

CONTENTS

TITLE	PAGE NO.
CHAPTER 1: INTRODUCTION	1-27
1.1 Novel analytical method development and validation	1
1.2 Stability indicating assay method	3
1.3 QBD (Quality by design) and statistical analysis for normal distribution of data	11
1.4 Degradation kinetics study and total error approach	12
1.5 Impurity profiling	13
1.6 Bio analytical method development and validation	15
1.7 Chemometric assisted analytical method development for counterfeit drugs	17
1.8 Microfluidic device fabrication and principles	20
1.9 References	21
CHAPTER 2: AIMS AND OBJECTIVES	28 - 29
CHAPTER 3: TO DEVELOP AND VALIDATE STABILITY INDICATING ANALYTICAL METHOD FOR CYPROHEPTADINE HCL ALONG WITH SEPARATION, ISOLATION AND CHARACTERIZATION OF MAJOR DEGRADATION PRODUCT.	30 - 79
3.1. Selection of drug	30
3.2 Drug profile	30
3.3 Literature review	31
3.4. Section –A	32-52
Development and validation of Stability Indicating HPLC method for Cyproheptadine HCl	
3.4.1 Experimental	32
3.4.1.1 Chemicals and materials	32
3.4.1.2 Equipments and analysis conditions (UV, HPLC-PDA)	32
3.4.1.3 Preparation of calibration samples and quality control samples	33

3.4.1.4 Preparation of buffer solution	33
3.4.1.5 Preparation of degradation Products (DP's)	34
3.4.1.6 HPLC method and sample preparation	35
3.4.1.7 Analysis of Formulation	36
3.4.1.8 Method validation	36
3.4.2 Results and discussion	36
3.4.2.1 Determination of suitable wavelength	36
3.4.2.2 Method optimization and development	36
3.4.2.3 Method validation using ICH Q2(R1) guideline	41
3.4.2.4 Stress degradation studies	43
3.4.2.5 Peak purity studies	47
3.4.2.6 Applicability of method	52
3.5 Section-B	53-76
Isolation and characterization of major degradation product of CPH	
3.5.1 Experimental	53
3.5.1.1 Equipments and Chromatographic Conditions (TLC, LC/MS/MS, NMR, FTIR and DSC conditions)	53
3.5.2 Results and discussion	54
3.5.2.1 Isolation of impurity, Data interpretation and characterization by LC/MS/MS, NMR, IR and DSC study	54
3.5.2.2 Proposed degradation pathway	76
3.6 Conclusion	77
3.7 References	77

CHAPTER 4: DEVELOPMENT OF VARIOUS CLASSICAL AND CHEMOMETRIC ASSISTED UV SPECTROPHOTOMETRIC AND LC-PDA METHODS FOR SIMULTANEOUS ESTIMATION OF CHLORHEXIDINE GLUCONATE AND CETRIMIDE	80- 155
4.1. Selection of drug combination	80
4.2 Drug profiles	80
4.3 Literature review	82
4.4 Section –A Development of various classical and chemometric assisted UV spectrophotometric for simultaneous estimation of Chlorhexidine gluconate and Cetrимide	83-122

4.4.1 Experimental approach UV methods developed are as follows: 4.4.1.1 Vieordt's method (Simultaneous equation method) 4.4.1.2 First derivative spectroscopy method 4.4.1.3 Absorption ratio spectra method 4.4.1.4 Mean centering of ratio spectra method 4.4.1.5 Multicomponent analysis method 4.4.1.6 Classical least squares 4.4.1.7 Inverse least squares 4.4.1.8 Principal component regression 4.4.1.9 Partial least squares 4.4.1.10 Statistical analysis	83
4.4.2 Experimental study	89
4.4.2.1 Chemicals and materials	89
4.4.2.2 Equipments and analysis conditions	89
4.4.2.3 Preparation of calibration samples and quality control samples	90
4.4.2.4 Methodology	90
4.4.2.5 Applicability of the method	92
4.4.2.6 Method Validation	93
4.4.2.7 Statistical analysis	94
4.4.3 Results and discussion	94
4.4.3.1 Method optimization and development	94
4.4.3.2 Method validation using ICH Q2(R1) guideline	111
4.4.3.3 Applicability of developed method for analysis of formulation	115
4.4.3.4 Statistical analysis using One-way Anova test and further by Tukey HSD test and Scheffe test.	115
4.5 Section –B	123-143
Development and validation of RP-HPLC method for Chlorhexidine gluconate and Cetrime drug combination	123
4.5.1 Experimental	
4.5.1.1 Chemicals and materials	123
4.5.1.2 Equipments and analysis conditions	123
4.5.1.3 Preparation of mobile phase buffer	123
4.5.1.4 Preparation of mobile phase	123
4.5.1.5 Preparation of stock solutions	123
4.5.1.6 Preparation of working standards and calibration curve solutions	123
4.5.1.7 Method Development	124
4.5.1.8 Applicability of the method	124
4.5.1.9 Method Validation	124

4.5.1.10 Statistical analysis	125
4.5.2 Results and discussion	125
4.5.2.1 Determination of suitable wavelength	125
4.5.2.2 Method optimization and development	125
4.5.2.3 Method validation using ICH Q2(R1) guideline and DOE	130
4.5.2.4 Peak purity studies	131
4.5.2.5 Robustness of the method was determined by using DOE approach	132
4.5.2.6 Applicability of developed method for analysis of formulation	139
4.5.2.7 Statistical analysis using Anderson darling normality test	140
4.6 Section – C	143-151
4.6.1 Experimental Development and validation of RP-HPLC method for Cetrimide	143
4.6.1.1 Chemicals and materials	
4.6.1.2 Equipments and analysis conditions	143
4.6.1.3 Preparation of mobile phase buffer	143
4.6.1.4 Preparation of mobile phase	143
4.6.1.5 Preparation of stock solutions	143
4.6.1.6 Preparation of working standards and calibration curve solutions	144
4.6.1.7 Method Development	144
4.6.1.8 Applicability of the method	144
4.6.1.9 Method Validation	144
4.6.1.10 Statistical analysis	144
4.6.2 Results and discussion	145
4.6.2.1 Determination of suitable wavelength	145
4.6.2.2 Method optimization and development	145
4.6.2.3 Method validation using ICH Q2(R1) guideline	148
4.6.2.4 Peak purity studies	149
4.6.2.5 Applicability of developed method for analysis of formulation	150
4.6.2.6 Statistical analysis using Anderson darling normality test	150
4.7 Conclusion	152
4.8 References	153

CHAPTER 5: DEVELOPMENT OF STABILITY INDICATING ANALYTICAL METHOD FOR POMALIDOMIDE USING DOE AND TOTAL ERROR APPROACH: APPLICATION TO DEGRADATION KINETICS STUDY	156 - 196
5.1. Selection of drug	156
5.2 Drug profile	156
5.3 Literature review	157

5.4 Section –A	152
Development and validation of HPLC method for Pomalidomide and application of total error approach for checking uncertainty of data distribution	157
5.4.1 Experimental	
5.4.1.1 Chemicals and materials	158
5.4.1.2 Equipments and analysis conditions	158
5.4.1.3 HPLC method and sample preparation	158
5.4.1.4 Analysis of Formulation	158
5.4.1.5 Method validation	158
5.4.1.6 Application of Total error approach	158
5.4.2 Results and discussion	159
5.4.2.1 Determination of suitable wavelength	159
5.4.2.2 Method optimization and development	159
5.4.2.3 Method validation using ICH Q2(R1) guideline	161
5.4.2.4 Total error approach	163
5.4.2.5 Applicability of developed method for analysis of formulation	165
5.5 Section – B Development of stability indicating HPLC method for Pomalidomide and application to degradation kinetics study	166
5.5.1 Experimental	
5.5.1.1 Chemicals and materials	166
5.5.1.2 Equipments and analysis conditions	166
5.5.1.3 Preparation of standard drug sample	166
5.5.1.4 Preparation of Degradation Products (DP's)	166
5.5.1.5 Method optimization and development utilizing Design of experiments	167
5.5.1.6 Preparation Degradation kinetics study samples	168
5.5.2 Results and discussion	168
5.5.2.1 Stability studies	168
5.5.2.2 Stress degradation studies	169
5.5.2.3 HPLC method optimization using Design of experiments	173
5.5.2.4 Peak purity studies	181
5.5.2.5 Degradation kinetics study	183
5.6 Conclusion	194
5.7 References	195

CHAPTER 6: BIOANALYTICAL METHOD DEVELOPMENT FOR CYPROHEPTADINE HCl	197 - 218
6.1. Selection of drug	197

6.2 Drug profile	197
6.3 Literature review	198
6.4 Section –A	198
6.4.1 Experimental Bionalytical method development for Cyproheptadine HCl in human plasma and application to rat pharmacokinetic study	198
6.4.1.1 Chemicals and materials	198
6.4.1.2 Equipments and analysis conditions	198
6.4.1.3 Sample preparation	198
6.4.1.4 Sample pre-treatment for extraction of drug from biological matrix	199
6.4.1.5 HPLC method	200
6.4.1.6 Preparation of buffer solution	200
6.4.1.7 Pharmacokinetic study	200
6.4.1.8 Method Validation	200
6.4.2 Results and discussion	200
6.4.2.1 Determination of suitable wavelength	200
6.4.2.2 Selection of internal standard	201
6.4.2.3 Sample extraction methods	201
6.4.2.4 Method optimization and development	202
6.4.2.5 Method validation using ICH Q2(R1) guideline	205
6.4.2.6 Peak purity studies	210
6.4.2.7 Stability studies	211
6.4.2.8 Pharmacokinetics study	213
6.4.2.9 Peak purity studies after pharmacokinetic studies	214
6.5 Conclusion	217
6.6 References	217

CHAPTER 7: TO DEVELOP CHEMOMETRIC ASSISTED AND HPLC ANALYTICAL METHODS FOR CHECKING THE ADULTERATION OF PHYTOPHARMACEUTICALS	219 - 267
7.1. Selection of phytopharmaceuticals prone to be adulterated	219
7.2 Drug profiles of synthetic analogues being counterfeited	219
7.3 Drug profiles of Ashwagandha and potent chemical constituents in Withanolides taken as herbal marker	222
7.4 Literature review	225

7.5 Section –A	225
7.5.1 Experimental Development and validation of simple RP-HPLC method for checking adulteration of Sildenafil citrate, Verdenafil, Tadalafil in Ashwagandha herbal tablets	225
7.5.1.1 Chemicals and materials	226
7.5.1.2 Equipments and analysis conditions	226
7.5.1.3 Sample preparation for HPLC method	227
7.5.1.4 Preliminary trials and need for application of risk based QBD approach	227
7.5.1.5 Risk based QBD approach for development of optimized HPLC method	228
7.5.1.6 Method Validation	238
7.5.2 Results and discussion	238
7.5.2.1 Selection of ATP	238
7.5.2.2 Application of CNX approach for risk assessment and selection of factors affecting the chromatographic method	239
7.5.2.3 Application of QBD for screening of factors for optimization of chromatographic method	239
7.5.2.4 QBD based HPLC method development using full factorial design	240
7.5.2.5 Chromatographic method optimization	246
7.5.2.6. Method validation using ICH Q2 (R1) guideline	247
7.5.2.7 Application of developed method for differentiating between counterfeit, placebo and marketed samples	248
7.6 SECTION –B Development of chemometric assisted analytical method for checking adulteration of Sildenafil citrate, Verdenafil, Tadalafil in Ashwagandha herbal tablets using NIR, Raman and ATR data	249
7.6.1 Experimental	
7.6.1.1 Chemicals and materials	249
7.6.1.2 Equipments and analysis conditions	249
7.6.1.3 Sample preparation and analysis by various Spectroscopic methods	249
7.6.1.4 Chemometrics	250
7.6.2 Results and discussion	251
7.6.2.1 Sample analysis using NIR, Raman and ATR	251
7.6.2.2 Statistical analysis by chemometrics techniques like PCA and HCA	253
7.6.2.3 Application of Savitzky Golay derivatization for chemometrics techniques like PCA and HCA	257
7.6.2.4 Summary of statistical analysis by chemometrics	263
7.7 Conclusion	263
7.8 References	264

CHAPTER 8: SMART PHONE BASED LOW-COST AND RAPID ESTIMATION OF ANALYTES BY USING PAPER MICROFLUIDIC DEVICE	268 - 296
8.1 Literature survey for selection of fabrication technique for development of microfluidic device	268
8.2 Highlights of paper microfluidic chip developed at our laboratory.	268
8.3 Section –A	269
8.3.1 Experimental Estimation of Tadalafil as adulterant in herbal formulation by paper microfluidic device	269
8.3.1.1 Brief outline for selection for herbal formulation prone to be adulterated and for the synthetic analogue prone to be counterfeited	270
8.3.1.2 Literature review	270
8.3.1.3 Drug profile	270
8.3.1.4 Chemicals and materials	270
8.3.1.5 Fabrication of paper microfluidic device	271
8.3.1.6 Reagents and sample preparation	272
8.3.2 Results and discussion	272
8.3.2.1 Development of colorimetric method for Tadalafil	273
8.3.2.2 Method optimization and development	274
8.3.2.3 Application of developed method in fabricated paper microfluidic device	276
8.3.2.4 Application of developed methods	277
8.3.2.5 Method validation	279
8.3.2.6 Comparison of both developed methods by statistical analysis	281
8.4 Section –B Estimation of methyl malonic acid as early biomarker for pernicious anaemia using paper microfluidic device	281
8.4.1 Introduction	
8.4.1.1 Underlying cause for pernicious anaemia	281
8.4.1.2 Selection of selective biomarker for pernicious anaemia	282
8.4.1.3 Literature review	282
8.4.1.4 Biomarker profile	282
8.4.1.5 Chemicals and materials	283
8.4.1.6 Paper chip fabrication	283
8.4.1.7 Reagents and sample preparation	283
8.4.2 Experimental	285
8.4.2.1 Development of colorimetric method for Methyl malonic acid	285

8.4.2.2 Sample preparation	285
8.4.2.3 Method optimization and development	286
8.4.2.4 Application of developed method in fabricated paper microfluidic device	287
8.4.2.5 Application of developed methods	288
8.4.2.6 Method validation using ICH Q2 (R1) guideline	289
8.4.2.7 Comparison of both developed methods by statistical analysis	290
8.5 Conclusion	290
8.6 References	291
Chapter 9: Summary	297 - 304
List of publications	305