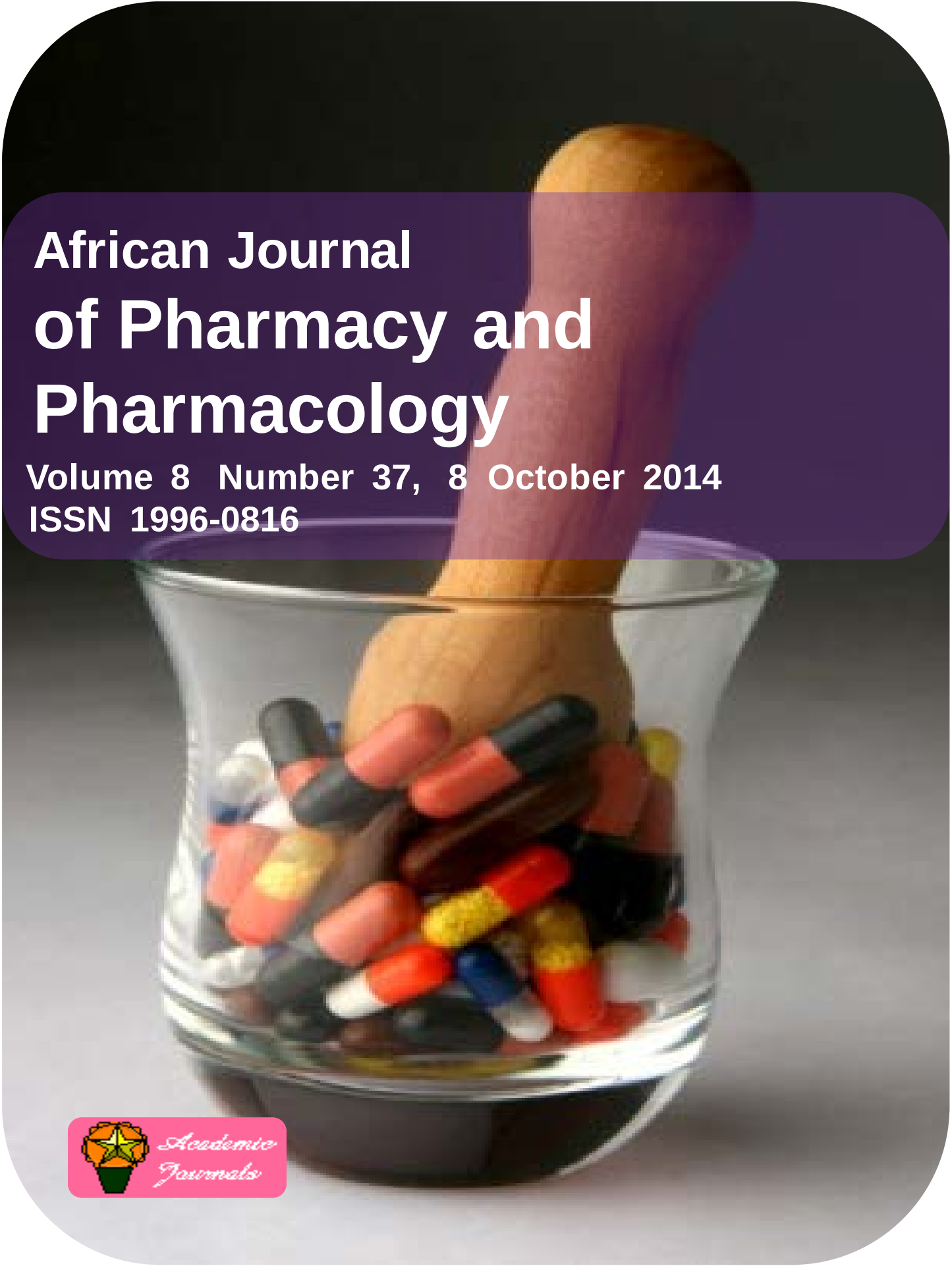

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Full Length Research Paper

The effect on potency of adding (-)-epicatechin to crude extracts of *Elephantorrhiza elephantina* and *Pentanisia prunelloides*

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***Elephantorrhiza elephantina* (*Ee*) and *Pentanisia prunelloides* (*Pp*) are two medicinal plants which are widely used by traditional healers to remedy various ailments including diarrhoea, dysentery, inflammation, fever, rheumatism, heartburn, tuberculosis, haemorrhoids, skin diseases, perforated peptic ulcers and sore joints in Southern Africa (South Africa, Swaziland, Botswana and Zimbabwe). Often, decoctions and infusions from these two plants are used in combination, specifically for stomach ailments. The following study was conducted to explore the possible mechanism underlying the synergistic interactions of the joint application of these two medicinal plant species.The checkerboard micro-dilution technique was used to determine the efficacy of (-)-epicatechin (EC): palmitic acid (PA) and (-)-epicatechin: *E. elephantina* or *P. prunelloides* combinations on five selected pathogenic bacteria. The results demonstrated that the combination of EC and PA exhibit either additive or synergistic but no antagonistic interactions. Of the 35 administered combinations, 11 were synergistic, 10 additive and 14 indifferent. The fractional inhibitory concentrations (FIC) indices for the combination of EC and *E. elephantina* for the three pathogens tested exhibited indifferent interactions with all FIC values above 1 while the FIC indices for the 1:1 combinations of EC and *P. prunelloides* exhibited additive interactions (FIC values between 1 and 0.50). This is the first report to explore the possible explanation underlying the synergistic interactions exhibited by the two medicinal plants.**

Key words: *Elephantorrhiza elephantina, Pentanisia prunelloides*, (-)-epicatechin, palmitic acid, efficacy, fractional inhibitory concentrations (FIC) index

INTRODUCTION

The use of plant extracts and mixtures is an ancient practice that has developed over thousands of years. It is referred to in Traditional Chinese Medicine (in the *Shen Nung Pen Tsao Ching* or *Divine Husbandman's Materia Medica,* ca. 3000 BC; Hamdard Pharmacopoeia of Eastern Medicine, 1970), Egyptian medicine (in the Ebers

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papyrus, 1550 BC; Chauncey, 1952), Ayurveda (based on the *Sushruta Samhita,* ca. 800 BC; Dwivedi and Dwivedi, 2007) as well as in *De Materia Medica* by Dioscorides (78 AD; Osbaldeston and Wood, 2000), to name a few. With recent emphasis on novel drug discovery, these age-old prescriptions are scientifically evaluated where efficacy is now being ascribed to possible synergistic interactions between extracts from different plants or components within the same plant extract, thus showing potential in multitarget therapy (Wagner*,* 2006). The driving hypothesis behind the idea of multi-drug therapy is to fight the pathogen via concerted action and not only through the direct destruction of the pathogen, but also by suppression, deactivation, interruption, diversion (or whatever the case may be) of various processes which are essential for the pathogen's survival. Potential benefits of using combination therapy include broad spectrum of efficacy, greater potency than either of the drugs used in monotherapy, improved safety and tolerability, and reduction in the number of resistant organisms (Lewis and Kontoyiannis, 2001). This multi-drug strategy is based on the proposition that many diseases have a multi-causal etiology and a complex pathophysiology, implying that it will be definitely advantageous to multiply targets in therapeutic efforts.

Bacterial multi-drug resistance efflux pumps (MDRs) are responsible for a significant level of resistance to antibiotics in pathogenic bacteria (Kumar et al., 2005). The mode of action for some antibiotics disrupts the capacity of these MDRs responsible for the extrusion of toxins across the permeability membrane barrier; hence, enhancing their efficacy. In southern Africa, plant extract combinations are also administered with the intention of attaining increased potency, as is implied with the term *imbiza* (that is, the generic Zulu name for plant mixtures that impart strength, health and vigour, normally as herbal preparations of a single plant or mixtures of plants which are administered orally for a purgative action, or as enemas) (Ngubane, 1977). One notable example of the combined administration of plant extracts to remedy stomach ailments and fevers comes from the traditional use of *Elephantorrhiza elephantina* together with *Pentanisia prunelloides* (Bryant, 1966). Such herbal mixtures may be obtained from muthi shops across South Africa, with a product by the name of '*Sejeso*' (Ingwe® brand) as a good example.

E. elephantina is known as elandsbean, *mupangara* (in Shona) or *intolwane* (in Xhosa and Zulu) (Phillips, 1917; Jacot, 1971). On its own, the root of this plant is known in Southern Africa for many traditional uses such the treatment of chest complaints and heart conditions (Watt and Breyer-Brandwijk, 1962), hypertension, syphilis, (Jacot, 1971) infertility in women, wasting in infants, fever, dysmenorrhea and haemorrhoids amongst others (Gelfand et al., 1985), and also as an aphrodisiac or emetic (to mitigate the anger of the ancestors or for fevers) (Hutchings et al., 1996). It is particularly known to be effective against stomach ailments such as abdominal pains, perforated peptic ulcers (bloody), diarrhoea and dysentery (Gelfand et al., 1985; Hutchings, 1989a; Pujol, 1990). *P. prunelloides* [syn. *P. variabilis* Harv. var. *intermedia* Sond, (Adeniji et al., 2000); common name: wild verbena (Van Wyk et al., 2009)] is an important traditional medicine in Southern Africa as a multi-purpose plant used for the treatment of several internal and external ailments (Rood, 1994; Maliehe, 1997; Grierson and Afolayan, 1999; Neuwinger, 2000). With stomach ailments in particular, the fresh root may be chewed and swallowed for the treatment of heartburn (Adeniji et al., 2000). Its vernacular names, that is, *setima-mollo* (Sotho) translated as "fire extinguisher" (Moteetee and Van Wyk, 2011), *icimamlilo* (Zulu) which means "putting out the fire" and *sooibrandbossie* (Afrikaans) translated as "little heartburn bush" (Van Wyk et al., 2009), emphasizes this longstanding traditional use. Root decoctions of *P. prunelloides* may also be taken orally as an emetic and for diarrhoea, dysentery, indigestion (Moteetee and Van Wyk, 2011).

The use of herbal remedies in the treatment of diarrhoeal diseases is a common practice in many communities of the world, including South Africa. A number of medicinal plants have been reported to be effective against diarrhoea and dysentery (Rouf et al., 2003). Diarrhoea, dysentery and cholera are some of the leading causes of morbitity and mortality in developing countries accounting for about 4.6 million deaths every year (Thaper and Sanderson, 2004). It is also alleged that about $4.3 million is spent every year on public and private direct health care costs due to diarrhoea alone (Pegram et al., 1998). It is against this background that rural dwellers rely on traditional medicine for their health care services due the prohibitive cost of orthodox medication. It is also reported that about 3 million South Africans use indigenous traditional plant medicine for primary health care purposes (Van Wyk and Gericke, 2000). It is therefore not surprising that 32 plant species derived from 26 families have been reported for the treatment of diarrhoea (Ippidii et al., 2008) in the Eastern Cape alone. Amongst the most frequently used plants for gastrointestinal problems are *E. elephantina* and *P. prunelloides.* Similar ethnobotanical studies have been reported in different South African provinces (Lewis et al., 2002; Mathabe et al., 2006) and other parts of the world (Mukharjee et al., 1998; Rahman et al., 2003). As a rule of the thumb, all these reports allude to the linkage of this disease to poor hygienic practices that are to a greater extent a function of poverty and poor service delivery (Obi et al., 2007).

In this study, we determined the antimicrobial activity of (-)-epicatechin (EC) and palmitic acid (PA) individually and in combination to probe the possible synergistic interactions between the two phytochemicals found in the two plant species as a validation of their possible contribution

to the enhanced potency of mixtures of *E. elephantina* and *P. prunelloides* especially for the remedy of stomach ailments in Southern African traditional medicine. Interaction between (-)-epicatechin with *E. elephantina* and *P. prunelloides* was also investigated to explore a possible explanation for the enhanced efficacy of the two plants administered in combination by traditional healers.

MATERIALS AND METHODS

Plant

Fresh plant rhizomes of *E. elephantina* and *P. prunelloides* were collected in June, 2010 from Kwazulu Natal Province, South Africa and were identified by Dr Anna Moteetee (Acting Dean of Faculty of Science University of Johannesburg). Voucher specimen numbers SJM-01 to SJM-2 were allotted and specimens were deposited in JRAU Herbarium, Department of Botany and Plant Biotechnology (Kingsway Campus) at the University of Johannesburg. Fresh plant rhizomes were washed with water, dried and marcerated and kept in the fumehood at room temperature. The dried plant materials were then ground into fine powders, extracted in solvent and water evaporated under reduced pressure and then stored in sample bottles and stored at -5°C until further use.

Plant extraction

Powdered material (100 g) of each plant was extracted with water and methanol, respectively. The methanol extracts were filtered under vacuum and evaporated to dryness under a stream of nitrogen at room temperature.The aqueous extracts were freeze dried then stored in tightly closed, sample bottles. Water was chosen especially as it is the solvent in which these medicinal plants are prescribed and administered by rural traditional healers while methanol is easier to dry apart from being a polar like water.

Determination of relative amounts of (-)-epicatechin in *E. elephantina* and *P. prunelloides* by Raman spectroscopy

Fine ground powders of fractions and extracts of *E. elephantina* and *P. prunelloides* were determined against (-)-epicatechin standard using the Raman instrument in Chemistry Department at the University of Johannesburg, Doornfontein Campus.

Microbiological testing

The minimum inhibitory concentrations (MIC) microdilution method was adopted from that reported in the literature (Eloff, 1998). All microbiological techniques, media and culture preparations were adopted in line with the CLSI/NCCLS (2003) guidelines. The antimicrobial activity was evaluated against two Gram-positive bacteria, *Bacillus cereus* (ATCC ll778) and *Staphylococcus aureus* (ATCC 6538) and three Gram-negative bacteria, *Escherichia coli* (ATCC 8739,) *Klebsiella pneumoniae* (ATCC l3883) and *Enterococcus faecalis* (ATCC 292l2). The bacteria were cultured in Tryptone soya broth (TSB) for 24 h. The yeast (*Cryptococcus neoformans*) was incubated for 48 h. Cultures were prepared for micro-dilution assays using 1:100 dilution, yielding an approximate inoculums size of 1×10^6 colony forming units (CFU)/ml (Van Vuuren and Viljoen, 2009). The microplates were sealed with seal-plate films and incubated at 37°C overnight to stimulate bacterial growth. A 40 µl volume of 4×10^{-1} mg/ml *p*-Iodonitrotetrazolium (INT) was added to all inoculated wells and left to stand for 6 h before plates were examined for bacterial growth.

MIC and FIC determination for palmitic acid and (-)-epicatechin combinations against five pathogens

Combinations of the stock solutions were prepared to represent the following ratios of EC/ PA, respectively: 9:1; 7:3; 6:4; 5:5; 4:6; 3:7; 1:9. The antimicrobial activities of the combinations of the two compounds against five pathogens selected on the bases of their susceptibility are shown on Table 1. This experimental procedure was undertaken to probe the effect of the two compounds (EC and PA) on the selected pathogenic agents especially as they were identified in *E. elephantina* and *P. prunelloidesl,* respectively. The corresponding FIC values from this experimental procedure were derived from the templates shown in Table 1 for *B. cereus* (ATCC ll778), *S. aureus* (ATCC 6538), *K. pneumoniae* (ATCC l3883), *E. faecalis* (ATCC 292l2) and Table 2 for *E. coli* (ATCC 8739,).

The templates used for the determination of MIC values for palmitic acid and (-)-epicatechin against the five pathogens.

Two different starting concentrations (1 mg/ml) for *E. coli* and (5 mg/ml) for the remaining four pathogens were used (Tables 1 and 2). The starting concentration for mixtures was adjusted to 1 mg/ml due to the high susceptibility of these pathogen higher concentrations.

Determination of MIC and FIC indices of 1:1 combinations of (-)-epicatechin against either crude extracts of *E. elephantina* or *P. prunelloides*

Stock solutions of 1:1 by mass of (-)-epicatechin with either crude *E. elephantina* or *P. prunelloides* were prepared and tested against three selected pathogens. The respective antimicrobial activities were probed starting with an effective concentration of 1.25 mg/ml then the MIC values recorded (Table 6). The corresponding FIC indices were calculated as shown in brackets in order to evaluate the effect of (-)-epicatechin on either of the crude extracts. The FIC index (FICI) is defined as the interaction of two agents where the concentration of each agent in combination is expressed as a fraction of the concentration that would produce the same effect when used independently (Berenbaum, 1977; Climo et al., 1999; Meletiadis, 2005; Guo et al., 2007). It is determined as the correlation between the two combined substances and can be classified as either synergistic when FICI (≤ 0.50), additive (> 0.5 to ≤ 1), independent (> 1 to ≥ 4) or antagonistic (> 4.00). The dose combinations are represented by geometric points with co-ordinates matching the dose rates of the separate components in combination (Van Vuuren, 2007; Hemaiswarya, 2008).

RESULTS

Comparative analysis of catechins in *E. elephantina* and *P. prunelloides* against (-)-epicatechin standard

Qualitative relative amounts of catechins in both *E. elephantina* and *P. prunelloides* as determined by Raman are shown on Figure 1. Spectra 1 is for catechin fraction form *E. elephantina* (Zimbabwe sample), spectra 2, *P. prunelloides* extract (KZN sample), spectra 3, (-)-epicatechin standard and spectra 4, *E. elephantina* extract (KZN sample). Considering absorption peaks 3196, 3071.8 and 2808.8 for (-)-epicatechin, the corresponding

Table 1. Template for palmitic acid (PA)/(-)-Epicatechin (EC) at 5 mg/ml (used against all the other pathogens).

100%	90 : 10		70 : 30		60 : 40		50 : 50		40 : 60		30 : 70		10 : 90		100%
PA	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	EC
1.25	1.125	0.125	0.875	0.375	0.750	0.500	0.625	0.625	0.500	0.750	0.375	0.875	0.125	1.125	1.25
0.63	0.563	0.063	0.438	0.188	0.375	0.250	0.313	0.313	0.250	0.375	0.188	0.438	0.063	0.563	0.63
0.313	0.282	0.031	0.219	0.094	0.188	0.125	0.156	0.156	0.125	0.188	0.094	0.219	0.031	0.282	0.313
0.156	0.141	0.016	0.105	0.047	0.094	0.0625	0.078	0.078	0.0625	0.094	0.047	0.105	0.016	0.141	0.156
0.078	0.705	0.0079	0.053	0.024	0.047	0.0313	0.039	0.039	0.0313	0.047	0.024	0.053	0.0079	0.705	0.078
0.039	0.353	0.0039	0.027	0.012	0.0235	0.0157	0.0195	0.0195	0.0157	0.0235	0.012	0.027	0.0039	0.353	0.039
0.0195	0.177	0.0020	0.014	0.006	0.0118	0.0078	0.0098	0.0098	0.0078	0.0118	0.006	0.014	0.0020	0.177	0.0195
0.0098	0.0089	0.0010	0.007	0.003	0.0059	0.0039	0.0049	0.0049	0.0039	0.0059	0.003	0.007	0.0010	0.0089	0.0098

Table 2. Template for palmitic acid (PA)/(-)-epicatechin (EC) at 1 mg/ml (used against *E. coli* only).

100%	90:10		70:30		60:40		50:50		40:60		30:70		10:90		100%
PA	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	PA	EC	EC
0.250	0.225	0.025	0.175	0.075	0.150	0.100	0.125	0.125	0.100	0.150	0.075	0.175	0.025	0.225	0.250
0.125	0.113	0.013	0.088	0.038	0.075	0.05	0.063	0.063	0.050	0.075	0.038	0.088	0.0125	0.113	0.125
0.063	0.057	0.007	0.044	0.019	0.038	0.025	0.032	0.032	0.025	0.038	0.019	0.044	0.0063	0.057	0.063
0.0313	0.029	0.0035	0.022	0.0095	0.019	0.013	0.016	0.016	0.013	0.019	0.0095	0.022	0.00315	0.0285	0.0313
0.0156	0.015	0.0018	0.011	0.00048	0.0095	0.0065	0.008	0.008	0.007	0.0095	0.0048	0.011	0.00158	0.0143	0.0156
0.0078	0.008	0.0009	0.0055	0.0024	0.00475	0.0033	0.004	0.004	0.0035	0.0048	0.0024	0.0055	0.00079	0.00715	0.0078
0.0039	0.004	0.0005	0.0028	0.0012	0.0024	0.00165	0.002	0.002	0.0018	0.0024	0.0012	0.0028	0.000395	0.00358	0.0039
0.00195	0.002	0.0003	0.0014	0.0006	0.0012	0.00083	0.001	0.001	0.0009	0.0012	0.0006	0.0014	0.000198	0.00179	0.00195

peaks for the three extracts of samples of *E. elephantina* and *P. prunelloides* showed less intensity with the *E. elephantina* peaks being more pronounced. The same trend was exhibited for the following sets of peaks with respect to standard (-)-epicatechin, (1616.3, 1341.7 and 1069.9) and (839.9, 723.4 and 547.4). If the intensities of peaks are related to the concentrations of the respective compounds in the referred extracts, it can be inferred that *P. prunelloides* extracts contain a higher concentration of catechins. Taking the KZN samples for the two medicinal plants, it can also be proposed that *E. elephantina* contains a greater concentration of catechins.

The MIC and FIC values for all the combinations of palmitic acid and (-)-epicatechin agsinst five tested pathogens

The MIC values for both EC and PA and the different combinations of the two compounds individually are shown in Table 3. Generally, most MIC values for the individual compounds were greater than the values for the corresponding mixtures (Table 3). The different combinations of palmitic acid and (-)-epicatechin exhibited predominently additive and synergic interactions. Of all the 35 possible interactions, 11 were synergistic, 10 additive and 14 indifferent (Figure 2). There were no antagonistic interactions observed for the combinations tested. The distribution of the synergistic interactions of the two compounds against a set of five pathogens is shown in Figure 2. Another notable enhanced efficacy of the combination of *E. elephantina* and *P. prunelloides* is the susceptibility of *B. cereus*. All the palmitic acid/epicatechin combinations exhibited indifference against this pathogen while the

combined aqueous extracts of *E. elephantina* and *P. prunelloides* exhibited at least two synergistic interactions (result not shown). This again alludes to the notion that it is not necessarily the presence of palmitic acid and epicatechin in the two plant species used in combination that accounts for the various synergistic interactions observed especially considering *B. cereus*. There could be other interactions involving other phytochemicals underlying this disperity.

Of great interest as well was the susceptibility of *E. coli* with the lowest FICi of 0.041 to the PA:EC combination of 7:3 (Table 4). Of the seven PA:EC combinations six were synergistic and only one combination being additive (Table 4). This observation suggests that the PA:EC combinations from the two plant species is conspiuously effective against *E. coli*, justifying the traditional use for the treatment of stomach ailments by traditional healers. A similar trend was also exhibited for *E. faecalis* that is also associated with gastrointestinal ailments (Table 4). The combination also showed synergy (FIC = 0.40) for the PA:EC combination of 4:6 against *K. pneumoniae*, one of the drug resistant Gram negative pathogens. This pathogen is implicated for chest problems for which *E. elephantina* and *P. prunelloides* are also used in traditional phytotherapy. *S. aureus* also showed marked susceptibility (Figure 2). Of the seven combinations administered to this pathogen, five were synergistic with the remaing two being additive, FIC = 1 (Table 5). This pathogen is also implicated for gastrointestinal ailments for which *E. elephantina* and *P. prunelloides* are administered. The susceptibility of this pathogen to the combination of these two compounds may be proposed as a justification for the use of *E. elephantina* and *P. prunelloides* to remedy stomach ailments as well.

Comparative efficacy of 1:1 combinations of (-)-epicatechin with *E. elephantina* and *P. prunelloides.*

The MIC values for both EC and 1:1 combinations of EC and either *E. elephantina* or *P. prunelloides* are shown in Table 6. Generally, MIC values for the individual EC and crude extracts of the two plants were greater than the values for the corresponding 1:1 mixtures (Table 6). All FIC values for the 1:1 combinations of *E. Elephantina* and (-)-epicatechin for the three pathogens tested exhibited indifferent interactions that is, all values were below 1 (Table 6). On the other hand all FIC indices for the 1:1 combinations of *P. prunelloides* and (-)-epicatechin demonstrated synergy that is, all values are between 0.38 and 0.50 depending on the pathogenic strain tested and this suggested enhanced potency (Table 6).

DISCUSSION

Both palmitic acid and (-)-epicatechin are common dietary phytochemicals and have been evaluated for several biological indications both *in vitro* and *in vivo*. Palmitic acid [CH_3 $(CH_2)_{14}COOH$] is a medium-length saturated fatty acid and is present as a major lipid in leaves and some seed oils (Harborne and Baxter, 1993). Previous studies have shown that palmitic acid is active against various bacterial strains (Hashem and Saleh, 1999) including *E. coli* (Yang et al., 2010), while (-)-epicatechin is an effective treatment for diarrhoea (Abhilash, 2010) and exhibits moderate antimicrobial activity (Pretorius et al., 2003). The primary mode of action of fatty acids is suggested to target cell membrane, (Tsuchido et al., 1985) and the proposed fatty acid-induced autolysis rather than large-scale solubilisation of the cell membrane is alleged to be detergent-like in character. Such antibacterial action could be explained through the insertion of the non-polar moieties of the fatty acids into the phospholipid layer of the bacterial cell membrane, resulting in a change in membrane permeability, alteration in function of membrane proteins responsible for maintenance of cellular functions and an uncoupling of the oxidative phosphorylation system (Saito and Tomioka, 1988). The antibacterial mode of action exerted by flavan-3-ols such as (-)-epicatechin and it is gallated derivatives on the other hand, including damaging the cytoplasmic membrane, as well as inhibiting nucleic acid synthesis, energy metabolism and cell membrane synthesis (Cushnie and Lamb, 2011).

The synergistic interactions of palmitic acid and (-)-epicatechin were demonstrated against the five pathogens (Table 4). Of particular interest was the demonstration of synergism towards both Gram positive and Gram negative bacteria, *K. pneumoniae* (0.40), S. *aureus* (0.25,), *E. faecalis,* (0.49), *E. coli*, (0.041). The results of this combination study show that *P. prunelloides* and *E. elephantina* display synergism or additive interactions subject to the test pathogens and the specific ratio in which the extracts were combined (Table 4). Since these two compounds have been identified in the two medicinal plants under study, it could be proposed that the synergistic interactions demonstrated in this study could also be effected by these two compounds among other undetected interactions. So interesting and conspicuous is the increased susceptibility of *E. faecalis* to the combinations of *E. elephantina* and *P. prunelloides* relative to that of palmitic acid and (-)-epicatechin administered individually (results not shown). The effects of different combinations of palmitic acid and (-)-epicatechin are just maginally synergistic with FIC indices approximately 0.5 (Figure 2) while most combinations of *E. elephantina* and *P. prunelloides* have been reported to have FIC values ranging between 0.18 and 0.33. This therefore suggests that there is far much more to the potency of *E. elephantina* and *P. prunelloides* other than the mere presence of palmitic acid and (-)-epicatechin in the two species.

Synergy or additivity in most combinations of EC and PA appeared in anti-bacterial activity against both Gram +

Table 4. Calculated FIC showing synergism between palmitic acid (PA; A) and (-)-epicatechin (EC; B) (stock solutions used were prepared in DMSO, concentrations given with pathogens).

Combination ratio	mg/ml extract contribution to combination		MIC contribution in combination[a]		Calculated FIC fractions		Calculated FIC[b]
	PA	EC	PA (MIC_A)	EC (MIC_B)	FIC_A	FIC_B	
***E. coli* (ATCC 8739) at 1 mg/ml**							
10:00	0.250	0.000	0.250	0.000	1.000	0.000	1.000
09:01	0.225	0.013	0.0140	0.0016	0.0562	0.0125	0.069
07:03	0.175	0.038	0.0055	0.0023	0.0218	0.0187	0.041
06:04	0.150	0.050	0.0047	0.0031	0.0187	0.0250	0.044
05:05	0.125	0.063	0.0078	0.0078	0.0312	0.0624	0.094
04:06	0.100	0.075	0.0500	0.0750	0.2000	0.6000	0.800
03:07	0.075	0.088	0.0189	0.0441	0.0756	0.3528	0.428
01:09	0.025	0.113	0.0063	0.0567	0.0252	0.4536	0.479
00:10	0.000	0.125	0.000	0.125	0.000	1.000	1.000

Table 5. FIC values for different combinations of palmitic acid and (-)-epicatechin against five pathogens.

PA	EC	*B. cereus* ATCC 11778	*S. aureus* ATCC 6538	*E. faecalis* ATCC 29212	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 8739
10	0	1.00	1.00	1.00	1.00	1.00
9	1	1.92^{I}	0.50^{S}	0.49^{S}	0.55^{A}	0.069^{S}
7	3	1.81^{I}	0.50^{S}	0.49^{S}	0.65^{A}	0.041^{S}
6	4	1.62^{I}	1.00^{A}	0.51^{A}	1.40^{I}	0.044^{S}
5	5	1.49^{I}	1.00^{A}	0.51^{A}	1.50^{I}	0.094^{S}
4	6	1.41^{I}	0.25^{S}	0.51^{A}	0.40^{S}	0.800^{A}
3	7	1.30^{I}	0.50^{S}	0.49^{S}	0.85^{A}	0.428^{S}
1	9	1.08^{I}	0.25^{S}	0.49^{S}	0.95^{A}	0.479^{S}
0	10	1.00	1.00	1.00	1.00	1.000

PA represents parts of palmitic acid and EC represents parts of (-)-epicatechin. Interpreting values: synergy (≤0.5), additive > 0.5 - 1.0), no interaction [>1.0 to ≤ (4.00) or antagonistic (> 4.0)].

Gram positive and Gram negative bacteria. Gram-negative bacteria have an effective permeability barrier composed of the outer phospholipidic membrane with lipopolysaccharide components which restricts penetration of amphipathic compounds (Tegos et al., 2002). Gram positive bacteria have an outer peptidoglycan layer which does not form a permeability barrier making them more susceptible to antimicrobial agents (Tadeg et al., 2005). Contrary to the structural differences of the pathogens tested, the combinations of palmitic acid and epicatechin or *P. prunelloides* with

Table 6. MIC and FIC values for 1:1 concentrations of crude extracts of *P. prunelloides* and *E. elephantina* against (-)-epicatechin at 1.25 mg/ml.

Crude *E. elephantina/P. prunelloides*	*B. cereus*	*E. faecalis*	*E. coli*
Crude *E. elephantina*	0.313	0.625	0.625
50:50 (Ee/EC)	1.25(2.5)	1.25(2.0)	1.25(2.0)
(-)-epicatechin	1.25	0.625	0.625
Crude *P. prunelloides*	1.25	0.625	0.625
50:50 (Pp/EC)	0.625(0.38)	0.625(0.50)	0.625(0.50)
(-)-epicatechin	0.625	0.625	0.625

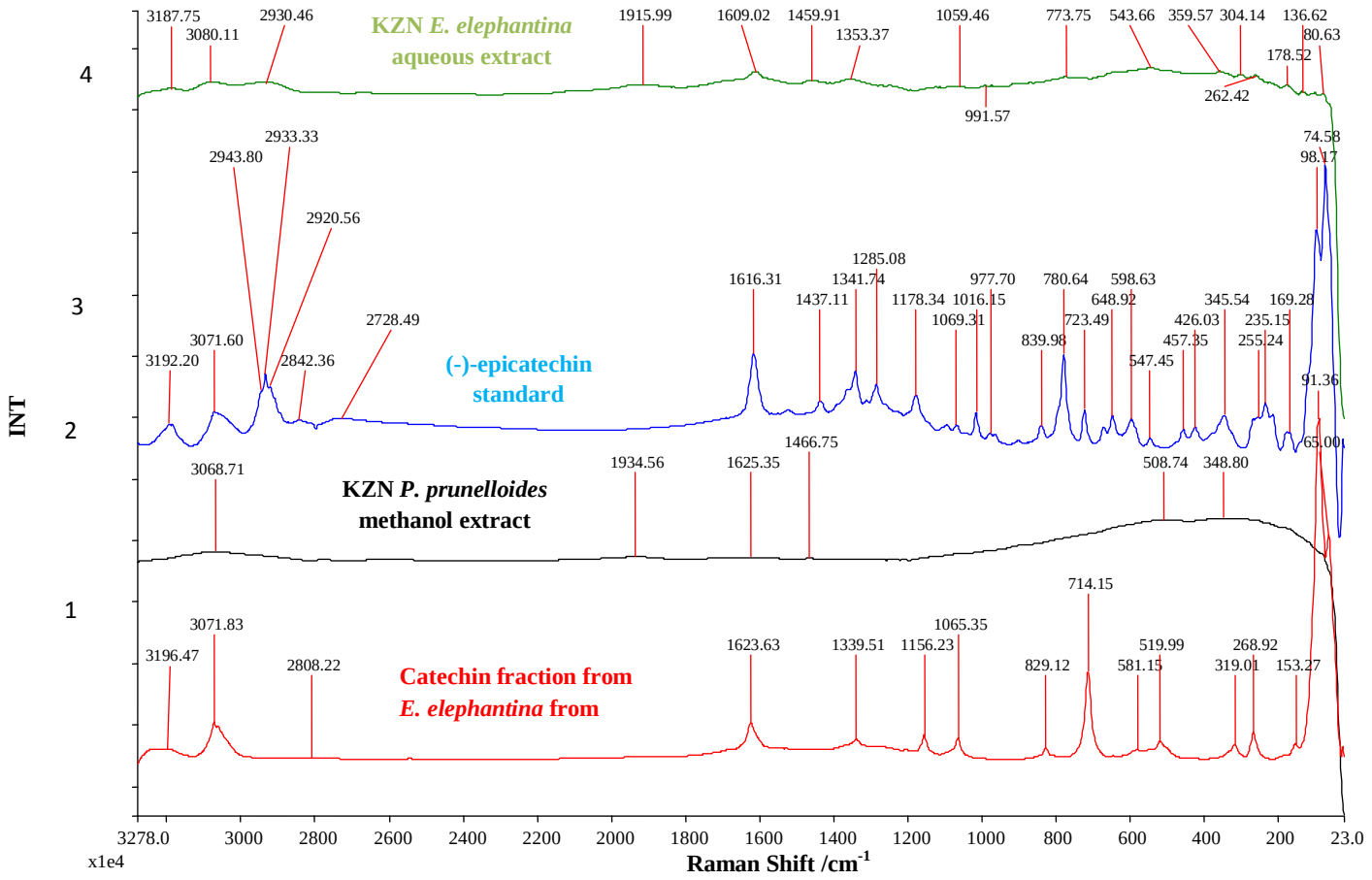

Figure 1. Determination of relative amounts of (-)-epicatechin in *E. elephantina* and *P. prunelloides* by Raman spectroscopy.

epicatechin exhibited activity against both strains of pathogens. The appearance of synergy in the activity against both Gram negative and Gram positive bacteria suggests that mixtures of components of *P. prunelloides* and *E. elephantina* can strongly enhance a sufficiently high bioavailability of anti-bacterial components within the cells effectively enhancing their potency.

The results of this study demonstrated a relatively greater content of (-)-epicatechin in *E. elephantina* compared to *P. prunelloides* (Figure 2) which also confirms reports in literature (Arotiba et al., 2013). *P. prunelloides* on the other hand has been reported to contain palmitic acid which is a known anti-microbial compound (Yff, 2002). The enhanced synergistic and additive effects that were observed with various ratios of plant administered imply that the phytochemicals from *P. pruneloides* and possibly some from *E elephantina* play different roles from a direct antibiotic one. It is most likely that the combination of *E. elephantina* and *P. prunelloides* would result in the epicatechin from *E. elephantina* enhancing the efficacy of

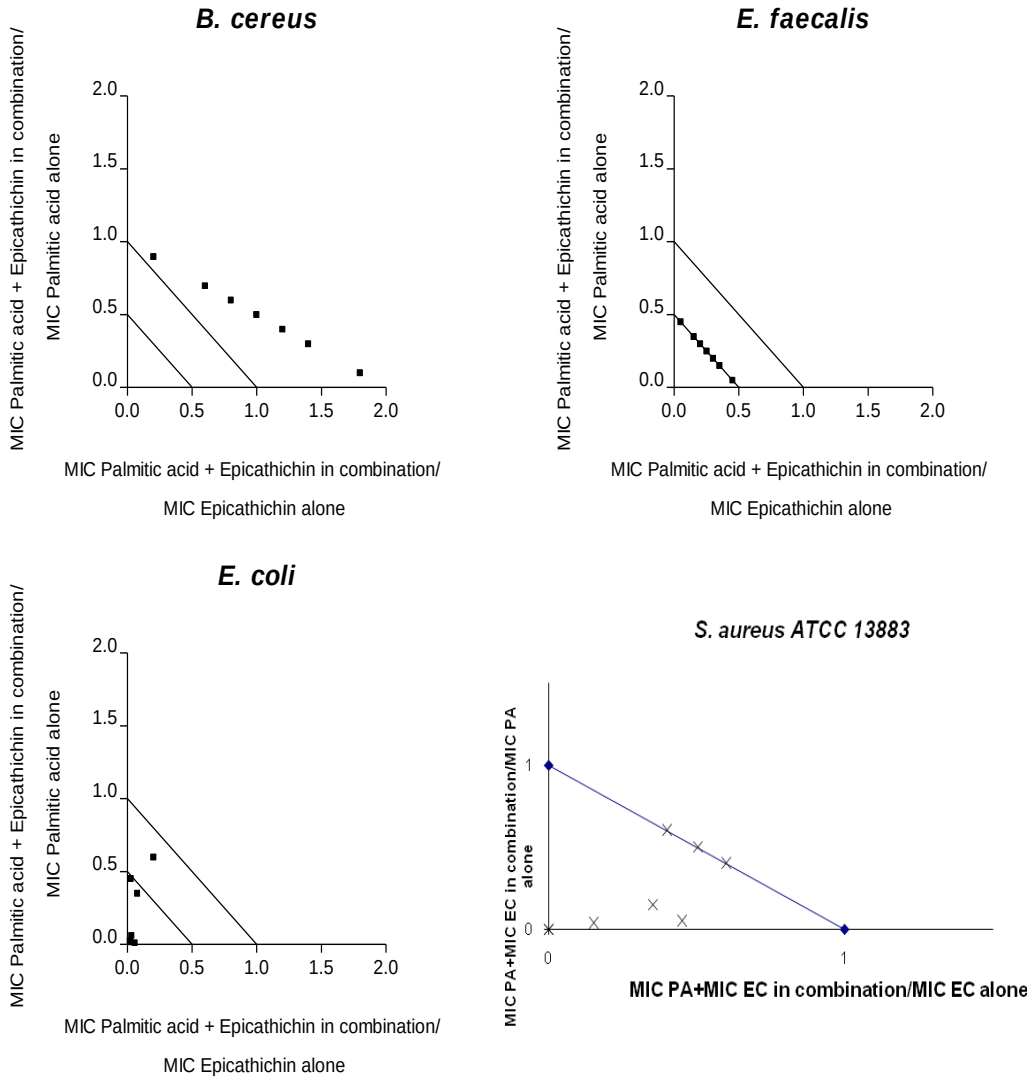

Figure 2. Isobolograms of interactions of palmitic acid and (-)-epicatechin.

phytochemicals in *P. prunelloides* resulting in synergistic interactions as reflected by the FIC indices below 0.50. On the other hand, the addition of (-)-epicatechin to *E. elephantina* that already contains a lot of this compound has no effect on the efficacy of the mixture (indifferent) as reflected by the FIC indices greater than 1 but less than 4. Of course, more combinations could have been carried out apart from the 50:50 combinations administered as a probe of the trend of interactions. More work is underway in our laboratories to further explore various combinations.

Conclusion

This study has demonstrated that the addition of (-)-epicatechin to crude *E. elephantina* has no effect but has a notable enhancement of the efficacy on crude *P. prunelloides* extracts. It could therefore be proposed that *E. elephantina* that contains a greater quantity of (-)-epicatechin enhances phytochemicals, especially palmitic acid in *P. prunelloides* when the two medicinal plants are jointly administered. Hence, justifying the synergistic and additive interactions exhibited by the two medicinal plants in this study.

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Conflict of interest

The authors declare that they have no competing interests.

REFERENCES

Abhilash M (2010). *In silico* analysis of cranberry proanthocyanidin epicatechin (4 beta-8, 2 beta-0-7) as an inhibitor for modeled afimbrial adhesion virulence protein of uropathogenic *Escherichia coli*. Int. J. Pharm. Biol. Sci. 1(1):1-10.

Adeniji KO, Amusan OOG, Dlamini PS, Enow-Orock EG, Gamedze ST, Gbile ZO, Langa AD, Makhubu LP, Mahunnah RLA, Mshana RN, Sofowora A, Vilane MJ (2000). Traditional medicine and pharmacopoeia contribution to ethnobotanical and floristic studies in Swaziland. The Scientific, Technical and Research Commission of the Organization of African Unity (OAU/STRC), Swaziland.

Appidi JR, Grierson DS, Afolayan AJ (2008). Ethno botanical studies of plants used for the treatment of diarrhoea in the Eastern Cape, South Africa. Pak. J. Biol. Sci. 11:1961-1963.

Arotiba O, Mpofu SJ, Ndinteh DT, Krause RWM, Hlekelele, L (2013). Determination of catechins from *E. elephantina* and *P. prunelloides* using a voltammetry and UV spectroscopy. J. Nat. Prod. Commun. 9(1):41-43.

Berenbaum MC (1977). Synergy, additivism and antagonism in immunosuppression: a critical review. Clin. Exp. Immunol. 28:1-18.

Bryant AT (1966). Zulu medicine and medicine-men. Struik, Cape Town.

Chauncey DL (1952). The old Egyptian medical papyri. University of Kansas Press, Lawrence, USA.

Climo MW, Patron RL, Archer GL (1999). Combinations of vancomycin and beta lactams are synergistic against staphylococci with reduced susceptibilities to vancomycin. Antimicrob. Chemother. 43:747-53.

CLSI/NCCLS (2003). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standards, (6th ed.)., CLSI document M7-A6, Pennyslvania, USA. pp.15-17.

Cushnie TPT, Land AJ (2011). Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 26(5):343-356.

Dwivedi G, Dwivedi S (2007). Sushruta-the clinician-teacher par excellence. Indian J. Chest Dis. Allied Sci. 49:243-244.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts from bacteria. Planta Med. 64:711-713.

Gelfand M, Mavi S, Drummond RB, Ndemera B (1985). The traditional medical practitioner in Zimbabwe: his principles of practice and pharmacopoeia. Mambo Press, Gweru.

Grierson DS, Afolayan AJ (1999). An ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. J. Ethnopharmacol. 67:327-332.

Guo XM (2007). Advanced Traditional Chinese Medicine. Series/ Chinese Materia Medica, Volume 1. Beijing, China: People's Medical Publishing House.

Harborne JB, Baxter H (1993). Phytochemical Dictionary: A handbook of bioactive compounds from plants. Taylor and Francis Ltd., London. pp. 34-38.

Hashem FA, Saleh MM (1999). Antimicrobial components of some Cruciferae plants. (*Diplotaxis harra Forsk* and *Erucaria microcarpa Boiss*). Phytother. Res. 13(4):329-332.

Hemaiswarya SH, Kruthivents AK, Doble M (2008). Synergism between natural products and antibiotics against diseases. Phytomedicine 15:9-652.

Hutchings A (1989a). A survey and analysis of traditional medicinal plants as used by the Zulu, Xhosa and Sotho. *Bothalia* 19(1):111-123.

Hutchings A (1989b). Observations on plant usage in Xhosa and Zulu medicine. Bothalia 19(2):225-235.

Hutchings A, Scott AH, Lewis G, Cunningham A (1996). Zulu Medicinal Plants. Natal University Press, Pietermaritzburg.

Jacot GA (1971). Flora of Lesotho. Cramer, Lehre.

Kumar A, Schweizer HP (2005). Bacterial resistance to antibiotics: Active efflux and reduced uptake. Adv. Drug Deliv. Rev. 57:1486-1513.

Lewis RE, Kontoyiannis DP (2001). Rationale for combination antifungal therapy. Pharmacotherapy 8(2):149-164.

Lewis RE, Dickema DJ, Messel SA, Pfaller MA, Klepser ME (2002). Comparison of E test, chequerboard dilution and time-kill studies for the detection of synergy or antagonism between antifungal agents tested against *Candida* species. J. Antimicrob. Chemother. 49:345-351.

Maliehe EB (1997). Medicinal Plants and Herbs of Lesotho. Mafeteng Development Project, Lesotho (in Sesotho).

Mathabe MC, Nikolova RV, Lall N, Nyazema NZ (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. J. Ethnopharmacol. 105(1-2):286-293.

Meletiadis J, Mouton JW, Meis JFGM, Verweij PE (2003). In vitro drug interaction modeling of combinations of azoles with terbinafine against clinical of *Scedosporium prolificam* isolates. Antimicrob. Agents Chemother 47:106-117.

Moteetee A, Van Wyk B.-E (2011). The medical ethnobotany of Lesotho: a review. Bothalia 41: 209–228.

Neuwinger HD, (2000). African Traditional Medicine – a dictionary of plant use and applications. Medpharm Scientific Publishers, Stuttgart.

Ngubane H (1977). Body and mind in Zulu medicine. An ethnography of health and disease in Nyuswa-Zulu thought and practice. Academic Press Inc. (London) Ltd, Oval Road, London NW1. pp. 24-28.

Obi CL, Ramalivhana J, Momba MNR, Onabolu B, Igumbor JO, Lukoto M, Mulaudzi TB, Bessong PO, Jansen van Rensburg EL, Green E, Ndou S (2007). Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDs patients with and without diarrhoea and their household drinking water in rural communities in Limpopo Province. South Africa. J. Biotechnol. 6(8):1035-1047.

Osbaldeston TA, Wood RPA, (2000). Dioscorides – De Materia Medica. Book 3. Ibidis press, Johannesburg, South Africa.

Pegram GC, Rollins N, Espey Q (1998). Estimating the costs of diarrhoea and epidemic dysentery in KwaZulu-Natal and South Africa. Water SA-Pretoria 24:11-20.

Phillips EP (1917). A contribution to the flora of the Leribe Plateau and environs: with a discussion on the relationships of the flora of Basutoland, the Kalahari, and the south-eastern regions. Annals of the South African Museum. Vol. XVI, 1.

Pretorius JC, Magama S, Zietsman PC (2003). Growth inhibition of plant pathogenic bacteria and fungi by extracts from selected South African plant species. South African J. Bot. 69(2):186-192.

Pujol J (1990). Naturafrica-the Herbalist Handbook. Jean Pujol Natural Healers Foundation, Durban.

Rahman MT, Khan OF, Saha S, Alimuzzaman M (2003). Antidiarrhoeal activity of the bark extract of *Careya arborea*, Roxb. Fitoterapia 74:116-118.

Rood B (1994). Uit die veldapteek. Tafelberg Publishers, Cape Town.

Rouf AS, Islam MS Rahman MT (2003). Evolution of antidiarrhoeal activity of *Rumex maritimus* roots, J. Ethnopharmacol. 84:307-310.

Saito H, Tomioka H, Sato K (1988). PSK, a polysaccharide from Coriolus vesicolor enhances oxygen metabolism of murine peritoneal macrophages and the host resistance to listerial infection. J. Gen. Microbiol. 134:1029-1035.

Tadeg H, Mohammed E, Asres K, Gebre-Mariam T (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. J. Ethnopharmacol. 100:168-175.

Tegos G, Stermitz FR, Lomovskaya O, Lewis K (2002). Multidrug Pump Inhibitors Uncover Remarkable Activity of Plant Antimicrobials. Antimicrob. Agents Chemother. 46(10):3133-3141.

Thaper N, Sanderson IR (2004). Diarrhoea in children: An interface

between developing and developed countries. Lancet 363:641-653.
Tsuchido M, Miura T, Mizutani K. Aibara K (1985). Fluorescent substances in mouse and human sera as a parameter of in vivo lipid peroxidation. Biochem. Biophys. Acta 834:196-204.
Van Vuuren SF (2007). The antimicrobial activity and essential oil composition of medicinal aromatic plants used in African traditional healing. PhD Thesis. The University of the Witwatersrand, Johannesburg.
Van Vuuren SF, Suliman S, Viljoen AM (2009). The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. Lett. Appl. Microbiol. 48(4):440-446.
Van Wyk BE, Gericke N (2000). People's plants: a guide to useful plants of Southern Africa. Pretoria: Briza.
Van Wyk BE, Van Oudtshoorn B, Gericke N (2009). Medicinal Plants of South Africa. Briza publications, Pretoria.
Wagner H (2006). Multitarget therapy – The future of treatment for more than just functional dyspepsia. Phytomedicine 13(Suppl 1):122-129.
Watt JM, Breyer-Brandwijk MG (1962). The medicinal and poisonous plants of Southern and Eastern Africa, 2nd edition. Livingston, London.
Yang J, Hou X, Mir PS, McAllister TA (2010). Anti-*Escherichia coli* O157:H7 activity of free fatty acids under varying pH. Can. J. Microbiol. 56:263-267.
Yff BTS, Lindsey KL, Taylor MB, Erasmus DG, Jäger AK (2002). The pharmacological screening of *Pentanisia prunelloides* and the isolation of the antibacterial compound palmitic acid. J. Ethnopharmacol. 79(1):101-107.

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Full Length Research Paper

Verification of pharmaceutical power of generic meropenem vs innovator trough assessment by microbial potency test

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This study presents the development of a microbiological test method with different microorganisms and different concentrations of antibiotics through "microbial potency," which established the dose-response concentrations and test conditions. The assay method was based on the plate cylinder with the result that there is equivalence between the pharmaceuticals antibiotic such as generic meropenem and innovator for the microorganisms such as *Bacillus subtilli* ATCC6633, *Staphylococcus aureus* ATCC 29737, *Pseudomonas aeruginosa* ATCC 25619 and *Escherichia coli* ATCC 10536, and pharmaceutical equivalence does not exist between the generic antibiotic meropenem and innovative meronem for *Microococcus luteus* ATCC 9341.

Key words: Microbial power, cylindrical methodin plate, antibiotics, pharmaceutical equivalence, *Bacillus subtillis* ATCC 6633, *Staphylococcus aureus* ATCC 29737, *Pseudomonas aeruginosa* ATCC 25619, *Escherichia coli* ATCC 10536, *Microococcus luteus* ATCC 9341.

INTRODUCTION

The market for medicines has been dramatically influenced by two major segments, innovative medicines which contain an innovative active ingredient, as a result of extensive research and backed by a patent, on the other side are generic drugs which according to the World Health Organisation/ Pan American Health Organisation (WHO/PAHO) are defined as follows: "Two products are pharmaceutically equivalent if they contain the same amount of the same substance or active ingredient, in the same pharmaceutical modality, if they have identical or comparable standards and if they are suitable for administration by the same route and same directions, if meet the same or comparable quality standards, although they have differences in the excipients or ways of processing" (WHO, 2011). A generic drug is the same as a brand name drug in dosage, safety, strength, how it is taken, quality, performance, and intended use. Before approving a generic drug product, the Food and Drug Administration (FDA) requires many rigorous tests and procedures to assure that the generic drug can be substituted for the brand

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name drug. The FDA bases evaluations of substitutability, or "therapeutic equivalence," of generic drugs on scientific evaluations. By law, a generic drug product must contain the identical amounts of the same active ingredient(s) as the brand name product. Drug products evaluated as "therapeutically equivalent" can be expected to have equal effect and no difference when substituted for the brand name product (USP 29, 2006).

It is believed that generic drugs are less expensive because they do not have the same benefits and efficiency as the innovator. Therefore it is important to determine and demonstrate the pharmaceutical equivalence between generic medications and the innovator, and thus to be sure of the quality in the use of these medications since the consequence can lead to serious health problems of patients if they do not have the same active principle and therefore do not act the same way. Meropenem is an antibiotic that has a broad spectrum of bacteria and is used to treat serious respiratory infections, nosocomial infections, intraabdominal infections, urinary tract infections, meningitis, septicemia, pediatric, gynecological infections, monotherapy in immunocompromised patients and others; it is marketed as an innovator product and as a generic product. This research will verify the pharmaceutical equivalence of the generic meropenem versus the innovator through microbial potency on the microbial strains of *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29737, *Pseudomonas aeruginosa* ATCC 25619, *Escherichia coli* ATCC 10536, and *Microococcus luteus* ATCC 9341, using the turbidimetric method, which will allow us to know the effect on the previously appointed microorganisms and if in fact there is pharmaceutical equivalence between the innovator meropenem and the generic meropenem (Alvarez, 2008).

MATERIALS AND METHODS

For this study, the standardization of the antibiotic means used for the power tests and the antibiotic concentrations was performed.

Inoculum preparation

For this study, the microorganisms *B. subtilis* ATCC 6633, *E. coli* ATCC10536, *M. luteus* ATCC 9341, *P. aeruginosa* ATCC 25619 and *S. aureus* ATCC 29737 were used, in accordance with the USP Pharmacopeia; which underwent 4 chimes of each strain in trypticase soy agar (TSA) agar, these organisms were submitted to analysis when they were in exponential phase; the organism suspension was prepared in sterile saline 0.9% at a transmittance of 25% + / - 0.5 to 600 nm.

Preparation and inoculation of the plate

20 ml of medium antibiotic and 0.6 ml of organism were served under sterile conditions. The solution was stirred to obtain a homogeneous solution, then it was allowed to solidify. In each box, 6 reservoirs were worked and 3 were for the S3 standard and the remaining three were for the corresponding sample and other standards as the case may be.

Preparation of the standard solution

A USP standard solution and a VICAR secondary standard, for which a weight of 36.97 and 34.53 mg respectively, was calculated in a 50 mL volumetric balloon and it was completed on volume with a phosphate buffer of pH 8, therefore, 5 ml in a 50 ml volumetric balloon were taken and was completed with phosphate buffer of pH 8, consequently, the concentrations in 0.2, 0.4, 0.8, 1.6 and 3.2 ug/ml were performed.

Sample preparation

For this study, the following tests were performed: Generic antibiotic of Meropenem 1g and 05 vs. USP standard and VICAR secondary standard; Meropenem 1 g innovator antibiotic and 0.5 vs. USP standard and VICAR secondary standard; meropenem 29.35 mg generic antibiotic and meronem 30 mg innovator antibiotic, each in volumetric balloon of 50 mL and was completed with a phosphate buffer of pH 8. Consequently, 5 ml were taken in volumetric balloon of 50 ml and it was completed with phosphate buffer pH 8. Consequently, the 0.2, 0.4, 0.8, 1.6 and 3.2 ug/ml concentrations were performed.

Procedure for the essay through the cylinder-plate method

Boxes were placed for the analysis on a level surface. 20 mL of antibiotic medium inoculated (0.3 ml per 20 ml of medium) were served in each box and were allowed to solidify. In each box, 6 reservoirs were worked of which 3 were for the standard S3 and the other three corresponded to the sample and other standards as applicable, each cylinder with 0.1 ml sample of the antibiotic. The plates were incubated 16-18 h at 37°C. After incubation time, the cylinders were removed from the agar and the diameters of the inhibition zones were measured (Kenneth, 1994).

Statistical design

A variance analysis was conducted on two factors with several samples per group to verify the pharmaceutical equivalence between generic meropenem and the innovator, compared with USP reference standard and secondary VICAR standard. The study posed the following three hypotheses:

First hypothesis

Ho: (null hypothesis). There is no pharmaceutical equivalence between the meropenem generic antibiotic and meronem.
Ha: (alternative hypothesis) There is pharmaceutical equivalence between the meropenem generic antibiotic and the meronem innovator.

Second hypothesis

Ho: The concentrations do not have effect on the diameter.
Ha: The concentrations have effect on the diameter.

Third hypothesis

Ho: There is a joint effect between the pharmaceutical equivalence of the antibiotics and the concentrations.
Ha: There is no joint effect between the pharmaceutical equivalence of the antibiotics and the concentrations.

The following probability was formulated: If $p \leq 0.05$, rejection of the Ho; If $p \geq 0.05$, acceptance of the Ho.

Table 1. Inhibition halos in Antibiotic media N° 1 and 11.

Microorganism	Antibiotic media No. 1	Antibiotic media No. 11	Ideal media
Staphylococcus aureus	Diffused and irregular halos	Defined and regular halos	Antibiotic media No. 11
Micrococcus luteus	Diffused and irregular halos	Diffused and irregular halos	Antibiotic media No. 1
Pseudomonas aeruginosa	Defined and regular halos	Diffused and irregular halos	Antibiotic media No. 1
Bacillus subtillis	Defined and regular halos	Diffused and irregular halos	Antibiotic media
Escherichia coli	Defined and regular halos	Diffused and irregular halos	Antibiotic media

Table 2. R^2 of the different microorganisms.

Microorganism	R^2 of each microorganism					
	Standard USP			Secondary standard		
	Meropenem	Meronem	Standard USP	Meropenem	Meronem	Secondary standard
Bacillus subtillis	0.99	0.98	0.98	0.98	0.98	0.98
Micrococcus luteus	0.98	0.98	0.99	0.99	0.99	0.99
Staphylococcus aureus	0.99	0.98	0.98	0.98	0.98	0.95
Pseudomona aeruginosa	0.98	0.98	0.99	0.98	0.99	0.98
Escherichia coli	0.99	0.99	0.98	0.98	0.98	0.99

RESULTS

The ideal media for growth of each microorganism was chosen, where a neat halo and regular growth was clearly shown (Table 1).

Determination of standard curve

For this study was considered two presentations for generic antibiotic and two presentations for these being innovative antibiotic 1 g and 0.5 mg, and prepared two curves: one standard and one standard USP secondary. Once the experiments were concluded by the standard deviation of antibiotics presentations 1 g and 0.5 mg yield the same results, so we proceeded to use for the entire study presentation of 1 g antibiotics. From the measurement of growth inhibition zones, averages were taken with antibiotic concentrations used and tracing the curve for each of the microorganisms from this equation was obtained from the subtraction and the coefficient of determination (r2) (Morales, 1957).

The r2 obtained for each of the curves studied both as the standard samples gave data ≥ 0.98 (Table 2) which fall within the acceptance range, showing a relationship between the values of both shafts (Fernandez et al 2006). By plotting standard curves of USP meropenem 1 g and meronem against microorganisms *B. subtilis*, *M. luteus*, *E. coli*, *P. aeruginosa*. (Figures 6, 7, 9 and 10), it appears that the stock is the left side of the standard, which means that the power stated is correct, however the values of *S. aureus* are located on the right side of the standard curve so the stated concentrations is not successful (Figure 8). The r^2 obtained for each of the studied curves both for standards and figures showed data of ≥0.98.

In the curve graphics of the VICAR secondary standard, the meropenem and meronem 1 g showed values below the standard for microorganisms such as *B. subtillis, M. luteus, S. aureus, E. coli* and *P. aeruginosa*. The averages obtained from the power percentages of all microorganisms, compared to the standard secondary curve were greater or equal to the amount labeled for the generic antibiotic and the innovator (Table 3). There is a pharmaceutical equivalence between the meropenem generic antibiotic and the meronem innovator for the *B. subtillis, S. aureus, P. aeruginosa* and *E. coli* microorganisms. There is no pharmaceutical equivalence between the meropenem generic antibiotic and the meronem innovator on the *M. luteus* microorganism.

Statistical analysis

In the first case, the null hypothesis was rejected for allmicroorganisms except for *M. luteus*. In the second case the null hypothesis was rejected for all microorganisms. In the third case the null hypothesis was rejected for all microorganisms except for *M. luteus* (Table 4). In the first case the null hypothesis was rejected for all the microorganisms except for *E. coli.* In the second case the null hypothesis was rejected for all microorganisms. In the third case, the null hypothesis was rejected for all microorganisms except for *E. coli* (Table 5).

Table 3. Power percentage for each microorganism.

Microorganism	Potency percentage			
	Standard USP		Secondary standard	
	Meropenem	Meronem	Meropenem	Meronem
Bacillus subtillis	78.18	78.47	90.1	92.1
Micrococcus luteus	86.87	86.87	85.1	89.1
Staphylococcus aureus	81.27	81.27	84.2	82.5
Pseudomona aeruginosa	80.38	88.14	82.1	91.3
Escherichia coli	91.97	95.72	86.5	86.5

Table 4. USP standard probabilities.

Microorganism	Probability		
	First hypothesis	Second hypothesis	Third hypothesis
Bacillus subtillis	5.24871E-25	5.7789E-123	2.56773E-50
Micrococcus luteus	0.999996725	1.2183E-118	0.999146312
Staphylococcus aureus	1.22157E-38	1.4671E-128	4.74343E-30
Pseudomonas aeruginosa	3.69496E-74	2.8246E-134	2.0361E-56
Escherichia coli	8.24422E-54	4.5048E-138	6.34906E-58

Table 5. Secondary standard probabilities.

Microorganism	Probability		
	First hypothesis	Second hypothesis	Third hypothesis
Bacillus subtillis	8.14039e-40	1.6195e-124	3.5956e-31
Micrococcus luteus	1.23425e-50	1.9309e-120	2.98384e-68
Staphylococcus aureus	3.85579e-14	1.043e-125	1.23678e-44
Pseudomonas aeruginosa	2.92389e-58	2.1076e-126	5.67466e-52
Esherichia coli	0.486602146	1.152E-118	0.621385479

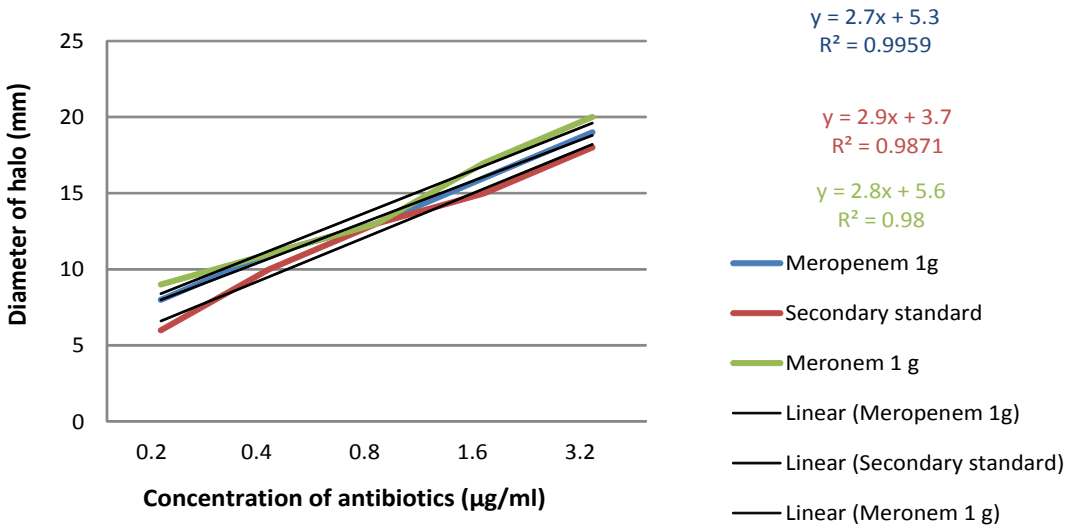

Figure 1. Secondary standar curve , meropenemand meronem with *Bacillus subtillis*.

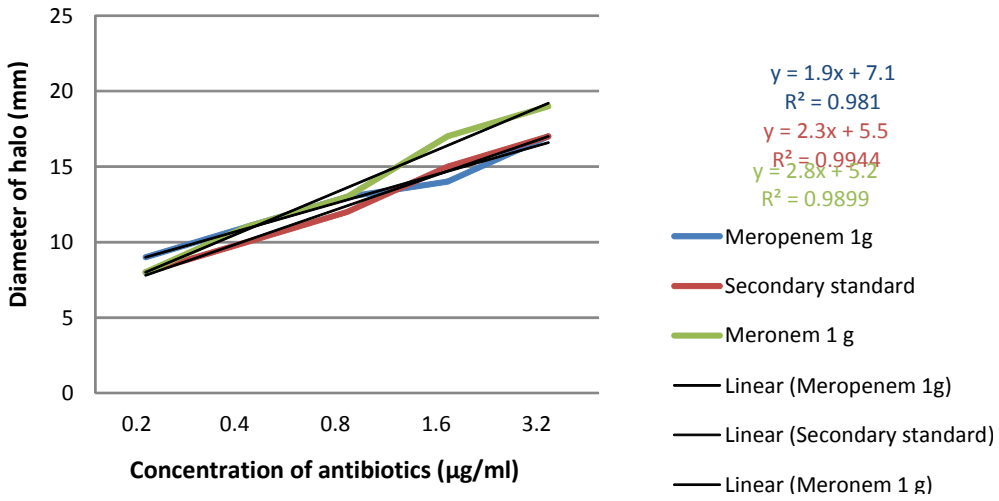

Figure 2. Secondary standard curve of meropenem and meronem with *Micrococcus luteus*.

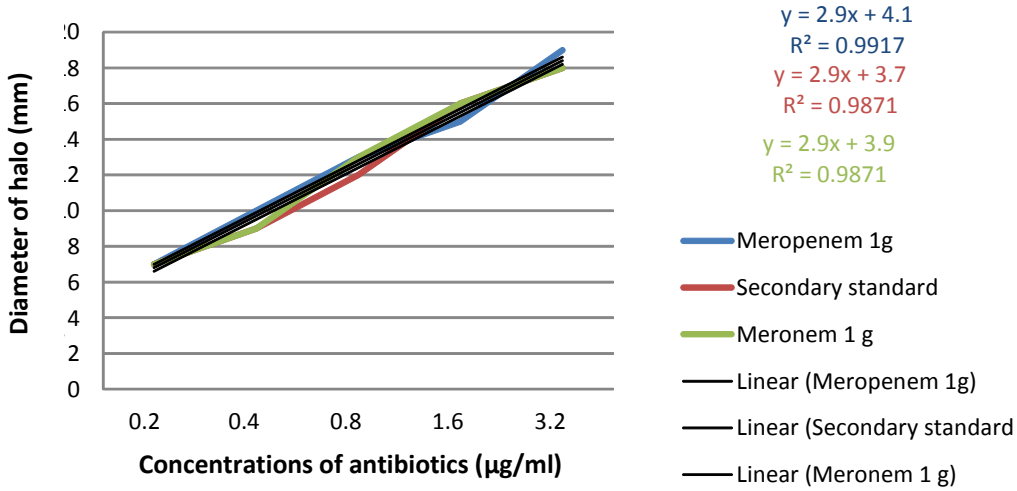

Figure 3. Secondary stand curve, meropenem and meronem with *Staphylococcus aures.*

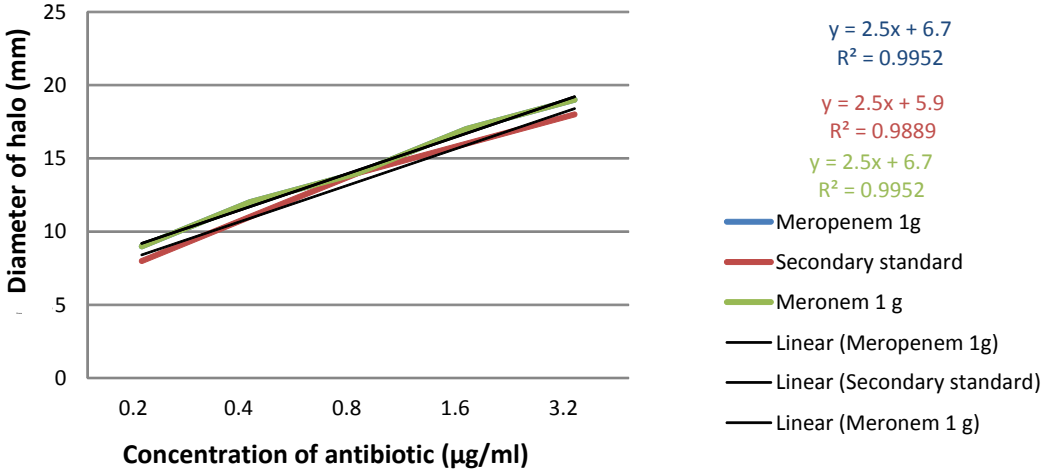

Figure 4. Secondary stándar curve VICAR , meropenem and meronem with *E. coli*.

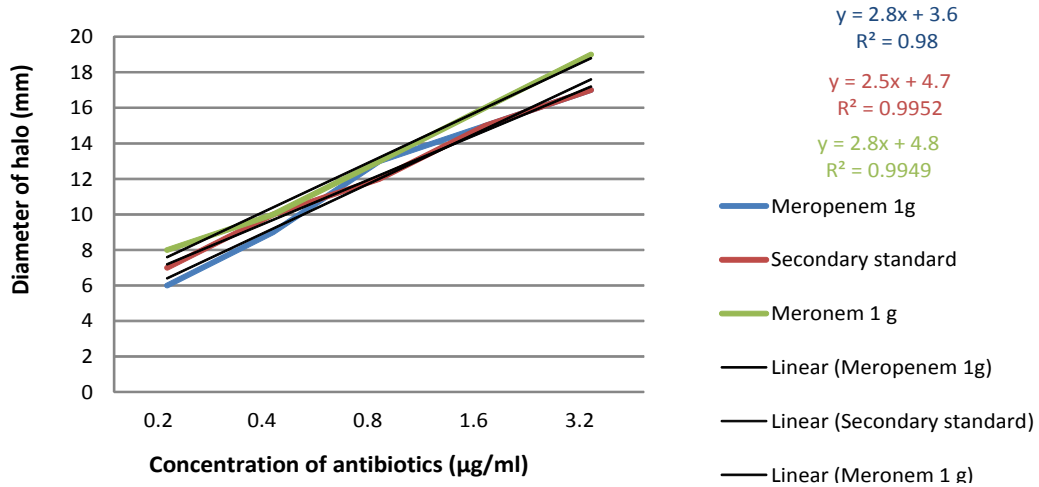

Figure 5. Secondary stándar curve VICAR, meropenem and meronem with *Pseudomona aeruginosa*.

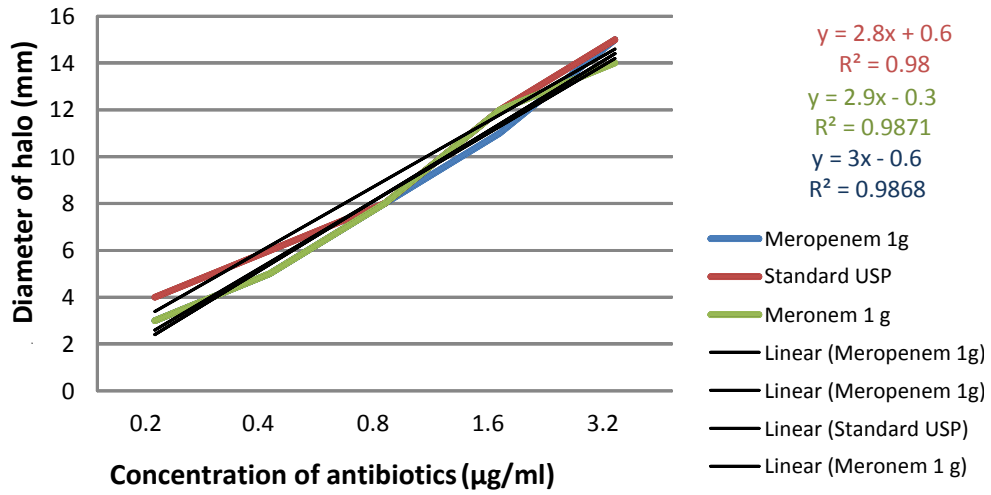

Figure 6. USP standard curve, Meropenem andMeronem with *Bacillus subtillis.*

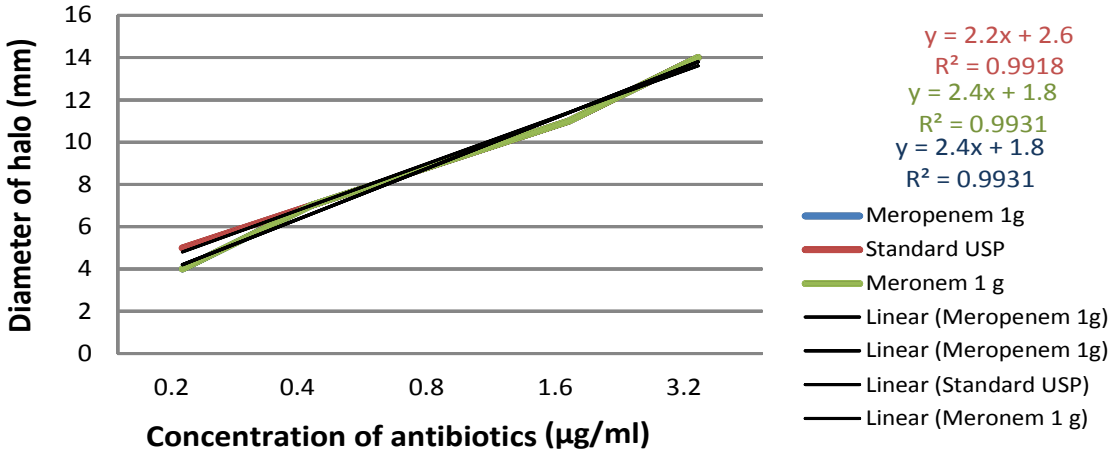

Figure 7. USP standard curve, meropenem and meronem with *Micrococcus luteus.*

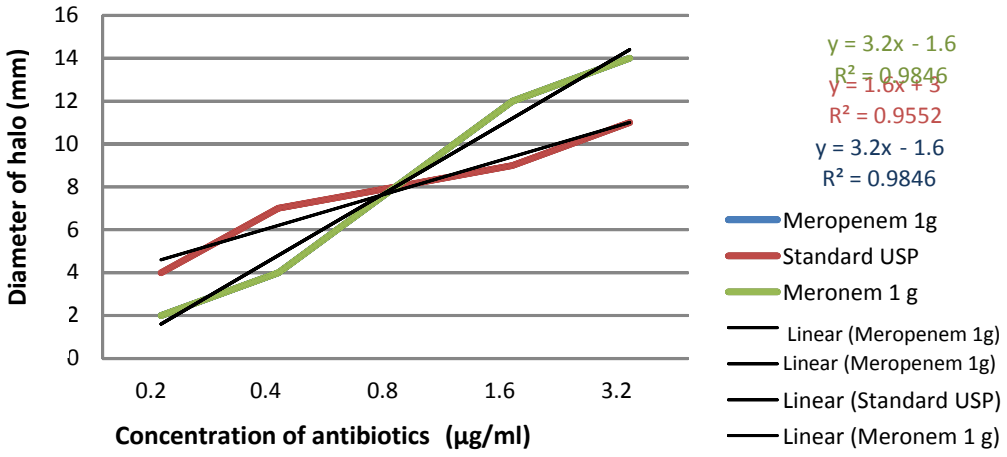

Figure 8. USP standar curve , meropenem and meronem with *S.aureus.*

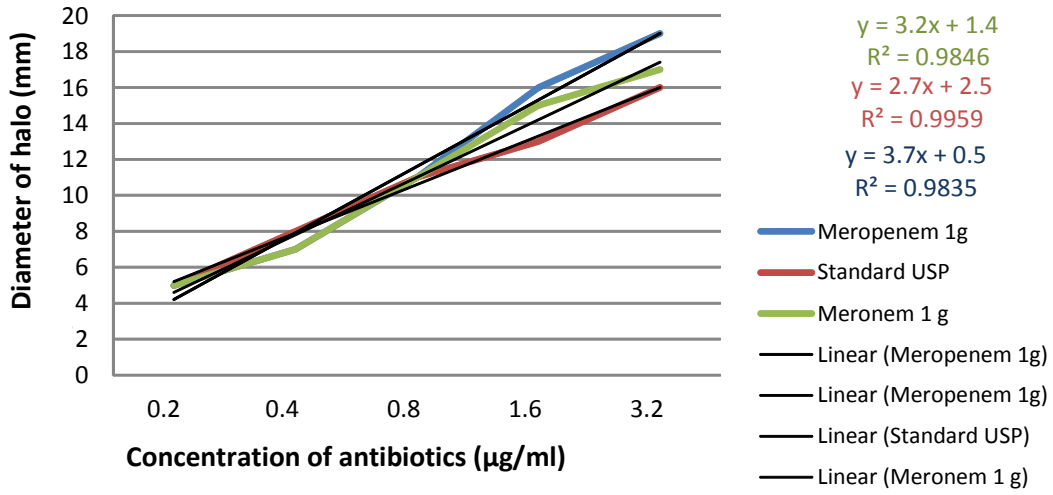

Figure 9. USP stándar curve, meropenem and meronem with *E.coli.*

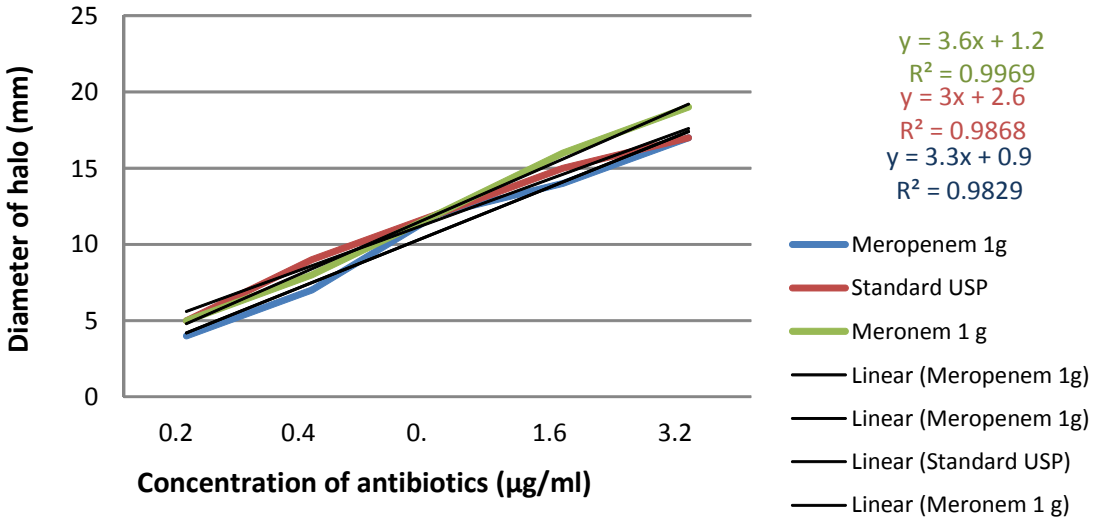

Figure 10. USP stándar curve, meropenem and meronem with *Pseudomona aeruginosa*.

DISCUSSION

The standardization of the antibiotic means that each of the microorganisms allows for a more accurate result of the inhibition zones, taking into account that each antibiotic medium (number 1 and number 11), only varies in pH, and thus it is possible to confirm that each evaluated microorganism holds varying conditions for its optimum growth. The r^2 obtained for each of the studied curves both of standards and the samples showed data of ≥0.98 which are placed within the acceptance range, showing that there is a relation between the values of both axis (Fernández et al., 2006).

Generic drugs generate much discussion about its competition with innovative medicines. The advantage of its use is its low cost, since it has no patent; in Colombia it is usual that health institutions and low-income users prefer generic drugs because they generate savings. The results of this research have great scientific and socio-economic impact, as it seeks to show that the generic drug is pharmaceutically equivalent to the innovator, demonstrating its quality and good manufacturing practices in their execution and therefore the same activity (Giner et al., 1995).

Taken together the data obtained by measuring halos in the standard curve is a reference on the feasibility of testing in statistical terms and this gives a criterion of analysis as valid ((Jones et al., 2004). By plotting the curves of the secondary standard VICAR, and meronem meropenem 1 g against microorganisms, *B. subtilis*, *M. luteus*, *S. aureus*, *E. coli* and *P. aeruginosa*, it is evident that the values are on the left side of the standard, which means that the declared capacity is accurate (Figures 1, 2, 3, 4 and 5). Taken together the data obtained by measuring halos in the standard curve is a reference on the feasibility of testing in statistical terms and this gives a criterion of analysis as valid.

According to the averages obtained from the potency percentage, there is no significant difference regarding the power of the different samples, since according to the USP XXXI (2008) for microbiological tests for all antibiotics, the percentage should be between 80 and 125%, since it is a test that has a lot of variables and possible sources of error. However, for the *Bacillus subtilis* microorganisms, the percentage of antibiotic potency of generic and innovative compared to the USP standard curve showed that there is a difference with the declared potency, because the values were 78.18% and 78.47% respectively. It is recommended that verifying the therapeutic equivalence to ascertain whether the generic antibiotic after administration of the molar dose, the effects on safety and efficacy are the same should be considered for further studies. In addition, understanding and manipulating various statistical programs and keeping concentrations antibiotics made for cooling and where light does not reach, for proper operation could also be ascertain.

Conclusion

This study verified the pharmaceutical equivalence of a generic meropenem vs. innovative by microbial power valuation. It was found that the coefficient of determination was equal to or greater than 0.98, determining the consistency of the results. Pharmaceutical equivalence was demonstrated for meropenem vs generic form through innovative technique plate cylinder. Pharmaceutical equivalence exists between the antibiotic and the innovative generic meronem meropenem for microorganisms *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli*.

Abbreviations

WHO/PAHO, World Health Organisation/ Pan American Health Organisation; **FDA,** Food and Drug Administration; **r2,** coefficient of determination.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Álvarez B, Álvarez F, Romero J, Fernández L, Ruiz F, Sancho H (2008). Optimización de la dosis de meropenem en infecciones graves. Resultados de un estudio multicéntrico observacional. Rev. Esp. Quim. 21(3):143-148.

Arias J (2006). Métodos en microbiología farmacéutica. Javegraf. Bogotá D.C., Colombia.

Fernández M, Palazón z, Gutiérrez M, Carrillo D, Bilbao O (2006). Estudio de la Actividad Antimicrobiana de la combinación Quitina-Gentamicina. Rev. Mex. Cienc. Farma. 4(37)21-29.

Giner S, Canos MY, Ferrer C (1995). Meropenem: un nuevo carbapenem en el arsenal terapéutico. Sección de Microbiología. Hospital Virgen de los Lirios. Alcoy (Alicante).Laboratorio de Salud Pública. Centro de Salud Pública de Castellón. Servicio de Medicina Interna. Hospital de Sagunto. Valencia. Farm Hosp. 19(2):109-113.

Jones R, Holly K, Douglas JB, Thomas R, Fritsche, Helio S (2004). Doripenem (s-4661), a novel carbapenem: comparative activity against contemporary pathogens including bactericidal action and preliminary in vitro methods evaluations J. Antimicrob. Chemother. (54):144–154.

Kenneth E, Natchez M, Denise D (1994). In Vitro Activity of Biapenem (L-627), a New Carbapenem, against Anaerobes. Antimicrob. Agents Chemother. (28):889-893.

Morales, J. y Velazco, C (1957). Curvas standard y determinación de potencia antibiótica. *Rev.* Perú. Med. Exp. Salud Púb. 11(1-2):92-103.

Pharmacopea (2006). The United States Pharmacopeia. The official compendial of standars. USP XXIX. Editorial United States Pharmacopeia Convention Inc.

Rivero E, Herrera M, Larrondo H, Lozano DY, León D (1998). Carbapenémicos y monobactámicos. Acta Méd. 8(1):66-70

USP (2006). NF24 Expert Committee monograph development antibiotics 30(3):1002

WHO (2011). Expert Committee on Specifications for Pharmaceutical Preparations. Forty-sixth report. Geneva. Pp.10–14.

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Review

Vascular endothelial growth factor (VEGF) in cancer

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Angiogenesis plays crucial part in the formation and progression of tumor, and vascular endothelial growth factor (VEGF) is one of the most important cytokine which aids in the growth of tumor cells. In various tumors whether locally invasive or metastasize to distal parts, elevated levels of VEGF is observed in blood through angiogenesis, this leads to progression of cancer.

Key words: Vascular endothelial growth factor (VEGF), cancer.

INTRODUCTION

Angiogenesis plays crucial part in the formation and progression of tumor and vascular endothelial growth factor (VEGF) is one of the most important cytokine which aids in the growth of tumor cells. VEGF is a polypeptide structurally associated to platelet-derived growth factor (PDGF). The gene for VEGF is present on chromosome 6p12. Angiogenesis, the process of new blood vessel formation, performs crucial role in both invasive tumor growth and noninvasive tumor growth in breast cancer and other benign and metastasized cancers. There are some benign and malignant tumors such as breast cancer, lung cancer, squamous cell carcinoma, thyroid cancer, etc., in which VEGF has been implicated as a key arbitrator of angiogenesis. Although scientists and clinicians have learned much about the character of VEGF and angiogenesis in breast cancer, many questions related to this phenomenon remain unanswered.

VEGF ISOFORMS AND RECEPTORS

Functions of different isoforms of VEGA are given here briefly:

1. VEGF-A: Angiogenesis, Chemotactic for macrophages and granulocytes, Vasodilation (indirectly by NO release)
2. VEGF-B: Embryonic angiogenesis (myocardial tissue, specifically)
3. VEGF-C: Lymphangiogenesis
4. VEGF-D: Needed for the development of lymphatic vasculature surrounding lung bronchioles

VEGF ROLES IN CANCER CELL PROLIFERATION

Bcl-2 is an anti-apoptotic protein that functions in cell survival, tumor progression and drug resistance, and is

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induced in response to VEGF in cancer cells (Saleem et al., 2013).

VEGF IN MALIGNANT AND NON-MALIGNANT DISEASE

Tumor develops as a result of abnormalities in the non-vascularizing tissues after certain consequences. Diagnosis of tumor is difficult as diagnosis of other complex diseases (Qadir, 2010; Nisar et al., 2011; Naz, 2012; Janbaz 2012). Extent, strength, aggressiveness and malignancy of tumor depend upon the type of tissue in which tumor arises. Tumor growth is nourished by the formation of blood vessels around the neoplastic cells. VEGF is one of the major growth factor present in the blood surrounding the tumor and causes tumor metastasis. It is an angiogenic cytokine stimulating the cancer propagation in the sera of patients suffering from benign and malignant diseases. Minimum level of VEGF in the healthy individuals than the tumor patients showed that VEGF performs an important role in tumor development, formation and progression of noninvasive and invasive effusions (Kraft et al., 1999).

Breast cancer patients

As a consequence of comparative study on patients having breast cancer and healthy woman, VEGF in breast cancer patients has surpassed and elevated to very high concentration. Breast cancer can be propagated and metastasize under the influence of elevated levels of VEGF and it also has an impact on tumor stages. Before initiating the therapy, VEGF level has surpassed in breast cancer patients (Berezov et al., 2009). VEGF has been indicated as a potent angiogenic factor that has an effect on the tumor progression. It has been measured in high concentrations in patients having breast cancer and other cancer types. After treating breast cancer by antiangenic agents combined with conventionally used drugs like temoxifen, methotrexate, flurouracil, adriamycin and cyclophosphamide as a first line therapy especially site specific (Khalid et al., 2009; Hussain et al., 2011; Ehsan et al., 2012; Naz et al., 2012), there is a low concentration of VEGF observed. Menopausal status not only affect the VEGF level in the serum, over expression of estrogen receptor and progesterone receptors have also an influence on its concentration in the serum of breast cancer patients (Foekens et al., 2001).

Tumor growth is dependent on angiogenesis that is carried out by the presence of VEGF. Over expression of estrogen receptor may enhance the concentration of VEGF in the plasma of breast cancer women. After testing on the tubular/ductal breast cancer women compared with those healthy, consequences that VEGF is a significant factor in the progression and further spreading of tumor to the distant parts and leads to malignancy. These findings may be helpful for further investigations on this issue (Heer et al., 2001). When there is an imbalance which occurs in the aniogenic factor and angiogenesis inhibitors then tumor cell becomes more invasive and malignant because it is supplied by blood vessels having excessive concentrations of growth stimulating cytokines that is VEGF, which propagate the further growth of tumor cells. Patients suffered from node-negative carcinoma have developed malignant condition due to the elevation of VEGF in the serum, so VEGF is considered as an important marker for its progression and propagation (Gasparini et al., 1997). VEGF and basic fibroblast growth factor (bFGF) are two major growth factors imperative role of the tumor growth via angiogenesis. Both of these angiogenic cytokines impart function in the spreading and differentiation of breast cancer into different parts which may be stimulated by over expression of estrogen receptor (Granato et al., 2004). Vascular angiogenesis and vascular permeability are regulated mainly by VEGF which is a growth factor and causes malignant growth of various tumors. Women with benign tumor have less concentration than patients having metastasized tumor growth. Ductal breast cancer patients have very low response towards chemotherapy than lobular or localized breast cancer women (Salven et al., 1999a).

VEGF in the serum of breast cancer patients may be increased. It's over storage and storage is done by tumor derived interleukin-6 (IL-6) in the platelets. This conforms the role of circulating platelets and IL-6 in the storage of VEGF, ultimately an elevated level of VEGF in the blood of breast cancer patients is seen (Benoy et al., 2002). Angiogenesis is very important and plays crucial role in the survival, and patient response towards therapy also depends on the degree of angiogensis. Temoxifen may also be related with higher VEGF level. Estrogen receptor over expression may enhance VEGF in the malignant effusions (Adams et al., 2000). VEGF enhances tumor growth to the distant parts in patients suffering from breast cancer. This VEGF is elevated to estrogen receptor over activity but is not affected by other serum components such as leptin and prolactin. So it is declared that leptin and prolactin in serum do not affect the tumor metastasis (Coskun et al., 2003). Tumor malignancy and propagation in breast cancer individual is also affected by intratumoral lymphoangiogenesis. VEGF-C in the lymph node performs crucial part in the succession and propagation of tumor cells. Therefore it is concluded that VEGF-C performs important part in tumor metastasis via lymphoangioangenesis. Over expression of estrogen receptor and mutation in the TP-53 gene status has a positive influence to elevate the level of VEGF which ultimately leads to cancerous cells metastasis in the breast cancer patients (Berns et al., 2003).

Under the influence of chemotherapy for breast cancer, there is a considerable low level of VEGF detected, which

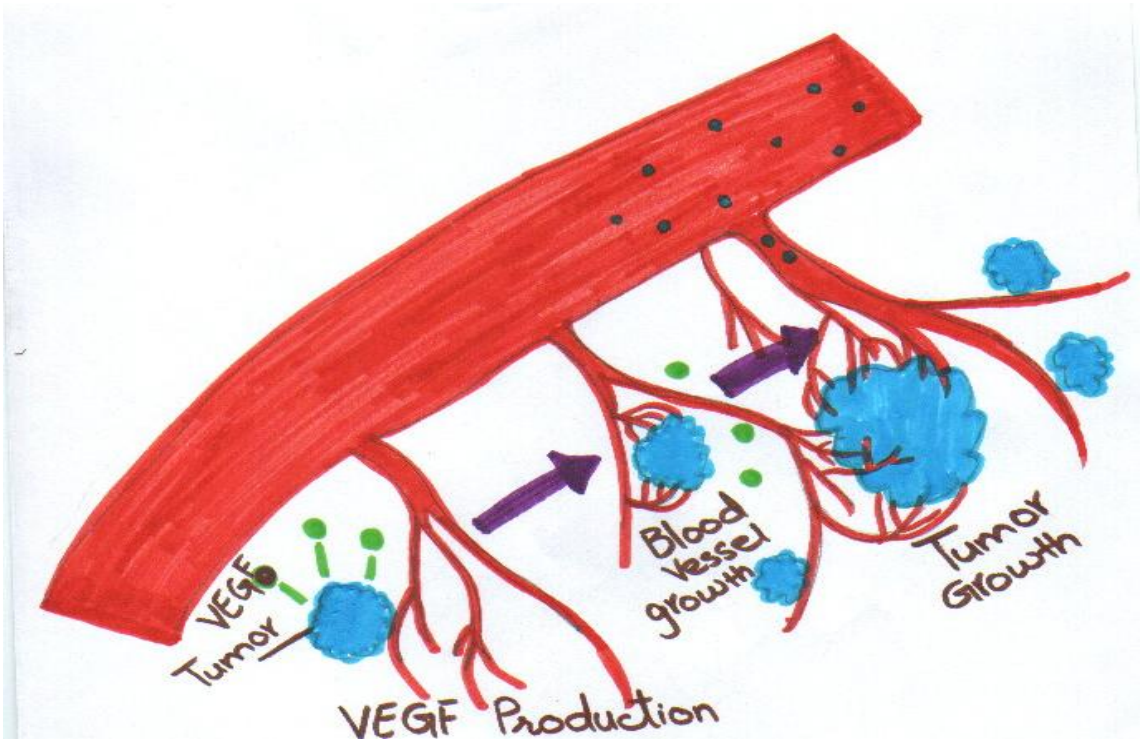

Figure 1. Elevated VEGF expression in newly produced blood vessels plays imperative role in tumor progression

shows that elevated VEGF has an impact on tumor progression Figure 1 after chemotherapy has become lowered. Pathological condition of breast cancer mainly surpassed to high degree due to various growth factors. Over stimulation of steroid hormone receptor status increased because of elevated concentration of VEGF in the patients having breast carcinoma (Manders et al., 2003). Therapeutic drugs work positively to control the tumor growth (Qadir and Malik, 2010; Masood et al., 2011; Javed et al., 2011; Qadir, 2011; Ameen et al., 2012), ultimately minimum concentrations of VEGF is detected in the serum of breast cancer patients after anticancer therapy (Kim et al., 2009; Colleoni et al., 2002). Pin-1 or VEGF in high concentrations is detected in the serum of patients having breast cancer. It is confirmed by investigations on different groups of patients suffering from breast cancer, the levels of VEGF at extreme point in the serum which leads to further proceeding of tumor cells (Quaranta et al., 2007). Platelets and leukocytes release VEGF and it leads to high concentration in the plasma. This elevated level of VEGF acts as an important angiogenic cytokine in the blood cells surrounding the neoplastic cells which ultimately leads to metastasis of tumor to distant parts. Physiological production of VEGF as an important angiogenic cytokine is by the uterine cells, but due to pathological changes in its secretion from uterus leads the tumor to propagate, causing its spread to distant parts.

Ultimately, it may lead to metastasis of tumor. High level of VEGF has key effect on breast cancer. VEGF level considerably decreases due to postmenopausal uterus which could prove protective against breast cancer progressing and spreading (Lowery et al., 2008).

METASTISIZED THYROID CANCER

Tumor formation, development and progression depend upon the concrete and distinct nutritive support that is supplied to thyroid tumor cells by the blood vessels formed around the cancerous area. VEGF is produced at the location of cancerous cells as a growth factor due to angiogenesis. Over expression of VEGF cause the aggressiveness and advance distribution of thyroid cancer. Elevated VEGF levels may also be due to stimulation by thyroid stimulated hormone (TSH). VEGF concentrations in the serum of patients distressed from differentiated thyroid cancer are more than those with non-differentiated thyroid cancer. On the basis of these findings, it is hypothesized that there would be drastically higher levels of VEGF in the serum of patients with persistent or intermittent thyroid cancer than those who had been cured from disease (Tuttle et al., 2002).

COLORECTAL CANCER

Concrete and explicit tumors progress and propagate due to the influence of VEGF which is an imperative angiogenic

cytokine. In colorectal cancer patients, VEGF is detected in the serum before and after the operative measures. After performing tumor surgical procedure, decrease of VEGF in the serum is observed. VEGF concentration in the patients suffering from colorectal cancer changes as the tumor progresses or metastasize which indicates that there is a solid relationship between the VEGF concentration and tumor stages, degree of tumor invasion and dissemination of tumor to the distant parts. High level of VEGF in the serum of patients with colorectal cancer has a significant role in the further progress, metastasis and sequence of tumor growth (Karayiannakis et al., 2002). Patients having colorectal cancer showed an unambiguous meditation of VEGF in their plasma. VEGF comes as a consequence of degranulation of granulocytes and platelets which are there in the plasma. The data collected after observing the VEGF serum level of cancer patients compared with vigorous individuals. Tumor cells nurture very swiftly under the influence of VEGF in the plasma of colorectal patients indicating a very crucial part before and after the surgery. Metastasis of cancerous cells occurs very hastily to the distant areas in colorectal cancer individuals due to extreme level of VEGF (Cubo *et al.*, 2004). Restoration of physical condition and survival, after resection is very difficult because of the high level of VEGF in the serum of colorectal cancer patients. This elevated concentration leads to progression and auxiliary scattering of cancerous cells and it is independent of tumor stages. After testing the serum level of patients anguish from colorectal cancer compared with individuals recovered from disease, it is suggested that VEGF in plasma has considerable importance in the headway and succession of tumor cell growth (Karayiannakis et al., 2002). Patients with elevated serum concentration of VEGF having gastrointestinal and colorectal carcinoma showed to be more progressive and tumor had metastasized in these patients. Tumor enlargement occurs very rapidly in these patients. Studies on the patients having gastrointestinal and colorectal cancers indicated the fact that patients with minimum concentration of VEGF will continue to exist for longer periods of times as compared to those with more VEGF and similarly these patients with low VEGF respond to chemotherapy better than those having high VEGF in their plasma. These findings advocate that due to high level of VEGF in the serum of colorectal as well as gastrointestinal cancer patients, there is less response towards chemotherapeutic treatment and fewer probability of their endurance (Hyodo et al., 1998).

OVARIAN CANCER

Before operating measures, VEGF in the serum of ovarian cancer patients is observed in very high concentrations. This high level of VEGF is detected in the localized tumors as well as in the adnexal masses of malignant tumors. Due to the presence of elevated level of VEGF in the serum of patients suffering from ovarian cancer, this leads to fatal condition because tumor is at its peak degree in the distant parts of the body (Cooper et al., 2002). To overcome the tumor angiogenesis, it is needed to regulate the normal concentration of VEGF in the serum. This approach is very helpful in improving the disease status in the patients having benign or metastasized ovarian tumors (Linderholm et al., 2009). Serum VEGF (S-VEGF) concentrations come under the influence of cyto-reductive treatment and it may be better to control VEGF levels in ovarian cancer patients. The survival of ovarian cancer patients do not depend upon this single factor VEGF in the serum (Salgado et al., 1999). VEGF may be transported in the serum by the circulating platelets. So platelets also have an important function in the regulation of VEGF in the serum (Oehler and Caffier, 2000). Ovarian cyst fluid has elevated level of VEGF which hastens the onset of disease towards malignancy. But high concentration of basic fibroblast growth factor (bFGF) has no remarkable function in the progression and succession of ovarian cancer. High level of VEGF in the ovarian cancer patients perform major role via tumor angiogenesis. So, it is an important target for chemotherapeutic agents (Verheul et al., 1997). Highest concentrations of VEGF in the ovarian cyst fluid indicated that the elevated level of VEGF plays important part in the progression and multiplication of ovarian cancer to the distant parts of body (Boss et al., 2001; Qadir and Malik, 2008).

Localized and metastasized tumors are well differentiated by the CA 125 and cystic VEGF in the serum. In ovarian cancer patients, having high level of VEGF, high level of CA 125 was observed in their ovarian cyst fluid. In patients with ovarian epithelial neoplasm, the angiogenesis and augmentation of vascular permeability caused by VEGF indicates for the discharge of the CA 125 antigen into the circulation of patients (Hazelton et al., 1999). There is no association between VEGF and the concentrations of VHL-associated cancers. High level of VEGF is related with the spreading of tumor growth to the distant parts of body.

DISSEMINATED CANCER

Serum VEGF (s-VEGF) may achieve an elevated point in the loco regional or benign tumors and in the metastasized cancers, related to the histological kind of tumors. This high level of VEGF is very important for the metastases of tumors to other areas of the body (Salven et al., 1997). In anemia and intratumoral hypoxia, there is an increased level of VEGF in the serum of patients having stumpy hemoglobin through hypoxia-induced VEGF secretions (Dunst et al., 1999; Qadir et al., 2007; Salven et al., 1999b).

BRAIN TUMOR CYSTS

In the brain tumor cysts, VEGF is released in brain tumors whether benign or malignant. VEGF in brain tumor cyst causes the tumor to progress to the distal parts and makes it malignant (Stockhammer et al., 2000). Metastasized carcinomas progress positively and lead to more severity due to the presence of VEGF and basic fibroblast growth factor in the serum (Lissoni et al., 2001; Weindel et al., 1994). The patients suffering from bacterial meningitis have comparatively low concentration of VEGF in the cyst fluid than those patients with brain tumors. VEGF is found much higher in patients having CM than those having bacterial meningitis (Stockhammer et al., 2000; Takano et al., 1996).

HEAD AND NECK CANCER

VEGF acts as an endocrine growth factor, a positive angiogenic cytokine. As clinical monitoring point of view, it develops solid and definite growth of head and neck cancer and causes the further proceeding of tumor, leads to malignancy condition (Stockhammer et al., 2000). Head and neck cancer may develop very rapidly in the presence of elevated concentration of VEGF due to the proliferation of cancer cells and endothelial cells of head and neck. This high level of VEGF in head and neck cancer is a pin point target for the therapeutic point of view (Riedel et al., 2000).

RENAL CELL CARCINOMA (RCC)

Cancerous cells in the patients suffering from renal cell carcinoma releases VEGF into the blood circulation which may be a crucial factor in the progression and further spreading of renal cell carcinoma. Over expression of VEGF receptor is detected in renal cell carcinoma patients. Chances of survival of patients in renal cell carcinoma will be increased, if VEGF level become low in the serum of patients having renal cell carcinoma (Eisma et al., 1997). In both the localized tumor and malignant tumor, an association has observed between VEGF and tumor cyst fluid (Sato et al., 1999). VEGF performs important role in the spreading of tumor cell growth in the tumor cyst wall, in areas of hyaline cystic degeneration, in stellate reticulated astrocytes around microcysts, in the biphasic squashed and loose areas. VEGF causes the formation of cysts, microcystic pattern, hyaline cystic degeneration, hyaline vessels and vascular proliferation (Demirkiran et al., 2003).

SOFT-TISSUE SARCOMA

Tumor-induced angiogenesis is mostly caused by VEGF and basic fibroblast growth factor. As an outcome of histological testing and by tumor grading, it is concluded that the elevated concentrations of VEGF and basic fibroblast growth factor (bFGF) detected in patients distress from soft tissue sarcoma. This higher level of both of these growth factors leads to the progression of cancerous cells whether it is malignant or nonmalignant circumstance (Graeven et al., 1999; Qadir et al., 2006).

ANEMIA AND ELEVATION OF VEGF

VEGF is regulated by a major stimulus that is tissue hypoxia. Due to impairment of tissue oxygenation, anemia has impact on angiogenesis which ultimately affect the tumor growth because angiogenesis provides nutritive support to the progression of tumor cells whether it is benign or malignant. Testing on patients suffering from malignant tumor showed elevated levels of VEGF as compared to those with nonmalignant diseases. Due to decreased hemoglobin level, there will be increase in VEGF in the serum of metastasized tumor patients. When hemoglobin level changes under erythropoietin treatment then there will be normalization in the VEGF level. This has indicated that anemia might have an important role in the progression and propagation of tumor growth (Dunst et al., 2002).

ANTI-VEGF THERAPIES

The emergence of resistance and tolerance to the existing drugs has created a decreased efficacy of these drugs in use. This problem has been tried to be overcome by increasing the drug delivery to the target site by the use of polymers (Khalid et al., 2009; Hussain et al., 2011) or through nanotechnology (Naz et al., 2012; Ehsan et al., 2012), synthesis of new drugs, either by the use of proteomics (Qadir, 2011; Qadir and Malik, 2011), or synthesis from lactic acid bacteria (Masood et al., 2011), or marine microorganisms (Javed et al., 2011). However, nowadays, the trend is being changed from synthetic drugs to the natural drugs either from plants or microbes to control the diseases. The natural products are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory (Qadir, 2009), hypotensive (Qadir, 2010), hepatoprotective (Ahmad et al., 2012; Ali et al., 2013), hypoglycaemic (Nisa et al., 2009; Qadir and Malik, 2010), amoebicidal (Asif and Qadir, 2011), anti-fertility, cytotoxic, antimicrobial (Amin et al., 2012), spasmolytic, bronchodilator (Janbaz et al., 2013a), antioxidant (Janbaz et al., 2012), anti-diarrheal (Janbaz et al., 2013b) and anti-cancer properties. With the passage of time and advancement in science and technology, new management tools have been emerged for the control of different diseases including cancer. Anti-VEGF therapies are important in

the treatment of certain cancers and in age-related macular degeneration. They can involve monoclonal antibodies such as bevacizumab (avastin), antibody derivatives such as ranibizumab (lucentis), or orally-available small molecules that inhibit the tyrosine kinases stimulated by VEGF: lapatinib (tykerb), sunitinib (sutent), sorafenib (nexavar), axitinib, and pazopanib.

CONCLUSION

The collected data show that in various tumors whether locally invasive or metastasize to distal parts, there are elevated levels of VEGF in blood through angiogenesis, which leads to progression of cancer. Anti-VEGF therapies are important in the treatment of certain cancers.

REFERENCES

Adams J, Carder PJ, Downey S, Forbes MA, MacLennan K, Allgar V, Kaufman S, Hallam S, Bicknell R, Walker JJ, Cairnduff F, Selby PJ, Perren TJ, Lansdown M, Banks RE (2000). Vascular endothelial growth factor (VEGF) in breast cancer: comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen. Cancer Res. 60:2898-2905.

Ahmad M, Mahmood Q, Gulzar K, Akhtar MS, Saleem M, Qadir MI (2012). Anti-hyperlipidaemic and hepatoprotective activity of *Dodonaea viscosa* leaves extracts in alloxan-induced diabetic rabbits (*Oryctolagus cuniculus*). Pak. Vet. J. 32:50-54.

Ali M, Qadir MI, Saleem M, Janbaz KH, Gul H, Hussain L, Ahmad B (2013). Hepatoprotective potential of Convolvulus arvensis against paracetamol-induced hepatotoxicity. Bangladesh J. Pharmacol. 8:300-304.

Ameen S, Qadir MI, Ahmad B (2012). Pharmacogenomic approaches in the treatment of breast cancer by tamoxifen. Pak. J. Pharm. Sci. 25:469-476.

Amin N, Qadir MI, Khan TJ, Abbas G, Ahmad B, Janbaz KH, Ali M (2012). Antibacterial activity of Vacuum liquid chromatography (VLC) isolated fractions of chloroform extracts of seeds of *Achyranthes aspera*. J. Chem. Soc. Pak. 34:589-592.

Asif MA, Qadir MI (2011). Molecular approaches for development of malarial vaccines. Rev. Pharmacol. 4:276-278.

Benoy I, Salgado R, Colpaert C, Weytjens R, Vermeulen PB, Dirix LY (2002). Serum interleukin 6, plasma VEGF, serum VEGF, and VEGF platelet load in breast cancer patients. Clin. Breast Cancer 2:311-315.

Berezov TT, Ovchinnikova LK, Kuznetsova OM, Karabekova ZK, Vorotnikov IK, Tuleuova AA, Katunina AI, Dvorova EK (2009). Vascular endothelial growth factor in the serum of breast cancer patients. Bull. Exp. Biol. Med. 148:419-424.

Berns EM, Klijn JG, Look MP, Grebenchtchikov N, Vossen R, Peters H, Geurts-Moespot A, Portengen H, van Staveren IL, Meijer-van Gelder ME, Biker B, Sweep FC, Foekens JA (2003). Combined vascular endothelial growth factor and TP53 status predicts poor response to tamoxifen therapy in estrogen receptor-positive advanced breast cancer. Clin. Cancer Res. 9:1253-1258.

Boss EA, Massuger LF, Thomas CM, Geurts-Moespot A, Boonstra H, Sweep CG (2001). Vascular endothelial growth factor in ovarian cyst fluid. Cancer 91:371-377.

Colleoni M, Rocca A, Sandri MT, Zorzino L, Masci G, Nolè F, Peruzzotti G, Robertson C, Orlando L, Cinieri S, de BF, Viale G, Goldhirsch A (2002). Low-dose oral methotrexate and cyclophosphamide in metastatic breast cancer: antitumor activity and correlation with vascular endothelial growth factor levels. Ann. Oncol. 13:73-80

Cooper BC, Ritchie JM, Broghammer CL, Coffin J, Sorosky JI, Buller RE, Hendrix MJ, Sood AK (2002). Preoperative serum vascular endothelial growth factor levels: significance in ovarian cancer. Clin. Cancer Res. 8:3193-3197.

Coskun U, Günel N, Toruner FB, Sancak B, Onuk E, Bayram O, Cengiz O, Yilmaz E, Elbeg S, Ozkan S (2003). Serum leptin, prolactin and vascular endothelial growth factor (VEGF) levels in patients with breast cancer. Neoplasma 50:41-46.

Cubo T, Padilla D, de la Osa G, Palomino T, García M, Pardo R, Martín J, Arévalo E, Hernández J (2004). Serum vascular endothelial growth factor levels in patients with colorectal cancer and its prognostic significance. Med. Clin. 122:201-204.

Demirkiran F, Kumbak B, Bese T, Arvas M, Benian A, Aydin S, Uzun H, Sanioglu C, Aydinli K, Kösebay D (2003). Vascular endothelial growth factor in adnexal masses. Int. J. Gynaecol. Obstet. 83:53-58.

Dunst J, Becker A, Lautenschläger C, Markau S, Becker H, Fischer K, Haensgen G (2002). Anemia and elevated systemic levels of vascular endothelial growth factor (VEGF). Strahlenther Onkol. 78(8):436-441.

Dunst J, Pigorsch S, Hansen G, Hintner I, Lautenschläger C, Becker A (1999). Low hemoglobin is associated with increased serum levels of vascular endothelial growth factor (VEGF) in cancer patients. Strahlenther Onkol. 175:93-96

Ehsan O, Qadir MI, Malik SA, Abbassi WS and Ahmad B (2012). Efficacy of nanogold-insulin as a hypoglycemic agent. J. Chem. Soc. Pak. 34:365-370.

Eisma RJ, Spiro JD, Kreutzer DL (1997). Vascular endothelial growth factor expression in head and neck squamous cell carcinoma. Am. J. Surg. 174:513-517.

Foekens JA, Peters HA, Grebenchtchikov N, Look MP, Meijer-van Gelder ME, Geurts-Moespot A, van der Kwast TH, Sweep CG, Klijn JG (2001). High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer. Cancer 61:5407-5414.

Gasparini G, Toi M, Gion M, Verderio P, Dittadi R, Hanatani M, Matsubara I, Vinante O, Bonoldi E, Boracchi P, Gatti C, Suzuki H, Tominaga T (1997). Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. J. Natl. Cancer Inst. 89:139-147.

Graeven U, Andre N, Achilles E, Zornig C, Schmiegel W (1999). Serum levels of vascular endothelial growth factor and basic fibroblast growth factor in patients with soft-tissue sarcoma. J. Cancer Res. Clin. Oncol. 125:577-581.

Granato AM, Nanni O, Falcini F, Folli S, Mosconi G, De Paola F, Medri L, Amadori D, Volpi A (2004). Basic fibroblast growth factor and vascular endothelial growth factor serum levels in breast cancer patients and healthy women: useful as diagnostic tools? Breast Cancer Res. 6:R38-45.

Hazelton D, Nicosia RF, Nicosia SV (1999). Vascular endothelial growth factor levels in ovarian cyst fluid correlate with malignancy. Clin. Cancer Res. 5:823-829.

Heer K, Kumar H, Read JR, Fox JN, Monson JR, Kerin MJ (2001). Serum vascular endothelial growth factor in breast cancer: its relation with cancer type and estrogen receptor status. Clin. Cancer Res. 7:3491-3494.

Hussain A, Khalid SH, Qadir MI, Massud A, Ali M, Khan IU, Saleem M, Iqbal MS, Asghar S, Gul H (2011). Water Uptake and Drug Release Behaviour of Methyl Methacrylate-co-itaconic acid [P(MMA/IA)] Hydrogels Cross-linked with Methylene Bis-acrylamide. J. Drug Deliv. Sci. Tech. 21:249-255.

Hyodo I, Doi T, Endo H, Hosokawa Y, Nishikawa Y, Tanimizu M, Jinno K, Kotani Y (1998). Clinical significance of plasma vascular endothelial growth factor in gastrointestinal cancer. Eur. J. Cancer 34:2041-2045.

Janbaz KH, Jan A, Qadir MI, Gilani AH (2013a). Spasmolytic, bronchodilator and vasorelaxant activity of methanolic extract of *Tephrosia purpurea*. Acta. Pol. Pharm. 79:261-269.

Janbaz KH, Nizsar U, Ashraf M and Qadir MI (2012). Spasmolytic, bronchodilator and antioxidant activities of *Erythrina superosa* Roxb. Acta. Pol. Pharm. 69:1111-1117.

Janbaz KH, Qadir MI, Ahmad B, Sarwar A, Yaqoob N, Masood MI (2012). Tuberculosis – Burning Issues: Multi-Drug Resistance and HIV-Coinfection. Critical Rev. Micro. 38:267–275.

Janbaz KH, Qadir MI, Jan A, Gilani AH (2013b). Anti-diarrheal activity of

methanolic extract of *Tephrosia purpurea*. Acta. Pol. Pharm. 79:345-347.
Javed F, Qadir MI, Janbaz KH, Ali M (2011). Novel drugs from marine microorganisms. Crit. Rev. Micro. 37:245-249.
Karayiannakis AJ, Syrigos KN, Zbar A, Baibas N, Polychronidis A, Simopoulos C, Karatzas G (2002). Clinical significance of preoperative serum vascular endothelial growth factor levels in patients with colorectal cancer and the effect of tumor surgery. Surgery 131:548-555.
Khalid SH, Qadir MI, Massud A, Ali M, Rasool MH (2009). Effect of degree of cross-linking on swelling and drug release behaviour of poly (methyl methacrylate-co-itaconic acid) [P(MMA/IA)] hydrogels for site specific drug delivery. J. Drug Deliv. Sci. Tech. 19:413-418.
Kim BC, Han SI, Lim SC (2009). Interpretation of Pin-1 and VEGF-C expression in breast infiltrating duct carcinoma. Oncol. Rep. 22:1381-1390.
Kraft A, Weindel K, Ochs A, Marth C, Zmija J, Schumacher P, Unger C, Marmé D, Gastl G (1999). Vascular endothelial growth factor in the sera and effusions of patients with malignant and nonmalignant disease. Cancer 85:178-187
Linderholm BK, Hellborg H, Johansson U, Elmberger G, Skoog L, Lehtiö J, Lewensohn R (2009). Significantly higher levels of vascular endothelial growth factor (VEGF) and shorter survival times for patients with primary operable triple-negative breast cancer. Ann. Oncol. 20:1639-1646
Lissoni P, Malugani F, Bonfanti A, Bucovec R, Secondino S, Brivio F, Ferrari-Bravo A, Ferrante R, Vigoré L, Rovelli F, Mandalà M,Viviani S, Fumagalli L, Gardani GS (2001). Abnormally enhanced blood concentrations of vascular endothelial growth factor (VEGF) in metastatic cancer patients and their relation to circulating dendritic cells, IL-12 and endothelin-1. J. Biol. Regul. Homeost. Agents 15:140-144.
Lowery AJ, Sweeney KJ, Molloy AP, Hennessy E, Curran C, Kerin MJ (2008). The effect of menopause and hysterectomy on systemic vascular endothelial growth factor in women undergoing surgery for breast cancer. BMC Cancer 8:279.
Manders P, Beex LV, Tjan-Heijnen VC, Span PN, Sweep CG (2003). Vascular endothelial growth factor is associated with the efficacy of endocrine therapy in patients with advanced breast carcinoma. Cancer 98:2125-2132.
Masood MI, Qadir MI, Shirazi JH, Khan IU (2011). Beneficial effects of lactic acid bacteria on human beings. Crit. Rev. Micro. 37:91-98.
Naz S, Qadir MI, Ali M, Janbaz KH (2012). Nanotechnology for imaging and drug delivery in cancer. J. Chem. Soc. Pak. 34:107-111.
Nisar L, Qadir MI, Malik SA, Tabassum N (2011). Characterization of the immunodominant regions within gp41 of env gene of HIV in Pakistan. J. Chem. Soc. Pak. 33:545-548.
Oehler MK, Caffier H (2000). Prognostic relevance of serum vascular endothelial growth factor in ovarian cancer. Anticancer Res. 20(6D):5109-5112.
Qadir MI (2009). Medicinal and cosmetological importance of *Aloe vera*. Int. J. Nat. Ther. 2:21-26.
Qadir MI (2010). Medicinal values of ginger. Int. J. Nat. Ther. 3:19-22.
Qadir MI (2011). Qadirvirtide. Pak. J. Pharm. Sci. 24:593-595.
Qadir MI, Malik SA (2009). Effect of *Eugenia jambolana* leaves extracts on blood glucose levels of experimental diabetic rabbits. Pharmacologyonline 3:829-835.
Qadir MI, Malik SA (2010). Anti-diabetic activity of inorganic metals *Eugenia jambolana* Lam. (Myrtaceae) flowers. Pharmacologyonline 2:979-985.
Qadir MI, Malik SA (2011). Genetic variation in the HR region of the env Gene of HIV: A perspective for resistance to HIV fusion inhibitors. AIDS Res. Hum. Retrovir. 27:57-63.
Quaranta M, Daniele A, Coviello M, Venneri MT, Abbate I, Caringella ME, Di Tardo S, Divella R, Trerotoli P, Di Gennaro M, Schittulli F, Fransvea E, Giannelli G (2007). MMP-2, MMP-9, VEGF and CA 15.3 in breast cancer. Anticancer Res. 27(5B):3593-600.
Riedel F, Götte K, Schwalb J, Wirtz H, Bergler W, Hörmann K (2000). Serum levels of vascular endothelial growth factor in patients with head and neck cancer. Eur. Arch. Otorhinolaryngol. 257:332-336.
Saleem M, Qadir MI, Perveen N, Ahmad B, Saleem U, Irshad T, Ahmad B (2013). Inhibitors of apoptotic proteins: New targets for anti-cancer therapy. Chem. Biol. Drug Desi. 82:243-251.
Salgado R, Vermeulen PB, Benoy I, Weytjens R, Huget P, Van Marck E, Dirix LY (1999). Platelet number and interleukin-6 correlate with VEGF but not with bFGF serum levels of advanced cancer patients. Br. J. Cancer 80:892-897.
Salven P, Mänpää H, Orpana A, Alitalo K, Joensuu H (1997). Serum vascular endothelial growth factor is often elevated in disseminated cancer. Clin. Cancer Res. 3(5):647-651
Salven P, Orpana A, Joensuu H (1999a). Leukocytes and platelets of patients with cancer contain high levels of vascular endothelial growth factor. Clin. Cancer Res. 5:487-491.
Salven P, Perhoniemi V, Tykkä H, Mäenpää H, Joensuu H (1999b). Serum VEGF levels in women with a benign breast tumor or breast cancer. Breast Cancer Res. Treat. 53:161-166.
Sato K, Tsuchiya N, Sasaki R, Shimoda N, Satoh S, Ogawa O, Kato T (1999). Increased serum levels of vascular endothelial growth factor in patients with renal cell carcinoma. Jpn. J. Cancer Res. 90:874-879.
Stockhammer G, Obwegeser A, Kostron H, Schumacher P, Muigg A, Felber S, Maier H, Slavc I, Gunsilius E, Gastl G (2000). Vascular endothelial growth factor (VEGF) is elevated in brain tumor cysts and correlates with tumor progression. Acta Neuropathol. 100:101-105.
Takano S, Yoshii Y, Kondo S, Suzuki H, Maruno T, Shirai S, Nose T (1996). Concentration of vascular endothelial growth factor in the serum and tumor tissue of brain tumor patients. Cancer Res. 56:2185-2190.
Tuttle RM, Fleisher M, Francis GL, Robbins RJ (2002). Serum vascular endothelial growth factor levels are elevated in metastatic differentiated thyroid cancer but not increased by short-term TSH stimulation. J. Clin. Endocrinol. Metab. 87:1737-1742.
Verheul HM, Hoekman K, Luykx-de Bakker S, Eekman CA, Folman CC, Broxterman HJ, Pinedo HM (1997). Platelet: transporter of vascular endothelial growth factor. Clin. Cancer Res. 3:2187-2190.
Weindel K, Moringlane JR, Marmé D, Weich HA (1994). Detection and quantification of vascular endothelial growth factor/vascular permeability factor in brain tumor tissue and cyst fluid: the key to angiogenesis? Neurosurgery 35:439-448.

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Full Length Research Paper

Quality assessment of different brands of Diclofenac tablets in some pharmacy stores in Abuja

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Diclofenac is one of the most commonly used non-steroidal anti-inflammatory drugs for the treatment of pain, rheumatism and other inflammatory conditions. The drug has analgesic, anti-pyritic and anti-inflammatory effects. Rapid and sensitive-reversed phase high performance liquid chromatography (HPLC) method was used to analyze the amount of Diclofenac in the samples. The calibration curve was linear with correlation coefficient (r^2) of 0.9999 at concentration range of 10 to 80 µg/ml and coefficient of variance (CV %) of less than 5%. Percentage content of Diclofenac from the different pharmaceutical preparations was within 97.5 to 115.5%, but 42.86% failed with over range, while 57.14% passed the British Pharmacopoeia (BP) specification range of 95 to 105.0% of the prescribed content. The drug release profiles were evaluated *in vitro* using a dissolution test apparatus. The USP paddle method was used to perform the dissolution profiles of Diclofenac Sodium. From the result, there is still need for the policy markers in the country to checkmate the imports of different brands of pharmaceutical products into the Nigerian market, since almost 50% of the drug analyzed is above the stated amount claimed by the manufacturers.

Key words: Diclofenac, reversed phase- high performance liquid chromatography (HPLC), ultraviolet (UV)-spectrophotometer, percentage content, Nigerian market.

INRODUCTION

Diclofenac sodium is 2-[{2, 6-dichlorophenyl} amino] benzene acetic acid or 2-(2, 6 -dichloroanilino) phenyl acetic acid (Figure 1), is a non-steroidal anti-inflammatory drug (NSAID) used for the treatment of different diseases such as rheumatoid arthritis, ankylosis spondylitis, osteoarthritis and sport injuries. Non steroidal anti-

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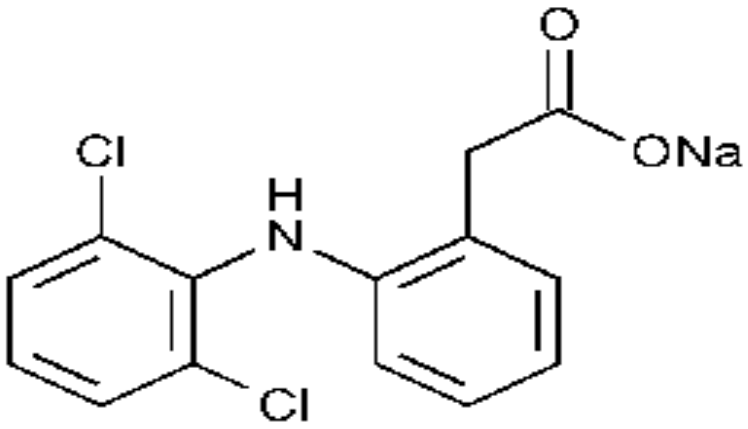

Figure 1. Structure of diclofenac.

inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs worldwide and are used for relief of inflammatory, chronic (e.g., rheumatoid arthritis, osteoarthritis, and gout), and acute (e.g., headache, postoperative pain, and orthopedic fractures) pain conditions (McCarberg and Gibofsky, 2012). The growing demand for NSAIDs stimulates higher level of quality control of these therapeutic substances and preparations.

Diclofenac is mostly available in tablets and injection as potassium or sodium salt with the later being common in Nigeria and are readily available as over-the counter pharmaceutical preparations.

However, owing to the importance of Diclofenac salts in pharmaceuticals and its widespread use, efforts have been made towards the development of simple and reliable analytical methods. Spectrophotometric methods have also been described for the determination of Diclofenac in pharmaceuticals (Sena et al., 2004; Khaskheli et al., 2009), showing reasonable sensitivity with significant economic advantages over other methods including HPLC (Hanysova et al., 2005; Choudhary et al., 2009), liquid chromatography (Senthie et al., 2006), capillary electrophoresis, LC-APCI-MS, differential scanning calorimetric and nuclear magnetic resonance, that are time consuming or require expensive and sophisticated instruments, and for this reason they are not suitable for routine analysis. HPLC method has been highly used for quality control of drug due to its sensitivity and high precision. UV method is very simple, rapid, and economical allows the determination of pharmaceuticals with enough reliability.

Conventional tablets and hard gelatin capsule dosage forms possess high disintegration time so patients obtain pharmacological effect after 30 to 45 min of dosage form administration that may result in high incidence of non-compliance and variable bioavailability (Seager, 1998). This can be achieved by addition of various super disintegrants like croscarmellose sodium, crospovidone, and sodium starch glycolate, alone or in various combinations. Due to the fast disintegration of dosage form, patients obtain quick pharmacological effect of active pharmaceutical ingredient (Chang et al., 2000; Dobetti, 2000; Kuchekar and Arumugam, 2001).

Diclofenac is well absorbed orally, 99% protein binding, metabolized and excreted both in urine and bile. The plasma half-life is 1 to 2 h. However, it has good tissue penetrability and concentration in the synovial fluid is maintained about three times more than in the plasma, thereby extending the therapeutic effects within the joints (Clarke, 1986).

Some patients experienced no relief from musculo-skeletal disorder and other pains after using various brands of Diclofenac sodium tablets. These prompted us to investigate the quality of the Diclofenac sodium tablet marketed in Abuja pharmacy stores, using high performance liquid chromatography and ultra-violet spectrophotometer.

MATERIALS AND METHODS

All reagents and solvents used in this study were of analaR and HPLC grade. The Diclofenac reference standard was also from Sigma. An Agilent 1100 series High Performance Liquid Chromatography System, with a C18 (25 cm length × 4.5 mm diameter and 5 µm particle size) column was used for the determination of Diclofenac content in the tablets.

Sample collection

The literature search and market survey indicate that in the Nigerian markets, there are nine brands of Diclofenac tablets. Seven brands were gotten of which five were normal and two were slow release. The different brands of the Diclofenac tablets were randomly purchased from reputable pharmacy stores within Abuja and subjected to the following assay procedures.

Weight variation

Twenty tablets were selected at random and average weight was

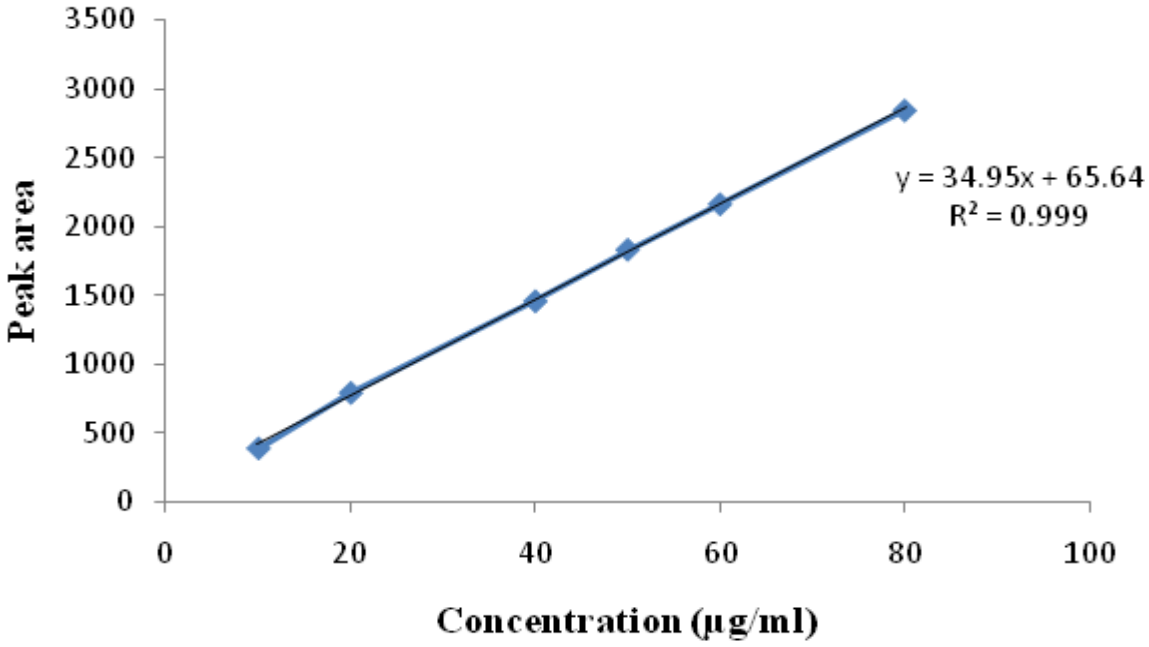

Figure 2. Calibration curve for Diclofenac.

determined. Then individual tablet was weighed and the weight was compared with the average weight (Chang et al., 2000).

Mobile phase

The column was equilibrated with a mobile phase composition of acetonitrile/water (60/40%) v/v filtered through 0.45 µm membrane, sonicated for 30 min and run at a flow rate 1 ml/min with run time of 5 min and UV detection at 278 nm.

Samples

Seven different commercial brands of Diclofenac sodium/Potassium tablets were purchased (5 fast released and 2 sustained released) from registered pharmacy shops in Abuja. The tablets samples contained 50 mg Diclofenac per tablet and 100 mg slow release tablets. Before purchase all tablets were checked for manufacturing license number, batch number and date of manufacture and expiring dates. These tablets were randomly coded (A, B, C, D, E, F, G).

Calibration curve

Standard solution of 1 mg/ml of the reference standard was prepared in a 50% methanol, from which different concentrations from 10 to 80 µg/ml were prepared in 50% methanol and 20 µl introduced into the column in triplicates.

Assay of pharmaceutical preparations

Twenty tablets of each brand were weighed individually and the average weighed calculated after which was finely powdered using glass mortar and pestle. An equivalent weight containing 50 mg of the powdered Diclofenac were accurately weighed and dissolved in a volume of 50% methanol. The solutions each were ultra sonicated for 15 min and then made up to mark in 50 ml volumetric flask with mobile phase. The solution was centrifuged for 10 min at 4500 rpm, the resulting supernatants were then filtered with 0.45 µm membrane filter paper and 20 µl of filtrate aliquot was introduced unto the column at 1 ml/min flow rate.

***In vitro* drug dissolution studies**

Dissolution test was performed on the tablets using an Erweka dissolution tester apparatus. The medium used was 900 ml 0.1 N HCl, and phosphate buffer (USP, 2003) thermostatically maintained at 37 ± 0.5°C at a paddle rotational speed of 50 rpm. 5 ml aliquot of dissolution medium was withdrawn at 5 min intervals using a syringe and needle and this was replaced with fresh 5 ml of the phosphate buffer medium after each withdrawal. The withdrawn samples were filtered and analyzed for Diclofenac using a Shimadzu UV spectrophotometer (Shimadzu Japan) at a pre-determined wavelength of 276 nm.

RESULTS AND DISCUSSION

The HPLC method was simple, selective and reproducible. The calibration curve reflects the linearity in the concentration range of 10 to 80µg/ml with a correlation coefficient (r) of 0.999 (Figure 2) and a coefficient of variance (CV %) for both inter-day and intraday assay were less than 5%. The quantification of Diclofenac was based on the calibrated curve constructed. The method of linear regression was used for the calculation and the linear regression equation was:

$y = 34.95x + 65.64$.

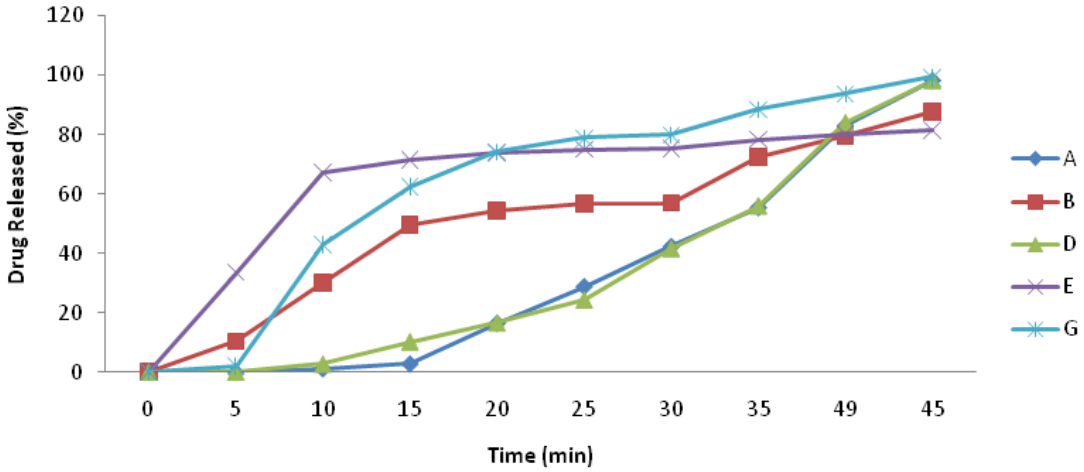

Figure 3. Percentage cumulative drug release against time.

Table 1. Percentage content and weight variation for the different tablets using HPLC method.

Sample code	Weight variation (g) ± SD	Nominal concentration (µg/ml)	Calculated Concentration (µg/ml)	Percentage content (%)	Remarks (BP, 2004)
A	0.2278±0.007	20	20.704	105.17	Passed
B	0.1852±0.003	20	22.394	111.98	Failed
C	0.2184±0.002	20	22.680	113.40	Failed
D	0.3195±0.006	20	23.101	115.50	Failed
E	0.2545±0.004	20	20.330	101.65	Passed
F	0.3083±0.004	20	19.500	97.51	Passed
G	0.2123±0.005	20	19.520	97.60	Passed

Result for percentage content shows that samples B, C and D failed out of the seven different brands with over range percentage drug content (Table 1). Percentage content of Diclofenac from the different pharmaceutical preparations were within 98 to 115.5%, where 42.86% failed with higher percentage content, while 57.14% passed the BP stipulated range (95 to 105.0% of the prescribed content) (BP, 2004a).

Dissolution test

Dissolution test is an important parameter for assessing drug release from pharmaceutical dosage forms. It is used as an indirect method of measuring drug availability (Mbah et al., 2012). A fast-dissolving tablet gets dispersed quickly and releases the drug easily. Figure 3 shows the cumulative percentage of Diclofenac released from the different samples. Samples E and G, show fast dissolution by releasing more than 50% of the drug content in 15 min, while samples A and E follow the same dissolution pattern. The samples were found to follow the following dissolution order E>G>B>D>A. The study shows the different dissolution profile of these different brands of Diclofenac sodium tablet retailed in pharmacy stores in Abuja.

The dissolution profile (Table 2) shows that formulations A, B, D, E and G released at least 80% of Diclofenac in 45 min, this conforms with the BP requirement that at 45 min, not less than 70% of the prescribed or slated amount of active ingredient should have been released at completion of test (BP, 2004b). The dissolution profile of brands C and F which are sustained release was not assessed.

Conclusion

All the brands of the samples passed the *in vitro* drug release analyses, but there was a 45% failure in drug content. This study confirms the need for constant surveillance on marketed drugs products within the country to ensure that commercially available drugs in markets confirmed with the Pharmacopeia standards, so as to meet up with national health delivery policy in Nigeria.

Table 2. Illustrate the cumulative percentage of drug release.

Time (min)	Cumulative drug release (%)				
	A	B	D	E	G
0	0	0	0	0	0
5	0	10.4	0	33.6	2.14
10	1.1	30.0	2.7	67.3	43.93
15	2.7	49.6	10.2	71.3	62.50
20	16.4	54.5	16.6	73.8	74.29
25	28.8	56.7	24.3	75.0	78.93
30	42.3	57.0	41.6	75.2	80.0
35	55.4	72.5	56.0	78.3	88.50
40	83.0	79.5	84.0	79.9	93.80
45	98.2	87.8	98.2	81.5	99.40

Conflict of interest

Authors declare that they have no conflicts of interest

REFERENCES

British Pharmacopeia (2004a). Diclofenac preparation. London: The Stationary office Vol. III. pp. 2354-2355.

British Pharmacopeia (2004b). Monograph A-Z. The Pharmaceutical Press, Her Majesty's Stationary Office, London. pp. 1-4:361,1306,2141.

Chang RK, Guo X, Burnside BA, Couch RA (2000). Fast dissolution tablets. Pharm. Tech. 24(6):150.

Choudhary B, Goyah A, Khokra SL, Kaushik D (2009). Simultaneous estimation of diclofenac sodium and rabprazole by high performance liquid chromatographic method in combined dosage forms. Int. J. Pharm. Sci. Drug Res. 1(1):43-45.

Clarke's Isolation and Identification of Drugs (1986). Diclofenac, 2nd edition. The Pharmaceutical Press. London. pp. 533-534.

Dobetti L (2001). Fast melting tablets: developments & technologies. Pharm. Tech. 56:44.

Hanysova L, Mokry M, Kastner P, Klimes J (2005). HPLC Evaluation of diclofenac in various forms of therapeutic preparations. Chem. Pap. 59(2):103-108.

Khaskheli AR, Abro SK, Serazi STH, Afridi HI, Mahesar SA, Saeed M (2009). Simpler and faster spectrophotometric determination of diclofenac sodium in tablets, serum and urine samples. Pak. J. Anal. Environ. Chem. 10(1&2):53-58.

Kuchekar BS, Arumugam V (2001). Fast dissolving tablets, Indian J. Pharm. Edu. 35:150.

Mbah CC, Emosariu CO, Builders PF, Isimi CY, Kunle OO (2012). Effect of process parameters on the properties of some metronidazole tablets and capsule formulations. Afr. J. Pharm. Pharmacol. 6(24):1719-1725.

McCarberg B, Gibofsky A (2012). "Need to develop new nonsteroidal anti-inflammatory drug formulations." Clin. Ther. 34(9):1954-1963.

Seager H (1998). Drug delivery product and the zydis fast dissolving dosage form. J. Pharm. Pharmacol. 50:375-382.

Sena MM, Choudhary ZF, Collins CH, Poppi RJ (2004). Direct determination of diclofenac in pharmaceutical formulations containing B vitamin by VU spectrophotometry and partial least square regression. J. Pharm. Biomed. Anal. 36:734-749.

Senthie M, Subarmanian G, Vaudevan M, Ravisankar S (2006). HPTLC estimation of tizanidine and diclofenac sodium in combined dosage forms. Indian Drugs 43:388-392.

United States Pharmacopeia (2003). Asian Edition. Vol. 26. pp. 595-596.

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Projections of pharmacokinetic parameter estimates from mid-dose plasma concentrations in individuals on efavirenz: A novel approach

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This work seeks to project individual pharmacokinetic (PK) parameter estimates of efavirenz (a drug with a long half life) from mid-dose concentrations and covariates, assuming full mass transfer of the drug. Gender, weight and CYP2B6, 516G>T genetic data of 61 patients on efavirenz containing highly active antiretroviral therapy (HAART) was collated and analysed. Models were derived to guide dose adjustment in patients predicted to have unsafe drug exposure, and new modelling methods and interpretations are suggested to estimate PK parameters. A new measure related to the uptake of the drug is incorporated in modelling of transportation (cumulative uptake volume). The cumulative uptake-volume associated with the full absorption of 600 mg of efavirenz was estimated to be 35.56 L whereas the volume of distribution was found to be 39.68 L. A sufficient relationship was established between estimated absolute oral bioavailability *(f)* and mid-dose concentration *(x)* at steady state $\{f = \frac{x^{1.121}}{x^{1.121}+3.135^{1.121}}, R^2 = 0.98\}$**. Patients who carry the CYP2B6 G516T TT genotype are projected to have high efavirenz exposure. The estimated bioavailability in this population ranges from (0.29; 0.86). Genotype, weight and gender based inference for dose adjustment proposition is evident for the drug efavirenz. The drug is projected to have been fully absorbed in 31 h in this population.**

Key words: Efavirenz, cumulative uptake-volume, bioavailability, volume of distribution, area under the curve (AUC), absorption rate.

INTRODUCTION

In addition to the enzyme CYP2B6, more studies have shown the potential role of other enzymes such as CYP2A6 and UGT 2B7 and drug transporters such as ABCB1 in efavirenz exposure levels (Habtewold et al., 2011;Mukonzo et al., 2009; Kwara et al., 2009; Ritchie et al., 2006). The study by Nyakutira et al. (2008) and those of others (Burger et al., 2006; Pedral-Sampaio et al., 2004; Mukonzo et al., 2009) observed a potential role of gender in efavirenz exposure levels. A study in Thailand observed that patient weight could also have an impact on exposure levels of efavirenz (Manosuthi et al., 2009). The pharmacokinetic (PK) and pharmacodynamic (PD)

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parameters of drugs used to treat human immuno-deficiency virus/acquired immune deficiency syndrome (HIV/AIDS) and/or tuberculosis (TB) have been shown to exhibit great inter-individual variability and interethnic variability (Friedland et al., 2006; Burger et al., 2006). Identifying subgroups occupying similar PK/PD clinical response space that requires modification of treatment strategy can greatly optimise the efficacy and safety in the use of current drugs.

Patient variability and changes occurring in the plasma concentrations affect the pharmacokinetics of efavirenz (Nyakutira et al., 2008; Sánchez et al., 2011). The differences in plasma concentrations are due to 'covariate' structure (in this work it is used as a variable that includes weight (W), genotype (CYP2B6 G516T) and gender (S)) and time. Weight, CYP2B6, and gender were considered the more likely covariates to influence efavirenz pharmacokinetics (Nyakutira et al., 2008; Rekić et al., 2011). The challenge then is to find a covariate structure that captures concentration differences. If one has relatively more information on the covariate structure that relates demographic, environmental and genetic factors to the dependent variable in this case concentration at mid-dose that is a strong correlation then a function can be formulated that relates the variables (Nemaura et al., 2012).

The time space has been used as the basis of the rate-defining variable in model formulation. In population pharmacokinetic modelling, covariates have been taken as additives and their inclusion into the structural model has commonly been investigated stepwise through backward/forward elimination regression methods (Ette and Williams, 2007). However, the developed covariate space is noted to be pivotal in the modelling and estimation of PK parameters in this work. It is used more importantly in the projection of bioavailability. The oral absorption of drugs is a complex phenomenon that manifests itself between drug and patient-specific variables that include, disease, genetics, age, sex and ethnicity (Ette and Williams, 2007). The Michaelis-Menten equation has had many biological applications from enzyme kinetics, membrane transport carrier to ligand-receptor binding and many more applications (Aksnes and Egge, 1991; Johnson and Goody, 2011; Portier et al., 1993). The body is assumed to have a finite volume where encountered particles can be handled. The Michaelis-Menten equation is used to model relative uptake. This is in order to mimic potential diffusion expected in the movement of the drug in the body as suggested by others (Yilmaz et al., 2012). There is no human data on absolute oral bioavailability of efavirenz to date (Cristofoletti et al., 2013). An important ratio the "relative uptake" (Total amount of drug reaching systemic circulation)/(cumulative uptake-volume at the point of full absorption) = A/V in modelling transportation is introduced. These results were extended and it was observed that the cumulative uptake volume could be modelled as a time dependent parameter.

A new way of modelling was introduced which enabled development of parameter estimates and this allowed better predictions of what possibly could occur within the body. Instead of using the time variable space only, there was the capturing of effects that are attributable to the covariate space as well. Furthermore, a proposition of an alternate way of interpretation of compartments and parameters is made.

MATERIALS AND METHODS

Patient data was obtained from Nyakutira et al. (2008) and only 61 patients were considered because they had the complete data for the following investigated independent variables, that is gender, weight and CYP2B6 G516T genotype. The patients were on TB treatment with a regimen containing rifampcin and also on stavudine and lamivudine as part of their HAART. Partial least squares regression (PLS) was used to generate a covariate function that was then projected into NONMEM for the estimation of middose concentration and other PK parameters. The following software packages were used to develop models in this work SIMCA, STATA, SPSS, NONMEM, and R. Non linear mixed effects modelling software brings data and models together, implementing an estimation method for finding parameters for the structural, statistical and covariate models that describe the data set (Mould and Upton, 2013). Clustering method(s) are used to identify subgroups that occupy different PK/PD response space. Clustering is a descriptive method that divides groups into a finite set of categories in order to capture the natural structure of data.

RESULTS

Primarily, estimations of mid-dose concentration in the sub-populations and the uptake-volume associated with the complete absorption of efavirenz were done. Sub-population in this work relates to a group of individuals in a population sample with the same characteristics that relates to the response variable and are projected to be in the same neighbourhood in the response space. The assumptions included the following:

time was considered initially constant, mid-dose plasma concentration(s) (x) were considered to be in the neighbourhood *(N)* of the point associated with complete absorption

$$(x_f)\{(x_f(t_a): t_a \geq t_{max})\ singleton, x(t) \in N(x_f) \subseteq (c_{min}, c_{max})\ for\ t \geq t_{max}\}$$

and were reflective of the total fraction absorbed in the systemic circulation, and the existence of a fixed finite carrying system (cumulative uptake-volume-enabling measurement of absorption time period) associated with the full dose of efavirenz. The assumption with respect to time was taken due to the fact that efavirenz has a long

Table 1. Predicted 12 h post plasma efavirenz concentration in relation to dose taken.

Covariate (φ)	Patient Description	n	Median Conc. (c_{md})	Predictions 600 mg		Prediction 400 mg		Prediction 200 mg	
				PLSR	NONMEM	PLSR	NONMEM	PLSR	NONMEM
0.05831	M,GG,W≥62	3	2.53	1.68	0.94	1.12	0.63	0.56	0.31
0.15339	M,GT,W≥62	5	3.27	2.53	2.30	1.69	1.53	0.84	0.77
0.23247	M,GG,W<62	5	3.52	3.24	3.28	2.16	2.19	1.08	1.09
0.23661	F,GG,W≥62	2	3.18	3.28	3.33	2.19	2.22	1.09	1.11
0.32754	M,GT,W<62	5	3.31	4.09	4.29	2.73	2.86	1.36	1.43
0.33169	F,GT,W≥62	9	3.2	4.13	4.33	2.75	2.89	1.38	1.44
0.41076	F,GG,W<62	3	3.64	4.84	5.05	3.22	3.37	1.61	1.68
0.50584	F,GT,W<62	13	4.9	5.69	5.78	3.79	3.85	1.9	1.93
0.58386	M,TT,W≥62	6	7.23	6.39	6.29	4.26	4.19	2.13	2.10
0.75802	M,TT,W<62	1	9.25	7.95	7.15	5.3	4.77	2.65	2.38
0.76216	F,TT,W≥62	4	8.17	7.98	7.17	5.32	4.78	2.66	2.39
0.93631	F,TT,W<62	5	9.14	9.54	7.72	6.36	5.15	3.18	2.57

half life (Almond et al., 2005; Yilmaz et al., 2012). This enabled the modelling of change in concentration post 12 h [in the 4 h-period sampled (12 to 16 h)] as being affected by the covariate structure only that excludes time. A relation that had been established as a link between genotype, gender and weight and the plasma efavirenz concentrations was used (Nemaura et al., 2012). Estimates for cumulative uptake-volume at the point of full absorption and estimated post 12 h concentrations were obtained. A one-compartment model (model 1a) was used:

$$\frac{dS_{PRE}}{d\varphi} = -g_d S_{PRE} \qquad (1.1)$$

$$\frac{dS_{FLOW}}{d\varphi} = g_d S_{PRE} - g_e S_{FLOW} \qquad (1.2)$$

Changes in concentration were considered to be affected by the covariate structure ($^{\varphi}$-a variable that define the potential trend in drug levels almost surely in differing sub-populations). The covariate structure included weight, gender and genetic information of CYP2B6 G516T. Where S_{PRE} represented the pre-uptake compartment for the drug and S_{FLOW} represent uptake compartment where depositing and elimination took place. The parameter g_d was defined as a depositing relative constant which was 1 per covariate (φ) unit. The part eliminated was defined by g_e. The results in Nemaura et al. (2012) were extended in derivation of an algorithm proposed therein (Table 1). The number of clusters (*ń(CYP 2B6 G516T)* ń (S)*ń(W)=3*2*2=12, ń* is the number of categories) formulated was exhaustive. The investigated variables spanned the population. It was sufficient to use the previous boundary of 4 µg/ml to estimate doses because it separated the data in an almost 1:1 ratio, in this case it was 56:44 and furthermore the clusters spanned the population. Otherwise the median plasma concentration would have been used for separation in the development of a monotone function. The extensions of projected plasma concentrations for 200 and 400 mg were linear extrapolations (proportions) projected from the 600 mg dose.

A large variation on cumulative uptake-volume associated with full absorption (Typical Value of 35.6 L, ω_v = 0.8451) was observed. It was postulated that this may have been due to an insufficient wealth of information in the covariate structure that also includes time variable and possibilities of errors arising from accumulation of the drug at steady state.

In this case inclusion of information on possible covariates that affect efavirenz metabolism like CYP2B6 T983C, CYP2A6 and UGT 2B7 (Habtewold et al., 2011; Jiang et al., 2013; Ribaudo et al., 2011) could help and also other demographic information.

NONMEM (model 1(a)) projected one group to be in sub therapeutic concentrations, this group is of males who carry the CYP2B6 G516T GG genotype and of weight above 62 kg for individuals taking 600 mg. A dose of 800 mg was projected to have a typical value of 1.25 µg/ml.

The two methods that is PLS and NONMEM-PLS (Table 1) converged to similar points with NONMEM-SIMCA (PLS) (model 1(a)), giving a slightly improved estimation with regards to relationship between the covariate structure and concentrations predicted at mid-dose that excluded the time variable.

Estimation of bioavailability

A one-compartment model (model 1b) was employed with a transformation for φ to $\hat{\varphi}$ in order to estimate the eliminated fraction (presystemic) for every full dose of efavirenz (that is to find the existence of g_e such that $0 \leq g_e \leq 1$). There exist a $\hat{\varphi}$ for the found φ (equation 1.3) with the following properties:

$$\{\hat{\varphi} \geq 0 | \rho(\hat{\varphi}, c_{md}) = -r, \rho(\varphi, c_{md}) = r\}$$

and

$$\hat{\varphi} = m_{\varphi, c_{md}} \left(0.5 - \frac{1}{m_{\varphi, c_{md}}} (\varphi - 0.5) \right) \qquad (1.3)$$

Where *r* (*r* = 0.95 in the case considered), was the correlation between φ and c_{md}-median sub-population plasma concentration (Table 1) and $m_{\varphi, c_{md}}$ was the gradient of the line φ on c_{md} with $x(\varphi = 0) \in N(x_{min})$, x_{min} was the minimum efavirenz concentration in the population a potential possible asymptote. A fixed value of uptake-volume associated with complete absorption was approximated and a depositing relative constant with a fixed value of 1 to ensure full mass transfer was used. It was important for the run to converge to a global minimum (to ensure convergence in the covariate space). The resultant estimations gave rise to the following estimation for bioavailability ($1 - g_e$) based on cumulative uptake-volume of 35.56 L. A sufficient relationship between the observed plasma concentration *(x)* at steady state (12 to 16 h) and estimated fraction f reaching systemic circulation from the population sample was fitted (equation 1.4). There was no patient below 1 µg/ml in this population sample.

$$f = \frac{x^{1.121}}{x^{1.121} + 3.135^{1.121}}, R^2 = 0.98. \qquad (1.4)$$

The projected function f was easily noted to be an increasing function with respect to plasma concentrations at mid-dose interval, consequently showing that individuals with higher concentrations had correspondingly higher bioavailability. However, it was to be noted that the resultant estimates for bioavailability could have relatively large errors for those with higher concentrations as it was difficult to extrapolate the accumulation factor in concentration at this stage.

Inclusion of time and covariates in models

The time variable space was then included in modelling the mid-dose plasma concentrations at steady state. A one-compartment model was employed, defined by the following equations.

$$\frac{dS_{PRE}}{dt} = -k_d S_{PRE} \qquad (1.5)$$

$$\frac{dS_{FLOW}}{dt} = k_d S_{PRE} - k_e S_{FLOW} \qquad (1.6)$$

Where $k_e = \frac{ER_v}{V} = \frac{ER}{V}$, ER was the elimination rate (measured in $\frac{L}{h}$) at the point of full absorption, k_d depositing rate constant and V was the cumulative uptake volume associated with full absorption. Below is a set of results for the two models one with the estimated bioavailability and the other model which retained the current modeling framework of a central compartment and the input compartment (Table 2).

Model 2a (Figures 1 and 2): was based on the current compartmental modelling framework where there is a central compartment (S_{FLOW} with *A*-amount reaching systemic circulation) which represented plasma concentration and S_{PRE} represents the gut *(Dose-A)* and the other is defined as follows: S_{PRE} represents pre-uptake compartment of the drug in the systemic circulation and S_{FLOW} represents uptake compartment of depositing and elimination (in systemic circulation) (model 2b (Figures 3 and 4)). Model 2a carried the assumption that cumulative uptake-volume at the point of full absorption was equal to the volume of distribution. Considering Model 2b, only *A*-(Amount reaching systemic circulation) was used in the structural models.

Model 2b:

$$x_u(t) = \frac{A}{V(1 - k_e)} (e^{-k_e t} - e^{-t})$$

$x_u(t)$: (a solution of 1.1 and 1.2) approximated $x(t)$ the plasma concentrations in the neighbourhood of the end of absorption phase and post absorption phase. Furthermore, $x_u(t)$ approximated $x(t)$ at terminal points for large t and small $k_e = \frac{ER}{V}$. At the point where absorption ended the following condition hold

Table 2. Parameter estimations for two models with different values of bioavailability.

Parameter/Description	Value/equation	
	Model 2a (with bioavailability of 1)	**Model 2b (estimated bioavailability)**
OFV[#]	205	136
V(L)	35.56 FIX	35.56 FIX
ER_v (L/h)	3.09	1.374
k_a/h (k_d/h)	0.19776	(1 FIX)
ω_{ER}	0.605	0.239
ω_v	0.03	0.03
Observed mid dose concentration *x* vs. population prediction *y*	*y=0.1368x+4.1773, R^2 = 0.1702*	*y=2.6494+0.4056x, R^2 =0.7754*

[#] OFV= Objective Functional Value.

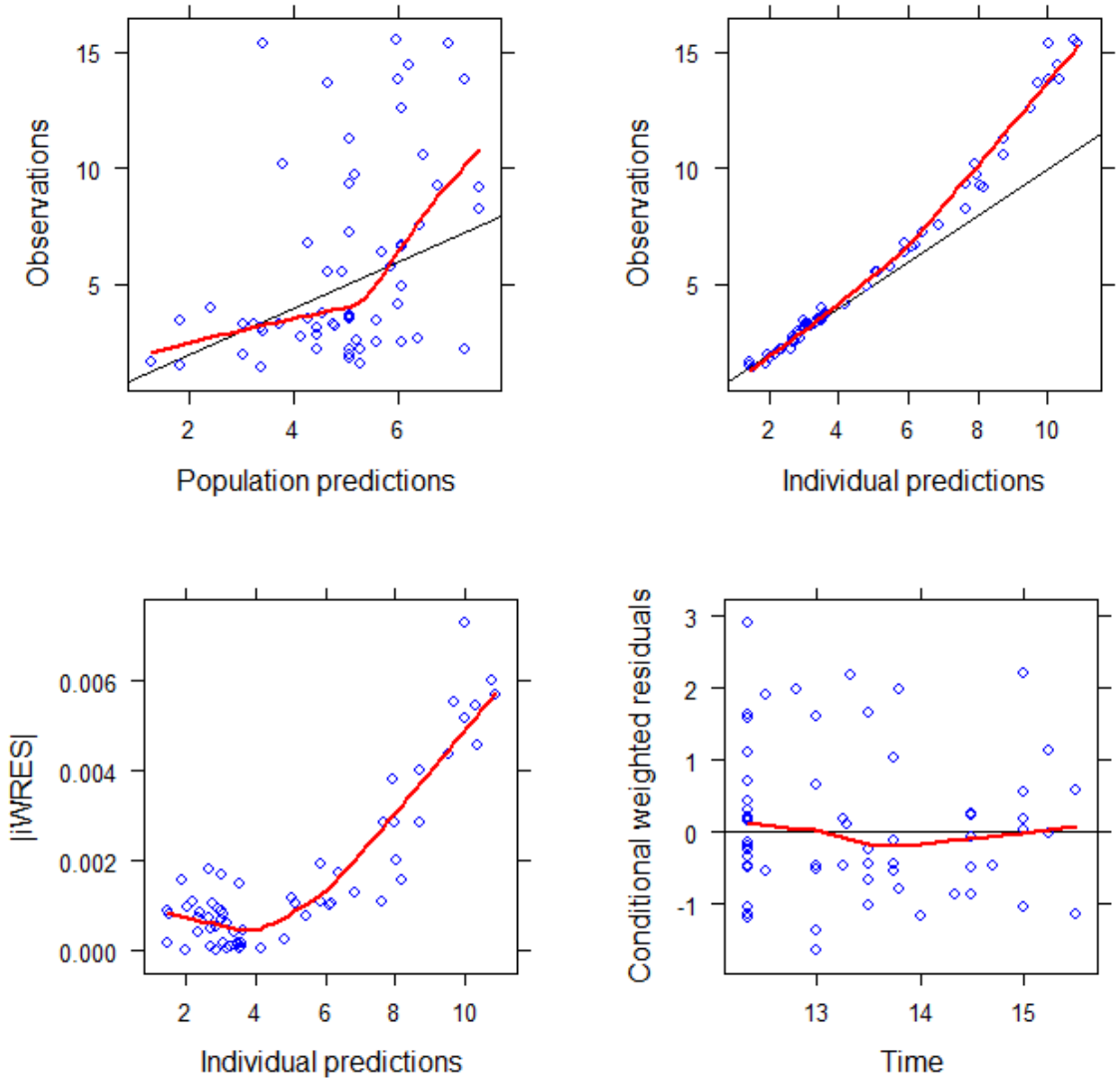

Figure 1. Goodness of fit plots for model 2a with bioavailability as 1.

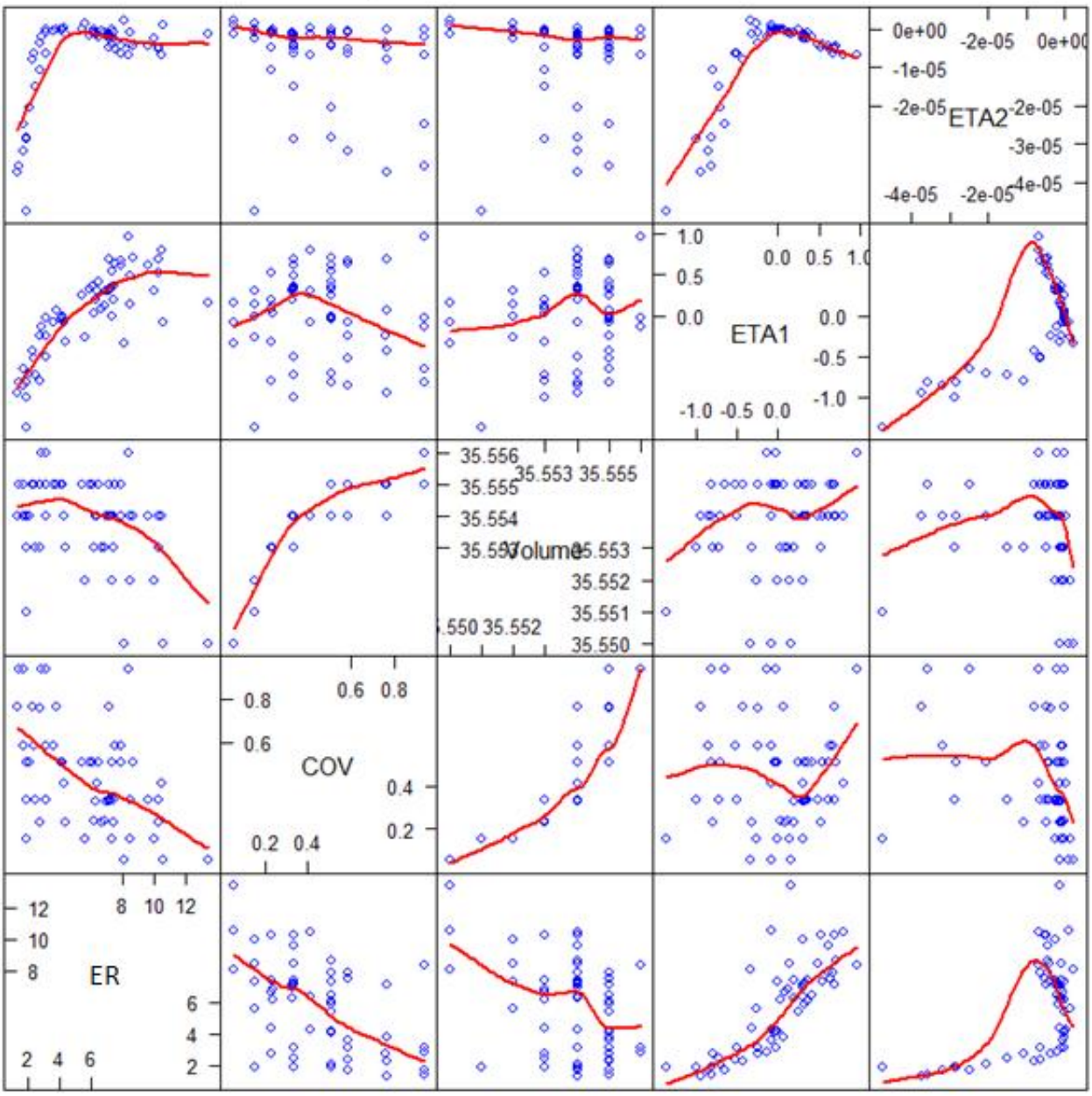

Figure 2. Scatter plot matrix showing how volume of distribution and elimination rate (clearance) and the covariate relation are related to each other model 2a.

$x_u(t_a) = x(t_a) = x_f$. *A* was the total amount that reached systemic circulation and *V* was the cumulative uptake volume associated with the complete absorption of *A* in the systemic circulation. The function $x_u(t)$ was used because of the two properties it exhibits. The definition and construction allowed the taking of the full fraction (total mass transfer of available drug) of the absorbed drug at any time t (A_t-the cumulative amount at time t) and per unit time. The ability to approximate the tail more favourably to the value $A/V * exp\ (-k_e t)$ since absorption was projected to be relatively low or close to zero after some time because of the decreasing amount of the drug in presystemic circulation, moreso for efavirenz with a long half life the terminal points of $x_u(t)$ then gravitates towards $A/V * exp\ (-k_e t)$.

Figures 1 to 4 show goodness of fit plots and scatter

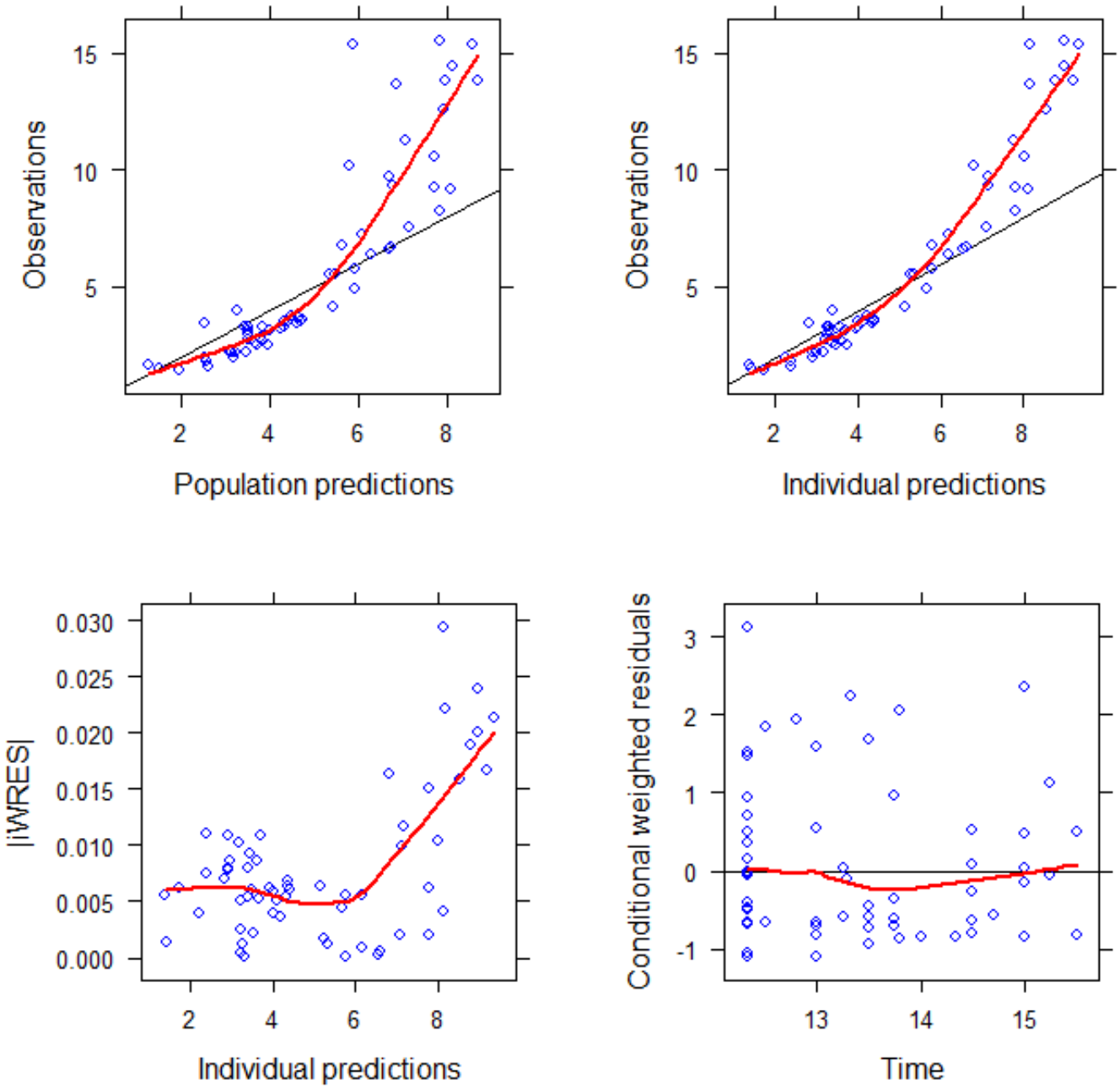

Figure 3. Goodness of fit plots for model 2b with the estimated varying bioavailability.

plots for the models 2a and b. The goodness of fit plots showed larger errors at higher concentrations. However, the models were formulated with the combined error model that takes into account both additional and proportional error. Model 2b was an improved model of 2a (a significant level of $p<0.01$ [$\Delta OFV=69>6.63$, *d f* (degrees of freedom) =*1*)].

The solid red line represents the median observed plasma concentration and the semi-transparent red field represents a simulation-based 95% confidence interval for the median (Figure 5). The observed 5 and 95% percentiles are represented with dashed red lines, and the 95% confidence intervals for the corresponding model predicted percentiles are shown as semi-transparent blue fields. Blue points represent the observed plasma concentrations. From the VPC, the model underestimated patients with high plasma efavirenz concentrations. The underestimation could be as a result of the data set not being corrected for accumulation, a consideration made in the next section

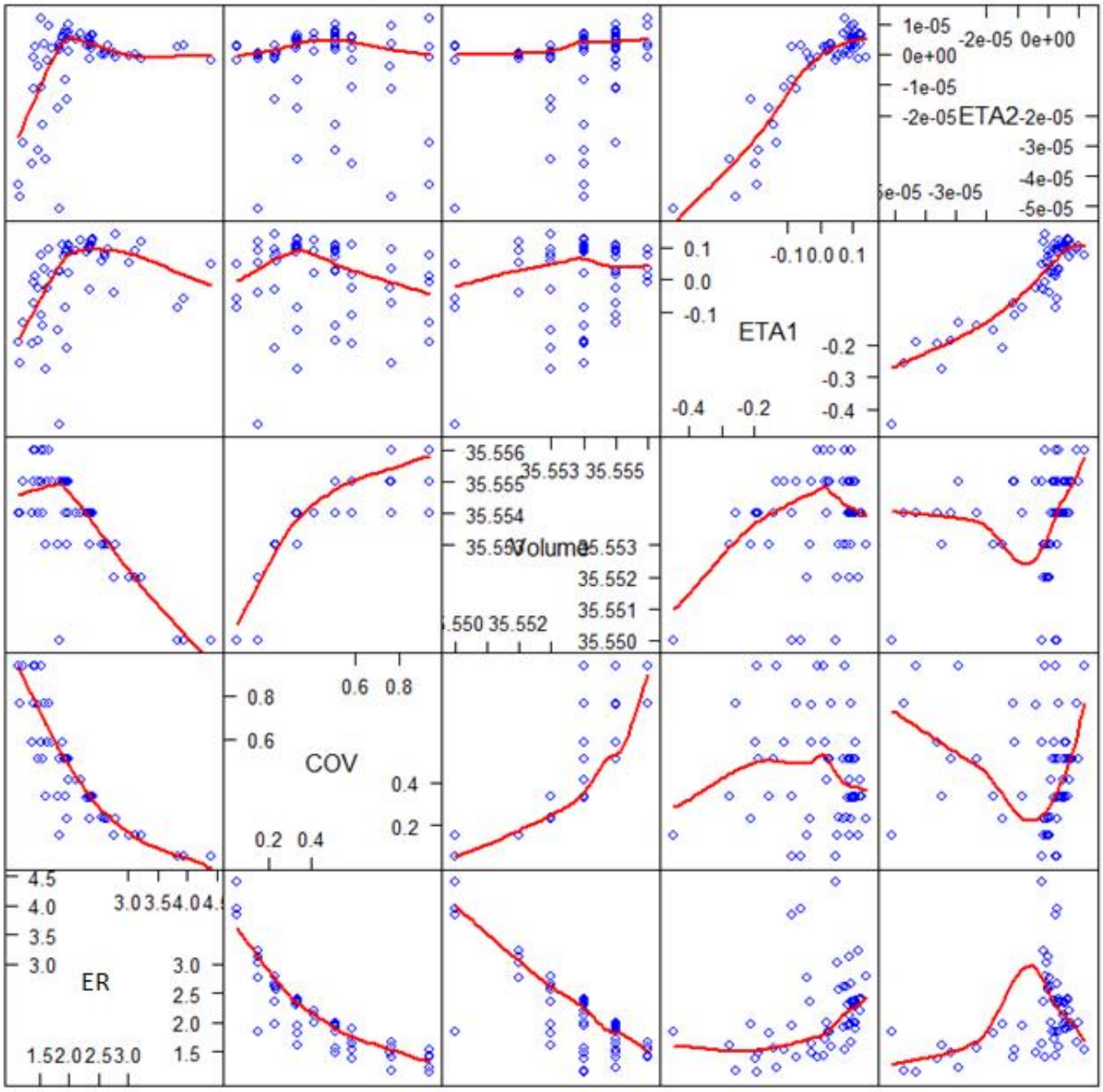

Figure 4. Scatter plot matrix showing how uptake-volume and elimination rate at the point of full absorption and covariate relation are related to each other model 2b.

(Toutain et al., 2004a) among other issues related to the drug efavirenz and its interaction with the body is made. Thus the patients with relatively higher concentrations with low elimination rates are expected to have higher accumulation thereby resulting in large errors. When the dosing interval is long comparative to the time required to eliminate the drug, accumulation is projected to be low. When the dosing interval is short in relation to the time needed to eliminate the drug, accumulation is high (this could be the case for individuals carrying the TT genotype). The projections of bioavailability, elimination rate constant and plasma concentrations are summarised including the elimination half life (Tables 3 and 4, Figures 6 and 7). These results are developed from model 2b.

The estimated amount of drug that reaches the targets (systemic circulation) for poor metabolisers is greater than that of fast metabolisers. A multiple linear regression model was developed for the three variables terminal

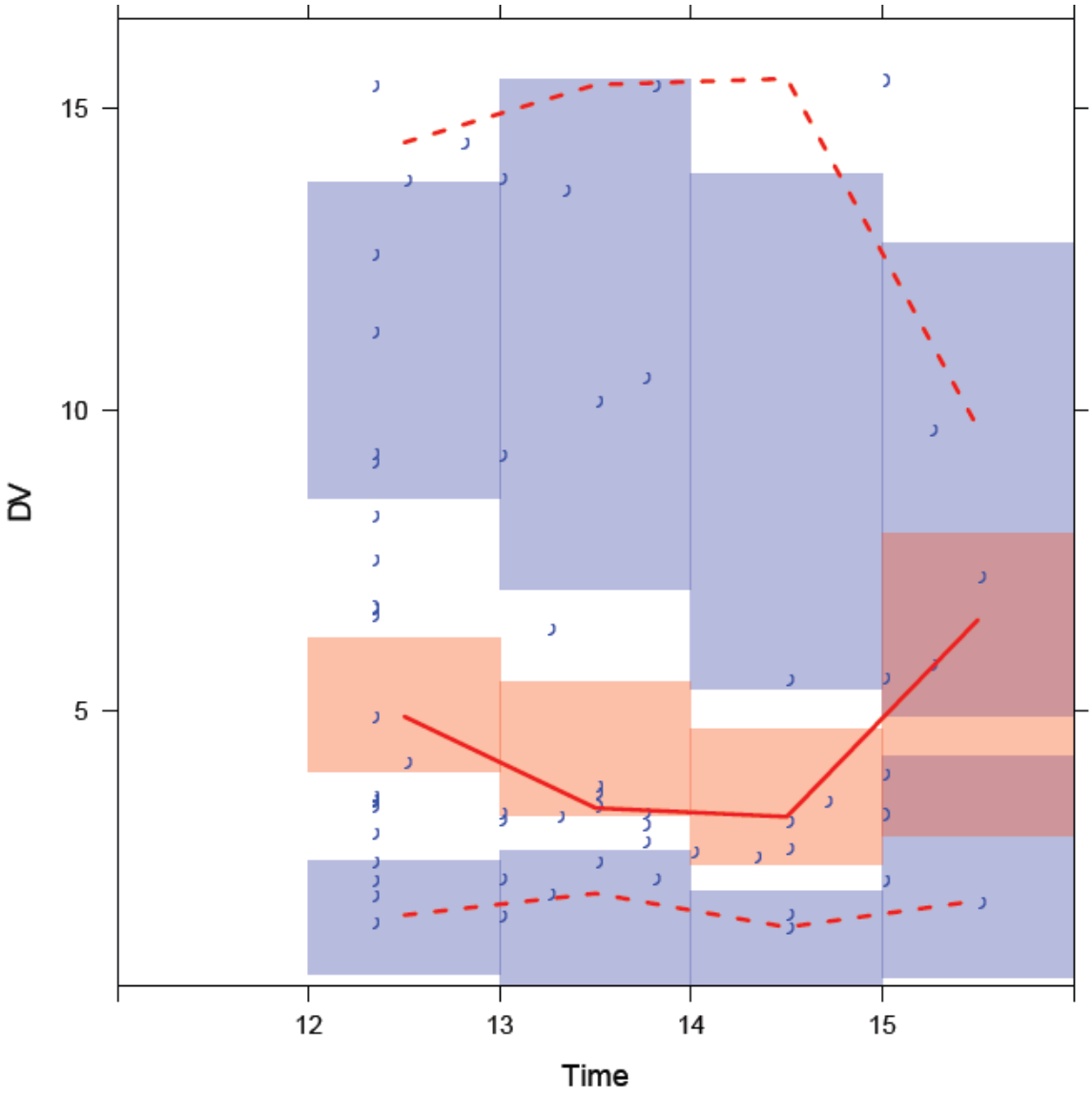

Figure 5. The value predictive check (VPC) for the terminal plasma concentration for model 2(b).

Table 3. Summary statistics showing median and interquartile ranges of plasma concentration at steady state, bioavailability, and elimination rate constant.

Variable	Median (IQR)
Plasma Conc. (µg/ml)	3.6 (2.72,8.25)
f.	0.65 (0.55,0.85)
k_e(/h)	0.056 (0.047,0.067)

plasma concentration *(x),* elimination rate constant *(k_e)* and oral bioavailability *(f); ln x = 3.3995f - 8.0614k_e* (Table 5). The rationale being that the concentrations observed are functions of both bioavailability and elimination related variables.

Factoring in correction of accumulation of the drug at steady state in modeling mid-dose concentrations-predicted results after only a single dose is taken

The results were extended by considering a model which corrected for accumulation brought about by continual taking in of the drug efavirenz at steady state. An accumulation factor of $a_{f,ss} = \frac{1}{(1-e^{-k_e\tau})}$ (where $\tau = 24hrs$ is a dosing interval) was used to correct for the mid-dose plasma concentrations at steady state. The elimination rate constant was estimated from model 2b, which had steady state conditions in order to form model 2bi (Table 6, Figures 8 and 9) which did negate accumulation. The solid red line represents the median

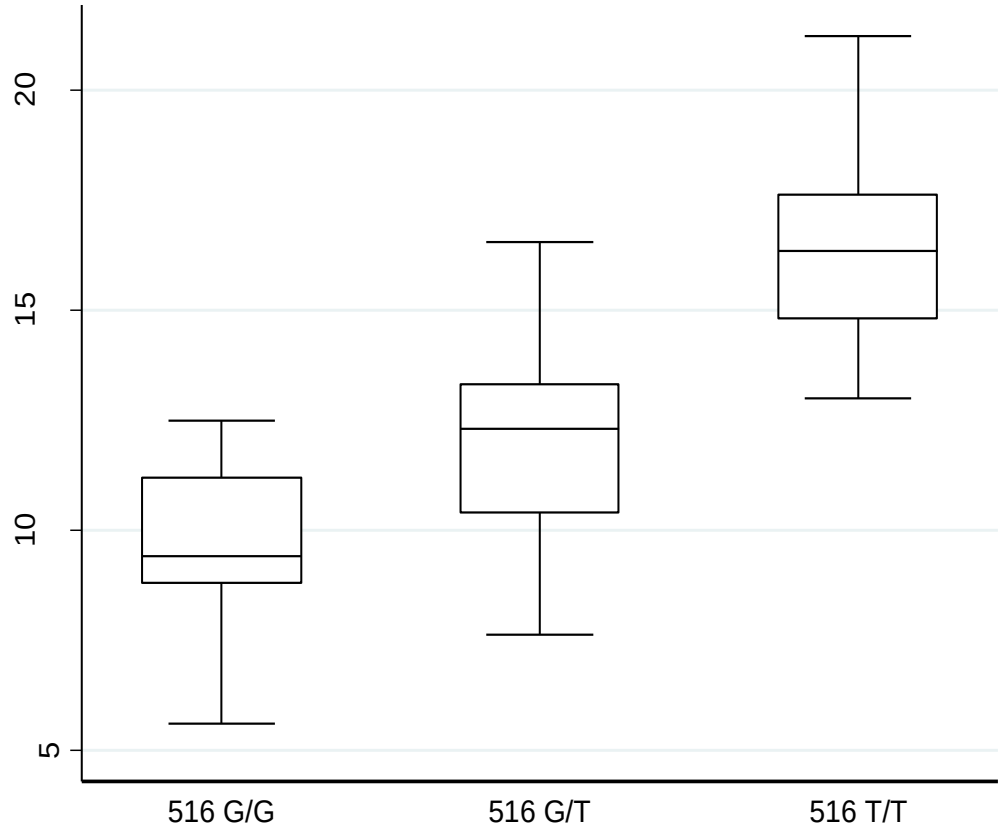

Figure 6. Estimated elimination half-life (h) for GG, GT, and TT genotypes of CYP2B6, the median (IQR; Interquartile range) times are as follows 9.4 (8.8, 11.2), 12.3 (10.4, 13.3) and 16.3 (14.8, 17.6), respectively.

Table 4. Correlations between *f, k_e*, and mid-dose efavirenz plasma concentration.

Variable	Plasma Conc.	f	k_e
Plasma Conc.	1	-	-
f.	0.92	1	
k_e	-0.64	-0.57	1

Table 5. Coefficients of the multiple linear regression model of 61 patients at steady state for plasma concentration (x) at t= (12 to 16 h), elimination rate constant (k_e) and oral bioavailability (f) (ln x=□$_1$f+□$_2k_e$ (Multiple R-square = 0.9975)).

Coefficients	Estimate	Standard Error	p-value
β_1	3.3995	0.0353	p<0.0001
β_2	-8.0614	0.3459	p<0.0001

observed plasma concentration and the semi-transparent red field represents a simulation-based 95% confidence interval for the median (Figure 10). The observed 5% and 95% percentiles are represented with *dashed red lines*, and the 95% confidence intervals for the corresponding model predicted percentiles are shown as semi-transparent blue fields. Blue points represent the observed plasma concentrations (corrected for accumulation). The most notable improvement was noted in the difference in objective functional value (OFV). Model 2bi was found to be an improvement of model 2b, the difference is highly statistically significant with an OFV difference of 75. The median and interquartile range (IQR) of the elimination rate constant of model 2bi was 0.0874(0.0758, 0.0933). The plasma concentration was shown to be positively correlated to bioavailability and negatively correlated with the elimination rate constant (Table 7). A multivariate model was developed for the relationship between *f, k_e,* and mid-dose interval efavirenz plasma concentration at steady state; $lnx = 3.609f - 7.1794k_e, R^2 = 0.9975$, established from model 2bi.

Extension

Transportation in the human body for an orally administered drug, volume of distribution and AUC

The results and extensions used in this section are mainly based on simulations from model 2b, because the concentrations were at steady state. At any given time t,

Table 6. Parameter estimation for model extension of model 2(b) that accounted/corrected for the accumulation at steady state.

Parameter	Value/equation
OFV[#]	61.34
V(L)	35.56 FIX
ER_v (L/h)	2.578
k_d/h	1 FIX
ω_{ER}	0.1748
ω_v	0.003
Observed mid dose concentration (corrected for accumulation) x_{acc} vs. population prediction y	$y=0.455x_{acc}+1.759$, $R^2 = 0.803$

Estimated bioavailability [model 2(bi)]. [#] OFV= Objective Functional Value.

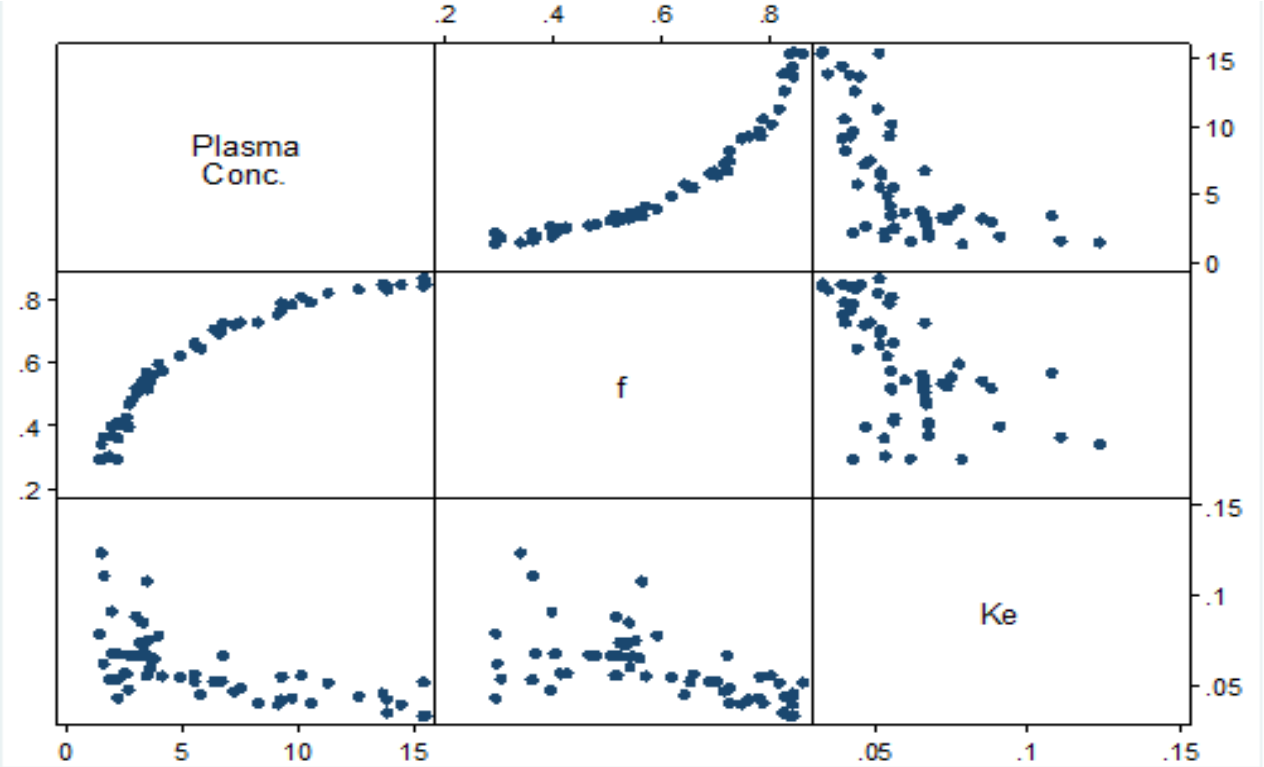

Figure 7. Scatter plot matrix of elimination rate constant k_e, 12 to 16 h post dose plasma concentration and bioavailability *f*.

$\frac{A}{V} = \frac{A_t}{V_t}$ and $\frac{ER}{V} = \frac{ER V_t}{V_t} = k_e$ are assumed to be constants (equation 1.7) (A_t was the accumulating drug in the systemic circulation and V_t was the uptake volume space covered by A_t, ER was the elimination rate at the point of full absorption, A was the total amount reaching the systemic circulation and V was the cumulative uptake volume associated with full absorption of A). The cumulative uptake-volume followed a saturation curve. The cumulative uptake volume moved from $V = 0L$, at $t = 0$ to a $V = V(A) \leq V(t \to \infty)$, where $V(A)$ was the cumulative uptake-volume associated with the full absorption of A. The cumulative uptake-volume V_t was observed to be a time dependent variable and the movement of A across V was facilitated by transportation mechanisms within the body (facilitated diffusion). The relation-ship between the drug uptake relative to volume-$\frac{A}{V}$ (dependent variable) and 'concentration' $x_u(t)$ (independent) was assumed to follow Michaelis-Menten

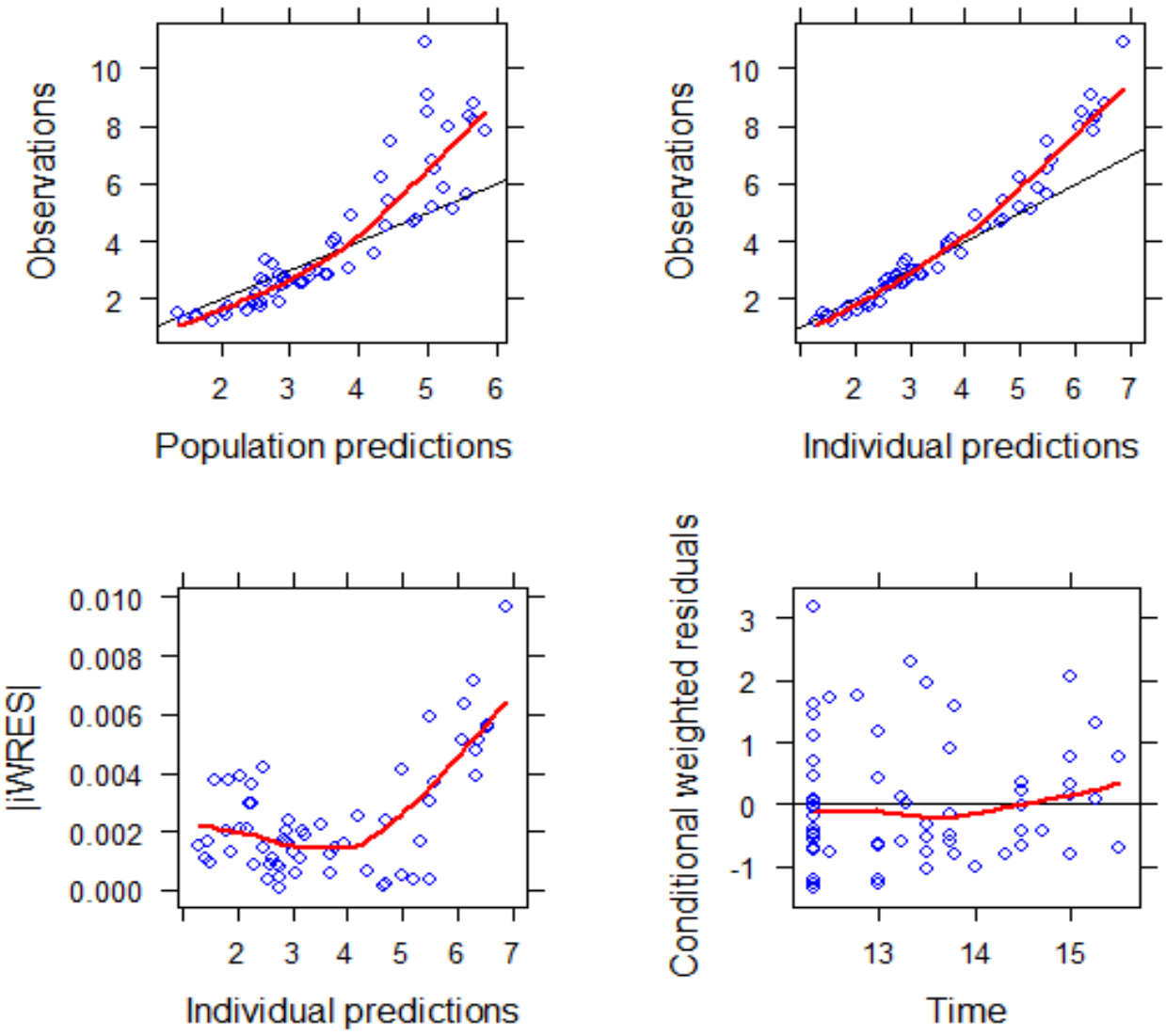

Figure 8. Goodness of fit plots for model 2bi with the estimated varying bioavailability and concentrations corrected for accumulation.

equation. Michaelis-Menten equation was used because of the saturation mechanism in relation to the cumulative uptake-volume associated with the uptake of the drug and the movement of A was projected to be facilitated (diffusion) by transportation mechanisms within the body. This was the main motivation for the use of the Michaelis-Menten equation below (equation 1.8). Primarily, the pharmacokinetic curve of an orally administered drug follows a biexponential curve with respect to time. A proposition was made such that for an oral dose that reached the systemic circulation, there exist a relationship for the uptake of A, defined by:

$$x_u(t) = \frac{A}{V(1-k_e)}(e^{-k_e t} - e^{-t}) \qquad (1.7)$$

for k_e small (small k_e improves convergence of $x_u(t)$ to $x(t)$-plasma concentration at terminal time points). The depositing rate constant was fixed to 1. The availability of occupation space (the uptake volume), elimination and transportation mechanism strongly influenced the availability of A in the systemic circulation. The relation between A/V and k_e at steady state is shown in Figure 11.

The Michaelis–Menten equation was used to describe the facilitated transport (a saturable process):

$$\frac{A}{V}=\frac{\left(\frac{Dose}{V}\right)_{max}x_u(t)}{k+x_u(t)}=\frac{A_t}{V_t}=\frac{\left(\frac{Dose}{V_t}\right)_{max}x_u(t)}{k+x_u(t)}=\frac{vx_u(t)}{k+x_u(t)},\text{where } V_t \leq \mathrm{V},\text{and } A_t \leq \mathrm{A} \quad (1.8)$$

The ratio $\frac{\mathrm{A}}{\mathrm{V}}$ is defined as the relative uptake and $\left(\frac{\mathrm{Dose}}{\mathrm{V}}\right)_{max}$ is the possible maximum relative uptake. This implies that for each t there exists a unique value of v. From v one could therefore obtain $\mathrm{V_t}$. It is important to stress that if the concentrations $x(t)$ are at steady state there is need to correct for accumulation by a factor of $\frac{1}{(1-\mathrm{e}^{-\mathrm{k_e}\tau})}$ where τ is a dosing interval. An asymmetrical sigmoid curve was then fitted for uptake volume against time, as this was sufficient for the data. In this case, two models were fit. The first model was for the whole sample of 61 patients' (model 3a) (where a naive assumption was made of equal transportation rates in all subjects) and the other one was where a relatively fast transportation at steady state was projected for one patient in the sample (model 3b) (an example of modelling one individual which could be extended to the whole group to obtain better estimated population parameters).

In the construction of model 3a, the facilitation here was assumed to be at the same rate in the group. The ratio $\frac{\mathrm{A}}{\mathrm{V}}$ being constant also implied that A and V may vary with respect to $time$ but their ratio did not. In other words A_t and uptake-volume were allowed to follow the same trajectory in terms of spread when A_t reached 100% (A) so did V_t as it assumed the value of cumulative uptake-volume associated with full absorption A. This was done primarily to capture the movement of A. The models estimated that generally in this population in 24 h, 90% of A (A is varying across individuals) will generally have reached systemic circulation (Table 8) at steady state. In this population the drug was projected to be fully absorbed after approximately 31 h (Figure 12 and Table 8). The accumulating percentage $A_{average}$ against $time$ was modelled. An asymmetrical sigmoid curve was found to be the best fit. This relationship could similarly have been observed for the cumulative uptake-volume.

A fit of a 5 parameter logistic regression (asymmetrical sigmoid curve (Figure 12)) was made and the following equation was obtained:

$$A_{average}=256.1598-\frac{255.9614}{\left(1+\left(\frac{t}{7.5247}\right)^{1.3786}\right)^{0.2365}},\qquad R^2=0.99999 \quad (1.9)$$

Points on the same line passing through the point $\left(0,\frac{Dose}{V}\right)$ share the same rate of percentage increase of drug absorbed relative to the full amount at any given time (that is the rate of uptake volume in m and w follow similar trends) (Figure 13). In this case the availability of A is directly linked/proportional to elimination rate that is:

$$A(=\mu ER_A+Dose)=\gamma ER_V+Dose \quad (1.10)$$

Dividing by $V\neq 0$ produces the linear relationships l_1, l_2 and l_3 (Figure 13). It can also be noted that:

$$AUC^{x_u(t)}{}_{\mathrm{m}}>AUC^{x_u(t)}{}_{\mathrm{n}}>AUC^{x_u(t)}{}_{\mathrm{u}}>AUC^{x_u(t)}{}_{\mathrm{v}}>AUC^{x_u(t)}{}_{\mathrm{w}}.$$

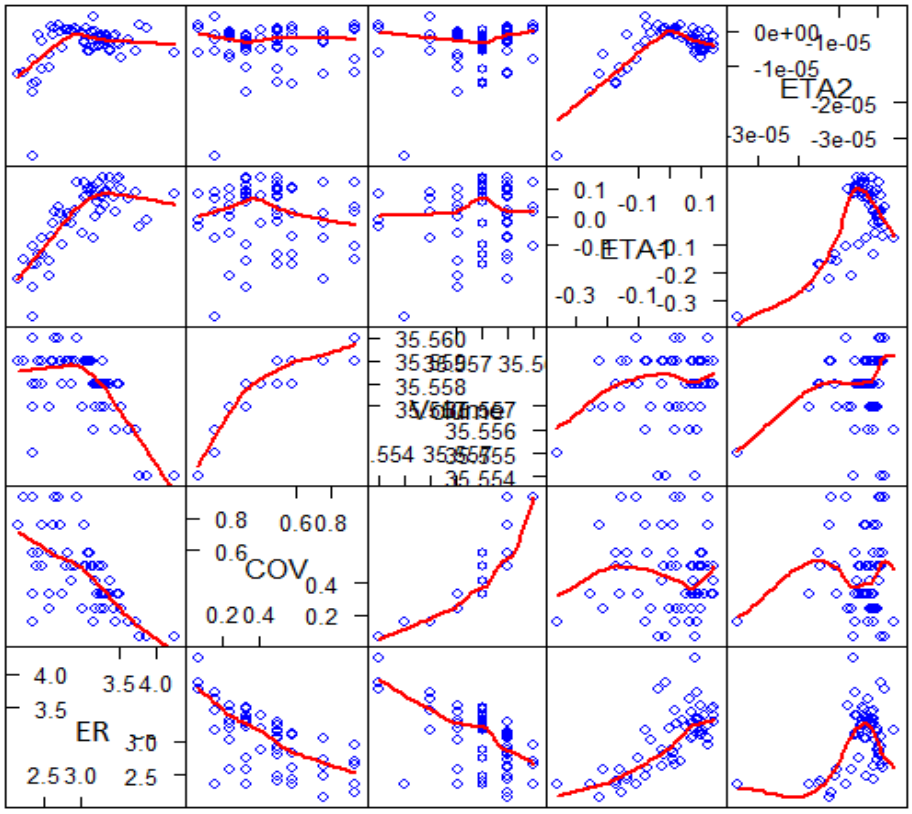

Figure 9. Scatter plot matrix showing how cumulative uptake-volume and elimination rate at the point of full absorption and covariate relation are related to each other for model 2b(i) with concentrations corrected for accumulation.

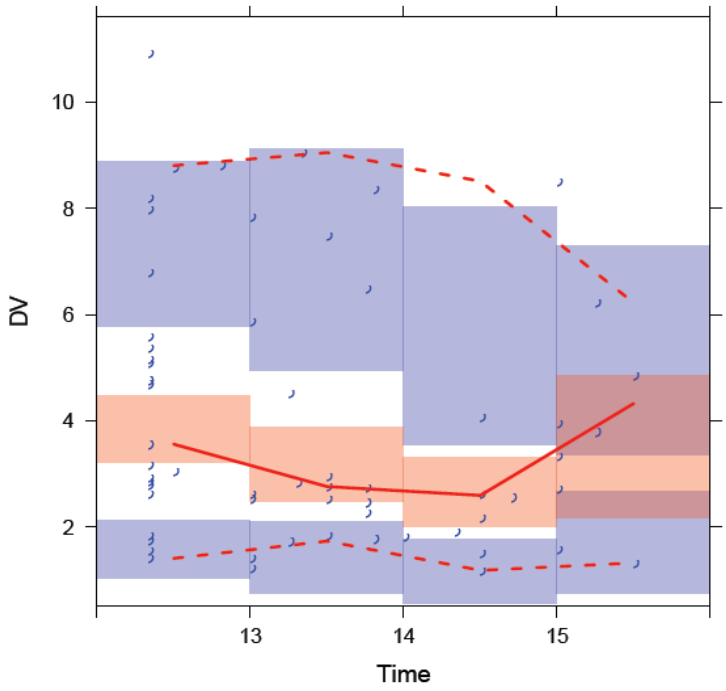

Figure 10. The value predictive check (VPC) for the terminal plasma concentration (corrected for accumulation) (500 samples were generated-bootstrapping method for the VPC).

Table 7. Correlations between *f*, k_e, and mid-dose interval efavirenz plasma concentration at steady state.

Variable	Plasma Conc.	f.	k_e
Plasma Conc.	1	-	-
f.	0.92	1	
k_e	-0.88	-0.83	1

$lnx = 3.609f - 7.1794k_e, R^2 = 0.9975$, established from model 2bi.

the individual P from eqn 1.7 using terminal points between C_{max} and C_{min} for the drug with the long half life.
2. Formulate the linear relationship such that the point $\left(k_e = 0, \frac{Dose}{V_P}\right)$ and $\left(k_{eP}, \frac{A}{V_P}\right)$ are on the line.
3. A range of points on the generated line are selected in the first quadrant (that is simulate individuals who have (share) the same transportation rates as P) (Table 9).
4. Equations 1.7 and 1.8 were then used to obtain the following results (Table 10).

$$A_{average} = 115.6782 - \frac{115.6568}{\left(1+\left(\frac{t}{13.1796}\right)^{1.1267}\right)^{3.3357}}, \quad R^2 = 0.999996 \qquad (1.11)$$

Points lying on $l\left\{l: \frac{A}{V} = \gamma k_e + \frac{Dose}{V} \overset{iff}{\Leftrightarrow} A = \gamma ER_V + Dose\right\}$ with the least gradient (γ) have the fastest transportation in the sampled population. The elimination rate of A for the drug that reaches systemic circulation follows from the relationship $k_e V_t = ER_{V_t} \leq ER_V$. This follows a similar relationship to that of cumulative uptake volume and $A_{average}$ with respect to time in an individual. For a monophasic PK curve, $\frac{A(mg)}{V(L)} = A_t(mg)/V_t(L) = c(mg/L)$

$$A_t = cV_t \qquad (1.12)$$

$$\frac{Amount\ cleared\ per\ unit\ time}{Total\ Amount\ at\ time\ t} = \frac{ER_{A_t}(mg/hr)}{A_t(mg)} = \frac{ER_{A_t}(mg/hr)}{cV_t(mg)} = \frac{\frac{ER_{A_t}(mg/hr)}{c(mg/L)}}{V_t(L)} = \frac{ER_{V_t}(L/hr)}{V_t(L)} = k_e. \qquad (1.13)$$

From equation 1.13 one notes that:

$$ER_{A_t} = k_e A_t \qquad (1.14).$$

It is noted that from Equations 1.13 and 1.14, and by definition, the elimination rate constant can also be used to define the ratio of the amount of drug (disregarding volume) cleared per unit time to total amount in the body at a particular time. The elimination rate is primarily dependent on the total amount in the system. Correspondingly, A_t accumulates with V_t (up to the case when $A_t = A$ and $V_t = V$). The uptake volume V_t can also be used as a dummy for A_t (Equation 1.13).

The parameter ER_{A_t} relates to the elimination of the actual amount in the systemic circulation and ER_{V_t} relates to elimination associated with the cumulative uptake volume occupied by A_t. An estimation of the amount of drug cleared (Table 11, Figures 14 and 15) associated with the individual on quicker transportation in this sample population was done.

$$D_t = \frac{A_t}{A} Dose = \frac{V_t}{V} Dose \qquad (1.15),$$

$$A_t = \frac{V_t}{V} A \qquad (1.16).$$

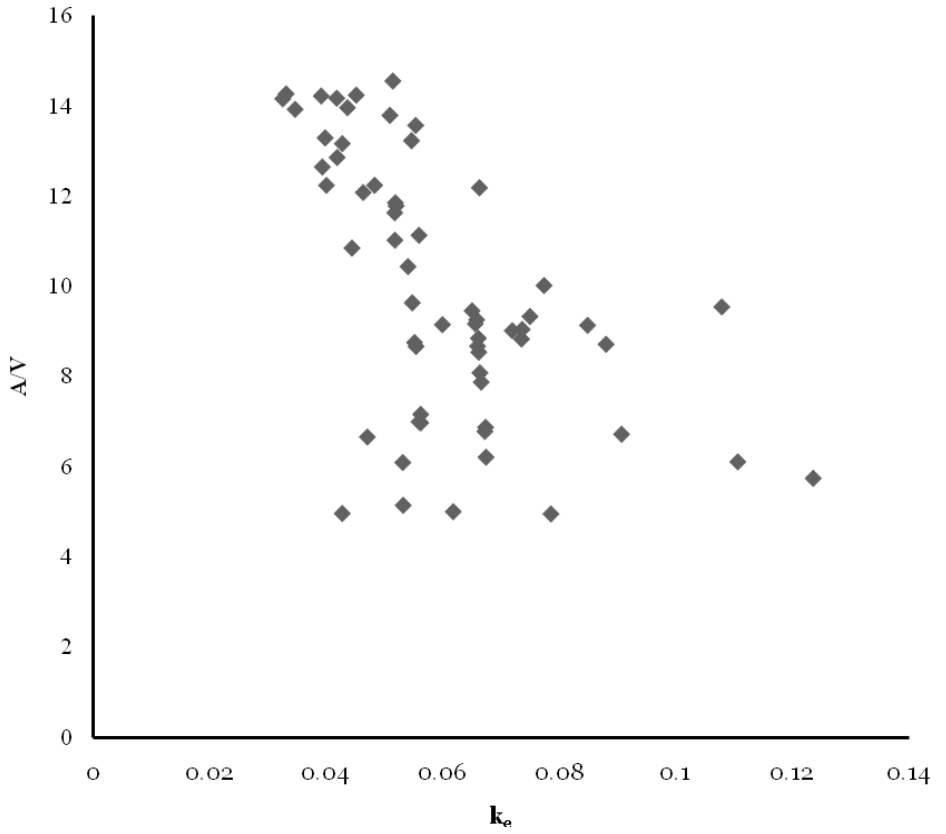

Figure 11. Graph showing evidence that of a negative correlation between $\frac{A}{V}$ and k_e for the 61 patients on efavirenz.

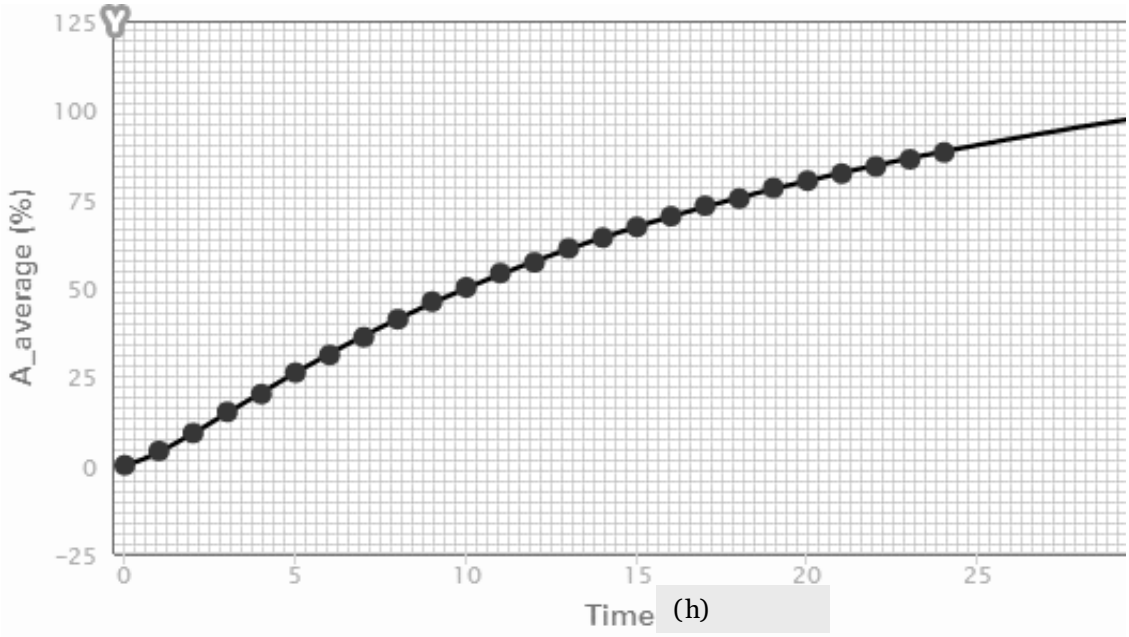

Figure 12. The five parameter logistic regression equation showing estimated average absorption time into the systemic circulation for the whole sample.

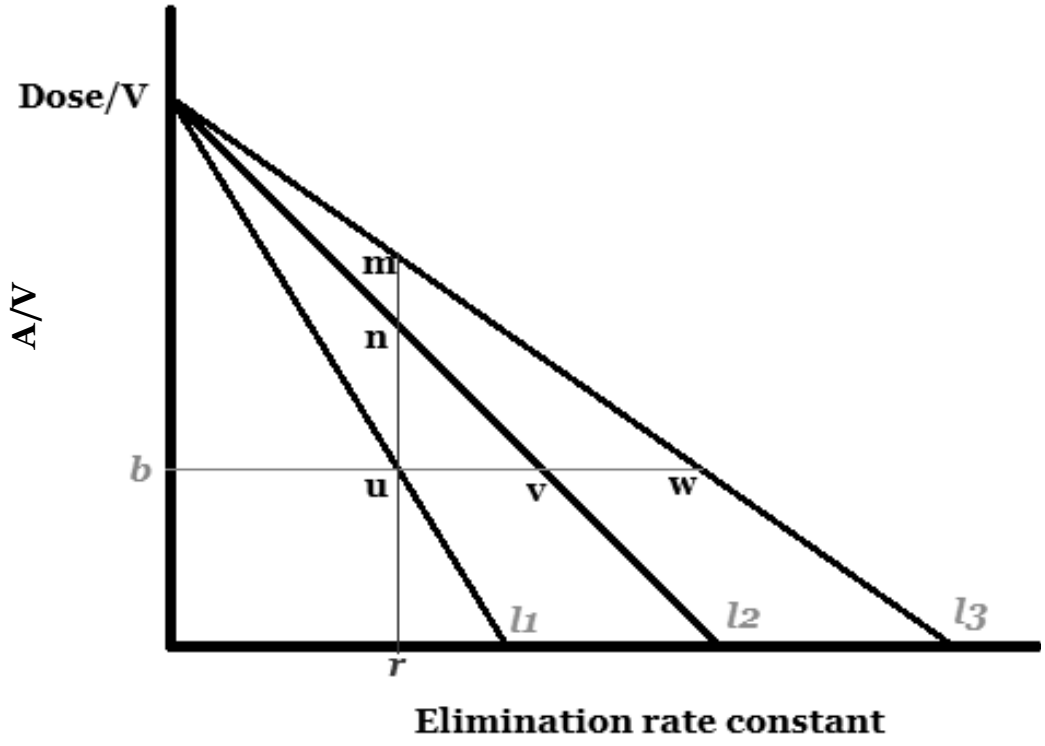

Figure 13. Illustration of relationships of the ratio $\frac{A}{V}$ and k_e between different individuals in a population.

A is full amount reaching systemic circulation and V is the corresponding cumulative uptake-volume associated with A's full absorption. The elimination rate constant is where one obtains the amount being eliminated from the systemic circulation please note $j = t$

$$A_1 k_e = Q_1$$
$$(A_2 - Q_1)k_e = Q_2$$
$$(A_j - Q_{j-1})k_e = Q_j$$

For patient P, points after absorption are well projected (Figure 16). Absorption was projected to end at the point reaching systemic circulation at time t) can be approximated by an asymmetrical sigmoid function. The estimated AUC's relation with mid-dose plasma I (x_f) in reference to patient P. The region in blue represents potential points where the concentration curve may have been laid (these may potentially be due to the volume occupied). All curves have to pass through the point I and point of origin (The assumption made was that there was no delayed absorption). The curve x represents the plasma concentration obtained by assuming maximum potential spread in the possible volume space (39.68 L) during the whole period. The patient's absorption rate at time t, it is found by $AR(t) = \frac{dA_t}{dt}$ where A_t (cumulative amount concentration at steady state projected using the volume of distribution (V_d) of 39.68 L per individual in this population was given by:

$$AUC = f.Dose/CL = 16.35x, r = 0.9767\ (AUC_{SS} = AUC.a_{f,ss} = 24.79x, r = 0.9763)$$

The results were generated from models 2b and 2b (i), where $CL = k_e * V_d$. The patients carrying the *CYP2B6 G516T TT* genotype were projected to have higher exposure levels (Table 12).

DISCUSSION

A proposition is made that a multifactorial approach of using 516G > T together with routine clinical measurements of weight and gender status, in decisions

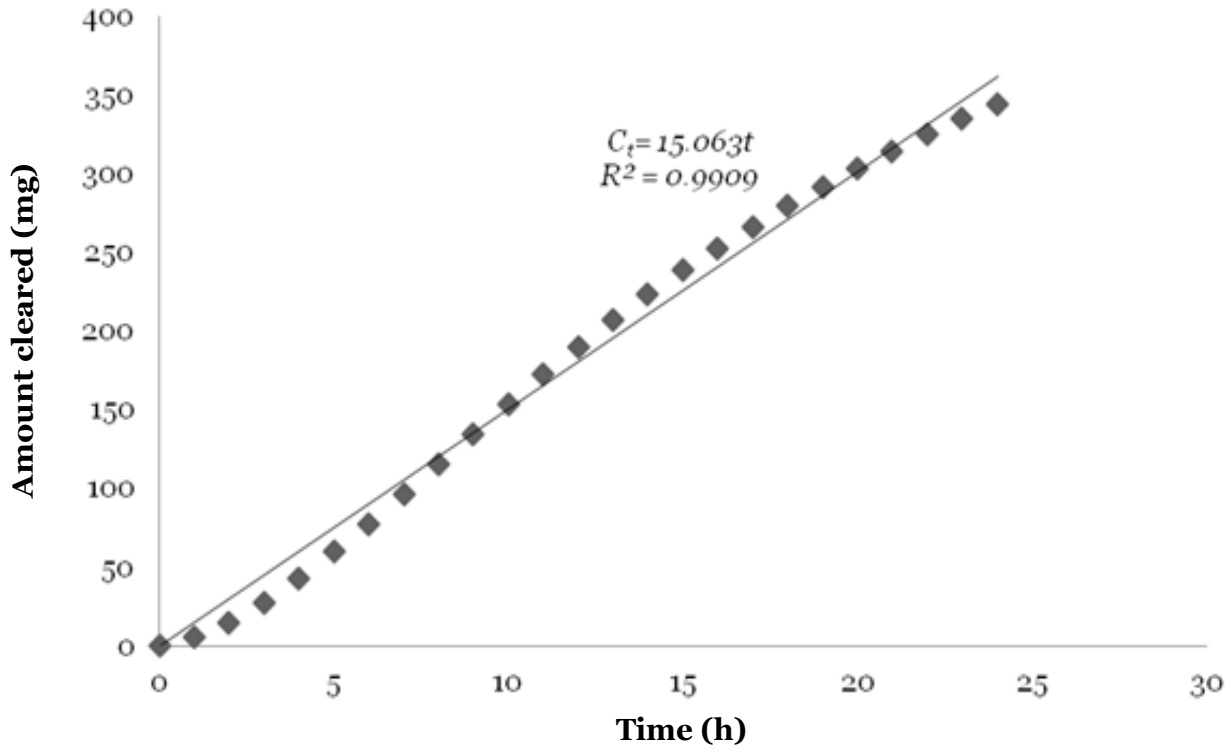

Figure 14. Estimated amount of drug cleared in systemic circulation in 24 h for patient P. The amount cleared was shown to be relatively constant (15 mg/h).

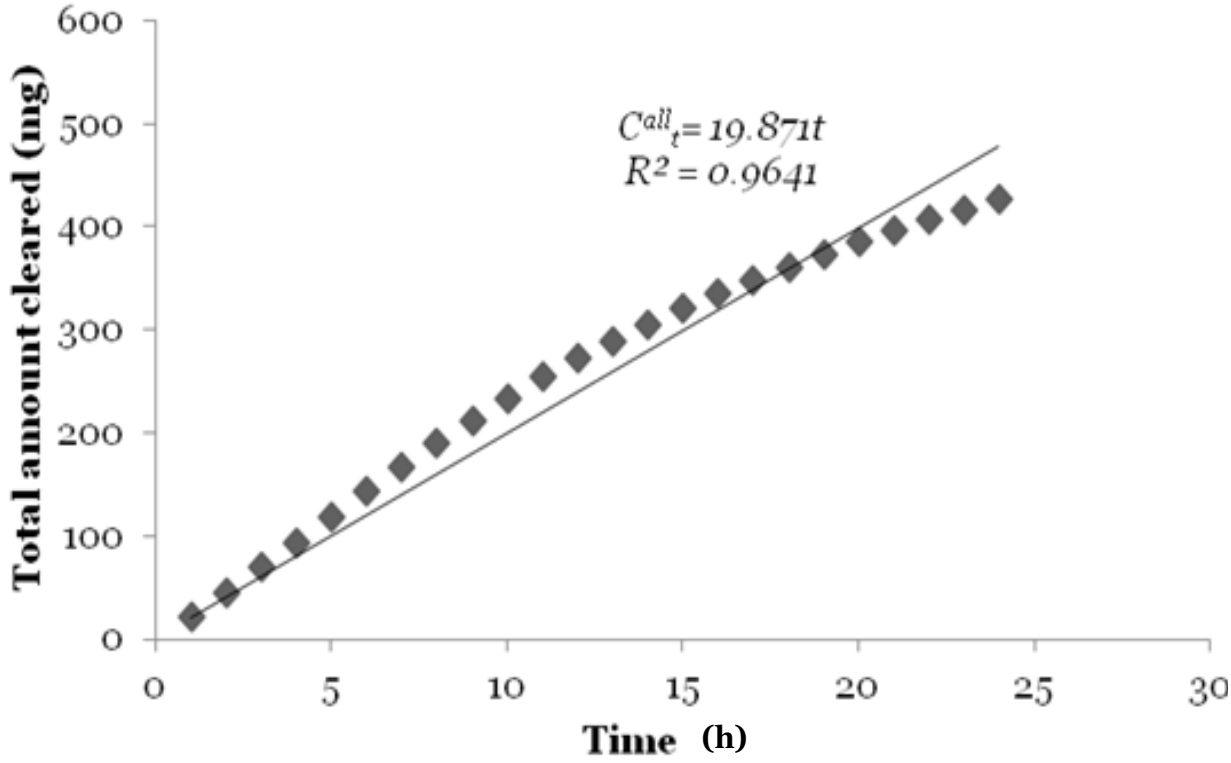

Figure 15. Estimated total amount of drug cleared in 24 h for patient P. The amount cleared was shown to be almost constant about 20 mg/h.

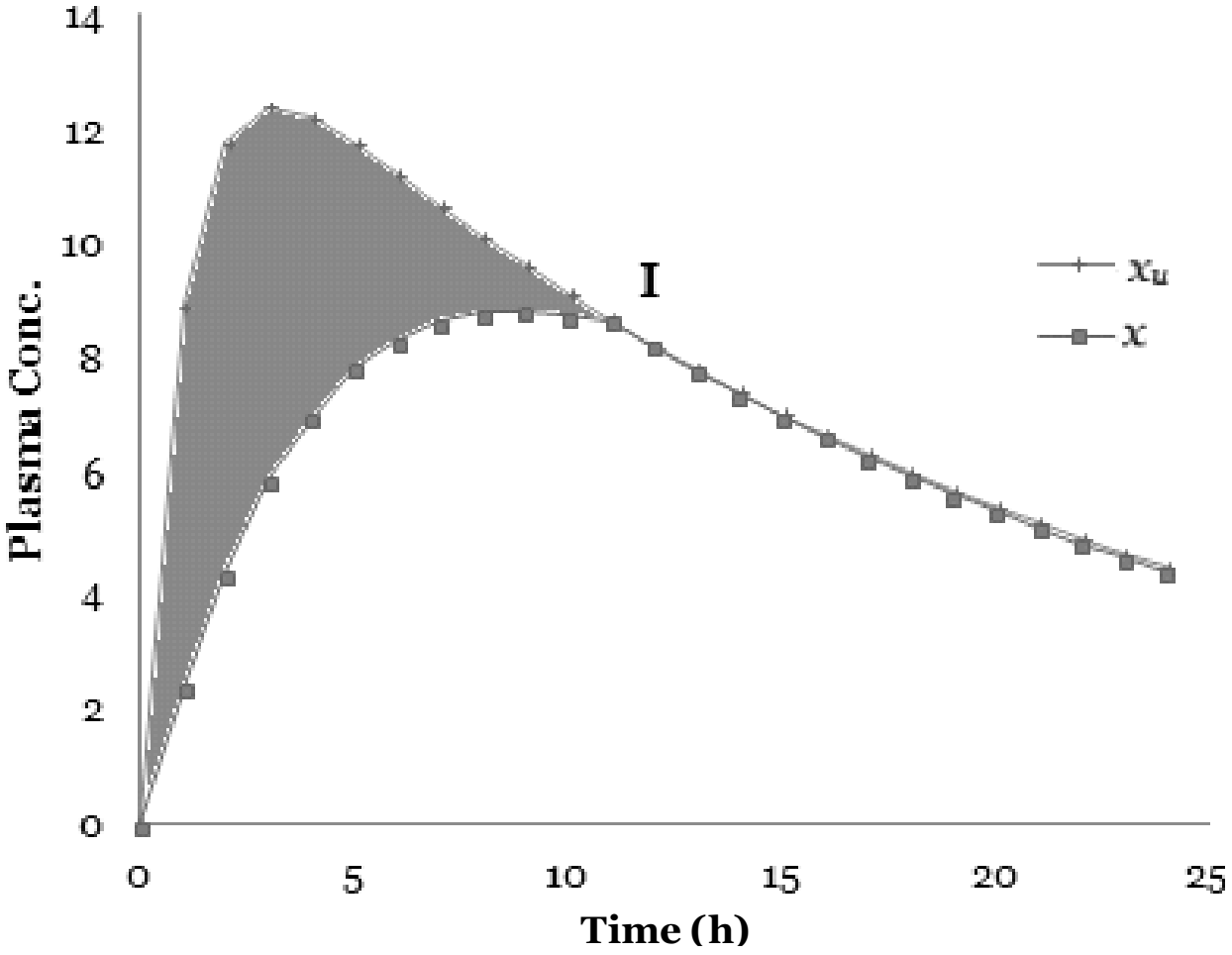

Figure 16. Projected plasma concentration- time curve for patient P.

on which patients are at risk of high efavirenz exposure (Table 1) and possibly CNS adverse drug reactions (Mukonzo et al., 2014). These patients with high efavirenz exposure may require dose reduction enabling less discontinuation of the drug efavirenz (Van Luin et al., 2009). The richer covariate information enables better estimates. Furthermore, more information on the covariate structure enables better correlations to be established between the covariates and the plasma concentration (Table 1). It is desirable to have full PK profiles (rich sampling) as these will guarantee monophasic profiles (one period). In addition, more robust estimates can be developed from the AUC generated from full profiles.

These results also point us towards an assumption that efavirenz is not a drug that hides in tissues due to the value of volume of distribution found but is a drug that is expected to distribute well within the body fluid volume system (Toutain et al., 2004b). This was supported by the fact that efavirenz is highly bound to plasma proteins (Cristofoletti et al., 2013; Rekić et al., 2011; Smith et al., 2001). However a different inference could be postulated from what others have found with regards to the volume of distribution (150 to 500 L) (Cristofoletti et al., 2013; Nyakutira et al., 2008; Ribaudo et al., 2006; Sánchez et al., 2011; Siccardi et al., 2012; Yilmaz et al., 2012), which could also be linked to the high lipophilicity of efavirenz (Cristofoletti et al., 2013).

The modelling not only improved estimates (Tables 2 and 6), but also the results showed how one can incurporate time and other variables that affect changes in concentration of efavirenz. As far as can be ascertained, these results in this work are the first that tried to estimate oral bioavailability based on an estimated value of a newly introduced cumulative uptake-volume associated with full absorption (and consider absorption as a cumulative process based on this cumulative uptake-volume). An asymmetric sigmoid relation was proposed for the relationship between efavirenz mid-dose concentration and bioavailability (equation 1.4). Other researchers have postulated on the potential impact which could be attributed to bioavailability on the esti-mation of efavirenz PK parameters (Csajka et al., 2003; Cabrera et al., 2009).

The current models in PK modelling define absorption as a continuous process that is not clearly defined as a

Table 8. Results showing average percentage of absorbed efavirenz in the systemic circulation in this population.

Time (t)	All patients (Model 3(a))				
	k	$(Dose/V)_{max}$	R_{square}	V_t	Average percentage absorbed relative to A $(A_{average})$
0	-	-	-	0	0
1	241.794	406.1381	0.9995	1.4773	4
2	139.172	184.1495	0.9974	3.2582	9
3	85.5356	114.8444	0.9935	5.2245	15
4	59.4248	82.7927	0.9877	7.2470	20
5	42.8447	64.9518	0.9803	9.2376	26
6	32.3415	53.8189	0.9716	11.1485	31
7	25.2901	46.3205	0.9618	12.9532	36
8	20.3153	40.9674	0.9512	14.6458	41
9	16.6609	36.9707	0.9399	16.2291	46
10	13.8876	33.8794	0.9282	17.7099	50
11	11.7264	31.4196	0.9161	19.0964	54
12	10.0056	29.4163	0.9037	20.3969	57
13	8.611	27.7530	0.891	21.6193	61
14	7.4637	26.3493	0.8781	22.771	64
15	6.5158	25.1641	0.8651	23.8435	67
16	5.714	24.13	0.8519	24.8653	70
17	5.0336	23.2268	0.8386	25.8322	73
18	4.4515	22.4306	0.8253	26.7492	75
19	3.9503	21.7232	0.8119	27.6202	78
20	3.516	21.09	0.7984	28.4495	80
21	3.1377	20.5195	0.7849	29.2405	82
22	2.8098	20.0126	0.7715	29.9811	84
23	2.5196	19.5444	0.7580	30.6993	86
24	2.2636	19.1163	0.7445	31.3869	88
31	1.1143	16.907	0.651	35.4883	100

Table 9. Simulated individuals carrying similar transportation rates as patient P.

k_e	$\frac{A}{V}$
0	16.85393
*(P=)*0.051531	14.55997
0.26703	4.966767
0.179937	8.843835
0.168722	9.343091
0.104649	12.19535
0.073601	13.57749
0.175802	9.027896
0.175151	9.056887
0.201422	7.887409
0.186481	8.552503
0.32379	2.44
0.356138	1
0.378602	0

Table 10. Results showing average percentage of absorbed efavirenz in the systemic circulation for the individual exhibiting fastest transportation in this population.

Time (t)	Patient (model 3(b))				
	k	$\frac{Dose}{V}_{max}$	R_{square}	V_t	Estimated Percentage absorbed relative to A $(A_{average})$
0	-	-	-	0	0
1	46.0809	89.6937	0.999998	6.6894	19
2	26.0169	46.7984	0.999966	12.8210	36
3	15.8841	33.3180	0.999795	18.0083	51
4	10.3502	27.0212	0.999314	22.2048	62
5	7.0993	23.4919	0.9984	25.5407	72
6	5.0644	21.2833	0.9968	28.1911	79
7	3.7221	19.7937	0.9947	30.3127	85
8	2.7976	18.7302	0.9920	32.0338	90
9	2.1392	17.9384	0.9888	33.4478	94
10	1.6577	17.3291	0.9851	34.6238	97
11	1.2981	16.8475	0.9811	35.6136	100

terminating process at some point in time. Furthermore, other researchers have noted the need to characterise drug absorption as most models lack physiological rationale (Ette and Williams, 2007). The work also introduced how one can incorporate two spaces that help us estimate changes in drug concentration in the body, that is the covariate space and the time space. A marked improvement was noticed in the estimation of the mid dosing interval concentrations for the model that took into consideration the estimated bioavailability (models 2a and b). Kwara et al. (2008) noted that efavirenz concentration at steady state was directly related to AUC (of up to 24 h) for individuals on both efavirenz and TB drugs at mid dose and at 24 h. They observed a strong correlation of 0.969 from full PK profile estimates and the work here obtained a correlation of 0.976 of projected estimates. Two main parameters were singled out, that is bioavailability and uptake-volume associated with full absorption in the estimation of absorption. Once absolute oral bioavailability was estimated consequently, the depositing rate constant was taken as 1, a 'parallel' function was used to model the uptake of the drug defined in this work as $x_u(t)$ (Table 11). Efavirenz was being taken up into the systemic circulation at the relative ratio defined by $\frac{A}{V}$ (equation 1.8). The limiting step was the amount of drug available for absorption. The cumulative uptake-volume associated with the uptake of A was shown to consequently follow an asymmetric sigmoid curve and similarly for elimination rate and accumulation of the drug. However, $\frac{A}{V}$ and $\frac{ER_V}{V}$ are constants for any monophasic PK curve.

As far as can be ascertained, this is also the first study which imposed a condition of the existence of an uptake-volume and using the Michaelis-Menten equation to a one compartmental model to relay the process of transportation. The amount of drug cleared per unit time in the dosing interval was noted to be relatively constant for one individual illustrated who had faster absorption (Figures 14 and 15 and Table 11). This could be extended to the whole group. On average in this population, 90% of A (total amount (mass) reaching systemic circulation) was projected to be absorbed in 24 h at steady state (Figure 12, Table 8). The patient who had the quickest transportation in this sample was estimated to have fully absorbed the drug into the systemic circulation in 11 h at steady state (Table 10). This work also highlighted the need to further investigate interpretation of the constants modelled by differential equations and implications (Table 2).

The limitation in this study that could improve estimates includes genetic information on other genotypes linked to efavirenz metabolism such as *CYP3A4, CYP2A6* and possibly transporters (Mukonzo et al., 2009; Kwara et al., 2009; Ritchie et al., 2006). Also, full profiles would ensure that the estimated PK curve is monophasic almost surely. Another improvement on estimates was to model the data above using the value of plasma concentrations in the neighbourhood of 24 h as well (Kwara et al., 2008).

Furthermore, increase in covariate information signifies increase in clusters to be formed which then requires a

Table 11. Estimated absolute amount(s) of the drug in the system and possibilities of its distribution in the patient P.

t	Cumulative uptake volume $V_t(L)$	Amount (mg) cleared per hr (Systemic) $Q_t = C_t - C_{t-1}$	Cumulative mg cleared (Systemic) C_t	$x_u(t)$	Projected (mg) cleared $(D_t - A_t)$	A_t (Systemic)	Total cleared per h (mg) $C^{all}{}_t - C^{all}{}_{t-1}$	Cumulative amount cleared (mg) $C^{all}{}_t$	Amount in systemic (AB_t)	Volume of distribution relative to $x_u(t)$ projected using $(AB_t)/x_u(t)$	Projected plasma Conc. $x(t)$
0	0	-	0	0	0	0		0	0	0	0
1	6.6894	5.0686	5.0686	8.9415	15.302	98.42	20.3706	20.3706	93.3514	10.4403	2.35243
2	12.821	9.3427	14.4113	11.7818	29.328	186.48	23.3687	43.7393	172.069	14.6047	4.33609
3	18.0083	12.8631	27.2744	12.4001	41.1939	264.18	24.729	68.4683	236.906	19.1051	5.96997
4	22.2048	15.1351	42.4095	12.2225	50.7934	321.16	24.7346	93.2029	278.751	22.8064	7.02445
5	25.5407	17.0234	59.4329	11.7724	58.4243	372.96	24.6542	117.857	313.527	26.6325	7.90081
6	28.1911	18.014	77.4469	11.2413	64.487	409.22	24.0768	141.934	331.773	29.5137	8.36061
7	30.3127	18.6869	96.1338	10.6989	69.3402	440.3	23.5401	165.474	344.166	32.1684	8.67291
8	32.0338	19.0584	115.192	10.1697	73.2772	466.2	22.9954	188.469	351.008	34.5151	8.84532
9	33.4478	19.144	134.336	9.6619	76.5117	486.92	22.3785	210.848	352.584	36.4922	8.88503
10	34.6238	18.9584	153.295	9.1777	79.2018	502.46	21.6485	232.496	349.165	38.0449	8.79889
11	35.6	18.7823	172.077	8.7172	82	518	20.927	253.423	345.923	39.6829	8.71718
12	-	17.815	189.892	8.2795	-	-	17.815	271.238	328.108	39.629	8.26825
13	-	16.8976	206.79	7.8637	-	-	16.8976	288.136	311.211	39.5755	7.84243
14	-	16.0273	222.817	7.4688	-	-	16.0273	304.163	295.183	39.5223	7.43855
15	-	15.2019	238.019	7.0936	-	-	15.2019	319.365	279.981	39.4693	7.05546
16	-	14.419	252.438	6.7374	-	-	14.419	333.784	265.562	39.4163	6.69211
17	-	13.6765	266.114	6.399	-	-	13.6765	347.461	251.886	39.3634	6.34746
18	-	12.9721	279.086	6.0776	-	-	12.9721	360.433	238.914	39.3106	6.02057
19	-	12.3041	291.39	5.7723	-	-	12.3041	372.737	226.61	39.2579	5.71051
20	-	11.6704	303.061	5.4824	-	-	11.6704	384.407	214.939	39.2052	5.41642
21	-	11.0694	314.13	5.2071	-	-	11.0694	395.477	203.87	39.1526	5.13747
22	-	10.4993	324.63	4.9455	-	-	10.4993	405.976	193.371	39.1001	4.87289
23	-	9.9586	334.588	4.6971	-	-	9.9586	415.935	183.412	39.0476	4.62194
24	-	9.4457	344.034	4.4612	-	-	9.4457	425.38	173.966	38.9952	4.38391

Table 12. The estimated and projected AUC and AUC_{SS} for efavirenz categorised according to the most significant variable *CYP2B6 G516T* investigated in the separation of observed plasma concentrations in this population sample for the 61 patients.

Genotype		Efavirenz plasma conc. µg/ml median (IQR)	AUC mg h/L median (IQR)	AUC_{ss} mg h/L median (IQR)
CYP2B6 G516T	**GG**	3.32 (1.62, 3.64)	84.16 (57.83, 101.50)	96.77 (65.21, 120.23)
	GT	3.49 (2.81, 6.14)	89.55 (77.51, 127.11)	115.42 (97.53,178.52)
	TT	8.70 (5.01, 11.40)	150.56 (98.91, 180.88)	240.69 (149.00, 297.11)

larger sample size. On validation for estimation of oral bioavailability by the method suggested in this work, an experiment using both intravenous doses and oral doses is required. It is also required to validate what has been estimated in this work with use of data outside this present population.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Aksnes DL, Egge JK (1991). A theoretical model for nutrient uptake in phytoplankton. Mar. Ecol Prog. Ser. 70:65–72.

Almond LM, Hoggard PG, Edirisinghe D, Khoo SH, Back DJ (2005). Intracellular and plasma pharmacokinetics of efavirenz in HIV-infected individuals. J. Antimicrob. Chemother. *56*(4):738–44.

Burger D, van der Heiden I, la Porte C, van der Ende M, Groeneveld P, Richter C, van Schaik R (2006). Interpatient variability in the pharmacokinetics of the HIV non-nucleoside reverse transcriptase inhibitor efavirenz: the effect of gender, race, and CYP2B6 polymorphism. Br. J. Clin. Pharmacol. 61(2):148–54.

Cabrera SE, Santos D, Valverde MP, Domínguez-Gil A, González F, Luna G, MJG (2009). Population Pharmacokinetics of Efavirenz in HIV patients: Influences of CYP2B6 Genotype. Antimicrob. Agents Chemother. 53:2791–2798.

Cristofoletti R, Nair A, Abrahamsson B, Groot DW, Kopp S, Langguth P (2013). Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Efavirenz. J. Pharm. Sci. 102(2):318–329.

Csajka C, Marzolini K, Fattinger L, De´costerd A, Fellay J, Telenti A, Biollaz J, Buclin T (2003). Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. Clin. Pharmacol. Ther. 73:20–30

Ette EI, Williams PJ (2007). Pharmacometrics. The Science of Quantitative Pharmacology, (1st ed.). Wiley: Hoboken, New Jersey, USA.

Friedland G, Khoo S, Jack C, Lalloo U (2006). Administration of efavirenz (600 mg/day) with rifampicin results in highly variable levels but excellent clinical outcomes in patients treated for tuberculosis and HIV. J. Antimicrob. Chemother. 58:1299-1302.

Habtewold A, Amogne W, Makonnen E, Yimer G, Riedel KD, Ueda N, Aklillu E (2011). Long-term effect of efavirenz autoinduction on plasma/peripheral blood mononuclear cell drug exposure and CD4 count is influenced by UGT2B7 and CYP2B6 genotypes among HIV patients. J. Antimicrob. Chemother. 66(10):2350-2361.

Jiang F, Desta Z, Shon JH, Yeo CW, Kim HS, Liu KH, Shin JG (2013). Effects of clopidogrel and itraconazole on the disposition of efavirenz and its hydroxyl metabolites: exploration of a novel CYP2B6 phenotyping index. Br. J. Clin. Pharmacol. *75*(1):244–53.

Johnson KA, Goody RS (2011). The original Michaelis constant: translation of the 1913 Michaelis-Menten paper. Biochemistry 50(39):8264–8269.

Kwara A, Lartey M, Sagoe KW, Xexemeku F, Kenu E, Oliver-Commey J, Boima V, Sagoe A, Boamah I, Greenblatt DJ, Court MH (2008). Pharmacokinetics of efavirenz when co-administered with rifampin in TB/HIV co-infected patients: pharmacogenetic effect of CYP2B6 variation. J. Clin. Pharmacol. 48(9):1032–1040.

Kwara A, Lartey M, Sagoe KW, Kenu E, Court MH (2009). CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. AIDS, 23(16):2101-2106.

Manosuthi W, Sungkanuparph S, Tantanathip P, Mankatitham W, Lueangniyomkul A, Thongyen S, Ruxrungtham K (2009). Body weight cutoff for daily dosage of efavirenz and 60-week efficacy of efavirenz-based regimen in human immunodeficiency virus and tuberculosis coinfected patients receiving rifampin. Antimicrob. Agents Chemother. 53(10):4545–8.

Mould DR, Upton RN (2013). Basic Concepts in Population Modeling, Simulation, and Model-Based Drug Development—Part 2: Introduction to Pharmacokinetic Modeling Methods. *CPT:* Pharmacometrics Syst. Pharmacol. *2*(4):e38.

Mukonzo JK, Röshammar D, Waako P, Andersson M, Fukasawa T, Milani L, Aklillu E (2009). A novel polymorphism in ABCB1 gene, CYP2B6*6 and sex predict single-dose efavirenz population pharmacokinetics in Ugandans. Br. J. Clin. Pharmacol. 68(5):690–699.

Mukonzo JK, Owen JS, Ogwal-Okeng J, Kuteesa JB, Nanzigu S, Sewankambo N, Thabane L, Gustafsson LL, Ross C, Aklillu E, (2014). Pharmacogenetic-Based Efavirenz Dose modification: Suggestions for an African population and the different CYP2B6 Genotypes. Plos one 9(1):e86919.

Nemaura T, Nhachi C, Masimirembwa C (2012). Impact of gender, weight and CYP2B6 genotype on efavirenz exposure in patients on HIV/AIDS and TB treatment: Implications for individualising therapy. Afr. J. Pharm. Clin. Pharmacol. 6(29):2188-2193.

Nyakutira C, Röshammar D, Chigutsa E, Chonzi P, Ashton M, Nhachi C, Masimirembwa C (2008). High prevalence of the CYP2B6 516G-->T(*6) variant and effect on the population pharmacokinetics of efavirenz in HIV/AIDS outpatients in Zimbabwe. Eur. J. Clin. Pharmacol. 64(4):357–365.

Portier C, Tritscher A, Kohn M, Sewall C, Clark G, Edler L, Lucier G (1993). Ligand/receptor binding for 2,3,7,8-TCDD: implications for risk assessment. Fundam. Appl. Toxicol. 20(1):48–56.

Pedral-Sampaio DB, Alves CR, Netto EM, Brites C, Oliveira AS, Badaro R (2004). Efficacy and safety of Efavirenz in HIV patients on Rifampin for tuberculosis. Braz. J. Infect. Dis. 8(3):211–216.

Rekić D, Röshammar D, Mukonzo J, Ashton M (2011). *In silico* prediction of efavirenz and rifampicin drug-drug interaction considering weight and CYP2B6 phenotype. Brit. J. Clin. Pharmacol 71(4):536–543.

Ritchie MD, Haas DW, Motsinger AA, Donahue JP, Erdem H, Raffanti S, Sterling TR (2006). Drug transporter and metabolizing enzyme gene variants and nonnucleoside reverse-transcriptase inhibitor hepatotoxicity. Clin. Infect. Dis. 43(6):779–782.

Ribaudo HJ, Haas DW, Tierney C, Kim RB, Wilkinson GR, Gulick RM, Acosta EP (2006). Pharmacogenetics of plasma efavirenz exposure after treatment discontinuation: an Adult AIDS Clinical Trials Group Study. Clin. Infect. Dis. 42(3):401–407.

Ribaudo HJ, Liu H, Schwab M, Schaeffeler E, Motsinger-reif AA, Ritchie MD, Haas DW (2011). Effect of CYP2B6, ABCB1, and CYP3A5 polymorphisms on efavirenz pharmacokinetics and treatment response: an AIDS Clinical Trials Group study. J. Infect. Dis. 202(5):717–722.

Sánchez A, Cabrera S, Santos D, Valverde MP, Fuertes A, Domínguez-Gil A, García MJ (2011). Population pharmacokinetic/ pharmacogenetic model for optimization of efavirenz therapy in Caucasian HIV-infected patients. Antimicrob. Agents Chemother. 55(11):5314–5324.

Siccardi M, Almond L, Schipani A, Csajka C, Marzolini C, Wyen C, Back D (2012). Pharmacokinetic and pharmacodynamic analysis of efavirenz dose reduction using an in vitro-*in vivo* extrapolation model. Clin. Pharmacol. Ther. *92*(4):494–502.

Smith PF, DiCenzo R, Morse GD (2001). Clinical uses of non-nucleoside reverse transcriptase inhibitors. Clin. Pharmacokinet. 10(4):217–229.

Toutain PL, Bousquet-Mélou A (2004a). Plasma terminal half-life. J. Vet. Pharmacol. Ther. 27(6):427–439.

Toutain PL, Bousquet-Mélou A (2004b). Volumes of distribution. J. Vet. Pharmacol. Ther. 27(6):441–453.

Van Luin M, Gras L, Richter C, Van Der Ende ME, Prins JM, De Wolf F,

Wit FW (2009). Efavirenz dose reduction is safe in patients with high plasma concentrations and may prevent efavirenz discontinuations. J. Acquir. Immune Defic. Syndr. 52:240–245.

Yilmaz A, Watson V, Dickinson L, Back D (2012). Efavirenz pharmacokinetics in cerebrospinal fluid and plasma over a 24-hour dosing interval. Antimicrob. Agents Chemother. 56(9):4583–4585.

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