect relationships demonstrated their potential influence on induction pharmacodynamics.

- Small increases in CYP3A4 activity as measured by the Erythromycin Breath Test from Day 1 to Day 40 of MMT were not correlated with changes in methadone clearance. CYP3A4 activities were informative but would be insufficient for use as a sole predictor of methadone clearance during MMT.

- Clinically significant respiratory depression occurred in 20% of subjects, at times of peak plasma R-methadone concentrations, after reports of withdrawal symptoms at pre-dose sampling times, and irrespective of illicit opioid use. Utilisation of both respiratory rate and blood oxygen saturation measurements provided a good indication of respiratory risk for individuals.
• Although prior opioid use was a strong predictor of continued use during MMT, adoption of a new equation ("abc") and comprehensive documentation of each individual’s MMT may increase prediction of MMT success.
• Even in light of recent advances in opioid substitution therapies, MMT’s advantages ensure it is still at the forefront of addiction treatment. Careful choice of methodology enabled narrowing of this investigation to those factors most relevant in methadone pharmacology and most responsible for MMT success or failure, and therefore extending previous knowledge of this area. Such data might be utilised to develop a clinically applicable model for MMT, and help provide clients with a safe and uncomplicated transition from heroin use to methadone induction in the future.

Some people dream of great accomplishments…
Others stay awake and get them done!

Successories, Illinois
heroin(e) - anonymous

stayed away my whole life long
for fear of getting burned
till that fateful night in smoke and song
my steadfast head was turned
a drug more potent than any other
an addiction truer than fact
it comes in many shapes and colours
this one in red and black
overcome by temptation
interest outweighs prudence
destined for lust upon creation
the drug welcomes my crudeness
defeated by its savoury smell
my pleasure-lust takes over
as effects take hold i can hardly tell
why i'd ever wish to be sober
i remember well, no need to try
why i found it so appealing
i still recall my first high
with vivid mem'ry of the feeling
for weeks it went on like this
i had to get my fix
and those weeks were nothing short of bliss
my mind played wond'rous tricks
but then one day it all ran out
no drug to fill my veins
and left me alone, empty, in doubt
no heroin(e) to soothe the pains
a year and one half has gone and came
and the addiction still remains
simple mention of the sweet substance' name
sends flashbacks 'cross my brain
and once every now and then
i'll try to get some more
but empty-handed again and again
i'm left hollow to the core
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Erin Brooke Morton, 17 January 2007
Acknowledgements

My sincere thanks go to my supervisors Andrew Somogyi, Felix Bochner, and Jason White, my nurses Amanda Mitchell and Charlotte Smith, and co-worker David Foster. I would not have been able to start, let alone complete my project without their help. I’d like to acknowledge my personal funding from the Royal Adelaide Hospital, Dawes Scholarship; the University of Adelaide, Methadone Induction Scholarship; and the NHMRC grant that provided funding for the research itself. Also to be thanked are the wide range of people and places involved. Many thanks go to the staff from Warinilla Clinic, Northern and Southern DASC clinics, with special thanks to Hayley at the DASC library. From the Brian Burdekin Clinic Drs John Foley and Damien Mead, from Willunga’s Moore St Clinic: Drs Philip Clarke and Rhys Henning, and from my past employer Hospital Pharmacy Services, Ben Stevenson and Camille Schubert. Within the prisons I would like to thank Drs Chris Holmwood, Chris Clohesy, and Greg Dayman, as well as Jan, Sue, Richard, Fay, Diane, Peter, Brian, Wayne, Joy, Bob, and especially Yasmin. From the RAH my thanks go to Max Bellon and Silvana from Nuclear Medicine, Virginia, Barbara and Kylie from Pharmacy Production, and to all the staff at CMAX. From the university financial department, I thank Genevieve for keeping track of all the cheques. From my own department, for their myriad times of help that enabled me to complete this project, as well as their pleasant company, I would very much like to thank Andy, David N, Aaron, Janet, Pete, Gordon, Mark, and Andrea.

Finally, to Harry and Lyn, the Morton family, and to my own extended family of grandmothers, aunts, uncles and cousins - thank you for your continual support.
Most importantly, I dedicate this thesis with love and thanks:

To my parents Neil and Andrea

You brought me up to believe in myself. To want to prove, if only to myself, that I left no barrier untried. Above all, to have the confidence to quit work and take the plunge.

Thank you.

To my brother Liam

We were blessed with such different talents, skills and interests. And yet at the core we still have so much in common. You breathe life into my creative side, and provide balance in my opposite.

This is for both of us.

To my husband Adam

I could not have done it without you. You anchored me- with your support, smiles and encouragement, and nagging to knuckle down – or lighten up – whenever I needed to hear it. You will always have my love, and my friendship also.

Forever yours.
Publications and presentations in support of this thesis

Somogyi, A.A., Morton, E.B., Bochner, F., White, J.M., Foster, D.J.R., Eichelbaum, M.


Additional publications and presentations associated with the work contained in this thesis

Foster, D.J.R., Morton, E.B., Heinkele, G., Mürdter, T., and Somogyi, A. Stereoselective quantification of methadone and a $d_6$-labelled isotopomer using high performance liquid chromatography-atmospheric pressure chemical ionization mass-spectrometry: Application to a pharmacokinetic study in a methadone maintained subject. Therapeutic Drug Monitoring 28 (4), 559-567.
Abbreviations, prefixes and symbols

δ  Delta
κ  Kappa
μ  Mu
σ  Sigma
AAG  α₁-acid glycoprotein / Alpha-1-acid glycoprotein
ABCB1  Multi-drug resistance gene (current name)
AEs  Adverse Effects
AIDS  Acquired Immuno-deficiency Syndrome
AIHW  Australian Institute of Health and Welfare
Ala  Alanine
ALT  Alanine transaminase
Asn  Asparagine
Asp  Aspartic acid
ASPD  Antisocial Personality Disorder
AST  Aspartate aminotransferase-serum
AUC  Area Under the plasma concentration-time Curve
BD  Becton Dickinson
C  Cytosine
Ca²⁺  Calcium Ion
CaMKII  Ca²⁺/calmodulin-dependent protein kinase II
cAMP  Adenosine 3',5'-cyclic monophosphate
CER  Percentage of $^{14}$C-Erythromycin dose released per minute as labelled carbon dioxide
CER$_{20\text{min}}$  CER at, for example, 20 minutes after $^{14}$C-Erythromycin dose
CLND  Chemiluminescent-nitrogen detector
CoA  Coenzyme A
CNS  Central Nervous System
CP  Cold Pressor (Test)
CV  Coefficient of Variation
CYP450  Cytochrome P450 enzymes
CYP3A4/CYP2D6 etc  Cytochrome P450 3A4/2D6 etc
CYP2D6  Cytochrome P450 2D6 gene
d₀  Unlabelled methadone
d₃  Deuterium labelled methadone (3 deuterium atoms)
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>d6</td>
<td>Deuterium labelled methadone (6 deuterium atoms)</td>
</tr>
<tr>
<td>D*</td>
<td>Day * of MMT</td>
</tr>
<tr>
<td>DAMGO</td>
<td>[D-Ala²,N-MePhe⁴,Gly-ol⁵] enkephalin</td>
</tr>
<tr>
<td>DASC</td>
<td>Drug &amp; Alcohol Services Council</td>
</tr>
<tr>
<td>DCS</td>
<td>Department of Correctional Services</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DPHM</td>
<td>Diphenhydramine</td>
</tr>
<tr>
<td>dpm</td>
<td>Decay per minute</td>
</tr>
<tr>
<td>DRD2</td>
<td>Dopamine D2 Receptor</td>
</tr>
<tr>
<td>DSM-III-R</td>
<td>Borderline Personality Disorder</td>
</tr>
<tr>
<td>EBT</td>
<td>Erythromycin Breath Test</td>
</tr>
<tr>
<td>EDDP</td>
<td>2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine</td>
</tr>
<tr>
<td>EMDP</td>
<td>2-ethyl-5-methyl-3,3-diphenpyrrole</td>
</tr>
<tr>
<td>EUT</td>
<td>Erythromycin Urine Test</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma Aminobutyric Acid</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas Chromatography – Mass Spectrometry</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>GRK</td>
<td>G-protein coupled receptor kinases</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HQC</td>
<td>High Concentration Quality Control</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>Concentration for 50 % inhibition</td>
</tr>
<tr>
<td>INR</td>
<td>International Normalized Ratio</td>
</tr>
<tr>
<td>IS</td>
<td>Internal Standard</td>
</tr>
<tr>
<td>iv</td>
<td>Intravenous</td>
</tr>
<tr>
<td>K⁺</td>
<td>Potassium Ion</td>
</tr>
<tr>
<td>LAAM</td>
<td>Levo-a-acetylmethadol / Levo-alpha-acetylmethadol</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid Chromatography – Mass Spectrometry</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of Quantification</td>
</tr>
<tr>
<td>LQC</td>
<td>Low Concentration Quality Control</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MDR1</td>
<td>Multi-drug resistance gene (old name)</td>
</tr>
<tr>
<td>MIA-</td>
<td>Methadone Induction Study A subject</td>
</tr>
<tr>
<td>MIB-</td>
<td>Methadone Induction Study B subject</td>
</tr>
<tr>
<td>MMT</td>
<td>Methadone Maintenance Treatment</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MOP</td>
<td>Mu Opioid (Peptide) Receptor</td>
</tr>
<tr>
<td>MQC</td>
<td>Medium Concentration Quality Control</td>
</tr>
<tr>
<td>MSC</td>
<td>Methadone Symptoms Checklist</td>
</tr>
<tr>
<td>MSCWYN</td>
<td>Methadone Symptoms Checklist – Withdrawal subscale, categorical scoring</td>
</tr>
<tr>
<td>NAS</td>
<td>Neonatal Abstinence Syndrome</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NTS</td>
<td>Tractus solitarius</td>
</tr>
<tr>
<td>OPRM1</td>
<td>Mu Opioid Receptor Gene</td>
</tr>
<tr>
<td>ORM</td>
<td>Orosomucoid</td>
</tr>
<tr>
<td>PAR</td>
<td>Peak Area Ratio</td>
</tr>
<tr>
<td>PD</td>
<td>Pharmacodynamics</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak Expiratory Flow</td>
</tr>
<tr>
<td>P-gp</td>
<td>P-glycoprotein</td>
</tr>
<tr>
<td>PhD</td>
<td>Doctorate of Philosophy</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetics</td>
</tr>
<tr>
<td>PKA</td>
<td>Cyclic AMP-dependent protein kinase (protein kinase A)</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein Kinase C</td>
</tr>
<tr>
<td>POMS</td>
<td>Profile of Mood States</td>
</tr>
<tr>
<td>QC</td>
<td>Quality Control</td>
</tr>
<tr>
<td>r²</td>
<td>Coefficient of Determination</td>
</tr>
<tr>
<td>R-</td>
<td>Right</td>
</tr>
<tr>
<td>Rac-</td>
<td>Racemic</td>
</tr>
<tr>
<td>RAH</td>
<td>Royal Adelaide Hospital</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Reverse-Phase High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>SA</td>
<td>South Australia</td>
</tr>
<tr>
<td>SAEs</td>
<td>Serious Adverse Effects</td>
</tr>
<tr>
<td>SAVIVE</td>
<td>South Australia Voice for IV Education</td>
</tr>
<tr>
<td>S</td>
<td>Sinister</td>
</tr>
<tr>
<td>S1/S2</td>
<td>Standard number 1/ Standard number 2</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>SROM</td>
<td>Slow Release Oral Morphine</td>
</tr>
<tr>
<td>Thy</td>
<td>Thymine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>Time of Maximum labelled carbon dioxide exhalation</td>
</tr>
<tr>
<td>TH</td>
<td>Alpha-tocopherol</td>
</tr>
<tr>
<td>TMD</td>
<td>Total Mood Disturbance</td>
</tr>
<tr>
<td>TQ</td>
<td>Alpha-tocopherolquinone</td>
</tr>
<tr>
<td>UCR</td>
<td>Urinary Cortisol Ratio</td>
</tr>
<tr>
<td>US/USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UV-HPLC</td>
<td>High Performance Liquid Chromatography with Ultraviolet Detection</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>