POST-MARKET ASSESSMENT OF THE QUALITY OF FIRST LINE REGIMEN FIXED-DOSE COMBINATION ANTIRETROVIRALS IN SOUTH AFRICA

Reem Abdallah S. Suleiman

A thesis submitted in partial fulfilment of the requirement for the degree of Magister in

Pharmaceutical Sciences at the School of Pharmacy

UNIVERSITY of the

University of the Western Cape

Supervisor: Dr Kim L. Ward

Co- supervisor: Dr Admire Dube

May 2017

POST-MARKET ASSESSMENT OF THE QUALITY OF FIRST LINE REGIMEN FIXED-DOSE COMBINATION ANTIRETROVIRALS IN SOUTH AFRICA

KEY WORDS

Antiretrovirals

Dissolution

Efavirenz

Emtricitabine

Fixed-dose combination

HPLC

Post-market quality

Quality tests

Tenofovir disoproxil fumarate

Validation

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

ABSTRACT

POST-MARKET ASSESSMENT OF THE QUALTIY OF FIRST LINE REGIMEN FIXED-DOSE COMBINATION ANTIRETROVIRALS IN SOUTH AFRICA

Reem Suleiman

MSc. Pharmaceutical thesis, School of Pharmacy, University of the Western Cape

UNIVERSITY of the
WESTERN CAPE

Background: The rapid increase in access to new antiretrovirals (ARVs) worldwide and, especially in sub-Saharan Africa, coupled with the well-documented problem of poor quality ARVs in developing countries has underscored the need for quality assessment of these medicines. South Africa has the worst human immunodeficiency virus (HIV) epidemic profile in the world; consequently, it has rolled out the world's largest antiretroviral ARV programme. With increasing market penetration of generic medicine in South Africa and especially ARVs, there is a call for stringent quality control mechanisms following the marketing approval (post-market quality control) of these medications. Unfortunately, evidence suggests that the World Health Organisation (WHO) recommendations for this aspect of quality assurance is not met by most Medicine Regulatory Authorities. In South Africa and many other countries this is attributed to a lack of physical and financial resources to enforce effective post-marketing surveillance (PMS) of all pharmaceuticals available in the country.

http://etd.uwc.ac.za/

Most of the generic industrial players lack facilities to attend to PMS and the Regulatory Authoritie seems to have not enforced it effectively.

Aim: The aim of this study was to evaluate and compare the post-market quality of a selected fixed-dose combination (FDC) product containing efavirenz (EFV 600 mg), emtiricitabine (FTC 200 mg) and tenofovir (TDF 300 mg) in tablet form with its originator counterpart. Four of these FDCs are currently on tender in the public health sector of South Africa.

Methods: Four generic FDC finished pharmaceutical products (FPPs) of FTC 200 mg, TDF 300 mg and EFV 600 mg in tablet dosage form were obtained from the Cape Antiretroviral Depot in the Province of the Western Cape. To ascertain the quality of these ARVs, the following tests were performed: identification by High performance or (pressure) liquid chromatography (HPLC), dissolution, assay, uniformity of weight and disintegration. Some of the tests were carried out according to the only available pharmacopoeial monograph for this FDC sourced from the WHO International Pharmacopoeia (IP). The HPLC method prescribed in the WHO IP monograph was found to be not suitable. Therefore, a reverse phase RP-HPLC method was developed and validated according to the International Conference on Harmonisation (ICH) requirements to carry out the identification, assay and the dissolution tests. Statistical analysis using One-way ANOVA, Tukey's multiple comparisons test was carried out to compare the release (dissolution testing) and the content (assay) of EFV, FTC and TDF between the originator and the generics and between the generics themselves.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Results: All the FDC samples passed the uniformity of weight tests, having less than 5% relative standard deviation (%RSD) of the average weight. The developed RP-HPLC method was successfully validated and met the ICH criteria. The three APIs were identified and quantified (content assay) in all the FPPs using the validated method. The percentage content of the three APIs in all sample FDCs was within 90% to 110%. For the dissolution tests all the FPP samples passed the specification except for one of the generic products (G2) which failed (at both stage 1 and 2) by releasing 62.23% with SD (20.43) of EFV in 30 minutes and this finding was significantly different when compared to other generics and the originator ($p < 0.0001$). The use of hypromellose in G2 might be responsible for the low Q-release values of EFV in the dissolution medium after 30 minutes. All the FPP samples passed the disintegration tests and disintegrated completely within 30 minutes.

Conclusion: The quality of the generic FPPs was generally good. All the assessed generic FPPs were within the WHO specification for the uniformity of weight, assay, identification and disintegration. All the FPPs were within the specification for the dissolution testing, except for one generic product (G2) which failed to release $\geq$ 80% of one of its APIs within 30 minutes. It is likely that the failure in dissolution of G2 was due to a difference in the formulation. This FPP included an excipient (hypromellose) which was not present in the other FDCs. Although there were some differences between the generics and the originator in the APIs quantities, there were no differences in the release of the three APIs in the generics and the originator except for G2. This study underscores the importance of post-market assessment of the quality of FDCs of ARVs.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

DECLARATION

I declare that POST-MARKET ASSESSMENT OF THE QUALITY OF FIRST LINE REGIMEN FIXED-DOSE COMBINATION ANTIRETROVIRALS IN SOUTH AFRICA is my own work, that it has not been submitted before for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

Reem Suleiman

May 2017

UNIVERSITY of the
WESTERN CAPE

Signed ……   …….

http://etd.uwc.ac.za/

DEDICATION

To my father, Mr. Abdallah Suleiman (عبدالله سليمان), mother Mrs. Ftahia Ali (فتحية علي) , and my twins, Muaath and Meehad Masoud

http://etd.uwc.ac.za/

ACKNOWLEDGEMENT

I wish to thank:

- Allah first and foremost.
- My supervisor, Dr. Kim Ward, for supervising the project and for her guidance and for proofreading the thesis.
- My co-supervisor, Dr. Admire Dube, for his supervision, encouragement, guidance and support.
- Mr. Yunus Kippie for his useful collaboration with the laboratory work, particularly the HPLC part.
- Dr. Gloudina Hon for her assistance in analysing the data statistically.
- The provincial government of the Western Cape, and the antiretroviral depot for providing the experiment samples.
- My husband, Mr. Mohamed Masoud, for his love, constant support and encouragement.
- My sisters and brothers for their love, prayer, " Doaa" and encouragement.
- The college students for their friendship and assistance.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

TABLE OF CONTENTS

KEY WORDS ii
ABSTRACT iii
DECLARATION vi
DEDICATION vii
ACKNOWLEDGEMENT viii
TABLE OF CONTENTS ix
LIST OF FIGURES xiv
LIST OF TABLES xv
LIST OF ACRONYMS xvi
CHAPTER 1 INTRODUCTION 1
1.1 Background 1
1.2 Problem statement 3
1.3 Aim and Objectives 4
CHAPTER 2 LITERATURE REVIEW 6
2.1 ARVs in the global and the local (South Africa) context 6
2.2 ARVs classifications 7
2.3 Chemical structure and definitions of the FDC of EFV+FTC+TDF 10
2.4 The manufacturing process of FDCs 12
2.5 Advantages and disadvantages of FDCs of ARVs 14
2.6 The medicines registration process 15
2.7 ARV supply chain in South Africa 18
2.8 Quality of FDCs 18
2.9 The need for quality assessment of FDCs 21
2.10 Quality control tests 23
2.10.1 Uniformity of weight test 23
2.10.2 Dissolution testing and its importance in the quality assessment of FDCs 23
2.10.4 Disintegration test 26

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

2.11 The theory of high performance liquid chromatography HPLC 26
2.11.1 Definition of HPLC 26
2.11.2 The HPLC system (apparatus) 27
2.11.2.1 Pumping systems 27
2.11.2.2 The injector 28
2.11.2.3 The chromatographic column and stationary phases 28
2.11.2.5 Mobile phases 29
2.11.2.6 Detectors 29
2.11.3 HPLC method validation 30
2.11.3.1 Specificity 30
2.11.3.2 System suitability testing 30
2.11.3.3 Linearity and range 33
2.11.3.4 Precision 33
2.11.3.5 Accuracy 34
2.11.3.6 Limits of detection (LOD) and Limits of quantitation (LOQ) 34
2.11.3.7 Robustness 35
2.11.4 The development and validation of chromatography and spectrophotometry methods to assess the quality of FDCs of ARVs 35
2.12 Methods of comparison 36
CHAPTER 3 METHODS 38
3.1 Study Design 38
3.2 Materials 38
3.2.1 FDC sampling 38
3.2.2 Chemicals and reagents 39
3.2.3 Materials 39
3.2.4 Instruments and equipment 39
3.3 Methods 41
3.3.1 Uniformity of weight 41
3.3.2 Validation of the WHO IP HPLC method 41
3.3.2.1 Preparation of the mobile phases 41

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

3.3.2.2 Preparation of standard solution 42
3.3.2.3 Preparation of FPP samples 43
3.3.3 Development and validation of the HPLC method for the FDC of FTC, TDF and EFV 43
3.3.3.1 Selection and optimization of the HPLC conditions 43
3.3.3.2 Method validation 44
3.3.3.2.1 Preparation of the mobile phases 44
3.3.3.2.2 Preparation of standard stock solution 45
3.3.3.2.3 Specificity 45
3.3.3.2.4 System suitability testing 46
3.3.3.2.5 Linearity 46
3.3.3.2.6 Precision 46
3.3.3.2.7 Accuracy 46
3.3.3.2.8 LOD and LOQ 47
3.3.3.2.9 Robustness 47
3.3.4 Identity tests 47
3.3.5 Assay test 47
3.3.6 HPLC validation for the dissolution testing 48
3.3.6.1 Preparation of standard stock solution 48
3.3.6.2 Linearity 48
3.3.6.3. Precision 48
3.3.6.4 Accuracy 49
3.3.6.5 LOD and LOQ 49
3.3.7 Dissolution tests 49
3.3.8 Disintegration test 50
3.3.9 Statistical analysis 50
3.4 Ethics 51
CHAPTER 4 RESULTS 52
4.1 The uniformity of weight test 52

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

4.2 WHO IP HPLC method for simultaneous identification and assay of the FDC of EFV, TDF and FTC 53
4.3 Development and validation of an HPLC method for the determination of FDC of FTC, TDF and EFV 55
4.3.1 Specificity 55
4.3.2 System suitability test 57
4.3.3 Linearity 58
4.3.4 Precision 60
4.3.5 Accuracy 60
4.3.6 LOD and LOQ 61
4.3.7 Robustness 61
4.4 Identity test 62
4.5 Assay test of the FDC tablets 64
4.5.1 The comparison of % content of EFV in the all FDCs 65
4.5.2 The comparison of % content of TDF in all the FDCs 66
4.5.3 The comparison of % content of TDF in all the FDCs 69
4.6 Validation of RP-HPLC for dissolution testing 70
4.6.1 Linearity 70
4.6.2 Precision 72
4.6.3 Accuracy 73
4.6.4 Limit of detection and limit of quantitation 73
4.7 Dissolution tests 74
4.7.1 The comparison of the Q-release of EFV in all the FPP FDCs 76
4.7.2 The comparison of the Q-release of TDF in all the FPP FDCs 78
4.7.3 The comparison of the Q-release of FTC in all the FPP FDCs 80
4.8 Disintegration test 82
CHAPTER 5 DISCUSSION 83
5.1 Uniformity of weight tests 83
5.2 The WHO IP HPLC method for simultaneous identification and assay of FTC, TDF and EFV FDC 84

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

5.3 Development and validation of an HPLC method for simultaneous detection and assay of FTC, TDF and EFV 85
5.4 Identity tests 85
5.5 Assay test of the FDC tablets 86
5.6 The validation of HPLC for dissolution testing 87
5.7 Dissolution tests 87
5.8 Disintegration test 90
5.9 Summary of the quality assessment 91
CHAPTER 6 CONCLUSIONS & RECOMMENDATIONS 92
6.1 CONCLUSIONS 92
6.2 RECOMMENDATIONS 93
REFERENCES 95
APPENDICES 105
APPENDIX 1: Certificate of analysis of FTC 105
APPENDIX 2: Certificate of analysis of TDF 107
APPENDIX 3: Certificate of analysis of EFV 109
APPENDIX 4: Certificate of analysis of Ascentis C18 column 110
APPENDIX 5: Disintegration tester 112

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

LIST OF FIGURES

Figure 2.1: ARV classifications and the mechanism of action 8
Figure 2.2: Chemical structure of efavirenz 10
Figure 2.3: Chemical structure of emtricitabine 11
Figure 2.4: Chemical structure of tenofovire disoproxil fumarate 11
Figure 2.5: Bi-layer FDC tablet manufacturing process 13
Figure 2.6: A general medicines registration process followed by NMRAs 17
Figure 2.7: HPLC system 27
Figure 2.8: Chromatographic separation of two substances 31
Figure 2.9: Asymmetrical chromatographic peak 33
Figure 4.1: Representative chromatogram for FTC, TDF and EFV RSs 53
Figure 4.2 Representative chromatogram for FTC, TDF and EFV in FDC tablet 54
Figure 4.3: Representative chromatogram of FTC (9.53), TDF (12.61) and EFV (14.25) RSs 56
Figure 4.4: Representative chromatogram of FTC (9.56), TDF (12.65) and EFV (14.37) in tablets 57
Figure 4.5: Calibration curves of FTC (a), TDF (b) and EFV (c) 59
Figure 4.6: Typical UV spectra for the FPP (originator) FTC (a), TDF (b) and EFV (c) and the reference standards FTC (d), TDF (e) and EFV (f) 63
Figure 4.7: The % content (w/w) of FTC, TDF and EFV in the originator and generic FDCs 64
Figure 4.8: The comparison of the % content of EFV in the originator and generic FDCs 65
Figure 4.9: The comparison of the % content (w/w) of TDF in the originator and generic FDCs 67
Figure 4.10: The comparison of the % content of FTC in the originator and generic FDCs 69
Figure 4.11: Calibration curves of FTC (a), TDF (b) and EFV (c) 72
Figure 4.12: Dissolution test of generic 2 (G2) at 30 minutes 75
Figure 4.13: Q-release of APIs from FDCs at 30 minutes 76
Figure 4.14: Comparison of the Q-release of EFV in the originator and the generic FDCs 77
Figure 4.15: Comparison of the Q-release of TDF in the originator and the generic FDCs 79
Figure 4.16: Comparison of the Q-release of FTC in the originator and the generic FDCs 81

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

LIST OF TABLES

Table 2.1: Availability of pharmacopoeial monographs and methods of dissolution tests of the FDC of FTC, TDF and EFV 20
Table 2.2: WHO IP specifications for uniformity of weight 23
Table 3.1: Mobile phases gradient of the WHO IP HPLC method 42
Table 3.2: Mobile phases gradient of the RP-HPLC method 45
Table 4.1: Uniformity of weight of the originator and generic FDCs 52
Table 4.2: System suitability parameters for FTC, TDF and EFV 58
Table 4.3: Regression analysis data for FTC, TDF and EFV 58
Table 4.4: The intra and inter-day data for FTC, TDF and EFV 60
Table 4.5: Accuracy data for FTC, TDF and EFV 60
Table 4.6: The limits of detection and quantitation of FTC, TDF and EFV 61
Table 4.7: Comparison of the % EFV in the originator and generic FDCs 66
Table 4.8: Comparison of the % TDF in the originator and generic FDCs 68
Table 4.9: Comparison of the % FTC in the originator and generic FDCs 70
Table 4.10: Regression analysis data for FTC, TDF and EFV 71
Table 4.11: Intra and inter-day data for FTC, TDF and EFV 73
Table 4.12: Accuracy data of FTC, TDF and EFV 73
Table 4.13: Limits of detection and quantitation of FTC, TDF and EFV 74
Table 4.14: Comparison of the Q-release of EFV in the originator and the generic FDCs . 78
Table 4.15: Comparison of the Q-release of TDF in the originator and generic FDCs 80
Table 4.16: Comparison of the Q-release of FTC in the originator and generic FCDs........ 82
Table 5.1: Inactive ingredients and film-coating materials in the originator and generic FDCs..88
Table 5.2: Summary of the assessment 91

http://etd.uwc.ac.za/

LIST OF ACRONYMS

AIDS	Acquired immunodeficiency syndrome
API	Active pharmaceutical ingredient
ARVs	Antiretrovirals
EFV	Efavirenz
FDA	Food and Drug Administration (of the United States)
FDC	Fixed-dose combination
FPP	Finished pharmaceutical product
FTC	Emtricitabine
G1	Generic 1
G2	Generic 2
G3	Generic 3
G4	Generic 4
HIV	Human immunodeficiency virus
HPLC	High performance or (pressure) liquid chromatography
ICH	International Conference on Harmonisation
IP	International Pharmacopoeia
mAU	Milli absorbance unit
MCC	Medicines Control Council
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
NTRTIs	Nucleotide reverse transcriptase inhibitors
O	Originator

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

PDA	Photodiode Array Detector
RP-HPLC	Revere phase-high performance or (pressure) liquid chromatography
RS	Reference standard
RSD	Relative standard deviation
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SLS	Sodium lauryl sulfate
TDF	Tenovofir disoproxil fumarate
USP	United States Pharmacopoeia
WHO	World Health Organization

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

CHAPTER 1 INTRODUCTION

This chapter provides the background to the study, the problem statement and the aim and objectives of the study.

1.1 Background

The rapid increase in access to new antiretrovirals (ARVs) worldwide and, especially in sub-Saharan Africa, coupled with the well-documented problem of poor quality ARVs in developing countries has underscored the need for quality assessment of these medicines (WHO, 2007). South Africa has one of the worst human immunodeficiency virus (HIV) epidemic profiles in the world, with an estimated 6.1 million South Africans living with HIV in 2012 (UNAIDS, 2012). Consequently, South Africa rolled out the world's largest antiretroviral ARV programme, which has achieved a 75 % increase in HIV treatment services between 2009 and 2011 (Shisana et al., 2014). More so, South Africa had 3.4 million people on the treatment, more than any other country in the world by the end of 2015 (UNAIDS, 2016). ARVs are the main treatment for HIV as it keeps the viral load in the body at a low level (Darbyshire, 1995). As a significant step forward for South Africa's national ARV programme, in April 2013, the South African Minister of Health launched a triple generic FDC as the first line treatment for HIV in adults. The FDC contains 200 mg emtricitabine of (FTC), 300 mg of tenofovir (TDF), and 600 mg of efavirenz (EFV) in a single tablet (WHO, 2008). An FDC is a combination of two or more active pharmaceutical ingredients in a single dosage form (Siew, 2015). It was anticipated that over 90% of new patients will be eligible to initiate the FDC treatment in the public sector (Davies, 2013).

http://etd.uwc.ac.za/

The FDC, which first reached the South African market in 2009, was registered by South Africa's Medicines Regulatory Authority. The registration of medicines is a function of the Medicines Control Council (MCC), that applies standards laid down by the Medicines and Related Substances Act, (Act 101 of 1965) which governs the manufacture, distribution, sale, and marketing of medicines (MCC, 2008). The MCC is responsible for compliance with international standards of good manufacturing practice (GMP) (Spencer, 2013). Although the generic ARVs dramatically reduce the therapy cost, there is still some concern about their quality. The quality of the generic medicines should be assured after market approval: during transportation, storage and dispensing (Del Tacca et al., 2009; Joshi et al., 2010; Kibwage, 2008; MOMS and MOPHS, 2012; WHO, 2007). Generics, also referred to as, interchangeable multi-source medicines are medicines that contain the same active pharmaceutical ingredients (APIs), are identical in concentration, dosage form and route of administration and meet the comparable standards, which comply with the requirements for therapeutic equivalence (MCC, 2003). The originator medicine is generally protected by a patent (can be produced and sold only by the company holding the patent), usually for a period of 20 years (Paveliu et al., 2011). When the patent protection for an originator product expires, generic versions of the medicine product can be offered for sale after a regulatory authority has approved them (Al-Jazairi et al., 2008). However, the quality of any drug product is determined by several stages of the product's life cycle, which could be from raw materials used, formulation, manufacturing tools, manufacturing conditions, packaging, storage and transportation conditions which may affect the quality of the product significantly. Some of the effects of these problems only appear in the consumption stage (Davies, 2013; Embrey and Management Sciences for Health, 2012).

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Therefore, it is important that any regulatory authority ensures the quality of products throughout their life cycle, which in turn reflects on the success of the treatment programmes.

1.2 Problem statement

In some developing countries, the ability of a regulatory authority to confirm the quality of a drug product through laboratory testing is still questionable, despite having a clear structure of legal requirements (Bartlett and Muro, 2007; Maigetter et al., 2015; Meredith, 2012). According to a survey of regulatory authorities in African countries, 63% of these countries were unable to evaluate the quality, safety and efficacy of new products due to a lack of physical and financial resources (WHO, 2007).

The MCC requirement for the registration of generic medicines is similar to regulatory authority requirements worldwide; they accept that if a generic product has a comparable standards to its originator counterpart, they are considered to be interchangeable. While the MCC receives samples of generic medicines from the manufacturer as part of medicines registration requirements, it does not conduct any independent quality tests, apart from visual inspections. Once the generic product has been approved and registered by the MCC, a manufacturer can distribute their product in SA (Hassim and Heywood, 2007; MCC, 2003) without any further regulatory requirements from the MCC for quality testing along the supply chain.

More so, when it comes to the manufacturing of FDC products, it is becoming increasingly complex. There are some complications that may arise when mixing two or three active

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

ingredients, which are related to stability, assay, physicochemical properties (dissolution rate) and bioequivalence. (WHO,2003; EMEA, 2007). Hence, random post-market testing by regulatory authorities is needed. The WHO recommendations for quality testing following the marketing approval (post-market quality control) are not met by most Medicine Regulatory Authorities, even in countries where procedures are well regulated (Bartlett and Muro, 2007; Meredith, 2012). The growth of the generic medicines industry worldwide has seen an influx of substandard products on the market and as such, regulatory authorities support post-market quality control testing in principle, although these are not always explicitly built into the regulatory framework and subsequently not enforceable. In South Africa and many other countries this is attributed to a lack of physical and financial resources (Hassim and Heywood, 2007; Hill and Johnson, 2004; Patel et al., 2012). It has been reported that, even though South Africa has a legal requirement for collecting ongoing medicine safety data post- market authorization, it lacked adequate capacity to monitor medicines and evaluate risks, according to the minimum standards of the WHO (Maigetter et al., 2015). Furthermore, routine quality control testing after market approval is not explicitly embedded in this regulation (Maigetter et al., 2015). To the best of our knowledge, no studies have been conducted in South Africa to assess the post-market quality of the generic FDC of EFV, FTC and TDF, hence the need for this study.

1.3 Aim and Objectives

The aim of this study is to evaluate and compare the quality of generic ARV FDC containing FTC, TDF and EFV to each other, and to the originator counterpart according to the IP specifications 2016.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

The objectives were to:

1. Identify the three active ingredients FTC, TDF and EFV in the FDC finished pharmaceutical products (FPPs).
2. Quantify the three active ingredients FTC, TDF and EFV in the FDC FPPs.
3. Evaluate the uniformity of weight for all the FDC FPPs
4. Evaluate the disintegration time of the FDC FPPs
5. Evaluate the extent of release (dissolution) of FTC, TDF and EFV from the FDC FPPs

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

CHAPTER 2 LITERATURE REVIEW

This section provides a review of literature concerning ARVs in the global and the local (South African) context, ARV classifications, the chemical structure and definitions of the FDC of EFV, FTC and TDF, the manufacturing process of FDCs, advantages and disadvantages of FDCs of ARVs, the medicines registration process, the ARV supply chain in South Africa, the need for quality assessment of ARV FDCs, quality control tests and the theory of high performance liquid chromatography HPLC.

2.1 ARVs in the global and the local (South Africa) context

ARVs were not available before 1987 as HIV management consisted of treating opportunistic infections and malignancies associated with acquired immune deficiency syndrome (AIDS). A nucleoside reverse transcriptase inhibitor (NRTI), zidovudine (AZT) was the first ARV approved in 1987 by the United States Food and Drug Administration (US-FDA) (Broder, 2010). In 2002, only 2.7% of the estimated 11 million adults who were eligible for ARV therapy were receiving it. Additionally, in sub-Saharan Africa, which has the highest number of people living with HIV, only 1% of those eligible were receiving treatment. However, access to ARVs has significantly improved in these countries as almost five million of the estimated 10.4 million eligible for treatment in the region were receiving ARVs by 2011 (UNAIDS, 2012). With the rapid recent increment in the amount of people receiving ARVs, most ARVs enter the market through accelerated approval based on changes in surrogate endpoints, primarily viral load, but also cluster of differentiation 4 (CD4) T-cells counts (Young et al., 2012).

http://etd.uwc.ac.za/

Since 2001 the South African government has provided ARV therapy in all provinces with the assistance of non-governmental international organisations (Coetzee et al., 2004; Kagee, 2008). Due to the continuous growth of the AIDS epidemic and high cost of these medications, many developing countries have introduced ARV programmes to achieve treatment objectives consistent with the WHO (Bartlett and Muro, 2007). Various treatment guidelines for HIV infected adults and adolescents exist in different countries or regions. The SA ARV treatment guidelines and its updates have provided a detailed explanation of the medicines, classified in terms of their use as first line or second line and patient eligibility criteria (Meintjes et al., 2014).

2.2 ARVs classifications

ARVs are classified by the retrovirus life-cycle that the drug inhibits (Smith et al., 2013). There are several classes of ARVs, which are usually used in combination (Meintjes et al., 2014):

UNIVERSITY of the
WESTERN CAPE

i. Nucleoside reverse transcriptase inhibitors (NRTIs): Act by interfering with the action of an HIV protein called reverse transcriptase, which the virus needs to make new copies of itself.

ii. Nucleotide reverse transcriptase inhibitor (NTRTIs): Work in the same way as NRTIs with a difference in the chemical structure

iii. Non-nucleoside reverse transcriptase inhibitors (NNRTIs): Inhibit HIV from replicating within cells by inhibiting the reverse transcriptase protein.

iv. Protease inhibitors (PI): Prevent HIV from being assembled and released from the infected CD4 cell.

http://etd.uwc.ac.za/

v. Integrase inhibitors: Interfere with the integrase enzyme, preventing the integration of HIV proviral DNA into human DNA

vi. Fusion or entry inhibitors: Prevent HIV from binding to entry by binding to glycoprotein on the viral envelope.

vii. CCR5 antagonists: Prevent entry into the host CD4 cell by binding to the chemokine co-receptor.

Figure 2.1 summarises the ARVs classifications.

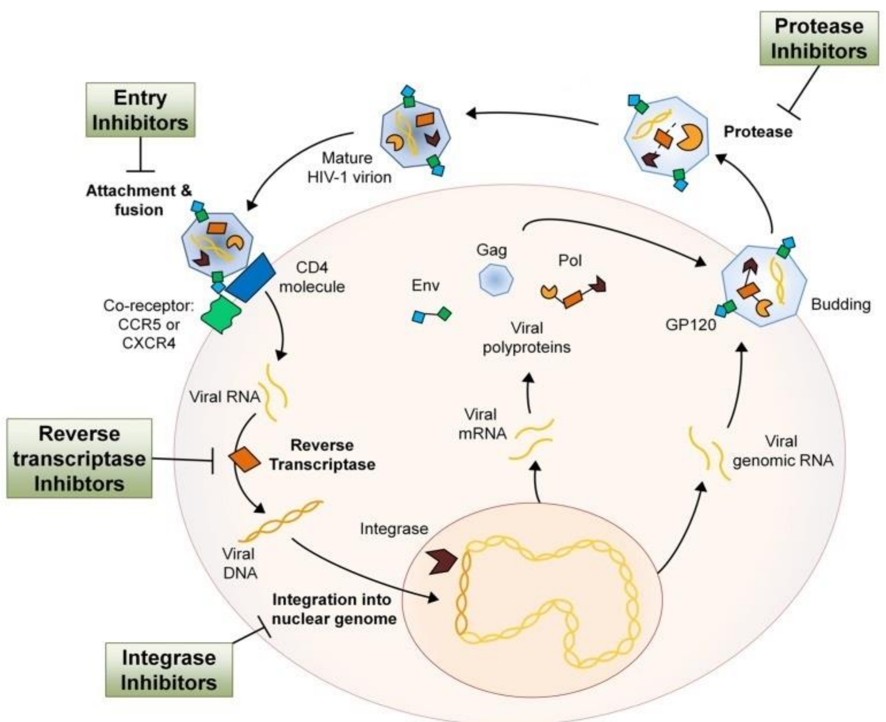


Figure 2.1: ARV classifications and the mechanism of action (Smith et al., 2013)

http://etd.uwc.ac.za/

The emergence of drug-resistant strains of HIV limited the efficacy of many ARVs and called for improved ways to fight the disease while ensuring the quality of life of users and reduced morbidity and mortality (Richman et al., 2004). This approach led to the introduction of highly active antiretroviral therapy (HAART) which involves taking a combination of two or more different types of ARV drugs for example, one or more NRTIs combined with a PI, NNRTI plus one or two NRTIs (Smith et al., 2013). However, ARV regimens imposed a high medication burden and the frequency of administration was seldom compatible with the patient's daily life. It has been reported that certain factors, *inter alia*, being away from home, difficulty with the dosing schedules, running out of medication and fear of being stigmatised by sexual partners, contributed towards dosing irregularity which, in turn decreased the adherence to therapy considerably (EMEA, 2007; Nachega et al., 2004). Therefore, the introduction of FDCs of ARV therapies became important. One of the leading recommendations for treating HIV/AIDS was published by the WHO in 2013, using two NRTIs plus an NNRTI as first line therapy (WHO, 2015). In 2006 the first version of the FDC of EFV, FTC and TDF was approved by the US-FDA. This version was manufactured by Bristol-Myers Squibb & Gilead Sciences LCC, under the trade name Atripla® and contains 600 mg of EFV which is an NNRTI and 300 mg of TDF and 200 mg of FTC, both of which are NRTIs in tablet dosage form. The recommended dose of this FDC is one tablet taken orally on an empty stomach once daily (FDA, 2006a). After the approval of the first version (originator), a generic version of the of the FDC of EFV, FTC and TDF was also approved by the FDA for sale outside the USA under the US President's Emergency Plan For AIDS Relief (PEPFAR). The PEPFAR plan

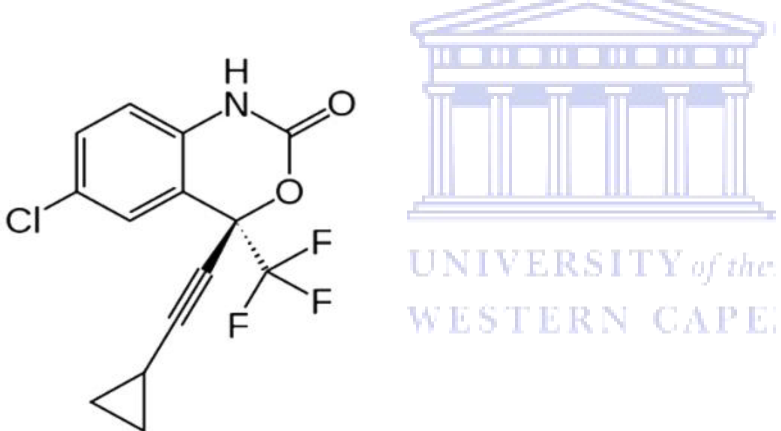
UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

has dramatically improved access to ARVs in Sub-Saharan Africa, thus reducing mortality (Holmes, 2010).

2.3 Chemical structure and definitions of the FDC of EFV+FTC+TDF

EFV is an NNRTI and binds directly and reversibly to the catalytic site of the reverse transcriptase enzyme (Manikanta Kumar et al., 2012). EFV is a white to slightly pink powder, practically insoluble in water and freely soluble in methanol. EFV is chemically described as (S)-6-chloro-4-(cyclopropylethynyl)-1, 4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one (Manikanta Kumar et al., 2012; Ramaswamy and Dhas, 2014) (Figure 2.2).



UNIVERSITY of the
WESTERN CAPE

Figure 2.2: Chemical structure of efavirenz

FTC is an NRTI which is a white to almost white crystalline powder, freely soluble in methanol and water. FTC is described chemically as 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl] cytosine (Raju and Begum, 2008; Ramaswamy and Dhas, 2014) (Figure 2.3).

http://etd.uwc.ac.za/

Figure 2.3: Chemical structure of emtricitabine

TDF is a fumaric acid salt, white to almost-white crystalline powder that is slightly soluble in water, soluble in methanol. Chemically TDF is described as {9-[(R)-2-[[bis [[isopropoxycarbonyl] oxy] methoxy] phosphonyl] methoxy] popyl] adenine fumarate}(Raju and Begum, 2008; Ramaswamy and Dhas, 2014) (Figure 2.4).

UNIVERSITY of the
WESTERN CAPE

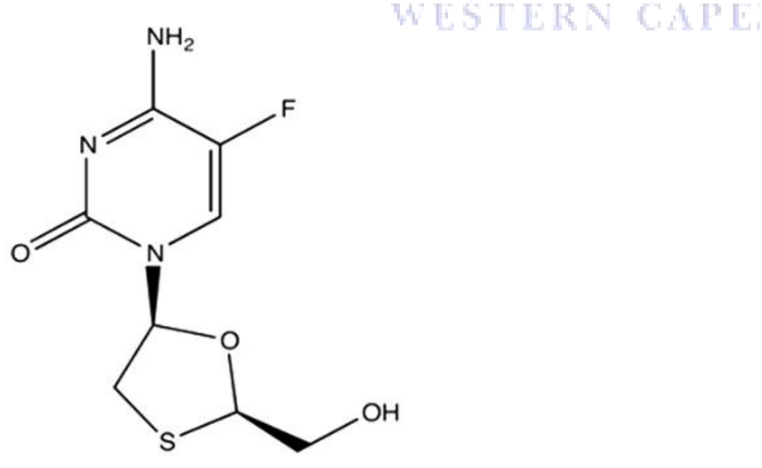


Figure 2.4: Chemical structure of tenofovir disoproxil fumarate

http://etd.uwc.ac.za/

2.4 The manufacturing process of FDCs

An FDC is an FPP containing a mixture of two or more active ingredients, provided the ingredients are physically and chemically compatible and stable with required excipients (Siew, 2014). Although the manufacturing process used for the formulation of bi-layer FDCs, three -layer, or tablet-in-a-tablet is complex, the basic manufacturing processes are the same as for single-layer tablets. The typical manufacturing process for a tablet dosage form includes the following: The first step is the milling and mixing (blending). Thereafter, granulation which imparts two primary requisites to formulate both wet and dry granulation. Then, drying it keeps the residual moisture low enough to prevent product deterioration. Thereafter, compression which creates the final tablet. Sometimes there is another process called coating. Actual tablet compression is required using a tumble blender and loading and unloading equipment. These may be subdivided into four stages:

UNIVERSITY of the
WESTERN CAPE

i. The filling refers to the transfer of granules which had been processed by either wet or dry granulation into a die. The die is a disc shape with a hole cut through its centre, and has two hardened steel punches that fit into the top and bottom of it.
ii. Temping (metering) is a stage of overfill removal from the compressing equipment.
iii. Compressing is bringing together the upper and lower punches under pressure within the die to form the tablet.

http://etd.uwc.ac.za/

iv. Ejection is the stage where the tablet removes from the lower punch-die (Gerhardt, 2010).

More so, the manufacturing of multi-layers FDCs involves multiple granulations and multiple compression of these layers using suitable multilayer tablet presses or a special press for tablet-in-a tablet (Desai et al., 2013). Figure 2.5 summarises the bi-layer FDC manufacturing process; a) the first layer fill, b) first-layer temping, c) upper punch withdrawal, d) second layer fill, e) main compression and f) the ejection (Koo, 2010).

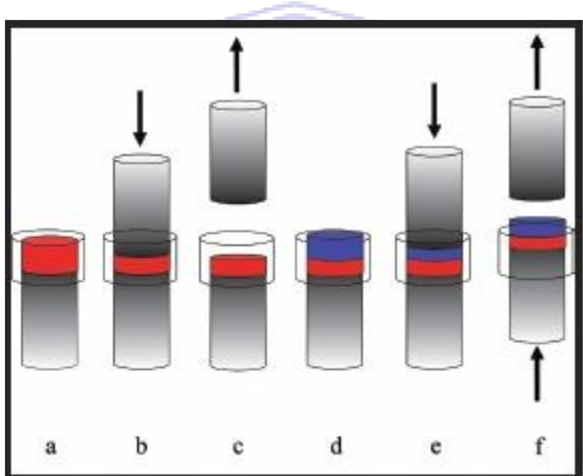


Figure 2.5: Bi-layer FDC tablet manufacturing process (Koo, 2010).

The manufacturing process that is used to prepare the originator FDC of FTC+TDF+EFV, involves the preparation of a wet granulation of EFV and a dry granulation of FTC and TDF. Furthermore, the granulations are blended separately with extra-granular magnesium stearate, compressed into bilayer tablets, and then film-coated (EMEA, 2007).

http://etd.uwc.ac.za/

2.5 Advantages and disadvantages of FDCs of ARVs

FDCs of ARVs are a significant advancement in ARV treatment simplification, contributing to increased compliance with complex chronic therapies, thus increasing the patient's quality of life. In addition, the advantages of FDCs of ARVs include the following:

- The simplification of stock management: FDCs can afford easy prescribing and dispensing because of the limitations of the number of tablets (Calmy et al., 2006; DeJesus et al., 2009; Llibre et al., 2010).
- Adherence: By reducing the medication burden to one tablet per day, FDCs largely improved adherence to ARVs (Kaposhi et al., 2015; Llibre et al., 2010).
- Efficacy: Many FDCs of ARVs have shown good therapeutic efficacy (Calmy et al., 2006) and FDCs also reduce the risk of mother to child transmission. Several FDCs have been approved by the WHO prequalification programme or other regulatory authorities (Bartlett and Muro, 2007).
- Cost-effectiveness: Many studies have confirmed that FDCs of ARVs are cost-effective as it was reported that generic FDCs reduced the cost of ARVs significantly compared to the originator (Bartlett and Muro, 2007; Calmy et al., 2006; Freedberg et al., 2001). For example, the SA government negotiated the cost of R89.37 per month for the FDC of EFV, FTC and TDF which makes it cost-effective (Davies, 2013).

On the other hand, FDCs also have disadvantages including the following:

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

- Some safety concerns have been raised because of the side effects of some compounds in the FDC, which may lead to poor adherence and drug resistance (Spencer, 2013).
- Difficulty in identifying the active ingredient responsible for causing an adverse drug reaction following FDC usage has also been reported (Subbaraman et al., 2007).
- Challenges in the formulation and the manufacturing of the FDC; some complications may arise during mixing two or more API leading to stability problems (WHO, 2003; EMEA, 2007).
- The complexity of the manufacturing of multi-layer FDCs (bi-layer, three-layer and tablet-in-a tablet) which require multiple granulations, multiple compression and sometimes a special press (Siew, 2014).

UNIVERSITY of the
WESTERN CAPE

2.6 The medicines registration process

In general, medicine registration is a system of standards that subjects all pharmaceutical products to pre-marketing evaluation, marketing authorisation, post-registration amendments and post-marketing review to ensure that they conform to required standards of quality, safety, and efficacy established by national regulatory authorities. The result of the drug registration process is the issuance or the rejection of a pharmaceutical product license or marketing authorisation (WHO, 1998).

The registration process can be explained using Figure 2.6. The first chart, which is the reception, describes the assessment of applications for new marketing authorisations, and provides a global description of the registration process. Not all the areas of assessment i.e.,

http://etd.uwc.ac.za/

those indicated in boxes in the chart, are relevant for all medicine products. For example, interchangeability applies only to generic products; safety and efficacy assessment is required for new chemical entities only and the price is not required for all countries as part of the assessment of an application for marketing authorisation. The second chart describes the assessment of imported, well established products. If the national medicines registration authority (NMRA) finds that the information submitted is incomplete or does not agree with the statements, conclusions, or proposals made by the applicant, an appropriate letter is usually sent to the applicant. Usually, such letters are requests for additional information or explanations on specific issues. They are referred to as the "correspondence loop" in the first chart. The third chart is the follow up assessment which describes the activities that should be done after a product has been approved (post-marketing assessment) such as, routine quality checks (e.g. dissolution rates, assay content, appearance), updated stability data, updated product information and pharmacovigilance (PV) studies (WHO, 1998).

UNIVERSITY of the
WESTERN CAPE

Furthermore, in order for a medicine to be registered in South Africa, it must meet all the MCC requirements for medicines registration. Guidelines were set to assist applicants in the preparation of documentation for the registration of medicines for human use. The types of medicine include a new medicine for a new chemical entity (NCE), a generic (interchangeable multisource) product, a biological medicine and a product line extension. Data submitted by the applicant should meet technical requirements of quality, safety and efficacy. For the registration of a generic medicine, appearance, physical parameters, impurity profiles and other relevant parameters of the test and reference/originator product, relevant physico-chemical parameters e.g. dissolution, uniformity of dosage units of the

http://etd.uwc.ac.za/

tablet and stability studies are required (MCC, 2014). Despite, the fact that there are local recommendations for follow-up medicines after marketing authorisation has been granted, South Africa is unable to follow those recommendations because of the lack of human and financial resources (Hassim and Heywood, 2007).

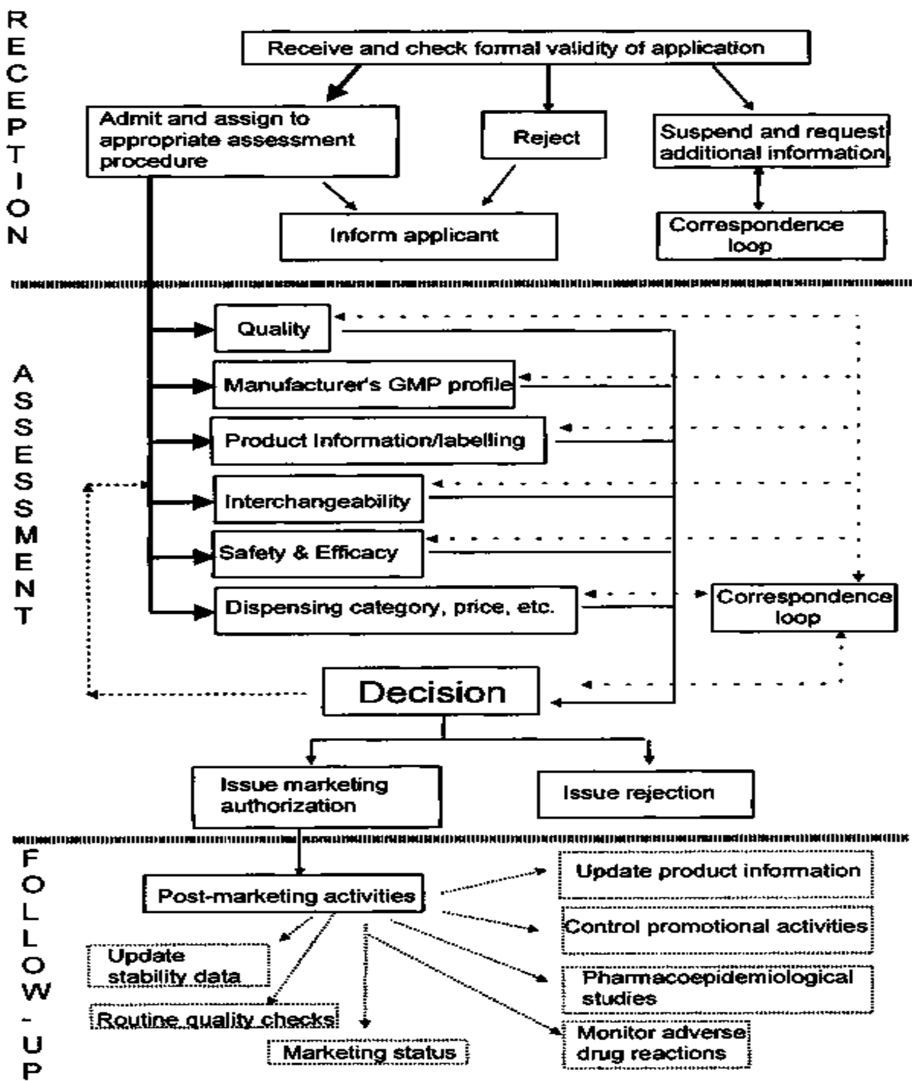


Figure 2.6: A general medicines registration process followed by NMRAs (WHO, 1998).

http://etd.uwc.ac.za/

2.7 ARV supply chain in South Africa

Understanding the dynamics of the ARV supply chain is important to ensure full efficiency and success in any treatment programme. Procurement is the process undertaken by a country to order drugs and other necessary items (Ripin et al., 2014), South Africa's National Department of Health spends about 12.3% of the public sector health funding on pharmaceuticals (Mahoro, 2013). South Africa's ARV supply chain encompasses: selection of medicines which need to be procured, preparation of tenders by the National Department of Health - procurement is done at the state's cost by the Central Procurement Agency (CPA), agency under the National Department of Health. Lastly, there is then the delivery of medicines to the provinces and the distribution of the medicines by the provincial depots to the primary health facilities (Mahoro, 2013). Due to the high demand for ARVs, the Department of Health has instituted mechanisms to mitigate risks in supply, *inter alia*, splitting the public sector tender to supply the generic FDC of FTC, TDF and EFV between four generic companies (Davies, 2013).

UNIVERSITY of the
WESTERN CAPE

2.8 Quality of FDCs

Quality is one of three important elements that must be assured during the registration of any drug product. Poor quality medicines are defined differently in different countries but, they can be mainly categorised into three types: counterfeit, substandard and degraded (Newton et al., 2010). "Counterfeit medicines are deliberately and fraudulently mislabeled with relation to identity and/or supply. Counterfeiting may include products with the correct or incorrect ingredients, without active ingredients, with insufficient active ingredients and false packaging" (WHO, 1999). Substandard medicines are manufactured

http://etd.uwc.ac.za/

by licensed manufacturers and do not meet the quality specifications. For example, they may contain less (or more) active ingredients than what is stipulated on the packaging (Newton et al., 2010). Degraded medicines on the other hand are approved medicines which might have been affected by light, heat, and humidity. However, it can be difficult to distinguish degraded medicines from those that left the factory as substandard, but the distinction is important as the causes and remedies are different (Newton et al., 2010). Despite studies on FDCs showing bioequivalence and WHO prequalification status, the quality of some FDCs of ARVs remain questionable (Laurent et al., 2007). In 2013, the WHO approved (prequalified) a generic FDC of FTC, TFD and EFV that was manufactured by Cipla® India. This FDC was subjected to several tests which involves the description, identification of APIs by HPLC and thin layer chromatography (TLC), uniformity of dosage units, dissolution and assay by HPLC, impurities, microbiological examination as well as stability testing (WHO, 2013). Although prequalification is a valuable tool for any regulatory system to supply good quality ARVs, the process does not guarantee the quality of the supplied products. Generic ARVs have the potential to cause harm if rigorous quality assurance processes are not followed in the pharmaceutical system given the high consumption as fundamental first line regimens (Joshi et al., 2010). Pharmaceuticals that meet the pharmacopoeial specifications throughout the supply chain offer great promise in the treatment of HIV/AIDS (Bartlett and Muro, 2007).

UNIVERSITY of the
WESTERN CAPE

For quality tests conducted by the procurement agencies, the results should be shared with national regulatory authorities. Furthermore, since manufacturers are not obliged to use the test methods of the local pharmacopoeia, they have to ensure that their products will meet

http://etd.uwc.ac.za/

the pharmacopoeial standards (WHO, 2003). For example, to test the FDCs, the availability of a pharmacopoeial monograph, either for the combination or its APIs, will simplify the task while on the other hand, the absence of the monographs means that regulatory agencies must commit more resources to assessment and testing (WHO, 2003). The availability of pharmacopoeial specifications of the FDC of FTC, TDF and EFV and the methods for quality testing are summarised in Table 2.1.

Table 1.1: Availability of pharmacopoeial monographs and methods of dissolution tests of the FDC of FTC, TDF and EFV

Sources	Notes
The WHO IP monograph of EFV, FTC and TDF tablets, 2015.	Available (WHO, 2016a)
The WHO draft monograph for the IP for EFV, FTC and TDF tablets, 2010.	Available (WHO, 2010)
The FDA dissolution methods for EFV 600 mg; FTC 200 mg; TDF 300 mg tablets	Available (FDA, 2007)
United States Pharmacopoeia (USP) monograph for the FDC of FTC, TDF and EFV 2015	Not available
British Pharmacopoeia (BP) monograph for the FDC of FTC, TDF and EFV 2015	Not available

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

2.9 The need for quality assessment of FDCs

Quality control is a system of maintaining standards in manufacturing products by testing a sample of the product against the specifications (Embrey and Management Sciences for Health, 2012).

Fourie and Spinaci, (1999) asserted that many FDCs which are on the market for the treatment of TB are of inferior quality and are unknowingly being used in tuberculosis treatment programs in low-income countries. An assessment of the post-market quality of rifampicin-containing fixed-dose combination (R-FDC) and rifampicin single formulation anti-TB drugs in Uganda, showed a high incidence of sub-standard R-FDC in drug outlets. Tests such as dissolution tests, uniformity of weight tests, assays and visual inspections were used to determine the quality of these medicines (Moses et al., 2013). A WHO report in 2003 issued an alert about the availability of a counterfeit version of FDC of zidovudine (200mg), lamivudine (150mg) and nevirapine (40mg) per capsule in the Ivory Coast. The product was manufactured by Selchi Pharmaceuticals, Namibia. The analysis of the product showed that the samples did not contain lamivudine or indinavir; they contained zidovudine 201 mg, stavudine 40 mg, and an unidentified substance (WHO, 2007), (Primo-Carpenter and McGinnis, 2007). Futhermore, a survey of the quality of antiretroviral medicines in selected African countries, found quality issues related to a FDC which contains stavudine 30mg, lamivudine 150mg, and nevirapine 200mg tablets. This product was collected at a public-sector treatment centre in Tanzania and despite, the fact that this FDC was pre-qualified by the WHO, it did not meet the specification in terms of assay, uniformity of weight and dissolution testing. The same survey indicated that a sample of zidovudine

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

50mg/5ml oral solution collected from a manufacturer in Nigeria had low content according to the label claim. In addition, zidovudine 300mg tablets collected at a public-sector procurement centre in Tanzania also did not meet the USP specification for dissolution testing (WHO, 2007). In a study conducted in Malawi, the pharmacokinetics and bioequivalence of generic and trade formulations of stavudine 40mg, lamivudine 150mg, and nevirapine 200mg were compared in HIV-infected adult Malawians. The patients were randomly assigned to receive either the generic or the trade formulation of the drugs. Although the exposure conditions were similar for the originator and the generic, the results indicated that Triomune-40™ (the generic) was not therapeutically equivalence to its trade formulation. It is, therefore, advisable for the pharmacokinetics and bioequivalence of generic formulations of ARV medicines to be evaluated in the local geographical contexts where they are being used (Hosseinipour et al., 2007).

On the other hand, Joshi et al., (2010) conducted a post-marketing *in vitro/in vivo* (stability studies and dissolution studies) assessment of FDCs of the generic and originator, lamivudine (3TC) 150 mg and zidovudine (AZT) 300 mg tablets that were available in the Nigerian public and private sectors. An isocratic HPLC-UV method was developed and validated to perform the assessment. The study results showed no difference between the dissolution profiles of the generic and the originator of the FDCs. Furthermore, the results emphasised the importance of assessing the quality of the combination drug products that would ensure the safety and efficacy of the generic drug products available on the market.

http://etd.uwc.ac.za/

2.10 Quality control tests

There are many pharmaceutical technical procedures which can be employed to assess the quality of a solid dosage form as stated below.

2.10.1 Uniformity of weight test

One of the pharmaceutical technical procedures followed to ensure the consistency of the mass of single-dose preparations is the uniformity of weight test. With uncoated tablets and film-coated tablets, when weighed individually, the deviation of individual masses from the average mass should comply with the specifications in Table 2.2 (WHO, 2016c).

Table 2.2: WHO IP specifications for uniformity of weight

Average weight of tablet	% Deviation	Number of tablets
Less than 80 mg	±10	Minimum 18
	±20	Maximum 2
80 mg to 250 mg	±7.5	Minimum 18
	±15	Maximum 2
More than 250 mg	±5	Minimum* 18
	±10	Maximum* 2

Minimum* 18 means the average weight of 20 to 18 tablets. Maximum* 2 is the average weight of 1 to 2 tablets.

2.10.2 Dissolution testing and its importance in the quality assessment of FDCs

Dissolution testing is considered one of the most important quality control tests performed on pharmaceutical dosage forms (Al Ameri et al., 2012; Guo et al., 2000). This test

UNIVERSITY of the WESTERN CAPE

http://etd.uwc.ac.za/

determines the amount of active ingredient(s) released from a solid oral dosage form, such as a tablet or a capsule, under controlled conditions using a known volume of dissolution medium within a predetermined length of time (WHO, 2016d). Dissolution is a valuable tool which can be used to assess post-approval changes, batch-to-batch consistency in routine quality control testing to identify interactions between drugs and between the drugs and excipient, as well as any physical changes such as API form and co-crystal formation (Zhang et al., 2015). Dissolution can also provide information about bioavailability and bioequivalence, under certain conditions, *in vitro* dissolution testing is used as a substitute for *in vivo* bioequivalence studies, to compare two formulations if they are therapeutically equivalent, in case they have the same dissolution profiles. A bioequivalence study is usually required by the health authority to compare the rate and extent of absorption of each therapeutic API in an FDC product with the rate and extent of absorption of each therapeutic API administered concurrently as separate single-ingredient products (FDA, 1997; Zhang et al., 2015).

UNIVERSITY of the
WESTERN CAPE

Moreover, the development of a dissolution method for FDC drug products may be challenging due to differences in the physicochemical properties of the active ingredients (e.g., form, pH-solubility profile and pH dependent stability profile) which could prevent the selection of a common dissolution medium. The use of a surfactant such as sodium lauryl sulfate (SLS) in dissolution media for poorly water-soluble drugs is based mainly on the solubilisation capacity of the synthetic surfactant. Nevertheless, SLS lacks the physiological relevance of the gastrointestinal tract. Dissolution profiles obtained using synthetic surfactants like SLS may or may not exhibit *in vitro in vivo* correlations (Jogia et

http://etd.uwc.ac.za/

al., 2009). According to the FDA, dissolution testing should ensure that the presence of two or more drugs does not affect dissolution performance testing (FDA, 2006b)

In addition, for FDC products, an HPLC method may be necessary for dissolution sample analysis due to challenges in selecting a unique wavelength that does not absorb other components by a traditional UV technique. An isocratic HPLC method is desirable, however, gradient HPLC may be necessary to achieve a shorter run time with adequate separation of multiple components. The criteria of a desirable detection method include robust performance, short sample analysis time, simple mobile phase and diluent preparation, and simple instruments that are available globally (Zhang et al., 2015). Therefore, a RP-HPLC method was developed and validated to evaluate a new dissolution profile of EFV tablet dosage form; therefore dissolution conditions are achieved using 900 ml of medium containing water with 2% of SLS. Moreover, Babu et al., (2014) conducted a study to develop different selected immediate release tablet formulation of EFV. Evaluation of the dissolution rate and the physicochemical parameters for the formulations were done. The results showed that, the release of the drug can be affected by the drug excipients. Furthermore, two different dissolution methods were developed to assess the release profiles to the FDC of FTC, TDF and Nevirapine (NVP). The first method was performed using 0.01N HCl as dissolution medium, whereas for NVP class II, phosphate buffer with 6% SLS (pH 6.8) was used as dissolution medium. Results confirmed that the method is suitable for routine quality control analysis and *in vitro* dissolution studies for the FDC (Jayapalu et al., 2014).

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

2.10.4 Disintegration test

A disintegration test is a pharmaceutical technical procedure which can be performed on tablets and capsules to determine whether they disintegrate within the prescribed time, when placed in a liquid medium under certain conditions. However, the disintegration of the tablet does not imply the availability for absorption of its active ingredient. Compliance with the limits on disintegration stated in the individual monographs is required except where the tablets or capsules are intended for use as chewing, or were extended or/and delayed-release dosage forms. The acceptable time for complete disintegration for film-coated tablets is ≤ 30 minutes (WHO, 2016e).

2.11 The theory of high performance liquid chromatography HPLC

The complexity of HPLC analysis increases with the number of active ingredients (WHO, 2003). Currently, HPLC is the most commonly the method of choice. Suitable instruments and columns are now widely available, although this may be less true in developing countries (Zhang et al., 2015).

UNIVERSITY of the
WESTERN CAPE

2.11.1 Definition of HPLC

HPLC is a separation technique that can be used for the analysis of organic molecules and ions. HPLC is based on the mechanisms of adsorption, partition and ion exchange, depending on the type of stationary phase used. Therefore, HPLC can be used to assess the purity and/or the quality of pharmaceutical products by determining the content of many pharmaceutical substances (WHO, 2016b; USP, 2006).

http://etd.uwc.ac.za/

2.11.2 The HPLC system (apparatus)

The apparatus consists of a pumping system, an injector, a chromatographic column, stationary and mobile phases, connecting tubing and fittings, a detector and a data collection device (computer, integrator or recorder) (WHO, 2016b). Figure 2.7 shows the HPLC system.

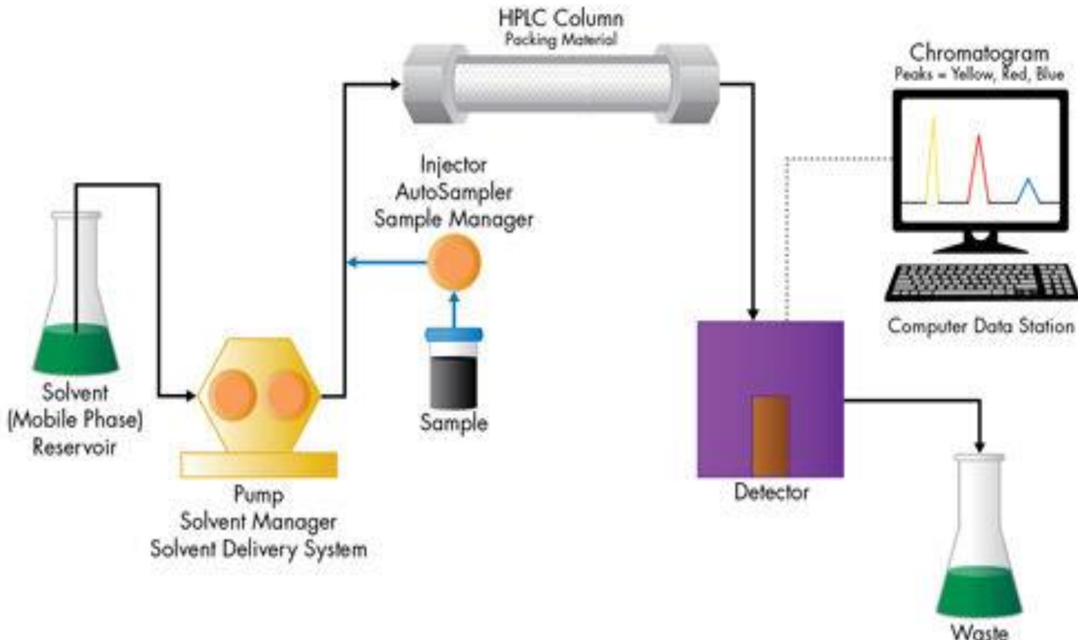


Figure 2.7: Diagram of HPLC instrumentation (Modi et al., 2016)

2.11.2.1 Pumping systems

HPLC pumping systems are required to deliver metered volumes of mobile phase at a constant flow rate. Computer- or microprocessor-controlled pumping systems are capable of accurately delivering a mobile phase of either constant (isocratic elution) or continuously

http://etd.uwc.ac.za/

changing the solvent composition during the run (gradient elution) composition, according to a defined programme (WHO, 2016b; USP, 2006).

2.11.2.2 The injector

The injector introduces the sample into the mobile phase. The injection system has a fixed-loop or a variable volume device which can be operated manually or by an auto-sampler. Manual filling of loops may lead to poorer injection volume precision. The sample is introduced into the loop when the valve is in the load position. At this stage the eluent flows from the pump to the column through another passage. When the valve is switched to inject, the loop is redirected to flow into the column conveying the sample into its destination (WHO, 2016b).

2.11.2.3 The chromatographic column and stationary phases

UNIVERSITY of the
WESTERN CAPE

The columns are made of highly polished stainless steel usually having a column length of 50 to 300 mm and an internal diameter of 2 to 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 μm. Stationary phases are the parts of the HPLC system which are responsible for the separation by partition, adsorption, or ion-exchange of compounds in the test solution with the mobile phase (WHO, 2016b). The most commonly used stationary phases are modified silica, unmodified silica, resins or polymers with acid or basic groups and porous silica or polymers. HPLC systems consisting of polar stationary phases and non-polar mobile phases are defined as normal-phase chromatography; those with non-polar stationary phases and polar mobile phases are defined as reversed-phase (RP-HPLC) (WHO, 2016b). In the RP-HPLC most separations are based on partition mechanisms using chemically modified silica. The surface of the

http://etd.uwc.ac.za/

support, e.g. the silanol groups of silica, is reacted with various silane reagents to produce covalently bonded silyl derivatives covering a varying number of active sites on the surface of the support. Some of commonly used bonded phases are octyl (C8), octadecyl (C18), phenyl (C6H5) and cyanopropyl (CN) (WHO, 2016b; USP, 2006).

2.11.2.5 Mobile phases

The mobile phase is a solvent or a mix of solvents. The selection of mobile phases is based on the desired retention behaviour and the physicochemical properties of the analyte as well as the type of detector chosen. In RP- HPLC aqueous mobile phases, with and without organic such as (methanol or acetonitrile) modifiers, are used (WHO, 2016b).

2.11.2.6 Detectors

One of the commonly used detectors in pharmaceutical analysis are Ultraviolet/visible (UV/vis) absorption spectrophotometers. A variant on the UV/vis type of detector, which can furnish detailed spectral information, is the diode array spectrophotometer. This type of detector acquires absorbance data over a certain UV/vis range and can provide chromatograms at multiple, selectable wavelengths, together with spectra for the eluted peaks. There are other types of detectors that can be used in specific cases such as fluorescence spectrophotometers, differential refractometers (RI), electrochemical detectors, evaporative light-scattering detectors (ELSD), charged aerosol detectors (CAD) and mass spectrometers (MS) (WHO, 2016b; USP, 2006).

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

2.11.3 HPLC method validation

The validation of an analytical procedure is done to demonstrate that it is suitable for its intended purpose (ICH, 2005). A brief description of the types of parameters considered to be validated is provided below.

2.11.3.1 Specificity

It is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Usually these might include, among others, matrix, impurities, degradants. The specificity of the HPLC method is demonstrated by the separation of the analytes from other potential components such as impurities, degradants or excipients which can be shown either in a representative chromatogram or by calculating the resolution of the two compounds which elute closest together.

2.11.3.2 System suitability testing

UNIVERSITY of the
WESTERN CAPE

System suitability specifications and tests are parameters that provide information about the behaviour of a chromatographic system and the quality of HPLC data collected. This test can be done by integrating part of the method which can be used to ensure the adequate performance of the chosen chromatographic system. Several parameters are normally used in assessing column performance such as the resolution factor which is the resolution between two peaks in a chromatogram (Figure 2.8). This can be calculated as shown in Equation 2.1.

$$R_S = \frac{1.18(t_{R2} - t_{R1})}{(W_{b1} + W_{b2})}$$

Equation 2.1

http://etd.uwc.ac.za/

Where: t_{R2} and t_{R1}= retention times or baseline distances between the point of injection and the perpendicular dropped from the maximum of each of the two peaks.

W_{b1} and W_{b2}= the respective peak widths determined at half peak height, measured in the same units as t_{R1} and t_{R2}.

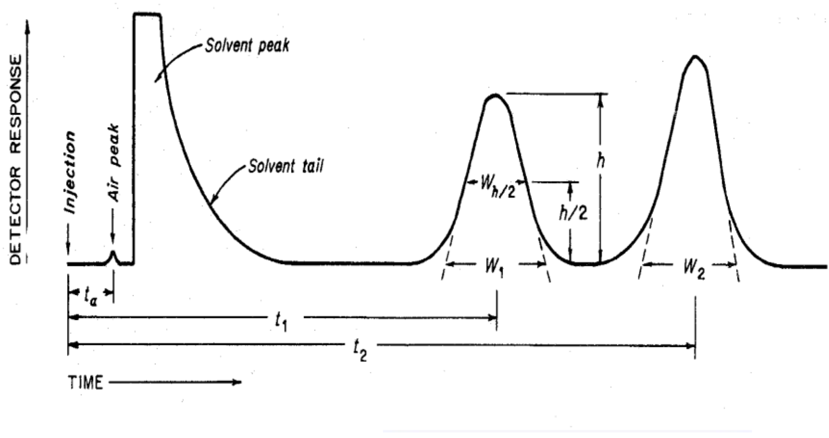


Figure 2.8: Chromatographic separation of two substances (USP, 2006)

UNIVERSITY of the
WESTERN CAPE

Efficiency is defined in terms of the number of theoretical plates (N). The efficiency of a chromatographic peak is a measure of the dispersion of the analyte band as it travels through the HPLC system and column. Thus, the number of theoretical plates N is a measure of the peak dispersion on the HPLC column, which reflects the column performance and can be calculated using Equation 2.2.

http://etd.uwc.ac.za/

$$N = 5.54 \frac{t_R^2}{W_h^2}$$

Equation 2.2

Where: t_R = retention time or the baseline distance between the point of injection and the perpendicular dropped from the maximum of the peak of interest. W_h= the width of the peak of interest determined at half peak height, measured in the same units as t_R.

Another parameter is the symmetry factor, also known as tailing factor (A_S) and is a peak which has a tail portion that is wider than the front portion (Figure 2.9). Tailing can happen if the concentration of the sample is too high or if the column is damaged. The A_S factor can be calculated as in Equation 2.3 below.

UNIVERSITY of the
WESTERN CAPE

$$A_S = \frac{W_X}{2d}$$

Equation 2.3

Where: W_X = peak width at 5% of peak height, measured from the baseline. d = baseline distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at 5% of the peak height, measured in the same units as W_X .

A symmetry factor of one signifies complete symmetry. Values of A_S which are greater than two may lead to incorrect integration, resulting in erroneous quantitation (WHO, 2016b; USP, 2006).

http://etd.uwc.ac.za/

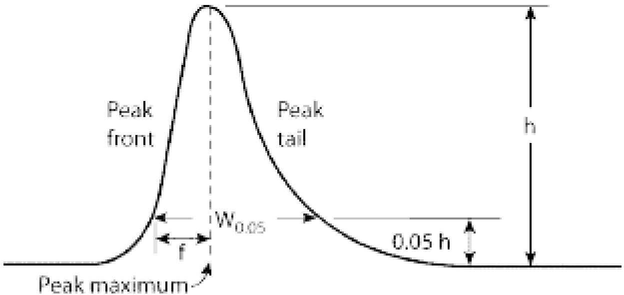


Figure 2.9: Asymmetrical chromatographic peak (USP, 2006)

2.11.3.3 Linearity and range

The linearity of an analytical procedure is its ability, within a specific range, to obtain test results that are directly proportional to the concentration of analyte in the sample. A linear relationship should be assessed across that specific range. That range depends on the intended application of the procedure. However, a minimum of five concentrations is recommended as per the ICH. Evaluation of linearity in an HPLC is conducted by determining the relationship between detector response (peak area or height) and sample concentrations. Data from the regression line such as, correlation coefficient, y-intercept slope of the regression line and the residual sum of squares, should be calculated (ICH, 2005).

UNIVERSITY of the
WESTERN CAPE

2.11.3.4 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements and values of the same homogeneous sample under prescribed conditions. It is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements, values of ≤ 2 % %RSD ensure the

http://etd.uwc.ac.za/

method's precision. Precision can be considered at three levels: repeatability, which expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. Intermediate precision is synonymous with the term ruggedness and expresses between laboratory variations different days when different analysts preform the experiment using different equipment. It is not necessary to study these effects individually. Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to the standardisation of methodology) (ICH, 2005 ;Walfish, 2006).

2.11.3.5 Accuracy

Accuracy, sometimes termed trueness, is an analytical procedure which expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy is usually evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample (a placebo). If the placebo is not available, it can be done by comparison of the result with a reference standard of known purity. Recovery values between 80% and 120% are usually acceptable (ICH, 2005 ;Walfish, 2006)

UNIVERSITY of the
WESTERN CAPE

2.11.3.6 Limits of detection (LOD) and Limits of quantitation (LOQ)

LOD is the lowest concentration of analyte in a sample which can be detected, but not necessarily quantitated as an exact value. The calculation of LOD can be done by determining the concentration of an analyte that yields a peak with a signal-to-noise ratio of three, which proves the presence of an analyte in the test sample with a probability larger than 99%. This method is often evaluated manually. On the other hand, LOQ is the lowest

http://etd.uwc.ac.za/

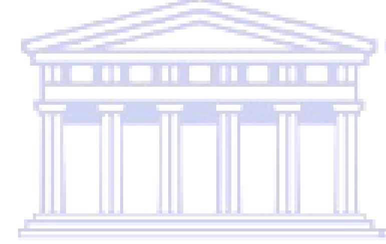
amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ can be calculated by determining the concentration of an analyte that yields a peak with a signal-to-noise ratio of ten. According to the ICH specifications (ICH, 2005) the LOQ and LOD are shown in equations 2.4 and 2.5.

$$LOD = \frac{3.3\sigma}{S}$$

Equation 2.4

Where σ = the standard deviation of the response and S = the slope of the calibration curve

$$LOQ = \frac{10\sigma}{S}$$



Equation 2.5

2.11.3.7 Robustness

UNIVERSITY of the
WESTERN CAPE

Robustness shows the reliability of an analysis with respect to deliberate variations in method parameters. In cases of HPLC, the variations of some parameters such as pH in a mobile phase, flow rate, temperature, and stability of analytical solutions, are recommended to assess robustness (ICH, 2005).

2.11.4 The development and validation of chromatography and spectrophotometry methods to assess the quality of FDCs of ARVs

Literature reveals that few RP-HPLC and normal HPLC methods, either in gradient or isocratic mode, are reported for assessment of the FDC of FTC, TDF, and EFV in pharmaceutical formulation (Raju et al., 2008; Raju and Begum, 2008; Ramaswamy and

http://etd.uwc.ac.za/

Dhas, 2014; WHO, 2016a). These methods were validated and can be applied for a routine quality control test. Similarly, Devrukhakar et al., (2013) developed and validated a RP-HPLC method to determine the stability and to quantify EFV, TDF and FTC in marketed FDCs. On the other hand, the literature also reveals that there are developed and validated available HPLC methods to estimate FTC, TDF, and EFV as individual compounds or in combination with one or two other ARVs (Bhavsar et al., 2012; Karunakaran et al., 2012; Kavitha et al., 2013). The results from the validated methods can be applied for quality tests. Equally, Hamrapurkar et al., (2009) developed an HPTLC method to quantify and estimate EFV from bulk drug and capsule dosage form. The validation parameters showed no interference between the capsules and the excipients, which meant the method could be used for routine testing for EFV. Anandakumar et al., (2011) developed a simple, rapid, precise, accurate spectrophotometric method for the estimation of FTC and TDF in pure and in FDC tablets. The result of the analysis was statistically validated and the method could be used for routine analysis.

UNIVERSITY of the
WESTERN CAPE

2.12 Methods of comparison

Various methods are used to compare the differences in the quality of generic and innovator product and this depends on the type of tests that are used. In the presence of certain minor changes, the single-point dissolution test may be adequate to ensure unchanged product quality and performance. For more major changes, such as post approval changes, manufacturing site changes, component and composition changes, and equipment and process changes, a dissolution profile comparison performed under identical conditions for the product before and after the changes, is recommended (FDA, 1997).

http://etd.uwc.ac.za/

Methods such as the model independent, model dependent, statistical (ANOVA, T-test) and graphical methods can be used in the case of dissolution profile comparison. While in the case of the single-point dissolution test, the use of statistical methods (ANOVA, T-test) and graphical methods are suitable.

http://etd.uwc.ac.za/

CHAPTER 3 METHODS

This chapter describes the design of the study and the materials and methods. To ascertain the quality of FDC ARVs, the following tests were performed: identification by HPLC, dissolution, assay, uniformity of weight and disintegration. Some of the tests were carried out according to the only available pharmacopoeial monograph sourced from the WHO IP. The HPLC method prescribed in the WHO IP monograph was found to be unsuitable. Therefore, RP-HPLC method was developed and validated according to the ICH requirements to carry out the identification, assay and the dissolution tests.

3.1 Study Design

This quantitative cross-sectional comparative study compared the quality profile of four generics of FDC of ARV drug containing EFV (600mg), FTC (200mg) and TDF (300mg). Furthermore, the generics were then compared to corresponding innovator product according to the WHO IP pharmacopoeia specifications (WHO, 2016a).

UNIVERSITY of the
WESTERN CAPE

3.2 Materials

3.2.1 FDC sampling

Four generic FDC FPPs of FTC, TDF and EFV in tablet dosage form were obtained from the Cape Antiretroviral Depot in the Western Cape, as free samples. The depot's most recent updated licence from the MCC was issued in March 2015, which gives an indication of compliance with Good Distribution Practice standards. The generic samples are listed in the Department of Health under tender list for the period 2015 to 2018 and are therefore available in the South African public sector. The tender for this FDC is split between four

http://etd.uwc.ac.za/

suppliers. The originator FDC was purchased from a local private sector community pharmacy in Cape Town (SA).

3.2.2 Chemicals and reagents

Samples of reference standard (RS) 100 mg of FTC (99.7 % m/m), 200 mg of TDF (98.8 % m/m) and 100 mg of EFV (99.8 % m/m) were purchased from the WHO, European Directorate for the Quality of Medicines & Healthcare (EDQM), France. The certificates of analysis of each reference standard are available in appendix 1 for FTC, appendix 2 for TDF and appendix 3 for EFV. Sodium dodecyl sulfate, sodium dihydrogen orthophosphate monohydrate and potassium phosphate dibasic, were purchased from Merck, Germany. Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Merck, SA; fumaric acid was obtained from Sigma Aldrich, SA. Distilled water was obtained from the Milli-RO 4 water purification system, USA and the O purity purification system, SA.

UNIVERSITY of the
WESTERN CAPE

3.2.3 Materials

HPLC vials 1.8 ml, AA Tech, USA; Nylon 0.22 µm syringe filters 25mm, Kim lab, India; 0.45 µm membrane filters Milipore, Ireland; parafilm Pechiney plastic packaging, Chicago, IL-6063; syringe 10 ml and syringe needles, Avacare, Sunray Medical CO., China as well as 10 ml test tubes, Plastpro scientific, SA.

3.2.4 Instruments and equipment

- SOTAX AT7 *SMART* dissolution system for USP apparatus 1,2,5 and 6 with Piston pump SOTAX CY7-50, Fraction collector SOTAX C613 with valve bar and software SOTAX PA 29A. SOTAX AG Basel, Switzerland.

http://etd.uwc.ac.za/

- Chromatographic equipment: Perkin Elmer model Flexar HPLC system (Shelton, CT 06484 USA) with; Flexar binary LC pump (N2910401, Singapore); Flexar FX (PDA) Photodiode Array UHPLC detector (N2920031, USA); Flexar Autosampler (N2930660, Netherland); Flexar solvent manager (N2600581, Singapore)

  Computer modelling software: Chromera® Chromatography data system N1473261600.

- Chromatographic equipment: Agilent HPLC-DAD analyses using an Agilent 1200 series HPLC equipped with an in-line degassing system (G1322A, Japan); quaternary pump G1311A; Thermostatted column compartment (G1316A/ G1316B, Germany); auto loading sampler (G1329A, Germany) and Diode Array and Multiple Wavelength Detector (G1315B, Germany).

- Computer modelling software: OpenLAB™ CDS ChemStation edition HPLC data acquisition software (Agilent Technologies, Palo Alto, CA, USA).

- Electrolab® disintegration tester ED 2AL with a basket-rack assembly consisting of six open-ended transparent tubes; a 1000 ml, low-form beaker for the immersion fluid; transparent plastic discs (Mumbai, India).

- Ascentis® C 18 Column 5 µm particle size, L × I.D. 25 cm × 4.6 mm from Sigma Aldrich (Cape Town, SA). The certificate of analysis for the column is available in appendix 4.

- Discovery® HS C18 Column 5µm particle size, L × I.D. 15cm × 4.6 mm.

- 4.0 L Ultrasonic bath with timer function 702, SA.

- Vortex mixer VM-300, Taiwan.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

- BUCHI VAC vacuum filter V-500, Switzerland.
- pH meter Model PL-700PV, Taipei, Taiwan.
- SHIMADZU electronic analytical balance max 220 g, USA.

3.3 Methods

3.3.1 Uniformity of weight

Twenty tablets (n=20) randomly selected from each container of the different FPPs were weighed individually using an electronic analytical balance and the average, standard deviation (SD) and the relative standard deviation (%RSD) of the twenty tablets were calculated. For a sample to pass the uniformity of weight test not more than two of the individual masses should deviate from the average mass by more than the percentage deviation as shown in Table 2.2 and none should deviate by more than twice the percentage (WHO, 2016c).

UNIVERSITY of the
WESTERN CAPE

3.3.2 Validation of the WHO IP HPLC method

The experiment was done using the Agilent HPLC-DAD system and by using Ascentis® C18 HPLC Column 5 µm particle size, L × I.D. 25 cm × 4.6 mm under the following conditions:

i. Detection wavelength: 280 nm
ii. Flow rate: 1.0 ml/min
iii. Injection volume: 20 µl
iv. Column oven temperature: 35Cº (WHO, 2016a)

3.3.2.1 Preparation of the mobile phases

http://etd.uwc.ac.za/

- Mobile phase A: was prepared by mixing 50 ml of potassium dihydrogen phosphate (27.2g/l) test solution (TS) and 950 ml of water R.
- Mobile phase B: was prepared by mixing 700 ml of acetonitrile R, 50 ml of potassium dihydrogen phosphate (27.2g/l) TS and 250 ml of water R. Both mobile phases were filtered by 0.45 µm membrane filter. After preparation, the solutions were kept in the fridge at about (6Cº) until used (WHO, 2016a). Table 3.1 shows the mobile phases gradient.

Table 3.1: Mobile phases gradient of the WHO IP HPLC method

Time Minutes	Mobile phase A % V/V	Mobile phase B % V/V
0 – 9.0	93	7
9.0 – 15.0	93 – 0	7 -100
15.0 – 19.0	0	100
19.0 – 19.1	0 – 93	100 – 7
19.1 – 30.0	93	7

UNIVERSITY of the
WESTERN CAPE

3.3.2.2 Preparation of standard solution

The solution containing 66.7 µg/ml of FTC RS, 100 µg/ml of TDF RS and 200 µg/ml of EFV RS was prepared. Each RS was accurately weighed and diluted in 80% methanol and water. Thereafter, the solutions were vortexed for two minutes, and filtered with a 0.22 µm

http://etd.uwc.ac.za/

syringe filter. 20 µl was injected into the HPLC system to determine the peak areas (WHO, 2016a).

3.3.2.3 Preparation of FPP samples

Twenty tablets of the FPP were weighed and the average weight of the tablets was determined. The tablets were ground into powder using a mortar and pestle. A quantity of powder containing about 10 mg of TDF was accurately weighed and transferred to a 100 ml flask. The drug powder was initially dissolved in 80% methanol and sonicated for 30 minutes and the solution was filtered using 0.22 µm nylon syringe filter. Each 1 ml of that solution contained 0.0667 mg/ml of FTC, 0.1 mg/ml of TDF and 0.2 mg/ml of EFV. Thereafter, a solution containing 0.02 % w/v of fumaric acid in water was prepared. 20 µl from each solution (RS solution, FPP solution and fumaric acid solution) was injected into the HPLC system to determine the peak areas (WHO, 2016a).

UNIVERSITY of the
WESTERN CAPE

3.3.3 Development and validation of the HPLC method for the FDC of FTC, TDF and EFV

3.3.3.1 Selection and optimization of the HPLC conditions

A reverse phase (RP-HPLC) method was developed and validated using the Perkin Elmer model Flexar HPLC system. A Discovery® HS C18 Column 5µm particle size, L × I.D. 15cm × 4.6 mm was selected after trying different available columns. The choice of a gradient programme with the two mobile phases, A (the puffer) and B (methanol in water 85:15%) was based on the literature (Raju and Begum, 2008). In addition, the use of a PDA detector has helped to select the appropriate detection wavelength for this study which is

http://etd.uwc.ac.za/

260 nm. Samples of RSs were tested at 247 nm, 260 nm, 265 nm and 280 nm in order to get the selected wavelength. Similarly, samples of RSs were injected into the HPLC at flow rates of 0.8 ml/min and 1.0 ml/min. A flow rate of 1.0 ml/min was found more robust than 0.8 ml/min. Then 1.0 ml/min was the flow rate that resulted in well-resolved peaks. The injection volume (10 µl) was chosen after trying to inject 10 µl and 5 µl and the temperature was ambient as per the ICH recommendation.

3.3.3.2 Method validation

The method has been validated according to the ICH specification 2005 which is the most recent ICH specifications (ICH, 2005).

3.3.3.2.1 Preparation of the mobile phases

- Mobile phase A: Sodium dihydrogen orthophosphate monohydrate (0.02M) was prepared by dissolving 2.75 g of buffer in 1000 ml of water and by adjusting the pH to 3.6 with dilute orthophosphoric acid.
- Mobile phase B: was prepared by mixing methanol and water in the ratio of 85:15v/v. Both mobile phases were filtered by 0.45 µm membrane filter before used. Table 3.2 describes the mobile phases gradient.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Table 3.2: Mobile phases gradient of the RP-HPLC method (Raju and Begum, 2008)

Time Minutes	Mobile phase A % V/V	Mobile phase B % V/V
0.01	90	10
5.00	90	10
6.00	35	65
9.00	10	90
11.00	10	90
13.00	90	10
15.00	90	10
15.01	Stop	Stop

UNIVERSITY of the
WESTERN CAPE

3.3.3.2.2 Preparation of standard stock solution

A standard stock solution containing 5 mg of EFV RS, 2.5 mg of TDF RS and 1.6 mg of FTC RS per 10 ml, was prepared. Each RS was accurately weighed and diluted in 100% methanol then the solutions were vortexed for two minutes, and filtered with a 0.22µm syringe filter.

3.3.3.2.3 Specificity

A solution containing a mixture of the tablet (excipients+ APIs) was prepared using the sample preparation procedure as reported in section 3.3.5 (Assay test) below and injected onto the HPLC, to evaluate possible interfering peaks.

http://etd.uwc.ac.za/

3.3.3.2.4 System suitability testing

System suitability tests were carried out on the freshly prepared stock solution of EFV, and TDF and FTC were prepared for the validation of the method. Parameters such as, retention time, resolution factor and symmetry factor (tailing factor) were obtained by integrating part of the method, using the Chromera® chromatography software.

3.3.3.2.5 Linearity

Aliquots of the stock solution were diluted with methanol to five different concentrations (0.05 mg/ml, 0.1 mg/ml, 0.15 mg/ml, 0.2 mg/ml and 0.25 mg/ml) for EFV, (0.025 mg/ml, 0.05 mg/ml, 0.075 mg/ml, 0.1 mg/ml and 0.125 mg/ml) for TDF and (0.0166 mg/ml, 0.0333 mg/ml, 0.05 mg/ml, 0.0667 mg/ml and 0.0838 mg/ml) for FTC. Calibration curves were plotted using the different concentrations against the peak area.

3.3.3.2.6 Precision

UNIVERSITY of the
WESTERN CAPE

The intra-day precision was evaluated by analysing six sample solutions (n=6), at the final concentration of analyses of 0.25 mg/ml of EFV, 0.125 mg/ml of TDF and 0.0838 mg/ml of FTC. The inter-day precision (intermediate precision) was evaluated in three consecutive days (n=18). The concentrations of EFV, TDF and ETC were determined and the %RSDs were calculated.

3.3.3.2.7 Accuracy

Accuracy was determined by adding known amounts of FTC, TDF, and EFV RS to the pre-analysed samples and subjected to the developed HPLC analysis. Samples were prepared in triplicate and the % recovery was determined.

http://etd.uwc.ac.za/

3.3.3.2.8 LOD and LOQ

LOD and LOQ were measured according to ICH on using the concentrations in the calibration curve.

3.3.3.2.9 Robustness

The robustness of the method was investigated by varying the pH of the mobile phase from 3.5 to 3.69 and the flow rate from 1.0 ml/min to 0.8 ml/min.

3.3.4 Identity tests

The identification of FTC, TDF and EFV in tablets was done by the validated HPLC method using the PDA detector at 260 nm and the retention times of the principal peaks due to FTC, TDF and EFV were compared with those in the reference standards.

3.3.5 Assay test

Twenty tablets of each FDC, were weighed and the average weight of the tablets was determined. The tablets were powdered finely using mortar and pestle. A quantity of powder containing about 10 mg of TDF was accurately weighed and transferred to a 100 ml standard flask. The drug powder was initially dissolved in 100% methanol and sonicated for 30 minutes and the solution was filtered using 0.22μm nylon syringe filter. Six replicates from each FDC, having a final concentration of 0.2 mg/ml of EFV, 0.1 mg/ml of TDF and 0.0667 m/ml g of FTC, was obtained. Thereafter, 10 μl from each sample was injected into the HPLC and the peak areas were determined. The average and the SD were calculated (WHO, 2016a). All the FPP FDCs were stored at 20 Cº until the time of the analysis, none of the products exceeded its expiry date before the end of the experiment.

http://etd.uwc.ac.za/

3.3.6 HPLC validation for the dissolution testing

3.3.6.1 Preparation of standard stock solution

A standard stock solution containing 5 mg of EFV RS, 2.5 mg of TDF RS and 1.6 mg of FTC RS per 10 ml, was prepared. Each RS was accurately weighed and diluted with (0.4% SDS in methanol) then, the solutions were vortexed for two minutes, and filtered with 0.22 μm nylon syringe filter.

3.3.6.2 Linearity

Aliquots of the stock solution were diluted with methanol to six different concentrations, (0.08mg/ml, 0.1 mg/ml,0.11 mg/ml, 0.12 mg/ml, 0.13 mg/ml and 0.14 mg/ml) for EFV, (0.04mg/ml, 0.05mg/ml, 0.055mg/ml, 0.06mg/ml, 0.065mg/ml and 0.07mg/ml) for TDF and (0.026mg/ml, 0.033mg/ml, 0.036mg/ml, 0.04mg/ml, 0.043/ml and 0.046mg/ml) for FTC. Calibration curves were plotted using the different concentrations against the peak area.

UNIVERSITY of the
WESTERN CAPE

3.3.6.3. Precision

The intra-day precision was evaluated by analysing six sample solutions at the final concentration of analyses of 0.14mg/ml of EFV, 0.07mg/ml of TDF and 0.046mg/ml of FTC. The inter-day precision (intermediate precision) was evaluated in three consecutive days (n=18). The concentrations of EFV, TDF and ETC were determined and the %RSD was calculated.

http://etd.uwc.ac.za/

3.3.6.4 Accuracy

Accuracy was determined by adding known amounts of FTC, TDF, and EFV RS to the pre analysed samples and subjected to the developed HPLC analytical method. Samples were prepared in triplicate and the % recovery was determined.

3.3.6.5 LOD and LOQ

LOD and LOQ were measured according to the ICH on using the concentrations in the calibration curve.

3.3.7 Dissolution tests

Single-point dissolution tests were carried out as described in the WHO IP pharmacopoeia (WHO, 2016a), to compare the quality of each generic FDC to the originator product. The test was conducted under the following conditions:

- Apparatus: USP type II (paddle)
- Medium: 1000 ml of 2% sodium dodecyl sulfate (SDS) R
- Speed: 100 RPM (revolutions per minute)
- Temperature: 37Cº± 0.5
- Sampling time point: 30 minutes

Six replicates from each FPP FDC were used. The test was done using SOTAX AT7 *SMART* dissolution system. The apparatus was pre- heated to 37 Cº (± 0.5) and the method of test was saved on the apparatus. Thereafter, the tablets were placed in dissolution vessels. Using a button at the side of the apparatus the tablets were placed inside the vessels and immediately the paddles started stirring. After 30 minutes, samples of 5ml each were

UNIVERSITY of the WESTERN CAPE

http://etd.uwc.ac.za/

automatically withdrawn from the vessel and placed into the collector. After allowing the samples to cool at room temperature, samples were filtered using 0.22µm syringe filters. Thereafter, the samples were diluted with methanol to obtain a final concentration of 0.4% w/v SDS. Moreover, by using the validated HPLC method for the dissolution test, 10 µl was injected into the HPLC to determine the peak areas. According to the IP monograph for this FDC, not less than 80% of the amount stated on the label from each active ingredient should be released in 30 minutes (stage 1), If the amount obtained for one of the six tablets is less than 80%, the test must be repeated using a further six tablets; the average amount for all 12 tablets tested should not be less than 75% and no tablet should release less than 60% (stage 2) (WHO, 2016d).

3.3.8 Disintegration test

The test was done using a disintegration apparatus A (Electrolab® disintegration tester ED 2AL) as per the IP general method (WHO, 2016e). The media was heated to 37 Cº and the timer was set at 30 minutes. Six tablets (n=6) were randomly selected from each sample bottle, and placed into each of the six tubes of the basket. The discs were added to the top of the tablets. The apparatus was operated using distilled water as the media. After 30 min the basket was lifted to observe the tablet disintegration.

3.3.9 Statistical analysis

A software program, Graph Pad Prism 6 (CA,USA, 2012), was used to analyse the collected data. Results are summarised as mean, SD and %RSD. One-way ANOVA, Tukey's multiple comparisons test, was used to compare differences between the quantities

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

and the release of the active ingredients in the originator and the four generics, and between the generics with a confidence interval (Cl) of 95% and significance level set at $p < 0.05$.

3.4 Ethics

The ethical considerations for this study are anonymity. Therefore, the names of the tender companies were concealed in the reports of study findings. They are referred to as an abbreviation namely, originator (O), generic 1 (G1), generic 2 (G2), generic 3 (G3) and generic 4 (G4).

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

CHAPTER 4 RESULTS

Chapter 4 presents the results of the study. Tables and graphs are used appropriately to describe the findings.

4.1 The uniformity of weight test

All generics and the originator samples passed uniformity of weight tests with all the samples having less than 5% RSD. The results are summarised in Table 4.1; and these results comply with the specifications stated in the IP for the film-coated tablets, which have more than 250 mg of the average weight.

Table 4.1: Uniformity of weight of the originator and generic FDCs

Samples	Weight of 20 tablets (g)	Average (g)	SD	Deviation by 5%
O	31.89	1.59	0.01	None
G1	32.63	1.63	0.01	None
G2	31.94	1.59	0.02	None
G3	32.34	1.61	0.01	None
G4	30.95	1.54	0.01	None

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

4.2 WHO IP HPLC method for simultaneous identification and assay of the FDC of EFV, TDF and FTC

It proved difficult to obtain a clear separation between the three active ingredients, either in the reference standards or in the tablet samples as shown in Figure 4.1 and 4.2

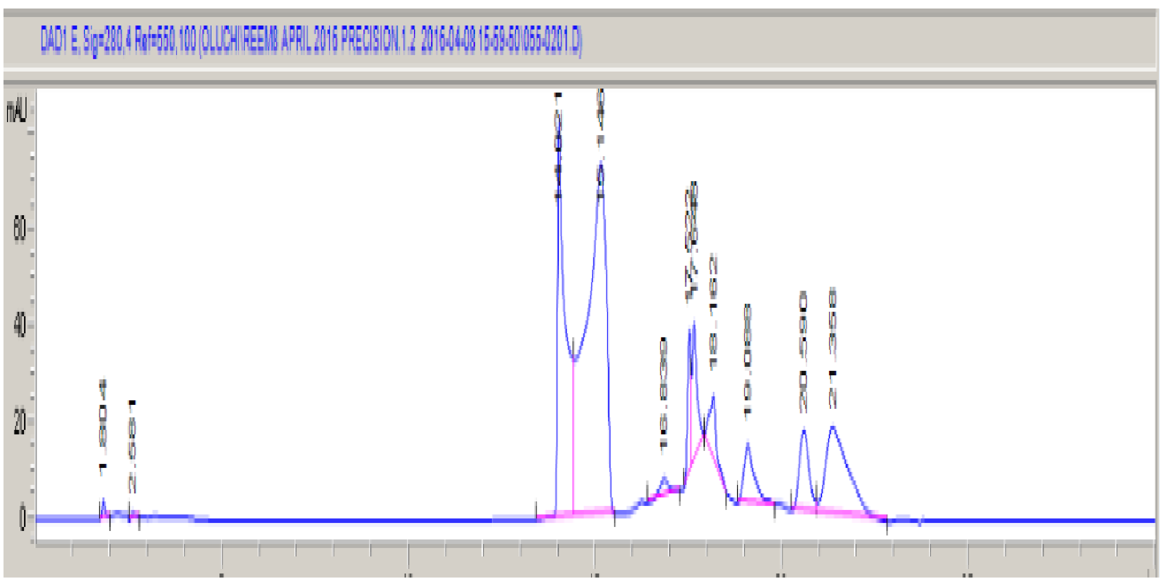


Figure 4.1: Representative chromatogram for FTC, TDF and EFV RSs

http://etd.uwc.ac.za/

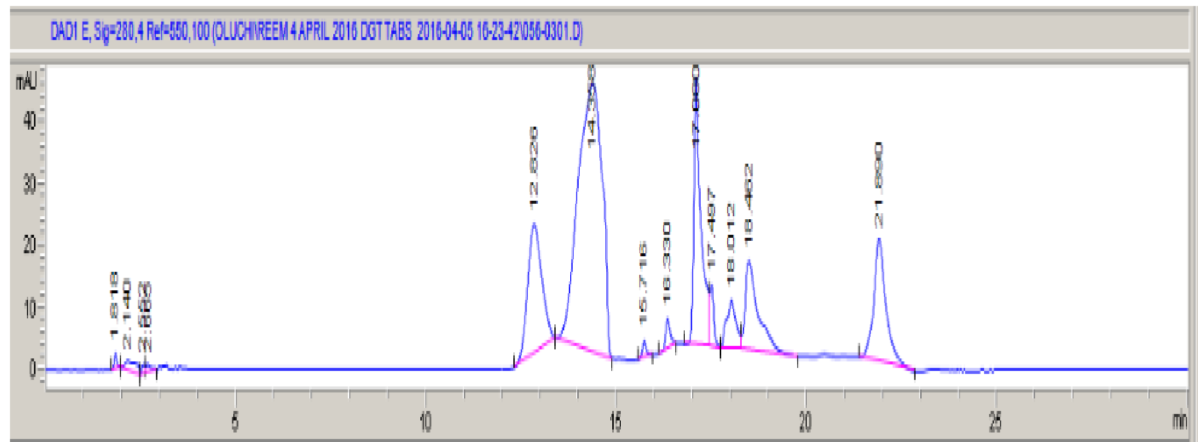


Figure 4.2 Representative chromatogram for FTC, TDF and EFV in FDC tablet

The analysis was performed on the HPLC system mentioned in section 3.3.2 with the aim of trying to match the condition stated in the IP monograph for this combination. According to the IP specifications, the test is not valid unless the resolution between the three peaks is at least 5 and this was not achieved. Solubility problems occurred with EFV RS after being diluted in 50% methanol, because it is widely known that EFV is insoluble in water and freely soluble in methanol in the pH range of 3 to 9 (Hamrapurkar et al., 2009). Therefore, a slight modification was performed on the sample diluents, by raising the percentage of the methanol from 50% to 80%. Although we tried to employ the same conditions that were indicated in the monograph, with a slight modification in the sample dilution, no change in the results was observed. Furthermore, the samples were tested at different wavelengths (280 nm, 260 nm, 254 nm and 210 nm) to selecte the best and highest molar absorptivity wavelength. The Ascentis® C18 HPLC Column (25cm×4.6mm, 5 µm) was a brand new column and the mobile phases were freshly prepared as indicated in the monograph. However, no significant change in the results was observed, as the resolution between the

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

three peaks was less than 1.5 which is not acceptable as per the ICH (ICH, 2005) and, with significant peak interference. Therefore, a new HPLC method was developed and validated to carry out the experiment.

4.3 Development and validation of an HPLC method for the determination of FDC of FTC, TDF and EFV

The RP-HPLC method was developed and validated to assess the post-market quality of generic FDCs of FTC, TDF and EFV available in the South Africa public sector. The experiment was performed using a Perkin Elmer model Flexar HPLC system and a Discovery® HS C18 Column (15cm × 4.6 mm, 5µm). The flow rate was 1.0 ml/min and the injection volume was10 µl at ambient temperature. The separation between the three active ingredients was achieved with a good and accepted resolution, within the limits as per the ICH. The value of the resolution factor which corresponds to a baseline separation between two symmetric peaks was greater than 1.5. Moreover, it was observed from the UV spectra that all three APIs have considerable absorbances at 260 nm wavelength. The wavelength of 260 nm was selected for detection due to the suitable molar absorptivity of FTC, TDF, and EFV and the higher selectivity and there were no interfering peaks of compounds or solvents in the sample at this wavelength. The method was Successfully validated and the results showed below.

UNIVERSITY of the
WESTERN CAPE

4.3.1 Specificity

The chromatogram obtained with the mixture of the tablet (excipients + APIs) showed no interfering peaks in the same retention time of EFV, TDF and FTC. Representative

http://etd.uwc.ac.za/

chromatograms are shown in Figure 4.3 for the reference standard and in Figure 4.4 for the samples of the FPPs.

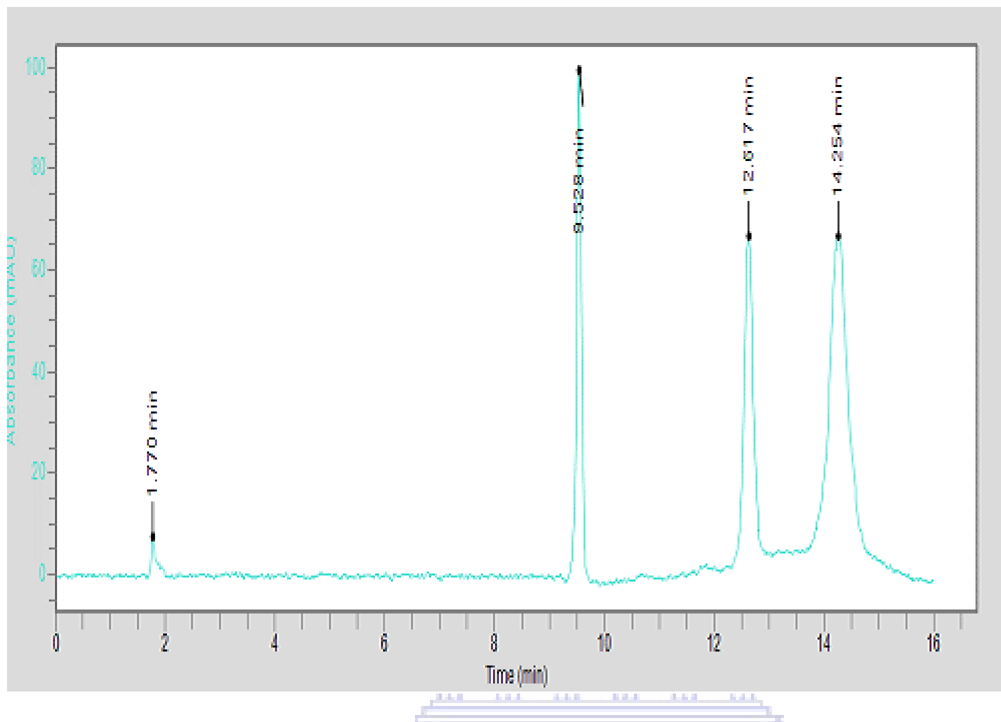


Figure 4.3: Representative chromatogram of FTC (9.53), TDF (12.61) and EFV (14.25) RSs

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

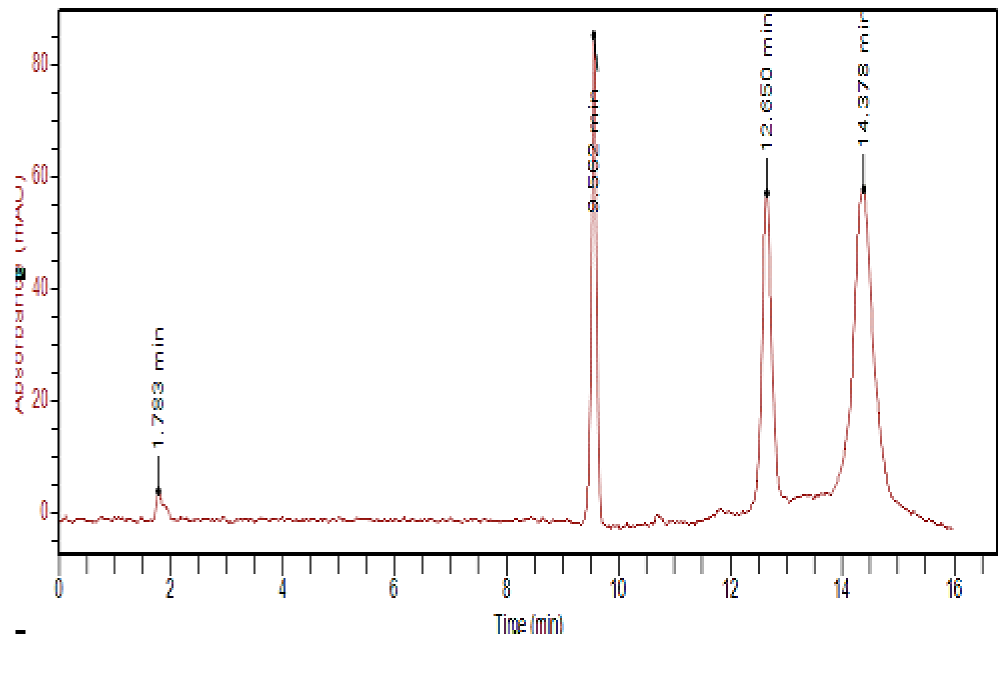


Figure 4.4: Representative chromatogram of FTC (9.56), TDF (12.65) and EFV (14.37) in tablets

4.3.2 System suitability test

The parameters that were studied to evaluate the suitability are presented in Table 4.2. The tailing factors were (≤ 1) which confirm and signify complete symmetry of the three peaks. The resolution factor which corresponds to a baseline separation between two symmetric peaks was greater than 1.5 of the all APIs; this is considered a good resolution.

http://etd.uwc.ac.za/

Table 4.2: System suitability parameters for FTC, TDF and EFV

Parameter	**FTC**	**TDF**	**EFV**
Retention time (min)	9.52	12.62	14.24
Tailing factor (As)	0.94	1.00	1.11
Resolution factor (Rs)	60.11	13.66	3.97

4.3.3 Linearity

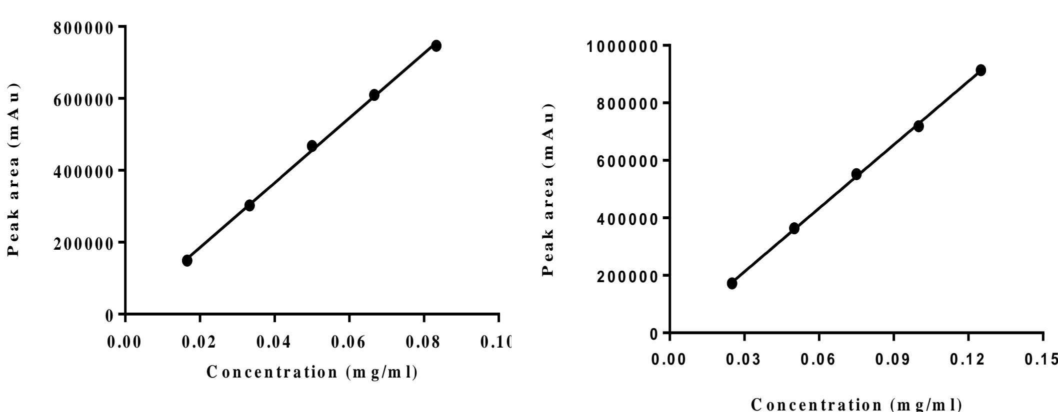
A linear correlation, i.e. $R^2 > 0.99$, was found between the peak areas and the concentration of FTC, TDF and EFV in the assayed range. The regression analysis data are presented in Table 4.3. Furthermore, the calibration graphs were obtained by plotting peak areas versus the concentrations of FTC (Figure 4.5; a), TDF (Figure 4.5; b) and EFV (Figure 4.5; c).

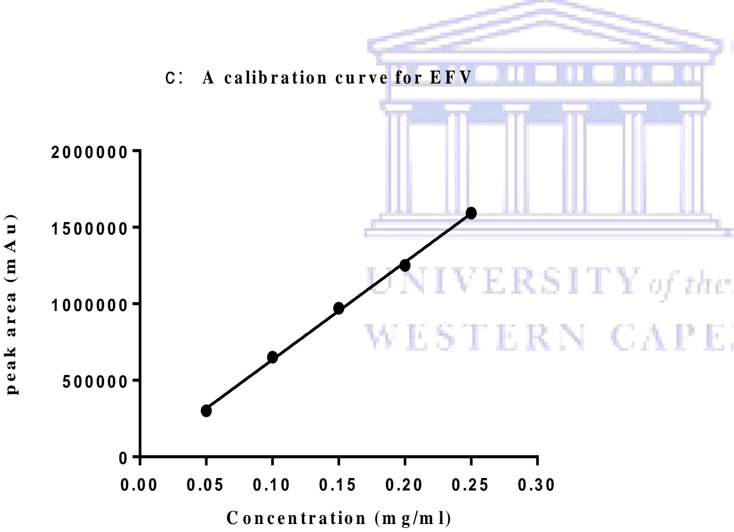
UNIVERSITY of the
WESTERN CAPE

Table 4.3: Regression analysis data for FTC, TDF and EFV

Regression parameters	**FTC**	**TDF**	**EFV**
R^2	0.9987	0.9995	0.9988
Regression equation	Y=9E+06×+4945.6	Y=7E+06× -7822.6	Y=6E+06×-2231
Linearity range (mg/ml)	0.05 to 0.25	0.025 to 0.125	0.016 to 0.083
Number of points	5	5	5

http://etd.uwc.ac.za/





UNIVERSITY of the
WESTERN CAPE

Figure 4.5: Calibration curves of a) FTC, b) TDF and c) EFV

http://etd.uwc.ac.za/

4.3.4 Precision

For the intra-day and the inter-day precision %RSD values lower than 2% were found which assure the precision of the method. The findings of intra and inter-day precision are presented in Table 4.4.

Table 4.4: The intra and inter-day data for FTC, TDF and EFV

API	Intra-day (%RSD)	Inter-day (%RSD)
FTC	1.12	1.21
TDF	0.75	1.36
EFV	1.08	1.53

4.3.5 Accuracy

The % recovery was within the limits and the %RSD of the three APIs ranged from 1.82 to 2.27. The results obtained are presented in Table 4.5.

UNIVERSITY of the WESTERN CAPE

Table 4.5: Accuracy data for FTC, TDF and EFV

API	Levels %	Sample amount (mg/ml)	Amount Added (mg/ml)	Amount recovered (mg/ml)	Mean % Recovery	% RSD
FTC	50	0.0667	0.016	0.0159	96.35*	0.21
	100	0.0667	0.0667	0.0671	100.73*	1.11
	120	0.0667	0.0833	0.0823	98.88*	1.23
TDF	50	0.1	0.025	0.025	100*	1.63
	100	0.1	0.1	0.1	100*	0.85
	120	0.1	0.125	0.13	104*	1.34
EFV	50	0.2	0.05	0.05	100*	2.25
	100	0.2	0.2	0.2	100*	0.40
	120	0.2	0.25	0.26	104*	0.52

(*) the mean of % recovery of three samples

http://etd.uwc.ac.za/

4.3.6 LOD and LOQ

The results of LOD and LOQ of FTC, TDF and EFV are presented in Table 4.6. The results should be considered as the limits of this study.

Table 4.6: The limits of detection and quantitation of FTC, TDF and EFV

API	LOD	LOQ
FTC	3.75 µg/ml	11.37 µg/ml
TDF	3.66 µg/ml	11.09 µg/ml
EFV	11.02 µg/ml	33.40 µg/ml

UNIVERSITY of the
WESTERN CAPE

4.3.7 Robustness

The method showed robustness with respect to changes in the pH of the mobile phases from 3.5 to 3.69 as these resulted in no significant difference between the results obtained, while the decrease of the flow rate from (1 ml/min to 0.8 ml/min) increases the retention times of all the APIs, especially for EFV. The retention time increased from 9.53 min to 10.56 min for FTC, 12.56 min to 13.88 min for TDF and from 14.25 min to more than 20 min for EFV. A decrease in flow rate generally increases the retention time of eluting compounds. In the case of EFV, the increase in retention time was significant. A significant increase in retention times may result in peak broadening, and lead to compromised chromatographic output.

http://etd.uwc.ac.za/

4.4 Identity test

The identification of FTC, TDF and EFV in the tablets was done by the validated HPLC method. The PDA detector wavelength was set at 260 nm. The UV spectra and the retention times of the peaks of FTC, TDF and EFV obtained for the reference standard are similar to the retention obtained with the tablets as shown in Figures 4.3 and 4.4. From the identification tests, the results indicated that the samples were the FDCs of EFV, TDF and FTC. Figures 4.6 (a-f) represent the UV spectra of FTC, TDF and EFV of the reference standards and the tablets. The results were same for all the FPPs generics and the originator.

http://etd.uwc.ac.za/

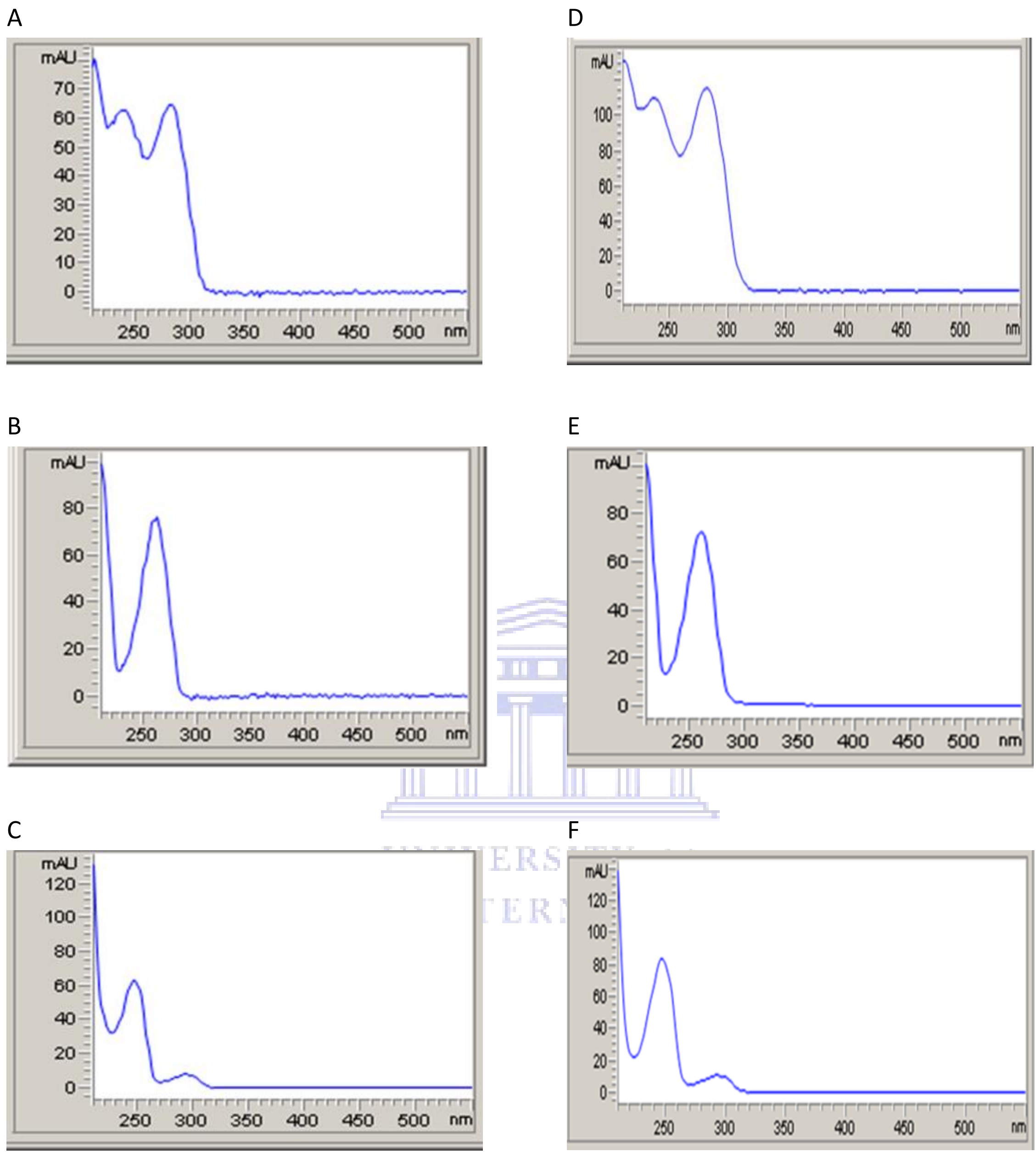


Figure 4.6: Typical UV spectra for the FPP (tablets) FTC (a), TDF (b) and EFV (c) and the reference standards FTC (d), TDF (e) and EFV (f)

http://etd.uwc.ac.za/

4.5 Assay test of the FDC tablets

All samples were analysed using the developed and validated RP-HPLC. All the analysed samples showed that, the EFV, TDF and FTC % content was very close to the label claims, and within the WHO IP limits as stated in the monograph for this combination which are from 90% to 110%. The results obtained are presented in Figure 4.7. The % content is the average of six samples. Furthermore, a comparison of the % content of the three APIs between the originator and the generics, and among the generics themselves, was carried out by Tukey's multiple comparisons test, with Cl of 95% and significance level of $p < 0.05$.

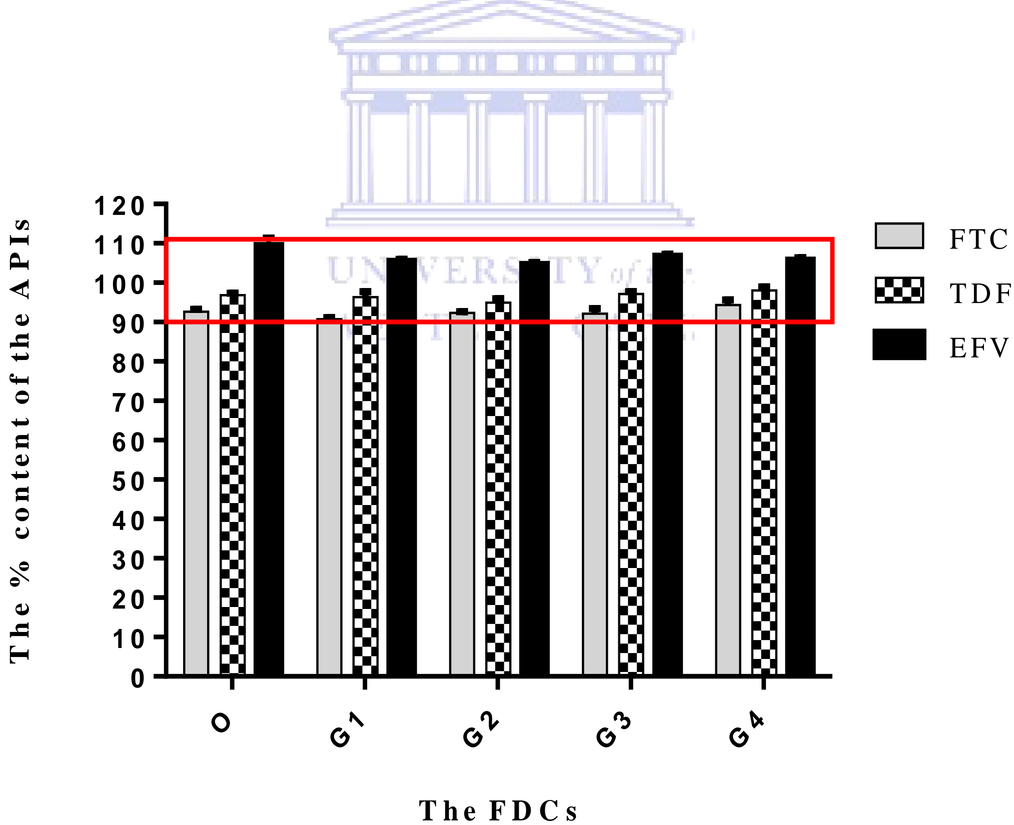


Figure 4.7: The % content (w/w) of FTC, TDF and EFV in the originator and generic FDCs

http://etd.uwc.ac.za/

Figure 4.7 shows the % content of the three APIs of all the FPP FDCs, and the WHO IP specification of the content assay for this FDC which is from 90 % to 110 %.

4.5.1 The comparison of % content of EFV in the all FDCs

The comparison of EFV in the originator and the four generics is summarised in Figure 4.8.

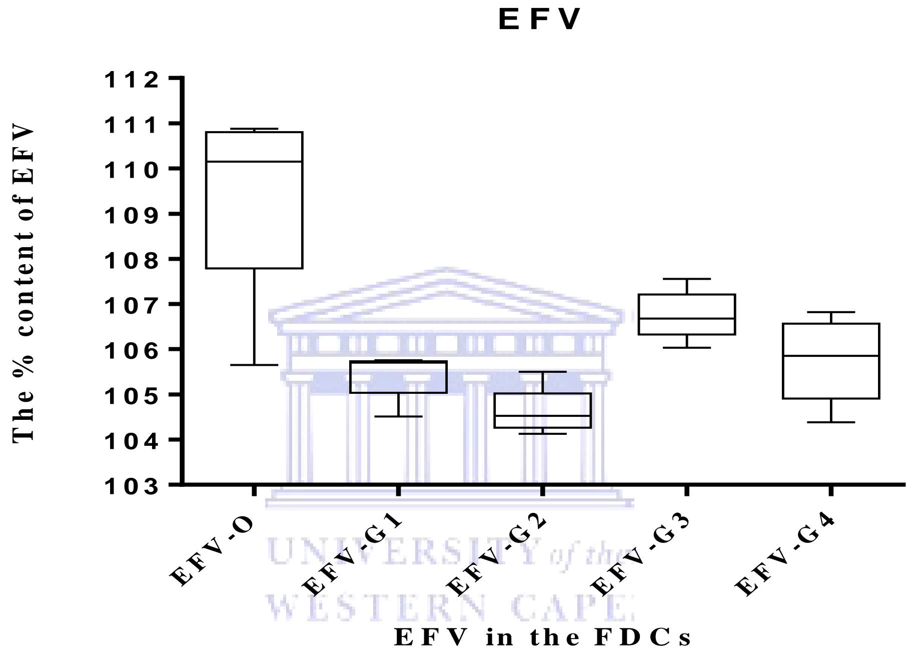


Figure 4.8: The comparison of the % content of EFV in the originator and generic FDCs

Figure 4.8 shows significant differences in the comparison of the % content of EFV in the originator and generics. See Table 4.7 for p-values. The results are summarised as mean and SD. The most significant differences were between EFV-O and EFV-G2

http://etd.uwc.ac.za/

Table 4.7: Comparison of the % EFV in the originator and generic FDCs

EFV-O[a]	EFV-G1[b]	EFV-G2[c]	EFV-G3[d]	EFV-G4[e]	p-value
Mean (SD)					
109.46 (2.17)	105.0 (0.52)	104.5 (0.52)	106.20 (0.54)	105.76 (0.92)	$p^{ab} = 0.0002$
					$p^{ac} < 0.0001$
					$p^{ad} = 0.0091$
					$p^{ae} = 0.0004$
					$p^{bc} = 0.7748$
					$p^{bd} = 0.3910$
					$p^{be} = 0.9917$
					$p^{cd} = 0.0518$
					$p^{ce} = 0.5172$
					$p^{de} = 0.6465$

UNIVERSITY of the
WESTERN CAPE

The results showed a significant difference between EFV-O and each of EFV-G1, EFV-G2, EFV-G3 and EFV-G4, ($p = 0.0002$, $p < 0.0001$, $p = 0.0091$ and $p = 0.0004$ respectively).

4.5.2 The comparison of % content of TDF in all the FDCs

The comparison of TDF in the originator and the four generics is summarised in Figure 4.9.

http://etd.uwc.ac.za/

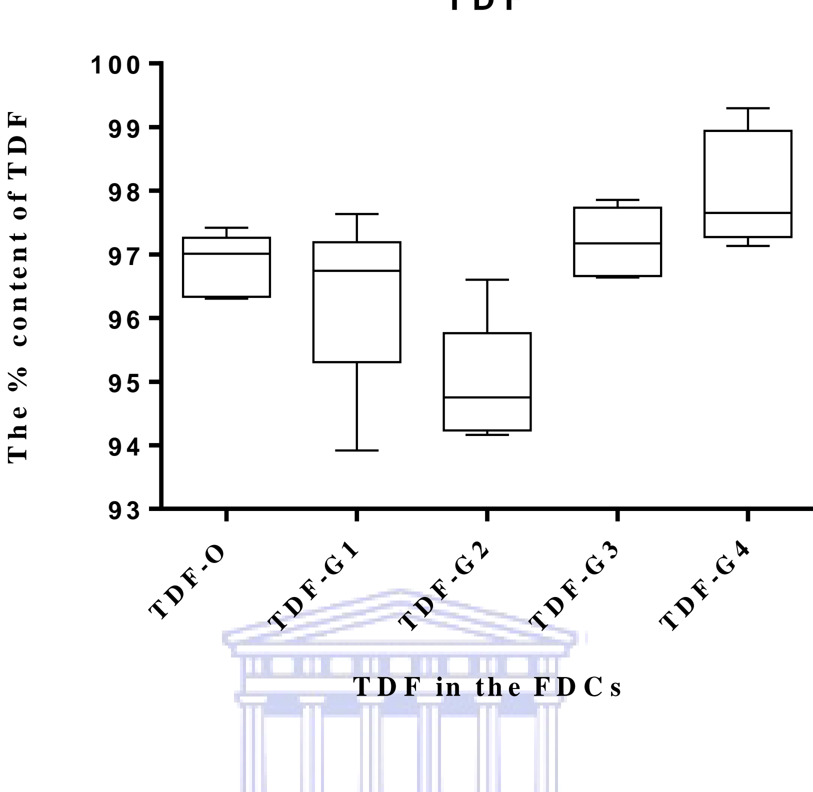


Figure 4.9: The comparison of the % content (w/w) of TDF in the originator and generic FDCs

UNIVERSITY of the
WESTERN CAPE

Figure 4.9 shows significant differences in the comparison of the % content of TDF in the originator and generics, see Table 4.8 for p-values. The results are summarised as mean and SD. The most significant differences were between TDF-G2 and TDF-G4.

http://etd.uwc.ac.za/

Table 4.8: Comparison of the % TDF in the originator and generic FDCs

TDF-O[a]	TDF-G1[b]	TDF-G2[c]	TDF-G3[d]	TDF-G4[e]	p-value
Mean (SD)					
96.84 (0.49)	96.35 (1.41)	94.95 (0.98)	97.19 (0.54)	98.02 (1.34)	$p^{ab} = 0.9161$
					$p^{ac} = 0.0308$
					$p^{ad} = 0.9728$
					$p^{ae} = 0.2985$
					$p^{bc} = 0.1591$
					$p^{bd} = 0.6105$
					$p^{be} = 0.0676$
					$p^{cd} = 0.0083$
					$p^{ce} = 0.0003$
					$p^{de} = 0.6328$

UNIVERSITY of the
WESTERN CAPE

Table 4.8 shows the % of TDF in O and G1, G2, G3, G4. There was a significant difference in %TDF between TDF-O and TDF-G2 ($p = 0.0308$). Also, a significant difference in % of TDF was found between TDF-G2 and TDF-G3 ($p=0.0083$), TDF-G2 and TDF-G4 ($p= 0.0003$).

http://etd.uwc.ac.za/

4.5.3 The comparison of % content of TDF in all the FDCs

The comparison of FTC in the originator and the four generics is summarised in Figure 4.10.

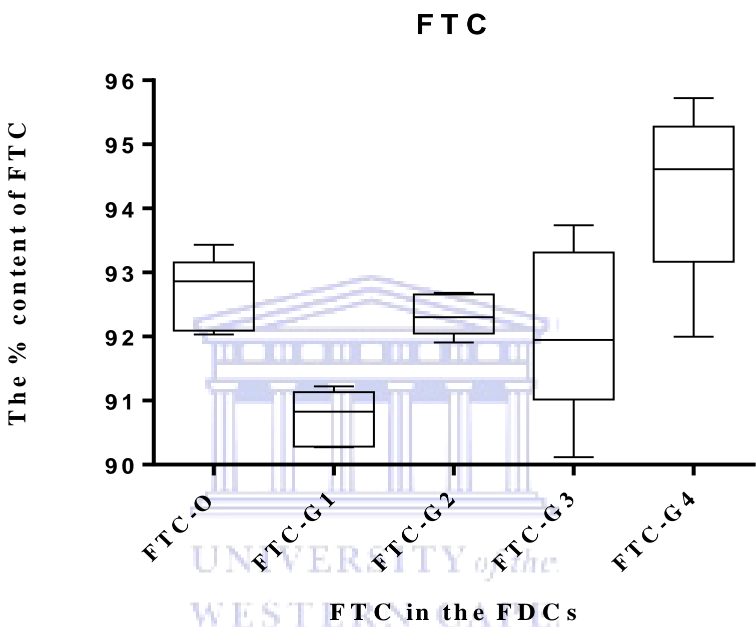


Figure 4.10: The comparison of the % content of FTC in the originator and generic FDCs

Figure 4.10 shows the significant differences in the comparison of the % content of the FTC of O and G1, G2, G3 and G4. See Table 4.9 for p-values. The % content values are summarised as mean and SD. The most significant differences were between FTC-G1 and FTC-G4 ($p < 0.0001$).

http://etd.uwc.ac.za/

Table 4.9: Comparison of the % FTC in the originator and generic FDCs

FTC-O[a]	FTC-G1[b]	FTC-G2[c]	FTC-G3[d]	FTC-G4[e]	p-value
Mean (SD)					
92.67 (0.58)	90.73 (0.43)	92.34 (0.32)	92.12 (1.35)	94.30 (1.38)	$p^{ab} = 0.0275$
					$p^{ac} = 0.9799$
					$p^{ad} = 0.8820$
					$p^{ae} = 0.0806$
					$p^{bc} = 0.0856$
					$p^{bd} = 0.1708$
					$p^{be} < 0.0001$
					$p^{cd} = 0.9955$
					$p^{ce} = 0.0257$
					$p^{de} = 0.0114$

UNIVERSITY of the
WESTERN CAPE

The % of FTC in O and the generic products, G1, G2, G3 and G4 are summarised in Table 4.9. There was a significant difference between FTC-O and FTC-G1 ($p= 0.0275$). Similarly, a significant difference was found between FTC-G4 and each FTC-G1, FTC-G2 and FTC-G3 ($p < 0.0001$, $p= 0.0257$, $p=0.0114$ respectively).

4.6 Validation of RP-HPLC for dissolution testing

4.6.1 Linearity

http://etd.uwc.ac.za/

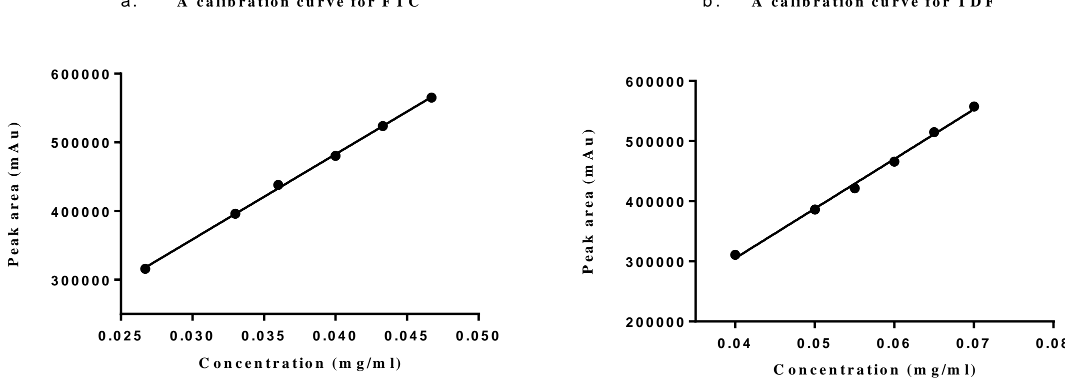
A linear correlation, i.e. $R^2 > 0.99$, was found between the peak areas and the concentration of FTC, TDF and EFV in the assayed range. The regression analysis data are presented in Table 4.10. Furthermore, the calibration graphs were obtained by plotting peak areas versus the concentrations of FTC (Figure 4.11; a), TDF (Figure 4.11; b) and EFV (Figure 4.11; c).

Table 4.10: Regression analysis data for FTC, TDF and EFV

Regression parameters	**FTC**	**TDF**	**EFV**
R^2	0.9992	0.9965	0.9966
Regression equation	Y= 1E+07X- 14039	Y=8E+06X-25140	Y= 7E+06X-122774
Linearity range (mg/ml)	0.026 to 0.046	0.04 to 0.07	0.08 to 0.14
Number of points	6	6	6

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/



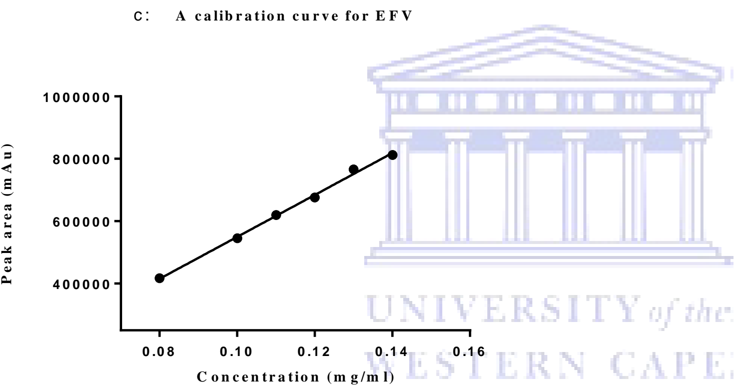


Figure 4.11: Calibration curves of a) FTC, b) TDF and c) EFV

4.6.2 Precision

For the intra-day and the inter-day precision %RSD values lower than 2% were found which assured the precision of the method. The findings are presented in Table 4.11.

http://etd.uwc.ac.za/

Table 4.11: Intra and inter-day data for FTC, TDF and EFV

API	Intra-day (%RSD)	Inter-day (%RSD)
FTC	0.32	0.62
TDF	0.86	1.30
EFV	1.39	1.62

4.6.3 Accuracy

The % recovery was within the limits and the %RSD of the three APIs ranged from 2 to 2.39. The results obtained are presented in Table 4.12.

Table 4.12: Accuracy data of FTC, TDF and EFV

API	Levels %	Sample amount (mg/ml)	Amount Added (mg/ml)	Amount recovered (mg/ml)	Mean % Recovery	% RSD
FTC	80	0.036	0.03	0.030	113.00*	1.69
	100	0.036	0.036	0.040	111.11*	0.17
	120	0.036	0.043	0.047	109.00*	0.96
TDF	80	0.05	0.045	0.043	95.63*	2.00
	100	0.05	0.055	0.050	90.90*	2.00
	120	0.05	0.065	0.060	92.30*	1.87
EFV	80	0.11	0.09	0.083	92.44*	1.98
	100	0.11	0.11	0.10	96.44*	0.86
	120	0.11	0.13	0.12	97.68*	0.37

(*) the mean of % recovery of three samples

4.6.4 Limit of detection and limit of quantitation

The results of LOD and LOQ of FTC, TDF and EFV are presented in Table 4.13. These results should be considered the limits of this study.

http://etd.uwc.ac.za/

Table 4.13: Limits of detection and quantitation of FTC, TDF and EFV

API	LOD	LOQ
FTC	1.81µg/ml	5.51µg/ml
TDF	5.61µg/ml	17.01µg/ml
EFV	11.06µg/ml	33.54µg/ml

4.7 Dissolution tests

A total of five products were tested for dissolution. All the products complied with the specifications except for one sample (G2). G2 was particularly insoluble in the dissolution medium after 30 minutes as shown in Figure 4.12.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Figure 4.12: Dissolution test of generic 2 (G2) at 30 minutes

UNIVERSITY of the
WESTERN CAPE

Despite G2 passing the specifications in terms of the Q-release of FTC and TDF, at 93.03% (1.90) and 94.94% (7.53) respectively, G2 failed to meet the specification in terms of EFV by 73.99% (10.78) Q-release and only two tablets of the six tested had a Q-release of 85.07% and 81.08% in stage 1. Therefore, another six tablets had to be tested for stage 2 dissolution. The average value of all the twelve tablets (stage 2) for the same API was 62.23% (20.43), which is less than 75% and more than one tablet released was less than 60%. The Q-release values of ten out of the twelve tablets were from 22.04% to78.73%. The results are presented in Figure 4.13. Comparisons between the originator and the

http://etd.uwc.ac.za/

generics and among the generics themselves, were carried out by Tukey's multiple comparisons test, with a Cl of 95% and significance level of $p < 0.05$.

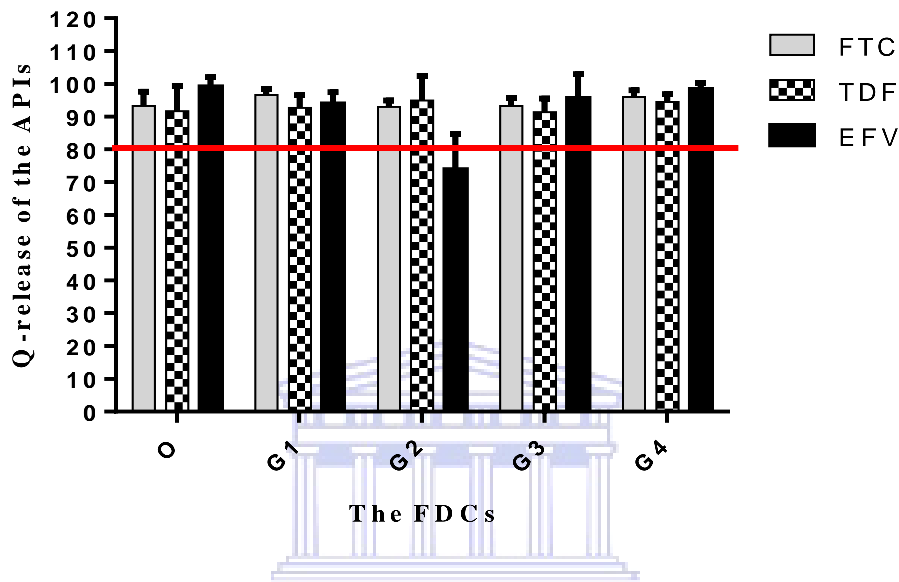


Figure 4.13: Q-release of APIs from FDCs at 30 minutes

UNIVERSITY of the
WESTERN CAPE

Figure 4.13: shows the Q-release of all the API of all the FDCs at 30 minutes, which must be ≥ 80 % as per the WHO IP specification of the dissolution test for this FDC.

4.7.1 The comparison of the Q-release of EFV in all the FPP FDCs

The comparison of the Q-release of EFV in the originator and the generic FDCs is summarised in Figure 4.14.

http://etd.uwc.ac.za/

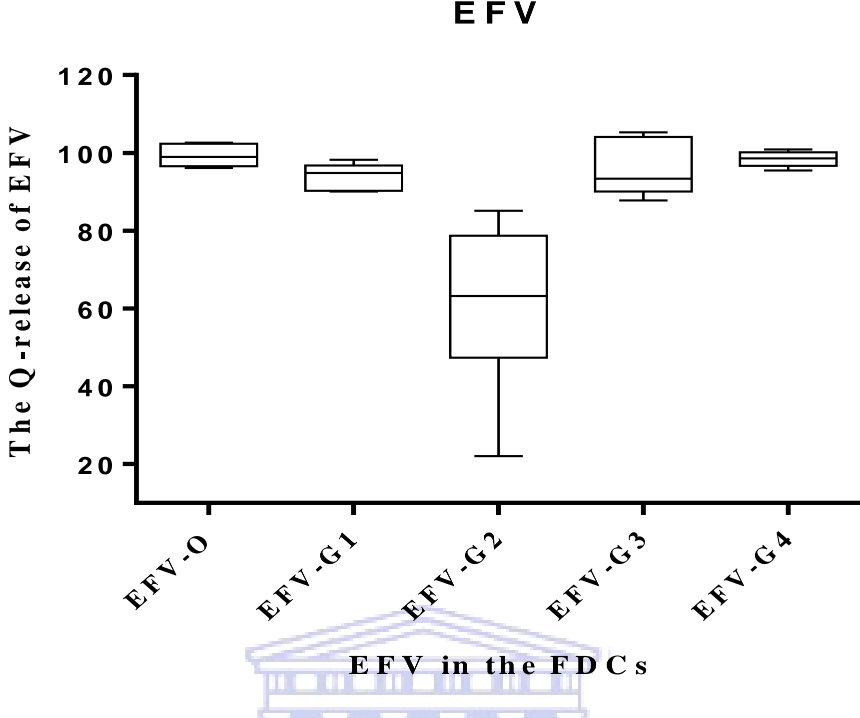


Figure 4.14: Comparison of the Q-release of EFV in the originator and the generic FDCs

UNIVERSITY of the
WESTERN CAPE

Figure 4.14 shows the significant differences in the Q-release of EFV in all products. See Table 4.14 for p-values. The results are summarised as mean and SD. The most significant differences were between EFV-G2 and all the other products.

http://etd.uwc.ac.za/

Table 4.14: Comparison of the Q-release of EFV in the originator and the generic FDCs

EFV-O[a]	EFV-G1[b]	EFV-G2[c]	EFV-G3[d]	EFV-G4[e]	p-value
Mean (SD)					
99.29 (2.73)	94.07 (3.33)	73.99 (10.78)	95.73 (7.17)	98.42 (1.96)	$p^{ab} = 0.9567$
					$p^{ac} < 0.0001$
					$p^{ad} = 0.9894$
					$p^{ae} > 0.9999$
					$p^{bc} = 0.0002$
					$p^{bd} = 0.9994$
					$p^{be} = 0.9775$
					$p^{cd} < 0.0001$
					$p^{ce} < 0.0001$
					$P^{de} = 0.9963$

UNIVERSITY of the
WESTERN CAPE

The results showed a significant difference between EFV-G2 and each of EFV-O, EFV-G1, EFV-G2, EFV-G3 and EFV-G4 ($p < 0.0001$ for all).

4.7.2 The comparison of the Q-release of TDF in all the FPP FDCs

The comparison of the Q-release of TDF in the originator and the four generics is summarised in Figure 4.15.

http://etd.uwc.ac.za/

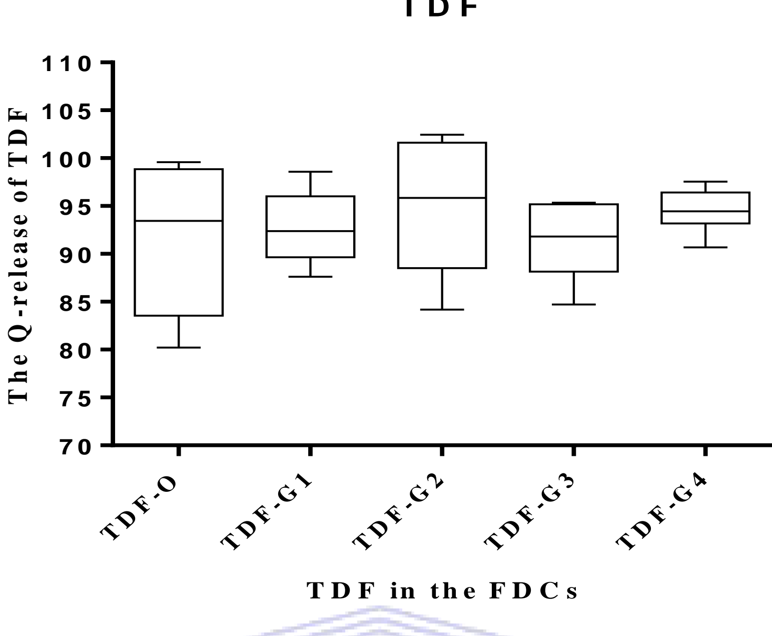


Figure 4.15: Comparison of the Q-release of TDF in the originator and the generic FDCs

Figure 4.15 shows no significant difference between the Q-release of TDF of all the products. See Table 4.15 for p-values. The Q-release values are summarised as mean and SD.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Table 4.15: Comparison of the Q-release of TDF in the originator and generic FDCs

TDF-O[a]	TDF-G1[b]	TDF-G2[c]	TDF-G3[d]	TDF-G4[e]	p-value
Mean (SD)					
91.66 (7.74)	92.74 (3.81)	94.94 (7.53)	91.36 (4.15)	94.54 (2.31)	$p^{ab} = 0.9969$
					$p^{ac} = 0.8408$
					$p^{ad} > 0.9999$
					$p^{ae} = 0.8940$
					$p^{bc} = 0.9576$
					$p^{bd} = 0.9922$
					$p^{be} = 0.9796$
					$p^{cd} = 0.7949$
					$p^{ce} > 0.9999$
					$p^{de} = 0.8558$

UNIVERSITY of the
WESTERN CAPE

The P values in Table 4.15 showed no significant difference in the Q-release of TDF in all products.

4.7.3 The comparison of the Q-release of FTC in all the FPP FDCs

The comparison of the Q-release of FTC in the originator and the four generics is summarised in Figure 4.16.

http://etd.uwc.ac.za/

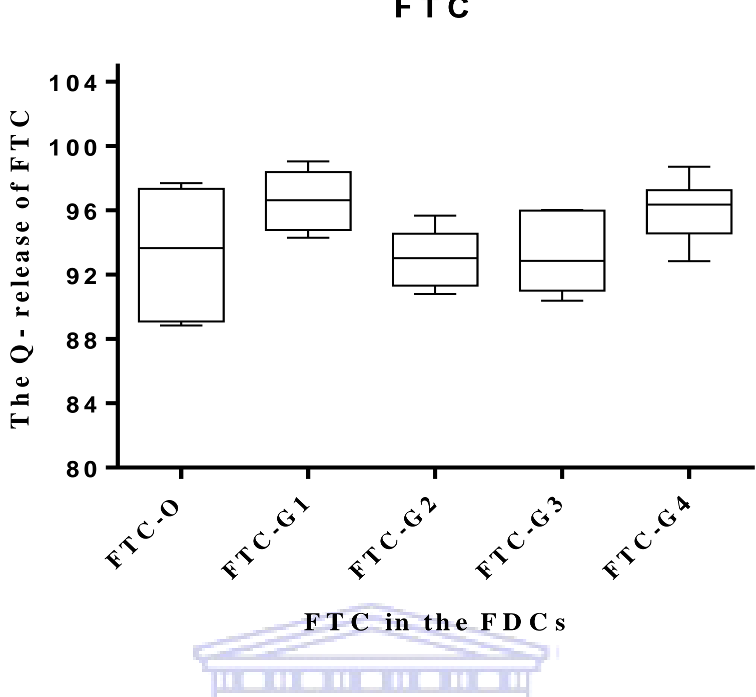


Figure 4.16: Comparison of the Q-release of FTC in the originator and the generic FDCs

Figure 4.16 shows no significant difference in the Q-release of FTC between all products. See Table 4.16 for p-values. The results are described as mean and SD.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Table 4.16: Comparison of the Q-release of FTC in the originator and generic FCDs

FTC-O[a]	FTC-G1[b]	FTC-G2[c]	FTC-G3[d]	FTC-G4[e]	p-value
Mean (SD)					
93.37 (4.29)	96.63 (1.81)	93.04 (1.90)	93.22 (2.56)	96.04 (1.96)	$p^{ab} = 0.2494$
					$p^{ac} = 0.9995$
					$p^{ad} > 0.9999$
					$p^{ae} = 0.4368$
					$p^{bc} = 0.1711$
					$p^{bd} = 0.2117$
					$p^{be} = 0.9954$
					$p^{cd} > 0.9999$
					$p^{ce} = 0.3215$
					$p^{de} = 0.3215$

UNIVERSITY of the
WESTERN CAPE

4.8 Disintegration test

The six selected tablets of each product disintegrated completely within 30 minutes and comply with the WHO IP specifications for film-coated tablets. The appendix 5 shows the empty basket of the disintegration tester after the tablets were disintegrated.

http://etd.uwc.ac.za/

CHAPTER 5 DISCUSSION

This chapter discusses the results obtained from the uniformity of weight tests, disintegration tests, the verification of the IP HPLC method, development and validation of an RP-HPLC method for identification and quantification as well as dissolution testing of the generics and originator FDCs of FTC, TDF and EFV.

The assessment of post-market quality of medicines is currently a matter of discussion, especially since this aspect of quality is ignored by regulatory authorities in developing countries because of the lack of resources. Generic ARV FDCs have been used as a first line regimen in some developing countries and are among the medicines that should be evaluated after being approved or pre-qualified (WHO, 2007). Based on these considerations, we performed a post-market quality assessment to compare generic FDCs of FTC, TDF and EFV available in the South African public sector with each other and against their originator counterpart. The results are discussed individually below.

5.1 Uniformity of weight tests

The uniformity of weight test is a method of determining whether proper mixing or compression of ingredients occurred throughout manufacture to avoid overdosing or low dosing which can both be fatal to a patient. Considering the average weight per tablet for each generic FDC and the originator, of the twenty tablets randomly selected, no 18 tablets should deviate by >5% and no 2 tablets by >10% (WHO, 2016c). All the sample generics and the originator passed uniformity of weight tests which infers the proper mixing, good flow of granules and accurate die filling of granules during manufacture.

http://etd.uwc.ac.za/

5.2 The WHO IP HPLC method for simultaneous identification and assay of FTC, TDF and EFV FDC

Although efforts were made to use the same conditions indicated in the WHO IP monograph as indicated in chapter 4, the IP HPLC method was not suitable for analysis of the three APIs in this study. Validation of HPLC methods is very important, since HPLC methods are not always specific for the target analyte. In addition, a long retention time does not in itself guarantee that an assay will separate the target analyte (WHO, 2003). For this study, the use of a 25cm length Ascentis® column did not help to separate the three APIs, while the 15cm length Discover® column did. However, there is another factor that may have contributed towards the failed reproducibility the IP HPLC in this study. It most likely that EFV RS was particularly insoluble in the recommended sample diluent which was 50 % v/v methanol in water. Thus, the percentage of the methanol was raised to 80 % to ensure complete solubility for EFV RS. Even after increase in the percentage of methanol, on separation was observed between the APIs. It is noted that this diluent had been changed from 80 % v/v acetonitrile in water in the WHO draft monograph for IP 2010 to, 50 % v/v methanol in water in the WHO IP monograph 2016 (WHO, 2010; WHO 2016a). This change in diluent is, therefore, questionable. Furthermore, in the IP HPLC method the required time to analysing one sample is 30 minutes. Thirty minutes considered long time when compared with other methods for analysing the same FDC (Raju et al., 2008; Raju and Begum, 2008). For quality control testing short sample analysis time, simple preparations for the method, and simple instruments that are available globally, are

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

very important and desired (Zhang et al., 2015). Hence, the need was for another HPLC method to carry out the study.

5.3 Development and validation of an HPLC method for simultaneous detection and assay of FTC, TDF and EFV

As the verification of the WHO IP HPLC method was not possible, it was necessary to develop and validate a new simple, short analysis time HPLC method to carry out the experiment. Different HPLC conditions were analysed based on several trials and previous studies (Raju et al., 2008; Raju and Begum, 2008; Ramaswamy and Dhas, 2014; WHO, 2016a), in order to obtain conditions with satisfactory resolution. Section 3.3.3.1 describes the final chromatographic conditions selected. Furthermore, the selection of the Discovery® HS C18 Column (15cm × 4.6 mm, 5µm) helped to achieve separation with a good resolution between the three APIs, and satisfactory peak shapes in less than 15 minutes per run. The developed method provided a simple and specific method with relatively good linearity, accuracy, precision and robustness in a short analysis time, which can be suitably employed in the quantification of FTC, TDF and EFV in an FDC tablet.

5.4 Identity tests

The use of the PDA detector at 260 nm of the developed HPLC method assisted in identifying the three active ingredients in all the FDC tablets. Furthermore, the retention factor values for all the FDC tablets corresponded to that of the RSs. Ramaswamy and Dhas, (2014) have found the 260 nm to be a suitable wavelength to detect FTC, TDF and EFV in FDC capsules which in turn, matches the findings of this study regardless of their

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

different HPLC conditions. On the other hand, other studies reported either 265 nm (Devrukhakar et al., 2013), (Raju and Begum, 2008) or 280 nm (WHO, 2016a) to be the best wavelengths for selectivity according to reported HPLC conditions. However, from the identification tests, the results indicated that the samples contained FTC, TDF and EFV.

5.5 Assay test of the FDC tablets

Despite the fact that all the FDCs complied with the specifications stated in the IP, statistical comparison of the % content of each API (EFV, TDF and FTC) in the originator with the generics, and among the generics themselves showed significant differences as indicated in section 4.2.4. A few studies have been carried out to quantify the three APIs of generic FDCs, as part of the development and validation of different HPLC methods (Raju et al., 2008; Raju and Begum, 2008; Ramaswamy and Dhas, 2014). These studies stated that they succeeded in quantifying three APIs in generic formulations and the finding were consistent with the label claim. However, none of these studies have performed using products sourced from South Africa and none of these studies compared the generics with their innovator product. The comparison between these products may provide valuable data about the possibility of interchangeability between the generics and the originator and between the generics themselves. According to Al-Jazairi et al., (2008) and Paveliu et al., (2011), the interchangeability of these products are an advisable practice only if there is a strong post-market assessment programme.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

5.6 The validation of HPLC for dissolution testing

For the validation of HPLC for the dissolution test samples of the RS of EFV, TDF and FTC were prepared in 0.4% w/v SDS in methanol. The same conditions as mentioned in section 3.3.3.1 describe the final (validated) chromatographic conditions for dissolution testing. Section 4.6 shows the parameters which have been validated. To the best of our knowledge, apart from the HPLC method indicated in the IP monograph, this study is the first to development and validate RP-HPLC for dissolution testing of FDCs in tablet dosage form of FTC, TDF and EFV. This method proved to be linear, precise and accurate as reported in chapter 4. This method can be suitably employed in single-point dissolution testing of the FDC of FTC, TDF and EFV.

5.7 Dissolution tests

The Q-release values for FTC, TDF and EFV from the generics and the originator are presented in Table 4.14 for EFV, Table 4.15 for TDF and Table 4.16 for FTC. With the exception of G2, all the samples passed the specifications with not less than 80% in 30 minutes (WHO, 2016a). G2 failed in terms of the Q-release of EFV only, but passed the specifications in terms of the Q-release of FTC and TDF. EFV-G2 was released in the dissolution medium with a low percentage and it showed a significant difference between EFV-G2 and all other samples. The dissolution conditions were similar for all the samples and complied with the specifications indicated in the monograph as shown in section 3.3.7. During the dissolution experiment it was observed that G2 had low solubility compared with the other products O, G1, G3 and G4 as shown in Figure 6 in the section 4.7, which could be the reason for the low Q-release values of EFV-G2. To investigate this further,

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

disintegration tests were performed on G2 and all the other products, i.e O, G1, G3 and G4. All these sample products disintegrated completely within the required time (30 minutes). A failed dissolution test could impact the efficacy of this FDC, potentially leading to therapeutic failure, development of drug resistance and toxic or adverse reactions (WHO, 2007). Consequently, further investigation comparing inactive ingredients of all the FPPs was done. The finding showed that, some of the inactive ingredients and film-coating materials were unique to G2, namely hypromellose and corn starch (Table 5.1).

Table 5.1: Inactive ingredients and film-coating materials in the originator and generic FDCs

Inactive ingredients and film-coating materials	O	G1	G2	G3	G4
Croscarmellose sodium	+	+	+	+	+
Hydroxypropyl cellulose	+	+	+	+	+
Magnesium stearate	+	+	+	+	+
Microcrystalline cellulose	+	+	+	+	+
Sodium lauryl sulfate	+	+	+	+	+
Hypromellose	-	-	+	-	-
Iron oxide red	-	+	+	+	+
Iron oxide black	-	+	-	+	-
Polyvinyl alcohol	+	+	-	+	-
Polyethylene glycol	+	+	-	+	-
Titanium dioxide	+	+	-	+	-

UNIVERSITY of the WESTERN CAPE

http://etd.uwc.ac.za/

Talc	+	+	-	+	-
Lactose monohydrate (sugar)	-	+	-	+	-
Opadry	-	-	-	-	+
Opadry pink			+		
Opadry brown			+		
Corn starch			+		

(+) included, (-) not included

Corn starch is usually used to speed up dissolution rate of drug substance, thus providing rapid disintegration, while hypromellose is mainly used as a tablet binder in film-coating, and as a matrix for use in extended-release and sustained release tablet formulations (Rowe et al., 2009). High-viscosity grades of hypromellose may be used to delay the release of drugs from a matrix at levels of 10–80% w/w in tablets and capsules (Rowe et al., 2009). Shoaib et al., (2006) indicated that an Ibuprofen sustained release matrix tablet was prepared successfully using hypromellose to delay the release and achieve a required dissolution profile. A matrix tablet prepared with hypromellose, and a granulating agent of a hydrophobic polymer was the most successful formulation for the once-daily sustained-release of Nicorandil (Reddy et al., 2003). Although the grade and quantity of hypromellose that has been used to prepare G2 was not confirmed, the use of hypromellose in G2 may be responsible for the low Q-release values of EFV in the dissolution medium after 30 minutes.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

In this study although the use of a single-point dissolution test was sufficient to assess the release of the generic and the originator FDCs, dissolution profiles at multi-points may give further information about the performance of FDCs. Because of the use of SDS as a dissolution medium, the multi-point dissolution testing (dissolution profile) was not performed. The use of a surfactant such as SDS or SLS as a dissolution medium was recommended for the FDC of FTC, TDF (highly soluble) and EFV (insoluble) to ensure the solubility of all APIs in routine quality control testing. SDS lacks the physiological conditions of the gastrointestinal tract and, the dissolution profiles obtained using synthetic surfactants like SDS may or may not exhibit in *vitro* in *vivo* correlations (Gowthamarajan and Singh, 2010; Jogia et al., 2009). The second reason for not using multi-point dissolution testing was because the WHO IP monograph recommends a single time point (30 minutes).

UNIVERSITY of the
WESTERN CAPE

5.8 Disintegration test

For a solid dosage form FPP to be absorbed after oral administration, it must initially be in solution, and therefore the initial vital step toward this condition is usually the break-up of the tablet - a method referred to as disintegration. This test was carried out after EFV-G2 showed a significant difference in the Q-release values when compared with EFV-O and the other generics. After observing that G2 tablets had low solubility in the dissolution media, disintegration tests had to be done on G2 tablets in water and 2% w/v SDS as media. G2 disintegrated completely within 30 minutes in both water and SDS, thereby complying with WHO IP specifications.

http://etd.uwc.ac.za/

5.9 Summary of the quality assessment

The assessment of the quality was performed on one batch of each product. Testing different batches of each product would infer batch-to-batch consistency. However, since the testing conditions must be the same for all products and the time factor was prohibitive, this was not undertaken. The sample products were supplied at one point in time from the Cape Antiretroviral Depot in the Western Cape. Table 5.2 shows the summary of all the quality tests that were carried out in the study. All quality tests were passed, except that G2 failed the dissolution test.

Table 5.2: Summary of the assessment

Test / Product	Uniformity of weight	Assay content	Dissolution test	Disintegration test
O	Passed	Passed	Passed	Passed
G1	Passed	Passed	Passed	Passed
G2	Passed	Passed	Fail*	Passed
G3	Passed	Passed	Passed	Passed
G4	Passed	Passed	Passed	Passed

Fail*: The product fails the specification for the EFV only and passed the specification for FTC and TDF.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

CHAPTER 6 CONCLUSIONS & RECOMMENDATIONS

6.1 CONCLUSIONS

According to the objectives stated in chapter one, and the results obtained, the following key findings were made:

- All the sample generics and the originator products passed uniformity of weight tests which infers the proper mixing, good flow of granules and accurate die filling of granules during manufacture.
- The three APIs in all FDCs were identified using a developed and validated RP-HPLC method.
- The three APIs were quantified using the validated RP-HPLC and all the samples passed the IP specification 2016 within 90 to 110% content.
- Dissolution tests at 30 minutes were carried out on all generic FDCs. Samples were tested using the developed RP-HPLC method. The method was successfully validated again using the dissolution medium as sample diluents. Apart from the WHO IP method for assessing dissolution using HPLC, this study produced the only other validated method for dissolution testing for the FDC of FTC, TDF and EFV. Q-release values (> 80%) for all the APIs in the generics and the originator were obtained except for one generic product. The product failed the specification of the Q-release values of EFV with 62.23% for 12 tablets (from one batch only) and more than one tablet had less (60%) of EFV. Statistical comparisons that were

UNIVERSITY of the
WESTERN CAPE
http://etd.uwc.ac.za/

carried out on the data showed significant differences between the EFV-G2 and all the EFV in the other products.

The quality of the generic FPPs was generally good. All the assessed generic FPPs were within the WHO specification for the uniformity of weight, assay, identification and disintegration. All the FPPs were within the specification for the dissolution testing except for one generic product (G2) which failed to release $\geq 80\%$ of one of its APIs within 30 minutes. It is likely that the failure in dissolution of G2 was due to a difference in the formulation. This FPP included an excipient (hypromellose) which was not present in the other FDCs. Although there were some differences between the generics and the originator in the APIs quantities, there were no differences in the release of the three APIs in the generics and the originator except for G2. There is a concern about G2 being used in the public sector. The delay of the release of EFV could negatively affect patients' health. This study underscores the importance of post-market assessment of the quality of FDCs of ARVs.

6.2 RECOMMENDATIONS

NMRAs should commit more resources to assess the post-market quality of medicines especially, ARV FDCs.

The results of this study should be shared with the National Department of Health and independent testing of ARV FDCs on tender should be undertaken by their quality control laboratories to confirm our findings.

http://etd.uwc.ac.za/

The choice of inactive ingredients and the film-coating materials for the manufacturing of generic FDCs of FTC, TDF and EFV should be carefully considered by manufacturers. The excipients might affect the dissolution rates of these FDCs leading to poor quality products.

International pharmacopoeial methods for ARV FDCs should be reproducible in order to facilitate routine post-market quality control. As such, further review of the HPLC method of the WHO IP monograph (2015 and 2016) for the FDC of EFV, TDF and FTC should be performed.

The developed and validated RP-HPLC method in this study is recommended to carry out post-market quality control assessment of the FDC of FTC, TDF and EFV in tablet dosage form.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

REFERENCES

Al Ameri, M.N., Aad, S.A., Tucker, A. & Johnston, A. 2012. Capecitabine: An in-vitro comparison between the Branded Xeloda® 500mg and its intended copy Capeda 500 mg. *Medicinal Chemistry*, 2: 112–118.

Al-Jazairi, A., Bhareth, S., Eqtefan, I. & Al-Suwayeh, S. 2008. Brand and generic medications: are they interchangeable? *Annals of Saudi Medicine*, 28(1): 33.

Anandakumar, K., Kannan, K. & Vetrichelvan, T. 2011. Development and validation of emtricitabine and tenofovir disoproxil fumerate in pure and in fixed dose combination by UV spectrophotometry. *Digest Journal of Nanomaterials and Biostructures*, 6(3): 1085–1090.

Babu, G.S., Kumar, D.V., Balakrishna, G., Naik, R.R. & Malathy, P.S. 2014. Development and in-vitro evaluation of immediate release tablets of efavirenz. *International Journal of Pharmaceutical, Biological and Chemical Sciences*, 3(3): 56–65.

Bartlett, J.A. & Muro, E.P. 2007. Generic and branded drugs for the treatment of people living with HIV/AIDS. *Journal of the International Association of Physicians in AIDS Care*, 6(1): 15–23.

Bhavsar, D., Patel, B. & Patel, C. 2012. RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined tablet dosage form. *Pharmaceutical Methods*, 3(2): 73–73.

British Pharmacopoeia (BP) .2015. Volume 4.London: Stationery Office; 2015.

Broder, S. 2010. The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic. *Antiviral Research*, 85(1): 1–18.

Calmy, A., Pinoges, L., Szumilin, E., Zachariah, R., Ford, N. & Ferradini, L. 2006. Generic fixed-dose combination antiretroviral treatment in resource-poor settings: multicentric observational cohort. *Aids*, 20(8): 1163–1169.

Coetzee, D., Hildebrand, K., Boulle, A., Maartens, G., Louis, F., Labatala, V., Reuter, H., Ntwana, N. & Goemaere, E. 2004. Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa. *Aids*, 18(6): 887–895.

Darbyshire, J. 1995. Perspectives in drug therapy of HIV infection. *Drugs*, 49(1): 1–3.

UNIVERSITY of the WESTERN CAPE

http://etd.uwc.ac.za/

Davies, N. 2013. Fixed-dose combination for adults accessing antiretroviral therapy: advice document. *Southern African Journal of HIV Medicine*, 14(1): 41–43.

DeJesus, E., Young, B., Morales-Ramirez, J.O., Sloan, L., Ward, D.J., Flaherty, J.F., Ebrahimi, R., Maa, J.-F., Reilly, K., Ecker, J. & McColl, D. 2009. Simplification of antiretroviral therapy to a single-tablet regimen consisting of efavirenz, emtricitabine, and tenofovir disoproxil fumarate versus unmodified antiretroviral therapy in virologically suppressed HIV-1-infected patients. *Journal of Acquired Immune Deficiency Syndromes*, 51(2): 163–174.

Del Tacca, M., Pasqualetti, G., Di Paolo, A., Virdis, A., Massimetti, G., Gori, G., Versari, D., Taddei, S. & Blandizzi, C. 2009. Lack of pharmacokinetic bioequivalence between generic and branded amoxicillin formulations. A post-marketing clinical study on healthy volunteers. *British Journal of Clinical Pharmacology*, 68(1): 34–42.

Desai, D., Wang, J., Wen, H., Li, X. & Timmins, P. 2013. Formulation design, challenges, and development considerations for fixed dose combination (FDC) of oral solid dosage forms. *Pharmaceutical Development and Technology*, 18(6): 1265–1276.

Devrukhakar, P.S., Borkar, R., Shastri, N. & Surendranath, K.V. 2013. A validated stability-indicating RP-HPLC method for the simultaneous determination of Tenofovir, Emtricitabine, and Efavirenz and statistical approach to determine the effect of variables. *International Scholarly Research Notices*, 2013: e878295.

Dunne, S., Shannon, B., Dunne, C. & Cullen, W. 2013. A review of the differences and similarities between generic drugs and their originator counterparts, including economic benefits associated with usage of generic medicines, using Ireland as a case study. *Biomedicine central Pharmacology and Toxicology*, 14(1): 1.

Embrey, M.A. & Management Sciences for Health. 2012. MDS-3: Managing access to medicines and health technologies. 3rd ed. Kumarian Press.

European Medicines Agency (EMEA). 2007. Atripla® : EPAR - scientific discussion. https://www.google.com/search?q=Atripla®+%3A+EPAR+-+Scientific+Discussion+&ie=utf-8&oe=utf-8 [5 March 2017].

Food and Drug Administration (FDA). 1997. Guidance for industry on dissolution testing of immediate release solid oral dosage forms, availability. *Federal Register*, 62(164): 44974–44975.

Food and Drug Administration (FDA). 2006a. Center for drug evaluation and research, new drug application 21-937: Medical review. Rockville, Maryland: Food and Drug

UNIVERSITY of the WESTERN CAPE

http://etd.uwc.ac.za/

Administration (FDA).
https://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/021937s000_MedR.pdf [15 April 2017].

Food and Drug Administration (FDA). 2006b. Guidance for industry fixed dose combinations, co-packaged drug products, and single entity versions of previously approved antiretrovirals for the treatment of HIV. Rockville, Maryland: Food and Drug Administration (FDA).

Food and Drug Administration (FDA). 2007. Drugs databases, dissolution methods, Efavirenz 600 mg; Emtricitabine 200 mg; Tenofovir Disoproxil Fumarate 300 mg tablets. Rockville, Maryland: Food and Drug Administration (FDA).
https://www.accessdata.fda.gov/scripts/cder/dissolution/dsp_SearchResults.cfm [5 March 2017].

Fourie, P.B. & Spinaci, S. 1999. Structures required, roles and responsibilities in maintaining laboratories for quality assurance of anti-tuberculosis fixed-dose combinations in accordance with the IUATLD/WHO statement. *The International Journal of Tuberculosis and Lung Disease: The Official Journal of the International Union Against Tuberculosis and Lung Disease*, 3(11 Suppl 3): S368-370-387.

Freedberg, K.A., Losina, E., Weinstein, M.C., Paltiel, A.D., Cohen, C.J., Seage, G.R., Craven, D.E., Zhang, H., Kimmel, A.D. & Goldie, S.J. 2001. The cost effectiveness of combination antiretroviral therapy for HIV disease. *New England Journal of Medicine*, 344(11): 824–831.

Gerhardt, A.H. 2010. Fundamentals of tablet compression. *Journal of GXP Compliance*, 14(1): 70-79

Guo, J.H., Harcum, W.W., Skinner, G.W., Dluzneski, P.R. & Trumbull, D.E. 2000. Validation of tablet dissolution method by high-performance liquid chromatography. *Drug Development and Industrial Pharmacy*, 26(3): 337–342.

Hamrapurkar, P., Phale, M. & Shah, N. 2009. Quantitative estimation of Efavirenz by high performance thin layer chromatography. *Journal of Young Pharmacists*, 1(4): 359.

Hassim, A. & Heywood, M. 2007. Health & Democracy: A guide to human rights, health law and policy in post-apartheid South Africa. Siber Ink.

Hill, S. & Johnson, K. 2004. Emerging challenges and opportunities in drug registration and regulation in developing countries. Department for International Development.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Holmes, C.B. 2010. Use of Generic Antiretroviral Agents and Cost Savings in PEPFAR Treatment Programs. *Journal of the American Medical Association*, 304(3): 313.

Hosseinipour, M.C., Corbett, A.H., Kanyama, C., Mshali, I., Phakati, S., Rezk, N.L., Van der Horst, C. & Kashuba, A.D. 2007. Pharmacokinetic comparison of generic and trade formulations of lamivudine, stavudine and nevirapine in HIV-infected Malawian adults. *Aids*, 21(1): 59–64.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2005. Validation of analytical procedures: text and methodology Q2 (R1). *International Conference on Harmonisation, Harmonised Tripartite Guideline*: 2–11.

Jayapalu, K., Malipeddi, H. & Chinnasamy, A. 2014. Chromatographic separation and in vitro dissolution assessment of Tenofovir disoproxil fumarate, Emtricitabine and Nevirapine in a fixed dose combination of antiretrovirals. *Journal of Applied Pharmaceutical Science*, 4(11): 76–80.

Jogia, H., Mehta, T. & Patel, M. 2009. Evaluation of dissolution media containing a novel synthetic surfactant by in vitro testing of BCS class II drugs. *Dissolution Technologies*, 8: 14–9.

Joshi, A., Esseku, F., Silva, L., Igwilo, C., Oqua, D., Kunle, B., Obodozie, O., Inyang, U. & Adeyeye, M.C. 2010. Postmarketing in vitro/in vivo assessment of fixed dose combination products of first line antiretrovirals. *Journal of Pharmaceutical Sciences*, 99(6): 2655–2663.

Kagee, A. 2008. Adherence to antiretroviral therapy in the context of the national roll-out in South Africa: Defining a research agenda for psychology. *South African Journal of Psychology*, 38(2): 413–428.

Kaposhi, B.M., Mqoqi, N. & Schopflocher, D. 2015. Evaluation of antiretroviral treatment programme monitoring in Eastern Cape, South Africa. *Health Policy and Planning*, 30(5): 547–554.

Karunakaran, A., Kamarajan, K. & Thangarasu, V. 2012. A Validated RP-HPLC method for simultaneous estimation of Lamivudine and Tenofovir disoproxil fumarate in pure and in tablet dosage form. *Eurasian Journal of Analytical Chemistry*, 7(2): 56–66.

Kavitha, K.Y., Geetha, G., Hariprasad, R., Venkatnarayana, R. & Subramanian, G. 2013. Development and validation of RP-HPLC analytical method for simultaneous estimation of

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Emtricitabine, Rilpivirine, Tenofovir Disoproxil Fumarate and Its pharmaceutical dosage forms. *Pharmacie Globale*, 4(1): 1–6.

Kibwage, I.O. 2008. Counterfeiting of drugs and the necessity of quality control systems in developing countries. *Interdisciplinary Courses on Development and Cultures, Katholieke University Leuven*: 1–12.

Koo, O. 2010. Manufacturing process considerations for fixed-dose combination drug products. *American Pharmaceutical Review*, 13(3): 71.

Laurent, C., Kouanfack, C., Koulla-Shiro, S., Njoume, M., Nkene, Y.M., Ciaffi, L., Brulet, C., Peytavin, G., Vergne, L., Calmy, A. & Mpoudi-Ngolé, E. 2007. Long-term safety, effectiveness and quality of a generic fixed-dose combination of nevirapine, stavudine and lamivudine. *Aids*, 21(6): 768–771.

Llibre, J.M., Antela, A., Arribas, J.R., Domingo, P., Gatell, J.M., López-Aldeguer, J., Lozano, F., Miralles, C., Moltó, J., Moreno, S., Ortega, E., Riera, M., Rivero, A., Villalonga, C. & Clotet, B. 2010. Role of fixed-dose combinations of antiretrovirals in HIV-1 therapy. *Enfermedades Infecciosas Y Microbiología Clínica*, 28(9): 615–620.

Mahoro, A. 2013. Examining the inventory management of antiretroviral drugs at community health centres in the Cape Metropole, Western Cape. Cape Town, South Africa: Universtiy of the Western Cape.

Maigetter, K., Pollock, A.M., Kadam, A., Ward, K. & Weiss, M.G. 2015. Pharmacovigilance in India, Uganda and South Africa with reference to WHO's minimum requirements. *International Journal of Health Policy and Management*, 4(5): 295–305.

Manikanta Kumar, A., Naga Sandhya, B., Mahesh, N., Prasad, V. & Prakash, V.D. 2012. Development and validation of UV Spectrophotometric method for simultaneous estimation of Lamivudine and Efavirenz in the Pharmaceutical dosage form. *Journal of Advanced Pharmacy Education & Research*, 2(4): 210–214.

Medicines control council (MCC). 2003. Acts, Regulations and govt notices: Medicines and related substances act, 1965 (ACT 101 OF 1965). http://www.mccza.com/documents/b335d1c0Act_101_of_1965_published_2003.pdf [11 April 2017].

Medicines control council (MCC). 2008. Guidelines for the registration of medicines, general information. http://www.mccza.com/documents/1d9c57df2.01_General_information_Jul12_v8_showing_changes.pdf [5 May 2017].

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Medicines control council (MCC). 2014. Guidelines for the registration of medicines, pharmaceutical and Analytical CTD /eCTD. http://www.mccza.com/documents/751d10ba2.25_PA_CTD_Aug14_v4.pdf [5May 2017].

Meintjes, G., Conradie, J., Cox, V., Dlamini, S., Fabian, J., Maartens, G., Manzini, T., Mathe, M., Menezes, C., Moorhouse, M. and Moosa, Y., 2014. Adult antiretroviral therapy guidelines 2014. *Southern African Journal of HIV Medicine*, 15(4), pp.121-143.

Meredith, D.P.A. 2012. Generic drugs. Drug safety, 15(4): 233–242.

Ministry of Medical Services (MOMS) & Ministry of Public Health and Sanitation (MOPHS). 2012. Post market survey of antiretroviral medicines in Kenya. http://apps.who.int/medicinedocs/documents/s21944en/s21944en.pdf [4 March 2017].

Modi, T., Patel, B. & Patel, J. 2016. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of Lignocaine HCl and Nifedipine in Cream. *Research & Reviews: Journal of Pharmaceutical Analysis*, 5(1): 2340-2347.

Moses, O., Patrick, V., Muhammad, N., Jasper, O.O. & Celestino, O. 2013. Substandard rifampicin based anti-tuberculosis drugs common in Ugandan drug market. *Journal of Pharmacy And Pharmacological Research*, 3(1): 11–21.

Nachega, J.B., Stein, D.M., Lehman, D.A., Hlatshwayo, D., Mothopeng, R., Chaisson, R.E. & Karstaedt, A.S. 2004. Adherence to antiretroviral therapy in HIV-infected adults in Soweto, South Africa. *AIDS Research & Human Retroviruses*, 20(10): 1053–1056.

Newton, P.N., Fernandez, F.M., Green, M.D., Primo-Carpenter, J. & White, N.J. 2010. Counterfeit and substandard anti-infectives in developing countries. In antimicrobial resistance in developing countries. *Springer*: 413–443.

Patel, A., Gauld, R., Norris, P. & Rades, T. 2012. Quality of generic medicines in South Africa: perceptions versus reality - a qualitative study. *BioMed Central Health Services Research*, 12(1): 297.

Paveliu, M.S., Bengea, S. & Paveliu, F.S. 2011. Generic substitution issues: Brand-generic substitution, generic-generic substitution, and generic substitution of narrow therapeutic index (NTI)/critical dose drugs. *Maedica*, 6(1): 52.

Primo-Carpenter, J. & McGinnis, M. 2007. Matrix of drug quality reports in USAID-assisted countries by the US Pharmacopeia drug quality and information program.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Raju, N.A. & Begum, S. 2008. Simultaneous RP-HPLC method for the estimation of Emtricitabine, Tenofovir disoproxil fumerate and Efavirenz in tablet dosage forms. *Research Journal of Pharmacy and Technology*, 1(4): 522–525.

Raju, N.A., Rao, J.V., Prakash, K.V., Mukkanti, K. & Srinivasu, K. 2008. Simultaneous estimation of Tenofovir disoproxil, Emtricitabine and Efavirenz in tablet dosage form by RP-HPLC. *Oriental Journal of Chemistry*, 24(2): 645–650.

Ramaswamy, A. & Dhas, A.S.A.G. 2014. Development and validation of analytical method for quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC. *Arabian Journal of Chemistry*.

Rathore, A.S., Sathiyanarayanan, L. & Mahadik, K.R. 2012. Stability-indicating high performance thin-layer chromatographic method for quantitative estimation of Emtricitabine in bulk drug and pharmaceutical dosage form. *International Scholarly Research Notices Chromatography*, 2012: 1–7.

Reddy, K.R., Mutalik, S. & Reddy, S. 2003. Once-daily sustained-release matrix tablets of nicorandil: Formulation and in vitro evaluation. *American Association of Pharmaceutical Scientists Pharmscitech*, 4(4): 480–488.

Richman, D.D., Morton, S.C., Wrin, T., Hellmann, N., Berry, S., Shapiro, M.F. & Bozzette, S.A. 2004. The prevalence of antiretroviral drug resistance in the United States. *Aids*, 18(10): 1393–1401.

UNIVERSITY of the
WESTERN CAPE

Ripin, D.J., Jamieson, D., Meyers, A., Warty, U., Dain, M. & Khamsi, C. 2014. Antiretroviral procurement and supply chain management. *Antiviral Therapy*, 19(supplement 3): 79–89.

Rowe, R.C., Sheskey, P.J. & Quinn, M.E. 2009. Handbook of Pharmaceutical Excipients. 6th ed. Pharmaceutical Press.

Shisana, O., Rehle, T., Simbayi, L.C., Zuma, K., Jooste, S., Zungu, N., Labadarios, D. & Onoya, D. 2014. South African national HIV prevalence, Incidence and behaviour survey, 2012. HSRC Press.

Shoaib, M.H., Tazeen, J., Merchant, H.A. & Yousuf, R.I. 2006. Evaluation of drug release kinetics from Ibuprofen matrix tablets using HPMC. *Pakistan Journal of Pharmaceutical Sciences*, 19(2): 119–124.

Siew, A. 2014. Tackling challenges in the development of fixed-dose combinations. *Pharmaceutical Technology*, 38(4): 57.

http://etd.uwc.ac.za/

Siew, A. 2015. Fixed-dose combinations. *Pharmaceutical Technology*, 39(12): 30–31.

Smith, R.L., de Boer, R., Brul, S., Budovskaya, Y., van der Spek, H., 2013. Premature and accelerated aging: HIV or HAART?

Spencer, D. 2013. A history of ART and fixed-dose combination pills. Medical chronicle. https://www.medicalchronicle.co.za/a-history-of-art-and-fixed-dose-combination-pills/ [4 March 2017].

Subbaraman, R., Chaguturu, S.K., Mayer, K.H., Flanigan, T.P. & Kumarasamy, N. 2007. Adverse effects of highly active antiretroviral therapy in developing countries. *Clinical Infectious Diseases*, 45(8): 1093–1101.

United Nations Programme on HIV/AIDS (UNAIDS). 2011. UNAIDS Word aids day report. Geneva, Switzerland.

United Nations Programme on HIV/AIDS (UNAIDS). 2012. Together we will end AIDS. Geneva, Switzerland.

United Nations Programme on HIV/AIDS (UNAIDS). 2016. Global AIDS update 2016. Geneva, Switzerland.

United States Pharmacopoeia (USP). 2006. General chapter <621> chromatography—system suitability. http://www.usp.org/usp-nf/official-text/accelerated-revision-process/accelerated-revisions-history/general-chapter-chromatography-system [5 March 2017].

WESTERN CAPE

United States Pharmacopoeia (USP). 2015. USP39-NF34, pending monographs, pending monograph for efavirenz, emtricitabine and tenofovir tablets, 2015 http://www.usp.org/usp-nf/pending-monographs [10 September 2015].

Walfish, S. 2006. Analytical methods: A statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods. *BioPharm International*, 19(12): 1–6.

World Health Organization (WHO). 1998. How to implement computer-assisted drug registration - a Practical guide for drug regulatory authorities - regulatory support series No. 002. http://apps.who.int/medicinedocs/en/d/Jh2994e/ [8 February 2017].

World Health Organization (WHO). 1999. Counterfeit and substandard drugs in Myanmar and Vietnam - report of a study carried out in cooperation with the governments of Myanmar and Vietnam - EDM Research Series N0. 029. http://apps.who.int/medicinedocs/en/d/Js2276e/ [8 February 2017].

http://etd.uwc.ac.za/

World Health Organization (WHO). 2003. Fixed-dose combinations for HIV/AIDS, tuberculosis, and malaria - report of a meeting held 16-18 December 2003 Geneva. http://apps.who.int/medicinedocs/en/d/Js6172e/ [7 February 2017].

World Health Organization (WHO). 2007. Survey of the quality of antiretroviral medicines circulating in selected African countries. September 2007. http://www.who.int/medicines/publications/ARV_survey.pdf [4 March 2017].

World Health Organization (WHO). 2008. Standard treatment guidelines and essential medicines for South Africa. Primary health care level. Fourth edition, 2008. http://apps.who.int/medicinedocs/en/d/Js17842en/ [4 March 2017].

World Health Organization (WHO). 2010. Draft monograph for the international pharmacopoeia, Efavirenz, Emtricitabine and Tenofovir tablets. http://www.who.int/medicines/services/expertcommittees/pharmprep/TDF-EmtricEfavTabs-QAS10-391_Sept10.pdf?ua=1 [4 March 2017].

World Health Organization (WHO). 2013. Essential medicines and health products: Prequalification of medicines, HA500 - Efavirenz/Emtricitabine/Tenofovir disoproxil fumarate - 600mg/200mg/300mg - tablets - Cipla Ltd - India WHOPAR part 6. https://extranet.who.int/prequal/sites/default/files/documents/HA500Part6v1.pdf [4 March 2017].

World Health Organization (WHO). 2015. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. apps.who.int/iris/bitstream/10665/186275/1/9789241509565_eng.pdf [15 April 2017]

World Health Organization (WHO). 2016a. International Pharmacopoeia (IP) - sixth edition. 2016. Monographs: Dosage forms: Specific monographs: Efavirenz, emtricitabine and tenofovir tablets (Efavirenzi, emtricitabini et tenofoviri compressi) http://apps.who.int/phint/pdf/b/Jb.6.2.2.52.pdf [4 January 2016].

World Health Organization (WHO). 2016b. International Pharmacopoeia (IP) - sixth edition. 2016. Methods of analysis: Physical and physicochemical methods: Chromatograph: High performance liquid chromatography. http://apps.who.int/phint/en/p/docf/ [8 March 2016].

World Health Organization (WHO). 2016c. International Pharmacopoeia (IP) - sixth edition. 2016. Methods of analysis: Pharmaceutical technical procedures: Uniformity of mass for single-dose preparations. http://apps.who.int/phint/en/p/docf/ [5 March 2017].

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

World Health Organization (WHO). 2016d. International Pharmacopoeia (IP) - sixth edition. 2016. Methods of analysis: Pharmaceutical technical procedures: Dissolution test for solid oral dosage forms. http://apps.who.int/phint/en/p/docf/ [5 March 2017].

World Health Organization (WHO). 2016e. International Pharmacopoeia (IP) - sixth edition. 2016. Methods of analysis: Pharmaceutical technical procedures: Disintegration test for tablets and capsules. http://apps.who.int/phint/en/p/docf/ [5 March 2017].

Young, J., Psichogiou, M., Meyer, L., Ayayi, S., Grabar, S., Raffi, F., Reiss, P., Gazzard, B., Sharland, M., Gutierrez, F. & others. 2012. Y CD4 Cell count and the risk of AIDS or death in HIV-infected adults on combination antiretroviral therapy with a suppressed viral load: A longitudinal cohort study from cohere. *PLoS Medicine*, 9(3) 1-10.

Zhang, L., Fiske, J., Zhao, H., Patel, H. & Jennings, S. 2015. Dissolution method development for fixed-dose combination drug products – challenges and strategies. http://www.americanpharmaceuticalreview.com/Featured-Articles/181886-Dissolution-Method-Development-for-Fixed-Dose-Combination-Drug-Products-Challenges-and-Strategies/ [7 February 2017].

Zongyun, H., Ruben, L., Robert, F., Anne-Francoise, A., Alyson, S. & Denis O., S. 2011. Development of a single in vitro dissolution method for a combination trilayer tablet formulation of Clopidogrel and pravastatin. Dissolution technologies. https://www.researchgate.net/profile/Zongyun_Huang/publication/259601649_Development_of_a_Single_In_Vitro_Dissolution_Method_for_a_Combination_Trilayer_Tablet_Formulation_of_Clopidogrel_and_Pravastatin/links/00b4952cda8581fe9c000000.pdf [14 September 2016].

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

APPENDICES

APPENDIX 1: Certificate of analysis of FTC

European Directorate for the Quality of Medicines & HealthCare
7, Allée Kastner CS 30026, F-67081 Strasbourg (France)
Tel. +33 (0)3 88 41 20 35   Fax. + 33 (0)3 88 41 27 71
For any questions: www.edqm.eu (HelpDesk)

Emtricitabine ICRS batch 1

1. **Intended use**

To be used

a. for identity tests according to the monographs of emtricitabine, emtricitabine capsules, emtricitabine and tenofovir tablets, efavirenz, emtricitabine and tenofovir tablets;

b. as external standard in the dissolution test by UV-spectrophotometry according to the monographs of emtricitabine capsules and efavirenz, emtricitabine and tenofovir tablets;

c. as external standard in the dissolution test by LC according to the monographs of emtricitabine and tenofovir tablets and efavirenz, emtricitabine and tenofovir tablets;

d. as external standard in the LC-assay test of the Ph. Int. monographs for emtricitabine capsules, emtricitabine and tenofovir tablets and efavirenz, emtricitabine and tenofovir tablets

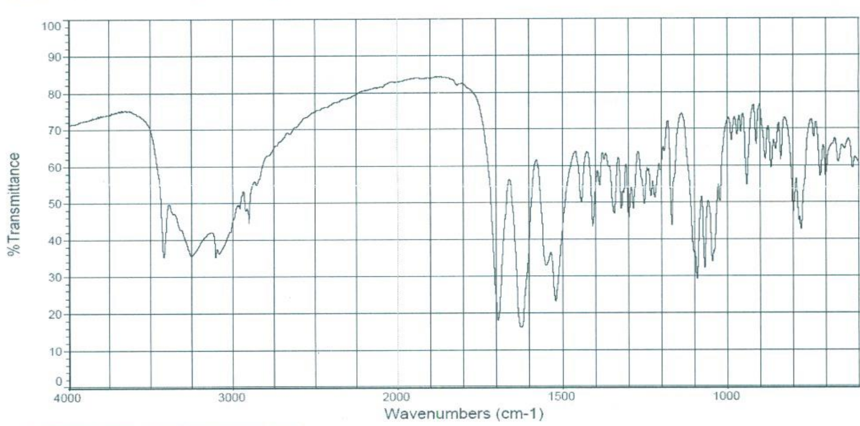
in *The International Pharmacopoeia.*

2. **Caution**

For laboratory use only. Handle in accordance with good occupational hygiene, safety and laboratory practices and take precautions to avoid exposure. This material is not for administration to humans or animals. The corresponding safety data sheet can be accessed via the EDQM website (Reference Standards Database) or is available upon request from the EDQM (Helpdesk-FAQ section).

3. **Analytical data**

Infrared absorption spectrophotometry (IR): A spectrum, about 2.3 mg of emtricitabine in about 343 mg of potassium bromide, is given in figure below:



Substance Name : Emtricitabine prop ICRS 1
File Name : 48743

http://etd.uwc.ac.za/

Loss on drying (LOD): 1.48 mg/g.

Sulfated ash: 0.2 mg/g.

Assigned content: 99.7% m/m of emtricitabine ($C_8H_{10}FN_3O_3S$).

4. **Storage**
Emtricitabine should be stored at 5°C ± 3°C, protected from light and the container should not be opened until required for use. Let the container equilibrate at room temperature just before opening to avoid uptake of moisture during handling of the substance.

5. **Reference**
This certificate is extracted from the report, which is the basis for the adoption of this International Chemical Reference Substance by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

6. **Citation**
The user has an obligation to ensure that any reference made to the present Standard in any publication, presentation or public document (ex. scientific articles, data sheets for kits) bears the correct name, and code of the Standard and the correct name and address of EDQM as given in the present leaflet.

7. **Product liability**
The Council of Europe makes no representation, contractual statement, or expression of opinion concerning the quality or safety of any item supplied the presence of any defect in it, or its fitness for any particular purpose. The product must be handled by professional persons having technical skill and at their own discretion and risk. It is for the purchasers of any such item who are responsible for persons in a workplace to determine independently the risks associated with the item according to the conditions of use and to take appropriate safety measures, including provision of appropriate information to persons working with the substance. Any liability of the Council of Europe for injury, loss or damage arising from the supply or use of any such item is in any event hereby excluded to the fullest extent permitted by law; in particular, no liability is accepted for loss of profits or indirect or consequential loss.

8. **Disputes**
In accordance with the provisions of article 21 of the General Agreement on the Privileges and Immunities of the Council of Europe, all disputes between the Council of Europe (EDQM) and the customer as regards the application of this contract shall be submitted, if a mutual agreement cannot be reached between the parties, to arbitration as laid down in Order No. 481 of the Secretary General, approved by the Committee of Ministers.

9. **Signature**
This document is electronically signed by:

Dr Pierre Leveau
Head of the Quality, Safety and Environment Division

http://etd.uwc.ac.za/

APPENDIX 2: Certificate of analysis of TDF

European Directorate for the Quality of Medicines & HealthCare
7, Allée Kastner CS 30026, F-67081 Strasbourg (France)
Tel. +33 (0)3 88 41 20 35 Fax. + 33 (0)3 88 41 27 71
For any questions: www.edqm.eu (HelpDesk)

Tenofovir disoproxil fumarate ICRS batch 1

1. Intended use

The International Chemical Reference Substance for tenofovir disoproxil fumarate is intended to be used in

a. identity tests according to the monographs on tenofovir disoproxil fumarate; efavirenz, emtricitabine and tenofovir tablets; emtricitabine and tenofovir tablets; tenofovir tablets
b. dissolution tests according to the monographs on efavirenz, emtricitabine and tenofovir tablets; emtricitabine and tenofovir tablets; tenofovir tablets
c. related substance tests according to the monographs on efavirenz, emtricitabine and tenofovir tablets; emtricitabine and tenofovir tablets;
d. assays according to the monographs on efavirenz, emtricitabine and tenofovir tablets; emtricitabine and tenofovir tablets; tenofovir tablets

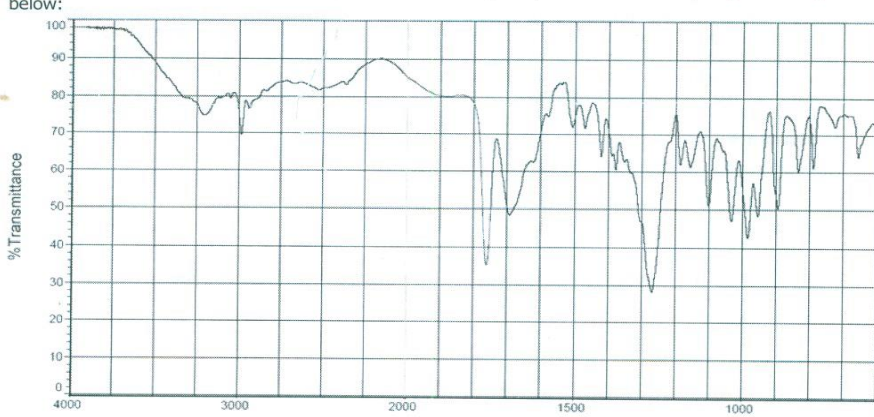
according to the monograph in *The International Pharmacopoeia*.

2. Caution

For laboratory use only. Handle in accordance with good occupational hygiene, safety and laboratory practices and take precautions to avoid exposure. This material is not for administration to humans or animals. The corresponding safety data sheet can be accessed via the EDQM website (Reference Standards Database) or is available upon request from the EDQM (Helpdesk-FAQ section).

3. Analytical data

Infrared absorption spectrophotometry (IR): A spectrum, about 1.7 mg of tenofovir disoproxil fumarate (recrystallized by methanol R) in about 276 mg of potassium bromide, is given in figure below:



IR File Name : 48744REC*1

http://etd.uwc.ac.za/

Liquid chromatography (LC): Total impurities 0.77%.
Water content (Karl Fischer): 0.04 mg/g.
Sulfated ash: no residue.
Assigned content: 98.8 % m/m of tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$).

4. Storage

Store the original container at +5°C ± 3°C, protected from light. The container should not be opened until required for use. Let the container equilibrate at room temperature just before opening to avoid uptake of moisture during handling of the substance. Shipping conditions are on the EDQM website (Reference Standards Database).

5. Reference

This certificate is extracted from the report, which is the basis for the adoption of this International Chemical Reference Substance by the WHO Expert Committee on Specifications for Pharmaceutical Preparations.

6. Citation

The user has an obligation to ensure that any reference made to the present Standard in any publication, presentation or public document (ex. scientific articles, data sheets for kits) bears the correct name, and code of the Standard and the correct name and address of EDQM as given in the present leaflet.

7. Product liability

The Council of Europe makes no representation, contractual statement, or expression of opinion concerning the quality or safety of any item supplied the presence of any defect in it, or its fitness for any particular purpose. The product must be handled by professional persons having technical skill and at their own discretion and risk. It is for the purchasers of any such item who are responsible for persons in a workplace to determine independently the risks associated with the item according to the conditions of use and to take appropriate safety measures, including provision of appropriate information to persons working with the substance. Any liability of the Council of Europe for injury, loss or damage arising from the supply or use of any such item is in any event hereby excluded to the fullest extent permitted by law; in particular, no liability is accepted for loss of profits or indirect or consequential loss.

8. Disputes

In accordance with the provisions of article 21 of the General Agreement on the Privileges and Immunities of the Council of Europe, all disputes between the Council of Europe (EDQM) and the customer as regards the application of this contract shall be submitted, if a mutual agreement cannot be reached between the parties, to arbitration as laid down in Order No. 481 of the Secretary General, approved by the Committee of Ministers.

9. Signature

This document is electronically signed by:

Dr Pierre Leveau
Head of the Quality, Safety and Environment Division

Cat. Code: ICRS48744 Date of issue: 17/11/2014 Rev.1 2/2

http://etd.uwc.ac.za/

APPENDIX 3: Certificate of analysis of EFV

European Directorate for the Quality of Medicines & HealthCare
7, Allée Kastner CS 30026, F-67081 Strasbourg (France)
Tel. +33 (0)3 88 41 20 35 Fax. + 33 (0)3 88 41 27 71
For any questions: www.edqm.eu (HelpDesk)

EFAVIRENZ International Chemical Reference Substance batch 2

1. **Intended use**

The International Chemical Reference Substance for efavirenz is intended to be used for

a. identity tests according to the monographs on efavirenz, efavirenz capsules, efavirenz tablets, efavirenz oral solution and efavirenz, emitricitabine and tenofovir tablets;

b. dissolution tests according to the monograph on efavirenz, emtricitabine and tenofovir tablets;

c. assays according to the monographs on efavirenz capsules, efavirenz tablets, efavirenz oral solution and efavirenz, emtricitabine and tenofovir tablets

d. related substances according to the monographs on efavirenz, efavirenz capsules, efavirenz tablets and efavirenz oral solution

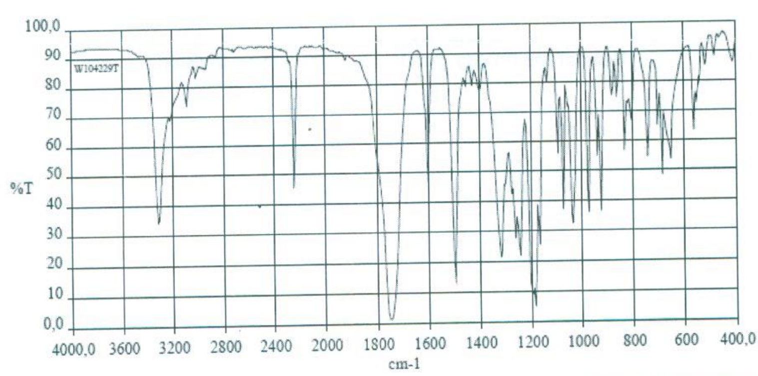
in *The International Pharmacopoeia*.

2. **Caution**

Danger. For laboratory use only. Handle in accordance with good occupational hygiene, safety and laboratory practices and take precautions to avoid exposure.
This material is not for administration to humans or animals. The corresponding Safety Data Sheet can be downloaded from the EDQM website (www.edqm.eu) or can be obtained from EDQM upon request.

3. **Analytical data**

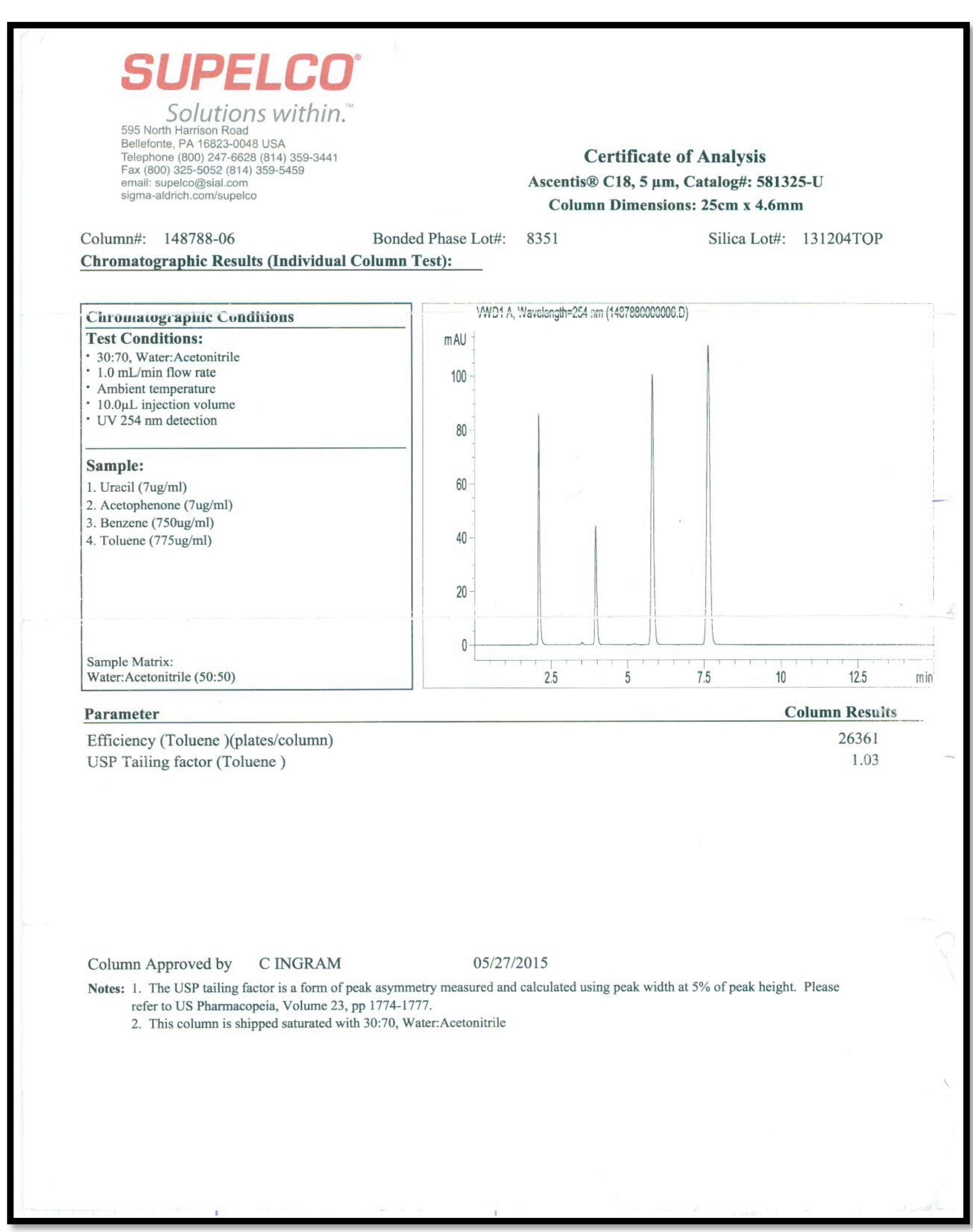
Infrared absorption spectrophotometry (IR): A spectrum, about 0.9 mg of efavirenz in about 300 mg of potassium bromide, is given in figure W104229T.



00ICRS1411

http://etd.uwc.ac.za/

APPENDIX 4: Certificate of analysis of Ascentis C18 column



SUPELCO
Solutions within.™
595 North Harrison Road
Bellefonte, PA 16823-0048 USA
Telephone (800) 247-6628 (814) 359-3441
Fax (800) 325-5052 (814) 359-5459
email: supelco@sial.com
sigma-aldrich.com/supelco

Certificate of Analysis
Ascentis® C18, 5 µm, Catalog#: 581325-U
Column Dimensions: 25cm x 4.6mm

Column#: 148788-06 Bonded Phase Lot#: 8351 Silica Lot#: 131204TOP

Chromatographic Results (Individual Column Test):

Chromatographic Conditions

Test Conditions:
- 30:70, Water:Acetonitrile
- 1.0 mL/min flow rate
- Ambient temperature
- 10.0µL injection volume
- UV 254 nm detection

Sample:
1. Uracil (7ug/ml)
2. Acetophenone (7ug/ml)
3. Benzene (750ug/ml)
4. Toluene (775ug/ml)

Sample Matrix:
Water:Acetonitrile (50:50)

Parameter	Column Results
Efficiency (Toluene)(plates/column)	26361
USP Tailing factor (Toluene)	1.03

Column Approved by C INGRAM 05/27/2015

Notes: 1. The USP tailing factor is a form of peak asymmetry measured and calculated using peak width at 5% of peak height. Please refer to US Pharmacopeia, Volume 23, pp 1774-1777.
2. This column is shipped saturated with 30:70, Water:Acetonitrile

http://etd.uwc.ac.za/

General Care and Use of Supelco HPLC Columns

E000922

This HPLC column has been extensively tested and inspected to ensure the highest quality possible. If you have any questions regarding this product, immediately notify your Supelco representative.

Column Information

The tag attached to the column indicates catalog number, packing type, column dimensions, particle size, flow direction, and column number. Keep this important information with the column at all times. The column number allows us to trace the manufacturing history of your column.

Connecting the Column to Your System

Supelco HPLC columns are provided with zero dead volume threaded end fittings. Flow direction during the column packing process is indicated on the column. Operate the column with the mobile phase flowing in this direction. Before connecting the column outlet to the detector, flush the column with mobile phase. This will prevent small particles, which can settle on the column frits during shipping and handling, from being washed into the detector.

Column Care

For optimum column performance and maximum life, the following conditions must be met.

pH — Operate silica and bonded silica columns within a pH range of 2.0 to 7.5. Higher pH will dissolve the silica, creating voids in the column. Lower pH can eventually strip away some of the bonded phase. These defects will cause changes in retention times.

Solvents — Silica-based reversed-phase columns can be used with all common HPLC grade organic solvents. Buffers made from acetate, citrate, formate, and phosphate salts at concentrations up to 0.2 M have been used without adverse effect. Organic modifiers and ion-pair reagents also present no problems as long as the appropriate pH range is not exceeded. Because ion-pair reagents are often difficult to completely flush from the column, columns used with these reagents should be dedicated to the particular application.

Limit the use of strong acids and bases to amounts needed to adjust the pH of the mobile phase. Be careful not to mix, or use in sequence, solutions that might precipitate or gel in the column or system.

Due to PEEK frit caps, it is not recommended to use solvents incompatible with PEEK.

Column Storage — To store the column, seal with the end plugs provided. Other plugs may allow the column to dry out, prolonging equilibration or reducing column performance. When storing the column for several days or longer, flush the column with 100 to 200 mL of the shipping solvent, then seal with the end plugs provided.

Column Protection — Protect all analytical and preparative columns with an in-line frit filter and a guard column. Supelco guard columns are supplied as stand-alone units (use with any HPLC column) or integrated. We recommend a filter with 2 µm porosity frits be used with a column containing a 5 µm packing. A filter with 0.5 µm pores is essential for adequate protection of a 3 µm packing.

Column Life — Column life is highly dependent on the sample and conditions, and cannot be generalized. To maximize column life, make sure samples and mobile phases are clean, particle-free, and use a guard column with an in-line frit filter.

Pressure — Continuous monitoring of system pressure will alert you to changes that may require you to perform preventative maintenance such as column washing, replacement of a guard column or filter, or cleaning of an inlet frit. A sudden increase in pressure usually means that there is a plugged frit at the column inlet. To unplug the frit, reverse the column and flush to waste with an appropriate solvent.

Temperature — Supelco silica-based columns have been used successfully at temperatures up to 75 °C. Prolonged use at temperatures above 75 °C can shorten column life.

T794165E
©1998 Sigma-Aldrich Co.

SUPELCO
Bellefonte, PA

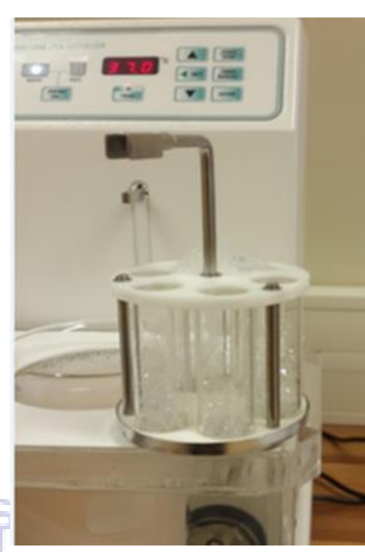
http://etd.uwc.ac.za/

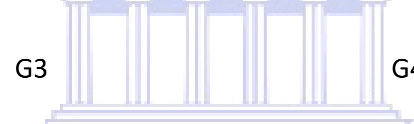
APPENDIX 5: Disintegration tester

O

G1

G2 in SDS





G2 in water

G3

G4

http://etd.uwc.ac.za/