A STUDY TO DETERMINE THE INFLUENCE OF PROBENECID (BENEMID®) ON THE URINARY EXCRETION OF ANDROSTERONE

HSL KRUGER

A Study To Determine The Influence Of Probenecid (Benemid®) On The Urinary Excretion Of Androsterone

Hester Susanna Lucia Kruger

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Supervisor: Dr PJ van der Merwe, Ph.D.
Co-supervisor: Prof JG Barnard, D.Phil.

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Central University of Technology, Free State

TABLE OF CONTENTS

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

APPENDICES

Central University of Technology, Free State

DECLARATION OF INDEPENDENT WORK

I, HESTER SUSANNA LUCIA KRUGER, do hereby declare that this research project submitted for the degree MAGISTER TECHNOLOGIAE: BIOMEDICAL TECHNOLOGY, is my own independent work that has not been submitted before to any institution by me or anyone else as part of any qualification.

SIGNATURE OF STUDENT

78-12-04

DATE

ABSTRACT

The aim of this study was to determine the influence of probenecid on the urinary excretion of anabolic-androgenic steroids. Androsterone is an endogenous steroid and since it is excreted in high concentrations in urine, it was used as a representative of anabolic steroids. It was also used because it would be unethical to give anabolic-androgenic steroids to healthy volunteers just to study the influence of probenecid on the excretion of anabolic steroids.

A literature survey of the relevant publications reveals a lack of information regarding the influence of probenecid on the urinary excretion of anabolic steroids as well as endogenous steroids. A pilot study performed at the FARMOVS Research Centre, Department of Pharmacology, Bloemfontein suggested a significant decrease on the urinary excretion of androsterone after the intake of probenecid.

Twenty (20) healthy, male volunteers participated in an open, single-dose, randomised, two way, cross-over study with a drug-free interval of 7 days between treatment phases. Each subject received each of the following 2 treatments once in a randomised order:

Treatment A: Placebo (control phase) + 200ml water.

Treatment B: 4 x 500mg Probenecid tablets (Benemid®) + 200ml water.

On the profile days (Day 1 and 8), the trial medication was taken at 08:00 with 200ml tap water. All subjects were required to take 200ml water hourly for the first four hours after drug administration. After administration urine collections were made at predetermined intervals over a peroid of 48 hours for the determination of androsterone and probenecid concentrations.

Androsterone was quantified using a gas chromatograph-mass spectrometric method. The results showed that the lowest excretion of androsterone was found between 2 - 3 hours after administration of probenecid. A statistically significant decrease in the excretion rate of

androsterone was seen from 2 to 8 hours after administration of a single dose of probenecid (4 x 500mg). The mean androsterone excretion rate for all fractional collection intervals in this period (1 - 2 h, 2 - 3 h, 3 - 4 h, 4 - 6 h, 6 - 8 h) were between 56% and 70% lower following administration of Benemid® compared to placebo. High doses of probenecid inhibit the urinary excretion of androsterone and therefore it will also inhibit the excretion of other anabolic steroids excreted as glucuronides in urine. Since the concentration of exogenous anabolic steroids in urine is usually very low, probenecid can decrease their concentration to below the limit of detection. Thus, probenecid can be used as a masking agent for the excretion of anabolic steroid glucuronides.

This inhibition of the renal excretion of anabolic steroids by probenecid represents a manipulation of the urine sample in doping control and therefore probenecid was banned by the Medical Commission of the International Olympic Committee.

OPSOMMING

Die doel van hierdie studie was om ondersoek in te stel na die invloed van probenesied op die urinêre uitskeiding van anaboliese steroïede. Aangesien androsteroon, 'n endogene steroïed, in hoë konsentrasies uitgeskei word, is dit as verteenwoordigend van die anaboliese steroïede gebruik. Hierdie benadering is gevolg omdat dit oneties sou wees om anaboliese steroïede aan gesonde vrywilligers toe te dien bloot om die invloed van probenesied op die uitskeiding daarvan te bestudeer.

'n Literatuuroorsig van toepaslike publikasies toon 'n gebrek aan inligting oor die invloed van probenesied op die urinêre uitskeiding van anaboliese steroïede asook endogene steroïede. 'n Loodsstudie, wat by die FARMOVS Navorsingssentrum, Departement Farmakologie, Bloemfontein uitgevoer is, het 'n betekenisvolle afname in urinêre uitskeiding van androsteroon aangetoon na inname van probenesied.

Twintig (20) gesonde manlik vrywilligers het aan 'n oop, enkel dosering, gerandomiseerde, tweerigting, oorkruis studie deelgeneem met 'n uitwas periode van 7 dae tussen behandelingsfases. Elke vrywilliger het elk van die volgende twee behandelings eenmalig ontvang op 'n ewekansige volgorde:

Behandeling A: Plasebo (kontrole fase) + 200ml water

Behandeling B: 4 x 500mg probenesied tablette (Benemied®) + 200ml water

Op profieldae (Dag 1 en 8) is proefmedikasie om 08:00 geneem met 200ml kraanwater. Van elke vrywilliger was verwag om daarna 200ml water uurliks vir die eerste vier uur te neem. Na geneesmiddel toediening is urine in fraksies versamel oor 'n tydperk van 48 uur vir die bepaling van androsteroon en probenesied konsentrasies.

Androsteroon is bepaal deur 'n gaschromatografiese-massa spektrometriese metode. Die resultate toon dat die laagste uitskeiding van androsteroon tussen 2 – 3 uur na toediening van

probenesied was. 'n Statisties betekenisvolle vermindering in die uitskeidingstempo van androsteroon kon tussen 2 en 8 uur waargeneem word. Die gemiddelde androsteroon uitskeidingstempo vir al die fraksionele versamelintervalle in hierdie tydperk (1 – 2h, 2 – 3h, 3 – 4h, 4 – 6h, 6 – 8h) was tussen 56% en 70% laer na die inname van Benemid® in vergelyking met plasebo. Aangesien hoë doserings probenesied die urinêre uitskeiding van androsteroon onderdruk kan dit verwag word dat dit ook die uitskeiding van ander anaboliese steroïede, wat as glukuroniedes uitgeskei word, sal onderdruk. Die konsentrasie van eksogene anaboliese steroïede is gewoonlik baie laag en dus kan probenesied hulle konsentrasie verlaag tot onder die deteksie limiet sodat hulle nie aangetoon kan word nie. Probenesied kan dus gebruik word as 'n maskeermiddel vir die uitskeiding van anaboliese steroïed glukuroniedes.

Hierdie onderdrukking van die renale uitskeiding van anaboliese steroïede deur probenesied word beskou as manipulasie van die urienmonster. Gevolglik het die Mediese Kommissie van die Internasionale Olimpiese Komitee probenesied as 'n verbode middel in sport verklaar.

Chapter 1

Introduction

CHAPTER 1

INTRODUCTION

INTRODUCTION

A literature survey of the relevant publications reveals a lack of information regarding the influence of probenecid on the urinary excretion of drugs in general and anabolic steroids in particular.

Probenecid is a uricosuric agent used in chronic gout to reduce the incidence and severity of attacks. Probenecid also reduces the renal tubular excretion of many other drugs, thereby increasing their plasma concentrations, and has therefore been used as an adjunct to antibacterial therapy (Reynolds *et al.,* 1996).

After oral intake, probenecid is rapidly absorbed from the gastro-intestinal tract and is extensively bound to plasma proteins (85 to 95%) (Reynolds *et al.,* 1996). Probenecid is almost completely metabolised in the liver and about 90% is excreted in the urine in the form of metabolites during the first 48 hours (Moffat *et al.,* 1986).

Adverse events most commonly experienced during treatment with probenecid include headache, nausea and vomiting, dizziness, flushing, skin rashes and urinary frequency (Reynolds *et al.,* 1996).

The oral formulation of probenecid (Benemid®) is tablets containing 500mg probenecid. It is usual to start treatment for gout with oral doses of 250mg twice daily, increased after a week to 500mg twice daily, if the therapeutic effects are unadequate followed by 500mg increments every 4 weeks up to 2g daily. The usual dosage for reducing tubular excretion of penicillins and cephalosporins is 500mg four times daily (Reynolds *et al.,* 1996).

In 1994, a pilot study was conducted with five healthy male volunteers at the Department of Pharmacology, University of the Orange Free State, to determine the influence of probenecid on the urinary excretion of androsterone. In this cross-over study doses of 500, 1000 and 2000mg were administered and it led to a decrease of urinary androsterone as illustrated in Figure 1.1.

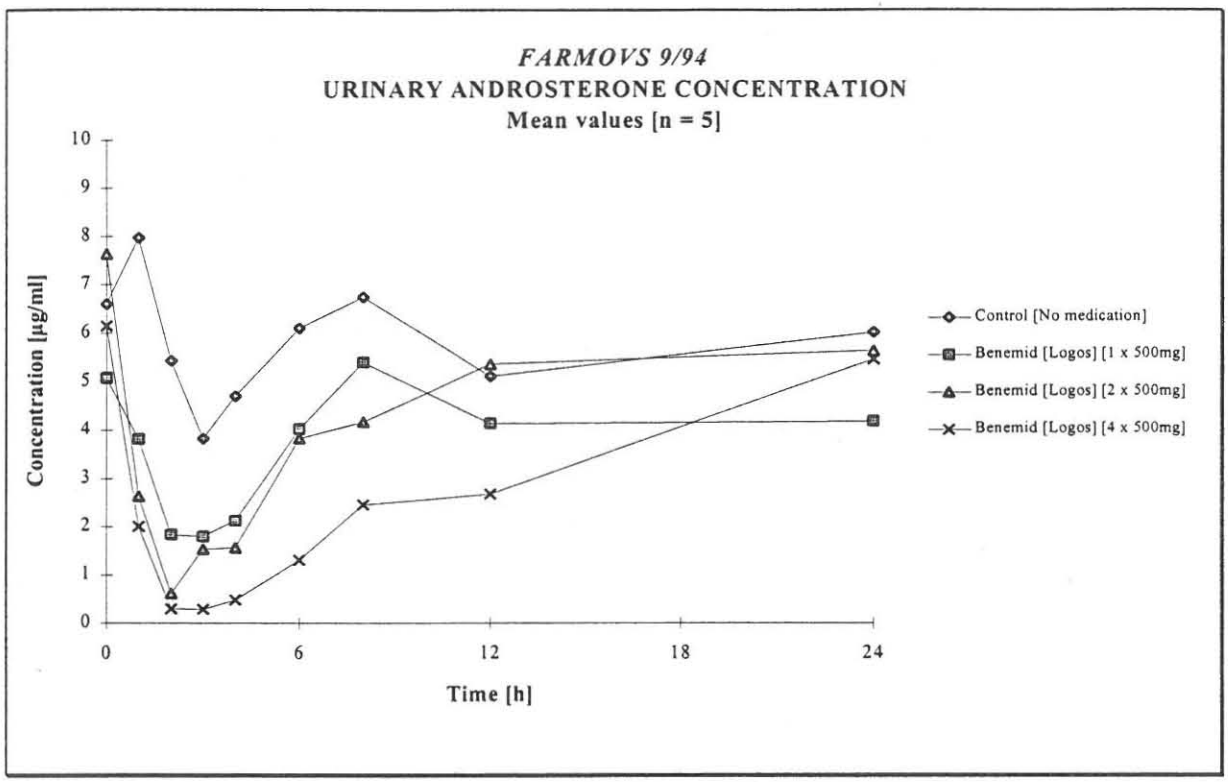


Figure 1.1 Graphic representative of mean urinary androsterone concentration (µg/ml) (n=5) against time (h) after a single administration of 500, 1000 and 2000mg probenecid (Benemid®) (FRM 9/94).

The use of probenecid in sport was first detected in 1987 in a routine control test of 5 athletes. In addition to the presence of probenecid unusually low concentrations of endogenous steroids in the urine samples were observed. The mean of the urinary androsterone concentration of the 5 athletes was 34ng/ml whereas the normal concentration of androsterone in urine of athletes ranges from 375ng/ml to 5323ng/ml.

Probenecid [p-(dipropylsulphamoyl) benzoic acid] occupies a central place in the field of organic anion transport because of its inhibitory potency as a classical competitive inhibitor of organic acid transport. Since most of the anabolic steroids, as well as their metabolites, are excreted in urine as their glucuronides, one may expect that probenecid inhibits the renal excretion of anabolic steroids. Therefore, competitors in sport taking exogenous anabolic steroids could use probenecid as a masking agent for the excretion of these compounds. Androsterone is an endogenous steroid and since it is excreted in high concentrations in urine, it was used as a representative of anabolic-androgenic steroids in this study. Androsterone

was also used because it will be unethical to give anabolic steroids to healthy volunteers just to study the influence of probenecid on the excretion of anabolic steroids.

The results of the pilot study necessitate a follow-up study with a sufficient number of volunteers to obtain statistical proof of the extent of the suppression of androsterone excretion by probenecid.

Chapter 2

Literature Survey

Chapter 2

Literature Survey

LITERATURE SURVEY

Probenecid is the classical competitive inhibitor of organic acid transport in the kidney and other organs. There are two primary clinical uses for probenecid: as a uricosuric agent in the treatment of chronic gout and as an adjunct to enhance blood levels of antibiotics. Most of the drug-drug interactions involving probenecid are due to an effect on the kidney-block of transport of acidic drugs. Similarly probenecid affects the tubular secretion of a number of acidic endogenous substances by the kidney (Cunningham *et al.,* 1981).

2.1 PHYSICAL AND CHEMICAL PROPERTIES

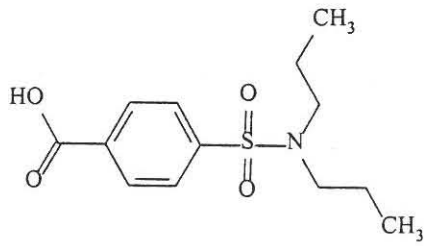
2.1.1 PROBENECID

Chemical name : [p-(dipropylsulphamoyl) benzoic acid]

Chemical composition : $C_{13}H_{19}NO_4S$

Molecular weight : 285.36

Structural formula :

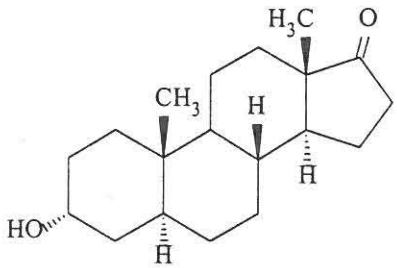


Physical properties: : A white crystalline powder with a melting point of 198° to 200°C. Practically insoluble in water; slightly soluble in ethanol and acetone; soluble in chloroform.

(Source: Moffat *et al.,* 1986; 923)

2.1.2 ANDROSTERONE

Chemical name	:	3α-hydroxy-5α-androstan-17-one
Chemical composition	:	$C_{19}H_{30}O_2$
Molecular weight	:	290.4
Structural formula	:	



Physical properties	:	A white crystalline powder with a melting point of 185°C. Practically insoluble in water; soluble in ethanol, ether and in most organic solvents.

(Source: Moffat *et al.*, 1986; 354)

2.2 PHARMACOKINETIC PROPERTIES

Probenecid is highly lipid-soluble; Kp (chloroform/0.15 M HCl) > 2000. The pKa of probenecid is 3.4 (aqueous medium) and 5.8 (aqueous-methanol). The binding of probenecid to human albumin and plasma is high (range of 83 to 95% for concentrations of 20 - 176µg/ml respectively); the binding to human globulins is lower (28 - 33%). Probenecid exhibits type I binding spectra when mixed with rat liver micorsomal fractions (Cunningham *et al.*, 1981).

2.2.1 ABSORPTION

Absorption of probenecid following oral administration (0.5 to 2g, as free acid in gelatin capsules or as tablets) is rapid with peak concentrations occurring in 1 to 5 hours (Cunningham *et al.*, 1981 , Selen *et al.*, 1982). Urinary elimination (>80% in 40 hours) of ^{14}C following oral administration of ^{14}C-probenecid further indicates completeness of absorption. It is to be noted that in children, peak plasma levels following oral administration on probenecid (2.5mg/kg) occur in 3 to 9 hours. The peak action (as determined by the effect on penicillin plasma levels) after an oral dose of probenecid occurs at about 2 hours and the action lasts for about 8 hours (Cunningham *et al.*, 1981).

2.2.2 DISTRIBUTION

After intravenous administration, probenecid distributes rapidly with an apparent volume of distribution (Vd) of about 11L. Cunningham *et al.*, (1981) published the following pharmacokinetic parameters of probenecid in patients:

Table 2.1 Pharmacokinetic parameters of probenecid in patients.

PATIENT	WEIGHT (kg)	IV DOSE (g)	$t_{½}$ (h)	Vd (L/kg)	K_{el}[1] (h^{-1})	Cl_{total}[2] (ml/min)
1	73	2.0	12	0.162	0.059	11.6
1	73	0.5	8	0.128	0.087	13.5
2	68	2.0	6	0.137	0.12	17.9
2	72	0.5	3	0.122	0.23	33.8
3	76	2.0	6	0.175	0.12	25.5
4	76	0.5	4	0.173	0.17	37.3

[1] K_{el} = rate constant of elimination from plasma.
[2] Cl_{total} = total body clearance. With normally acid urine, the renal clearance of probenecid is about 1 to 2 ml/min; faecal excretion is low (<20% of dose). Thus, metabolism accounts for about 90% of the total body clearance of probenecid.

Following oral administration (500mg, q 6h) for 4 weeks, the mean plasma level of probenecid was 22.5µg/ml (of n = 19 patients). The drug binds extensively to plasma proteins. In man, probenecid may inhibit the efflux of endogenous compounds and co-administered drugs from the eye. The kidney, and especially renal tubules, may accumulate probenecid since renal cortical slices are known to concentrate the drug (Cunningham *et al.*, 1981).

2.2.3 METABOLISM

2.2.3.1 PROBENECID

In man, the biotransformation of probenecid involves oxidation of alkyl side chains (about 70%) and glucuronide conjugation (about 20%). Cunningham *et al.*, (1981) published the following metabolism of probenecid:

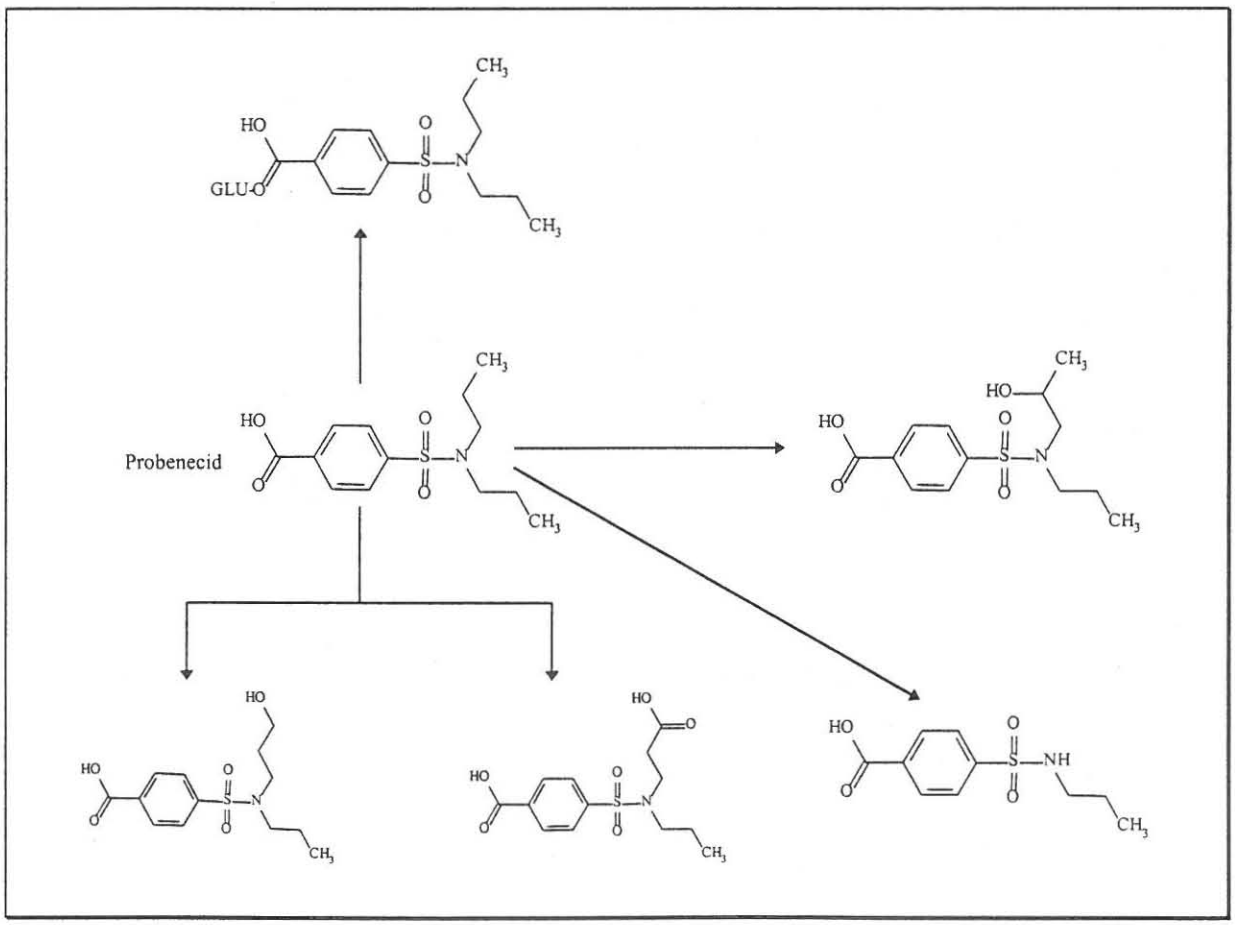


Figure 2.1 Metabolism of probenecid (Cunningham *et al.*, 1981).

The concentrations of oxidised metabolites are much lower (about 10% of the circulating dose) than the parent drug in human plasma. The metabolites have lower binding (59 to 81%) to plasma proteins and lower lipid solubility than probenecid; this accounts for the rapid clearance of probenecid metabolites. Self-stimulation of probenecid metabolism has been reported in animals. Although the fate of probenecid in man appears to be totally explained by side chain metabolism, glucuronide conjugation and excretion of the unchanged drug, the possibility of aromatic ring oxidation was investigated (Cunningham *et al.*, 1981).

2.2.3.2 ANDROSTERONE

Androsterone is a naturally occurring androgen which may be isolated from male urine. It is a major metabolite of testosterone (Moffat *et al.*, 1986). In contrast to the diol metabolites of testosterone with a 17β-hydroxy structure (D-ring), the main metabolites, androsterone (3α-hydroxy-5α-androstane-17-one) and etiocholanolone (3α-hydroxy-5β-androstane-17-one), have a 17-keto-D-ring structure (Schänzer *et al.*, 1997). Schänzer *et al.*, (1997) published the following metabolism of testosterone:

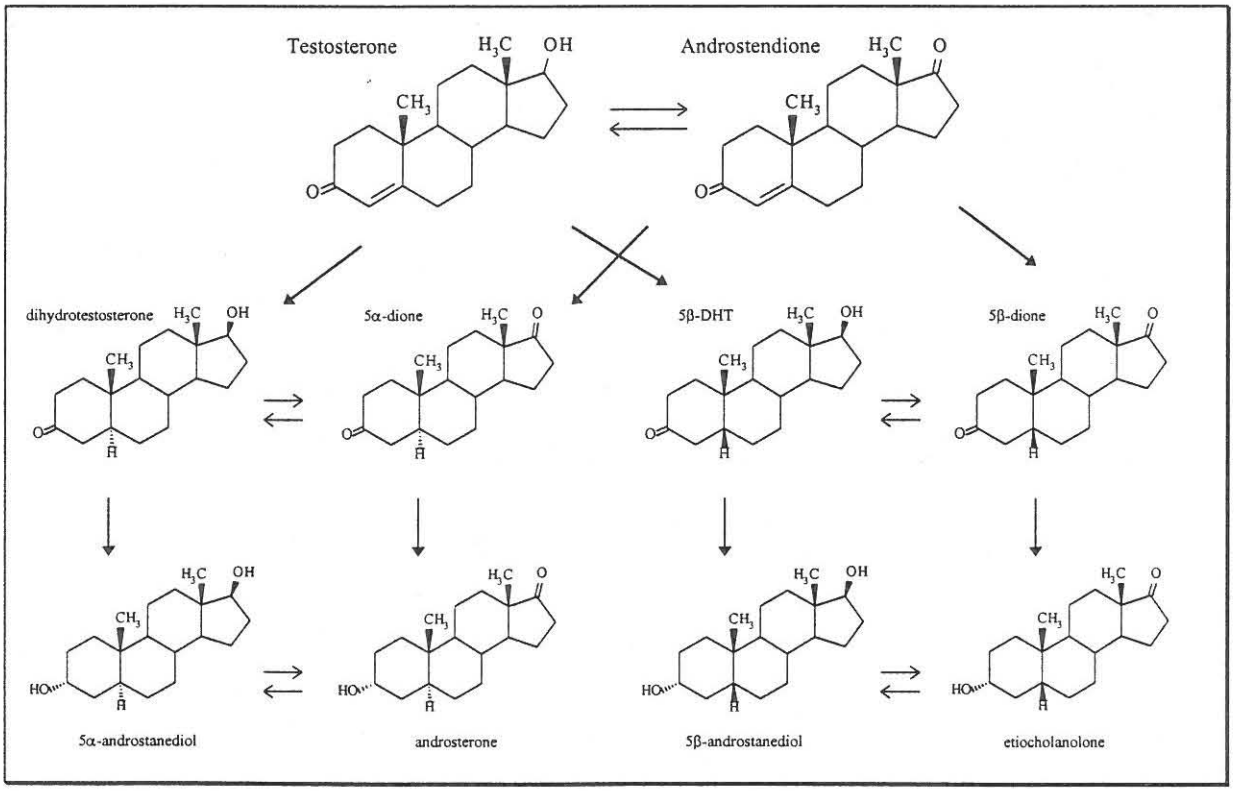


Figure 2.2 Metabolism of testosterone (Schänzer *et al.*, 1997).

2.2.4 HALF-LIFE

In most individuals the half-life of probenecid following a 2g dose (oral or intravenous) ranged between 6 to 12 hours. In man, dose dependence was observed in the elimination half-life given intravenously or orally. The plasma concentration decline being rapid at lower doses (half-life: 2 to 6 hours after 0.5 to 1g and 4 to 12 hours after 2g) (Cunningham *et al.*, 1981).

2.2.5 ELIMINATION

After a single doses of 0.5 to 2g of probenecid, 75 to 88% of the drug was recovered in urine in 4 days with only a small amount (5 to 11%) as the intact drug. The major metabolic product was excreted as probenecid-acyl glucuronide (16 to 47%) while the remainder of the metabolised drug was recovered in approximately equal amounts of the other metabolites (mono N-propyl, secondary alcohol, carboxyl and primary alcohol) in the free form (Cunningham *et al.*, 1981). Cunningham *et al.*, (1981) found the renal clearance of probenecid to be independent of dose but dependent on the pH and flow rate of urine.

2.3 PHARMACODYNAMICS

Probenecid is actively secreted into the kidney tubules, but is efficiently reabsorbed into the peritubular capillaries, and only a small fraction of the drug is cleared by this route (Selen *et al.*, 1982). It seems important that sufficiently high doses of probenecid must be used to produce a nearly complete blockade of transport. Probenecid is a competitive inhibitor of active transport processes by which acidic metabolites of noradrenaline, dopamine and serotinin are removed from the brain and cerebrospinal fluid. This active transport process is similar to that found in the kidney. The interaction of probenecid with drugs that influence urate transport are of particular interest. They suggested that two separate and simultaneous mechanisms are involved when antagonism of uricosuric activity occurs a) competition for transport, decreasing delivery of the second drug to the active site, and b) inhibition of urate

secretion. Other interactions of drugs with probenecid possibly involve competition for binding to plasma and tissue proteins, as well as effects on microsomal drug metabolising enzymes (Cunningham *et al.*, 1981).

2.3.1 PROBENECID AS A URICOSURIC AGENT

The uricosuric activity of probenecid was discovered in 1950 and the drug found to be of value in the treatment of chronic gout. At a dose of 1 to 2g of probenecid, uric acid clearance increases of 4- to 6-fold were found in man (Dayton *et al.*, 1963; Sirota *et al.*, 1952). The contribution of the metabolites of probenecid to the overall uricosuric activity of the drug is probably less significant than that of probenecid, due to the high renal clearance and elimination half-life of metabolites as shown in dogs (Cunningham *et al.*, 1981).

2.3.2 PROBENECID INTERACTION WITH ANTIBIOTICS

The co-administration of probenecid with a number of acidic compounds, particularly antibiotics, generally causes an increase in peak plasma concentrations and half-life and decrease in distribution half-life and urinary excretion of these compounds. The earliest clinical application of probenecid was as an adjunt to high dose penicillin therapy and in the treatment of turberculosis with para-aminosalicylic acid. The latter antibiotic is extensively conjugated in the liver with glycine; the conjugate is therapeutically active and is rapidly excreted by the kidney with renal clearance approximating that of renal blood flow. More detailed clinical studies of blood levels and excretion patterns of ampicillin, nafcillin and cephaloridine with and without administration of probenecid, showed that the action of probenecid at the renal level was not sufficient to account for several fold serum level elevations encountered (Cunningham *et al.*, 1981).

Cunningham *et al.*, (1981) also suggested that probenecid causes a significant decrease in the distribution volume of the antibiotic. Only in this way could one rationalise the marked effect on renal retention. This decrease in distribution volume would indicate that probenecid probably competes for drug binding sites in the kidney, liver and other tissues and/or blocks

transport of antibiotics into tissues. Although this hypothesis has not been fully tested, probenecid continues to be co-administered with many antibiotics. The tacit assumption is that enhanced tissue levels will follow when plasma levels are increased by whatever means.

Probenecid has also been co-administered with certain antibiotics of short biological half-life (cefoxitin, cefazolin and cephalothin) to prolong their plasma concentrations so that patients may be given doses of the antibiotic less frequently (8 hourly or longer). The possibility that target tissues may be exposed to lower concentrations of antibiotic than might be predicted from blood levels (when probenecid is concomitantly administered) has been pointed out by Cunningham *et al.* It has been shown, however, that probenecid does not affect the entry of antibiotics into fibrin clots in vitro (Cunningham *et al.,* 1981).

2.3.3 PROBENECID INTERACTION WITH FUROSEMIDE

Probenecid decreased the oral clearance, the renal clearance and the calculated non-renal clearance of furosemide, which may indicate that active secretion into bile, or the rate of hepatic glucuronidation is decreased. All three clearance parameters were reduced to approximately 35% of their baseline value. Impaired kidney function will reduce the renal secretion of both parent drug and conjugate. Reduction of kidney function by probenecid did not affect the percentage of the dose recovered as furosemide glucuronide; however, it slowed the elimination processes (Vree *et al.,* 1995).

2.3.4 PROBENECID INTERACTION WITH DYPHYLLINE

Dyphylline has been shown to be primarily eliminated by the kidneys, with 80 to 85% appearing unchanged in the urine. The kinetic parameters of dyphylline elimination in control subjects clearly implicate both active secretion and glomerular filtration. In spite of the concomitant use of probenecid, it is likely that a significant fraction of a dyphylline dose is still eliminated by the kidneys in unchanged form. Further studies on the interaction of probenecid and dyphylline are necessary to determine the clinical utility of concomitant dyphylline - probenecid use (May and Jarboe, 1983).

2.3.5 PROBENECID INTERACTION WITH ANABOLIC STEROIDS

The fact that the renal excretion of androsterone can be depressed by probenecid confirms the tubular secretion of this steroid, since probenecid is known to block the renal tubular secretion of compounds such as para-aminosalicylic acid and penicillin. The renal clearance of androsterone in man has been shown to be much greater than that of dehydroepiandrosterone. This explains the preponderance of dehydroepiandrosterone in human plasma on the basis of renal factors rather than on the basis of an inordinately high production of this steroid. The rapid renal clearance of androsterone accounts for its greater concentration in human urine and indicates that its over-all production is greater than that of dehydroepiandrosterone in man. The renal excretion of androsterone is inversely proportional to the elevated blood levels produced by its administration, and is inhibited by probenecid; therefore, androsterone is eliminated by tubular secretion rather than glomerular filtration. It appears unlikely that dehydroepiandrosterone is reabsorbed by the renal tubules after significant glomerular filtration, since its urinary excretion is not enhanced by probenecid. The renal clearance of beta-cortolone was also determined. Administration of this substance appeared to diminish the plasma levels of Porter-Silber chromogens in a single experiment (Bongiovanni and Eberlein., 1957).

Gardner *et al.*, (1951) reported that Benemid in doses of 2g per day per adult produced approximately 50% decrease in urinary excretion of 17-ketosteroids. Urinary 11-oxycorticosteroids did not appear to be affected by Benemid.

2.4 ADVERSE AVENTS AND ABUSE OF PROBENECID

Probenecid is generally well tolerated, though nausea, vomiting, anorexia, headache, sore gums, flushing, dizziness and urinary frequency may occasionally occur. Hypersensitivity reactions, with fever, dermatitis, prutitus, urticaria and rarely anaphylaxis have occurred. When used in chronic gout, probenecid may precipitate an acute attack, and renal calculi or renal colic with or without haematuria, may occur. It has been alleged that some athletes

using banned anabolic steroids have been taking probenecid in an attempt to inhibit the urinary excretion of steroid metabolites in order to avoid detection by urine screening tests (Reynolds *et al.*, 1996).

2.5 ANALYTICAL METHODS

2.5.1 PROBENECID

Conway and Melethil, (1975) developed a gas chromatograhic method for the detection of probenecid and its metabolites in biological fluids. The method involves direct extraction of the free acids from acidified urine by methylene dichloride or acid hydrolysis of conjugated metabolites prior to extraction, addition of N,N-dibenzyl-(2,5-dimethylbenzenesulfonamide) as an internal standard, followed by gas chromatography using flame ionization detection.

Hekman *et al.*, (1980) felt the need to develop a rapid and sensitive quantitative method for the detection of probenecid in plasma and urine, because of the various older spectrophotometric methods that have been reported lack the required sensitivity and specificity. They used high performance liquid chromatography and the chromatography is performed in a soap chromatography mode using a C_8 hydrocarbon stationary phase. As internal standards the diethyl analog (diethylsulfamoyl benzoic acid) and the diisobutyl analog (diisobutylsulfamoyl benzoic acid) of probenecid were applied. This method of probenecid analysis was successfully applied to pharmacokinetic studies.

Campíns-Falcó *et al.*, (1994) used a column-switching technique for the screening of probenecid in urine samples. This method was based on high performance liquid chromatography using column-switching and a pre-column for the on-line sample cleanup and enrichment. Untreated urine samples were directly injected and the pre-column flushed with water to eliminate polar matrix components. The method is selective and sensitive enough to detect probenecid in urine and the proposed column-switching system leads to a considerable saving of both chemicals and time.

2.5.2 ANDROSTERONE

The first comprehensive and sensitive gas chromatographic-mass spectrometric screening procedure for the detection of anabolic as well as endogenous steroids was developed by Donike (Donike *et al.*, 1993).

Massé *et al.*, (1989) also developed a gas chromatographic-mass spectrometric method for anabolic and endogenous steroids in urine. This method involved solid phase extraction with 5α-androstan-17-one as external standard. For the specific purpose of gas chromatographic-mass spectrometric screening, where specificity and sensitivity were the major analytical requirements, trimethylsilyl enol-trimethylsilyl ether derivatives were prepared using trimethylsilyl iodide as catalyst.

Central University of Technology, Free State

CHAPTER 3

STUDY METHODS

STUDY METHODS

This study was conducted as an open, single-dose, randomised, two way, cross-over study with a drug-free interval of 7 days between treatment phases. Subjects were randomly assigned to treatment order.

3.1 STUDY POPULATION

3.1.1 NUMBER OF SUBJECTS

Twenty (20) healthy, male subjects who fulfilled the inclusion criteria (Section 3.1.2), did not meet any of the exclusion criteria (Section 3.1.3), and who gave written informed consent, entered the study. Subjects were recruited from the Department of Pharmacology.

3.1.2 INCLUSION CRITERIA

a) Healthy male volunteers between 18 and 45 years of age.

b) Body weight within 10% of the ideal weight according to the Body Mass Index (BMI) (Appendix 2).

c) Findings within the range of clinical acceptability in medical history and physical examination and laboratory results within the normal ranges for the relevant laboratories, unless the clinical investigator considers the degree of abnormality to be clinically irrelevant for the purpose of the study.

d) Normal vital signs or abnormalities which the clinical investigator does not consider a disqualification for participation in the study.

e) Willing to undergo pre- and post-study medical examinations and laboratory investigations.

f) Subjects willing and able to sign voluntarily an informed consent form at the initial screening visit.

3.1.3 EXCLUSION CRITERIA

a) Evidence of psychiatric disorder, antagonistic personality, poor motivation, emotional or intellectual problems likely to limit the validity of consent to participate in the study, or the ability to comply with protocol requirements.

b) Abuse or regular use of alcohol or medication, use of any medication up to 2 weeks, or participation in another study with an experimental drug witin 8 weeks before the study.

c) Treatment within the previous 3 months with any drug with a well-defined potential for toxicity in a major organ or system (for example, chloramphenicol, which may cause bone marrow suppression).

d) A major illness during the 3 months before commencement of study-related procedures.

e) History of hypersensitivity to the study drug or any related drug.

f) History of presence of gastro-intestinal, liver, or kidney disease, or other conditions known to interfere with the absorption, distribution, metabolism or excretion of drugs.

g) History of bronchial asthma.

3.1.4 WITHDRAWAL CRITERIA

a) Subjects not wishing to continue with the study, irrespective of the reason.

b) Unwanted effects from the study drug.

c) Abnormal findings in laboratory investigations of physical examinations.

d) Intercurrent illness requiring medication or invalidating data.

e) Protocol violation by subjects.

f) Pathologically raised body temperature on clinic days.

3.1.5 PRE-STUDY SCREENING

Subjects were examined within 2 weeks before the study and assessed for their ability to participate. The examinations and investigations included:

(i) Medical history and physical examination.

(ii) Measurements of height and body mass.

(iii) **Haematology:** leucocytes, erythrocytes, haemoglobin, haematocrit, platelets, neutrophils, eosinophils, lymphocytes.

(iv) **Clinical chemistry:** potassium, urea, creatinine, uric acid, calcium, protein, albumin, total bilirubin, ALP, GGT, AST, ALT, glucose.

(v) **Urinalysis (Multistix® SG):** pH, specific gravity, protein, glucose, ketones, bilirubin, blood, urobilinogen.

3.1.6 POST-STUDY CLINICAL AND SAFETY EVALUATIONS

Haematological and clinical investigations were repeated within 72 hours of completion of the final phase of the study.

3.1.7 RESTRICTIONS ON SUBJECTS

With the exception of the study drugs, all subjects had to refrain from taking any medicines, including those sold over the counter, for 2 weeks before the study started and for the duration of the study (Section 3.1.3). Ingestion of foods and beverages containing alcohol or caffeine, as well as hazardous, strenuous or athletic activities were not permitted for 24 hours before and until 24 hours after drug administration on clinic days. Subjects were, as far as possible, recruited from members of the Department of Pharmacology, who proceeded with their normal daily work after drug administration in the FARMOVS Research Centre. Food and fluid intake was standardized to minimize variations within and among individual subjects until 13:00 on the profile day. Subjects received 200ml water hourly for the first four hours after drug administration.

3.1.8 DROP-OUTS

One subject failed to complete the study. A total of nineteen (19) volunteers completed the study.

3.2 STUDY DRUGS

3.2.1 TREATMENTS AND DOSAGES

Each subject received each treatment once. Subjects were allocated to treatment order according to the randomisation schedule (Appendix 1).

The two treatments were:

Treatment A : Placebo (control phase)

Treatment B : Probenecid

Source

Trade name : Benemid®

Manufacturer : Logos Pharmaceuticals [LOGOS]

Dosage form : Tablets containing 500mg probenecid

Dose : 2000mg once

3.2.2 DOSAGE INSTRUCTIONS

After an overnight fast of at least 10 hours, subjects received the relevant product (4 tablets) with 200ml tap water according to the randomisation schedule. Dosing was staggered at specific intervals, starting at 08:00 with the first subject.

3.2.3 RANDOMISATION SCHEDULE

The randomisation schedule was generated using RANDOM (Version 1.1), a locally developed program written in MS FORTRAN (Version 5.1) which runs under the MS-DOS operating system and is included in Appendix 1.

3.2.4 SUPPLY, STORAGE AND DISPENSING

All study drugs were stored in a limited access area in which the temperature was regulated. Administration of the drug on clinic days was supervised by the clinical investigator.

3.2.5 PACKAGING, LABELLING AND RANDOMISATION

Packaging, labelling and randomisation of the study was carried out by the pharmacist from FARMOVS Research Centre. Each single dose was retained in a separate container with a label indicating the study number, subject's number, treatment and treatment phase.

3.3 STUDY PERFORMANCE

The study was carried out in the FARMOVS Research Centre, University of the Orange Free State, Bloemfontein, Republic of South Africa and was designated as FARMOVS 9/96. Subjects received detailed instructions of the study performance, restrictions, obligations, remunerations and possible adverse events that may be experienced as a result of taking the study drugs.

3.3.1 TREATMENT PHASES

The study consisted of 2 treatment phases. Each included a single-dose run-in period of 2 days (Days 1 and 8 - profile days).

Run-in peroid (profile days)

During the run-in peroids, placebo or Benemid® (2000mg), as laid out in the randomisation plan, was administered to subjects at 08:00, swallowed with 200ml tap water at room temperature.

Subjects were, as far as possible, recruited from members of the Department of Pharmacology, who proceeded with their normal daily work after drug administration in the FARMOVS Research Centre. Food and fluid intake were standardized to minimize variations within and among individual subjects until 13:00 on the profile day, thereafter food and fluid intake was allowed ad libitum. Subjects received 200ml water hourly for the first four hours after drug administration.

Urine sampling

Urine was collected in large labelled plastic bottles. Immediately before drug administration, subjects emptied their bladders completely at completion of the -12-0 interval. Urine collections were made during the following intervals before and after drug administration: -12-0, 0-1, 1-2, 2-3, 3-4, 4-6, 6-8, 8-12, 12-24, 24-30, 30-36 and 36-48 hours (12 samples per subject per treatment phase). The actual urine sampling times were documented in the case report forms (CRF's). From each fraction the pH, SG and creatinine were measured. The volumes of collected urine were recorded within 30 minutes and 2 aliquots (20ml) of each urine sample were transferred to labelled tubes. All samples were handled at room temperature and stored at -20°C until probenecid and androsterone were assayed.

3.3.2 SPECIAL INVESTIGATIONS

All clinical chemistry investigations were performed in FARMOVS Research Centre and haematological investigations in the Department of Haematology, University of the Orange Free State, Bloemfontein, Republic of South Africa.

3.4 MANDATORY CONSIDERATIONS

3.4.1 ETHICAL AND INSTITUTIONAL REVIEW

This project was reviewed and approved by a Faculty Research Committee at the Technicon Free State. Approval by the Ethics Committee of the Faculty of Health Sciences of the University of the Orange Free State was given before commencement of the study-related procedures.

3.4.2 SUBJECT INFORMATION AND INFORMED CONSENT

Before the study, the nature, purpose and risk of the research involved were explained to all subjects. They were informed that they may withdraw from the screening procedures or the study-specific procedures at any time and for any reason. They signed both FARMOVS informed consent forms in the presence of the clinical investigator and a witness after sufficient time for deliberation had been provided.

3.4.3 ADVERSE EVENTS/ SERIOUS ADVERSE EVENTS

Clinical adverse events or serious clinical adverse events are any undesirable experience occuring in a subject during a clinical study, whether or not it is thought to be associated with the study drug. These include illnesses, subjective and objective signs or symptoms (including important abnormal laboratory values as well as significant shifts from baseline within the range of normal, which the investigator considers to be clinically important), which have appeared or worsened during the course of a study.

3.4.4 GOOD CLINICAL PRACTICE/QUALITY ASSURANCE

The conduct of the study conformed with the recommendations for clinical studies in man as set out in the 1989 Hong Kong Revision of the "Declaration of Helsinki" (Appendix 4), the local legal requirements, and in accordance with the guidelines on "Good Clinical Practice for

Trials on Medicinal Products in the European Community" (CPMP Working Party on Efficacy of Medicinal Products, 1990). Internal auditing by the Quality Control and Quality Assurance officers of FARMOVS was carried out at all stages of the study. A Quality Assurance Statement is included in Appendix 5.

3.4.5 INDEMNITY

Insurance was arranged to cover the subjects in the event of death or any deterioration in health or well-being caused by participation in the study. The certificate of insurance is presented in Appendix 3.

3.4.6 CONFIDENTIALITY

All information obtained during the conduct of the study with respect to the subject's state of health is regarded as confidential. An agreement for disclosure of any such information was obtained in writing and was included in the informed consent form.

3.4.7 REMUNERATION OF SUBJECTS

Compensation was made for loss of time and inconvenience as a result of participation in the study.

Chapter 4

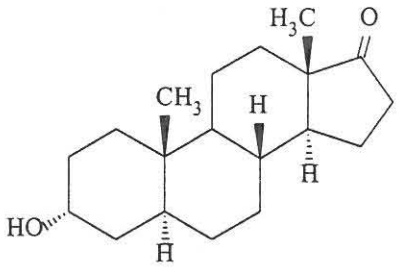
Analytical Methods

ANALYTICAL METHODS

4.1 CHEMICAL OVERVIEW

4.1.1 ANALYTE I - ANDROSTERONE

Chemical name	:	3α-hydroxy-5α-androstan-17-one
Chemical composition	:	$C_{19}H_{30}O_2$
Molecular weight	:	290.4
Structural formula	:	

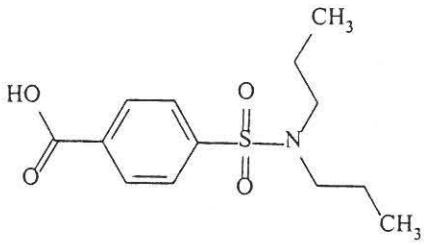


Analytical standard	:	Androsterone, Department of Pharmacology, pure substance.

4.1.2 ANALYTE II - PROBENECID

Chemical name	:	[p-(dipropylsulphamoyl) benzoic acid]
Chemical composition	:	$C_{13}H_{19}NO_4S$
Molecular weight	:	285.36

Structural formula :



Analytical standard : Probenecid, Department of Pharmacology, pure substance.

4.1.3 INTERNAL STANDARD FOR ANDROSTERONE

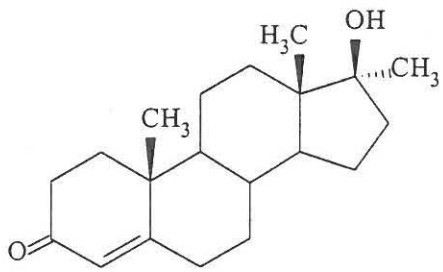
17α-METHYLTESTOSTERONE

Chemical name : 17α-methyl-4-androsten-17β-ol-3-one

Chemical composition : $C_{20}H_{30}O_2$

Molecular weight : 302.2

Structural formule :

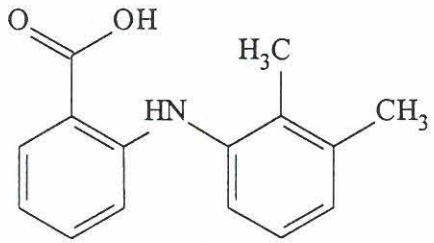


Analytical standard : 17α-methyltestosterone, Department of Pharmacology, pure substance.

4.1.4 INTERNAL STANDARD FOR PROBENECID

MEFENAMIC ACID

Chemical name	:	2-[(2,3-dimethyl(phenyl)amino]-benzoic acid
Chemical composition	:	$C_{15}H_{15}NO_2$
Molecular weight	:	241.3
Structural formula	:	



Analytical standard	:	Mefenamic Acid, Department of Pharmacology, pure substance.

4.2 MATERIALS

4.2.1 CHEMICALS AND REAGENTS

Water was purified by RO 20SA (Millipore) reverse osmosis and Milli-Q® (Millipore) polishing system (Waters Assoc., Milford, MA, U.S.A.). All listed chemicals are of Pro Analyse quality or better, unless stated otherwise (Table 4.1).

Table 4.1 List of chemicals and reagents used.

REAGENT/CHEMICAL	SUPPLIER	REF. NO.
Acetone	Merck	000014.25
Ammonium iodide	Merck	1173
Diethylether	Merck	UN 1863040
1,4-Dithioerythritol	Merck	24511
Ethyl acetate	Merck	9623
β-glucuronidase [E.Coli]	Boehringer	127680
Methanol	Merck	6009
N-methyl-N-trimethylsilyl-trifluroacetamide	Sigma	M7891
Potasium carbonate	Merck	4928
Potasium dihydrogen phosphate	Merck	4873
Sodium hydrogen carbonate	Merck	Art. 6329
Sodium hydrogen phosphate	Merck	6586
Toluene	Merck	8325

4.2.2 BUFFERS

4.2.2.1 PHOSPHATE BUFFER PH 7

- Dissolve 14.196g sodium hydrogen phosphate in distilled water and dilute to 1 liter (1).
- Dissolve 13.609g potasium dihydrogen phosphate in distilled water and dilute to 1 liter (2).
- To 1 liter of (1) add sufficient of (2) to bring the pH to 7. Store at 4°C and boil before use.

4.2.2.2 POTASIUM CARBONATE BUFFER PH 9.6

- Dissolve 20g potassium carbonate and 20g sodium hydrogen carbonate in 160 ml distilled water.

4.3 APPARATUS

Standard laboratory glassware and equipment were used, with in addition:

- Aluminium caps
- Balances
  - Precisa (6200 D SCS)
  - Mettler (M3)
  - Mettler (PM 400)
- Disiccator with vacuum facility
- Disposable glass pipettes (Pasteur ISO 7712)
- Electric heating block with adjustable thermostat (Thermolyne Type 17600)
- Glass injector vials with glass inserts (Anatech)
- Glass syringes (Hamilton)
- Heating module thermostat adjustable with nitrogen facility (Techne Dri-Block® DB-3D)
- Heraeus Labofuge 400 centrifuge (Function Line)
- Horizontal shaker (Kotterman)
- 5ml Open amber ampoules (Anchor Rand)
- pH-meter (Mettler Toledo)
- Semi-automatic pipettes (Adjustable sample systems)
- Sep-Pack C_{18} cartridge (Waters, Millipore)
- Vortex mixer (Thermoline Type 16700)

4.4 EXTRACTION PROCEDURES

4.4.1 ANDROSTERONE

One (1) ml urine and one (1) ml distilled water were pipetted into a 5ml ampoule. To the mixture, 1ml phosphate buffer pH 7 and 25µl β-glucuronidase enzyme (E.Coli) were added and hydrolysis was performed for 2 hours at 50°C. A Sep-Pak C_{18} cartridge was activated by washing with 5ml methanol followed by 5ml distilled water. One (1) ml of the hydrolised

urine solution was added to Sep-Pak C_{18} cartridge. The column was washed with 5ml of distilled water and the absorbed fraction was eluted with 3ml of methanol. As internal standard, 50µl 17α-methyltestosterone was added to the solution. The methanolic eluate was evaporated to dryness and the residue was dissolved in 1ml of phosphate buffer pH 7. The buffered solution was alkalified with 200µl potasium carbonate solution to pH 9 - 10 and the androsterone was extracted with 4ml of diethyl ether by vortexing for 30 seconds. After centrifuging for 5 minutes at 2000 rpm the organic layer was transferred to a 5ml ampoule and evaporated to dryness under a stream of high purity nitrogen. The ampoules were left in an evacuated desiccator for at least 60 minutes.

Derivatization

40µl of the derivatization solution [10ml N-methyl-N-(trimethylsilyl)-trifluoroacetamide + 20mg ammonium iodide + 40mg 1,4-dithioerythritol (1000:2:4 v/m/m)] was added to the dry residue, the ampoule was sealed and 15 minutes reaction time was allowed at 60°C. 200µl toluene was added and 1µl of the solution was injected onto the GC/MSD in splitless mode (time to purge 0.5 min).

4.4.2 PROBENECID

A Sep-Pak C_{18} cartridge was activated by washing with 5ml methanol followed by 5ml distilled water. A half (0.5ml) ml urine was added to Sep-Pak C_{18} cartridge. The column was washed with 5ml of distilled water and the absorbed fraction was eluted with 2ml of methanol. The methanolic eluate was evaporated to dryness and the residue was dissolved in 1ml of phosphate buffer pH 7. To the buffer solution, 25µl β-glucuronidase enzyme (E.Coli) was added and hydrolysis was performed for 1 hour at 50°C. As internal standard, 50µl mefenamic acid was added to the buffered solution and the probenecid was extracted with 1.5ml ethylacetate and 2.5ml diethyl ether by vortexing for 30 seconds. After centrifuging for 5 minutes at 2000 rpm, the organic layer was transferred to a 5ml ampoule and evaporated to dryness under a stream of high purity nitrogen. The ampoules were left in an evacuated desiccator for at least 60 minutes. The residue was dissolved in 200µl acetone, 50mg potassium carbonate and 25µl iodomethane were added. The ampoule was sealed and 30

minutes reaction time was allowed at 60°C. The solvent was transferred to another ampoule and evaporated to dryness under a stream of high purity nitrogen. The residue was dissolved in 500µl toluene and 1µl was injected into a gas chromatograph.

4.5 INSTRUMENTAL AND CHROMATOGRAPHIC CONDITIONS

4.5.1 ANDROSTERONE: GAS CHROMATOGRAPH-MASS SELECTIVE DETECTOR

A Hewlett-Packard 6890 gas chromatograph with 6890 mass selective detector (GC/MSD), fitted with a ultra-1 crosslinked methyl silicone column: 16.5m x 0.2mm i.d., 0.11µm film thickness was used (Hewlett-Packard).

- Injection was performed by a Hewlett-Packard Model 6890 auto sampler.
- Injection port temperature : 250°C
- Injection mode : Splitless
- Oven program :

Initial temp 1	:	100°C
Initial time 1	:	0.0
Rate 1	:	30°C/min
Int temp 2	:	180°C
Int time 2	:	0.0
Rate 2	:	4°C/min
Int temp 3	:	250°C
Int time 3	:	0.0
Rate 3	:	30°C/min
Final temp	:	300°C
Final time	:	0.5min
Oven max	:	325°C

- Carrier gas : Helium (20ml/min)
- Capillary column head pressure : 8.8 psi
- Flow : Constant flow

Recording and integration were done by means of a Hewlett-Packard Vectra Workstation and the results printed on a Hewlett Packard Laser Jet 4 Printer. All data were captured and stored, both in electronic form and as paper print-outs.

Retention times

Androsterone	:	11.04 - 11.21 min
17α-Methyltestosterone	:	14.45 - 14.64 min

4.5.2 PROBENECID : GAS CHROMATOGRAPH

A Hewlett-Packard 5890 A gas chromatograph, fitted with a Chrompack, WCOT fused silica, CP SIL 19CB column: 15m x 0.32mm i.d., 0.25µm film thickness was used (Hewlett-Packard).

- Injection was performed by a Hewlett-Packard Model 6890 auto sampler.
- Injection temperature : 220°C
- Injection mode : Splitless
- Oven program :

Initial temp 1	:	80°C
Initial time 1	:	0.0
Rate 1	:	30°C/min
Final temp	:	280°C
Final time	:	0.5min
Oven max	:	300°C

- Detector : Nitrogen-phosphorous
- Detector temperature : 280°C

Recording and integration were done by means of a Hewlett-Packard Vectra Workstation and the results printed on a Hewlett Packard Deskjet 600 Printer. All data were captured and stored, both in electronic form and as paper print-outs.

Retention times

Probenecid	:	5.125 - 5.179 min
Mefenamic Acid	:	4.533 - 4.544 min

4.6 STABILITY

4.6.1 STABILITY ON STANDARD SOLUTIONS

4.6.1.1 ANDROSTERONE

Standard solutions were freshly made up in methanol and used immediately to spike phosphate buffer pH 7 for the preparation of calibration standards and quality controls. Phosphate buffer was used instead of urine because androsterone is an endogenous steroid and therefore present in all urine samples. 17α-methyltestosterone (internal standard) was also made up in methanol and stored at -20°C.

4.6.1.2 PROBENECID

Standard solutions were freshly made up in methanol and used immediately to spike urine for the preparation of calibration standards and quality controls. Mefenamic acid (internal standard) was also made up in methanol and stored at -20°C.

4.6.2 ON-INSTRUMENT STABILITY

4.6.2.1 ANDROSTERONE

The on-instrument stability was obtained from the quality control validation for androsterone (Table 4.2).

4.6.2.2 PROBENECID

The on-instrument stability was also obtained from the quality control validation for probenecid (Table 4.3). The downward trend of about 35% in the peak areas over nearly 12 hours was attributed to a change of sensitivity of the NPD detector. This change did not influence the ratio of probenecid:internal standard as can be seen in Table 4.3.

Table 4.2 Summary of on-instrument stability of androsterone.

ON-INSTRUMENT STABILITY OF ANDROSTERONE

QUALITY CONTROL B

	PEAK AREA OF ANALYTE	PEAK AREA OF INT. STD.	PEAK AREA RATIO	TIME OF INJ.
	106	3475	0.0305	18:01
	109	3459	0.0315	22:26
	127	3941	0.0322	2:51
	127	3896	0.0326	7:16
	122	3696	0.0330	11:39
n	5	5	5	
Mean	118	3693	0.032	
SD	10	226	0.001	
CV(%)	8.49	6.13	3.09	

QUALITY CONTROL E

	PEAK AREA OF ANALYTE	PEAK AREA OF INT. STD.	PEAK AREA RATIO	TIME OF INJ.
	12406	3811	3.2553	19:21
	13039	3990	3.2679	23:46
	13648	4146	3.2918	4:11
	14368	4483	3.2050	8:35
	13182	3988	3.3054	12:58
n	5	5	5	
Mean	13329	4084	3.265	
SD	731	253	0.039	
CV(%)	5.49	6.19	1.19	

QUALITY CONTROL G

	PEAK AREA OF ANALYTE	PEAK AREA OF INT. STD.	PEAK AREA RATIO	TIME OF INJ.
	51836	4236	12.2370	20:14
	54618	4300	12.7019	00:39
	55281	4202	13.1559	05:04
	51040	4111	12.4155	09:28
	51563	4027	12.8043	13:51
n	5	5	5	
Mean	52868	4175	12.663	
SD	1936	107	0.356	
CV(%)	3.66	2.57	2.81	

Table 4.3 Summary of on-instrument stability of probenecid.

ON-INSTRUMENT STABILITY OF PROBENECID

QUALITY CONTROL A

	PEAK AREA OF ANALYTE	PEAK AREA OF INT. STD.	PEAK AREA RATIO	TIME OF INJ.
	206230	1184476	0.1741	19:08
	147061	811530	0.1812	21:02
	147163	791121	0.1860	22:56
	134097	715516	0.1874	0:50
	132254	696470	0.1899	2:33
n	5	5	5	
Mean	153361	839823	0.184	
SD	30372	198721	0.006	
CV(%)	19.80	23.66	3.40	

QUALITY CONTROL C

	PEAK AREA OF ANALYTE	PEAK AREA OF INT. STD.	PEAK AREA RATIO	TIME OF INJ.
	625792	1069257	0.5853	18:45
	580647	1049926	0.5530	20:39
	472981	853869	0.5539	22:33
	396095	707820	0.5596	0:27
	365205	642756	0.5682	2:10
n	5	5	5	
Mean	488144	864726	0.564	
SD	113270	193740	0.013	
CV(%)	23.20	22.40	2.37	

QUALITY CONTROL F

	PEAK AREA OF ANALYTE	PEAK AREA OF INT. STD.	PEAK AREA RATIO	TIME OF INJ.
	3995866	940803	4.2473	18:01
	3761157	919869	4.0888	20:05
	3847918	912626	4.2163	21:59
	3187401	766345	4.1592	23:53
	2978492	687263	4.3338	01:36
n	5	5	5	
Mean	3554167	845381	4.209	
SD	444452	112276	0.092	
CV(%)	12.51	13.28	2.19	

4.7 PRE-STUDY VALIDATION

For this study, the assay methods were validated on the 13th of November 1996 for androsterone and on the 21th of July 1997 for probenecid with limited validation batches described here after.

4.7.1 PREPARATION OF CALIBRATION STANDARDS AND QUALITY CONTROLS

4.7.1.1 ANDROSTERONE

Calibration standards were prepared in phosphate buffer by preparation of a stock solution in a suitable solvent and spiking a certain volume to phosphate buffer pH 7 which was serially diluted with phosphate buffer to obtain the desired concentrations. All volumetric operations were performed by weighing and the masses of the phosphate buffer were converted to volumes when calculating concentrations. Quality controls were prepared in phosphate buffer by a competent person by the same method used for the calibration standards. Calibration standards and quality controls were divided into aliquotes and stored under the same conditions as the trial samples, approximately -20°C. The same calibration standards and quality controls as prepared for the method validation were used during the analysis of the study samples. The preparation data are reproduced in Table 4.4, Table 4.5 and Table 4.6.

CALIBRATION STANDARDS STOCK SOLUTION

Solvent : Phosphate buffer

Specific gravity : 1 kg/l

Concentration of std K : 27.490µg/ml

ANALYTE (mg)	SOLVENT (g)	SOLVENT (ml)	CONC. (µg/ml)
6.765	246.09	246.09	27.490

6.765mg Androsterone was dissolved in 1ml of methanol and diluted with 245.09ml of phosphate buffer.

CALIBRATION STANDARDS

Table 4.4 Concentrations of calibration standards prepared in phosphate buffer.

STD CODE	A (g)	B (g)	C (g)	D (µg/ml)
K	110.690	356.780		27.490
J	117.510	248.000	390.520	14.351
I	118.550	238.540	358.530	7.175
H	126.840	246.830	366.850	3.588
G	109.890	229.890	349.890	1.794
F	110.910	230.920	351.040	0.897
E	110.560	230.570	350.650	0.449
D	115.560	235.560	355.670	0.225
C	110.400	230.400	350.380	0.112
B	110.960	230.940	350.940	0.056

A = Mass of empty container.
B = Mass of container and phosphate buffer.
C = Total mass of container plus normal plus spiked phosphate buffer.
D = Concentration of analyte in the phosphate buffer.

(I) QUALITY CONTROL STOCK SOLUTION FOR QC G TO QC E

Solvent : Phosphate buffer

Specific gravity : 1 kg/l

Concentration of QC G : 25.156µg/ml

ANALYTE (mg)	SOLVENT (g)	SOLVENT (ml)	CONC. (µg/ml)
6.289	250.00	250.00	25.156

6.289mg Androsterone was dissolved in 1ml of methanol and diluted with 249.00ml of phosphate buffer.

QUALITY CONTROLS

Table 4.5 Concentration of quality controls, prepared in phosphate buffer.

QC CODE	A (g)	B (g)	C (g)	D (µg/ml)
G	118.080	368.080		25.156
F	119.900	264.900	409.900	12.578
E	109.070	254.080	399.080	6.289

A = Mass of empty container.
B = Mass of container and phosphate buffer.
C = Total mass of container plus normal plus spiked phosphate buffer.
D = Concentration of analyte in the phosphate buffer.

(II) QUALITY CONTROL STOCK SOLUTION FOR QC D TO QC A

Solvent : Methanol

Specific gravity : 0.791 kg/l

ANALYTE (mg)	SOLVENT (g)	SOLVENT (ml)	CONC. (µg/ml)
1.418	0.831	1.051	1.35

QUALITY CONTROLS

Solvent : Phosphate buffer

Specific gravity : 1kg/l

Volume spiked in QC D : 50µl

Table 4.6 Concentration of quality controls, prepared in phosphate buffer.

QC CODE	A (g)	B (g)	C (g)	D (µg/ml)
D	103.080	363.090		0.260
C	111.150	131.060	186.160	0.191
B	103.390	163.380	223.360	0.130
A	104.610	144.620	179.620	0.061

A = Mass of empty container.
B = Mass of container and normal phosphate buffer.
C = Total mass of container plus normal plus spiked phosphate buffer.
D = Concentration of analyte in the phosphate buffer.

4.7.1.2 PROBENECID

Calibration standards were prepared in urine by preparation of a stock solution in a suitable solvent and spiking a pool of normal urine which was serially diluted with normal urine to obtain the desired concentrations. All volumetric operations were performed by weighing and the masses of the biological fluid were converted to volumes when calculating concentrations. Quality controls were prepared in normal urine by a competent person by the same method used for the calibration standards. Calibration standards and quality controls were divided into aliquotes and stored under the same conditions as the trial samples, approximately -20°C. The same calibration standards and quality controls as prepared for the method validation were used during the analysis of the study samples. The preparation data are reproduced in Table 4.7, Table 4.8 and Table 4.9.

CALIBRATION STANDARDS STOCK SOLUTION

Solvent	:	Normal urine
Specific gravity	:	1 kg/l
Concentration of std J	:	1182µg/ml

ANALYTE (mg)	SOLVENT (g)	SOLVENT (ml)	CONC. (µg/ml)
45.818	38.775	38.775	1182

45.818mg Probenecid was dissolved in 1ml of methanol and diluted with 37.775ml of normal urine.

CALIBRATION STANDARDS

Table 4.7 Concentrations of calibration standards prepared in normal urine.

STD CODE	A (g)	B (g)	C (g)	D (µg/ml)
J	104.980	143.755		1182
I	110.550	123.000	147.790	787
H	55.480	58.740	68.420	588
G	39.740	54.320	68.930	394
F	64.380	80.620	96.870	197
E	109.060	127.650	140.040	78.8
D	63.600	80.420	87.660	59.3
C	108.440	126.440	144.450	39.4
B	125.900	137.920	149.960	19.7

A = Mass of empty container.
B = Mass of container and normal biological fluid.
C = Total mass of container plus normal plus spiked biological fluid.
D = Concentration of analyte in the biological fluid.

(I) QUALITY CONTROL STOCK SOLUTION FOR QC G TO QC E

Solvent : Normal urine

Specific gravity : 1 kg/l

Concentration of QC G : 1060µg/ml

ANALYTE (mg)	SOLVENT (g)	SOLVENT (ml)	CONC. (µg/ml)
50.048	47.221	47.221	1060

50.048mg Probenecid was dissolved in 1ml of methanol and diluted with 47.221ml of normal urine.

QUALITY CONTROLS

Table 4.8 Concentration of quality controls, prepared in normal urine.

QC CODE	A (g)	B (g)	C (g)	D (µg/ml)
G	81.150	128.371		1060
F	64.380	85.310	111.510	589
E	64.920	80.620	96.320	295

A = Mass of empty container.
B = Mass of container and normal biological fluid.
C = Total mass of container plus normal plus spiked biological fluid.
D = Concentration of analyte in the biological fluid.

(II) QUALITY CONTROL STOCK SOLUTION FOR QC D TO QC A

Solvent : Methanol

Specific gravity : 0.791 kg/l

ANALYTE (mg)	SOLVENT (g)	SOLVENT (ml)	CONC. (µg/ml)
40.452	2.392	3.024	13.38

QUALITY CONTROLS

Solvent : Normal urine

Specific gravity : 1kg/l

Volume spiked in QC D : 500µl

Table 4.9 Concentration of quality controls, prepared in normal urine.

QC CODE	A (g)	B (g)	C (g)	D (µg/ml)
D	109.990	179.990		94.9
C	109.390	117.230	140.780	71.2
B	103.380	118.630	133.950	47.5
A	62.810	72.970	83.310	24.0

A = Mass of empty container.
B = Mass of container and normal biological fluid.
C = Total mass of container plus normal plus spiked biological fluid.
D = Concentration of analyte in the biological fluid.

4.7.2 CALIBRATION CURVE

4.7.2.1 ANDROSTERONE

Calibration standards used	:	STD K - STD B
Calibration range	:	0.056 - 27.490 µg/ml
Regression equation	:	$\ln(y) = a(\ln x)^2 + b(\ln x) + c$
r^2	:	0.9996

4.7.2.2 PROBENECID

Calibration standards used	:	STD I - STD B
Calibration range	:	20.14 - 758.26 µg/ml
Regression equation	:	$\ln(y) = a(\ln x)^2 + b(\ln x) + c$
r^2	:	0.9998

4.7.3 ACCURACY AND PRECISION OF THE QUALITY CONTROLS

Accuracy is measured as % bias and precision is measured as coefficient of variation (CV%).

4.7.3.1 ANDROSTERONE

Table 4.10 Accuracy and precision as determined during the method validation.

CODE	ACTUAL (µg/ml)	DETERMINATION					MEAN	BIAS (%)	CV (%)
		1	2	3	4	5			
G	25.20	20.817	21.588	22.341	21.113	21.758	21.5232	-14.590	2.7
F	12.60	11.573	11.690	11.467	11.284	11.328	11.4682	-8.983	1.5
E	6.289	5.964	5.985	6.025	5.879	6.048	5.9800	-4.913	1.1
D	0.260	0.289	0.234	0.238	0.249	0.278	0.2576	-0.923	9.6
C	0.191	0.194	0.198	0.195	0.186	0.217	0.1979	3.613	5.8
B	0.130	0.120	0.123	0.125	0.126	0.127	0.1242	-4.462	2.4
A	0.061	0.073	0.050	0.057	0.085	0.075	0.068	11.475	21.2

4.7.3.2 PROBENECID

Table 4.11 Accuracy and precision as determined during the method validation.

CODE	ACTUAL (µg/ml)	DETERMINATION					MEAN	BIAS (%)	CV (%)
		1	2	3	4	5			
G	1060	904.19	989.68	1061.29	1116.79	1123.59	1039.107	-1.971	8.9
F	589	641.37	613.93	635.99	626.10	656.45	634.766	7.770	2.5
E	295	290.61	317.42	319.67	327.12	324.24	322.113	9.191	1.4
D	94	91.40	100.38	99.70	99.35	100.31	99.937	6.316	0.5
C	71.2	74.83	70.62	70.74	71.48	72.60	72.055	1.201	2.4
B	47.50	44.39	47.20	47.93	46.55	47.24	46.662	-1.764	2.9
A	24.00	22.44	23.32	23.92	24.09	24.40	23.635	-1.521	3.3

4.7.4 CALIBRATION RANGE

For the assignment of a valid calibration range, bias is taken as measure of accuracy, and coefficient of variation (CV%) is taken as measure of precision. Accuracy and precision for a valid range must be within 20% over the whole calibration range. Results from the validation assays above indicate a valid calibration range of 0.061 - 25.156 µg/ml for androsterone and 24.0 - 1060.0 µg/ml for probenecid. For this study, the LLOQ (lower limit of quantification) was set to the lower limit of the calibration range, which was taken to be the calibration standard (0.056 µg/ml) for androsterone and calibration standard (19.7 µg/ml) for probenecid.

4.7.5 REPRESENTATIVE CHROMATOGRAMS

4.7.5.1 ANDROSTERONE

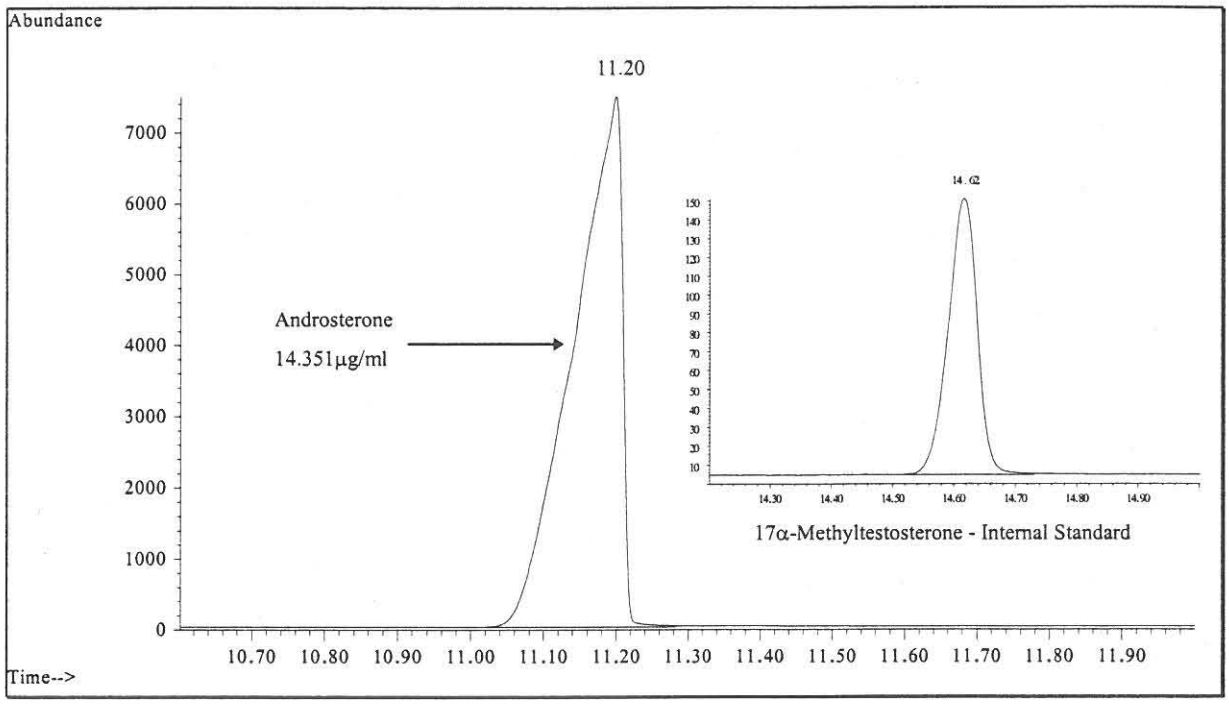


Figure 4.1 Representative chromatogram of androsterone for calibration standard J.

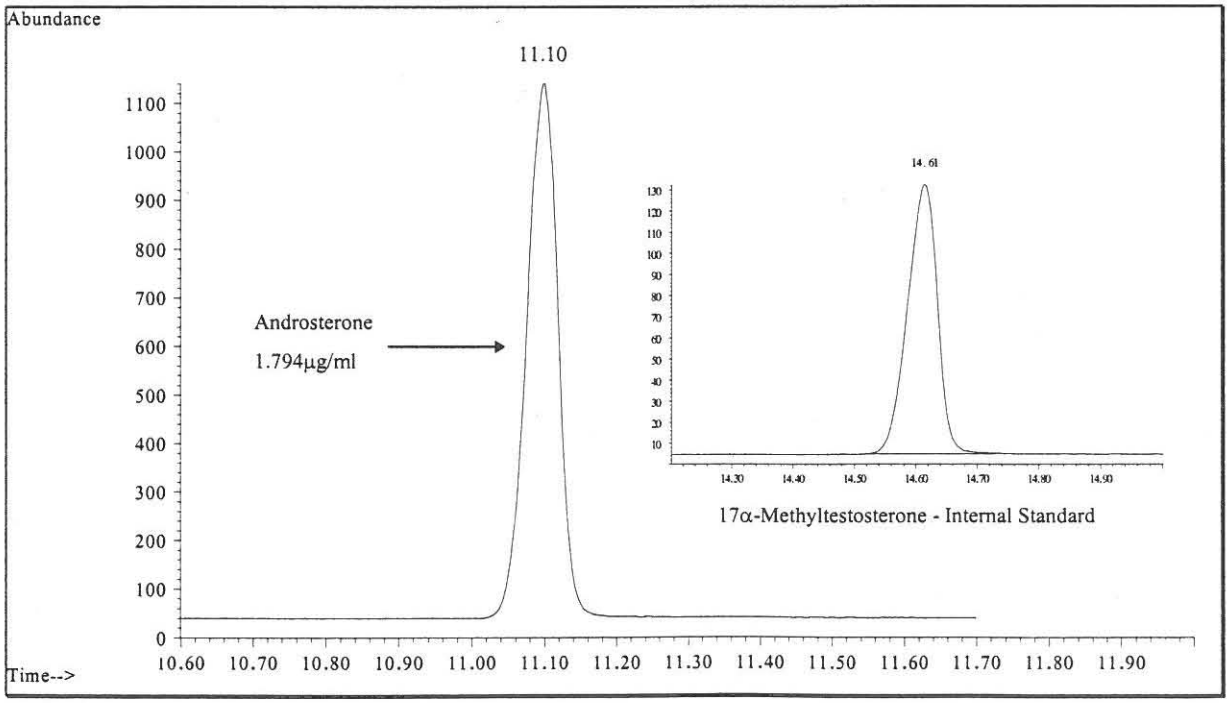


Figure 4.2 Representative chromatogram of androsterone for calibration standard G.

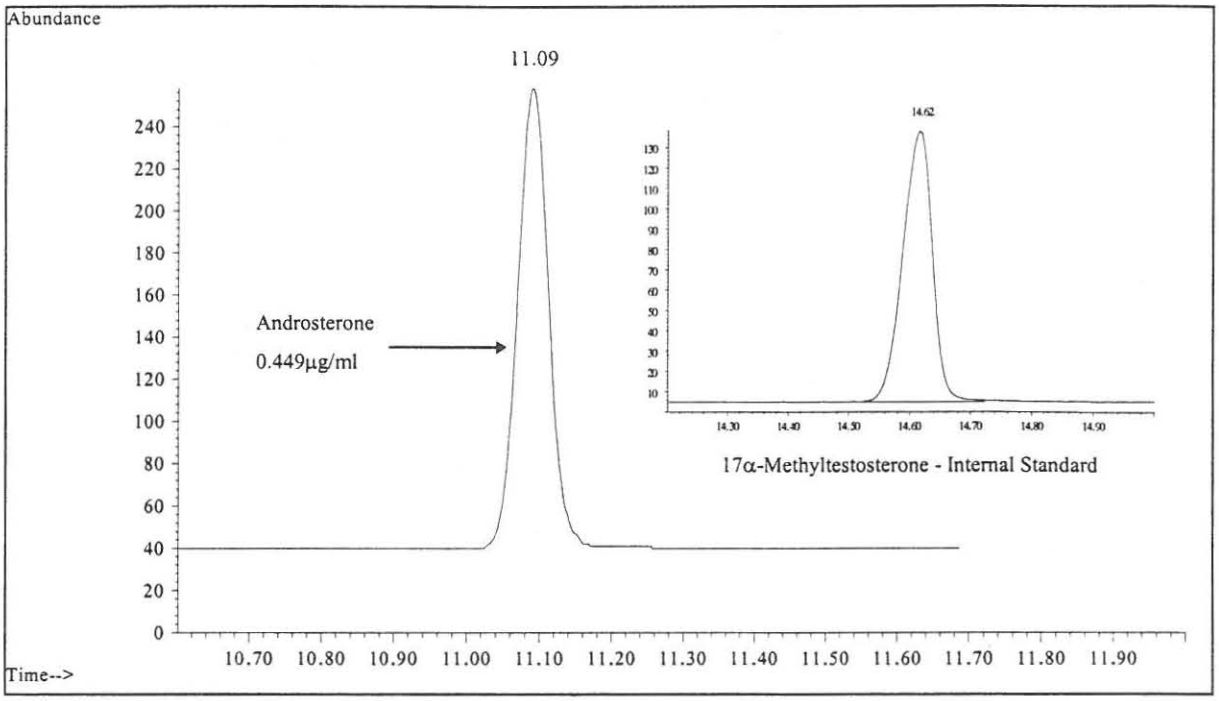


Figure 4.3 Representative chromatogram of androsterone for calibration standard E.

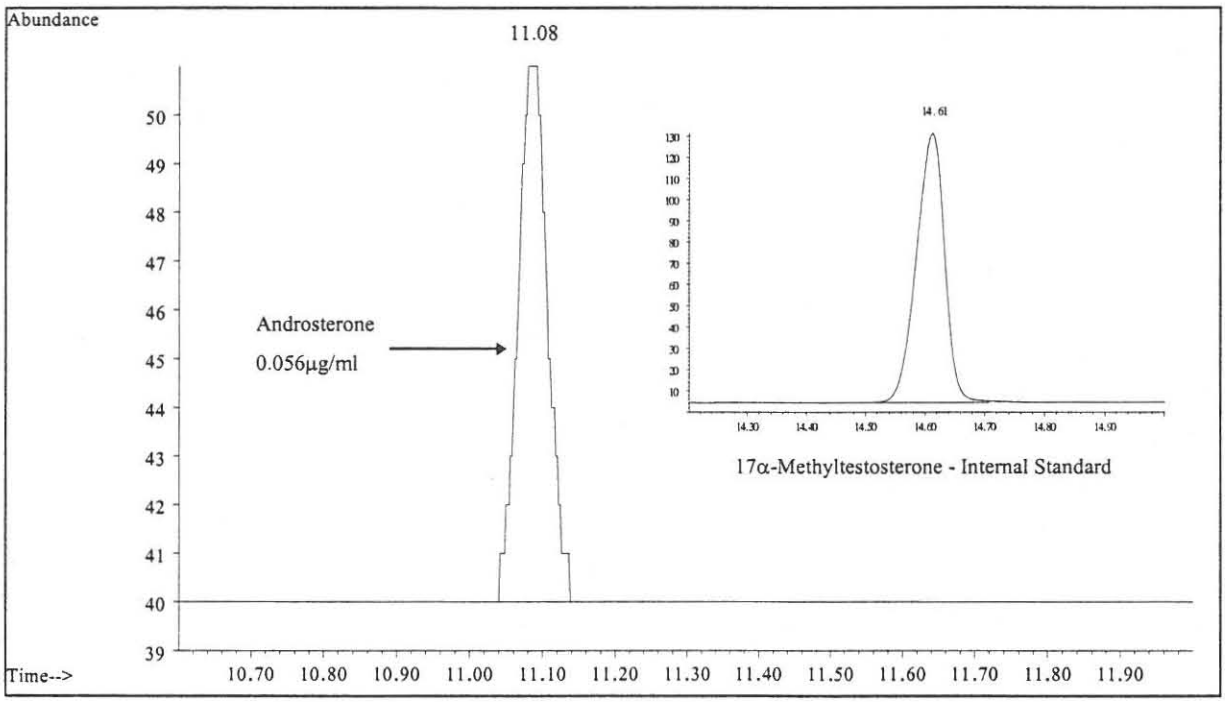


Figure 4.4 Representative chromatogram of androsterone for calibration standard B.

4.7.5.2 PROBENECID

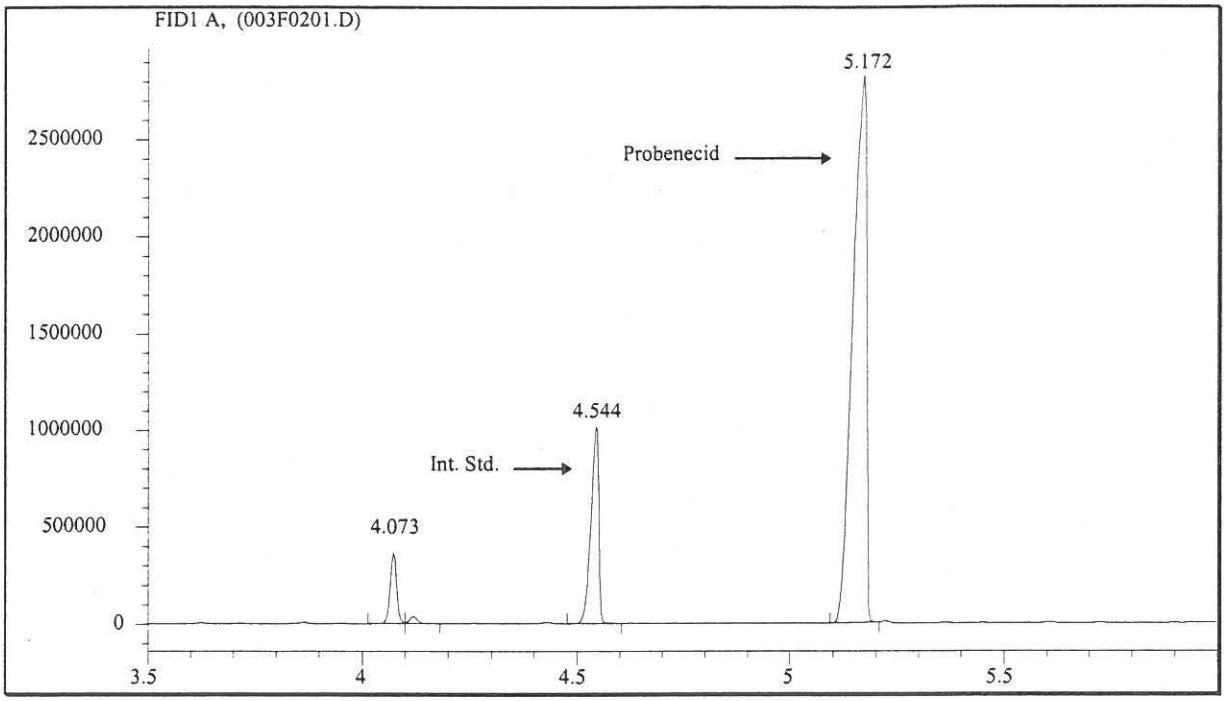


Figure 4.5 Representative chromatogram of probenecid for calibration standard I.

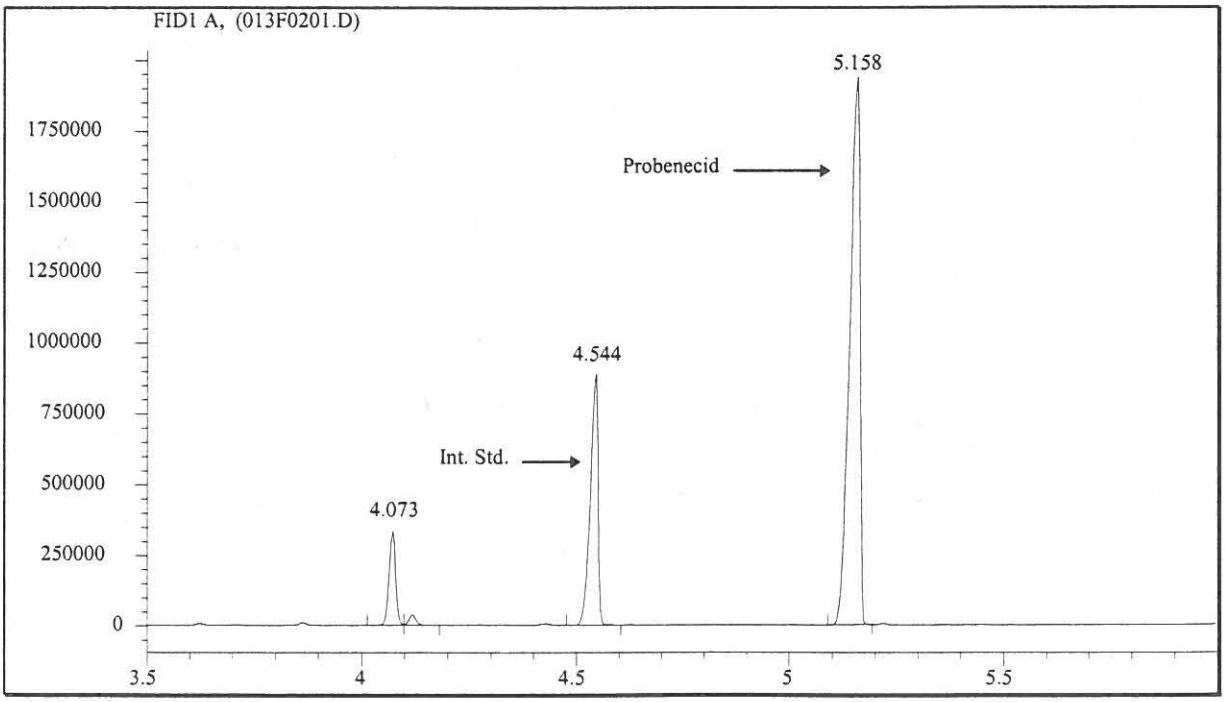


Figure 4.6 Representative chromatogram of probenecid for calibration standard G.

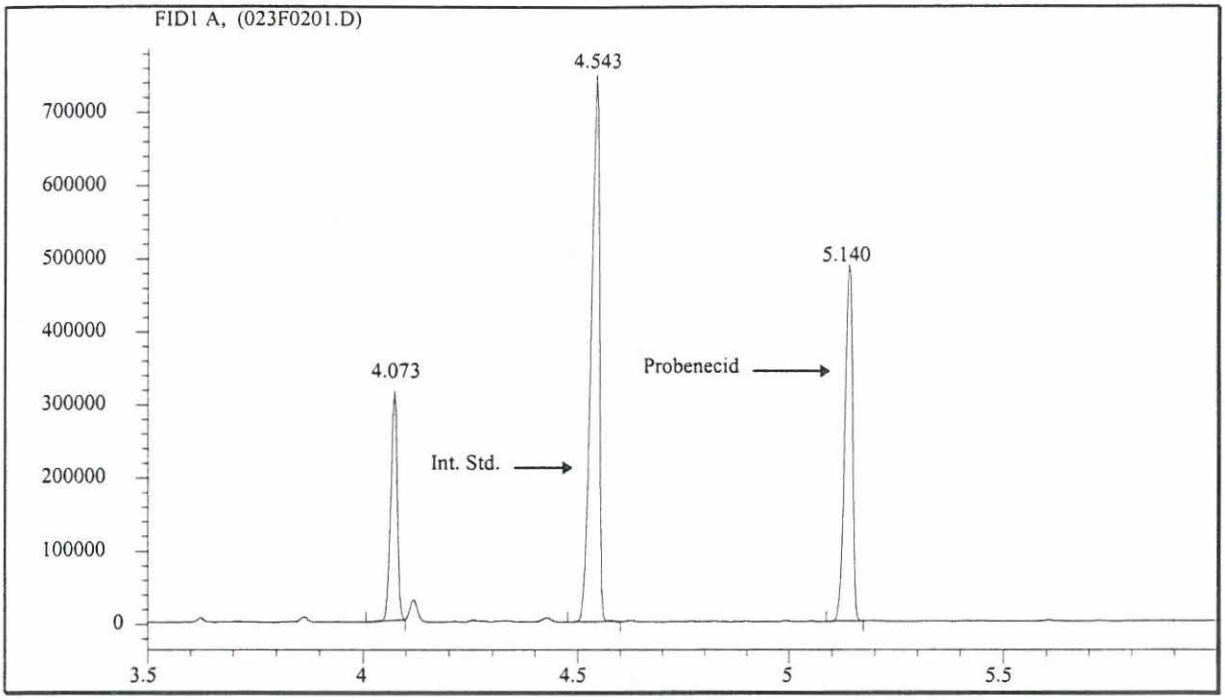


Figure 4.7 Representative chromatogram of probenecid for calibration standard E.

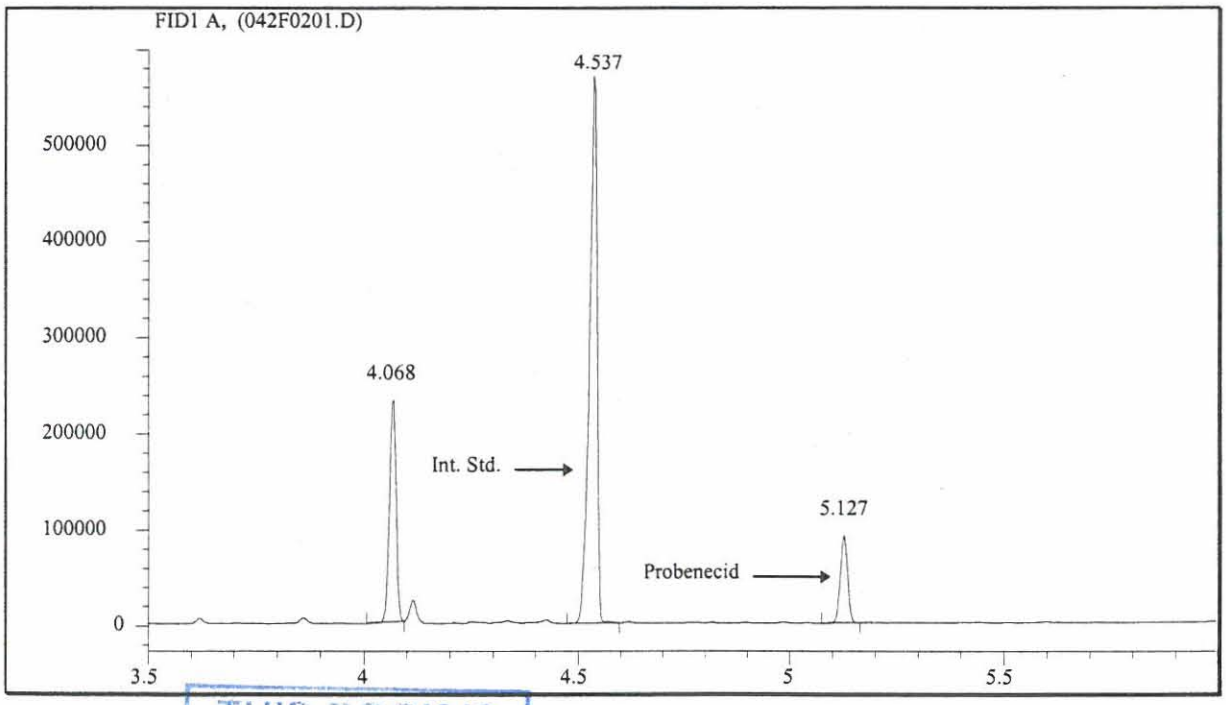


Figure 4.8 Representative chromatogram of probenecid for calibration standard B.

Chapter 5

With-in Study Assay Performance

WITH-IN STUDY ASSAY PERFORMANCE

5.1 STUDY EXECUTION

Samples were assayed in batches. A batch consisted of calibration standards (usually 6 to 10), quality controls (at high, medium and low concentrations), and study samples. Attempts were always made to process complete profiles of a subject for each treatment in a batch. Thus in a given batch, profiles of treatments were alternated whenever possible. The calibration standards and quality controls were interspersed among study samples in a predetermined manner, tabulated in a batch sample logistics table. The quality controls which were processed in each batch comprised duplicates near the maximum and near the mean concentration and three controls at respectively LLOQ, 2 x LLOQ and 3 x LLOQ (where LLOQ represents the lower limit of quantification determined during the validation of the assay method). After the batch had been run the chromatograms were inspected and checked against documented acceptance criteria.

The calibration curves were plotted, regression equations determined, and the quality controls calculated as unknowns using the regression equation giving the best overall results throughout the study.

5.1.1 PREPARATION OF CALIBRATION STANDARDS AND QUALITY CONTROLS

The same calibration standards and quality controls as prepared for the pre-study validations were used during the analysis of the study samples.

5.1.2 TYPICAL BATCH STRUCTURE

Samples were designated in the run sheet table by a three digit code seperated by commas consisting of subject number, sampling time (h) and period. The typical batch structure is detailed in Tables 5.1 and Table 5.2.

Table 5.1 Typical batch structure for androsterone.

INJ. NO.	SAMPLE	INJ. NO.	SAMPLE	INJ. NO.	SAMPLE
1	QC A	15	QC E	29	STD G
2	QC B	16	15,3.0,1	30	15,24.0,1
3	STD C	17	15,3.0,2	31	15,24.0,2
4	STD C	18	STD E	32	QC F
5	15,0.0,1	19	15,4.0,1	33	15,30.0,1
6	15,0.0,2	20	15,4.0,2	34	15,30.0,2
7	QC C	21	15,6.0,1	35	QC F
8	15,1.0,1	22	15,6.0,2	36	15,36.0,1
9	15,1.0,2	23	STD J	37	15,36.0,2
10	STD B	24	15,8.0,1	38	STD I
11	STD B	25	15,8.0,2	39	15,48.0,1
12	15,2.0,1	26	STD F	40	15,48.0,2
13	15,2.0,2	27	15,12.0,1	41	QC D
14	QC E	28	15,12.0,2	42	QC D

Table 5.2 Typical batch structure for probenecid.

INJ. NO.	SAMPLE	INJ. NO.	SAMPLE	INJ. NO.	SAMPLE
1	Response	16	QC D	31	QC C
2	STD B	17	20,3.0,1	32	19,24.0,1
3	19,0.0,1	18	QC D	33	20,24.0,1
4	STD B	19	19,4.0,1	34	STD H
5	20,0.0,1	20	20,4.0,1	35	19,30.0,1
6	QC F	21	STD I	36	20,30.0,1
7	19,1.0,1	22	19,6.0,1	37	QC B
8	QC F	23	20,6.0,1	38	19,36.0,1
9	20,1.0,1	24	STD D	39	20,36.0,1
10	STD G	25	19,8.0,1	40	STD E
11	19,2.0,1	26	20,8.0,1	41	19,48.0,1
12	QC E	27	STD C	42	20,48.0,1
13	20,2.0,1	28	19,12.0,1	43	QC A
14	QC E	29	STD C	44	STD F
15	19,3.0,1	30	20,12.0,1		

5.1.3 REPRESENTATIVE CHROMATOGRAMS - STUDY SAMPLES

5.1.3.1 ANDROSTERONE

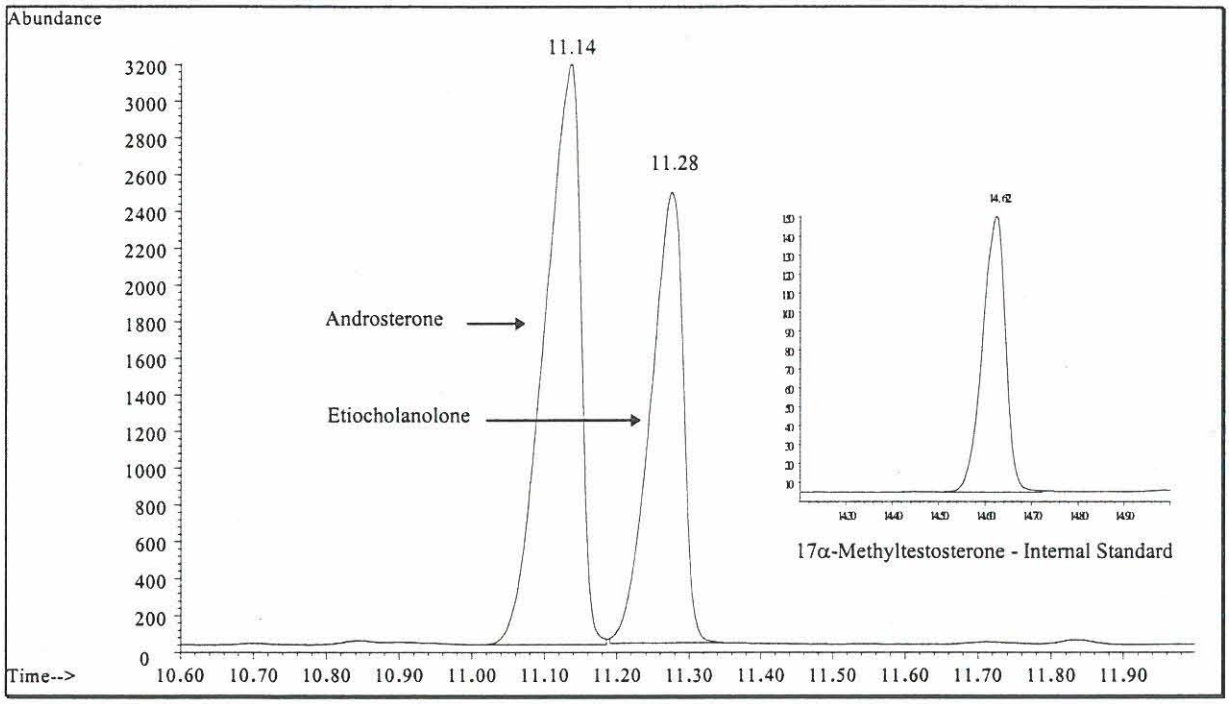


Figure 5.1 Representative chromatogram for Subject 1, period 1, before start of profile.

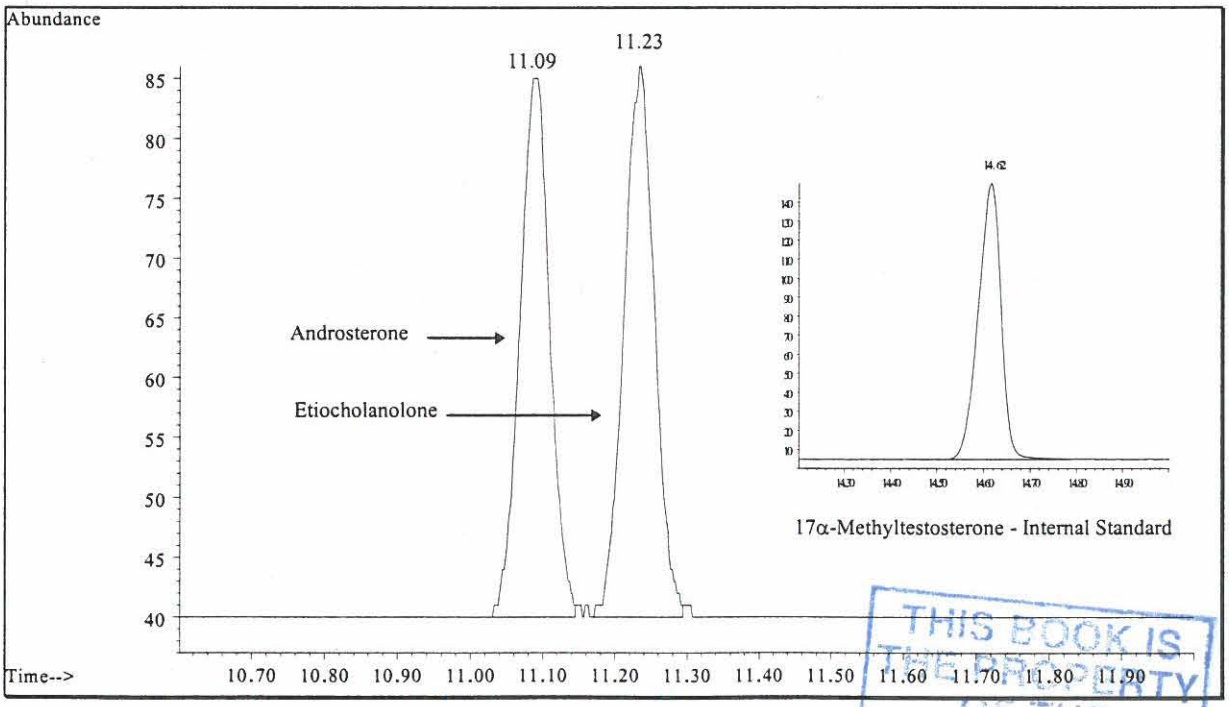


Figure 5.2 Representative chromatogram for Subject 1, period 1, at 4 hours after start of profile.

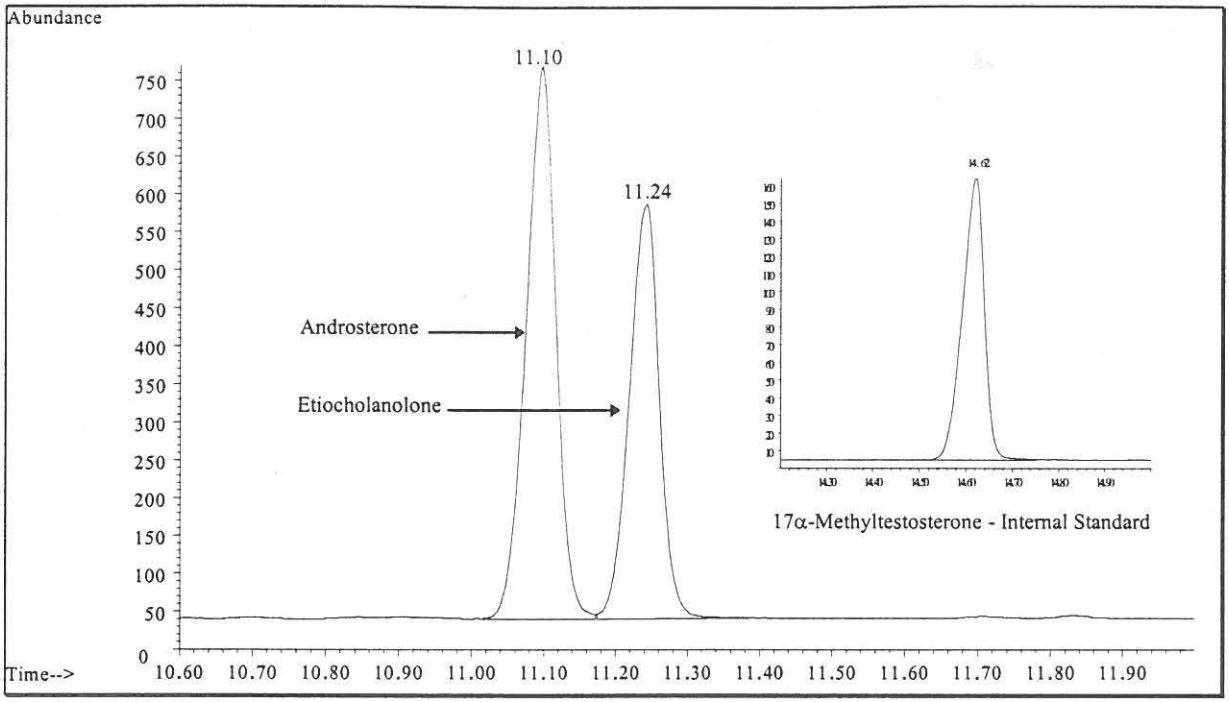


Figure 5.3 Representative chromatogram for Subject 1, period 1, at 12 hours after start of profile.

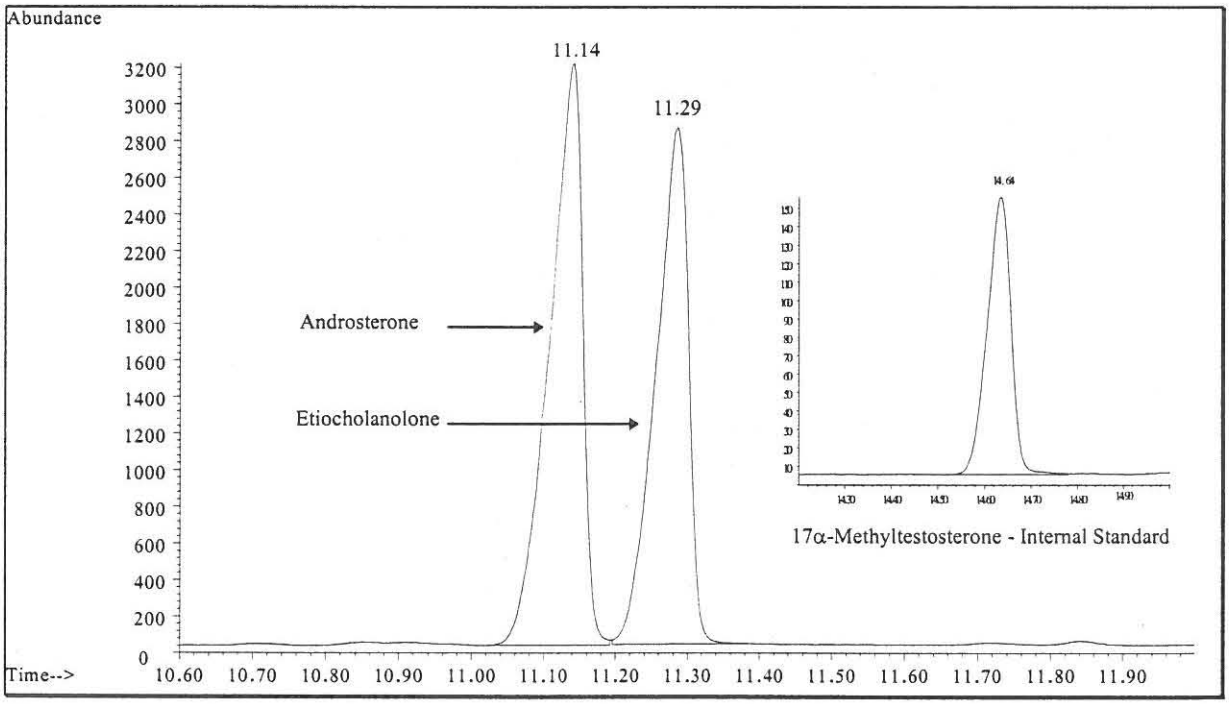


Figure 5.4 Representative chromatogram for Subject 1, period 1, at 48 hours after start of profile.

5.1.3.2 PROBENECID

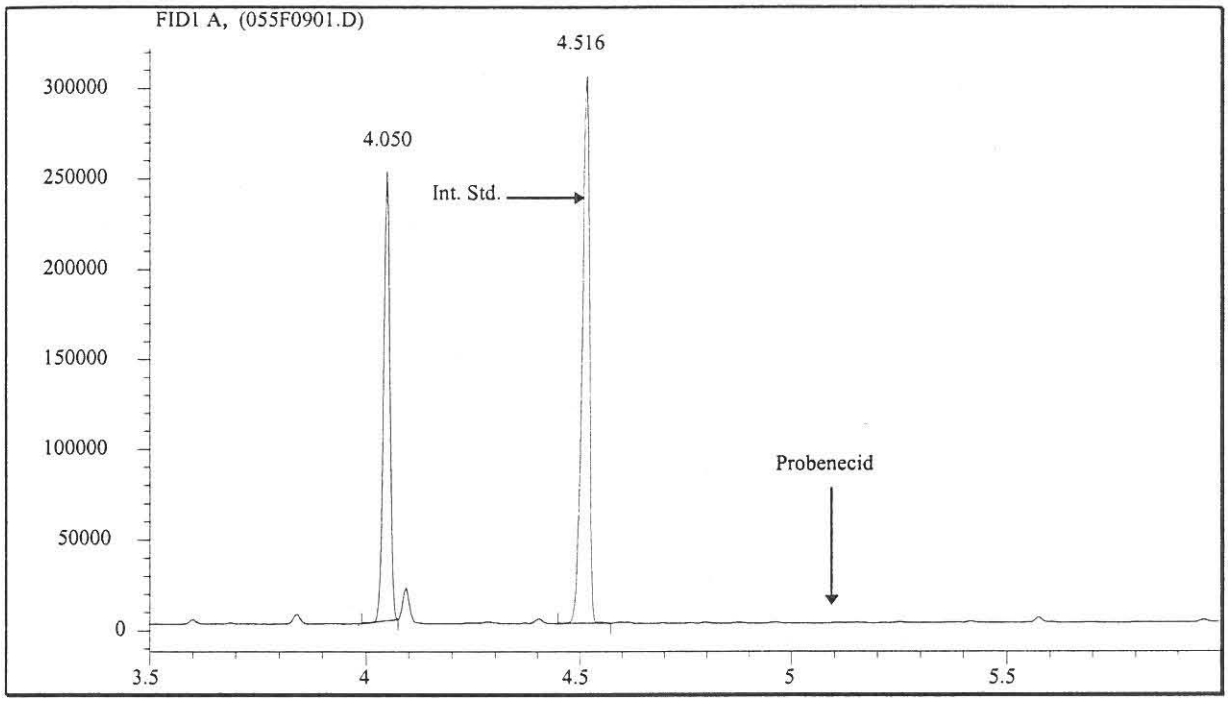


Figure 5.5 Representative chromatogram for Subject 17, period 2, before start of profile.

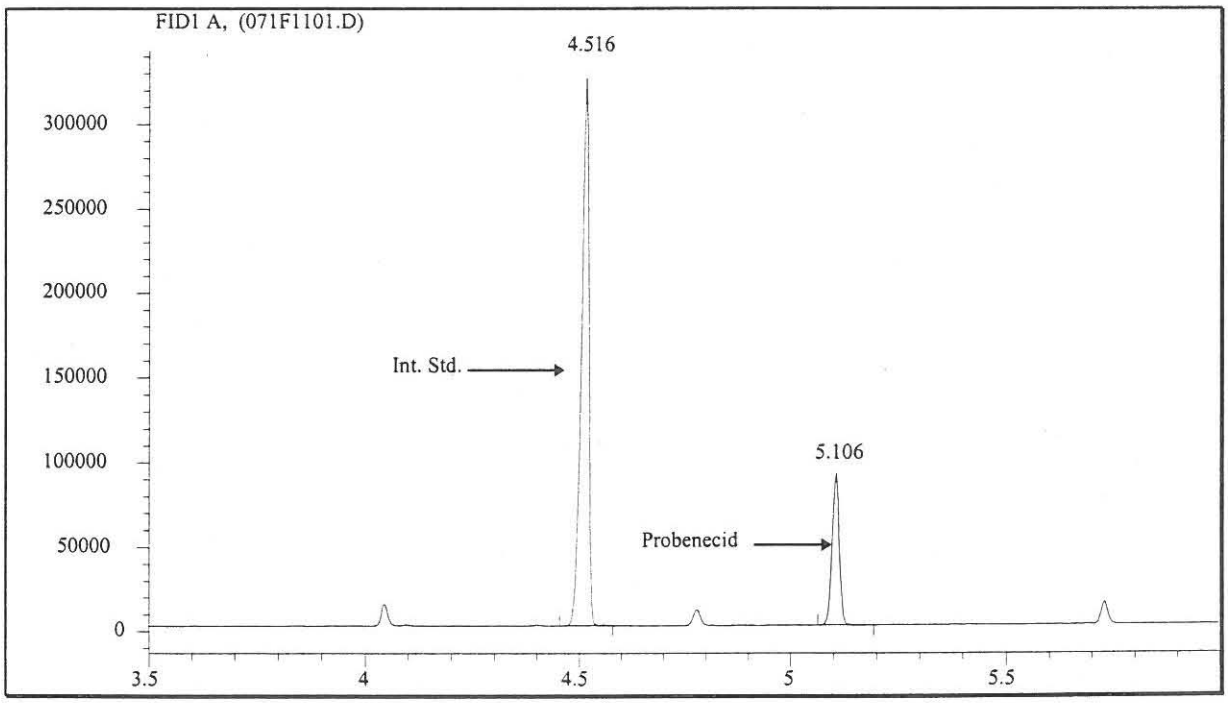


Figure 5.6 Representative chromatogram for Subject 17, period 2, 4 hours after start of profile.

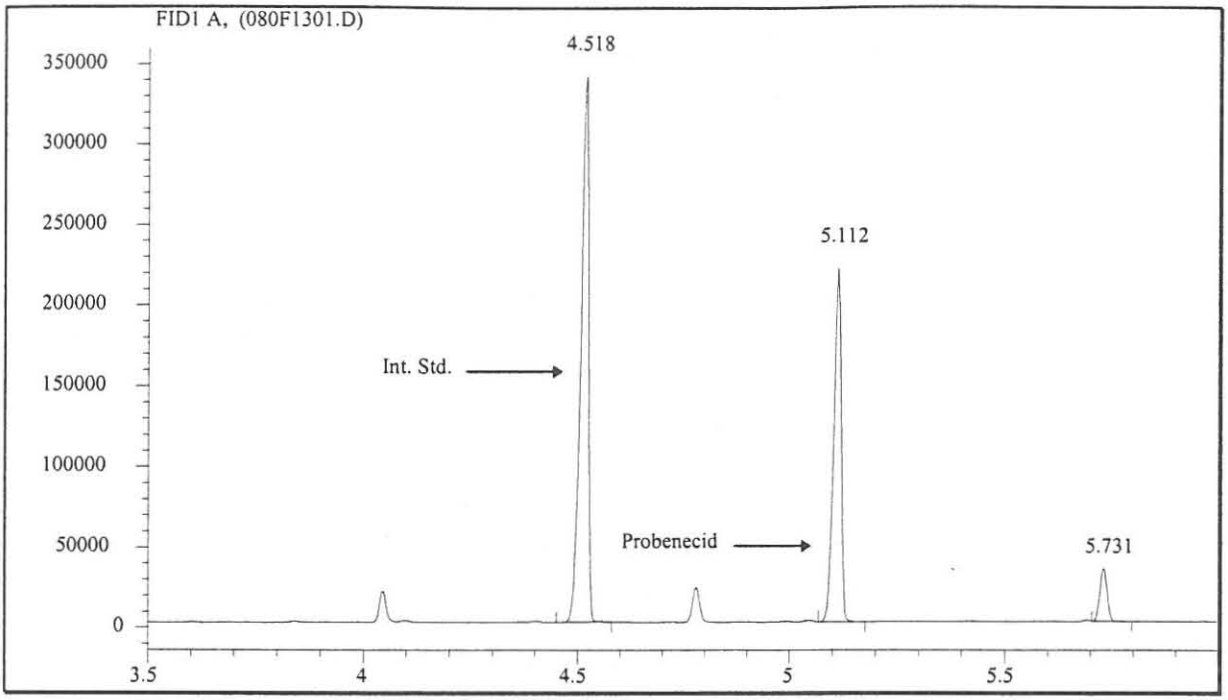


Figure 5.7 Representative chromatogram for Subject 17, period 2, 12 hours after start of profile.

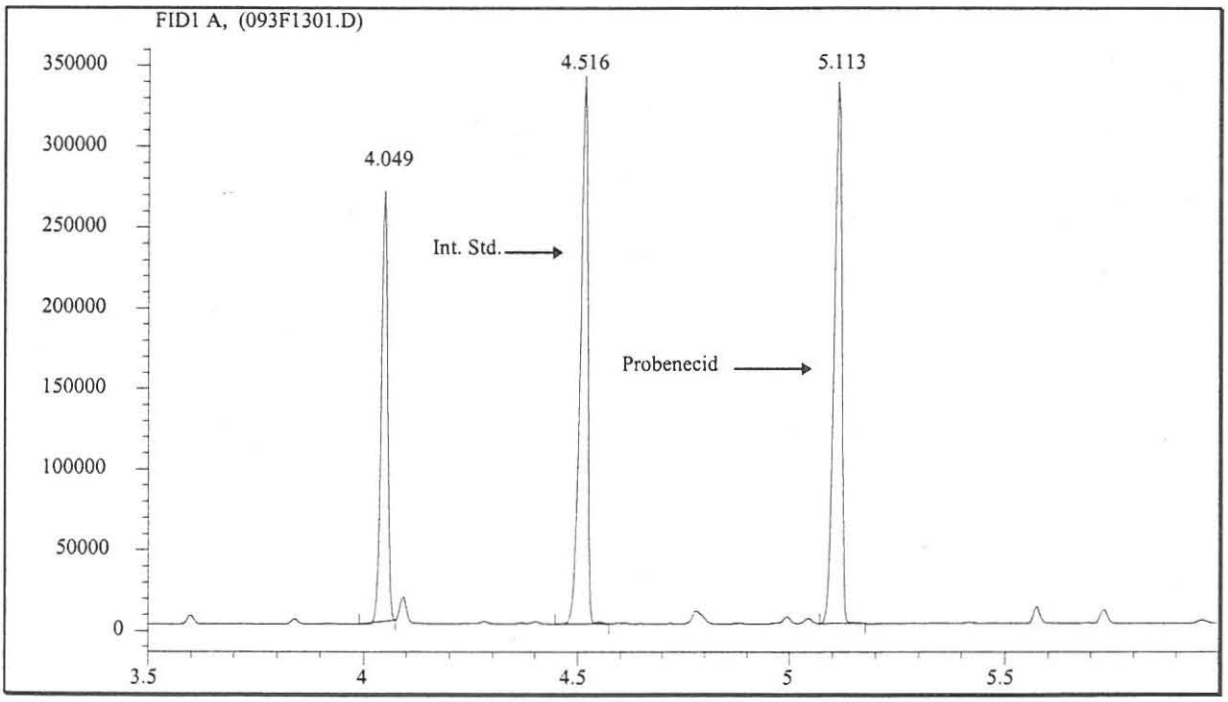


Figure 5.8 Representative chromatogram for Subject 17, period 2, 48 hours after start of profile.

5.2 CALCULATION OF RESULTS

Results were calculated using PhIRSt chromatographic data reporting package (Product of Phoenix International Life Sciences, Montreal, Canada). Peak areas were electronically and automatically read from the report files generated by Winner on Windows. Data were automatically summarised, calibration curves calculated according to pre-set regression equations and concentrations interpolated by the program. Results were presented in printed ordered tables with performance statistics per batch and later summarized to give overall study statistics. This package has been validated in Canada by the manufacturer to Federal Drug Administration (FDA) requirements Phoenix International Life Sciences.

Chapter 6

Results and Discussion

RESULTS AND DISCUSSION

6.1 BIOMETRIC ANALYSIS

6.1.1 SAMPLE SIZE

The results of a pilot study (FRM 9/94) indicated that n = 20 subjects will be sufficient to obtain results with satisfactory precision.

6.1.2 PHARMACOKINETIC AND SAFETY VARIABLES

The fractional and cumulative urine volumes (-12-48h) and urinary probenecid and androsterone excretion (-12-48h), as well as the actual sampling times, were tabulated for each subject, product and collection interval, together with the following descriptive statistics for each product and collection interval:

- The mean (MEAN).
- The standard deviation (SD).
- The geometric mean (GEOM MEAN).
- The geometric standard deviation (GEOM SD).
- The coefficient of variation (CV).
- The standard error of the mean (SEM).
- The minimum (MIN).
- The maximum (MAX).
- The median value (MEDIAN).
- The number of observations (n).

6.1.3 ANALYSIS OF SAFETY DATA

All haematological and clinical chemistry results from pre- and post-study examinations were summarized by descriptive statistics. The number of subjects with laboratory values outside the normal range, as well as individual data for all variables, were reported, but are not included in this dissertation.

6.2 RESULTS AND DISCUSSION

Individual results are reported in Appendix 6.1 and 6.2. Mean results are tabulated in this section.

6.2.1 DEMOGRAPHIC DATA

Table 6.1 Demographic data of subjects participating in FARMOVS 9/96.

SUBJECT NO.	AGE (YEAR)	BODY MASS (kg)	HEIGHT (cm)
1	30	72	191
2	28	87	187
3	25	76	176
4	19	75	179
5	24	80	182
6	36	84	189
7	34	68	179
8	45	65	179
9	30	70	176
10*	27	92	184
11	24	69	176
12	25	77	184
13	27	72	182
14	24	69	176
15	27	78	178
16	23	66	188
17	20	74	174
18	23	79	175
19	25	74	183
20	22	73	175
MEAN	27	75	180.7
SD	5.99	6.96	5.19
MIN	19	65	174
MAX	45	92	191
MEDIAN	25	74	179
n	20	20	20

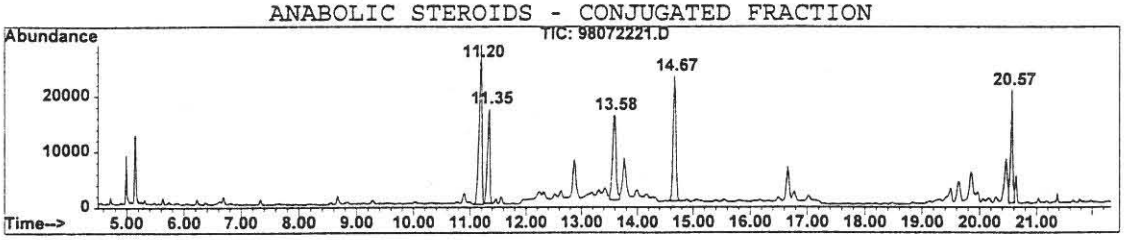
*Subject 10 failed to complete the study due to flu.

6.2.2 DETECTION OF PROBENECID IN THE SCREENING METHOD FOR ANABOLIC STEROIDS

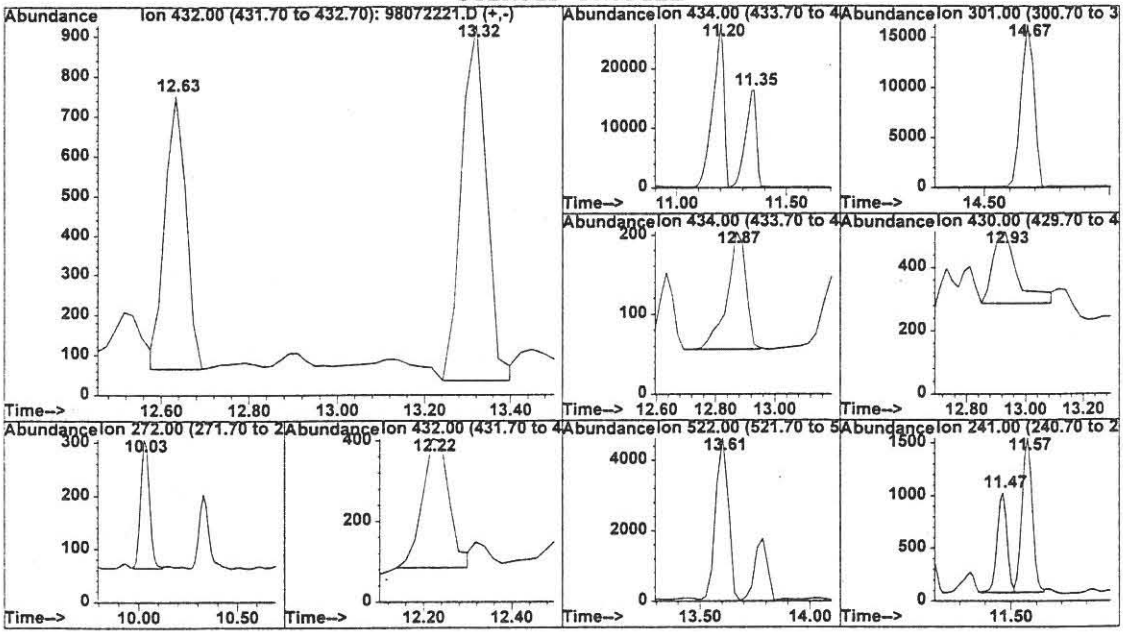
File : u:\sport\msdata\obelix\98072221.D
Method file : S-STER-C
Sample name : Blank

Operator : E Kruger
Date acquired : 22 Jul 98 8:30 pm

ANABOLIC STEROIDS - CONJUGATED FRACTION



STEROID PROFILE



SUBSTANCE	RT	AREA	ng/ml	NORMAL RANGE Male	Female
Androst	11.20	95779	2709	942 - 6027	404 - 5439
Etiochol	11.35	55206	1736	652 - 4385	473 - 6107
Epitestos	12.63	2269	19	10 - 89	1 - 42
Testos	13.32	3650	27	5 - 90	1 - 57
DHT	12.87	647	21	8 - 82	7 - 52
5-a-diol	11.47	2501	60	19 - 136	5 - 91
5-b-diol	11.57	4092	101	29 - 372	9 - 366
Androst_dione	12.93	1713	****	*** - ****	*** - ****
17-alpha-MT	14.67	58223			

Figure 6.1 Printout of a normal steroid profile obtained form the screening method for anabolic steroids.

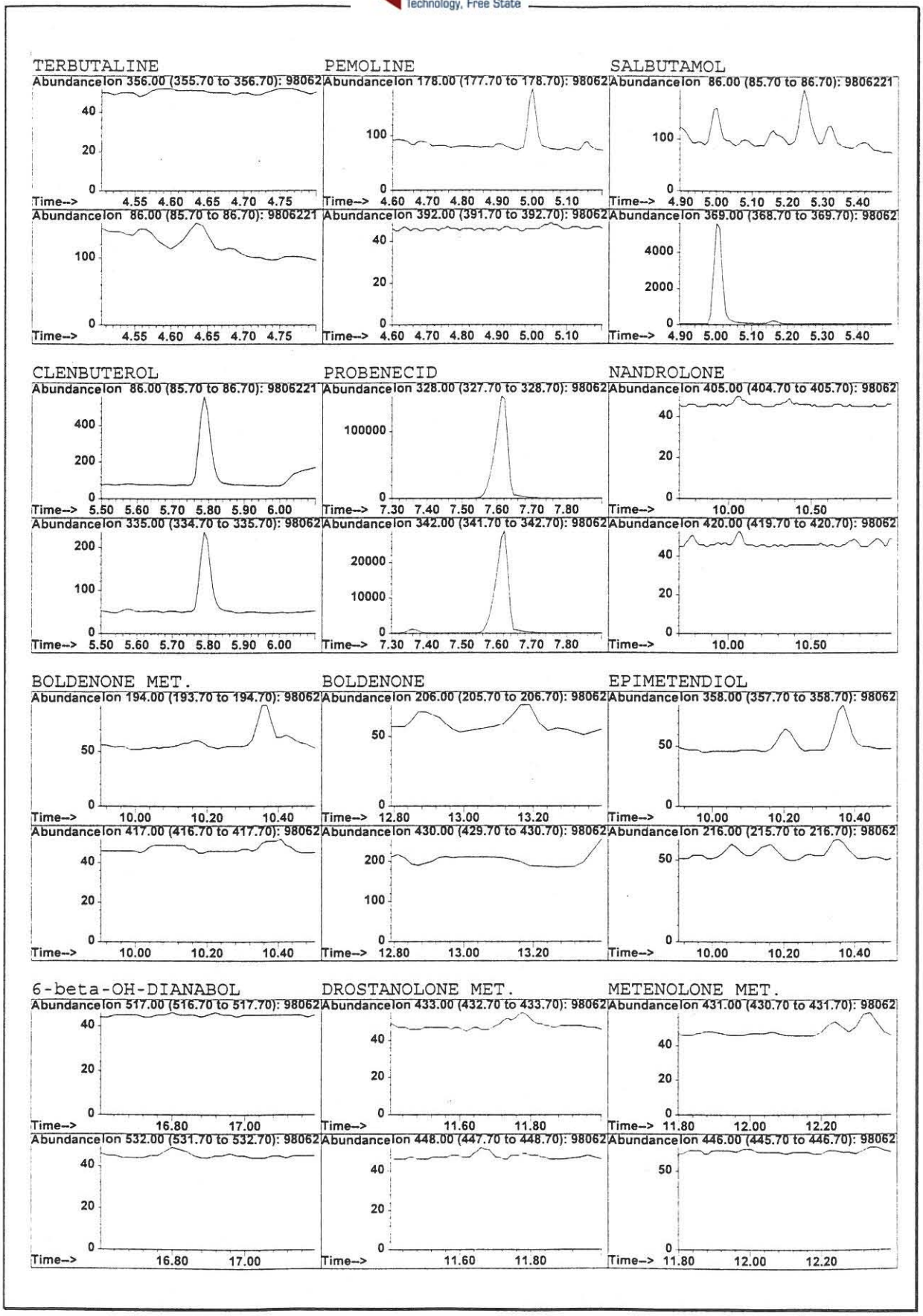
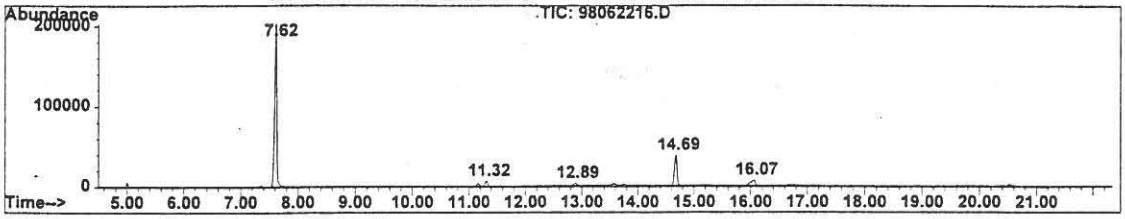


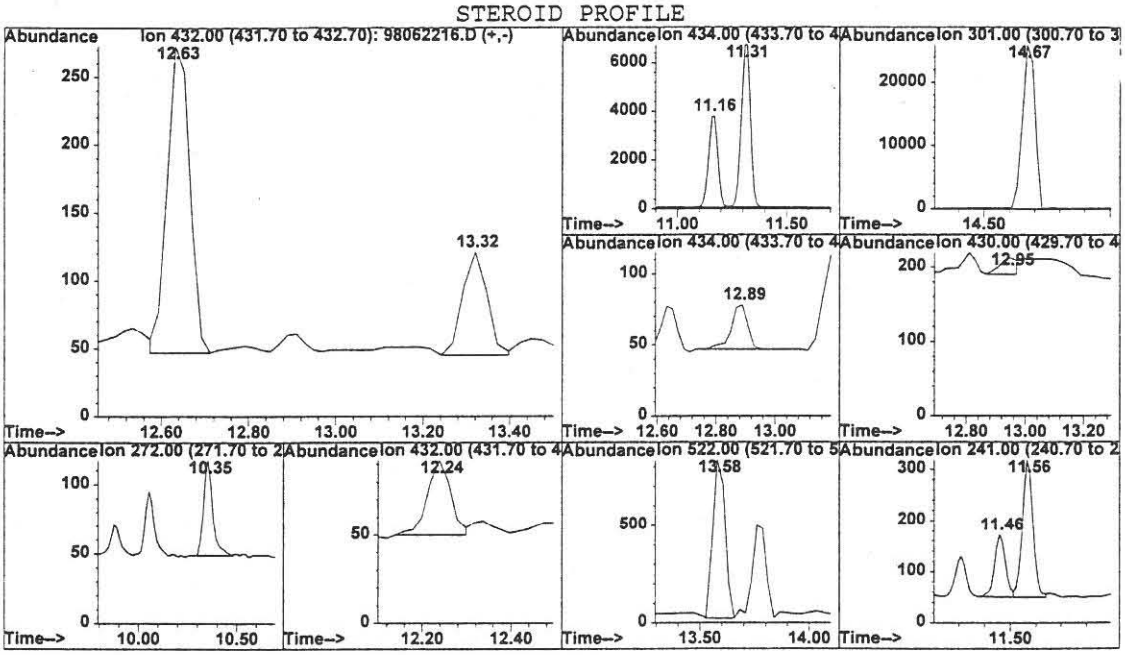
Figure 6.2 Printout of a steroid profile after the application of probenecid (Benemid®), obtained form the screening method for anabolic steroids.

File : u:\sport\msdata\obelix\98062216.D
Method file : S-STER-C
Sample name : 100 354
Baseball
Operator : E Kruger
Date acquired : 22 Jun 98 9:57 pm

ANABOLIC STEROIDS - CONJUGATED FRACTION



STEROID PROFILE



SUBSTANCE	RT	AREA	ng/ml	NORMAL RANGE Male	Female
Androst	11.16	11018	144	942 - 6027	404 - 5439
Etiochol	11.31	19189	319	652 - 4385	473 - 6107
Epitestos	12.63	812	5	10 - 89	1 - 42
Testos	13.32	304	2	5 - 90	1 - 57
DHT	12.89	117	2	8 - 82	7 - 52
5-a-diol	11.46	357	5	19 - 136	5 - 91
5-b-diol	11.56	768	10	29 - 372	9 - 366
Androst_dione	12.95	90	****	*** - ****	*** - ****
17-alpha-MT	14.67	89668			

Figure 6.2 (cont.) Printout of a steroid profile after the application of probenecid (Benemid®), obtained form the screening method for anabolic steroids.

Figures 6.1 and 6.2 are representative examples of printouts obtained form the screening method for anabolic steroids. Steroid profiling is a valuable tool when performed routinely on urinary doping control samples under carefully controlled analytical conditions. The

parameters of the urinary steroid profile are very stable but can be influenced by the application of probenecid, diuretics, ethanol etc. Also bacterial activities in the urine and side activities during the enzymatic hydrolysis may cause changes. All these factors lead to a characteristic pattern of the steroid profile. The ranges of the endogenous steroids that can be expected in a normal steroid profile, are also indicated in Figure 6.1. In Figure 6.2 probenecid can be seen at retention time 7.62 (ions: $^{m}/_{z}$ = 328 , $^{m}/_{z}$ = 342). The presence of probenecid is further indicated by the suppression of the endogenous steroids in the steroid profile. Figure 6.3 is a representative example of a mass spectrum of probenecid.

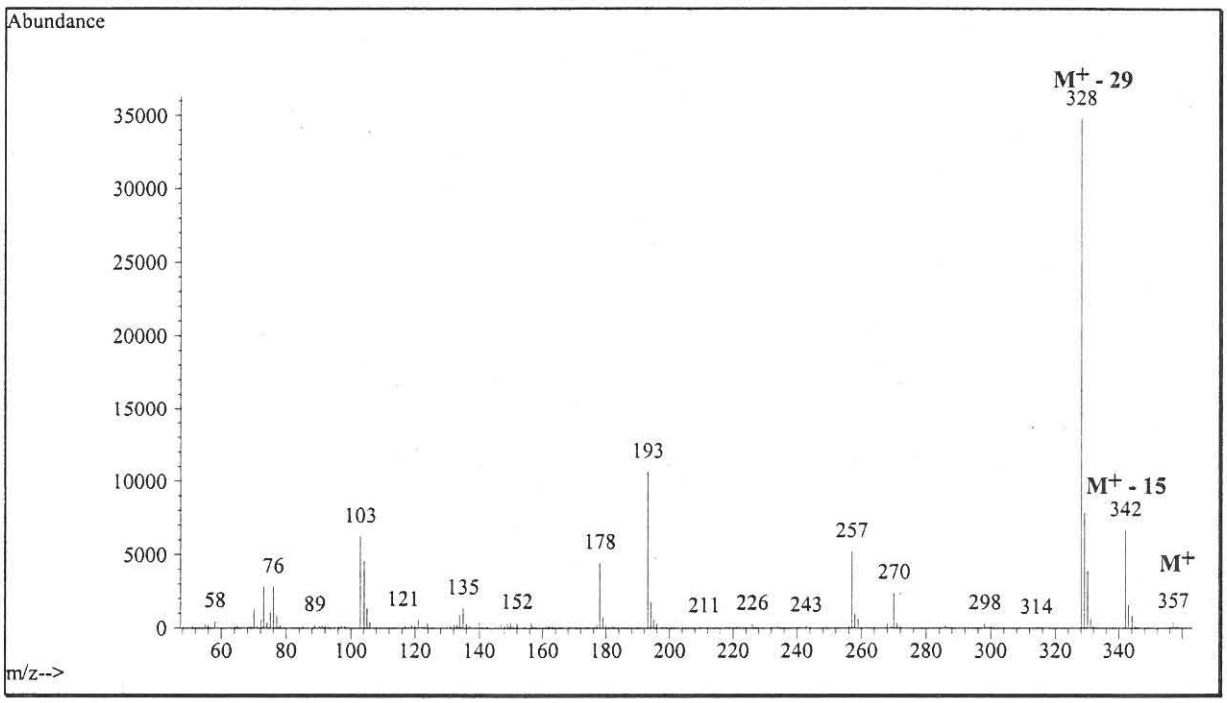


Figure 6.3 Electron impact ionization mass spectrum of a trimethylsilyl derivative of probenecid.

6.2.3 FRACTIONAL URINARY EXCRETION OF ANDROSTERONE

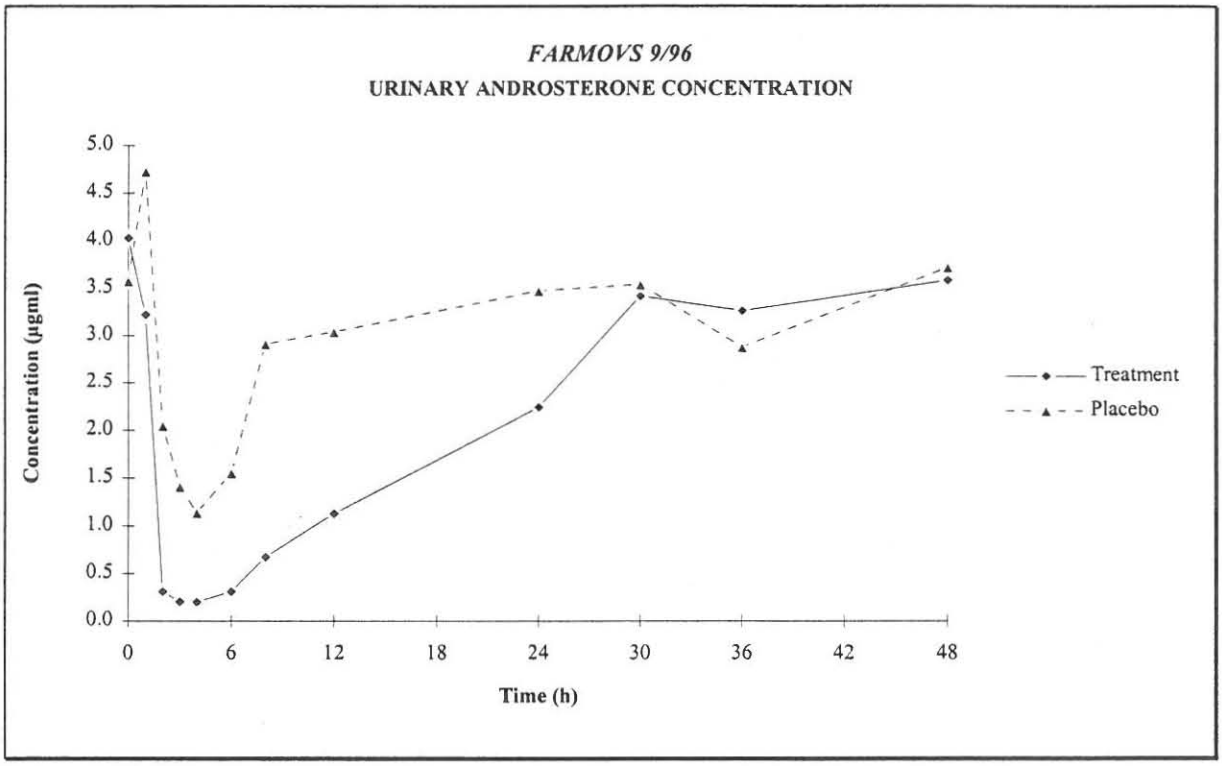


Figure 6.4 Geometric mean values (n = 19) of the urinary androsterone concentration over 48 hours.

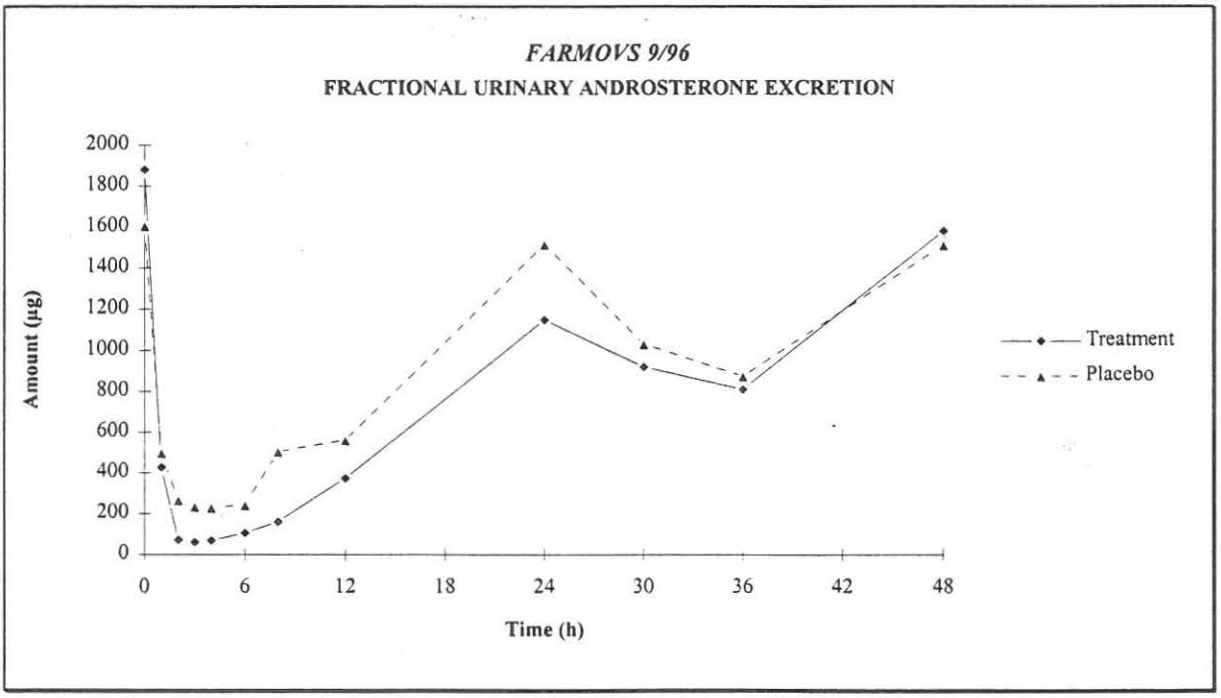


Figure 6.5 Geometric mean values (n = 19) of fractional urinary androsterone excretion over 48 hours.

The administration of probenecid leads to a significant decrease of the urinary concentration of androsterone (Figure 6.4). The lowest excretion (65.712µg) was found between 2 - 3 hours after the administration of 4 x 500mg probenecid (Figure 6.5). The average excretion of androsterone in all the volunteers in the treatment phase (2 to 8 hours) was reduced to about 6% of the average pretest levels. The decrease in the androsterone excretions in the placebo phase was due to the dilution of the urine by the intake of 200ml water hourly during the first four hours after treatment. This decrease was 33% of the average pretest levels (2 to 8 hours).

6.2.4 ANDROSTERONE CONCENTRATION EXPRESSED PER MMOL CREATININE

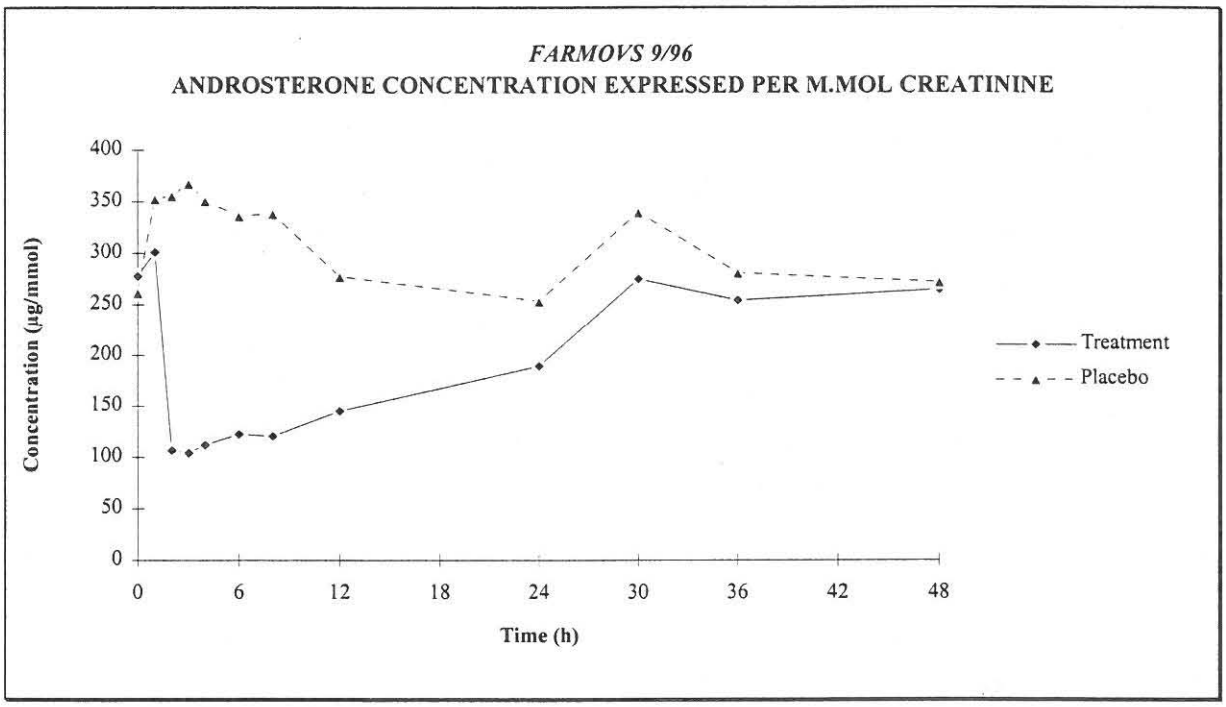


Figure 6.6 Geometric mean values (n = 19) of androsterone expressed per mmol creatinine over 48 hours.

In order to compensate for the dilution effect of the urine the creatinine concentration of each fraction was measured and the androsterone concentration was expressed per mmol creatinine. From Figure 6.6 it is clear that the excretion of androsterone in the placebo phase remained constant, in contrast with the reduced excretion of androsterone in the treatment phase. This clearly demonstrates that probenecid suppressed the excretion of androsterone.

6.2.5 URINARY EXCRETION RATE OF ANDROSTERONE

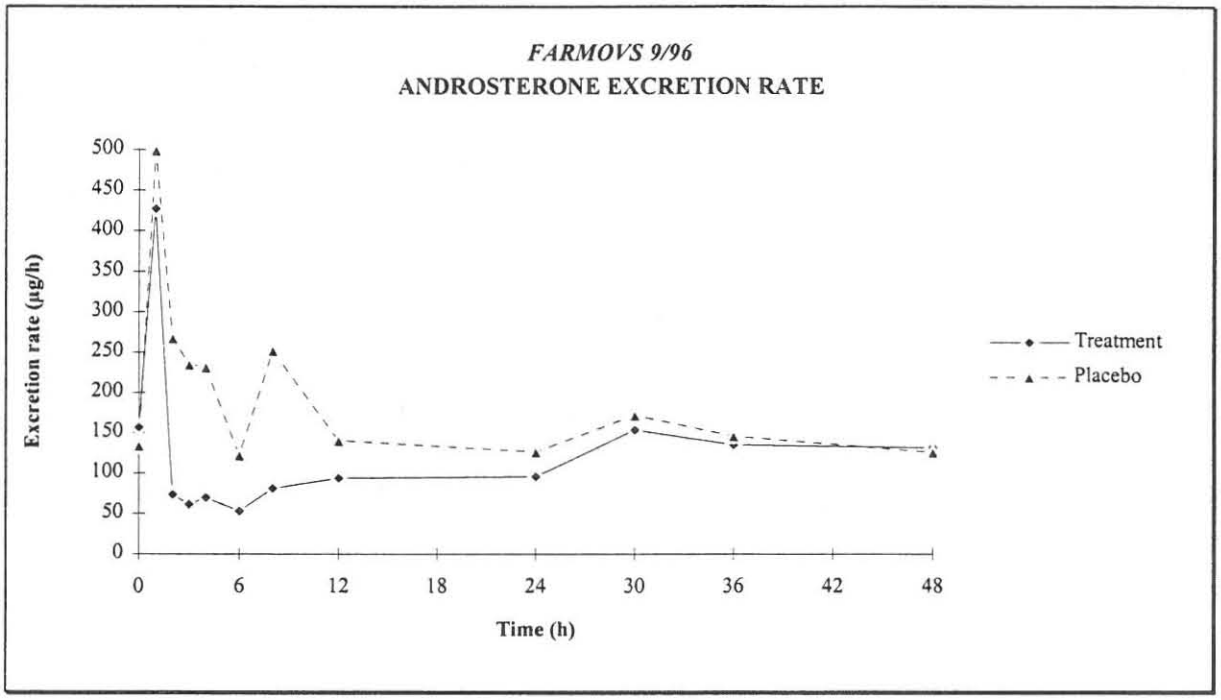


Figure 6.7 Geometric mean values (n = 19) of androsterone excretion rates (µg/h) over 48 hours.

The data on the urinary excretion rate of androsterone are presented in Figure 6.7. The data were subjected to analysis of covariance, with the (-12 - 0 h) excretion rate as covariate. A statistically significant decrease in the excretion rate was seen from 2 to 8 hours after administration of a single dose of probenecid (4 x 500mg). The data on the percentage decrease of the excretion rates of the treatment phase relative to the control phase are presented in Table 6.2. The mean androsterone excretion rate for all fractional collection intervals in this period (1 - 2 h, 2 - 3 h, 3 - 4 h, 4 - 6 h, 6 - 8 h) were between 56% and 70% lower following administration of Benemid® than placebo.

These results were confirmed by the cumulative urinary excretion of androsterone (Figure 6.8). The mean cumulative urinary androsterone excretion (0 - 48 h) was 8027.8mg following placebo, and 6192.3mg following Benemid®, i.e. the mean excretion (0 - 48 h) following Benemid® was 77% of that following placebo. An analysis of covariance (with the baseline androsterone excretion as covariate), showed that the true 0 - 48 h androsterone excretion following Benemid® administration lies between 16% and 29% below that following placebo, with 90% certainty (90% confidence interval for the mean ratio "benemid/placebo": 71-84%).

Table 6.2 Average decrease in the excretion rate of androsterone after administration of probenecid.

TIME INTERVAL (h)	PERCENTAGE DECREASE RELATIVE TO CONTROL
0 - 1	24
1 - 2	72
2 - 3	72
3 - 4	68
4 - 6	56
6 - 8	70
8 - 12	34
12 - 24	23
24 - 30	11
30 - 36	11
36 - 48	-11*

* A negative value indicates an increase relative to control.

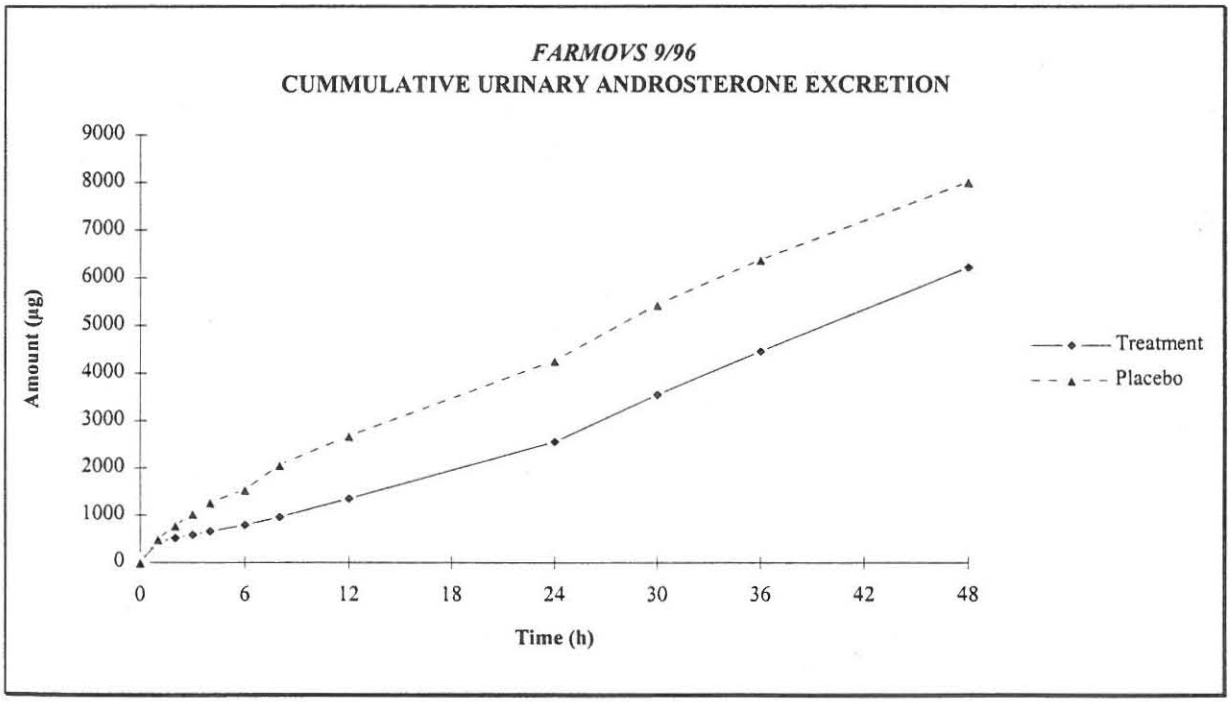


Figure 6.8 Geometric mean values (n = 19) of the cumulative urinary androsterone excretion(µg) over 48 hours.

6.2.6 URINARY EXCRETION OF PROBENECID

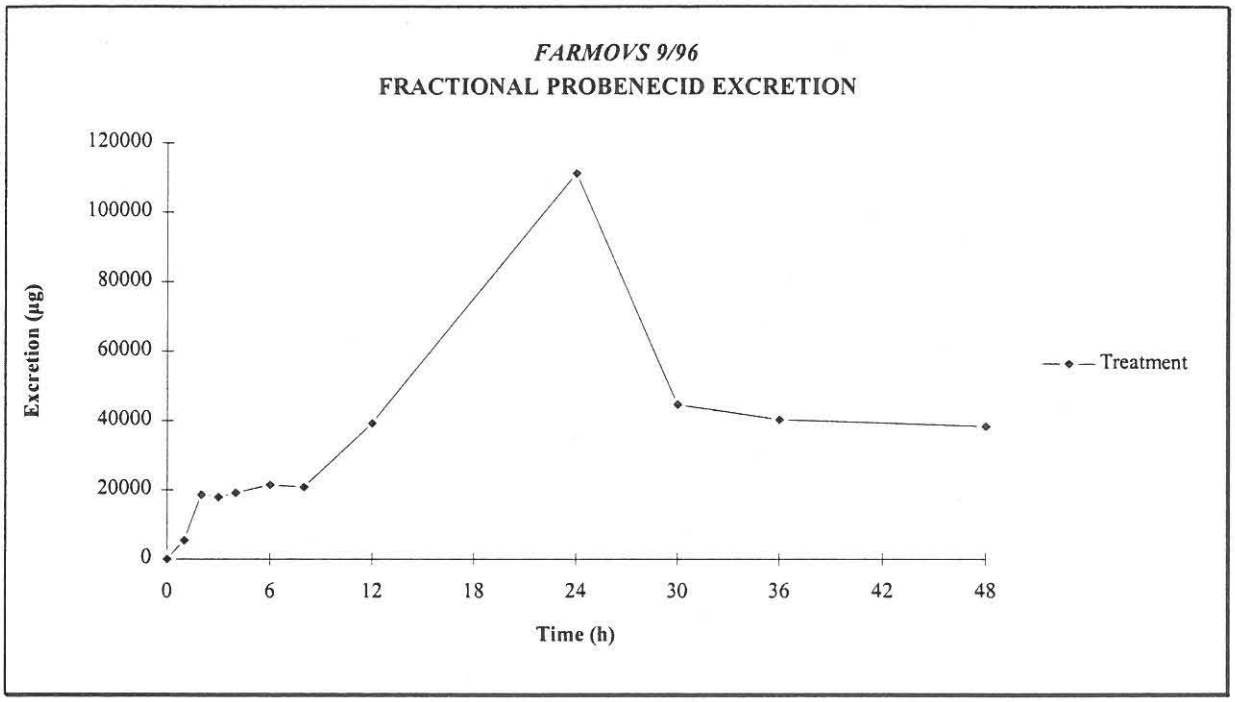


Figure 6.9 Geometric mean values (n = 19) of fractional urinary probenecid excretion over 48 hours.

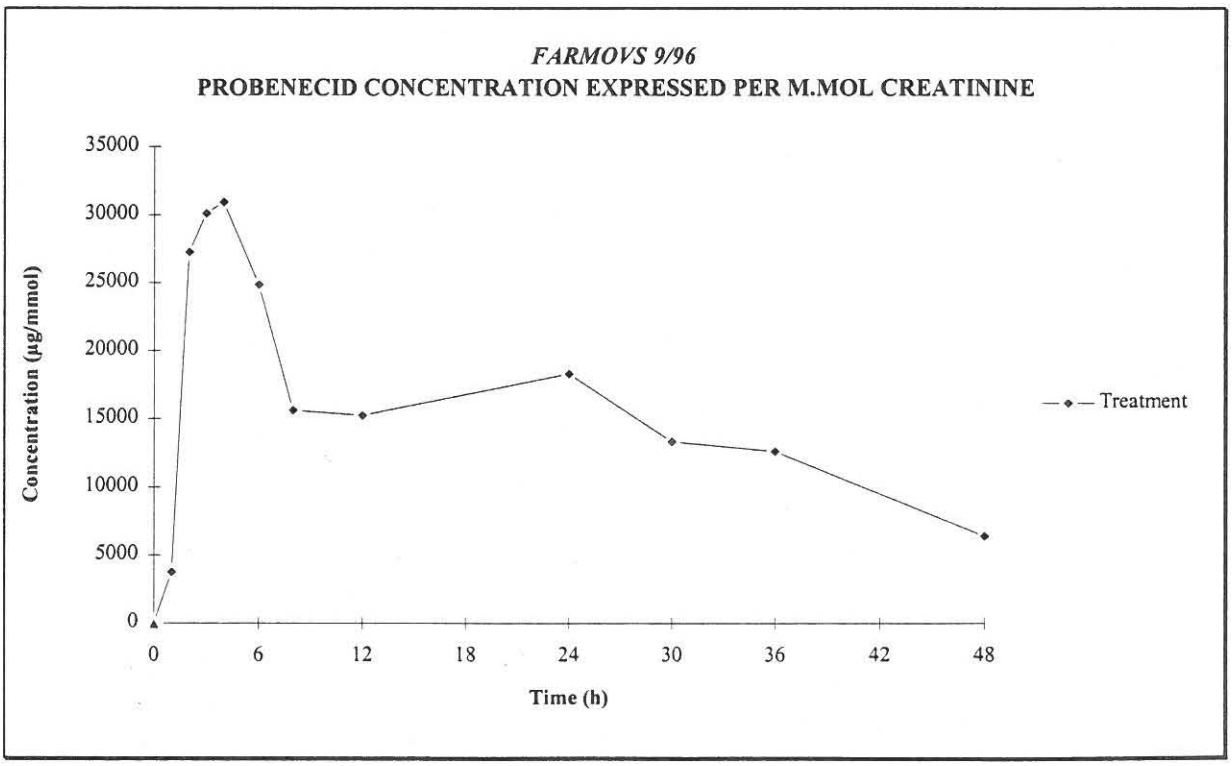


Figure 6.10 Geometric mean values (n = 19) of probenecid concentration expressed per mmol creatinine over 48 hours.

The data on the excretion of probenecid are presented in Figure 6.9 and Figure 6.10. In order to compensate for the dilution effect of the urine the creatinine concentration of each fraction was measured and the probenecid concentration was expressed per mmol creatinine (Figure 6.10). Although a decrease in the excretion of androsterone, as a result of the administration of probenecid, was observed, the excretion of probenecid itself increased over the same observation period (2 to 8 hours) (Figure 6.11).

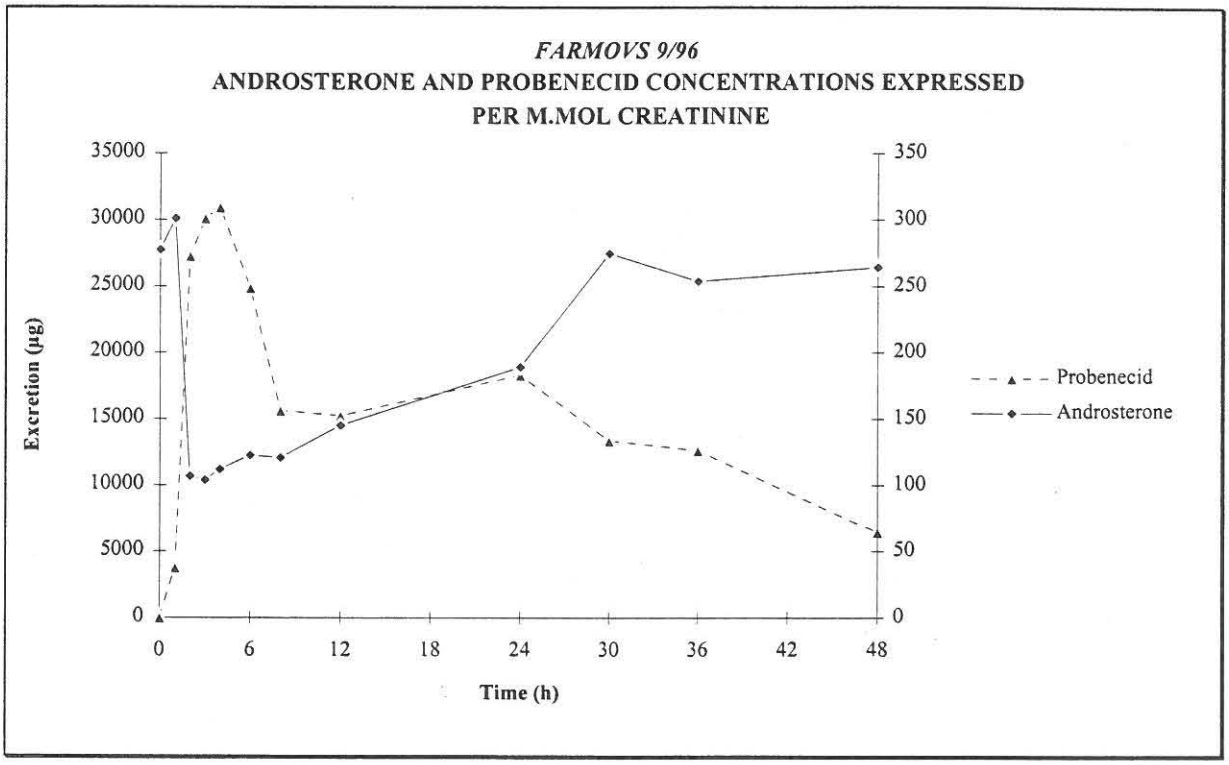


Figure 6.11 Comparison between the geometric mean values of androsterone and probenecid concentrations expressed per mmol creatinine over 48 hours.

6.2.7 CONCLUDING REMARKS

The results from this study showed that the lowest excretion of androsterone was found between 2 - 3 hours after administration of probenecid. A statistically significant decrease in the excretion rate of androsterone was seen from 2 to 8 hours after administration of a single dose of probenecid (4 x 500mg). The mean androsterone excretion rate for all fractional collection intervals in this period (1 - 2 h, 2 - 3 h, 3 - 4 h, 4 - 6 h, 6 - 8 h) were between 56% and 70% lower following administration of Benemid® compared to placebo. These results are

in agreement with those of Gardner *et al.*, (1951) who reported that Benemid over a single 24-hour period (1g each 6 hours) produced approximately 50% deminution in urinary 17-ketosteroids (androsterone is also a 17-ketosteroid).

The results of this study are also in agreement with Cunningham *et al.*, (1981) who suggested that the co-administration of probenecid with a number of acidic compounds, particularly antibiotics, generally causes an increase in peak plasma concentrations and half-life and decrease in distribution half-life and urinary excretion of these compounds.

Studies by Vree *et al.*, (1995) also demonstrated that the oral clearance of furosemide, the renal clearance and the calculated non-renal clearance of furosemide were decreased by probenecid to approximately 35% of their baseline value.

High doses of probenecid inhibit the urinary excretion of androsterone glucuronide and therefore it will also inhibit the excretion of other anabolic steroids excreted as glucuronides in urine. Since the concentrations of exogenous anabolic steroids in urine are usually very low, probenecid can decrease their concentration to below the limit of detection. Thus, probenecid can be used as a masking agent for the excretion of anabolic steroid glucuronides.

This inhibition of the renal excretion of anabolic steroids by probenecid represents a manipulation of the urine sample in doping control and therefore probenecid was banned by the Medical Commission of the International Olympic Committee.

For doping analysis only single urine samples, collected after a sporting event or out of competition are available. Normally, no information detailing the exact volume and time interval during which the urine was collected is available. Therefore, the urine concentration (μg/ml) of doping agents is the only measureable parameter. The excretion of non-conjugated metabolites of anabolic steroids is not affected by probenecid administration.

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APPENDICES

APPENDIX 1

APPENDIX 1

RANDOMISATION SCHEDULE FOR FARMOVS 9/96

SUBJECT	TREATMENT PHASE I	TREATMENT PHASE II
1	B	A
2	A	B
3	A	B
4	B	A
5	B	A
6	A	B
7	B	A
8	A	B
9	B	A
10	A	B
11	B	A
12	B	A
13	A	B
14	A	B
15	A	B
16	B	A
17	A	B
18	B	A
19	B	A
20	B	A

Treatment

A: Placebo *(Control)*

B: Benemid® 2000mg *(Probenecid)*

APPENDIX 2

APPENDIX 2

BODY MASS INDEX (BMI) AS IMPLEMENTED BY FARMOVS

BODY WEIGHT* IN KILOGRAMS ACCORDING TO HEIGHT** AND BODY MASS INDEX						
	AGE GROUP *(yr)*					
Height *(cm)*	18-24yr	25-34yr	35-44yr	45-54yr	55-64yr	65+yr
140.0	33.5 - 51.7	35.3 - 53.9	37.1 - 56.1	38.8 - 58.2	40.6 - 60.4	42.3 - 62.5
141.0	34.0 - 52.5	35.8 - 54.7	37.6 - 56.9	39.4 - 59.1	41.2 - 61.3	42.9 - 63.5
142.0	34.5 - 53.2	36.3 - 55.4	38.1 - 57.6	40.0 - 59.8	41.8 - 62.2	43.6 - 64.3
143.0	34.9 - 54.0	36.8 - 56.2	38.6 - 58.5	40.5 - 60.7	42.4 - 63.0	44.2 - 65.2
144.0	35.5 - 54.8	37.4 - 57.0	39.2 - 59.3	41.0 - 61.6	42.9 - 63.9	44.8 - 66.1
145.0	36.0 - 55.6	37.9 - 57.9	39.8 - 60.2	41.7 - 62.5	43.6 - 64.8	45.5 - 67.1
146.0	36.5 - 56.3	38.3 - 58.6	40.3 - 60.9	42.2 - 63.4	44.1 - 65.7	46.1 - 68.0
147.0	37.0 - 57.1	38.9 - 59.5	40.9 - 61.8	42.8 - 64.2	44.7 - 66.6	46.7 - 69.0
148.0	37.4 - 57.9	39.4 - 60.3	41.4 - 62.7	43.4 - 65.0	45.4 - 67.4	47.3 - 69.8
149.0	38.0 - 58.6	40.0 - 61.2	42.0 - 63.6	44.0 - 66.0	46.0 - 68.4	48.0 - 70.8
150.0	38.5 - 59.4	40.5 - 61.9	42.6 - 64.3	44.6 - 66.9	46.6 - 69.3	48.6 - 71.8
151.0	39.0 - 60.2	41.0 - 62.8	43.1 - 65.2	45.2 - 67.8	47.3 - 70.3	49.2 - 72.8
152.0	39.5 - 60.9	41.6 - 63.6	43.7 - 66.1	45.7 - 68.6	47.8 - 71.2	49.9 - 73.7
153.0	40.1 - 61.8	42.1 - 64.5	44.3 - 67.0	46.4 - 69.5	48.4 - 72.2	50.6 - 74.7
154.0	40.6 - 62.6	42.7 - 65.2	44.8 - 67.9	47.0 - 70.4	49.1 - 73.0	51.2 - 75.7
155.0	41.1 - 63.5	43.3 - 66.1	45.5 - 68.8	47.6 - 71.3	49.8 - 74.0	51.9 - 76.7
156.0	41.6 - 64.2	43.8 - 66.9	46.0 - 69.6	48.2 - 72.3	50.4 - 74.9	52.6 - 77.7
157.0	42.1 - 65.1	44.4 - 67.8	46.6 - 70.5	48.8 - 73.3	51.0 - 75.9	53.3 - 78.7
158.0	42.7 - 65.9	44.9 - 68.6	47.2 - 71.4	49.4 - 74.1	51.7 - 76.9	53.9 - 79.6
159.0	43.2 - 66.8	45.5 - 69.5	47.8 - 72.4	50.0 - 75.1	52.4 - 77.9	54.6 - 80.6
160.0	43.7 - 67.5	46.1 - 70.4	48.4 - 73.3	50.7 - 76.0	53.0 - 78.9	55.3 - 81.6
161.0	44.4 - 68.4	46.7 - 71.3	49.1 - 74.1	51.3 - 77.0	53.7 - 79.9	56.0 - 82.7
162.0	44.9 - 69.3	47.3 - 72.2	49.6 - 75.0	51.9 - 78.0	54.4 - 80.8	56.7 - 83.7
163.0	45.5 - 70.2	47.9 - 73.0	50.2 - 76.0	52.7 - 79.0	55.1 - 81.8	57.4 - 84.8
164.0	46.0 - 71.1	48.4 - 73.9	50.9 - 76.9	53.3 - 79.9	55.7 - 82.8	58.1 - 85.8
165.0	46.6 - 71.8	49.1 - 74.9	51.5 - 77.9	53.9 - 80.8	56.4 - 83.9	58.8 - 86.9
166.0	47.2 - 72.7	49.6 - 75.8	52.1 - 78.8	54.5 - 81.8	57.1 - 84.9	59.5 - 87.9
167.0	47.7 - 73.6	50.2 - 76.8	52.7 - 79.8	55.3 - 82.8	57.8 - 85.9	60.2 - 88.9
168.0	48.2 - 74.5	50.8 - 77.7	53.4 - 80.7	55.9 - 83.8	58.4 - 86.9	60.9 - 90.0
169.0	48.9 - 75.5	51.4 - 78.5	54.0 - 81.7	56.6 - 84.8	59.1 - 88.0	61.7 - 91.1
170.0	49.4 - 76.3	52.0 - 79.5	54.6 - 82.6	57.2 - 85.8	59.9 - 89.0	62.5 - 92.2
171.0	50.0 - 77.2	52.7 - 80.5	55.3 - 83.6	57.9 - 86.9	60.6 - 90.0	63.2 - 93.3
172.0	50.6 - 78.1	53.3 - 81.4	55.9 - 84.6	58.6 - 87.9	61.2 - 91.1	63.9 - 94.4
173.0	51.2 - 79.0	53.9 - 82.3	56.6 - 85.6	59.2 - 88.9	61.9 - 92.2	64.6 - 95.5
174.0	51.8 - 80.0	54.5 - 83.3	57.2 - 86.6	59.9 - 89.9	62.6 - 93.3	65.4 - 96.6
175.0	52.4 - 80.8	55.2 - 84.3	57.9 - 87.6	60.6 - 91.0	63.4 - 94.4	66.2 - 97.7
176.0	53.0 - 81.7	55.8 - 85.1	58.5 - 88.6	61.3 - 92.0	64.1 - 95.4	66.9 - 98.8
177.0	53.6 - 82.7	56.4 - 86.1	59.2 - 89.7	62.0 - 93.1	64.9 - 96.5	67.7 - 99.9

APPENDIX 2 *(continued)*

BODY MASS INDEX (BMI) AS IMPLEMENTED BY FARMOVS *(continued)*

BODY WEIGHT* IN KILOGRAMS ACCORDING TO HEIGHT** AND BODY MASS INDEX						
	AGE GROUP *(yr)*					
Height *(cm)*	**18-24yr**	**25-34yr**	**35-44yr**	**45-54yr**	**55-64yr**	**65+yr**
178.0	54.2 - 83.6	57.1 - 87.1	59.9 - 90.6	62.7 - 94.1	65.6 - 97.6	68.4 - 101.1
179.0	54.8 - 84.6	57.7 - 88.1	60.6 - 91.6	63.5 - 95.2	66.3 - 98.7	69.2 - 102.3
180.0	55.4 - 85.6	58.3 - 89.1	61.2 - 92.6	64.2 - 96.3	67.1 - 99.8	70.0 - 103.4
181.0	56.1 - 86.6	59.0 - 90.1	61.9 - 93.7	64.9 - 97.3	67.8 - 100.9	70.8 - 104.6
182.0	56.6 - 87.5	59.6 - 91.1	62.6 - 94.7	65.6 - 98.3	68.6 - 102.0	71.6 - 105.7
183.0	57.2 - 88.4	60.3 - 92.1	63.3 - 95.8	66.3 - 99.4	69.4 - 103.2	72.4 - 106.9
184.0	57.9 - 89.4	60.9 - 93.1	64.0 - 96.8	67.1 - 100.5	70.1 - 104.3	73.2 - 108.0
185.0	58.5 - 90.4	61.7 - 94.2	64.7 - 97.9	67.8 - 101.6	70.9 - 105.4	74.0 - 109.2
186.0	59.1 - 91.3	62.3 - 95.2	65.4 - 98.9	68.5 - 102.7	71.6 - 106.6	74.7 - 110.3
187.0	59.9 - 92.3	63.0 - 96.3	66.2 - 100.0	69.2 - 103.8	72.4 - 107.8	75.5 - 111.5
188.0	60.5 - 93.3	63.6 - 97.2	66.8 - 101.1	70.0 - 104.9	73.2 - 108.9	76.3 - 112.8
189.0	61.1 - 94.3	64.3 - 98.2	67.5 - 102.2	70.7 - 106.2	74.0 - 110.1	77.1 - 114.0
190.0	61.7 - 95.3	65.0 - 99.3	68.2 - 103.3	71.5 - 107.3	74.7 - 111.2	77.9 - 115.2
191.0	62.4 - 96.4	65.7 - 100.4	68.9 - 104.3	72.3 - 108.4	75.5 - 112.4	78.8 - 116.4
192.0	63.0 - 97.3	66.3 - 101.4	69.7 - 105.4	73.0 - 109.5	76.3 - 113.5	79.7 - 117.6
193.0	63.7 - 98.3	67.1 - 102.5	70.4 - 106.5	73.7 - 110.7	77.2 - 114.7	80.5 - 118.8
194.0	64.3 - 99.3	67.8 - 103.5	71.1 - 107.7	74.5 - 111.8	78.1 - 115.9	81.3 - 120.0
195.0	65.1 - 100.4	68.5 - 104.6	71.8 - 108.8	75.3 - 113.0	78.8 - 117.2	82.2 - 121.3
196.0	65.7 - 101.4	69.1 - 105.6	72.6 - 109.9	76.1 - 114.1	79.6 - 118.4	83.0 - 122.5
197.0	66.4 - 102.5	69.8 - 106.7	73.3 - 111.0	76.8 - 115.3	80.4 - 119.6	83.9 - 123.9
198.0	67.1 - 103.5	70.6 - 107.8	74.1 - 112.1	77.6 - 116.5	81.2 - 120.8	84.7 - 125.1
199.0	67.8 - 104.6	71.3 - 108.9	74.9 - 113.3	78.4 - 117.7	82.0 - 122.0	85.6 - 126.4
200.0	68.4 - 105.6	72.0 - 110.0	75.6 - 114.4	79.2 - 118.8	82.8 - 123.2	86.4 - 127.6

* Weight: wearing indoor clothing

** Height: without shoes

APPENDIX 3

APPENDIX 3

CERTIFICATE OF INSURANCE

Price Forbes (Pty) Ltd
International Insurance Brokers and
Risk Managers
Reg No; 85/02410/07

Forbes Financial Services Group (Pty) Ltd
Price Forbes House 25 Sauer Street Ext
Johannesburg 2001
P O Box 61689 Marshalltown 2107
Tel. (011) 378 3000 Fax. (011) 378 3003
Telex. 4-84392 SA
Reg No: 69/18487/07

CERTIFICATE OF INSURANCE

Contract Number 622 **Certificate Number CA/A02/622/481**

Lloyd's of London

This Insurance is effected with certain Underwriters at Lloyd's London.

This Certificate is issued in accordance with the authorisation granted to Forbes Financial Services Group (Pty) Ltd, by certain Underwriters at Lloyd's, London whose syndicate numbers and the proportions underwritten by them can be ascertained from the office of Forbes Financial Services Group (Pty) Ltd and in consideration of the premium specified herein, the Underwriters do hereby bind themselves severally and not jointly, each for his own part and not for one another, their executors and administrators to insure in accordance with the terms and conditions described in this Certificate.

Certificate Provisions

1. All premiums are payable to Forbes Financial Services Group (Pty) Ltd at the address stated above in the currency of the Republic of South Africa.
2. All claims are payable by the Underwriters, care of Forbes Financial Services Group (Pty) Ltd at the address stated above.
3. This insurance shall be governed by the law of the Republic of South Africa, whose courts shall have jurisdiction in any dispute arising hereunder, and any summons, notice or process to be served upon the Underwriters for the purpose of instituting any legal proceedings against them in connection with this insurance may be served upon Mr R S Napier, c/o Messrs Webber, Wentzel, Bowens, 2nd Floor, 60 Main Street, Johannesburg.
4. It is expressly understood and agreed by the Insured by accepting this Certificate that Forbes Financial Services Group (Pty) Ltd is not one of the Underwriters hereunder and neither is nor shall be in any way or to any extent liable for any loss or claim whatsoever, but the Underwriters hereunder are only those Underwriters whose names can be ascertained in the above mentioned Contract.
5. This insurance is made and accepted subject to all the provisions, conditions and warranties set forth herein or in any forms or endorsements attached hereto, all of which are to be considered as incorporated herein and any provisions or conditions appearing in any forms or endorsements attached hereto which alter the Certificate provisions stated above shall supercede such Certificate provisions insofar as they are inconsistent therewith.
6. This Certificate shall not be assigned either in whole or in part without the written consent of Forbes Financial Services Group (Pty) Ltd endorsed hereon.
7. If you have any complaints concerning your insurance, please contact Forbes Financial Services Group (Pty) Ltd.
8. This certificate shall not be valid unless signed by Forbes Financial Services Group (Pty) Ltd.

Enclosures: Personal Accident/Illness Policy of Insurance.

Dated at Johannesburg this 21st day of June 1998

PRICE FORBES (PTY) LTD
MANAGERS FOR FORBES FINANCIAL SERVICES GROUP (PTY) LTD

APPENDIX 4

APPENDIX 4

DECLARATION OF HELSINKI

Recommendations guiding medical physicians in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964. Amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983 and the 41st World Medical Assembly, Hong Kong, September 1989.

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words "the health of my patient will be my first consideration" and the International Code of Medical Ethics declares that: "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient".

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research, a fundamental distinction must be recognised between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity. The World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

1. BASIC PRINCIPLES

1.1 Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

1.2 The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that his independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

1.3 Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

1.4 Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objectives is in proportion to the inherent risk to the subject.

1.5 Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interest of science and society.

1.6 The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimise the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

1.7 Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

1.8 In publication of his or her results, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration, should not be accepted for publication.

1.9 In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferable in writing.

1.10 When obtaining informed consent for the research project, the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

1.11 In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with the legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

1.12 The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

2. MEDICAL RESEARCH COMBINED WITH PROFFESIONAL CARE

(Clinical Research)

2.1 In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, re-establishing health or alleviating suffering.

2.2 The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

2.3 In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method.

2.4 The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

2.5 If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (1.2).

2.6 The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

3. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS

(Non-clinical Biomedical Research)

3.1 In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

3.2 The subjects should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3.3 The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.

3.4 In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

APPENDIX 5

APPENDIX 5

QUALITY ASSURANCE STATEMENT

A STUDY TO DETERMINE THE INFLUENCE OF PROBENECID (BENEMID®) ON THE URINARY EXCRETION OF ANDROSTERONE

This report has been audited by die Quality Assurance Division of the FARMOVS Research Centre. It is considered to be an accurate presentation of the findings as well as a precise account of the procedures and practices employed during the performance of the study.

The following audits were performed:

Pre-study audit : 18/09/96

Post-study audit (clinical) : 06/12/96

The following guidelines were in use:

* In-house SOP's
* Declaration of Helsinki
* Good Clinical Practice for Trials on Medicinal Products in the European Community. In: The Rules Governing Medicinal Products in the European Community. Volume III. Addendum no 2. Guidelines on the quality, safety and efficacy of medicinal products for human use. Commission of the European Communites, Luxenbourg, 1992 149-164.

SIGNATURE

06 December 1998

DATE

APPENDIX 6

Central University of Technology, Free State

6.1 Raw Data

6.1.1 Androsterone

RAW DATA - ANDROSTERONE

FARMOVS 9/96
URINARY VOLUMES (ml)
PLACEBO

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	341.19	135.73	59.38	36.38	40.07	67.00	71.15	146.77	368.59	226.32	212.42	299.31
2	448.46	102.15	434.36	290.20	359.20	175.59	399.67	215.50	395.87	130.99	649.34	487.08
3	393.78	50.57	29.00	296.65	695.16	519.19	346.36	269.00	306.39	139.86	459.40	783.19
4	481.76	56.12	77.33	228.60	82.46	75.27	396.89	144.06	101.90	659.10	265.84	590.60
5	575.15	231.33	314.30	166.81	164.00	82.33	137.36	219.00	970.69	512.47	307.12	400.00
6	224.40	40.92	22.27	30.20	47.76	60.09	115.36	271.22	314.59	177.88	182.39	426.26
7	920.65	140.34	253.24	126.57	119.68	134.63	358.77	308.13	922.66	379.00	356.51	595.77
8	347.00	141.99	376.00	357.10	348.79	398.98	230.83	128.64	265.07	566.00	285.39	204.37
9	716.27	136.31	71.50	203.26	152.08	187.65	251.89	545.75	466.13	369.90	914.31	733.45
11	409.64	77.66	85.54	52.00	377.09	257.14	130.30	83.90	481.36	585.43	832.62	706.69
12	477.66	498.68	572.18	367.53	340.40	99.50	94.46	407.00	191.91	202.90	273.38	316.16
13	678.49	127.39	42.41	171.63	723.27	837.02	190.30	425.13	654.77	408.79	320.39	391.88
14	747.47	45.69	30.57	44.99	240.28	26.59	68.22	41.49	1382.32	256.09	401.81	346.56
15	486.00	128.75	167.71	218.03	290.12	88.52	147.74	298.74	452.13	533.34	208.70	437.38
16	454.76	95.00	294.74	177.83	68.66	166.10	403.32	261.24	460.23	245.90	328.82	202.52
17	345.25	66.59	273.79	444.35	268.00	435.78	120.15	470.45	512.99	390.12	208.48	314.13
18	293.80	151.38	101.12	295.95	138.77	70.99	156.10	81.73	431.34	123.86	74.93	421.74
19	261.90	141.70	140.80	325.35	250.71	252.10	122.35	82.70	559.22	158.00	232.70	293.59
20	486.83	69.35	472.00	198.12	408.72	351.07	121.54	48.31	413.82	270.67	174.26	343.27
MEAN	478.445	128.297	200.960	212.187	269.222	225.555	203.303	234.145	507.999	333.506	352.043	436.524
SD	180.544	101.739	170.267	120.764	194.454	205.661	118.885	149.092	301.033	171.822	221.510	171.724
GEOM MEAN	448.630	105.495	130.579	166.483	202.985	156.972	173.264	184.543	435.993	291.339	299.390	406.217
GEOM SD	1.445	1.836	2.805	2.279	2.296	2.435	1.789	2.149	1.791	1.727	1.792	1.479
CV %	37.736	79.299	84.727	56.914	72.228	91.180	58.477	63.675	59.258	51.520	62.921	39.339
SEM	41.420	23.340	39.062	27.705	44.611	47.182	27.274	34.204	69.062	39.419	50.818	39.396
MIN	224.400	40.920	22.270	30.200	40.070	26.590	68.220	41.490	101.900	123.860	74.930	202.520
MAX	920.650	498.680	572.180	444.350	723.270	837.020	403.320	545.750	1382.320	659.100	914.310	783.190
MEDIAN	454.760	127.390	140.800	203.260	250.710	166.100	147.740	219.000	452.130	270.670	285.390	400.000
N	19	19	19	19	19	19	19	19	19	19	19	19

FARMOVS 9/96
URINARY VOLUMES (ml)
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	497.20	125.33	123.64	451.87	684.95	785.34	142.00	274.16	586.30	259.68	500.25	424.59
2	443.75	309.39	540.30	400.12	298.10	328.35	220.80	280.66	495.76	199.28	169.02	489.45
3	364.84	51.34	73.50	268.30	575.29	1187.71	965.33	358.87	413.27	173.73	332.82	595.10
4	806.59	107.80	336.12	231.75	365.74	125.88	172.00	161.38	294.40	488.00	431.00	599.88
5	647.25	140.77	134.55	413.19	305.32	226.99	222.00	303.30	519.00	348.55	220.97	1004.53
6	298.65	112.66	147.21	268.77	398.62	345.20	163.75	351.14	385.65	241.54	230.65	301.64
7	518.35	98.55	375.37	453.91	500.90	284.18	373.59	312.15	702.65	248.99	275.69	632.08
8	342.70	162.68	149.84	72.39	62.51	100.60	100.00	1327.59	576.09	211.66	146.95	311.78
9	951.83	141.19	618.96	699.31	791.84	1468.81	1024.52	667.85	995.46	777.58	277.60	452.13
11	247.41	127.45	752.72	674.82	690.72	357.69	86.00	272.50	385.44	277.99	398.10	360.89
12	127.67	258.82	396.45	441.05	425.69	335.59	233.53	292.60	737.00	137.62	286.40	506.50
13	770.30	114.16	117.00	226.72	453.04	636.00	177.23	395.25	898.84	228.25	137.22	581.30
14	508.89	49.93	65.99	50.40	236.12	200.26	166.69	264.61	438.96	187.17	170.77	241.94
15	758.02	234.00	382.16	365.75	259.79	317.05	154.90	323.29	581.93	644.93	289.68	668.02
16	467.26	156.59	277.55	488.30	190.75	373.26	590.16	375.15	258.00	256.86	353.00	655.06
17	266.16	96.14	520.78	544.00	283.00	494.21	950.09	471.92	403.27	250.07	154.04	366.24
18	516.91	203.85	549.81	345.26	435.70	434.78	87.00	380.38	467.29	750.61	129.48	753.37
19	464.42	251.45	139.10	156.11	85.37	119.10	350.53	198.26	464.47	208.51	Missing	56.18
20	866.80	80.08	104.10	378.72	748.37	235.80	134.38	165.54	708.66	90.54	334.70	493.71
MEAN	519.211	148.536	305.534	364.776	410.096	439.832	332.342	377.716	542.760	314.819	268.797	499.705
SD	227.776	72.104	211.704	177.025	213.664	359.207	311.954	256.555	194.492	200.901	108.749	210.536
GEOM MEAN	466.726	132.484	235.330	308.156	344.303	341.790	238.856	331.676	511.854	268.894	248.094	440.588
GEOM SD	1.655	1.654	2.167	1.991	1.977	2.044	2.203	1.608	1.422	1.746	1.519	1.838
CV %	43.870	48.543	69.290	48.530	52.101	81.669	93.865	67.923	35.834	63.815	40.458	42.132
SEM	52.255	16.542	48.568	40.612	49.018	82.408	71.567	58.858	44.620	46.090	25.632	48.300
MIN	127.670	49.930	65.990	50.400	62.510	100.600	86.000	161.380	258.000	90.540	129.480	56.180
MAX	951.830	309.390	752.720	699.310	791.840	1468.810	1024.520	1327.590	995.460	777.580	500.250	1004.530
MEDIAN	497.200	127.450	277.550	378.720	398.620	335.590	177.230	312.150	495.760	248.990	276.645	493.710
N	19	19	19	19	19	19	19	19	19	19	18	19

FARMOVS 9/96
CUMULATIVE URINARY VOLUMES (ml)
PLACEBO

SUBJECT	0 to 1 h	0 to 2 h	0 to 3 h	0 to 4 h	0 to 6 h	0 to 8 h	0 to 12 h	0 to 24 h	0 to 30 h	0 to 36 h	0 to 48 h
1	135.730	195.110	231.490	271.560	338.560	409.710	556.480	925.070	1151.390	1363.810	1663.120
2	102.150	536.510	826.710	1185.910	1361.500	1761.170	1976.670	2372.540	2503.530	3152.870	3639.950
3	50.570	79.570	376.220	1071.380	1590.570	1936.930	2205.930	2512.320	2652.180	3111.580	3894.770
4	56.120	133.450	362.050	444.510	519.780	916.670	1060.730	1162.630	1821.730	2087.570	2678.170
5	231.330	545.630	712.440	876.440	958.770	1096.130	1315.130	2285.820	2798.290	3105.410	3505.410
6	40.920	63.190	93.390	141.150	201.240	316.600	587.820	902.410	1080.290	1262.680	1688.940
7	140.340	393.580	520.150	639.830	774.460	1133.230	1441.360	2364.020	2743.020	3099.530	3695.300
8	141.990	517.990	875.090	1223.880	1622.860	1853.690	1982.330	2247.400	2813.400	3098.790	3303.160
9	136.310	207.810	411.070	563.150	750.800	1002.690	1548.440	2014.570	2384.470	3298.780	4032.230
11	77.660	163.200	215.200	592.290	849.430	979.730	1063.630	1544.990	2130.420	2963.040	3669.730
12	498.680	1070.860	1438.390	1778.790	1878.290	1972.750	2379.750	2571.660	2774.560	3047.940	3364.100
13	127.390	169.800	341.430	1064.700	1901.720	2092.020	2517.150	3171.920	3580.710	3901.100	4292.980
14	45.690	76.260	121.250	361.530	388.120	456.340	497.830	1880.150	2136.240	2538.050	2884.610
15	128.750	296.460	514.490	804.610	893.130	1040.870	1339.610	1791.740	2325.080	2533.780	2971.160
16	95.000	389.740	567.570	636.230	802.330	1205.650	1466.890	1927.120	2173.020	2501.840	2704.360
17	66.590	340.380	784.730	1052.730	1488.510	1608.660	2079.110	2592.100	2982.220	3190.700	3504.830
18	151.380	252.500	548.450	687.220	758.210	914.310	996.040	1427.380	1551.240	1626.170	2047.910
19	141.700	282.500	607.850	858.560	1110.660	1233.010	1315.710	1874.930	2032.930	2265.630	2559.220
20	69.350	541.350	739.470	1148.190	1499.260	1620.800	1669.110	2082.930	2353.600	2527.860	2871.130
MEAN	128.297	329.257	541.444	810.666	1036.221	1239.524	1473.669	1981.668	2315.175	2667.217	3103.741
SD	101.739	242.516	318.632	395.740	519.292	539.627	604.717	596.431	621.383	697.623	754.186
GEOM MEAN	105.495	255.693	447.817	703.072	890.336	1102.395	1337.102	1883.319	2223.310	2563.176	3003.998
GEOM SD	1.836	2.139	1.988	1.827	1.850	1.719	1.620	1.412	1.360	1.369	1.315
CV %	79.299	73.655	58.849	48.817	50.114	43.535	41.035	30.097	26.840	26.155	24.299
SEM	23.340	55.637	73.099	90.789	119.134	123.799	138.732	136.831	142.555	160.046	173.022
MIN	40.920	63.190	93.390	141.150	201.240	316.600	497.830	902.410	1080.290	1262.680	1663.120
MAX	498.680	1070.860	1438.390	1778.790	1901.720	2092.020	2517.150	3171.920	3580.710	3901.100	4292.980
MEDIAN	127.390	282.500	520.150	804.610	893.130	1133.230	1441.360	2014.570	2353.600	2963.040	3303.160
N	19	19	19	19	19	19	19	19	19	19	19

FARMOVS 9/96
CUMULATIVE URINARY VOLUMES (ml)
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	0 to 1 h	0 to 2 h	0 to 3 h	0 to 4 h	0 to 6 h	0 to 8 h	0 to 12 h	0 to 24 h	0 to 30 h	0 to 36 h	0 to 48 h
1	125.330	248.970	700.840	1385.790	2171.130	2313.130	2587.290	3173.590	3433.270	3933.520	4358.110
2	309.390	849.690	1249.810	1547.910	1876.260	2097.060	2377.720	2873.480	3072.760	3241.780	3731.230
3	51.340	124.840	393.140	968.430	2156.140	3121.470	3480.340	3893.610	4067.340	4400.160	4995.260
4	107.800	443.920	675.670	1041.410	1167.290	1339.290	1500.670	1795.070	2283.070	2714.070	3313.950
5	140.770	275.320	688.510	993.830	1220.820	1442.820	1746.120	2265.120	2613.670	2834.640	3839.170
6	112.660	259.870	528.640	927.260	1272.460	1436.210	1787.350	2173.000	2414.540	2645.190	2946.830
7	98.550	473.920	927.830	1428.730	1712.910	2086.500	2398.650	3101.300	3350.290	3625.980	4258.060
8	162.680	312.520	384.910	447.420	548.020	648.020	1975.610	2551.700	2763.360	2910.310	3222.090
9	141.190	760.150	1459.460	2251.300	3720.110	4744.630	5412.480	6407.940	7185.520	7463.120	7915.250
11	127.450	880.170	1554.990	2245.710	2603.400	2689.400	2961.900	3347.340	3625.330	4023.430	4384.320
12	258.820	655.270	1096.320	1522.010	1857.600	2091.130	2383.730	3120.730	3258.350	3544.750	4051.250
13	114.160	231.160	457.880	910.920	1546.920	1724.150	2119.400	3018.240	3246.490	3383.710	3965.010
14	49.930	115.920	166.320	402.440	602.700	769.390	1034.000	1472.960	1660.130	1830.900	2072.840
15	234.000	616.160	981.910	1241.700	1558.750	1713.650	2036.940	2618.870	3263.800	3553.480	4221.500
16	156.590	434.140	922.440	1113.190	1486.450	2076.610	2451.760	2709.760	2966.620	3319.620	3974.680
17	96.140	616.920	1160.920	1443.920	1938.130	2888.220	3360.140	3763.410	4013.480	4167.520	4533.760
18	203.850	753.660	1098.920	1534.620	1969.400	2056.400	2436.780	2904.070	3654.680	3784.160	4537.530
19	251.450	390.550	546.660	632.030	751.130	1101.660	1299.920	1764.390	1972.900	1972.900	2029.080
20	80.080	184.180	562.900	1311.270	1547.070	1681.450	1846.990	2555.650	2646.190	2980.890	3474.600
MEAN	148.536	454.070	818.846	1228.942	1668.773	2001.115	2378.831	2921.591	3236.410	3491.059	3990.764
SD	72.104	246.842	384.456	499.545	735.371	932.763	968.832	1060.453	1154.555	1182.348	1234.811
GEOM MEAN	132.484	384.631	721.730	1123.414	1511.938	1810.065	2222.995	2771.729	3082.109	3335.143	3825.007
GEOM SD	1.654	1.869	1.743	1.584	1.612	1.602	1.451	1.385	1.365	1.328	1.351
CV %	48.543	54.362	46.951	40.648	44.067	46.612	40.727	36.297	35.674	33.868	30.942
SEM	16.542	56.629	88.200	114.604	168.706	213.991	222.265	243.284	264.873	271.249	283.285
MIN	49.930	115.920	166.320	402.440	548.020	648.020	1034.000	1472.960	1660.130	1830.900	2029.080
MAX	309.390	880.170	1554.990	2251.300	3720.110	4744.630	5412.480	6407.940	7185.520	7463.120	7915.250
MEDIAN	127.450	434.140	700.840	1241.700	1558.750	2056.400	2377.720	2873.480	3246.490	3383.710	3974.680
N	19	19	19	19	19	19	19	19	19	19	19

FARMOVS 9/96
URINARY ANDROSTERONE CONCENTRATION (µg/ml)
LLOQ = 0.06µg/ml
PLACEBO

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	3.578	3.453	2.689	3.487	3.509	4.737	4.977	3.577	4.034	4.379	5.333	4.604
2	3.245	4.552	0.485	0.925	0.697	0.778	1.228	2.572	3.281	3.523	2.008	3.816
3	6.930	10.470	9.511	1.005	0.549	0.707	1.317	3.660	5.415	6.994	3.038	2.556
4	5.915	12.886	5.399	1.517	4.348	2.568	2.424	7.201	6.618	6.703	5.335	6.143
5	2.518	3.882	1.178	1.338	1.487	4.558	4.028	2.340	2.377	2.584	2.108	4.595
6	8.997	13.443	11.803	7.940	3.666	7.509	5.296	3.166	4.974	8.958	7.800	4.022
7	1.797	3.630	0.871	1.100	1.188	1.797	0.522	1.193	1.620	2.503	1.900	2.383
8	3.505	2.289	0.479	0.509	0.452	0.398	1.434	2.929	3.938	1.309	2.701	4.136
9	1.403	2.854	2.807	1.017	1.081	0.568	2.400	0.861	2.631	1.928	1.213	2.365
11	5.868	5.514	4.958	2.544	0.512	1.304	5.399	4.004	3.808	2.201	1.633	3.757
12	1.327	0.578	0.770	1.381	1.257	3.669	4.455	2.050	7.870	8.086	3.062	1.662
13	2.745	3.889	3.807	0.819	0.232	0.303	1.980	1.765	2.177	2.708	2.405	3.991
14	2.489	7.408	5.756	3.533	0.829	10.573	6.732	6.568	1.644	5.109	4.328	6.153
15	5.052	7.882	2.108	1.539	1.101	2.928	7.417	5.852	4.981	5.394	8.109	7.883
16	7.781	5.131	1.364	2.483	6.012	1.268	1.794	5.351	6.982	7.231	6.054	3.894
17	4.686	12.408	0.860	0.518	0.869	0.603	4.310	2.143	2.488	0.978	0.792	5.658
18	2.819	3.920	2.849	1.666	1.587	2.174	2.324	3.486	1.851	1.228	3.357	1.369
19	3.789	3.170	2.392	1.179	1.861	2.143	5.933	4.934	4.420	6.848	Missing	3.922
20	3.312	4.382	0.530	0.585	0.301	0.286	3.454	2.527	2.667	3.209	1.518	4.032
MEAN	4.092	5.881	3.190	1.847	1.660	2.572	3.549	3.483	3.883	4.309	3.483	4.050
SD	2.162	3.810	3.138	1.728	1.579	2.711	2.035	1.783	1.868	2.538	2.205	1.626
GEOM MEAN	3.578	4.728	2.047	1.410	1.138	1.552	2.911	3.041	3.476	3.539	2.878	3.718
GEOM SD	1.722	2.091	2.706	2.025	2.444	2.919	2.021	1.751	1.630	1.978	1.913	1.560
CV %	52.826	64.781	98.352	93.553	95.105	105.399	57.354	51.196	48.119	58.901	63.297	40.165
SEM	0.496	0.874	0.720	0.396	0.362	0.622	0.467	0.409	0.429	0.582	0.520	0.373
MIN	1.327	0.578	0.479	0.509	0.232	0.286	0.522	0.861	1.620	0.978	0.792	1.369
MAX	8.997	13.443	11.803	7.940	6.012	10.573	7.417	7.201	7.870	8.958	8.109	7.883
MEDIAN	3.505	4.382	2.392	1.338	1.101	1.797	3.454	3.166	3.808	3.523	2.870	3.991
N	19	19	19	19	19	19	19	19	19	19	18	19

FARMOVS 9/96
URINARY ANDROSTERONE CONCENTRATION (µg/ml)
LLOQ = 0.06µg/ml
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	4.265	2.983	0.611	0.162	0.131	0.195	0.549	0.993	1.329	1.676	2.559	3.783
2	3.382	1.106	0.122	0.092	0.146	0.172	0.670	1.142	1.823	2.417	3.447	4.113
3	4.358	6.091	0.962	0.262	0.176	0.189	0.231	1.064	4.209	6.430	4.824	4.306
4	3.540	7.873	0.359	0.252	0.633	1.296	1.915	4.164	3.433	4.666	7.998	5.869
5	3.421	4.805	0.667	0.254	0.178	0.370	1.397	1.521	3.366	4.102	6.359	2.088
6	5.665	4.466	0.982	0.356	0.227	0.523	1.438	1.519	4.249	5.196	6.620	7.633
7	2.629	3.829	0.096	0.100	0.079	0.240	0.408	0.664	1.642	3.146	3.437	1.957
8	3.393	1.670	0.595	1.080	1.703	1.907	1.039	0.249	1.137	2.220	2.919	3.022
9	1.592	2.601	0.161	0.116	0.097	0.133	0.189	0.528	2.038	0.945	2.708	2.812
11	6.256	3.417	0.096	0.108	0.106	0.131	0.497	1.367	2.675	3.009	1.838	6.218
12	16.229	2.195	0.175	0.145	0.220	0.301	0.874	1.186	1.384	8.258	4.748	1.141
13	2.333	2.748	0.262	0.260	0.151	0.293	0.704	0.819	1.146	2.759	2.693	3.888
14	3.628	2.698	0.506	0.570	0.195	0.287	0.579	1.181	2.463	2.919	3.341	4.215
15	5.016	3.083	0.365	0.237	0.341	0.638	1.724	2.141	3.245	4.400	1.810	3.321
16	7.264	3.671	0.177	0.197	0.492	0.478	0.262	1.550	9.562	7.119	5.145	5.210
17	5.693	3.957	0.936	Missing	0.126	0.103	0.267	0.956	2.292	4.334	1.336	4.547
18	1.979	1.554	0.088	0.116	0.092	0.134	0.983	0.827	0.671	1.726	2.231	1.705
19	5.726	9.911	0.507	0.408	0.590	1.027	1.108	2.395	3.707	8.199	Missing	6.231
20	2.528	2.073	0.234	0.073	0.067	0.150	0.915	0.962	1.305	2.112	2.067	3.347
MEAN	4.679	3.723	0.416	0.266	0.303	0.451	0.829	1.328	2.720	3.981	3.671	3.969
SD	3.202	2.212	0.306	0.240	0.379	0.475	0.511	0.858	1.988	2.200	1.881	1.717
GEOM MEAN	4.027	3.221	0.310	0.205	0.201	0.309	0.674	1.126	2.242	3.418	3.262	3.583
GEOM SD	1.701	1.728	2.279	2.009	2.293	2.307	2.007	1.817	1.866	1.796	1.646	1.633
CV %	68.433	59.411	73.468	90.252	125.217	105.327	61.666	64.623	73.090	55.263	51.229	43.266
SEM	0.735	0.507	0.070	0.057	0.087	0.109	0.117	0.197	0.456	0.505	0.443	0.394
MIN	1.592	1.106	0.088	0.073	0.067	0.103	0.189	0.249	0.671	0.945	1.336	1.141
MAX	16.229	9.911	0.982	1.080	1.703	1.907	1.915	4.164	9.562	8.258	7.998	7.633
MEDIAN	3.628	3.083	0.359	0.217	0.176	0.287	0.704	1.142	2.292	3.146	3.130	3.888
N	19	19	19	18	19	19	19	19	19	19	18	19

FARMOVS 9/96
FRACTIONAL URINARY ANDROSTERONE EXCRETION (µg)
PLACEBO

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	1220.778	468.676	159.673	126.857	140.606	317.379	354.114	524.996	1486.892	991.055	1132.836	1378.023
2	1455.253	464.987	210.665	268.435	250.362	136.609	490.795	554.266	1298.849	461.478	1303.875	1858.697
3	2728.895	529.468	275.819	298.133	381.643	367.067	456.156	984.540	1659.102	978.181	1395.657	2001.834
4	2849.610	723.162	417.505	346.786	358.536	193.293	962.061	1037.376	674.374	4417.947	1418.256	3628.056
5	1448.228	898.023	370.245	223.192	243.868	375.260	553.286	512.460	2307.330	1324.222	647.409	1838.000
6	2018.927	550.088	262.853	239.788	175.088	451.216	610.947	858.683	1564.771	1593.449	1422.642	1714.418
7	1654.408	509.434	220.572	139.227	142.180	241.930	187.278	367.599	1494.709	948.637	677.369	1419.720
8	1216.235	325.015	180.104	181.764	157.653	158.794	331.010	376.787	1043.846	740.894	770.838	845.274
9	1004.927	389.029	200.701	206.715	164.398	106.585	604.536	469.891	1226.388	713.167	1109.058	1734.609
11	2403.768	428.217	424.107	132.288	193.070	335.311	703.490	335.936	1833.019	1288.531	1359.668	2655.034
12	633.855	288.237	440.579	507.559	427.883	365.066	420.819	834.350	1510.332	1640.649	837.090	525.458
13	1862.455	495.420	161.455	140.565	167.799	253.617	376.794	750.354	1425.434	1107.003	770.538	1563.993
14	1860.453	338.472	175.961	158.950	199.192	281.136	459.257	272.506	2272.534	1308.364	1739.034	2132.384
15	2455.272	1014.808	353.533	335.548	319.422	259.187	1095.788	1748.226	2252.060	2876.836	1692.348	3447.867
16	3538.488	487.445	402.025	441.552	412.784	210.615	723.556	1397.895	3213.326	1778.103	1990.676	788.613
17	1617.842	826.249	235.459	230.173	232.892	262.775	517.847	1008.174	1276.319	381.537	165.116	1777.348
18	828.222	593.410	288.091	493.053	220.228	154.332	362.776	284.911	798.410	152.100	251.540	577.362
19	992.339	449.189	336.794	383.588	466.571	540.250	725.903	408.042	2471.752	1081.984	Missing	1151.460
20	1612.381	303.892	250.160	115.900	123.025	100.406	419.799	122.079	1103.658	868.580	264.527	1384.065
MEAN	1758.018	530.696	282.437	261.583	251.432	268.991	545.064	676.267	1627.006	1297.512	1052.693	1706.432
SD	760.115	202.269	95.518	125.616	109.014	118.244	223.197	419.816	634.130	964.772	535.592	843.798
GEOM MEAN	1605.153	498.758	267.288	234.807	231.031	243.674	504.327	561.110	1515.443	1030.985	873.711	1510.296
GEOM SD	1.564	1.427	1.409	1.613	1.519	1.603	1.508	1.918	1.479	2.078	2.045	1.694
CV %	43.237	38.114	33.819	48.021	43.357	43.958	40.949	62.079	38.975	74.356	50.878	49.448
SEM	174.382	46.404	21.913	28.818	25.010	27.127	51.205	96.313	145.479	221.334	126.240	193.580
MIN	633.855	288.237	159.673	115.900	123.025	100.406	187.278	122.079	674.374	152.100	165.116	525.458
MAX	3538.488	1014.808	440.579	507.559	466.571	540.250	1095.788	1748.226	3213.326	4417.947	1990.676	3628.056
MEDIAN	1617.842	487.445	262.853	230.173	220.228	259.187	490.795	524.996	1494.709	1081.984	1120.947	1714.418
N	19	19	19	19	19	19	19	19	19	19	18	19

FARMOVS 9/96
FRACTIONAL URINARY ANDROSTERONE EXCRETION (µg)
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	2120.558	373.859	75.544	73.203	89.728	153.141	77.958	272.241	779.193	435.224	1280.140	1606.224
2	1500.763	342.185	65.917	36.811	43.523	56.476	147.936	320.514	903.770	481.660	582.612	2013.108
3	1589.973	312.712	70.707	70.295	101.251	224.477	222.991	381.838	1739.453	1117.084	1605.524	2562.501
4	2855.329	848.709	120.667	58.401	231.513	163.140	329.380	671.986	1010.675	2277.008	3447.138	3520.696
5	2214.242	676.400	89.745	104.950	54.347	83.986	310.134	461.319	1746.954	1429.752	1405.148	2097.459
6	1691.852	503.140	144.560	95.682	90.487	180.540	235.473	533.382	1638.627	1255.042	1526.903	2302.418
7	1362.742	377.348	36.036	45.391	39.571	68.203	152.425	207.268	1153.751	783.323	947.547	1236.981
8	1162.781	271.676	89.155	78.181	106.455	191.844	103.900	330.570	655.014	469.885	428.947	942.199
9	1515.313	367.235	99.653	81.120	76.808	195.352	193.634	352.625	2028.747	734.813	751.741	1271.390
11	1547.797	435.497	72.261	72.881	73.216	46.857	42.742	372.508	1031.052	836.472	731.708	2244.014
12	2071.956	568.110	69.379	63.952	93.652	101.013	204.105	347.024	1020.008	1136.466	1359.827	577.917
13	1797.110	313.712	30.654	58.947	68.409	186.348	124.770	323.710	1030.071	629.742	369.533	2260.094
14	1846.253	134.711	33.391	28.728	46.043	57.475	96.514	312.504	1081.158	546.349	570.543	1019.777
15	3802.228	721.422	139.488	86.683	88.588	202.278	267.048	692.164	1888.363	2837.692	524.321	2218.494
16	3394.177	574.842	49.126	96.195	93.849	178.418	154.622	581.483	2466.996	1828.586	1816.185	3412.863
17	1515.249	380.426	487.450	Missing	35.658	50.904	253.674	451.156	924.295	1083.803	205.797	1665.293
18	1022.965	316.783	48.383	40.050	40.084	58.261	85.521	314.574	313.552	1295.553	288.870	1284.496
19	2659.269	2492.121	70.524	63.693	50.368	122.316	388.387	474.833	1721.790	1709.573	Missing	350.058
20	2191.270	166.006	24.359	27.647	50.141	35.370	122.958	159.249	924.801	191.220	691.825	1652.447
MEAN	1992.728	535.626	95.632	65.712	77.563	124.021	184.956	397.944	1266.225	1109.434	1029.684	1802.022
SD	736.822	508.202	100.955	23.210	44.063	64.998	94.452	143.650	546.644	681.359	779.573	849.633
GEOM MEAN	1879.623	426.751	72.976	61.278	69.309	105.728	161.020	373.617	1147.614	919.166	809.404	1578.533
GEOM SD	1.412	1.874	1.975	1.498	1.591	1.841	1.765	1.450	1.617	1.937	2.057	1.782
CV %	36.976	94.880	105.567	35.321	56.809	52.409	51.067	36.098	43.171	61.415	75.710	47.149
SEM	169.038	116.589	23.161	5.471	10.109	14.912	21.669	32.956	125.409	156.315	183.747	194.919
MIN	1022.965	134.711	24.359	27.647	35.658	35.370	42.742	159.249	313.552	191.220	205.797	350.058
MAX	3802.228	2492.121	487.450	104.950	231.513	224.477	388.387	692.164	2466.996	2837.692	3447.138	3520.696
MEDIAN	1797.110	377.348	70.707	67.123	73.216	122.316	154.622	352.625	1031.052	1083.803	741.724	1665.293
N	19	19	19	18	19	19	19	19	19	19	18	19

FARMOVS 9/96
CUMULATIVE URINARY ANDROSTERONE EXCRETION (µg)
PLACEBO

SUBJECT	-12 to 0 h	0 to 1 h	0 to 2 h	0 to 3 h	0 to 4 h	0 to 6 h	0 to 8 h	0 to 12 h	0 to 24 h	0 to 30 h	0 to 36 h	0 to 48 h
1	1220.778	468.676	628.349	755.206	895.811	1213.190	1567.304	2092.300	3579.192	4570.247	5703.083	7081.106
2	1455.253	464.987	675.651	944.086	1194.449	1331.058	1821.853	2376.119	3674.968	4136.446	5440.321	7299.018
3	2728.895	529.468	805.287	1103.420	1485.063	1852.130	2308.286	3292.826	4951.928	5930.109	7325.766	9327.600
4	2849.610	723.162	1140.667	1487.453	1845.989	2039.283	3001.344	4038.720	4713.094	9131.042	10549.298	14177.354
5	1448.228	898.023	1268.268	1491.460	1735.328	2110.588	2663.874	3176.334	5483.665	6807.887	7455.296	9293.296
6	2018.927	550.088	812.940	1052.728	1227.817	1679.032	2289.979	3148.661	4713.432	6306.881	7729.523	9443.941
7	1654.408	509.434	730.006	869.233	1011.413	1253.343	1440.621	1808.220	3302.929	4251.566	4928.935	6348.655
8	1216.235	325.015	505.119	686.883	844.536	1003.330	1334.340	1711.127	2754.973	3495.867	4266.705	5111.979
9	1004.927	389.029	589.729	796.445	960.843	1067.428	1671.964	2141.855	3368.243	4081.410	5190.468	6925.078
11	2403.768	428.217	852.325	984.613	1177.683	1512.993	2216.483	2552.419	4385.437	5673.969	7033.637	9688.672
12	633.855	288.237	728.816	1236.375	1664.257	2029.323	2450.142	3284.492	4794.824	6435.473	7272.563	7798.021
13	1862.455	495.420	656.875	797.440	965.238	1218.855	1595.649	2346.004	3771.438	4878.441	5648.979	7212.972
14	1860.453	338.472	514.432	673.382	872.574	1153.710	1612.967	1885.474	4158.008	5466.372	7205.405	9337.789
15	2455.272	1014.808	1368.340	1703.888	2023.310	2282.497	3378.285	5126.511	7378.571	10255.407	11947.755	15395.621
16	3538.488	487.445	889.470	1331.022	1743.806	1954.421	2677.977	4075.872	7289.198	9067.301	11057.977	11846.590
17	1617.842	826.249	1061.708	1291.881	1524.773	1787.549	2305.395	3313.570	4589.889	4971.426	5136.542	6913.890
18	828.222	593.410	881.500	1374.553	1594.781	1749.113	2111.890	2396.801	3195.211	3347.311	3598.851	4176.213
19	992.339	449.189	785.983	1169.570	1636.142	2176.392	2902.294	3310.336	5782.089	6864.073	6864.073	8015.533
20	1612.381	303.892	554.052	669.952	792.977	893.383	1313.182	1435.261	2538.919	3407.499	3672.026	5056.090
MEAN	1758.018	530.696	813.133	1074.715	1326.147	1595.138	2140.202	2816.469	4443.474	5740.986	6738.274	8444.706
SD	760.115	202.269	246.071	314.308	391.889	442.311	607.609	950.046	1343.779	2010.031	2360.736	2913.016
GEOM MEAN	1605.153	498.758	780.892	1031.488	1270.313	1534.079	2058.899	2671.265	4264.664	5437.749	6375.879	8006.682
GEOM SD	1.564	1.427	1.334	1.344	1.356	1.340	1.333	1.398	1.340	1.397	1.418	1.397
CV %	43.237	38.114	30.262	29.246	29.551	27.729	28.390	33.732	30.242	35.012	35.035	34.495
SEM	174.382	46.404	56.453	72.107	89.905	101.473	139.395	217.955	308.284	461.133	541.590	668.292
MIN	633.855	288.237	505.119	669.952	792.977	893.383	1313.182	1435.261	2538.919	3347.311	3598.851	4176.213
MAX	3538.488	1014.808	1368.340	1703.888	2023.310	2282.497	3378.285	5126.511	7378.571	10255.407	11947.755	15395.621
MEDIAN	1617.842	487.445	785.983	1052.728	1227.817	1679.032	2216.483	2552.419	4385.437	5466.372	6864.073	7798.021
N	19	19	19	19	19	19	19	19	19	19	19	19

FARMOVS 9/96
CUMULATIVE URINARY ANDROSTERONE EXCRETION (µg)
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	-12 to 0 h	0 to 1 h	0 to 2 h	0 to 3 h	0 to 4 h	0 to 6 h	0 to 8 h	0 to 12 h	0 to 24 h	0 to 30 h	0 to 36 h	0 to 48 h
1	2120.558	373.859	449.403	522.606	612.335	765.476	843.434	1115.675	1894.868	2330.091	3610.231	5216.455
2	1500.763	342.185	408.102	444.913	488.436	544.912	692.848	1013.362	1917.132	2398.792	2981.404	4994.512
3	1589.973	312.712	383.419	453.714	554.965	779.442	1002.433	1384.271	3123.724	4240.808	5846.332	8408.832
4	2855.329	848.709	969.376	1027.777	1259.291	1422.431	1751.811	2423.798	3434.473	5711.481	9158.619	12679.315
5	2214.242	676.400	766.145	871.095	925.442	1009.428	1319.562	1780.882	3527.836	4957.588	6362.736	8460.194
6	1691.852	503.140	647.700	743.382	833.869	1014.408	1249.881	1783.262	3421.889	4676.931	6203.834	8506.252
7	1362.742	377.348	413.383	458.774	498.346	566.549	718.973	926.241	2079.992	2863.315	3810.861	5047.842
8	1162.781	271.676	360.830	439.012	545.466	737.310	841.210	1171.780	1826.795	2296.680	2725.627	3667.826
9	1515.313	367.235	466.888	548.008	624.816	820.168	1013.802	1366.427	3395.174	4129.988	4881.728	6153.118
11	1547.797	435.497	507.758	580.638	653.855	700.712	743.454	1115.962	2147.014	2983.485	3715.193	5959.207
12	2071.956	568.110	637.489	701.441	795.093	896.105	1100.211	1447.234	2467.242	3603.708	4963.535	5541.452
13	1797.110	313.712	344.366	403.313	471.722	658.070	782.840	1106.550	2136.620	2766.362	3135.895	5395.990
14	1846.253	134.711	168.102	196.830	242.873	300.348	396.862	709.366	1790.525	2336.874	2907.416	3927.193
15	3802.228	721.422	860.910	947.593	1036.182	1238.459	1505.507	2197.671	4086.034	6923.726	7448.047	9666.541
16	3394.177	574.842	623.968	720.163	814.012	992.431	1147.053	1728.535	4195.531	6024.117	7840.302	11253.165
17	1515.249	380.426	867.876	867.876	903.534	954.438	1208.112	1659.267	2583.562	3667.365	3873.163	5538.456
18	1022.965	316.783	365.166	405.216	445.301	503.561	589.082	903.657	1217.208	2512.761	2801.631	4086.127
19	2659.269	2492.121	2562.645	2626.338	2676.706	2799.022	3187.409	3662.241	5384.032	7093.605	7093.605	7443.663
20	2191.270	166.006	190.365	218.012	268.153	303.523	426.480	585.730	1510.531	1701.752	2393.576	4046.024
MEAN	1992.728	535.626	631.257	693.511	771.073	895.094	1080.051	1477.995	2744.220	3853.654	4829.144	6631.167
SD	736.822	508.202	518.601	522.798	528.503	542.803	620.084	715.594	1087.118	1646.823	2030.522	2569.752
GEOM MEAN	1879.623	426.751	517.493	582.239	662.240	785.191	957.778	1344.403	2552.335	3543.507	4450.326	6212.796
GEOM SD	1.412	1.874	1.838	1.780	1.716	1.670	1.629	1.551	1.480	1.521	1.506	1.440
CV %	36.976	94.880	82.154	75.384	68.541	60.642	57.412	48.417	39.615	42.734	42.047	38.753
SEM	169.038	116.589	118.975	119.938	121.247	124.527	142.257	164.168	249.402	377.807	465.834	589.541
MIN	1022.965	134.711	168.102	196.830	242.873	300.348	396.862	585.730	1217.208	1701.752	2393.576	3667.826
MAX	3802.228	2492.121	2562.645	2626.338	2676.706	2799.022	3187.409	3662.241	5384.032	7093.605	9158.619	12679.315
MEDIAN	1797.110	377.348	466.888	548.008	624.816	779.442	1002.433	1366.427	2467.242	3603.708	3873.163	5541.452
N	19	19	19	19	19	19	19	19	19	19	19	19

FARMOVS 9/96
ANDROSTERONE EXCRETION RATE (µg/hr)
PLACEBO

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	101.731	468.676	159.673	126.857	140.606	158.690	177.057	131.249	123.908	165.176	188.806	114.835
2	121.271	464.987	210.665	268.435	250.362	68.305	245.397	138.567	108.237	76.913	217.312	154.891
3	227.408	529.468	275.819	298.133	381.643	183.534	228.078	246.135	138.258	163.030	232.610	166.819
4	237.468	723.162	417.505	346.786	358.536	96.647	481.031	259.344	56.198	736.325	236.376	302.338
5	120.686	898.023	370.245	223.192	243.868	187.630	276.643	128.115	192.278	220.704	107.901	153.167
6	168.244	550.088	262.853	239.788	175.088	225.608	305.473	214.671	130.398	265.575	237.107	142.868
7	137.867	509.434	220.572	139.227	142.180	120.965	93.639	91.900	124.559	158.106	112.895	118.310
8	101.353	325.015	180.104	181.764	157.653	79.397	165.505	94.197	86.987	123.482	128.473	70.440
9	83.744	389.029	200.701	206.715	164.398	53.293	302.268	117.473	102.199	118.861	184.843	144.551
11	200.314	428.217	424.107	132.288	193.070	167.655	351.745	83.984	152.752	214.755	226.611	221.253
12	52.821	288.237	440.579	507.559	427.883	182.533	210.410	208.588	125.861	273.442	139.515	43.788
13	155.205	495.420	161.455	140.565	167.799	126.809	188.397	187.589	118.786	184.501	128.423	130.333
14	155.038	338.472	175.961	158.950	199.192	140.568	229.629	68.127	189.378	218.061	289.839	177.699
15	204.606	1014.808	353.533	335.548	319.422	129.593	547.894	437.057	187.672	479.473	282.058	287.322
16	294.874	487.445	402.025	441.552	412.784	105.307	361.778	349.474	267.777	296.350	331.779	65.718
17	134.820	826.249	235.459	230.173	232.892	131.388	258.923	252.044	106.360	63.590	27.519	148.112
18	69.019	593.410	288.091	493.053	220.228	77.166	181.388	71.228	66.534	25.350	41.923	48.114
19	82.695	449.189	336.794	383.588	466.571	270.125	362.951	102.010	205.979	180.331	Missing	95.955
20	134.365	303.892	250.160	115.900	123.025	50.203	209.900	30.520	91.971	144.763	44.088	115.339
MEAN	146.501	530.696	282.437	261.583	251.432	134.495	272.532	169.067	135.584	216.252	175.449	142.203
SD	63.343	202.269	95.518	125.616	109.014	59.122	111.599	104.954	52.844	160.795	89.265	70.316
GEOM MEAN	133.763	498.758	267.288	234.807	231.031	121.837	252.164	140.278	126.287	171.831	145.618	125.858
GEOM SD	1.564	1.427	1.409	1.613	1.519	1.603	1.508	1.918	1.479	2.078	2.045	1.694
CV %	43.237	38.114	33.819	48.021	43.357	43.958	40.949	62.079	38.975	74.356	50.878	49.448
SEM	14.532	46.404	21.913	28.818	25.010	13.564	25.602	24.078	12.123	36.889	21.040	16.132
MIN	52.821	288.237	159.673	115.900	123.025	50.203	93.639	30.520	56.198	25.350	27.519	43.788
MAX	294.874	1014.808	440.579	507.559	466.571	270.125	547.894	437.057	267.777	736.325	331.779	302.338
MEDIAN	134.820	487.445	262.853	230.173	220.228	129.593	245.397	131.249	124.559	180.331	186.824	142.868
N	19	19	19	19	19	19	19	19	19	19	18	19

FARMOVS 9/96
ANDROSTERONE EXCRETION RATE (µg/hr)
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	176.713	373.859	75.544	73.203	89.728	76.571	38.979	68.060	64.933	72.537	213.357	133.852
2	125.064	342.185	65.917	36.811	43.523	28.238	73.968	80.128	75.314	80.277	97.102	167.759
3	132.498	312.712	70.707	70.295	101.251	112.239	111.496	95.459	144.954	186.181	267.587	213.542
4	237.944	848.709	120.667	58.401	231.513	81.570	164.690	167.997	84.223	379.501	574.523	293.391
5	184.520	676.400	89.745	104.950	54.347	41.993	155.067	115.330	145.580	238.292	234.191	174.788
6	140.988	503.140	144.560	95.682	90.487	90.270	117.736	133.345	136.552	209.174	254.484	191.868
7	113.562	377.348	36.036	45.391	39.571	34.102	76.212	51.817	96.146	130.554	157.924	103.082
8	96.898	271.676	89.155	78.181	106.455	95.922	51.950	82.642	54.585	78.314	71.491	78.517
9	126.276	367.235	99.653	81.120	76.808	97.676	96.817	88.156	169.062	122.469	125.290	105.949
11	128.983	435.497	72.261	72.881	73.216	23.429	21.371	93.127	85.921	139.412	121.951	187.001
12	172.663	568.110	69.379	63.952	93.652	50.506	102.053	86.756	85.001	189.411	226.638	48.160
13	149.759	313.712	30.654	58.947	68.409	93.174	62.385	80.927	85.839	104.957	61.589	188.341
14	153.854	134.711	33.391	28.728	46.043	28.737	48.257	78.126	90.097	91.058	95.090	84.981
15	316.852	721.422	139.488	86.683	88.588	101.139	133.524	173.041	157.364	472.949	87.387	184.875
16	282.848	574.842	49.126	96.195	93.849	89.209	77.311	145.371	205.583	304.764	302.698	284.405
17	126.271	380.426	487.450	Missing	35.658	25.452	126.837	112.789	77.025	180.634	34.300	138.774
18	85.247	316.783	48.383	40.050	40.084	29.130	42.761	78.644	26.129	215.925	48.145	107.041
19	221.606	2492.121	70.524	63.693	50.368	61.158	194.194	118.708	143.483	284.929	Missing	29.171
20	182.606	166.006	24.359	27.647	50.141	17.685	61.479	39.812	77.067	31.870	115.304	137.704
MEAN	166.061	535.626	95.632	65.712	77.563	62.010	92.478	99.486	105.519	184.906	171.614	150.169
SD	61.402	508.202	100.955	23.210	44.063	32.499	47.226	35.913	45.554	113.560	129.929	70.803
GEOM MEAN	156.635	426.751	72.976	61.278	69.309	52.864	80.510	93.404	95.635	153.194	134.901	131.544
GEOM SD	1.412	1.874	1.975	1.498	1.591	1.841	1.765	1.450	1.617	1.937	2.057	1.782
CV %	36.976	94.880	105.567	35.321	56.809	52.409	51.067	36.098	43.171	61.415	75.710	47.149
SEM	14.087	116.589	23.161	5.471	10.109	7.456	10.834	8.239	10.451	26.052	30.625	16.243
MIN	85.247	134.711	24.359	27.647	35.658	17.685	21.371	39.812	26.129	31.870	34.300	29.171
MAX	316.852	2492.121	487.450	104.950	231.513	112.239	194.194	173.041	205.583	472.949	574.523	293.391
MEDIAN	149.759	377.348	70.707	67.123	73.216	61.158	77.311	88.156	85.921	180.634	123.621	138.774
N	19	19	19	18	19	19	19	19	19	19	18	19

FARMOVS 9/96
URINARY CREATININE CONCENTRATIONS (mmol/l)
PLACEBO

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	17.97	13.99	10.22	13.65	14.73	17.69	17.70	15.45	17.12	16.95	20.92	20.26
2	16.96	15.51	1.92	2.36	1.63	2.22	4.50	11.99	15.63	11.38	6.26	16.08
3	18.31	25.15	20.42	2.06	1.18	1.74	2.68	10.44	14.19	16.01	9.76	7.45
4	15.88	25.30	10.92	3.24	9.19	5.29	5.25	18.70	19.29	15.28	13.69	15.48
5	8.73	10.35	2.62	3.03	3.79	12.04	10.33	7.61	7.38	7.36	13.91	17.82
6	29.76	38.26	36.20	23.85	10.76	23.65	13.16	9.46	19.28	21.93	19.77	17.80
7	7.29	12.50	2.81	3.68	4.70	8.25	2.17	6.15	8.16	10.64	10.06	15.35
8	17.76	7.81	1.34	1.52	1.52	1.25	4.78	12.94	22.15	4.74	11.03	17.88
9	9.48	11.56	11.09	3.65	3.69	1.86	8.73	3.51	12.58	7.19	4.32	11.27
11	20.71	15.62	12.52	6.65	1.24	3.28	17.12	14.15	15.34	5.96	4.86	13.79
12	4.14	1.17	1.51	2.78	2.38	8.29	11.72	5.35	20.47	17.30	6.54	3.10
13	16.28	15.39	17.43	3.76	1.15	1.40	7.17	8.74	12.32	10.31	16.77	18.85
14	8.06	19.07	17.04	10.49	2.57	30.18	18.66	21.92	5.67	12.98	10.43	14.71
15	15.60	13.84	3.97	3.20	2.37	6.73	16.47	14.49	15.60	10.30	17.14	19.41
16	16.66	8.01	1.82	3.57	8.90	1.93	2.87	10.85	14.75	12.49	14.46	10.05
17	14.32	30.46	2.72	1.63	2.27	1.64	13.49	9.00	9.33	2.83	4.21	28.03
18	16.49	17.45	15.80	6.26	9.54	16.64	8.72	20.18	16.93	7.18	16.02	8.55
19	9.59	7.51	4.73	2.14	3.53	4.44	15.72	14.04	13.98	17.15	18.73	15.53
20	20.50	18.10	1.94	2.17	1.30	1.18	17.77	13.60	15.39	12.80	7.24	12.25
MEAN	14.973	16.161	9.317	5.247	4.549	7.879	10.474	12.030	14.503	11.620	11.901	14.929
SD	5.985	8.777	9.130	5.482	4.018	8.461	5.764	4.945	4.479	5.016	5.432	5.504
GEOM MEAN	13.677	13.414	5.756	3.838	3.243	4.618	8.605	10.964	13.719	10.420	10.578	13.686
GEOM SD	1.595	2.096	2.846	2.077	2.298	2.919	2.022	1.596	1.439	1.675	1.683	1.614
CV %	39.971	54.309	97.998	104.488	88.326	107.394	55.027	41.106	30.885	43.163	45.646	36.865
SEM	1.373	2.014	2.095	1.258	0.922	1.941	1.322	1.134	1.028	1.151	1.246	1.263
MIN	4.140	1.170	1.340	1.520	1.150	1.180	2.170	3.510	5.670	2.830	4.210	3.100
MAX	29.760	38.260	36.200	23.850	14.730	30.180	18.660	21.920	22.150	21.930	20.920	28.030
MEDIAN	16.280	15.390	4.730	3.240	2.570	4.440	10.330	11.990	15.340	11.380	11.030	15.480
N	19	19	19	19	19	19	19	19	19	19	19	19

FARMOVS 9/96
URINARY CREATININE CONCENTRATIONS (mmol/l)
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	17.32	11.15	5.06	1.44	1.11	1.62	6.28	10.66	10.75	9.21	11.50	14.82
2	16.60	4.12	1.23	1.11	1.72	1.71	6.46	9.97	14.02	12.71	17.23	20.65
3	10.66	15.23	9.63	2.06	1.16	1.02	1.13	5.95	16.51	17.17	17.55	12.63
4	9.20	14.47	2.14	1.39	3.02	6.19	8.12	18.17	12.08	13.87	16.01	11.23
5	10.53	11.28	4.93	1.80	1.22	3.05	9.61	8.08	13.61	11.90	16.35	6.94
6	17.95	12.26	5.48	2.25	1.33	3.25	8.62	7.59	17.63	15.37	19.95	23.01
7	10.02	15.24	1.28	1.22	1.19	2.75	5.54	5.26	11.14	15.93	16.67	13.26
8	20.30	6.72	3.37	5.62	8.18	9.41	9.15	1.28	8.69	13.04	16.48	18.41
9	7.88	10.62	1.36	1.13	1.00	1.05	1.56	3.82	10.05	3.56	9.97	13.63
11	24.07	12.59	1.15	1.18	1.14	1.44	4.80	11.82	19.57	15.06	7.49	24.84
12	39.53	5.97	1.34	1.22	1.86	2.18	7.42	8.09	8.34	23.35	9.87	2.35
13	12.90	13.90	6.32	3.52	1.37	2.22	7.41	7.39	6.80	9.84	9.81	15.78
14	11.63	11.21	7.78	8.67	2.30	2.71	5.31	10.86	15.41	11.34	11.51	14.45
15	15.09	6.92	1.96	1.86	2.67	4.77	11.71	11.01	12.25	9.43	15.49	14.38
16	13.59	6.58	1.29	1.72	3.01	2.81	1.42	7.86	13.99	13.36	10.22	9.77
17	21.52	14.81	5.04	1.42	1.55	0.95	2.01	5.53	11.38	12.73	11.07	19.99
18	9.61	7.41	1.42	1.85	1.35	1.89	12.59	8.05	5.80	10.30	12.14	11.28
19	16.52	29.06	4.74	4.23	5.30	8.92	6.60	12.23	17.38	22.05	Missing	16.45
20	13.89	14.04	4.70	1.30	1.10	2.34	16.48	12.37	10.79	11.74	10.37	20.26
MEAN	15.727	11.767	3.696	2.368	2.188	3.173	6.959	8.736	12.431	13.261	13.316	14.954
SD	7.301	5.475	2.529	1.936	1.793	2.472	4.013	3.756	3.763	4.495	3.600	5.535
GEOM MEAN	14.515	10.703	2.903	1.939	1.798	2.527	5.586	7.761	11.857	12.439	12.853	13.556
GEOM SD	1.483	1.571	2.081	1.792	1.784	1.947	2.154	1.766	1.384	1.486	1.318	1.695
CV %	46.424	46.524	68.441	81.770	81.946	77.919	57.661	42.992	30.270	33.895	27.032	37.011
SEM	1.675	1.256	0.580	0.444	0.411	0.567	0.921	0.862	0.863	1.031	0.848	1.270
MIN	7.880	4.120	1.150	1.110	1.000	0.950	1.130	1.280	5.800	3.560	7.490	2.350
MAX	39.530	29.060	9.630	8.670	8.180	9.410	16.480	18.170	19.570	23.350	19.950	24.840
MEDIAN	13.890	11.280	3.370	1.720	1.370	2.340	6.600	8.080	12.080	12.730	11.825	14.450
N	19	19	19	19	19	19	19	19	19	19	18	19

FARMOVS 9/96
URINARY ANDROSTERONE CONCENTRATION EXPRESSED PER MMOL CREATININE (µg/mmol)
PLACEBO

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	199.110	246.819	263.112	255.458	238.221	267.778	281.186	231.521	235.631	258.348	254.924	227.246
2	191.333	293.488	252.604	391.949	427.607	350.450	272.889	214.512	209.917	309.578	320.767	237.313
3	378.482	416.302	465.769	487.864	465.254	406.322	491.418	350.575	381.607	436.852	311.270	343.087
4	372.481	509.328	494.414	468.210	473.123	485.444	461.714	385.080	343.079	438.678	389.701	396.835
5	288.431	375.072	449.618	441.584	392.348	378.571	389.932	307.490	322.087	351.087	151.546	257.856
6	302.319	351.359	326.050	332.914	340.706	317.505	402.432	334.672	257.988	408.482	394.537	225.955
7	246.502	290.400	309.964	298.913	252.766	217.818	240.553	193.984	198.529	235.244	188.867	155.244
8	197.354	293.086	357.463	334.868	297.368	318.400	300.000	226.352	177.788	276.160	244.878	231.320
9	147.996	246.886	253.111	278.630	292.954	305.376	274.914	245.299	209.141	268.150	280.787	209.849
11	283.341	353.009	396.006	382.556	412.903	397.561	315.362	282.968	248.240	369.295	336.008	272.444
12	320.531	494.017	509.934	496.763	528.151	442.581	380.119	383.178	384.465	467.399	468.196	536.129
13	168.612	252.697	218.417	217.819	201.739	216.429	276.151	201.945	176.705	262.658	143.411	211.724
14	308.809	388.464	337.793	336.797	322.568	350.331	360.772	299.635	289.947	393.606	414.957	418.287
15	323.846	569.509	530.982	480.938	464.557	435.067	450.334	403.865	319.295	523.689	473.104	406.131
16	467.047	640.574	749.451	695.518	675.506	656.995	625.087	493.180	473.356	578.943	418.672	387.463
17	327.235	407.354	316.176	317.791	382.819	367.683	319.496	238.111	266.667	345.583	188.124	201.855
18	170.952	224.642	180.316	266.134	166.352	130.649	266.514	172.745	109.333	171.031	209.551	160.117
19	395.099	422.104	505.708	550.935	527.195	482.658	377.417	351.425	316.166	399.300	Missing	252.543
20	161.561	242.099	273.196	269.585	231.538	242.373	194.373	185.809	173.294	250.703	209.669	329.143
MEAN	276.370	369.327	378.426	384.486	373.352	356.315	351.614	289.597	268.065	354.989	299.943	287.397
SD	91.758	118.963	140.444	122.334	131.593	119.634	103.779	88.597	90.203	106.541	107.812	102.201
GEOM MEAN	261.599	352.463	355.594	367.497	350.916	336.154	338.280	277.311	253.363	339.601	280.832	271.652
GEOM SD	1.413	1.365	1.436	1.358	1.447	1.440	1.327	1.352	1.424	1.365	1.465	1.407
CV %	33.201	32.211	37.113	31.817	35.246	33.575	29.515	30.593	33.650	30.013	35.944	35.561
SEM	21.051	27.292	32.220	28.065	30.189	27.446	23.808	20.326	20.694	24.442	25.411	23.447
MIN	147.996	224.642	180.316	217.819	166.352	130.649	194.373	172.745	109.333	171.031	143.411	155.244
MAX	467.047	640.574	749.451	695.518	675.506	656.995	625.087	493.180	473.356	578.943	473.104	536.129
MEDIAN	288.431	353.009	337.793	336.797	382.819	350.450	319.496	282.968	257.988	351.087	296.029	252.543
N	19	19	19	19	19	19	19	19	19	19	18	19

FARMOVS 9/96
URINARY ANDROSTERONE CONCENTRATION EXPRESSED PER MMOL CREATININE (µg/mmol)
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	246.247	267.534	120.751	112.500	118.018	120.370	87.420	93.152	123.628	181.976	222.522	255.263
2	203.735	268.447	99.187	82.883	84.884	100.585	103.715	114.544	130.029	190.165	200.058	199.177
3	408.818	399.934	99.896	127.184	151.724	185.294	204.425	178.824	254.936	374.490	274.872	340.934
4	384.783	544.091	167.757	181.295	209.603	209.370	235.837	229.169	284.189	336.410	499.563	522.618
5	324.881	425.975	135.294	141.111	145.902	121.311	145.369	188.243	247.318	344.706	388.930	300.865
6	315.599	364.274	179.197	158.222	170.677	160.923	166.821	200.132	241.010	338.061	331.830	331.725
7	262.375	251.247	75.000	81.967	66.387	87.273	73.646	126.236	147.397	197.489	206.179	147.587
8	167.143	248.512	176.558	192.171	208.191	202.657	113.552	194.531	130.840	170.245	177.124	164.150
9	202.030	244.915	118.382	102.655	97.000	126.667	121.154	138.220	202.786	265.449	271.615	206.310
11	259.909	271.406	83.478	91.525	92.982	90.972	103.542	115.651	136.689	199.801	245.394	250.322
12	410.549	367.672	130.597	118.852	118.280	138.073	117.790	146.601	165.947	353.662	481.054	485.532
13	180.853	197.698	41.456	73.864	110.219	131.982	95.007	110.825	168.529	280.386	274.516	246.388
14	311.952	240.678	65.039	65.744	84.783	105.904	109.040	108.748	159.831	257.407	290.269	291.696
15	332.406	445.520	186.224	127.419	127.715	133.753	147.225	194.460	264.898	466.596	116.850	230.946
16	534.511	557.903	137.209	114.535	163.455	170.107	184.507	197.201	683.488	532.859	503.425	533.265
17	264.545	267.184	185.714	Missing	81.290	108.421	132.836	172.875	201.406	340.456	120.687	227.464
18	205.931	209.717	61.972	62.703	68.148	70.899	78.078	102.733	115.690	167.573	183.773	151.152
19	346.610	341.053	106.962	96.454	111.321	115.135	167.879	195.830	213.291	371.837	Missing	378.784
20	182.001	147.650	49.787	56.154	60.909	64.103	55.522	77.769	120.945	179.898	199.325	165.202
MEAN	291.836	319.022	116.866	110.402	119.552	128.621	128.598	151.881	210.150	292.077	277.110	285.757
SD	97.143	114.018	47.141	39.355	45.006	41.328	47.017	45.271	126.850	105.282	120.751	120.606
GEOM MEAN	277.461	300.958	106.834	104.018	111.971	122.402	120.692	145.136	189.086	274.798	253.837	264.289
GEOM SD	1.384	1.419	1.578	1.429	1.450	1.387	1.448	1.372	1.534	1.433	1.542	1.493
CV %	33.287	35.740	40.337	35.647	37.645	32.132	36.561	29.807	60.362	36.046	43.575	42.206
SEM	22.286	26.158	10.815	9.276	10.325	9.481	10.786	10.386	29.101	24.153	28.461	27.669
MIN	167.143	147.650	41.456	56.154	60.909	64.103	55.522	77.769	115.690	167.573	116.850	147.587
MAX	534.511	557.903	186.224	192.171	209.603	209.370	235.837	229.169	683.488	532.859	503.425	533.265
MEDIAN	264.545	268.447	118.382	107.577	111.321	121.311	117.790	146.601	168.529	280.386	258.504	250.322
N	19	19	19	18	19	19	19	19	19	19	18	19

6.1.2 PROBENECID

RAW DATA - PROBENECID

FARMOVS 9/96
PROBENECID CONCENTRATIONS (µg/ml)
LLOQ = 0.06µg/ml
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	24.760	99.090	32.650	27.640	39.400	99.210	189.830	145.150	215.240	226.770	193.770
2	20.490	59.090	57.530	76.980	79.370	75.050	99.500	143.160	267.760	303.520	264.780
3	32.860	472.790	76.290	37.350	22.730	24.060	71.330	454.660	378.410	140.800	73.730
4	26.330	67.870	47.400	66.060	58.450	101.380	188.870	170.570	184.590	170.920	37.570
5	23.650	153.470	62.980	41.220	32.890	296.650	84.910	425.470	91.140	206.500	24.240
6	57.920	218.740	84.980	43.980	104.240	127.700	158.750	385.300	95.090	244.770	179.960
7	39.150	36.410	38.150	40.740	56.420	114.380	84.300	181.440	295.000	294.730	49.660
8	30.210	128.940	314.870	443.210	471.970	386.010	21.690	271.320	456.210	503.590	224.180
9	51.030	30.120	26.050	25.780	156.160	21.390	43.080	161.480	19.900	78.790	55.550
11	24.680	26.650	31.640	33.360	46.870	39.490	203.140	337.000	87.960	85.010	184.220
12	34.010	20.570	28.320	91.760	36.070	58.820	166.860	93.100	369.730	134.250	23.710
13	49.860	53.960	61.070	47.980	82.300	116.930	138.180	131.570	89.920	196.140	100.420
14	42.680	97.210	155.090	42.790	36.370	92.640	264.530	329.140	212.450	157.500	278.370
15	34.430	73.950	92.960	82.550	79.290	306.060	259.110	272.910	79.960	179.960	63.270
16	34.320	36.820	52.820	82.130	93.200	29.020	111.060	660.260	115.830	63.100	47.940
17	128.340	56.620	30.280	42.640	24.490	45.340	101.980	249.610	277.250	156.700	164.690
18	42.210	51.410	62.990	44.720	37.400	125.970	78.880	71.260	165.090	56.910	45.610
19	62.670	143.420	89.680	115.790	207.290	53.320	80.060	287.190	255.460	Missing	82.260
20	133.040	402.860	44.450	34.660	35.050	170.720	393.850	94.900	245.890	174.950	81.730
MEAN	46.981	117.368	73.168	74.807	89.472	120.218	144.206	256.078	205.415	187.495	114.508
SD	31.753	124.501	66.294	92.546	104.009	102.819	91.052	151.050	118.741	106.366	83.392
GEOM MEAN	40.487	79.143	58.364	55.622	62.855	87.286	118.318	217.102	166.143	162.314	87.049
GEOM SD	1.677	2.391	1.870	1.924	2.169	2.304	1.982	1.827	2.127	1.758	2.193
CV %	67.588	106.078	90.605	123.713	116.248	85.527	63.140	58.986	57.806	56.730	72.826
SEM	7.285	28.563	15.209	21.232	23.861	23.588	20.889	34.653	27.241	25.071	19.132
MIN	20.490	20.570	26.050	25.780	22.730	21.390	21.690	71.260	19.900	56.910	23.710
MAX	133.040	472.790	314.870	443.210	471.970	386.010	393.850	660.260	456.210	503.590	278.370
MEDIAN	34.430	67.870	57.530	43.980	56.420	99.210	111.060	249.610	212.450	172.935	81.730
N	19	19	19	19	19	19	19	19	19	18	19

FARMOVS 9/96
FRACTIONAL PROBENECID EXCRETION (µg)
LLOQ = 0.06µg/ml
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	3103.171	12251.488	14753.556	18932.018	30942.396	14087.820	52043.793	85101.445	55893.523	113441.693	82272.804
2	6339.401	31926.327	23018.904	22947.738	26061.140	16571.040	27925.670	70973.002	53359.213	51300.950	129596.571
3	1687.032	34750.065	20468.607	21487.082	26996.648	23225.840	25598.197	187897.338	65741.169	46861.056	43876.723
4	2838.374	22812.464	10984.950	24160.784	7357.686	17437.360	30479.841	50215.808	90079.920	73666.520	22537.492
5	3329.211	20649.389	26022.706	12585.290	7465.701	65856.300	25753.203	220818.930	31766.847	45630.305	24349.807
6	6525.267	32200.715	22840.075	17531.308	35983.648	20910.875	55743.475	148590.945	22968.039	56456.201	54283.134
7	3858.233	13667.222	17316.667	20406.666	16033.436	42731.224	26314.245	127488.816	73452.050	81254.114	31389.093
8	4914.563	19320.370	22793.439	27705.057	47480.182	38601.000	28795.427	156304.739	96561.409	74002.551	69894.840
9	7204.926	18643.075	18217.026	20413.635	229369.370	21914.483	28770.978	160746.881	15473.842	21872.104	25115.822
11	3145.466	20059.988	21351.305	23042.419	16764.930	3396.140	55355.650	129893.280	24452.000	33842.481	66483.156
12	8802.468	8154.977	12490.536	39061.314	12104.731	13736.235	48823.236	68614.700	50882.243	38449.200	12009.115
13	5692.018	6313.320	13845.790	21736.859	52342.800	20723.504	54615.645	118260.379	20524.240	26914.331	58374.146
14	2131.012	6414.888	7816.536	10103.575	7283.456	15442.162	69997.283	144479.294	39764.267	26896.275	67348.838
15	8056.620	28260.732	34000.120	21445.665	25138.895	47408.694	83767.672	158814.516	51568.603	52130.813	42265.625
16	5374.169	10219.391	25792.006	15666.298	34787.832	17126.443	41664.159	170347.080	29752.094	22274.300	31403.576
17	12338.608	29486.564	16472.320	12067.120	12103.203	43077.081	48126.402	100660.225	69331.908	24138.068	60316.066
18	8604.509	28265.732	21747.927	19484.504	16260.772	10959.390	30004.374	33299.085	123918.205	7368.707	34361.206
19	15758.372	19949.722	13999.945	9884.992	24688.239	18690.260	15872.696	133391.139	53265.965	Missing	4621.367
20	10653.843	41937.726	16834.104	25938.504	8264.790	22941.354	65197.929	67251.834	22262.881	58555.765	40350.918
MEAN	6334.593	21330.745	18987.711	20242.149	33548.940	24991.432	42886.835	122797.339	52158.864	47503.080	47413.174
SD	3720.338	10277.978	6221.757	6848.391	49248.834	15421.265	18331.512	50113.114	29289.729	26215.298	28939.052
GEOM MEAN	5363.882	18624.677	17985.355	19150.962	21483.347	20848.716	39243.406	111124.702	44674.764	40269.206	38352.994
GEOM SD	1.836	1.771	1.417	1.418	2.341	1.923	1.552	1.639	1.797	1.895	2.120
CV %	58.730	48.184	32.767	33.832	146.797	61.706	42.744	40.810	56.155	55.187	61.036
SEM	853.504	2357.930	1427.369	1571.129	11298.457	3537.881	4205.537	11496.737	6719.525	6179.005	6639.074
MIN	1687.032	6313.320	7816.536	9884.992	7283.456	3396.140	15872.696	33299.085	15473.842	7368.707	4621.367
MAX	15758.372	41937.726	34000.120	39061.314	229369.370	65856.300	83767.672	220818.930	123918.205	113441.693	129596.571
MEDIAN	5692.018	20059.988	18217.026	20413.635	24688.239	20723.504	41664.159	129893.280	51568.603	46245.681	42265.625
N	19	19	19	19	19	19	19	19	19	18	19

FARMOVS 9/96
CUMULATIVE PROBENECID EXCRETION (µg)
LLOQ = 0.06µg/ml
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	3103.171	15354.658	30108.214	49040.232	79982.628	94070.448	146114.241	231215.686	287109.209	400550.901	482823.706
2	6339.401	38265.728	61284.632	84232.370	110293.509	126864.549	154790.219	225763.221	279122.434	330423.384	460019.955
3	1687.032	36437.097	56905.704	78392.786	105389.434	128615.274	154213.471	342110.809	407851.979	454713.035	498589.758
4	2838.374	25650.838	36635.788	60796.573	68154.259	85591.619	116071.459	166287.267	256367.187	330033.707	352571.199
5	3329.211	23978.599	50001.305	62586.596	70052.297	135908.597	161661.800	382480.730	414247.577	459877.882	484227.689
6	6525.267	38725.983	61566.057	79097.365	115081.013	135991.888	191735.363	340326.308	363294.346	419750.547	474033.681
7	3858.233	17525.454	34842.121	55248.787	71282.222	114013.447	140327.692	267816.508	341268.558	422522.671	453911.764
8	4914.563	24234.932	47028.372	74733.429	122213.611	160814.611	189610.038	345914.777	442476.185	516478.736	586373.576
9	7204.926	25848.001	44065.026	64478.662	293848.031	315762.514	344533.492	505280.373	520754.215	542626.319	567742.140
11	3145.466	23205.454	44556.759	67599.178	84364.108	87760.248	143115.898	273009.178	297461.179	331303.660	397786.816
12	8802.468	16957.445	29447.981	68509.295	80614.026	94350.261	143173.497	211788.197	262670.440	301119.640	313128.755
13	5692.018	12005.338	25851.128	47587.987	99930.787	120654.291	175269.936	293530.315	314054.555	340968.886	399343.032
14	2131.012	8545.900	16362.436	26466.011	33749.467	49191.629	119188.912	263668.207	303432.473	330328.748	397677.586
15	8056.620	36317.352	70317.472	91763.137	116902.031	164310.725	248078.397	406892.913	458461.516	510592.329	552857.954
16	5374.169	15593.560	41385.566	57051.863	91839.695	108966.139	150630.298	320977.378	350729.471	373003.771	404407.348
17	12338.608	41825.171	58297.491	70364.611	82467.814	125544.895	173671.296	274331.521	343663.429	367801.497	428117.562
18	8604.509	36870.241	58618.168	78102.672	94363.444	105322.834	135327.208	168626.294	292544.499	299913.206	334274.411
19	15758.372	35708.094	49708.038	59593.031	84281.270	102971.529	118844.225	252235.364	305501.329	305501.329	310122.696
20	10653.843	52591.569	69425.673	95364.177	103628.967	126570.321	191768.250	259020.084	281282.965	339838.730	380189.648
MEAN	6334.593	27665.338	46653.049	66895.198	100444.138	125435.569	168322.405	291119.744	343278.608	388281.525	435694.699
SD	3720.338	11869.424	15125.964	16476.045	51384.562	53398.799	53231.473	83575.082	73778.692	77427.877	82023.345
GEOM MEAN	5363.882	25024.669	43926.560	64592.811	92314.997	117471.566	162253.603	280292.734	336430.426	381406.342	428371.686
GEOM SD	1.836	1.620	1.459	1.336	1.495	1.433	1.301	1.327	1.224	1.209	1.209
CV %	58.730	42.904	32.422	24.630	51.157	42.571	31.625	28.708	21.492	19.941	18.826
SEM	853.504	2723.033	3470.134	3779.864	11788.427	12250.525	12212.137	19173.439	16925.993	17763.173	18817.446
MIN	1687.032	8545.900	16362.436	26466.011	33749.467	49191.629	116071.459	166287.267	256367.187	299913.206	310122.696
MAX	15758.372	52591.569	70317.472	95364.177	293848.031	315762.514	344533.492	505280.373	520754.215	542626.319	586373.576
MEDIAN	5692.018	25650.838	47028.372	67599.178	91839.695	120654.291	154213.471	273009.178	314054.555	367801.497	428117.562
N	19	19	19	19	19	19	19	19	19	19	19

FARMOVS 9/96
CUMULATIVE PROBENECID EXCRETION AS PERCENTAGE OF DOSE (%)
LLOQ = 0.06µg/ml
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	0.155	0.768	1.505	2.452	3.999	4.704	7.306	11.561	14.355	20.028	24.141
2	0.317	1.913	3.064	4.212	5.515	6.343	7.740	11.288	13.956	16.521	23.001
3	0.084	1.822	2.845	3.920	5.269	6.431	7.711	17.106	20.393	22.736	24.929
4	0.142	1.283	1.832	3.040	3.408	4.280	5.804	8.314	12.818	16.502	17.629
5	0.166	1.199	2.500	3.129	3.503	6.795	8.083	19.124	20.712	22.994	24.211
6	0.326	1.936	3.078	3.955	5.754	6.800	9.587	17.016	18.165	20.988	23.702
7	0.193	0.876	1.742	2.762	3.564	5.701	7.016	13.391	17.063	21.126	22.696
8	0.246	1.212	2.351	3.737	6.111	8.041	9.481	17.296	22.124	25.824	29.319
9	0.360	1.292	2.203	3.224	14.692	15.788	17.227	25.264	26.038	27.131	28.387
11	0.157	1.160	2.228	3.380	4.218	4.388	7.156	13.650	14.873	16.565	19.889
12	0.440	0.848	1.472	3.425	4.031	4.718	7.159	10.589	13.134	15.056	15.656
13	0.285	0.600	1.293	2.379	4.997	6.033	8.763	14.677	15.703	17.048	19.967
14	0.107	0.427	0.818	1.323	1.687	2.460	5.959	13.183	15.172	16.516	19.884
15	0.403	1.816	3.516	4.588	5.845	8.216	12.404	20.345	22.923	25.530	27.643
16	0.269	0.780	2.069	2.853	4.592	5.448	7.532	16.049	17.536	18.650	20.220
17	0.617	2.091	2.915	3.518	4.123	6.277	8.684	13.717	17.183	18.390	21.406
18	0.430	1.844	2.931	3.905	4.718	5.266	6.766	8.431	14.627	14.996	16.714
19	0.788	1.785	2.485	2.980	4.214	5.149	5.942	12.612	15.275	15.275	15.506
20	0.533	2.630	3.471	4.768	5.181	6.329	9.588	12.951	14.064	16.992	19.009
MEAN	0.317	1.383	2.333	3.345	5.022	6.272	8.416	14.556	17.164	19.414	21.785
SD	0.186	0.593	0.756	0.824	2.569	2.670	2.662	4.179	3.689	3.871	4.101
GEOM MEAN	0.268	1.251	2.196	3.230	4.616	5.874	8.113	14.015	16.822	19.070	21.419
GEOM SD	1.836	1.620	1.459	1.336	1.495	1.433	1.301	1.327	1.224	1.209	1.209
CV %	58.730	42.904	32.422	24.630	51.157	42.571	31.625	28.708	21.492	19.941	18.826
SEM	0.043	0.136	0.174	0.189	0.589	0.613	0.611	0.959	0.846	0.888	0.941
MIN	0.084	0.427	0.818	1.323	1.687	2.460	5.804	8.314	12.818	14.996	15.506
MAX	0.788	2.630	3.516	4.768	14.692	15.788	17.227	25.264	26.038	27.131	29.319
MEDIAN	0.285	1.283	2.351	3.380	4.592	6.033	7.711	13.650	15.703	18.390	21.406
N	19	19	19	19	19	19	19	19	19	19	19

FARMOVS 9/96
PROBENECID EXCRETION RATE (µg/h)
LLOQ = 0.06µg/ml
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

SUBJECT	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1	3103.171	12251.488	14753.556	18932.018	15471.198	7043.910	13010.948	7091.787	9315.587	18906.949	6856.067
2	6339.401	31926.327	23018.904	22947.738	13030.570	8285.520	6981.418	5914.417	8893.202	8550.158	10799.714
3	1687.032	34750.065	20468.607	21487.082	13498.324	11612.920	6399.549	15658.112	10956.862	7810.176	3656.394
4	2838.374	22812.464	10984.950	24160.784	3678.843	8718.680	7619.960	4184.651	15013.320	12277.753	1878.124
5	3329.211	20649.389	26022.706	12585.290	3732.851	32928.150	6438.301	18401.578	5294.475	7605.051	2029.151
6	6525.267	32200.715	22840.075	17531.308	17991.824	10455.438	13935.869	12382.579	3828.006	9409.367	4523.595
7	3858.233	13667.222	17316.667	20406.666	8016.718	21365.612	6578.561	10624.068	12242.008	13542.352	2615.758
8	4914.563	19320.370	22793.439	27705.057	23740.091	19300.500	7198.857	13025.395	16093.568	12333.758	5824.570
9	7204.926	18643.075	18217.026	20413.635	114684.685	10957.241	7192.745	13395.573	2578.974	3645.351	2092.985
11	3145.466	20059.988	21351.305	23042.419	8382.465	1698.070	13838.913	10824.440	4075.333	5640.414	5540.263
12	8802.468	8154.977	12490.536	39061.314	6052.366	6868.117	12205.809	5717.892	8480.374	6408.200	1000.760
13	5692.018	6313.320	13845.790	21736.859	26171.400	10361.752	13653.911	9855.032	3420.707	4485.722	4864.512
14	2131.012	6414.888	7816.536	10103.575	3641.728	7721.081	17499.321	12039.941	6627.378	4482.713	5612.403
15	8056.620	28260.732	34000.120	21445.665	12569.447	23704.347	20941.918	13234.543	8594.767	8688.469	3522.135
16	5374.169	10219.391	25792.006	15666.298	17393.916	8563.222	10416.040	14195.590	4958.682	3712.383	2616.965
17	12338.608	29486.564	16472.320	12067.120	6051.601	21538.540	12031.600	8388.352	11555.318	4023.011	5026.339
18	8604.509	28265.732	21747.927	19484.504	8130.386	5479.695	7501.094	2774.924	20653.034	1228.118	2863.434
19	15758.372	19949.722	13999.945	9884.992	12344.120	9345.130	3968.174	11115.928	8877.661	Missing	385.114
20	10653.843	41937.726	16834.104	25938.504	4132.395	11470.677	16299.482	5604.320	3710.480	9759.294	3362.577
MEAN	6334.593	21330.745	18987.711	20242.149	16774.470	12495.716	10721.709	10233.112	8693.144	7917.180	3951.098
SD	3720.338	10277.978	6221.757	6848.391	24624.417	7710.633	4582.878	4176.093	4881.622	4369.216	2411.588
GEOM MEAN	5363.882	18624.677	17985.355	19150.962	10741.674	10424.358	9810.852	9260.392	7445.794	6711.534	3196.083
GEOM SD	1.836	1.771	1.417	1.418	2.341	1.923	1.552	1.639	1.797	1.895	2.120
CV %	58.730	48.184	32.767	33.832	146.797	61.706	42.744	40.810	56.155	55.187	61.036
SEM	853.504	2357.930	1427.369	1571.129	5649.229	1768.940	1051.384	958.061	1119.921	1029.834	553.256
MIN	1687.032	6313.320	7816.536	9884.992	3641.728	1698.070	3968.174	2774.924	2578.974	1228.118	385.114
MAX	15758.372	41937.726	34000.120	39061.314	114684.685	32928.150	20941.918	18401.578	20653.034	18906.949	10799.714
MEDIAN	5692.018	20059.988	18217.026	20413.635	12344.120	10361.752	10416.040	10824.440	8594.767	7707.613	3522.135
N	19	19	19	19	19	19	19	19	19	18	19

FARMOVS 9/96
PROBENECID CONCENTRATION EXPRESSED PER MMOL CREATININE (µg/mmol)
LLOQ = 0.06µg/ml
BENEMID
DOSE: 4 X 500 MG PROBENECID TABLETS

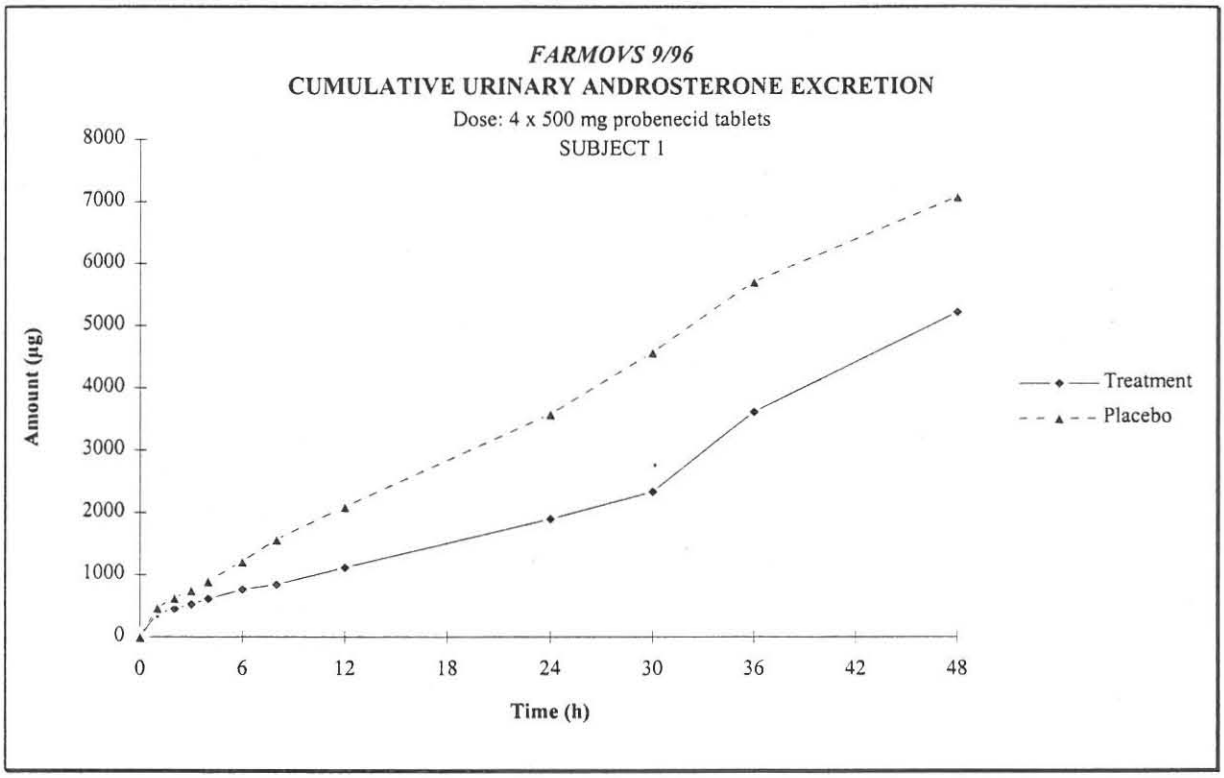
SUBJECT	-12 to 0 h	0 to 1 h	1 to 2 h	2 to 3 h	3 to 4 h	4 to 6 h	6 to 8 h	8 to 12 h	12 to 24 h	24 to 30 h	30 to 36 h	36 to 48 h
1		2220.628	19583.004	22673.611	24900.901	24320.988	15797.771	17807.692	13502.326	23370.250	19719.130	13074.899
2		4973.301	48040.650	51828.829	44755.814	46415.205	11617.647	9979.940	10211.127	21066.876	17615.786	12822.276
3		2157.584	49095.535	37033.981	32198.276	22284.314	21292.035	11988.235	27538.462	22039.022	8022.792	5837.688
4		1819.627	31714.953	34100.719	21874.172	9442.649	12485.222	10394.606	14120.033	13308.580	10675.828	3345.503
5		2096.631	31129.817	34988.889	33786.885	10783.607	30868.887	10508.663	31261.572	7658.824	12629.969	3492.795
6		4724.307	39916.058	37768.889	33067.669	32073.846	14814.385	20915.679	21854.793	6186.727	12269.173	7820.947
7		2568.898	28445.313	31270.492	34235.294	20516.364	20646.209	16026.616	16287.253	18518.519	17680.264	3745.098
8		4495.536	38261.128	56026.690	54182.152	50156.217	42186.885	16945.313	31222.094	34985.429	30557.646	12177.078
9		4805.085	22147.059	23053.097	25780.000	148723.810	13711.538	11277.487	16067.662	5589.888	7902.708	4075.569
11		1960.286	23173.913	26813.559	29263.158	32548.611	8227.083	17186.125	17220.235	5840.637	11349.800	7416.264
12		5696.817	15350.746	23213.115	49333.333	16545.872	7927.224	20625.464	11163.070	15834.261	13601.824	10089.362
13		3587.050	8537.975	17349.432	35021.898	37072.072	15780.027	18698.241	19348.529	9138.211	19993.884	6363.752
14		3807.315	12494.859	17888.120	18604.348	13420.664	17446.328	24358.195	21358.858	18734.568	13683.753	19264.360
15		4975.434	37729.592	49978.495	30917.603	16622.642	26136.635	23534.060	22278.367	8479.321	11617.818	4399.861
16		5215.805	28542.636	30709.302	27285.714	33167.260	20436.620	14129.771	47195.139	8669.910	6174.168	4906.858
17		8665.766	11234.127	21323.944	27509.677	25778.947	22557.214	18441.230	21934.095	21779.262	14155.375	8238.619
18		5696.356	36204.225	34048.649	33125.926	19788.360	10005.560	9798.758	12286.207	16028.155	4687.809	4043.440
19		2156.573	30257.384	21200.946	21847.170	23238.789	8078.788	6546.198	16524.166	11585.488	Missing	5000.608
20		9475.783	85714.894	34192.308	31509.091	14978.632	10359.223	31839.127	8795.181	20944.634	16870.781	4034.057
MEAN		4268.357	31451.256	31866.477	32063.110	31467.308	17388.173	16368.495	20008.904	15250.451	13844.917	7376.265
SD		2181.861	17604.388	11284.305	9129.706	30553.140	8761.393	6266.434	9275.469	7815.579	6073.016	4323.653
GEOM MEAN		3782.777	27265.912	30097.293	30939.060	24871.292	15627.070	15244.578	18309.402	13356.174	12628.830	6421.320
GEOM SD		1.661	1.758	1.412	1.312	1.880	1.597	1.484	1.530	1.723	1.573	1.688
CV %		51.117	55.974	35.411	28.474	97.095	50.387	38.284	46.357	51.248	43.865	58.616
SEM		500.553	4038.724	2588.797	2094.498	7009.371	2010.001	1437.619	2127.938	1793.017	1431.424	991.914
MIN		1819.627	8537.975	17349.432	18604.348	9442.649	7927.224	6546.198	8795.181	5589.888	4687.809	3345.503
MAX		9475.783	85714.894	56026.690	54182.152	148723.810	42186.885	31839.127	47195.139	34985.429	30557.646	19264.360
MEDIAN		4495.536	30257.384	31270.492	31509.091	23238.789	15780.027	16945.313	17220.235	15834.261	13115.897	5837.688
N		19	19	19	19	19	19	19	19	19	18	19

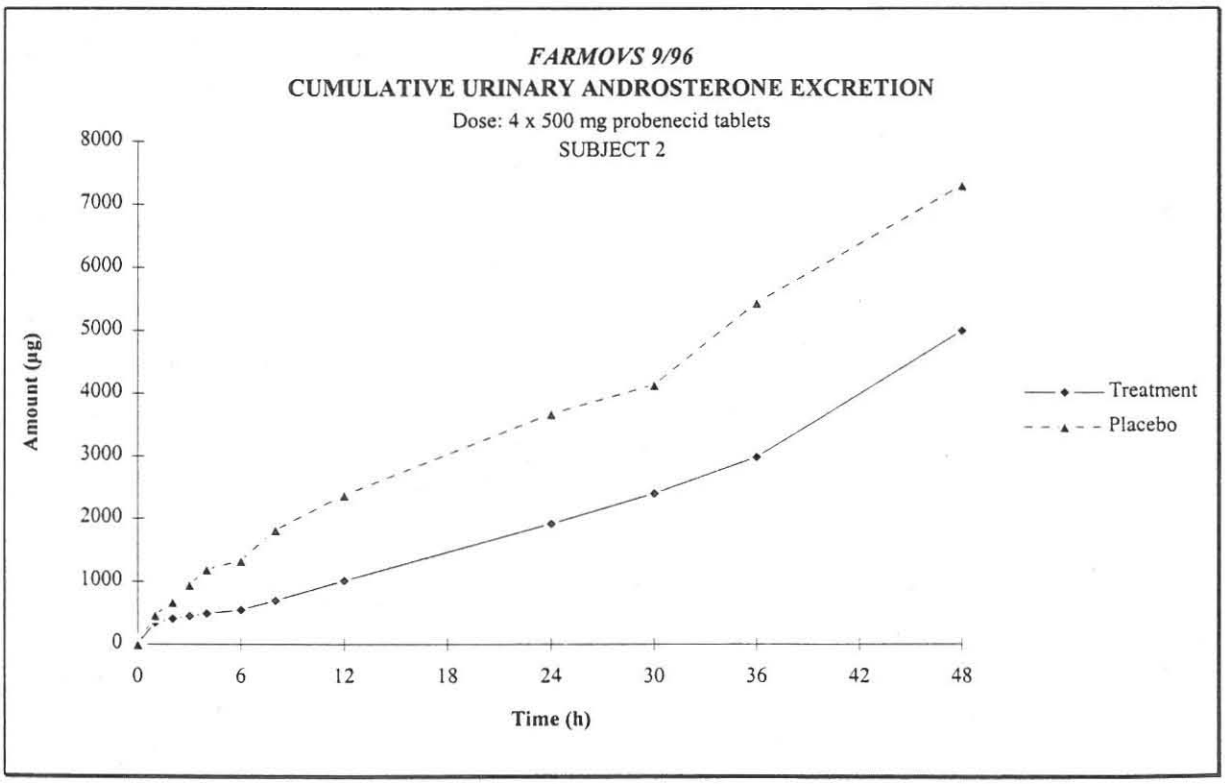
Central University of Technology, Free State

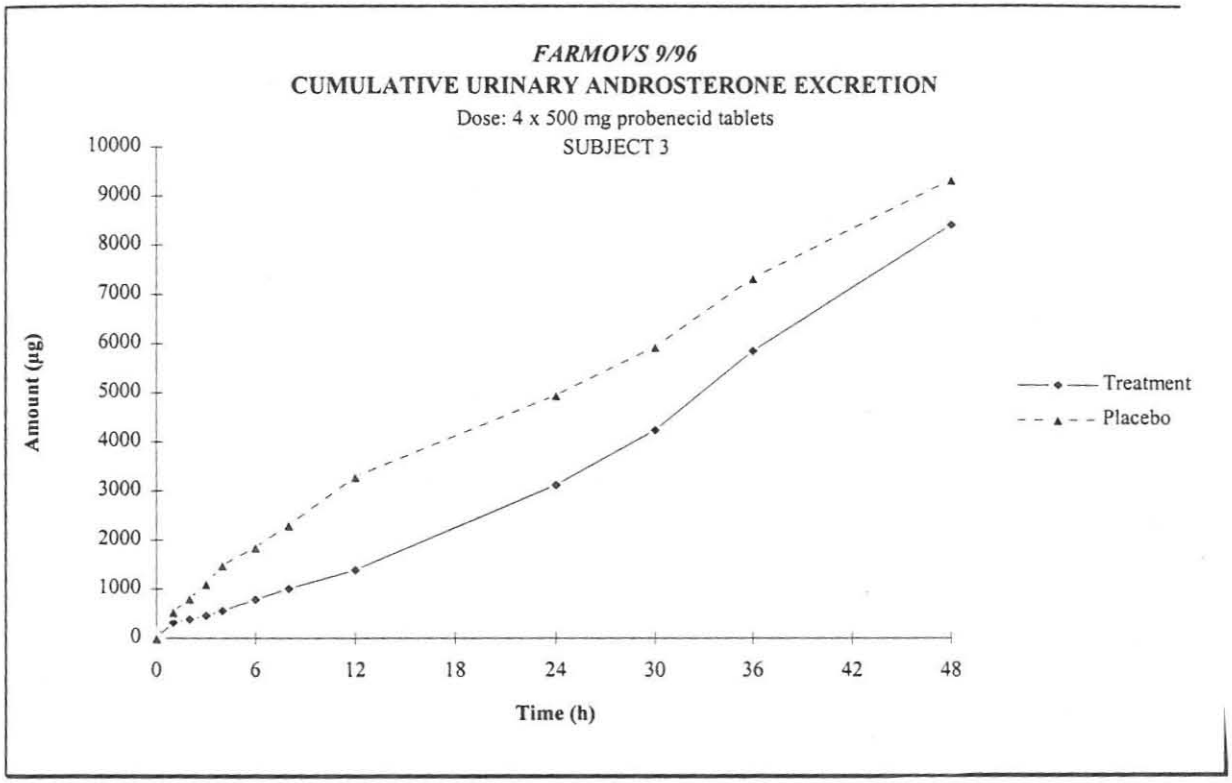
6.2 Individual graphs

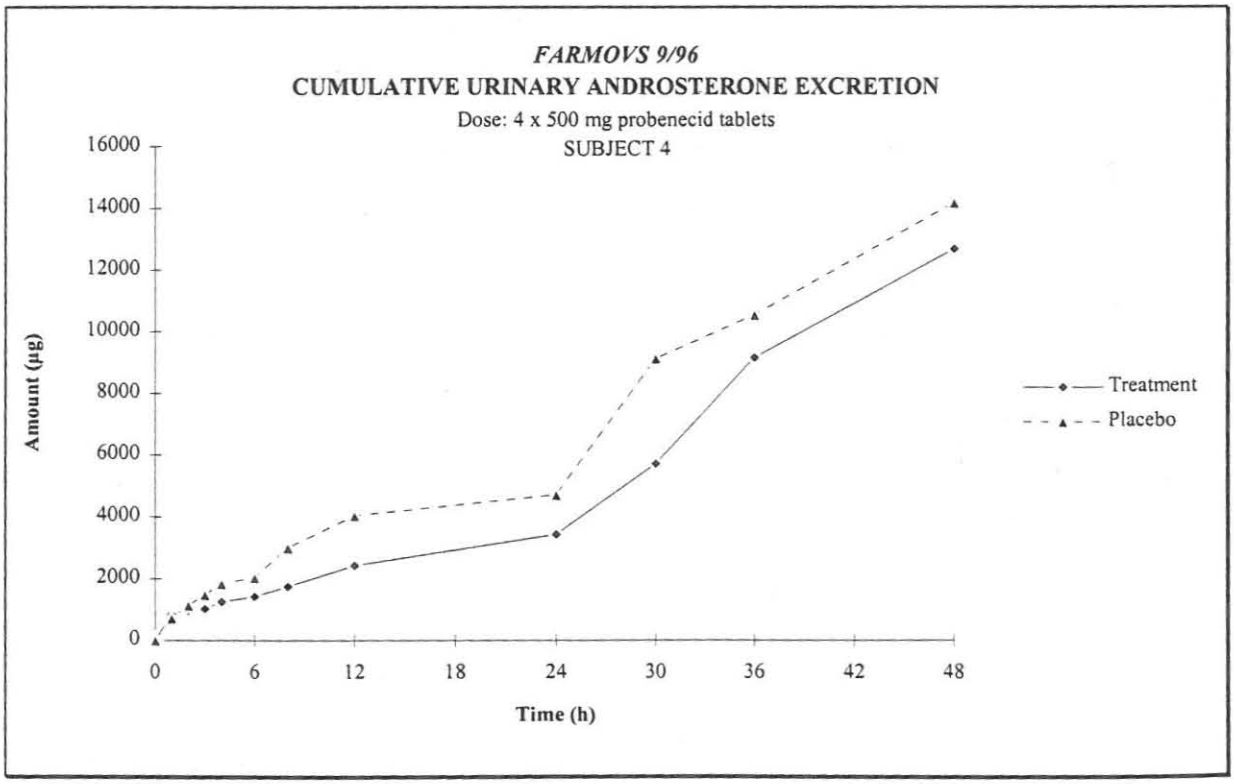
6.2.1 Androsterone

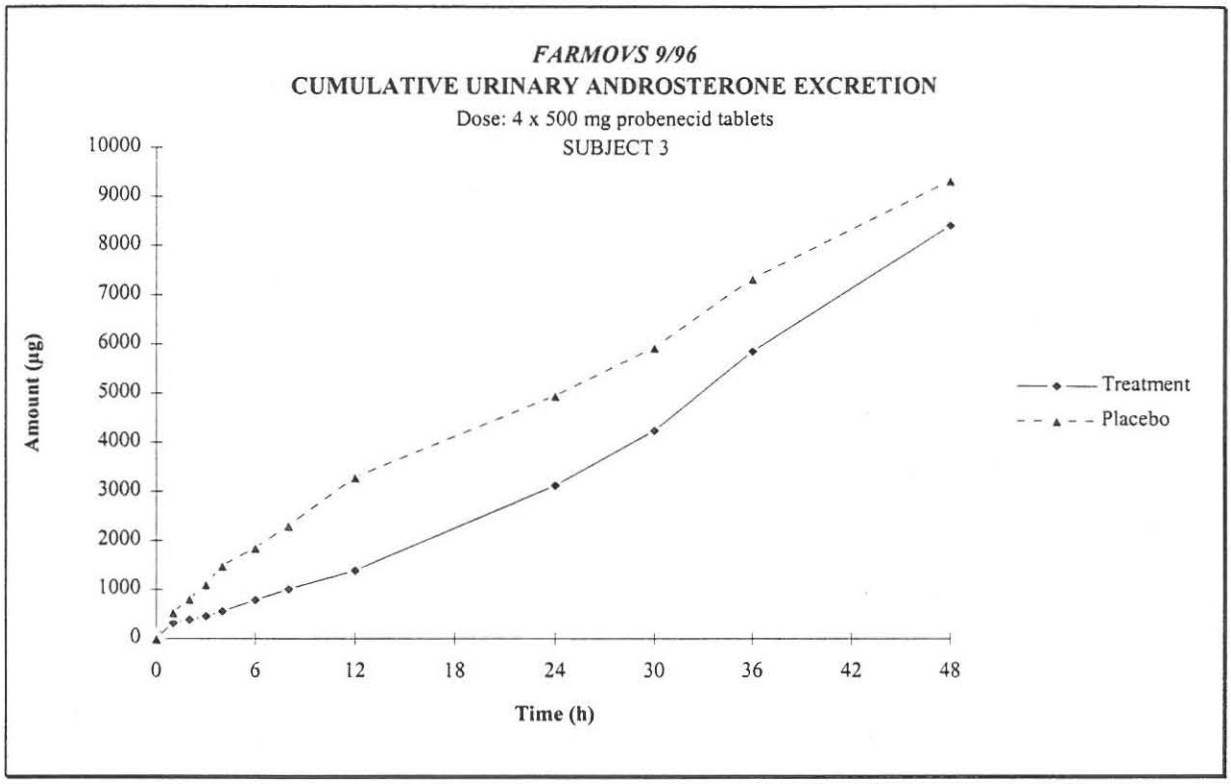
INDIVIDUAL CUMULATIVE URINARY ANDROSTERONE EXCRETION FOR EACH SUBJECT AND TREATMENT

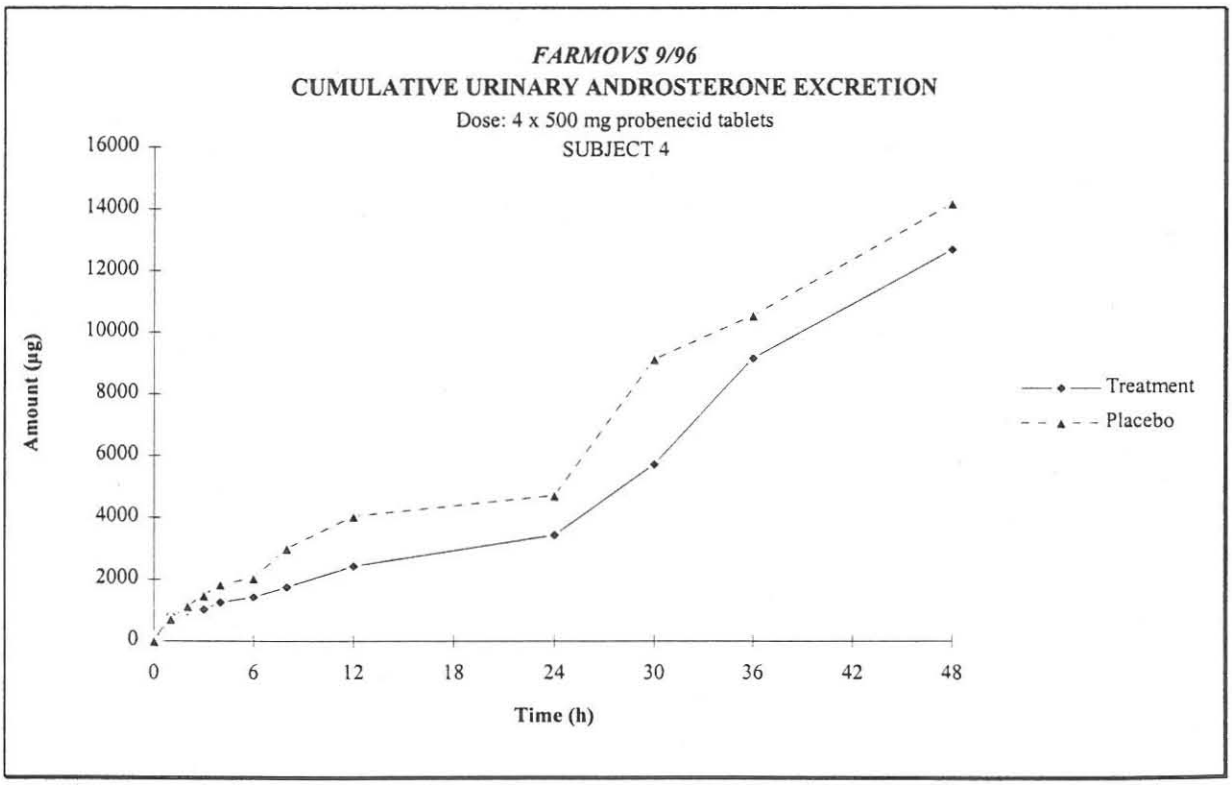


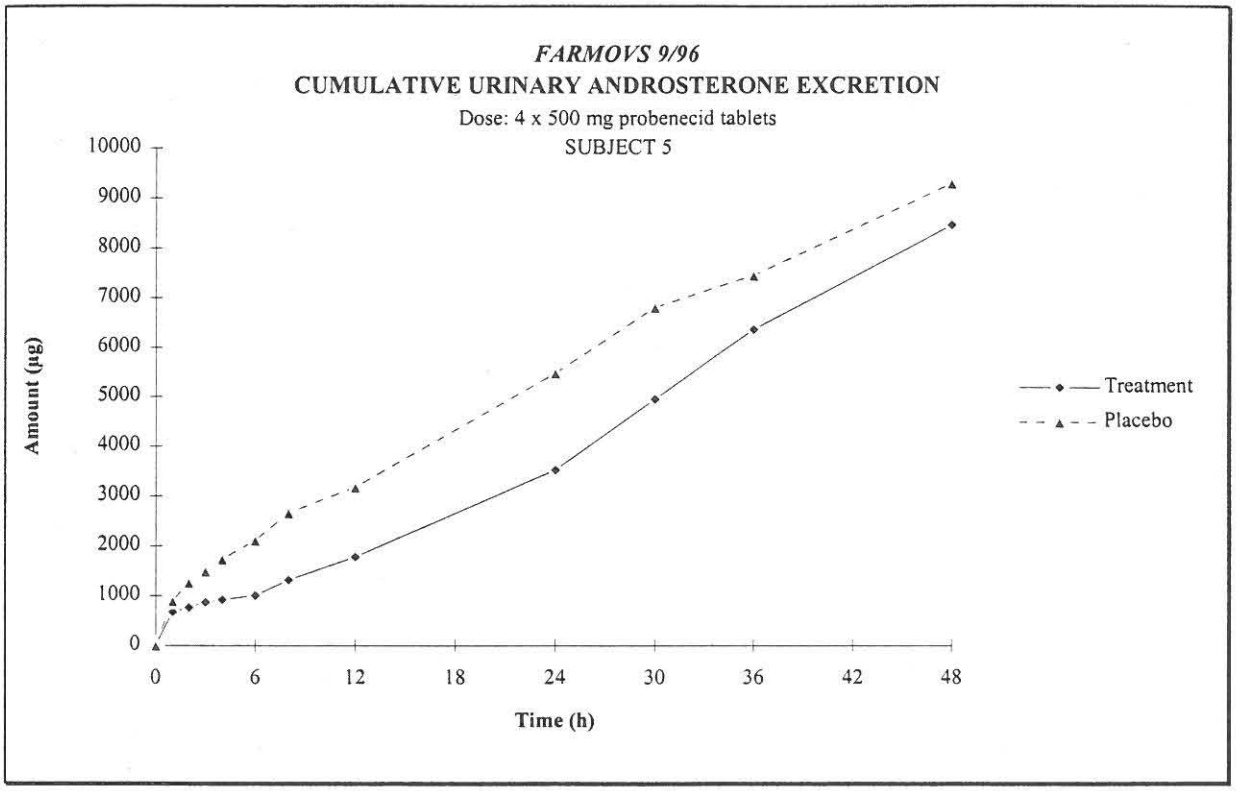


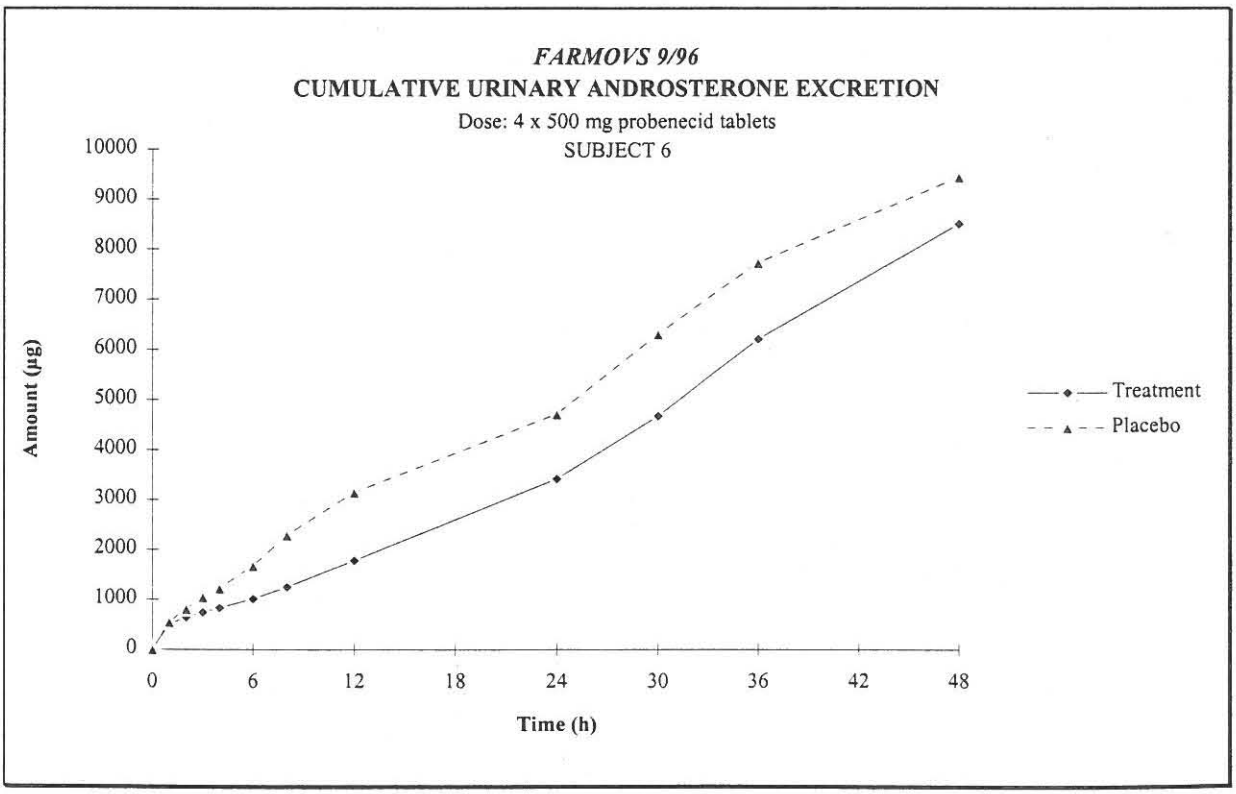


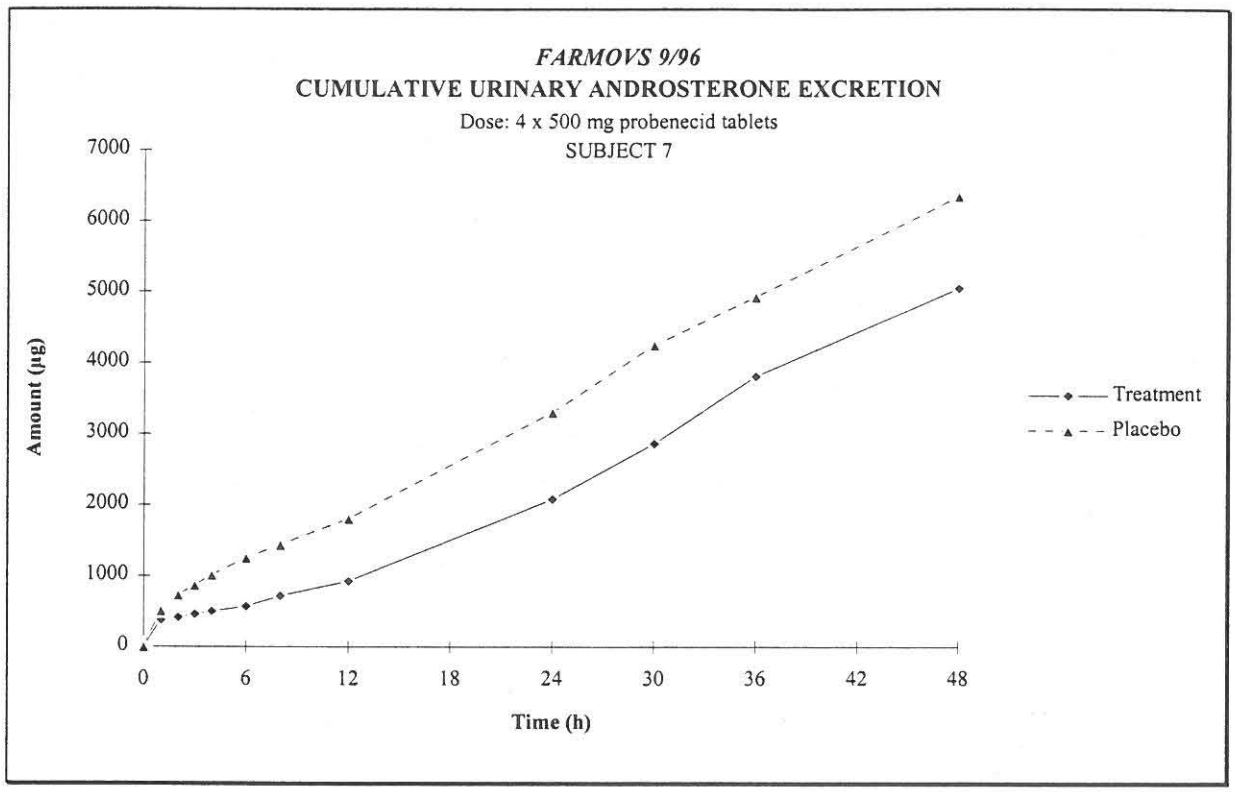


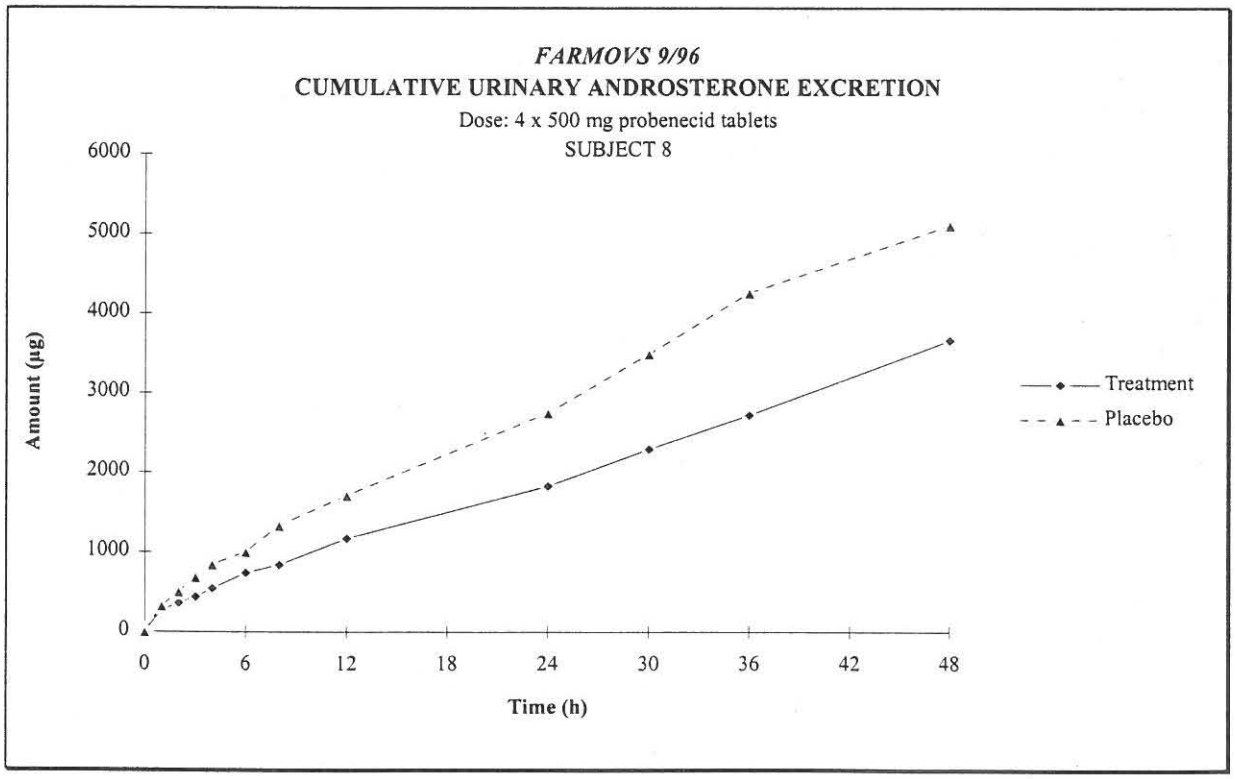


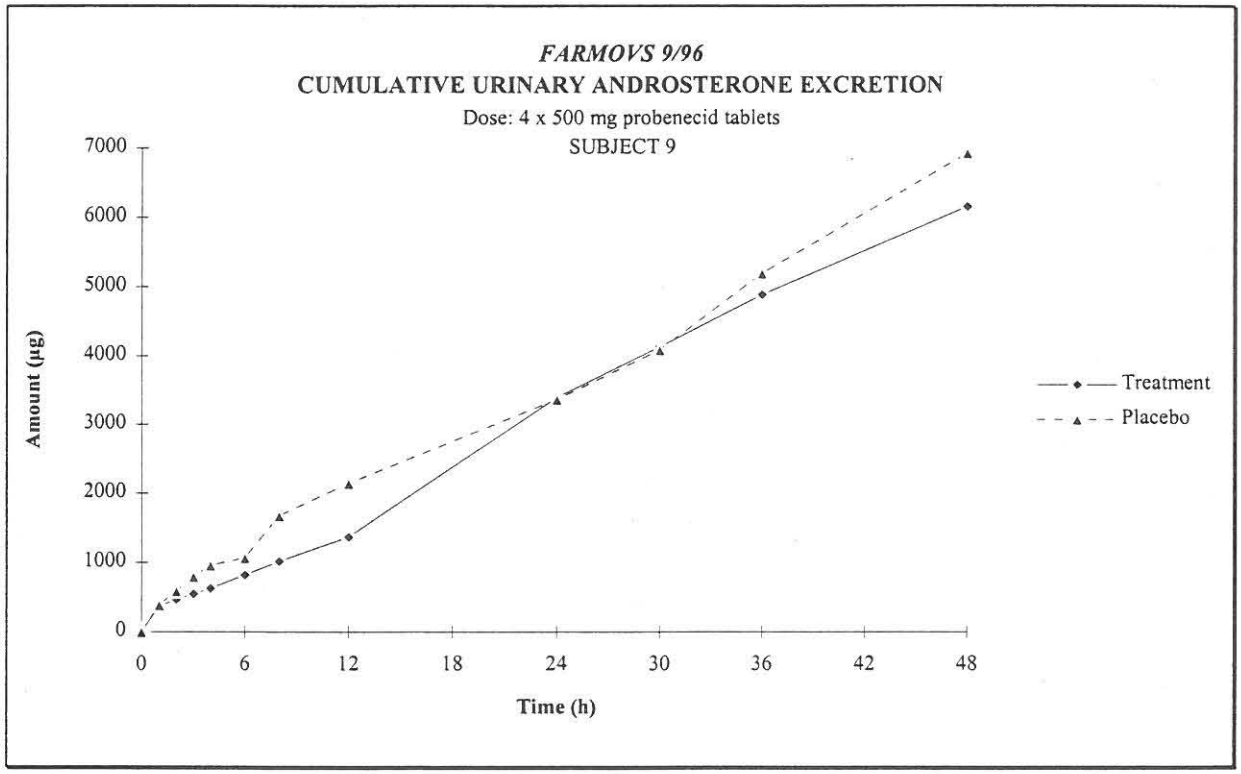


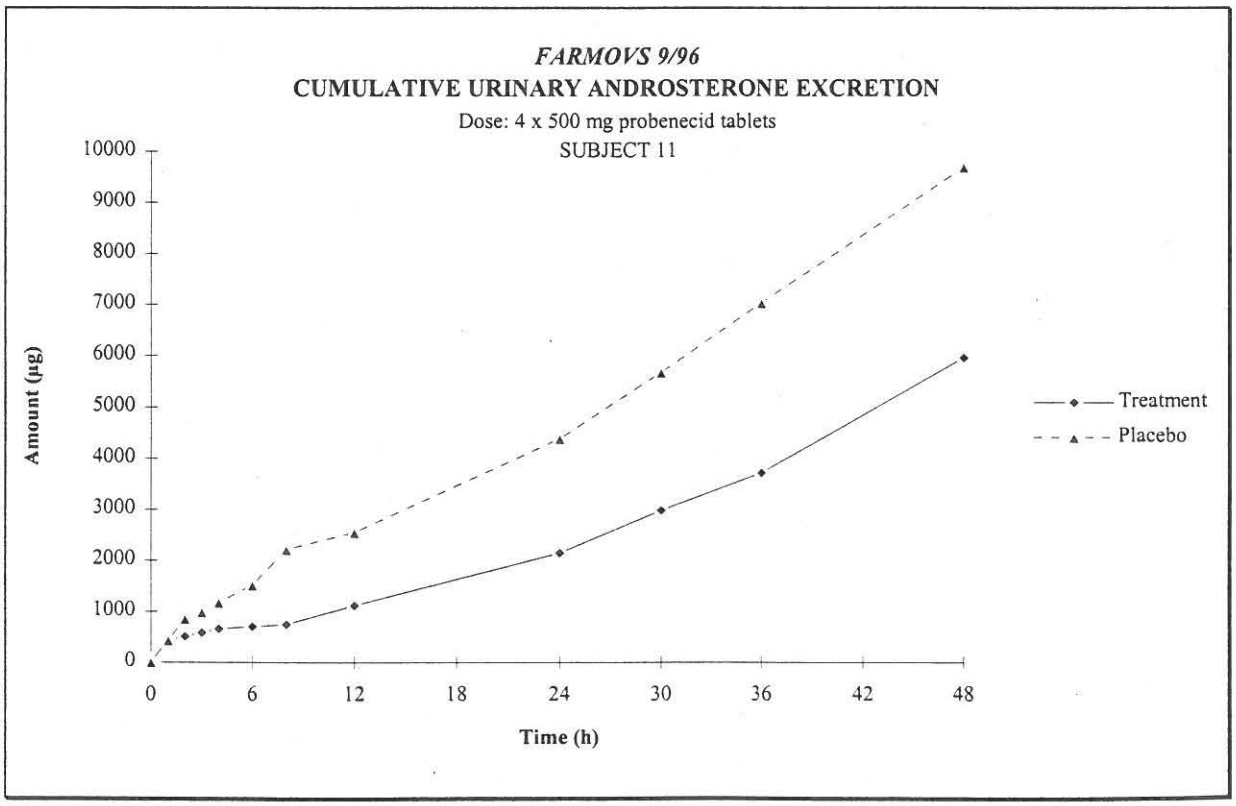


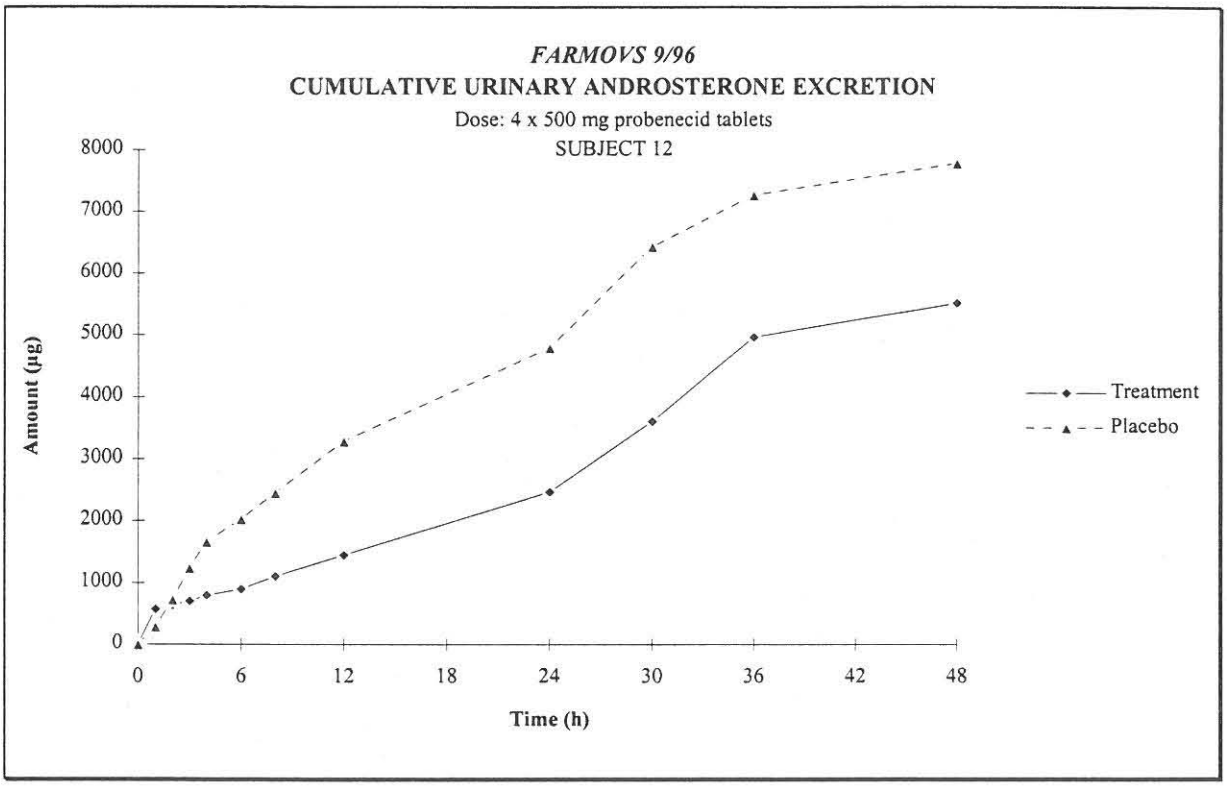


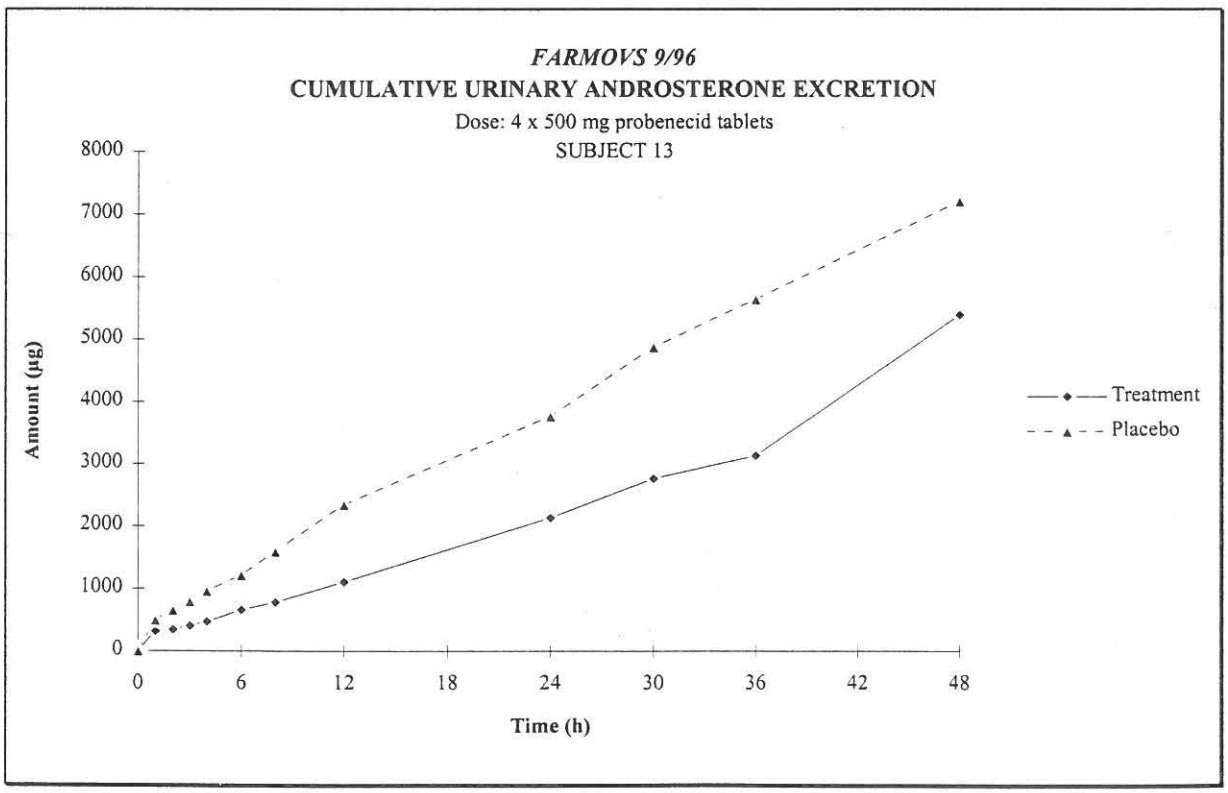


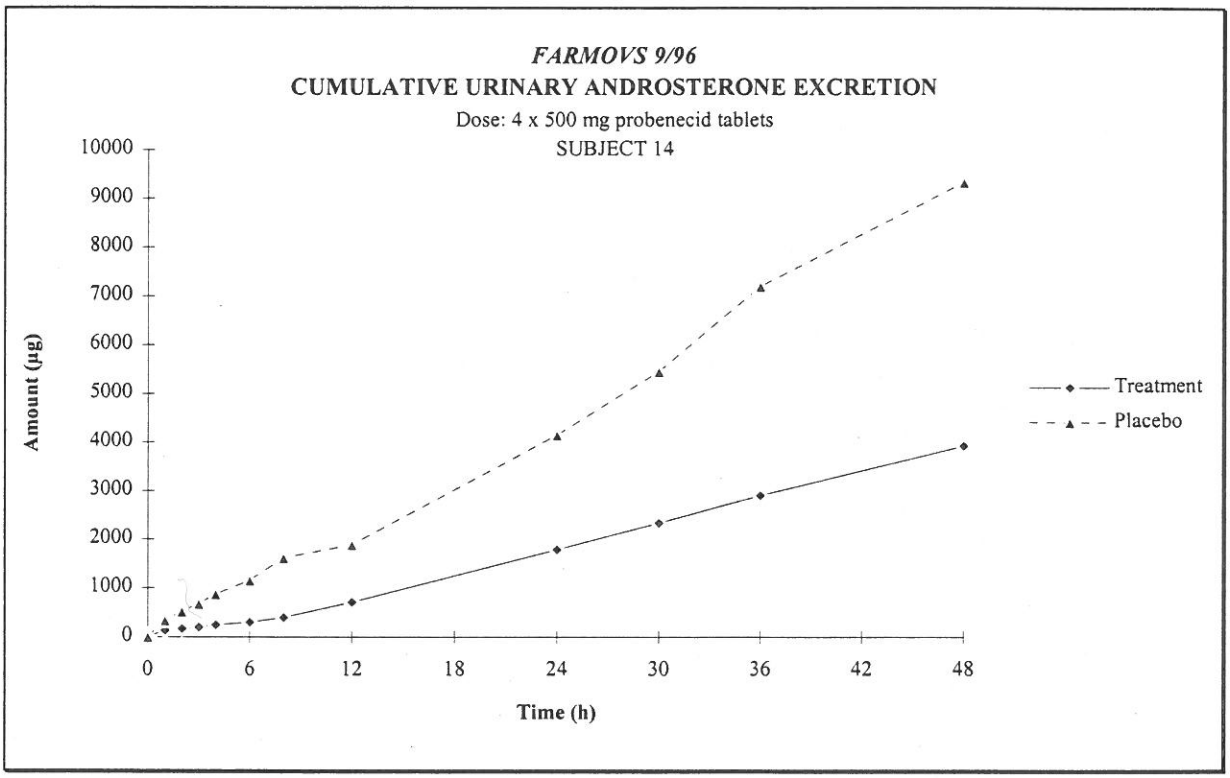


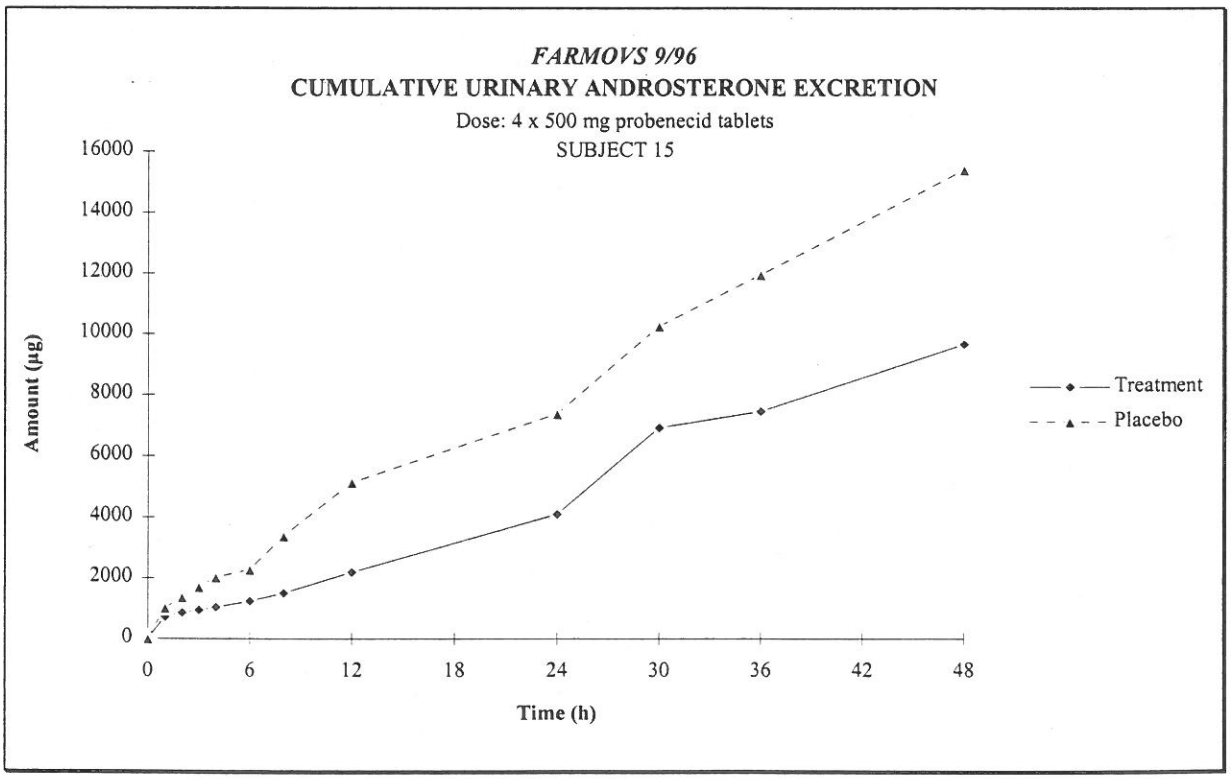


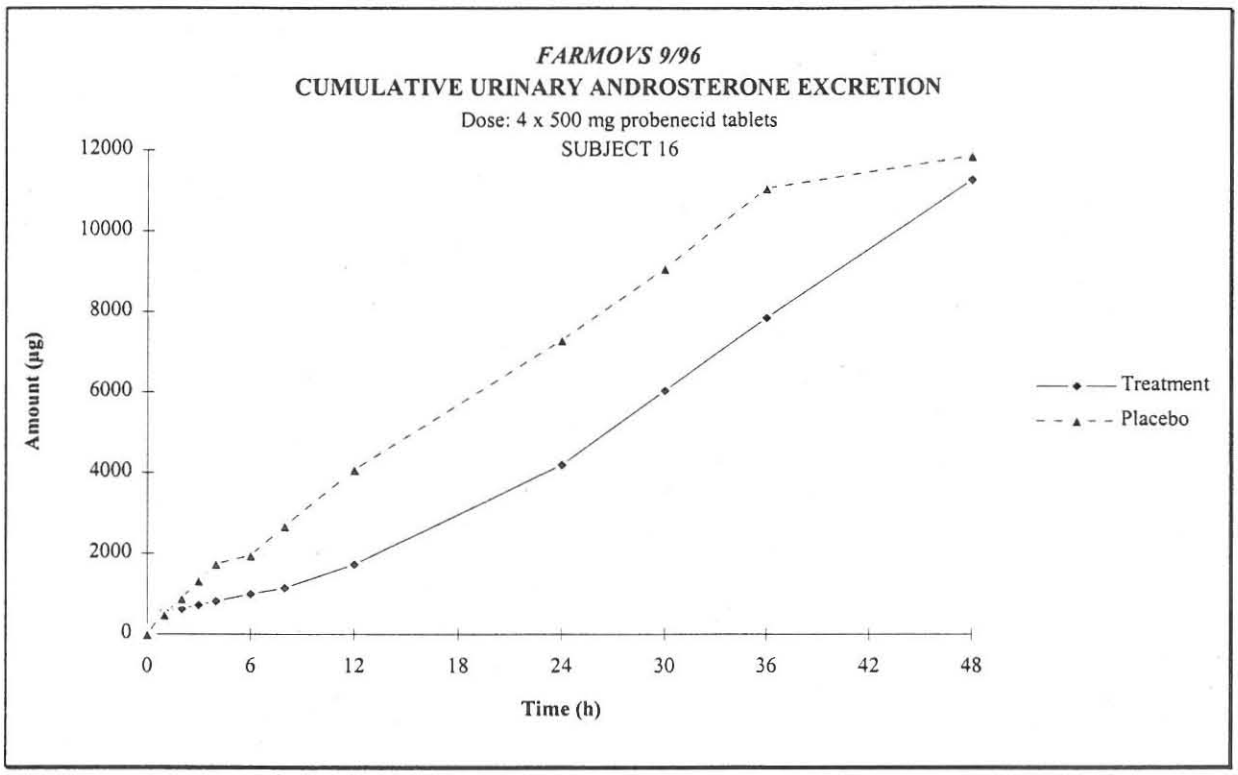


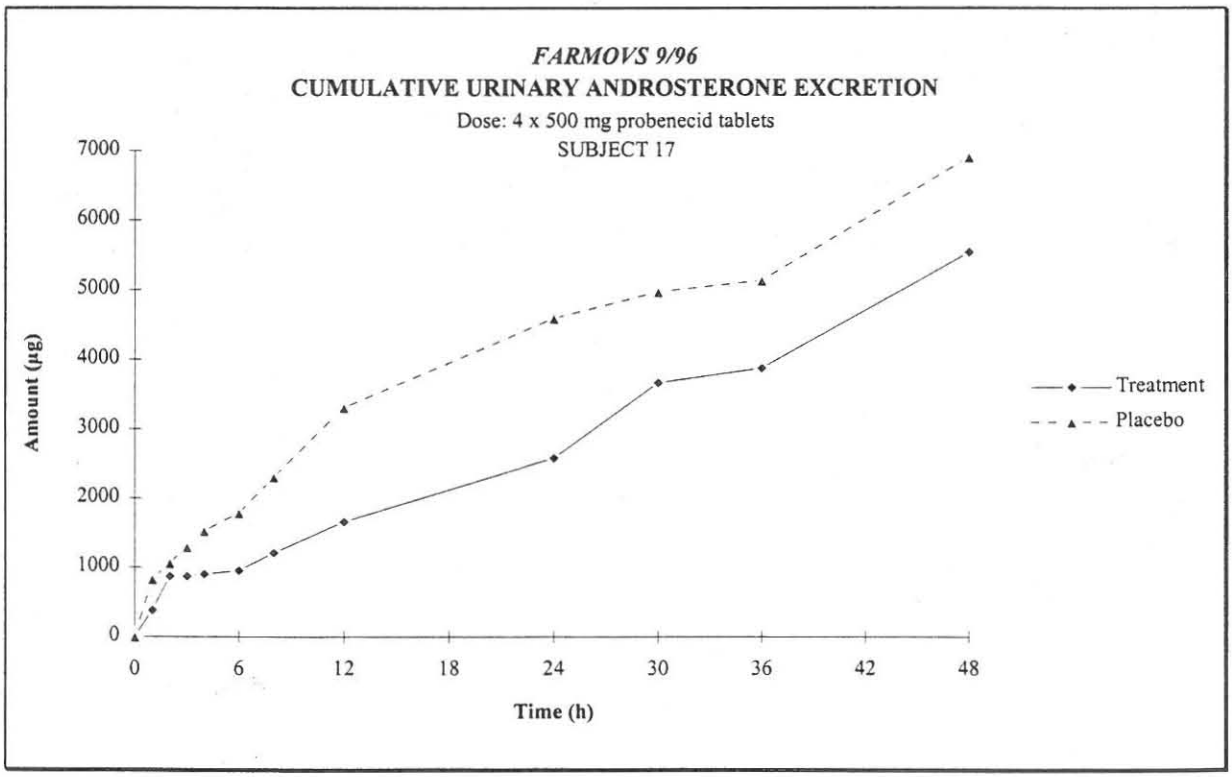


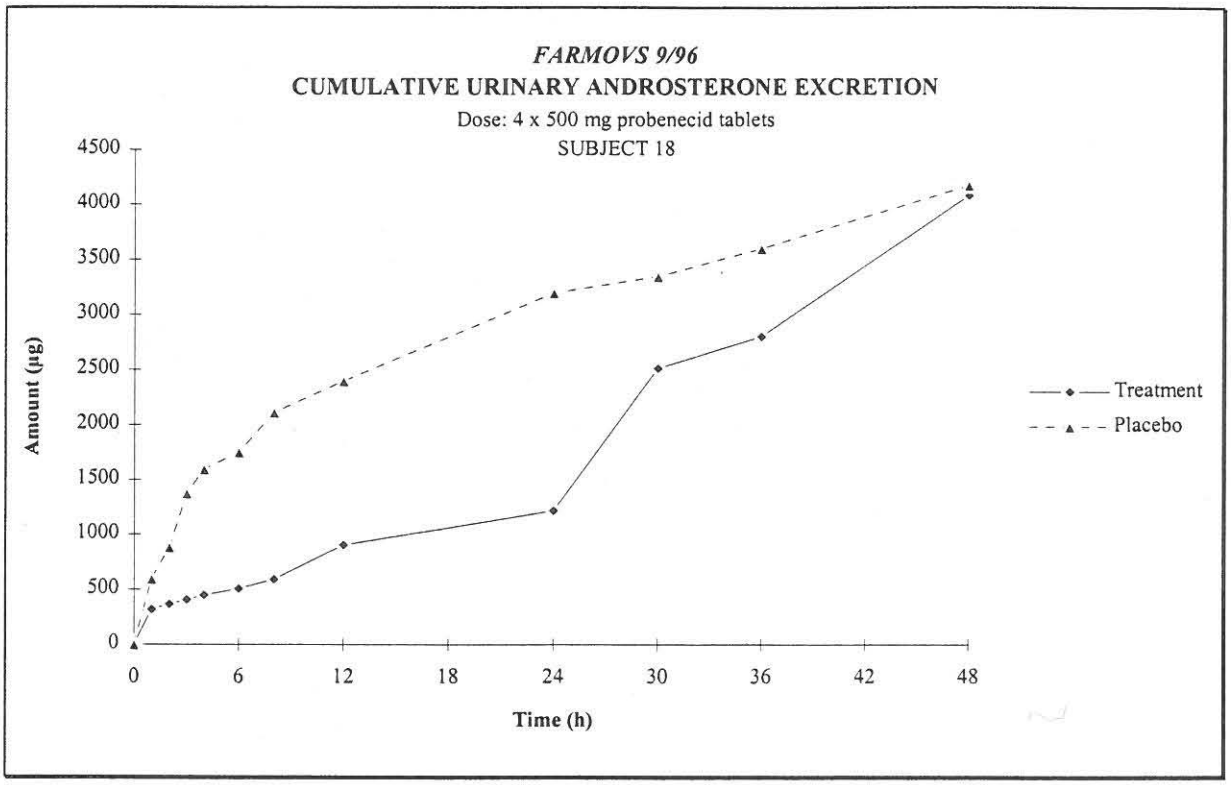


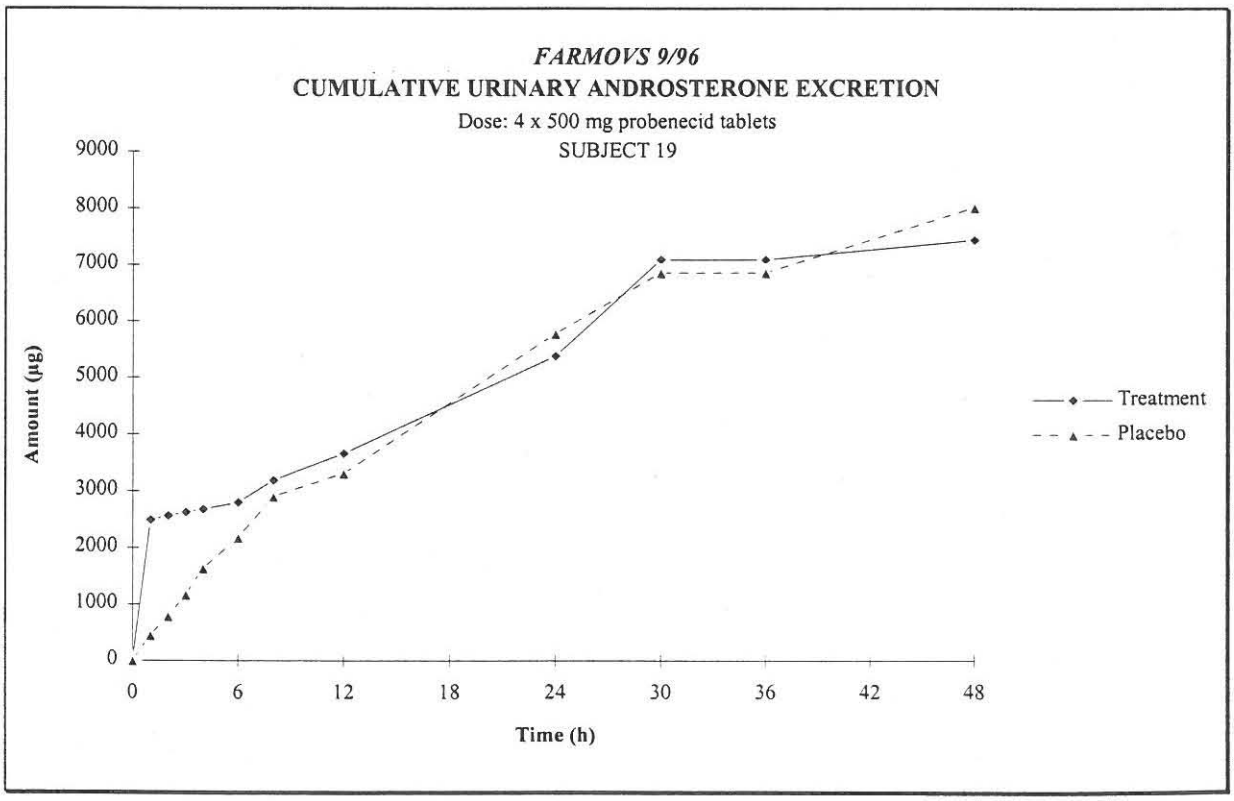


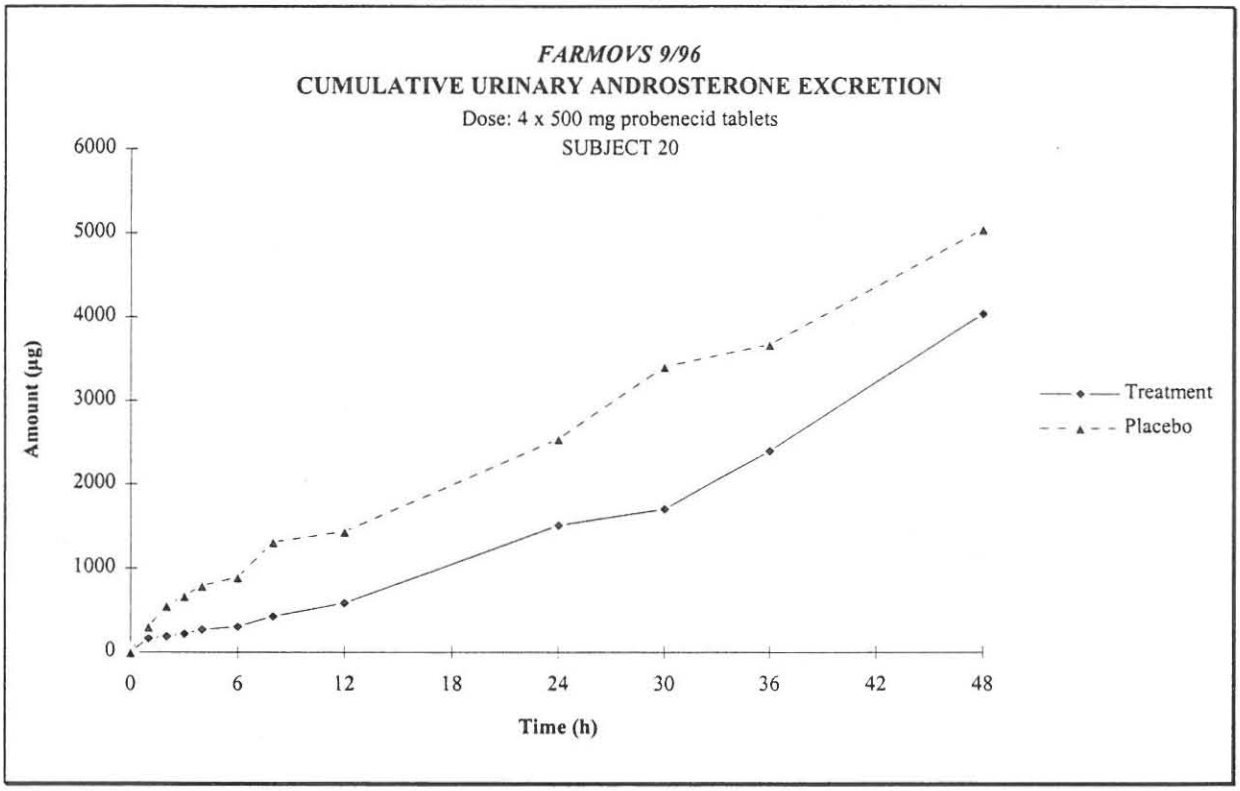




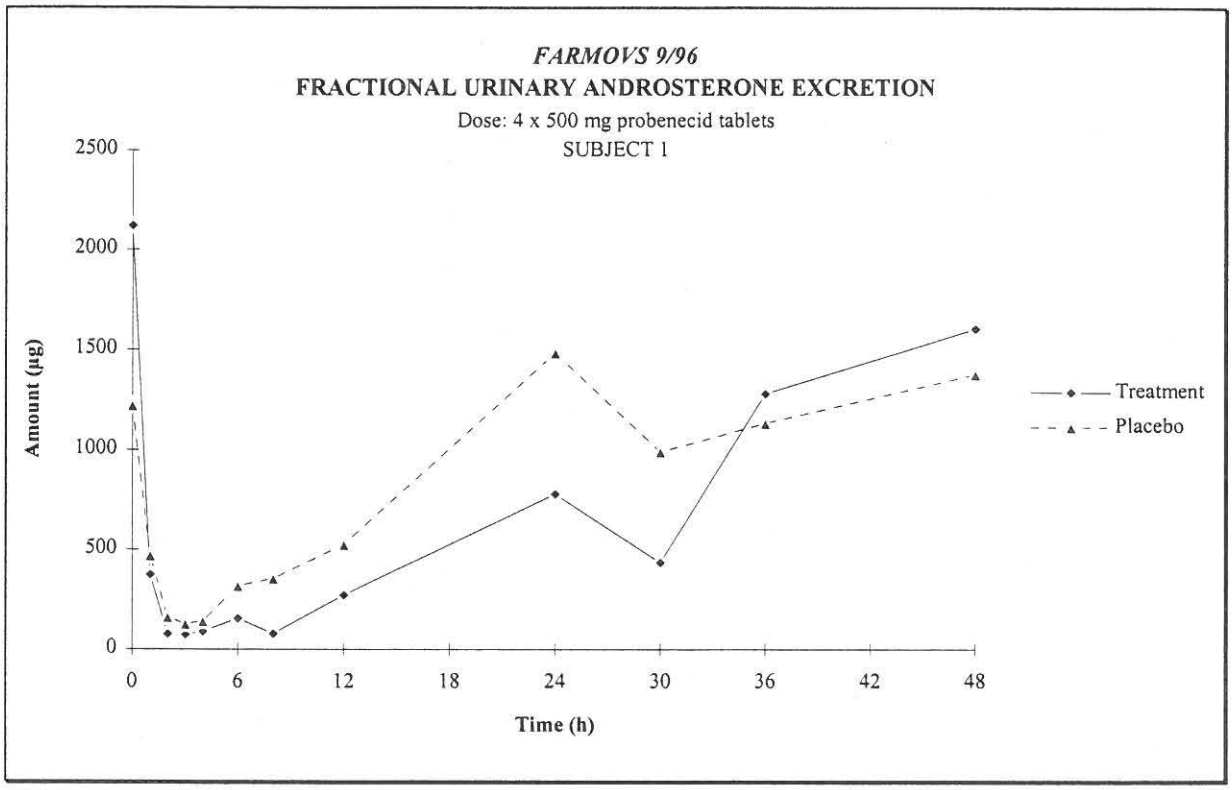


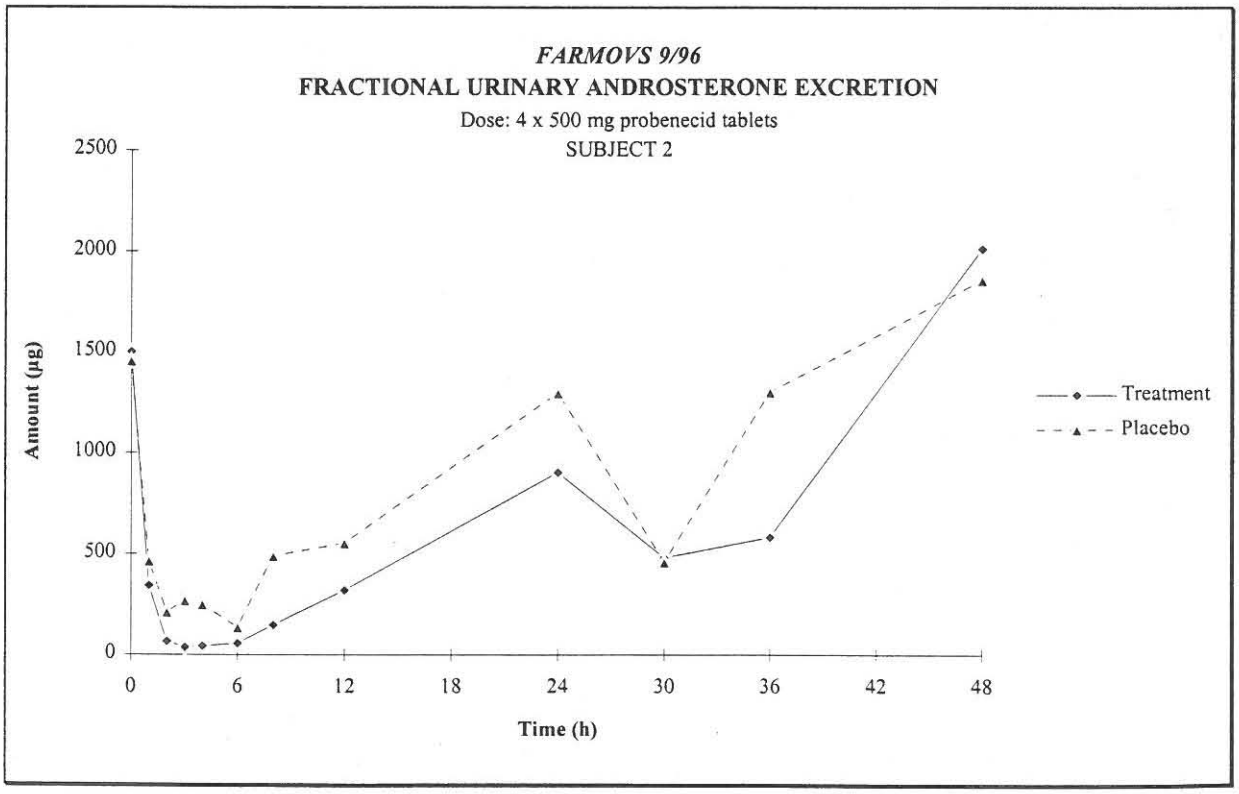


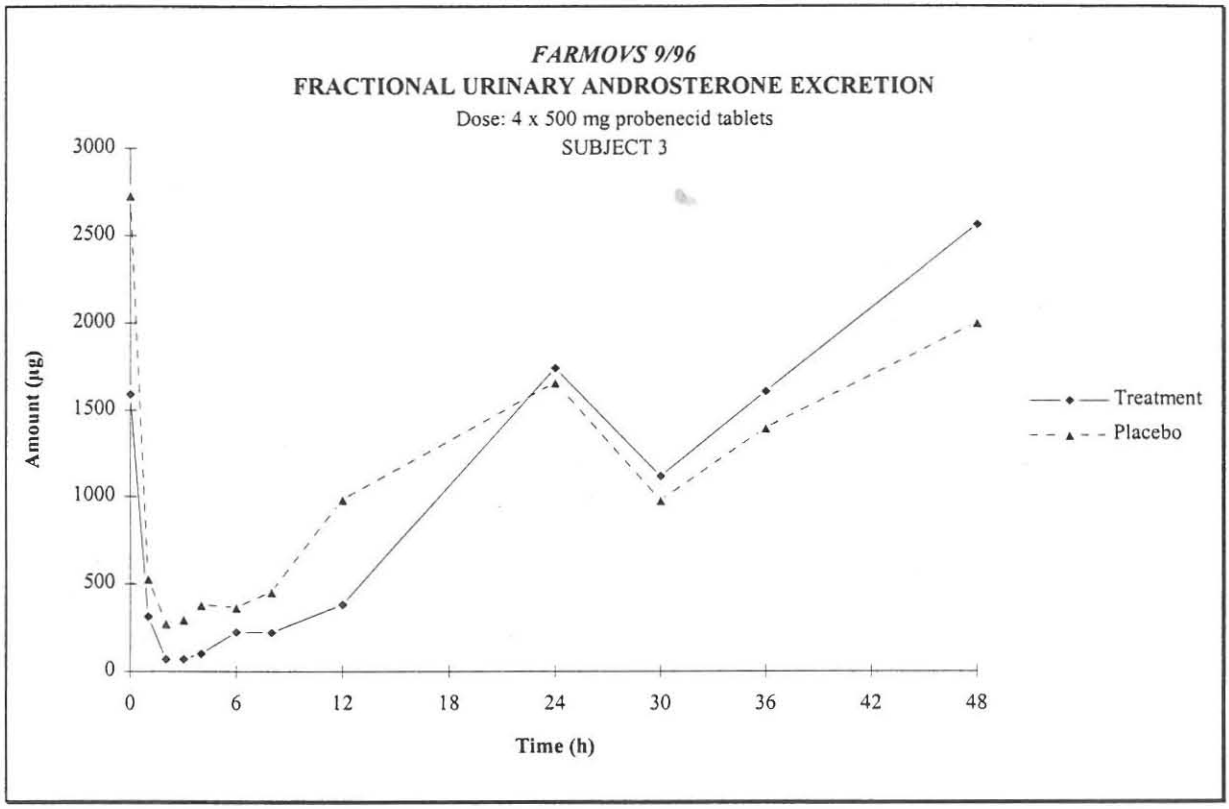


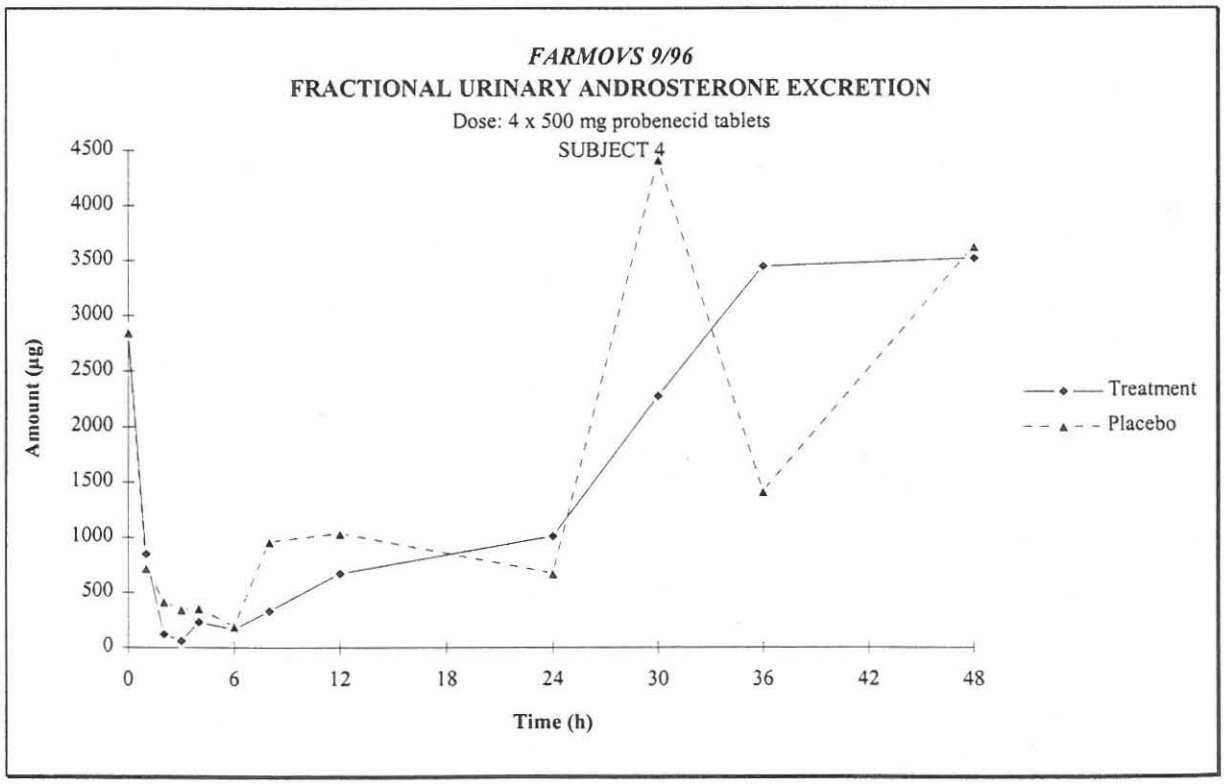


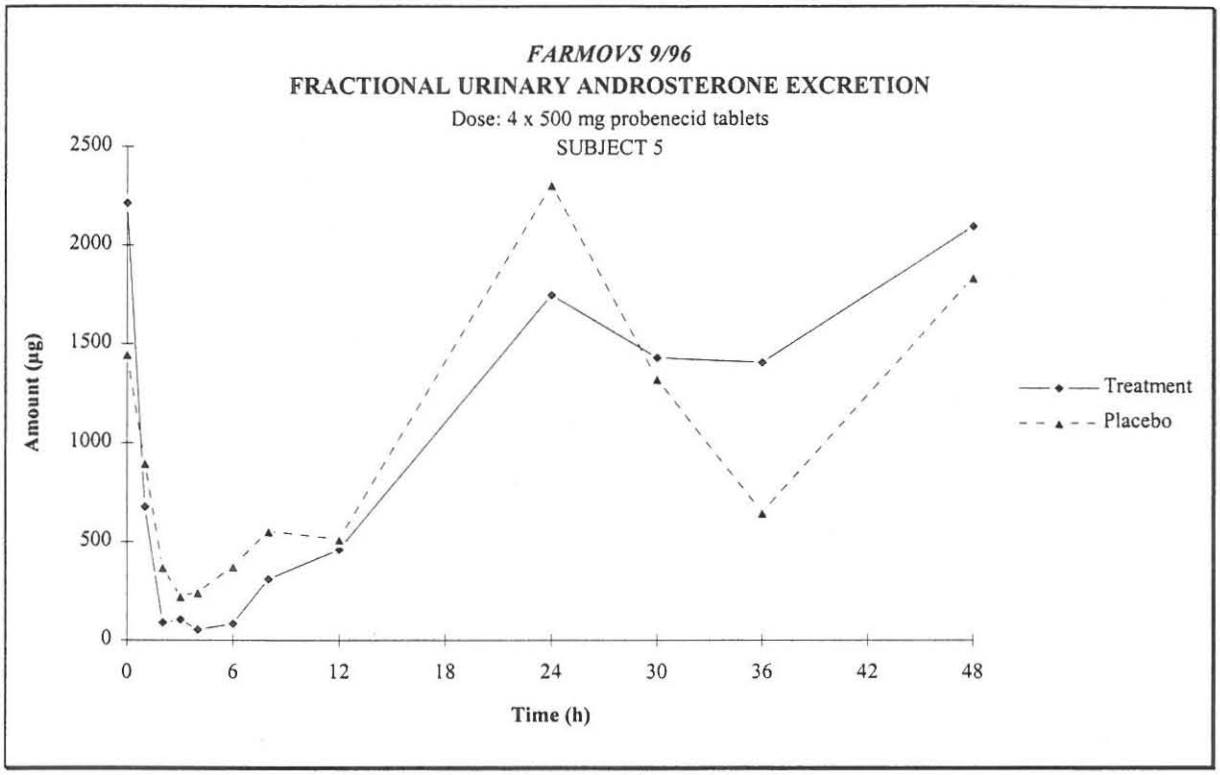
INDIVIDUAL FRACTIONAL URINARY ANDROSTERONE EXCRETION FOR EACH SUBJECT AND TREATMENT

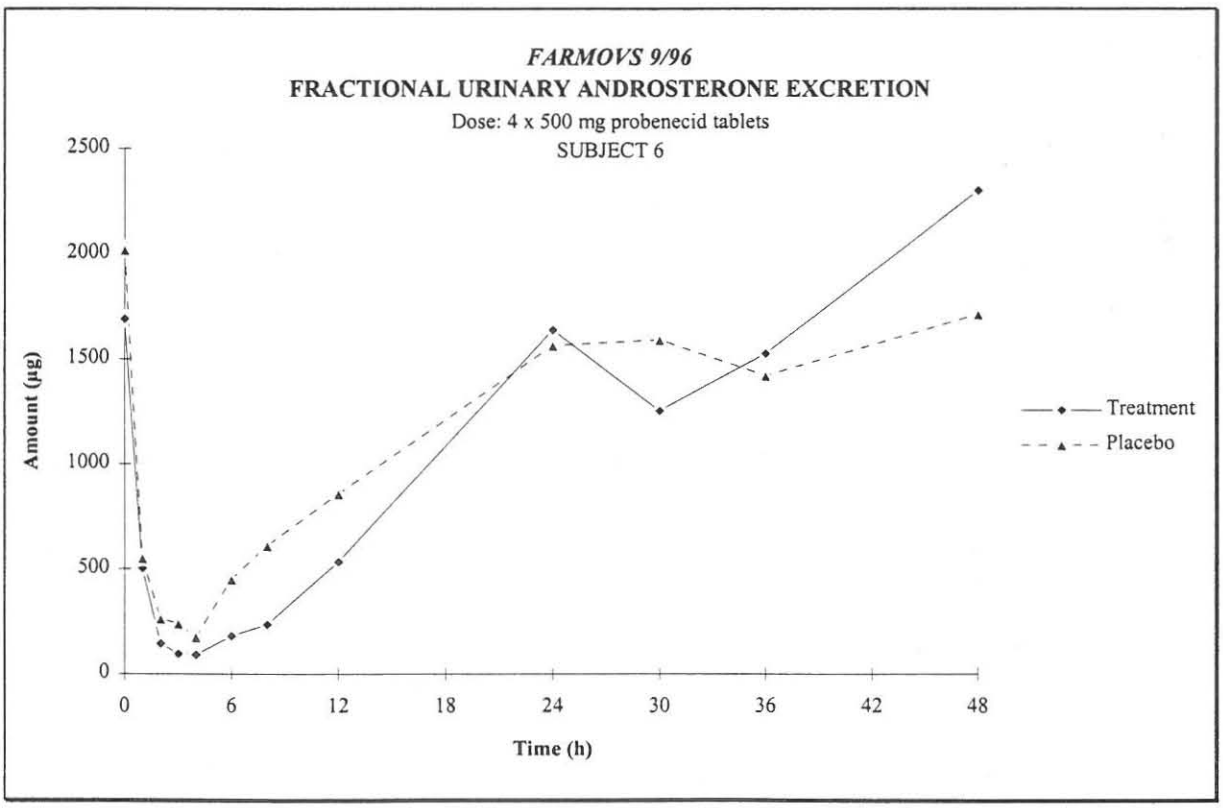


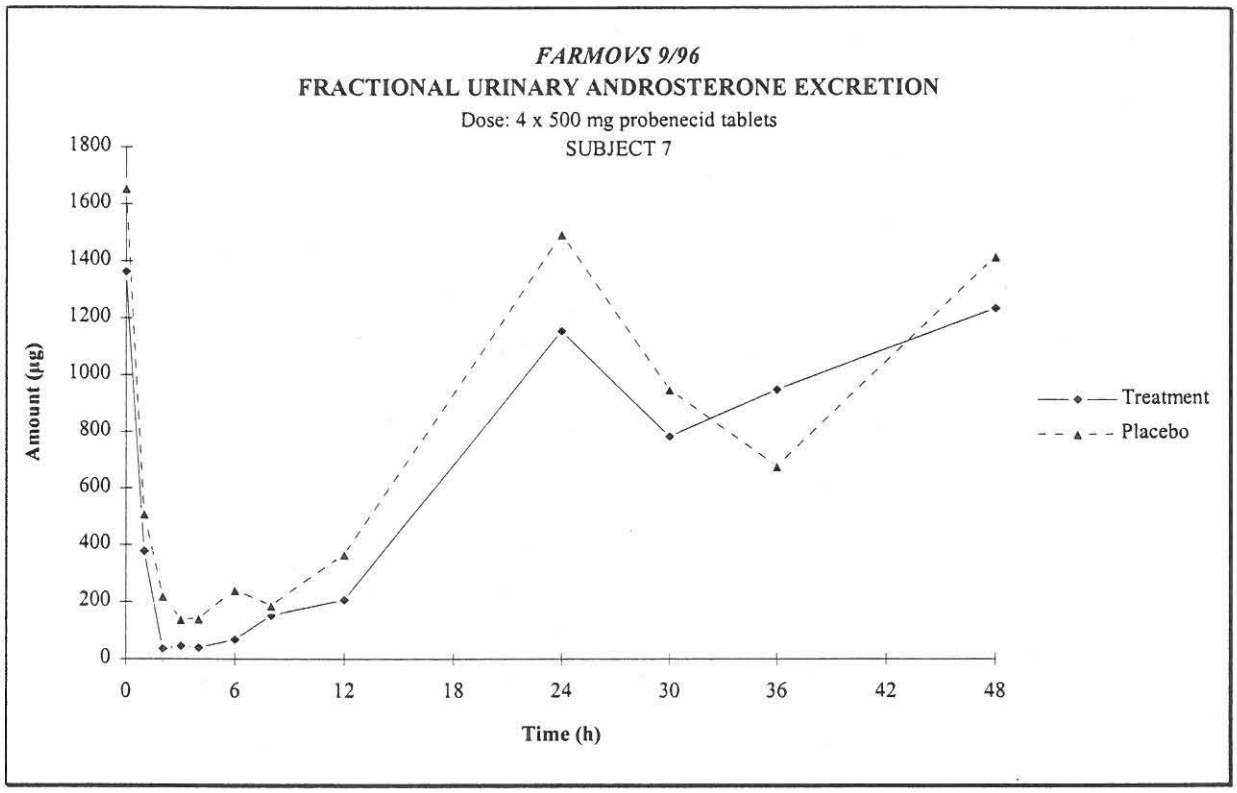


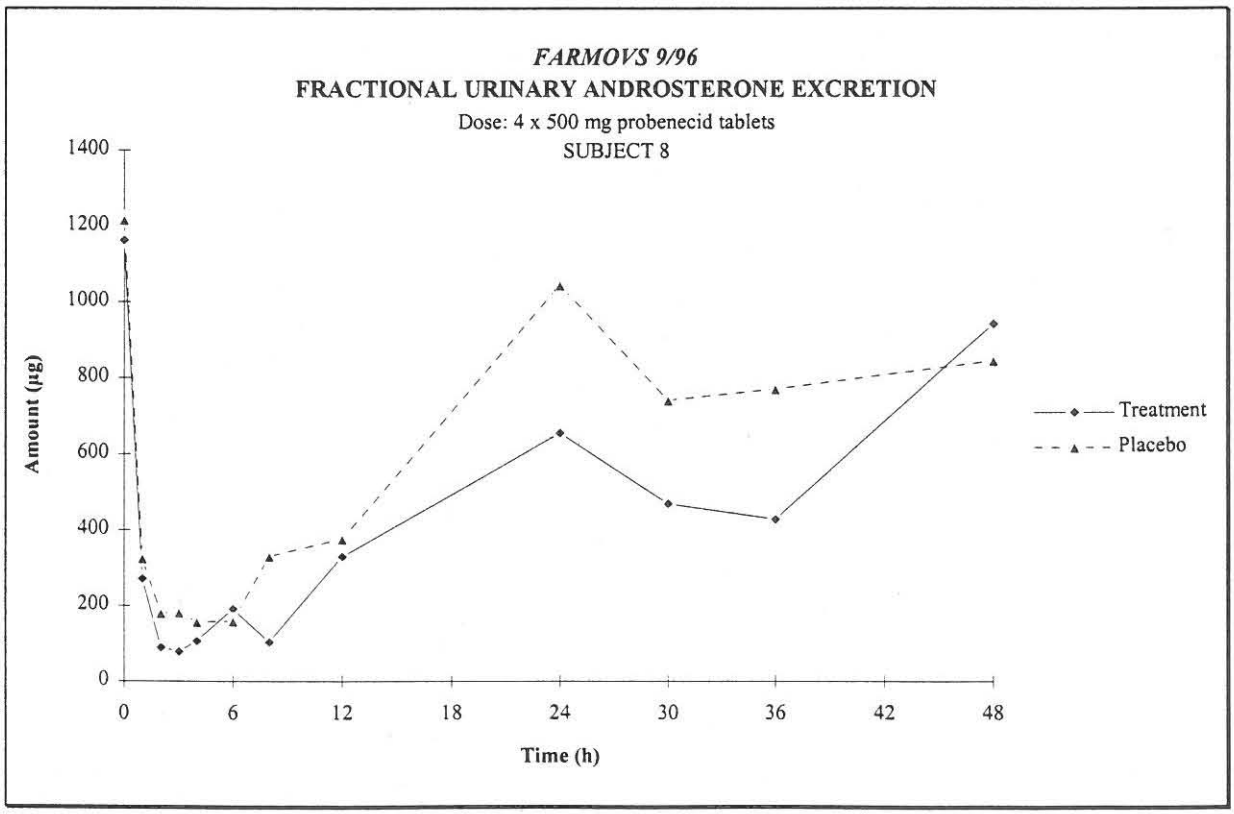


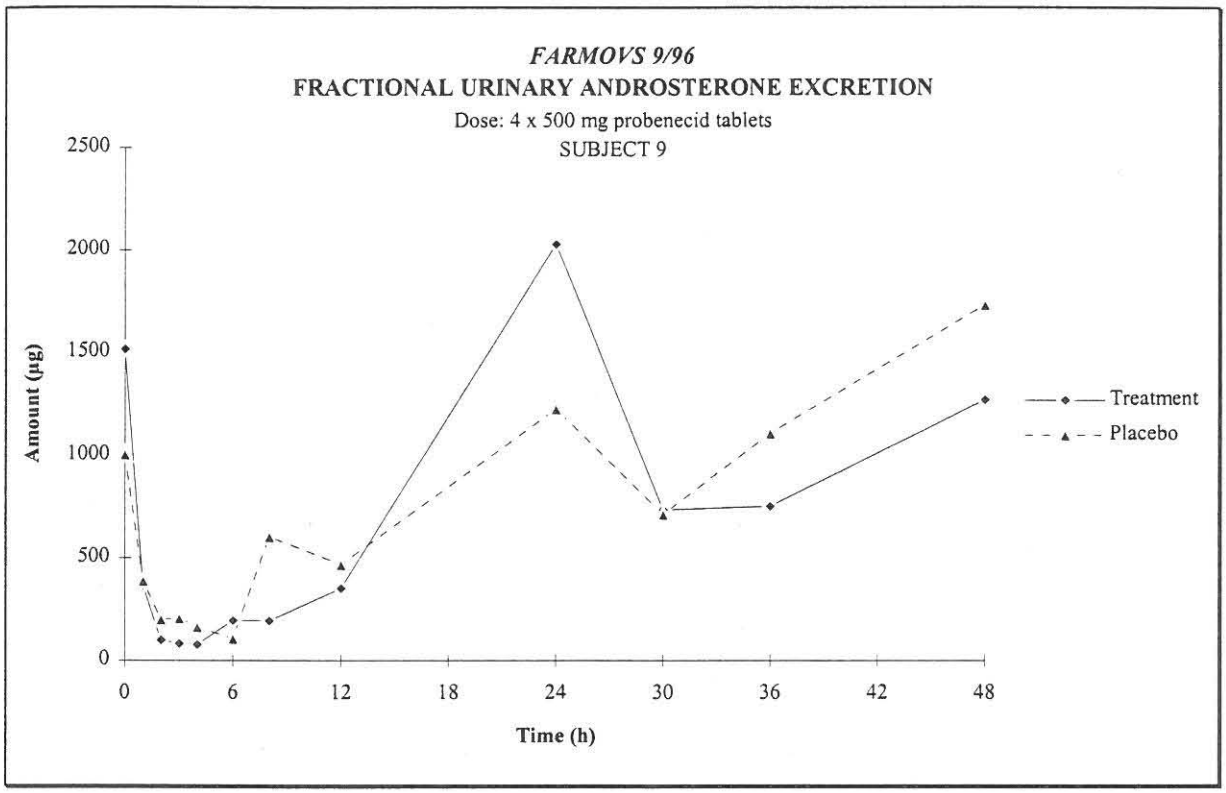


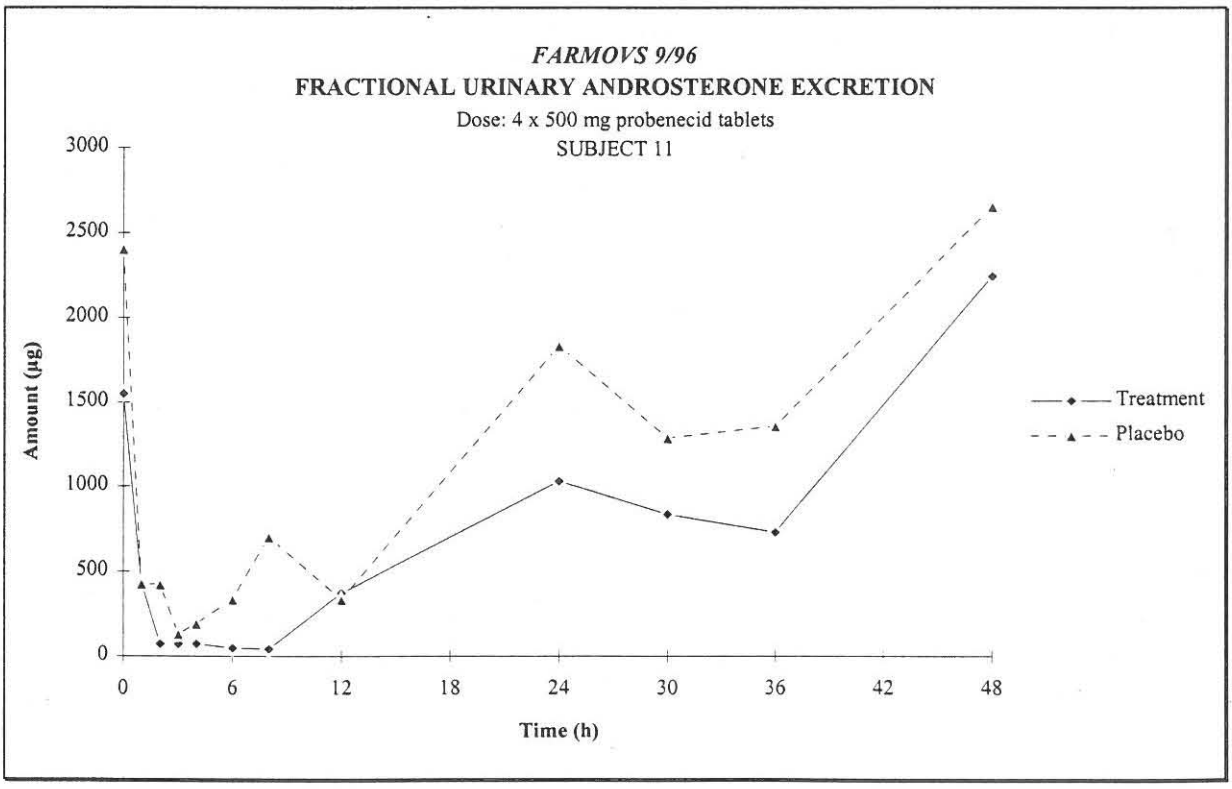


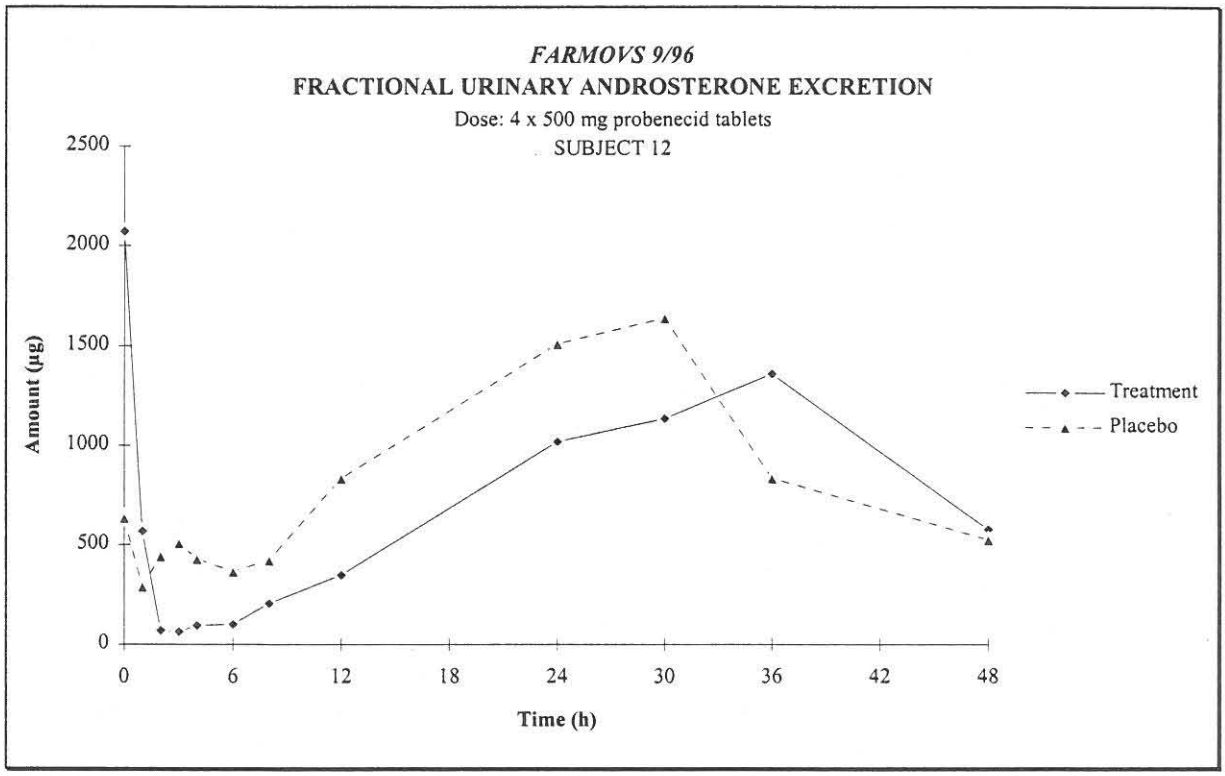


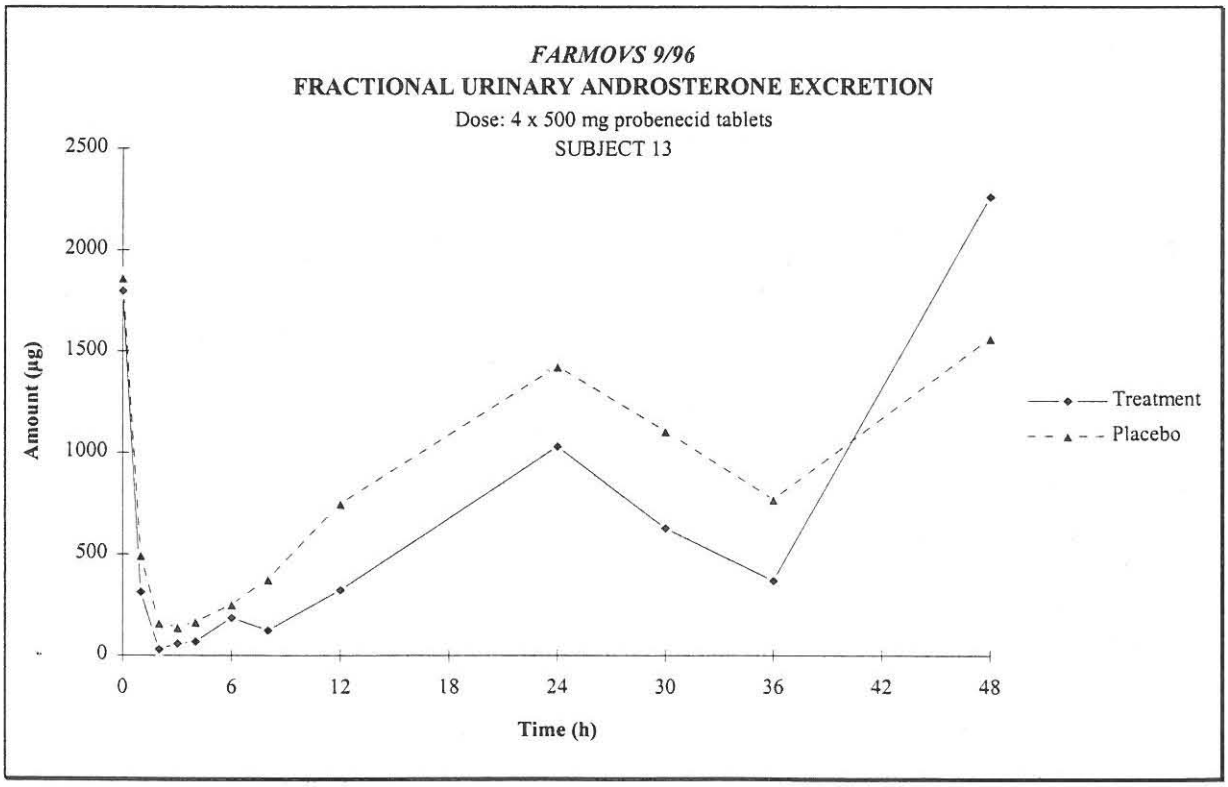


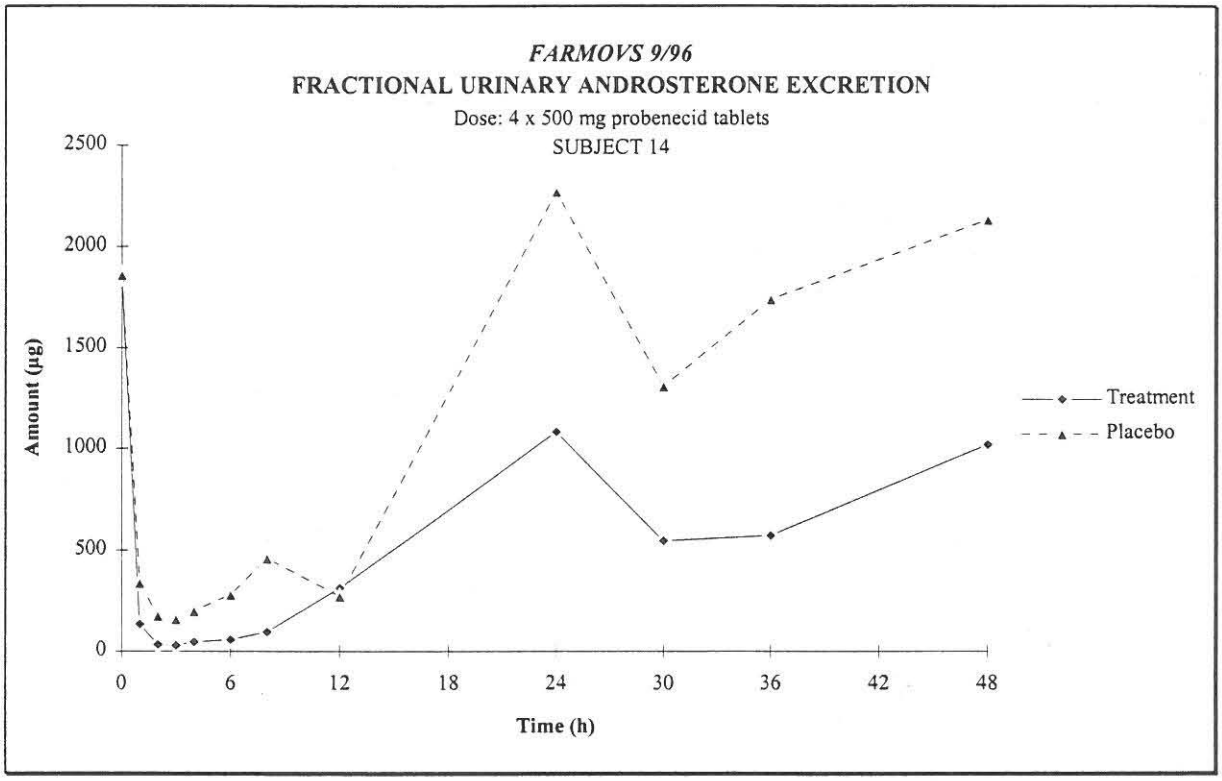


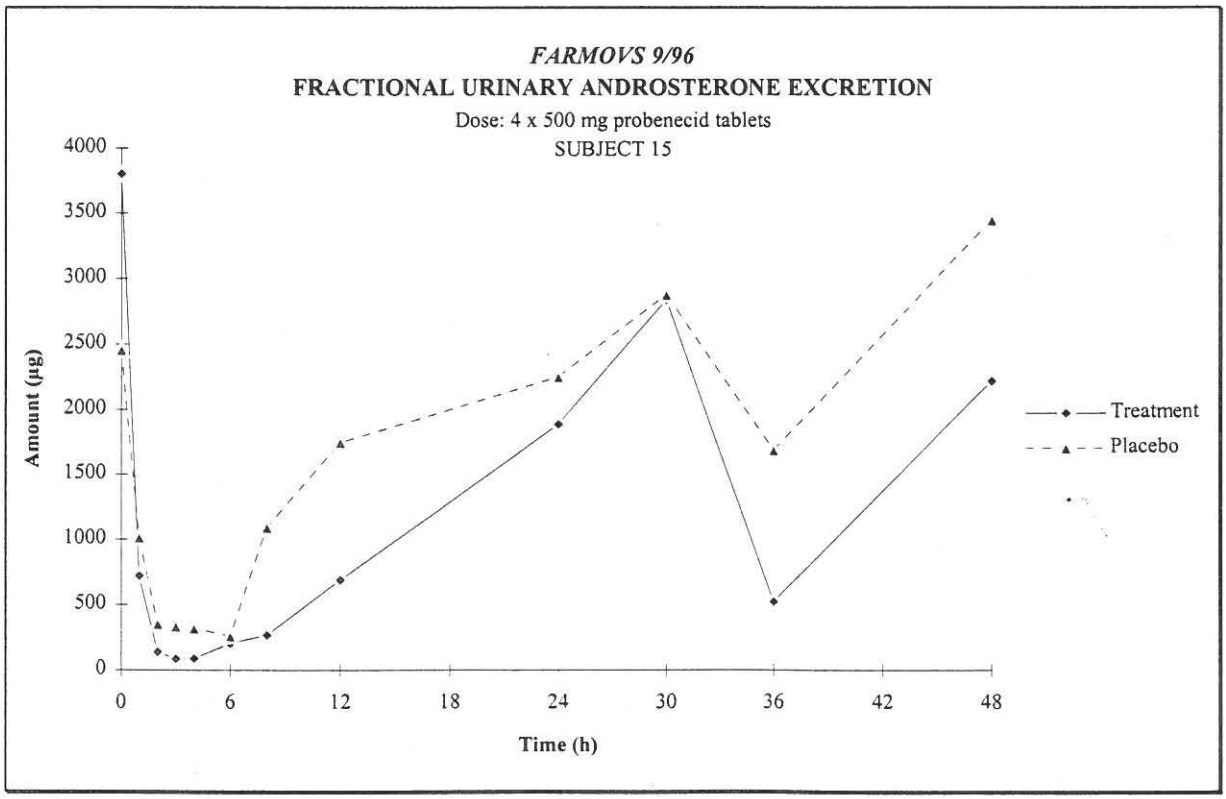


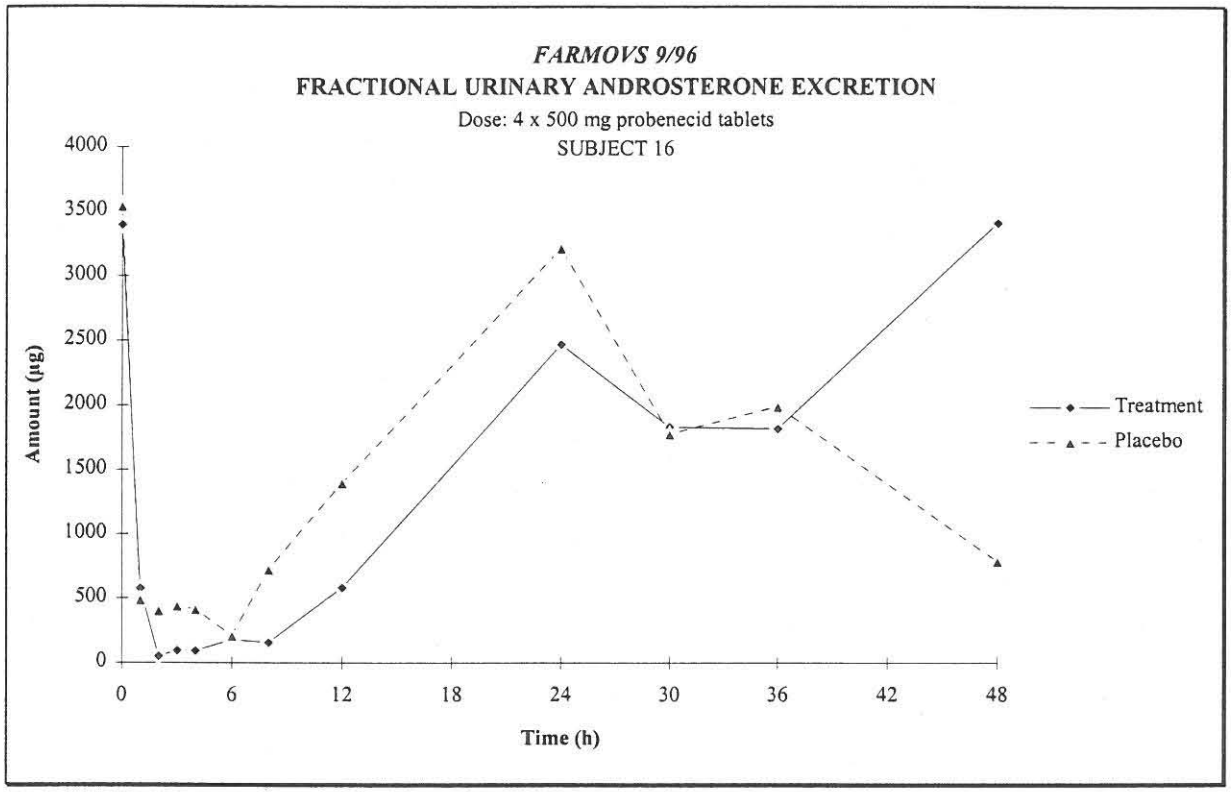


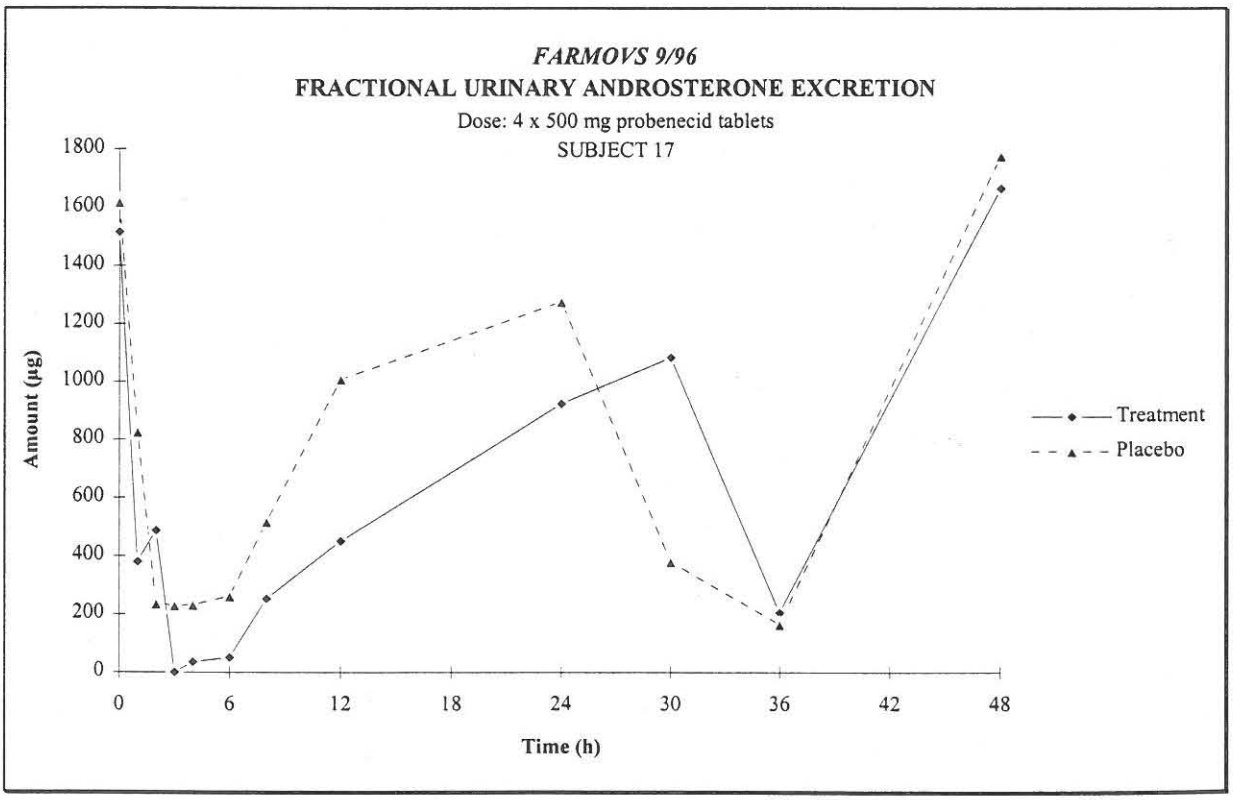


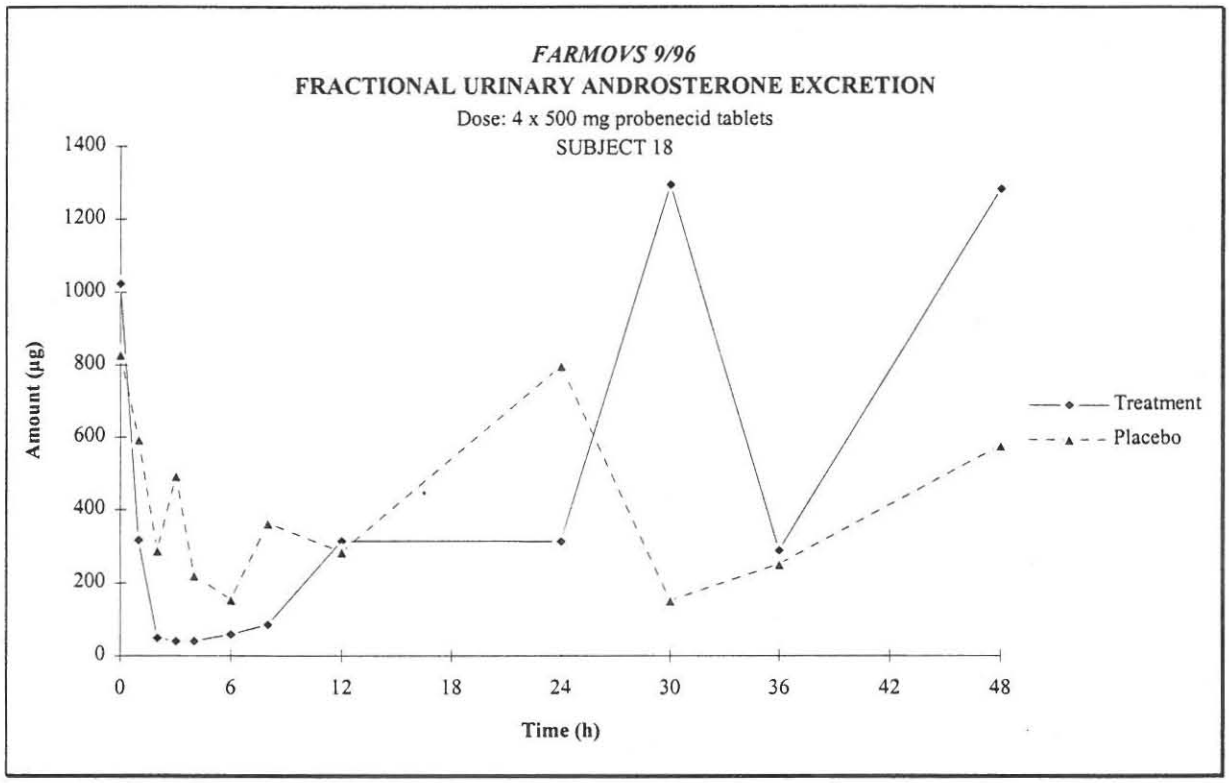


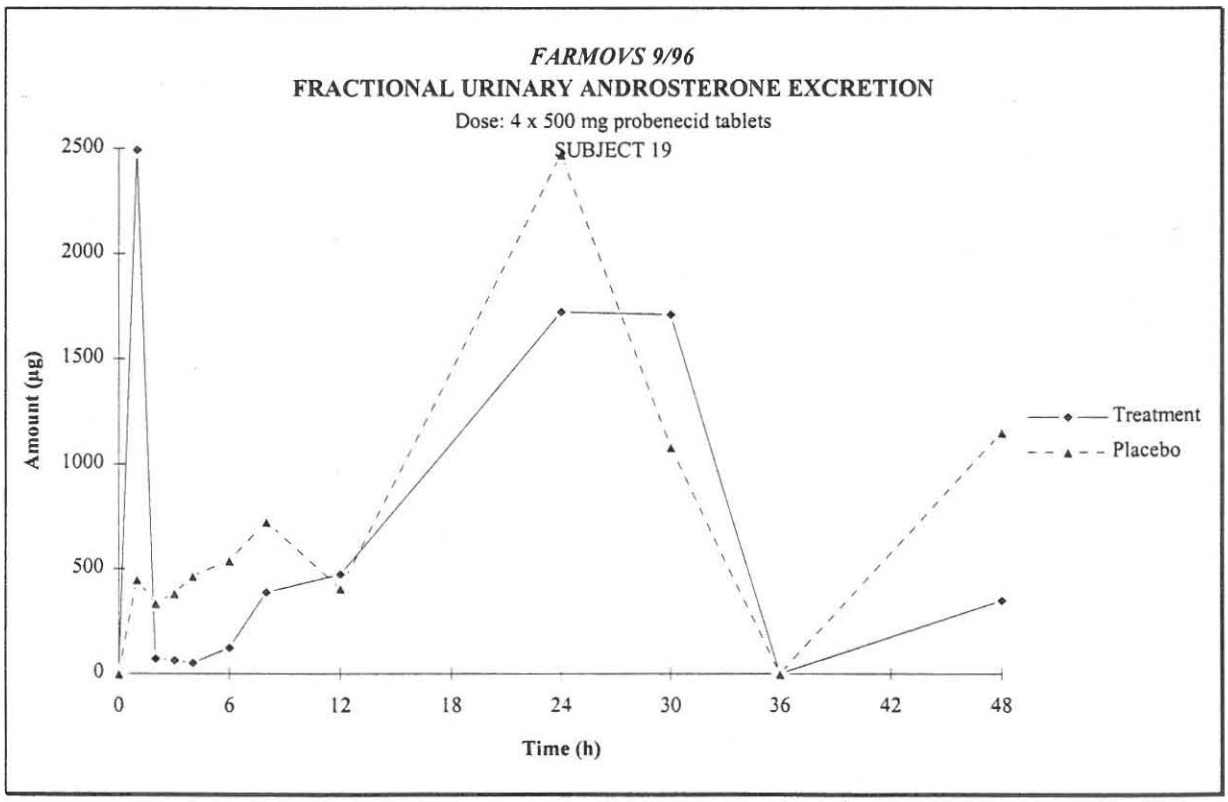


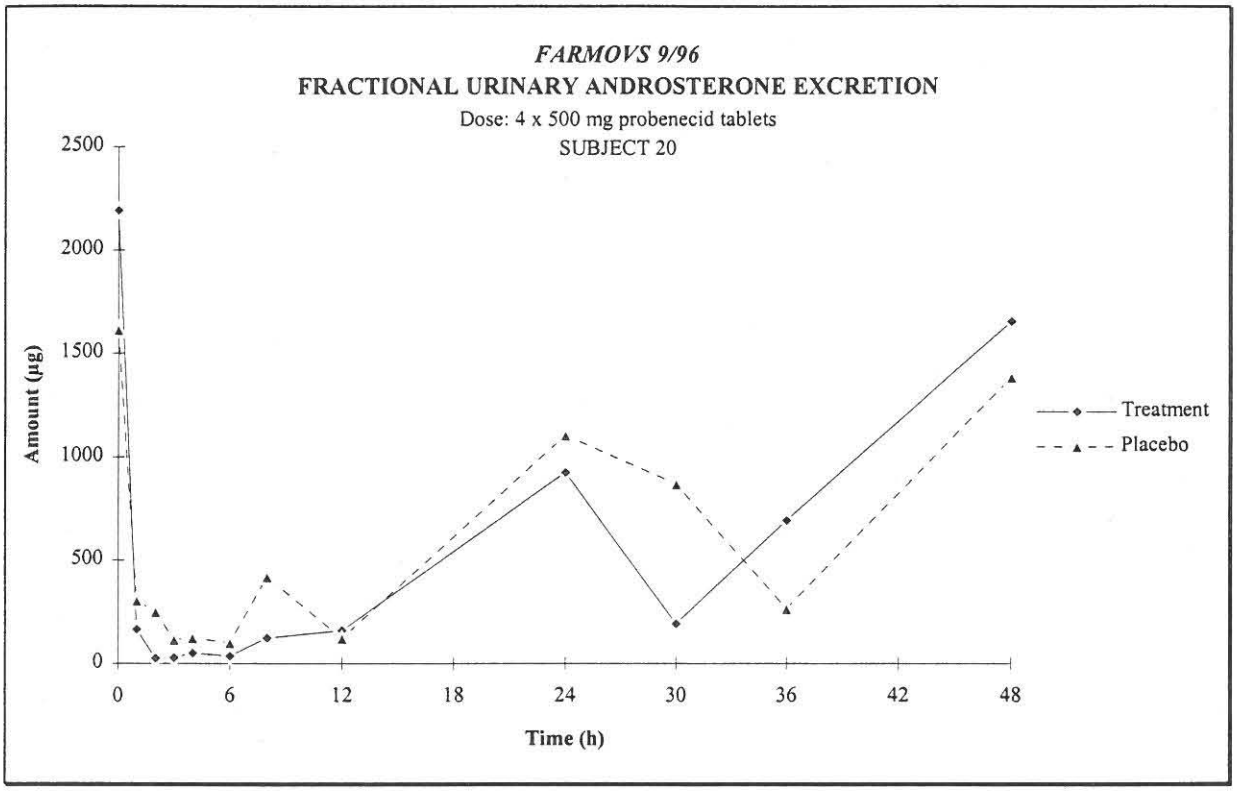






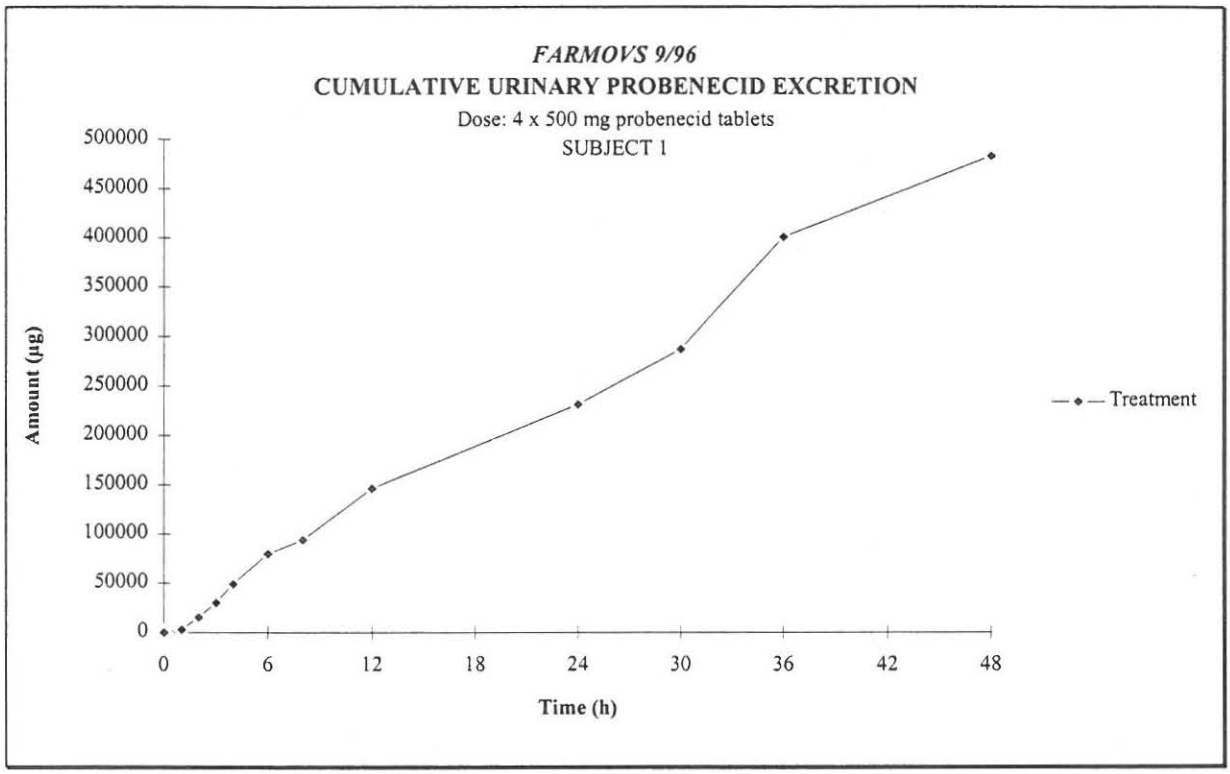


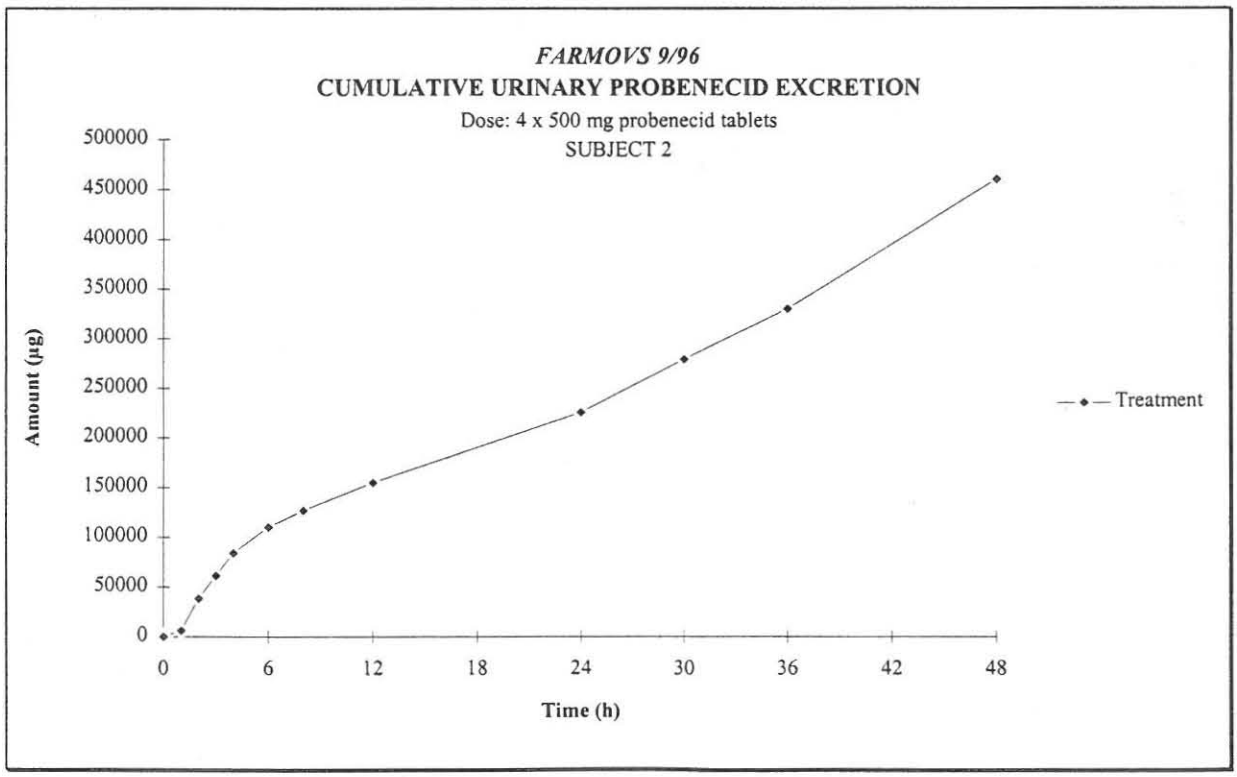


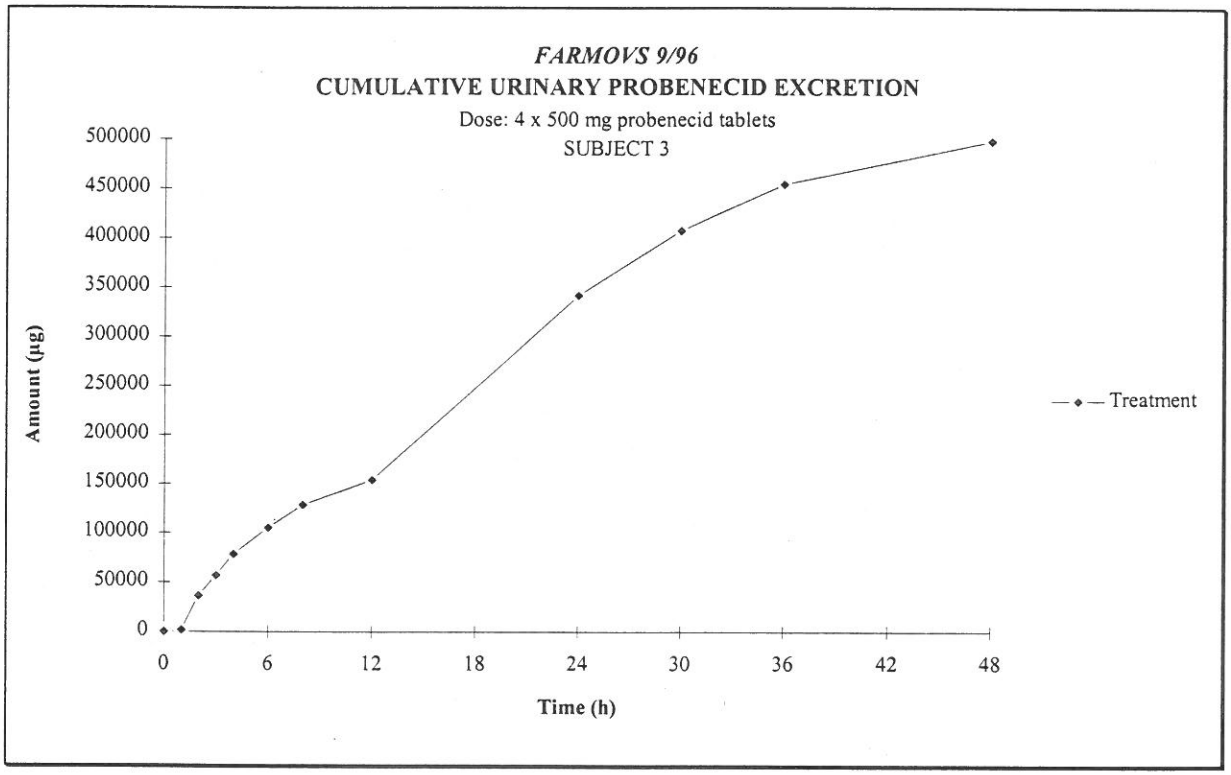


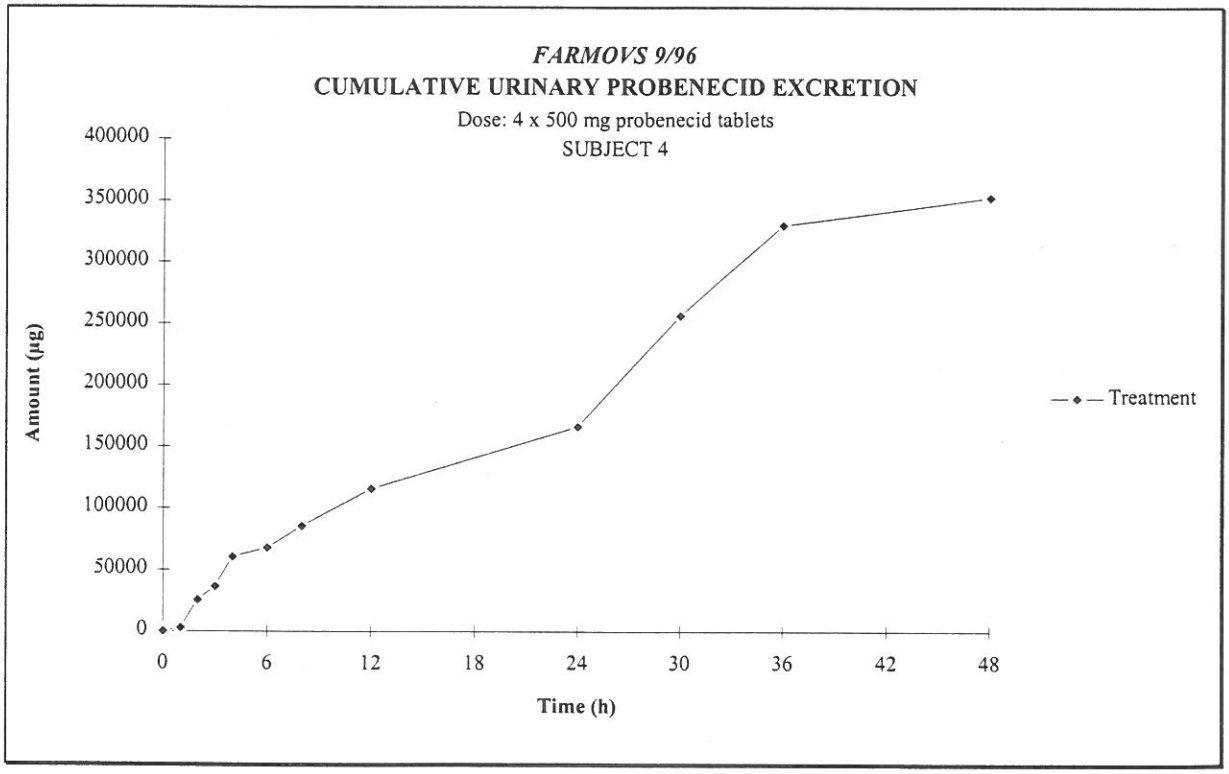
6.2.2 Probenecid

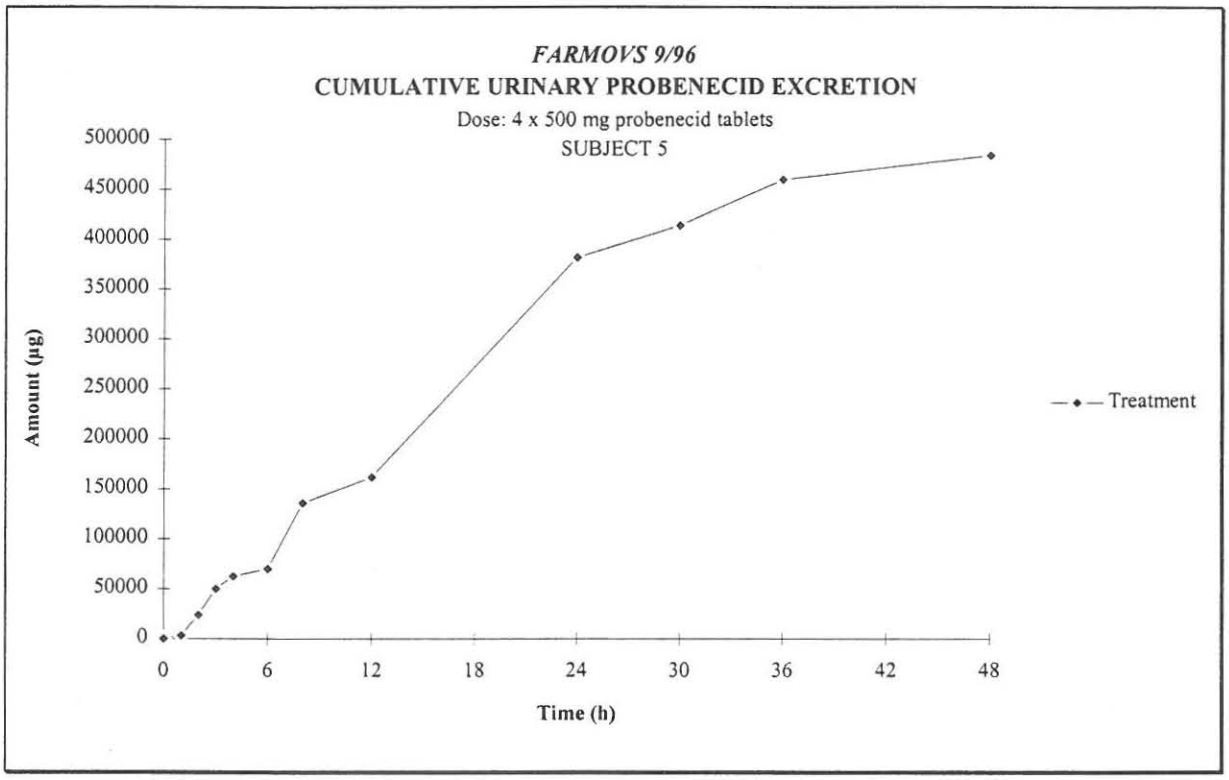
INDIVIDUAL CUMULATIVE URINARY PROBENECID EXCRETION FOR EACH SUBJECT AND TREATMENT

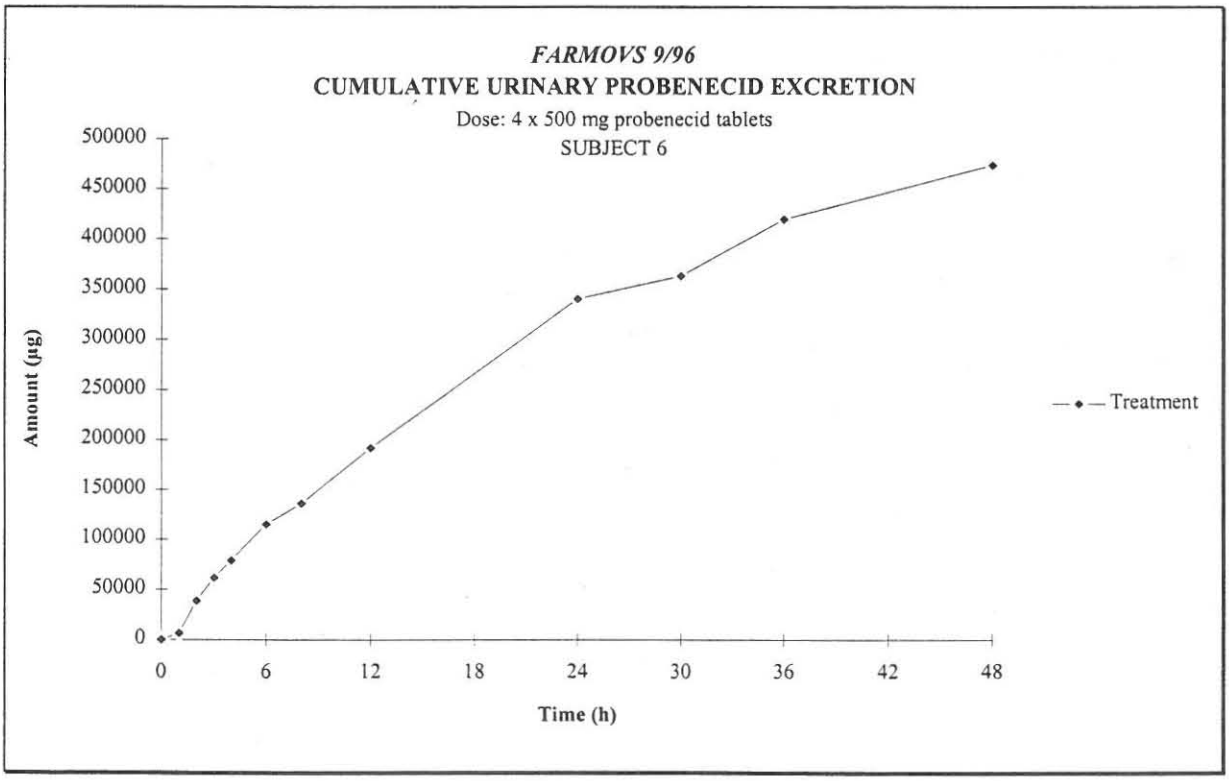


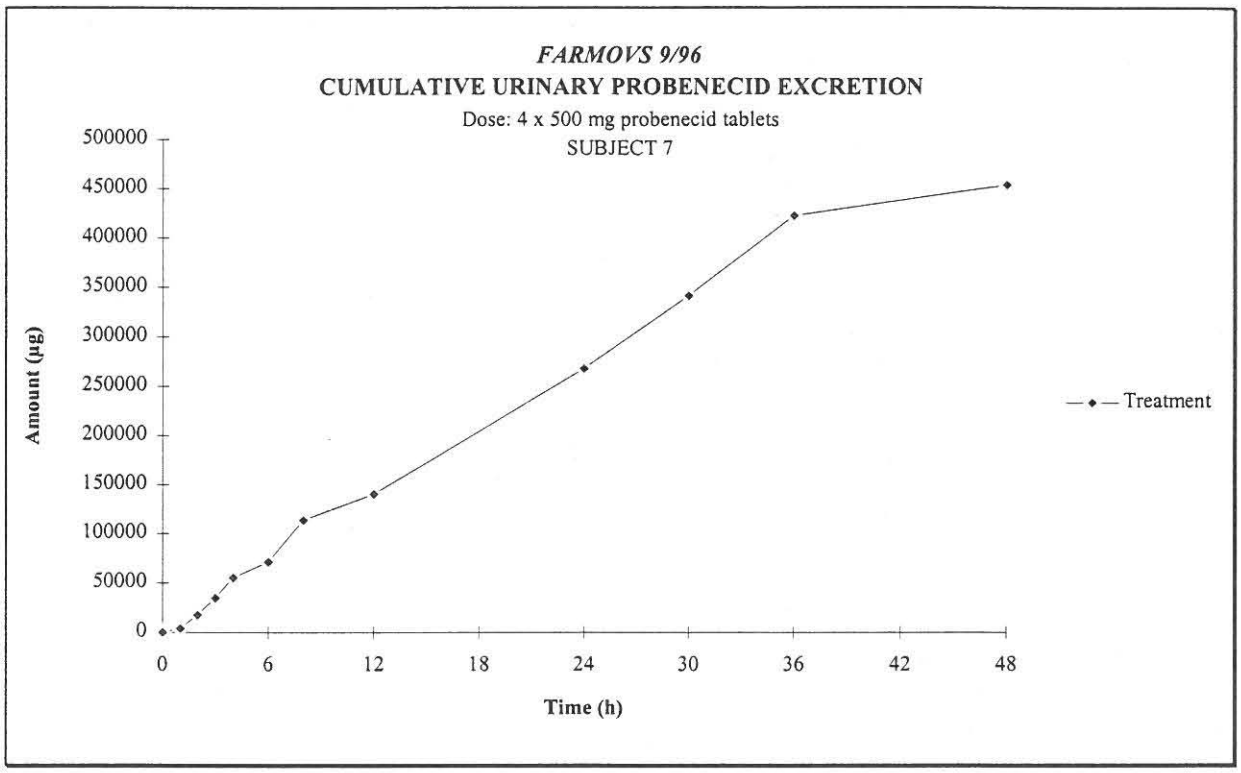


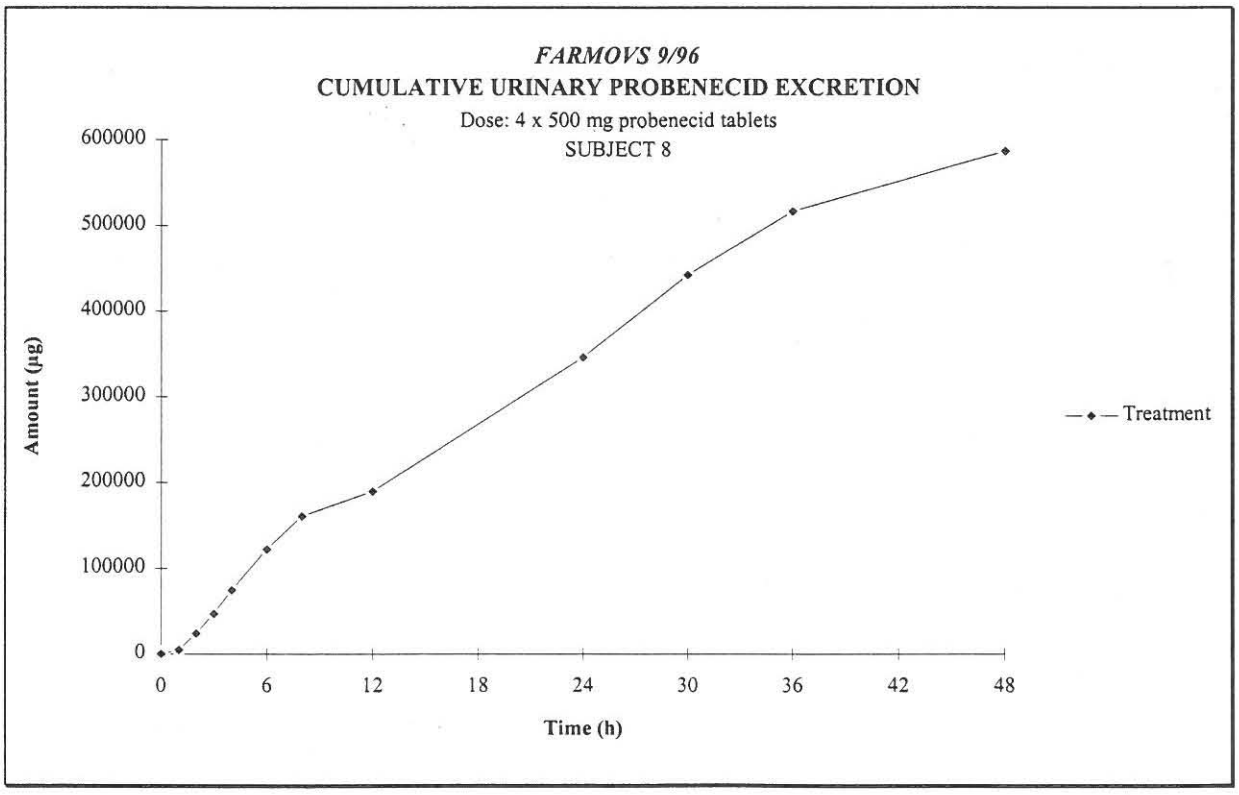


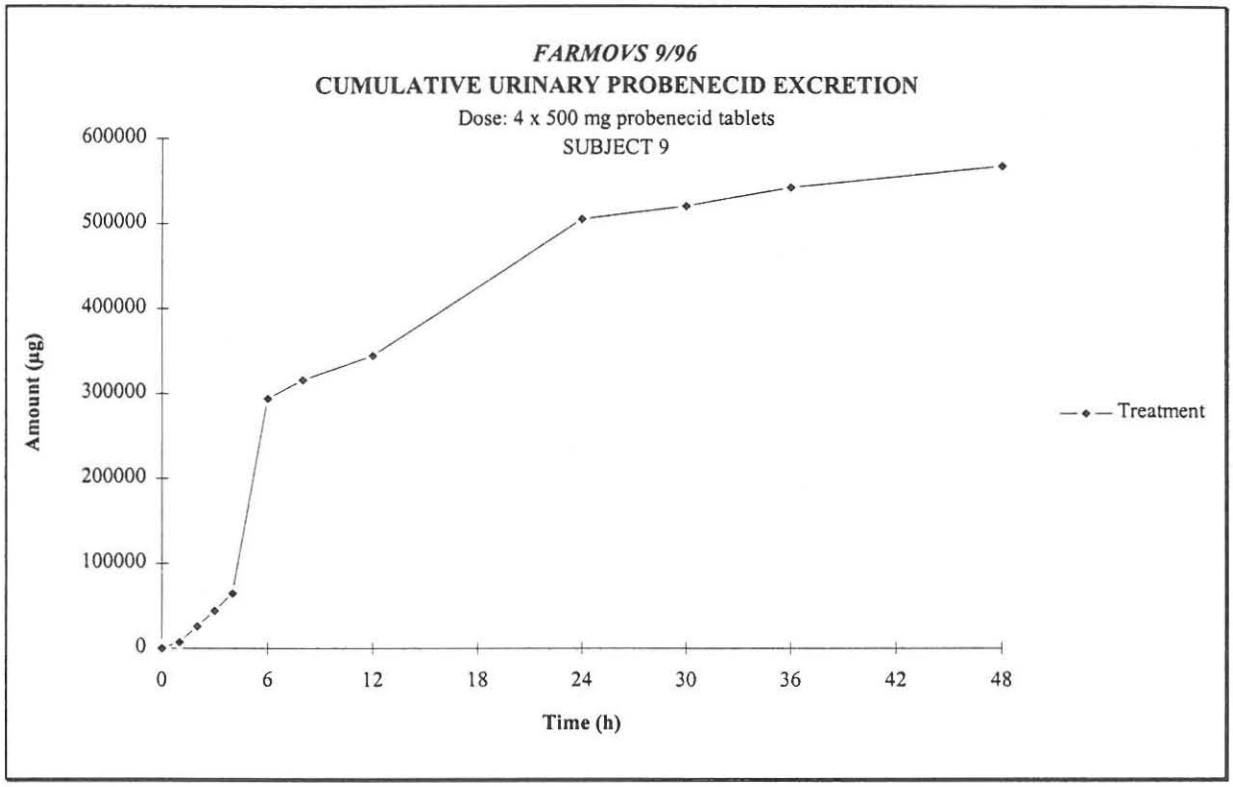


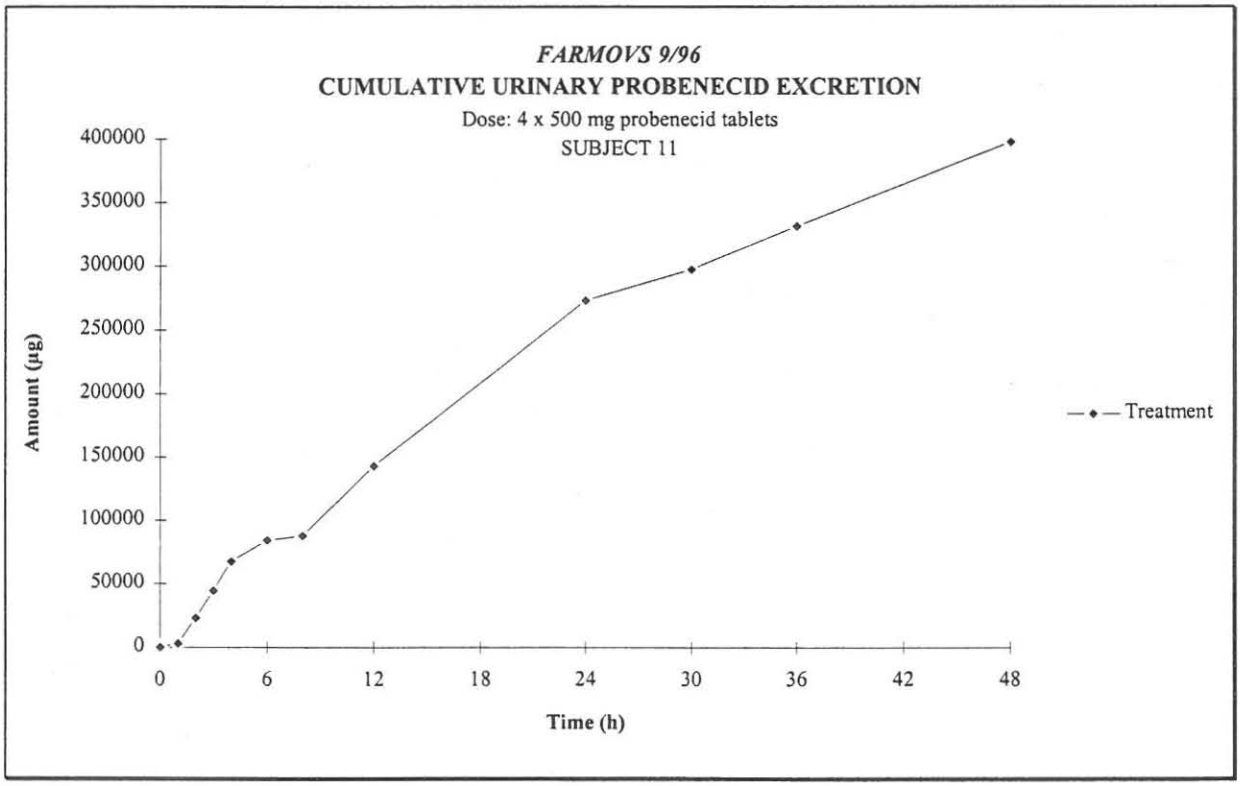


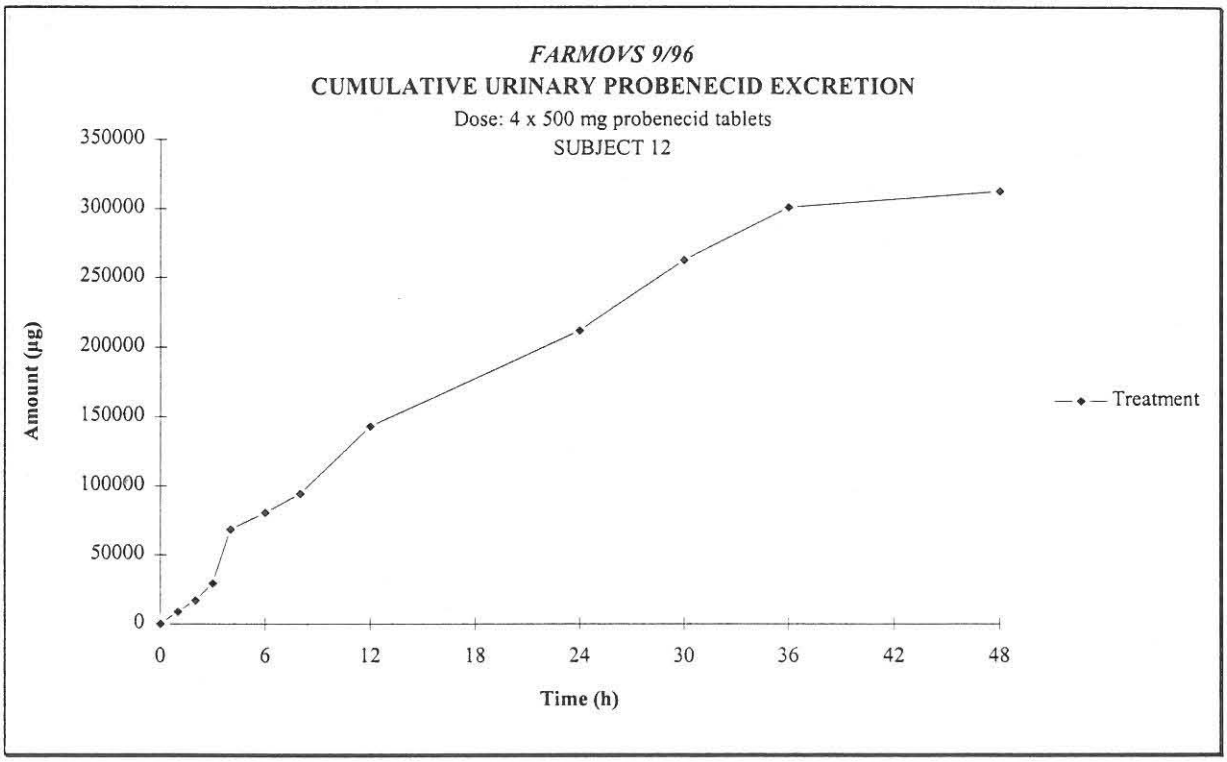


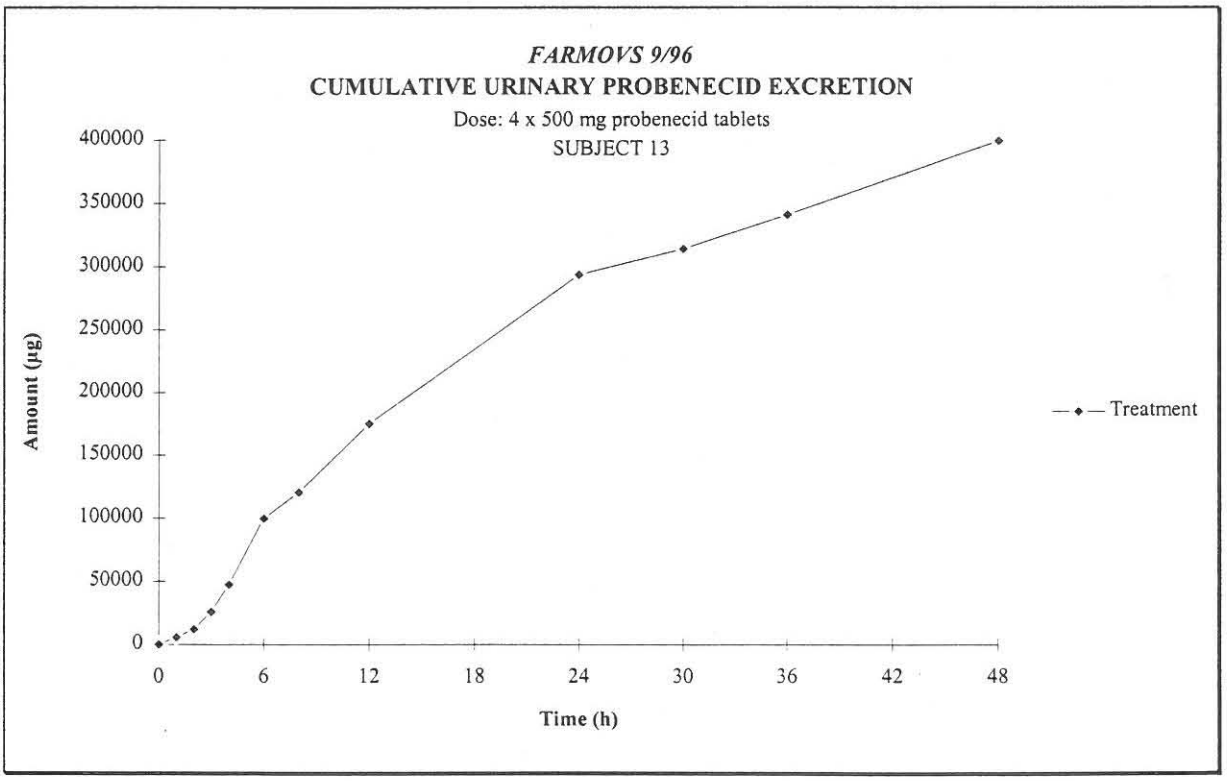


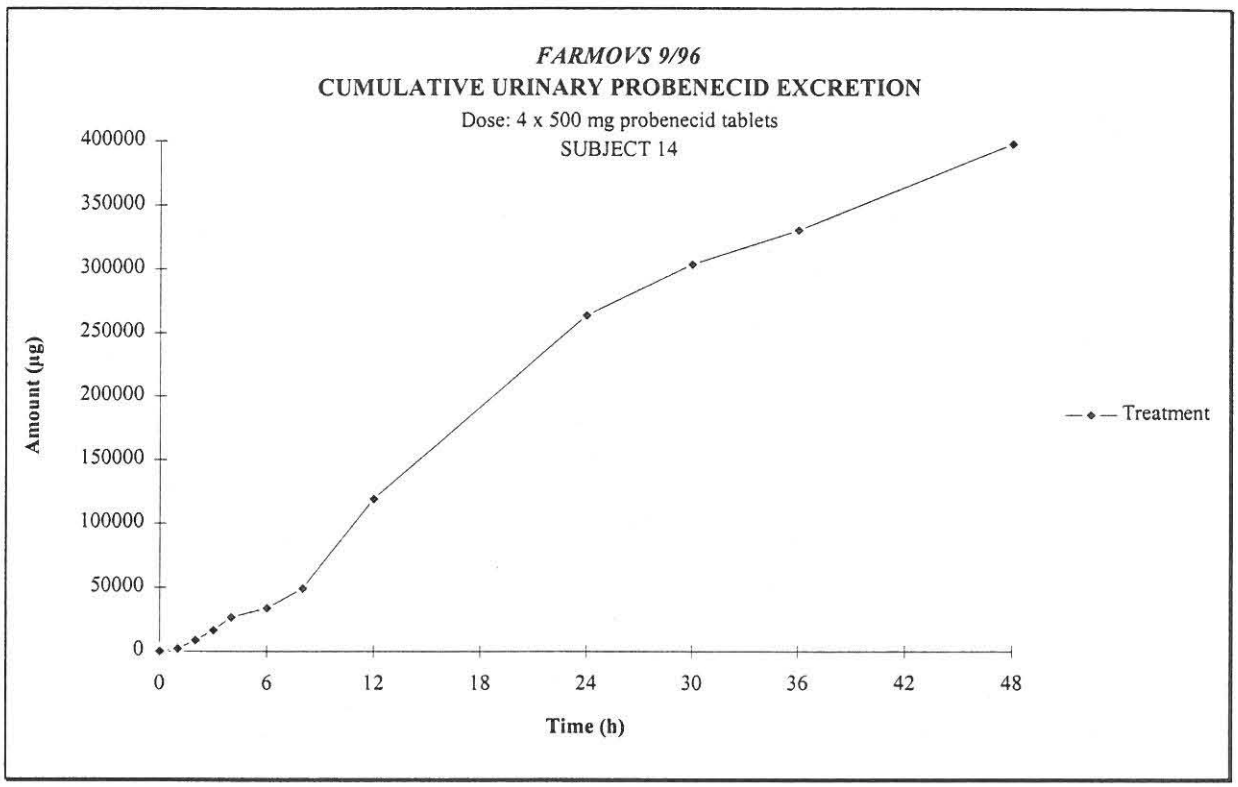


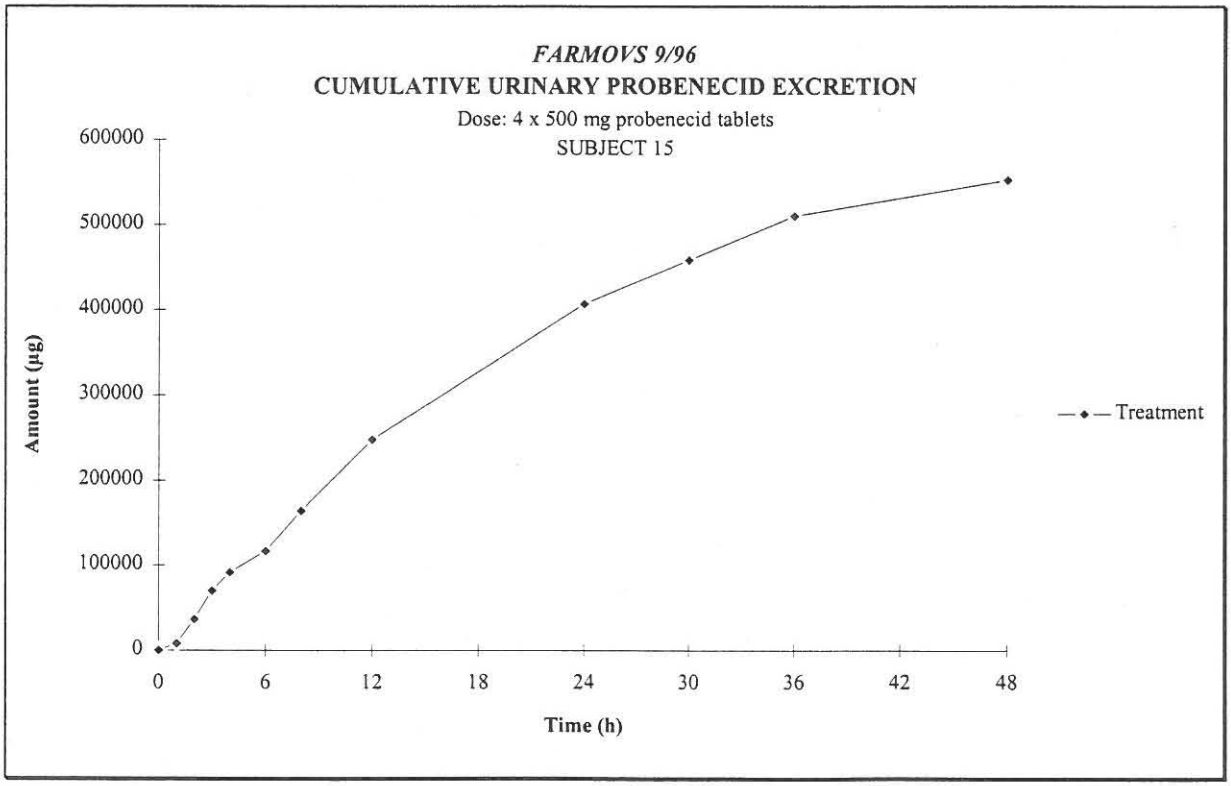


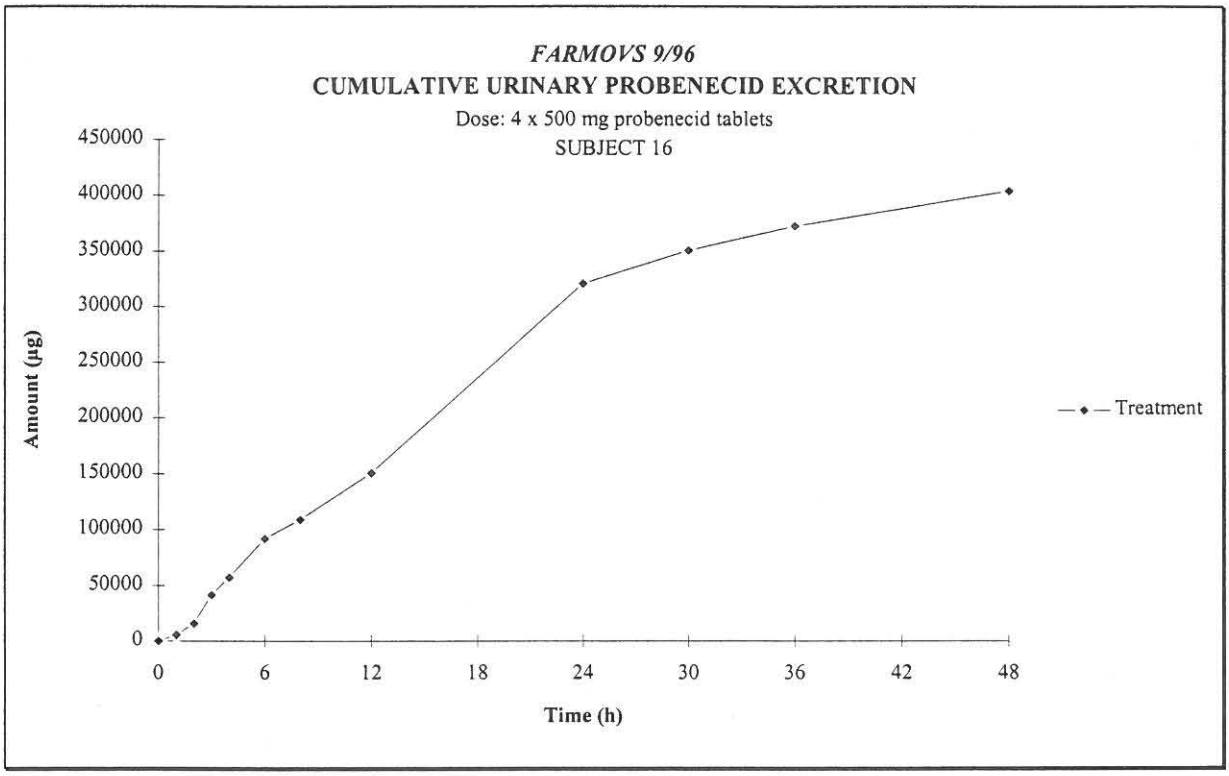


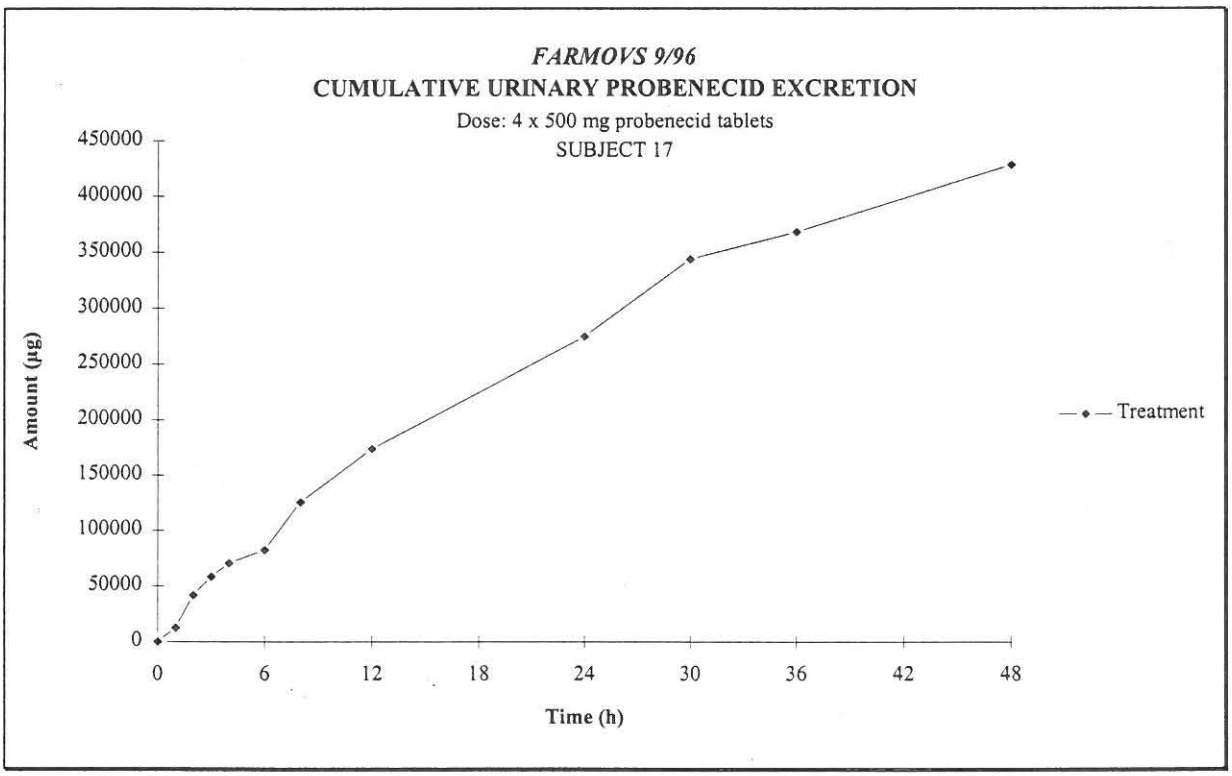


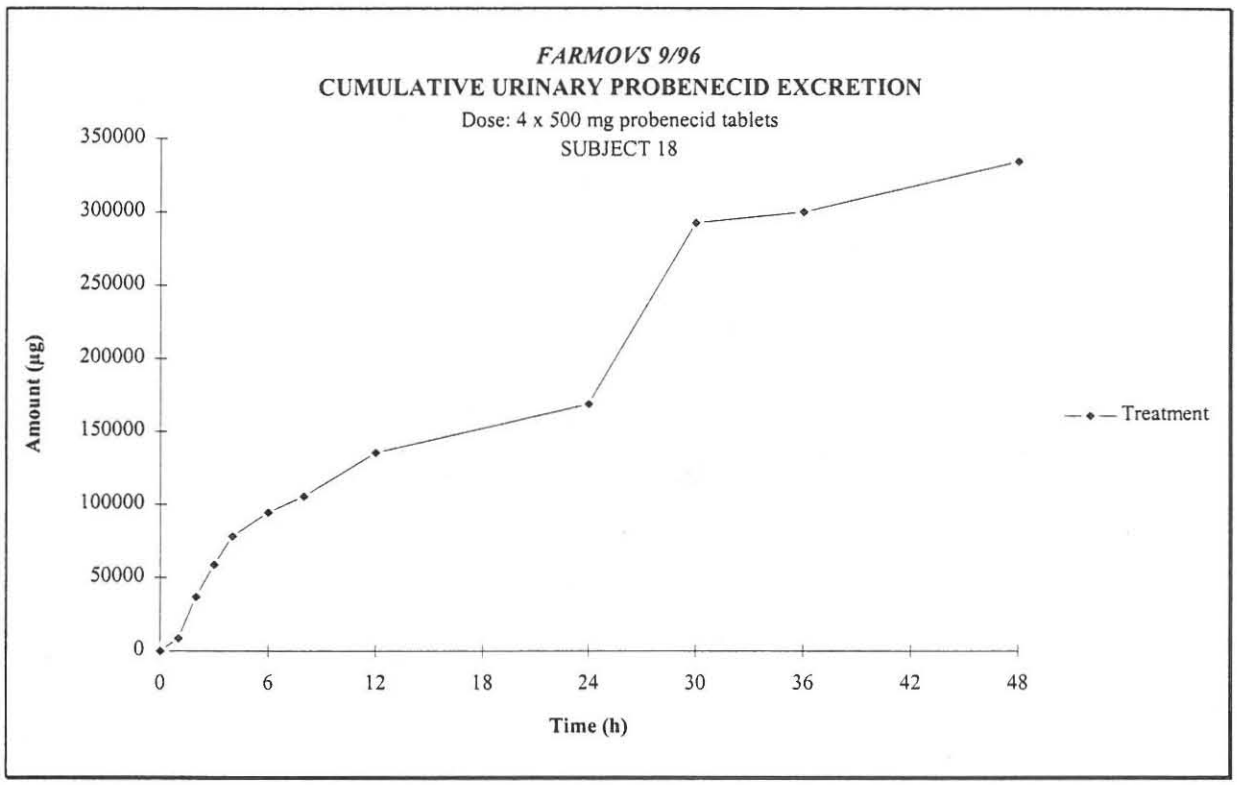


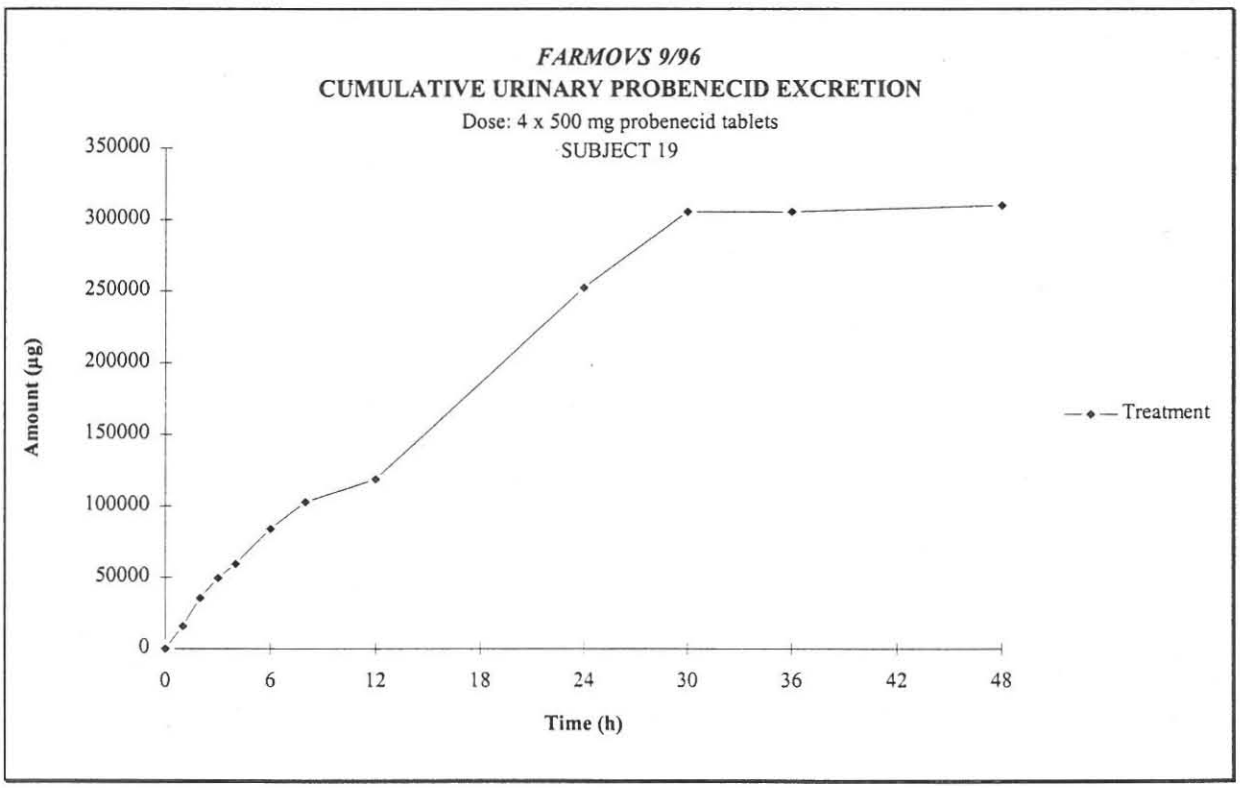


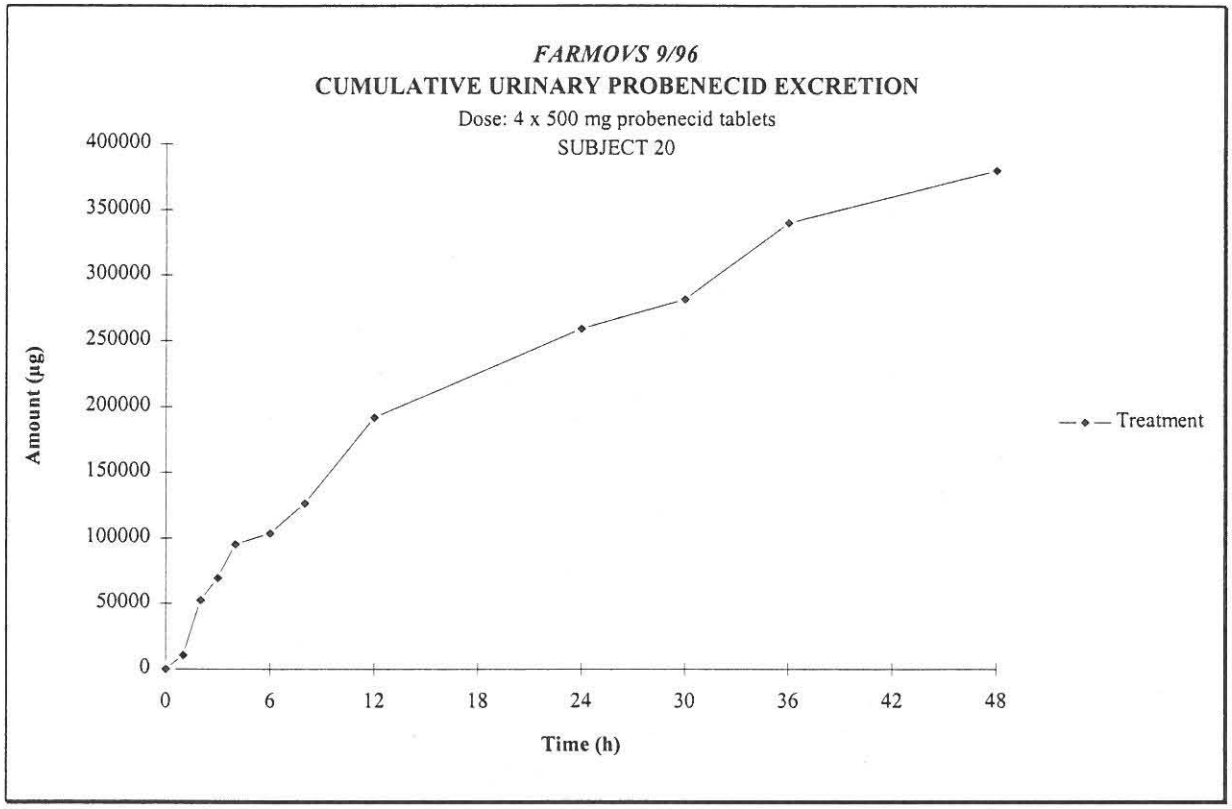












INDIVIDUAL FRACTIONAL URINARY PROBENECID EXCRETION FOR EACH SUBJECT AND TREATMENT